Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

A large-scale panmictic population: phylogeography of Onchidella floridana (Gastropoda: Onchidiidae) across the Brazilian coastline

Flávia Bezerra

flaoliver@ib.usp.br

Universidade de São Paulo: Universidade de Sao Paulo https://orcid.org/0000-0003-3312-693X

Thainá Cortez

USP: Universidade de Sao Paulo

Sónia Andrade

USP: Universidade de Sao Paulo

Research Article

Keywords: molluscs, rocky shore, mitochondrial genes, dispersal, demography

Posted Date: February 2nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8523052/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Molluscs are among the most abundant inhabitants of rocky shores and represent the second-largest animal phylum in species richness. Within this group, the genus Onchidella occurs in eulittoral zones of sandy, muddy, and rocky shores, as well as estuarine environments. Onchidella floridana Dall, 1885, which lacks a planktonic larval phase, has been reported from the Gulf of Mexico, the Caribbean Sea, and the western Atlantic. Although the absence of larvae is often associated with restricted dispersal, genetic structure in rocky-shore molluscs can also be shaped by alternative dispersal mechanisms, oceanographic features, local adaptation, and life-history strategies. To evaluate whether the absence of a planktonic larval stage necessarily promotes strong genetic differentiation, we investigated the phylogeographic patterns of the direct-developing O. floridana along the Brazilian coast using two mitochondrial markers—Cytochrome c oxidase subunit I (COI) and 16S rRNA. Analyses across 16 localities revealed a panmictic population (F_{ST} = 0), high genetic diversity (16S rRNA average θπ = 2.2; COI average θπ = 4.3), and shared haplotypes across ~ 3,600 km. Demographic analyses indicated gradual increases in effective population size over time, with a more pronounced rise in the recent past. These findings suggest that larval developmental mode alone does not reliably predict genetic diversity or gene flow. By showing that O. floridana maintains connectivity across broad spatial scales even in periods encompassing climatic fluctuations such as the Last Glacial Maximum, this study contributes to understanding how life-history traits influence genetic structure in marine systems.

Introduction

Rocky shores represent key components of marine systems. By being positioned at the land–sea interface, they undergo strong daily fluctuations in abiotic conditions (Satyam & Ganesh, 2018). The spatial distribution and evolutionary diversification of marine organisms are largely shaped by biological and physiological traits that enable survival and dispersal under such fluctuations. More broadly, dispersal ability, productivity, ocean circulation, and habitat complexity are major determinants of population abundance and biogeographic ranges (Costello & Chaudhary, 2017). Among the most abundant inhabitants of rocky shores, molluscs represent the second-largest animal phylum in terms of species richness (Zhang, 2011). Population genetic structure in rocky shore molluscs results from the combined effects of dispersal ability, larval traits, local adaptation, oceanographic barriers and life-history strategies (Palumbi, 2003; Kinlan & Gaines, 2003; Siegel et al., 2003; Ayre et al., 2009; Shanks, 2009; Cortez et al., 2021). Beyond larval duration, factors such as effective population size, selection, and ocean current patterns may also shape genetic structure (Chiu et al., 2013; Hedgecock, 1986; Johnson & Black, 1998; Miller et al., 2013; Palumbi, 1994). Additionally, juveniles or adults may disperse passively across distant populations depending on species-specific life-history traits (Winston, 2012).

The family Onchidiidae Rafinesque, 1815 is one of the most diverse clades of pulmonate gastropods, comprising 143 species across 19 genera (Dayrat, 2009). The genus *Onchidella* J. E. Gray, 1850 inhabits eulittoral zones of sandy, muddy, and rocky shores, as well as estuaries (Arey & Crozier, 1921; Watson, 1925; Fretter, 1943; Wägele et al., 2006; Dayrat, 2009). Its members are distributed worldwide, with the

exception of polar regions and the Indo-West Pacific (Stringer, 1969; Kenny & Smith, 1987; Barker, 2001; Dayrat, 2009; Dayrat et al., 2011b). Two contrasting reproductive strategies occur in *Onchidella* species: direct and planktonic development (Stringer, 1969). Among the direct-developing species, *Onchidella floridana* (Dall, 1885) has been reported from the Gulf of Mexico, the Caribbean Sea (De la Cruz-Francisco, 2025), and the Western Atlantic (Marcus, 1978) ranging from the Tropical Northwestern Atlantic to the Warm Temperate Southwestern Atlantic province (Spalding et al., 2007). Although the reproductive mode of *O. floridana* has not yet been formally documented, its morphological and ecological resemblance to the brooding species *O. campbelli*, *O. flavescens*, and *O. marginata* strongly suggests that it likewise exhibits direct development. The species is morphologically characterized by an oval body, longer than wide, with an elevated dorsal region. The mantle surface is densely covered with conical and/or hemispherical tubercles exhibiting a rough texture. Two tentacles bearing eyes at their tips are present, and dorsal gills are absent (De la Cruz-Francisco, 2025). So far, only a handful of studies have addressed *O. floridana*, focusing mainly on glandular morphology (Arey & Barrick, 1942) and its taxonomic placement within Onchidiidae (Dayrat et al., 2016). Although the absence of a larval stage is often linked to limited dispersal (e.g. Burton, 2009; Collin, 2001; Kyle & Boulding, 2000; Bucklin, 2000; Cowen, 2000; Launey, 2002; Taylor, 2003; Rynearson & Armbrust, 2004; Sotka et al., 2004; Carini & Hughes, 2006; Kramarenko & Snegin, 2015; Tang et al., 2025), no investigations have explored its ecological or populational evolutionary dynamics.

To test the hypothesis that the absence of a planktonic larval phase is not the sole driver of genetic differentiation in rocky shore molluscs, we investigated the phylogeographic patterns of the direct-developing *O. floridana* along the Brazilian coast using two mitochondrial regions (cytochrome c oxidase subunit I and 16S rRNA). We analyzed the genetic variation from 16 localities distributed across ~ 3.600 km and provided new insights into the gene flow dynamics of direct-developing species, contributing to a broader understanding of evolutionary processes in rocky shore organisms.

