

Comparison of segment development among annelids

Mariana Lobato de Carvalho Martins¹

Biol 533: Evolution and Development of Metazoans
Summer 2011

Núcleo em Ecologia e Desenvolvimento Sócio-Ambiental de Macaé, Federal
University of Rio de Janeiro, Macaé, RJ 27971-550

Keywords: annelids, segmentation, *engrailed*.

Contact information:

Mariana Martins

Núcleo em Ecologia e Desenvolvimento Sócio-Ambiental de Macaé

Universidade Federal do Rio de Janeiro

Av. São José do Barreto s/n (atrás do Centro de Convenções)

São José do Barreto

Macaé, RJ 27971-550

Comparison of segment development among annelids

Mariana Lobato de Carvalho Martins
Federal University of Rio de Janeiro

Introduction

We know there are some genes responsible for the body segmentation through research done with *Drosophila melanogaster*. Some examples of these genes are *engrailed* and *wingless*, that belong to the segment-polarity genes class, and *even-skipped*, *hairy* and *fushi-tarazu* belonging to pair-rule gene class (Davis, G. k. and Patel, N. H., 1999). *Engrailed* is conserved among arthropods and has been shown to function like a segment formation gene in annelids. The expression of segment-polarity genes has been characterized in few annelid species and the expression patterns vary among the species characterized (Seaver, E. C. and Kaneshige, L. M., 2006).

I intended to compare the development of segment formation among three annelid species, *Boccardia proboscidea*, *Notomastus sp.* (Capitellidae family) and *Spirorbis sp.* Beyond the comparison of segment development, I intended to see if they have similar expression patterns, or vary among the species.

These three species has the same form of embryo development, all have trochophore larvae although they have different methods of feeding. *Notomastus sp.* has planktotrophic larvae, feeding on plankton, while the *Spirorbis sp.* has lecithotrophic larvae, or, in other words, *Spirorbis sp.* eats its own yolk (Shanks, A., 2001). *Boccardia proboscidea* has 'poecilogony phenomenon', which means that the *Boccardia proboscidea* has more than one strategy of reproduction (Handley, S. J., 2000). The larvae can be lecithotrophic or planktotrophic, depending of environment conditions. I worked with larval stage of all three species.

Materials and Methods

Collecting specimens

Each species was collected on San Juan Island. *Boccardia proboscidea* was collected in False Bay, *Spirorbis sp.* on the Friday Harbor Laboratories dock and the *Notomastus sp.* was provided by Bruno Pernet from laboratory 1.

All specimens were placed in tanks on laboratory 4. I could not find any eggs or larvae from *B. proboscidea* so I was not able to examine this species.

Molecular data

DNA and RNA were extracted with DNeasy blood & tissue and RNeasy® project mini kit from Qiagen from 30mg of tissue of each adult species following the protocol provided by the manufacturer. *Engrailed* was amplified with polymerase chain reaction (PCR) using the promega Taq polymerase. cDNA was made following the Retroscript® kit protocol. I used three different forward primers and one reverse primer. EnEH2, en1-1 from Seaver, E. C. and Kaneshige, L. M. (2006) and Mollusc-5, mollusc-3 from Kevin's Kocot data, respectively. First I followed the following temperature cycles: initial denaturation of 4min at 94°C, 35x (1 min at 94°C, 30 sec at 46.5°C, 1min at 72°C) and a final extension of 10 min at 72°C. However this did not work, so I tried a touchdown PCR temperature: initial denaturation of 1min at 94°C, 35x (30 sec at 94°C, start annealing temperature of 60°C for 30 sec decreasing 0.3°C per cycle, 1min at 72°C) and a final extension of 10 min at 72°C. DNA and cDNA were run on 2% agarose gel.

Phylogenetic analysis

Alignments of protein sequences from mollusks and annelids *engrailed* sequences were made on Mega5. Gene trees were made on Mega5 also with Maximum Likelihood with a 100 bootstrap.

Phalloidin staining

Notomastus sp. larvae at eleven and forty days were relaxed with MgCl₂ for one hour and then fixed with 4% paraformaldehyde overnight. After overnight in paraformaldehyde, I washed with 50% ethanol and 80% ethanol and stored in the freezer.

Phalloidin Staining was conducted as follows. First the embryos were rehydrated, returning to 50% ethanol and washed 3 times with phosphate buffered saline (PBS). Then I incubated the embryos in 200µL PBST solution with 5µL phalloidin for one hour. After incubation I washed three times with PBS.

