Establishing the identity and assessing the dynamics of invasion in the Mediterranean Sea by the dusky sweeper, *Pempheris rhomboidea* Kossmann & Räuber, 1877 (Pempheridae, Perciformes)

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Abstract We investigate the genetic diversity of the sweeper *Pempheris,* a biological invader that entered the Mediterranean Sea via the Suez Canal. Two mitochondrial regions and one nuclear region were sequenced and topological reconstructions investigated from samples collected from the eastern Mediterranean Sea, Red Sea and three Indo-Pacific localities. Morphological and molecular analyses assigned samples from this study to three distinct species of *Pempheris* in the Red Sea (*P. flavicyla*, *P. rhomboidea,* and *P. tominagai*) and confirmed a misidentification of the Mediterranean sweepers, previously identified as *P. vanicolensis* and now recognized as *P. rhomboidea. Pempheris rhomboidea* clustered in a single clade including specimens from Madagascar and South Africa. Similarly to most other studied Lessepsian bioinvaders, no evidence of a genetic bottleneck in its invasive Mediterranean population was found. Yet, lowered gene flow levels were observed between Red Sea and Mediterranean populations in this species. These findings highlight the importance of molecular tools to the proper identification of morphologically challenging alien organisms and contribute to the understanding of the dynamics of Lessepsian invasions.

Keywords Pempheridae · *Pempheris* · Red Sea · Mediterranean · Lessepsian bioinvasion

Introduction

Human activity has increased the volume and rates of biological invasions with often dramatic effects in some habitats (Mack et al. 2000). The consequences of biological invasions are considerable, including negative impacts on local ecosystems and economic costs

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(Sax et al. 2005a, b). Studies of bioinvasions increased with the urgent demand to detect, understand and manage these events and the need to comprehend the underpinnings of successful invasions (Simberloff 2011; Azzurro et al. 2014). The difficulty of early detection and proper identification of marine bioinvaders, the logistic problems of field work, and the presence of cryptic species are but a few of the common problems faced by the study of marine bioinvasions.

The opening of the Suez Canal in 1869 resulted in the introduction of Red Sea biota into the Mediterranean (Por 1978). The Canal has undergone major enlargements in order to maintain its market share: its typical cross-sectional area, which was 304 m^2 in 1869, 1,800 m^2 in 1962 and 3,600 m^2 in 1980, is at present 5,200 m^2. The recent expansion, completed in January 2010, increased its depth to allow passage of vessels up to a draft of 20 m, and already the Suez Canal Authority is evaluating the feasibility of increasing the Canal's depth and doubling its width to attract larger vessels (Galil et al. 2014). To date, 90 bony fishes are considered to have passed through the Suez Canal (Golani 2010; Bariche and Heemstra 2012; Belmaker et al. 2013; Galil and Goren 2014). These species are acknowledged as a major driver of biodiversity change in the Mediterranean Sea (Galil 2007, 2009, 2012). The Lessepsian bioinvasions present some unique scientific opportunities since the date of vector initiation, the opening of the Canal (1869), and the route of invasion are known.

Species introductions are expected to result from transport of individuals to a new location where they thrive, in part due to being partially released from competition (see Azzurro et al. 2014 for Lessepsian fish), and natural enemies (Sax et al. 2007). The recent population explosion of lionfishes in the Caribbean is a spectacular example (Betancur-R et al. 2011). Theoretically, founding effects should be revealed as a decrease in genetic diversity resulting in a genetic bottleneck (e.g. Azzurro et al. 2006). The genetics of the majority of Red Sea bioinvasions are counterintuitive. A founder effect pattern was originally reported for the Mediterranean population of the bluespotted cornetfish, *Fistularia commersonii* Ruppell 1838, where a genetic bottleneck was observed (Golani et al. 2007). However, subsequent analyses performed on a number of mitochondrial and nuclear regions revealed the presence of additional genotypes in that species (Sanna et al. 2010; Tenggardjaja et al. 2014)

making it a less clear-cut example. All other Suez Canal introductions studied to date display a level of genetic diversity that is essentially indistinguishable from their Red Sea populations (Azzurro et al. 2006; Golani et al. 2007; Bernardi et al. 2010). Since these studied species had been first recorded in the Mediterranean between 1902 and 1964, the lack of a genetic bottleneck was explained by successive introductions, which slowly built up the genetic diversity in the invasive populations (Bucciarelli et al. 2002; Hassan et al. 2003; Hassan and Bonhomme 2005; Azzurro et al. 2006). The discrepancy between predictions and observations may be explained by assuming that species that underwent severe bottlenecks are not successful invaders and soon disappear before being detected. Indeed, while a number of invaders are initially observed in small numbers, they don't appear to reach critical mass or diversity to establish themselves (Bariche and Saad 2005; Bariche and Bernardi 2009). However, the difficulty of detecting marine species is a significant problem that may obscure the dynamics of the invasion. The presence of cryptic species, namely species that are morphologically indistinguishable but genetically different, adds further complication. Cryptic species are common in the marine realm (Knowlton 1993), and in that respect Lessepsian invaders are no exception (Bucciarelli et al. 2002; Golani and Bernardi 2012).

