

Evolutionary relationships of the Tehuelche scallop *Aequipecten tehuelchus* (Bivalvia: Pectinidae) from the south-western Atlantic Ocean

BERENICE TROVANT¹, LUCIANO E. REAL¹, ANA M. PARMA², J.M. ORENSANZ² AND NÉSTOR G. BASSO¹

¹Instituto de Diversidad y Evolución Austral (CCT CONICET – CENPAT), Bv. Almirante Brown 2915, Puerto Madryn, Chubut, Argentina, ²Centro para el Estudio de Sistemas Marinos (CCT CONICET – CENPAT), Bv. Almirante Brown 2915, Puerto Madryn, Chubut, Argentina

This study addresses aspects of the phylogenetic relationships of the commercial Tehuelche scallop, Aequipecten tehuelchus s.l. (Bivalvia: Pectinidae), from southern South America using molecular techniques. The Tehuelche scallop presents two different putative subspecies, A. t. tehuelchus and A. t. madrynsis, and a potentially related sympatric species, Flexopecten felipponei. The Tehuelche scallop is a very important component of ecosystems and is the target of artisanal fisheries in the northern Patagonian gulfs of Argentina. Despite its importance, the systematic relationships of these taxa have not been fully addressed. The main goal of this study is to place the Tehuelche scallop within a partial phylogenetic framework of the family Pectinidae. Scallops were sampled at 10 localities distributed along the south-western Atlantic Ocean. Phylogenetic reconstructions were carried out from two mitochondrial (12S rRNA and 16S rRNA) and two nuclear markers (28S rRNA and H3) using Bayesian, maximum likelihood and maximum parsimony analyses. Our phylogenetic analysis indicates that the two putative subspecies of the Tehuelche scallop together with F. felipponei form a monophyletic clade, without differentiating at the specific level. Observed differences would be the result of phenotypic plasticity, probably caused by environmental factors. However, further analysis using genes with faster evolution rate are needed to corroborate it. Our phylogenetic analysis resolved to Aequipecten as polyphyletic. The Tehuelche scallop has a basal position within the Argopecten group and we recommend that it should be transferred to this genus. The relationship between the hypotheses about the origin of the Tehuelche scallop implicit in the literature and our results are discussed.

Keywords: Southern Ocean, scallop, phylogeny

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INTRODUCTION

The family Pectinidae is one of the most conspicuous groups of Bivalvia and an important component of ecosystems, constituting the support of artisanal and industrial fisheries (Waller, 1991). The phylogenetic relationships of pectinids have been explored using morphological (e.g. Thiele, 1935; Hertlein, 1969; Waller, 1986, 1991, 2006) and molecular data (e.g. Canapa *et al.*, 2000; Barucca *et al.*, 2004; Saavedra & Peña, 2006; Puslednik & Serb, 2008; Alejandrino *et al.*, 2011; Feng *et al.*, 2011; Krause & von Brand, 2016). The most accepted classification system derived from morphological characters is the one of Waller (2006), who divided the family Pectinidae into three subfamilies: Camptonectinae, Chlamydiae and Pectininae, using microsculptural shell features and morphological characteristics of the juveniles. Waller's hypothesis of subfamily relationships generally matched the molecular phylogeny of Alejandrino *et al.* (2011), the molecular phylogenetic study with the

most comprehensive taxon sampling to date, with only the placement of Aequipectini as a major difference (Serb, 2016). This molecular study and other contributions published in the last two decades (see Waller, 2006; Krause & von Brand, 2016; Serb, 2016), although extensive, did not include taxa from the south-western Atlantic.

In the south-western Atlantic Ocean, scallops are represented by *Aequipecten* (*Chlamys*) *tehuelchus* (d'Orbigny 1842), *Austrochlamys natans* (Philippi 1845), *Cyclopecten falklandicus* Dell, 1964, *Flexopecten felipponei* (Dall 1922) and *Zygochlamys patagonica* (King & Broderip 1832). Only two of these species, *Z. patagonica* and the Tehuelche scallop *A. tehuelchus*, are commercially exploited in the south-western Atlantic (Orensanz *et al.*, 1991). The Tehuelche scallop is the target of small inshore fisheries that operate within the northern Patagonian gulfs, involving dredging and commercial diving, and they are of considerable significance for the local economies (Orensanz *et al.*, 1991; Soria *et al.*, 2016). Despite their importance, the status and phylogenetic relationships of these taxa have not been fully elucidated.

