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ORIGINAL ARTICLE

Phylogeography of *Agathistoma* (Turbinidae, Tegulinae) snails in tropical and southwestern Atlantic

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Abstract

The rocky intertidal gastropods *Agathistoma viridulum* and *A. hotessierianum* occur from the Caribbean to southern Brazil, with a gap in the equatorial region, giving them an anti-tropical distribution. We used sequences from mitochondrial genes to elucidate the phylogeography of *A. viridulum* and *A. hotessierianum* and to infer their relationships to other species of *Agathistoma*. For *A. hotessierianum*, haplotype networks and phylogenetic analyses split samples into two distinct groups: one (*A. hotessierianum*) in the Caribbean region (Greater and Lesser Antilles; Venezuela: Sucre and Isla Margarita) and a new species that we describe from northeastern Brazil. For *A. viridulum*, genetic analyses split the samples into three groups (Caribbean, northeastern Brazil and southeastern Brazil), but genetic divergence among these was too low for them to be considered species, and morphological differences were not significant. The mtDNA tree identified two clades of eastern Pacific *Agathistoma*, but many lower-level relationships within *Agathistoma* were not well resolved, suggesting that more complete taxon sampling and additional genetic data will be needed to establish more robust relationships among Tegulinae.

KEYWORDS
allopatry, gastropods, marine biogeography, systematics

1 | INTRODUCTION

Gastropods of the genus *Tegula* Lesson, 1832 inhabit intertidal and shallow subtidal waters of the Americas and East Asia, living among rocks and grazing on algae. They have a lecithotrophic larval stage that is planktonic for 4–14 days before settlement (Guzmán del Próo et al., 2011; Moran, 1997). *Tegula* as a whole has about 40–45 species (DeVries, 2007; Hellberg, 1998; Keen, 1971; McLean, 1969; Rios, 2009), roughly half of which have been ascribed to the genus *Agathistoma* (Olsson & Harbison, 1953).

Agathistoma was originally described as a subgenus based on shared shell features. Primary characteristics include a deep, open umbilicus and a strong tooth at the end of the columella bordered below by a small notch, which forms the end of a spiral cord (Olsson & Harbison, 1953). The type species of *Agathistoma* is *Trochus viridulus* Gmelin, 1791 (Olsson & Harbison, 1953 by original designation),

with no type specimen designed. DeVries (2007) later revised the Late Cenozoic tegulines from southern Peru and elevated the *Agathistoma* and *Chlorostoma* Swainson, 1840 to genus level. Two phylogenetic studies aiming to resolve teguline relationships (Table 1) have been conducted using molecular (Hellberg, 1998) and morphological data (Dornellas et al., 2021). The relationships of *Tegula s.l.* inferred from two mitochondrial gene regions (cytochrome c oxidase I [COI] and 12S) recovered *Agathistoma* as the sister group to *Tegula pellisserpentis* (Wood, 1828), the type species of *Tegula*, with a strong support (Hellberg, 1998). Cladistic analysis of the Tegulinae based on external and internal morphological characters (Dornellas et al., 2021) included Tegulinae within the subfamily Turbinidae and also recovered *Agathistoma* supported by four synapomorphies, albeit with low bootstrap support.

Hellberg (1998) concluded that sister species of *Tegula* often occur along the same coast or even sympatrically, calling to question the need for strong and lasting geographical barriers to form new species. Sampling for that study, however, was not as comprehensive for the largely tropical *Agathistoma* as it was for more temperate species, potentially missing instances where major barriers may have been important.

Two species of *Agathistoma*, *A. viridulum* and *A. hotessierianum* (d'Orbigny, 1842), have especially broad latitudinal ranges when compared with other western Atlantic species of the genus. *Agathistoma viridulum* occurs from Costa Rica and Venezuela south to Brazil (Ceara to Santa Catarina states), while *A. hotessierianum* occurs from the Florida Keys, United States, throughout the Caribbean (Bahamas, Cuba, Dominican Republic, Virgin Islands and Saint Lucia), south to Venezuela and Brazil (Maranhao to Bahia states). Species with such wide latitudinal distributions sometimes have disjunct distributions across the tropics, creating an anti-tropical

TABLE 1 Taxonomic information and species of *Agathistoma* analysed in literature and herein

Taxon *Agathistoma*	Hellberg (1998)	Dornellas et al. (2021)	This study	Locality
T. bergeroni (McLean, 1970)	Not analysed	Not analysed	Not analysed	Panama to Colombia—Pacific Ocean
T. cooksoni (Smith, 1877)	Not analysed	Not analysed	Not analysed	Galapagos Is.
T. corteziana McLean, 1970	✓	✓	✓	Mexico, Gulf of California
T. corvus (Philippi, 1850)	Not analysed	Not analysed	Not analysed	Peru
T. eiseni Jordan, 1936	✓	✓	✓	EUA, Los Angeles to Mexico, Baja California
T. excavata (Lamarck, 1822)	✓	✓	✓	Caribbean
T. fasciata (Born, 1778)	✓	✓	✓	Caribbean
T. felipens McLean, 1970	✓	Not analysed	✓	Mexico, Baja California
T. globulus (Carpenter, 1857)	Not analysed	Not analysed	✓	Mexico—Pacific Ocean
T. gruneri (Philippi, 1849)	Not analysed	Not analysed	Not analysed	Puerto Rico
T. hotessieriana (d'Orbigny, 1842)	Not analysed	✓	✓	Caribbean to Brazil
T. ligulata (Menke, 1850)	Not analysed	Not analysed	✓	Mexico, Mazatlan
T. lividomacula (Adams, 1845)	Not analysed	✓	✓	Caribbean
T. mariana (Dall, 1919)	✓	✓	✓	Mexico, Gulf of California
T. malaleucos (Jonas, 1844)	Not analysed	Not analysed	Not analysed	Ecuador
T. panamenis (Philippi, 1849)	Not analysed	Not analysed	✓	El Salvador to Peru
T. patagonica (d'Orbigny, 1835)	Not analysed	✓	✓	South Brazil to Argentina, Patagonia
T. picta McLean, 1970	Not analysed	Not analysed	Not analysed	Ecuador
T. quadricostata (Wood, 1828)	✓	Not analysed	✓	Peru
T. snodgrassi (Pilsbry & Vanatta, 1902)	Not analysed	✓	Not analysed	Galapagos Is.
T. tridentata (Poetiz & Michaud, 1838)	✓	Not analysed	✓	Peru
T. verdispira (McLean, 1970)	Not analysed	Not analysed	Not analysed	Mexico—Pacific Ocean
T. verrucosa (McLean, 1970)	✓	✓	✓	El Salvador to Peru
T. viridula (Gmelin, 1791)	Not analysed	✓	✓	Costa Rica to south Brazil

distribution that often separates closely related taxa (Burridge, 2002; Hubbs, 1952; Ludt et al., 2015; Randall, 1981). Such anti-tropical species pairs include several western Atlantic species distributed to the north-west and south-west of the Amazon and Orinoco river deltas, among them coral reef fishes, crustaceans and mussels (Trovant et al., 2016). In addition to their anti-tropical distributions, *A. viridulum* and *A. hotessierianum* are good models to study the biogeographic patterns of the Western Atlantic region. In addition, populations of *A. hotessierianum* and *A. viridulum* separated by the tropical gap also show morphological differences in their shells and radulae (Dornellas et al., 2021).

