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PHYLOGEOGRAPHY OF SOUTHERN HEMISPHERE BLUE MUSSELS OF THE GENUS *MYTILUS*: EVOLUTION, BIOSECURITY, AQUACULTURE AND FOOD LABELLING

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Abstract Smooth-shelled blue mussels of the genus *Mytilus* have long been a major focus of research because such mussels have an interesting global (antitropical or bipolar) distribution, have a complex phylogeographic history, are ecosystem engineers, are economically important as an aquaculture product, are very successful as bioinvaders, hybridise readily both naturally and after accidental introduction, and have a complex and still incompletely resolved taxonomy. Historically, most research has been focussed on Northern hemisphere mussels, and investigation has spanned a range of different methodological approaches that have set the foundation for our current understanding of the global situation. However, research into native blue mussels in the Southern hemisphere has tended to lag behind that in the Northern hemisphere. The result has been that native Southern hemisphere mussels have often been viewed only in the perspective of their Northern hemisphere congeners. Recently, however, the application of new molecular markers – single nucleotide polymorphisms, SNPs – has substantially improved our understanding of the taxonomy of Southern hemisphere blue mussels, their biogeography and indirectly their evolutionary histories. Based largely on new SNP surveys of native and non-native blue mussels from all major Southern hemisphere regions, we highlight the need for recognition of distinct species that are consistent with a large body of evidence, both SNP-based and other, and can be understood in the context of Southern hemisphere oceanography. We also highlight with the new SNPs-based data the ongoing difficulty of agreeing on what constitutes a distinct species by emphasising different interpretations of the data, and discussing how the use of species delimitation models may remove some of the qualitative assessment that is so often applied to the problem. The recognition of new species has implications for management, including the identification of bioinvasive mussels, the conservation of native genetic diversity, aquaculture production statistics, food labelling and traceability.

Keywords: Blue Mussels; *Mytilus*; Southern Hemisphere; Phylogeography; Taxonomy and Systematics; Hybridisation and Introgression; Bioinvasions; Aquaculture; Food Labelling and Traceability.

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The global distribution of smooth-shelled mussels of the genus *Mytilus*

The first scientific description of mussels of the genus *Mytilus* dates to 1758 and is attributable to the great Swedish biologist, Carl Linnaeus (Linnaeus 1758). He described *M. edulis* from the European coast of the North Atlantic Ocean and the Baltic Sea (the exact location does not seem to have been recorded), and subsequently, the great French naturalist Jean-Baptiste Lamarck described *M. galloprovincialis* from the Mediterranean Sea (Lamarck 1819). During an unprecedented period of exploration and collecting, from approximately 1750 to 1850, a substantial number of biological samples were collected from many regions of the world, both Northern hemisphere and Southern hemisphere (the genus is found naturally on all continents except Antarctica). Many of these blue mussel samples were returned to European museums, where they were catalogued and often given new names (reviewed by Lamy 1936, Soot-Ryen 1955). What soon became apparent is that the genus, like many other marine taxa, has an antitropical distribution, sometimes also referred to as a bipolar distribution (Ekman 1953, Briggs 1974, Santelices 1980, Lindberg 1991, Hilbish et al. 2000). Attempting to explain this distribution and the evolutionary relationships between Northern and Southern hemisphere mussels has been a challenge for biologists, and has been aided and hindered by the tangle of taxonomy that developed from the earliest days.

For blue mussels, which are very variable in their shell phenotypes (Figure 1), it was often the case that even only limited morphometric difference was viewed as being enough to erect a new species or subspecies or variety. For example, the World Registry of Marine Species (WoRMS Editorial Board 2020) lists 21 synonymised names for *M. edulis*, 25 synonymised names for *M. galloprovincialis*, and eight synonymised names for *M. trossulus* (Gould, 1850). As subsequent large spatial scale studies of shell trait and shell shape variation have demonstrated (e.g., McDonald et al. 1991, Gardner & Thompson 2009), many of the shell characters (traits and shape) do have genuine taxonomic resolving power, which means that disentangling the complexities of the old nomenclature against today's thinking has, at times, been fraught (e.g., as discussed by Larraín et al. 2018).

The decade of the mid-1980s to the mid-1990s saw an incredible explosion of new knowledge that changed the way researchers thought about the taxonomy and biogeography of smooth-shelled blue mussels of what was and still is called the *Mytilus edulis* species complex, and laid the foundation for much of today's interpretation of the taxonomy and systematics of the genus (Koehn et al. 1984, McDonald & Koehn 1988, Varvio et al. 1988, McDonald et al. 1991 and references therein). While most of this body of work was carried out using allozymes (protein variation), a lot of it was supported by increasingly sophisticated analyses of shells, including trait and shape variation. In addition, this research was being conducted at a time when newly developed molecular methods such as the analyses of mtDNA (mitochondrial DNA) RFLPs (restriction fragment length polymorphisms) were being developed and applied, and at a time when much attention in biological sciences was focussed on the challenge of defining a species (reviewed by Cracraft 1983, 1989, Mishler & Brandon 1987, Templeton 1989, Avise & Ball 1990, Wallace & Willis 1994, Mallet 1995, Zink & McKitrick 1995).

The application of new molecular markers such as single nucleotide polymorphisms (SNPs) and the development of new analytical approaches have significantly advanced our understanding in many areas of biology, forensics, medicine and agricultural production. While the taxonomy, evolutionary origins and genetic diversity of blue mussels in the Northern hemisphere are now reasonably well understood, this is not the case for the Southern hemisphere mussels. However, recent analysis of native blue mussels from the Southern hemisphere using SNPs (usually involving a panel of reference Northern hemisphere mussels) has profoundly improved our understanding of the global situation. Because SNPs are high-definition markers (i.e., they are easy to interpret and generally provide higher species-level and population-level differentiation than other marker types), they are co-dominantly inherited and they can be found throughout the genome in very high numbers; they provide a new level of detail about regional genetic differentiation, hybridisation and introgression,

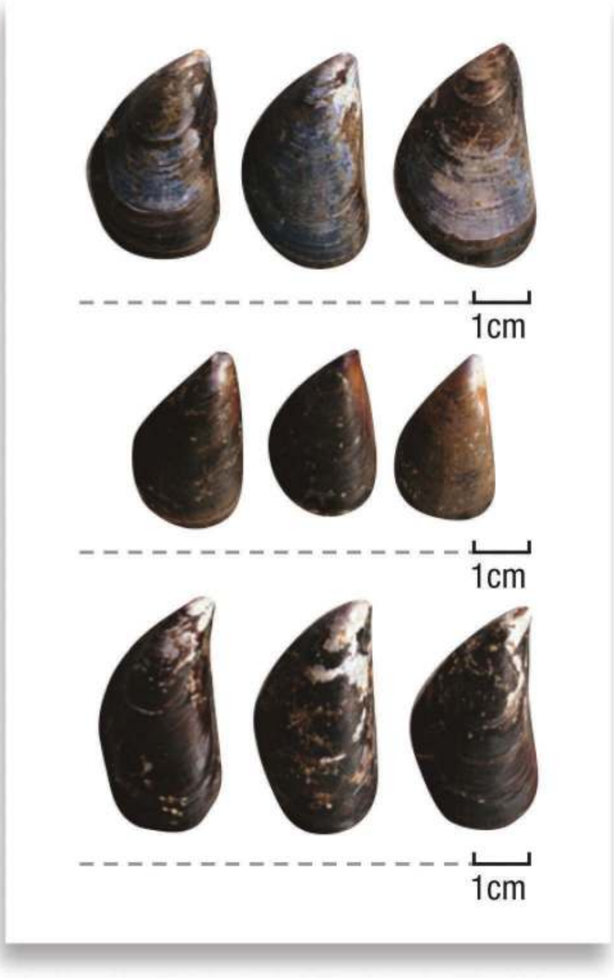

Figure 1 Examples of *Mytilus edulis* Linneaus, 1758 (top panel) collected from Magdalen Island, Quebec, Canada, of *M. galloprovincialis* Lamarck, 1819 (middle panel) collected from Posjet Bay, USSR, and *M. trossulus* Gould, 1850 (bottom panel) collected from Magadan, USSR. Shells deposited in the British Museum of Natural History (accession number 2377) from the study of McDonald et al. (1991). Scale bars at bottom of each panel (photo credit – Jonathan Gardner).

genetic integrity of independent lineages, evolutionary origin, and ultimately the taxonomy and systematics of Southern hemisphere blue mussels. This new knowledge has a direct bearing on other aspects of the study of blue mussels, including biosecurity, aquaculture production and food labelling. This review, the first of its kind with a focus on Southern hemisphere blue mussels, builds on previous reviews (e.g., Koehn 1991, Gardner 1992, Gosling 1992a,b, Seed 1992). The work that we describe, from the earliest days to the present, is truly a global effort, with many different research teams from across the world having contributed. Progress has often been limited and incremental – one small step at a time – but is also occasionally characterised by a profound leap that has changed the world view. The story also highlights the natural tension that exists within the science community about what constitutes a species, especially in light of extensive hybridisation and introgression. There are also questions of taxonomic priority and, dare we say it, of national identity being linked to a native blue mussel. The ongoing process of blue mussel speciation in the sea is rarely clear-cut

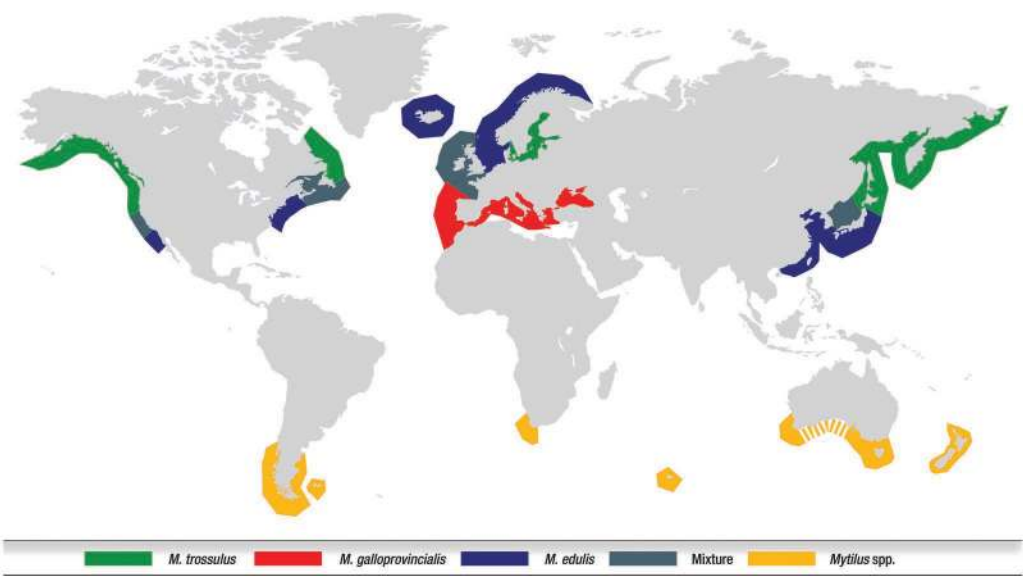

Figure 2 Map of broad regional differences in distributions of smooth-shelled mussels of the genus *Mytilus*. The three Northern hemisphere species (*M. edulis*, *M. galloprovincialis*, *M. trossulus*) and areas of sympatry between them are shown. All Southern hemisphere blue mussels (native and introduced) are shown in yellow – specific details for each region are discussed below.

and has been substantially muddied by anthropogenic activities that have deliberately and accidentally moved blue mussels around the world, in particular during the last 300 years. This chapter focusses on the native mussels from South America and Australasia (blue mussels are not native to southern Africa), including remote offshore islands in the Southern Ocean, but in the context of Northern hemisphere species (Figure 2). We review the fossil and midden records to establish where blue mussels are likely to be native, review the extensive body of literature, examine Southern hemisphere mussel phylogeography based on markers before SNPs, and then describe the recent work using SNPs and how this has confirmed earlier interpretations or added a new view of Southern hemisphere mussel phylogeography. In an evolutionary context, we question how many different native species may be said to exist in the Southern hemisphere, we examine the role of hybridisation in speciation, we discuss the role of new approaches to recognising species – the species delimitation (SD) models, and we seek to determine whether physical oceanography may contribute to or even explain species distributions in the Southern hemisphere. In a broader context, because blue mussels are ecologically and economically important throughout the world, we then review biosecurity threats, old and new, natural and manmade, before finally taking a look at aquaculture production of blue mussels and the importance of correct taxonomy to food labelling, marketing, traceability and production statistics. We conclude with a brief section that focusses on our view of some important future research directions.

The evolutionary origin of modern smooth-shelled blue mussels

The earliest attempts to understand the origin of modern blue mussels focussed on fossil evidence and interpretations of the timing of the opening of major sea passages or major basins such as the Bering Strait, the Mediterranean Sea and the Baltic Sea. From this work, hypotheses were ultimately developed to allow for molecular testing (DNA sequence analyses) of the natural range expansion of blue mussels within the Northern hemisphere and from the Northern to the Southern hemisphere.

Evidence suggests that speciation among the three closely related Northern hemisphere species of the *Mytilus edulis* species complex – that is, *M. edulis*, *M. galloprovincialis* and *M. trossulus* – was most likely allopatric (Figure 3). The molecular evidence shows that *M. trossulus* is the oldest of the three Northern hemisphere smooth-shelled species (Hilbish et al. 2000, Gérard et al. 2008). *Mytilus trossulus* (or its immediate ancestor – some form of proto-*trossulus*) was restricted in its range to the North Pacific region (Lindberg 1991, Vermeij 1991). The first major range expansion, which led to the first allopatric speciation event giving rise to North Atlantic *M. edulis*, occurred ~3.5 M ybp (years before present) with the opening of the Bering Strait between the North Pacific Ocean and the North Atlantic Ocean (Lindberg 1991, Vermeij 1991, Dunton 1992, Cunningham & Collins 1994). This was a period of major biotic interchange, with most movement being west to east, but some also occurring east to west (Vermeij 1991). According to Vermeij (1991), *Mytilus* fossils first appear in the North Atlantic in the early Pliocene. This earliest form of *M. edulis* spread throughout the North Atlantic region during periods of sea level change and colonised the Mediterranean Sea by the Pleistocene (Vermeij 1991). Barsotti & Meluzzi (1968) suggest that the mussels of the North Atlantic were separated from and then reconnected to the mussels of the Mediterranean Sea something like 17 times during periods of major sea level change over a period of ~1.7 M years. More recently, Roux et al. (2014) date the time of divergence of *M. galloprovincialis* from *M. edulis* at 2.5 M ybp. During this period, sea level dropped by as much as 100 m (Lindberg 1991), and this phenomenon is known to have given rise to separation among groups of mussels or between major geographic regions such as the North Atlantic Ocean and the Mediterranean Sea. This separation among mussel regional groups gave rise to genetic differences among members of the different groups. Ultimately, speciation between the northern North Atlantic *M. edulis*, the southern North Atlantic lineage of *M. galloprovincialis* and the Mediterranean Sea lineage of *M. galloprovincialis* was therefore vicariant (Barsotti & Meluzzi 1968, Riginos & Cunningham 2005).

A second natural range expansion from the North Pacific Ocean to the North Atlantic Ocean, again via the Bering Strait, has occurred more recently, during the Pleistocene or the Holocene (Riginos & Cunningham 2005). This invasion gave rise to *M. trossulus* (i.e., this was not a speciation event) on the North American Atlantic coast with a centre of distribution in the Canadian Maritime provinces (Koehn et al. 1984, McDonald & Koehn 1988, McDonald et al. 1991) and in the Baltic Sea (Varvio et al. 1988, Väinölä & Hvilsom 1991). These two disjunct distributions of North Atlantic *M. trossulus* appear to be separate (genetically differentiated) lineages of the original North Pacific form of *M. trossulus* (Riginos & Cunningham 2005) and display different environmental tolerances of, for example, salinity variation (e.g., Kautsky et al. 1990, Gardner & Thompson 2001, Qiu et al. 2002, Braby & Somero 2006).

The molecular and the fossil evidence points very strongly to a Northern hemisphere origin of modern smooth-shelled blue mussels. The North Pacific Ocean *M. trossulus* form is the oldest form (the ancestral lineage), and the two North Atlantic species, *M. edulis* and *M. galloprovincialis*, are much more recent forms. Importantly, there are now two clearly recognised lineages of Northern hemisphere *M. galloprovincialis*, one which is from the Mediterranean Sea and the other from the North Atlantic Ocean, and which shows greater affinity to North Atlantic *M. edulis* than the former (e.g., Sanjuan et al. 1997, Daguin & Borsa 2000, Hilbish et al. 2000, Larraín et al. 2018, Zbawicka et al. 2019, 2021, Popovic et al. 2020). An understanding of the distributions and ages of the Northern hemisphere species is important for our understanding of the origin of modern-day Southern hemisphere *Mytilus*. It is important to note that there is increasing evidence of *Mytilus* taxa (e.g., *M. trigonus*) from southern South America on both the Pacific Ocean and Atlantic Ocean coasts, in particular around Patagonia, which dates to the Miocene epoch and, as such, may predate the origin of modern *M. trossulus* (del Río et al. 2001). This fossil form needs further investigation, given that it may be the ancestral form of modern-day smooth-shelled blue mussels.

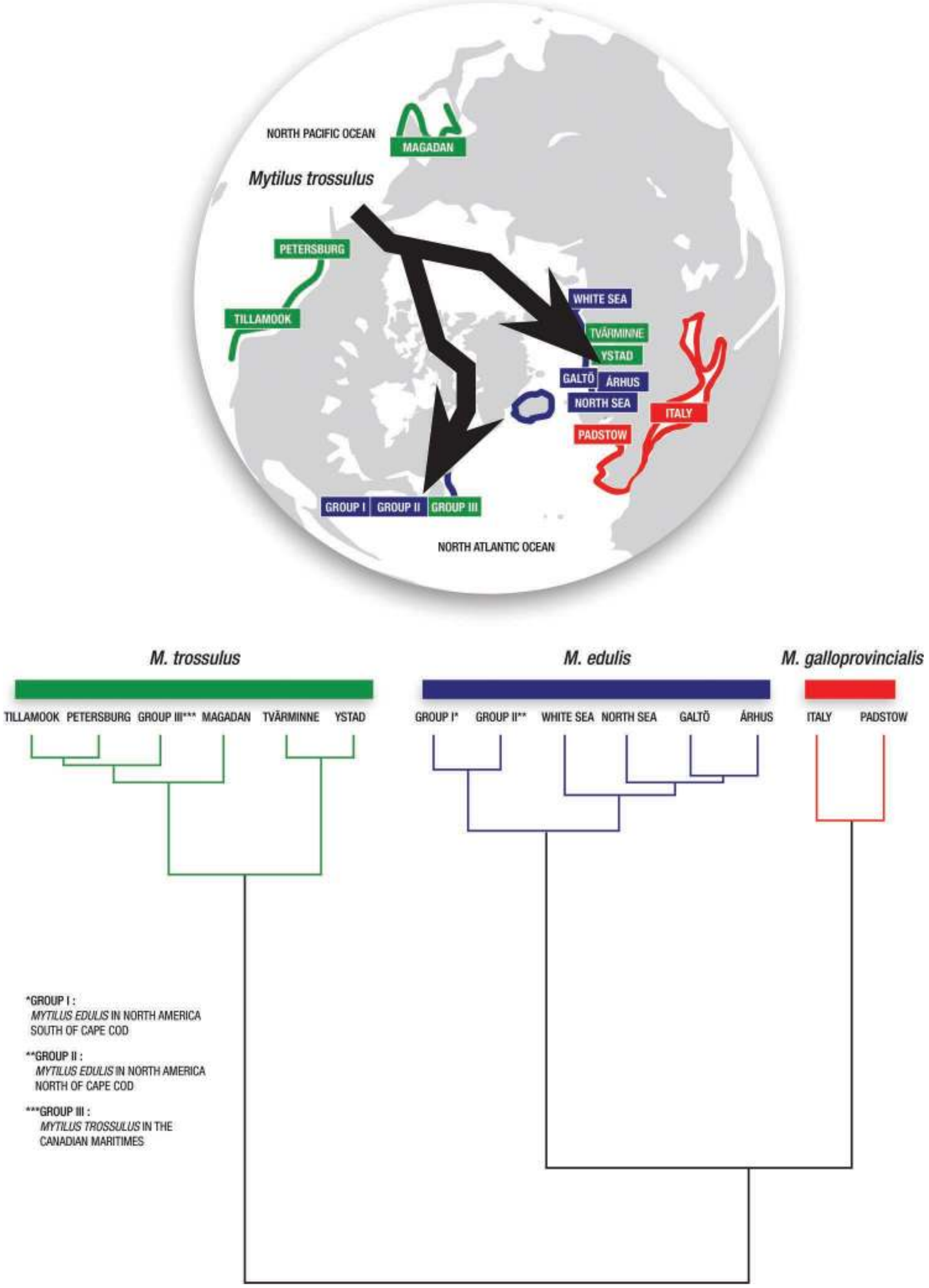

Figure 3 Evolutionary relationships among *Mytilus* species as shown by a UPGMA tree based on Cavalli-Sforza chord distances from five allozyme loci (*Ap*, *Gpi*, *Lap*, *Mpi*, and *Pgm*). Populations are as follows: *M. trossulus* – (1) Tillamook, (2) Petersburg, (3) Magadan, (4) Group III (*Mytilus trossulus* in the Canadian Maritimes), (5) Tvärminne, (6) Ystad (note separation of North Atlantic Ocean from Baltic Sea populations; *M. edulis* – (7) Group I (*Mytilus edulis* in North America south of Cape Cod), (8) White Sea, (9) North Sea, (10) Galtö, (11) Group II (*M. edulis* in North America north of Cape Cod), (12) Århus; *M. galloprovincialis* – (13) Italy, and (14) Padstow. Arrows on the globe represent the recent invasion(s) of Pacific *M. trossulus* into the North Atlantic Ocean. Modified from Riginos & Cunningham (2005).

Establishing the foundation – early genetics-based studies of biogeography and taxonomy

Early work based on allozyme and shell trait and/or shape variation identified three distinct groupings of smooth-shelled blue mussels in the Northern hemisphere: *M. edulis* from the North Atlantic, including the coasts of Europe and North America, *M. galloprovincialis* from the Mediterranean Sea and extending north to SW England and southern Ireland, and south at least as far as southern Morocco on the Atlantic Ocean, and *M. trossulus* from the Baltic Sea and also from parts of the Atlantic seaboard of North America (Koehn et al. 1984, McDonald & Koehn 1988, Varvio et al. 1988, McDonald et al. 1991). During this period, several authors noted that despite extensive hybridisation and varying levels of introgression between pairs of species, each maintained its genetic integrity across large parts of the world and as such they all warranted recognition as distinct species (e.g., McDonald & Koehn 1988, Varvio et al. 1988).

By the early 1990s, there were already five separate reviews of the taxonomy of one or more of the *Mytilus edulis* species complex in the Northern hemisphere (Gosling 1984, 1992a, Koehn 1991, Gardner 1992, Seed 1992). Subsequently, as summarised in Table 1, the application of more modern approaches such as mtDNA and nuclear DNA (nDNA) RFLP analysis (Edwards & Skibinski 1987, Gardner & Skibinski 1991, Inoue et al. 1995, Toro 1998a, Santaclara et al. 2006, Westfall et al. 2010), DNA sequencing (Geller et al. 1993, Hilbish et al. 2000, Gérard et al. 2008), sperm protein analysis (Riginos & McDonald 2003) and microsatellites (Presa et al. 2002, Varela et al. 2007) all confirmed the interpretation of the earlier allozyme-based surveys that three distinct species (*M. trossulus*, *M. edulis* and *M. galloprovincialis*) exist in the Northern hemisphere.

In comparison to the Northern hemisphere, much less work of a similar nature was conducted in the Southern hemisphere, despite the occurrence of blue mussels of the *Mytilus edulis* species complex existing in all Southern continents except Antarctica. The interpretation of the *Mytilus edulis* complex problem in the Southern hemisphere was very much influenced by the very large body of research being conducted in the Northern hemisphere at the time and the major taxonomic advances being made there.

Table 1 Summary table of molecular markers that have been used in the identification of *Mytilus edulis* complex species

DNA Markers	Species	References
Me15/16 (n)	Me, Mg, Mt	Inoue et al. (1995)
Me15/16 RFLP assay (n)	Mc[a], Me, Mg, Mt	Santaclara et al. (2006)
COIXba RFLP assay (mt)	Mc,Mg	Fernández-Tajes et al. (2011)
Myti RFLP assay (n)	Mc, Me, Mg, Mt	Fernández-Tajes et al. (2011)
16S RFLP assay (mt)	MgS, MgN, MgN/Me, Mt	Westfall et al. (2010)
M7 (n)	Me, Mg, Mt	Kijewski et al. (2009)
Mac-1 (n)	Me, Mg	Daguin et al. (2001)
EFbis (n)	Me, Mg	Bierne et al. (2003)
EFbis RFLP assay (n)	Me, Mg, Mt	Kijewski et al. (2009)
16S (n)	Me, Mg, Me/Mg	Bendezu et al. (2005)
ITS RFLP assay (n)	Me/Mg, Mt	Heath et al. (1995)
PLIIa RFLP assay (n)	Me, Mg, Mt	Heath et al. (1995)

DNA type – n, nuclear; mt, mitochondrial; Mc, *Mytilus chilensis*; Me, *M. edulis*; Mg, *M. galloprovincialis*; Mt=*M. trossulus*; MgS, Southern hemisphere *M. galloprovincialis*; MgN, Northern hemisphere *M. galloprovincialis*; MgN/Me, "North Atlantic" haplotype as defined by Hilbish et al. (2000).

[a] This is the same as the MgS pattern of Westfall et al. (2010).

Levinton & Koehn (1976) compared allele frequencies at three allozyme loci (LAP, GPI, AP) in mussels (which, at the time, they called *M. edulis*) from Melbourne, Australia, with mussels from five locations in the Northern hemisphere. They noted several regional differences in the occurrence of alleles at highest frequency that today reflect what we know about the occurrence of *M. edulis*, *M. galloprovincialis* and *M. trossulus* in the Northern hemisphere. Levinton & Koehn (1976) concluded by stating that the three locus-specific alleles at highest frequency in the Australian mussels were the same as those at highest frequency in their sample from south of Cape Cod (*M. edulis* from the Atlantic coast of North America), all of which showed considerable difference to samples from the Gulf of Maine (*M. trossulus* from the Atlantic coast of North America).

The first study of genetic variation focussing specifically on Southern hemisphere blue mussels was that carried out by Grant & Cherry (1985). This work was of interest and importance because the authors demonstrated that blue mussels found in South Africa are not native, but are introduced Northern hemisphere *M. galloprovincialis*, as based on both shell morphometric trait analysis and allozyme electrophoresis. They also noted the absence of *Mytilus* sp. from two large shell deposits, both of which predate European arrival. Subsequently, Blot et al. (1988) examined allozyme variation in native blue mussels from the Kerguelen Islands (southern Indian Ocean, 70°E, 49°S) that, at the time, were called *Mytilus desolationis* Lamy, 1936. They compared *M. desolationis* with reference samples of Northern hemisphere *M. edulis* and *M. galloprovincialis*. Correspondence analysis (CA) (Figure 4) revealed that the two Kerguelen populations were very clearly differentiated from the Northern hemisphere *M. edulis* and *M. galloprovincialis*, but other analyses based on genetic distance revealed limited differentiation among all three mussel types, such that Blot et al. (1988) concluded that *M. desolationis* does not exhibit enough differentiation from *M. edulis* to warrant the rank of species. Blot et al. (1988) noted that Thiriot-Quiévreux (1984) had previously demonstrated the existence of karyotypic differences among *M. desolationis*, *M. edulis* and

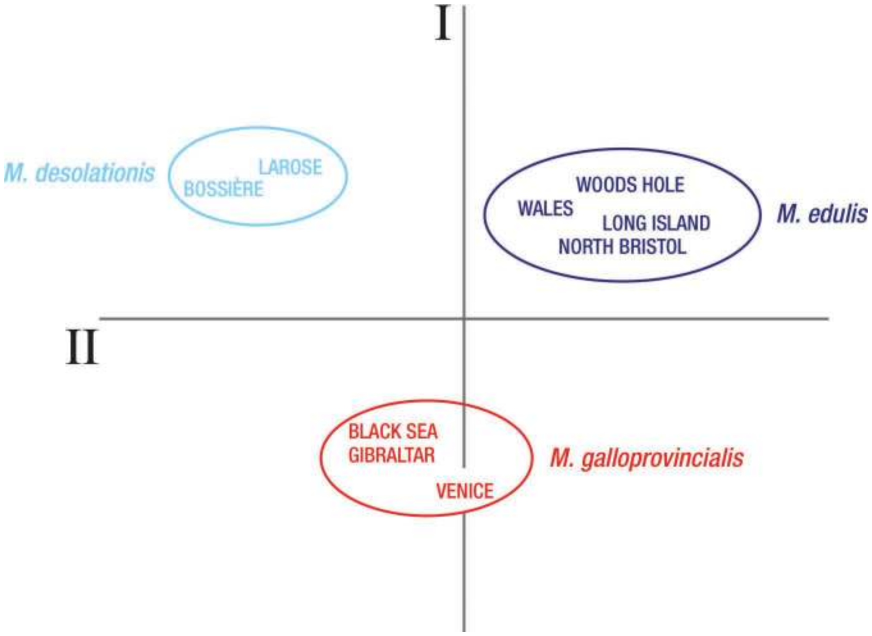

Figure 4 Correspondence analysis plot of allozyme data for mussels from the Kerguelen Islands (*Mytilus desolationis* – Bossière, Larosse), the Mediterranean Sea (*M. galloprovincialis* – Gibraltar, Venice, Black Sea) and the North Atlantic Ocean (*M. edulis* – Long Island, Woods Hole, North Bristol, Wales). Modified from Blot et al. (1988).

M. galloprovincialis (i.e., alongside their own allozyme data, there were additional karyotypic data pointing to what might be interpreted as subspecies differences). Blot et al. (1988) concluded that *M. desolationis* is best considered as a semi-species (following Mayr 1970) of the super-species *M. edulis*. Significantly, the potential limitations of this conclusion based on the best methodology of the day are recognised by the authors, who state that this semi-species status should hold "… until species-specific characters are found …" (Blot et al. 1988, p. 246).

A new global perspective – the 1990s to the early 2000s

Taking a more global view of the *Mytilus* taxonomic problem, which was at least starting to be resolved in the Northern hemisphere by now, McDonald et al. (1991) identified two distinct groups of Southern hemisphere mussels – a South America group including mussels from Chile, Argentina, the Falkland Islands and the Kerguelen Islands, and an Australasian group including mussels from Australia and New Zealand (Figure 5). The former group was most similar to Northern hemisphere *M. edulis*, whereas the latter group was most similar to Northern hemisphere *M. galloprovincialis*. The allozyme-based results were confirmed by analyses of shell trait variation, although not to the same extent of differentiation because the South American group was intermediate between Northern hemisphere *M. edulis* and *M. trossulus*.

Subsequent work based on allozymes and then on molecular markers tended to confirm the earlier interpretation of three species in the Northern hemisphere and also the results of McDonald et al. (1991) for the Southern hemisphere. Sanjuan et al. (1997) reviewed published allozyme data for the mussel *Mytilus galloprovincialis*. At a global scale, their analyses revealed three groups, including (1) Mediterranean, Asian and North American Pacific populations, (2) a group of European North Atlantic populations and (3) a group of Australasian samples that the authors said did not constitute a well-defined cluster. Multi-dimensional scaling (MDS) analysis showed that the two Australian samples were, in fact, quite distinct from the one New Zealand sample (Figure 6). Subsequently, Daguin & Borsa (2000) analysed variation at two nuclear DNA markers, the polyphenolic adhesive protein gene *Glu-5′* and the first intron of the actin gene *mac-1*, to examine the three recognised groups (based on allozyme variation – McDonald et al. 1991, Sanjuan et al. 1997) of *M. galloprovincialis* in the world at the time – a NE Atlantic group, a Mediterranean group, and an Australasian group (invasive *M. galloprovincialis* had already been reported in both of the first two named groups by this time and also from South Africa). It was hoped that the use of molecular markers would provide a new, more in-depth view of *M. galloprovincialis* than that developed only three years previously by the allozyme variation review of Sanjuan et al. (1997). While the *Glu-5′* marker was fixed, or nearly so, for the diagnostic *G* (*M. galloprovincialis*) marker, the *mac-1* marker confirmed the existence of the three already recognised groups, and also identified the likelihood of introduction of Northern hemisphere *M. galloprovincialis* to Chile. Significantly, the analysis of allele variation at the *mac-1* locus highlighted the distinctness of the Australasian *M. galloprovincialis*-like mussels relative to the two Northern hemisphere groups of *M. galloprovincialis* (Figure 7). This led Daguin & Borsa (2000) to suggest that Australasian mussels (i.e., those from both Australia and New Zealand) are derived from what they called a proto-*M. galloprovincialis* population that was already introgressed by *M. edulis*-like genes. They argued that this Australasian mussel should be considered as a subspecies of *M. galloprovincialis*; that is, it is native and is not introduced from the Northern hemisphere. Clustering analyses revealed clear separation of the Southern from the Northern hemisphere mussels, but statistics of differentiation (e.g., F_{ST} or Φ_{ST} or G_{ST}) produced low values (often < 0.03) that did not tend to support the idea of different species. The *mac-1* locus did not provide any evidence of substantive differentiation between the Australian and the New Zealand samples, and therefore tended to confirm that the Australasian mussels all fall within one group.

While interpreting the results from these studies, it is important to appreciate that individual sample sizes were often not very large, that a very small spatial extent of the Southern hemisphere

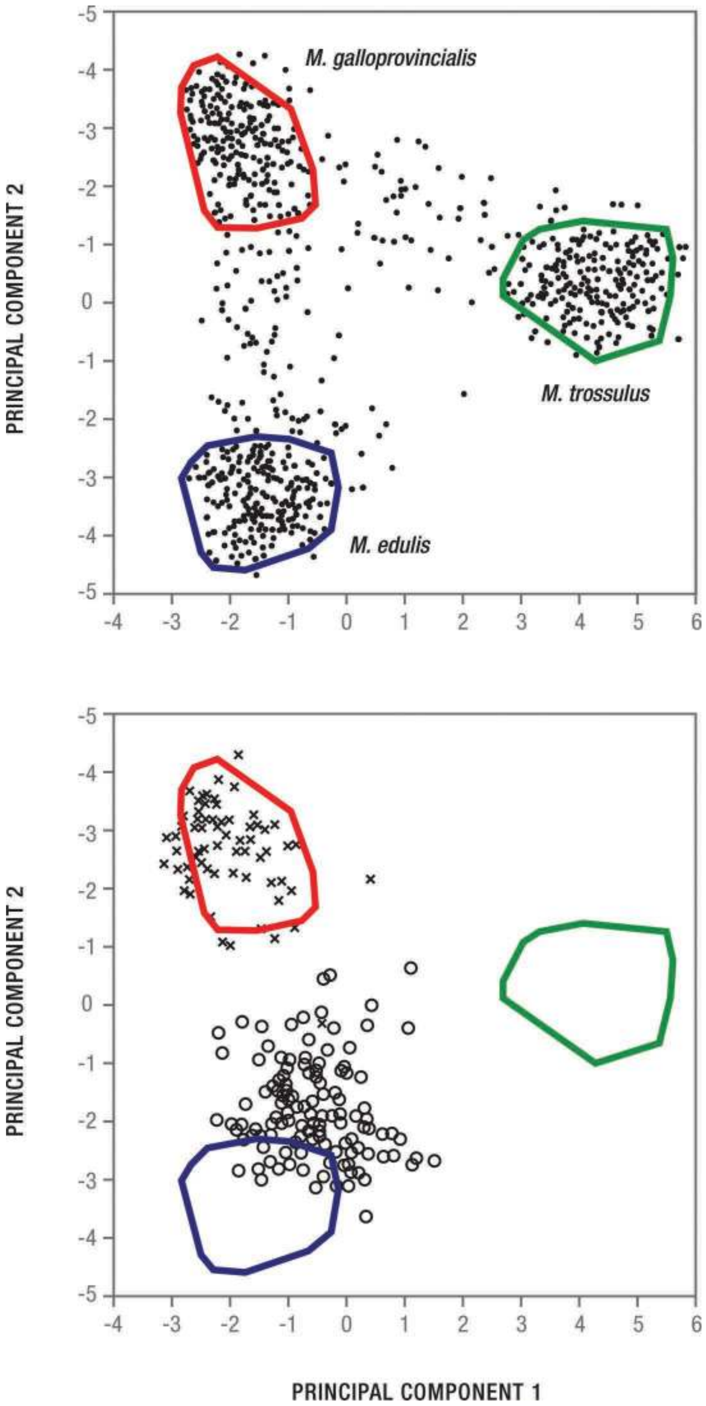

Figure 5 Principal components analysis of allozyme data for mussels from Northern and Southern hemispheres. Top panel showing only individuals from the Northern hemisphere (that is, reference Northern hemisphere samples). Bottom panel same as top panel except only individuals from the Southern hemisphere are shown with polygons for reference Northern hemisphere taxa. (o) Mussels from Chile, Argentina, the Falkland Islands and the Kerguelen Islands; (x) mussels from Australia and New Zealand. Modified from McDonald et al. (1991).

