Potential Pitfalls in the Definition of Lessepsian Migrants: The Case of *Brachidontes*

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Abstract Nonindigenous species are considered among the major threats to biodiversity. A deep understanding of the processes underlying invasion is impeded, among others, by insufficient taxonomic and distributional knowledge. The Mediterranean Sea is experiencing a large invasion by Red Sea species as a result of the opening of the Suez Canal, at a rate which far exceeds those in any other open marine system. Here, we focus on a potential Red Sea marine invader into the Mediterranean, the mytilid *Brachidontes pharaonis*, as a case study, and review alternative explanations for its present distribution in the Mediterranean, Red Sea, and the Indo-Pacific. A previous study showed a significant difference in haplotype composition among the Mediterranean and the northern Red Sea and that most Mediterranean Sea haplotypes were unique (up to 80%). We present updated analyses using mitochondrial DNA (mtDNA) samples from the Persian Gulf (Arabian Sea) and compare them to populations in the Mediterranean, Red Sea, and Sri Lanka. We found that haplotypes collected in the southern Red Sea, Qatar, and Sri Lanka were significantly distinct from all those sampled in the eastern Mediterranean and northern Red Sea. In contrast, the haplotypes from Abu Dhabi, a large, active port, clustered within those from the Mediterranean Sea and the northern Red Sea. Together, the Persian Gulf and the Indian Ocean do not seem to be the sources of the Mediterranean and northern Red Sea populations. The presence of many haplotypes within the Mediterranean and northern Red Sea that are not traceable to other populations (apart from Abu Dhabi) suggests that *B. pharaonis* is not truly a nonindigenous to the Mediterranean but, rather, is a previously undetected indigenous species. The

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establishment of the Suez Canal may have brought together divergent lineages and resulted in an admixture of populations, but this scenario alone cannot explain the rich assembly of unique haplotypes in the Mediterranean Sea. Our results lead to a better understanding of the genetic structure of this species. The results of our study suggest that detailed empirical and careful evaluations are needed in order to resolve the correct origin of those classified as nonindigenous species.

1 Introduction

Worldwide, biological invasions are profoundly reshaping species composition, and nonindigenous species are considered among the most pervasive and immediate threats to biodiversity (Clavero and García-Berthou 2005; Molnar et al. 2008; Walther et al. 2009). Nonindigenous species have been shown to outcompete indigenous populations and to induce profound phase shifts (Golani 1998; Edelist et al. 2013; Sala et al. 2011; Simberloff et al. 2013; Givan et al. 2017).

A deep understanding of these invasion processes depends on an accurate classification of nonindigenous species. However, due to a general insufficiency of taxonomic and distributional knowledge (the Linnaean and Wallacean shortfalls), a definitive classification of a species as nonindigenous is problematic. Many taxa lack a firm historical baseline to which new occurrence data can be compared. Thus, in many cases, the identification of a species as nonindigenous is based on very limited evidence with alternative explanations for the appearance of a "new" species rarely being considered. Here, we review the possibility of such alternative explanations, focusing on a Red Sea marine invader into the Mediterranean (Lessepsian migration; Por 1971, 1978).

The Mediterranean Sea is a species-rich yet highly impacted sea (Bianchi and Morri 2000; Coll et al. 2010; Lejeusne et al. 2010). Nonindigenous species in the Mediterranean now total above 950 (Zenetos et al. 2010) and are of particular concern in the eastern Mediterranean, where a large migration of Red Sea species followed the opening of the Suez Canal in 1869 (Galil 2009; Golani and Appelbaum-Golani 2009; Ben Rais Lasram and Mouillot 2009; Rilov and Galil 2009). These nonindigenous species constitute as much as 50–90% of total fish biomass in some habitats in the eastern Mediterranean (Goren and Galil 2005; Edelist et al. 2011; Buba et al. 2017). The current rate of nonindigenous species invasion and the consequent changes in species composition far exceed those in any other open marine system (Edelist et al. 2013). Do all of these recorded Lessepsian species, however, represent true nonindigenous species? We would like to highlight two problems:

First, a species might have been classified as nonindigenous if it had not been detected in the past due to restricted sampling effort or lack of taxonomic expertise. For many groups there is a serious lack of baseline information on species historical records. Thus, we are lacking the necessary comprehensive knowledge on indigenous species that could serve as a reference point. For example, even for

Mediterranean fish, which are relatively well studied, the number of species records is still increasing (Belmaker et al. 2009). Moreover, the number of species does not increase linearly but in a stepwise fashion, representing the major survey efforts. There is probably an even greater under-sampling of indigenous species in regard to other, lesser known groups such as invertebrates.

