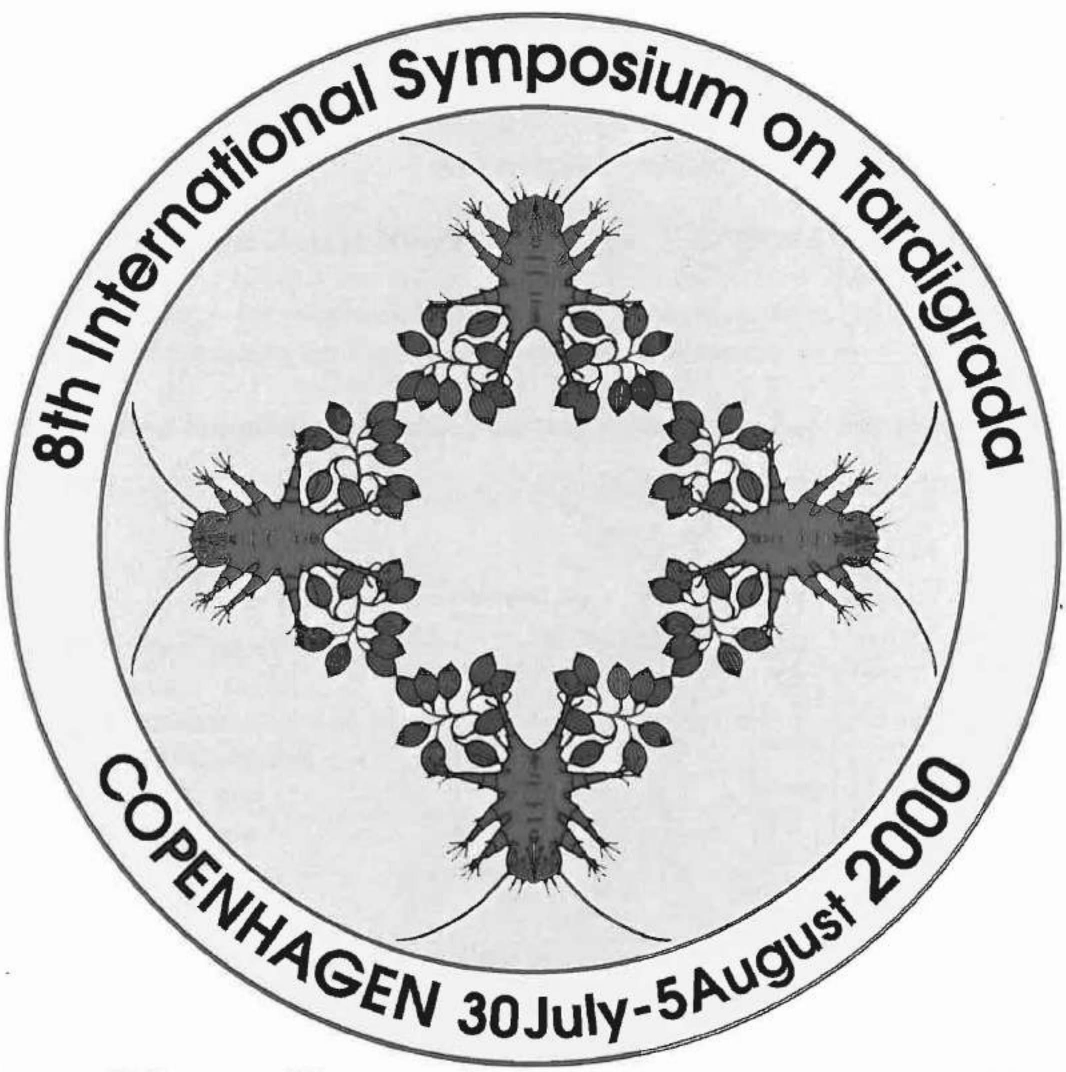
# PROGRAMME AND ABSTRACTS

## THE 8TH INTERNATIONAL SYMPOSIUM ON TARDIGRADA, COPENHAGEN, DENMARK 30 July - 5 August 2000




Zoological Museum, University of Copenhagen
Universitetsparken 15
DK-2100 Copenhagen Ø, Denmark

**Frontpage:**
The logo is made by the scientific illustrator Mrs. Birgitte Rubæk. Zoological Museum.
The four tardigrades are a new species of *Tanarctus* with bouyant bodies.

**Editor:**
Jesper Guldberg Hansen, Zoological Museum, University of Copenhagen.

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Copenhagen Ø, Denmark

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# 8th International Symposium on Tardigrada

Copenhagen, 30 July - 5 August 2000

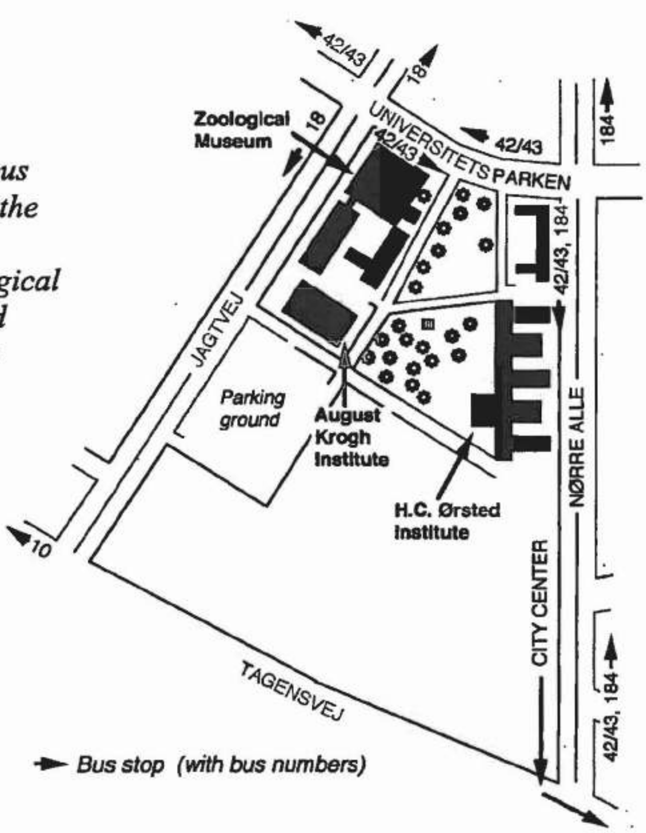
## Contents



## Acknowledgments


We are thankful to the August Krogh Institute, the H.C. Ørsted Institute and the Zoological Museum (ZMUC) for hosting the symposium. We especially thank the staff of the Department of Invertebrate Zoology for secretarial support, Mr. Jens Mogensen for book-keeping and Mrs. Birgitte Rubæk for creating the logo. The symposium was sponsored by:

**Carlsberg Foundation, Danish Research Agency, Dr. Bøje Benzons Støttefond.**


*Map of University Campus showing the location of the August Krogh Institute (lecture Hall), the Zoological Museum (Workshop) and the H.C. Ørsted Institute (cafeteria for lunch).*

## Foreword and general information

Copenhagen, 19. july 2000

Dear colleague,

Thank you for your participation in the Eigth International Symposium on Tardigrada. Like its predecessors, this symposium presents oral presentations and posters on a wide variety of tardigradological themes. Fifty four abstracts have been received. While structuring the scientific programme it appeared that the invited and registered oral presentations could be grouped into 12 main themes (sessions) which might have been given more or less fanciful names by us. The two poster sessions have mixed themes and therefore we have not given them any scientific names. The present booklet contains the final programme and all abstracts received, as well as various information which you will need when you arrive in Copenhagen. Furthermore, towards the back of the booklet we have included the programme of "Workshop on Arctic Tardigrades". We hope to have covered everything but please do not hesitate to contact the organizers if you need more information.

## General information

**Homepage**

The second and final Announcement will be available on the homepage of the Zoological Museum, University of Copenhagen under http://www.zmuc.dk until 5 August and will then be deleted. It will not be updated!

**Date and place of the Symposium**

The symposium takes place from Sunday, 30 July to Saturday, 5 August 2000 (both days included). The scientific sessions will be held in the big lecture hall of the August Krogh Institute, Universitetsparken 13, 2100 Copenhagen Ø

**Dates and place of Workshop on Arctic Tardigrades**

*Planning Meeting for Participants:* Sunday, 6 August 2000 at 10:00 am: Zoological Museum, Universitetsparken 15.

The Workshop on Arctic Tardigrades will take place at Arctic Station, Qeqertarsuaq, West Greenland. We leave Copenhagen in the evening Monday, 7 August and return to Copenhagen in the evening Friday, 18 August 2000.

**Correspondence**

Please address all correspondence concerning registration, scientific programme, presentation of papers and posters, excursion, Workshop on Arctic Tardigrades and proceedings to:

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**How to reach Copenhagen and the August Krogh Institute**

Copenhagen's International Airport Kastrup is connected with direct flights to numerous cities worldwide. The airport is situated quite close to the city center. The train departs directly from Terminal 3, International Arrivals, and takes you to the Central Railway Station (Hovedbanegården, 16 DKK if you buy a 2-zone 10-trip card, see below). Alternatively, you can board the ordinary bus (<30 mins by bus) line 32 to the City Hall Square (Rådhuspladsen, not far from the Central Railway Station, same price as above).

To reach the August Krogh Institute from the Central Railway Station, take the urban train ("S-tog") to Nørreport. Nørreport is the second station from the Central Railway Station and is served by all trains heading towards Holte, Hillerød, Hellerup, Farum and Klampenborg. From Nørreport enter bus 42 or 43 towards Bagsværd or bus 184 towards Kokkedal. Ask the driver for Zoologisk Museum/Universitetsparken (a city map will be given to you together with the receipt for your payment).

For the local train and buses you can buy 10-trip cards ("Rabatkort"). The price depends on the number of zones you have to cross. To get from the center of the city to the August Krogh Institute, a 2-zone card (blue, 80 DKK) is sufficient. "Rabatkort" can be bought at the railway stations, including the one in Kastrup, and in most kiosks and supermarkets. If you use the train or bus 32 from the airport to the City Hall Square, you must punch your 2-zone card twice.

Participants arriving by car will be pleased to know that there is a big parking lot next to the August Krogh Institute (no parking fee).

**The weather in Copenhagen in July-August**

The Danish summer is known for its unpredictability, anything from bright sunshine to heavy rainfall is possible. The temperature varies between 12-25°, and at night it may feel rather cold.

**Contributed lectures and posters**

Participants were invited to present lectures or posters on all aspects of Tardigrada. Furthermore a few invited lectures are included on following aims: Molecular Biology, General Phylogeny, Fossil Proarthropoda, Onychophora and Cryptobiosis. Overhead and slide projectors will be available in the lecture room. "Power point" and computers will only be available if you specificly ask for it. Lectures and posters must be presented in *English*. See, however, below under "Proceedings".

**The Zoological Museum**

The public exhibition of the Zoological Museum will be open without charge for symposium participants wearing their badge from Tuesday 1 July to Friday 4 August, 1100-1700. Participants wishing to study the scientific collection of the museum should preferably indicate their interest before their arrival at the symposium.

**Meals**

Lunch: The symposium lunches will be held at H.C. Ørsted Institute. They are included in the symposium fee. If spouses want to join us they have to pay 50 DKK per lunch.

There are very few restaurants or café's close to the university campus, although there are plenty in central Copenhagen.

The symposium dinner will be held at **Tivoli Gardens**. Price per person: 300 DKK (see registration form).

**Excursion**

A whole-day excursion (lunch included) will be arranged to various localities on the island of Zealand, including Louisiana (Museum of Modern Art), Helsingør and Kronborg (Hamlet's Castle), and Frederiksborg Castle and Gardens (former Royal residence, now museum).

**Louisiana** is an internationally renowned museum of modern art, placed in beautiful and pictorial surroundings. It was built in 1958 and is an example of modern Danish architecture. The permanent exhibition is Danish Art after the Second World War, supplemented by changing exhibitions of the highest quality.

**Kronborg Castle**, rebuilt 1574 on the foundations of the old Elsinore Castle (Krogen), is the setting of Shakespeare's Hamlet. It was thought to be a fortification of the narrow seaward approach and a protection of the 'Sound Duty', a duty paid by any ship entering the sound. Even today remains of the medieval Krogen can be seen in the walls, and the road still leads through a system of fortifications. The castle today houses the Danish Maritime Museum and is situated at Helsingør.

**Helsingør**, an interesting old town with outstanding buildings dating back to the Middle Ages, like St. Olai's Church and the Carmelite Monastry from ca. 1400, the best preserved of the North, and the adjacent Carmelite Hospital, today the Town Museum.

**Frederiksborg Castle**, Denmark's most magnificent Renaissance castle, rebuilt 1620-22 by Christian IV, now housing a historical collection of paintings and portraits from the last 400 years. The Baroque Gardens were reconstructed during the 1990's and are well worth a visit.

For further reading a guide 'Tourist in Copenhagen and North Zealand' are included in your welcome package.

**Proceedings**

The proceedings of the symposium will be published in Zoologischer Anzeiger. Manuscripts must be submitted to R. M. Kristensen no later than 4 November (**new deadline**) and must conform to the following standards:

-The text must be written in English. A summary in another major language may be included. Captions for illustrations and tables may also be submitted in English + the language of the summary.

-Each contribution must take up no more than 20 printed pages (including illustrations and tables). A page in Zoologischer Anzeiger will hold approx. 950 characters.

-The "Instructions to authors" (see the enclosed page from Zoologischer Anzeiger) must be strictly adhered to.

-The original paper version of the manuscript (including illustrations and tables) must be submitted together with two copies *and with a PC-disk containing the manuscript.* Please include two versions of the manuscript on the disk: one version in a word-processing language (WordPerfect is preferred) and one version in pure ASCII text.

All manuscripts will be sent out for peer review, and authors may be requested to make substantial changes to their manuscripts. Manuscripts may even in extreme cases be rejected. Authors with a native language other than English should seek professional linguistic help.

Looking forward to receiving your manuscript, we are pleased to welcome you in Copenhagen,

**The organizing committee**

Reinhardt Møbjerg Kristensen Hans Ramløv Jette Eibye-Jacobsen

Aslak Jørgensen Anne Marie Richardy Warfield

# Programme of Speakers and Posters

For co-authored papers the underline name is the presenting author. The number in **bold** is the abstract in the alphabetic order after the first author.

**Sunday 30 July 2000**

| | |
|---|---|
| Arrival: | Kastrup Airport - Copenhagen Hotels and Youth Hostel |
| 19.00 - 20.00 | Start mounting posters - August Krogh Institute |
| 20.00 - 22.00 | Video from the Ice Cap of Greenland and Icebreaker Party - August Krogh Institute |

**Monday 31 July 2000**

| | |
|---|---|
| 09.00 - 10.30 | Registration at August Krogh Institute, Universitetsparken 13 |

**WELCOMING ADDRESS**

| | | |
|---|---|---|
| 10.30 - 10.45 | Dean of the Science Faculty | H. Jeppesen |
| 10.45 - 11.00 | Director of the Zoological Museum | H. Enghoff |
| 11.00 - 11.30 | Morning tea or coffee | |

**SESSION 1: GENERAL PHYLOGENY** Chairman: D.R. Nelson

| | | |
|---|---|---|
| 11.30 - 12.30 | **Keynote lecture**: Garey, J. & Nichols, P.B.: Molecular analysis and tardigrade systematics | **(14)** |
| 12.30 - 14.00 | Lunch | |

**SESSION 1 (Continued)** Chairman: D.R. Nelson

| | | |
|---|---|---|
| 14.00 - 14.30 | Nielsen, C.: Panarthropods: articulates or ecdysozoans? | **(44)** |
| 14.30 - 15.00 | Sørensen, M.V.: Modern computer cladistics: Cladogram of the Animal Kingdom | **(52)** |
| 15.00 - 15.30 | Afternoon tea or coffee | |

**SESSION 2: PHYLOGENIC POSITION OF TARDIGRADA** Chairman: H. Greven

| | | |
|---|---|---|
| 15.30 - 16.00 | Schmidt-Rhaesa, A.: Tardigrades - are they really miniaturized dwarfs? | **(51)** |
| 16.00 - 16.30 | Dewel, R.A. & Dewel, W.C.: A new hypothesis for the origin of the Bilateria: Implications for understanding the body plan of tardigrades | **(12)** |
| 16.30 | Bus departure for Town Hall | |
| 17.00 - 18.00 | Reception at the Copenhagen Town Hall | |

**Tuesday, 1 August 2000**

**SESSION 3: ALPHA-TAXONOMY OF TERRESTRIAL TARDIGRADES** Chairman: C.W. Beasley

| | | |
|---|---|---|
| 10.00 - 10.30 | Romano, F.A.III, Barreras-Borrero, B. & Nelson, D.R.: Ecological distribution and community analysis of Tardigrada from Choccolocco Creek, Alabama, with the description of a new species of *Echiniscus* | **(48)** |
| 10.30 - 11.00 | Claxton, S.K.: *Antechiniscus* in Australia: Description of *Antechinicus* sp.n. and redescription of *Antechiniscus parvisentus* (Horning & Schuster, 1983) (Heterotardigrada: Echiniscidae) | **(10)** |
| 11.00 - 11.30 | Morning tea or coffee | |

**SESSION 3 (Continued)**

| | | |
|---|---|---|
| 11.30 - 12.00 | Nichols, P.B., Romano, F.A.III, & Nelson, D.R.: Seasonal and altitudinal variation in the distribution and abundance of Tardigrada on Dugger Mountain, Alabama | **(42)** |

12.00 - 13.00 **Round Table Discussion**: Techniques for whole-mount preparations of Tardigrada.
Chairs: Bertolani, R., Claxton, S.K., Gallo D'Addabbo, M., and McInnes, S.J.
13.00 - 14.00 Lunch

