

## The recent colonization of south Brazil by the Azores chromis *Chromis limbata*

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The damselfish *Chromis limbata* is native to the Macaronesian Archipelagos (Azores, Madeira and Canaries) and the western coast of Africa between Senegal and Angola. During the austral summers of 2008 and 2009 the species was recorded for the first time in the south-western Atlantic Ocean around Campeche and Xavier Islands, in Florianópolis, Santa Catarina State, Brazil. Here, the progression of *C. limbata* in southern Brazilian waters is described using visual counts and genetic surveys and changes in the density of the native congener *Chromis multilineata* were also investigated. Underwater visual censuses of both *Chromis* species were carried out from 2009 to 2014. *Chromis limbata* tissue samples were collected and the mtDNA control region was sequenced and compared with mtDNA haplotypes from the natural range to confirm species identity, compare genetic diversity and to infer connectivity between newly established Brazilian populations. The Brazilian population of *C. limbata* increased significantly over the past 5 years and the effect on *C. multilineata* is still an open question, longer time-series data will be necessary to clarify possible interactions. The molecular analyses confirmed species identity, revealed strong haplotype connectivity among Brazilian study sites and showed a low genetic diversity in Brazil when compared with the native populations, suggesting few individuals started the invasion. Four hypotheses could explain this colonizing event: *C. limbata* was released by aquarium fish keepers; larvae or juveniles were transported *via* ship ballast water; the species has rafted alongside oil rigs; they crossed the Atlantic Ocean through normal larval dispersal or naturally rafting alongside drifting objects. The rafting hypotheses are favoured, but all four possibilities are plausible and could have happened in combination.

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Key words: biogeography; dispersal; invasion genetics; invasive species; reef fishes; south-western Ocean.

## INTRODUCTION

Biological invasions are considered one of the main threats to biodiversity worldwide, with detrimental consequences for native community ecology and local economies (Elton, 1958; Cardinale *et al.*, 2006; Lurgi *et al.*, 2014). Many alien species constitute drivers of major environmental change, affecting nutrient cycling, food webs and

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even causing changes in evolutionary trajectories (Strauss *et al.*, 2006; McDonald *et al.*, 2008; Chown *et al.*, 2015). Therefore, much attention has been given to understanding the invasion process and the mechanisms that are involved in each stage of human-mediated invasions (*i.e.* transport, colonization and establishment) (Blackburn *et al.*, 2011; Chown *et al.*, 2015). Natural range expansions however, are poorly understood, mostly because they are rare and hard to detect and monitor (Gillespie *et al.*, 2012).

The Azores chromis *Chromis limbata* (Valenciennes 1833) is native to the Macaronesian islands (Azores, Madeira and Canaries) and the western coast of Africa between Senegal and Angola (Wood, 1977; Edwards, 1986; Domingues *et al.*, 2006; Wirtz, 2012). This species inhabits rocky reefs from 3 to 50 m depth, where it forms aggregations in the water column and feeds on zooplankton (Domingues *et al.*, 2006; Leite *et al.*, 2009). During the summer, nesting males defend territories and take care of eggs, which are attached to the substratum (Domingues *et al.*, 2006; Leite *et al.*, 2009). The duration of *C. limbata*'s larval stage is unknown, but it lasts 18–19 days in the closely related *Chromis chromis* (L. 1758) (Domingues *et al.*, 2006; Leite *et al.*, 2009).

In March and April of 2008, two individuals of *C. limbata* were repeatedly observed at Campeche Island (27° 70' 38" S; 48° 46' 83" W), state of Santa Catarina, southern Brazil by Leite *et al.* (2009). In May, a single individual was encountered and in June none was sighted. All encounters occurred in the same 12 m<sup>2</sup> boulder area, suggesting a small home range. Both specimens were observed feeding together with a single individual of *Chromis multilineata* (Guichenot 1853), a look-alike species native to the area. Both species feed on zooplankton and share a similar niche. In December 2008 and March 2009, three to five individuals were observed at Xavier Island (27° 41' 57" S; 48° 28' 05" W) located 12 km north of Campeche Island, 3 km from the east coast of Florianópolis Island, Santa Catarina State (Leite *et al.*, 2009).

The increasing number of records of *C. limbata* at Santa Catarina since 2009 indicated an important ecological event (Anderson *et al.*, 2015). Here, the progression of *C. limbata* in southern Brazilian waters is described using visual counts and genetic surveys and changes in the density of the native congener *C. limbata* that shares a similar niche were also investigated, to answer the following questions: has the Brazilian *C. limbata* persisted over this period and are the populations expanding in space and time? Are *C. multilineata* population densities decreasing due to the presence of *C. limbata*? If the populations of the invasive species increase in densities, it would indicate the persistence of its populations. In addition, a decrease in density of *C. multilineata* native populations could indicate detrimental effects (*e.g.* density-dependent antagonistic mechanisms) induced by *C. limbata*'s population expansion. What are the levels of genetic connectivity among Brazilian *C. limbata* populations? What is their relationship with West African populations, can we pinpoint a specific source? Did the invasion originate from a single, few or multiple introductions? To test the above, phylogenetic relationships between native and invasive populations were studied and patterns of genetic diversity compared. If nucleotide and haplotype diversity are low relative to the native populations it suggests that the invasion was from a few individuals, if the genetic diversity and number of haplotypes are similar to the native populations it suggests that there have been multiple invasions and probably from different sources. In addition, a genetic comparison would allow confirmation of the taxonomic status of the Brazilian invaders as *C. limbata*.

