

AMPHIOXUS- BRANCHIOSTOMA (CHORDATA: CEPHALOCHORDATA) : FIRST REPORT OF ITS OCCURRENCE FROM THE INTERTIDAL SOFT SEDIMENT BENTHIC HABITAT OF CLIFTON BEACH, KARACHI, PAKISTAN (NORTHERN ARABIAN SEA)

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ABSTRACT

This communication reports the first evidence of the presence of cephalochordate Amphioxus genus *Branchiostoma* from the intertidal sandy beach of Clifton, Karachi, Pakistan. During the analysis of macrobenthic fauna, individuals of lancelets were observed and separated for identification. Their morphometric characters were studied and measured. Most of the specimens were juvenile or in immature phase. The average body length of *Branchiostoma* sp. was 16.87 ± 4.53 mm. No previous records of this genus are available in literature and it is the first report of *Branchiostoma* sp. from the sandy beach of Clifton, Karachi.

Key words: Branchiostoma, Sandy beach, Benthic fauna, Northern Arabian-Sea, Ecology, Intertidal zone

INTRODUCTION

Amphioxus which is also known as lancelets is a representative of subphylum Cephalochordata. This organism has been under focus of scientists studying phylogenetic relationships of the chordates (Gee, 2006). The subphylum consists of two families with three representative genera namely: *Branchiostoma*, *Epigonichthys*, and *Asymmetron* (Kon *et al.*, 2007). The lancelets for the first time described by Pallas (1774) and placed in Phylum Mollusca, named as *Limax lanceolatum*. Later in 1834 they were renamed as *Branchiostoma lubricus* (Costa, 1834) and classified as animals closely related to vertebrates and commonly called as *Amphioxus* by William Yarrell in 1836 (Bertrand and Escriva, 2011). They commonly represent cephalochordates and are 6.35- 8.9 cm long. They appear to be more similar to a fish, but lacks complex organs. Their primary feeding structures are concentrated in the front side of the body (Babu *et al.*, 2013). The primal features of amphioxus include the naked notochord and myotomic segmentation from end to end. The jaws and paired fins are absent. They have ciliary method of feeding with a single layered epidermis and a simple tubular intestine with simple liver diverticulum. The circulatory system is simple and lack specialized heart. The gonads and nephridia are segmental and are without ducts. There are separate dorsal and ventral roots of spinal nerves. The eggs are small and almost all are yolkless with hollow and spherical blastula. (Anonymous, 2016)

About 30 species of these soft bottom dwelling small marine creatures belonging to two families and three genera have been identified from coastal waters around the world (Poss and Boschung, 1996; Nishikawa, 2004; Chen, 2007; Anaya, 2010; Vergara *et al.*, 2011). The lancelets genera were differentiated on the basis of sexual organ (gonadic pouches). In genus *Branchiostoma* paired sexual organ, whereas, in genera *Epigonichthys* and *Asymmetron* unilateral gonads are present (Anonymous, 1911; Bertrand and Escriva, 2011). *Branchiostoma* is distinguishable further in addition to paired gonads, by its bilateral metapleural folds that terminate immediately posterior to the atriopore. The species within *Branchiostoma* have been identified on meristic variation such as myomere counts, counts of fin chambers, position of atriopore and anus, rostral fin and caudal fin shape etc. In lancelets early stages are pelagic and larval stage span over a period of one week to one month in shallow tropical and temperate seas (Wickstead, 1970; Wu *et al.*, 1994; Stokes and Holland, 1995). The adults are usually found buried inside sandy or coarse sandy substratum and selectively filter feed from the water column (Chen *et al.*, 2008). Because of their distribution in near shore benthic waters, the occurrence and biomass of lancelets (Rota *et al.*, 2009; Barboza *et al.*, 2013), and their associated benthic communities (Antoniadou *et al.* 2004; Chen *et al.* 2013), have been used to monitor anthropogenic impacts like sewage disposal, industrial waste, construction and developmental work in the coastal areas (Da Silva *et al.*, 2008) and their effects on biological diversity (Raveen *et al.*, 2016).

MATERIALS AND METHODS

The specimens of *Branchiostoma* sp. have been separated out of the macro faunal samples which were collected on monthly basis for two years (June 2005 to May 2007) from three stations China Town (A), Casino Clifton (B) and Corniche Point Sea view (C) at Clifton beach, Karachi covering an area of approximately 2 km (24° 47.733N 067° 02.326E to 24° 48.563N 067° 00.992E) during low tides. A metallic quadrat of 30 x 30 cm was inserted into the bottom sediment up to 20 cm deep to excavate the sediment. The sediments were sieved through 0.5 mm mesh size and the lancelets thus collected along with other macrobenthic fauna were sorted and fixed in borax buffered formaldehyde solution (5 to 10%). The identification and morphometric measurements of lancelets specimens were carried out under stereo zoom microscope with the help of available literature (Anonymous, 1911; Poss and Boschung, 1996). Micrograph paper (one small square equals to 1 mm) imaged on a transparent plastic sheeth was used to take length and width measurements.