**SESSION 4: REPRODUCTIVE STRATEGY** Chairman: J. Eibye-Jacobsen

| | | |
|---|---|---|
| 14.00 - 14.30 | Bertolani, R.: Evolution of reproductive mechanisms in tardigrades | **(6)** |
| 14.30 - 15.00 | Greven, H. & Kristensen, R.M.: Notes on spermiogenesis of *Echiniscoides sigismundi* | **(16)** |
| 15.00 - 15.30 | Altiero, T., Rebecchi, L. & Bertolani, R.: Banding techniques on tardigrade chromosomes | **(1)** |
| 15.30 - 16.00 | Afternoon tea or coffee | |

**SESSION 5: PHYLOGENY OF TARDIGRADA** Chairman: R.A.Dewel

| | | |
|---|---|---|
| 16.00 - 16.30 | Rebecchi, L.: Spermatozoon in tardigrades: Evolution and relationships with the environment | **(47)** |
| 16.30 - 17.00 | Guidetti, R. & Bertolani, R.: New data on the phylogeny of the Macrobiotidae (Tardigrada, Eutardigrada) | **(19)** |

**POSTER SESSION 1**

| | | |
|---|---|---|
| 17.00 - 18.00 | Greaves, P.M.: Royal tardigrades – The fauna of Buckingham Palace, London | **(15)** |
| | Greven, H. & Schüttler, L.: How to crawl and dehydrate on moss | **(17)** |
| | Guidetti, R. & Bertolani, R.: Long-term ecological studies on tardigrades in a beech leaf Litter | **(20)** |
| | Guidetti, R. & Bertolani, R.: Tardigrades of Piane di Mocogno (Northern Apennines, Italy) | **(21)** |
| | Ito, M. & Abe, W.: Depth distribution of soil inhabiting tardigrades in a subalpine coniferous forest of Japan. II | **(25)** |
| | Meyer, H.A.: Tardigrades of Arkansas and Louisiana, U.S.A. | **(37)** |
| | Miller, W.R. & Case, S.B.: KanCRN: a collaborative research model using tardigrades | **(39)** |
| | Miller, W.R. & Miller, J.D.: Return to Terra Incognita: more giant tardigrades | **(40)** |
| | Peters, T. & Dumjahn, P.: Moss-living tardigrades from two altitudinal transects at Disko Island, West Greenland | **(45)** |

**Wednesday 2 August 2000**

**SESSION 6 : ULTRASTRUCTURAL AND PHYLOGENETIC RESEARCH** Chairman: W.C. Dewel

| | | |
|---|---|---|
| 09.00 - 10.00 | **Keynote lecture**: Ruhberg, H.: Recent Onychophora: are they a sister group of the Tardigrada? | **(49)** |
| 10.00 - 10.30 | Kristensen, R.M., Møbjerg, N. & Jørgensen, A.: The cephalic sensory structures and the brain of *Actinarctus doryphorus* (Arthrotardigrada) | **(30)** |
| 10.30 - 11.00 | Eibye-Jacobsen, J.: A new method for making SEM-preparations of the tardigrade buccopharyngeal apparatus | **(13)** |
| 11.00 - 11.30 | Morning tea or coffee | |

**SESSION 7: GENERAL ECOLOGY AND ZOOGEOGRAPHY** Chairman: W.R.Miller

| | | |
|---|---|---|
| 11.30 - 12.00 | Bateman, L. & Collins, M.: Tardigrades of Newfoundland, Canada | **(3)** |
| 12.00 - 12.30 | Collins, M. & Bateman, L.: The biogeography of tardigrades of Newfoundland, Canada | **(11)** |
| 12.30 - 13.00 | Nelson, D.R. & Adkins, R.G.: Distribution of tardigrades within a Moss Cushion: Do tardigrades migrate in response to changing moisture conditions? | **(41)** |
| 13.00 - 14.00 | Lunch | |

**SESSION 8: TERRESTRIAL ZOOGEOGRAPHY** Chairman: S.J. McInnes

| | | |
|---|---|---|
| 14.00 - 14.30 | Marley, N.J.: Tardigrade assemblages from an altitudinal transect in Venezuela | (32) |
| 14.30 - 15.00 | Nickell, K.J. & Miller, W.R.: Tardigrades of South America: Macchu Picchu and Ollantaytambo, Peru | (43) |
| 15.00 - 15.30 | Afternoon tea or coffee | |

**POSTERS SESSION 2**

| | | |
|---|---|---|
| 15.30 - 16.30 | Altiero, T. & Rebecchi, L.: Results and problems in rearing tardigrades | (2) |
| | Brockmann, C. & Ruhberg, H.: *Ooperipatellus decoratus* (Peripatopsidae): Hatching observations in an oviparous onychophoran | (8) |
| | De Zio Grimaldi, S. & Gallo D'Addabbo, M.: Further data on the Mediterranean Sea tardigrade fauna | (18) |
| | Jönsson, K.I., Borsari, S., & Rebecchi, L.: Do populations within tardigrade species differ in anhydrobiotic capacity? | (27) |
| | Jørgensen, A.: A review and graphical presentation of the African tardigrade fauna using GIS with the description of *Isohypsibius* nov.sp. (Eutardigrada: Hypsibiidae) from Lake Malawi | (29) |
| | McInnes, S.J., Chown, S.L., Dartnall, H.J.G., & Pugh; P.J.A.: *Milnesium* cfr *tardigradum* a monitor of high altitude micro-invertebrates on sub-Antarctic Marion Island | (34) |
| | McInnes, S.J.: Is it real? | (35) |
| | McInnes, S.J.: Exposed: Danger lurking in the Maritime Antarctic freshwater algal mats for rotifers and tardigrades | (36) |
| | Middleton, R.: Ecology of tardigrades in southern Africa: A preliminary report | (38) |
| | Russell, P.M., Marley, N.J. & Hockings, M.E.: Do confocal microscopy and tardigrades have a future together? | (50) |

**Thursday, 3 August 2000**

**EXCURSION**

| | |
|---|---|
| 09.30 | Bus departure from August Krogh Institute. |
| 10.00 - 12.00 | Visit to Louisiana Museum of Modern Art |
| 12.00 - 12.30 | Bus drive to Kronborg (The castle of Hamlet) |
| 12.30 - 14.00 | Lunch at Kronborg Castle |
| 14.00 - 16.00 | Sightseeing on the site of Shakespeare's Hamlet and the Casemates. |
| 16.00 - 16.30 | Bus drive to Hillerød |
| 16.30 - 17.30 | Sightseeing: the fountains, gardens and castle of Frederiksborg. |
| 17.30 - 18.00 | Bus drive back to Copenhagen |

**Friday, 4 August 2000**

**SESSION 9: FOSSIL PROARTHRODA** Chairman: H.Ruhberg

| | | |
|---|---|---|
| 09.00 - 10.00 | **Keynote lecture:** Bergström, J. & Hou, X.-g.: Cambrian Onychophora or Tardipolypoda | (5) |
| 10.00 - 10.30 | Budd, G.: Tardigrades as "stem-group" arthropods: The evidence from the Cambrian fauna | (9) |
| 10.30 - 11.00 | Maas, A. & Walossek, D.: Fossil stemline arthropods – tardigrades, lobopodes and pentastomids from the Cambrian – An 'Orsten' perspective | (31) |
| 11.00 - 11.20 | Morning tea or coffee | |

**SESSION 10: MARINE TARDIGRADA** Chairman: G. Budd

| | | |
|---|---|---|
| 11.20 - 11.50 | Jørgensen, A. & Kristensen, R.M.: A new tanarctid arthrotardigrade with buoyant bodies | (28) |

| | | |
|---|---|---|
| 11.50 - 12.20 | McInnes, S.J.: Biodiversity and biogeography of the global marine tardigrade fauna | **(33)** |
| 12.20 - 12.50 | Boesgaard, T., Jørgensen, A. & Kristensen, R.M.: Tardigrades from Australian marine caves | **(7)** |
| 12.50 - 13.10 | Hansen, J.G. , Jørgensen, A. & Kristensen, R.M.: Preliminary studies of the tardigrade fauna of the Faroe Bank | **(22)** |
| 13.10 - 14.00 | Lunch | |

**SESSION 11: GENERAL ECOLOGY** Chairman: K.J. Nickell

| | | |
|---|---|---|
| 14.00 - 14.30 | Hohl, A.M. & Miller, W.R.: Tardigrades of North America: As possible bio-indicators relative to a power plant | **(24)** |
| 14.30 - 15.00 | Herbert, M. & Miller, W.R.: Tardigrades of North America: A study of populations in Southern Indiana | **(23)** |
| 15.00 - 15.30 | Afternoon tea or coffee | |
| 15.30 - 16.00 | **SYMPOSIUM PHOTOGRAPH** | |
| 16.00 - 17.00 | **Round Table Discussion**: The phylogeny of Tardigrada<br>Chairs: Bertolani, R., Budd, G., Greven, H., Dewel, R.A. and Nelson, D.R. | |
| 19.00 - 21.00 | Symposium Dinner at Tivoli Gardens | |

**Saturday 5 August 2000**

**SESSION 12: PHYSIOLOGY AND CRYPTOBIOSIS** Chairman: R. Bertolani

| | | |
|---|---|---|
| 09.00 - 10.00 | **Keynote lecture**:<br>Wright, J.: Cryptobiosis 300 years on from van Leeuwenhoek: What have we Learned about tardigrades? | **(54)** |
| 10.00 - 10.30 | Jönsson, K.I. & Rebecchi, L.: Natural selection on cryptobiotic capacity in tardigrades | **(26)** |
| 10.30 - 11.00 | Ramløv, H. & Westh, P.: Aspects of cryptobiosis in the eutardigrade *Adorybiotus (Richtersius) coronifer* | **(46)** |
| 11.00 - 11.20 | Morning tea or coffee | |

**SESSION 12 (continued)** Chairman: H. Ramløv

| | | |
|---|---|---|
| 11.20 - 11.50 | Westh, P.: Bound water and cryptobiosis | **(53)** |
| 11.50 - 12.20 | Beasley, C.W.: Response of *Macrobiotus hufelandi* (Tardigrada) to light | **(4)** |
| 12.20 - 14.00 | Lunch | |

**CLOSING ADDRESS and NEXT SYMPOSIUM**

14.00 - 15.00 Kristensen, R.M. (convener)

## (1)

# Banding Techniques on Tardigrade Chromosomes

**Tiziana ALTIERO, Lorena REBECCHI, and Roberto BERTOLANI**

*Department of Animal Biology, University of Modena and Reggio Emilia*
*Via Campi 213/d, I - 41100 Modena, Italy*

**Lecture**

Data on chromosome banding are completely absent in tardigrades. This fact is probably due to the small size of the animals and of their chromosomes, and overall to the limited number of cell divisions. Recently, chromosome preparations by air-drying technique were applied for the first time to the eutardigrade *Xerobiotus pseudohufelandi* obtaining permanent slides. This allowed the first attempt to define the karyotype of tardigrades using banding techniques. In particular, C-banding followed by staining with fluorochrome (DAPI) and/or Giemsa and Ag-NOR staining were used. As a model, *Macrobiotus richtersi* was considered, a eutardigrade with amphimictic diploid and thelytokous triploid cytotypes. Mostly oocyte chromosomes were analyzed, because they are much larger than those of spermatocytes and mitotic chromosomes. The metaphasic chromatids of the 6 bivalents of the diploid cytotype and those of the 17-18 univalents of the triploid cytotype appear as short rod- or flame-shaped elements, very similar in the same plate and in the two cytotypes. In the diploid strain, a chiasma is generally present in each bivalent at diplotene, whereas no chiasmata was observed in the oocyte prophases of the triploid strain. Both in diploid and triploid cytotypes, C-banding evidences a heterochromatic centromeric band on the telomere towards the spindle pole of each chromosome, indicating that they are all acrocentric. Tardigrades are in agreement with the hypothesis that the karyotype evolves towards the acrocentric condition and not with that which supposes an evolution towards metacentric chromosomes. DAPI staining after C-banding reveals a positive band in the middle region of two bivalents of the diploid cytotype and in some univalents of the triploid cytotype. Silver staining indicates NOR in only one pair of chromosomes of the diploid cytotype and probably three of the triploid one, in both cases very close to the centromeric C-banded site. NOR is particularly evident in the oocyte prophase and is not heteromorphic for number, size, or position. Other silver-positive regions are located in all other chromosomes on the extremity towards the spindle pole, and they correspond to the kinetochore.

## (2)

# Results and Problems in Rearing Tardigrades

**Tiziana ALTIERO and Lorena REBECCHI**

*Department of Animal Biology, University of Modena and Reggio Emilia*
*Via Campi 213/d, I - 41100 Modena, Italy*

**Poster**

Culture methods for tardigrades were only occasionally dealt with in the sixties. Literature data referred to rearing on algae (*Chlorella* or *Pseudochlorella*) as food. Species of *Hypsibius, Ramazzottius* and *Dactylobiotus* were reared for almost one year or more with this method. Other data refer to attempts to grow *Milnesium tardigradum* and *Hypsibius myrops* utilizing bacteria, protozoa, nematodes and rotifers as food source. We report here the first results of attempts to rear several species of eutardigrades inhabiting different substrates, feeding on different kind of food and characterized by different sexual and reproductive conditions. Several attempts were carried out with terrestrial carnivorous (*Macrobiotus richtersi, M. joannae*) and limnic herbivorous (*Diphascon* cf. *scoticum*; *Hypsibius* sp.; *Isohypsibius monoicus*) species. Carnivorous leaf litter-dwelling species were reared on small agar Petri dishes using bacteriophagous nematodes as food and kept at different temperature. Several generations were obtained with the diploid amphimictic strain and with the triploid thelytokous strain of *M. richtersi*, whereas at least two generations were obtained with the hermaphroditic species *M. joannae*. The time of hatching was quite variable, even at the same temperature, and nonetheless, always very long, up to 40 days. Some problems with anoxia were found, other obstacles were related to bacteria and fungi. These problems were absent with freshwater species. Algae were used as food: *Scenedesmus acutus* for *Hypsibius* sp. and *D.* cf. *scoticum*, *Selenastrum* sp. for *I. monoicus*. Several generations were obtained for all three species. The embryos of *Hypsibius* develop over a longer period than those of the other two limnic species. Males were never found in either *Hypsibius* sp. or *D.* cf. *scoticum*. Instead, *I. monoicus* was hermaphroditic, and its specimens maintained isolated from hatching reproduced several times. The time to first oviposition was evaluated for all species.

(3)

# The Tardigrades of Newfoundland, Canada

**Lois BATEMAN** [1] **and Michael COLLINS** [2]

[1] *Division of Science, Sir Wilfred Grenfell College, Memorial University of Newfoundland, Corner Brook, NF A2H 6P9*

[2] *Biology Department, Memorial University of Newfoundland, St John's, NF A1C 5S7*

**Lecture**

The few extensive studies of tardigrades in Canada have been limited to mainly terrestrial sites in three geographical areas namely New Brunswick, Ontario and British Columbia, with a few species reported from elsewhere, mainly in the Arctic and Quebec. Up until the time of this study there had been no published reports of tardigrades in Newfoundland and Labrador.

A cross-island sampling programme of tardigrades in terrestrial mosses and lichens was undertaken throughout the summers (June to August) of 1995-1998. Some thirty-one species have been identified so far, including four new records for Canada. A complete description of these species, their distributions, and their variations with respect to previously reported specimens is under preparation. This paper will give an overview of the island's various ecosystems and will deal with the species identified so far, their distributions within the province, and comparisons of the Newfoundland tardigrades with other Canadian tardigrades reported.

Although there is some overlap of species with those found in earlier Canadian studies, there are some interesting discrepancies. The rare occurrence and restricted distribution of the very few heterotardigrades (three species and 30 specimens) found in Newfoundland is noteworthy, as is the occurrence of several tardigrade species whose former records have been for Greenland.

Previous authors have classified one-half (15) of the species found in Newfoundland as being cosmopolitan in distribution and so it is not a great surprise to find them here. A recent classification system for tardigrades, based on habitat distribution, groups a number of species as being arctic, sub-arctic, montane, tundra, or holarctic, all categories which might be expected to be represented in Newfoundland. However, none of the species classified in the first four categories has been found in Newfoundland. Two of the holarctic species, namely *Diphascon pingue* and *D. prorsisrostre* are found in Newfoundland and two of six species which had been previously recorded as limited to Greenland have now been found in a number of Newfoundland sites. This may indicate that the environmental conditions in Newfoundland can support a small number of species that would normally have a more northerly distribution.

A comparison of the species found in Newfoundland with those of a number of other northern countries suggests that the Newfoundland species are most similar to those found in Greenland and mainland Canada, since twenty-one of Newfoundland's species are also found in either Greenland or on the Canadian mainland.

(4)

# Response of *Macrobiotus hufelandi* (Tardigrada) to Light

**Clark W. BEASLEY**

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**Lecture**

*Macrobiotus hufelandi* was tested for response to light. Size groups of less than 120 μm and of 120 μm or more were used. It was found that the smaller, younger size group exhibited a statistically significant negative response to light. This is hypothesized to function in conservation of body moisture and be more important to the smaller individuals because of the greater surface area-to-volume ratio. Experiments with geotaxis were done in an effort to determine if this was a more complex type of behavior. These experiments did not detect a significant difference in geotaxic responses in either of the size groups. Observation of the bacterial trails left by the tardigrades indicates that this behavior is not a true negative phototaxis, but rather photokinesis, a nondirected, random movement in which the animal increases its speed or direction when exposed to unfavorable conditions.

## (5)
## Cambrian Onychophora or Tardipolypoda

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**Key-note Lecture**

Finds of well-preserved Cambrian fossils tend to raise the hope that we can fill in some of the holes in the evolutionary tree. The lobopodian xenusians found in the last 15 years are no exception. They are superficially similar to onychophorans and have been suggested to be marine relatives. Despite the difference in size, they could also be related to tardigrades. The problem is that uniquely shared characters are few if any, and similarities may as well be caused by convergence. The appearance in the Cambrian of still another partly lobopodian group, the true anomalocaridids and their relatives, is a further complication. We encounter the classical problem: the material raises more questions than it resolves. Then we have the possibility of a relationship with arthropods. Ultimately, some structures of potential value can not be observed in the fossils.