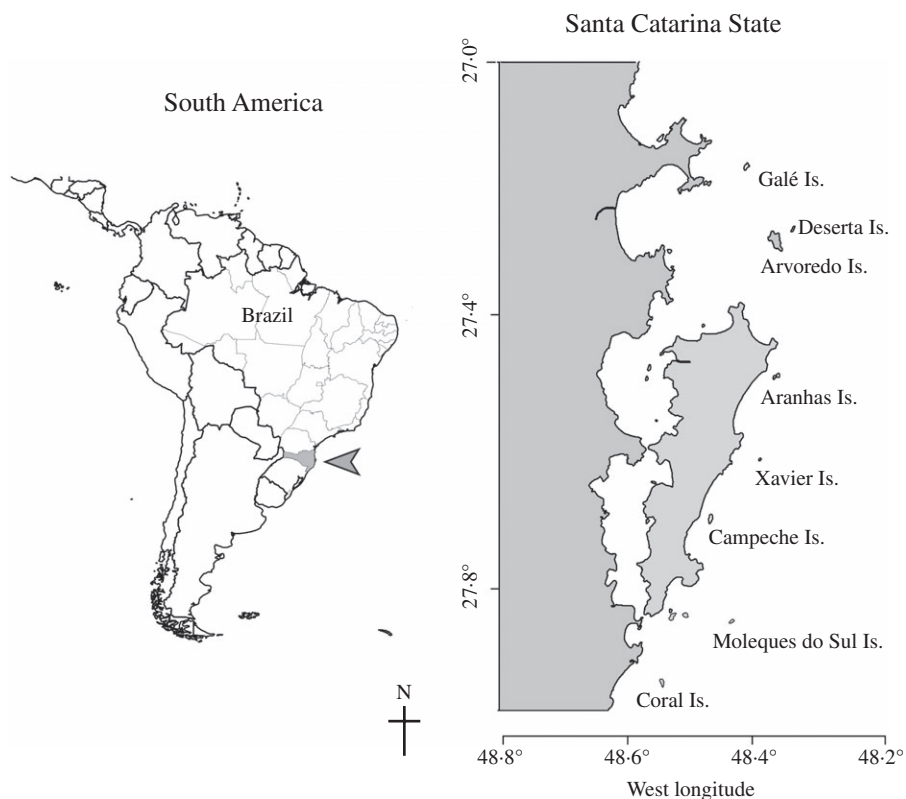


FIG. 1. Location of sampling sites (◄) in the state of Santa Catarina, southern Brazil.

## MATERIALS AND METHODS

### STUDY AREA

This study was carried out on subtropical reefs at Florianópolis, Santa Catarina, southern Brazil ( $27^{\circ} 35' 41.08''$  S;  $48^{\circ} 32' 38.96''$  W). Reefs in the area are mostly composed of steep granitic rocks, interfacing with sandy bottoms, generally 10–15 m deep (Anderson *et al.*, 2014). The study was conducted during austral summers, from 2010 to 2016. During this period, water temperatures ranged from 22 to 28°C and underwater visibility ranged from 4 to 15 m.

*Chromis limbata* was recorded on eight islands: Arvoredo, Deserta, Galé, Aranhas, Xavier, Campeche, Moleques do Sul and Corais (Fig. 1). To infer the progress of the *C. limbata* colonization, its potential influence on local congener populations of *C. multilineata* and to study the population genetics of *C. limbata*, five islands were selected for sampling: Arvoredo, Deserta, Galé, Aranhas and Xavier. These islands were selected for logistical reasons (*e.g.* boat availability and short distance from the shore).

### FIELD-DATA COLLECTION

Underwater visual censuses [ $20 \times 2$  m strip transects ( $40 \text{ m}^2$ )] were used to quantify fish density across the five sites. For this methodology, a scuba diver swam 1 m above the substratum along 20 m, recording fish 1 m to each side of the transect. At each study site, two depth strata were sampled: slope and interface. Slope (*S*) was considered the area comprising the water surface and half of the total depth ( $D_T$ ). Thus, if  $D_T = 12$  m, the slope would be = from 0 to 6 m

[i.e.  $D_T$  (12 m depth) divided by 2 (stratum) = slope (6 m)]. The interface ( $I$ ) corresponded to the transition zone between the rocky reef and the non-consolidated substratum, typically sandy bottom (Anderson *et al.*, 2015). Total depth ( $D_T$ ) varied from 7 m at Arvoredo to 24 m at Xavier. For each depth stratum, 15 transects were carried out each year, totaling 30 transects (1200 m<sup>2</sup>) year<sup>-1</sup> site<sup>-1</sup>.