For the slides I put one drop of Vectashield Mountin Medium for Fluorescence and one drop with the embryos in PBS and covered with a cover slip.

Results

The alignments of *engrailed* in annelid and mollusk and the primers succeeded in showing a conserved area where the primers were aligned (figure 6). However the PCR amplifications for *engrailed* and a positive control (18S, a ribosomal gene) did not work with DNA, even with cDNA (figure 7). The tree don't have significant bootstrap values, but shows *Drosophila* how an external group, annelids on a clade as mollusks (fig 8).

If I had more time I would do the alignments again and pick another primers for *engrailed* and do *in situ* hybridization with the larvae to see the difference of expression of *engrailed* in segments among these three species.

Acknowledgments

I wish to thanks Ken, Billie, Joe, Kevin and Max for the help on the lab and with the computer programs, and Bruno for grant the adults and larvae of *Notomastus sp.* And Robert L. Fernald Endowed Fellowship for give to me the opportunity to come to the course and my advisor Rodrigo Nunes for show me this course. I would like to thanks my classmates for all strong and competence that we have to do this course, thanks René, Diane, Ayako, Elija, Neem and Nathan!

References

Davis, G. k. and Patel, N. H., (1999) The origin and evolution of segmentation. *Millennium issue* (M68 – M73).

Dray, N.; Tessmar-Raible, K.; Gouar, M. Le; Vibert, L.; Christodoulou, F.; Schipany, K.; Guillou, A.; Zantke, J.; Snyman, H.; Béhague, J.; Michel, V.; Arendt, D. and Balavoine, G. (2010) Hedgehog signaling regulates segment formation in the annelid *Platynereis*. *Science* vol.329 (339-342)

Gibson, G. D. and Smith, H. L. (2004) from embryos to juveniles: morphogenesis in the spionid *Boccardia proboscidea* (Polychaete). *Invertebrate Biology* 123(2) (136-145)

Handley, S. J. (2000) Larval development of *Boccardia knoxi*, a shell-infesting spionid polychaete. *New Zealand Journal of Marine and Freshwater Research*, Vol. 34 (681-687)

Irvine, S. M. and Martindale, M. Q. (1996) Cellular and molecular mechanisms of segmentation in annelids. *Cell and developmental biology* vol.7 (593-604)

Seaver, E. C. and Kaneshige, L. M. (2006) Expression of ‘segmentation’ genes during larval and juvenile development in the polychaetes *Capitella sp. I* and *H. elegans*. *Developmental Biology* 289 (179-194)

Shanks, A. (2001) An identification guide to the larval marine invertebrates of the pacific northwest. Oregon State University Press (chapter 6 – Polychaete)



By Read, Geoffrey B.

Fig 1: Adult of *Boccardia proboscidea*.



http://www.nwmarinelife.com/htmlswimmers/n_tenuis.html

Fig 2: Adult of *Notomastus sp.*



<http://www.biology.ualberta.ca/palmer/People/People.htm>

Fig 3: Adults of *Spirorbis* sp.