RESULTS AND DISCUSSION

This study for the first time reports the occurrence of Cephalochordate genus *Branchiostoma* from the intertidal sandy beach of Clifton, Karachi. Due to the unavailability of taxonomic literature or reference material for comparing our specimen the specimens were not identified to the species level. There is no earlier record of this genus available in literature from Pakistan. In the present study the lancelets were found to be a part of the intertidal macrobenthic community of soft sandy sediment of Clifton beach. A total of 15 sexually immature specimens except one individual probably female of *Branchiostoma* were collected. The genus *Branchiostoma* belongs to the Branchiostomatidae family. The family Branchiostomatidae Bonaparte, 1846 characterized by having mouth nearly median, with oral cirri, a closed atrial chamber, lateral and paired gill-slits; undivided pharynx; gonad pouches developed on both epipleura; metapleura of each side terminating just behind atriopore (Hubbs, 1922). The lancelets body is elongated, laterally compressed and tapering at both ends. Their body is somewhat transparent or slightly opaque due to which the body structures are quite visible. The observed and measured morphometric characters are given in Table 1, and illustrated through Fig. 1. The body length and width were in the range of 5.5 to 22.5 mm and 1.0 to 2.0 mm, respectively. The number of myotomes, dorsal fin chambers, and ventral fin chamber were in the range of 56-68, 208-290 and 46-57, respectively. Though specimens were identified as *Branchiostoma* sp., however, few morphological characteristics of *B. belcheri* reported by Henmi and Yamaguchi (2003) from Japan and Zhang *et al.* (2006) from China resemble our specimen such as: number of dorsal fin-ray chambers (both male and female), number of pre-anal fin-ray chamber (male & female), total number of myotomes (male & female) and gonads number on both sides. However, the body length reported for Japanese lancelet is 30-56 mm for male specimens and 25-53 mm for female individuals, whereas, in our collected specimen the body length varied from 5.5 to 22.5 mm.

Table 1. *Branchiostoma* sp.: Measurements of morphometric characteristics.

Morphometric characters	Present study	Japan (<i>B. belcheri</i>)	China (<i>B. belcheri</i>)
	Mean ± SD	Mean ± SD	Mean ± SD
Body length (mm)	16.87±4.53	M= 43.2±6.0 F= 41.3±7.0	47.63±4.73
Body height (mm)	1.43±0.41	-	
Buccal cirri (No)	25±1.04	-	
Total myotomes (No.)	63.92±3.20	63.8±0.8 /63.9±0.8 63.6±0.9/ 63.8±0.8	64.48±0.59
Dorsal fin-ray chambers (No)	267.86±18.11	282.5±12.5 282.4±10.5	333.07±11.89
Ventral fin-ray chambers (No)	50.89±2.12	55.4±4.9 55.4±3.5	92.44±5.83
Gonads (No)	L=23.67±3.06	-	L=26.35±1.56
	R=25±1.73	-	R= 27.90± 1.01

