




# Adding the Molecular Diversity Information of the Common Fouling Barnacle *Amphibalanus amphitrite* (Darwin, 1854) (Crustacea: Cirripedia) from the Persian Gulf and Gulf of Oman to the Global Diversity Pattern

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Received 8 March 2022 / Accepted 18 January 2023 / Published 25 April 2023

Communicated by Benny K.K. Chan

The balanid barnacle, *Amphibalanus amphitrite*, is known as one of the most common fouling species in the world. A phylogenetic study using material from around the world recovered three distinct clades for this species. Material from the Persian Gulf (PG) and the Gulf of Oman (GO) were not included in that survey. In the present study, we aimed to assess the genetic diversity of the balanid barnacles of these two gulfs and to evaluate their phylogeography. In total, 94 *COI* DNA sequences were obtained from the PG and the GO material. Most of these sequences clustered into a single clade, corresponding to clade I of the previous global study. However, two sequences, one from the PG and one from the GO, fell into a separate clade corresponding to clade III of the previous study. These two gulfs share some common haplotypes, but host several unique ones that are separated from the most common haplotype mainly by a single mutation. Based on various indices, the genetic diversity of the PG material was higher than that of the GO. Low values of  $\Phi_{ST}$  show a regular gene flow among the stations and the two gulfs. The Bayesian skyline plots and the mismatch distribution analyses both showed signs of a recent population expansion in the PG and the GO. We also modeled the potential distribution areas for *A. amphitrite* to reveal the separate suitable habitats for the clades. The current phylogeographic status and genetic diversity of *A. amphitrite* in the PG and GO appears to have been shaped by both historical events and recent human activities.

**Key words:** Barnacle, *Amphibalanus amphitrite*, Phylogeography, Population Genetic, Gene flow.

## BACKGROUND

Knowledge of the distribution patterns of marine species is important for understanding their ecology and biogeography (Robinson et al. 2011). These patterns may be changed by human-mediated dispersal on a global scale (Gallardo et al. 2015), leading to the possible homogenization of the world's biota, including those associated with plate tectonics or glacial cycles (Brown and Lomolino 1998; Bank et al. 2015). In coastal and estuarine habitats, most structural and functional modifications of communities in the recent decades were caused by the introduction and establishment of non-native species (Ruiz et al. 1999; Levin and Crooks 2011). By creating movement corridors, transport networks have promoted the dispersal of non-native species to new regions and thus caused stress to native species by altering their habitats (Hulme 2009). International marine shipping networks, which account for 90% of world trade, can catalyze the spreading of many marine organisms and the establishment of their populations far beyond their native home range (Hulme 2009; IMO 2021).

Biofouling significantly promotes the dispersal of organisms, including organisms with low inherent dispersal capacity, and the consequence is increased gene flow between populations, making a homogenized genetic structure (Olden et al. 2004; Crispo et al. 2011). For instance, despite limited larval dispersal of polychaetes *Hydroides elegans* (Haswell 1883), microsatellites studies revealed a high level of genetic similarity between seven sub-populations from the Atlantic, Pacific, and Indian Oceans and the Mediterranean Sea (Pettengill et al. 2007). Although this implies that few migrants may be sufficient to homogenize local genetic variation (Hartl and Clark 2007; Hellberg 2009; Pannacciulli et al. 2009 2017), genetic differentiation sometimes remains even for species with high dispersal capacity over long distances. The maintenance of distinct genetic profiles of the cosmopolitan marine planktonic diatom *Pseudo-nitzschia pungens* (Casteleyn et al. 2010), the pedunculate and acorn barnacles *Pollicipes* sp. (Quinteiro et al. 2007) and *Tetraclita* sp. (Tsang et al. 2012; Reynolds et al. 2014), may have been due to patterns of oceanic currents, natural selection, geographic distance or historical events, respectively.

Barnacles are highly conspicuous for having a wide distribution caused largely by ship hull fouling or ballast water (Carlton et al. 2011; Gollasch and David 2011). These animals settle on and colonize diverse types of hard substrates including rocks, man-made marine structures and sailing vessels. Some species are “sessile voyagers” on turtles, sea snakes,

or whales (Kim et al. 2020; Dreyer et al. 2020) and can be found on floating and drifting objects such as timber, cuttlefish bones, bottles, cans, and light plastic sheets. Barnacles will affect economic loss by causing increased fuel consumption of vessels and by damaging submarine structures (Holm 2012). The widespread barnacle species, *Amphibalanus* (= *Balanus*) *amphitrite* (Darwin 1854) from the family Balanidae (Pitombo 2004; see Chan et al. 2021 for the latest barnacle classification) is commonly used as a model organism for ecological studies (Clare et al. 1994; Holm et al. 2000; Khandeparker et al. 2002; Lagersson and Høeg 2002; Leslie et al. 2005; Wong et al. 2011; Ip et al. 2021; Campanati et al. 2016), and antifouling assays (Rittschof et al. 1992; Hirota et al. 1996; Hellio et al. 2004; Maréchal and Hellio 2011). This barnacle is a common intertidal fouling species in tropical and warm temperate waters worldwide (Henry and McLaughlin 1975; Chen et al. 2014), including the Persian Gulf (PG) and the Gulf of Oman (GO) (Shahdadi et al. 2014). Its cosmopolitan distribution seems to be largely related to anthropogenic activities, especially recently increased shipping traffic affecting transmission of larvae and adult barnacles globally (Seebens et al. 2013; Banks et al. 2015). This transmission causes changes in the genetic diversity of populations. The changes in genetic diversity can be investigated by various approaches including DNA barcoding.

DNA barcoding is a useful approach in uncovering genetic diversity, population structures and phylogenetic patterns (DeSalle and Goldstein 2019; Kim et al. 2019). This method can be used to reveal the genetic structure of non-indigenous species and their evolutionary potential and to assist with the management of introduced/invasive species (Strayer et al. 2006). Using the mitochondrial *COI* marker, Chen et al. (2014) investigated genetic differentiation in *A. amphitrite* in various regions of the world. They found three clades for the examined specimens including two widely distributed clades and one with only two representatives from North Carolina (USA) and Singapore (see Table 1 in Chen et al. 2014). While most sampling sites hosted members of only one clade, some localities harbored members of two clades and had higher genetic diversity (see Fig. 1 and Table 1 in Chen et al. 2014). This sympatry was assigned to both historical events and recent human activities (Chen et al. 2014). However, a very important part of the species global distribution, the PG and the GO, were not included in their study.