The Tehuelche scallop is a warm-temperature species endemic to the Argentine biogeographic province, inhabiting

Corresponding author:

B. Trovant

Email: trovant@cenpat-conicet.gob.ar

shallow shelf bottoms from Rio de Janeiro, Brazil (23°S) to the north of the San Jorge Gulf, Argentina (45°S) (Castellanos, 1971). It exhibits two geographic variants which differ in shell morphology (Figure 1). The 'tehuetchus' phenotype is distributed in the warm-temperate Argentine biogeographic province (north of 42°30'S), typically has 14–18 'squamous' and relatively rounded ribs, and is the only one that forms large banks which support important fisheries mostly confined to the northern Patagonian gulfs (Orensanz *et al.*, 1991). The 'madrjensjs' phenotype is distributed in the cold-temperate Magellanic province (42°30'–45°S), presents 11–14 comparatively softer and more pronounced ribs (the number of ribs is not correlated with the size of the valve) and lives at low densities (Castellanos, 1971). This southern geographic variant has been described as a separate species, *Aequipecten madrynsjs* (Lahille 1906) (Castellanos, 1971) or as a subspecies, *A. tehuelchus madrynsjs* (Bavay 1906) (Orensanz *et al.*, 1991). The correlation between morphological and genetic differences of the two forms was explored by Real *et al.* (2004), who found no evidence of interspecific genetic differentiation using allozymes. Another related species that lives in sympatry with *tehuetchus* and *madrjensjs* is the non-commercial pectinid *Flexopecten felipponei* (Dall 1922) (Figure 1), which has rarely been recorded in the south-western Atlantic Ocean (Waller, 1991), distributed in rocky and sandy bottoms from 36°S to San Matias and Nuevo Gulfs (43°S) (Castellanos, 1970, 1971). Orensanz *et al.* (1991) proposed that *F. felipponei* would be a phenotypic variant of *A. tehuelchus*, based on examination of soft tissue of numerous individuals with intermediate characters and the presence of simultaneous hermaphroditism. The

phylogenetic relationships among the two geographic variants of the Tehuelche scallop and *F. felipponei* remains unknown.

The main goal of this study was to place the two putative subspecies (*tehuetchus* and *madrjensjs*), as well as the sympatric *F. felipponei*, within a partial phylogenetic framework of the family Pectinidae and to investigate their taxonomic status using four molecular markers. The phylogenetic reconstruction will allow us to infer relationships among these taxa within the pectinid family and to explore the hypothesis that *A. t. tehuelchus*, *A. t. madrynsjs* and *F. felipponei* correspond to different phenotypes of the same species. This study utilized the phylogenetic species concept (Nixon & Wheeler, 1990).

MATERIALS AND METHODS

Sample collection

Scallops were obtained from the subtidal of 10 localities distributed along the south-western Atlantic Ocean (Figure 2, Table 1) covering the distribution of the three taxa. All *Aequipecten* scallops collected north of 42°30'S were of the 'tehuetchus' variant while all collected from Golfo Nuevo to the south (up to 45°S) were of the 'madrjensjs' variant. All specimens were fixed in 96% ethanol.

DNA extraction, amplification and sequencing

Genomic DNA was isolated from the adductor muscle using the phenol-chloroform protocol (Sambrook *et al.*, 1989). We



Fig. 1. Phenotype variation of the Tehuelche scallop. To the left: *Flexopecten felipponei* of two different sizes; to the right at the top, *Aequipecten tehuelchus* 'tehuetchus' and below, *Aequipecten tehuelchus* 'madrjensjs'.

