

On the distribution of the invasive long-spined echinoid *Diadema setosum* and its expansion in the Mediterranean Sea

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ABSTRACT: Sea urchins from the genus *Diadema* are some of the most widespread and ecologically important species in tropical marine environments. Two distinct clades of *Diadema setosum* have been previously recognized based on DNA sequence data: the widespread clade a (distributed throughout the Indo-West Pacific) and clade b (native to the Arabian Peninsula). We use published and novel molecular data to assert the distribution of the 2 *D. setosum* clades and compile a comprehensive, georeferenced occurrence list for both clades throughout their range. These data are then used to model the global distribution of *D. setosum* with respect to the 2 molecularly inferred clades and evaluate their invasive potential throughout the Mediterranean. We combine morphological with molecular data to assert the taxonomic identification of the single Mediterranean *Diadema* recovered to date and provide an updated phylogenetic analysis of this genus. Species distribution modeling predicts suitable habitats for the 2 clades, including some along the Egyptian and Israeli coasts of the Levantine Basin, and in restricted areas in the Aegean and Adriatic Seas. Genetic data show that the Mediterranean *Diadema* derives from mitochondrial clade b of *D. setosum*. The spatio-temporal pattern of *D. setosum*'s Mediterranean invasion does not follow a gradual succession of directional population expansion as may be expected from an unmediated larval transport through the Suez Canal, indicating potential anthropogenic involvement. Without intervention, further spread of *D. setosum* in the Mediterranean seems likely, elevating the risk of a population outbreak that may have far-reaching anthropogenic and environmental consequences.

KEY WORDS: Alien species · Environmental niche modeling · Molecular diagnostics · Mediterranean Sea · MaxEnt · *Diadema setosum*

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INTRODUCTION

Alien (i.e. nonindigenous) species introductions are widely recognized as one of the most prevalent threats to biodiversity (Molnar et al. 2008, Walther et al. 2009, Belmaker et al. 2013). The term 'invasive' is used to denote alien populations that spread beyond their point of introduction without the aid of humans (adopting the terminology of Galil & Goren 2014). In the marine environment, invasive species have been shown to transform marine habitats and obliterate

indigenous communities (Byrnes et al. 2007, Sala et al. 2011, Edelist et al. 2013). Invasive marine species are often also associated with profound economic consequences as some species may diminish fisheries, foul ship hulls or clog intake pipes (see review by Molnar et al. 2008), while others may even pose a direct threat to human health (Ruiz et al. 1997).

The Mediterranean Sea, and in particular its eastern basin (the Levantine Sea), stands out as one of the most heavily invaded marine regions to date (Belmaker et al. 2013, Edelist et al. 2013). The rapid and

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unparalleled scale of species introduction into the eastern Mediterranean is predominantly attributed to the opening of the Suez Canal in 1869 (Golani 2010, Galil & Goren 2014). The influx of Red Sea organisms into the Mediterranean via the Suez Canal is referred to as Lessepsian Migration (Por 1971). Red Sea invaders are causing fundamental changes to the marine environment of the eastern Mediterranean and have far reaching ecological and economic impacts (Galil 2009, Gewing et al. 2016). Lessepsian invaders already constitute more than 90% of the total fish biomass in some areas of the eastern Mediterranean (Goren & Galil 2005, Edelist et al. 2011). Hundreds of alien marine species have so far been recorded in the Mediterranean Sea (World Register of Introduced Marine Species, www.marinespecies.org/introduced/; Galil & Goren 2014). Galil (2012) provided an overview of the alien species recorded in the Mediterranean and characterized their identity, regional setup and pathways of introduction. She noted that 31% of the documented alien species in the Mediterranean are mollusks, 17% crustaceans, 16% bony fish, 14% macrophytes, 9% annelid polychaetes, 3% bryozoans, and 3% cnidarians. The remaining 7% are other taxa, and include the phylum Echinodermata. Indeed, this group of benthic organisms demonstrates a remarkably low rate of invasion and only 7 recognized Lessepsian alien echinoderms have so far been reported: *Aquilonastra burtoni* (Gray, 1840) (Tortonese 1966), *Amphioplus (Lymanella) laevis* (Lyman, 1874) (Tortonese 1953–1954), *Ophiactis macrolepidota* (Marktanner-Turneretscher, 1887) (Tom & Galil 1991), *O. savignyi* (Müller & Troschel, 1842) (Mortensen 1926), *Amphiodia (Amphisipina) obtecta* Mortensen, 1940 (Mortensen 1940b, Stöhr et al. 2010), *Synaptula reciprocans* (Forsskål, 1775) (Cherbonnier 1986), and *Diadema setosum* (Leske, 1778) (Yokes & Galil 2006).

Sea urchins of the genus *Diadema* are some of the most abundant and ecologically important shallow water echinoids (Lawrence & Sammarco 1982, Birke-land 1989, Bronstein & Loya 2014, Bronstein et al. 2016). They occur in all tropical seas and are markedly common throughout their range (Lessios et al. 2001, Muthiga & McClanahan 2013). They typically inhabit shallow rocky and reef habitats, occurring on corals, reefal rubble or sand (Bauer 1980), although some may also occur in the channels of mangroves and estuaries (Muthiga & McClanahan 2013). *Diadema* are omnivore grazers and detritus feeders consuming mainly algae and seagrass (Randall et al. 1964, Ogden et al. 1973, Bronstein & Loya 2014) although some species have been reported to occasionally prey on

live coral as well as coral spat (Bak & van Eys 1975, Glynn et al. 1979, Carpenter 1981). This dietary flexibility, coupled with their great abundance at the sites where they occur, place *Diadema* as prominent species in tropical marine environments. For humans, *Diadema* are notorious for their long, venomous, needle-like spines. Although, like in other venomous echinoids, their venom is not considered fatal to humans, their (unknown) toxin may cause substantial swelling and pain. In addition, their brittle spines easily break off, making them exceptionally challenging to remove from the wound (Williamson et al. 1996).

Lessios et al. (2001) summarized the geographical distributions of the different *Diadema* species and provided a global scale phylogeographic analysis of this genus. They showed that the Indo-Pacific *D. setosum* was the first to diverge from all other extant *Diadema* sometime during the Miocene, and that the *D. setosum* lineage split into 2 clades 3 to 5 million years ago: one common throughout the Indo-West Pacific (*D. setosum* clade a) and the other (*D. setosum* clade b) confined to the areas surrounding the Arabian Peninsula (from the Red Sea through the Gulf of Aden and along the Arabian Sea coastline of Oman to the Persian Gulf). While these 2 clades receive strong molecular support from DNA sequence data, currently no morphological characters are known that allow them to be confidently distinguished.

D. setosum is a nonindigenous (alien) species in the Mediterranean that was first reported in 2006 off the Kaş Peninsula, on the south-western coast of Turkey (Yokes & Galil 2006). In June 2007, this species was observed again on Konyaaltı Beach, Antalya, Turkey (Gokoglu et al. 2007). Later, in 2009 it was observed in the coastal waters of the town of Monsef, along the Lebanese coastline (Nader & El Indary 2011), and in 2010 at Yayladagi, Antakya Bay on the south-eastern coast of Turkey (Turan et al. 2011). By 2014 this species had spread westward up to Gökova Bay, Aegean Sea, Turkey (Katsanevakis et al. 2014 and references therein), and by late 2014, it had reached the coastal area of the Greek island of Kastelorizo, where it was encountered again in 2015 (Tsiamis et al. 2015 and references therein). Most recently, on 2 August 2016, *D. setosum* reappeared in the coastal waters of Konyaaltı Beach, Antalya, Turkey (M. Gökoglu pers. comm.). Given the vast range of *D. setosum* (from Eastern Africa to the Western Pacific), the question of origin of the introduced individuals arises. While the majority of invasive species in the eastern Mediterranean are Lessepsian (Galil 2009), there are many non-Lessepsian immigrants (Check-list of exotic spe-

cies: CIESM Atlas of Exotic Fishes in the Mediterranean Sea; www.ciesm.org/atlas/appendix1.html [last accessed on 31.03.2017]). Uniquely, the presence of the 2 geographically distinct mtDNA lineages in *D. setosum* facilitates distinction between Lessepsian and non-Lessepsian migration, which may provide insights into the underlying process of invasion for this species.

