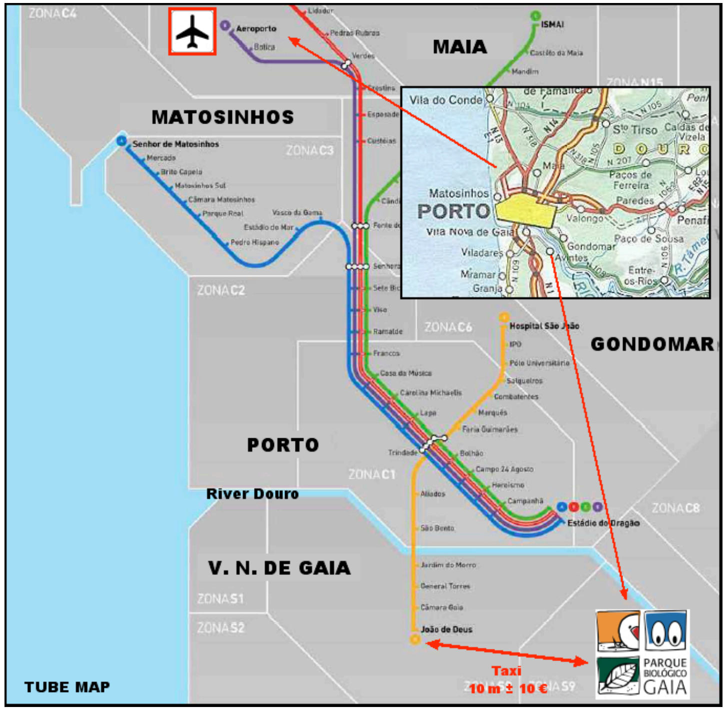

Conference Program and Abstract Booklet

12th

INTERNATIONAL SYMPOSIUM ON TARDIGRADA

V. N. Gaia | Portugal

23-26 July 2012

Co-financiamento:
ON.2 O NOVO NORTE
QUADRO DE REFERÊNCIA ESTRATÉGICO NACIONAL
UNIÃO EUROPEIA
Fundo Europeu de Desenvolvimento Regional
Município de V. N. Gaia
ÁGUAS E PARQUE BIOLÓGICO DE GAIA, EEM
U.PORTO
FACULDADE DE CIÊNCIAS UNIVERSIDADE DO PORTO

Conference Program and Abstract Booklet

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INTERNATIONAL SYMPOSIUM ON TARDIGRADA

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FICHA TÉCNICA

Titulo: 12th International Symposium on Tardigrada

Edição: Águas e Parque Biológico de Gaia, EEM / Faculdade de Ciências da Universidade do Porto

Coordenação: Paulo Fontoura, Nuno Gomes Oliveira

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WELCOME ADDRESS

Dear Colleagues

Tardigrada Symposia take place every three years and are unique opportunities for tardigrade scientific researchers, students and other tardigrade enthusiasts from all over the world to get together and to promote scientific exchange and also friendship.

It is with great satisfaction that we can announce the presence of a record number of attendants, proceeding from twenty one countries from all over the world, presenting their last findings on much diversified topics on tardigrade biology. The scientific quality of the meeting is also ensured by the contribution of some worldwide well known tardigradologists present.

In addition to the promotion of the scientific discussion and cooperation, symposia are events also providing the contact with new cultures and moments of diversion. We have done our best to honor the traditional hospitality and authenticity of the Portuguese people, offering you the opportunity to discover the charm, natural beauty and history of the region and to taste the wonderful Portuguese gastronomy.

We are very pleased to welcome you to the 12th International Symposium on Tardigrada.

Profit our Symposium and enjoy your stay in Vila Nova de Gaia.

Paulo Fontoura (Faculdade de Ciências da Universidade do Porto)
Nuno Gomes Oliveira (Parque Biológico de Gaia)
Alexandre Valente (Faculdade de Ciências da Universidade do Porto)
Cristina Neves (Parque Biológico de Gaia)

GENERAL INFORMATION

REGISTRATION

All the delegates can register at the registration desk at the Parque Biológico de Gaia from Monday, 23th July, open from 8:30.
The Registration Desk and the Secretary will be located in the Parque Biológico de Gaia. They will be open during the Symposium from 8:30 to 17:30. Please check regularly the information board at the Registration Desk during the Symposium for any change in the program, special announcements or other messages.
Important notice:
The Badge Identification is required for admission to all the Symposium sessions, activities and facilities.

PRESENTATIONS

Oral Presentations

All oral presentations will be conducted at the Auditorium of the Parque Biológico de Gaia. Oral presentations will be 15 minutes long, followed by a 5 minutes discussion period. 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, video, 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 (PowerPoint presentations are favored). Facilities for alternative presentation material may be available with advanced request. Please check the compatibility of your software/hardware prior to your presentation.

Poster presentations

Posters will be displayed in the Exhibition Room of the Parque Biológico de Gaia. Posters (dimension A0: 840 wide mm x 1189 mm high) will be attached to boards by means furnished by the organizers. Posters will be displayed for the whole period of the symposium (23th to 25th July). You are kindly requested to display your poster as soon as possible on the numbered board allocated to you (see poster program and the board number). Please do not remove your poster before the end of the poster session on Wednesday 25th. Delegates presenting posters should be next to their poster during the poster session.

LUNCH

Lunch (23^{th} to 25^{th} July) is included in the registration fee for all registrants (regular, student, accompanying person and children). Lunch will be served in the Self-Service Restaurant of the Parque Biológico de Gaia.

MICROSCOPY

During the Symposium a Zeiss microscope, kindly provided by Grupo Taper, will be available at the Parque Biológico de Gaia. Delegates interested in the examination of microscope slides should request this facility at any time at the Registration Desk.

CONFERENCE DINNER

The Conference Dinner will be held in the evening of 25^{th} July, at the Restaurant Parque da Aguda (Av. Gomes Guerra – Parque Municipal da Aguda, Arcozelo, Vila Nova de Gaia, 00 351 227 622 929). Details about the shuttle service for this event (departure and arrival time, meeting point…) will be posted at the Registration Desk.

SHUTTLE SERVICE

To reach the Parque Biológico a special shuttle service (included in the registration fee) from the Hotel/Parque Biológico de Gaia/Hotel will be available daily (see the timetable in the conference program). However, only hotels with a negotiated special rate are covered by this shuttle service. Delegates staying in other hotels are requested to arrange their own transport to/from the conference room. The Parque Biológico can be reached from Porto and Vila Nova de Gaia by metro and taxi. Delegates are recommended to use the Line D (Yellow Line), to *D. João II*, and then by taxi about 10 minutes) to the Parque Biológico (see the map available in this booklet).

Important notice 1: The meeting point for the shuttle hotel – Parque Biológico will be at the main door of the hotel. Delegates not on time should arrange their own transport to the conference room.

Important notice 2: During Sunday, 22^{nd} July 2012, delegates arriving by air will be offered a special shuttle service from the Airport Francisco Sá Carneiro to the Hotel (only hotels with negotiated rate are included in this service). The timetable of this special shuttle service will be arranged according to the arrival time of the delegates. The shuttle service may not be available for arrivals after 20:00.

Friday morning, 27th July 2012, the same special shuttle service will be available from the Hotel to the Airport. Delegates interested in this service should check the timetable at the symposium reception desk.

EXCURSION

All participants and accompanying guests are welcome to attend an all-day excursion (26st July). We will go by boat from Porto to Régua (breakfast and lunch on board) and we will return (expected time to arrive at Porto – 19:00, however time table can change) by train (or bus). Boarding at 8:00 am at Cais de Gaia "Cais da Rota do Douro". Shuttle departure time at the hotels covered by the shuttle service: 7.00 am.

The River Douro valley, rewarded in the 18th century by the Marquis of Pombal with the title of the first demarcated wine region in the world, is considered World Heritage by UNESCO since 2001. During this Cruise we will admire a beautiful landscape combining natural wonders, lovely rural scenarios, picturesque villages and vineyards shaped by human sweet.

For the excursion we strongly recommend the use of solar protection (e.g. hat, cap, sunscreen lotion).

PROCEEDINGS

The proceedings of the Symposium will be published in a special issue of the *Journal of Limnology* (http://www.jlimnol.it). The manuscripts of all topics of both oral and poster presentations will be considered for peer-review. However, it will be possible to submit only one manuscript per participant (or two with a collaborator who is also a registrant of the Symposium). Only manuscripts of high quality will be accepted for publication and authors may be requested to make substantial changes to comply with the standards of the Journal. The Guest Editors reserve the right to reject manuscripts of poor quality. All manuscripts must be submitted electronically (as a Microsoft Word document: doc, txt or rtf) no later than 30 August 2012. After that date manuscripts WILL NOT BE ACCEPTED. Manuscripts must be in English and must not have been published or accepted for publication elsewhere. Each manuscript must have no more than eight (8) printed pages, including abstract, introduction, methods, results, discussion, conclusions, acknowledgements, references and legends (As an example, eight pages correspond to about 26,000 characters, including blanks, plus two tables and two figures of medium size). Before submitting your manuscript, you are requested to consult the "Guide for the preparation of manuscripts", available in the website of the

Symposium (http://www.tardigrada2012.com) or in the Journal of Limnology (http://www.jlimnol.it) and confer with the Guest Editors if the guidelines are not strictly followed.

YOUNG SCIENTIST AWARDS

Presentations considered for the Young Scientist Awards will be evaluated by a Jury constituted by Senior Scientists and the winners will be chosen on the basis of the originality and quality of the work and of the presentation. The winners will be announced during the Conference Dinner.

CONFERENCE PROGRAM

Monday 23rd July

08:30 – Shuttle to the Conference Room

08.30 – 09:30 **Registration**

09:30 – 09:45 **OPENING CEREMONY**

09:45 – 10:15 **OPENING LECTURE:** L. REBECCHI (Italy)- Dry and survive: the role of the antioxidant metabolism in anhydrobiotic organisms.

10:15 – 10:45 Coffee break

TAXONOMY & PHYLOGENY

Session I. Chairpersons: R. BERTOLANI (Italy) & P. BARTELS (USA)

10:45 – 11:05 G. PILATO - Past, present and future of Eutardigrade taxonomy

11:05 – 11:25 P. FONTOURA, A. JØRGENSEN, R. M. KRISTENSEN & P. CENTENA – An illustrated dichotomous key to the genera of the marine heterotardigrades (Tardigrada)

11:25 – 11:45 Ł. MICHALCZYK & Ł. KACZMAREK - What should be the modern standards of tardigrade taxonomic descriptions?

11:45 – 12:05 R.M. KRISTENSEN, N. MØBJERG, S. FAURBY, D. PERSSON, K.A. HALBERG, A.W. NIELSEN, D. GRANN & A. JØRGENSEN - A new revision of the tidal genus *Echiniscoides* and its morphological variability (Heterotardigrada: Echiniscoidea)

12:05 – 12:25 N.J. MARLEY - What do you do with a problem like ~~Maria~~ Milnesiidae?

12:25 – 12-45 N. GUIL & A. MACHORDOM - The importance of being *Milnesium tardigradum*. Preliminary results about morphological and molecular variability among *Milnesium tardigradum*-like specimens

12:45 – 14:30 Lunch

Session II. Chairpersons: R.M. KRISTENSEN (Denmark) & R, GUIDETTI (Italy)

14:30 – 14:50 G. MAYER - Neural markers in onychophorans and tardigrades help unravel the phylogenetic position of Tardigrada

14:50 – 15:10 T. PRASATH, H. GREVEN, JOCHEN D'HAESE - EF-hand proteins in Tardigrades and Onychophorans
(Presentation submitted to the Young Scientist Awards)

15:10 – 15:30 M. CESARI, L. REBECCHI, R. GUIDETTI, I. GIOVANNINI, L. MORI & R. BERTOLANI - A DNA barcoding approach in the study of tardigrades

15:30 – 15:50 T. MARCHIORO, L. REBECCHI, J. GULDBERG HANSEN, R. BERTOLANI, & R. GUIDETTI – Comparative analysis and phylogenetic implications of tardigrade musculature architectures

15:50 – 16:10 Coffee break

16:10 – 16:30 F. BEMM, M. A. GROHME, M. FROHME, R. O. SCHILL, M. EBELING, R. SCHMUCKI, L. BURLEIGH, U. CERTA, T. DANDEKAR, J. SCHULTZ, F. FÖRSTER – The genome sequence of *Milnesium tardigradum* reveals a condensed genome and supports the tardigrades as sister taxon to the arthropods. (Presentation submitted to the Young Scientist Awards)

16:30 – 16:50 A. JØRGENSEN, S. FAURBY, D. PERSSON, K.A. HALBERG, R.M. KRISTENSEN & N. MØBJERG – Local and small scale genetic diversity in clonal lineages of the asexually reproducing tardigrade *Echiniscus testudo* (Heterotardigrada: Echiniscoidea)

17:00 – 19:30 **Visit to the Port Wine Cellars** (Caves Calém). The visit offers all the participants and accompanying persons the opportunity to taste this national world famous drink and to know how it is made and its course over the ages.

19:30 – Shuttle to the Hotel

Tuesday 24th July

8:30 – Shuttle to the Conference Room

BIOGEOGRAPHY, ECOLOGY & MORPHOLOGY

Session III. Chairpersons: W.R. MILLER (USA) & S. McINNES (UK)

09:00 – 09:20 B. P. L. RAMSAY – Tardigrada communities from the high-altitude wetlands in Volcan Chiles and El Angel, Carchi Province, Ecuador
(Presentation submitted to the Young Scientist Awards)

09:20 – 09:40 B. V. TRYGVADÓTTIR & R. M. KRISTENSEN - Eohypsibiidae (Eutardigrada, Tardigrada) and other tardigrade records from the Faroe Islands

09:40 - 10:00 V. INSHYNA – The structure of the Tardigrada communities in the “Cape Martyan” Nature Reserve (Crimean Peninsula, Ukraine)
(Presentation submitted to the Young Scientist Awards)

10:00 – 10:30 Coffee break

10:30 – 10:50 P.J. BARTELS & D.R. NELSON - An analysis of the distribution and diversity of soil tardigrades in the Great Smoky Mountains National Park (NC/TN, USA) using maximum entropy modelling

10:50 – 11:10 S. QUIROGA, M. CAICEDO, R. LONDOÑO, J. MAZENET, A. DAZA, Ł. KACZMAREK – Water bears (Tardigrada) from the lower Manzanares river basin, Santa Marta, Colombia

11:10 – 11:30 MICHALA BRYNDOVÁ, MILOSLAV DEVETTER – Long term effect of salvage logging on soil population of Tardigrada in mountain spruce forest in the Czech Republic (Presentation submitted to the Young Scientist Awards)

11:30 – 11:50 H.A. MEYER, J.G. HINTON & C. SAMLETZKA – Water Bears in the Anthropocene: A Comparison of Urban and Woodland Tardigrade Communities in Southwestern Louisiana, USA

11:50 – 12:10 S.J. McINNES & P.J.A. PUGH – The role of human activity in the distribution and population dynamic of soil Tardigrada on the maritime Antarctic Peninsula

12:10 – 12:30 Group photo

12:30 – 14:30 Lunch

Session IV. Chairpersons: H. GREVEN (Germany) & N. MØJBERG (Denmark)

14:30 – 14:50 M. DEVETTER & J. FROUZ – Primary succession of Tardigrade community on brown coal post – mining dumps.

14:50 – 15:10 E.J.C. NILSSON, K. I. JÖNSSON & J. PALLON – Element analysis of the eutardigrades *Richtersius coronifer* and *Milnesium tardigradum* using PIXE

15:10 – 15:30 C. SCHULZE & A. SCHMIDT-RHAESA – The brain architecture of *Echiniscus testudo* (Echiniscoidea, Heterotardigrada)

15:30 – 16:00 Coffee break

16:00 – 16:20 D.K. PERSSON, K.A. HALBERG, A. JØRGENSEN, N. MØBJERG, R.M. KRISTENSEN – Comparative morphology of the brain structure of Eutardigrada and Arthrotardigrada

(Presentation submitted to the Young Scientist Awards)

16:20 – 16:40 S. FUJIMOTO & K. MIYAZAKI – Musclular Architecture of Marine Heterotardigrades

(Presentation submitted to the Young Scientist Awards)

17:00 – 19:30 – **Porto Guided City Tour:** all the participants and accompanying persons are cordially invited to discover the charm and natural beauty of the city. We will do a panoramic bus trip through Gaia and Porto, to admire the river banks and bridges, the historical center and some of the most beautiful monuments. The trip will finish at the “Ribeira” (however, if they want, participants accommodated in hotels with a special price can be returned to their hotel), in the old city, a place full of typical restaurants and bars offering the opportunity to taste the wonderful Portuguese gastronomy and to admire the hospitality and authenticity of the people.

19:30 – Shuttle to the Hotel

Wednesday 25th July

08:30 – Shuttle to the Conference Room

PHYSIOLOGY, MOLECULAR BIOLOGY & OTHER TOPICS

Session V. Chairpersons: C.W. BEASLEY (USA) & R.O. SCHILL (Germany)

09:00 – 09:20 T. KUNIEDA, K. ARAKAWA, T. KATAYAMA, A. TOYODA, H. KUWAHARA, D.D. HORIKAWA, Y. SUZUKI, K. ABE, T. SHIN-I, K. OHISHI, A. MOTOYAMA, T. AIZU, Y. KOHARA AND A. FUJIYAMA – Genome annotations and transcriptomic analysis in *Ramazzottius varieornatus*

09:20 – 09:40 E. BELTRAN, R. BERMÚDEZ, K. I. JÖNSSON, A. WOJCIK, S .HAGHDOOST, M. HARMS-RINDAHL & J. BERNAL – Tardigrades: characterization of recombinase rad51, important protein of the DNA repair system
(Presentation submitted to the Young Scientist Awards)

09:40 – 10:00 K. I. JÖNSSON, E. BELTRAN, S. HAGHDOOST, A. WOJCIK & M. HARMS-RINGDAHL – Radiation tolerance of tardigrade eggs

10:00 – 10:20 T. ALTIERO, V. ROSSI, R. BERTOLANI, L. REBECCHI – Diversified bet-hedging strategy in the desiccation tolerant tardigrade *Paramacrobiotus richtersi*

10:20 – 10:50 Coffee break

Session VI. Chairpersons: T. KUNIEDA (Japan) & I. JÖNSSON (Sweden)

10:50 – 11:10 F. BEMM, F. FÖRSTER, T. DANDEKAR, J. SCHULTZ – Inside the genome of *Milnesium tardigradum*: LEA, Trehalose, and stress response proteins
(Presentation submitted to the Young Scientist Awards)

11:10 – 11:30 D. D. HORIKAWA, J. CUMBERS, D. ROGOFF, S. LEUKO, R. HARNOTO, K. ARAKAWA, T. KATAYAMA, A. TOYODA, T. KUNIEDA, L. J. ROTHSCHILD - UVC Radiation Tolerance in the Tardigrade *Ramazzottius varieornatus*

11:30 – 11:50 N. MØBJERG, K.A. HALBERG, D. PERSSON, R.M. KRISTENSEN & A. JØRGENSEN - On the current knowledge of osmoregulation in tardigrades

11:50 – 12:10 K. ARAKAWA, T. ITO, T. KUNIEDA, D. HORIKAWA, T. SOGA, & M. TOMITA - Metabolomics of tardigrade *Ramazzottius varieornatus* reveals dynamic metabolic response during anhydrobiosis

12:10 – 12:30 R. SCHUSTER & H. GREVEN - A 17-month study of the reproductive cycle of *Macrobiotus hufelandi* in a lawn moss with notes on reproduction of *Macrobiotus richtersi* and *Diphascon pingue* (Eutardigrada)

12:30 – 12:50 ATSUSHI C. SUZUKI, HIROSHI KAGOSHIMA & SATOSHI IMURA - Antarctic tardigrade culture from moss samples near Syowa Station, East Antarctica

12:50 – 14:30 Lunch

14:30 – 16:00 **POSTER SESSION**

16:00 – 16:30 – Coffee break

16:30 – 17:30 **CONCLUSIONS** – D. NELSON (USA) and **CLOSING CEREMONY**

17:30 – Shuttle to the coast; walk in the Park Dunes of Aguda (meeting point: parking of the Biological Park)

19:30 – Conference Dinner at Aguda

23:00 – Shuttle to the hotels (meeting point: parking of the Restaurant)

Thursday 26th July

07.00 – Shuttle from the hotel to Cais de Vila Nova de Gaia (Cais da Rota do Douro)

08:00 – 19:00 – **Boat Cruise on the River Douro:** Boarding at 8:00 at Cais de Vila Nova de Gaia (Cais da Rota do Douro). We will go by boat from Porto to Régua (breakfast and lunch on board) and return by train (expected time to arrive at Porto – 19:00, however timetable can change).

