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Invasion Genetics of Botryllid Colonial Ascidians in North America

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INVASION GENETICS OF BOTRYLLID COLONIAL ASCIDIANS IN NORTH AMERICA

By

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Declaration of Co-Authorship / Previous Publication

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research undertaken under the supervision of Dr. Melania Cristescu (University of Windsor) as follows: Chapter 2 contains material from a manuscript entitled " Looking at both sides of the invasion: patterns of colonization in the violet tunicate *Botrylloides violaceus* " that has been submitted to *Molecular Ecology*. This manuscript is coauthored by Bock DG, Zhan A, Lejeusne C, MacIsaac HJ and Cristescu ME. The Appendix contains the manuscript “Polymorphic microsatellite markers for two highly invasive colonial ascidians *Botryllus schlosseri* and *Botrylloides violaceus*" published in *Molecular Ecology Resources*. This manuscript is coauthored by Bock DG, Adebayo AA, Egbosimba EE and Cristescu ME. In all chapters, the main ideas, primary contributions, experimental designs, data analysis and interpretation, were performed by the author, and the contribution of co-authors was primarily through provision of guidance with analytical, field and lab work, as well as helping with revising early drafts.

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II. Declaration of Previous Publication

This thesis includes two original papers that have been previously published/submitted for publication in peer reviewed journals, as follows:

Thesis Chapter	Publication title/full citation	Publication status
Chapter 2	Bock DG, Zhan A, Lejeusne C, MacIsaac HJ, Cristescu ME. Looking at both sides of the invasion: patterns of colonization in the violet tunicate *Botrylloides violaceus.* In review at *Molecular Ecology*	Submitted with revisions
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ABSTRACT

This thesis analyzes genetic patterns across botryllid tunicate invasions in North America - encompassing the violet tunicate *Botrylloides violaceus* and the golden-star tunicate *Botryllus schlosseri*. I investigate these species' entry and spread on the continent by using the mitochondrial cytochrome *c* oxidase subunit I (COI) gene, and 13 (*B. violaceus*) and 12 (*B. schlosseri*) nuclear polymorphic microsatellite loci. Considerable genetic differentiation was detected both within and among East and West coast locales. Also, there was substantial variation in the degree of genetic diversity maintained in introduced populations, which showed, in general, signatures of long-distance dispersal. Taken together, these results indicate the invasions were founded from multiple source regions. Also, post-introduction spread along the coasts appears to occur predominantly through human-mediated dispersal of sexually-produced propagules. I relate these findings to knowledge of the life-history attributes of *B. schlosseri* and *B. violaceus*, and to available records of their introductions to North America.

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1.0 - GENERAL INTRODUCTION

Invasive species and the role of genetics in revealing patterns of invasion

The introduction of nonindigenous species (NIS) to new environments, one of the most pervasive and damaging effects of human activities, is now recognized as a fundamental aspect of global environmental change (Carlton & Geller 1993; Ricciardi 2007). The alarming ecological and economic consequences of species invasions have prompted a surge in invasion-related research devoted to the identification of the factors associated with the persistence and spatial expansion of NIS (Lockwood *et al*. 2007). With the integration of genetics to the study of biological invasions (i.e. invasion genetics), much progress has been made. For example, genetic approaches have successfully been used to identify the sources of introduction and pathways of spread in widely distributed invaders (e.g. Cristescu *et al*. 2001; Roman 2006; Brown & Stepien 2009). Additionally, comparisons of native and introduced populations have yielded insights into the extent of genetic variation that is lost during an invasion process (reviewed by Roman & Darling 2007). This information has allowed researchers to infer if the founding populations were small or large, and if they were supplemented by later independent release events (e.g. Kolbe *et al*. 2004; Colautti *et al*. 2005). More recently, genetic studies have also been used to advance our understanding of the post-establishment spread patterns of non-native species across areas of introduction (e.g. Darling & Folino-Rorem 2009; Rollins *et al*. 2009). Collectively, by providing a more complete representation of the invasion process, this information is expected to lead to the development of accurate risk assessment, and the implementation of sound, science-informed control measures. The motivation for using genetics to investigate biological invasions can also be theoretical. As such, NIS

introductions are increasingly seen as 'natural' colonization experiments that might be used to explore and refine theories regarding the properties of range expansions (e.g. Estoup *et al*. 2004) or to address the speed and predictability of evolutionary processes in different geographical settings (e.g. Huey *et al*. 2000).

Invasive ascidians - a growing global concern

Among the numerous NIS recorded to date, ascidians have recently attracted a lot of attention, as they are becoming increasingly abundant in marine coastal areas worldwide (Lambert 2002) . Their accelerated spread has been attributed to transport by a number of anthropogenic dispersal vectors, among which vessel ballast water, hull fouling, and aquaculture trade are considered the most important (Carver *et al*. 2006; Dijkstra *et al.* 2007). Once introduced, ascidians can quickly establish, and in more extreme cases become dominant members of recipient ecosystems (Lambert 2005; Blum *et al*. 2007). In aquaculture facilities, ascidians are considered a serious biofouling concern, as they suffocate target species and cover any available substrate, thereby making harvesting difficult and increasing the costs of operations (Coutts & Forrest 2007; Howes *et al*. 2007). Invasive ascidians comprise both solitary and colonial forms, several of which are now regarded as some of the most successful NIS. These include the solitary species *Ciona intestinalis* and *Styela clava,* as well as the colonial species *Botrylloides violaceus* and *Botryllus schlosseri* (Lambert & Lambert 2003; Lambert 2005). All of these species have been introduced in North America and are spreading along the coasts. In this context, a comprehensive genetic analysis of North American non-indigenous ascidians is timely. Information on these species' population genetic structure on the East and West coasts would constitute an important resource not only to those tasked to mitigate the

negative effects of these invasions in North America, but also to a more general audience of invasion and evolutionary biologists.

Thesis objectives

In the current study, I focus on the botryllid colonial species *B. violaceus* and *B. schlosseri*, with the main goal of exploring patterns of genetic diversity and differentiation across these species' invasions on the East and West coasts of North America. Specifically, in chapter 2, I aim to i) investigate the genetic diversity of *B. violaceus* populations introduced on the West and East coasts, ii) compare the patterns of gene flow within and between coasts, and iii) evaluate the relative contribution of sexual and asexual reproduction to this species' rapid population expansion. In addition, characterization of genetic structure in established populations allowed me to make inferences on the colonization history of this species in the two invaded ranges in North America. In chapter 3, I aim to i) explore the phylogeographic history of native and invasive *B. schlosseri* populations, ii) determine the post-introduction level of genetic variation maintained in North American populations, and iii) identify patterns of gene flow and fine-scale genetic subdivision within the native, East and West coast ranges. To meet these goals, I use the mitochondrial (mt) cytochrome c oxidase I (COI) gene as well as 13 (*B. violaceus*) and 12 (*B. schlosseri*) polymorphic microsatellite loci, while drawing on recent advances in the analysis of hypervariable genetic data based on Bayesian clustering and assignment methods.

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2.0 - LOOKING AT BOTH SIDES OF THE INVASION: PATTERNS OF COLONIZATION IN THE VIOLET TUNICATE *BOTRYLLOIDES VIOLACEUS**

2.1 INTRODUCTION

Despite growing interest to identify factors that determine the success of widespread invaders, we still have a limited understanding of how patterns of colonization vary across the large spectrum of environments typically encountered by widely introduced species. However, such information is essential not only for recognizing how dispersal vectors and life history traits contribute to the spread of invasive species, but also for understanding the evolutionary forces that drive invasion success, and ultimately for developing effective management strategies.

Evidence that post-establishment dispersal varies not only between but also within species has started to accumulate with the recent expansion in the geographical scale of NIS genetic surveys (e.g. Voisin *et al*. 2005; Darling & Folino-Rorem 2009). Collectively, these studies suggest that fine scale population genetic analyses performed at multiple spatial scales or settings might be the key to understanding how region-specific attributes can drive within-species invasion patterns. In this context, studies of widespread invaders such as colonial ascidians provide excellent opportunities for exploring how invasion history, vector availability, and life history traits influence colonization dynamics in different areas of introduction.

*modified from: Bock DG, Zhan A, Lejeusne C, MacIsaac HJ, Cristescu ME. Looking at both sides of the invasion: patterns of colonization in the violet tunicate *Botrylloides violaceus.* In review at *Molecular Ecology*

Botrylloides violaceus, also known as the violet tunicate, is an invasive colonial ascidian, commonly recognized as a biofouling nuisance species. In natural ecosystems, it has been shown to overgrow and outcompete indigenous species, at times becoming dominant in subtidal benthic communities (Berman *et al*. 1992). In aquaculture facilities, it smothers target species, limits food availability, and covers available substrate making harvesting difficult (Carver *et al.* 2006). *B. violaceus* is considered native to the Northwest Pacific, most likely Japan (Saito *et al*. 1981). Outside of its native range, it occurs on both coasts of North America (Carver *et al.* 2006) as well as the coasts of Australia, Italy, the United Kingdom, Ireland and the Netherlands (Zaniolo *et al.* 1998; Gittenberger 2007; Minchin 2007; Perez-Portela *et al.* 2009). The first non-indigenous population of *B. violaceus* was reported on the coast of California (Lambert & Lambert 2003). However, due to taxonomic confusion, the exact date of this introduction - either 1945 (Van Name 1945, unconfirmed) or the 1970s (Fay & Vallee 1979, confirmed) - remains uncertain. At present, *B. violaceus* occupies much of the West coast, achieving high abundance from Ensenada, Mexico to Alaska (Lambert & Sanayam 2001; Lambert & Lambert 2003). On the East coast, the first population is believed to have established in 1972 in Massachusetts following an unintentional release of *B. violaceus* colonies (Carlton 1989; Yund & Feldgarden 1992). It has been suggested that the founding individuals descended from West coast stocks, most likely from southern California (Yund & Feldgarden 1992; Grosholz 2001). Since 1972, the East coast invasion spread rapidly south to Connecticut and north to Prince Edward Island (Carver *et al.* 2006) and Newfoundland (Callahan *et al*. 2010). Multiple vectors are considered responsible for dispersal of *B. violaceus* along the West and East coasts. Hull fouling and aquaculture trade are considered the primary means (Dijkstra *et al.* 2007), although ballast water discharge (Dijkstra *et al.* 2007),

rafting of fragmented colony parts (Carver *et al.* 2006), and spread as epibionts on large crustaceans (Bernier *et al*. 2009) have also been suggested to facilitate dispersal of the species.

Biological characteristics linked to the high invasiveness of *B. violaceus* include the ability to engage in both sexual and asexual reproduction. In particular, asexual budding of individual zooids often leads to the formation of large mats which commonly fragment and regenerate, leading to an increased local abundance and dispersal potential (Carver *et al.* 2006). *B. violaceus* is highly effective at reattaching when dislodged from substrates (Bullard *et al.* 2007), thus the common aquaculture management technique of high pressure washing is largely ineffective and may, in fact, lead to an increase in colony fragmentation and subsequent infestation (Paetzold & Davidson 2010). Although the distribution, impacts, and ecology of *B. violaceus* have been extensively studied, a comprehensive examination of genetic patterns associated with this species' rapid range expansion is still lacking. Interestingly, the only study that investigated genetic structure of populations established in North America (Lejeusne *et al*. in press) provides mitochondrial based evidence for the occurrence of successive founder effects from the West to the East coasts. Extensive genetic divergence between identified cytochrome *c* oxidase subunit I (COI) haplotypes suggested that multiple introductions have occurred on the Northwest coast of North America. However, the dramatically reduced levels of mitochondrial genetic diversity indicated that a thorough investigation of the invasion history of *B. violaceus* in North America requires the additional use of polymorphic microsatellite markers, coupled with intensive sampling efforts.

Here, I use mitochondrial COI sequences and 13 polymorphic nuclear microsatellite loci to characterize the genetic structure of 25 introduced populations of *B. violaceus* sampled along the West and East coasts of North America. The primary goals of this chapter are to: i) investigate the genetic diversity of introduced populations on the West and East coasts, ii) compare the patterns of gene flow within and between coasts, and iii) evaluate the relative contribution of sexual and asexual reproduction to this species' rapid population expansion. Characterization of genetic structure in established populations further allowed me to make inferences on the colonization history of *B. violaceus* in the two invaded ranges in North America.

2.2 MATERIALS AND METHODS

Sampling and DNA extraction

A total of 673 colonies of *B. violaceus* were sampled from 25 North American locations: 344 (50.7%) colonies from the West coast and 335 (49.3%) from the East coast (Fig. 2.1; Table 2.1). A fine-scale sampling scheme was employed for the northern part of the current distribution range where rapid spread of *B. violaceus* is challenging aquaculture operations. Sampling was undertaken by SCUBA diving and/or by excising colonies from submerged ropes and buoys in harbours, marinas and infested aquaculture facilities. To prevent re-sampling of the same colonies, all specimens were taken at least 1 m apart from one another. Samples were preserved in 95% ethanol at -20 ℃ prior to genetic analyses. Genomic DNA (gDNA) was extracted from single zooids using the protocol of Elphinstone *et al.* (2003).

Mitochondrial DNA amplification and sequencing

A fragment of the COI gene was initially amplified using the universal primer pair LCO1490 and HCO2198 (Folmer *et al*. 1994). Because these primers failed to amplify consistently, species-specific primers (BvCOIF: 5'-TTTGTATTTTATTTTTAGGGTTTGG-3' and BvCOIR: 5'-TCAAAATAAATGTTGATAAAGTACAGG-3') which amplify a 659-bp fragment were designed and used. The 25 µL PCR volume consisted of 1 µL (~50 - 100 ng) gDNA, 1x PCR buffer with 1.5 mM $MgCl_2$ (Genscript), 0.2 mM dNTPs, 0.4 µM of each primer, and 0.5 U of *Taq* DNA Polymerase (Genscript). PCR cycling parameters consisted of an initial denaturation step at 94 ºC for 5 min, followed by 35 amplification cycles (94 ºC for 30 s, 50 ºC for 30 s, 72 ºC for 45 s), and a final elongation step at 72 ºC for 5 min. Sequencing reactions were performed using the reverse primer (BvCOIR), BigDye Terminator 3.1 chemistry, and an ABI 3130XL automated sequencer (Applied Biosystems, Foster City, CA). The forward primer (BvCOIF) was used to confirm all sequences that contained ambiguous sites.

Microsatellite genotyping

All 673 *B. violaceus* samples - representing 25 populations - were genotyped for 13 polymorphic microsatellite markers (Bvm2, Bvm4 - 9, Bvm12 - 13, Bvm15 - 18; Molecular Ecology Resources Primer Development Consortium *et al.* 2010). PCR cocktails (10 µL) contained 50 ng of gDNA, 1x PCR buffer with 1.5 mM $MgCl_2$ (Genscript), 0.125 mM of each dNTP, 0.5 µM of each primer and 0.2 U of *Taq* DNA Polymerase (Genscript). Forward primers were labelled with one of four fluorophores (6FAM, VIC, NED or PET) according to Schuelke (2000). The cycling PCR profile

consisted of an initial denaturation at 95 ℃ for 3 min, 10 cycles of 35 s at 95 ℃, 35 s at an initial annealing temperature of 60 ℃ that decreased by 1 ℃ in each of 10 cycles, and 45 s at 72 ℃ followed by 35 cycles of 35 s at 95 ℃, 35 s at 52 ℃, 45 s at 72 ℃, and a final extension for 10 min at 72 ℃. Amplified fragments were separated on an ABI 3130XL automated sequencer (Applied Biosystems, Foster City, CA), with GeneScan™-500 LIZ™ (Applied Biosystems, Foster City, CA) internal size standard. The alleles were scored using GENEMAPPER® software v.4.0 (Applied Biosystems, Foster City, CA). To confirm genotyping accuracy, 3% of the samples, chosen at random, were rerun.

Mitochondrial DNA analysis

Sequence data was aligned and edited using CodonCode Aligner v. 2.0.6 (CodonCode Corporation, Dedham, MA). DNASP v.5 (Rozas *et al.* 2003) was used to identify individual *B. violaceus* haplotypes, calculate the number of haplotypes (*Nh*), haplotype diversity (*h*) and nucleotide diversity (π) and to test whether the sequences evolved under neutrality according to Tajima's *D* statistic. Neighbor joining (NJ) and maximum-likelihood (ML) phylogenetic analyses were conducted in PAUP* v.4b10 (Swofford 2001) and PHYML v. 2.4.4 (Guidon & Gascuel 2003), respectively. To determine the best fit nucleotide substitution model, I used MODELTEST v.3.7 (Posada & Crandall 1998) under the Akaike Information Criterion (AIC). Phylogenetic reconstructions were rooted using the congeneric species *B. fuscus* (GenBank accession number: GQ365690). Relationships among the COI haplotypes were further examined using a statistical parsimony haplotype network generated at the 95% connection limit with TCS v.1.21 (Clement *et al.* 2000).

Population pairwise Φ_{ST} was calculated with 10,000 permutations in ARLEQUIN v.3.1 (Excoffier *et al.* 2005) using the Tamura & Nei (TrN) substitution model. To assess genetic differentiation among sampling sites, I conducted a hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) in ARLEQUIN. Molecular variance was partitioned into three levels: between coasts, among populations within coasts, and within populations. Isolation by distance (IBD) within the West and East coasts was further examined by testing the correlation between genetic distance [$\Phi_{ST} / (1 - \Phi_{ST})$] and geographical distances using a Mantel test with 10,000 permutations implemented in GENEPOP v. 3.4 (Raymond & Rousset 1995). Geographical distances were calculated as the minimum coastline distances between adjacent sampling locations using GOOGLE EARTH v.4.3 (beta).

Microsatellite DNA analysis

The number of repeated multilocus genotypes was calculated using GENECAP software (Wilberg & Dreher 2004). For all putative clones, I estimated the probability of identical genotypes arising by chance *via* sexual reproduction. I computed the lower bound of this probability, P_{HW} under Hardy-Weinberg expectations, and the more conservative upper bound P_{sib}, under strict sibling reproduction, as recommended by Waits *et al.* (2001). Calculations for P_{HW} and P_{sib} consider the observed allele frequencies in the population within which clones were identified (Wilberg & Dreher 2004).

Microsatellite data was checked for departures from Hardy-Weinberg equilibrium (HWE) using 10,000 permutations in GENEPOP, with levels of significance adjusted by sequential Bonferroni corrections (Rice 1989). The total number of alleles (N_A), allelic

richness (*A*), mean observed and expected heterozygosities (H_O and H_E), and the inbreeding coefficient (F_{IS}) were calculated using FSTAT v.2.9.3.2 (Goudet 2002). FSTAT incorporates a rarefaction method (Mousadik & Petit 1996) that compensates for unequal sample sizes to calculate *A.* The degree of genetic differentiation between pairs of populations was assessed using pairwise F_{ST} values computed with 10,000 permutations in ARLEQUIN. Additionally, population structure was determined by conducting a three dimensional factorial correspondence analysis in GENETIX v. 4.05 (Belkhir *et al*. 2004) and by the Bayesian clustering approach implemented in STRUCTURE v. 2.3.1 (Pritchard *et al.* 2000). For the STRUCTURE analysis, for each value of *K* (population clusters), I carried out five independent Markov Chain Monte Carlo (MCMC) runs with 10^5 generations discarded as burn-in followed by an additional 10^6 generations. The simulated *K* values ranged from 1 to 25 (total sites) when pooling individuals from all localities and from 1 to 10 and 1 to 15 when using individuals previously grouped into a single cluster based on the initial global analysis. The optimal number of clusters was estimated by comparing the log-likelihood of the data given the number of clusters [ln $P(X|K)$] (Pritchard *et al.* 2000) and by examining the standardized second order rate change of ln $P(X|K)$, ΔK (Evanno *et al.* 2005).

Contemporary gene flow was assessed by individual-based assignment using GENECLASS v. 2.0 (Piry *et al.* 2004). I used the partially Bayesian method of Rannala & Mountain (1997); this method is preferred when not all possible source populations have been sampled (Berry *et al*. 2004). Genotype assignments were determined by assessing probabilities through 10,000 MCMC simulations (Paetkau *et al.* 2004). The sample with the highest probability of assignment was considered the most likely source

for the assigned genotype. Individuals not assigned to any population with a probability of $P > 0.05$ were assumed to be from an unsampled location. To test for a pattern of isolation by distance, I used a Mantel procedure with 10,000 permutations implemented in GENEPOP to assess the dependence between Rousset's (1997) genetic distance [F_{ST} / $(1 - F_{ST})$] and geographic distances. A hierarchical analysis of molecular variance (AMOVA) was performed in ARLEQUIN based on microsatellite genetic distances between populations, and partitioning variance between the West and East coasts, among populations within coasts, and within populations.

2.3 RESULTS

Mitochondrial DNA analyses

The final 558 bp COI alignment contained a total of 27 polymorphic sites (9 parsimony-informative), with 26 synonymous substitutions and 1 nonsynonymous substitution corresponding to a valine - isoleucine change. Within all 604 sequences, I identified only seven distinct haplotypes. Among these, 4 are new (Bv8 - Bv11; GenBank accession numbers GU946476 - GU946479) and three (Bv1 - Bv3; GenBank accession numbers GQ365691 - GQ365693) were previously reported by Lejeusne *et al.* (in press). Most haplotypes were shared between two or more sampling locations. Only haplotypes Bv10 and Bv11 were restricted to the Deep Bay (DPB) and Lemmens Inlet (LMI) populations, respectively (Appendix S2.1). The most abundant haplotype on the West coast was Bv1 (66.8%) followed by Bv3 (20.2%) and Bv2 (6.2%). On the East coast 98.6% of colonies sampled shared haplotype Bv1 and only 1.4% haplotype Bv3 (Table 2.1). Haplotype diversities were typically much higher in West coast (range: 0 to 0.610; mean 0.313) than

in East coast (range: 0 to 0.166; mean 0.038; Table 2.1) populations. Tajima's *D* statistic was not significant (D = 1.076; $P > 0.10$) for the entire data set, suggesting that selection was not acting on this locus and that a neutral model of evolution cannot be rejected.

The neighbour-joining and maximum likelihood phylogenetic analyses revealed that all North American *B. violaceus* haplotypes correspond to two phylogroups (Appendix S2.2). This finding was confirmed by the 95% parsimony haplotype network, where two groups of haplotypes separated by 9 substitution steps were identified (Fig. 2.2). One group included the dominant Bv1 haplotype, shared between the two coasts, and Bv8, restricted to the West coast. The second group consisted of four haplotypes that occur on the West coast (Bv2, Bv9, Bv10 and Bv11), and one (Bv3) detected on both coasts (Fig. 2.2).

Pairwise Φ_{ST} values indicated genetic structure exists within the West coast, with 43.60% of comparisons significant after Bonferroni corrections (Appendix S2.3). The Lemmens Inlet (LMI) and Bodega Bay (BBY) populations were differentiated from most populations sampled on the West coast, with Φ_{ST} values ranging from 0.12 to 0.84. By contrast, all pairwise Φ_{ST} values between East Coast populations were low and not significant. Within the entire data set, the highest pairwise Φ_{ST} values (0.86) were attained between West and East coast populations. Hierarchical AMOVA based on mitochondrial data revealed that most of the genetic variance was found within sampling sites (48.46 %). Significant partitioning occurred among populations within each coast and between the West and East coasts, accounting for 33.30% and 18.24% of the variation, respectively ($P < 0.05$; Table 2.2). Mantel tests failed to reveal relationships

between genetic distance and coastline distances between populations on both the West (r^2 = 0.012, P = 0.619) and East (r^2 = 0.002, P = 0.338) coast.

Microsatellite DNA analyses

I identified 27 multilocus repeated genotypes across all populations (C1 - C27; Appendix S2.4). Most putative clones were restricted to single populations; only genotype C4 was shared between Deep Bay (DPB) and Ladysmith (LSM). The repeated genotypes were encountered between one and seven times depending on the location (Appendix S2.4). The probability of separate occurrences of the same genotype arising *via* sexual recombination under Hardy-Weinberg assumptions (P_{HW}) was extremely low, ranging from 5.34 x 10^{-6} to 1.93 x 10^{-12} (Appendix S2.4). In addition, the more conservative estimate (P_{sib}) remained low and nonsignificant ($P < 0.05$), suggesting that repeated genotypes resulted from resampling of fragmented colonies. Data analysis was performed with and without clones, producing comparable results. I present results obtained after removing clonal genotypes from the data set.

A total of 620 *B. violaceus* colonies were analyzed at 13 microsatellite loci. In total I identified 169 alleles across North America, of which 155 (91.2%; mean 12 alleles/locus) were detected in West coast populations, and 110 (65%; mean 8.5 alleles/locus) in East coast samples (Appendix S2.5). The number of private alleles also differed between the West coast (22 private alleles) and the East coast (7 private alleles; Table 2.1). The allelic richness and expected heterozygosity varied from 3.2 to 4.4 (mean 3.7) and 0.554 to 0.704 (mean 0.611), respectively, on the West coast, and from 3.0 to 3.9 (mean 3.4) and 0.559 to 0.662 (mean 0.600), respectively, on the East coast. West coast populations

sampled at Lemmens Inlet (LMI), Neah Bay (NHB), and Sequin (SQN) had the highest genetic diversity. Conversely, the lowest variation was identified on the East coast at Chester (CHT), Methegan (MTG), and Mystic Harbor (MYH; Table 2.1). While most loci conformed to HWE, 35 of 325 cases exhibited significant deviations after sequential Bonferroni corrections (Appendix S2.6). However, no systematic deviations were observed for loci across all populations or at all loci within populations. Microsatellite data showed highly significant genetic differentiation after Bonferroni correction between most pairs of samples (Appendix S2.7), with the exception of one pair of sites on the West coast (DPB - LSM) and five more on the East coast (APY - SPB; CRV - SHB; BKH - MCV; BKH - LNB; LNB - MCV).

The two different approaches used to identify population structure, factorial correspondence analysis and Bayesian clustering, provided a largely concordant picture. Three-dimensional factorial correspondence analysis (3D-FCA) illustrated high genetic distances between geographically proximate West coast populations (Fig. 2.3). For the East coast, however, neighbouring samples generally grouped together. Component 1 explained 27.25% of genetic variance and nearly perfectly separated West from East coast populations (Fig. 2.3). The only exception was Bodega Bay (BBY), which clustered with East coast populations. The Bayesian algorithm implemented in STRUCTURE indicated that all *B. violaceus* individuals could be assigned to two main genetic clusters ($K = 2$). This partitioning was supported by the evaluation of both $\ln P(X|K)$ and ΔK. The likelihood of the data was lowest for $K = 1$ and the largest difference of successive likelihoods was between $K = 1$ and $K = 2$. After $K = 2$, the likelihood of the data plateaued and standard deviations increased (Appendix S2.8). When $\ln P(X|K)$ only

marginally increases above a certain value of *K*, the smallest value of *K* before the plateau (here $K = 2$) is considered the best model (Pritchard *et al*. 2007). The estimation of ΔK as per Evanno *et al*. (2005) also showed a clear pattern for $K = 2$ (Appendix S2.8), confirming that this is the most parsimonious model for the global data set. Two genetic clusters, hereafter S1 and S2, corresponded remarkably well with the West versus East coast geographic partitioning of the data (Fig. 2.4A). For West coast populations, membership coefficients (*Q*) to cluster S1 averaged 92% with the exception of Bodega Bay (BBY) which averaged only 18%. For East coast populations, *Q* to S2 averaged 93% (Fig. 2.4A). When clustering analysis was performed separately for S1 and S2, two genetic clusters were identified for each data subset. The substructure within S1 revealed the distinctiveness of proximate populations such as Victoria (VIC) – Sydney (SYD) or Sequin (SQN) – Brinnon (BRN; Fig. 2.4B). Within S2, the partition in two clusters revealed that individual genotypes assign to different clusters in the northern and the southern parts of the East coast, with assignment ratios following a gradual transition along the north-south axis (Fig. 2.4C). Additionally, southern locations (MYH – CHT) appeared to be most similar to Bodega Bay (BBY).

The analysis for detection of migrants showed high overall assignment success, approximately 99.7 % (603 of 605 individuals were definitively classified at $P > 0.05$). On the West coast, the inferred migration events appeared to be distributed throughout the sampling region, and only one population (BRN) displayed 100% self-assignment (Table 2.3). On the East coast, the majority of putative migrant genotypes (32/38) were restricted to the northern part of the sampling region (SPB; SHB; CRV and APY). Outside this area, there was limited indication of migration (Table 2.3). Ten individuals were

identified as potential inter-coastal dispersers. The inferred migration events were highly directional, from the West to the East coast (Table 2.3). The pattern of dispersal on the West coast did not reflect a correlation between genetic differentiation and geographical distances (Fig. 2.5A). The Mantel test remained nonsignificant after excluding the genetically distinct Bodega Bay (BBY) population from analysis (Fig. 2.5B). Conversely, for East coast populations, the Mantel test suggested highly significant genetic isolation-by-distance (Fig. 2.5C). A strong correlation between genetic and geographic distance was supported at the large (1,706 km) and smaller (504 km) scales for East coast populations. Hierarchical AMOVA of microsatellite data revealed that most variation was attributed within sampling sites (82.65%). Variation within coasts (10.93%) and between coasts (6.42%) was also statistically significant (Table 2.2).