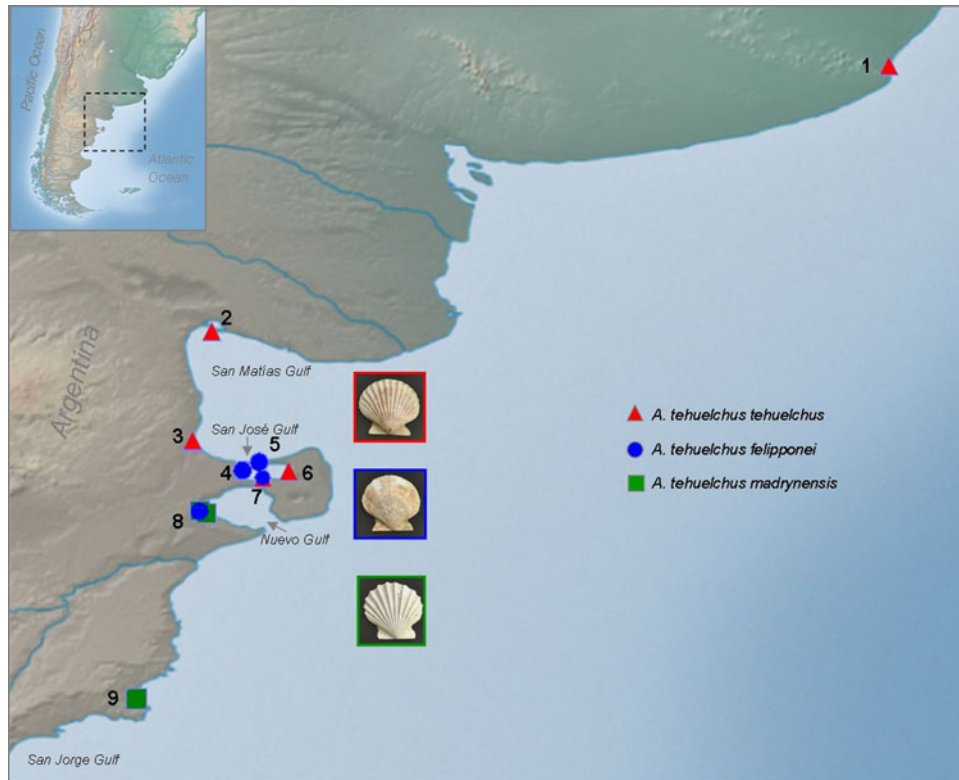


Fig. 2. Sampling localities of each phenotype along the south-western Atlantic coast: (1) Mar del Plata; (2) Villarino beach; (3) Puerto Lobos; (4) La Tapera; (5) Larralde beach; (6) Punta Conos; (7) Punta Buenos Aires; (8) Puerto Madryn I, (9) Puerto Madryn II and (10) Camarones (for details see Table 1).

amplified fragments of two mitochondrial ribosomal genes, 12S (domain III) and 16S (domain IV and V) rRNA, of 394 bp and 465 bp (aligned length), and two nuclear genes, the large ribosomal subunit (28S), of 745 bp (aligned length), and the Histone H3 of 295 bp (aligned length). We chose these genes due to the high availability of related species sequences to compare in the GenBank database. In addition, to efficiently distinguish these three taxa, we tried hard to amplify, with numerous combinations of primers and conditions, the cytochrome *c* oxidase subunit I (COI), but we did not have success. The primers used for both amplification and sequencing of the selected genes are listed in Table 2. When possible, we sequenced two specimens per locality for each gene for a total of 93 sequences (26 for 16S rRNA, 23 for 12S rRNA, 20 for 28S rRNA and 24 for H3). To amplify the genes we used Tsg DNA polymerase (Bio Basic Inc., Canada). The protocol used included an initial denaturing temperature of 94°C for 1 min; followed by 30 cycles of 94°C for 30 s; an annealing temperature of 50°C for 30 s; 72°C for 1 min; and a final extension at 72°C for 10 min. After extraction and amplification the DNA was visualized by UV transillumination in 1% agarose gels stained with green gel (BIOTUM). Extractions, amplifications, purification of PCR products and sequencing of both strands of DNA samples were performed in the Laboratory of Molecular Biology (IDEAus – CONICET, Argentina). DNA sequences were edited in CodonCode Aligner v 2.0.4 and aligned using default parameters with Clustal W version 1.75 (Thompson *et al.*, 1994). All DNA sequences were deposited in GenBank and their accession numbers (KY055443–KY055527, KY070308–KY070314) are detailed in Table 1. The estimates of evolutionary divergence over sequence

pairs within and between *A. 'tehuelchus'*, *A. 'madrynsensis'* and *F. 'felipponei'* were calculated using 'p-distance' (Kimura, 1980) in MEGA v5 (Tamura *et al.*, 2011).

Phylogenetic analysis

To assess the degree of saturation of nucleotide saturations of mitochondrial gene regions, a test of substitution saturation (Xia & Lemey, 2009) was performed in DAMBE v5 (Xia, 2013). To determine if different partitions of the data have significantly different signals and following the concatenation of all genes, a partition homogeneity test with 1000 replications in PAUP (Swofford, 1998) was carried out. Sequences of related species were downloaded from GenBank (Table 3). *Zygochlamys amandi* Soot-Ryen 1959, *Z. patagonica* (King 1832) and *Veprichlamys jousseaumei* (Bavay, 1904) (Pectinoidea: Pectinidae) were selected as outgroups. This selection was based on the robust phylogeny of Alejandrino *et al.* (2011).

Three methods were utilized for phylogenetic reconstruction of each gene (Supplementary material SM1) and also of the concatenated dataset: Maximum parsimony (MP), Maximum likelihood (ML) and Bayesian inference (BI).

