diversity

MDPI

Article

Species-Specific *Mytilus* Markers or Hybridization Evidence?

Hardy S. Guzmán [1], Jorge E. Toro [2,*], Pablo A. Oyarzún [3], Alex Illesca [2], Xiomara Ávila [2] and Jonathan P. A. Gardner [4]

[1] Departamento de Biología, Facultad de Química y Biología (FQyB), Universidad de Santiago de Chile, Santiago 9170022, Chile; hardy.guzman@usach.cl
[2] Instituto de Ciencias Marinas y Limnológicas (ICML), Universidad Austral de Chile, Valdivia 5090000, Chile; illescaalex@gmail.com (A.I.); xiomara.avila@alumnos.uach.cl (X.Á.)
[3] Centro de Investigación Marina Quintay (CIMARQ), Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago 7550196, Chile; pablo.oyarzun@unab.cl
[4] School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington 6140, New Zealand; jonathan.gardner@vuw.ac.nz
* Correspondence: jorgetoroyagui@gmail.com

Abstract: The development of molecular methods to detect *Mytilus* hybrids is important for food authentication, conservation, and the sustainable management of shellfish aquaculture as accurate food labeling is a legal requirement, and because introgression may promote undesirable phenotypes or displace native species. However, nuclear and mitochondrial markers can segregate independently, compromising diagnostic congruence between these markers. This study aimed to detect hybrids in the *Mytilus edulis* species complex using a multi-locus approach, including RFLP-PCR assays for Me 15/16, 16S rRNA, and COIxba, in samples collected from five continents. We used a model-based Bayesian method for hybrid and pure species detection to analyze the diagnostic potential of nuclear and mitochondrial markers in mussel samples from South America, North America, Africa, Oceania, and Europe. Our results showed that the combined use of markers can differentiate between *M. trossulus*, *M. edulis*, *M. galloprovincialis*, and *M. chilensis*. The combined use of nuclear and mitochondrial molecular markers also improves hybrid detection and allows us to identify introgression using Bayesian analysis.

Keywords: cryptic; hybrid; marine diversity; *Mytilus edulis* species complex; mitochondrial; nuclear markers

check for updates

Academic Editors: Jasenka Sremac, Marija Bošnjak and Bert W. Hoeksema

Received: 10 December 2024
Revised: 10 January 2025
Accepted: 17 January 2025
Published: 23 January 2025

Citation: Guzmán, H.S.; Toro, J.E.; Oyarzún, P.A.; Illesca, A.; Ávila, X.; Gardner, J.P.A. Species-Specific *Mytilus* Markers or Hybridization Evidence? *Diversity* **2025**, *17*, 82. https://doi.org/10.3390/d17020082

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Hybridization, or interspecific crossing, is a phenomenon that favors the gene flow that occurs between species during their divergence or by secondary contact [1]. It is a microevolutionary force that homogenizes the allelic distributions between populations; however, it also allows the emergence of genetic novelties more rapidly than mutations alone [2,3]. In this sense, hybridisation may favor the incorporation of alleles from one species into another by backcrossing [3]. However, anthropogenic intervention has made it easier for some species to increase their native distributions, invading new niches and negatively affecting local biodiversity and species-specific genetic diversity [4]. Often, hybridisation and backcrossing give rise to species complexes, i.e., groups of closely related taxa that may or may not show complete morphological differentiation due to incomplete reproductive isolation [5]. These are referred to as cryptic species [6], and their study is an excellent opportunity for understanding evolutionary processes and macroevolutionary trends [7].

The genus *Mytilus* (Linneaus, 1758) is a group of filter-feeding, semi-sessile bivalve mollusks that is of great economic and gastronomic interest [8]. They are considered a cryptic group due to their high phenotypic plasticity and their ability to generate stable hybrid zones that form with complex patterns [9–11]. They exhibit a double uniparental inheritance (DUI) system of mitochondria, which can generate mussels with two different mitochondrial genomes, or heteroplasmy [12–21].

The *Mytilus edulis* complex was initially composed of three formally recognized species: *M. edulis* (Linneaus, 1758), *M. galloprovincialis* (Lamarck, 1819), and *M. trossulus* (Gould, 1850) [22–24]. Over the years, more species belonging to the complex have been added, such as *M. platensis*, *M. aoteanus*, *M. planulatus*, and *M. chilensis* in the Southern hemisphere [25]. This increase in the number of species is due to the use of molecular tools in conjunction with the application of species delimitation methods [26].

The genus *Mytilus* is found on all continents, specifically inhabiting the temperate and boreal regions of the Northern and Southern hemispheres, now including the Antarctic continent due to anthropogenic factors [24,26–28]. Given their wide distribution, blue mussel species can generate hybrid zones where their distributions overlap, which has been documented in the literature [29–32]. The low reproductive isolation among most of the taxa has been extensively studied by directed crosses, which explains their morphological and genetic similarities [9,10,29,33–35]. This has allowed researchers to test questions about the taxonomic status of some taxa of the genus [26].

