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Iranian *Branchiostoma* species (Cephalochordata, Branchiostomatidae) inhabiting Chabahar Bay (Gulf of Oman), with remarks on habitat preferences

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Abstract: Variations in the population of lancelets inhabiting Chabahar Bay were surveyed statistically. Multivariate analysis of the Iranian *Branchiostoma* population using 5 morphometric characteristics indicates no separation of populations by locality in the study area (ANOSIM global; R = 0.2). Principal component analysis of the mean values of the 5 meristic characteristics of the present population with those of conspecifics of other populations around the world showed that Iranian lancelet populations are very similar to *B. lanceolatum* and completely different from the *B. arabia* and *B. belcheri* previously reported in adjacent waters (ANOSIM for pairwise tests; R = 1). Cluster analysis indicates that the Iranian *B. lanceolatum* shows greater affinity with its conspecifics in the Mediterranean than in the Indian Ocean. The distribution of lancelet populations correlates more closely with sand particles; however, this correlation was not significant (P = 0.28). The novel aspect of this study is the information presented about *B. lanceolatum* populations in the Iranian Gulf of Oman and its greater affinity with the species reported with Mediterranean conspecifics.

Key words: Lancelets, identification, Makran Sea, *Branchiostoma lanceolatum*

1. Introduction

The amphioxus, or lancelet, is a filter-feeding marine organism that is widely distributed in tropical and temperate seas. Adult lancelets are benthic and prefer to live in soft bottom sediment from intertidal zones to depths of 0–230 m (Wickstead, 1975; Nishikawa, 2004; Da Silva et al., 2008). Their larvae may be nektonic and live in a variety of coastal habitats, estuaries, coastal lagoons, open coasts, and river deltas (Laudien et al., 2007; Chen, 2008; Bertrand and Escriva, 2011; Meerhoff et al., 2016).

Lancelets were systematically described for the first time in 1774 (Pallas, 1774); they were classified as mollusks and were named *Limax lanceolatus*. In 1834, they were renamed *Branchiostoma lubricus* (Costa, 1834) and classified as animals closely related to vertebrates. Recent phylogenetic studies (Kon et al., 2007) have shown that the cephalochordate subphylum (Schubert et al., 2006) is divided into 3 genera: *Branchiostoma* Costa 1834 with 23 valid species; *Epigonichthys* Peters 1876 with 5 valid species; and *Asymmetron* Andrews 1893 with 2 valid species (Poss and Boschung, 1996; Nishikawa, 2004; Kon et al., 2007; http://www.marinespecies.org/).

The diagnostic characteristics of the genera are the different positions of the gonads, the symmetric or asymmetric endings of the metapleural fold (Poss and Boschung, 1996), and the presence of a urostyloid process (Nishikawa, 2004). Traditionally, myotome counts, fin chambers, position of the atriopore and anus, and qualitative differences in the notochord and caudal fin shape are diagnostic characteristics used for species identification. Multivariate analysis of meristic variation has shown considerable intraspecific variability in which the range of variation differs depending on the species (Poss and Boschung, 1996).

Temperature, salinity, sediment characteristics (grain size, total organic matter), and ocean currents have been suggested as habitat preferences and geographical distribution factors affecting the population structure of lancelets (DaSilva et al., 2008; Lin et al., 2015). Globally, *Branchiostoma* and *Asymmetron* are found along the warm coasts of the world's oceans, whereas *Epigonichthys* is restricted to the coasts of the Indian Ocean and the western and central Pacific Ocean (Poss and Boschung, 1996). A literature survey shows that *B. belcheri*, *E. maldivensis*, *B. arabiae*, *B. lanceolatum*, and *E. lucayanus* (accepted as *Asymmetron lucayanum*) are distributed in the western Indian Ocean, which includes the Arabian Sea, Red Sea, Sea of Oman, and Persian Gulf (Poss and Boschung, 1996). Among these, the last 3 species mentioned have been reported in the Persian Gulf and Sea of Oman (Webb, 1957;

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Dawson, 1964; fig. 2 of Poss and Boschung, 1996).