Methods

Sampling

Approximately 90 individuals of *O. floridana* were manually collected from barnacle beds on rocky shores 16 sites spanning 2,400 km along the Brazilian coast between August 2017 and January 2020 (Figure 1, Table 1). Specimens were fixed in 99% ethanol and maintained at −20 °C at the Laboratório de Diversidade Genômica, University of São Paulo, Brazil, until further molecular analyses.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from the entire animal using the DNeasy Blood & Tissue Kit (Qiagen, Brasil) following the manufacturer instructions. DNA integrity was verified by 1% agarose gel electrophoresis and quantified with a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Mitochondrial regions cytochrome c oxidase subunit I and 16S ribosomal RNA (hereafter COI and16S rRNA, respectively) were amplified by polymerase chain reactions (PCRs) using the primers ONCH-

COI/H7005mod1 (Cumming et al., 2014) and 16SAf/16SKr (Norenburg, unpublished). Primer sequences were: ONCH-COI, 5'- ATT GGG GCT CCN GAT ATA AG -3'; H7005mod1, 5'- ART GNG CAN CAN CRT ART ANG TRT CRT G -3'; 16SAf, 5'-TCG TCT GTT TAT CAA AAA CAT AGY-3'; 16SKr, 5'-AAT AGA TAG AAA CCA ACC TGG C-3'. PCRs were carried out in 15 µL reactions containing 1× PCR buffer, 2.5 U Taq DNA polymerase, 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.35 µM of each primer, and ultrapure water. Cycling conditions included an initial denaturation at 94 °C for 1 min; 35 cycles of 94 °C for 1 min, annealing at 52 °C (COI) or 49 °C (16S rRNA) for 1 min, and 72 °C for 1 min; followed by a final extension at 72 °C for 10 min. PCR products were purified with a 15% polyethylene glycol solution (PEG; http://labs.icb.ufmg.br/lbem/protocolos/peg.html) and sequenced with a BigDye Terminator Cycle Sequencing Kit v.3.1 (Thermo Fisher Scientific) at ACTgene Análises Moleculares LTDA (Alvorada, RS, Brazil).

Chromatograms were inspected and consensus sequences generated in Geneious 9.1.8 (Biomatters, Auckland, New Zealand). The final alignments of COI and 16SrRNA were concatenated and analyzed as a single dataset. Alignments were performed with MAFFT using the Q-INS-i structural strategy (Katoh & Standley, 2013).

Phylogenetic relationships and species identification

A phylogenetic tree was constructed using available *Onchidella* sequences from public databases to confirm species identification and assess evolutionary relationships with closely related taxa. The tree was inferred under the maximum likelihood (ML) framework using IQ-TREE v.1.6.12 (Nguyen et al., 2015), with substitution models selected via ModelFinder (Kalyaanamoorthy et al., 2017). The best-fit models were TIM2+F+G4 according to the Bayesian Information Criterion (BIC). The species *O. nigricans* (COI: KF258109.1;16SrRNA: KF258006.1), *Onchidella marginata* (COI: MK160632.1; 16SrRNA: JX430933.1), *Onchidella patelloide* (COI: MG970878.1; 16SrRNA: MG970944.1), *Onchidella hildae* (COI: HQ660036.1; 16SrRNA: HQ659904.1), and *Onchidella floridana* (COI: HQ660035.1; 16SrRNA: HQ659903.1) were incorporated into the phylogenetic tree. Only species with both COI and 16S rRNA sequences available on GenBank were included. The species *Peronina zulfigari* (Onchidiinae: Onchidiidae) was used as an outgroup (COI: MG958778.1; 16SrRNA: MG958834.1).

Genetic diversity, population structure and demographic history

To explore the relationships among haplotypes and assess gene flow, a minimum spanning network was constructed in PopART v.1.7 (Leigh & Bryant, 2015). Local populations represented by a single individual (MBO and SBO for 16S rRNA; MBO, SBO, GAO and JBO for COI) were excluded from subsequent analyses due to insufficient sample size. To assess intrapopulation genetic diversity, we estimated genetic variation using Arlequin v.3.5 (Excoffier & Lischer, 2010). The intrapopulational parameters analysed were: the percentage of diversity representing the proportion of haplotypes (S) relative to the number of individuals sampled per population, the theta estimate for nucleotide diversity ($\theta\pi$), and the sizes average number of segregating sites (θ_S). Analysis of molecular variance (AMOVA) was also conducted in Arlequin to assess genetic structuring, with significance tested through 10,000 permutations and

100,000 Markov chain Monte Carlo (MCMC) iterations. Fixation indices (F_{ST}) (Weir & Cockerham, 1984) were calculated at three hierarchical levels: (i) among geographical regions (Northeast, Southeast, South), (ii) among locations (beaches) within regions, and (iii) within locations.

To investigate the demographic history of the populations Tajima's D (Tajima, 1989) and Fu's F_S (Fu, 1997) were calculated using Arlequin v.3.5 (Harpending et al., 1993) to detect deviations from neutrality, where significant negative values suggest population growth. Historical changes in effective population size were inferred with Extended Bayesian Skyline Plots (eBSP) in BEAST v.1.10.4 (Suchard et al., 2018), using the same dataset, partitions and substitution models as in the phylogenetic analysis. The MCMC chain length was set to 300,000,000, logging every 30,000. Convergence was evaluated in Tracer v.1.7.5 (Rambaut et al., 2018), ensuring effective sample sizes (ESS) > 200. Graphs were obtained through R v. 4.1.2 (R core, 2021)

Results

Datasets

We obtained 78 COI and 90 16SrRNA sequences of *O. floridana* from 16 localities across the Brazilian coast. The partial sequences of 16S rRNA presented 454 pb with 44 polymorphic sites, COI presented sequences 663 pb long with 68 polymorphic sites. The final concatenated dataset was 1,117 bp, with 112 polymorphic sites, 38 parsimony-informative sites and 74 singletons. Sequences are publicly available in GenBank, with accession numbers provided in Supporting Information (Table S1).

Genetic diversity

The concatenated phylogenetic tree (COI + 16S rRNA, Figure 2), as well as the individual gene trees (supporting information figures S1 and S2) consistently recovered a monophyletic *O. floridana* clade comprising samples from this study and public databases. Furthermore, all the recovered trees showed no clear geographic structuring, as sequences from multiple localities clustered together. Regarding the haplotype networks, the one based on COI (Figure 3) revealed 12 haplotypes shared among at least two localities and several star-like patterns, with a single predominant haplotype shared by PCO, MBO and ITO forming the central group. The remaining haplotypes differed by only a few substitutions (one to six), encompassing both shared and site-restricted haplotypes. In addition, distant regions such as TRO (South) and BSM (Northeast) also shared haplotypes. The 16S rRNA haplotype network (Figure 3) showed a large central haplotype comprising samples from 12 of the 16 localities, from which several unique and shared haplotypes radiated in a star-shape network. Overall, both markers showed no clear geographic structuring: haplotypes were broadly shared among both nearby and distant localities, and no isolated populations were detected.

