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Phylogeography of the reef-building polychaetes of the genus *Phragmatopoma* in the western Atlantic Region

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Abstract :

Aim

To verify the synonymy of the reef-building polychaete *Phragmatopoma caudata* (described for the Caribbean) and *Phragmatopoma lapidosa* (described for Brazil) using molecular data. To evaluate the patterns of genetic diversity and connectivity among populations from Florida to South Brazil.

Location

Intertidal zone in the western Atlantic biogeographical Region: Brazil, eastern Caribbean and Florida (USA).

Methods

DNA sequence data from one mitochondrial (cox-1) and one nuclear ribosomal (ITS-1) loci were obtained from 11 populations of *P. caudata* spanning the coasts of Brazil, eastern Caribbean and Florida. Phylogenetic relationships among populations of *P. caudata* and other members of the genus were inferred by Bayesian methods. Population differentiation was evaluated by Bayesian analysis of population structure (baps), AMOVA and pairwise ϕ_{st} . Demographic history was inferred by Bayesian skyline plots.

Results

Phylogenetic inference supported the interpretation of a single species of *Phragmatopoma* spanning the Brazilian and Caribbean Provinces of the western Atlantic Region. Little population structure was observed across the species distribution, with the exception of the Florida population. The baps analysis supported a 2-population model, with population differentiation being strong and significant between Florida and all other Atlantic populations for *cox-1*, and significant between Florida and most populations for *ITS-1*. Differences in genetic diversity were not significant between Caribbean and Brazilian populations, although several populations in Brazil had low values for diversity indices. Bayesian skyline plots indicate population expansion starting at c. 200 ka.

Main conclusions

Phragmatopoma caudata is able to maintain genetic connectivity across most of its geographical range, with population differentiation being observed only between Florida and all other localities, possibly due to ecological speciation in the transition zone between tropical and subtropical environments. Long-distance connectivity across much of the species range is likely the result of long-lived larvae that are tolerant to a wide range of environmental conditions.

51 **Introduction**

52 Many benthic marine invertebrates have patchy distributions as a result of the interaction among
53 abiotic and biotic variables that limit dispersal, settlement and survival. Discontinuous distributions can
54 affect population connectivity even in species with a long planktonic larval stage, having consequences for
55 gene flow, genetic diversity and speciation. Thus, benthic marine invertebrates provide interesting models
56 for addressing questions related to how species distributions reflect the interplay among dispersal dynamics,
57 environmental conditions, biotic interactions or historical isolation (Awise, 1992; Palumbi, 1994). The
58 tropical Atlantic fauna are affected by five major biogeographical barriers: the Mid-Atlantic Barrier, the
59 Terminal Tethyan Event, the Amazon-Orinoco Barrier, the Isthmus of Panama and the Benguela Barrier
60 (Floeter *et al.*, 2008). But while these barriers are effective for numerous species, exceptions exist for each
61 one, providing opportunities for understanding the variables that contribute to species distributions, their
62 delimitations and connectivity among their populations.

63 Some marine polychaetes from the family Sabellariidae Johnston, 1865 are gregarious and are
64 important reef-building organisms in coastal environments (Goldberg, 2013). These ecosystem engineers
65 create complex habitats supporting high levels of biodiversity, and provide ecosystem services such as
66 coastal protection (Dubois *et al.*, 2002; Noernberg *et al.*, 2010; Ataide *et al.*, 2014). While most sabellariids
67 are solitary, the species that are reef-building typically construct biogenic structures in intertidal or shallow
68 subtidal environments (Faroni-Perez *et al.*, 2016). Reefs of *Phragmatopoma caudata* Krøyer in Mörch, 1863
69 are broadly distributed along the intertidal zone in the western Atlantic coastline, from Florida (USA)
70 (34°N) to Santa Catarina (Brazil) (27°S), including many localities in the Caribbean (Kirtley, 1994).
71 Although *P. caudata* reefs are known to exist at various locations along the Brazilian coast (Pagliosa *et al.*,
72 2014), records in new localities continue to be reported. For instance, new reefs formed in Fortaleza (north
73 Brazil) following the construction of a harbour (Fournier & Panizza pers. obs.). Currently, the northernmost
74 known occurrence of the species in Brazil is in the state of Piauí (Santos *et al.*, 2012). Beyond the Amazon
75 and Orinoco Rivers, the species has also been recorded in Venezuela (Liñero-Arana, 2013). Discontinuities
76 in the range cannot at present be stated with certainty, as field observations in this geographical region are
77 far from exhaustive, and areas with confirmed absences have yet to be described. The abundance and

78 distribution of sabellariid reefs depend on the availability of a hard substrate, suspended sediments, and
79 appropriate levels of turbulence (Main & Nelson, 1988). *Phragmatopoma caudata* (as *P. lapidosa* Kinberg,
80 1866) reproduces by external fertilization of gametes that are produced year-round (Eckelbarger, 1976).
81 Spawning and recruitment are highest in the summer months, from June to August in Florida, USA
82 (McCarthy *et al.*, 2003) and February to April in São Paulo State, Brazil (Faroni-Perez, 2014). Fecundity is
83 high, as the average female can spawn 1500-2000 oocytes (McCarthy *et al.*, 2003), indicating a high
84 potential for dispersal. During development, planktonic larvae drift in the water column from two to four
85 weeks (Mauro, 1975; Eckelbarger, 1976). Larvae tolerate a wide temperature range (15.5°C-29.5°C), but
86 beyond these extremes, development and survival are compromised (Eckelbarger, 1976). While tolerance to
87 salinity has not yet been quantified for larvae, adults can tolerate brackish waters of up to 30-40% seawater
88 (Mauro, 1977). When metatrochophore larvae are competent for metamorphosis, they settle onto hard
89 substrate, usually a conspecific pre-existing reef, induced by chemical cues (Pawlik, 1988) and mediated by
90 the larval sensory organs, such as the dorsal hump and palps (Faroni-Perez *et al.*, 2016). Finally, *P. caudata*
91 has an estimated lifespan of one to two years (McCarthy *et al.*, 2003).

92 Systematics of the genus have been recently revised (Drake *et al.*, 2007; Capa *et al.*, 2012).
93 Notwithstanding, the brief original descriptions for *P. caudata* and *P. lapidosa* and the disappearance of the
94 type material led to uncertain taxonomic status. Hartman (1944) questioned whether the two species were
95 distinct, and upon revision of Sabellariidae, Kirtley (1994) synonymized *P. lapidosa* with *P. caudata*. More
96 recently, molecular phylogenetics supported a single Caribbean species, with distinct populations in Florida
97 and West Indies (Drake *et al.*, 2007). However, patterns of oogenesis in individuals from Florida differed
98 from those in Brazil, reopening the debate on plasticity or speciation (Faroni-Perez & Zara, 2014).
99 Moreover, intraspecific variability in the composition of the cement used for reef construction was found
100 along the Brazilian coast, suggesting potential differences among populations (Fournier *et al.*, 2010).
101 Currently, no molecular study has taken Brazilian populations into consideration, and the question remains
102 whether a single species is distributed from Florida to South Brazil.

103 The aims of this study are i) to examine if a single *Phragmatopoma* species occurs in the Western
104 Atlantic Region, ii) to assess the genetic connectivity among populations of *P. caudata* in the Caribbean and
105 Brazilian biogeographical provinces and iii) to assess the effectiveness of putative biogeographical barriers
106 on the connectivity of *P. caudata*.

107

108 **Materials and Methods**

109 *Study sites and sampling method*

110 *Phragmatopoma caudata* was collected from seven sites spanning its distribution along the coast of
111 Brazil: Fortaleza (FOR), Tamandaré (TAM), Peracanga (PER), Ubatuba (UBA), Ilha Porchat (POR),
112 Itanhaém (ITA) and Ilha do Mel (MEL). In addition, four sites in the Caribbean were analysed: three
113 previously sampled sites in Florida, USA (FLO), Puerto Rico (PRI) and Virgin Islands (VIL), and one new
114 site in Guadeloupe Island (GUA) (Figure 1, Appendix S1). Specimens were collected at low tide by
115 breaking off small blocks of reef and removing 1-3 worms from each block. At each locality, several reef
116 blocks were collected, separated by tens of meters, to ensure good representation of genetic diversity at each
117 site. Individual specimens were fixed in 70% ethanol and stored at -20°C.

118

119 *DNA extraction and sequencing*

120 DNA was extracted using the CTAB method (Denis *et al.*, 2009). Two loci were used in order to
121 make comparisons with previously studied Caribbean populations (Drake *et al.*, 2007): the mitochondrial
122 cytochrome c oxidase subunit I (*cox-1*) and the first internal transcribed spacer region (*ITS-1*) of the
123 ribosomal DNA. New primers were designed for *cox-1*: PHRALCO: 5' -
124 TTTATATTTTGG AATTTGGTCAGG -3'; PHRAHCO: 5' - TAAAGAACTGGGTCTCCACC-3'.
125 Published primers were used for *ITS-1* (ITS1-fw: 5'-CACACCGCCCGTCGCTACTA-3', ITS3r: 5'-
126 TTCGACSCACGAGCCRAGTGATC-3') (Denis *et al.*, 2009). Amplification was performed with the
127 Ready-to-Go PCR kit (GE Healthcare, Little Chalfont, UK) using 0.1 µg of DNA. Thermal cycler conditions
128 included an initial denaturation step at 96°C for 2 min, 40 cycles at 96°C for 30s, 52°C for 30s and 72°C for
129 1 min, with a final extension at 72°C for 5 min. After electrophoresis, PCR products were extracted from the

130 agarose gel and purified using the Wizard SV Gel System (Promega, Fitchburgh, WI, USA). Cloning was
131 carried out for a subset of the *ITS-1* amplifications (23 individuals), to confirm the phase of heterozygous
132 alleles. PCR products were ligated into the pGEMT Easy vector (Promega, Fitchburgh, WI, USA) and
133 transformed into JM 109 competent cells (Promega, Fitchburgh, WI, USA). Five colonies were sequenced
134 for each individual. Sequencing reactions used the BigDye v.3.1 chemistry (Applied Biosystems, Waltham,
135 MA, USA) and was analysed on an ABI 3130 automated sequencer.

