11th International Symposium on Tardigrada

Conference Guide

Tübingen, Germany,
3-6 August 2009

Nikon

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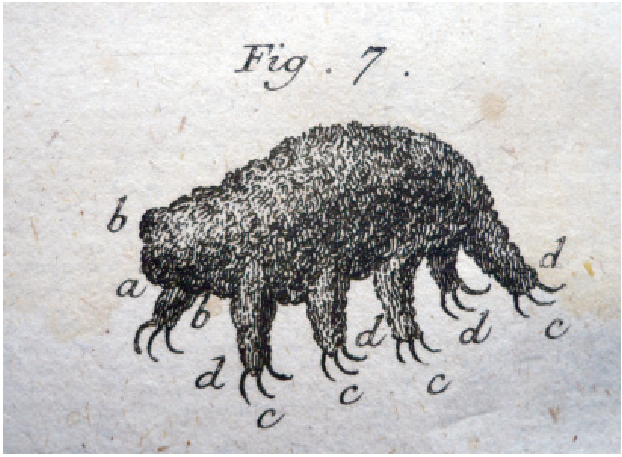
Welcome address of the organizers

Dear colleagues,

Symposia are by definition meetings of like-minded people to present new findings and to correlate available data and ideas concerning a specific theme. Just now, you have the abstracts of the 11th International Symposium on Tardigrada in your hand, which may serve as guide for the next days.

The abstracts cover a very broad spectrum of topics ranging from ecology to physiology of water bears and from α-taxonomy to systematics and phylogenetic hypotheses. At present, tardigradology is not at all a playing field of a few specialist. Rather, a growing number of followers takes possession of these fascinating creatures to study their biology with a set of established and novel techniques. This interest in tardigrades is reflected also by the growing number of attendants.



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. Halle: Gebauer.

Thus, an important goal of the symposium is to gather the active workers, i.e. known experts in specific fields of tardigradology, but also those, who want to become experts, to discuss the various topics presented. We believe that a common forum will lead to a better communication and cooperation.

Enjoy our meeting and Tübingen,

Hartmut Greven, Karin Hohberg & Ralph O. Schill

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Conference program		Monday, 03.08.2009		Tuesday, 04.08.2009		Wednesday, 05.08.2009	Thursday, 6.8.09
08:00-09:00		Registration & Poster Installation					
09:00-09:15		Opening Ceremony	Session 3: ecology & others	Romano *et al.*	Session 4: physiology & molecular biology	Persson *et al.*	all day excursion **Castles & Caves**
09:15-09:30		Opening Lecture		Accogli *et al.*		Cesari *et al.*	
09:30-09:45				Sewell & Romano		Altiero *et al.*	
09:45-10:15		*Coffee break*		*Coffee break*		*Coffee break*	
10:15-10:30	Session 1: Phylogeny	Jørgensen *et al.*		Hohberg		Frohme *et al.*	
10:30-10:45		Hansen & Guil		Dafoe & Romano		Hengherr *et al.*	
10:45-11:00		Guil *et al.*		Degma *et al.*		Reuner *et al.*	
11:00-11:15		Marchioro *et al.*		Jönsson		Grohme *et al.*	
11:15-11:30		Guidetti *et al.*		Ramsay & Marley		Schokraie *et al.*	
11:30-11:45		Mayer		Marley		Dandekar *et al.*	
11:45-13:15		*Lunch*		*Lunch*		*Lunch*	
13:15-13:30	Session 2: Taxonomy & Biogeography	Bartels *et al.*		Vicente		Horikawa *et al.*	
13:30-13:45		Fontoura & Morais	Sess-ion 4	Møbjerg *et al.*		Kunieda *et al.*	
13:45-14:00		Kristensen *et al.*		Halberg *et al.*		Katayama *et al.*	
14:00-14:15		Lundbye *et al.*	Poster session	Poster session		Arakawa *et al.*	
14:15-14:30		Keyence Mikroskopie				Resumée	
14:30-15:00		*Coffee break*		*Coffee break*		*Coffee break*	
15:15-15:30		Faurby *et al.*		Poster session	type material	Examination of type material	
15:30-15:45		McInnes & Sands					
15:45-16:00		Miller & Beasley					
18:00-19:00		*Guided city tour*		*Boat trip*			
19:00-20:00		*Guided city tour*		*Beer garden*			
20:00						*Conference dinner*	

Conference program   Monday, 03.08.2009

08:00-09:00	Registration & Poster Installation
09:00-09:15	Opening Ceremony
09:15-09:45	Opening Lecture by D. Nelson & R. Bertolani
09:45-10:15	Coffee break

Session 1: Phylogeny (Chair: R. M. Kristensen)

10:15-10:30	Jørgensen *et al.*
10:30-10:45	Hansen & Guil
10:45-11:00	Guil *et al.*
11:00-11:15	Marchioro *et al.*
11:15-11:30	Guidetti *et al.*
11:30-11:45	Mayer
11:45-13:15	Lunch

Session 2: Taxonomy & Biogeography (Chair: D. Nelson)

13:15-13:30	Bartels *et al.*
13:30-13:45	Fontoura & Morais
13:45-14:00	Kristensen *et al.*
14:00-14:15	Lundbye *et al.*
14:15-14:30	„Keyence Mikroskopie“
14:30-15:00	Coffee break
15:15-15:30	Faurby *et al.*
15:30-15:45	McInnes & Sands
15:45-16:00	Miller & Beasley
18:00-19:45	Guided city tour

Changes in scientific approach over the past 35 years of Tardigrada Symposia

R. Bertolani (University of Modena and Reggio Emilia, Italy),
D. R. Nelson (East Tennessee State University, Tennessee, U.S.A.)

The story of the Tardigrada Symposia began 35 years ago in Pallanza, Italy. It was the idea of professor Livia Tonolli, who wanted to honor professor Giuseppe Ramazzotti, at that time the authority in the tardigrade scientific field and also her friend. Given that tardigrades were a neglected group of metazoans (and still so to date), this particular event could have ended with that occasion, but this was not the case. With some gaps, the meetings have continued, promoting scientific exchange among the researchers and improving the research quality. By examining the topics of the symposia over these 35 years, we can understand the changes in the scientific approach to studying tardigrades. Taxonomy and faunal reports were the predominant topics of the first symposium, but also ultrastructural morphology, ecology, physiology, cytotaxonomy and reproductive biology were represented. Moreover, the first studies utilizing SEM began with that occasion. Faunal and taxonomic studies often represented the main topics during the years, but also other kinds of research involving phylogeny, ecology, physiology, ultrastructure of organs and cells always accompanied them. From these points of view, often tardigrades were compared with other animal groups, giving a broader scope to the research. Molecular aspects were first considered in the third symposium (1980, Tennessee), but reconsidered only in the symposium in Cambridge (1994) and developed further from the symposium in Denmark (2000). The last two symposia have seen an increased number of participants and scientific contributions. In line with the improved means of international publications, the quality of the proceedings has also increased, with the use in the last ten years of qualified international journals with peer review and a high impact factor.

The phylogeny of Echiniscidae (Tardigrada, Heterotardigrada) inferred from molecules and morphology

A. Jørgensen (University of Copenhagen, Denmark); N. Møbjerg (University of Copenhagen, Denmark); R.M. Kristensen (University of Copenhagen, Denmark).

The heterotardigrade family Echiniscidae consists of 12 currently recognized genera that are generally represented by few species with the exception of the genera *Echiniscus* and *Pseudechiniscus*, which have numerous species. The echiniscids inhabit a wide variety of different moss and lichen substrates.

Phylogenetic relationships within the Echiniscidae have been investigated by Kristensen (1987) and Jørgensen (2000) using morphological characters primarily based on cuticular plates, sense organs and buccal apparatus. Although both studies were based on the characters gathered by Kristensen (1987) substantial differences occur in the resulting cladograms. Jørgensen (2000) did not infer the *Echiniscus* evolutionary lineage proposed by Kristensen (1987) and the "*Pseudechiniscus* lineage" did not include *Pseudechiniscus* and had a different topology. The present study includes molecular data from the nuclear 18S and 28S genes and the mitochondrial COI gene, as well as morphological data to investigate the incongruences between the studies by Kristensen (1987) and Jørgensen (2000) and between morphology and molecules. *Echiniscoides* and *Oreella* have been included as outgroups and ten genera of echiniscids as the ingroup in the phylogenetic analyses. The data sets were analysed individually and combined with Bayesian inference. In the present study Echiniscidae was always inferred to be monophyletic. Various analyses without COI was conducted following the indication of substitutional saturation in COI by a transition/transversion plot. Analysis of the combined data set (18S, 28S and morphology) inferred a "*Pseudechiniscus* lineage" not including *Mopsechiniscus*, which surprisingly was inferred as the second most basal echiniscid. Furthermore the inferred "*Pseudechiniscus* lineage" only included part of the *Pseudechiniscus* species included in the present study making the genus polyphyletic. The five Echiniscus species included in the analysis were monophyletic and sister-group to *Testechiniscus* (COI had *Echiniscus* paraphyletic). *Parechiniscus* was inferred to be the basal taxon of *Echiniscidae* a phylogenetic position that corresponds well with its weakly sclerotized dorsal plates.

Jørgensen, A. 2000. Cladistic analysis of the Echiniscidae Thulin, 1928 (tardigrada: Heterotardigrada: Echiniscoidea). Steenstrupia 25: 11-23.

Kristensen, R.M. 1987. Generic revision of the Echiniscidae (Heterotardigrada), with a discussion of the origin of the family. In: R. Bertolani (Ed.), Biology of Tardigrades. Selected Symposia and Monographs U. Z. I., 1, Mucchi, Modena. pp. 261-335.

Morphological phylogeny among heterotardigrade families and subfamilies

J.G. Hansen, N. Guil (University of Copenhagen)

The number of investigations of the relationships among tardigrades has steadily increased in recent years due to the implementation of computer analyses and molecular sequencing techniques. Within Heterotardigrada, the advancement has been most prominent for the order Echiniscoidea, using both molecular and morphological data sets. On the contrary, computerized analyses have not yet been utilized in morphological phylogenies of Arthrotardigrada. In addition, difficulties associated with specialized sampling techniques as well as with the accurate species identification have caused arthrotardigrade phylogeny research to be neglected and only a few species have been included in molecular phylogenetic studies within Tardigrada. Moreover, Heterotardigrada has never been included when invertebrate phyla are studied. With the prospect of tomorrows accelerated advancements in molecular sequencing techniques, it becomes more and more urgent to improve our knowledge of character evolution and phylogeny in marine tardigrades, in order to understand the evolution within the whole phylum as well as among invertebrate phyla. Except for a single study, relationships between heterotardigrade families and their subfamilies have not been systematically evaluated. In the present study, we assemble a set of morphological characters that are informative at family and subfamily level within Heterotardigrada. Characters selected are related with surface structures (lobes, plates, sensory structures), morphology of claws and buccopharyngeal apparatus, as well as characteristics associated with development and reproductive system. Characters previously used in high taxonomic level analyses are critically evaluated and new characters are proposed. The suitability of the characters selected for phylogenetic reconstruction is discussed and a data matrix to be used as reference in future investigations of the phylogeny within Tardigrada is generated.

A molecular approach to the phylogenetic status of *Echiniscus* (Tardigrada, Heterotardigrada, Echiniscidae)

N. Guil (University of Copenhagen, Denmark); A. Jørgensen (University of Copenhagen, Denmark); G. Giribet (Harvard University, USA); R. M. Kristensen (University of Copenhagen, Denmark)

Echiniscus was the first described Heterotardigrada genus, the second for the phylum Tardigrada, after *Macrobiotus*. These two genera have become the most diverse ones in species within the phylum although some doubts have arisen about their monophyletic status. Morphological features that characterize Echiniscus are related to body plates and buccopharyngeal apparatus morphology, mainly having to do with cuticle ornamentation and surface appendages when distinguishing among *Echiniscus* species. However, morphological and molecular approaches have demonstrated limitations in the use of these morphological characteristics in taxonomy within the *Echiniscus blumi-canadensis* series. We investigated, in a taxonomically broader perspective, the monophyletic status of the genus *Echiniscus* using molecular information. To do so, we extracted DNA from single specimens for diverse *Echiniscus* species (collected in different geographic areas) and related genera

(*Bryodelphax*, *Proechiniscus*, *Pseudechiniscus*) of Echiniscidae, with the aim of testing monophyly of *Echiniscus* as well as for genetic diversity comparisons. In addition, information about heterotardigrades from GenBank was included. Widely amplified fragments (present in majority of species from GenBank) from 18S rRNA and 28S rRNA were sequenced for phylogenetic and genetic structure analyses. Differences in haplotype variation with 18S rRNA data were due to among genus variability (85%) more than within genus variation (15%). On the contrary, among genus variation (48%) explained almost the same genetic variability than within genera (52%) when 28S rRNA information was used. Special discussion is offered on the validity of *Echiniscus* and *Pseudechiniscus* since monophyly status of the latter was questioned by the data. Monophyly of *Echiniscus* is discussed from parsimony and Bayesian results analysing both, each gene separated and combined. Moreover, some haplotypes are shared among different Echiniscus species. Possible sister groups are discussed. Problems with GenBank information and its impact on the monophyletic status of *Echiniscus* are discussed.

The phylogeny of Eutardigrada, a molecular approach and its morphological support

T. Marchioro, M. Cesari, R. Bertolani, L. Rebecchi, R. Guidetti
(University of Modena and Reggio Emilia, Italy)

The phylogeny of the different taxa of Tardigrada was not often studied in the previous century, except for a few praiseworthy and very important exceptions. The lack of phylogenetic studies was possibly due to the difficulty of many tardigradologists to interpret the low number of characters associated with often very small structures. The result was that alphabetical order was used very frequently, and is often still employed, in citing tardigrade taxa. In the last few decades, the advent of molecular methods has produced a large number of phylogenetic studies not only in the animal kingdom but also with tardigrades (mostly eutardigrades). Results obtained from molecular studies confirm part of the phylogenetic interpretation based on morphology and partly disagree with it. Classes and orders have been confirmed, but this has not always happened for the families. Nonetheless, many genera, possibly important for phylogenetic evaluations, still have not been considered or have only been partly taken into account. Moreover, in our opinion, to date too little attention has been placed on the relationships between molecular and morphological data. Therefore, we have analyzed several genera and species of eutardigrades from a molecular point of view, utilizing our experience with morphology to correlate morphology with the molecular results, and discussing the erection of superfamilies, within a coherent morphological framework.

The buccal-pharyngeal apparatuses in tardigrades: anatomy, function, and phylogenetic significance

R. Guidetti, L. Sarzi Amadè, L. Rebecchi, R. Bertolani
(University of Modena and Reggio Emilia, Italy)

Sclerified structures of the buccal-pharyngeal apparatus (BPA) are key structures for tardigrade taxonomy, and also provide data for understanding tardigrade evolution. However, there is a paucity of data regarding the relationships between anatomy and function of BPA structures. The anatomy of the BPA of several eutardigrade species belonging to several genera (*Milnesium, Dactylobiotus, Murrayon, Adorybiotus, Richtersius, Macrobiotus, Paramacrobiotus, Minibiotus, Bertolanius, Ramazzottius, Doryphoribius, Isohypsibius, Thulinius, Hypsibius, Borealibius, Diphascon, Astatumen, Platicrista*) and of some heterotardigrade species belonging to three genera (*Echiniscus, Pseudechiniscus, Cornechiniscus*) have been studied with different techniques. BPAs have been extracted from the animals and the sclerified structures observed by scanning electron microscopy (SEM) or the BPAs of in vivo or mounted specimens have been observed by light microscopy. In addition, since the presence of $CaCO_3$ in the BPA is used for phylogeny in Heterotardigrada, we performed a chemical analysis by a microanalysis system (SEM X-EDS) of the BPA sclerified structures to detect the presence of Ca. New morphological data have been obtained, increasing our knowledge of the structures and working mechanisms of the BPA. Phylogenetic relationships of the studied taxa have been also hypothesized based on the BPA anatomies.

New data on the onychophoran brain composition and their bearing on the phylogenetic position of tardigrades

G. Mayer (University of Melbourne, Australia)

Understanding the composition of the arthropod “head” has caused much controversy in the past. In particular, the homology of modified “head” appendages and their innervation from the brain are the most contentious issues in evolutionary biology. As the closest relatives of arthropods, onychophorans (velvet worms) play an important role in this debate. However, the relationship of the onychophoran “head” segments to those of arthropods is far from clear. Here, I present evidence from neural development and neuroanatomy in Onychophora that only two anterior-most body segments contribute to onychophoran brain formation. These results contradict previous assumptions of a tri-partite brain composition in Onychophora and suggest that the tritocerebrum is an evolutionary novelty of arthropods. These findings indicate a gradual increase

of complexity in the organization of the “head” within the Panarthropoda during evolution and have an impact on the phylogenetic position of tardigrades.

