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RESEARCH PAPER

Range expansions across ecoregions: interactions of climate change, physiology and genetic diversity

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ABSTRACT

Aim Climate change is expected to drive range shifts among a wide array of organisms. Non-indigenous species (NIS) provide a unique opportunity to observe the establishment of range boundaries in a way that cannot be directly seen for native species. Recent studies have indicated that climate change facilitates biological invasions at local scales. However, the generality of these effects is unclear, as there is a dearth of comparative studies that assess how rapid environmental change affects species ranges across taxa and biogeographic provinces.

Location The South African coast and other coastlines across the world.

Methods We first studied the distribution of shallow-marine benthic organisms along the South African coastline and analysed the global distribution of NIS. We then obtained DNA sequence data from a suite of co-occurring NIS from along the studied coastline and compared these data with available genetic information from other regions of the world. Subsequently, we conducted physiological experiments to assess how thermal tolerance was related to species distribution. Finally, we analysed ship-based seawater temperature records and compared these with past changes in the range size and abundance of NIS. These records were used to estimate shipping intensity and NIS propagule pressure.

Results We found that NIS with a variety of thermal tolerances and distributions have expanded their ranges and increased in abundance as seawater temperature regimes have changed. We found little interannual variation in shipping transport intensity. Most haplotypes of the studied NIS in South Africa were shared with other regions.

Main conclusions This study provides empirical evidence that NIS, regardless of their thermal tolerance, range size and genetic variability, are expanding their ranges and increasing in abundance. This trend is uncorrelated with levels of human-mediated NIS transport but concurrent with changes in seawater temperature, which suggests that climate change fosters the spread and abundance of NIS across multiple spatial scales.

Keywords

Ascidians, biogeography, ecotones, invasive species, naturalization, non-native, performance curve, population expansion, thermal sensitivity.

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INTRODUCTION

Species ranges are historically affected by climatic fluctuations (Roy *et al*., 2001) that may result in range expansions or contractions, with major changes in species borders and ecotones (Parmesan *et al*., 2005). Temperature is one of the most important abiotic factors determining the distribution of the world's biota (Belanger *et al*., 2012), as it influences physiological processes and species interactions across a wide range of taxa (Somero, 2012). For example, temperature affects the ability of propagules and juveniles to disperse and complete development, and thus may determine the geographic ranges of species (Bonte *et al*., 2008) and intraspecific genetic lineages (Teske *et al*., 2008). Consequently, temperature and shifts in range boundaries are inextricably linked.

Our perception of species ranges becomes considerably more complex when non-indigenous species (NIS) are involved, as they do not share an evolutionary history with the native community and, once introduced, establish new range limits that can remain labile for decades (e.g. Crisp & Southward, 1959). Thus, NIS provide an unparalleled opportunity to observe the establishment of species ranges in a way that cannot be directly observed for native species with long-established boundaries (Sax *et al*., 2007). Understanding how NIS ranges are determined, therefore, represents a rich source of knowledge, especially at a time when human-induced climate change and disturbances are expected to alter species ranges world-wide (Walther *et al*., 2009). However, it is important to be aware that NIS ranges may (at least initially) be set in ways that are fundamentally different from the natural boundaries of native species.

The introduction of NIS is generally attributed to a transient window of opportunity (Davis *et al*., 2005). Each new colonization event results from the arrival of only a tiny fraction of the source population (founder event) and that fraction will carry only a subset of the overall genetic diversity (Sakai *et al*., 2001). However, single colonizations are rare and multiple introductions may be more common (e.g. Kolbe *et al*., 2004), allowing introduced populations to escape bottleneck effects. Once a NIS is naturalized, the next step is the invasive period. This generally includes a sudden geographic expansion or a series of saltatory expansions, after which the rate of spread drops and range size eventually stabilizes within new boundaries (Prentis *et al*., 2008).

Climate change is expected to alter temperature regimes and generate poleward and upward range shifts of native species globally (Parmesan *et al*., 2005). The population dynamics and impacts of NIS will also respond to climate change (Walther *et al*., 2009), and recent research suggests that such change will disproportionately facilitate NIS at local scales (Stachowicz *et al*., 2002; Sorte *et al*., 2010). However, the generality of these effects is unclear as there is a dearth of comparative studies that assess how rapid changes in environmental conditions affect species ranges across taxa and biogeographic provinces.

Here we investigated historical range shifts of multiple co-occurring NIS across divergent biogeographic coastal regions to understand the role of environmental filtering, range size, genetic signatures and climatic variability in shaping and maintaining species ranges. We began by documenting the distribution of shallow-marine benthic organisms along a coastline comprising several biogeographic provinces, and analysed the global distribution of NIS. We then compared regional and global genetic signatures of a suite of NIS. Subsequently, we investigated the effects of temperature on individual performance of a subset of species. Finally, we analysed long-term temperature records for the studied coast and evaluated historical changes in species ranges and abundance. Specifically, this research addressed the following questions:

1. Are the studied NIS similar in terms of range size and physiological performance?

2. Is the genetic composition of the studied populations representative of the genetic pool of the global species range?

3. Is there evidence that NIS are expanding their ranges and increasing in abundance? If so, could climate change be responsible for facilitating the success of NIS at regional and global scales?

We inferred that given that the studied species are most likely to be adapted to different temperature regimes, their temperature tolerances would differ. We predicted that comparisons between regional and global genetic signatures would show similar composition among different regions within the introduced range as a result of human-mediated transport. We hypothesized that range expansions of NIS would occur across ecoregions, and that historical temperature variation would be consistent with increases in abundance and rates of spread of NIS.

MATERIALS AND METHODS

Studied taxa

Interest in marine NIS has increased not only because they have a great ability to displace native species and alter ecosystem processes, but also because they have economic impacts on human activities. Shipping and aquaculture activities are the main vectors for the introduction of marine NIS world-wide (McQuaid & Arenas, 2009), and consequently these species are concentrated in harbours, marinas and bays. As the dispersal capabilities, niche occupation strategies and response to environmental factors vary widely among taxa, we chose as our model system the Class Ascidiacea (Tunicata, Chordata), a group containing conspicuous members of coastal benthic and fouling communities world-wide, including key bioengineering species with disjunct distributions (e.g. Teske *et al*., 2011). Ascidians are sessile as adults, and the motile stages (embryonic and lecithotrophic larval stages) can last from just minutes to a few days, which allows for short-distance dispersal (Millar, 1971). Therefore, transoceanic dispersal of these species is solely human mediated.

