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Magellan mussels *Aulacomya atra* from the South African coast show high diversity within populations but a lack of geographic differentiation

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The Magellan mussel *Aulacomya atra* is a bivalve mollusc found along parts of the South African and Namibian coastline. Its numbers were low historically compared with other indigenous species but have decreased further since the 1970s owing to habitat invasion by Mediterranean mussels *Mytilus galloprovincialis.* We studied sequences of the mitochondrial cytochrome oxidase subunit 1 (CO1) and the nuclear internal transcribed spacer (ITS1) of *A. atra* to determine patterns of differentiation among three localities on the South African coastline and the phylogenetic position of these populations relative to other populations of *Aulacomya* species in the Southern Hemisphere. Results from both mitochondrial and nuclear genes revealed a high level of diversity within South African populations from the west and south coast, with little to no geographic differentiation among these populations. Phylogenetic trees constructed using maximum likelihood and haplotype network analysis show that individuals from all three regions sampled are intermingled in groups with low bootstrap support. Our CO1 sequences exhibited a phylogeographic structure concordant with the spatial distribution in South Africa, Argentina, Chile and New Zealand. However, results for ITS1 showed a lack of differentiation over a large spatial scale stretching from South Africa to New Zealand. Future studies should include additional samples from across the species' distributional range and the examination expanded to include genetic markers with adaptive significance.

Keywords: cytochrome oxidase, genetic differentiation, haplotype networks, internal transcribed spacer, phylogenetic tree, ribbed mussel, South Africa

Introduction

The Magellan mussel (or ribbed mussel) *Aulacomya atra* is a marine bivalve that occurs along the coastlines of several countries in the Southern Hemisphere. While most genera in the family Mytilidae have relatively smooth shells, members of the genus *Aulacomya*, along with the genus *Geukensia* from the Northern Hemisphere, have prominently ribbed shells. *Aulacomya atra* occurs along the southern African coastline from Rocky Point in northern Namibia to Port Alfred on the southeastern coast of South Africa (Figure 1, based on Caza et al. [2016]). Further afield, *A. atra* occurs along the Pacific coast of South America from Peru to Chile, and along the Atlantic coast from southern Argentina to southern Brazil (Castilla and Guiñez 2000), as well as in the Falkland and Kerguelen islands (Caza et al. 2016). In Chile, the species is commercially cultivated from wild seed (Molinet et al. 2021). The New Zealand species *A. maoriana* has a finer radial sculpture than its South African and South American counterparts (Caza et al. 2016). Representative examples of those species from South Africa, Chile and New Zealand are shown in Figure 2. Colour differences reflect age differences, with the shells of *Aulacomya* changing from light brown in younger individuals to dark brown or black in older mussels (Day 1969).

Aulacomya atra is most likely descendent from *A. anderssoni* which occurred in Antarctica during the Paleocene–Early Oligocene (Crame 1999, cited in Caza et al. 2016). Its colonisation of present habitats is thought to be through natural mechanisms involving kelp-rafting or through larval dispersal, with *A. atra* larvae spending up to 4 weeks in the free-swimming planktotrophic stage (Castilla and Guiñez 2000). Historically, it appears that *A. atra* always formed a small component of overall mussel diversity in the distribution range along the South African coast. Grant and Cherry (1985) investigated species diversity using 750 shells from middens of Koi-San hunter gatherers (spanning 10 000 to 1 500 years ago), as well as raised-beach deposits (from the last interglacial period 120 000 years ago). Those authors found that *A. atra*, easily identifiable by its ribbed shell, made up <1% of the shells examined. In more recent times, its numbers have come under increased pressure, as the habitat of *A. atra* along the South African coastline has been heavily colonised by the invasive Mediterranean mussel *Mytilus galloprovincialis*. That species entered South African waters through anthropogenic actions in the 1970s (Grant and Cherry 1985; Zardi et al. 2007) and spread rapidly to become the dominant intertidal mussel along the west

