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Short Communication

Lack of a genetic bottleneck in a recent *Lessepsian bioinvader*, the blue-barred parrotfish, *Scarus ghobban*

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ABSTRACT

The present study investigates the genetic diversity of *Scarus ghobban*, a recently introduced parrotfish in the Mediterranean Sea via the Suez Canal. Two mitochondrial and one nuclear DNA regions were sequenced and phylogenetic relationships investigated, from samples collected from Lebanon and across its natural range. *Scarus ghobban* clustered in two major clades, Pacific Ocean and Indian Ocean, indicating strong population structure, or cryptic speciation. Expectedly, Mediterranean samples clustered with Indian Ocean-Red Sea individuals. However, unlike other recent Lessepsian invaders, *S. ghobban* displayed high genetic diversity. These results underscore that genetic diversity is a poor predictor of success of an invasive species.

1. Introduction

Biological invasions are becoming the focus of attention due to their potential for shedding light on fundamental issues in ecology, and more importantly for their growing impact on native organisms, which often result in disastrous ecological and economical outcomes (Sax et al., 2005). Thus, at many levels, understanding the dynamic of biological invasions is essential to accurately predict the potential and effectiveness for a given species to invade. Empirical and theoretical data have been used to model how biological invasions may proceed. In general, few individuals are involved in a given biological invasion, which should result in a genetically bottlenecked invading population. Such lowered diversity tends to decrease the genetic fitness of the invading population, yet this may be offset by the release from much ecological pressure, such as competition, predation, and parasitic load.

Biological invasions are difficult to study, mostly because the time and source of invasion are poorly known, and the early stages of invasion are usually not recorded. This situation is even more challenging in the marine environment, where direct observations are logistically complicated and few systems are amenable to investigation. Yet, one marine system seems to be ideal for the study of marine bioinvasions: the Mediterranean Sea.

The opening of the Suez Canal in 1869 resulted in a movement of marine organisms, termed Lessepsian migration (after the Canal engineer Ferdinand de Lesseps), and initiated a process of invasion from the Red Sea into the Mediterranean Sea. In this system, the source (Red Sea) and route (Suez Canal) of the invaders are known. This phenomenon is very significant as out of the 558 species of exotic metazoans recorded from the Mediterranean, 64% are introduced via the Suez Canal (Galil, 2008). To date, three cartilaginous and about 70 bony fishes are considered to be Lessepsian migrants (Galil, 2008; www.ciesm.org/atlas).

In general, it is expected that invading populations be genetically depressed due to the small number of effective original migrants, a phenomenon known as founder effect resulting in a genetic bottleneck (Azzurro et al., 2006). In contrast, all Lessepsian species, except for the recently recorded blue-spotted cornetfish *Fistularia commersonii*, showed very high levels of genetic diversity, which were essentially indistinguishable from their source (Red Sea) populations (Golani et al., 2007). Since all studied species were early Lessepsian migrants (first Mediterranean record between 1902 and 1964) one simple explanation was that the lack of genetic bottleneck resulted from multiple invasions, which slowly built up the genetic diversity observed in Mediterranean populations (Bucciarelli et al., 2002; Hassan et al., 2003; Hassan and Bonhomme, 2005; Azzurro et al., 2006). This explanation was also consistent with the extreme bottleneck observed in *F. commersonii*, a very recent invader (first observed in 2000), where only two haplotypes

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(possibly corresponding to as few as two females) were observed in the Mediterranean (Golani et al., 2007).

In this study we aimed to test the hypothesis that the very recently invader and rare Lessepsian migrant, the blue-barred parrotfish *Scarus ghobban*, shows low levels of genetic diversity. The blue-barred parrotfish is a widespread species in the Indo-Pacific, where it is abundant from the Red Sea to the tropical Eastern Pacific. The first record of *S. ghobban* in the Mediterranean dates back to 2001 (Goren and Aronov, 2002). Since then, very few individuals have been seen and collected from the eastern Mediterranean (Bariche and Saad, 2005; Golani and Levy, 2005). Together, the very recent occurrence of the species in the Mediterranean, and its rarity (very few individuals have been sighted), make *S. ghobban* unique among the studied Lessepsian migrants, and predict a low genetic diversity in the invading population.