Japan: Henmi and Yamaguchi, 2003; China: Zhang *et al.*, 2006

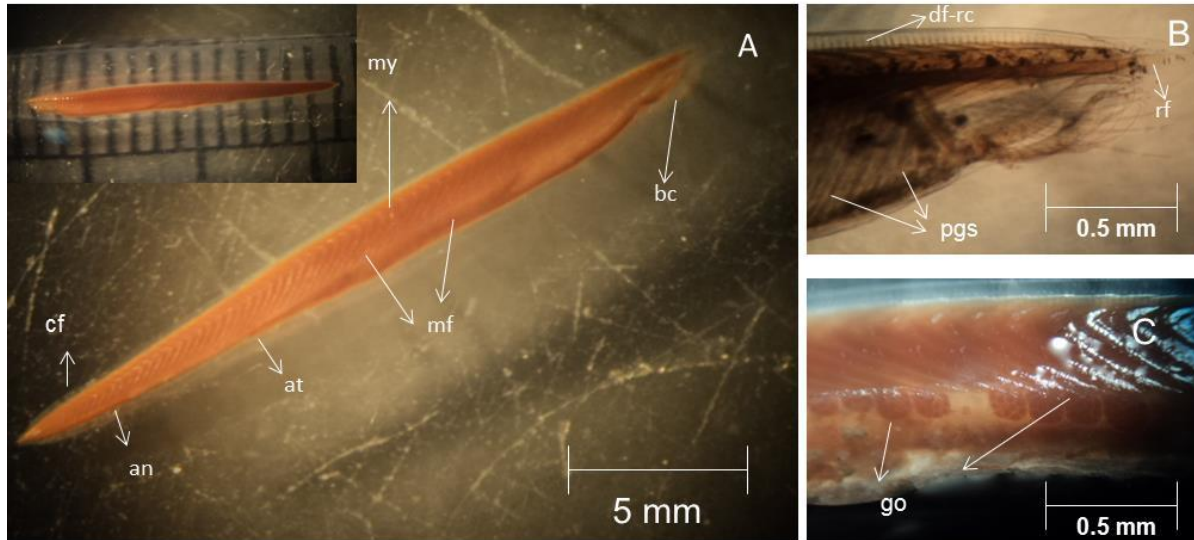


Fig. 1. Genus *Branchiostoma*; A. lateral view of an individual (original size (mm) shown in-set picture on top left corner), an: anus, at: atriopore, bc: buccal cirri, cf: caudal fin, my: myotomes, mf: metapleural fold; B. Close-up view of anterior region showing head, of: rostral fin, notochord, df-rc: dorsal fin-ray chambers and pgs: pharyngeal gill-slits; C. middle part of the right side of body in bottom view showing mature go (gonads) having granular appearance.

The earlier study of Babu *et al.* (2013) reported for the first time the distribution and abundance of Amphioxus from the southeast coast of India. They described four species of *Branchiostoma*, in relation with sediment characteristics. This report of lancelets (especially *Branchiostoma*) from northern Arabian sea will extend its distribution and biogeography in the Indo-West Pacific from middle, eastern and southern Indian Ocean to extreme northern limit i.e., northern Arabian Sea. This finding of lancelets from our area will provide an opportunity for opening the molecular and phylogenetic research for its being an emerging model organism for vertebrate evolutionary studies (Bertrand and Escriva, 2011; Yang *et al.*, 2016). To better understanding of its role in ecosystem, identification to species level will be of greater significance (Zhang *et al.*, 2006).

REFERENCES

- Anaya, J.P. (2010). *Los complejos Hox como modelo de evolución genómica en cordados: caracterización y regulación de la expresión del clúster Hox en el anfioxo Europeo*. PhD Thesis. Universidad de Barcelona, Spain.
- Anonymous (1911). *Encyclopedia Britannica* 11th 1: 887. Available from www.en.wikisource.org/wiki/Page:EB1911_-_Volume_01.djvu/938. [Accessed on 9/27/2015]
- Anonymous (2016). Basic idea about Amphioxus (= Branchiostoma). available from <http://www.biology-today.com/general-zoology/vertebrate-zoology/basic-idea-about-amphioxus-branchiostoma/>. [Accessed on 6/7/2016]
- Antoniadou, C., Y. Krestenitis and C. Chintiroglou (2004). Structure of the 'amphioxus sand' community in Thermaikos Bay (Eastern Mediterranean). *Fresen. Environ. Bull.*, 13: 1122- 1128.
- Babu, A., P. Sampathkumar, T. Balasubramanian, D. Varadharajan and T. Manikandarajan (2013). Distribution and abundance of amphioxus with relation to sediment characteristics from south east coast of India. *J. Mar. Sci. Res. Dev.*, 3: 121. doi: 10.4172/2155-9910.1000121.
- Barboza, C. A., H.L. Hadlich, L. Sandrini-neto, C. Martins CDE and C. Lana PDA (2013). Is the distribution of the lancelet *Branchiostoma caribaeum* affected by sewage discharges? An analysis at multiple scales of variability. *Mar. Poll. Bull.*, 69: 178- 188.
- Bertrand, S. and S. Escriva (2011). Evolutionary crossroads in developmental biology: amphioxus. *Development*, 138: 4819-4830.
- Chen, Y. (2007). *The ecology and biology of amphioxus in Hong Kong*. PhD Thesis. City University of Hong Kong, Hong Kong.
- Chen, Y., S.G. Cheung and P. K. S. Shin (2008). The diet of amphioxus in subtropical Hong Kong as indicated by fatty acid and stable isotopic analyses. *J. Mar. Bio. Assoc. U.K.*, 88: 1487- 1491.