Misidentifications

In order to fully assess and understand the dynamics of invasions, researchers need to study the ecology, biology, behaviour and distribution of biological invaders (Sax et al. 2005a). Foremost, introduced species must be accurately identified (Holland 2000). Traditionally, taxonomic identification has been accomplished by morphological analyses, but molecular tools have been increasingly used to identify morphologically challenging alien and cryptic species (Armstrong and Ball 2005; Bickford et al. 2007). Occasionally, introduced species have been erroneously identified and the history of Lessepsian invasion is no exception (McGlashan et al. 2007; Galil 2009). While morphological keys are arguably more complete in fishes, misidentifications have also occurred in this group (Belmaker et al. 2009) including *Epinephelus coioides* and *Epinephelus malabaricus*, both misidentified as *E. tauvina* (Ben Tuvia and Lourie 1969; Heemstra and Golani 1993); *Apogon*

(Nectamia) taeniatus, initially misidentified as *Apogon nigripinnis* (Tortonese 1986) and then reassessed as *Apogonichthyoides pharaonis* (Fraser and Allen 2010); *Lagocephalus suezensis* recorded as *L. sceleratus* (Mouneimne 1977) and *Sphoeroides cutaneous* (Avşar and Çiçek 1999); *Upeneus moluccensis*, first recorded as *Mulloides auriflamma* (Haas and Steinitz 1947) and more recently, *Nemipterus randalli* initially reported as *N. japonicus* (Golani and Sonin 2006; Lelli et al. 2008).

The sweeper puzzle

Sweepers (Pempheridae), also known as bullseyes, are commonly found on rocky outcrops and coral reefs of the tropical and temperate Indo-Pacific and western Atlantic oceans. They typically stay in tight schools in caves during the daytime, feeding at night (Mooi 2000; Annese and Kingsford 2005). Sweepers currently include 48 nominal species, 30 of which being considered valid (Eschmeyer 2013). This family is today represented by two genera: *Parapriacanthus* Steindachner 1870, which includes four species, and *Pempheris* Cuvier 1829, which includes 26 recognized species (Koeda et al. 2013).

The superficial resemblance of pempherids was long the cause of taxonomic confusion. Consequently, only ca 38 out of the 48 nominal species of *Pempheris* are currently considered valid (Mooi 2000; Koeda et al. 2014; Mooi and Randall 2014). Even within a geographical region, taxonomic issues occur, for example, "one cannot tell how many species of *Pempheris* are present in the Red Sea" (Goren 1986). Dor considers that four species occur there (*P. mangula*, *P. oualensis*, *P. schwenkii*, *P. vanicolensis*) (Dor 1984), Golani and Diamant acknowledge only one, *P. vanicolensis* (Golani and Diamant 1991), and later Goren and Dor consider five species, by adding *P. molucca* to Dor 1984 list (Goren and Dor 1994). In a recent checklist of Red Sea fishes, only two species are retained, *P. schwenkii* and *P. rhomboidea* (Golani and Bogorodsky 2010). The presence of *P. vanicolensis* in the Red Sea being attributed, according to Mooi, to a misidentification of *P. rhomboidea* (Golani and Bogorodsky 2010). Most recently, a new Red Sea species previously identified as *P. vanicolensis* was described as *P. flavicycla*, with a subspecies endemic to the Red Sea, *P. flavicycla marisrubri* (Randall et al. 2013). Finally, Koeda et al. determined that four

sweeper species are present in the Red Sea, *P. adusta, P. mangula, P. nesogallica,* and a newly described species, *P. tominagai*, which is very closely related to *P. schwenkii* (Koeda et al. 2014). Yet, recent papers clearly show that some of these conclusions are unwarranted (Mooi and Randall 2014; Randall and Bineesh 2014). Here, we consider that only three species are present in the Red Sea, *P. flavicycla, P. rhomboidea,* and *P. tominagai* (Koeda et al. 2014; Randall et al. 2014).