## POPULATION DENSITY ANALYSES

To test the effect of time (year) on the density of *C. limbata* and its close relative and potential competitor *C. multilineata*, two-way ANOVA was used. Mean density per site was used as a dependent variable and time and site as the fixed factors. To evaluate differences in the mean densities of *C. limbata* and *C. multilineata*, across the five islands and throughout the years, a mixed design ANOVA was used. Mean densities of both species were used as response variables and year and island (space and time) as fixed factors. Two-way ANOVA was also used to evaluate differences in species vertical distribution (depth effect) in two depth strata [slope ( $S$ ) and interface ( $I$ )]. In this case, only, the sum of mean densities of species in all islands were aggregated and used as a response variable and strata used as fixed factor. When significant differences were found, the Tukey honest significant difference *post hoc* test was used to verify sources of variation. Before the analyses, assumptions of normality and homoscedasticity were assessed with Kolmogorov-Smirnov–Lilliefors and Bartlett tests (Underwood, 1981; Snedecor & Cochran, 1989; Zar, 1999). Analyses were run in R environment ([www.r-project.org](http://www.r-project.org)) with the package agricolae (De Mendiburu, 2016).

## SPECIMEN COLLECTION, DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

In 2012 and 2013, 29 specimens of *C. limbata* were collected with a spear while scuba diving. The left pectoral fin of each individual was removed, labelled and preserved in 95% ethanol. The samples were stored at  $-4^{\circ}\text{C}$  until DNA extraction. Genomic DNA was extracted using a standard phenol-chloroform protocol (Sambrook & Russell, 2006). A fragment of the mitochondrial (mt)DNA control region (d-loop) was amplified using universal primers *Cr-a* and *Cr-b* (Lee *et al.*, 1995). Brazilian *C. limbata* sequences were compared with mtDNA haplotypes from the natural range to confirm species identity, compare genetic diversity and to infer connectivity between newly established Brazilian populations. The fast evolving control region was used because it is useful for population genetic comparisons and because it allows comparison with data already available from native populations (Domingues *et al.*, 2006). PCR products were visualized on 1% agarose gel, purified with ExoSapIT (ThermoFisher; [www.thermofisher.com](http://www.thermofisher.com)) and cycle-sequenced in both forward and reverse direction on an ABI 3130 automated sequencer (Applied Biosystems; [www.appliedbiosystems.com](http://www.appliedbiosystems.com)). PCR amplification was performed in 15  $\mu\text{l}$  reactions using *c.* 10 ng of template DNA, 1.5  $\mu\text{l}$  of  $\times 10$  PCR buffer, 0.75 MgCl<sub>2</sub> (50 mM), 0.3  $\mu\text{l}$  of each forward and reverse primers (10  $\mu\text{M}$ ), 0.75  $\mu\text{l}$  of 10 mM deoxynucleotide triphosphate (dNTP) and 0.3  $\mu\text{l}$  of DNA polymerase (5 units  $\mu\text{l}^{-1}$  Invitrogen; [www.invitrogen.com](http://www.invitrogen.com)). PCR conditions were as follows: initial denaturation at  $94^{\circ}\text{C}$  for 2 min, followed by 30 cycles of denaturing at  $94^{\circ}\text{C}$  for 60 s,  $54^{\circ}\text{C}$  annealing for 60 s,  $72^{\circ}\text{C}$  extension for 2 min and a final extension of  $72^{\circ}\text{C}$  for 10 min.

## GENETIC ANALYSES

Analyses were performed using samples collected by the team as well as sequences downloaded from GenBank (*C. limbata* d-loop sequences from eastern Atlantic Ocean locations and a sequence of *C. chromis*, from Domingues *et al.*, 2006). Sequences were aligned and trimmed to 328 bp in Geneious 5.4 (Kearse *et al.*, 2012) using ClustalW (Thompson *et al.*, 1994) with free end gaps and an IUB cost matrix. Phylogenetic relationships of *C. limbata* native and invasive individuals were assessed using the Bayesian method. Trees were constructed using the MrBayes (Ronquist & Huelsenbeck, 2003) plug-in available in Geneious with *C. chromis* as an out-group and the following settings: 1 000 000 Markov chain Monte-Carlo chain length,

subsampling frequency 1000, burn-in length 50 000, four heated chains and temperature 0.2. jModelTest (Guindon & Gascuel, 2003) was used to explore the most likely model of evolution, which was TrN + G. Since this model is not available in MrBayes, the second most likely model HKY + G was used. In addition, a haplotype network was created using the R package pegas (Paradis, 2010) to illustrate the relationships among haplotypes. Nucleotide and haplotype diversity were calculated using Arlequin 3.5.1.2 (Excoffier & Lischer, 2010).

## RESULTS

### POPULATION DENSITY ANALYSES

A total of 480 transects were performed from 2010 to 2014 at all sites, covering an area of 19 200 m<sup>2</sup> and corresponding to c. 64 h of underwater observation. Populations of *C. limbata* grew significantly over time. From zero individuals in 2010, it went to 14 in 2011, 11 in 2012, 113 in 2013 and 257 individuals in 2014 ( $F = 12.40_{4,745}$ ,  $P < 0.05$ ). Populations of the local congener *C. multilineata* also showed significant variations in time: from 50 individuals in 2010 to 101 in 2011, decreasing to nine in 2012, 43 in 2013 and finally 53 individuals detected in 2014 ( $F = 4.86_{4,745}$ ,  $P < 0.05$ ) (Table I, Fig. 2 and Fig. S1, Supporting information). Mean densities of *C. limbata* and *C. multilineata* during the period of 5 years showed significant differences for the year 2014 ( $F = 37.79_{4,745}$ ,  $P < 0.05$ ) (Fig. 2 and Fig. S1, Supporting information).