Study region

The *c*. 3600 km of the South African coastline contains multiple biogeographic regions and a broad gradient in thermal condi-

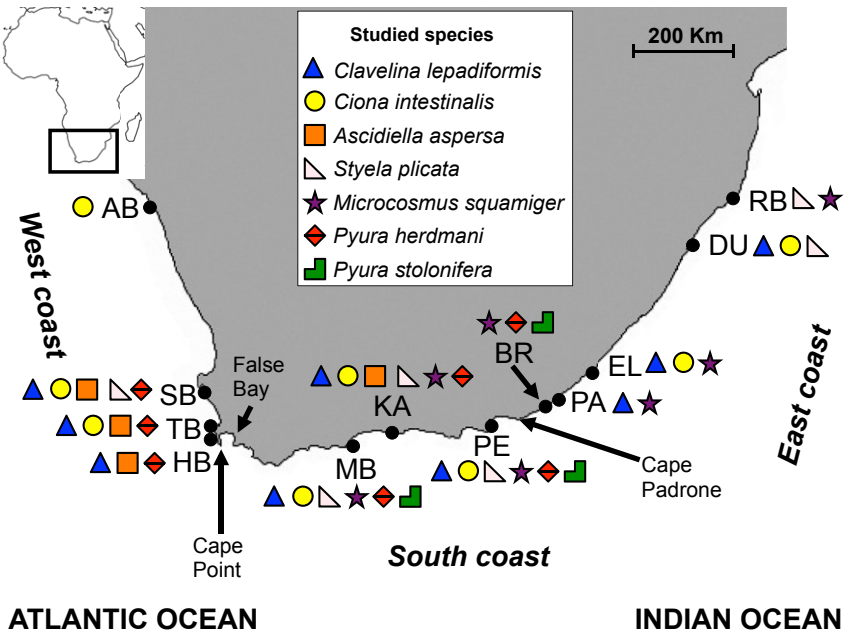

Figure 1 Map of the South African coastline with the sampled sites indicated. The distribution of the studied species found during the field surveys is indicated with symbols. Site abbreviation names and details can be found in Table 1, except for RB, which indicates Richard's Bay – for this site we were unable to conduct a thorough survey but collected samples of *Styela plicata* and *Microcosmus squamiger* for genetic analyses (see main text).

Table 1 Sampled sites included in the present study. The site name abbreviations (Code), the geographic position and the characteristics (Type) of each site are indicated.

Name of the site	Code	Latitude (S)	Longitude (E)	Type
Alexander Bay	AB	28°46′33″	16°34′23″	Oyster farm
Saldanha Bay	SB	33°00′18″	17°56′53″	Small harbour
Table Bay	TB	33°55′22″	18°26′36″	Large harbour
Hout Bay	HB	34°02′60″	18°20′53″	Recreational marina
Mossel Bay	MB	34°10′42″	22°08′40″	Small harbour
Knysna	KA	34°02′29″	23°02′48″	Recreational marina
Port Elizabeth	PE	33°58′02″	25°38′07″	Large harbour
Bushman's River	BR	33°40′47″	26°39′22″	Recreational marina
Port Alfred	PA	33°35′38″	26°53′31″	Recreational marina
East London	EL	33°01′22″	27°53′45″	Small harbour
Durban	DU	29°51′49″	31°01′23″	Large harbour

tions, from tropical waters on the east coast to cool-temperate waters on the west coast (Emanuel *et al.*, 1992), providing an ideal system for examining mechanisms shaping species distributions. The region has been a crossroads for several major transoceanic trading routes (Kaluza *et al.*, 2010) since the 10th century (Yap & Man, 1996), and has an active though limited aquaculture industry (Rius *et al.*, 2011).

Surveys

We surveyed all main harbours along the South African coastline, plus five recreational marinas and an oyster farm (Fig. 1; see Table 1 for details). We chose these sites because they cover the entire coastline and include virtually all the main entry points for NIS. We considered the three traditional major biogeographic provinces proposed for South Africa, namely the west, south and east coasts (Fig. 1) (Stephenson & Stephenson, 1972) (see details in Appendix S1 in Supporting Information). The surveys were conducted twice (2007 and 2009) during the austral winter (details of sampling methodology and species identification can be found in Appendix S2).

Genetic study

Specimens of the widespread NIS *Clavelina lepadiformis*, *Ciona intestinalis*, *Styela plicata* and *Microcosmus squamiger* (see details about these species in Appendix S3) were collected from the same sites during the 2009 survey, and in addition we obtained samples of *S. plicata* and *M. squamiger* from Richard's Bay harbour (28°47′39″ S, 32°04′45″ E) (Fig. 1, Table S1). Sites where fewer than five individuals were found were excluded from the analyses. Samples were collected by hand from harbour ropes or floating pontoons and fixed in absolute ethanol. In addition, we obtained samples from other biogeographic regions including individuals from the Azores and Madeira (see details in Table S2). To maximize information for other regions from GenBank, we targeted a section of the mitochondrial DNA (mtDNA; cytochrome oxidase subunit I, COI). The

smaller effective population size and high mutation rate of mitochondrial markers make them extremely useful for geographic genetic studies (Avise, 2009), particularly studies of biological invasions (e.g. Pineda *et al.*, 2011). In addition, it has been shown that the mutation rate of mtDNA is conservative enough to retain information on the origins and range expansion of introduced populations (Rius *et al.*, 2008). Sequences were obtained using primers described in Table S3 (see general genetic methods in Appendix S4) and aligned in BioEdit v.7.0.5.2 (Hall, 1999). We then used DnaSP v.5.10 (Librado & Rozas, 2009) to determine the number of haplotypes and standard diversity indices (haplotype and nucleotide diversities), as well as the number of unique haplotypes. We excluded GenBank COI sequences that did not align with our haplotypes because they covered a different section of the target gene or the final alignment was unacceptably short. Parsimony haplotype networks were generated using the programme tcs v.1.21 (Clement *et al.*, 2000), which creates an absolute distance matrix by calculating all possible pairwise comparisons among haplotypes, considering a parsimony probability of 0.95.

