

Research Article

Molecular phylogeny of bivalve families (Arcidae, Chamidae, Margaritidae, Ostreidae, Veneridae) in the Persian Gulf

Masaeli Sh.¹; Ghavam Mostafavi P.^{2*}; Hosseinzadeh Sahafi H.¹;
Tamadoni Jahromi S.³; Nabinejad A.⁴; Noaman V.⁵

Received: March 2016

Accepted: July 2016

Abstract

Bivalvia is one of the least studied classes of the Persian Gulf. A survey and molecular analysis was conducted to determine bivalve species diversity in the Persian Gulf. To the best of our knowledge, this is the first report of barcoding data on bivalves of the Persian Gulf. We examined 40 individuals representing 8 species, 6 genera and 5 families. We collected samples from Hengam, Larak and Qeshm Islands and Bandar Lengeh in Persian Gulf, Iran. After DNA extraction, mitochondrial 16S ribosomal DNA (16SrDNA) and Cytochrome Oxidase subunit I were amplified by polymerase chain reaction (PCR). Based on obtained 16SrDNA and COI gene sequences and maximum parsimony, neighbor joining and maximum likelihood trees of these genes there was no overlap between maximum Kimura 2- parameter distance among conspecifics. Most species formed agglutinate sequence units with a small amount of changes. Eventually, comparison of the 8 selected studied species with metadata from India, Brazil, Japan, China, and America exposed that these species in Persian Gulf are classified in sister clades with high bootstraps except *Pinctada*. Since there is not much work on bivalves identification in the Persian Gulf, larger sampling and more research is needed to investigate mollusc diversity in this area.

Keywords: Bivalvia, Persian Gulf, 16SrDNA, DNA sequencing, COI

1-Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization, Tehran, Iran.

2-Department of Marine Biology, Faculty of Natural Science and Environment, Science and Research Branch, Islamic Azad University, Tehran, Iran

3-Persian Gulf and Oman Sea Ecology Research Center, Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Abass, Iran.

4-Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization, (AREEO), Karaj, Iran .

5-Department of Animal Parasitic Disease, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), karaj, Iran

*Corresponding author's Email: Mostafavi_pa@srbiau.ac.ir

Introduction

One powerful use of molecular characters in systematics is to examine basal divergences for the groups which morphology could not identify in species level. Many scientists have used sequence data to investigate the relationships between animal phyla and the classes within them (Philippe *et al.*, 1994; Boore *et al.*, 1995; Halanych *et al.*, 1995; Winnepeninckx *et al.*, 1994). Of the protostome phyla, mollusca are among the most ancient and diverse classes. Living representatives of this phylum are usually divided into seven classes. Bivalves and gastropods are the most common classes in which most species are placed in the modern fauna (Halanych *et al.*, 1995). To date, molecular investigations of the molluscs have focused primarily on relationships of other phyla to Mollusca, demonstrated by the classes Gastropoda, Bivalvia, and the Polyplacophora, the latter being considered as basic (Canapa *et al.*, 2000). Bivalvia is a class of marine and freshwater molluscs that have laterally compressed bodies enclosed by a shell consisting of two hinged parts. The first bivalve fossils are found in Lower Cambrian sediments. But changes were not found in the fossil records both taxonomically and ecologically until the Lower Ordovician. These changes

continued non-stop throughout the Phanerozoic, with relatively small reductions during the end-Permian and end-Cretaceous extinction incidents. Gradually diversified and expanded molluscs species became dominant in most marine ecosystems (Waller, 1998).

Approximately, 20,000 species of marine bivalves exist worldwide, so this class offers a rich diversity of life (Pearse *et al.*, 1987). Following that, studies in the Persian Gulf continued by Melvill (1904), Smythe (1972) and Hosseinzadeh Sahafi *et al.* (2001). Most of these studies have focused on identification of bivalves.

According to the available literature, in the Arabian side of the Persian Gulf some of the molluscs including bivalves are studied by a small number of conchologists (Basson *et al.*, 1977).

