

An integrated approach to the origin and diversification of protostomes

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<http://www.mc.z.harvard.edu/Departments/InvertZoo/tol/>

Project Goals (for 2-year funding):

1. Generate a collection of tissues for 265 species belonging to all protostome phyla. Having such a collection available for research will ensure gathering of different sources of data for the same organisms across laboratories worldwide.
2. Generate sequence data (about 10 markers that have shown to resolve metazoan deep divergences) for 50% of the metazoan lineages proposed in the previous section.
3. Generate new cDNA libraries for 8 protostomes.
4. Generate sequence data for about 100 molecular markers by EST technology for the ca. 15 taxa for which the cDNA libraries are generated/available.
5. Generate new embryological cell lineage studies for a selected collection of protostome phyla for which cDNA libraries have been generated.
6. Generate gene expression data for several genes implied in segmentation, mesoderm, and body plan organization.
7. Compile a comprehensive dataset on protostomes morphology/anatomy coded for exemplar taxa, including key fossil species (see accessory Table).

Goal 1:

- Fieldwork has been conducted in Bermuda, Florida, France, Japan, Australia and Sweden
- Tissues of more than 200 species belonging to Acocela, Xenoturbellida, Myzostomida, Nemertea, Mollusca, Annelida, Sipuncula, Entoprocta, Brachiopoda, Phoronida, Platyhelminthes, Gnathostomulida, Gastrotricha, Rotifera, Cyclophora, Micrognathozoa, Pripipula, Kinorhyncha, Tardigrada, Loricifera, Oryctophora, Arthropoda have been collected and preserved in RNAlater, 96% EtOH, formalin, and glutaraldehyde
- Online database of species, modes of preservation, and status of work is under development. A preliminary version can be viewed at: <http://collections.oeb.harvard.edu/Invertebrate/atol/species.cfm>



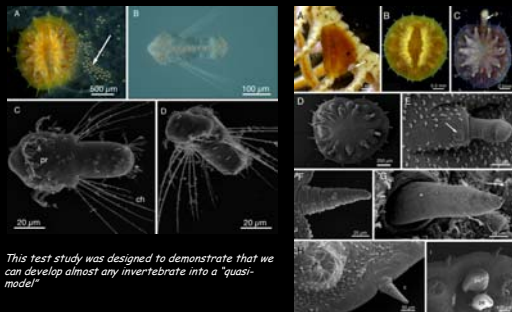
Xenoturbella bocki dredged in Tjarno, Sweden

Collecting protostomes from marine sediments in Sweden and Florida. Clockwise: Fred Pleijel, Greg Rouse and Gonzalo Giribet dredging for *Xenoturbella* in Kristineberg; F. Pleijel and Akiko Okusu with the skipper in Kristineberg; Stephanie Huff sorting sediments in Kristineberg; Martin Sørensen collecting sediments for metazoans in Florida.

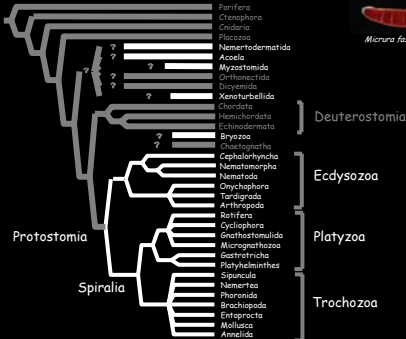
Study case: An unknown protostome species is discovered and studied: The "quasi-model" approach

- Description and morpho-anatomical characterization of animal (light microscopy, SEM, TEM)
- Spawning for study of larvae and developmental stages
- Generation of cDNA library
- Sequencing of ca. 1,400 ESTs
- Study of expression patterns

We will apply this integral approach to a number of key species to understand protostome evolution, including *Xenoturbella*, acocels, and others.