The analyses revealed an increase in populations of *C. limbata* in all five sites during the past 5 years (sites:  $F = 2.55_{4,745}$ ,  $P < 0.05$ ; time:  $F = 12.40_{4,745}$ ,  $P < 0.05$ ) (Table I, Fig. 2 and Fig. S1, Supporting information).

The analyses also revealed significant differences among populations of the invader *C. limbata* in space (factor, site) and also in the populations of the local congener *C. multilineata*. Significant differences in populations of *C. limbata* were detected between two islands: Deserta (highest total density 156 individuals) and Arvoredo (lowest 21 individuals) ( $F = 2.55_{4,745}$ ,  $P < 0.05$ ) (Fig. 2 and Fig. S1, Supporting information). Considering the populations of *C. multilineata* significant differences among sites were higher, also, among the two Islands Deserta (highest total density 89 individuals) and Galé (lowest total density 8 individuals) ( $F = 5.49_{4,745}$ ,  $P < 0.05$ ) (Fig. 2 and Fig. S1, Supporting information). Exponential population growth was detected for *C. limbata* populations at all five sites (Fig. 3 and Table I).

Considering the habitat affinity of *C. limbata*, significant differences were detected for the depth strata (interface). The analyses of total sums of species density means in time and the two depth strata biotopes (slope and interface) used as factors showed significant differences only for *C. limbata* at the interface ( $F = 38.12_{4,745}$ ,  $P < 0.05$ ; Fig. 4). *Chromis limbata* showed significant preference for deeper and colder waters (>10 m deep and 2° C below sea surface temperature) mostly when searching for shelter and during reproduction. It did not differ from the native *C. multilineata* in the slope. Both species were observed feeding together in large schools in shallow water.

### GENETIC ANALYSES

The mitochondrial control region of 29 individuals from Brazilian islands was sequenced and compared with 26 sequences from the Azores, 18 from Madeira and

TABLE I. Total density of specimens of the invasive *Chromis limbata* and the native congener *Chromis multilineata* detected per island in the years from 2010 to 2014

	2010		2011		2012		2013		2014		Total	
	<i>C. limbata</i>	<i>C. multilineata</i>	<i>C. limbata</i>	<i>C. multilineata</i>	<i>C. limbata</i>	<i>C. multilineata</i>	<i>C. limbata</i>	<i>C. multilineata</i>	<i>C. limbata</i>	<i>C. multilineata</i>	<i>C. limbata</i>	<i>C. multilineata</i>
Arvoredo Is.	0	27	0	19	11	4	6	4	4	10	21	64
Gale Is.	0	0	0	0	0	0	35	4	56	4	91	8
Xavier Is.	0	23	4	26	0	0	6	16	21	9	31	74
Deserta Is.	0	0	10	56	0	5	66	19	80	9	156	89
Aranhas Is.	0	0	0	0	0	0	0	0	96	21	96	21
Totals	0	50	14	101	11	9	113	43	257	53		