Mollusca with almost 100,000 species organize the second largest animal phylum (Barnes *et al.*, 2009). Many mollusc species are used as bioindicators (Astani *et al.*, 2012; Saeedi, 2012). In terms of biodiversity and ecology, regardless of great variety in Persian Gulf molluscs, little is known about subtidal species. Some studies on molluscs of the Iranian coasts of Persian Gulf are conducted but are mostly centralized on specimens greater than about 5 mm in size, therefore small species are often neglected (Tadjallipour, 1974;

Rezai Marnani *et al.*, 1994; Hosseinzadeh Sahafi *et al.*, 2001; Nassaj *et al.*, 2010; Asgari *et al.*, 2012). Morphological studies are sometimes unable to perform reliable species identification; therefore molecular studies are necessary to be done (Ardura *et al.*, 2010).

In the past, species were identified primarily on the basis of morphology, a main problem here was that the border line between intra-specific variation and inter-specific morphological similarities were sometimes unclear (Kyle and Wilson, 2007). Accurate and relatively simple identification of species is based on the nucleotide sequence of widely used species-level, usually a short DNA fragment. DNA barcoding is introduced in a bold decision to overcome some of these shortcomings (Hebert *et al.*, 2003). Sometimes, short sequences of different markers such as a mitochondrial or nuclear target gene confirm the COI sequence data (Monaghan *et al.*, 2006; Sonnenberg *et al.*, 2007). These findings allow barcoding tools to be used not only for species identification, but also for biodiversity related issues. After two hundred years of morphology based taxonomic studies, species are now identified in a more effective molecular method (Waugh, 2007). In addition to

systematics, such protocols can potentially be applied to important subjects in ecology, conservation and issues related to economy (Armstrong and Ball, 2005; Markmann and Tautz, 2005; Savolainen *et al.*, 2005; Smith and Fisher, 2009). The phylogenetic reconstruction of a bivalve family (Pectinidae) based on mitochondrial 12S and 16SrRNA and nuclear histone H3 sequence study (Puslednik and Serb, 2008), was quite in contrast with the then morphological hypothesis of Pectinid evolution (Waller, 2006). An important difficulty in Pectinidae systematics remains the evolutionary relationships of the subfamilies and major tribes (Puslednik and Serb, 2008). Various tools, allowing phylogenetic investigation of RNA and protein sequences at the sequence structure level, are developed (Jow *et al.*, 2002; Smith *et al.*, 2004; Seibel *et al.*, 2006). DNA barcoding is introduced as a faster and more accessible method for species identification (Hebert *et al.*, 2004; Blaxter *et al.*, 2005; Kress *et al.*, 2005; Saunders, 2005). The feasibility of identifying species by DNA barcodes is related to the sequence variation among living groups. Bivalvia is a great and diverse class among animals, but little is known about molecular level variations within this class of molluscs (Smith *et al.*, 2004). It is

estimated that only half of the existing species of molluscs are studied (Coleman, 2007). Additionally, dominance of mollusc species is grossly underestimated in ecological surveys (Bouchet *et al.*, 2008). The incidence of secret species also makes identification more difficult. A quicker and easier way for species recognition would therefore be useful for molecular level identification of bivalves (Schander and Willassen, 2005).

Materials and methods

Marine molluscs were sampled from several sites in Bandar Abbas, Bandar Lengeh, Qeshm, Larak and Hengam Islands in Persian Gulf, during May and July 2015 by wading and scuba diving (Fig. 1). The stations and number of replicates are shown in Table 1.

All specimens were kept in refrigerator of agriculture research center. Species were identified based on conchology. DNA was extracted from living bivalves or frozen somatic tissue (Barber *et al.*, 2006). Three DNA extraction methods were used for DNA extracting. DNA was extracted with a DNeasy tissue extraction kit from CINAGEN and MBST kit, using the tissue protocol as recommended by the manufacture. A piece of tissue (150-200mg) was removed and placed in 850µl extraction buffer (Tris-HCl, EDTA, SDS, NaCl) and 5 µl proteinase K was added into a micro centrifuge tube, then incubated in water bath at 55°C for 12 hr (overnight). Then

