Tenth International Symposium on Tardigrada

18-23 June 2006, Catania

(http://www.tenth.tardigrada.symposium.unimo.it)

Organizing Committee:

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Under the Patronage of:
- Accademia Gioenia di Scienze Naturali, Catania
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Acknowledgements:
The Organizing Committee is especially thankful to:
Tiziana Altiero, Edy Bonaccorso, Deborah Boschini, Vincenzo Costa, Lucia Di Mauro, Agata Esposito, Michele Failla, Giuseppe Fassari, Giuseppina Fassari, Silvana Ferrari, Fabio Marino, Alessandro Marletta, Giuseppe Montesanto, Angelo Ronsisvalle, Ignazio Corbello, Giuseppe Fabrizio Turrisi, Fabio Viglianisi, Fiorella Zappalà for their precious help.
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GENERAL INFORMATION

REGISTRATION DESK AND WELCOMING (Sunday 18th)
On Sunday 18th from 16:30 to 19:00 delegates can register at “Dipartimento di Biologia Animale” (Department of Animal Biology), University of Catania, via Androne 81, Catania.
In the meantime delegates can meet colleagues and can visit the “House of butterflies” located in the garden of the Department.

REGISTRATION DESK AND SECRETARY (from Monday 19th to Thursday 22nd)
During the Symposium, the registration desk will be located in the Auditorium of Benedettini, Piazza Dante 32, Catania. Delegates should collect their conference information at the registration desk, open from 8.30 to 12.45 and from 14.30 to 17.00.

BADGE IDENTIFICATION
A congress badge is required for admission to all congress sessions, activities and facilities.

ORAL PRESENTATION
All oral presentations are conducted in the Auditorium of Benedettini, Piazza Dante 32, Catania. Speakers have 20-25 minutes for their presentation (including questions and changeover). Speakers using the entire time for their presentation will leave no time for questions. Chairpersons are under strict instructions to keep delegates on time.
Before the beginning of each session, speakers should make sure that their presentation support media (computer based, slides, transparencies) have been handed to the operator at the registration desk, to allow technical setup. For computer based presentations the Auditorium is equipped with PC Windows based system. The presentation could be recorded on the following devices: CD-rom, DVD, USB Data Key. PowerPoint presentations are favored. Slides projectors and overhead projectors are also available. Even if personal laptops can be connected to media system of the Auditorium, it is kindly recommended to commit to the operator all the material for projection.

POSTER PRESENTATION
Posters are displayed in the Auditorium of Benedettini. Posters are divided into two sessions. Please display your poster on the numbered board allocated to you (see poster program and the board number). Delegates presenting posters should be next to their poster during the designated viewing session.

LUNCH DURING THE SYMPOSIUM
From Tuesday to Thursday, it is possible to have lunch in the Auditorium of Benedettini. It is necessary to book and to pay at least one day before at the registration desk, if you are interested.

CONFERENCE BULLETIN / INFORMATION BOARD
Please check the bulletin board at the registration desk each day for any change in the program, announcements or messages.

SOCIAL DINNER (Thursday, 22nd)
The conference dinner will be at Restaurant “I Crociferi”, Piazza San Francesco d’Assisi 14, Catania.
CONFERENCE EXCURSION (Friday, 23rd)
Details of departure (time, place, etc.) for the conference excursion to Volcano Etna are posted on the conference information board at the registration desk. The excursion will take all day. For the excursion we recommend bringing a wind jacket and suitable shoes for a short walk.

T-SHIRT
At the registration desk it is be possible to buy a nice T-shirt with the logo of the Symposium.

MANUSCRIPT SUBMITTAL AND PROCEEDINGS
During the symposium, delegates can present a variable number of talks/posters, but it will be possible to submit only one manuscript per participant (or two with a collaborator who is also a registrant) for the proceedings, which will be published in the “Journal of Limnology” (http://www.iii.cnrt.cnr.it/pubblicaz/jour_lim.htm).
All topics on tardigrades will be considered, but manuscripts will be peer-reviewed and authors may be requested to make substantial changes in the manuscript. Only manuscripts of high quality will be accepted for publication. Guest Editors of the journal (Giovanni Pilato, Lorena Rebecchi) reserve the right to reject manuscripts of poor quality. Manuscripts must be submitted no later than 23 June 2006 to the Organizing Committee via e-mail <tardigrada_symp@unimore.it> (a paper copy and a copy of manuscript on CD-ROM can be also submitted to registration desk during the symposium).
Manuscripts must conform to the following standards:
- They must not have been published or accepted for publication elsewhere, and must be in English.
- Each contribution must have no more than 8 printed pages in the “Journal of Limnology” including abstract, introduction, methods, results, discussion, acknowledgments, references, tables, figures and legends. As an example, 8 pages correspond about to 25,000 characters (including blanks) plus two-three tables and two-three figures of medium size.
- The “Instructions to authors” of the “Journal of Limnology”, available on line at the web site of the symposium (file in PDF), must strictly followed.
- The original paper version of the manuscript (including illustrations and tables) must be submitted together with a copy on CD-ROM (MS Word for Windows), or an electronic copy by e-mail sent before the Symposium.
The deadline for submitting manuscripts is no later than 23 June 2006 (last day of the symposium). After the end of the symposium, manuscripts will NOT be accepted.
Conference Programme

Sunday 18th

16.30-19.00  Registration and Welcoming at the **Department of Animal Biology** (Via Androne 81). Visit to the “House of Butterflies” located in the garden of the Department

Monday 19th

Conference Centre: Auditorium of Benedettini (Piazza Dante 32, Catania)

09.00-10.00  Poster Installation
10.00-11.00  Opening Ceremony
11.00-12.00  Opening Lecture: R. M. KRISTENSEN, N. MOBJERG, A. JORGENSEN, J. G. HANSEN - Theories about Tardigrada phylogenetic position. (pag. 13)
12.30  Welcoming Cocktail

Session Chairperson: Hartmut Greven - Germany

14.55-15.20  S. FAURBY, P. FUNCH - First comparative study on anhydrobiosis and cryobiosis in a heterotardigrade. (pag. 15)
15.20-15.45  K.I. JONSSON - How does experimental manipulation of the frequency of dry conditions affect development of anhydrobiotic tardigrade populations? (pag. 16)
15.45-16.00  Coffee break
16.00-16.25  R.O. SCHILL, B. MCGEE, A. TUNNACLIFFE - Stressprotein and LEA proteins: molecular adaptations to anhydrobiosis in tardigrades. (pag. 17)
16.25-16.50  T. KUNIEDA, T. KUBO - Identification and analysis of threalase from Milnesium tardigradum (Eutardigrada). (pag. 18)
16.50-17.15  D. BOSCHINI, R. BERTOLANI, L. REBECCHI - Thermotolerance and thermal acclimation in active tardigrades. (pag. 19)

Tuesday 20th

Session Chairperson: Lorena Rebecchi - Italy

08.45-09.10  K. HOHBERG, W. TRAUNSPURGER - The foraging behaviour of Macrobiotus richtersi. (pag. 20)
09.10-09.35  T. ALTIERO, R. GUIDETTI, R. BERTOLANI, L. REBECCHI - Energy allocation in the reproductive events of Macrobiotus richtersi and Hypsibius convergens (Eutardigrada). (pag. 21)
09.35-10.00  N.J. MARLEY - Biodiversity within the Milnesiidae. (pag. 22)
10.00-10.25  S.J. McINNES - Milnesium tardigradum; heterogeneity or speciation in action. (pag. 23)
10.25-10.50 G. PILATO, G. COSTA, E. CONTI, M. G. BINDA, O. LISI - Morphometric analysis of some metric characters of eutardigrades. (pag. 24)

10.50-11.05 Coffee break

Session Chairperson: Clark W. Beasley - U.S.A.

11.5-11.30 G. PILATO, P. FONTOURA, O. LISI - Remarks on the *Echiniscus viridis* group, with the description of a new species (Tardigrada, Echiniscidae). (pag. 25)


12.20-12.45 S.J. MCINNES, P.J.A. PUGH - Biogeography of limno-terrestrial Tardigrada, revisited. (pag. 28)

12.45-13.00 Picture Group

13.00 Lunch

14.30-16.00 POSTER SESSION I: Authors presenting poster should be next to their poster

16.15-17.30 Time for examination of types and other material of the tardigrade collections

**Wednesday 21st**

09.00-11.00 Visit to the “Kitchen house” of Benedettini and to Civic Library "Biblioteche Riunite Civica e A. Ursino Recupero"

11.00-11.15 Coffee break

Session Chairperson: Diane R. Nelson - U.S.A.


11.40-12.05 A. JØRGENSEN, N. MØBJERG, R.M. KRISTENSEN - Molecular investigation of the phylogenetic relationship of Arthrotardigrada. (pag. 30)

12.05-12.30 N. GUIL, G. GIRIBET - Molecular approach to the taxonomy of the *Echiniscus blumi-canadensis* series (Heterotardigrada, Tardigrada). (pag. 31)


12.55 Lunch
Session Chairperson: **Nadja Møbjerg - Denmark**

14.30-14.55 L. Michalczyk, L. Kaczmarek - Scanning electron microscope observations of Tardigrada with emphasis on the egg shell variability in the genus *Macrobiotus* Schultze, 1834. (pag. 33)


**Wednesday 21st**

15.20-15.45 K.-T. Kurz, W. Klepal, R.M. Kristensen - Ultrastructural study on the female (resp. hermaphrodite) reproductive system and some aspects of oogenesis in five species of marine Arthrotardigrada (Heterotardigrada). (pag. 35)


16.10-16.20 L. Michalczyk, L. Kaczmarek - Tardigrada newsletter - www.tardigrada.net (pag. 37)

16.30-17.30 Round Table on the taxonomic and systematic criteria of Tardigrada

**Thursday 22nd**

Session Chairperson: **Sandra J. McInnes - United Kingdom**

08.45-09.10 P.J. Bartels, D.R. Nelson - Diversity and distribution of soil tardigrades in the Great Smoky Mountains National Park, TN and NC, USA. (pag. 38)

09.10-09.35 P.J. Bartels, DR. Nelson - Tardigrade biodiversity in the Great Smoky Mountains National Park, TN and NC, USA: inventory update. (pag. 39)

09.35-10.00 R. Schuster, H. Greven - A long-range study of population dynamics of tardigrades in the moss *Rhytidiadelphus squarrosus* and their correlation to climatic factors. (pag. 40)

10.00-10.25 R. Dafoe, F. A. Romano III - A seasonal and transect survey of leaf litter tardigrade communities from a Gulf of Mexico barrier island (Dauphin Island, Alabama, USA). (pag. 41)

10.25-10.50 B.P.L. Ramsay - The first results of a study on the tardigrade communities in *Polylepis* woodlands from the Mantanay Valley, Cordillera de Vilcanota, Peru. (pag. 42)

10.50-11.05 Coffee break

Session Chairperson: **K. Ingemar Jönsson - Sweden**

11.05-11:30 C.R. Mitchell, F.A. Romano III - A life history study of a natural population of *Echiniscus mauci* (Tardigrada; Heterotardigrada) in Alabama. (pag. 43)


12.45 Lunch

14.30-16.00 POSTER SESSION II: Authors presenting poster should be next to their poster

16.15-17.30 Time for examination of types material and tardigrade collections

20.30 Conference Dinner at Restaurant “I Crociferi”, Piazza San Francesco d’Assisi 14, Catania

**Friday 23rd**

08.45 Excursion to Etna Volcano. The excursion will take all day

**PROGRAM FOR ACCOMPANYING PERSONS**

**Sunday 18th**

16.30-19.00 Registration and Welcoming at the Department of Animal Biology (Via Androne 81). Visit to the “House of Butterflies” located in the garden of the Department.

**Monday 19th**

12.30 Welcoming cocktail.

**Tuesday 20th**

From Tuesday to Thursday, every morning from 9.00 to 11.00 a Guided Tour through Catania will be available (information at Registration Desk)

08.30 Visit to the marine reserve: “Area Marina Protetta Isole dei Ciclopi”. Those interested should book for the excursion Sunday, during the registration (departure from Piazza Dante).

**Wednesday 21st**

09.00-11.00 Visit to the “Kitchen house” of Benedettini and to Civic Library “Ursino-Recupero”.

June 18-23, 2006 – Catania, Italy
Thursday 22nd

20.30 Conference Dinner at Restaurant “I Crociferi”, Piazza San Francesco d’Assisi 14, Catania.

Friday 23rd

08.45 Excursion to Etna Volcano. The excursion will take all day.
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ORAL PRESENTATION

Opening lecture

THEORIES ABOUT TARDIGRADA PHYLOGENETIC POSITION

REINHARDT MØBIEG KRISTENSEN¹, NADJA MØBIEG², ASLAK JØRGENSEN³, JESPER GULDBERG HANSEN¹

¹ Department of Invertebrate Zoology, Zoological Museum, University of Copenhagen, Copenhagen, Denmark;
² Institute of Molecular Biology and Physiology, The August Krogh Building, University of Copenhagen, Denmark
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In many textbooks Tardigrada are placed in Panarthropoda (Arthropoda, Onychophora and Tardigrada) and Panarthropoda is regarded as a sister-group to Annelida in the taxon Articulata, but this view is not supported by molecular data. Recently the older theory of Rauther from 1909 hypothesizing a relationship between arthropods and Cycloneuralia (the former included in Aschelminthes) has gained support, primarily based on analysis of 18S rRNA gene sequences. Comparisons of these molecular data suggest that all taxa with ecdysis (Nematoda, Nematomorpha, Priapulida, Kinorhyncha, Loricifera and Panarthropoda) should be included in a monophylum Ecdysozoa. The general body plan of tardigrades based on TEM-data is very central in this discussion. Traditionally, the following tardigrade characteristics indicate arthropod relationships: segmented legs, procuticle containing chitin, a very complex moulting cycle, sensillae of arthropod type, cross-striated muscles, malphigian tubules, rectal pads and a peritrophic membrane. Furthermore, the tardigrades are strictly segmented animals consisting of three fused head segments and always four trunk segments with legs. However, some Cycloneuralia also show many of these so-called arthropod characters, e.g. Kinorhyncha, Priapulida and Loricifera have chitin (shown by the WGA-gold labelling technique) in the procuticle and Kinorhyncha displays metamery in the nervous system, in the muscular arrangement, and in the distribution of cuticular plates.