Maximum parsimony analysis was carried out using TNT (Goloboff *et al.*, 2008). We used the traditional search, including a tree bisection–reconnection (TBR) as swapping algorithm and 10 trees saved by replication. Then, a strict consensus tree was calculated. Branch support was evaluated through bootstrapping (500 replicates).

The Akaike Information Criterion (AIC), implemented in jModelTest v 2.1.10 (Darriba *et al.*, 2012), was applied to each dataset to find the models of evolution that best fit the data. The General Time Reversible model (GTR + I + G,

Table 1. General information on samples analysed as part of this study.

Morphotype	Locality	N	Latitude/ Longitude	Mitochondrial		Nuclear		Histone H3
				12S rRNA	16S rRNA	28S rRNA	Histone H3	
<i>Aequipecten tehuelchus</i>	Mar del Plata, Buenos Aires Province	2	38°00'S, 57°33'W	KY055443, KY055444	KY055464, KY055465	KY055488, KY055489	KY055507, KY055508	
	Playa Villarino, Río Negro Province	3	40°53'S, 64°48'W	KY055445-KY055447	KY055466-KY055468	KY055490, KY055491	KY055509-KY055510, KY055525	
<i>Aequipecten madrynensis</i>	Playa Larralde, San José Gulf, Chubut Province	2	42°24'S, 64°18'W	KY055451, KY055452	KY055479, KY055480	KY055495	KY055518, KY055519	
	Puerto Lobos, Chubut Province	3	41°01'S, 65°04'W	KY055448-KY055450	KY055469-KY055471	KY055492-KY055494	KY055511-KY055513	
	Puerto Madryn I (Piedra Buena Deck), Chubut Province	1	42°46'S, 65°00'W	-	KY055475	-	KY055517	
	Puerto Madryn II (Punta Este), Chubut Province	4	42°47'S, 64°57'W	KY055458-KY055461	KY055477, KY055478, KY055481-KY055485	KY055501-KY055504	KY055520-KY055523	
<i>Flexopecten felipponei</i>	Camarones, Chubut Province	2	44°46'S, 65°41'W	KY055462, KY055463	KY055486, KY055487	KY055505, KY055506	KY055524, KY055527	
	Puerto Madryn I, Chubut Province	1	42°45'S, 65°01'W	KY055456	-	KY055499	-	
<i>Zigochlamys patagonicus</i>	Playa Larralde, San José Gulf, Chubut Province	1	42°24'S, 64°18'W	KY055454	KY055474	KY055500	KY055516	
	Punta Conos, San José Gulf, Chubut Province	1	42°19'S, 64°03'W	KY055453	KY055472	KY055497	KY055515	
	Punta Buenos Aires, San José Gulf, Chubut Province	1	42°14'S, 64°21'W	KY055457	KY055473	KY055496	KY055526	
	La Tapera, San José Gulf, Chubut Province	1	42°21'S, 64°34'W	KY055455	KY055476	KY055498	KY055514	
San Matías Gulf, Río Negro Province; (Holberg 02) ^a	2	41°30'S, 64°15'W	KY070308, KY070309	KY070310, KY070311	KY070312	KY070313, KY070314		

^aGolfo San Matías, Río Negro: Specimens were collected in sublittoral zone during 'Holberg 02' campaign of Patagonian scallop prospection and by-catch with a bottom trawl.

Tavaré, 1985) was the best-fit substitution model in the four datasets (Supplementary material SM1, Table 1). The selected model was used in ML analysis on the concatenated dataset, conducted with RAxML 7.4.2 (Stamatakis, 2006) implemented in raxmlGUI 1.3 (Silvestro & Michalak, 2012), with a rapid bootstrapping analysis (500 replicates).

The phylogeny reconstructed with BI was estimated with different substitution (HKY + G + I, Hasegawa *et al.*, 1985, and GTR + G + I, Tavaré, 1985) and clock (strict and relaxed) models (Supplementary material SM1). The marginal-likelihood scores of the posterior distributions were compared using Bayes factors (BFs, Kass & Raftery, 1995) with two different methods: harmonic mean estimation (HME, Newton & Raftery, 1994) and a posterior simulation-based analogue of the Akaike information criterion through Markov Chain Monte Carlo (MCMC) analysis (AICM, Raftery *et al.*, 2007) implemented in Tracer v1.6 (Rambaut *et al.*, 2014). Bayesian reconstructions were conducted under a Yule process for species-level phylogenies as a tree prior, using BEAST v. 1.8.3 (Drummond *et al.*, 2012) with a MCMC simulation for 60 million generations for the concatenated dataset, sampling trees every 1000 generations with a burn-in of 10%. Convergence diagnostics were conducted in Tracer and reliable ESS values (>200) were ensured. Then, the maximum clade credibility tree was generated from the combined trees in TreeAnnotator v 1.6.1 (Drummond *et al.*, 2012). Finally, the editing of the trees was carried out in Figtree v 1.4 (Morariu *et al.*, 2008).