Clarified identification of the Lessepsian *Pempheris*

The first record of the sweeper in the Mediterranean was collected off Lebanon (Mouneimne 1977). Subsequently it was recorded in 1979 in Israel, in 1983 in Turkey, in 1986 in Greece, in 1991 in Syria, in 2001 in Tunisia, in 2004 in Libya, and in 2007 in Egypt (Golani 2010; Halim and Rizkalla 2011). Soon after its arrival, its populations experienced explosive growth (Golani and Diamant 1991; Bradai et al. 2004; Shakman and Kinzelbach 2007). It was initially identified as *P. molucca* Cuvier 1829 and subsequently as *P. vanicolensis* (Golani and Ben Tuvia 1986), since the color pattern and some meristic and morphometric characters (D VI–VII (usually VI 9); A 32–41; V 1 5; P 16–18; L.L. scales; Snout 16.6–19.1; eye 39.7–42.9, all % head length) were considered to be congruent with the description of this species (Randall 1983; Golani and Diamant 1991). All successive authors adopted the name of *P. vanicolensis* for this species. The identity of the Lessepsian *Pempheris* reflects the complex taxonomy of the genus, now under revision (Koeda et al. 2013, 2014; Randall et al. 2014). Owing to its success in the Mediterranean Sea, the sweeper was included among the 100 worst invasive species in this sea (Streftaris and Zenetos 2006). Recently doubts have been raised concerning its identity. In order to resolve them we examined specimens collected in the Mediterranean, Red Sea, and Indo Pacific Ocean deposited in the fish collections of the Smithsonian Institute Washington D.C. (USNM) and the Steinhardt Museum of Natural History, Tel Aviv University (TAU).

Aims

Our primary goal in this study was to clarify the identity of the pempherid fish established in the

Mediterranean Sea. Molecular tools were used to determine the modes and dynamics of this invasion.

Materials and methods

Material for morphological analyses

We examined the following material from Museum collections.

P. flavicycla Randall et al. 2013

Red Sea (around Sinai Peninsula): USNM 402211, Straits of Jubal (1 spec.); USNM 402218, Near Ras Burka (4 spec.); TAU 6624 Marsa Bareika (1 spec.); TAU 14763 Ras Garra (3 spec.); Southern Red Sea (Eritrea) USNM 402260 (1 spec.); Seychelles (Alphonse Island) TAU 11787 (1 spec.); Comoros Anjouan Island (Anjouan Island): USNM 402278 (1 spec.).

P. rhomboidea Kossmann & Räuber 1877

Red Sea (around Sinai Peninsula): TAU 1921 Eilat (1 spec.); USNM 402214, Straits of Jubal (1 spec.); TAU 9401 Eilat (2 spec.); TAU 15999 Eilat (15 spec. vouchers); TAU 3327 Ras Garra (12 spec.); TAU 6291 Ras Garra (4 spec.); TAU 6838 Ras Garra (7 spec.); TAU 3326 Ras Tanaka (4 spec.); TAU 9540 Ras el Millan (1 spec.); Central Red Sea USNM 402272 off Kosier.

Mediterranean (off the Israeli coast): TAU 12871 Hazrot Yasaf (2 spec.); TAU 10679 Shiqmona (3 spec.); TAU 14673 Haifa Bay (1 spec.); TAU 14800 Haifa Bay (1 spec.); TAU 9879 Herzliya (3 spec.);- TAU 15047 Ashdod (6 spec.); TAU 14797 Ashqelon (1 spec.).

P. tominagai Koeda et al. 2014

Red Sea (around Sinai Peninsula): USNM 343766 Eilat (1 spec.); TAU 5285 Eilat (2 spec.); TAU 5279 Eilat (6 spec.); TAU 5282 Eilat (20 spec.); TAU 5286 Eilat (20 spec.); TAU 5424, Eilat (10 spec.); TAU 5284, Dahab, (5 spec.); TAU 5313, Dahab, (1 spec.); TAU 14761, Ras Garra (20 spec.); TAU 14762, Ras Garra (20 spec.); TAU 5710 Ras Garra (2 spec.); TAU 11831 Denis Island (4 spec.). Mauritius (Albatross Island): USNM 343768 (8 spec.). Indonesia, Makuyn Island USNM 180510 (3 spec.).

Material for molecular/genetic analyses

Pempherid specimens (Table 1) were collected by spear while free diving. Samples were collected in the Mediterranean (Haifa, Israel; Alexandria, Egypt), Red Sea, (Eilat, Israel; Al Lith and Farasan Island, Saudi Arabia), Western Indian Ocean (Europa I., Mozambique Channel; Madagascar), Pacific Ocean (Ulithi, Micronesia), Atlantic Ocean (San Blas Is, Panama; Curaçao, Netherland Antilles; Trindade I. Brazil). Fin clips were preserved in 95 % ethanol at room temperature and DNA was extracted following standard protocols (Sambrook et al. 1989).

PCR amplification and sequencing

Two mitochondrial markers (ATPase and cytochrome oxydase 1, CO1) and one nuclear marker (RAG2) were used in this study. All sequences were deposited in GenBank (Accession numbers KJ609322- KJ609479). The amplification of CO1 used fish specific primers VF2T1 and VR1dT1 (Ward et al. 2005). After purification following the manufacturer's protocol (ABI, Perkin-Elmer), sequencing was performed with the primers used in the PCR amplification on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA). In addition, available GenBank CO1 (DNA barcode) sequences for Pempheridae were included in our analyses. These included sequences for *Parapriacanthus ransonneti* Steindachner 1870, which was used as an outgroup. All available *Pempheris* sequences were used, including *Pempheris adusta* Bleeker 1877 from Kwa-Zulu Natal, South Africa and India, *P. schwenkii* from Kwa-Zulu Natal, South Africa, and *P. vanicolensis* from the Seychelles. Two sequences from Indonesia (one from Bali and one from Java) that correspond to unidentified fishes were also included, as they clearly belong to *Pempheris*. Two additional unpublished sequences of *P. oualensis* Cuvier and Valenciennes 1831, were obtained from the Biocode project in Moorea, French Polynesia. It is in fact very likely that many of these identifications are incorrect. We are not changing them here in the text, nor in our figures, because our purpose is not to revise the genus. By maintaining the names, we both highlight the complexity of the question, and also keep a record of the original assignment.

