The discovery of 3 new phyla: Loricifera, Cyclophora and Micrognathozoa
Department of Environmental Sciences, Basel, 4 March 2013
By Reinhardt Møbjerg Kristensen
Discovery of the first phylum: Loricifera Kristensen, 1983

Station Biologique, Roscoff, France, July 1975
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*Nanalaricus mysticus* Kristensen, 1983. From Roscoff, France. The new phylum is common in the Deep-sea.
First larva of *Limnognathia maerski*

First seen 2010

Cycliophora Funch and Kristensen, 1995

Micrognathozoa Kristensen and Funch, 2000
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Three new phyla: The cladogram of Sørensen et al. 2000

Totally outdated
Dunn et al., 2008: EST 140 genes and "new" 34 metazoans

ATOL-NSF programme 2008 “Assembling the Tree of Life” in Copenhagen
Interstitial fauna from carbonate sand (2002)

1. Nematoda
   G. Gad

2. Gastrotricha
   C. Clausen

3. Kinorhyncha
   M.V. Sørensen & R.P. Higgins

4. Loricifera
   I. Heiner; R. Neves

5. Polychaeta
   K. Worsaae

6. Tardigrada
   J.G. Hansen & A. Jørgensen

7. Copepoda & Tantalocarida
   R. Huys & P. Funch

8. Aplacophora
   A. Jørgensen
Mentor: Robert P. Higgins, Smithsonian Institution

Samplings on Faroe Bank, BIOFAR Project 1989

1. Anchor dredge
2. Box core
3. Higgins’ Meiobenthic sledge
Pliciloricus enigmaticus

The first known Loricifera from the Eastward-Expedition, USA (1974)
Station Biologique, Roscoff, France, May 2011

Search for live Loricifera
Higgins-larva of *Nanaloricus* from Roscoff, 2005

Trenzen ar Skoden, 50 m
Live adult female of *Nanaloricus* nov. sp.1
Jumping like a flea

Trezen ar Skoden, Roscoff 2011
The life cycle of the family Nanaloricidae

Nanaloricus mysticus, Roscoff
**Rugiloricus bacatus** and a new type of reduced larva – a ghost-larva.

The life cycle of Pliciloricidae is more complicated than first assumed in 2002.

Especially concerning the asexual (parthenogenetical) loops.

**Parthenogenesis:** reproduction from a female gamete without fertilization by a male gamete.

(Heiner 2008)
Photos of Yoshihisa Shirayama: The first deep sea loriciferans

_Pliciloricus hadalis_ Kristensen and Shirayama, 1988: 8260 m depth
Armorloricus elegans Kristensen and Gad, 2004, Trezen ar Skoden, Roscoff, France

Male, dorsal view

Higgins larva, ventral view
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New loriciferans from Roscoff, France:
*Armorloricus elegans* and *A. davidi* Kristensen and Gad, 2004

*Adults with spermatozoa*                          *Higgins-larvae with flippers*
TEM of *N. mysticus*
Transmission Electron Microscopy (TEM) of *Nanaloricus mysticus* from Roscoff

Buccal tube retractors and buccal tube

Mouth tube and buccal tube
How many nerve fibres?

TEM photo of *Armorloricus elegans*: Cross section of the brain (neuropile)
Armorloricus elegans ♂

Cross-section through trunk

Gonad with protonephridia

Ultrastructure of the protonephridia of Loricifera (Neuhaus and Kristensen 2007)
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Loricifera from deeper water at Faroe Bank, North Atlantic Sea

*Rugiloricus* nov. sp

New Family: *Urnaloricicidae*

Head

Embryo
New family from the Faroe Bank

Urinaloricidae: Ghost- or Megalarva
The life cycle of the New Order

Pedogenesis:
The cyst-like ghost larva retains the cuticles of the postlarva and the adult. Out of the cyst-like ghost larva comes a normal Higgins-larva.

