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Comparative population genetic study of an important marine parasite from New Zealand flat oysters

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Abstract The comparative genetic structure of hosts and parasites can reveal constraints acting on parasite dispersal among host populations and the evolution of local adaptation. We sampled New Zealand flat oysters *Ostrea chilensis* from 12 sites throughout New Zealand to (1) determine the distribution and prevalence of the haplosporidian parasites *Bonamia exitiosa* and *B. ostreae*, and (2) test for congruent patterns of host and parasite genetic structure. *B. exitiosa* was detected at three sites: Hauraki Gulf (5% prevalence), Marlborough Sounds (30%), and Foveaux Strait (7%), whereas *B. ostreae* was only detected in the Marlborough Sounds (37%). Using nuclear internal transcribed spacer (ITS) rDNA sequences of *B. exitiosa,* as well as mitochondrial cytochrome *c* oxidase subunit 1 gene (CO1) sequences of *O. chilensis* from the same sites plus other key *O. chilensis* growing areas (Tasman Bay and Chatham Islands), we compared the genetic structure of host and parasite. *B. exitiosa* displayed genetic structure across all three sites which were reflected in populations of *O. chilensis* except for gene flow between Tasman Bay-Marlborough Sounds-Chatham Islands. The observed patterns reflect the host specificity of *Bonamia* parasites and the limited dispersal capability of oysters. *O. chilensis* may experience long distance dispersal which is likely influenced by oceanographic factors. Nonetheless, a failure to detect *Bonamia* parasites among genetically connected *O. chilensis* populations suggests natural long distance co-dispersal of *Bonamia* parasites with *O. chilensis* is unlikely. Instead, the dispersal of *Bonamia* parasites is likely influenced by anthropogenic factors.

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Introduction

The comparative genetic structure of host and parasite is important for understanding parasitic dispersal among host populations as well as the evolution of local host–parasite adaptations (Keeney et al. 2009). Dispersal among populations maintains gene flow, which is largely determined by the dispersal capabilities of a species and the presence or absence of barriers to dispersal, e.g., salinity gradients (Haskin and Ford 1982). The lifecycle of a parasite is intimately linked with its host; therefore, gene flow among parasite populations is often largely dependent on the host, and for parasites with complex lifecycles, population genetic structure is determined by the vagility of the most mobile host (Feis et al. 2015). Host and parasite population structure is often congruent (Criscione and Blouin 2007); however, different processes can contribute to different dispersal routes for parasites that are reflected in their population structure (Blasco-Costa et al. 2012). Indeed, a recent meta-analysis of comparative studies of host–parasite genetic structure has indicated that host-limited dispersal is not always the rule (Mazé-Guilmo et al. 2016).

The Haplosporidian genus *Bonamia* contains three described species that are small (typically 1–3 µm), uninucleate, and infect oyster haemocytes causing a disease known as bonamiosis (Arzul and Carnegie 2015). Due to the severity of disease that these parasites can cause *Bonamia*

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exitiosa and *B. ostreae* area listed as pathogens notifiable to the World Organisation of Animal Health. Unique within the haplosporidians, *B. exitiosa* and *B. ostreae* are directly transmitted between oysters, although whether the *Bonamia* lifecycle is solely characterised by direct transmission or if these parasites use alternate hosts is uncertain (Engelsma et al. 2014). A sexual lifecycle has not been documented for either parasite and intrahaemocytic reproduction is asexual (Montes et al. 1994). Under direct transmission, a parasite must be able to survive in the water column for a period of time. In vitro experiments found that up to 58% of *B. ostreae* cells were alive after 1 week in water at 15 °C, and survival rate declined with increasing temperature and decreasing salinity (Arzul et al. 2009); similar results were found with *B. exitiosa* (Audemard et al. 2008). Under the appropriate environmental conditions, *Bonamia* spp. may, therefore, be capable of being dispersed by water currents to new areas. However, environmental dispersal of *B. exitiosa* within the Foveaux Strait, which separates Stewart Island from the South Island of New Zealand, was found to be less than the movement of water during one tidal cycle, and the movement of disease particles does not appear to contribute much to the diffusion of *B. exitiosa* in this area (Cranfield et al. 2005). Recently, Arzul et al. (2011) and Flannery et al. (2016) detected *B. ostreae* from oyster larvae, which could have implications for the parasite's dispersal during the planktonic phase of the oyster lifecycle. To date, no data exist on *B. exitiosa* infection in oyster larvae, however, considering the similarities of these two parasites, it is likely *B. exitiosa* is also capable of larval infection and, thus, oyster larvae should be viewed as a possible source of infection and dispersal route.

In January 2015 the exotic Northern Hemisphere pathogen *B. ostreae* was reported from two New Zealand flat oyster *Ostrea chilensis* aquaculture farms in the Marlborough Sounds, New Zealand, where it was associated with high levels of mortality (Lane et al. 2016). *Ostrea chilensis* is a developing aquaculture species within New Zealand and also supports commercial dredge fisheries in the South Island. In response to the report of *B. ostreae*, New Zealand's government enforced a Controlled Area Notice (CAN) at the top of the South Island to prevent the spread of this parasite to other key *O. chilensis* growing areas (see Fig. 2). The CAN restricts movements on certain bivalve molluscs, however, this movement control only takes into account intentional translocation of stock and does not account for natural dispersal events. Indeed, accounting for natural dispersal within a management plan would not be straightforward, however, determining how *Bonamia* sp. are genetically connected within New Zealand can be useful in evaluating the risk of *B. ostreae* dispersal to other oyster beds.