2.4 DISCUSSION

Contrasting patterns of genetic structure and regional spread on the West and East coasts

Overall, the genetic diversity estimates for *B. violaceus* in North America are lower than those reported for other invasive ascidians. For example, mitochondrial genetic diversity estimates computed for 25 invasive populations (seven haplotypes, mean *h* of 0.176) were much lower than those reported for the solitary ascidian *Microcosmus squamiger* (30 haplotypes, mean *h* of 0.712 estimated in 9 invasive populations sampled worldwide; Rius *et al*. 2008). Also, microsatellite gene diversity estimates (mean H_E of 0.606, computed using 13 microsatellites) were lower than those identified for the closely related colonial species *Botryllus schlosseri* in North America (mean H_E of 0.845, computed over

seven invasive populations using four microsatellites, Stoner *et al*. 2002), and comparable to those reported for the solitary ascidian *Styela clava* in North America (mean H_E of 0.536, computed over four invasive localities using six microsatellites, Dupont *et al*. 2010).

Despite the overall low level of genetic diversity, I observed strong geographic partitioning of genetic variance between North American *B. violaceus* samples, with West coast populations exhibiting higher levels of genetic diversity than those on the East coast (Table 2.1). This pattern was supported by the mitochondrial number of haplotypes (7 vs. 2), mean haplotype diversity (0.313 vs. 0.038), and nucleotide diversity (0.005 vs. 0.001). This difference in genetic diversity was consistent with that observed for microsatellite markers, mostly in the total number of alleles (155 vs. 110) and private alleles (22 vs. 7) sampled. The genetic partitioning between the two coasts might reflect bottleneck events associated with the introduction of this species to North America. Historical records indicate that the invasion proceeded in a 'stepping stone' fashion, and that East coast *B. violaceus* populations were likely seeded from West coast stocks (Yund & Feldgarden 1992; Grosholz 2001). As expected, the bottleneck signature on the East Coast is more apparent in the mitochondrial genome, which is more sensitive to demographic population fluctuations than the nuclear genome, due to its smaller effective population size, more rapid extinction of lineages, and lack of recombination (Avise 2000).

Overall, the pattern of regional spread and resultant population genetic structure differed sharply in the two main sampling regions analysed, as revealed by microsatellite genetic variation. On the West coast, almost all pairwise F_{ST} values were high and significant

(Appendix S2.7). Also, factorial correspondence analysis (Fig. 2.3) revealed high genetic divergence between most sampling locales. On the other hand, increased levels of genetic connectivity were detected between few distant sites such as DPB - LSM, situated 90 km apart (F_{ST} = 0.03; Appendix S2.7; see also Table 2.3), suggestive of long distance (likely human-mediated) dispersal. Consistent with this observation, there was no indication of isolation-by-distance for West coast populations (r^2 = 0.2686, P = 0.2420; Fig. 2.5A). The pattern of genetic structure observed on the West coast may reflect the combined effects of low levels of natural dispersal coupled with long distance spread (most likely human-mediated) between key locations. Similarly complex patterns of connectivity have been reported in other invasive taxa, such as the solitary ascidian *Styela clava*, the anemone *Nematostella vectensis*, and the hydrozoan *Cordylophora caspia*, that possess limited natural dispersal capability and spread mainly *via* anthropogenic vectors (Dupont *et al*. 2009; Darling *et al*. 2009; Darling & Folino-Rorem 2009).

On the East coast, genetic differentiation increases with increasing spatial separation between sites (Figs. 2.3, 2.4C, Appendix S2.7). High genetic similarity was detected between adjacent sites (i.e. 1.5 km apart) such as MCV - BKH (F_{ST} = 0.01) or LNB - BKH (F_{ST} = 0.02; Appendix S2.7), while high genetic divergence was observed between more spatially separated sites (i.e. 150 - 250 km apart) such as CRV - SBR (F_{ST} = 0.12) or PDG - SBR (F_{ST} = 0.16; Appendix S2.7). Also, STRUCTURE analysis revealed that East coast genotypes were assigned to different clusters in the northern and the southern parts of the sampling region, with assignment ratios following a gradual transition along the north-south axis (Fig. 2.4C). Consequently, a highly significant pattern of isolation-by-distance was detected for East coast populations (r^2 = 0.4667; P < 0.001; Fig. 2.5C).

This finding was surprising considering that strong associations between the two metrics are typically considered indicative of systems under migration-drift equilibrium (Hutchinson & Templeton 1999). However, historical evidence suggests that East coast *B. violaceus* is unlikely to have achieved such equilibrium. The species is thought to have been introduced in 1972 at Woods Hole, Massachusetts (Carlton 1989), and was not reported in Nova Scotia until approximately 30 years later, in 2001 (Carver *et al*. 2006). Therefore, most populations analysed here likely represent relatively recent introductions, and the assumption of equilibrium is unreasonable. The strong isolation-by-distance pattern is most likely a result of stepping-stone spread. Historical records also indicate that *B. violaceus* spread gradually along the East coast, facilitated by human-mediated vectors (Carver *et al*. 2006; Dijkstra *et al*. 2007; Locke *et al*. 2009). Still, I did not observe a cline in microsatellite genetic variation along the coast. The bottleneck signature expected at the periphery of the invasion front was most likely obscured as a result of gene flow between sites in the northern part of the East coast (discussed below).

Evidence presented here indicates that natural dispersal is not a major contributor to ascidian spread on either coast. Most populations show high levels of genetic differentiation, reflecting a general restriction to natural spread (Table 2.3; Appendix S2.7). Also, the observed regional invasion patterns do not appear to be associated with the dynamics of marine currents. Most sites on the West coast were located in the Strait of Georgia, which is characterized by currents of low intensity (LeBlond 1983) and thus not likely to drive long distance dispersal. Likewise, on the East coast, the southern flowing Nova Scotia current (Hannah *et al*. 2001) appears to be at odds with

observational records indicating the invasion spread northward on the coast (Dijkstra *et al*. 2007; Locke *et al*. 2009).

Therefore, the divergent patterns of spread observed between the two coasts appear to be influenced at least partially by differences in anthropogenic vector dynamics. More specifically, evidence for long distance dispersal was more frequently observed throughout the sampling region on the West coast (Table 2.3; Fig. 2.5). Although evidence for human-mediated 'jump' dispersal was also detected on the East coast, these events appear to be more spatially restricted. Low F_{ST} values (0.01 - 0.02; Appendix S2.7) were detected between four northern sites separated by 15 - 200 km (SPB, SHB, CRV and APY; Fig. 2.1), which also share the majority of identified East coast migrant genotypes (32/38; Table 2.3). Previous studies of other prominent aquatic invaders, such as the zebra mussel (*Dreissena polymorpha*) have shown that patterns of long distance dispersal can be correlated to the spatial and temporal variation of human-mediated vectors like recreational vessels (Bossebroek *et al*. 2007). Indeed, a possible explanation for my findings might be that the number of recreational vessels currently registered in British Columbia is significantly higher than in Nova Scotia (Dr. T. Therriault, personal communication) providing more opportunities for long distance spread. Also, ice formations during winter in Nova Scotia significantly limit vessel traffic between sites, whereas in British Columbia shipping occurs year round, providing more opportunities for dispersal of *B. violaceus* propagules *via* hull fouling or ballast water transfer. Apart from vessel-mediated vectors, aquaculture is also considered a primary means of introduction and spread of *B. violaceus* (Dijkstra *et al*. 2007). This vector most likely is responsible for long distance dispersal events observed in northern range areas on the

East coast. Previous studies have suggested that transfer of living material and equipment between mussel growers in the region is the main contributor to the spread of invasive tunicates, including *B. violaceus* (Locke *et al*. 2009).

Contribution of vegetative proliferation to the spread of *B. violaceus*

Theory predicts that asexual reproduction can substantially facilitate invasion success, especially during the introduction stage, when population size is small (Sakai *et al*. 2001). Although successful colonization by clonal lineages has been demonstrated in a number of widespread invaders (e.g. *Daphnia pulex* in Africa, Mergeay *et al*. 2006), in other cases asexual reproduction has a more limited role in post-establishment spread (e.g. *Cordylophora caspia* in North America, Darling & Folino-Rorem 2009). Dispersal of colonial ascidians can occur either through sexual propagules (larvae and adult colonies) or asexually-derived fragments. Since the larval stage is very short, often lasting only minutes to a few hours (Lambert & Lambert 2003), and settled adults have limited mobility (Lambert 2005), the generation and spread of colony fragments has often been cited as a potentially important mechanism of secondary spread (Lambert 2005; Carver *et al*. 2006). Additionally, a recent study demonstrated that three of the most notorious invasive colonial ascidians (*Botrylloides violaceus*, *Botryllus schlosseri* and *Didemnum vexillum*) can easily reattach to substrata following fragmentation under laboratory conditions (Bullard *et al*. 2007).

My genetic analyses of 25 established populations suggest that although dispersal of *B. violaceus* fragments is limited, it can be prevalent near infested aquaculture facilities. The sampling design allowed me to investigate the extent of fragment dispersal mostly at

regional (i.e. along approximately 500 km of coastline on the West and East coasts; Fig. 2.1) and finer scales (i.e. within sampling locations). At the regional scale, the importance of fragment dispersal was limited. Only genotype C4 was sampled independently at Deep Bay and Ladysmith, locations situated approximately 90 km apart (Appendix S2.4). Since these two populations were collected from local marinas, hull fouling is the most likely vector that could have facilitated exchange of colonies between sites. Ships heavily fouled with *B. violaceus* have been observed in other locations, such as Prince Edward Island (Locke *et al*. 2009) and Newfoundland (Callahan *et al*. 2010), and are a primary vector responsible for the spread of colonial ascidians (Dijkstra *et al.* 2007).

The majority of multilocus genotypes (26/27) were restricted at smaller spatial scales, within populations, where each clone was encountered between one and seven times (Appendix S2.4). The highest number of clones (7) was identified for the Lemmens Inlet (LMI) population on the West coast, which was sampled in an infested aquaculture facility. The same pattern was observed on the East coast, where most clones were detected in aquaculture sites such as Cardigan River (CRV; 4 clones) and Saint Peter's Bay (SPB; 3 clones). This pattern may be a consequence of the removal of *B. violaceus* colonies (and other fouling organisms) from contaminated aquaculture gear using high-pressure seawater, a common practice that may in fact facilitate fragmentation (Paetzold & Davidson 2010). An increase in local infestations and spread through surviving asexual propagules may, therefore, be expected if this management strategy is used to counteract problems associated with ascidian invaders in aquaculture facilities.

On the invasion history of *B. violaceus* in North America

The observed distribution of genetic variation between the West and East coasts allows me to formulate several conclusions regarding the invasion history of *B. violaceus* in North America. On the West coast, California and Northwest populations are highly genetically diferentiated (Fig. 2.4A, Appendices S2.3, S2.7). Two competing scenarios are compatible with this strong genetic structure: multiple, independent introductions from genetically diferentiated populations from the native range, or *in situ*, gradual divergence following a single colonization. Overall, historical records appear to support the scenario of multiple West coast introductions. Although the first confirmed North American occurrence of the species is attributed to southern California in the early 1970s (Fay & Vallee 1979), *B. violaceus* was recorded at about the same time in surveys conducted approximately 1,500 km north at Puget Sound (Dr. G. Lambert, personal communication). Alternatively, the limited natural dispersal capacity of *B. violaceus* could provide ample opportunities for *in situ* differentiation following a single introduction, driven by genetic drift. This scenario is less likely, given that similar genetic differentiation did not evolve between the California and East coast samples (also founded in the 1970s, Carlton 1989), which, on the contrary, show a high degree of genetic resemblance (see below).

On the East coast, all sampled populations were genetically similar to Bodega Bay (Figs. 2.3, 2.4A). This finding suggests that the East coast invasion was founded either from California or from a native population that is genetically similar to Bodega Bay. Historical records support a West coast origin of the East coast invasion, and indicate the initial introduction occurred in 1972, when approximately 20 microscope slides with *B.*

violaceus from southern California were suspended from the Marine Biological Laboratory Dock in Woods Hole, Massachusetts, for studies of historecognition (Carlton 1989; Yund & Feldgarden 1992; Grosholz 2001). Considering that prior to this date the conspicuous bright orange colonies of the species were not recorded in the region (Van Name 1945), it is likely that this experiment coincided with the actual introduction event. Although the assignment test indicated that contemporary transport of *B. violaceus* propagules from the West to the East coast might still occur (Table 2.3), this possibility needs to be investigated further by studies undertaking a broader geographic coverage of samples, including a detailed, comprehensive coverage of the native range. Results presented here should serve as stimulus for future research aiming to resolve this issue and clarify the colonization history of this globally invasive species.

2.5 CONCLUSIONS

My results provide strong evidence that the invasion of *B. violaceus* in North America was linked to highly contrasting patterns of post-establishment spread within the two sampling regions analysed. Since similar patterns may very well have been shaped in other invasive species, I highlight the necessity of considering multiple invaded spatial ranges in genetic surveys of NIS. Evidence presented here indicates that colony fragmentation and regeneration may have a limited contribution to the regional dispersal of colonial ascidians. However, in aquaculture facilities, the treatment of fouled equipment with high pressure seawater, unless performed on land, may inadvertently lead to an increase in local infestations and should be avoided.

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Table 2.1: Locations of *Botrylloides violaceus* sampling and genetic diversity indices for mitochondrial and microsatellite markers with N_C, sample size including clonal genotypes; *N*, sample size after removal of clones; *Nh*, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity; N_A, number of alleles; N_{AP}, the number of private alleles for each sampling site; *A*, allelic richness; H_O, mean observed heterozygosity; H_E, mean expected heterozygosity.

Location	**ID**	**mtDNA**						**Microsatellite**						
		N_C	*N*	*Nh*	**Haplotype codes**	*h*	π	N_C	*N*	N_A	N_{AP}	*A*	H_O	H_E
West Coast														
Deep Bay, BC	DPB	22	18	3	Bv1; Bv2; Bv10	0.386	0.007	19	15	53	2	3.3	0.568	0.576
French Creek, BC	FRC	24	21	2	Bv1; Bv2	0.095	0.001	17	14	51	1	3.3	0.571	0.569
Lemmens Inlet, BC	LMI	48	37	4	Bv1; Bv2; Bv3; Bv11	0.417	0.004	53	42	90	3	4.0	0.511	0.612
Ladysmith, BC	LSM	26	21	4	Bv1; Bv2; Bv8; Bv9	0.610	0.010	20	15	49	0	3.2	0.429	0.601
Sydney, BC	SYD	32	31	1	Bv1	0.000	0.000	32	31	61	1	3.4	0.434	0.554
Victoria, BC	VIC	31	30	1	Bv1	0.000	0.000	32	31	68	2	3.7	0.562	0.643
Lopez Island, WA	LPI	30	30	3	Bv1; Bv2; Bv3	0.522	0.008	30	30	77	2	3.8	0.472	0.596
Neah Bay, WA	NHB	30	30	3	Bv1; Bv2; Bv3	0.503	0.008	30	30	89	1	4.4	0.545	0.704
Sequin, WA	SQN	30	30	1	Bv1	0.000	0.000	30	30	83	1	4.0	0.553	0.641
Brinnon, WA	BRN	30	30	3	Bv1; Bv3; Bv9	0.549	0.009	28	28	75	5	4.0	0.517	0.621
Bodega Bay, CA	BBY	35	35	2	Bv1; Bv8	0.363	0.001	34	34	86	4	3.9	0.487	0.604
Total		**338**	**313**	**7**		**0.313**	**0.005**	**325**	**300**	**155**	**22**	**3.7**	**0.514**	**0.611**
East Coast														
St. Peter's Bay, PEI	SPB	38	33	1	Bv1	0.000	0.000	44	39	57	0	3.3	0.468	0.561
Savage Harbour, PEI	SHB	45	43	1	Bv1	0.000	0.000	50	50	62	0	3.5	0.613	0.610

Cardigan River, PEI	CRV	42	38	1	Bv1	0.000	0.000	45	41	64	1	3.5	0.531	0.600
Aspy Bay, NS	APY	29	29	2	Bv1; Bv3	0.133	0.002	29	29	57	0	3.4	0.545	0.601
South Bar, NS	SBR	30	29	2	Bv1; Bv3	0.133	0.002	28	27	59	1	3.5	0.484	0.599
Petit de Grat, NS	PDG	23	23	2	Bv1; Bv3	0.166	0.002	24	24	60	0	3.5	0.567	0.604
Chester, NS	CHT	12	12	1	Bv1	0.000	0.000	13	13	49	0	3.2	0.518	0.561
Martin's Cove, NS	MCV	15	15	1	Bv1	0.000	0.000	17	17	51	0	3.4	0.526	0.621
Black Harbour, NS	BKH	13	12	1	Bv1	0.000	0.000	16	15	55	3	3.6	0.554	0.652
Lunenburg, NS	LNB	20	20	2	Bv1; Bv3	0.100	0.002	22	22	67	1	3.9	0.515	0.662
Methegan, NS	MTG	7	7	1	Bv1	0.000	0.000	7	7	39	0	3.0	0.478	0.576
Lockeport, NS	LKP	8	8	1	Bv1	0.000	0.000	8	8	54	1	3.8	0.461	0.612
Ingomar, NS	IGM	7	7	1	Bv1	0.000	0.000	10	10	51	0	3.5	0.472	0.579
Mystic Harbor, CT	MYH	17	15	1	Bv1	0.000	0.000	20	18	52	0	3.2	0.532	0.559
Total		**306**	**291**	**2**		**0.038**	**0.001**	**335**	**320**	**110**	**7**	**3.4**	**0.518**	**0.600**

Table 2.2: Analysis of molecular variance (AMOVA) results on *Botrylloides violaceus* mtDNA and microsatellite data for East vs. West coast population grouping. All fixation indices are statistically significant.

Source of variation	d.f.	Variance components	% variation	Fixation indices	*P* value
mtDNA					
Between coasts	1	0.295 Va	18.25	F_{CT}: 0.182	0.0332
Among populations within coasts	23	0.538 Vb	33.30	F_{SC}: 0.407	0.0000
Within populations	579	0.782 Vc	48.46	F_{ST}: 0.515	0.0000
Microsatellite					
Between coasts	1	0.122 Va	6.42	F_{CT}: 0.064	0.0000
Among populations within coasts	23	0.208 Vb	10.93	F_{SC}: 0.117	0.0000
Within populations	1215	1.570 Vc	82.65	F_{ST}: 0.173	0.0000

Table 2.3: Results of assignment test, with source populations listed by column and recipient populations by row. Populations on the West coast are separated by a box in the upper left corner. Individuals assigned to the sampling site where they were collected are indicated in bold along the diagonal. Populations with sample size of less than 10 individuals ($N < 10$) were not included.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
1. DPB	**14**	1																					
2. FRC		**13**		1																			
3. LMI			**38**				1	1		2													
4. LSM	6			**9**																			
5. SYD	2			2	**24**					2													
6. VIC	1					**29**		1															
7. LPI	2						**26**			2													
8. NHB								**29**	1														
9. SQN								2	**28**														
10. BRN										**28**													
11. BBY								1			**32**					1							
12. SPB												**33**	3	3									
13. SHB								1				1	**43**	3	2								
14. CRV												4	1	**36**									
15. APY												9	6		**14**								
16. SBR												1				**26**							
17. PDG																	**22**		1				
18. CHT																		**13**					
19. MCV																			**17**				
20. BKH																				**14**	1		
21. LNB								2			2								2		**16**		
22. IGM											1											**9**	
23. MYH											3												**15**

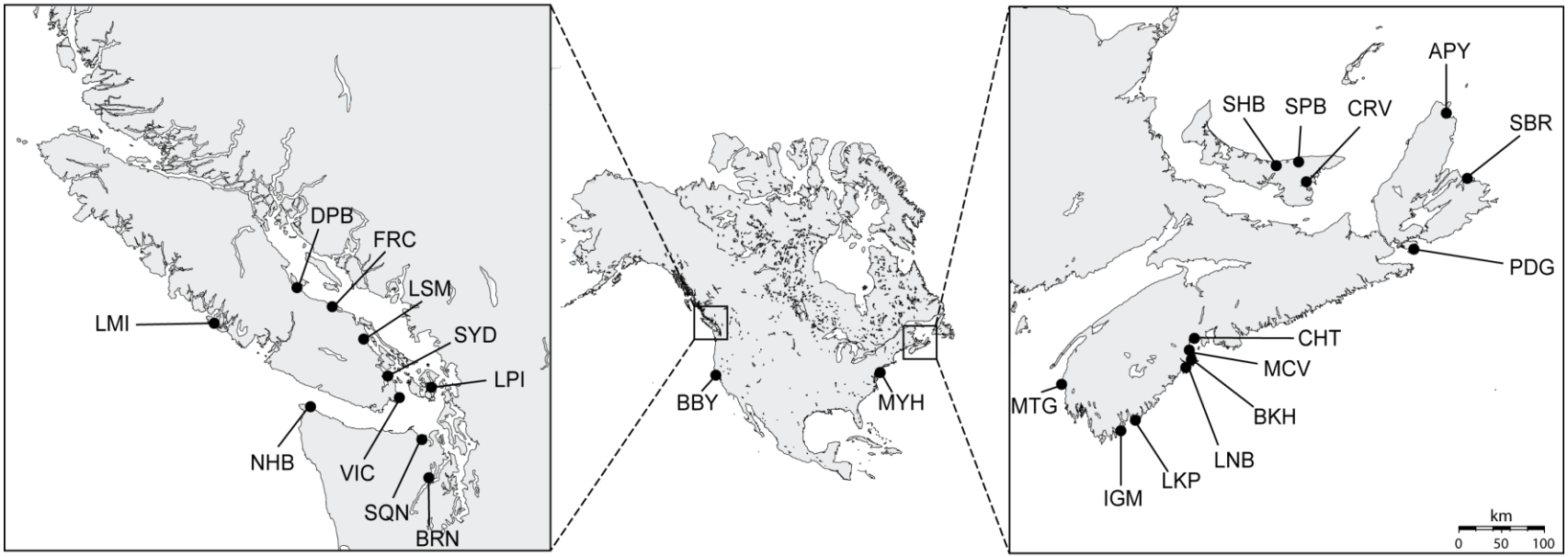


Figure 2.1 - Sampling locations for the violet tunicate *Botrylloides violaceus* on the West and East coasts of North America, with locality names defined in Table 2.1. Inset maps show the focal sampling regions on each coast.

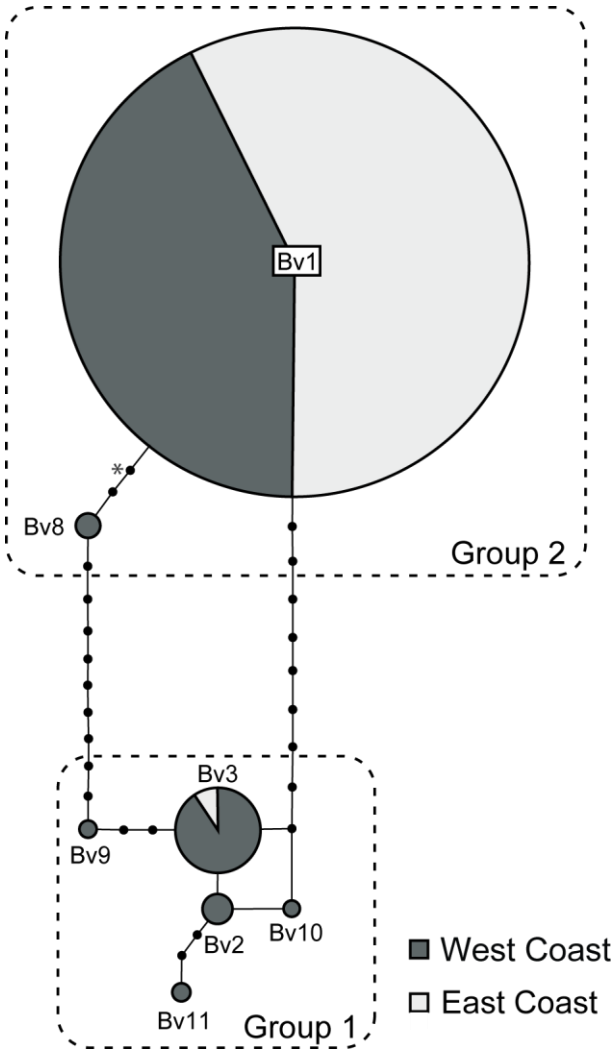


Figure 2.2 - Statistical parsimony network of *Botrylloides violaceus* cytochrome *c* oxidase subunit I (COI) haplotypes. Circle size is proportional to haplotype frequency, and small black dots indicate unsampled haplotypes inferred from the data. The nonsynonymous substitution is indicated by an asterisk. Groups 1 and 2 correspond to clades well supported by the neighbour-joining and maximum likelihood phylogenetic analyses (Appendix S2.2).

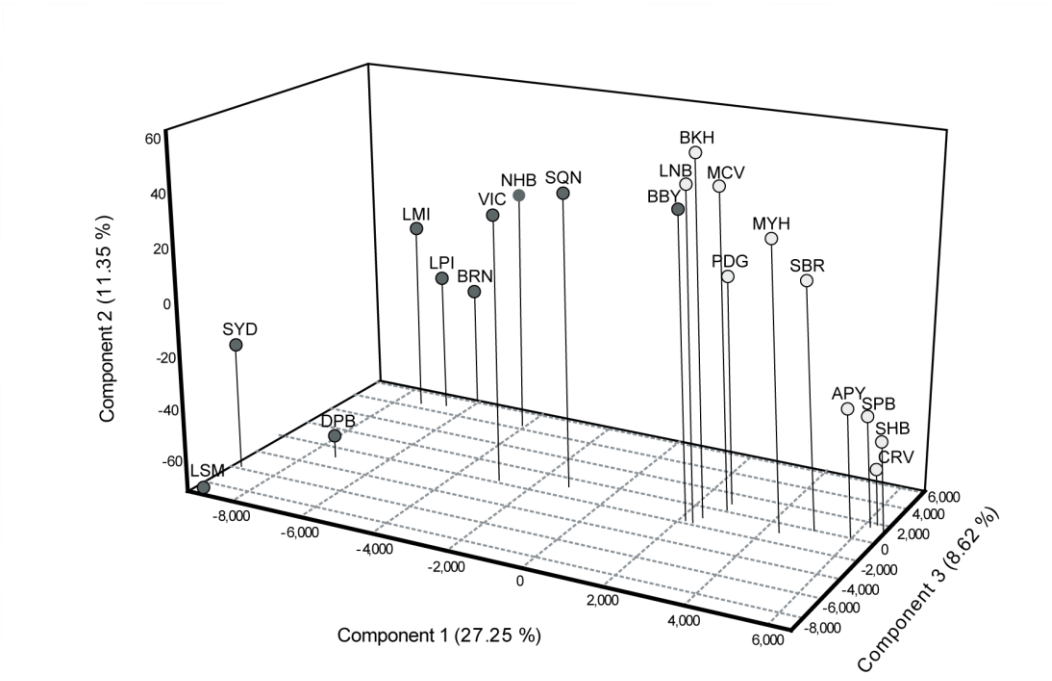


Figure 2.3 - Three dimensional factorial correspondence analysis (3D-FCA) of *Botrylloides violaceus* microsatellite data showing clustering between North American West coast (dark grey) and East coast (light grey) sites. Bodega Bay population (BBY) clusters tightly in the analysis with East coast populations. Sampling sites with less than 15 individuals ($N < 15$) were not considered for this analysis.

