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Genetics of the early stages of invasion of the Lessepsian rabbitfish *Siganus luridus*

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Abstract

Information on the initial stages of dispersal and settlement are of great interest in understanding the dynamics of biological invasions and in designing management responses. A newly settled population of the Lessepsian rabbitfish migrant *Siganus luridus,* that arrived in Linosa Island (Sicily Strait) in 2000, offered a unique opportunity to examine the genetic variability of the early phase of invasion and the starting point to test genetic variation within and between colonist and source populations.

Demographics and dynamic aspects of *S. luridus* in the Mediterranean were evaluated by using phylogeographic and demographic (coalescent) methods based on DNA sequences of the mitochondrial control region. Sequences from 95 *S. luridus*, 25 *Siganus rivulatus*, and one of *Siganus (Lo) vulpinus* and *S. doliatus* were used. Samples were collected in one locality in the Red Sea (Eilat) and three localities in the Mediterranean (Israel, Greece and Linosa, Italy). Data showed (for the first time in a Lessepsian migrant) a lowering of the genetic diversity of the invading population (Mediterranean) (haplotype diversity 0.879, nucleotide diversity 0.592) compared to the parental one (Red Sea) (haplotype diversity 0.978, nucleotide diversity 0.958).

Within the Mediterranean populations, there was no pattern of regional separation and mitochondrial diversity appeared to be preserved during the Linosa colonization, with no traces of founder events. Such evidence agrees with the idea that Lessepsian migration involves many individuals from its earliest stages.

Keywords: Genetic differentiation; Lessepsian migration; Mitochondrial DNA; *Siganus luridus*

1. Introduction

Biological invasions and the ensuing homogenization of the world's biota pose one of the greatest contemporary threats to biodiversity and ecosystem function (Vitousek et al., 1996; Bright, 1999; Van Driesche and Van Driesche, 2000; Occhipinti-Ambrogi and Savini, 2003) leading to serious ecological and economical consequences (Groves and Burdon, 1986; Sax et al., 2005). In recent years, much of the research efforts have been focused in identifying the factors that allow successful invasions, with the promising goal to predict the identity of future invaders and vulnerable ecosystems (Mack, 1996; Ricciardi and Rasmussen, 1998; Goodwin et al., 1999; Alcaraz et al., 2005). Generally the success of invasion is thought to be

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influenced by intrinsic attributes of the invaders, such as genetic variability, growth rates, widening of the feeding niche, reproductive strategies, and tolerance to abiotic factors (Ehrlich, 1989; Byers and Goldwasser, 2001). In other cases, the role of external factors, and most importantly the attributes of the recipient community, such as species diversity (Elton, 1958; Lodge, 1993; Moyle and Light, 1996; Tilman, 1997; Chapin et al., 1998; Levine and D'Antonio, 1999) or the release from natural controls including predators, competitors and parasites (Ugarteburu and McQuaid, 1998; Mitchell and Power, 2003; Torchin et al., 2002, 2003; Colautti et al., 2004) have been suggested as the primary cause of invasion success.

Genetic studies, even if relatively rare, are thought to offer insights into the mechanisms of invasions, namely the relationship of the genetic structure of the invasive populations and their ability to respond to natural selection (Lee, 2002; Allendorf and Lundquist, 2003). Ecological conditions in the newly colonized areas are usually considerably different than the native ones so that new physiological stresses (temperature and salinity, for example) would force colonizers to adapt to their new environment based on the existence of sufficient genetic variability. For these reasons, high genetic variability of invasive populations has been listed among the prerequisites for successful colonization (Safriel and Ritte, 1980; Ehrlich, 1989; Golani, 1998; Tsutsui et al., 2000). Yet, the role of genetic variation in predisposing invasive species to successful establishment is a widely debated issue (Holland, 2001). Thus far, no clear model has emerged to encompass the range of different genetic characteristics exhibited by invasive populations (Holland, 2000) and the history of invasions is rich of bottlenecked populations (with reduced genetic diversity) with great invasive potential (Tsutsui et al., 2000; Frankham, 2005).

The complexity of these issues necessitates a thorough understanding of the invasion process (Hastings et al., 2005; Puth and Post, 2005) which is particularly difficult to achieve in aquatic systems where cryptic species, recruitment variability, and poor direct observation add a layer of complexity (Wonham et al., 2000). One system, the Lessepsian bioinvasion, has proven more tractable due to its confined historical and geographic context (Por, 1978) becoming a case study for marine bioinvasion research. In 1869, the excavation of the Suez Canal, engineered by Ferdinand de Lesseps, opened the gate to a large number (at least 300) of Red Sea species that invaded the Mediterranean Sea generating dramatic modifications in the local communities (Por, 1978; Galil, 2000). These species, called Lessepsian migrants, comprise approximately 62 fishes, with new species regularly being added to the list (Golani et al., 2004). Among them, rabbitfishes are considered one of the most invasive taxa, presenting high abundances in the eastern Mediterranean (Ben-Tuvia, 1964; Bariche, 2002).