Bonamia exitiosa is endemic to New Zealand and has been retrospectively detected in *O. chilensis* sampled from Foveaux Strait in 1964 (Hine and Jones 1994). In the same area, two significant *B. exitiosa* epizootic events occurred between 1986–1992 and 2000–2005 that reduced the oyster population to 9% of its pre-disease level (Cranfield et al. 2005). Elsewhere in New Zealand, *B. exitiosa* has been detected from the Hauraki Gulf (Hill et al. 2014), Tasman Bay and Marlborough Sounds (Hine 1997), and Wellington Harbour (Jones unpublished data), although no epizootic event has been reported from these locations, and whether *B. exitiosa* occurs at other beds in New Zealand is likely but has not yet been explicitly addressed.

Oysters have a bi-phasic life history, being sedentary as adults, but capable of dispersal as larvae. Adult *O. chilensis* brood their larvae for a period ranging 15–38 days prior to releasing them into the water column (Jeffs and Creese 1996). Fully developed larvae are capable of settling within a few minutes of release (DiSalvo et al. 1983), although a small proportion may remain pelagic for up to 20 days. A population genetic rule-of-thumb for marine organisms is that the longer the larval phase, the further larvae are likely to be dispersed (Shanks 2009). However, despite larval duration and population connectivity of New Zealand marine fauna being directly proportional, this relationship broke down for larval durations shorter than 10 days (Ross et al. 2009). Therefore, any assumption regarding *O. chilensis* dispersal based on life-history traits may not accurately reflect actual population connectivity.

Ostrea chilensis larval dispersal simulations found only a small portion of larvae settle more than 1 km from their natal location (Broekhuizen et al. 2011). However, modelling fails to determine whether immigrants to a stock will survive and reproduce, whereas genetic markers sample inherited characteristics that are shared by individuals that are part of the same reproductive process, thereby providing more accurate estimates of connectivity (Faurby and Barber 2012). *Ostrea chilensis* are genetically differentiated between Hauraki Gulf and Foveaux Strait based on mitochondrial DNA (mtDNA) cytochrome *c* oxidase subunit 1 gene (CO1) markers. (Foighil et al. 1999) and Thomas (2015) detected genetic structure among southern South Island sites using random amplifiable polymorphic DNA markers. Therefore, an a priori expectation for our study is that *O. chilensis* will have genetically structured populations. Furthermore, Hill-Spanik et al. (2015) found genetic differentiation of *B. exitiosa* between Hauraki Gulf and Foveaux Strait based on nuclear internal transcribed spacer (ITS) rDNA markers. Accurate inferences of *Bonamia* dispersal around New Zealand by *O. chilensis* cannot be made from these studies. Simultaneously collecting genetic information from a parasite and the corresponding host enables a specific comparison of population genetic structures that these previous studies cannot. Moreover, because of the small number of sample sites used in these

studies, it is likely that some level of genetic diversity or genetic structuring was missed.

In this study, we sample *O. chilensis* populations throughout New Zealand to determine the distribution and prevalence of the enzootic *B. exitiosa* and exotic *B. ostreae* within oyster beds. We then compare the genetic structure of *B. exitiosa* with that of *O. chilensis* across New Zealand, using ITS rDNA and mtDNA CO1 markers, respectively. Compared with other commercial New Zealand species, there is little genetic information available for *O. chilensis*, including of stock structure. Mitochondrial DNA is a good first choice marker for a genetic assessment of a stock structure, because primers are comparatively cheap and easy to develop, and loci such as the CO1 are hypervariable enabling elucidation of intraspecific relationships. Similarly, the limited genetic data of *Bonamia* spp. limit marker choice: no *Bonamia* mitochondrial sequence data are available and conserved loci including actin genes (Lopez-Flores et al. 2007; Prado-Alvarez et al. 2015) and a heat shock protein (Prado-Alvarez et al. 2013) are unsuitable for resolving intraspecific relationships. The ITS locus accumulates mutations quickly (Hillis and Dixon 1991), which makes it useful for characterising intraspecific diversity, and has previously been used to infer relationships among *Bonamia* species (see Hill-Spanik et al. 2015) and other important marine oyster parasites, e.g., *Perkinsus marinus* (Brown et al. 2004) and *Mikrocytos mackini* (Abbott et al. 2011).

Considering that *B. exitiosa* and *B. ostreae* have no reported spore, are directly transmissible, and have no other known vector, as well as the short pelagic larval duration of *O. chilensis*, it is hypothesised that both host and parasite will display congruent genetically structured populations. The comparative nature of the data enables us to test the hypothesis that natural dispersal of *Bonamia* spp. within New Zealand is achieved through short distance dispersal of *O. chilensis* and any longer distance *Bonamia* dispersal is unlikely to be facilitated by the natural dispersal of its oyster hosts.

Materials and methods

Sample collection

Adult *Ostrea chilensis* were collected from 12 sites throughout New Zealand (Fig. 1): nine sites were wild oyster beds, and three were from *O. chilensis* aquaculture farms, including Tory Channel (sample site 6) and Port Underwood (7) (hereafter collectively referred to as Marlborough Sounds), and one site from Stewart Island (12). Sample collections were made between March and April 2015 (austral autumn), which is the time of the year when *B. exitiosa* is typically at its highest prevalence and, therefore, most likely to be detected (Hine 1991). Sample collections aimed for 150 oysters per site on the assumption of 2% prevalence of