The distribution and ecology of lancelets in the Iranian waters of the Persian Gulf and the Gulf of Oman is unknown. This study was undertaken to investigate the lancelet populations along a small part of the vast southern Iranian coast. Information about the habitat preferences of the observed *Branchiostoma* species has been obtained. Reports about *B. lanceolatum* in Iranian waters of the Gulf of Oman and the greater affinity of the reported species with Mediterranean conspecifics are the novelties of this study.

2. Materials and methods

2.1. Study area

Chabahar Bay is a horseshoe-shaped subtropical bay on the southeastern coast of Iran. The bay surface area is 290 km^2; it is 14 km wide and is connected to the Indian Ocean by the Gulf of Oman (Figure 1).

Materials were collected in 3 replicates using a 0.25-m^2 van Veen grab on 15.01.2016 at 3 subtidal and 3 intertidal stations. One additional sample was collected for sediment analysis. The specimens of amphioxi were preserved in 5% buffered formalin and stained with Rose Bengal solution (0.1 g in 100 mL of distilled water) in order to better sort them from other macrobenthos. The samples were preserved in 75% alcohol for further identification.

The density of the amphioxus specimens is expressed as the number of individuals per m^2 (Table 1). Nine morphometric (meristic/metric) characteristics were measured for 42 specimens (Table 2) using a stereomicroscope (Nikon; SMZ 500). Taxonomic identification was based on morphological characteristics after Poss and Boschung (1996) and multivariate analysis. The materials were deposited in the Iranian National Institute for Oceanography and Atmospheric Science collection as INIOC-2-6S, INIOC-2-7S, and INIOC-2-8S. The following is a list of abbreviations of the meristic and metric characteristics used in the present study: AAD, atriopore–anal distance; AAM, atriopore–anal–myotome; DFC, dorsal fin chamber; DFT, dorsal fin tallest/broad; PAM, pre-atriopore myotome; PFC, preanal fin chamber; POM, postanal myotome; TL, total length; TM, total myotome.