The GO is the northwestern extension of the Arabian Sea (Indian Ocean) and expectedly shares common biota with that sea (Owfi et al. 2016). In contrast, the PG is a semi-closed and relatively young sea connected to the GO and Arabian Sea through the

Strait of Hormoz (Lambeck 1996). Until the early Holocene, the PG basin was almost dry (Sheppard et al. 2010). Its current marine community started to form only around 15 ka BP, receiving its biota from

the northwestern part of the Indian Ocean (Teller et al. 2000). The PG reached its current sea level about 6–8 ka BP (Lambeck 1996). Currently, the PG is among the major destinations of oil tankers from all over the world

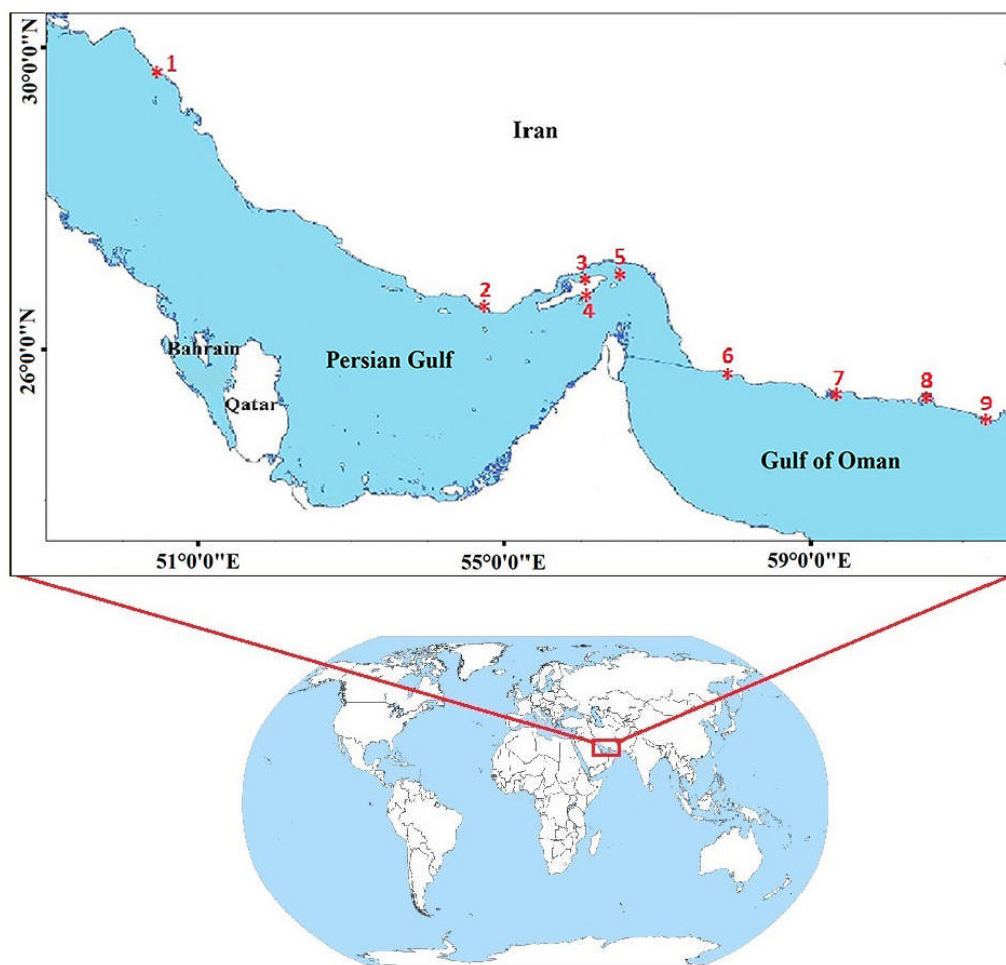


Fig. 1. Map of the sampling localities.

Table 1. Sampling localities and summary of statistics of genetic variability for *Amphibalanus amphitrite*

#	Location	Latitude	Longitude	N	Nh	Np	h	$\pi$
1	Bushehr Province, Bandar Genaveh (GV)	29°33'17"N	50°29'08"E	3	-	-	-	-
2	Hormozgan Province, Bandar Charak (BC)	26°43'32"N	54°16'51"E	2	-	-	-	-
3	Qeshm Island, Toola and Hamoon harbors (TH)	26°55'01"N	55°56'13"E	15	11	17	0.9	0.0047
4	Qeshm Island, Messen and Kandaroo (MK)	26°41'53"N	55°54'49"E	14	10	22	0.89	0.0091
5	Hormozgan Province, Hormoz Island (HR)	27°02'33"N	56°29'38"E	2	-	-	-	-
<b>Persian Gulf (PG) (total)</b>				36	24	46	0.93	0.0077
6	Hormozgan Province, Bandar Jask (JS)	25°42'18"N	57°47'07"E	10	7	8	0.87	0.0037
7	Sistan and Baluchestan Province, Jod (JD)	25°27'04"N	59°30'17"E	5	-	-	-	-
8	Sistan and Baluchestan Province, Chabahar, Tis, Chabahar (CH)	25°21'12"N	60°36'08"E	30	19	25	0.89	0.0058
9	Sistan and Baluchestan Province, Gwatr Bay (GU)	25°10'34"N	61°36'14"E	13	6	35	0.92	0.0102
<b>Gulf of Oman (GO) (total)</b>				58	35	63	0.87	0.0066

N, sample size; Nh, number of haplotypes; Np, number of polymorphic sites; h, haplotype diversity;  $\pi$ , nucleotide diversity.

with about 53,000 visits annually by oil transporting ships (Al-Yamani et al. 2015). Therefore, the present biota of the PG and its genetic composition are expected to reflect natural historic events as well as anthropogenic activities (Spalding et al. 2007). To test this hypothesis and to fill the void of knowledge pertaining to the PG and the GO, the present work aimed to study the genetic variation within the populations of *A. amphitrite* from these two gulfs using the mitochondrial *COI* gene. The study also investigated the genetic diversity within each Gulf and compared the diversity indices with those of other populations globally.

## MATERIALS AND METHODS

### Sample collection

Specimens of *Amphibalanus amphitrite* were collected from nine locations along the PG and the GO (Table 1, Fig. 1). In total, 94 individuals were collected from both artificial substrata (*e.g.*, human-made structures, piers, small vessel hulls and floating objects) and natural habitats (*e.g.*, intertidal rocks, mollusk shells, crab carapaces and mangrove trunks). The specimens were kept in 96% or absolute ethanol immediately upon collection and transferred to the Molecular Systematics Laboratory at the University of Tehran for molecular analysis.

### DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscles using the salt precipitation method (Katouzian et al. 2016). A 576-bp fragment of the cytochrome *c* oxidase subunit *I* gene (*COI*) was amplified by the polymerase chain reaction using primer pairs LCO1490-JJ and HCO2198-JJ (Astrin and Stüben 2008) as described in Chen et al. (2014). The PCR products were outsourced for sequencing to LGC Genomics GmbH (Berlin, Germany) and Macrogen Europe, Amsterdam using the same forward primers. Sequences were proofread via Chromas Lite (v. 2.1.1) (Technelysium Pty Ltd, Queensland, Australia). Sequences of all unique haplotypes/genotypes were submitted to GenBank (<http://www.ncbi.nlm.nih.gov>) and are available under accession numbers (OQ119797–OQ119890). *COI* sequences of *A. amphitrite* from previous study (Chen et al. 2014) were also retrieved from GenBank and included in the analyses (accession numbers KC138445, KM211362–KM211497). *Amphibalanus reticulatus*, *A. variegatus* and *Balanus glandula* were used as outgroups (GenBank accession numbers JQ035518.1, JQ035522.1 and KU204282.1, respectively).

### Sequence data analyses

Sequences were aligned using Clustal W (Thompson et al. 1994; Villesen 2007) implemented in BioEdit 7.0.5 (Hall 1999). The ML tree was obtained using raxmlGUI, v. 1.3 (Silvestro and Michalak 2012) with 1000 bootstrap pseudoreplicates. The selected evolutionary model was GTR+G (Rodriguez et al. 1990). Two maximum parsimony haplotype networks (Templeton et al. 1992) were constructed with PopART (Leigh and Bryant 2015), one for clade *I* at the global level (Chen et al. 2014) taken from the tree, and one for the sequences of the present study.