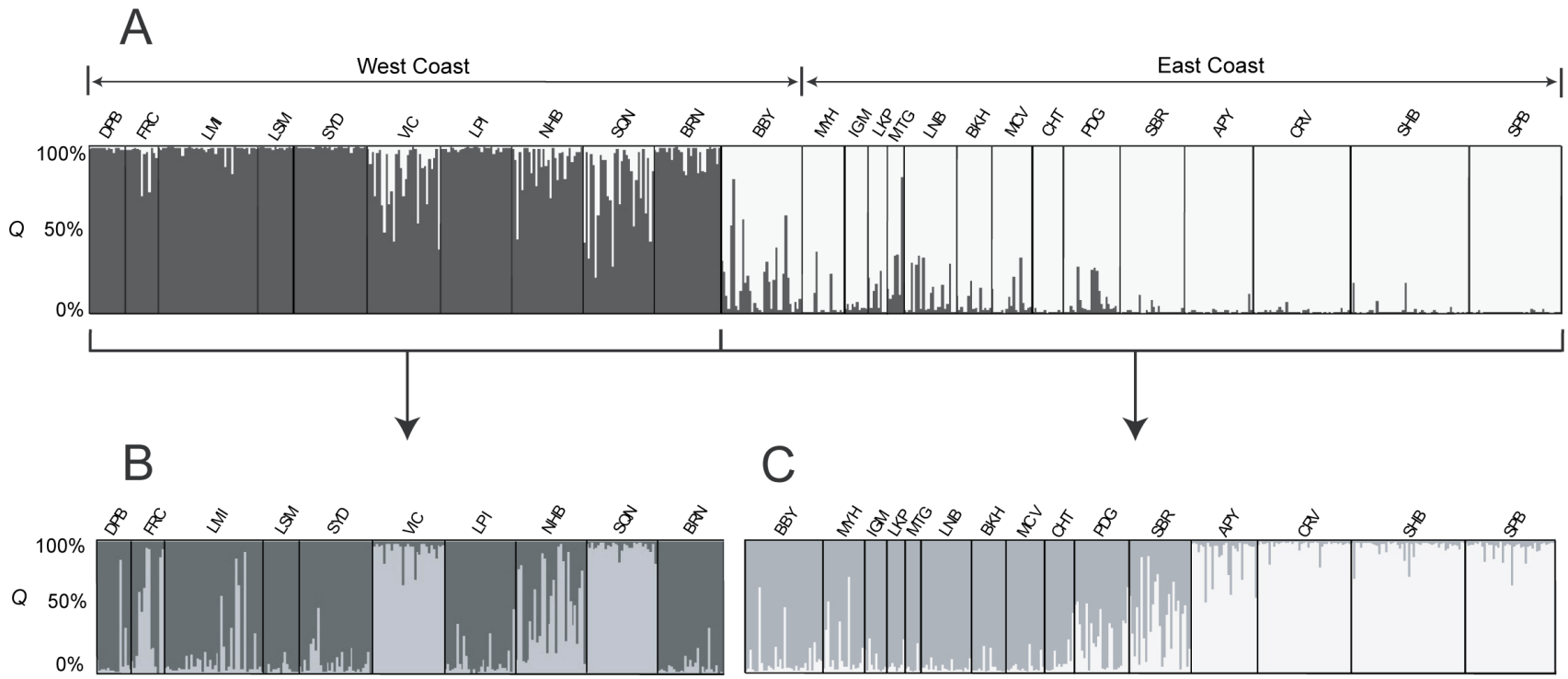


Figure 2.4 - Bayesian clustering of *Botrylloides violaceus* genotypes performed in STRUCTURE for all samples (A) and each of the two main genetic clusters (B; C). Each individual is represented by a thin vertical line, which is partitioned into $K = 2$ segments representing the individual's estimated proportional membership (Q). Sampling sites are separated by black lines. For (A) dark grey corresponds to cluster S1 and light grey to cluster S2. Different shades of grey are used to represent two genetic clusters in each data subset.

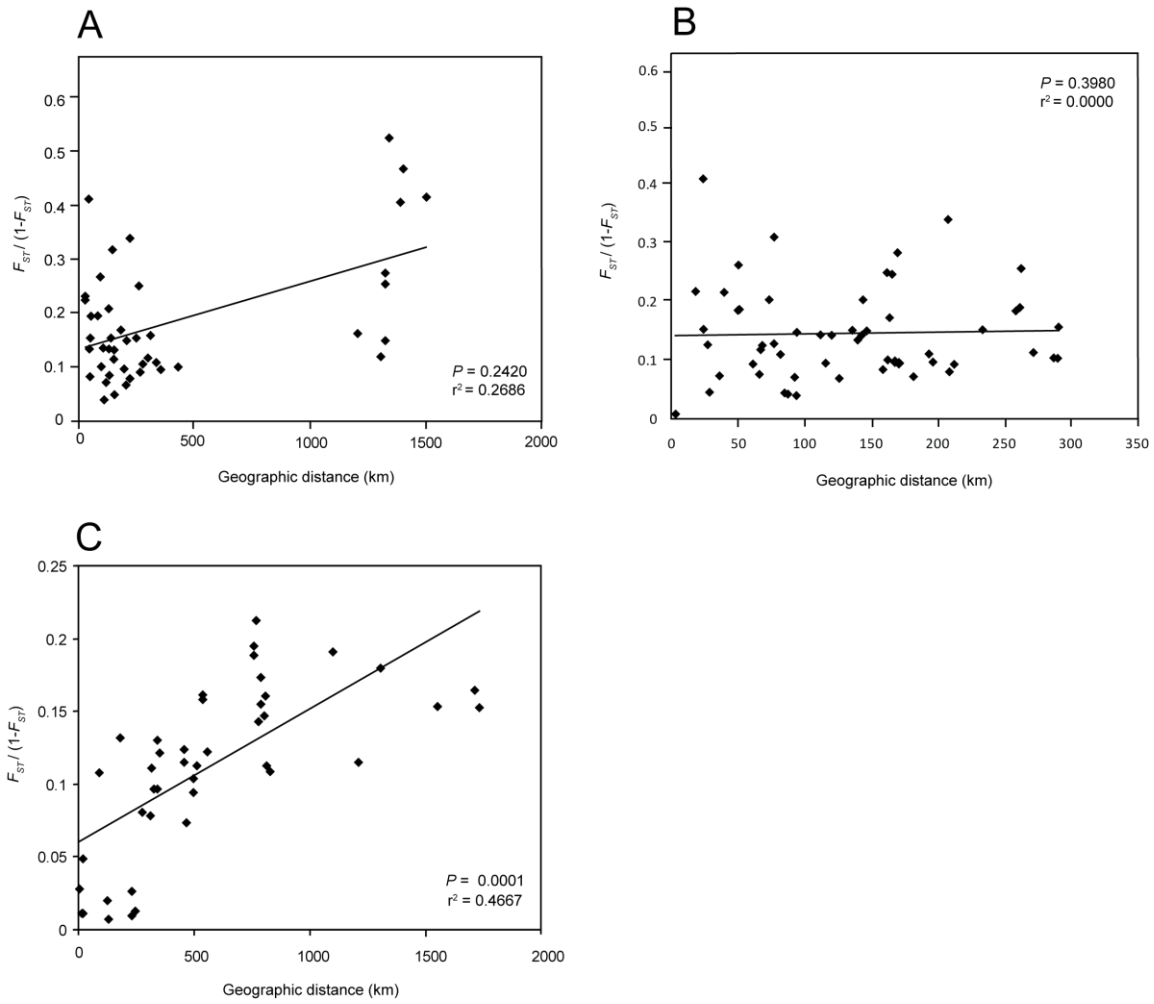
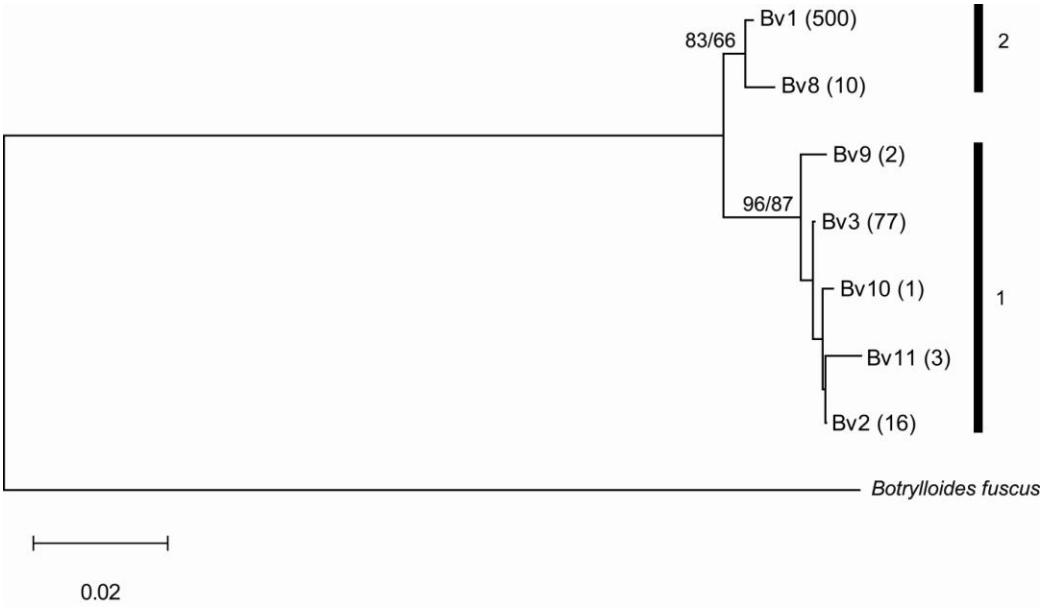


Figure 2.5 - Results of isolation by distance (IBD) analysis performed using microsatellite data for West coast (A), Northwest coast (B), and East coast (C) *Botrylloides violaceus* populations.

Appendix S2.1: Occurrence and frequency of *Botrylloides violaceus* mtDNA COI haplotypes for 25 North American locations.

Location	Haplotype						
	Bv1	**Bv2**	**Bv3**	**Bv8**	**Bv9**	**Bv10**	**Bv11**
West Coast							
Deep Bay, BC	0.78	0.17	-	-	-	0.05	-
French Creek, BC	0.95	0.05	-	-	-	-	-
Lemmens Inlet, BC	0.11	0.05	0.76	-	-	-	0.08
Ladysmith, BC	0.57	0.28		0.10	0.05	-	-
Sydney, BC	1.00	-	-	-	-	-	-
Victoria, BC	1.00	-	-	-	-	-	-
Lopez Island, WA	0.37	0.03	0.60	-	-	-	-
Neah Bay, WA	0.63	0.03	0.33	-	-	-	-
Sequin, WA	1.00	-	-	-	-	-	-
Brinnon, WA	0.50	-	0.47	-	0.03	-	-
Bodega Bay, CA	0.77	-	-	0.23	-	-	-
East Coast							
St. Peter's Bay, PEI	1.00	-	-	-	-	-	-
Savage Harbour, PEI	1.00	-	-	-	-	-	-
Cardigan River, PEI	1.00	-	-	-	-	-	-
Aspy Bay, NS	0.93	-	0.07	-	-	-	-
South Bar, NS	0.93	-	0.07	-	-	-	-
Petit de Grat, NS	0.91	-	0.09	-	-	-	-
Chester, NS	1.00	-	-	-	-	-	-
Martin's Cove, NS	1.00	-	-	-	-	-	-
Black Harbour, NS	1.00	-	-	-	-	-	-
Lunenburg, NS	0.95	-	0.05	-	-	-	-
Methegan, NS	1.00	-	-	-	-	-	-
Lockeport, NS	1.00	-	-	-	-	-	-
Ingomar, NS	1.00	-	-	-	-	-	-
Mystic Harbor, CT	1.00	-	-	-	-	-	-

Appendix S2.2: Neighbor-joining phylogenetic reconstruction of *Botrylloides violaceus* cytochrome *c* oxidase subunit I (COI) haplotypes. Numbers at phylogenetic nodes indicate the neighbor-joining and maximum-likelihood bootstrap support with 1,000 replicates. The number of samples possessing each haplotype is presented in brackets.



Appendix S2.3: Pairwise Φ_{ST} comparisons for *Botrylloides violaceus* populations using the COI marker. *Significant ($P < 0.05$); ** remains significant after sequential Bonferroni correction (Rice 1989). Populations on the East coast are separated by a box in the upper left corner.

	SPB	SHB	CRV	APY	SBR	PDG	CHT	MCV	BKH	LNB	MTG	LKP	IGM	MYH	DPB	FRC	LMI	LSM	SYD	VIC	LPI	NHB	SQN	BRN
SPB																								
SHB	0.00																							
CRV	0.00	0.00																						
APY	0.04	0.06	0.05																					
SBR	0.04	0.06	0.05	-0.04																				
PDG	0.07	0.09	0.08	-0.04	-0.04																			
CHT	0.00	0.00	0.00	-0.01	-0.01	0.00																		
MCV	0.00	0.00	0.00	0.00	0.00	0.02	0.00																	
BKH	0.00	0.00	0.00	-0.01	-0.01	0.00	0.00	0.00																
LNB	0.03	0.04	0.03	-0.04	-0.04	-0.04	-0.03	-0.02	-0.03															
MTG	0.00	0.00	0.00	-0.05	-0.05	-0.04	0.00	0.00	0.00	-0.07														
LKP	0.00	0.00	0.00	-0.04	-0.04	-0.02	0.00	0.00	0.00	-0.06	0.00													
IGM	0.00	0.00	0.00	-0.05	-0.05	-0.04	0.00	0.00	0.00	-0.07	0.00	0.00												
MYH	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	-0.02	0.00	0.00	0.00											
DPB	0.25*	0.29*	0.27*	0.07	0.07	0.03	0.13	0.15	0.13	0.08	0.07	0.09	0.07	0.15										
FRC	0.02	0.04	0.03	-0.04	-0.04	-0.03	-0.03	-0.02	-0.03	-0.05	-0.07	-0.06	-0.07	-0.02	0.08									
LMI	0.85**	0.86**	0.86**	0.76**	0.76**	0.74**	0.79**	0.80**	0.79**	0.77**	0.77**	0.78**	0.77**	0.80**	0.59**	0.77**								
LSM	0.34**	0.39**	0.36**	0.18*	0.18*	0.14*	0.21*	0.23*	0.21*	0.18*	0.15	0.17	0.15	0.23	-0.01	0.18*	0.44**							
SYD	0.00	0.00	0.00	0.04	0.04	0.07	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.24*	0.02	0.84**	0.33**						
VIC	0.00	0.00	0.00	0.04	0.04	0.06	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.24*	0.02	0.84**	0.33**	0.00					
LPI	0.63**	0.67**	0.65**	0.50**	0.50**	0.45**	0.52**	0.54**	0.52**	0.49**	0.48*	0.49*	0.48*	0.54**	0.26*	0.50**	0.13*	0.13*	0.62**	0.62**				
NHB	0.35**	0.39**	0.37**	0.20*	0.20*	0.16*	0.24*	0.26*	0.24*	0.21*	0.20*	0.21*	0.20*	0.26*	0.01	0.21*	0.42**	-0.01	0.35**	0.34*	0.10			
SQN	0.00	0.00	0.00	0.04	0.04	0.06	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.24*	0.02	0.84**	0.33**	0.00	0.00	0.62**	0.34**		
BRN	0.49**	0.53**	0.51**	0.34*	0.34*	0.29*	0.37*	0.39*	0.37*	0.34*	0.32*	0.33*	0.32*	0.39*	0.12*	0.35*	0.27*	0.03	0.48**	0.47**	0.00	0.00	0.47**	
BBY	0.20*	0.23*	0.21*	0.12*	0.12*	0.12*	0.12	0.13	0.12	0.12*	0.08	0.09	0.08	0.13	0.22*	0.12*	0.81**	0.28**	0.19*	0.19*	0.59**	0.33**	0.19*	0.45**

Appendix S2.4: Clonal genotypes observed in the dataset with *N*, number of times the genotype appears in the dataset; N_{gen}, number of genotypes in the population of origin; P_{sib}, probability of identity considering strict sibs reproduction; P_{HW}, probability of identity under Hardy-Weinberg equilibrium. Clonal genotypes shared between locations are indicated with an asterisk.

Genotype	Location	*N*	N_{gen}	P_{sib}	P_{HW}
West Coast					
C1 - DPB	Deep Bay	3	19	0.007	6.88×10^{-8}
C2 - DPB	Deep Bay	2	19	0.005	9.27×10^{-9}
C3 - DPB	Deep Bay	2	19	0.010	1.63×10^{-6}
C4 - DPB*	Deep Bay	1	19	0.006	3.75×10^{-8}
	Ladysmith	1	21		
C5 - FRC	French Creek	2	17	0.004	1.29×10^{-8}
C6 - FRC	French Creek	2	17	0.009	7.06×10^{-7}
C7 - FRC	French Creek	2	17	0.003	4.66×10^{-11}
C8 - LMI	Lemmens Inlet	2	53	0.009	9.31×10^{-7}
C9 - LMI	Lemmens Inlet	2	53	0.011	3.41×10^{-7}
C10 - LMI	Lemmens Inlet	2	53	0.010	5.97×10^{-7}
C11 - LMI	Lemmens Inlet	8	53	0.012	2.60×10^{-6}
C12 - LMI	Lemmens Inlet	2	53	0.007	1.89×10^{-8}
C13 - LSM	Ladysmith	2	21	0.002	3.70×10^{-11}
C14 - LSM	Ladysmith	2	21	0.004	1.06×10^{-9}
C15 - LSM	Ladysmith	2	21	0.007	2.36×10^{-7}
C16 - LSM	Ladysmith	2	21	0.012	4.73×10^{-7}
C17 - LSM	Ladysmith	2	21	0.005	3.48×10^{-9}
C18 - SYD	Sydney	2	32	0.006	1.38×10^{-8}
C19 - VIC	Victoria	2	32	0.009	1.00×10^{-6}
East Coast					
C20 - SPB	Saint Peter's Bay	2	44	0.012	5.22×10^{-6}
C21 - SPB	Saint Peter's Bay	3	44	0.008	2.69×10^{-6}
C22 - SPB	Saint Peter's Bay	3	44	0.009	1.19×10^{-6}
C23 - CRV	Cardigan River	4	45	0.006	3.04×10^{-7}
C24 - CRV	Cardigan River	2	45	0.005	2.90×10^{-8}
C25 - SBR	South Bar	2	28	0.010	4.47×10^{-6}
C26 - BKH	Black Harbour	2	16	0.002	1.93×10^{-12}
C27 - MYH	Mystic Harbour	3	20	0.011	5.34×10^{-6}

Appendix S2.5: Microsatellite allele frequency for *Botrylloides violaceus* from 25 locations in the North American invaded range

Locus	Allele	West Coast											East Coast													
		DPB	FRC	LMI	LSM	SYD	VIC	LPI	NHB	SQN	BRN	BBY	SPB	SHB	CRV	APY	SBR	PDG	CHT	MCV	BKH	LNB	MTG	LKP	IGM	MYH
Bv2	188	0.07	-	-	-	0.02	-	0.03	-	-	-	-	-	0.03	0.03	-	-	-	-	-	-	-	-	-	-	-
	191	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	197	0.04	-	0.01	-	-	-	0.03	0.02	0.06	0.04	0.03	-	-	0.01	-	0.02	-	-	0.07	0.11	0.05	0.29	-	0.10	-
	200	0.04	-	-	-	-	-	-	-	0.02	-	-	0.04	-	-	-	-	-	-	-	0.07	-	-	-	0.05	-
	203	0.04	-	0.01	-	0.02	0.48	-	0.08	0.17	-	0.52	0.64	0.50	0.53	0.64	0.69	0.94	0.65	0.70	0.39	0.41	0.36	0.56	0.45	0.75
	207	-	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	210	-	-	-	-	-	-	0.02	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	212	0.32	0.27	0.64	0.27	0.42	0.18	0.47	0.38	0.11	0.30	0.40	0.32	0.29	0.21	0.26	0.29	0.04	0.35	0.23	0.43	0.55	0.36	0.44	0.40	0.25
	215	-	-	0.07	-	0.31	-	-	-	0.15	0.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	218	0.11	0.27	0.04	0.27	0.23	0.24	0.05	0.26	0.46	0.07	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	221	0.39	0.46	0.23	0.47	0.02	0.10	0.40	0.24	0.04	0.35	0.03	-	0.18	0.22	0.10	-	0.02	-	-	-	-	-	-	-	-
Bv4	172	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	178	0.11	-	0.21	0.03	0.11	0.18	0.45	0.35	0.13	0.61	0.14	0.12	0.15	0.28	0.03	0.11	0.06	-	-	-	0.05	0.29	0.25	0.20	0.25
	180	0.07	0.27	0.07	0.20	0.29	0.03	0.15	0.06	0.04	0.13	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-
	182	-	-	0.04	-	-	0.05	-	0.07	0.04	-	0.03	-	-	-	-	0.09	0.25	0.62	0.24	0.10	0.23	0.07	0.38	0.05	0.25
	184	0.21	0.08	0.17	0.23	0.05	0.07	0.23	0.11	0.43	0.09	0.11	0.42	0.48	0.43	0.62	0.02	0.40	0.19	0.24	0.23	0.16	-	0.25	0.15	0.06
	186	0.50	0.62	0.51	0.53	0.55	0.68	0.17	0.39	0.38	0.18	0.70	0.46	0.35	0.24	0.35	0.69	0.29	0.19	0.53	0.67	0.57	0.64	0.13	0.60	0.33
	188	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	194	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	0.07	-	-	-	-	-	-	-	-	-
	196	0.04	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	198	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	200	-	-	-	-	-	-	-	-	-	-	-	-	0.02	0.01	-	-	-	-	-	-	-	-	-	-	0.06
	212	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	0.06
Bv5	187	-	0.15	0.10	-	0.02	0.11	0.20	0.22	0.10	0.19	0.27	0.15	0.11	0.04	0.09	0.18	0.04	0.08	-	0.04	0.10	-	0.07	-	0.09
	189	-	-	-	-	-	0.13	-	0.05	0.38	-	0.13	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-
	191	-	-	-	-	-	0.05	-	-	0.02	-	0.08	0.28	0.44	0.34	0.41	0.05	0.13	0.33	0.41	0.29	0.28	-	-	0.11	0.12
	193	-	-	0.04	-	-	-	0.04	-	0.02	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	195	0.50	-	0.02	0.50	0.10	0.03	0.04	0.08	0.12	0.19	0.03	0.05	0.11	0.22	-	-	-	0.21	-	-	0.05	-	-	0.06	0.06

	197	0.50	0.55	0.72	0.42	0.65	0.45	0.68	0.43	0.24	0.35	0.12	0.01	0.03	-	0.07	0.33	0.44	0.04	0.09	0.18	0.33	0.50	0.29	0.17	0.21
	199	-	-	0.04	0.08	0.24	-	-	-	-	-	0.10	-	-	-	-	0.08	0.07	0.17	0.03	0.14	0.10	-	-	0.39	-
	201	-	0.30	0.09	-	-	0.23	0.05	0.22	0.12	0.23	0.27	0.50	0.29	0.41	0.43	0.38	0.33	0.17	0.47	0.36	0.15	0.50	0.64	0.28	0.53
Bv6	173	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	203	-	-	0.07	-	-	0.03	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	215	0.46	0.15	0.46	0.21	0.13	0.44	0.73	0.28	0.10	0.45	0.02	0.18	0.18	0.24	0.17	0.08	0.17	0.04	0.38	0.37	0.16	-	0.14	-	-
	217	0.21	0.23	0.40	0.50	0.76	0.42	0.10	0.52	0.87	0.55	0.72	0.44	0.25	0.34	0.31	0.60	0.59	0.77	0.44	0.47	0.46	0.67	0.71	0.85	0.59
	219	0.14	0.04	0.01	0.11	0.11	-	-	0.10	-	-	0.09	-	-	-	-	-	0.15	0.04	0.09	0.17	0.02	0.17	-	0.05	-
	221	0.18	0.27	0.01	0.18	-	0.11	0.05	-	-	-	0.03	0.10	0.15	0.08	0.22	0.25	0.04	-	-	-	0.16	0.08	0.07	-	0.15
	223	-	0.08	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	229	-	0.23	0.04	-	-	-	0.12	0.10	-	-	0.10	0.28	0.43	0.34	0.29	0.08	0.04	0.15	0.09	-	0.21	0.08	0.07	0.10	0.27
Bv7	112	0.10	0.38	0.10	0.07	0.08	0.19	0.29	0.08	0.17	0.29	0.14	0.10	0.03	0.05	-	-	-	-	-	-	-	-	-	-	0.08
	114	-	-	-	-	-	-	0.04	0.02	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	118	0.05	-	0.05	-	-	0.13	-	0.20	0.07	-	0.05	0.19	0.38	0.30	0.39	0.06	0.31	0.08	0.06	0.17	0.05	0.29	0.19	0.17	0.08
	120	0.15	-	0.06	0.03	-	-	0.21	0.08	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	124	-	-	0.05	-	-	-	-	0.02	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	128	0.15	-	0.05	0.03	-	0.29	0.02	0.07	0.02	-	0.61	0.16	0.19	0.09	0.22	0.39	0.19	0.19	0.18	0.10	0.27	0.29	0.50	0.06	0.36
	136	0.35	0.46	0.31	0.37	0.68	-	0.19	0.10	0.02	0.33	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	140	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	142	-	-	-	0.20	0.13	0.02	0.02	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	144	-	-	-	-	-	-	0.13	0.10	0.02	0.02	0.06	-	-	-	-	-	0.23	0.04	0.09	0.17	0.18	0.14	0.19	0.61	-
	146	-	-	0.20	0.20	0.08	0.29	0.02	0.10	0.21	-	0.05	0.15	0.19	0.23	0.09	0.26	0.04	0.12	0.15	0.17	0.32	0.29	-	0.17	0.11
	148	-	-	0.04	-	-	0.08	-	-	0.05	-	0.03	0.25	0.18	0.20	0.17	0.24	0.23	0.58	0.53	0.40	0.16	-	0.13	-	0.33
	150	-	-	0.09	-	-	-	0.02	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	152	-	-	0.05	-	-	-	0.02	0.12	0.05	0.08	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03
	154	-	-	-	-	-	-	0.02	0.02	0.17	0.06	-	0.04	0.03	0.01	0.07	0.02	-	-	-	-	-	-	-	-	-	-
	156	-	-	0.01	-	0.02	-	-	0.10	0.17	0.02	-	0.09	-	0.04	0.06	0.04	-	-	-	-	0.02	-	-	-	-	-
	158	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	160	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	162	-	0.04	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	172	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	176	0.15	0.08	-	0.07	-	-	-	-	-	-	-	0.02	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-
	186	0.05	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bv8	95	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	97	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	99	-	-	0.07	-	0.15	0.15	0.08	0.26	0.28	0.04	0.27	0.26	0.35	0.45	0.28	0.28	0.13	0.08	-	-	0.09	-	0.56	0.30	0.27
	101	1.00	1.00	0.77	0.83	0.77	0.60	0.60	0.35	0.62	0.92	0.46	0.74	0.57	0.49	0.66	0.44	0.58	0.46	0.81	0.70	0.75	0.64	0.38	0.50	0.53
	103	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	105	-	-	0.02	-	0.03	0.05	0.04	-	-	-	0.05	-	-	-	0.05	0.04	-	-	-	-	-	0.36	-	0.15	-
	106	-	-	0.01	-	0.05	0.05	0.04	0.14	0.02	-	0.11	-	0.08	0.07	0.02	0.13	0.29	0.08	0.16	0.13	0.07	-	0.06	0.05	-
	107	-	-	0.04	0.17	-	-	0.15	0.10	0.08	-	0.08	-	-	-	-	0.11	-	0.39	0.03	0.17	0.09	-	-	-	0.21
	109	-	-	-	-	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	110	-	-	0.02	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	111	-	-	0.05	-	-	0.02	-	0.05	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	113	-	-	-	-	-	0.08	0.04	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bv9	88	-	-	-	-	-	-	-	-	-	-	0.02	0.19	0.13	0.13	0.10	-	0.10	-	-	-	-	-	-	-	-	-
	100	0.04	0.06	0.14	-	0.08	0.02	-	0.03	-	0.03	0.16	0.03	0.09	0.03	-	-	-	-	-	-	0.05	-	0.36	0.13	0.03
	102	0.31	-	-	0.04	0.19	0.31	0.05	-	0.02	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	104	-	-	0.05	-	-	0.02	0.10	0.18	0.30	0.05	0.25	0.47	0.46	0.54	0.47	0.29	0.38	0.69	0.09	0.30	0.26	0.17	0.14	0.06	0.86
	106	-	-	0.10	-	0.15	-	-	0.03	-	0.05	-	-	0.01	-	-	-	-	-	-	-	0.02	-	-	-	-
	110	-	-	0.01	-	0.05	0.10	-	0.20	0.02	0.05	0.03	-	-	-	-	-	0.21	0.04	-	-	-	0.17	-	0.06	-
	112	-	-	0.04	-	0.03	0.07	0.03	0.02	0.05	0.05	-	-	-	-	-	-	-	-	0.13	-	0.02	-	-	-	-
	114	-	0.06	0.23	0.08	-	0.10	0.25	0.13	0.17	0.11	0.18	0.22	0.12	0.14	0.24	0.25	0.27	-	0.50	0.20	0.21	0.67	0.29	0.75	-
	116	-	-	0.11	-	-	0.13	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	120	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	126	0.58	0.06	0.11	0.39	0.36	0.02	0.43	0.10	0.03	0.32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	128	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	0.07	-	-	-	-	-	-
	130	-	-	-	-	-	-	-	0.02	0.02	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	134	-	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	138	-	0.22	0.06	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	140	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-
	142	-	-	-	0.04	-	-	-	0.02	-	-	0.06	-	0.02	-	0.02	0.06	-	0.12	0.03	0.07	0.07	-	-	-	0.06
	144	0.08	0.33	0.08	-	0.05	0.24	0.08	0.20	0.18	0.05	0.24	0.07	0.07	0.08	0.12	0.15	-	0.12	0.19	0.37	0.21	-	0.07	-	-
	146	-	-	-	-	-	-	0.05	0.02	0.05	0.13	-	-	0.01	0.03	-	-	0.02	0.04	-	-	-	-	-	-	-	-
	148	-	-	-	-	-	-	0.03	-	-	-	-	0.03	0.09	0.06	0.05	0.04	0.02	-	-	-	-	-	0.07	-	0.06
	152	-	-	0.06	0.46	0.03	0.02	-	0.02	-	-	0.03	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-
	154	-	0.17	0.02	-	0.07	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	162	-	-	-	-	-	-	-	-	0.13	-	0.03	-	-	-	-	0.21	-	-	-	-	0.10	-	0.07	-	-
Bv12	177	-	-	-	-	-	0.05	0.03	-	0.06	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	179	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-
	181	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-
	183	-	0.45	0.06	0.50	0.18	-	0.17	0.21	0.52	0.02	0.30	0.17	0.11	0.04	0.31	0.02	0.11	0.21	0.27	0.27	0.11	0.29	0.06	0.50	0.31
	187	0.75	0.50	0.78	0.50	0.73	0.65	0.65	0.35	0.33	0.71	0.32	-	0.06	0.04	0.07	0.14	0.30	0.17	0.60	0.65	0.46	0.64	0.63	0.35	0.09
	189	0.25	-	0.16	-	0.08	0.27	0.15	0.40	0.06	0.14	0.05	-	0.01	0.05	0.07	-	0.02	-	0.13	-	0.30	0.07	-	-	0.47
	193	-	0.05	-	-	0.02	0.03	-	0.04	0.02	0.14	0.32	0.83	0.82	0.88	0.55	0.84	0.57	0.63	-	-	0.14	-	0.31	0.15	0.13
Bv13	116	-	-	-	-	-	-	-	-	-	-	-	0.05	0.14	0.22	0.03	0.06	0.02	-	-	-	-	-	-	0.06	0.06
	120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-
	122	0.17	0.18	0.30	0.20	0.34	0.47	0.27	0.55	0.38	0.11	0.73	0.69	0.54	0.60	0.48	0.44	0.48	0.50	0.18	0.27	0.36	0.57	0.44	0.61	0.25
	124	-	-	0.01	-	-	0.18	0.02	0.02	0.37	-	0.18	0.06	0.21	0.01	0.07	0.13	0.04	0.27	0.50	0.50	0.41	0.07	0.38	0.17	0.33
	126	0.83	0.82	0.33	0.80	0.61	0.36	0.67	0.30	0.12	0.32	0.08	0.09	0.05	0.15	0.33	-	0.46	0.23	0.27	0.10	0.14	0.14	0.06	0.11	0.19
	128	-	-	0.36	-	0.05	-	0.05	0.13	0.13	0.20	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-
	130	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	132	-	-	-	-	-	-	-	-	-	0.36	0.02	0.10	0.06	0.02	0.09	0.35	-	-	0.06	0.10	0.09	0.21	0.13	0.06	0.17
Bv15	173	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	175	-	0.04	-	-	-	-	-	-	0.02	-	-	0.03	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-
	177	0.68	0.89	0.42	0.27	0.48	0.69	0.45	0.59	0.83	0.43	0.99	0.90	0.80	0.86	0.83	0.85	0.63	0.73	0.59	0.63	0.77	0.50	0.88	0.85	0.94
	179	-	-	0.13	-	0.07	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-
	181	0.32	0.07	0.43	0.53	0.39	0.31	0.53	0.41	0.08	0.57	0.02	0.08	0.10	0.07	0.05	-	0.33	0.27	0.41	0.37	0.21	0.50	0.06	0.15	-
	183	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-