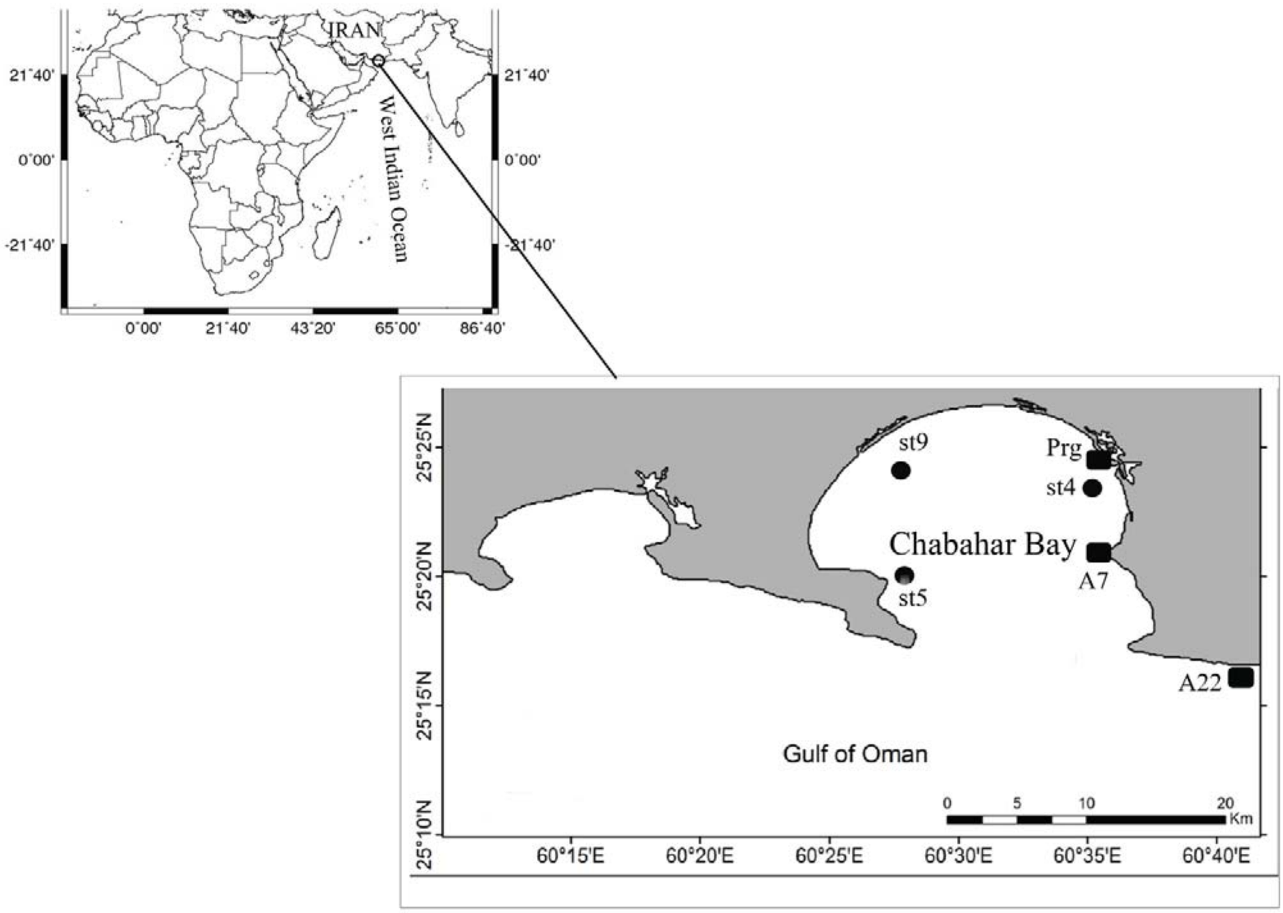

Figure 1. Sampling area containing 3 subtidal (circle) and 3 intertidal (square) stations.

Table 1. Station characteristics and sediment analysis.

Station	Depth	Temperature (°C)	Salinity (ppt)	Sand %	Silt %	Clay %	TOM %	Density/m^2
A22	0	23.5	36.5	100	0	0	3.25	4
A7	0	23.2	36.5	93	7	0	3.5	4
Prg	0	23.4	36.6	97.5	2.5	0	3.1	8
st4	2.7	23.4	36.5	100	0	0	2.4	80
st5	6.8	23.6	36.4	98.2	1.8	0	3.7	68
st9	6.4	23.7	36.4	11.6	85	3.4	6.3	4

Table 2. Summary of the mean and standard deviation found for each character. Of the 42 specimens collected, 37 intact specimens were measured.

Characters*	Mean (n = 37)	Std. deviation	Maximum value	Minimum value
DFC	209.16	18.44	260	178
PFC	37.57	4.87	48	28
PAM	39.11	4.34	46	26
AAM	15.62	1.93	18	12
POM	8.19	0.94	11	6
TM	62.9	5.2	71	44
DFT_B	1.86	0.17	2.1	1.68
TL	14.8	3.2	18	13
AAD	2.78	0.79	2.6	2.1

*Character abbreviations listed in Section 2.

The total organic matter of the sediment was measured by the loss on ignition method (LOI: Heiri et al., 2001). Sediment particle size distribution was measured using a Horiba laser scattering particle size distribution analyzer (LA-950).

2.2. Statistical analysis

Factor analysis was done with SPSS 17 and was used to calculate mean values and the standard deviation (Table 2) for each meristic/metric characteristic and to prepare a correlation matrix and significance level for the different characteristics. Primer (v. 6.1.12) and Permanova (v. 1.0.2) were used for multivariate analysis (PCA, ANOSIM) of the Iranian *Branchiostoma* populations, and also to test similarities between Iranian *Branchiostoma* and other congeners. The meristic characteristics used in multivariate analysis were selected based on the 5 characteristics (except for total myotome) listed in Table 2 from Poss and Boschung (1996) for world *Branchiostoma* species, plus the characteristics of *B. japonicum* from Zhang et al. (2006). Because of the abnormal distribution of the data before and after transformation, the nonparametric Spearman's test was done in SPSS to examine correlations between the numbers of specimens per station with sediment characteristics.