Here, we provide a broad-scale habitat suitability model for the widespread, long-spined echinoid *D. setosum*. Initially, we evaluated the phylogeographic hypothesis of Lessios et al. (2001) pertaining to the distribution of the 2 lineages by analyzing all available sequence data from the literature in addition to newly generated sequences. We then used this information to generate distribution models for the 2 clades and evaluated the invasive potential of *D. setosum* throughout the Mediterranean. We combined morphological observations with molecular data to assert the taxonomic identification of available Mediterranean *Diadema*, investigated its genetic affiliation and provide the first phylogenetic analysis incorporating *Diadema* from this region. Consequently, our molecular diagnostics together with the habitat suitability models enable the testing of alternative hypotheses concerning the modes of introduction and invasive potential of *D. setosum* in the Mediterranean.

MATERIALS AND METHODS

Material

The first reported specimen of *Diadema setosum* in the Mediterranean Sea was observed off the Kaş Peninsula (36° 08.45' N, 29° 39.30' E), Turkey, on August 12, 2006, at a depth of 18 m (Yokes & Galil 2006). This specimen, 58 mm in diameter, is stored in 70% ethanol and deposited at The Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies, Tel Aviv University under specimen voucher number SMNH_EC_25437 (formerly the National Collections, Tel Aviv University [TAU], Israel, under specimen voucher number TAU EC 25437). To our knowledge, this is the only available specimen of *D. setosum* from the Mediterranean, none of the other specimens recorded were collected and/or deposited in a public collection. We ratified the specimen's identification following the criteria of Coppard & Campbell (2006). Several tube-feet and spines were removed from the specimen for DNA extraction. Additional *D. setosum* specimens from the northern Red Sea were collected in shallow reefs

of the Gulf of Aqaba (see Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m583p163_supp.pdf for details).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from tube feet and spine muscles using the DNeasy® Blood and Tissue Kit (Qiagen) following the manufacturer's instructions. Two mitochondrial regions were targeted and amplified using the polymerase chain reaction (PCR) following Lessios et al. (2001): (1) a fragment of the lysine-tRNA, ATPase-6 and 8 region (*LYS*) and (2) a fragment of the cytochrome *c* oxidase subunit I (*COI*) gene. Primers *LYS*a 5'-AAG CTT TAA ACT CTT AAT TTA AAA G-3' (fwd) and *ATP6b* 5'-GCC AGG TAG AAC CCG AGA AT-3' (rev) were used to amplify a 595 bp long sequence. Primers *COI*f 5'-CCT GCA GGA GGA GGA GAY CC-3' (fwd) and *COI*a 5'-AGT ATA AGC GTC TGG GTA GTC-3' (rev) were used to amplify a 663 bp long sequence. PCR amplifications were conducted using the Top-Taq DNA Polymerase (Qiagen) using 1 µl of extracted genomic DNA (approximately 10 to 15 ng) in each reaction. Reaction conditions for the *COI* fragments were 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 57°C and 60 s at 72°C. For the *LYS* fragment, the annealing temperature was adjusted to 55°C, while the other steps of the reaction were kept as above. Amplified fragments were purified using ExoSAP-IT (Affymetrix) and sequenced (in both directions) at Microsynth (Vienna) using the PCR primers. The new sequences have been deposited in GenBank under the accession numbers: KX600494, KY817842–KY817844 (*LYS*); KX600495, KY817839–KY817841 (*COI*).

Data assembly and phylogenetic analyses

An additional 60 *COI* sequences and 395 *LYS* sequences comprising all extant species of *Diadema* were recovered from GenBank (see Table S1 in the Supplement) and used to compile a comprehensive phylogenetic tree of *Diadema*. The biogeographical distribution of the 2 mitochondrial clades as suggested by Lessios et al. (2001) was ratified based on all available *D. setosum* sequences in the phylogeny. Sequences were primarily aligned using MAFFT v. 7.245 (Kato & Standley 2013) and subsequently adjusted by eye using Bioedit v. 7.1.3 (Hall 1999). After ambiguous site removal, the final *LYS* dataset

contained 399 samples and was 524 bp long, while the *COI* dataset contained 64 samples and was 526 bp long. *Echinothrix diadema* and *Astropyga radiata* were used as outgroups for the *COI* dataset and *E. calamaris*, *A. pulvinata*, and *A. radiata* served as outgroups for the *LYS* dataset (see Table S1 in the Supplement). A median-joining haplotype network of *D. setosum* was calculated using the *LYS* dataset including both clades (a and b) and the Mediterranean sequence. This network comprised 96 sequences (524 bp long) and was constructed with PopArt v. 1.7 (Leigh & Bryant 2015) applying the default settings.

Phylogenetic analyses were conducted using both maximum likelihood (ML) and Bayesian inference (BI) approaches. The best fitting models for the ML and BI analyses were selected using PartitionFinder2 (Lanfear et al. 2017) based on the Bayesian information criterion (BIC) (Schwarz 1978). The selected models for the 2 different datasets and phylogenetic-reconstruction strategies were GTR+G+I (ML and BI) for the *LYS* dataset and GTR+G (ML) and HKY+G (BI) for the *COI* dataset.

ML analysis was performed using the program RAxML GUI v. 1.5b1 (Silvestro & Michalak 2012). Settings were 'ML+thorough bootstrap', 100 runs, 1000 replicates, applying the best-fit models as inferred from PartitionFinder. Bayesian analysis was carried out using the program MrBayes v. 3.2.2 (Ronquist et al. 2012). We ran 2 independent runs of 3 'heated' and 1 'cold' chain for 10 million (*COI* dataset) and 20 million (*LYS* dataset) generations, and sampled parameters and trees every 100 generations. Convergence was assessed according to the standard deviation of split frequencies (<0.01). The runs were also visually checked by plotting generations versus likelihood scores and in Tracer v. 1.6 (Rambaut et al. 2013) to assess whether the 2 runs had converged and when the stationary phase was reached. In a conservative approach, the first 25% (*COI* dataset) and 50% (*LYS* dataset) of trees were discarded as burn-in and a 50% majority rule consensus tree was calculated from the remaining trees. Bayesian posterior probabilities (PP) were obtained from the 50% majority-rule consensus of the trees sampled during the stationary phase.

Species distribution modeling

Ambiguities over the correct identification of the different *Diadema* species are thought to be common (Clark 1925). In particular, the morphological distinctions between *D. setosum* and *D. savignyi* are so

slight that considerable confusion exists as to the true distributions of these species (Pearse 1998). To mitigate this shortcoming and avoid incorporating erroneous data in our models, we restricted data collection to studies with sound taxonomical background, specimens with verified taxonomic evaluation present in public collections, and publications providing sufficient data to make unambiguous identifications possible. Consequently, a comprehensive set of georeferenced presence data for *D. setosum* throughout its range was extracted from the literature and supplemented with unpublished data derived from the Steinhardt Museum of Natural History and National Research Centre (SMNH, Tel Aviv University, Israel), and the National Natural History Collections (The Hebrew University of Jerusalem, Israel) (see Table S2 in the Supplement). Additional georeferenced localities for *D. setosum* clade a, confirmed by unpublished DNA sequence data, were provided by S. Coppard, G. Paulay and N. Shahid. *D. setosum* occurrences in the Mediterranean (except for the molecularly validated specimen from the Kaş Peninsula, Turkey) were excluded from our list and not used in the creation of the models to facilitate their unbiased performance. Consequently, we compiled a list of 325 records comprising both clades (see Table S2 in the Supplement for the list of sources). After exclusion of localities not covered by the environmental data (see following section) and multiple records per grid cell, 105 and 29 points remained for *D. setosum* clades a and b, respectively.

