

TO STUDY THE MOST INVINCIBLE MICROANIMAL TARDIGRADE

(IDENTIFICATION AND SURVIVABILITY)

NIBEDITA PRADHAN

Registration No:-180705180111

Under the guidance of

SUNITA SATHPATY

DEPARTMENT OF ZOOLOGY

SCHOOL OF APPLIED SCIENCES

CENTURIAN UNIVERSITY OF TECHNOLOGY AND MANGEMENT,

ODISHA, INDIA.

Abstract

The tardigrades are popularly known as water bears, are micrometazoans with four pairs of Lobopod legs. Tardigrades survive in lichens and mosses, usually associated with water film on mosses, liverworts and lichens. More species are found in milder environments such as meadows, ponds and lakes. These are closely related to Arthropoda and Nematodes based on their morphology. They are the microanimals which can live in extreme conditions. Knowing about their adpatation, they are quite investigating among the most stress tolerant animals known. When experiencing adverse environment conditions, they enter a reversible ametabolic state termed as anhydrobiosis and is known to confer tolerance to a variety of stresses.

INTRODUCTION

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

Water bears resemble small bears (0.1-1mm) complete with claws, but a few too many legs (4 pairs).They are either armored (Eutardigrada).

Each of eight legs has claws, which when combined with their slow gait, makes them look very much like miniature polar bear with some extra legs.

Kingdom: Animalia

Scientific name: Tardigrade

Phylum: Tardigrada

Super phylum: Ecdysozoa

Rank: Phylum

Tardigrades are first describe by the German Zoologist Johan August Ephraim Goeze in 1773. Who called then little Water bears. In 1777, the Italian biologist Lazzaro Spallanzani named them Tardigrada which means slow steppers. Tardigrades are found everywhere, from mountaintops to the deep sea and mud volcanoes and from tropical rainforest to the Antarctic. These are among the most resilient animals known, with individual species able to survive extreme condition such as exposure to extreme temperatures, extreme pressures (both low and high), air deprivation, radiation and starvation—that would kill most like others known forms of life. Tardigrades have survived exposure to outer space. It withstand large amount radiation, it withstand temperatures ranging from 300°F to near absolute zero, it possibly withstand the pressures of the deepest trenches of the oceans. It can endure extreme temperatures ranging from more than 100°C down to 196 degrees below zero. They can exposed to 5000 grays of radiation and to be just fine while 5 to 10 grays are fatal to human. Recently scientists studied the genome to find out the mechanism by which they are so tolerant to radiation. It was originally thought that Tardigrades simply had robust DNA repair mechanism to help them survive extreme radiation damage, this might still be the case, but researchers found a novel Tardigrade protein named Dsup which stands for damage suppressors, that wraps itself around the Tardigrade DNA and act as a radiation shield. To demonstrate that Dsup has protective properties, they took human kidney cells and inserted the Dsup gene into them when they cultured those human. Dsup cells and exposed them to radiation they found that they experienced 40% less DNA damage than control cells.

The phylum Tardigrada is often classified as one of the “lesser-known” groups of protostomes. Although much has been learned about these organisms, as evidenced by publications of international symposia (Higgins, 1975; Węglarska, 1979; Nelson, 1982a; Bertolani, 1987a; McInnes and Norman, 1996; Greven, 1999; Kristensen, 2001), the tardigrades present an exceptional opportunity for teaching and research, especially in the areas of development, evolution, and ecology. These micrometazoans may play an

important role in the elucidation of metazoan phylogeny, particularly with respect to the evolution of the arthropods. Tardigrades have a ubiquitous distribution, being found in a diversity of niches in terrestrial, freshwater, and marine environments throughout the world, ranging from the abyss in the deep sea to the highest mountains (Ramazzotti and Maucci, 1983; Kinchin, 1994).

SUITABILITY OF BRYOPHYTES AS HABITAT

In water, algae as well as bryophytes, provides homes. The terrestrial tardigrades depends on the water drops that adhere to mosses and liverworts (Hingley 1993) and therefore of ten termed limno-terrestrial.

Water bears are found in habitats from hot springs to layer under ice (in cryoconite holes in glaciers) and occupy every continent of the world.