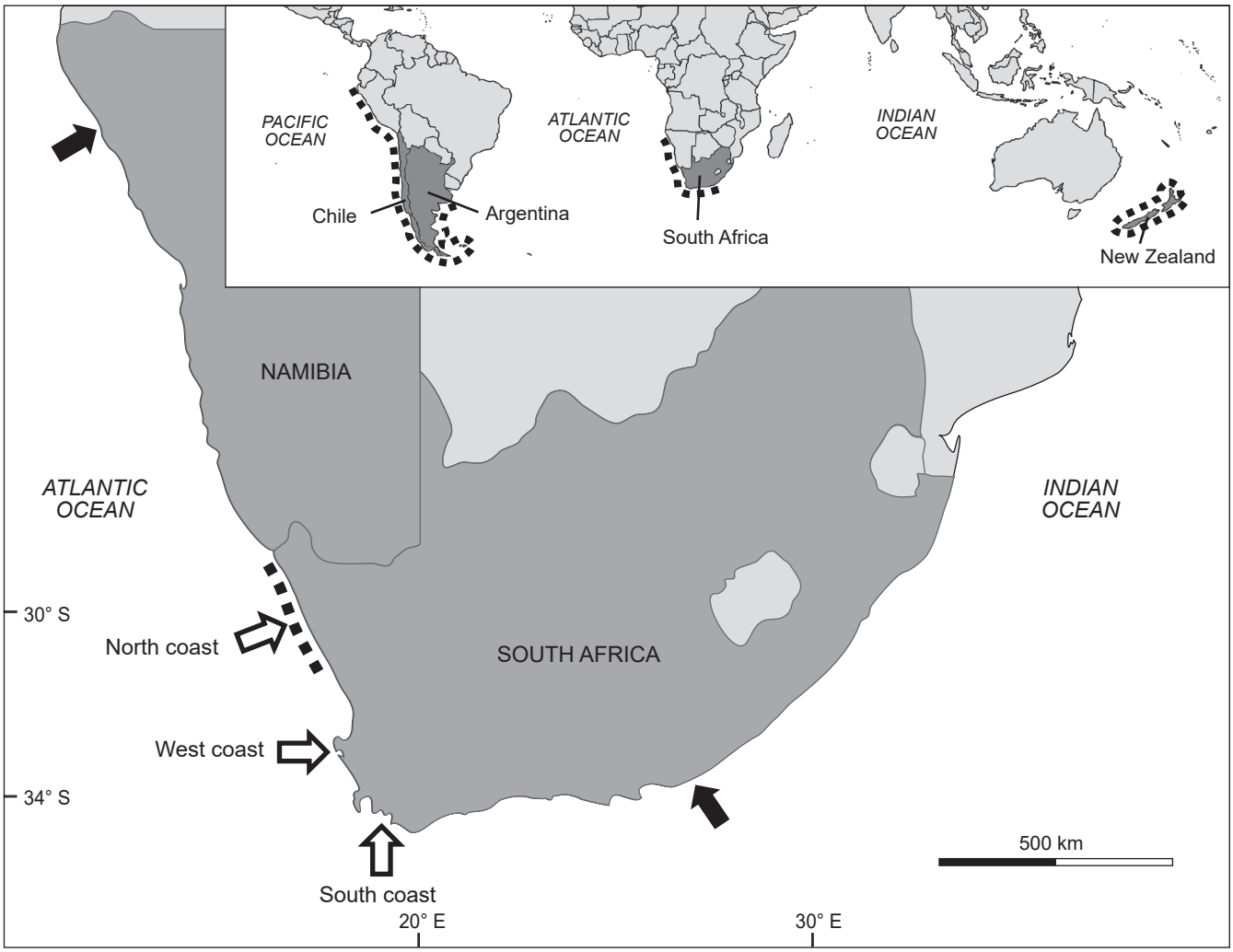

Figure 1: Distribution of the genus *Aulacomya*. The main figure shows distribution and collection sites for *Aulacomya atra* along the southern African coastline. Solid arrows indicate the northern and eastern limits of the African distribution range, according to Caza et al. (2016). Open arrows indicate sites sampled during the current study. Inset shows the coastal distributions of *A. atra* and *A. maoriana* across the full distributional range. (Maps adapted from D-maps, https://d-maps.com)

coast of South Africa. The invasive mussel has now largely displaced *A. atra* along the western and southern coasts of South Africa (Herbert 1998; Caza et al. 2016). A more recent immigrant that further invaded typical *A. atra* habitat along the west coast of South Africa and Namibia is the bisexual mussel *Semimytilus algosus*, native to Chile (de Greef et al. 2013; Ma et al. 2020). *Aulacomya atra* is now largely absent from intertidal sites but persists subtidally where the invasive species do not occur (de Greef et al. 2013).

Few genetic studies have been reported on the genus *Aulacomya* across its distributional range. Santaclara et al. (2006) developed methods for the genetic identification of a range of marine mussels found on the coast of Chile, to support legislation on the labelling of processed products and the protection of consumer rights. Wood et al. (2007) sampled specimens of *A. maoriana* on the coast of New Zealand to serve as the outgroup in a study focused on the molecular phylogeny of species of *Perna*. These studies yielded sequences for the mitochondrial DNA (mtDNA) cytochrome oxidase subunit 1 region (CO1) and the nuclear ITS1 gene. We note that Wood et al. (2007) use the taxonomic name *Aulacomya atra maoriana* to describe their specimens, thus regarding *A. maoriana* as a subspecies of *A. atra* rather than a separate species.

There are no published studies on the genetic status of *A. atra* from the South African coastline. The aims of this study were therefore to determine i) the genetic structure of *A. atra* based on selected populations along its distributional range, and ii) the genetic position of South African *A. atra* populations relative to congeneric populations in the Southern Hemisphere.

Materials and methods

Mussel samples

Individuals of *A. atra* were collected at three localities along the South African coastline (Figure 1). Twenty-five