	185	-	-	-	-	-	-	-	-	0.07	-	-	-	0.10	0.05	0.10	0.15	0.04	-	-	-	0.02	-	0.06	-	0.06
	187	-	-	0.01	0.20	0.07	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bv16	99	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	0.02	-	0.04	-	-	0.02	-	-	-	-
	105	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-
	108	-	-	0.03	-	-	0.10	-	0.07	0.05	-	0.02	-	-	-	-	-	0.02	-	-	-	0.02	-	0.06	-	-
	114	-	0.04	-	0.11	-	-	0.02	0.02	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	117	-	-	-	-	-	-	-	0.02	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	0.06	-
	120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15	-	-	0.10	-	-	-	0.06	-
	123	-	-	0.08	-	-	0.02	0.04	-	0.07	0.17	-	0.59	0.57	0.50	0.66	0.28	0.27	-	0.03	0.07	-	0.14	0.06	0.11	0.03
	126	-	-	-	-	-	-	0.06	-	-	0.07	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	129	0.04	-	0.01	-	-	0.12	0.19	0.02	0.14	0.04	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-
	134	0.14	0.11	-	-	-	-	0.04	0.07	-	-	0.05	0.13	0.06	0.21	0.05	0.15	0.13	0.04	0.35	0.03	0.11	0.21	0.06	0.11	0.03
	137	0.04	0.14	-	0.07	0.13	0.30	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	140	-	-	0.03	-	-	-	0.17	0.10	-	-	0.02	-	-	-	-	-	-	0.08	-	-	0.07	-	-	-	-
	143	-	-	0.11	-	-	-	0.19	0.20	-	0.04	-	-	-	-	-	-	0.04	-	-	-	-	-	0.13	-	-
	146	0.04	0.04	0.06	0.07	0.04	-	0.02	0.03	0.02	0.04	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	148	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	-	-	-	-	-	-	-	-
	150	0.25	-	-	0.07	-	-	0.02	0.02	-	-	0.03	0.12	0.21	0.12	0.12	-	0.02	-	-	-	0.02	-	-	-	-
	152	-	-	0.13	-	-	0.02	-	0.02	-	0.17	0.09	-	-	-	-	-	0.10	-	0.18	0.07	0.05	0.29	0.06	0.11	-
	155	-	-	-	-	-	-	-	0.02	-	0.07	-	-	0.04	0.09	0.02	-	-	-	-	-	-	-	-	-	-	-
	158	-	-	0.15	-	0.21	-	-	0.10	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	161	-	0.21	-	0.21	-	0.04	0.04	-	0.04	0.20	0.09	0.10	-	0.08	-	-	-	0.12	0.06	0.07	0.07	-	-	-	0.56
	167	0.04	-	-	-	-	-	-	-	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	170	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	173	-	-	0.13	-	-	-	-	0.07	0.02	0.02	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	176	0.04	0.18	0.11	-	0.16	0.14	-	0.03	0.38	0.04	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-
	179	-	-	-	-	-	-	0.04	-	0.02	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	182	-	-	0.01	0.18	0.07	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	185	-	-	0.05	-	0.11	0.04	-	0.08	0.07	0.02	0.33	-	-	-	-	0.17	0.02	0.65	0.21	0.23	0.21	0.29	0.25	0.28	0.25
	188	-	-	0.06	-	-	0.02	0.10	0.13	0.14	-	0.12	0.04	0.06	-	0.14	0.17	0.15	-	0.18	0.40	0.43	0.07	0.19	0.28	0.03

	191	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-
	193	0.07	-	0.03	0.04	-	0.10	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06
	195	0.11	0.04	-	0.11	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	197	0.25	0.25	0.01	0.14	0.21	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-
	200	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	202	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-
	204	-	-	-	-	-	-	-	-	0.02	-	0.08	0.01	-	-	0.02	0.15	-	-	-	-	-	-	0.06	-	0.03
	207	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	210	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	0.03
	246	-	-	-	-	-	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bv17	223	0.87	0.79	0.76	1.00	0.82	0.50	0.80	0.63	0.50	0.71	0.57	0.55	0.56	0.60	0.57	0.41	0.65	0.50	0.34	0.43	0.57	0.86	0.75	0.70	0.41
	225	-	-	-	-	-	-	-	-	-	0.05	0.05	0.08	0.05	0.02	0.07	-	0.15	-	0.25	0.32	0.07	0.14	0.13	0.05	-
	227	-	-	-	-	0.02	-	0.05	0.12	0.03	0.07	0.02	0.03	-	0.05	-	-	-	-	-	-	0.02	-	-	-	-
	229	-	0.21	0.08	-	0.16	0.50	0.15	0.13	0.43	0.13	0.30	0.22	0.29	0.24	0.24	0.50	0.10	0.27	0.31	0.07	0.11	-	-	0.25	0.59
	231	-	-	-	-	-	-	-	0.03	0.03	-	-	-	-	-	-	-	0.10	0.23	0.09	0.18	0.23	-	0.06	-	-
	233	0.13	-	0.08	-	-	-	-	0.08	-	0.04	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	239	-	-	0.09	-	-	-	-	-	-	-	0.02	0.12	0.10	0.09	0.12	0.09	-	-	-	-	-	-	-	-	-
	241	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-
Bv18	149	-	0.04	-	-	-	-	-	-	-	0.06	0.10	0.26	0.31	0.34	0.15	0.02	0.08	0.27	0.25	0.35	0.22	-	0.07	0.19	-
	155	0.35	0.11	0.50	0.23	0.26	0.35	0.32	0.67	0.28	0.29	0.37	0.03	-	0.04	0.10	0.17	0.29	-	0.15	0.30	0.28	0.67	0.43	0.44	0.50
	157	0.60	0.07	0.46	0.69	0.62	0.45	0.64	0.16	0.35	0.59	0.23	0.35	0.28	0.34	0.39	0.37	0.61	-	0.15	0.05	0.19	0.33	0.14	0.38	-
	159	0.05	0.79	0.04	0.08	0.12	0.21	0.04	0.17	0.37	0.06	0.31	0.36	0.42	0.28	0.37	0.44	0.03	0.73	0.45	0.30	0.31	-	0.36	-	0.50

Appendix S2.6: Genetic diversity at 13 microsatellite loci for 23 sites of the violet tunicate, *Botrylloides violaceus*. *N*, sample size; N_A, number of alleles; *A*, allele richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS} value; P_{HW}, exact *P*-value for Hardy-Weinberg equilibrium. Significant departures from equilibrium after sequential Bonferroni correction are indicated in bold.

Sites		**Bv 2**	**Bv 4**	**Bv 5**	**Bv 6**	**Bv 7**	**Bv 8**	**Bv 9**	**Bv 12**	**Bv 13**	**Bv 15**	**Bv 16**	**Bv 17**	**Bv 18**
DPB	N_A /*A*	7/4.8	7/4.8	2/2.0	4/3.8	7/5.9	1/1.0	4/3.2	2/1.0	2/1.9	2/2.0	10/6.5	2/1.9	3/2.6
N = 15	H_O	0.286	0.857	0.000	0.714	0.900	NA	0.692	0.500	0.333	0.643	0.929	0.267	0.700
	H_E	0.749	0.709	0.545	0.712	0.837	NA	0.588	0.400	0.287	0.452	0.862	0.239	0.542
	F_{IS}	0.627	-0.219	1.000	-0.004	-0.080	NA	-0.187	-0.273	-0.167	-0.444	-0.080	-0.120	-0.313
	P_{HW}	**0.000**	0.885	0.021	0.002	0.076	-	0.019	1.000	1.000	0.220	0.011	1.000	0.276
FRC	N_A /*A*	3/3.0	4/3.2	3/3.0	6/5.1	5/3.8	1/1.0	7/5.9	3/2.6	2/2.0	3/2.1	8/6.0	2/2.0	4/2.9
N = 14	H_O	0.545	0.615	0.600	1.000	0.833	NA	1.000	0.200	0.357	0.071	0.928	0.416	0.285
	H_E	0.675	0.563	0.616	0.822	0.667	NA	0.837	0.574	0.304	0.204	0.855	0.344	0.378
	F_{IS}	0.200	-0.097	0.027	-0.228	-0.264	NA	-0.210	0.664	-0.182	0.658	-0.090	-0.222	0.252
	P_{HW}	0.015	1.000	0.038	**0.000**	0.107	-	0.118	0.019	1.000	0.034	0.001	1.000	0.090
LMI	N_A /*A*	6/3.2	5/3.9	6/3.5	6/3.3	12/6.5	8/3.3	12/7.1	3/2.5	4/3.1	5/3.1	16/7.8	4/3.0	3/2.4
N = 42	H_O	0.310	0.512	0.537	0.634	0.683	0.310	0.738	0.317	0.476	0.643	0.600	0.450	0.436
	H_E	0.535	0.667	0.468	0.624	0.848	0.396	0.889	0.366	0.681	0.633	0.914	0.405	0.542
	F_{IS}	0.425	0.234	-0.149	-0.017	0.196	0.220	0.171	0.136	0.303	-0.016	0.346	-0.113	0.199
	P_{HW}	0.004	0.046	0.639	0.688	0.004	0.037	0.003	0.137	0.033	0.981	**0.000**	0.941	0.032
LSM	N_A /*A*	3/3.0	4/3.4	3/2.8	4/3.8	8/5.4	2/2.0	5/3.6	2/2.0	2/2.0	3/3.0	9/7.0	1/1.0	3/2.7
N = 16	H_O	0.133	0.533	0.167	0.500	0.800	0.333	0.308	0.000	0.400	0.667	0.857	NA	0.462
	H_E	0.662	0.641	0.594	0.685	0.800	0.303	0.655	0.545	0.331	0.625	0.894	NA	0.480
	F_{IS}	0.804	0.173	0.728	0.278	0.000	-0.111	0.541	1.000	-0.217	-0.069	0.043	NA	0.040
	P_{HW}	**0.000**	0.001	0.006	0.002	**0.000**	1.000	**0.000**	0.022	1.000	0.151	0.058	-	0.051
SYD	N_A /*A*	6/3.5	4/3.3	4/2.9	3/2.6	5/3.4	4/2.7	9/5.7	4/2.8	3/2.5	4/3.2	9/6.2	3/2.1	3/2.8
N = 31	H_O	0.419	0.613	0.226	0.387	0.233	0.419	0.483	0.452	0.645	0.645	0.571	0.355	0.200
	H_E	0.690	0.610	0.524	0.402	0.510	0.382	0.811	0.442	0.516	0.618	0.861	0.302	0.544
	F_{IS}	0.396	-0.005	0.573	0.039	0.546	-0.099	0.408	-0.022	-0.257	-0.045	0.340	-0.179	0.637
	P_{HW}	**0.000**	0.829	**0.000**	0.430	**0.000**	0.703	**0.000**	0.372	0.141	0.553	0.001	0.633	**0.000**

VIC	N_A /A	4/3.6	5/3.3	6/4.4	4/3.1	6/4.6	7/4.3	10/5.7	4/2.8	3/2.9	2/2.0	12/6.7	2/2.0	3/3.0
N = 31	H_O	0.645	0.548	0.581	0.645	0.645	0.613	0.839	0.467	0.516	0.290	0.600	0.677	0.241
	H_E	0.677	0.510	0.724	0.631	0.783	0.617	0.820	0.511	0.634	0.432	0.862	0.508	0.649
	F_{IS}	0.048	-0.076	0.201	-0.023	0.179	0.007	-0.023	0.089	0.189	0.332	0.308	-0.340	0.632
	P_{HW}	0.159	0.456	0.013	0.869	**0.000**	0.288	0.955	0.085	0.083	0.091	**0.000**	0.079	**0.000**
LPI	N_A /A	6/3.5	4/3.8	5/3.2	4/3.1	12/5.9	7/4.4	8/5.1	4/3.2	4/2.7	3/2.2	14/7.4	3/2.4	3/2.4
N = 30	H_O	0.345	0.800	0.500	0.400	0.458	0.500	0.450	0.433	0.300	0.567	0.833	0.300	0.250
	H_E	0.632	0.705	0.505	0.444	0.833	0.613	0.754	0.535	0.490	0.521	0.895	0.341	0.491
	F_{IS}	0.458	-0.138	0.009	0.100	0.455	0.187	0.409	0.193	0.392	-0.088	0.071	0.121	0.495
	P_{HW}	**0.000**	0.957	0.153	0.095	**0.000**	0.023	0.001	0.002	0.009	0.583	0.176	0.005	0.009
NHB	N_A /A	6/4.1	6/4.2	5/4.1	4/3.5	12/7.4	7/5.1	13/6.5	4/3.4	4/3.0	2/2.0	17/7.9	5/3.7	3/2.8
N = 30	H_O	0.600	0.741	0.633	0.533	0.700	0.483	0.867	0.115	0.500	0.483	0.800	0.567	0.069
	H_E	0.738	0.717	0.721	0.644	0.902	0.790	0.868	0.684	0.596	0.494	0.913	0.569	0.503
	F_{IS}	0.190	-0.034	0.123	0.174	0.227	0.393	0.002	0.834	0.164	0.022	0.126	0.004	0.865
	P_{HW}	0.071	0.877	0.136	0.407	**0.000**	0.001	0.730	**0.000**	0.050	1.000	0.002	0.848	**0.000**
SQN	N_A /A	7/4.8	5/3.6	7/4.8	3/2.1	12/6.6	4/2.9	12/6.0	5/3.4	4/3.7	4/2.5	13/6.2	4/2.7	3/3.0
N = 30	H_O	0.370	0.893	0.793	0.267	0.931	0.367	0.800	0.167	0.733	0.333	0.679	0.667	0.200
	H_E	0.732	0.669	0.771	0.242	0.871	0.541	0.837	0.622	0.699	0.299	0.817	0.569	0.674
	F_{IS}	0.499	-0.342	-0.030	-0.105	-0.071	0.326	0.045	0.736	-0.050	-0.118	0.172	-0.174	0.707
	P_{HW}	**0.000**	0.018	0.530	1.000	0.010	0.075	0.760	**0.000**	0.611	1.000	0.048	0.869	**0.000**
BRN	N_A /A	6/4.5	4/3.5	5/4.4	2/2.0	11/5.7	3/1.8	11/6.9	4/3.0	5/3.9	2/2.0	13/7.3	5/3.4	4/3.2
N = 28	H_O	0.444	0.679	0.692	0.393	0.667	0.154	0.526	0.231	0.821	0.571	0.852	0.464	0.235
	H_E	0.758	0.586	0.782	0.503	0.801	0.148	0.862	0.466	0.732	0.499	0.894	0.473	0.578
	F_{IS}	0.418	-0.161	0.118	0.223	0.170	-0.042	0.396	0.510	-0.125	-0.149	0.048	0.020	0.600
	P_{HW}	**0.000**	0.792	0.647	0.274	0.001	1.000	0.011	0.002	0.136	0.470	0.045	0.376	0.001
BBY	N_A /A	6/3.0	5/3.3	7/5.4	7/3.5	8/4.4	6/4.3	10/5.7	5/3.6	4/2.8	2/1.2	16/7.0	6/3.4	4/3.7
N = 34	H_O	0.441	0.576	0.667	0.471	0.469	0.667	0.588	0.364	0.455	0.030	0.455	0.700	0.462
	H_E	0.584	0.490	0.822	0.467	0.607	0.709	0.831	0.714	0.439	0.030	0.856	0.593	0.723
	F_{IS}	0.247	-0.178	0.192	-0.008	0.231	0.060	0.295	0.495	-0.037	0.000	0.473	-0.184	0.366
	P_{HW}	0.004	1.000	0.317	0.426	0.014	0.803	0.038	**0.000**	0.239	1.000	**0.000**	0.517	0.024

SPB	N_A /A	3/2.4	3/2.8	5/3.5	4/3.7	8/5.8	2/2.0	6/4.1	2/1.9	5/3.5	3/1.9	7/4.1	5/3.7	4/3.4
N = 39	H_O	0.305	0.538	0.378	0.641	0.853	0.323	0.459	0.133	0.513	0.205	0.692	0.568	0.483
	H_E	0.495	0.602	0.653	0.697	0.845	0.389	0.697	0.282	0.502	0.190	0.618	0.633	0.694
	F_{IS}	0.386	0.107	0.424	0.081	-0.009	0.174	0.344	0.532	-0.022	-0.078	-0.122	0.104	0.308
	P_{HW}	0.004	0.593	**0.000**	0.736	0.004	0.370	0.002	0.015	0.058	1.000	0.246	**0.000**	0.005
SHB	N_A /A	4/3.2	4/3.1	6/4.0	4/3.8	6/4.5	3/2.7	9/5.1	4/2.5	5/3.8	3/2.5	7/4.0	4/3.2	3/3.0
N = 50	H_O	0.600	0.620	0.668	0.894	0.800	0.540	0.600	0.244	0.760	0.367	0.638	0.714	0.532
	H_E	0.639	0.631	0.701	0.712	0.761	0.552	0.743	0.320	0.645	0.349	0.620	0.596	0.663
	F_{IS}	0.062	0.017	0.049	-0.259	-0.052	0.021	0.194	0.241	-0.180	-0.052	-0.029	-0.200	0.200
	P_{HW}	0.112	0.070	0.187	0.144	0.676	0.811	**0.000**	0.037	0.059	0.502	0.341	0.327	0.024
CRV	N_A /A	5/3.4	5/3.5	4/3.4	4/3.6	8/5.4	3/2.6	7/4.5	4/2.2	5/3.3	5/2.4	5/4.1	5/3.4	4/3.4
N = 41	H_O	0.526	0.439	0.568	0.541	0.763	0.500	0.641	0.146	0.610	0.211	0.816	0.634	0.514
	H_E	0.636	0.687	0.682	0.716	0.813	0.566	0.671	0.227	0.580	0.265	0.686	0.580	0.699
	F_{IS}	0.175	0.363	0.170	0.247	0.062	0.118	0.045	0.357	-0.053	0.206	-0.192	-0.094	0.268
	P_{HW}	0.435	**0.000**	**0.000**	0.039	0.005	0.325	0.308	0.001	0.180	0.026	0.319	0.051	0.001
APY	N_A /A	3/2.8	3/2.4	4/3.4	4/3.9	6/4.8	4/2.7	6/4.3	4/3.2	5/3.7	4/2.5	6/3.6	4/3.4	4/3.6
N = 29	H_O	0.586	0.379	0.636	0.724	0.852	0.241	0.621	0.379	0.862	0.276	0.379	0.655	0.500
	H_E	0.525	0.503	0.648	0.751	0.769	0.500	0.709	0.600	0.658	0.307	0.543	0.609	0.699
	F_{IS}	-0.120	0.250	0.018	0.036	-0.110	0.522	0.127	0.372	-0.318	0.102	0.306	-0.077	0.289
	P_{HW}	0.434	0.272	0.882	0.727	0.043	0.001	0.454	0.001	0.264	0.431	0.033	0.057	0.132
SBR	N_A /A	3/2.2	6/3.6	5/4.1	4/3.3	6/4.1	5/4.0	6/4.9	3/2.1	5/3.6	2/1.9	7/5.4	3/2.7	4/3.2
N = 27	H_O	0.385	0.259	0.700	0.615	0.667	0.852	0.542	0.080	0.741	0.222	0.522	0.455	0.261
	H_E	0.446	0.513	0.733	0.581	0.732	0.708	0.799	0.280	0.671	0.257	0.829	0.588	0.658
	F_{IS}	0.139	0.499	0.047	-0.060	0.091	-0.208	0.327	0.718	-0.106	0.138	0.376	0.231	0.609
	P_{HW}	0.741	0.001	0.380	0.868	0.260	0.277	0.067	0.001	0.945	0.455	0.010	0.055	**0.000**
PDG	N_A /A	3/1.7	4/3.6	5/3.9	5/3.7	5/4.3	3/2.8	6/4.2	4/3.1	4/2.7	3/2.5	10/6.4	4/3.4	4/3.0
N = 24	H_O	0.125	0.417	0.739	0.522	0.792	0.542	0.959	0.435	0.750	0.522	0.750	0.458	0.368
	H_E	0.121	0.707	0.697	0.612	0.777	0.571	0.746	0.588	0.570	0.505	0.862	0.551	0.558
	F_{IS}	-0.030	0.416	-0.063	0.150	-0.020	0.052	-0.292	0.265	-0.325	-0.033	0.132	0.172	0.345
	P_{HW}	1.000	0.002	0.063	0.041	0.238	0.757	0.045	0.198	0.198	1.000	0.125	0.078	0.068

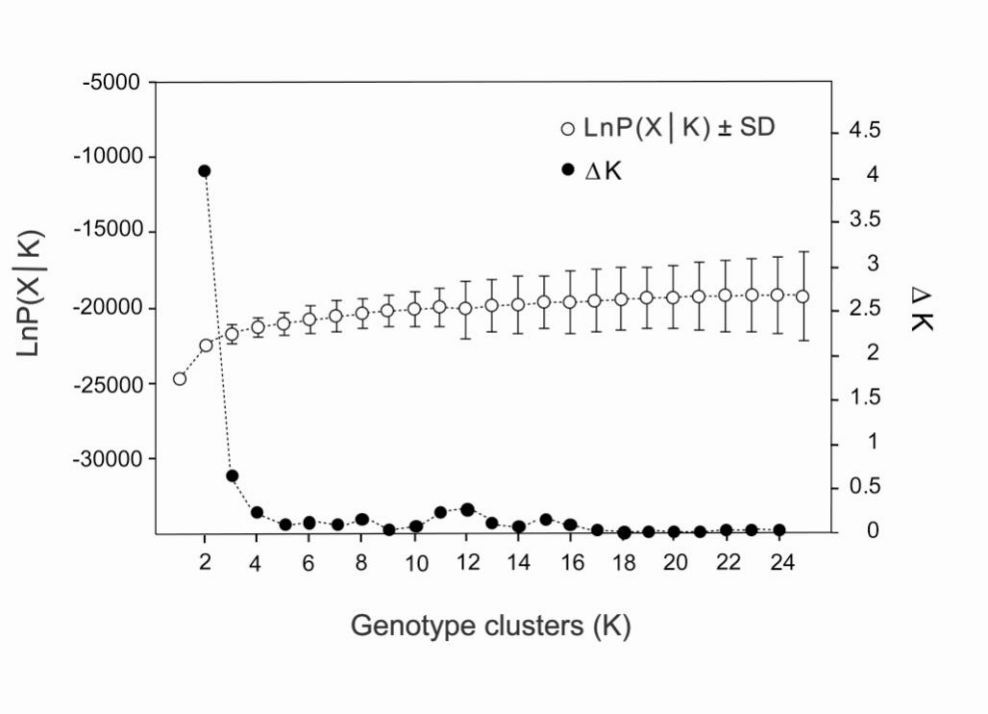
CHT	N_A/A	2/2.0	3/2.9	6/5.1	4/2.9	5/4.0	4/3.4	5/3.6	3/2.9	3/3.0	2/2.0	7/4.4	3/3.0	2/2.0
N = 13	H_O	0.538	0.769	0.500	0.462	0.769	0.308	0.615	0.417	0.615	0.231	0.615	0.538	0.364
	H_E	0.471	0.569	0.815	0.397	0.634	0.652	0.511	0.562	0.649	0.409	0.569	0.649	0.416
	F_{IS}	-0.151	-0.371	0.397	-0.171	-0.224	0.538	-0.215	0.267	0.054	0.446	-0.085	0.176	0.130
	P_{HW}	1.000	0.278	**0.000**	1.000	1.000	0.003	1.000	0.001	0.018	0.166	0.544	0.053	1.000
MCV	N_A/A	3/2.6	3/3.0	4/3.1	4/3.5	5/4.2	3/2.3	6/4.6	3/2.9	4/3.5	2/2.0	6/4.8	4/3.8	4/3.9
N = 17	H_O	0.333	0.529	0.411	0.563	0.647	0.375	0.750	0.733	0.529	0.235	0.706	0.625	0.400
	H_E	0.467	0.627	0.619	0.671	0.676	0.325	0.708	0.570	0.665	0.499	0.790	0.736	0.726
	F_{IS}	0.293	0.160	0.341	0.167	0.043	-0.161	-0.062	-0.300	0.209	0.536	0.109	0.155	0.463
	P_{HW}	0.207	0.076	0.156	0.407	0.223	1.000	0.185	0.614	0.135	0.045	0.305	0.642	0.014
BKH	N_A/A	4/3.5	3/2.8	5/4.3	3/2.9	5/4.6	3/2.8	5/4.3	4/2.9	5/4.0	2/2.0	8/5.5	4/3.6	4/3.6
N = 15	H_O	0.357	0.333	0.429	0.467	0.667	0.333	0.933	0.462	0.800	0.467	0.867	0.500	0.600
	H_E	0.669	0.508	0.765	0.641	0.772	0.480	0.752	0.517	0.680	0.480	0.786	0.701	0.732
	F_{IS}	0.476	0.352	0.449	0.279	0.141	0.314	-0.252	0.111	-0.183	0.030	-0.106	0.295	0.188
	P_{HW}	0.003	0.046	**0.000**	0.380	0.661	0.173	0.406	0.050	0.079	1.000	0.853	0.073	0.257
LNB	N_A/A	3/2.5	4/3.4	6/5.0	5/4.1	6/4.6	4/3.1	10/6.0	4/3.7	4/3.6	3/2.2	9/5.3	5/3.7	4/3.9
N = 22	H_O	0.381	0.500	0.450	0.409	0.682	0.318	0.810	0.227	0.636	0.455	0.545	0.727	0.556
	H_E	0.547	0.612	0.794	0.717	0.781	0.426	0.841	0.690	0.689	0.369	0.763	0.622	0.763
	F_{IS}	0.309	0.187	0.439	0.435	0.130	0.258	0.038	0.676	0.078	-0.239	0.290	-0.175	0.278
	P_{HW}	0.264	0.011	0.005	0.003	0.038	0.009	0.045	**0.000**	0.064	0.627	0.007	0.334	**0.000**
MTG	N_A/A	3/3.0	3/2.9	2/2.0	4/4.0	4/4.0	2/2.0	3/3.0	3/2.9	4/3.8	2/2.0	5/4.8	2/2.0	2/2.0
N = 7	H_O	0.429	0.429	0.333	0.500	0.714	0.429	0.333	0.429	0.857	0.429	0.714	0.286	0.333
	H_E	0.714	0.538	0.545	0.561	0.791	0.495	0.545	0.538	0.648	0.538	0.824	0.264	0.485
	F_{IS}	0.419	0.217	0.412	0.118	0.104	0.143	0.412	0.217	-0.358	0.217	0.143	-0.091	0.333
	P_{HW}	0.202	1.000	0.480	0.521	0.689	1.000	0.192	1.000	0.778	1.000	0.822	1.000	1.000
LKP	N_A/A	2/2.0	4/3.9	3/2.9	4/3.7	4/3.9	3/2.8	6/5.6	3/2.8	4/3.7	3/2.5	10/8.2	4/3.5	4/3.8
N = 8	H_O	0.375	0.625	0.571	0.571	0,500	0.125	0.571	0.250	0.500	0.250	0.875	0.500	0.286
	H_E	0.525	0.767	0.538	0.495	0.708	0.575	0.813	0.542	0.692	0.242	0.917	0.442	0.714
	F_{IS}	0.300	0.195	-0.067	-0.171	0.309	0.794	0.314	0.556	0.291	-0.037	0.049	-0.143	0.619
	P_{HW}	0.529	0.366	0.330	1.000	0.345	0.007	0.130	0.079	0.151	1.000	0.725	1.000	0.038

IGM	N_A /A	4/3.5	4/3.5	5/4.5	3/2.5	4/3.6	4/3.6	4/3.5	3/3.0	5/4.2	2/2.0	7/6.0	3/2.6	3/3.0
N = 10	H_O	0.200	0.700	0.667	0.300	0.444	0.500	0.250	0.500	0.667	0.100	0.889	0.300	0.625
	H_E	0.658	0.605	0.771	0.279	0.601	0.668	0.442	0.637	0.614	0.268	0.850	0.468	0.675
	F_{IS}	0.707	-0.167	0.143	-0.080	0.273	0.262	0.451	0.224	-0.091	0.640	-0.049	0.372	0.079
	P_{HW}	0.006	1.000	0.493	1.000	0.051	0.200	0.135	0.207	1.000	0.158	0.866	0.137	0.777
MYH	N_A /A	2/2.0	6/4.7	5/4.1	3/2.9	6/4.6	3/3.0	4/2.5	4/3.6	5/4.4	2/1.6	8/4.2	2/2.0	2/2.0
N = 18	H_O	0.500	0.833	0.471	0.647	0.722	0.529	0.278	0.500	0.778	0.111	0.556	0.588	0.412
	H_E	0.386	0.776	0.672	0.579	0.752	0.626	0.259	0.679	0.779	0.108	0.640	0.499	0.515
	F_{IS}	-0.308	-0.076	0.306	-0.121	0.041	0.158	-0.076	0.271	0.002	-0.030	0.135	-0.185	0.206
	P_{HW}	0.523	0.020	0.072	0.181	0.075	0.200	1.000	0.184	0.476	1.000	0.641	0.623	0.627

Appendix S2.7: Pairwise F_{ST} comparisons for *Botrylloides violaceus* populations using 13 microsatellite markers. *Significant ($P < 0.05$); ** remains significant after sequential Bonferroni correction (Rice 1989). Populations on the East coast are separated by a box in the upper left corner. Populations with sample size of less than 15 individuals ($N < 15$) were not included in this analysis.