Phenol chloroform isoamyl alcohol (25:24:1) was added into the tube and inverted 5-6 times (Selig *et al.*, 2008). After centrifuge, the same steps were repeated two times. NaCl and cold absolute ethanol was added and was kept in -20°C overnight. After spin and pouring out the ethanol, the DNA pellets stayed at the bottom of the tube under the following thermal profile: 94°C for 10 min; five cycles of 94°C for 45 s, 45°C for 45 s, 72°C for 45 s; 40 cycles of 94°C for 45 s, 51°C for 45 s, 72°C for 45 s; 72°C for 10 min (Gil, 2007). Each reaction mixture contained 25 µL of 10% Trehalose, 5 µL of 10-PCR buffer, 2 µL of 50 mM MgCl₂, 1 µL of 10mM dNTP mix, 0.5 µL of each primer (10pmol), 1-2 µL of DNA and 1-2 U *SmarTaq* DNA polymerase (Cinnagen Tehran, Iran). Those were also identified in our study. Deionized water was added to obtain a reaction volume of 50 µL (Gil, 2007). PCR products were visualized on 1% agarose gels. Sequencing was performed on samples that produced a single band.



Figure 1: Map of sampling locations in the Persian Gulf.

Table1: Sample sites, location and molecular identification of each specimen.

Code	Sampling Location	Map Coordinates	Sampling Date	Species Molecular Identification
A	Hengam Island	26°40'19.67°N- 55°51'42.16°E	24.06.2015	<i>Saccostrea cucullata</i>
B	Larak Island	26°53'16.36°N- 56°22'11.25°E	15.07.2015	<i>Crassostrea virginica</i>
C	Qeshm Island	26°54'38.85°N- 56°13'44.08°E	25.07.2015	<i>Paratapes undulatus</i>
D	Bandar Lengeh	26°33'26.95°N- 54°54'11.83°E	14.08.2015	<i>Pinctada imbricata radiata</i>
E	Bandar Lengeh	26°33'26.95°N- 54°54'11.83°E	14.08.2015	<i>Pinctada imbricata fucata</i>
F	Qeshm Island	26°54'38.85°N- 56°13'44.08°E	25.07.2015	<i>Barbatia obliquata</i>
G	Larak Island	26°53'16.36°N- 56°22'11.25°E	15.07.2015	<i>Chama</i> sp.
H	Hengam Island	26°40'19.67°N- 55°51'42.16°E	24.06.2015	<i>Saccostrea scyphophilla</i>

A band of almost 500 bp in addition to the band of expected size was observed in a few cases. In this condition, DNA in the 1000 bp band was removed using a gel extraction kit (Fermentas International Inc., ON, Canada) and sequenced. Sequencing was carried out using the ABI Big Dye terminator chemistry and an ABI prism 3700 instrument, Applied Biosystems, Korea (Wolf *et al.*, 2008). To further query existence of cryptic species flagged by COI sequences, 16srDNA fragments were used. For amplification of the 500 bp fragment encoding 16srRNA, the 16Sar-5 and 16Sbr-3 primers were used (Ivanova *et al.*, 2007).

Barcode sequence analysis

Chromatograms were first examined visually to avoid reading mistakes, sequencer software (Technelysium, Australia) was used. Sequences were then aligned in Mega 6.0 (Tamura *et al.*, 2007). Fasta alignment was checked onto the NCBI website. Computing of Kimura 2-parameter (K2P) distances (Kimura, 1980) and output of neighbor-joining (NJ) tree (Blaxter *et al.*, 2005) of K2P distances were done using the BOLD Management and Analysis System. Computing K2P intervals and output of NJ tree of 16srDNA sequences were done using MEGA 6.0 (Nassaj *et al.*, 2010).

Meta data analysis were done using BOLD management and analysis system to contrast 16s sequences of selected species of the present study with conspecifics from Australia, India,

China and South Africa. Two types of dividing values were calculated. A common global divergence value was calculated for each species which was the average of all pairwise intervals of sequences depending to the identical species regardless of location of origin. Territorial divergences were computed as mean interval of all con special sequences from the same position (Selig *et al.*, 2008). A number of experimental molecular operational taxonomic units (MOTUs) were derived based on patterns of sequence clustering and grade of variegate for each nominal species. NJ tree covering for all sequences was built. Finally degrees of observed divergence at different taxonomic levels of samples of the present study were compared with those of BOLD project encompassing broader geographical ranges (Blaxter *et al.*, 2005).