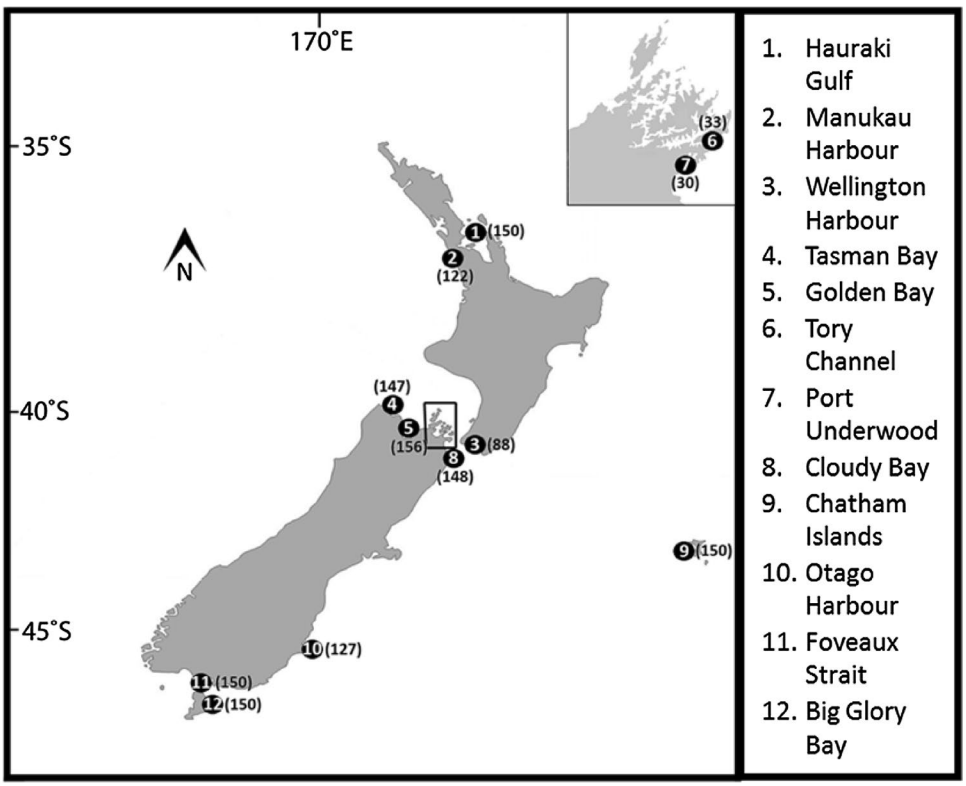

Fig. 1 New Zealand *Ostrea chilensis* sample sites. The sample number collected is noted in parentheses. The locations corresponding to the numbers in the closed circles are listed in the vertical panel

Bonamia spp., however, this was not achieved at every site due to low oyster abundance. All oysters were shipped on ice to the laboratory. After shucking, any gross pathologies were noted before gill, mantle, and heart tissue was sampled for molecular analyses from the first 150 adult oysters. All sampled tissue was placed in 1.5 mL microcentrifuge tube and preserved in 70% EtOH. All remaining tissue was stored at − 70 °C. An additional 36 *B. exitiosa*-positive *O. chilensis* collected by the National Institute of Water and Atmospheric Research Ltd. (NIWA) during the 2014 Foveaux Strait *B. exitiosa* survey were added to our data set and used in analyses.

DNA extraction and PCR

DNA was extracted from ~ 25 mg of combined gill, mantle, and heart tissue using an automated QiaXtractor 96-well robot following the manufacturer's instructions. From each 96-well extraction plate, 12 representative samples were quantified using a Qubit® 2.0 fluorometer and stored at 4 °C. All samples were confirmed for amplifiable nucleic acid by an 18S rDNA Internal Control real-time PCR (Applied Biosystems).

All samples were tested for *B. exitiosa* and *B. ostreae* using species-specific conventional PCR assays (Ramilo et al. 2013). *B. ostreae* was only detected in one sample site; therefore, we only used *B. exitiosa* samples for comparison with the *O. chilensis* hosts. ITS rDNA *B. exitiosa* sequences were determined using a newly designed *B. exitiosa* species-specific ITS rDNA conventional PCR. The forward primer Exitiosa_F (5′-ACTTCACTTGAGACTTTTGTATG-3′) was paired with the reverse Bonamia_R (5′-GGAAGGAAAAGT CGTAACAA-3′) to produce an amplicon ~ 600 bp in length. All PCRs consisted of Kapa2G Fast Hotstart Mix (Kapa Biosystems), which consists of proprietary reaction buffer including 0.2 mM of each dNTP at 1X, 1.5 mM $MgCl_2$, 0.5 U of Kapa2G Fast Hotstart DNA Polymerase, 0.4 uM of forward and reverse primer, and 2 µL of DNA template (~ 20 ng μL^{-1}). Thermal cycling was performed on a VeritiR Dx Thermal Cycler (Applied Biosystems): 95 °C for 2 min, followed by 35 cycles of 95 °C for 10 s, 58 °C for 10 s, and 72 °C for 1 s. All PCR products were electrophoresed on 1.5% gel and visualised under a UV light source. All products were purified prior to sequencing using a ZYMO genomic DNA kit.

Selected sites were assayed for *O. chilensis* mtDNA CO1 sequencing. Sites were selected if either: (1) *B. exitiosa* was detected at that site during this study, or (2) the site is key *O. chilensis* growing area in New Zealand. Newly designed primers, Ochil_F (5′-TCGGTAGCTTATACATAGTT-3′) and Ostrea_R (5′-ACAGGATCAAAGAAGGAT-3′) were paired to amplify a ~ 600 bp CO1 fragment of *O. chilensis*. The PCR reaction mix and protocol used was similar to that described above, except primer concentrations were 0.3 µM and the annealing temperature was 54 °C. Gel electrophoresis and purification for sequencing was achieved as described above. All DNA sequencing was performed by Macrogen, Korea.