Rabbitfish currently form a monogeneric family (family Siganidae, genus *Siganus*) that comprises 27 marine species divided in two subgenera, *Siganus* and *Lo* (Woodland, 1990; Tyler and Sorbini, 1991; Kuiter and Debelius, 2001). Early on, *Siganus rivulatus* (Forsskål, 1775) was recorded in the Levantine area of the Mediterranean (1927), followed in 1956 by a second species, the dusky rabbitfish *Siganus luridus* (Rüppell, 1828) (Ben-Tuvia, 1964). The natural range of the dusky rabbitfish is encompassed by two populations that can be distinguished morphologically, one is restricted to the Red Sea and the other to the western Indian Ocean (Woodland, 1983, 1990). Detailed morphological analysis showed that Lessepsian Mediterranean rabbitfishes are derived from the Red Sea population (Woodland, 1990).

In the western Mediterranean, rabbitfishes have been recorded as far as Tunisia (Ktari-Chakroun and Bouhlal, 1971) and no subsequent evidence of geographical expansion has been documented (Quignard and Tomasini, 2000; Bradai et al., 2004). Recently, two adult *S. rivulatus* individuals were recorded in the southern Adriatic (Dulčić and Pallaoro, 2004) and a newly settled population of *S. luridus* was also recorded in the Italian Island of Linosa (Azzurro and Andaloro, 2004), in the Sicily Strait. The situation of *S. luridus* individuals in Linosa is particularly interesting because it represents a cohort that apparently arrived in June 2000 in an area considered to be the western boundary to Lessepsian migration (Por, 1978; Quignard and Tomasini, 2000). Hence, the Linosa population provides a unique opportunity to examine the genetic variability of the early phase of invasion.

Biological invasions are often seen as the success of few individuals capable of invading, surviving, reproducing, and eventually overwhelming a new habitat. Such a scenario should leave a genetic signature of "founder effect", or of "population bottleneck". Indeed, the genetic structure of an invasive population depends on several factors, including the effective population size of the introduction event and the genetic diversity of the source population (Holland, 2000). In the case of Lessepsian rabbitfishes, however, previous studies have shown that genetic diversity, in Mediterranean populations, was very high and that there was no evidence of founder effect or bottleneck (Bonhomme et al., 2003;

Hassan et al., 2003). Analogous outcomes were also drawn for others Lessepsian migrants as *Atherinomorus lacunosus* (Bucciarelli et al., 2002), *Upeneus pori* and *Upeneus moluccensis* (see Golani and Ritte, 1999; Hassan and Bonhomme, 2005).

The goal of the present study was to capitalize on the unique situation of the recently founded Linosa population by using molecular markers to determine: 1) the mode of invasion of the newly arrived *S. luridus* in Linosa (i.e. few individuals, with founder effect, or many diverse individuals) and 2) if it is possible to reconstruct the route and timing of this invasion. To approach these questions, phylogeographic and demographic (coalescent) methods based on DNA sequences of the mitochondrial control region were used.

2. Materials and methods

2.1. Collections and DNA samples

Samples were collected at one site in the Red Sea and seven sites in the Mediterranean (Fig. 1). Fishes were collected using traps in the Red Sea, trammel nets at the Israel sites of the Mediterranean Sea, and free diving with spearguns at the remaining sites. After collection, tissue samples were immediately placed in 95% ethanol and stored at ambient temperature in the field, and then at 4 °C in the lab. Total genomic DNA was prepared from 75 to 150 mg of tissue by proteinase K digestion in lysis buffer (10 mM Tris, 400 mM NaCl, 2 mM EDTA, 1% SDS) overnight at 55 °C. This was followed by purification using chloroform extractions and alcohol precipitation (Sambrook et al., 1989). Sample numbers and locations are presented in Table 1.