Second, we believe often overlooked, reason for misclassification of species as nonindigenous refers to the long-term population fluctuations and regime shifts. There are large fluctuations in biological and oceanographic data on interdecadal time scales, and periods of apparently stationary conditions separated by abrupt shifts are commonly observed (Hare and Mantua 2000; Rothschild and Shannon 2004; Overland et al. 2008). Although it remains unclear whether this variability represents true regime shifts or interdecadal fluctuations (Rudnick and Davis 2003; Hsieh et al. 2005; Overland et al. 2006; Overland et al. 2008; Di Lorenzo and Ohman 2013), it is clear that long-term monitoring is needed in order to gain a complete understanding of the natural range of environmental and biological states.

In this chapter we focus on the mytilid *Brachidontes pharaonis* as a case study. This species is one of the first Red Sea species to be recorded in the Mediterranean as long ago as 1876 (Galil 2006). From around the 1980s, this species became extremely abundant along the eastern Mediterranean coast, replacing the previously dominant *Mytilaster minimus* (Felsenburg and Safriel 1974; Safriel et al. 1980; Safriel and Sasson-Frostig 1988; Rilov et al. 2002). In recent years *B. pharaonis* has also been spreading westward in the Mediterranean (Sara et al. 2000, 2008).

A previous study had identified the presence of two major phylogenetic lineages of *B. pharaonis* in the northern Red Sea and Mediterranean (Shefer et al. 2004). One possible explanation for these two lineages is that the genetic makeup of the Mediterranean and northern Red Sea comprises a mix of an original Red Sea population and a more recent population of Arabian Sea origin that has entered the Red Sea after the Ice Age when sea levels rose (Dolby et al. 2018). Another possibility is that the current presence of two major lineages in the Mediterranean and northern Red Sea is the result of human introduction of specimens from other, potentially distant, populations (Shefer et al. 2004).

Here, we would like to suggest yet another possibility. Perhaps *B. pharaonis* is not truly nonindigenous to the Mediterranean but, rather, an indigenous species that had simply remained previously undetected? The establishment of the Suez Canal may have brought together two divergent lineages, which left their mark in the mixed genetic makeup of the populations in the Mediterranean and northern Red Sea. However, to support this hypothesis, it was necessary to sample other populations of *B. pharaonis* and to demonstrate that the haplotypes present in the Mediterranean and northern Red Sea do not appear in other Indo-Pacific populations. We therefore updated the genetic analyses made by Shefer et al. (2004) with additional mitochondrial DNA (mtDNA) data from populations from the Persian Gulf (Arabian Sea). These combined sources are employed here in order to better understand the genetic structure of this species and to identify the origin of the Mediterranean and northern Red Sea populations of *B. pharaonis*.

2 Methods

2.1 Study Species

The Indo-Pacific mytilid *Brachidontes pharaonis* (Fischer 1870; =*Brachidontes variabilis* Kraus 1848) was selected as an ideal "Lessepsian-invasion model" due to its success in the eastern Mediterranean Sea, where it is widely distributed and proliferating in a variety of tidal and shallow subtidal habitats. Since its presumed invasion into the Mediterranean, this mussel has become a dominant species along the rocky intertidal habitats of the Israeli Mediterranean coast, forming mats of very high densities that are displacing the indigenous mytilid *Mytilaster minimus* and other intertidal species (Felsenburg and Safriel 1974; Rilov et al. 2001). The successful invasion of *B. pharaonis* can be attributed to several features that are typical of invading species, such as tolerance to variable environmental conditions, including exposure to pollution, high fecundity, and dispersal abilities (Shefer 2003).

B. pharaonis has a wide Indo-Pacific distribution, spanning a variety of tidal and subtidal zone habitats, indicating, beyond tolerance, its dispersal abilities. Mytilid larvae, in general, are planktotrophic, spending considerable time in the water column (at least 10–20 days, Olson and Olson 1989; Widdows 1991). During the planktonic phase, larvae are carried away by large-scale currents and can settle at remote locations from their place of origin. Moreover, in the Levantine Sea, *B. pharaonis* is reproductive throughout most of the year, and larvae are released into the water even during midwinter (Shefer 2003). The potentially massive expansion mode, enormous population size, and large dispersal distance exhibited by *B. pharaonis* mussels suggest that the two lineages of haplotypes of *B. pharaonis* are unlikely to be an outcome of a random process (Shefer et al. 2004).

