*Zoological Journal of the Linnean Society*, 2020, **190**, 54–64. With 2 figures.

# Phylogeny of the comb-tooth blenny genus *Scartella* (Blenniiformes: Blenniidae) reveals several cryptic lineages and a trans-Atlantic relationship

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*Received 2 January 2019; revised 26 September 2019; accepted for publication 17 October 2019*

Here we present the first phylogeny of the genus *Scartella* based on mitochondrial data. The analysis strongly corroborates the validity of all species of the genus and shows that *Scartella cristata*, a species with a disjunct distribution, is a lineage complex comprising five clades: two in Caribbean waters, another in the East Atlantic/Mediterranean and two in Brazil. Brazilian clades occur in sympatry from Rio de Janeiro to Rio Grande do Sul states (southern Brazil). One clade (BRA 1) is unique to Brazil, while the other (BRA 2) is closely related to the eastern Atlantic lineage. Possible explanations for this pattern include both natural and anthropic mechanisms.

ADDITIONAL KEYWORDS: reef fish – molecular phylogeny – cryptic species – mtDNA – intertidal.

## INTRODUCTION

Blenniidae (blennies) is one of the most diverse families of Teleostei with 404 known species (Fricke *et al.*, 2018). Due to various general sets of life-history traits, blennies are considered cryptobenthic reef fishes (Brandl *et al.*, 2018). Although they are sometimes the most abundant reef fishes (Thomson & Gilligan, 2002; Griffiths, 2003), and may comprise more than 40% of species (Ackerman & Bellwood, 2000), cryptobenthic fishes are probably among the most neglected group of reef vertebrates (Brandl *et al.*, 2018).

The genus *Scartella* Jordan, 1886 contains typical species of Blenniidae. They are small fishes without scales, with restricted mobility as adults, adhesive and benthic spawning, and typically inhabit shallow, marine environments such as mangroves, shallow areas of rocky shores, tide pools and coral reefs (Greenfield & Johnson, 1981; Springer, 1993). Some species also live in warm-temperate and subtropical regions such as the Mediterranean Sea and the coasts of South Africa and southern Brazil (Penrith & Penrith, 1972; Balma & Delmastro, 1984; Rangel & Guimarães, 2010).

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Currently, there are seven recognized species described in the genus: *S. caboverdiana* Bath, 1990, endemic to the Cabo Verde Archipelago; *S. cristata* (Linnaeus, 1758) in the western Atlantic from Bermuda and Florida to Rio Grande do Sul (including the Gulf of Mexico), and in the eastern Atlantic on the west coast of Africa and the Mediterranean Sea (Bath, 1990; Rangel, 2003); *S. emarginata* (Günther, 1861), mostly occurring in the Indian Ocean, from False Bay, South Africa, to south-east Asia and north-western Australia (Williams, 2014); *S. itajobi* Rangel & Mendes, 2009, endemic to the Fernando de Noronha Archipelago, Rocas Atoll and St Paul's Rocks; *S. nuchifilis* (Valenciennes, 1836), endemic to Ascension Island; *S. poiti* Rangel *et al*., 2004, endemic to the Trindade-Martin Vaz insular complex; and *S. springeri* (Bauchot, 1966), endemic to St. Helena Island (Fig. 1).

*Scartella cristata* presents the largest geographic distribution among Atlantic species of the genus. This distribution range encompasses a number of biogeographic provinces and crosses several recognized biogeographic barriers. It is interesting to note that among the approximately 350 species of blennioids known from coastal waters of the New World, only three are considered native to other regions, all of them being trans-Atlantic: *Labrisomus nuchipinnis* (Quoy & Gaimard, 1824), *Parablennius pilicornis* (Cuvier, 1829) and *S. cristata* (Hastings, 2009). This overall picture, along with the clear endemism of *Scartella* species to the oceanic islands of the Atlantic Ocean, suggests the existence of peripheral isolation of populations of *S. cristata*, from both the western and eastern Atlantic, including the Mediterranean Sea (Rangel, 2003). In addition, characteristics of the