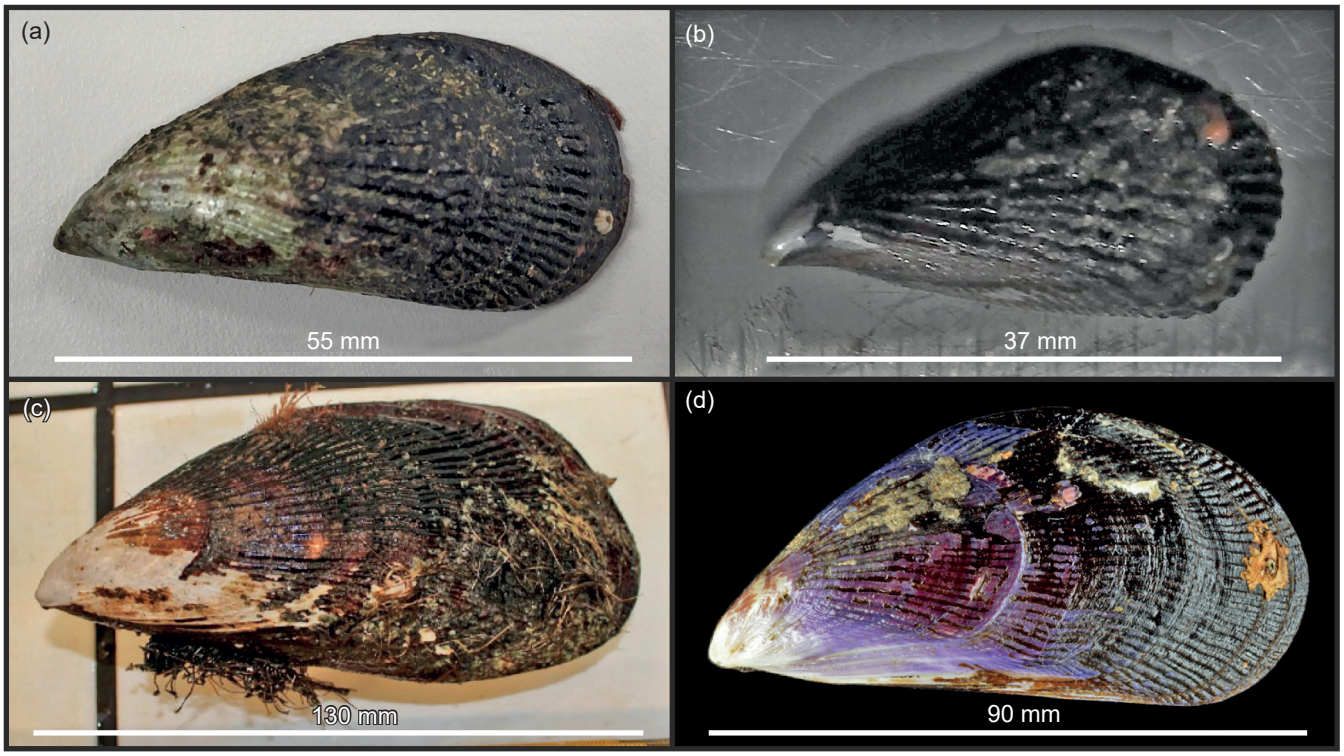

Figure 2: Comparative morphology of *Aulacomya* species from South Africa, Chile and New Zealand: (a) *A. atra* from the west coast (current study); (b) *A. atra* from the north coast (current study); (c) *A. atra* from Chile (Caza et al. 2016); and (d) *A. maoriana* from New Zealand (supplied by A Spurgeon, private collector, New Zealand)

mussels were sampled from the St Helena Bay area in the Western Cape Province, in the Benguela ecoregion (as defined by Sink et al. 2012) on the Atlantic coast (here designated 'west coast': 32°43′38″ S, 17°55′00″ E). A further 24 mussels were collected from the Hermanus area on the south coast of the Western Cape in the Agulhas ecoregion near the eastern edge of the Atlantic Ocean ('south coast': 34°26′13″ S, 19°13′19″ E). Individuals in these west and south coast populations were each sampled within a ~100-m radius, and the sample sets were therefore considered suitable for population genetic analysis. We also collected four mussels from farther north along the coast of the Northern Cape Province ('north coast'), in a zone stretching over a range of several hundred kilometres from latitude 28°69′ S to 31°14′ S along the Benguela ecoregion, to use for the phylogenetic analysis.

All mussels were collected from the intertidal zone, suggesting that *A. atra* is persisting in that environment. *Aulacomya atra* was nevertheless outnumbered by the invasive *M. galloprovincialis* at all localities sampled. Mantle tissue was collected and stored in 95% ETOH. DNA was isolated using the Roche© High Pure PCR Template preparation kit and following the manufacturer's instructions. Digestion time was increased to allow for complete digestion of the tough mantle-edge tissues. DNA quantification was performed with a NanoDrop® 1000 Spectrophotometer V3.7 (Thermo Fisher Scientific, Waltham, Massachusetts) to evaluate the success of the extraction procedures, by measuring both the quantity and quality of isolated DNA.

Genetic variation

Levels of genetic diversity and differentiation were determined using the mitochondrial DNA cytochrome c oxidase subunit 1 (CO1) region and the nuclear internal transcribed spacer (ITS1). The CO1 region was amplified and sequenced using the modified primers described by Geller et al. (2013), after results from the original CO1 primers by Folmer et al. (1994) yielded unsatisfactory results. The Geller et al. (2013) primer pair amplifies the same CO1 region as the Folmer et al. (1994) primers but contains several degenerate sites to allow for annealing in a wider range of marine invertebrates. Primer sequences for amplification of the CO1 region were jgLCO1490: 5′-TIT CIA CIA AYC AYA ARG AYA TTG G-3′ and jgHCO2198: 5′-TAI ACY TCI GGR TGI CCR AAR AAY CA-3′. Primer sequences for the amplification of ITS1 were XelaITS1_F: 5′-AAG TAA AAG TCG TAA CAA GGT TTC CGT AGG-3′ and OnmyITS1_R: 5′-CAA GCC GAG TGA TCC ACC GC-3′, as reported by Pérez et al. (2004) and Santaclara et al. (2006).

All PCR amplification reactions were carried out in a volume of 12.5 µl, containing 6.25 µl of TEMPase Hot Start 2x Master Mix A (Ampliqon), 0.313 µl of each primer (at 10 µM, providing a final concentration of 1 µM), 3.625 µl of ddH_2O, and 2 µl of template DNA. Reaction conditions were: 95 °C for 15 min, followed by 35 cycles of 30 sec at 95 °C, 40 sec at the optimal annealing temperature and a 90-sec extension step at 72 °C, and with a final extension step of 5 min at 72 °C. The optimal annealing temperatures,

determined through experimental amplification at 2-degree intervals, were 54 °C for CO1 and 67 °C for ITS1. Products from PCR were cleaned using the Biospin PCR Purification Kit (Bio-Rad), followed by sequencing using the ABI Prism® BigDye® Terminator 3.1 Cycle Sequencing Kit, standard conditions and the same annealing temperature used for the initial PCR amplification. Products were cleaned using BigDye® Xterminator, and sequences were then resolved on an ABI 3500 Genetic Analyser.

Sequences were inspected, aligned and trimmed using Geneious Prime 2021.2.2 (https://www.geneious.com). Unique haplotypes for the mtDNA CO1 region were identified using DnaSP 5.10.01 (Rozas et al. 2017). For the diploid nuclear ITS1 region, visual inspection showed heterozygotes at various positions, which were coded using ambiguity characters. The dataset was then phased into haplotypes using a Bayesian method in Phase 2.1 (Stephens et al. 2001; Stephens and Scheet 2005) as implemented in the DnaSP software, to get a complete picture of genetic variation. The phased haplotypes were used for all subsequent analyses involving the ITS1 region.

For population genetic analysis, the genetic diversity within each population was determined using the number of distinct haplotypes, haplotype diversity (Hd) and nucleotide diversity (π). The frequencies of each haplotype per population were also determined. Genetic differentiation between populations was quantified using the F_{ST} statistic and the average number of pairwise differences between populations (Pi), with Arlequin software version 3.5.2.2 (Excoffier et al. 2005). The average number of nucleotide substitutions per site between populations (D_{xy}) and the number of net nucleotide substitutions per site between populations (D_a) were determined using the software package DnaSP. The population genetic analysis was restricted to the west and south coast populations, with only haplotype frequencies calculated for the north coast sample.