Genetic diversity

Bonamia exitiosa and *O. chilensis* sequences were imported and assembled in Geneious 9.1.6, and then aligned using MAFFT v7.222 (Katoh et al. 2002; Katoh and Standley 2013). Specific amplification was confirmed by submitting the consensus sequence of *B. exitiosa* to BLAST and the consensus sequence of *O. chilensis* to the validated CO1 database, BOLDsystems (Ratnasingham and Hebert 2007).

Published *B. exitiosa* ITS sequences from Hauraki Gulf (GenBank accession numbers JF831639–JF831657) and Foveaux Strait (JF831658–JF831677) were added to the sequences generated from our study for the following analyses unless otherwise stated. The summary statistics of the level of genetic diversity in each population for both *B. exitiosa* and *O. chilensis* sequence sets that included the number of haplotypes (N_h), haplotype diversity (h), nucleotide diversity (π), the number segregating sites (S), and the average of number nucleotide differences (K) were calculated in Arlequin v.3.1.5.3 (Excoffier and Lischer 2010). Neutrality of the data sets was determined using Tajima's D test (Tajima 1989) calculated in Arlequin v.3.1.5.3 using 1000 permutations.

Genetic structure

Pairwise genetic differences (ϕ_{st}) were estimated as indicators of gene flow between all sampled sites for *B. exitiosa* and *O. chilensis*. The conventional ϕ_{st} were calculated using Arlequin 3.1.5.3 and the significance was assessed using 1000 permutations. For the *O. chilensis* data set, an analysis of molecular variance (AMOVA) was conducted on the clusters of sampling sites to determine the maximum proportion of the total genetic variation that could be attributed to differences between groups. Two different structures were tested, (1) three groups were tested based on pairwise comparisons: (a) Hauraki Gulf, (b) Foveaux Strait, and (c) Tasman Bay-Marlborough Sounds-Chatham Islands; (2) three groups were tested based on the detection of *B. exitiosa*: (a) Tasman Bay, (b) Chatham Islands, and (c) Hauraki Gulf-Marlborough Sounds-Foveaux Strait.

Haplotype frequencies were calculated as proportion of the total number of haplotypes within each site for each data set in FaBox v.1.41 (Villesen 2007), graphed in Microsoft Excel and visually represented in Adobe Illustrator. A 95% statistical haplotype network was constructed using TCS (Clement et al. 2000) for the *B. exitiosa* ITS data set. The

resulting network was edited in tcsBU (Santos et al. 2015) and Adobe Illustrator.

Migration

Migration rates among sampling sites were estimated using the Bayesian inference method of Migrate v. 3.6.11 (Beerli and Felsenstein 2001; Beerli 2006). Two analyses were run for the migration of *O. chilensis* and *B. exitiosa.* Input parameters incorporated the transition/transversion ratio acquired from jModelTest (Posada 2008). Migrate runs included one long chain, 1,000,000 visited genealogies, and 50,000 recorded genealogies with a burn-in of 10,000. Convergence of the data set was determined by effective MCMC sample sizes being greater than 200. An adaptive heating scheme with default start temperatures of 1.00, 1.50, 3.00, and 1000.00 was used to estimate boundaries of Θ ($2N_{ef}\mu$ for *O. chilensis*; N_{ef} = female effective population size; μ = mutation rate; and $2N_e\mu$ for *B. exitiosa*; N_e = population size); and *M* (m μ^{-1}; m = migration rate). The difference in the chosen theta value between the two species is based on the assumption that the extracted life stage of *B. exitiosa* is haploid. Migration rates were then estimated as $N_e m_i = 0.50 \times \Theta_i \times M_{j \to i}$, where i = receiving site and j = source site. The 95% confidence intervals incorporated the 2.5 and 97.5% estimates of both Θ_i and $M_{j \to i}$.

Results

This study generated 69 new *B. exitiosa* ITS rDNA sequences from three sample sites: 8 from Hauraki Gulf, 14 from Marlborough Sounds, and 47 from Foveaux Strait (Table 1) (GenBank accession numbers KY680577–KY680645). Only 14 Marlborough Sounds out of 19 samples were analysed, because five sequences from *B. exitiosa* to *B. ostreae* concurrently infected samples had poor sequence quality and were omitted. *Bonamia ostreae* was only detected from the Marlborough Sounds. Summary genetic diversity statistics are presented in Table 2 for all three sites. Four haplotypes

Table 1 Collection date and summary of the number of *Ostrea chilensis* with detected *Bonamia exitiosa* or *B. ostreae* as well as the prevalence of *B. exitiosa* at each sampling site

Location	Collection date	*B. exitiosa*	*B. ostreae*	*B. exitiosa* prevalence (%)
1. Haurakai Gulf (HG)	9 April 2015	8	0	8/150 (5)
2. Manakau Harbour	23 March 2015	0	0	0/122 (0)
3. Wellington Harbour	31 March 2015	0	0	0/88 (0)
4. Tasman Bay (TB)	26 March 2015	0	0	0/156 (0)
5. Golden Bay	13 April 2015	0	0	0/147 (0)
6. Tory Channel (MS)	12 February 2015	9[a]	18	1/33 (27)
7. Port Underwood (MS)	12 February 2015	10[a]	5	10/30 (33)
8. Cloudy Bay	17 April 2015	0	0	0/148 (0)
9. Chatham Island (CI)	13 April 2015	0	0	0/150 (0)
10. Otago Harbour	17 March 2015	0	0	0/127 (0)
11. Foveaux Strait (FS)	10 March 2015	11 (+ 36)[b]	0	11/155 (7)
12. Big Glory Bay	12 March 2015	0	0	0/150 (0)
Total		38 (+ 36)	23	