19:30 – Shuttle to the Hotel

ADDITIONAL PROGRAM FOR ACCOMPANYING PERSONS

Monday 23rd July

10:30 – 12:30 Guided visit to the Parque Biológico and its exhibitions

An opportunity to admire some fascinating secrets of the Portuguese fauna and flora and to get in touch with the old lovely rural architecture and traditions that explain the symbiosis between man and nature. **Meetingpoint** – Parque Biológico

Tuesday 24th July

09:00 – 12:30 Seaside of Vila Nova de Gaia and Museum Teixeira Lopes

A sightseeing tour at the seaside of Vila Nova de Gaia to admire the picturesque landscape, and some stunning beaches, not forgetting the fishing port of Afurada and the Local Nature Reserve of the Douro Estuary. An interesting visit of the Museum Teixeira Lopes (1866-1942), one of the most famous Portuguese sculptors, is also included in this event. **Meetingpoint** – Parque Biológico

POSTER PROGRAM

Taxonomy

1 - P. DEGMA - A new *Milnesium* species (Tardigrada, Eutardigrada, Milnesiidae) from Tanzania

2 - P. DEGMA & R.O. SCHILL - A new *Echiniscus* species (Tardigrada, Heterotardigrada, Echiniscidae) from the Alpi Marittime natural park (Maritime Alps Mts, NW Italy)

3 - YE.O. KIOSYA, R. BERTOLANI, L. REBECCHI, T. ALTIERO & M. CESARI - Systematic position of *Macrobiotus glebkai* within the "*hufelandi group*", based on morphology and molecular analysis of a population from Ukraine

4 - M. RUBAL, P. VEIGA, P. FONTOURA & I. SOUSA-PINTO - A new genus of an intertidal arthrotardigrade (Tardigrada: Heterotardigrada), from the North of Portugal (Atlantic Ocean)

5 - KAREL JANKO, JERZY SMYKLA, ŁUKASZ, MICHALCZYK & ŁUKASZ KACZMAREK - Tardigrades from the Antarctic Peninsula with a description of a new species of the genus *Ramajendas* (Hypsibiidae)

Biogeography and Ecology

6 - H.A. MEYER - Freshwater and Terrestrial Tardigrada of the Americas

7 - J.G. HINTON, H.A. MEYER, N.D. McDANIEL, C.B. BERGERON, S.J. KEELY & A.M. MATTE - The Tardigrada of Big Thicket National Preserve, Texas, USA: Final Results of an All Taxa Biological Inventory

8 - J.G. HINTON, H.A. MEYER, B.N. SOILEAU & A.P. DUPUIS - Tardigrada of Dominica, West Indies

9 - I. SATKAUSKIENE - A preliminary survey of tardigrades (Tardigrada) in Lithuania

10 - A. ROCHA & C. CLAPS - Preliminary analysis of urban tardigrades of Rafaela, a medium sized city in the province of Santa Fe (Argentina)

11 - DA ROCHA, C. M. C., SANTOS, E. C. L., GOMES JÚNIOR, E. L., GOMES, E. P. S., PUGAS, R. A. F. & LUZ, B. R. A. - Brazilian Marine Tardigrades

12 - G.T. GROTHMAN - Preliminary survey of tardigrades from Alberta and British Columbia, Canada

13 - K. ZAWIERUCHA, J. SMYKLA, J. CYTAN & Ł. KACZMAREK - Preliminary studies on the tardigrade fauna of the Arctic archipelago of Svalbard (Poster submitted to the Young Scientist Awards)

14 - R.O. SCHILL & P. DEGMA - Census of tardigrades in the French and Italian nature reserves Parc national du Mercantour and Parco Naturale delle Alpi Marittime

15 - P.J. BARTELS & D.R. NELSON - A summary of the large-scale inventory of tardigrades in the Great Smoky Mountains National Park (NC/TN, USA)

16 - ŁUKASZ KACZMAREK, ŁUKASZ MICHALCZYK & KRZYSZTOF ZAWIERUCHA - Costa Rican "bears" – what we do not know

17 - ŁUKASZ KACZMAREK, ŁUKASZ MICHALCZYK & BARTŁOMIEJ GOŁDYN - Invertebrates biodiversity of astatic waters of Costa Rica in relation to the hypothesis of "The Great American Biotic Interchange"

18 - J. SMYKLA, N. IAKOVENKO, D. PORAZINSKA, M. DEVETTER, K. JANKO & Ł. KACZMAREK - Diversity and abundance of tardigrades, nematodes and rotifers in different soil habitats of the Edmonson Point area (Northern Victoria Land, Continental Antarctica)

19 - YE.O. KIOSYA & N.YU. KHUDAEVA - Preliminary data on terrestrial tardigrade diversity and small-scale distribution in the park 'Yalivshchina' (Chernihiv, Ukraine). (Poster submitted to the Young Scientist Awards)

20 - F. VICENTE, M. CESARI, A. SERRANO, R. BERTOLANI - The impact of fire on terrestrial tardigrade biodiversity: a case-study from Portugal

Phylogeny

21 - AZUSA KUME, ATSUSHI C. SUZUKI & KOJI TOJO - A study into the dispersion pattern of *Milnesium* cf. *tardigradum*: A molecular phylogenetic approach

22 - R. BERTOLANI, M. CESARI, O. LISI, L. REBECCHI, I. GIOVANNINI & G. PILATO - Morphology, DNA barcoding and phylogeny of *Macrobiotus persimilis* and *Macrobiotus polonicus*

23 - S KOMINE, K., ARAKAWA,T. KUNIEDA, T. KATAYAMA, A. TOYODA & M. TOMITA - Identification of Hox genes in the draft genome sequence of *Ramazzottius varieornatus* (Poster submitted to the Young Scientist Awards)

24 - T. MARCHIORO, O. ROTA-STABELLI, L. REBECCHI, R. GUIDETTI, & D. PISANI - Dating tardigrade evolution and early terrestrialization events

25 - M. L. CHRISTOFFERSEN & C.M.L. ASSUNÇÃO - Tardigrades and emergent clades: a new framework

Morphology

26 - R. GUIDETTI, T. MARCHIORO, T. ALTIERO, R. BERTOLANI, & L. REBECCHI - Comparative analyses of the cuticular and muscular structures of the buccal-pharyngeal apparatus of tardigrades

27 - M.M. ROST-ROSZKOWSKA, I. POPRAWA, M. KSZUK-JENDRYSIK, M. MAREK-SWĘDZIOŁ, Ł. KACZMAREK - The fine structure of the midgut epithelium in *Xerobiotus pseudohufelandi* (Iharos, 1966) (Tardigrada, Eutardigrada, Macrobiotidae) with the special emphasis on midgut degeneration

28 - J. CYTAN, K. ZAWIERUCHA, J.Z. KOSICKI & Ł. KACZMAREK - Morphometric differentiation of Spitsbergen populations of *Macrobiotus islandicus islandicus* Richters, 1904 (Eutardigrada, Macrobiotidae). (Poster submitted to the Young Scientist Awards)

29 - V. GROSS & R. HOCHBERG - Bearly There: Differences in tardigrade muscular organization reflect locomotory adaptations to different habitats (Poster submitted to the Young Scientist Awards)

30 - K. A. HALBERG, D. PERSSON, A. JØRGENSEN, R. M. KRISTENSEN & N. MØBJERG - Comparative study on tardigrade myoanatomy: the use of muscular body plans in tardigrade phylogeny

Physiology and Molecular Biology

31 - T. HASHIMOTO, Y. SAITO, M. OYAMA, H. HATA, H. KUWAHARA, D. D. HORIKAWA, A. TOYODA, T. KATAYAMA, K. ARAKAWA, A. FUJIYAMA, T. KUBO & T. KUNIEDA - Proteomic analysis of chromatin fraction in an anhydrobiotic tardigrade, *Ramazzottius varieornatus* (Poster submitted to the Young Scientist Awards)

32 - S. TANAKA, A. YAMAGUCHI, S. YAMAGUCHI, H. KUWAHARA, C. TAKAMURA, S. OHMI, D. D. HORIKAWA, A. TOYODA, T. KATAYAMA, K. ARAKAWA, A. FUJIYAMA, T. KUBO & T. KUNIEDA - Two families of novel abundant heat-soluble proteins in an anhydrobiotic tardigrade. (Poster submitted to the Young Scientist Awards)

33 - L. REBECCHI, T. ALTIERO, A.M. RIZZO, M. CESARI, G. MONTORFANO, T. MARCHIORO, R. BERTOLANI, R. GUIDETTI - Two tardigrade species on board of the STS-134 space flight

34 - R.O. SCHILL, B. POST & B. SATTLER - Freezing tolerance in the cryoconital tardigrade *Hypsibius klebelsbergi*

35 - A. BONIFACIO, R. GUIDETTI, T. ALTIERO, V. SERGO & L. REBECCHI - Raman imaging study on living tardigrades: origin, nature and function of pigments in *Echiniscus blumi*

36 - MARKUS A. GROHME, BRAHIM MALI, WERONIKA WEŁNICZ, STEPHANIE MICHEL, RALPH O. SCHILL & MARCUS FROHME - Aquaporin channel proteins in the tardigrade *Milnesium tardigradum*

Other topics

37 - R.O. SCHILL & S. HOOS - Life-history traits in the tardigrade species *Paramacrobiotus kenianus* and *Paramacrobiotus palaui*

38 - CZERNEKOVÁ MICHAELA & DEVETTER MILOSLAV - Comparison of different methods for quantitative extraction and sample handling of soil Tardigrada (Poster submitted to the Young Scientist Awards)

39 - WILLIAM R. MILLER & CLARK W. BEASLEY - The Tardigrade Reference Center

40 - T. TISCHER & C. SCHULZE - The heat is on - different staining protocols for tardigrades (Poster submitted to the Young Scientist Awards)

ABSTRACTS

OPENING LECTURE

Dry and survive: the role of the antioxidant metabolism in anhydrobiotic organisms

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Although evolution of life has turned oxygen into a vital compound for most organisms, this element can also have deleterious effects on living systems. Oxidative stress is a process resulting from an imbalance between excessive production of reactive oxygen species (ROS) and limited action of antioxidant defenses. It is a particularly harmful health risk factor, common to the development of several chronic human pathologies (e.g. cancer, Alzheimer's and Parkinson's) and believed to play a major role in the ageing process. Thus antioxidant protection is essential for survival under an aerobic environment.

Water too is essential for life, but some organisms have the ability to survive extreme desiccation by entering into a state of suspended animation called anhydrobiosis. These organisms are widespread throughout nature, including bacteria, protists, yeasts, plants and animals. The loss of water involves important biological processes such as changes in metabolism, alterations of cell membranes, and production of oxidative stress. Therefore, the maintenance of life in the absence of water requires a complex set of mechanisms working in close coordination, such as the accumulation of bioprotectant molecules, the activation of molecular repair mechanisms and of antioxidant and molecular chaperone systems.

Oxidative stress seems to be one of the most deleterious effects of water depletion, since the susceptibility to oxidative damage may increase with dehydration. Anhydrobiotes seem to apply two main strategies to cope with the danger of oxygen toxicity, namely an increasing efficiency of antioxidant defences and a metabolic control of both energy-production and energy-consuming processes. Tardigrades are here presented as model system to evaluate the effective damages induced by an increase of ROS production during desiccation and to understand the role of antioxidant systems to ensure survival of living beings when in the anhydrobiotic state.

Even though desiccation does not seem to have an effect on tardigrade longevity, damages are accumulated in proportion to the time spent in the desiccated state, leading to animal death. High temperatures, high humidity and high oxygen partial pressure are all factors that negatively affect tardigrade survival during long-term anhydrobiosis since they are involved in the production of oxidative stress. These abiotic conditions also directly influence the time required by animals to recover active life after a period of desiccation. Experimental studies produced evidence that enzymes (e.g. peroxidases, catalases, superoxide dismutase) and antioxidants (e.g. glutathione and carotenoids) represent a key group of molecules required for desiccation tolerance in tardigrades. The action of these molecules emphasises the need for redox balancing in anhydrobiotic tardigrades.

ORAL PRESENTATIONS

Past, present and future of Eutardigrade taxonomy

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The Author recalled the past of eutardigrade taxonomy and indicated the main facts that for a long time restrained its progress: many descriptions were short and defective; traditionally, only some characters were taken into consideration while others were not considered; often, permanent slides were not mounted; the type material of many species got lost; some authors, in the descriptions of new species, stressed the characters for which a species differed from the known ones and overlooked the other characters, this making difficult afterwards to recognize new species different for the overlooked characters.

As a consequence, a very wide individual variability has been erroneously attributed to many species and this has become the main problem for tardigradologists. This traditional mistake has been transferred into Marcus's (1928, 1936), Ramazzotti's (1962, 1972) and, partially, also into Ramazzotti & Maucci's monograph of 1983. Those authors scrupulously reported for each species the notices present in the literature with the impossibility to verify personally whether they were correct or not. Those monographs, certainly very useful, constituted for decennia an important landmark for tardigrade taxonomy which in this way remained based on schemes of the past.

Since 1969 the situations began to change because of the first attempts to eliminate the above mentioned problems. Characters before overlooked began to be taken into consideration and exchanges of slides and notices between tardigradologists become more frequent. This allowed to compare different opinions about most species and to eliminate many misunderstandings. Above all, it appeared evident that the individual variability in eutardigrades is not very wide but, on the contrary, very narrow. These novelties gave impulse to the taxonomy and renewed it, and the actual systematic, finally having phylogenetic base, became popular. The new evaluation of the characters had also consequences on the heterotardigrade taxonomy.

Today, researches also on DNA sequences are more and more in fashion; they are surely useful and allow to distinguish species morphologically very similar, today even not distinguished one from the another; they also will help to reconstruct the phylogeny of a taxon. However, in the author's opinion, this progress will be possible only if the molecular studies are always associated to careful morphological studies, which, in the meantime, will be more and more detailed also thanks to the use of the S.E.M.

An illustrated dichotomous key to the genera of the marine heterotardigrades (Tardigrada)

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The interest devoted to marine tardigrades is low when compared with the efforts given their limnoterrestrial relatives. Actually, the knowledge of the diversity, ecology and geographical distribution of marine tardigrades is still poor. Only about 160 species of marine tardigrades have currently been described which represent less than 15 percent of all known tardigrade species. Nevertheless, marine tardigrades are present in all oceans, ranging from the intertidal zone to abyssal depths, inhabiting a great diversity of sediments from fine mud to coarse sediments, rocks and algae. The small size of marine tardigrades, the low number of specimens frequently found and the need of specialized methods of study, such as sampling procedures, are some of the difficulties responsible for the paucity of knowledge regarding their biology. On the other hand, an accurate identification of the specimens at the species level is a task restricted to experts.

The main goal of the present work is to make available an easy to use dichotomous key to be used not only by experts but especially by all those interested in marine fauna with a basic knowledge in Biology. It is expected that the key can be a useful tool for an easy identification of the different genera of marine tardigrades and, by this reason, a source of motivation for marine researchers, and thereby contributing to trigger new studies about these intriguing organisms.

The morphological characters used to discriminate the 41 currently known genera of marine heterotardigrades and consequently to design the key were carefully chosen. A special concern has been devoted to the selection of the more conspicuous discriminating characters. In order to facilitate an easy and objective identification, more than one discriminating character was used, whenever possible, at each step in the key. In addition, all the details referring to the discriminating characters were illustrated, according to the original descriptions. To increase the probability of correct identifications, diagnosis of the orders, families, subfamilies and genera of the marine heterotardigrades were updated.

What should be the modern standards of tardigrade taxonomic descriptions?

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This talk is addressed to every one involved in the process of publishing tardigrade taxonomic descriptions, i.e. authors, reviewers and journal editors. We would like to put forward a set of unified standards for descriptions and redescriptions of tardigrade taxa and, most importantly, invite the community of tardigradologists to a thorough discussion on the subject. As a result we hope to agree on recommendations that would facilitate not only comprehensive descriptions but should also make it easier to compare taxonomic accounts written by different authors. We are convinced that such action is long overdue and strongly needed in order to standardise both the type and form of information provided in modern tardigrade taxonomic descriptions.

We will describe morphometric methods (including relative indices) available from tardigrade literature, discuss their importance and propose sets of methods for specific tardigrade taxa. Furthermore, we will list most important original papers where methods are described and illustrated. We will also advocate a unification of morphological and morphometric terminology in tardigrade taxonomy. Finally, we will try to list most common mistakes and things that are frequently omitted or overlooked in taxonomic descriptions as well as provide more general guidelines to improve the quality of taxonomic papers (e.g. regarding graphic illustration, which is often unfoundedly neglected).

A new revision of the tidal genus *Echiniscoides* and its morphological variability (Heterotardigrada: Echiniscoidea)

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The marine family Echiniscoididae was established in 1980 (Kristensen and Hallas, 1980) comprising the genera *Anisonyches* Pollock, 1975 and *Echiniscoides* Plate, 1889; however, the family is polyphyletic. The *Anisonyches* with reduced or small median cirrus should be removed to Arthrotardigrada close to the subfamily Archechiniscinae in the family Halechiniscidae. Currently Echiniscoides consists of 8 species and 7 subspecies of E. *sigismundi. Echiniscoides* has been found on different substrates: barnacles, polychaete tubes, algae, lichen and interstitial in sand. Especially the interstitial forms (*E. higginsi*-group) with isonych claws (same numbers and type of claws on each leg) and with pillars in the epicuticle differ a lot from other members of the genus *Echiniscoides* morphologically. Therefore, we suggest a new genus for the *E. higginsi*-group. This group seems to be incapable of entering cryptobiosis as the tidal form of *Echiniscoides*, and it might be the sister-group to all other *Echiniscoides*, where the pillars in the epicuticle are not present.

Recently, DNA was extracted from 465 specimens of the genus *Echiniscoides* from 48 localities world-wide (Faurby et al. 2012). The ectoparasitic *E. hoepneri*, previously described from barnacles (W. Greenland), was recovered from *Semibalanus balanoides* from Island and Faroe Islands, but the genetic and morphological variations were low. This is in strong contrast to morphospecies of the type species *Echiniscoides sigismundi*, which had high genetic variation and consist of at least two cryptic species.