Modeling *Diadema* distribution

Species distributions were modeled using the 22 environmental layers provided by Bio-ORACLE (Tyberghein et al. 2012; available at www.oracle.ugent.be) at the spatial resolution of 5 arcmin (ca. 9.2 km). This dataset offers a variety of geophysical, biotic, and climatic data suitable for modeling current and future distributions of marine species. We used the cropped dataset (70° N to 70° S) as suggested by Tyberghein et al. (2012) to avoid biases caused by missing or incorrect data in the polar regions. In addition, the Baltic Sea (including the Gulfs of Bothnia, Finland and Riga and the Kattegat) and Black Sea (including the Sea of Azov) were excluded from the study as no echinoids occur in these regions.

We employed the variance inflation factor (VIF) to control for collinearity among the 22 predictor variables. In general, values greater than 10 indicate strong correlations between the variables (Quinn &

Keough 2002) and thus these variables were not used in the final models. Consequently, only 13 variables were included in the models (Table 1).

The machine-learning program MaxEnt (v. 3.3.3k, Phillips et al. 2006) was employed to predict spatial distributions and explore potential habitat suitability for the 2 *D. setosum* clades (i.e. clades a and b). MaxEnt predicts the distribution of an organism extrapolated from environmental parameters at point locations where the organism has been observed (Phillips et al. 2006). This algorithm has been proven to perform well with presence-only data and small sample sizes (Elith et al. 2006, Phillips & Dudík 2008), and has often been employed to model marine species distributions (e.g. Pierrat et al. 2012, Giannoulaki et al. 2013, Anderson et al. 2016). We used the default settings and the logistic output format which provides an estimate of probability of occurrence (Phillips & Dudík 2008). To reduce model overfitting and retain realistic predictions, we set the regularization multiplier to 2 according to Radosavljevic & Anderson (2014). For each clade, 10-fold replicates were created, and cross-validation was used to randomly split the occurrence data in training and test.

The performance of the MaxEnt models was evaluated with the area under the curve (AUC) of the receiver operating characteristic (ROC) plot (Fielding & Bell 1997). Although the AUC has been criticized as a measure to evaluate species distribution models (SDMs) by some researchers (see Lobo et al. 2008), it nevertheless remains one of the most widely used threshold-independent means for SDM evaluation (Elith et al. 2006, Merow et al. 2013). AUC values range between 0 and 1, where values above 0.75 are indicative of sufficiently robust models (see Elith et al. 2006). We used the average test AUC of the 10-fold replicates to evaluate the MaxEnt generated models.

In addition, the predictive accuracy of the models was evaluated with the true skill statistic (TSS), a threshold dependent measure ranging from -1 to 1. TSS is used when probability values of the models are converted to presence-absence information (e.g. see Georgopoulou et al. 2016). Values above 0.75 indicate excellent agreement between predicted and observed presences/absences (see Allouche et al. 2006 for details), or in our case pseudo-absences, i.e. artificial absence data used in SDMs when true absences are unavailable. We used the maximum sensitivity plus specificity test threshold (MST) to transform the habitat suitability maps to presence-absence maps. MST, generated by MaxEnt, is a frequently used threshold and has been proven to pro-

Table 1. Predictor variables used in MaxEnt models. Data were derived from Bio-ORACLE (Tyberghein et al. 2012)

Predictor variables	Abbreviation	Units
Mean calcite concentration	calcite	mol m ⁻³
Chlorophyll <i>a</i> range	chlorange	mg m ⁻³
Maximum cloud cover	cloudmax	%
Mean diffuse attenuation	damin	m ⁻¹
Mean dissolved oxygen	dissox	ml l ⁻¹
Mean nitrate	nitrate	µmol l ⁻¹
Maximum photosynthetically available radiation	parmax	Einstein m ⁻² d ⁻¹
Mean photosynthetically available radiation	parmean	Einstein m ⁻² d ⁻¹
Mean pH	ph	
Mean phosphate	phos	µmol l ⁻¹
Mean salinity	salinity	PSS
Mean silicate	silicate	µmol l ⁻¹
Maximum sea surface temp.	sstmax	°C

duce accurate predictive models in comparison to other thresholds (for details see Liu et al. 2005, Jiménez-Valverde & Lobo 2007). For the TSS calculation, we randomly selected points equal to the number of sample records to use as pseudo-absences. These points were taken from the 10 000 background points generated by MaxEnt and used for the prediction models. For each clade, TSS was averaged for the 10-fold model replicates. The calculations were carried out in R v. 3.2.2 (R Core Team 2013).

RESULTS

Morphological examination

Re-examination of the specimen of Yokes & Galil (2006), the first record of the species in the Mediterranean, confirms its specific identification (Fig. 1). The preserved specimen clearly shows a hemicyclic apical disc (ocular plate III exert, plate II barely insert, others fully insert), 23% test-diameter (TD) in size, with large periproct (14.5% TD in diameter), slightly raised ambulacra adapically, 1 to 2 median rows of interambulacral tubercles and relatively small gonopores (18.1 to 21% of genital plate height). Of the original coloration (see also Yokes & Galil 2006, their Fig. 1, for live images of this specimen), the presence of an orange ring on a small anal cone without platelets can still be observed (Fig. 2a). The interradial white spots that are so prominent in living specimens of this species can still be seen in the form of small unpigmented areas in the middle of the aboral interambulacra (Fig. 2a). The peristomal mem-

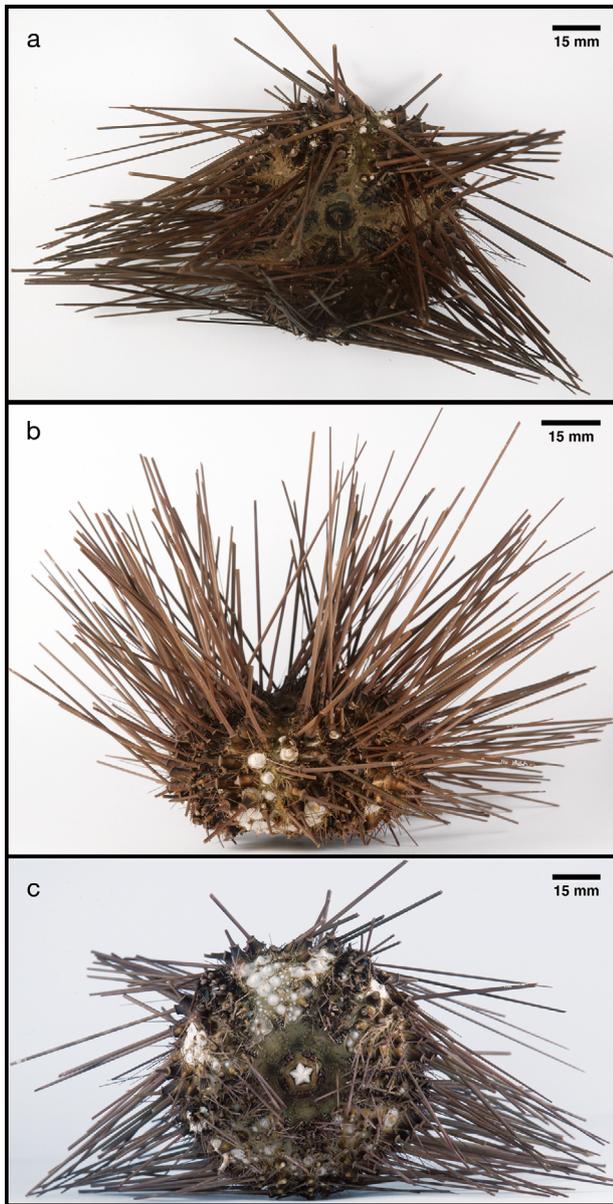


Fig. 1. Images of the preserved *Diadema setosum* specimen reported by Yokes & Galil (2006) from the Kaş Peninsula (36° 08.45' N, 29° 39.30' E), Turkey, on 12 August 2006, at a depth of 18 m (specimen is deposited at The Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies, Tel Aviv University under voucher number SMNH_EC_25437). (a) Apical, (b) lateral, and (c) oral views