The phylogenetic position of Tardigrada has always been controversial. One school (“the American School”) placed the tardigrades within the polyphyletic group Aschelminthes, based on the triradiated myo-epithelial pharyngeal bulb, the second school (“the European school”) placed them within the Arthropoda, based on nervous system and segmentation. The Ecdysozoa concept elegantly solves the apparent riddle of the Tardigrada. Instead of the dilemma of whether tardigrades are “aschelminthes” or Arthropoda, this group can now be seen as a very close relative of the Arthropoda that retains many primitive (plesiomorphic) ecdysozoan traits.
CRYOBIOSIS SURVIVAL IN TARDIGRADES

ROBERTO BERTOLANI, LORENA REBECHI, DEBORAH BOSCHINI, ROBERTO GUIDETTI

Department of Animal Biology, University of Modena and Reggio Emilia, Modena, Italy
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Eight species of eutardigrades, collected in different terrestrial and freshwater habitats and characterized by different anhydrobiotic capabilities, have been subjected to freezing and thawing experiments. The aim was to test: i. their interspecific ability to survive from cryobiosis and the differences in recovery time to return to active life; and ii. the relationship between survival and cooling rates. Starting from a uniform condition of tardigrades maintained for 24 h in water at 14°C, the cooling was performed according to three different protocols. Experiment 1: replicates for each species considered were put in 4 ml of water, frozen at three temperatures (-9°C, -20°C and -80°C) and kept frozen for six days. Experiment 2: replicates of Ramazzottius oberhaeuseri and Amphibolus volubilis were put in 4 ml of water, frozen in liquid nitrogen for two minutes and then stored at –9°C. Experiment 3: replicates of R. oberhaeuseri were placed in different amounts of water (2 ml, 1 ml and 0.5 ml), frozen at three different temperatures as in experiment 1 and kept frozen for six days. Before thawing, all frozen animals were first put or maintained at –9°C and then thawed at 14°C. Animals were examined both after 2.5 h and 24 h, and they were considered alive if they displayed evident and coordinated body movements.

In experiment 1, R. oberhaeuseri, A. volubilis, Macrobiotus areolatus and Macrobiotus richtersi showed high survival at all tested temperatures. Hypsibius dujardini, Borealibius zetlandicus and Diphascon cf. scoticum showed lower survival and differences in survival among the temperatures. Dactylobiotus parthenogeneticus did not survive at any tested temperatures. In most cases, the recovery time increased with a decrease in temperature and differed among the species. The differences among the species seem clearly related to their habitat and above all to their ability to carry out anhydrobiosis, suggesting a strong relationship between anhydrobiosis and cryobiosis. In experiment 2, all specimens of both species did not survive freezing at –196°C. In experiment 3, R. oberhaeuseri showed high survival also when the animals were frozen in less than 4 ml of water, even though a survival decrease was recorded between –9°C and –80°C for all tested water amounts. In general, the survival was lower when the water freezing time was shorter (corresponding to a higher cooling rate). Moreover, a negative relationship between the water freezing time and the recovery time was found: the shorter the water freezing time, the longer the recovery time. If the water freezing time is too short, the animals do not survive. These results can be easily explained with the need to produce a sufficient amount of protectants or to not be exposed to too much damage.
FIRST COMPARATIVE STUDY ON ANHYDROBIOsis AND CRYOBIOsis IN A HETEROTARDIGRADE

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Cryptobiosis can be defined as the ability of an organism to reversible halt it’s metabolism under unfavourable conditions. So far most studies on cryptobiosis in tardigrades have focused on the biochemical aspects in semiterrestrial species. In order to elucidate some ecological and evolutionary aspects of cryptobiosis we studied the ability of a marine heterotardigrade to cope with desiccation (anhydrobiosis) and subzero temperatures (cryobiosis). This is the first such study on cryobiosis and the first study on anhydrobiosis of a heterotardigrade. Different populations of the tidal tardigrade Echiniscoides sigismundi sigismundi s.s. collected throughout its known distribution was used. Animals from each population were divided into three experimental treatments; an anhydrobiosis group, which was dried under controlled temperature and humidity, a control group, which was kept at the same temperature for the same duration, and a cryobiosis group, which was slowly frozen and thawed. This analysis enables us to investigate whether local adaptations towards greater or lower cryptobiotic abilities are found and how tight the coupling between cryobiotic and anhydrobiotic abilities is. A tight coupling between these two processes is generally presumed and selection on cryobiosis has been suggested to explain differences in anhydrobiotic abilities but no experiments have analysed whether his tight coupling is actually found. Furthermore we analysed how the size of the animals is influencing their cryptobiotic abilities since earlier studies have given contrasting results on this relationship. The results are discussed in line with previous studies on cryptobiosis.
HOW DOES EXPERIMENTAL MANIPULATION OF THE FREQUENCY OF DRY CONDITIONS AFFECT DEVELOPMENT OF ANHYDROBIOTIC TARDIGRADE POPULATIONS?

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Anhydrobiotic tardigrade populations are known to exhibit very variable population densities in seemingly identical substrates. In addition, substrates exposed to frequent desiccation often seem to exhibit generally high densities of tardigrades. The factors underlying these patterns are largely unknown. However, the frequency of dry periods, under which tardigrade populations are unable to grow and reproduce, obviously is expected to influence development of tardigrade populations, but the actual effect of perturbing conditions has never been investigated. I will present an experimental study where populations of the moss-living eutardigrade *Richtersius coronifer* and other tardigrades sharing the same microhabitat were exposed to drier than normal, and wetter than normal, conditions, respectively. The experiment was run from spring 2000 until autumn 2001, and samples were taken at the beginning and at the end of the experiment. Treatment samples were compared to unmanipulated control populations. I will report the results for animal and egg density in *R. coronifer*, and for animal densities of other tardigrades sharing the same microhabitat. The results will provide a starting point for discussing population dynamics and selection processes in populations of anhydrobiotic tardigrades.
STRESSPROTEIN AND LEA PROTEINS: MOLECULAR ADAPTATIONS TO ANHYDROBIOsis IN TARDIGRADES

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Tardigrades are among the most resistant of all invertebrates to extremes of environmental stress. They are able to survive adverse conditions including dehydration due to the ability to enter into an anhydrobiotic state in which they arrest metabolic activity and contract their bodies into the “Tönnchen” state. When environmental conditions are adequate, tuns rehydrate and the animals resume metabolic activity. Anhydrobiosis in tardigrades and other invertebrates probably requires major biochemical adaptations, but these have not yet been identified definitively. Some evidence shows that several families of heat shock proteins serve as molecular chaperones to assist the folding of newly synthesized proteins, protect them from stress-associated denaturation and aggregation, aid in their renaturation and influence the final intracellular location of mature proteins. In addition, “late embryogenesis abundant” (LEA) proteins, which were first identified 20 year ago in cotton and wheat seeds during acquisition of desiccation tolerance, seem to be further keys in understanding anhydrobiotic mechanisms. In this study, we describe experiments on the roles of stress genes of the \textit{hsp70} family of heat shock proteins and of putative late embryogenesis abundant proteins. We show differential expression of the different \textit{hsp70} gene isoforms during the cycle of dehydration, anhydrobiosis and rehydration, and demonstrate the presence of putative LEA proteins in tardigrades.
IDENTIFICATION AND ANALYSIS OF TREHALASE FROM MILNESIUM TARDIGRADUM (EUTARDIGRADA)

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Many species of tardigrades can tolerate the desiccation by entering an ametabolic state called ‘tun’ (anhydrobiosis). To enter the tun state, desiccation should be done slowly to afford animals enough time to prepare for coming complete dehydration. The most known in preparation is the accumulation of compatible solutes like sugars. Many anhydrobiotic animals are known to accumulate and use trehalose as such solute. In tardigrades, one species, Richtersius coronifer, was also reported to accumulate trehalose during transition to tun-state. This species accumulate trehalose during dehydration and lose quickly on rehydration. Surprisingly, diminution of trehalose precedes restart of protein synthesis. To achieve this, trehalose production and destruction should be finely regulated. However the gene network that regulates the anhydrobiosis is almost totally unknown.

To elucidate the molecular basis of desiccation tolerance of tardigrades, we isolated a gene fragment encoding trehalase which catalyze the hydrolysis of trehalose from terrestrial tardigrades, Milnesium tardigradum by RT-PCR. Degenerated primers were designed from highly conserved motifs called trehalase signature region. Total RNA was extracted from active state Milnesium tardigradum and subjected for reverse transcription. Sequences of amplified gene fragments were determined and the predicted protein sequence shows high similarity with other species’ trehalases. Based on the determined sequence, we designed specific primers for Milnesium tardigradum trehalase and examined its expression profile during dehydration by real-time PCR. Relative expression level of trehalase is calculated by dividing by expression level of actin whose sequence was determined experimentally by a similar way. As a result, highest expression of trehalase was observed in active state and relatively weak in drying or tun state. Trehalase most likely participate in hydrolysis of trehalose during rehydration process and is expected to accumulate advance in tun state. The result was opposite to the expectation and suggested that transcriptional control of trehalase gene might not play an important role for trehalose regulation through dehydration/rehydration. Otherwise, Milnesium tardigradum might utilize another mechanism than trehalose in the main part of desiccation tolerance.
THERMOTOLERANCE AND THERMAL ACCLIMATION IN ACTIVE TARDIGRADES

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The ability of desiccated (anhydrobiotic) tardigrades to survive and to resist high temperature stresses (up to 100°C) is well-known, while the tolerance of active (hydrated) tardigrades to heat-shocks is still very little known. In order to increase our knowledge on thermal adaptations of active tardigrades, we have carried out lab experiments on three eutardigrade species to evaluate the ability to survive to heat-shock (considering lethal temperature - LT₅₀ - and lethal thermal maximum - LTₘₐₓ), and the possibility of thermo-tolerance induction. We considered two terrestrial species, the moss-dwelling Amphibolus volubilis and the lichen-dwelling Ramazzottius oberhaeuseri, and one limnic species, Borealiibius zetlandicus. These species differ each other in anhydrobiotic and cryobiotic abilities, and in substrate colonized.

Starting from a uniform condition of tardigrades maintained for 24 h in water at 16°C or 10°C (according to the species), groups of hydrated tardigrades have been exposed for 1 hour to a heat-shock (different experiments from 25°C to 42°C). The presence of active animals (body movements) was evaluated immediately after heat-shock (t₀), after 1h (t₁) and after 24 h (t₂₄) from heat-shock. Survival was represented by active animals at t₂₄.

For A. volubilis and R. oberhaeuseri the presence of active animals and survival was evaluated also after acclimation of 1 h (the first species at 28°C, the latter at 30°C) and subsequent heat-shocking to temperatures higher than 33°C.

All species look thermotolerant, even though their survival significantly decreases with the increase of the stress temperatures. Both LTₘₐₓ and LT₅₀ are species-specific. The first one is 39.0°C for A. volubilis and 37.0°C for R. oberhaeuseri and B. zetlandicus. The latter is 35.1°C for A. volubilis, 33.6°C for R. oberhaeuseri and 33.0° for B. zetlandicus. The percentage of active animals changes according to the shock temperature and differs among the species. The percentage of animals with active movements often significantly increases between t₀ and t₁ for some temperatures, apart R. oberhaeuseri which shows a decrease at 28°C. There are not significant differences between t₁ and t₂₄ in the percentage of active animals, with the exception of an increase in A. volubilis at 30°C and 33°C. Both in A. volubilis and R. oberhaeuseri the acclimation produces significant survival increases for heat-shock temperature from 33°C to 39°C, while at 40°C and 42°C there is not survival.

These results demonstrate that tardigrades, even when active, have evident ability to survive heat stresses, even though sampled in different habitats and characterized by quite different cryptobiotic performances.
THE FORAGING BEHAVIOUR OF MACROBIOTUS RICHTERSI

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Little is known about the feeding mode and foraging behaviour of tardigrades. According to present knowledge, limno-terrestrial species may pierce and suck the contents of living cells (fungi, algae, yeasts) or tissue (animals, plant roots) or swallow whole cells (bacteria, protozoa) or entire animals (nematodes, rotifers, other tardigrades). These findings are mainly based on observations, which are not experimentally quantified. In a series of laboratory experiments, we assessed quantitative and qualitative aspects of foraging behaviour of Macrobiotus richtersi (Tardigrada, Macrobiotidae) when feeding on nematode prey.

J1 juveniles of M. richtersi were not feeding on nematodes, but on detritus, probably consuming bacteria. The smallest juvenile sucking on a living nematode was 220 µm long. A single pre-starved, adult tardigrade consumed nematode prey with up to 4.6 µg in four hours, that is 43% of its own body mass. Predation rate is positively correlated to prey density, temperature, predator and prey body mass. Also, the soil matrix (particle size) affected the tardigrade-nematode feeding-interaction. Fine sand, for example, decreased consumption rates as it improved nematode agility and possibly provided small pores as refuge for the nematodes.

In preference experiments under direct observation, we noted that M. richtersi did not select a prey type but attacked whatever prey came in contact with the circum-oral field of the tardigrade. Instead of selecting the optimal prey, the tardigrades varied the time spent per prey, thus, maximising energy uptake according to optimal foraging theory. When searching time was short or overall food quality was high, they consumed only the first, easily available sip of the captured prey and abandoned food item for the next prey when sucking on nematode body contents became slower. The observed behaviour was in rather good fit with optimal foraging theory, astonishingly so, as it requires that the tardigrade may assess environmental prey quality and quantity. The tardigrade nervous system and possible chemical clues are discussed.
ENERGY ALLOCATION IN THE REPRODUCTIVE EVENTS OF 
MACROBIOTUS RICHTERSI AND HYPSIBIUS CONVERGENS 
(EUTARDIGRADA)

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The knowledge of life histories in tardigrades is still limited, while an evaluation of the energy allocation for their reproduction has seldom been examined. To improve our knowledge on these topics, we have studied two species differing in evolutionary histories, diet and ways of oviposition. Macrobiotus richtersi (Macrobiotidae) is carnivorous and lays “free” ornamented eggs, while Hypsibius convergens (Hypsibiidae) is certainly not carnivorous and lays smooth eggs within the exuvium. For both species we examined a bisexual population dwelling in the same substrate, beech leaf litter collected on the Apennines (Piane di Mocogno, Modena, Italy) at 1200 m a.s.l.

Both species are iteroparous. In M. richtersi, the maturative patterns of male and female gonads follow the respective models proposed by Rebecchi & Bertolani (1994). In H. convergens the male germ cell maturation is continuous and follows the previous male model, whereas the female germ cell maturation does not strictly follow the stages described for M. richtersi and other eutardigrade species.

With regard to the energy allocation, males with the testis rich in spermatozoa and females with the ovary containing oocytes in advanced vitellogenesis have been examined in both species. The age of those specimens has been estimated according to the buccal tube length. Their body and gonad areas have been determined with an image analysis program.

In both species females reach a larger size than males. Macrobiotus richtersi has statistically longer buccal tube and greater body area than H. convergens. Statistical analysis reveals that the buccal tube length is positively related to the body area and to the gonad area.