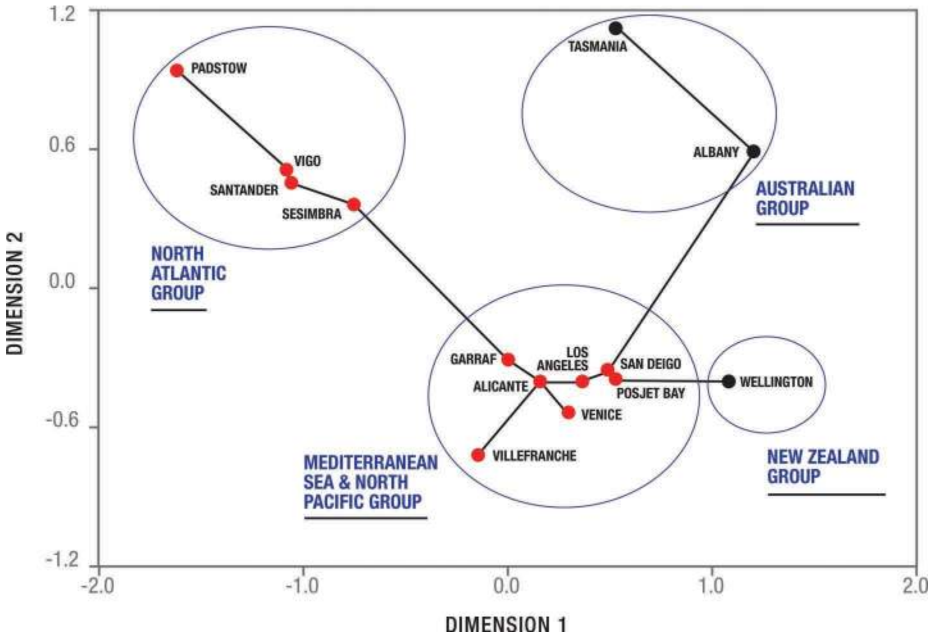

Figure 6 Linear multi-dimensional scaling (MDS) plot of genetic distance among *Mytilus galloprovincialis* populations, with minimum spanning tree superimposed on the MDS plot. Populations: **North Atlantic group** – Padstow, SW England; Santander, Spain; Vigo, Spain; Sesimbra, Portugal: **Mediterranean Sea and North Pacific group** – Alicante, Spain; Garraf, Spain; Villefranche, France; Venice, Italy; Los Angeles, USA; San Diego, USA; Posjet Bay, Russia: **Australian group** – Albany, Western Australia; Tasmania, Australia: **New Zealand** – Wellington, New Zealand. Note that samples from Los Angeles, San Diego and Posjet Bay are now viewed as being introduced (non-native). Modified from Sanjuan et al. (1997).

had been sampled and that today we recognise that Northern hemisphere *M. galloprovincialis* has invaded many areas in the Northern and Southern hemispheres. Nonetheless, what was rapidly becoming apparent was that native Southern hemisphere mussels were different across a range of different marker types to their Northern hemisphere counterparts and that two distinct groups of Southern hemisphere mussels could be identified (South America and Australasia). All native Southern hemisphere mussels were judged to be derived from the Northern hemisphere and to be (1) members of *M. edulis* or *M. galloprovincialis* (note that there was no evidence of *M. trossulus* from the Southern hemisphere), or (2) *M. edulis*-like or *M. galloprovincialis*-like, or (3) subspecies of these two species – e.g., *M. edulis chilensis* Hupé, 1854 (Pacific coast of South America) or *M. edulis platensis* d'Orbigny, 1842 (Atlantic coast of South America) or *M. galloprovincialis* subspecies unspecified (Australasia) or *M. edulis planulatus* Lamarck, 1819 (Australia). Note that WoRMS (http://www.marinespecies.org/) does not accept *M. edulis chilensis*, *M. edulis platensis* or *M. edulis planulatus*.

The evolutionary origin of Southern hemisphere blue mussels

Understanding the origin of Southern hemisphere blue mussels, and thereby perhaps explaining the antitropical distribution of the genus, has been a challenge. A full explanation of the origin of Southern hemisphere blue mussels should shed light on the timing or timings of the origin or origins as well as on the route or routes taken to reach the present-day distributions. Ideally, the methodological approach taken will test hypotheses that permit clear differentiation among putative

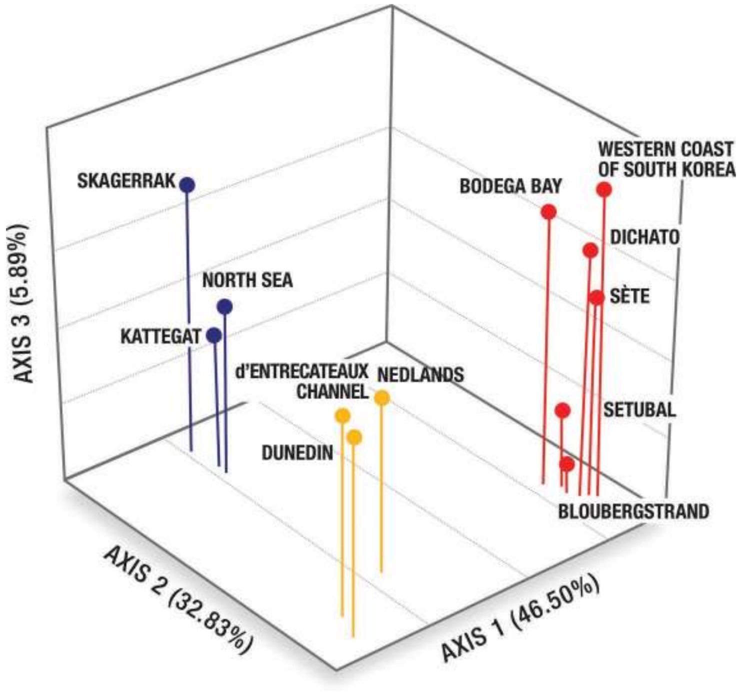

Figure 7 Three-dimensional plot of *mac-1* allelic frequency variation for Northern and Southern hemisphere mussels. Reference *Mytilus edulis* (in blue – Skagerrak, Kattegat, North Sea); Australasian mussels (in yellow – Nedlands (Western Australia), d'Entrecasteaux Channel (Tasmania), Dunedin (New Zealand); *M. galloprovincialis*, both native and introduced (in red – Bodega Bay (California, USA), Dichato (central Chile), Setubal (Portugal), Sète (France), Bloubergstrand (South Africa), western coast of South Korea. Modified from Daguin & Borsa (2000).

timings of colonisation and putative routes of range expansion. We would expect that molecular results are supported by the fossil and midden information available from each major geographic location. Beyond this, we would also like to see an approach that is able to identify recent anthropogenic introductions and differentiate these from natural range expansion events.

Perhaps not surprisingly, there are relatively few predictions about the colonisation of the Southern hemisphere by blue mussels based on non-molecular analyses. Building on earlier work that looked at bipolarity (e.g., Powell 1965), in his evaluation of the marine biotic exchange between the Northern and Southern hemispheres, Lindberg (1991) suggested that Australasian *Mytilus edulis planulatus* may have diverged in the Pliocene (2.58–5.33 M ybp) and might have been dispersed from the eastern North Pacific to Australia and New Zealand by circumpolar currents (the West Wind Drift) as has been hypothesised for several different groups, including the nudibranch *Acanthodoris*, the gastropod *Fusitriton* and the crab *Cancer*. One possible mechanism for this range expansion across the tropics, which is normally a barrier to movement for cooler water species such as *Mytilus*, is increased upwelling along the east Pacific margin and the establishment of a series of stepping stone like cold water refugia (Lindberg 1991). However, Lindberg (1991) notes that whether the ~100 m drop in sea level at the time (~1 M ybp) increased or decreased the number of upwelling sites along the eastern Pacific coast remains unknown, and this may influence the spreading success of certain genera.

This earlier body of work, based on fossil or midden remains, provided a set of testable hypotheses for the molecular researchers who were to follow. The fossil record also provides a reasonably robust framework against which to build molecular hypotheses and to make further predictions. This approach was not, however, implemented until some years later when molecular advances permitted the testing of specific hypotheses, often of a nuanced nature.

In some of the earliest DNA sequencing work of its type, Kenchington et al. (1995) examined 18S sequence variation among representatives of the Northern hemisphere *Mytilus edulis* complex, with one representative from Australia that was recognised as *M. e. planulatus* (collected from Cloudy Bay Lagoon, Tasmania). As we recognise today, the 18S gene is a slowly evolving region that is better suited to exploration of more ancient speciation events than to recent (e.g., less than 3–4 M ybp) speciation events and as such may not be particularly informative for the *Mytilus edulis* species complex. However, Kenchington et al. (1995) concluded that their phylogeny strongly suggested an early separation of Northern hemisphere *M. galloprovincialis* from the other smooth-shelled *Mytilus* spp., including *M. edulis*, *M. trossulus* and Australasian *M. galloprovincialis*. Subsequently, Sanjuan et al. (1997), in their review of published allozyme variation for *M. galloprovincialis* at a global scale, suggested that their data indicated the existence of two main events: first, a natural and ancient transequatorial migration through the Pacific Ocean, and second, a subsequent trans-Arctic migration. They note that these hypotheses assumed a Pacific origin of *M. galloprovincialis*. These two studies were soon superseded by more sophisticated analyses using more informative markers, which illustrates both the rapid pace of marker and data analysis development, as well as how our understanding of “the *Mytilus* problem” could change so rapidly and dramatically.

In the first paper to explicitly test the origin of Southern hemisphere blue mussels, and arguably the one that advanced our understanding the most, Hilbish et al. (2000) used mitochondrial DNA (mtDNA – the 16S rRNA mitochondrial gene) in a phylogenetics framework to test four separate hypotheses (Figure 8). They were able to reject hypotheses indicating that Southern hemisphere blue mussels migrated via the northern equatorial Pacific region to the southern Pacific Ocean region, and that what they viewed as being native blue mussels had been accidentally introduced into the Southern hemisphere. They confirmed the Northern hemisphere origin of native blue mussels, highlighted the route of colonisation being via the Atlantic Ocean from the north to the south and dated the primary migration to ~1.2 M ybp, during the Pleistocene. They went on to note, however, that overall, their data supported the hypothesis of two separate migration events, rather than one, both from the Northern to the Southern hemisphere, with most haplotypes being derived from the first migration 1.2 M ybp, and far fewer being derived from a more recent migration event, also via the Atlantic Ocean route. Unfortunately, Hilbish et al. (2000) were unable to determine the exact origin or timing of this second event, but did note that it predates human activity (i.e., native Southern hemisphere mussels are genuinely native and not a result of human introduction). This two-invasion scenario is best interpreted as representing the older colonisation of South America, including the Falkland Islands and the Kerguelen Islands, and the more recent colonisation of Australasia, including Australia, mainland New Zealand and its offshore islands. This molecular interpretation is consistent with the findings of McDonald et al. (1991) and Sanjuan et al. (1997) based on allozyme variation, and also of Daguin & Borsa et al. (2000) based on two nDNA markers. In addressing the Pleistocene-Pacific hypothesis (e.g., as proposed by Lindberg 1991), Hilbish et al. (2000) were very clear that their results reject any suggestion of transequatorial migration through the Pacific and that the greater affinity of Southern hemisphere mussel mtDNA lineages with those found in Northern hemisphere *M. edulis* and *M. galloprovincialis* clearly indicates an Atlantic route of migration for colonisation of the Southern hemisphere. If this is the case, then the primary colonisation event of South America (via a North Atlantic to South Atlantic Ocean route) seems sensible because it is direct and geographically nearest to the North Atlantic centre of origin, and the subsequent secondary colonisation event of Australasia is also intuitively appealing because it is more remote from the North Atlantic centre of origin, and Australasia is connected to South America via the West Wind Drift.

While the work of Borsa et al. (2007) using *Glu-5′*/*Glu-3′* and *mac-1* (both are nuclear DNA markers) did not specifically examine the timing of origin nor the routes of colonisation of Southern

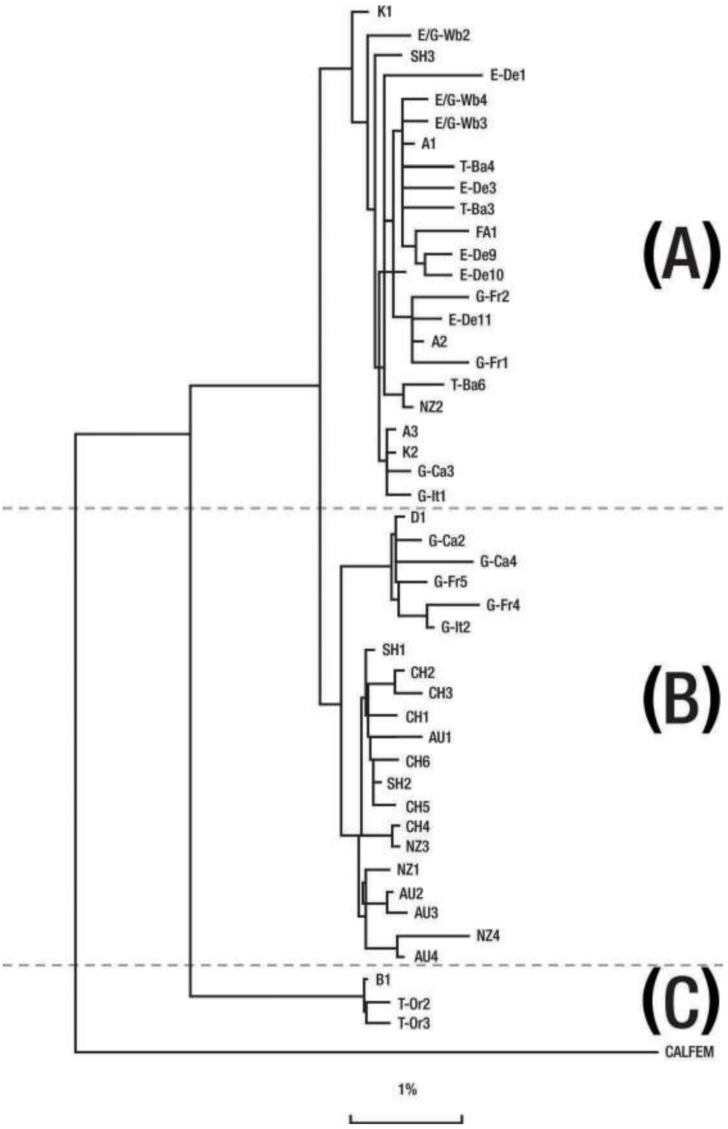

Figure 8 Neighbour-joining phylogeny for the *Mytilus* 16S rRNA maternal mitochondrial lineage sequences from Northern and Southern hemisphere mussel populations, taken from Hilbish et al. (2000). **Clade A** – Northern hemisphere *M. edulis* and *M. galloprovincialis*, but also including some mussels from the Falkland Islands, Kerguelen Islands, Western Australia and New Zealand. On average, these sequences are 0.3% divergent from other A haplotypes found in the northern hemisphere. **Clade B** – Northern hemisphere *M. galloprovincialis* mussels and all Southern hemisphere mussels except those from the Kerguelen Islands. The parsimony and maximum-likelihood analyses, however, both showed that the Northern hemisphere B clade is significantly distinguished from Southern hemisphere B haplotypes. This Southern hemisphere clade is, on average, 1.4% divergent from the next closest B clade of *M. galloprovincialis*. **Clade C** – Northern hemisphere *M. trossulus*. Outgroup (CALFEM) = female *M. californianus*. Modified from Hilbish et al. (2000).

hemisphere mussels, their work did, nonetheless, shed new light on the matter. The authors confirmed the native (endemic) status of mussels from Tasmania and the Kerguelen Islands (i.e., these mussels are not accidentally introduced), and they suggested that the great affinity of Australasian mussels with *Mytilus galloprovincialis* from the western Mediterranean (the type locality for this species) indicates a rapid expansion from the Mediterranean Sea to Australia and New Zealand with very little time for genetic exchange. This interpretation is consistent with the idea of a second, more recent colonisation event of the Southern hemisphere as proposed by Hilbish et al. (2000).

The following year, Gérard et al. (2008) analysed both 16S and COI (both are mtDNA regions) sequence variation, but focussed on COI because it is more variable and therefore more informative. They highlighted pronounced genetic differentiation among the mussels from South America and the Kerguelen Islands, Australia (Tasmania), and New Zealand, consistent with two independent colonisation events (e.g., Hilbish et al. 2000), confirmed the divergence between mussels from the Northern and Southern hemispheres, and suggested that this divergence predated the divergence of Northern hemisphere *M. edulis* and *M. galloprovincialis* (Figure 9). Using the trans-Arctic interchange as a molecular clock calibration, Gérard et al. (2008) estimated the time since divergence to be between 0.5 M and 1.3 M ybp (average 0.84 M ybp). This estimate dates from the Pleistocene (0.01–1.8 M ybp) and is consistent with the estimate produced by Hilbish et al. (2000) of 1.2 M ybp based on sequence variation of the 16S gene. In terms of identifying a route or routes of invasion, Gérard et al. (2008) noted that results obtained with the single mitochondrial genome do not permit the inference of a general history of population divergence (Figure 10). They also noted that comparison of published nuclear and mitochondrial results suggested two different scenarios, the first involving two separate colonisation events and the second involving one event but biased by taxonomic preconception. This latter point has been, and continues to be, a key point of contention in the interpretation of *Mytilus* spp. in the Southern hemisphere.

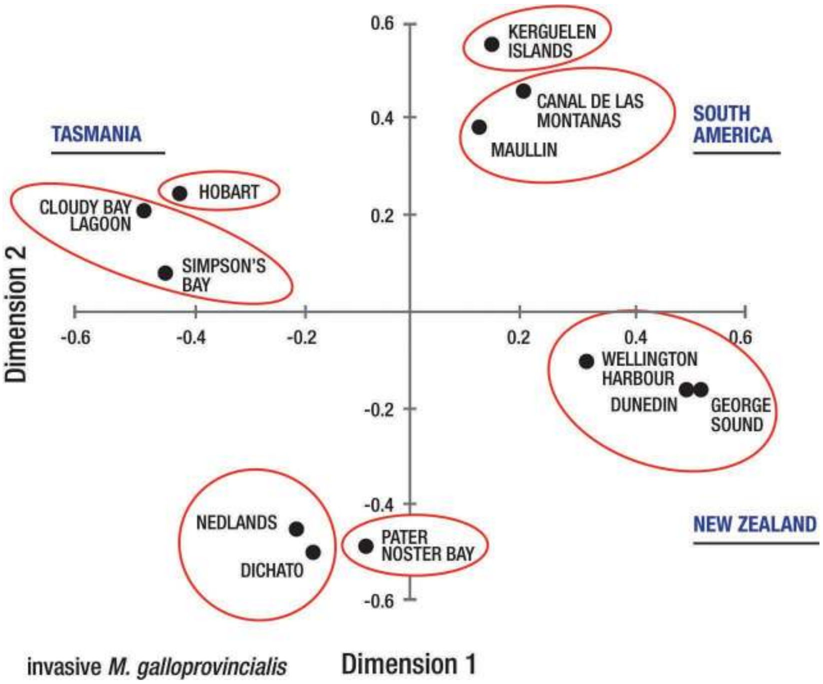

Figure 9 Four groups of differentiated mussels based on COI sequence variation. Tasmania group – Cloudy Bay Lagoon, Tasmania; Hobart, Tasmania; Simpson's Bay, Tasmania. South America group – Kerguelen Islands; Maullin, Chile; Canal de las Montañas, Patagonia, Chile. New Zealand group – Dunedin, South Island; George Sound, South Island; Wellington Harbour, North Island. Invasive Northern hemisphere *M. galloprovincialis* – Pater Noster Bay, South Africa; Nedlands, Australia; Dichato, Chile. Samples within the same circle are not significantly different ($P > 0.05$), whereas samples in different circles are different ($P < 0.05$) based on Φ_{ST} values. Modified from Gérard et al. (2008).

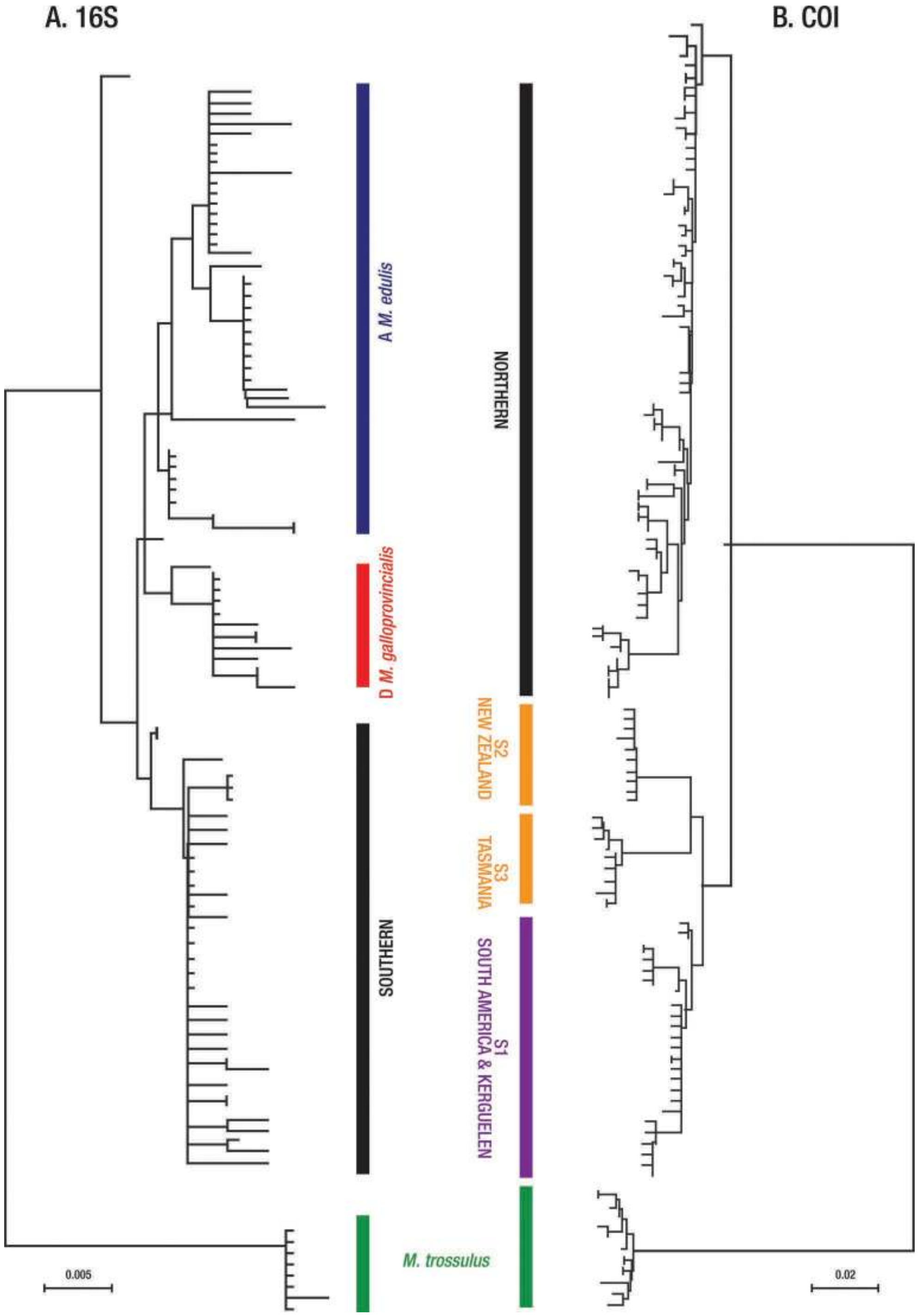

Figure 10 Phylogenetic relationships of *Mytilus* spp. mussels based on (A) 16S rDNA and (B) COI haplotypic variation. Bootstrap scores > 50% are indicated on branches for NJ (neighbour-joining) and ML (maximum-likelihood) trees, respectively. Tree-length scales given at the bottom of each tree. Note that the scale of the 16S tree is four times larger than that of the COI tree. Clade interpretations are given down the centre of the figure. Modified from Gérard et al. (2008).

Pickett & David (2018) failed to detect a Northern versus Southern hemisphere split based on COI sequence variation alone (360-bp fragment with 157 variable sites – Figure 31) in sequences downloaded from GenBank (we note that all such DNA sequence data are only as good as the meta-data). The authors did, however, detect four well-differentiated haplogroups (Australasia, including all New Zealand samples, all Tasmanian samples and some mainland Australia samples; Turkey; southern Korea; southern China) that diverged from a central cluster of haplotypes that showed no real evidence of geographic structure. They suggested that the absence of a distinct Northern versus Southern hemisphere split as previously reported by Hilbish et al. (2000) and Gérard et al. (2008) and as also indicated by Westfall et al. (2010) might be explained by dilution of the signal due to what they called cryptic dispersal, that is frequent and ongoing anthropogenic movement of mussels. Nonetheless, Pickett & David (2018) did identify a very strong Australasian grouping that is quite distinct from all other groups (separated by at least 20 mutational steps from its nearest haplogroup neighbour), as well as provide confirmation of the presence of invasive Northern hemisphere *M. galloprovincialis* in several Southern hemisphere locations such as Chile, South Africa and eastern Australia.

Most recently, Popovic et al. (2020) have used transcriptome-wide markers (a panel of 20,509 SNPs) in an Approximate Bayesian Computing (ABC) framework to test multiple hypotheses of the origin of native Australian (but not New Zealand) mussels. They showed that native mussels from Tasmania are representative of the endemic Australian taxon (*M. planulatus*) and that these mussels exhibit the greatest affinity to Northern hemisphere *M. galloprovincialis*. Based on model testing, Popovic et al. (2020) suggested that Australian *M. planulatus* diverged in allopatry from Northern hemisphere *M. galloprovincialis* between 0.1 and 0.6 M ybp, under a model of historical gene flow followed by divergence in isolation. These findings are consistent with earlier work (e.g., Hilbish et al. 2000, Gérard et al. 2008, Pickett & David 2018), in particular with the suggestion of a second, more recent, range expansion by Mediterranean Sea *M. galloprovincialis* into Australasia (e.g., Hilbish et al. 2000).

While the interpretation of the evolution of Southern hemisphere blue mussels is still not entirely clear, what is apparent is that most studies, regardless of the approach that they have taken, have tended to arrive at similar conclusions. The big picture is relatively clear, but the details are still not always either known or agreed upon by all workers in the field. We can summarise this body of work in five key points: (1) Southern hemisphere mussels are native and do not originate from recent human transport, either accidental or deliberate; (2) Southern hemisphere mussels are derived from Northern hemisphere mussels; (3) the colonisation of the Southern hemisphere first occurred ~1 M ybp (1.2 M ybp according to Hilbish et al. 2000 based on 16S variation; 0.84 M ybp according to Gérard et al. 2008 based on COI variation) during the Pleistocene; (4) the route of invasion was via the Atlantic Ocean and not via the Pacific Ocean; and (5) there is evidence of a second, more recent, colonisation event – unable to be dated by Hilbish et al. (2000) but more recent than 1.2 M ybp, supported by the work of Popovic et al. (2020) who reported a date of 0.1–0.6 M ybp for Australian mussels. It remains unclear, however, whether the two invasions are linked or are independent events, although increasingly the body of evidence is now pointing to two independent events. For example, it is possible that (1) there was one invasion from the Northern hemisphere that gave rise to South American mussels, which subsequently and at an unknown date then gave rise to the Australasian mussels, versus (2) there were two independent invasion events from the Northern hemisphere, the first of which (derived from *M. edulis*) gave rise to the South American mussels and the second of which (derived from *M. galloprovincialis*) gave rise to the Australasian mussels. A recent analysis of transcriptome sequencing and identification of putative biomineralisation genes might provide a new insight (Malachowicz & Wenne 2019). The phylogenetic relationships within some, but not all, members of the genus *Mytilus* were defined on the basis of concatenated, aligned nucleotide sequences of 202 homologous genes using the neighbour-joining (NJ) method (Figure 11). As expected, *M. californianus* and *M. coruscus* are genetically more distant from the other taxa, being equivalent to outgroups for their smooth-shelled mussel congenerics. Again, as expected, *M. trossulus* is the oldest and most distinct species among the smooth-shelled mussels. Detail within the NJ tree

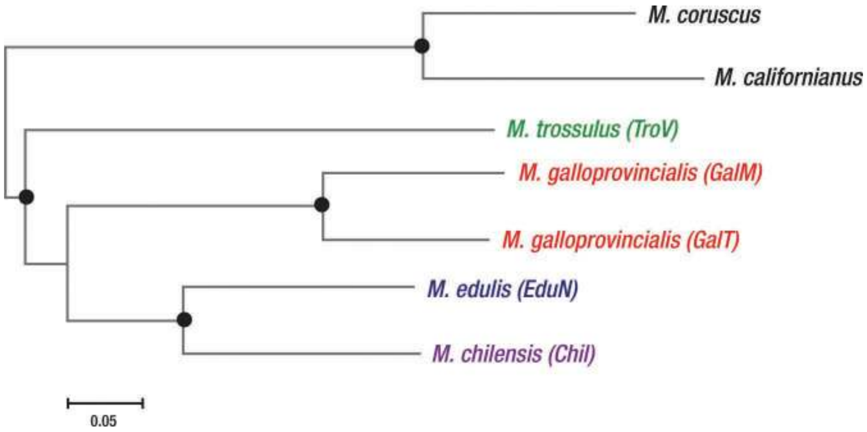

Figure 11 Phylogenetic relationships of six *Mytilus* taxa based on alignment of 202 homologous DNA sequences. A black dot at the node represents bootstrap values >90%. TroV – *M. trossulus* (Vancouver, Pacific coast of Canada), GalM – *M. galloprovincialis* (Trieste and Chioggia, Mediterranean Sea), GalT – *M. galloprovincialis* (Spring Bay, Tasmania, Australia), EduN – *M. edulis* (Oosterschelde estuary, North Sea), Chil – *M. chilensis* (Punta Arenas and Concepción, Chile). Modified from Malachowicz & Wenne (2019).

reveals that *M. galloprovincialis* from the Mediterranean Sea clusters with Southern hemisphere *M. galloprovincialis* from Tasmania (Australia), whereas *M. edulis* from the North Sea clusters with *M. chilensis* from Chile (Malachowicz & Wenne 2019). Thus, this NJ tree provides evidence of two separate invasions of the Southern hemisphere, the first involving Northern hemisphere *M. edulis* that gave rise to the South American native mussels and the second invasion involving Northern hemisphere *M. galloprovincialis* that gave rise to the Australasian native mussels (Malachowicz & Wenne 2019). This interpretation is consistent with the findings of Hilbish et al. (2000), the suggestion of Gérard et al. (2008) and the dating of the origin of the Australian native mussels by Popovic et al. (2020). The presence of two distinct Southern hemisphere groups of native mussels (South America versus Australasia) in the NJ tree is consistent with multiple reports from numerous different authors of this apparent biogeographic split. The inclusion of other native taxa (e.g., from New Zealand, Argentina, the Falkland Islands, the Kerguelen Islands, western Australia) in this sort of analysis would be informative and may help to clarify the one-invasion or two-invasion scenarios that, at present, remain unresolved. On the weight of evidence available, we support the two-invasion scenario.

The recent and ongoing application of SNPs to mussels from all regions of the Southern hemisphere, and the use of reference Northern hemisphere taxa for comparative purposes, sheds new light on the situation in the Southern hemisphere. While markers such as SNPs do not have great utility for determining either the timing of the colonisation or the number of such events (but see Popovic et al. 2020), they are nonetheless particularly informative in terms of providing new information about genetic affinities (relatedness) of Southern and Northern hemisphere mussels, of genetic differentiation among mussels from different Southern hemisphere regions, and may also provide new insights into evolutionary processes such as hybrid speciation (reticulate evolution – Borsa et al. 2007, Arnold & Fogarty 2009, Mallet et al. 2016) and bioinvasions.

Southern hemisphere mussel taxonomy has long been embedded in Northern hemisphere thinking and interpretation

Because the *Mytilus edulis* species complex was much better known and understood at any given time in the Northern hemisphere than in the Southern hemisphere, the nomenclature applied in the north was generally widely applied to the south as well. That is, the species-level taxonomy of Southern hemisphere mussels was often not viewed as being separate from the north, but was viewed as being part of the north. This situation had not always been the case (e.g., taxonomies based on shell characters alone – d'Orbigny 1846, Hupé 1854, Lamy 1936, Powell 1958), but increasing evidence of species-level regional differences when allozyme and the first generations of molecular markers were applied started to change this interpretation.

In considering the taxonomic status of mussels of the Southern hemisphere, it is important to appreciate that taxonomic interpretation is only as good as the traits or the markers being used, in particular given that very few, if any, anatomical or behavioural differences exist among the species. If the marker in question is not informative, then the interpretation has to be that there is no difference at a taxonomic level between the two samples being examined. It is also important to appreciate that the first colonisation event giving rise to Southern hemisphere mussels (*M. edulis* to South America) is judged to be reasonably recent (~1 M ybp) and therefore profound (species-specific diagnostic) differences between Southern and Northern hemisphere mussels may not have had sufficient time to evolve. This will be particularly true if Australasian mussels (*M. planulatus* and *M. aoteanus*) are derived from a much more recent (perhaps in the range 0.1–0.6 M ybp) range expansion from the Northern hemisphere, meaning that even less time has occurred for them to differentiate from *M. galloprovincialis*. Thus, many markers of whatever type may not yet be diagnostically different because of ongoing speciation. Taken in isolation, as they usually were, a single marker may not contain enough information to differentiate between species, but in conjunction with other markers, perhaps the multi-marker approach is sufficient to point to a level of differentiation that is enough to identify different evolutionary lineages. Dealing with incipient speciation is therefore challenging given that in an evolutionary context, not much time may have passed since a split, and also given that for blue mussels, the processes of hybridisation and introgression may blur or retard the formation of new species boundaries. The question of whether speciation occurred without gene flow (allopatrically) or in the face of ongoing gene flow is also important, in this regard.