Ramazzoti and Maucci (1983) considered mosses suitable habitat based on three needs of 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

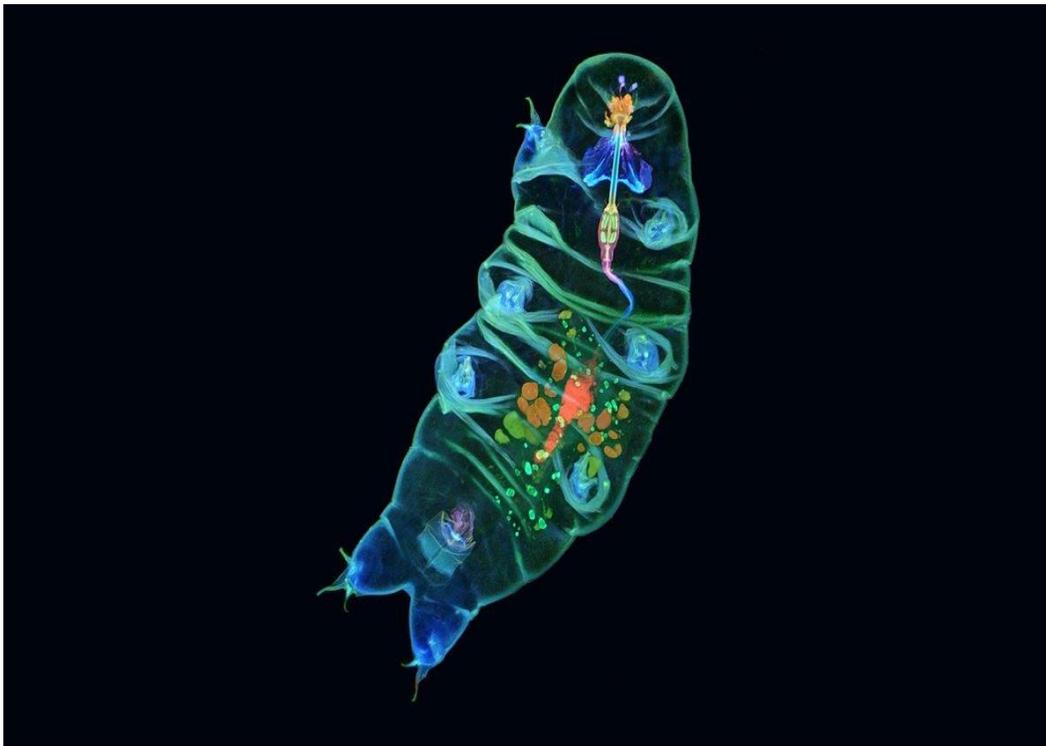
Based on these criteria, bryophyte are particularly good habitats for tardigrades in several ways.

Bryophytes experience drying, which most do slowly, permitting the tardigrades like wise dry slow and both have a tolerance to dehydration that permits them to survive in adverse condition (Kinchin 1994).Span when it is interrupted by such dormancy period.

ADAPTATION OF TARDIGRADES:

Their greatest adaptation that permits them to live. They live in a habitat that permits them to dry slowly and go into a dormant state a kind of behavioral/physiological adaptation.

Tardigrade wins international photo prize in the year 2019



A colorful photo of a tardigrade, popularly known as a “water bear,” has won a top prize in the first-ever Olympus Global Image of the Year Award that was created to honor the best life science microscopy images.

OBJECTIVES

The objective of present study are as follows :

The identification of tardigrades from moss as well as from lichen in rainy season and in winter season. Its survivability condition in exposure to freezing that is cryobiosis a form of cryptobiosis, when the temperature decreases and water in the cell has frozen. Molecular mobility stops the result is tun, tardigrade survive for decade. Its survivability condition in salt solution that is osmobiosis that permits tardigrade to tolerate high salinity and form a tun, but tardigrades have already high salt tolerance.

MATERIALS AND METHODS

Area of Study :

Mosses and lichens selected for this experimental study, which was collected from Centurion University (Bbsr campus), Jatni and some sample was collected from backyard of my house in Bhubaneswar during rainy and winter season.