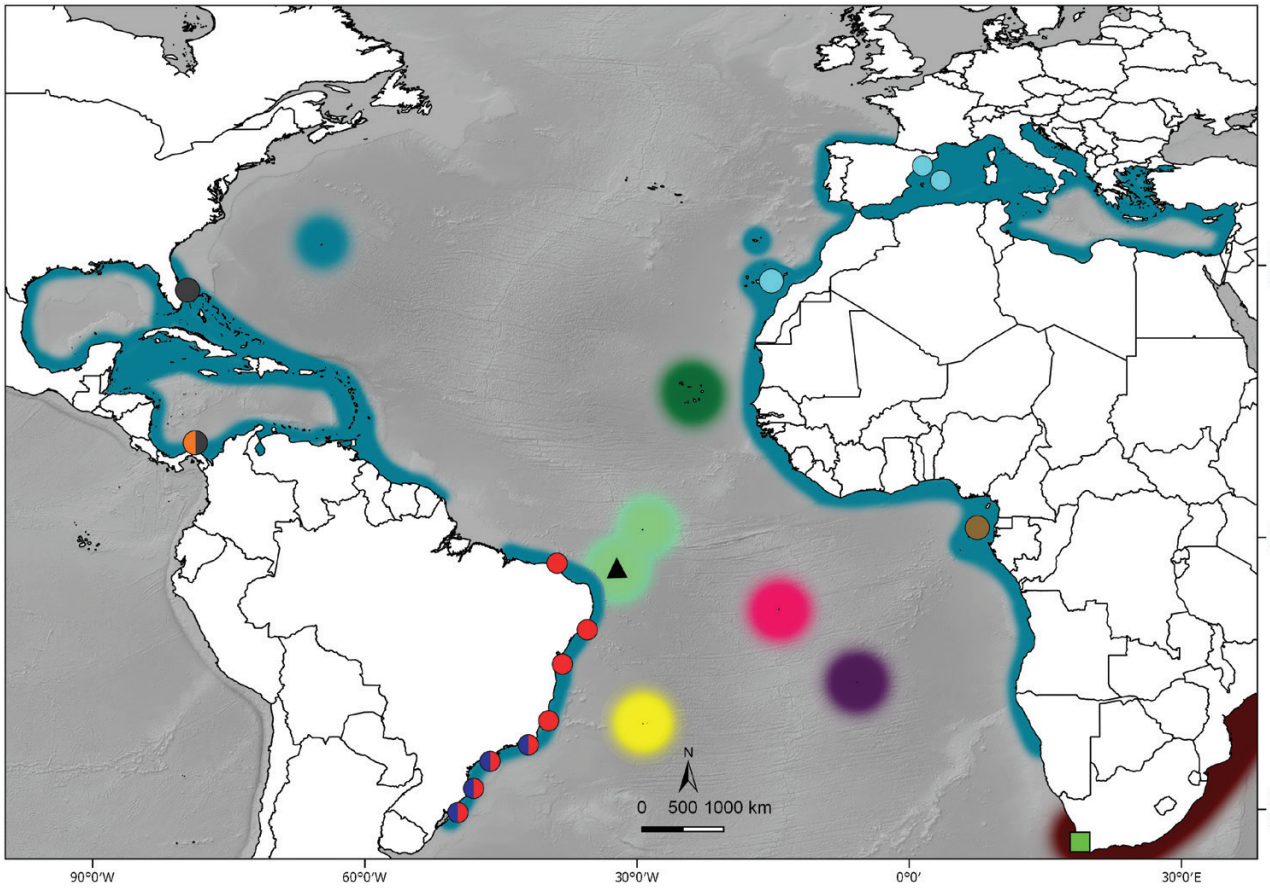


**Figure 1.** Species range and sample locations of *Scartella* in the Atlantic Ocean. Green area, *Scartella caboverdiana*; garnet area, *Scartella emarginata* (green square = sampled site); light-green area, *Scartella itajobi* (black triangle = sampled site); pink area, *Scartella nuchifilis*; yellow area, *Scartella poiti*; purple area, *Scartella springeri*; blue area, *Scartella cristata*; sampled sites: brown circle, São Tomé and Príncipe complex; light-blue circle, Mediterranean Sea (Spain, Barcelona and Ibiza) and Canaries; dark-grey circle, United States (Florida); orange/dark-grey circle, Caribbean (Panama and Florida); red circle, Brazil (Ceará to Espírito Santo States); red/blue circle, Brazil (Rio de Janeiro to Rio Grande do Sul States). The area representing the distribution of insular species (except *Scartella itajobi*), also represents the sampled site.

life history of the genus, mainly sedentary habits and spawning strategy, suggests a restriction on dispersal and gene flow between populations from different localities.

Such putative isolation was surprisingly not found in previous studies that investigated the taxonomy of *S. cristata* based on morphology. Bath (1970) found little morphometric variation between specimens on both sides of the Atlantic and concluded that individuals from the western and eastern Atlantic, besides the Mediterranean Sea, should be recognized as *S. cristata*. In his thesis, Rangel (2003) proposed a taxonomic revision of the genus based on the external morphology of Atlantic species. For that study, specimens of *S. cristata* from the Brazilian coast, Caribbean Sea, Mediterranean Sea and west Africa were analysed. The comparisons of individuals from these different localities did not reveal significant morphological differences between them, corroborating previous findings (Bath, 1970).