Results

In this research 5 bivalve families including Arcidae, Chamidae, Margaritidae, Ostreidae and Veneridae were studied. Also intra specific variation and inter specific divergence in six genera of marine bivalves was investigated. 16srRNA from species *Pinctada imbricata radiata*, *Pinctada imbricata fucata* (family Margaritidae), *Paratapes undulatus* (family Veneridae), *Barbatia obliquata* (family Arcidae), *Chama sp.* (family Chamidae), *Saccostrea cucullata*, *Saccostrea scyphophilla*, *Crassostrea virginica* (family Ostreidae) was

sequenced to compare divergence within and between the species. The resulting phylogenetic tree for the

aligned mitochondrial 16s ribosomal DNA sequences is shown in "Figure 2".

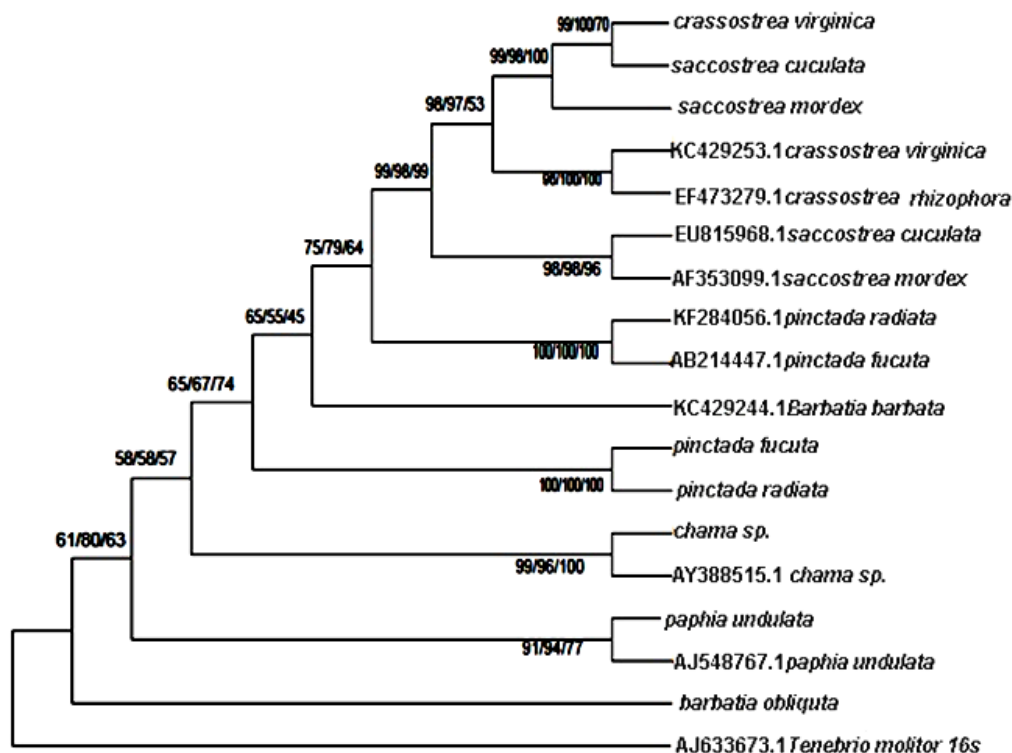


Figure 2: Neighbor joining, maximum parsimony and maximum likelihood tree for specimens in the Persian Gulf from mitochondrial 16S ribosomal DNA sequences. First numbers on left show maximum likelihood, second numbers in middle show neighbor joining, third numbers on right show maximum parsimony.