Nucleotide diversity indices indicated generally high genetic diversity, with the highest values observed in TRO (COI: θs = 4.91 and θπ = 4.50; 16S rRNA: θs = 8.57 and θπ = 6.57) and the lowest values detected in RB (COI: θs = 1.92 and θπ = 1.80) and ANO (16S rRNA: θs = 1.00 and θπ = 1.00) (Figure 4). When

accounting for differences in sample size, haplotype diversity—expressed as the proportion of haplotype diversity within each locality—did not differ significantly among regions.

Population structure and demographic history

The AMOVA revealed statistically significant but weak genetic structuring among regions for the 16S rRNA marker (F_{CT} = 0.05, p < 0.03). Similarly, the population differentiation index (F_{ST} was low despite being significant (F_{ST} = 0.06, p = 0.05). More than 99% of the genetic variation was allocated within locations. By contrast, COI did not show significant population structure, with non-significant F_{CT} (F_{CT} = 0, p = 0.83) and F_{ST} values (F_{ST} = 0.02, p = 0.277).

Pairwise FST comparisons among localities revealed only a few significant values, all of which were low, for both markers. For COI, significant differentiation was detected only between ITO – BSM and ITO – ARO. For 16S rRNA, low but significant values were observed between BSM – PCO, BSM – RBO, PCO – MRO, PCO – STO, and ANO – MRO (Supplementary Information, Figure S3).

Among the neutrality tests used for demographic inference, statistically significant results were limited to a few localities (Table 3), all exhibiting negative values, suggesting departures from mutation–drift equilibrium consistent with recent population expansion or the action of purifying selection in these populations. For COI, both Tajima's D and Fu's Fs were significant in PRN and RBO, while only Fu's Fs was significant in ITO. For 16S rRNA, significant values for both statistics were detected only in ITO, whereas PRN, ARO, ITN, and STO showed significance only for Fu's Fs. The extended BSP analysis (Figure 5) also indicates historical population stability with a slight increase in effective population size (Ne) in the recent past, a pattern supported by the 95% highest posterior density (HPD) intervals.

Discussion

In this study, we provide the first phylogeographic assessment of *O. floridana*, a common pulmonate gastropod that inhabits rocky shores along the Brazilian coast (personal obs.). Despite the absence of a larval stage, our results revealed a panmictic population along the Brazilian coast, historically stable and characterized by high genetic diversity, shared haplotypes across ~3,600 km of coastline, and genetic variation patterns indicative of recent demographic expansion. These findings are particularly relevant, as they reinforce a growing body of evidence indicating that larval developmental mode alone is insufficient to predict patterns of genetic diversity and gene flow in marine species.

Phylogeographic studies on molluscs with larval development frequently report weak population structure and moderate to low genetic diversity. For instance, *Crassostrea rhizophorae* and *C. brasiliana* (Lazoski et al., 2011), *Littoraria flava* (Cortez et al., 2021), *L. subvittata* and *L. pallescens* (Lee & Boulding, 2009; Nehemia et al., 2019), *Echinolittorina punctata* (Evangelist et al., 2017), and *L. scabra* and *L. glabrata* (Silva et al., 2013) exhibited either moderate nucleotide diversity or limited haplotype richness, typically associated with high gene flow across broad geographic distances. Conversely, some studies have revealed strong genetic differentiation despite the presence of a larval stage, as reported for

Saccostrea mordax (Tang et al., 2025), the bivalves *Mytilopsis leucophaeata* (Fernandes et al., 2022) and *Anomalocardia brasiliana* (Arruda et al., 2009). Within Onchidiidae, *O. campbelli* and *O. flavescens*, revealed widespread distribution patterns in New Zealand, subantarctic, and South American populations, even in the absence of a larval stage (Cumming et al., 2014). These patterns were most likely driven by passive rafting on *Durvillaea* kelp mediated by circumpolar currents. In addition, species of the genus *Peronia*, which also exhibit direct development, showed geographically restricted distributions, except for *P. verruculata* (Maniei et al., 2020) Thus, genetic diversity and gene flow appear to vary independently of developmental type, across species with planktonic larvae as well as those with direct development.

Our findings on *O. floridana* are broadly consistent with patterns mainly observed in species with indirect development, with all analyses supporting a single panmictic population across the Tropical Southwestern Atlantic and the Warm Temperate Southwestern Atlantic ecoregions (Spalding et al., 2007). The high number of shared haplotypes indicates a lack of population isolation among the sampled localities, suggesting ongoing gene flow. This pattern was further supported by genetic diversity indices, which showed generally high values, as well as by the phylogenetic reconstruction revealing no clear geographic structure and AMOVA revealing low but statistically significant differentiation among regions for the 16S rRNA marker. Overall, the global F_{ST} = 0 alongside the few and low significant pairwise F_{ST} values fully support a high level of genetic similarity among populations. Therefore, the wide distribution of *O. floridana* along the Brazilian coast, despite the absence of a planktonic larval stage, suggests that alternative dispersal mechanisms may play a role, such as transport via coastal currents or stepping-stone dispersal across continuous intertidal habitats, as documented for the blue mussel *Mytilus edulis* in the North Sea (Coolen et al., 2020) and for wood-boring bivalves of the family Xylophagaidae in the northeastern Atlantic Ocean (Romano et al., 2020).

The demographic patterns described for other mollusc species show both similarities and contrasts with those observed in *O. floridana*, reflecting differences in dispersal potential, life-history traits, and species-specific responses to late Quaternary environmental changes. In many coastal molluscs, demographic shifts have been associated with glacial–interglacial cycles, particularly the Last Glacial Maximum (LGM). For example, *Echinolittorina punctata*, which has indirect development, exhibited evidence of a demographic bottleneck followed by population expansion, potentially linked to post-glacial sea-level rise and habitat re-expansion (Evangelist et al., 2017). Similarly, several Littorinidae species showed signals of demographic expansion supported by negative Fu's Fs values, suggesting population growth following glacial phases (Silva et al., 2013; Nehemia et al., 2019, Cortez et al. 2021). In *S. mordax*, BSP and neutrality tests indicated population growth during the transition from the Last Interglacial (LIG) to the LGM in the western Pacific and Persian Gulf, highlighting the sensitivity of rocky-shore molluscs to major climatic transitions (Tang et al., 2025). In contrast, the demographic history inferred for *O. floridana* along the Brazilian coast suggests a more gradual demographic response to historical climatic oscillations. Although neutrality tests revealed significant deviations from neutrality in a limited number of localities, the overall signal points to weak and spatially heterogeneous demographic expansion. This

interpretation is supported by the EBSP, which indicates a slow and continuous increase in effective population size through time, with a slightly more pronounced rise in the recent past, together with the presence of numerous unique and star-like haplotypes. Rather than indicating abrupt demographic changes associated with the LGM, these patterns are consistent with progressive population growth, possibly driven by the gradual expansion or long-term persistence of suitable intertidal habitats along the Brazilian coast. The relatively stable availability of rocky shores and the absence of extensive glacial impacts in the South Atlantic (Fernandez Iriarte et al., 2020) may have reduced the magnitude of demographic contractions during glacial periods, resulting in a smoother demographic trajectory compared to that reported for some other mollusc species.