There are also taxonomic inconsistencies surrounding *Scartella cristata*. Its description dates back to *Blennius cristatus* Linnaeus (1758), which was based on the description of *Blennius crista setacea longitudinali inter oculus* in Gronovius' *Museum Ichthyologicum* (1754). In the original description, there is no reference to the type locality and Linnaeus (1758: 256) wrote only '*Habitat in Indiis*', although Gronovius provides the vernacular name *punaru brasiliensium*. Pinto (1954) redescribed *B. cristatus* and assumed that the specimens from Rio de Janeiro State came from the region where the type material of Gronovius was collected. More recently, some authors indicated that the type locality of *S. cristata* was probably Ascension Island (the same of *S. nuchifilis*) (Menezes *et al.*, 2003; Fricke *et al.*, 2019). The holotype is deposited in the Natural History Museum of London, but unfortunately consists only of a skin on paper and lacks locality information.

The monophyly of the genus was proposed in previous studies (Levy *et al.*, 2012; Hundt *et al.*, 2014). *Scartella* was recovered as monophyletic in both analyses, and was found to be sister to *Salaria* Forsskål, 1775 [despite the apparent paraphyly of *Salaria* in relation to *Scartella* in Hundt *et al.* (2014)]. However, no complete phylogenetic analyses were performed on the relationships of the species within the genus. In this study, we conduct the first phylogenetic analysis including all currently recognized species of the genus *Scartella*, in order to (1) investigate the systematics of the genus and (2) test the validity of *S. cristata*, exploring the existence of possible cryptic species along the geographic distribution indicated for the species.

## MATERIAL AND METHODS

Individuals of all *Scartella* species were collected using hand-nets or plastic bags in shallow water near the shore and from tidal pools. Samples of *S. cristata* were obtained from: Brazil – Trairi, Ceará State [CE] ($N$ = 3), Marechal Deodoro, Alagoas State [AL] ($N$ = 3), Salvador, Bahia State [BA] ($N$ = 3), Guarapari, Espírito Santo State [ES] ($N$ = 3), Arraial do Cabo, Rio de Janeiro State [RJ] ($N$ = 6), Ilhabela, São Paulo State [SP] ($N$ = 5), Bombinhas, Santa Catarina State [SC] ($N$ = 6) and Torres, Rio Grande do Sul State [RS] ($N$ = 6). North-western Atlantic – Colón, Panama [CAR] ($N$ = 5) and Florida, USA [FL] ($N$ = 3). Eastern Atlantic – Canary Islands, Spain [CAN] ($N$ = 5) and São Tomé and Príncipe [STP] ($N$ = 5). Mediterranean – Barcelona, Spain [BAR] ($N$ = 4) and Ibiza, Spain [IBZ] ($N$ = 1). This gives a total of 58 individuals (Supporting Information, Table S1; Fig. 1).

Specimens of all six congeners of *Scartella cristata* were collected from different localities. We used a total of 22 samples: three of *S. caboverdiana* (Cabo Verde Archipelago), two of *S. emarginata* (Cape Town, South Africa), five of *S. itajobi* (Fernando de Noronha Archipelago), four of *S. nuchifilis* (Ascension Island), four of *S. poiti* (Trindade Archipelago) and four of *S. springeri* (Saint Helena) (Supporting Information, Table S1; Fig. 1.).

Genomic DNA was extracted from muscle tissue on the left side of each specimen through a modified salt-extraction protocol of Miller *et al.* (1988). Amplifications of mitochondrial control region were performed using the primers LPro1 (5′- ACT CTC ACC CCT AGC TCC CAA A -3′) and HDL1 (5′- CCT GAA GTA GGA ACC AGA TGC CAG - 3′) (Osteralli *et al.*, 1996). Polymerase chain reactions (PCR) were performed with the following parameters: initial denaturation at 94 °C for 7 min, followed by 35 cycles of denaturation (30 s at 94 °C), annealing (30 s at 55 °C) and extension (1 min at 72 °C), ending with a final extension at 72 °C for 7 min. DNA was sequenced using the same primers as for the PCRs and in both directions.

Electropherograms were visually checked and edited manually in the program SEQMAN (DNAstar Inc., http://www.dnastar.com), as well as the corresponding consensus sequences formed by the forward and reverse sequences. The sequences were aligned in MEGA 7.0 (Kumar *et al.*, 2015) through the Clustal W algorithm (Thompson *et al.*, 1994). All polymorphic sites that were present in the final alignment were carefully inspected in order to minimize read errors that would result in an overestimated number of polymorphisms. All the sequences were deposited in GenBank (Supporting Information, Table S1). Genetic divergence was estimated in MEGA 7.0, through

Kimura 2-parameter distance model (K2P) (Kimura, 1980).

The best nucleotide substitution model was achieved using jModelTest 2.1 (Darriba *et al.*, 2012). Among the strategies implemented in the selection, we used Akaike information criterion (AIC) and Bayesian information criterion (BIC). For both criteria, the chosen model was HKY+I+G.