Allometry and the removal of body size effects in the morphometric analysis of tardigrades

P. J. Bartels (Warren Wilson College, USA); D. R. Nelson (East Tennessee State University, USA); R. P. Exline (Warren Wilson College, USA)

Quantitative traits are an important part of tardigrade taxonomy for both heterotardigrades and eutardigrades. Because most quantitative traits vary as a function of body size, differences in body size complicate comparisons between individuals or populations. Thus, body size effects must be eliminated in morphometric analysis. Although ratios (size of character / body size) are often used to attempt this, they only work for the specific case of isometry (i.e., when a structure grows proportionally to body size). Ratios do not eliminate body size effects for allometric (disproportionate) growth. We measured 27 traits in 97 specimens of *Macrobiotus tonollii* and 14 traits in 100 specimens of *Echiniscus virginicus* and found that many traits are allometric rather than isometric. Thorpe’s normalization (Y* = Yi [Xo/Xi]b, where Yi = the individual measure of a quantitative trait, Xo = mean body size, Xi = the corresponding measure of individual body size, and b = slope of the regression of log Y vs log X) provides a way to eliminate body size effects for any trait regardless of its relationship to body size. Using the data from *Macrobiotus tonollii* and *Echiniscus virginicus*, we show that Thorpe’s normalization does successfully remove body size effects while pt indices generally do not. Furthermore, we demonstrate the effectiveness of Thorpe’s normalization in species delineations of *Macrobiotus recens* and *M. hufelandi*, two species that differ primarily in a few quantitative traits and overall body size. From these illustrations, we propose that the allometric exponent (b) and the Y-intercept (a*) of the regression line of Thorpe normalized traits vs. buccal tube length or body length are invaluable metrics in tardigrade systematics.

Assessment of traditional and geometric morphometrics for discriminating cryptic species of the *Pseudechiniscus suillus* complex (Tardigrada: Echiniscidae)

P. Fontoura, P. Morais (Eco-Ethology Research Unit FCT-331/94 and Department of Zoology and Anthropology, Faculty of Sciences, University of Porto, Portugal)

Tardigrade taxonomy is mainly based on the analysis of morphological characters. However the low number of useful taxonomic characters and the remarkable degree of phenotypic plasticity exhibited by a considerable number of species are responsible for great problems in the identification process. It is consensual among taxonomists that more characters, namely quantitative characters, are needed to solve this problem. Despite difficulties to obtain precise measurements because of the orientation and deformation of the mounted small sized specimens, the introduction of morphometric analysis in the description of Eutardigrade species (e.g., pt indices) showed to be a useful tool for specific diagnosis. This kind of analysis also showed that intraspecific variability was much more restricted than previously thought and what was considered a single species is now considered a complex of species (e.g., *Macrobiotus hufelandi* group). Unfortunately, in Heterotardigrades (Fam. Echiniscidae) this problematic is especially pronounced. The situation is particularly fluid in the so-called *Pseudechiniscus suillus* complex, a group of species very difficult to distinguish from one another that needs a complete revision. In this study traditional morphometric and geometric morphometric relationships among five populations of the *P. suillus* group, from four different Atlantic islands (three in the Azores and S. Tomé) and from the Portuguese mainland, were investigated to explore their potential for discriminating cryptic species. Eleven traditional morphometric variables were used to describe variation by means of principal component analysis. In addition, two traits, claws and dorsal plates, were analyzed using a geometric morphometric approach which has the ability to evidence very subtle differences in shape. The results of both analyses suggest that four morphs exhibiting different sizes and shapes of cirrus A, claws and dorsal plates could be recognized. The high taxonomic value of those characters allied to some other qualitative aspects, such as the cuticle sculpture seems to indicate that these morphs correspond to different species.

Tardigrades from Easter Island, Rapa Nui (Pacific Ocean, Chile) with the description of a new species of *Bryodelphax* (Heterotardigrada: Echiniscidae)

R. M. Kristensen (Københavns Universitet, Denmark); Ł. Michalczyk (University of East Anglia, UK); Ł. Kaczmarek (A. Mickiewicz University, Poland)

Easter Island or Rapa Nui is the most isolated island inhabited by humans in the World and its nearest inhabited neighbour is Pitcairn Island, about 2250 km away. Rapa Nui is also the easternmost inhabited island of Polynesia. The nearest point in South America is Conception in Chile about 3872 km away. The terrestrial and tidal tardigrade fauna of Rapa Nui is from a biogeographic point of view very interesting, i.e. the main question is the relationship between the Rapa Nui tardigrade fauna and the faunas of Polynesia and South America. A new heterotardigrade, *Bryodelphax* sp. nov. is under description from a moss sample collected in the Rano Kau Crater on Easter Island (Rapa Nui) in 1989. More than 200 specimens were found in the very small moss sample. The description is mainly based on DIC and TEM observations. The new species belongs to the group of species within the genus that have ventral plates. *B*. sp. nov. is similar to *B. weglarskae* (Pilato, 1972), *B. sinensis* (Pilato, 1974) and *B. iohannis* Bertolani, Guidi & Rebecchi, 1995 but differs from them mainly by a different number and arrangement of ventral plates. This is the first record of the genus *Bryodelphax* from Easter Island/Rapa Nui.
The other species we found in the same moss sample was *Macrobiotus hufelandi* and *Milnesium tardigradum*, both already known from Easter Island (Rahm 1936). In 2009 new collections of both marine and terrestric tardigrades were made. Eleven species has until now been sorted out. The marine genus *Echiniscoides* from Anakena Beach has been used for molecular data by Søren Faurby. The tardigrade fauna of Rapa Nui seems to be more similar to the fauna of South America than to that of Polynesia which suggests that the main source of immigrants was/is South America.

Rahm, G. (1936). Tardigraden der Osterinsel (Rapa Nui). Zoologischer Anzeiger 115: 27-28

New morphological and biogeographical data of the marine genus *Archechiniscus (*Arthrotardigrada: Halechiniscidae)

H. Lundbye, J. G. Hansen, R. M. Kristensen (University of Copenhagen, Denmark)

Controversy of the phylogenetic position of the genus *Archechiniscus* through the years has primarily been due to its remarkable claw configuration, possessing a combination of Halechiniscidae-like internal claws (crescent shaped) and Echiniscoidea-like external claws (J-shaped with a secondary spur). Furthermore, the median cirrus, a character traditionally used to separate the two heterotardigrade orders Echiniscoidea and Arthrotardigrada, is present in only one out of three described species. Archechiniscus spp. has been reported from all around the world, with the majority being assigned to *A. marci* Schulz, 1953. In this study, material from the Azores, Australia, New Zealand, Solomon Islands, Egypt, Japan, Norway (from a tropical aquarium with coral sand) and U.S Virgin Islands was examined by light microscopy and eight new species were identified. Additionally type material of *A. minutus* from Italy, was included in the examination. All species, including *A. minutus*, have median cirrus or a median pore that we interpreted as median cirrus. The different species displayed an unusual high variation on cephalic, leg- and trunk appendages. Furthermore two different types of seminal receptacle structures were observed. A biogeographical analysis of the genus revealed a tropical to subtropical distribution and on the basis of morphology of the genital complex and common habitat, two evolutionary lines were proposed: one tidal on barnacles and the other interstitial in carbonate sand.

Speciation in *Echiniscoides:* The role of geographic isolation and climate

S. Faurby (Aarhus University, Denmark), A. Jørgensen (University of Copenhagen, Denmark), R. M. Kristensen (Natural History Museum, Denmark) , P. Funch (Aarhus University, Denmark)

Speciation can follow two main routes: 1) As a neutral event when a barrier impedes gene flow between two populations and over time differences accumulate and results in speciation; 2) Under selection if two adjacent populations diverge to specialise to different environments. The importance of these two routes to speciation has rarely been tested in microscopic animals since cosmopolitanism was previously thought to be the rule and isolation as a speciation factor therefore seemed very unlikely. Recent genetic studies indicate that cosmopolitanism is not as common as once thought and therefore both factors deserve attention. In this study we tested the importance of these factors using species from the genus *Echiniscoides*. If historical isolation was the only driver of speciation, species from adjacent regions should be sisters regardless of climatically differences, whereas species in similar climatic but geographically distant regions should be sisters if speciation was entirely climatically driven. Up to 40 genetic based taxa of *Echiniscoides* depending on the threshold and mode of lineage separation were collected from 48 sites worldwide, most of which likely representing undescribed species. Each taxon had a rather restricted geographic distribution and there are no indications that cosmopolitanism exists within the genus, since no taxon was collected from separate oceans or hemispheres. A Bayesian phylogeny of the genus was constructed and compared with genetic and environmental matrices of the differences between each taxon. The correlation between the genetic data and the various matrices was made in a modified Mantel-test we developed to take phylogenetic uncertainties into account instead of testing on the single most likely tree as is the most usual approach. Our results showed no correlation between habitat and genetic distance but significant correlations between genetic distance and both physical distances and similarities in temperature between sites containing each taxon. A correlation between physical distances and temperature similarities makes it difficult to separate the effects of each factor but our preliminary results indicate that speciation within the genus was mainly climatically driven.

Macrobiotus furciger group complex along the Scotia Arc and Antarctic Peninsula

S. J. McInnes (British Antarctic Survey, UK); C. J. Sands (British Antarctic Survey, UK);

In this paper we present a new species of the *Macrobiotus furciger* group which is distinguished from others in the group by having a simple stellate egg rather than the bifurcated/castellated egg processes. We also discuss the problems that have arisen from the re-description of *Macrobiotus furciger* Murray 1907 that did not involve type specimens or type locality. Finally, we explore the biogeography and the potential for speciation or population groups of the furciger-group through the Scotia Arc and islands of the Antarctic Peninsula using molecular techniques and morphology.
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The Planetary Biodiversity and Biogeography of the Phylum Tardigrada

W. R. Miller (Baker University, U.S.A.); C. W. Beasley (McMurry University, U.S.A.)

We would like to invite everyone who works on tardigrades to collaborate in applying for and conducting a five year grant to describe and document the diversity and distribution of tardigrades on a planetary scale. The National Science Foundation of the United States has solicited applications for such projects. The NSF has reviewed the international scope of the proposal being presented here and concurs that it meets the intent of their program. The project has three objectives:1) image and re-describe to modern standards all species, 2) establish DNA sequences (bar codes) for all species, and 3) explore the areas of the world where the occurrence of tardigrades is under documented. Finally, we propose to assemble a dynamic electronic and paper based monograph to the tardigrades of the world. We will outline a program, in which we may all work together without sacrificing our individual independence and right to publish our own work. We propose to ask NSF for 2.5 million dollars over five years to bring our entire phylum up to modern standards as a starting point for the new discoveries of the 21st century.

Conference program   Tuesday, 04.08.2009

Session 3: Ecology (Chair: N. J. Marley & S. J. McInnes)

09:00-09:15	Romano et al.
09:15-09:30	Accogli et al.
09:30-09:45	Sewell & Romano
09:45-10:15	Coffee break
10:15-10:30	Hohberg
10:30-10:45	Dafoe & Romano
10:45-11:00	Degma et al.
11:00-11:15	Jönsson
11:15-11:30	Ramsay & Marley
11:30-11:45	Marley
11:45-13:15	Lunch
13:15-13:30	Vicente

Session 4: Physiology & molecular biology (Chair: T. Kunieda)

13:30-13:45	Møbjerg et al.
13:45-14:00	Halberg et al.
14:00-14:30	Poster session
14:30-15:00	Coffee break
15:15-16:00	Poster session
18:00-19:00	Boat trip
from 20:00	Beer garden

Deep sea tardigrades in the northern Gulf of Mexico with a description of a new species of Coronarctidae

F. A. Romano, III (Jacksonville State University, U.S.A.), M. Gallo, R. D'Addabbo, R. Sanduli (University of Bari, Italy), J. Baguley (University of Nevada-Reno, U.S.A.) and P. Montagna (Texas A&M University, U.S.A).

To date, few data are available about deep-sea tardigrades. Thiel (1966) provided information on the first deep-sea tardigrades collected from the abyssal zone 2500- 5000 m depth in the north–western Indian Ocean. Renaud- Mornant described for the first time in 1974-1975 a new family of the Arthrotardigrada, Coronarctidae, with genus and species types *Coronarctus tenellus*. Further data from the NE and NW Atlantic Ocean and the Mozambique channel, Indian Ocean, and Pacific Ocean with the description of many other new tardigrades from both abyssal and bathial depths by Renaud Mornant (1980, 1981, 1983, 1984, 1987, 1988) and by Kristensen & Renaud- Mornant (1983). During May and June 2000 benthic samples were collected from 43 stations in the northern Gulf of Mexico (This research was funded in part by the U.S. Department of Interior, Minerals Management Service, Contract No. 1435-01-99-CT-30991). A total of 54 tardigrades were extracted from samples ranging in depth from 625 m to 3150 m of which 35 tardigrades were mounted onto permanent glass slides (glycerol mounting medium) and identified to species using standard morphological characteristics. Five genera of tardigrades were collected; *Angursa* (2 specimens - 1401m and 2020m), *Coronarctus* (29 specimens - 625m to 3150m), *Euclavarctus* (1 specimen - 2743m), Proclavarctus (1 specimen - 2600m), and *Styraconyx* (2 specimens - 1565m and 2600m). *Coronarctus* was the most prevalent genera and was represented by 4 species; *C. laubieri* (16 specimens – 1875 to 3150m), *C. stylisetus* (1 specimens - 3000m), *C. disparilis* (10 specimens – 763 to 2600m) and *C. mexicus* (12 specimens – 625 to 3000m). Samples from only 17 stations (31.5%) contained tardigrades. Most samples had only a few individuals (1-2). Tardigrades were most prevalent in the basin and west-central portion of the Gulf and they tended to be in deep water. No abyssal tardigrades were found. Further specimens were collected from the northern Gulf of Mexico during October and November 2007 and 2008 from shallower waters (125-700m). Collected tardigrades belong to three genera: Angursa, Euclavarctus, and Coronarctus. *Coronarctus* was the most prevalent genera in this collection and was represented by 2 species *C. laubieri* and *C. disparilis.*

Diversity and Ecology of the marine Tardigrades along the Apulian Coast

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A comprehensive study on the composition, structure and diversity of tardigrade communities along the Southern Apulian coast was carried out in March and September 2000. The Apulian coastline counts about 820 km and includes a wide variety of natural environments such as Torre Guaceto, Laghi Alimini and Porto Cesareo and areas with high human impact such as Mattarelle (Enel power Station), Brindisi Harbour and Torre Mozza with its fish processing industry. The research was carried out only in the subtidal zone but in different kinds of sediments: coarse, medium, fine, and very fine sand, sand in Posidonia meadows, coarse organogenous debris and mud. 17 stations were sampled at 3 different depths in two seasons; March and September, resulting in 86 samples of which 58 samples contained tardigrades. A total of 4287 specimens (2972 in March and 1315 in September) where identified to 53 species (51 in March and 38 in September). The species found in Southern Italy are Heterotardigrada, belonging to the arthrotardigrade families Neoarctidae (1 species), Stygarctidae (4 species), Halechiniscidae (42 species) and Batillipedidae (6 species). Halechiniscidae are the most abundant family followed by Batillipedidae, Stygarctidae and Neoarctidae. The diversity, density and relative abundance were recorded and the spatial variation of species diversity was statistically evaluated.

Structure of a natural population of *Dactylobiotus ambiguus*

S. M. Sewell and F. A. Romano, III (Jacksonville State University, U.S.A.)

The tardigrade, *Dactylobiotus ambiguus*, is characterized by large claws, with short to extremely short secondary branches and bases connected to each other. *D. ambiguus* (Murray, 1907) has a smooth cuticle, normally colorless, eyes in the posterior position, and a pharynx with two macroplacoids (1st is up to twice as long as the 2nd and may appear to be divided into two by a narrow constriction) and no microplacoids. Free eggs are spherical with pointed tubercle projections that have contacting polygonal bases. An almost pure population of this species was found among algae growing on sandstone rocks at Cold Water Falls in Tuscumbia, Al. Sampling began on 12 February 2008 and continued every two weeks until 27 March 2008 (the sampling area became completely dry after this date). Three replicate samples (10.75 cm2) using a 3.7 cm (ID) PVC cylinder were taken during each collection period. All

samples were returned to the laboratory for processing the same day. After sample processing, tardigrades and eggs were removed, mounted in Hoyer's on glass slides, and were measured (length and width where possible and egg diameter). A total of 2,116 tardigrades, 69 free eggs and 62 pregnant females were captured. Mean length is 336.4 + 92.1 µm (range = 135.5-601.8), mean width is 107.8 + 49.2 µm (range = 35.6-216.2). Mean egg diameter is 73.4 + 15.7 µm (range = 35.0-122.7). Mean clutch size is 5.5 + 2.1. Mean free egg diameter is 72.9 + 13.5 µm (range = 46.4 – 93.8 µm). Sampling resumed after the sample area became wet again in April 2008 and no animals were found for the next 4 months. At this time, August 2008, 22 adults were returned to the site from our bulk cultures. Monthly sampling was initiated in October 2008. From October through April 2009 575 animals, 2 free eggs, and 19 gravid females were collected. Mean length is 344.4 µm + 62.4 (range = 152.9 – 534.2 µm). Mean clutch size is 4.3 + 1.1. Mean egg diameter is 66.9 + 9.3 µm (range = 42.5 – 87.4 µm). Average free egg diameter is 74.8 µm.