In the current study maximum parsimony (MP), neighbor joining (NJ) and maximum likelihood (ML) trees based on 16srRNA data were used for the families of bivalvia. In this study *Paratapes undulatus* from Persian Gulf with *Paratapes undulatus* from Northern Atlantic Ocean were classified in sister groups (MP:77 ,NJ:94,ML:91). The Iranian species of *Chama* sp. with (MP: 100 bootstrap, NJ: 96, ML: 99) was similar to the same species from the Western North Atlantic and both were classified in the same sister group and classified in separate clade with

high bootstrap from *Pinctada* clade and classified in almost similar sister groups. Iranian *Pinctada imbricata fucata* from Bandar Lengeh were totally different from *Pinctada imbricata fucata* from Australia and Japan, so they were classified in two different clades. In this research *Crassostrea virginica* and *Saccostrea cucullata* of Persian Gulf (MP: 70, NJ: 100, ML: 99) were classified in one sister clade. They were similar to *Saccostrea scyphophilla* from Persian Gulf with MP: 100, NJ: 98, ML: 99. These species were similar to the same species from Brazil and

were relatively close to each other; so they were located in the same sister clade. In current study, *Saccostrea cucullata* from South China Sea with *Saccostrea scyphophilla* from the Indo-

West Pacific were classified in a sister groups (MP: 96, NJ: 98, ML: 98). High quality photographs of bivalves are shown in Figure 3.

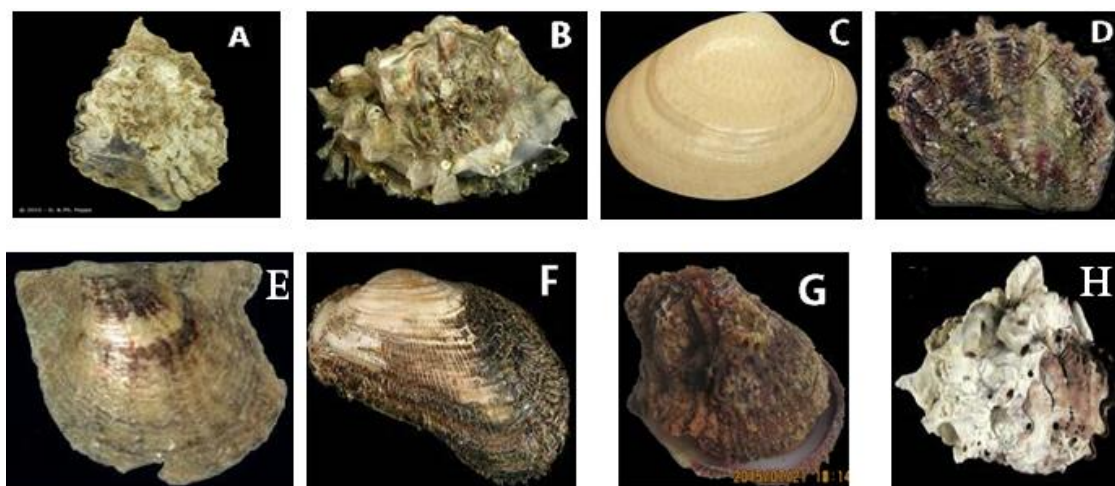


Figure 3: *In situ* images of bivalve specimens collected in this study; A: *S. cucullata*, B: *C. virginica*, C: *P. undulatus*, D: *P. imbricata radiata*, E: *P. imbricata fucata*, F: *Barbatia obliquata*, G: *Chama sp.*, H: *S. scyphophilla*.

Discussion

Reduction in aquatic resources in many parts of the world stresses the importance of fisheries and aquaculture resources management. Before any kind of action, study and determining the genetic structure of valuable species via molecular methods is necessary. This is very important in sustainable utilization programs of marine reserves, aquatic industry and breeding programs (Lin *et al.*, 2002). The data related to DNA sequence is used to determine ancestral relations in development of most animals. These data are less influenced by selection index and phylogenetic relations; as a result the real genetic structure is demonstrated (Saavedra and Peña, 2006).

The reason for this difference can be special condition of the geographical area, i.e. existence of estuaries and mangrove forests. These factors can affect genetic diversity of the area. For example mangrove forests are important nursery and feeding areas for larva of crustaceans and molluscs (Barber *et al.*, 2006). One of the main causes of isolation of species is geographical distance which can affect the genetic distance due to physical and natural barriers which can reduce genetic transfer (Bisby *et al.*, 2010). It is reported that animal immigration behavior is an important factor on the transfer of gene and change in population structure. Mollusca often select nests in different areas in their life cycle and for the completion of

their life cycle have to move to different areas (Lin *et al.*, 2002). Geographic movement of bivalves into each area is related to the environmental conditions, especially due to variability of salinity, or other important factors such as the seabed and hydrological conditions, existence of estuaries and also dispersion of the bivalves which can have an effect on gene transfer (Weersing and Toonen, 2009).