brane is naked, bearing only tridentate pedicellariae and 5 pairs of buccal tube feet (Fig. 2b). All of these features support the initial identification as *Diadema setosum*. Unlike typical *D. setosum* (see Coppard & Campbell 2006), however, the studied specimen has only a single tubercle on each genital plate along the periproctal margin (none on genital plate 2) and has a genital plate height to width ratio ranging from



Fig. 2. Close up images of the (a) apical system and (b) peristome of preserved *Diadema setosum* (see Fig. 1 for details). (a) Apical view showing relict orange pigment around the anal cone and the hemicyclic apical disc (indicated by arrow). White arrow heads indicate 2 of the 5 characteristic inerradial white spots of *D. setosum*. (b) Oral view showing the peristomal membrane

0.84:1 to 1.04:1. However, the examination of hundreds of individuals of various *Diadema* and *Echinothrix* species by Coppard & Campbell (2006) showed that many test characters show considerable variation.

Phylogenetic analysis

BI and ML analyses for each marker (i.e. *LYS* and *COI*) produced congruent topologies for all major clades and subclades. Consequently, the BI-inferred topologies are presented with both posterior probabilities and bootstrap support for each clade (Fig. 3, see also Figs. S1 & S2 in the Supplement). The current phylogenetic reconstruction reaffirms the distinction of the 2 *D. setosum* clades according to their geographical distribution (Lessios et al. 2001): clade a being widely distributed throughout the Indo-Pacific while excluded from the Red Sea and Persian Gulf, and clade b showing an opposite trend. Our novel sequence data for the Mediterranean *Diadema* supports the identification of this specimen as *D. setosum* clade b (i.e. belonging to the Red Sea/Arabian Peninsula clade) (Fig. 3). In agreement with the results of Lessios et al. (2001), in both the *COI* and the *LYS* datasets, *D. setosum* clade b (including the Mediterranean specimen) was resolved as sister to *D. setosum* clade a, both clades diverging early from the other *Diadema* species.

The molecular diversity of *D. setosum* was further explored by displaying the distribution patterns of the *LYS* haplotypes through a median-joining haplotype network (Fig. 4). Similar to the topologies inferred from the trees, the *LYS* network was clearly divided into 2 clusters of haplotypes: (1) the group of Indo-Pacific haplotypes (corresponding to *D. setosum* clade a), and (2) the group formed by the Arabian Peninsula haplotypes including the Mediterranean material (corresponding to *D. setosum* clade b) (Fig. 4). The Mediterranean *Diadema* is part of the most common haplotype of *D. setosum* clade b, equally shared between Red Sea and Persian Gulf representatives, preventing assignment of the Mediterranean specimen to any distinct geographic subset within clade b. Within the large variety of *D. setosum* clade a,

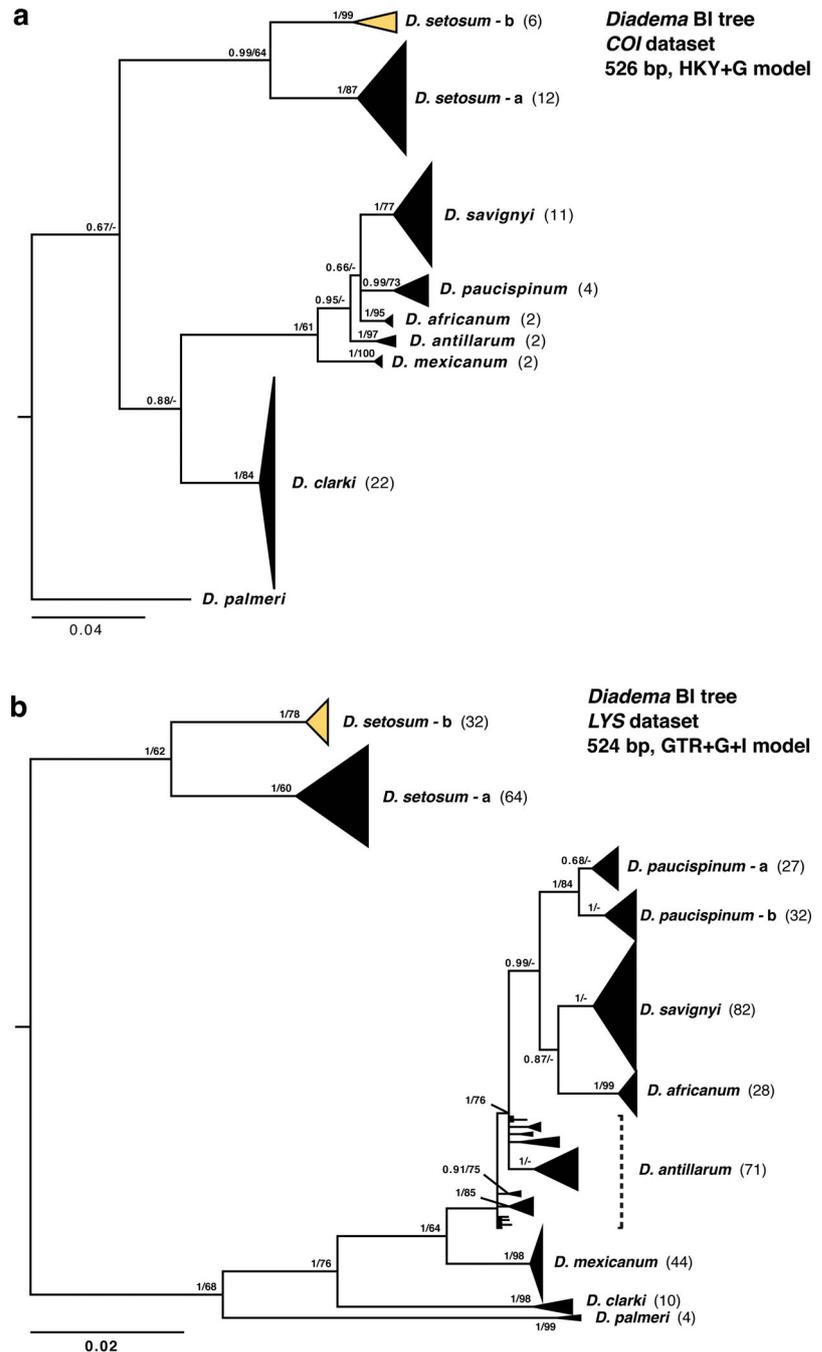


Fig. 3. Phylogenetic relationships of the genus *Diadema*. Bayesian phylogenetic tree estimates are based on the (a) *COI* and (b) *LYS* datasets. Sequences of both datasets represent all extant species of *Diadema* and are rooted on *Echinothrix diadema* and *Astropyga radiata* in the *COI* dataset and *E. calamaris*, *A. pulvinata*, and *A. radiata* in the *LYS* dataset. Major clades were collapsed using congruent nodal support by both tree estimation methodologies. The clades including the Mediterranean *Diadema* sequences are highlighted in yellow (*D. setosum* clade b). Supporting values (>0.6 posterior probabilities and >60% maximum likelihood [ML] bootstrap values) are shown above the nodes (before and after the slash, respectively). ML bootstrap support was calculated from 1000 replications and Bayesian inference (BI) posterior probabilities from 7.5 and 15 million generations (after burn-in) for the *COI* and *LYS* trees, respectively. Details on the sequences used for these trees are given in Table S1 in the Supplement. Scale bars reflect number of changes per site