Our results show that a major taxonomic revision of the genus *Echiniscoides* is needed, and support our view that most subspecies of *E. sigismundi* should be evaluated to species level. The Easter Island form of *Echiniscoides* with up to 12-13 claws on each legs has a hairy cuticle not seen in other *E. sigismundi* subspecies. Therefore we suggest that this is also a new species.

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What do you do with a problem like ~~Maria~~ Milnesiidae?

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Milnesium tardigradum Doyère, 1840, was one of the first species of tardigrade to be described. It is also one of the most cited tardigrade species, having been reported in most terrestrial habitats, from the tops of the highest mountains down to sea level. However it is now believed that the majority of these reports refer to sibling (cryptic) species and not to *M. tardigradum s.str*.

I will summarise my initial work that reported on a global survey of specimens of Milnesiidae, and present additional data comprising and utilizing molecular data that had not been accessible when this research started. Also highlighted are some of the perceived relative values for different morphological characters of the buccal apparatus and the claws. Similar discussions on these had been previously aired for Parachela but had been somewhat side-stepped for the Apochela.

Exploiting a global dataset of specimens, the biogeography of the seven Milnesiidae genera will be discussed, together with an hypothesis for the Gondwanan origin for the Apochela.

Finally, the statuses for a number of Milnesiidae taxa are re-evaluated and an open question placed before the international symposium on the proposed suppression of one recently described taxon.

The importance of being *Milnesium tardigradum*. Preliminary results about morphological and molecular variability among *Milnesium tardigradum*-like specimens

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Milnesium tardigradum has been recently re-described using more morphological information than in the original description (Doyère, 1840), and molecular information (COI -cytochrome oxidase I-, and ITS2 -internal transcribed spacer 2-). An intensive field work in three mountain systems located in the centre of the Iberian Peninsula (*Sistema Central, Montes de Toledo, and Serranía de Cuenca*), collecting several habitats and substratums, has provided us numerous *Milnesium* populations with specimens with *tardigradum* appearance. So, we had the opportunity to study the suspected cryptic speciation among specimens, which morphologies resemble *Milnesium tardigradum*, with morphological and molecular information obtained individually, and compare them to the newly described *Milnesium tardigradum* neotype and paratypes.
Morphological characteristics from the buccopharyngeal apparatus, the claws and the cuticle have been analysed and photographed. Besides, fragments from four genes have been sequenced individually, three nuclear (18S rRNA, 28S rRNA and ITS2), and one mitochondrial (COI). Relationships among different *Milnesium* specimens found and with the re-described *Milnesium tardigradum* have been analysed using molecular information with different approaches (maximum likelihood, maximum parsimony, and Bayesian inference). Moreover, levels of connectivity among mountain systems and habitats and substratums, as well as possible morphological traits describing different clades will be evaluated and discussed, due to huge differentiation found among lineages, especially with ITS2 variability even within some individuals.

Neural markers in onychophorans and tardigrades help unravel the phylogenetic position of Tardigrada

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Onychophora (velvet worms) and Tardigrada (water bears) are key groups for understanding the evolution of arthropods, but their exact phylogenetic relationship remains unresolved. In particular, the position of tardigrades is controversial since in different studies they are regarded as the sister group of Onychophora, Arthropoda, Onychophora plus Arthropoda, or Nematoda and allies. Extant onychophorans and tardigrades share some ancestral features with Cambrian fossils called lobopodians, such as the unjointed limbs and the soft body (missing exoskeleton). Notably, segmentation of the body is less advanced in these animals as compared to extant arthropods. Hence, analysing the features associated with body segmentation, in particular the nervous system, in onychophorans and tardigrades may play an important role for resolving their relationships. In this talk, I present new neuroanatomical data from onychophorans and tardigrades and discuss their implications for the phylogenetic position of Tardigrada.

EF-hand proteins in Tardigrades and Onychophorans

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Recently we have determined and analyzed the complete cDNA sequences of proteins of the EF--hand superfamily (calmodulin, troponin C, essential myosin light chain and regulatory myosin light chain) of the eutardigrade *Hypsibius klebelsbergi*. These proteins are typified by a helix-loop--helix motif and trigger the actin–myosin interaction in muscle tissue by coordinative binding of calcium ions. We have compared our data with corresponding data available in GenBank covering a wide spectrum of organisms (Prasath et al. 2012). Concerning the most recent molecular data, which support the view that the ecdysozoan taxa Tardigrada, Onychophora and Arthropoda belong to the clade Panarthropoda and that tardigrades are the sistergroup of Onychophora + Arthropoda, and the fact that EF-hand protein sequence data for onychophorans practically do not exist in GenBank, we have analyzed the same set of proteins in the velvet worm *Peripatoides novaezealandiae* (Onychophora). Our current sequence data show that within Ecdysozoa the sequences are most similar between onychophorans and tardigrades. Although such data might primarily reflect the evolution of these proteins rather than phylogenetic relationships, we think that they might be useful within a given taxon as shown here for Ecdysozoa.

A DNA barcoding approach in the study of tardigrades

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DNA barcoding is a technique proposed by Hebert and coworkers in 2003 and it aims to discriminate biological entities through analysis of a single gene barcode locus. The DNA barcoding system promised a better taxonomic resolution than that achieved through morphological studies, with a partial solution to the decline in taxonomic knowledge. Today DNA barcoding is a global enterprise, and the implementation of the idea has seen a rapid rise (more than 450 papers published to date on different organisms). Nonetheless, controversy still arises regarding barcoding and taxonomy. It is important to note that DNA barcoding does not focus on building a tree-of-life or on doing DNA taxonomy, even though sometimes it has been used for these purposes. DNA barcoding rather focuses on producing a universal molecular identification key based on strong taxonomic knowledge that should be included in the barcode reference library.

In Phylum Tardigrada, DNA barcoding represents a recent approach to species identification and for helping to solve taxonomic problems, especially considering the diminutive size of these animals and the paucity of morphological characters useful for taxonomy. In the framework of the MoDNA Project (Morphology and DNA), carried out by our research group in collaboration with several colleagues, we are combining the study of a fragment of the mitochondrial cytochrome c oxidase subunit I gene (*cox1*) with morphological data, in a wide sense, to form an integrative taxonomy of tardigrades.

Building of a database of reference sequences is of paramount importance for a correct application of DNA barcoding in tardigrades. Without verified reference sequences from voucher specimens that have been authenticated by qualified taxonomists, there is no reliable library for newly generated sequences with which to be compared. Methods and protocols for standardized results are focused on obtaining tight correspondence between molecular sequence and animal morphology, possibly both LM and SEM images (and egg shell morphology, when useful). This approach is particularly useful in describing new species, and important when applied on material collected in type localities.

Results using this approach are presented, focusing primarily on a number of species from the so--called "*Macrobiotus hufelandi group*".

Comparative analysis and phylogenetic implications of tardigrade musculature architectures

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Most knowledge on tardigrade musculature architecture dates back to the end of XIX century, and the beginning of XX century. It has been only in the last five years that a great deal of new information on tardigrade musculature system has become available, mainly thanks to the use of rhodamine-phalloidin staining of F-actin in combination with three-dimensional microscopical techniques such as confocal laser scanning microscopy (CLSM). In spite of all these information, only few and fragmentary evolutionary considerations on tardigrade musculature system have been done. This is probably due to the relatively low number of analyzed taxa, and to the difficulty in the comparisons of data that often have been obtained with different degree of accuracy, and are presented using different terminologies.

In this study we increased the number of analyzed species, by studying CLSM the musculature architectures of 7 species representative of most tardigrade higher taxa: the heterotardigrades *Batillipes bullacaudatus* (Arthrotardigrada), and *Echiniscus testudo* (Echiniscoidea), and the eutardigrades *Paramacrobiotus richtersi, Dactylobiotus parthenogeneticus* (Parachela, Macrobiotoidea), *Bertolanius volubilis* (Parachela, Eohypsibioidea), *Acutuncus antarcticus* (Parachela, Hypsibioidea) and *Milnesium tardigradum* (Apochela, Milnesiidae). We were able to define all the muscular fibers associated with the body movement. The number of fibers and their organization changed among taxa, with heterotardigrades being the least complex. Muscular fibers have been schematically organized into three systems: dorsal, lateral, and ventral. The ventral system was the most conservative, showing a clear metameric pattern and only few differences among taxa, while the lateral system was the most derived and so precise homologies were not always well defined.

Using these new morphological information and literature data on *Halobiotus crispae* (Parachela, Isohypsibioidea), it was possible to analyse the phylogenetic signal of the musculature system. Two matrixes were constructed: a morphological matrix of 94 characters based on musculature data, and a matrix for a total evidence analysis combining the previous data with molecular data (18S, 28S). Both matrixes have been analyzed in Bayesian and parsimony frameworks. The phylogenetic trees, obtained by both analyses using both matrixes, differ only for the position of Eohypsibioidea. Heterotardigrade taxa were the sister group of all Eutardigrada, within this last class, Apochela and Parachela were sister taxa; among parachelan superfamilies the Isohypsibioidea was the most basal, the Macrobiotoidea the most derived, while the Eohypsibioidea changed position according to the analyses. Our data demonstrated that musculature architecture can be used for phylogenetic purposes, but it is only applicable at higher taxonomic levels. Indeed, the musculature of *Paramacrobiotus* and *Dactylobiotus*, although belonging to different families of Macrobiotoidea, showed the same muscular organization.

The genome sequence of *Milnesium tardigradum* reveals a condensed genome and supports the tardigrades as sister taxon to the arthropods

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With their exceptional physiological features, tardigrades are unique throughout the metazoa. Since these features are encoded within the genome, questions about their genetic basis and evolution could be answered with a genome at hand. Therefore, we have sequenced *Milnesium tardigradum* Doyère 1840 using 454 technology. A set of 4.5 million reads was assembled into a genome draft. It has a total consensus length of approximately 120 Mbp, a N50 of 26 kbp and a number of 38,023 contigs. As a first step, genomic features like repeats, low complexity regions and transposable elements were annotated. With these features *M. tardigradum* is significantly underrepresented, when compared to other metazoan genomes and it indicates an excessively condensed genome.

Based on the genome, the gene complement of *M. tardigradum* was annotated using an iterative evidence based gene annotation pipeline. To allow high quality annotations, two EST data sets were assembled from 5.5 million reads and used as evidence. As these covered different life cycle stages, most transcripts should be present in the data sets. We identified 16,061 genes of which 1,093 transcribed more than one splice variant, in total. To get a first glimpse of the encoded functionality, protein families and domains were identified using Interpro, functional classifications predicted using Blast2GO and enzymes searched using PRIAM and Iprscan. The results allowed us to obtain functional information for more than 90 % of the encoded genes and will enable researchers to establish *M. tardigradum* as experimental platform for postgenomics in the nearest future.

Additionally, with the complete set of coding genes at hand, the genome provides the possibility for phylogenomic analysis for the first time. We identified one-to-one orthologs of 18 metazoan species covering Arthropoda, Nematoda, a Cnidaria and *Trichoplax adherens*. A following maximum likelihood based supertree approach placed *M. tardigradum* as sister group to the Arthropoda with the Nematoda as outgroup, consistently. Thus, our phylogenomic approach further corroborates previous studies based on single or few selected markers.

On the road to a molecular characterization of the exceptional physiology of tardigrades, the genome is the first step towards a better understanding of the whole phylum. But obviously, one genome is not sufficient to characterize the whole phylum in detail. We therefore envision the sequencing of additional genomes to unravel key steps in the evolution of tardigrades with comparative genomics approaches.

Local and small scale genetic diversity in clonal lineages of the asexually reproducing tardigrade *Echiniscus testudo* (Heterotardigrada: Echiniscoidea)

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Echiniscus testudo (Echiniscoidea: Echiniscidae) belongs to a large cosmopolitan genus of terrestrial tardigrades comprising 150+ species. It is a common tardigrade in mosses in the temperate part of the Northern Hemisphere, and exhibits a high desiccation tolerance.

Genetic variation in microscopic animals living in larger water bodies is fairly well studied especially with regard to rotifers (*Brachionus*) and crustaceans (*Daphnia*). However, the genetic structure of microscopic animals from mosses and lichens is still largely unknown. A few studies have dealt with geographic variation in tardigrades from mosses and barnacles on a large geographical scale (Jørgensen et al. 2007; Faurby et al. 2008, 2011), and the intrapopulation or local clonal lineage variation (Guil & Giribet 2009). Interpreting the results of phylogeographic investigations of clonal organisms on the assumption of neutrality is conceptually difficult, or perhaps even impossible, since all markers are totally linked for exclusively clonal organisms.

Jørgensen et al. (2007) reported a maximum of 1.28 % sequence variation in COI haplotypes between clonal lineages covering a large geographical area. The present study investigates the COI sequence variation and haplotype diversity of individual *Echiniscus testudo* at three Danish localities focusing on local (80 m apart) and small geographic scales (186 km apart). The 185 COI sequences constituted 14 COI haplotypes, and only three of the 11 haplotypes (Et2, Et3 and Et9) reported in Jørgensen et al. (2007) were recovered. The average COI sequence diversity was 0.5%, ranging between 0-3.5%, with haplotype Et21 accounting for the high end value. The second most variable haplotypes (Et14, Et15, Et17 and Et19) showed a diversity of 1.7% to the other sequences. Our data could indicate an ecological restriction of some haplotypes based on sun exposure, and raises the possibility that common, apparently generalized species such as E. testudo could contain lineages with much more restricted habitats (non-neutral distribution). Furthermore, the current study emphasizes that numerous sequences from single specimens are needed to describe the local genetic diversity, especially within species evolving in clonal lineages.

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Tardigrada communities from the high-altitude wetlands in Volcan Chiles and El Angel, Carchi Province, Ecuador

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Little information has been published about the high-altitude Tardigrada communities of Ecuador. This is the first study of wetland areas in Ecuador. The study presents results from three wetlands in two sites at three different altitudes 3676, 3856 and 4249m in Volcan Chiles and El Angel, Carchi Province, North of Ecuador. Representative samples of bryophytes and lichens were collected from different parts of these wetlands, and transported to Plymouth University in a preserved, dry state. Characteristics of the samples, including dry weight and indicators of structural complexity, were noted. An attempt was made to extract and identify all Tardigrada, eggs and exuvia from the samples, by detailed searching. Some interesting taxa were discovered. The community-level data were analysed using multivariate techniques to characterize the Tardigrada communities associated with wetlands.

Eohypsibiidae (Eutardigrada, Tardigrada) and other tardigrade records from the Faroe Islands

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The Faroe Islands archipelago is a group of 18 small islands situated in the Northeast Atlantic Ocean between Iceland and the Shetland Islands. During a zoogeographic investigation of the limnoterrestrial tardigrade fauna of the islands in 2001-2004, three new species were discovered in a new genus, *Austeruseus faeroensis, A. balduri and A. rokuri*, belonging to the family *Eohypsibiidae, Eutardigrada*. The new genus was found in mosses and lichens primarily in high altitudes up to 726 m a.s.l. and differentiates from Eohypsibius and Bertolanius in the apophyses of the buccal tube. Amongst other findings the investigation also showed a first record of *Bertolanius weglarskae* and a new record of *Eohypsibius nadjae*. So far the tardigrade fauna on the Faroe Islands is the richest in the world with regard to the family Eohypsibiidae.

In all 49 species were sampled whereof 34 are new records. The tardigrade fauna on the Faroe Islands represents members from both classes of the Tardigrada: Heterotardigrada and Eutardigrada. There are two families of Heterotardigrada represented i.e. Echiniscoididae and Echiniscidae. Within the Eutardigrada there are 6 families represented i.e. Milnesiidae, Eohypsibiidae, Calohypsibiidae, Hypsibiidae, Macrobiotidae and Murrayidae.

The central position of the Faroe Islands in the middle of the Northatlantic Ocean makes an investigation of the tardigrade fauna very interesting in relation to the surrounding Islands and countries. A faunistic comparison was done with Iceland, Svalbard Islands, Disko Island (Greenland), Greenland (West- and East Greenland excl. Disko), Scotland, England and with Newfoundland as an out group. On the basis of presence/absence data of the tardigrade species from the different countries, a cluster analysis was computed based on the Bray-Curtis similarities and a dendrogram based on group-average linking was constructed. The interesting results showed that the Faroese tardigrade fauna is mostly similar with Iceland, which could be expected, but is also very similar to the tardigrade fauna on Disko Island (Greenland).

The structure of the Tardigrada communities in the “Cape Martyan” Nature Reserve (Crimean Peninsula, Ukraine)

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Water bears (Tardigrada) of Crimean Peninsula are very poorly known and 50 species were reported from this region. In present studies moss and lichen samples were collected in “Cape Martyan” Nature Reserve (Crimean Peninsula; Ukraine). “Cape Martyan” N. R. is the smallest National Reserve on Ukraine and it protects part of the relict of the Mediterranean type forests.

76 moss and lichen samples were collected from different types of forest habitats during the dry season. All samples were characterized with the details like: moisture, altitude above ground level, elevation and plant habitat.

All tardigrades their eggs and exuvia were carefully extracted and counted. In the studying material two new species for Crimean Peninsula were found.

The percentage of positive samples, numbers of specimens per sample, co-occurrence of tardigrade species, and tardigrade and plant species relationships were studied.

All the data were analyzed using multivariate techniques to characterize the Tardigrada communities associated with different types of habitats as well as different types of inhabiting substrates.

An analysis of the distribution and diversity of soil tardigrades in the Great Smoky Mountains National Park (NC/TN, USA) using maximum entropy modelling

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A large database now exists for tardigrades in the Great Smoky Mountains National Park (GSMNP) consisting of 780 samples, 15618 specimens, and 81 species including 15 new to science. For this study, tardigrades found in soil/leaf litter samples (40 species) were analyzed using Maxent. Maxent is a GIS-based, machine language method for modeling species distributions. Maxent generates predictive distributions given a set of occurrence data and known environmental variables (GIS data layers) at those locations. Advantages of Maxent compared to traditional multivariate statistical analysis are that i) it requires only presence data, ii) any environmental data available in GIS format can be analyzed including categorical variables, iii) output is continuous and interpolated over the entire study area rather than only at specific collection sites, and iv) it seems to perform well with small sample sizes. GSMNP is perhaps the best studied national park in the USA, and comprehensive GIS data are available including standard physiographic measures (elevation, slope, etc.), as well as detailed soil data, microhabitat temperature patterns, land cover, and anthropogenic variables such as forest disturbance history, NO_2 and SO_2 deposition. A comprehensive model was developed including all relevant environmental variables, and theoretical distributions were produced for all soil-dwelling species. An additional analysis was run to isolate significant soil variables. Some soil variables and some other environmental variables were correlated with species distributions. Individual Maxent species distributions were "stacked" yielding a map of soil tardigrade species richness in the park. Additional environmental variables were correlated with species richness.