3. Results

A total of 42 amphioxi were found from intertidal (5 individuals) and subtidal areas (38 individuals). The minimum calculated density was 4 ind./m^2 and the maximum was 80 ind./m^2. The water depth of the collection sites varied from 0 to 6.8 m. The sediment structure was mainly fine sand with a total organic matter (TOM) ranging from 2.4 to 6.3 (Table 1). The surface water temperature was 23.4 to 23.7 °C, and salinity was 36.4 to 36.6 ppt.

Subphylum: Cephalochordata

Class: Leptocardii

Family: Branchiostomatidae Bonaparte 1846

Genus: *Branchiostoma* Costa 1834

Species: *Branchiostoma lanceolatum* (Pallas, 1774) (Figures 2 and 3)

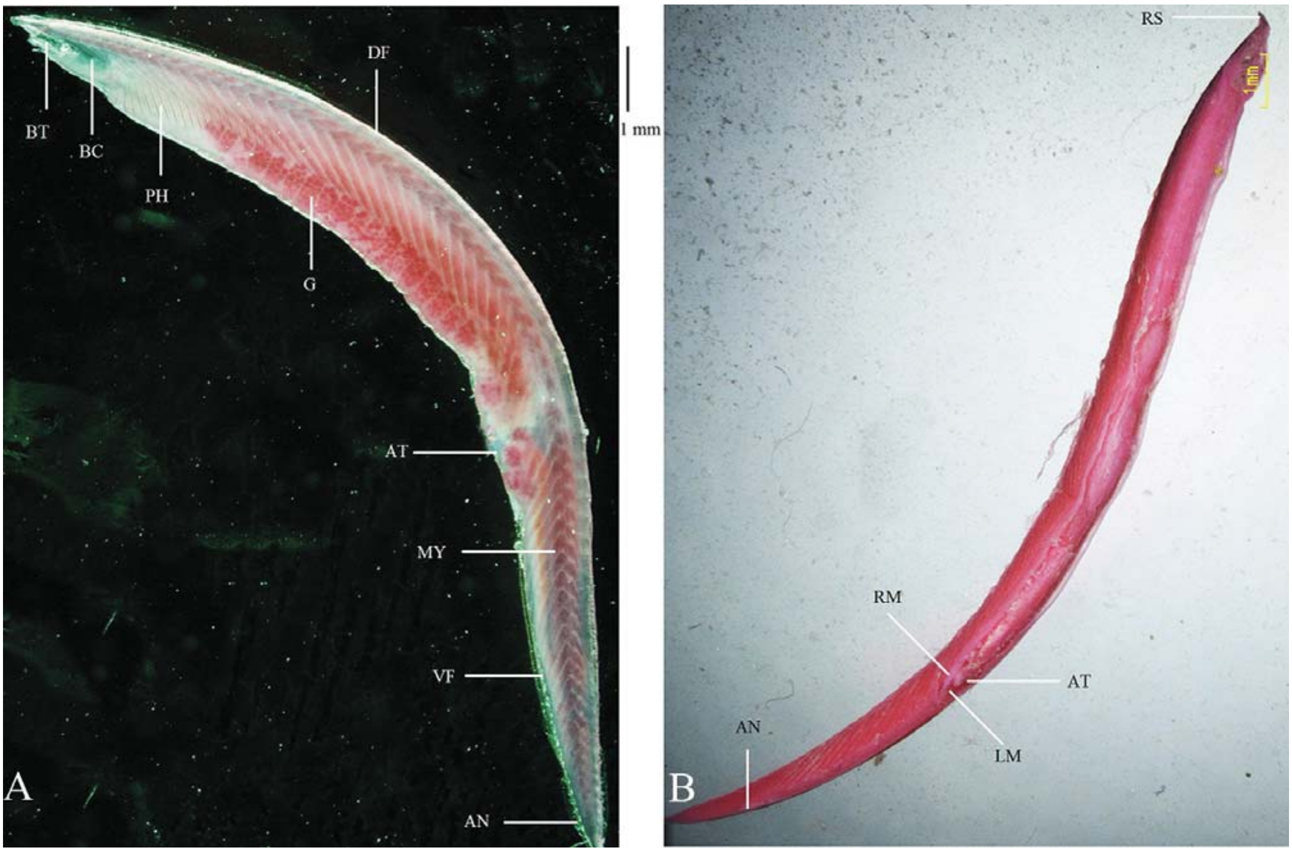

Figure 2. *Branchiostoma lanceolatum* collected in this study: A (INIOC-2-7S); B (INIOC-2-6S). AT, Atriopore; AN, anus; BT, buccal tentacle; BC, buccal cavity; DF, dorsal fin; G, gonad; PH, pharynx; LM, left metaplural; MY, myotome; RM, right metaplural; RS, rosterum; VF; ventral fin.