The cyst-like larva’s armature
Since its discovery

- About 30 species described
  - assigned to 3 families:
    - Nanaloricidae
      - Nanaloricus, Armorloricus, Phoeniciloricus, Spinoloricus, Culexiregiloricus, Australoricus
    - Pliciloricidae
      - Pliciloricus, Rugiloricus, Titaniloricus
    - Urnaloricidae
      - Urnaloricus

(Heiner, 2008)
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The new generation of researchers: Gunnar Gad and Iben Heiner
Molecular data does not support Scalidophora

- Kinorhyncha
- Priapulida
- Nematoda
- Loricifera
- Nematomorpha

18S rRNA

Scalidophora

Nematoida

- Introvert + mouth cone
- Scalids
- Papillar sense organs (flosculi)
- Protonphridia

- Unpaired dorsal and ventral nerve strings
- Loss of circular muscles
- Loss of sperm flagellum
- Both sexes with cloaca
- Vermiform habitus

Plesiomorphies?

Adult morphology

Sørensen et al., 2008
Loriciferan – Nematomorph sister groups?

- Hexaradial symmetry patterns in pharynx, opposed to pentaradial symmetry patterns in Kinorhyncha and Priapulida

Martin Vinther Sørensen’ theory
Loriciferan – Nematomorph sister groups?

Evolution through Progenese

1. Original cycle maintained
2. Recent Nematoda
3. Recent Nematomorpha
4. Recent Loricifera

Loss of larva
Loss of adult stage through progenesis
Nanalaricus nov sp. from Trezen ar Skoden

Ricardo Neves: Roscoff 2011

Myoanatomy: Phallacidin  DAPI-staining
Why do we study them?

- **Myoanatomy** (phalloidin staining)
  
  *Nanalaricus* nov. sp.1 from Roscoff
Extremophilic loriciferans as the dominating meiofauna animals at l’Atalante Basin: (Danovaro et al., 2010.)

Hell on Earth: l’Atalante Basin
Extremophilic loriciferans as the dominating meiofauna animals at l’Atalante Basin: (Danovaro et al, 2010)

The first adult specimen of the genus *Spinoloricus*

Female of *Spinoloricus* from l’Atalante Basin
The animal is stained with Rose Bengal
Holotypic female of *Spinoloricus* nov. sp. from l’Atalante Bassinet
The DHABs

- The deep hypersaline anoxic basins Bannock, Urania, Discovery, l’Atalante represent unique deep-sea environments:

- Remains of hypersaline waters of the Miocene period (23x10^6 yrs ago)
- Separated for several thousand years from the surrounding SW environment
- Characterized by “extreme values” of the following parameters:
  - High salinity (above 30%)
  - Absence of light
  - Elevated pressure (> 300 atm)
  - Variable pH values (from acidic to alkaline, depending on the basin)
  - Gas emission (Urania, l’Atalante basin)
Expeditions to L’Atalante in 1998, 2005 and 2008

The research vessel: R/V Urania

Modus – the ROW who took all the samples
Evaporit containing *Synechoccus*

Photomicrograph of *Haloarcula* in a NaCl crystal (Nature, 2001)

Gypsum crystal

Expedition to the Urania Basin in 1998
1. Light Microscopy

In the first expedition, specimens belonging to three animal Phyla were observed inner part of the L’Atalante basin:

- **Copepoda**
- **Nematoda**
- **Loricifera**

All specimens were initially stained with Rose Bengal. All copepods were empty exuviae, nematodes were only weakly stained whereas *all of the loriciferans were intensely colored*.
Ultra-structural analyses revealed the lack of mitochondria, which are replaced by hydrogenosome-like organelles H (a-c).

TEM revealed:

the presence of hydrogenosome fields (c) similar to those reported in anaerobic ciliates;

the presence of rod-shaped structures (d-f), like prokaryotes P, in proximity to the hydrogenosome-like organelles.
Sirius Passet in 2011 (Photo: Martin Stein)
Third Workshop on Scalidophora 2012

Holotype of *Siriloricus carlbergi* Peel, 2010
New material from 2011: *S. carlsbergi* with muscle attachments to the plicae and a thin layer of epidermis preserved.
Ricardo Neves: Roscoff 2011

IMARIS- reconstruction of Myoanatomy

*Nanaloricus* nov. sp. 1
Martin Stein’s 3-D reconstruction of holotype
Discovery of the second phylum: Cycliophora
Funch and Kristensen, 1995

Tjärnö 1997: *Symbion pandora* on the mouth limbs of Norway Lobster
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Kaldbak Marine Biological Laboratory, 1998, Faroe Islands

**Symbion pandora** was believed to be a marine Rotifera.

- The overall myoanatomy of cycliophorans resembles that found in bdelloid rotifers.