Here, we were able to obtain five specimens of *S. ghobban* from the Mediterranean, and also a panel of specimens from the Indo-Pacific. The goal of our study was to determine the degree of genetic diversity in the Mediterranean population based on two mitochondrial and one nuclear gene regions, and to test the prediction that a recent invader is likely to exhibit the genetic signature of a bottleneck.

2. Materials and methods

Samples were collected for this study by spear, or at fish markets, in localities described in Table 1. Samples from Egypt (Red Sea) were provided by E. Beck and L. van Herwerden, James Cook University, Townsville, Australia. Fin or gill tissues were preserved in 95% ethanol at room temperature and DNA was extracted following standard protocols (Sambrook et al., 1989). In addition, twenty one sequences (20 ATPase and 1 D-loop) were obtained from GenBank (Table 1).

Two mtDNA (control region, ATPase 6 and 8) and one nuclear DNA (1st intron of the ribosomal protein S7) regions were used in this study. All sequences were deposited in GenBank (Accession Numbers: GQ396165–GQ396220). Amplification of the 5′ hypervariable portion of the mitochondrial control region (also called D-loop), the ATPases 6 and 8, and the 1st intron of the nuclear S7 ribosomal protein (S7) used the primers and protocols of Azzurro et al. (2006), Quenouille et al. (2004) and Chow and Hazama (1998), respectively. After purification following the manufacturer's protocol (ABI, Perkin-Elmer), sequencing was performed in both directions with the primers used in the PCR amplification on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA). We used the computer program Clustal V implemented by Sequence Navigator (Applied Biosystems) to align the DNA sequences. Number of haplotypes, haplotype diversity, and nucleotide diversity were calculated using the software package DNAsp (Rozas et al., 2003).

Phylogenetic relationships were assessed by maximum likelihood (ML, GARLI software, Zwickl, 2006), maximum parsimony (MP, PAUP* software, Swofford, 2003), and neighbor-joining (NJ, PAUP software), methods. Substitution models that best fitted our data were obtained with Modeltest (HKY + G) (Posada and Crandall, 1998), and used for ML and NJ reconstructions. For ML analyses, 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. The highest likelihood of those runs was retained and is presented here. Maximum parsimony searches included 100 random addition replicates and TBR branch swapping with the Multrees option. Statistical confidence in nodes was evaluated using 2000 non-parametric bootstrap replicates (Felsenstein, 1985) (100 replicates for maximum likelihood in GARLI, using the automated stopping criterion set at 10,000 generations). Topological differences were tested using a Shimodaira and Hasegawa test (Shimodaira and Hasegawa, 1999) implemented by PAUP*, based on resampling of estimated log-likelihoods tests (RELL, 1000 replicates).

3. Results and discussion

Sequencing of the control region resulted in 360 aligned base pairs. Of those, 82 were variable and 68 were phylogenetically informative. The sequencing of the ATPase resulted in 842 aligned bases, with 45 variable and 40 informative positions. A random subset of each mitochondrial clade was amplified for the nuclear marker. The nuclear 1st intron of the ribosomal S7 protein fragment was 540 base pairs long, with 19 variables and 15 informative positions. None of the samples showed any heterozygous position at the nuclear locus, making the direct reading of the sequence straightforward and cloning unnecessary. The different phylogenetic methods yielded topologies that were not statistically significantly different (SH test, $p > 0.2$), thus we decided to present results based on the Maximum Likelihood method (Fig. 1). Although the exact topologies differed across the three molecular regions, the Indian Ocean and Pacific Ocean samples of *S. ghobban* were consistently reciprocally monophyletic (100% bootstrap support in most cases, Fig. 1). Since both mitochondrial and nuclear markers consistently separated such samples, it is likely that the species called *S. ghobban* is either a complex of very divergent populations, or even a complex of cryptic species. This warrants further investigation, which is currently underway (Beck

Table 1
Collection localities for *Scarus ghobban*. Columns represent the number of individuals included in the study, and the abbreviations used in Fig. 1. Individuals from Egypt were obtained from James Cook University (JCU). Twenty-one sequences were obtained from GenBank. A summary of data for three regions, Mediterranean Sea, Indian Ocean, and Pacific Ocean is also given for each locus. Summary data correspond to: number of individuals, number of haplotypes, haplotype diversity, and nucleotide diversity, respectively.