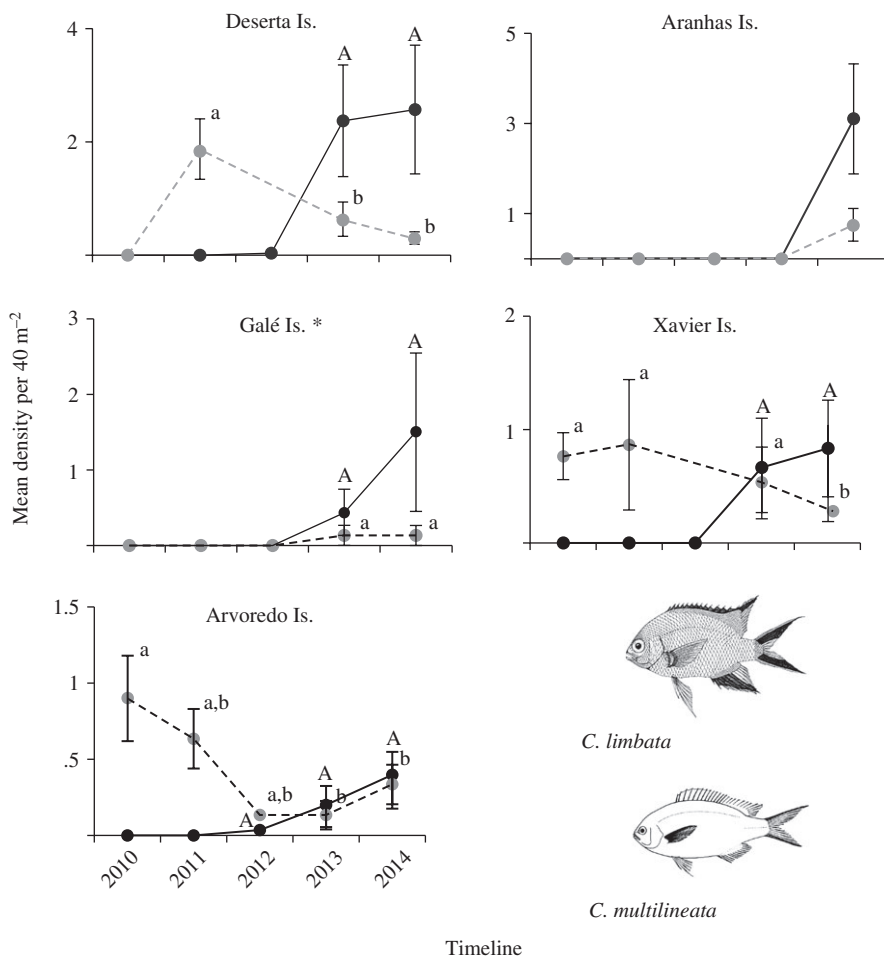


FIG. 2. Temporal variations (ANOVA) in population density ( $n$ , mean  $\pm$  S.E.) of *Chromis limbata* (—●—) and native *Chromis multilineata* (---●---). Different letters above bars indicate significant differences (Tukey HSD,  $P < 0.05$ ) within species in time; upper case indicate *C. limbata* and lower case *C. multilineata*. \*Significant differences (Tukey HSD,  $P < 0.05$ ) between species in time per site.

17 from the Canaries, totalling 90 sequences (Table II). The 29 samples from southern Brazil were confirmed as *C. limbata*, with close relatives in the Macaronesian islands (Fig. 5). GenBank locus accession numbers are from KT844434 to KT844464.

Only three haplotypes were detected in southern Brazil, a number much lower than that observed in the original *C. limbata* range (46 haplotypes). Such low haplotype diversity is consistent with the hypothesis of dispersal consisting of just a few individuals (Table II and Fig. 5). The phylogenetic tree and haplotype network suggest that these individuals could have come from any of the sampled Macaronesian islands, with the available information it is not possible to pinpoint a specific source. As expected due to proximity, southern Brazilian populations showed a high level of connectivity among sites and all three haplotypes are shared among Galé, Xavier and Arvoredo (Table II and Fig. 5).



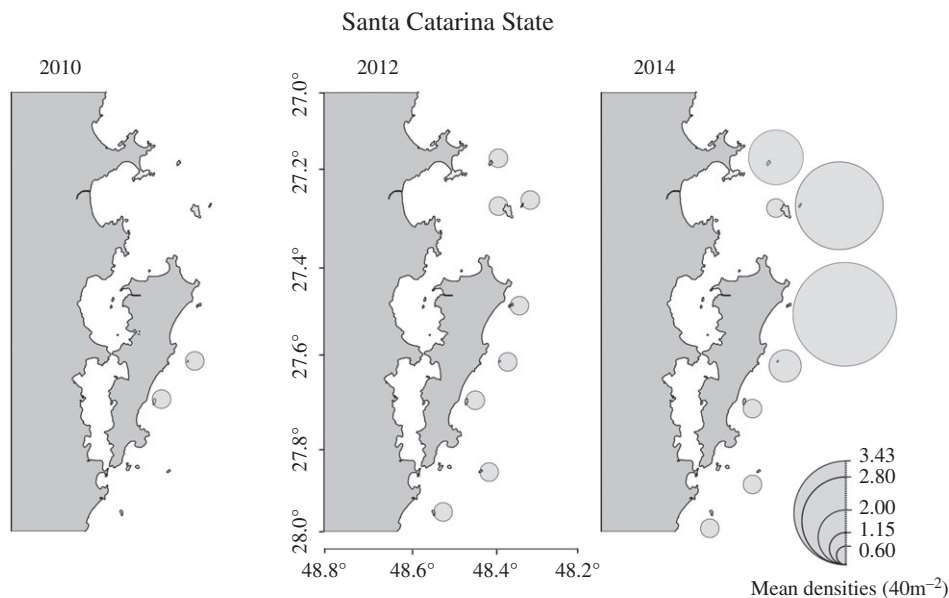


FIG. 3. Range expansion of *Chromis limbata* into south-western Atlantic Ocean reefs from 2010 to 2014 with an indication of local population density ( $n$ ).

## DISCUSSION

The success of a new population depends on several factors such as mating success, new mutations, elimination of deleterious alleles and repeated inflow of new genotypes, which may reduce the effects of inbreeding and further loss of genetic variation (Kaňuch *et al.*, 2014). Successful colonizations are often started by large numbers of

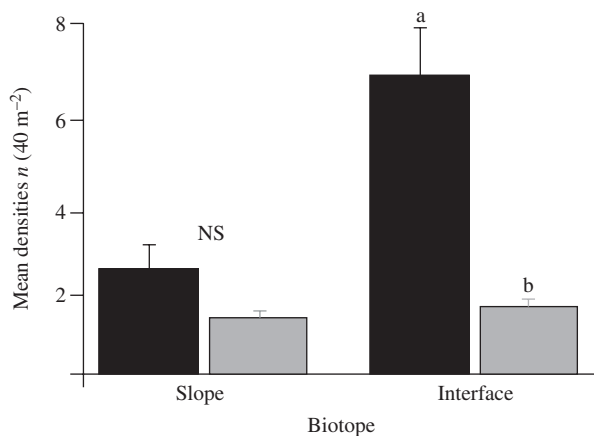


FIG. 4. Mean densities ( $n$ , +s.e.) of *Chromis limbata* (■) and *Chromis multilineata* (■) in the sampled biotopes. Different letters above bars indicate significant differences (Tukey HSD,  $P < 0.05$ ); NS, no significant difference.