RESULTS

Phylogenetic analysis

The observed saturation indexes were significantly different and lower than the expected indexes, $I_{ss.c}$ ($0.139 < 0.369-0.6756$; $P < 0.05$) for the 12S rRNA and $I_{ss.c}$ ($0.115 < 0.3847-0.6912$; $P < 0.05$) for the 16S rRNA, suggesting that our mitochondrial data show little saturation. The partition homogeneity test was not significant ($P = 0.01$) suggesting that there is no conflict between partitions, so all genes were combined in the subsequent analyses.

The MP analysis resulted in four equally parsimonious trees with 2441 steps for the concatenated dataset. The General Time Reversible models (GTR + I + G) were selected for ML estimation based on the AIC criterion. In the case of Bayesian inference, Bayes factors based on HME and AICM favoured the GTR + I + G and the strict clock models over the HKY + I + G and the relaxed clock models for the concatenated dataset (Supplementary material SM2).

The phylogenetic reconstruction based on maximum parsimony, maximum likelihood and Bayesian analyses of the concatenated dataset (Figure 3) and of separate genes (Supplementary material SM1) supports the hypothesis that the two putative subspecies of the Tehuelche scallop, *A. t. tehuelchus* and *A.t. madrynensis*, and *F. felipponei* form a monophyletic clade, without differentiating at the specific level. The genetic distances between and within the 16S rRNA sequences from the three forms were 0.1%. This monophyletic clade is more closely related to the

Table 2. Primers used in this study.

Locus	Primer names	Sequence 5'→3'	References
12S rRNA	12S F	AGA CAT GGA TTA GAT ACC C	Barucca <i>et al.</i> (2004)
	12S R	ACC CCT ACC TTG TTA CGA CTT	Barucca <i>et al.</i> (2004)
16S rRNA	16S arL	CGC CTG TTT AAC AAA AAC AT	Palumbi <i>et al.</i> (1991)
	16S rbH	CCG GTT TGA ACT CAG ATC ACG T	Palumbi <i>et al.</i> (1991)
28S rRNA	sc28S_70F	CAG CAC CGA ATC CCT CAG CCT TG	Alejandrino <i>et al.</i> (2011)
	sc28S_950R	TCT GGC TTC GTC CTA CTC AAG CAT AG	Alejandrino <i>et al.</i> (2011)
Histone H3	H3 F	ATG GCT CGT ACC AAG CAG AC(ACG) GC	Colgan <i>et al.</i> (1998)
	H3 R	ATA TCC TTR GGC ATR ATR GTG AC	Colgan <i>et al.</i> (1998)

genus *Argopecten* than to the other members of the genus *Aequipecten*. *Argopecten* and *Lectopecten* are monophyletic, while *Aequipecten* is resolved as polyphyletic. From a phylogenetic point of view, *Aequipecten* is an artificial group, therefore does not reflect the phylogeny.

DISCUSSION

We have placed the Tehuelche scallop within a partial phylogenetic framework of the family Pectinidae outlined by Alejandrino *et al.* (2011). The hypothesis that *Aequipecten tehuelchus*, *A. madrynsensis* and *Flexopecten felipponei* correspond to different phenotypes of the same species was supported by the results of this study. The estimated interpopulational genetic distances obtained for the Tehuelche scallop were similar to the intraspecific variation expected

for Pectinids (0.1% average, 1% threshold, Feng *et al.*, 2011). Our results are consistent with those of Real *et al.* (2004), who found no evidence of interspecific genetic differentiation using allozymes. Of their 10 loci, five had significant F_{ST} values, averaging $F_{ST} = 0.032$, suggesting some genetic differentiation along the Patagonian coast. However, the level of genetic differentiation was not sufficient to justify the classification of the phenotypes 'tehuelchus' and 'madrynsensis' as different species or subspecies, as had been proposed by Castellanos (1971) and Orensanz *et al.* (1991). Consistently, Huber (2010), using morphological characters (number of ribs, strength of sculpture on the main ribs and colour), concluded that there was only one highly variable species of *Aequipecten* from southern Brazil to southern Argentina. The current evidence suggests the occurrence of high phenotypic plasticity, resulting in three different phenotypes which are genetically similar.

Table 3. DNA sequences used in this study.