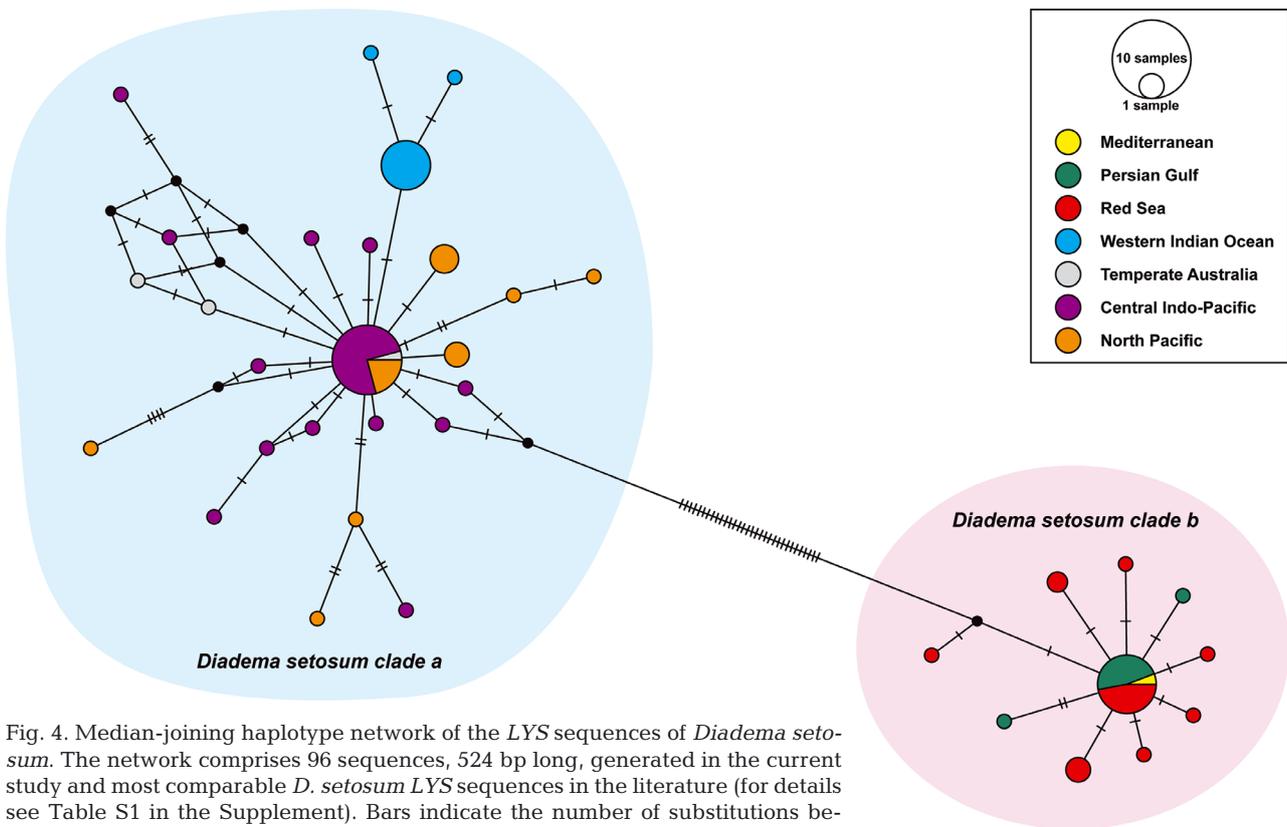


Fig. 4. Median-joining haplotype network of the *LYS* sequences of *Diadema setosum*. The network comprises 96 sequences, 524 bp long, generated in the current study and most comparable *D. setosum* *LYS* sequences in the literature (for details see Table S1 in the Supplement). Bars indicate the number of substitutions between nodes. Colors represent localities of the different haplotypes, and the frequency of each haplotype is indicated by size (see key, top right). Shaded zones denote the different clades of *D. setosum*: the Indo-West Pacific clade a, and the Arabian Peninsula (i.e. Red Sea to Persian Gulf) clade b

with haplotypes shared from the North Pacific to temperate Australia, only haplotypes from the Western Indian Ocean seem to form a distinctive geographical cluster.

Species distribution modeling

Average AUC values for the test data indicated excellent model performance ($AUC_{\text{clade-a}} = 0.984 \pm 0.009$ (SD), $AUC_{\text{clade-b}} = 0.998 \pm 0.001$). Similarly, TSS results showed excellent agreement between predicted and observed pseudo-absences ($TSS_{\text{clade-a}} = 0.894 \pm 0.071$, $TSS_{\text{clade-b}} = 0.899 \pm 0.162$).

The potential distribution of *D. setosum* clade a was mainly explained by mean nitrate concentration (70.1%) and maximum sea surface temperature (7.8%) based on permutation importance (see Table S3 in the Supplement). In detail, the average MaxEnt model of *D. setosum* clade a shows higher relative probability of presence in areas with low nitrate concentration (less than $5 \mu\text{mol l}^{-1}$) and high maximum surface temperature (more than 30°C). For *D. setosum* clade b,

high permutation importance in the average MaxEnt model is denoted for maximum sea surface temperatures (74.8%) and salinity (23.9%) (see Table S3 in the Supplement). The relative probability of occurrence of *D. setosum* clade b is higher in areas with higher maximum sea surface temperatures (more than 30°C) and highly saline waters (more than 38).

Suitable habitats for *D. setosum* clade a were mainly detected along the coasts of the islands between southern Japan, Indonesia, Papua New Guinea and northern Australia, western India, and between eastern Africa and Madagascar (Fig. 5). Predictive habitat suitability for *D. setosum* clade a dropped sharply in the north-western Indian Ocean from the Seychelles and coast of Somalia to the north-western coast of India, although moderate to high suitability was estimated along the central and southern coasts of western India (Fig. 5). Although the species has not been recorded from western Africa and the Caribbean, our model indicates that potential suitable habitats for this species also exist in these regions. For *D. setosum* clade b, suitable areas are mostly found in the central and northern Red Sea, parts of