SAMPLING AND EXTRACTION OF EXPERIMENTAL MICROANIMAL :

Some lichens scrapped off from a rock in the backyard of my house . There are several colours of lichens in the area ,they all may have different diversities of tardigrades in winter season in the month of december.



Then the moss leafy green stuff ,again their may be several types.As i get proficient start to fan out and explore other environments . I found moss on the wall and from a nearby mango tree inside cutm campus .



Moss collected from a wall in CUTM campus in winter season
(In the month of December)

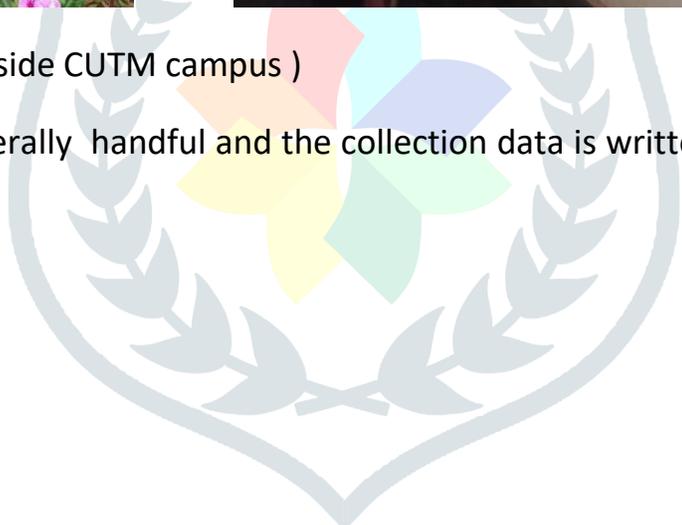


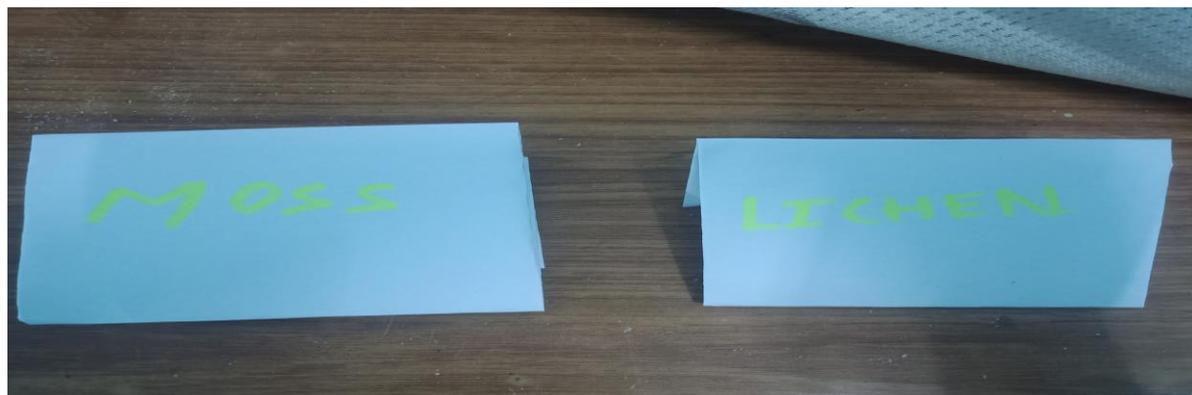
Moss collected from a nearby mango tree in CUTM campus in rainy season (In the month of August)



(Collection of moss inside CUTM campus)

The sample size is generally handful and the collection data is written on the paper with a marker.