We implemented maximum likelihood (ML) and Bayesian inference (BI) methods to reconstruct phylogenetic relationships. The ML analyses were performed in the program PhyML 3.0 (Guidon *et al.*, 2010), with 1000 replicates to evaluate the reliability of the nodes. The BI were executed in MrBayes 3.2.0 (Ronquist *et al.*, 2012), with two independent runs of four concomitant Markov chain Monte Carlo (MCMC) for 15 million generations and sampling parameters every 1000 generations. The first 25% of trees were discarded (burn-in) and a 50% majority-rule consensus tree was estimated. A Bayesian analysis was conducted in the software BEAST 2.0 (Bouckaert *et al.*, 2014) to estimate the divergence time, employing a strict clock and the Yule Model, with mutation rate of 3% per million years (Myr) (Lessios, 2008). Four MCMC were used, with a chain length of 20 million generations. Trees and parameters were sampled every 1000 generations and, lastly, the first 20% of the samples were discarded as burn-in. The software TRACER 1.5 was used to check the parameters of the run, and a maximum clade credibility tree was obtained through TreeAnnotator 1.8.2 (Bouckaert *et al.*, 2014). The blenny *Parablennius tentacularis* (Brünnich, 1768) was used as an outgroup in all analyses.

To test the validity of putative species resulting from the analyses, we performed two species delimitation tests, the Generalized Mixed Yule Coalescent (GMYC) and the Multi-rate Poisson Tree Processes (mPTP) (Pons *et al.*, 2006; Fujisawa & Barraclough, 2013; Kapli *et al.*, 2017). The GMYC uses an ultrametric tree and attempts to detect the predicted difference in branching rate under two processes of lineage evolution (within and between species) evaluating the point of highest likelihood of the transition. The mPTP is an improved method that alleviates shortcomings of Poisson Tree Processes (PTP), mainly by incorporating the potential divergence in intraspecific diversity and does not require an ultrametric tree (Kapli *et al.*, 2017).

## RESULTS

An alignment of 360 base pairs (bp) from the mitochondrial control region was used for the analyses. All three methods recover similar topologies (Fig. 2; Supporting Information, Figs. S1, S2). *Scartella caboverdiana* is sister to all other species of *Scartella,* although its monophyly is not recovered in ML (Supporting Information, Fig. S1). Analyses then revealed a topology containing three major clades: one clade [basal in Bayesian analysis of divergence time and in ML (Fig. 2; Supporting Information, Fig. S1)] with *S. emarginata* together with the Mid-Atlantic Ridge species (Clade 1); a second clade including south-western Atlantic representatives, with the Brazilian insular species plus one lineage from the Brazilian coast (Clade 2); and a third clade including two lineages from Caribbean waters, sister to a group with individuals from the eastern Atlantic and Mediterranean, and another lineage from southern south-east Brazil (Clade 3). Based on a mutation rate of 3%, the estimated time of divergence between Clades 2 and 3 is approximately 3.2 Myr and about 3.7 Myr between those and Clade 1 and 4.9 Myr between *S. caboverdiana* and the other species of *Scartella* (Fig. 2). These results strongly corroborate the validity of almost all insular species of *Scartella* plus *S. emarginata*, with all these lineages presenting support values above 80% (most above 90%) in the three analyses. Although non-monophyletic in ML, both bayesian approaches indicate *S. caboverdiana* as monophyletic, with high support (>80%).

The same is not true for *Scartella cristata*, with all trees showing *S. cristata* as non-monophyletic, being split into four groups: three in the western Atlantic and one in the eastern Atlantic (Fig. 2; Supporting Information, Figs S1, S2). In the western Atlantic, samples from Panama and Florida cluster in a clade, and two clades are also found in Brazil. One clade (BRA 1) includes specimens collected along the Brazilian coast (samples from Ceará to Rio Grande do Sul States), and another clade (BRA 2) is restricted to the southern south-east regions (Rio de Janeiro to Rio Grande do Sul States). Thus, according to our data, there are two distinct lineages (BRA 1 and BRA 2) occurring in sympatry in parts of the Brazilian coast. The genetic divergence (K2P) between those lineages (15.6%) is greater than any comparisons among western Atlantic lineages, suggesting a deep separation among them (Table 1).

Across the Atlantic, analyses reveal a lineage that includes samples from the Mediterranean Sea, the Canary islands and São Tomé and Príncipe. Surprisingly, this clade is sister to the BRA 2 group in all analyses, with high support (>98%). Levels of genetic divergence between BRA 2 and Brazilian lineages (BRA 1, *S. poiti* and *S. itajobi*) range from 14.7 to 17.9%, while the greatest divergence between samples of BRA 2 and the eastern Atlantic lineages is 6.1% (Table 1).