Laboratory observation of mature specimens shows gonads on both sides of the body (Figure 2A) and a clean termination of the metapleural folds immediately posterior to the atriopore (Figure 2B); therefore, the specimens collected belong to the genus *Branchiostoma* (Poss and Boschung, 1996). Observation of the anterior and posterior ends of the body also indicated a well-developed dorsal fin chamber (Figure 3A), ventral fin chamber, and caudal fin (Figure 3B).

Statistical analysis of 9 metric and meristic characteristics is given as mean and standard deviation in Table 2.

Pairwise correlation matrix of morphometric characters examined in this study showed significant correlation between AAM and PFC (preanal fin chamber) (P = 0.005), POM and DFC (P = 0.01), POM and TM (P = 0.003), TM and PAM (P = 0.0001), AAD and PFC (P = 0.02), and TL and AAD (P = 0.0001) and high significant correlations between TL and AAM (P = 0.007) and TL and PFC (P = 0.001).

The meristic characteristic means (except for that of total myotome) as recommended by Poss and Boschung (1996) were analyzed using principal component analysis (PCA) to investigate similarity within the Iranian population and also between Iranian *Branchiostoma* populations and other congeners around the world (species listed in Table 2 of Poss and Boschung, 1996, plus relevant characteristics of *B. japonicum* from Zhang et al., 2006). PCA analysis (Figure 4) showed similarities between all collected Iranian populations. The analysis also showed the similarity of Iranian *Branchiostoma* to *B. lanceolatum* (Figure 5). ANOSIM pairwise testing indicated that the Iranian *B. lanceolatum* is completely different from *B. arabia* (ANOSIM for pairwise tests; R = 1), which has previously been reported (Webb, 1957; Dawson, 1964) for waters adjacent to the present study area.

Cluster analysis also demonstrated the closer affinity of Iranian *B. lanceolatum* with congeners from the Mediterranean Sea, especially from Naples, than to other European congeners from the English Channel and North Sea or from Indian Ocean populations (Figure 5). Spearman's correlation coefficient showed greater correlation between the distribution of lancelet populations with sand particles; however, this correlation was not significant (P = 0.28). No