The development of methodologies to detect *Mytilus* hybrids is important for the conservation and sustainable management of mussel culture, because introgression may promote undesirable phenotypes or displace native species [32,36,37]. Beginning with the development of allozyme-based markers (e.g., [9,38]), a range of molecular markers of different types has been developed and applied to the question of species identification within the *Mytilus* species complex, including RFLP-PCR assays such as Me15/16, 16S rRNA, and COIxba [39–42], followed more recently by more in-depth molecular markers such as microsatellites [43] and single nucleotide polymorphisms known as SNPs [44]. Most of these RFLP-PCR markers have traditionally been used in isolation, with the result that, despite the widespread availability of multiple different marker types, very little multi-marker testing has been carried out to assess the performance of a panel of markers. Recent studies have reported the need for multi-locus approaches to assess the natural populations of *Mytilus* [45] due to diagnostic inconsistency between markers for pure lineage assignment and in regions of active interbreeding [31,45,46]. Thus, this study aimed to detect hybrids of the *Mytilus edulis* species complex using a multi-locus approach, employing the RFLP-PCR assays of Me 15/16, 16S rRNA, and COIxba on specimens collected from five continents.

2. Materials and Methods

Samples were collected from sites across five continents to assess the effectiveness of RFLP-PCR in detecting hybrids of smooth-shelled blue mussels (Figure 1 and Table 1). The sampling sites were chosen by literature review [24,26,27]. Table 1 details the species expected to be found at each of the sites studied and their expected genotypes.

A piece of the mantle tissue of each specimen was dissected and stored in ethanol at −80 °C. DNA extraction was performed using the E.Z.N.A® Tissue DNA kit (Omega Bio-Tek, Norcross, GA, USA), following the protocol indicated by the manufacturer. PCR and RFLP assays were performed with three markers: Me15/16, COIxba, and 16S rRNA, with their respective PCR conditions and restriction digests carried out following the authors' instructions [39–42]. COIxba was applied when the Me 15/16 marker showed a 126 bp

allele due to its specificity in recognizing *M. chilensis*. PCR-RFLP products were visualized on a 2% agarose gel and put into a matrix for further analysis.

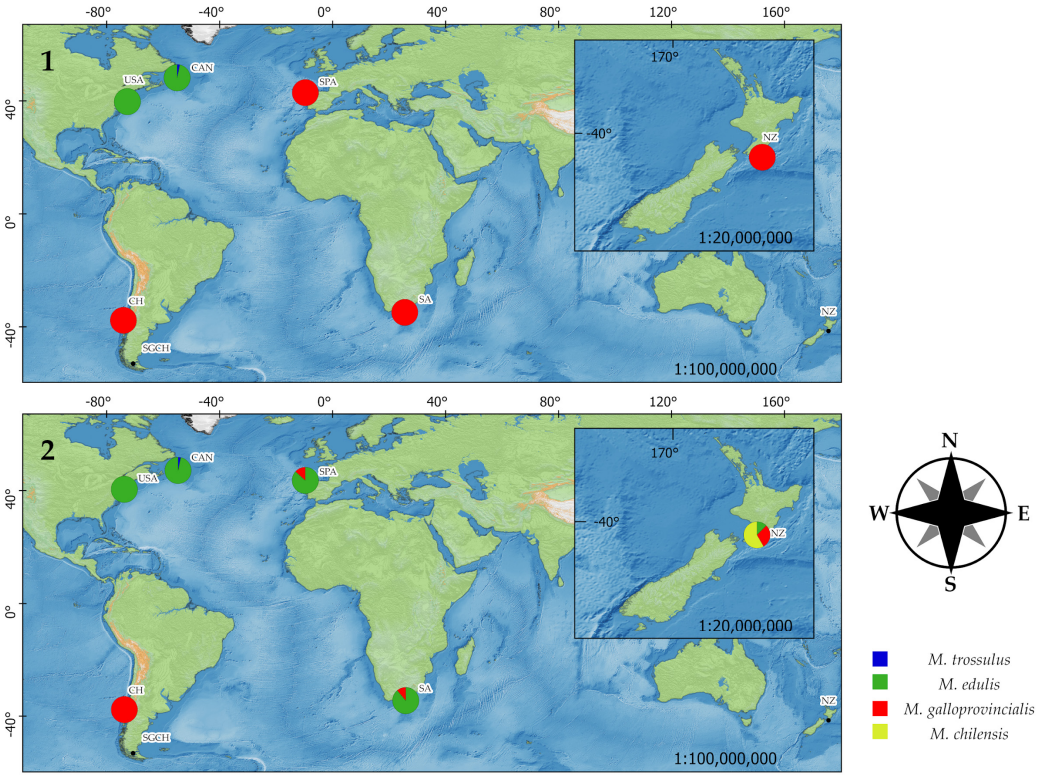


Figure 1. Map of sampled areas showing the results for the Me 15/16 (**1**) and 16S rRNA (**2**) molecular markers in the *Mytilus* complex. San Gregorio, Chile (SGCH) data were obtained from Oyarzún et al. [32]. See code details in Table 1. No map of results for the COIxba marker is provided because the results for this marker are very similar to those for the Me15/16 marker, except for the SGCH and NZ populations used for hybrids analysis (refer below).