2.2 Sampling and Analyses

We sequenced 592 bp of mtDNA cytochrome oxidase subunit I (COI) from 290 individuals of *Brachidontes pharaonis*, collected at 16 sites. We sampled 77 individuals from the eastern Mediterranean Sea (i.e., Levantine Sea), 56 from the Gulf of Suez, 78 from the northern Red Sea, 32 from the southern Red Sea, 34 from the Persian Gulf, and 13 from the Pacific Ocean (Sri Lanka; Fig. 1). Four sites were sampled in the eastern Mediterranean Sea, four in the Gulf of Suez, four in the northern Red Sea, one in the southern Red Sea, two in the Persian Gulf, and one in the Pacific Ocean (Fig. 1, Table 1). Our sampling design followed the common route of cargo ships between the Far East and Europe (Kaluza et al. 2010).

Most of the sequence data we used had been published in Shefer et al. (2004), and for the present analysis, we only added samples from two sites in the Persian Gulf (Table 1). We used the DNA extraction, amplification, and sequence protocols outline in Shefer et al. (2004). We used Clustal X (Version 2.1, Thompson et al.

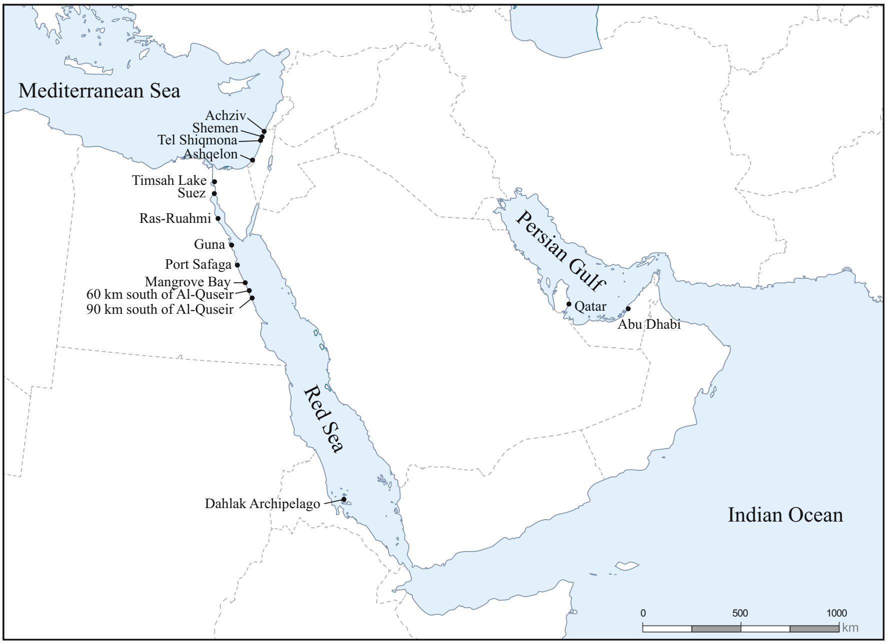

Fig. 1 Map of all sampling sites (excluding Sri Lanka)

Table 1 Sample size (N), number of haplotypes (N_H), haplotype diversity ($\widehat{H}$), nucleotide diversity ($\widehat{\pi}_n$), Tajima's test of selective neutrality (D_Tajima), and the P value for the Tajima's test (D_Tajima P) for the 16 sites sampled

