Journal of Biogeography
July 2017, Volume 44, Issue 7, Pages 1612-1625
http://dx.doi.org/10.1111/jbi.12938
http://archimer.ifremer.fr/doc/00363/47376/

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 φst. 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.

Introduction

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

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

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

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

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

Materials and Methods

Study sites and sampling method

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

DNA extraction and sequencing

DNA was extracted using the CTAB method (Denis *et al.*, 2009). Two loci were used in order to make comparisons with previously studied Caribbean populations (Drake *et al.*, 2007): the mitochondrial cytochrome c oxydase subunit I (*cox-1*) and the first internal transcribed spacer region (*ITS-1*) of the ribosomal DNA. New primers were designed for *cox-1*: PHRALCO: 5' - TTTATATTTTGGAATTTGGTCAGG -3 '; PHRAHCO: 5' - TAAAGAACTGGGTCTCCACC-3'. Published primers were used for *ITS-1* (ITS1-fw: 5'-CACACCGCCCGTCGCTACTA-3', ITS3r: 5'-TTCGACSCACGAGCCRAGTGATC-3') (Denis *et al.*, 2009). Amplification was performed with the Ready-to-Go PCR kit (GE Healthcare, Little Chalfont, UK) using 0.1µg of DNA. Thermal cycler conditions 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 1 min, with a final extension at 72°C for 5 min. After electrophoresis, PCR products were extracted from the

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

Molecular data analysis

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

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

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

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

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

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

Results

Properties of the DNA sequences

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

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

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

Phylogenetic analysis

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

Pairwise genetic distances and divergence time estimates are shown in Table 1. Based on *cox-1*, *P. caudata* diverged from *P. californica* at 8.6±1.0 Ma and from *P. virgini* at 9.5±1.0 Ma. Divergence times estimated with *ITS-1* indicate an older split between *P. caudata* and *P. californica*, at 28.2±5.4 Ma, and of 33.1±5.9 Ma between *P. caudata* and *P. virgini*. *P. caudata* from Florida diverged from the remaining *P. 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

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

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

Population differentiation

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

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

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

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

Discussion

Phylogenetic analysis based on *cox-1* and *ITS-1* confirms the monophyly of three species of *Phragmatopoma* (*P. caudata, P. californica* and *P. virgini*). In addition, our analyses which included one published sequence of *P. moerchi*, also indicate that this may be a separate species, as suggested by Drake *et al.* (2007). In contrast to previous work, however, the results presented here do not show conclusive phylogenetic relationships among *P. caudata*, *P. californica* and *P. virgini*, as trichotomies were observed in 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 (Parnaiba, 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 15.5–29.5ºC, 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/m^{-2} (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 Fs and Tajima's D are indicative of recent population expansion (dating to *c.* 200 ka). Each

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

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

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

Conclusions

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

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

Acknowledgements

The authors would like to thank C. Bouchon (Université des Antilles et de la Guyane, Guadeloupe, France) for providing specimens, A.C. Panizza (CNPq and Federal University of Ceará, Fortaleza, Brazil) for assistance in the field in Brazil, C.A. Drake (Utah State University, USA) for providing sequence data information for Caribbean populations. This project was supported by the Muséum National d'Histoire Naturelle of Paris (BQR HYDROGENE 2006-2008 and ATM "Formes Possibles, Formes Réalisées" 2013-2014) to JF. FLDN was supported by the "Laboratoire d'Excellence" LabexMER (ANR-10-LABX-19) and co-funded by a grant from the French government under the program "Investissements d'Avenir", and by a grant from the Regional Council of Brittany. LFP was supported by the São Paulo Research Foundation (FAPESP 07/56340-3) and the National Council for Scientific and Technological Development, Brazil (CNPq – SWE 201233/2015-0).

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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.

Editor: Michelle Gaither

Data accessibility

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

FIGURES

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).