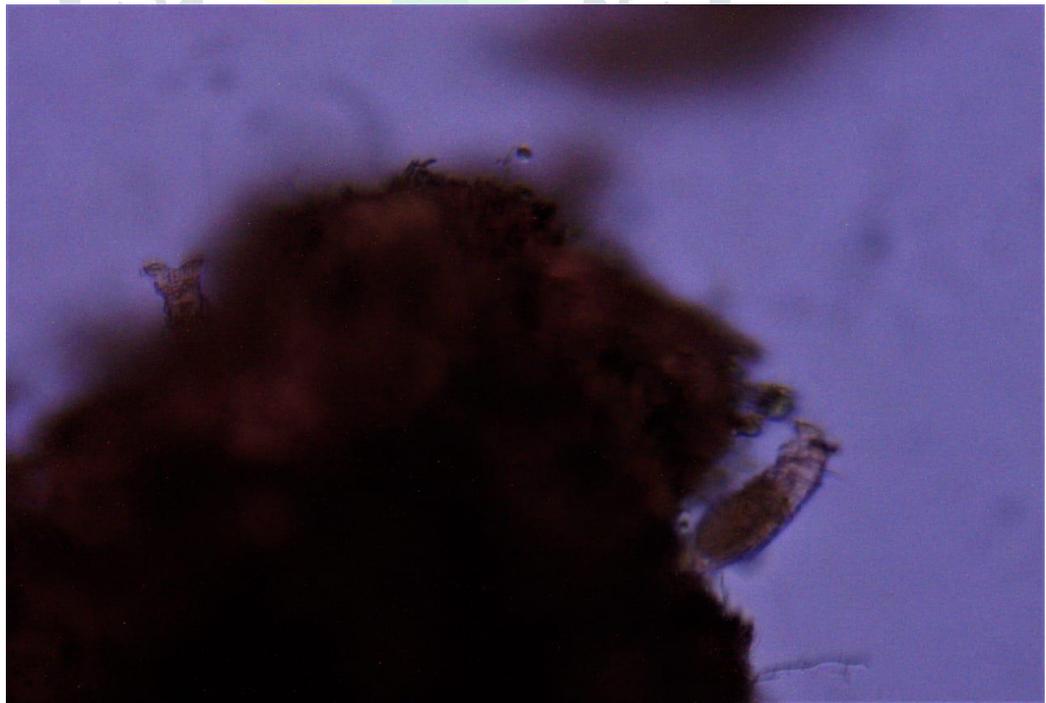


(Collection of moss and lichen was marked up in a marker)

Then the moss was soaked overnight (24 hours) in a paper cup which was scrapped off from the wall during rainy season.



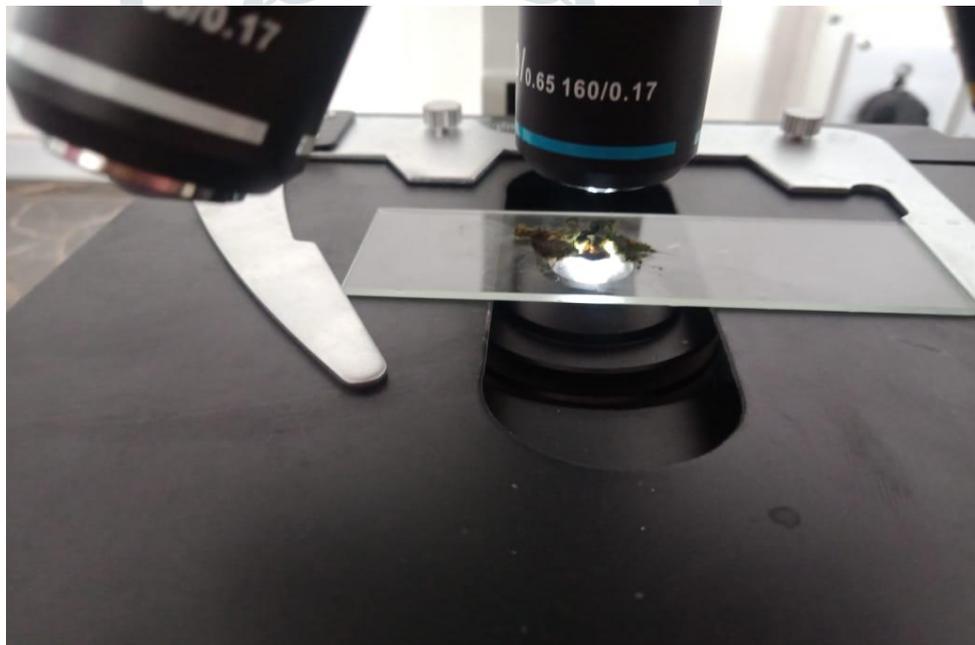
In the next morning in lab ,debris pipette off from the bottom of cuo and small amount of moss is taken in a slide and examined under a compound microscooe to study the microanimal (Tardigrade)