Acronyms of sampling sites used in analyses are shown in parentheses

[a]Concurrent *B. exitiosa* and *B. ostreae* infections

[b]2014 Foveaux Strait sequences

Table 2 Sampling sites where *Bonamia exitiosa* was detected, number of *B. exitiosa* sequences analysed (*N*), the number of haplotypes (N_h), haplotype diversity (*h*), the average number of nucleotide differences (*K*), segregating sites (*S*), nucleotide diversity (π), Tajima's *D* (*D*)

Location	*N*	N_h	*h*	*K*	*S* (SD)	π	*D*
Hauraki Gulf	27	18	0.949 ± 0.027	1.253 ± 0.817	3.892 (1.546)	0.002 ± 0.002	− 2.339*
Marlborough Sounds	14	8	0.868 ± 0.068	1.723 ± 1.070	1.887 (0.996)	0.003 ± 0.002	− 0.328
Foveaux Strait	67	24	0.773 ± 0.052	0.905 ± 0.637	3.980 (1.355)	0.002 ± 0.001	− 2.345*
Total	108	50	0.908 ± 0.023	1.242 ± 0.793	7.041 (2.009)	0.002 ± 0.002	− 2.584*

*Significant $p < 0.001$

were shared across at least two of sampling sites as well as a high number of private haplotypes observed from all three locations (Fig. 2); there was no distinct geographic pattern among *B. exitiosa* haplotypes. One haplotype made up 46% of all detected haplotypes from Foveaux Strait (Fig. 2), whereas 74% of haplotypes detected in the Hauraki Gulf were not detected from any other site.

A 551 bp CO1 fragment was analysed from 162 *Ostrea chilensis* from five different sites. Overall, 13 *O. chilensis* CO1 sequences were detected across all sites, with low haplotype and nucleotide diversity (Table 3). Marlborough Sounds displayed only one haplotype which was also the dominant haplotype in Tasman Bay and Chatham Islands. Different dominant haplotypes were detected from Hauraki Gulf and Foveaux Strait (Fig. 2). Genetic sequence neutrality was rejected at Hauraki Gulf and Foveaux Strait for *B. exitiosa* (Table 1) and accepted for all *O. chilensis* locations (Table 2).

Genetic structure

Significant genetic variation ($p < 0.05$) was detected among all three sites where *B. exitiosa* was found (Fig. 3a). Genetic differentiation was detected among all *O. chilensis* populations except for the pairwise comparisons involving Marlborough Sounds, Tasman Bay, and Chatham Islands (Fig. 3b). Significant genetic variation ($p < 0.001$) was detected among three regions broadly comprising of northern *O. chilensis,* mid-latitude *O. chilensis*, and southern *O. chilensis* (Table 4a). No genetic differentiation was detected among regions where *B. exitiosa* was detected and sites where it was not, despite showing a significant *p*

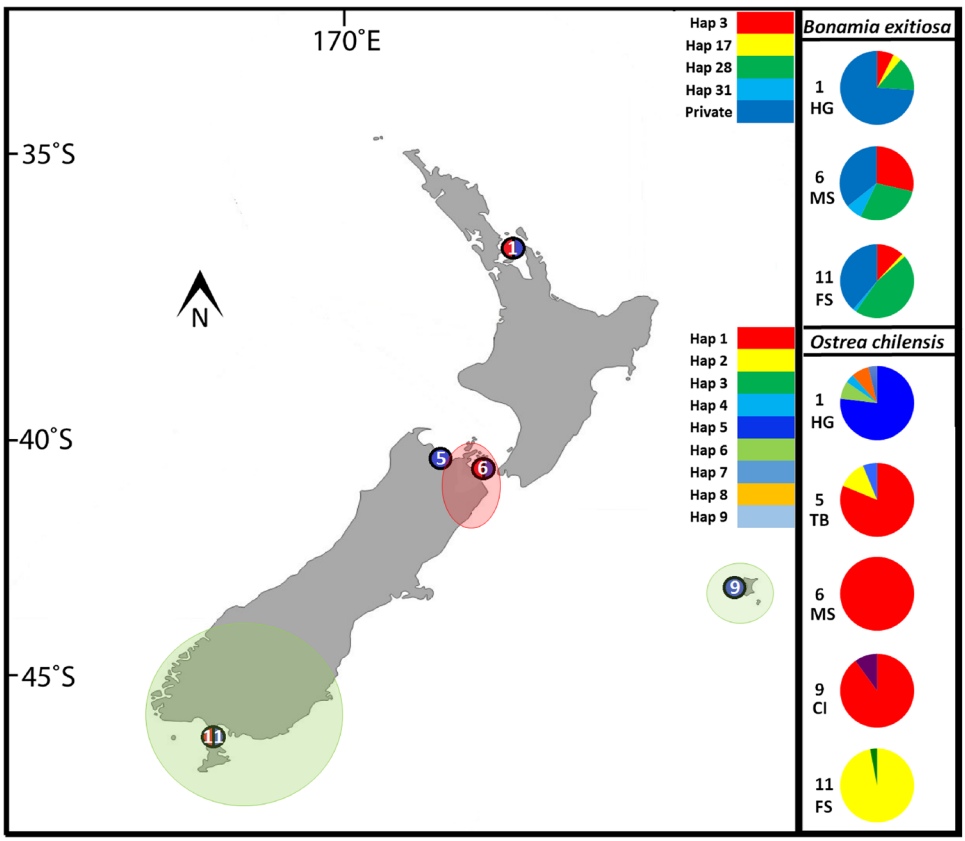

Fig. 2 Sampling locations where *Bonamia exitiosa* was detected and *Ostrea chilensis* cytochrome *c* oxidase subunit 1 gene (CO1) were sequenced. Red and blue closed circles indicate that *B. exitiosa* and *O. chilensis* were analysed (sample sites 1, 6, 11); closed blue circles indicate only *O. chilensis* was analysed (sample sites 5, 9). The vertical panel on the right shows the haplotype frequency for each sampling location. For *B. exitiosa* only the four haplotypes shared among at least two sites are shown, because private haplotypes are too numerous to be shown individually. The shaded green and red circles indicate the approximate protected zone and controlled zone of the Controlled Area Notice (CAN), respectively