Xenusians share with tardigrades the presence of segmentation, lobopods with claws, and a cuticle that is moulted. Segmentation occurs in such different animals as annelids and vertebrates, and moulting of a cuticle is common in aschelminth worm phyla as well. It has been suggested that the claws are fundamentally different. Is there any reason to believe that lobopods could not also be formed independently in different groups? Dissimilarities appear to include the structure of the mouth and pharynx, with circularly arranged lamellae and a buccal apparatus in tardigrades, but despite nice preservation no sign of such hard structures in the xenusians. The circular arrangement of lamellae, however, has its counterpart in anomalocaridids. The evidence for a xenusian tardigrade therefore is meagre. On the other hand, one may say that the evidence against such a relationship is non-conclusive.

Similarities between xenusians and onychophorans are include segmentation, lobopods, ringed cuticle, moulting, non-circular mouth, and 'particle' feeding (rather than mud ingestion, as in early arthropods). Still, there are striking differences, such as in the mode of moulting, with a dorsal splitting of the cuticle in onychophorans but ventral in xenusians. The segmentation of the head is quite different in onychophorans and Aysheaia, but it does not rule out a shared origin of onychophorans and xenusians. On balance, this appears more likely than a shared origin of the latter and tardigrades, and tardigrades may be closer to anomalocaridids.

(6)

# Evolution of Reproductive Mechanisms in Tardigrades

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**Lecture**

Tardigrades reproduce only through gametes. Their capability of regeneration is limited to a physiological restoration of a few cells in some tissues, and therefore they do not have vegetative reproduction. Nevertheless, they exploit several modes of reproduction, often in close relationship with the environment that is colonized. In the sea, where tardigrades probably originated, the species (mainly heterotardigrades) are gonochoristic (only one case of hermaphroditism is cited). As in most marine animals, parthenogenesis is unknown. Males are often smaller than females, and they are probably semelparous. In many cases females mature one egg at a time. In limnic and "terrestrial" species, gonochorism is still present, even if amphimixis is no longer the most common mode of reproduction. Evidently, amphimixis is maintained for genetic recombination. In all eutardigrade and heterotardigrade species, females are certainly iteroparous and lay groups of eggs (free or in the exuvium), utilizing the small body space alternately for food storage and oocyte maturation. In males the situation may be different. In freshwater sediments, species with semelparous males are found, like in the sea, whereas in mosses or in leaf litter males are iteroparous, with a continuous or cyclical maturation of the spermatozoa. Amphimixis is also present in several hermaphroditic species, found in different habitats, such as freshwater, mosses, leaf litter and turf. Egg maturation is similar to that of gonochorisric species. The spermatozoa begin to mature in appreciable numbers before the oocytes, and subsequently they mature continuously and in small numbers over the life of the animal. Laboratory data point out the possibility of self-fertilization. However, in limnic and "terrestrial" species the most diffuse mode of reproduction is parthenogesesis. It appears always continuous (heterogony is unknown in tardigrades). Some cases (eutardigrades of several families) present morphospecies with both amphimictic (diploid) and thelytokous (often but not always polyploid) cytotypes. Other cases, such as in Murrayinae (Eutardigrada, Macrobiotidae), have only females, or as in *Echiniscus*

(Heterotardigrada, Echiniscidae), most of the more than 120 species have no males. We can hypothesize that parthenogenesis and self-fertilization may be favored in less stable environments by the appearance of cryptobiosis and passive dispersal. These two modes of reproduction allow colonization of a new territory by a single individual. One species is known to have gonochoristic and hermaphroditic strains, but no species have parthenogenetic and hermaphroditic strains. We can suppose that hermaphroditism can be favorably selected only when parthenogenesis has never occurred.

(7)

# Tardigrades from Australian Marine Caves

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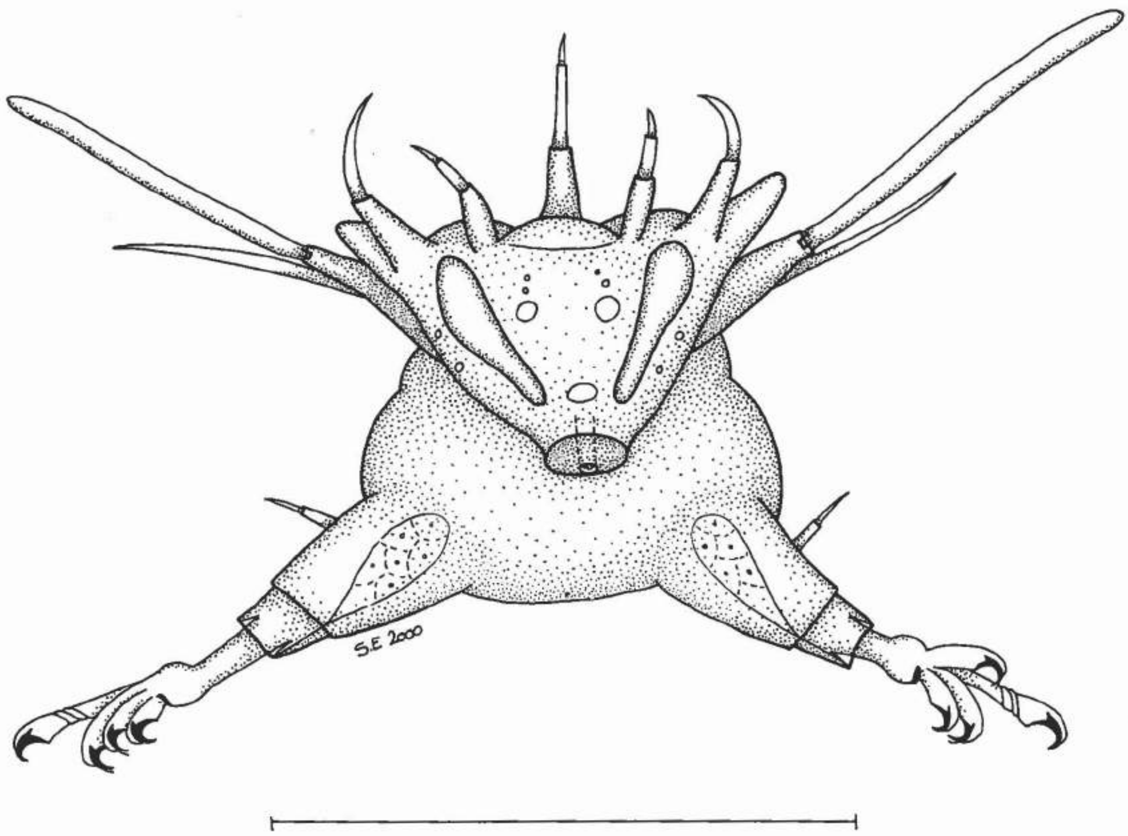
**Lecture**

Sublittoral marine caves have been objects of intensive macrofauna investigations, which have resulted in the discovery of new major groups of Crustacea, e.g. Remipedia described from an anchialine cave in Bahamas and Mictacea from marine cave in Bermuda. Few meiofauna investigations from eumarine caves have been published until now. A marine cave study on tardigrades was carried out on the Italian coasts describing four new species of Arthrotardigrada, and more recently a new genus, *Trogloarctus* of the deep sea family Coronarctidae from a submarine cave in the French Mediterranean Sea was described. The last finding supports the theory that the marine caves may serve as refugees for an old Tethyan fauna or as biota for Mesozoic relict ties to the deep sea.

Several caves in Australia have been investigated for meiofauna using the freshwater technique to shock large sediment samples. Several species of nematodes, gastrotrichs, crustaceans, polychaetes and aplacophorans were found. Two new species of loriciferans and one new species of kinorhynch from marine caves in New South Wales, Australia are right now being described. This paper is the third in a series describing the unique meiofauna in submarine caves and inland anchialine caves of Australia. The paper is dealing with the tardigrade fauna of two caves, Jim's Cave and Fish Rock Cave, both located off the coast of New South Wales. The sediment consist of carbonate sediments (shell-hash to shell-gravel) mixed with organic detritus.

The abundance of tardigrades is very low in the two caves, but the species diversity is very high. Until now species of the following arthrotardigrade genera are found: *Actinarctus*, *Batillipes*, *Dipodarctus*, *Halechiniscus*, *Raiarctus*, *Styraconyx*, *Tanarctus*, *Tholoarctus*, and *Wingstrandarctus*. The cave fauna seems not to be related to the well-investigated tidal to subtidal tardigrade fauna along the East Coast of Australia. Most surprising is the finding of the species *Actinarctus neretinus* known from the Italian cave and a new species of *Tanarctus* from the carbonate sediments in North Atlantic and Danish Waters. There has not been found any deep-sea tardigrades in the Australian caves until now, but the search is still going on.



(8)

# *Ooperipatellus decoratus* (Peripatopsidae): Hatching observations in an oviparous onychophoran

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**Poster**

While the majority of the Onychophora is viviparous or ovoviviparous, representatives of only 12 genera from Australia and New Zealand are oviparous. Females of oviparous onychophorans are characterized by the presence of an ovipositor and large, thick-walled, yolky eggs in the uteri. From cultured specimens of the Tasmanian *Ooperipatellus decoratus* (Baehr, 1977) eggs were obtained. From the deposited eggs, 15 juveniles hatched. The majority of these juveniles had only a short life expectancy of less than 28 days. 33% of the hatched juveniles reached a longer life expectancy from 36 days to 167 days. Two hatchings were recorded by video using a CCD-camera and infrared lights. The hatching period was 28 minutes and 43 minutes, respectively. Both juveniles hatched with their posterior part of the body first. Some pairs of legs held on to the egg and to the substrate while the body was gradually pulled out of the egg with slow winding movements. Peristaltic body movements and walking backwards supported this process. During the hatching, periods of stronger body movements are followed by resting periods. Finally the head is pulled out of the egg in one quick movement. Both hatchlings stood for four minutes on their eggs touching their vicinity with the antennae, before they left the empty eggshells. Additionally four attempted hatchings were noted. In these cases the juveniles failed to leave the eggshells and died. Two of these eggs showed a juvenile attempted to hatch with its head first, in the other two cases the posterior part of the body got out of the egg first.

The developmental period of the laid eggs until hatching was about six to seven months. When first found, these eggs did not show any differentiation. This is the first time that under laboratory conditions hatched juveniles of oviparous Onychophora were successfully cultured and demonstrates the difficulty of obtaining regular breedings for extended studies.

The collection of specimens from Tasmania was supported by a grant from the DFG (Ru 358/2-5).

(9)

# Tardigrades as 'stem-group' arthropods: the evidence from the Cambrian fauna

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**Lecture**

The Cambrian fauna can now reasonably be seen as containing many taxa that lie in the stem-groups of the extant phyla. As such, these fossils suggest how both the 'body plans' of extant phyla were assembled, and also various 'minor' phyla relate to the larger groupings of today such as the arthropods and annelids.

The various arthropod and lobopod taxa of the Cambrian faunas have been controversial and have generally been considered either as lying in the crown or (occasionally) stem groups of the arthropods, onychophorans and tardigrades. However, phylogenetic analysis strongly suggests that many of even the most arthropod-like taxa do not lie within the arthropod crown-group but are more basal. Further, the commonly expressed view that Cambrian lobopods are in effect stem- or crown-group onychophorans also seems not to be well supported. Lobopods in the Cambrian appear to be diverse and not particularly closely related to one another, and certainly cannot be combined in a monophyletic clade.

Both these advances offer hope that the tardigrades (placed as the sister group to the arthropods in many analyses of extant taxa) may be more closely related to some of these Cambrian taxa than others. The challenge for both neontologists and palaeontologists is to refine the systematic analysis of both living fossil taxa in order to maximise the usefulness of the (admittedly few) characters that unite tardigrades to their Cambrian forbears.

## (10)

# *Antechiniscus* in Australia: Description of *Antechiniscus* sp. n. and redescription of *Antechiniscus parvisentus* (Horning & Schuster, 1983) (Heterotardigrada: Echiniscidae)

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**Lecture**

The genus *Antechiniscus* Kristensen, 1987 has, at present, a quite specific distribution, being found only in the *Nothofagus* rainforests of South America and New Zealand. Five species have been described, two from South America and three from New Zealand.

Males, half the size of females, have been found in the type species, *Antechiniscus lateromamillatus* Ramazzotti, 1964 but were not mentioned in the descriptions of the other four species. The three New Zealand species were described on the basis of very few specimens each and the descriptions are very brief and unsatisfactory.

A population of the New Zealand species, *Antechiniscus parvisentus* (Horning & Schuster 1983), has been found in and around a *Nothofagus* rainforest in northern New South Wales and has provided the opportunity to describe this species more thoroughly. Males, on average about 20% shorter than females, have been found. They have a long spine above the third pair of legs which is absent in females.

*Antechiniscus* sp. n. was found in liverwort in a regenerating *Nothofagus* forest in Tasmania. Males are, on average, about 20% shorter than females and exhibit some sexual dimorphism. This species has a buccal tube with a flexible (but not annulated) posterior section observed in live specimens, a character requiring further observation in other species of the genus. *Antechiniscus* sp. n. also has an extensive series of ventral plates, a character which it shares with *A. pulcher* (Murray, 1910) which will be redescribed at a later date.

Members of this genus occur in Australia, as they do in New Zealand and South America, in habitats dominated by the Southern hemisphere genus *Nothofagus* which favours aseasonal climates with moderate temperatures, low variability and high humidity.

## (11)

# The Biogeography of the Tardigrades of Newfoundland, Canada

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**Lecture**

The previous paper dealt with an overview of the various types of ecosystem occurring on the island of Newfoundland and the thirty-one species of tardigrades located so far in samples of moss and lichens from across the island, and a comparison of the Newfoundland species with species found elsewhere in Canada. This paper is an account of the various interesting biogeographical features of the Newfoundland tardigrade species assemblage, including their distribution with respect to ecosystem, vegetation type, geological bedrock formation, altitude, and relationship to environmental water content and dessication.

A comparison was made with the tardigrade species in the various bedrock and altitudinal categories constructed for Polish tardigrades. Over one-half of the Newfoundland species (eighteen) fall into categories strongly associated with non-carbonate rocks, which represents over one-half of the species classified as such for Poland. None have yet been found in Newfoundland from the Polish groups strongly associated with carbonate rocks. Of the twenty-three species listed for the Polish tychoalpine group, which occur in all altitudinal zones, thirteen do occur in Newfoundland. However, of the sixty-two Polish species found in the categories associated with more specific altitude ranges only eight have so far been located in Newfoundland.

It has been suggested that the distribution of some tardigrades is related to the ability to resist dessication. Three ecotypes have been proposed elsewhere, each with two or three representative species. The effect of competitive exclusion and carnivory on the distribution of the same group of species was analysed in the same study. Four of those representative species, including at least one from each ecotype, are found in Newfoundland, namely *Milnesium tardigradum* (a xerophile), *Hypsibius dujardini* and *Macrobiotus* *hufelandi* (hygric ecotypes), and *Isohypsibius- prosostomus* (eurytopic). The distribution in Newfoundland of these and other species is examined with relation to each other and to potential dessication of the habitat in which they were found.

## (12)

# A New Hypothesis for the Origin of the Bilateria: Implications for Understanding the Body Plan of Tardigrades

**Ruth Ann DEWEL and William C. DEWEL**

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**Lecture**

The currently accepted concept for the origin and early diversification of the Metazoa has been formulated with an alarming lack of concrete evidence and rests as a consequence on an unsteady foundation of unproven assumptions. The most pervasive and persistent of these assumptions is that morphological complexity arose gradually and relatively late in the radiation of the Metazoa. Small and simple larva or flatworm-like organisms have been posited as the last common ancestors of the Eumetazoa, Bilateria, and even protostomes and deuterostomes. For example, the Bilateria are considered by some to have experienced a long prehistory as micrometazoans similar to the primary larvae of extant echinoderms and lophotrochozoans. Neither the underlying similarity in the construction of macroscopic bilaterians nor the fossil record have been taken seriously or interpreted literally.

Nevertheless, a rich fossil record from the Late Neoproterozoic, the Ediacaran fauna, appears to reveal with stunning clarity the early events of metazoan evolution. The earliest Ediacarans, dating from approximately 600 Ma, are centimeter-sized discs or annuli, while later forms from about 565 Ma are complex and diverse. Many of the latter are highly modular. Modularity is the defining attribute of colonial organisms, and this fact is a compelling reason to consider that modular Ediacarans evolved from the bud colonies of a simple disc-like ancestor. Some of the modular forms have extramodular structures and may display frond- or slug-like morphologies. Such forms exhibit the same well-documented trend toward higher levels of integration that typifies colonial organisms. Because this trend can culminate in the evolution of colonial superorganisms, within which the former individuals of a colony function as organs, we propose that bilaterians arose through the integration or "individuation" of an Ediacaran colony. If correct, this proposal will have profound implications for our understanding of the stem species of the Ecdysozoa. For example, if a macroscopic body size and "segmentation" arose before the origin of the Bilateria, they may be plesiomorphic for the entire ecdysozoan clade.

## (13)

# A new method for making SEM-preparations of the tardigrade buccopharyngeal apparatus.

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**Lecture**

The buccopharyngeal apparatus of several species of tardigrades, both eutardigrades and heterotardigrades, has been prepared for scanning electron microscopy (SEM) by using a new method developed and refined for this purpose. The live or lightly formaldehyde fixed specimens are submerged directly into a small drop of diluted NaClO-solution on a round coverslip. After a few minutes the specimens are torn apart mechanically using fine metal needles. In this way the buccopharyngeal apparatus is freed from the body cuticle. The preparation is rinsed several times using distilled water to remove any salt precipitate arising from the decomposition of the NaClO. The extracted buccopharyngeal apparatus is allowed to dry in situ on the coverslip and no further drying is necessary. The coverslip is finally glued to an SEM specimen holder.