Water bears (Tardigrada) from the lower Manzanares river basin, Santa Marta, Colombia

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Among animals, water bears have been one of the most neglected taxa in Colombia. Up to now only few studies have been carried out and approximately 30 species have been reported (Ritchers 1911; Heinis 1914 and 1928; Rham 1931 and 1932; Marcus 1936 and 1939; Barros 1942; Jerez & Narvez 2000; Degma *et al*. 2008). This study represents a modern contribution to the knowledge of the biodiversity of tardigrades and to date, we have identified 13 species from the lower Manzanares river basin in Santa Marta, Colombia. Manzanares is a rather short river, only 33.4 km long, located in an alpine zone known as "La Sierra Nevada de Santa Marta". The river's source is at 2450 m asl. The riverside vegetation, on the lower river basin, consists of tropical dry forest; however, about the 70% of the original flora of the basin has been altered by anthropogenic activities (De Arco & De León 2006). Samples of epiphytic mosses from different trees such as *Mangifera indica* and *Anacardium excelsum* were collected at an altitude range between 150 and 200 m asl. For this, a metal tube of 2 inches was used to mark a known area of moss (20.26 cm^2); then, it was scratched into paper bags for drying and storage. In the laboratory samples were rehydrated with spring water for a period of 48 hours. Examination was done under the dissecting microscope at 24h and 48h. Tardigrades were mounted directly in Hoyer's medium. All specimens were measured and photographed using a Phase Contrast Microscopy associated with digital camera. Species identification were made based on Ramazzotti & Maucci (1983) and modern literature (Nelson 2011, Jerez *et al*. 2002, Kaczmarek & Michalczyk 2004, Degma *et al*. 2010, Michalczyk et al. 2012). In studied material 13 species belonging to four genera (*Doryphoribius, Macrobiotus, Milnesium* and *Paramacrobiotus*) were identified. This is the first record of genus *Doryphoribius* from Colombia. *Paramacrobiotus* sp.1 with a density of about 0.8 ind./cm^2, followed by *Paramacrobiotus* cf. *richtersi* and *Macrobiotus* sp.1 with similar densities of about 0.2 ind./cm^2, were the most abundant species. A single sample of 36 specimens representing 9 species was the most diverse (1.8 ind./cm^2). These preliminary results are part of a bigger on-going project titled "Morphological and Molecular Characterization of Tardigrades from the Sierra Nevada de Santa Marta, Colombia".

Long term effect of salvage logging on soil population of Tardigrada in mountain spruce forest in the Czech Republic

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The effect of logging after bark beetle breaktrough on the soil population of *Tardigrada* was investigated after 15 years and more from the treatment. Sampling is realized twice a year – in spring and autumn and is performed in three types of plots (clearings after breakthrough, breakthroughs and wet breaktroughs) and under three types of vegetation cover at each plot (grass, blueberry and moss).

The total number of individuals differs significantly among plots only in one season, but the trend of higher numbers in clearings is similar in all seasons. Till now 7 tardigrade species have been found. The most frequent species are *Diphascon scoticum, Diphascon spitzbergense* and *Macrobiotus hufelandi*. Species composition differs in favour of wet breaktroughs, but logging has no significant effect. But the trend of decreased number of species in breaktrough plots is notable. Vegetation cover somehow influences numbers of certain species.

Data on numbers and species of Tardigrada are furthermore correlated with data on abiotic conditions at sites (temperature, humidity, soil nutrient composition) and population parameters of another soil animal groups (*Diplopoda, Chilopoda* and *Collembola*).

Water Bears in the Anthropocene: A Comparison of Urban and Woodland Tardigrade Communities in Southwestern Louisiana, USA

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Humans have had such a profound effect on global ecosystems, including biodiversity, that "Anthropocene" is being increasingly used as a chronological term to mark the period of greatest human impact. No areas show the effect of human impact on the environment more than cities, which often have novel combinations of species in unique communities. Tardigrades have often been collected in cities, but studies dedicated to urban tardigrade biodiversity are few (Tokyo and other Japanese cities; Nice, France; Cincinnati and Fresno, USA; Calgary, Canada, and General Pico and Santa Rosa, Argentina), and those comparing urban diversity with nearby rural or "natural" sites even fewer (Nice, Cincinnati, and Fresno). In this paper we compare the diversity and abundance of tardigrade species in Lake Charles with a nearby non-urban site.

Lake Charles is a city of 70,000 in southwestern Louisiana, USA. Sam Houston Jones State Park (SHJSP) is a 440 ha expanse of second-growth bottomland forest less than 15 km from Lake Charles. Lichen, moss and leaf litter samples were collected from Lake Charles and SHJSP in the spring of 2011 (50 samples total at each site). Urban sampling stations were classified in five categories: single family residential, undeveloped lot, light commercial district, heavy commercial district, and industrial area).

Although tardigrade density (specimens per gram substrate) did not differ markedly between Lake Charles and SHJSP, species richness and diversity were greater in SHJSP (16 species, H_1=3.06) than in Lake Charles (8 species, H_1=1.42). No heterotardigrades were found in Lake Charles, but four species were present in SHJSP. Seven tardigrade taxa (*Macrobiotus* cf. *echinogenitus, Macrobiotus* cf. *hufelandi, Minibiotus acadianus, Paramacrobiotus areolatus, P. richtersi* and two species of *Milnesium*) were common to both sites. Almost three quarters of all specimens found in Lake Charles belonged to a single species, *Minibiotus acadianus*; the most abundant species in SHJSP, *Macrobiotus* cf. *echinogenitus*, represented slightly over a third of all specimens there. Both species of *Milnesium* (a new species with claw configuration [3-3]-[3-3] and a new species most similar to *M. reticulatum*) were widespread in both Lake Charles and SHJSP; they co-occurred in two samples. In Lake Charles the highest species richness occurred in single family residential and industrial areas.

The number of species found in Lake Charles lies within the range (5–10) found in previous urban surveys. All tardigrade studies comparing urban with nearby nonurban habitats have found lower species richness in cities. The most widely-reported taxa in urban surveys are *Milnesium* sp. and *Ramazzottius oberhaeuseri* (the latter was not found in Lake Charles). Heterotardigrades have only rarely been recorded in urban surveys.

The role of human activity in the distribution and population dynamic of soil Tardigrada on the maritime Antarctic Peninsula

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Tardigrades are important members of the Antarctic biota yet little is known about the soil fauna or whether the impact of tourism to the region affects the tardigrade populations. As part of a German Federal Environment Agency commissioned research to assess the role of human activities on soil meiofauna in the maritime Antarctic, we examined the population dynamics of soil tardigrades from 14 localities in the Antarctic Peninsula (ten frequented by tourists, four by researchers) over the austral summers 2009/2010 and 2010/2011. A block design of sampling was used to compare areas of high and low human impact, abiotic parameters and data on vegetation cover was collected for each site, and it was noted that wildlife densities were equal in both area types. Specimens were extracted using Baermann funnel technique, which favoured mobile subjects. Analysis of the data was carried out using simple ordination cluster techniques to identify which parameters, factors and variables were biologically relevant and whether any had anthropogenic significance. Differences within communities were greatest between the localities and the two years, rather than anthropogenic influence, which needed to be above the influence of local fauna (birds and seals). No 'alien' imports had arrived with human traffic. However, there were indications of anthropogenic influences in the population dynamics. Some species were negatively associated with tourist impact and others were tolerant of the conditions. The study also explored new sites and habitats around the maritime Antarctic and has therefore increased the knowledge of species richness for some of the localities. This presentation communicates some of the findings of this study.

Primary succession of Tardigrade community on brown coal post – mining dumps

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The changes of rotifer soil communities along primary succession chronosequence was studied on brown coal post mining areas near Sokolov, NW part of Czech Republic. The alcalic clays of *Cypris* formation covering coal layers are excavated and deposited on spoil dumps. The chronosequence of unreclaimed plots was 2, 11, 14, 20, 43 years old and were subjected for natural succession. Pioneer plant species occupied 2 and 11 year old plots, 14-20 year old plots were covered by Salix caprea shrubs and a forest formed by *Betula pendula* and *Populus tremuloides* developed on the 43 year old plot. Tardigrades were extracted from substrate quantitatively, using modified Baermann funnel and combined gradient of light and temperature.

11 taxa of *Tardigrada* were identified through the project. Most common were *Hypsibius convergens, Macrobiotus richtersi* and *M. harmsworthi*. The total abundances vary in order from 103 to 105 individuals per square meter. Along the age of the locality, the total abundances increase. Highest abundance is on 20 years old plot and decrease later. Tardigrade species diversity follows similar pattern. Depressions of the surface relief host higher numbers of tardigrades than peaks.

The pioneer species on chronosequence are *M. richtersi* and *M. harmsworthi*. *Hypsibius convergens* coming later together with *Diphascon* cf. *rugosum* and other *Diphascon* species in consequence. Although *M. richtersi* inhabit initial successional stage, It persist through the chronosequence in regular abundance. The pattern of primary succession of tardigrates follows simillar pattern given by other soil hydrobionts such as rotifers and nematods. All these groups are significantly positively affected by development of soil organic horizons and negatively by development of strong canopy of young forest.

Element analysis of the eutardigrades *Richtersius coronifer* and *Milnesium tardigradum* using PIXE

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We will present results of a qualitative as well as quantitative Particle Induced X-ray Emission (PIXE) analysis of the tardigrade species *Richtersius coronifer* and *Milnesium tardigradum*, using the Lund Nuclear Microprobe. A PIXE analysis can, in a non-distructive manner, reveal information about element content, distribution and concentration of the investigated sample. This PIXE analysis is, to our knowledge, the first of its kind carried out on tardigrades.

The analysis was conducted to learn more about the composition of tardigrades, to pinpoint possible elemental differences between the two selected species and also to investigate a possible link between elemental distribution in the animals and tolerance to desiccation and radiation, as has been previously reported in the bacteria *Deinococcus radiodurans*. Desiccated as well as freeze-dried (resembling active, hydrated) specimens have been analyzed, to investigate possible changes in the element distribution between the two states. Results of the PIXE analysis will be presented, along with a comparison with similar investigations of other radiation tolerant species and a possible interpretation of the element content.

The brain architecture of *Echiniscus testudo* (Echiniscoidea, Heterotardigrada)

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The nervous system of *Echiniscus testudo* (Doyère, 1840) was investigated by the means of immunohistochemistry in conjunction with confocal laserscanning microscopy. Antibodies against serotonin (5-HT), FMRFamide and tyrosinated α-tubulin provided a positive immunoreactivity. 5-HT-like immunoreactivity (lir) showed the lowest degree of a signal. The only neurites stained are the connectives between the ventral ganglia. Additionally, few cells in the brain and the ventral ganglia could be detected. FMRFamide-lir showed a totally different picture, staining distinctly more nervous structures. A massive brain structure is present together with a number of cells in each ganglion and one cell in each leg. Several connectives and commissures in the brain and the trunk region were detectable by FMRFamide-lir. With the application of anti-tyrosinated α-tubulin again a massive brain structure in contrast to the 5-HT-lir could be detected. Also connectives and commissures were visible. It seems that the application of these three antibodies can give quite a good overview of the brain architecture of *E. testudo*.

Comparative morphology of the brain structure of Eutardigrada and Arthrotardigrada

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The position of Tardigrada within the tree of life has always been problematic, and is still a matter of much debate. Neither molecular nor morphological investigations have so far provided any definite conclusion. In order to shed more light on the phylogenetic relationships of tardigrades, morphological investigations of their neuroanatomy have been implemented. Especially the organization of the central nervous system has been considered important, in order to understand not only the phylogenetic relations of Tardigrada, but also to understand the evolution of the brain and cephalisation in bilaterians.

Despite the alleged phylogenetic importance of the organization of the tardigrade nervous system, there are still doubts as to the existence of certain nervous structures. Here we bring new insight to the morphology of the brain of *Actinarctus doryphorus* Schulz, 1935 (Arthrotardigrada), and compare these data with the brain of the eutardigrade *Halobiotus crispae* Kristensen, 1982 (Parachela). Because arthrotardigrades are considered to exhibit more plesiomorphic characters than eutardigrades, understanding their brain structure will aid in 1) understanding the organisation of the eutardigrade brain 2) suggesting a possible brain configuration of the tardigrade ancestor. This could assist in resolving the sister group relationships of Tardigrada.

We combine transmission electron microscopy with confocal laser scanning microscopy and immunocytochemical staining, to achieve an increased level of detail and spatial understanding of brain structure. Both tardigrade species have at least three paired brain lobes, though their arrangement is very different. It is clear that the brain of *A. doryphorus* exhibits a more pronounced segmentation compared to the brain of *H. crispae*. The first brain lobe of *A. doryphorus* is located anteriodorsally, with the second lobe just below it in an anterioventral position. The two lobes are located anterior to the buccal tube. The third pair of brain lobes is situated posterioventrally to the first two brain lobes, and flanks the buccal tube. In *H. crispae* the first and second brain lobes are located dorsal to the buccal tube, and the second brain lobes are positioned in between and somewhat dorsal to the first brain lobes. The third pair of brain lobes is arranged ventral to the first and second lobes, and also flanks the buccal tube. In addition, both species possess a subpharyngeal ganglion, which is connected with the first ventral trunk ganglion. The first and second brain lobes of *A. doryphorus* innervate the clavae and cirri of the head. These external structures are reduced in *H. crispae*; however, innervations are present in the cuticle or epidermis in the areas termed temporalia and papilla cephalica, as well as in the area of the median cirrus. The innervations of these areas suggest a homology between the clavae and cirri of *A. doryphorus* and the temporalia and papilla cephalica of *H. crispae*.

Musclular Architecture of Marine Heterotardigrades

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There have been only a few studies shedding light on the muscular architecture of tardigrades. Two recent studies (Schmidt-Rhaesa & Kulessa, 2007; Halberg *et al*., 2009) examined the muscular architecture of several eutardigrade species in detail utilizing florescent-coupled phalloidin. They showed that the eutardigrades have a comparable pattern in the muscular architecture, which could be informative as a morphological character for taxonomy.
In the present study, we investigate the muscular architecture in marine heterotardigrades utilizing the same staining method. We have observed the following twelve species in one class, two orders, five families, six subfamilies and ten genera: *Batillipes similis* (Arthrotardigrada: Batillipedidae); *Archechiniscus* cf. *symbalanus* (Arthrotardigrada: Halechiniscidae: Archechiniscinae); *Florarctus glareolus* (Arthrotardigrada: Halechiniscidae: Florarctinae); *Halechiniscus remanei*, *H*. sp. (Arthrotardigrada: Halechiniscidae: Halechiniscinae); *Raiarctus aureolatus, Styraconyx* sp. (Arthrotardigrada: Halechiniscidae: Styraconyxinae); *Tanarctus* sp. 1, *T.* sp. 2 (Arthrotardigrada: Halechiniscidae: Tanarctinae); *Renaudarctus* sp. (Arthrotardigrada: Renaudarctidae); *Stygarctus bradypus* (Arthrotardigrada: Stygarctidae: Stygarctinae); *Echiniscoides sigismundi* (Echiniscoidea: Echiniscoididae).

Among the marine heterotardigrades observed, some evident differences in the muscular architecture were detected (e.g. the lack of median ventral attachment points, the existence of muscle strands stained in a broken line pattern, and the existence of apparently segmented muscle strands). Such a higher morphological diversity has not been reported in the previous studies on eutardigrades. The evolutionary significance for marine heterotardigrades is discussed in relation to the muscular architecture diversity and its bringing differences in locomotion.

Genome annotations and transcriptomic analysis in *Ramazzottius varieornatus*

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Till now, more than thousand species were described for phylum Tardigrada and each species was classified according to a diversified morphology. But, of course, all tardigrades share a common body plan such as one head segment with four body segments, four pairs of legs, and small size. The secret for these key characteristics distinguishing tardigrades from other phyla should reside in their genome. In addition, various limno-terrestrial tardigrades show anhydrobiotic ability and extremotolerance in dehydrated state. Elucidation of whole gene repertoire of an anhydrobiotic tardigrade, provides a essential foundation for understanding their unique body plan and mechanisms of anhydrobiosis or extremotolerance.

Here, we report the construction of more than 19,000 high quality gene models based on our genome project of *Ramazzottius varieornatus*, using combination of refined informatic pipelines tuned by numerous trials, and various transcriptomic data including Sanger-sequencing and high-throughput sequencing. Extensive transcriptomic analyses supported approximately 70% of our gene models experimentally. As for molecules related to anhydrobiosis, our gene models contained gene-set required for synthesis and metabolism of trehalose supporting the presence of trehalose in this species. Genes encoding late embryogenesis abundant (LEA) proteins were also discovered, but its number is limited. Instead, larger numbers of members were found for two novel abundant heat soluble (AHS) protein families. Some members of AHS genes formed tandem cluster in the genome with the same orientation, suggesting their origin by recent gene duplication during evolution. Our high quality gene models will provide solid basis for wide spectrum of further tardigrade researches.

Tardigrades: characterization of recombinase rad51, important protein of the DNA repair system

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Tardigrades can resist extreme conditions as desiccation and ionizing radiation in doses above 1 kGy. It has been reported that tolerance to radiation is similar in both dehydrated and hydrated tardigrades, then it has been suggested that rather than preventing biochemical damage, repair mechanisms may be responsible for the tolerance observed. According to the current hypothesis, tardigrades must have evolved an effective DNA repair system, but none of their proteins has been characterized. For this reason, the study of the DNA repair systems in tardigrades could aid to understand the radiation resistant mechanism, and also contribute with more information about this important system in the genome integrity maintenance.

There are two important pathways in DNA repair; Homologous repair (HR) and Non-homologous end joining (NonHEJ). Studies of cancer genomes support the conclusion that NonHEJ is more prone to giving rise to genomic rearrangements than HR. On the other hand, HR is a key pathway, as it leads to precise repair of DNA damage using the sister chromatid as the repair template. Also, restrictions on HR, including sequence heterology and crossover suppression, attenuate potentially deleterious HR events in the genomes of somatic cells emphasizing the key role that HR has in promoting precise repair in lieu of mutagenic repair by other pathways (Moynahan, 2010).

Based on this information, we decided to start the DNA repair system characterization of tardigrades studying the homologous repair pathway. Among the numerous proteins known to participate in the DNA repair process, RAD51 is a very important recombinase that is highly conserved. This protein is in charge of promoting strand exchange during homologous recombination, thus maintaining genomic stability. In order of characterize the Rad51 homolog in tardigrades MtRad51 (from *Milnesium tardigradum*), different sets of primers were designed. Different PCR products were obtained, cloned and sequenced. They were joined together and the full predicted protein sequence was analyzed by BLAST against NCBI database, showing an identity of 71% with the RadA (Rad51 homolog) of the nematode *T. spiralis*. Also the structural analysis of the MtRad51putative protein sequence has shown a high structural similarity with *Saccharomyces cerevisiae* Rad51. This confirms the high conservation of the protein among other phylum. On the other hand, the application of the western blot technique with a heterologous antibody specific against Rad51, revealed, as preliminary data, that Rad51 protein levels increase after 45 min after 70 Gy radiation exposure, and this tendency is maintained up to 3h. As expected, Rad51 levels were up regulated in response to ionizing radiation in tardigrades, this may suggest that homologous recombination could be the immediate response to double strand breaks, at least partly.

Radiation tolerance of tardigrade eggs

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Tardigrades represent one of the most radiation tolerant animals on Earth, surviving doses of ionizing radiation well above 1 kGy. This tolerance has been confirmed in several species of tardigrades, but mainly in adults, and there are few studies on the effect of ionising radiation on the eggs of tardigrades. We present some results from experiments where eggs of the eutardigrades *Richtersius coronifer* and *Milnesium tardigradum* were irradiated with gamma ray (Cs-137).
Initial studies in *R. coronifer* suggested a relatively linear dose response at low doses, from ca. 70% survival in the controls to about 25% at 400 Gy, followed by a stable response up to 1 kGy and thereafter a declining response up to 4 kGy where only ca. 5% of the eggs hatched. However, since developmental stage was not controlled in these initial studies we made more detailed experiments where different developmental stages were separated.