Table 3 Sampling sites of *Ostrea chilensis*, number of *O. chilensis* sequences analysed (*N*), the number of haplotypes (N_h), haplotype diversity (*h*), the average number of nucleotide differences (*K*), segregating sites (*S*), nucleotide diversity (π), and Tajima's *D* (*D*)

Location	*N*	N_h	*h*	*K*	*S* (SD)	π	*D*
Hauraki Gulf	26	5	0.409 ± 0.117	0.880 ± 0.637	1.572 (0.785)	0.002 ± 0.001	− 1.448
Marlborough Sounds	31	1	0.000 ± 0.000	0.000 ± 0.000	0.000 (0.000)	0.000 ± 0.000	0
Tasman Bay	32	3	0.331 ± 0.099	0.347 ± 0.350	0.496 (0.367)	0.001 ± 0.001	− 0.606
Foveaux Strait	33	2	0.061 ± 0.056	0.061 ± 0.134	0.246 (0.246)	0.001 ± 0.000	− 1.140
Chatham Islands	40	2	0.185 ± 0.076	3.138 ± 1.661	3.997 (1.474)	0.006 ± 0.003	− 0.696
Total	162	13	0.837 ± 0.010	5.318 ± 2.581	6.005 (1.662)	0.009 ± 0.005	− 0.341

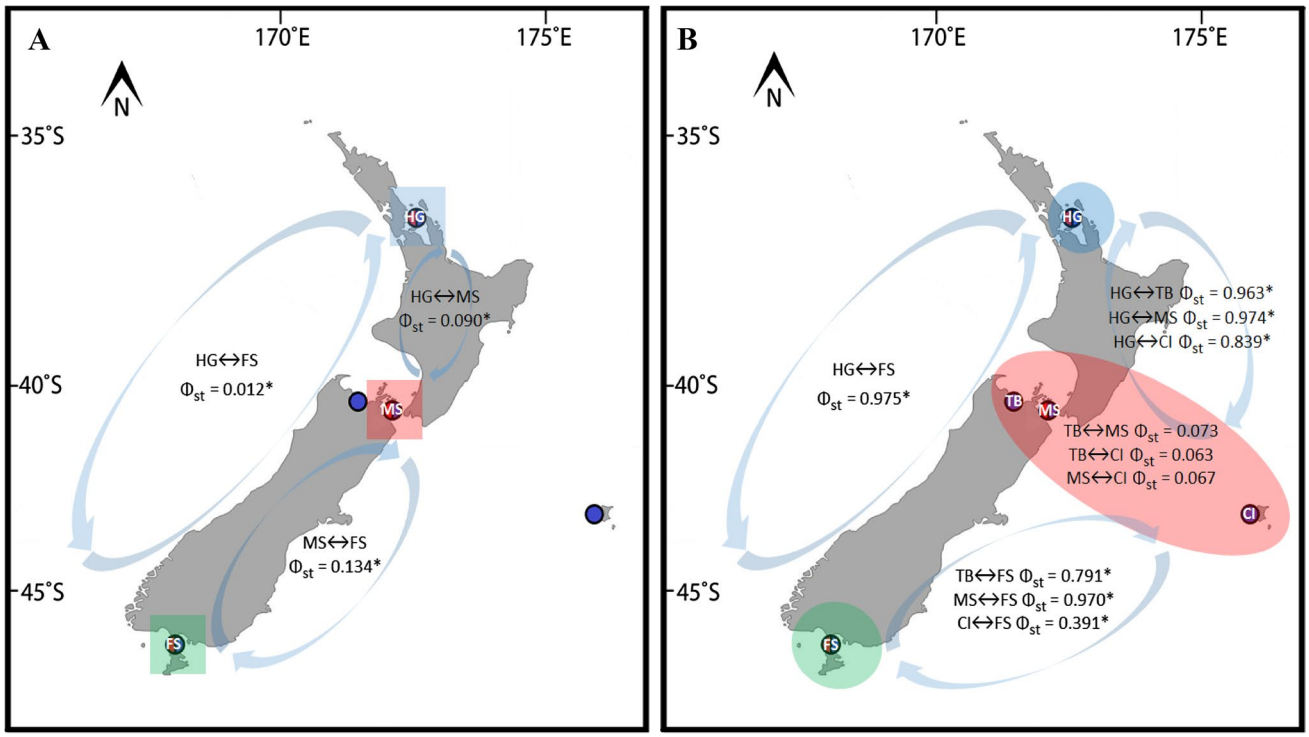

Fig. 3 Pairwise (ϕ_{st}) genetic differentiation between samples sites for **a** *Bonamia exitiosa* and **b** *Ostrea chilensis*. The shaded squares (*B. exitiosa*) and shaded ovals (*O. chilensis*) represent putative sequence groups based on ϕ_{st} results. *Significant at $p < 0.001$