Using this method, excellent preparations of the buccopharyngeal apparatus from several tardigrade species are now available for description and further investigation. Three well known species are used as examples and the new findings are compared to studies from the literature. The species used are: *Milnesium tardigradum*, *Halobiotus crispae* and *Echiniscus testudo*. For each species a redescription of the buccopharyngeal apparatus, in particular the stylets, the stylet supports, the stylet sheets and their interrelations, is presented. For *H. crispae* the cuticular elements of the pharynx are furthermore redescribed from the new material and a comparison is made to results in Kristensen (1982) and Eibye-Jacobsen (1997).

The potential new information available with this method is discussed, together with possible artifacts and difficulties with the method.

**Eibye-Jacobsen, J.** 1997. Development, ultrastructure and function of the pharynx of *Halobiotus crispae* Kristensen, 1982 (Eutardigrada). Acta Zoologica (Stockholm) 78: 329-347.
**Kristensen, R.M.** 1982. The first record of cyclomorphosis in Tardigrada based on a new genus and species from arctic meiobentos. Zeitschrift für zoologische Systematik und Evolutionsforschung 20: 249-270.

## (14)

# Molecular Analysis and Tardigrade Systematics

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**Key-note Lecture**

DNA sequence analysis can be useful in the study of tardigrades. DNA suitable for Polymerase Chain Reaction (PCR) amplification can be obtained from a single or just a few specimens as long as formalin was not used as a preservative.

The analysis of the 18S rRNA gene has been used in my laboratory to show that tardigrades belong to the clade Panarthropoda which includes arthropods and onychophorans. Panarthropoda is part of Ecdysozoa, and includes all molting animals. Ecdysozoa includes many of the taxa previously included in Aschelminthes which explains why tardigrades have characters reminiscent of both arthropods and aschelminths. In other laboratories, studies are underway to analyze a series of protein coding genes from tardigrades, and the mitochondrial genome of a tardigrade has recently been completed. We can expect to see the publication and analysis of tardigrade Hox gene clusters in the near future as well.

The 18S rRNA gene has also been used to investigate high level phylogeny within tardigrades and thus far has been remarkably congruent with morphological data. To date, there has not been a large-scale cladistic analysis of the phylogenetic relationships encompassing more than just a single family of tardigrades. We have begun a long-term project to sequence the 18S rRNA gene and two mitochondrial protein coding genes (Cytochrome Oxidase I and Cytochrome b) from at least 48 tardigrade species representing all extant families and sub-families. These data will be combined with a study of morphological characters to determine a phylogenetic framework for Tardigrada. As part of this project, we will produce a web-based database of sequence tags linked to morphological characters that can be used to confirm the identity of tardigrade species for which we have data.

**(15)**

# Royal Tardigrades – The Fauna of Buckingham Palace, London

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**Poster**

There have been comparatively few studies of tardigrades of urban sites and those studies that have been performed have reported a low diversity of species compared to rural sites. The characteristics of urban sites that are thought to reduce diversity include reduced or rapidly changing site humidity and aïr quality, particularly sulphur dioxide pollution.

Results from a single day of sampling in the gardens of Buckingham Palace, London, UK are therefore surprising in the richness of tardigrade species found, especially when compared to neighbouring, more rural, locations. A total of 11 species from 7 genera were found, including *Diphascon bullatum* (first record for England) and *Isohypsibius sattleri* (first record for UK). For comparison, more intensive sampling over a two year period at Bookham Common, a semi-rural woodland site approximately 25 miles south of central London, yielded only 12 species of 6 genera.

Buckingham Palace has been a royal residence since 1761 and the gardens have been maintained since before that time. Remodelled of the gardens has occurred a number of times in the past 250 years and much material (plants, wood and organic mulches) is imported on a regular basis. It is therefore likely that the tardigrade population has largely been imported with incoming materials, accounting for the high number of species. Of interest, however, is that these species can survive in an urban environment. Although the gardens are located in an area of low air quality (due to traffic), the use of organic mulches in the gardens helps to maintain humidity levels and it may be this that is the key factor in ensuring survival.

## (16)

## Notes on spermiogenesis of *Echiniscoides sigismundi*

**Hartmut GREVEN[1] & Reinhardt Møbjerg KRISTENSEN[2]**

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[2] *Zoological Museum, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark*

**Lecture**

Some stages of spermiogenesis of *Echinscoides sigismundi* (Heterotardigrada) are described using conventional transmission electron microscopy. Spermiogenesis from spermatogonia to early spermatids does not show any unusual features. Spermatids possess two large mitochondria that become closely attached. A large vesicle is formed by the Golgi-apparatus and the rough endoplasmic reticulum that is also present in the mature spermatozoon and my be taken for the acrosome at first glance. The functional acrosome develops later in close association with the nucleus. The Golgi-apparatus appears not to be involved in its formation. A special arrangement of microtubules ("Saturn rings") is associated with the centriole and the nucleus. Late spermatids and spermatozoa are characterized by three elongations: 1) large "free" mitochondria, 2) the flagellum, and 3) the nucleus with the acrosome. Compared with Hetero- and Eutardigrada investigated in this respect, spermiogenesis shows characters of both in *E. sigismundi*.

## (17)

## How to crawl and dehydrate on moss

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**Poster**

We observed *Echiniscus testudo* and *Macrobiotus* spec. partly by videotechniques, when crawling and dehydrating on single moss plants (*Polytrichum formosum, Encalyptra contorta*). In accordance with Marcus (1929) specimens here gave up in many cases their rhythmic locomotion pattern. Water forms a continuous layer between leaflets along the entire stem in fully hydrated *E. contorta*. In *P. formosum* , however, water runs off, leaving a drop of water in the leaf axil at best. Due to the low specific weight of the tardigrades (ca. 1.04) walking on the leaf surfaces , even if structures as in the leaves of *P. formosum*, is not easily in fully hydrated mosses. Claws appear to be ineffective in holding on. When crawling beyond the edge of a leaflet in a fully hydrated moss plant, tardigrades reach the drop of water under the leaflet falling down on the next leaflet and so on. During dehydration animals do not search selectively for moist places, e.g. between the stem and the leaflets nestling against the stem (*E. contorta*) or in "tubes" produced by rolling up leaflets (*P. formosum*). We suggest that mosses as secondary habitats for tardigrades do not offer optimal conditions when fully dehydrated for crawling.

## (18)

# Further Data on the Mediterranean Sea Tardigrad Fauna


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**Poster**

Information about Mediterranean Tardigrades, has become more numerous in the last 15 years, not only as regards the known species geographical distribution, but with the finding of many new species, also in taxonomy. The new data confirm the old ones about the ecology of this group and suggest a hypothesis abuot its biogeographic history.

The available data regard the italian coasts, the Sicily Channel and the Alboran Sea. The Mediterranean Tardigrades, about 70 species, are Heterotardigrada, mainly Arthrotardigrada, with Neostygartidae, Neoarctidae, Stygarctidae, Halichiniscidae, Batillipedidae, and Echiniscoidea with Echiniscoididae. Halechiniscidae are the prevailling taxon i Arthrotardigrada, followed by Batillipedidae and Stygarctidae.

Studying tardigrades coming from different kinds of sediments, reveals that subtidal species are more frequently associated to carbonatic sediments, mainly coarse organeous detritus.

The frequent precense of Stygarctidae in submarine caves, where, on the contrary, Batillipedidae are, as yet unknown, is very interesting. The latter are frequent in the intertidal zone.

The precense of Stygarctidae in submarine caves, which are, in contrast to the open sea, a well sheltered and conservative environment, agrees with the primitivity of the family, which is supplied by numerous plesiomorphic characters. It is furthermore interesting, that the most primitive species til now known, have only been found in the Mediterranean Sea. This allows us to formulate, with the necessary prudence, the hypothesis of a probable origin of the group in the ancient Tethys from which the Meditarranean Sea originated.

**(19)**

# New data on the phylogeny of the Macrobiotidae (Tardigrada, Eutardigrada).

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**Lecture**

The systematics of the Eutardigrada is mainly based on the morphological characters of the sclerified parts of the animals (claws structure and bucco-pharyngeal apparatus). Ultrastructural studies and cladistic analyses were used in the systematics of the Macrobiotidae leading to the identification of two subfamilies (Guidetti et al., 2000). The satisfactory and precise results obtained by this method of analysis prompted us to continue with this method.

For our study on the phylogeny of the Macrobiotidae, several different characters were examined, related to the claws, bucco-pharyngeal apparatus, cuticular structure and ultrastructure. The polarities can be clearly defined only in some cases. The combined use of these characters clarifies or suggests some important phyletic relations within the Macrobiotidae. The relationships among the genera of the Murrayinae can be better recognized. Instead, among the genera of Macrobiotinae, even if at least 5 evolutionary lines were identified, the data do not allow us to precisely detect their phyletic relationships. This situation is probably influenced by the presence of one or more polyphyletic genera inside this subfamily.

Guidetti, R., Rebecchi, L. & Bertolani, R. (2000). Cuticle structure and systematics of the Macrobiotidae (Tardigrada, Eutardigradi). Acta Zool. **81**: 27-36.

## (20)

# Long-term Ecological Studies on Tardigrades in a Beech Leaf Litter

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**Poster**

To date the sinecology of tardigrades has not received a great deal of attention. Data on the population dynamics are decidedly scanty, mostly due to the difficulty in obtaining homogeneous and comparable samples. This problem can be overcome utilizing the leaf litter, a relatively homogeneous and thick substrate, until recently neglected but often rich in tardigrades. We studied the tardigrade community in a litter for two years, in a beech forest (*Fagus sylvatica*) on the Northern Apennines, 1,200 m asl, at Piane di Mocogno, Modena, Italy. The study was carried out by bi-monthly samplings, each constituted by 8 standardized replicates in a circumscribed area. An upper (first 3 cm of litter) and deep (subsequent 3 cm made up of litter+humus) layers were considered. Only eurytopic and hygrophilic species were found. Some species occupied only one layer of the leaf litter, others were found in both, and still others were alternately rich in the upper or in the deep layer. The population dynamics of the species appear closely related to meteorological factors (temperature and rainfall). These relationships may be related to the tolerance of the species to dehydration, the duration of a constant and high level of relative humidity, and the availability of food. In particular, considering the two most represented species, *Macrobiotus richtersi* increased mainly in spring and summer, whereas *Hypsibius convergens* decreased in the same period. These species have different population dynamics, with even opposing seasonal cycles and trends; therefore, they evidently have different ecological needs. All species with great seasonal variations of population density had a high reproductive rate, being able to achieve drastic increases in population in a relatively short period (*r* strategy). The two most represented species produced a higher mean number of eggs per animal when the population was increasing, probably exploiting the favorable periods.

## (21)

# Tardigrades of Piane di Mocogno (Northern Apennines, Italy)

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**Poster**

Samples of leaf litter, mosses and lichens were collected in a beech forest of Piane di Mocogno (Emilia, Italy) at 1,200 asl, an area already studied. The possibility of finding a certain species in a sample is related to different factors: i) the kind of substrate, ii) the sampling season, iii) the amount of the analyzed substrate, iv) the abundance of a species in the substrate v) the layer of substrate.

The tardigrade community in leaf litter was typical of this substrate. *Macrobiotus richtersi*, *M. harmsworthi*, *Hypsibius convergens* and *H. dujardini*, *Amphibolus weglarskae*, *Astatumen trinacriae*, *Itaquascon placophorum* and several species of *Diphascon* and *Isohypsibius* were found. Moreover, the findings of *Eohypsibius nadiae* (first record in leaf littêr) and *Microhypsibius bertolanii* (first record in leaf litter and in Italy) were particularly significant from a faunistic point of view. Species associations were found similar in mosses and lichens but different from those of the leaf litter, confirming that different substrates are characterized by very different species.

As a consequence of this faunistic analysis, nine species of tardigrades were found only in this second study of the area. Among them *Minibiotus foratus* is new to science. For *Hypsibius scabropygus*, its eggs were found in an exuvium. They appeared ornamented, similar to the eggs of *Hypsibius roanensis*.

(22)

# Preliminary Studies of the Tardigrade Fauna of the Faroe Bank

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**Lecture**

Four cruises to the Faroe Bank have collected bottom samples for qualitative analysis of the meiofauna. The preliminary results show a very species rich tardigrade fauna with a large proportion of species new to science.

The Faroe Bank is situated south-west of the Faroe Islands. It rises from the sea floor at approximately 1000 m until 90-100 m. The sediment is changing successively from the top where the sediment is coarse shell-gravel through calcareous sand with different grain sizes on the slopes and finally mud at the bottom. Scattered over the surface of the Bank is *Lophelia* reefs, "Ostebunde" (sponges) and stone reefs composed of basalt rocks.

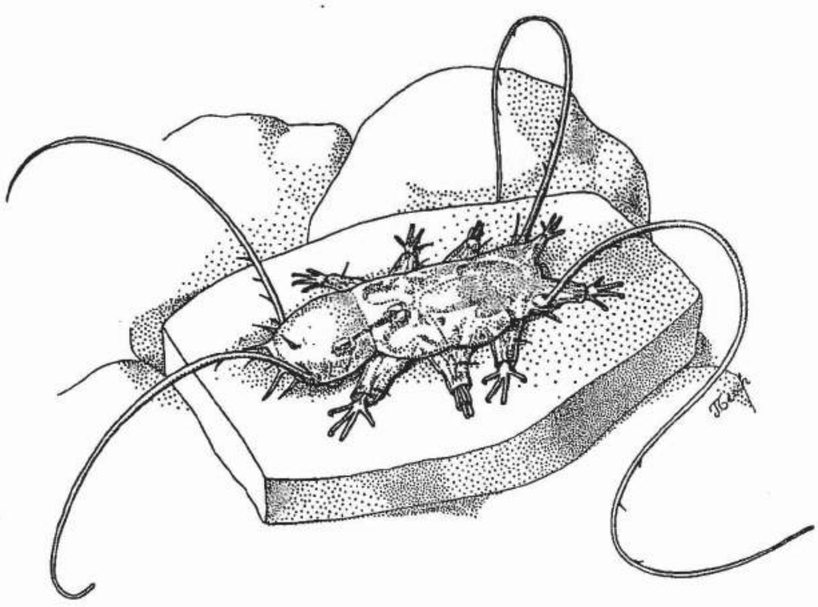
The four cruises were conducted in 1989 (October), '90 (April), '92 (April) and '98 (September) and are thus representative for spring and autumn while the summer and winter periods are still unexplored. Samplings were conducted with various sampling gear between 90 m and 1040 m, and the sediment varied from basalt cobble and coarse shell-gravel to mud. A total of 21 stations were sampled for meiofauna analysis at the Faroe Bank during the cruises. Subsamples have been treated with fresh water, $MgCl_2$ or airbubbeling to extract the meiofauna which were then fixed in formaldehyde, DMSO, ethanol, or trialdehyde. Meiofauna samples have also been taken at the other Banks in the area (Bill Bailey, Lucy and Rosemary) and at the Faroe plateau, thus representing important localities against which the fauna at the Faroe Bank can be compared.

Partially sorting of samples from 13 stations have been conducted, most of them during screening for loriciferans. At present 34 species of tardigrades belonging to 4 families (6 subfamilies) have been found, of these are 20 new to science (58.8%). The 34 species comprise more than 20% of all known marine tardigrades. Halechiniscidae is represented by 28 species (11 Styraconyxinae, 10 Tanarctinae, 3 Halechiniscinae, 2 Florarctinae, 1

Euclavarctinae and 1 Dipodarctinae). This family comprises 84.8% of the specimens sorted so far. Specimens from the subfamilies Tanarctinae (50.47%) and Styraconyxinae (44.34%) are dominating. Batillipedidae is represented with 4 species (12.0% of the specimens) and Coronarctidae and Stygarctidae with a single species each (1.6% of the specimens each). Nine species are known only from a single specimen (singletons) and of these are 6 species new to science. The new species are 1 Florarctinae nov. gen. nov. sp., 1 *Wingstrandarctus* nov. sp., 1 *Parmursa* nov.sp, 7 *Tanarctus* nov. sp., 1 *Halechiniscus* nov. sp., 1 *Paradoxipus* nov. sp., 1 *Angursa* nov. sp., 1 *Raiarctus* nov. sp., 1 *Rhomboarctus* nov. sp, 2 *Styraconyx* nov. sp., 2 *Batillipes* nov. sp. and a neotene *Pseudostygarctus* nov. sp. A total of 248 specimens have until now been assigned to the level of species (or nov. sp.) and an equal number has been sorted out awaiting further preparation.

Samples of similar sediment from 104-260 m depth have similar species diversity. This implicates that the sediment is the key factor involved in the species distribution and that depth is less important. The calcareous sediment is a unique substrate and the fauna of the Faroe Bank can be compared with sub-tropical and tropical coralline sand. The interstitial meiofauna exihibits a strong taxonomic affinity with the meiofauna from more southern latitudes, i.e. the Mediterranean Sea and the southeastern coast of USA, and a lesser similarity with the rich meiofaunal community found at the coast of Britanny.

The associated meiofauna is dominated by interstitial Nematoda and harpacticoid Copepoda. Turbellaria, Gastrotricha, Polychaeta, Ostracoda and Halacarida were also abundant. Cnidaria, Kinorhyncha, Loricifera and interstitial Mollusca were less abundant.



## (23)

# Tardigrades of North America: A Study of Populations in Southern Indiana

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**Lecture**

Significant differences in the density, diversity, and distribution of tardigrades living in lichens inhabiting rock, soil, and tree substrates were found. The rock-lichen habitat was the most productive while soils were barren. The statistical differences are reported.