Mass occurrence of algal-feeding tardigrade *Apodibius* sp. in the young soils of a post-mining site

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In a coal-mining site at Spremberg, Germany, the soil tardigrade community was investigated. *Apodibius* sp. occurs with densities of up to 2733 tardigrades in the uppermost 5 cm of 10 cm² soil. Algivore *Apodibius* (99 % of overall tardigrade numbers) first appeared in October 2007, 2 years after the coal-mining site was established. Together with large algivore nematode species of *Aporcelaimellus* and *Ecumenicus monohystera, Apodibius* contributed 32.3 % (October 2007), 52.5 % (April 2008), and 68.1 % (October 2008) of the overall tardigrade-nematode biomass. In contrast to all other trophic groups, where feeding mode is derived from potential food sources used in successful laboratory cultures, actual algivore species may be recognized in the soil samples by their green intestines. The high biomass of this trophic group indicates that soil algae are a very important food source in these young soils. This concurs with the findings of earlier studies on primary soil succession that unicellular algae are one of the first colonisers and food sources in young soils. At the moment we aim at culturing Apodibius. sp. in order to provide life history data and alternative food sources of the species.

Tardigrade species diversity changes on a barrier island (Dauphin Island, U.S.A.) and the introduction of two new species of *Calcarobiotus*

R. C. Dafoe and F. A. Romano, III (Jacksonville State University, U.S.A.)

Dauphin Island, a barrier island in the Gulf of Mexico just south of Mobile Bay, AL. The Eastern forested areas of the island were survey for tardigrade communities. Leaf litter samples were collected from October 1999 - January 2001 and from July 2006 - January 2007. A total of 1,731 specimens were found, 1,011 from 1st sample period that included 6 genera and 16 species and 720 from the 2nd collection that included 5 genera and 8 species. The genus *Macrobiotus* (84.5 % in the 1st & 85% in the 2nd) dominated the collection. Other genera found were *Hypsibius, Milnesium, Minibiotus, Diphascon* and *Calcarobiotus*. *Macrobiotus richtersi* (6% & 10.8%) *M.* cf. *hufelandi* (45.2% & 44.6%) , *M.* cf. *echinogenitus* (10.9% & 24.3%), and *Diphascon pingue* (9% & 3.3%) were the dominant species from both collections. Species diversity indices (Simpson & Shannon-Weiner) and community similarity indices (Jaccard's & Stander's) were calculated. The 1st collection (with 16 species) had a much higher species diversity (0.75 vs. 0.39) than the 2nd collection (with 8 species). Community similarity was relatively the same (0.91 vs. 0.75), since the dominating species on the island didn't change much between the two collections (rarer species were generally missing from the 2nd collection). A large number of perturbations to the island in the form of hurricanes (7) and tropical storms (8) occurred between January 2001 and July 2006 which altered the island and may have been responsible for the change in species diversity. Dauphin Island was cut into 2 islands by Hurricane Katrina (2005 – H4), the Western end of was moved northwards by approximately 10 meters, and the beach front was greatly disturbed (golf course was nearly destroyed) during Hurricane Ivan (H4). During these collections 2 new species of Calcarobiotus were discovered. Further analyses were accomplished using Nonparametric Multidimensional Scaling (NMS) and Detrended Correspondence Analysis (DCA) for the species that were found in both collections. These ordinations (based on species presence/absence) separated the island into 4 distinct clusters. The least disturbed, middle, forested portions of the island seem to separate from the more disturbed sand dune portions.

Horizontal distribution of moisture and Tardigrada in a single moss cushion

P. Degma, S. Katina, L. Sabatovičová
(Comenius University in Bratislava, Slovakia)

We carried out a study of the moisture distribution and the horizontal distribution of Tardigrada in a single moss cushion. During one field trip we collected 25 equal samples (in a square of five rows x five columns) from a growth of the moss *Hypnum cupresiforme* Hedwig. Using two-way ANOVAs without replications and linear regression analyses we determined that there was no significant gradient of absolute or relative moisture along the moss slope. We isolated 224 specimens of seven Tardigrada species (*Milnesium tardigradum* Doyère, 1840, *Hypsibius convergens* (Urbanowicz, 1925), *H. microps* Thulin, 1928, *Diphascon pingue* (Marcus, 1936), *Astatumen trinacriae* (Arcidiacono, 1962), *Macrobiotus hufelandi* C.A.S. Schultze, 1833 and *Minibiotus* sp.) from the 25 samples. Using both Chi-square tests of independence and Chi-square goodness of fit tests as well as by calculations of the coefficients of dispersion we found that the horizontal distribution of tardigrade specimens in general, as well as the distribution of each species, was agreggated. By contrast, species number was random in the observed moss samples. Based on the comparison of all polynomial regression models (third, second and first order) with the null model and between each other, the distribution of Tardigrada specimens as a whole as well as the distribution of *Macrobiotus hufelandi* were related neither to absolute nor relative amount of water in the moss cushion. Based on these results, we formulated a hypothesis explaining tardigrade heterogeneity in randomly sampled mosses. According to this hypothesis there are two processes occurring at the same time: 1) random recruitment of specimens or eggs on a substratum, 2) subsequent establishment of their own micro-populations and gradual increase of their density in time of active periods with slow radiating of these micro-populations into the surroundings. The consequences of these processes are: a) larger substrata usually contain more tardigrades than smaller ones, and b) some parts of larger substrata can be without any tardigrades while other parts can host rich tardigrade population(s). We hypothesize that aggregated Tardigrada distribution in each moss cushion is the most likely reason for the large variability in tardigrade abundance in random samples taken from different moss cushions.

Temporal variation in population density of moss-living tardigrades at Ölands Alvar, southern Sweden

K. I. Jönsson (Kristianstad University College, Sweden)

Population dynamics of tardigrade populations have received little attention and there are few studies documenting changes in population density over longer time periods. Such studies are important for understanding both the life history strategies of tardigrade populations and the way environmental conditions affect their numbers and age-structure dynamics. In this study, moss samples with tardigrade populations of several species were collected from a carbonate rock fence at Ölands Alvar in south-east Sweden. Samples were collected monthly during a 2-year period. The samples contained the tardigrade species Richtersius coronifer, Milnesium tardigradum, Macrobiotus cf. hufelandi, and Echiniscus spiniger. Number of animals and eggs were recorded, and the body-size of extracted animals measured. The study will report the patterns of variation over the year in numbers, reproduction and age-structure for these tardigrade species. Results will be discussed in the context of environmental influence over ecological and evolutionary time.

Tardigrada communities from the high-altitude forest to grassland transition in the Cordillera de Vilcanota Peru

B. P. L. Ramsay, N. J. Marley (University of Plymouth, United Kingdom)

Little information has been published about the high-altitude Tardigrada communities of Peru. This study presents results from a 135 m-long transect at 4,150 m altitude in the Mantanay Valley, Cordillera de Vilcanota, approximately 40 km NNW of Cusco, Peru. The transect includes Polylepis woodland, puna grassland and the transition between these two key habitats. Representative samples of mosses and lichens were collected from different parts of these habitats along the transect, in the dry season, and transported to the University of Plymouth in a preserved, dry state. Characteristics of the samples, including dry weight and indicators of structural complexity, were noted.

An attempt was made to extract and identify all Tardigrada, eggs and exuvia from the samples, by detailed searching. Some interesting taxa were discovered. The community-level data were analysed using multivariate techniques to characterize the Tardigrada communities associated with forest, grassland and the transition zone, as well as different parts of habitat structure.

The Tardigrada of Devon and Cornwall, United Kingdom, including three species new to science and seven new additions to the U.K. fauna

N. J. Marley (University of Plymouth, United Kingdom)

There has been very little data published on the Tardigrada in the counties of Devon and Cornwall, southwest United Kingdom. Prior to the author's work only 10 species had previously been reported from this large, geographically diverse area. This paper collates the sparse published records and the previously unpublished results from nearly 20 years of planned and ad hoc sampling.

With these two large counties containing such diverse habitats, sampling has encompassed many terrestrial, freshwater and marine sites; from the sub-tidal zone to the top of the moorland. It was therefore hypothesized that these habitats would support a much richer Tardigrada fauna than previously reported and that the limited records only reflected the previous tiny sampling efforts. The combined results of this and other smaller studies within the country is aimed at providing a new baseline dataset for future U.K. Tardigrada research projects and to provide a basis for monitoring the national Tardigrada fauna in a revamped national reporting scheme.

More than forty species were recorded from twenty-three genera. Three species were new to science, seven species were new additions to the U.K. fauna and twenty-two species were first reports for the region. Specimens were examined using a range of light microscopy, confocal microscopy and scanning electron microscopy techniques, with some specimens also being included in molecular research studies.

Do tardigrades need a conservation strategy

F. Vicente (Faculty of Sciences, University of Lisbon, Portugal)

Tardigrada is a poorly studied phylum of microscopic animals, whose ecological importance is still poorly understood. Potential use of these animals in scientific research in fields such as biotechnology or medicine could be significant, but are still in their beginnings. This lack of knowledge should suffice to launch the discussion on the possible need of a conservation strategy for these animals, as well as for others in similar situation. Given its reduced size and the short number of specialists worldwide, tardigrades are rarely, if ever, considered in studies regarding biodiversity indexes or environmental impacts. Nonetheless, threats to their survival may be considerable and should therefore be evaluated. One major threat would be habitat destruction, e.g., in terrestrial environments mosses and lichens with inhabiting tardigrades can easily be destroyed by means of fires or by conversion of natural land for agriculture or human occupation. Also air pollution is a threat since this is known to kill lichens and prevent their proliferation. In marine beaches, diverse tardigrade species are distributed along differently tide affected zones. With the rising of the sea level, this zonation could be severely reduced or even vanished in cases where beaches will be submerged – one can only speculate on the effects on tardigrades biodiversity. Considering that the great majority of animal groups are invertebrates and that they are still the neglected son of conservationists, we think tardigrades can be an interesting phylum to help explore the theme. Threats, possible conservation strategies and future perspectives are discussed.

Halobiotus crispae - a model organism of non-cryptobiotic survival in extreme environments

N. Møbjerg, A. Jørgensen, K. Halberg, D. Persson and
R. Møbjerg Kristensen (University of Copenhagen, Denmark)

Whereas heterotardigrades are commonly found in both marine and limno-terrestrial environments, eutardigrades are almost exclusively limno-terrestrial. However, two genera, within the Isohypsibius clade - *Halobiotus* in the Northern Hemisphere and *Ramajendas* in the Southern Hemisphere - have entered the marine environment. Molecular data show that *Halobiotus* evolved within the currently paraphylelic genus *Isohypsibius*. *Ramajendas* apparently evolved independently in the Subantarctic. The genus *Halobiotus* comprises five species colonising tidal and subtidal habitats at numerous localities on the Northern Hemishere. One species, *Halobiotus crispae*, has been extensively studied. Thus, detailed information is available on the life cycle and sample localities of this species. In addition thorough structural analyses have been performed on the Malpighian tubulus, the muscle system, and the buccopharyngeal apparatus with special emphasis on the pharynx as well as descriptions of selected developmental stages.

While tardigrades are well known for their abilities to cope with extreme environmental conditions by entering cryptobiosis, little focus has been on their ability to sustain metabolism and remain active in environmental extremes. Halobiotus crispae is an excellent model for such studies. The transition zone between land and sea is characterized by large fluctuations in abiotic factors; most noticeably alterations are seen in salinity and temperature. In order to remain active, to sustain growth and reproduction, physiological and molecular mechanisms need to be in place to cope with these factors. *H. crispae* has an extensive ability to supercool, enabling the animal to withstand subzero temperatures without freezing. In addition animals in the pseudosimplex 1 stage are freeze tolerant. Tidal habitats may pose a challenge for osmoregulation and *H. crispae* has a large capacity to tolerate perturbations in ambient salinity making it ideal for the study of osmo- and volume regulation in tardigrades. Thus, *H. crispae* handles environmental extremes not by entering cryptobiosis but by withholding metabolism, expending energy on active regulatory mechanisms and by entering cyclomorphosis. In the present presentation we summarize our current knowledge of *H. crispae* and discuss its use as model organism for non-cryptobiotic survival in extreme environments.

Osmo- and volumeregulation in the marine eutardigrade *Halobiotus crispae*

K. Halberg, D. Persson (University of Copenhagen, Denmark); H. Ramløv, P. Westh (University of Roskilde, Denmark); R. M. Kristensen, Nadja Møbjerg (University of Copenhagen, Denmark)

The ability to respond and adapt to an osmotic challenge is an essential prerequisite for cellular and animal life. Accordingly, osmoregulation has been extensively studied in numerous organisms however major gaps still exists in our understanding of how many animals deal with osmotic stress. In the present study, the osmo- and volume regulatory capacities of the marine tardigrade *Halobiotus crispae* in the active stage were investigated over a period of 48 hours in response to severe osmotic shock at external salinities of 2, 10 and 40 ppt. The salinity at the sample locality is 20 ppt. Total body volume of individual specimens was calculated from light microscopical images by measuring median length and average width of the animal and hind legs and by assuming an approximate geometric shape of a cylinder. Nanoliter samples of hemolymph were collected from individual specimens and the osmotic pressure of the body fluids were measured by melting point depression in a nanoliter osmometer. Hemolymph osmolality of control animals at 20 ppt were 926 ± 29 mOsm/kg (n=6). Independent verification of osmolality during control conditions was performed using differential scanning calorimetry. Exposure of specimens to hypotonic stress caused rapid swelling followed by a regulatory volume decrease, reaching a new steady state after a period of 48 hours. New steady state body volume during 2 ppt and 10 ppt were 114 ± 14% (n=10) and 110 ± 7% (n=11) while body fluid osmolality stabilized at 330 ± 50 mOsm/kg (n=6) and 584 ± 68 mOsm/kg (n=6), respectively. Conversely, exposure to hypertonic stress caused an initial rapid shrinkage, resulting in a regulatory volume increase, reaching a new steady state after 24 hours of 82 ± 9% (n=11) of initial total body volume. Body fluid osmolality increased from 925 ± 29 mOsm/kg to 1297 ± 32 mOsm/kg (n=6) during this period. At any investigated external salinity active stage *H. crispae* hyper-regulate indicating the excretion of dilute urine.

Conference program   Wednesday, 05.08.2009

Session 4: Physiology & molecular biology
(Chair: T. Kunieda, N. Møbjerg & R. Guidetti)

09:00-09:15	Persson *et al.*
09:15-09:30	Cesari *et al.*
09:30-09:45	Altiero *et al.*
09:45-10:15	Coffee break
10:15-10:30	Frohme *et al.*
10:30-10:45	Hengherr *et al.*
10:45-11:00	Reuner *et al.*
11:00-11:15	Grohme *et al.*
11:15-11:30	Schokraie *et al.*
11:30-11:45	Dandekar *et al.*
11:45-13:15	Lunch
13:15-13:30	Horikawa *et al.*
13:30-13:45	Kunieda *et al.*
13:45-14:00	Katayama *et al.*
14:00-14:15	Arakawa *et al.*
14:15-14:30	Resumée
14:30-15:00	Coffee break
15:15-18:00	Examination of type material
from 20:00	Conference dinner

Extreme stress tolerance in tardigrades – Surviving space conditions in low earth orbit

D. Persson (Københavns Universitet, Denmark); C. Ricci (University of Milano); K. A. Halberg , R. M. Kristensen (Københavns Universitet, Denmark)

Some tardigrades posses the ability to enter a reversible ametabolic state termed anhydrobiosis as a response to desiccating conditions. In the anhydrobiotic state tardigrades display an incredible capacity to tolerate extreme environmental stress, not necessarily encountered in their natural habitat. The aim of this thesis was to determine the effect of different extreme stresses, regarding initial survival, long-term survival and fecundity. Tardigrades were exposed to extreme cold and vacuum in the laboratory at the University of Copenhagen. Results reveal that neither initial nor long-term survival of *Richtersius coronifer* in anhydrobiosis is affected by instant freezing to -195.8° C or vacuum (96-100% survival). Though, it seems that fecundity is affected by instant freezing of active animals, and by extended periods of anhydrobiosis. An assessment of the effect of cosmic radiation was achieved through collaboration with the Danish research group and the group from the University of Milano in the RoTaRad project. RoTaRad was part of the BIOPAN 6 mission run by ESA, but funding came from ASI (Agenzia Spaziale Italiana), Italy. To test their tolerance of space environment, tardigrades were sent into low earth orbit and exposed to cosmic radiation and microgravity. Experiments on Whatman 3 filters show little effect of cosmic radiation on survival of the eutardigrade *R. coronifer* (67.9% ±21.9%, 89.5% ±7.4%, 82.5% ±24.8%). In a microcosmos experiment the tardigrades *R. coronifer*, *Ramazzottius oberhauseri* and *Echiniscus testudo* were desiccated on a moss substrate together with rotifers and nematodes. Very low survival rates were obtained from this experiment (0-22.5%), likely due to faults in the desiccation protocol.