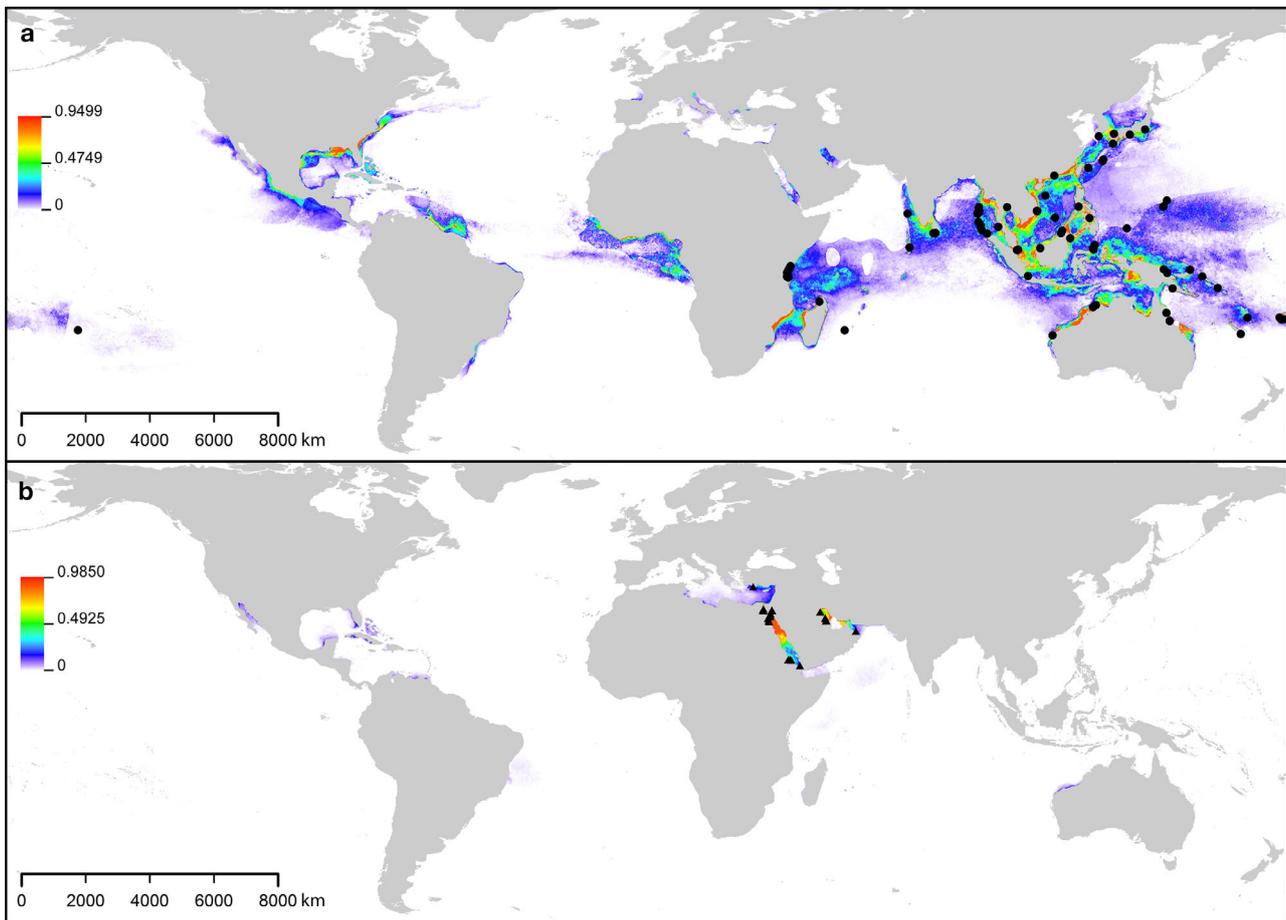


Fig. 5. Habitat suitability map generated using MaxEnt. Environmental variables used are listed in Table 1. (a) *Diadema setosum* clade a and (b) *D. setosum* clade b. Presence records: (●) *D. setosum* clade a and (▲) *D. setosum* clade b. Color scale bars represent predictive relative habitat suitability

the Persian Gulf, and along the eastern shores of the Mediterranean (Figs. 5 & 6). Although moderate habitat suitability for *D. setosum* clade b is predicted for most parts of the Mediterranean (Fig. 6), high habitat suitability was detected in the eastern Mediterranean (Figs. 6 & 7), from Egypt through Israel, Lebanon, Syria, and Cyprus to the Levantine coast line of Turkey and Greece. In contrast, for *D. setosum* clade a, low suitability is predicted for most of the Mediterranean and almost none in the Levantine Basin (Figs. 6 & 7), with only a few isolated patches of suitable habitats for this clade in parts of the Aegean and Adriatic Seas (Fig. 7).

DISCUSSION

Invasive species are considered by many as major drivers of species extinction (Clavero & García-Berthou 2005). Although some researchers question

the link between species invasions and extinction of native species (Gurevitch & Padilla 2004), mounting evidence from across the animal kingdom clearly demonstrates the contribution of biological invasions to the decline of biodiversity worldwide (McGeoch et al. 2010, Pyšek & Richardson 2010). While a variety of interactions may occur between native and invasive species (including predation, hybridization and introgression; see Mooney & Cleland 2001), competitive exclusion and niche displacement are most prominent between closely related species or species sharing a similar ecological niche (Čuda et al. 2015). To date, only a single indigenous diadematoid sea urchin is known from the Mediterranean (Guallart & Templado 2012). This species, *Centrostephanus longispinus* (Philippi, 1845), is widely distributed throughout the Mediterranean and eastern Atlantic (Pawson & Miller 1983) and is among the most common echinoids of the Levantine Basin (Tortonese 1946, Özyaydin et al. 1995, Koukouras et al. 2007, Özgür et al. 2008).

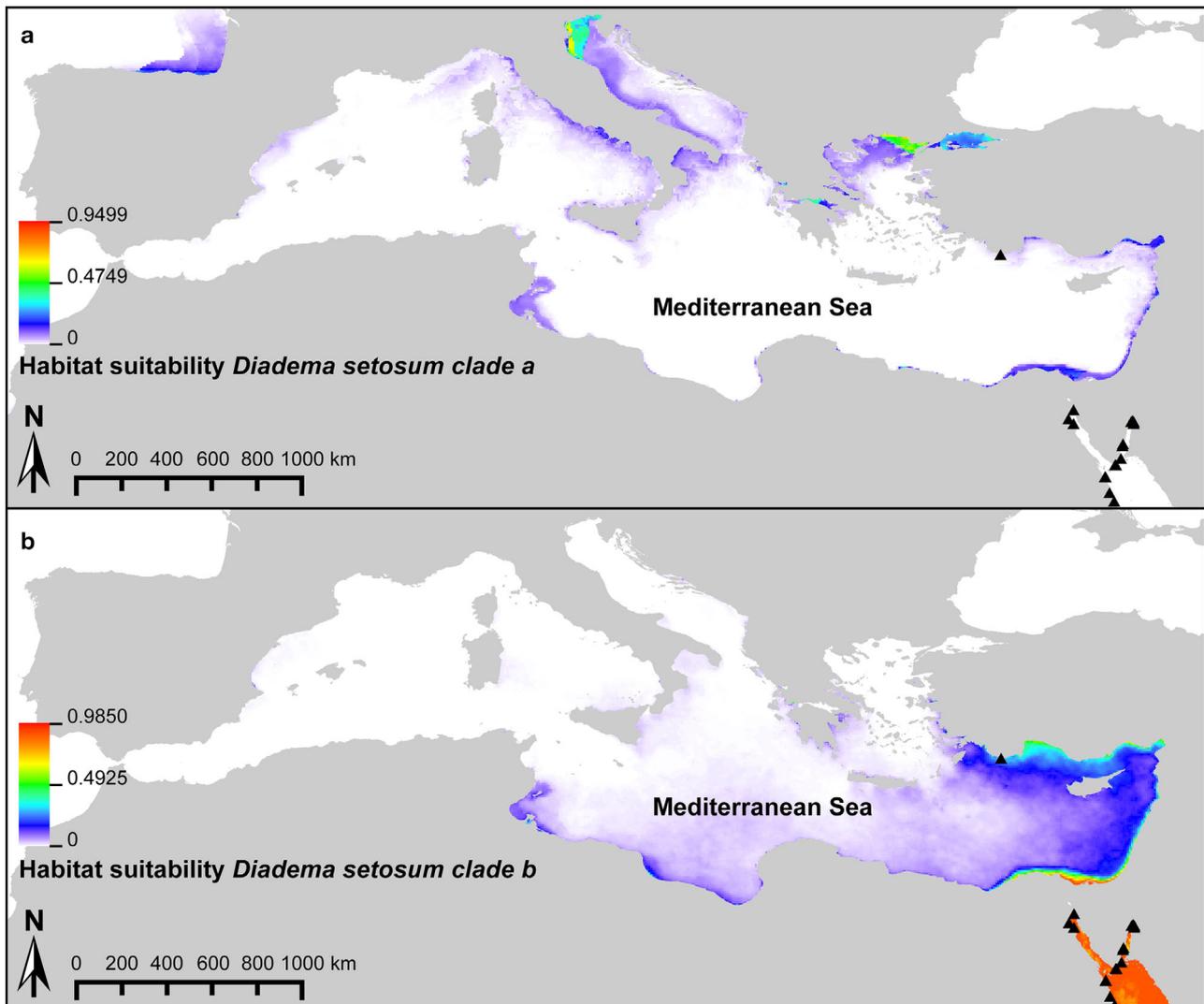


Fig. 6. Habitat suitability map of (a) *Diadema setosum* clade a and (b) *D. setosum* clade b in the Mediterranean Sea based on the MaxEnt model. Environmental variables used are listed in Table 1. (▲) Site locality of *D. setosum* clade b as confirmed by DNA sequence data. Mediterranean localities lacking molecular support have been excluded from the model. Color scale bars represent predictive relative habitat suitability