(24)

# Tardigrades of North America: As Possible Bio-Indicators Relative to a Power Plant

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**Lecture**

Significant differences in the density, diversity, and distribution of tardigrades, rotifers, and nematodes were identified up and down wind from a coal burning power plant. Changes included both macro and micro vegetation as oak tree and lichen diversity shifted. Tardigrade and rotifer density was greatest upwind where nematode density was lower. One species of tardigrade was unique to each the up wind (*Ramazzottius oberhaeuseri*) and the down wind (*Echiniscus* sp.) areas while three species (*Macrobiotus* sp., *Minibiotus intermedius*, and *Milnesium tardigradum*) were found in both directions though in different densities. Five genera of lichen (*Physcia, Xanthoria, Candelaria, Parmelia*, and *Perusaria* ) were found up wind of the power plant and only three genera (*Parmelia, Physica*, and *Xanthoria*) were found down wind. The study establishes a base line for using tardigrades as bio-indicators to measure change.

## (25)

# Depth distribution of soil inhabiting tardigrades in a subalpine coniferous forest of Japan. II.

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**Poster**

At the time of last Symposium, we (Abe, Ito & Amano) already reported the outline of depth distribution of soil inhabiting tardigrades in a subalpine coniferous forest of Japan. In the first report, we took soil samples of every 5cm layer (0-5cm, 5-10cm ------) from 40cm depth and revealed that many individuals of soil tardigrades inhabiting in deep soil (under 10cm). We reported some deep soil dwelling species of tardigrades.

The purposes of this study are to reexamine the results of first report and detect microdistribution of soil tardigrades in the depth of 0-10cm. Study area is typical subalpine coniferous forest dominated by single tree species, *Abies veitchii* Lindl., altitude 1,850m.asl. and located in the slope of Mt. Yatsugatake (2,899m). The soil type of this area is Wet Podzols. Litter accumulate thick on the ground with moss vegetations. We took soil samples of every 1cm layers (0-1cm, 1-2cm ----) to 10cm depth. In the depth of 10-30cm, we took samples of every 5cm layers as the first report. Tardigrades were extracted from the soil samples by using the Baermann funnels.

Density of soil tardigrades in the depth 0-10cm are summarized in Table 1. Maximum densities are occurred in the 3~6cm depth. Same study on soil arthropods were already done. Maximum densities of arthropods were in the depth of 0~3cm. Tardigrades preferred to deeper layers than arthropods generally. Distribution patterns of dominant species will be discussed.

Table 1. Densities of soil tardigrades of every 1cm layer. No. of individuals / $25.5 cm^2$.

| Core No. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 0-1cm | 4 | 1 | 6 | 29 | 9 | 5 |
| 1-2cm | 36 | 3 | 7 | 49 | 22 | 24 |
| 2-3cm | 37 | 19 | 24 | 36 | 31 | 48 |
| 3-4cm | 71 | 11 | 20 | 80 | 26 | 35 |
| 4-5cm | 86 | 12 | 13 | 63 | 54 | 45 |
| 5-6cm | 50 | 11 | 7 | 51 | 18 | 69 |
| 6-7cm | 18 | 8 | 3 | 26 | 11 | 44 |
| 7-8cm | 5 | 3 | 1 | 15 | 8 | 48 |
| 8-9cm | 5 | 2 | 0 | 7 | 7 | 15 |
| 8-10cm | 8 | 3 | 1 | 3 | 9 | 6 |

## (26)

# Natural Selection on Cryptobiotic Capacity in Tardigrades

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**Lecture**

In spite of a long-term interest in the ability of tardigrades and some other micro-metazoans to survive in a cryptobiotic state, microevolutionary aspects of cryptobiosis have never been studied. As a consequence, the phenotypic and genetic constraints underlying the evolution of cryptobiosis as a life history tactic have remained unexplored. We report a study on phenotypic predictors of anhydrobiotic survival in individual tardigrades, using the eutardigrade *Richtersius coronifer*, found at the island Öland in the Baltic sea, as study organism. This species has a well documented cryptobiotic capacity.

The experimental design consisted of pre-anhydrobiosis analysis of phenotypic traits in hydrated specimens, dehydration and 12-days period of anhydrobiosis, rehydration, and post-anhydrobiosis recording of survival or death. In the surviving animals the phenotypic characteristics were re-analysed. Predictive effects of variables on anhydrobiotic survival were analysed using logistic regression analysis.

Our results show that body size, energy stores (storage cells size), and reproductive condition (stage of oocyte vitellogenesis) seem to be involved in determining the probability to survive a period of anhydrobiosis. The largest animals had a dramatically reduced probability to survive, particularly if they were at an advanced stage of the reproductive cycle. For the large animals, the chance to survive increased with the size of their storage cells, indicating energy limitation as a cause of mortality. However, for small and medium sized animals anhydrobiotic survival was inversely related to storage cell size, a result that remains to be explained. Our study also shows that the size of storage cells declines over a period of anhydrobiosis, suggesting that energy contained in these cells are utilised in the anhydrobiotic process.

(27)

# Do populations within tardigrade species differ in anhydrobiotic capacity?

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**Poster**

Differences among tardigrade species in the ability to survive desiccation have earlier been documented, and seem to relate to the capacity of body surface reduction during tun formation. Such differentiation among species may represent adaptations to conditions of the species habitat. When substantial gene flow is prevented, also separate populations within tardigrade species may be expected to differentiate as a result of local adaptations, given that the environmental conditions differ.

We report some preliminary results along this line from a study on the eutardigrades *Richtersius coronifer* and *Ramazzottius oberhaeuseri*. In each of these species, the anhydrobiotic survival of two populations, one from Sweden and one from Italy, were investigated. The animals were dehydrated individually on wet sand and kept dry for 12 days at 23° C and 65 % RH, followed by rehydration and revival monitoring.

We found that anhydrobiotic survival was similar in the Swedish and the Italian populations of both species, indicating that no divergence with respect to anhydrobiotic capacity has occurred. The two species, however, differed in anhydrobiotic survival. *Ramazzottius oberhaeuseri* had a considerable higher survival (66 %) than *R. coronifer* (40 %). This result is in line with earlier studies which found *R. oberhaeuseri* to have a very high water-retentive capacity.

## (28)

# A New Tanarctid Arthrotardigrade with Buoyant Bodies

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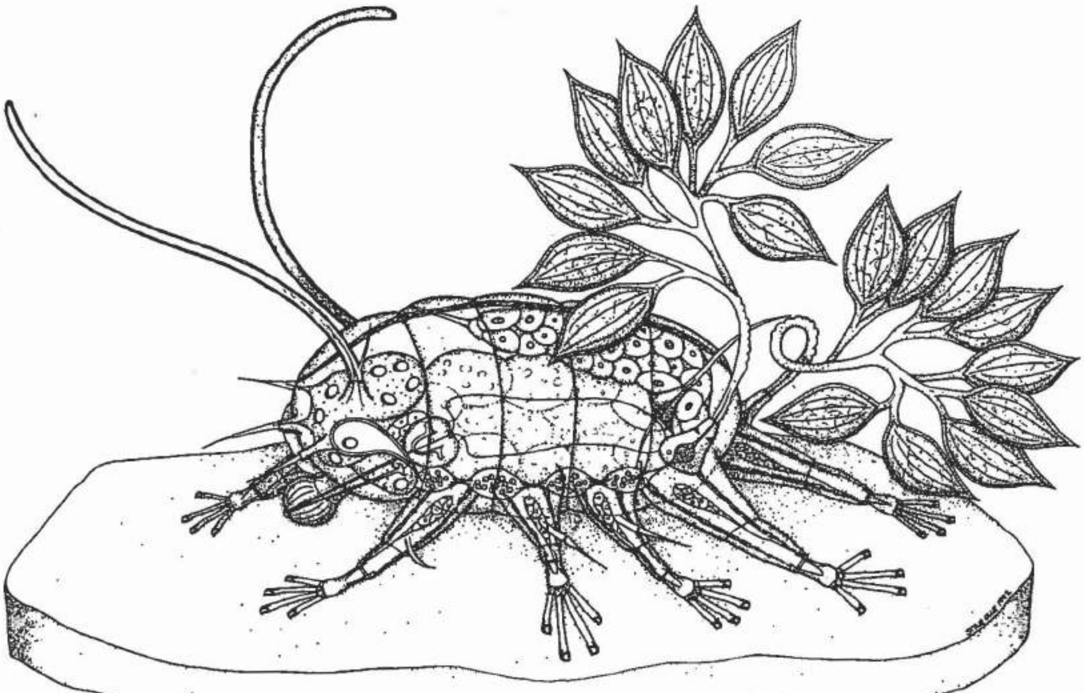
**Lecture**

Four cruises have revealed a very rich tardigrade fauna inhabiting the shell gravel at the Faroe Bank and Bill Bailey Bank in the North Atlantic. This study presents the description of *Tanarctus* n. sp., which is one of the many new tanarctids from the Faroe Bank. Currently 10 species of *Tanarctus* are described from various localities around the world. At the Faroe Bank 9 species new to science have been found together with *T. gracilis* Renaud-Mornant, 1980 and *T. heterodactylus* Renaud-Mornant, 1980 originally described from the Western Atlantic. The new species is found from 104 m to 160 m (BIOFAR stations: 571, 573, 591, 786, 787 and 2013) and always in clean carbonate sand to shell gravel.

*Tanarctus* n. sp. ("the balloon animal") has been investigated using LM, SEM and TEM and is characterised by the sense organs on the fourth pair of legs (P4) which are terminating in nine balloons each. The surface of the balloons is adhesive and in whole-mount preparations the balloons attach strongly to the coverslip. The epicuticle consists only of a very thin membrane-like layer, which may be folded or smooth, depending of the degree of extension of the balloon. The stalk of P4 is hollow and seems to lack sensory structures. The internal of the balloon is also hollow with a secondary membrane (internal trilaminary layer?). The balloon is filled with liquid which may be different from sea water. It is hypothesised that the balloons are used as bouancy devices, which allow the animal to move easily in the detritus layer on top of the sediment. Live animals are observed upside-down in the water column floating with the head downwards. The dorsal surface of the animal is often covered with coccoliths of the species *Emiliania huxleyi*, camouflaging the animal in the white sediment.

*Tanarctus* n. sp. is most closely related to the other tanarctid species without club-shaped secondary clavae and possessing leaf-like terminations of the sense organ on the fourth pair of legs, i.e. *T. velatus* McKirdy, Schmidt & McGinty-Bayly, 1976 (Galapagos Islands), *T. helleouetae* Renaud-Mornant, 1984 (Guadeloupe) and *Zioella pavonina* Renaud-Mornant, 1987 (Guadeloupe). The last tree mentioned species are all found in tropical waters at low water depths (intertidal). The subtidal finding of *Tanarctus n. sp.* in the North Atlantic is surprising. Coarse sediment, primarily carbonate in origin, on the slope of Faroe Bank and Bill Bailey Bank, make these banks unique habitats. Such sediments are usually associated with much more shallow biotopes at southern latitudes. The two localities may be strongly influenced by the Gulf Stream, which results in high bottom temperature (9-11°C) on the top of the banks also during the winter.



## (29)

# A review and graphical presentation of the African tardigrade fauna using GIS with the description of *Isohypsibius* nov. sp. (Eutardigrada: Hypsibiidae) from Lake Malawi.

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**Poster**

The tardigrade fauna of the African continent is reviewed and presented graphically using WorldMap a Geographical Information System (GIS) developed for exploring geographical patterns in diversity in large biological datasets. References to the African tardigrade fauna have been gathered in the literature and supplemented with unpublished species information from the collection of Prof. Reinhardt M. Kristensen, Zoological Museum, University of Copenhagen.

154 species belonging to 34 genera of tardigrades are present. They are constituted of 103 species of eutardigrades and 51 species of heterotardigrades of which 42 are semiterrestrial and 9 are marine. 20 countries report the presence of tardigrades, but of these are 10 countries represented by a single reference only. It is evident that the research has been concentrated on the countries of North Africa (Morocco, Algeria and Tunisia; 10 references), and South Africa and Tanzania (10 references each). Especially the marine tardigrades have been neglected with no reports from the shores of the African continent. Species data from the RMK collection from Egypt is included in the analysis.

Several species, especially from the older literature, have been omitted from the GIS analysis due to imprecise locality data. The grid or resolution of the current analysis is one degree.

The scattered and sparse knowledge of the distribution of the African tardigrade fauna makes general conclusions difficult, but large regions which should be investigated is easily recognised. Also regions where GIS could be further used to illustrate ecological preferences are pointed out by the analysis. The distribution of investigated sites corresponds with easy accessible or "tourist" locations.

*Isohypsibius* nov. sp. is described from Lake Malawi. It has a smooth body surface and lacks eyes. The length of the first macroplacoid is longer than the second macroplacoid, which is only slightly longer than the third. The microplacoids are absent. The new species is interstitial and common in coarse sediment with no other tardigrade species present.

(30)

# The cephalic sensory structures and the brain of *Actinarctus doryphorus* (Arthrotardigrada).

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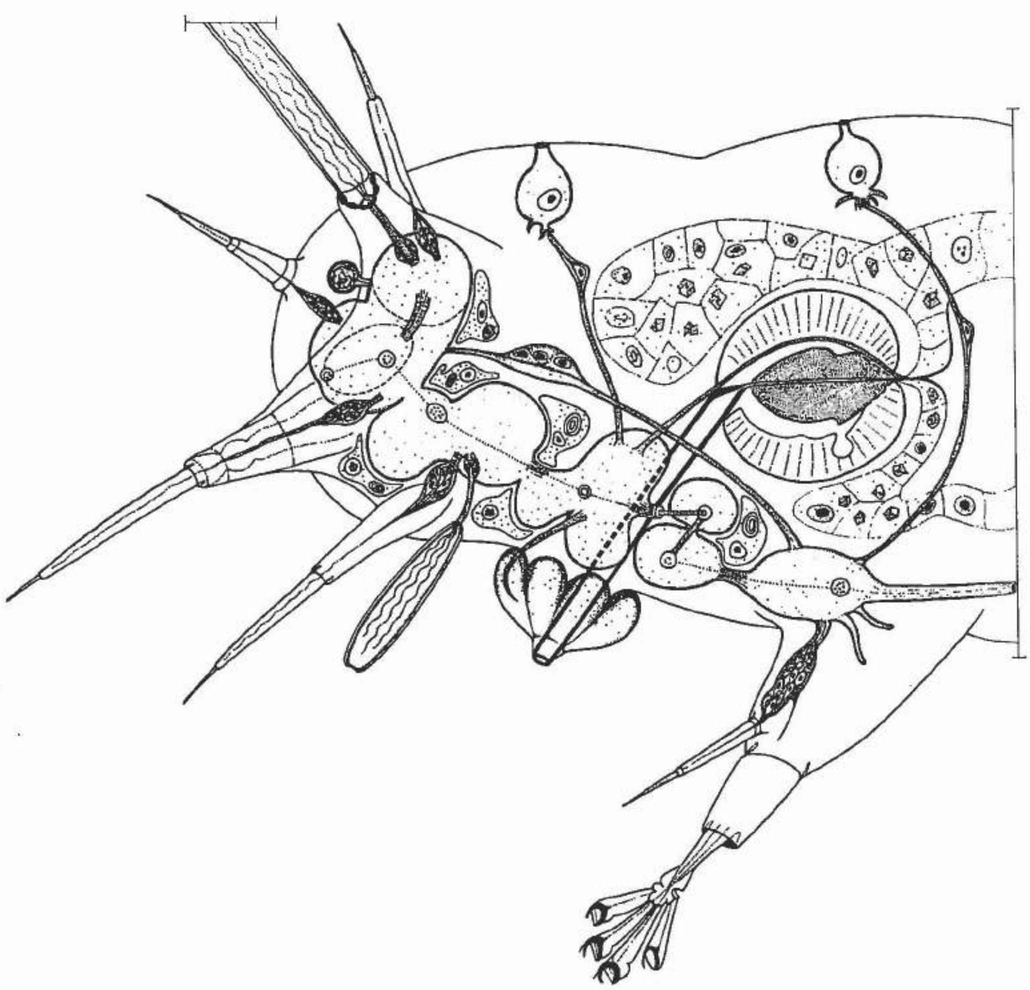
**Lecture**

The cephalic sensory structures of *Actinarctus doryphorus* consist of 11 external structures (seven cirri and four clavae), several internal sensorial fields around the mouth cone, and a pair of internal phaosome-like ocelli. The three pairs of cirri (lateral, internal and external) and the unpaired median cirrus may be mechanoreceptors. The sensory part of each cirri consists of one or two very modified ciliary structures surrounded by microvilli from a sheath cell. The two pairs of clavae may be chemoreceptors. The primary clava is very long and vermiforme with a large pore close to the tip of the sense organ. The sensory structure of this clava consists of both microvilli and ciliary structures. The primary clava and the lateral cirri on each side have a common cirrophore. During the molting cycle the new clava and the new cirrus are formed in the same invagination of the epidermis. In the cirrophore an osmiophilous structure, called the van der Land's body is located proximal to the primary clava. The van der Land's body is a cuticular closure surrounding the large sensoric nerve from the clava. The so-called buccal clavae are serial homologous with the primary clavae. They are located close to the ventral external cirri and not close to the rostral internal cirri; therefore the buccal clavae probably represent the tertiary clavae. If so the secondary clavae are absent.

The brain is large and consist of 1) a protocerebrum that innervates the ocelli, the lateral cirri, the median cirrus and the large primary clavae; 2) a deutocerebrum that seems to innervate both the internal and external cirri and the buccal clavae; 3) a tritocerebrum that innervates the mouth cone, and buccal apparatus. Futhermore, this third part of the brain innervates a pair of dorsal unicellular glands. However, this interpretation of the innervation may be wrong, as the cephalic sensory structures secondarily can move from the ventral to the dorsal side. The stylets and stylet supports are most likely innervated by the subpharyngeal ganglion, which consists of two elements. The commensure between the protocerebrum and the first trunk ganglion has its own small ganglion. The nine "ocelli" described by Renaud-Mornant are amoebocytes permanently fixed to different parts of the brain.