Sites	N	N_H	$\widehat{H}$ (SD)	$\widehat{\pi}_n$ (SD)	D_Tajima	D_Tajima P
Achziv	17	11	0.941 ± 0.036	0.026 ± 0.014	−0.71	0.272
Tel Shikmona	16	11	0.942 ± 0.041	0.044 ± 0.023	0.64	0.779
Shemen	15	9	0.924 ± 0.044	0.055 ± 0.029	1.50	0.955
Ashkelon	29	7	0.768 ± 0.057	0.047 ± 0.024	3.34	0.999
Timsah Lake	13	8	0.923 ± 0.050	0.034 ± 0.018	0.30	0.684
Suez	10	7	0.933 ± 0.062	0.027 ± 0.015	1.00	0.883
Ras Ruahmi	19	14	0.965 ± 0.028	0.036 ± 0.019	−0.21	0.441
Guna	14	7	0.879 ± 0.058	0.028 ± 0.015	−0.36	0.415
Port Safaga	21	9	0.824 ± 0.060	0.020 ± 0.010	−1.31	0.089
Mangrove Bay	25	10	0.887 ± 0.035	0.028 ± 0.015	0.25	0.673
60 km south of Al-Qusir	18	7	0.869 ± 0.047	0.040 ± 0.021	1.73	0.983
90 km south of Al-Qusir	14	8	0.890 ± 0.060	0.051 ± 0.026	1.89	0.981
Eritrea	32	7	0.869 ± 0.021	0.006 ± 0.003	1.05	0.871
Abu Dhabi	13	13	0.999 ± 0.030	0.052 ± 0.027	0.17	0.643
Qatar	21	15	0.943 ± 0.036	0.011 ± 0.006	−0.40	0.388
Sri Lanka	13	8	0.923 ± 0.050	0.008 ± 0.005	−1.25	0.098

1997) to align the Persian Gulf samples to the 93 haplotypes in Shefer et al. (2004). The 34 samples from the Persian Gulf were collapsed into 25 unique haplotypes (Table 1). We examined the phylogenetic relationship between haplotypes by constructing a maximum likelihood tree and a minimum spanning network. We tested for the best fitted nucleotide substitution model using MEGA (version 7, Tamura et al. 2011). The maximum likelihood tree was constructed using PhyML (version 3, Guindon and Gascuel 2003), with the TN93 substitution model (best fitted model), and the Ts/Tv ratio, proportion of invariable sites, and the Gamma distribution parameter estimated by the program. The support level for each node was estimated by 100 bootstraps. The minimum spanning tree for haplotypes was estimated by Arlequin (version 3.5, Excoffier et al. 2005) and plotted by Netdraw (version 1, ONA Surveys).

For each site we sampled, we calculated the number of haplotypes, haplotype diversity ($\widehat{H}$; Nei 1987), nucleotide diversity ($\widehat{\pi}_n$; Nei 1987), and the Tajima's test of selective neutrality (Tajima 1989). We also calculated the average number of pairwise differences between populations (D_A; Nei and Li 1979) as a distance measure between populations or basins. This measure takes into account both the difference in haplotype sequences and frequency. All these genetic diversity measures were calculated using the program Arlequin. The P values associated with the D_A distances were calculated by a permutation test. We used the D_A distance matrix to construct a minimum spanning network for the sampled sites. We used the program MINSPNET (Excoffier 1993) to construct the minimum spanning network and the program Netdraw to plot it out.

3 Results

In this analysis, we examined genetic differences between haplotypes and between sites, but we also examined differences at the basin level. We partitioned the sites into five basins: the eastern Mediterranean Sea (i.e., Levantine Sea), the northern Red Sea (including the Gulf of Suez and the Suez Canal), the southern Red Sea (the coast of Eritrea), the Persian Gulf (Qatar and Abu Dhabi), and the Pacific Ocean (Sri Lanka). This geographical division into five distinct basins that are sequentially situated was essential in order to test for long-range migration of individuals.

Both the maximum likelihood phylogeny and the minimum spanning network of haplotypes revealed that haplotypes collected in Qatar and Sri Lanka were significantly distinct from all those sampled in the Mediterranean and the Red Sea (Fig. 2 a, b). However, the haplotypes from Abu Dhabi resembled those from the Mediterranean Sea and the northern Red Sea and were found in both subsections of the phylogeny and network (Fig. 2 a, b) that were recognized by Shefer et al. (2004).