Evolution of anhydrobiosis in Tardigrada: analysis of the tps gene

M. Cesari, A. Frigieri, L. Rebecchi (University of Modena and Reggio Emilia, Italy)

Extreme habitats are highly selective and require organisms to possess specific adaptations to stressors. Habitats that unpredictably desiccate and rehydrate can be considered extreme. Some animals can withstand desiccation by entering anhydrobiosis. Tardigrada represent one of the animal phyla where desiccation tolerance is widespread requiring often, but not always, the accumulation of trehalose, a non-reducing disaccharide. Trehalose allows anhydrobiosis by acting as a water replacement molecule and vitrifying agent. It has been demonstrated that trehalose accumulation is different in various tardigrade species. Therefore, the analysis of a gene involved in its metabolic pathway, such as the trehalose-6-phosphate synthase (tps) gene, could prove very useful to elucidate the evolutionary relationships among taxa. The presence of the gene and potential differences in its sequencewere investigated since many tardigrade species do not have the ability to enter anhydrobiosis. Sequences of the tps gene were determined by amplification using degenerate primers based on conserved protein sequence and cloning. Once a tps sequence was obtained, specific primers were designed and used to amplify it in different species, which were chosen for their different anhydrobiotic response and phylogenetic position. The origin and evolution of anhydrobiosis in Tardigrada are discussed.

Tardigrade resistance to space flight: LIFE – TARSE mission on FOTON-M3 spacecraft

T. Altiero, R. Guidetti, M. Cesari, R. Bertolani (University of Modena and Reggio Emilia, Italy), M. Negroni, A.M. Rizzo (University of Milan, Italy), L. Rebecchi
(University of Modena and Reggio Emilia, Italy)

The TARSE (TArdigrade Resistance to Space Effects) project, part of the mission LIFE on FOTON-M3 spacecraft, analyzed the effects of the space environment on desiccated and active tardigrades. Four kinds of experiments were conducted in which the eutardigrade *Paramacrobiotus richtersi* was used as a model species. Desiccated (in leaf litter, Exp. F1 and on paper, Exp. F2) and hydrated tardigrades (fed, Exp. F3 and starved, Exp. F4) were flown in a low-earth orbit at an altitude between 250-290 km for 12 days in September 2007. These experiments allowed, for the first time, a comparison of the effects of the space environment on desiccated and on active metazoans. The TARSE project also included post-flight controls on Earth, using both simulated microgravity and the temperature profile experienced by tardigrades during the flight mission. In all experiments, desiccated animals had the highest survival rates, but a relatively high survival was also found for starved animals. For both physiological conditions, no significant differences in survival were recorded between flight and control tardigrades. During the flight mission, starved tardigrades moulted and females laid eggs. Several eggs hatched and the newborns exhibited normal morphology and behaviour. The relative levels of Hsp70 and Hsp90 of desiccated flight tardigrades deviated significantly from the controls, whereas no differences were detected in Hsps expression between starved flight tardigrades and controls. No visible damages to double strand genomic DNA were observed in any samples. In both active starved tardigrades and in desiccated ones, differences were found in antioxidant enzyme contents and activities between flight and control animals.

This work was supported by the Italian Space Agency (A.S.I.), MoMa – ASSC Grants to L. Rebecchi and A.M. Rizzo.

FUNCRYPTA – a systems biology approach to understand cryptobiosis in tardigrades

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Cryptobiosis and in particular anhydrobiosis in tardigrades is a phenomenon known since the times of van Leeuwenhoek and Spallanzani. Yet it is not understood until now. In the research program QuantPro the aim is to understand dynamic processes in living systems with the help of quantitative analyses. FUNCRYPTA – the functional analysis of cryptobiotic tardigrades - comprises a consortium of five groups in this systems biology program. Their goal is to understand and make use of the anhydrobiotic properties of tardigrades and to establish them as model organisms. The strength of this group is the broad approach based on an effective animal facility, a laboratory for functional genomics, a proteome group, bioinformatics resources, metabolite analyses and an industrial partner. The people in the group search for genes, proteins, metabolites and pathways which can explain the outstanding properties of tardigrades. The vision is to get a comprehensive model of cryptobiosis and to transfer basic knowledge to application. The latter may improve various aspects of conservation in biomedicine, white industry, food production or else. Thus we currently investigate the potential of various substances to improve cryoconservation.

The project (www.FUNCRYPTA.de) is funded by the German Federal Ministry of Education and Research, BMBF (0313838).

Trehalose and vitrification as essential aspect for desiccation tolerance in tardigrades and other aquatic metazoans

S. Hengherr; A. G. Heyer; F. Brümmer; R.O. Schill (Universität Stuttgart, Germany)

Several aquatic or semi aquatic organisms are able to withstand extreme desiccation, commonly known as anhydrobiosis. During this state they show no detectable metabolism but resume activity after rehydration. To explain how the cellular structures are protected during dehydration, two hypotheses have been proposed: Water replacement by compatible solutes and vitrification. Several studies on encysted embryos of Artemia and the chironomid larvae Polypedilum have shown that the non reducing disaccharide trehalose is accumulated to high levels (up to 20% d.w.) and involved in these protecting mechanisms. The trehalose levels of 6 species of the class Eutardigrada, therefrom one species of the Family Milnesidae (Order Parachela) and five species of the Family

Macrobiotidae (Order Apochela) and 2 species of the Class Heterotardigrada containing two species of the family Echiniscidae were observed quantitatively during anhydrobiosis. The eutardigrades of the order Parachela show a low trehalose accumulation of up to 0.5% d.w., but still present a vitreous state at temperatures up to 85°C, measured by Differential scanning calorimetry. No trehalose and vitreous state was detected in the eutardigrade of the order Apochela and the two heterotardigrade species. Quantitative analysis of trehalose levels in desiccation tolerant states of the freshwater sponge *Trochospongilla* sp., the bryozoan *Cristatella mucedo*, the turbellarian *Mesostoma thamagai* and the crustaceans *Daphnia magna, D. pulex, Triops longicaudatus, T. cancriformis* and *T. australiensis* also show low trehalose concentrations of up to 0.02% d.w. Regarding the remarkable desiccation tolerance of the tested organisms and the low levels or absence of trehalose, high trehalose concentrations during the dormant state seem to be rather special to some anhydrobiotes than a basic principle in desiccation tolerant metazoans. Therefore, the predominant role of trehalose as protecting agent should be re-evaluated.

The project (www.FUNCRYPTA.de) is funded by the German Federal Ministry of Education and Research, BMBF (0313838A).

Are heat shock proteins important during anhydrobiosis in tardigrades?

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The habitat of the tardigrade *Milnesium tardigradum* Doyère 1840 undergoes frequent dehydration. Active life of *M. tardigradum* is dependent on water, and to survive the adverse dry conditions the animals reduce the body surface and form a tun. In this anhydrobiotic tun-state tardigrades are able to endure complete desiccation until they revive again when they are rehydrated. The tun-state goes in hand with a high tolerance against physical and chemical extremes.

Drying of cells generally leads to damage of proteins, membranes and DNA often leading to death of the cells and consequently to death of the organism. To check if heat shock proteins act as stabilising agents due to their ability to refold proteins or to prevent aggregation of misfolded proteins, the expression changes of a set of different heat shock protein transcripts was analysed. Sequences were derived from EST data of M. tardigradum and expression changes of the following hsp transcipts were measured: hsp10, hsp17.2, hsp19.5, hsp90 and three hsp70. The only heat inducible hsp was hsp17.2. During the process of dehydration one hsp70 gene was up regulated, in anhydrobiotic animals the hsp90 transcript was more abundant than in active animals. Because most of the analysed hsp genes were down regulated and of the comparatively slight up regulation of hsp90 and hsp70, it is suggested that heat shock proteins play a minor role in the process of anhydrobiosis in tardigrades.

The Project www.FUNCRYPTA.de is funded by the German Federal Ministry of Education and Research, BMBF (0313838).

The transcriptome of *Milnesium tardigradum* and generation of a gene expression atlas for studying anhydrobiosis in tardigrades

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We are using *Milnesium tardigradum* as a model for the transcriptional regulation underlying anhydrobiosis. Tuns of *M. tardigradum* show a remarkable resistance to environmental stresses, frequently outperforming other tardigrade species. In order to understand the underlying genetic basis of this resistence, we are generating extensive sequence resources as a fundamental basis for transcriptional profiling experiments. We have employed classical Sanger-sequencing of cDNA clones and next-generation pyrosequencing to establish a comprehensive transcriptome map for this species. Our initially gathered transcriptome data consisted of two non-normalized cDNA clone libraries from active and inactive animals. This data was complemented with four normalized cDNA libraries of active, inactive and two intermediate stages that have been sequenced using 454 GS-FLX pyrosequencing, generating an extensive catalog of genes. The gathered data suggest a good transcriptome coverage, thus a comprehensive metabolic pathway map could be contructed. Also genes that are known to be involved in anhydrobiotic survival have been identified and are being evaluated. We are now emplyoing digital gene expression tag profiling to generate a high resolution gene expression atlas. This will allow us to compare expression levels across different states during anhydrobiosis. Earlier experiments emplyoing cDNA representational difference analysis (cDNA-RDA) identified differentially expressed transcripts between active and inactive (tun) animals. Candidate transcripts with various functions have been cloned and their expression evaluated via quantitative real-time PCR. These genes have diverse cellular functions and are probably reflecting complex biological processes that anhydrobiotic tardigrades undergo.

The Project www.FUNCRYPTA.de is funded by the German Federal Ministry of Education and Research, BMBF (0313838).

Towards a two-dimensional protein reference map of *Milnesium tardigradum*

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Tardigrades are well known because of their extraordinary capability to undergo cryptobiosis in extreme environmental conditions. Even though tardigrades have been found for more than 100 years, little is known about their protective molecular mechanisms. To gain insight into the unique adaptation capabilities of tardigrades on the protein level we aimed to establish a comprehensive proteome reference map of *Milnesium tardigradum*. Therefore we optimized protocols from protein preparation and generation of high-resolution 2D gels to high-throughtput protein identification by electrospray ionization tandem mass spectrometry (ESI-MS/MS). We developed a reference protein map of Milnesium tardigradum in the active stage by searching the acquired MS/MS data in two different databases, the publicly available NCBInr database and a protein database developed from 3000 EST sequences from *Milnesium tardigradum*. So far, a total number of 603 protein spots which were picked from a preparative 2D gel have been analysed by mass spectrometry. 252 spots could be identified in the tardigrade protein database and the NCBInr database. Furthermore it was possible to identify 130 spots by searching in the nucleotide clustered database, which could not be annotated by homology search.

In summary, we were able to identify 382 (63%) out of 603 protein spot. 252 spots yielded 145 unique proteins with distinct functions whereas 130 spots corresponding to 35 unique proteins were identified as proteins with yet unknown function. The identified proteins with known sequence or motif/domain homologies were further classified into groups according to their molecular function and biological process using Blast2GO program. The result shows a total of 9 different molecular function groups on GO level 2. The major groups belong to binding (45%) and catalytic activity (47%). The result shows also 16 different groups for biological process on level 2 with majorities in cellular process (23%) and metabolic process (18%). This study represents the first comprehensive proteomic analysis of tardigrades to date and provides a reference proteome map for future comparative proteomic studies.

The Project www.FUNCRYPTA.de is funded by the German Federal Ministry of Education and Research, BMBF (0313838).

Tardigrade adaptation potential: a bioinformatics perspective

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Tardigrade physiology shows their unique adaptation potential in their tun stage against extreme temperature and other harsh environmental conditions. However, there is still little known about the molecular basis for these adaptations.
We will summarize several studies on this adaptation potential and the bioinformatical analysis of involved sequences, interactions and pathways. Examples include a general overview of protein families with similar function or similar sequence involved in tardigrade stress response and their general adaptations, specific heat shock and other stress response proteins in *Milnesium tardigradum*, as well as a comparison between the predicted protein inventory of *Hypsibius dujardini* and *M. tardigradum*.
Thus we could identify more then 50 different clusters of sequence similar proteins in tardigrades, and several of these are tardigrade specific (10) others involve clusters of proteins involved in protection against oxidative stress, DNA protection, and protein protection. Heat shock proteins are present in different tardigrades and we find that in *M. tardigradum* several ones (hsp10, hsp60, hsp70, hsp90, small hsp) are present and we look in detail at the differences in their regulation. Further protective protein functions include aquaporins, DNA protection including Helicases, DNA repair (DNA J family), cold-shock like proteins and specific membrane protection. Though in functional terms the protein inventory of *M. tardigradum* and *H. dujardini* involves similar protein, DNA and stress protective functions, we can show that both have several specific and independent protein families for these tasks and that Milnesium tardigradum has a richer inventory of such species-specific families which correlates with its higher resistance for instance against extreme temperatures.
We will furthermore discuss current software to analyze such extreme adaptations and in particular in tardigrades as well as future directions of research including modelling of time courses of tardigrade adaptation switches.

The Project www.FUNCRYPTA.de is funded by the German Federal Ministry of Education and Research, BMBF (0313838).

Life history of the tardigrade *Ramazzottius varieornatus* under artificial culture conditions

D. Horikawa (NASA Ames Research Center, USA); T. Kunieda (The University of Tokyo, Japan); W. Abe (The University of Tokyo, Japan); S. Higashi (Hokkaido University, Japan); T. Okuda (National Institute of Agrobiological Sciences, Japan)

Limno-terrestrial tardigrades have fascinated researchers with their considerable tolerance to extreme environmental conditions and then have been expected as a potential model animal for research on mainly desiccation tolerance and radiation tolerance. However, contrary to the expectation, there seems to have been relatively a few investigations in which researchers use tardigrades as materials for research on their extremotolerance. We, however, have established a relatively simple culture system for the extremotolerant tardigrade Ramazzottius varieornatus in the laboratory. R. varieornatus, originally collected from the dry moss vegetation in Sapporo, Japan, was cultured with the green alga Chlorella vulgaris as food. Under the culture conditions at 25ºC, the average life span of R. varieornatus was approximately 35 d, and the maximum life span was 87 d. The first egg laying was observed 9 d after hatching, and the average number of eggs produced by a single individual was 7.85. Of the eggs produced, 83% hatched, and the average embryonic stage was 5.7 d. R. varieornatus produced the next generation under solitarily cultured conditions, indicating that this species is parthenogenetic or hermaphroditic. Based on this research, we suggest that R. varieornatus will be a potential model organism for research on extremotolerance of animals.

The tardigrade genome of an anhydrobiotic extremotolerant species, *Ramazzottius varieornatus*

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Phylum Tardigrada consists of approximately a thousand species. Some species, especially which habit in limno-terrestrial area, have tolerant ability against desiccation by entering an ametabolic state known as anhydrobiosis. Anhydrobiotic ability is sparsely distributed among metazoa and its appearance does not well coincide with systematic lineage. During evolution, many genomic mutations might accumulate to provide anhydrobiotic ability in the limited number of species by modification of genes or their regulatory elements and/ or even by the generation of new genes, which cooperate together to afford the ability. Although anhydrobiosis in these animals was found more than 200 years ago, little is known about genomic basis of this ability. To address this, we have conducted whole genome sequencing of anhydrobiotic extremotolerant tardigrade, *Rammazzottius varieornatus*, whose efficient culture system was recently established. We performed a large-scale whole genome shotgun analysis with the library constructed from approximately 20,000 individuals. To avoid genetic variation, all animals used in this study are derived from a single individual (YOKOZUNA-1 strain). To facilitate assembly and reorientation of contigs, we also constructed fosmid library and performed paired-end sequencing. In this symposium, we will report the overview and recent progress of our tardigrade genome project. Genome sequence of this species provides a good clue to analyze the evolution of anhydrobiotic ability, as well as a solid foundation to analyze other omics data including transcriptome, proteome, and metabolome.