We calculated the distribution of pairwise differences (*i.e.*, mismatch distribution; Rogers and Harpending 1992) to trace population size change in DnaSP v.5.10 (Librado and Rozas 2009).

To assess how mtDNA effective population size changed through time, we analyzed historical demography using coalescent based Bayesian Skyline Plot (BSP) in BEAST 2.4.7 (Bouckaert et al. 2014). We selected GTR+G as the best model of nucleotide substitution and adopted a substitution rate of 3.1% per MY for *COI* (according to Tsang et al. 2008). We set a strict clock model as prior and ran three independent MCMC analyses with 60 million generations, sampling every 6,000 steps, to verify the consistency of the results. The initial 25% of the samples were discarded as burn-in. The convergence of all parameters was tested and BSP produced in Tracer 1.6 (Rambaut et al. 2014).

Standard genetic indices were calculated to determine the genetic diversity within the species based on *COI* sequences. The haplotype diversity (*h*), nucleotide diversity ( $\pi$ ), number of polymorphic sites (*N<sub>p</sub>*) and number of haplotypes (*N<sub>h</sub>*) were calculated using DnaSP v.5 (Librado and Rozas 2009) for each local population and for the complete dataset of each Gulf. Overall mean *p*-distance was analyzed with MEGA v.6 (Tamura et al. 2013). We computed pairwise  $\Phi_{ST}$  with 1,000 permutations in Arlequin v.3.5.2.2 (Excoffier and Lischer 2010) to investigate population differentiation patterns among local populations (only populations with  $n \geq 8$  were included).

### Species distribution modelling

To construct the distribution modelling of *A. amphitrite*, we included localities of specimens of clade *I* and *III* used in worldwide phylogenetic analysis compiled from field observations and available data based on Chan et al. (2014). The ocean climate layers were downloaded from Bio-ORACLE (Ocean Rasters for Analysis of Climate and Environment) data set

(Tyberghein et al. 2012). Variables were selected based on their ecological meaningfulness and contribution rate species in distribution model. To avoid the effect of high correlation among layers, we first examined all layers using OpenModeller 1.0.7 (De Souza Muñoz et al. 2011) and then used the Pearson correlation method to obtain higher correlative layers ( $> 0.7$ ). Layers with lower correlation ( $< 0.7$ ) were selected for further analyses: Temperature (Mean and Range), Phytoplankton Mean, Salinity (Mean and Range), Current Velocity Mean, Min., Max., Ltmax, dissolved oxygen range and Phosphate Mean. All the data were downloaded as raster format with a 5-arc-minute from Bio-ORACLE data set (Tyberghein et al. 2012).

## RESULTS

Five out of the nine sampling localities were located within the PG, and four were located in the GO (Fig. 1, Table 1). Here, we postulated two main populations, namely the PG and GO populations. 36 specimens from the PG and 58 specimens from the GO were studied. Each of the 94 *COI* sequences contained approximately 576 base pairs (bp), and none of these contained a stop codon. In total, 53 polymorphic sites were found. 136 additional sequences of previous studies on the species were obtained from GenBank and included into the dataset for tree and haplotype network construction.

### Sequence data analyses

In the ML tree, three distinct and well separated clades were recovered (Fig. 2). Sequences of the present study (green circles) are well distributed in the tree, with representatives in two clades. Most sequences were placed in clade *I* and only two specimens of the present study were significantly different from the others (Fig. 2) namely one from the PG (Kandaloo) and one from the GO (Gwatr) (Table 1). The phylogenetic tree showed 186 sequences, including 92 sequences of the present study placed in clade *I*. The haplotype network constructed for the clade *I* recovered 101 haplotypes, with 36 unique haplotypes for the PG and GO (Fig. 3).

In the haplotype network (Fig. 4) of the Iranian samples (clade *I*), some haplotypes were present in only one of the two gulfs, while other haplotypes were found in both gulfs. Except for some outlying haplotypes, the network is nearly star-shaped and most of the haplotypes remained close to the main haplotype with only one mutation step. However, the haplotype network (Fig. 4) showed no clear patterns of isolation between specimens from the two gulfs.

The mismatch distributions showed a clear unimodal pattern in the populations of the PG and the GO. The distribution of pairwise haplotype differences was skewed to the left (Fig. 5).

An exponential growth in effective population size, shown in Bayesian Skyline Plots (Fig. 6), was consistent with the results of the mismatch distribution analysis. However, the BSP inferred slightly different timings of expansion from the mismatch analyses. The two populations shared a broadly similar timing of demographic growth, which began at 200 ka BP (Fig. 6). The number of sequences available for the GO produced a plot showing a tendency to demographic expansion, truncated at 200 ka BP.

Diversity indices were calculated for material from the sampling localities with 10 or more sequences, as well as for each gulf in total. The analyses showed that the specimens from both gulfs had high genetic diversity, and that the diversity within the PG was higher than the diversity within the GO (*i.e.*, haplotype diversity in the PG = 0.93; the GO = 0.87). The individual sampling localities of both gulfs also showed high genetic diversity (Table 1).

The  $\Phi_{ST}$  value was calculated only between populations with a sample size  $> 10$  (Table 2). Most pairwise  $\Phi_{ST}$  values between populations were small, and only two pairwise comparisons were significant ( $p < 0.05$ ) (Table 2).