One of the key points made by Gérard et al. (2008) was that the combined published multi-marker mitochondrial and nuclear DNA data point to two different scenarios. They defined the first scenario as two separate invasion events from the north to the south and the second as "… a single trans-equatorial migration and a view of the composition of the nuclear genome **biased by taxonomic preconception**" (Gérard et al. 2008, p. 84 – emphasis added in bold). They concluded by stating that for the single colonisation event "Under this scenario the distinction, based on nuclear markers, of *M. edulis*-like and *M. galloprovincialis*-like mussels in the Southern Hemisphere would be merely virtual, constrained by human's wish to always assign new samples to reference populations assumed to represent the genetic composition of a given taxon …" (Gérard et al. 2008, p. 90). That is, that genetic differences of Southern hemisphere mussels at the species level might not be recognised as such and would only be interpreted in terms of existing Northern hemisphere species. Perhaps not surprisingly, because the history of marker development and application and the fact that most of the *M. edulis* species complex research has been carried out in the Northern hemisphere, a lot of the interpretation of the Southern hemisphere situation has been strongly embedded in the interpretation of the species in the Northern hemisphere. For example, based on marker types available at the time Blot et al. (1988) concluded that native mussels from the Kerguelen Islands (*M. desolationis*) are part of the *M. edulis* species complex, and many authors noted the similarities between Northern hemisphere *M. edulis* or *M. galloprovincialis* and mussels from different regions and continents in the Southern hemisphere (e.g., McDonald et al. 1991, Sanjuan et al. 1997, Daguin & Borsa 2000). However, they also noted several differences, but often of a subtle nature that was difficult to interpret. In the end, all of these authors tended to favour a taxonomic interpretation of similarity to Northern hemisphere species rather than a separate identity for the Southern hemisphere mussels. This seems to have been as true of mussels from Australasia as it was for mussels from South America (both coasts) and from remote island locations. Even more recently, although a 16S RFLP revealed a clear difference between Northern and Southern hemisphere mussels, a nomenclature based on similarity rather than difference – Northern hemisphere *M. galloprovincialis* and Southern hemisphere *M. galloprovincialis* – was employed (Westfall & Gardner 2010, Westfall et al. 2010, Dias et al. 2014, Ab Rahim et al. 2016). These examples highlight the difficulties faced by workers when using only partially diagnostic markers (often one at a time) that cover only a tiny fraction of the genome to allow the recognition of new species that are endemic to the Southern hemisphere.

In more recent work, Borsa et al. (2007) noted that allozyme and/or *mac-1* (nuclear DNA marker) variation separated placing Southern hemisphere mussels into two clusters, consistent with earlier interpretation (e.g., Hilbish et al. 2000). One cluster was from South America and included the Falkland and Kerguelen populations, having greatest affinity to Northern hemisphere *M. edulis*. The second cluster was composed of populations from mainland Australia, Tasmania and New Zealand (Australasia), having the greatest affinity to *M. galloprovincialis* from the western Mediterranean (the type locality for this species). Borsa et al. (2007) noted that this latter affinity suggests rapid expansion of *M. galloprovincialis* from the Mediterranean to Australasia, presumably with sufficient time for genetic differentiation of the two lineages. Gérard et al. (2008) provided strong evidence of separation among regional groups (New Zealand versus Australia versus South America and the Kerguelen Islands) within the Southern hemisphere and between taxa of the two hemispheres. Ultimately, Gérard et al. (2008) were cautious in their interpretation, noting that gene trees are not species trees (*sensu* Nichols 2001), particularly in the *Mytilus* species complex where hybridisation and introgression are common. Westfall et al. (2010) developed a single marker (16S rRNA RFLP) assay that was able to differentiate Northern hemisphere *M. galloprovincialis*, *M. edulis* and *M. trossulus* from what they called native Southern hemisphere *M. galloprovincialis*. This assay could not, however, differentiate between or among native Southern hemisphere mussels from Chile, Argentina, Australia and New Zealand. The authors suggested that sequencing of each 16S fragment may be helpful in differentiating among regional Southern hemisphere mussels and may be informative in terms of their phylogeography. Thus, the interpretation at the time was still that Southern hemisphere mussels were all similar because they could not be differentiated based on this assay alone (Westfall & Gardner 2010) and were most like Northern hemisphere *M. galloprovincialis*, but that they were clearly different from all Northern hemisphere species (Westfall et al. 2010). Most recently, Astorga et al. (2015) analysed both COI and 16S sequence variation in mussels from South America based on newly collected material (from Chile, Argentina and Uruguay) and GenBank sequence data, including reference Northern hemisphere taxa. As expected, *M. trossulus* was most different from all other groups. Beyond this, COI sequence divergence estimates were of similar magnitudes, such that all four groups (South American *Mytilus*, Northern hemisphere *M. galloprovincialis*, Southern hemisphere *M. galloprovincialis* and *M. edulis*) were equally differentiated. For 16S sequence divergence, again there was evidence of divergence among all pairwise comparisons of the four groups, but the South American *Mytilus* were more similar to other Southern hemisphere mussels than any other pairwise comparison (refer to Table 2 for details). A minimum spanning network of COI haplotypes clearly resolved separate groups: Northern hemisphere *M. trossulus* (black), *M. edulis* (dark blue) and *M. galloprovincialis* (light blue) from their Southern hemisphere counterparts of South American mussels (red) and other Southern hemisphere (Australia and New Zealand) mussels (green) (Figure 12). Consistent with previous analyses (e.g., Hilbish et al. 2000, Gérard et al. 2008), Astorga et al. (2015) reported that there are great differences between Southern hemisphere mussels (two distinct groups were recognised, South America and Australasia) and that samples from South America, including the Kerguelen Islands "… are taxonomically independent" (Astorga et al. 2015, p. 924). Finally, Astorga et al. (2015) noted the complex and, at times, tangled taxonomy of the global *M. edulis* species complex (they dismissed use of regional-specific subspecific status (*M. edulis edulis*, *M. edulis galloprovincialis*, *M. edulis planulatus* and *M. edulis platensis*) and concluded somewhat uncertainly that "In the southern hemisphere, *Mytilus planulatus* should be the name used for specimens from South America, Kerguelen, and

Table 2 Percentage genetic distance between regional taxa of *Mytilus*, as reported by Astorga et al. (2015)

	South American *Mytilus*	Northern hemisphere *M. galloprovincialis*	Southern hemisphere *M. galloprovincialis*	*Mytilus edulis*	*Mytilus trossulus*
South American *Mytilus*	–	0.035	0.033	0.030	0.438
Northern hemisphere *M. galloprovincialis*	0.019	–	0.041	0.015	0.472
Southern hemisphere *M. galloprovincialis*	0.005	0.021	–	0.039	0.490
Mytilus edulis	0.034	0.038	0.036	–	0.448
Mytilus trossulus	0.098	0.103	0.107	0.100	–

The number of base substitutions per site averaged over all sequence pairs between groups are shown. For COI, analyses were conducted using the Tamura-Nei 93 model+G. The rate variation among sites was modelled with a gamma distribution (shape parameter=0.241). For 16S, analyses were conducted using the Tamura-3-parameters model. The rate variation among sites was modelled with a gamma distribution (shape parameter=0.319).
Values for COI above and values for 16S below the diagonal.

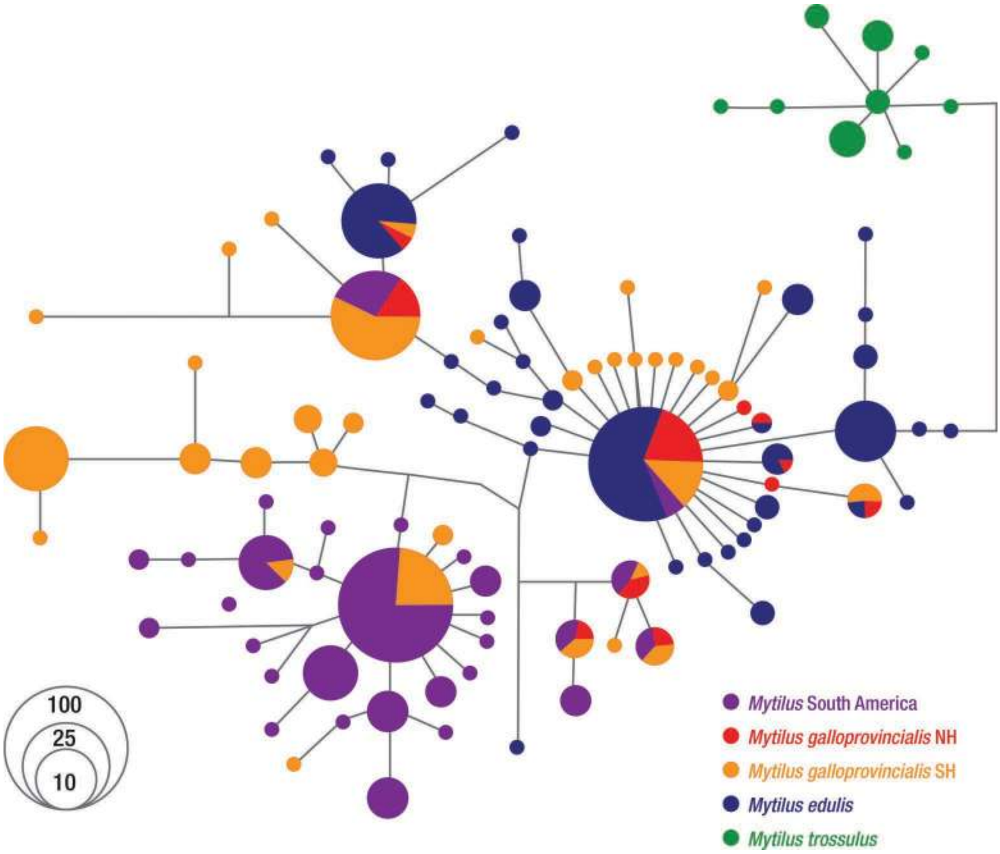

Figure 12 Minimum spanning network of haplotypes derived from variation at the COI gene for *Mytilus* species. The size of each circle is proportional to the absolute haplotype frequency, and the total number of individuals in each haplotype is indicated inside the circle (see graphic bottom left). The branch lengths are proportional to the mutational steps. Modified from Astorga et al. (2015).

the southwest Pacific (eastern Australia, Tasmania and New Zealand); or perhaps two differentiated groups should exist for the southern hemisphere, namely *planulatus* and *platensis*" (Astorga et al. 2015, pp. 927–928). Thus, based on taxonomic priority, the name *M. chilensis* would cease to be used for mussels from the Pacific coast of South America (Astorga et al. 2015).

Overall, there has long been evidence of differentiation between mussels from the Northern and Southern hemispheres (all studies have reported this, to greater or lesser degrees) and of differentiation between South America and Australasia within the Southern hemisphere (most, but not all studies have reported this). However, there was still, at this time, no definitive evidence that quite clearly pointed to differentiation among between-region or within-region mussel groups in the Southern hemisphere consistent with mussels of different species.

What constitutes a species within the *Mytilus edulis* species complex?

Understanding the diversity and biogeography of native Southern hemisphere blue mussels requires an understanding and appreciation of what constitutes a species. The idea and application of the species concept is, of course, a topic in its own right and one that has been debated over many decades. The detail of this debate is beyond the scope of the present review, and the reader is directed towards other papers for further discussion of this topic (e.g., Mallet 1995, Hey 2001a,b, de Queiroz 2007, Häuser 2009, Hausdorf 2011, Chambers 2012, Harrison & Larson 2014, Hohenegger 2014, Stanton et al. 2019). However, much of the longstanding debate about the taxonomy of the *Mytilus edulis* species concept in a global sense can really only be understood in the light of what various authors consider to be a species. As noted by Hey (2001a,b) and also indirectly by Gérard et al. (2008), this may be a personal view, rather than a purely objective view.

Changing views of the taxonomy of Southern hemisphere blue mussels

Many, but not all, of the native smooth-shelled blue mussels from the Southern hemisphere were named in the nineteenth century, during one of the great periods of global exploration and scientific discovery. Of course, these different mussels were named based on shell shape variation and with reference to descriptions provided for Northern hemisphere taxa (e.g., *M. edulis* Linnaeus, 1758, *M. galloprovincialis* Lamarck, 1819 and *M. trossulus* Gould, 1850) as these existed at the time. Many different taxa were described (refer to WoRMS for more detail – http://www.marinespecies.org/) and often on what may best be described as minor shell morphological differences. While this classification of global blue mussels may not seem to be of great importance, ultimately it is very relevant to the recognition of Southern hemisphere species, at least in part because of the concept of taxonomic priority (International Commission for Zoological Nomenclature 1999) and also as taxonomy relates to food labelling and biosecurity issues (see subsequent sections). These factors – the recognition of geographically isolated species and the concept of taxonomic priority – combined with individual views of what constitutes a species, have led to a series of debates and disagreements in the literature about the global taxonomic status of smooth-shelled blue mussels.

Two of the most important reviews of the subject of native Southern hemisphere mussel taxonomy are provided by Lamy (1936) and Soot-Ryen (1955), in the days before the application of genetic markers. In some respects, these two reviews provide an important framework, based on morphometric differences, for testing using modern molecular approaches. Lamy (1936) recognised the three Northern hemisphere taxa *Mytilus edulis* Linnaeus, 1758, *M. galloprovincialis* Lamarck, 1819 from the Mediterranean Sea, *M. trossulus* Gould, 1850 from the Pacific coast of North America, and also several Southern hemisphere taxa, including *M. chilensis* Hupé, 1854 from Chile, *M. platensis* d'Orbigny, 1846 from Argentina and Uruguay, and *M. planulatus* Lamarck, 1819 from Australia (but no mention of New Zealand), and then also described *M. desolationis* from the

Kerguelen Islands (Lamy 1936). In contrast, Soot-Ryen (1955) considered most of the above listed taxa as subspecies of Northern hemisphere *M. edulis*. In his extensive review of the littoral ecology and biogeography of the Southern oceans, Knox (1960, pp. 591–592) notes that while the blue mussels of the Southern hemisphere had formerly been split into separate species, they are now "… but subspecies of the cosmopolitan *Mytilus edulis*". Knox (1960, his figure 64) recognised five distinct subspecies – *M. e. chilensis*, *M. e. platensis*, *M. e. planulatus*, *M. e. aoteanus* Powell, 1958 and *M. e. desolationis* – consistent with the suggestions of the time made by Soot-Ryen (1955, 1957) and Fleming (1959) that had superseded the interpretation of Lamy (1936).

While the concept of the species may be in the eye of the beholder (Hey 2001a,b), there is one line of thinking that seems to be shared among most workers in the field in terms of species-level recognition and that is the concept of differentiated evolutionary lineages that maintain their identity even in the face of hybridisation and introgression. Thus, distinct genetic groupings with different evolutionary histories, despite gene flow between them, are the most commonly accepted interpretation of a "species" within the *M. edulis* species complex (e.g., Varvio et al. 1988, Koehn 1991, Daguin & Borsa 2000, Riginos & Cunningham 2005). Numerous more modern reviews of the species problem and of what constitutes a species boundary tend to agree with this interpretation. For example, Harrison & Larson (2014, p. 795) note in their review that species may be defined as "… populations that are diagnosably distinct, reproductively isolated, cohesive, or exclusive groups of organisms", that species boundaries are not uniform in space, in time or across the genome, and that species boundaries may be maintained despite hybridisation and introgression (i.e., species boundaries may be semi-permeable). However, the application of this framework does not guarantee a consistent approach across all workers. For example, when applied to mussels from Chile, Borsa et al. (2012) recognised the distinction between what they called Southern hemisphere *M. edulis* and Northern hemisphere *M. edulis* that gave rise to a "… separate, geographically isolated entity" (Borsa et al. 2012, p. 7) and concluded that these Southern hemisphere mussels warranted subspecific status as *M. edulis platensis* d'Orbigny 1846 and that the name *chilensis* (as in *M. chilensis* or *M. edulis chilensis*) should be dropped. In a similar vein, they argued that what they called Southern hemisphere *M. galloprovincialis* (e.g., so-called *M. desolationis* Lamy, 1936 for mussels from the Kerguelen Islands) should be called *M. galloprovincialis planulatus* Lamarck, 1819. Their argument was based, quite correctly, on the principle of taxonomic priority, but their interpretation of subspecific status for Southern hemisphere mussels was not widely accepted or applied. This example highlights the problem of taxonomic designations within the *M. edulis* species complex, and how, as noted above, the concept of the species may be in the eye of the beholder (Hey 2001a, b).

In their appraisal of blue mussels from the Pacific coast of North America, McDonald & Koehn (1988) make the point about taxonomy and specific status for Northern hemisphere smooth-shelled blue mussels that scientific clarity (i.e., the avoidance of confusion) is an important consideration. Revisions of taxonomy are based on rules (International Commission for Zoological Nomenclature 1999), but nonetheless revisions of taxonomy need to be mindful of longstanding practice and must not generate confusion. Given the difficulty of recognising distinct species in a species complex where speciation is recent and ongoing, and where both hybridisation and introgression often occur at high frequencies, an interpretation based on distinct evolutionary histories and genetic differences that are maintained despite gene flow seems to be entirely appropriate. We suggest that this approach now needs to be applied to Southern hemisphere mussels in the light of newly published data based on SNPs.

Interpretation of Southern hemisphere species and problems with taxonomy

Borsa et al. (2012) make the point that "Given the morphological variation encountered within Northern-Hemisphere *M. edulis* (McDonald et al. 1991), it remains to be proven that the reportedly flatter shell of Hupé's *M. chilensis* constitutes a character strong enough to distinguish it from

M. edulis and assign it specific rank" (Borsa et al. 2012, p. 3). The same reasoning about shell morphometry also applies to the recognition of other putatively endemic Southern hemisphere taxa (e.g., *M. platensis* in Argentina and Uruguay, *M. planulatus* in Australia, *M. aoteanus* in New Zealand, *M. desolationis* at the Kerguelen Islands) and their differentiation from Northern hemisphere taxa and, of course, among themselves. To some extent, the answer to this question will depend on the species concept applied, but it will also depend on the supporting evidence derived from other sources, such as new molecular markers.

While applying their 16S RNA RFLP assay to native Southern hemisphere mussels, Westfall & Gardner (2010, 2013) noted that all native Southern hemisphere mussels that they tested (from Australia, Chile and New Zealand) were distinct from Northern hemisphere mussels. However, the 16S RFLP assay could not differentiate among the different Southern hemisphere regional populations. This led them to apply the concept of different evolutionary lineages of mussels (i.e., a Southern hemisphere *M. galloprovincialis* versus a Northern hemisphere *M. galloprovincialis*). While this approach was informative for identifying non-native Northern hemisphere mussels in the Southern hemisphere, it was not informative in terms of identifying native Southern hemisphere region-specific differences.

Those who consider the differences to be important (e.g., Lamy (1936) who was more of a "splitter") and those who consider the similarities to be more important (e.g., Soot-Ryen (1955) who was more of a "lumper") may help to explain the absence of consensus about the specific status of Southern hemisphere mussels (refer to species delimitation review by Stanton et al. 2019). In some respects, molecular workers have also tended towards one or other of these schools of thought (lots of different regional differentiated taxa versus a relatively few species of Northern hemisphere origin, into which all Southern hemisphere taxa are fitted as subspecies). This dichotomy reflects, in the minds of some workers, what has been called "taxonomic inflation" (Isaac et al. 2004) and has real consequences across a range of different fields and management options. These two contrasting viewpoints provide an opportunity for testing ideas about speciation and biogeography, and this is very much the background that helped spur the development and application of a panel of new SNP markers (Zbawicka et al. 2012) to Southern hemisphere mussels (Gardner et al. 2016, Larraín et al. 2018, Zbawicka et al. 2018, 2019, 2021).

The fossil and midden records of *Mytilus* spp. in the Southern hemisphere

A number of authorities have noted that *Mytilus* shells are found as fossils or in middens that predate European arrival in all Southern hemisphere countries so far examined, with the notable exception of South Africa (e.g., McDonald et al. 1991 and references therein). The coverage for fossil or midden valves of *Mytilus* sp. is patchy, with several important regions having no information about them at all. Thus, interpretation needs to be made with care, but the evidence overwhelmingly supports the view that fossils and/or midden valves that predate European arrival in all regions point to the native status of blue mussels in the Southern hemisphere.

Surprisingly, few analyses of shell trait or shape variation among Southern hemisphere shells in the context of reference Northern hemisphere shells have been carried out, despite the fact that shell trait and shape variability may be informative at the species level (e.g., McDonald et al. 1991, Gardner 2004, Gardner & Thompson 2009, Illesca et al. 2018). Thus, interpretation of Southern hemisphere shells may, at times, be difficult given the absence of reference samples.

Intriguingly, there is evidence from both coasts of South America of what appear to be very old deposits (Miocene epoch, ~5.333–23.030 M ybp) that contain native blue mussels. The specific status of these mussels is often unclear, but their presence on both coasts suggests that blue mussels may be older than much of the present literature suggests. Given a presumed origin of *M. trossulus* approximately 3.5 M ybp, which is thought to be the ancestral species of contemporary smooth-shelled blue mussels in the North Pacific Ocean region (Riginos & Cunningham 2005), the occurrence of a relative in Chilean middens can be explained, but the presence of a relative in Argentina that predates *M. trossulus* is harder to explain. Such fossil remains need further investigation, and new interpretation may, once again, revise our thinking about the evolutionary origin of the *Mytilus edulis* species complex.

Chile

Mytilus sp. fossils have been reported from several sites on the Chilean coast. The oldest records date from the early Miocene, in the Navidad formation (33°55′S) on Ipún Island (Los Chonos – 44°36′S) (Kiel & Nielsen 2010), at Lo Abarca in central Chile – (33°31′S) (Covacevich & Frassinetti 1990) and at Tubul (37°S) an early Pleistocene *Mytilus* fossil has been recorded (Nielsen & Valdovinos 2008, Kiel & Nilsen 2010). In the south, near the Strait of Magellan, molluscs of the genus *Mytilus* are the most important macroinvertebrates of the modern benthic fauna (Aldea & Rosenfeld 2011). They have been a key ecological component of the coastline since at least the Holocene period (Estevez et al. 2001, Rabassa et al. 2009, Gordillo et al. 2010), and the fossil record indicates that a smooth-shelled blue mussel (*Mytilus* sp.) may date back to the late Miocene (~10 Myr BP) in this region (Martínez & del Río 2002, Aguirre et al. 2008). The molecular data (Bayesian Skyline Plot) indicate that population increase of *Mytilus* in Chile happened 20,000 years ago after the Last Glacial Maximum (Figure 13). It is likely that the colonising populations settled in discrete refuges (e.g., Ipún Island) and subsequently colonised the south as the glaciers retreated. This would indicate that the fossil records of *Mytilus* in South America are

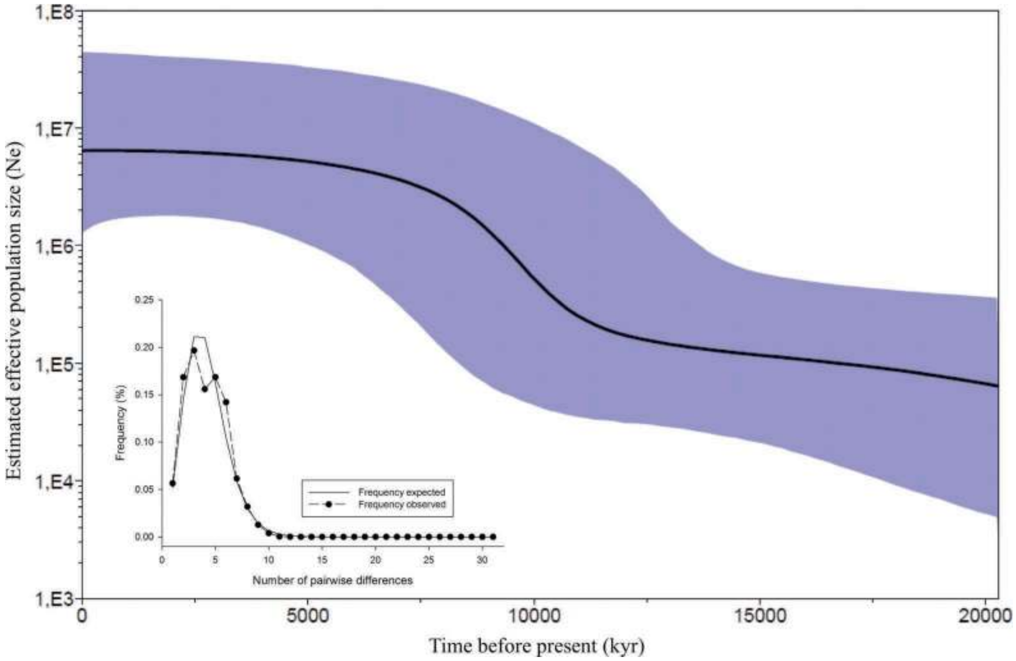

Figure 13 Bayesian skyline plot showing the demographic history of *Mytilus chilensis* on the Chilean coast. The dark line (solid black line) represents the median value of the population size (Ne), and the blue area represents the 95% highest probability density interval. Inset, lower left – the mismatch analysis represents the frequency distribution of pairwise differences among cytochrome oxidase subunit I (COI) haplotypes in *Mytilus*. Modified from Oyarzún et al. (2019).

older than those reported in the Northern hemisphere (e.g., Vermeij 1991). This interpretation may have some bearing on the origin of modern smooth-shelled blue mussels, but does not, as things presently stand, argue against or disprove the molecular interpretation of the origin of modern smooth-shelled blue mussels.

Argentina and Uruguay

McDonald et al. (1991) noted that pre-Columbian fossil shell deposits and/or middens are reported from Uruguay (Sprechemann 1978) and Argentina (Johnson 1976). More recently, del Río et al. (2001) have reported the presence of *M. trigonus* that inhabited the Miocene sea in northern Patagonia, Argentina. Subsequently, Savoya et al. (2015) used Fourier analysis to show that pre-European and modern *Mytilus* sp. shells from Patagonia are different from one another, possibly suggesting that modern native blue mussels in Argentina are, in fact, invaders rather than genuinely native. However, the authors note that alternative explanations also exist, including the fact that environmental change may result in a change in shell shape over evolutionary time. The question of a possible cryptic invasion requires the application of modern molecular markers to answer it and thereby to inform management (e.g., protection) practices (Savoya et al. 2015). We are unaware of any evidence of fossil or midden valves from the Falkland Islands.

New Zealand

Pre-European fossil shell deposits and/or middens are reported from numerous locations throughout mainland New Zealand (Fleming 1959, Fleming & Suggate 1964, McDonald et al. 1991, Gardner 2004). However, we are unaware of any such records from the numerous offshore islands in the Southern Ocean, although these were infrequently inhabited (e.g., as summer fishing and hunting camps).

In New Zealand, the fossil record for *Mytilus* dates back a little over 1.0 M ybp, to the early Castlecliffian (Sutherland et al. 1995). An analysis of one fossil valve and 83 midden valves from New Zealand (Gardner 2004) in comparison to reference Northern hemisphere valves, indicated that native New Zealand mussels were best interpreted as *M. galloprovincialis*-like. That is, consistent with genetic markers being employed at the time, native New Zealand mussels showed the greatest affinity in terms of shell trait variation to Northern hemisphere *M. galloprovincialis*. Interestingly, the Bay of Islands region (far north of New Zealand) was identified by the analysis of shell trait variation to have the greatest affinity with reference Northern hemisphere *M. edulis*. Subsequently, this region has been shown to have been very heavily invaded by Northern hemisphere *M. galloprovincialis* (Gardner & Westfall 2012, Gardner et al. 2016), so we speculate that it is possible that the NZ shells with the greatest affinity to *M. edulis* were non-native Northern hemisphere mussels or perhaps were hybrids and/or backcrosses between native and introduced mussels.

Australia

McDonald et al. (1991) note that pre-European fossil shell deposits and/or middens are reported from mainland Australia and Tasmania (Hope et al. 1977, Donner & Jungner 1981, Colhoun et al. 1982, Kerrison & Binns 1984). However, all of these reports relate to sites in eastern Australia (New South Wales, Victoria, South Australia, Tasmania), and there appear to be no records of such shells from Western Australia. Svane (2011) noted that because blue mussel valves may be difficult to identify (even to genus level) and to age from archeological material, the identification of the shells and fragments has not been independently verified. In the context of blue mussels in Australia, all authors have noted that the presence of valves in middens that predate European arrival strongly supports the contention that such mussels are native to Australia or at least some parts of it.

South Africa

Several different authors have noted that blue mussel valves are not found in fossil or shell midden deposits (e.g., Grant & Cherry 1985, McDonald et al. 1991, and references therein). This information has been interpreted as meaning that the genus is naturally absent from southern Africa. This interpretation is consistent with the physical oceanographic data that explains contemporary blue mussel distributions in the Southern hemisphere.

Offshore islands

Reports of fossils from remote offshore Southern hemisphere islands are harder to find, but nonetheless, Fletcher (1938) has reported marine fossils, including a recent *Mytilus* sp., from a late Tertiary bed at the Kerguelen Islands (Powell 1965). The natural (i.e., pre-human) distributions of blue mussels across the many islands in the Southern Ocean are hard to establish, and there is only limited evidence that clearly points to their native presence. Nonetheless, it appears to be widely accepted or believed that blue mussels are native to many, but not all, remote offshore islands in the Southern hemisphere.

Southern hemisphere mussel phylogeography based on markers before the use of SNPs

Chile

In South America, blue mussels occur naturally from approximately the latitude of Concepción, Chile (36°49′S; 73°03′W) on the Pacific coast, along the southern Chilean coast line and around Cape Horn (55°58′S; 67°17′W), and then extend north along the Atlantic coastline of Argentina, to a northern limit at approximately Punta del Este, Uruguay (34°58′S; 54°57′W) (Hernández & González 1976, Oyarzún 2016).

The native blue mussel from the Pacific coast of South America (Chile) was described by Hupé in 1854 as *M. chilensis*. This was soon after the description of the native blue mussel from the Atlantic coast of South America (Uruguay), described by d'Orbigny in 1846 as *M. platensis*. As noted by Borsa et al. (2012) when addressing this point "… Hupé mentioned the presence of *M. chilensis* '*en la costa*, *en Valparaíso*, *etc*.' and recognised that *M. chilensis* '*tiene enteramente el aspecto del Mytilus edulis de las mares de Europa*' except that '*su forma es más aplastada*'" (Borsa et al. 2012, p. 3). In other words, Hupé (1854) described a mussel that he felt was different from *M. edulis* as found in Europe (this is specifically mentioned), and presumably also from *M. platensis* described only eight years earlier by d'Orbigny (1846) from Uruguay. So although Hupé does not specifically mention *M. platensis* in his description of *M. chilensis*, it is reasonable to assume that he was aware of d'Orbigny's (1846) description of *M. platensis* (Larraín et al. 2018). Consistent with the scientific practices of the day, it warranted specific status – *M. chilensis*.

Mussel phylogeography in the Southern hemisphere, specifically in Chile, but also in Argentina and Uruguay, has been based on the use of markers such as allozymes (McDonald et al. 1991, Toro et al. 2006, Borsa et al. 2012), diagnostic nuclear DNA regions (Daguin & Borsa 2000), RAPDs (Toro et al. 2004a), RFLPs (Toro 1998b, Toro et al. 2005, Westfall et al. 2010, Larraín et al. 2012, Oyarzún et al. 2016), microsatellites (Ouagajjou et al. 2011, Larraín et al. 2015), F mtDNA sequencing (Gaitán-Espitia et al. 2016), 16S rRNA sequencing (Astorga et al. 2015) and COI sequencing (Pickett & David 2018, Astorga et al. 2018) to obtain evidence about the macro- and micro-distributions of species. Based on this body of research, and very much depending on its interpretation, there is evidence of three *Mytilus* species on the Pacific coast and into the Strait of Magellan region. First, the Chilean mussel *Mytilus chilensis* (Hupé, 1854) has been reported from many locations (from 36°S to 54°S) along the southern Pacific coast of Chile (Toro et al. 2006, Santaclara et al. 2006, Larraín et al. 2012, Śmietanka & Burzyński 2017) and the Strait of Magellan (Oyarzún et al. 2016). Second, the mussel *Mytilus edulis* (Linnaeus, 1758) has been reported in southern Chile (McDonald et al. 1991, Hilbish et al. 2000, Santaclara et al. 2006, Fernández-Tajes et al. 2011). Oyarzún et al. (2016) reported a cline with a high proportion of mussel samples being classified as *M. edulis* from the east of the Strait of Magellan declining towards the west of the Magellan Channel. Third, the invasive Northern hemisphere *Mytilus galloprovincialis* (Lamarck, 1819) has been reported from the central coast of Chile (Daguin & Borsa 2000, Toro et al. 2005, Westfall & Gardner 2010, Borsa et al. 2012, Tarifeño et al. 2012, Pickett & David 2018) and also in the Strait of Magellan (Oyarzún et al. 2016). Despite all these data, the interpretation of the spatial distribution of native blue mussels around the coasts of South America is still controversial, complicated by a dispersive larval pelagic stage (four to five weeks), the ability of mussels to hybridise and the contribution that processes such as rafting may have on natural range expansion and gene flow between sites or regions (e.g., Ó Foighil et al. 1999, Miller et al. 2018). In addition, some species have been used for aquaculture, and the transfer of juveniles from a few sites of spat collection to the grow-out sites is another important human-mediated form of gene flow (Holmberg 2012, Astorga et al. 2018).

Argentina and Uruguay

On the Atlantic coast of South America, smooth-shelled blue mussels are present from the south of Brazil (Klappenbach 1965), along the coasts of Uruguay and Argentina south to Tierra del Fuego (Castellanos 1962, Amaro-Padilla 1967) and around Cape Horn. This Atlantic Ocean natural (native) distribution also includes the Falkland Islands (Davenport et al. 1984), but not apparently any other South Atlantic Ocean islands.

The native blue mussel from the Atlantic coast of South America (Uruguay) was first described by d'Orbigny in 1846 as *M. platensis*. As described previously, the situation on the Atlantic coast of South America and into the Strait of Magellan has received a lot of attention (but less than for Chilean mussels), based on a variety of different marker types. The native Argentine mussel *Mytilus platensis* (d'Orbigny, 1846) has been reported from Montevideo, Uruguay (34°50′S; 56°10′W) (Astorga et al. 2015), Mar del Plata, Argentina (38°10′S; 57°27′W) (Gaitán Espitia et al. 2016), Puerto Deseado, Argentina (47°45′S; 65°53′W) (Astorga et al. 2015) and along much of the Atlantic coast. *Mytilus edulis* has been reported from southern Argentina (McDonald et al. 1991, Hilbish et al. 2000, Santaclara et al. 2006, Fernández-Tajes et al. 2011), where (as noted previously) it forms a natural hybrid zone with *M. chilensis* in the Strait of Magellan (Oyarzún et al. 2016). Invasive Northern hemisphere *Mytilus galloprovincialis* has now also been reported from the central coast of Argentina (42°46′S; 64°59′W) (Zbawicka et al. 2018).

New Zealand

Smooth-shelled blue mussels of the genus *Mytilus* are distributed throughout New Zealand, from the Bay of Islands in the north (35°S), as far south as the Campbell Islands (52°S). This distribution includes all offshore (sub-Antarctic) islands, the Chatham Islands to the east and the three main islands – Stewart Island, the South Island and the North Island (Powell 1955, Morton & Miller 1968, Gardner & Westfall 2012, and references therein). This distribution spans 17° of latitude (1,800 km) and includes subtropical, warm temperate, cold temperate and subantarctic waters (Gardner 2004). For reasons that remain unclear (possibly related to environmental conditions), there is a major discontinuity in mussel distribution from 41°S to 35°S (Morton & Miller 1968, Gardner & Westfall 2012). A single New Zealand fossil valve has been dated to ~1 M ybp (references in Gardner 2004), an age that is consistent with molecular phylogenetic analyses that place the Southern hemisphere origin of so-called *M. galloprovincialis* between 0.84 M ybp (Gérard et al. 2008) and 1.2 M ybp

(Hilbish et al. 2000). Interestingly, this fossil valve considerably predates the estimate of Popovic et al. (2020) of an Australian (and therefore, by extension, a New Zealand?) origin of native blue mussels dating to 0.1–0.6 M ybp.