	SPB	SHB	CRV	APY	SBR	PDG	MCV	BKH	LNB	MYR	DPB	LMI	LSM	SYD	VIC	LPI	NHB	SQN	BRN
SPB																			
SHB	0.02**																		
CRV	0.02**	0.01																	
APY	0.02*	0.02**	0.02**																
SBR	0.07**	0.10**	0.12**	0.11**															
PDG	0.10**	0.12**	0.11**	0.08**	0.16**														
MCV	0.12**	0.10**	0.14**	0.11**	0.12**	0.09**													
BKH	0.12**	0.11**	0.15**	0.13**	0.13**	0.14**	0.01												
LNB	0.07**	0.07**	0.10**	0.09**	0.08**	0.12**	0.05*	0.02											
MYH	0.10**	0.09**	0.11**	0.10**	0.05**	0.14**	0.09**	0.15**	0.08**										
DPB	0.23**	0.19**	0.18**	0.17**	0.29**	0.21**	0.19**	0.18**	0.17**	0.28**									
LMI	0.18**	0.17**	0.16**	0.18**	0.23**	0.21**	0.16**	0.12**	0.12**	0.24**	0.08**								
LSM	0.27**	0.23**	0.22**	0.21**	0.32**	0.22**	0.20**	0.19**	0.19**	0.31**	0.03	0.09**							
SYD	0.21**	0.22**	0.20**	0.21**	0.23**	0.20**	0.19**	0.16**	0.15**	0.24**	0.11**	0.09**	0.07**						
VIC	0.10**	0.10**	0.10**	0.11**	0.09**	0.12**	0.06**	0.08**	0.08**	0.10**	0.14**	0.13**	0.16**	0.13**					
LPI	0.26**	0.21**	0.19**	0.22**	0.34**	0.26**	0.22**	0.22**	0.22**	0.31**	0.06**	0.08**	0.11**	0.18**	0.18**				
NHB	0.12**	0.11**	0.09**	0.13**	0.17**	0.15**	0.13**	0.11**	0.09**	0.17**	0.09**	0.04**	0.09**	0.07**	0.07**	0.10**			
SQN	0.13**	0.13**	0.14**	0.15**	0.15**	0.19**	0.14**	0.14**	0.12**	0.13**	0.25**	0.20**	0.24**	0.16**	0.11**	0.29**	0.11**		
BRN	0.24**	0.21**	0.19**	0.23**	0.26**	0.23**	0.19**	0.18**	0.19**	0.25**	0.13**	0.08**	0.12**	0.13**	0.17**	0.09**	0.07**	0.21**	
BBY	0.05**	0.10**	0.11**	0.12**	0.06**	0.16**	0.16**	0.13**	0.07**	0.10**	0.29**	0.21**	0.31**	0.20**	0.10**	0.34**	0.13**	0.12**	0.28**

Appendix S2.8: The log probability of the data, LnP (X| *K*) ± SD, and the rate of change in the probability between successive runs (Δ*K*), as a function of *K* for the 25 *Botrylloides violaceus* populations.



3.0 - GENETIC ANALYSIS OF THE INVASIVE COLONIAL TUNICATE *BOTRYLLUS SCHLOSSERI* IN NORTH AMERICA: EVIDENCE FOR HIGH POPULATION GENETIC DIFFERENTIATION

3.1 INTRODUCTION

The colonial ascidian *Botryllus schlosseri*, also known as the golden star tunicate, is currently one of the most widely introduced species, having established populations globally, mainly through the transportation of colonies attached on the hulls of ships (Van Name 1945). Considered native to the Mediterranean Sea (Berril 1950; Brunetti *et al.* 1980; Carver *et al.* 2006), this species was first introduced on the East coast of North America in the 1830s (Van Name 1945; Stoner *et al.* 2002). It was reported in New Zealand and Australia in 1928 (Van Name 1945) and in Japan in 1929 (Tokioka 1953). On the West coast of North America, *B. schlosseri* was first observed in California in the San Francisco Bay area in the mid-1940s (Van Name 1945). Since then, it has established throughout the coast, where it can often be found on artificial structures in harbours, marinas, and aquaculture sites (Lambert & Lambert, 1998). The impact of *B. schlosseri* in areas of introduction has been touted mostly in marine aquaculture facilities. Here, this species is considered a serious biofouling concern, competing for food and space with target species, arguably causing crop losses and increasing the costs of operations (Carver *et al*. 2006; McNair *et al*. 2006).

The global invasion success of *B. schlosseri* has commonly been linked to several of its biological characteristics. First, high growth rates and tolerance to a wide range of

environmental conditions are thought to facilitate establishment of pioneering populations (Lambert & Lambert, 1998; Carver *et al*. 2006). Second, the capacity to regenerate a fully-functional organism from minute tissue fragments suggests that dispersal of this species within regions of introduction might be achieved not only through sexually-derived adults and larvae, but also through vegetative (asexual) propagules (Carver *et al*. 2006). Third, frequent association of *B. schlosseri* with anthropogenic dispersal vectors like recreational vessels and aquaculture trade, favours rapid secondary spread (Carver *et al*. 2006). However, other life history traits of *B. schlosseri* are not typically associated with colonizing species. For example, the free-swimming larval stage is short, often lasting for less than one hour (Rinkevich *et al*. 1998). Also, gregarious settlement of kin *B. schlosseri* larvae, a phenomenon that may promote inbreeding and loss of genetic variation, has been demonstrated in the field (Grosberg & Quinn, 1986). These contrasting traits make *B. schlosseri* an excellent system for investigating the interplay between microgeographical population structure, genetic diversity, and human-mediated dispersal in a widely distributed NIS.

In the current study I use a multiple-marker approach to characterize the genetic structure of native Mediterranean and introduced North American populations of *B. schlosseri*. The main objectives of this chapter are to i) explore the phylogeographic history of native and invasive *B. schlosseri* populations, ii) determine the level of genetic variation maintained in introduced populations in North America, and iii) identify patterns of gene flow and fine-scale genetic subdivision within the native, East and West coast ranges. To meet these goals, I use the mitochondrial (mt) cytochrome *c* oxidase I gene (COI) as well as 12 polymorphic microsatellite loci, while drawing on recent advances in the analysis of

hypervariable genetic data based on Bayesian clustering and assignment methods. I relate the results of these analyses to historical records of *B. schlosseri* invasions as well as to previous studies exploring the genetic aspects of this species' extensive range expansions.

3.2 MATERIALS AND METHODS

Sampling, DNA extraction and amplification

I analysed 5 native European (N = 79) and 18 introduced North American (N = 510) *B. schlosseri* populations (Table 3.1; Fig. 3.1). Sampling was undertaken either by SCUBA diving or by excising colonies from submerged ropes, buoys, and PVC settling plates in harbours and marinas. To prevent re-sampling of the same colonies, all specimens were taken at least 1 m apart from one another. Samples were preserved in 95% ethanol at -20 ℃ prior to genetic analyses.

Genomic DNA (gDNA) was extracted from single zooids using the protocol of Elphinstone *et al*. (2003). Amplification for the mitochondrial COI gene was initially performed using the universal primer pair LCO1490/HCO2198 (Folmer *et al*. 1994). Because these primers failed to amplify consistently, a species specific primer (BsCOIR: 5'-GTATTTTATTTTTAGAATTTGG TCAAG-3') was designed and used with the universal HCO2198 primer. The 25 µL PCR reactions consisted of 1 µL (~50 - 100 ng) gDNA, 1x PCR buffer with 1.5 mM $MgCl_2$ (Genscript), 0.2 mM dNTPs, 0.4 µM of each primer, and 0.5 U of Taq DNA Polymerase (Genscript). PCR cycling parameters consisted of an initial denaturation step at 94 ºC for 5 min, followed by 35 amplification cycles (94 ºC for 30 s, 50 ºC for 30 s, 72 ºC for 45 s), and a final elongation step at 72 ºC for 5 min. Sequencing reactions were performed using the HCO2198 primer, BigDye

Terminator 3.1 chemistry, and an ABI 3130XL automated sequencer (Applied Biosystems, Foster City, CA). The BsCOIR primer was used to confirm all sequences that contained ambiguous sites. Sequence data was edited and aligned using CodonCode Aligner 2.0.6 (CodonCode Corporation, Dedham, MA).

For all sampled individuals, genetic variation was further assessed using 12 microsatellite loci previously developed for this species including BS321, BS531, BS811 (Pancer *et al.* 1994), PB29, PB 49, PB41, PBC1 (Stoner *et al.* 1997), Bsm1, Bsm2, Bsm4, Bsm6, Bsm9 (Molecular Ecology Resources Primer Development Consortium *et al.* 2010). PCR cocktails (10 μL) contained 50 ng of gDNA, 1x PCR buffer with 1.5 mM $MgCl_2$ (Genscript), 0.125 mM of each dNTP, 0.5 μM of each primer, and 0.2 U of Taq DNA Polymerase (Genscript). Forward primers were labelled with one of four fluorophores (6FAM, VIC, NED or PET) according to Schuelke (2000). The PCR cycling profile consisted of initial denaturation at 95 ℃ for 3 min, 10 cycles of 35 s at 95 ℃, 35 s at an initial annealing temperature of 60 ℃ that decreased by 1 ℃ in each of 10 cycles, and 45 s at 72 ℃ followed by 35 cycles of 35 s at 95 ℃, 35 s at 52 ℃, 45 s at 72 ℃, and a final extension for 10 min at 72 ℃. Amplified fragments were separated on an ABI 3130XL automated sequencer (Applied Biosystems, Foster City, CA), with GeneScan - 500 LIZ (Applied Biosystems, Foster City, CA) internal size standard. The alleles were scored manually using GENEMAPPER® software v.4.0 (Applied Biosystems, Foster City, CA).

Data analysis

DNASP v.5 (Rozas *et al*. 2003) was used to identify individual *B. schlosseri* mtDNA haplotypes, calculate the number of haplotypes (*Nh*), haplotype diversity (*h*) and

nucleotide diversity (π) and to test whether the sequences evolved under neutrality according to Tajima's D statistic. For the microsatellite data, the number of repeated multilocus genotypes was calculated using GENECAP software (Wilberg & Dreher 2004). For all putative clones, I estimated the probability of identical genotypes arising by chance *via* sexual reproduction. I computed the lower bound of this probability, P_{HW} under Hardy-Weinberg expectations, and the more conservative upper bound P_{sib}, under strict sibling reproduction, as recommended by Waits *et al*. (2001). The total number of alleles (N_A), allelic richness (A), observed and expected heterozygosities (H_O and H_E), and the inbreeding coefficient (F_{IS}) were calculated using FSTAT v.2.9.3.2 (Goudet 2002). Conformance to Hardy-Weinberg equilibrium (HWE) expectations was tested using 10,000 permutations in GENEPOP v. 3.4 (Raymond & Rousset 1995), with levels of significance adjusted for sequential Bonferroni corrections (Rice 1989). To test for the presence of null alleles in the data set, the relationship between the number of nonamplifying samples for each locus and F_{IS} was examined, as recommended by Beaumont *et al*. (2001). A positive correlation would indicate that F_{IS} is affected by amplifying failure, due to null homozygotes.

Phylogenetic analyses were performed on COI sequence data using neighbor joining (NJ) and maximum-likelihood (ML) algorithms implemented in PAUP* v.4b10 (Swofford 2001) and PHYML 2.4.4 (Guidon & Gascuel 2003), respectively. *Botryllus tyreus* (GenBank accession number: DQ365851) was used as outgroup for the reconstructions. MODELTEST v.3.7 (Posada & Crandall 1998) was used to select the best-fit model of sequence substitution under the Akaike Information Criterion (AIC). Phylogeographic relationships among the COI haplotypes were further examined with a statistical

parsimony haplotype network generated at a 95% confidence level with TCS v.1.21 (Clement *et al*. 2000).

Population pairwise genetic differentiation was estimated based on Φ_{ST} (COI) and F_{ST} (microsatellites), using the Tamura & Nei (TrN) substitution model and 10,000 permutations in ARLEQUIN v.3.1 (Excoffier *et al*. 2005). ARLEQUIN was also used to conduct a hierarchical analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) for all *B. schlosseri* populations. Molecular variance was partitioned among populations and among the three biogeographic regions represented in the dataset: Europe (native range), East coast of North America, and West coast of North America (introduced ranges). A similar AMOVA was conducted with the microsatellite data, based on the same hierarchical geographic structure. Isolation by distance (IBD) was examined using COI and microsatellite data by testing the correlation between Rousset's (1997) genetic distance and geographical distances using a Mantel test with 10,000 permutations implemented in GENEPOP v. 3.4 (Raymond & Rousset 1995). Geographical distances were calculated as the minimum coastline distances between adjacent sampling locations using GOOGLE EARTH v.4.3 (beta).

Three dimensional factorial correspondence analysis (3D-FCA) performed in GENETIX v.4.05 (Belkhir *et al.* 2004) was used to further explore population relationships using the microsatellite data. Additionally, population structure was determined by using the Bayesian clustering approach implemented in STRUCTURE v.2.3.2 (Pritchard *et al.* 2000). For the STRUCTURE analysis, I carried out five independent Markov Chain Monte Carlo (MCMC) runs with 10^5 generations discarded as burn-in followed by an

additional 10^6 generations for each value of *K* (population clusters). I conditioned the data on values of *K* ranging from 1 to 23 (total sites). The optimal number of clusters was estimated by comparing the log-likelihood of the data given the number of clusters [ln P(X|*K*)] (Pritchard *et al*. 2000) and by examining the standardized second order rate change of ln P(X|*K*), Δ*K* (Evanno *et al.* 2005). Because simulations indicate that STRUCTURE detects only the uppermost level of genetic structure in a dataset (Evanno *et al.* 2005), I used a hierarchical approach, and performed separate analyses using individuals grouped into single clusters based on the global analysis.

Contemporary gene flow was assessed by individual-based assignment using BAYESASS v.1.3 (Wilson & Rannala 2003). This method was preferred over other individual-based assignment tests available as it does not require populations to be in HWE. Mean migration rates among populations were estimated by 3×10^6 MCMC iterations, with the first 10^6 discarded as burn-in. Samples were collected every 2000 generations to infer posterior probability distributions of parameters of interest.

3.3 RESULTS

Identification of clonal genotypes

Only five genotypes were repeated in the dataset, occurring between one and two times in their population of origin (Appendix S3.1). There was no repeated genotype shared between collection sites. For each replicate, the probability of two occurrences arising *via* random sexual recombination under HWE (P_{HW}), considering the microsatellite allele frequencies in the populations of origin was extremely low and ranged from 6.15×10^{-10} to 1.02×10^{-6}. Also, the more conservative estimate (P_{sib}) remained low and

nonsignificant ($P < 0.05$), suggesting that repeated genotypes resulted from resampling of fragmented colonies. Data analysis was performed with and without clones, producing similar results. I present results obtained after removing clonal genotypes from the data set.

Geographic patterns of genetic diversity

The 524 bp final COI alignment contained a total of 97 polymorphic sites, among which 96 were parsimony-informative. Most positions had synonymous substitutions, and only one exhibited a non-synonymous change. Within the total of 524 *B. schlosseri* individuals analysed, 17 distinct haplotypes were identified: 13 (HA, HC, BR, Bs1-Bs5; Bs8-Bs12) that were previously reported by Lejeusne *et al*. (in press), and 4 (Bs13-Bs16) that are new (Table 3.1; Appendix S3.2). Among the 17 haplotypes, 14 (82.4%) are found in multiple individuals and 3 (17.6%) are singletons. Native range samples contained seven haplotypes (Bs3, Bs5, Bs9, Bs11, Bs12, HA, HC), including two common ones (HA and HC; 88.6 % of native samples) that were shared with invasive populations (Table 3.1; Appendix S3.2). In the introduced range, 10 haplotypes were recovered, with Bs2, BR and Bs1 collectively accounting for 92.5% of all individuals analysed. Native populations averaged 3.2 COI haplotypes (range 2 - 5), while in the invaded range, East coast populations averaged 2.6 (range 1 - 4) and West coast populations 2.0 (range 1 - 4). Also, mean haplotype diversity in the native range was 0.541, compared to 0.398 on the East coast (mean loss of 26.4 %) and 0.323 on the West coast (mean loss of 40.3 %; Table 3.1).

Overall, 190 alleles were discerned among the 12 microsatellite loci analysed for *B. schlosseri* populations in Europe and North America (Appendix S3.3). Of those, 132 (69.5%, mean: 11 alleles / locus) characterize native populations, 106 (55.8%, mean: 8.8 alleles per locus) East coast populations and 108 (56.8%, mean: 9 alleles per locus) West coast populations. The number of private alleles was higher for native populations (23 alleles) than either East coast (13 alleles) or West coast (11 alleles) locales. Also, mean gene diversity (H_E) for native populations (0.690) was higher than East coast (0.559; mean loss of 18.9 %) or West coast (0.577; mean loss of 16.4 %; Table 3.1) sites. Mean allelic richness (A), corrected for differences in sample size by rarefaction to 11 individuals, was higher for native populations (5.7) than either East coast (3.8; mean loss of 33.3 %) or West coast (3.7; mean loss of 35.08 %) samples (Table 3.1; Appendix S3.4).

Significant deviations from HWE were observed in 54 out of the 204 analysed cases (Appendix S3.4). Heterozygote deficiencies were observed at most loci in all of the populations sampled, resulting in positive F_{IS} values in 157 out of 194 cases (80.9%, Appendix S3.4). Null alleles did not appear to have a major contribution, due to the lack of relationship between F_{IS} and nonamplification ($P > 0.05$) for all of the 12 loci used.

Phylogeographic structure

The parsimony haplotype network generated in TCS identified three distinct groups of haplotypes at the 95% confidence level, separated by a maximum of 10 connecting mutational steps (Fig. 3.2). Additionally, in the native range, two highly divergent haplotypes were identified (Bs11 and Bs12), separated, respectively, by a minimum of 67

and 64 mutational steps from all other haplotypes sampled in this study. In the introduced range, samples were characterised by different dominant haplotypes on each coast: Bs2 and BR on the East coast, and Bs1 on the West coast. Haplotype sharing between coasts was extremely reduced and consisted of a single occurrence of the dominant East coast haplotype Bs2 in a colony sampled at Ladysmith (LSM) on the West coast (Table 3.1; Fig. 3.2). The phylogenetic analyses confirmed these findings. The four clades identified (Fig. 3.3) corresponded to the grouping suggested by the parsimony network. Within the native range, clade 1, comprising haplotypes Bs11 and Bs12, was highly divergent from all other clades inferred from the data (mean 10% sequence divergence, Tamura-Nei model; Fig. 3.3). Clades 2 - 4 contained all remaining haplotypes found in the locations sampled. All major clades had high overall bootstrap support (Fig. 3.3).

Population genetic structure

Hierarchical AMOVA based on the COI data revealed that most of the genetic variation is attributable to differences between groups when populations were grouped as native and introduced (46.73%, $P < 0.0001$) or native, East coast, and West coast (43.72%; $P < 0.0001$). However, for both types of population groupings, a significant fraction of genetic variation was distributed within groups and within sampling sites (Table 3.2). A strong geographic partitioning of COI haplotypes was also supported by Φ_{ST} estimates, indicating high and significant genetic differentiation for population pairwise comparisons between the native, East coast and West coast sampling areas (Appendix S3.5). The only exceptions were comparisons between two native populations (ETQ and BLN) and two West coast populations (SQN and BBY), which were low and not significant (Appendix S3.5). Within each region, Φ_{ST} estimates were generally low and

not significant. For the microsatellite data, AMOVA tests indicated that most of the genetic variance was partitioned within sampling sites, although significant structure was also inferred between and within the population groups considered (Table 3.2). With the exception of native range samples, almost all pairwise F_{ST} estimates were high and significant, demonstrating pronounced genetic structure exists between most of the sites analysed (Appendix S3.6).

The 3D-FCA highlighted the distinctiveness of native, East coast and West coast populations, which formed three distinct clusters (Fig. 3.3). Within this general structure, component 1 of the analysis, accounting for most of the genetic variation (23.32%), indicated West coast populations are more similar to native range populations than East coast ones. This finding was confirmed by the Bayesian algorithm implemented in STRUCTURE. Both methods of determining the most likely value of *K* indicated the presence of two main distinct clusters for the global dataset (Fig. 3.4, Level 1). The likelihood of the data was lowest for $K = 1$ and the largest difference of successive likelihoods was between $K = 1$ and $K = 2$. After $K = 2$, the likelihood of the data plateaued and standard deviations increased. When ln $P(X|K)$ only marginally increases above a certain value of *K*, the smallest value of *K* before the plateau (here $K = 2$) is considered the best model (Pritchard *et al*. 2007). The estimation of ΔK as per Evanno *et al*. (2005) also showed a clear pattern for $K = 2$, confirming that this is the most parsimonious model for the data. The two hypothetical clusters correspond to: i) native and West coast samples, and ii) East coast populations. In subsequent hierarchical analyses, substantial subdivision was revealed for each of the two main genotype clusters (Fig. 3.4, Levels 2 & 3).

The BAYESASS analysis supports the conclusion that *B. schlosseri* populations are exchanging migrants only within each of the three main sampling regions analysed (native, East coast and West coast; Table 3.3). Within the native range, migration events were inferred to occur from Alicante (ALC), or a genetically similar population, to Arenys de Mar (ADM) and Blanes (BLN). Within the East coast, migration seems to occur to Point Tupper (PTR), Ballentyne's Cove (BTC), Guysborough (GBR) and Digby (DGB) from a population genetically similar to Petit de Grat (PDG). Within the West coast, migration was inferred from French Creek (FRC) to Ladysmith (LSM) and Brinnon (BRN). There was no significant relationship between geographical and genetic distances for either COI or microsatellite data in native, East coast, or West coast ranges.

3.4 DISCUSSION

Genetic structure within the native Mediterranean range

In the current study, reduced genetic differentiation was indicated between the native *B. schlosseri* populations sampled. This finding was supported by the observation of extensive haplotype sharing between collection sites (Table 3.1), and the generally low and not significant pairwise Φ_{ST} and F_{ST} estimates (Appendices S3.3 and S3.7). Additionally, high migration rates were inferred between several native locales by the Bayesian assignment test (Table 3.3). Collectively, these observations suggest the possibility of long-distance dispersal events (most likely human-mediated) occurring in the region. Given the intensity of recreational sailing in the Mediterranean and the propensity of *B. schlosseri* to associate with vessel-related vectors of dispersal (Dijkstra *et al.* 2007), this is a highly probable scenario.

The only exceptions to the high genetic resemblance observed between native populations were given by comparisons between Sete (SET) and Arenys de Mar (ADM), and SET and Alicante (ALC). The genetic distinctiveness of the SET population, as revealed by the significant pairwise Φ_{ST} comparisons (Appendix S3.5), is most likely caused by the presence in that population of two haplotypes (Bs11 and Bs12) that show substantial sequence divergence from all other haplotypes sampled (Figs. 3.2 and 3.3). Previous studies of *B. schlosseri* populations from the Atlanto-Mediterranean coast have also indicated the presence of few highly divergent haplotypes in harbours and marinas (Lopez-Legentil *et al*. 2006; Lejeusne *et al*. in press). The most likely explanation for this pattern is the occurrence of multiple introduction events from genetically differentiated areas (native or introduced), a phenomenon previously described in the Mediterranean for other systems such as the green crab *Carcinus maenas* (Darling *et al*. 2008). Again, given the high intensity of sea trade connections between the Mediterranean Sea and most global regions, this is a highly plausible scenario.

Genetic structure within the invaded North American range

Analysis of mtDNA and microsatellite data demonstrated that the East and West coast *B. schlosseri* populations are highly genetically differentiated. Pairwise Φ_{ST} and F_{ST} estimates (Appendices S3.3 and S3.7), 3D-FCA (Fig. 3.3) and STRUCTURE analysis (Fig. 3.4) all strongly supported this conclusion. Additionally, the assignment test suggested that migration events between the two biogeographical regions are limited (Table 3.3). These findings are in concordance with those reported in a preliminary microsatellite survey of eight North American *B. schlosseri* populations (Stoner *et al*. 2002), and a recent mitochondrial COI survey (Lejeusne *et al*. in press), indicating strong

genetic partitioning between the two coasts. Collectively, these observations exclude the possibility of an East coast origin for the more recent West coast introduction, and suggest different source regions might have seeded the two invasions.

The strong partitioning of genetic variance observed between the East and West coasts is accompanied by substantial population genetic structure at the intracoastal level as well. Two major genotype clusters were detected in each region, with further genetic subdivision indicated by subsequent hierarchical analyses in STRUCTURE (Fig. 3.4). Overall, population genetic differentiation was more pronounced on the East coast (95 % of pairwise F_{ST} estimates significant) than on the West coast (50 % of pairwise F_{ST} estimates significant; Appendix S3.6). Most likely, this distinction is a consequence of the different chronologies of the East and West coast invasions, since the former occurred at least 110 years prior to the latter (Van Name 1945). During this period, limited natural dispersal capacity of *B. schlosseri* and/or additional introductions, could have determined the higher genetic differentiation between East coast sites.

The patterns of spread across the two regions appear to be dominated by long distance dispersal. The assignment test indicated migration occurs only between key locations on each coast (Table 3.3). Also, the lack of isolation-by-distance patterns suggests that *B. schlosseri* is spreading mainly *via* human-mediated 'jump' dispersal events, in accordance with its limited ability to disperse independently (Carver *et al*. 2006). Furthermore, the low numbers of clonal genotypes identified, and their apparent confinement to the populations they were sampled in (Appendix S3.1), indicate spread is

achieved mostly through sexually-derived propagules, and less through the fragmentation and subsequent regeneration of colonies.

Genetic diversity of introduced *B. schlosseri* populations

A widespread belief in invasive species research has been that introduced populations commonly experience loss of genetic variation due to founder effects and bottlenecks (Holland 2000). In accordance with this expectation, a number of experimental studies have featured low levels of genetic variation in introduced populations of NIS (see for example Chandler *et al*. 2008, Peacock *et al*. 2009). However, recent empirical evidence suggests invasive species may circumvent such reductions, mainly as a result of multiple introductions from genetically differentiated sources (e.g. Kolbe *et al*. 2004; reviewed by Roman & Darling 2007).