For an estimate of the relative energy allocated for reproduction in one reproductive event (here called RRE = relative reproductive effort), we have used the ratio of gonad area/body area. In males of both species, the absolute amount of energy and, above all, the RRE is statistically lower than that of females. In M. richtersi, the RRE of both males and females is directly related to the age of the animal, whereas in H. convergens this direct relationship is not detectable. This means that in M. richtersi the energy allocation for a reproductive event increases during the life of the animal. In each reproductive event, due to their smaller size, in absolute value, females of H. convergens allocate a lower amount of energy with respect to M. richtersi, but if we consider the RRE, their energy investment is higher than that of M. richtersi.
BIODIVERSITY WITHIN THE MILNESIIDAE

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In recent years many new taxa of the Milnesiidae have been described. With this paper the author considers the significance and variability of some morphological taxonomic characters. This will be illustrated using both historic and modern published works, biogeographical data, and with examples of populations from several continents. Finally the relationships between the four genera of the Milnesiidae is considered.
MILNESIUM TARDIGRADUM: HETEROGENEITY 
OR SPECIATION IN ACTION

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The genus Milnesium had for many years been considered mono-specific and 
cosmopolitan. More recently closer examination of the genus has resulted in several 
papers describing new species as specimens from a variety of geographical sites, though 
mainly from the Palaearctic and Nearctic regions. A recent review of “Milnesium 
tardigradum” collected from a variety of sites around the Antarctic and sub-Antarctic 
islands have shown that there are potentially a number of different species present. 
Closer examination has shown that there are major differences and some novel 
characters not previously seen, or recorded, in Northern hemisphere specimens.
MORPHOMETRIC ANALYSIS OF SOME METRIC CHARACTERS OF EUTARDIGRADES

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The specific diagnosis of eutardigrades is often very problematic. It is necessary to take into consideration as many characters as possible and to know their intraspecific variability. The knowledge about the individual variability of metric characters is very scanty and rigorous statistical studies are almost lacking.

A study on individual variability was carried out on some metric characters of two species of Eutardigrada Macrobiotidae: *Macrobiotus diffusus* Binda & Pilato, 1987 and *Macrobiotus areolatus* Murray, 1907.

The following metric characters were studied: body length; length and width of buccal tube; value of the $pt$ index relative to the width of the buccal tube and the insertion point of the stylet supports on the buccal tube.

Other than the range of variability of the various characters considered, correlation analysis and the linear regression for some of them were carried out by means of binary comparison.

By means of confidence ellipses, which were always determined with a probability of $P = 99.9\%$, it was possible to hypothesize – with an error risk of 0.1\% - if an individual whose data were outside the confidence interval belonged to a species. The possibility of finding ranges of variability that are similar, even identical, in different species for some metric characters, is not be excluded.

The value of the $pt$ index relative to the point of insertion of the stylet supports is particularly significative, showing variations with extremely limited and independent dispersion both from body length and from the length of the buccal tube.

The resemblance between metric characters must still be evaluated to see if they can be considered indicative of some kind of phylogenetic affinity.
REMARKS ON THE ECHINISCUS VIRIDIS GROUP, WITH THE DESCRIPTION OF A NEW SPECIES (TARDIGRADA, ECHINISCIDAE)

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Very few species of Echiniscus are green, and some of them, namely E. viridis Murray, 1910, E. rufoviridis Du Bois-Reymond Marcus, 1944, Echiniscus viridissimus Péterfi, 1956 and Echiniscus perviridis Ramazzotti, 1959 which share the presence of filament A, lack other lateral and dorsal appendages, and have claws unusually large, are traditionally considered elements of the so-called “viridis group”.

We examined specimens from North America and from the Azores attributable, according to the literature, to E. viridis, and specimens of all other species of the “viridis group” - typical material of E. viridis (unfortunately lost) and E. rufoviridis excluded - and we noted that the specimens from North America and the Azores belong to a new species, named E. viridianus sp. n. We also noted some problems that we consider opportune to point out.

The colour green is not exclusive of the species of the “viridis group” and, on the other hand, not all the species of this group are truly green, but sometimes grey-violet or almost black. Therefore the colour green, alone, does not seem to be a certain index of phylogenetic affinity.

The terminal plate is faceted in E. rufoviridis, slightly faceted or unfaceted in the other species.

As far as the plate ornamentation of E. viridis is concerned some confusion exists; we ascertained that in E. viridissimus, E. perviridis and in E. viridianus sp. n., the model of the cuticular ornamentation is the same (with very small differences), while in E. rufoviridis important differences are present.

The filament A in some species of the “viridis group” is short (30-40 µm), in one species it is of intermediate length (80 µm); in another species it is long (150-170 µm).

In conclusion, in our opinion, the viridis group is well characterized by a particular cuticular ornamentation, by the absence of lateral and dorsal appendages (filament A excluded), by well developed claws and by a green or grey-violet or almost black colour over all the body or in a portion of it; but notwithstanding these shared characters, the group is more heterogeneous than traditionally considered, and it seems that probably E. rufoviridis should not be considered an element of the “viridis group”.

June 18-23, 2006 – Catania, Italy
TERRESTRIAL TARDIGRADA OF THE NEARCTIC REALM: BIOGEOGRAPHY, DISTRIBUTION, AND ECOLOGY

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In 2001 Pilato and Binda analyzed the global biogeography of limno-terrestrial tardigrades, proposing that the proportion of cosmopolitan species to those of more limited distribution is lower than previously believed. We examined all available records of terrestrial (i.e., in unsubmerged cryptogams, anthophytes, soils, and leaf litter) tardigrade distribution in the Nearctic realm, both to compare this fauna with other realms and to investigate distribution within North America. Of the 177 terrestrial species of tardigrade found in the Nearctic realm, 35 were cosmopolitan sensu Pilato and Binda, while 51 were unique to the Nearctic. The Nearctic tardigrade fauna is most similar to the Palearctic, with 122 species in common, 39 of which have not been reported in other realms. Sixty-nine of the Nearctic species were also present in the Neotropical realm, but only three of these were not also present in other realms. These data are consistent with the geological history of the three realms, and the distinction between Laurasian and Gondwanan tardigrade faunas suggested by McInnes and Pugh (1998). Although little if anything is known about terrestrial tardigrade distribution in much of North America, there are several excellent regional or local surveys. Available data range from studies in which extensive details of sampling, species per sample, and substrate are provided, to those which indicate only the presence of a species somewhere in a state or province. Many species are distributed widely throughout the continent, but approximately 40% of the 177 Nearctic species have been reported from a single site or sample. Distinctive regional faunas in the Pacific coastal states and provinces, the southeastern United States, and northernmost North America can be recognized. Inconsistent sampling methodology and inadequate habitat description make ecological analysis difficult. The number of species present per site-substrate combination ranges from 1 to 8, with a mean of 2.3. Little substrate specificity is indicated, other than that some species are more likely to be found in cryptogams and some in soils or leaf litter. Species distributions in the United States do not clearly correlate with ecological regions defined by landform and vegetation.
BIOGEOGRAPHY OF ANTARCTIC TARDIGRADES: PROBLEMS, PRACTICE AND PATIENCE

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The remote and isolated continent of Antarctica and the islands of the Southern Ocean provide exciting opportunities for biologists, particularly in regards to the origins and diversification of species. Tardigrades are found on islands throughout the sub-Antarctic and ice free regions of the Antarctic continent. Intensive sampling and morphological based studies are providing detailed biogeographic information on southern tardigrade fauna, including implications that elements are included with ancient and vicariant regional origins. By using the heritable information available in DNA sequences we intend to examine fine-scale phylogeographic patterns within and between terrestrial tardigrade species to assess biodiversity and to gain insights into the historical processes that have affected the evolution of Antarctic and sub-Antarctic taxa. However, Antarctic biology is often hampered by the very conditions that make the continent a fascinating field site. Furthermore, from a molecular perspective, tardigrades themselves are notoriously difficult to work with. Here we share some of the trials and triumphs of studying Antarctic and sub-Antarctic tardigrades.
The Tardigrada is a cosmopolitan phylum of Pangaeon (Triassic or even Permian) origin, yet tardigrade families and genera show distinct biogeographic components isolated by two major geological events. Separate Laurasian and Gondwanan familial clusters correlate with the Triassic/Jurassic disintegration of Pangaea, while discrete Antarctic, Australian and New Zealand familial/generic clusters relate to the subsequent Jurassic/Cretaceous disintegration of Gondwana.

A combination of numerous new (particularly Antarctic) records and recent advances in the computer analysis of biogeographic data has prompted us to re-examine the global tardigrade fauna. We now have sufficient data to not only apply a range of clustering techniques but to corroborate these with principal co-ordinates analysis and parsimony analysis of endemicity (PAE).

Preliminary analysis of these new data confirm the Laurasian, East and West Gondwana elements suggested in our original 1998 study, but implies that these element very dissimilar. The Laurasian cluster is corroborated by Dollo PAE and so largely vicariant, East Gondwana by Wagner PAE and so largely a product of dispersal, while West Gondwana appears to be a chimera but largely vicariant. These findings imply that tardigrades probably evolved in the Permian/Triassic northern hemisphere, colonised East Gondwana during the early Tertiary, while the Antarctic/Southern Ocean fauna is probably late Tertiary/Quaternary, but certainly derived from West Gondwana.
THE PHYLOGENY AND MORPHOLOGICAL DIVERSITY OF THE 
STYGARCTIDAE (ARTHROTARDIGRADA, TARDIGRADA)

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During the last three decades several species of stygarctids, new to science, have 
accumulated in the tardigrade collection at the Zoological Museum, University of 
Copenhagen. The major aims of this investigation were to describe some of the new 
species to further increase the knowledge of the morphological diversity and to conduct 
phylogenetic analyses of all currently described species within the family Stygarctidae. 
The major objectives of the phylogenetic analyses were to investigate the internal 
phylogenetic relationships of Stygarctidae, the relationship to closely related families 
(Neostygarctidae and Renaudarctidae), and the character evolution within the 
Stygarctidae. The outgroups were chosen to represent presumed ancestral 
arthrotardigrades (Coronarctus and Neoarctus) and arthrotardigrades (Neostygarctus and 
Renaudarctus) with a close phylogenetic relationship to Stygarctidae.

The present account uses the classification scheme suggested by Bello & Grimaldi de 
Zio (1998) for setting up the cladistic analysis. In order to evaluate the significance of 
the morphological diversity within the Stygarctidae, all species currently described have 
been personally examined by the authors and species new to science were described in 
each of the genera: nov. gen. 1, Mesostygarctus, Parastygarctus, Pseudostygarctus and 
Renaudarctus. The character matrix consists of 31 species and 80 morphological 
characters. The characters have been scored from six main characters systems, i.e. the 
arrangement of head lobes, the cuticular segmental plates (head, body and caudal plates), 
the seminal receptacles, the legs and claws, the sense organs and the buccal apparatus. 
All 80 characters were parsimony informative and 51 are multistate characters.

The most notable results from the phylogenetic analyses are: 1) Neostygarctidae is the 
sister group to Stygarctinae; 2) nov. gen. 1 is the most basal member of Stygartinae; 3) 
Mesostygarctus is the sister group to Pseudostygarctus; 4) S. spinifer is the most basal 
member of Stygarctus.

In our opinion Megastygarctides is very different from the members of Stygarctinae and 
its phylogenetic position as sister group to (Neostygarctidae + Stygarctinae) might 
justify recognition higher in the taxonomic hierarchy. Not surprisingly the 
Renaudarctidae are basal to the clade (Mega stygarctidinae + (Neostygarctidae + 
Stygarctinae)).
MOLECULAR INVESTIGATION OF THE PHYLOGENETIC RELATIONSHIP OF ARTHROTARDIGRADA

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Morphological as well as molecular data suggests that tardigrades are ecdysozoans, closely related to arthropods and onychophorans. Previous studies of a few 18S rRNA sequences from echiniscids, Batillipes and Halechiniscus have inferred the monophyly of Heterotardigrada. However, these molecular studies have only included a single arthrotardigrade species and the monophyly of Arthrotardigrada, therefore, still needs to be investigated. Recently a study based on morphological characters has suggested that the arthrotardigrades are paraphyletic within a monophyletic Heterotardigrada.

In the present study we have increased the gene and taxon sampling of heterotardigrades (including arthrotardigrades) compared to our previously published study in order to infer their phylogenetic relationships. New partial sequences of 18S rRNA and 28S rRNA have been collected from various tardigrade genera most notably the arthrotardigrades Batillipes (Batillipedidae), Riaractus and Tanarctus (Halechiniscidae) and the echiniscoid Echiniscoidea (Echiniscooididae). This is the first time 28S rRNA is used for the inference of phylogenetic relationships within Tardigrada.

In our preliminary analyses we have used parsimony, maximum likelihood and Bayesian inference to infer the phylogenetic relationships within Tardigrada. The analyses of 18S rRNA partly support the monophyly of Arthrotardigrada, Echiniscoidea and Heterotardigrada. The analyses of 28S rRNA and the combined genes always support Heterotardigrada and very often Echiniscoidea, but Arthrotardigrada is paraphyletic. The paraphyly was apparently an artifact of poor 28S rRNA sampling within Halechiniscidae, and the topology of the heterotardigrade clade inferred by 28S rRNA and the combined genes received no or weak branch support according to bootstrap analyses. The 28S rRNA from Tanarctus was inferred to be basal to Echiniscoidea in the different inference methods and also when gaps were analyzed as a fifth character. Assuming that the obtained Tanarctus 28S rRNA sequence is similar to the missing 28S rRNA sequence of Halechiniscus and Riaractus the Tanarctus 28S rRNA sequence was copied to Halechiniscus and Riaractus. In the subsequent analyses the Arthrotardigrada became monophyletic.

The results from the present study need to be verified through increased gene and taxon sampling. Complete 18S and 28S rRNA sequences and additional arthrotardigrade taxa are needed in order to investigate the phylogeny within Arthrotardigrada. This might be a difficult task since our preliminary data suggest that PCR amplification using the highly conserved 18S rRNA primers is problematic at least with regard to the stygarctids and renaudarctids.

June 18-23, 2006 – Catania, Italy
MOLECULAR APPROACH TO THE TAXONOMY OF THE ECHINISCUS BLUMI-CANADENSIS SERIES (HETEROTARDIGRADA, TARDIGRADA)

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One persistent issue in tardigrade taxonomy is the definition of species and species complexes of forms that are morphologically similar or almost identical. Species definition is certainly a problem in the Echiniscus blumi-canadensis series, composed of five species (Echiniscus blumi, E. canadensis, E. mediantus, E. bisetosus and E. trisetosus) characterized by the presence of dorsal filaments and spines in dorsal positions C and D, respectively. All these species also share modified external claws in the fourth leg with basal spines, and lack dorso-lateral appendages. They have been traditionally distinguished based on the absence or presence, as well as the shape and position of the lateral appendages. However, intermediate morphologies both in adults and juveniles — in addition to the typical described forms — have been reported in the literature. Several authors have recommended merging the five forms into the nominal species, E. blumi, although no formal taxonomic amendment was proposed.