Based on shell morphometric differences, Powell (1958) described the native blue mussel from New Zealand as the endemic species, *M. aoteanus*. Powell (1958) differentiated the New Zealand *Mytilus* (the type locality is Wellington Harbour, in the North Island, which is important in the use of this binomial for New Zealand mainland and also offshore island locations) from native Australian *Mytilus* by variation in several shell traits, including dorsal slope (longer in New Zealand shells), hinge teeth (fewer in New Zealand shells), shape of the posterior retractor scar (narrower in New Zealand shells) and the anterior adductor scar (larger in New Zealand shells) (Figure 14). These are all relatively minor differences but were consistent enough across samples to convince Powell of a specific difference between native New Zealand and Australian shells. This status was subsequently reduced to subspecific status – *M. edulis aoteanus* – by Fleming (1959), who followed Soot-Ryen (1955) in the application of trinomial taxonomy, in large part because of the close similarity of shell form, habitat preference and zonal distribution of both Northern hemisphere *M. edulis* Linnaeus, 1758 and the many Southern hemisphere varieties. Many workers have employed this changing taxonomy over the years (e.g., Duff 1967, Morton & Miller 1968, Kennedy 1977).

Subsequent work based on both allozyme and nuclear DNA marker variation (e.g., McDonald et al. 1991, Sanjuan et al. 1997, Daguin & Borsa 2000) and also on fossil and middens remains (Gardner 2004) tended to highlight a degree of separation of native New Zealand mussels from reference Northern hemisphere *M. galloprovincialis*, but greater affinity to *M. galloprovincialis* than to any other Northern hemisphere species (Figures 5–7). Consistent with this, Hilbish et al. (2000) and Gérard et al. (2008), using 16S and COI sequencing, respectively, both noted the separation of New Zealand native mussels from Northern hemisphere mussels within a clade composed of all *M. galloprovincialis* (Figures 8 and 9). Overall, these findings resulted in calls for native New Zealand mussels to be recognised as a subspecies of Northern hemisphere *M. galloprovincialis* or as equivalent to (indistinguishable from) Northern hemisphere *M. galloprovincialis*. This taxonomy has been followed reasonably closely by most workers (e.g., Gardner & Kathiravetpillai 1997, Gardner 2000, Rogers 2003, Petes et al. 2007). However, on occasions, a broader geographic interpretation of New Zealand blue mussel taxonomy has been applied. For example, Morley & Hayward (2010), who noted the unstable taxonomy of New Zealand *Mytilus* sp., used the trinomial *M. galloprovincialis planulatus*, following Crowe (2010) who recorded this particular trinomial for mussels from Tasmania and southern Australia. Interestingly, Morley & Hayward (2010) describe an apparent range expansion of native New Zealand blue mussels around Auckland (36.8485°S, in northern New

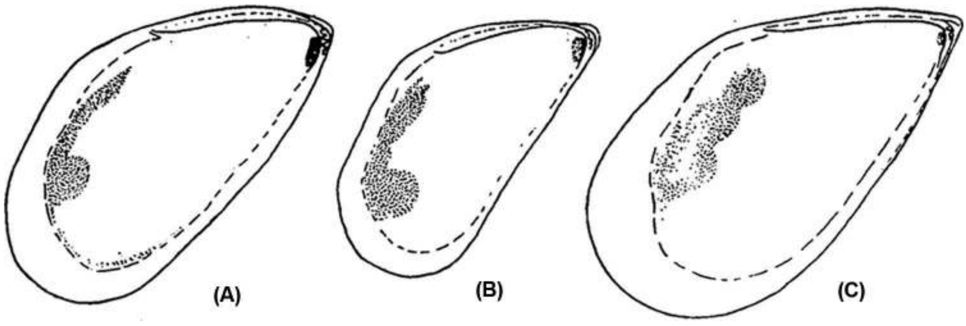

Figure 14 Examples of interior shell characteristics of native blue mussels from the Campbell Islands (at 52°S in the Southern Ocean), New Zealand (A), the Atlantic coast of the United States of America (B) and Tasmania, Australia (C). Taken from Fleming (1959).

Zealand), an area where it has traditionally been found in low abundance or not at all. It cannot be determined from their work whether this range expansion refers to native New Zealand mussels or to the morphologically very similar form of the invasive Northern hemisphere *M. galloprovincialis*: this would need to be tested with molecular markers.

Westfall et al. (2010), using a 16S RFLP assay, reported the presence of native New Zealand *M. galloprovincialis*-like mussels that were different from Northern hemisphere *M. galloprovincialis*. Based on Me15/16 results, they also reported the presence of two *M. galloprovincialis*/*M. edulis* hybrid genotypes, both from the Auckland Islands in the Southern Ocean (Westfall & Gardner 2010). A significant proportion of invasive Northern hemisphere *M. galloprovincialis* were reported from a number of different sites and regions, principally in the north of the country. Subsequently, using the Me15/16 and 16S RFLP assays, Gardner & Westfall (2012) reported a new meta-population of native New Zealand blue mussels (Southern hemisphere lineage of *M. galloprovincialis*) in the far north of the country. They also noted the presence of invasive Northern hemisphere *M. galloprovincialis* at many of these far north sites. Spencer et al. (2009), in their “Key to New Zealand molluscs” (last updated May 2017), and following a number of authorities, list the native New Zealand blue mussels as *M. galloprovincialis*.

Australia

In Australia, *Mytilus* is common in the temperate waters of southern Australia and around Tasmania, and extends into northern New South Wales (Cape Hawk or Port Stephens ~ 32.4°S) on the east coast, and to Perth at ~ 32.2°S on the west coast (Gardner & Westfall 2012, Dias et al. 2014, Ab Rahim et al. 2016 and references therein). At the moment, it is unclear whether this distribution is continuous or whether there exists a discontinuous distribution, with centres of distribution around southeastern and southwestern Australia, and an absence along the Great Australian Bight. This point needs checking and clarifying. Svane (2011) notes that in Australia, the blue mussel may be found on the rocky coastline of the temperate region “… but the species has until recently only been found in environments associated with marinas and harbours” (Svane 2011, p. 134).

Lamarck (1819) described multiple *Mytilus* species from Australian waters that at the time were called Nouvelle-Hollande (present day Albany, southwestern Australia), including *M. angustanus*, *M. corneus*, *M. planulatus* and *M. ungularis* (cited by McDonald & Koehn 1988). Because of morphological similarity of shells, Australian populations of *Mytilus*, including those from Tasmania, were, at times, considered to be *M. edulis* Linnaeus, 1758 (Wallis 1975). However, it is the name *M. planulatus* Lamarck, 1819 (Lamarck’s type locality was King George Sound, Western Australia) that was extensively used, at least until the assessment of allozyme variation (e.g., Lamy 1936 in McDonald et al. 1991, McDonald et al. 1991, Sanjuan et al. 1997) and nuclear DNA markers (Daguin & Borsa 2000) for Australian mussels, at which time differences between Northern hemisphere *M. galloprovincialis* and native Australian mussels became less clear and often resulted in calls for subspecific status (i.e., *M. galloprovincialis planulatus*).

Allozyme variation in mussels from Australia was more similar in allele frequencies to *M. galloprovincialis* from the Northern hemisphere than to *M. edulis* (McDonald et al. 1991 – Figure 5). Sanjuan et al. (1997 – Figure 6) and Daguin & Borsa (2000 – Figure 7) both reported the separation of Australian mussels from their Northern hemisphere counterparts based on global analyses of *M. galloprovincialis* allozyme alleles. Daguin & Borsa (2000) also examined two nuclear DNA markers (*Glu-5′* and *mac-1*) in their study of global *M. galloprovincialis* populations and found that the *mac-1* allele *G* was diagnostic for Australian mussels, which led them to suggest that Australian *M. galloprovincialis* had mixed genetic architecture, with a high frequency of *M. edulis*-like and *M. galloprovincialis*-like alleles (“patchy genetic architecture”) and was distinct from northern *M. galloprovincialis*. They suggested that native Australian *Mytilus* populations should be considered as a “regional subspecies of *M. galloprovincialis*”. Sequencing of mitochondrial DNA led both Hilbish et al. (2000) and Gérard et al. (2008) to highlight the distinctness of native Australian mussels and their greater affinity to *M. galloprovincialis* than any other species among the Northern hemisphere reference taxa. Analysis of what was called native *M. galloprovincialis* from Tasmania revealed the presence of both *M. edulis* (at the *mac-1* locus) and *M. galloprovincialis* (at the *Glu-5′/Glu-3′* locus) allele frequencies that were nearly fixed, indicating a possible hybrid origin of these mussels (Borsa et al. 2007).

Westfall & Gardner (2010) combined the use of the Me15/16 and 16S RFLP assays to describe native mussels from Port Arthur (Tasmania) and Melbourne (Victoria) as Southern hemisphere *M. galloprovincialis*, and also reported the presence of introduced Northern hemisphere *M. galloprovincialis* at 10% and 20%, respectively. Phylogenetic analysis of COI sequence variation revealed the occurrence of Northern hemisphere *Mytilus* haplotypes in southeastern Australia populations (Colgan & Middelfart 2011) and based on F-type COI variation, in mussels from three hatcheries in South Australia, Victoria and Tasmania, and in four wild populations from Western Australia (Dias et al. 2014). Most recently, Ab Rahim et al. (2016) identified both native and introduced Northern hemisphere *M. galloprovincialis* in Australian populations. Svane (2011) hypothesised that Northern hemisphere *M. galloprovincialis* was introduced into Australia with the first ships that arrived from Europe, most probably into Western Australia which is the type location of *Mytilus* in Australia (i.e., *Mytilus planulatus* Lamarck 1819). Interestingly, Svane (2011) went on to suggest that native mussels in South Australia might have died out as a consequence of post-Ice Age climate change, only to be replaced by what we would now consider to be invasive *M. galloprovincialis* from Europe. However, as noted above, the results of several studies do not support this contention because all report a mix of what appears to be native and what are identified as invasive mussels. More recently, Pickett & David (2018) who analysed global records of COI variation noted that Tasmanian mussels did not share haplotypes with any other population and were even genetically isolated from nearby South Australian and New Zealand individuals (refer to Figure 31). Overall, these results are difficult to interpret, but strongly suggest that native blue mussels in Australia were originally only found in the southeast of the country, in particular in the island state of Tasmania. Today's disjunct distribution of blue mussels (if that is, in fact, what it is), in the southeast and the southwest of the country may reflect native (southeast) and introduced (southwest) mussels.

Offshore islands

Offshore islands in the Southern Ocean are usually characterised by high levels of endemism. However, this is not always the case for marine species capable of dispersal over large geographic distances. So the question of island endemism for mussels of the genus *Mytilus* remains unresolved, despite the fact that mussels have been reported from many, but not all, remote Southern Ocean islands, including some near to Antarctica. Whereas offshore islands such as the Falklands Islands (52°S, 58°W) have reasonably clear affiliations both in terms of geographic distance and biogeography with the neighbouring continent of South America (in particular with the Atlantic coast of Argentina), the situation is much less clear for the more remote Kerguelen Islands (49°S, 70°E) in the South Indian Ocean. To the best of our knowledge, there is no information about native blue mussels from any other island or island groups in the Southern Ocean.

Mussels from the Kerguelen Islands were described as belonging to the endemic species *Mytilus desolationis* by Lamy in 1936 and then *M. kerguelensis* by Fletcher in 1938 (if they are an endemic species, then Lamy's nomenclature will have priority). As discussed previously, the comparison of allozyme variation of the native Kerguelen mussels with *M. edulis* and *M. galloprovincialis* from the Northern hemisphere (Blot et al. 1988) revealed that all alleles found in Kerguelen Islands populations were also observed in Northern hemisphere *Mytilus* taxa, and the only real difference was in the number of rare alleles. No unique alleles were found in Kerguelen Island mussels. According to Blot et al. (1988, p. 246), these results indicated that *M. desolationis* is not a separate taxon, but rather a “semi-species in the super-species *M. edulis*”. These allozyme results contrasted with the earlier description of karyotypic differences among *M. desolationis*, *M. edulis* and *M. galloprovincialis* (Thiriot-Quiévreux 1984). Later, McDonald et al. (1991) reported that mussels from the Kerguelen Islands and the Falkland Islands were not different from *Mytilus* populations in Chile and Argentina based on allozyme variation, and referred to them being *M. edulis*-like. These mussels were morphologically intermediate between Northern hemisphere *M. edulis* and *M. trossulus*, but they contained alleles characteristic of all three Northern hemisphere species but with the greatest similarity to *M. edulis* from the Northern hemisphere.

Analysis of 16S sequence variation of Kerguelen Island and Falkland Island mussels in comparison to Northern hemisphere *Mytilus* populations was inconclusive and indicative of the necessity of using markers with much higher resolution power (Hilbish et al. 2000). In contrast, analysis of COI sequence variation revealed that Kerguelen Islands mussels grouped with South American populations and created one subclade (Gérard et al. 2008). Analysis of what was called native *M. edulis* from the Kerguelen Islands by Borsa et al. (2007) revealed the presence of both *M. edulis* (at the *mac-1* locus) and *M. galloprovincialis* (at the *Glu-5′/Glu-3′* locus) alleles, indicating a possible hybrid origin of these mussels (i.e., reticulate evolution). The taxonomy of mussels from islands groups such as the Falkland Islands and, in particular, the Kerguelen Islands, has therefore been tangled and represents both a major challenge and a major opportunity to better understand Southern hemisphere phylogeography and also, possibly, hybrid speciation.

SNP markers applied to Southern hemisphere mussels – phylogeography, taxonomy and cryptic species

Single-nucleotide polymorphisms

SNPs are, as the name suggests, single base pair changes in a length of DNA (see reviews by Davey et al. 2011, DeFaveri et al. 2013, da Fonseca et al. 2016). Several different sets of SNPs have now been developed for use with *Mytilus* spp. (e.g., Zbawicka et al. 2012, Saarman & Pogson 2015, Araneda et al. 2016, Fraïsse et al. 2016, Mathiesen et al. 2017, Wilson et al. 2018, Popovic et al. 2020) and the number of papers describing results from SNP analyses, both in the Northern and Southern hemispheres, is increasing rapidly. SNPs are, most usually, nuclear DNA co-dominant variants, and as such may be very informative in terms of genetic variation, population genetic structure, gene flow (connectivity) and evolutionary processes such as hybridisation and introgression (Wenne et al. 2020), whereas the non-neutral SNPs may be informative about adaptation to environmental variation. In particular, SNPs are appropriate markers for the identification and analysis of genetic relationship between closely related species (Daïnou et al. 2016 and references therein). The application of SNPs to resolve taxonomic and biogeographic uncertainty within the *Mytilus edulis* species complex may well overcome the low genomic representativeness of markers such as allozymes, RFLPs and even DNA sequencing.

Chile

SNP markers have been applied to nine populations of mussels from Chile, spanning almost the entire natural distribution of ~2,500 km (Larraín et al. 2018). Consistent with the approach employed in other studies (Gardner et al. 2016, Zbawicka et al. 2018, 2019, 2021), a set of reference Northern hemisphere mussels (*M. trossulus* from the Pacific coast of Canada, *M. edulis* from the United Kingdom (Northern Ireland), *M. galloprovincialis* from Italy and Spain) and Southern hemisphere mussels (*M. galloprovincialis*-like from New Zealand) was also tested. In total, 338 mussels were assayed for variation at 49 informative SNP loci. Both neighbour-joining

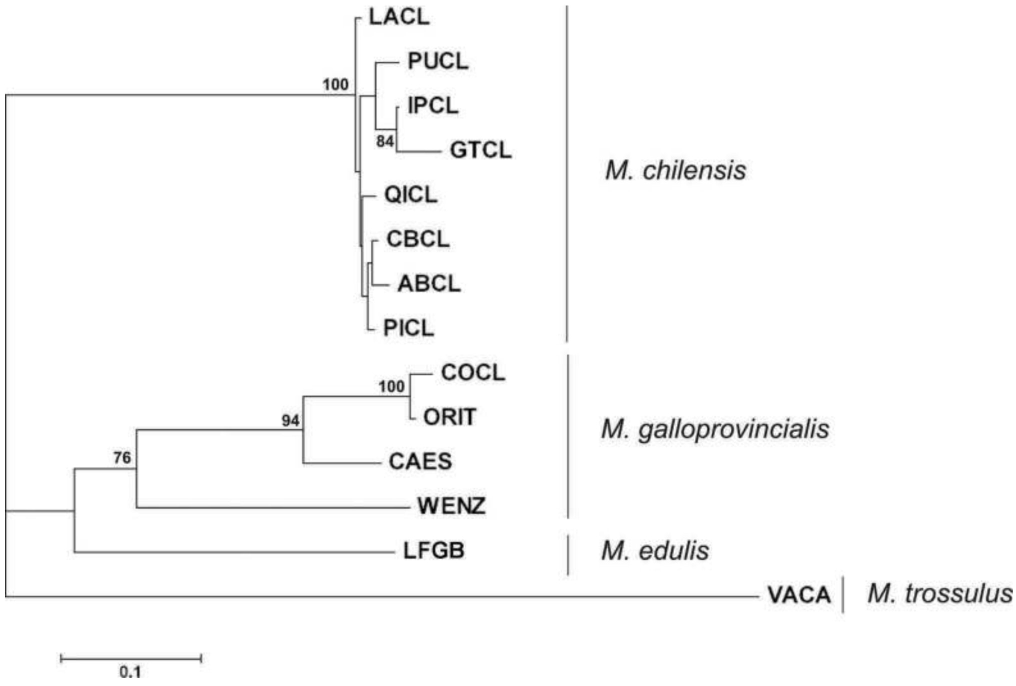

Figure 15 Neighbour-joining tree of *Mytilus* populations based on F_{ST} distance matrix for the analysis of nine Chilean populations using SNP markers. Taken from Larraín et al. (2018).

tree analysis (Figure 15) and also DAPC (not shown) revealed clear differences among the putative mussel species. The Northern hemisphere reference *M. trossulus* (VACA), *M. edulis* (LF) and the *M. galloprovincialis* (ORIT – Mediterranean Sea lineage, CAES – North Atlantic Ocean lineage) and the Southern hemisphere reference *M. galloprovincialis*-like mussels (WENZ) formed very distinct groupings. The Chilean population of Cocholgue (COCL) displayed clear evidence of being recently introduced Northern hemisphere *M. galloprovincialis*, most likely from the Mediterranean Sea. The eight "pure" Chilean populations formed a distinct group with 100% bootstrap support, which Larraín et al. (2018) identified as being native *M. chilensis*. Similarly, clear-cut results were reported for other analysis methods, including DAPC, assignment testing and Structure (Figure 16).

Depending on the criterion used (the value of minor allele frequency, MAF, ranging from >0.1 to > 0.4) as few as three SNP loci could be employed to accurately assign random individual mussels to their correct species designation.

Consistent with a range of reports from different workers employing different approaches, Larraín et al. (2018) concluded that their SNP markers provided unequivocal evidence of the differentiation of native Chilean mussels from all other reference mussels. That is, that the native mussel of the Pacific coast of South America is indeed *M. chilensis*, consistent with the designation provided by Hupé in 1854. Consistent with their application elsewhere, e.g., New Zealand (Gardner et al. 2016) and more recently Argentina (Zbawicka et al. 2018), Southern Ocean islands (Zbawicka et al. 2019) and also Australia (Zbawicka et al. 2021), the SNP panel quite clearly differentiates the native reference Northern hemisphere species (and the two *M. galloprovincialis* lineages), it identifies known cases of invasion (e.g., the Northern hemisphere *M. galloprovincialis* on northern Chile), it detects hybrids and backcrosses and it clearly identifies a distinct group of native Chilean mussels that must now be recognised as a separate species, *M. chilensis*.

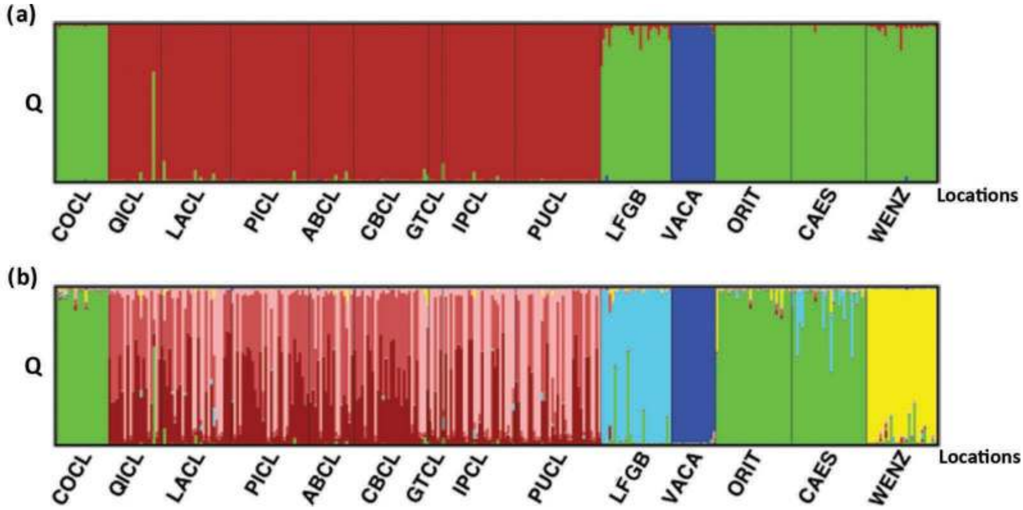

Figure 16 Structure plot showing proportional membership (Q) of each of three (panel a) and seven (panel b) clusters inferred from the analysis of nine Chilean populations using SNP markers. Population codes – COCL (Cocholgue, Chile), QICL to PUCL (Chile), LFGB (Lough Fyne, Northern Ireland, United Kingdom), VACA (Vancouver, Pacific coast of Canada), ORIT and CAES (Italy Mediterranean Sea and Spain Atlantic Ocean, respectively), WENZ (New Zealand). Colour codes for the groups – green is Northern hemisphere *M. galloprovincialis*, red-brown is *M. chilensis*, light blue is Northern hemisphere *M. edulis*, dark blue is Northern hemisphere *M. trossulus*, and yellow is Southern hemisphere *M. galloprovincialis*-like from New Zealand. Taken from Larraín et al. (2018).

Argentina and Uruguay

Fifty-one polymorphic SNP loci have recently been used to examine native smooth-shelled mussels along the Atlantic coast of South America and from Patagonia (Zbawicka et al. 2018). *Mytilus* spp. samples from ten localities in Argentina were analysed alongside reference samples from Northern and Southern hemisphere locations (e.g., Gardner et al. 2016, Larraín et al. 2018, Zbawicka et al. 2019, 2021). Correspondence analysis (CA) revealed clear distinction among five major groupings: reference *M. edulis* and *M. galloprovincialis* from the Northern hemisphere, *M. chilensis* from Chile, *M. galloprovincialis*-like Southern hemisphere mussels from New Zealand and *M. platensis* from the Atlantic coast of Argentina (Figure 17). The Argentinian Atlantic coast population of Puerto Madryn (PMD) sat in the middle of the plot, with no obvious affiliation to any of the five main groupings. Structure analysis (Figure 18) revealed that eight populations from Argentina clustered together (these are interpreted as being *M. platensis*). The sample from Ushuaia (Strait of Magellan) clustered with *M. chilensis* from Chile, consistent with interpretation of other workers (e.g., Oyarzún et al. 2016) of the existence of this species in the far south. All individuals in the Puerto Madryn (PMD) sample were identified as F2 hybrids (*M. platensis*×*M. galloprovincialis*), except one which was classified as invasive Northern hemisphere *M. galloprovincialis*. Zbawicka et al. (2018) suggested that European *M. galloprovincialis* had probably been introduced by ship traffic. Overall, *Mytilus* from Argentina is composed of three groups: native *M. platensis* (Atlantic coast south of Rio de La Plata), native *M. chilensis* in the south (area of Tierra del Fuego, Strait of Magellan) and hybrid individuals (native *M. platensis*×introduced *M. galloprovincialis*) in the vicinity of Puerto Madryn.

Neighbour-joining tree analysis (Figure 19) confirmed the differentiation of *M. trossulus* from all other groups, the known Northern versus Southern hemisphere split within the *M. galloprovincialis*-like mussels, the identity of the reference *M. edulis* and the separation of *M. chilensis* from *M. platensis*.

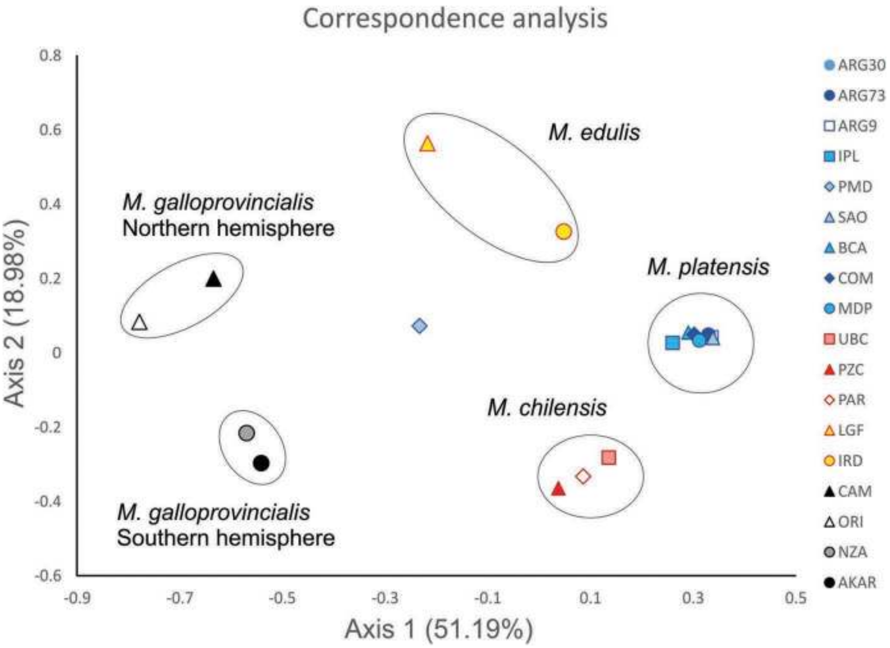

Figure 17 Correspondence analysis plot of mussel populations from the Atlantic coast of South America, with reference Northern hemisphere populations of *M. edulis* and *M. galloprovincialis* plus reference Southern hemisphere populations from Chile (Pacific coast of South America) and New Zealand, both the mainland and the offshore islands (Southern Ocean). Population codes – *M. platensis* ARG30, ARG73, ARG9, IPL, SAO, BCA, COM, MDP; mixed *M. galloprovincialis* and *M. platensis* – PMD; native mussels from Chile, *M. chilensis* – UBC, PZC, PAR; reference Northern hemisphere *M. edulis* – LGF, IRD; reference Northern hemisphere *M. galloprovincialis* – CAM, ORI; native New Zealand mussels, mainland – AKAR, offshore islands – NZA. Taken from Zbawicka et al. (2018).

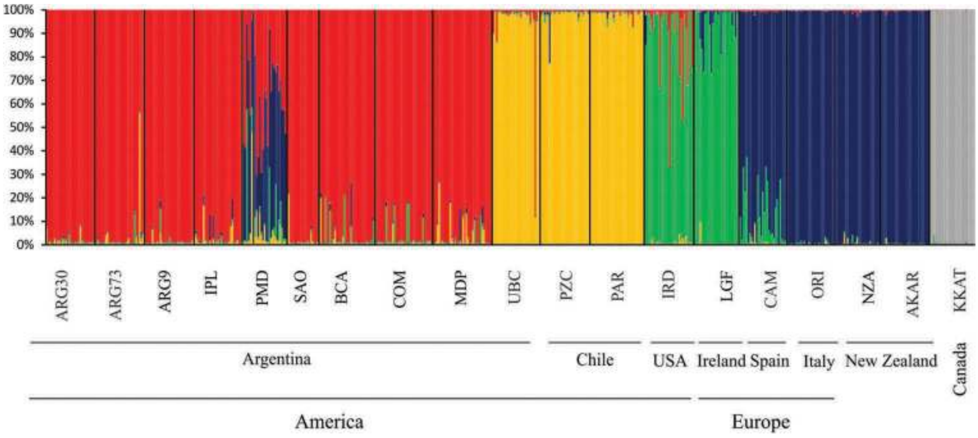

Figure 18 Structure plot ($k=5$) for ten Argentinian mussel populations (ARG30 to UBC), with the inclusion of reference populations. Colour groups: red=native *M. platensis* from Argentina; yellow=native *M. chilensis* from Chile (PZC, PAR); green=native Northern hemisphere *M. edulis* (IRD, LGF); blue=native Northern hemisphere *M. galloprovincialis* (CAM, ORI) plus introduced Northern hemisphere *M. galloprovincialis* in the Argentinian population of PMD and also native Southern hemisphere *M. galloprovincialis*-like mussels from New Zealand (NZA, AKAR); grey=native Northern hemisphere *M. trossulus* (KKAT). Taken from Zbawicka et al. (2018).

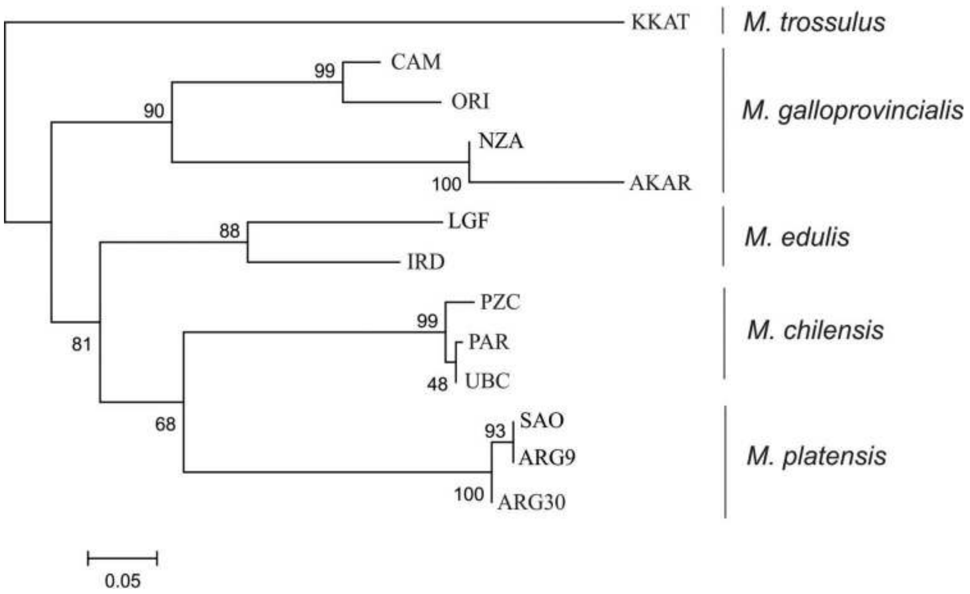

Figure 19 Neighbour-joining tree of *Mytilus* spp. samples from Argentina and reference populations of *M. edulis*, *M. trossulus*, *M. galloprovincialis* and *M. chilensis* from North America, Europe and New Zealand based on F_{ST} values. Tree constructed using individuals without admixture ($q>0.8$ or $q<0.2$) identified by Structure analysis ($K=5$). Taken from Zbawicka et al. (2018).

Overall, this study demonstrates that *M. platensis* and *M. chilensis* are clearly distinguishable native taxa in South America, the former from the Atlantic coast and the latter from the Pacific coast. In total, 19 SNP loci were involved in differentiating between native Argentine *M. platensis* and the mussels of all other regions. Five SNPs were identified as highly informative – BM106B, BM12A, BM151A, BM21B and BM6C – indicating that even relatively small panels of SNPs can be used to differentiate among species.

New Zealand

SNPs were applied to Southern hemisphere blue mussels for the first time from New Zealand (Gardner et al. 2016). This work built on the earlier use of microsatellite markers (Westfall 2010) and the application of the Me15/16 and 16S RFLPs to clarify the status of the native blue mussel and also the presence of invasive Northern hemisphere blue mussels (Westfall & Gardner 2010, Westfall et al. 2013). Mussels from 39 sites (= populations) were collected from throughout mainland New Zealand and also from the offshore islands, and their SNP profiles at 44 loci were compared against reference Northern hemisphere blue mussels (refer also to Larraín et al. 2018, Zbawicka et al. 2018, 2019, 2021). In total, 57 loci were assayed, but 13 were dropped because they were variable only in *M. trossulus* mussels and were therefore uninformative in the Southern hemisphere context.

A range of different analyses revealed clear and consistent differences among individual mussels and populations from different regions, with the New Zealand mussels being differentiated from Northern hemisphere reference taxa (Figure 20). The New Zealand mussels do not form a single homogeneous group, but in fact, three groups are apparent: (1) the remote offshore island mussels collected from the Auckland Islands and Campbell Islands are differentiated from all other mussels, (2) the Chatham Island mussels, all the South Island mussels and a limited number of North Island populations form a separate group and (3) several North Island populations, in particular those from the far north (the Bay of Islands) plus Wellington Harbour, are partially differentiated

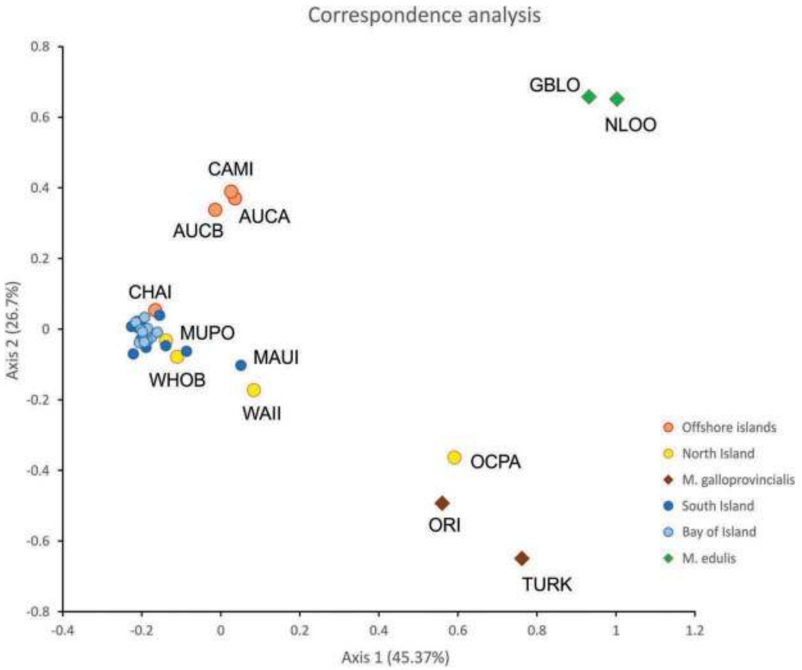

Figure 20 Correspondence analysis plot computed from the SNP data of two reference *Mytilus* spp. taxa (*M. edulis* – GBLO and NLOO – green diamonds; *Mytilus galloprovincialis* – ORI and TURK – dark brown diamonds), with all New Zealand mussel populations (offshore islands=orange circles; North Islands samples=yellow circles; South Island samples=blue circles). Each dot denotes a site sample. Note that in this figure, the reference *M. trossulus* from the Northern hemisphere have not been included because they are very different from all other mussels. OCPA=a moveable oil rig with Northern hemisphere *M. galloprovincialis* as biofouling. Taken from Gardner et al. (2016).

from all other mussels (refer to NJ tree – Figure 21). One sample collected from a moveable oil rig (OCPA) that had recently arrived in New Zealand from South Africa via Australia had biofouling mussels that were identified as Northern hemisphere *M. galloprovincialis*.

These analyses, with or without the inclusion of the reference *M. trossulus*, highlight the considerable differentiation of the New Zealand mussels from all reference mussels. The New Zealand mussels are at least as differentiated from the reference taxa as the three reference taxa are differentiated among themselves. This degree of separation raises questions about the specific status of the native New Zealand mussels. If the three reference Northern hemisphere taxa are now widely recognised as distinct species, then should not also the native New Zealand mussels be recognised as a separate species. And what then is the status of the remote offshore mussels, given that they too form a distinct grouping? While these two questions were not explicitly answered by Gardner et al. (2016), the application of SNPs to mussels from other Southern hemisphere locations helps to develop the picture and draws into focus the uncertain status of the native New Zealand mussels, both on the mainland and on the offshore islands.

