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Clam fishing along 1300 km Chilean coastline: A morphometric and genetic survey of *Mulinia edulis* King, 1832

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ABSTRACT

Mulinia edulis, also known as Taquilla clam, is a fishery resource, and one of the 6 species of clams commercially exploited in Chile. Valuable advances have been made for its culture, but there is still uncertainty regarding the species associated with the main commercial banks. The hypothesis was that, if the different harvested banks of *M. edulis* correspond to a single species along the Chilean coast, when comparing individuals from the north, central and south, they should not present significant morphological differences in their valves. The aim of this paper is to analyze and compare the morphology of *M. edulis* valves using geometric morphology and genetic comparison by sequencing the mitochondrial gene Cytochrome oxidase I and 16S RNA gene. Samples were obtained in three main fishery grounds, Tongoy Bay (northern), Arauco Gulf (central) and Ancud Bay (southern). In the inner side of the right valve 28 morphological landmarks were set, using TPS Util and R package geomorph, landmarks settings were overlaid through the generalized Procrustes analysis. Principal Components Analysis (PCA), using residuals were conducted among locations and sex. The results show no in-fluence of size or sex on shape of the valves. Through PCA analysis banks were differentiated by their shape. Abductor muscle and pallial sinus show the most relevant differences, especially when comparing northern and southern clams, where Arauco Gulf shows an intermediate morph. The three localities show low genetic diversity and little differentiation between them. This finding suggests that the fishery of taquilla clam is sustained only by *M. edulis*.

1. Introduction

The geographical distribution of the Mactridae family Lamarck, 1809 extends from the northwestern Atlantic Ocean to the Caribbean Sea and the Pacific coast of North America to the Gulf of California in Mexico. In the South Pacific, it is distributed between Ecuador and Chile and between Argentina and Brazil in the Atlantic Ocean. This family also occur in the Red Sea, Asia (Singapore; China) and Oceania (Australia) (Smith, 1914; Cruz, 1983; Avila-Serrano et al., 2006; Wong, 2009; Signorelli and A Pastorino, 2012; Signorelli and Pastorino, 2012; Signorelli and Pastorino, 2011). The following features characterized the family from a morphological point of view: The equivalent shells with two cardinal teeth fused in the left valve that makes the tooth "V" shaped, the right valve presents unfused cardinal teeth, the anterior and posterior lateral teeth generally have a single cusp. In turn, the lobes of the mantle are more or less free underneath, joined at the front and back, and extended into two retractable siphons (Signorelli and Pastorino, 2011).

Signorelli (2019) described that within the family Mactridae, four

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subfamilies are included, where the subfamily Mactrinae (Lamarck, 1809) have 25 genera, including the genus *Mulinia* Gray, 1837. This genus has a wide distribution range, extending from the northern Atlantic to northern Pacific and eastern Africa. They inhabit sandy sediments or substrates with high silt and clay content (such as estuarine areas and protected bays). *Mulinia* is characterized by a trigonal shell, with the anterior and posterior subangular end, the ligaments are exclusively internal, and it has a well-developed chondrophore (Signorelli and Pastorino, 2011; MolluscaBase et al., 2024; Signorelli, 2019). In the southeastern Pacific, five species are recorded: *Mulinia bicolor* Gray, 1837; *Mulinia byronensis* Gray, 1837; *Mulinia levicardo* (Smith, 1881); *Mulinia exalbida* (Gray, 1837) and *Mulinia edulis* (King, 1832) (Signorelli and Pastorino, 2011; González et al., 2002), which are differentiated by shell morphology.

M. edulis, commonly known as "sweet clam" or "taquilla clam", is distributed in the southeastern Pacific from Guayaquil in Ecuador to the Strait of Magellan and Tierra del Fuego in Chile and is also found in Ushuaia, Argentina in the southwest Atlantic (Signorelli and Pastorino, 2011; WORMS World Register of Marine, 2024). This species has a trigonal to subcircular, inflated, opaque white, yellowish and sometimes grayish shell. The umbos are slightly inflated, external surface smooth and with concentric growth lines. It has a curved ventral border without acute angles, the pallial sinus is separated from the ventral border, U-shaped, horizontal and approximately half the length of the shell. The right valve has short, small lateral teeth and the cardinal teeth are unfused (Signorelli and Pastorino, 2011).

The "taquilla clam" is one of the 6 clam's species that are commercially harvested in Chile (Oliva et al., 2013); the fishery started in the 1980s and is regulated by a minimum catch size of 5.5 cm in valvar length (D.S. No. 683, of 1980) and a 5-year extractive ban (regulation D. S. No. 12/2023) for Ancud Bay in Los Lagos Region. The extraction is carried out by hookah diving and the resource is exported canned, frozen and fresh-refrigerated to countries such as Spain, the United States, Argentina and Mexico (Díaz and Cancino, 2015; SUBPESCA, 2020). In Chile the main fishery grounds are in the Coquimbo (northern), in the Biobío (central) and in the Los Lagos Region (southern). The largest landings were recorded in the 2000s with 8000 t per year. Currently, the fishery is concentrated in the Biobío Region with a landing of 1421 t in 2022 (SUBPESCA, 2020; SERNAPESCA, 2022). The banks of Tubul in the Arauco Gulf and Quetalmahue in Ancud Bay are described as the main fishing areas for the Biobío and Los Lagos Regions, respectively. In the northern zone, Stotz et al. (2008), studied geometric morphometry, DNA and re-productive aspects (reproductive cycle, size of first sexual maturity and sperm morphology). In the Central and Southern zone Jaramillo et al. (2008) analyzed the reproduction (reproductive cycle and sperm morphology), morphology and growth of the taquilla clam.

On the other hand, advances in the development of technology for the commercial cultivation of *M. edulis* have been made. Oliva et al. (2014), evaluated the effect of culture density and food ration on the growth and survival of veliger, pediveliger larvae and postlarvae (Oliva et al., 2013) and Oliva et al. (2020) and Abarca et al. (2012), developed the technology for fattening *M. edulis* seeds in suspended and bottom cultures.

Chile has an established fishery for the "taquilla clam" with developed international markets for its export and important advances in the technology for its cultivation. However, it is still not clear which species are associated with the main banks, since, according to Jaramillo et al. (2008), the characters used to separate the nominal species of *Mulinia* are not valid as diagnostic characters and there is insufficient morphometric evidence to separate the different taquilla clam populations.

