

# The amphioxus *Epigonichthys maldivensis* (Forster Cooper, 1903) (Cephalochordata Branchiostomatidae) larvae in the plankton from Rapa Nui (Chile) and ecological implications

Erika Meerhoff<sup>1,2\*</sup>; David Veliz<sup>2,3</sup>; Caren Vega-Retter<sup>2,3</sup> & Beatriz Yannicelli<sup>1,2</sup>

<sup>1</sup>Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, Chile

<sup>2</sup>Millennium Nucleus for Ecology and Sustainable Management of Oceanic Islands (ESMOI), Universidad Católica del Norte, Lar-rondo 1281, Coquimbo, Chile

<sup>3</sup>Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile

\*Corresponding author, e-mail: erikameerhoff@udec.cl

---

## ABSTRACT

We report the first record of amphioxus larvae in the plankton from Rapa Nui island (Chile). Zooplankton was sampled using an oblique Bongo net during an oceanographic survey in April and September 2015. A total of four larvae were collected in the coastal area of Rapa Nui in April and 13 in September. The larvae were identified as *Epigonichthys maldivensis* (Forster Cooper, 1903) (Cephalochordata Branchiostomatidae) using both morphological and genetic characters. The water column in this area presented a mean temperature of 21.2°C, a mean salinity of 35.7 ‰ and 4.94 ml/L dissolved oxygen in April, and 20°C and 35.75 ‰ mean salinity in September. Amphioxus have been reported as playing a key role in marine food webs transferring important amounts of microbial production to higher trophic levels, due to this their role in the Rapa Nui plankton and benthos as adults could be interesting because Easter island is located in the oligotrophic gyre of the South Pacific ocean where a microbial trophic web is expected to dominate. This record increases the biodiversity of Rapa Nui plankton and widens the geographic distribution of *E. maldivensis* that was restricted only to the Western and Central Pacific and Indian Ocean.

## KEY WORDS

amphioxus larvae; Pacific Ocean; plankton.

Received 03.12.2015; accepted 19.01.2016; printed 30.03.2016

---

## INTRODUCTION

The Amphioxus or lancelets (Chordata) comprise the subphylum Cephalochordata (Schubert et al., 2006); which is formed by three genera: *Branchiostoma* Costa, 1834, *Epigonichthys* Peters, 1876 and *Asymmetron* Andrews, 1893 (Kon et al., 2007). The amphioxus are filter-feeding marine organisms that as adults burrow in the sand, gravel or shell deposits in tropical and/or temperate waters around the world ocean (Bertrand & Escriva, 2011). The filtering is performed through jawless ciliated mouths (Vergara et al., 2011).

Amphioxus are found in general in shallow waters close to the shore (0.5 to 40 m depths) and many species prefer habitats of coarse sand and gravel (Desdevises et al., 2011). They live in a variety of coastal habitats, estuaries, coastal lagoons, open coasts and river deltas (Laudien et al., 2007; Chen, 2008). However, little is known about the ecological role of these organisms (Vergara et al., 2011). In addition, some amphioxus have been considered as endangered species (Kubokawa et al., 1998). Environmental factors as temperature and salinity changes are determinant in the life cycle of some amphioxus species (Webb, 1956a; Webb,

1956b; Webb & Hill, 1958). As a consequence, the amphioxus populations migrate between winter and summer (Webb, 1971), and the larvae are described as restricted to waters of high salinity and temperature (Webb & Hill, 1958). The duration and timing of the spawning season varies between species (Stokes & Holland, 1996; Holland, 2011). When the gametes are released in the water, fecundation occurs and the embryos persist in the plankton (Bertrand & Escriva, 2011) until metamorphosis, when they migrate to the sand and become benthic adults.

Some authors have studied the zooplankton and meroplankton around Easter Island (Castro & Landaeta, 2002; Palma & Siva, 2006; Mujica, 2006) most zooplankton results are from CIMAR islands cruise in November 1999. However, there are no records of amphioxus larvae or adults in the area. In this work we describe the presence of amphioxus larvae from Rapa Nui plankton (Chile) for the first time. Larvae were found in stations close to the coast around the island in April and September 2015.

## MATERIAL AND METHODS

Zooplankton samples and hydrographic measurements were gathered in the coastal area of Easter Island or Rapa Nui (27°13' S - 109°37' W), Chile, in April and September 2015. The hydrographic characterization of the water column was done using a set of CTD profiles in both months (Seabird 18).