Population genetic studies of marine mollusks in the Brazilian Biogeographical Province have increased over the last decade, providing insights into species delimitation, cryptic species and polymorphic species (Arruda et al., 2009; Barroso et al., 2020; Claremont et al., 2011; Lazoski et al., 2011; Padula et al., 2016; Sales et al., 2013; Trovant et al., 2016). However, no studies have yet addressed connectivity patterns within the Brazilian Province.

Here, we use partial sequences from three mitochondrial genes along with morphological characters to assess the differentiation of *A. viridulum* and *A. hotessierianum* populations spanning the Amazon/Orinico delta and to place these species in the context of others in the subgenus *Agathistoma*. Based on our findings, we describe one new species for northeastern Brazil, postulating that *A. hotessierianum* is actually separated by the combined plumes of the Orinoco and Amazon rivers. Subdivision was less deep in *A. viridulum*, although the major phylogeographic break in this nominal species occurred in the same region.

2 | MATERIAL AND METHODS

2.1 | Sample collection

Specimens of *A. viridulum* were collected from eight localities along the coast of Brazil. Specimens of *A. hotessierianum* were collected from three localities in northeastern Brazilian coast (Table S2). All samples were preserved in 96%–100% EtOH. We also included five individuals of *A. hotessierianum* and two individuals of *A. viridulum* from Venezuela deposited in the Museu de Zoologia da Universidade de São Paulo, SP (MZSP) and from MEH's collection at Louisiana State University. We also included 24 sequences from GenBank of 10 species of *Agathistoma* and 13 other tegulines (Table S1), from Aktipis and Giribet (2010); Collado et al. (2012); Hellberg (1998); Hellberg and Vacquier (1999); Williams (2012); Lee et al. (2016) and Uribe et al. (2016).

We tried but failed to sequence teguline samples from other museums' collections (National Museum of Natural History, Smithsonian Institution; Natural History Museum of Los Angeles). The only specimen sequenced from MZSP collection used herein belongs to *A. verrucosum* MZSP 63,839. The detailed list of material examined is in (Table S2).

2.2 | Morphological data

Morphological data were extracted from Dornellas et al. (2021), except for samples of *A. viridulum* from the Caribbean (Table S2). The features of the head–foot, esophagus and odontophore muscle were examined, but radulae could not be inspected. The shells of *A. viridulum* and *A. hotessierianum* were examined and photographed at Instituo Oceanográfico da Universidade de São Paulo (IOUSP) and MZSP. Photographs and measurements were obtained using a pachymeter and a Zeiss Stereo Discovery V8 stereomicroscope with the program ZEN and also with a Nikon D500 camera with 60 mm macro lens. The following abbreviations are used throughout the text for shell measurements: H, shell height; W, shell width. The taxonomical information extracted from the shells comprises the colour, number of body whorls, sculpture pattern, number and shape of beaded spiral cords, umbilicus, columellar teeth and shell profile.

2.3 | DNA extraction, PCR amplification and sequencing

DNA was obtained from 5–20 individuals from each population by breaking the shell for the larger specimens or pulling the snail out, while for the smaller ones up to 30–50 mg of foot tissue was cut off. The preserved shells were retained as vouchers and deposited in the Museu Nacional, Universidade Federal do Rio de Janeiro, RJ (MNRJ) and MZSP. Genomic DNA was evaluated using the Qiagen DNeasy Blood & Tissue Kit, and the quality of the DNA obtained was evaluated by micro-volume spectrophotometry.

Portions of two mitochondrial genes were amplified: a 655-bp region of the COI using the primers HCO1490 and LCO2198 (Folmer et al., 1994) and 600 bp of the 16S ribosomal gene using the primers 16SA-L and 16SB-H (Palumbi et al., 1991). Sequences of the 12S ribosomal gene were obtained from the GenBank. PCR amplifications were performed using GoTaq Green Master Mix (Promega Corporation) in 25 μl volumes that contained 12.5 μl of GreenTaq, 1 μl of each primer (25 μM stock) and about 100 ng of DNA template. The PCR cycles for

COI and 16S amplification consisted of an initial denaturation step at 95°C for 3 min followed by 40 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 45 s and extension at 72°C for 2 min, and a final extension at 72°C for 10 min. The PCR products were examined using gel electrophoresis on 1.3% Tris-Borate-EDTA-agarose gel stained with SYBR safe DNA Gel Stain (Invitrogen). The PCR products showed in gel electrophoresis were purified with IllustraExoProStar—1 step (GE Healthcare Life Sciences), following its standard protocol, and sent for Sanger sequencing (Macrogen Inc.).

2.4 | Alignment and phylogenetic inference

Sequences were aligned using MAFFT 1.3.6 (Katoh & Standley, 2013) as implemented in Geneious under the E-INS-I algorithm. Default parameters were used for gap opening and extension. COI sequences were checked to verify if all sequences remained in an open reading frame.

PartitionFinder 2 (Lanfear et al., 2016) was used to choose the combined sets of partitioning schemes and models of molecular evolution. The data matrix was divided into five partitions (COI by codon positions and each rRNA gene separately) and performed a search using the greedy option. Models of molecular evolution were selected among (a) those implemented in RAxML using the Akaike Information Criterion with correction (Supplementary Material, Table S3) and (b) those implemented in MrBayes 3.1.2 using the Bayesian Information Criterion with correction (Table S4). PartionFinder was not allowed to select models with correction for proportion of invariant sites (P-Invar), as suggested in RAxML's manual, to avoid correlation between values of alpha and P-Invar (Stamatakis, 2014).

The maximum likelihood (ML) analysis was performed using RAxML 8.2.3 (Stamatakis, 2014). The ML tree was estimated using the RAxML algorithm that conducts a rapid bootstrap analysis and searches for best scoring ML tree in the same run (option-*f a*). We ran 1,000 bootstrap replicates, and the best scoring ML tree was estimated 200 times using as starting tree each fifth bootstrap tree.

Bayesian inference (BI) analysis was performed using MrBayes 3.2.7 (Ronquist et al., 2012). We used four incrementally heated Markov chains (standard initial temperature with heating parameter set to 0.05) and the chains for 500 million generations, sampling every 1,000th generation. Maximum clade credibility tree and values of Bayesian posterior probability (PP) were estimated from sampled trees after discarding as burn-in the trees sampled before posterior trace convergence. Tracer 1.6.1 (Rambaut et al., 2018) was used to check for trace convergence and values of effective sample size and TreeAnnotator v 1.5.4 (Rambaut & Drummond, 2010) to perform the burn-in and summarize the tree distribution and the estimated parameters.