Table 1. Site survey information, number of *Mytilus* spp. specimens collected (*N*) and map code corresponding to Figure 1. The expected band pattern for each pure species is also detailed according to the locus analyzed.

Location	Map Code	Species	Expected Genotype (bp)			*N*
			Me 15/16-AciI	16S	COIxbaI	
Ria de Vigo (Spain)	SPA	*M. galloprovincialis*	126	342, 195	230	30
Bellevue (Canada)	CAN	*M. trossulus*	168	370, 85, 82	230	30
Long Island (U.S.A)	USA	*M. edulis*	180	342, 85, 82, 28	230	30
Port Elizabeth (South Africa)	SA	*M. galloprovincialis*	126	342, 195	230	18
Wellington (New Zealand)	NZ	*M. galloprovincialis* Northern and Southern hemispheres	69, 57 *	342, 167, 28 *	134, 99 *	24
Tumbes (Chile)	CH	*M. galloprovincialis*, Northern hemisphere	126	342, 195	230	25
San Gregorio (Chile)	SGCH	Hybrid zone				35

The symbol * indicates the expected genotype after a treatment with restriction enzyme of a 126 bp amplicon for the native Southern hemisphere *M. galloprovincialis* type [31,41] which is *M. aoteanus*.

Data Analysis

To determine whether the three markers could distinguish between pure lineages, a principal component analysis (PCA) was performed using the r package "adegenet", with samples from SA, CAN, SPA, and the USA (Table 1). Sampling sites were chosen based on published results of pure lineage distribution of each species in these samples, which were used as reference populations.

For hybrids analysis, we used published data from the San Gregorio, Chile (SCGH) population that was previously described as a hybrid population of *M. edulis*, *M. galloprovincialis*, and *M. chilensis* by Oyarzún et al. [32]. Genotype data from the newly sampled New Zealand (NZ) and the previously sampled SGCH populations were analyzed with NewHybrids v.1 [47] because putative hybrids have been reported in these two locations by Gardner and Westfall [31] and Oyarzún et al. [32], respectively. We performed 100,000 burn-in iterations and 1,000,000 MCMC replicates to estimate the Bayesian posterior probability for each of the six genotypic categories: backcrosses to both parental types (0 Bx and 1Bx), F1, F2, and the pure parental types (0 and 1). Parental types were defined based on the alleles found within each mussel.

3. Results

In total, 192 mussels from Port Elizabeth (SA), Ría de Vigo (SPA), Long Island (USA), Bellevue (CAN), Wellington (NZ), Tumbes (CH), and San Gregorio (SGCH) were analyzed (Table 1). Only two mussels (1.85%) exhibited null alleles, and this was for the 16S marker.

3.1. Pure Lineage Analysis

The single locus approach showed heterozygote absence, but nuclear and mitochondrial marker discordance was found (Figure 1), mainly in sampling areas where *M. galloprovincialis* is the predominant species. Although COIxba showed 100% concordance with expected pure species within almost all sampling sites, some inconsistencies in sympatric zones were observed (NZ and SGCH). PCA results showed that the combined use of Me 15/16, 16S rRNA, and COIxba did not affect the discrimination of pure lineages, clustering the genotypes to their corresponding taxonomic category (Figure 2). Other than the reference population of Bellevue (CAN) *M. trossulus* which were found only one mussel.

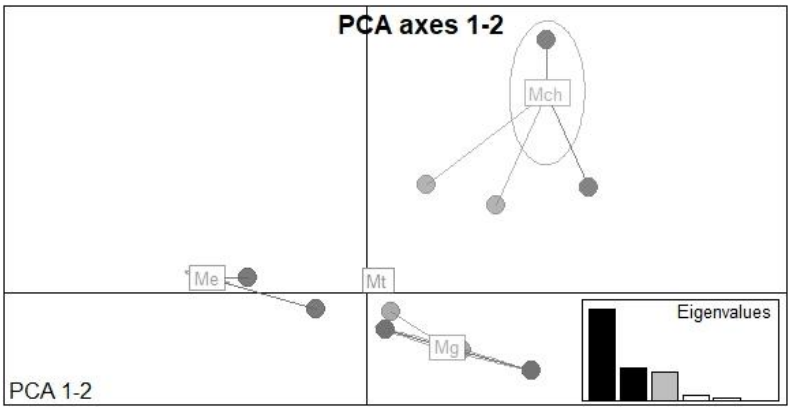


Figure 2. Principal component analysis (PCA) plot of the use of the three markers simultaneously: each point represents a multi-locus genotype. Eigenvalues are indicated in black (bottom right) and indicate that the two axes explain 80% of the variance. *Mytilus chilensis* (Mch), *M. edulis* (Me), *M. galloprovincialis* (Mg), *M. trossulus* (Mt).