Table 1 Sampling localities, sample numbers and sample codes of *Pempheris* used in this study

Species	Site, country	n	Sample code	Lat–Lon	
P. schomburkii	San Blas, Panama	2	PSC_PAN	N9° 18.964	W78° 14.941
	Curacao, Netherland Antilles	9	PSC_CUR	N12° 12.666	W69° 5.766
P. poeyi	Trindade, Brazil	1	PPO_TRI	S20° 30.033	W29° 20.259
P. flavicycla	Al Lith, Saudi Arabia	4	PSC_KSA_615, 616, 137, 138	N20° 8.744	E40° 15.102
	Farasan Island, Saudi Arabia	8	PSC_KSA_1181-1188	N16° 40.948	E42° 6.055
	Eilat, Israel	1	PVA_EIL	N29° 32.753	E34° 57.460
P. tominagai	Shi'b al Bay, Saudi Arabia	1	PSH_BAY	N22° 38.336	E38° 53.642
	Eilat, Israel	4	PVA_EIL	N29° 32.753	E34° 57.460
P. rhomboidea	Eilat, Israel	34	PRH_EIL	N29° 32.753	E34° 57.460
	Haifa, Israel	43	PRH_HAI	N32° 50.118	E34° 59.265
	Alexandria, Egypt	8	PRH_ALE	N31° 13.406	E29° 53.708
	Nosy Be, Madagascar	1	PRH_NBE	S13° 24.173	E48° 12.405
	Nosy Iranja, Madagascar	1	PRH_NIR	S13° 36.217	E47° 48.691
P. sp	Europa, Mozambique Channel	1	PVA_EUR	S22° 19.808	E40° 21.615
P. sp	Gelub, Ulithi Atoll, Micronesia	4	POU_ULI	N9° 55.855	E139° 49.457
P. sp	Cook Islands	4	POU_COO	S21° 12.056	W159° 45.671

Phylogenetic analyses

We used the computer program MAFFT (Katoh et al. 2002) implemented by the Geneious software package (Drummond et al. 2010) to align the DNA sequences. Phylogenetic relationships were assessed by Maximum Likelihood (ML, GARLI software)((Zwickl 2006), and Maximum Parsimony (MP, PAUP* software Swofford 2003) methods. For Maximum Likelihood topologies, we conducted 10 independent runs in GARLI, using default settings and the automated stopping criterion, terminating the search when the ln score remained constant for 20,000 consecutive generations. Maximum Parsimony searches included 100 random addition replicates and TBR branch swapping with the Multrees option. Statistical confidence in nodes was evaluated using 2,000 non-parametric bootstrap replicates (Felsenstein 1985) (100 replicates for Maximum Likelihood in GARLI, using the auto-mated stopping criterion set at 10,000 generations). Topological differences were tested using a Shimo-daira and Hasegawa test (Shimodaira and Hasegawa 1999) implemented by PAUP, based on resampling of estimated log-likelihoods tests (RELL, 1,000 repli-cates). Genetic distances between clades were calcu-lated using the pairwise distance of individuals between clades minus the pairwise distance of indi-viduals within clades.

Population analyses

Number of haplotypes, haplotype diversity, and nucleotide diversity were calculated using the soft-ware package DNAsp (Librado and Rozas 2009). Population structure (F_{st} and Φ_{st}) and their associated statistical significance were calculated using Arlequin (Excoffier and Lischer 2010) with 1,000 random permutations. Phylogenetic relationships between haplotypes were assessed using a Minimum Spanning Network, MSN or Haplotype Network (Excoffier and Smouse 1994). Haplotype networks were generated in R using HaploNet in the APE package (Paradis et al. 2004) combined with pie diagrams of haplotype frequencies obtained with APE and ARLEQUIN (Excoffier and Lischer 2010).

Results

Morphological identifications

Examination of Red Sea *Pempheris* revealed three species: *P. flavicycla*, *P. rhomboidea* and *P. tomina-gai,* which can be easily distinguished (see key). *Pempheris mangula* may also be present in the Red Sea, but we did not find this species in collections or in the field.