Zooplankton samples were collected by oblique tows from a depth of 300 m up to the surface, using a Bongo net with 300 µm mesh and 60 cm mouth diameter. The volume of sampled water was estimated using a mechanical flowmeter (General Oceanics) attached to the net. Samples were preserved in 96% ethanol, until laboratory identification and quantification. In these samples seventeen amphioxus larvae were found. Considering that no information about amphioxus larvae morphology is available, three larvae were used to perform the genetic identification. After that a simple morphological description of the larvae is also supplied.

### *Genetic identification*

Three larvae were used for the genetic analysis. The DNA extraction was conducted using the Qiagen QIAamp kit (Mississauga, Canada). The mitochondrial COI gene was amplified using the protocol and

primers described by Folmer et al. (1994) with 56°C as annealing temperature. Forward and reverse sequencing was performed at Pontificia Universidad Católica de Chile and aligned by eye using the ProSeq v.2.9 software (Filatov, 2002). The haplotype was deposited in Genbank (Accession Number: KU201542). The Blast tool was used to determine similarities with sequences deposited in Genbank.

In order to determine the nucleotide relationship among lancelets, a neighbour-joining based phylogenetic (NJ) analysis was performed using Mega 6.0 software (Tamura et al., 2013). Using a bootstrap of 10,000 replicates, the analysis tested the consistency of each branch in the tree, grouping sequences with similar nucleotide composition. Using this method, unidentified sequence obtained in this study could be grouped with conspecific sampled in other geographical areas.

## RESULTS AND DISCUSSION

A total of 4 amphioxus larvae were found in the coastal area of Rapa Nui in April and 13 in September 2015. The larvae were identified as *Epigonichthys maldivensis* (Forster Cooper, 1903) (Cephalochordata Branchiostomatidae) (Fig. 1). In April, in the south station, larvae were found up to 200 m depth and the abundance was 0.8 individual per 1000 m<sup>3</sup>, while in the south-east station, the abundance of *E. maldivensis* larvae was 2 individuals per 1000 m<sup>3</sup> and were found between 300 m depth and surface. The amphioxus larvae mean abundance in September was 2 individuals per 1000 m<sup>3</sup> and they were found in the south station. The environmental characteristics of the area were mean water temperature of 21.2°C, mean salinity of 35.7 ‰ and 4.94 ml/L dissolved oxygen in April, and 20°C and 35.75 ‰ mean salinity in September.

Genetic identification. One haplotype of 550 bp was obtained for the larvae. The analysis of the COI gene showed a clear relationship of our sequence with *Epigonichthys maldivensis* (Fig. 2). The Blast analysis showed a similarity of 99% with one sequence of *E. maldivensis* (Accession Number: AB110093.1), deposited by Nohara et al. (2005) and obtained from one individual collected in the Kuroshira Island, Japan. Both sequences differ only in 6 bp.

*Epigonichthys maldivensis* is a tropical species whose distribution was restricted only to the Western and Central Pacific and Indian Ocean

(Richardson & McKenzie, 1994; Poss & Boschung, 1996; Lin et al., 2015), the present results expand the geographic range of this species to Rapa Nui island. Lancelets exhibit a week- to month long planktonic larval stage (Wickstead, 1970; Wu et al., 1994; Stokes & Holland, 1996) and in Eastern Island these were present in April and September 2015.

The benthic communities from Rapa Nui are extremely species-poor compared with reefs in the central and western Pacific (Friedlander et al., 2013), the presence of the amphioxus larvae, implies that amphioxus adults probably live in the benthos that would contribute to the benthos species richness. Moreover, anecdotal histories from the local fisherman of Rapa Nui reporting, in some areas and dates, the presence of white filaments like hairs in the bottom, are likely to corroborate our findings; these filaments could be the adult amphioxus. This record increases the biodiversity value of Rapa Nui. In addition, since amphioxus have been reported as playing a key role in marine food webs transferring important amounts of microbial production to higher trophic levels (Chen et al., 2008), their role in the Rapa Nui plankton and benthos as adults could be interesting since Easter island is located in the oligotrophic gyre of the South Pacific ocean where a microbial trophic web is expected to dominate. Finally, new amphioxus genome sequences will be of great importance for comparative genomics at the inter and intra species levels.

**ACKNOWLEDGEMENTS**

Authors acknowledge the support from the Chilean army at Easter Island and the ORCA diving center to conduct the samplings. EM acknowledges the support from Postdoctoral-FONDECYT/Chile 3150419. EM acknowledges the support of Millennium Nucleus for Ecology and Sustainable Management of Oceanic Islands (ESMOI). CV acknowledges the support of Fondecyt de Iniciación N° 11150213. Authors also acknowledge Carolina Paz Concha Molina from CFRD University of Concepcion for her contribution with the drawing.