2.5 | Populational structure and phylogeography history

Haplotype networks were built for each gene and for a concatenated dataset under an infinite site model (i.e., uncorrected or Hamming distance) using R package *pegas* (Paradis, 2010). The model-based Bayesian analysis of population structure (BAPS) v6.0 (Corander et al., 2008) was performed to investigate population structure. Five runs were performed for each $K = 1–10$ and another 100 replicates run with K fixed at the inferred number of genetic clusters. The best fitting K was assessed using highest log marginal likelihood value. An admixture model was then run for the inferred number of clusters.

Analysis of molecular variance (AMOVA: Excoffier et al., 1992) was assessed using Arlequin v 3.5. Two alternative scenarios of biogeographic subdivision were compared for *A. viridulum* and *A. hotessierianum*: (a) Caribbean Province (VNZ) versus Brazilian Province (BRA) and (only for *A. viridulum*) the Brazilian Province was split into: (b) North–Northeastern area versus Southern area. Population pairwise F_{ST} comparisons were calculated using Arlequin v 3.5, with statistical significance determined using 10,000 replicates.

3 | RESULTS

3.1 | Phylogenetic relationships of Agathistoma

Our concatenated alignment totaled 1,581 base pairs (638 bp for COI, 514 bp for 16S and 429 bp for 12S). PartitionFinder selected five partitions with the GTR + G model for the ML analysis. For the BI analysis, PartitionFinder selected five partitions, and the best-fit model for each selected partition is presented in Table S3.

Both BI (Figure 1) and ML analyses of the concatenated (COI + 16S + 12S) mtDNA dataset recovered *Agathistoma* (PP = 0.82), excluding the clade of three temperate South America species: *Tegula patagonica* (d'Orbigny, 1835), *T. quadricostata* (Wood, 1828) and *T. tridentata* (Poetiz & Michaud, 1838; analyses of individual genes in Figures S1-S3). In BI and ML trees, topologies were consistent with one another in the clade with high support nested *Agathistoma* and *Tegula pellisserpentis* + *T. excavata* (PP = 0.92).

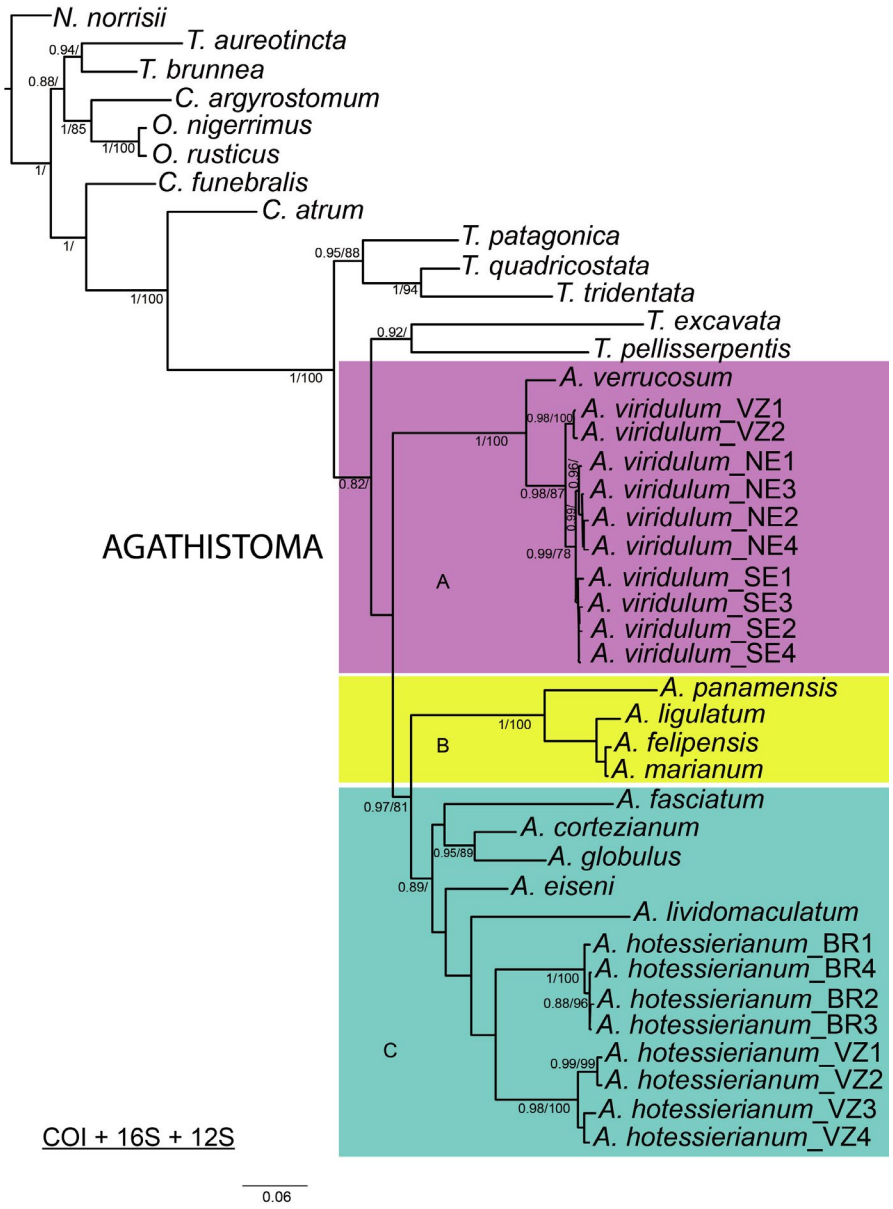

FIGURE 1 Bayesian inference tree generated by MrBayes of the concatenated data including cytochrome c oxidase I (COI), 16S and 12S sequences. The numbers on branch, on the left, represent posterior probability > 0.80, and the numbers on the branch, on the right, represent bootstrap values > 75%

Within *Agathistoma*, Clade A, containing the trans-Isthmian sister species *A. viridulum* and *A. verrucosum*, was well resolved, as was a clade including all remaining *Agathistoma* (Clade B + C). Clade B includes four species from the eastern Pacific and is well supported (PP = 1; BV = 100%), while Clade C includes a well-supported clade of two other species that are adjacently distributed in Pacific Mexico, *A. cortezianum* and *A. globulus*. Most other lower-level relationships are not well resolved.

Both *A. viridulum* and *A. hotessierianum* clades were split into two clades with high support: one including the Caribbean region samples and other Brazilian samples. Among the Brazilian samples of *A. viridulum*, the BI analysis recovered separate clades for the northeastern (NE1-4) and southeastern (SE-S1–4) populations.

3.2 | Genetic differentiation and population structure

Haplotype networks and the BAPS analysis were based on only COI for *A. viridulum* and on 16S for *A. hotessierianum* due to inconsistent amplification of other gene regions and the need for complete datasets. For *A. viridulum* (Table 2), the alignment for COI mitochondrial gene totaled 484 bp, with 38 variable and 25 parsimony informative sites. The 16S dataset totaled 440 bp: five of these were variable and two were parsimony informative sites. The concatenated COI and 16S alignment totaled 924 bp, with 31 variable and 13 parsimony informative sites. For *A. hotessierianum* (Table 3), our alignment for network analysis totaled 466 bp for COI, with seven variable and three parsimony informative sites and 490 bp for

TABLE 2 Summary statistics and neutrality tests computed in the DNAsp software for the *Agathistoma viridulum*

Genes	S	H	bp	*N*	% Nd (SC)	% Hd (*SD*)	Tajima's D	Fu and Li's D	Fu and Li's F	Fu's Fs
COI	38	29	638	83	0.00946	0.897	−1.268	−1.55782	−1.78296	−11.868
16S	5	6	534	76	0.00047	0.176	−1.778	−2.01654	−2.27995	−6.179

Note: Statistically significant values are presented in bold.