2.2. PCR amplification and sequencing

Amplification of the mitochondrial control region was accomplished using the primers CR-A and CR-E (Lee et al., 1995). All amplifications (25 ml) contained 10 to 100 ng of DNA, 10 mM Tris HCL (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 units of Taq DNA Polymerase (Perkin-Elmer, Norwalk, Connecticut), 150 mM of each dNTP, and 0.3 mM of each primer, and used a cycling

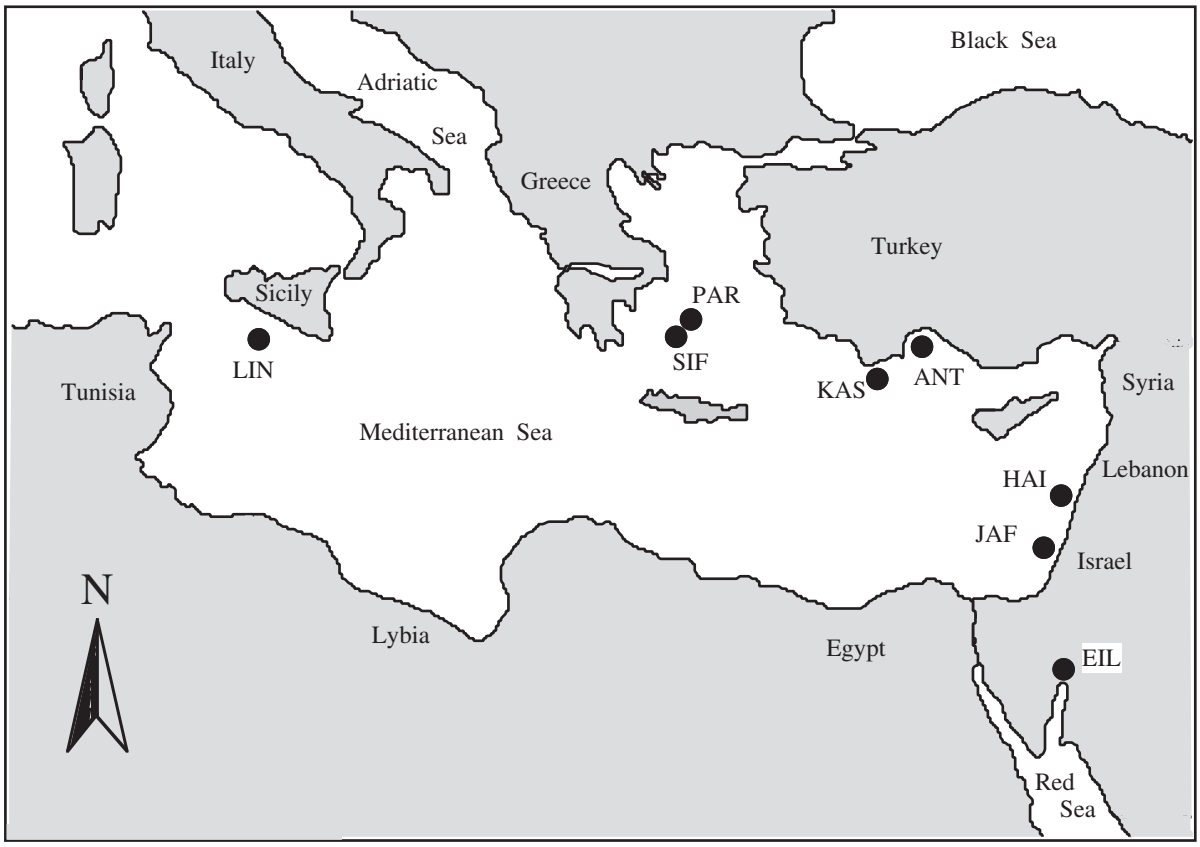

Fig. 1. Sampling locations of *S. luridus* and *S. rivulatus*. Labels refer to sampling locations described in Table 1.

Table 1
Collection localities for *Siganus* spp

Species		Sampling site	*n*	nh	hd	π	Label
Siganus luridus			95	32	0.918	0.720	SLU
Red Sea							
	Israel	Eilat	26	21	0.978	0.958	EIL
Mediterranean			69	15	0.879	0.592	
	Israel	Jaffa	01	–	–		JAF
		Haifa	19	09	0.871	0.576	HAI
	Turkey	Antalya	01	–	–		ANT
	Greece		22	09	0.853	0.645	
		Kastellorizon	09	–	–		KAS
		Sifnos	08	–	–		SIF
		Paros	05	–	–		PAR
	Italy	Linosa	26	08	0.883	0.522	LIN
Outgroup							
Siganus rivulatus			23	12	0.870	0.611	SRI
Red Sea							
	Israel		04	04	1.000	0.911	EIL
Mediterranean			19	09	0.853	0.533	
	Israel	Jaffa	07	–	–		JAF
	Turkey	Antalya	11	–	–		ANT
	Greece	Kastellorizon	01	–	–		KAS

Columns represent the number of individuals per population (*n*), number of haplotypes (nh), haplotype diversity (hd), and nucleotide diversity (π). The locality abbreviations as in Fig. 1, and the acronyms used in Fig. 2 are in the right column (label).

profile of 45 s at 94 °C, 45 s at 48 °C, 1 min at 72 °C, for 35 cycles. Automated sequencing was performed in both directions with the primers used in the amplification using an ABI 3100 automated sequencer (Applied Biosystems, Foster City, California).