Effects of temperature on ontogenetic stages

Temperature may not determine species ranges only through its effects on adult performance (Gilman, 2006), as other life stages may be more sensitive (Pineda *et al.*, 2012). Therefore, distributional ranges can be set by the tolerance levels of sexual and asexual propagules rather than adult fitness. To test ontogenetic effects of temperature, we studied the effects of seawater temperature on development of all pre-adult life-history stages. We selected four different NIS (*C. intestinalis*, *Ascidiella aspersa*, *S. plicata* and *M. squamiger*) that have widespread distributions along the world's coastlines (see below) and two species (*Pyura stolonifera* and *Pyura herdmani*) that are native but have a sister species that has been reported as a highly invasive species elsewhere (Teske *et al.*, 2011) (see Sampling Sites and Field Methodology in Appendix S5). We conducted laboratory experiments under a range of temperatures and measured embryonic development time, and the success of larval development, larval settlement and settler metamorphosis (for details of methods see Appendix S6).

Given the nonlinear nature of rate–temperature relationships (Janion *et al.*, 2010) and the fact that most species embryos did not develop above 20 °C (see Results), we only statistically analysed the linear portion of the reaction norm, i.e. from 10 to 20 °C, to evaluate interspecific differences. Therefore, we implemented a linear model with mean embryonic development time as the response variable, and species and temperature as predictors. Interactions between species and temperature indicated differences in reaction norm slopes among species. Given the proportional nature of developmental success data, a generalized linear model using a binomial error structure, and a logit link function was used to assess the effects of species, temperature and their interactions on development success. This model was checked for overdispersion and scaled deviances were obtained when necessary. Species differences were determined by examining least squares means and overlap of the Wald 95% confidence limits from the generalized linear model outputs. The same statistical model type was used to assess the effects of species and temperature on the number of successful post-metamorphs at day 3 after fertilization (i.e. those that had completed the larval, settlement and post-metamorphic stages, providing the most complete measure of success). We also investigated the effects of species, temperature and day after fertilization (first and third day) on the proportion of attached settlers and the proportion of floating (detached from the substratum) settlers in relation to the total number of initial larvae. Finally, we investigated the effects of species, temperature and day after fertilization on the proportion of larvae that failed to settle. All analyses were done with sas v.9.1 (SAS Institute, Cary, NC, USA) and Statistica v.10 (StatSoft, Tulsa, OK, USA).

Seawater temperature data

We obtained sea surface temperature recordings from the South African Data Centre for Oceanography (SADCO) (see Appendix S7 and Table S4 for details). We calculated the mean annual temperature, the mean of summer months (January–March), winter months (July–September) and the difference between the annual maximum and minimum temperature recorded each year. Inter-annual trends were tested using linear regression with a critical value of 0.05. In addition, these data provide an indirect estimate of shipping intensity through time, which we calculated by measuring the number of temperature recordings per year. Data were analysed and plotted using R v.2.10.0 (R Development Core Team, 2011).

Measuring range and abundance shifts of NIS

To understand the recent range shifts of NIS, we combined biogeographic information from taxonomic studies (references in Appendix S3) that included extensive surveys along the South African coast, and our own data. We then plotted the distances among sites where species were recorded to visualize changes in species ranges. We considered five NIS: *Clavelina lepadiformis*, *Ciona intestinalis*, *A. aspersa*, *S. plicata* and *M. squamiger*. All are highly conspicuous and abundant, and are unlikely to have been unnoticed by a specialist. We excluded for this analysis the remaining four NIS obtained in the field surveys (see below). These were two colonial (*Diplosoma listerianum*, *Botryllus schlosseri*) and two solitary NIS (*Ascidia sydneiensis* and *Asterocarpa humilis*). For these the taxonomy is in debate, so they may contain cryptic species or have been misidentified as closely related species. In order to compare abundance trends, we obtained abundance data for all ascidian species from our field surveys and compared the mean values among status types (native, cryptogenic and NIS) and sampling years (i.e. 2007 and 2009).