3.2. Analysis of Hybrid Genotypes

To test for the presence of putative hybrids we applied Bayesian analysis to two sympatric populations (SGCH and NZ, Figure 1) with the three molecular markers (Me

15/16, 16S rRNA, and COIxba). In SGCH, we found two types of putative hybrids: (1) heterozygous genotypes with Me 15/16, and (2) homozygous with nuclear and mitochondrial incongruence. With the Bayesian approach, the first type of putative hybrid composed of *M. edulis* x *M. chilensis* was classified as pure lineage *M. chilensis* with over 80% assignment probability (Table 2). A total of 29 individuals representing 93.5% of the SGCH mussels were classified as *M. chilensis*. In the second case, we found only one animal with a 50% assignment probability as an F2 hybrid (*M. edulis* x *M. chilensis*) in the SGCH population.

Table 2. Putative hybrid genotypes found in the SGCH and NZ populations. Posterior Bayesian probabilities for each genotype category were obtained for the following classes; pure 0 and pure 1 (the two parental types) are reference populations of non-hybrid samples, F1 and F2 hybrid genotypes, and 0 Bx and 1 Bx are backcrosses with pure 0 or pure 1 parental types, respectively. The pure lineages for this hybrid analysis were: *M. edulis* (Me); *M. chilensis* (Mch); *M. galloprovincialis* (Mg); *M. trossulus* (Mt). *N* is the number of individuals for each genotype. Note that SGCH data were obtained from Oyarzún et al. [32].

Location	Genotype (bp)			Pure 0	Pure 1	F1	F2	0 Bx	1 Bx	*N*	
	Me15/16	16S	COIxba	Me	Mch	F1	F2	Mg Bx	Mch Bx		Loci *N*
SGCH	126/180			0.07517	0.80299	0.03930	0.02133	0.01687	0.04433	6	3
	126/180	167		0.00001	0.96095	0.00035	0.00741	0.00007	0.03121	4	3
	126/180		134	0.00001	0.96095	0.00035	0.00741	0.00007	0.03121	1	3
				Me	Mch	F1	F2	Me Bx	Mch Bx		
SGCH	180	167		0.14656	0.29935	0.00057	0.50532	0.01469	0.03351	1	3
				Mg	Mch	F1	F2	Mch Bx	Mg Bx		
SGCH	126	167	134	0.00000	0.99731	0.00000	0.00045	0.00223	0.00000	7	3
	126		134	0.00009	0.99373	0.00003	0.00176	0.00437	0.00002	8	3
	126	167		0.00009	0.99373	0.00003	0.00176	0.00437	0.00002	3	3
				Mg	Mt	F1	F2	Mt Bx	Mg Bx		
SGCH	168/126			0.30865	0.15148	0.13768	0.11560	0.18249	0.10410	1	3
				Mg	Mch	F1	F2	Mg Bx	Mch Bx		
NZ	126	195	134	0.02136	0.36400	0.00020	0.59269	0.00171	0.02005	4	3

The underlined numbers indicate which is the higher posterior probability in a hybrid category.

In the NZ population, four mussels showed >50% assignment probability assignments as F2 hybrids, corresponding to a *M. galloprovincialis* x *M. chilensis*. None of the mussels analyzed were assigned to either backcross category. It is important to appreciate that the suite of three independent markers employed here do not differentiate amongst the native Southern hemisphere mussel species, so the putative *M. chilensis* designation of mussels in the NZ sample are not actually *M. chilensis* but are considered to be native New Zealand mussels (i.e., *M. aoteanus*–[8,25,31,44]).

4. Discussion

Most molecular approaches to the analysis of the *M. edulis* species complex have involved single loci, specifically RFLP-PCR, because it is cheaper and faster than other methods [48,49]. In the present study, we performed a comparative test of three molecular markers for *Mytilus* taxa from five continents. We found that nuclear and mitochondrial markers can exhibit some incongruences; this is particularly the case for 16S rRNA in conjunction with Me 15/16. Similar 16S rRNA and Me 15/16 marker variation outcomes were reported by Larraín et al. [45]. However, Westfall et al. [41] stated that, in some cases, these incongruences are because the mitochondrial lineage of *M. edulis* shares an allelic

pattern with *M. galloprovincialis*-referred to as the *M. edulis/M. galloprovincialis* allelic pattern-and which often leads to a misinterpretation of the mitochondrial marker 16S rRNA. To avoid misinterpretation of 16S results the same authors used a second marker, the Me15/16 assay. The *M. edulis/M. galloprovincialis* allelic pattern, found mostly in Northern hemisphere mussels, could represent an introgressed allele due to the independent segregation of nuclear and mitochondrial markers which may affect the diagnostic congruence among the markers [50]. This pattern, which has been well described by Bierne et al. [51], is typically found in mussels from the Atlantic coast of Europe, that is, in the North Atlantic lineage of *M. galloprovincialis*.

Incongruence between nuclear and mitochondrial marker results may be due to hybridization events, which have been studied in Southern hemisphere *Mytilus* species. In this context, Westfall and Gardner [31] quantified these phenomena by analyzing cytonuclear disequilibrium, finding asymmetric mitochondrial-exchange sex links, which could be driven by mitochondrial inheritance systems or DUIs (Doubly Uniparental Inheritances). Larraín et al. [45] discussed this scenario with a multi-locus approach, using five molecular markers; however, their analysis and sampling were not focused on hybrid detection.