Key for the Red Sea species of *Pempheris*

1a. Black margin on anal fin (Fig. 1a); 57–63 (54–61) lateral line pored scales; 39–43 (38–44) anal segmented rays *P. flavicycla*

1b. Margin on anal fin transparent or dusky; lateral line with 59 or fewer pored scales....2a

2a. Black base of anal fin (Fig. 1b). Lateral-line pored scales 45–51 (47–52); 36–39 (36–39) anal segmented rays *P. tominagai*

2b. Anal fin dusky (Fig. 1c) Lateral-line pored scales 54–57 (52–59); 32–39 (36–42) anal segmented rays *P. rhomboidea*

Number between parentheses provide counts for *P. flavicycla, P. tominagai,* and *P. rhomboidea* from previously published papers (Randall et al. 2013; Koeda et al. 2014).

All samples collected in the Mediterranean were identified as *P. rhomboidea*, while the three species identified from the Red Sea were present and collected at Eilat. In central Saudi Arabia (Yanbu to Thuwal), we saw and collected a single *P. tominagai* individual. All other observed and collected individuals were identified as *P. flavicycla*.

DNA sequences

We analyzed two sets of data. One set was used for a phylogenetic assessment of *Pempheris* based on CO1 using a combination of our data and Genbank sequences. This alignment resulted in 617 aligned base pairs, with 217 variable and 190 phylogenetically informative positions, respectively. The second dataset included our own Lessepsian samples with two mitochondrial markers (CO1 and ATPase) and one nuclear marker (RAG2). The alignment of CO1 resulted in 617 base pairs with 6 variable sites and 5 informative ones, the alignment of ATPase resulted in 677 base pairs, with 7 variable and 2 informative ones, and the alignment of RAG2 resulted in 749 aligned base pairs, with 2 variable and 1 informative one. Thus the entire dataset of the Lessepsian fishes corresponded to an alignment of 2,043 base pairs, with 15 variable sites and 8 informative ones. None of the samples showed any heterozygous position at the nuclear locus (RAG2), making the direct reading of the sequence straightforward and cloning unnecessary.
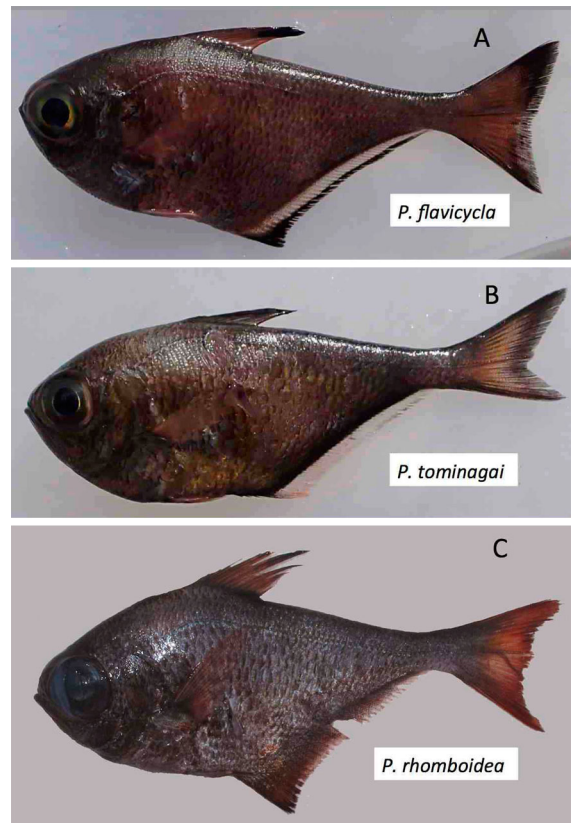

Fig. 1 *Pempheris* species from the Red Sea. *P. flavicycla* (**a**), *P. tominagai* (**b**), and the Lessepsian *P. rhomboidea* (**c**). Samples were collected in central Red Sea (Yanbu, *P. flavicycla* and *P. tominagai*), and northern Red Sea, Gulf of Aqaba (Joey's wreck, *P. rhomboidea*)

Phylogenetic relationships

The analyses of our data were done in two steps. We first proposed a simplified phylogenetic hypothesis for the *Pempheris* samples at hand, in order to identify the species introduced in the Mediterranean. Capitalizing on the presence of CO1 sequences in GenBank, we established a phylogeny of *Pempheris* based on that locus. Below we describe our results presented on Fig. 2.

Using *Parapriacanthus* as an outgroup, basal lineages include the Caribbean samples of *Pempheris*, *P. schomburgkii* Müller and Schomburgk 1848 and *P. poeyi* Bean 1885. Then, Indo-Pacific samples group into three major clades.

The first major clade includes the Red Sea *P. flavicycla* that contains samples we obtained from the

central Red Sea (Saudi Arabia) and one individual from Eilat. Two GenBank sequences from individuals collected in the Seychelles, labelled as *P. vanicolensis,* are closely related to this group. These may correspond to the two recently described subspecies of *P. flavicyla,* one inside the Red Sea and the other outside of the Red Sea (Randall et al. 2013). A group of individuals collected in French Polynesia (labelled

P. oualensis, but possibly *P. otaitensis,* Randall, pers. comm.), Micronesia, and the Cook Islands clusters with this major clade as well. Two GenBank samples collected in Indonesia are also part of this clade.