The number of haplotypes for each site ranged from 7 to 15, and the haplotype diversity per site ranged from 0.77 to 0.99 (Table 1). We did not detect the selection signature at any of the sites we sampled ($P > 0.09$ at all sites; Table 1). Taking into account both haplotype nucleotide difference and frequency between sites, Eritrea,

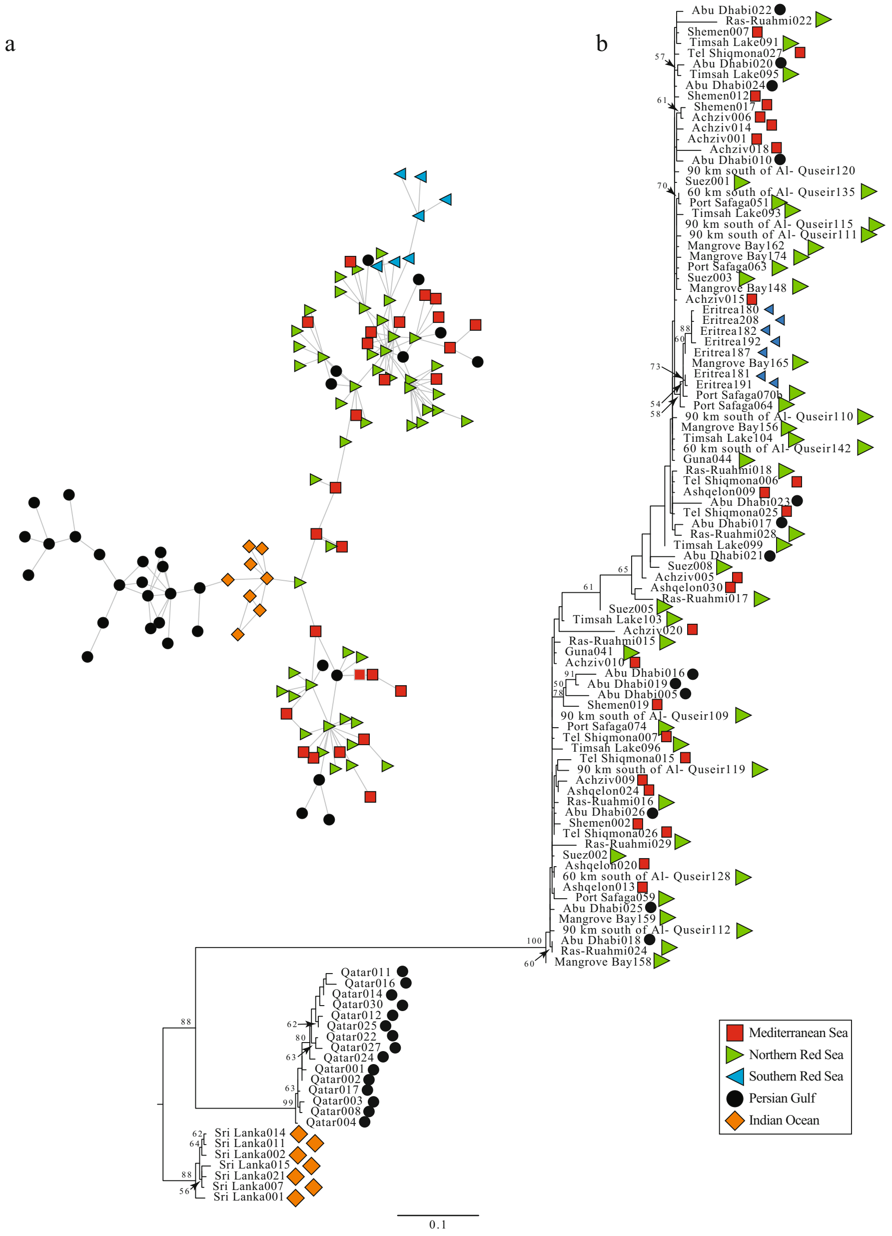

Fig. 2 Minimum spanning network (**a**) and maximum likelihood tree (**b**) of all haplotypes identified at all sites. Colors and shapes denote basins. The numbers at the nodes of the maximum likelihood tree are ≥50% bootstrap values

Qatar, and Sri Lanka were significantly distinct from each other and from all other sites (Table 2). We detected several other sites that were also significantly distinct. Ashqelon and Shemen in the Mediterranean Sea were distinct in their haplotype composition from Guna, Port Safaga, and Mangrove Bay in the northern Red Sea. Guna, Port Safaga, and Mangrove Bay were also significantly distinct from the sampling site 90 km south of Al-Qusir and from Abu Dhabi (Table 2). All other sites did not significantly differ in haplotype composition.