This test study was designed to demonstrate that we can develop almost any invertebrate into a "quasi-model"



Working hypothesis of metazoan relationships highlighting the protostomes and other putative protostomes covered in this study

Goal 2:

- Markers that amplify via PCR or rPCR
- 18S rRNA (ca. 1.8 Kb)
 - 28S rRNA (ca. 3 Kb)
 - Myosin heavy chain II (ca. 800 bp)
 - RNA Polymerase II
 - Elongation factor 1 α
 - Elongation factor 2
 - Histone H3 (ca. 350 bp)
 - Cytochrome c oxidase subunit I (ca. 1,200 bp)

We are sequencing other more traditional markers via PCR or rPCR amplification.

Goals 3 and 4:

- cDNA libraries have been built for 5 protostomes:
- *Myzostoma seymourcollegensis* sp. (Myzostomida)
 - *Cerebratulus lacteus* (Nemertea)
 - *Themiste lageniformis* (Sipuncula)
 - *Xenoturbella bocki* (Xenoturbellida)
 - *Phoronis vancouverensis* (Phoronida)

- ESTs have been sequenced for 5 animals:
- *Mnemiopsis leidyi* (Ctenophora), 1,000 clones
 - *Capitella* sp. I (Annelida), 1,000 clones
 - *Myzostoma seymourcollegensis* sp. (Myzostomida), 1,400 clones
 - *Cerebratulus lacteus* (Nemertea), 900 clones
 - *Themiste lageniformis* (Sipuncula), 100 clones (in progress)
 - *Xenoturbella bocki* (Xenoturbellida), 3,000 clones (in progress)

We have obtained homologues of several markers that could be useful for phylogenetics such as troponomyosin, elongation factors, α -ubulin, Dynein, Notch, myosines, etc.

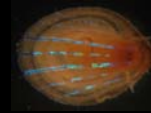
Brief protocol: Specimens are collected in the field, preserved in RNAlater, transferred to Kewalo for generation of cDNA, plates are sent to the sequencing facilities of the AMNH or Harvard.



Glossoscolex sp.

Paripatopsis maseleyi

Aysheaia pedunculata



Helcion pellucidus



Micrura fasciolata

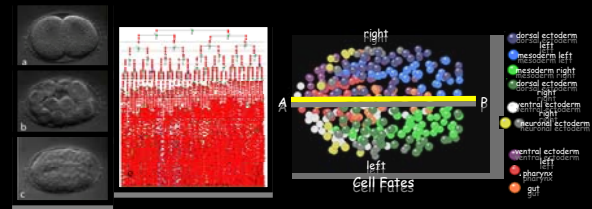
Convoluta rosaffensis



The ATol-protostome team met at Harvard, January 2004. A second meeting is scheduled at Kewalo, January 2005.

Goal 5:

Cell lineages for selected species of metazoans. An example from the gastrotrich *Lepidodermella squamata*. A new technique using a 4D microscope has been developed to study cell lineages in small invertebrates. With this technique we are able to expand the number of embryos that can be studied for cell lineages without the hassle of cell injections.



Goal 6:

Generate expression patterns of developmental regulatory genes potentially involved in segmentation, body plan formation and mesoderm formation

The following genes have been rescued so far:

Gene	ATol	Myzostoma	Cerebratulus	Themiste	Xenoturbella	Phoronis
segmentation						
axial patterning						
mesoderm						
paradox						

In situ hybridization of *Capitella* sp. I, ventral view:



ax. Axial patterning gene often marking the anterior end of the animal (expressed in the brain)



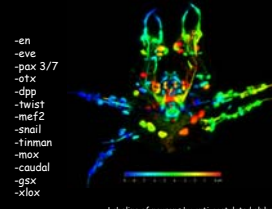
max. Involved in mesoderm patterning. It shows a very complex pattern of expression in some of the mesoderm.

segmentation

axial patterning

mesoderm

paradox



Labeling of neurons by anti-acetylated alpha-tubulin immunoreactivity in the pyrosoma (*Allopiodes* sp. parvulus) protoplanarian. Whole mount, dorsal view with depth-coding.