**REFERENCES**

Bertrand S. & Escriva H., 2011. Evolutionary crossroads in developmental biology: amphioxus. *Development*,

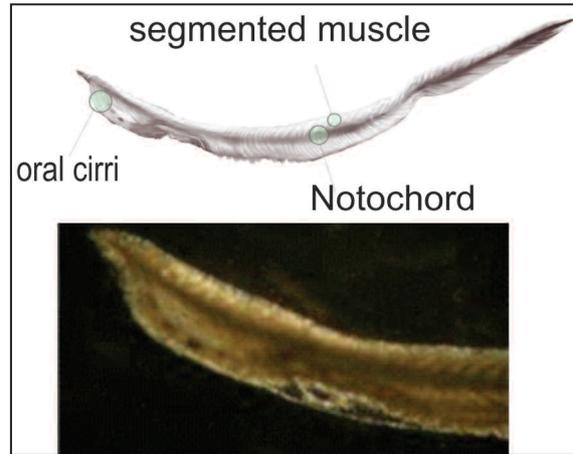


Figure 1. Above: schematic views of the amphioxus larva, basic anatomy the oral cirri, the segmented muscles, and the notochord are signaled. Below: *Epigonichthys maldivensis* larval individual collected from Rapa Nui.

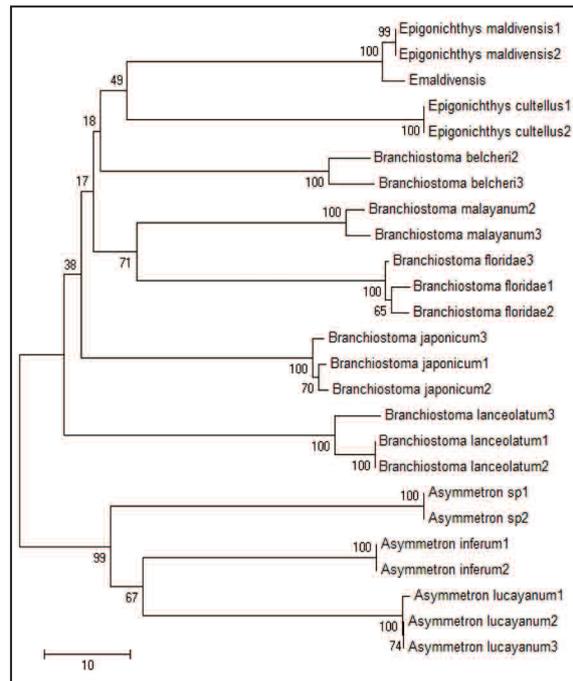


Figure 2. Neighbour-joining tree of the COI sequences for the Branchiostomidae species. The number at the tree nodes indicates the bootstrap values from 10,000 replicates. The figure shows also the GenBank Accession Numbers.

138: 4819–4830.  
 Castro L.R. & Landaeta M.F., 2002. Patrones de distribución y acumulación larval en torno de las islas oceánicas: Islas de Pascua y Salas y Gómez. *Ciencia y Tecnología del Mar*, 25: 131–145.