Geometric morphometry has been used as a technique to discriminate between different species, since it allows interpretations of high biological significance. This technique is based on Rohlf & Slice (Rohlf and Slice, 1990), who indicate that shape is all the geometric information that results from removing the effects of the position, scale and rotation of an object, complemented by genetic techniques.

According to Adams et al. (2004) and Toro et al. (Toro Ibacache et al., 2010), geometric morphometric methods based on landmarks start with the collection of two-dimensional (x, y) or three-dimensional (x, y, z) coordinates of biologically definable landmarks that describe their position in space. Direct analysis of these coordinates as variables would be inappropriate as the effects of variation on the position, orientation, and scale of specimens are still present. Therefore, non-form dependent variation must be mathematically eliminated before analyzing such variables. Once the nonshape variation has been eliminated, the variables are converted to shape variables and can be used to statistically compare samples, and shape plots can be generated for comparison.

Adams et al. (2004) pointed out that overlapping methods eliminate formless variation in cue point configurations by overlapping them according to some optimization criteria. Several methods have been proposed, each with slightly different protocols and optimization criteria. The Generalized Procrustes Analysis (GPA) overlays set-point configurations using least squares estimates for translation and rotation parameters. First, the centroid of each configuration is translated to the source, and the configurations are scaled to a common unit size (dividing by the size of the centroid). Finally, the configurations are optimally rotated to minimize squared differences between the corresponding reference points. In other words, Procrustes' analysis allows to obtain variables of size and shape from Cartesian coordinates of anatomical points and then analyze them using multivariate statistical techniques (Torcida and Pérez, 2012).

Based on Signorelli et al. (2013), the morphological analysis of mollusk shells plays an important role in various fields, such as taxonomy and evolution or fisheries management. Several authors have used geometric morphometry as a technique to discriminate between different species of mollusks (e.g., [25,26).

On the other hand, as a complement to morphometric analysis, genetic analyses using DNA sequencing allow the characterization of populations, measuring their levels of diversity, product of possible selection processes by fisheries, among other issues. To date, there are no genetic studies in *M. edulis* for population comparison, nor for the species of the genus, so it is an understudied group in this field. This genetic study is the first approach to the understanding of the genetic-population processes that occur in this species along over 1300 km of the Chilean coast, due to the lack of genetic studies on this species and even on other species of the *Mulinia* genus.

Therefore, this paper evaluates if the banks of the taquilla clam *M. edulis* correspond to the same species along 1300 km the Chilean coast, by comparing individuals from the northern, central and southern areas of Chile. With this aim the morphology of the valves is analyzed and compared, and genetic characterization is carried out by DNA sequencing of individuals of taquilla clam *M. edulis* (King, 1832) from the banks of the northern, central and southern areas of the Chilean coast, which are the base of the fishery and source of broodstock for aquaculture.

2. Materials and methods

2.1. Sampling

A total of 384 samples of *M. edulis* valves were collected in Tongoy Bay (Playa Tongoy and Puerto Aldea) (30°17′56″ S, 71°36′23″ W) in the Coquimbo Region, in the northern part of the country, with 369 samples gathered between 1999 and 2000, and 15 in 2021. For the central zone, 34 samples were obtained from Punta Lavapié bank in the Gulf of Arauco (37°08′ 59″ S, 73°34′56″ W.) in the Biobío region in 2022. In the southern zone, 135 individuals were collected from the Quetalmahue bank (41°51′31″ S, 73°58′36″ W) in Ancud Bay on Chiloé Island, Los Lagos Region, with 120 samples collected in 2017 and 15 in 2020 (Fig. 1). Samples gathered from 2020 to 2022 were used in genetic

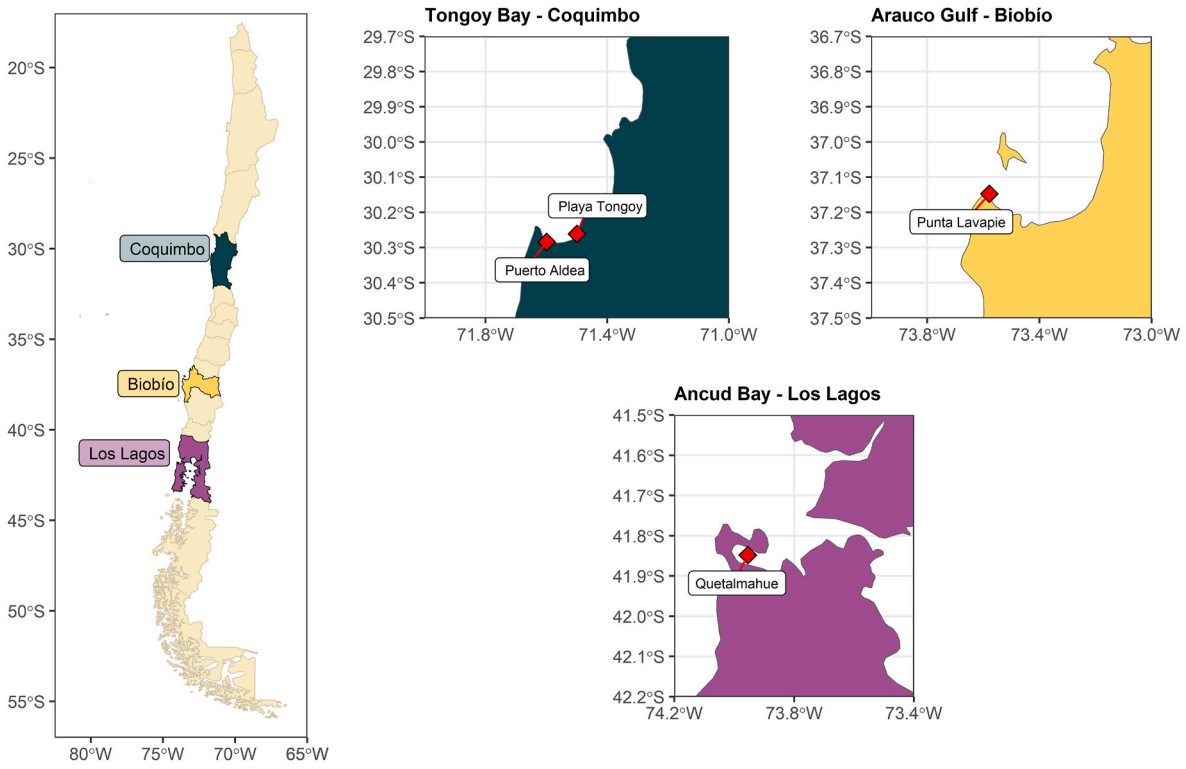


Fig. 1. Location of the sampling sites in Tongoy Bay (30°17′56″ S, 71°36′23″ W.), Arauco Gulf (37°08′ 59″ S, 73°34′56″ W.) and Ancud Bay (41°51′31″ S, 73°58′36″ W.) in northern, central and southern Chile.

analyses.