**(31)**

# Fossil stemline arthropods - tardigrades, lobopodes and pentastomids - from the Cambrian – An 'Orsten' perspective

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**Key-note Lecture**

'Orsten'-type preservation is an exceptional secondary phosphatisation of the cuticular surfaces without further deformation, hence yielding three-dimensional fossils. This rare type seems to affect mainly arthropods at the very small scale from approx. 100 to 1000 µm in body size. Records are now known from several continents and ranging from the Lower Cambrian (approx. 540 Mio. years old) to Lower Cretaceous. Among the Cambrian records, also representatives of the stemline of Arthropoda have been found, such as early larval stages of pentastomids, tongue worms, which today are parasites of various tetrapods. Not unexpectedly, also a representative of the minute tardigrades could be discovered in Middle Cambrian rock from the Siberian platform which is currently under description and possibly represents the sister taxon to all extant groups. New, not yet described in detail are the fragments of small tubular, finely annulated organisms, which are presented as the first lobopodians in an `Orsten´-type preservation. They share with known Lower to Middle Cambrian lobopodes the annulated segmental limbs (lobopodia) and segmental paired dorsal outgrowths on the finally annulated tubular body, such as known from the Lower Cambrian *Xenusion auerswaldae* Pompeckij, 1927, which has been found in drift boulders in northern Germany, and several other loboponians from the Burgess-shale and Chengjiang faunas. In addition these Orsten fossils provide significant new data due to their preservation of cuticular details undetectable on flattened fossils. All three so-called "protarthropods" still lack or partly lack characteristic features known from forms belonging to the later evolutionary line of Arthropoda, such as details of the tagmatisation (head formation), the clear arthrodisation (segmental sclerotisations) and clearly arthropodial limbs with stem and rami. Among the three taxa, the pentastomids seem to be the latest offshoots due to their possession of articulated limbs with pivoted joints between the three articles. The Cambrian record of the "protarthropods" suggests that their ancestral line started even earlier, most likely even in Pre-Cambrian times.

## (32)

# Tardigrade assemblages from an altitudinal transect in Venezuela.

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**Lecture**

Little is known about the tardigrade fauna in Venezuela with only one previous paper on heterotardigrades published. The current work is based on material from Mount Roraima, one of the tepuis of the Gran Sabana region in southern Venezuela. The tepuis of this region represent remnants of the eroded Guyana Shield, and are well-known for their endemic fauna & flora. Tepuis are high sandstone massifs with sheer or almost sheer sides, which emerge up to 800m above the surrounding lowlands.

Mount Roraima is situated on the international borders between Guyana, Brazil and Venezuela. All samples were collected from the Venezuela territory. Material was collected at three altitudes, 1500m, 2300m and 2800m, and consisted of assorted vegetation and plant debris. The batched samples from each altitude were separated into sub-samples of similar plant morphotypes and aired dried for storage. Sub-samples were rehydrated in tap water for at least 24 hours before tardigrades were extracted.

The species composition of the tardigrade communities varied with altitude, being more diverse at the middle and highest sites. The community composition at the lower site was quite different from the other two sites, which were very similar to each other.

Many species from the current study represent new records for S. America, including *Milnesoides exsertum* and *Calohypsibius schusteri* reported in sites other than their type localities in Australia and the USA respectively. No eutardigrades had previously been reported from Venezeula, so all reports are first records. Heterotardigrade diversity was also high with species from four genera found.

## (33)

# Biodiversity and Biogeography of the global marine tardigrade fauna

**Sandra J McINNES**

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**Lecture**

Limno-terrestrial Tardigrada contain three generic/ familial biogeographic clusters which correlated with plate tectonic events. These are the early division of Pangaea into Laurasia (Europe & North America) and Gondwana, and subsequent West (South America/ Africa) versus East (Antarctica/ Australasia) divisions of Gondwana. This begs two questions. (1) Are Laurasian and E/ W Gondwanan tectonic divisions also apparent in the marine Tardigrada? (2) Do limno-terrestrial and marine Tardigrada represent 'sister' taxa of comparable antiquity, or is one a younger derived 'daughter'?

These questions were addressed via a complementary systematic/ biogeographic database containing all records of extant marine tardigrades. The database currently contains two major flaws. The verified taxa (130 species and 33 genera), are too few for precise biogeographic analysis, and the data are clearly focused on a few well-studied areas, with maximal diversity, co-occurrence and clusters centred on the Mediterranean, North Atlantic and Indo-Pacific.

Four features are however apparent from the data. (1) Higher taxa are not confined to prescribed biogeographic provinces. (2) Species are either deepwater or coastal, but genera can occur in both. (3) Coastal species dominate the database. (4) Possible evidence of coastal species being anthropogenically dispersed between biogeographic provinces, via bio-fouling of ship hulls and/ or in ballast water.

The value of this database can be vastly improved if all unpublished data are made available and if future studies focus on sites other than the Mediterranean, North Atlantic Provinces. Data compilation and species identification can be made more even more effective via a central (web site?) 'guide' to marine Tardigrada. Finally, re-examination of critical species, and the historical/ biogeographical congruence between the two groups can resolve the relationship between the marine and limno-terrestrial tardigrades.

(34)

# *Milnesium* cfr *tardigradum* a monitor of high altitude micro-invertebrates on sub-Antarctic Marion Island.

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**Poster**

Sub-Antarctic Marion Island in the South Indian Ocean is a *c.* 4 million years old shield volcano, with the remnants of a summit Pleistocene glacial ice cap. Katedraalkrans is a potential high altitude (*c.* 700m a.s.l.), glacial refuge and 'wet' oasis in a plain of small (<20 mm diameter) free draining and mobile scoria particles. Samples of moss and *c.* 400 kg. of scoria were washed through 90 µm sieve and the meiofauna extracted with kerosene.

Extraction yielded a meiofauna (*c.* 20000 individuals) of spiders, mites, insects and snails, together with an unexpected *c.*200 micro-faunal specimens. The latter included large forms of *Milnesium* cfr. *tardigradum* (>660 µm), *Echiniscus* sp. (>300 µm), and *Macrobiotus* sp. (>420 µm). The guts of mounted *Milnesium* cfr. *tardigradum* contained remains of other smaller micro-fauna, including the trophi of bdelloid rotifers, mouthparts, and entire individuals, of *Diphascon* sp. (Tardigrada). Additional micro-fauna and meiofauna were subsequently extracted, via a colloidal suspension, from particularly rich samples. These data confirmed the *Milnesium* gut content data by revealing additional smaller tardigrades (including *Calohypsibius* cfr *ornatus*, and *Diphascon* sp. nov.), four bdelloid rotifer species and nematodes.

*Milnesium* cfr. *tardigradum* is the top micro-faunal predator of Katedraalkrans scoria and mosses that ingests its prey entire. This feeding strategy, in contrast with macrobiotids body fluid ingestion, makes this tardigrade an essential monitor of the smaller micro-fauna occurring at high altitude Marion Island.

# (35)

## Is it real?

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**Poster**

How true is the statement: "The camera never lies"?

'Trick' photography has a long and varied history, from ghost (double exposure) images to 'fairies at the bottom of the garden'. Good 'fakes' are extremely difficult to refute.

There are often problems in copying images from the microscope for publication. Early attempts were hindered by poor optics that have rendered some diagrams and taxonomic descriptions unusable. Optics have improved but, while the *camera lucida* can minimise some basic drawing problems, the final image is dependent on the illustrator's skills, which also influences the interpretation of structures.

Modern computer technology allows laboratory images to be 'grabbed' from cameras or scanned from photomicrographs into powerful graphic manipulating software, where "real" images can be directly converted into line drawings. This should, in theory, make life easier but, as with *camera lucida*, the resulting images are again dependent upon operator skill and observation.

With new computer software it is now possible to move beyond the "simple" 'live'/ photographic image to 'line drawing' aspect of taxonomy to extensive manipulation of a photographic original. From an original Scanning Electron Micrograph of a non-cleaned tardigrade image-manipulation was initially confined to removal of large background debris. Subsequent retouching edited out the dirt overlying the specimen, but it is now possible to take that very short step to make major morphological changes and thereby editing a 'new species'.

The computer is, like the camera, an imaging tool, which still requires careful observation. Use of image manipulation software can accidentally or wilfully distort reality, by falsifying taxonomic details in drawings and photographs. The temptation to use image manipulation to show a 'clean' specimen particularly if only one photograph is available should perhaps be avoided. The camera itself may not lie, but computer software can edit the truth.

(36)

# Exposed: Danger lurking in the Maritime Antarctic freshwater algal mats for rotifers and tardigrades.

**Sandra J McINNES**

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**Lecture**

Whilst several terrestrial nematophagus fungi have been reported from Antarctica, this is the first report of a Hyphomycetes that traps freshwater rotifers and tardigrades. A new species, *Cephaliophora* sp. nov., was isolated from cyanobacterial mats and sediments in the shallows of lakes and pools of Signy Island, in the Antarctic South Orkney archipelago. A trapped tardigrade, *Hypsibius antarcticus* (Richters, 1904) (Hypsibiidae; Tardigrada), and the husks of several bdelloid rotifers were found attached to the vegetative hyphae of this predacious fungus.

Trapped rotifers and tardigrades where observed being assimilated by a convoluted knot of irregular hyphae, grown from the tips of the adhesive pegs into the prey. Once the prey was digested these assimilating hyphae were re-absorbed into the main hyphal branch, or occasionally re-emerged as vegetative hyphae. The prey husk was further degraded by bacterial action.

*Cephaliophora* spp. has been isolated from animal dung, decaying wood, mosses, organic debris and soils, on all continents except Antarctica. *Cephaliophora* sp. nov., represents the first generic record from the Maritime Antarctic and from freshwater habitats.

(37)

# Tardigrades of Arkansas and Louisiana, U.S.A.

**Harry A. MEYER**

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**Poster**

Outside of the states of Texas and Alabama, the distribution of limnoterrestrial tardigrades in the states of the American Gulf Coast is poorly known. There are no records of tardigrades from the states of Louisiana and Mississippi, while in Arkansas only one species has been reported. I began sampling for Louisiana and Arkansas tardigrades in the spring of 2000, as part of a planned long-term study of tardigrade distribution and ecology in the region.

I sampled mosses and lichens from three sites in Calcasieu Parish in southwest Louisiana. No tardigrades were present in ground mosses (*Sphagnum* sp.). From mosses and lichens on trees I identified six species of tardigrade, belonging to four genera (*Milnesium, Echiniscus, Macrobiotus,* and *Minibiotus*). No sample contained more than two species of tardigrade.

Mosses and lichens obtained in the Ouachita and Ozark Mountain regions of Arkansas are currently being processed.

## (38)

# Ecology of tardigrades in southern Africa: A preliminary report

**Roger MIDDLETON**

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**Poster**

Limno-terrestrial tardigrades were sampled from a range of habitats in southern Botswana and Lesotho, southern Africa. Terrestrial habitats included mosses (*Pleurocarpous* and *Acrocarpous* spp.) and lichens growing on trees and rocks; soil and leaf litter. Botswana has a semi-arid climate with generally ephemeral aquatic habitats and thus limited opportunities for obtaining freshwater tardigrades. Collections focused on algae and substrates from temporary streams, rock pools and other novel, transitory habitats such as salt pans and the Okavango Delta.

The tardigrade records from this study represent the first reports from both Botswana and Lesotho. These will be compiled into a database with three primary objectives; 1) elucidate the biodiversity of sub-Saharan African tardigrades; 2) identify ecological, in particular substrate association, of the limno-terrestrial fauna; and 3) associations with other co-occurring meiofauna.

There is both an ecological and a biogeographic significance to the tardigrade faunas of Botswana and Lesotho. First, both countries represent marginal environments, including the Kalahari Desert, suggesting many species will have novel life history strategies to cope with these very challenging conditions. Second, both countries are part of the South African biogeographic province, which has well documented unique endemic macro-faunas and macro-floras but a poorly documented microfauna. The aim of this study is to redress the problem by forming an essential baseline for the Tardigrada.

(39)

# KanCRN: A Collaborative Research Model using Tardigrades

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**Poster**

The design, operation, and success of the Kansas Collaborative Research Network's use of tardigrades as a classroom tool to teach real world research at the secondary level is discussed. Interviews with teachers, students, organizers and mentors are included. The success of two students in using tardigrades in science fair competition is highlighted as examples of what can be achieved when collaboration is a team objective.

(40)

# Return to Terra Incognita: More Giant Tardigrades

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**Poster**

After the journal of the late Dr. Harry Johnson that described an encounter with giant tardigrades was published, additional information has come to light about the events described. Two other members of the trip have come forward to collaborate the events of the phenomena. They also describe a second expedition, designed to capture and display two of the giant tardigrades. The events and fate of that trip are reported in an interview with the authors and are supported by some interesting pictures. For the first time, the interviewees advance an explanation for the phenomena they claim to have encountered on both trips. The authors draw no conclusions.

## (41)

# Distribution of Tardigrades within a Moss Cushion: Do Tardigrades Migrate in Response to Changing Moisture Conditions?

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**Lecture**

The distribution of tardigrades within the layers of the moss *Grimmia alpicola* Hedw. was investigated. The aim of this study was to determine the tardigrade species present within the moss layers during both wet and dry moisture conditions and to determine if migration occurred in response to changing moisture conditions.

Samples of the moss were removed from concrete caps on brick fence post caps during wet and dry periods and separated into two sections (top and bottom). The tardigrade species from each location and moisture condition were identified to species. Data for each species were statistically analyzed with a two-way ANOVA to compare the numbers of individuals present in the top and bottom layers of the moss under both wet and dry moisture conditions.

Five tardigrade species were identified, including 2 species new to science: *Macrobiotus* sp. nov.; *Milnesium tardigradum*, Doyère, 1840; *Echiniscus viridissimus* Peterfi, 1956; *Echiniscus perviridis* Ramazzotti, 1959; and *Echiniscus* sp. nov. The first identification of a male in the *viridis* group of the genus *Echiniscus* (*E. viridissimus*) was recorded. No significant differences were found in the numbers of individuals of 4 of the 5 species at each location within the moss or for each moisture condition. Only 1 species, *E. viridissimus*, was significantly more frequent in the top layer of the moss, regardless of moisture condition.

Migration within the moss cushion was not exhibited by any of these 5 species as a result of changes in moisture conditions. In xeric moss species, it may not be beneficial for tardigrades to migrate to avoid desiccation. Instead, they apparently undergo cryptobiosis in both the top and bottom layers of the moss cushion.

## (42)

# Seasonal and Altitudinal Variation in the Distribution and Abundance of Tardigrada on Dugger Mountain, Alabama

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**Lecture**

A survey of the distribution of terrestrial tardigrades on Dugger Mountain, Alabama, was conducted seasonally during the time period from April 1997 through June 1998. Five trees (*Quercus alba*) with cryptogams, 3 on north-facing slopes and 2 on south-facing slopes, were sampled seasonally at 3 sites (head-waters, mid-waters, mouth-waters) along an unnamed tributary of the South Fork of Terrapin Creek. Trees were chosen based on their location outside the riparian zone at the peak, mid-point, or base of the north-facing and south-facing slopes along the creek. Tardigrades were extracted from the samples, mounted individually in Hoyer's medium, and identified to species using phase microscopy. Seasonal and altitudinal variations in the distribution of the populations on the north- and south-facing slopes were determined. Present on Dugger Mountain were tardigrades belonging to 2 classes, 7 genera, and 11 species (*Macrobiotus areolatus/tonollii*, *Macrobiotus* cf. *echinogenitus*, *Macrobiotus islandicus*, *Minibiotus intermedius*, *Milnesium tardigradum*, *Diphascon pingue*, *Hypsibius pallidus*, *Echiniscus* n. sp., *Echiniscus virginicus*, *Pseudechiniscus ramazzottii*, and *Pseudechiniscus suillus*). Significant seasonal and altitudinal differences were found in tardigrade abundance from samples collected at specific sites and between north- and south-facing slopes. Pooled data showed no differences in the overall abundance or number of species at each altitude. However, significant seasonal differences in both abundance and number of species were seen in pooled samples.

## (43)

# Tardigrades of South America: Machu Picchu and Ollantaytambo , Peru.

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[1]*Department of Biology, Chestnut Hill College, Philadelphia, PA 19118, USA*

**Lecture**

During July 1999, a study group from the University of Kansas visited the ancient Inca ruins in and around Machu Picchu and Ollantaytambo, Peru. They collected lichens and mosses from the rock walls around the ruins. The samples contained four genera and six species of tardigrades. Their associational patterns and relationships are reported.

## (44)

# Panarthropods: articulates or ecdysozoans ?

**Claus NIELSEN**

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**Lecture**

The monophyly of the Panarthropoda (Onychophora + Tardigrada + Arthropoda) seems not to be questioned now, but their phylogenetic position is hotly debated. The more "classical" view is that they show so many morphological similarities, i.e. synapomorphies, with annelids that the two groups are regarded as closely related, often as sister groups, whereas the Cycloneuralia (Gastrotricha + Nematoda + Nematomorpha + Priapula + Kinorhyncha + Loricifera) are regarded as primarily unsegmented and therefore only distantly related. The so-called "new phylogeny", which is mainly based on gene sequencing and HOX genes, considers the panarthropods as members of a larger clade, Ecdysozoa, comprising all protostomes which moult, i.e. the Cycloneuralia except the Gastrotrichs.

The morphological characters used in assessing the phylogenetic interrelationships within these groups will be discussed, the conclusion being that there are several striking similarities between annelids and panarthropods, whereas the similarities between panarthropods and nematodes (the only phylum where sufficient information is available) seem less well supported.

(45)

# Moss – living tardigrades from two altitudinal transects at Disko Island, West Greenland

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**Poster**

Little is known about the factors leading to habitat preferences in limnoterrestrial tardigrade species. Numerous observations from a variety of heights have suggested that some species prefer certain altitudes and / or certain kinds of bedrock.

In this study, 15 tardigrade species were collected out of moss samples from alcaline and non-carbonate bedrock along two vertical transects in a Low Arctic and a High Arctic environment. Ten samples were taken per height from rocks along two vertical gradients from 0 m above sea level (a.s.l.) up to 400 m a.s.l. every 100 metres.