- Chen Y., Shin P.K.S. & Cheung S.G., 2008. Growth, secondary production and gonad development of two co-existing amphioxus species (*Branchiostoma belcheri* and *B. malayanum*) in subtropical Hong Kong. *Journal of Experimental Marine Biology and Ecology*, 357: 64–74.
- Desdevises Y., Maillet V., Fuentes M. & Escriva H., 2011. A snapshot of the population structure of *Branchiostoma lanceolatum* in the Racou Beach, France, during its spawning season. *PLoS ONE* 6, e18520.
- Folmer S.C., Black M., Hoek R., Lutz R.A. & Vrijenhoek R., 1994. DNA primers for amplification for mitochondrial cytochrome c oxidase subunit 1 from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3: 294–299.
- Filatov D.A., 2002. ProSeq: a software for preparation and evolutionary analysis of DNA sequence data sets. *Molecular Ecology Notes*, 2: 621–624.
- Friedlander A.M., Ballesteros E., Beets J., Berkenpas E., Gaymer C.F., Gorny M. & Sala E., 2013. Effects of isolation and fishing on the marine ecosystems of Easter Island and Salas y Gómez, Chile. *Aquatic Conserv: Marine and Freshwater Ecosystems*, 23: 515–531.
- Holland N.D., 2011. Spawning periodicity of the lancelet, *Asymmetron lucayanum* (Cephalochordata), in Bimini, Bahamas. *Italian Journal of Zoology*, 78: 478–486.
- Kon T., Nohara M., Yamanoue Y., Fujiwara Y., Nishida M. & Nishikawa T., 2007. Phylogenetic position of a whale-fall lancelet (Cephalochordata) inferred from whole mitochondrial genome sequences. *BMC Evolutionary Biology*, 7 (127). DOI: 10.1186/1471-2148-7-127.
- Kubokawa K., Nobuyuki A. & Tomiyama M., 1998. A new population of the amphioxus (*Branchiostoma belcheri*) in the Enshu-Nada Sea in Japan. *Zoological Sciences*, 15: 799–803.
- Laudien J., Rojo M., Oliva M., Arntz W. & Thatje S., 2007. Sublittoral soft bottom communities and diversity of Mejillones Bay in northern Chile (Humboldt Current upwelling system). *Helgoland Marine Research*, 61: 103–116.
- Lin H.C., Chen J.P., Chan B.K.K. & Shao K.T., 2015. The interplay of sediment characteristics, depth, water temperature, and ocean currents shaping the biogeography of lancelets (Subphylum Cephalochordata) in the NW Pacific waters. *Marine Ecology*, 36: 780–793.
- Mujica A., 2006. Larvas de crustáceos decápodos y crustáceos holoplanctónicos en torno a la isla de Pascua. *Ciencia y Tecnología del Mar*, 29: 123–135.
- Nohara M., Nishida M. & Nishikawa T., 2005. Evolution of the mitochondrial genome in cephalochordata as inferred from complete nucleotide sequences from two epigonichthys species. *Journal of Molecular Evolution*, 60: 526–537.
- Palma S. & Silva N., 2006. Epipelagic siphonophore assemblages associated with water masses along a transect between Chile and Easter Island (eastern South Pacific Ocean). *Journal of Plankton Research*, 28: 1143–1151.
- Poss S.G. & Boschung H.T., 1996. Lancelets (Cephalochordata: Branchiostomatidae): how many species are valid? *Israel Journal of Zoology*, 42: S13–S66.
- Richardson B.J. & McKenzie A.M., 1994. Taxonomy and distribution of Australian Cephalochordates (Chordata: Cephalochordata). *Invertebrate Taxonomy*, 8: 1443–1459.
- Schubert M., Escriva H., Xavier-Neto J. & Laudet V., 2006. Amphioxus and tunicates as evolutionary model systems. *Trends in Ecology & Evolution*, 21: 269–277.
- Stokes M.D. & Holland N.D., 1996. Reproduction of the Florida lancelet (*Branchiostoma floridae*): spawning patterns and fluctuations in gonad indexes and nutritional reserves. *Invertebrate Biology*, 115: 349–359.
- Tamura K., Stecher G., Peterson D., Filipowski A. & Kumar S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, 30: 2725–2729.
- Vergara M., Oliva M.E. & Riascos J.M., 2011. Population dynamics of the amphioxus *Branchiostoma elongatum* from northern Chile. *Journal of the Marine Biological Association of the United Kingdom*, 92: 591–599.
- Webb J.E., 1956a. A note on the lancelets of Singapore, with a description of a new species of *Branchiostoma*. *Proceedings of The Zoological Society of London*, 127: 119–123.
- Webb J.E., 1956b. On the populations of *Branchiostoma lanceolatum* and their relations with the West African lancelets. *Proceedings of The Zoological Society of London*, 127: 125–140.
- Webb J.E. & Hill M.B., 1958. The ecology of Lagos lagoon. IV. On the reactions of *Branchiostoma nigeriense* Webb to its environment. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 241: 355–391.
- Webb J.E., 1971. Seasonal changes in the distribution of *Branchiostoma lanceolatum* (Pallas) at Helgoland. *Vie et Milieu*, 22: 827–839.
- Wickstead J.H., 1970. On a small collection of *Acrania* (Phylum Chordata) from New Caledonia. *Cahiers du Pacifique*, 14: 237–243.
- Wu X.-H., Zhang S.-C., Wang Y.-Y., Zhang B.-I., Qu Y.-M. & Jiang X.-J., 1994. Laboratory observation on spawning, fecundity and larval development of amphioxus (*Branchiostoma belcheri* Tsingtaunese). *Chinese Journal of Oceanology and Limnology*, 12: 289–294.