Our findings underscore the importance of considering alternative dispersal mechanisms in marine invertebrates and further demonstrate that the presence of a larval stage is not necessarily the primary driver of species distribution, particularly in taxa lacking a planktonic phase. By showing that *O. floridana* maintains high gene flow across broad geographic scales and relatively stable population sizes over evolutionary timescales, this study advances our understanding of how life-history traits modulate genetic structure in marine systems.

Declarations

ETHICS APPROVAL

This study did not involve experiments on humans or vertebrate animals. All sampled organisms are marine invertebrates, for which ethical approval is not required under current national and institutional regulations. Field sampling was conducted in accordance with local environmental guidelines and permits.

AUTHOR CONTRIBUTIONS

SCSA and TC designed the study. FOB performed computational data analysis and drafted the manuscript. TC performed specimen collections, computational and molecular analyses. SCSA supervised the study, secured funding, and assisted with computational analyses. All authors revised and approved the manuscript.

ACKNOWLEDGEMENTS

We are grateful to the Instituto de Biociências – USP and the Centro de Biologia Marinha da Universidade de São Paulo (CEBIMar) staff; to all colleagues at the Laboratório de Diversidade Genômica. We are especially grateful to Cecilli B. Mendes, Stephanie Von Ungern Sternberg, Gabriel Piedade, Gabriel Sonoda, Paulo P.G. Pachelle e Henrique Cardilli for helping with the sampling. This work

was supported by CAPES (process: 88887.626442/2021−00) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, process 2015/20139−9). The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Arey LB, Barrick LE (1942) The structure of the repugnatorial glands of. J Morphol 71:493–521
2. Arey LB, Crozier WJ (1921) On the natural history of. J Exp Zool 32:443–502
3. ARRUDA CCB et al (2009) Significant genetic differentiation among populations of. (Gmelin 1791): bivalve planktonic larval dispersion Genet Mol Biology 32:423–430
4. Ayre D, Minchinton T, Perrin C (2009) Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? Mol. Ecol 18:1887–1903
5. Barker GM (2001) Gastropods on land: phylogeny, diversity and adaptive morphology. Biology of Terrestrial Mollusks. CABI Publishing, pp 1–146
6. Britton KM (1984) The Onchidiacea (Gastropoda, Pulmonata) of Hong Kong with a worldwide review of the genera. J Molluscan Stud 50:179–191
7. Bucklin A (2000) Population genetic variation of. Icelandic waters ICES J Mar Sci 57:1592–1604
8. Burton RS (2009) Molecular markers, natural history, and conservation of marine animals. Bioscience 59:831–840
9. Carini G, Hughes J (2006) Subdivided population structure and phylogeography of an endangered freshwater snail. Biol J Linn Soc 88:1–16
10. Chiu YW, Bor H, Tan MS, Lin HDU, Jean CT (2013) Phylogeography and genetic differentiation among populations of the moon turban snail. (Gmelin 1791) Int J Mol Sci 14:9062–9079
11. Collin R (2001) Effects of developmental mode on phylogeography of North Atlantic. Mol Ecol 10:2249–2262
12. Coolen JW et al (2020) Marine stepping-stones: connectivity of. populations between offshore energy installations Mol Ecol 29:686–703
13. Cortez T, Amaral RV, Sobral-Souza T, Andrade SC (2021) Genome-wide assessment of. Biol J Linn Soc 133:999–1015
14. Cowen R (2000) Connectivity of marine populations: open or closed? Science 287:857–859
15. Cumming RA, Nikula R, Spencer HG, Waters JM (2014) Transoceanic genetic similarities of kelp-associated sea slugs: rafting dispersal? J Biogeogr 41:2357–2370
16. Dalton Mc P Pe (2019) Direct development in the intertidal shell-less. Molluscan Res 39:373–376
17. Dayrat B (2009) Systematics of Onchidiidae with a checklist of species. Zootaxa 2068, 1–26
18. Dayrat B, Goulding TC, Apte D, Bhave V, Comendador J, Qua NX, Tan SH (2016) Integr taxonomy Onchidium ZooKeys 636:1

19. Dayrat B, Zimmermann S, Raposa M (2011) Systematic revision of Onchidiidae from the Tropical Eastern Pacific. J Nat Hist 45:939–1003
20. De la Cruz-Francisco V (2025) Nuevas adiciones de babosas marinas. Novitates Caribaea 25:32–53
21. Doyle JJ, Doyle JL (1987) DNA isolation for small quantities of tissue. Phytochem Bull 19:11–15
22. Evangelisti F, Bellucci A, Sabelli B, Albano PG (2017) tracked warming of the Mediterranean. Mar Biol 164:34
23. Excoffier L, Lischer HE (2010) Arlequin ver 3.5. Mol. Ecol Resour 10:564–567
24. Fernandes Mr, Salgueiro F, Miyahira I (2022) Genetic investigation of. Estuaries Coasts 45:812–826
25. Fernandez Iriarte PJ, González-Wevar CA, Segovia NI, Rosenfeld S, Hüne M, Fainburg L, Poulin E (2020) Quaternary ice sheets and sea level regression drove divergence in a marine gastropod along Eastern and Western coasts of South America. Scientific Reports, 10(1), 844
26. Fretter V (1943) Functional morphology and embryology of. J Mar Biol Assoc U K 25:685–720
27. Fu Y (1997) Statistical tests of neutrality. Genetics 147:915–925
28. Gabe M, Prenant M (1950) Histology of the repulsive gland of. Bull Soc Zool France 76:78–83
29. Harpending H et al (1993) Genetic structure of ancient human populations. Curr Anthropol 34:483–496
30. Hedgecock D (1986) Pelagic larval dispersal and adaptation. Bull Mar Sci 39:550–564
31. Ho SY, Shapiro B (2011) Skyline-plot methods for demographic history. Mol Ecol Resour 11:423–434
32. Je Lee HYUK, Boulding EG (2009) Genetic structure of littorinid gastropods. Mol Ecol 18:2165–2184
33. Johnson MS, Black R (1998) Genetic subdivision of Littoraria cingulata. Mar Biol 132:295–303
34. Kalyaanamoorthy S et al (2017) ModelFinder Nat Methods 14:587–589
35. Katoh K, Standley DM (2013) MAFFT software improvements. Mol Biol Evol 30:772–780
36. Kenny R, Smith A (1987) Distribution of. Pac Sci 41:21–30
37. Kinlan BP, Gaines SD (2003) Propagule dispersal in marine systems. Ecology 84:2007–2020
38. Kyle CJ, Boulding EG (2000) Population structure of. spp Mar Biol 137:835–845
39. Launey S (2002) Genetic structure of. J Hered 93:331–341
40. Lazoski C, Gusmão J, Boudry P, Solé-Cava AM (2011) Phylogeny and phylogeography of oysters. Mar Ecol Prog Ser 426:197–212
41. Leigh JW, Bryant D (2015) POPART software. Methods Ecol Evol 6:1110–1116
42. Marcus EDBR (1978) Western Atlantic species of. Sarsia 63:221–224
43. Marcus EDBR (1979) The Atlantic species of. Part 2 Bol Zool 4:1–38
44. Miller AD et al (2013) Ocean currents influence an intertidal mollusc. Ecol Evol 3:1248–1261
45. Nehemia A, Ngendu Y, Kochzius M (2019) Population structure of. L pallescens J Exp Mar Biol Ecol 514:27–33
46. Nguyen LT, Schmidt HA, von Haeseler A et al (2015) IQ-TREE algorithm. Mol Biol Evol 32:268–274