In *R. coronifer,* eggs were irradiated with 500 Gy and almost all eggs in the early and middle stage of development died after irradiation while eggs in late stage had a hatching rate of 70%.
In *M. tardigradum* we investigated effects of both developmental stage and three different doses (50, 200, and 500 Gy). At 50 Gy, there was no significant effect of developmental stage on hatchability. In contrast, at both 200 Gy and 500 Gy, hatchability in the early developmental stage was significant lower compared to the middle and late stages, while the latter did not differ from each other, or from the controls. Thus, only eggs in the early developmental stage were affected by irradiation. The sensitivity of the early stage was also expressed when developmental stages were analyzed separately for the effect of dose. Only in the early developmental stage there was an effect of dose, with lower hatchability at higher doses. The hatching time of eggs in different developmental and dose categories was also evaluated. Irradiated eggs in all developmental stages showed a delay in development compared to controls, but a dose response was observed only in the early developmental stage.

These results show that in both *R. coronifer* and *M. tardigradum* eggs in the early part of the development are more sensitive to radiation compared to eggs in late development. In addition, eggs of *R. coronifer* seem to be more sensitive than eggs of *M. tardigradum* in the mid-stage of development. This provides additional evidence that these two species differ in their patterns of tolerance to extreme physical conditions, as earlier indicated in exposures to, e.g., space conditions and high temperature. The delay in the hatching time may indicate a cell cycle arrest that allows the DNA repair system to get activated, to correct the damage, and to continue with the development later, or to stop it if the damage is too high.

Diversified bet-hedging strategy in the desiccation tolerant tardigrade *Paramacrobiotus richtersi*

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In variable environmental conditions a genotype may reduce the risk associated with reproduction by two main types of bet-hedging strategies: conservative and diversified bet-hedging. The former involves an avoidance of extremes to minimize fluctuations in a life-history trait that will assure an optimal mean for energetic constraints. The latter entails probabilistic *risk spreading* among individuals *of the same genotype* that express a range of diversified phenotypes to sample a range of different environments through time. Diversified bet-hedging is generally considered the most viable strategy for a long-term performance in a habitat varying in an unpredictable way. To date, only a few empirical studies provide evidence that bet-hedging occurs in nature.

The timing of many phenological events (e.g. egg hatching) results from a complex interplay among organism genotype, environmental factors and maternal effects (e.g. egg size). The relationship between egg size and development time is known and it is generally accepted that larger eggs take longer to develop than smaller ones. The production of eggs with variable size may represent a strategy by which a mother spreads the risk connected with life in a temporary habitat.

As regards tardigrades, they evolved a large variety of dormant stages that can be ascribed to diapause (encystment, resting eggs) and cryptobiosis (anhydrobiosis, cryobiosis, anoxibiosis). Therefore, their life-cycle can be generally divided into two very distinct stages: the active stage and the dormant stage, which are characterized by substantially different requirements and risks. During the active stage, tardigrades may exhibit high plasticity in life history traits (e.g. egg number, egg size and hatching time), as an adaptive measure to cope with risks linked to unpredictable habitat conditions.

Clonal lineages from an apomictic population of *Paramacrobiotus richtersi* (Eutardigrada, Macrobiotidae) may produce up to four kinds of eggs: subitaneous eggs, delayed-hatching eggs, abortive eggs and diapause resting eggs, the last ones require a stimulus to hatch (e.g. rehydration after a period of desiccation). The lack of genetic variation expected among clonal organisms make them ideal material for investigating diversified bet-hedging that is, by definition, life history trait variance expressed within genotypes, and maternal effects.
We compared the proportion of different kinds of eggs and analysed the correlation between egg size and hatching time within clonal eggs.

Our preliminary results provide possible empirical support of the occurrence of diversified bet-hedging strategy due to maternal effect in tardigrades living in stochastic environments.

Inside the genome of *Milnesium tardigradum*: LEA, Trehalose, and stress response proteins

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Animals of the species *Milnesium tardigradum* Doyère 1840 evolved an anhydrobiotic state which withstands the desiccation of their life habitats. During this so called tun stage, the animals are not only resistant against desiccation, but also against other abiotical stress factors like temperature, pressure, or radiation. The exact molecular mechanisms behind this adaptability are so far unknown. The complete genome sequence of *M. tardigradum* enabled us to search for genomic adaptations behind its unusual physiological features.

Several mechanisms for water stress resistance have been described and some have been posited for tardigrades. This includes the late embryogenesis abundant (LEA) proteins. Recently we demonstrated the existence of LEA proteins in *M. tardigradum* on protein level, but lacked genomic or transcriptomic evidence. This gap can now be closed by two LEA proteins identified within the genome sequence.

Another widely discussed compound to withstand desiccation is the sugar trehalose. Contrasting other anhydrobiotic tardigrades, no accumulation of trehalose at the tun state was found so far in *M. tardigradum*. Indeed, the genome harbours only an incomplete set of enzymes of the trehalose metabolism. Thus, trehalose is not the main molecular mechanism behind desiccation tolerance in *M. tardigradum*.

Complementary to the identification of known mechanisms, the genome sequence allows searching for novel adaptations on the genomic level. Therefore, we compared *M. tardigradum* with the published genomes of nematodes and arthropods. Indeed, the tardigrade has a larger inventory of proteins involved in stress response reactions than the other analysed species. Additionally, we found expanded protein families which could hint for tardigrade specific adaptations.

In summary, with the whole genome sequence of *M. tardigradum* in our hands we identified genes which have been postulated to provide desiccation stress tolerance. Other proposed mechanisms, namely trehalose, could be rejected. On the global level, we found expanded protein families and a rich set of stress response proteins. The identified genes are good candidates for the further detailed experimental characterisation of the molecular mechanisms behind the unusual stress tolerance of *M. tardigradum* and tardigrades in general.

UVC Radiation Tolerance in the Tardigrade *Ramazzottius varieornatus*

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Tardigrades have been shown to tolerate massive doses of UV radiation and ionizing radiation. However, it was not known whether this tolerance was due to protecting an organism from radiation-induced damage or an extraordinary repair mechanism. In the present study, we examined tolerance of the tardigrade *Ramazzottius varieornatus* strain YOKOZUNA-1 to UVC (254 nm) radiation in hydrated and desiccated anhydrobiotic states. *R. varieornatus* in the hydrated state survived and produced next generation after exposure to 2.5 kJ/m^2 of UVC radiation. We found that *R. varieornatus* has efficient systems for repairing detrimental photoproduct thymine dimers in DNA following exposure to the corresponding dose of UVC radiation. In R. varieornatus accumulation of thymine dimers in DNA induced by irradiation with 2.5 kJ/m^2 of UVC radiation disappeared 18 h after the exposure when the animals were exposed to fluorescent light. A putative homolog of the *D. melanogaster* photo repair gene (*phrA*) was found in the *R. varieornatus* genome, implying that this gene is partly responsible for repairing damaged DNA after UVC irradiation. Much higher UVC radiation tolerance was observed in the anhydrobiotic *R. varieornatus* compared to the hydrated specimens. Progeny was produced from tardigrades exposed to even 20 kJ/m^2 of UVC radiation in the anhydrobiotic state. The anhydrobiotes of *R. varieornatus* accumulated much less UVC-induced thymine dimers in DNA than hydrated one. It suggests that anhydrobiosis efficiently avoids accumulation of DNA damage in *R. varieornatus* and confers better UV radiation tolerance on this species. In conclusion, it is proposed that UV radiation tolerance in tardigrades is due to high capacities of DNA damage repair and DNA protection.

On the current knowledge of osmoregulation in tardigrades

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Tardigrades are well-known for their ability to thrive in habitats characterized by large fluctuations in abiotic factors. Especially, the semi-terrestrial species are renowned for their ability to enter cryptobiosis, and thereby cope with periods of dehydration. Yet, tardigrades may be highly tolerant of ambient perturbations, even in their active state. The latter is closely linked to another strategy for surviving extremes, in contrast to the ametabolic, cryptobiotic state, namely to rely on metabolism, and handle e.g. large fluctuations in external salt concentrations by osmoregulation.

We have shown that two eutardigrades, the marine *Halobiotus crispae* and the semi-terrestrial *Richtersius coronifer* both display a hyper-osmoregulatory strategy, keeping their body fluids hyperosmotic at a range of external salinities [1]. Experiments on H. crispae have further revealed that this eutardigrade may expel large quantities of body fluid, regulating body volume, following experimentally induced volume changes. These data would imply that eutardigrades have a high water turnover, and the ability to excrete hypotonic and isotonic fluid. It has been suggested on numerous occasions that the Malpighian tubules, found in eutardigrades (and mesotardigrades), are renal organs. Recent data, however, shows that the midgut rather than Malpighian tubules are involved in excretion of organic anions [2]. Even so, the tubules could be involved in extracellular fluid homeostasis, working in conjunction with the gut, representing the site for formation and excretion of dilute and isotonic fluid.

Interestingly, preliminary experiments on *Echiniscoides sigismundi* (from Lynæs, Denmark) suggest that this heterotardigrade may lack the ability to volume regulate. A crude experiment was performed in which approximately 90 animals were transferred from seawater (app. 20 ‰) to distilled water. Survival was subsequently followed for a period of 98 hours, by stochastically removing 10 animals every 12-14 hours, and monitoring activity following re-transfer to seawater. Mortality was low during the entire experimental period, showing that this species tolerates exposure to distilled water for at least 4 days. However the tardigrades remained inactive and swelled during the entire period, which could indicate that this heterotardigrade has a limited ability to regulate body volume. Quantifications of body volume as well as further experiments in solutions less hypotonic are necessary to clearly establish whether this species has the ability to regulate body volume.

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Metabolomics of tardigrade *Ramazzottius varieornatus* reveals dynamic metabolic response during anhydrobiosis

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Limno-terrestrial tardigrades can withstand almost complete desiccation through a mechanism called anhydrobiosis, and several of these species have been shown to survive the most extreme environments through exposure to space vacuum. Molecular mechanism for this tolerance has so far been studied in many anhydrobiotic metazoans, leading to the identification of several key molecules such as the accumulation and vitrification of trehalose as well as the expression of LEA proteins to prevent protein aggregation. On the other hand, the understanding of comprehensive molecular mechanisms and regulation machinery of metabolic compounds during anhydrobiosis is yet to be explored. To this end, we have conducted a comprehensive metabolome analysis using the tardigrade *Ramazzottius varieornatus*, which is a potential model species for anhydrobiosis. In order to analyze the metabolic changes in the active and dehydrated states, we measured the metabolome in both conditions using two types of high-throughput mass spectrometry (MS) systems, liquid chromatography time-of-flight MS (LC-TOFMS) for lipids and sugars and capillary electrophoresis TOFMS (CE-TOFMS) for primary metabolite, with three biological replicates for each state. As a result, increase, but no significant accumulation of trehalose in this species suggests a more complex mechanism for anhydrobiosis in comparison to other metazoans. While changes in gene expression profiles are limited in between active and tun states, dynamic changes were observed in the metabolism of this species in response to desiccation. Changes in the metabolic profiles suggests complex intracellular responses to oxidative and osmotic stress.

A 17-month study of the reproductive cycle of *Macrobiotus hufelandi* in a lawn moss with notes on reproduction of *Macrobiotus richtersi* and *Diphascon pingue* (Eutardigrada)

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During a three year study on population dynamics of tardigrades in the moss *Rhytidiadelphus squarrosus* in the Black Forest, Germany (see Schuster & Greven 2007, J Syst. Evol. Res.27, 269-276), we also recorded the size and reproductive status of the females of *Macrobiotus hufelandi, Macrobiotus richtersi* and *Diphascon pingue* extracted twice per month from March 2001 to November 2002 (except for August). Data obtained from the most abundant *M. hufelandi* (1109 females were collected during the 17 month-study) showed: (1) Females with mature and immature oocytes were found throughout the whole year. (2) Reproductive activity (calculated as number and percentage of ovigerous females) was highest in February, March, and April. (3) Fecundity (calculated as number of recognizable oocytes and length of gravid females) was negatively correlated with temperature and drought (i.e. the sum of sun-shine hours and number of dry days), but positively correlated with humidity and rainfall. (4) Presence of juveniles (< 260 µm) was positively correlated with the sunshine-hours before the collecting date and negatively correlated with the mean air humidity. The collected number of females of *M. richtersi* (n= 225) and *D. pingue* (n= 245) was much smaller, but as in *M. hufelandi* ovigerous females were found throughout the year, partly, however with different maxima.

Antarctic tardigrade culture from moss samples near Syowa Station, East Antarctica

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A tardigrade species was isolated from moss samples collected from Langhovde and Skarvsnes, near Syowa Station, East Antarctica, and propagated in petri dishes with green algae which also come from the same moss. This culture can be maintained at both 4 ℃ and 10 ℃, but the latter temperature apparently suitable for their growth. Their eggs are laid freely, not in the exuvia. Homology search of the 18S rRNA sequence of this tardigrade revealed that it is identical to that of *Acutuncus antarcticus*. The morphology of this tardigrade was studied using light microscopy and scanning electron microscopy.

POSTER PRESENTATIONS

A new *Milnesium* species (Tardigrada, Eutardigrada, Milnesiidae) from Tanzania

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A new *Milnesium* species is described from two moss samples collected in Tanzania. It belongs to the *granulatum* species group. Species of this group have dorsal cuticle covered with a system of shallow depressions forming a reticular design. Having two points on secondary branches of all claws, the new species is most similar to *Milnesium katarzynae* Kaczmarek, Michalczyk & Beasley, 2004 (the only member of the group with two points on all secondary claws branches). The new *Milnesium* species differs from *M. katarzynae* in the following characters: absolutely and relatively (in relation to a body length) shorter buccal tube, relatively wider buccal tube (i.e. greater *pt* of this measurement), relatively longer main and secondary branches of hind legs claws (thus greater *pt* of these structures), absolutely and relatively shorter distance to a stylet support insertion point. The new *Milnesium* species is the first member of the *granulatum* group found in continental Africa and only third one known from the eastern hemisphere, together with *M. katarzynae* (known from China only) and *M. reticulatum* Pilato, Binda & Lisi, 2002 (known from Seychelles only).

This study was partly supported by the Slovak Scientific Grant Agency (VEGA) as a Project No. 1/0294/09.

A new *Echiniscus* species (Tardigrada, Heterotardigrada, Echiniscidae) from the Alpi Marittime natural park (Maritime Alps Mts, NW Italy)

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We collected more than 300 moss cushions from the Parc national du Mercantour and Parco Naturale delle Alpi Marittime (both in Maritime Alps Mts.) which were established as the first European pilot sites of the "All Taxa Biodiversity Inventories+Monitoring" project stimulated by the "European Distributed Institute of Taxonomy". Two samples collected in two localities of the latter protected area contained specimens of a new *Echiniscus* species. It belongs to the *reticulatus* species group. Species of this group have the only trunk appendages (in the position A) and plate ornamentation composed of a system of round or polygonal units (depressions or thickenings) while space between them has an appearance of more or less distinct reticulation. The surface of these structural units themselves is covered by granulation which is different in different species. The new species differs from other members of the *reticulatus* group mainly by unique cuticular pattern on its plates – a system of darker polygonal patches delimited from each other by a rosette of several dark spots on their perimeters and with or without central spot. More differences between the new species and others having same number of trunk appendages and similar character of a cuticular ornamentation are also discussed.

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Systematic position of *Macrobiotus glebkai* within the "*hufelandi* group", based on morphology and molecular analysis of a population from Ukraine

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The "*Macrobiotus hufelandi* group" is characterized by species which share some distinguishing animal and egg shell characters. In general, they have two rod-shaped macroplacoids and an evident microplacoid, eye spots, pores on the cuticle, medium sized claws, egg shell with inverted goblet-like processes, and spermatozoa with corkscrew-shaped head. We had the opportunity to analyse one species, *Macrobiotus glebkai* Biserov, 1990, found in Eastern Ukraine, which shows characters of the group in the animals, but a peculiar egg shell morphology.
We have carried out a phylogenetic analysis on the 18S nuclear gene in order to verify the systematic position of this species. This analysis has been followed by an integrated study on morphology by LM and SEM, sex ratio, karyology and DNA barcoding (mtDNA *cox1*).
The phylogenetic tree based on 18S sequences grouped *M. glebkai* with *M. hufelandi* and other related species with inverted goblet-like processes on the egg shell. This evidences that the egg shell morphology can sometimes be more variable than that of the animals. In depth morphological analysis allowed us to define details of both animals and eggs of *M. glebkai*, which showed the same characters as the Russian type material (that we also investigated). We verified that the population from Eastern Ukraine was composed by females and males and that the spermatozoon morphology is in line with that found in the various species of the "*Macrobiotus hufelandi* group". The oocytes contain bivalents, but their number has to be specified, as oocytes of some specimens appeared to contain more than 6 bivalents (6 bivalents is the usual pattern for amphimictic species of the "*M. hufelandi* group"). The DNA barcoding has evidenced the presence of three haplotypes (Kimura 2-parameter distances: 0.2-0.3%) belonging to the same haplogroup, well differentiated (more than 20%) from *M. hufelandi* and the other considered species of the group.

A new genus of an intertidal arthrotardigrade (Tardigrada: Heterotardigrada), from the North of Portugal (Atlantic Ocean)

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A new arthrotardigrade n. gen., n. sp., is described from intertidal meiobenthos samples collected at the mid-tide level at Ofir Beach, Esposende, North of Portugal. The Ofir Beach is an Atlantic dissipative exposed beach of well sorted medium sand sediments with very low organic matter content.

The new species is an armored tardigrade with four tubular claws on each leg, a complete set of cephalic cirri and two pairs of clavae. The dorsal cuticle, forming five segmental plates and inter-segmental plates, is strongly sculptured. Ventral plates are also present. Leg sense organs, only present on the fourth pair, are bulb-shaped papillae.

The new arthrotardigrade is distinguished from all the described species of armored marine arthrotardigrades particularly by its peculiar caudal apparatus and by the presence of dorsal spines directed backwards and arranged in pairs. Furthermore, the frontal lobe of the head plate is subdivided into four smaller lobes and a pair of well developed ventral spines is present anteriorly to the third pair of legs.

Some traits, considered primitive, such as the poor sclerotized basal trait of the tubular claws and the presence of dorsal spines, are shared by the new species and *Neostygarctus acanthophorus* Grimaldi de Zio, D'Addabbo Gallo, & Morone De Lucia, 1987, from the monospecific family Neostygarctidae. However, strong affinities between the new arthrotardigrade with the family Stygarctidae can also be considered. Actually, species of the genus *Pseudostygarctus* McKirdy, Schmidt & McGinty-Bayly, 1976, have a similar morphology of the exoskeleton, namely a deeply grooved cephalic plate and the presence of two pairs of lateral processes in the body plates, and also the same shape of the secondary clavae that is almost completely enclosed in the frontal edge. Therefore, because of the exclusivity of some characters exhibited by the new species and its strange combination, the establishment of the new genus is justified. A renewed reflection about the taxonomic arrangement of the armored arthrotardigrades is also suggested.

Tardigrades from the Antarctic Peninsula with a description of a new species of the genus *Ramajendas* (Hypsibiidae)

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The Antarctic region is divided into biogeographic zones that correspond to different climatic regions: the Subantarctic zone (small groups of islands scattered in the Southern Ocean), the maritime Antarctic (west coast of Antarctic Peninsula and neighbouring archipelagos) and the continental Antarctic (east and south part of the Antarctic Peninsula and the Antarctic continent).

Tardigrada (water bears) are microscopic invertebrates inhabiting numerous ecosystems from polar to tropic regions. Tardigrades are known as one of the most extreme-climate resistant animals in the world and they are also able to survive in Antarctica. Studies on Antarctic tardigrades were initiated at the beginning of the 20th century, but progressed very slowly up to the 1990s and 2000s, when more research was conducted in Antarctica. Currently, up to 50 tardigrade species have been reported from the Antarctic region, mostly from islands in the maritime Antarctic.