A second major clade includes individuals collected from the Red Sea/Indian Ocean. There are four subclades that include (1) Samples from Reunion Island (labelled *P. oualensis*) and Europa Island, (2)
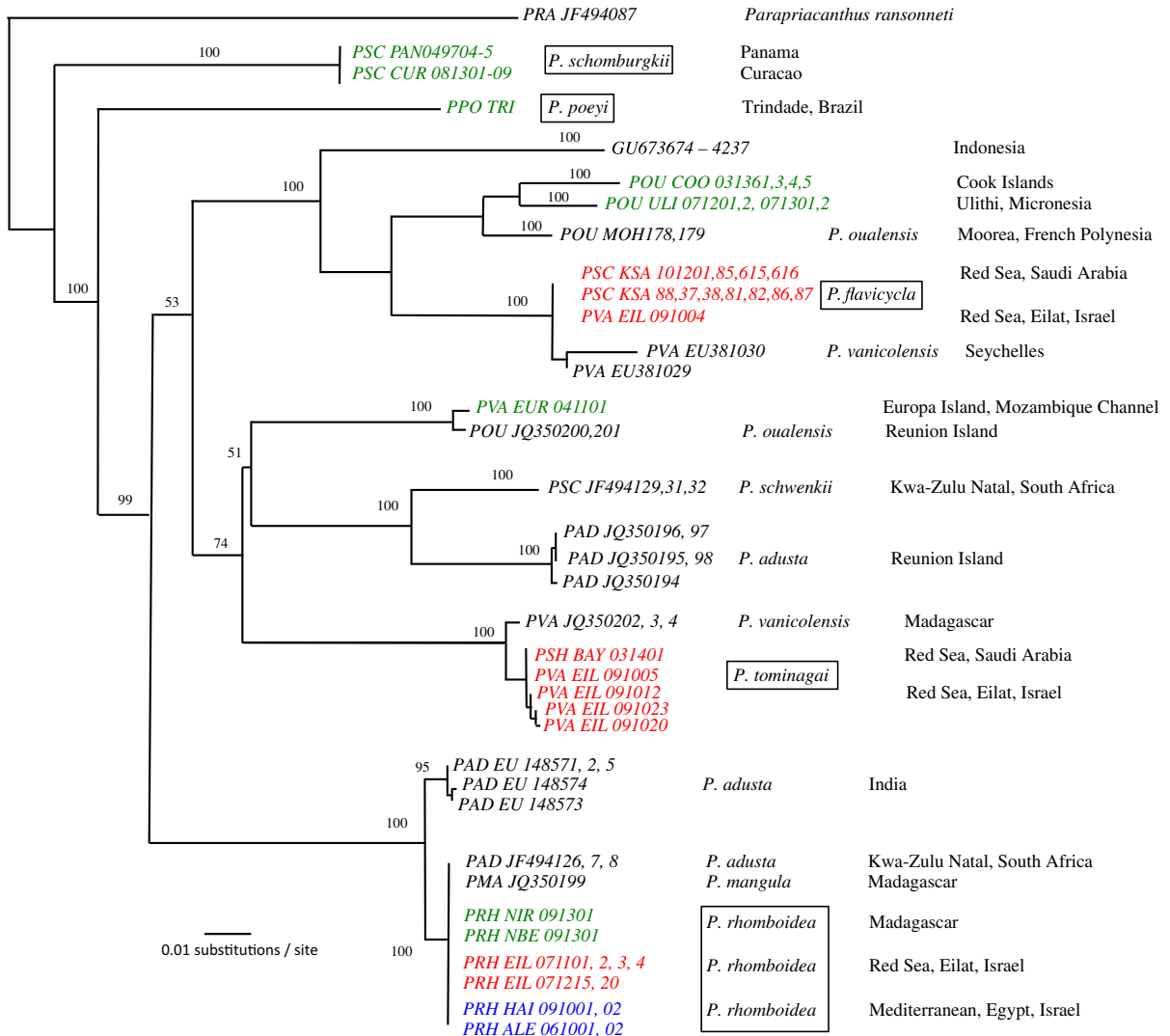

Fig. 2 Phylogenetic relationships of *Pempheris* species. Maximum Likelihood phylogenetic reconstruction based on the mitochondrial cytochrome oxidase 1 (CO1) marker. Labels are from Table 1. Samples labeled in *black* correspond to sequences from GenBank. Names from those samples are from the original authors, in many cases these are misidentified specimens, but are kept here to allow the reader to cross-reference them with

GenBank accessions and provide a understanding of the complexity of identifying species in this genus. Samples from this study were labeled in *red* and collected in the Red Sea, samples labeled in *blue* were collected in the Mediterranean. Other samples are labeled in *green*. Names that are *boxed* correspond to our own identifications