2.3. Phylogenetic analyses

Phylogenetic analyses were performed on *S. luridus* control region sequences using *S. rivulatus*, *Siganus (Lo) vulpinus* and *S. doliatus* sequences as outgroups. *S. rivulatus* sequences were from this study (Table 1), while *S. (Lo) vulpinus*, and *S. doliatus* sequences were from Genbank (AY057327 and AY057324). We used the computer program Clustal V implemented by Sequence Navigator (Applied Biosystems) to align the DNA sequences. Phylogenetic relationships were assessed using Maximum Parsimony, and Neighbor-Joining (Saitou and Nei, 1987) (Kimura 2 distance, Kimura, 1980) methods implemented by the Software package PAUP (Phylogenetic Analyses Using Parsimony, version 4.0, Swofford, 1998). Topological confidence was evaluated with 1000 bootstrap replicates (Felsenstein, 1985) for Neighbor-Joining and also using the Fast-Step method for Maximum Parsimony (only one tree kept at each replicate). In both Neighbor-Joining and Maximum Parsimony, bootstrapping analysis was performed with equal weighting of transitions and transversions.

2.4. Gene flow and genetic divergence

Genetic divergence was estimated using Kimura 2 parameter distance. In order to account for polymorphism in each population (or species), divergence was estimated as the average pairwise distance between populations minus the average pairwise distance within a population. Genetic divergence (as a proxy for population divergence) estimates based on a single mitochondrial locus, however, may differ from the actual time of separation between populations (see Edwards and Beerli, 2000). Number of haplotypes (nh), haplotype diversity (hd), and nucleotide diversity (π), were calculated using the software package DNAsp (Rozas and Rozas, 1997). Population structure was estimated by an analysis of molecular variance (AMOVA; Excoffier et al., 1997) using ARLEQUIN (vers. 2.000; Schneider et al., 2000). Populations were grouped in four regions (Israel–Red Sea, Israel–Mediterranean, Greece, and Linosa–Italy) and two alternative groupings were tested (Red Sea versus Mediterranean, and Eastern versus Western Mediterranean). Some levels of selection may have become established since the presence of *S. luridus* in the Mediterranean, thus Tajima's *D* test of neutrality (Tajima, 1989) was performed on the same four populations, using the software package ARLEQUIN (vers. 2.000; Schneider et al., 2000). Importantly, Tajima's *D* test is classically used to test neutrality, but

it can also test population growth as a population that has been experiencing expansion may result in a rejection of the null hypothesis of neutrality (significant negative *D*-values).

2.5. Historical demography

Population parameters Θ (Theta=$2N\mu$, where μ is the mutation rate for mitochondrial DNA) and g (the exponential growth parameter in units of μ^{-1}) were estimated using a coalescent approach with FLUCTUATE 1.4 (Kuhner et al., 1998). The parameter Θ was estimated with population growth (parameters are estimated jointly) or with growth kept constant ($g=0$). Both estimates were obtained by running 10 replicates, which generated a mean value and its associated standard deviation. Analysis of each dataset was done with 10 short Monte Carlo chains of 4000 steps each and 5 long chains of length 20,000, with a sampling increment of 20. Fluctuate generated a random topology for initial searching.

Migration between the four groups (Israel–Red Sea, Israel–Mediterranean, Greece, and Linosa) was analyzed with the software MIGRATE 2.0.3 (Beerli, 2003), which is a maximum likelihood estimator based on the coalescent theory. It uses a Markov Chain Monte Carlo approach to investigate possible genealogies with migration events. Analysis of each dataset was also done with 10 short Monte Carlo chains of 4000 steps each and 5 long chains of length 20,000, with a sampling increment of 20. For each estimate, 10 replicates were used to generate a mean value of migration (Nm) and its associated standard deviation.

3. Results

3.1. DNA sequences

In the present study, 95 samples of *S. luridus* were collected from 8 sampling sites (Table 1, Fig. 1). Additionally, we used 23 samples of *S. rivulatus*, 1 sequence of *S. (Lo) vulpinus*, and 1 sequence of *S. doliatus* as outgroups. A 390 bp portion of the mitochondrial control region was sequenced for all sampled individuals. No insertions or deletions (indels) were observed. Out of the 390 base pairs, 28 were variable and 14 were phylogenetically informative, resulting in 32 haplotypes. As expected, transitions were more frequent than transversions (ratio=3.2), a plot of transition and transversions versus genetic distance also confirmed that substitutions had not reached saturation (not shown). Thus transitions and transversions were equally weighed in our phylogenetic analysis.