Region Sampling site	Label	ATPase	D-loop	S7	Source
Mediterranean Sea		5, 2, 0.6, 0.001	5, 5, 1, 0.03	2, 2, 1, 0.005	
Lebanon	LEB	5	5	2	This study
Indian Ocean		7, 4, 0.8, 0.001	5, 5, 1, 0.03	1, 1, 1, -	
Zanzibar	ZA	4	2	1	This study
Egypt (Red Sea)	EGY	3	3	–	JCU
Pacific Ocean		35, 9, 0.8, 0.03	11, 10, 0.98, 0.02	4, 3, 0.83, 0.01	
Taiwan	TAI	–	1	–	GenBank
Christmas Isl.	XMA	10	–	–	GenBank
Christmas Isl.	XMA	3	2	1	This study
Galapagos Isl.	FLO	5	3	2	This study
Mexico (S. of Cortez)	AVE, VEN	6	2	1	This study
Panama	MMP	1	3	–	This study
Panama	PM	10	–	–	GenBank

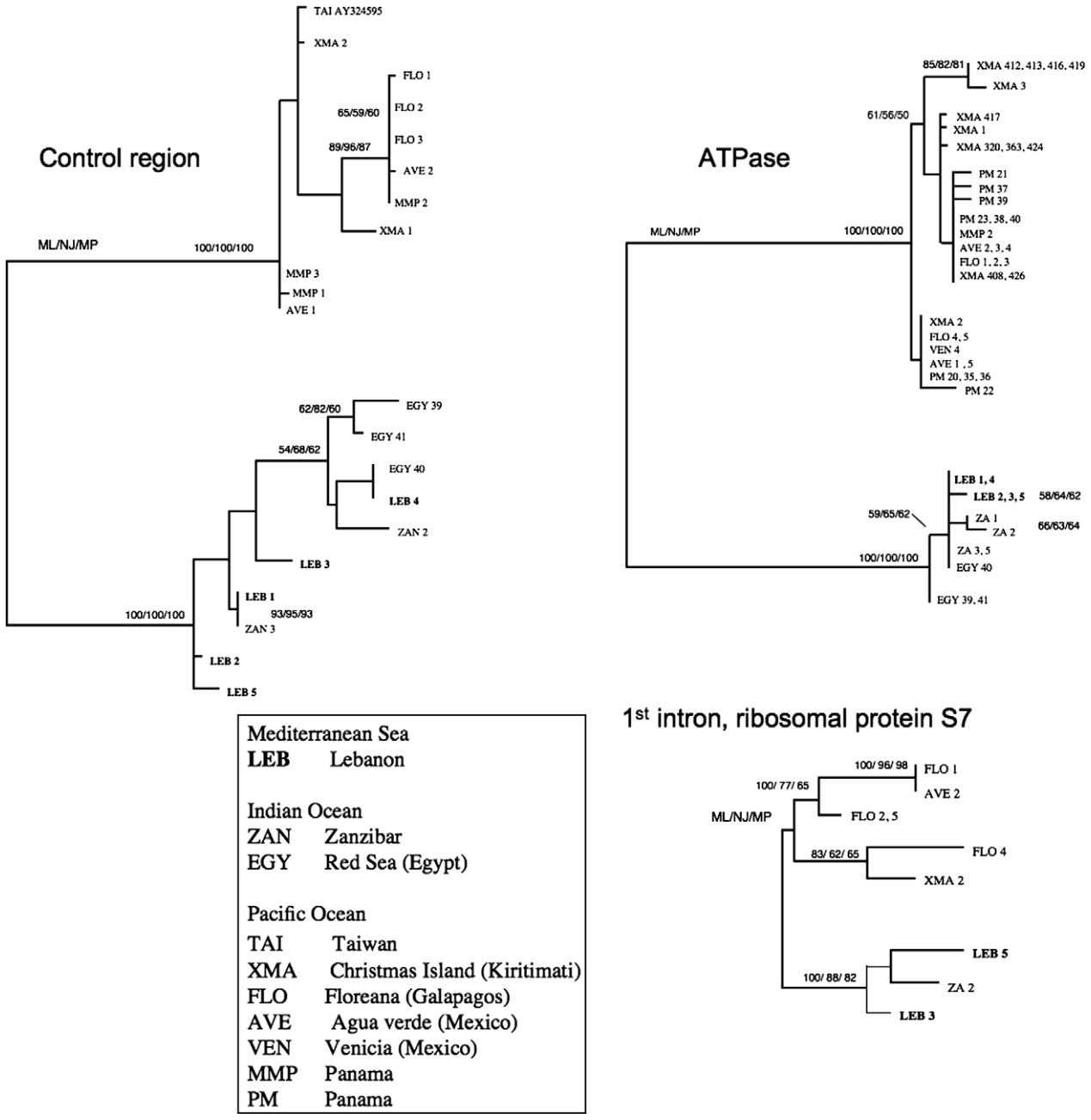

Fig. 1. Phylogenetic relationships of *Scarus ghobban* individuals based on control region, ATPase, and 1st intron of the ribosomal protein S7 sequences. Three reconstruction methods were used, maximum likelihood (shown here), neighbor-joining, and maximum parsimony, which resulted in identical topologies. Bootstrap support above 50% for each of the methods used is shown next to the corresponding node.

and van Herwerden, pers. com.). Mediterranean Sea samples of *S. ghobban* always clustered with Indian Ocean samples (Red Sea and Zanzibar samples) for both nuclear and mitochondrial molecular markers. In addition, while three Mediterranean samples showed the same ATPase sequence, none of the samples shared a sequence at the more variable control region or the nuclear S7 region (Fig. 1).

Marine bioinvasions are becoming increasingly devastating, and a clear understanding of their dynamics is essential to manage and protect native resources. Here, we focused on a recent *Lessepsian bioinvader*, the blue-barred parrotfish, *S. ghobban,* to test a specific hypothesis: that Mediterranean Sea samples are expected to show low levels of diversity due to the very recent invasion and rarity of the species. Following the opening of the Suez Canal, marine organisms started to invade the Mediterranean, an area with relatively few competitors, when compared with the species-rich Red Sea. The success of hundreds of Lessepsian species, in