2.2. Morphometric analysis

For shape analysis, the inner surface of the right valve was used. Images were captured at a fixed distance with a Nikon COOLPIX L340 digital camera positioned at a right angle. A total of 28 landmarks were digitized to describe the inner surface of the valve following the description provided by Signorelli et al. (2013) and Aguirre et al. (2016) using tpsUtil 6.4 v1.82 and tpsDig2 v2.31 software (Fig. 2).

The landmarks coordinates were processed using generalized Procrustes analysis (GPA) (Rohlf and Slice, 1990), in which the landmarks configuration was translated and rotated to a common origin and scaled to a unit centroid size.

Subsequently, all analyses were performed based on the residuals of multivariate regressions to rule out allometric effects, using Ordinary Least Square (OLS) with 1000 per-mutations as an estimation method.

A principal component analysis (PCA) was performed on the covariance matrix of the individual after the allometry correction, to simulate the morphospace. Shape changes were visualized with displacement vector graphs for each locality.

To determine if shape variation was influenced by sex, a subsample of 101 specimens with known sex was selected from Quetalmahue bank, 50 males and 51 females. For sexing, the adductor muscles of the valves were cut, and the gonad evaluated by color: reddish in the case of females, and yellow in the case of males. The sex determination was confirmed by the observation under optical microscope of the gonadal smear.

Following the previously described procedure, a second PCA was performed using the residual values of multivariate regressions. Finally, a discriminant function was performed in order to identify if there was any clear variation be-tween male and female. All morphometric analyses were performed using R (v4.2.3) and RStudio (v2023.3.0), using the geomorph and ggplot2 packages (Baken et al., 2021).

2.3. Genetic analysis

For genetic analysis a portion of the mantle from each individual was removed for DNA extraction using the EZ.N.A. commercial mollusk extraction kit, following the manufacturer's instructions (Omega Bio-Tek, USA).

The gene regions for the 16S ribosomal RNA subunit (16S) and Cytochrome Oxidase I (COI) were amplified through polymerase chain reaction (PCR). For 16S gene the primers 16Sar-L and 16Sbr-H (Palumbi et al., 1991) were used and for COI gen the universal primers LCO1490 and HCO2198 (Folmer et al., 1994) were used. The amount of each reagent for the PCR reactions was standardized to 23 μL. The PCR program for amplification included an initial denaturation at 95 °C for 9 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min.

The samples were sequenced using the Sanger method in both directions at Macro-gen Inc. (Korea) The sequences obtained from each sample were edited by eye and using the chromatogram aligned forward and reverse sequences using the BIOEDIT V7.0 program (Hall, 2024) and all sequences aligned using the ClustalW method (Thompson et al., 2003). The sequences were deposited in GenBank under Accession Numbers: PV177469 to PV177563.

The genetic characterization of the banks was carried out by estimating genetic diversity indicators using the DnaSp v6 program (Rozas et al., 2017) and the number of haplotypes (k), haplo-typic diversity (H), polymorphic sites (S), average number of differences between sequence pairs (Π), and nucleotide diversity (π) were obtained. The genetic differentiation analysis was performed by estimating FST values and their significance by means of a permutation test carried out in the Arlequin software (Excoffier and Lischer, 2010). Finally, a haplotype network was constructed using DnaSp and plotted using PopArt (Leigh and Bryant, 2015). Phylogenetic reconstruction was performed using Neighbor-Joining method implemented in MEGA 11 (Tamura et al., 2021) with bootstrap support for clade weighting. These phylogenetic

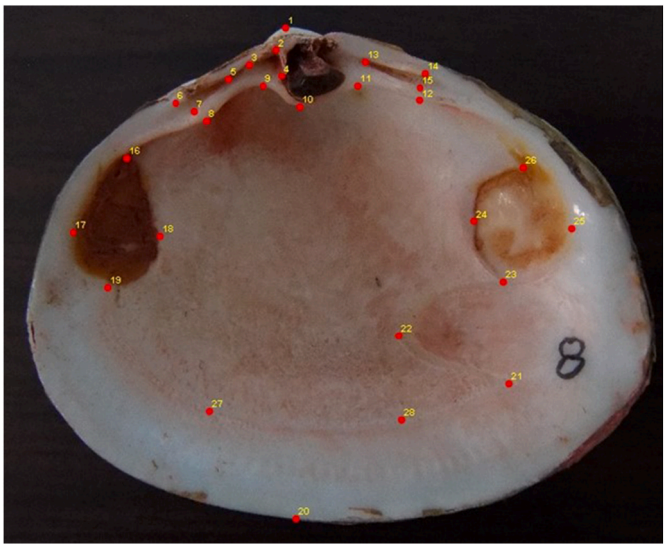


Fig. 2. Internal view of the right valve of *Mulinia edulis*, with 28 landmarks (LM). LM1, Dorsal margin; LM2, upper limit between cardinal teeth; LM3-4, lower limit of the cardinal teeth; LM5, upper limit anterior lateral tooth; LM6, lower limit lateral tooth; LM7, the most anterior distal limit of the dorsal anterior lateral tooth; LM8, the most posterior distal limit of the dorsal anterior lateral tooth; LM9, anterior valve cleft; LM10, distal boundary under the internal ligament; LM11, posterior valve cleft; LM12, external distal limit of the posterior lateral tooth; LM13, upper limit posterior lateral tooth; LM14, lower limit posterior lateral tooth; LM15, inner distal boundary of the posterior lateral tooth; LM16, upper limit of the anterior abductor muscle scar; LM17; outer side of the anterior abductor muscle scar; LM18, inner side of the anterior abductor muscle scar; LM19, lower limit of the anterior abductor muscle scar; LM20, ventral margin; LM21, external limit of the pallial sinus; LM22, inner boundary of the pallial sinus; LM23, lower limit of the posterior abductor muscle scar; LM24, inner side of the posterior abductor muscle scar; LM25, outer side of the pos-terior abductor muscle scar; LM26, upper limit of the posterior abductor muscle scar; LM27 and LM28, line of the pallial sinus.