3.2. Phylogenetic analysis

The four rabbitfish species used in this study formed a trichotomy. One branch grouped the two closely related species, *S. luridus* and *S. rivulatus* (Kimura 2 sequence divergence, 9.7%), the other two branches corresponded to *S. doliatus* and *S. (Lo) vulpinus*. These latter species differed from each other by 19% sequence divergence, and, respectively, by 22% and 19.6% with the *S. luridus–S. rivulatus* clade (not shown). Individuals from the two species of Lessepsian rabbitfishes, *S. luridus* and *S. rivulatus* grouped in two well-supported clades corresponding to the nominal species (Fig. 2). In the case of our focal species, *S. luridus*, samples did not partition into Red Sea and Mediterranean geographic groups in the phylogenetic analysis (Red Sea samples are italicized in Fig. 2), which was not surprising considering the previously reported high levels of gene flow between these two regions (Bonhomme et al., 2003). Similarly, Linosa samples did not partition in a defined clade, but were scattered throughout the phylogenetic tree (bolded individuals, Fig. 2).

3.3. Genetic diversity and structure among regions and populations

Haplotype diversity (hd) was found to be higher in Eilat, Red Sea (hd=0.978) than in any Mediterranean populations, as well as in the combined Mediterranean samples (hd=0.879) (Table 1). In fact, 26 samples from Eilat separated into 21 haplotypes, while a larger number of Mediterranean samples (69) comprised less haplotypes (15). Within the Mediterranean, no obvious trend was apparent, with the diversity of the youngest westernmost population at Linosa (hd=0.883) being similar to the diversity observed on the Mediterranean coast of Israel (hd=0.871). Nucleotide diversity followed the same trend, with a higher diversity in the Red Sea ($\pi=0.958$) than in the Mediterranean ($\pi=0.592$), and similar diversity levels within the Mediterranean (Table 1). An Analysis of Molecular Variance (AMOVA) also revealed a higher structure level between Red Sea and Mediterranean than within the Mediterranean. However, structure in all cases was very low, with 10% of the variance being attributable to the structure between Red Sea and the Mediterranean, and only 2% of the variance being attributable to the structure between eastern and western Mediterranean. The conditions in the Mediterranean, particularly in its