### Species distribution modelling

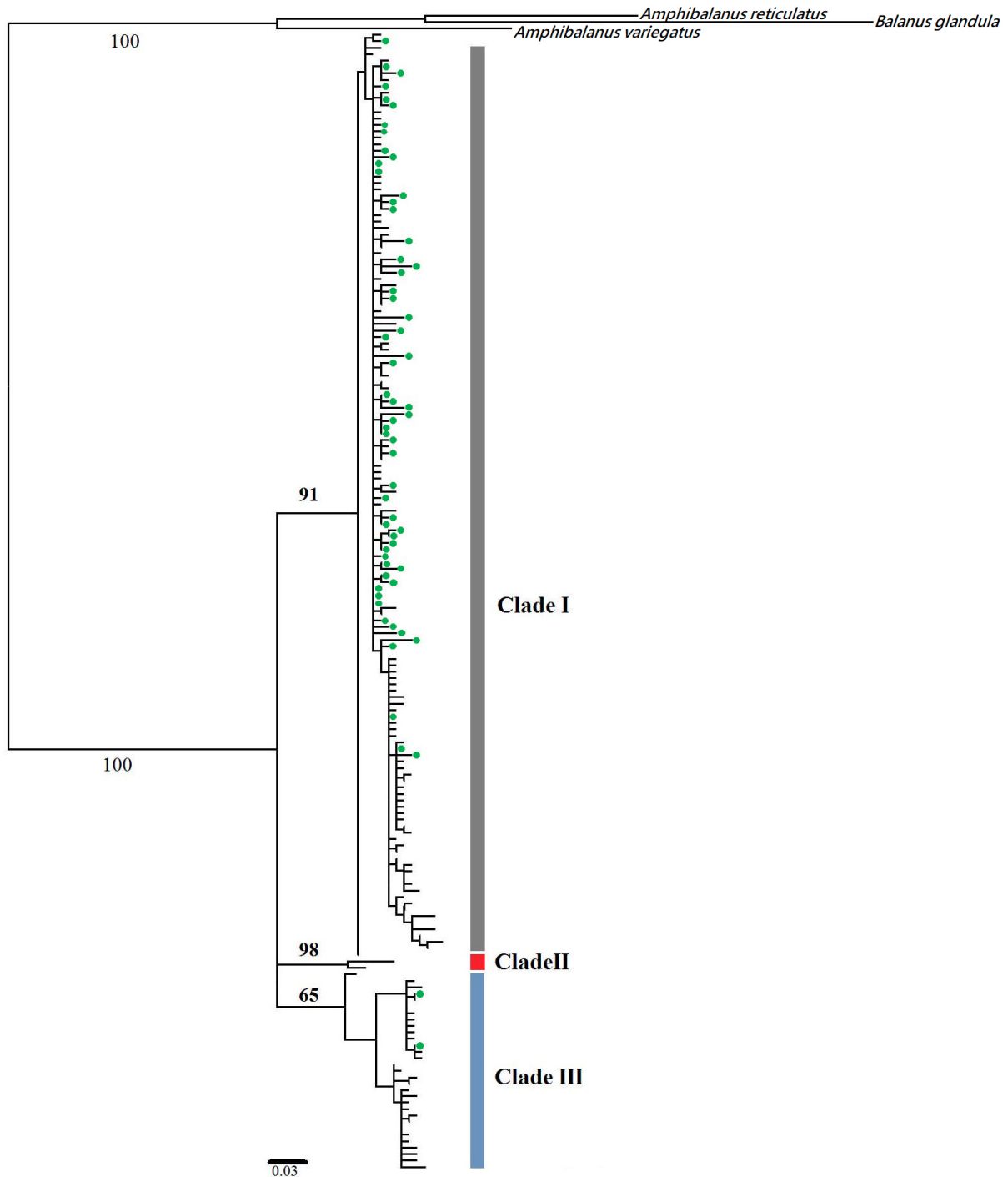
All models were run in ten replicates for the current time. The potential distribution models of *A. amphitrite* showed perfect Area Under Curve (AUC) test values, with an index of  $0.89 \pm 0.03$  and  $0.96 \pm 0.03$  for clade *I* and clade *III*, respectively. This showed significance for the binomial omission test, hence the maps were evaluated as very good (more than 0.850). The contribution performance of layers for each period is presented in table 3. Based on the results, temperature mean and range for clade *I* and temperature mean and current velocity min. for clade *III* made the largest contributions to current habitat suitability in the predictions modeling. Accordingly, the suitable habitats for *A. amphitrite* were the tropical and subtropical areas (Fig. 7).

## DISCUSSION

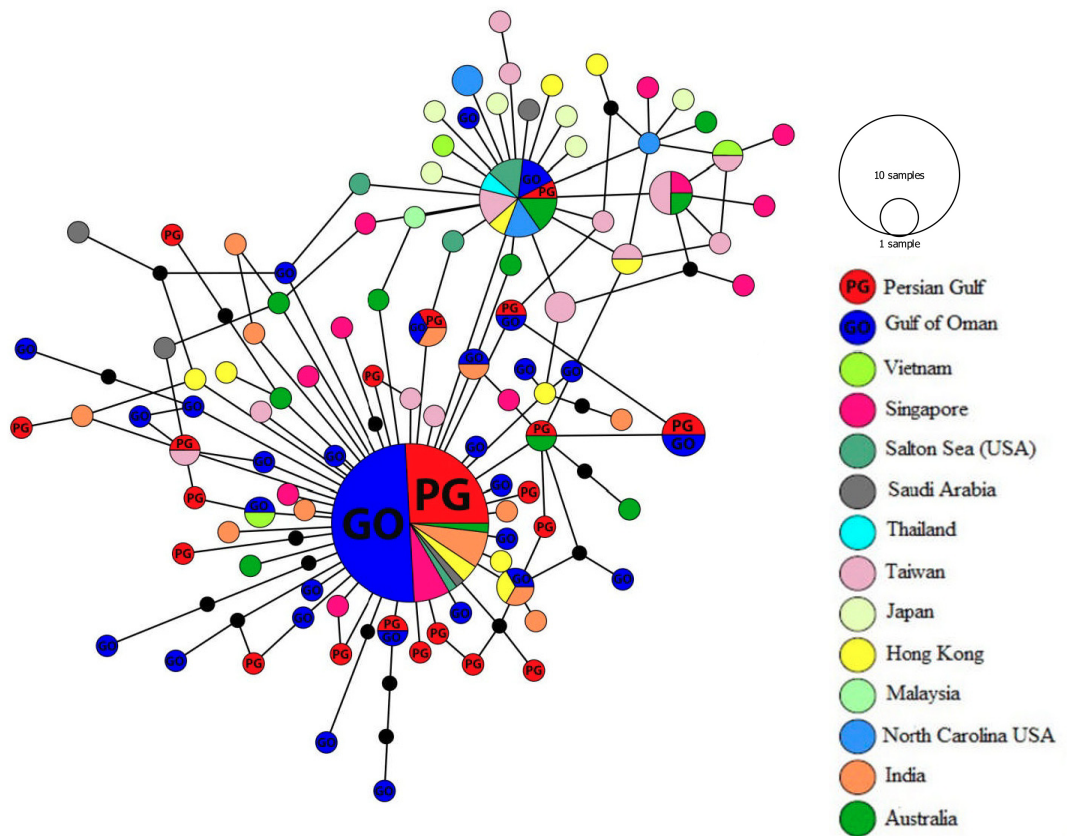
In the framework of biogeography, barnacles are among the most interesting invertebrates. During their pelagic larval stages, they are able to disperse under the influence of oceanic currents. By the end of

the larval phase and after dramatic changes through metamorphosis, they become sessile which is expected to limit their active distribution, gene flow among populations, and cause decreased genetic homogeneity. Their fouling behavior, however, has greatly

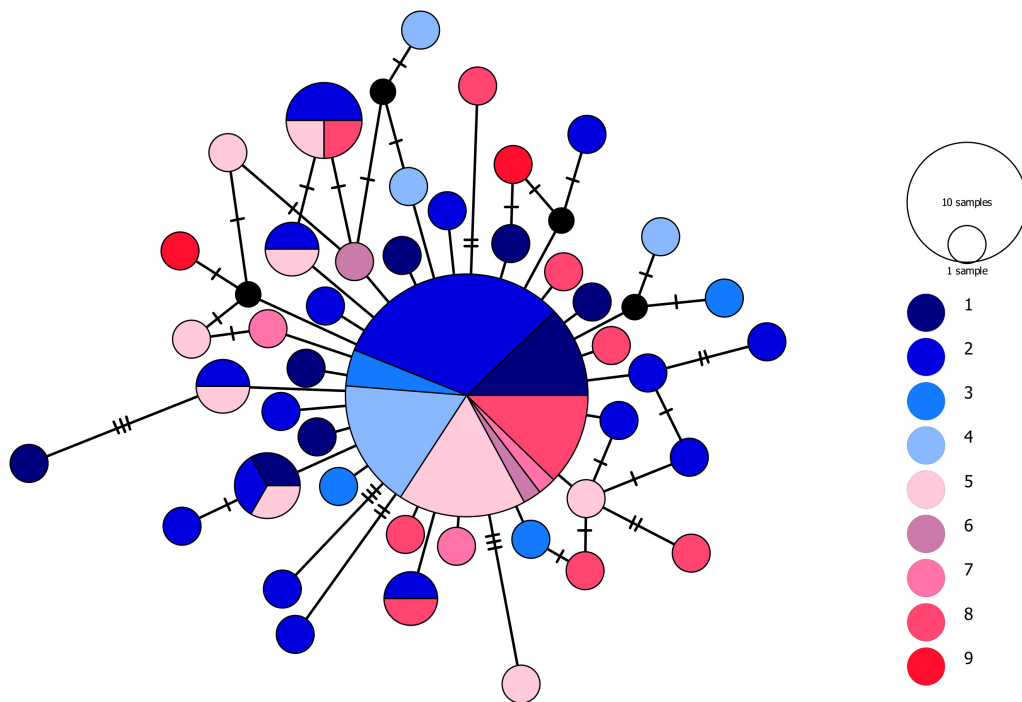
counteracted their natural dispersal ecology in recent decades (Holm 2012). It seems that ships have played a great role in their dispersal throughout the open waters (Yamaguchi et al. 2009). The fouling behavior increases gene flow among distant populations and therefore