analyses were conducted using COI gene sequences, which are commonly applied for species identification in the Marine Species DNA Barcoding Project. This analysis was performed on the COI sequences analyzed and also compared with those of the genus *Mulinia* obtained from GenBank for *M. edulis* (Accession No. JF301798 to JF301804) and *Mulinia lateralis* (Accession No. MN207094 to MN207099). A phylogenetic analysis was also carried out, including an external group of the family Mactridae the species *Mactra veneriformes* (Accession No. AY874531). The same analysis performed using the 16S sequences, which are provided in supplementary material 1, comparing sequences of *M. lateralis* and *Mactra corallina*. Since no *M. edulis* sequences are available in GenBank, it was not possible to compare our data with any previously existing sequences. Consequently, these 16S gene sequences represent the first entries for this species in the GenBank database."

3. Results

3.1. Morphological analysis

Residuals of shape, after removing the allometric effect, were extracted from the multivariate regression to eliminate any allometric influence on the data. The principal component analysis (PCA) shows a clear differentiation between three Chilean banks of *M. edulis* (Fig. 3). Where the first three components explain 57,7 % of the shape variation (PC1: 33,3 %, PC2: 14,4 %, PC3: 9,95 %). PC1 on the x-axis segregated the Ancud Bay (negative values) and Tongoy Bay (positive values) valves and the valves of Arauco Gulf are grouped in a middle position.

The displacement vectors indicate that landmarks 3 and 4, which describe the lower limit of the cardinal teeth, are more separated in Tongoy Bay compared to those in Ancud Bay and Arauco Gulf. The landmarks describing the upper and lower limits of the lateral teeth (LM 5, 6, 7 and 13, 14, 15) on the anterior and posterior ends, along with landmarks 8, 9, 10, 11 and 12 which correspond to the most posterior distal limit of the dorsal anterior lateral tooth, the distal limit under the internal ligament, the posterior cleft of the valve and external distal limit of the posterior lateral tooth, respectively, showed greater dis-placement in Ancud Bay (Fig. 4).

A difference was observed in the arrangement of landmarks 16–19 and 23–26, which describe the insertion points of the anterior and posterior abductor muscles, respectively, across the three localities. The most notable differences were observed in landmarks 18, 19, 23, and 24 for Ancud Bay, where these landmarks showed greater displacement from those describing the abductor muscles compared to Tongoy Bay. Arauco Gulf exhibited an intermediate level of displacement. Similarly, landmarks 21, 22, 27, and 28, which outline the outer and inner limits and the line of the pallial sinus, display considerable variation. This allowed us to observe a sharper and more extended shape in Ancud Bay, contrasting with Tongoy Bay, which showed a more open angle and less extended pallial sinus. Arauco Gulf again presented an intermediate separation of the vectors describing the pallial sinus, positioned between the northern and southern localities (Fig. 4).

For sexual dimorphism, the PCA of the sexed subsample showed that males and females share a very similar shape (PC1: 33.3 %, PC2: 14.4 %, and PC3: 9.95 %). Males exhibited slightly more variation in morpho-space, but the average shape did not differ between the groups (Fig. 5A). The discriminant function analysis showed a peak of variation from the males after maximizing the variance between groups showing some slightly differences between sexes (Fig. 5B).

3.2. Genetic analysis

A total of 95 sequences of the 16S gene were obtained from the three analyzed localities of *M. edulis*. The 16S gene sequence consisted of 366 nucleotides. In contrast, only 10 COI gene sequences of 489 nucleotides were obtained across the three localities, which limited their use for comparative population analysis; however, they were still used for phylogenetic reconstruction.

The genetic results for the samples from the three sectors showed low genetic diversity and low genetic differentiation, using 16S. Genetic diversity for the molecular marker 16S is presented in Table 1, showing high similarity between localities. However, Ancud Bay exhibited the highest genetic diversity across all indicators, while Arauco Gulf had the lowest.

Genetic diversity indices for marker 16S, for Tongoy Bay, Arauco Gulf and Ancud Bay are represented by 5, 4 and 6 haplotypes respectively. The population of Ancud Bay had the highest haplotypic diversity index (H) ($H = 0.32$) and the lowest was found in the population of Arauco Gulf ($H = 0.18$). On the other hand, the average number of differences between pairs of sequences was higher in the Ancud Bay (0.344) and lower in Arauco Gulf (0.18). Also, 5 polymorphic sites were observed for Ancud Bay, 4 for Tongoy Bay and 3 for Arauco Gulf. The nucleotide diversity ranged from 0.00050 to 0.00094 (Table 1).

The genetic differentiation estimator (FST) does not show significant differences between all localities ($P = 0.365$) and the analysis between pairs of localities confirms this pattern, shown in Table 2. The values of genetic differentiation estimated by the FST statistics are observed below the diagonal and over the diagonal the p-value that shows the absence of significant differences between pairs of localities. The value of FST indicated a trend of less differentiation between Tongoy Bay and Arauco Gulf and a greater difference between both extreme localities (Tongoy vs Ancud Bay).

The haplotype diversity and distribution of 16S gene sequences, obtained by the network, revealed low overall diversity, with only 12 haplotypes identified. A predominant central haplotype was observed, from which low frequency haplotypic variants are derived (Fig. 6).



Fig. 3. Principal components analysis of *Mulinia edulis* shell shape, the convex hull represents the shape space of every locality.