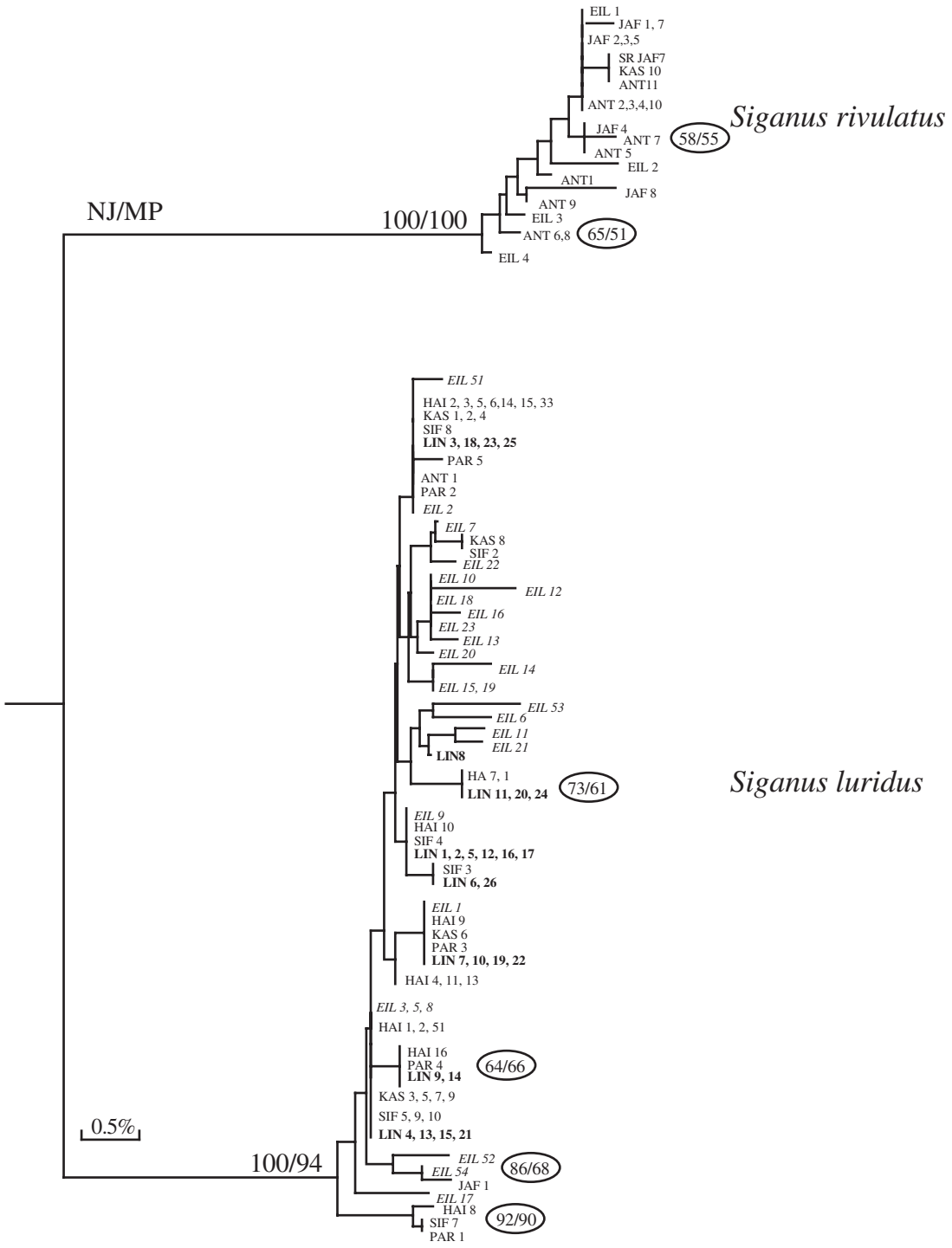

Fig. 2. Molecular phylogeny of *S. luridus* based on the mitochondrial control region marker using the Neighbor-Joining and Maximum Parsimony methods. Labels are described in Table 1. Bootstrap support is shown when above 50%, for both methods used, Neighbor-Joining, and Maximum Parsimony, in that order. Scale bar represents 0.5% sequence divergence. *Siganus (Lo) vulpinus*, *S. doliatus*, and *S. rivulatus* were used as outgroups. Sequences for *Siganus (Lo) vulpinus*, and *S. doliatus* were from Genbank (see Materials and methods section). These sequences are not shown here as they were very distantly related to the other two species (see Results section). Linosa individuals are in bold, Red Sea individuals are italicized.

western region, are very different than in the Red Sea, thus potentially resulting in strong selective pressure. However, Tajima's D test did not uncover departure from neutrality in any of the three Mediterranean populations, and a marginally significant negative value for the Red Sea population (Tajima's $D=-1.42$, $p=0.055$).

3.4. Historical demography, coalescence and gene flow

Demographic factors were estimated for both population size (T) and population growth (Table 2). These two estimates were found to be consistent among replicates (low standard deviations). Population growth was highest in the Red Sea population (Eilat, $g=1300$) (which explains the Tajima's D test result presented above), and lowest in the westernmost Mediterranean population (Linosa, $g=66.8$), with intermediate values between these geographic extremes (Table 2). A proxy for population size, T, followed the same trend (Table 2). Values of directional gene flow (immigration) are presented in Table 3. In this case, no clear trend was observed. For example the number of migrants per generation from Eilat into the Mediterranean sites of Israel was, as expected, very high (503) compared to the reverse (181 migrants from the Mediterranean Israel to the Red Sea). However, between Eilat and Linosa, those expected trends were reversed, with 64 migrants from the Red Sea to Linosa, v.s. 248 for the reverse migration, (Table 3).

4. Discussion

As mentioned above, earlier genetic work showed high levels of gene flow between the Red Sea and the Mediterranean populations of rabbitfishes, and did not uncover evidence of founding effects or bottlenecks in the invading populations from the Mediterranean (Bonhomme et al., 2003; Hassan et al., 2003). Actually, in these studies, samples were restricted to the eastern region of the Mediterranean (Israel and Syria), where individuals are very abundant. These populations, closest to the source of new individuals (the Suez Canal), may also represent older and successive waves of invaders, a situation that may obliterate specific signatures of any single recruitment event, making a genetic study of invading populations difficult.