Abbreviations: bp, number of base pairs; COI, cytochrome c oxidase I; H, number of unique haplotypes; Hd, haplotype diversity; *N*, number of sequences; Nd, nucleotide diversity; S, number of variable sites.

TABLE 3 Summary statistics and neutrality tests computed in the DNAsp software for the *Agathistoma hotessierianum*

Genes	S	H	bp	*N*	% Nd (SC)	% Hd (*SD*)	Tajima's D	Fu and Li's D	Fu and Li's F	Fu's Fs
COI	7	5	693	15	0.00446	0.676	−0.128	−0.97212	−0.85358	−0.128
16S	39	6	582	20	0.0629	0.747	0.531	**1.41068**	1.33768	8.032

Note: Statistically significant values are presented in bold.

Abbreviations: bp, number of base pairs; COI, cytochrome c oxidase I; H, number of unique haplotypes; Hd, haplotype diversity; *N*, number of sequences; Nd, nucleotide diversity; S, number of variable sites.

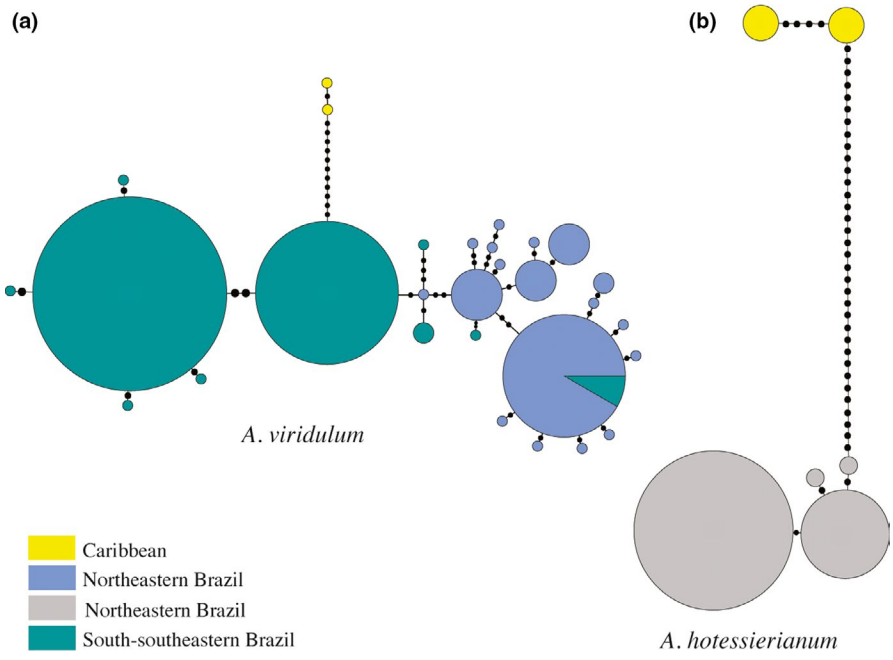

FIGURE 2 Networks of (a) cytochrome c oxidase I of *Agathistoma viridulum* and (b) 16S haplotypes from *A. hotessierianum*. Each circle represents a different haplotype, with their size proportional to relative frequency. Populations are represented by colours. The small black circles are nucleotide substitutions inferred in that branch

16S, with 39 variable and 38 parsimony informative sites. For concatenated COI + 16S, we found 956 bp, with eight variable and four parsimony informative sites.

Neither marker showed consistent departure from neutrality for multiple tests (Tables 2 and 3 and Tables S5-S7), except for Fu's test of *A. hotessierianum* for 16S. The ML analysis of the COI tree of *A. viridulum* resulted in a polytomy of Brazilian populations and a clade of Caribbean population nested within a Brazilian polytomy. The analysis of the 16S tree of *A. hotessierianum* resulted in two well-supported clades, one Brazilian and other Caribbean (Figures S4 and S5).

The two Venezuelan haplotypes in the *A. viridulum* COI haplotype network (Figure 2b) were separated by 11 mutational steps from the 27 Brazilian haplotypes. The Brazilian populations were further subdivided between the northeastern region (NE) and the southeastern region (SE-S). The BAPS analysis of COI (Figure 3) indicated an optimal partition of $k = 4$, matching the clades recovered in the phylogenetic analysis (Figure 1). Mapping the distributions of the four BAPS clusters shows a Caribbean cluster, two clusters occurring in NE Brazil (CE-RN and AL-BA) and a SE-S Brazil cluster. *A. hotessierianum* 16S haplotypes (Figure 2a) separated into two distinct clusters:

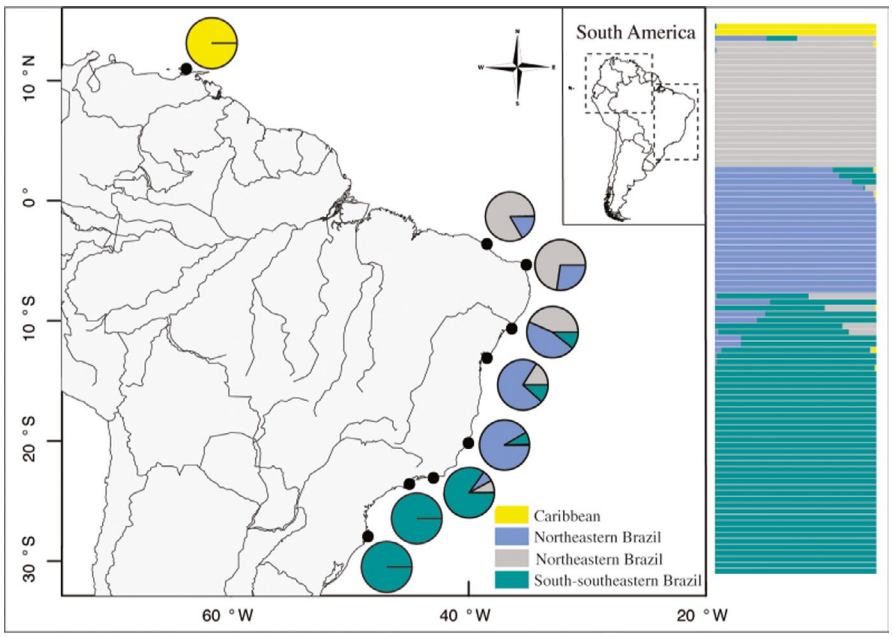

FIGURE 3 Genetic clusters of *Agathistoma viridulum* inferred by Bayesian analysis of population structure from mitochondrial cytochrome c oxidase I sequences. Map with the locations of the samples used in the dataset. Colours represent each cluster