For the present study we examined 14 soil samples in which we found 837 tardigrade specimens and 32 eggs. The most abundant species was *Acutunctus antarcticus* which was found in 11 samples (733 specimens and 17 eggs). Moreover, we also found: *Echiniscus jenningsi* (1 specimen), *Diphascon victoriae* (3), *Hypsibius dujardini* (6), *Macrobiotus* cf. *harmsworthi* (13) and *Ramajendas* sp. nov. (81 specimens and 15 eggs). The new species differs from other known congeners mainly by the presence of smooth gibbosities on the dorso-caudal end of the body (a character not present in previously described species of the genus). *Echiniscus jenningsi* and *Diphascon victoriae* are rare species and are known only from a few localities in the Antarctic.

Freshwater and Terrestrial Tardigrada of the Americas

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The Americas (North America, Central America, South America, and associated islands; also known as America, the Western Hemisphere or the New World) cover 8.3% of the Earth's total surface area (28.4% of its land area) and contain about 14% of the human population (about 900 million people). They comprise two biogeographical ecozones, the Nearctic and Neotropical. In the Americas studies of tardigrades date back to the mid-Nineteenth Century. Although there have been several recent compilations of Western Hemisphere tardigrades at the national level, no comprehensive list since McInnes' global survey (1994) has covered all the Americas. This paper provides a comprehensive list of the freshwater and terrestrial tardigrade fauna reported from the Americas, their distribution in the Americas, and the substrates from which they have been reported.
I consulted 280 theses, dissertations, books and published papers for data on the distribution of freshwater and terrestrial tardigrades and the substrates from which they have been collected. Authors' identifications were accepted at face value unless subsequently emended (as, for example, with *Milnesium tardigradum*). Taxa were assigned to Greenland, USA states, territories and districts, Canadian provinces and districts, Mexican states, West Indian islands and Central and South American countries. Many areas, in particular large portions of Central America and the West Indies, have no reported tardigrade fauna.
The presence of 52 genera and 368 species has been reported for the Americas; 235 species have been collected in the Nearctic ecozone and 244 in the Neotropical ecozone. Among the tardigrade species found in the Americas, 51 are currently considered cosmopolitan, while 149 species have known distributions restricted to the Americas. Based on recent taxonomic revision of the genus *Milnesium*, the vast majority of records of *M. tardigradum* in the Americas should now be reassigned to *Milnesium* sp., either because the provided description differs from *M. tardigradum* sensu stricto or because insufficient description is provided to make a determination; the remainder should be considered *Milnesium* cf. *tardigradum*.
Most terrestrial tardigrade sampling in the Americas has focused on cryptogams (mosses, lichens and liverworts), and 89.9% of the species have been collected in such substrates. The proportion of species collected in other habitats is lower: 13.9% in leaf litter, 19.6% in soil, and 23.8% in aquatic samples (in other terrestrial substrates the proportion never exceeds 5%). Most freshwater tardigrades are collected from aquatic vegetation and sediment. For nine species in the Americas no substrates have been reported.

The Tardigrada of Big Thicket National Preserve, Texas, USA: Final Results of an All Taxa Biological Inventory

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We have completed an All Taxa Biological Inventory (ATBI) for water bears (Phylum Tardigrada) in the Big Thicket National Preserve (BTNP) of southeastern Texas, USA. Our inventory is based on methods used in a tardigrade ATBI of the Great Smoky Mountains National Park (GSMNP). BTNP consists of geographically separated units comprising about 39,941 hectares; elevation ranges from 0 to 1m. Eight major communities are recognized: longleaf pine uplands, pine savannah wetlands, beech-magnolia loblolly pine, palmetto-hardwood flats, cypress-tupelo swamps, acid bog/baygall, and an arid sandyland. Plants present range from hydrophilic to xerophylic species.
We sampled lichens, mosses, liverworts, leaf litter, soil, and aquatic vegetation in nine units. Tardigrades were present in 47% of terrestrial and 3% of freshwater samples. We identified 631 tardigrade specimens and 63 eggs, representing 13 genera and 37 species. Ten species are new to the fauna of Texas. Two (*Pseudechiniscus juanitae* and *Pseudobiotus longiunguis*) are new records for North America. Mean estimates of tardigrade species richness at BTNP using seven estimators (EstimateS 8.2.0) ranged from 41 to 68; tardigrade species richness in BTNP is approximately half that in GSMNP. Neither ATBI used quantitative sampling; nevertheless it is worth noting that the number of specimens collected in BTNP was an order of magnitude lower than in GSMNP.

Lower diversity in BTNP is partly explained by the absence of the altitudinal variation characteristic of GSMNP. Freshwater tardigrades were especially poorly represented at BTNP. Streams and lakes in BTNP are eutrophic and muddy, and may be poor habitats for tardigrades. This study emphasizes that the high tardigrade diversity found in mountainous areas may not be typical of other landscapes. In North America, tardigrades are less numerous and diverse in southern coastal plains and flatlands than in northern forests and mountains.

Tardigrada of Dominica, West Indies

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The first investigations of water bears (Phylum Tardigrada) in the West Indies were made in the mid-Twentieth Century in the Netherlands Antilles. Since then there have been few studies of terrestrial and freshwater tardigrades from the islands of the Caribbean Sea. These studies have been limited to Puerto Rico, the Dominican Republic, Cuba and Grand Cayman in the Greater Antilles, and Curaçao, Los Testigos, Saint Lucia and Barbados in the Lesser Antilles. This paper presents the results of a survey of the terrestrial Tardigrada of Dominica, the most northerly of the Windward Islands of the Lesser Antilles in the West Indies. Dominica (750 km2) is a volcanic island of very rugged topography. Unlike many Caribbean islands, it retains large expanses of virgin tropical wet rainforest. Yearly rainfall in the mountains ranges from 5,000 to 9,000 mm.

108 samples of moss, lichen, liverwort and leaf litter were collected in June 2009 from seven sites in Dominica. Specimens were found in 35 samples, representing nine genera, and 23 species: *Echiniscus barbarae, E. cavagnaroi, Pseudechiniscus brevimontanus, Pseudechinscus suillus, Milnesium* cf. *tardigradum, Milnesium* sp. (with claw configuration [3-3]-[3-3]), *Diphascon (Diphascon) pingue, Hypsibius convergens, Astatumen trinacriae, Doryphoribius flavus, Doryphoribius quadrituberculatus, Doryphoribius taiwanus, Macrobiotus echinogenitus, Macrobiotus* cf. *harmsworthi* (two species), *Macrobiotus* cf. *hufelandi, Macrobiotus occidentalis, Macrobiotus* cf. *polyopus, Paramacrobiotus* cf. *areolatus, Paramacrobiotus richtersi, Minibiotus* sp. nov., *Minibiotus fallax, Minibiotus furcatus* and *Minibiotus* cf. *intermedius.*

A preliminary survey of tardigrades (Tardigrada) in Lithuania

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The distribution of tardigrades in Lithuania is poorly known. Eleven species of tardigrades have been recorded (four are still at the genus level identification) in this study. Samples of eleven species of mosses and lichens (*Xanthoria parietina*) were collected from the ground and trees in variety regions of Lithuania during 2009 - 2010. From these samples 628 tardigrades and 350 their eggs were extracted. The collected tardigrades are terrestrial eutardigrades belong to 7 genera and 4 families. The most genera and species of tardigrades (except *Ramazottius oberhaeuseri* and genus *Macrobiotus*, that were mentioned in previously study of microfauna of Lithuania) are the first records for Lithuania. *Macrobiotus* was the genus with the most species among mosses. *Ramazzottius* dominated in lichens. Some ecological notice with relation to diversity and abundance of tardigrades in different biotopes are presented.

Preliminary analysis of urban tardigrades of Rafaela, a medium sized city in the province of Santa Fe (Argentina)

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The Knowledge of the tardigrades fauna inhabiting in the Neotropical region cities is poorly known. A study on the composition, structure and diversity of tardigrades communities was carried out in Rafaela, a medium sized city of Santa Fe province, in december 2008. In the study area five sites were selected with different traffic intensities: urban areas with high, middle and lower traffic, industry and surrounding rural areas as well. For each site type, three sites were selected and in each site, three trees. From each of them nine subsamples of lichens and/or moss, 11 mm in diameter, were taken with cylindrical steel cores. The diversity, density and relative abundance of tardigrades were recorded. Statistical and multivariate analyses (CCA) were driven.

The tardigrades were present in 93% of the samples, a total of 2756 specimens were indentified within 6 genera: *Echiniscus, Milnesium, Macrobiotus, Paramacrobiotus, Minibiotus* and *Ramazzottius*. Density ranges varied between urban and rural sites, being maximum at high traffic sites. The number of genus per sample ranged from 1 to 4 and per site between 4 to 5. The maximun number of genus was found at the rural site and the abundance was maximum at the high traffic site. Species of *Macrobiotus* and *Echiniscus* were most frequent. The genus Macrobiotus dominates in all sites, except in industrial sites, where *Echiniscus* was dominant. The genera *Ramazzottius* and Minibiotus are uncommon in the city, but they were only present in industrial and rural areas respectively but always very few specimens. *Milnesium* and *Paramacrobiotus* are present in all sites.

In the city of Rafaela, the species richness found in sites exposed to traffic exceeds the estimated for the other cities of Argentina (Santa Rosa, General Pico, La Plata). The *Macrobiotus* species complex behaves as highly poleotolerantes whereas *Echiniscus rufoviridis* manifests a medium--low tolerance to urbanization.

Future analysis will allow to answer objectives more especifically about the spatial-temporal distribution of tardigrades in urban ecosystems.

Brazilian Marine Tardigrades

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Studies on the marine tardigrades of Brazil initiated with Ernest Marcus in 1946, however, until 2006 only eight species had been recorded for the country: *Batillipes mirus* Richters, 1909; *B. pennaki* Marcus, 1946; *B. tubernatis* Pollock, 1971; *Chrysoarctus briandi* Renaud-Mornant, 1984; *Orzeliscus belopus* Marcus, 1952; *Opydorscus fonsecae* Renaud-Mornant, 1990; *Tanarctus heterodactylus* Renaud-Mornant, 1980 and *Echiniscoides sigismundi* Schultze, 1865. Of these, only *Batillipes pennaki* and *Opydorscus fonsecae* had been observed in the northeastern coast of Brazil. From 2006 on, research carried out by the Federal Rural University of Pernambuco (UFRPE) along the coast and on the continental platform of northeastern Brazil, as well as at the Saint Peter and Saint Paul Archipelago, expanded the known distribution for five species (*Batillipes tubernatis, Chrysoarctus briandi, Orzeliscus belopus, Opydorscus fonsecae* and *Tanarctus heterodactylus*) and allowed over 20 taxa to be identified and recorded for Brazilian territorial waters: *Batillipes annulatus* De Zio, 1963; *B. dicrocercus* Pollock, 1970; *B. lesteri* Kristensen & Mackness, 2000; *Archechiniscus marci* Schulz, 1953; *Dipodarctus subterraneus* Renaud-Mornant, 1959; *Florarctus hulingsi* Renaud-Mornant, 1976; *Wingstrandarctus intermedius* Renaud-Mornant, 1967; *Halechiniscus perfectus* Schulz, 1955; *H. tuleari* Renaud-Mornant, 1979; *Angursa lingua* Bussau, 1992; *Raiarctus aureolatus* Renaud-Mornant, 1981; *Actinarctus doryphorus doryphorus* Schulz, 1935; *A. doryphorus occelatus* Renaud-Mornant, 1971; *Tanarctus dendriticus* Renaud--Mornant, 1980; *T. velatus* McKirdy, Schmidt & McGinty-Bayly, 1976; Neostygarctidae Grimaldi de Zio, D'Addabbo Gallo & Morone De Lucia, 1987; Neoarctidae Grimaldi de Zio, D'Addabbo Gallo & Morone De Lucia, 1992; *Parastygarctus sterreri* Renaud-Mornant, 1970; *Pseudostygarctus intermedius* Renaud-Mornant, 1979; and *Stygarctus bradypus* Schulz, 1951. Thus, the knowledge on this group's taxonomy in Brazil increased significantly, as there are now more than 28 species of marine tardigrades which occurrence has been recorded for the country.

Preliminary survey of tardigrades from Alberta and British Columbia, Canada

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Canada is a large country with diverse habitats and considerable seasonal variation in temperature and precipitation. Most tardigrade surveys have been conducted near the West and East coasts, with relatively little sampling done inland. To date, 92 species have been identified in Canada (Boeckner *et al.* 2006, Collins 2010, Grothman 2011), comprising 23 Heterotardigrada and 69 Eutardigrada.
I have begun surveying tardigrades in the provinces of British Columbia and Alberta. British Columbia borders the Pacific Ocean, and is divided from Alberta to the East by the Rocky Mountains. Alberta tends to be drier and colder and to have greater seasonal variation than British Columbia. Most sampling has focused on moss, lichen, and leaf litter. From 140 samples, 1220 specimens (adults and eggs) have been recovered. Identification is not complete, but the specimens include 17 species, including 15 Eutardigrada one of which, *Murrayon stellatus*, is a new record for Canada.

Preliminary studies on the tardigrade fauna of the Arctic archipelago of Svalbard

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Svalbard is an Arctic archipelago comprising one large (Spitsbergen) and many smaller islands located between Norway and the North Pole. The archipelago is surrounded by the Arctic Ocean on the north, Norway Sea on the south, Greenland Sea on the west and Barents Sea on the east. The west coast of Svalbard is strongly influenced by warm waters of the West-Spitsbergen current. In consequence, these areas are characterised by ameliorated climatic conditions and their coastal terrestrial ecosystems are covered by relatively rich arctic tundra dominated by mosses and lichens. On the other hand, climate in the east part of Svalbard, which is determined by a cold ocean current from Barents Sea, is harsh and the coastal areas of this region are covered mostly by polar deserts.

The first studies on tardigrade fauna of the Svalbard Archipelago took place as early as in 1897 and were continued by a number of researchers throughout the XX century. Despite over of a more than a two centuries long history of research, tardigrade fauna of this area remains poorly known. In total only 24 papers were published and 91 species were reported from the whole Archipelago. This includes 16 species described as new for science, of which four are known only from the Archipelago and thus are considered as endemic for this region.

In order to deepen our knowledge of the tardigrade biodiversity in the Arctic, during the last several years 613 samples of bryophytes, lichens, soil and water sediments were collected from the Svalbard Archipelago area. The majority of the samples were collected from West Spitsbergen (Hornsund and Magdalenfjorden). Some of the samples were collected also from smaller islands: Prince Karl Foreland, Barentsøya, Edgeøya and Nourdastlandet. Up to now 87 samples have been analysed (79 from Spitsbergen, 5 from Edgeøya and 3 from Nordaustlanded). In these samples a total of 40 species were found including four new for science (*Bryodelphax parvuspolaris, Isohypsibius coulsoni* and two freshwater species (from cryoconite holes) from the genus *Isohypsibius*) and five new for the Svalbard Archipelago (*Milnesium eurystomum, M. asiaticum, Diphascon (Adropion) prorsirostre, Microhypsibius bertolanii* and *Halobiotus crispae*). Additional new species for tardigrade fauna were reported also for Edgeøya (*Testechiniscus spitsbergensis* and *Hypsibius convergens*) and Nourdastlandet (*Testechiniscus spitsbergensis, Tenuibiotus voronkovi* and *Macrobiotus islandicus islandicus*).

At present, based both on literature data and the current study, the list of tardigrade fauna from the Svalbard Archipelago consists of 91 species. However, up to now only ca. 15% of all the samples collected during the present sampling were analysed. Therefore, it is very likely that more species will be found in the remaining samples.

Census of tardigrades in the French and Italian nature reserves Parc national du Mercantour and Parco Naturale delle Alpi Marittime

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The "European Distributed Institute of Taxonomy" (EDIT) stimulates "All Taxa Biodiversity Inventories+Monitoring" (ATBI+M) in the Parc national du Mercantour and Parco Naturale delle Alpi Marittime as the first European pilot sites to apply the science of taxonomy to the conservation of biodiversity. ATBI include intensive community efforts to identify and record all living species that exist within a given area. Together with the parks' management boards, EDIT coordinates activities from scientists from over 27 mainly EU-based, scientific institutions that thus cooperate to work together on a baseline biodiversity assessment of the two parks.

We collected more than 300 moss cushions from the two nature reserve sampling sites. In 79 samples we found eutardigrades and heterotardigrades of the species *Milnesium tardigradum tardigradum* Doyère, 1840, *Hypsibius* cf. *convergens* (Urbanowicz, 1925), *Hypsibius* cf. *dujardini* (Doyère, 1840), *Diphascon (Diphascon) pingue* (Marcus, 1936), *Diphascon (Diphascon)* cf. *pingue* (Marcus, 1936), *Diphascon (Adropion)* prorsirostre Thulin, 1928, Diphascon *(Adropion)* cf. *mauccii* Dastych & McInnes, 1996, *Macrobiotus harmsworthi* group, *Macrobiotus hufelandi* group s.l., *Paramacrobiotus areolatus* group, *Paramacrobiotus richtersi* group, *Minibiotus* sp., *Echiniscus canadensis* Murray, 1910, *Echiniscus granulatus* (Doyère, 1840), *Echiniscus* cf. *granulatus* (Doyère, 1840), *Echiniscus merokensis merokensis* Richters, 1904, *Echiniscus cf. merokensis merokensis* Richters, 1904, *Echiniscus quadrispinosus cribrosus* Murray, 1907, *Echiniscus trisetosus* Cuénot, 1932, *Testechiniscus spitsbergensis* (Scourfield, 1897) and a new species of *Echnisicus*.

This first results of the Tardigrada inventory in the first European site reflects the high biodiversity due to the geographic positions of the territory and the alpine, mediterranean and ligurian climatic influences.

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A summary of the large-scale inventory of tardigrades in the Great Smoky Mountains National Park (NC/TN, USA)

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The multi-habitat inventory of tardigrades begun in 2000 in the Great Smoky Mountains National Park (GSMNP) is now complete. Habitats investigated included moss, lichen, leaf litter/soil, and streams. The resultant database consists of 780 samples, 15618 specimens, and 81 species including 15 new to science. The species list is presented with habitat associations. We also compare overall species richness of the GSMNP with other large scale inventories in Italy, Vancouver, Poland, Spain, and Costa Rica. Soil-dwelling tardigrades are compared with existing data from Japan using the Chao1 species richness estimate.

Costa Rican "bears" – what we do not know

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Costa Rica is a small Caribbean country (*ca.* 50 000 km^2) situated in Central America between the Caribbean Sea on the East and the Pacific Ocean on the West. On the north, Costa Rica borders on Nicaragua and on the south with Panama. Costa Rica is situated in seismically active area and as a result it is a mountainous region with many volcanoes. Because the country is one of the world's biodiversity hot spots, it is an interesting place for biological research. Moreover, thanks to a comprehensive programme of biodiversity conservation many unchanged (or only moderately affected) by humans areas are well preserved. As much as 27% of Costa Rica's territory are under various forms of environmental protection (e.g. National Parks, reserves, refugia, buffer zones). Based on climatic zones as many as 12 forest types have been defined.

So far, only some questions regarding Costa Rican tardigrades have been answered, e.g. we know that there are *ca.* 75 tardigrade species inhabiting mosses and lichens, moreover, for some of the species environmental preferences (such as altitude or forest type) have been established. However, virtually nothing is known about species living in soil, temporary waters and tree crowns. Also, preferences of the majority of species remain unidentified. The origins of Costa Rican tardigrade fauna are unknown too, with only a successful delineation of the fauna from both the Nearctic and Neotropical faunas. Nevertheless, without the detailed knowledge on tardigrades inhabiting all environments any analyses will be prone to a considerable error. A separate challenge constitute marine tardigrades of the Costa Rican shores that have not been studied at all. To sum up, our knowledge of the Tardigrada fauna of Costa Rica remains unsatisfactory and more research is desirable.