Similarly, our samples did not partition into Red Sea and Mediterranean clades in the phylogenetic analysis. However, based on a wider geographic sampling, our data showed differences between Red Sea and invading *S. luridus*, with a lower mitochondrial diversity in the Mediterranean than in the Red Sea. In addition, an analysis of Molecular Variance (AMOVA) did uncover a weak structure between Red Sea and Mediterranean populations (10% of the variance was assigned to the difference among those regions). These results possibly indicate the effects of natural selection in the Mediterranean, however Tajima's D test of neutrality did not show any departure from neutrality in these populations. More likely, a random sorting of haplotypes occurred, where not all genetic diversity of the native population migrated through the Suez Canal. This sorting, however, was not severe enough to result in a proper bottleneck. It simply resulted in a detectable lowering of the genetic diversity of the invading population compared to the parental one.

4.1. A foray into the demographics of invasion

Population fluctuations, following birth rates, death rates, immigration and emigration, have been modeled using ideal natural populations. Estimates of gene flow are usually given either using statistical values (Fst, or Nm) or more recently using coalescent models (Beerli and Felsenstein, 1999). In all cases, several assumptions are made, these are sometimes violated, but due to the robustness of the methods, gene flow values are still indicative of true biological trends. In the case of biological invasions, almost all assumptions are violated,

Table 2
Historical demography of *S. luridus*

Region	Population	n	Theta (constant)	Theta (variable)	Growth
Red Sea					
	Eilat	26	0.044 (±0.001)	2.368 (±1.178)	1300.7 (±233.5)
Mediterranean					
	Israel	20	0.015 (±0.000)	0.055 (±0.020)	671.0 (±253.0)
	Turkey/Greece	23	0.011 (±0.000)	0.023 (±0.004)	450.2 (±195.5)
	Linosa	26	0.011 (±0.000)	0.014 (±0.003)	66.8 (±55.1)

Columns represent the regions investigated, populations, number of individuals, Theta with no growth, Theta with growth, and growth. The latter three columns are averages of 10 replicates, between parentheses are their standard deviations.

Table 3
Migration estimates among each of the *Siganus luridus* populations

	Red Sea	Israel	Greece	Linosa
Red Sea	–	181.4 (±216.7)	3.1 (±6.9)	248.2 (±196.6)
Israel	503.7 (±351.6)	–	11.8 (±27.2)	964.8 (±559.1)
Greece	30.8 (±42.4)	8.4 (±14.4)	–	18.4 (±28.0)
Linosa	65.4 (±144.8)	737.0 (±939.1)	25.7 (±15.3)	–

Immigration is given in Nm, the number of migrants per generation into populations. Estimates of migration from columns (source) to rows (recipient) are based on a coalescent approach using the computer program migrate. Values are the mean of ten replicates with their associated standard deviations given between parentheses (e.g. there were 503.7 migrants from the Red Sea into Israel's Mediterranean coast).

because mutations in the populations are not natural, and populations are almost by definition not in any sort of equilibrium. In this study, we have decided to present data both on population fluctuations and directional gene flow to determine if these values are at all indicative of invasion histories. Expectations are relatively simple, we know where the populations originally came from (the Red Sea), we also know where the individuals have most recently arrived (Linosa). We assume that populations in between these two geographic extremes are likely to be genetically somewhere in between also.

As far as population size is concerned, we expect values for the Red Sea population to be the highest, since Mediterranean populations are likely to be a subsample of it. Indeed, Theta values were highest in the Red Sea population (Table 2). Population growth was also found to be highest in the Red Sea. This high value may be due to the relatively recent recolonization of the northern Red Sea by southern Red Sea populations, following the last glaciations. Growth rates in Mediterranean populations are fairly high, with highest values in the eastern region, decreasing towards the west. This may be due to the environmental gradient that this warm-adapted species is encountering, with colder and more marginal environments being encountered as the species is moving westward in the Mediterranean. An alternative factor could possibly due to the on-going input of new colonizers that preferentially arrive from the east, with new genotypes, giving a signature of population expansion. Immigration is expected to be directional, going from the Red Sea into the Mediterranean, and within the Mediterranean, from the eastern region to the west. This trend, however, was not apparent from our data (Table 3). Thus, such approaches show some promise in the arena of biological invasions, but may need additional information. Much larger sampling, more loci, and different theoretical development would likely improve the power of the analysis (Bowen et al., 2005).

4.2. The Linosa invasion

Many biological invasions proceed as dynamic series of sequential invasion called "metainvasion" (Davies et al., 1999). Each of these invasive processes follows three subsequent phases: initial dispersal, establishment of self-sustaining populations within the new habitat and spread of the organism to nearby habitats (Puth and Post, 2005). The recent invasion of the westernmost Island of Linosa by *S. luridus* should be viewed as a secondary (at least) invasion event, the likely source populations were themselves only recently established as part of the primary invasion. At the genetic level, unique sets of mutations are not expected in such recently founded populations because there has been little time for them to occur, the relationship among haplotypes would therefore reflect evolutionary events in the ancestral range of the species rather than in their history of newly occupied areas (Villablanca et al., 1998; Davies et al., 1999). This renders difficult the task to identify the source of marine bioinvasions and to track their invasion pathways.