TABLE II. Sampling sites used in the present study and diversity indices for mitochondrial control region of *Chromis limbata*

Islands	<i>n</i>	Private haplotypes	Shared haplotypes	Total haplotypes	$H_D$ (mean $\pm$ S.D.)	$\pi$ over loci (mean $\pm$ S.D.)
Azores (Portugal)	26	12	0	12	0.914 $\pm$ 0.023	0.036 $\pm$ 0.019
Madeira (Portugal)	18	17	0	17	0.993 $\pm$ 0.021	0.058 $\pm$ 0.030
Canaries (Spain)	17	17	0	17	1.000 $\pm$ 0.017	0.050 $\pm$ 0.026
Deserta (Brazil)	1	0	1	1	1.000 $\pm$ 0.000	0.000 $\pm$ 0.000
Aranhas (Brazil)	3	0	2	2	0.667 $\pm$ 0.314	0.033 $\pm$ 0.026
Galé (Brazil)	8	0	3	3	0.714 $\pm$ 0.123	0.029 $\pm$ 0.017
Xavier (Brazil)	12	0	3	3	0.667 $\pm$ 0.091	0.027 $\pm$ 0.015
Arvoredo (Brazil)	5	0	3	3	0.700 $\pm$ 0.218	0.029 $\pm$ 0.019
Macaronesian	61	46	0	46	0.984 $\pm$ 0.007	0.049 $\pm$ 0.025
Brazilian	29	3	0	3	0.638 $\pm$ 0.056	0.026 $\pm$ 0.139
Total	90					

*n*, Number of fish;  $H_D$ , haplotype diversity;  $\pi$ , nucleotide diversity

individuals in multiple events during a long period avoiding loss of genetic diversity in the newly founded population (Roman, 2006; Dlugosch & Parker, 2008; Kaňuch *et al.*, 2014). The low genetic diversity detected in Brazilian *C. limbata*, suggests a small larval pulse, or the arrival of a small group of individuals that could compromise the establishment, in time, of a genetically healthy population (Roman, 2006; Dlugosch & Parker, 2008; Kaňuch *et al.*, 2014) (Table II and Fig. 5). Four hypotheses could explain this colonization event: *C. limbata* was released by aquarium fish keepers; larvae may have been transported in ship ballast water; the species might have rafted in few numbers alongside oil rigs; natural colonization *via* larval transport and natural rafting across the Atlantic Ocean.

All four hypotheses may be possible, but two of them seem more plausible than the others. Aquarium release is an unlikely source since this species is not particularly appealing to aquarists, very rarely exported from the north-eastern Atlantic region and never seen for sale in Brazil; also, considering the number of haplotypes found in Brazilian populations, a release of a large number of individuals would be necessary to establish this population (see Table II). Introduction *via* ship ballast water is also unlikely since most fish larvae (especially those of demersal pomacentrids) do not survive for long periods in such an environment (Carlton, 1996; David *et al.*, 2007).

The transport of larvae and adults rafting alongside towed oil rigs seems plausible and it has been responsible for several odd occurrences worldwide (Langeneck *et al.*, 2012; Dulčić & Dragičević, 2013; Pajuelo *et al.*, 2016). Moreover, the commerce and trade in oil, the infrastructure involved in drilling operations and oil refining in Brazil, the Macaronesian Archipelago and African countries has been frequent since 2003 (Frynas & Paulo, 2007). Specimens of *C. limbata* have recently (April 2016) been detected in São Tomé, Príncipe and Angola (Vasco-Rodrigues *et al.*, 2016), indicating that this species might have a wider range in the eastern Atlantic Ocean and more potential source populations.

The natural dispersal hypotheses also seems plausible considering that *C. limbata* has been observed rafting associated with patches of algae and debris (Bortone *et al.*,