Phylogenetic analysis

For the phylogenetic analysis we first downloaded all CO1 and ITS1 sequences available for *A. atra* on GenBank. For CO1, we used MN454335.1–MN454337.1 from Argentina; JF301750.1, JF301751.1 and JF301757.1 from Chile; and DQ917614.1 and DQ917615.1 from New Zealand. Sequences of the Atlantic ribbed marsh mussel *Geukensia demissa* from the Northern Hemisphere (KU905866.1 and KU906097.1) were downloaded to use as the outgroup. For the ITS1 region, the only GenBank material available were two sequences for *A. maoriana* from New Zealand (DQ924557.1 and DQ924558.1). Available sequences for *G. demissa* showed only 22% identity with those of *A. atra*, but haplotype AY621962.1 was nevertheless used to root trees in the subsequent analyses after a BLAST search yielded no material on GenBank showing better identity with *A. atra*. MEGA11 software (Tamura et al. 2021) was first used to determine the best model of nucleotide substitution for each marker. Results showed that the CO1 data are best described by a Tamura–Nei model of substitution, with the ITS1 data following a Kimura 2-parameter model. A maximum likelihood tree was then generated for each region using MEGA, with 1 000 bootstrap replications and implementing the relevant model of nucleotide substitution. Data from CO1 and ITS1 were analysed separately rather than as concatenated haplotypes, considering the different modes of inheritance of these mtDNA and nuclear markers.

Genetic differentiation among the haplotypes resolved were further investigated using haplotype network analysis. A minimum spanning approach was used, as implemented in Network 10.2.0.0 (Fluxus Engineering 2022). The South African samples as well as GenBank-sourced sequences of *Aulacomya* were used during the construction of haplotype networks.

Results

After alignment and trimming, a total length of 583 base pairs (bp) was available for the CO1 region, and 362 bp for the ITS1 region. Results yielded 36 unique haplotypes for CO1, and 12 unique phased haplotypes for ITS1. A representative of each unique haplotype was uploaded to the NCBI GenBank database, with accession numbers OP161078–OP161113 for CO1, and OP204639–OP204650 for ITS1.

The genetic diversity values based on CO1 haplotypes (Table 1) were comparable in the *A. atra* populations from the west and south coasts. Results from ITS1 showed that this region is slightly less diverse in the west coast population compared with in the south coast population. Haplotype frequencies for CO1 and phased ITS1 haplotypes are presented in Table 2. For CO1, only three haplotypes out of 36 resolved occurred in more than one sampled region, with only a single haplotype found in all three regions. Of the remaining haplotypes, 2 were unique to the north coast, 18 to the west coast, and 14 to the south coast. For the ITS1 region, three out of 12 haplotypes occurred in all three regions, with 5 unique to the north coast, 1 unique to the west coast, and 3 unique to the south coast.

Calculation of population differentiation based on the CO1 data yielded an F_{ST} value of 0.010 (p = 0.198) between the west and south coast populations. Based on the nuclear ITS1 data, the F_{ST} value was 0.002 (p = 0.316). Results from both CO1 and ITS1 also showed nonsignificant (p > 0.05) levels of differentiation between the west and south coast populations based on the average number of pairwise differences between populations, with Pi = 1.175 (p = 0.255) from CO1 haplotypes, and Pi = 3.664 (p = 0.318) from ITS1. The average number of nucleotide substitutions per site between populations and the number of net nucleotide substitutions per site between populations were D_{xy} = 0.006 and D_a = 0.00006 from CO1, with D_{xy} = 0.003 and D_a = 0.00001 based on ITS1.

After alignment and trimming to match the GenBank-sourced reference sequences for phylogenetic analysis, a total length of 480 bp was available for the CO1 region. The length of the ITS1 sequences remained at the full 362 bp resolved after alignment with reference sequences.

A phylogenetic tree generated from the CO1 data (Figure 3) shows no clustering based on geographic origin among the South African samples, with haplotypes from the north coast, west coast and south coast intermingled, and with weak (≤64%) bootstrap support for most nodes. Among the reference populations, *A. atra* from Argentina formed a clade closest to the South African populations, with

95–97% bootstrap support. Second-closest to the South African groups were a clade comprising samples from Chile (42% bootstrap support), with *A. maoriana* from New Zealand the most distant (99% bootstrap support). Samples of *Geukensia demissa* from the Northern Hemisphere clustered distantly from samples of the *Aulacomya* populations, with 100% bootstrap support. Since 103 bp of the CO1 sequences were lost during alignment with the reference samples, the analysis was re-run using only the South African samples and the full 583 bp. Results (not shown) were similar to those from the shorter sequences, with no signature of geographic differentiation.

As with CO1, the ITS1 haplotypes showed no geographic clustering among South African populations that correlate with geographic distribution, and with individuals from all three regions sampled also sharing a number of haplotypes (Figure 4). Furthermore, *A. maoriana* from New Zealand clustered with South African haplotypes, and there was more identity between South African haplotypes ITSHap-3 and ITSHap-9 and the New Zealand samples than there was between the two South African haplotypes and the remaining haplotypes resolved from the South African populations. *Geukensia demissa* from the Northern Hemisphere clustered well away from the *Aulacomya* populations.

Minimum spanning networks based on the number of mutational events between unique haplotypes are presented in Figure 5a,b. The results mirrored the trends in the phylogenetic analysis. There was no evidence of geographic structure among the South African populations. The CO1-based data confirms progressively increasing genetic distance that correlated with spatial patterns of distribution of the South African populations and samples from Argentina, Chile and New Zealand. Conversely, samples from South Africa and New Zealand were intermingled based on ITS1 haplotypes.