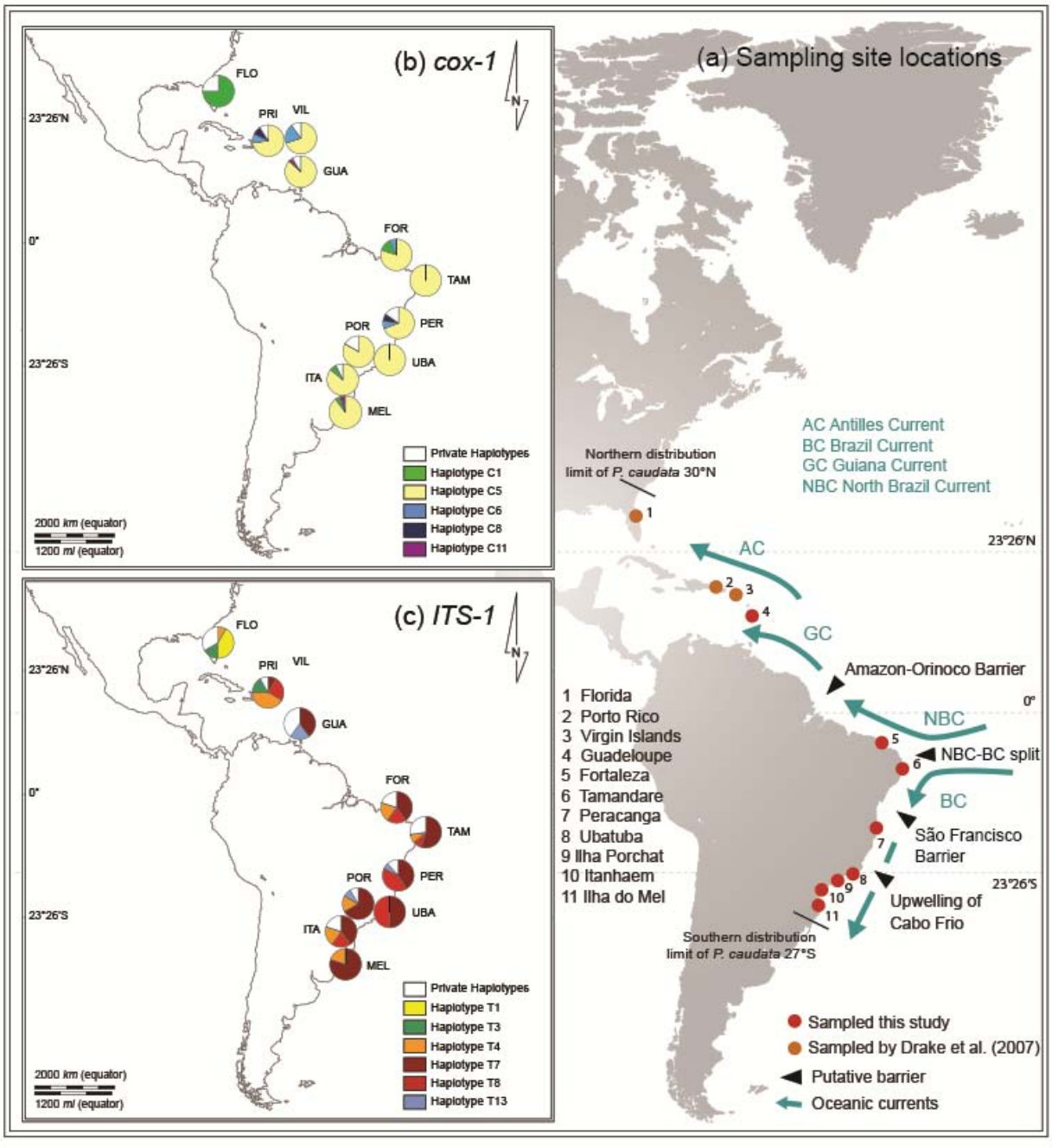
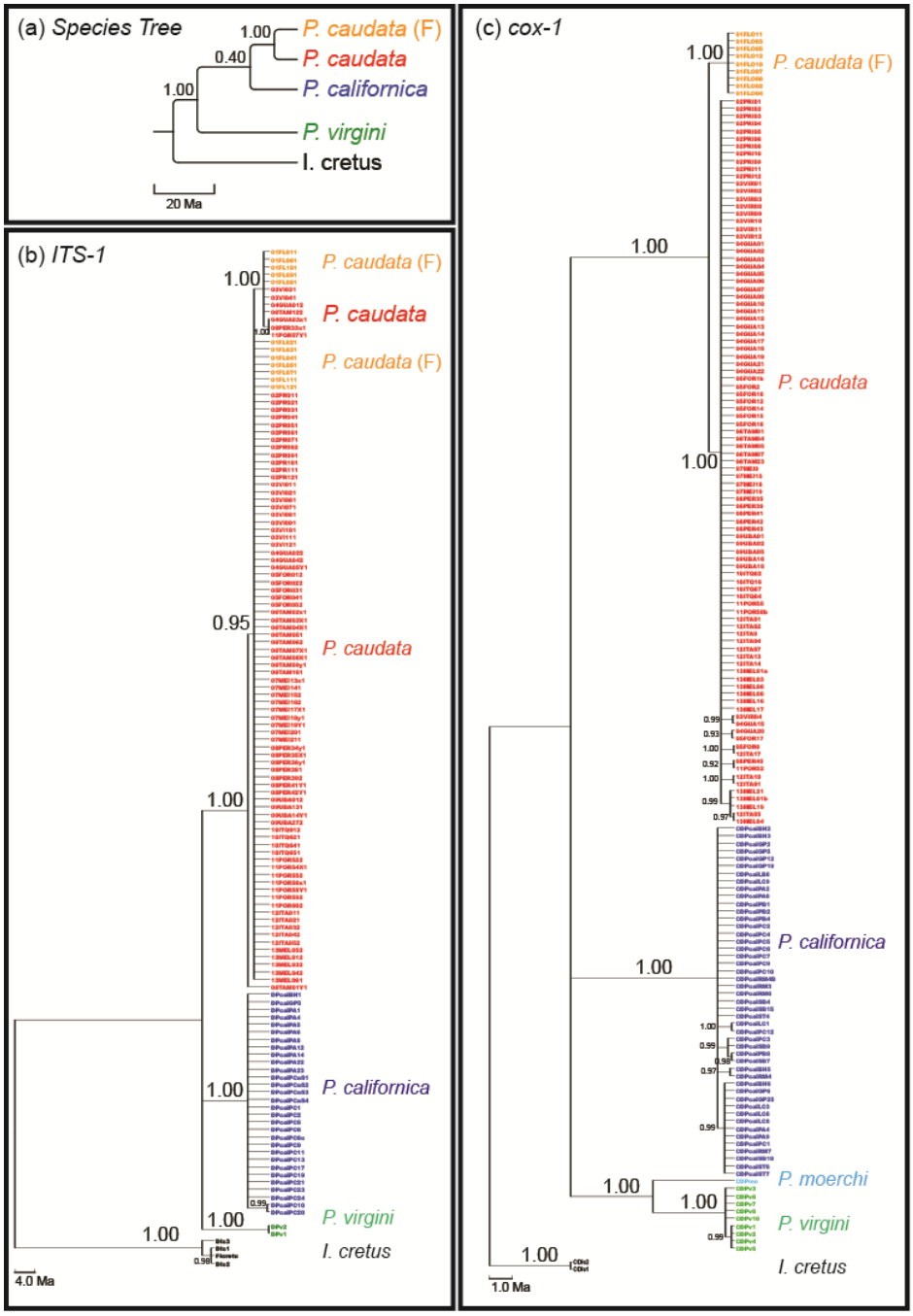