Both species delimitation tests agree with the phylogenetic results. The number of well-defined species is 12 for both tests, strongly supporting the validity of the species presented in the analyses,

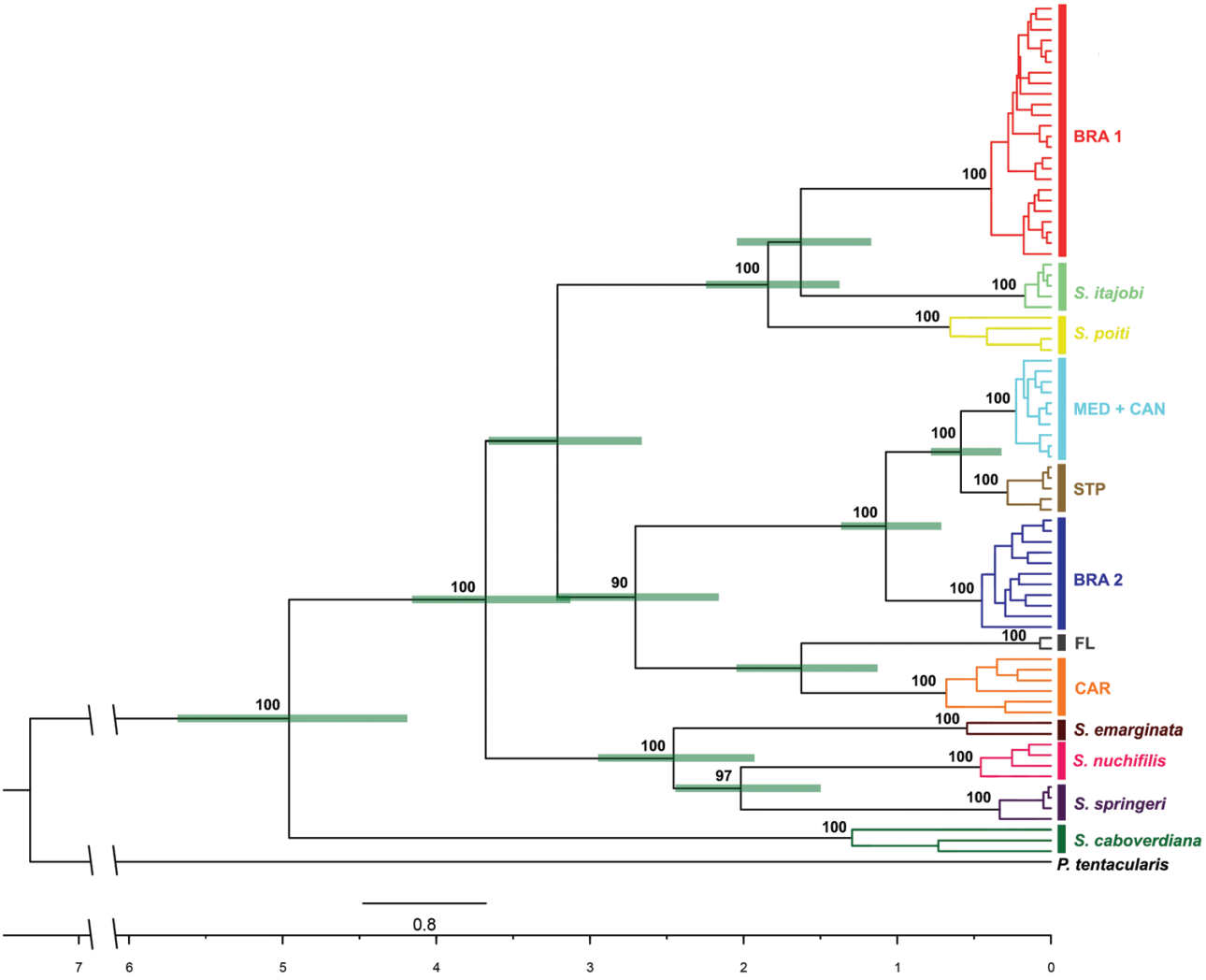


**Figure 2.** Estimates of divergence time for the genus *Scartella* based on Bayesian inference for the mitochondrial D-loop region. Scale bar in Myr. The 95% credible intervals for node ages are shown with horizonal green bars. Bayesian posterior probability are shown only for nodes with over than 70% support values. BRA 1 (Brazil, Ceará to Rio Grande do Sul States); BRA 2 (Brazil, Rio de Janeiro to Rio Grande do Sul States); FL (United States, Florida); CAR (Florida and Panama); MED (Spain, Barcelona and Ibiza); CAN (Spain, Canaries) and STP (São Tomé and Príncipe complex).

including all lineages formerly united under *S. cristata*. However, the results are not identical. For GMYC, *S. caboverdiana* is separated into two putative species. This result may be due to sampling bias, as well as inherent artefacts of the method itself. On the other hand, mPTP distinguishes the eastern Atlantic sequences into two species: one with the lineage from the Mediterranean Sea plus the Canary Islands, and another with the lineage from São Tomé and Príncipe. Notably, this was the only node with a support value different than 1 (0.58) in mPTP.