Species	12S rRNA	16S rRNA	28S rRNA	Histone H3	References
<i>Argopecten ventricosus</i>	AM039765.1	HM630408.1	HM630410.1	HM630409.1	Saavedra & Peña (2006); Alejandrino <i>et al.</i> (2011)
<i>Argopecten purpuratus</i>	AM039763.1	NC_027943.1	HM630495.1	EU379526.1	Saavedra & Peña (2006); Li unpublished; Puslednik & Serb (2008); Alejandrino <i>et al.</i> (2011)
<i>Argopecten gibbus</i>	EU379389.1	EU379443.1	HM622698.1	EU379497.1	Puslednik & Serb (2008); Alejandrino <i>et al.</i> (2011)
<i>Argopecten nucleus</i>	EU379406.1	EU379461.1	HM630528.1	EU379515.1	Puslednik & Serb (2008); Alejandrino <i>et al.</i> (2011)
<i>Argopecten irradians</i>	EU379392.1	EU379432.1	HM622700.1	EU379486.1	Puslednik & Serb (2008); Alejandrino <i>et al.</i> (2011)
<i>Leptopecten latiauratus</i>	EU379393.1	EU379447.1	HM622714.1	EU379501.1	Puslednik & Serb (2008); Alejandrino <i>et al.</i> (2011)
<i>Leptopecten bavayi</i>	EU379381.1	EU379435.1	HM540102.1	EU379487.1	Puslednik & Serb (2008); Alejandrino <i>et al.</i> (2011)
<i>Pecten novaezelandiae</i>	EU379405.1	EU379459.1	HM630530.1	EU379513.1	Puslednik & Serb (2008); Alejandrino <i>et al.</i> (2011)
<i>Pecten maximus</i>	EU379400.1	EU379454.1	HM630545.1	EU379508.1	Puslednik & Serb, (2008); Alejandrino <i>et al.</i> (2011)
<i>Pecten fumatus</i>	HM622693.1	HM622694.1	HM622696.1	HM622695.1	Alejandrino <i>et al.</i> (2011)
<i>Amusium pleuronectes</i> Isolate 1	EU379415.1	EU379469.1	HM630508.1	HM630502.1	Puslednik & Serb (2008); Alejandrino <i>et al.</i> (2011)
<i>Amusium pleuronectes</i> Isolate 2	HM630500.1	HM630501.1	HM630503.1	EU379523.1	Alejandrino <i>et al.</i> (2011); Puslednik & Serb (2008)
<i>Nodipecten subnodosus</i>	HM630430.1	HM630431.1	HM630433.1	HM630432.1	Alejandrino <i>et al.</i> (2011)
<i>Euvola chazaliei</i>	EU379382.1	EU379436.1	HM561999.1	EU379490.1	Puslednik & Serb (2008); Alejandrino <i>et al.</i> (2011)
<i>Euvola vogdesi</i>	HM630387.1	AJ972432.1	HM630390.1	HM630389.1	Alejandrino <i>et al.</i> (2011); Saavedra & Peña (2006)
<i>Euvola perulus</i>	HM630516.1	HM630517.1	HM630514.1	HM630518.1	Alejandrino <i>et al.</i> (2011)
<i>Cryptopecten vesiculosus</i>	HM630399.1	HM630400.1	HM630406.1	HM630401.1	Alejandrino <i>et al.</i> (2011)
<i>Aequipecten glyptus</i>	EU379391.1	EU379445.1	HM622699.1	EU379499.1	Puslednik & Serb (2008); Alejandrino <i>et al.</i> (2011)
<i>Aequipecten opercularis</i>	JQ611462.1	AM494408.1	HM630527.1	EU379516.1	Moreno <i>et al.</i> , unpublished; Alejandrino <i>et al.</i> (2011); Malkowsky & Klusmann-Kolb (2012)
<i>Flexopecten glaber</i>	HQ197870.1	GU320272.1	AJ307545.1	HQ197861.1	Pujolar <i>et al.</i> (2010); Chrysaidei <i>et al.</i> , unpublished; Hammer, unpublished
<i>Veprichlamys jousseaumei</i>	HM622710.1	HM622711.1	HM622676.1	HM622712.1	Alejandrino <i>et al.</i> (2011)
<i>Zygochlamys amandi</i>	HM535651.1	HM535652.1	HM535654.1	HM535653.1	Alejandrino <i>et al.</i> (2011)