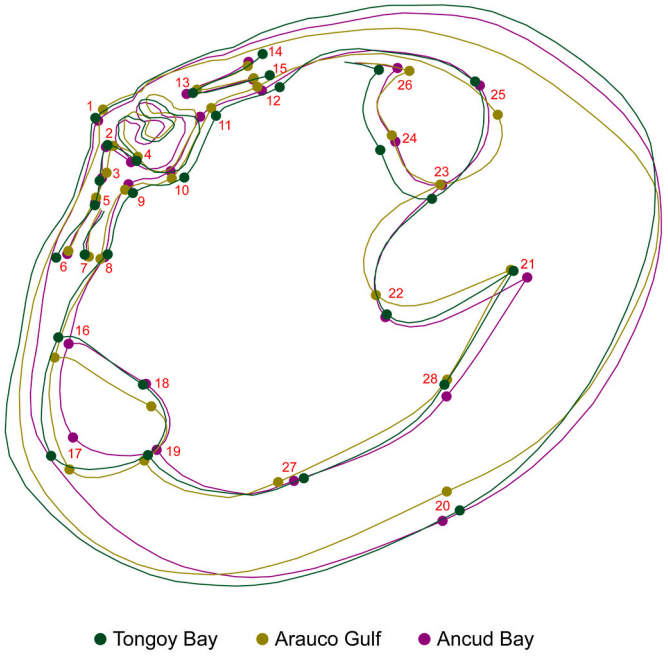


Fig. 4. Average shape of the shells of *Mulinia edulis* from Tongoy Bay, Arauco Gulf and Ancud Bay.

Noteworthy that the central haplotype, or common haplotype, is equally represented in the three localities analyzed.

On the other hand, using the COI gene sequences, for identification of the species using barcode DNA samples through phylogenetic reconstruction. This marker is suitable for species identification analysis. Furthermore, due to the low number of sequences per locality, obtained from this marker, no comparative population analysis was carried out between areas, and it was only used for what allows the data to be obtained with certainty, which corresponds to identification or confirmation of the species. This was due to a limited collection of high-quality sequences, not allowing a more exhaustive population analysis from being carried out.

The phylogenetic tree generated from the COI gene sequences confirms that the analyzed samples correspond to the species *M. edulis*, based on a comparison with sequences from two *Mulinia* species and one species of Mactridae family *M. veneriformes*, obtained from GenBank, thus confirming the species identification in cluster with boostrap of 99. This result can be seen in Fig. 7, where the samples cluster with *M. edulis* but are not separated into distinct clades based on their geographical origin. Furthermore, the results of the phylogenetic analysis obtained through the 16S gene show the same pattern, confirming the high genetic similarity between localities which is shown in the supplementary material 1.

4. Discussion

The objective of this study was to provide new information regarding the morphology of the clam *M. edulis* through the analysis of geometric morphometry of the shell and genetic analysis of fishery grounds in the

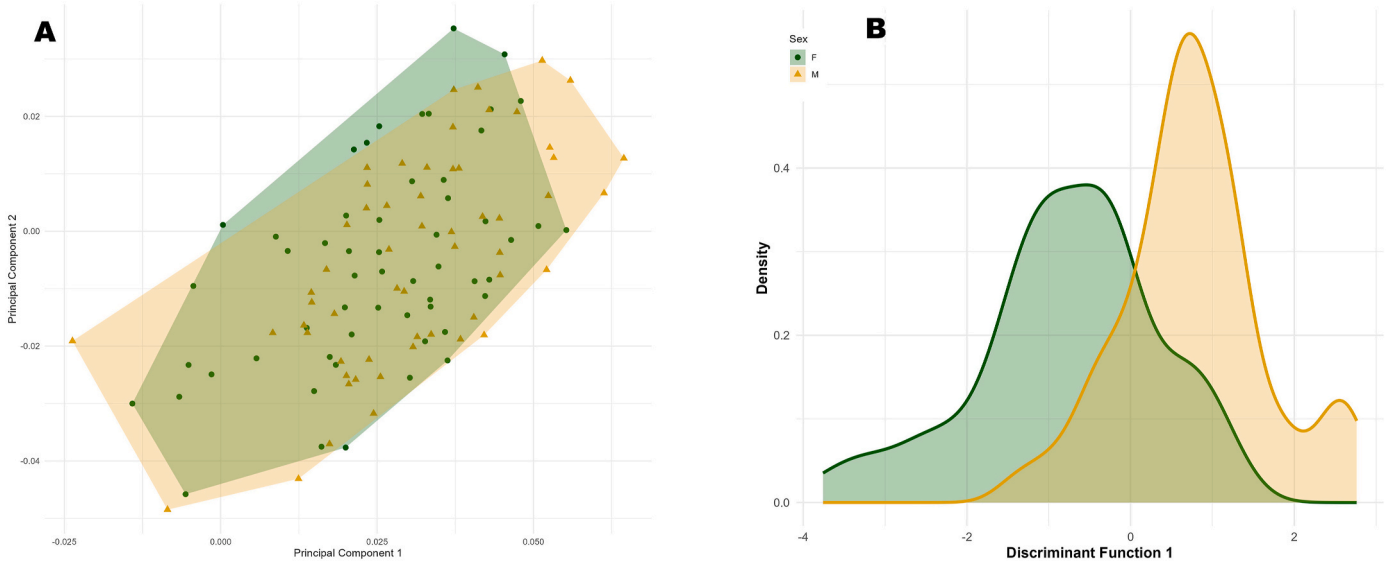


Fig. 5. A: Scatterplot of Principal Component analysis valve shape between sexes in *Mulinia edulis* B: Graphical representation of the discriminant analysis for sexes.

Table 1
Genetic diversity Indicators for the population of *Mulinia edulis* from Tongoy Bay, Arauco Gulf and Ancud Bay using the marker 16S. n: number of individuals; k: number of haplo-types; H: Haplotypic diversity; Π: average number of differences between pairs of sequences; S: Polymorphic sites; π: Nucleotide diversity.

Locality	n	k	H	Π	S	π
Tongoy Bay	33	5	0.28220	0.29924	4	0.00082
Arauco Gulf	33	4	0.17614	0.18182	3	0.00050
Ancud Bay	29	6	0.32020	0.34483	5	0.00094

Table 2
Genetic differentiation between pairs of localities using the 16S marker. Population-pairwise FST values presented below the diagonal and significance values (P) shown over the diagonal.