47. Palumbi S (2003) Demographic connectivity and marine reserves. Ecol Appl 13:146–158
48. Palumbi SR (1994) Genetic divergence and marine speciation. Annu Rev Ecol Syst 25:547–572
49. Pepe PJ, Pepe SM (1985) Activity pattern of. Veliger 27:375–380
50. R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
51. Rambaut A, Drummond AJ, Xie D et al (2018) Posterior summarisation in Tracer. Syst Biol 67:901–904
52. Romano C et al (2020) Wooden Stepping Stones: Diversity and Biogeography of Deep-Sea Wood Boring Xylophagaidae (Mollusca: Bivalvia) in the North-East Atlantic Ocean, With the Description of a New Genus. front. Mar Sci 7:579959
53. Rynearson T, Armbrust VE (2004) Genetic differentiation of. J Phycol 40:34–43
54. Satyam K, Thiruchitrambalam G (2018) Habitat ecology of rocky shore fauna. In: Biodiversity and climate change adaptation in tropical islands. Academic Press, pp 187–215
55. Shanks AL (2009) Pelagic larval duration and dispersal. Biol Bull 216:373–385
56. Siegel DA, Kinlan BP, Gaylord B, Gaines SD (2003) Lagrangian descriptions of larval dispersion. Mar Ecol Prog Ser 260:83–96
57. SILVA SE et al (2013) Genetic and morphological variation in littorinid gastropods. Biol J Linn Soc 108:494–508
58. Sotka E et al (2004) Genetic clines in. Mol Ecol 13:2143–2156
59. Spalding MD et al (2007) Marine ecoregions of the world. Bioscience 57:573–583
60. Stringer BL (1969) Species of New Zealand Onchidiidae. N Z J Mar Freshw Res 3:29–45
61. Suchard MA et al (2018) Bayesian phylogenetics using BEAST 1.10. Virus Evol 4:16
62. Tajima F (1989) Testing the neutral mutation hypothesis. Genetics 123:585–595
63. Tang Z et al (2025) Phylogeography of Saccostrea mordax. J Oceanol Limnol, 1–15
64. Taylor M (2003) Local retention of pelagic larvae in a reef fish. Science 299:107–109
65. Wägele H, Ballesteros M, Avila C (2006) Defensive glandular structures in opisthobranchs. Oceanogr Mar Biol Annu Rev 44:197–276
66. Watson H (1925) South African species of. Ann S Afr Mus 20:237–309
67. Weiss K, Wägele H (1998) Morphology and histology of. Arch Moll 127:69–91
68. Winston JE (2012) Dispersal in marine organisms without a pelagic larval phase. Integr Comp Biol 52:447–457
69. Zhang ZQ (2011) Animal biodiversity: higher-level classification outline. Magnolia

Tables

Tables are available in the Supplementary Files section.