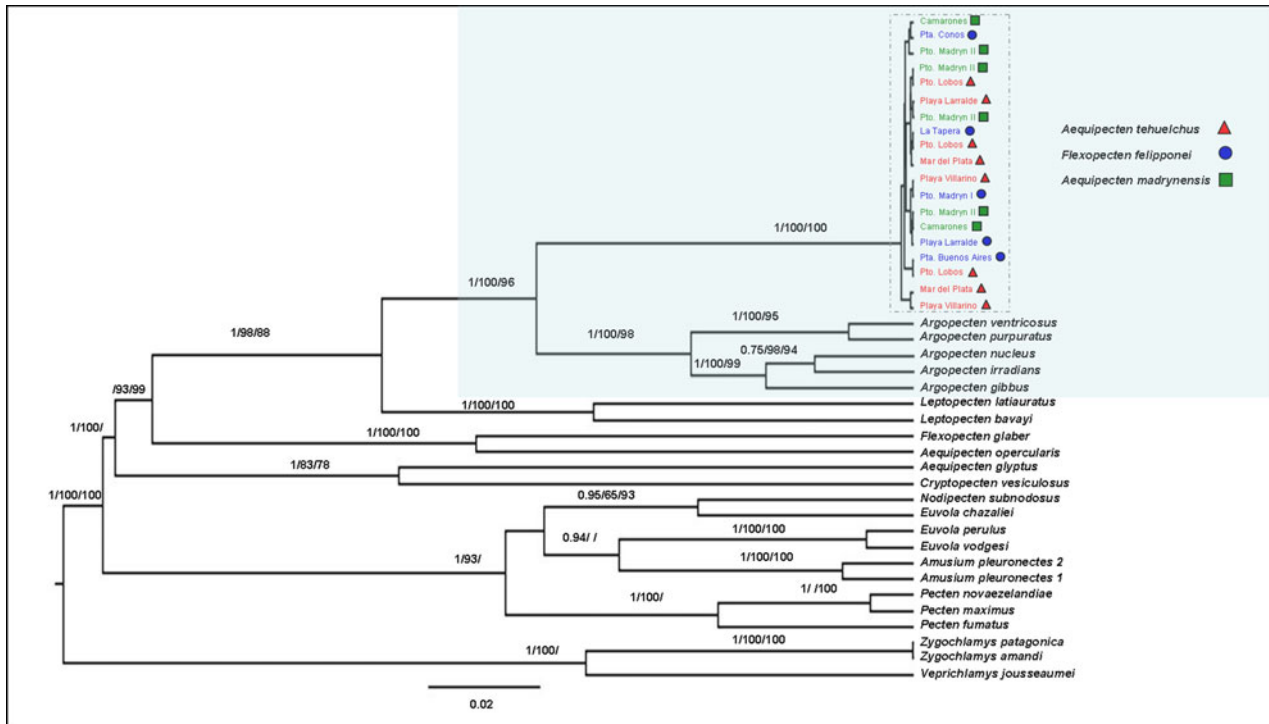


Fig. 3. Bayesian tree for the Tehuelche scallop based on the concatenated dataset of 12S rRNA + 16S rRNA + 28S rRNA -H₃. Numbers above the branches represent the Maximum parsimony and Maximum likelihood bootstrap values and Bayesian posterior probabilities (>60 only) for the supported nodes.

Phenotypic plasticity, i.e. the capacity of a genotype to produce different phenotypes under varying environmental conditions (Via, 1994), is a common trait in marine molluscs (e.g. Vermeij, 1973; Melatunan *et al.*, 2013) and has been used to explain differences in shell shapes in several bivalve populations (Krapivka *et al.*, 2007; Leyva-Valencia *et al.*, 2012). The occurrence of morphological adaptation of shell shapes to different local environmental conditions appears likely in the Tehuelche scallop given the geographic segregation of the two phenotypes (Figure 1). This, however, cannot be the case of the smooth phenotype *F. felipponei*, which has a sympatric distribution with the other two phenotypes. An alternative explanation that we cannot entirely discard is that the molecular markers used in this study may not be sufficiently variable to resolve the status of these three forms. Future studies should include more variable markers such as the cytochrome c oxidase subunit I (COI), which we failed to amplify.