136

137 *Molecular data analysis*

138 DNA sequence chromatograms were inspected for errors and edited with SEQUENCHER 4.5 (Gene
139 Codes Corp, Ann Arbor, MI, USA). Published sequences of *Phragmatopoma caudata* [Genbank accession
140 numbers: DQ172733- DQ172763, DQ172801-DQ172810], *Phragmatopoma californica* (Fewkes, 1899)
141 [DQ172682-DQ172732, DQ172768-DQ172800], *Phragmatopoma moerchi* Kinberg, 1866 [DQ172764] and
142 *Phragmatopoma virgini* Kinberg, 1866 [DQ172813-DQ172822, DQ172811-DQ172812] were added to the
143 dataset, and sequences of *Idanthysus cretus* Chamberlain 1919 [DQ172680-DQ172681, DQ172765-
144 DQ172767] were used for the outgroup (Drake *et al.*, 2007). Sequence alignment was performed using
145 CLUSTALX in MEGA 6.0.6 using default parameters (Tamura *et al.*, 2013). For *ITS-1* sequences containing
146 double peaks in both sequencing directions, cloned sequences and the sequences of homozygous individuals
147 were used for haplotype reconstruction using PHASE implemented in DNASP 5 (Librado & Rozas, 2009).
148 For phylogenetic analysis, all redundant sequences were removed, such that phylogenetic inference was
149 made with unique sequences. A gene tree was constructed for each locus in BEAST 1.8.2 and species tree
150 ancestral reconstruction was estimated combining both *cox-1* and *ITS-1* with the *BEAST algorithm (Heled
151 & Drummond, 2010). The best-fit model of nucleotide substitution was determined by hierarchical
152 likelihood ratio test in MRMODELTEST 2.2 (<https://github.com/nylander/MrModeltest2>). The GTR+ Γ +I
153 substitution model was used for *cox-1* and the HKY+ Γ model was used for *ITS-1*. A strict molecular clock
154 was employed with a fixed substitution rate of 2.1% per Myr for *cox-1* and 0.25% per Myr for *ITS-1*. In the
155 species tree, clock rates were estimated relative to *cox-1*. Substitution rates vary across species and genes,

156 and depend on the accurate timing of vicariant events or fossil occurrences, which can incur considerable
157 uncertainties. However, averaging the rates obtained for the same gene over several closely related taxa can
158 improve the confidence of molecular clock estimates. The substitution rate selected for *cox-1* corresponds to
159 the average rate across 27 transisthmian crustacean species pairs (Lessios, 2008). In addition, a rate of 2.1%
160 per Myr (based on crustaceans) has also been employed in previous work on *Phragmatopoma* spp. (Drake *et*
161 *al.*, 2007). Fewer estimates of substitution rates are available for the invertebrate *ITS-1* locus. The rate of
162 0.25% per Myr used for *Phragmatopoma* was estimated for a marine gastropod (Coleman & Vacquier,
163 2002).

164 For phylogenetic analysis, the Markov chain Monte Carlo (MCMC) ran for 30 million generations
165 with sampling at every 1000 steps. The results of three independent runs were verified for convergence using
166 TRACER 1.5 and combined after discarding a burn-in of 20% using LOGCOMBINER 1.8.2. Target trees used
167 the maximum clade credibility criterion in TREEANNOTATOR 1.8.2. Nodes with a posterior probability inferior
168 to 0.90 were collapsed. Estimates of genetic distance between species pairs were calculated using the
169 Kimura 2-parameter model in MEGA 6. For *cox-1*, positions containing missing data were eliminated, while
170 for *ITS-1* positions containing gaps between sequence pairs were removed. Divergence time estimates based
171 on genetic distances used the same substitution rates listed above.

172 Relationships among haplotypes (including redundant sequences) were inferred with a haplotype
173 network based on maximum parsimony, constructed with TCS 1.21 (Clement *et al.*, 2000). Haplotype
174 frequencies, the number of unique haplotypes (H), segregating sites (s), haplotype diversity (h) and
175 nucleotide diversity (π) were calculated for each sampling site using ARLEQUIN 3.1 (Excoffier *et al.*, 2005).
176 Deviations from neutrality were assessed with Tajima's D and Fu's Fs statistics in ARLEQUIN. Population
177 demographic history was inferred by Bayesian skyline plots implemented in BEAST (Drummond *et al.*,
178 2005). The population size function of the Bayesian skyline plots were fitted using a piecewise constant
179 function, with 10 groups. In order to obtain an effective sampling size of at least 200, the MCMC chain ran
180 for 50 million generations and was sampled every 100 for *cox-1*, and for *ITS-1*, the MCMC ran for 40
181 million generations sampled every 1000.

182 Population structure was explored for both loci using Bayesian analysis of population genetic
183 structure (BAPS; Corander *et al.*, 2008), a clustering algorithm which uses a Bayesian predictive model to
184 estimate the number of genetically diverged groups based on molecular data. A population mixture analysis
185 was run using the “clustering with linked loci” option. Single-locus sequence data are expected to be
186 genetically linked because of their close proximity along the chromosome. This option therefore takes into
187 consideration the non-independence of linked loci. The clustering of groups with the lowest log likelihood
188 was selected.

189 Population differentiation was assessed by analysis of molecular variance (AMOVA) and pairwise
190 ϕ_{st} in ARLEQUIN (Excoffier *et al.*, 2005). The significance level of pairwise tests was adjusted by a
191 Bonferroni correction. For the three-level hierarchical AMOVAs, the results of the BAPS analysis were used
192 to select biogeographical divisions.

193

194 **Results**

195 *Properties of the DNA sequences*

196 After sequence quality screening and trimming, a total of 146 sequences of 497 bp length were
197 obtained for *cox-1* and 99 sequences of 403 bp length were obtained for *ITS-1* for *P. caudata*. Sequences
198 were deposited in Genbank (accession numbers: KT182639 - KT182784 for *cox-1* and KT182785 -
199 KT182883 for *ITS-1*). For the *cox-1* locus, population genetic analyses were calculated (1) considering all
200 three codon positions, and (2) with the third codon position excluded. Because of high polymorphism in the
201 third codon position, 74% of sequences were unique, leading to a haplotype network characterized by
202 numerous loops, indicating homoplasy. Moreover, the AMOVA and population differentiation results were
203 similar whether the third codon position was kept or excluded from the analysis. Therefore, results shown
204 for haplotype network, allele frequencies, genetic diversity, AMOVA, pairwise ϕ_{st} and BAPS consider only
205 the first and second codon positions, while all three codon positions were kept for phylogenetic analysis and
206 Bayesian skyline plots.

207 Previous work has shown that multiple alleles (>2) can be observed for *ITS-1* (Drake *et al.*, 2007).
208 Among all sequences, multiple peaks were found in 23 nucleotide sites. More than one allele was observed

209 for nearly all individuals (identified either by cloning or by haplotype reconstruction), but only eight
210 individuals had more than two sites with multiple peaks. Because the present study combined published data
211 (from Drake *et al.*, 2007) with new data, only one allele per individual was kept in the analysis as was done
212 previously. A random number generator was used to select the haplotype kept in the analysis, to ensure that
213 allele selection did not bias the dataset towards lower diversity by selecting the most common allele, with
214 the caveat that this approach does not discriminate paralogous from orthologous alleles. Few mutational
215 differences were observed among intra-individual alleles (maximally nine mutations); therefore inadvertent
216 selection of paralogous alleles likely had only a small effect on estimates of genetic diversity or
217 differentiation.

218

219 *Phylogenetic analysis*

220 All three species of *Phragmatopoma* (*P. caudata*, *P. californica* and *P. virgini*) were monophyletic
221 for *cox-1*, *ITS-1* and the species tree combining both loci (Figure 2). However, all phylogenetic
222 reconstructions found the relationship between the three species to be unresolved. Low support was found
223 for *P. caudata* being sister taxon to *P. californica* in the species trees (pp = 0.40; Figure 2a), for *P.*
224 *californica* being sister to *P. virgini* in the *ITS-1* tree (pp=0.21; node collapsed; Figure 2b), and for *P.*
225 *caudata* being sister to *P. virgini* in the *cox-1* tree (pp=0.86; node collapsed; Figure 2c). Sequences of
226 individuals of *P. caudata* from Brazil belonged to the same clade as those from the Caribbean (Figure 2 b,c)
227 and identical sequences were observed among some individuals from Brazil and the Caribbean. However, *P.*
228 *caudata* from Florida formed a reciprocally monophyletic clade with *P. caudata* from the rest of the
229 Caribbean+Brazil, having high support in the analyses using *cox-1* and the combined loci (pp=1.00 for both
230 trees; Figure 2 a,c).

231 Pairwise genetic distances and divergence time estimates are shown in Table 1. Based on *cox-1*, *P.*
232 *caudata* diverged from *P. californica* at 8.6 ± 1.0 Ma and from *P. virgini* at 9.5 ± 1.0 Ma. Divergence times
233 estimated with *ITS-1* indicate an older split between *P. caudata* and *P. californica*, at 28.2 ± 5.4 Ma, and of
234 33.1 ± 5.9 Ma between *P. caudata* and *P. virgini*. *P. caudata* from Florida diverged from the remaining *P.*
235 *caudata* populations at approximately 1.5 ± 0.3 Ma (*cox-1*) to 3.2 ± 1.2 Ma (*ITS-1*).