Given the limitations of morphology in solving this question, we adopted a molecular approach to test the validity of this informal recommendation. With this aim we collected all forms in the blumi-canadensis series from multiple populations in Sierra de Guadarrama (Madrid, Spain), spanning 1,265 km². Specimens with the typical morphologies as well as “intermediate” morphotypes were obtained and a 815 bp fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI hereafter) was sequenced. This molecular marker was chosen because it shows appropriate variation at the population/species level in most bilateria, including some tardigrades. Our results show that COI variation between the Echiniscus individuals from all populations ranged between 0 and 3%, considering all studied morphotypes. In comparison, variation among three closely related members of the genus Macrobiotus ranges from 12 to 19%; variation between Echiniscus and Macrobiotus is 24-31%. COI showed ca. 3.8% polymorphic sites among the Echiniscus sequences examined, and individual haplotypes relative sequences ranged between 0.045 and 0.273. No haplotypes was found in all populations or all morphotypes. Among the widespread haplotypes, one was sequenced from individuals from three populations, and assigned to two species. Other haplotypes are shared between two morphotypes. The phylogenetic pattern obtained after parsimony analysis of the sequence data showed no resolution. Given that the proposed species in the Echiniscus blumi-canadensis series cannot be recognized using population-level molecular analyses (as morphological studies indicated), we propose that this group of species is probably the consequence of artificial taxonomic splitting. Analysis of specimens from more distantly related populations will aid to further test this issue and propose an amended classification for the group.
A cDNA library was constructed from the glacier-dwelling eutardigrade Hypsibius klebelsbergi. This species inhabits cryoconite holes in great numbers. We used > 2000 individuals from samples collected on the Rotmoosferner glacier (Nordtirol, the Austrian Central Alps) in September 2005. Samples were stored in the refrigerator at 4°C for some months until processing. RNA, DNA and proteins were successively isolated using Trizol®. The technique used for library construction leads to full length cDNAs including complete 5’-ends. The cDNA was inserted in the expression vector λTriplEx2. The quality of the library was verified by the presence of the highly conservative actin sequences. Currently, we use the library to examine the variability of proteins of putative phylogenetic relevance (e.g. the actin binding protein gelsolin) and functional significance with respect to cryobiosis.
SCANNING ELECTRON MICROSCOPE OBSERVATIONS OF TARDIGRADA WITH EMPHASIS ON THE EGG SHELL VARIABILITY IN THE GENUS MACROBIOTUS SCHULTZE, 1834

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A number of structures in a few Tardigrada genera (Calcarobiotus, Diphascon, Echiniscus, Macrobiotus, Minibiotus, Pseudechiniscus and Ramazzottius) were observed using Scanning Electron Microscopy (SEM). The talk is composed mainly of SEM photomicrographs. Comparative and functional interpretation is proposed. The photomicrographs include the claws of Ramazzottius, cuticular granulation of some Calcarobiotus and Macrobiotus species (revealing the complex structure of granulation), intraspecific variability of egg processes in the Macrobiotus hufelandi and the Macrobiotus richtersi group and the oral cavity armatures of some Calcarobiotus, Diphascon and Minibiotus species. We also provide the first SEM micrographs of premature eggs isolated form the ovary, body cavity cells and muscles of some Macrobiotus species.
NOTES ON THE STRUCTURE OF THE MALPIGHIAN TUBULES IN THE GLACIER EUTARDIGRADE HYPSIBIUS KLEBELSBERGI MIHELJCIC, 1959

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The glacier-dwelling eutardigrade Hypsibius klebelsbergi Miheljcic, 1959 is known almost exclusively from cryoconite holes, i.e., water-filled micro-caverns on the glacier surface. This highly specialized environment is characterized, among the others, by near zero temperatures and a low mineral content. These special conditions might be also reflected by the structure of the excretory and osmoregulatory system represented in eutardigrades by the “Malpighian tubules” situated at the transition of the midgut and rectum. Hypsibius klebelsbergi possesses three such tubules largely identical in structure. Each tubule can be differentiated in a large three-lobed distal, a thin transitional portion and a short proximal portion. The distal portion consists of three cells with large nuclei. Cells are characterized by a basal labyrinth, interdigitating plasma membranes and large nuclei. The transitional portion lacks nuclei; it represents offshoots of the proximal portion. Here some nuclei can be found. The transitional as well as the proximal portion have intercellular channels and only some basal infolding. Intercellular channels are often filled with granular masses or whirls of membrane-like structures. In the region where tubules open, the midgut largely lacks microvilli. Thus, the overall aspect of the Malpighian tubules in H. klebelsbergi fits very well in the pattern known from other Eutardigrada, i.e. short largely monomorphous tubules as common in freshwater and semiterrestrial hydrophilous species, and, apart from the large amounts of depositions in the intercellular channels, a largely corresponding ultrastructure. Clear evidence for an increased osmoregulatory demand was not obtained from ultrastructure.
ULTRASTRUCTURAL STUDY ON THE FEMALE (RESP. HERMAPHRODITE) REPRODUCTIVE SYSTEM AND SOME ASPECTS OF OOGENESIS IN FIVE SPECIES OF MARINE ARTHROTARDIGRADA (HETEROTARDIGRADA)

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There is little information on the ultrastructural anatomy and oogenesis in marine Arthrotardigrada. In this masters thesis the ultrastructural anatomy of the female, respectively hermaphroditic, reproductive tract of five marine Arthrotardigrada (Heterotardigrada) is described and compared. The structures which are depicted are: gonopore, receptaculum seminis, gonoduct and ovary. Additionally the stages of oogonia, oocytes and ova which were available inside the ovary are described. Microvilli-like structures which were found surrounding advanced stages of ova of some species are related with the formation of the chorion. Intercellular bridges between oocytes and nurse cells found in most of the species refer to an alimentary and nutrimentary mode of oogenesis, as defined by Marcus in 1929. The five species which were examined are Halechiniscus greveni, Actinarctus doryphorus, Styraconyx nov. sp. (Halechiniscidae); Batillipes noerrevangi (Batillipedidae) and the hermaphroditic species Orzeliscus belopus (Orzeliscidae).
ULTRASTRUCTURAL STUDY ON OOGENESIS OF ACTINARCTUS DORYPHORUS (ARTHROTARDIGRADA: HALECHINISCIDAE)

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The ultrastructure of the ovaries of Actinarctus doryphorus was studied by transmission electron microscopy (TEM) with four specimens of different stages. 3-D reconstructions from two series of them improved our understanding on the whole structure. The ovary is held upon the digestive tract by basement membranes and a pair of longitudinal dorsal muscle bundles each of which consists of three lines of muscle. Immature oocytes are located in the anterior part of the ovary and the more posterior part includes more matured oocytes.

The constituents of the adult ovary were as follows. Most cells near the anterior pole show the characteristic synaptonemal complexes suggesting these cells to be in meiotic prophase during zygotene and pachytene stage. The number of oogonia in the adult ovary appears to be very limited. Each oocyte in the anterior area has a nucleus with a rather small nucleolus and the cytoplasm contains no yolk granules. They are followed by other oocytes with larger nucleoli but without yolk granules yet. These previtellogenic oocytes are connected to each other by cytoplasmic bridges. Posterior large oocytes have prominent nucleoli and their cytoplasm contain more or less yolk granules with a number of small vacuoles. The cell borders among these vitellogenic oocytes are so weakly developed that they are believed to become a partial syncytium. However, the most posterior oocyte is well defined and develops as the next egg. The total number of nuclei surrounding the cytoplasm with yolk granules may be eight although it is still uncertain due to incompleteness of the serial sections. These cells other than the developing oocyte appear to function as nurse cells. This vitellogenic unit is attached by another type of cells with prominently developed ER in their cytoplasm. Many electron dense granules are observed between the developing oocyte and the ER-rich cells, which appear to supply some material to the oocyte. At the posterior periphery in the ovary, there are several degenerating cells with tremendous size of secondary lysosomes. They are likely previous nurse cells or ER-rich cells because such cells are not observed in the ovary of a juvenile.
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Tardigrada Newsletter is not a conventional newsletter. It is a website that provides news and references of currently published papers on Tardigrada, Tardigradologists’ directories and news from the water bear world. The references are being added to the Newsletter as we receive them. The Newsletter is a non-commercial initiative and it is completely free to use. We warmly welcome your help with adding the references and putting important news on the web. All you need to do is e-mail either one of us. We hope that Tardigrada Newsletter will facilitate access to any information on Tardigrada. Remember that the more people who know your papers, the higher the chances are that they will cite you. The web address of Tardigrada Newsletter is www.tardigrada.net
DIVERSITY AND DISTRIBUTION OF SOIL TARDIGRADES IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK, TN AND NC, USA

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For the past 5 years, we have been collecting tardigrades in the Great Smoky Mountains National Park as part of the All Taxa Biodiversity Inventory (ATBI) (see www.discoverlifeinamerica.org). Our basic collection includes soil, lichen, and moss samples from each of 19 permanent ATBI plots established throughout the park, as well as aquatic samples. The terrestrial permanent plots are 100 m X 100 m representing all major land-cover types and various elevations. Fourteen of the 19 plots have now been analyzed for soil tardigrades. Four samples (10 cm x 10 cm x top 1 cm of soil) were collected randomly from each of the 14 plots. Non-decomposed leaf-litter was removed before collection, so the material collected included the transition from O layer (organic layer) to A layer (topsoil). The samples were placed in paper bags, and allowed to air dry in the laboratory. Specimens were isolated using Ludox™ centrifugation, and up to 50 specimens per sample (plus eggs) were individually mounted in Hoyer’s medium and identified with phase contrast and DIC microscopy. A total of 58 samples were analyzed, yielding 1071 identifiable specimens. Our soil samples contained 41 species in 17 genera and 6 families. About half of all species found in soil are exclusively or predominantly found there: Amphibolus cf. smreczimskii, Astatumen trinacriae, Calohypsibius schusteri, Diphascon belgicae, Diphascon cf. carolae, Diphascon higginsi, Diphascon nobilei, Diphascon patanei, Diphascon pingue, Diphascon scoticum, Doryphoribius n. sp., Hypsibius convergens, Isohypsibius cf. basalovoi, Isohypsibius cf. brevispinosus, Isohypsibius lunulatus, Macrobiotus cf. liviae, Macrobiotus harmsworthi, Macrobiotus richtersi, Macrobiotus sp. 8, and Platicrista angustata. Eleven species were extremely rare (<1% of all tardigrades collected in soil): Amphibolus cf. smreczimskii, Diphascon belgicae, Diphascon cf. carolae, Diphascon nobilei, Diphascon patanei, Doryphoribius n. sp., Isohypsibius cf. basalovoi, Isohypsibius cf. brevispinosus, Isohypsibius lunulatus, Macrobiotus sp. 8, and Ramazzottius cf. oberhaeuseri group. To investigate overall diversity, EstimateS 7.0 (viceroy.eeb.uconn.edu/estimates) software was used to calculate species richness estimates. The Chao1 estimator indicates that a total of 53.1±9.72 (mean ± SD) species may occur in soil throughout the park. Indices of similarity indicate some significant differences in tardigrade community structure among plots. To investigate ecological variables associated with tardigrade distribution, a multivariate statistical analysis was performed using JUMP (Mac OS X). Variables analyzed included elevation, slope, aspect, land cover, basic soil type, and a moisture index. The results of this analysis will be discussed.
TARDIGRADE BIODIVERSITY IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK, TN AND NC, USA: INVENTORY UPDATE

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For the past 5 years we have been conducting a large-scale, multi-habitat inventory of the tardigrades in the Great Smoky Mountains National Park as part of the All Taxa Biodiversity Inventory (ATBI) (see www.discoverlifeinamerica.org). In terrestrial habitats, we collected moss, lichen, and soil samples from 19 permanent ATBI plots, representing all major land cover types within the park. Each ATBI plot is 100 m X 100 m. In each plot when available 16 moss samples, 16 lichen samples, and 4 soil samples were collected in paper bags and air dried in the laboratory. Specimens were isolated with Ludox™ centrifugation, and for each sample up to 50 adults plus eggs were individually mounted on microscope slides in Hoyer’s medium and identified using phase contrast and DIC microscopy. Additional collections were made in the limestone caves of the Cades Cove region of the park, bird nests, and 13 different streams. To date (February 2006), 589 samples have been collected, and of these 376 have been analyzed, yielding a total of 8242 identifications. Many of these are identified only to species groups. A total of 70 species have been found in the park, 13 of which we believe are new to science. Seven species richness estimators have been developed to predict total species richness (see EstimateS 7.0 software, viceroy.eeb.uconn.edu/estimates), and these were evaluated by comparing predictions from half of our data to the actual numbers from the total database. The results of this comparison indicate that different estimators work best in different habitats. Using the best estimators in each habitat, EstimateS 7.0 indicates that a total of 96 species are likely to occur throughout the park. Thus, Great Smoky Mountains National Park tardigrade diversity represents 10% of the world’s known tardigrade fauna, higher than that found in other large scale inventories in Italy and Poland.
A LONG-RANGE STUDY OF POPULATION DYNAMICS OF TARDIGRADES IN THE MOSS *RHYTIDIADELPHUS SQUARROSUS* AND THEIR CORRELATION TO CLIMATIC FACTORS

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The population dynamics of tardigrades were studied in the moss *Rhytidiadelphus squarrosus* over a period of four and a half years (October 1999 to July 2004, with the exception of August) in the Black Forest (Germany). Altogether approximately 20,000 specimens were collected belonging to 24 species. Most abundant were *Macrobiotus hufelandi*, *Macrobiotus richtersi* and *Diphascon pingue*. In the whole investigation period these species reached dominances of 56%, 18%, and 12%, respectively. Dominances of the remaining varied between 0.32 to 2.15%. Species diversity (Shannon-Index and evenness) was highest during the winter. In general, however, the moist season supported species considered as hygrophilous, whereas the dry sunny season promoted the relative increase of euryhygric species. Despite some changes in the species composition during the season probably due to species-specific demands, the composition of the tardigrade community and the dominances of the species were largely robust over the years. In addition, for the most dominant ten species (dominance > 1) correlations were established with temperature (positive: *Macrobiotus hufelandi*, *Macrobiotus patiens*; negative: *Hypsibius dujardini*, *Hypsibius convergens*, *Hypsibius scabropygus*, *Diphascon rugosum*), atmospheric humidity (positive: *Hypsibius dujardini*, *Hypsibius convergens*, *Hypsibius scabropygus*; negative: *Macrobiotus richtersi*, *Macrobiotus patiens*, *Isohypsibius prosostomus*), precipitation (positive: *Macrobiotus richtersi*, *Diphascon pingue*, *Hypsibius dujardini*, *Hypsibius convergens*, *Hypsibius pallidus*, *Isohypsibius prosostomus*; negative: *Macrobiotus hufelandi*, *Macrobiotus patiens*, *Hypsibius scabropygus*), and dry days (positive: the species with negative correlations to precipitation; negative: the species with positive correlations to precipitation and *Diphascon rugosum*).
A SEASONAL AND TRANSECT SURVEY OF LEAF LITTER TARDIGRADE COMMUNITIES FROM A GULF OF MEXICO BARRIER ISLAND (DAUPHIN ISLAND, ALABAMA, USA)