Draft genome sequence assembly and preliminary annotations of *Ramazzottius varieornatus* genome

T. Katayama (University of Tokyo, Japan), K. Arakawa, Y. Hasebe, N. Kido (Keio University, Japan), T. Kunieda (University of Tokyo, Japan), A. Toyoda, T. Shin-I (National Institute of Genetics, Japan), D. D. Horikawa (NASA Ames Research Center, USA), H. Kuwahara (University of Tokyo, Japan), K. Ohishi, A. Motoyama, T. Aizu (National Institute of Genetics, Japan), M. Kanehisa (Kyoto University, Japan), Y. Kohara (National Institute of Genetics, Japan), A. Fujiyama (National Institute of Informatics, Japan)

We have recently sequenced the genome of anhydrobiotic extremotolerant tardigrade, *Rammazzottius varieornatus*. The first draft genome sequence is assembled from 283,286 whole genome shotgun reads and additional paired-end reads of 15,000 fosmid clones. Total amount of the assembled sequence is about 57Mb with a N50 size of 520Kb. It corresponds approximately to 80% of the estimated 70Mb genome size; however, we assume the actual coverage is slightly lower than that because of a certain amount of overlaps in contigs. The quality of the sequence is considerably high, where 86% of the bases has quality value of 97 or above, whereas only 3% are 50 or below. The average GC-content is 47.5% which is relatively higher than that of D. melanogaster (41%) and C. elegans (35%). Based on the assembled sequences, we then applied sequence similarity search against UniProt and KEGG databases to estimate conserved protein sequences. Additionally, rRNA and tRNA sequences are predicted by RNAmmer and tRNAscan SE programs respectively. We also performed ab initio gene prediction using GlimmerHMM and SNAP whereby we obtained about 20,000 candidate genes. These results are stored in our annotation database including a dedicated genome browser based on the

GMOD/GBrowse. We will report the preliminary results of our analysis on functional annotation and phylogenetic study.

Comparative metabolome profiling of active and anhydrobiotic states of tardigrade *Ramazzottius varieornatus*

K. Arakawa, T. Ito (Keio University, Japan); T. Kunieda (University of Tokyo, Japan); D. Horikawa (NASA Ames Research Center, USA); T. Soga, M. Tomita (Keio University, Japan)

Limno-terrestrial tardigrades can withstand almost complete desiccation through anhydrobiosis, and these species can tolerate extreme environments under this ametabolic state. Metabolic changes during anhydrobiosis have been studied in many anhydrobiotic organisms including tardigrades, yeasts, nematodes, rotifers, and sleeping chironomid, and also in plants under dehydration, leading to the identification of several key molecules. On the other hand, previous researches mostly focused on the changes in specific compounds such as trehalose that is reported to accumulate in several species upon dehydration, and thus the understanding of comprehensive molecular mechanisms and regulation machinery of metabolic compounds during anhydrobiosis is still limited. To this end, we have conducted a comprehensive metabolome analysis using the tardirade *Ramazzottius varieornatus*, which is a potential model species for anhydrobiosis, for its ability to survive extremely rapid dehydration (<30 min.), and for which a rearing system was recently established. In order to analyze the comprehensive metabolic changes in the active and dehydrated states, 20000 individuals (approximately 10 mg dry weight) of *R. varieornatus* were measured in both conditions using two types of high-throughput mass spectrometry (MS) systems, liquid chromatography time-of-flight MS (LC-TOFMS) for lipids and sugars and capillary electrophoresis TOFMS (CE-TOFMS) for primary metabolite. In this talk, we wish to report the current progress from these metabolome analyses. Proline accumulation was not observed unlike previous reports of plant anhydrobiosis metabolomics, and increase, but not significant accumulation of trehalose was observed, suggesting a novel anhydrobiosis mechanism in this species.

List of Poster

T-taxonomy, BC-biochemistry, BG-biogeography, P-physiology, E-ecology, MB-molecular biology

Tardigrade fauna of marine caves

G. Accogli, M. Gallo, R. D'Addabbo (University of Bari, Italy)

There are very few data regarding marine cave tardigrade fauna and they refer to some Mediterranean Sea cave and the coasts of New South Wales, Australia. This work contributes to the MoDNA Project, for the biomolecular, taxonomic and ecological study of marine tardigrada. The aim is to improve the information about the phylogeny and biodiversity of marine tardigrada in the Mediterranean Sea. Furthermore, we would like to extend this kind of study to tardigrade fauna in Indian Ocean caves of Velassaru (Maldive Atolls). New data refer to a further sampling at Corvine Cave (P.to Cesareo Lecce, Mediterranean Sea) and a first sampling in Velassaru Cave (Maldive Atolls, Indian Ocean). Samples mostly consisted of coarse organogenous debris, and only samples from Velassaru Cave were fine coralline sand. Interesting information about density, diversity and composition of taxa collected, was obtained by observing data referring to sediment type. In Porto Cesareo Corvine Cave there are 12 species belonging to 4 families Neostygarctidae (1%), Stygarctidae (2%), Halechiniscidae (80%) and Batillipedidae (17%), 721 specimens in total. In Velassaru 8 species were collected belonging to 3 families, Stygarctidae (1%), Halechiniscidae (51%) Batillipedidae (48%), 104 specimens in total. Most surprising is the discovery of the open sea species *Florarctus* n. sp. and *Florarctus antillensis* only in Corvine Cave, and *Orzeliscus belopus*, *Tanarctus helleouetae* and *Batillipes philippinensis* only in Velassaru Cave.

Antioxidant metabolism is induced in the desiccated state of the tardigrade *Paramacrobiotus richtersi*

T. Altiero (University of Modena & Reggio Emilia), M. Negroni, G. Montorfano, P. Corsetto, P. Berselli (University of Milano, Italy), R. Guidetti, M. Cesari, L. Rebecchi (University of Modena & Reggio Emilia, Italy), A.M. Rizzo (University of Milano, Italy)

Oxygen, due to its nuclear elasticity and electron configuration, readily accepts single electron transfers generating several derivatives collectively known as reactive oxygen species (ROS). ROS can react with the non-radical molecules, leading to oxidative damage of lipids, proteins and DNA, involved in various diseases. A number of defence mechanisms, including antioxidant enzymes, have developed to protect the non-radical molecules from radical attack by ROS, thus limiting the damage. Tolerance to desiccation is correlated with an increase in antioxidant potential in different organisms. However, regulation of antioxidant defense system is complex and its role in desiccation tolerance is not yet firmly established. Until now, no data have been available on tardigrades related to this issue. Here we report the activities of various antioxidant enzymes to determine if *Paramacrobiotus richtersi* has an antioxidant metabolism able to counteract ROS both in dehydrated and active animals. In hydrated tardigrades, superoxide dismutase (SOD) and catalase had comparable activities, while in desiccated ones SOD activity increased 6-fold, in addition to a decrease in total proteins. Catalase increase was mainly due to total protein decrease. The glutathione peroxidase scavenger activity was less than that of catalase. On the contrary, GSH peroxidase was induced by desiccation together with glutathione (GSH) content. The increase of GSH reductase in desiccated specimens was probably related to the decrease in total proteins. Thiobarbituric Acid Reactive Substances (TBARS) content was much higher in dehydrated animals than in hydrated ones. Particularly relevant was the increase in SOD activity that appeared to be the main protective enzyme for desiccated animals, as the absence of water prevented the formation of hydroperoxides that are usually scavenged by catalase and GSH peroxidase. These results indicate that possession of antioxidant metabolism could represent a crucial strategy to avoid damages in desiccation tolerant organisms.

This work was supported by Italian Space Agency A.S.I., MoMa – ASSC Grants to A.M. Rizzo and L. Rebecchi.

Status report on the multihabitat inventory of tardigrades in the Great Smoky Mountains National Park (Tennessee and North Carolina, USA)

P. J. Bartels (Warren Wilson College, USA); D. R. Nelson (East Tennessee State University, USA)

The inventory of the Tardigrada for the All Taxa Biodiversity Inventory in the Great Smoky Mountains National Park is nearing completion. We have collected 778 samples from streams, soil and leaf litter, moss, and lichens. Processing and identifications have been completed for approximately 700 of these samples, and a total of 13,716 tardigrades have been identified thus far. The total species list now stands at 77 (compared to 3 prior to this inventory), and species richness estimators suggest that approximately 96 species may occur in the park. Our collection includes 18 species we believe are new to science. Six of these new species have been described in the literature, and one additional description is in preparation. The species list with corresponding habitat associations will be presented, and species richness will be compared with other large scale tardigrade inventories.

Additional Tardigrada of Hubei Province, China, with the description of *Doryphoribius barbarae* sp. n. (Eutardigrada: Parachela: Hypsibiidae)

C. W. Beasley (McMurry University, U.S.A.); W. R. Miller (Baker University, U.S.A.)

Three previous papers (Yang 1999, 2003, 2007) have reported on Tardigrada from Hubei Province, China, including a total of 11 species. This paper reports an additional 13 species from Hubei, which includes four new records for China, two new records for mainland China, and one species new to science. Doryphoribius barbarae sp. n., has two macroplacoids, no microplacoid, nine rows of gibbosities (2-4-4-4-4-6-4-4-2), cuticle and gibbosities with irregularly shaped and arranged tubercles. It differs from similar species in the number of rows of gibbosities, the number of gibbosities per row, the cuticular pattern, or the size of the macroplacoids. Other species reported from Hubei Province are *Cornechiniscus lobatus, Echiniscus* cf. *perviridis, Echiniscus reticulatus, Echiniscus viridissimus, Pseudechiniscus papillosus, Doryphoribius huangguoshuensis, Doryphoribius qinlingense, Doryphoribius taiwanus, Astatumen bartosi, Macrobiotus* cf. *lorenae, Macrobiotus mauccii,* and *Macrobiotus tenuiformis*.

Searching for rad51 homologue in tardigrade *Richtersius coronifer* preliminar data

E. Beltran-Pardo E (Universidad Javeriana, Colombia), R. Bermudez-Cruz (Cinvestav, México); I. Jönsson (Kristianstad University, Sweden), J. Bernal-Villegas (Universidad Javeriana, Colombia)

Tardigrades possess exceptional abilities to resist extreme conditions such as ionizing radiation, several investigations have indicated that such capacities are probably based primarily on a highly efficient DNA repair system. Rad51 is a highly conserved recombinase among many living organisms with a very important function during DNA repair. Considering this and the fact that tardigrades classification has placed them phylogenetically close to phylum Arthropoda and Nematoda, we compared the nucleotide sequence of Rad51 among several genus: *C. elegans, D. melanoganster, Homo sapiens, Arabidopsis thaliana* and *Gallus gallus*, confirming a high conservation level, as reported by Lin et al. 2006, Takami et al, 1998 with aminoacid sequences. Based on previous studies we detected the active site in the protein as well as the highly conserved Walker A and B motifs in the nucleotide sequence. Degenerate primers based on the 5th and 7th exon of the C. elegans and 2nd exon of *D. melanoganster* Rad51 gene were designed. Genomic DNA extraction conditions from R. conifer were standardized in our laboratory. Genomic DNA from *R. coronifer, C. elegans* y *D. melanoganster*, was amplified under the same conditions. For DNA quality ribosomal 18S RNA primers were used as the positive control. We obtained a 1000-bp and 400-bp PCR fragments from *C. elegans* and *D. melanoganster* Rad51 gene; respectively, however no signal was detected from *Richtersius coronifer.* All genomic DNAs tested were amplified with the 18S RNA primers. Considering that the tardigrade genome database is not nearly complete and annotation is still on the way, we figured that the exon/intron arrangement could be different for *R. conifer*, we decided to carry out RT-PCR with the designed degenerate primers. A 424-bp PCR product was obtained from *C. elegans* cDNA, but no signal was observed for *R. coronifer*. These findings indicate that likely Rad51 gene from tardigrades may not have conserved DNA sequences at least where primers were designed.

DNA barcoding in Ramazzottius and cryptic diversity in Tardigrada

M. Bigi, M. Cesari, R. Guidetti, R. Bertolani, L. Rebecchi
(University of Modena and Reggio Emilia, Italy)

DNA barcoding has proved useful in studying metazoan meiofauna. It has recently been successfully applied to the eutardigrade *Macrobiotus macrocalix* as the first case study. Therefore, a DNA barcoding project on tardigrades, called MoDNA (Morphology and DNA), has been developed, representing a novel approach to the taxonomy of this animal group. We believe that the results will be useful not only for the tardigrade specialists, but also for applications in studies on biodiversity, ecology and evolution. This project aims to analyze different tardigrade species, when possible from the locus typicus, from both the molecular and morphological point of view, as requested by the Barcode of Life Data System. One objective of the MoDNA project has been to utilize DNA barcoding to verify the variability in the characteristics of cryptic species in the genus *Ramazzottius*, based on evidence from our preliminary data. Specimens from different populations on lichens from rocks and trees were sampled and analysed by means of morphological (light and electron microscopy), karyological and molecular (mtDNA COI) approaches; the sex ratio was assessed for each population analysed. Voucher specimens were obtained by mounting both adults and eggs/eggshells in Faure-Berlese fluid, while DNA was extracted from newborns and adults. At least four different cryptic species could be attributed to *R. oberhaeuseri*, whose diversity was supported by different haplotypes and different degrees of ploidy, but not by a different eggshell and adult morphology.

Do buildings in Cambridge, United Kingdom act as "islands" for Tardigrada and associated Meiofauna?

C. Bromley (Anglia Ruskin University, Cambridge, England)

I mapped the distribution of tardigrades on the roofs of buildings within the English city of Cambridge. I treated individual buildings as "islands" and then applied the MacArthur & Wilson 1967 "Theory of Island Biogeography" model to my data in order to address the following questions. (1) Are larger buildings' areas more taxon rich? (2) Is there a relationship between tardigrade taxa and age of a building? (3) Which tardigrade genera are present at what sites? (4) Does building height or aspect influence colonisation? (5) Are some sites subject to more disturbance than others? Cambridge provides an excellent platform for such a study, as the city has buildings ranging from 15th Century mediaeval ornate stone to modern concrete within a relatively compact area, offering a variety of substrata and thus potential tardigrade (and other meiofauna) habitats. Moss samples were collected from the measured roof areas of exemplar buildings of known ages, height and construction. Tardigrades, and other meiofauna, were then extracted from these samples and presented as 'classic' island models in terms of diversity versus building/island age and size.

Analysis of the 18srDNA gene from individual tardigrades.

L. Chen, S.M. Sewell, F.A. Romano, III, and C.A. Murdock (Jacksonville State University, U.S.A.)

Tardigrades were isolated in samples collected from Alabama by washing through sieves (150 micron, 45 micron). Tardigrades were initially identified by stereomicroscope followed by characterization under phase contrast microscopy. Individual DNA samples were isolated from single tartigrades and regions of the 18S rDNA gene were amplified via PCR for each sample. The resulting PCR products were subsequently cloned and recombinant plasmids were isolated and sequenced. Five tardigrade sequences (Dactylobiotus ambiguus) were obtained and compared to existing sequences available in GenBank database (NCBI) using the Basic Local Alignment Search Tool (BLAST). Analyses of sequences generated from 18S1 and 18S2 primers, yielded alignments of 718 bases, with one transitions and no transversions observed. Analyses of sequences generated from 18S4 and 18S5 indicated five transitions and no transversion. The region amplified by primers S4 and S5 showed more variability per base than the region amplified by primers S1 and S2. Whether this is indicative of the S4/S5 region having more variability or from random chance is not forthcoming. Additionally, there is very little sequence data for this region of the 18S rDNA for the genus *Dactylobiotus*, where only 8 others sequences are available from

GenBank (none spanning the entire S4/S5 region). The presence of single nucleotide polymorphisms (SNPs) was noted in the sample, where a variation in 18S rDNA sequence was present between individual tardigrades. Another observation, focused on the transition located at position 112 is interesting in that the difference can be correlated to the collection time, where samples showing 5'AAC 3' were collected in April 2008 and 5'AGC 3' originated from samples in March 2009.

Tardigrade fauna from two biogeographic regions of Patagonia (Río Negro province, Argentina)

M. Claps, G. Rossi (La Plata University, Argentina); A. Rocha (La Pampa University, Argentina)

Samples of lichens and mosses were obtained in two protected areas of Río Negro Province located at different biogeographic regions of Argentina: subantartic forests and Patagonian steppe. Fourteen samples were collected in locations of the Nahuel Huapi National Park and nine in the Reserve Natural of Somuncurá Plateau. The subantartic region is characterized by the presence of Nothofagus forests. The annual mean precipitation is approximately 800 mm and the mean temperature 8.3 ºC (mean warm temperature: 12.9 and mean cold temperature: 1.3). The Somuncurá plateau is a massive 25,000 km2 volcanic plain conformed by superimposed layers of basalt flows and elevated between 600 – 1,600 m above the sea level. Annual mean temperature is around 10 °C and precipitation around 200 mm. At highest elevations, winter temperature is -25 °C and summer temperature is above 35 °C. A total of twenty eight species belonging to ten genera were found. Eighteen species were collected in the samples of Nothofagus forests meanwhile in those obtained in the plateau thirteen species were identified. The assemblages recorded in the mentioned regions were clearly different. A cluster analysis (UPGMA technique) perfumed with Baroni Urbani-Buser coefficient revealed similarities between some locations. *Ramazzottius baumanni* and *Milnesium tardigradum* are present in some samples of both sectors. Milnesioides exsertum is recorded for the first time after its discovery in Nothofagus forests of Australia and New Zealand in three samples of Nahuel Huapi. Four new species obtained in samples of Somuncurá plateau are described for science (three species of *Macrobiotus* and one of genus *Echiniscus*).