236

237 *Haplotype networks and haplotype frequencies*

238 Haplotype frequencies for *cox-1* and *ITS-1* are shown in Figures 1b-c, and maximum
239 parsimony haplotype networks are shown in Figure 3. Among the 17 haplotypes sequenced for *cox-1*, two
240 were abundant among the sampled populations (C5 and C1), three were present in more than one population
241 at low frequencies (C6, C8 and C11), with the remainder being observed in only one individual (singletons)
242 and being restricted to one population (private haplotypes) (Figure 3a). Haplotype C5 was abundant in most
243 populations, ranging in frequency from 69 – 100%, except in Florida, where it was absent. Haplotype C1
244 was abundant only in Florida (75%) and was found in three other Brazilian populations at low frequencies
245 (Figure 1b). Overall, nearly all populations had similar haplotype frequencies for *cox-1* across the range of
246 *P. caudata*, including populations in Caribbean and Brazil, with the exception of the Florida population.

247 Among the 22 haplotypes sequenced for *ITS-1*, six were present in more than one population
248 (in order of abundance: T7, T8, T4, T1, T3 and T13), and the remainder were private haplotypes (Figure 3b).
249 The most common haplotype (T7) was abundant in all Brazilian populations (40-80%) and one Caribbean
250 population (GUA, 40%). Haplotype T7 was also present in Puerto Rico and the Virgin Islands, but at lower
251 frequencies (8-9%), and was absent in Florida (Figure 1c). Haplotypes T1 and T3 were found only in the
252 Caribbean Province (including Florida). In sum, haplotype frequencies for *ITS-1* were similar among
253 Brazilian populations, with greater variability being observed among the Caribbean populations. The Florida
254 population had the most divergent pattern, with a high abundance of haplotype T1 (42%), which was absent
255 from most other populations, except for the Virgin Islands (18%).

256

257 *Genetic Diversity*

258 Genetic diversity indices were usually greater in the Caribbean populations (FLO, PRI, VIL, GUA)
259 relative to the Brazilian populations (FOR, TAM, PER, UBA, POR, ITA, MEL). For example, gene
260 diversity among Caribbean populations was greater than among Brazilian populations for both *cox-1*
261 (0.426 ± 0.121 compared to 0.243 ± 0.195) and *ITS-1* (0.854 ± 0.06 compared to 0.692 ± 0.178), although these
262 differences were not statistically significant ($p=0.125$ and $p=0.119$ respectively). Likewise, average

263 nucleotide diversity was greater in the Caribbean rather than Brazilian populations for both *cox-1* and *ITS-1*,
264 but again, the difference was not significant ($p=0.166$ and $p=0.07$ for *cox-1* and *ITS-1* respectively)
265 (Appendix S2). Within both regions, genetic diversity was variable across populations, but in Brazil,
266 variability was greater, with some populations having values similar to the Caribbean, while others had
267 much lower diversity.

268 Deviations from neutral expectation were also observed. Tajima's D was negative for five out of 11
269 populations in *cox-1* and Fu's Fs indicated significant deviation from neutrality for seven populations for
270 *cox-1* and three populations for *ITS-1* (Appendix S2). These large negative values for Fu's Fs suggest recent
271 population expansion in the Caribbean, and in several Brazilian populations. Bayesian skyline plots for both
272 *cox-1* and *ITS-1* also support an interpretation of recent population expansion, dating to ~200 ka (Figure 4).
273 The age estimate for population expansion of *P. caudata* is concordant for both loci, even though
274 independent molecular clocks were used. Population structure has been shown to have a confounding effect
275 on demographic history (Heller *et al.*, 2013). Because strong differentiation was observed with respect to the
276 Florida population, Bayesian skyline plots were also estimated after excluding sequences from Florida
277 (Figure 4b, d). Regardless of whether individuals from Florida were excluded or kept in the analysis, a
278 signature of population expansion was observed, all dating to ~200 ka.

279

280 *Population differentiation*

281 BAPS found two genetically distinct groups for *cox-1*, among the sampled *P. caudata* localities
282 ($\log_{ML}=-1905.8$). All individuals assigned to one group were sampled from Florida, while the remaining
283 individuals, sampled across all other populations in the Caribbean and Brazil, were assigned to the second
284 group (Appendix S3). In order to examine whether further population structure occurred within the second
285 group, an analysis was conducted excluding individuals from Florida. However, no further genetically
286 distinct groups were identified, as all individuals from this reduced dataset were still all assigned to the same
287 group ($\log_{ML}=-1607.3$). For *ITS-1*, BAPS found four genetically distinct groups ($\log_{ML}=-352.4$); but, only
288 one individual was assigned to two of the four groups. The log likelihood for two groups was similar to four
289 groups ($\log_{ML}=-393.5$), but there was no clear geographic pattern in the assignment of individuals to either

290 group. For example, one group contained individuals from Florida, the Caribbean (in PRI and GUA) and
291 Brazil (TAM, PER and POR) (Appendix S3). The BAPS analysis therefore did not indicate any strong
292 geographical trend in population differentiation for *ITS-1*.

293 The hierarchical population structure design in the AMOVA considered two groups (group 1: FLO;
294 group 2: all other populations). This scenario was selected based on the results of the BAPS analysis (for
295 *cox-1*), and patterns in haplotype frequencies and haplotype networks for both loci. Differentiation among
296 populations (F_{ST}) was significant for both *cox-1* ($F_{ST}=0.721$, $p<0.00001$) and *ITS-1* ($F_{ST}=0.21338$,
297 $p<0.00001$). Differentiation among groups was also significant for *ITS-1* ($F_{CT}=0.196$, $p=0.00098$). Although
298 the F_{CT} value was high for *cox-1* ($F_{CT}=0.721$), it was not significant ($p=0.088$) (Table 2). These results
299 indicate that there is some significant population structure among *P. caudata* populations, and that much of
300 this structure is due to the Florida population.

301 Values of pairwise ϕ_{st} for *cox-1* clearly indicate strong differentiation of the Florida population with
302 respect to all other Caribbean and Brazilian populations (ϕ_{st} ranges from 0.606–0.784) (Table 3). For all
303 other pairwise comparisons, ϕ_{st} was small and not significant, suggesting that connectivity is maintained
304 among populations along the coast of Brazil and among the eastern Caribbean Islands. For *ITS-1*, nearly all
305 pairwise ϕ_{st} comparisons were non-significant after Bonferroni correction (except PER compared to FLO
306 and PRI), indicating that for this locus, although some population structure can be detected, connectivity
307 appears to be maintained among most populations.

308

309 **Discussion**

310 Phylogenetic analysis based on *cox-1* and *ITS-1* confirms the monophyly of three species of
311 *Phragmatopoma* (*P. caudata*, *P. californica* and *P. virgini*). In addition, our analyses which included one
312 published sequence of *P. moerchi*, also indicate that this may be a separate species, as suggested by Drake *et*
313 *al.* (2007). In contrast to previous work, however, the results presented here do not show conclusive
314 phylogenetic relationships among *P. caudata*, *P. californica* and *P. virgini*, as trichotomies were observed in
315 the *cox-1*, *ITS-1* and in the species trees. Sequencing of additional loci or sampling of additional species in

316 the genus (such as *P. attenuata* from the Pacific, or more individuals of *P. moerchi*) may help to clarify
317 phylogenetic relationships within the genus.

318 Phylogenetic analysis also indicates the existence of a single species – *Phragmatopoma caudata* –
319 from the eastern Caribbean to southern Brazil. These are the first molecular data to support a single species
320 spanning this broad geographical range, confirming Kirtley’s (1994) synonymization of *P. lapidosa*
321 (originally described from Brazil) and *P. caudata* (originally described from the Caribbean). However, two
322 genetically differentiated lineages were also identified - one that spans the Brazilian coast and part of the
323 Caribbean and another that is restricted to Florida. Genetic differentiation with respect to Florida is
324 congruent with contrasting patterns of oogenesis observed between *P. caudata* from Brazil and Florida
325 (Faroni-Perez & Zara, 2014). For instance, the ovaries in *P. caudata* from Brazil were composed of oogonia
326 and oocytes attached to blood vessels during early development, whereas in Florida, oocytes were associated
327 with blood vessels until the end of vitellogenesis. Several additional features of oogenesis differed between
328 individuals from either locations, including the type of oogenesis (intra- versus extra-ovarian), the nature of
329 oocyte development (auto versus heterosynthetic), and the locations of the oocyte mitochondria cloud, Golgi
330 complexes and ovary capsules (Faroni-Perez & Zara, 2014). These findings show various distinctive aspects
331 of gametogenesis between Florida and Brazil. Characterization of reproductive traits in individuals from
332 Puerto Rico and Virgin Islands (geographically close to Florida, but genetically more similar to Brazil)
333 could help elucidate whether reproductive differences are associated with the genetic differentiation
334 observed here, and whether species-level distinction is warranted with respect to *P. caudata* from Florida.