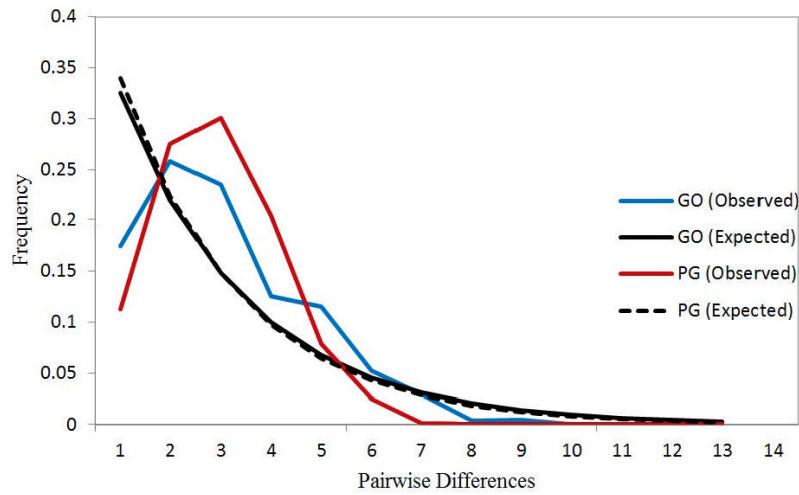
**Fig. 2.** Phylogenetic tree constructed with Maximum Likelihood (ML) based on the *COI* gene for selected sequences of *A. amphitrite*, focusing on Iranian populations (green circles). *Amphibalanus reticulatus* (JQ035518.1), *A. variegatus* (JQ035522.1) and *Balanus glandula* (KU204282.1) were used as outgroups. Numbers show the bootstrap values after 1000 pseudo-replicates. Clades I, II and III are corresponding to those in Chen et al. (2014).



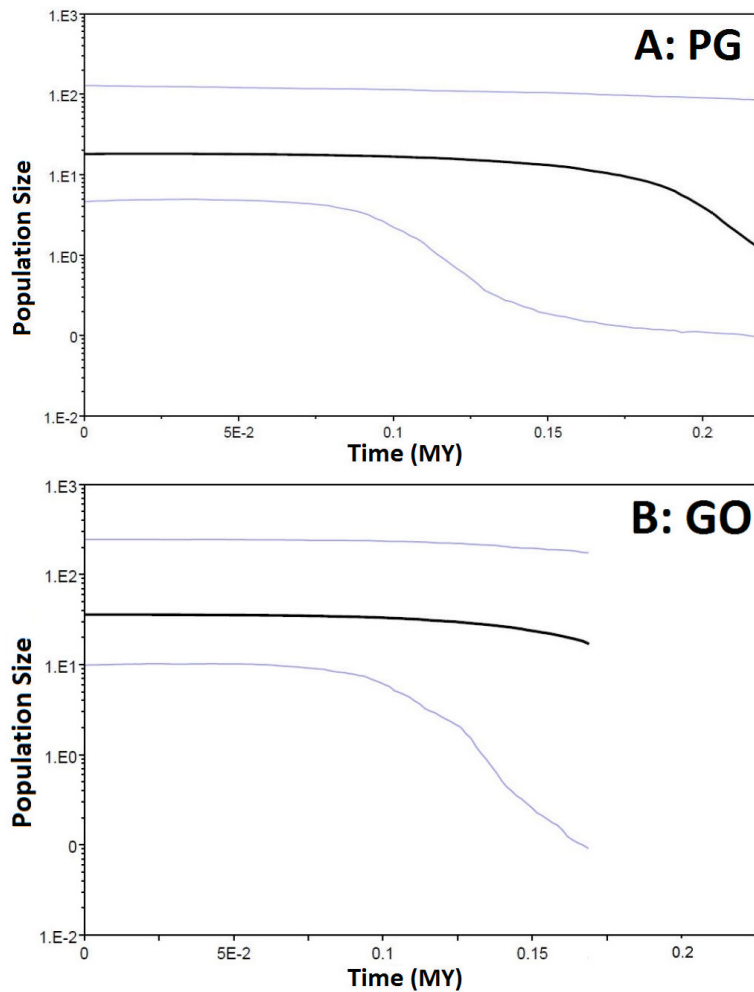
**Fig. 3.** Maximum-parsimony mitochondrial DNA haplotype networks for material from the PG and the GO, and the retrieved sequences from the GenBank (only sequences of the clade *I* in Chen et al. (2014) phylogenetic tree are included).



**Fig. 4.** Maximum-parsimony of mitochondrial DNA haplotype networks for the PG and the GO populations constructed in PapArt. Hatch marks represent mutations; numbers in the figure legend refer to sampling sites in table 1 and figure 1.



**Fig. 5.** Frequency distribution of the number of pairwise nucleotide differences (mismatch) between *COI* haplotypes in the two populations of *Amphibalanus amphitrite*. The solid line shows the theoretical distribution under the model of demographic expansion.



**Fig. 6.** Bayesian skyline plots of effective population size through time in *Amphibalanus amphitrite* from two biogeographical areas (A: PG and B: GO), based on the 576 bp sequences of *COI* and a nucleotide substitution rate of 3.1%/MY. The bold black curve is the median of the parameter  $N_eT$ , which is proportional to the effective population size; the blue lines delimit the 95% highest posterior density. For comparison, all x-axes have the same scale. The plots are truncated to the median estimate of each area's TMRCA.