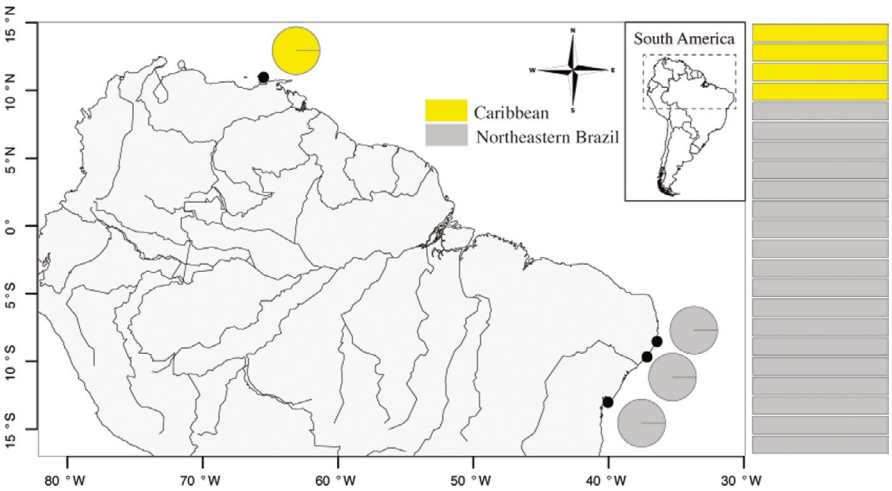

FIGURE 4 Bayesian analysis of population structure chart generated with cytochrome c oxidase I and 16S mitochondrial genes dataset of *Agathistoma hotessierianum*, showing the genetic clusters. Map with the locations of the samples used in the dataset

samples from the Caribbean were at least 34 mutational steps from any Brazilian population. The BAPS analysis of 16S mitochondrial gene for *A. hotessierianum* (Figure 4) showed an optimal partition of $k = 2$, again matching the clades from the phylogenetic trees (Figure 1).

For the AMOVA, populations of *A. viridulum* and *A. hotessierianum* were grouped in two different ways: by clusters from the BAPS analysis (Tables 4 and 5) and by sampling locality.

For *A. viridulum*, the four populations clustered by BAPS analysis of COI were grouped in three different ways (Table 4): (a) one group including the four populations; (b) two groups defined by biogeographical provinces (Caribbean and Brazilian); and (c) three groups defined by areas (Caribbean and Brazilian areas: northeastern and southeastern). Alternatively, nine populations sampled by their own localities (Brazilian states) were grouped by biogeographical provinces, by areas and by localities (Tables S8–S10). The percentage of variation was stronger for populations analysed in two groups (Caribbean and Brazilian provinces).

For *A. hotessierianum*, the analyses were based on the 16S gene. The populations clustered by BAPS ($k = 2$) were grouped only in one group, the Western Atlantic. The three populations clustered by localities were grouped according to the biogeographical provinces (one for Caribbean and two for Brazilian) (Table 5; Tables S11 and S12). The AMOVA with percentage of variation attributed to among groups component was found with high value (97.18%), and the percentage attributed to among populations was higher for those clustered in the BAPS analysis than for those clustered by their localities.

TABLE 4 Analysis of molecular variance of populations of *Agathistoma viridulum* clustered in BAPS analysis ($K = 4$) for the COI mitochondrial gene showing the scenarios of biogeographical provinces and its areas

Composition	Groups/populations	Source of variation	*df*	Sum of squares	Variance components	Percentage of variation	Φ	*p*
Western Atlantic	One group/four populations	Among populations	4	127.514	2.36124 Va	75.62	—	—
		Within populations	78	60.124	0.76106 Vb	24.37	FST: 0.75625	.00
Provinces								
Caribbean/Brazilian	Two groups/four populations	Among groups	1	22.102	4.04460 Va	58.88	FCT: 0.58880	.25
		Among populations within groups	2	105.413	2.06355 Vb	30.04	FSC: 0.73056	.00
		Within populations	79	60.1241	0.76106 Vc	11.08	FST: 0.88921	.00
Areas								
VNZ; BRA/*N*-NE; BRA/SE-S	Three groups/four populations	Among groups	2	104.504	1.52190 Va	44.79	FCT: 0.44785	.17
		Among populations within groups	1	23.010	1.11524 Vb	32.82	FSC: 0.59438	.00
		Within populations	79	60.124	0.76106 Vc	22.40	FST: 0.77604	.000

Abbreviations: BAPS, Bayesian analysis of population structure; BRA, Brazil; COI, cytochrome c oxidase I; *N*-NE, north and north-eastern; SE-S, south-eastern and south; VNZ, Venezuela.

TABLE 5 Analysis of molecular variance for the 16S mitochondrial gene of *Agathistoma hotessierianum*, showing the scenarios of the sample's localities

Composition	Groups/populations	Source of variation	*df*	Sum of squares	Variance components	Percentage of variation	Φ	*p*
Western Atlantic	One group/two populations	Among populations	2	123.650	19.22700 Va	96.98	FST: 0.96987	—
		Within populations	17	10.750	0.59722 Vb	3.01	—	—
Caribbean/Brazilian	Two groups/three populations	Among groups	1	123.65	19.27087 Va	97.18	FCT: 0.97177	.33
		Among populations within groups	1	0.205	−0.06048 Vb	−0.30	FSC: −0.10802	.71
		Within populations	17	10.545	0.62032 Vc	3.13	FST: 0.96872	.0002

3.3 | Taxonomy

Based on our population genetic analysis of *A. viridulum* and *A. hotessierianum* and on morphological data from their shells, radulae and soft parts, we present a new taxonomic arrangement for the latter as follows: (a) *A. hotessierianum* (Figure 5) is distributed in the Caribbean region (Greater and Lesser Antilles; Venezuela: Sucre and Isla Margarita) and (b) *Agathistoma* sp. n. from northeastern Brazil is recognized herein as a new species (Figure 6). *A. viridulum* (Figures 7 and 8), while phylogenetically split across the Amazon/Orinoco delta, remains distributed from the Caribbean region (Costa Rica to Venezuela) to Brazil (Ceara to Santa Catarina).

Below, we present the synonymic list, diagnosis, description, geographical distribution and discussion for the new species of *Agathistoma*.

Agathistoma nordestinum sp.n. Dornellas et al., 2021

(urn:lsid:zoobank.org:pub:C40300D4-7339-4F82-B75B-2586B84E07DD)

(urn:lsid:zoobank.org:act:73AEB38F-4F37-4F76-981D-5BB784ADA63F).

(Description of taxa in Appendix S1).