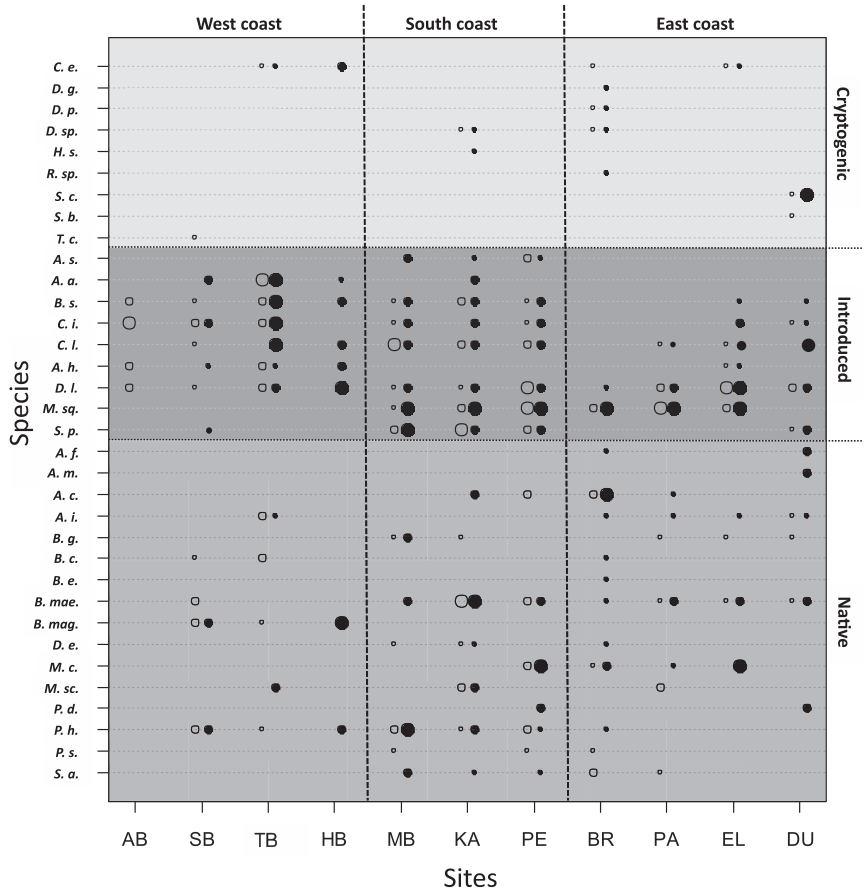

Figure 2 Distribution and abundance of 34 taxa collected during the field surveys of 2007 (open circles) and 2009 (filled circles). The size of the circles indicates relative abundances as follows: scarce, common and dominant as small, medium and large circles, respectively (for details see Appendix S2). Site name abbreviations correspond to those given in Table 1, and are plotted from west to east. Note that Alexander Bay and Hout Bay were sampled in only one year. The biogeographic provinces (west, south and east coasts) and species status (cryptogenic, introduced and native) are indicated. Species names: *C. e.*, *Corella eumyota*; *D. g.*, *Didemnum granulatum*; *D. p.*, *Didemnum psammathodes*; *D. sp.*, *Didemnum* sp.; *H. s.*, *Halocynthia spinosa*; *R. sp.*, *Rhodosoma* sp.; *S. c.*, *Styela canopus*; *S. b.*, *Symplegma brakenhielmi*; *T. c.*, *Trididemnum cerebriforme*; *A. s.*, *Ascidia sydneiensis*; *A. a.*, *Ascidiella aspersa*; *B. s.*, *Botryllus schlosseri*; *C. i.*, *Ciona intestinalis*; *C. l.*, *Clavelina lepadiformis*; *A. h.*, *Asterocarpa humilis*; *D. l.*, *Diplosoma listerianum*; *M. sq.*, *Microcosmus squamiger*; *S. p.*, *Styela plicata*; *A. f.*, *Aplidium flavolineatum*; *A. m.*, *Aplidium monile*; *A. c.*, *Ascidia canaliculata*; *A. i.*, *Ascidia incrassata*; *B. g.*, *Botryllus gregalis*; *B. c.*, *Botryllus closionis*; *B. e.*, *Botryllus elegans*; *B. mae.*, *Botryllus maeandrius*; *B. mag.*, *Botrylloides magnicoecum*; *D. e.*, *Didemnum epikelp*; *M. c.*, *Molgula conchata*; *M. sc.*, *Molgula scutata* (small form); *P. d.*, *Polyandrocarpa durbanensis*; *P. h.*, *Pyura herdmani*; *P. s.*, *Pyura stolonifera*; *S. a.*, *Styela angularis*.

RESULTS

Field surveys

Combining the results from the sampling in 2007 and 2009, we identified 16 species endemic to South African shores, nine NIS that are widely distributed around the world (Fig. S1), and nine species for which the status could not be confirmed and that were included as cryptogenic (Table S5). NIS were the most widespread group, followed by the native species and, finally, the most narrowly distributed group were the cryptogenic species (Fig. 2). All NIS were found in at least two biogeographic provinces, with *C. intestinalis* being the most widely distributed species (Fig. 1). In general, ascidian species richness marginally increased eastwards (Fig. 2).

Comparing regional and global genetic signatures

We obtained a total of 764 COI sequences (Tables S1 & S2) with fragment lengths of 546, 786, 639, 655 base pairs (bp) for *Clavelina lepadiformis*, *Ciona intestinalis*, *S. plicata* and *M. squamiger*, respectively. *Clavelina lepadiformis* showed the lowest haplotype diversity while *M. squamiger* was the most diverse (Fig. S2, Table S1). Haplotype diversity increased eastwards for *C. lepadiformis* and *Ciona intestinalis* (Fig. S2, Table S1). When we compared the haplotypes generated in our

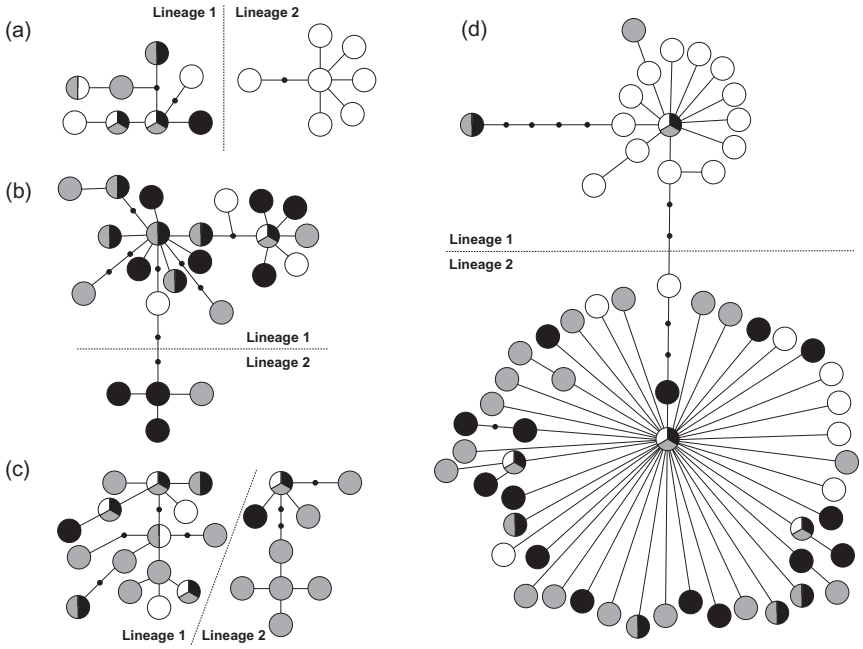

Figure 3 Haplotype network of the species studied (a, *Clavelina lepadiformis*; b, *Ciona intestinalis*; c, *Styela plicata*; d, *Microcosmus squamiger*) indicating the presence of each haplotype in: 1, South Africa (in black); 2, the rest of the introduced range (in grey); 3, native or cryptogenic ranges (in white). The smaller black circles represent unsampled or extinct haplotypes. Branch sections delimited by two circles indicate single mutational steps, irrespective of their branch length. Note that *Clavelina lepadiformis* Lineage 1 is the Atlantic clade and Lineage 2 is the Mediterranean clade of Turon *et al.* (2003); *Styela plicata* Lineage 1 is group 1 and Lineage 2 is group 2 of Pineda *et al.* (2011); *Microcosmus squamiger* Lineage 1 is group H1 and Lineage 2 is group H2 of Rius *et al.* (2008).