Finally, in the Greater Caribbean clade, both delimitation tests divide the Caribbean sequences into two species. One lineage with sequences from Florida and another with sequences from Panama plus Florida. The estimated time of separation is nearly 1.6 Myr and its genetic divergence is 8.8%, higher than some interspecific values (Table 1). As on the Brazilian coast, there is apparently a geographical overlap between the Caribbean lineages.

## DISCUSSION

Phylogenetic analyses presented in this study are consistent with a monophyletic *Scartella*, as proposed

**Table 1.** K2P distance values for *Scartella* species sampled. Acronyms relative to lineages formerly recognized as *Scartella cristata*. BRA 1 include samples from Brazilian coast (Ceará to Rio Grande do Sul States); BRA 2 include samples from southern south-east Brazil (Rio de Janeiro to Rio Grande do Sul States); FL include samples from Florida (United States); CAR include samples from Panama and Florida; MED include samples from Barcelona and Ibiza (Spain); CAN include samples from Canaries (Spain) and STP include samples from São Tomé and Príncipe complex

| | *Scartella caboverdiana* | BRA 1 | BRA 2 | FL | CAR | CAN | MED | STP | *Scartella emarginata* | *Scartella itajobi* | *Scartella nuchiflis* | *Scartella poiti* | *Scartella springeri* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Scartella caboverdiana* | - | | | | | | | | | | | | |
| BRA 1 | 19.8% | - | | | | | | | | | | | |
| BRA 2 | 21.0% | 15.6% | - | | | | | | | | | | |
| FL | 19.9% | 14.4% | 15.3% | - | | | | | | | | | |
| CAR | 19.9% | 12.7% | 14.8% | 8.8% | - | | | | | | | | |
| CAN | 20.2% | 16.0% | 5.9% | 14.4% | 14.9% | - | | | | | | | |
| MED | 19.7% | 16.0% | 5.6% | 13.8% | 14.3% | 0.5% | - | | | | | | |
| STP | 20.1% | 15.8% | 6.1% | 15.2% | 14.2% | 3.5% | 3.2% | - | | | | | |
| *Scartella emarginata* | 22.5% | 18.6% | 21.5% | 20.3% | 19.2% | 22.7% | 22.3% | 22.1% | - | | | | |
| *Scartella itajobi* | 18.5% | 7.0% | 14.7% | 14.9% | 13.6% | 16.5% | 16.0% | 14.7% | 20.7% | - | | | |
| *Scartella nuchiflis* | 23.2% | 17.9% | 20.2% | 18.7% | 17.9% | 21.1% | 20.4% | 20.1% | 15.7% | 19.5% | - | | |
| *Scartella poiti* | 22.0% | 8.8% | 17.9% | 17.1% | 16.0% | 19.1% | 18.6% | 18.5% | 18.2% | 9.9% | 17.9% | - | |
| *Scartella springeri* | 21.0% | 14.6% | 17.7% | 18.0% | 16.5% | 17.3% | 17.3% | 19.5% | 15.1% | 17.1% | 12.3% | 16.8% | - |

in previous studies (Levy *et al.*, 2012; Hundt *et al.*, 2014) and recovers the non-monophyletic nature of *Scartella cristata*, as presumed, but not previously tested. This study suggests that the number of *Scartella* species is underestimated, with the possibility of the existence of at least 12 species in the Atlantic, rather than the seven species recognized to date.

Our data also place *S. caboverdiana* in a basal position, with the remaining species clustering into three main groups: (1) one with species from the Mid-Atlantic Ridge plus *S. emarginata* (Clade 1); (2) another with species from the south-western Atlantic (Clade 2) and, lastly, a group (3) with species from the eastern Atlantic plus Greater Caribbean (Clade 3). The grouping of BRA 2 with the lineages from the eastern Atlantic is an important topic that will be discussed below.

Almost all groups recovered in the phylogenies agree with patterns of relationship between regions proposed by Floeter *et al.* (2008): species from the Brazilian Province clustering together (except BRA 2), the grouping of eastern Atlantic species and the close relationship of the Mid-Atlantic Ridge species and South Africa (*S. emarginata*). Only the close connection between the Caribbean and the eastern Atlantic lineages is not aligned with the overall patterns suggested by Floeter *et al.* (2008). Indeed, we show evidence for a greater connectivity between the Caribbean and eastern Atlantic than between the Caribbean and Brazil. This relationship, although not so common, has been reported in other species of fish and corals (Casey *et al.*, 2004; Souza *et al.*, 2017).