Table 4 AMOVA results of *Ostrea chilensis* populations (A) grouped by genetic differentiation results (i) HG, (ii) FS, and (iii) MS-TB-CI; (B) grouped based on whether *Bonamia exitiosa* was detected (i) TB, (ii) CI, and (iii) HG-MS-FS

	df	Percent of variation	*p* value
(A) Source of variation			
Among regions	2	87.8	< 0.0001
Among populations within regions	2	1.16	< 0.05
Within populations	157	11.04	0.09
(B) Source of variation			
Among regions	1	− 25.64	< 0.0001
Among populations within regions	3	108.48	< 0.0001
Within populations	157	17.18	1.0000

value, because negative percent of variation is common in the absence of genetic structure (Table 4b). *Bonamia exitiosa* displayed a complex genealogy with sequences from all three sites mixed (Fig. 4). *B. exitiosa* sequences generated from this study belong to the Southern Hemisphere group of *B. exitiosa* sequences that include haplotypes from Australia and Argentina in addition to all three New Zealand sites (see Hill-Spanik et al. 2015; Lane 2017).

Migration

Mean migration estimates were broadly consistent with the results obtained from the genetic differentiation analyses. *O. chilensis* had a low number of effective migrants in all comparisons. There was no evidence of skewed, unidirectional gene flow between any sites. There was a higher level of unidirectional migration observed from *B. exitiosa* of Hauraki Gulf and Foveaux Strait into Marlborough Sounds (Table 5).

Discussion

Distribution of *Bonamia exitiosa* in New Zealand

In our study, we sampled flat oyster beds throughout New Zealand for *Bonamia exitiosa* and *B. ostreae*. *Bonamia ostreae* was not detected in any site other than the Marlborough Sounds, and *B. exitiosa* was only detected from three sites: Hauraki Gulf, Marlborough Sounds, and Foveaux Strait where it has been reported before. The prevalence of *B. exitiosa* from Foveaux Strait in this study is lower than previously recorded from the same area (Michael et al. 2013). Oysters collected for this study were sampled from an oyster bed located in the north-west area of Foveaux Straitt where *B. exitiosa* is less prevalent than other more eastern oyster

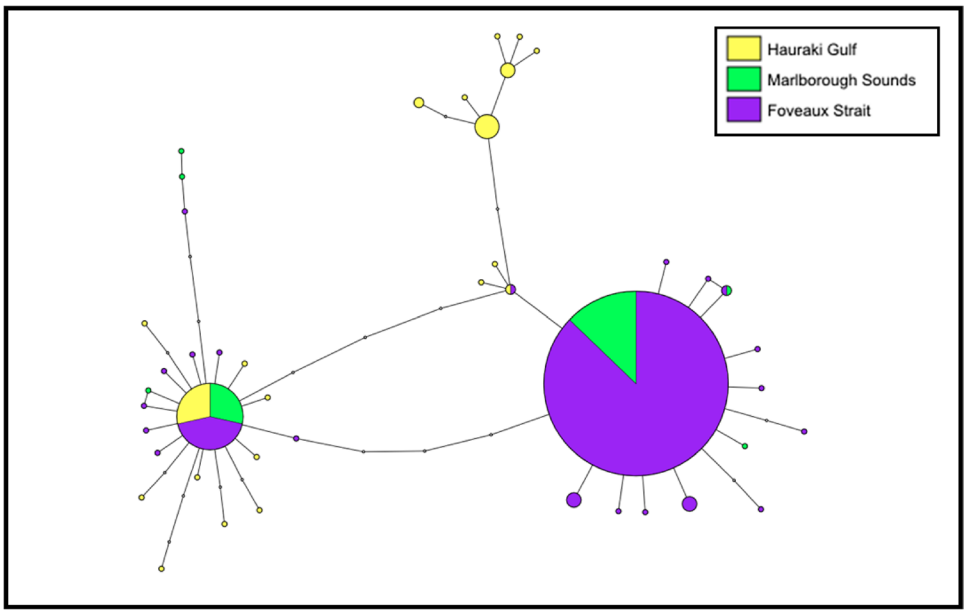

Fig. 4 TCS analysis of ITS rDNA *Bonamia exitiosa* sequences. The small enclosed circles represent putative mutational steps. The size of the circle corresponds to the number of individuals with that respective DNA sequence

Table 5 Bayesian estimates of Θ and pairwise migration rates of *Ostrea chilensis* and *Bonamia exitiosa*

Site	*Ostrea chilensis*	*Bonamia exitiosa*
Hauraki Gulf Θ	0.00404 (0, 0.00900)	0.00116 (0, 0.00400)
Marlborough Sounds Θ	0.00026 (0, 0.00200)	0.02172 (0, 0.08150)
Tasman Bay Θ	0.00210 (0, 0.00700)	–
Foveaux Strait Θ	0.00027 (0, 0.00200)	0.00071 (0, 0.00300)
Chatham Islands Θ	0.00181 (0, 0.00500)	–
MS → HG	0.20 (0, 1.42)	0.25 (0, 1.81)
TB → HG	0.32 (0, 2.36)	–
FS → HG	0.25 (0, 1.78)	0.35 (0, 2.00)
CI → HG	0.38 (0, 2.61)	–
HG → MS	0.06 (0, 0.88)	6.82 (0, 40.75)
TB → MS	0.06 (0, 0.93)	–
FS → MS	0.06 (0, 0.90)	7.00 (0, 40.75)
CI → MS	0.07 (0, 1.00)	–
HG → TB	0.33 (0, 2.89)	–
MS → TB	0.58 (0, 3.50)	–
FS → TB	0.57 (0, 3.48)	–
CI → TB	0.47 (0, 2.80)	–
HG → FS	0.05 (0, 0.90)	0.19 (0, 1.50)
MS → FS	0.06 (0, 0.84)	0.15 (0, 1.25)
TB → FS	0.06 (0, 0.90)	–
CI → FS	0.06 (0, 0.93)	–
HG → CI	0.25 (0, 1.95)	–
MS → CI	0.48 (0, 2.49)	–
TB → CI	0.56 (0, 2.50)	–
FS → CI	0.45 (0, 2.45)	–