the Mediterranean, is a bit of a puzzle. The lack of genetic bottlenecks in most investigated Lessepsian species suggested that successive waves of invasions were the source of genetic diversity of the invaders (Hassan et al., 2003; Hassan and Bonhomme, 2005; Azzurro et al., 2006). A genetic bottleneck was not observed in the recent invasion of the Italian island of Linosa by *Siganus luridus* (Azzurro et al., 2006), but was indeed observed in the blue-spotted cornetfish, *Fistularia commersonii* (Golani et al., 2007), presenting conflicting results about this issue.

The presence of a very recent invader, such as *Scarus ghobban,* gave us the unique opportunity to determine if early invasions

are indeed the result of few "lucky" individuals. Data presented here indicate that all five sampled individuals are genetically different from each other, and that haplotype and nucleotide diversity is not lowered when considering the Mediterranean Sea population (Table 1). This is particularly remarkable when one compares these results with another Lessepsian migrant, the blue-spotted cornetfish *Fistularia commersonii*, where the same molecular marker (control region) was used. There, while in its natural range genetic diversity was very high (haplotype diversity = 0.997), out of 52 individuals sampled in the Mediterranean, only two haplotypes were observed, suggesting at most two invasion events (Golani et al., 2007). *Fistularia commersoni* also invaded the Mediterranean very recently, 1 year before *S. ghobban*, in 2000 (Golani, 2000), yet unlike *S. ghobban*, its population exploded and rapidly expanded across the entire Mediterranean (Golani et al., 2007). The five *S. ghobban* samples exhibited more genetic diversity than all *F. commersonii* put together, resulting from anything between a single to five separate invasion events. It is likely that these results indicate that genetic diversity alone cannot be taken as a gauge of success in the invasion event.

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