Table 1: Genetic diversity within three regional populations of *Aulacomya atra* along the South African coastline, based on haplotypes observed in the CO1 and ITS1 regions. Values for ITS1 are based on the phased haplotypes resolved in each population, with the phased sample size shown in parentheses

Population	Sample size	No. of distinct haplotypes	Haplotype diversity (Hd)	Nucleotide diversity (π)
		CO1 region		
North coast	4	4	–	–
West coast	22	20	0.984	0.007
South coast	23	16	0.961	0.006
Overall	49	36	0.974	0.006
		ITS1 region		
North coast	4 (8)	8	–	–
West coast	21 (42)	4	0.573	0.003
South coast	21 (42)	6	0.715	0.003
Overall	46 (92)	12	0.683	0.005

Discussion

The results of the study are characterised by high levels of genetic diversity within South African populations of *A. atra*, but with a weak signature of geographic differentiation. The level of haplotype diversity from CO1 haplotypes (0.961–0.984, overall Hd = 0.974) is higher than the values reported for populations of the indigenous brown mussel *Perna perna* by Barker (2021), who estimated population-specific values of 0.378–0.933 (overall Hd = 0.846) across 10 populations over two seasons. De Oliveira et al. (2017) estimated Hd = 0.457 and 0.901 in two haplogroups of *P. perna* from Brazil, Venezuela and the African coast, which is also lower than values for the South African *A. atra* in the current study. The estimated levels of nucleotide diversity from CO1 haplotypes (0.006–0.007, overall π = 0.006) is within to slightly below the range for *P. perna*, for which Barker (2021) reported values of generally 0.001–0.014 (average 0.017). The values for *A. atra*, however, are higher compared with π = 0.002–0.006 reported for *P. perna* by de Oliveira et al. (2017) from CO1. The comparatively high level of CO1-based diversity in South African *A. atra* populations is notable, considering the low extant density and the threats

Table 2: Haplotype frequencies for the CO1 and ITS1 regions in *Aulacomya atra* from three regions on the South African coastline

Haplotype	CO1 region			Haplotype	ITS1 region		
	North coast	West coast	South coast		North coast	West coast	South coast
Hap1–2	0.250	–	–	Hap1–2 (2)	0.100	–	–
Hap3	0.250	0.043	0.091	Hap3	0.100	0.52381	0.375
Hap4	0.250	0.043	–	Hap4–5 (2)	0.100	–	–
Hap5–9	–	–	0.045	Hap5	0.100	–	–
Hap10	–	0.130	0.182	Hap6	0.300	0.40476	0.375
Hap11	–	–	0.045	Hap7	0.100	–	–
Hap12	–	–	0.091	Hap8	0.100	0.04762	0.125
Hap13–14	–	–	0.045	Hap9	–	0.02381	–
Hap15	–	–	0.091	Hap10	–	–	0.050
Hap16–19	–	–	0.045	Hap11	–	–	0.025
Hap20–21	–	0.043	–	Hap12	–	–	0.050
Hap22	–	0.087	–				
Hap23–36	–	0.043	–				