Invertebrates biodiversity of astatic waters of Costa Rica in relation to the hypothesis of "The Great American Biotic Interchange"

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Biodiversity, and especially its protection, became a particularly attractive topic after 1992 when the UN leaders at the Conference on Environment and Development in Rio de Janeiro worked out general rules for sustainable development, which included the development of national strategies for the conservation and sustainable use of biological diversity.

Tropical rain forests, jungles and coral reefs constitute one of the greatest centres of biological diversity. To date, 25 main centres of biodiversity have been identified, mainly in the tropical and subtropical regions. Costa Rica is placed within the internal part of one of the most important centres of biodiversity (Region: Mesoamerica). According to the predominant opinion, Central America owes its biodiversity to the mixed influence of zoographical elements of the neighbouring lands. An interchange of fauna took place approximately 3-5 million years ago, when a connection between the two Americas was formed. A corridor connecting previously isolated Neotropical and Nearctic faunas was created and a fast interchange occurred between them, known as the "Great American Biotic Interchange" (GABI).

Temporary water bodies belong to one of the most endangered ecosystems, quickly reacting to human activity. The following organisms: water bears (Tardigrada), wheel animals (Rotifera) and large branchiopods (Anostraca, Notostraca, Laevicaudata, Spinicaudata), which are characteristic taxa of astatic reservoirs, will be used as model groups.

The study aims at:

1. A verification of the hypothesis that a general schema of interchange of the Nearctic and Neotropic fauna elements (Great American Biotic Interchange) explains the distribution and taxonomic composition of invertebrates inhabiting astatic waters in Costa Rica.
2. Establishing to what extent Costa Rica constitutes a "biodiversity hotspot" in relation to invertebrates of astatic waters.

The project was funded by the Polish Ministry of Science and Higher education by a grant within the programme "Iuventus Plus" no. IP2010 015570: „*Bioróżnorodność bezkręgowców wód okresowych Kostaryki a hipoteza 'Great American Biotic Interchange*".

Diversity and abundance of tardigrades, nematodes and rotifers in different soil habitats of the Edmonson Point area (Northern Victoria Land, Continental Antarctica)

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Different soil habitats at Edmonson Point (74°20'S, 165°08'E) located in Wood Bay, Northern Victoria Land, Ross Sea Sector of the Continental Antarctic were investigated for the diversity and abundance of soil invertebrates. Forty-one soil samples were collected from five different soil habitats (barren, moss, cyanobacteria, and active and relict penguin rookeries) during two consecutive austral summers: 2003/04 and 2004/05.

Altogether 23 species of soil invertebrates taxa have been identified. Tardigrades were represented by two species: *Acutuncus antarcticus* (Richters, 1904) and *Milnesium antarcticum* Tumanov, 2006. Nematodes represented four species: *Eudorylaimus antarcticus* (Steiner 1916) Yeates 1970, *Panagrolaimus davidi* Timm 1971, *Plectus murrayi* Yeates & de Man 1970 and *Scottnema lindsayae* Timm 1971. Rotifers represented 17 species: *Adineta grandis* Murray, 1910, *Encentrum* sp.1, *Habrotrocha constricta* (Dujardin, 1841), *Habrotrocha* cf. *elusa vegeta* Milne, 1916, *Habrotrocha* sp. 1, *Habrotrocha* sp. 2, *Macrotrachela* cf. *insolita* De Koning, 1947, *Macrotrachela* sp.1, *Macrotrachela* sp.2, *Macrotrachela* sp.3, *Macrotrachela* sp.4, *Macrotrachela* sp.5, *Macrotrachela* sp.6, *Mniobia burgeri* Bartoš, 1951, *Philodina gregaria* Murray, 1910, *Philodina* cf. *australis* Murray, 1911, *Rotaria rotatoria* (Pallas, 1766).

In general, soil invertebrate communities were dominated by rotifers (mean = 981.9 individuals/100g soil) while nematodes (mean = 162.5) and tardigrades (mean = 112.4) were significantly less numerous. Abundances of all invertebrates varied and ranged from 0 to 7 760, 2 312, and 1 824 individuals per 100g of soil for rotifers, nematodes, and tardigrades, respectively. Invertebrates were completely absent in 22% of all soil samples, majority of which represented soils from active and relict penguin rookeries. Although the physical and chemical characteristics of the soil habitats differed, high soil water content (>20%) seemed to be the major factor determining distribution and abundance of invertebrates in the Antarctic soils.

Preliminary data on terrestrial tardigrade diversity and small-scale distribution in the park 'Yalivshchina' (Chernihiv, Ukraine)

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Despite significant progress in understanding tardigrade distribution patterns and environmental preferences over the past decades, there is still few answers to some important questions. Firstly, it is not clear, how many samples should be taken from a site, given the patchiness and possible seasonal variation of tardigrade distribution. Secondly, it is unknown, how long do the local tardigrade populations survive. To answer these questions and optimize tardigrade sampling protocol we started a long-term study of tardigrade small-scale distribution and now report the first results. A test site was established in 'Yalivshchina' park, Chernihiv, northern Ukraine. To date tardigrades samples have been collected three times, autumn 2009, summer 2010 and spring 2011. Moss and lichen patches (1 g. dry weight) were taken from the ground, tree base, and from tree trunks (up to the height of 2.5 m). Additional, occasional samples of soil and leaf litter were also collected. For each sample the numbers of individuals of each taxon were assessed. A total of 350 samples have been studied revealing ca. 11500 individuals, eggs and exuvia, belonging to 10 species of Eutardigrada. Total abundance of tardigrades was high and rather uniform in all categories of samples, except those of soil. However, species diversity and the number of individuals per sample were substantially higher in samples taken from trees.

Most samples taken from tree trunks contained numerous individuals of *Ramazzottius* sp. and *Milnesium* sp., while *Paramacrobiotus* sp. occurred mostly in ground mosses and leaf litter. Nevertheless, the most numerous species (*Hypsibius* and *Macrobiotus* spp.) have shown no association with a particular category of microhabitats. Our findings have confirmed the great patchiness of tardigrade species distribution within the sampled habitats. No substantial differences between seasons have been found except that specimen numbers of almost all tardigrade species was lower in summer 2010. An evaluation of the extraction techniques used across different substrates is presented, as well as a measure of the repeatability for the sampling results across the test site. The study will be continued for several years to estimate longer-term dynamics of tardigrade populations.

The impact of fire on terrestrial tardigrade biodiversity: a case-study from Portugal

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Nowadays, loss of habitat is the greatest threat to biodiversity, yet little is known about its effect on microscopic animal taxa, such as Tardigrada. One of the causes behind habitat destruction are forestal fires, both naturally occurring and manmade. The latter type is a very common method used in agriculture, as a way for killing insect plagues or for soil preparation as well as in conservation, being used for creating habitat mosaics. In Portugal, 42% of fire frequency is due to human activities. The impact of fires in biodiversity is not consensual, with studies pointing towards different conclusions. Different methodologies and target taxonomic study groups may partly explain this paradigm.

This study is a first approach to possible effects caused by habitat destruction on tardigrade populations. For this, we have analyzed the taxonomic and genetic variations of tardigrades from a fire affected location in a Portuguese natural park. Sampling was performed during a 10 year period, from 2000 to 2010. The location was affected by a small fire in 1998 and a big fire in 2003. A total of 12 species from nine separate genera were recorded, and 26 cox1 haplotypes were found.

Our data show a pattern which suggests a negative effect of forestal fire over tardigrade populations. Taxonomic and genetic richness, as well as animal abundance show lower levels in the years after a fire, when compared with the years that preceded it. Additionally, the population recovered visibly faster after the small fire than after the bigger one. This is consistent with the fact that bigger fires destroy larger forestal areas, leaving lesser animals available for re-colonization and at a longer distance. Most species found before the main fire are also found after it, indicating a high capability to re-colonize by these tardigrades. However, less than 10% of all recorded haplotypes were found both pre and post the main fire, which indicates genetic diversity loss by direct consequence of fire. Therefore, we conclude that habitat destruction by means of forestal fire has detrimental effect over tardigrade biodiversity, and may have similar effects over other small animals. More studying would be useful to elucidate this matter, focused on this and other understudied taxa.

A study into the dispersion pattern of *Milnesium* cf. *tardigradum*: A molecular phylogenetic approach

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Typical habitats of many terrestrial tardigrades (*i.e.*, interstitial spaces within mosses and lichens) are generally unstable environments, subjected to fluctuation such as repeated wetting and drying. Under the unsuitable environmental conditions that periodically come upon such habitats, many tardigrades enter into a dormant state, cryptobiosis, eliminating most of their metabolic activity. In this condition, they become 'tun' state in which they contract their body and appendages. It has been often considered that the tun has a high potential of passive airborne dispersal. However, any direct evidence of such wind facilitated dispersal has not been obtained yet.

From this background, we have been conducting a molecular phylogeographic analysis of a tardigrade in order to clarify the extent of the dispersion, utilizing the world wide distributed species *Milnesium* cf. *tardigradum*. Based on the comparison of genetic structure within and among local populations, we tried to examine the state of gene flow and the degree of genetic differentiation at both the intra- and inter-population levels.

About 230 specimens of *Milnesium* cf. *tardigradum* were collected from 40 localities throughout the Japanese Archipelago and the Korean Peninsula, and the total genomic DNA was extracted from each individual. As the result of the analysis of their nuclear ITS2 region and mitochondrial COI gene, nine genotypes and twelve haplotypes have been detected, respectively. It has been confirmed that the differentiation pattern of ITS2 genotypes and the COI haplotypes almost completely matched. Although three clades were identified with clear genetic difference, the degree of genetic diversity within each clade was extremely low. The dominant genotype was detected in about 78% of all of the examined specimens. This genotype was found among 35 localities, separated geographically by up to 1,300km or more. Furthermore, only a slight difference was genetically observed between populations. As the most remarkable case, the genotype detected from Asahikawa (Hokkaido Isl.) and Tendo, Yamagata (Honshu Isl.) populations was identical to the sequences registered in GenBank of a specimen collected in Tübingen, Germany. Although this might be an extremely rare situation, such an inter-continental long-range dispersal mechanism apparently exists.

Morphology, DNA barcoding and phylogeny of *Macrobiotus persimilis* and *Macrobiotus polonicus*

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Macrobiotus polonicus and *Macrobiotus persimilis* are two species of the "*Macrobiotus hufelandi* group" which are distinguished one from the other for the presence in *M. polonicus* of one lateral gibbosity on each hind leg, the size of cuticular pores (smaller and less evident in *M. polonicus*), the buccal tube size (larger in *M. persimilis*) and the egg processes (larger terminal discs and with more evident indentation in *M. polonicus*). However, the two taxa share peculiar characteristics: similar values of the pt index relative to the insertion point of the stylet supports on the buccal tube, first macroplacoid clearly longer than the second and with a central constriction, lunules of the hind legs clearly larger than those of the first three pairs and with an indented margin, egg shell smooth or with faint dots but without a reticular design. Therefore, it could be hypothesized that they belong to a single peculiar group, namely the "*Macrobiotus polonicus/persimilis* group".

In order to verify this hypothesis, a morphological and DNA barcoding analysis (*cox1*) was carried out on tardigrades attributable to these two taxa and coming from five different localities in Italy and France.

This study revealed differences, both morphological and molecular, between some populations apparently belonging to the same species, which in some cases turned out to be cryptic species. A phylogenetic analysis using rDNA 18S was performed in order to verify the relationships among the species of this group and more in general among those of the "*M. hufelandi* group".

The DNA barcoding analysis revealed a high divergence, with very high values of genetic distance among some populations (more than 18%). In one case (Enna, Sicily) at least two different and very distinct entities are present together. The morphological and morphometrical investigation, also based on type material, confirms this variety, showing that at least four species related to *M. polonicus* and three related to *M. persimilis* should be distinct. In several cases the relationship between morphology and cox1 sequence has been ascertained.

The dendrogram computed on 18S showed that there are two different sequences pertaining to the "*M. polonicus/persimilis* group" clustered in a single evolutionary line inside the main *M. hufelandi* cluster.

Identification of Hox genes in the draft genome sequence of *Ramazzottius varieornatus*

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Tardigrades are microscopic metazoans comprising a distinct phylum Tardigrada, harboring approximately 1000 described species. These animals are exceptional in their adaptation to the most extreme environments through the ability to enter a reversible ametabolic state known as cryptobiosis. However, their precise phylogenetic position is still debated, whether they are more closely related to arthoropods and onychophorans, or to nematodes and nematomorphs. To this end, here we report the identification of ANTP class *Hox* genes in the draft genome of *Ramazzottius varieornatus*. Hox genes, arranged in clusters and in collinear order with regard to temporal expression pattern, play pivotal roles in anteroposterior patterning by specifying positional information along the main body axis of animals. While the use of similarity search alone is not sufficient in the precise identification of these genes in *R. varieornatus* due to low sequence conservation to known *Hox*, we were able to identify six genes, namely, *Hox1*, *Hox3*, *Hox4*, *Antp* and two *Abd-Bs*, through careful study of the conserved sequence motifs located upstream and downstream of the homeodomain. Surprisingly, clustering of these *Hox* genes was not observed in this species, and the conserved set suggests that tardigrades are closer to arthropods rather than to nematodes. Furthermore, since *Abd-B* plays instructive role in male cell fate determination in somatic gonad, we also discuss the possible sex determination pathways of *R. varieornatus*.

Dating tardigrade evolution and early terrestrialization events

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The tempo and mode of cryptobiosis evolution within tardigrades are still unknown, but it is clear that comprehending this process is the key to better understand evolutionary history and ecology of this phylum, and the process of animal terrestrialization, given the ubiquitous continental distribution of tardigrades. Our study was mainly aimed at timing tardigrade radiation and key events in tardigrade evolution. Given the existence of two lineages of continental tardigrades, i.e. Eutardigrada and Echiniscidae, we decided to implement a relaxed molecular clock based approach to attempt to derive a minimal time interval for tardigrade terrestrialization.

Data from new and Genbank partial 18S and 28S rDNA sequences of 41 specimens, belonging to 31 species from 26 genera, representing all known tardigrade orders and superfamilies, were acquired. Phylogenetic inference was achieved using Bayesian mixture models that have allowed the most accurate estimates of evolutionary rates. The following molecular clock analyses were then based on 3 tardigrade calibration points derived from fossil records and improved by the addition of 5 new calibrations points pertaining to Arthropoda, Priapulida and Kinoryncha, emerged from paleontological studies. To clarify the evolutionary history of cryptobiosis, and to evaluate whether its origin might have played a role in the process of tardigrade terrestrialization, a maximum--likelihood based ancestral character state reconstruction was used.

Molecular phylogeny analyses yielded a robustly supported and well-resolved evolutionary tree for all considered tardigrade taxa. Our results suggest the origin of the tardigrade stem group in the Ediacaran age (~620 Million years ago -Mya), with the two major extant tardigrade lineages (Eutardigrada and Heterotardigrada) split quite recently ~443 Mya. Ancestral character state reconstruction indicated a probability of ~30% for cryptobiosis to have been present in the last common ancestor of Heterotardigrada and Eutardigrada. Hence, it is most likely that this adaptive trait evolved twice independently within this phylum. Cryptobiosis most likely emerged in the time interval between 443 and 359 Mya, in the stem Eutardigrada lineage that existed for ~84 My. This is in accordance with available fossil evidence suggesting terrestrial ecosystems first flourished in the Silurian. The second independent origin (within Heterotardigrada) was within the Echiniscoidea clade and can be dated to an interval included between 238 and 141 Mya (Middle Triassic to early Cretaceous). In both cases, existence of a link between cryptobiosis and terrestrialization was clear. All the eutardigrade superfamilies originated within a short-time span of ~257-200 Mya, suggesting an early Mesozoic eutardigrade radiation. Moreover, within each parachelan superfamily a great mid Jurassic to Early Cretaceous process of diversification was recorded.

Tardigrades and emergent clades: a new framework

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This first Hennigian analysis of the Tardigrada as a whole provides a fully resolved cladogram for 13 terminal higher taxa. The 78 morphological characters obtained from the primary literature produced the following system with Hennig86 (c.i. 0.81, length 83 steps, for 59 unordered transformation series): Pan-Tardigrada (unnamed Cambrian larvae + Tardigrada (Neoarctidae + **Archaeotardigrada nov. (Macrocephala nov.** (Stygarctidae + **Digitopoda nov.**) + **Prototardigrada nov.** (Coronarctidae + **Paratardigrada nov.** (*Anisonyches* + **Hemitardigrada nov.** (Echiniscoididae + **Limnotardigrada nov.** (Oreellidae + **Neotardigrada nov.** (Echiniscidae + ***Metatardigrada nov.*** (Carphanidae + **Holotardigrada nov.** (Mesotardigrada + Eutardigrada (Apochela + Parachela))))))))))).

The traditional higher taxa Heterotardigrada, Arthrotardigrada, and Echiniscoidea are not supported. The Macrocephala contain the main pool of diversity of the marine tardigrades, reuniting 37 genera with four head metameres. From these taxa, 32 genera with digitate claws comprise the Digitopoda. The other main pool of tardigrade diversity (Prototardigrada), with 65 genera, contains the single major adaptive radiation of continental forms (Limnotardigrada), with 61 genera.

Key words: Tardigrade system; phylogenetic analysis; molting animals; water babies; systemic approach; post-modern perspective; Hennigian method.

Comparative analyses of the cuticular and muscular structures of the buccal-pharyngeal apparatus of tardigrades

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In spite of the great importance of the tardigrade buccal-pharyngeal apparatus in taxonomic and phylogenetic studies, this structure received little attention as regards its evolution and operating mechanism. To understand the relationships between form and function of the structures of buccal-pharyngeal apparatus (i.e. cuticular structures, muscular fibers, pharynx), and to increase our knowledge on this apparatus, a comparative analyses using different investigation techniques was performed.

The buccal-pharyngeal apparatuses of three species have been studied, *Echiniscus trisetosus*, *Milnesium tardigradum* and *Paramacrobiotus richtersi*, as representative of the two classes and of three orders of tardigrades. The cuticular structures of the buccal-pharyngeal apparatus have been analyzed from a morphological (light microscopy, scanning electron microscopy – SEM, and confocal laser scanner microscopy -CLSM) and chemical (energy dispersive X-ray spectroscopy) point of view. The musculature associated to the sclerified structures of the buccal-pharyngeal apparatus has been analyzed by CLSM to identify the muscular fibers and their relationships with the cuticular structures.

The differences in the general anatomy of the buccal-pharyngeal apparatuses among the three species were high, even though homologous structures were recognizable. The higher differences among species were found in the organization of the muscular system responsible of the stylet movements. The detailed analyses of the buccal-pharyngeal apparatus allowed a new interpretation of the stylet system organization, and to understand the muscular system related to the feeding.

The chemical analyses showed that the piercing stylets were formed by calcium, in form of $CaCO_3$. Heterotardigrada were differentiated from Eutardigrada for the presence of high concentration of $CaCO_3$ encrustations in the buccal tube. Within Eutardigrada, Apochela differs from Parachela since they are characterized by the absence of $CaCO_3$ in the buccal tube.