The Structure plot (Figure 22) and the NewHybrids analysis both highlight the extent of hybridisation and introgression that is occurring within New Zealand between the native mussels and introduced Northern hemisphere *M. galloprovincialis*. While the majority of mussels (~90%) were identified as being native, ~10% were identified as being non-native or admixed (individuals of mixed ancestry). Nine individuals (~2%) were identified as pure Northern hemisphere *M. galloprovincialis*, and while no F1 hybrids were detected, ~9% of mussels were identified as F2 hybrids, and nine mussels were identified as being backcrosses to native New Zealand mussels.

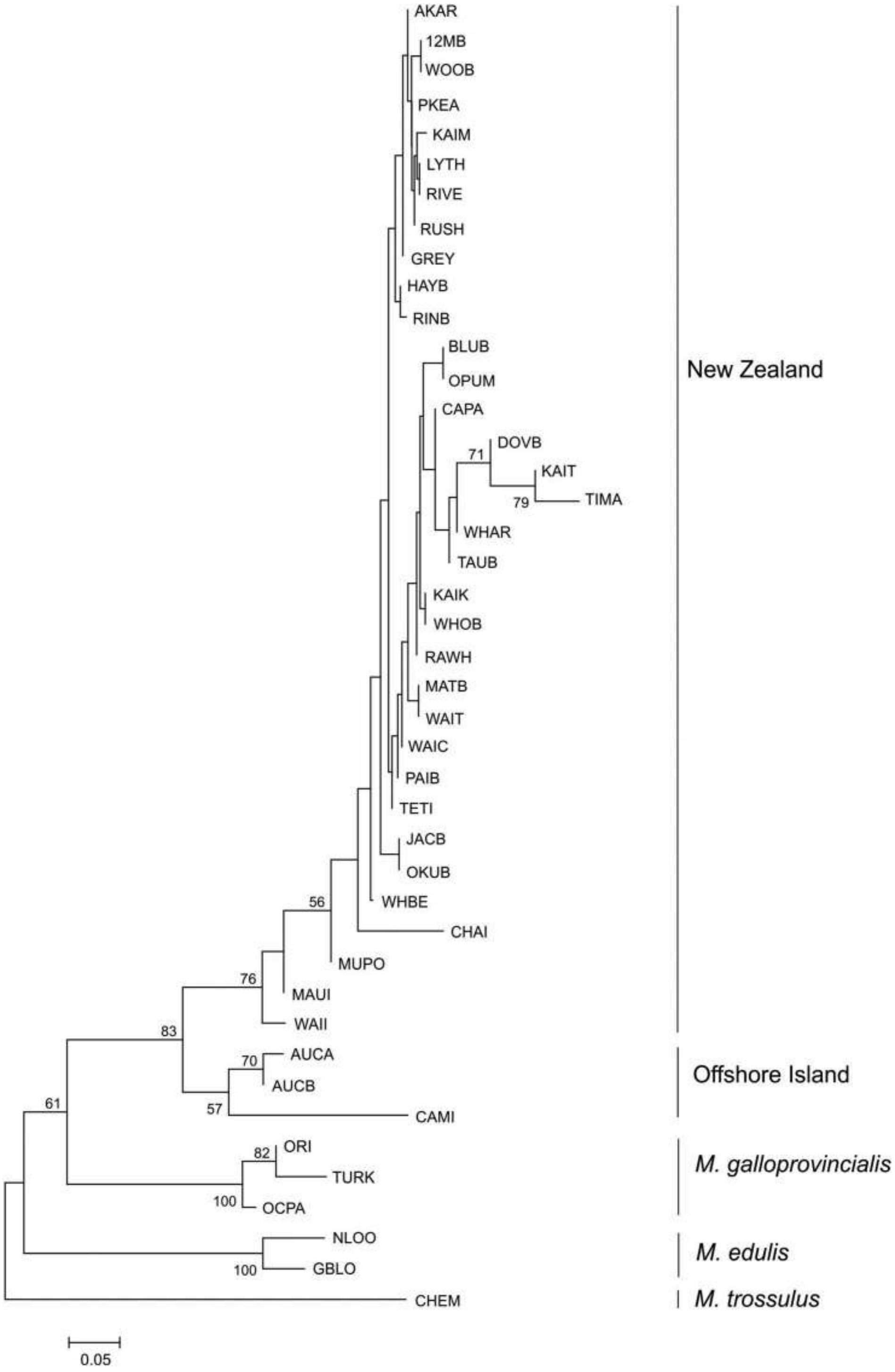

Figure 21 Neighbour-joining tree of 43 *Mytilus* populations from New Zealand and reference samples of *Mytilus edulis*, *Mytilus galloprovincialis* and *Mytilus trossulus* based on the F_{ST} distance matrix from allele frequencies of the SNP loci. Tree shows clear separation of all mainland and offshore New Zealand population samples from the three reference taxa. Taken from Gardner et al. (2016).

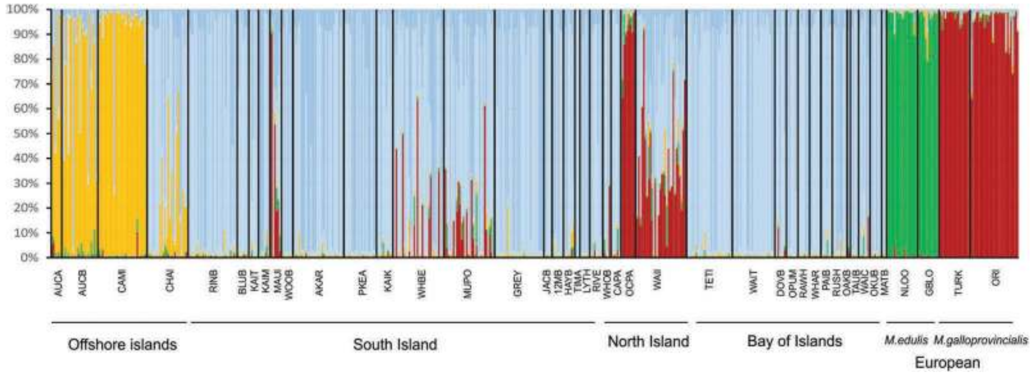

Figure 22 Plot from Structure analysis at $K=5$ showing group affinities of all New Zealand populations (the Southern Ocean offshore islands, South Island, North Island and the Bay of Islands), the two reference *Mytilus edulis* populations and the two reference *Mytilus galloprovincialis* populations. Taken from Gardner et al. (2016).

Interestingly, there was limited evidence of the presence of both *M. trossulus* and *M. edulis* alleles (but not actual mussels) within the populations of the two remote offshore island groups in the Southern Ocean, although an explanation of common ancestry for the SNP alleles in question is probably most likely.

Overall, the SNP analyses of the New Zealand mussels highlighted the considerable separation of the native mussels from the reference Northern hemisphere taxa and also the prevalence of hybridisation and introgression between the native mussels and the introduced Northern hemisphere *M. galloprovincialis*, in particular, in the north of the country, but also in proximity to the major port cities of Auckland, Wellington and Nelson.

Australia

Popovic et al. (2020) applied a panel of 20,509 SNPs to representative samples of Australian mussels from one site in Tasmania and two sites in New South Wales, to test for bioinvasion and to estimate the timing of origin of native Australian blue mussels. They identified the presence of invasive Mediterranean Sea lineage *M. galloprovincialis* in Sydney Harbour and North Atlantic lineage *M. galloprovincialis* at Batemans Bay, and also highlighted the distinct nature of the native Australian mussel, *M. planulatus*, from Tasmania. Unfortunately, this study did not include samples from Australia's west coast. The authors estimated the time of divergence of *M. planulatus* from Northern hemisphere *M. galloprovincialis* to 0.1–0.6 M ybp must likely under a model of historical gene flow followed by divergence in isolation.

SNP testing has been applied to mussel samples from ten localities in Australia, in comparison with reference taxa, to examine native Australian blue mussel species diversity, biogeography and taxonomy (Zbawicka et al. 2021). In total, results were obtained for 53 polymorphic SNPs for samples collected from the states of Western Australia, Victoria, New South Wales, South Australia and Tasmania.

CA revealed that native Australian mussels clustered together and distinct from the reference mussels (Figure 23). In fact, the Australian mussels exhibit the greatest affinity to Northern hemisphere *M. galloprovincialis* from the Mediterranean Sea, and then about equal affinity to Northern hemisphere *M. galloprovincialis* and to what is called Southern hemisphere Northern hemisphere *M. galloprovincialis*-like mussels from mainland New Zealand. Structure analysis (Figure 24) revealed that *Mytilus* in Australia is composed of two groups, native Southern hemisphere *M. galloprovincialis*-like (one sample from Tasmania that showed affinity with the reference

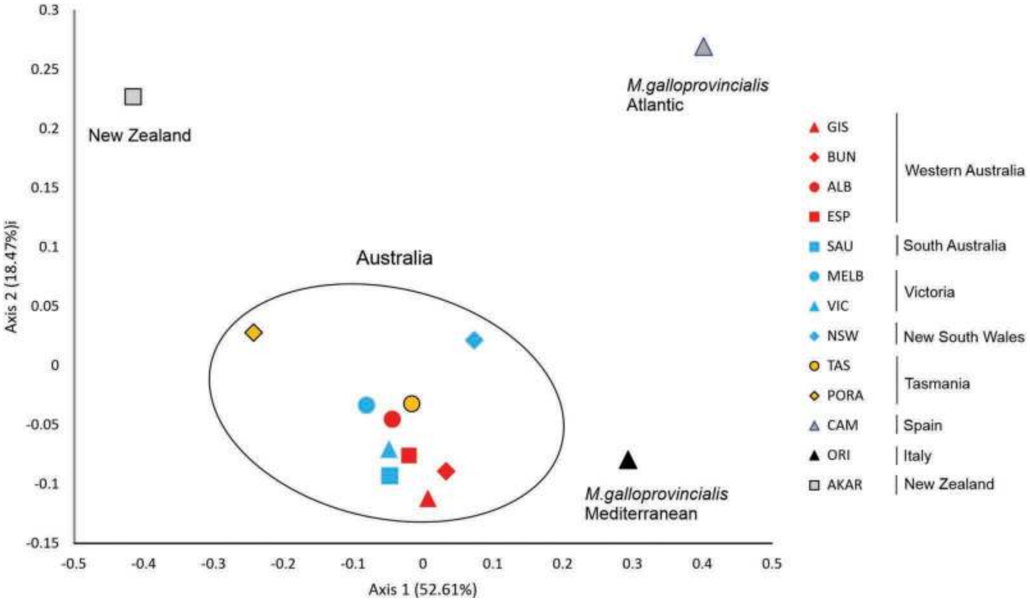

Figure 23 Correspondence analysis plot of mussel populations from mainland Australia and Tasmania, with inclusion of reference populations from the Northern hemisphere and New Zealand. Population codes: GIS = Garden Island, Western Australia; BUN = Bunbury, Western Australia; ALB = Albany, Western Australia; ESP = Esperance, Western Australia; SAU = Port Lincoln, South Australia; MELB = Melbourne, Victoria; VIC = Portarlington, Victoria; NSW = Eden, New South Wales; TAS = Spring Bay, Tasmania; PORA = Port Arthur, Tasmania; CAM = Camarinal, reference Northern hemisphere *M. galloprovincialis* from the Spanish Atlantic coast; ORI = Oristano, reference Northern hemisphere *M. galloprovincialis* from the Mediterranean Sea, Italy; AKAR = Akaroa, New Zealand mainland. Taken from Zbawicka et al. (2021).

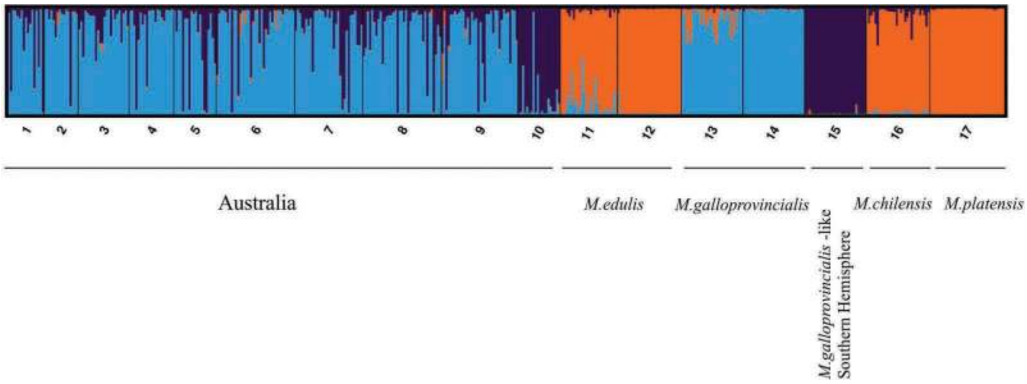

Figure 24 Structure plot ($K=2$) of SNP analysis of Australian mussels. Population codes: 1 = ALB, Albany, Western Australia, 2 = BUN, Bunbury, Western Australia, 3 = ESP, Esperance, Western Australia, 4 = GIS, Garden Island, Western Australia, 5 = MELB, Melbourne, Victoria, 6 = NSW, Eden, New South Wales, 7 = SAU, Port Lincoln, South Australia, 8 = VIC, Portarlington, Victoria, 9 = TAS, Spring Bay, Tasmania, 10 = PORA, Port Arthur, Tasmania, 11 = reference Northern hemisphere *M. edulis* from Lough Foyle, Northern Ireland, UK, 12 = reference Northern hemisphere *M. edulis* from Indian River, Delaware, USA, 13 = reference Northern hemisphere *M. galloprovincialis* from Camarinal, Spain, 14 = reference Northern hemisphere *M. galloprovincialis* from Oristano, Italy, 15 = reference Southern hemisphere mussels from Akaroa, mainland New Zealand, 16 = reference *M. chilensis* from Chiloé, Chile, 17 = reference *M. platensis* from Comodoro, Argentina. Modified from Zbawicka et al. (2021).

native mussels from New Zealand) and hybrid individuals that showed very high levels of admixture in the other nine populations, that is Southern hemisphere *M. galloprovincialis*-like × Northern hemisphere *M. galloprovincialis*. GeneClass2 analysis of hybrid individuals confirmed the admixed status of the nine populations from the Australian mainland. NewHybrid analysis identified most admixed individuals from Australia as F2 hybrids. These SNP results tend to confirm the results of many of the earlier studies which suggest that the native mussels from Tasmania are distinct from mainland populations and that mainland Australian populations have experienced substantial introgression from invasive (introduced) Northern hemisphere *M. galloprovincialis*. The SNP analyses do, however, clearly point to the difference of the native Australian mussels from reference Northern hemisphere taxa.

Offshore islands

Having investigated native mussels from Chile, Argentina/Uruguay, New Zealand and Australia in the context of reference Northern hemisphere mussels using SNPs (Gardner et al. 2016, Larraín et al. 2018, Zbawicka et al. 2018, 2021), the situation for the remote offshore islands in the Southern Ocean is of particular interest. Zbawicka et al. (2019) examined mussels from the Falkland Islands, the Kerguelen Islands, the Auckland Islands, the Campbell Islands and also Tasmania, and obtained results from 53 polymorphic loci, in the context of SNP variation in reference Northern and Southern hemisphere mussels.

CA of the Chile, Argentina, Falkland Island and Kerguelen Island mussels revealed clear separation of samples based on geography (Figure 25a). Native mussels from the Falkland Islands and the Kerguelen Islands exhibited greater affinity to *M. platensis* from Argentina than to *M. chilensis* from Chile and were clearly separated from all other blue mussel groups of the Northern and Southern hemispheres. The native Tasmanian mussels (*M. planulatus*) clustered with native mainland New Zealand mussels (*M. aoteanus*) in a Southern hemisphere *M. galloprovincialis*-like group, distinct from all other groupings, while the New Zealand Southern Ocean island individuals formed their own cluster distinct from all other groupings, but sitting between the Southern hemisphere *M. galloprovincialis*-like mussels from mainland New Zealand and from Tasmania, and the *M. chilensis* cluster. CA carried out for individuals revealed that mussels from the Kerguelen Islands exhibited overlap with the *M. platensis* and *M. chilensis* individuals, whereas mussels from the Falkland Islands exhibited more overlap with *M. platensis* than with *M. chilensis* (Figure 25b). Finally, CA carried out for only reference *M. chilensis*, *M. platensis* and the mussels from the Falkland Islands and the Kerguelen Islands (these four groups were included to reveal maximum detail – Figure 25c) showed clear separation of the four mussel groups, but with samples from the Falkland Islands and the Kerguelen Islands showing greater affinity to *M. platensis* than to *M. chilensis*.

In the Structure analysis, the individual assignments of mussels from the Falkland Island, Kerguelen Island and Tasmanian samples were often revealed to be influenced by introgression (Figure 26). Most individuals from the Falkland Islands and Kerguelen Islands were assigned to *M. platensis*, while other individuals were considered potentially admixed (*M. chilensis* × *M. platensis*). In total, 94% of individuals were correctly assigned to their location of origin using GeneClass2, indicating the extent of genetic differentiation that exists among them. Potentially admixed individuals from the Falkland Islands and the Kerguelen Islands were assigned to their original location or to the Argentinian sample (*M. platensis*). These results point to a different evolutionary history for New Zealand Southern Ocean island plus Tasmanian mussels compared to the South American (Atlantic and Pacific coasts), Falkland Island plus Kerguelen Island mussels, consistent with earlier suggestions of two separate migration events from the Northern hemisphere into the Southern hemisphere (Hilbish et al. 2000; Gérard et al. 2008). Overall, these results reveal that Southern hemisphere island mussels have mixed genome ancestry and are native, not introduced by human activities. Zbawicka et al. (2019) suggested that the offshore island groups should be treated as separate

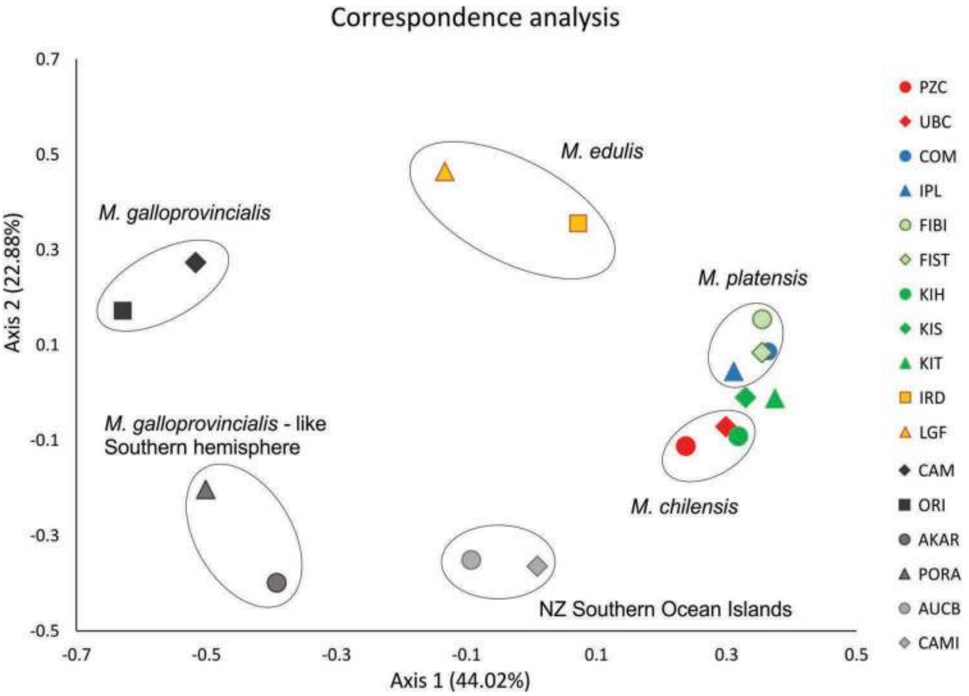

Figure 25a Correspondence analysis plot of Southern hemisphere island populations of native blue mussels, with inclusion of reference taxa populations from the Northern hemisphere. Population codes – Reference Northern hemisphere *M. edulis* – IRD=Indian River, Delaware, USA, Atlantic coast; LGF=Lough Foyle, United Kingdom, Atlantic Ocean; Reference Northern hemisphere *M. galloprovincialis* – CAM=Camarinal, Spain, Atlantic Ocean; ORI=Oristano, Italy, Mediterranean Sea; *M. chilensis* – PZC=Chiloé Island, Chile, Pacific Ocean; UBC=Ushuaia, southern Argentina, Strait of Magellan; *M. platensis* – MYBI – Bense Island, Falkland Islands, Atlantic Ocean; MYST – Port Stanley Wharf, Falkland Islands, Atlantic Ocean; KIH – Henri Bossiere Fjord, Kerguelen Islands, Indian Ocean; KIS – Ile Suhm, Kerguelen Islands, Indian Ocean; KIT – Ilot des Trois Bergers, Kerguelen Islands, Indian Ocean; Southern hemisphere *M. galloprovincialis* – PORA=Port Arthur, Tasmania, Australia, Pacific Ocean; AKAR=Akaroa, New Zealand, Pacific Ocean; Offshore islands – AUCB=Auckland Islands, New Zealand, Southern Ocean; CAMI=Campbell Islands, New Zealand, Southern Ocean; Note – in this figure there are no reference Northern hemisphere *M. trossulus* because all Southern hemisphere mussels were not found to be similar enough to them. Taken from Zbawicka et al. (2019).

evolutionary significant units for the purposes of protecting *Mytilus* taxa of the Southern hemisphere. The invasive Northern hemisphere *M. galloprovincialis* was identified only in Tasmania, among native mussels of a distinct Australian lineage. The preservation of distinct evolutionary lineages (or Southern hemisphere species) needs to be an ongoing focus of conservation efforts, given that population sizes on some of the remote offshore oceanic islands will be small and may be more easily adversely affected by invasion and subsequent hybridisation and introgression than larger populations elsewhere (Gardner et al. 2016).

In conclusion, the application of SNP markers to Southern hemisphere mussels has, as expected, provided much greater detail to the elaboration of smooth-shelled blue mussel diversity. While the earliest study of New Zealand mussels (Gardner et al. 2016) may not have been able to explicitly address the question of taxonomic rank for mainland or for offshore island mussels because of a lack of immediate context, subsequent studies (e.g., Larraín et al. 2018, Zbawicka et al. 2018, 2019, 2021) using both the same reference Northern hemisphere taxa and also some of the same Southern mussels as reference have provided much more context and greater certainty around taxonomic

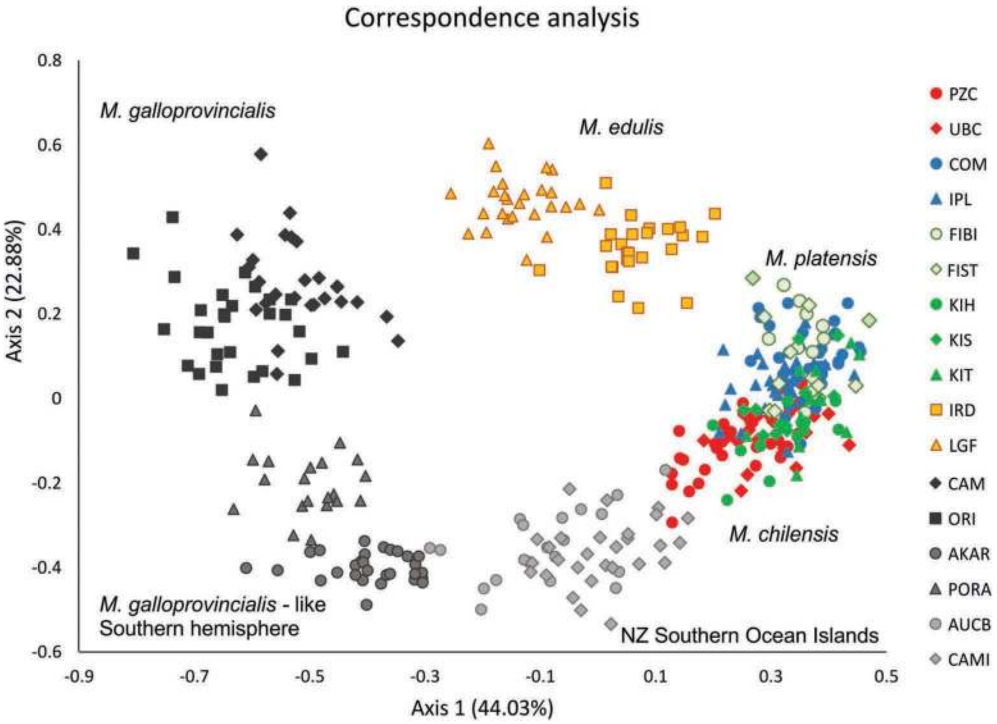

Figure 25b Correspondence analysis plot of Southern hemisphere island individuals of native blue mussels, with inclusion of reference taxa from the Northern hemisphere. Population codes – Reference Northern hemisphere *M. edulis* – IRD=Indian River, Delaware, USA, Atlantic coast; LGF=Lough Foyle, United Kingdom, Atlantic Ocean; Reference Northern hemisphere *M. galloprovincialis* – CAM=Camarinal, Spain, Atlantic Ocean; ORI=Oristano, Italy, Mediterranean Sea; *M. chilensis* – PZC=Chiloé Island, Chile, Pacific Ocean; UBC=Ushuaia, southern Argentina, Strait of Magellan; *M. platensis* – MYBI – Bense Island, Falkland Islands, Atlantic Ocean; MYST – Port Stanley Wharf, Falkland Islands, Atlantic Ocean; KIH – Henri Bossiere Fjord, Kerguelen Islands, Indian Ocean; KIS – Ile Suhm, Kerguelen Islands, Indian Ocean; KIT – Ilot des Trois Bergers, Kerguelen Islands, Indian Ocean; Southern hemisphere *M. galloprovincialis* – PORA=Port Arthur, Tasmania, Australia, Pacific Ocean; AKAR=Akaroa, New Zealand, Pacific Ocean; Offshore islands – AUCB=Auckland Islands, New Zealand, Southern Ocean; CAMI=Campbell Islands, New Zealand, Southern Ocean; Note – in this figure there are no reference Northern hemisphere *M. trossulus* because all Southern hemisphere were not found to be similar enough to them. Taken from Zbawicka et al. (2019).

distinctiveness, as well as hybridisation and introgression, and the presence of non-native taxa. The recent study of Popovic et al. (2020) has also highlighted the distinctness of native Australian mussels and shed light on the likely timing and mechanism of their origin.

How many Southern hemisphere mussel species are there?

Given the vast expanse of the Southern Ocean and the remoteness of many of its landmasses and small islands, it is not surprising that the marine biogeography of the Southern hemisphere is not as well understood as that of the Northern hemisphere. For example, a recent biogeographic study of Antarctic and sub-Antarctic intertidal communities reveals that species accumulation curves for the 11 best sampled regions are not even close to reaching an asymptote (Griffiths & Waller 2016). That is, there are many more new species (across all taxa and phyla) yet to be described.

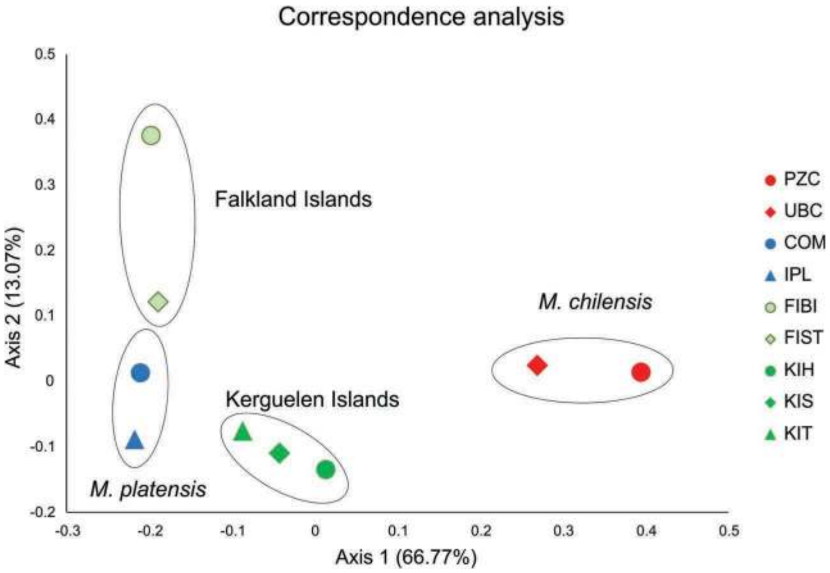

Figure 25c Correspondence analysis plot of Southern hemisphere island populations of native blue mussels from the Falkland Islands (Atlantic Ocean) and the Kerguelen Islands (Indian Ocean), with reference to native *M. chilensis* and *M. platensis* from South America. Population codes: *M. chilensis* – PZC=Chiloé Island, Chile, Pacific Ocean; UBC=Ushuaia, southern Argentina, Strait of Magellan; *M. platensis* – COM=Comodoro Rivadavia, Argentina, Atlantic Ocean; IPL=Isla de los Pajaros, Argentian, Atlantic Ocean; Falkland Islands – MYBI – Bense Island, Falkland Islands, Atlantic Ocean; MYST – Port Stanley Wharf, Falkland Islands, Atlantic Ocean; Kerguelen Islands – KIH – Henri Bossiere Fjord, Kerguelen Islands, Indian Ocean; KIS – Ile Suhm, Kerguelen Islands, Indian Ocean; KIT – Ilot des Trois Bergers, Kerguelen Islands, Indian Ocean. Taken from Zbawicka et al. (2019).

Held (2014) has noted the prevalence of cryptic marine species in the Southern hemisphere and how frequently such cryptic species may be recognised when molecular techniques are employed (e.g., Held 2003, Held & Wägele 2005, Janosik & Halanych 2010, González-Wevar et al. 2019). The general problem of crypticity in biological invasions has been reviewed by Jarić et al. (2019). As noted by Held (2014), even before the advent of molecular techniques, cryptic species were reported from the Southern Ocean using morphological evidence, and of course, this is as true of members of the genus *Mytilus* in the Southern hemisphere (e.g., Lamy 1936, Soot-Ryen 1955, Powell 1958) as it is for many other taxa. In fact, it has been suggested that the identification of cryptic species is now one of the main contributors to the discovery of new species in the Southern Ocean and elsewhere (Bickford et al. 2007). While such newly discovered and described species are an important contribution to our understanding of biogeographic patterns and evolutionary processes such as speciation, and perhaps also hybridisation and introgression, new taxonomies may have implications that extend far beyond taxonomy and systematics (Held 2014), reaching, for example, into fields such as conservation, biodiversity protection, aquaculture and food labelling, and biosecurity (e.g., Gardner et al. 2016, Larraín et al. 2018, Stanton et al. 2019, Zbawicka et al. 2021).

Three Northern hemisphere species are now universally recognised, *M. edulis*, *M. galloprovincialis* and *M. trossulus*. Based largely but not exclusively on the new SNP data, and applying the concept of evolutionary different lineages that maintain some form of genetic distinctness (identity) in the face of hybridisation and introgression as is now widely applied by many different workers, we suggest the following for blue mussels in the Southern hemisphere: *Mytilus chilensis* on the Pacific coast of South America, *M. platensis* on the Atlantic coast of South America, and *M. planulatus* from Australia should be recognised as three distinct species. The native mussels from the Kerguelen

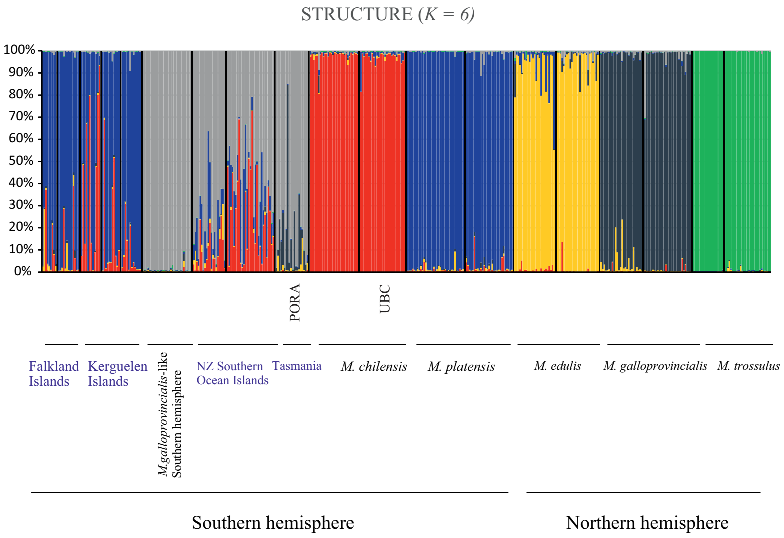

Figure 26 Structure plot (K=6) for 13 native Southern hemisphere and six reference Northern hemisphere mussel populations. Population codes – reference Northern hemisphere *M. edulis* – IRD=Indian River, Delaware, USA, Atlantic coast; LGF=Lough Foyle, United Kingdom, Atlantic Ocean; reference Northern hemisphere *M. galloprovincialis* - CAM=Camarinal, Spain, Atlantic Ocean; ORI=Oristano, Italy, Mediterranean Sea; *M. chilensis* – PZC=Chiloé Island, Chile, Pacific Ocean; UBC=Ushuaia, southern Argentina, Strait of Magellan; *M. platensis* – MYBI – Bense Island, Falkland Islands, Atlantic Ocean; MYST – Port Stanley Wharf, Falkland Islands, Atlantic Ocean; KIH – Henri Bossiere Fjord, Kerguelen Islands, Indian Ocean; KIS – Ile Suhm, Kerguelen Islands, Indian Ocean; KIT – Ilot des Trois Bergers, Kerguelen Islands, Indian Ocean; Southern hemisphere *M. galloprovincialis* – PORA=Port Arthur, Tasmania, Australia, Pacific Ocean; AKAR=Akaroa, mainland New Zealand, Pacific Ocean; Offshore islands – AUCB=Auckland Islands, New Zealand, Southern Ocean; CAMI=Campbell Islands, New Zealand, Southern Ocean. Taken from Zbawicka et al. (2019).

Islands and the Falkland Islands appear to have some sort of hybrid origin (they appear to be derived from reticulate evolution, e.g., Borsa et al. 2007, Zbawicka et al. 2019), are clearly different from populations of the geographically nearest land masses and appear to maintain separate genetic identities. As such, mussels from the Kerguelen Islands and from the Falkland Islands may now warrant specific status as *M. desolationus* Lamy, 1936. Mussels from the New Zealand subantarctic islands also show evidence of a hybrid origin, but it is different from that shown by the Falkland and the Kerguelen mussels (Zbawicka et al. 2019). The New Zealand offshore island mussels have previously been accepted as being the same as mussels from mainland New Zealand – that is, there has been, as far as we are aware, no separate binomial or trinomial applied to the New Zealand mainland versus the offshore island mussels. As such, the island mussels may warrant a new specific name, different from that applied to mussels that are native to mainland New Zealand and the Chatham Islands to the east. We note that Powell (1958) applied the binomial *M. aoteanus* to mussels collected from Wellington Harbour (type locality) as well as the Campbell Islands. Following the principle of avoiding taxonomic confusion (McDonald & Koehn 1988) we suggest that all New Zealand blue mussels (i.e., mainland plus offshore islands) are recognised as *M. aoteanus*. Future work may reveal that the hybrid origin of the offshore mussels requires taxonomic revision. Under this scenario, there are as many as six species of native Southern hemisphere mussels now to be recognised. We acknowledge that this will need further work and confirmation, and that not everyone will agree with this interpretation, but the data from multiple independent studies on which this conclusion is based are very strong.