Direct competitive interactions with the invasive *Diadema setosum*, however, are expected to be low as the 2 species inhabit different depth ranges. *C. longispinus* mostly inhabits deep water of 40 to 208 m (Pawson & Miller 1983, Koukouras et al. 2007), with most occurrences in the eastern Mediterranean deeper than the 60 m mark (based on records from the TAU and Hebrew University of Jerusalem collections). *D. setosum* in the Mediterranean, in contrast, only occurs in shallow depths of up to 20 m. Even when considering the depth range of *D. setosum* in its native habitat, it rarely exceeds depths of 40 m. While this species has been reported to occur down to 70 m (Mortensen 1940a), recent echinoderm surveys in the Red Sea from the shoreline to a depth of

60 m show rapid decline in densities below 20 m (O. Bronstein unpubl. data). Nonetheless, direct interaction in the overlapping depth zone, as well as indirect interactions such as hybridization, may affect the future of these species in ways hard to predict. Nevertheless, one must recognize that, currently, the population size of *Diadema* in the Mediterranean is marginal. As such, competitive exclusion of local species (being diadematoids or other) or interactions of any kind may simply not yet be visible. It is only possible to speculate how these interactions will unfold in the future. As competition may evolve even between distantly related taxa (McClanahan 1988), the drastic reduction in *Paracentrotus lividus* populations along the eastern Mediterranean (Yeruham et

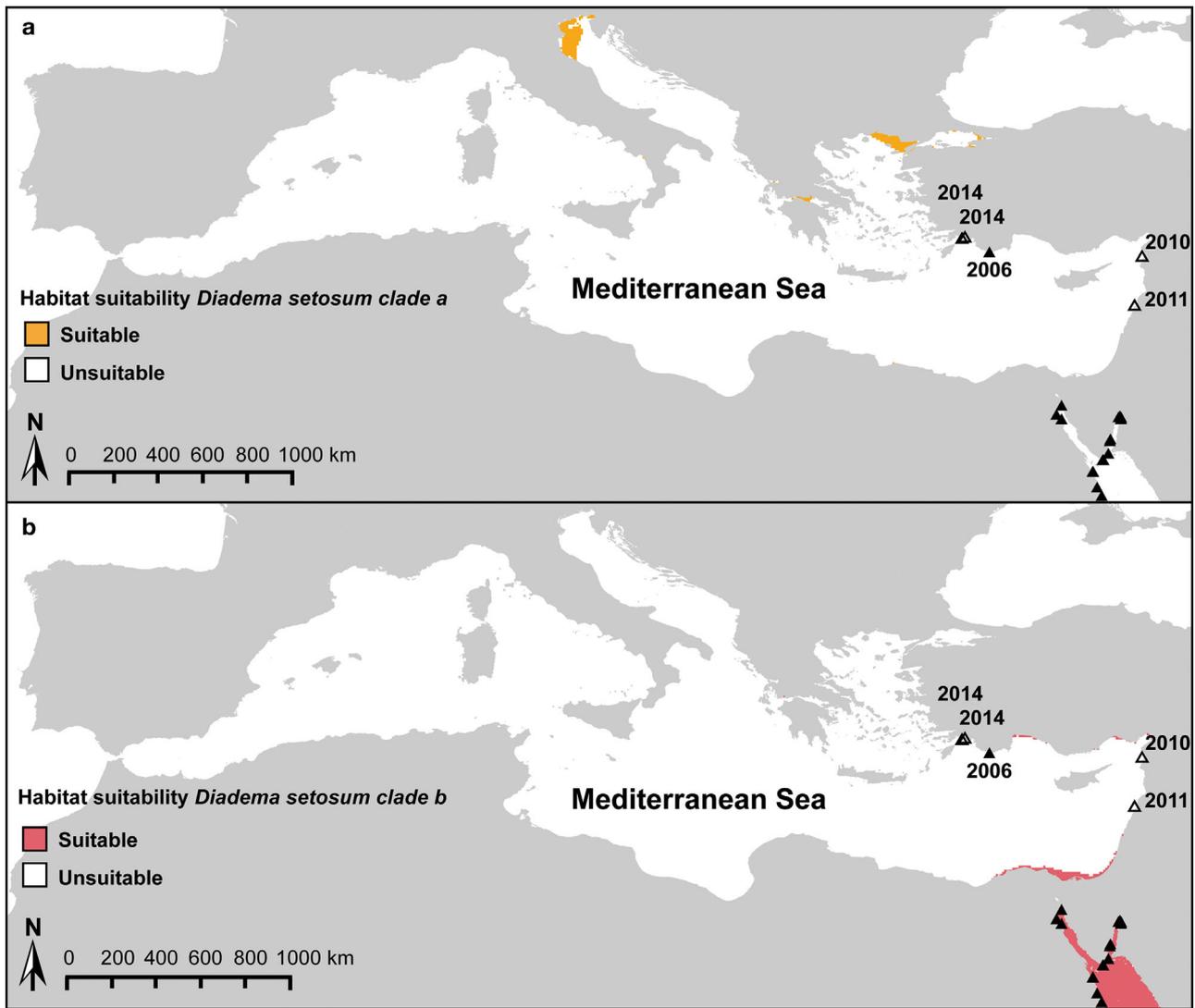


Fig. 7. Suitable predicted range of (a) *Diadema setosum* clade a and (b) *D. setosum* clade b in the Mediterranean Sea based on the MaxEnt model. Suitability maps for the 2 *D. setosum* clades were converted to binary presence–absence maps based on the maximum sensitivity plus specificity test threshold (MST) of MaxEnt. (▲) Site locality of *D. setosum* clade b as confirmed by DNA sequence data; (△) confirmed occurrences of *D. setosum* where no DNA sequence data is available for clade distinction. Mediterranean localities lacking molecular support have been excluded from the model. Numbers next to the Mediterranean presence sites refer to year of first record

al. 2015) may contribute to the expansion of *Diadema* to niches previously occupied by the former. Recognizing the magnitude of *Diadema*'s ecological footprint in other regions (Carpenter 1981, Birkeland 1989, Muthiga & McClanahan 2013), it is likely that, on proliferation, *Diadema* will become a prominent component of eastern Mediterranean shallow benthic communities.

The genetic loci analyzed here demonstrate that the Mediterranean *D. setosum* collected in Turkey belongs to the mitochondrial clade b of this species, native to the Red Sea, Arabian Peninsula, and the Persian Gulf. Nevertheless, as these data are cur-

rently based on a single specimen, future collections of additional material from diverse localities throughout the Levantine Basin are needed to assert whether this pattern is consistent, or whether additional *Diadema* lineages co-occur. Genetic differentiation between the Persian Gulf and Red Sea populations of *D. setosum* is negligible in the markers employed, which is not surprising since marine intrusion in the Persian Gulf started only approximately 12 500 yr ago (Lessios et al. 2001). Longer fragments contributing additional phylogenetic signal, additional rapidly evolving markers (such as the mitochondrial control region, see Bronstein et al. 2017) of both mitochondr-

ial and nuclear loci, as well as comprehensive sampling of *D. setosum* populations in its native range (and in particular in the Persian Gulf) and the Mediterranean would be needed to pinpoint the origin of the introduced specimens or larvae.