4 | DISCUSSION

Our results indicate that Caribbean populations of *Agathistoma hotessierianum* are genetically differentiated from Brazilian populations. Our phylogenetic analysis recovered *A. hotessierianum* + *A. nordestinum* sp.n. (referred herein as *A. hotessierianum*_VZ1–4: Figure 1) as sister taxa, with high support for each species clade. These species had previously been regarded as a single species with a disjunct, anti-tropical distribution, with populations to either side of the Orinoco and Amazon

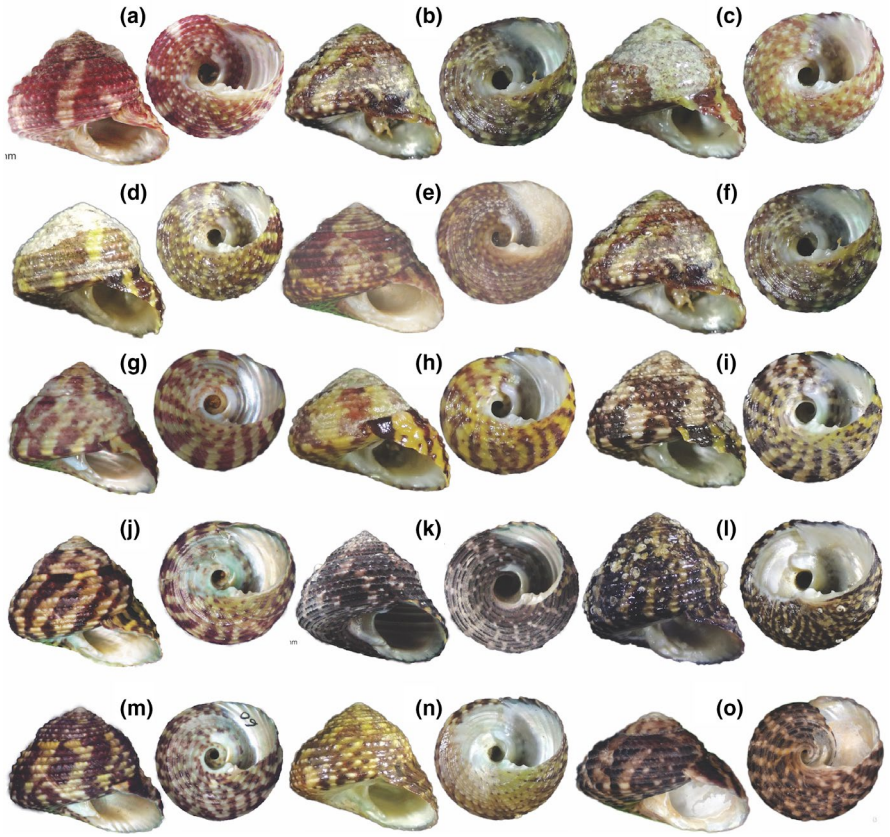

FIGURE 5 Frontal and umbilical views of *Agathistoma viridulum* from Brazil. (a) Northeastern, MZSP 63,583, h: 15.3 mm, w: 16 mm. (b) Ceara, Fortaleza, MNRJ 23,651, h: 16 mm, w: 19 mm. (c) Rio Grande do Norte, Maxaranguape, MNRJ 23,652 hr: 13.9 mm, w: 16 mm. (d) Rio Grande do Norte, Maxaranguape, MNRJ 23,652, h: 15.4 mm, w: 17.3 mm. (e) Bahia, Costa do Sauipe, MZSP 147,380, h: 15.5 mm, w: 16.8 mm. (f) Bahia, Salvador, MNRJ 23,654, h: 15.9 mm, w: 18.5 mm. (g) Espirito Santo, Manquinhos, MZSP 24,197, h: 15.4 mm, w: 17 mm. (h) Espirito Santo, Guarapari, MNRJ 23,655, h: 12.9 mm, w: 16.9 mm. (i) Espirito Santo, Guarapari, h: 15 mm, w: 17 mm. (j) Rio de Janeiro, Ilha Grande, MZSP 180,241, h: 17.5 mm; w: 18.9 mm. (k) Rio de Janeiro, Jorge Grego Island, MZSP 91,748, h: 18.7 mm; 19 mm. (l) Rio de Janeiro, Niteroi, MNRJ 23,656, h: 21.5 mm, w: 21 mm. (m) Sao Paulo, Santos, MZSP 131,760, h: 18.8 mm, w: 19.5 mm. (n) Sao Paulo, Ubatuba, MNRJ23657, h: 19 mm, w: 21.8 mm. (o) Parana, Matinhos, MZSP 152,658, h: 12.3 mm, w: 15.8 mm

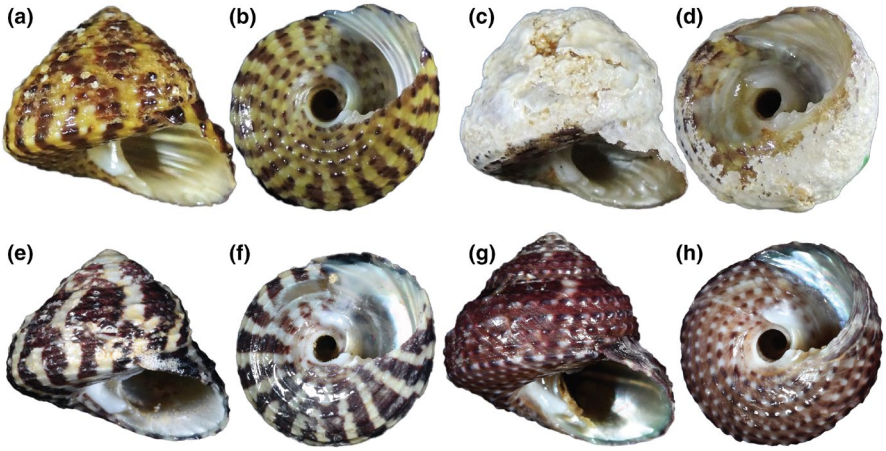

FIGURE 6 Frontal and umbilical views of *Agathistoma viridulum* from Caribbean. (a–d) East Panama, Bocas del Toro, Cayo del Tigre, MZSP 69,168. (a,b) h: 16.9 mm, w: 18.3 mm. (c,d) h: 18.5 mm, w: 20 mm. (e–h) Venezuela, Isla Margarita. (e,f) La Mula Beach, MZSP 61,498, h: 17 mm, w: 19.5 mm. (g,h) Caracola Beach, MZSP 57,402, h: 16 mm, w: 18.2 mm