In conclusion, results obtained from the present study, as the first study on diversity and genetic structure of bivalvia in coastal waters of Hormozgan using mt DNA sequencing 16srRNA, demonstrated that molecular markers, especially mt marker, can be a suitable parameter for separation of aquatic populations such as bivalvia. These kinds of markers can be used in the study of species and related common ancestor (Puslednik and Serb, 2008). In this study haplotype diversity, in a limited extent, and high nucleotide diversity is found in species of different studied areas (Selig *et al.*, 2008).

In order to complete this research further studies like application of mitochondrial gene with other methods such as microsatellite, RFLP, AFLP are needed. Because of intensive reduction of populations and due to reduced genetic diversity, evaluation of this study and the application of results of further studies can help in rebuilding the population of these valuable species (Asgari *et al.*, 2012).

Acknowledgement

The authors are grateful to Dr. Asgharian, University of Southern California for his useful advice and his answers to all our questions during this study and we also thank Majid Afkhami and Hossein Rameshi for collecting the samples.

References

- Ardura, A., Linde, A.R., Moreira, J.C. and Garcia-Vazquez, E., 2010.** DNA barcoding for conservation and management of Amazonian commercial fish. *Biological Conservation*, 143(6), 1438-1443.
- Armstrong, K.F. and Ball, S.L., 2005.** DNA barcodes for biosecurity: invasive species identification. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 360(1462), 1813-1823.
- Asgari, M., Yekta, F.A. and Izadi, S., 2012.** Dominant intertidal crustacean and gastropod species in Qeshm Island, Iran, northern Persian Gulf. *Marine Biodiversity Records*, 5(3), e87.
- Astani, M., Vosoughi, A.R., Salimi, L. and Ebrahimi, M., 2012.** Comparative study of heavy metal (Cd, Fe, Mn, and Ni) concentrations in soft tissue of gastropod *Thais mutabilis* and sediments from intertidal zone of Bandar Abbas. *Advances in Environmental Biology*, 6(1), 319-327.

- Barber, P.H., Erdmann, M.V. and Palumbi, S.R., 2006.** Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. *Evolution*, 60(9), 1825-1839.
- Barnes, R.S.K., Calow, P.P., Olive, P.J.W., Golding, D.W. and Spicer, J.I., 2009.** *The invertebrates: a synthesis*, 3rd edition. John Wiley and Sons, USA.
- Basson, P., Burchard, J., Hardy, J. and Price, A., 1977.** Biotopes of the western Persian Gulf: Marine Life. 284P.
- Bisby, F.A., Roskov, Y.R., Orrell, T.M., Nicolson, D., Paginawan, L.E., Bailly, N., Kirk, P.M., Borrgoin, T. and Baillargeon, G., eds 2010.** Species 2000 and ITIS Catalogue of Life: 2010. Annual Checklist. Digital resource at [http://www.catalogueoflife.org/annual-checklist/2010.Species 2000](http://www.catalogueoflife.org/annual-checklist/2010.Species%2000): Reading, UK.
- Blaxter, M., Mann, J., Chapman, T., Thomas, F., Whitton, C., Floyd, R. and Abebe, E., 2005.** Defining operational taxonomic units using DNA barcode data. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 360(1462), 1935-1943.
- Boore, J.L., Collins, T.M., Stanton, D., Daehler, L.L. and Brown, W.M., 1995.** Deducing the pattern of arthropod phylogeny from mitochondrial-DNA rearrangements. *Nature*, 1995 Jul 13;376(6536), 163-165. doi: 10.1038/376163a0.
- Bouchet, P., Héros, V., Lozouet, P. and Maestrati, P., 2008.** A quarter-century of deep-sea malacological exploration in the South and West Pacific: where do we stand? How far to go?, in: HÉROS V. *et al.* eds, *Tropical deep-sea Benthos*, 25, Mémoires du Muséum national d'Histoire naturelle (1993), 196, 9-40.
- Canapa, A., Barucca, M., Marinelli, A. and Olmo, E., 2000.** Molecular data from the 16S rRNA gene for the phylogeny of Pectinidae (Mollusca: Bivalvia). *Journal of Molecular Evolution*, 50, 93-97.
- Coleman, A.W., 2007.** Pan-eukaryote ITS2 homologies revealed by RNA secondary structure. *Nucleic Acids Research*, 35(10), 3322-3329.
- Gil L.A. 2007.** PCR-based methods for fish and fishery products authentication. *Trends in Food Science and Technology*, 18(11), 558-566.
- Halanych, K.M., Bacheller, J.D., Aguinaldo, A.M., Liva, S.M., Hillis, D.M. and Lake, J.A., 1995.** Evidence from 18S ribosomal DNA that the lophophorates are protostome animals. *Science*, 267(5204), 1641-1643.
- Hebert, P.D.N., Cywinska, A., Ball, S.L. and deWaard J.R., 2003.** Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B:*