  - However, the insertion in the body integument is clearly different.

  ![Diagram](image1.png) ![Diagram](image2.png) ![Diagram](image3.png)

Feeding stage of Cycliophora

True cuticula
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*Symbion pandora* on Norway Lobster, Kaldbak Fjord, Faroe Islands, 1990

*Symbion americanus* on American Lobster, Maine, USA, 1998
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Pandora larva

Dwarf female
1. The phylum Cycliophora

- The proposed life cycle:
  - Metagenesis

(From Obst & Funch, 2003 after Kristensen, 2002)
Symbion americanus from Maine, USA

Chordoid larva stained with phallacidin. Reconstruction by confocal laser microscopy (After Ricardo Neves)
2. The second described species

- *Symbion americanus* lives attached to the mouthparts of *Homarus americanus*.

(Adapted from Obst et al., 2006)
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The free-living stages of
*Symbion americanus*
Obst et al., 2006
3. Results

- The gross morphology

- *S. americanus*

  - frontal ciliated field (fc)
  - lateral sensoria (la)
  - penis (pe)
  - ventral ciliated field (vc)
3. Results

- The ultrastructure

  - *S. pandora*

  - brain (ga)
  - cerebral glands (ce)
  - medial glands (mg)
  - penial sheath (ps)
  - testis (te)
  - Prometheus larva (pl)
Are there more cycliophorans?

Nephropsis atlantica
900 - 1400 m

Nephropidae
(52 described species)

12 from the continental shelf
48 from continental slopes and deeper
Discovery of the third phylum: Micrognathozoa
Kristensen and Funch, 2000

Danish Arctic Station, Qeqertarsuaq, June 2004
Limnognathia maerski, Kristensen and Funch, 2000
(Micrognathozoa)
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Discovery of a Shangri-La in Greenland – The Valley of Angelica (Kvandalen).

Arctic Biological Field Course to Mudderbugten and Kvandalen August 1994.

More than 30 new homothermic springs were discovered.
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Homothermic spring in Østerlien, summer and winter
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The first sledge-expedition to Mudderbugten, April 1978.

Field Course in Arctic Biology, base camp at Isunngua-Spring, August 1994.

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Royal Lecture
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The species has recently been found in Sub-Antarctic (Crozet Islands) and Wales.

The animal is only about 0.1 mm
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Scanning electron microscopy of *Limnognathia maerski*

Lateral view

Ventral view

Winter egg
The Secret of Greenlandic Springs

Transmission Electron Microscopy (TEM) of *Limnognathia maerski*
The Secret of Greenlandic Springs

SEM of *Limnognathia maerski* Jaws treated with sodium hypochlorite
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3-D reconstruction of the jaws of *Limnognathia maerski*

Martin V. Sørensen
Homology between the rotifer incus and the articularium in Gnathostomulida and Micrognathozoa:

- Similar location
- Similar appearance: paired sclerites that join caudally into unpaired pedicle
- Scleropili or sclero fibrillae present
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Phylogeny of Kristensen and Funch, 2000
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- Basal plate and fibularium
- Basal plate and suspensorium present
- Reversal to monociliated cells
- Cellular epidermis with intracellular lamina
- Jaws composed on lucent rods
  Incus/main jaw/articularium present

- Syncytial epidermis
  Loss of body ciliation
  Wheel organ
- Mallei present

Sørensen’s phylogeny
The Secret of Greenlandic Springs

Giribet et al. 2004
new molecular data
Arctic Workshop 2010: ”The tree of Life/ATol-team”
Arctic Workshop 2010: Photo of *Limnognathia maerski*
Arctic Workshop 2010: *Limnognathia maerski*

Bergen-connection

Katrine Worsaae: Myoanatomy (phalloidin, depth-coded)
Arctic Workshop 2010: Tree of Life/ATol

K. Worsaae 2011: cilia, nephridia, gonoducts & nerves
anti-acetylated α-tubulin (depth-coded)
K. Worsaae 2011: anti-serotonin (red), anti-acetylated α-tubulin (blue), F-actin (green)
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