Overall, the current study has confirmed the theoretical prediction that introduced populations of *B. schlosseri* show less genetic variation than native sites (Table 3.1). In the native range, mitochondrial genetic diversity estimates of haplotype (0.541) and nucleotide (0.023) diversity were comparable to those previously reported by Lopez-Legentil *et al*. (2006) and Lejeusne *et al*. (in press) for populations on the Atlanto-Mediterranean coast. On average, these estimates were higher than those observed for introduced populations on the East (0.398 and 0.013) or West coasts (0.323 and 0.008). Microsatellite genetic diversity parameters of gene diversity (H_E) and allelic richness (A) were also noticeably higher for native sites (0.690 and 5.7) than either East (0.559 and 3.8) or West (0.577 and 3.7) coast ones (Table 3.1). However, this general trend conceals substantial heterogeneity in the level of genetic diversity retained in populations within

the two North American invaded ranges. On the East coast, Port La Tour (PLT), Sydney (SYD), Point Tupper (PTR), and Yarmouth (YMT) exhibited levels of genetic variation comparable to native sites. By contrast, the most depressed levels of genetic diversity were identified for the Little Narrows (LTN) population, which was also markedly different from nearby sites according to the STRUCTURE analysis (Fig. 3.4). Jointly, these results suggest LTN most likely resulted from a recent colonization event, by a limited number of propagules. On the West coast, the pattern observed provides an unexpectedly clear illustration of successive bottleneck events, with genetic variation decreasing precipitously in a South-North direction along the coast. The highest genetic diversity was detected in the south, at Bodega Bay (BBY). Other West coast sites, such as Sequin (SQN), Brinnon (BRN), and Ladysmith (LSM) were of intermediate diversity, while the northernmost samples, French Creek (FRC) and Deep Bay (DPB), displayed significant reductions in variation. Therefore, the pattern observed on the West coast is consistent with the generally accepted idea that the *B. schlosseri* invasion began in San Francisco Bay, California (Van Name 1945). From there, the invasion spread northward along the coast, most likely facilitated by anthropogenic vectors of dispersal.

Heterozygote deficiencies in *B. schlosseri*

In the current study, significant heterozygote deficits were detected in all populations surveyed, across multiple microsatellite loci (Appendix S3.4). These results are in accordance with previous studies that have used microsatellite markers to analyse the genetic structure of *B. schlosseri* populations (e.g. Ben-Shlomo *et al*. 2001; Stoner *et al*. 2002; Ben-Shlomo *et al*. 2006). Three factors can be considered to explain this pattern: microsatellite null alleles, inbreeding, and subpopulation structure (i.e. Wahlund effect).

Among these, microsatellite null alleles appear to be the least likely factor, as indicated by two independent lines of evidence: excess homozygosity was not observed consistently at any one locus in the present study, and no positive relationship between F_{IS} and nonamplification was detected at any of the loci used.

Therefore, inbreeding and subpopulation structure appear to be more likely candidates. Although inbreeding has been previously reported in other ascidian species such as the solitary *Corella eumyota* (Dupont *et al*. 2007), in *B. schlosseri*, a protogynous hermaphrodite, self-fertilization is effectively prevented by the different maturation times of sperm and ovules of the same colony (Yund *et al*. 1997). However, because larvae usually settle close to the parental colony and other sibling larvae (Grosberg & Quinn 1986), mating between kin *B. schlosseri* colonies could occur (Grosberg 1988; Rinkevich *et al*. 1998). Such aggregations have also been shown to lead to subpopulation structure and differentiation over very small spatial scales (< 5 m; Yund & O'Neil 2000). The results presented here, indicating substantial intraspecific admixture even within populations (see Fig. 3.4), support this conclusion. I should note that similarly high levels of heterozygote deficiency have not been observed in the colonial ascidian *Botrylloides violaceus* (Chapter 2.0). These patterns suggest there may be substantial variation in dispersal-related life history traits between closely related invasive colonial ascidians.

3.5 CONCLUSIONS

Evidence presented in the current study corroborate with previous genetic surveys of invasive *B. schlosseri* populations, and indicate the likelihood that different introduction events occurred on the East and West coasts of North America. However, due to the long

history of anthropogenic range expansions of this species and its current cosmopolitan distribution, inferences on the origins of the introduction events could not be made. Still, the study dataset helped to substantiate a high degree of genetic structure for populations on each coast while revealing great heterogeneity in levels of genetic variation retained among populations, even at small spatial scales. Our data and analyses additionally provided clear evidence of long distance dispersal occurring between sites, most likely assisted by anthropogenic vectors of dispersal. As *B. schlosseri* continues to spread and colonize new environments, these are important information to consider in the management actions that are to be taken.

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Table 3.1: Locations of *Botryllus schlosseri* sampling and genetic diversity indices for mitochondrial and microsatellite markers with N_C, sample size including clonal genotypes; *N*, sample size after removal of clones; *Nh*, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity; N_A, number of alleles; N_{AP}, the number of private alleles for each sampling site; *A*, allelic richness; H_O, mean observed heterozygosity; H_E, mean expected heterozygosity.

Location	**ID**	**mtDNA**						**Microsatellite**						
		N_C	*N*	*Nh*	**Haplotype codes**	*h*	π	N_C	*N*	N_A	N_{AP}	*A*	H_O	H_E
Native range														
Estaque, France	ETQ	5	5	2	HA, HC	0.600	0.014	7	7	53	4	-	-	-
Sete, France	SET	6	6	4	HA, HC, Bs11, Bs12	0.867	0.079	6	6	37	0	-	-	-
Blanes, Spain	BLN	13	13	3	HA, HC, Bs3	0.603	0.012	14	14	69	2	5.4	0.427	0.684
Arenys de Mar, Spain	ADM	19	19	2	HA, HC	0.199	0.005	19	19	72	3	5.3	0.419	0.692
Alicante, Spain	ALC	28	28	5	HA, HC, Bs3, Bs5, Bs9	0.434	0.007	33	33	102	14	6.3	0.454	0.695
Total		**71**	**71**	**7**		**0.541**	**0.023**	**79**	**79**	**132**	**23**	**5.7**	**0.433**	**0.690**
Introduced range														
East Coast														
Sydney, NS	SYD	30	30	3	BR, Bs2, Bs16	0.522	0.016	30	30	58	2	3.9	0.404	0.548
Little Narrows, NS	LTN	64	63	1	Bs2	0.000	0.000	61	60	50	0	3.1	0.294	0.447
Petit de Grat, NS	PDG	40	40	2	Bs2, BR	0.385	0.013	29	29	57	0	4.1	0.235	0.539
Point Tupper, NS	PTR	17	17	3	Bs2, Bs13, BR	0.559	0.018	17	17	54	1	4.2	0.392	0.628
Ballantyne's Cove, NS	BTC	36	36	2	Bs2, BR	0.386	0.013	24	24	48	1	3.7	0.337	0.605
Guysborough, NS	GBR	28	28	2	Bs2, BR	0.254	0.009	27	27	50	0	3.6	0.216	0.539
Shining Waters, NS	SWM	29	29	3	HA, Bs2, BR	0.394	0.014	20	20	39	1	3.0	0.259	0.526
Lunenburg, NS	LNB	40	40	3	Bs2, Bs14, BR	0.381	0.009	29	29	48	5	3.5	0.310	0.551
Digby, NS	DGB	22	22	3	HA, Bs2, BR	0.177	0.006	21	21	52	0	4.0	0.331	0.568
Ingomar, NS	IGM	8	8	2	Bs2, BR	0.429	0.015	8	8	44	0	-	-	-

Port La Tour, NS	PLT	8	8	4	Bs2, Bs4, BR, HA	0.750	0.024	8	8	38	1	-	-	-
Yarmouth, NS	YMT	26	26	3	HC, Bs2, BR	0.542	0.016	31	31	66	2	4.6	0.406	0.640
Total		**348**	**347**	**8**		**0.398**	**0.013**	**305**	**304**	**106**	**13**	**3.8**	**0.318**	**0.559**
West Coast														
Deep Bay, BS	DPB	9	9	1	Bs1	0.000	0.000	8	8	31	0	-	-	-
French Creek, BC	FRC	28	24	1	Bs1	0.000	0.000	27	23	48	1	3.5	0.397	0.577
Ladysmith, BC	LSM	17	15	2	Bs1, Bs2	0.133	0.002	15	13	41	1	3.3	0.485	0.567
Brinnon, WA	BRN	11	11	2	Bs1, Bs8	0.436	0.007	11	11	42	0	3.5	0.374	0.539
Sequin, WA	SQN	6	6	2	Bs1, Bs10	0.600	0.017	5	5	39	1	-	-	-
Bodega Bay, CA	BBY	36	36	4	Bs1, Bs8, Bs10, Bs15	0.767	0.019	36	36	71	8	4.6	0.469	0.623
Total		**107**	**101**	**4**		**0.323**	**0.008**	**102**	**96**	**105**	**11**	**3.7**	**0.431**	**0.577**

Table 3.2: Analysis of molecular variance (AMOVA) results on *Botryllus schlosseri* mtDNA and microsatellite data for native vs. invasive and native vs. East coast vs. West coast population groupings. All fixation indices are statistically significant.

Source of variation	d.f.	Variance components	% variation	Fixation indices	*P* value
mtDNA					
Among groups (native vs. invasive)	1	4.322 Va	46.73	F_{CT}: 0.467	0.000
Among populations within groups	22	2.038 Vb	22.04	F_{SC}: 0.414	0.000
Within populations	514	2.888 Vc	31.23	F_{ST}: 0.688	0.000
Among groups (native vs. East coast vs. West coast)	2	3.317 Va	43.72	F_{CT}: 0.437	0.000
Among populations within groups	21	1.382 Vb	18.21	F_{SC}: 0.324	0.000
Within populations	524	2.888 Vc	38.06	F_{ST}: 0.619	0.000
Microsatellite					
Among groups (native vs. invasive)	1	0.302 Va	9.71	F_{CT}: 0.097	0.001
Among populations within groups	15	0.458 Vb	14.70	F_{SC}: 0.163	0.000
Within populations	857	2.355 Vc	75.59	F_{ST}: 0.244	0.000
Among groups (native vs. East coast vs. West coast)	2	0.380 Va	12.38	F_{CT}: 0.124	0.000
Among populations within groups	14	0.334 Vb	10.89	F_{SC}: 0.124	0.000
Within populations	857	2.355 Vc	76.73	F_{ST}: 0.234	0.000

Table 3.3: Mean immigration rates among 17 *Botryllus schlosseri* localities, with source populations listed by column and recipient populations by row. Values along the diagonal are self-recruitment rates for each locality (bold). Only the most likely immigrant sources, with means > 0.1, are presented. Populations with less than 10 individuals ($N < 10$) were not included.

	Native			**East coast**										**West coast**			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1. BLN	**0.689**		0.214														
2. ADM		**0.682**	0.248														
3. ALC			**0.982**														
4. SYD				**0.988**													
5. LTN					**0.994**												
6. PDG						**0.989**											
7. PTR						0.181	**0.684**										
8. BTC						0.249		**0.680**									
9. GBR						0.211			**0.678**								
10. SWM										**0.981**							
11. LNB											**0.988**						
12. DGB						0.168						**0.681**					
13. YMT				0.185									**0.676**				
14. FRC														**0.986**			
15. LSM														0.173	**0.689**		
16. BRN														0.168		**0.692**	
17. BBY																	**0.991**

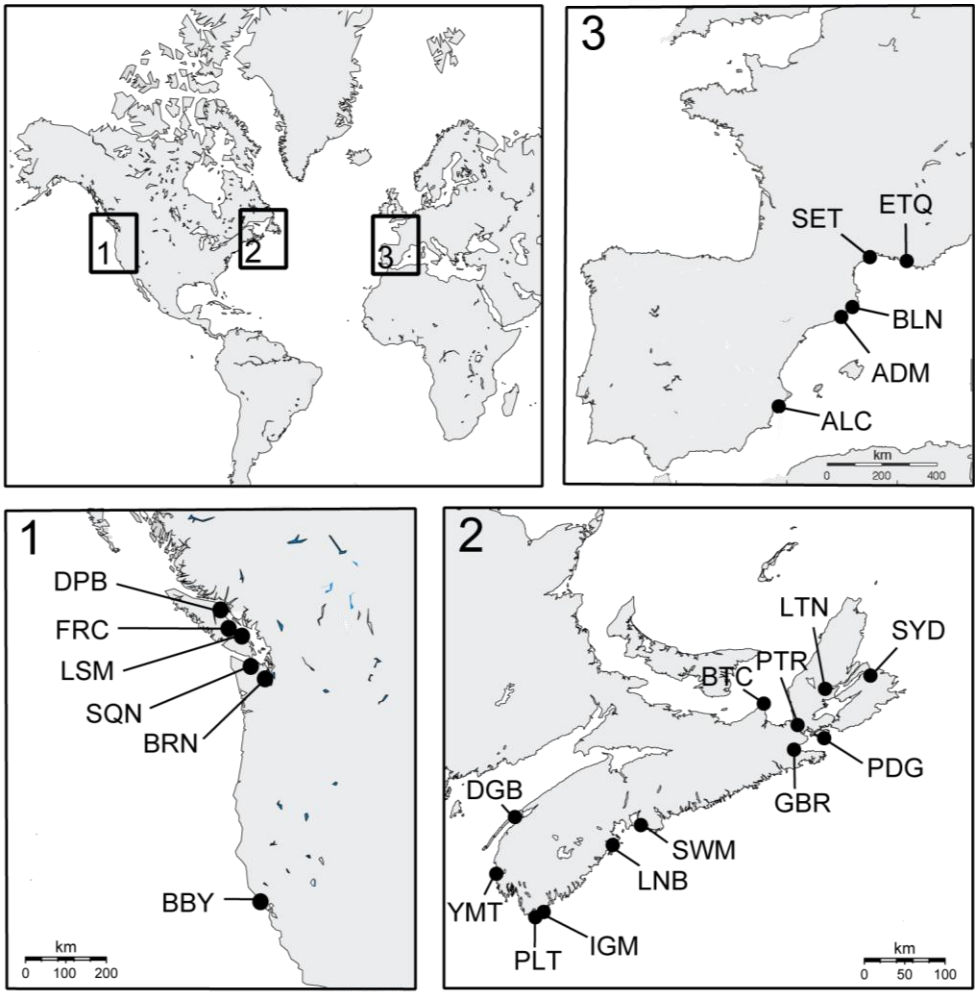

Figure 3.1: Map of the sampling locations for the golden star tunicate *Botryllus schlosseri* with locality names defined in Table 3.1.

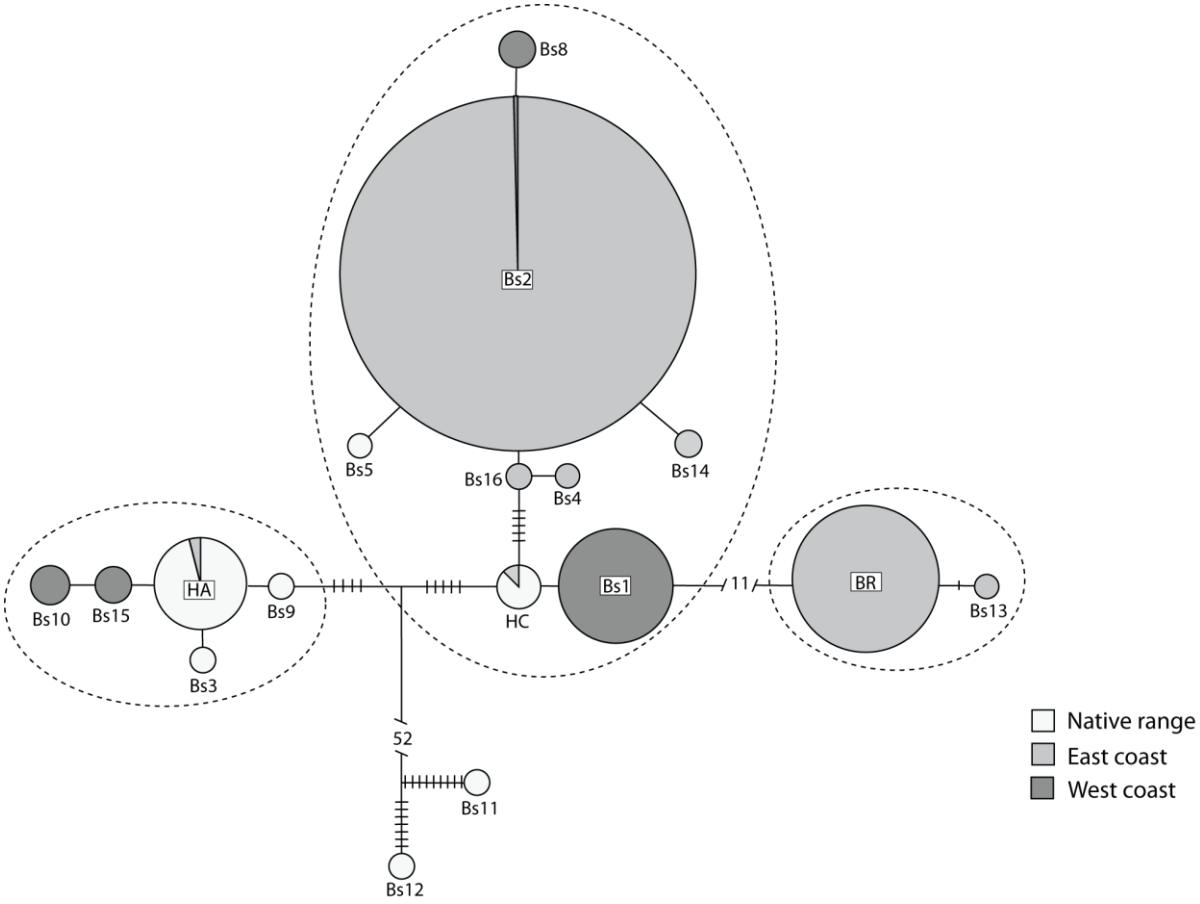

Figure 3.2: Statistical parsimony network of *Botryllus schlosseri* cytochrome *c* oxidase subunit I (COI) haplotypes. Circle size is proportional to haplotype frequency. Unsampled haplotypes inferred from the data are indicated by hashes, or by numbers in the case of widely divergent haplotypes. Dashed lines indicate clades well-supported in maximum parsimony analysis (> 95% majority rule consensus support).

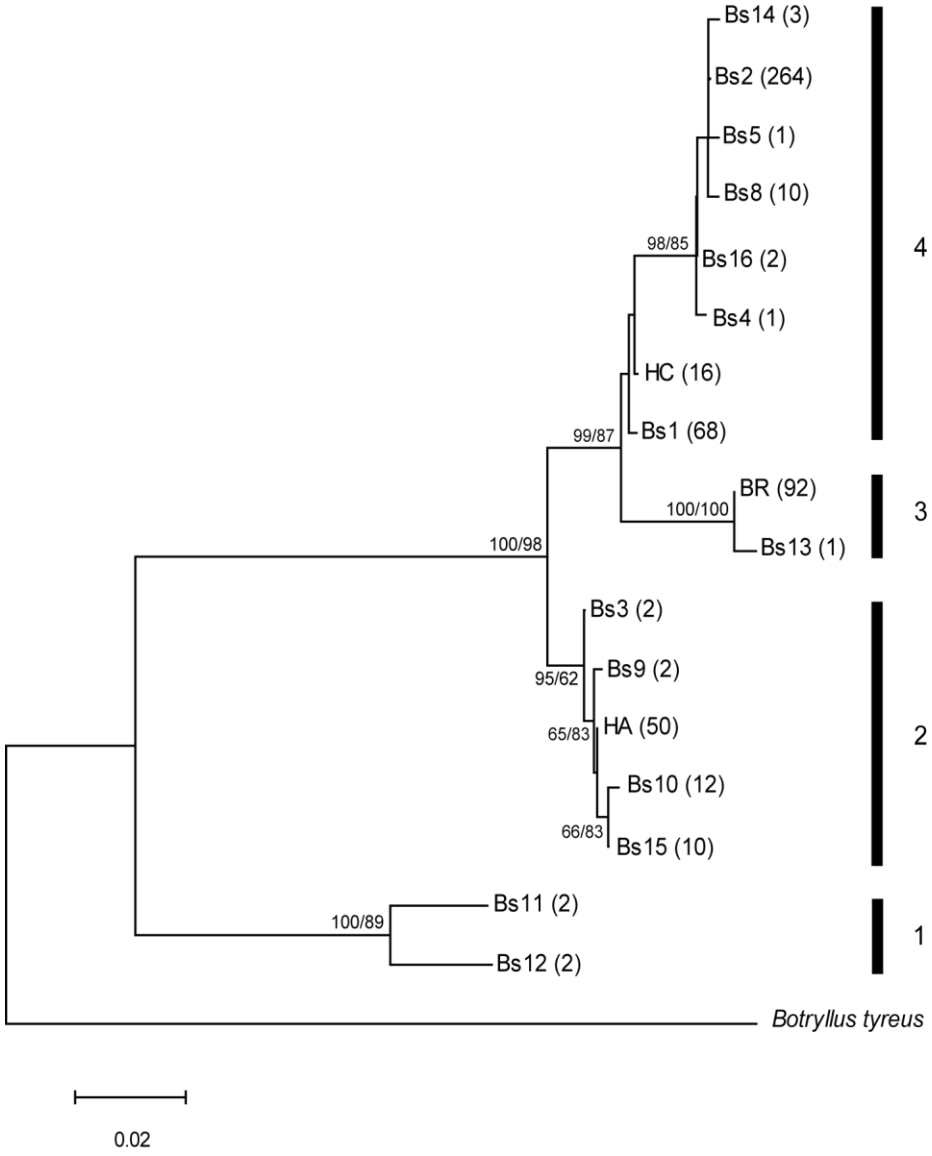

Figure 3.3: Neighbor-joining phylogenetic reconstruction of *Botryllus schlosseri* cytochrome *c* oxidase subunit I (COI) haplotypes. Numbers at phylogenetic nodes indicate the neighbor-joining and maximum-likelihood bootstrap support with 1,000 replicates. The number of samples possessing each haplotype is presented in brackets.

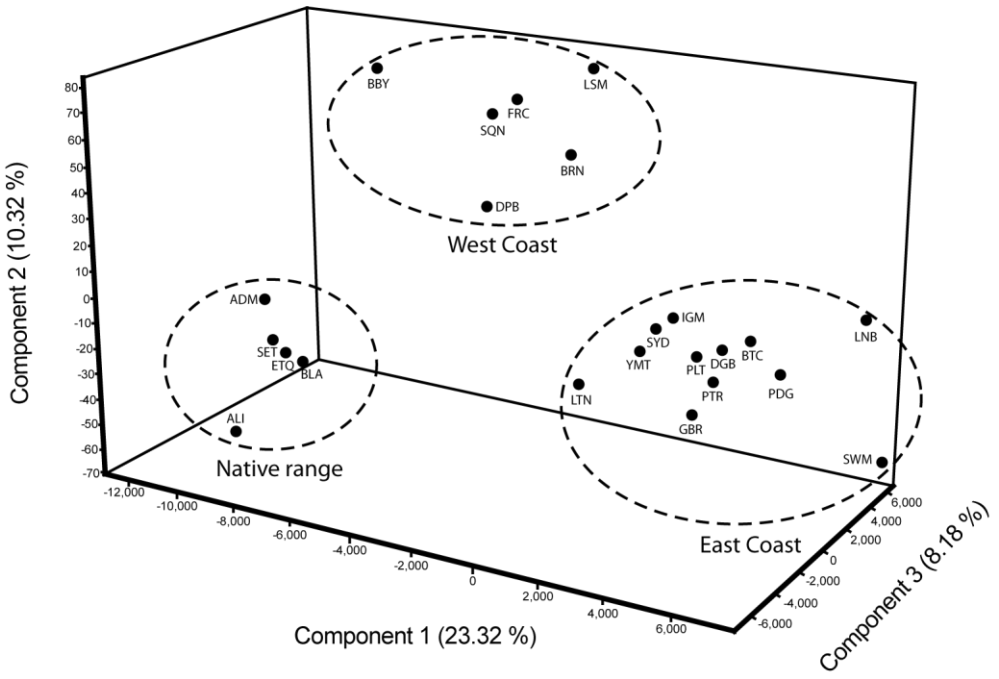

Figure 3.4: Three dimensional factorial correspondence analysis (3D-FCA) of *Botryllus schlosseri* microsatellite data showing clustering between native, West coast and East coast sites.

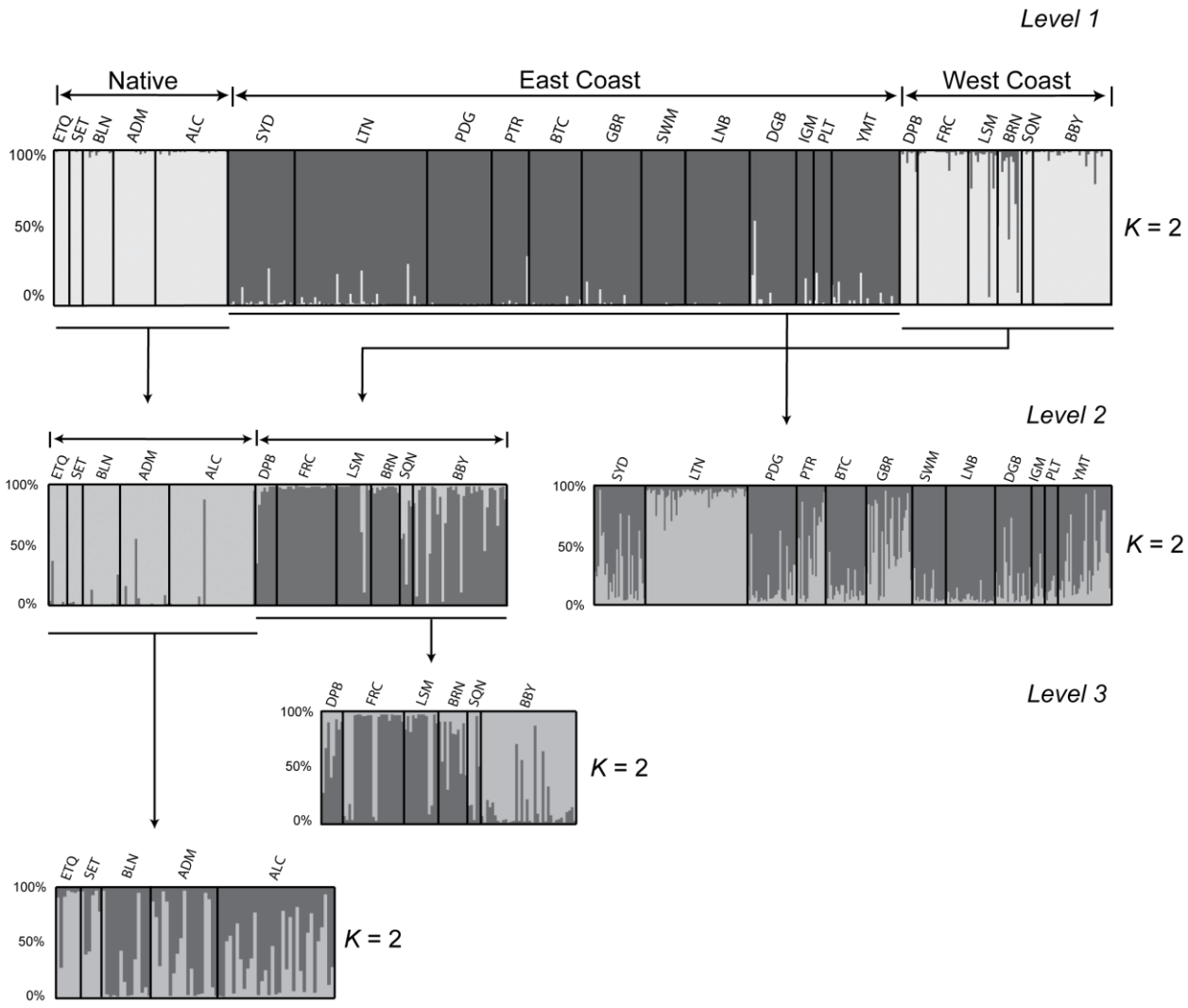

Figure 3.5: Bayesian clustering of *Botryllus schlosseri* genotypes performed in STRUCTURE for all samples (Level 1) and each of the main genetic clusters (Levels 2 and 3). Each individual is represented by a thin vertical line, which is partitioned into *K* = 2 segments representing the individual's estimated proportional membership (*Q*). Sampling sites are separated by black lines. Different shades of grey are used to represent the genetic clusters in each data subset.

Appendix S3.1: Clonal genotypes observed in the dataset with *N*, number of times the genotype appears in the dataset; N_{gen}, number of genotypes in the population of origin; P_{sib}, probability of identity considering strict sibs reproduction; P_{HW}, probability of identity under Hardy-Weinberg equilibrium

Genotype	**Location**	N	N_{gen}	P_{sib}	P_{HW}
East Coast					
C1 - LTN	Little Narrows	2	60	0.003	1.02×10^{-6}
West Coast					
C2 - FRC	French Creek	3	23	0.004	1.29×10^{-7}
C3 - FRC	French Creek	3	23	0.004	1.50×10^{-7}
C4 - LSM	Ladysmith	2	13	0.001	6.15×10^{-10}
C5 - LSM	Ladysmith	2	13	0.001	1.04×10^{-8}

Appendix S3.2: Occurrence and frequency of *Botryllus schlosseri* COI haplotypes in 24 native and introduced locations.