	Tongoy Bay	Arauco Gulf	Ancud Bay
Tongoy Bay	–	0.694	0.324
Arauco Gulf	0.0002	–	0.234
Ancud Bay	0.0060	0.0010	–

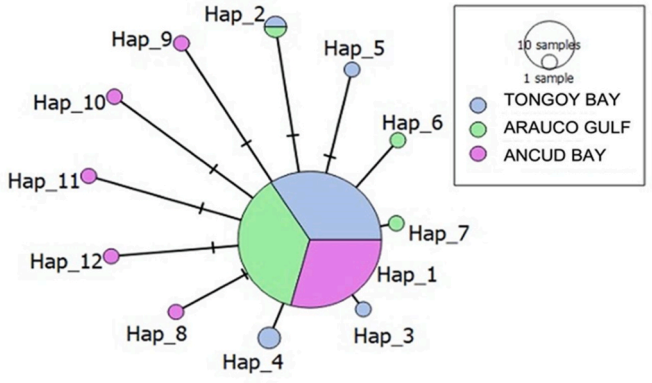


Fig. 6. Haplotype network of *Mulinia edulis* samples from Tongoy Bay, Arauco Gulf and Ancud Bay, by sequencing the 16S gene.

northern, central and southern areas of Chile.

4.1. Morphometric analysis

Allometric effects can explain a high and statistically significant proportion of morphological variation (Signorelli et al., 2013), although multivariate regression analysis of regression variation (shape) as a function of centroid size (size), did not reveal a significant relationship between size and shape (allometry). This suggests that the differences in the shape of the valves in the three studied locations are not driven by variation in size. To compare these results with those of Stotz et al. (2008) and Jaramillo et al. (2008) the analysis of the effect of size on shape should be considered.

On the other hand, the principal component analysis (PCA) from multivariate regression separated the locations, where principal components 1 and 2 explained 47,75 % of the total variation in the shape of the valves.

The valves showed morphological differences between the localities in the landmarks that described the lower limit of the cardinal teeth (LM 3 and 4), anterior cleft of the valve (LM 9), anterior and posterior muscles insertions (LM 16–19 and LM 23–26), external (LM 21) and internal (LM 22) limits of the pallial sinus. Stotz et al. (2008) and Jaramillo et al. (2008), did not find significant differences in the shape between samples of northern and south-ern Chile, using ten 10 landmarks on the external face of the valves.

Morphological changes can be related to factors such as latitude, water temperature, depth and type of sediment as mentioned by Signorelli et al. (2013) and Aguirre et al. (2016). So, latitude could be an explanation for the changes in shape observed in the present study. Similarly, no morphological differences were found between males and females, which supports that the species does not present external sexual dimorphism, coinciding with the results of Oliva et al. (2014).

The landmarks pattern accurately described the taquilla clam's anatomical characteristics and showed that the fishery banks analyzed belong to the same species with inter-site variability. Genetic analyses strengthened the morphometric results suggesting that the population of northern, central and southern Chile belong to the same species, *M. edulis*. In addition, the genetic estimators of diversity, in general, showed a high similarity between localities with a small tendency for greater genetic diversity in the locality of Ancud compared to the other two localities. On the other hand, the genetic differentiation indicator shows values that confirm a high genetic similarity between localities, so

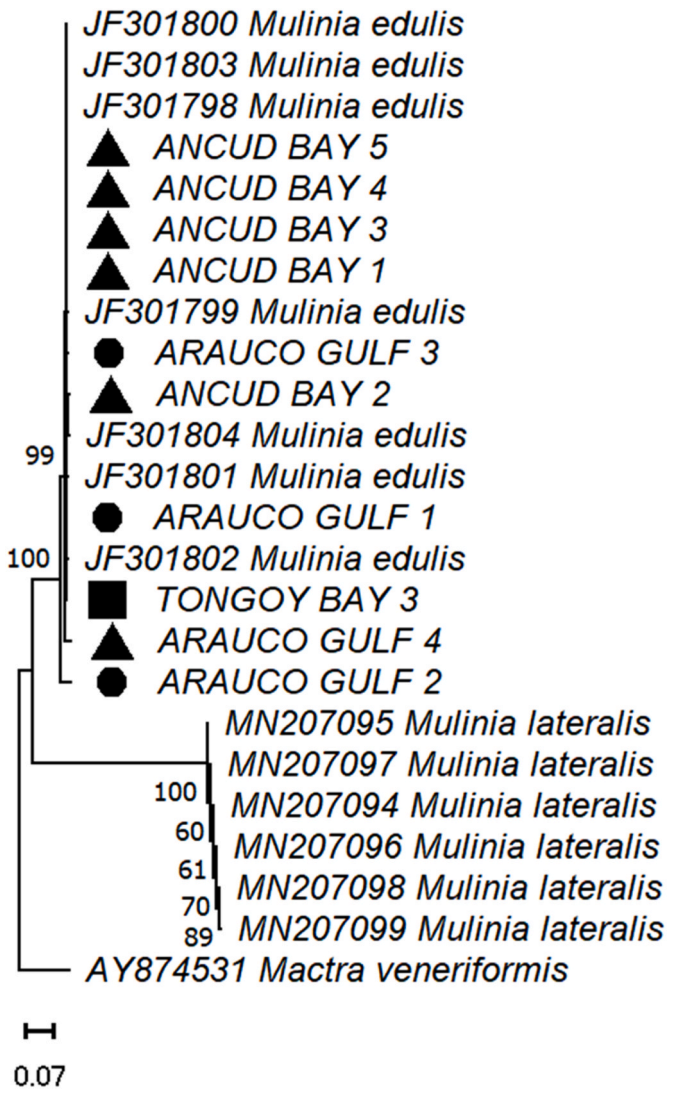


Fig. 7. Phylogenetic reconstruction of *Mulinia edulis* samples sequenced with the Cytochrome Oxidase I (COI) gene and their comparison with *Mulinia edulis*, *Mulinia lateralis* sequences and *Mactra veneriformes* like outgroup obtained from GenBank.

morphological differences observed by the morphometric indicator could be explained as a result of a process of phenotypic plasticity, in response to changes in environmental conditions between localities distributed in a wide latitudinal range and separated by more than 1500 km of differentiation and environmental conditions differentiated oceanographic systems. This suggests that the morphological differences observed cannot be explained by genetic differentiation of individuals by locality.

4.2. Genetics analysis

Molecular genetic markers are valuable tools for identifying species, populations, and estimating differentiation between groups. To this end, the cytochrome oxidase I gene has been standardized as a universal marker for species through the DNA barcoding project (Hebert et al., 2003). The use of a standardized DNA barcoding approach involves identifying a specimen as belonging to a specific animal species based on a single universal marker: the DNA barcode sequence. In this study, the use of this marker enabled the confirmation of the species *M. edulis* corresponding to the analyzed samples.