D. setosum has not so far been observed along the Mediterranean coast of Israel and Egypt, despite the geographical proximity of these regions to the Mediterranean opening of the Suez Canal. The absence of *D. setosum* from the Israeli coast is supported by extensive field surveys dating back to the mid-20th century (e.g. Gilat-Gottlieb 1959, Gilat 1964, Galil & Lewinsohn 1981) and recent monitoring programs (Yahel 2016) that covered broad areas of potential habitats suitable for this species. Yokes & Galil (2006) have suggested 3 alternative vectors for the introduction of *D. setosum* into the Mediterranean: (1) larval transport through the Suez Canal, (2) shipping, and (3) aquarium trade. The spatio-temporal pattern of *D. setosum* occurrences in the Mediterranean does not follow a gradual succession of directional population expansion (referred to as 'stepping stones') originating at the Mediterranean opening of the Suez Canal, as may be expected from an unmediated larval transport. Two alternative hypotheses may thus explain the absence *D. setosum* from the Israeli coast: (1) longshore counterclockwise currents along the Israeli coastline (Millot & Taupier-Letage 2005) coupled with *Diadema*'s relatively long larval life span (estimated at several weeks; Rahman et al. 2015, Dautov & Dautova 2016) could facilitate their northward transport for several hundreds of kilometers, or (2) a more direct anthropogenic intervention may have been the driver of this species' introduction. Although the preliminary means of introduction cannot currently be unambiguously resolved, this may change in the future with studies applying population genetic tools. Such studies may allow us to identify discrete populations within the natural range of *D. setosum* clade b and, given adequate sampling, assign the Mediterranean population (or populations) to one of them. The range expansion of Mediterranean *D. setosum* observed in the past few years is expected to continue, as suitable habitats are predicted by the SDMs along the Israeli and Egyptian coastline. This expansion, however, may be slowed down by the prevailing counterclockwise current in the Levantine Basin (Millot & Taupier-Letage 2005), which may prevent rapid larval transport in a south-eastern direction.

The concept of time lags in invasion biology is an increasingly recognized aspect of both plant and animal invasions (Crooks 2011, Azzurro et al. 2016). Lags typically refer to either the dormant period or

slow growth of an invader population preceding population outbreaks (see Azzurro et al. 2016). While in some instances an invading species may experience an ecological release causing it to rapidly dominate the newly invaded grounds (e.g. the brown tree snake *Boiga irregularis* on Guam and the zebra and quagga mussels *Dreissena* spp. in North America; Crooks 2011), in other cases an ecological explosion may only come after a long and seemingly dormant period that may last years and even centuries (Rilov et al. 2004, Whitney & Gabler 2008). Records now show that *D. setosum* has already persisted in the eastern Mediterranean for more than a decade. Although the true scale of *D. setosum* proliferation throughout the eastern Mediterranean is currently unknown, the paucity and limited available information for much of the Levantine Basin may have caused severe underestimations. Nonetheless, despite the currently limited ecological data, it is clear that *D. setosum* is well into the establishment phase of its invasion (see Azzurro et al. 2016, their Fig. 2, for details). Consequently, for *Diadema* to persist, reproduction and generation of offspring must take place. As *Diadema*, like most echinoids, are broadcast spawners that release gametes into the seawater (Muthiga & McClanahan 2013, Bronstein et al. 2016), their reproductive success is highly dependent on environmental regulation and the population densities required to achieve synchronous spawning and reach the sperm concentrations needed for fertilization (Levitan & Petersen 1995). Temperature is one of the key environmental variables regulating *Diadema* reproduction and approximately 25°C has been suggested as the minimum threshold needed to complete gametogenesis (Pearse 1970, Muthiga & McClanahan 2013). In the eastern Mediterranean, average monthly sea-water temperatures reach 25°C and above for 5 months a year (www.israelweather.co.il/english/page3.asp?topic_id=76&topic2_id=73&page_id=62), from June through October. Consequently, temperatures seem favorable for *Diadema* reproduction in that region. Inevitably, as local populations keep growing, reproductive success is predicted to increase, and, given suitable habitat availability, the population growth rate is expected to accelerate and possibly lead to population outbreaks.

Ironically, one factor that may contribute to the relatively slow spread of *D. setosum* in the eastern Mediterranean may in fact be related to the proliferation of other Indo-Pacific alien species. Although no gut analysis or direct observations of predation on *D. setosum* have so far been reported, 15 species of finfish, 2 species of gastropods, and the spiny lobster

Panulirus argus are known to prey on species of *Diadema* (Randall et al. 1964, Muthiga & McClanahan 2013). Thus, of the more than 100 Lessepsian alien fish species currently reported from the Mediterranean (Check-list of exotic species: CIESM Atlas of Exotic Fishes in the Mediterranean Sea; www.ciesm.org/atlas/appendix1.html [last accessed on 31.03.2017]), some may potentially be predators of *Diadema* exerting top-down regulation on the latter populations.

Although *D. setosum* is currently unknown from the west coast of mainland India (Clark & Rowe 1971, Sastry 2007), our models predict that some regions, in particular along the south-western Indian coast, exhibit moderate to high relative suitability for this species (Fig. 5). That no records of this species are currently available from these localities may be attributed to the paucity of data from that region and warrants further investigation. Nevertheless, both clades of *D. setosum* seem to be absent from large parts of the north-western Indian Ocean, from the Seychelles through the coast of Somalia to the northern-most part of the Arabian Sea (see, for example, Tortonese 1949, Sloan 1982, Rowe & Richmond 2004). To date, no geographic overlap is known for these 2 mitochondrial clades and a vast potential buffer zone seems to exist between them. While the current biogeographic setup may contribute to maintaining these 2 clades in allopatry, the historical conditions leading to their speciation more than 3 million years ago are currently unknown. One may speculate that a buffer zone and lack of direct contact similar to the one we see today may have existed between the 2 clades, limiting gene flow between them, and setting the stage for their allopatric speciation. Potential barriers, such as the cold water upwelling at the boundary of the Gulf of Aden and the Arabian Sea which is often mentioned as a prominent barrier in that region, could have prevented the transport of both adults and larvae (DiBattista et al. 2016). However, to truly understand the molecular structure we see today, one must gain knowledge of the history of the barrier over the course of the evolution of this species, spanning 3 to 5 million years. More samples are needed, in particular from presumable 'boundary' populations along the coasts of Oman and Somalia, to facilitate detailed population genetic studies. Genetic divergence between the 2 *Diadema* clades is much more distinct than that of most of the other species of this genus (Fig. 3). Indeed, further taxonomic studies as well as the use of additional nuclear markers and additional samples are needed to shed new light on the marine barriers in the Arabian Sea and to con-

clude whether these 2 geographically and genetically distinct clades warrant the distinction of 2 separate species.

Increased sampling efforts along the coast of Oman may help resolve another peculiarity concerning the distribution of *D. setosum* clade b: although the widespread species *D. paucispinum* has been reported from the south coast of Oman (Lessios et al. 2001), *D. setosum* has not. The apparent absence of *D. setosum* from this region is intriguing, as if this gap in the distribution of *D. setosum* clade b truly does exist (and is not an artifact of insufficient sampling), it will be challenging to explain how the Red Sea and Persian Gulf populations of clade b are able to maintain connectivity.

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