study with those from GenBank (Table S2), we used a final alignment of 366, 692, 560 and 561 bp for *Clavelina lepadiformis*, *Ciona intestinalis*, *S. plicata* and *M. squamiger* respectively. The haplotype networks examined the relationships among haplotypes at a global scale and showed two separate lineages for each species (Fig. 3). These lineages showed different levels of genetic divergence. Firstly, topologies connecting haplotypes with a cumulative probability of being correct of greater than 95% were constrained to divergence levels of less than 10 and 12 steps for *C. intestinalis* and *M. squamiger*, respectively. Since the number of mutational steps between haplotype pairs did not exceed these values, the two lineages could be connected (Fig. 3). Secondly, the two lineages of *Clavelina lepadiformis* and *S. plicata* exceeded the maximum number of steps, eight and ten steps respectively, and therefore the two lineages could not be connected with 95% probability (Fig. 3). We obtained a total of 15 haplotypes for *C. lepadiformis*, 23 for *Ciona intestinalis*, 24 for *S. plicata* and 63 for *M. squamiger*. Many haplotypes found in South Africa were also detected elsewhere within the introduced range of the species (Fig. 3). For *Clavelina lepadiformis*, three South African haplotypes were shared with other regions around the world (Fig. 3), of which one had not been sampled before and one was shared with the Azores (Table S2). For *Ciona intestinalis*, all individuals collected in South Africa were Type A (*sensu* Nydam & Harrison, 2007) and we found nine South African haplotypes that were unique and six that were shared with other regions. For *S. plicata*, South African haplotypes were found across the two global lineages that had been formerly reported (Pineda *et al.*, 2011) with one previously undiscovered South African haplotype found in each lineage (Fig. 3). *Microcosmus squamiger* had 15 private haplotypes from South Africa (eight new from our study) and nine shared with other regions (Fig. 3). In addition, two clear lineages were recovered, of which Lineage 1 contained most haplotypes found in South Africa. The haplotypes obtained from the Azores and Madeira had been previously recorded in other regions (Table S2).

Effect of temperature on development, settlement and metamorphosis

Development rate increased slowly up to 20 °C, with the slopes of *C. intestinalis* and *A. aspersa* (Fig. S3a) being lower than for the other four species (linear model, species–temperature interaction effects, $F_{(6,78)} = 296.8$, $P < 0.001$). This difference in slope was mostly due to the development success and relatively long development time of these two species at 10 °C. At 25 °C, *C. intestinalis*, *S. plicata* and *M. squamiger* showed successful development, while no development occurred for *A. aspersa* or either *Pyura* species (Fig. S3a,b). No species showed larval development at 30 °C (Fig. S3b). Temperature, species and their interaction all had significant effects on larval development success (generalized linear model; temperature, d.f. = 1, Wald chi-square = 8.66, $P = 0.003$; species, d.f. = 6, Wald chi-square = 45.46, $P < 0.001$; temperature × species interaction, d.f. = 5, Wald chi-square = 19.15, $P = 0.002$). The 95% Wald confidence limits for each species indicated that the development success of *A. aspersa* and *C. intestinalis*, and of *A. aspersa* and *S. plicata* were not significantly different from each other, but estimates for *A. aspersa* and *C. intestinalis* were significantly higher than for *P. stolonifera*, *P. herdmani* and *M. squamiger* (Table 2).

In the experiment testing the effect of temperature on settlement and metamorphosis, the proportion of successful settlers (defined here as those that completed metamorphosis or post-metamorphs) at day 3 was generally highest at higher temperatures (20–25 °C), but the number of total settlers (successful and non-metamorphosed settlers) showed the lowest numbers for

Table 2 Estimates and Wald 95% confidence limits from the generalized linear models testing for the effects of species on larval development success. 'Chi-square' tests whether the estimate is different from zero, alpha is set at 0.05. Significance of pairwise comparisons was determined by non-overlapping Wald 95% confidence intervals.

Species effect	Estimate	SE	DF	Chi-square	*P*	Wald 95% confidence limits	
Ascidiella aspersa	−0.136	0.273	1	0.25	0.618	−0.672	0.399
Ciona intestinalis	0.561	0.279	1	4.05	0.044	0.015	1.108
Styela plicata	−1.229	0.331	1	13.82	< 0.001	−1.877	−0.581
Microcosmus squamiger	−3.874	0.961	1	16.23	< 0.001	−5.758	−1.989
Pyura herdmani	−2.053	0.228	1	81.35	< 0.001	−2.499	−1.607
Pyura stolonifera	−2.155	0.349	1	38.11	< 0.001	−2.840	−1.471

most species at the highest temperature (30 °C) (Fig. S4). *Pyura stolonifera* showed higher settlement success at 20 °C, while other species (e.g. *M. squamiger* and *S. plicata*) performed better at 25 °C. A noteworthy exception was *A. aspersa*, which, at high temperatures (e.g. 25 °C; Fig. S4a) produced settlers despite poor larval developmental success at these temperatures (Fig. S3b). Settlement data showed a significant effect of temperature on all variables analysed: successful settlers, floating settlers and failed larvae (Table 3). When we analysed the effect of each factor and their interactions for each species separately (Table S6), most interactions between the factors 'day' and 'temperature' were significant. *Pyura* species were the only species for which temperature did not have consistent major effects. Floating settlers (i.e. settled to the water surface pellicle or settlers that started metamorphosis while in the water column) and failed larvae were not considered viable. We found an increase of floating settlers with temperature and time, especially at 30 °C for day 3, which indicated that temperature stress affected their final success (Fig. S4c). Lower temperatures resulted in the highest proportion of failed larvae, although this trend weakened with time (Fig. S4d). Regarding post-metamorphic stages, we found that most species achieved metamorphosis at three different temperatures, while native species did so at only one or two temperatures (Fig. 4). *Ascidiella aspersa* and *C. intestinalis* were able to complete metamorphosis at 15 °C, and only *S. plicata* completed metamorphosis at 30 °C (Fig. 4).