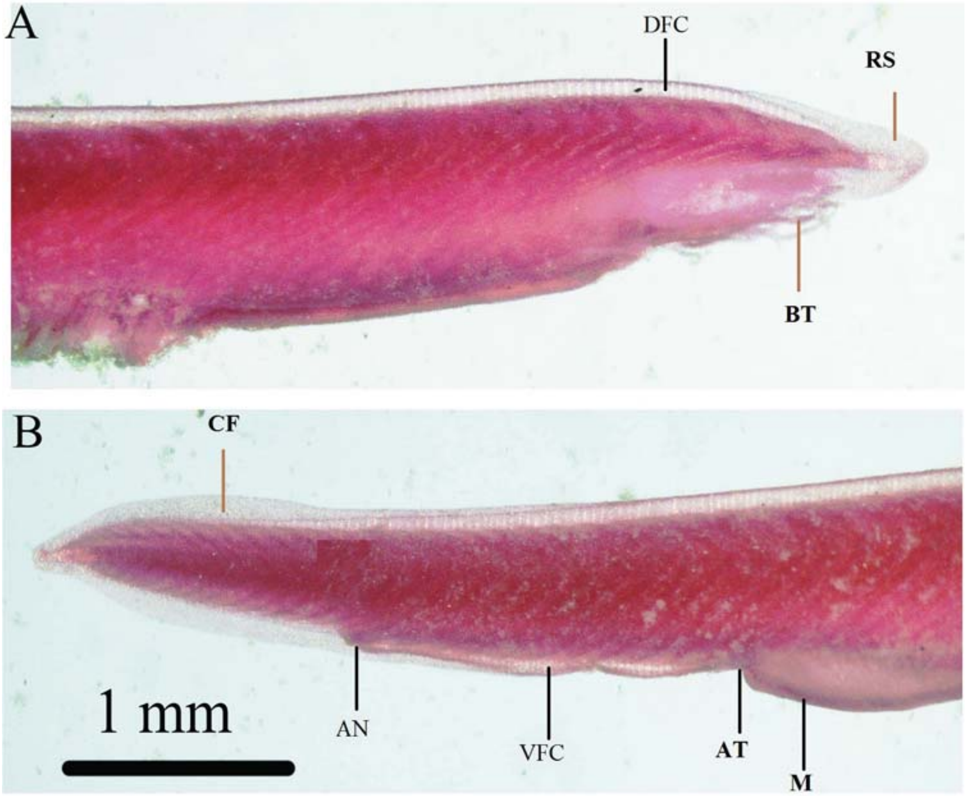

Figure 3. Anterior (A) and posterior (B) end of the *Branchiostoma lanceolatum* (INIOC-2-8S) of the present study. AN, Anus; AT, atriopore; BT, buccal tentacle; CF, caudal fin; DFC; dorsal fin chamber; M, metaplural fold; RS, rosterum; VFC, ventral fin chamber.

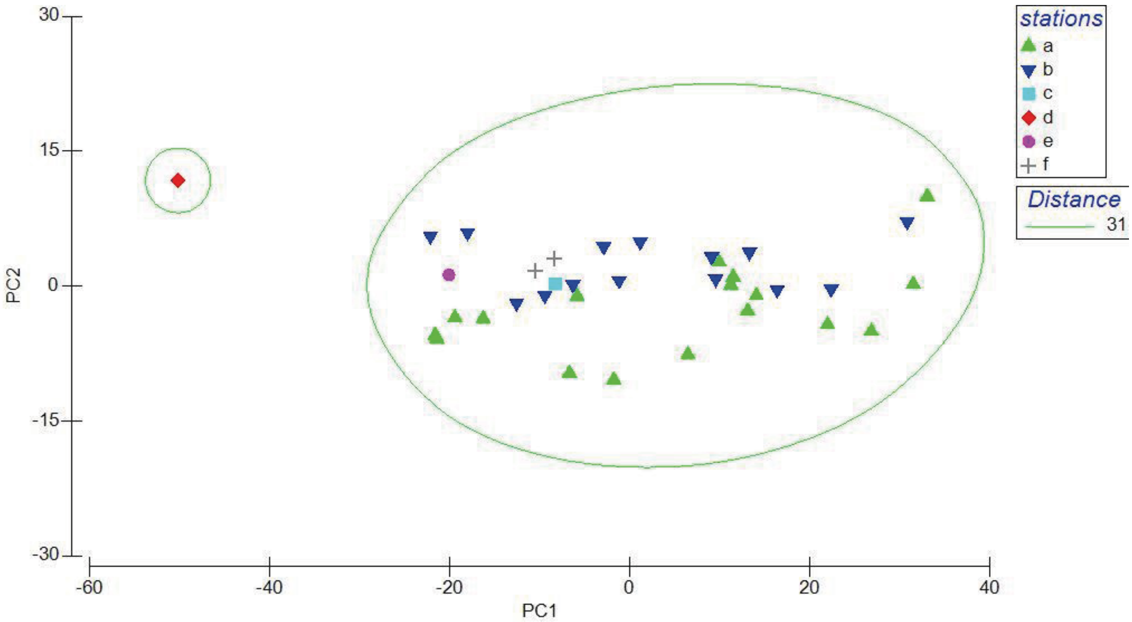

Figure 4. PCA analysis of Iranian *Branchiostoma* populations based on the mean of 5 meristic values recommended by Poss and Boschung (1996). Legend for stations: St4 = a, St5 = b, St9 = c, A22 = d, A7 = e, Prg = f. List of abbreviations mentioned above for each variable. Only one specimen being collected at Station d is considered to be a possible reason for the isolation of Station d from the other stations.