The basins were also significantly distinct from each other (Table 3). The genetic distances between the sites in the Pacific Ocean and the Persian Gulf to all the other basins were very large (>42). In contrast, the genetic distance between the northern Red Sea and eastern Mediterranean Sea was small (1.3; Table 3). These results reflect the high frequency of unique haplotypes in each of the basins. We found that 80.3% of samples and 80.6% of haplotypes were unique to the Mediterranean Sea. Likewise, 77.6% of samples and 88.5% of haplotypes were unique to the northern Red Sea. Only two haplotypes (Tel Shikmona 027 and Achziv 009) were shared between the Mediterranean Sea and the Persian Gulf, and a single haplotype (Ras Ruahmi 022) was shared between the northern Red Sea and the Persian Gulf. Thus, the Persian Gulf also demonstrated a high level of uniqueness (91.2% of samples and haplotypes). No haplotypes were shared with the southern Red Sea site or with Sri Lanka. These results are reflected in the minimum spanning network for sites (Fig. 3). The Mediterranean sites are clearly clustered together (Fig. 3), but a more complex pattern, composed of two subclusters, can be observed in the northern Red Sea.

4 Discussion

This study extended the genetic sampling of *B. pharaonis* to sites in the Persian Gulf (i.e., Qatar and Abu Dhabi), a major hub for cargo ships moving from the Mediterranean Sea to North-East Asia and vice versa (Kaluza et al. 2010). By means of this extra sampling, we sought to better understand the origin of the two genetic lineages reported in Shefer et al. (2004) for both the northern Red Sea and eastern Mediterranean populations.

We found that haplotypes collected in the southern Red Sea, Qatar, and Sri Lanka were significantly distinct from all those sampled in the eastern Mediterranean and northern Red Sea. Hence, both the Persian Gulf and the larger Indian Ocean do not seem to be the sources of the Mediterranean and northern Red Sea populations. In contrast, the haplotypes from Abu Dhabi clustered within those from the Mediterranean Sea and the northern Red Sea. These findings could be derived from one of the several underlying processes.

First, it is possible that the source of the Mediterranean and northern Red Sea *B. pharaonis* lineages is from migrants from around Abu Dhabi, whose *B. pharaonis* populations are divergent from the populations in other regions of the Persian Gulf (e.g., Qatar). Individuals of this population may have been transported by ship to the

Table 2 Corrected average number of pairwise differences (D_A) between sites (below diagonal) and their *P* values (above diagonal). *P* values were calculated by permuting individuals among sites

Sites	Achziv	Tel Shikmona	Ashqelon	Shemen	Timsah Lake	Suez	Ras Ruahmi	Guna	Port Safaga	Mangrove Bay	60S Al-Qusir	90S Al-Qusir	Eritrea	Abu Dhabi	Qatar	Sri Lanka
Achziv		0.509	0.136	0.182	0.300	0.364	0.336	0.118	0.036	0.127	0.264	0.309	>0.001	0.364	>0.001	>0.001
Tel Shikmona	−0.573		0.236	0.127	0.482	0.400	0.655	0.145	0.236	0.182	0.509	0.445	>0.001	0.327	>0.001	>0.001
Ashqelon	1.333	0.266		0.282	0.073	0.436	0.245	0.009	>0.001	>0.001	0.173	0.491	>0.001	0.427	>0.001	>0.001
Shemen	1.104	1.113	0.173		0.082	0.545	0.118	0.036	>0.001	>0.001	0.100	0.345	>0.001	0.236	>0.001	>0.001
Timsah Lake	−0.122	−0.752	1.754	3.309		0.191	0.627	0.400	0.227	0.455	0.500	0.391	>0.001	0.136	>0.001	>0.001
Suez	−0.142	−0.535	−0.737	−0.470	0.589		0.355	0.082	0.055	0.055	0.300	0.582	>0.001	0.436	>0.001	>0.001
Ras Ruahmi	−0.028	−0.831	0.541	2.117	−0.597	−0.382		0.355	0.136	0.191	0.509	0.309	>0.001	0.455	>0.001	>0.001
Guna	1.273	0.574	3.986	5.896	−0.282	2.566	0.013		0.464	0.264	0.218	0.055	>0.001	0.064	>0.001	>0.001
Port Safaga	2.064	1.207	5.238	7.483	0.159	3.758	0.986	−0.272		0.309	0.127	0.045	>0.001	0.036	>0.001	>0.001
Mangrove Bay	1.516	0.408	3.707	6.111	−0.234	2.752	0.579	0.140	0.094		0.309	0.045	>0.001	0.064	>0.001	>0.001
60S Al-Qusir	0.108	−0.808	0.916	2.534	−0.713	0.040	−0.597	0.385	0.880	−0.005		0.318	>0.001	0.191	>0.001	>0.001
90S Al-Qusir	0.237	−0.775	−0.611	−0.379	0.398	−1.196	−0.144	2.390	3.383	2.019	−0.253		>0.001	0.536	>0.001	>0.001
Eritrea	7.868	6.390	11.295	14.099	5.243	10.565	6.513	5.029	3.918	4.291	5.853	9.195		>0.001	>0.001	>0.001
Abu Dhabi	0.038	−0.351	−0.418	−0.256	0.706	−0.959	−0.409	2.068	3.696	2.773	0.403	−0.784	9.370		>0.001	>0.001
Qatar	117.119	117.208	112.532	112.146	117.835	113.982	116.627	123.050	124.365	123.208	117.301	114.008	125.939	114.018		>0.001
Sri Lanka	106.732	108.800	105.360	106.307	109.004	105.343	107.099	112.631	113.086	111.566	107.643	105.947	115.523	106.912	64.836	