Cytonuclear disequilibrium is associated with successive outcrossing and backcrossing events of sympatric populations and has been related mainly to geographical factors rather than to taxonomic relationships [51]. For instance, in the Strait of Magellan, an important area of interspecific hybridization of the genus *Mytilus* in the Southern Hemisphere, a significant asymmetry in the exchange of mitochondrial haplotypes has been observed, which has a sex-linked polarity (i.e., direction of introgression depending on the sex of the parents), indicating a higher frequency of crossing between a native female parent (*M. chilensis*) and a non-native male parent (*M. galloprovincialis*; [52]).

The frequency of putative hybrid mussels from the Strait of Magellan reported here is lower than that reported by Oyarzún et al. [32]; thus, this method is proven to be a promising method for detecting introgression within the population. Additionally, including a second nuclear marker could give a more reliable classification of hybrids and pure lineages. For instance, an individual mussel may be an F2 hybrid and yet have a homozygous genotype for one of the species-specific alleles at the nuclear marker as well as mitotype from that same species.

Hybrid animals in the aquaculture industry and in food authentication programs may cause major problems within the *Mytilus* genus; our results support using a multi-locus approach for such work in the *Mytilus* genus, as suggested by Larraín et al. [45]. Bayesian analysis based on the results of the three markers used in this study could be an effective method for analyzing *Mytilus* populations that coexist sympatrically, which is common in some areas (e.g., Chile [32,53]). Nevertheless, it would be interesting to test the performance of SNPs, focusing on sympatric zones to evaluate the hybrid detection performance. Hence, multi-locus markers can give researchers detailed information on mosaic hybrid zones of mussels in order to evaluate their biogeographic history and determine how anthropogenic factors affect mussel distribution [54,55]. In addition, improving these tools could give us a better understanding of mitochondrial inheritance in *Mytilus* by tracking the mitochondria from both sexes to the offspring, because various models of mitochondrial inheritance have been proposed for DUI [56,57].

5. Conclusions

The segregation of mitochondrial species-specific markers does not appear to be linked to nuclear markers, resulting in diagnostic inconsistencies or incongruence. These diagnostic incongruities have been studied as a product of backcrossing. When analyzing the probability of assignment of the heterozygotes of the Me 15/16 nuclear marker as

hybrids, their assignment as F1 or F2 hybrids decreased in the combined analysis of the three markers evaluated (Me15/16, 16S and COIxbaI). These genotypes correspond with higher probability of assignment to the product of backcrosses. Comparative identification allows us to evaluate diagnostic congruence between nuclear and mitochondrial markers, which show better performance due to the use of the combined data of all three RFLP-PCR assays from a Bayesian point of view. These methods can be useful in identifying interbreeding processes or putative hybrids, and provides a higher resolution for a better understanding of hybridization patterns in *Mytilus*.

Author Contributions: Conceptualization, J.E.T., P.A.O. and H.S.G.; methodology, J.E.T. and P.A.O.; validation, H.S.G., X.Á. and A.I.; writing—original draft preparation, J.E.T., P.A.O. and H.S.G.; writing—review and editing, J.P.A.G., J.E.T., P.A.O. and H.S.G.; investigation and formal analysis, H.S.G.; funding acquisition, J.E.T. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financed by FONDECYT 1230212—ANID (Toro, J. E).

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the Universidad Austral de Chile.