With regard to within-population differences, Real *et al.* (2004) found that the San José Gulf population of Tehuelche scallops was the most distant genetically, which they attributed to different hydrodynamic conditions of the study areas. According to Orensanz (1986), the Tehuelche scallops from San José Gulf have an outline that is more typical of sedentary species, characterized by higher valves with more asymmetric auricles than those from San Matías Gulf. Such sedentary outline might improve scallop attachment to the bottom and thus be favoured by selective pressures in high-current environments (Stanley, 1970, 1972; Orensanz *et al.*, 1991). Further efforts to understand the effect of the environment on the genetic structure of the Tehuelche scallop would require the use of microsatellites. Although some advances have been made in the development of microsatellites in this species (Domínguez-Contreras *et al.*,

2017), the genetic structure of the population has not been studied to date. Based on a model of water circulation in the south-western Atlantic Ocean proposed by Tonini & Palma (2017), we hypothesized that the northward coastal current described could connect the scallop populations along the Argentinean coast. Amoroso *et al.* (2011), postulated a physical mechanism capable of dispersing larvae over long distances from San José Gulf into the adjacent San Matías Gulf. It would be interesting to investigate, using microsatellites, if there are intraspecific genetic differentiations among the populations in relation with these patterns of circulation.

Phylogenetic relationships

The non-commercial *Flexopecten felipponei* formed a monophyletic group with the two subspecies of *Aequipecten tehuelchus*. Waller (1991, 2006) concluded that *Flexopecten s.s.* would be related to *Aequipecten* and restricted to the Mediterranean and adjacent eastern Atlantic with a single 'outlier' species, *F. felipponei*, occurring in the western South Atlantic. Orensanz *et al.* (1991), on the examination of soft tissue and simultaneous hermaphroditism of numerous individuals with intermediate characters, proposed that '*F. felipponei*' would be only a phenotypic variant of *A. tehuelchus*, a hypothesis supported by our results. We found that *Flexopecten glaber* is grouped together with *Aequipecten opercularis* within the Pectinidae, both being phylogenetically distant from *F. felipponei*. This result is in agreement with those of Canapa *et al.* (2000), but in contrast with the results of Waller (1991) who placed *Flexopecten* as a member of the *Decatopecten* group, distant from *Aequipecten*.

Our phylogenetic reconstruction suggests that the clade formed by the three morphotypes of the Tehuelche

scallop distributed along the south-western Atlantic Ocean is more closely related to the genus *Argopecten* from the Eastern Pacific and North and Central-Western Atlantic than to the other members of the genus *Aequipecten*, including the type species of the genus, *Aequipecten opercularis* (Linnaeus 1758), a species distributed in the Norwegian and Mediterranean seas (Huber, 2010). *Aequipecten tehuelchus* was originally classified in *Chlamys* (d'Orbigny 1842) and considered a species of this genus until Waller (1991) and Del Río (1992) transferred it to *Aequipecten*, even though *Aequipecten* is primarily distributed to the North Atlantic. A more comprehensive phylogenetic reconstruction of the family Pectinidae (Alejandrino *et al.*, 2011; but see also: Saavedra & Peña, 2006; Puslednik & Serb, 2008; Feng *et al.*, 2011) showed that the monophyletic genus *Chlamys*, including the type species *Chlamys islandica* (O. F. Müller, 1776), is very distant to both genera *Argopecten* and *Aequipecten*. In our phylogeny, the Tehuelche scallop is located in a basal position within the clade formed by the species belonging to the genus *Argopecten*. Therefore, we recommend transfer of the species including the forms: *A. t. tehuelchus*, *A. t. madrynensis* and *Flexopecten filipponei*, to the genus *Argopecten* Monterosato, 1889.

Future directions to test species origins

Two alternative hypotheses about the origin of the Tehuelche scallop are implicit in the literature. The extant species '*Aequipecten tehuelchus*' (d'Orbigny 1846) originated in the south-western Atlantic from an endemic stock of '*Aequipecten paranensis tehuelchus*' (d'Orbigny 1842), a species from the Miocene, which was classified as a subspecies of *A. tehuelchus* by del Río (1992). Alternatively, *A. tehuelchus* is an austral vicariant of a genus primarily distributed in the North Atlantic (Waller, 1991). Our phylogenetic results give support to a third hypothesis: the Tehuelche scallop is part of the genus *Argopecten*, which is primarily distributed in the eastern Pacific and North and Central western Atlantic. The evaluation of these hypotheses requires the use of palaeontological and morphological data, as well as a molecular clock to estimate the separation time of '*Aequipecten tehuelchus*' from the other species of the genus. These would allow formulation of possible scenarios for the Tehuelche scallop evolutionary history, including potential causes of speciation such as geological or historical events that may have intervened (e.g. formation of the Isthmus of Panama).

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at <https://doi.org/10.1017/S0025315418000292>

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Correspondence should be addressed to:

Berenice Trovant

Instituto de Diversidad y Evolución Austral (CCT CONICET – CENPAT), Bv. Almirante Brown 2915, Puerto Madryn, Chubut, Argentina

email: trovant@cenpat-conicet.gob.ar