Figures

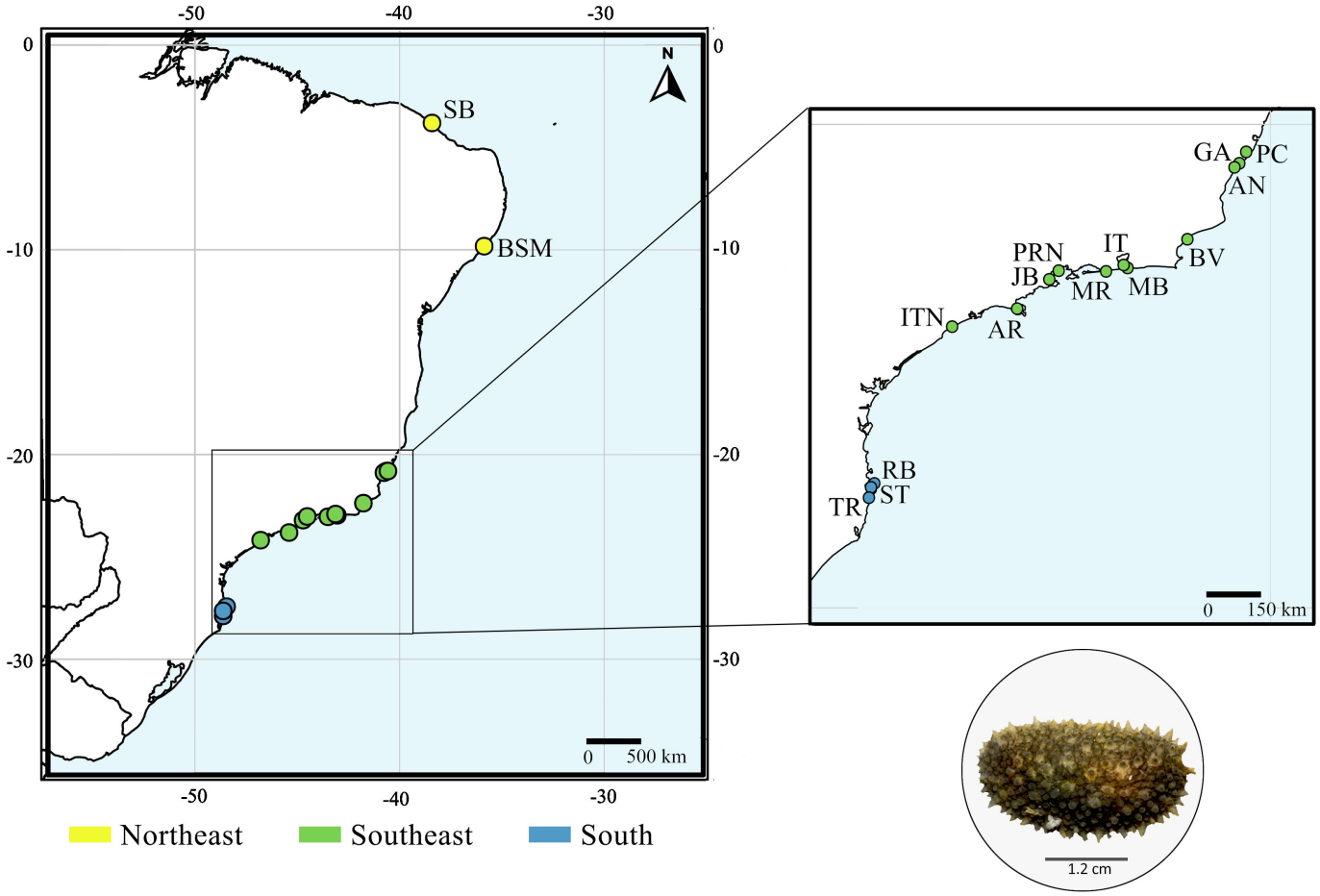

Figure 1

Figure 1: Onchidella floridana sampling localities along the Brazilian coast. Colors indicate coastline regions according to the legend. At the bottom left, a photograph of a representative specimen collected at Araçá (AR). Locations abbreviation as follow: SBO = Sabiaguaba; BSM = Baía São Miguel; ANO = Anchieta; PCO = Praia dos Coqueiros; GAO = Gamboa; BVO = Boa Viagem; ITO = Itaipu; MBO = Mambuacaba; MRO = Marechal; PRN = Prainha; JBO = Jabaquara; ARO = Araçá; ITN = Itanhaém; RBO = Ribeirão da Ilha; STO = Santo Antônio, TRO = Trapiche da Ilha. Coordinates and sampling sizes are presented in Table 1.

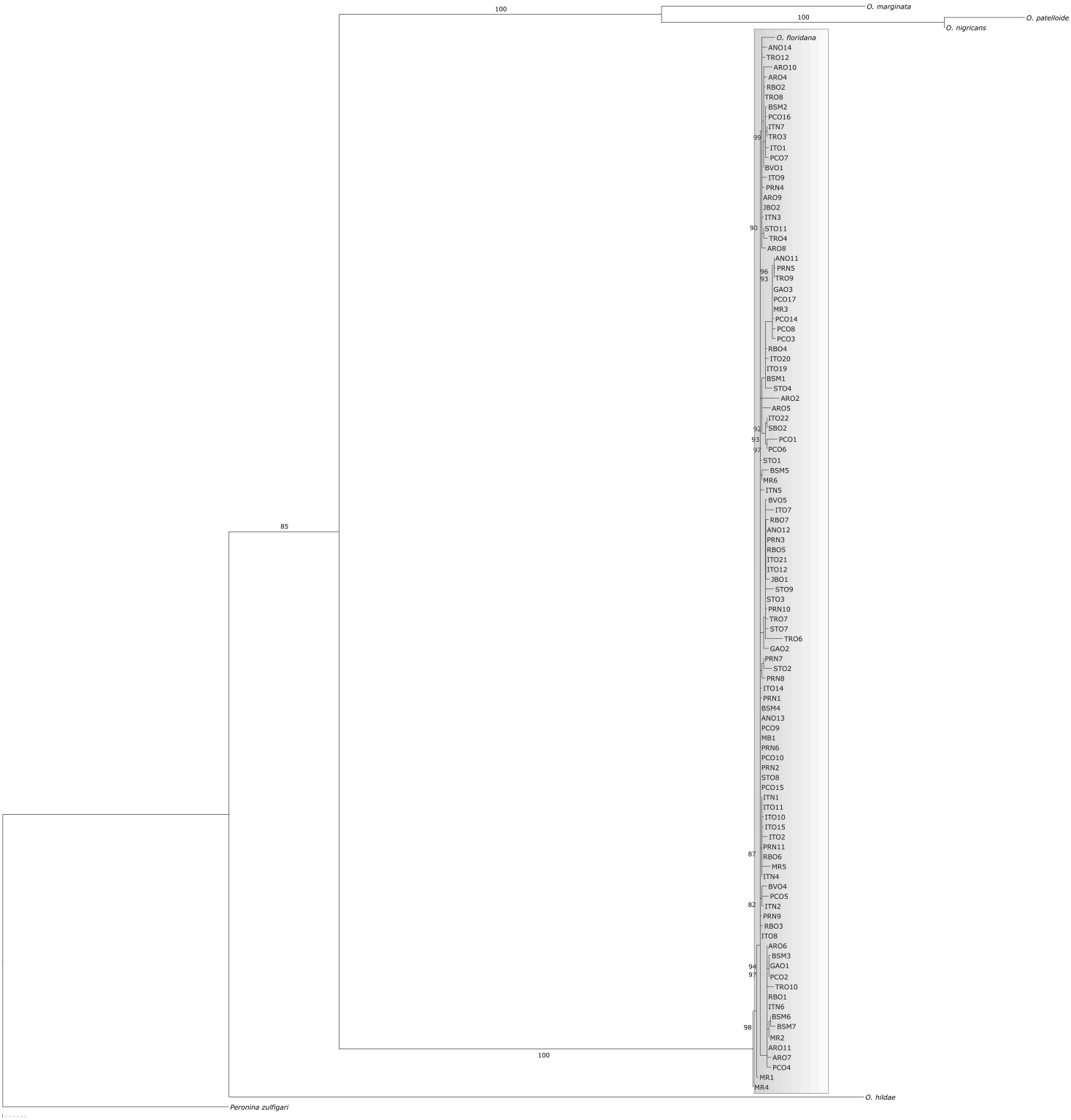

Figure 2

Figure 2: Maximum likelihood phylogenetic tree based on COI and 16S rRNA concatenated sequences of Onchidella species (-lnL -2545.825). The species Onchidella floridana are highlighted in bold. Only bootstrap support values > 80% are shown. Locations abbreviation

as follow: SBO = Sabiaguaba; BSM = Baía São Miguel; ANO = Anchieta; PCO = Praia dos Coqueiros; GAO = Gamboa; BVO = Boa Viagem; ITO = Itaipu; MBO = Mambuacaba; MRO = Marechal; PRN = Prainha; JBO = Jabaquara; ARO = Araçá; ITN = Itanhaém; RBO = Ribeirão da Ilha; STO = Santo Antônio, TRO = Trapiche da Ilha. Coordinates and sampling sizes are presented in Table 1.