Genetic studies on *M. edulis* have been very limited, with few studies conducted. These include research using isoenzyme markers from over 20 years ago (González et al., 2002), as well as other studies using molecular markers, which are mostly found only in project reports (Stotz et al., 2008; Jaramillo et al., 2008). Therefore, the gene sequence results for this species presented here are novel data. González et al. (2002) conducted an electrophoretic study of *Mulinia* in ten localities between Bahía Tongoy (Coquimbo) and Puerto Engaño (Magallanes), where they found very low genetic variation in 13 enzymatic loci. Genetic similarity between populations was extremely high, around 99 %, and no statistically significant genetic structuring was found (FST = 0.0057). However, a generalized deficiency of heterozygotes (FIS = 0.6947) was observed. These results suggest significant global gene flow and high larval dispersal, which prevents differentiation by genetic drift. Thus, the authors suggest that the *Mulinia* group on the Chilean coast should be reconsidered as a homogeneous unit, rather than five distinct species. Signorelli and Pastorino (2011) highlight that the genus *Mulinia* clearly dominates the mactrid fauna in Magallanes Region, with five species represented. This area must be included in future studies.

The gene sequences confirm low genetic diversity of the species and low differentiation between localities, confirming the pattern of a population unit based on the analyzed distribution range. These results coincide with Jaramillo et al. (2008), who, through their phylogenetic and population genetics analysis, concluded that there is no significant molecular differentiation using mitochondrial nucleotide sequences between different populations of the clams on the coast of the Biobío and Los Lagos Regions. On the other hand, Stotz et al. (2008) found two morphs of *M. edulis* for the Bay of Coquimbo, considering both as discrete stocks. These identified morphs cannot be explained by genetic differences, since the high similarity between localities is confirmed. Both authors note that the fishery is based on a single species, *M. edulis*.

In the present work, the use of morphometric markers has been complemented by using molecular analysis of two DNA regions, in addition, our results confirm that this species would correspond to a large and unique population, which implies that these separate fishing banks can have gene flow through migration in their larval stages that generates a panmixmatic reproduction throughout the distribution of the species.

5. Conclusions

Through the use of multivariate regression analysis, no influence of size on the shape of the clam valves of the northern, central and southern Chilean coast was observed.

The principal component analysis performed from the regressions allowed to separate the individuals from the sampled banks by their morphology. This was corroborated by visualizing changes in shape by means of displacement vectors.

No sexual dimorphism was detected in the clam *M. edulis*.

This study allowed us to verify that there is a differentiation in the morphology of the valves for the clam fishery banks in the northern, central and southern areas of the Chilean coast.

Molecular marker sequencing revealed a high genetic similarity among the banks, indicating that all analyzed samples belong to the species *M. edulis* and form a single population ranging from Coquimbo to Ancud. Genetic analyses show that the three localities exhibit low diversity and minimal differentiation, with Ancud Bay displaying the highest diversity and Arauco Gulf the lowest. FST values further confirm this strong genetic similarity.

A predominant central haplotype, common to all three localities, underscores the region's low haplotypic diversity and high genetic homogeneity.

Results from the COI marker, as a DNA barcode for species identification, confirmed that the samples are indeed *M. edulis*.

Finally, it is important to indicate that the current fishery of the taquilla clam in the country is carried out on *M. edulis* and cultivation experiments have been done on this same species.

CRediT authorship contribution statement

Doris Oliva: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Valentina Salgado:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Hugo A. Benítez:** Writing – review & editing, Visualization, Validation, Software, Methodology, Formal analysis. **Alejandro Abarca:** Writing – review & editing, Conceptualization. **L. René Durán:** Writing – original draft, Resources, Project administration, Funding acquisition, Conceptualization. **Anahi Canto:** Visualization, Software, Formal analysis, Data curation. **Camila Barría:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Marcela P. Astorga:** Writing – review & editing, Validation, Software, Resources, Methodology, Investigation, Formal analysis.

Declaration of competing interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcz.2025.03.001.

Data availability

Data will be made available on request.

References

Abarca, A., Oliva, D., Gutiérrez, R., Celis, Á., Durán, L.R., 2012. Grown-out of seeds of the taquilla clam *Mulinia edulis* (king & Broderip, 1832) in the subtidal zone in northern Chile and in the intertidal zone in southern Chile. Lat Am J Aquat Res 40, 694–704. https://doi.org/10.3856/vol40-issue3-fulltext-17.

Adams, D.C., Rohlf, F.J., Slice, D.E., 2004. Geometric Morphometrics: ten years of progress following the 'Revolution. Ital. J. Zool. 71, 5–16. https://doi.org/10.1080/11250000409356545.

Aguirre, M.L., Richiano, S., Álvarez, A., Farinati, E.A., 2016. Reading shell shape: implications for palaeoenvironmental recon-structions. A case study for bivalves from the marine quaternary of Argentina (South-Western Atlantic). Hist. Biol. 28, 753–773. https://doi.org/10.1080/08912963.2015.1026898.

Avila-Serrano, G.E., Flessa, K.W., Téllez-Duarte, M.A., Cintra-Buenrostro, C.E., 2006. Distribución de la macrofauna intermareal del Delta del Río Colorado, Norte del Golfo de California, México. Cienc. Mar. 32, 649–661.

Baken, E.K., Collyer, M.L., Kaliontzopoulou, A., Adams, D.C., 2021. Geomorph v4.0 and GmShiny: enhanced analytics and a new graphical interface for a comprehensive morphometric experience. Methods Ecol. Evol. 12, 2355–2363. https://doi.org/10.1111/2041-210X.13723.

Cruz, M., 1983. Bivalvos del Golfo de Guayaquil. Acta Oceanográfica del Pacífico. INOCAR 2, 735–821.

Díaz, C., Cancino, J., 2015. Estudio de emplazamiento de áreas de acuicultura de pequeña escala en la zona Sur (VI a XIV Regiones). In: Informe Final Proyecto FIP 2013-24. Universidad Católica de La Santísima Concepción, Concepción, pp. 1–223.

Excoffier, L., Lischer, H.E.L., 2010. Arlequin Suite Ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x.