Data Availability Statement: All data generated or analyzed during this study are included in the published article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. McLaughlin, J.F.; Faircloth, B.C.; Glenn, T.C.; Winker, K. Divergence, gene flow, and speciation in eight lineages of trans-Beringian birds. *Mol. Ecol.* **2020**, *29*, 3526–3542. [CrossRef] [PubMed]
2. Twyford, A.D.; Ennos, R.A. Next-generation hybridization and introgression. *Heredity* **2012**, *108*, 179–189. [CrossRef]
3. Harrison, R.G.; Larson, E.L. Hybridization, introgression, and the nature of species boundaries. *J. Hered.* **2014**, *105*, 795–809. [CrossRef] [PubMed]
4. Zbawicka, M.; Wenne, R.; Dias, P.J.; Gardner, J.P.A. Combined threats to native smooth-shelled mussels (genus *Mytilus*) in Australia: Bioinvasions and hybridization. *Zool. J. Linn. Soc.* **2022**, *194*, 1194–1211. [CrossRef]
5. Duminil, J.; Kenfack, D.; Viscosi, V.; Grumiau, L.; Hardy, O.J. Testing species delimitation in sympatric species complexes: The case of an African tropical tree, *Carapa* spp. (Meliaceae). *Mol. Phylogenetics Evol.* **2012**, *62*, 275–285. [CrossRef] [PubMed]
6. Bickford, D.; Lohman, D.J.; Sodhi, N.S.; Ng, P.K.; Meier, R.; Winker, K.; Ingram, K.K.; Das, I. Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* **2007**, *22*, 148–155. [CrossRef] [PubMed]
7. Struck, T.H.; Feder, J.L.; Bendiksby, M.; Birkeland, S.; Cerca, J.; Gusarov, V.I.; Kistenich, S.; Larsson, K.H.; Liow, L.H.; Nowak, M.D.; et al. Finding evolutionary processes hidden in cryptic species. *Trends Ecol. Evol.* **2018**, *33*, 153–163. [CrossRef]
8. Larraín, M.A.; Zbawicka, M.; Araneda, C.; Gardner, J.P.A.; Wenne, R. Native and invasive taxa on the Pacific coast of South America: Impacts on aquaculture, traceability and biodiversity of blue mussels (*Mytilus* spp.). *Evol. Appl.* **2018**, *11*, 298–311. [CrossRef]
9. Toro, J.; Thompson, R.J.; Innes, D.J. Fertilization success and early survival in pure and hybrid larvae of *Mytilus edulis* (Linnaeus, 1758) and *M. trossulus* (Gould, 1850) from laboratory crosses. *Aquac. Res.* **2006**, *37*, 1703–1708. [CrossRef]
10. Toro, J.E.; Oyarzún, P.A.; Peñaloza, C.S.; Alcapán, A.; Videla, V.; Tilleria, J.; Astorga, M.; Martinez, V. Production and performance of larvae and spat of pure and hybrid species of *Mytilus chilensis* and *M. galloprovincialis* from laboratory crosses. *Lat. Am. J. Aquat. Res.* **2012**, *40*, 243–247. [CrossRef]
11. Wenne, R.; Bach, L.; Zbawicka, M.; Strand, J.; McDonald, J.H. A first report on coexistence and hybridization of *Mytilus trossulus* and *M. edulis* mussels in Greenland. *Polar Biol.* **2016**, *39*, 343–355. [CrossRef]
12. Fisher, C.; Skibinski, D.O.F. Sex-biased mitochondrial DNA heteroplasmy in the marine mussel *Mytilus*. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **1990**, *242*, 149–156. [CrossRef]
13. Hoeh, W.R.; Blakley, K.H.; Brown, W.M. Heteroplasmy suggests limited biparental inheritance of *Mytilus* mitochondrial DNA. *Science* **1991**, *251*, 1488–1490. [CrossRef]
14. Skibinski, D.O.F.; Gallagher, C.; Beynon, C.M. Sex-limited mitochondrial DNA transmission in the marine mussel *Mytilus edulis*. *Genetics* **1994**, *138*, 801–809. [CrossRef]