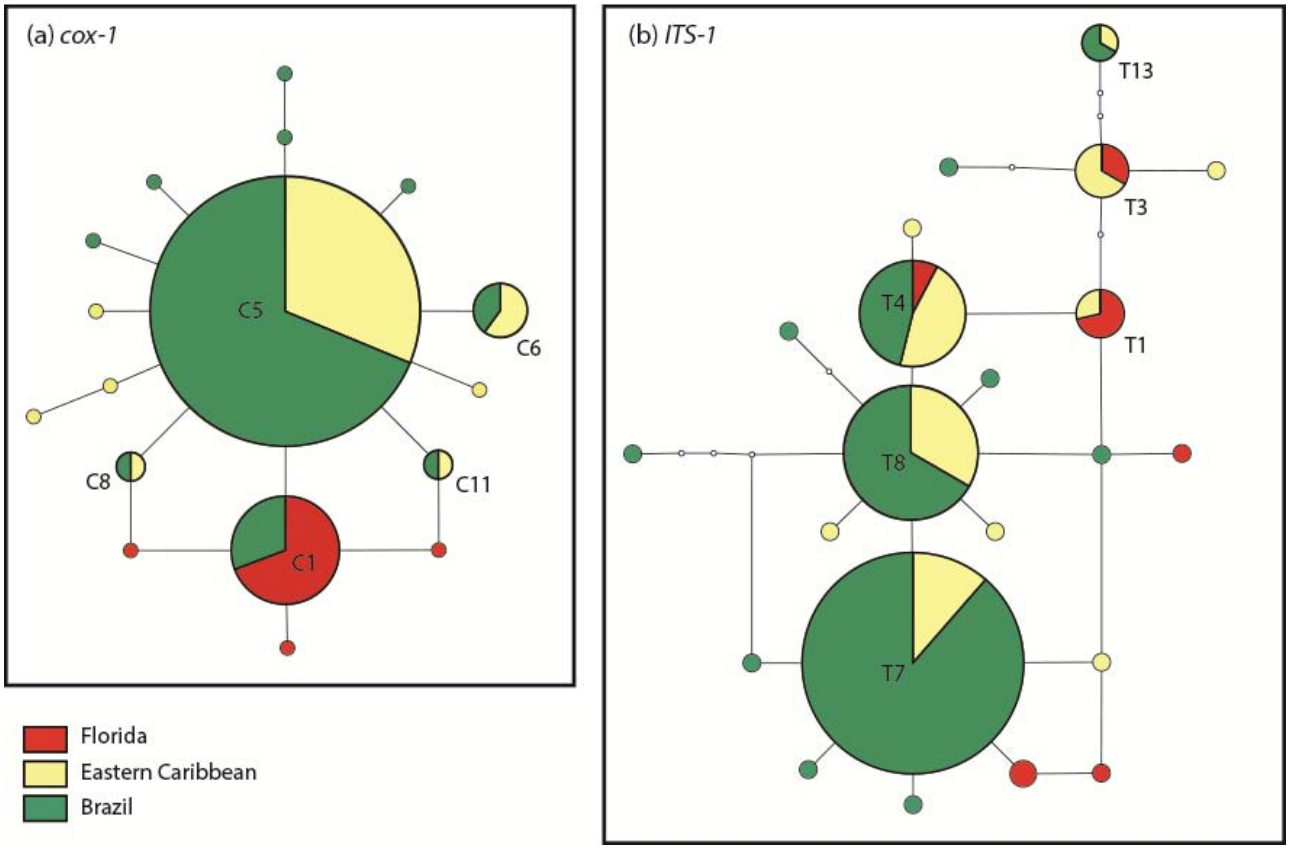