335 Recent reassessments in Atlantic biogeography find the marine fauna within the Greater Caribbean
336 to be relatively homogeneous, with the Caribbean Province being comprised of all the northern Western
337 Atlantic tropics, including the southern tip of Florida and the West Indian islands (Floeter *et al.*, 2008;
338 Briggs & Bowen, 2012). *Phragmatopoma caudata*, however, differs from this general trend, and shows a
339 split between south Florida and nearby West Indian islands. The isolation of the Florida population may
340 have three possible explanations. Firstly, the fast flowing currents in the Florida Straits may hinder larval
341 dispersal between Florida and the West Indies (Briggs, 1995), as has been suggested for *Symbiodinium*
342 harboured by the octocoral *Gorgonia ventalina* (Andras *et al.*, 2011). A second possibility is long-term

343 divergence between Caribbean and Brazilian lineages, followed by recent dispersal from Brazil into the
344 West Indies, as suggested for the rock hind *Epinephelus adscensionis* (Carlin *et al.*, 2003). Finally, the
345 Florida population may be a case of incipient or recent speciation. Phylogenetic analysis based on *cox-1* and
346 the species tree based on both loci show high support for a Florida clade (Figure 2), indicating a possible
347 cryptic species. Because phylogenetic analysis based on *ITS-1* alone does not identify a Florida clade, these
348 results require verification from additional molecular markers and/or morphological comparisons between
349 specimens from Florida and the rest of the range of *P. caudata*. However, differences in gonad development
350 between *P. caudata* from Brazil and Florida support the interpretation of a cryptic species (Faroni-Perez &
351 Zara, 2014). Florida is a transition zone between the tropics and subtropics, where ecological speciation
352 could take place as different genotypes become adapted to contrasting environmental conditions in different
353 habitat types. The wrasse *Halichoeres bivittatus* provides a compelling example of ecological speciation in
354 the marine environment (Rocha *et al.*, 2005). In this species, genetic connectivity is maintained across
355 >2400 km, from Belize to the Lesser Antilles, but strong differentiation is observed between tropical
356 Bahamas and subtropical Florida, separated by only 300 km. In the Florida Keys, where tropical and
357 subtropical habitats exist in close proximity, subtropical genotypes of this species were found in cooler
358 inshore channels while tropical genotypes were found in warmer offshore reefs (Rocha *et al.*, 2005).
359 Ecological speciation in *P. caudata* is an intriguing hypothesis for the genetic break observed between the
360 West Indies and Florida, and future work examining contrasting habitats along the coast of Florida and
361 adjacent areas may help to clarify the mechanisms that have led to genetic isolation in this location.

362 While genetic differentiation between Florida and the eastern Caribbean has previously been
363 documented in *P. caudata* (Drake *et al.*, 2007), our work reveals continued genetic connectivity across the
364 Amazon-Orinoco Barrier, among populations separated by as much as 9000 km. The Amazon plume is an
365 important barrier to dispersal for a variety of marine species such as corals (Nunes *et al.*, 2009, 2011),
366 crustaceans (Terossi & Mantelatto, 2012), echinoderms (Lessios *et al.*, 2003), and reef fish (Mendonça *et al.*,
367 2013). However, it is considered a “soft barrier” or “filter” because of the large number of shared fish
368 species on either side of the barrier (Floeter *et al.*, 2008). Indeed, connectivity between the Caribbean and
369 Brazilian Provinces has been observed in several marine species, such as ascidians (Nóbrega *et al.*, 2004),

370 sea urchins (Zigler & Lessios, 2004), sponges (Lazoski *et al.*, 2001) and fish (Floeter *et al.*, 2008). Similarly,
371 the Amazon-Orinoco Barrier does not appear to be an effective barrier for dispersal for *P. caudata*, even
372 though occurrences on either side of the Amazon and Orinoco Rivers (Parnaíba, Brazil and Puerto Viejo,
373 Venezuela) indicate that populations may be separated by up to 2700 km. Connectivity among populations
374 of *P. caudata* in Brazil and the West Indies may be maintained by the North Brazil and Guiana Currents,
375 both flowing northward from the north-eastern point of Brazil towards the Amazon and onwards to the
376 Caribbean (Figure 1a). In addition, the Amazon River discharge varies seasonally, and is weakened from
377 January to April (Molleri *et al.*, 2010), potentially allowing larval permeability from North Brazil to the
378 eastern Caribbean Islands. Larvae of *P. caudata* develop over two to four weeks (Eckelbarger, 1976), likely
379 contributing to the ability to disperse broadly and to maintain connectivity across great distances. Moreover,
380 larvae of *P. caudata* can develop normally between 15.5–29.5°C, a relatively wide temperature range
381 (Eckelbarger, 1976). Tolerance to salinity in larvae of *P. caudata* is currently unknown, but could be an
382 additional parameter favouring long-distance dispersal. Further experiments are needed to determine
383 tolerance to environmental variability in larvae, but such traits could explain dispersal across the Amazon-
384 Orinoco Barrier.

385 At the intra-specific level, *cox-1* was characterized by a high number of private alleles (haplotypes
386 restricted to one population), and singletons (haplotypes observed in only one individual) in all populations
387 of *P. caudata*. Interestingly, all singleton mutations were synonymous (i.e. did not alter the amino acid
388 sequence of a protein). A large number of singletons could be due to a high mutation rate in the
389 mitochondrial genome, to a large effective population size, or recent population expansion. While data to
390 estimate a mutation rate specific to *P. caudata* are currently unavailable, large population size and/or recent
391 population expansion may explain the large number of singletons in *cox-1*. *P. caudata* likely has large
392 population sizes, as the density of individuals has been estimated at ~65,000 individuals/m² (Faroni-Perez,
393 2014). Given that the generation time of *P. caudata* is of one year, a large fraction of individuals potentially
394 contribute to the gene pool each year. In addition, Bayesian skyline plots (Figure 4) and significant negative
395 values for Fu's F_s and Tajima's D are indicative of recent population expansion (dating to *c.* 200 ka). Each

396 of these factors may explain, alone or in combination, the high polymorphism observed in the mitochondrial
397 locus.

398 Within the Brazilian Province, no significant population structure was observed for *P. caudata*.
399 Long-distance connectivity along the coast of Brazil is known for other invertebrates, including broadcasting
400 corals (Nunes *et al.*, 2009, 2011) and fiddler crabs (Laurenzano *et al.*, 2013; Wieman *et al.*, 2014).
401 Nevertheless, the lack of genetic differentiation along >5000 km from Fortaleza to Ilha do Mel was
402 unexpected. For example, the “coastal/island” species of the fireworm *Eurythoe complanata* shows
403 significant population structure along the coast of Brazil (Barroso *et al.*, 2010), despite having a similar
404 larval duration to *P. caudata*. The data presented here suggest that *P. caudata* can overcome various barriers
405 to dispersal that are known for other marine organisms within the Brazilian Province, such as the split
406 between the north-flowing North Brazil Current and south-flowing Brazil Current (Santos *et al.*, 2006), the
407 São Francisco Barrier (Floeter *et al.*, 2001; Picciani *et al.*, 2016; Souza *et al.*, 2017) and the upwelling at
408 Cabo Frio (for the coral *M. hispida*, L. Peluso, UFRJ, pers. comm.).

409 South of the Point of Natal, the Brazil Current is a powerful western-boundary current that may
410 facilitate larval transport and gene flow (Figure 1a). Currently only a few studies have addressed genetic
411 connectivity in annelids in the Brazilian Province (Barroso *et al.*, 2010; Ahrens *et al.*, 2013). Future work on
412 other annelid species may help identify traits that favour or hinder connectivity in this biogeographical
413 region. Finally, for a better understanding of *P. caudata* population connectivity, the use of higher resolution
414 markers such as such as microsatellites or SNPs derived from RAD-Seq could be used to examine finer-
415 scale population structure and dispersal dynamics.

416

417 **Conclusions**

418 Molecular data from two loci (*cox-1* and *ITS-1*) confirms the occurrence of a single species,
419 *Phragmatopoma caudata*, from Florida to South Brazil. High levels of connectivity are implied across the
420 species range, possibly due to high gamete density upon spawning, long pelagic larval stage, and larvae that
421 are tolerant to a wide range of temperatures, and possibly salinity. The Amazon plume, other major rivers
422 along the coast of Brazil or the upwelling in Cabo Frio are not effective barriers for dispersal for this species,

423 as connectivity is maintained along the entire coast of Brazil and between Brazil and the eastern Caribbean.
 424 Population structure is observed only in comparisons with the Florida population, possibly due to ecological
 425 speciation in the transition zone between tropical and subtropical environments. Additional sampling within
 426 the Caribbean is needed to identify whether other barriers to dispersal occur within this biogeographical
 427 region.

428

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440

441 **References**

442

- 443 Ahrens J.B., Borda E., Barroso R., Paiva P.C., Campbell A.M., Wolf A., Nugues M.M., Rouse G.W., &
 444 Schulze A. (2013) The curious case of *Hermodice carunculata* (Annelida: Amphinomidae): evidence
 445 for genetic homogeneity throughout the Atlantic Ocean and adjacent basins. *Molecular Ecology*, **22**,
 446 2280–2291.
- 447 Andras J.P., Kirk N.L., & Harvell C.D. (2011) Range-wide population genetic structure of *Symbiodinium*
 448 associated with the Caribbean Sea fan coral, *Gorgonia ventalina*. *Molecular Ecology*, **20**, 2525–2542.
- 449 Ataíde M.B., Venekey V., Rosa Filho J.S., & Santos P.J.P. (2014) Sandy reefs of *Sabellaria wilsoni*
 450 (Polychaeta: Sabellariidae) as ecosystem engineers for meiofauna in the Amazon coastal region, Brazil.
 451 *Marine Biodiversity*, **44**, 403–413.
- 452 Avise J.C. (1992) Molecular population structure and the biogeographic history of a regional fauna - a case
 453 history with lessons for conservation biology. *Oikos*, **63**, 62–76.
- 454 Barroso R., Klautau M., Solé-Cava A.M., & Paiva P.C. (2010) *Eurythoe complanata* (Polychaeta:
 455 Amphinomidae), the “cosmopolitan” fireworm, consists of at least three cryptic species. *Marine*
 456 *Biology*, **157**, 69–80.