- Biological Sciences*, 270(1512), 313-321.
- Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S. and Francis, C.M., 2004.** Identification of birds through DNA barcodes. *PLoS Biol*, 2(10): e312. <https://doi/10.1371/journal.pbio.0020312>.
- Hosseinzadeh sahafi, H., Daghoghi, B. and Rameshi, H., 2001.** Atlas of the Persian Gulf molluscs. Iranian Fisheries Research Organization, Tehran, Iran.
- Ivanova, N.V., Zemlak, T.S., Hanner, R.H. and Hebert, P.D.N., 2007.** Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, 7(4), 544-548.
- Jow, H., Hudelot, C., Rattray, M. and Higgs, P.G., 2002.** Bayesian phylogenetics using an RNA substitution model applied to early mammalian evolution. *Molecular Biology and Evolution*, 19(9), 1591-1601.
- Kimura, M., 1980.** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular evolution*, 16(2), 111-120.
- Kress, W.J., Wurdack, K.J., Zimmer, E.A., Weigt, L.A. and Janzen, D.H., 2005.** Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America*, 102(23), 8369-8374.
- Kyle, C.J. and Wilson, C.C., 2007.** Mitochondrial DNA identification of game and harvested freshwater fish species. *Forensic Science International*, 166(1), 68-76.
- Lin, Y.S., Poh, Y.P., Lin, S.M. and Tzeng, C.S., 2002.** Molecular techniques to identify freshwater eels: RFLP analyses of PCR-amplified DNA fragments and allele-specific PCR from mitochondrial DNA. *Zoological Studies*, 41(4), 421-430.
- Markmann, M. and Tautz, D., 2005.** Reverse taxonomy: an approach towards determining the diversity of meiobenthic organisms based on ribosomal RNA signature sequences. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 360(1462), 1917-1924.
- Melvili, J.C., 1904.** Descriptions of twenty three species of Gastropoda from the Persian Gulf, Gulf of Oman, and Arabian Sea, Dredged by Mr. FW Townsend, of the Indoeuropean Telegraph Service, in 1903. *Journal of Molluscan Studies*, 6(1): 51-60.
- Monaghan, M.T., Balke, M., Pons, J. and Vogler, A.P. 2006.** Beyond barcodes: complex DNA taxonomy of a South Pacific Island radiation. *Proceedings of the Royal Society of London B: Biological Sciences*, 273(1588), 887-893.
- Nassaj, S.M.S., Nabavi, S.M.B., Yavari, V., Savari, A. and Maryamabadi, A., 2010.** Species