- Chen, Y., S. G. Cheung and P. K. S. Shin (2013). A baseline study of benthic community associated with Amphioxus sand in subtropical Hong Kong. *Mar. Poll. Bull.*, 72: 274- 280.
- Costa, O. G. (1834). Ceeni zoologici, ossia descrizione somariadelie specie nuove di animali disopteri in diverse contrade del regno neell'anno 1834. In *Annuario Zoollogico*, 12: 49- 50. Napoli, Italy: Tipografia di Azzolino e Comp.
- Da Silva, L. F. B., M. Tavares and A. Soares-Gomes (2008). Population structure of the lancelet *Branchiostoma caribaeum* (Cephalochordata: Branchiostomidae) in the Baia de Guanabara, Rio de Janeiro, southeastern Brazil. *Rev. Bras. Zool.*, 25: 617 - 623.
- Gee, H. (2006). Careful with that amphioxus. *Nature*, 439: 923-924.
- Henmi, Y. and T. Yamaguchi (2003). Biology of the amphioxus *Branchiostoma belcheri* in the Ariake Sea Japan I. Population structure and growth. *Zool. Sci.*, 20: 887-906.
- Hubbs, C. L. (1922). A list of the lancelets of the world with diagnosis of five new species of *Branchiostoma*. Occasional papers of the Museum of Zoology, University of Michigan, Ann Arbor, Michigan, No. 105, 16.
- Kon, T., M. Nohara, Y. Yamanoue, Y. Fujiwara, M. Nishida and T. Nishikawa (2007). Phylogenetic position of a whale-fall lancelet (Cephalochordata) inferred from whole mitochondrial genome sequences. *BMC Evol. Biol.*, 7: 127.
- Nishikawa, T. (2004). A new deep-water lancelet (Cephalochordata) from off Cape Nomamisaki, SW Japan, with a proposal of the revised system recovering the Genus *Asymmetron*. *Zool. Sci.*, 21: 1131- 1136.
- Pallas, P. S. (1774). *Limax lanceolatus*. In: *Spicilegia zoologica*, Vol. I (Quadrupedum, avium, amphibiorum, piscium, insectorum, molluscorum, aliorumque marinarum). Fascicle 10 (quibus novae imprimus et obscurae animalium species iconibus, descriptionibus atque comentariis illustrantur cura P. S. Pallas). Berlin, Germany: G. A. Lange.
- Poss, S. G. and H. T. Boschung (1996). Lancelets (Cephalochordata: Branchiostomatidae): how many species are valid? *Israel J. Zool.*, 42 Suppl.: 13-66.
- Raveen, R., V. D. Samuel and T. Samuel (2016). Disappearance of *Branchiostoma lanceolatum* (Pallas) from Pulicat Lake area, Tamil Nadu, India. *Int. J. Fauna Biol. Stud.*, 3 (1): 55- 57.
- Rota, E., G. Perra and S. Focardi (2009). The European lancelet *Branchiostoma lanceolatum* (Pallas) as an indicator of environmental quality of Tuscan Archipelago (Western Mediterranean Sea). *Chem. Ecol.*, 25: 61- 69.
- Stokes, M. D. and N. D. Holland (1995). Embryos and larvae of a lancelet, *Branchiostoma floridae*, from hatching through metamorphosis: growth in the laboratory and external morphology. *Acta Zool.*, 76: 105- 120.
- Vergara, M., M.E. Oliva and J.M. Riascos (2011). Population dynamics of the amphioxus *Branchiostoma elongatum* from northern Chile. *Jour. Mar Biol Assoc. UK* DOI:10.1017/S0025315411000804
- Wickstead, J. H. (1970). On a small collection of Acrania (Phylum Chordata) from New Caledonia. *Cah. Pacifique*, 14: 237- 243.
- Wu, X.H., S.C. Zhang, Y.Y. Wang, B.I. Zhang, Y.M. Qu and X.J. Jiang (1994). Laboratory observation on spawning, fecundity and larval development of amphioxus (*Branchiostoma belcheri* Tsingtaunese). *Chinese J. Oceanol. Limnol.*, 12: 289- 294.
- Yang, K. Y., Y. Chen, Z. Zhang, P.K. Ng, W. J. Zhou, Y. Zhang, M. Liu, J. Chen, B. Mao and S. K. Tsui (2016). Transcriptome analysis of different developmental stages of amphioxus reveals dynamic changes of distinct classes of genes during development. *Sci. Rep.*, 6: 23195. DOI: 10.1038/srep 23195(2016)
- Zhang, Q., J. Zhang, S. Fang and Y. Wang (2006). *Branchiostoma japonicum* and *Branchiostoma belcheri* are distinct lancelets (Cephalochordata) in Xiamen waters in China. *Zool. Sci.*, 23: 573- 579.

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