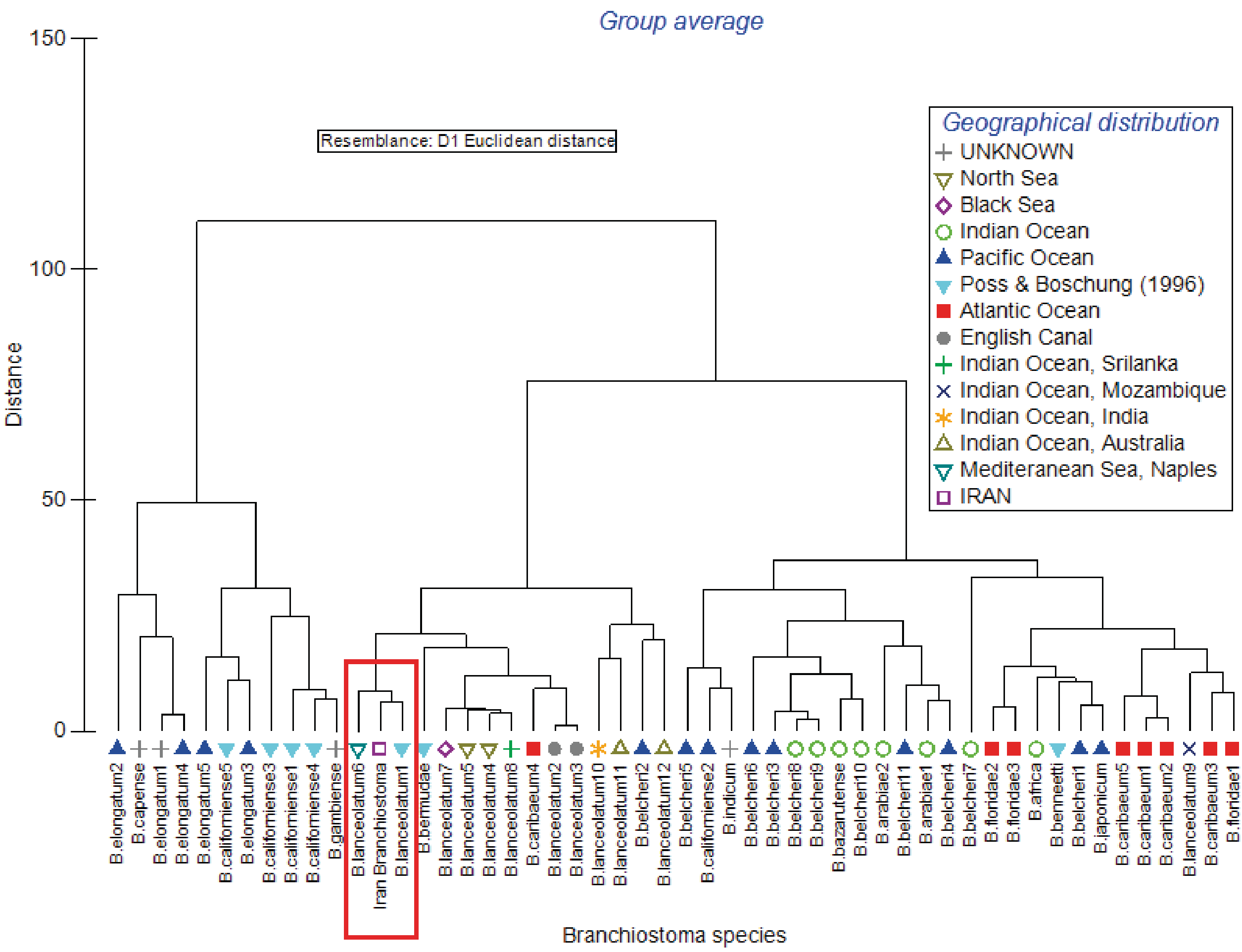

Figure 5. Cluster analysis shows similarity of *Branchiostoma lanceolatum* collected in the present study with congeners from the Mediterranean Sea, based on the 5 characters (except total myotome) listed in Table 2 of Poss and Boschung (1996) plus *B. japonicum* (Zhang et al., 2006).

significant correlation was observed between TOM and the frequency of lancelets or sediment grain sizes.