The highest number of species and individuals was found in the sample from 0 m a.s.l. of the transect in the Lyngmarken area (Low Arctic), where 97 tardigrades of 8 genera were collected.

The greater part of the species collected showed no particular altitudinal distribution pattern but some species showed a wider ecological amplitude than those described in earlier studies.

These results were obtained during the 1998 Danish-German Excursion to Disko Island, West Greenland.

(46)

# Aspects of cryptobiosis in the eutardigrade *Adorybiotus* (*Richtersius*) *coronifer*.

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**Lecture**

*Adorybiotus coronifer* is a bryophilic tardigrade found in extreme environments throughout the world (it is for example found in Greenland, Sweden and the high parts of the Himalayas). During dry spells it is found in the dry mosses in the anhydrobiotic tun state. *Adorybiotus coronifer* collected at Øland, Sweden has previously been shown to survive severe desiccation and temperatures down to -196°C both in the anhydrobiotic and in the active state. During desiccation it accumulates the disaccharide trehalose in concentrations of up to approximately 1.6% dw.

Dry mosses containing *Adorybiotus coronifer* in anhydrobiosis were collected at Øland, Sweden and kept dry in the laboratory until use.

**Resistance to alcohols of varying polarity.**

To investigate the role of hydrophilicity (polarity) of organic solvents on the survival of anhydrobiotic *A. coronifer*, animals in anhydrobiosis were exposed to 3 alcohols of different polarities for various periods of time.

Extraction of animals was carried out by rinsing lightly crushed moss cushions through a sieve system after which animals were collected from the 63 um fraction with an Irwin sling. Groups of 70 animals were transferred 30 um netting attached to a small plastic tube. The tubes were placed in moist sand, with the netting touching the sand, in a petri disc and capped with aluminium foil and the sand was allowed to dry for 48 hr at 20°C and approximately 60% RH.

Animals were exposed to each of the three alcohols; 96% ethanol, 1-butanol and 1-hexanol by transferring the tubes with the dry animals to the alcohols and exposing them for various time periods. After exposure the tubes were dried on blotting paper for 15 min at 40°C and placed in water at 5°C for 24 hr after which survival was calculated. Results showed and increasing survival rate with decreasing polarity. Animals exposed to 96% ethanol died within minutes whereas animals exposed to 1-hexanols did not show any decline in survival over a period of 7 days and the survival in this group did nor differ from survival in the controls not exposed to the alcohols.

**Resistance to high temperatures.**

It has previously been shown that anhydrobiotic animals can survive exposure to high temperatures (e.g. the chironomid larvae *Polypedilum vanderplanki* survives temperatures up to 106°C for 3 hr). To investigate if this is a general property in anhydrobiotic animals, anhydrobiotic *A. coronifer* were exposed to various temperatures between 50 and 100°C.

Small moss cushions containing A. coronifer in anhydrobiosis were exposed to 50, 60, 70, 80, 85, 90 and 100°C for 60 min. After exposure the moss cushions were hydrated in tap water for 2-4 hr and live and dead animals were counted. Results showed no increase in mortality at temperatures up to about 70°C, after that survival decreased to about 20% at 80°C and no animals survived exposure to 100°C.

***De novo* protein synthesis after anhydrobiosis.**

Various proteins are likely to be denatured during anhydrobiosis as a consequence of changes in pH and ion strength during drying. *De novo* protein synthesis may therefore occur in anhydrobiotic animals during dehydration. This protein may be the synthesis of stress proteins (chaperone, heat shock proteins), which "help" the cells in refolding or degrading denatured proteins. Proteins that protect parts of the cells during dehydration may also be synthesized as a response to dehydration.

Groups of 60 to 80 animals were extracted from the moss and within 15 min after rehydration had begun transferred to an incubation chamber and incubated with $^{3}$H-leucin for 1/2, 1 and 2 hr. After incubation the animals were homogenised, centrifuged and the proteins in the supernatant were separated by SDS gel electrophoresis and scintillation autoradiography was carried out.

Results showed that there is indeed *de novo* synthesis of a 67 kDa protein during dehydration of *A. coronifer*. The role of this protein is not known and further investigations will be carried out with the aim of investigating the role of *de novo* protein synthesis in anhydrobiosis.

# (47)

# Spermatozoon in Tardigrades: Evolution and Relationships with the Environment

**Lorena REBECCHI**

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**Lecture**

Our research group has studied the ultrastructure of tardigrade spermatozoon for more than ten years. These studies have allowed us to collect information on a large number of species belonging to several taxa and inhabiting different environments. An evaluation of male gamete evolution can be made in this phylum, considering that tardigrades most likely originated in the sea and subsequently colonized freshwater and land. The spermatozoon ultrastructure (SEM and TEM) of several moss-dwelling Echiniscidae (Echiniscoidea, Heterotardigrada) and of terrestrial and freshwater species of various families of eutardigrades was analyzed. The general morphology of the spermatozoon in five genera of Echiniscidae is similar. It is morphologically quite similar to those of the tidal species of *Echiniscoides* (Echiniscoididae, Echiniscoidea) but differs from those of the Arthrotardigrada. This confirms the close phyletic relationships between marine and terrestrial Echiniscoidea and underscores that the change of environment has a low influence on the organization of the sperm cell, at least in this group. These data are in agreement with the large number of conservative characters present in the phylum Tardigrada.

The spermatozoa of Echiniscoidea differ markedly from those of eutardigrades. In the latter, the male gamete is always of the derived type and has undergone remarkable evolutionary modifications. Several different structural organizations are known; they can be related to the family but also to the environment. The land-dwelling species have spermatozoa with different shape and organization. Many have in common a relatively short length and a thick shape (besides some long and thread-shaped spermatozoon of some Macrobiotidae species), evident acrosome and mid-piece. The freshwater Hypsibiinae with *Isohypsibius*-type claws and the related moss-dwelling species have a very long and filiform male gamete, with a very short acrosome and without mid-piece. The other eutardigrades most represented in freshwater belong to the subfamily Murrayinae and to *Hypsibius*

(Hypsibiinae). Males were never found in the first group. The spermatozoon structure of *Hypsibius convergens* here described was described in a population collected from a leaf litter (beech). Its shape recalls that of the land-dwelling species.

**(48)**

# Ecological Distribution and Community Analysis of Tardigrada from Choccolocco Creek, Alabama, with the Description of a New Species of *Echiniscus*


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**Lecture**


A seasonal survey of tardigrade populations in the riparian zone of the Choccolocco Creek, Alabama, was undertaken from August 1994 through December 1995. Choccolocco Creek is 113.6 km (71 miles) long, beginning at the southern terminus of the Appalachian Mountain Chain (Dugger Mountain area) and flowing southwesterly to join the Coosa River near Pell City, Alabama. Six stations within the riparian zone were sampled in different portions of this creek: headwaters (stations 1 and 2), mid-waters (stations 3 and 4), and mouth-waters (stations 5 and 6). At each sampling site, 3 trees with cryptogams were selected within the riparian zone, within 10 m of the stream. Six collections were made during the seasons for a total of 108 samples, and 1,588 tardigrades were extracted, individually mounted on slides in Hoyer's medium, and identified using phase contrast microscopy. Two classes, 7 genera, and 11 species were represented (*Echiniscus* n. sp., *Echiniscus mauccii, Pseudechiniscus suillus, Hypsibius dujardini, Itaquascon trinacriae, Macrobiotus areolatus/tonollii, Macrobiotus* cf. *echinogenitus, Macrobiotus islandicus, Macrobiotus richtersi, Minibiotus intermedius,* and *Milnesium tardigradum*). The community was dominated (86%) by specimens in the genus *Macrobiotus*. One species of *Echiniscus* was new to science and is described in this paper. It differs from other *Echiniscus* species by the presence of a papilla on the proximal outer portion of legs II and III. No significant difference was found between tardigrade occurrence and season, moss genera, or tree species. However, there was a significant relationship between the number of tardigrades and site (habitat). Tardigrades seemed to prefer areas with a closed canopy and/or heavy subcanopy that decreased insolation and increased relative

humidity. Simpson's and Shannon-Wiener species diversity indices within the riparian zone communities along Choccolocco Creek suggested that species evenness was lower than species richness; there were more rare individual species than common species. Jaccard's and Standard's indices of community similarity suggested that the communities within the riparian zone were dissimilar along Choccolocco Creek. The communities with shared species had similar relative abundances and those communities with similar species occurrences had different relative abundances.

(49)

# Recent Onychophora: are they a sister group of the Tardigrada?

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**Key-note Lecture**

Since their discovery both taxa the Onychophora and the Tardigrada have been assigned to various positions in the phylogenetic system. Their origins as well as their affinities still remain controversial and are under discussion.Traditionally both groups are regarded as separate phyla within the Articulata, but their sistergroup-relationships and their exact position within the animal kingdom remain uncertain.

In the past it was common use to integrate three taxa of uncertain systematic position (namely the Onychophora, the Tardigrada and the Pentastomida) into artificial groupings like the ‚Pararthropoda' or the ‚Pro(t)arthropoda'. Some authors united them as the ‚Malacopoda' or the ‚Oncopoda' because of the unique structure of their appendages. Today the old concept of.the ‚Pararthropoda' is rejected as the Pentastomida are considered to be Euarthropoda. The remaining taxa, viz. the Onychophora and the Tardigrada are sometimes considered as sistergroups.

In order to discuss the origin and the relationships of onychophorans and tardigrades the authoress aims to summarize the recent literature and to present proposals to help resolve the question of how closely these taxa might be allied. This discussion is based on her long-lasting scientific research into one of the the groups, the Onychophora. In order to question their possible sistergroup-relationship common and special features of the morphology and development of both taxa will be discussed. Suggestions as to the reconstruction of their possible ancestors will be presented.

## (50)

# Do Confocal Microscopy and Tardigrades have a future together?

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**Poster**

Due to the inherent transparency of the majority of tardigrades they have been routinely examined, unstained, using light microscopy contrast techniques such as phase contrast and differential interference contrast with the occasional dark field examination. Surface morphology has also been examined for many tardigrades using scanning electron microscopy.

With all internal examinations of intact specimens, using optical techniques, there is the problem of depth of field. A tardigrade 40µm in diameter would be, at X400 examination, approximately 10% in focus with the 90% out of focus image causing a blurring of the final image.

There have been examples of tardigrades sectioned for light microscopy examination in either wax or resin plus those who have sectioned for transmission electron microscopy. The difficulty of sectioning for subsequent light microscopy is the size of the specimen and the imperious nature of their exoskeletons.

The ability of confocal microscopy to focus in the z direction and achieve numerous in focus images is a powerful tool. The resultant images can be subsequently manipulated to create a montage, stereo image or give a three-dimensional reconstruction. Studies using confocal imaging to elucidate the morphology of specimens are less frequent than those studying the location of a fluorescent label for a specific chemical e.g. $Ca_{2+}$, or specific structure e.g. chromosomes.

This study used Acridine orange with four different protocols and fluorescent Feulgen reaction in combination with different fixation protocols. Once the most informative techniques had been established serial optical sectioning using confocal microscopy (Leica

UK) was used to acquire up to 40 images through the tardigrade *Echiniscus testudo*. The results indicate that there is a future for confocal microscopy in the study of intact tardigrades.

(51)

# Tardigrades – are they really miniaturized dwarfs?

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**Lecture**

A close relationship of tardigrades to Onychophora and Euarthropoda in a taxon Panarthropoda is usually accepted. However, tadigrades share some puzzling resemblances to nemathelminth ("aschelminth") taxa such as a myoepithelial sucking pharynx with a triradiate lumen and the terminal mouth opening. In the traditional concept of a close relationship of Panarthropoda to Annelida (= Articulata) a large annelid-like ancestor seems likely and therefore tardigrades are often interpreted as miniaturized animals that lost features such as a coelom, excretory organs or a heart secondarily. The current hypothesis of a closer relationship of Panarthropoda to Cycloneuralia, a subset of Nemathelminthes (including Nematoda, Nematomorpha, Loricifera, Kinorhyncha and Priapulida), together named Ecdysozoa, motivates to recheck character evolution in panarthropods. It is most parsimonious to assume for Cycloneuralia a small benthic marine organism with direct development. In an outgroup comparison this would argue for tardigrades being the basal panarthropod taxon. Several tardigrade characters would be interpreted as plesiomorphies (such as pharyngeal characters, terminal mouth opening and small body size) rather than as convergences. The absence of coelom and heart would be primary rather than a secondary loss. The debate about the validity of the Articulata- and the Ecdysozoa-hypothesis is still in process, but it is evident how these concurring hypotheses influence the interpretation of tardigrade characters.

## (52)

# Modern computer cladistics: Cladogram of the Animal Kingdom

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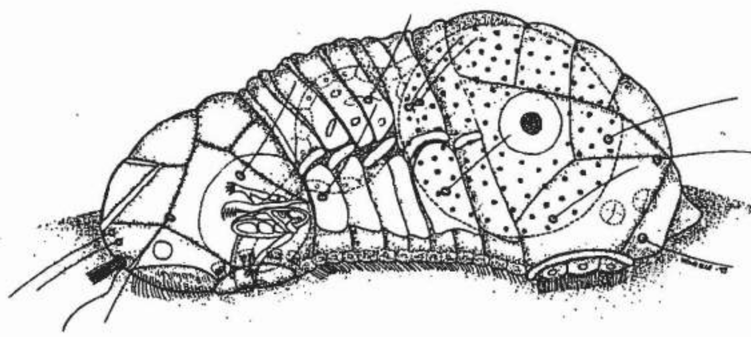
**Lecture**

Modern computer cladistics has become a well-incorporated discipline in zootaxonomy. Computer generated cladistic analyses of morphological as well as molecular data have been considered the most objective method to establish hypothesis of relationships between taxa. However, neither morphological nor molecular cladistic analyses can be performed without setting up some initial assumptions. The choice of characters, character coding, treatment of polymorphic taxa, number of taxa, and for molecular analyses choice of coding sequences and alignment options, are all based on assumptions and the authors subjective view.

In the following some of the general lines and pitfalls in computer cladistics are discussed with offset in a newly produced analysis of Animal Kingdom. The data matrix is a modification of the matrix presented by Nielsen et al. (1996). It is based on morphological data and holds 37 taxa, including Echiura, Gnathostomulida, Cycliophora and a new, yet undescribed, rotifer-like animal from a cold spring on Greenland.

References:

Nielsen, C., Scharff, N. & Eibye-Jacobsen, D. (1996): Cladistic analyses of the animal kingdom. Biol. J. Linn. Soc. **57**: 385-410.



## (53)

# Bound water and cryptobiosis

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### Lecture

The disaccharide trehalose has been shown to accumulate in tardigrades and numerous drought tolerant species from other phyla upon entry to the anhydrous state. This observation, along with some success achieved in the use of trehalose as a protective agent in technical desiccation trials, suggest that trehalose plays a key role in the resistance towards dehydration. However, the mechanism through which this effect in brought about remains to be resolved. Important evidence has suggested that the hydroxy groups of the saccharide interacts with polar groups of the biopolymers and hence provides structural stabilization by "replacing" the bound or structured water, which is lost during dehydration.

This presentation will discuss some properties of bound water, which has recently emerged from work with simple model systems such as lipid bilayer membranes and purified proteins. These results suggest that water near the surface is in fact less ordered than water in the bulk; *i.e.* this water is "released from the bulk" rather than "bound to the surface". In the light of this, possible roles of protectants such as trehalose as water replacing agents is discussed.

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# Cryptobiosis 300 Years on from Van Leeuwenhoek: what have we learned about tardigrades?

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**Key-note Lecture**

Biochemical and ultrastructural advances in the latter part of the 20$^{th}$ C. have revolutionized our understanding of cryptobiosis since Anton Van Leeuwenhoek gave the first formal description of the phenomenon at a Royal Society lecture in 1702. Keilin coined the term cryptobiosis in 1959 to describe the entry into a reversible ametabolic state and recognized that such 'latent life' could encompass processes induced by dehydration, cooling, and perhaps osmotic stress and anoxia. True cryptobiosis, as now understood, depends on the loss of a liquid water phase and can be induced by desiccation or freezing. Loss of 'bulk' or liquid water may result directly from evaporation, or arise through vitrification promoted by the formation of a carbohydrate matrix. The carbohydrates in question appear to be ubiquitous in cryptobiotes and serve multiple additional roles: as compatible intracellular osmolytes during desiccation or freeze-dehydration; as stabilizers of protein quaternary structure and lipid bilayer integrity with declining free water activity; and as supercoolants. Plants tend to rely on oligosaccharides such as stachyose and raffinose, while yeasts, spores, and metazoans depend primarily on disaccharides, particularly sucrose and trehalose, and on glycerol. As in many other anhydrobiotes, a metabolic preparatory stage in which these carbohydrates are synthesized from glycogen reserves appears essential for anhydrobiosis in tardigrades, and thus limits physiological tolerance of desiccation rate. Adaptive processes such as tun-formation in tardigrades and bdelloids, coiling in nematodes, and gradient-dependent changes in integumental permeability, retard water losses during preliminary desiccation and exert important influence on survival in xeric extremes. In tardigrades, cryobiosis, or cold-induced cryptobiosis, differs from anhydrobiosis in several important details. Tun formation is not essential for survival, and tolerance of cooling rate depends on the ability to inhibit intracellular freezing. Unlike many cold-tolerant arthropods, tardigrades are freeze-tolerant. Extracellular freezing is promoted by one or more ice-nucleating proteins in *Adorybiotus coronifer*, and occurs at high temperatures close to 0 °C. Tolerance of variable cooling rates to sub-freezing temperatures in this species does not seem to depend on trehalose synthesis, although a role of other possible intracellular cryoprotectants is likely. It is presently unclear whether cryobiotic tardigrades undergo cytoplasmic vitrification, or whether freeze-dehydration and colligative lowering of cytoplasmic water activity renders the remaining water unfreezable. The profound tolerance of environmental extremes displayed by cryptobiotic organisms apparently depends on the loss of a liquid water phase with accompanying metabolic depression as elegantly described by Clegg's vicinal network model. Thus protected from temperature- and solute-dependent effects on reaction kinetics, and (in part) from destructive free-radical oxidation, cryptobiotes can retain viability in a near-inert state for decades. Disruption of multi-subunit enzyme reactions accompanying loss of the vicinal water fraction may control the decline in metabolism, but also eliminates important free-radical scavenging pathways. Glycerol partly offsets this, acting as an antioxidant, but progressive free-radical oxidation in cryptobiotes may set the upper limit to longevity under aerobic conditions. While we can make many inferences of the physiology of cryptobiosis in tardigrades based on information gathered from other cryptobiotic organisms, specific studies on tardigrades are few, and encompass only a small number of species. This should prove a fruitful field for future research.