The results of the laboratory experiments and resulting temperature tolerance breadth for all stages are summarized in Table 4, and broadly show that the eggs and larvae of all species were able to develop, settle and metamorphose at 20 °C, but only *A. aspersa* and *C. intestinalis* were able to do so at 15 °C, and *C. intestinalis*, *M. squamiger* and *S. plicata* at 25 °C.

Table 3 Output of generalized linear models reflecting the effects of species and temperature on (a) overall successful settlers/metamorphs and (b) settlers including incomplete metamorphs. Same models were run including the effects of species, temperature and days on (c) floating settlers and (d) failed larvae. Models (a) and (b) only include data from day 3 after fertilization whereas (c) and (d) examine the effect of day of observation.

Effect	d.f.	Wald chi-square	*P*
(a) Proportion of successful settlers/complete metamorphs			
Temperature	1	8.43	**< 0.01**
Species	6	65.44	**< 0.001**
Temperature × species	5	4.36	0.50
(b) Proportion of settlers including incomplete metamorphs			
Temperature	1	0.09	0.77
Species	6	71.37	**< 0.001**
Temperature × species	5	46.88	**< 0.001**
(c) Proportion of floating settlers			
Temperature	1	13.49	**< 0.001**
Species	6	87.01	**< 0.001**
Days	1	7.19	**0.007**
Temperature × species	5	26.55	**< 0.001**
Species × days	5	22.24	**< 0.001**
Temperature × days	1	6.09	**0.01**
Temperature × species × days	5	32.74	**< 0.001**
(d) Proportion of failed larvae			
Temperature	1	82.33	**< 0.001**
Species	6	126.28	**< 0.001**
Days	1	23.76	**< 0.001**
Temperature × days	1	31.16	**< 0.001**
Temperature × species	5	8.91	0.11
Species × days	5	8.04	0.15
Temperature × species × days	5	15.93	**< 0.01**

Significant results ($P < 0.05$) are indicated in bold.

Temperature records

The SADCO records indicated that seawater temperature during the period 1960 to 2010 has significantly increased in six sites and remained relatively stable in the remaining five sites (Fig. S5). Most sites showed significant positive slopes in mean winter temperatures, with the exception of the northern sites of Alexander Bay and Durban, which lie on opposite coasts (Fig. 1). Only four sites showed significant positive regressions for the summer months (Fig. S5). The magnitude of such change in mean values ranged from +0.5 to +1.5 °C over the five-decade period. All sites showed an increasing trend when annual differences between maximum and minimum temperatures were plotted, although only three sites showed significant positive slopes (Fig. S6). At these sites increases in temperature of 2–3 °C were observed during this period.

There was an increase in the frequency of temperature readings in the late 1960s and early 1970s which is likely to reflect an

Table 4 Summary results of experiments testing the success of egg development (D), larval settlement (S) and settler metamorphosis (M) 3 days after fertilization at different temperatures for the studied species.

Species temperatures	10	15	20	25	30
Developmental stage	D / S / M	D / S / M	D / S / M	D / S / M	D / S / M
Ciona intestinalis	☑ / ☒ / ☒	☑ / ☑ / ☑	☑ / ☑ / ☑	☑ / ☑ / ☑	☒ / ☑ / ☒
Ascidiella aspersa	☑ / ☑ / ☒	☑ / ☑ / ☑	☑ / ☑ / ☑	☒ / ☑ / ☑	☒ / ☑ / ☒
Styela plicata	☒ / ☒ / ☒	☑ / ☑ / ☒	☑ / ☑ / ☑	☑ / ☑ / ☑	☒ / ☑ / ☑
Microcosmus squamiger	☒ / ☑ / ☒	☑ / ☑ / ☒	☑ / ☑ / ☑	☑ / ☑ / ☑	☒ / ☑ / ☒
Pyura herdmani	☒ / ☑ / ☒	☑ / ☑ / ☒	☑ / ☑ / ☑	☒ / ☑ / ☑	☒ / ☑ / ☒
Pyura stolonifera	☒ / ☑ / ☒	☑ / ☑ / ☒	☑ / ☑ / ☑	☒ / ☑ / ☒	☒ / ☑ / ☒

☑ and ☒ indicate success or failure, respectively. Overall failure (indicated in grey) was considered when larval development could not be achieved or when metamorphosis was not completed.

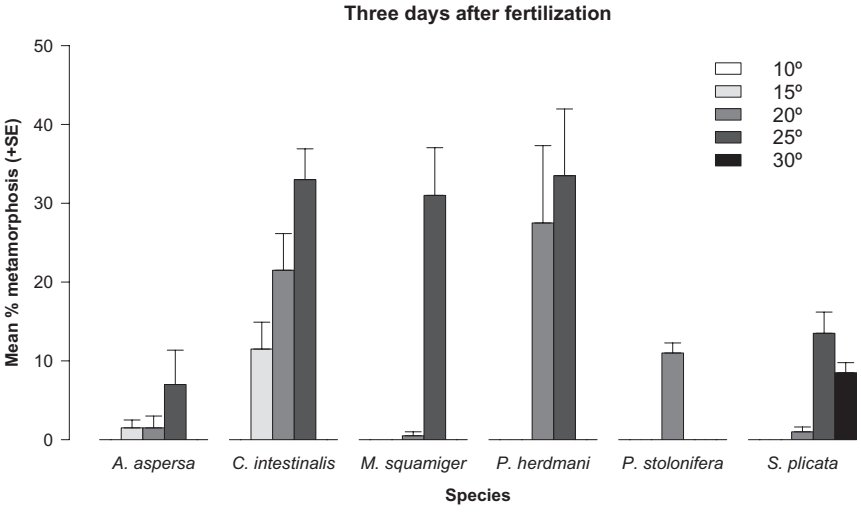

Figure 4 Mean percentage of individuals that successfully completed metamorphosis (means ± SE) for each species (*x*-axis) and temperatures (grey scale) at day 3 after fertilization.

increase in shipping intensity coinciding with the closure of the Suez Canal (Fig. S7). Before and after this period the number of temperature readings remained relatively constant with a gradual decline towards the early 21st century. This is taken as indirect evidence that shipping intensity, a known vector of NIS correlated with propagule pressure, did not increase significantly over the studied period.