Table 3 Corrected average number of pairwise differences (D_A) between basins (below diagonal) and their *P* values (above diagonal). *P* values were calculated by permuting individuals among basins

Basin	Eastern Mediterranean Sea	Northern Red Sea	Southern Red Sea	Persian Gulf	Indian Ocean
Eastern Mediterranean Sea		0.018	<0.001	<0.001	<0.001
Northern Red Sea	1.333		<0.001	<0.001	<0.001
Southern Red Sea	10.034	5.673		<0.001	<0.001
Persian Gulf	42.542	46.258	53.627		<0.001
Indian Ocean	106.282	109.185	115.524	53.182	

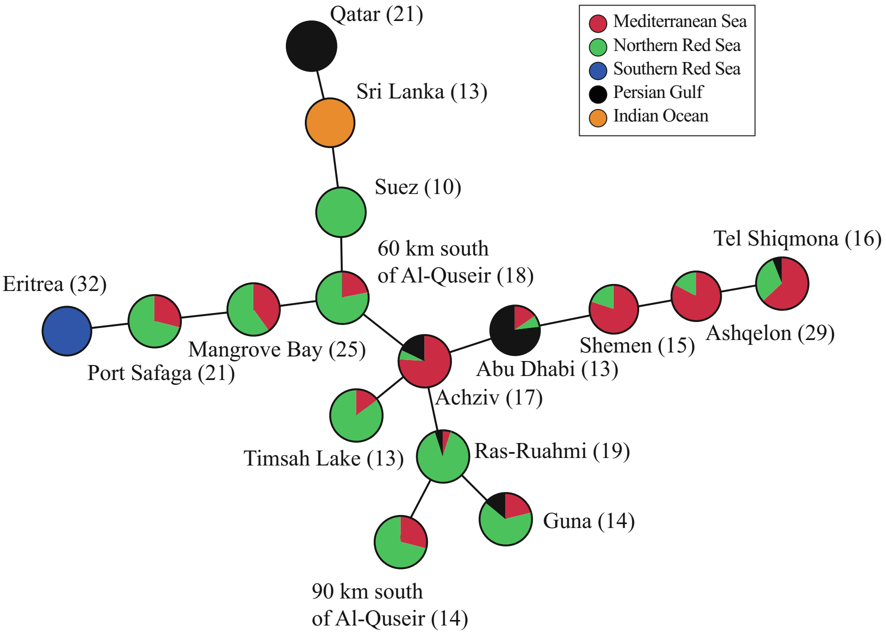

Fig. 3 Minimum spanning network between sampling sites. The site pie charts show the proportion of haplotypes shared by basins. Sample size is presented in parenthesis

Mediterranean and northern Red Sea. However, given the low chance of deep population divergence over such a small distance as that between Qatar and Abu Dhabi, which features no apparent biogeographic barriers, this scenario is unlikely.

It is also interesting to note that the sampling in Abu Dhabi was in a large and active port. Hence, the population sampled there may actually contain individuals from other sources, perhaps even lineages from the eastern Mediterranean and northern Red Sea, representing a complex pattern of genetic mixture derived from the secondary transport of individuals from their potentially invasive range back to their native range.