- 457 Briggs J.C. (1995) *Global Biogeography*. Elsevier, Amsterdam.
- 458 Briggs J.C. & Bowen B.W. (2012) A realignment of marine biogeographic provinces with particular
459 reference to fish distributions. *Journal of Biogeography*, **39**, 12–30.
- 460 Capa M., Hutchings P., & Peart R. (2012) Systematic revision of Sabellariidae (Polychaeta) and their
461 relationships with other polychaetes using morphological and DNA sequence data. *Zoological Journal
462 of the Linnean Society*, **164**, 245–284.
- 463 Carlin J.L., Robertson D.R., & Bowen B.W. (2003) Ancient divergences and recent connections in two
464 tropical Atlantic reef fishes *Epinephelus adscensionis* and *Rypticus saponaceus* (Percoidei:
465 Serranidae). *Marine Biology*, **143**, 1057–1069.
- 466 Clement M., Posada D., & Crandall K. a. (2000) TCS: A computer program to estimate gene genealogies.
467 *Molecular Ecology*, **9**, 1657–1659.
- 468 Coleman A.W. & Vacquier V.D. (2002) Exploring the phylogenetic utility of ITS sequences for animals: a
469 test case for abalone (*Haliotis*). *Journal of Molecular Evolution*, **54**, 246–257.
- 470 Corander J., Marttinen P., Tang J., Sirén J., & Tang J. (2008) Enhanced Bayesian modelling in BAPS
471 software for learning genetic structures of populations. *BMC Bioinformatics*, **9**, 359.
- 472 Denis F., Ravallec R., Pavillon J.-F., & Van Wormhoudt A. (2009) Genetic differentiation of Atlantic
473 populations of the intertidal copepod *Tigriopus brevicornis*. *Scientia Marina*, **73**, 579–587.
- 474 Drake C.A., McCarthy D.A., & Von Dohlen C.D. (2007) Molecular relationships and species divergence
475 among *Phragmatopoma* spp. (Polychaeta: Sabellaridae) in the Americas. *Marine Biology*, **150**, 345–
476 358.
- 477 Drummond A.J., Rambaut A., Shapiro B., & Pybus O.G. (2005) Bayesian coalescent inference of past
478 population dynamics from molecular sequences. *Molecular Biology and Evolution*, **22**, 1185–1192.
- 479 Dubois S., Retiere C., & Olivier F. (2002) Biodiversity associated with *Sabellaria alveolata* (Polychaeta:
480 Sabellariidae) reefs: effects of human disturbances. *Journal of Marine Biology Association of the
481 United Kingdom*, **82**, 817–826.
- 482 Eckelbarger K.J. (1976) Larval development and population aspects of the reef-building polychaete
483 *Phragmatopoma lapidosa* from the east coast of Florida. *Bulletin of Marine Science*, **26**, 117–132.
- 484 Excoffier L., Laval G., & Schneider S. (2005) Arlequin (version 3.0): An integrated software package for
485 population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.
- 486 Faroni-Perez L. (2014) Seasonal variation in recruitment of *Phragmatopoma caudata* (Polychaeta,
487 Sabellariidae) in the southeast coast of Brazil: validation of a methodology for categorizing age classes.
488 *Iheringia*, **104**, 5–13.
- 489 Faroni-Perez L., Helm C., Burghardt I., Hutchings P., & Capa M. (2016) Anterior sensory organs in
490 Sabellariidae (Annelida). *Invertebrate Biology*, **135**, 423–447.
- 491 Faroni-Perez L. & Zara F.J. (2014) Oogenesis in *Phragmatopoma* (Polychaeta: Sabellariidae): evidence for
492 morphological distinction among geographically remote populations. *Memoirs of Museum Victoria*, **71**,
493 53–65.
- 494 Floeter S.R., Guimaraes R.Z.P., Rocha L.A., Ferreira C.E.L., Rangel C.A., & Gasparini J.L. (2001)
495 Geographic variation in reef-fish assemblages along the Brazilian coast. *Global Ecology and
496 Biogeography*, **10**, 423–431.
- 497 Floeter S.R., Rocha L.A., Robertson D.R., Joyeux J.C., Smith-Vaniz W.F., Wirtz P., Edwards A.J., Barreiros
498 J.P., Ferreira C.E.L., Gasparini J.L., Brito A., Falcón J.M., Bowen B.W., & Bernardi G. (2008)
499 Atlantic reef fish biogeography and evolution. *Journal of Biogeography*, **35**, 22–47.
- 500 Fournier J., Etienne S., & Le Cam J.-B. (2010) Inter- and intraspecific variability in the chemical
501 composition of the mineral phase of cements from several tube-building polychaetes. *Geobios*, **43**,
502 191–200.
- 503 Goldberg W.M. (2013) *The Biology of reefs and reef organisms*. University of Chicago Press, Chicago.
- 504 Hartman O. (1944) Polychaetous Annelids. Part VI. Paraonidae, Magelonidae, Longosomidae,
505 Ctenodrilidae, and Sabellariidae. *Allan Hancock Pacific Expeditions*, **10**, 311–389, NaN-342.
- 506 Heled J. & Drummond A.J. (2010) Bayesian inference of species trees from multilocus data. *Molecular
507 Biology and Evolution*, **27**, 570–580.
- 508 Heller R., Chikhi L., & Siegmund H.R. (2013) The confounding effect of population structure on Bayesian
509 skyline plot inferences of demographic history. *PLOS ONE*, **8**, e62992.

- 510 Kirtley D.W. (1994) *A review and taxonomic revision of the family Sabellariidae Johnston 1865 (Annelida;*
511 *Polychaeta)*. Sabecon Press, Science Series, Vero Beach.
- 512 Laurenzano C., Mantelatto F.L.M., & Schubart C.D. (2013) South American homogeneity versus Caribbean
513 heterogeneity: population genetic structure of the western Atlantic fiddler crab *Uca rapax* (Brachyura,
514 Ocypodidae). *Journal of Experimental Marine Biology and Ecology*, **449**, 22–27.
- 515 Lazoski C., Solé-Cava A., Boury-Esnault N., M K., & Russo C.A.M. (2001) Cryptic speciation in a high
516 gene flow scenario in the oviparous marine sponge *Chondrosia reniformis*. *Marine Biology*, **139**, 421–
517 429.
- 518 Lessios H.A. (2008) The great American schism: divergence of marine organisms after the rise of the
519 Central American isthmus. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 63–91.
- 520 Lessios H.A., Kane J., & Robertson D.R. (2003) Phylogeography of the pantropical sea urchin *Tripneustes*:
521 contrasting patterns of population structure between oceans. *Evolution*, **57**, 2026–2036.
- 522 Librado P. & Rozas J. (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism
523 data. *Bioinformatics*, **25**, 1451–1452.
- 524 Liñero-Arana I. (2013) New records of Sabellariidae (Annelida: Polychaeta) from the Caribbean sea.
525 *Interciencia*, **38**, 382–386.
- 526 Main M.B. & Nelson W.G. (1988) Sedimentary characteristics of sabellariid worm reefs (*Phragmatopoma*
527 *lapidosa* Kinberg). *Estuarine, Coastal and Shelf Science*, **26**, 105–109.
- 528 Mauro N.A. (1975) The premetamorphic developmental rate of *Phragmatopoma lapidosa* Kinberg, 1867,
529 compared with that in temperate sabellariids (Polychaeta: Sabellariidae). *Bulletin of Marine Science*,
530 **25**, 387–392.
- 531 Mauro N.A. (1977) Variations in osmoregulatory capacity in two species of intertidal sabellariids (Annelida:
532 Polychaeta) from tropical and mediterranean habitats. *Comparative Biochemistry and Physiology Part*
533 *A: Physiology*, **56A**, 375–377.
- 534 McCarthy D. a., Young C.M., & Emson R.H. (2003) Influence of wave-induced disturbance on seasonal
535 spawning patterns in the sabellariid polychaete *Phragmatopoma lapidosa*. *Marine Ecology Progress*
536 *Series*, **256**, 123–133.
- 537 Mendonça F.F., Oliveira C., Gadig O.B.F., & Foresti F. (2013) Diversity and genetic population structure of
538 the Brazilian sharpnose shark *Rhizoprionodon lalandii*. *Aquatic Conservation: Marine and Freshwater*
539 *Ecosystems*, **23**, 850–857.
- 540 Moller G.S.F., Novo E.M.L. de M., & Kampel M. (2010) Space-time variability of the Amazon River
541 plume based on satellite ocean color. *Continental Shelf Research*, **30**, 342–352.
- 542 Nóbrega R., Solé-Cava A.M., & Russo C. a. M. (2004) High genetic homogeneity of an intertidal marine
543 invertebrate along 8000 km of the Atlantic coast of the Americas. *Journal of Experimental Marine*
544 *Biology and Ecology*, **303**, 173–181.
- 545 Noernberg M.A., Fournier J., Dubois S., & Populus J. (2010) Using airborne laser altimetry to estimate
546 *Sabellaria alveolata* (Polychaeta: Sabellariidae) reefs volume in tidal flat environments. *Estuarine,*
547 *Coastal and Shelf Science*, **90**, 93–102.
- 548 Nunes F., Norris R.D., & Knowlton N. (2009) Implications of isolation and low genetic diversity in
549 peripheral populations of an amphi-Atlantic coral. *Molecular Ecology*, **18**, 4283–97.
- 550 Nunes F.L.D., Norris R.D., & Knowlton N. (2011) Long distance dispersal and connectivity in amphi-
551 Atlantic corals at regional and basin scales. *PLOS ONE*, **6**, e22298.
- 552 Pagliosa P.R., Doria J.G., Misturini D., Otegui M.B.P., Oortman M.S., Weis W.A., Faroni-Perez L., Alves
553 A.P., Camargo M.G., Amaral A.C.Z., Marques A.C., & Lana P.C. (2014) NONATObase: a database
554 for Polychaeta (Annelida) from the Southwestern Atlantic Ocean. *Database*, **2014**, bau002.
- 555 Palumbi S.R. (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of*
556 *Ecology and Systematics*, **25**, 547–572.
- 557 Pawlik J.R. (1988) Larval settlement and metamorphosis of Sabellariid polychaetes, with special reference
558 to *Phragmatopoma lapidosa*, a reef-building species, and *Sabellaria floridensis*, a non-gregarious
559 species. *Bulletin of Marine Science*, **43**, 41–60.
- 560 Picciani N., Seiblit I.G.L., Paiva P.C., Castro C.B., & Zilberberg C. (2016) Geographic patterns of
561 *Symbiodinium* diversity associated with the coral *Mussismilia hispida* (Cnidaria, Scleractinia) correlate
562 with major reef regions in the Southwestern Atlantic Ocean. *Marine Biology*, **163**, 236.