The actin gene of *Hypsibius klebelsbergi* (Eutardigrada) - complete sequence and comparison with actin from related taxa

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and H. Greven (Institut für Zoolmorphologie und Zellbiologie der
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Actin is a highly conserved cytoskeletal proteins in eucaryotic cells and numerous sequences are available for a comparative study. The actin filaments are formed by polymerization of globular actin subunits consisting of 374 – 376 amino acids. In most organisms multigene families code for several muscle and non-muscle isoforms, which slightly differ especially at their N-terminus. To analyse and sequence the actin gene of tardigrades, we used a directional λ ZAP Express cDNA library, previously constructed by us from the eutardigrade Hybsibius klebelsbergi. We obtained the complete actin coding sequence of one isoform (1128 bp; 375 amino acids) together with parts of the 3`-and 5`-UTR region. Comparison with GenBank data of the twelve EST actin sequence fragments of Hybsibius dujardini indicates that the *Hybsibius klebelsbergi* actin sequence probably represents the most abundant muscle isoform. Ten of these clones show minor differences in codon usage and identical amino acid compositions to the *H. klebelsbergi* actin. Only two clones show amino acid variations in one and five positions compared to the *H. klebelsbergi* and *H. dujardini* actin and represent isoforms, though their N-terminal amino acids are identical. Interestingly, there exists a considerable conformity of the 5`- and 3'- UTR regions (56 and 80% of about 100 and 300 nucleotides, respectively). Generally, the *H. klebelsbergi*-actin sequence corresponds largely to the β-actin of vertebrates as known from muscles of other invertebrates. When compared with the actin of several Ecdysozoa, there is a more pronounced similarity to the arthropod than to the nematode actin.

Functional protein clusters and regulatory motifs in *Hypsibius dujardini* und *Milnesium tardigradum*

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Functional protein clusters and regulatory motifs do not only mediate the unique adaptation of tardigrades against extreme temperature and other harsh environmental conditions, but are important markers to distinguish species and taxonomic units. We show here in detail results of our current comparison between *Hypsibius dujardini* and *Milnesium tardigradum*. We found 50 different clusters of sequence similar proteins between both tardigrades of which 10 are tardigrade specific. Proteins of other clusters are involved in DNA and protein protection and protection against oxidative stress. Heat shock proteins were found in both tardigrades. Several ones (hsp10, hsp60, hsp70, hsp90, small hsp) were only present in *M. tardigradum*. Further we investigate differences in their regulation. Additionally protective protein functions include aquaporins, DNA protection including Helicases, DNA repair (DNA J family), cold-shock like and specific membrane protection proteins. In functional terms the protein inventory of *M. tardigradum* and *H. dujardini* involves similar protein, DNA and stress protective functions. However, results indicate that both species have several specific and independent protein families for these tasks and more *M. tardigradum*-specific families became apparent from the ongoing sequencing project. Furthermore, the high resistance of *M. tardigradum* against environmental stress may result from the comprehensive capabilities of such species-specific families.

The Project www.FUNCRYPTA.de is funded by the German Federal Ministry of Education and Research, BMBF (0313838).

Distinguishing species in *Paramacrobiotus* (Tardigrada, Macrobiotidae)

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Analyses of the internal transcribed spacer 2 (ITS2) secondary structures were performed for five morphologically almost identical individuals from *Paramacrobiotus* (Tardigrada). Individuals were sampled from four different continents and sequenced. Sequences were annotated and their secondary structures predicted. A phylogenetic Profile Neighbor-Joining tree was reconstructed on a combined sequence-structure alignment. The topology obtained is consistent with a tree derived from a distance matrix of compensatory base changes (CBCs). CBCs were calculated between all ITS2 sequence-structure pairs of the global multiple sequence-structure alignment. A CBC represents enough evolutionary distance to distinguish between species with a confidence level of 93%.Therefore the CBC analysis indicates that *Paramacrobiotus* consists of at least three species distinguishable with molecular evidence.

The Project www.FUNCRYPTA.de is funded by the German Federal Ministry of Education and Research, BMBF (0313838).

Preliminary Survey on the Freshwater Tardigrades from the Kamo River, Kyoto, Japan

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The freshwater tardigrade fauna has been poorly studied in Japan. 28 species have been recorded (seven are still at the genus-level identification), but more species are expected to be discovered. The first author (S.F.) has been surveying the tardigrades in the Kamo River, Kyoto from 2008 and has collected over 80 specimens. We can count at least four species: two belonging to the Hypsibiidae and other two to the Macrobiotidae. The most abundant one belongs to the exclusively aquatic genus *Dactylobiotus* Schuster, 1980 based on the characteristic double claws and the chitinous bar connecting them at their base. In Japan, species of this genus has been recorded from the Tama River, Tokyo (Sudzuki, 1975), the Imperial Palace, Tokyo (Abe & Takeda, 2000), and the Lake Biwa, Shiga (Biserov et al. 2001). We investigated both the adults and eggs of the present *Dactylobiotus* specimens by light and scanning electron microscopy and identified them as *D. dispar* Murray, 1907. Although the species is said to be parthenogenetic, we found one possible male specimen with a curious form of claws. Besides, we are making further investigations on the remaining three species.

UV-B tolerance in two species of tardigrades

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The ability of tardigrades to enter cryptobiosis in a desiccated habitat could allow anhydrobiotic tardigrades to become much more resistant to extreme chemical and physical stresses than active ones, even if desiccation lasts for quite long periods. So far, most studies on the resistance of desiccated tardigrades have focused on their responses to very low or high temperatures, chemical molecules, high pressure and ionizing radiations. However, there is a paucity of data regarding the tardigrade's ability to tolerate ultraviolet radiation. Considering also the future perspectives offered by space flights, we evaluated the UV-B resistance of tardigrades. Here we report on the ability of two eutardigrade species (*Paramacrobiotus richtersi* and *Ramazzottius oberhaeuseri*) to withstand UV-B radiation in two different physiological conditions, namely desiccated and hydrated states. For both species, hydrated and desiccated tardigrades were exposed to seven different UV-B doses (from 47.8 to 406.1 kJ/m2) in a controlled environment chamber. In addition, the UV-B resistance of *P. richtersi* specimens under different values of temperature or relative humidity were analysed. In all experiments non-irradiated tardigrades were used as controls. Both species showed similar resistance to UV-B radiation both in the hydrated and desiccated state, indicating that tolerance to physical extremes is not an exclusive property of desiccated tardigrades. Our results further demonstrate that tardigrades are a good animal model for astrobiological research.

This work was supported by the Italian Space Agency A.S.I., MoMa – ASSC Grant to L. Rebecchi.

Freeze tolerance in embryonic stages of the eutardigrade *Milnesium tardigradum*

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Subzero temperature survival commonly found among tardigrades allows them to cope with low temperatures in their environment. While the ability to tolerate freezing body water has been demonstrated in several tardigrade species, freeze tolerance of eggs and embryonic stages has been little studied, though this is an important ecological aspect.

We evaluated the subzero temperature survival of five different developmental stages of the eutardigrade species *Milnesium tardigradum* after freezing to -30°C. Eggs and embryos were exposed to five different cooling rates between room temperature and –30 ºC at 1 ºC/h, 3 ºC/h, 5 ºC/h, 7 ºC/h, and 9 ºC/h followed by a warming period at 10ºC/h. The results show that the developmental stage and the cooling rate have a significant effect on the hatching rate. Less developed embryonic stages were more sensitive to freezing at higher freezing rates than further developed stages. Differential Scanning Calorimetry was used to determine the temperature of crystallization (Tc) in single embryos of the different developmental stages and revealed no differences between the stages. Evaluating the calorimetric data we also conclude that the ice nucleation is homogeneous in embryonic stages in tardigrades and not triggered by nucleating agents as it also has been shown for fully developed tardigrades recently.

The Project www.FUNCRYPTA.de is funded by the German Federal Ministry of Education and Research, BMBF (0313838).

Ecological factors determining distribution of Tardigrada in Costa Rican rain forests

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Over 700 moss, lichen and hepatic samples were collected from the whole territory of Costa Rica. Tardigrades were present in nearly half of the samples. Overall, more than 7000 tardigrades were found. All specimens were identified as 64 species belonging to 18 genera. The number of species per sample varied between 1 and 10 but on average it did not exceed 3. The most important factor influencing distribution of Tardigrada in Costa Rica was altitude. Tardigrades most often inhabited mosses, lichens and hepatics collected from 2000-2400 m asl and above 3200 m asl (more than 70% positive samples). The highest Tardigrada diversities were found between 1400-2000 m asl (35 species, 54.7%), and the lowest between 2400-2800 m asl (18 species, 28.1%). The second strongest factor that influenced tardigrade distribution was the type of environment. A significantly higher proportion of positive samples was found in urban and agricultural areas (about 60%) than in natural habitas (only about 30%). The highest frequency of positive samples was found in humid habitats. At least 24 species (37.5%) showed very clear habitat preferences and only 7 (10.9 %) did not. Surprisingly, the substrate and plant type had the lowest influence on tardigrade distribution.

Zoogeography of Costa Rican rain forests tardigrades

Ł. Kaczmarek (A. Mickiewicz University, Poland), Ł. Michalczyk (University of East Anglia, UK), B. Gołdyn (A. Mickiewicz University, Poland) & W. Wełnicz (Technische Fachhochschule Wildau, Germany)

Over 700 moss, lichen and hepatic samples were collected from the whole territory of Costa Rica. Tardigrades were present in nearly half of the samples. Overall, more than 7000 tardigrades were found. All specimens were identified as 64 species belonging to 18 genera. Within the tardigrade Costa Rican fauna (75 species in total: 64 species found in this study and another 11 from other papers) there can be distinguished five zoogeographical groups of species: cosmopolitan (23 species), holoarctic (3 species), palearctic (2 species), neotropical (5 species), and mesotropical (19 species). Remaining 23 species were found in single localities, therefore their zoogeographical ranges are unknown. Most of species found in Costa Rica belong to two groups: cosmopolitan species (known from many localities throughout the world) and mesotropical species (inhabiting tropical lands of the Americas between 30° South and 30° North). Holoarctic and Palearctic species were found in Costa Rica in single samples in urban areas and were probably transported to Costa Rica by humans.

Preliminary account of the tardigrades of Ukraine

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Tardigrades of Ukraine are imperfectly studied. The main body of research was conducted in the thirties, just before the Second World War. In 1931-1941 E. Bartoš, Gy. Iharos and L. Rodewald performed extensive studies on tardigrades in Carpathian Mountains Zakarpattya (Transcarpathia), and Bukovina, whereas M.P. Bozhko studied tardigrades in in Kharkiv region, and examined occasional samples from Crimea. Since then only few researchers have contributed to the present knowledge on the fauna. V.V. Polishchuk studied tardigrades in freshwater pools of Danube basin (1974, identification by L. Rudescu). N.E. Kovalchuk carried out a research on freshwater tardigrades in Dniester basin (1987). N.G. Sergeeva et al. investigated marine tardigrades in the Black Sea coastal zone of the Crimea (2006). We attempted to summarize the results of these past studies and proceeded to a new research on tardigrade fauna. We investigated 250 samples of mosses and lichens and 50 samples of leaf litter and soil from various regions of Ukraine and studied some specimens of marine tardigrades from the Black Sea deposited in the collection of the Institute of Biology of Southern Seas. Literature data were updated to modern taxonomy of tardigrades (where it was possible). Tardigrades from our samples were extracted by the use of standard methods, mounted on the slides in glycerol or Faure's medium and studied by the means of light microscopy. Keys used for identification were those given in the 3-rd edition of 'Il Phylum Tardigrada', 'Tardigrada of Poland' (Dastych, 1987) and in revisions of some genera of tardigrades. Tardigrade taxa were named according to the actual checklist of Tardigrada (Guidetti & Bertolani, 2005; Degma & Guidetti, 2007), except for the species of the *Echiniscus canadensis-blumi* series that were considered as a single species *E. blumi*. As a result, a checklist of tardigrade species known from Ukraine has been compiled. It includes 84 species of limno-terrestrial and 7 species of marine tardigrades. 10 species were registered in Ukraine for the first time. The checklist displays data deficiency and contains some dubious records and records of species complexes. More intense sampling and research on the collected specimens is required.

Life history studies of bisexual tardigrades

M.-L. Lemloh, F. Brümmer, R. O. Schill (Universität Stuttgart, Germany)

Although tardigrades were studied for a long time there is still less known about their way of life and until now only few studies are dedicated exclusively to tardigrade life history traits. However, an improvement of rearing methods during the last decade resulted in more detailed life cycle studies for parthenogenetic tardigrades whereas the present work is the first one presenting life history studies of bisexual tardigrades under laboratory conditions. Two bisexual tardigrade strains (*Macrobiotus tonollii* and *Macrobiotus sapiens*) were examined analysing the following life history traits: active life span, body lengths, age at first oviposition, egg-laying intervals, clutch size, hatching time and hatching percentages. The received data supplement our knowledge of tardigrades in general and enable further research into factors which may influence life history of these species.

The Project www.FUNCRYPTA.de is funded by the German Federal Ministry of Education and Research, BMBF (0313838A).

Investigation of movement behaviour of tardigrades by a custom-made automated video tracking system

D. Shcherbakov (University of Hohenheim, Germany), R. O. Schill, F. Brümmer, M. Nissen (Universität Stuttgart, Germany) & M. Blum (University of Hohenheim, Germany)

Locomotion behaviour is one of the most important forms of tardigrades adaptation to different environmental conditions. In such way animals can find the places with optimal environmental conditions to get their nutrition (for example through prey hunting), avoid danger and adverse environmental areas. A deeper understanding of tardigrades behaviour makes possible a better understanding of their complete biology. The investigation of locomotion behaviour of tardigrades is difficult because of their small and relative transparent body. Their slow locomotion level often requires long term observation. To overcome these difficulties an automated video tracking system for short and long term investigation of the locomotion behaviour of tardigrades was developed. Using this system automated experiments were performed to investigate the movement behaviour in 32 individuals of *Milnesium tardigradum* under infrared as well as under visible light conditions. The results are based on digital analysis of 754 hours of recorded video material. The mean velocity in the experiments reached 23.3 mm/h, with a maximum of 1166.4 mm/h. There were no significant differences in the movement activity of specimens between infrared and visible light conditions.

The effect of the insecticide imidacloprid on the leaf litter/soil tardigrade community in a hemlock woolly adelgid infested forest in the Great Smoky Mountains National Park, Tennessee, USA

A. M. Lyons (Massachusetts Institute of Technology, USA); D.R. Nelson (East Tennessee State University, USA); P.J. Bartels (Warren Wilson College, USA)

The invasive hemlock woolly adelgid (*Adelges tsugae* Annand) (HWA) currently threatens to destroy the ecologically important native hemlock forests (*Tsuga canadensis* (L.) Carrière and *Tsuga caroliniana* Engelmann) throughout eastern North America. Among treatment options, the systemic insecticide imidacloprid can provide resistance to the adelgid for several years when applied to infested trees by soil drenching. The effects of imicacloprid on soil microclimates and meiofauna, however, are largely unknown. To determine if soil-applied imidacloprid affected leaf litter/soil tardigrade populations, a field study was conducted in a highly impacted forest in the Great Smoky Mountains National Park, Tennessee, USA. Two leaf litter/soil samples were collected at the base of each of 20 infested Eastern hemlocks (*T. canadensis*): 10 trees that had been treated with imidacloprid and 10 that had been left untreated, for a total of 40 samples. In the laboratory, the samples were processed with Ludox, and the tardigrades were extracted and mounted in Hoyer's for identification. The numbers of tardigrades, nematodes, mites, rotifers, springtails, and other organisms were not significantly different between treated and untreated trees. However, there was a significant difference in tardigrade species diversity between treated and untreated hemlocks, indicating that soil-applied imidacloprid may affect the population dynamics of non-target soil microorganisms.