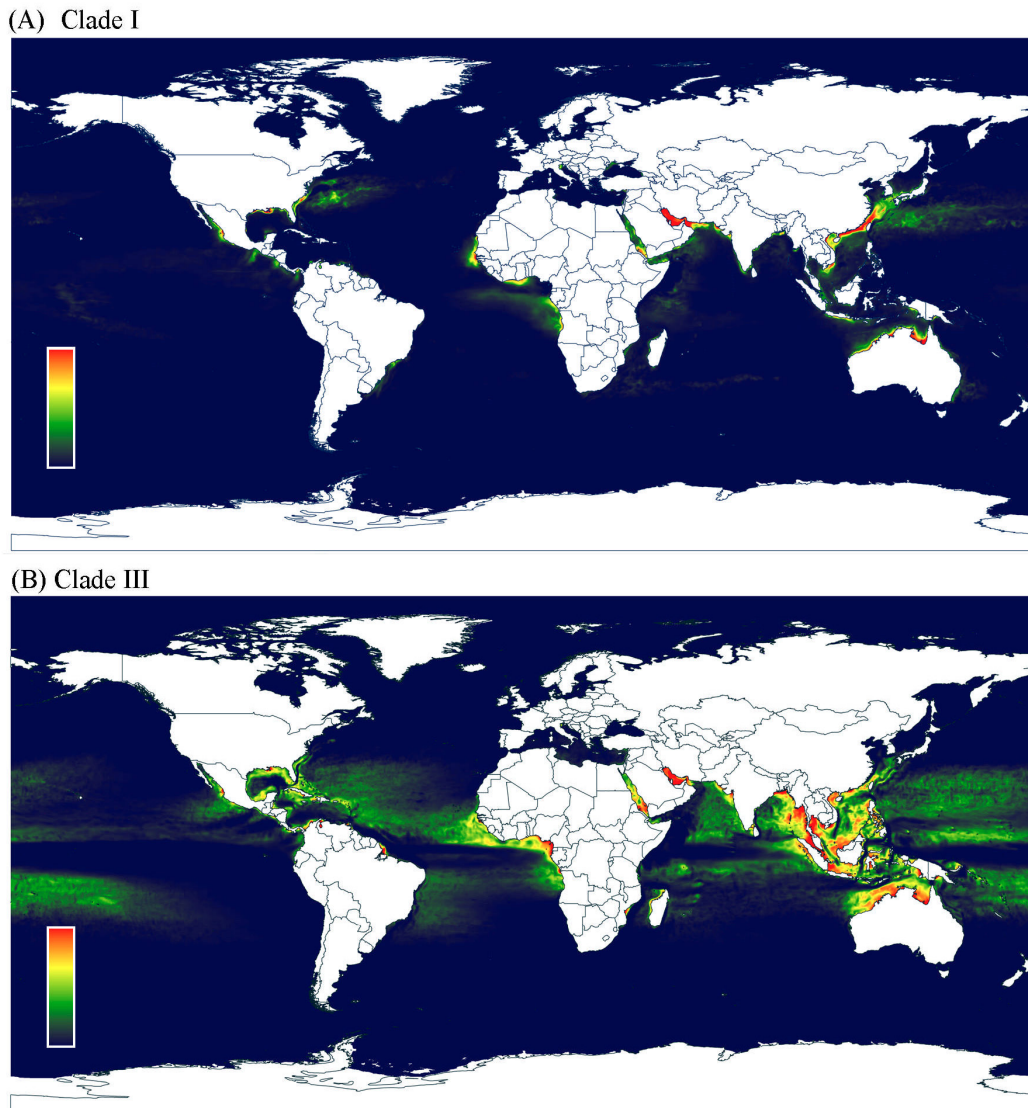


Fig. 7. MAXENT reconstruction for clade I (A) and clade III (B) of *Amphibalanus amphitrite* in the world representing current distribution models.

**Table 2.** Pairwise estimates of *COI* genetic divergence ( $\Phi_{ST}$ ) for *Amphibalanus amphitrite* among five biogeographical areas. The significance of  $\Phi_{ST}$  values was tested by a permutation test with 1000 replicates

	MK(PG)	TH(PG)	GU(GO)	CH(GO)	JS(GO)
MK(PG)	0				
TH(PG)	0.00748	0			
GU(GO)	0.00728	0.03051*	0		
CH(GO)	0.03196*	0.00921	0.01027	0	
JS(GO)	0.01838	0.00420	0.00528	0.02062	0

\*  $p < 0.05$ .

**Table 3.** The frequency of contribution of environmental variables in predicting the clades geographic distribution models

Environmental variable (unit)	Clade I	Clade III
Temperature Mean	39.8	56.8
Temperature Range	25.7	4.9
Phytoplankton Mean	15.3	5.9
Salinity Mean	5.5	1.4
Salinity Range	3.9	6.2
Current Velocity Mean	2.7	3.7
Current Velocity Ltmax	2	0
Dissolved oxygen Range	1.9	1.6
Phosphate Mean	1.6	5.3
Current Velocity Min	1	14.2
Current Velocity Max	0.6	0

homogenizes their genetic structure; this in turn makes it difficult to pinpoint the origin of their establishment (Ardura et al. 2016). One of the most common fouling barnacles is *Amphibalanus amphitrite*, an important species not only in marine ecology but also in the global economy (Holm 2012).

Using the mitochondrial *COI* marker, Chen et al. (2014) studied global phylogeography of this species with material from 25 localities. They found three distinct morphologically similar clades (with a 4% divergence between clades) indicating historical isolation between populations or possibly a cryptic species complex. These include clade *I*, as the most common and globally distributed clade along tropics and warm temperate areas, clade *II* with only two sequences, one from Singapore and one from North Carolina (USA) and clade *III* with more representatives, but restricted to the Indo-West Pacific tropics. It is commonly stated that the native range of *A. amphitrite* is the Indo-West Pacific (Jones et al. 2000; Shahdadi et al. 2014). This is a huge water body with high diversity that is occupied by all three clades. However, centuries of hitchhiking with human-mediated vectors or corridors has made it difficult to determine the autochthonous population distribution throughout the Indo-West Pacific region (Carlton 2011). The study of Chen et al. (2014) also included two localities in the northwest of the Indian Ocean, namely Mumbai (East of India) and Saudi Arabia (the Red Sea), which were dominated by representatives of clade *I*, with only one sequence of clade *III* in Mumbai.

In the previous studies, no specimens from the Persian Gulf (PG), a major commercial and oil transportation destination, were investigated. In the present study, in addition to the sequences of the specimens collected from the PG and the GO, the sequences reported in Chen et al. (2014) were included in the phylogenetic analyses. The distribution pattern of the PG and the GO samples was similar to that previously reported for samples from the east of India (Mumbai in Chen et al. 2014); most specimens were placed in clade *I* and only one sequence from each gulf was placed in clade *III* (Fig. 2). Clade *I* specimens included barnacles from Japan to Malaysia and also from Australia, India, Saudi Arabia, Hawaii, California and North Carolina (Chen et al. 2014). Within clade *I*, the PG and the GO samples clustered with sequences from Australia, West India, Taiwan, Hong Kong, Vietnam, Salton Sea (USA) and Singapore.

Clade *III* was only present in tropical Indo-West Pacific waters (Chen et al. 2014). In the phylogenetic tree (Fig. 2), only two specimens from current study showed great affinity with clade *III* together with sequences from Australia, Taiwan, Hong Kong

and Singapore. This clade had limited distribution compared to clade *I*. In the case of a complex species, it is expected to have one species or subspecies with a cosmopolitan distribution and other specimens restricted to specific areas (Chan et al. 2007; Zhan et al. 2010; Keshavmurthy et al. 2013). We demonstrated that this clade is mostly distributed and mapped on the shipping routes. The results of the current study clearly confirm that anthropological, mainly shipping activities, are the most likely explanations for the present global distribution of *A. amphitrite*. This information is expected to help shipping policy makers in the designing of effective measures for protection of ships as well as in the maintenance of the biodiversity of barnacles (Carlton et al. 2011; IMO 2021).