Although previous studies did not find significant morphological differences between individuals of *S. cristata* from distinct localities (Rangel, 2003; Bath, 1970), data obtained in this study strongly suggest the existence of at least six different lineages identified within this 'species complex'. Previous studies with marine fishes have already demonstrated the absence of morphological distinction between individuals from different regions (*Osmerus*, Taylor & Dodson, 1994; *Aulostomus*, Bowen *et al.*, 2001; *Macrodon*, Santos *et al.*, 2006). Nevertheless, the aforementioned studies dealt with organisms that have remarkable dispersal capabilities and which in general appear to inhabit the same niches along their geographic distribution. This could explain the absence of morphological differentiation (Santos *et al.*, 2006). The absence of readily discernible diagnostic features, such as differences in coloration or non-overlapping meristic counts, is common in cryptobenthic reef fishes (Brandl *et al.*, 2018). This is particularly true for species of *Scartella*, where there is overlap between the diagnostic characters of the different species, and where distinction between species is only possible through combinations of characters (Rangel *et al.*, 2004; Rangel & Mendes, 2009).

In the western Atlantic, allopatric speciation seems to be the most likely scenario to explain the separation between lineages of the Brazilian coast and the Caribbean. The barrier responsible for this separation might naturally be the discharge of fresh water from several rivers present on the north-eastern coast of South America, especially the Orinoco and Amazon, which are the largest. The Orinoco–Amazon filter has proven to be effective for several other species of reef fishes (Muss *et al.*, 2001; Rocha, 2003; Floeter *et al.*, 2008), notably demersal-spawning with small-bodies, cryptobenthic species, like *Scartella*, which appear to be most affected by conditions of low salinity and high sedimentation (Floeter *et al.*, 2008). Therefore, the separation between Brazilian and Caribbean lineages is expected, given that species of the genus are inhabitants of intertidal zones and tide pools, and are permanent residents of shallow waters (to 3 m) (Macieira *et al.*, 2015).

The existence of two distinct lineages in Brazilian waters with an overlap zone (from the State of Rio de Janeiro to the State of Rio Grande do Sul), apparently represents a case of sympatric separation. However, the tree topologies suggest that another mechanism may have played a role in the evolutionary history of these lineages. Considering the close relationship between BRA 2 and eastern Atlantic species, in addition to the low genetic divergence among their sequences, it is likely that the mechanism responsible for the two distinct lineages on the southern south-east Brazilian coast was a possible colonization from the eastern Atlantic towards the western Atlantic. In addition, the phylogenies recovered the origin of the genus in the eastern Atlantic, as reported for *Parablennius*, *Salaria* (sister genus of *Scartella*) (Levy *et al.*, 2012) and suggested by Floeter *et al.* (2008). Therefore, it is possible that the diversification in the genus was a result of dispersal events from the eastern Atlantic (particularly Cabo Verde) to the other regions, a scenario that may have occurred in other taxa (e.g. in *Diplodus*, Summerer *et al.*, 2001).

Two recent cases of reef fishes following the same pattern reinforce this hypothesis: the recent arrival and establishment of Azores chromis, *Chromis limbata* (Valenciennes, 1833), a native damselfish of the eastern Atlantic, in southern Brazil (Anderson *et al.*, 2017), and the recognition of two distinct lineages of *Ophioblennius* (Gill, 1860) in Brazil: one on the north-east coast (Bahia as the southern boundary) plus oceanic islands, *Ophioblennius trinitatis* Miranda Ribeiro, 1919, and another an undescribed species occurring along the southern coast of Brazil (Santa Catarina) and in São Tomé and Príncipe in the Gulf of Guinea (Lastrucci *et al.*, 2018).

The case of *Ophioblennius* is similar to the present study, because, like *Scartella,* it belongs to the Blenniidae and shares similar life-history characteristics. The explanation invoked by the authors for the occurrence of *Ophioblennius* in southern Brazil and in the Gulf of Guinea includes natural dispersal of larvae and rafting on floating objects, from the southern Gulf of Guinea to the south of Brazil, via the South Equatorial Current or the Benguela Current (Lastrucci *et al.*, 2018).

Indeed, the currents of the Atlantic Ocean play an important role in the dispersal of marine organisms (Lumpkin & Garzoli, 2005; Cunha *et al.*, 2014). The South Atlantic current system consists of an equatorial gyre formed by the Equatorial Northern Countercurrent, the Guinean Current and the Equatorial South Current with its three branches (north, central and south), that form the Northern Brazilian Current and Brazilian Current (Lumpkin & Garzoli, 2005; Cunha *et al.*, 2014). These currents, which usually bring water from the eastern Atlantic towards Brazil and from north to south below the Equator (Matano, 1993; Molina-Schiller *et al.*, 2005; Matano *et al.*, 2010), may be important forces that boost the exchange of larvae between the two sides of the Atlantic.