Second, given that, apart from Abu Dhabi, we did not find a clear source of Mediterranean and northern Red Sea haplotypes, it is possible that one of these lineages originates from a distant and yet-to-be-sampled population. We believe, however, that this is not the case, as it is unlikely that a successful human-mediated introduction would be composed exclusively of individuals from very distant populations, while closer populations (such as the Indian Ocean and southern Red Sea) have remained unshuffled by human activities.

Finally, we would like to suggest that the presence of two distinct lineages within the Mediterranean and northern Red Sea that are not traceable to other populations (apart from Abu Dhabi) attests to a secondary contact between the two populations. For the eastern Mediterranean and northern Red Sea populations of *B. pharaonis*, a logical explanation is that these genetic lineages represent two distinct populations (one in the Mediterranean and one in the Red Sea) that came into contact following the construction of the Suez Canal. The immediate implication of this hypothesis is that *B. pharaonis* is not nonindigenous to the Mediterranean but, rather, a wide-ranging species with an unrecognized native population within the Mediterranean. Support for this hypothesis comes from Late Cretaceous fossil records of the genus *Brachidontes* found at sites around the Mediterranean area (Egypt: El Qot 2006; Mekawy 2007; Tunisia: Peron 1890; Algeria: Peron 1890; France: d'Orbigny 1844; Valentin et al. 2014).

This hypothesis immediately raised the question: If *B. pharaonis* is indeed indigenous to the Mediterranean, why was it not discovered prior to the twentieth century and what might explain its dramatic increase over recent years (Felsenburg and Safriel 1974; Safriel et al. 1980; Safriel and Sasson-Frostig 1988; Sara et al. 2008)? While we acknowledge that we cannot firmly either refute or support the above-posited hypothesis at this stage, we believe that this pattern could be explained by long-term population fluctuations. Interdecadal fluctuations separated by periods of apparently stationary conditions are common in marine systems (Hare and Mantua 2000; Overland et al. 2008; Rothschild and Shannon 2004). Such a pattern of boom-and-bust seems to be a common phenomenon in many taxa and is associated with planktotrophic larval development (Uthicke et al. 2009). This pattern may more generally explain the high frequency of species exhibiting population fluctuations in the marine realm, as planktotrophic larval development is the most common type of larval development among benthic invertebrates such as *B. pharaonis*. The implications of such fluctuations for marine biodiversity assessments are dramatic. It is important to note that in many cases these cycles in density can span several decades. Typical biological surveys are thus very likely to overlook low-density species or very long-term fluctuations. Thus, natural fluctuations in species abundances will hinder our ability to differentiate true nonindigenous species from indigenous but previously rare species.

Moreover, marine environments worldwide are subject to directional environmental forces such as changing climate, water acidification, pollution, and overfishing (Doney et al. 2012). These will probably induce persistent trends in species composition and abundance, with low-density species possibly demonstrating population size increases and becoming visible in biological samples. Thus, human-induced changes will make it even harder to differentiate changes in the abundance of indigenous species from the establishment and spread of nonindigenous species.

Examples of long-term fluctuation in the abundance of marine species are ubiquitous. A recent study suggests that the oft-cited jellyfish bloom of recent years may actually represent a natural waxing and waning in abundance over a ~20-year time scale (Condon et al. 2013). Similarly, the corallivorous crown-of-thorns starfish (*Acanthaster planci*) exhibits periodic outbreaks in abundance, with this species alternating between being a relatively rare reef inhabitant and being a superabundant pest species capable of destroying large tracks of coral reefs (De'ath et al. 2012; Kayal et al. 2012; Osborne et al. 2011). Our model animal, *B. pharaonis*, also exhibits such extreme population fluctuations. In the 1970s, this mytilid species was rare along the Mediterranean coast of Israel, but its density increased dramatically during 1990s–2000s (Shefer et al. 2004; Galil 2006). However, in recent years (2016–18), its population has crashed, and it is again becoming rare (S. Shefer, personal observation). In the Gulf of Aqaba, it was abundant during the 1980s (Safriel et al. 1980) but very rare from the early 2000s (Shefer et al. 2004) until now (2018, A. Abelson, personal observation).

We believe that these examples demonstrate that empirical evaluations are needed in order to resolve the correct status of newly identified species. In the end, only detailed species-specific studies can reveal the true origin of potentially alien species. We argue that in order to advance our knowledge of invasion biology, it is vital to compile independent evidence verifying the status of previously unknown species, rather than simply assuming that they are nonindigenous.

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