Location ID	Haplotypes																
	HA	**HC**	**BR**	**Bs1**	**Bs2**	**Bs3**	**Bs4**	**Bs5**	**Bs8**	**Bs9**	**Bs10**	**Bs11**	**Bs12**	**Bs13**	**Bs14**	**Bs15**	**Bs16**
Native																	
ETQ	0.60	0.40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SET	0.17	0.17	-	-	-	-	-	-	-	-	-	0.33	0.33	-	-	-	-
BLN	0.54	0.38	-	-	-	0.08	-	-	-	-	-	-	-	-	-	-	-
ADM	0.89	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ALC	0.71	0.14	-	-	-	0.04	-	0.04	-	0.07	-	-	-	-	-	-	-
Introduced																	
East Coast																	
SYD	-	-	0.63	-	0.30	-	-	-	-	-	-	-	-	-	-	-	0.07
LTN	-	-	-	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-
PDG	-	-	0.25	-	0.75	-	-	-	-	-	-	-	-	-	-	-	-
PTR	-	-	0.35	-	0.59	-	-	-	-	-	-	-	-	0.06	-	-	-
BTC	-	-	0.25	-	0.75	-	-	-	-	-	-	-	-	-	-	-	-
GBR	-	-	0.14	-	0.86	-	-	-	-	-	-	-	-	-	-	-	-
SWM	0.03	-	0.76	-	0.21	-	-	-	-	-	-	-	-	-	-	-	-
LNB	-	-	0.15	-	0.78	-	-	-	-	-	-	-	-	-	0.07	-	-
DGB	0.03	-	0.03	-	0.94	-	-	-	-	-	-	-	-	-	-	-	-
IGM	-	-	0.25	-	0.75	-	-	-	-	-	-	-	-	-	-	-	-
PLT	-	-	0.57	-	0.29	-	0.14	-	-	-	-	-	-	-	-	-	-
YMT	-	0.07	0.31	-	0.62	-	-	-	-	-	-	-	-	-	-	-	-
West Coast																	
DPB	-	-	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
FRC	-	-	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
LSM	-	-	-	0.93	0.07	-	-	-	-	-	-	-	-	-	-	-	-
BRN	-	-	-	0.73	-	-	-	-	0.27	-	-	-	-	-	-	-	-
SQN	-	-	-	0.50	-	-	-	-	-	-	0.50	-	-	-	-	-	-
BBY	-	-	-	0.28	-	-	-	-	0.19	-	0.25	-	-	-	-	0.28	-

Appendix S3.3: Microsatellite allele frequencies for *Botryllus schlosseri* from 24 global locations

Locus	Allele	Native range					Introduced (East Coast)												Introduced (West Coast)					
		ETQ	SET	BLN	ADM	ALC	SYD	LTN	PDG	PTR	BTC	GBR	SWM	LNB	DGB	IGM	PLT	YMT	DPB	FRC	LSM	BRN	SQN	BBY
BS321	115	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-
	119	-	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	121	-	-	0.07	0.13	0.34	0.14	0.31	0.05	0.55	-	-	-	-	-	-	-	0.18	-	-	-	-	0.10	-
	127	0.58	1.00	0.64	0.46	0.38	0.81	0.14	0.93	0.32	0.33	0.67	1.00	0.53	0.97	1.00	0.81	0.68	1.00	0.50	0.70	0.85	0.70	0.37
	143	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-
	145	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-	0.10	-
	147	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01
	159	-	-	0.07	0.33	0.13	-	0.42	-	0.09	0.33	-	-	-	0.03	-	-	0.05	-	0.48	0.30	0.05	0.10	0.51
	161	-	-	0.07	0.08	0.06	0.03	0.14	0.02	-	-	-	-	-	-	-	0.06	0.02	-	0.03	-	0.10	-	0.10
	163	-	-	-	-	-	-	-	-	-	-	0.33	-	0.29	-	-	-	-	-	-	-	-	-	-
	165	0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	183	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	185	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-	-
	209	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-
	223	-	-	-	-	-	-	-	-	-	0.33	-	-	-	-	-	-	-	-	-	-	-	-	-
	255	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	267	-	-	0.07	-	0.06	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-
	301	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-
	307	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-	-	-	-	-	-	-
BS531	125	-	-	-	-	-	0.02	0.03	-	0.19	-	0.03	0.07	-	-	-	-	0.05	-	0.03	-	-	-	-
	129	-	-	-	-	-	-	-	0.04	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-
	135	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-
	143	-	-	-	0.07	0.02	-	-	-	-	-	-	-	0.14	-	-	0.36	0.16	0.06	0.07	0.13	-	-	-
	151	-	-	-	-	-	-	-	0.04	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-
	153	0.14	0.10	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	159	-	-	0.08	0.10	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	161	0.29	0.30	0.17	0.23	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	163	0.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	167	0.14	0.50	0.42	0.37	-	0.33	-	0.11	0.08	0.47	-	-	-	-	0.20	0.07	0.18	0.88	0.70	0.71	0.94	0.40	0.33

169	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
173	-	-	-	-	0.02	-	-	0.04	-	0.03	-	0.21	0.05	0.07	-	-	-	-	-	-	-	-	-	-
179	0.14	-	0.08	0.07	0.15	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	0.08	-	-	0.14	
193	-	-	-	0.03	0.04	-	-	-	0.04	-	-	-	-	-	-	-	-	-	0.10	-	-	-	0.02	
197	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	
203	-	-	-	-	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
209	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
211	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
213	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
217	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.11	
219	-	-	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
221	-	-	0.04	-	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
223	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.30	0.03	
225	-	0.10	-	-	0.04	0.19	0.06	0.28	0.35	0.05	0.30	0.07	0.20	0.43	0.40	0.07	0.34	-	-	-	0.06	-	0.03	
229	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
231	-	-	-	-	-	-	-	0.09	-	-	0.03	0.07	0.13	0.11	0.20	-	-	-	-	-	-	-	-	
233	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
239	0.14	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
243	-	-	-	-	0.02	-	-	0.09	-	-	0.08	0.36	0.07	0.07	-	0.43	-	-	-	-	-	-	-	
245	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
255	-	-	0.08	0.03	0.02	0.24	0.89	0.13	0.04	0.05	0.43	0.14	-	0.14	-	-	0.21	0.06	-	0.04	-	-	0.28	
259	-	-	-	-	0.10	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
263	-	-	0.04	-	-	0.13	-	0.07	0.27	0.40	0.08	0.07	0.38	0.11	0.20	-	0.05	-	-	-	-	0.30	0.03	
269	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
271	-	-	-	-	-	-	-	-	-	-	0.03	-	-	0.07	-	0.07	-	-	-	-	-	-	-	
279	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
281	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	
287	-	-	-	-	-	0.09	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
291	-	-	-	0.03	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
295	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
297	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

	300	-	-	-	-	-	-	-	0.02	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-
BS811	136	-	-	-	-	-	-	0.12	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	150	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	0.10	-	-	-	-	-
	154	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	0.04	-	-	-
	160	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	-	-	0.20	0.03	-	-	-	-
	162	0.07	0.20	0.04	0.06	-	-	-	-	-	-	-	-	-	-	0.10	0.07	-	-	-	-	0.08	-	-
	164	-	-	0.15	-	0.05	-	0.03	0.11	0.18	0.33	0.20	-	-	-	0.10	0.07	0.15	-	-	-	0.17	-	0.09
	166	-	0.20	0.27	0.03	0.05	0.25	-	-	-	0.33	-	-	-	-	-	-	0.04	0.20	0.03	0.29	-	-	0.09
	170	-	-	-	-	0.07	-	0.03	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	0.03
	172	-	-	-	-	0.17	0.05	-	-	-	-	-	-	-	-	0.10	-	0.08	0.10	-	-	-	-	0.03
	174	-	-	-	0.09	0.10	0.03	-	-	0.05	-	-	-	0.33	-	-	-	0.12	-	0.56	-	-	0.25	-
	176	-	-	-	0.03	-	-	-	0.11	0.05	-	-	-	-	-	0.10	-	0.04	-	-	0.46	-	-	0.25
	180	-	-	-	-	0.02	0.03	0.06	-	0.09	-	-	-	-	0.06	0.20	-	0.04	-	-	-	-	-	-
	182	-	-	-	-	-	-	-	-	-	-	-	-	-	0.11	-	-	-	-	0.06	0.04	0.08	-	-
	184	-	-	-	-	-	0.65	0.77	0.78	0.59	0.17	0.80	1.00	-	0.78	0.20	0.71	0.46	-	-	0.17	0.58	-	0.25
	190	-	-	-	-	-	-	-	-	-	0.17	-	-	-	-	0.10	-	-	-	-	-	-	-	0.03
	192	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	0.07	-	-	-	-	-	-	-
	202	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.07	-	-	-	-	-	-	-
	204	-	-	-	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	206	0.07	-	0.12	0.19	0.07	-	-	-	-	-	-	-	-	-	-	-	-	0.20	-	-	-	-	-
	208	0.14	-	0.08	0.22	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	0.09	-	-	-	-
	210	0.14	0.20	0.12	0.06	0.17	-	-	-	-	-	-	-	-	-	-	-	-	0.10	0.09	-	-	-	-
	212	0.50	0.20	0.12	0.06	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	0.13	0.09
	214	-	-	-	0.06	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	0.13	-
	216	-	-	0.04	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	224	-	-	-	0.09	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	226	0.07	0.20	0.04	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.08	0.13	0.03
	228	-	-	0.04	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	230	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	0.04	0.10	-	-	-	0.25	0.09
	232	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.13	-
	234	-	-	-	-	-	-	-	-	-	-	-	-	0.67	-	-	-	-	-	-	-	-	-	-

PB29	153	0.07	0.08	0.04	0.17	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.13	-	0.21
	154	0.29	-	0.04	-	-	-	-	-	0.07	-	-	-	-	-	-	-	-	-	0.10	-	-	-	0.03
	155	0.36	0.50	0.32	0.44	0.59	-	-	-	-	-	0.11	-	0.02	0.06	-	-	0.03	-	0.25	0.38	0.06	0.30	0.22
	157	0.14	-	0.11	-	-	0.74	0.85	0.55	0.23	0.50	0.73	0.11	0.66	0.47	0.81	0.43	0.63	0.43	0.40	0.25	0.56	-	0.25
	158	0.14	0.42	0.46	0.39	0.33	0.03	0.12	0.03	0.10	-	-	0.11	-	0.12	-	0.29	0.10	0.57	0.23	0.38	0.13	0.70	0.29
	160	-	-	-	-	0.02	0.02	0.01	-	0.03	-	0.02	-	-	-	0.06	-	0.18	-	-	-	-	-	-
	164	-	-	0.04	-	-	0.21	0.03	0.41	0.57	0.50	0.14	0.79	0.33	0.35	0.13	0.29	0.07	-	-	-	0.13	-	-
	167	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-
PB49	194	-	-	-	-	-	-	-	0.07	-	-	-	-	-	-	-	-	0.13	-	-	-	-	-	-
	200	-	-	0.18	0.15	0.04	0.02	0.05	0.17	0.23	0.07	0.06	0.71	0.14	0.08	0.13	0.06	0.16	-	-	-	0.05	-	0.03
	202	-	-	-	-	-	0.02	-	0.06	-	0.05	0.02	-	-	-	-	-	-	-	-	-	-	-	-
	204	-	-	-	-	-	0.12	0.59	0.13	0.42	0.07	0.38	-	0.02	-	-	-	0.14	-	-	-	-	-	-
	206	-	-	-	-	-	0.42	-	0.04	0.04	-	0.02	-	0.02	0.28	0.19	-	0.05	-	-	0.04	-	-	-
	208	-	-	-	-	0.02	0.18	0.03	0.32	0.15	0.48	0.20	0.11	0.55	0.14	0.44	0.06	0.23	-	0.72	0.79	0.75	0.30	0.22
	210	0.08	-	0.29	0.09	0.22	0.18	0.34	0.15	0.08	0.09	0.24	0.11	0.11	0.19	0.25	0.88	0.27	0.17	-	-	0.05	-	0.07
	212	-	-	-	-	-	-	-	-	-	0.07	-	0.03	-	-	-	-	-	-	0.03	0.17	-	-	0.07
	216	0.42	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	220	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	222	-	-	0.04	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	0.17	-	-	-	-	-
	224	-	-	0.04	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	0.67	0.06	-	-	-	0.10
	226	0.08	0.50	-	0.06	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	0.10	-
	228	0.08	-	0.07	0.12	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-	0.10	-
	230	0.17	-	0.14	0.03	0.19	0.07	-	0.07	0.08	0.18	0.08	0.05	0.16	0.31	-	-	0.02	-	0.08	-	0.10	0.50	0.26
	232	0.08	0.30	-	0.06	0.20	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-	-	0.04
	234	0.08	-	-	0.18	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01
	236	-	-	-	0.12	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.19
	238	-	-	0.07	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	240	-	-	0.07	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	244	-	0.20	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PB41	169	-	-	-	0.03	-	0.23	0.13	0.45	0.47	0.17	0.64	0.35	0.38	0.44	-	-	0.12	-	-	-	-	-	-
	171	-	-	-	-	-	0.43	0.30	0.29	0.19	0.06	-	0.65	0.50	0.32	0.43	1.00	0.22	-	0.05	0.17	0.40	-	-

	173	0.29	0.17	0.21	0.34	0.36	0.30	0.56	0.26	0.31	0.78	0.36	-	0.13	0.18	0.43	-	0.62	0.20	0.18	0.33	0.40	0.30	0.38
	175	0.36	0.33	0.14	0.11	0.13	-	0.01	-	0.03	-	-	-	-	-	-	-	0.02	0.20	0.64	0.33	0.20	0.50	0.59
	177	0.07	0.17	0.11	0.16	0.06	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.20	-
	179	0.29	0.33	0.50	0.34	0.39	0.04	-	-	-	-	-	-	-	0.06	0.14	-	0.02	0.60	0.14	0.17	-	-	0.03
	181	-	-	0.04	0.03	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PBC1	176	0.08	-	0.25	0.12	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-
	182	0.17	0.20	0.13	0.19	0.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	184	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	186	0.17	0.20	0.25	0.04	0.14	0.12	0.01	0.08	0.20	0.11	0.10	-	0.05	0.21	0.06	-	0.11	-	-	-	-	-	0.13
	188	0.08	-	-	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	0.02
	192	-	0.10	-	0.08	0.14	0.04	0.32	0.08	0.17	0.33	0.43	0.08	0.08	0.05	0.13	-	0.18	-	-	0.06	-	-	-
	194	0.25	-	-	-	0.09	-	0.02	0.02	-	0.06	-	-	-	0.05	-	0.50	0.04	-	-	-	-	-	-
	198	-	0.10	-	-	0.14	0.31	0.10	0.04	-	0.06	0.07	0.81	0.30	-	-	-	-	0.50	0.29	-	0.05	-	-
	200	-	-	-	-	-	0.12	0.16	0.14	0.30	0.03	0.21	0.04	0.35	0.26	0.31	-	0.11	-	0.10	0.28	0.10	0.25	0.02
	202	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.07
	203	0.25	0.40	0.38	0.39	0.05	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	-	0.48
	206	-	-	-	0.08	0.02	0.06	0.05	0.08	0.10	0.19	0.05	0.04	-	0.16	-	0.25	0.13	-	0.38	0.44	0.45	0.75	0.04
	208	-	-	-	0.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05
	212	-	-	-	-	-	0.21	0.30	0.50	0.20	0.22	0.07	0.04	0.23	0.11	0.44	0.25	0.32	0.44	0.24	0.22	0.25	-	0.20
	214	-	-	-	-	-	0.02	-	0.06	0.03	-	0.07	-	-	0.13	0.06	-	0.13	-	-	-	-	-	-
	218	-	-	-	-	-	0.02	0.03	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-
	221	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	223	-	-	-	-	-	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bsm1	211	0.08	0.08	0.32	0.18	0.40	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	0.20	0.07
	215	-	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	225	0.75	0.92	0.43	0.71	0.45	0.47	0.92	0.52	0.50	0.43	0.67	0.53	0.29	0.63	0.44	0.38	0.45	1.00	0.61	0.67	0.30	0.70	0.50
	231	0.08	-	-	-	0.02	0.22	0.05	0.35	0.35	0.27	0.19	0.18	0.59	0.29	0.25	-	0.33	-	0.37	0.29	0.55	0.10	0.14
	287	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01
	291	0.08	-	0.11	0.03	0.10	0.31	0.03	0.13	0.15	0.30	0.15	0.29	0.12	0.08	0.31	0.63	0.22	-	-	0.04	0.05	-	0.11
	293	-	-	0.07	0.08	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	-	0.17
Bsm2	162	-	-	0.07	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	165	0.21	0.25	0.18	0.29	0.23	0.09	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	0.05	0.10	0.16
	171	0.21	0.25	0.36	0.29	0.26	0.45	0.28	0.26	0.29	0.46	0.39	0.39	0.14	0.24	0.58	0.14	0.19	0.06	-	0.08	-	-	0.06
	174	-	-	-	0.08	0.10	0.02	0.01	0.30	-	0.10	0.13	0.17	0.04	0.17	0.08	0.29	0.36	-	-	0.04	-	-	-
	180	-	-	-	-	0.07	-	-	-	0.06	0.06	-	0.08	-	-	-	0.07	0.02	-	-	-	-	-	-
	183	0.50	0.50	0.11	0.26	0.26	0.41	0.70	0.17	0.44	0.23	0.24	0.08	0.54	0.50	0.25	0.07	0.23	0.50	0.77	0.62	0.64	0.50	0.56
	186	0.07	-	0.07	0.08	0.05	-	0.01	0.09	0.06	-	0.06	-	0.27	0.07	-	0.36	0.05	0.44	0.18	0.27	0.32	0.40	0.21
	192	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	0.02
	201	-	-	0.21	-	-	0.03	0.01	0.17	0.15	0.15	0.19	0.28	0.02	0.02	0.08	0.07	0.13	-	-	-	-	-	-
	204	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-
Bsm4	95	-	-	-	0.03	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	0.07	0.13	-	-	0.03
	99	0.07	0.17	-	0.05	0.13	0.40	0.40	0.41	0.44	0.48	0.30	0.80	0.38	0.41	0.25	0.50	0.27	0.44	-	0.29	-	0.40	0.18
	101	0.36	0.25	0.57	0.55	0.34	0.30	0.19	0.20	0.18	0.17	0.19	0.05	0.18	0.33	0.19	0.19	0.37	0.13	0.41	0.25	0.55	0.20	0.61
	103	0.36	0.08	0.18	0.18	0.27	0.18	0.42	0.23	0.27	0.08	0.50	0.03	0.16	0.14	0.25	0.31	0.27	0.44	0.37	0.04	0.32	0.20	0.18
	105	-	-	-	-	-	0.03	-	0.04	-	-	-	-	-	0.07	0.19	-	0.05	-	-	-	-	-	-
	107	0.07	0.25	0.14	0.13	0.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.09	-	-
	109	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-
	135	-	-	-	-	-	0.08	-	0.13	0.12	0.27	0.02	0.10	0.29	0.05	0.13	-	0.03	-	0.15	0.29	0.05	0.20	-
	139	0.07	0.25	0.11	0.05	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	141	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bsm6	208	-	-	-	-	0.08	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	0.02
	210	1.00	1.00	0.40	1.00	0.88	0.90	0.93	0.92	0.97	0.79	0.93	1.00	0.90	0.88	0.88	0.94	0.79	1.00	1.00	0.96	1.00	1.00	0.99
	214	-	-	0.60	-	0.04	0.08	0.07	0.08	0.03	0.21	0.04	-	0.10	0.10	0.06	-	0.18	-	-	-	-	-	-
	216	-	-	-	-	-	0.02	-	-	-	-	-	-	-	0.02	0.06	0.06	0.03	-	-	0.04	-	-	-
Bsm9	171	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03
	189	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01
	192	-	-	-	-	-	0.03	0.42	0.98	0.57	0.88	0.78	1.00	0.97	0.67	-	-	0.21	-	-	-	-	-	-
	195	1.00	1.00	0.88	0.89	0.83	0.97	0.58	-	0.43	0.10	0.22	-	0.03	0.33	1.00	1.00	0.79	1.00	1.00	1.00	1.00	0.33	0.69
	198	-	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	204	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	228	-	-	0.13	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.22
	237	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01

240	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03
243	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
261	-	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
264	-	-	-	-	0.03	-	-	0.02	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	0.33	-
276	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.17	-
285	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.17	-

Appendix S3.4: Genetic diversity at 12 microsatellite loci for 24 sites of *Botryllus schlosseri*. *N*, sample size; N_A, number of alleles; *A*, allele richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS} value; P_{HW}, exact *P*-value for Hardy-Weinberg equilibrium. Significant departures from equilibrium after sequential Bonferroni correction are indicated in bold.

Sites		BS321	BS531	BS811	PB29	PB49	PB41	PBC1	Bsm1	Bsm2	Bsm4	Bsm6	Bsm9
BLN	N_A /*A*	6/6.0	9/8.3	10/9.0	6/5.1	10/8.8	5/4.7	4/4.0	5/4.8	6/5.8	4/4.0	2/2.0	2/2.0
N = 14	H_O	0.571	0.500	0.462	0.571	0.571	0.571	0.000	0.429	0.429	0.571	0.200	0.250
	H_E	0.604	0.804	0.886	0.690	0.873	0.696	0.767	0.717	0.802	0.632	0.505	0.228
	F_{IS}	0.059	0.389	0.489	0.178	0.354	0.184	1.000	0.411	0.475	0.100	0.617	-0.100
	P_{HW}	0.676	0.006	**0.000**	0.032	0.001	0.327	**0.000**	0.006	0.005	0.510	0.081	1.000
ADM	N_A /*A*	4/4.0	9/7.7	13/10.5	3/3.0	11/9.6	6/5.0	7/6.7	4/3.4	5/4.8	6/5.1	1/1.0	3/2.6
N = 19	H_O	0.333	0.400	0.500	0.667	0.353	0.474	0.462	0.474	0.368	0.579	NA	0.000
	H_E	0.685	0.811	0.907	0.641	0.914	0.748	0.806	0.467	0.771	0.654	NA	0.210
	F_{IS}	0.524	0.516	0.457	-0.041	0.621	0.373	0.438	-0.016	0.529	0.118	NA	1.000
	P_{HW}	0.037	**0.000**	0.001	0.655	**0.000**	0.011	0.017	0.198	0.002	0.102	-	0.001
ALC	N_A /*A*	6/5.3	25/14.2	15/11.2	4/3.1	11/8.1	5/4.5	9/7.7	5/3.8	7/6.1	6/5.0	3/2.6	6/3.6
N = 33	H_O	0.375	0.708	0.476	0.531	0.407	0.594	0.318	0.233	0.742	0.813	0.083	0.167
	H_E	0.740	0.956	0.926	0.544	0.864	0.706	0.855	0.637	0.811	0.764	0.231	0.303
	F_{IS}	0.501	0.263	0.492	0.024	0.533	0.161	0.633	0.638	0.086	-0.065	0.643	0.455
	P_{HW}	0.004	**0.000**	**0.000**	1.000	**0.000**	0.066	**0.000**	**0.000**	0.044	0.079	0.001	0.003
SYD	N_A /*A*	4/2.9	6/5.2	5/3.8	4/2.9	7/5.4	4/3.6	10/7.6	3/3.0	5/3.8	5/4.4	3/2.2	2/1.6
N = 30	H_O	0.172	0.630	0.300	0.276	0.567	0.036	0.654	0.483	0.828	0.700	0.200	0.000
	H_E	0.328	0.785	0.524	0.413	0.753	0.681	0.837	0.648	0.630	0.720	0.186	0.066
	F_{IS}	0.480	0.201	0.434	0.336	0.251	0.948	0.222	0.258	-0.322	0.029	-0.077	1.000
	P_{HW}	0.012	**0.000**	0.001	0.003	0.013	**0.000**	0.005	0.040	0.158	0.282	1.000	0.017
LTN	N_A /*A*	4/4.0	4/2.7	5/4.0	4/2.6	4/3.1	5/3.3	9/5.8	3/2.2	5/2.5	3/3.0	2/1.8	2/2.0
N = 60	H_O	0.269	0.077	0.235	0.125	0.500	0.173	0.627	0.167	0.500	0.712	0.138	0.000
	H_E	0.704	0.215	0.408	0.269	0.545	0.590	0.775	0.158	0.438	0.640	0.130	0.491
	F_{IS}	0.622	0.645	0.431	0.537	0.083	0.709	0.192	-0.058	-0.143	-0.114	-0.065	1.000
	P_{HW}	**0.000**	**0.000**	0.036	**0.000**	0.880	**0.000**	0.006	1.000	0.772	0.057	1.000	**0.000**

PDG	N_A /A	3/2.2	11/9.0	3/3.0	3/2.6	8/7.0	3/3.0	8/6.5	3/3.0	6/5.3	5/4.6	2/1.9	2/1.4
N = 29	H_O	0.048	0.304	0.000	0.345	0.074	0.048	0.160	0.296	0.852	0.500	0.154	0.038
	H_E	0.138	0.877	0.392	0.532	0.834	0.661	0.720	0.602	0.795	0.735	0.145	0.038
	F_{IS}	0.661	0.658	1.000	0.356	0.913	0.930	0.781	0.512	-0.073	0.324	-0.064	0.000
	P_{HW}	0.024	**0.000**	0.004	0.010	**0.000**	**0.000**	**0.000**	0.002	0.417	0.022	1.000	-
PTR	N_A /A	4/3.9	7/6.3	6/5.7	5/4.5	6/5.7	4/3.6	6/5.6	3/3.0	5/4.7	4/4.0	2/1.7	2/2.0
N = 17	H_O	0.091	0.692	0.364	0.333	0.385	0.375	0.400	0.471	0.882	0.647	0.067	0.000
	H_E	0.619	0.791	0.632	0.630	0.760	0.667	0.818	0.622	0.711	0.711	0.067	0.508
	F_{IS}	0.859	0.129	0.437	0.480	0.504	0.446	0.520	0.249	-0.250	0.093	0.000	1.000
	P_{HW}	0.000	**0.000**	0.023	0.024	0.000	0.001	0.005	0.422	0.035	0.789	-	**0.000**
BTC	N_A /A	3/3.0	5/4.1	4/4.0	2/2.0	7/6.2	3/2.8	7/6.1	3/3.0	5/4.7	4/3.9	2/2.0	3/2.4
N = 24	H_O	0.000	0.474	0.000	0.364	0.318	0.000	0.389	0.500	0.750	0.583	0.417	0.250
	H_E	0.800	0.630	0.788	0.512	0.732	0.375	0.805	0.667	0.716	0.676	0.337	0.228
	F_{IS}	1.000	0.253	1.000	0.294	0.571	1.000	0.524	0.255	-0.048	0.140	-0.243	-0.100
	P_{HW}	0.067	0.305	0.001	0.217	**0.000**	**0.000**	**0.000**	0.305	0.454	0.612	0.540	1.000
GBR	N_A /A	2/2.0	9/6.3	2/2.0	4/3.4	7/5.5	2/2.0	7/6.3	3/3.0	5/4.7	4/3.4	3/2.2	2/2.0
N = 27	H_O	0.000	0.250	0.000	0.136	0.280	0.000	0.190	0.222	0.667	0.556	0.074	0.222
	H_E	0.533	0.733	0.356	0.449	0.762	0.476	0.762	0.509	0.751	0.639	0.143	0.352
	F_{IS}	1.000	0.665	1.000	0.701	0.638	1.000	0.755	0.568	0.114	0.133	0.485	0.373
	P_{HW}	0.202	**0.000**	0.112	**0.000**	**0.000**	0.000	**0.000**	0.001	0.228	0.301	0.038	0.081
SWM	N_A /A	1/1.0	7/6.7	1/1.0	3/2.9	5/4.2	2/2.0	5/4.3	3/3.0	5/4.8	5/3.7	1/1.0	1/1.0
N = 20	H_O	NA	0.000	NA	0.211	0.316	0.000	0.154	0.211	0.778	0.400	NA	NA
	H_E	NA	0.815	NA	0.364	0.482	0.471	0.351	0.622	0.751	0.355	NA	NA
	F_{IS}	NA	1.000	NA	0.429	0.351	1.000	0.571	0.667	-0.037	-0.130	NA	NA
	P_{HW}	-	**0.000**	-	0.035	0.097	**0.000**	0.005	0.000	0.095	1.000	-	-
LNB	N_A /A	5/4.2	8/6.2	2/2.0	3/2.3	6/4.6	3/3.0	5/4.6	3/3.0	5/4.0	4/4.0	2/2.0	2/1.6
N = 29	H_O	0.176	0.643	0.000	0.379	0.321	0.000	0.050	0.552	0.577	0.821	0.200	0.000
	H_E	0.636	0.790	0.533	0.471	0.647	0.606	0.747	0.566	0.630	0.733	0.186	0.068
	F_{IS}	0.729	0.189	1.000	0.198	0.508	1.000	0.935	0.025	0.085	-0.123	-0.077	1.000
	P_{HW}	**0.000**	0.003	0.200	0.261	**0.000**	**0.000**	**0.000**	0.651	0.398	0.466	1.000	0.018
DGB	N_A /A	2/1.6	7/6.7	4/4.0	4/3.8	5/4.9	4/3.8	8/7.0	3/2.9	5/4.3	5/4.6	3/2.4	2/2.0