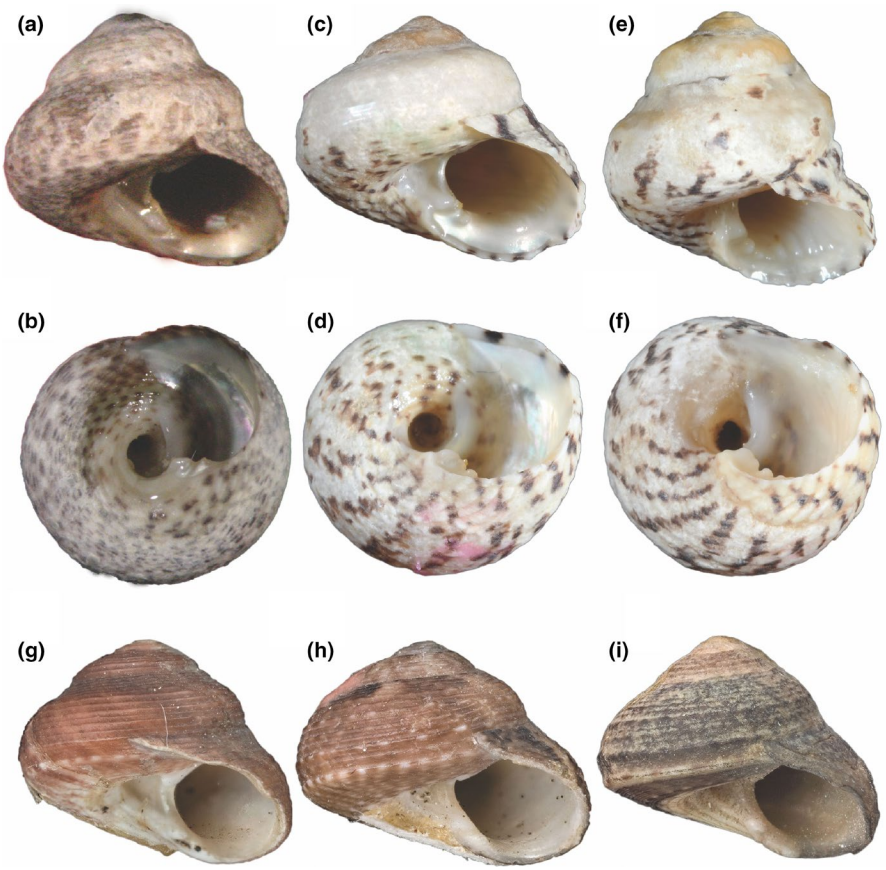

FIGURE 7 *Agathistoma hotessierianum* in frontal and umbilical views, Venezuela, Isla Marguerita, MZSP 59,088. (a,b) h: 9 mm, w: 10 mm. (c,d) h: 12 mm, w: 11 mm. (e,f) h: 9 mm, w: 9 mm. (g–i) Syntypes. Cuba, St. Lucie, New Orleans. NHMUK 185.10.4.261. D'Orbigny collection. (g) h: 10.9 mm, w: 13.2 mm. (h) h: 10.5 mm, w: 13.5 mm. (i) h: 9.6 mm, w: 12.4 mm

Rivers, mouths. The shells and radulae of this pair, *A. nordestinum* and *A. hotessierianum*, also differed between the Caribbean and Brazil (Dornellas et al., 2021). Previous morphological analysis had also placed *A. hotessierianum* and *A. nordestinum* sp.n. (which correspond to *A. hotessierianum* 2 and *A. hotessierianum* 1, respectively, in Dornellas et al., 2021: figures 18–20) in different clades.

Dornellas et al. (2020) also included *A. viridulum* from northeastern and southeastern Brazil but not specimens from the Caribbean region. Here, upon adding Caribbean samples, we found no significant morphological differences between an *A. viridulum* population from the Caribbean and those from Brazil (for anatomical data, see Dornellas et al., 2021), with the exception of slight shell variation (Figures 7 and 8, discussed previously). While the Caribbean population we analysed was genetically distinct from those from Brazil (Figures 2a and 3), neither AMOVA nor summary statistics were significant (Tables 2 and 4), and sequence divergence between the clades was

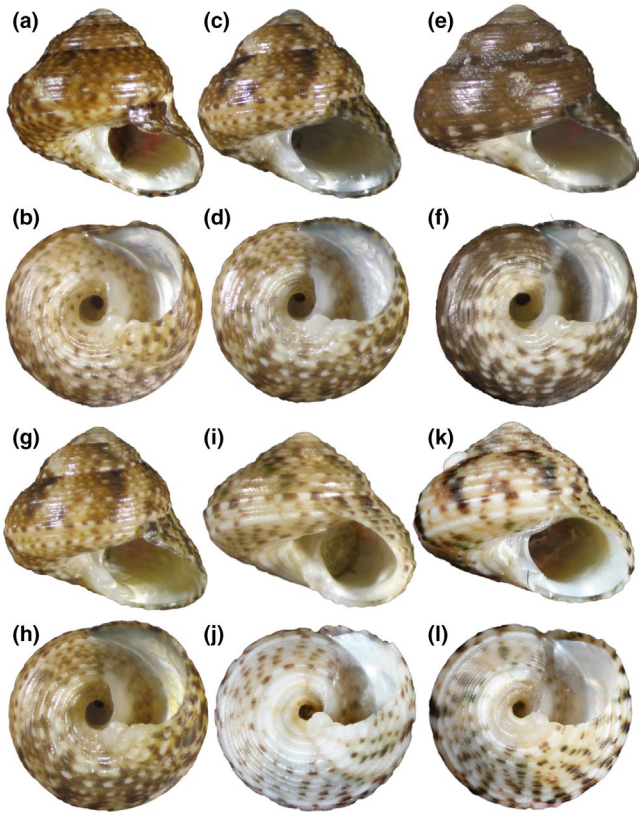

FIGURE 8 *Agathistoma nordestinum* sp.n. (a,b) Holotype in apertural and umbilical views, MZSP 153,719, h: 8.8 mm, w: 9.1 mm. (c–h) Paratypes from type locality, apertural and umbilical views, MZSP 153,720. (c,d) h: 8.5 mm, w: 10.3 mm. (e,f) h: 8.4 mm, w: 10 mm. (g,h) h: 8.4 mm, w: 9.2 mm. (i–l) Paratypes in apertural and umbilical views, MZSP 153,721, juveniles, Brazil, Alagoas, Maceio, Pajuçara beach. (i,j) h: 5 mm, w: 6 mm. (k,l) h: 5 mm, w: 6 mm

modest (Figure 1). For now, we still consider the populations of *A. viridulum* from Caribbean and Brazil as the same species.

Several factors influence the effectiveness of the Orinoco/Amazon as a barrier or filter: changing sea levels, sedimentation, current speeds, salinity and the availability of marine habitats beneath the freshwater river plume (Figueiredo et al., 2009; Floeter et al., 2008; Rocha, 2003). Because different species experience these factors differently, the Orinoco/Amazon is a selective barrier, affecting different species to varying degrees (Liedke et al., 2020; Trovant et al., 2016). The Orinoco/Amazon barrier seems to drive speciation for several coastal taxa, including reef fishes, lobsters and mussels (Floeter et al., 2008; Rocha, 2003; Tourinho et al., 2012; Trovant et al., 2016). On the other hand, many reef fishes show high genetic connectivity between populations to either side of the Orinoco/Amazon (Floeter et al., 2008; Liedke et al., 2020; Rocha et al., 2005), as do some invertebrates, including the marine gastropods *Stramonita* and *Neritina* (Barroso et al., 2020; Claremont et al., 2011). The existence of truly marine ecosystems located off the Amazon's mouth at depths below the influence of the river plume may act as a corridor for many species adapted to subtidal conditions (Cordeiro et al., 2015; Moura et al., 1999, 2016).