Changes in species ranges and abundance

We found evidence of range expansion between years for NIS (Figs 2 & 5). Observed expansions ranged between *c.* 1000 and 2500 km, with *C. intestinalis* showing the widest range and the greatest range expansion. In contrast, the distribution of both native and cryptogenic species did not vary (Fig. 2), with some species showing small range contractions and others expanding. NIS were on average more abundant than native and cryptogenic species in both 2007 and 2009 (Fig. 6), and increased significantly in abundance between years (*t*-test; $t = -2.035$, d.f. = 176, $P = 0.043$).

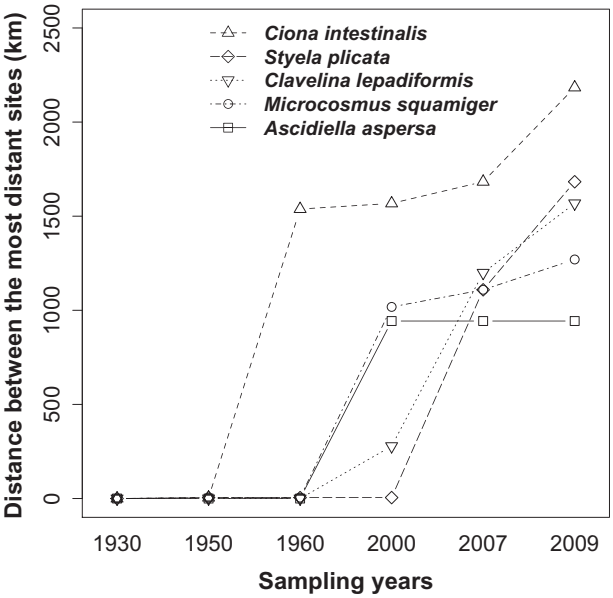

Figure 5 Range expansion estimates of the studied non-indigenous species along the South African coast. We included data from taxonomic records (1950, 1960 and 2000) and our surveys (2007 and 2009) to calculate the distance between the most distant sites where each species has been documented.

DISCUSSION

We found that NIS both expanded their ranges and increased in abundance across diverse biogeographic regions. This trend was

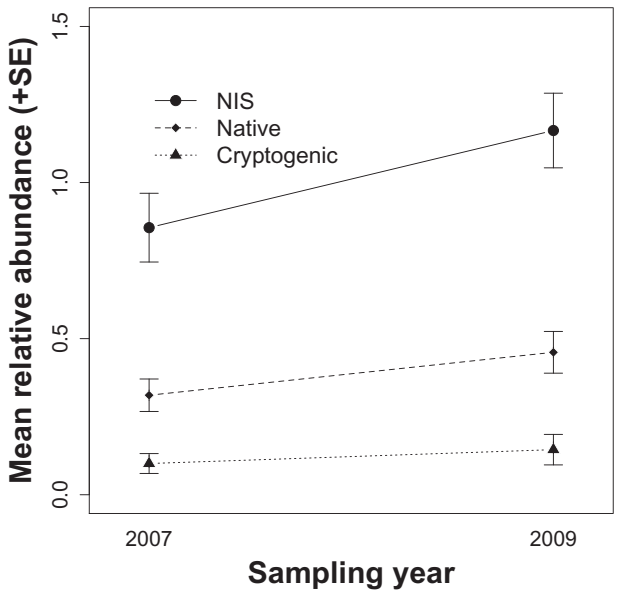

Figure 6 Changes in species abundance along the South African coast between sampled years. We used mean relative abundance of native, cryptogenic and non-indigenous species (NIS), pooling data from all sites and species.

independent of species differences in optimal temperature, range size or genetic variability at both regional and global scales. In line with this, interannual variation of shipping intensity was unrelated to the expansion of NIS. Although other factors not included in this analysis (e.g. transport via recreational boating) cannot be dismissed, the increase in range and abundance of NIS was synchronized with a trend in seawater temperature over the last 50 years towards warmer mean temperatures and a wider thermal range. This suggests that the facilitating effects of climate change on biological invasions advocated by previous studies at local scales (Stachowicz *et al*., 2002; Sorte *et al*., 2010) also occur at regional and perhaps global levels.

Integrating genetic patterns across different spatial scales

Our study revealed two divergent mtDNA lineages in each of the four species studied, with extensive geographical mixing and sympatry of widespread genotypes. This indicates multiple secondary contacts of ancestral lineages at both regional and global scales (for specific details see Appendix S8). Such processes have the potential to generate adaptive evolutionary changes in invasive populations (Chun *et al*., 2009). In addition, these patterns could be explained by within-species physiological differences among lineages, and/or limited connectivity among certain harbours. However, this requires further investigation using a more multilocus dataset (e.g. Rius *et al*., 2012). When we placed the South African populations within a global context, we found an intricate distribution of haplotypes. This suggests a scenario of continuous interchange of propagules due to exchange through shipping (Kaluza *et al*., 2010), which has a homogenizing effect on the genetic composition of introduced populations (e.g. Pineda *et al*., 2011). Thus, the genetic composition of the introduced range most likely consists of a mixture of diverging genotypes from the native range, and the picture is one of a trend towards global genetic panmixia. The widespread regional and global distribution of these species indicates their adaptation to several climatic regions, and our data suggest that individuals containing certain haplotypes are more widespread and perhaps more adaptable than other individuals of the same species. The range shifts observed for the studied NIS were independent of their global level of genetic diversity (Table S1, Figs 3 & S2).

Effects of temperature on early life-history stages

In the sea the planktonic larval stage has a major influence on enabling dispersal and population connectivity (McQuaid, 2010). The large diversity of evolutionary strategies in the sea has resulted in a wide range of propagule forms. This gives rise to varying degrees of planktonic periods and dispersal capabilities, and can lead to high levels of intraspecific phenotypic plasticity (but see Ling *et al*., 2008) due to variable conditions. Our experimental results indicate that higher-temperature treatments induced earlier settlement and metamorphosis. Some species could not complete egg development at higher temperatures, even though their larvae performed well and completed metamorphosis at these temperatures, which suggests that initial development stages are more sensitive. In contrast, cold environments delayed or constrained larvae settlement and metamorphosis (see also Dybern, 1965; Thiyagarajan & Qian, 2003).