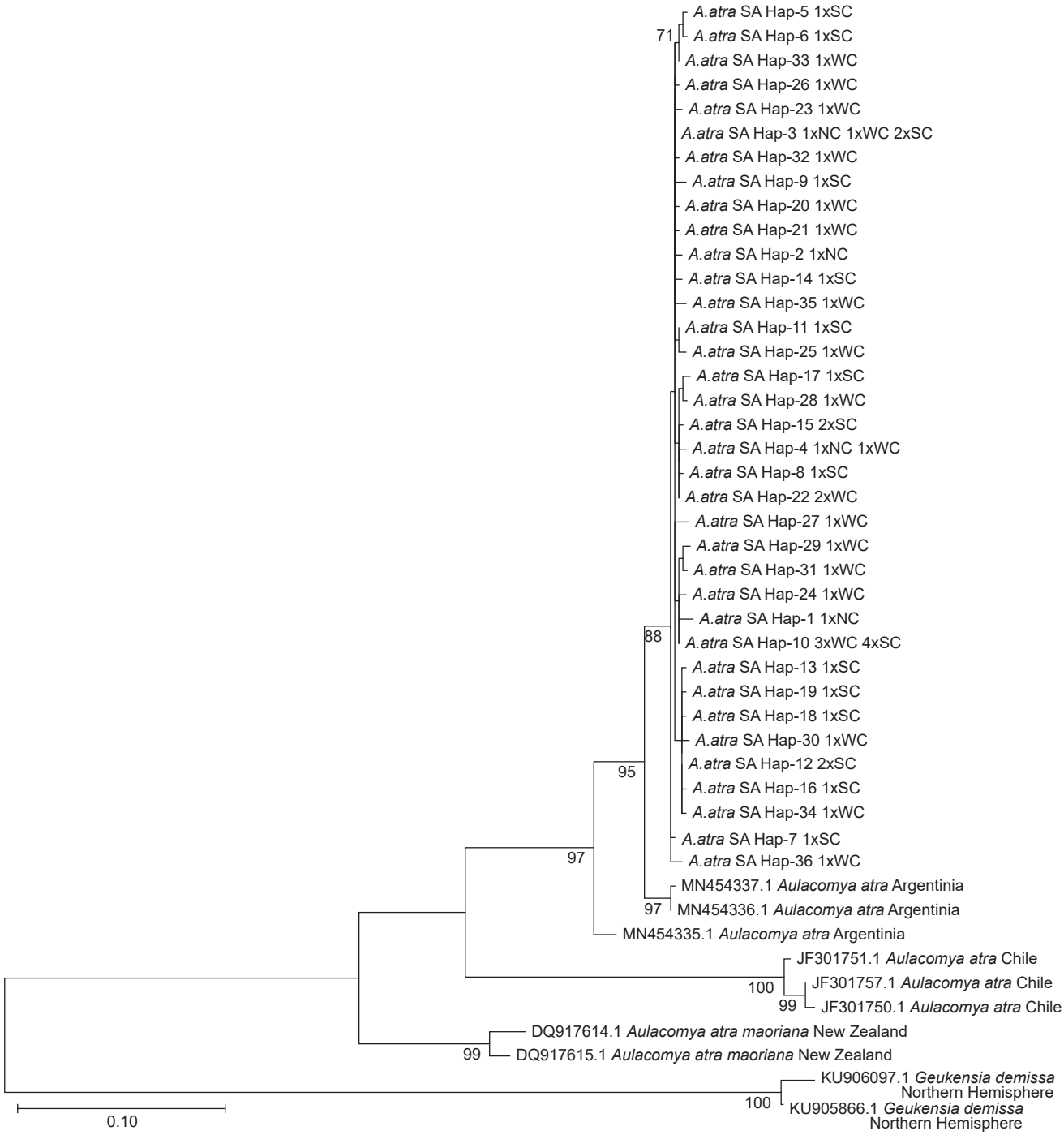

Figure 3: Evolutionary history inferred between *Aulacomya atra* from six regions in the Southern Hemisphere, with *Geukensia demissa* from the Northern Hemisphere used to root the tree, based on haplotypes of the CO1 region. A maximum likelihood method and Hasegawa–Kishino–Yano model was used and the tree with the highest log likelihood is shown, following 1 000 bootstrap replications. The percentage of the tree in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap values below 70% are not shown. This analysis involved 46 nucleotide sequences, with a total of 583 bp positions in the final dataset

to numbers of this species along the South African coastline. This could indicate that the population size of *A. atra* historically may have been relatively large, despite recent demographic declines.

Results from the population genetic analysis provide no evidence of significant differentiation between the west and south coast populations, notwithstanding some differences among populations suggested by the presence and

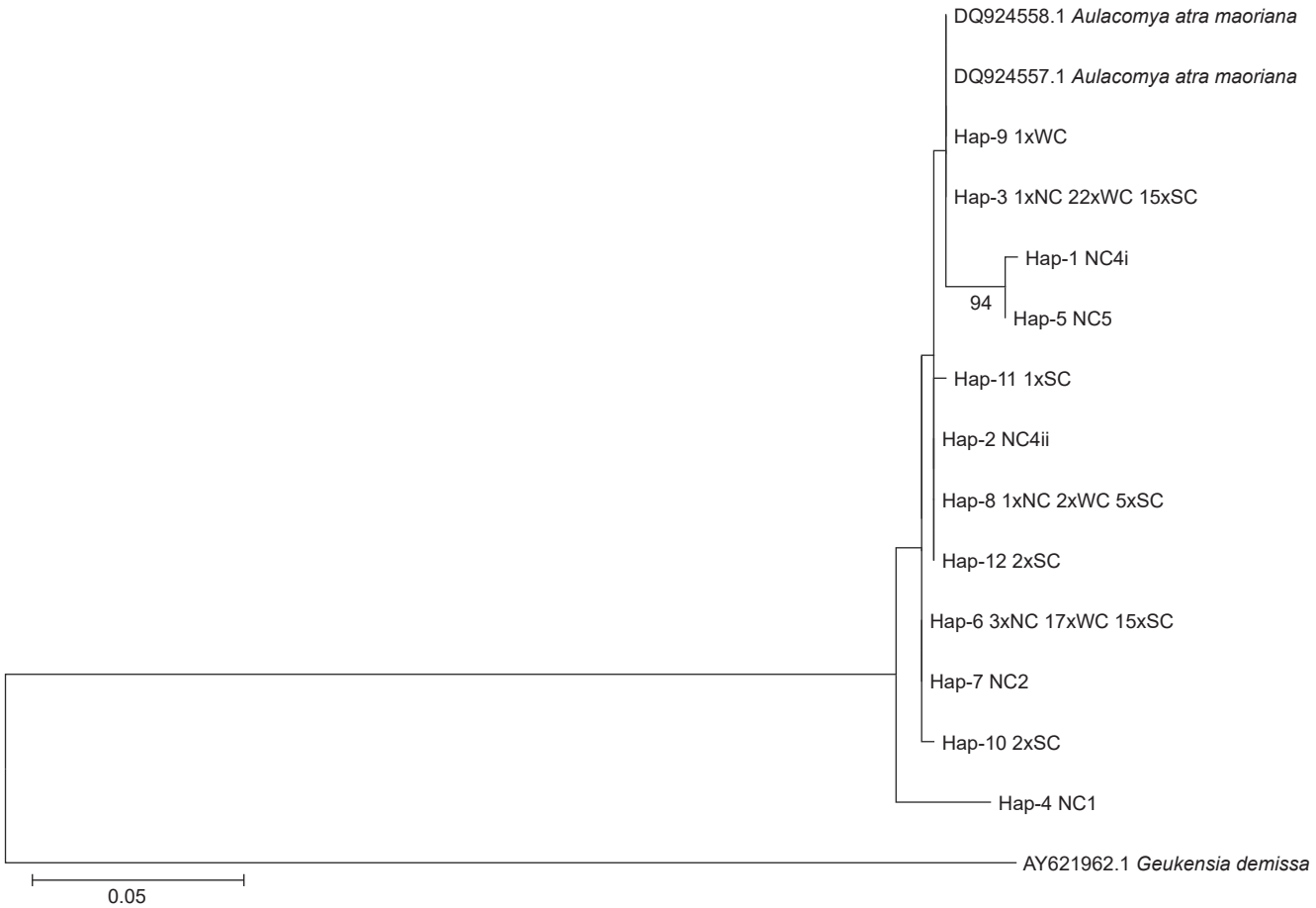

Figure 4: Evolutionary history inferred between *Aulacomya atra* and *A. maoriana* from four localities in the Southern Hemisphere, and with *Geukensia demissa* from the Northern Hemisphere used to root the tree, based on haplotypes of the ITS1 region. A maximum-likelihood method and Kimura 2-parameter model was used and the tree with the highest log likelihood is shown, following 1 000 bootstrap replications. The percentage of the tree in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap values below 70% are not shown. This analysis involved 15 nucleotide sequences and a total of 433 bp positions in the final dataset

absence of specific haplotypes in the respective populations (Table 2). This is evident from the F_{ST} values (p = 0.200) and the average number of pairwise differences between populations (p = 0.318) based on the CO1 haplotypes resolved. For ITS1 data, differentiation between the west coast and south coast populations is also not significant using these measures (with p = 0.316 and p = 0.255, respectively). Indeed, the F_{ST} values of 0.010 and 0.002 suggest that more than 99% of variation occurs within the studied populations from the west and south coasts. These trends are supported by results of the phylogenetic analysis and the haplotype networks constructed from CO1 and ITS1 haplotypes, which also show little pattern of phylogeographic structure along the South African coastline, with haplotypes for all three regions intermingled in the phylogenetic tree and haplotype networks.