- 563 Rocha L.A., Robertson D.R., Roman J., & Bowen B.W. (2005) Ecological speciation in tropical reef fishes.
 564 *Proceedings of the Royal Society B: Biological Sciences*, **272**, 573–579.
- 565 Santos M.V.Q.B., Aquino-Souza R., & Gomes-Filho J.G.F. (2012) Ocorrência, grau de ocupação do
 566 substrato e tamanhos das colônias de *Phragmatopoma caudata* na região entremarés da Praia da Pedra
 567 do Sal, Parnaíba-PI.
- 568 Santos S., Hrbek T., Farias I.P., Schneider H., & Sampaio I. (2006) Population genetic structuring of the
 569 king weakfish, *Macrodon ancylodon* (Sciaenidae), in Atlantic coastal waters of South America: deep
 570 genetic divergence without morphological change. *Molecular Ecology*, **15**, 4361–4373.
- 571 Souza J.N., Nunes F.L.D., Zilberberg C., Sanchez J.A., Migotto A.E., Hoeksema B.W., Serrano X.M., Baker
 572 A.C., & Lindner A. (2017) Contrasting patterns of connectivity among endemic and widespread fire
 573 coral species (*Millepora* spp.) in the tropical Southwestern Atlantic. *Coral Reefs*, in press.
- 574 Tamura K., Stecher G., Peterson D., Filipski A., & Kumar S. (2013) MEGA6: Molecular evolutionary
 575 genetics analysis version 6.0. *Molecular Biology and Evolution*, **30**, 2725–2729.
- 576 Terossi M. & Mantelatto F.L.A. (2012) Morphological and genetic variability in *Hippolyte obliquimanus*
 577 Dana, 1852 (Decapoda, Caridae, Hippolytidae) from Brazil and the Caribbean Sea. *Crustaceana*, **85**,
 578 685–712.
- 579 Wieman A.C., Berendzen P.B., Hampton K.R., Jang J., Hopkins M.J., Jurgenson J., McNamara J.C., &
 580 Thurman C.L. (2014) A panmictic fiddler crab from the coast of Brazil? Impact of divergent ocean
 581 currents and larval dispersal potential on genetic and morphological variation in *Uca maracoani*.
 582 *Marine Biology*, **161**, 173–185.
- 583 Zigler K.S. & Lessios H.A. (2004) Speciation on the coasts of the new world: phylogeography and the
 584 evolution of binding in the sea urchin genus *Lytechinus*. *Evolution*, **58**, 1225–1241.

587 **Supporting Information**

588 Additional Supporting Information may be found in the online version of this article:

589 **Appendix S1.** Sampling coordinates and location details.

590 **Appendix S2.** Genetic diversity indices for (a) *cox-1* and (b) *ITS-1*

591 **Appendix S3.** BAPS assignments for (a) *cox-1* and (b) *ITS-1*.

592

593

594 **Biosketches**

595 **Flavia Nunes** is an evolutionary biologist interested in population connectivity, speciation and adaptation in
 596 marine invertebrates

597 **Author contributions:**

598 AVW and JF conceived the project; JF and LFP collected the samples; AVW and FLDN did the molecular
 599 analyses, AVW, FLDN and JF analysed the data. FLDN, AVW, LFP and JF contributed to writing the
 600 manuscript.

601 **Editor:** Michelle Gaither

602

603 **Data accessibility**

604 DNA sequences produced during this study have been deposited in Genbank (see Methods and Materials for
605 details). Raw data can be requested by contacting the corresponding author (Flavia.nunes@ifremer.fr)

606

607

FIGURES

608

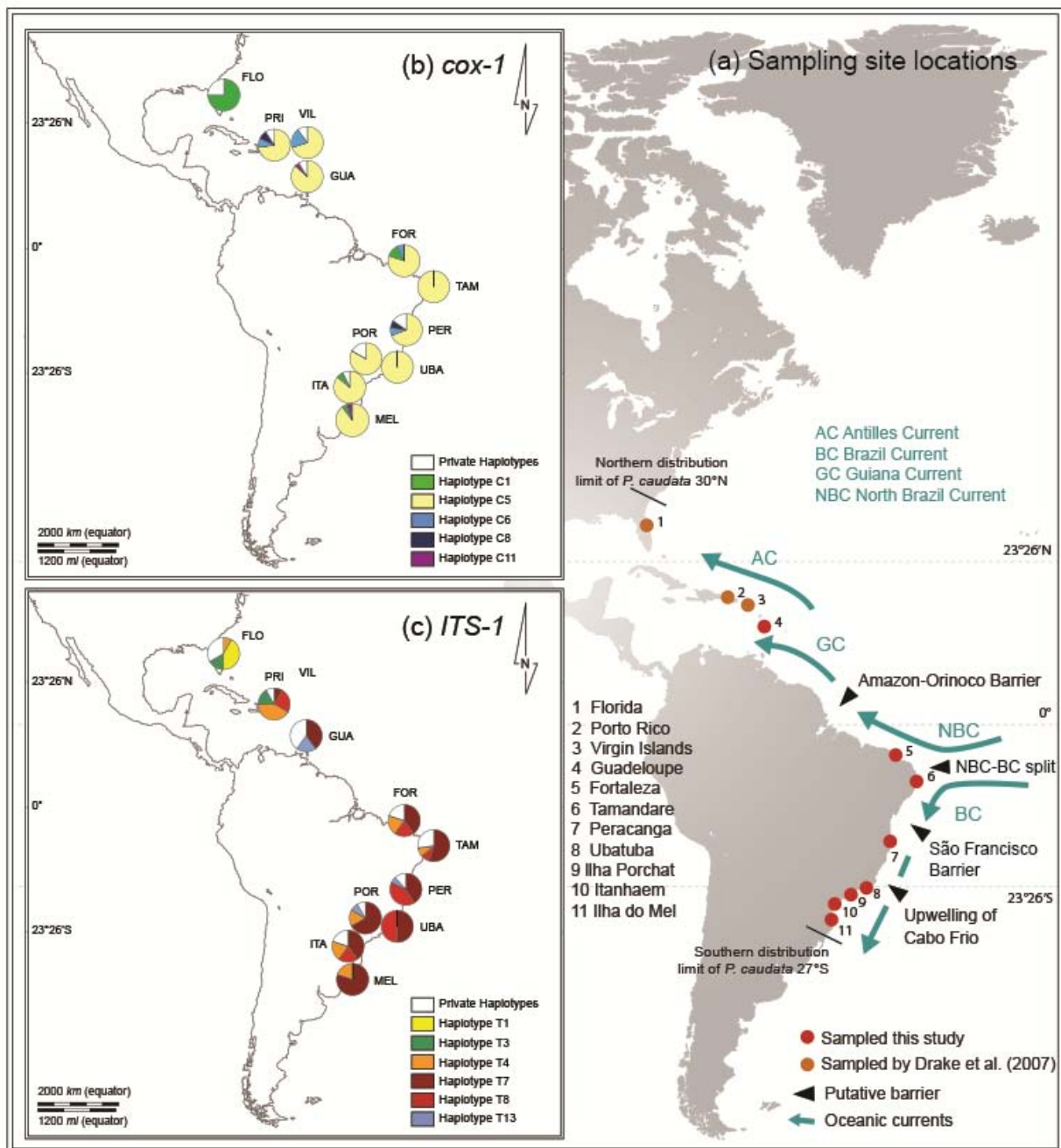
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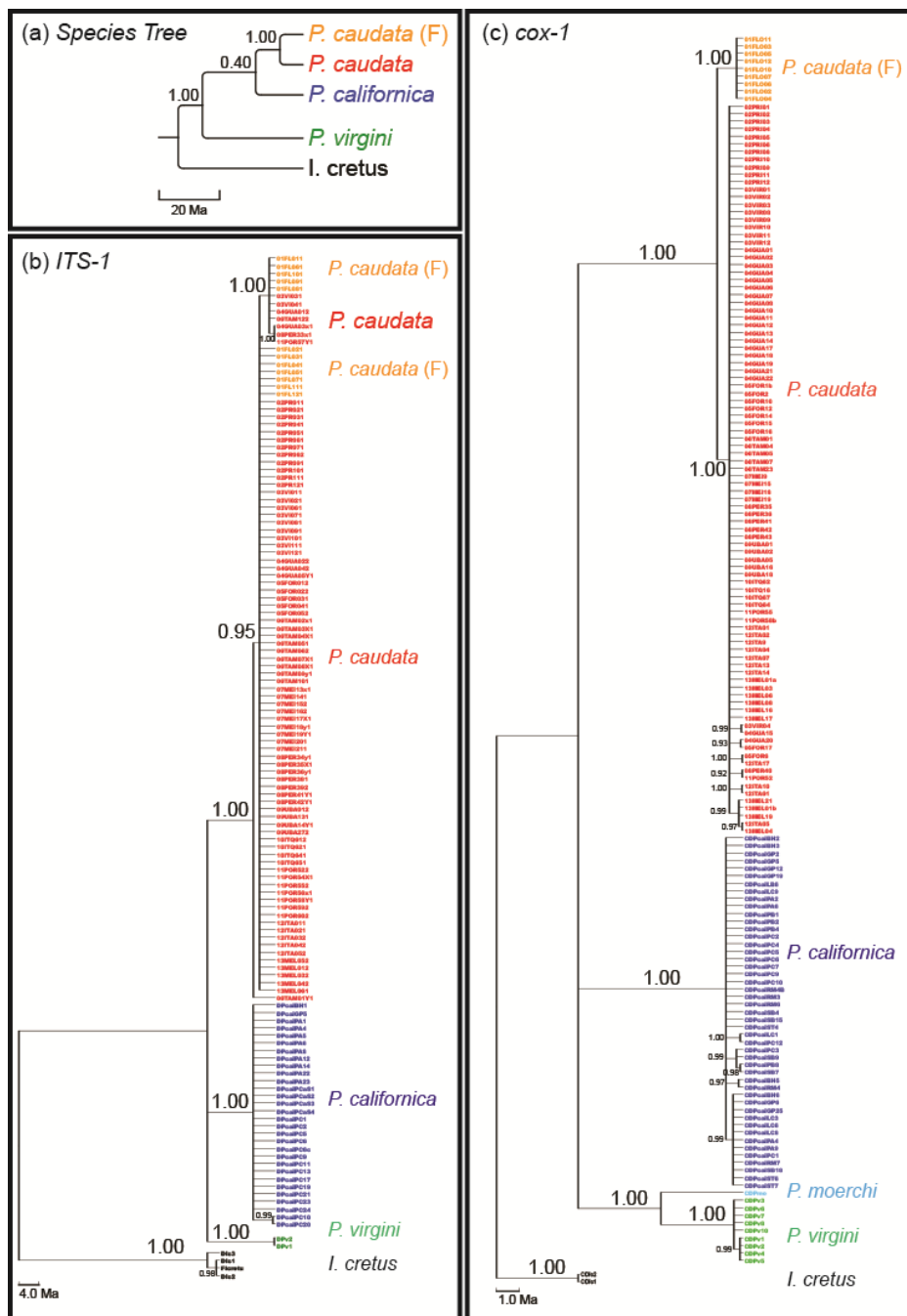
612