Our laboratory experiments indicate thermal limitation during early life-history stages, especially at extreme temperatures (10 and 30 °C). This suggests that coastal regions that experience such temperatures during reproductive periods will be unable to support these species. This could explain the absence of some species on the west coast (e.g. *M. squamiger*), where strong upwelling periods can reduce sea surface temperatures to 8–9 °C. However, it is known that some groups of organisms can shift their phenology and seasonal thermal tolerance with changing environmental conditions (Millar, 1971; Yang & Rudolf, 2009). Thus, such species are able to adapt or demonstrate plastic responses when facing different thermal conditions or latitudes. Our physiological results showed that the optimal temperature for the studied species was between 15 and 20 °C, which is in accordance with previous studies (Thiyagarajan & Qian, 2003). However, even if development is assumed to be ideal at such optimal temperatures, suboptimal conditions may also play an important role in species establishment. For instance, although the embryonic development of *C. intestinalis* was improved between 15 and 20 °C (Fig. S3) (see also Dybern, 1965; Bellas *et al*., 2003, for performance curves in other regions), this species was capable of settling and completing metamorphosis in both warmer (> 20 °C) and colder conditions (Figs 4 & S4). In general, all the studied NIS show widespread distributions around the world (Fig. S1), suggesting

a broad range of temperature tolerance. The link between thermal tolerance ranges and geographic success requires careful interpretation, especially when extrapolating physiological outcomes to other regions without accounting for microsite temperature variability (Clusella-Trullas & Chown, 2011) or ecological interactions with the receiver community.

Thermal tolerance and changes in temperature regimes

In terrestrial ecosystems, temperature has been used to predict both extinctions and the spread of species by considering different scenarios of climate change driven by anthropogenic effects (Deutsch *et al.*, 2008). For example, organisms with restricted thermal tolerance have moved to higher elevations and latitudes in response to recent climate change (Angert *et al.*, 2011). For ascidians, temperature exerts a strong influence on reproduction, development, energy requirements and feeding across all life-history stages (Millar, 1971; Thiyagarajan & Qian, 2003). Thus, a slight change in seawater temperature has the potential to affect species survival significantly through lethal and sublethal effects. Warming is believed to have the most deleterious consequences on organisms that are relatively sensitive to temperature change and are currently living in conditions close to their optimal temperature or 'safety margin' (Deutsch *et al.*, 2008). This might be even more critical during the most sensitive ontogenetic stages, for which optimal temperature ranges are narrower (Pineda *et al.*, 2012). Therefore, the biological consequences of rising temperatures depend on the physiological sensitivity of each organism (Somero, 2012) and, as demonstrated here, the cumulative effects through multiple life-history stages. Our results indicate higher thermal tolerances of NIS during the developmental stages than for native species. However, this requires further investigation by including a larger number of phylogenetically dissimilar native species and broader sampling of conspecifics of different origins to evaluate the role of local adaptation. Empirical evidence is especially needed in aquatic environments, as there is a paucity of studies that analyse the influence of altered environmental conditions on performance of both native species and NIS in these ecosystems compared with terrestrial ones (Sorte *et al.*, 2013).

Climate change and species invasions

The analysis of ship-based temperature data collected over the last 50 years revealed a significant positive trend at most sites, indicating that temperatures are predominantly increasing. This trend was supported by the annual mean temperature and most especially by the mean temperature of winter months (Fig. S5). Analyses of time-series of infrared satellite imagery suggest a more complex picture, with cooling on the south and southwest coasts of South Africa, and warming on the east coast (Rouault *et al.*, 2010). This is supported by minor, but telling, changes in the distribution of cold-water kelps (Bolton *et al.*, 2012). In either event, the situation is one of changing conditions. The differences between maximum and minimum temperature revealed a positive trend towards more extreme annual temperatures at most sites (Fig. S6). In line with this, extreme climatic events, which are expected to increase in the future, have recently been identified as potential factors enhancing species invasions (Diez *et al.*, 2012). This suggests that species with a wider thermal niche have the potential to benefit from more extreme conditions in the future. Correspondingly, our field data show that biogeographic patterns are related to the thermal-response results obtained during the early life-history stages of the studied species – the most widespread species had greater developmental thermal tolerances, while the lowest and highest experimental temperatures were generally correlated with the range boundaries of the studied species.

The warming of seawater temperature has been identified as an important driver of community change (e.g. Sorte *et al.*, 2010). Warmer seawater temperatures in winter have been shown to enhance the earlier seasonal arrival of invasive species at local scales, by inducing earlier recruitment (Stachowicz *et al.*, 2002). In contrast, resident species might become increasingly poorly adapted to the local environment, opening colonization opportunities for NIS. Our study provides empirical evidence of range expansions of NIS at multiple spatial scales during a period of change of temperature regimes, which indicates a trend towards global biotic homogenization.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Figure S1 Global distribution of the non-indigenous species found in the present study.

Figure S2 Haplotype frequencies for each site and species.

Figure S3 Embryonic development and larval development at different temperatures.

Figure S4 Settlement and metamorphosis rates across temperature treatments and between days.

Figure S5 Time series of mean seawater surface temperature recordings.

Figure S6 Time series using the largest difference between maximum and minimum annual temperatures.

Figure S7 Shipping intensity estimated indirectly from the temperature records.

Table S1 Name and genetic characteristics of the sampled sites.

Table S2 GenBank accession numbers for all the sequences included in our study.

Table S3 Primers (5′–3′) used for each of the studied species.

Table S4 Sample sizes of seawater temperature recordings for each site and year.

Table S5 Species found during the field samplings considering species status.

Table S6 Models testing the effects of temperature on settlement and metamorphosis.

Appendix S1 Biogeographic provinces found along the South African coastline.

Appendix S2 Methodology used in the field surveys and species identification.

Appendix S3 Species used for the genetic analyses.

Appendix S4 Genetic methods. DNA extraction, amplification and sequencing.
Appendix S5 Sampling sites and general field methodology.
Appendix S6 Larval culture methods and experimental methodology.
Appendix S7 Methodology used to obtain seawater temperature recordings.
Appendix S8 Interpretation of the genetic patterns per species.

BIOSKETCH

Marc Rius is interested in the underlying mechanisms that determine and maintain species ranges and how alterations such as anthropogenic disturbances and biological invasions affect the composition of native assemblages. His research includes biogeography, population genetics, community ecology and conservation biology, with a special focus on marine foundation species.

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