- Diversity of Macrobenthic Communities in Salakh Region, Qeshm Island, Iran. *World Journal of Fish and Marine Sciences*, 2(6), 539-544.
- Pearse, V., Pearse, J., Buchsbaum, M. and Buchsbaum, R., 1987.** *Living invertebrates*, Blackwell Scientific Publications, USA.
- Philippe, H., Chenail, A. and Adoutte, A., 1994.** Can the Cambrian explosion be inferred through molecular phylogeny? *Development for Advances in Developmental Biology and Stem Cells*, 1994, 15-25.
- Puslednik, L. and Serb, J.M., 2008.** Molecular phylogenetics of the Pectinidae (Mollusca: Bivalvia) and effect of increased taxon sampling and outgroup selection on tree topology. *Molecular Phylogenetics and Evolution*, 48(3), 1178-1188.
- Rezai Marnani, H., Sanjabi B., Rameshi, H., Ranai Rad, E., Dianat S. and Ghanbarzadeh H., 1994.** Distribution of benthic molluscs in shallow waters around some Iranian Islands in the Persian Gulf. *Iranian Fisheries Research Organization, Research Report in Persian, Tehran, Iran*.
- Saavedra, C. and Peña, J.B., 2006.** Phylogenetics of American scallops (Bivalvia: Pectinidae) based on partial 16S and 12S ribosomal RNA gene sequences. *Marine Biology*, 150(1), 111-119.
- Saedi, H., 2012.** Availability of Venerid Clam, *Amiantis umbonella* as potential metal bioindicator in Bandar Abbas coast, the Persian Gulf. *The Egyptian Journal of Aquatic Research*, 38(2), 93-103.
- Saunders, G.W., 2005.** Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 360(1462), 1879-1888.
- Savolainen, V., Cowan, R.S., Vogler, A.P., Roderick, G.K. and Lane, R., 2005.** Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 360(1462), 1805-1811.
- Schander, C. and Willassen, E., 2005.** What can biological barcoding do for marine biology? *Marine Biology Research*, 1(1), 79-83.
- Seibel, P.N., Muller, T., Dandekar, T., Schultz, J. and Wolf, M., 2006.** Software 4SALE - a tool for synchronous RNA sequence and secondary structure alignment and editing. *BMC Bioinformatics*, 7(1), 498, <http://www.biomedcentral.com/1471-2105/7/498>.
- Selig, C., Wolf, M., Muller, T., Dandekar, T. and Schultz, J., 2008.** The ITS2 Database II: homology modelling RNA structure for molecular systematics. *Nucleic Acids Research*, 36 (Database Issue): D377-D380.

- Smith, A.D., Lui, T.W.H. and Tillier, E.R.M., 2004.** Empirical models for substitution in ribosomal RNA. *Molecular Biology and Evolution*, 21(3), 419-427.
- Smith, M.A. and Fisher, B.L., 2009.** Invasions, DNA barcodes, and rapid biodiversity assessment using ants of Mauritius. *Frontiers in Zoology*, 6(31), doi: 10.1186/1742-9994-6-31.
- Smythe, K.R., 1972.** Marine Mollusca from Bahrain Island, Persian Gulf. *J. Conch*, 27(7), 491-496.
- Sonnenberg, R., Nolte, A.W. and Tautz, D., 2007.** An evaluation of LSU rDNA D1-D2 sequences for their use in species identification. *Frontiers in Zoology*, 4(6) Doi: 10.1186;1742-9994-4-6.
- Tadjallipour, M., 1974.** *Contribution à l'étude de la systématique et de la répartition des mollusques des côtes iraniennes du Golfe Persique*, Académie de Montpellier, Univ. des Sciences et Techniques du Languedoc.
- Tamura, K., Dudley, J., Nei, M. and Kumar, S., 2007.** MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24(8), 1596-1599.
- Waller, T.R., 1998.** Origin of the molluscan class Bivalvia and a phylogeny of major groups. *Bivalves: An eon of Evolution*, 1, 5.
- Waller, T.R., 2006.** Chapter 1 New phylogenies of the Pectinidae (Mollusca: Bivalvia): reconciling morphological and molecular approaches. *Developments in Aquaculture and Fisheries Science*, 35, 1-44.
- Waugh, J., 2007.** DNA barcoding in animal species: progress, potential and pitfalls. *BioEssays*, 29(2), 188-197.
- Weersing, K.A. and Toonen, R.J., 2007.** Population genetics, larval dispersal, and demographic connectivity in marine systems. *Marine Ecology Progress Series*, 393, 1-12.
- Winnepenninckx, B., Backeljau, T. and De Wachter, R., 1994.** Small ribosomal subunit RNA and the phylogeny of Mollusca. *The Nautilus, Supplement*, 2, 98-110.
- Wof, M., Ruderisch, B., Dandekar, T., Schultz, J. and Muller, T., 2008.** ProfDistS: (profile-) distance based phylogeny on sequence--structure alignments. *Bioinformatics*, 24(20), 2401-2402.