N = 21	H_O	0.063	0.429	0.333	0.235	0.500	0.059	0.316	0.474	0.667	0.667	0.238	0.000
	H_E	0.063	0.786	0.399	0.656	0.787	0.686	0.849	0.525	0.676	0.714	0.220	0.457
	F_{IS}	0.000	0.464	0.172	0.648	0.372	0.917	0.635	0.100	0.014	0.068	-0.087	1.000
	P_{HW}	-	0.001	0.335	**0.000**	0.031	**0.000**	**0.000**	0.826	0.388	0.614	1.000	**0.000**
YMT	N_A /A	7/4.5	6/5.6	9/7.8	5/4.3	7/6.0	5/3.8	7/6.4	3/3.0	7/5.5	5/4.2	3/2.5	2/2.0
N = 31	H_O	0.182	0.263	0.462	0.290	0.429	0.200	0.679	0.500	0.806	0.677	0.355	0.032
	H_E	0.510	0.795	0.766	0.567	0.824	0.563	0.824	0.650	0.778	0.720	0.348	0.337
	F_{IS}	0.649	0.675	0.407	0.492	0.484	0.650	0.179	0.234	-0.037	0.060	-0.019	0.906
	P_{HW}	**0.000**	**0.000**	0.001	**0.000**	**0.000**	**0.000**	0.066	0.308	0.001	0.175	1.000	**0.000**
FRC	N_A /A	3/2.5	5/4.5	9/7.1	5/4.4	6/4.9	4/3.9	4/3.9	3/2.4	3/2.7	4/3.8	1/1.0	1/1.0
N = 15	H_O	0.200	0.400	0.375	0.300	0.167	0.182	0.762	0.783	0.364	0.435	NA	NA
	H_E	0.537	0.501	0.675	0.735	0.475	0.567	0.725	0.503	0.376	0.680	NA	NA
	F_{IS}	0.634	0.208	0.453	0.598	0.655	0.690	-0.053	-0.575	0.034	0.366	NA	NA
	P_{HW}	0.003	0.005	**0.000**	**0.000**	**0.000**	0.001	**0.000**	0.006	0.041	**0.000**	-	-
LSM	N_A /A	2/2.0	5/4.6	5/4.7	3/3.0	3/2.8	4/4.0	4/4.0	3/2.8	4/3.7	5/4.8	2/1.8	1/1.0
N = 13	H_O	0.400	0.500	0.500	0.250	0.417	0.667	1.000	0.167	0.692	0.667	0.077	NA
	H_E	0.442	0.493	0.703	0.750	0.359	0.867	0.712	0.489	0.563	0.783	0.077	NA
	F_{IS}	0.100	-0.015	0.298	0.700	-0.170	0.273	-0.440	0.669	-0.241	0.154	0.000	NA
	P_{HW}	1.000	0.561	0.001	0.142	1.000	0.467	0.038	0.010	0.109	**0.000**	-	-
BRN	N_A /A	3/3.0	2/2.0	5/5.0	5/5.0	5/5.0	3/3.0	6/6.0	4/4.0	3/2.9	4/3.9	1/1.0	1/1.0
N = 11	H_O	0.100	0.111	0.500	0.250	0.200	0.000	0.800	0.600	0.545	0.636	NA	NA
	H_E	0.279	0.111	0.667	0.675	0.442	0.711	0.747	0.626	0.515	0.619	NA	NA
	F_{IS}	0.654	0.000	0.268	0.646	0.561	1.000	-0.075	0.044	-0.062	-0.029	NA	NA
	P_{HW}	0.052	-	0.148	0.005	0.009	0.010	0.839	0.326	1.000	0.756	-	-
BBY	N_A /A	4/3.2	9/6.3	10/8.3	5/4.5	9/6.9	3/2.5	8/5.8	6/5.0	5/4.0	4/3.5	2/1.3	6/3.5
N = 36	H_O	0.486	0.594	0.438	0.333	0.861	0.353	0.500	0.556	0.618	0.472	0.030	0.389
	H_E	0.596	0.790	0.863	0.770	0.833	0.514	0.716	0.695	0.625	0.568	0.030	0.473
	F_{IS}	0.187	0.251	0.501	0.570	-0.034	0.317	0.306	0.203	0.011	0.171	0.000	0.180
	P_{HW}	0.108	**0.000**	**0.000**	**0.000**	0.159	0.007	0.001	0.011	0.026	0.015	-	0.016

Appendix S3.5: Pairwise Φ_{ST} comparisons for *Botryllus schlosseri* populations using the mitochondrial COI marker. *Significant ($P < 0.05$); ** remains significant after sequential Bonferroni correction (Rice 1989).

	Native					**Introduced (East Coast)**												**Introduced (West Coast)**				
	ETQ	SET	BLN	ADM	ALC	SYD	LTN	PDG	PTR	BTC	GBR	SWM	LNB	DGB	IGM	PLT	YMT	DPB	FRC	LSM	BRN	SQN
ETQ																						
SET	0.47																					
BLN	-0.16	0.61*																				
ADM	0.16	0.72**	0.16																			
ALC	0.01	0.74**	0.05	-0.02																		
SYD	0.50**	0.72**	0.53**	0.69**	0.65**																	
LTN	0.96**	0.92**	0.92**	0.97**	0.92**	0.73**																
PDG	0.53*	0.76**	0.56**	0.71**	0.67**	0.25*	0.29**															
PTR	0.44*	0.66**	0.50**	0.69**	0.64**	0.05	0.61**	0.02														
BTC	0.53*	0.76**	0.56**	0.72**	0.67**	0.24*	0.30**	-0.03	0.02													
GBR	0.65**	0.77**	0.65**	0.80**	0.74**	0.39**	0.19*	0.00	0.14	0.00												
SWM	0.56**	0.74**	0.59**	0.72**	0.69**	0.01	0.83**	0.40**	0.20*	0.40**	0.54**											
LNB	0.64**	0.80**	0.65**	0.78**	0.73**	0.39**	0.17**	0.01	0.14*	0.01	-0.03	0.54**										
DGB	0.80**	0.84**	0.77**	0.88**	0.82**	0.56**	0.03	0.13*	0.35**	0.14*	0.03	0.69**	0.04									
IGM	0.50*	0.59**	0.55**	0.78**	0.70**	0.19	0.58*	-0.08	-0.04	-0.08	-0.04	0.37*	-0.04	0.17								
PLT	0.46*	0.56*	0.53**	0.76**	0.69**	-0.09	0.87**	0.17	-0.04	0.16	0.36*	0.00	0.35*	0.62**	0.09							
YMT	0.45*	0.71**	0.49**	0.68**	0.63**	0.14*	0.45**	-0.01	-0.03	-0.01	0.06	0.29*	0.07	0.24*	-0.07	0.05						
DPB	0.67*	0.64**	0.58*	0.86**	0.72**	0.45**	1.00**	0.46**	0.41*	0.47**	0.62**	0.55**	0.59**	0.81**	0.58**	0.54**	0.38*					
FRC	0.83**	0.80**	0.72**	0.91**	0.79**	0.55**	1.00**	0.54**	0.55**	0.55**	0.71**	0.65**	0.66**	0.85**	0.74**	0.73**	0.49**	0.00				
LSM	0.67**	0.71**	0.60**	0.85**	0.73**	0.47**	0.97**	0.45**	0.42**	0.45**	0.60**	0.57**	0.56**	0.78**	0.57**	0.57**	0.38**	-0.04	0.03			
BRN	0.50**	0.64**	0.50**	0.78**	0.68**	0.36*	0.89**	0.26*	0.23*	0.26*	0.38*	0.49**	0.37*	0.59**	0.27*	0.35*	0.19*	0.17	0.33*	0.09		
SQN	-0.09	0.48*	0.00	0.36*	0.22*	0.47**	0.94**	0.51**	0.41*	0.51**	0.62**	0.53**	0.61**	0.77**	0.46*	0.41*	0.42*	0.49*	0.69*	0.52*	0.39*	
BBY	-0.04	0.67**	0.03	0.21*	0.15*	0.43**	0.70**	0.42**	0.37**	0.42**	0.47**	0.49**	0.48**	0.55**	0.37**	0.39**	0.36**	0.32*	0.41**	0.34**	0.28*	-0.06

Appendix S3.6: Pairwise F_{ST} comparisons for *Botryllus schlosseri* populations using 12 microsatellite markers. *Significant ($P < 0.05$); ** remains significant after sequential Bonferroni correction (Rice 1989). Populations with sample size of less than 10 individuals ($N < 10$) were not included in this analysis.

	Native			**Introduced (East Coast)**										**Introduced (West Coast)**		
	BLA	ADM	ALI	SYD	LTN	PDG	PTR	BTC	GBR	SWM	LNB	DGB	YMT	FRC	LSM	BRN
BLA																
ADM	0.01															
ALI	0.03*	0.01														
SYD	0.12**	0.08**	0.07**													
LTN	0.24**	0.21**	0.16**	0.14**												
PDG	0.37**	0.38**	0.34**	0.39**	0.24**											
PTR	0.20**	0.20**	0.15**	0.15**	0.03*	0.11**										
BTC	0.34**	0.36**	0.32**	0.34**	0.22**	0.03*	0.07**									
GBR	0.27**	0.29**	0.24**	0.29**	0.14**	0.04**	0.04*	0.07**								
SWM	0.51**	0.51**	0.45**	0.48**	0.35**	0.09**	0.20**	0.06**	0.17**							
LNB	0.43**	0.42**	0.37**	0.42**	0.22**	0.07**	0.11**	0.07**	0.12**	0.20**						
DGB	0.23**	0.21**	0.18**	0.20**	0.07**	0.09**	0.01	0.08**	0.06**	0.20**	0.09**					
YMT	0.08**	0.07**	0.05**	0.08**	0.13**	0.25**	0.10**	0.25**	0.18**	0.37**	0.31**	0.11**				
FRC	0.23**	0.16**	0.15**	0.17**	0.20**	0.50**	0.28**	0.48**	0.39**	0.64**	0.48**	0.30**	0.17**			
LSM	0.20**	0.14**	0.12**	0.09**	0.18**	0.44**	0.20**	0.39**	0.35**	0.57**	0.42**	0.23**	0.12**	0.08**		
BRN	0.16**	0.09**	0.11**	0.16**	0.21**	0.48**	0.26**	0.46**	0.36**	0.63**	0.47**	0.27**	0.14**	0.00	0.09*	
BBY	0.09**	0.06**	0.07**	0.13**	0.16**	0.37**	0.17**	0.36**	0.30**	0.49**	0.37**	0.17**	0.11**	0.10**	0.10**	0.05*

4.0 - GENERAL DISCUSSION

A decade after Holland's (2000) timely review on the application of genetics to the study of marine biological invasions, the field has advanced tremendously, and genetics is now regarded as a key addition not only to the study of NIS in the marine realm (reviewed by Geller *et al*. 2010) but also to invasion biology research in general (Lockwood *et al*. 2007). The two empirical studies presented here offer yet another illustration of the value of using genetic approaches for addressing issues of species invasions. For both *B. violaceus* and *B. schlosseri*, I was able to identify genetically distinct groups of populations at the continental and intra-coastal scales, using both equilibrium-based (i.e. pairwise F_{ST} and Φ_{ST} estimates) and non-equilibrium-based methods (i.e. multilocus genotype clustering), which were largely congruent. I inferred the post-introduction level of neutral genetic variation maintained in West vs. East coast populations (for *B. violaceus*) and in invasive vs. native populations (for *B. schlosseri*). Moreover, by relying on multilocus genotyping data, correlations of IBD, and assignment tests, I was able to identify the regional patterns of spread for these species in the East and West coast invaded ranges. This information has important implications, and should be considered in future management actions taken to alleviate the impacts of *B. violaceus* and *B. schlosseri* in regions of introduction.

Several of the genetic patterns emerging from this study highlight the importance of using multiple marker systems in genetic surveys of introduced populations. Uniparentally-inherited markers such as the mitochondrial COI gene have great potential in revealing bottleneck events associated with biological invasions while also being readily available

through the use of versatile universal primers (Simon *et al*. 1994). However, the genetic variability retained in such markers for recently founded populations may be severely reduced as a result of the introduction event itself and subsequent drift effects (Freeland 2005). Although not always the case, it is possible that all introduced populations share a single haplotype (e.g. Chandler *et al*. 2008) or very few haplotypes (as illustrated here in the case of East coast *B. violaceus* populations), therefore preventing interpretations on patterns of genetic structure and population connectivity. In this situation, microsatellite markers may provide an advantage over mitochondrial markers due to the greater genetic variation retained. However, microsatellites are significantly less practical when inferences on the evolutionary history and origin of introduced populations are to be made. Therefore, as it has been shown in this thesis, both marker systems should be used in combination. Although still relatively uncommon in the field of invasion genetics, this ideal approach will provide a more comprehensive representation of patterns of genetic structure, population connectivity, and invasion history for NIS.

On a more restricted note, discrepancies were also observed between the two study species analysed here. For example, clonal genotypes were identified more frequently for *B. violaceus* (27 clones; Appendix S2.4) than for *B. schlosseri* (5 clones; Appendix S3.1). Three possible explanations can be provided for this pattern. First, laboratory experiments have previously indicated that reattachment capabilities (and hence survivability) of fragments differ between the two ascidian species, with the highest success (100 %) recorded in *B. violaceus* (Bullard *et al*. 2007). Second, the colony structure for the two species is markedly different, with *B. violaceus* possessing a brittle morphology and thin tunic that could potentially facilitate fragmentation. By contrast, *B. schlosseri* colonies

are fleshy and enveloped in a thicker tunic that could effectively prohibit frequent fragmentation in this species. Third, the differences observed may be influenced by certain aspects of this study's design. More specifically, the availability of colonies from aquaculture facilities for *B. violaceus* but not for *B. schlosseri* could represent a source of bias in my results, since aquaculture management techniques such as equipment handling, maintenance, and cleaning, most likely contribute regularly to the fragmentation of fouling colonial ascidians.

The patterns of spread and resultant population genetic structure inferred for the two species in North America were generally congruent, indicative of long-distance dispersal. The only exception was detected along the Nova Scotia coastline, on the East coast. Here, while *B. violaceus* populations displayed evidence for stepping-stone dispersal, *B. schlosseri* populations appeared to be spreading mostly *via* 'jump' dispersal events. Considering that both species have limited abilities to spread independently (Carver *et al*. 2006), it is likely that both of these patterns, although highly divergent, are a result of transport by human-mediated vectors. Given the 100-years difference in the chronologies of these species' introductions in Nova Scotia (Carver *et al*. 2006), the disagreement is difficult to interpret. It is possible that human-mediated vectors have had more time to disperse propagules of *B. schlosseri* over long distances compared to *B. violaceus*. Also, the dynamics of vector traffic along the Nova Scotia coast may have suffered temporal changes between these introductions. Finally, the sampling strategy for the study, which occasionally included different harbours and marinas for each of the two study species, could have influenced the patterns observed.

4.1 FUTURE DIRECTIONS

To expand on results presented here, future studies should aim to increase sampling coverage in the native range for both species. In the case of *B. violaceus*, this approach might lead to the identification of specific regions in the Western Pacific that seeded the introduction(s) of this ascidian to North America. However, this outcome can be expected only if contemporary anthropogenic mixing of populations is not extensive enough to homogenize the genetic structure of *B. violaceus* populations in the native range. In the case of *B. schlosseri*, a better coverage of the ancestral distributional region would allow a re-evaluation of the taxonomic status of this species. Subsequent investigations could then explore if particular haplotypes/species differ in their invasive potential. In this context, future research could use the *B. schlosseri* species/species complex as a model system for the study of the genetic basis of invasiveness. Other similar model systems, also among widely distributed ascidians, have now been proposed (e.g. *Ciona intestinalis* species complex; Zhan *et al*. in press). Recent advances in DNA sequencing technology will provide the means to explore these exciting avenues of research at a genomic scale.

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APPENDIX S1- POLYMORPHIC MICROSATELLITE MARKERS FOR TWO HIGHLY INVASIVE COLONIAL ASCIDIANS, *BOTRYLLUS SCHLOSSERI* AND *BOTRYLLOIDES VIOLACEUS* *

The colonial ascidians *Botryllus schlosseri* and *Botrylloides violaceus* are highly invasive species that have been introduced into coastal marine ecosystems throughout the world as a result of human activities (Lambert 2005). In spite of their rapid spread, population genetics studies have been hampered by the limited number of polymorphic markers available (Pancer et al. 1994; Stoner et al. 1996). In this study, we developed 28 novel microsatellite markers (9 for *B. schlosseri* and 19 for *B. violaceus*) that are useful for conducting detailed population genetics studies.

Two microsatellite libraries were developed for each species using the magnetic bead-based enrichment method described by Glenn & Schable (2005) with modifications. Initially, individual zooids were isolated from the colony tunic and stored in 95% ethanol until further use. Following DNA extraction, genomic DNA (gDNA) was fragmented using *Rsa*I (New England Biolabs) and DNA fragments of 300 to 1000 bp ligated to double-stranded SNX-24 linkers were hybridized to a mixture of $(AG)_{12}$ $(TG)_{12}$ $(AAC)_6$ $(AAG)_8$ $(AAT)_{12}$ $(ACT)_{12}$ $(ATC)_8$ biotinylated oligonucleotide probes. Probe-annealed DNA fragments were captured with streptavidin-coated magnetic beads (Dynabeads® M-280, Invitrogen). The enriched library was cloned into *E. cloni*® 10G cells using a

* Molecular Ecology Resources Primer Development Consortium, Abdoullaye D, Acevedo I *et al*. (2010) Permanent Genetic Resources added to Molecular Ecology Resources Database 1 August 2009-30 September 2009. *Molecular Ecology Resources*, **10**, 232-236. DOI: 10.1111/j.1755-0998.2009.02796.x

pSMART® GC HK vector (Lucigen, Middleton, WI. USA). Bacterial clones were sequenced using the SR2 primer (5' GGTCAGGTATGATTTAAATGGT CAGT-3') and BigDye Terminator 3.1 chemistry on an ABI 3130XL automated sequencer (Applied Biosystems, Foster City, CA). Because many of the microsatellites were located at the end of the insert sequence, greatly impeding primer design, a second microsatellite library was developed for each species by mechanically shearing the gDNA with a Sonic Dismembrator 60 (Fisher Scientific, Pittsburg, PA). Out of a total of 480 clones sequenced, 133 contained microsatellite repeats, 77 of which (30 for *B. schlosseri* and 47 for *B. violaceus*) had sufficient flanking regions that allowed primer design. Primer pairs were designed using Primer 3 software (Rozen & Skaletsky 2000). A universal M13 tail was added to the 5' end of each forward primer to allow fluorescent labeling, according to Schuelke (2000). PCRs were performed with tailed forward primer, reverse primer and one of four fluorophore 6FAM, VIC, NED or PET (Applied Biosystems, Foster City, CA). PCR cocktails (10 µL) contained 10 ng of gDNA, 1 x PCR buffer (1 mM Tris HCl pH 8.3, 5 mM KCl, 1.5 mM MgCl2), 0.125 mM of each dNTP, 0.5 µM of each primer and 0.2 U of TopTaq DNA Polymerase (QIAGEN). The cycling PCR profile was performed on an Eppendorf Mastercycler epGradient S thermocycler (Eppendorf, Hamburg, Germany) and consisted of a touchdown method with initial denaturation at 95 ℃ for 3 minutes, 10 cycles of 35 s at 95 ℃, 35 s at an initial annealing temperature of 60 ℃ that decreased by 1 ℃ in each of the 10 cycles, and 45 s at 72 ℃. The first 10 cycles were followed by 35 cycles of 35 s at 95 ℃, 35 s at 50-54 ℃, 45 s at 72 ℃, and a final extension for 10 min at 72 ℃. For *B. schlosseri*, 22 out of 30 primer pairs gave consistent amplifications while for *B. violaceus* 39 out of 47 primer pairs could be used for polymorphism screening.

We tested the degree of polymorphism of the selected microsatellite loci by genotyping 12 individuals from 10 distantly isolated populations from Canada, Japan and Europe. To assess the Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) for each polymorphic locus, 24 individual colonies from one population were genotyped (Arenys de Mar, Spain for *B. schlosseri* and Cardigan River, Prince Edward Island, Canada for *B. violaceus*). Amplified fragments were separated on an ABI 3130XL automated sequencer (Applied Biosystems, Foster City, CA), with GeneScan™-500 LIZ™ (Applied Biosystems, Foster City, CA) internal size standard. The alleles were scored using Genemapper® software version 4.0 (Applied Biosystems, Foster City, CA), and data was analyzed using GenePop software (Raymond & Rousset, 1995).

For *B. schlosseri*, 9 out of 22 loci were polymorphic with 2 to 9 alleles per locus. For *B. violaceus*, 19 out of 39 loci were polymorphic with 3 to 11 alleles per locus (Table 1). Twenty one of the 28 polymorphic markers were in HWE. Most loci that departed from HWE revealed significant heterozygote deficiency (Table 1). Such deviations are characteristic of *B. schlosseri* (Stoner *et al*. 2002), and can be attributed to life-history traits of colonial tunicates such as non random mating, limited gene flow and population substructure. Significant LD was found between two pairs of loci (Bs3-Bs7, Bv6-Bv17) after standard Bonferroni correction. Cross amplification of the markers was tested between the two species and two other related colonial tunicates: *Botrylloides fuscus* and *Botrylloides simodensis*. Twenty-one of the 28 developed markers produced fragments of the expected size in more than one botryllid species (Table 1).

These new microsatellite markers are useful tools for population genetics studies of *B. schlosseri* and *B. violaceus*. Understanding the invasion history of these ascidians requires accurate insight into the spatial and temporal genetic architecture of invasive and native populations and identifying potential sources and vectors of invasion.

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Table A1 Characterization of polymorphic microsatellite loci for *Botryllus schlosseri* and *Botrylloides violaceus*: T_a, annealing temperature used in the touchdown protocol (see text); N_A, number of alleles; *S*, allele size range (bp); H_O, observed heterozygosity; H_E, expected heterozygosity; *P* value from exact tests for Hardy-Weinberg equilibrium. Additional information is presented for the loci that were successfully cross-amplified in *B. schlosseri* (a), *B. violaceus* (b), *B. fuscus* (c) and *B. simodensis* (d).

Locus	GenBank Accession no.	Primer sequences (5'-3')	Motif	T_a	N_A	*S*	H_O	H_E	*P* value	Cross-species amplification
Botryllus schlosseri										
Bs 1	GQ272527	F: ACTGCGCTAATCAGTAGAC R: CACTTCGCAATTTAAACTTCGG	$(AC)_8$ imperfect	54	2	211-224	0.33	0.48	0.1951	-
Bs 2	GQ272528	F: GAGCGAGAAGAGTGATG R: ATTTCCTGATTTGGTCTAAC	$(AAC)_8$	54	7	165-201	0.54	0.82	0.0097	c
Bs 3**	GQ272529	F: GCGATCAAAAACGAACTGCT R: ACCTGTTTGATGCTGGTGTG	$(AG)_9$	54	3	217-237	0.50	0.39	0.4442	b, c
Bs 4	GQ272530	F: CCAGATTTGATGCTTGAGTGG R: TGTCGTCGTCACTCGTCAGT	$(GA)_{11}$ imperfect	54	5	99-141	0.58	0.66	0.0893	b
Bs 5	GQ272531	F: CGGCGGTTAGCCACAC R: GAATCGTTAGCACGATGGGTA	$(CA)_9$	54	2	194-200	0.88	0.50	0.0002*	b, c, d
Bs 6	GQ272532	F: TGTTCAAGTGACCCCATCAA R: ACAATGCGTGCAGGGTATTC	$(TG)_5...(ATTG)_5$	54	4	208-264	0.33	0.61	0.0008*	b
Bs 7**	GQ272533	F: TGGTCTTAGGCCCAATGAAG	$(CAGA)_2(CA)_4$	54	3	180-196	0.13	0.26	0.0126	b, c

		R: AATAAACGGATTTGCGTTCG								
Bs 8	GQ272534	F: GCGCCAATTCCTACTAGGTG R: CGTAGATCCGAGGCTGGTAA	$(TA)_2(TG)_4$	54	9	181-277	0.17	0.74	0.0000*	-
Bs 9	GQ272535	F: CGCATGCCTCCTCTTTACAT R: TCACAGGATAAACTGGATTTCG	$(TGA)_6$	54	6	192-261	0.50	0.70	0.0663	b
Botrylloides violaceus										
Bv 1	GQ272536	F: CACTGTTTGACATTTCGGGATA R: ACACAGGCCCACAGTGGTA	$(CT)_9$	50	3	115-119	0.63	0.53	0.4119	a, d
Bv 2	GQ272537	F: TTACGCGACCCATAACTTCC R: CCGGTATAGTCAGTCAACACCA	$(TGA)_9$	50	4	200-221	0.58	0.63	0.4317	c
Bv 3	GQ272538	F: ATTGCCCACTGAAAGGTGAG R: AGCAAGCTCGATAACCTCCA	$(GA)_{20}$ imperfect	50	4	177-251	0.58	0.61	0.8653	a, c
Bv 4	GQ272539	F: TGCGATACTGTATGTTGTTTGG R: CTAATGTCACTAACTTTCCCTTCTG	$(TC)_{19}$ interrupted	50	4	176-186	0.33	0.73	0.0000*	-
Bv 5	GQ272540	F: TTTTTATGCTGCACCAAGCA R: CCTTCGCTGTTTGTGACGTA	$(TC)_{13}$	50	5	191-219	0.54	0.71	0.0026	a, c
Bv 6**	GQ272541	F: TCGCCGTCTCCATTTTCT R: GTGGAAGTAGCGGGTTCTGT	$(TC)_{11}$	50	5	207-229	0.58	0.74	0.0801	a
Bv 7	GQ272542	F: CGGTCTGTAGTGCTCTCTCTCTC R: GTGTCATTAGCGTCGGGAGT	$(CT)_{27}$	50	8	118-176	0.54	0.81	0.0000*	-
Bv 8	GQ272543	F: AAATGGAGTCGATGGAGTTTG R: TTTTGCTGCAGTCACACAGA	$(GT)_{11}$	50	6	97-119	0.17	0.16	1.0000	d
Bv 9	GQ272544	F: GCGCACCTATACCAAGCAAT	$(CA)_{30}$ imperfect	50	6	88-152	0.29	0.33	0.0844	-

		R: CCCGAGTAATGTGTGCTGTG								
Bv 10	GQ272545	F: CAACAGTATAATGGAGTCAAAGTGC R: GTGGGAGAGTGAGGGAGACA	$(TC)_{17}$ interrupted	50	3	177-199	0.46	0.49	1.0000	c, d
Bv 11	GQ272546	F: TTGGCACTGGTCGATAATCA R: TGCTAACGTAAATCAATACACACA	$(TG)_{11}$	50	4	154-164	0.42	0.41	0.4566	c
Bv 12	GQ272547	F: CCGTCAATAACGGAAACGAT R: CCAACCCACCTCTCATGATT	$(CT)_{15}$ imperfect	50	5	179-189	0.38	0.49	0.1115	-
Bv 13	GQ272548	F: GCACAGTGGTGAGTTGTCAAA R: AACACTCCTTCCCAGAGACATC	$(GA)_{11}$	50	5	116-126	0.75	0.58	0.1569	a
Bv 14	GQ272549	F: TCTCTCGCACGGTACATCAG R: CCCTCATTCTGCATACCTTTG	$(GA)_{12}$ imperfect	50	3	171-189	0.42	0.63	0.0000*	a, c, d
Bv 15	GQ272550	F: AAAAACAGGGAGAGGGTGGT R: TGCCGAATGTTTCTTTTCTAA	$(GA)_{8}$	50	5	175-183	0.21	0.23	0.2249	d
Bv 16	GQ272551	F: TAAGCCTTCTCTTGGGCAGA R: TGCTACAGCCATGGCCTACT	$(TAG)_{13}$	50	11	123-204	0.79	0.67	0.8731	a, c
Bv 17**	GQ272552	F: CACCCTGTCAATACCGGAAA R: CGGGAGTGACCTTACGAGAC	$(CT)_{8}$	50	6	221-237	0.54	0.58	0.6718	a
Bv 18	GQ272553	F: ATTATTACGCACCCCCAGTG R: TAATACAAAAAGAAATTTGACCAAAAG	$(GT)_{9}$	50	4	149-159	0.58	0.72	0.0006*	a, c
Bv 19	GQ272554	F: ACAAAAGCAACAGGAACAAACA R: GGTCAGCATGTCATTTATGAGAGA	$(TC)_{9}$	50	3	202-206	0.42	0.38	1.0000	-

*Did not conform to Hardy-Weinberg equilibrium after standard Bonferroni correction ($P < 0.004$ for *B. schlosseri* and $P < 0.002$ for *B. violaceus*)

**Linkage disequilibrium

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