Samples from South Africa (labelled *P. schwenkii*), (3) Samples from Reunion Island (labelled *P. adusta*), and (4) Our own samples from Eilat, identified as *P. tominagai*, which cluster with GenBank samples collected in Madagascar and labelled *P. vanicolensis.*

A third major clade includes two subclades, one subclade containing samples from India and labelled *P. adusta*, the other including samples from Genbank collected in South Africa (labelled *P. adusta*) and Madagascar (labelled *P. mangula*) and our own samples collected in Madagascar, Eilat, and the Mediterranean, that we assigned to *P. rhomboidea.*

For individuals that we collected, we also sequenced an additional mitochondrial marker (ATP-ase) and one nuclear marker (RAG1). The phylogenetic topologies described above were upheld when using those additional molecular markers (not shown).

Population structure

All individuals collected in the Mediterranean clustered in a single clade, identified as *P. rhomboidea,* referred to here as the "Lessepsian Clade". Of the samples collected in the Red Sea, 15 specimens (out of 23, 65 %) from Eilat belonged to *P. rhomboidea*. In contrast, none of the 12 individuals collected in Saudi Arabia belonged to the Lessepsian clade.

In this section of the study, we analyzed the Lessepsian individuals using two mitochondrial markers and one nuclear marker. We found no evidence of lowered genetic diversity or "bottleneck" in the Mediterranean populations. Indeed, haplotype diversity was similar in Mediterranean and Red Sea samples (0.790 and 0.733, respectively, Table 2). Evidence of genetic differences between Mediterranean and Red Sea samples was not seen visually with a haplotype network reconstruction (Fig. 3), however population genetic metrics (Fst and Nm) (Table 3) did suggest low levels of gene flow and migration (overall number of migrants per generation, Nm = 45.3; Fst = 0.011).

Discussion

Identification of the Lessepsian *Pempheris*

Our molecular analyses validated the existence of at least three distinct species of *Pempheris* in the Red

Table 2 Genetic characteristics of Red Sea and Mediterranean populations of *P. rhomboidea* based on combined mitochondrial markers, mitochondrial markers taken independently, the nuclear marker RAG2, and all markers taken together

Locality/locus	n	NH	HD	π
CO1				
Red Sea Eilat, Israel	15	5	0.562	0.00191
Eilat, Israel	40	10	0.623	0.00164
Mediterranean	51	8	0.729	0.00228
Alexandria, Egypt	8	5	0.786	0.00342
Haifa, Israel	43	5	0.715	0.00210
All samples	66	9	0.704	0.00228
ATPase				
Red Sea Eilat, Israel	15	6	0.571	0.00172
Mediterranean	51	3	0.353	0.00053
Alexandria, Egypt	8	2	0.250	0.00037
Haifa, Israel	43	3	0.375	0.00057
All samples	66	8	0.408	0.00080
RAG2				
Red Sea Eilat, Israel	15	1	0.000	0.00000
Mediterranean	51	3	0.115	0.00015
Alexandria, Egypt	8	1	0.000	0.00000
Haifa, Israel	43	3	0.135	0.00018
All samples	66	3	0.0089	0.00012
CO1 + ATPase				
Red Sea Eilat, Israel	15	8	0.733	0.0018
Mediterranean	51	10	0.751	0.00174
Alexandria, Egypt	8	4	0.643	0.00174
Haifa, Israel	43	7	0.748	0.00130
All samples	66	16	0.754	0.00189
CO1 + ATPase + RAG2				
Red Sea Eilat, Israel	15	8	0.733	0.00115
Mediterranean	51	13	0.784	0.00095
Alexandria, Egypt	8	4	0.643	0.00110
Haifa, Israel	43	10	0.790	0.00091
All samples	66	19	0.779	0.00100

n number of samples, *nH* number of haplotypes, *HD* haplotype diversity, *π* molecular diversity