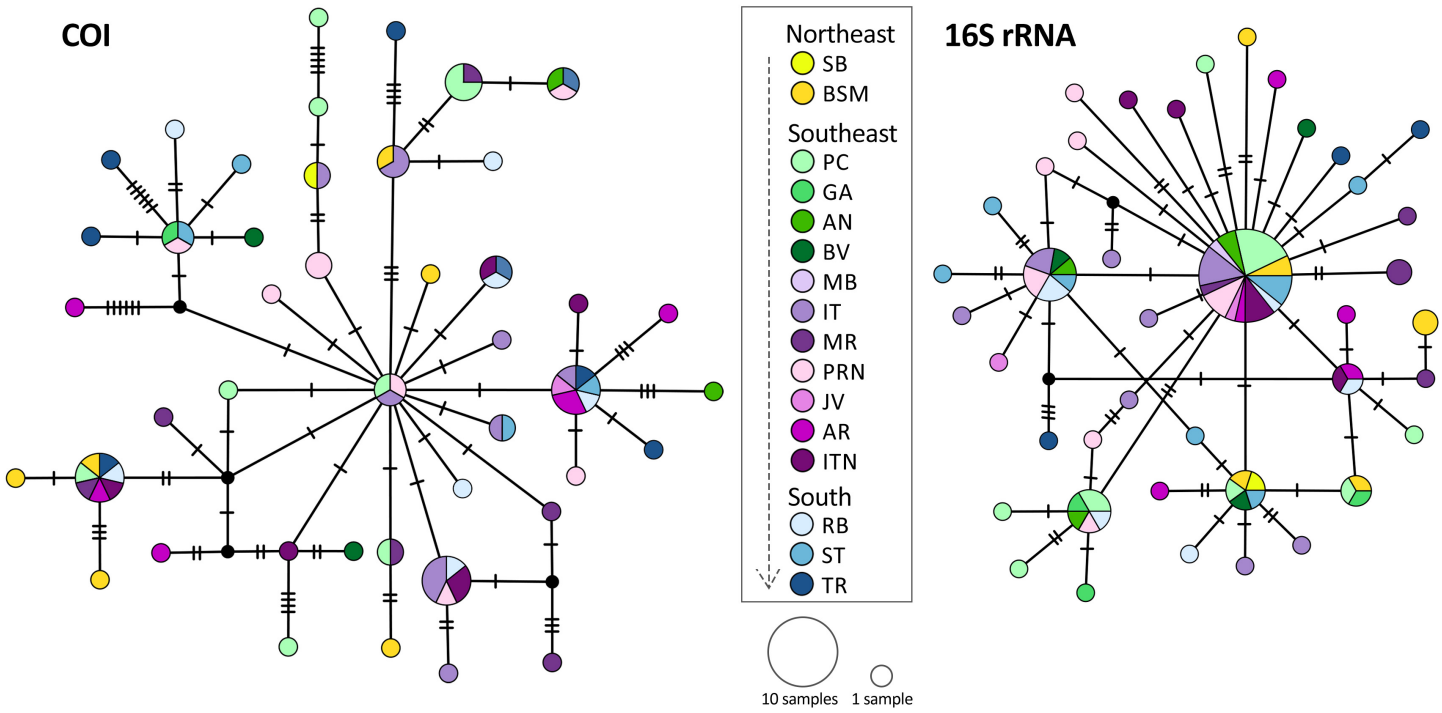

Figure 3

Figure 3: Haplotype networks for COI (n = 78), left, and 16S rRNA (n = 90), right. Each node corresponds to a haplotype, with node size proportional to its frequency and colors indicating sampling localities. Hatch marks on branches represent mutational steps. Locations abbreviation as follow: SBO = Sabiaguaba; BSM = Baía São Miguel; ANO = Anchieta; PCO = Praia dos Coqueiros; GAO = Gamboa; BVO = Boa Viagem; ITO = Itaipu; MBO = Mambuacaba; MRO = Marechal; PRN = Prainha; JBO = Jabaquara; ARO = Araçá; ITN = Itanhaém; RBO = Ribeirão da Ilha; STO = Santo Antônio, TRO = Trapiche da Ilha. Coordinates and sampling sizes are presented in Table