Figure 1. (a) Map of the sampling site locations of *P. caudata*, showing the direction of major ocean currents in January for the western Atlantic Ocean and Caribbean Sea. Haplotype frequencies are shown for each population for (b) of *cox-1* and (c) *ITS-1*. Population codes: Florida (FLO), Puerto Rico (PRI), Virgin Islands (VIL), Guadeloupe (GUA), Fortaleza (FOR), Tamandaré (TAM), Peracanga (PER), Ubatuba (UBA), Porchat (POR), Itanhaém (ITA) and Ilha do Mel (MEL).

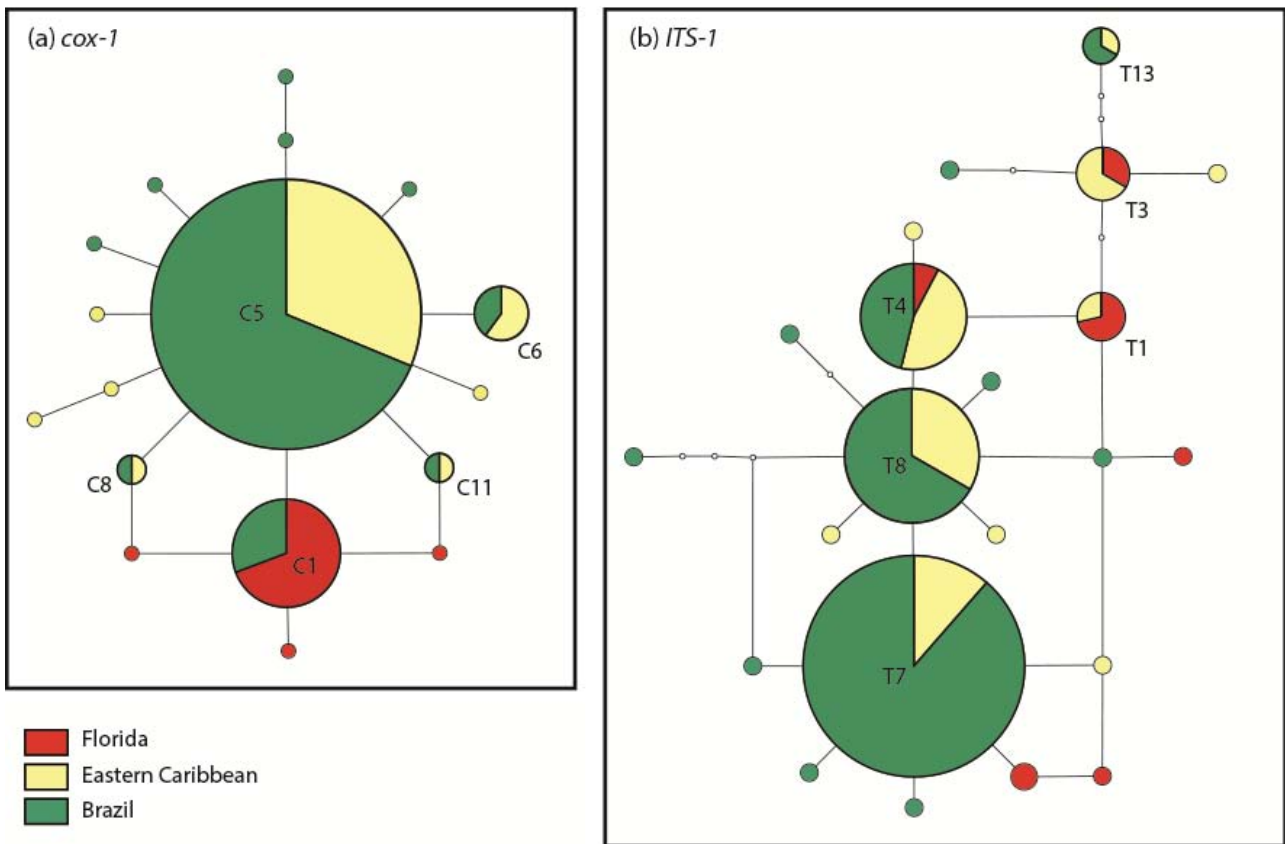


613

614 **Figure 2.** Phylogenetic reconstruction of relationships among species of *Phragmatopoma*. (a) Species tree
 615 based on combined data from *cox-1* and *ITS-1*; (b) based on sequences of *ITS-1* and (c) based on sequences
 616 of *cox-1*. Posterior probabilities are shown for nodes with support >0.90. Species are colour-coded as
 617 follows: red: *P. caudata*, orange: *P. caudata* from the Florida population, blue: *P. californica*; green: *P.*
 618 *virgini*; cyan: *P. moerchi*; black: *I. cretus*. (F) denotes individuals of *P. caudata* from the Florida population.