Transcriptome data of Milnesium tardigradum and differential gene expression during anhydrobiosis

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In order to understand anhydrobiosis on an expressional level we have generated a comprehensive transcriptome map of *Milnesium tardigradum*. Our initially gathered transcriptome data consisted of ~10.000 ESTs from two non-normalized cDNA libraries from active and inactive (un state) animals resulting in about 3200 putatively unique transcripts. This data was complemented with 4 normalized cDNA libraries of active, inactive and two intermediate stages that have been sequenced using 454 GS-FLX pyrosequencing generating about 150 Megabases of transcriptome data.

To generate robust quantitative real-time PCR data a set of reference genes from different functional classes has been evaluated regarding their stability during anhydrobiosis. Most of the tested reference genes exhibited suitable expression stability whereas the commonly used reference GAPDH (glyceraldehyde-3-phosphate dehydrogenase) showed significant regulation during anhydrobiosis. Additionally we used cDNA representational difference analysis to identify differentially expressed transcripts between active and inactive (tun) animals. Transcripts with various functions have been cloned and evaluated via quantitative real-time PCR. Among them are genes from known stress and protein folding responses, probably as a result of anhydrobiosis.
These resources will serve as a basis for gene expression studies based on digital gene expression tag profiling and cDNA representational difference analysis. Together a comprehensive gene expression atlas will be generated that will allow to identify pathways that are involved in anhydrobiosis.

The Project www.FUNCRYPTA.de is funded by the German Federal Ministry of Education and Research, BMBF (0313838).

A comparison of tardigrade diversity between the Big Thicket National Preserve and the Great Smoky Mountain National Park

H. A. Meyer, J. G. Hinton (McNeese State University, Lake Charles, Louisiana USA)

For the past 2 years, we have been collecting tardigrades (water bears) in the Big Thicket National Preserve in southeast Texas as part of an All Taxa Biodiversity Inventory (ATBI). Our inventory is based on methods used by Bartels and Nelson in their survey of tardigrades in the Great Smoky Mountains National Park (GSMNP). The Big Thicket lacks the altitudinal variation and habitat diversity of the GSMNP. Our experience in Louisiana, Florida, and Mississippi suggests that terrestrial tardigrade diversity in southern habitats may be lower than the Appalachians. Therefore, we do not anticipate finding as many species new to Texas or to science as Bartels and Nelson have found in GSMNP, but a doubling of the reported Texas tardigrade species richness is likely. In any event, comparing the GSMNP diversity with a survey in the Big Thicket of comparable sampling intensity and methodology will be instructive. Our collection includes lichen, moss, leaf litter, and aquatic samples from the 15 units of the Big Thicket. Of the 15 units, 7 have been sampled and analyzed for tardigrades. Terrestrial samples were placed in paper bags and aquatic samples were placed in plastic bags. Tardigrades were extracted from samples, mounted on microscope slides with polyvinyl lactophenol, and studied with phase contrast and DIC microscopy. A total of 113 samples have been analyzed: 105 moss, lichen, and leaf litter; and 8 periphyton. No tardigrades have been found in the 8 aquatic samples or in 31of the terrestrial samples. Samples yielded 303 identifiable specimens. Moss, lichen, and leaf litter samples yielded 4 families and 6 genera. Species found thus far are: *Macrobiotus echinogenitus, Macrobiotus* cf. *harmsworthi, Macrobiotus* cf. *hufelandi, Macrobiotus* cf. *liviae, Macrobiotus richtersi, Macrobiotus tonolli, Milnesium tardigradum, Minibiotus intermedius, Echiniscus virginicus, Echiniscus tamus, Diphascon* (*Adropion*) *scoticum*, and *Pseudechiniscus brevimontanus*. Species new to Texas are *Macrobiotus tonolli, Echiniscus virginicus, Diphascon* (*Adropion*) *scoticum*, and *Pseudechiniscus brevimontanus*. Overall, the diversity of species and higher taxa found in the BTNP so far is much lower than that reported from the GSMNP. This is consistent with other surveys of tardigrades in the Gulf Coast region of the Deep South.

Terrestrial Tardigrada of Michigan, USA

H. A. Meyer, J. G. Hinton (McNeese State University, USA)

The presence of more than 200 freshwater and terrestrial species has been reported in North America. The provinces and states surrounding the Great Lakes have been among the more poorly surveyed areas; only 31 species have been recorded from the region. Isle Royale and two peninsulas make up the state of Michigan. The Lower Peninsula is largely flatland, consisting of deciduous forest, extensive farmland, and large cities; the hilly Upper Peninsula remains heavily forested. Two journal articles and one PhD dissertation have reported the presence of five species in Michigan: *Isohypsibius prosostomus* and *Macrobiotus hufelandi* on Isle Royale, and *Diphascon* (*Diphascon*) *belgicae, Macrobiotus echinogenitus* and *Parhexapodibius pilatoi* in the Lower Peninsula. In 2005 we collected lichen, moss, and leaf litter samples from nine Lower Peninsula counties and all fourteen Upper Peninsula counties. These samples contained 606 specimens and 94 eggs, belonging to 8 genera and 25 species. *Echiniscus wendti, Pseudechiniscus facettalis, P. suillus, Diphascon* (*Diphascon*) sp., *Fractonotus caelatus, Macrobiotus areolatus, Macrobiotus richtersi, Minibiotus hufelandioides,* and *Astatumen trinacriae* were present only in material from the Lower Peninsula. *Echiniscus* cf. *arctomys, E. merokensis, D.* (D.) *alpinum, Hypsibius arcticus, H. calcaratus, Ramazzottius baumanni, Macrobiotus echinogenitus,* and *Minibiotus furcatus* were found only in the Upper Peninsula. *Milnesium tardigradum*, *D.* (D.) *nodulosum, R. oberhaeuseri, Macrobiotus* sp., *Macrobiotus* cf. *hufelandi, Macrobiotus tonollii, Minibiotus intermedius*, and *Hypsibius* sp. were present in both peninsulas. Some of the samples were analyzed in 2005. Unfortunately, our university and laboratory were severely disrupted by the effects of Hurricane Rita in September 2005, and therefore laboratory analysis of most samples could not be completed before 2008. The delay in processing some samples probably reduced the number and diversity of tardigrades extracted.

Studies on olfactory cognition in tardigrades by video tracking

M. Nissen, F. Brümmer (Universität Stuttgart, Germany), D. Shcherbakov (University of Hohenheim), R.O. Schill, (Universität Stuttgart, Germany)

Most known tardigrade taxa represent species which live in moist environments. These habitats undergo seasonal changes. However, locomotion is essential for reacting to fast changing environmental conditions. Further more, carnivore species like *Milnesium tardigradum* have to move in order to hunt their prey, i.e. other small invertebrates like nematodes and rotifers. For a better understanding of the way of interaction with the environment a defined analysis of basic reaction patterns of tardigrades is needed. We performed different experiments to investigate the change in movement behavior of 21 individuals of *M. tardigradum* caused by depriving of food and their reaction on odorous substances made of an extracts of their prey organisms. A custom-made automated video tracking software was used for the behavioral analysis. The reaction on the depriving of food depended on the time between the experiment and the last molt.

Remarks on tardigrade diversity in the city of La Plata (Argentina) with descriptions of two new species of *Macrobiotus* Schultze.

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(Universidad Nacional de La Pampa, Argentina)

As part of ongoing research on tardigrades living in Neotropical cities, this study includes tardigrade faunas of La Plata (34 55‘ S, 57 17‘ W), Argentina. Near the estuary of the Río de La Plata, the city is home to a population of c. 700,000. Average annual relative humidity is 77.6 %, and annual average rainfall is 1023 mm. Sample sites included paved urban locations with different traffic intensities. At each site, lichens and moss samples were taken from sidewalk trees. Previous studies on tree-dwelling tardigrades in South American cities (Santa Rosa and General Pico, Argentina) revealed a low species richness (few species per city, and fewer still at the same location) and similar results were published from samples in the northern hemisphere such as Nice, Zürich and Cincinnati. Generally, only one of the species present in each city was highly tolerant to intense vehicle traffic conditions. Samples from the city of La Plata rendered surprising results. Species richness at locations with moderate to high vehicle traffic is significantly higher than that of other cities. On the other hand, at some high traffic sites tardigrade density reaches 38/cm2. Many species behave as highly poleotolerant. Some of these belong in the heterotardigrades. Such is the case of *Echiniscus rufoviridis*, previously considered poorly tolerant. Others belong in the eutardigrades, i.e., several species of *Macrobiotus*. Two of the latter - *Macrobiotus urbanensis* sp. nov. of the *harmsworthi* group, and *Macrobiotus platensis* sp. nov. of the hufelandi group – are described herein. Each one of these species can be separated from other related ones mainly by the features of the buccal armature and egg morphology. The two of them behave as highly tolerant to urbanized conditions, as they were found at diverse locations in the city, including those with intense vehicle traffic. On the other hand, those most frequent species at locations with moderate to high vehicle traffic in cities of central Argentina are rare in the city of La Plata. Results of this study pose interesting questions that should be addressed by future research.

New dates and new methods for research of freshwater Tardigrada fauna of city Szekesfehervar

A. Eles (Eszterhazy Karoly College, Hungary); L. Repas (Fejerviz ZRT., Hungary)

In Hungary there is not official, professional research of freshwater Tardigrades currently. We have started investigating with wonderful, tiny creatures on amateur level few years ago. Based on Vargha's article we presupposed that the incidence of the freshwater Tardigrada maybe indicate the water pollution. We took samples in 46 sampling points from surface waters, slow streams and lakes on Szekesfehervar area. (regio litoralis+pellagialis, submersiherbosa: mosses, algae, other water plants we selected four points from these (two contaminated, two less contaminated), which we often examine. The control samples were taken from stair epilitoralis of zone paralimnolitoralis. We developed a new filtering-washing procedure to the isolation from the aquatic samples. We often did the compaction of the samples on field already. We tried many methods between lab environmental, what we present in a pictorial report on our poster. Onto the facilitation of the isolation we fixed on inverse-microscope a homemade micro-manipulator too; and we often substitute for the expensive devices with cheap solutions, inventiveness. We have used modified polivinillactofenol instead of Hoyer's medium, based an own recipe, onto fixing. We observed when biodiversities of water biocoenosis increase in the zone litoralis, then the water bear cannot be found in the water. But they reappear in a winter period (when biodiversities decrease). Onto this appearance the wash down cannot give an explanation. In stair infra-, and eulitoralis we found lesser and different groups than in the taller, dry region. Presumably in egg and inactive state are in the bentos, in stair epilitoralis, or on the submersiherbosa. We prove the aquatic and terrestrial species' ethological and morphological differences with photographs. Freshwater Tardigrades specimens have faster motion in the streams. And their claws are longer than the terrestrial specimens. They defend themselves against drifting.

We prove with photoseries, the terrestrial specimens under water asphyctic state bring they to, in what they are able to exist until short time. However the aquatic species are stored under thick waterlayer they life is long. It seems that the aquatic individuals are more tolerant opposite the relative less oxygen. In our aquatic samples we have seen mostly genus *Dactilobyotus*.

Effects of starvation and anhydrobiosis on storage cell size in three tardigrade species

A. Reuner, S. Hengherr, F. Brümmer, R. O. Schill (Universität Stuttgart, Germany)

In the three tardigrade species *Milnesium tardigradum, Macrobiotus tonollii* and *Macrobiotus sapiens* the impact of starvation and anhydrobiosis on number and size of the storage cells was investigated. Those cells are free floating within the body cavity of tardigrades and are presumed to store and release energy in form of glycogen, protein and fat to maintain a constant nutrient regime for the other tissues. The body size of the animals was not correlated with the size of the storage cells, however, *M. tardigradum* (801.74 ± 37.63 µm) the largest species analysed also had the largest storage cells (214.14 ± 70.39 µm2). Seven days of starvation led to a reduction of the size of the cells, a similar period of anhydrobioses caused decrease in cell size in *M. tardigradum* but not in *M. tonollii* and *M. sapiens*. Although *M. sapiens* was raised on green algae, and *M. tardigradum* and *M. tonollii* were fed with rotifers and nematodes this difference in nourishment was not reflected in the response of the storage cells to anhydrobiosis. The differerent energetics during anhydrobiosis, displayed by the storage cell size variability during anhydrobiosis, indicate differences coping with anhydrobiosis in tardigrades.

International Barcode of Life Project (iBOL) – Tardigrada

R.O. Schill, C. Vogel, F. Brümmer (Universität Stuttgart, Germany)

The phylum Tardigrada has been expanded by several new taxa and increased to around 1000 species. They are subdivided into 2 classes (Eutardigrada and Heterotardigrada), 4 orders, 21 families, 105 genera and more than 960 species, but the number of described species increases quickly each year. They can be found in a diversity of (extreme) habitats including marine, freshwater and terrestrial ecosystems ranging from the deep sea to the highest mountains. Despite their overall abundance tardigrades have received little attention in the last 200 years. However, the study of tardigrade species presents a general problem which is frequently encountered in the study of small invertebrates; small size, phenotypic plasticity, and genomic variability in the characters, may not permit a definite identification of the species. Further more, morphological keys are often only effective for a particular life stage. Cysts or single parts of tardigrades seem to be quite difficult to distinguish by classical morphological methods. The new Tardigrade Barcoding Database (www.tardigradebarcoding.org) is the prime access point for DNA signature sequences together with information on conventional morphological taxonomic characters of tardigrades, and part of the International Barcode of Life Project (iBOL).

Comparative proteome analysis of desiccation stress response in *Milnesium tardigradum* using DIGE technology

E. Schokraie (Functional Proteome Analysis, German Cancer Research Center, Heidelberg, Germany); R. O. Schill (Department of Zoology, University Stuttgart, Stuttgart, Germany); M. Schnölzer (Functional Proteome Analysis, German Cancer Research Center, Heidelberg, Germany)

For the better understanding of molecular processes during anhydrobiosis especially on the protein level, we performed a comparative proteome study based on 2D gel electrophoresis. The emphasis was to detect changes in the protein expression of tardigrades (*Milnesium tardigradum*) in active versus anhydrobiotic (tun) stage. Two dimensional differential in gel electrophoresis (2D DIGE) minimizes gel-to-gel variation by allowing the separation of two different sample which are labelled with different fluorescent dyes on the same gel. Especially the minimal labelling approach allows using an internal standard (a pool of two different samples), which offers many advantages. The most important one is accurate quantification and spot statistics between the gels.
To perform 2D DIGE analysis we pooled 1600 active tardigrades (pool 1) and 1600 anhydrobiotic tardigrades (pool 2). A total of six technical replicates and one preparative gel were performed. Protein extracts of active and anhydrobiotic tardigrades were labelled alternately with Cy3 and Cy5. The internal standard was prepared by mixing protein extract of tardigrades in the two different stages (active and tun) and labelling with Cy2. These three different labelled protein extracts were mixed at a ratio of 1:1:1 and separated by their isoelectric point in the first dimension and by molecular weight in the second dimension. For image acquisition we used Typhoon Variable Mode Imager. We analysed images with Decyder Differential Analysis Software, which allows differential in-gel analysis (DIA) and biological variation analysis (BVA). In BVA module we used a protein filter with following parameter: student t-test: <0.05, average ratio: >1.2 or <-1.2 and appearance in at least 75% images. These parameters delivered 90

regulated proteins. So far 40 proteins were analysed by electrospray ionization tandem mass spectrometry (ESI-MS/MS). Proteins like Arginin kinase, paramyosin, malate dehydrogenase, Glycerol-3-phosphate dehydrogenase and methyl-accepting chemotaxis sensory transducer could be identified as upregulated in anhydrobiotic tardigrades. In summary we performed differential analysis of tardigrades in the active and anhydrobiotic stage using 2D DIGE system. We could not detect regulations above 1.5fold or below -1.5fold. This indicates that de novo protein synthesis might not play an important role during anhydrobiosis.

Microclima and vertical distribution of tardigrades in a moss cushion

R. Schuster* (Instiut für Zoomorphologie und Zellbiologie, Heinrich-Heine Universität Düsseldorf, Germany); I. Spiertz, R. Lösch (Geobotanik der Heinrich-Heine Universität Düsseldorf, Germany); H. Greven (Instiut für Zoomorphologie und Zellbiologie, Heinrich-Heine Universität Düsseldorf, Germany); *present address: Schule Birklehof, Hinterzarten

Microclimatic conditions (temperature, light intensity, oxygen, carbon dioxide) and distribution of tardigrades were studied in a cushion of the moss Rhytidiadelphus loreus. Temperature varied with the climate, but in deeper layers of the moss it was lower during the day and higher during the night compared to the environment indicating a temperature conserving effect. As expected, intensity of light decreased toward the most basal layer of the moss cushion. Oxygen was saturated in the entire cushion, whereas a marked increase of CO2 was found towards the depth. Tardigrades (six species) were predominantly found in the green brown b-layer of the moss. We suggest that apart from a possibly species-specific vertical distribution, migration of tardigrades in the upper regions of the moss is mainly influenced by temperature. The elevated CO2 concentration in deeper layers may be a limiting factor for most species. In mosses of the *R. loreus* – type, light intensity and in particular oxygen seems to be of minor significance regarding the distribution of tardigrades.