Mitochondrial diversity appeared to be preserved during the Linosa colonization and no traces of founder events have been observed. This translated in the scattered positions of the Linosa samples in the phylogenetic tree (bolded individuals, Fig. 2) rather than grouping in a single clade with a single, or few haplotypes. Such evidences agree with the idea that Lessepsian migration involves many individuals since its beginning (Hassan and Bonhomme, 2005) and may be explained by both a simultaneous introduction from multiple, genetically diverse individuals or the Linosa population being founded by a very large number of individuals. According to Holland (2000), a large genetically diverse assortment of individuals would be composed by numbers of over 1000 specimens and actually, this circumstance do not disagree with preliminary censuses (Azzurro, 2005).

The invasive process of *S. luridus* in the Strait of Sicily exhibited a wide temporal lag since Linosa

rabbitfishes appeared three decades after their first appearance in the Gulf of Tunis (Ktari-Chakroun and Bouhlal, 1971). Such delay between the initial establishment of colonist and subsequent expansion is a common feature of biological invasion (Sakai et al., 2001; Lee, 2002; Rilov et al., 2004) and can be explained as an evolutionary phenomenon: the time needed for genetic adaptation to the new environment (Sakai et al., 2001). Yet, the genetic analyses do not provide any further support to this hypothesis and open to alternative ecological explanations due, for instance, to early stage of exponential growth or to the direct influence of environmental changes. As a matter of fact, large scale changes in the distribution pattern of fish species may reflect changes in the oceanographic–climatic conditions (Stephens et al., 1988) and recently many displacements of tropical affinity species in the Mediterranean have been related to environmental changes (Bianchi and Morri, 1994; Francour et al., 1994; Astraldi et al., 1995; Guidetti and Boero, 2001). This hypothesis would be better evaluated with the support of environmental data and the knowledge of the spreading pattern of *S. luridus*.

4.3. Patterns of spreading and invasion of S. luridus in the Mediterranean

Very little information is available on the mode of invasion for Lessepsian fish species so that their invasive dynamics and the pattern of spreading remains mostly unknown. This situation mirrors a more widespread lack of knowledge on the initial stages of dispersal and settlement in both terrestrial and aquatic habitats (Holland, 2001; Puth and Post, 2005). Such information would be essential in understanding the dynamic of invasion and in designing management responses (Davies et al., 1999; Wadsworth et al., 2000).

Our results indicated that genetic variability was maintained during the early phases of colonization but a spreading mode of *S. luridus* in the Mediterranean cannot be defined with certainty. The dusky rabbitfish could disperse during the larval stage but little is known about their pelagic larval phase (May et al., 1974; Bryan and Madraisau, 1977; Popper et al., 1979). Generally, siganids produce large numbers of benthic adhesive eggs (Lam, 1974; Popper et al., 1979) that hatch within 26–32 h of fertilization. Larvae then swim actively near the water surface (Popper et al., 1979). Data from reared larvae, as well as from the time elapsed between spawning time and first settlement bouts, suggest that the pelagic larval duration lasts approximately 30 days (Bariche et al., 2004). During this time, surface currents may transport these larvae for up to 1000 km (Woodland, 1999) allowing them to potentially settle in new areas.

Alternatively, *S. luridus* may migrate through long distances by adult active swimming or even by association with drifting seaweeds, a behavior known for other rabbitfish species (Cho et al., 2001). Parasitological evidence suggested that Lessepsian migration of another rabbitfish, *S. rivulatus*, was undertaken by adult active swimmers and not by pelagic larvae (Diamant, 1998).

5. Conclusions

For the first time in a Lessepsian migrant, our findings uncovered a slight but detectable lowering of the *S. luridus* genetic diversity in the invading (Mediterranean) population compared with the parental one (Red sea). These results stressed the importance to encompass wide geographic samplings in this kind of studies, so far limited to the eastern Mediterranean. The analysis of the youngest and westernmost population of Linosa allowed to conclude that mitochondrial diversity is maintained during the early invasions, thus avoiding suboptimal assortments of genotypes and preserving the needed genetic plasticity to adapt to the new habitat. This information accorded with the idea that Lessepsian migration involves many individuals since its beginning and emphasized the need to explore the causes which trigger migration itself, as for instance changes in the oceanographic–climatic conditions.

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