The recent application of SNP markers to Southern hemisphere mussels has been enlightening. Despite the relatively recent evolutionary origin of all native Southern hemisphere mussels from their Northern counterparts, there are more clearly recognisable Southern hemisphere lineages that maintain their evolutionary identities despite hybridisation and the potential of natural gene flow among them than are recorded in the Northern hemisphere. The most likely explanation for this is not solely to do with evolutionary time, but is also a function of geographic distance. That is, building on a large body of research from many different workers, we suggest that *M. edulis* newly arrived from the Northern hemisphere settled somewhere on the modern-day coastline of Uruguay and/or Argentina and due to a relative absence of gene flow with their Northern hemisphere counterparts gave rise, via genetic drift and mutation, to *M. platensis*. This is the first (oldest) range expansion described by Hilbish et al. (2000). Either the original *M. edulis* or the more newly formed *M. platensis* then spread from the Atlantic to the Pacific coastline of South America, via the Strait of Magellan. The separation between the Pacific and Atlantic coasts, in conjunction with changes in global sea level and regional ice coverage, subsequently gave rise to *M. chilensis* in southern South America (the Strait of Magellan in Argentina and Chile) and along the Pacific coastline. The Atlantic *M. platensis*, with a small input from *M. chilensis* in southern South America, then gave rise to mussel populations on the Falkland Islands and the Kerguelen Islands. Subsequently, and consistent with previous suggestions (e.g., Hilbish et al. 2000, Gérard et al. 2008), there was a second independent invasion event from the Northern hemisphere involving *M. galloprovincialis* that gave rise to *M. planulatus* in Australia (e.g., Popovic et al. 2020) and, we speculate, also to *M. aoteanus* in New Zealand (the modern-day remote offshore Auckland Islands and Campbell Islands populations arose via reticulate evolution after the colonisation of the New Zealand mainland). The similarity of native Australasian mussels to Northern hemisphere *M. galloprovincialis*, and their difference to native South American mussels, as reported by multiple different studies over the years, provides strong supporting evidence for the original hypothesis proposed by Hilbish et al. (2000) of two independent invasion events from the Northern hemisphere.

Distances between the South American and the Australasian landmasses are sufficiently large (1000s of km), and the pelagic larval duration (PLD) of blue mussels is sufficiently short (typically four to five weeks) that gene flow between these regions was rare at best, but could have occurred infrequently via rafting and island hopping. This sort of pattern of island hopping has been described for other Southern Ocean species, including those that are strictly benthic and lacking a pelagic larval stage (Leese et al. 2010), and illustrates the impact and biodiversity importance of what has been called "founder takes all" (Waters et al. 2013). This genetic isolation across the Southern hemisphere allowed for the build-up of regional-specific genetic lineages that in time became new species (*sensu* Held 2014). This model of allopatric speciation for up to four species, in many ways, mirrors the model of allopatric speciation noted for the three North Atlantic species, after the loss of the connection with the North Pacific approx. 3.5 M ybp (Vermeij 1991), while the model of reticulate evolution (speciation by hybridisation) for up to two species on remote Southern Ocean islands is novel.

The role of hybridisation in speciation

It is frequently reported that wherever two or more smooth-shelled *Mytilus* species occur in sympatry they hybridise. The extent of this hybridisation, and any introgression and backcrossing that may follow, varies from region to region, for reasons that are not always understood (e.g., Rawson et al. 1999, Borsa et al. 2007, Brannock et al. 2009, Brannock & Hilbish 2010). Hybridisation as a process is potentially important in the evolution of new species, at least in part because it is thought to generate new genetic combinations (Darwin 1868, Harrison 1990, Mallet 2007). While hybrid unfitness may, in some cases, act against the formation of hybrid individuals (hybridisation often results in co-adapted gene complexes being broken up), in other cases the beneficial combination of genes from two different species may result in viable offspring and a new evolutionary lineage. Hybridisation in the sea is a reasonably common phenomenon across many marine phyla (Gardner 1997), and hybridisation within the genus *Mytilus* has been reported from many regions of the world wherever two or more species, native or introduced, co-exist (Skibinski et al. 1978, Skibinski 1983, Gardner & Skibinski 1988, Väinölä & Hvilsom 1991, Gardner 1994, 1997, Suchanek et al. 1997, Comesaña 1999, Rawson et al. 1999, Daguin et al. 2001, Gardner & Thompson 2001, 2009, Skurikhina et al. 2001, Penney et al. 2002, 2007, 2008, Toro et al. 2002, 2004a,b, 2006, 2012, Bierne et al. 2003, Gilg & Hilbish 2004, Riginos & Cunningham 2005, Braby & Somero 2006, Beaumont et al. 2008, Elliot et al. 2008, Shields et al. 2010, Oyarzún et al. 2013, 2016, Westfall & Gardner 2013, Roux et al. 2014, Zbawicka et al. 2014, 2018, Saarman & Pogson 2015, Ab Rahim et al. 2016, Wenne et al. 2016, to name but a few). The reason for the frequency of blue mussel hybridisation is likely to be the high degree of genetic similarity between pairs of species (or lineages) that results because of the young evolutionary age of the group. That is, the split since the most common recent ancestor (~3.5 M ybp) which is thought to be *M. trossulus* from the North Pacific Ocean (Riginos & Cunningham 2005) has not yet given rise to large and disruptive species-specific differences (i.e., non-compatible genomic combinations) that prevent hybridisation via pre- or post-fertilisation mechanisms. This ongoing divergence of the different lineages (sometimes defined as incipient speciation – e.g., Lawniczak et al. 2010, Andrew & Rieseberg 2013) allows for hybridisation and in many cases also introgression between pairs of species.

The role of reticulate evolution (i.e., introgressive hybridisation) as a mechanism promoting speciation (divergence) in the sea has been questioned given the often large body of evidence of extensive gene flow for many marine species (reviewed by Arnold & Fogarty 2009). Given the prevalence of hybridisation and introgression between pairs of species within the *Mytilus edulis* species complex, it is of interest to know just how important reticulate evolution may be for blue mussels (e.g., Borsa et al. 2007, Zbawicka et al. 2019), and it is also of interest to understand which parts of the genome are most actively involved. Several studies have described the karyotypes of different blue mussel taxa (the diploid number = 28) using a range of different approaches (e.g., Thiriot-Quiévreux 1984, 2002, Dixon & Flavell 1986, Martínez-Lage et al. 1995, 1996, Pérez-Garcia et al. 2014). The first report of polyploidy within the genus *Mytilus* was published for *M. trossulus* (González-Tizón et al. 2000) and as far as we aware this remains the only such report. It is surprising that little work has focussed on the role that chromosomal rearrangements may play in promoting or retarding interbreeding and/or speciation in blue mussels when so much research in this area has been carried out for other taxa (refer to Searle 1998, Edwards et al. 2016, Mallet et al. 2016, Potter et al. 2017, Fuller et al. 2018 and references therein). Notably, with the exception of the work by Thiriot-Quiévreux (1984), all analyses of *Mytilus* karyotypes have been conducted on taxa from the Northern hemisphere, meaning that we know nothing about the karyotypes of most Southern hemisphere blue mussel lineages/taxa. This is clearly an area that needs immediate attention, in particular given the role that chromosomal rearrangements may play in speciation.

Based on an assessment of allozyme (protein) and nuclear DNA (the *mac-1* locus) variation among mussels from the Kerguelen Islands and Tasmania (Australia) relative to reference Northern hemisphere taxa, Borsa et al. (2007) reported extreme interlocus variation in allelic composition. They interpreted this as evidence for the native origin of these Southern hemisphere mussels and also for past introgressive hybridisation that has given rise to the distinct differences between mussels from Tasmania (the Australasian grouping) and the Kerguelen Islands (the South American grouping). While it remains unclear how these patterns of introgression actually arose, the authors suggested that factors such as the founder effect, small effective population size, pseudo-selection (selection at a locus not specifically studied, but linked to the assayed locus) and partial (incomplete) reproductive isolation may have contributed to the hybrid origin of these present-day Southern hemisphere populations (Borsa et al. 2007).

Based on SNP variation, most regional meta-populations of native *Mytilus* spp. in the Southern hemisphere display clear differentiation from one another and also from reference Northern hemisphere taxa. Mussels of the Falkland Islands (South Atlantic Ocean), the Kerguelen Islands (South Indian Ocean), and the Auckland Islands and the Campbell Islands (South Pacific Ocean) all exhibit some form of intermediate (hybrid) status compared to mussels of the nearest main land masses. For example, mussels of the Falkland Islands have the greatest affinity to mussels from Argentina (*M. platensis*), but clearly exhibit a degree of introgression with mussels from Chile (*M. chilensis*) (Zbawicka et al. 2019). Similarly, mussels from the Kerguelen Islands exhibit the greatest affinity to mussels from Argentina (*M. platensis*), but exhibit a larger degree of introgression with mussels from Chile (*M. chilensis*), a finding which is surprising given the greater proximity of the Falkland Islands than the Kerguelen Islands to South America (Zbawicka et al. 2019). Mussels of the New Zealand Auckland Islands and the Campbell Islands exhibit the greatest affinity to the native mainland New Zealand group (*M. aoteanus*) but with lesser affinity to both *M. platensis* and *M. chilensis* (Zbawicka et al. 2019) (Figure 27).

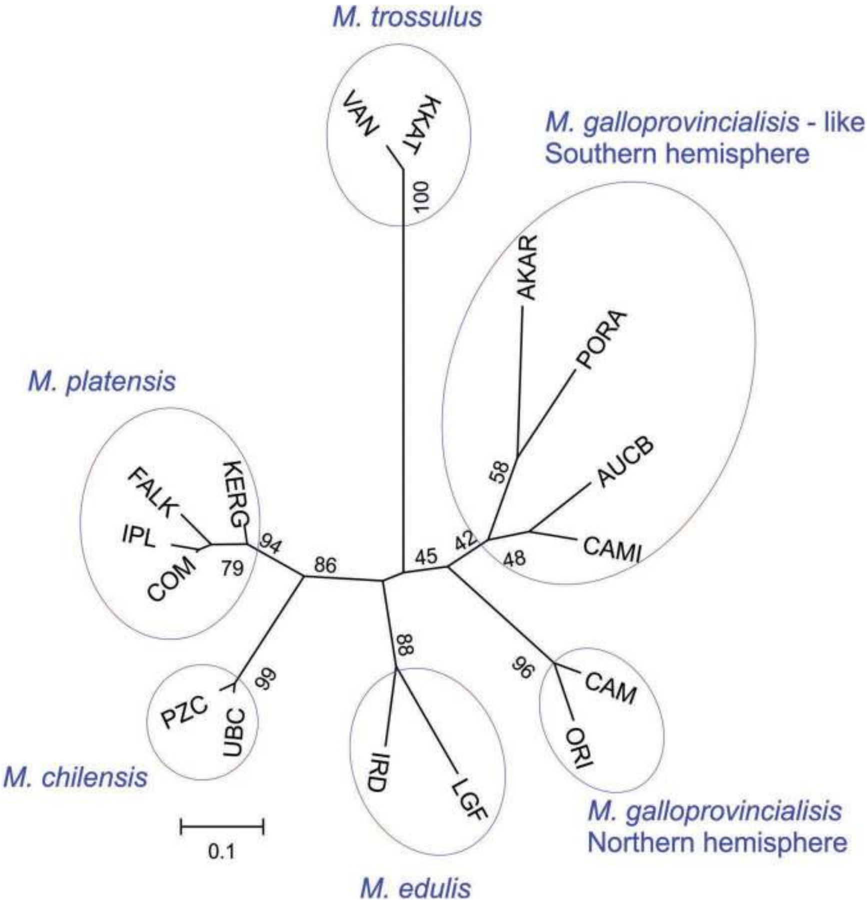

Figure 27 Neighbour-joining phylogenetic tree of SNP variation for Southern hemisphere mussel samples and reference Northern hemisphere samples based on F_{ST} variation. Population codes: *M. platensis* – KERG=Kerguelen Islands, Indian Ocean; FALK=Falkland Islands, Atlantic Ocean; IPL=Isla de los Pajaros, Argentian, Atlantic Ocean; COM=Comodoro Rivadavia, Argentina, Atlantic Ocean; *M. chilensis* – PZC=Chiloé Island, Chile, Pacific Ocean; UBC=Ushuaia, southern Argentina, Strait of Magellan; *M. edulis* – IRD=Indian River, Delaware, USA, Atlantic coast; LGF=Lough Foyle, United Kingdom, Atlantic Ocean; Northern hemisphere *M. galloprovincialis* – CAM=Camarinal, Spain, Atlantic Ocean; ORI=Oristano, Italy, Mediterranean Sea; Southern hemisphere *M. galloprovincialis* – CAMI=Campbell Islands, New Zealand, Southern Ocean; AUCB=Auckland Islands, New Zealand, Southern Ocean; PORA=Port Arthur, Tasmania, Australia, Pacific Ocean; AKAR=Akaroa, New Zealand, Pacific Ocean; *M. trossulus* – KKAT=Halifax, Canada, Atlantic Ocean; VAN=Vancouver, Canada, Pacific Ocean. Taken from Zbawicka et al. (2019).

The SNP analyses identify the existence of unique mussel lineages on the remote offshore islands that span the Southern Ocean in the higher latitudes (Falkland Islands ~ 51°S, Kerguelen Islands ~ 49°S, Auckland Islands ~ 50°S, Campbell Islands ~ 52°S). Levels of introgression for the Falkland Island, Kerguelen Island and Auckland Island plus Campbell Island mussels were estimated to be 23%, 25% and 73%, respectively (Zbawicka et al. 2019), which strongly suggests that hybridisation and the subsequent introgression of the different lineages (*M. chilensis* × *M. platensis* for the Falkland Islands and Kerguelen Islands populations; *M. aoteanus* × *M. chilensis* and also *M. aoteanus* × *M. platensis* for the Auckland Islands and Campbell Islands mussels) are moderately to very common. None of this reported hybridisation and introgression involves introduced Northern hemisphere species, but it all involves native Southern hemisphere species. However, despite this fact, it is not yet apparent whether all Southern hemisphere species arrived at these remote island locations independently (e.g., via rafting as adults or larval dispersal with the anti-clockwise flow of the Southern Ocean – Zbawicka et al. 2019) or whether they benefitted from human-mediated (accidental) transfer. The latter is possible, given both the reasonably long history of sailing ship movement for trade and for whaling around the Southern Ocean and the reasonably frequent strandings or wrecking of ships on these remote islands (Gardner 2004, Gardner et al. 2016 and references therein).

The status of the Kerguelen Island mussels is particularly interesting, and several different studies using a range of different markers have highlighted their difference from reference Northern taxa and/or from other Southern hemisphere mussels (e.g., Thiriot-Quiévreux 1984, Blot et al. 1988, Hilbish et al. 2000, Borsa et al. 2007, Roux et al. 2014, Fraïsse et al. 2018). Lamy (1936) described Kerguelen mussels as a distinct species, *M. desolationis*, based on shell characters alone. Several studies have placed the Kerguelen mussels within or closest to the South American mussel grouping, and distinct from Australasian mussels. This mixed ancestry led Borsa et al. (2007) to suggest that the Kerguelen mussels are a product of reticulate evolution (i.e., they have a hybrid origin), a finding that is strongly supported by the most recent SNP analyses (Zbawicka et al. 2019). We can add to this short list the mussels of the Falkland Islands which show similar mixed ancestry, although this seems to be slightly less complicated than the story for the Kerguelen mussels (Borsa et al. 2007) and also the mussels for the New Zealand offshore islands (Zbawicka et al. 2019).

Ultimately, the role that hybridisation and/or introgression may have played in the speciation process of Southern hemisphere blue mussels remains unclear, but there is now a body of evidence that shows that hybridisation and introgression have occurred and are important, although the timing of these events is unknown. Thus, Southern hemisphere mussels from these remote island groups are presently defined by different lineages that are clearly derived from hybridisation and some extent of introgression. As such, the island groups provide a rare opportunity to examine reticulate evolution among evolutionarily young lineages to better understand speciation in the sea. Whether the mussels of the Falkland Islands, the Kerguelen Islands and the NZ offshore islands are distinct species or not, remains to be seen. This matter may be resolved by the use of species delimitation models, the application of new molecular markers (e.g., Ryu et al. 2012, Quattrini et al. 2017, 2019) or greater depth of coverage of SNPs, perhaps involving hundreds or even thousands of loci (e.g., Smith et al. 2020).

Traces of M. trossulus *in the Southern hemisphere*

The traditional view, based on a range of different analyses, is that *M. trossulus* is the oldest of the three *Mytilus edulis* species complex species and that this species is absent from the Southern hemisphere (e.g., McDonald et al. 1991, Hilbish et al. 2000, Gérard et al. 2008). However, there is a small, but growing body of evidence that points to the existence of *M. trossulus*, or at least *M. trossulus*-like alleles, in mussels of the Southern hemisphere. The first report is from Fernández-Tajes et al. (2011) who noted the presence of two *M. chilensis* × *M. trossulus* hybrids in a can of mussels purchased from a local supermarket in Spain and labelled as *M. galloprovincialis* from Galicia, northern Spain. It is unclear what exactly has happened here, but it very much looks as if mussels from Chile (hence the *M. chilensis* component) have been canned and sold in Spain as *M. galloprovincialis*. The confirmed presence of *M. chilensis*×*M. trossulus* hybrids in the can therefore raises the possibility of *M. trossulus* or *M. trossulus*-like genes being present in Chile. Given that there may be only 10 or 12 mussels in a can, this puts the abundance of *M. chilensis*×*M. trossulus* hybrids at ~16%–20%. This might be as far as the story goes, had it not been for the fact that soon after this event, Larraín et al. (2012), using the Me15/16 RFLP assay, reported the presence of *M. chilensis*×*M. trossulus* hybrids from wild mussel populations in Chile. They reported the hybrids (two individuals in each case) from four separate sites (two sites are mussel collecting centres, two are mussel on-growing centres), three on the Chilean mainland and one on the island of Chiloé, which is a major aquaculture centre. Within any given population, the *M. chilensis*×*M. trossulus* hybrids reached a maximum frequency of 4%, but across all 11 populations, they reached only ~1.5% frequency. Subsequently, Astorga et al. (2015) failed to record *M. trossulus* or its hybrids in their survey of Chilean mussel populations, whereas Oyarzún et al. (2016) noted the presence of *M. trossulus*×*M. galloprovincialis* and also *M. trossulus*×*M. chilensis* hybrids at very low frequencies at two separate sites in the Strait of Magellan region. These records, from a range of different groups, working at different sites and using different molecular markers suggest that *M. trossulus* or at least its alleles may be found at very low frequencies in some locations in southern Chile. Clearly, this topic warrants further attention, but the most simple explanation right now is that the supposed "*trossulus*" alleles are not definitively from *M. trossulus*, but actually represent an ancestral shared state.

At New Zealand's remote offshore islands in the Southern Ocean, Gardner et al. (2016) reported the presence of SNP alleles present in *M. trossulus* and reaching moderately high frequencies at the Auckland Island and the Campbell Island sampling locations. They noted, however, that no hybrids involving *M. trossulus* were observed. They highlighted the difficulty faced by workers when trying to determine the presence of "invasive" alleles from, for example Northern hemisphere *Mytilus* populations, because such alleles may not be invasive at all but may be an ancestral polymorphism that reflects the close evolutionary histories of the species. On balance, Gardner et al. (2016) concluded that there was no evidence of *M. trossulus* or its hybrids in New Zealand. Similarly, no evidence was found of *M. trossulus* or its hybrids in mussel samples collected from the Atlantic coast of South America (Zbawicka et al. 2018), from Southern Ocean islands including the Falkland and Kerguelen islands (Zbawicka et al. 2019), or from mainland Australia or Tasmania (Zbawicka et al. 2021).

In conclusion, the range of analyses carried out to date across the Southern hemisphere points to a limited presence of *M. trossulus* alleles and/or hybrids in the Southern hemisphere. It remains to be determined whether this is a reflection of anthropogenically mediated invasion, whether it reflects a natural and historical range expansion, perhaps from the North Pacific Ocean into the South Pacific Ocean (e.g., Lindberg 1991) or whether it is simply a matter of a shared ancestral polymorphism that has not yet been completely recognised as such. Further work addressing these different points will help to clarify the situation and may well provide new insight into Southern hemisphere phylogeography or invasion dynamics (natural or anthropogenically mediated) from the Northern hemisphere.

Are any more new Mytilus *species likely to be identified in the Southern hemisphere?*

Native blue mussels are widely distributed throughout the Southern Ocean, being present on all major land masses except Antarctica and South Africa (Figure 28). While recording the presence of native blue mussels is easy on major land masses, recording their presence or absence on each of the numerous small, isolated islands of the Southern hemisphere is much harder. Knox (1960, p. 592)

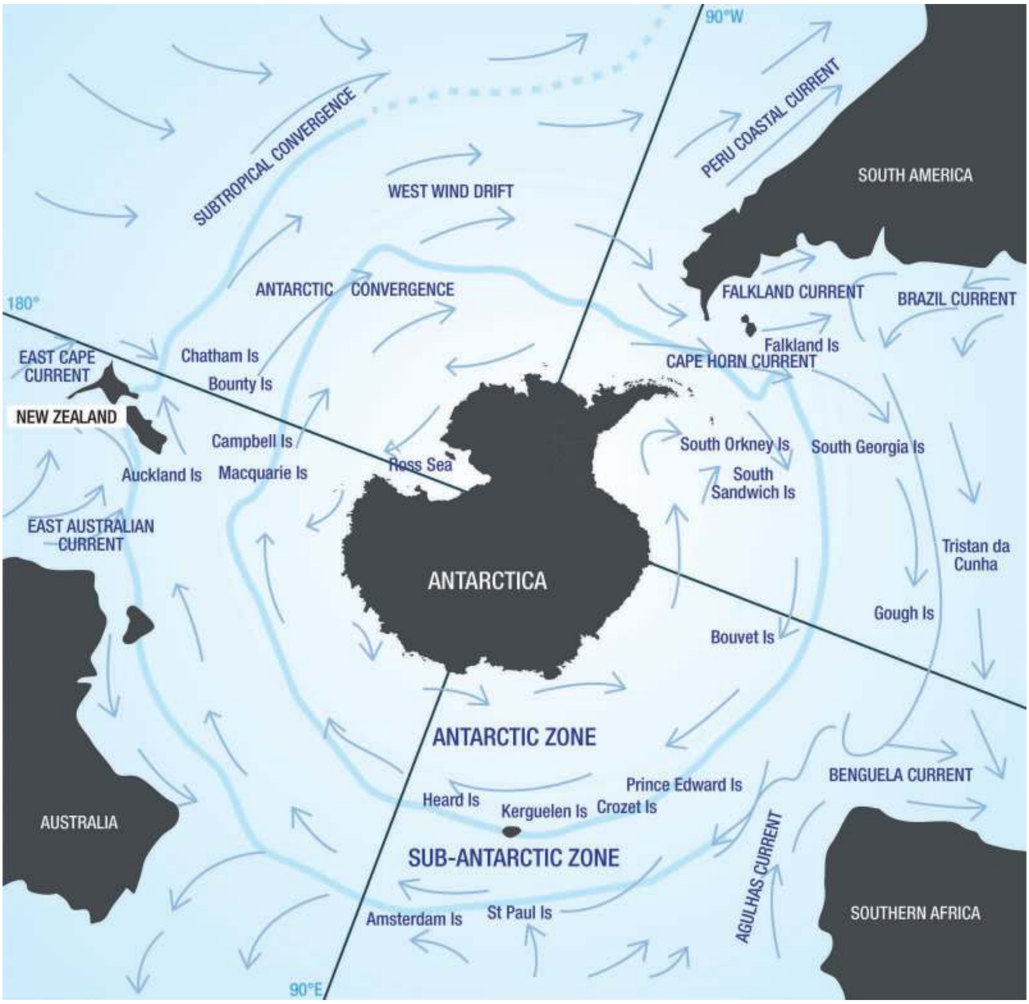

Figure 28 Map of the Southern Ocean showing major landmasses and remote offshore islands in the context of major oceanographic features (Antarctic Convergence, Subtropical Convergence) and major currents.

notes that Southern hemisphere blue mussels occur "… throughout the subantarctic cold temperate of South America and in Kerguelen and the Auckland and Campbell Islands. It appears to be absent from all the other subantarctic islands". Powell (1965) notes that blue mussels are common in most subantarctic waters but are conspicuously absent from Antarctic Seas. The absence of blue mussels has been specifically recorded from Heard Island (Dell 1964) and from Macquarie Island, the Australian territory and the subantarctic island closest to Antarctica (Powell 1957, Dell 1964, Powell 1965). Finding any sort of record of the presence or absence of native blue mussels on the other remote Southern Ocean islands is very difficult, and it seems likely that our knowledge of native *Mytilus* sp. biogeography in the Southern hemisphere is still incomplete, despite recent new work on the subject (e.g., Griffiths & Waller 2016). Thus, there exists the possibility of further new discovery of native blue mussels on remote Southern Ocean islands, although, on balance, we suggest that the likelihood of this appears to be low.

Examination of the biotas of the remote Southern Ocean islands presents new opportunities for hypothesis testing about the biogeography and routes of distribution of native blue mussels in the Southern hemisphere. For example, a large body of data indicate that general patterns of current flow, and therefore of colonisation route, are from west to east, following the Antarctic Circumpolar Current (ACC) (Arnaud 1974, Waters 2008, Leese et al. 2010, Zbawicka et al. 2019). For example, Powell (1965) suggests that the Kerguelen, Crozets, Marion and Prince Edward, Macquarie and possibly Heard and Bouvet islands all belong to the Kerguelenian province within the subantarctic region. Thus, the presence of native blue mussels on one island (e.g., the Kerguelen Islands) may be viewed as a likely source population for blue mussels to the east. With the application of the most recent SNP markers and new analyses testing for unidirectional gene flow (e.g., Sundqvist et al. 2016), it is now possible to test this hypothesis, both in terms of direct migration and colonisation by one mussel group, and in terms of the role of hybridisation (reticulate evolution) in the speciation process.

The use of species delimitation models

Much of biological systematics is based on phylogenetic inference and the theory of species concepts. Thus, the main objectives in systematics have usually been (1) to discover monophyletic groups (clades) and relationships within them at all hierarchical levels above species and (2) to discover lineages (i.e., species) at lower levels (Sites & Marshall 2003). While much of the discipline of systematics has been devoted to the first objective, the second, until very recently, has been largely ignored (Wiens 2007), even though species are routinely used as the basic units of analysis in biogeography, ecology and conservation biology (e.g., Agapow et al. 2004, Padial et al. 2009). However, delimiting species is difficult and is often based on qualitative assessment (Hey 2001a,b) rather than on any robust (i.e., repeatable) quantitative assessment. To help address this problem, Sites and Marshall (2003) proposed several empirical ways of delimiting species. This arises because of the need to distinguish between an ontological definition of the species (non-operational) versus what is operative, with data necessary to prove its reality (Frost & Kluge 1994). However, it was only reasonably recently that de Queiroz (2007) emphasised the distinction between a General Lineage Concept (GLC = metapopulational lineages that evolve separately, or more specifically, segments of such lineages) and the secondary biological attributes or properties of organisms that permit the empirical quantification of the status of a species. This is a crucial distinction because it clearly separates the conceptual problem from the methodological issues to delimit the species. This differentiation greatly aided the growth of the empirical species delimitation (SD) concept.

Species delimitation (SD) is a topic of growing interest in evolutionary biology (e.g., Pons et al. 2006, Knowles & Carstens 2007, Flot et al. 2010, Ence & Carstens 2011, Puillandre et al. 2012, Zhang et al. 2013, Jones et al. 2014, Yang & Rannala 2014, Leaché et al. 2014, Kapli et al. 2017). The incorporation of coalescence models to SD has been one of the most significant advances in this area (Carsten & Dewey 2010, Harrington & Near 2012). By applying probabilistic models, coalescent-based SD provides clear and objective testing of alternative hypotheses of evolutionary independence. In the last decade, several methods of coalescent-based species delimitation (CSD) have been developed, which have been adopted quickly by researchers and have facilitated the discovery and description of an important number of cryptic species (Camargo & Sites 2013), when cryptic species have long been a challenge for systematics and taxonomists (reviewed by Bickford et al. 2007). However, the increase in the findings of cryptic species has prompted further searches for them often targeted at groups where there are known taxonomic problems (e.g., bivalves of the family Pinnidae – Lemer et al. 2014; freshwater mussels – Huang et al. 2019). As such, smooth-shelled blue mussels are also a group that would benefit from the SD approach (Oyarzún et al. 2021).

Although CSD has been widely used in recent years, reproductive isolation-based species delimitation (RISD – following Mayr 1942, Dobzhansky 1970) has been the approach with which the largest number of metazoan species has been delimited (Coyne & Orr 2004). These methods focus on the quantifiable characters of reproductive isolation (i.e., success of fertilisation, viability of offspring, gametic differences, etc.). For example, in molluscs, the morphological characters of spermatozoa have been used in systematic and phylogenetic studies because the ultrastructure of the gametes is highly conserved at the species level (e.g., in the Mytiloidea (Bivalvia) – Kafanov & Drozdov 1998; differences of the sperm ultrastructure in the *Mytilus edulis* complex – Oyarzún et al. 2014). The theoretical basis is that the process of speciation involves acquiring reproductive barriers and that during this process, there are intermediate states where inter-specific hybrids may eventually emerge (Mallet 2005). So, based on information to hand today, what approach should we use for delimiting or identifying smooth-shelled blue mussels or any other group? It is probably more convenient to study the limits of species using molecular methods than by estimating the characteristics of reproductive isolation, since the generation and analysis of molecular data do not require any prior knowledge of the anatomy, ecology or behaviour of the taxa involved. Nevertheless, both approaches should be able to detect species in groups such as the *Mytilus edulis* species complex.

A species complex is a concept referring to a group of closely related species that share morphological and physiological characteristics, to the extent that the boundaries between them are unclear (Steyskal 1972). Several terms are used as synonyms to refer to a species complex, although some of them may be slightly different such as “cryptic species” or “sibling species” or “ring species” (Alcaide et al. 2014, Pereira & Wake 2015). In a phylogenetic context, a complex of species is a group that has a common ancestor (it is not always so – Steinfartz et al. 2000) and that is characterised by the fact that the time of divergence between the lineages is recent, as has happened with the *Mytilus edulis* complex (e.g., Hilbish et al. 2000, Gérard et al. 2008, Popovic et al. 2020). These species have few inter-specific differences; therefore, the lineages of these groups are located within the first speciation criterion in the so-called grey zone of the divergence representation (Figure 29) (*sensu* de Queiroz 2007). These are the characteristics that have been observed in the *Mytilus edulis* complex in the Northern and Southern hemispheres, and which have contributed to the absence of a universally accepted taxonomy of the different evolutionary lineages.

The “species” is considered one of the most important units in biology because the knowledge of a group of organisms is built on this unit (Gascon et al. 2015). As such, the empirical SD has not been exclusively of interest to evolutionary biologists and taxonomists, but is also of interest to other disciplines. For example, the species delimitation concept has direct relevance in many different areas of management. Thus, biosecurity measures are imposed by governments to protect countries from the threat of invasive species (e.g., Northern hemisphere *Mytilus galloprovincialis* – GISD 2012) linked to the high traffic caused by trade (Cook et al. 2015). In this sense, cryptic species categorised as invasive have been a problem for biosecurity authorities, particularly due to the lack of taxonomic knowledge in some groups (Armstrong & Ball 2005). As discussed elsewhere in this review, invasive blue mussels pose ecological, economic and evolutionary problems on arrival and establishment (Gardner et al. 2016 and references therein). Beyond this, there is also the question of food labelling and traceability that relies on accurate descriptions of species for production statistics, reporting and consumer protection and confidence (e.g., European Normative, Regulation (CE) No. 104/2000 and No. 2065/2001 – Ogden 2008, Muehlbauer et al. 2014) given that smooth-shelled blue mussels are the basis of the aquaculture industry in many countries (Smaal 2002, FAO 2015, Larraín et al. 2018, Zbawicka et al. 2021). Finally, from a biodiversity protection and conservation perspective the description of species contributes to the understanding of ecosystem biodiversity and relationships between communities (Gazis et al. 2011). For example, Rissler and Apodaca (2007) identified cryptic species and then developed a method to assess the ecological suitability of the contact zone between lineages to understand how appropriate a habitat is and to predict certain ecological events. It is expected that the application of SNP data to species delimitation will dramatically improve the power, cost-effectiveness and utility of the SD process (e.g., Leaché et al. 2014) across a range of disciplines. In the context of the recognition of regional species within the global *Mytilus edulis* species complex (e.g., Oyarzún et al. 2021), we anticipate that the SD approach will provide much more clarity, given its solid theoretical framework and also the objective and

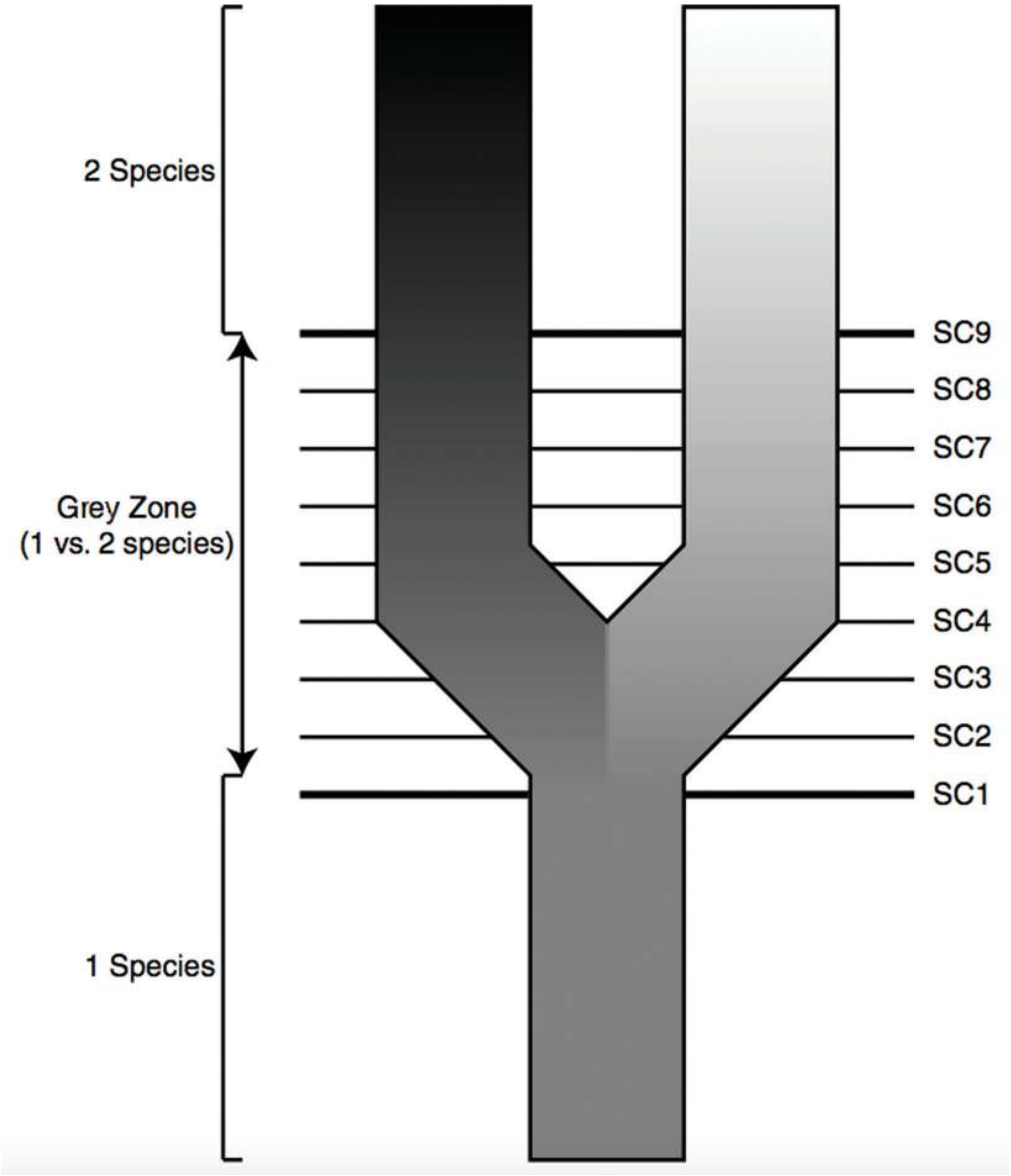

Figure 29 Graphic illustrating the concept of lineage separation and divergence (speciation). This highly simplified diagram represents a single lineage (species) splitting to form two lineages (species). The gradations in shades of grey represent the daughter lineages diverging through time, and the horizontal lines labelled SC (species criterion) 1–9 represent the times at which they acquire different properties (i.e., when they become phenetically distinguishable, diagnosable, reciprocally monophyletic, reproductively incompatible, ecologically distinct, etc.). The entire set of properties forms a grey zone within which alternative species concepts come into conflict. On either side of the grey zone, there will be unanimous agreement about the number of species. Before the acquisition of the first property, everyone will agree that there is a single species, and after the acquisition of the last property, everyone will agree that there are two. In between, however, there will be disagreement. The reason is that different contemporary species concepts adopt different properties (represented by the horizontal lines) as their species criteria – that is, as their cut offs for considering a separately evolving lineage to have become a species. Taken from de Queiroz (2007).

repeatable decision-making mechanisms that underpin its use. While it may be wishful thinking to believe that the SD approach will have all the answers or that all researchers will agree with SD outcomes, we suggest that this approach will prove to be an important next step in recognising species of the smooth-shelled blue mussel complex.