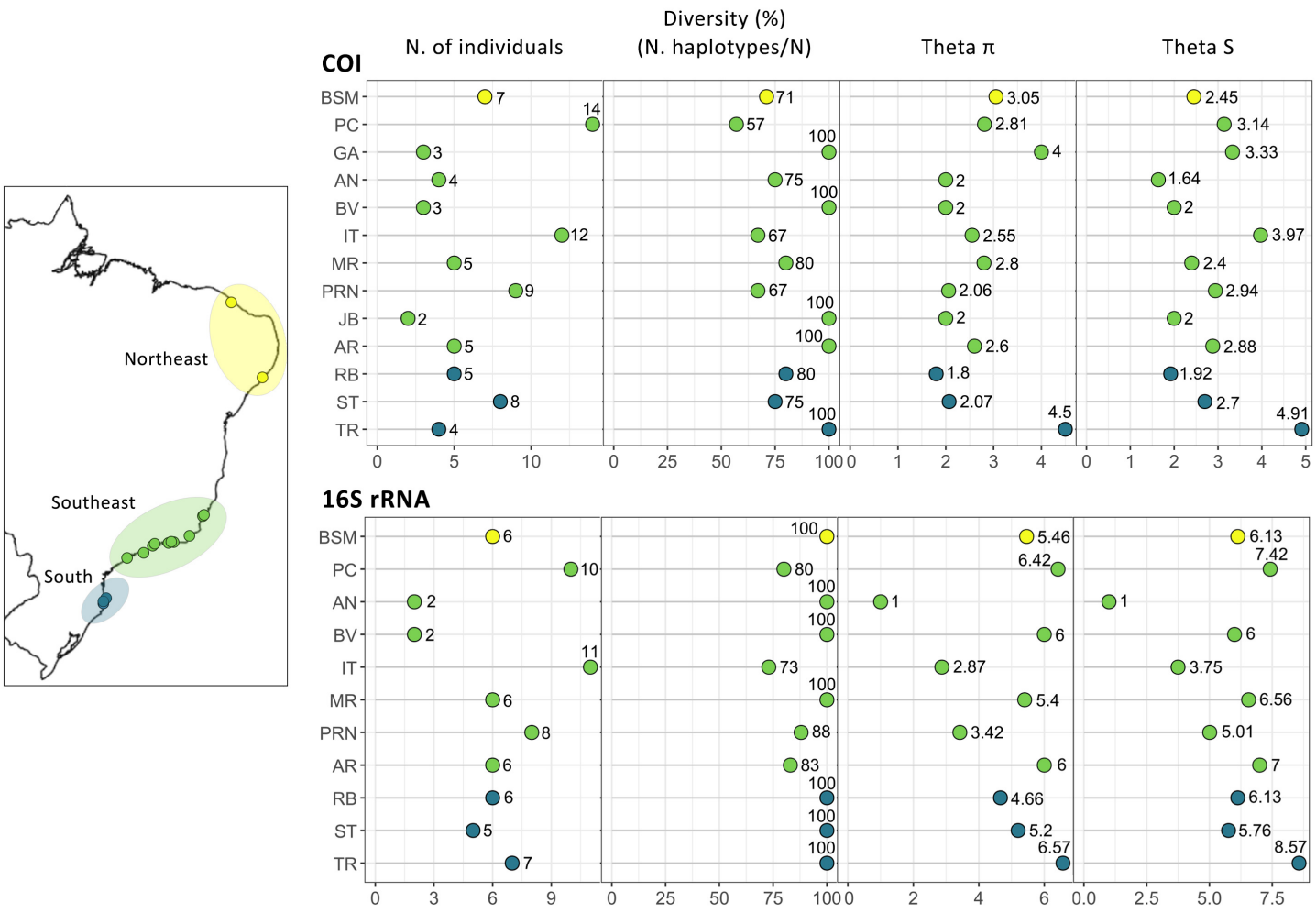

Figure 4

Figure 4: Lollipop plots for intrapopulation diversity indices computed for the COI and 16S rRNA markers of Onchidella floridana. Θπ = mean number of pairwise differences, Θs = number of segregating sites. Each panel displays the indices for one marker. Circles represent populations and are colored according to their corresponding region along the Brazilian coast, as indicated in the map on the left. Locations abbreviation as follow: SBO = Sabiaguaba; BSM = Baía São Miguel; ANO = Anchieta; PCO = Praia dos Coqueiros; GAO = Gamboa; BVO = Boa Viagem; ITO = Itaipu; MBO = Mambuacaba; MRO = Marechal; PRN = Prainha; JBO = Jabaquara; ARO = Araçá; ITN = Itanhaém; RBO = Ribeirão da Ilha; STO = Santo Antônio, TRO = Trapiche da Ilha. Coordinates and sampling sizes are presented in Table 1.

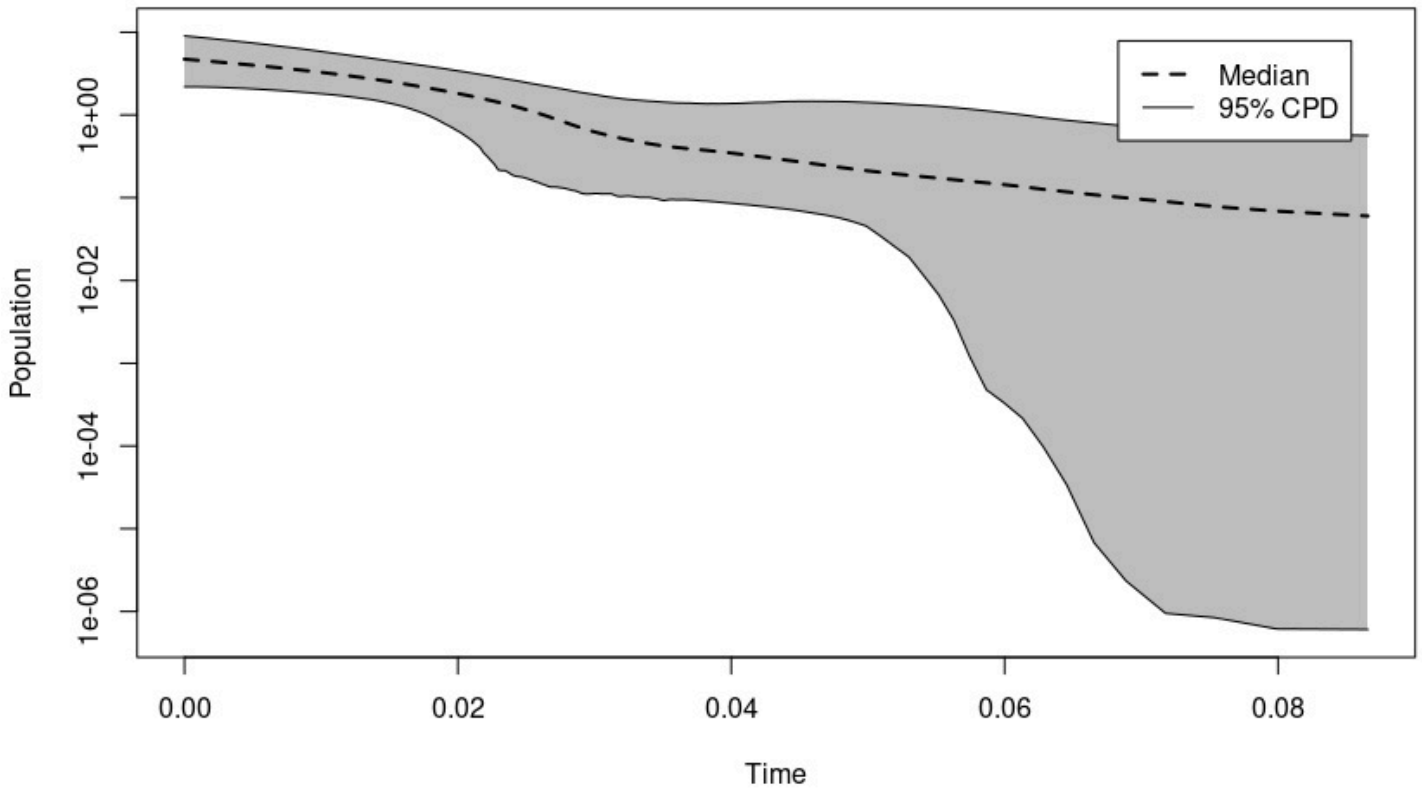

Figure 5

Figure 5: Onchidella floridana Extended Bayesian Skyline Plot. Solid lines represent the mean estimates of effective population size (Neτ), the shaded area indicates the 95% credible posterior density (CPD) interval, and the dashed line marks its limits. The x-axis shows time (in Myears) and the y-axis shows effective population size on a log scale.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FOBtables.pdf
- FOBsupplementary.pdf