The fine structure of the midgut epithelium in *Xerobiotus pseudohufelandi* (Iharos, 1966) (Tardigrada, Eutardigrada, Macrobiotidae) with the special emphasis on midgut degeneration

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The digestive system of *Xerobiotus pseudohufelandi* (Eutardigrada, Macrobiotidae) is composed of the foregut, midgut and hindgut. Midgut epithelium of both females and males is formed by flat or cubic digestive cells. Regionalization in organelles distribution, which is commonly observed in midgut epithelia of invertebrates, has not been found. The cytoplasm of the digestive cells possesses numerous electron-dense and electro-lucent granules. Histochemical studies have revealed that mainly lipids with small amounts of sugar and proteins form them. The amount of the storage material is the same in females and males. The relationship between the storage material and stages of oogenesis has been analyzed in order to decide if the midgut epithelium of *X. pseudohufelandi* plays a role in vitellogenin synthesis. The cytoplasm of the digestive cells is rich in small and large multivesicular bodies which take part in water accumulation - *X. pseudohufelandi* lives in a dry environment that is devoid of water. The number of multivesicular bodies in females was higher what might be caused by the fact that water is necessary for many metabolic processes including the process of vitellogenin synthesis. One of the processes which commonly occurs in the cytoplasm of digestive cells is autophagy, which is responsible for cell survival. However when many degenerated mitochondria appear in the cytoplasm, the apoptosis is activated. Eventually the apoptotic cell is discharged into the midgut lumen.

Morphometric differentiation of Spitsbergen populations of *Macrobiotus islandicus islandicus* Richters, 1904 (Eutardigrada, Macrobiotidae)

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Spitsbergen is the largest island of the Svalbard Archipelago. One of the most abundant species found in many localities on Spitsbergen was *Macrobiotus islandicus islandicus* (Richters, 1904). In the present study we tested for intraspecific differences in taxonomically important morphometric traits between populations of *M. islandicus islandicus* from different localities. We assayed three different populations from Spitsbergen (Revdalen (67 m asl), Rotjesfjellet (201 m asl) and Fugleberget (569 m asl)) and one from Greenland.

All morphometric traits were highly correlated with each other (all $p<0.001$). Such co-linearity prevented their joint inclusion in the analysis. Therefore, we calculated a "body size index (BSI)" using a Principal Component Analysis (PC). Obtained BSI expressed as PC1 was correlated negatively with all measurements (all $p<0.0001$) and explained 89% of variance. Next, to test for differences in the BSI between populations analysis one-way ANOVA was used.
We found that specimens in populations from higher altitudes (lower average temperatures) were lower BSI index than populations from lower altitudes (higher average temperatures). The results correspond well with Bergmann's rule for invertebrates.

Bearly There: Differences in tardigrade muscular organization reflect locomotory adaptations to different habitats

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The general body plan and muscular organization of several marine heterotardigrades and a limnoterrestrial eutardigrade, *Isohypsibius* sp., were examined and compared. Specimens were analyzed with scanning electron microscopy and also stained with Alexa Fluor® 488-conjugated phalloidin for use in visualizing the musculature with confocal laser scanning microscopy. A 3-dimensional reconstruction of the muscular system was performed for each species and analyzed using Volocity 5.0 software package and ImageJ v1.46i. The muscular organization of *Batillipes mirus* revealed by CLSM is largely consistent with Marcus' (1929) original description, with some minor variations. Within *Batillipes*, more notable deviations exist between *B. mirus* and *B. pennaki*, where the latter species has two pairs of dorsal longitudinal muscles that span the length of the body compared to only one pair in *B. mirus*. Several differences are also present between species of Batillipes and the eutardigrades examined thus far; the most notable difference occurs in the 4th pair of legs. *B. mirus* has significantly more leg muscles on the ventral side of the appendages than Isohypsibius, while the number of dorsal leg muscles is similar. We hypothesize that this difference in the ventral leg muscles is functionally correlated to their methods of locomotion. Species of *Batillipes* regularly use their 4th pair of legs for rearward movement and for gaining anchorage to the substrate, while species of *Isohypsibius* do not engage in such locomotion. Neither species appear to use their 4th pair of legs while crawling forward. Based on these and other observations using both videomicroscopy and SEM, we hypothesize that the organization of the tardigrade muscular system has strong potential for future analyses of functional morphology regarding both locomotion and feeding, and once taxon sampling has increased, may provide additional evidence for inferring phylogenetic relationships.

Comparative study on tardigrade myoanatomy: the use of muscular body plans in tardigrade phylogeny

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Molecular phylogenetics is a growing discipline in tardigrade research; however, comparative morphology is still fundamental in tardigrade phylogeny, and structures such as the bucco-pharyngeal apparatus, cuticular ornamentation as well as claw and egg morphology are important diagnostic characters.

In order to expand the current morphological framework, and to promote the use of muscular body plans in elucidating tardigrade phylogeny, the organization of the musculature of *Actinarctus doryphorus* Schulz, 1935 (Arthrotardigrada), *Echiniscoides sigismundi* Plate, 1889 (Echiniscoidae) and *Richtersius coronifer* Richters, 1903 (Parachela) were examined by means of fluorescent coupled phalloidin in combination with confocal laser scanning microscopy and computer aided three-dimensional reconstruction. Our data confirm that the tardigrade musculature is organized into structurally independent muscle fibers, which can be divided into a dorsal, ventral, dorsoventral and a lateral musculature, in addition to a distinct leg musculature. However, when comparing our data with existing information on both heterotardigrade and eutardigrade species [1, 2, 3], it becomes apparent that tardigrades differ in significant details of their myoanatomy, and that these differences may be informative on both higher and lower taxon levels. Specifically, the dorsal leg musculature, the number and arrangement of individual dorsal attachment sites as well as the lateral muscles may be informative on a generic level (variation between members of *Richtersius, Hybsibius* and *Halobiotus*), the number of ventral and intermediate ventral attachment sites may be informative on family level (revealed by comparing members of Milnesiidae, Macrobiotidae and Hypsibiidae), whereas the relative position of ventromedian attachment sites as well as the associated ventral leg musculature appears to be informative on higher taxon level (as evidenced by differences between Heterotardigrades and Eutardigrades). In addition, cross striation of the somatic musculature is especially pronounced in Arthrotardigrada (based on *A. doryphorus* and *B. pennaki*). Accordingly, the current study supports the use of muscular body plans in resolving tardigrade phylogeny, but emphasizes the need for additional high-quality data.

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Proteomic analysis of chromatin fraction in an anhydrobiotic tardigrade, *Ramazzottius varieornatus*

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Tardigrades can tolerate a wide range of extreme environments including high dose of irradiation in anhydrobiosis. Dehydration itself provides a protection from radiation by preventing a formation of reactive radicals from water. Recent analyses revealed that some tardigrades could tolerate high dose of irradiation even in hydrated state with similar extent to that in dehydrated state, suggesting the presence of active mechanisms providing protection of biomolecules from radiation or repair of radiation-induced damages. Molecular basis for these mechanisms, however, are totally unknown. DNA is one of the main targets of radiation and especially double strand breaks of DNA cause severe effect on survival. To identify molecules involved in protection of DNA and/or repair of damages by radiation, we performed selective proteome analysis of chromatin fraction using *Ramazzottius varieornatus*. Based on the primary structure of proteins detected by MS/MS analyses, several candidate genes were selected and introduced to cultured cells as GFP-fusion protein to visualize their subcellular localization. One of them, designated S261, showed almost perfect colocalization with whole genomic DNA. Although BLASTP search and Pfam motif search were performed, neither similar sequence nor known motif was found. Thus, S261 would be a novel protein. As isoelectric point is highly basic, S261 could directly bind to DNA. Considering no similar genes in other phyla and subcellular localization pattern, S261 could be a good candidate protein contributing to tardigrade-unique mechanisms for protection or repair of DNA.

Two families of novel abundant heat-soluble proteins in an anhydrobiotic tardigrade

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The molecular basis of anhydrobiotic ability of limno-terrestrial tardigrades is hardly understood. Trehalose has long been suggested to play an important role in desiccation tolerance in other anhydrobiotic nematodes, insects and crustaceans. However, tardigrades accumulate much less trehalose in anhydrobiotic state, which suggests that tardigrades may apply other molecule to tolerate dehydration. Another molecular candidate is late embryogenesis abundant (LEA) protein family. LEA proteins retain the water-soluble state even after the boiling treatment and are proposed to prevent protein aggregation in other anhydrobiotic organisms. In tardigrades, heat-soluble proteins such as LEA have not been found previously.

Here, we identified five major heat-soluble proteins from an anhydrobiotic tardigrade, *Ramazzottius varieornatus*. All of them turned out to be poorly-matched with LEA proteins in primary sequences, and formed two distinct novel protein families with different subcellular localizations, Cytoplasmic Abundant Heat Soluble (CAHS) and Secretory Abundant Heat Soluble (SAHS), both of which are conserved in tardigrades. Furthermore, both proteins changed their conformation to alpha-helical structure upon water-deficit. These findings suggest that tardigrades may evolve distinct protein family in order to tolerate dehydration.

Two tardigrade species on board of the STS-134 space flight

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The TARDIKISS project (Tardigrades in Space) investigated the effects on physiology and molecular aspects of space stresses on live desiccated tardigrades, as representatives of multicellular organisms. It was part of BIOKIS (Biokon in Space), a set of multidisciplinary experiments of DAMA (DArk MAtter) mission on board of STS-134 space flight, the last of the shuttle Endeavour, in the frame of a joint between Italian Space Agency (ASI) and Italian Air Force.

In TARDIKISS, experimentally desiccated (anhydrobiotic) specimens of *Paramacrobiotus richtersi* and *Ramazzottius oberhaeuseri* have been used. Both species have very good anhydrobiotic ability, but differ for several biological and ecological characters. The experiment units of TARDIKISS were hosted into the Biokon, a standard transportable container designed and manufactured by Kayser Italia. In May 2011, the Biokon containing the TARDIKISS experiment unit was integrated on Middeck Locker of International Space Station (ISS) and flown for 16 days at Low Earth Orbit. Two sample sets were used as controls: the former (Laboratory Control) was maintained in Modena laboratory for the duration of the flight, and the latter (Temperature Control) was a post-flight control in which samples were exposed to the temperature profile experienced by tardigrades the days immediately before, during, and just after the flight mission.

For both species, the flight animals did not show differences in survival with regard to Laboratory control and Temperature control animals. Only in *R. oberhaeuseri* differences have been recorded between Flight and Temperature Control samples. Live specimens of *P. richtersi* from Flight samples, Laboratory control and Temperature control samples have been reared in lab. Flight females laid eggs with normal shape; several eggs have been able to hatch, and newborns exhibited normal morphology, behaviour and, when adult, normal capability to reproduce. Moreover, a comparative analysis of the antioxidant metabolism between Flight samples and Temperature control samples has been done. No differences have been evidenced between the two groups, with the exception of the reductase activity: significant differences in reductase activity between Flight and Temperature control were evidenced ($p<0.05$) in *R. oberhaeuseri*. These first results lead us to deduce that during the DAMA mission, microgravity and cosmic radiations did not significantly affect survival of tardigrades in flight, confirming that tardigrades represent a useful animal tool for space research.

Freezing tolerance in the cryoconital tardigrade *Hypsibius klebelsbergi*

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The cryoconital tardigrade *Hypsibius klebelsbergi* Mihelčič, 1959 live in water-filled small holes on surface of glaciers. These small habitats originate by the melting of surface ice caused by fine dark inorganic and organic debris under the impact of solar radiation. This cryoconite holes are quite unstable habitats where *H. klebelsbergi* experience extreme environmental conditions such as subzero temperatures, but they are well adapted to great daily temperature fluctuations without dehydration. Survival of subzero temperatures in an active state requires either the ability to tolerate the freezing of body water or mechanisms to decrease the freezing point. We studied the survival rate of this tardigrade species originating from different glaciers of the Alps in Austria by different cooling rates, and measured the supercooling points by Differential Scanning Calorimetry (DSC). These results expand our current understanding of freeze tolerance in tardigrades and will lead to a better understanding of their ability to survive subzero temperature conditions.

Raman imaging study on living tardigrades: origin, nature and function of pigments in *Echiniscus blumi*

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Tardigrades exhibit a variety of pigmentations. Several species are translucent or whitish, but many others are markedly colored with red-orange, brown, green and yellow pigments. These pigments may be found in the body cavity, storage cells, epidermis, eye-spots and, according to literature, in cuticle. The genus *Echiniscus* is composed of many species. Despite occurrence of pigments is well documented for many of them, the chemical nature, source and function (especially in relation to their resistance to harsh physical and chemical conditions) of these pigments remain unknown. Some hypotheses were formulated about them, but to the best of our knowledge no direct and conclusive experimental proof has been reported to date. Therefore, we are taking this opportunity to attempt a resolution for these open questions using Raman spectroscopy on living individuals of *Echiniscus blumi*.

Raman spectroscopy is a non-destructive and (semi)-quantitative analytical technique based on scattering laser radiation by vibrating molecules, which proved to be an ideal tool for studying living cells and biological tissues. Moreover, by incorporating Raman micro-spectroscopy it is possible to obtain an image of the spatial distribution of the main biochemical constituents of a biological sample (i.e. Raman mapping or imaging).

In our analyses, pigments in *E. blumi* are identified as carotenoids. Their spectra well match those available in literature for β-carotene, zeaxanthin and β-cryptoxanthin, all having 11 conjugated C=C bonds, but not that of lutein, which has only 10. Previously reported chemical reactivity data of *Echiniscus* pigments suggest β-carotene as the most likely candidate pigment in the genus. Moreover, pigment distribution within the animal body cavity is imaged with Raman mapping. The dietary origin of the pigments (from the moss, *Grimmia orbicularis*) is demonstrated, as well as their presence in the eggs and in eye-spots, and their absence in the animal cuticle. Using *in-vivo* Raman imaging, a decrease in carotenoid content is detected after the induction of oxidative stress on animals, supporting the hypothesis of an antioxidant function of these pigments during anhydrobiosis.

Considering the lack of methods to directly study antioxidant function of carotenoids *in vivo*, pigmented tardigrades, investigated with Raman imaging, could be used as model organisms for this purpose opening new perspectives of research in living organisms.

Aquaporin channel proteins in the tardigrade *Milnesium tardigradum*

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Tardigrades and other small invertebrates are known for their capability of surviving complete desiccation by undergoing anhydrobiosis. This evolutionary adaptation has allowed them to colonize extreme habitats in which they are frequently subjected to drastic changes in water availability. During the course of desiccation and rehydration their cellular water content changes drastically. These changes in water content are likely to be tightly regulated to ensure survival of the organisms during anhydrobiosis. We therefore chose to study aquaporins (AQP) in tardigrades as they are likely to be involved in such physiological adaptations. Aquaporins and aquaglyceroporins are a ubiquitous family of channel forming membrane proteins that facilitate the flow of water and other small solutes across cellular membranes. The have been found in prokaryotic as well as eukaryotic organisms. Here we present the first study on AQPs in the limno-terrestrial tardigrade *Milnesium tardigradum* (Doyère, 1840). Using a combination of data mining, targeted sequence assembly and cDNA cloning we have isolated and characterized eleven AQPs, of which seven contain two canonical "asparagine-proline-alanine" (NPA) motifs in their pore region, three have at least partially deviating pore regions and one belongs to the class of aquaporin-like proteins. Sequence and structure analysis of these proteins predict, that most of the identified channels are putative aquaglyceroporins, allowing the passage of larger solutes such as glycerol. Using quantitative real--time PCR we analyzed the transcriptional regulation of these AQPs in three different stages. To some extent, *M. tardigradum* AQPs show expression changes during dehydration, rehydration or both. Interestingly, tardigrades seem to possess a similar number of these channel proteins as the nematode *Caenorhabditis elegans*. As both organisms are capable of anhydrobiosis and inhabit similar environments, a large repertoire of AQPs might be favorable in efficiently regulating cellular water content when subjected to desiccation.

Life-history traits in the tardigrade species *Paramacrobiotus kenianus* and *Paramacrobiotus palaui*

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Until now more than one thousand tardigrade species are described, including approximately 700 living in limno-terrestrial habitats. Although tardigrades were studied for a long time there is still less known about their way of life, and until now only few studies are dedicated exclusively to tardigrade life history traits. It is known that reproduction modes of tardigrades include parthenogenesis and sexual reproduction, however the type of reproduction varies not only between the species but also between populations of the same species. Improvement of rearing methods during the last decade resulted in more detailed life cycle studies for parthenogenetic tardigrades. The present work is presenting life history traits in two populations of the african tardigrade species *Paramacrobiotus kenianus*, and also in the species *Paramacrobiotis palaui* from the pacific islands of Palau under laboratory conditions. Animals were examined analysing the following life--history traits: active life span, age at first oviposition, egg-laying intervals, clutch size, hatching time and hatching percentages. The received data supplement our knowledge of tardigrades in general and enable further research into factors, which may influence life history of these species.

Comparison of different methods for quantitative extraction and sample handling of soil Tardigrada

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Quantitative analysis of soil tardigrades is dependent on relevant method of extraction organisms. Different authors use several methods, which never has been compared yet. This study compares four recently used methods of extraction and their modifications. Extractors based on own activity of animals under light and temperature gradient (L-C extractor, Baermann funnel, high-gradient funnel) as well as flotation method were used. Base of L-C extractor was cooled board kept at constant temperature in plexiglas box supported by source of cool light of luminosity 1600 lm. The soil samples were placed on cellulose covered sieve and put on a petri dish filled with distilled water on a cooled board. The high-gradient funnel was a Baermann funnel in plexiglas box with a common light making strong light and heat in the upper part and cooled to 10 °C in lower part. The samples were placed on cellulose covered sieve and put into a funnel filled with distilled water. There was a constant temperature in this box. The centrifugal - flotation methods was compared. Both temperature and a light were determined as important factors for efficiency of extraction. Preliminary results shows, that the method, which gives the highest extraction results, was the high gradient funnel in contrast to inappropriate centrifugal-flotation method and Baermann funnel which looks to be inappropriate for quantitative analysis.

The heat is on – different staining protocols for tardigrades

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Immunohistochemistry (IHC) in conjunction with confocal laser scanning microscopy normally is an easy way to get information of the nervous system and the musculature. In small animals, that can be used as whole mounts, IHC can give an overview of the nervous system and the musculature in relation to the whole body. It is therefore less labour-intensive than the usage of traditional histology and the access to required equipment is still wider spread than the access to micro--computed tomography (µ-CT). The gained information from IHC is suitable for morphological descriptions and in a further attempt can be applied to phylogenetic discussions. As stated above, especially due to their size and often easy penetrable cuticle microscopical small animals usually are perfectly suited for the method of immunohistochemistry. Not so tardigrades! Due to their armoured cuticle and other black box mysteries it is very hard to stain the nervous system of tardigrades. Nevertheless, did we take the challenge! Different staining protocols were used to get closer to the "perfect" staining. Here we present some results of different staining protocols and try to show which parameters have more or less impact and which procedures are obligatory. It appeared that the most crucial points are the permeability and the temperature at which the stainings were operated. Experience showed that individuals of the Heterotardigrada are even more difficult to stain than individuals of the Eutardigrada. Therefore we used the heterotardigrade *Echiniscus testudo* that can easily be found in moss around Hamburg.

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ADDITIONAL INFORMATION

IMPORTANT PHONE NUMBERS

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Police: 112

Taxi Service: 00 351 965 809 968

Parque Biológico: 00 351 227 878 120 (24h)

Organizing Committee (for urgent contact): 00 351 917 888 272

MAPS

How to arrive to the Parque Biológico