Physical oceanography explains Southern hemisphere blue mussel biogeography

The SNP analyses of native mussels from Chile (Larraín et al. 2018), Argentina (Zbawicka et al. 2018), New Zealand (Gardner et al. 2016), remote offshore islands (Zbawicka et al. 2019) and from Australia (Popovic et al. 2020, Zbawicka et al. 2021) reveal distinct native blue mussel species based on genetic differences maintained in the face of gene flow. The physical oceanography of the Southern Ocean explains these regional distributions.

As a brief recap, we note that the molecular data suggest that native Northern hemisphere blue mussels (most probably *M. edulis*) migrated into the Southern hemisphere ~1 M ybp (1.2–0.8 M ybp) via a North Atlantic route (Hilbish et al. 2000, Gérard et al. 2008). Based on geographic proximity, this strongly suggests that the first major region to be colonised in the Southern hemisphere was South America, and most likely that would have been along the Atlantic coast of modern-day Uruguay and Argentina (giving rise to *M. platensis*). Spread from this point of invasion and establishment would have occurred, most likely involving range extension into Patagonia, through the Strait of Magellan, and into modern-day Chile (giving rise to *M. chilensis*). A second major blue mussel invasion (this time of *M. galloprovincialis*), also via the North to South Atlantic Ocean route, is indicated by the molecular data, but its timing is uncertain. Popovic et al. (2020) have recently suggested that this event occurred between 0.1 and 0.6 M ybp. This gave rise to the Australasian group of mussels (*M. planulatus* in Australia and *M. aoteanus* in New Zealand), but this invasion bypassed South Africa. At some stage, offshore islands such as the Falkland Islands, the Kerguelen Islands, the Auckland Islands and the Campbell Islands were colonised, while others such as Macquarie Island and South Georgia Island were not. This hemisphere-wide pattern of distribution, as well as the natural absence of *Mytilus* mussels from South Africa, can be explained by the physical oceanography of the Southern Ocean.

In the Southern hemisphere, the predominant direction of oceanic flow is west to east (Figure 28) and numerous authors have commented on the effect that this has on biogeographic patterns across many different taxa and also on patterns of gene flow within species. This flow may promote migration directly when larvae are transported during a pelagic dispersal phase or indirectly when post-metamorphic individuals are distributed via rafting (e.g., Mortensen 1933, Fell 1962, Edgar 1987, Helmuth et al. 1994, Ó Foighil et al. 1999, Hobday 2000, Barnes 2002, Thiel & Gutow 2005a,b, Thiel & Haye 2006, Waters 2008, Leese et al. 2010). Numerous studies have described patterns of species, abundance that are consistent with a pattern of west to east flow and that these greatly outnumber examples of east to west flow (e.g., Griffiths et al. 2009, Leese et al. 2010, Griffiths & Waller 2016). Numerous authors have also noted how features such as the ACC and the Polar Front (PF) explain, at least in part, patterns of separation between regions (i.e., the ACC and the PF may often act as barriers to gene flow and connectivity). For example, Gérard et al. (2008) noted that despite the fact that the Kerguelen archipelago is isolated by the ACC and the PF, this effect is not permanent, with the result that occasional latitudinal shifts in the locations of the two features may have allowed for connection between the archipelago and South America (Hollyday and Read 1998). Thus, despite the very large area of the Southern Ocean and the remoteness of many of its islands, temporal changes to the location or the strength of the ACC or PF, or events with a low probability of success such as a raft with associated biota arriving at a new location, may be particularly important, if infrequent, occurrences that contribute to contemporary patterns of biogeography.

Most of the Southern hemisphere current flow is at the mid-latitudes – typically in the region 40–50°S – and as such it connects all major landmasses in the Southern hemisphere, except South Africa, the southern tip of which (Cape Agulhas) is located at 34°49′20″S20°1′0″E. That is, Cape Agulhas is too far north of the predominant west to east flow in the Southern Ocean to have received mussels from South America as they expanded their Southern hemisphere distributions < 1 M ybp (or if mussels did arrive in modern-day South Africa, they did not survive and left no record of their arrival).

The Southern hemisphere's only natural *Mytilus* spp. hybrid zone – the Strait of Magellan

Hybrid zones are locations of high genetic diversity and may give rise to novel genetic variants that may ultimately contribute to the process of speciation (Harrison 1990, Gardner 1997, Abbott et al. 2013). Because of the circumstances that give rise to natural hybrid zones (a zone of sympatry between two reproductively compatible species), these are of evolutionary importance as well as being a conservation management challenge.

Wherever two smooth-shelled blue mussel species co-occur, they hybridise and hybrid zones between *Mytilus* species that naturally occur and those resulting from the anthropogenic introduction of a non-native species have been described extensively in the Northern hemisphere, including from the Pacific and Atlantic coasts of North America (McDonald & Koehn 1988, Bates & Innes 1995, Comesaña et al. 1999, Rawson et al. 1999, Elliott et al. 2008, Toro et al. 2004a), from northwestern Europe and the Baltic Sea (Skibinski et al. 1978, Gardner & Skibinski 1988, Väinölä & Hvilsom 1991, Wilhelm & Hilbish 1998, Bierne et al. 2003, Gilg & Hilbish 2004, Hilbish et al. 2012, Simon et al. 2019), from Japan (Inoue et al. 1997, Brannock et al. 2009, Brannock & Hilbish 2010), and the White Sea region of Russia (Skurikhina et al. 2001).

The Magellan Region in southern Chile is characterised by a unique system of fjords and channels. Specifically, the Strait of Magellan is a complex natural channel that connects the Pacific and Atlantic Oceans. Along this Strait, molluscs of the genus *Mytilus* are the most important macroinvertebrates of the intertidal and subtidal benthic fauna (Aldea & Rosenfeld 2011). This southern region of South America has been little studied, is an area of high conservation value given the biotic differences between the Pacific and Atlantic oceans, and is one of the three recognised Antarctic provinces (Thatje & Mutschke 1999).

Using three nuclear DNA molecular markers (ITS, Glu-5′/Glu-3′ and Me15/16), Toro et al. (2005) reported the presence of what they called *M. edulis*, *M. galloprovincialis* and their hybrids from locations within the middle part of the Strait of Magellan. Larraín et al. (2012), using the Me15/16 RFLP, reported the presence of only *M. chilensis* from one site (Isla Peel) in southern Chile. More recently, Oyarzún et al. (2016) used two mitochondrial-DNA and one nuclear-DNA RFLP assays (16S, COI and Me15/16, respectively) that allowed the identification of the native Pacific Ocean blue mussel, *Mytilus chilensis*, the native Atlantic Ocean blue mussel, which they called *Mytilus edulis*, as well as invasive mussel haplotypes, *M. galloprovincialis* and *M. trossulus* to assay mussels from the Strait of Magellan. The native South American mussel of the Pacific coast, *Mytilus chilensis*, occurred at high frequency (up to 100%) at eight of nine locations, within the Strait of Magellan. The form of the hybrid zone followed a classic cline model. The percentage of *M. chilensis* decreased from 97% in the west to 0% in the most eastern location, reflecting the diminishing influence of gene (larval) flow from the Pacific towards the Atlantic. In contrast, a high percentage of *M. edulis* (now recognised to be *M. platensis*) was detected towards the Atlantic side of the Strait of Magellan, from 14% to 97% in three sampled sites within 100 km. It is hypothesised that *M. edulis* on the Atlantic coast of South America have migrated westward as larval stages, carried by the Atlantic-derived coastal currents from the east entrance into the Strait (Piola & Falabella 2009). The abiotic and topographic conditions of the east entrance of the Strait are the main factors that keep *M. edulis* at those locations. The Strait of Magellan is an inter-oceanic channel which is characterised by the presence of three micro-basins, the Atlantic water body of the eastern micro-basin penetrates through the Strait to approximately Bahía Zenteno (Valdenegro & Silva 2003, Salinas et al. 2004), which is the most westerly site in which *M. edulis* genotypes were observed (Oyarzún et al. 2016). Sampling within the Strait of Magellan indicated that the hybrid zone is ~125 km in length. Consistent with the locations of other natural *Mytilus* hybrid zones (Gardner 1996), this zone is located at an environmental ecotone between two major biogeographic regions, the southern Atlantic and the southern Pacific oceans. A unique feature of the *M. chilensis*×*M. platensis* hybrid zone is that it occurs not just between two biogeographic regions, but also within a region (the Magellan Region) that is itself recognised as being biogeographically different from the Atlantic and Pacific regions, with close affinity to the Antarctic province (Thatje & Mutschke 1999). Further assessment of this hybrid zone with SNPs may be informative, both in terms of increasing our knowledge of the spatial and temporal stability of the zone, and in terms of understanding gene flow and introgression between *M. chilensis* and *M. platensis* (which was called *M. edulis* at the time).

In this region, the importance of detailed information about sampling site location and the history of the sampling site is highlighted. For example, Oyarzún et al. (2016), Larraín et al. (2018) and Zbawicka et al. (2018) all sampled from Caleta de Pescadores which is an artisanal fishery site with a pier, and all reported finding 100% *M. chilensis* here. However, ~1 km east of Caleta de Pescadores, at Muelle Loreto, is an old and unused pier that was used to transport coal and for international commerce (it was the most important pier in Punta Arenas from 1900 to 1950). At Muelle Loreto, Oyarzún et al. (2016) reported finding 70% *M. chilensis*, 23% *M. edulis* and 7% hybrids (Figure 30). Thus, the presence of hybrids appears to be strongly linked to shipping trade

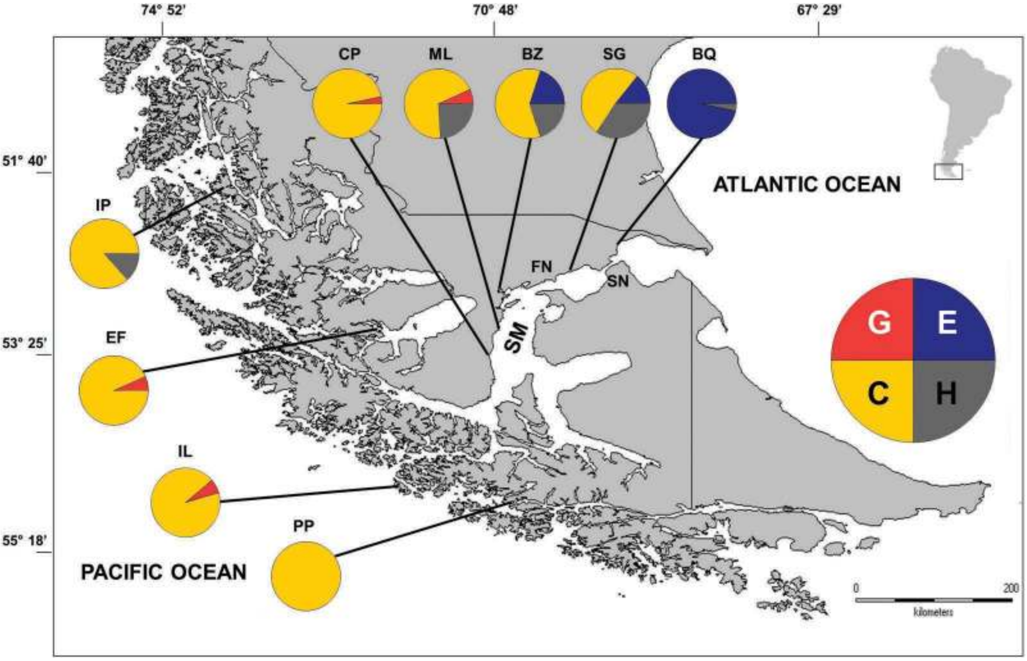

Figure 30 Location of sampling sites within the blue mussel natural hybrid zone of the Magellan Region, Chile. Allele compositions of *Mytilus* samples are shown as frequencies of alleles for the species-specific nuclear and mitochondrial DNA RFLP assays: C=*Mytilus chilensis* or Southern hemisphere *Mytilus galloprovincialis*; G=Northern hemisphere *Mytilus galloprovincialis*; E=*Mytilus edulis*; H=hybrid mussels. Sampling sites: BQ=Buque Quemado, SG=San Gregorio, BZ=Bah_ıa Zenteno, ML=Muelle Loreto (Punta Arenas), CP=Caleta Pescadores, IP=Isla Piazzi, EF=Estero Fanny, IL=Isla London, PP=Paso Pomar. Other codes – SM=Strait of Magellan, FN=First Narrow, SN=Second Narrow. Modified from Oyarzún et al. (2016).

(i.e., human-mediated bioinvasions) as well as to natural processes. During the course of their study, Oyarzún et al. (2016) also reported the presence of six individual *Mytilus galloprovincialis* (i.e., ~3% of all mussels analysed) and three *M. trossulus*×*M. galloprovincialis* hybrids (but no pure *M. trossulus*) from the Strait of Magellan. In conjunction with the native *M. chilensis* and *M. platensis*, the Strait of Magellan therefore has the highest reported genetic diversity of blue mussel species anywhere in the world. This genetic diversity results for two main reasons, one anthropogenically mediated and one natural. The Strait is a very busy shipping route with an international port at Punta Arenas that is the likely source for introduced species (e.g., Northern hemisphere *M. galloprovincialis*, *M. trossulus*) from all over the world. In addition, the Strait facilitates the exchange of waters, mostly from east to west, from the Atlantic and Pacific oceans with the result that marine organisms native to both oceans can meet and mix along the channel within the Strait of Magellan (Oyarzún et al. 2016).

In the context of hybrid zones, it may be helpful to reiterate that mussels from the Falkland Islands, the Kerguelen Islands and the New Zealand offshore islands all exhibit a hybrid background. That is, they appear to have been formed by reticulate evolution or hybrid speciation (Borsa et al. 2007, Zbawicka et al. 2019). However, the important distinction here between the situation described above for the Strait of Magellan and the situation described previously for the offshore islands is that the former involves a hybrid zone (genetic cline) between two "pure" species, whereas the latter does not involve a hybrid zone because all mussels (at least as far as we can tell) exhibit a mixed or hybrid origin. That is, outside a hybrid zone, we find "pure" parental species on either side, but "pure" species do not exist on the remote islands because over time hybridisation has produced mixed or backcrossed genotypes everywhere.

Biosecurity threats to Southern hemisphere blue mussels

Many authors have highlighted the role that anthropogenic activities such as shipping and aquaculture play in the accidental or deliberate movement of marine organisms around the world (e.g., Gardner 1997, Dias et al. 2014, Crego-Prieto et al. 2015, Grosholz et al. 2015, Gardner et al. 2016, Michalek et al. 2016, Larraín et al. 2018, Zbawicka et al. 2018, 2021, McFarlane & Pemberton 2019). Not surprisingly, human-mediated activities have resulted in the introductions of many different groups of marine organisms – algae, invertebrates and vertebrates – to many different regions of the world (Pederson 2003, Ojaveer et al. 2018). Such introductions often result in substantial ecological change to the receiving habitat, with pronounced associated ecological and economic costs (Grosholz 2002, Bax et al. 2003, Ojaveer et al. 2015).

Smooth-shelled blue mussels have been described as invasive from many different locations over, at least, the last 40 years. In the Northern hemisphere, *M. galloprovincialis* of Mediterranean origin or of Atlantic origin (usually the lineage in question is not specified) has been described as being invasive in many locations, including Japan, Hong Kong, the west coast of North America from southern California (USA) to British Columbia (Canada), Norway, Greenland, northern China, eastern Russia and possibly Hawaii (e.g., Wilkins et al. 1983, Lee & Morton 1985, McDonald et al. 1991, Geller et al. 1994, Apte et al. 2000, Anderson et al. 2002, Elliott et al. 2008, Brannock et al. 2009, Shinen & Morgan 2009, Hilbish et al. 2010, Han et al. 2014). In some instances, the invader has been so successful that it has effectively displaced the native cogener, for example, with *M. trossulus* on parts of the Pacific coast of the United States of America (Geller 1999) and in Japan (Brannock et al. 2009). In both cases, it has taken researchers a long time to identify these invasions because of the phenotypic similarity of the invader to the native mussel (this is often classed as a cryptic invasion). Because of this invasion success, Northern hemisphere *M. galloprovincialis* is listed as one of the world's most successful invasive species (Lowe et al. 2000). As far as we are aware there is no evidence for the invasion success of other smooth-shelled blue mussels in the Northern hemisphere, although Beaumont et al. (2006) note that there is anecdotal evidence that *M. edulis* from the North

Atlantic region have been introduced to the Mediterranean coast of France for aquaculture. In contrast to invasion success, Crocetta (2012) lists *M. edulis* in Italy as a possible introduction, but this is impossible to verify for sure, and Casoli et al. (2016) report the failed establishment of *M. edulis* in the Mediterranean Sea after the wreck of the *Costa Concordia* on Italian shores. It seems more than likely that successful invasions or ingressions have occurred elsewhere, but they have not been noticed or cannot be verified.

In the Southern hemisphere, it is again Northern hemisphere *M. galloprovincialis* that has been most often described as the invader. Using a range of different genetic markers, sometimes supported by shell morphological analyses, invasive Northern hemisphere *M. galloprovincialis* has been described from South Africa (Grant & Cherry 1985, Robinson et al. 2007a, Hanekom 2008, Pickett & David 2018, Zardi et al. 2018), southern Namibia (Branch & Steffani 2004, Zardi et al. 2018), from locations in central and southern Chile and from locations in central and southern Argentina (Daguin & Borsa 2000, Toro et al. 2005, Westfall & Gardner 2010, Borsa et al. 2012, Larraín et al. 2012, Tarifeño et al. 2012, Oyarzún et al. 2016, Larraín et al. 2018, Pickett & David 2018, Zbawicka et al. 2018), in much of Australia including Tasmania and also eastern and western mainland sites (Hilbish et al. 2000, Borsa et al. 2007, Gérard et al. 2008, Westfall & Gardner 2010, Colgan & Middelfart 2011, Dias et al. 2014, Ab Rahim et al. 2016, Pickett & David 2018, Popovic et al. 2020, Zbawicka et al. 2021), and in much of New Zealand, particularly in the north (Hilbish et al. 2000, Gérard et al. 2008, Westfall & Gardner 2010, Gardner & Westfall 2012, Gardner et al. 2016). In addition to this, the presence of Northern hemisphere shell types, taxon-specific alleles or haplotypes has also been reported in Southern hemisphere populations. For example, based on morphometric analysis of shells from middens, Gardner (2004) highlighted the similarity of some shells from northern New Zealand to Northern hemisphere *M. edulis*, but was careful not to state that such mussels are (or were) *M. edulis*. Westfall & Gardner (2010) reported the occurrence of two *M. edulis*×*M. galloprovincialis* hybrids from the subantarctic Auckland Islands and noted that although this particular hybrid combination was rare in New Zealand (a total of 484 mussels were examined), it was quite common at this site (two of 34 mussels examined). As noted previously, the presence of *Mytilus trossulus*-like alleles in wild mussel populations of the Southern hemisphere has also been reported by Larraín et al. (2012) at four sites in central Chile and from the Strait of Magellan region by Oyarzún et al. (2016). Larraín et al. (2018), using SNPs, found no evidence of *M. edulis* or *M. trossulus* alleles in Chile. Thus, the status of *M. trossulus* in the Southern hemisphere remains unclear. It requires further research to determine whether these reports of its presence are actually cases of ancestral polymorphisms, or whether it is a natural arrival, or whether it has been spread via anthropogenic activity.

The threat of introduction of non-native mussels to remote Southern hemisphere islands and even to Antarctica has long been recognised (Lewis et al. 2003, Lee & Chown 2007, Shaw et al. 2014, Gardner et al. 2016, Oyarzún et al. 2016), but at the moment, there are still few documented examples of invasion. Ralph et al. (1976) reported the presence of a single, very large (shell length = 83 mm, shell height = 49 mm) blue mussel collected from jetty piles at King Edward Point, South Georgia Island, a region from which blue mussels are naturally absent (Knox 1960, Dell 1964, Powell 1965). Ralph et al. (1976) noted that the nearest population of *M. edulis* (they state that the actual species designation of the mussel remains unclear, but that it is definitely a smooth-shelled *Mytilus* species) is the Falkland Islands, and they go on to discuss the possible ship-borne routes that such an introduction might take, given the history of the South Georgia Islands as both a whaling station and then, more recently, as a science station and stop-over point. Ralph et al. (1976) conclude by stating that it is surprising that this species has not established itself at South Georgia. The fate of this single mussel is unknown (presumably it died a lonely death!), but the threat of future invasion remains. Following on from this, Cárdenas et al. (2020) have recently reported the successful settlement of a newly arrived cohort of *Mytilus* cf. *platensis* (mean shell length 2.0 mm ± 0.1 [mean ± SD]) in a shallow subtidal habitat of the South Shetland Islands in 2019. This is the first report of smooth-shelled blue mussels from the Antarctic region, and while these mussels may not yet have reached the mainland of Antarctica, it appears that they will do so very soon. Cárdenas et al. (2020) note that their genetic analyses, in conjunction with the available shipping records, indicate that the presence of the mussel is consistent with the dominant vectors and pathways linking southern Patagonia with the Antarctic Peninsula, exactly as predicted previously by Oyarzún et al. (2016).

It is now very clear that the Southern hemisphere has been extensively invaded by Northern hemisphere mussels (we do not know of an invasion event in the other direction, but this may well be related more to the fact that no one has looked for it than that it has not occurred). Of the Southern hemisphere invasions, all major land masses have now been colonised. The invasion of South Africa and subsequently southern Namibia is particularly interesting because this is the only Southern hemisphere location where *Mytilus* sp. does not naturally exist (Grant & Cherry 1985) but where other mussels are abundant and an important component of the local system. In all other locations, as far as we can tell, naturally occurring blue mussel populations have been invaded by one or perhaps both lineages of Northern hemisphere *M. galloprovincialis*, and, as noted above, there is some evidence of the presence of other Northern hemisphere taxa such as *M. trossulus* and *M. edulis* in the Southern hemisphere based on RFLP and SNP markers, but these refer to alleles only (i.e., introgression), not to actual mussels. As noted by Gardner et al. (2016), the occurrence of such alleles does not necessarily reflect the occurrence of invasive mussels, it may simply reflect co-ancestry of the marker in question. The very recent description of blue mussels from islands off Antarctica (Cárdenas et al. 2020) is both worrying and fascinating. Antarctica has been described as the "final frontier for marine biological invasions" (McCarthy et al. 2019), and with it having no native intertidal or shallow subtidal mussels, the spread of invasive blue mussels is likely to be rapid and extensive, once established on the mainland.

The usual explanation for the occurrence of Northern hemisphere mussels at Southern hemisphere sites is that they have arrived accidentally via maritime vectors, either as hull fouling or in ballast water (e.g., Apte et al. 2000, Williams et al. 2013, Bailey 2015). Dating introductions can be very difficult, given the reasonably long history of shipping connections across many parts of the globe (e.g., Carlton & Hodder 1995, Gardner 2004, Svane 2011), although we note that it is possible to use the software Structure to estimate the time (number of generations) since admixture between two taxa (Falush et al. 2003). The occurrence of two *M. edulis*×*M. galloprovincialis* hybrid mussels from the remote New Zealand subantarctic Auckland Islands in the Southern Ocean may be attributable to historic attempts at colonisation of this inhospitable islands at least 100 years ago and/or the islands' use as a base for whaling (with numerous associated ship wrecks) dating back 200 years (O'Connor 1999, Westfall & Gardner 2010). Not surprisingly, many different authors have noted that invasions are associated with the presence of ports or harbours, suggesting a significant role for hull fouling (and ballast water to a lesser extent) as the vector of introduction (e.g., Branch & Steffani 2004, Westfall & Gardner 2010, Gardner & Westfall 2012, Oyarzún et al. 2016, McCarthy et al. 2019, Simon et al. 2019, Cárdenas et al. 2020). In addition, deliberate introductions for aquaculture have also been reported (e.g., Crego-Prieto et al. 2015, Michalek et al. 2016, Gurney-Smith et al. 2017), sometimes after the arrival and establishment of *M. galloprovincialis*, for example in South Africa (Branch & Steffani 2004). There is also anecdotal information that *M. galloprovincialis* from NW Spain was transferred to Chile for aquaculture purposes, after the first report of *M. galloprovincialis* in Chile.

What this record shows is that Northern hemisphere *M. galloprovincialis* is a particularly successful invader, in both the Northern and Southern hemispheres. It is very competitive and can increase its invasive distribution in the face of competition from native mussels and also against the predominant oceanographic flow (e.g., McQuaid & Phillips 2000). It is, however, worth noting that in line with problems of recording invasions generally, it is the successful invasion events that we know about, while unsuccessful invasion events are usually unknown and therefore unrecorded. While *M. galloprovincialis* is undoubtedly a very successful invader, there are cases of invasion collapse. For example, Robinson et al. (2007b) report the complete die-off of invasive *M. galloprovincialis* from the sand banks of Langebaan Lagoon on the west coast of South Africa. This report is unusual given that there are, to the best of our knowledge, no other reports of invasion die off for *M. galloprovincialis* (but as noted above there is one for *M. edulis* in the Mediterranean Sea – Casoli et al. 2016), but this was a small spatial scale event in the context of a much larger, ongoing and successful, invasion of southern Africa. Nonetheless, the report highlights the importance and difficulty of understanding invasion events and invasion collapses. The global connectivity patterns of invasive *M. galloprovincialis* based on COI variation have recently been reviewed by Pickett & David (2018). Their results (Figure 31) highlight the complex and multi-directional routes of invasion as documented by 360 bp of the COI region from a large central group of haplotypes to numerous regions in the world.

The biosecurity and management implications of invasive blue mussels have been discussed extensively. There are two main concerns associated with a blue mussel invasion (e.g., Geller et al. 2010, Gardner & Westfall 2012, Gardner et al. 2016, Bourne et al. 2018, Larraín et al. 2018). The first relates to the ecological and environmental outcomes of the bioinvasions, in particular to the displacement of native biota and the subsequent change in ecosystem structure and function (Wilkins et al. 1983, Grant & Cherry 1985, Lee & Morton 1985, Geller, Carlton & Power 1994, Geller 1999, Schneider & Helmuth 2007, Elliott et al. 2008, Hanekom 2008, Shinen & Morgan

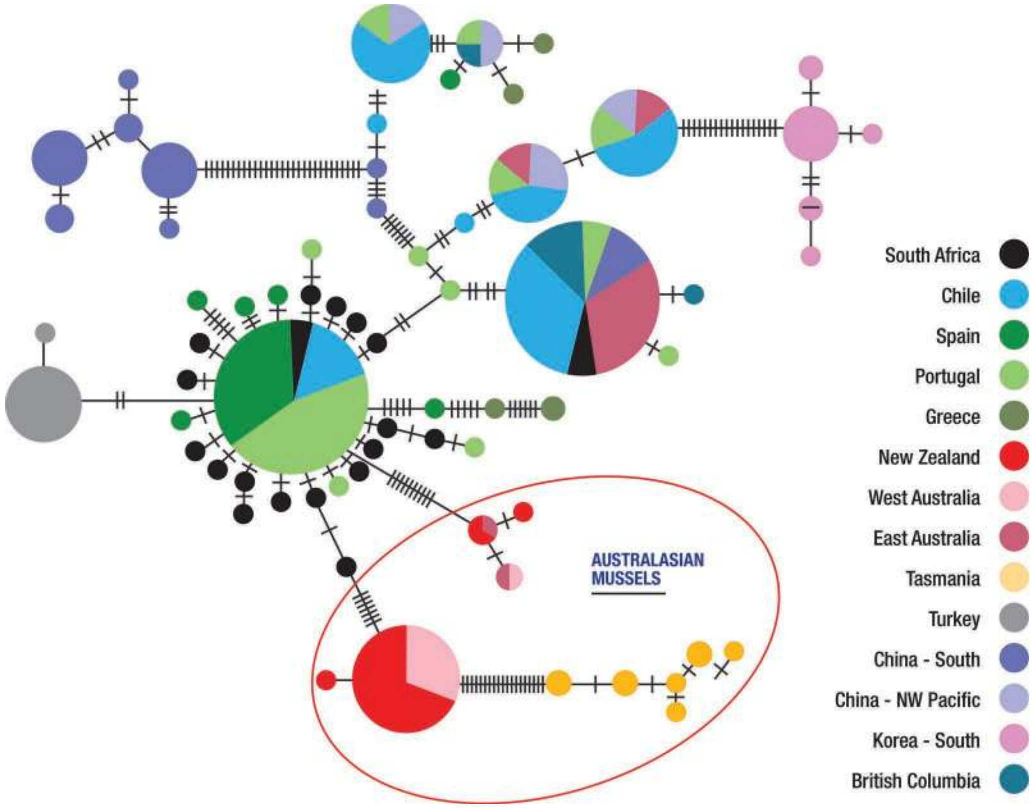

Figure 31 Haplotype network for *Mytilus galloprovincialis* based on mtDNA COI sequence data. Size of circles is representative of individuals with that haplotype. The smallest circles represent a haplotype frequency of one. Each connecting line between haplotypes represents one mutational step and perpendicular lines represent an additional mutational change. Dashed circles indicate distinct haplogroups. Modified from Pickett & David (2018).

2009, Gardner & Westfall 2012, Gardner et al. 2016, Cárdenas et al. 2020). The second relates to the extensive hybridisation that may occur when two or more taxa co-exist and the subsequent introgression that may occur (e.g., Skibinski 1983, McDonald & Koehn 1988, Gardner & Skibinski 1990a, McDonald et al. 1991, Väinölä & Hvilsom 1991, Hilbish et al. 1994, Gardner 1996, Suchanek et al. 1997, Rawson et al. 1999, Bierne et al. 2002, Brannock et al. 2009, Simon et al. 2020, Wenne et al. 2020). The fear of the loss of the genetic integrity of the native taxon in the face of gene flow from the invader, mostly usually Northern hemisphere *M. galloprovincialis*, has been highlighted several times (e.g., Gardner & Westfall 2012, Gardner et al. 2016, Oyarzún et al. 2016, Larraín et al. 2018; Zbawicka et al. 2018, 2019, 2021, Popovic et al. 2020). How this hybridisation and any associated introgression may translate into fitness differences among individuals (pure native taxon, F_N hybrids, backcrosses and pure invasive taxon) remains largely unexplored, in particular in the context of field-based and laboratory-based comparisons of fitness among naturally occurring mussels that have been made in an evolutionary perspective (e.g., Gardner & Skibinski 1988, Gardner & Skibinski 1990a,b, 1991, Wilhelm & Hilbish 1998, Toro et al. 2006, Schneider & Helmuth 2007, Dutton & Hofmann 2009, Elliott et al. 2008, Shields et al. 2010, Dias et al. 2009, Shinen & Morgan 2009). Fitness comparisons and genomic interactions may be key to better understanding the ecological consequences of mussel invasions and the interactions of native and invasive mussels.

Dealing with a mussel invasion most usually means recognising that it has occurred and then accepting that there is little or nothing that can be done about it. Most authorities seem to believe that the relatively rapid growth rate, young age at first reproduction, the production of huge numbers of gametes, considerable larval dispersal ability and tolerance of environmental variation are likely to mean that once established, a mussel invasion cannot not be wound back (Coutts & Forrest 2007, Forrest et al. 2009, Gardner & Westfall 2012). Whether this is true or not, will depend to some extent on how soon after its establishment the invasion is found, the specific context (geographic location) of the invasion and the will of the government of the day to spend money on eradication. Successful invasive mussel eradication programmes have occurred. For example, the black striped mussel, *Mytilopsis sallei*, was successfully eradicated from Darwin Harbour in northern Australia in 1999 (https://nt.gov.au/marine/for-all-harbour-and-boat-users/biosecurity/aquatic-pests-marine-and-freshwater/black-striped-mussel; Ferguson 2000), the invasive *M. galloprovincialis* was prevented from establishing in Pearl Harbor, Hawaii, following its arrival there (Apte et al. 2000), and the brown mussel, *Perna perna*, was eradicated from Tasman Bay, New Zealand, in 2007 (Hopkins et al. 2011). However, the two non-*Mytilus* examples are, of course, very different from the invasion of a blue mussel taxon, in particular if that invasion occurs into a region already home to a blue mussel where it will be difficult to detect and eradicate.

Biosecurity – future threats

In the modern world, where nearly everything is interconnected (Williams et al. 2013), geographic isolation does not guarantee the protection from invasion that it was, until fairly recently, assumed to provide (McCarthy et al. 2019). Even small and remote Southern Ocean islands are not now immune to unwelcome visitors. The two main threats to Southern Ocean biodiversity as posed by an invasive species have been identified as maritime traffic and rafting, either naturally on substrata such as kelp rafts or on anthropogenic substrata such as plastics. In an area as large as the Southern Ocean, the concept of island hopping is important (Leese et al. 2010) because an invader may arrive from its natal region and then proceed to expand its new, invasive range in a series of steps, consistent with patterns of colonisation already recorded for native Southern Ocean species with a benthic life style and no pelagic larval stage (Leese et al. 2010). With an increasing number of reports of the northward expansion towards the Arctic region of the invasive *M. galloprovincialis* in the Northern hemisphere, a spread that may be aided by global climate change and warming temperatures (e.g., Berge et al. 2005, Wenne et al. 2016), there is a similar and related fear that *M. galloprovincialis* or one of its congeners may soon reach the mainland of Antarctica, from where it (and all other *Mytilus* species) is presently and historically absent (e.g., Clark 1996, Lee & Chown 2007, Griffiths et al. 2009, Gardner et al. 2016, Oyarzún et al. 2016, Cárdenas et al. 2020).

Maritime traffic

Lewis et al. (2003) and Lee & Chown (2007) have suggested that hull and sea chest fouling assemblages are of particular concern for the antarctic region because of long winter port layover times, slower cruising speeds and the lack of antifouling agents used on ship hulls breaking through sea-ice in the Southern Ocean and polar seas. Lewis et al. (2003) and Lee & Chown (2007) also cite future increases in the numbers of ships for tourism, fisheries and science as possible vectors for introduction of non-native species to Southern Ocean islands and even Antarctica itself.