Our results suggest that the species pair *Agathistoma hotessierianum* + *A. nordestinum* sp.n. arose via vicariance. Salinity and habitat are likely factors responsible for the disjunct distribution shown by these marine rocky shore species because large tropical rivers typically exclude carbonate reef builders from continental shelves (Cordeiro et al., 2015; Moura et al., 1999, 2016) and these herbivorous *Agathistoma* species require exposed intertidal rocks.

Dispersal mediated by pelagic larvae also plays a role in the divergence and isolation of marine populations (Álvarez-Noriega et al., 2020). The lecithotrophic larvae of tegulines spend 4–14 days in the plankton (Guzmán del Próo et al., 2011; Kulikova & Omel'yanenko, 2000; Moran, 1997; Yamazaki et al., 2019). The modest dispersal capabilities endowed by such development should enhance the effectiveness of Amazon/Orinoco barrier/filter. However, even invertebrates with long pelagic larval duration sometimes diversify within the Atlantic basin. Despite a long pelagic larval duration of 2–3 months, a complex of *Stramonita haemastoma* species has speciated within the Atlantic both in response to barriers operating at the largest geographical scale, the width of Atlantic (although not the Amazon barrier) and at a smaller scale within the western Atlantic (see also *Strombus*, Claremont et al., 2011; Latiolais et al., 2006).

Agathistoma viridulum has a broad distribution, from the southern Caribbean to Santa Catarina state Brazil, over 9,000 km. Morphologically, the shells of the Caribbean population differ from those in Brazil by having two well-pronounced and more strongly beaded cords on the shell periphery. The axial blotches are larger, almost covering the spire background colour, and coloured dark brown, while in *A. viridulum* they range from red-brownish or red- to purple-brownish or purple (Figure 8). Regarding the populations in Brazil, specimens from the north-east are small, and their shells have purple or red axial blotches (Figure 7a–h). This pattern is observed south to Rio de Janeiro state (southeastern Brazil). From Espirito Santo state to Santa Catarina, specimens have higher and heavier shells with purple to greenish axial blotches (Figure 7i–o).

Habitat preference varied among colour morphs of *T. xanthostigma* in Japan, suggesting that habitat as well as the colour pattern may be useful for identification and classification of these species (Yamazaki et al., 2019). Although the habitat varies along the Brazilian coast, with

sandstone reefs and beach rocks in the north-east and more rocky shores in the south-east, colour morphs nonetheless overlap from the north-east to Rio de Janeiro.

The genetic differentiation of the north-east population of *A. viridulum* (Caribbean, Figure 3) may be explained by the Amazon and Orinoco river mouths. Genetic subdivision along the Brazilian coast (Figures 2a and 3; Figure S6) may be explained by major oceanic currents, as the North Brazil Current separates the northeastern Brazilian population from Ceara and Rio Grande do Norte states. The North Brazil Current has not been suggested as driving the genetic structure of marine organisms in previous biogeographic studies (Floeter et al., 2008; Spalding et al., 2007); however, the same pattern that we see has also been reported for other shallow-water "prosobranch" gastropods (Barroso et al., 2016). The next shift between *A. viridulum* clusters, separating the populations from the states of Alagoas and Bahia from southern populations, is potentially influenced by the mouth of the Rio Doce (Espirito Santo) and the Last Glacial Maximum (~21,500 Mya) that exposed the continental shelf (Leite et al., 2016). The southernmost population defined by BAPS occurs from Guarapari (ES) to Santa Catarina (Figure 3). This southern limit may be influenced by cold water temperatures, limiting expansion into the Argentina Province.

Our phylogenetic analysis indicates that the faunas of Caribbean and Brazilian coasts comprise well-supported sister clades. The first *Agathistoma* record appears in the Early-Middle Miocene, Navidad Formation, central Chile, about 24–20 Mya. (DeVries, 2007; Gutiérrez et al., 2013; Nielsen et al., 2004; Williams et al., 2008). Based on this record, *Agathistoma* species may already have been distributed along the western Atlantic coast when the Amazon river began to form about 11 Mya, in the Middle-Late Miocene at the end of the uplift of the Andes (Hoorn et al., 1995, 1996), although the genetic divergence between the Caribbean and Brazilian clades seems too limited to date back so far. Another Miocene occurrence, *Tegula patagonica*, is reported from Argentina (del Río, 1998). These records of *Agathistoma* fossils may indicate a Miocene austral origin for the Tegulinae, rather than the Pliocene western Atlantic origin suggested by Hickman and McLean (1990) (DeVries, 2007; Nielsen et al., 2004).

At higher levels of our phylogenetic tree (Figure 1), the concatenated (16S + COI + 12) data recovered the *Agathistoma*, excluding *Tegula patagonica*, *T. quadricostata* and *T. tridentata*. As previously reported (Hellberg, 1998), smaller south Pacific species (*T. tridentate* + *T. quadricostata*) may be the sister group to the tropical *Agathistoma*. As with the morphological phylogenetic analysis (Dornellas et al., 2021), *Agathistoma* appears to be more closely related to *T. pellisserpentis* and *T. excavata* than to other tegulines (Figure 1; Hellberg, 1998: Figure 3; Dornellas et al., 2021: figure 18). Our results also indicate that the non-*Agathistoma Tegula* are not monophyletic. *Tegula pellisserpentis* (type species of the genus) forms a clade with just *T. excavate*; thus, all tegulines need a systematic rearrangement.

Tegula aureotincta (Forbes, 1852) was placed in a new genus, *Intistoma*, by DeVries (2007). Previous phylogenies (Dornellas et al., 2021; Hellberg, 1998) have likewise placed *T. aureotincta* far outside the *T. pellisserpentis* + *Agathistoma* clade and indeed outside all other *Tegula*, supporting that this taxon might be a different genus. Non-monophyly of *Chlorostoma* was also indicated by previous molecular phylogenies (Hellberg, 1998; Yamazaki et al., 2019), with East Asian and Californian clades ascribed to the subgenus being independent.

Two eastern Pacific clades (Clade B and *A. cortezianum* + *A. globulus*) are well supported, consistent with the idea that strong geographical barriers to gene flow may not always be required for speciation in the sea and that the speciation in Tegulinae often occurs along single coastlines (Hellberg, 1998). This seems especially so for the quartet of Clade B species, which replace each other geographically from the head of the Gulf of California south to Peru (Keen, 1971). While this clade's distribution is continuous, rocky intertidal habitat is not (Riginos & Nachman, 2001), creating the possibility of speciation during transient bouts of allopatry, a prospect boosted by the rapid divergence of gamete recognition proteins in tegulines (Hellberg et al., 2012; Hellberg & Vacquier, 1999).

5 | CONCLUSIONS

Our results suggest that speciation between one pair of tropical and southwestern Atlantic species occurred by vicariance and the plume formed by Amazon and Orinoco rivers seems the most likely barrier that drove this process. The same biogeographic barrier acts as a filter for well-structured populations of *Agathistoma viridulum*. Shell colour morphotypes in this species were not associated with the populations' limits and were genetically indistinguishable. While more genetic data will be required to robustly discern relationships among subgenera within the Tegulinae, this study provides new insights into speciation in *Agathistoma* and contributes to phylogeographic studies of marine mollusks in Brazil.

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