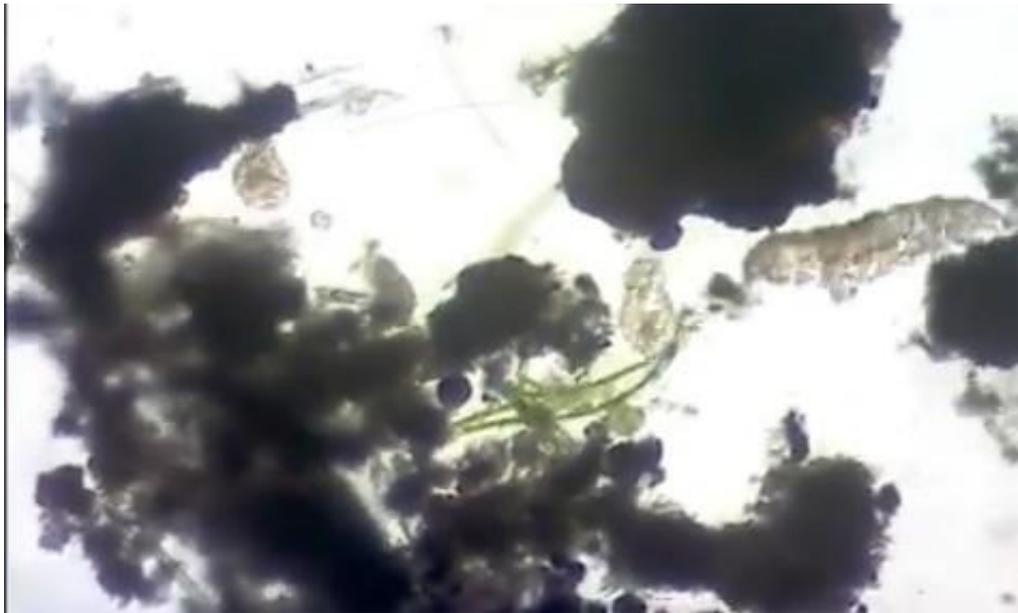
Then finally the microanimal tardigrade appears with 5x objective ,10x eye piece ,40x magnification .



Lichen soaked overnight (24 hours)



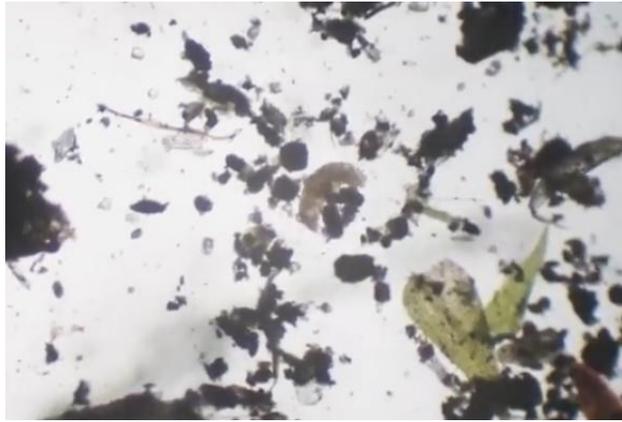
Next morning ,it examined under the microscope and by facing many difficulties after 2 hours the tardigrade appears.



(Tardigrades appears from lichen in winter season)