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Dauphin Island is a barrier island in the Gulf of Mexico just south of Mobile Bay, Alabama, USA. The island is approximately 27 km long and 1.5 – 2.0 km at its widest. The widest part of the island is at the eastern end and the easternmost 6 - 7 km is forested. Also, only 12 km of the eastern end of the island has homes. The remainder of the island is a narrow band of sand. Six seasonal collections (Oct. '99 – Feb. '01) were taken along 4 transects (east to west) across the island, from Bay to Gulf, through the eastern forested areas of the island to survey tardigrade communities. Within each transect samples of mosses/lichens growing on trees and leaf litter (upper & lower separated) were collected. We hypothesized that a gradient across the island would be found, with the least number of tardigrades found near the Gulf coast, a higher number near the Mobile Bay coast, and the most in the middle of the island. The moss/lichen samples from tree trunks yielded only a very few tardigrades, thus the emphasis was switched to leaf litter. A total of 1,169 tardigrades were collected from the upper leaf litter. Seasonal samples yielded no obvious patterns. Fall samples (Oct. '99 and '00) had 123 and 382 tardigrades respectively. Winter samples (Jan. '99 and Feb. '00) yielded 89 and 301 tardigrades respectively. Transect 1 was the eastern most transect, through the Audubon forest, and 3 trees were sampled (2 live oak and tupelo) and produced 330 tardigrades. Transect 2 extended through Cadillac Square had 5 trees were sampled (4 live oak and 1 tupelo) and produced 508 tardigrades. Transect 3, just west of Rt. 193 and crossed through the Isle du Dauphine golf course, had 5 trees sampled (4 live oak and 1 hardwood) and produced 222 tardigrades. Transect 4, just east of the Dauphin Island elementary school, had 3 trees sampled (2 live oak and pine). An average of 96 tardigrades were found in Gulf coast dune leaf litter. An average of 61 tardigrades were found from middle island samples and an average of 41 tardigrades were found in the Mobile Bay samples. Most tardigrades (78.8 %) were in the genus Macrobiotus. Others genera found were Diphascon/Itaquascon (9.1 %), Hypsibius (5.7%), Milnesium (4.5%), Echiniscus (0.76%) and Calcarobiotus (0.57%).
THE FIRST RESULTS OF A STUDY ON THE TARDIGRADE COMMUNITIES IN *POLYLEPIS* WOODLANDS FROM THE MANTANAY VALLEY, CORDILLERA DE VILCANOTA, PERU

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Little information has been published on the high altitude tardigrade communities of Peru. The study area was located in the Mantanay valley, situated in the Cordillera de Vilcanota, approximately 40 km NNW of Cusco, Peru. Presented are the first results of a study into the tardigrade communities along a transect from the centre of the *Polylepis* woodland to the surrounding grassland. The transect was 135 m long with collecting stations every 15 m, at an altitude of 4,150 m. A range of representative samples of mosses and lichens were collected from each sampling station along the transect. All samples were collected in the dry season and returned to the University of Plymouth in a preserved dried state. The weight of each sample was recorded so that a quantitative analysis of species density could be calculated along the transect. An attempt to extract and identify every tardigrade specimen from each sample including eggs and exuvia was made. These initial results were then examined for any trends across the transition from the centre of the woodland to its edge and then on to the grassland. Further replicated samples along the transect from a range of other sample substrates are to be processed to help model the changes in the composition of the tardigrade communities.
A LIFE HISTORY STUDY OF A NATURAL POPULATION OF ECHINISCUS MAUCCI (TARDIGRADA; HETEROTARDIGRADA) IN ALABAMA

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This fifteen month study (December 2002 though February 2004) of a meiofaunal community living in moss and lichen from a Pecan tree (Carya illinoensis) on the campus of Jacksonville State University reports 9,791 microinvertebrates. *Echiniscus maucci* was the most prevalent tardigrade species (1,327 specimens). The length of all the specimens (adults, juveniles) for each month was determined. The average length of identifiable males and females in the study was also determined. A histogram of all of *Echiniscus maucci* specimens was constructed to determine the life stages of this species. Three major life stages were identified for *E. maucci*, juvenile, pre-reproductive, and reproductive. Life stage divisions were based upon the presence of juvenile and adult sexually dimorphic characteristics. It was determined that this population is an opportunistic sexual reproducer. Adults have a mean clutch size of two and may breed more than once in a life span. An analysis of variance was run on the monthly means of *E. maucci*. Significant difference between monthly means was found. To further analyze the data a multiple comparison test was run using the Fisher's Least Significant Difference (LSD) test. When a regression was performed for all specimens there was no statistically significant relationship observed. When it was performed for adults only, a statistically significant negative relationship was observed between the average adult size for each month and the number of adult *E. maucci* collected in that month. Male and female lengths were compared with Student’s t-Test and females were found to be significantly larger than the males. A regression between average temperature and precipitation and the numbers of *E. maucci* was performed and no statistically significant relationship exists.
CRYPTIC OCCURRENCE AND NEW RECORDS OF THE MARINE FAMILY RENAUDARCTIDAE (ARTHROTARDIGRADA)

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The family Renaudarctidae has remained monotypic since the description in 1984. The type species Renaudarctus psammocryptus Kristensen and Higgins, 1984 was originally described from Pepper State Park, Fort Pierce and Red Reef Park, Florida. On both localities the specimens were found at 70-100 cm depth close to the mean low-water level in coarse carbonate sediment consisting of shell hash. The collections were made in 1982-1983. Unpublished data from Pollock and Higgins indicate the same species was collected already in 1980 from Cap Hatien, Republic of Haiti and Monte Criste, Dominican Republic. After the description of the family, Pollock sent scanning electron microscopy (SEM) photos of specimens from the Caribbean Sea to the senior author. Later in 1984 Renaud-Mornant published records of the species from Martinique and Guadeloupe (Antilles).

Noda published during the “Sixth International Symposium on Tardigrada, Cambridge, 1994 an abstract on two species of renaudarctids from Ryukyu Archipelago, Japan. He also showed a poster with drawings of what he called Renaudarctus psammocryptus and gen. (?) sp. The new genus had only two digits on leg IV. However, Noda could not describe the new genus on the single specimen he found. The coralline sandy sediments were collected only 15 cm from the surface in the middle intertidal zone. Noda stated that the occurrence of R. psammocryptus from Western Pacific far from the type locality (the East coast of North America) suggests that this species has a worldwide distribution.

From 13 August 1995 to 14 June 1996 an Australian Biological Research Study was carried out along the whole Eastern coast of Australia. About 80 marine localities were investigated. Only at one locality, Tannum Sand, Gladstone, Queensland that was visit three times were a species of Renaudarctus found. All specimens were found in shell hash at mean high-water level from 20 to 50 cm sediment depth close to the freshwater groundwater. The species look very similar to R. psammocryptus; however, in a few characters the new species differs.

Collecting marine tardigrades for molecular data at Seychelles from 16 to 28 March 2005 at Praslin and Mahé several beaches were investigated. Only at Beau Vallon, Mahé a species of Renaudarctus was found in carbonate sand 100-130 cm depth in the sediment close to the mean low-water level. Our investigations using light microscopy and SEM show that this Indian Ocean species also is very close to R. psammocryptus.

Conclusion: The genus Renaudarctus has a worldwide distribution; however it has a very cryptic occurrence: Often found very deep in the carbonate sediment close to the groundwater. On all localities the species is very rare.
It is known that specimens of *Amphibolus* can carry out both cryptobiosis (anhydrobiosis and cryobiosis) and encystment and that they produce two types of cysts (type 1 and type 2). In *Amphibolus nebulosus* from Greenland, the type 1 cyst is related to winter and the type 2 to summer. In *Amphibolus volubilis*, found in Apennines (Monte Rondinaio, Italy), a detailed description of the encystment processes has been done, but not of the relationships between climatic factors and dynamics of the cyst 1 and cyst 2. Therefore, a study on seasonal dynamics of the *A. volubilis* cysts has been carried out with monthly samplings, from March 2003 up to March 2005. For each sampling, all specimens present in five or six replicates of 0.5 g of moss have been collected and analyzed. In all samplings, non-encysted and encysted animals (type 1 or type 2 cysts) have been found. The cyst trends are similar in the two considered years. Type 1 cysts have been found from November to March-April, but they were present in a very low percentage with respect to the total animals. Type 2 cysts have been found from June to October and are often more abundant than the non-encysted tardigrades. The two types of cysts never overlap. Correlation tests have evidenced that dynamics of type 2 cyst is positively related to the air temperature (T) and negatively related to the relative humidity (RH) of the air. The trend of type 1 cyst is negatively related to T, while no relationship to RH has been evidenced. The presence of non-encysted animals is negatively related to T and positively related to RH.

Experiments of encystment induction have been performed in the laboratory. In the same experimental conditions (same temperature, photoperiod, food and oxygen availability), non-encysted animals collected in April form type 2 cysts, whereas animals collected in November form type 1 cysts. The number of days to enter each type of cyst is related to experimental temperature. At the same temperature, the animals spent less time to enter in type 1 cyst than in type 2 cyst. In the lab, the animals emerge from type 2 cyst, if they do not die, sooner or later encyst again and always enter the type 1 cyst.

The data from nature and those from the laboratory lead to the conclusion that, differently from what happens in anhydrobiosis and cryobiosis, climatic conditions do not directly affect encystment; but seasonal changes induce still unknown endogenous factors responsible of that kind of dormant stage.
NEW RECORDS ON CYCLOMORPHOSIS IN THE MARINE EUTARDIGRADE HALOBIOTUS CRISPAE

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Although seasonal cyclic changes in morphology has been suggested for several tardigrades, Halobiotus crispae remains the only tardigrade for which cyclomorphosis has been clearly established. The eutardigrade genus Halobiotus belongs to Hypsibiidae. H. crispae was described from Disko Island, Greenland by Kristensen in 1982. We now have sampling data from several other localities on the Northern hemisphere. These localities include Vellerup Vig (Denmark), Kristineberg (Sweden), Tromsø (Norway), Igloolik (Canada), Alaska (U.S.A.) and the Ikait columns from Ilka Fjord (Greenland). H. crispae from the type locality at Nipissat Bay, Disko Island is found in the subtidal zone and occurs in four cyclomorphic stages: 1. The active stage characterized by fully developed gonads and bucco-pharyngeal apparatus; 2. A simplex or moulting stage lacking stylets and placoids; 3. The pseudosimplex 1 stage or hibernation stage characterised by a double cuticle, immature gonads and closed mouth; 4. The pseudosimplex 2 stage, a sexually ripening stage enclosed by a single cuticle and characterized by a functional, but aberrant, bucco-pharyngeal apparatus.

In this study we compare the originally described seasonal occurrence of cyclomorphic stages at Nipissat with stages found at other localities. Emphasis is on seasonal appearance of cyclomorphic stages at the southernmost locality, Vellerup Vig, Denmark. All four cyclomorphic stages have been found at Vellerup. However, when comparing lifecycles of Halobiotus crispae at Nipissat and Vellerup profound differences are found in time of year, as well as period in which, these stages appear. Noticeably, at Nipissat the pseudosimplex 1 stage is a hibernating stage occurring during the long Arctic winter. In contrast, at Vellerup, this stage appears during summer. Thus, while pseudosimplex 1 seems to be an adaptation to withstand low temperatures in Greenland, this stage possibly enables the animal to tolerate periods of oxygen depletion and heat stress during the Danish summer. The environmental or endogenous signals underlying the transition between different stages remain unknown.

Our molecular data suggest that Halobiotus has evolved within Isohypsibius. Further investigations on the lifecycle of members of the Halobiotus genus as well as other members of Hypsibiidae is needed in order to establish whether cyclomorphosis is i) a general theme among members of Hypsibiidae or ii) an autapomorphy for Halobiotus, developed as an adaptation to life in the subtidal zone characterized by large fluctuations in environmental parameters such as salinity and temperature.
FIRST POSTER SESSION

FIRST SCANNING ELECTRON MICROSCOPE OBSERVATIONS OF PYXYDIUM TARDIGRADUM VAN DER LAND, 1964 LIVING ON TARDIGRADES

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*Pyxidium tardigradum* is thought to be an external symphoriant of tardigrades. Here we provide first Scanning Electron Microscope photomicrographs of *P. tardigradum* attached to *Ramazzottius oberhaeuseri* and *Macrobiotus* sp. collected respectively from Kirghizia (Asia) and Costa Rica (Central America). The SEM observations revealed that the surface of the *Pyxidium* cell is not smooth, as it appears to be in the Light Microscope, but is covered with distinct parallel ridges. The cytostome is located on the top of the cell. No morphological differences were found between specimens form Asia and America, both in SEM and in Light Microscope.
MORPHO-FUNCTIONAL CHARACTERISTICS OF THE DIGESTIVE SYSTEMS OF RAMAZZOTTIUS TRIBULOSUS AND MACROBIOTUS RICHTERSI (EUTARDIGRADA)

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The morphology of the bucco-pharyngeal apparatus is correlated with the food utilized. Nevertheless, even though the ultrastructure of the digestive system of tardigrades was already described in some species, it was not studied in depth, and not in relationship to the feeding. Therefore, comparative ultrastructural analyses of the digestive system of the phytophagous Ramazzottius tribulosus and the zoophagous Macrobiotus richtersi were made.

In R. tribulosus within the cuticle of the buccal ring there are distinct transverse pillar-like structures. Posterior to the mouth, the cuticle of the buccal tube becomes electron-dense and compact, without pillar-like structures and vacuoles, even though holes or groups of holes are sometime present anterior to the stylet supports. At each side of the buccal tube there are thin processes of the salivary glands, which form a large reservoir where secreted mucous is accumulated, like in the salivary sac of arthropods. Under the reservoir wall there are thin muscle processes and neuromuscular contacts. Stylets and stylet supports are located in the salivary reservoirs. Each stylet has a basal cuticular part and an apical hard calcium part divided by a well visible border. The beginning of the pharynx is characterized by thin valves and cuticular apophyses; the latter are homogenous ultrastructure so as the posterior part of the buccal tube and placoids. The lumen of the pharynx is limited by a clearly tri-layered thin cuticle. At the transition from pharynx to oesophagus there is one valve formed by folds of the gut which hinder the food return. The increase of midgut surface is realized by both large folds of the gut wall and microvilli. Microvilli are relatively short and thick but have well developed glycocalyx and in cross section look disposed regularly forming very compact hexagons (111 per μm²). Epithelial cells of the midgut have large and small vacuoles. In tardigrades fixed after feeding, the midgut lumen exhibits a peritrophic membrane. In the cross section the lumen of the rectum looks as a big heart-like cavity with some narrow cell evaginations. The lumen is lined by a thin cuticle.