This hypothesis may serve as an explanation for the case of *Scartella* on the Brazilian coast. However, other scenarios can also be explored, such as human-mediated invasion through transportation as ship ballast water and via oil rigs. The hypothesis of transport by ship seems unlikely, because ballast water tanks have filters that mostly exclude fish larvae, being more permeable to larvae of smaller invertebrates (Wonham *et al.*, 2000). Although it was the second most common family recorded as introduced by ballast water or alternative dispersion modes, such as fouling in the hull of the ship, blennies were collected only once from ballast water (Wonham *et al.*, 2000). Transport mediated by oil rigs seems more likely. In fact, several studies have already shown the close relationship between blennies and oil rigs, with records of distinct species of the family (including species of *Scartella*) closely associated with platforms (Ferreira *et al.*, 2006; Wanless *et al.*, 2010; Falcon *et al.*, 2015; Pajuelo *et al.*, 2016). This scenario is not surprising since platforms are suitable places for blennies, and harbour a biofouling community, including corals, barnacles and bivalves (Wanless *et al.*, 2010), which are widely used by blennies as shelter and nests (McEachran & Fechhelm, 1998). In addition, Ferreira *et al.* (2006) already recorded 22 exotic species on oil platforms on the south-eastern coast of Brazil. However, due to the uncertainty regarding the type of dispersion of BRA 2, any deduction alluding to the source of the supposed link between southern Brazil and the eastern Atlantic is tenuous.

Although on a smaller scale, the scenario in the Greater Caribbean has similarities with that of the Brazilian coast: two lineages with considerable genetic divergence occurring in sympatry. Some genetic and biogeographic data have already shown a division in the Caribbean Province, e.g. at the Mona Passage, between Puerto Rico and Hispaniola (Dennis *et al.*, 2005; Taylor & Hellberg, 2006) and between the Caribbean and Florida (Robertson & Cramer, 2014). The Caribbean lineages of *Scartella* appear to be sensitive to those environmental breaks. Nonetheless, the presence of individuals from the two lineages in Florida suggests a faunistic exchange. Analyses with more individuals from Florida and other regions in the Caribbean would help clarify this scenario.

Our work strongly corroborates the validity of the *Scartella* species and indicates that the unusual distribution of *S. cristata* is an artefact, because individuals in each region are representatives of distinct lineages. Although the results are conspicuous, studies exploring more genomic areas as an evolutionary source of information, and with a variation greater than that exhibited by the control region, are necessary to give more robustness to the findings of the study. Next-generation sequencing (NGS) data have these attributes and can, therefore, elucidate the issues that remained open, especially the link between the southern south-east of Brazil and the eastern Atlantic. Resolution of this relationship is indispensable for understanding the evolutionary forces that act in the Atlantic, especially the depth of anthropic action. From this, it is possible to find the resolution of taxonomic uncertainties, which is one of the main targets of conservation genetics (Frankhan *et al.*, 2010), because it is essential in the definition of management units within species (or group of species). The elucidation of these ambiguities is crucial for solving the nomenclatural issues of the group and contribute by increasing the accuracy in a future taxonomic investigation on this subject. Finally, it is clear that, although cryptobenthic fishes constitute a large proportion of species richness in reefs (Brandl *et al.*, 2018), the actual diversity of this group of fishes is still underestimated.

## ACKNOWLEDGEMENTS

We thank D. Almeida, V. Brito, N. Dias, R. Dias, R. Freitas, J. L. Gasparini, L. Neto, L. A. Rocha, B. Victor and P. Wirtz for fieldwork assistance and sharing collected specimens. S. Lima for the loan of some specimens. K. Morelli and FIOCRUZ for help in obtaining some sequences. This work is in part result of the first author's dissertation at the PPGZoo (Programa de Pós-Graduação em Zoologia), Museu Nacional, Universidade

Federal do Rio de Janeiro; supported by CNPq (Conselho Nacional de Desenvolvimento Científico) grant number 131703/2016-1. MRB is supported by CNPq process 305955/2015-2. We also acknowledge funding from The Rufford Foundation (Grant No. 18424-1), California Academy of Sciences, CNPq grants 05358/2015-4 (SRF) and 490531/2007-5 (Pro-Africa). The authors have no conflicts of interest to disclose.

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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.

**Table S1.** Species, localities and GenBank accession numbers.

**Figure S1.** Phylogenetic tree of the genus *Scartella* based on maximum likehood for the mitochondrial D-loop region. Maximum likehood bootstrap are shown only for nodes with over than 70% support values. BRA 1 (Brazil, Ceará to Rio Grande do Sul States); BRA 2 (Brazil, Rio de Janeiro to Rio Grande do Sul States); FL (United States, Florida); CAR (Florida and Panama); MED (Spain, Barcelona and Ibiza); CAN (Spain, Canaries) and STP (São Tomé and Príncipe complex).

**Figure S2.** Phylogenetic tree of the genus *Scartella* based on Bayesian inference for the mitochondrial D-loop region. Bayesian posterior probability are shown only for nodes with over than 70% support values. BRA 1 (Brazil, Ceará to Rio Grande do Sul States); BRA 2 (Brazil, Rio de Janeiro to Rio Grande do Sul States); FL (United States, Florida); CAR (Florida and Panama); MED (Spain, Barcelona and Ibiza); CAN (Spain, Canaries) and STP (São Tomé and Príncipe complex).