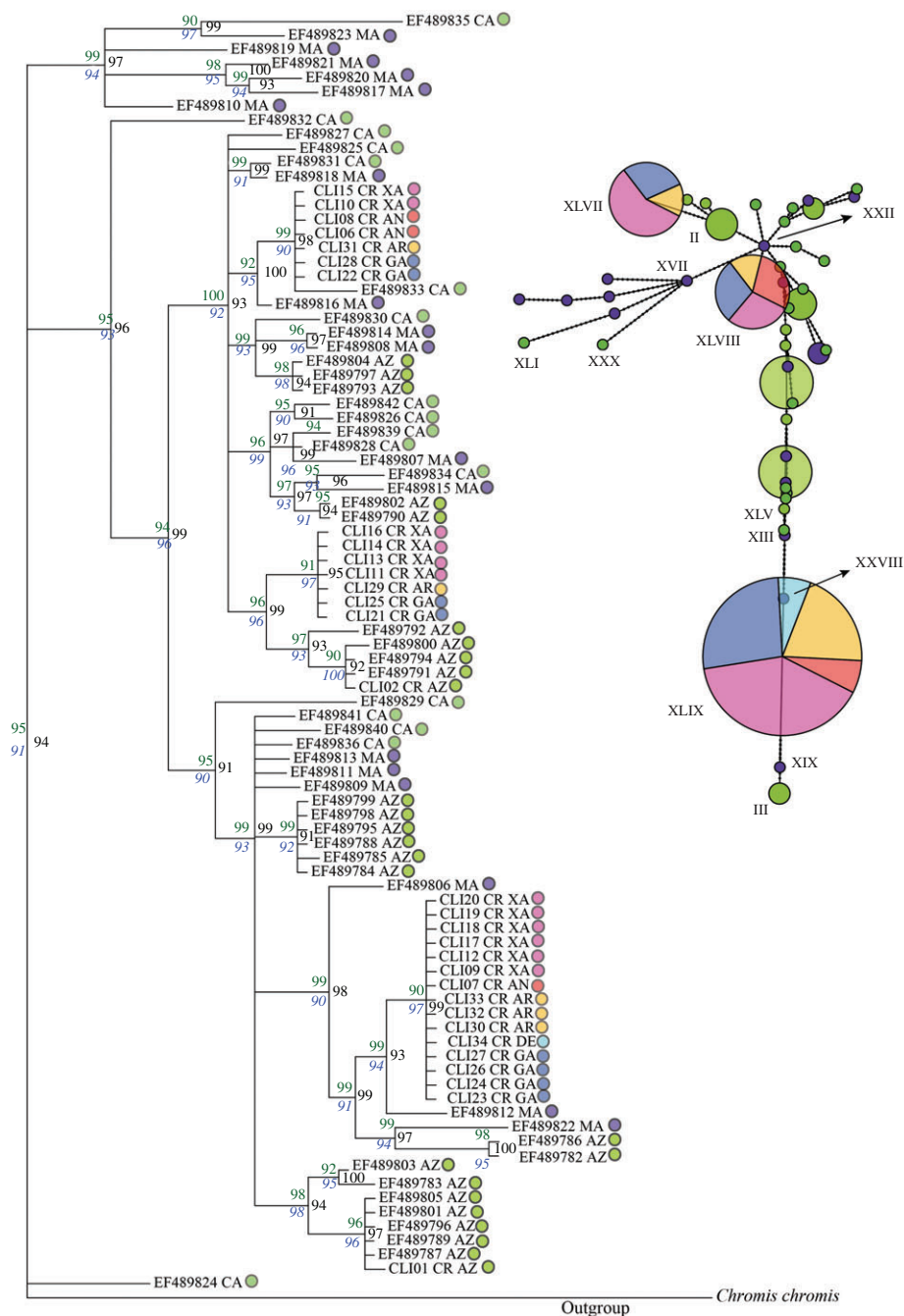


FIG. 5. (a) Bayesian tree showing relationships among *Chromis limbata* haplotypes from the Macaronesian Islands (●, Madeira; ●, Azores; ●, Canaries) and Brazil (●, Deserta; ●, Gale; ●, Xavier; ●, Avoredo; ●, Aranhas). The numbers listed at each fork in the tree show posterior probability. (b) Haplotype network showing the spatial distribution among populations and haplotype sharing. Circles represent the haplotypes, and their sizes are proportional to the number of individuals with that haplotype. The three biggest circles and their colours represent the haplotypes shared among Brazilian sites.

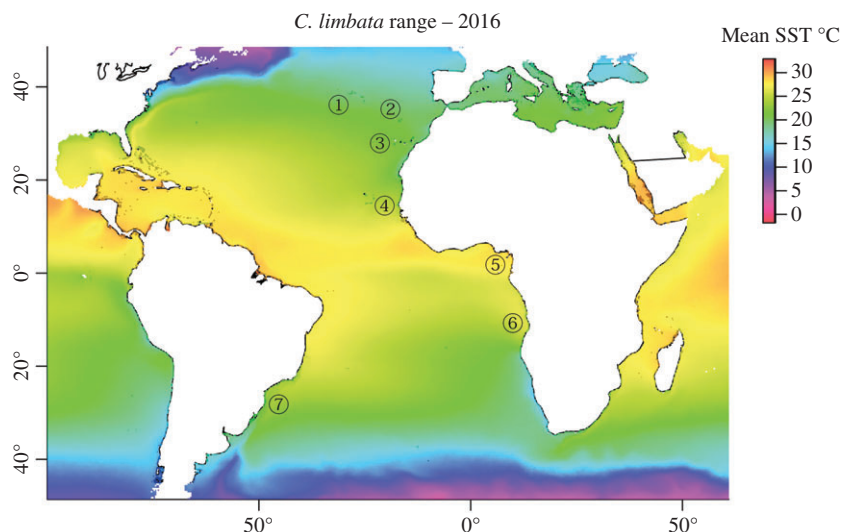


FIG. 6. Distribution of *Chromis limbata* and mean sea surface temperature [raster source Bio-ORACLE; Tyberghein *et al.* (2011)]. 1, Azores; 2, Madeira; 3, Canaries; 4, Senegal; 5, St Tomé–Príncipe; 6, Angola; 7, Brazil.

1994). In addition, the closely related *C. multilineata* also shows a history of successful long-distance dispersal. The African populations of this species are unique, but date back to only a few tens of thousands of years, indicating that they crossed the Atlantic Ocean recently (Rocha *et al.*, 2008), albeit in the opposite direction that *C. limbata* now shows. The sister species of *C. limbata*, *C. chromis* also rafts alongside floating objects in the open ocean (Bortone *et al.*, 1994) and species that can raft are generally better dispersers (Luiz *et al.*, 2015).