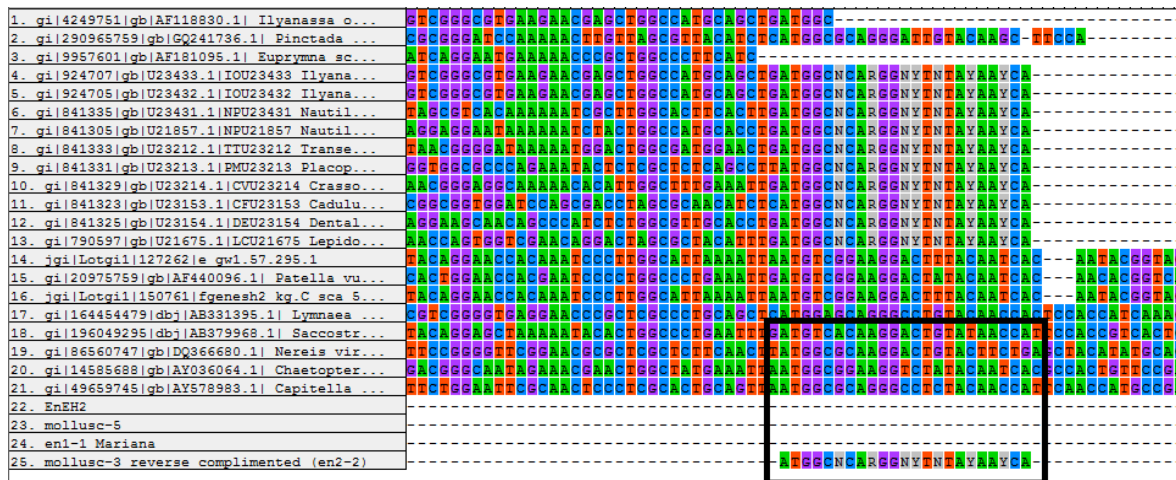
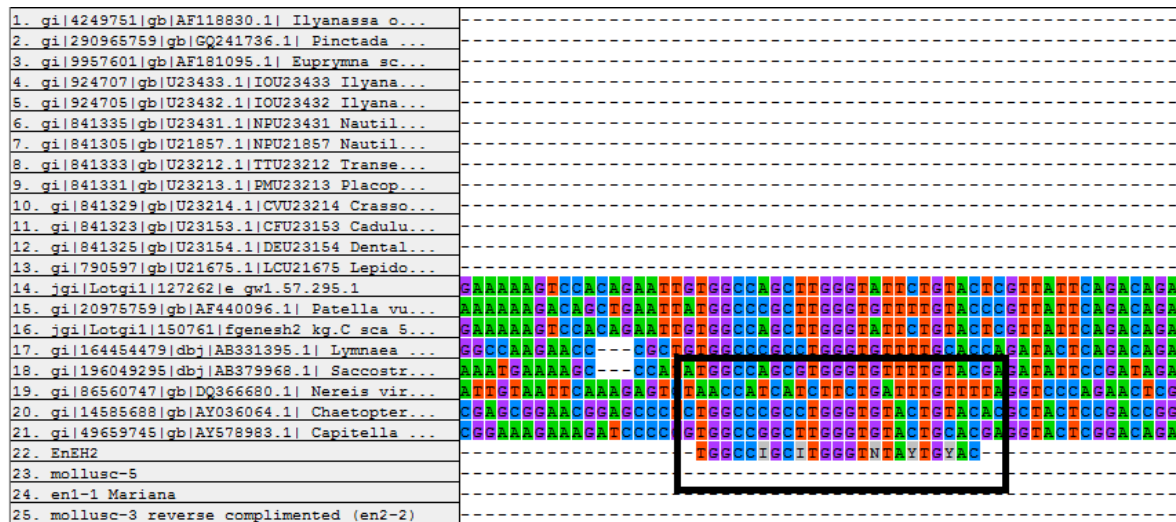
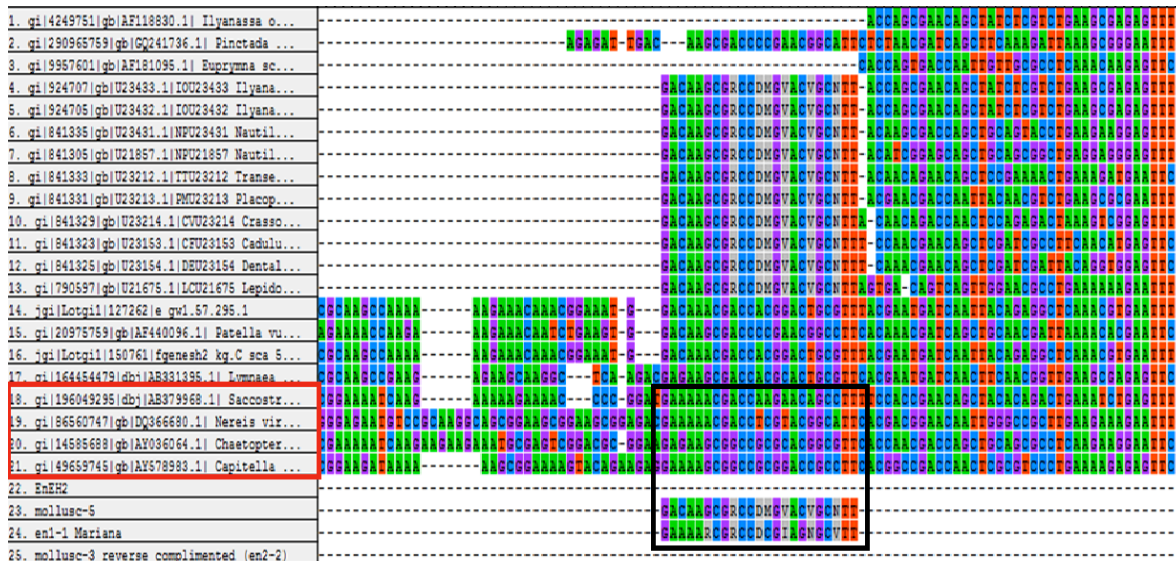


Fig 4: DNA alignments made on Mega5 with the primers, mollusks and annelids sequences for *engrailed*. Circulated in red are the annelids, in black the primers and the respective annelids sequences.