Figure 2. Phylogenetic reconstruction of relationships among species of *Phragmatopoma*. (a) Species tree based on combined data from *cox-1* and *ITS-1*; (b) based on sequences of *ITS-1* and (c) based on sequences of *cox-1*. Posterior probabilities are shown for nodes with support >0.90. Species are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



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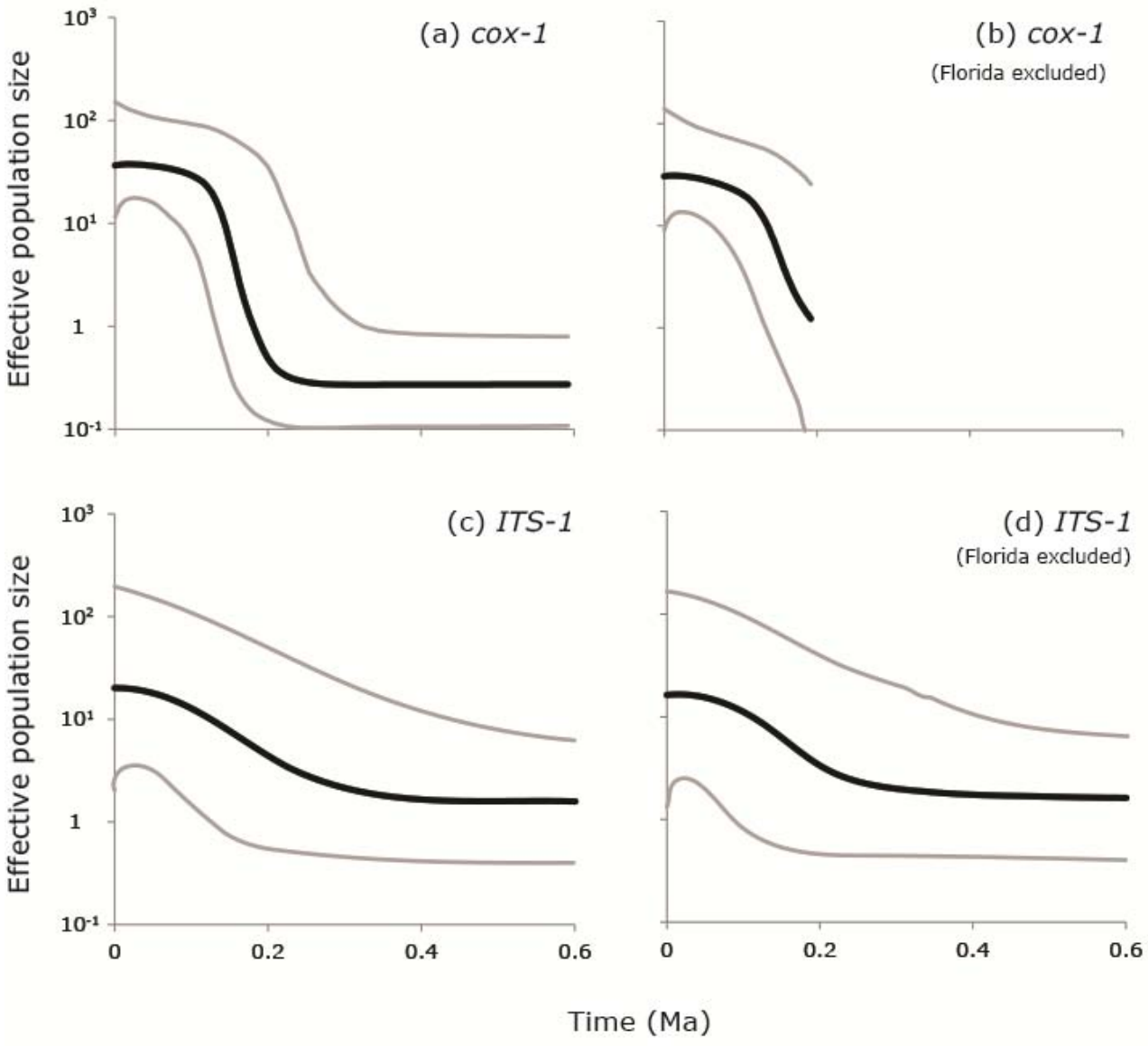
627 **Figure 4.** Bayesian skyline plots showing changes in effective population size of *P. caudata* over time based
628 on sequences from: (a) *cox-1*; (b) *cox-1* excluding individuals from Florida; (c) *ITS-1* and (d) *ITS-1*
629 excluding individuals from Florida.