The geographic structure of *A. atra* could be determined by larval dispersal, isolation by distance, and barriers or enablers of gene flow, as well as through selection. The adults of *A. atra* attach to rocks and vegetation and generally are stationary during their lifetime. However, the larvae are planktonic and can travel kilometres along the coastline, drifting on currents prior to settlement (Solís et al. 2019). The three localities sampled are affected differently by the currents occurring off South Africa, which could affect dispersal as well as temperature tolerance. The warm Agulhas Current flows southwestwards down the eastern to southern coastline of South Africa, terminating at the edge of the Agulhas Bank, whereas the western coastline is influenced by the cold Benguela Current, which flows northwards from the Cape of Good Hope (Zardi et al. 2007). The western and northern populations sampled in the Benguela ecoregion are located within the influence of the colder Benguela Current. The southern population, originating from the Hermanus area in the Agulhas ecoregion, lies within the influence of the warm current despite being relatively close to the Cape of Good Hope. It is thus possible that *A. atra* from the western and southern sample sites could be subjected to different environmental selective forces. Zardi et al. (2015) stated that these environmental discontinuities along the coastline may be mirrored in phylogeographic breaks in species

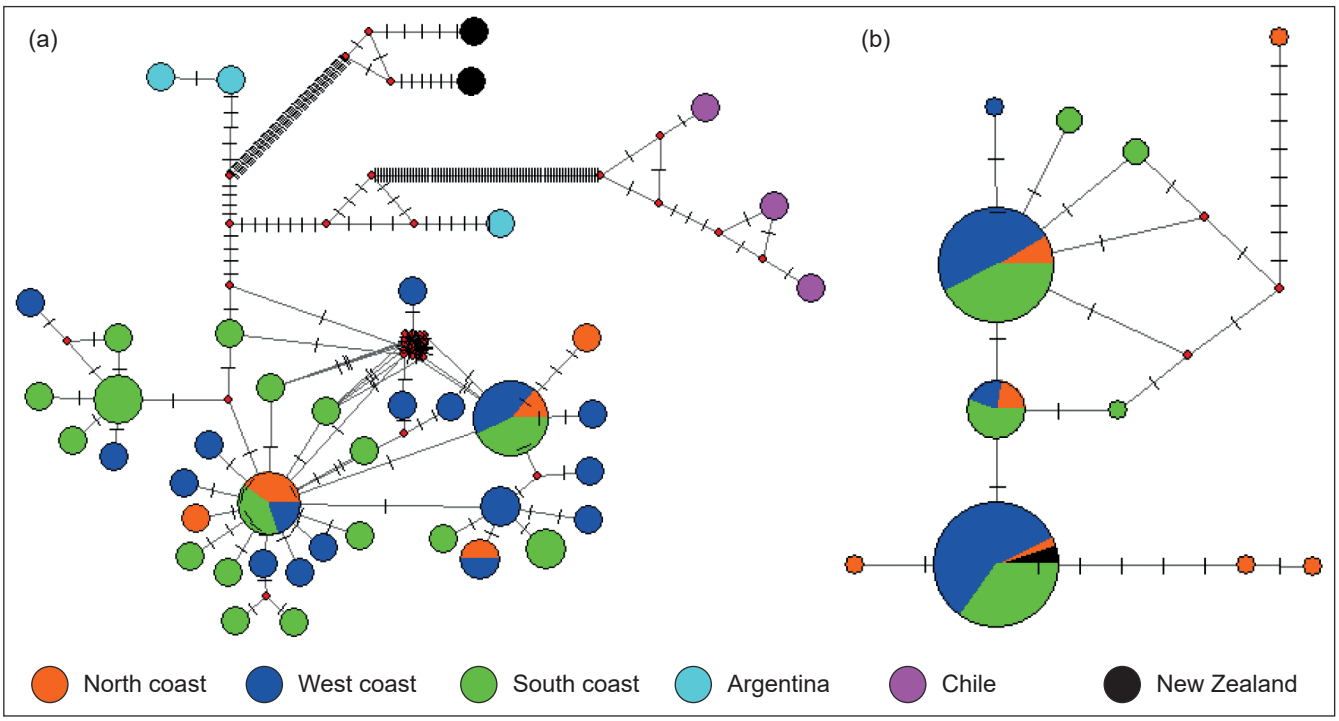

Figure 5: Haplotype network showing the relationships of haplotypes among groups of mussels, from haplotypes of (a) the CO1 region, and (b) the ITS1 region. Each unique haplotype is represented by a circle, with the size of the node proportional to the number of individuals displaying the haplotypes. Lines between nodes indicate mutations, with diagonal lines on connecting lines indicating the number of mutations between haplotypes. Coloured zones in nodes indicate the localities(s) where each haplotype was observed

that occur along the shores of more than one province. Such differentiation was not observed during the current study but may possibly be detected using markers with adaptive significance. Differentiation may also be found in populations farther north and east of the confluence of the Benguela and Agulhas currents.