The spread of *A. amphitrite* may appear unidirectional toward the PG depending upon vectors, local hydrographic conditions and exploration history of larval stages. The vectors are important in transferring this species especially to the regions with high shipping traffic such as the PG and the GO. The PG produces around 46% of the world's oil consumption, and more than 90% of this oil is transported annually in thousands of oil tankers (Haapkylai et al. 2007; Al-Yamani et al. 2015).

The dispersal pattern of barnacle larvae and consequently the distribution range of the adults, are expected to reflect the interaction of larvae with oceanographic currents (Keith et al. 2011; Tsang et al. 2012). The spatial structure of a species may mirror the major oceanographic systems and environmental conditions. Additionally, the Indian Ocean tsunami off the west coast of northern Sumatra, Indonesia in 2004 (Lay et al. 2005; Okal et al. 2006) and, the Pangandaran tsunami off the west and central coast of Java, an island in the Indonesian archipelago in 2006 (Fritz et al. 2007), affected major parts of the Indian Ocean. This resulted in the transport of vast bulks of water within only a few days to different parts of the Indian Ocean including the GO and the PG.

The currents of the PG and the GO are also anticipated to play important roles in determining the present dispersal pattern of *A. amphitrite* (Ghanbarifardi et al. 2018; Sepahvand et al. 2021). For example, the anticlockwise currents that circulate from the GO to the PG (Yao 2008; Thoppil and Hogan 2010) most likely facilitate the transfer of the species' larvae (larval duration 7–17 days in 20–26°C) in the planktonic stages (Costlow and Bookhout 1958). These passive transports will spread individuals of *A. Amphitrite* to the PG through different means, namely, natural larval distribution, transport and release of larvae by ballast water or carrying ovigerous barnacles on ship hull to new regions.

Temporal patterns of introduction of *A. amphitrite* to the PG and the GO should take into account dates of first records. The increased diversity of barnacle invasions in the last half of the twentieth century is in close synchronization with general global observations of increasing invasions of marine invertebrates, fish, and algae after World War II associated with vastly expanded global trade facilitated by more, larger, and faster ships. For example, *A. amphitrite* together with *A. improvisus* were the first barnacles introduced to Americas for the first 100 years of invasion history at 1853–1955 (Carlton et al. 2011). In California, the first record of *A. amphitrite* is in 1914 (Henry and McLaughlin 1975).

The first record of *A. amphitrite* from the PG was given by Nilsson-Cantell (1938) as *Balanus amphitrite hawaiiensis* from an unknown locality and then by Stubbings (1961) as *B. amphitrite* var. *communis*, and *B. amphitrite* var. *hawaiiensis*, from Kuwait; by Utinomi (1969) as *B. amphitrite* from Hormoz Island; and by Jones (1986) as *B. amphitrite* var. *communis* from Kuwait. A recent checklist of Iran barnacles recorded *A. amphitrite* from all over the coasts of Iran (Shahdadi et al. 2014). Introduction of this species to the PG and the GO fell within a well-known global pulse of invasions related to an earlier surge of shipping, particularly in special economic zones. This widespread distribution is more related to clade I. Comparatively, clade III from the Indo-West Pacific has not expanded geographically. This distribution pattern reveals the major role of human-mediated vectors and corridors in introduction and dispersion of barnacles.

Present results revealed high levels of genetic diversity in the mitochondrial marker *COI* in the PG ( $h = 0.9$ ) and the GO populations ( $h = 0.8$ ). These values are also notable when compared to the population of several other invertebrate species in the PG and the GO, such as the fiddler crab *Uca sindensis* ( $h = 0.63$ ; Shih et al. 2015), Jinga shrimp *Metapenaeus affinis* ( $h = 0.0–0.33$ ; Tamadoni-Jahromi et al. 2016) and pearl oyster *Pinctada radiata* ( $h = 0.0–0.47$ ; Al-Saadi 2013).

Some other barnacle species from other parts of the world also demonstrated similar levels of genetic diversity in *COI* such as *Amphibalanus improvisus* ( $h = 0.75–0.96$ ; Wrangé et al. 2016), *Chthamalus proteus* ( $h = 0.9–1$ ; Zardus and Hadfield 2005) and *Tetraclita serrata* ( $h = 0.9$ ; Reynold et al. 2014).

The PG is a geologically young, semi-enclosed basin with a harsh environment, including high temperature and salinity. These extreme indices are believed to be largely responsible for the lower biodiversity of the PG (Naderloo 2017). The conditions of the PG may allow a recent selective sweep for fixation of particular haplotype. In contrast, according to

Shahdadi et al. (2014), the number of barnacle species in the PG is 33, which compares to 26 species in the GO. The *A. amphitrite* populations from two gulfs share several haplotypes including the most common one. The genetic diversity in the PG population is higher than the diversity of the GO and many other previously studied populations from around the world (see Table 1 in Chen et al. 2014).

Owing to the young age of the PG, the *A. amphitrite* population seems to have been introduced to this region naturally. This establishment was before species discovery by Darwin (1854). Its transport into the gulfs by ships occurred later. Therefore, according to common hypothesis, this population is expected to present low genetic diversity compared to native population. Our data revealed high genetic diversity among the PG barnacles. This is indicative of multiple episodes of introduction of barnacles to the PG from different sources (such as traditional Indian and Chinese shipping, tsunamis, tropical cyclones, and earthquake induced waves).

The high genetic diversity within the PG population and presence of common haplotypes among the PG and other populations of the world are therefore likely to be partially resulted from the anthropogenic introduction of haplotypes from around the world. The same reason could justify the high genetic diversity in some previously studied areas like Singapore and Hong Kong (see Chen et al. 2014). This could be well explained by the fact that ships from all over the world travel to these waters, stay in harbors for some time and subsequently transfer barnacles with different haplotypes from multiple sources to this area, providing their larvae with enough time for settlement and permanent sessile life in the region.

In this case, *A. amphitrite* has experienced multiple modes of dispersal including transportation of barnacle larvae by currents and ballast water and transportation of adults on ships hulls as fouling organisms to other parts of the globe. This could explain the presence of some of these newcomer haplotypes that are very distinct from the local haplotypes. However, intensive human-mediated gene flow has probably counteracted these processes. The result of all has been colonization of new areas at different scales and the presence of the dominant species on most coasts in the PG and the GO (Shahdadi et al. 2014; Shabani et al. 2019; Al-Khayat et al. 2021). Transport has been mediated via most natural and anthropogenic substrates including floating marine debris of various types and surface texture. For example, plastics have been common and pervasive anthropogenic debris in marine environments (Rech et al. 2018) and as floating objects they provide opportunities to alter the abundance, distribution and invasion potential

of this species for effective colonization (Goldstein et al. 2014). These transport opportunities may have been the key elements for successful distribution of this species. The knowledge about different types of dispersal dynamics of non-native species is crucial to our understanding of both evolutionary aspects of colonization as well as future management of biological introductions and invasions. Human-mediated dispersal has increased transmission of haplotypes from all over the world to these regions. This has caused increased genetic diversity within populations. On the other hand, with increasing gene flow, genetic differentiation has decreased as evidenced by genetic divergence ( $\Phi_{ST}$ ) between sampling sites that generally do not exceed 3% (Table 2). The values suggest high gene flow and absence of or weak genetic subdivision between the populations of the biogeographical areas (see Guo and Wares 2017).