Natural rafting

Numerous authors have highlighted the importance of natural rafting, most usually of biota on kelp raft holdfasts, for the dispersal of species around the Southern hemisphere, both among the major continental landmasses and also to/from Antarctica. The earliest report of kelp rafting that we are aware of dates back almost 90 years (Mortensen 1933 cited in Leese et al. 2010), and this has been followed by multiple reports or reviews (e.g., Fell 1962, Edgar 1987, Hobday 2000, Thiel & Gutow 2005a,b, Thiel & Haye 2006, Waters 2008, Leese et al. 2010, Nikula et al. 2010, Fraser et al. 2011). The importance of kelp rafting to dispersal and long-range expansion is highlighted by Ó Foighil et al. (1999) for the brooding oyster *Ostrea chilensis* (from New Zealand to Chile) and by Griffiths & Waller (2016) who note that patterns of kelp rafting are likely to explain many of the biogeographic patterns of different groups in the Southern Ocean. Based on ship-board transect counts, Smith (2002) estimated that at any one time, there may be ~70 million kelp rafts in the Southern Ocean (mostly *Durvillaea antarctica*), of which ~20 million had a holdfast with associated fauna. Tala et al. (2019) note that more rafting kelps occur at higher latitudes (50–60°S) than at lower latitudes (30–40°S), another factor that will facilitate transport of species such as blue mussels to/from Southern Ocean islands and Antarctica. Given that even if only a very small proportion of these kelp rafts are derived from a location with invasive blue mussels, it is still possible that such invaders may be moved around the Southern hemisphere on natural kelp rafts and will tend to go with the flow, mostly travelling west to east on the Antarctic Circumpolar Current and dispersal through West Wind Drift (Arnaud 1974, Waters 2008, Leese et al. 2010, Zbawicka et al. 2019). While there is a low probability that any given raft will make landfall at a location with suitable conditions for the establishment of its hitch-hikers (Griffiths & Waller 2016), once established the early colonisers are likely to experience little competition for space and resources, and may therefore thrive (Waters et al. 2013, Cárdenas et al. 2020). In their description of the biodiversity and biogeography of Antarctic and sub-Antarctic intertidal communities, Griffiths & Waller (2016) note that all of the significant pattern-driving species of the Antarctic and sub-Antarctic are molluscs or macroalgae. Specifically, they report that distributions of several of the key animal species (*Laevilitorina caliginosa*, *Kerguelenella lateralis* and the genera *Nacella* and *Mytilus*) correspond to the distribution of large kelps (mostly *Durvillaea antarctica* but also *Macrocystis pyrifera*). Interestingly, while both *D. antarctica* and *M. pyrifera* are absent from the Antarctic region, another species, *Himantothallus grandifolius*, is circumpolar and may play a role in transporting shallow water species around the continent. From a management perspective, it is, of course, impossible to protect against rafting as a vector of transport, not only because of the sheer number of such rafts that are on the move at any given time, but also because many of the island locations where the rafts may arrive are simply not staffed to deal with this sort of potential incursion. What this suggests is that in the near future, we must expect a number of range expansions of native and non-native mussels in the Southern Ocean.

Rafting on manmade substrata

Many species, including mussels of the genus *Mytilus*, have a prodigious ability to settle and then grow rapidly on a wide range of manmade substrata. This is one reason why blue mussels are such a problem in terms of fouling of wharf piling, ships, power station cooling water intake pipes, and indeed just about any substratum in the water column. With the rapid increase in the amount of plastics in the oceans over the last 50 or 60 years, and the general tendency of this material to float near the surface and to degrade very slowly, multiple different taxa have been recorded on manmade debris found in the oceans, in particular on plastics (Barnes 2002, Barnes & Fraser 2003, do Sul et al. 2011, Griffiths & Waller 2016). Do Sul et al. (2011) report that fishing operations in the Southern Ocean are the main source of manmade marine debris and that plastics from lower latitudes have the ability to cross the PF. They suggest that the most likely route of invasion of Antarctica by fouled plastic debris is from South America, given its proximity to Antarctica. The authors highlight an alarming lack of knowledge about the problem of marine debris and call for more research. In contrast to this view, Griffiths & Waller (2016) suggest that because rafting on debris, like kelp rafting, is a passive process under the control of ocean currents, it is unlikely to promote a wider range of species to colonise Antarctica or the sub-Antarctic region.

The role of anthropogenic debris in the spread of many species has recently been discussed by Carlton et al. (2017) in the context of the huge volume and very large number of individual pieces of debris resulting from the 2011 East Japan tsunami. The authors documented 289 living Japanese coastal marine species from 16 phyla, transported over six years from Japan to Hawaii and the Pacific coast of the USA. *M. galloprovincialis* (itself originally invasive in Japan) was recorded as being a long-term survivor of this trans-ocean dispersal event. Most of the dispersal occurred on manmade non-biodegradable objects, highlighting the role that manmade materials may play in future marine invasions. More recently, Miller et al. (2018) reported that *M. galloprovincialis* was present on >50% of the pieces of biofouled debris (*M. trossulus* at 2.7% and *M. coruscus* at 0.2% occurrence were also reported) and that the majority of mussels (79%) had developing or mature gametes. That is, the mussels were not only viable but ready to reproduce. While we cannot protect in a management sense against natural events such as earthquakes, submarine slumps and tsunamis, it is now apparent that we need to be thinking in terms of manmade debris from such events as a vector for invasive species, in particular for blue mussels given their tolerances of environmental variation.

Climate change and possible new invasions or range expansions

With global climate change and in particular with increasing sea surface temperatures, many intertidal and shallow water species are expected to move poleward, in both hemispheres. Sea water temperate has, of course, long been known to influence species' distributions and to (partially) explain many biogeographic patterns. For example, Ekman (1953) noted that sea water temperature during the post-glacial warm period (approx. 7000–4000 ybp) was ~2°C warmer than at the time of his writing and that *M. edulis* had at that time lived near Spitsbergen and other Arctic localities outside its (then) present distribution. More recently, *M. edulis* has again been reported from Svalbard after 1000 years of absence (Berge et al. 2005). Thus, sea surface temperature changes, in particular in polar regions, are expected to result in range shifts for many species over the next few decades (e.g., Diez et al. 2012, Poloczanska et al. 2013, Early et al. 2016, McCarthy et al. 2019) and have already done so in the Northern hemisphere for species such as *M. edulis* (Berge et al. 2005), *M. galloprovincialis* (Beaumont et al. 2006, Mathiesen et al. 2017) and *M. trossulus* (Feder et al. 2003, Mathiesen et al. 2017). It is interesting to note that this poleward expansion of blue mussels may, in some cases, be matched by catastrophic mortality at the southern (equatorward) limit of distribution associated with increased summer warming above a species' thermal limit (Jones et al. 2010).

Based on fairly extensive, but still incomplete, surveys of many Southern hemisphere regions, smooth-shelled blue mussels are known to be absent from many subantarctic islands including Heard Island (Dell 1964) and Macquarie Island (Powell 1965), and also from the Antarctic mainland (Clark 1996, Griffiths et al. 2009, McCarthy et al. 2019 but see Cárdenas et al. (2020) for a report of introduced mussels on Antarctic offshore islands). They are, however, naturally present in regions such as southern South America (e.g., Oyarzún et al. 2016) and offshore islands in the Southern Ocean (e.g., Gardner et al. 2016, Zbawicka et al. 2019) that are either in immediate proximity to Antarctica (e.g., the Antarctic Peninsula and South America) or are relatively close to Antarctica. As such, both Antarctica itself and the most southern islands that do not have native mussels are likely to be subject at some stage to climate-change-mediated range expansion of smooth-shelled blue mussels and numerous other taxa (e.g., Aronson et al. 2007). As noted by Leese et al. (2010), changing temperatures and associated shifts in the Polar Frontal Zone are likely to mean that higher latitude marine habitats become suitable for invading species. The remote Antarctic and sub-Antarctic islands are therefore expected to act as stepping stones that promote entry of species into Antarctica's coastal systems. The question then may well be what can be done to minimise or perhaps even prevent this, given that many countries have limited capacity to respond to invasions (Early et al. 2016). Antarctica's independent political status may, in fact, hinder the likelihood of invasion identification and subsequent eradication given that no one country has legal responsibility for the continent. While several authorities have advocated for increased monitoring and surveillance (e.g., Gardner et al. 2016, Carlton & Fowler 2018, Ojaveer et al. 2018), others have taken a completely different approach. For example, Beaumont et al. (2006) make the point that short-term (5–10 years) crisis management may well not be worth the effort in the face of longer term (50–100 years) climate change factors. Both views may be correct depending on the geography and the spatial context. Identification of an invasion on, for example, Macquarie Island may be possible and may even be followed up by a successful eradication programme, given the comparatively small scale of the island and the commitment of the Australian Government to prevent bioincursions. However, once established on Antarctica a blue mussel invasion is unlikely to be eradicated successfully given the lack of individual governmental responsibility (with associated cost implications), the remoteness of the location and also the scale of the invasion once established and its potential for subsequent spread.

Management – the need for monitoring and rapid decision-making

As noted, invasive blue mussels are difficult to identify quickly (e.g., when they first arrive at a new destination – but see Ralph et al. 1976, Cárdenas et al. 2020) and are therefore difficult to eradicate (we are unaware of any successful attempts to eradicate invasive blue mussels). Once established, a new invader is likely to spread relatively rapidly and its spread may be aided, at least in part, by its ability to hybridise and interbreed with the native taxon. Alternatively, if an invader arrives at a location where mussels are not native, it may be able to spread rapidly in the absence of biotic resistance. Generally speaking, once an invader is established it will be impossible to eradicate it (Coutts & Forrest 2007, Forrest et al. 2009, Gardner & Westfall 2012). Many authors over the years have highlighted these and other problems associated with bioinvasions, the risks associated with various forms of vectors, and have also suggested a range of different management options aimed at minimising the establishment potential and/or the impact of an invader (e.g., Lewis et al. 2003, Lee & Chown 2007, Gardner & Westfall 2012, Gardner et al. 2016). Not surprisingly, blue mussel bioinvasions are often associated with aquaculture operations (Beaumont et al. 2006, McKindsey et al. 2007, Dias et al. 2014, Grosholz et al. 2015, Crego-Prieto et al. 2016, Michalek et al. 2016. Gurney-Smith et al. 2017), with working ports (e.g., Gardner et al. 2016, Oyarzún et al. 2016, Larraín et al. 2018, Simon et al. 2020), and it has been suggested that many introductions may be relatively old and associated with seventeenth- or eighteenth-century sailing vessels (e.g., Carlton & Hodder 1995, Gardner 2004, Svane 2011). Recent work has highlighted that geographic remoteness is no longer a protection from bioinvasions (e.g., Larraín et al. 2012, Gardner et al. 2016, Oyarzún et al. 2016, Wenne et al. 2016, McCarthy et al. 2019, Cárdenas et al. 2020).

The need for ongoing monitoring is clearly an important step in protecting native biodiversity and identifying an invader (Ojvaeer et al. 2015, 2018, Gardner et al. 2016). Monitoring may be time-consuming and is expensive, in particular if molecular tools are needed to identify an invader and if many samples need to be processed. Typically, existing monitoring is focussed on high-risk areas such as primary ports (e.g., routine surveys in Australia and New Zealand), but is not routinely carried out by all countries (e.g., not in Chile, Argentina and Uruguay). However, specific monitoring of mussel settlement is sometimes carried out. For example, in Chile, from Tongoy (IV Region) in the north to Porvenir (XII Region) in the south, across a latitudinal range of ~3200 km, nine sites are presently being monitored using spat collectors that are replaced every month, over a three-year period (Jorge Toro, pers. obs.). Regardless of which country we are talking about, because routine monitoring cannot be carried out at all locations (e.g., smaller ports, marinas and remote offshore islands), it is easy to miss an invasion, in particular one that does not originate at a primary port. Furthermore, because of the difficulty of differentiating between or among blue mussel taxa based on morphometric criteria alone (Geller 1999, Krapivka et al. 2007, Gardner & Thompson 2009, Illesca et al. 2018), many invasions will not be identified at all because molecular testing for Northern hemisphere blue mussels is not routinely carried out by biosecurity agencies in the Southern hemisphere.

The need for baseline monitoring to establish the present situation for native blue mussel presence/absence and also for native blue mussel genetic identity and integrity has been highlighted by Ovajeer et al. (2015, 2018), Gardner et al. (2016), Oyarzún et al. (2016) and Larraín et al. (2018). The rationale for the Southern hemisphere is clear – until we know what we have and where it is, it is going to be very difficult to protect native biodiversity, native ecosystem structure and function, and native genetic variation.

Given the financial and logistical difficulties associated with monitoring, this activity will, at best, only be targeted to certain areas or events. For example, towing of the *USS Missouri* from Bremerton, Washington State on the Pacific coast of the USA, approximately 4200 km to the state of Hawai'i was known to carry with it some risk of movement of marine invaders. Because of this, an extensive programme was set in place to monitor the vessel and any associated biofouling on arrival in June 1998 into Pearl Harbor (Apte et al. 2000 and references therein). Blue mussels were noted to initiate spawning activity almost immediately upon arrival in Pearl Harbor, and subsequently, mussels were collected from the ballast tanks of a US Navy submarine in the port. Molecular analysis identified these mussels as invasive *M. galloprovincialis* (Apte et al. 2000). While this series of events may be unusual, they highlight the need for targeted monitoring and how management actions may reduce or prevent the likely spread of an invader, even when via an apparently unsuitable habitat or region.

Gardner & Westfall (2012) note that following the identification of non-native blue mussels, it may be possible to model the likely spread of the invader (providing, of course, that sufficient environmental, habitat distribution and physical oceanography data exist) to help predict the spread and the taxon's likely distributional pattern. Such information might then be used in the context of designing a targeted monitoring or eradication programme at sites of particular interest, for example, of high conservation value or of high economic value (e.g., aquaculture production sites). Such an approach may be based on the concept of internal borders (Forrest et al. 2009), which are loosely defined as natural barriers within a country or region that may prevent or slow the spread of the invader. For blue mussels, examples include deep water between islands, long stretches of unsuitable habitat type (e.g., long sandy beaches that separate rocky reef environments) or river outflow that creates a freshwater barrier to dispersal. Identification of such natural barriers to dispersal and range expansion by the invader and the subsequent management planning around such barriers is a well-developed concept in biosecurity, but has not been that widely applied in marine environmental science (Forrest et al. 2009). The concept is rather like a military plan in which it is acknowledged that certain territory has been lost to the invader but that a line exists at a given point, past which the invader will not cross. But for this strategy to be successful, there is a need for ongoing monitoring and the capacity for a rapid incursion response.

In conclusion, it seems likely that there are a number of steps that are required to protect native Southern hemisphere ecosystems and also to protect the integrity and uniqueness of Southern hemisphere genetic lineages of mussels. In particular, we are thinking here of the island-specific lineages, e.g., Borsa et al. (2007), Gardner et al. (2016) and Zbawicka et al. (2019). Gardner et al. (2016, p. 3193) state that "… there is a need for (i) a greater understanding of biosecurity threats, (ii) more baseline information about native (endemic) species plus their genetic uniqueness, (iii) an increased understanding of the likely extents and effects of hybridization and introgression and what has been called 'the invasion of the genome' (Mallet 2005), and (iv) ongoing monitoring and surveillance, plus the political will to act in the event of an incursion".

Aquaculture

Marine mussels of the genus *Mytilus* are one of the most widely cultivated and sold molluscs in the world (FAO 2016, Ferreira & Bricker 2016). In the Southern hemisphere, the major blue mussel producer is Chile, with Australia and to a much lesser extent both Argentina and South Africa also farming *Mytilus* species. Understanding which species is being cultivated is important to growers, processers and marketers alike given that correct labelling of food products is now a legal requirement in many parts of the world and as a point of commercial difference among the many countries around the world that grow and sell blue mussels.

Blue mussel aquaculture is associated with several problems that contribute to production issues (e.g., more fragile shells of *M. trossulus* in comparison to other species in North America – Penney et al. 2007 and also in Europe – Beaumont et al. 2008) and also to biosecurity issues (e.g., accidental and deliberate introductions of species – Branch & Steffani 2004, McKindsey et al. 2007, Crego-Prieto et al. 2015, Grosholz et al. 2015). Associated with this latter point, there may be subsequent issues in terms of inter-specific hybridisation and introgression of non-native genes into the local, native stock (reviewed by Michalek et al. 2016), with concomitant problems in terms of reduced fitness of aquaculture mussels when compared to locally adapted stocks (e.g., Perez et al. 2003, Beaumont et al. 2006, Penney et al. 2006, Toro et al. 2006, Oyarzún et al. 2013). As a consequence, in many parts of the world, legislation now exists that governs aquaculture transfers to help safeguard the consumer, the local industry, the genetic integrity of locally adapted stocks and also the local environment (e.g., Dias et al. 2014, Muehlbauer et al. 2014, Larraín et al. 2018). Despite this, invasive blue mussels in countries like Australia, Canada and Chile may now pose a threat to the well-being of the local industry, even if only in certain regions (Dias et al. 2014, Crego-Prieto et al. 2015, Ab Rahim et al. 2016, Gurney-Smith et al. 2017, Larraín et al. 2018). Zbawicka et al. (2021) have recently suggested that mussel aquaculture (e.g., based on hatchery production) in the Australian island state of Tasmania for the native mussel, *M. planulatus*, could help enhance the status of threatened native mussel in the wild, as well as providing a sales point of difference for the state's mussel farmers.

The importance and difficulty of differentiating among mussel species in an aquaculture context are particularly well highlighted by a recent example from Chile. Coelho-Caro et al. (2018) note that the classification, counting and sorting of mussel seed for aquaculture production is currently performed by human experts (this is time-consuming and labour-intensive). They described the development of an automatic mussel classifier system that uses machine learning to differentiate with ~95% success among five mussel species of four genera, including the native *M. chilensis* and the introduced Northern hemisphere *M. galloprovincialis*. The aquaculture industry's move towards the use of such technology, in particular to differentiate among different species at the spat/seed stage that are derived from wild settlements, illustrates how important it now is to industry to know which species is being cultivated to maximise the economic return. The use of this sort of technology, which is cost-effective to develop and deploy, may help mitigate some of the problems that the mussel aquaculture industry faces in certain regions in terms of differentiating between a native and an introduced species or between a strong shell shape and a weak shell shape, something that is particularly important at the post-harvest processing stage.

Chile

Chile is now the world's second largest mussel (*Mytilus* spp.) aquaculture producer at 365,595 tonnes, behind only China at 880,000 tonnes (FAO 2006–2019, FAO 2017, FAO 2018c). Production is concentrated in the Gulf of Reloncaví and along the coastline of Chiloé Island (Los Lagos region) and is based on the native blue mussel, *Mytilus chilensis* (Larraín et al. 2018). There has been a steady pattern of industry growth since 1993 from 3,864 tonnes production to 338,847 tonnes production in 2017 (SERNAPESCA 2017), rising to 365,595 tonnes in 2018 (FAO 2018c). The contribution of mussel production to total Chilean aquaculture increased from 6.1% in 2001 to 27.8% in 2017, and mussel (*M. chilensis*) production was 97.5% of total mollusc production in Chile (SERNAPESCA 2017). While China is producing mainly for its domestic market, Chile exports almost all of its production, with most going to the EU (Fernández-Tajes et al. 2011), such that Chile is rapidly becoming the top mussel exporter in the world (FAO 2018a,b).

The industry is almost totally dependent on the supply of seed from natural (wild) populations (Uriarte 2008). Therefore, for the ongoing sustainable exploitation of *M. chilensis* it is important to recognise native from introduced (*M. galloprovincialis*) mussels and to understand regional population dynamics of the species (Astorga et al. 2015). Aquaculture activity in Chile has an enormous impact on anthropogenic-mediated gene flow via the transfer of juveniles from two or three major spat collection sites to a broad number of sites for grow-out to commercial size (Holmberg 2012). As noted above, Chile is a world leader in the development of image analysis technology and machine learning to differentiate among wild-caught seed (Coelho-Caro et al. 2018), and as noted below, it is also a world leader in terms of mussel food forensics (Fernández-Tajes et al. 2011, Larraín et al. 2014, Jilberto et al. 2017).

Argentina

Despite its very long coastline and the presence of two native mussel species (*M. platensis* in the north and *M. chilensis* in the south), Argentina is not a major player in world mussel aquaculture terms. Bivalve molluscs occupy the third place in aquaculture production statistics in Argentina. *Mytilus platensis* is produced at Río Negro and Chubut, while *Mytilus chilensis* is produced in Tierra del Fuego. All of this production is based on the suspended culture techniques. Commercial production for the domestic market commenced in 1996. By 2014, a total of 11.2 tonnes were produced, which is equivalent to 0.33% of total aquaculture production in Argentina (Dirección de Acuicultura, Ministerio de Agroindustria 2016).

Uruguay

Mytilus platensis, the native mussel in Uruguay, inhabits the intertidal and subtidal rocky shore of the Atlantic coast (Riestra & Defeo 2000). This species is the dominant organism in these coastal systems, and the natural mussel beds have been exploited for over 40 years by artisanal fishers. No mussel aquaculture exists so far in Uruguay, although there are moves to develop a new industry.

Falkland Islands

There is a small local aquaculture industry based on the production of *M. platensis* in the Falkland Islands, and all of this production is used for domestic consumption. The FAO does not yet report annual production values for the islands (FAO 2018c). The coastal topography of the Falkland Islands with its many sheltered bays and its clean water is ideal for mussel aquaculture.

Low human population density is, however, a problem that will limit further development of the industry. Nonetheless, the feasibility of developing an export industry has been explored – https://en.mercopress.com/2004/11/16/prospects-for-falklands-mussel-industry-examined

South Africa

Mussel aquaculture of two species – the native *Choromytilus meridionalis* and the introduced *M. galloprovincialis* – occurs on a limited scale in Saldanha Bay, Western Cape, where four farms exist (Louw 2020). The FAO reports production of 2182 tonnes in 2018 for *M. galloprovincialis*, up from 682 tonnes in 2009 (FAO 2018c). Mussel culture method is based on ropes suspended from longlines in the cool and highly productive waters of Saldanha Bay (e.g., https://blueocean-mussels.com/; https://www.vikingaquaculture.co.za/mussels/) that are well known for periods of wind-driven upwelling that bring cooler, nutrient-rich waters from deep to the surface, a regular phenomenon that enhances mussel growth.

New Zealand

New Zealand is unique among the larger Southern hemisphere countries with a native blue mussel because at present it does not have an aquaculture industry for *Mytilus* sp., in this case *M. aoteanus*. New Zealand's mussel aquaculture industry is very well developed, but is entirely focussed on another native species, the greenshell mussel *Perna canaliculus* (Hickman 1991, Jeffs et al. 1999, Alfaro et al. 2011). Native (*M. aoteanus*) and introduced (Northern hemisphere *M. galloprovincialis*) blue mussels settle and grow on the suspended culture (long line) system that is employed to grow *P. canaliculus*. These blue mussels, which tend to settle on the tops of the ropes and can outcompete the native greenshell mussels for space and access to particulate food, are more resilient to salinity fluctuations than are the green mussels (Forrest & Atalah 2017). At harvest, the blue mussels are collected at the same time as the greenshell mussels, but they are separated from the greenshells, and are treated as fouling and a waste product. Historically, blue mussels have been sent to landfill sites or used as pig food. For the last 40 years or so, the New Zealand mussel aquaculture industry has been solely focussed on the production of *P. canaliculus* (2018 production was 86,176 tonnes – FAO 2018c) because this is a major point of difference for New Zealand from all other producers, and because the New Zealand greenshell mussel often sells for twice the price per kg of blue mussels on international markets (mostly Europe and North America, but SE Asia as well). However, over the last few years, there has been recognition within New Zealand that these non-target blue mussels can be used in soups and chowders, and it seems likely that a new, but small value, blue mussel aquaculture industry will develop in New Zealand. In taste tests, international consumers often prefer blue to green mussels, and international chefs have commented that they are not used to working with big mussels (often 7 cm shell length and larger for *P. canaliculus*) but prefer smaller blue mussels (5–7 cm shell length). Much of the information above is taken from Aquaculture New Zealand, which is the industry trade magazine (https://www.aquaculture.org.nz/).

One final point relates to monitoring of the occurrence of the invasive Northern hemisphere *M. galloprovincialis* (e.g., Gardner et al. 2016) on New Zealand greenshell mussel farms, and whether or not aquaculture farms are important manmade surfaces that inadvertently promote the further spread of this invader. A preliminary study is presently underway testing this idea at five sites within the Marlborough Sounds, New Zealand's major centre for mussel aquaculture.

Australia

Blue mussels in Australia are naturally distributed from approximately Cape Hawke, in New South Wales on the east (Pacific Ocean) coast, along the southern coastline including the island state of

Tasmania, to Perth in Western Australia on the west (Indian Ocean) coast (Love & Langenkamp 2003, Dias et al. 2014). The biggest producer is the state of Victoria followed by Western Australia (Dias et al. 2014, Ab Rahim et al. 2016), but all regions except Queensland and Northern Territory have an industry (Dias et al. 2014). According to Dias et al. (2014), Western Australia produced 365 tonnes of mussels in 2011. By world standards, the Australian mussel industry is small, but it is growing rapidly: the FAO lists production of *M. planulatus* as 3781 tonnes in 2018 (FAO 2018c). Until recently, all seed were wild caught, but the unpredictable supply of spat and the requirement to develop the industry has seen the advent of hatchery seed production, with particular emphasis in the states of South Australia and Victoria (Hickman et al. 2005; Jahangard et al. 2010; Nguyen et al. 2011).

In Australia, the native mussel is recognised as *M. planulatus* following recent SNP-based work (Popovic et al. 2020, Zbawicka et al. 2021), which until recently was widely recognised as a native Southern hemisphere lineage of *M. galloprovincialis* (e.g., Westfall & Gardner 2010, Dias et al. 2014, Ab Rahim et al. 2016). In addition, several authors have recorded the presence of the invasive Northern hemisphere *M. galloprovincialis*, in particular in Western Australia, but also at lesser frequencies elsewhere (e.g., Gérard et al. 2008, Westfall & Gardner 2010, Colgan & Middelfart 2011, Dias et al. 2014. Ab Rahim et al. 2016). In a broad survey across Australia, Ab Rahim et al. (2016) reported that 56.2% of all mussels were native Southern hemisphere haplotype, 10.3% were putatively introduced Northern hemisphere mussels, and 32% of all mussels had genotypes consistent with either Northern or Southern hemisphere lineages. In the context of seed supplied from hatcheries by the states of South Australia, Victoria and Tasmania, Dias et al. (2014) reported that most mussels were native Southern hemisphere lineage mussels (i.e., *M. planulatus*), but also noted that a significant proportion of the seed supply was of Northern hemisphere *M. galloprovincialis* stock (South Australia = 43%, Victoria = 48% and Tasmania = 30%). Consistent with earlier reports of very high frequencies of Northern hemisphere *M. galloprovincialis* in Western Australian populations, Dias et al. (2014) reported frequencies of 65%, 88%, 60% and 24% of this non-native mussel at four separate aquaculture sites in Western Australia. Thus, blue mussel aquaculture in the state of Western Australia is largely, but not exclusively, based on the production of the introduced Northern hemisphere *M. galloprovincialis*, whereas blue mussel aquaculture in the eastern and southern states is largely, but not exclusively, based on the production of the native Southern hemisphere *M. planulatus*. However, subsequent movement of stocks and hatchery-produced spat around the country will contribute to further mixing, as will hybridisation and introgression between the two species.

Importance of correct taxonomy for food labelling, marketing, traceability and production statistics

Taxonomy has a key role to play in the protection and sustainable exploitation of species (Mace 2004, Larraín et al. 2018). Correct product identification (taxonomy) underpins a lot of aquaculture at the post-harvest stage of production (Beaumont et al. 2008). This is more than just getting the species name correct on the can: this is about differentiating the product of one country or one region from others, protecting the consumer, preventing commercial fraud by substitution, recognising biosecurity concerns when the product is grown in one country but processed in another, understanding regional production statistics, monitoring temporal change in production dynamics and the effective use of marketing to sell more product. While this aspect of mussel taxonomy has not been a major focus of global efforts to better understand the taxonomy and phylogeography of the world's blue mussels, its relevance to aquaculture and to national economies has long been recognised. In the Southern hemisphere, this is most applicable to major mussel producing countries such as Chile, and to a lesser extent to Australia and Argentina, but not presently applicable to New Zealand.

Identification of mussel species and their hybrids is of economic importance for different reasons, some of which are not immediately obvious. For example, while mixed species can be sold in some regions of the world (e.g., Canada – Penney et al. 2002) the more fragile-shelled *M. trossulus* can cause problems at the sorting and processing stage in the factory that may render rope growth of *M. edulis* and *M. trossulus* uneconomical for some farmers. In another example, Beaumont et al. (2008) describe how biosecurity fears may directly impinge on aquaculture production. They note that most bottom-cultured mussels in the United Kingdom are sent to the Netherlands for processing, but the identification of *M. edulis*, *M. galloprovincialis* and *M. trossulus*, as well as their hybrids, at the main growing site in Loch Etive, Scotland, raised concerns from the Dutch Government who lodged legal challenges against the importation of Scottish mussels on biosecurity grounds. In this case, correct mussel taxonomy may have helped protect the Netherlands (its mussel growers and its native ecosystem), but at the time it cost Scottish growers, at least in the short term, until the problem was resolved.

Seafood traceability has three levels: species identification, geographic location of origin, and supply chain tracking and tracing (Ogden 2008, Larraín et al. 2014). Numerous authors have pointed out the difficulties of identifying aquaculture-produced mussels by species or even by genera, most often because of the absence of shells and/or because mussel flesh is in some processed (unrecognisable) form. As a consequence, several studies have either applied existing molecular markers or developed new markers to test species identity and/or the provenance of mussels (Santaclara et al. 2006, Fernández-Tajes et al. 2011, Larraín et al. 2014). Because these sorts of approaches are informative about mussel species identity and perhaps provenance, they may contribute to supply chain tracking. Compared to other industries (e.g., beef), the seafood tracking component of the aquaculture industry is still young, poorly developed and not that widely applied, but it is increasing and contributes to meeting labelling obligations (e.g., the European decision concerning labelling, Regulation [CE] 104/2000 (Santaclara et al. 2006), the more recent Regulation (EU) n. 1379/2013 (D'Amico et al. 2016) and the *Codex Alimentarius* regulations CE.N°104/2000 and CE.N°178/2002 (Larraín et al. 2014)).

In the Southern hemisphere, Chile is by far the largest blue mussel producer, and much of this product is exported to the European Union (EU) in frozen or canned form (Fernández-Tajes et al. 2011). Perhaps not surprisingly then, all blue mussel traceability studies from the Southern hemisphere are from Chilean laboratories. DNA-based methods to assess canned (heat-treated) and frozen products have been developed to identify different mussel species and also different genera (Fernández-Tajes et al. 2011). Examination of four cans of mussels from Galicia (northwest Spain) that were supposed to be Northern hemisphere *M. galloprovincialis* revealed that three cans were correctly labelled and that one can contained *M. chilensis* from Chile, as well as the fact that two mussels were hybrids of *M. chilensis*×*M. trossulus*. This study highlights the value of molecular assays designed for taxonomic (species delimitation) purposes that are applied to food testing with outcomes directly relevant to labelling, fraud by substitution and to consumer protection. Subsequently, Larraín et al. (2014) used microsatellite markers to test the assignment success of blue mussels to populations from southern Chile. Their different assignment approaches showed varying levels of success, up to only ~50%, highlighting the difficulty of identifying the correct production site when population-level genetic differentiation is not pronounced because of the relatively close proximity of the sites and the apparently high levels of gene flow among them (and the possibility of human-mediated transfers). Additionally, the high frequency of occurrence of null alleles in most/all shellfish is likely to hinder the use of microsatellites in the field of food forensics (e.g., Vera et al. 2010). Most recently, in an attempt to move beyond traditional DNA-based PCR methods that are laborious and time-consuming, Jilberto et al. (2017) and more recently Quintrel et al. (2021) have developed a high-resolution melting point analysis that can differentiate among *M. chilensis*, *M. galloprovincialis*, *M. edulis* and their hybrids with high sensitivity, specificity and precision. Increasingly, we will see these sorts of approaches applied to seafood generally, as countries seek to protect their industries and products.

Reference to the results of many different population genetics studies of Southern hemisphere blue mussel species based on nuclear or mitochondrial DNA markers (e.g., Inoue et al. 1995, Santaclara et al. 2006, Westfall et al. 2010, Fernández-Tajes et al. 2011, Larraín et al. 2014) suggests that in terms of aquaculture traceability and species identifications, these markers are powerful enough to differentiate among species and to identify hybrids, but are unlikely to be powerful enough to pinpoint a specific production site when samples from a single species are analysed with samples from other nearby sites within a single region. The application of new SNP markers, as applied to mussels in both the Northern and Southern hemispheres, is expected to substantially increase diagnostic power for traceability and provenance testing. One caveat is that generally speaking SNP analysis requires high-quality DNA, something that may usually be obtained from frozen samples, but may not be so easily obtained from heat-treated (e.g., canned) product and/or product that is sold in wine vinegar (acetic acid) or tomato-based sauce (e.g., Quintrel et al. 2021). Application of the SNP markers to mussels as a food product is an exciting new step forward, and one that may substantially increase traceability and provenance analyses (Vera et al. 2010, Larraín et al. 2014, 2018, Jilberto et al. 2017).

Future research directions

The application of SNPs to Southern hemisphere smooth-shelled mussels has provided a new insight into the evolutionary history and biogeography of this important model group. Some of the results provide a new verification of the specific status of native mussels (e.g., Chile, Argentina/Uruguay, Australia, New Zealand), a subject that has been hotly debated for many years, other results confirm the findings of several different studies that have pointed to the distinct status of native mussels between regions (e.g., South America versus Australasia) and also within regions (e.g., the Falkland Islands, the Kerguelen Islands), while still other findings suggest the existence of new, previously unrecognised lineages (e.g., the New Zealand offshore islands). Below, we highlight in bullet points some areas of research that new generations of molecular markers and also new generations of analytical tools and software may be able to shed light on. This is by no means an exhaustive list. Examples include an improved understanding of

- Species and evolutionary lineages present in the Southern hemisphere and their evolutionary relationships with Northern hemisphere congeners
- The extent of co-ancestry of SNP alleles across all species to clarify the situation for *M. trossulus* in the Southern hemisphere
- The genetic basis of selection to environmental variation, for example at the Kerguelen Islands or at the New Zealand offshore islands compared to the New Zealand mainland
- The genetic architecture of hybridisation between two taxa, whether they are naturally occurring or introduced
- The role that hybridisation may play in the speciation process (i.e., reticulate evolution)
- Karyotype differences between the taxa and lineages and the role that chromosomal differences play in promoting or retarding interbreeding
- The role of cytonuclear incompatibilities in promoting or retarding hybridisation and speciation
- The detection of non-native mussels in the context of biosecurity management and biodiversity protection
- Range expansions and how genetic processes promote or retard these as new areas are colonised by blue mussels in the Southern hemisphere
- The timing of natural range expansions and also invasions by non-natives
- The extent of introgression following hybridisation and which genes or gene complexes are involved

- Species delimitation as quantitative analytical approaches are developed and applied to complex “problem” groups such as smooth-shelled blue mussel
- Population genetic diversity and gene flow, and how connectivity is mediated by, for example, patterns of coastal and oceanic flow and/or rafting on natural and manmade substrata
- How best to apply new markers such as SNPs to food products (frozen, canned, vacuum-packed) and how best to protect producers and consumers via the process of labelling
- How best to apply new markers such as SNPs to counter food substitution (counter-feiting) and to demonstrate and guarantee provenance.

Concluding remarks

Smooth-shelled blue mussels of the genus *Mytilus* have long been a favourite model group, in large part because of their almost cosmopolitan distribution (e.g., the Mussel Watch Program established in 1986 by NOAA as part of the National Status and Trends monitoring programme), their ecological and economic importance, and their intriguing evolutionary history. As a group, these mussels provide new insights into the process of speciation (often in the face of gene flow), into hybridisation and introgression (including speciation by hybridisation), and also into one of the biggest single threats to global biodiversity – bioinvasions. Recent SNP-based work on Southern hemisphere blue mussels has provided a new layer of detail and a new level of confidence to our understanding of this group’s evolutionary origin, phylogeography and their taxonomy and systematics. The SNP markers have helped provide clarity among the many different interpretations provided in earlier times by researchers who did not have the benefit of working with such high-definition markers, as well as providing new insights that simply were not previously possible. SNP markers have not, of course, answered all the questions or resolved all the uncertainties, but coupled with further developments and new applications (e.g., species delimitation models, whole-genome sequencing), smooth-shelled blue mussels will doubtless continue to be an excellent study group to help us better understand the process of speciation in the sea, with all that this entails for global aquaculture, biosecurity and conservation.

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