On a large spatial scale, the results from CO1 are congruent with the geographic positions of the populations studied. *Aulacomya atra* from Argentina is more closely related to the South African populations than to the remaining reference populations. Caza et al. (2016) hypothesised that *A. atra* originated from the coast of Antarctica to colonise coastlines on the western and eastern shores of the Atlantic, which is in agreement with the patterns observed in our CO1-based phylogenetic trees and haplotype networks. Colonisation of the Chilean coastline on the Pacific side of South America would have involved a different route, contributing to more differentiation from South African and Argentinian populations. The New Zealand population is farthest removed from the remaining populations and is in fact regarded as a different species (*A. maoriana*), and it also grouped as the most distant during our analyses. Conversely, there was no differentiation between *A. atra* from South Africa and *A. maoriana* from New Zealand based on the ITS1 region. This should, however, be further investigated using a larger sample size for New Zealand, since only two sequences for ITS1 from *A. maoriana* are available on GenBank.

This study provides the first data on genetic diversity in *A. atra* from the South African coastline, and the position of South African populations relative to congeneric populations from Argentina, Chile and New Zealand. We suggest that the following be incorporated in a follow-up study: first, more southern African populations should be sampled intensively, specifically towards the limits of the distribution area in the northern and eastern parts of the distribution range. This will enable population genetic analysis along the full distribution area on the South African and Namibian coastlines and indicate whether the low level of differentiation observed between populations close to the confluence of the Agulhas and Benguela currents holds true for populations farther removed from this area. In addition, the range of markers can be expanded to include genes with adaptive significance, to determine whether populations in the Agulhas and Benguela ecoregions are impacted by different selective influences relating to water temperature and nutrition. Finally, obtaining larger sample sizes for members of the genus from the wider distribution range, particularly in New Zealand, will increase phylogenetic accuracy and allow for analyses of a more detailed pattern of genetic connectivity between the populations as discussed here.

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References

Barker C. 2021. Phylogeography and reproductive isolation of the brown mussel, *Perna perna*, on the South African coastline. MSc thesis, Rhodes University, South Africa.

Castilla JC, Guiñez R. 2000. Disjoint geographical distribution of intertidal and nearshore benthic invertebrates in the Southern Hemisphere. *Revista Chilena de Historia Natural* 73: 585–603.

Caza F, Cledon M, St-Pierre Y. 2016. Biomonitoring climate change and pollution in marine ecosystems: a review on *Aulacomya ater*. *Journal of Marine Biology* 2016: article 7183813.

Day JH. 1969. *A guide to marine life on South African shores*. Cape Town, South Africa: Balkema.

de Greef K, Griffiths CL, Zeeman Z. 2013. Deja vu? A second mytilid mussel, *Semimytilus algosus*, invades South Africa's west coast. *African Journal of Marine Science* 35: 307–313.

de Oliveira MJS, Beasley CR, Barros NGV, do Socorro Marques-Silva N, de Simone LRL, Lima ES, Tagliaro CH. 2017. Two African origins of naturalized brown mussel (*Perna perna*) in Brazil: past and present bioinvasions. *Hydrobiologia* 794: 59–72.

Excoffier L, Laval G, Schneider S. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47–50.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.

Geller J, Meyer C, Parker M, Hawk H. 2013. Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources* 13: 851–861.

Grant WS, Cherry MI. 1985. *Mytilus galloprovincialis* Lmk. in southern Africa. *Journal of Experimental Marine Biology and Ecology* 90: 179–191.

Herbert DG. 1998. Molluscan conservation in South Africa: diversity, issues and priorities. *Journal of Conchology Special Publication* 2: 61–76.

Ma KCK, Zardi GI, McQuaid CD, Nicastro KD. 2020. Historical and contemporary range expansion of an invasive mussel, *Semimytlius algosus*, in Angola and Namibia despite data scarcity in an infrequently surveyed region. *PLoS ONE* 15: e0239167.

Molinet C, Astorga M, Cares L, Diaz M, Hueicha K, Marín S et al. 2021. Vertical distribution patterns of larval supply and spatfall of three species of Mytilidae in a Chilean fjord used for mussel farming: insights for mussel spatfall efficiency. *Aquaculture (Amsterdam, Netherlands)* 535: article 736341.

Pérez M, Cabado AG, Vieites JM, Presa P. 2004. Experimental assessment of a new rDNA-based method for the identification of *Merluccius capensis* and *Merluccius paradoxus* in commercial products. *Journal of Aquatic Food Product Technology* 13: 49–57.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution* 34: 3299–3302.

Santaclara FJ, Espiñeira M, Cabado AG, Aldasoro A, Gonzalez-Lavín N, Vieites JM. 2006. Development of a method for the genetic identification of mussel species belonging to *Mytilus, Perna, Aulacomya*, and other genera. *Journal of Agricultural and Food Chemistry* 54: 8461−8470.

Sink K, Holness S, Harris L, Majiedt P, Atkinson L, Robinson T et al. 2012. National Biodiversity Assessment 2011. Technical Report, vol. 4: marine and coastal component. Pretoria, South Africa: South African National Biodiversity Institute.

Solís MA, Ballesteros M, Riascos JM. 2019. The early life-history transitions of the bivalve *Aulacomya atra* from the Humboldt current system off Peru are affected by human exploitation and modulated by El Niño–La Niña cycle. *Frontiers in Marine Science* 6: article 496.

Stephens M, Scheet P. 2005. Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. *The American Journal of Human Genetics* 76: 449–462.

Stephens M, Smith NJ, Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. *The American Journal of Human Genetics* 68: 978–989.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution* 38: 3022–3027.

Wood AR, Apte S, MacAvoy ES, Gardner JPA. 2007. A molecular phylogeny of the marine mussel genus *Perna* (Bivalvia: Mytilidae) based on nuclear (ITS1&2) and mitochondrial (COI) DNA sequences. *Molecular Phylogenetics and Evolution* 44: 685–698.

Zardi GI, McQuaid CD, Teske PR, Barker NP. 2007. Unexpected genetic structure of mussel populations in South Africa: indigenous *Perna perna* and invasive *Mytilus galloprovincialis*. *Marine Ecology Progress Series* 337: 135–144.

Zardi GI, Nicastro KR, McQuaid CD, Castilho R, Costa J, Serrão EA, Pearson GA. 2015. Intraspecific genetic lineages of a marine mussel show behavioural divergence and spatial segregation over a tropical/subtropical biogeographic transition. *BMC Evolutionary Biology* 15: article 100.

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