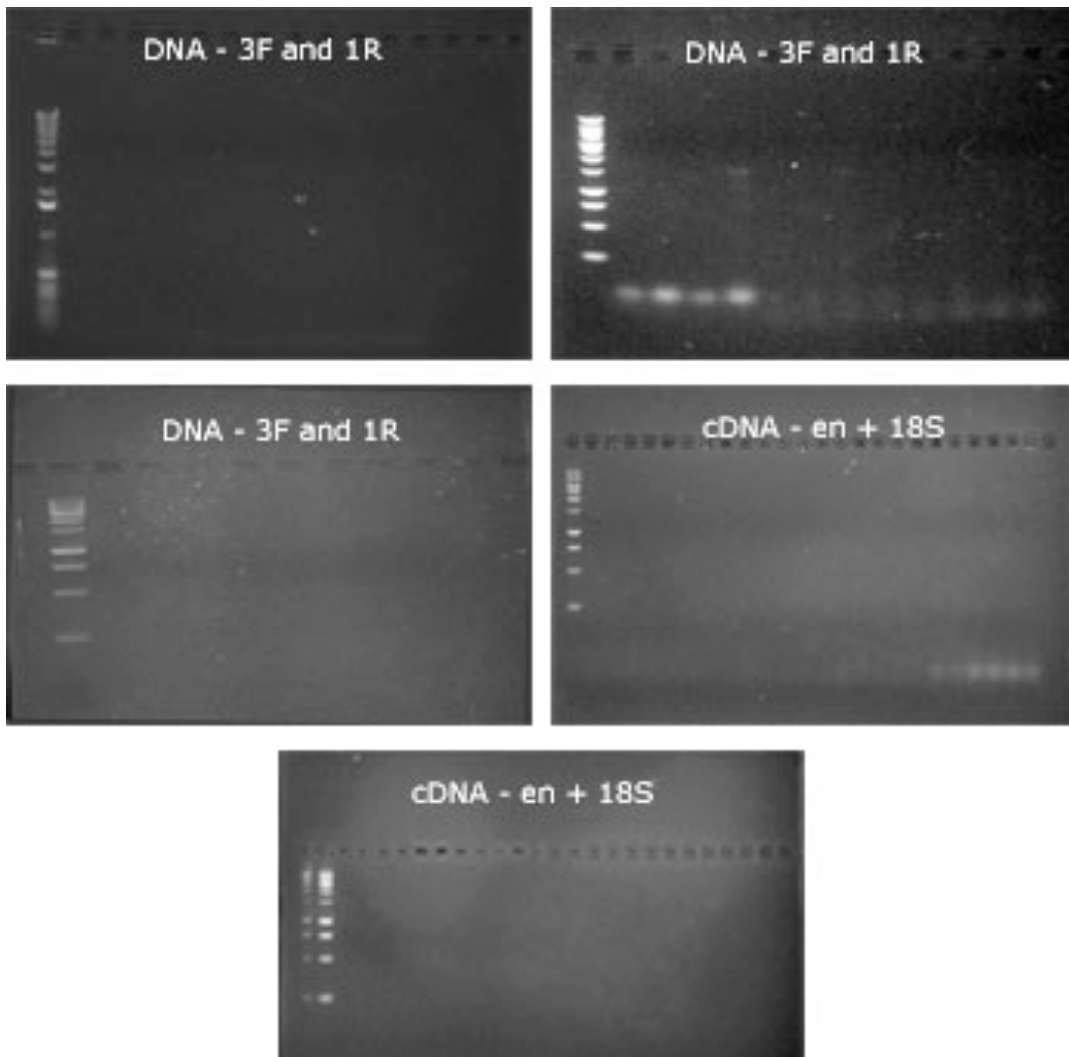


Fig 5: 2% Agarose gels with no band for either *engrailed* or 18S. These results show that my positive central (18S) was not working. Unfortunately, I was not able to get a band for *engrailed*.