The smaller values of  $\Phi_{ST}$  observed in the present study may derive from higher gene flow between individuals of *A. amphitrite* from different localities. The real distribution and connectivity of intertidal animals in the region and closely related areas (*i.e.*, West Indian Ocean) are evidently determined by oceanographic regimes, environmental conditions and historical events (Tsang et al. 2012; Afkhami et al. 2016; Rahimi et al. 2016; Ghanbarifardi et al. 2018).

The unimodal right skewed mismatch distribution curves (Fig. 5) confirm the recent population expansion for individuals collected both from the PG and the GO. The Bayesian Skyline Plot analysis (BSP) displayed the demographic expansion curve for the populations of the PG and the GO areas with truncation possibly due to the smaller sample size (Fig. 6). Despite more truncation for the GO, the curve shape was consistent with that of the PG. The results uncovered that the demographic expansion of *A. amphitrite* in the PG and the GO areas started approximately before 200 ka BP (Fig 6). This dating could not be in accordance with the history of the PG, which appeared about 18 ka years ago, and the fact that just about 8–10 ka BP, the northeastern margin of the PG approached its present position in several localities (Lambeck 1996; Marko et al. 2010). Thus, the expansion time of *A. amphitrite* in the PG and the GO had likely been at the early Holocene, concurrent with the re-flooding of the PG. This historical data seems to be related to gene flow in the Indian Ocean prior to the appearance of the PG. Consequently, it is not possible to precisely define the beginning of population expansion for this area. However, it is plausible that human factors played a role in introducing haplotypes to this region in recent decade.

Distribution modelling maps showed the presence of potential localities for geographical distributions

of two clades based on natural environment factors. From this perspective, both clades are common in the China Sea, Northern Australia especially the Gulf of Carpentaria, PG, GO, the Red Sea, West African coastal areas, the east coast of the United States and the western coasts of Mexico. These are potential distribution locations for both clades, but clade *III* in the Indo-Pacific Ocean presents a noticeably broader distribution. With ever growing human intervention (Seebens et al. 2013), we can expect changes in distribution of *A. amphitrite* clades in the future.

The shape of the population genetic structure of marine invertebrate species, in addition to historical demography and selection regimes, is affected by environmental factors (Bohonak 1999; O’Riordan et al. 2004). According to previous records, *A. amphitrite* is widely distributed globally around tropical and subtropical coasts (Henry and McLaughlin 1975; Chen et al. 2014). In the present study, distribution modelling showed the importance of environmental factors such as temperature, phytoplankton availability and salinity in the distribution of this species. Distribution of the fouling species is related to fouling type. The extent of fouling on ships’ hulls depended on many factors, *e.g.*, water salinity, light, temperature, pollution, geographical location and nutrient availability (Pettengill et al. 2007; Hulme 2009). Based on studies on invasions in marine environments (Gallardo et al. 2015), the distribution of invasive species is influenced by nutrients (40%), temperature (26%), human footprint (21%) and other factors (13%) that support a relevant role of the match between physicochemical characteristics of the donor and receiving waters for species introductions (Seebens et al. 2013). It seems that temperature plays the most important role in species distribution, specifically in tropical or subtropical waters. The polar zones are subjected to the most severe fouling attack, particularly in more shallow, coastal waters where there is greater abundance of light, heat and nutrients, resulting in prolific reproduction of the fouling species (Ubagan et al. 2021). One of the important factors in future distribution of *A. amphitrite* is climatic warming scenarios (Carlton 2000). The southward spread of *A. amphitrite* from Brazil to Argentina is a remarkable example (Carlton et al. 2011), and therefore, distribution maps of modelling can help to understand the role of potential climatic changes on expansion (Howard 1997; Poloczanska et al. 2008; Southward 1991).

The species displays several life history traits including broad environmental tolerance such as with temperature (Qiu and Qian 1999; Piazza et al. 2016). In their study, Khosravi et al. (2019) suggested that during global warming, *A. amphitrite* has maximum biofouling coverage in the PG and overtakes more

rivals. Furthermore, this species can survive in the high salinity of the PG (Simpson and Hurlbert 1998) better than the closely related mesohaline barnacle, *A. improvises*. The latter species is often restricted to low salinity environments and does not show a structured population genetic pattern globally (Wrangle et al. 2016). However, *A. amphitrite* has displayed three clades with potentially different physiological properties which display distribution restrictions (Chan et al. 2014). This tolerance ability has granted *A. amphitrite* a competitive advantage over rivals. Remarkably, Bishop (1947) reported that three species of live barnacles, including *A. amphitrite*, arrived in Liverpool, England, after a 30-day voyage from Australasia, via the Panama Canal.

## CONCLUSIONS

Molecular phylogeny of the common fouling barnacle, *A. amphitrite*, recovered three distinct clades using the mitochondrial *COI* marker. In the present study, specimens of the PG and the GO were placed into two different clades. Most specimens clustered in one clade which is mainly distributed globally, and two sequences fell in a separate clade. Genetic diversity of *A. amphitrite* revealed high genetic diversity in the PG and the GO populations and also the presence of common haplotypes of other regions of the world, indicating high gene flow between populations via shipping and other anthropogenic activities. Despite the young age of the PG, there are several unique haplotypes including 13 in the PG and 20 in the GO.

The contribution of anthropological activities to the transportation of this species into the PG and the GO seem evident. The implications of the broad environmental tolerance and high dispersal capacity of *A. amphitrite* for its ability to adapt to local conditions and tolerate future environmental changes remain to be elucidated.

Global homogenization of biota is underway through worldwide introduction and establishment of non-indigenous (exotic) species. The fouling organisms on ship hulls are continuously in transit and can affect communities through biodiversity loss and serious damage to economy and public health.

Through continuous human interference in the transportation of marine species, particularly through intense global shipping, it seems a high admixture between populations of *A. amphitrite* in the PG and the GO has occurred. Hence, *A. amphitrite*'s genetic structure determination has systematically vital importance for discovering species invasions and planning for the future control of species introduced via ship.

Naturally, even widely dispersed animals such as planktons can show significant population differences at macro-geographical scales (Barber et al. 2002), indicating geographical distributional constraints. However, human intervention interferes with the separation of the clade boundaries. Therefore, it is difficult to predict what the distribution composition of this species will be in the future. The distribution maps (using modelling) will help to model future patterns for better control and management of fouling organisms such as barnacles.

**Acknowledgments:** The authors would like to thank the anonymous referees for their valuable comments. This work was supported by Iran National Science Foundation (Grant number, 91058724) to Alireza Sari. We are grateful to the University of Tehran office of research affairs for their partial funding and laboratory support. The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

**Authors' contributions:** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Alireza Sari], [Asma Moeinadini], [Adnan Shahdadi], [Ahmad-Reza Katouzian] [Alimorad Sarafrazi] and [Elahe Elahi]. The first draft of the manuscript was written by [Asma Moeinadini] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Competing interests:** The authors have no relevant financial or non-financial interests to disclose.

**Availability of data and materials:** The datasets generated and/or analyzed during the current study will be available in the GenBank repository, <http://www.ncbi.nlm.nih.gov>. The receiver operating characteristic (ROC) curves are presented as figure S1.

**Consent for publication:** Not applicable.

**Ethics approval consent to participate:** Not applicable.

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## Supplementary materials

**Fig. S1.** The receiver operating characteristic (ROC) curves. (download)