15. Comesaña, A.S.; Toro, J.E.; Innes, D.J.; Thompson, R.J. A molecular approach to the ecology of a mussel (*Mytilus edulis–M. trossulus)* hybrid zone on the east coast of Newfoundland, Canada. *Mar. Biol.* **1999**, *133*, 213–221. [CrossRef]
16. Zouros, E. The exceptional mitochondrial DNA system of the mussel family Mytilidae. *Genes Genet. Syst.* **2000**, *75*, 313–318. [CrossRef] [PubMed]
17. Xu, J. The inheritance of organelle genes and genomes: Patterns and mechanisms. *Genome* **2005**, *48*, 951–958. [CrossRef] [PubMed]
18. Breton, S.; Beaupre, H.D.; Stewart, D.T.; Hoeh, W.R.; Blier, P.U. The unusual system of doubly uniparental inheritance of mtDNA: Isn't one enough? *Trends Genet.* **2007**, *23*, 465–474. [CrossRef]
19. Doucet-Beaupré, H.; Breton, S.; Chapman, E.G.; Blier, P.U.; Bogan, A.E.; Stewart, D.T.; Hoeh, W.R. Mitochondrial phylogenomics of the Bivalvia (Mollusca): Searching for the origin and mitogenomic correlates of doubly uniparental inheritance of mtDNA. *BMC Evol. Biol.* **2010**, *10*, 50. [CrossRef]
20. Capt, C.; Renaut, S.; Ghiselli, F.; Milani, L.; Johnson, N.A.; Sietman, B.E.; Stewart, D.T.; Breton, S. Deciphering the link between doubly uniparental inheritance of mtDNA and sex determination in bivalves: Clues from comparative transcriptomics. *Genome Biol. Evol.* **2018**, *10*, 577–590. [CrossRef] [PubMed]
21. Varvio, S.L.; Koehn, R.K.; Väinölä, R. Evolutionary genetics of the *Mytilus edulis* complex in the North Atlantic region. *Mar. Biol.* **1988**, *98*, 51–60. [CrossRef]
22. McDonald, J.H.; Seed, R.; Koehn, R.K. Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. *Mar. Biol.* **1991**, *111*, 323–333. [CrossRef]
23. Gosling, E. (Ed.) *The Mussel Mytilus: Ecology, Physiology, Genetics and Culture*; No. 594.1 MUS; Elsevier: Amsterdam, The Netherlands, 1992.
24. Gaitán-Espitia, J.D.; Quintero-Galvis, J.F.; Mesas, A.; D'Elía, G. Mitogenomics of southern hemisphere blue mussels (Bivalvia: Pteriomorphia): Insights into the evolutionary characteristics of the *Mytilus edulis* complex. *Sci. Rep.* **2016**, *6*, 26853. [CrossRef]
25. Oyarzún, P.A.; Toro, J.E.; Nuñez, J.J.; Suárez-Villota, E.Y.; Gardner, J.P.A. Blue mussels of the *Mytilus edulis* species complex from South America: The application of species delimitation models to DNA sequence variation. *PLoS ONE* **2021**, *16*, e0256961. [CrossRef]
26. Koehn, R.K. The genetics and taxonomy of species in the genus *Mytilus*. *Aquaculture* **1991**, *94*, 125–145. [CrossRef]
27. Hilbish, T.J.; Mullinax, A.; Dolven, S.I.; Meyer, A.; Koehn, R.K.; Rawson, P.D. Origin of the antitropical distribution pattern in marine mussels (*Mytilus* spp.): Routes and timing of transequatorial migration. *Mar. Biol.* **2000**, *136*, 69–77. [CrossRef]
28. Cárdenas, L.; Leclerc, J.C.; Bruning, P.; Garrido, I.; Détrée, C.; Figueroa, A.; Astorga, M.; Navarro, J.; Johnson, L.; Carlton, J.; et al. First mussel settlement observed in Antarctica reveals the potential for future invasions. *Sci. Rep.* **2020**, *10*, 5552. [CrossRef]
29. Sanjuan, A.; Zapata, C.; Alvarez, G. *Mytilus galloprovincialis* and *M. edulis* on the coasts of the Iberian Peninsula. *Mar. Ecol. Prog. Ser.* **1994**, *113*, 131–146. [CrossRef]
30. Toro, J.E.; Ojeda, J.A.; Vergara, A.M.; Castro, G.C.; Alcapan, A.C. Molecular characterization of the Chilean blue mussel (*Mytilus chilensis*, Hupe 1854) demonstrates evidence for the occurrence of *Mytilus galloprovincialis* in southern Chile. *J. Shellfish Res.* **2005**, *24*, 1117–1121. [CrossRef]
31. Westfall, K.M.; Gardner, J.P.A. Genetic diversity of Southern hemisphere blue mussels (Bivalvia: Mytilidae) and the identification of non-indigenous taxa. *Biol. J. Linn. Soc.* **2010**, *101*, 898–909. [CrossRef]
32. Oyarzún, P.A.; Toro, J.E.; Cañete, J.I.; Gardner, J.P.A. Bioinvasion threatens the genetic integrity of native diversity and a natural hybrid zone: Smooth-shelled blue mussels (*Mytilus* spp.) in the Strait of Magellan. *Biol. J. Linn. Soc.* **2016**, *117*, 574–585. [CrossRef]
33. Toro, J.E.; Thompson, R.J.; Innes, D.J. Reproductive isolation and reproductive output in two sympatric mussel species (*Mytilus edulis*, *Mytilus trossulus*) and their hybrids from Newfoundland. *Mar. Biol.* **2002**, *141*, 897–909. [CrossRef]
34. Valenzuela, A.; Astorga, M.P.; Oyarzún, P.A.; Toro, J.E. Caracterización genética de híbridos entre las especies *Mytilus edulis platensis* y *Mytilus galloprovincialis* (Mytilidae: Bivalvia) en la costa Chilena. *Lat. Am. J. Aquat. Res.* **2016**, *44*, 171–176. [CrossRef]
35. Michalek, K.; Ventura, A.; Sanders, T. *Mytilus* hybridisation and impact on aquaculture: A minireview. *Mar. Genom.* **2016**, *27*, 3–7. [CrossRef] [PubMed]
36. Zhang, W.; Li, R.; Chen, X.; Wang, C.; Gu, Z.; Mu, C.; Song, W.; Zhan, P.; Huang, J. Molecular identification reveals hybrids of *Mytilus coruscus* × *Mytilus galloprovincialis* in mussel hatcheries of China. *Aquac. Int.* **2020**, *28*, 85–93. [CrossRef]
37. Michalek, K.; Vendrami, D.L.; Bekaert, M.; Green, D.H.; Last, K.S.; Telesca, L.; Wilding, T.A.; Hoffman, J.I. *Mytilus trossulus* introgression and consequences for shell traits in longline cultivated mussels. *Evol. Appl.* **2021**, *14*, 1830–1843. [CrossRef]
38. Skibinski, D.O.F.; Beardmore, J.A.; Cross, T.F. Aspects of the population genetics of *Mytilus* (Mytilidae; Mollusca) in the British Isles. *Biol. J. Linn. Soc.* **1983**, *19*, 137–183. [CrossRef]
39. Inoue, K.; Waite, J.H.; Matsuoka, M.; Odo, S.; Harayama, S. Interspecific variations in adhesive protein sequences of *Mytilus edulis*, *M. galloprovincialis*, and *M. trossulus*. *Biol. Bull.* **1995**, *189*, 370–375. [CrossRef] [PubMed]
40. Santaclara, F.J.; Espiñeira, M.; Cabado, A.G.; Aldasoro, A.; Gonzalez-Lavín, N.; Vieites, J.M. Development of a method for the genetic identification of mussel species belonging to *Mytilus*, *Perna*, *Aulacomya*, and other genera. *J. Agric. Food Chem.* **2006**, *54*, 8461–8470. [CrossRef] [PubMed]