For M. richtersi, at this moment we have considered only the ultrastructure of midgut and hindgut. Microvilli of the midgut are shorter and thinner than those of R. tribulosus. They form less dense hexagons (73 per μm²). Glycocalyx is present but not particularly developed. The ultrastructure of the midgut (epithelial cells, peritrophic membrane) and that of the rectum, is similar to what is found in R. tribulosus.

Comparing these results among them and with published data on the zoophagous Isohypsibius prosostomus, several similarities has been found, such as valves, cuticle lining, ultrastructure of epithelial cells, and peritrophic membrane. They can be related to common feeding processes. The presence of peritrophic membrane indicates that in tardigrades extracellular digestion occurs. Nevertheless, we can identify differences that can be related to the food utilized. Mainly, there is more absorption surface of the midgut in the phytophagous R. tribulosus than in zoophagous M. richtersi and I. prosostomus.

Investigations supported by INTAS Grant n. 04-83-3807.

June 18-23, 2006 – Catania, Italy
APPEARANCE OF MALES FROM A THELYTOKOUS STRAIN OF 
MILNESIUM TARDIGRADUM (EUTARDIGRADA)

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Tardigrades are generally gonochoristic and almost all marine species have both males and females. Hermaphrodites are known from several limno-terrestrial and one marine species. However, many moss-dwelling tardigrades have a thelytokous mode of parthenogenesis. Although Milnesium tardigradum also has both sexes, and some populations are indeed known to have a sex ratio close to 1:1, males are nevertheless usually rare and many populations appear to have parthenogenetic reproduction. I have maintained a strain originated from one female of Milnesium tardigradum since 2000. Individuals of this strain are, effectively, all females and propagate by thelytokous parthenogenesis. Recently, however, it has been elucidated that males are found from this strain at a very low frequency. In this presentation, I will report on the morphology of the rare males with results from light microscopy and SEM, comparing with those of the females of the strain. The modified claws of the first pair of legs as a secondary sex character are recognized in these males, as already shown repeatedly in other populations. The body size of a male is smaller than that of a female. The posterior part of the male body, after the third pair of legs, is more slender and longer than that of the female. Additionally, males appear to be slower than females in locomotive activity in this strain. The frequency of the appearance of males in the cultural strain is so low that any environmental effect to generate males has not been known yet. Rare males in hermaphroditic C. elegans have been known to be a result of accidental loss of an X chromosome. Sex chromosomes are unknown in tardigrades so far, and even if the existence of XX-XO system of sex determination in tardigrades might be thought, the similar mechanism as in hermaphrodites could not take part in generating rare males in a thelytokous strain. The reason for occasional males in the thelytokous strain is very difficult to answer, and any sexual behavior has not yet been observed in the culture. It is also unknown if the hero of this tragedy could play a role as a sexual male in other populations. However, they might be the representatives that show some possibility of genetic exchange of the clonal population.
SOME MORPHOLOGICAL AND MOLECULAR DATA ON THE RELATIONSHIP OF HYPSIBIUS KLEBELSBERGI MIHELCIC, 1959 WITHIN THE EUTARDIGRADA

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Currently, Hypsibius klebelsbergi is considered as a member of the genus Hypsibius (Hypsiibiidae). First molecular data (rDNA sequences) analysed by a neighbour joining tree (ClustalW) suggest, however, a closer relationship to the genus Ramazzottius than to other Hypsibiidae (Thulinius, Halobiotus). Owing to the type of the anterior tube apophyses and the structure of claws it was speculated in a recent redescription that H. klebelsbergi might not belong to either Isohypsibius or Hypsibius (see Dastych et al., 2003). In addition to the 18S rDNA, we now use the highly variable sequences of Internal Transcribed Spacer (ITS1 and ITS2) of the rDNA. The sequences of ITS1 and ITS2 are easily accessible, because they are flanked by conserved regions of the rDNA. This facilitates primer design and has been shown to be suitable for species differentiation.
3-DIMENSIONAL FLUORESCENCE IMAGEING OF THE BUCCAL APPARATUS AND CLAWS FOR SPECIES IDENTIFICATION OF EUTARDIGRADES

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Studies of eutardigrades at the specific level face several problems because species are differentiated by few characteristics, which are sometimes difficult to observe. The buccal apparatus with placoids are among the most important characters for species identification. Placoids alternate in position with three equal cuticular apophysis, which are posterior to the end of the buccal tube. The larger anterior placoids, called macroplacoids, are present in either two or three transvers rows. Smaller, posterior placoids are termed microplacoids. Furthermore, size, shape and number of claws are additional important characters which have commonly been used in tardigrade systematics. The buccal apparatus and claws show (after excitation with blue or green light) sufficient auto-fluorescence to be detected at a range of wavelengths (green to red). New techniques for optical sectioning and 3-dimensional reconstruction with increased positional accuracy and spatial resolution allow the reproduction of the real 3D structure of the buccal apparatus. Thus, modern epi-fluorescence techniques provide easy and fast access to important taxonomic features of tardigrades and at the same time produce demonstrative visualizations. We investigated several eutardigrade species with this new methods and displayed the 3-dimensional buccal apparatus and claws for species determination and description. As an outlook: the 3-dimensional image data can be used for the production of spatial models.
COLD TOLERANCE IN MILNESIUM TARDIGRADUM

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Cold tolerance in ectothermic animals is thought to be an important adaptation for overwintering. In this study cold tolerance of Milnesium tardigradum from Hokkaido (43°N, 141°E), northern Japan and Jawa Island (6°S, 106°E), central Indonesia were investigated. After an exposure to subzero temperature conditions, the mean survival rate of the Japanese population was significantly higher than that of the Indonesian one, although more than 40% of test animals survived freezing at -196°C for 20 days even in the Indonesian population. In both populations freezing and melting processes in their body were observed using a cryomicroscope and a Differential Scanning Calorimeter (DSC). These results suggest that there is an intraspecific variation in freezing tolerance in M. tardigradum and that this species has the capability to survive extreme low temperature even in the tropics. In conclusion, it is possible that cold tolerance in terrestrial tardigrades has evolved as not a strategy to overcome severe winter but a secondary character of their desiccation tolerance because both traits are thought to be based on similar physiological mechanisms.
INDUCTION OF THE STRESS PROTEIN HSP70 BY DESICCATION, IONISING RADIATION AND HEAT-SHOCK IN THE EUTARDIGRADE RICHTERSIUS CORONIFER (EUTARDIGRADA)

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The role of stress-proteins in desiccation tolerant organisms has recently become a field of increasing interest. Extensive evidence shows that several families of heat shock proteins serve as molecular chaperones to assist the folding of newly synthesized proteins, protect them from stress-associated denaturation, aid in their renaturation, influence the final intracellular location of mature proteins, and interact with other compounds such as sugars during desiccation. Such interactions are believed to play an important role in protecting cell components from damage during the desiccation or rehydration processes. We report a study on the induction of heat-shock protein Hsp70 in the eutardigrade Richtersius coronifer, after exposure to desiccation, heating (37°C), and ionising radiation (500 Gy). The immuno-western blot method was used to quantify the amount of Hsp70 induced by different treatments. Expression of Hsp70 was investigated in six different experimental groups: (1) hydrated unstressed animals, (2) hydrated animals 1 h after anhydrobiosis, (3) hydrated animals 1 h after heat-shock, (4) dry anhydrobiotic animals, (5) hydrated animals irradiated in the anhydrobiotic state, 1 h after rehydration, (6) hydrated animals irradiated in the active state, 1 h after irradiation. As expected, heat stress induced a strong Hsp70 response, with induced levels of more than twice that of the untreated active tardigrades. Also irradiation by 500 Gy induced a strong response. Animals irradiated in an active state showed higher Hsp70 levels than those irradiated in a dry state. Elevated Hsp70 levels were also found in active tardigrades shortly after a period of desiccation, while dry samples had reduced levels. These results suggest that, if Hsp70 is part of the protection mechanisms against damage induced by desiccation, its role is probably connected to repair processes rather than to prevention of cellular damage.
NEW DATA ON TARDIGRADES OF SARDINIA (ITALY)

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The first records of marine tardigrades in Sardinia date back to 1964 when Batillipes pennaki Marcus, 1946 was found in the sandy beaches of S. Marinella Gulf and S. Reparata Bay near Sassari (De Zio, 1964; Grimaldi, 1965). Many new data about tardigrades of this Mediterranean island were successively collected in different coastal localities from both intertidal and subtidal zones, revealing a high richness of species mainly in the subtidal (Grimaldi de Zio et al., 1983, 1988, 1990a, b, 1992; D’Addabbo Gallo et al., 1989, 1992; de Zio Grimaldi & D’Addabbo Gallo, 2001; de Zio Grimaldi et al., 2003).

Our present research expanded to 4 further localities of the northern and western coast and to 4 new sites inside the Orosei Gulf, adding more information about Sardinian tardigradofauna, that presently account for 9 species new to science. Overall from the new data it looks evident that the subtidal is richer than the intertidal zone (16 species and 7 species, respectively). In terms of individuals abundance, Batillipedidae (6 species) are the most important family, whereas Halechiniscidae (7 species) are less abundant. Furthermore, the finding of the new species of Batillipedidae, Batillipes spinicauda (see Gallo D’Addabbo et al., 2005) is quite interesting because it shows the highest densities of all taxa in the more recent sediment samples. In the subtidal sediments, Halechiniscidae shows the highest number of species compared to the remaining taxa (8 species), while Stygarctidae, present only in subtidal sediments, accounts only for 2 species. The differences between intertidal and subtidal tardigradofauna could not only depend on the physical conditions, but also on the sediments chemical composition; the intertidal sands are in fact mainly siliceous, whereas the subtidal ones are calcareous.

Considering all data collected, the Sardinian tardigradofauna appears very rich and includes 43 species belonging to the family Neoarctidae (1 species), Neostygarctidae (1 species), Stygarctidae (3 species), Halechiniscidae (27 species) Batillipedidae (9 species) and Echiniscoiodidae (2 species).

The discovery in 1992 of Neoarctus primigenius is to be considered quite remarkable from the phylogenetic and taxonomic point of view, because of its several plesiomorphic characters, determining the institution of the new family Neoarctidae (Bello & de Zio Grimaldi, 1998; de Zio Grimaldi et al., 1992).
SOME TARDIGRADES FROM USHKANYE ISLANDS (LAKE BAIKAL, RUSSIA), WITH THE DESCRIPTION OF A NEW MINIBIOTUS SPECIES (EUTARDIGRADA: MACROBIOTIDAE)

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In 34 bryophytes samples collected in Ushkanye Islands (Lake Baikal) in years 1997 – 1999, 21 species of Tardigrada (2 species of Heterotardigrada and 19 ones of Eutardigrada) including a new species Minibiotus sp. 1 were found. The new species is most similar to Minibiotus floriparus Claxton, 1998 and Minibiotus weglarskae Michalczyk, Kaczmarek and Claxton, 2005 in accordance with the shape of eggs. Minibiotus sp. 1 differs from M. weglarskae mainly by absence of cuticular pores and from M. floriparus by shape of claws as well as by pt ratio of some structures. Eggs of the new species differ from those in both species in shape and measurements of their processes.

This study was partly supported by the Slovak Scientific Grant Agency (VEGA) as a Project No. 1/3265/06.
TARDIGRADA OF XINJIANG PROVINCE, CHINA

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Relatively little is known of the Tardigrada fauna of China, and some areas of the country have not been investigated. There are no previous tardigrade records for Xinjiang, China’s largest province. The moss specimens in the Missouri Botanical Garden Herbarium were used as a source of tardigrades from this province. Of the 258 moss specimens sampled, 66 yielded tardigrades. Species found from Xinjiang Province are \textit{Cornechiniscus holmeni, Echiniscus blumi, E. canadensis, E. granulatus, E. testudo, E. trisetosus, Isohypsibius qinlingensis, Macrobiotus maucci, Milnesium asiaticum, Milnesium katarzynae, and Milnesium tardigradum}. Heterotardigrada are better represented in the results than the Eutardigrada, which is related to the environment of the province and the altitude at which the samples were collected. Many of the \textit{Echiniscus} species are considered to be xerophilic, and Xinjiang has an annual average precipitation of 150 mm. Some of the \textit{Echiniscus} are considered montane species, and the samples containing tardigrades were collected at 1,140-4,100 m a.s.l.
A NEW SPECIES OF THE GENUS *PARASCON* (TARDIGRADA, HYPSIBIIDAE) FROM CHINA

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*Parascon qinlingensis* sp. nov. from Qinling Mountains located in the central part of Shaanxi Province, China is described as new to science. *Parascon qinlingensis* sp. nov. differs from *P. schusteri* and *P. nichollsae* in having no stylet support, in having stylet furcae without branches, and with the external claws not forked at the base. Photomicrographs and drawings are provided to illustrate the morphological characters of this new species. Type specimens are deposited at the College of Life Sciences, Shaanxi Normal University, Xian, P. R. China.
NEARCTIC FRESHWATER TARDIGRADA

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The distribution and ecology of limnoterrestrial Tardigrada in the Nearctic realm remain poorly known. This is especially true of freshwater tardigrades (i.e., species found in more-or-less permanently submerged habitats), who have received much less attention than terrestrial species. We reviewed the published literature on North American freshwater tardigrades, and included data from our own unpublished Louisiana collections. The number of tardigrade species identified in freshwater collections is almost an order of magnitude less than the nearly 200 known Nearctic terrestrial species. Nearctic freshwater tardigrades have been collected from sediments and aquatic vegetation in streams, rivers, lakes, and groundwater. Several studies focus on intensive sampling of one or a few sites, and have provided valuable insights into local spatial and temporal variation in diversity and abundance. Comparison among studies is hampered by inconsistent sampling methodology. Most other records of freshwater tardigrade distribution are from opportunistic samples taken during terrestrial surveys. While there are a number of regional collections of terrestrial tardigrade distribution in North America, such studies are lacking for freshwater habitats. Although some Nearctic freshwater species are known from only single collections, most appear to be widely distributed. Apart from the distinctive fauna of some unusual habitats, such as Greenland’s homothermic springs, available data do not suggest regional variation in the distribution of Nearctic freshwater Tardigrada.
THE DIVERSITY OF INDIAN OCEAN HETEROTARDIGRADA

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Marine tardigrades bibliography is not too wide, nevertheless references on the species found in the Indian Ocean are numerous and mainly relate to Arthrotardigrada from abyssal muds, and coastal coralline sands (Renaud–Debyser, 1965a,b; Renaud-Mornant, 1974, 1975, 1979a,b, 1981, 1984, 1987; Kristensen & Renaud-Mornant, 1983; Grimaldi de Zio et al., 1987; de Zio Grimaldi et al., 1991, 1999).