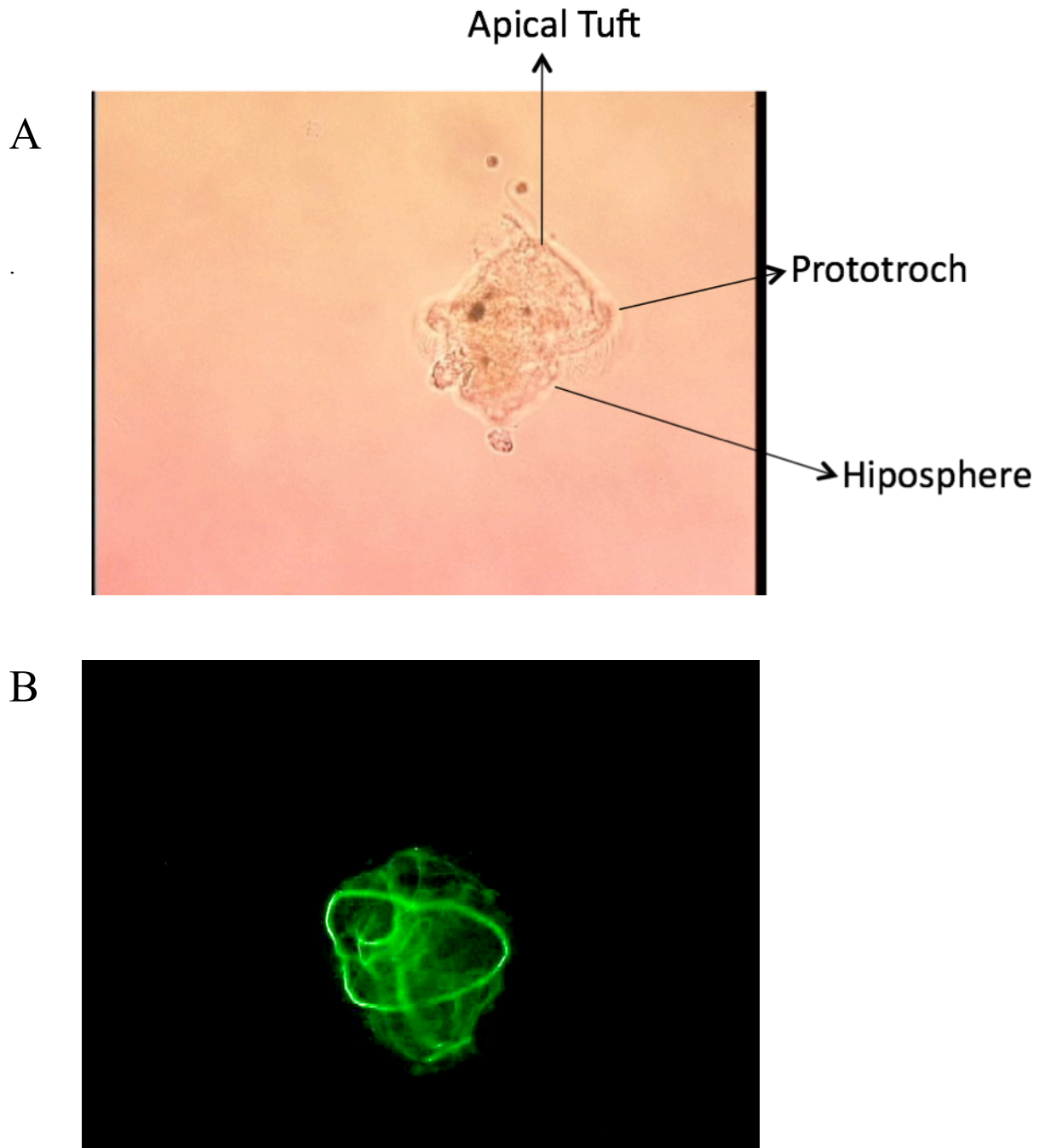


Fig 6: Trochophore larvae of *Notomastus sp.* with 11 day post fertilization in A and B. On B we see the Prototroch staining with phalloidin.