41. Westfall, K.M.; Wimberger, P.H.; Gardner, J.P.A. An RFLP assay to determine if *Mytilus galloprovincialis* Lmk. (Mytilidae; Bivalvia) is of Northern or Southern hemisphere origin. *Mol. Ecol. Resour.* **2010**, *10*, 573–575. [CrossRef] [PubMed]
42. Fernández-Tajes, J.; Longa, A.; García-Gil, J.; Chiu, Y.W.; Huang, Y.S.; Méndez, J.; Lee, R.S. Alternative PCR–RFLP methods for mussel *Mytilus* species identification. *Eur. Food Res. Technol.* **2011**, *233*, 791–796. [CrossRef]
43. Ouagajjou, Y.; Presa, P.; Astorga, M.; Pérez, M. Microsatellites of *Mytilus chilensis*: A genomic print of its taxonomic status within *Mytilus* sp. *J. Shellfish Res.* **2011**, *30*, 325–330. [CrossRef]
44. Gardner, J.P.A.; Zbawicka, M.; Westfall, K.M.; Wenne, R. Invasive blue mussels threaten regional scale genetic diversity in mainland and remote offshore locations: The need for baseline data and enhanced protection in the Southern Ocean. *Glob. Change Biol.* **2016**, *22*, 3182–3195. [CrossRef] [PubMed]
45. Larraín, M.A.; González, P.; Pérez, C.; Araneda, C. Comparison between single and multi-locus approaches for specimen identification in *Mytilus* mussels. *Sci. Rep.* **2019**, *9*, 19714. [CrossRef] [PubMed]
46. Kijewski, T.; Wijsman, J.W.; Hummel, H.; Wenne, R. Genetic composition of cultured and wild mussels *Mytilus* from The Netherlands and transfers from Ireland and Great Britain. *Aquaculture* **2009**, *287*, 292–296. [CrossRef]
47. Anderson, E.C.; Thompson, E. A model-based method for identifying species hybrids using multilocus genetic data. *Genetics* **2002**, *160*, 1217–1229. [CrossRef] [PubMed]
48. Dellicour, S.; Flot, J.F. The hitchhiker's guide to single-locus species delimitation. *Mol. Ecol. Resour.* **2018**, *18*, 1234–1246. [CrossRef] [PubMed]
49. Alexander, J.L.; Malham, S.K.; Smyth, D.; Webb, J.; Fidler, D.; Bayford, P.; McDonald, J.; Le Vay, L. Improving quantification of bivalve larvae in mixed plankton samples using qPCR: A case study on *Mytilus edulis*. *Aquaculture* **2021**, *532*, 736003. [CrossRef]
50. Rawson, P.D.; Hilbish, T.J. Asymmetric introgression of mitochondrial DNA among European populations of blue mussels (*Mytilus* spp.). *Evolution* **1998**, *52*, 100–108. [CrossRef]
51. Kijewski, T.; Śmietanka, B.; Zbawicka, M.; Gosling, E.; Hummel, H.; Wenne, R. Distribution of *Mytilus* taxa in European coastal areas as inferred from molecular markers. *J. Sea Res.* **2011**, *65*, 224–234. [CrossRef]
52. Westfall, K.M.; Gardner, J.P.A. Interlineage *Mytilus galloprovincialis* Lmk. 1819 hybridization yields inconsistent genetic outcomes in the Southern hemisphere. *Biol. Invasions* **2013**, *15*, 1493–1506. [CrossRef]
53. Oyarzún, P.A.; Toro, J.E.; Nuñez, J.J.; Ruiz-Tagle, G.; Gardner, J.P.A. The Mediterranean mussel *Mytilus galloprovincialis* (Mollusca: Bivalvia) in Chile: Distribution and genetic structure of a recently introduced invasive marine species. *Animals* **2024**, *14*, 823. [CrossRef]
54. Simon, A.; Arbiol, C.; Nielsen, E.E.; Couteau, J.; Sussarellu, R.; Burgeot, T.; Bernard, I.; Coolen, P.W.J.; Robert, S.; Skazina, M.; et al. Replicated anthropogenic hybridisations reveal parallel patterns of admixture in marine mussels. *Evol. Appl.* **2020**, *13*, 575–599. [CrossRef] [PubMed]
55. Simon, A.; Fraïsse, C.; El Ayari, T.; Liautard-Haag, C.; Strelkov, P.; Welch, J.J.; Bierne, N. How do species barriers decay? Concordance and local introgression in mosaic hybrid zones of mussels. *J. Evol. Biol.* **2021**, *34*, 208–223. [CrossRef]
56. Zouros, E. Doubly uniparental inheritance of mitochondrial DNA: Might it be simpler than we thought? *J. Zool. Syst. Evol. Res.* **2020**, *58*, 624–631. [CrossRef]
57. Stewart, D.T.; Robicheau, B.M.; Youssef, N.; Garrido-Ramos, M.A.; Chase, E.E.; Breton, S. Expanding the search for sperm transmission elements in the mitochondrial genomes of bivalve mollusks. *Genes* **2021**, *12*, 1211. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.