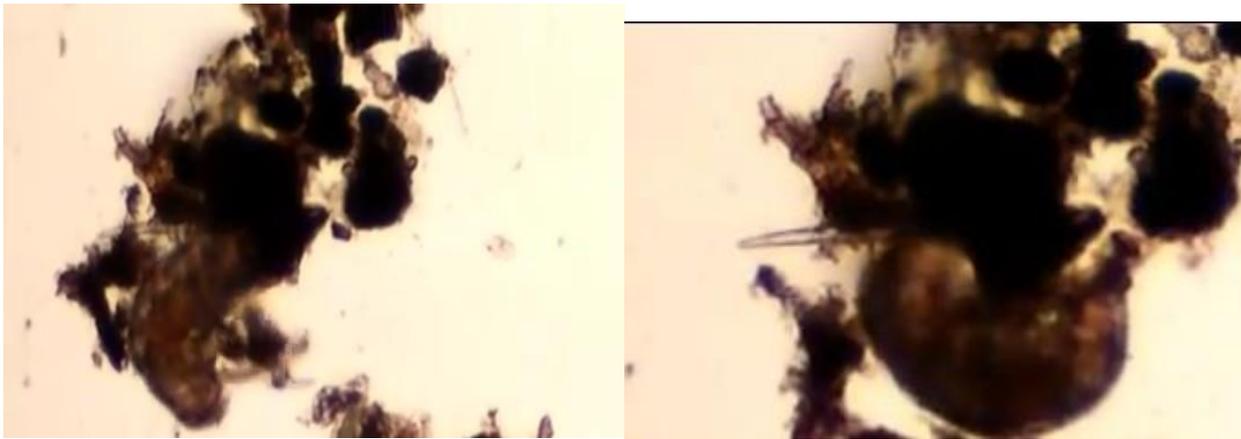
Moss are submerged with salt solution in a petridish for 48 hours ,then it examined under the microscope .



Tun formation in salt solution (osmobirosis)



Moss exposure to cold temperature (-18 degree Celcius in freezer)



Tun formation due to decreasing of temperature (cryobiosis)

RESULT

Tardigrades were identified in eastern zone (Bhubaneswar,Odisha) in rainy season and in winter season from moss and lichen.

Survivability after exposure to low temperature (cryobiosis) freezing at -18 degree celcius (approx) in freezer,the microanimal formed as tun.

In salt solution (osmobiosis) tardigrade was turned to tun.

DISCUSSION

In both experiments , the tardigrades is in cryptobiotic state that is cryobiosis and osmobiosis. In cryobiosis the temperature decreases and water in the cell has frozen ,molecular mobility stops permitting the tardigrade to survive in low temperature and it formed as tun.Glycerol act as antioxidant ,being embedded in ice probably also prevents oxidation damage . In this cryobiotic state ,the tardigrades can survive for decades. In osmobiosis ,that permits some species to tolerate high salinity and to form tun. It is initiated when the animal experiences an external salt concentration.

Determining if an animal is viable or dead can be problematic with tardigrades as they tend to become passive under unfavorable conditions such as asphyxia. In an attempt to avoid unfavorable conditions and maintain a somewhat identical post experimental environment, the animals were regularly supplied with clean water and using a Pasteur pipette oxygen was “bubbled” into the water to avoid low oxygen levels. Fundamentally two conditions can be recognized 1) dead or 2) living, though the living can be subdivided into two sub conditions. Active: exhibiting coordinated movement; and passive: exhibiting no coordinated movement, a twitch of a claw or jerk of a leg at most. In the results only

dead or living animals is presented. The terms active and passive are used for interpretation of behavior during observation. A major problem during the post experimental observation period was an inevitable fungal infestation and the massive propagation of their hyphae, sometimes covering everything in the embryo dishes. To diminish effect on mortality the fungi had to be removed, or “weeded” out, as often as possible.

CONCLUSION

The animals of the phylum Tardigrada remain a little-known, little-studied group despite their overall abundance. ... At the same time, tardigrades present a unique opportunity for research. They are easy to work with, forgiving about collection, and their removal does little environmental damage. They have been applied in transplantology because of their cryptobiotic effect. Though there are studies emphasizing on their identification and importance, there is a need for more research to understand their relevance in different fields of science.

REFERENCE

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 highaltitude 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 Raman imaging study on a living multicellular organism. Accessed 20 May 2012 at <http://www.cognicao.pt/AbstractsECSBM/index_arquivos/089_Bonifacio.pdf>.

Bordenstein, Sarah. 2008. Tardigrades (Water Bears), Microbial Life, Educational Resources. Accessed on 9 February 2008 at <<http://serc.carleton.edu/microbelife/topics/tardigrade/>>. Brave New Biosphere. 1999. The Why Files. University of Wisconsin. Accessed on 7 January 2008 at <<http://whyfiles.org/022critters/meiofauna.html>>. Brueggemann, 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., Baganá, 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 point