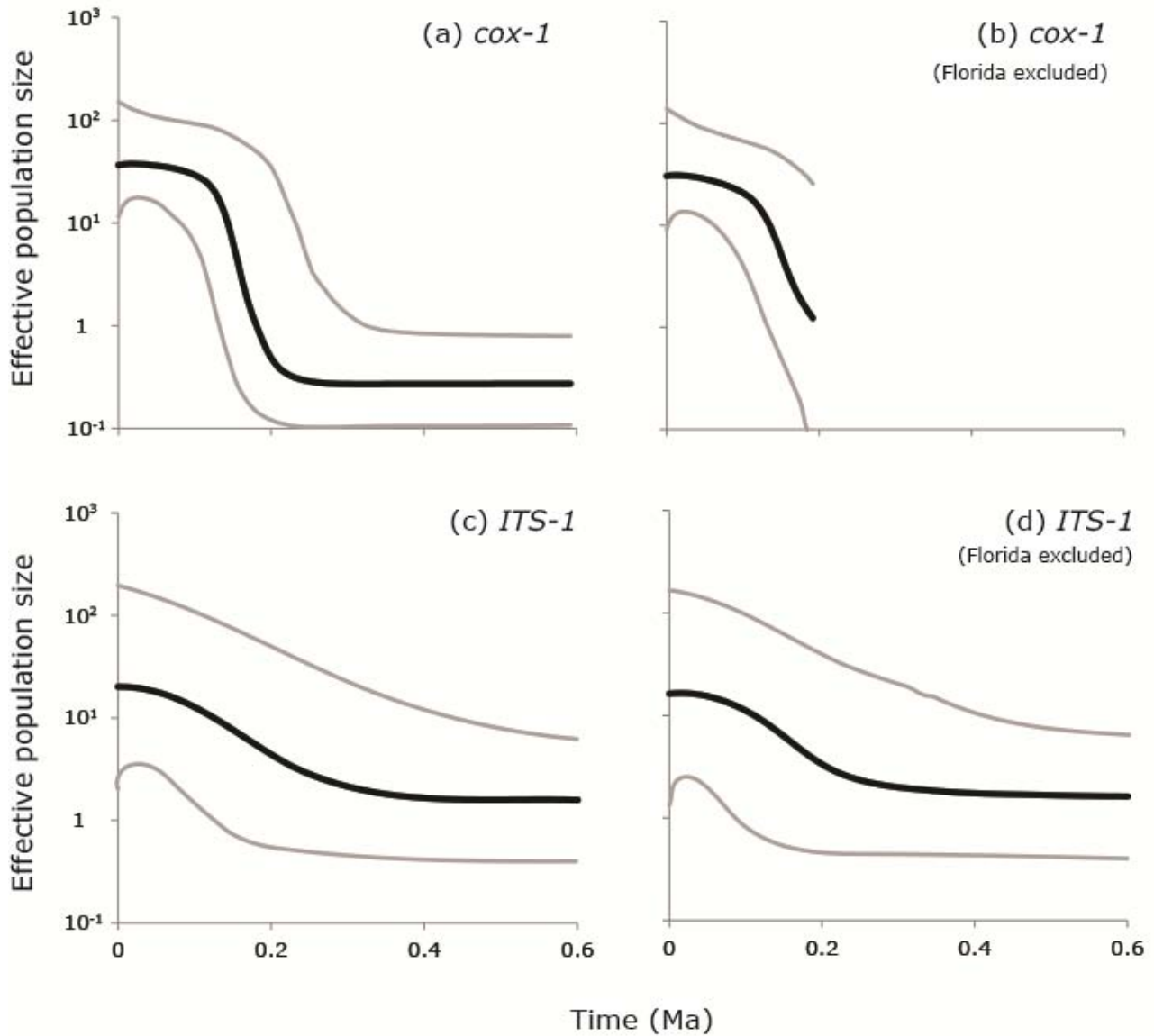


620 **Figure 3.** Haplotype networks based on sequences of *P. caudata* for (a) *cox-1* and (b) *ITS-1*. Each circle
 621 represents a haplotype and its size is proportional to the frequency of the haplotype across all populations.
 622 Empty circles represent mutational steps between sampled haplotypes. Haplotypes are colour-coded by
 623 geographic region: red = Florida (FLO); yellow = eastern Caribbean (PRI, VIL, GUA); green = Brazil (FOR,
 624 TAM, PER, UBA, POR, ITA, MEL). See Figure 1 for population code names.
 625



627 **Figure 4.** Bayesian skyline plots showing changes in effective population size of *P. caudata* over time based
628 on sequences from: (a) *cox-1*; (b) *cox-1* excluding individuals from Florida; (c) *ITS-1* and (d) *ITS-1*
629 excluding individuals from Florida.

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TABLES

634 **Table 1.** Estimates of evolutionary divergences between species pairs of *Phragmatopoma*, and outgroup, *I.*
 635 *cretus*. The number of base substitutions per site from averaging over all sequence pairs between species is
 636 shown below the diagonal, and standard error estimates are shown above the diagonal for (a) *cox-1* and (c)
 637 *ITS-1*. Divergence time estimates using locus-specific substitution rates are shown for each species pairs for
 638 (b) *cox-1* and (d) *ITS-1*. (F) denotes the Florida population of *P. caudata*.

(a) Genetic distances between species pairs based on *cox-1*

	1	2	3	4	5	6
1 <i>I. cretus</i>		0.019	0.022	0.022	0.024	0.024
2 <i>P. californica</i>	0.180		0.021	0.022	0.021	0.020
3 <i>P. moerchi</i>	0.192	0.186		0.016	0.021	0.020
4 <i>P. virgini</i>	0.203	0.202	0.121		0.021	0.021
5 <i>P. caudata</i> (F)	0.216	0.193	0.194	0.194		0.007
6 <i>P. caudata</i>	0.218	0.181	0.183	0.200	0.032	

(b) Divergence time estimates between species pairs based on a 2.1% substitution rate for *cox-1*

	1	2	3	4	5	6
1 <i>I. cretus</i>		0.9	1.0	1.1	1.1	1.1
2 <i>P. californica</i>	8.6		1.0	1.0	1.0	1.0
3 <i>P. moerchi</i>	9.2	8.8		0.8	1.0	1.0
4 <i>P. virgini</i>	9.7	9.6	5.8		1.0	1.0
5 <i>P. caudata</i> (F)	10.3	9.2	9.2	9.3		0.3
6 <i>P. caudata</i>	10.4	8.6	8.7	9.5	1.5	

(c) Genetic distances between species pairs based on *ITS-1*

	1	2	3	4	5
1 <i>I. cretus</i>		0.036	0.036	0.036	0.036
2 <i>P. californica</i>	0.353		0.013	0.014	0.013
3 <i>P. virgini</i>	0.339	0.075		0.015	0.015
4 <i>P. caudata</i> (F)	0.340	0.073	0.085		0.003
5 <i>P. caudata</i>	0.339	0.070	0.083	0.008	

(d) Divergence time estimates between species pairs based on a 0.25% substitution rate for *ITS-1*

	1	2	3	4	5
1 <i>I. cretus</i>		14.6	14.2	14.4	14.3
2 <i>P. californica</i>	141.2		5.3	5.4	5.4
3 <i>P. virgini</i>	135.5	29.9		6.0	5.9
4 <i>P. caudata</i> (F)	136.0	29.2	33.9		1.2
5 <i>P. caudata</i>	135.5	28.2	33.1	3.2	

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640 **Table 2.** Analysis of molecular variance (AMOVA) for (a) *cox-1* and (b) *ITS-1*; based on two groups of
 641 populations of *P. caudata*: Group 1: Florida (FLO); Group 2: Puerto Rico (PRI), Virgin Islands (VIL),
 642 Guadeloupe (GUA), Fortaleza (FOR), Tamandaré (TAM), Peracanga (PER), Ubatuba (UBA), Porchat
 643 (POR), Itanhaém (ITA) and Ilha do Mel (MEL). F_{CT} : variation among groups; F_{SC} : variation among
 644 populations within groups; F_{ST} : variation within populations. Significant values ($P < 0.05$) are highlighted in
 645 bold.

(a) Analysis of Molecular Variance for *cox-1*

Source of variation	df	Sum of Squares	Variance Components	% of Variation	
Among groups	1	10.556	0.47092	Va	72.07
Among populations within groups	9	1.649	0.00006	Vb	0.01
Within populations	135	24.623	0.18239	Vc	27.92
Total	145	36.829	0.65338		
Fixation Indices		p-value			
F_{SC} (Vb)	0.00035	0.37146			
F_{ST} (Vc)	0.72085	0.00000			
F_{CT} (Va)	0.72075	0.08798			

(b) Analysis of Molecular Variance for *ITS-1*

Source of variation	df	Sum of Squares	Variance Components	% of Variation	
Among groups	1	12.894	0.24476	Va	19.57
Among populations within groups	9	10.559	0.02209	Vb	1.77
Within populations	88	86.567	0.98372	Vc	78.66
Total	98	110.02	1.25057		
Fixation Indices		p-value			
F_{SC} (Vb)	0.02196	0.13881			
F_{ST} (Vc)	0.21338	0.00000			
F_{CT} (Va)	0.19572	0.00098			

Population Structure:

Population 1

FLO

Population 2

PRI, VIL, GUA, FOR, TAM, PER, UBA, POR, ITA, MEL

647 **Table 3.** Pairwise ϕ_{ST} among populations of *P. caudata*. Values in the upper triangle were calculated based on *ITS-1*, while values in the lower triangle were
 648 calculated based on *cox-1*. Statistically significant values ($P < 0.05$) are highlighted in bold. Underlined values indicate significance after Bonferroni correction
 649 ($P < 0.00091$).

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			FLO	PRI	VIL	GUA	FOR	TAM	PER	UBA	POR	ITA	MEL
			1	2	3	4	5	6	7	8	9	10	11
Florida	FLO	1		0.234	0.060	0.001	0.284	0.207	<u>0.339</u>	0.305	0.305	0.284	0.329
Puerto Rico	PRI	2	<u>0.615</u>		-0.005	0.229	0.204	0.132	<u>0.195</u>	0.260	0.249	0.204	0.337
Virgin Islands	VIR	3	<u>0.661</u>	-0.024		0.051	0.150	0.090	0.173	0.164	0.205	0.150	0.241
Guadeloupe	GUA	4	<u>0.746</u>	0.009	0.082		0.071	0.026	0.163	0.085	0.069	0.071	0.097
Fortaleza, BR	FOR	5	<u>0.632</u>	0.003	0.018	0.034		-0.091	-0.049	-0.166	-0.086	-0.167	-0.146
Tamandaré, BR	TAM	6	<u>0.753</u>	-0.046	0.028	-0.070	-0.022		-0.012	-0.123	-0.024	-0.110	-0.073
Peracanga, BR	PER	7	<u>0.606</u>	-0.039	-0.017	0.019	0.002	-0.055		-0.142	0.030	-0.049	0.013
Ubatuba, BR	UBA	8	<u>0.784</u>	-0.009	0.074	-0.043	0.012	0.000	-0.022		-0.065	-0.166	-0.053
Porchat, BR	POR	9	<u>0.706</u>	0.003	0.053	0.005	0.025	-0.051	-0.026	-0.016		-0.086	-0.125
Itanhaém, BR	ITA	10	<u>0.691</u>	0.009	0.062	0.001	-0.030	-0.058	0.003	-0.026	0.001		-0.146
Ilha do Mel, BR	MEL	11	<u>0.735</u>	0.025	0.086	-0.021	-0.012	-0.067	0.016	-0.038	0.008	-0.029	

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