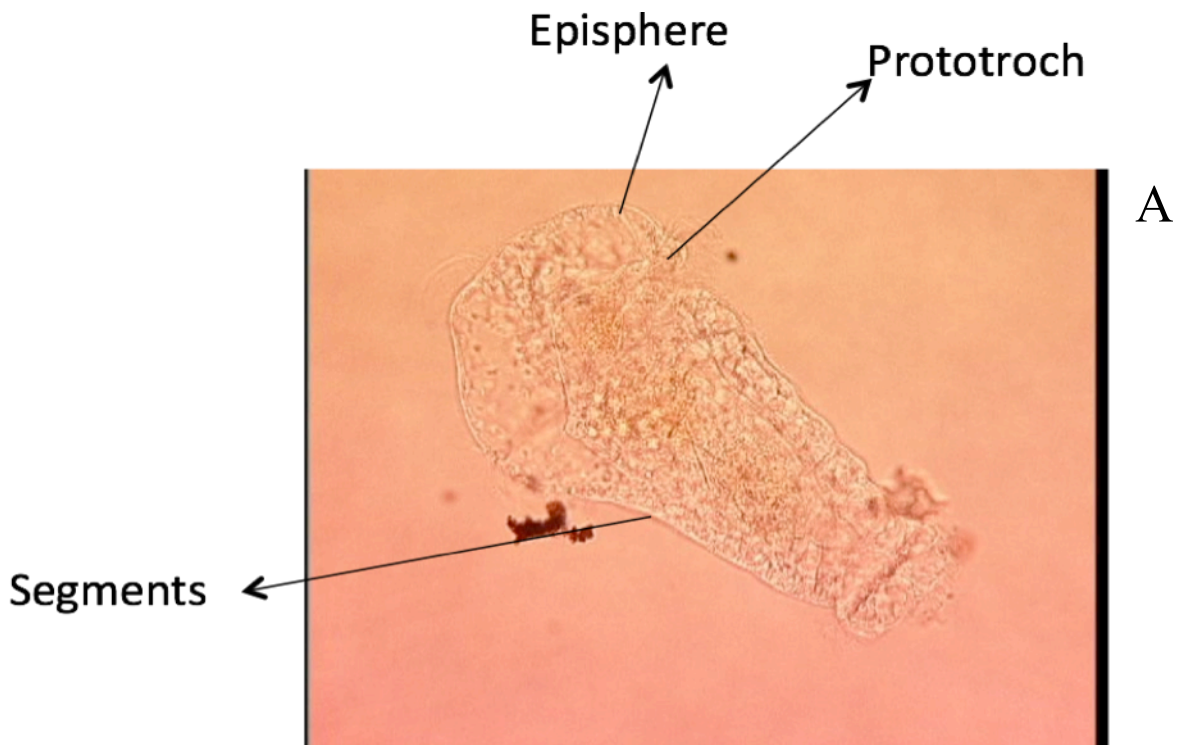


Fig 7: Trochophore larvae of *Notomastus sp.* with 40 day post fertilization in A and B. On B we see the muscles staining with phalloidin.

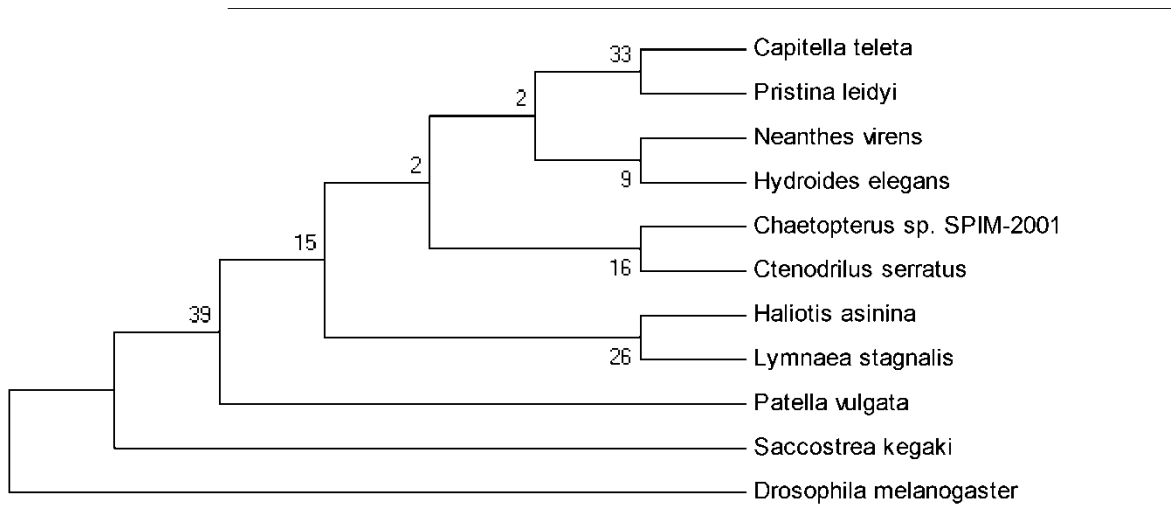


Fig 8: The six first animals are annelid in a single calde and the others, except *Drosophila melanogaster*, are mollusks.