630



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633 **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. cretus***		0.019	0.022	0.022	0.024	0.024
2	***P. californica***	0.180		0.021	0.022	0.021	0.020
3	***P. moerchi***	0.192	0.186		0.016	0.021	0.020
4	***P. virgini***	0.203	0.202	0.121		0.021	0.021
5	***P. caudata* (F)**	0.216	0.193	0.194	0.194		0.007
6	***P. caudata***	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. cretus***		0.9	1.0	1.1	1.1	1.1
2	***P. californica***	8.6		1.0	1.0	1.0	1.0
3	***P. moerchi***	9.2	8.8		0.8	1.0	1.0
4	***P. virgini***	9.7	9.6	5.8		1.0	1.0
5	***P. caudata* (F)**	10.3	9.2	9.2	9.3		0.3
6	***P. caudata***	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. cretus***		0.036	0.036	0.036	0.036
2	***P. californica***	0.353		0.013	0.014	0.013
3	***P. virgini***	0.339	0.075		0.015	0.015
4	***P. caudata* (F)**	0.340	0.073	0.085		0.003
5	***P. caudata***	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. cretus***		14.6	14.2	14.4	14.3
2	***P. californica***	141.2		5.3	5.4	5.4
3	***P. virgini***	135.5	29.9		6.0	5.9
4	***P. caudata* (F)**	136.0	29.2	33.9		1.2
5	***P. caudata***	135.5	28.2	33.1	3.2	

639

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

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

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