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. Mol. Mar. Biol. Biotechnol. 3, 294–299.

González, C.A., Mena, C.P., Gallardo, M., Clasing, E., 2002. Variabilidad genética en los miembros del género *Mulinia*, Gray, 1837 (Bivalvia, Mactridae) a lo largo de la costa chilena. Revista Ciencia y Tecnología del Mar 25, 147–156.

Hall, T., 2024. BioEdit version 7.0. 0. Tom Hall [software]. https://bioedit.software.informer.com/7.0/.

Hebert, P.D.N., Ratnasingham, S., deWaard, J.R., 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc Roy Soc London, Ser B, Biol Sci 270. https://doi.org/10.1098/rsbl.2003.0025.

Jaramillo, E., Contreras, H., Garrido, O., Gallardo, C., Nuñez, J., Jeréz, G., 2008. Estudio de reproducción y crecimiento del recurso Taquilla (*Mulinia sp*) en la VIII y X Región. Informe Final Proyecto FIP, 2006-51. Universidad Austral de Chile, Valdivia, pp. 1–141.

Leigh, J.W., Bryant, D., 2015. POPART: full-Feature software for haplotype network construction. Methods Ecol. Evol. 6, 1110–1116. https://doi.org/10.1111/2041-210X.12410.

MolluscaBase, Mactridae Lamarck, 1809, 2024. World register of marine species. https://www.molluscabase.org/aphia.php?p=taxdetails&id=230/.

Oliva, D., Abarca, A., Gutiérrez, R., Celis, Á., Herrera, L., Pizarro, V., 2013. Effect of stocking density and diet on growth and survival of post-larvae of the Taquilla Clam *Mulinia edulis* cultivated in sand in a hatchery. Rev. Biol. Mar. Oceanogr. 48, 37–44. https://doi.org/10.4067/S0718-19572013000100004.

Oliva, D., Abarca, A., Gutiérrez, R., Herrera, L., Celis, Á., Durán, L.R., 2014. Effect of stocking density and food ration on growth and survival of veliger and pediveliger larvae of the Taquilla clam *Mulinia edulis* reared in the laboratory. Rev. Biol. Mar. Oceanogr. 49, 567–575. https://doi.org/10.4067/s0718-19572014000300013.

Oliva, D., Abarca, A., Pizarro, M., Durán, L.R., 2020. Pre-engorda de semillas de cultivo de la almeja Taquilla, *Mulinia edulis*, en un sistema suspendido en el mar. Rev. Biol. Mar. Oceanogr. 55, 183–190. https://doi.org/10.22370/rbmo.2020.55.3.2579.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. The Simple Fool's Guide to PCR. University of Hawaii Press, Honolulu.

Rohlf, F.J., Slice, D., 1990. Extensions of the Procrustes Method for the optimal superimposition of landmarks. Syst. Biol. 39, 40–59. https://doi.org/10.2307/2992207.

Rozas, J., Ferrer-Mata, A., Sanchez-Del Barrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sanchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol. Biol. Evol. 34, 3299–3302. https://doi.org/10.1093/molbev/msx248.

SERNAPESCA, 2022. Anuario Estadístico de Pesca. https://www.sernapesca.cl/informacion-utilidad/anuarios-estadisticos-de-pesca-y-acuicultura/2022. (Accessed 19 November 2024).

Signorelli, J.H., 2019. The Superfamily Mactroidea (Mollusca:Bivalvia) in American Waters: an Illustrated Catalogue of Recent Species. Springer International Publishing, New York.

Signorelli, J.H., A Pastorino, G., 2012. Revision of the living Mactridae (Bivalvia: autobranchia) from northern Argentina and Uruguay. Am. Malacol. Bull. 30, 85–101. https://doi.org/10.4003/006.030.0107.

Signorelli, J.H., Pastorino, G., 2011. Revision of the magellanic Mactridae Lamarck, 1809 (Bivalvia: heterodonta). Zootaxa 2757, 47–67.

Signorelli, J.H., Pastorino, G., 2012. Taxonomic revision of Brazilian Mactridae Lamarck, 1809 (Bivalvia: cardiida). Zootaxa 3245, 30–53.

Signorelli, J.H., Márquez, F., Pastorino, G., 2013. Phenotypic variation of south-western atlantic clam *Mactra isabelleana* (Bivalvia: Mactridae). J. Mar. Biol. Assoc. U. K. 93, 511–517. https://doi.org/10.1017/S0025315412000410.

Smith, E.A., 1914. List of Australian Mactridae, with a description of a new species. J. Molluscan Stud. 11, 137–151.

Stotz, W., Valdebenito, M., Romero, M., Bernal, G., Caillaux, L., Aburto, J., Contreras, H., Lancellotti, N., Urriola, N., Guajardo, P., Baro, S., Aquea, G., Cerda, C., 2008. Estudio reproductivo del recurso almeja en la IV Región. In: Informe Final Proyecto FIP 2006-46. Universidad Católica del Norte, Coquimbo, pp. 1–140.

SUBPESCA, 2020. Estado de situación de las principales pesquerías chilenas, años 2019, Departamento de Pesquería. División de Administración Pesquera. Subsecretaría de Pesca y Acuicultura, Gobierno de Chile. Valparaíso. https://www.subpesca.cl/portal/publicaciones//110503. (Accessed 6 March 2025).

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38, 3022–3027. https://doi.org/10.1093/molbev/msab120.

Thompson, J.D., Gibson, T.J., Higgins, D.G., 2003. Multiple sequence alignment using ClustalW and ClustalX. Curr Protoc Bioinform 2–3.

Torcida, S., Pérez, S.I., 2012. Análisis de procrustes y el estudio de la variación morfológica. Revista argentina de antropología biológica 14, 131–141.

Toro Ibacache, M.V., Manríquez Soto, G., Suazo Galdames, I., 2010. Morfometría geométrica y el estudio de las formas biológicas: De la morfología descriptiva a la morfología cuantitativa. Int. J. Morphol. 28, 977–990. https://doi.org/10.4067/S0717-95022010000400001.

Wong, H.W., 2009. The Mactridae (Mollusca: Bivalvia) of east coast park. Nature in Singapore 2, 283–296.

WORMS World register of marine species (WORMS). https://www.marinespecies.org, 2024. July 2024.