Our new findings of several different species in the intertidal and subtidal sands of Maldive and Seychelles Islands, add further information on previous data. The sediment samples studied are very similar and consist of white coralline sands, generally moderately and poorly sorted, with small amounts of coarse detritus. Tardigrades represent always one of the less abundant taxa of meiofauna present in all samples, nevertheless Diversity (H’) and Evenness (J) indices are always rather high. The 22 species found In the Maldive Islands belong to Neostygarctidae, Stygarctidae, Halechiniscidae and Batillipedidae, and H’ values range between 1.82 and 2.59, whereas J values range between 0.65 and 1. The species found in the Seychelles Islands are 12 and belong mainly to Halechiniscidae, but also to Renaudarctidae, Stygarctidae, Batillipedidae ed Echiniscoididae, and average H’ and J values are respectively 2.78 and 0.78.

The present data add faunistic and biogeographic information about the Indian Ocean Tardigradofauna, including 1/3 of the known species of Heterotardigrada. Arthrotardigrada show the highest densities, with Halechiniscidae heavily contributing to the high values of the diversity. The finding of Renaudarctus psammocriptus, previously recorded only in the Atlantic Ocean, and of Tanarctus velatus and Florartctus heimi, found only in the Pacific Ocean (McKirdy et al., 1976; Renaud Mornant, 1965; Kristensen & Higgins, 1984), along with the presence of Neostygarctus acanthophorus, Archechiniscus minutus and Styraconyx tyrrhenus, known to date only in the Mediterranean Sea (Grimaldi de Zio & D’Addabbo Gallo, 1987; de Zio Grimaldi & Gallo D’Addabbo, 2001; de Zio Grimaldi et al., 2004), is quite remarkable.

Our data on the present geographical distribution of marine tardigrades strongly support Sterrer’s ideas about meiofauna speciation and dispersion mechanisms, mainly by means of geological events (Sterrer, 1973). In fact, due to the very limited capability of locomotion and dispersal of tardigrades, the distribution of many cosmopolitan species can only be explained taking into account the geological evolution of the seas. Furthermore, the old origin of the group and the affinities between the Mediterranean Sea tardigradofauna and that of the Indian and Atlantic Oceans, could be considered as a document of their consistent presence in the old Tethys Sea.
TARDIGRADE EGGS IN SEDIMENTS OF ANTARCTIC LAKES

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Tardigrade eggs have rarely been recorded from lake or peat bog sediments, with only occasional, often casual, observations of eggs from high northern latitude locations. Here we report and discuss the occurrence of tardigrade eggs in lake sediments from two widespread areas of Antarctica. Eggs of *Macrobiotus furciger* and *Dactylobiotus cfr ambiguus*, identified by details of surface processes, were abundant in sediments from Lake Boeckella (63°24’S, 56°59’W), with the latter species also occurring in Limnopolar Lake on nearby Livingston Island (62°36’S, 60°30’W). Eggs of *Macrobiotus blocki* and *Minibiotus weinerorum* were found in sediments from Lake Terrasovoje, located in the Amery Oasis (70°33’S, 68°02’E). Further, smooth eggs, most likely of the widespread Antarctic species *Acutuncus antarcticus*, were recorded from all sites, as well as Waterfall Lake in the Vestfold Hills (68°33’S, 78°20’E). In each case the eggs were distributed throughout the sediments, which in most lakes dated from the present back to early in the Holocene (<10 000 years old). No degradation of the external architecture of the eggs with age was apparent. These dates provided the earliest evidence for colonisation of Antarctica for these species. The distribution of the eggs in the sediments also allowed investigation of population dynamics. In Lake Boeckella, lacustrine *D. cfr ambiguus* was the dominant species from soon after the lake was formed. This species disappeared soon after eutrophication of the lake due to the arrival of breeding penguins in the lake’s drainage basin. *Macrobiotus furciger*, which probably inhabited the littoral zone of the lake and the surrounding vegetation, was present in low numbers early in the lake’s history, but became more abundant after the disappearance of *D. cfr ambiguus*. Productivity in the lake has dropped over the last few hundred years, but *D. cfr ambiguus* has not been able to recolonise the lake. While it appears that the preservation of the tardigrade eggs may be limited to high latitude sediments, they provide important insights into both the colonisation of these habitats as well as changes in the population dynamics of these communities over periods of thousands of years.
MEIOFAUNAL ABUNDANCE, DIVERSITY AND SIMILARITY IN A UNIQUELY RICH BATILLIPES MIRUS TARDIGRADE COMMUNITY

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A clone library of 18S rRNA gene fragments derived from a sediment sample with a uniquely high concentration of tardigrades from Dauphin Island, Alabama yielded 126 sequences. The abundance of Batillipes mirus from this site is unusually high, often with counts nearing 1000 individuals per 10 cm² core samples. Twenty molecular groups (OTUs) and 12 morphological groups were identified from the samples. Rarefaction curves were calculated and indicated that the sample sizes were large enough to adequately assess the communities. Each of the sorting techniques (molecular and manual) was treated as an individual community for the comparisons. The species diversity of the two communities were similar with Shannon’s $H'$ and Simpson’s $C$ being 0.837 and 0.231 respectively for the OTUs and 0.733 and 0.167 respectively for the manually sorted samples. Community similarity was compared using Jaccard’s and Stander’s coefficients. Jaccard’s coefficient which simply accounts for the number of taxa shared between the two communities was 58.3%, while Stander’s coefficient, which is a function of the number of species shared and their relative distributions, was 98.6%. The molecular and phylogenetic methods used here to assess a community of marine tardigrades were successful in discriminating between the meiofaunal groups (OTUs). The results indicate that rarefaction curves can reach saturation and that groups can be identified from small sample sizes. The dominant species in both communities was tardigrades.
SECOND POSTER SESSION

FOOD PREFERENCE IN TARDIGRADES, DETECTED BY A PCR-BASED APPROACH WITH PARTIAL RBCL SEQUENCES

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Most of the more than 960 known tardigrade species are terrestrial and live in moist microhabitats, such as soil and leaf litter, mosses, lichens, cushion-shaped plants and between superficial roots. Mosses are such an excellent habitat for tardigrades due to their ability to ensure high humidity, and especially rich food supply for carnivorous and herbivorous species. Feeding preferences seem to be correlated with the morphology of the buccal apparatus and consequential the distribution is sometimes linked to food availability like, nematodes, rotifers, plant cells, algae and bacteria. In many species matter containing chlorophyll is often observed in the midgut. But actual food preferences remained a puzzling secret so far. Trophic interactions within soil food webs are difficult to study. Therefore, a polymerase chain reaction (PCR)-based approach was used as a highly sensitive detection method. The study was carried out to investigate the apparently chlorophyll matter in the gut of active and anhydrobiotic specimens, based on phylogenetic analyses of the chloroplast ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene data from mosses and algae. Partial rbcL sequences, 390 bp long, were detectable and identified in the heterotardigrade species Echiniscus granulatus and eutardigrade species Macrobiotus persimilis, Macrobiotus sapiens, and Richtersius coronifer. The moss in which E. granulatus and M. persimilis were coexistent has been identified as Schistidium sp. In E. granulatus as well as in M. persimilis moss which belong to the Bryopsida were detected. Macrobiotus sapiens has been found between the mosses Didymodon sinuosus, Schistidium sp., Orthotrichum diaphanum and Tortula muralis. The gut contained moss which belong to the Bryopsida, too. In contrast, R. coronifer inhabits moss pads of the genus Orthotrichum cupulatum and the gut contained green algae. This first result showed the necessity to investigate more species, as well as different phases of the life history for a better understanding of habitat and food preferences, distribution and population dynamics.
THE ULTRASTRUCTURE OF THE TRUNK GANGLIA IN
MACROBIOTUS RICHTERSI (TARDIGRADA)

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The current importance of investigating the anatomical and ultrastructural organization of Tardigrada nervous system is determined in relationship to the early evolution of the arthropods. According to the data by Dewel and Dewel (1996, 1999), the organization of the Tardigrada brain could be best understood by analyzing the pattern of the nervous system in the trunk. On this basis, we analyzed the ultrastructure of the trunk ganglia in the eutardigrade Macrobiotus richtersi.

The trunk ganglion is located ventrally under the midgut beneath the delicate visceral muscles and surrounded by the body-cavity. The ganglion has an oval compact shape and distinct basal lamina. Eight perikarya have been found in the investigated portion of the ganglion (25 serial sections were examined). Most of them are located at the periphery and they contact basal lamina directly. At least one of the neurons is situated to some extent deeper and it contacts fringe cells. This neuron projects the process to the center of the neuropile and then passes into the lateral nerve. Usually, perikarya have an oval nucleus and neuroplasm with dense packed organelles such as mitochondria, reticulum and vesicles. Only one cell has irregular-shaped nucleus and a lucent cytoplasm. It is unclear if the Tardigrada nervous system has glial cells or not. Perhaps this lucent cell has a glia-like function in the trunk ganglion of M. richtersi.

A pair of nerves goes out from the ganglion and innervates the lateral area of the segment. These nerves have no neuron cell bodies and any other cells like glial cells. The nervous processes are slim, dense-packed and contain mitochondria and synaptic vesicles. A basal lamina surrounds not only the ganglion but nerves too, and consists of fine dense filaments. Rough fibres have not been found. There are numerous neurites containing small clear and rare dense vesicles in the neuropile. Besides in the ganglion there is one large axon which contains clear cytoplasm and dense-core vesicles. Its perikaryon is not found in the ganglion. This axon goes through the ganglion and passes out into a nerve. Many processes in the neuropile have a specific dense neuroplasm with clear small vesicles. We have found a similar pattern in neurites innervating the salivary duct and muscles of the salivary glands and perhaps in the circumesophageal connective of another eutardigrade, Ramazzottius tribulosus.

Investigations partially supported by INTAS Grant n. 04-83-3807.
COMPARATIVE MORPHOMETRICS OF POPULATIONS OF MACROBIOTUS AREOLATUS MURRAY (EUTARDIGRADA, MACROBIOTIDAE) FROM TWO NEOTROPICAL CITIES

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Macrobiotus areolatus Murray, 1907, a cosmopolitan species, has been recently recorded in urban environments of the Neotropical Region. Although it has been known for a long time, its pattern of geographic variation remains uncertain or poorly defined. This is especially so for the South American populations (as occurs generally for all tardigrade taxa). The need of detailed morphologic and morphometric studies of tardigrades and their eggs – with emphasis in the sclerotized parts and their proportions - has been pointed out. This information should allow to improve the taxonomic discrimination within of species groups such as that of Macrobiotus harmsworthi, to which has been previously related M. areolatus. Consequently, the purpose of this study is to contribute new morphometric data towards a better characterization of M. areolatus and its morphometric features, especially of its Neotropical populations. Specimens of Macrobiotus areolatus were obtained from two medium-sized cities of central Argentina: Santa Rosa (36° 39' S, 64° 17' W) and General Pico (35°40' S; 63°44' W). At the two cities, samples were collected from lichens and mosses growing on sidewalk trees of the urban and periurban area. Samples were treated following the usual methodology and specimens were mounted in polyvinyl-lactophenol. The animals and eggs were measured and eleven size-parameters were recorded in the specimens and three in the eggs. Besides, meristic and qualitative characters were recorded in the latter. Percentage ratios between the length of the structure considered and the buccal tube length \( pt \) were calculated. Univariate and multivariate analyses were performed on data from the two localities. Analysis of variance revealed significant population differences in \( pt \) of nearly all characters of the buccal apparatus. Conversely, the null hypothesis could not be rejected in the case of all claw and eggs parameters. Therefore, \( pt \) ratios of the buccopharyngeal apparatus may be suitable variables in order to detect levels of phenotypic variability among populations of tardigrades. Only characters showing significant variation among the cities were subject to multivariate analysis. With six characters, the PCA resolved most of the variation (>85%) in low multidimensional space (first three components). However, population segregation was poor. Further studies should be focused on whether the populations differences are due to genetic causes or phenotypic plasticity caused by environmental variations.
OSMOTIC STRESS TOLERANCE IN THE MARINE EUTARDIGRADE
HALOBIOTUS CRISPÆ

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The eutardigrade genus Halobiotus is secondarily adapted to the marine environment. The transition from freshwater to seawater must have required the possession of a large osmoregulatory capacity. Halobiotus crispæ Kristensen, 1982 colonize sublitoral habitats, often characterized by large fluctuations in salinity, making osmoregulation imperative to survival. The ability to cope with fluctuations in salinity might be adaptively correlated with the large size of the excretory organs, the Malpighian tubules, found in this genus. In H. crispæ these organs compose up to one third of the total body cavity, the size of the tubules, however, varying with the annual cycle of cyclomorphosis. In addition, the ability to cope with varying salinities seems to be related to the cyclomorphic stage.

In the present study we investigate osmotic stress tolerance in Halobiotus crispæ collected at Vellerup Vig, Denmark. The salinity at the sampling locality was 20‰. The limits of the osmoregulatory capacity of H. crispæ in the active stage were assessed by exposing groups of animals to gradual changes in salinity and concomitantly observing their behaviour. Using lack of mobility as the parameter of this limitation, our results demonstrated that H. crispæ is able to tolerate salinities ranging from 0‰ to 60‰ for longer periods of time, while still retaining some level of activity. A very similar situation was applicable to animals in the pseudosimplex 2 stage. When animals in the active stage were exposed to salinities from 60‰ to 80‰ they became inactive and reduced body volume significantly. Animals in the pseudosimplex 2 stage seemed less tolerant, becoming inactive around 40‰. Animals in both stages resumed activity following transfer to 20‰. Experimental protocols, in which animals were exposed to total desiccation, demonstrated a lack of ability to enter anhydrobiosis.