Sea: *P. tominagai*, *P. flavicycla* and the Lessepsian *P. rhomboidea*. The morphological analysis of the Mediterranean individuals confirmed their identification as *P. rhomboidea*. They are clearly distinguished from *P. vanicolensis*. *Pempheris vanicolensis* is unique in the genus *Pempheris* in having the following combination of characters: '57–63 pored lateral line scales; 40–43 anal fin rays; 5½ scale rows above lateral line; bright
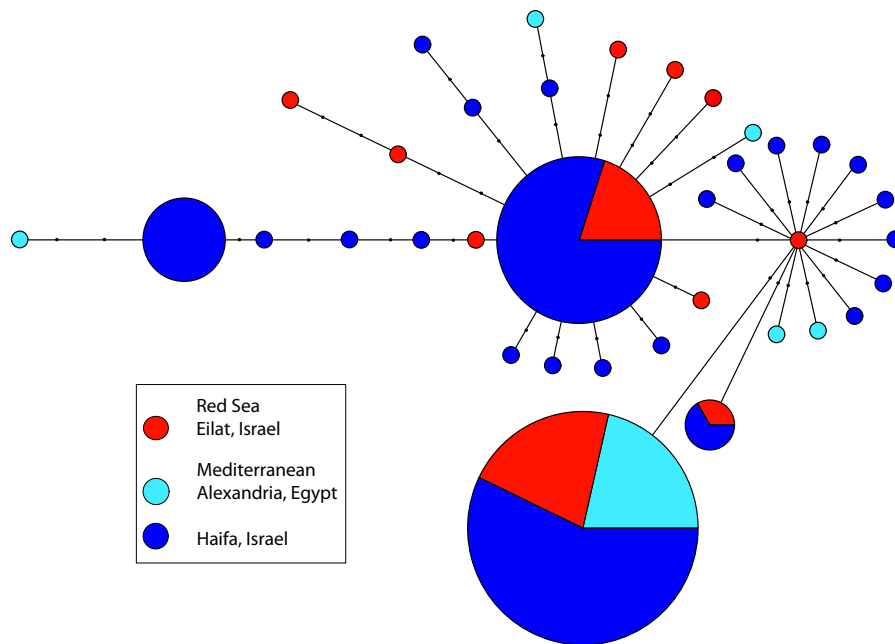
Fig. 3 Haplotype network of *P. rhomboidea* based on combining one nuclear (RAG2) and two mitochondrial (CO1 and ATPase) markers. Haplotype networks based on individual molecular markers are shown in Supplementary Figure. 1 (Figure S1)

Table 3 Population differentiation between Red Sea and Mediterranean populations of *P. rhomboidea*

	Fst	Nm
CO1	0.02370	20.59
CO1 (larger dataset)	0.07358	6.30
ATPase	0.00098	509.63
RAG2	0.0133	37.00
Mt DNA (CO1 + ATPase)	0.01085	45.58
All markers	0.01092	45.30

yellow pectoral fin in fresh specimens; no black spot on pectoral fin base; clearly keeled prepelvic area' and an anal fin with a black base (Koeda et al. 2010). Instead *P. rhomboidea* has 54–57 lateral-line pored scales; 32–39 anal segmented rays and a dusky anal fin (Fig. 1c) and this can be taken as morphological evidence of a long lasting misidentification of this invasive species.

Further studies based on secondary inspections of morphological characters of individuals associated with DNA sequencing and a larger sample of Red Sea material is needed to ascertain the number and identity of species occurring in the Red Sea.

Southern Red Sea samples

The absence of *P. rhomboidea* in the Saudi Arabia samples is not due to a sampling bias. Examination of hundreds of individuals underwater revealed that all but a single individual were identified as *P. flavicycla*. One individual, that was collected, was identified as *P. tominagai* (Figs. 1, 2). *Pempheris rhomboidea* is likely to be very rare in central Saudi Arabia. Interestingly, the GenBank samples from the Seychelles, an area geographically close, groups with Saudi Arabia samples of *P. flavicycla*. A single sample from Eilat (northern Red Sea) also clusters with this group, indicating that this species ranges at least from Eilat to the Seychelles, but may not be abundant in Eilat (1 individual out of 23).

Population structure of Lessepsian invaders

Pempheris rhomboidea ranges from the northern Red Sea to at least South Africa, since samples from Madagascar and South Africa cluster with this clade (Fig. 2). This finding extends the distribution of this species to the Indian ocean, in agreement with the general hypothesis that successful Lessepsian invaders

have a relatively large geographical range (Belmaker et al. 2013). Its abundance within the Red Sea deserves close attention, as it seems to be more common in the north, and quite rare in the central and southern part. This is also consistent with the idea that abundant species in the northern Red Sea may be better candidates for Lessepsian invasions (Golani 1993). The lack of evidence of a genetic bottleneck in the Mediterranean population is not unusual; it is the norm for Lessepsian migrants with very few notable exceptions (Bernardi et al. 2010). In contrast, the lowered gene flow levels observed between Red Sea and Mediterranean populations in this species are unusual. Further work is needed to identify its possible reasons.

Differential invading potential in cryptic species

We collected all three Red Sea species of *Pempheris* in the northern Red Sea (Eilat), yet only one species was identified in the Mediterranean. This may be due to the existence of different ecological traits and dispersal capabilities among Red Sea Pempheridae (Belmaker et al. 2013), but also to stochastic events that may occur in the traversing of the Suez Canal. The species that invaded the Mediterranean, *P. rhomboidea*, was also the most commonly caught species in Eilat (65 % of individuals). While we did not specifically target this species, our sampling method may have been biased towards it. Further work to properly estimate the relative abundance of each of the three species in the northern Red Sea, especially in the Gulf of Suez that is effectively the launching pad for Suez Canal introductions (Golani 1993), may shed light on the mechanisms involved in the successful invasion.

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