Tardigrade culturing at Jacksonville State University.

S. M. Sewell and F. A. Romano, III (Jacksonville State University, U.S.A.).

The difficulty of culturing tardigrades has played a major obstacle in gaining information on life histories (Altiero & Rebecchi 2001). Since a major difficulty in rearing tardigrades is that their food source is mostly unknown, much of the culturing that has been done is with carnivorous tardigrades (Sayre 1969, Baumann 1964, Altiero et al. 2006). Others have had some success raising herbivorous tardigrades for a few weeks or months in small crucibles, small aquaria or small glass containers (Baumann 1961, Baumann 1966, Weglarska 1957). More recently 4 species of tardigrades were reared successfully through several generations in small plastic culture dishes in a desciation controlled environment by Altiero & Rebecchi (2001). Very few attempts to culture individuals in microcosms have been made since there are so many inherent problems. Beginning in May 2007 several types of culturing techniques were attempted with *Echiniscus mauccii* and *Dactylobiotus ambiguous*. Varying numbers of tardigrades (2-12), placed in microaquaria (as microcosms) filled with spring water and small pieces of moss, were observed frequently. Very little success in following individual tardigrades was found with these microaquaria and *E. mauccii* was difficult to keep alive for long periods. At this time a second type of microcosm was used. Five animals (or less) were placed into glass depression slides (depressions coated with 2% agar) kept in covered Petri dishes with saturated filter paper and kept at room temperature. Since this had limited success (*D. ambiguus* encysted) these microcosms were placed into a growth chamber at environmental temperature (16.6oC) and light cycle. Lastly, plastic depression slides were used as microcosms (kept in the same manner). These depression slides (deeper wells were less likely dehydrate) and the plastic allowed tardigrades to walk, thus, agar was not needed. Gravid females were used in an attempt to track information on egg laying and hatching, juvenile characteristics, as well as other life cycle information. The longest an individual survived was 22 days. Although not successful at obtaining complete life cycle information, useful information was obtained pertaining to egg size, clutch size, hatching time, as well as juvenile size.

Marine tardigrades from Shimabara Bay, Japan

A. C. Suzuki (Keio University, Japan)

Although several tardigrades have been described around Japanese coast so far, information on marine species in Japan has still been very limited. The author recently began to faunistic research of marine tardigrades in Japan, and this presentation will show some preliminary results obtained from sediment samples collected in 2007 at several locations of Shimabara Bay. Subtidal samples, consisted of shelly gravel, were collected with a Smith-McIntire grab at about 15 m depth of the bay. An intertidal sample was collected near Aitsu Marine Station, Kumamoto University. The samples were treated with fresh-water and decanted through a 32 µm mesh net. Arthrotardigrada from these samples comprise at least 14 species in the 3 Families: Stygarctidae, Halechiniscidae and Batillipedidae. Echiniscoidea in subtidal sediments include at least one species of *Anisonyches*; *Echiniscoides* cf. *sigismundi* was found from barnacles near the Marine Station. This collection includes first records of two genera, *Pseudostygarctus* and *Actinarctus*, from Japan.

Tardigrades of China: an analysis of habitat Chemistry

J. Vaughn, T. Clark (Baker University, U.S.A.)

Tardigrades are aquatic animals, thus it is the chemistry of the water that determines if a habitat is suitable for living. For limno-terrestrial tardigrades found in the moss and lichen habitat of a given area the water chemistry is a product of the chemicals in the rain (or snow) that occasionally hydrates the habitat for the animals. This water mixes with the surface film accumulated on the habitat while dry, the chemicals leached from the substrate (tree bark, rock, or soil) below the habitat and the chemicals of the habitat itself. Several hundred samples of moss and lichen from China and North America have been tested for their pH, conductivity, nitrate, ammonium, and calcium content. The results present some significant differences and suggest tolerance ranges for several species of tardigrades.

An optimized protocol for tardigrade and rotifer muscular staining

F. Vicente (Faculty of Sciences, University of Lisbon, Portugal)

Tardigrada is a phylum of microscopic animals that can play an important role in early metazoan phylogeny clarification. Their muscular structure is, for this matter, an appealing study issue. Taking advantage of computer software and laser confocal microscopy, the specific interaction of phalloidin with f-actin has been used to create 3D models of some species musculatures (Halberg *et al.*, 200?; Schmidt-Rhaesa, & Kulessa, 2007). However interesting and cutting-edge these studies are, I think that the protocols used to prepare animal specimen is unnecessarily long and can considerably be shortened. Here I demonstrate how good muscular staining results can be obtained with a protocol reduced to less than 24 hours.

Molecular relationships inside the *Macrobiotus*"*hufelandi* group" (Eutardigrada, Macrobiotidae) based on 18S rDNA and ITS2 Designing molecular markers of rDNA

W. Wełnicz (University of Applied Science Wildau, Germany; Adam Mickiewicz University Poznań, Polen); Ł. Kaczmarek (Adam Mickiewicz University Poznań, Polen); M. Grohme, B. Mali (University of Applied Science Wildau, Germany); W. Niedbała (Adam Mickiewicz University Poznań, Polen); M. Frohme (University of Applied Science Wildau, Germany)

Molecular techniques are a widely used tool in phylogenetic analyses especially when morphological diversity is limited or the homology of morphological features are unclear. DNA sequence information is currently the main source of data for molecular phylogeny based on the hypothesis that related species share a common ancestor. Our aim is to design molecular markers which enable to determine molecular relationships within Tardigrada especially the current position of the "*hufelandi* group" and clarifiy the molecular relationships inside this group. We have studied three species from this group, which show a very low level of morphological diversity: *Macrobiotus hufelandi, Macrobiotus kaźmierski*, and *Macrobiotus polonicus*. Based on morphology and morphometric characters of eggs they have been divided into two subgroups, where *M. polonicus* and *M. kaźmierski* lack of egg shell reticulated which is present in M.hufelandi. In order to determine the phylogenetic relationships inside the hufelandi group the nucleotide sequences from the nuclear ITS-2 and 18S rDNA were utilized and our studies indicate that they are informative markers for resolving tardigrade phylogenetic relationships.

General Information

Conference venue

The conference venue will be the „Hörsaalzentrum“ at the „Morgenstelle“ of the Eberhard-Karls-University of Tübingen, conference room N1. Some events will take place at other venues, which will be explicitly named in the text.

Map

On the last pages, a plan of the lecture hall at the „Morgenstelle“ can be found.

Language

The official language of the meeting is English.

Registration Desk

Meeting documents and badges will be handed out at the registration desk. The registration desk will be in the „Hörsaalzentrum“ at the „Morgenstelle“ in front of the conference room N1. the registration starts on Monday 3rd August at 8:00 am.

Oral presentations

Speakers, please submit your files at the day before your talk at least two hours prior to beginning of the session in which you will present. This will give us a chance to make you aware of last minute instructions or changes concerning your session. We cannot guarantee that your presentation will be available in time if you check-in late. The stuff will provide you with audio and visual support, i.e. you can check on a local computer if your animations etc. work properly. Your files will be uploaded and are available in the conference room when needed.

Important numbers for Tübingen

direct connect to police	110
fire service	112
doctor on call	112
ambulance	19222
taxi in Tübingen	07071 9 20 55-5
	07071 2 43 02
	07071 2 43 01

Social program

Guided city tour (Monday 3rd August)
In the evening, after the first day of the conference, we will meet at the market place in the centre of the old town for a guided tour (in English) through the historical old town. Meetingpoint at the market place - 6:00 pm.

Boat trip (Tuesday, 4th August)
After the second day of the conference we will meet at the Hölderlin Tower and make a boat trip down the Neckar in a famous „Stocherkahn“ - a typical Tübingen boat that is propelled by a long wooden pole. The boat ride offers a scenic view of the picturesque Neckar waterfront and the famous Hölderlin Tower. After the boat trip we let the evening end at the German beer garden (drinks not included in the registration fee) as harmoniously as it has begun. Meetingpoint at the Hölderlin Tower - 6:00 pm.

Conference dinner (Wednesday 5th August)
All participants and accompanying guests are cordially invited to attend the conference dinner, which will be held in the evening of the last day of the conference, 5th August. It will begin at 8:00 pm at the „Casino am Neckar“ in Tübingen, which is located in the old town centre. The cost for the conference dinner is included in the registration fee. If you have any restriction for meals, please let us know so that we will try to accommodate your request. Meetingpoint at the „Casino am Neckar“ - 8:00 pm.

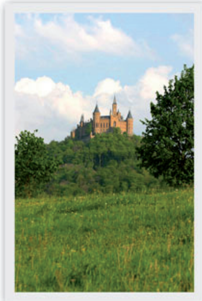
One-day excursion

All participants and accompanying guests are welcome to attend an all-day excursion starting in the morning at 9:00 am on the 6st August. The cost of the excursion is not included in the registration fee. Participants and accompanying guests who want to attend the excursion should indicate this at the registration desk until 4th August. We are arranging an all-day coach trip to:

The Bears' Cave and Charles' Cave - Cave of Erpfingen
The Karlshöhle (Charles' Cave), the first part of the Bärenhöhle (Bears' Cave), was discovered in 1834 by Karl Wilhelm Fauth, a school teacher from Erpfingen. The original entrance can be seen 20m behind today's entrance as a cleft, about 50cm wide, which resulted from the collapse of a piece of the ceiling. It is though that plague victims were probably thrown into this cleft during the Middle Ages as several skeletons were found in the debris under the cleft. At the time that Charles' Cave was discovered there were huge amounts of cave bear (*Ursus spelaeus*) bones on the floor. The bears had entered the cave not by the Fauthsloch, but by an entrance at the other end of the cave, near the artificial exit, though this entrance collapsed many thousand years ago. Included in the costs of the day trip is a guided tour of the caves (in English). Outside the caves are many places for collecting moss with tardigrades.

Lichtenstein Castle & Forester´s lodge
Lichtenstein Castle can be described, without any exaggeration, as the, „fairy tale castle of Württemberg". The novel „Lichtenstein" by Wilhelm Hauff, published in 1826, provided the inspiration for Count Wilhelm of Urach when building this beautiful castle complex. The former hunting lodge on the steep rock above the Echaz Valley was rebuilt during 1840-1842 and contains a large collection of historic weapons, armour and treasures. Here, at the Forester's lodge beside the Castle, we will have our lunch. (If you have any dietary restrictions for meals, please let us know so that we can try to accommodate your request). After lunch everybody will have time to visit the castle (for an additional fee not included in day trip costs).

Hohenzollern Castle

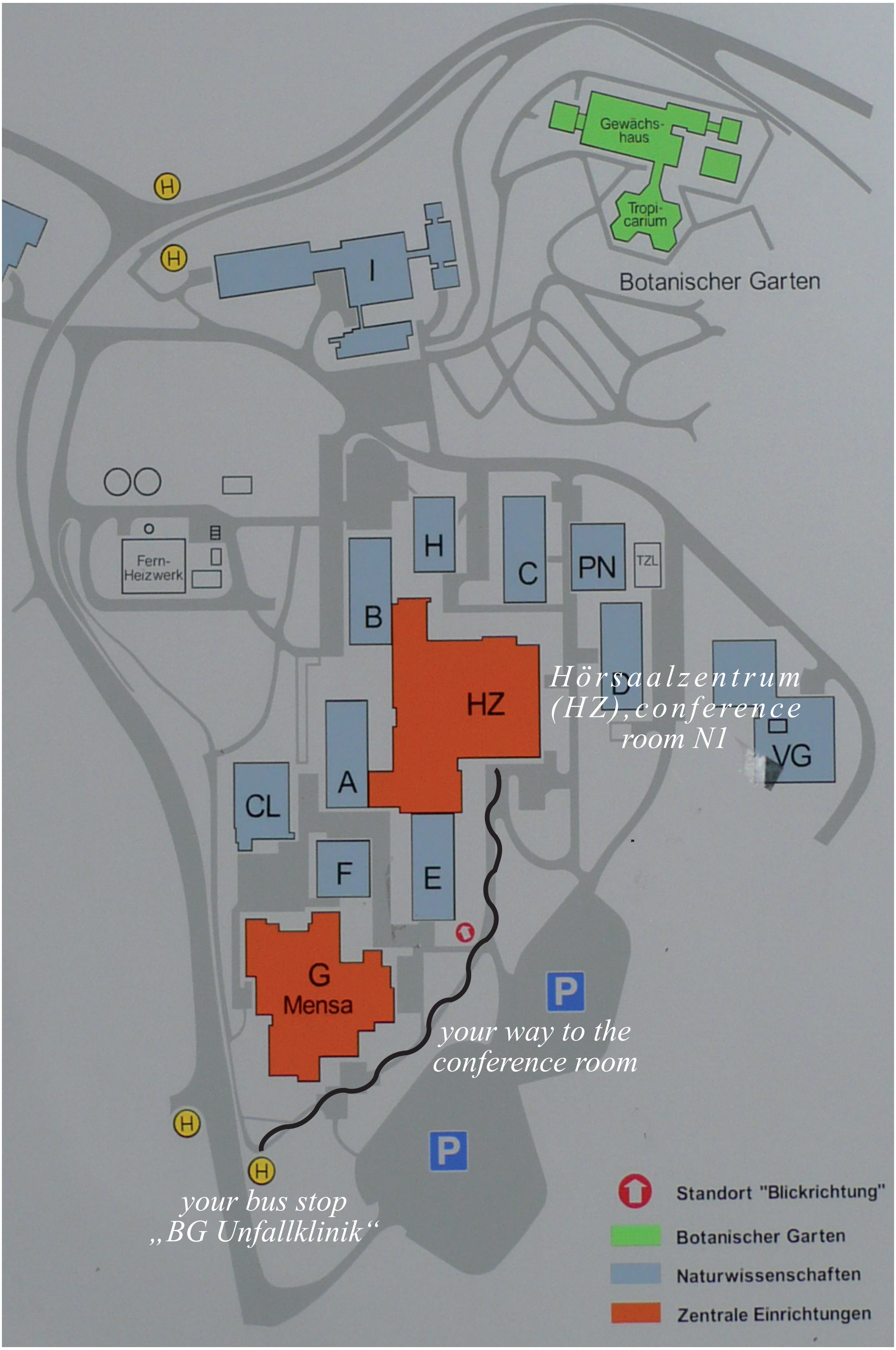


After lunch we will go by coach to the Hohenzollern Castle. Hohenzollern Castle is the ancestral seat of the Prussian Kings and German Emperors as well as the Swabian branch of the Princes of Hohenzollern. It ranks among Europe‘s most famous and popular castles. The stunning panorama of the picturesque landscape was admired by Emperor William II (1859-1941) who visited the castle several times and is said to have commented, „The view from up here is truly worth the voyage“. Again, included in the costs, we will have a guided tour (in English) of the castle.

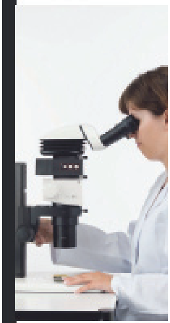
The day will finish at around 6:00 pm when we will have return to Tübingen.

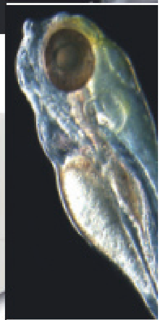
2h excursion Bebenhausen Monastery and Palace

Bebenhausen Monastery and Palace (Wednesday 5th August)
In the middle of Schönbuch, amidst charming scenery, lies the former Cistercian Monastery of Bebenhausen, which was probably founded around 1183/84 by Palgrave Rudolph von Tübingen. The monastery experienced its heyday in the 13th to 15th centuries, and was one of the wealthiest in Württemberg. Later it was used as a protestant monastery school and as a hunting palace for the Württemberg kings. In the years 1947 to 1952 it served as the seat of the „Landtag“ (state parliament) of Württemberg-Hohenzollern. On Wednesday afternoon we will offer transport and a guided tour (in English). Meetingpoint at the central bus station - 3:00 pm.



Gewächs-
haus
Tropi-
carium
Botanischer Garten
I
H
C
PN
TZL
Fern-
Heizwerk
B
HZ
Hörsaalzentrum
(HZ), conference
room N1
VG
A
CL
F
E
G
Mensa
your way to the
conference room
your bus stop
„BG Unfallklinik“
Standort "Blickrichtung"
Botanischer Garten
Naturwissenschaften
Zentrale Einrichtungen





Die richtige Basis für Ihre Arbeit

Biologische Proben stellen hohe Anforderungen an ein Stereomikroskop. Neben optimaler Bildqualität sind hoher Beobachtungskomfort und einfache Bedienung Grundvoraussetzung für präzise und schnelle Probenmanipulation.

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