We are at present expanding these observations on osmotic stress tolerance in H. crispæ. Quantitative assessments of the osmoregulatory capacity are performed by evaluating changes in body volume following exposure to various salinities. Our preliminary results indicate that large volume changes may take place during exposure to very dilute as well as concentrated saltwater solutions. The time course of these volume changes will be investigated. Furthermore, we have extracted total protein from specimens exposed to salinities of 0‰ and 20‰. These samples will be used for investigations of potential changes in gene expression following osmotic stress.
IDENTIFICATION OF HETEROTARDIGRADA AND EUTARDIGRADA SPECIES USING 18S rDNA RESTRICTION SITE VARIATION

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In the last decades, the number of known tardigrade species has considerably increased to more than 960 species with new ones being discovered every year. However, the study of tardigrade species presents a general problem which is frequently encountered during the work with invertebrates: small size and remarkable degrees of phenotypic plasticity may sometimes not permit a definite identification of the species. In this investigation we have used riboprinting, a tool to study rDNA sequence variation, in order to distinguish tardigrade species from each other. The method combines a restriction site polymorphism approach of ribotyping with amplified DNAs. The 18S rDNA of different species of the families Echiniscidae, Macrobiotidae and Milnesiidae were sequenced and analysed together with 18S rDNA sequences of the families Echiniscidae, Macrobiotidae, Hybsibiidae, and Milnesiidae available on GenBank. On the basis of the obtained sequences, restriction fragment patterns can be predicted. We were able to show that the variation in positions and numbers of restriction sites obtained by standard restriction fragment analysis on agarose gels can be used successfully for taxonomic identification on different taxonomic levels. The simple restriction fragment analysis provides a fast and convenient method of molecular barcoding for species identification in tardigrades.
SUB-FREEZING TEMPERATURE TOLERANCE OF DIFFERENT SPECIES OF TARDIGRADES

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Tardigrades exhibit extraordinary tolerance to physical extremes, including low temperatures. Micro-environments, inhabited by tardigrades during winter, can be exposed to great daily temperature fluctuations, especially at unsheltered sites. These fluctuations depend on snow cover and absorption of solar radiation, resulting in exposure to freeze/thaw cycles. The survival rate after freezing to -30°C of the species *Echiniscus granulatus*, *Macrobiotus tonollii* and *Macrobiotus sapiens* was investigated. Specimens were exposed to five different cooling rates between +4°C and –30°C at 1°C/h, 3°C/h, 5°C/h, 8°C/h, and 10°C/h followed by a warming period at 10°C/h. In terms of mortality, *M. sapiens* was more sensitive to freezing than *E. granulatus* and *M. tonollii*. Cooling rates had strong effects on the rate of mortality. Lower cooling rates resulted in an increasing survival rate of the species *M. tonollii*. The highest survival was found at a cooling rate of 1°C/h. In contrast, *E. granulatus* showed a survival at a high level, independent from cooling rates. Therefore, survival of animals seems to be depending on cooling rates. Little is known about the survival mechanisms allowing tardigrades to persist in the face of extreme physical challenges. However, they have a variety of strategies that ensure survival in a harsh and variable environment. We need to more fully understand the conditions to which they are exposed during cold periods. Concurrently a more detailed study design is needed including a wider spectrum of natural rates of freezing and thawing.
DESICCATION TOLERANCE AND HEAT-SHOCK PROTEINS EXPRESSION IN ACTIVE AND DORMANT *AMPHIBOLUS VOLUBILUS* (EUTARDIGRADA)

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Invertebrates living in extreme habitats at high altitudes or latitudes, as those living in ephemeral habitats, must be able to survive environmental stresses such as desiccation and freezing. In this contest several important scientific issues are emerging. Organisms can have four responses to overcome environmental stresses: regulative, acclimation, developmental, and evolutionary responses. Regulative and acclimation responses are carried out by the organisms in short or very short time to maintain their internal conditions for appropriate optimal functioning. Both responses are reversible in such a way as to follow the environmental fluctuations. They are also measurable according to a space-time scale depending on the size and the length of the life cycles of the organisms.

Considering tardigrades, their persistence in unpredictable habitats is due to two widespread adaptive strategies, which are regulative responses: ability to enter cryptobiosis (both anhydrobiosis and cryobiosis) and/or to enter cyst. Anhydrobiosis and encystment are certainly characterized by several molecular events only partly identified. Other than the disaccharide trehalose, several stress proteins seem to be further keys to understand anhydrobiotic mechanisms. In particular, heat shock proteins (Hsps) and their molecular partners, which play diverse roles, including that of molecular chaperons, even in unstressed cells, in successful folding, assembly, intracellular localization, secretion and degradation of other proteins.

A moss-dwelling eutardigrade collected in Northern Apennines (Modena, Italy; 1700 m a.s.l.), *Amphibolus volubilis*, has been utilized in our lab to evaluate the survival strategies in unpredictable habitats. It is known that this species is able to enter anhydrobiosis and cryobiosis and to form cysts. Lab experiments on desiccation tolerance have been carried out considering three different values of air relative humidity (RH). Survival resulted directly related to the RH values, and high survival (about 80%) was obtained only with the highest RH value tested (85%).

Desiccated animals were utilized to evaluate the Hsp70 and Hsp90 expression by means of SDS-Page and Western blotting analysis. Hsp expression was also evaluated in active animals and (only Hsp70) in encysted (type 2 cyst) animals. Quantitative comparisons of protein expression have been made among these three conditions. The level of both Hsp70 and Hsp90 is higher in desiccated animals than in active ones. Encysted animals have a Hsp70 level lower than both active and desiccated animals. These results indicate that Hsp induction due to desiccation is necessary to preserve cells from desiccation damages. Instead, the lower Hsp level found in cysts with respect to active animals could be explained hypothesizing a little need of these proteins in animals already protected from environmental stresses by strong cuticular layers.
FIRST RECORD OF TARDIGRADES AT THE SINAI PENINSULA IN NORTHEASTERN EGYPT

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The mountains at the Sinai Peninsula in north-eastern Egypt and their extensions form a network of semi-isolated desert valleys (wadis) which create extreme ecosystems and habitats. Also a lot of research has been done on Sinai in the last decades, no research so far has focused on the tardigrade fauna, and information about species composition is missing. The aim of this study was to investigate if there are species of tardigrades present within the terrestrial environment and to determine the distribution of each species within the study site and their relative abundance. Three different areas in the north of the St. Katherine protectorate, near St. Katherine mountain were sampled. Each sampling location was recorded with a GPS tracking system and the altitude, exposure, humidity, species occurrence, number and life stage of the collected tardigrade, sample types and soil type with each sample were taken. Three different species of tardigrades have been found and collected exclusively from different moss at a higher altitude wadi systems (> 1500 m above sea level) in the St. Katherine Protectorate, South Sinai, Egypt. Samples contained the tardigrade species \textit{Echiniscus testudo} (Doyère 1840), \textit{Cornechiniscus lobatus} (Ramazzotti 1943), and \textit{Isohypsibius} sp. Further investigations in Egypt are needed to give a statement about diversity that is representative for the region.
TARDIGRADES FROM STELVIO NATIONAL PARK, CENTRAL ALPS (ITALY)

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Tardigrades have been collected on different habitats in an Alpine glacial valley (Val de la Mare, Stelvio National Park, Trentino, Italy), between 2200 and 2300 m a.s.l. Submerged mosses of streamlets (2 samples), turf (1 sample) and mosses on rocks (2 samples) have been considered. These habitats hosted a very rich and diversified tardigrade fauna. Particularly rich in tardigrades were the submerged mosses, which comprised 9 species of eutardigrades belonging to 3 different families and 8 genera. Worthy of note are the first record of Borealibius zetlandicus in Italy (species with a boreo-alpine geographic distribution) and the presence of two kinds of eggs in Murrayon pullari, situation reported only once in tardigrades. Within the turf, a poorly known habitat, we found only one species, a Macrobiotus belonging to the “hufelandi group”, certainly new to science. Mosses on rocks contain 8 species (three echiniscid heterotardigrades and 5 belonging to two families of eutardigrades), different from those found in the other two habitats; three of them (two Ramazzottius and one Hypsibius) are probably new to science. In total, 18 species have been identified, four of which new two science, one new to Italy and three new to the Trentino province. These results confirm the biogeographic meaning of many tardigrades and suggest the need for more specific studies on tardigrade distribution focused on high altitudes and glacial areas of the temperate zones, which should be considered as biogeographic islands.
THREE NEW SPECIES OF MACROBIOTUS
(MACROBIOTIDAE, TENUIS-GROUP)

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An illustrated description of *Macrobiotus voronkovi* sp. n., *Macrobiotus danilovi* sp. n. and *Macrobiotus tenuiformis* sp. n. from the moss cushions of Spitsbergen and Tien Shan (Kirghizia) is given. All new species belongs to the *tenuis*-group. *Macrobiotus danilovi* and *Macrobiotus tenuiformis* (Tien Shan) differs clearly from all known species of these group in details of buccal armature. *Macrobiotus voronkovi* (Spitsbergen) is most similar to *Macrobiotus mongolicus* Maucci, but differs clearly from this species in details of buccal armature and characters of the egg processes.
THE TARDIGRADES FROM SOME MOSSES OF SHENNONG FRAME STATE FOREST PARK IN THE WEST OF HUBEI PROVINCE, CHINA

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Shennong Frame is “the ridge of roof” in center China and its area is 3250 square Km in the west of Hubei Province. During the fourth season glacier, many species of animals and plants escaped death by sheer luck in this area. So Shennong Frame is channeled into “human and biosphere” protect region project by UNESCO in 1990 and it is a demonstrated region of biological diversity in Asia. Our knowledge of Tardigrada from this area was rather meager. Shennong Frame State Forest Park closes to the Muyu Town (31.4°N, 110.7°E) and includes Jinhou Ridge, Tiansheng Bridge, Shennong Altar, Xiangxi River Source and other sceneries. In June of 2005, I have collected more than 20 paper bags of mosses from above sceneries. Using sedimented method, I have gotten more tardigrade specimens from these mosses. The following known species were found: Echiniscus japonicus Morikawa, 1951; Pseudechiniscus facettalis Petersen, 1951; Milnesium tardigradum Doyère, 1840; Milnesium katarzynae Kaczmarek et al., 2004; Dactylobiatus aquatilis Yang, 1999; Macrobiotus terricola Mihelečič, 1949; Macrobiotus shennongensis Yang, 1999; Macrobiotus adelges Dastych, 1977; Macrobiotus richtersi Murray, 1911. Echiniscus bigranulatus Richters, 1907 were found in this region and is new record for China. It has 108.9 – 298.4 µm body length, filamentous lateral cirrus A of 48.4 – 72.6 µm, two granular sculptures on the dorsal plates, dentate collars on the fourth pair of legs, sickle claws with dagger spurs. Isohypsibius jinhouensis sp. nov. were found in Jinhou Ridge and differs from other described members of genus in having 338.8 – 423.5 µm body length, fine armores on the dorsal cuticle, large eyes, rectangular and oval macroplacoids, circular microplacoids, two double furcate claws of similar size and shape, concentric circle bars in wide claw bases and lunulae. So it is new for science.
BIOGEOGRAPHY AND ECOLOGY OF TERRESTRIAL TARDIGRADA IN THE GULF COAST STATES OF THE UNITED STATES OF AMERICA

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Our understanding of terrestrial tardigrade distributions in the states along the Gulf Coast of the United States continues to improve. Currently the number of species reported in publications or theses for the region is 44. Statewide surveys have been conducted in Alabama, Florida, and Texas, while sampling in Georgia, Louisiana, and Mississippi has been more localized. Currently, the number of known terrestrial tardigrade species stands at 18 in Texas, 15 in Louisiana, 9 in Mississippi, 36 in Alabama, 3 in Georgia, and 23 in Florida. These tardigrades have been collected from cryptogams (mosses, lichens, and liverworts) on trees or rocks, and from soil and leaf litters. Fourteen species or species-complexes are widely distributed in the region (found in ≥2 non-contiguous states), while 27 were found in only one state. Several species are unique to the Nearctic fauna, while others are cosmopolitan. Five species, widespread in the Gulf Coast states but unknown or rare elsewhere in the Nearctic – *Echiniscus kofordi*, *Echiniscus cavagnaroi*, *Echiniscus virginicus*, *Minibiotus fallax*, and an undescribed *Macrobiothus* cf. *hufelandi* – may represent a fauna distinctive of cryptogams in the southern United States. The available data suggest that tardigrade species richness in cryptogams and leaf litter in the region is lower than in other Nearctic areas. More work is needed to determine whether this lower diversity is real or an effect of insufficient sampling.
PRELIMINARY REPORT ON TARDIGRADA OF THE COSTA RICAN TROPICAL RAINFORESTS (CENTRAL AMERICA)

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1340 moss, lichen and algae samples were collected in December and January 2002/2003 in a variety of habitats in Costa Rica (Central America). Until present 320 (23.9%) samples were examined and only 182 (56.9%) contained tardigrades. From these samples, 3530 tardigrades and their eggs were extracted (19.4 specimens per sample on average). Forty Tardigrada species from 15 genera were identified but 7 of them were identified only to the genus level. One of the species, Doryphoribius quadrituberculatus was new for science and has been described in 2004. Next three species Calcarobiotus longinoii sp. nov., Macrobiotus huziori sp. nov. and Macrobiotus magdalenae sp. nov. are also new to science and the manuscripts with their descriptions are being reviewed or they are in press.
AN ELECTRONIC FIELD GUIDE AND KEY TO THE TARDIGRades OF THE GREAT SMOKY MOUNTAINS NATIONAL PARK, TN AND NC, USA

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Taxonomic keys to the tardigrades are almost exclusively text-based and non-pictorial. Additionally, they are scattered among obscure and difficult to find publications, and many are outdated due to the upsurge in publications of new species in the past 10 years. Updated, user-friendly, pictorial, web-based keys would be useful for people new to tardigrade taxonomy and experts alike. Several computer-based software programs now exist for developing pictorial, multichotomous (or non-linear) keys. We used Lucid 3.3 (www.lucidcentral.com) developed by the Centre for Biological Information Technology at the University of Queensland, Australia to develop a key for the tardigrades found during our inventory of the Great Smoky Mountains National Park. To date, this includes 70 species. In addition to the key itself, Lucid allows the attachment of photomicrographs, species accounts, and range maps, thus providing a complete electronic field guide. A laptop computer running our key and field guide will be available at the poster session. The poster will include complete instructions and some photographic enlargements of specimens to use with the software.
BRYOPHYTE HERBARIA AS A SOURCE OF TARDIGRADA

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Herbarium collections of mosses are a potential source of tardigrades. Although the probability of obtaining living tardigrades may not be high, good specimens can be extracted from such dried moss samples. The bryophyte collection of the Missouri Botanical Garden Herbarium was used as a source of Chinese tardigrades. Ten provinces plus Taiwan were represented in this project. The mosses were soaked in water approximately 24 hours, then the moss was removed and the sample was fixed and preserved. These samples were later examined, and slides were made of the tardigrades present. A total of 1,850 moss samples were examined; 113 of these yielded tardigrades. Over 25% of the samples from Xinjiang Province contained tardigrades, but the yield from other provinces was much lower, ranging from 0-17.4%. Moss samples containing soil or other particulate matter were more likely to contain tardigrades than clean moss samples were. Although the yield of tardigrades was low, tardigrades were obtained from areas of China in which tardigrade collecting has not been done. Although the monetary cost of such a project may seem high, it is competitive with the cost of field collecting in China.
The Tardigrade Reference Center is a web site being developed under a National Science Foundation grant and hosted by the Academy of Natural Sciences, Philadelphia for the purpose of collecting and making available the biology of tardigrades. The site will have two sides, one for education and one for science. The education side will be for the general public, student and teachers who want to learn about tardigrades, it will include “what is a tardigrade”, general descriptions of common species, and how to work with tardigrades. The science side is for advanced students and researchers and is based on the literature. It is built on a triad of data files: a taxonomic file, a publications file, and a geographic file. These three main files and their supporting files are cross linked such that they support mapping of tardigrades by location, by taxa, by document, by key word, or by author. Each species has a page with its history, and will soon include descriptions and images.
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