4. Discussion

Multivariate analysis of meristic variation has shown considerable intraspecific variability in which the range of variation differs depending on the species (Poss and Boschung, 1996). Pairwise correlation testing of the meristic/metric characteristics also indicates highly significant differences between total length (TL) and the atriopore–anal myotome (AAM; P = 0.007) and preanal fin chamber (PFC; P = 0.001). The literature review revealed that *B. belcheri*, *E. maldivensis*, *B. arabiae*, *B. lanceolatum*, and *E. lucayanus* (accepted as *A. lucayanum*) are distributed in the western Indian Ocean, including in the Arabian Sea, Red Sea, Sea of Oman, and Persian Gulf (Poss and Boschung, 1996). Among them, the last 3 species have been reported along the non-Iranian coasts of the Persian Gulf and in the Arabian Sea (Webb, 1957; Dawson, 1964; fig. 2 of Poss and Boschung, 1996). The present study area (Chabahar Bay) is connected to the Indian Ocean by the Gulf of Oman (Figure 1). There is no report on the occurrence of lancelets either in the present study area or along the other Iranian coasts of the Persian Gulf and the Gulf of Oman.

As shown by the results, the lancelets collected belong to *Branchiostoma* and show greater affinity to *B. lanceolatum* (Figure 5). The species also known as the European lancelet was originally described in the North Sea and the Mediterranean. The recorded range of the species is extensive: from the eastern Atlantic and Arabian Sea to East Africa and India, in the Indian Ocean (Webb, 1957; Poss and Boschung, 1996). The greatest abundance is in the Mediterranean near Naples and Messina (Rota et al., 2009).

Webb (1957) suggested that the occurrence of a single specimen of *B. lanceolatum* in the Persian Gulf is of particular interest, because it confirmed that this species had spread from the Mediterranean to the Arabian Sea (Lessepsian migration), and also because *B. lanceolatum* may now be considered sympatric with *B. arabiae*. He also stated that there is no notable difference between *B. lanceolatum* from the Arabian Peninsula and the European *B. lanceolatum*.

The results of the current study indicate that the Iranian *B. lanceolatum* has affinity with its Mediterranean congener from Naples (Figure 5) and is different from European congeners from the English Channel and North Sea, as well as Indian Ocean populations. This finding confirms the hypothesis of Poss and Boschung (1996) that if Indian Ocean populations proved to represent a different species distinct from the European *B. lanceolatum*, the name *Branchiostoma haeckelii* would be available. Molecular studies of different specimens of *B. lanceolatum* from the Indian Ocean and European waters will help solve this problem.

The habitat preference of lancelets has been studied, but all reports have focused on the species of *Branchiostoma*, which is much larger than the other 2 genera. Globally, *Branchiostoma* and *Asymmetron* are found along the warm coasts of the world's oceans, while *Epigonichthys* is restricted to the coasts of the Indian Ocean and the western and central Pacific Ocean (Poss and Boschung, 1996; Lin et al., 2015). Locally, the sediment structure has been stated to determine the distribution of lancelets (Babu et al., 2013; Lin et al., 2015). *B. arabiae* and *A. lucayanum* in the western Persian Gulf have been found in areas where more than half of the sedimentary elements were larger than 0.5 mm (Futch and Dwinell, 1977). For the present contribution, no significant correlation between the distribution of lancelet populations and sediment structures was observed ($P > 0.3$).

The typical habitat for *B. lanceolatum* is sublittoral coarse-grained sediment in high-energy environments (amphioxus sand) (Futch and Dwinell, 1977; Rota et al., 2009). However, laboratory studies have suggested that the substratum might not be critical for lancelet culturing (Fuentes et al., 2007; Yasui et al., 2007). The small number of specimens collected (42 individuals) in the present study may be associated with sampling time. Webb (1971) suggested that amphioxus populations migrate between winter and summer. It also may be related to the environmental conditions of the sampling area (Chabahar Bay), where tidal currents are very low (Shirinmanesh and Chegini, 2014). In seas swept by strong tides, like the English Channel, the species can occupy large areas. In the Mediterranean, which is almost tideless, this type of habitat is mainly found in infralittoral deposits exposed to bottom currents (Rota et al., 2009).

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