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**Update July 19, 2000**

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**Workshop:**
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# WORKSHOP ON ARCTIC TARDIGRADES

## Danish Arctic Station, Qeqertarsuaq, Greenland, 7 - 18 August 2000

***Aslak Jørgensen & Reinhardt Møbjerg Kristensen***

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**Introduction**

Link to the homepage of Dept. of Invertebrates: http://www.zmuc.dk/inverweb/index3.htm

Sponsored by: **Carlsberg Fondation** (Grant no. 990457/10 – 1268) and **Danish Arctic Station**, Faculty of Science, University of Copenhagen.

The "Workshop on Arctic Tardigrades" is scheduled to take place immediately after the 8th International Symposium on Tardigrada in Copenhagen. Planning Meeting for participants will be held Sunday, 6 August 2000 at 10:00 am, Zoological Museum, Universitetsparken 15. The exact departure from Kastrup, Copenhagen Airport to Kangerlussuaq/Sdr Stromfjord is at the moment scheduled for 20:00 hrs. You must be at the Copenhagen Airport for check-in two hours before departure. We arrive local time in Kangerlussuaq at 20:40. Already 21:50 we depart on a small flight (DASH 7) and at 22:35 we arrive in Ilulissat (Jakobshavn). In the midnight sun we sail on the former Police cutter, "Esle", to the island Disko where the Danish Arctic Station is based. The cruise across the Disko Bay may be rough, but under good weather conditions there will be a good chance to see lots of whales and seals. We arrive early in the morning in the harbour of Qeqertarssuaq. Our luggage is transported by the car of the Arctic Station, but we have to walk the two kilometers through the town to the Arctic Station which is located outside Qeqertarssuaq. We hope to fly back in a helicopter to Ilulissat on the 17 August 2000. The weather can be very foggy for helicopters in August, and we have therefore included one day in Ilulissat/Jakobshavn on our way back to ensure that we are not late for our flight back to Denmark. The Ilulissat area is very special for tardigradiologists. Rare species of *Proechiniscus, Amphibolus, Adorybiotus, Microhypsibius* and *Thulinia* may be found here. The largest glacier of the Northern Hemisphere (same size as the Amazonas River) is also found here. We have a one night stay at the Youth Hostel at Ilulissat. Friday the 18 August we are flying back to Kangerlussuaq on the small plane (DASH 7) and then directly back to Copenhagen. We should arrive at Copenhagen Airport at 21:05, Friday, 18 August 2000.

**Preliminary tour programme**

Check-in Kastrup, Copenhagen Airport 18:00 hrs, 7 August 2000.

Departure Kastrup, Copenhagen Airport 20:00 hrs, 7 August 2000.

Flight SAS Boeing 727, flight time about 4 hours.

Arrival Kangerlussuaq/Sdr.Strømfjord 20:40 (local time) , 7 August 2000.

Departure Kangerlussuaq 21:50, 7 August 2000.

Flight DASH 7, flight time about 1 hour.

Arrival Ilulissat 22:45, 7 August 2000.

Departure Ilulissat 23:00, 7 August 2000.

Cruise on the vessel "Esle " across the Disko Bay.

Arrival Qeqertarsuaq/Arctic Station 7:00, 8 August 2000.

Stay at Arctic Station, Disko Island from 8 to 17 August 2000.

The Workshop includes a one-day cruise on the research vessel "Porsild" to Disko Fjord, Kangerluk or to Isunngua, Mudderbugten. See the paragraphs about Arctic Station and "The Tardigrada of Disko".

Departure from Qeqertarsuaq 16:10, 17 August 2000 in a helicopter.

Flight Sikorsky S-61N, flight time half an hour.

Arrival Ilulissat 16:40, 17 August 2000.

Sightseeing of the largest Northern glacier in the world, nature and historical tour of the Ilulissat area.

Accomodation: Night between 17 and 18 August, Ilulissat Youth Hotel.

Departure from Ilulissat 10:30, 18 August 2000.

Flight DASH 7, flight time one and half an hour.

Arrival Kangerlussuaq 12:00, 18 August 2000.

Departure from Kangerlussuaq 12:45, 18 August 2000, flight time 4 hours.

Arrival Kastrup, Copenhagen Airport, 21:05 (local time), 18 August 2000.

**The weather at Disko Island in August**

The Arctic summer is known for its unpredictability, anything from snowing, foggy weather to the "Godhavn summer" with bright sunshine during all the day´s 24 hours. When it is sunny weather the mosquitoes may be a problem, otherwise there are no dangerous animals (polar bears) on the island. Bring a thick sweater, welly boots and rain gear with you. Do not forget your camera: Disko Island is the pearl of the Arctic.

**List of Participants:**

1. Reinhardt Møbjerg Kristensen, Copenhagen, Denmark
2. Aslak Jørgensen, Copenhagen, Denmark
3. Jette Eibye-Jacobsen, Copenhagen, Denmark
4. Anne Marie Richardy Warfield, Copenhagen, Denmark
5. Jesper Hansen, Copenhagen, Denmark
6. K. Ingemar Jönsson, Lund, Sweden
7. Diane R. Nelson, Johnson City, USA
8. William R. Miller, Philadelphia, USA
9. Amber Hohl, Philadelphia, USA
10. Clark Beasley, Abilene, USA
11. Roberto Bertolani, Modena, Italy
12. Silvana Ferrari, Modena, Italy
13. Roberto Guidetti, Modena, Italy
14. Sandra McInnes, Cambridge, UK
15. Nigel Marley, Plymouth, UK

| **Estimate of prices for one person:** | |
|---|---|
| **Price for group tour to Qeqertarsuaq-Copenhagen (return tickets) app.** | **10.500 DKK** |
| **Accommodation at Arctic Station (150 DKK per night) x 9** | **1.350 DKK** |
| **Accommodation at Ilulissat Youth Hostel and Food** | **550 DKK** |
| **Rent of "Porsild" for one day** | **400 DKK** |
| **Total** | **12.800 DKK** |
| **PLEASE NOTE: FOOD IS ONLY INCLUDED AT ARCTIC STATION** | |

**Proposed research programme at Arctic Station:**

**Airborne tardigrades.**
**Tidal tardigrades.**
**Life-cycles studies of the family Eohypsibiidae.**
**Interstitial tardigrades at Flakkerhuk.**
**Chromosomes investigation of the genus *Amphibolus*.**
**SEM-preparations of the bucco-pharyngeal apparatus of Eutardigrada**
**Tardigrades in radioactive springs**
(But all kinds of research programmes on tardigrades is still open!)

## ARCTIC STATION

**Link to the homepage of Arctic Station: http://www.geogr.ku.dk/as**

**The physical surroundings:**

The Arctic Station (69°15.146'N, 53°31.271'W) is situated on the south coast of Disko Island at the outer part of Disko Bay in an area of West Greenland which shows the greatest variation in the natural environment. Disko Island is the largest island in Greenland. The interior part of the island is dominated by a local ice-cap called "Storbræen" with many glaciers, but none of the glaciers reach the sea. Just behind the station the dominant plateau-basalt mountain "Lyngmarkfjeldet" rises up to 900 m with its own small ice cap.

The climate is Arctic and by 8 August the midnight sun will disappear. From December until May the sea enclosing the town Qeqertarsuaq/Godhavn is ice-covered, while the surrounding fjords are only navigable from June to November. Sudden changes in the weather are common, and it may even snow in the summer, but usually, at the beginning of August, Disko still has warm and sunny weather (Godhavn-summer).

The old building of the Arctic Station is situated on a marine foreland of sand formed after the last Ice Age. The laboratory building is resting on Precambrian bedrock, which is more than 1800 mill. years old. The bedrock just behind the station consist of basalt formed of Tertiary lavas which are only 70 mill. years old. The whole area around the station is a protected nature reserve. The reason for that is the many cold homothermal springs in the valley of "Østerlien" just behind the station. One of these springs "Vandelven" secures the water supply to the station and enters a lagoon in front of the station. An exuberant number of plant species are found around the homothermal springs, many of the species have their northernmost occurrence here, e.g. three species of orchids.

Disko and the surrounding area have a rich bird life and several bird cliffs. In Disko Bay there are several shrimps fields and rich fishing grounds and the town Qeqertarsuaq have a modern shrimp factory. Many species of sea mammals are still hunted in the traditional way, but the large whales, such as the Fin whale, Sperm whale and the rare Bowhead whale are now protected.

**The history of the station:**

The Arctic Station at Qeqertarsuaq was founded in 1906 by the botanist Morten P. Porsild, and it has since functioned as an arctic scientific station. Porsild was the scientific leader of the station for the first 40 years and due to Porsild's many-sided interests and his personality the station worked both as a scientific and a cultural centre for North West Greenland. In 1953 the station was placed as a biological station under the Botanical Central Institute. Today the Arctic Station is placed directly under the Faculty of Science and a board of scientists in botany, geology, geography and zoology is responsible for the running of the station. A new laboratory building, where the library, darkroom and herbarium are situated, was ready in 1966. In 1980 a thorough restoration of Porsilds original two story building was finished. Now the station offers modern facilities for up to 26 scientists or participants in courses. In 1994 the old wooden cutter "Porsild" was replaced with a modern research vessel also called "Porsild".

**The station:**

Arctic Station is situated about one kilometer outside the town Qeqertarsuaq/ Godhavn. Please see the folder. Today the station comprises four buildings:

(1) Main building where the manager lives in the western wing of the ground floor, while the rest of the building forms the accommodation for the guests.

(2) The laboratory building contains two laboratories, room for equipment, library, herbarium, thermo-room and darkroom.

(3) The scientific leader's house with the automatic climate station.

(4) Garage and workshop.

**The town Qeqertarsuaq:**

Earlier the town was called the capitol of North Greenland. During whale hunting periods it was the main center in North Greenland before Thule was colonized at the beginning of the last century. The reason for founding the town as a Danish colony town in 1773 was the good natural harbour (Godhavn means good harbour). The harbour has served ever since and several coast boats call twice a week at Qeqertarsuaq. Today Qeqertarsuaq is a small town with approximately 1000 inhabitants. The town has three groceries, a baker, and several smaller shops. There is also a hospital, a school, a church, and a youth hostel outside the town. The town lacks an airport and the only connection is the helicopter service via Ilulissat. The island was much more densely populated in former times. The coal mine town Qullissat with more than 1400 inhabitants was deserted as late as in 1972. The only other inhabited place on Disko is now a small settlement, Kangerluk, in Disko Fjord with 90 inuits living a traditional Greenlandic way of life.

**Tardigrades of Disko Island**

Several reports about the fauna of tardigrades have been published from the student field courses (Kristensen 1973; Pape 1986; Kristensen 1987; Hansen et al., 1989; Grøngaard et al. 1992; Heide-Jørgensen and Kristensen 1999; Peters & Dumjahn 1999; Stark & Kristensen 1999). Unfortunately nearly all these reports have been in Danish. Except from the Danish reports a few papers have been published about the tardigrades in the homothermal springs (Heide-Jørgensen & Kristensen 1999; Kristensen 1977, 1982a) and the marine tardigrades

(Kristensen and Hallas 1980; Kristensen 1982b; Kristensen and Higgins 1984). During the workshop we want to work with all different groups of tardigrades, but especially the true freshwater species will be of high priority. Approximately 80 species are known in the neighborhood of Arctic Station. Many of these species are related to those of the cold homothermal springs or the "Tinten-streifen" (bluegreen bacteria on the basalt wall of the cliff). Very few species are endemic for the warm, radioactive springs. Other very interesting localities for tardigrades at Disko Island are the cryoconite holes in the ice cap or in the glaciers. Tardigrades related to bird colonies with lichen *Xanthoria elegans* has the highest density of Arctic tardigrades but only few species are found. These species are very large compared with the same species in Europe. The same tendency is seen in the freshwater species in ponds and small lakes on Disko, e.g. the Arctic specimens of *Amphibolus nebulosus* can grow to a length of more than one millimeter. In fact some of the arctic tardigrades could have polytene chromosomes. The marine tardigrade fauna is especially rich in shell gravel or bryozoan sand, but the tidal fauna holds only few species which are well adapted to the harsh environment. The temperature gradient pro tide can vary at winter from +2°C (sea water) to -35°C (air temperature).

The illustration shows a transect from the local ice cap of Disko (Lyngmarksfjeldet, 875 m o.s.l) to the deep Godhavn shrimp field (500 m water depth). Eleven genera of tardigrades from the transect are indicated. Furthermore are the different characteristic biotas shown semi-schematic, e.g. cryoconite holes at the ice cap, bird cliff, radioactive springs, lakes influenced by cold homothermal waters and the arctic mud on the shrimp field.

We hope the illustration of the "Disko Island Transect" will give the participants of the "Arctic Workshop" some ideas about the unique environment just around the station. The purpose of the workshop is to have different experts to work on as many different groups of tardigrades as possible. The results may be published in a special volume of Bioscience, Meddelelser om Grønland to honour late Dr. Børge Petersen´s pioneering achievement in Arctic tardigrade research (Petersen 1951).

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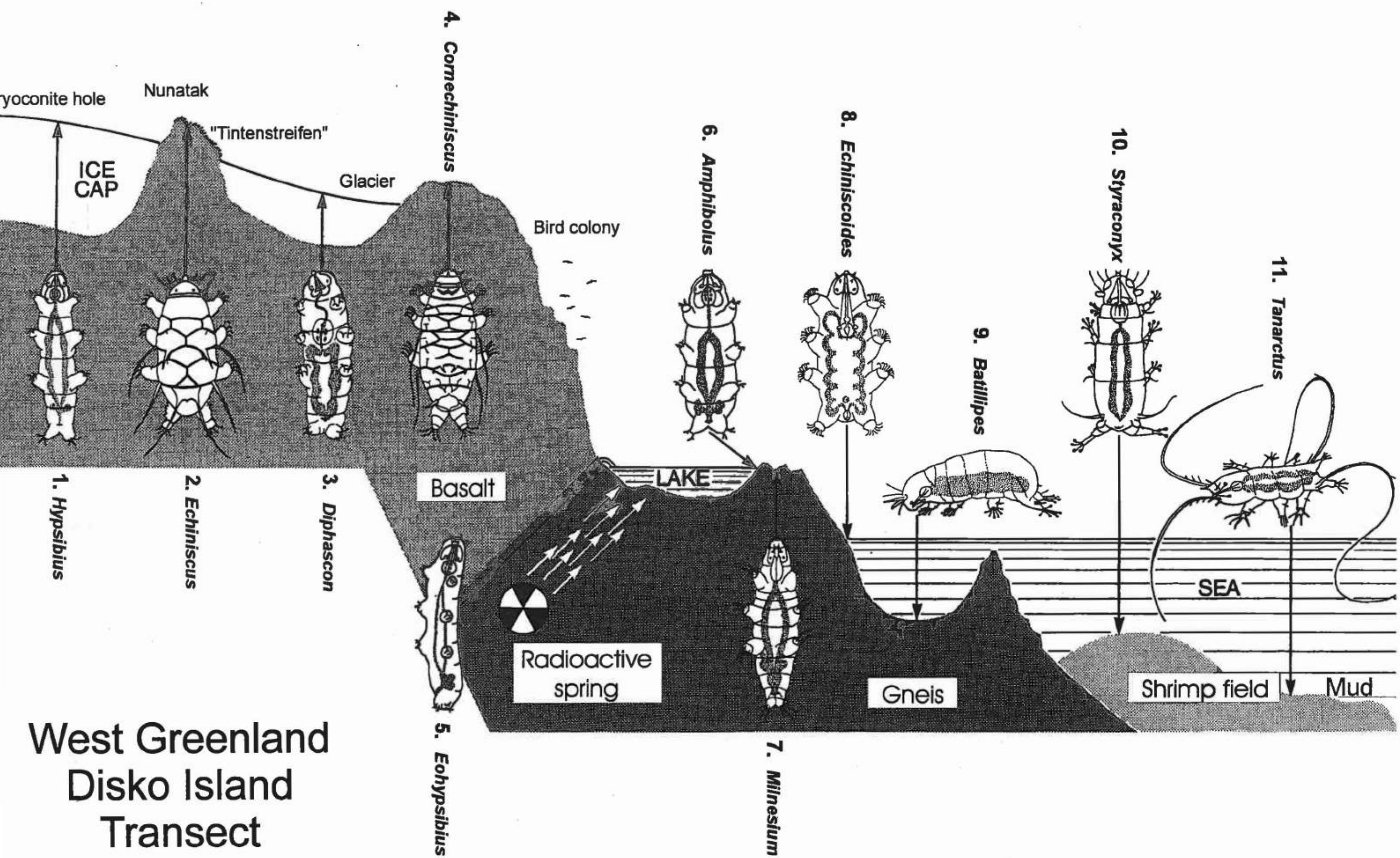

yoconite hole
Nunatak
"Tintenstreifen"
ICE CAP
Glacier
Bird colony
Basalt
LAKE
Radioactive spring
Gneis
SEA
Shrimp field
Mud
1. Hypsibius
2. Echiniscus
3. Diphascon
4. Cornechiniscus
5. Eohypsibius
6. Amphibolus
7. Milnesium
8. Echiniscoides
9. Batillipes
10. Styraconyx
11. Tanarctus
West Greenland
Disko Island
Transect