The *C. limbata* long distance dispersal event seems to be a recurrent phenomenon in reef fishes in the Atlantic and other species from the eastern Atlantic and have recently been detected in southern Brazilian waters (*e.g.* Monrovia surgeonfish *Acanthurus monroviae* Steindachner 1876, native from south Morocco to Angola; Luiz-Júnior *et al.*, 2004; Anderson *et al.*, 2015). Moreover, the Caribbean invasive lionfish *Pterois volitans* (L. 1758) has been detected in Brazil and seems to have arrived as a result of natural dispersal from Caribbean waters (Ferreira *et al.*, 2015).

The Atlantic Ocean currents play an important role in the dispersal of marine organisms (Lumpkin & Garzoli, 2005; Cunha *et al.*, 2014) and may function, in synergy with anthropogenic influences (*e.g.* oil rigs, cargo ship traffic, *etc.*), as an important driver to long-distance dispersal events and the disjunct distributions discussed in this work. The surface circulation in the South Atlantic Ocean is complex and consists of an equatorial gyre formed by the North Equatorial Counter Current (NECC), the Guinea Current and three branches (north, central and south) of the South Equatorial Current (SEC), which form the North Brazil Current (NBC) and the Brazil Current (BC) (Lumpkin & Garzoli, 2005; Cunha *et al.*, 2014). These currents, which generally bring water from Africa towards Brazil and then from north to south below the equator (Matano, 1993; Molina-Schiller *et al.*, 2005; Matano *et al.*, 2010), may help larvae and rafters cross the Atlantic Ocean and also may be driving the unusual colonization events discussed here.



FIG. 7. Behaviour of *Chromis limbata* in the south-western Atlantic: (a) schools at Galé Island, January, 2014; (b) juvenile (►) c. 3 cm total length ( $L_T$ ) using a sea urchin as shelter, Deserta Island, February 2014; (c) blue male c. 15 cm  $L_T$  defending its nest, Xavier Island, May 2014; (d) *C. limbata* and *Chromis multilineata* feeding together, Xavier Island, May 2014; (e) school in Arvanhas Island, April 2014; (f) juveniles c. 5 cm  $L_T$  at Arvoredo Island, 2013.

Other examples of disjunct distributions that were possibly driven by these same oceanographic processes include *Epinephelus marginatus* (Lowe 1834) and *Parablennius pilicornis* (Cuvier 1829). Their current distributions reflect that of *C. limbata*: the north-eastern Atlantic Ocean (including the Macaronesian Islands) and southern Brazil (Froese & Pauly, 2016). Such similarity in distribution patterns suggests that, during their range expansion across the Atlantic Ocean, these species may have dispersed using similar routes and that in the future, *C. limbata* may establish a permanent population in Brazil.



## POTENTIAL CONSEQUENCES

Despite significant differences in mean densities among sites for *C. multilineata*, they do not seem to have been affected by the increase in the *C. limbata* population (Fig. 2). It is important to note that *C. multilineata* is a tropical species at the southern limit of its distribution, whereas *C. limbata* is inhabiting its optimum environment (subtropical, warm-temperate rocky reefs). Given its ecological preferences in the eastern Atlantic (Fig. 6), it is predicted that *C. limbata* will be more abundant than *C. multilineata* on the south and south-eastern coast of Brazil and may even expand further south to Uruguay and Argentina. The dusky grouper *Epinephelus marginatus* (Lowe 1834) for example, expanded southwards recently (Irigoyen *et al.*, 2005).

Even though *C. limbata* and *C. multilineata* have a similar zooplankton diet (Froese & Pauly, 2016), the high productivity of south Atlantic waters owing to upwelling and consequent high abundance of plankton (Barua, 2005), indicates that these species may not be competing for food (Fig. 7; Green *et al.*, 2012; Elleouet *et al.*, 2014; Anderson *et al.*, 2015; Chown *et al.*, 2015). Schools of *C. multilineata* and *C. limbata* feeding together have been observed in the past 2 years at all studied sites. In addition to *C. multilineata*, *C. limbata* also interacts with other natives and its aggressive behaviour during reproduction may affect local territorial species [*e.g.* *Stegastes* spp., *Abudefduf saxatilis* (L. 1758)]. Shelter for example, may become a limiting resource as their densities in the new environment increase (Green *et al.*, 2012; Elleouet *et al.*, 2014; Chown *et al.*, 2015). So far, however, there has been no evidence of detrimental effects to native species. With the current data, it could not be determined if *C. limbata* is affecting *C. multilineata*, but the data form an important baseline. Long term monitoring of this recent arrival will be important and could constitute a valuable tool for a better understanding the genetics, ecology and consequences of species range expansions.

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## Supporting Information

Supporting Information may be found in the online version of this paper:

**FIG. S1.** (a) Spatial variations in sum of mean population densities ( $n$ , + S.E.) and (b) temporal variations of mean population densities ( $n$ , + S.E.) of *Chromis limbata* (■) and *Chromis multilineata* (▒). Letters above bars indicate significant differences; upper-case letters indicate *C. limbata* and lower-case letters *C. multilineata*; \* significant differences among species (Tukey HSD,  $P < 0.05$ ).

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