Sample site acronyms can be found in Table 1

beds (see Michael et al. 2013). Previously, *B. exitiosa* has also been detected in Wellington Harbour and Tasman Bay (Hine 1997; Jones unpublished data). Reasons for not detecting it from these sites in our study include: (1) *B. exitiosa* is at a prevalence lower than 2%; (2) *B. exitiosa* infections could be below the limit of detection of the PCR used; or (3) The correct tissue was not sampled to enable detection—very low intensity *Bonamia* infections only occur beneath the basement membrane of the gut (Hine 1991) and no histology was performed in our study to confirm this. Although unconfirmed, it seems likely that *B. exitiosa* still exists in these locations albeit at very low levels (Van Banning 1987). *Bonamia exitiosa* was not detected in the Chatham Islands; however, interestingly, Michael et al. (2015) suggest that the Chatham Islands may not be free of *B. exitiosa*. During validation of a *B. exitiosa* real-time PCR, the authors used Chatham Island *O. chilensis* for *B. exitiosa*-negative controls and experienced some late amplification. This requires further investigation and if *B. exitiosa* is present within the Chatham Islands, sequences from there could be useful in elucidating *Bonamia* parasite dispersal around New Zealand. Biotic factors might explain the failure to detect *B. exitiosa* from Stewart Island despite being adjacent to Foveaux Strait, although reasons for failed detection could be as outlined above. *Bonamia* spp. have higher infection intensities in higher salinity water (Audemard et al. 2008, 2014; Arzul et al. 2009) and the salinity in some Stewart Island inlets can be as low as 3–5 ppm because of freshwater runoff (Buroker et al. 1983). Disease patterns along environmental gradients are documented (e.g., Bushek et al. 2012) and identifying spatial disease refuges could be beneficial to a developing *O. chilensis* aquaculture industry.

Comparative genetic structure

The three populations of *B. exitiosa* detected in our study displayed significant genetic differentiation and low levels of gene flow among them. Populations of *O. chilensis* displayed a similar pattern of genetic structure, except that gene flow is occurring between the top of the South Island and the Chatham Islands. The predominant cell form of *B. exitiosa* and *B. ostreae* in host tissue is a uninucleate naked cell and it is unknown what form this cell takes when released into the environment. Some studies have detected *B. ostreae* in other marine organisms (e.g., the brittlestar *Ophiothrix fragilis*, the Pacific oyster *Crassostrea gigas*, and the blue mussel *Mytilus edulis*), which suggests that the parasite might be able to use additional routes of transmission (Lynch et al. 2007, 2010; Flannery 2014). Nonetheless, *Bonamia* parasites mainly depend on oysters for survival and dispersal (Hill-Spanik et al. 2015). Therefore, the results from our study suggest that natural co-dispersal of *Bonamia* spp. with *O. chilensis* could be likely over shorter distances, but no evidence presented suggests that co-dispersal occurs over longer distances. Indeed, the markers used in this study are inherently different, i.e., ITS is a nuclear marker and CO1 is cytoplasmic, and, perhaps, comparable markers may present higher levels of congruence between host and parasite. Nonetheless, with the clear observed patterns of connectivity, inferences of dispersal can still be made. Certainly, the development of other genomic markers for *O. chilensis* and *Bonamia* spp. will build upon this study's data.

The pattern of genetic differentiation among *O. chilensis* populations suggests that life history traits and ocean currents have influenced their distribution. *Ostrea chilensis* are brooders and the time spent as pelagic larvae ranges from minutes to 20 days (Jeffs and Creese 1996). This likely explains the significant genetic differences of pairwise comparisons between Hauraki Gulf and Foveaux Strait with all sites, which are consistent with other genetic data for New Zealand *O. chilensis* (Foighil et al. 1999; Thomas 2015). Despite modelling results for the Tasman Bay *O. chilensis* population indicating that only a small proportion of larvae travel more than one km from their natal location (Broekhuizen et al. 2011), our study presents evidence of gene flow between Tasman Bay, Marlborough Sounds, and Chatham Islands. Although the migration data suggest a bi-directional flow, in all likelihood, it will be in a west–east direction which aligns with Foighil et al. (1999), who reported *O. chilensis* rafted from New Zealand to Chile. Similar patterns of genetic structure have been found with other New Zealand marine fauna including the clam *Austrovenus stutchburyi* (Ross et al. 2012), where the easterly flow of the subtropical convergence is believed to facilitate the transport of larvae from mainland to Chatham Islands (Heath 1985).

Bonamia exitiosa displayed genetic structure across all three sites, despite sharing haplotypes among them. Genetic structuring is expected from a parasite with a presumed simple direct life cycle and high host specificity, and is concordant with a meta-analysis that found a parasite's lifecycle was the best predictor of genetic structure, highlighting the dependence of host mobility for dispersal (Blasco-Costa and Poulin 2013; but see Mazé-Guilmo et al. 2016). In this light and considering the *O. chilensis* data presented, we would expect *B. exitiosa* to be detected in the Chatham Islands. Moreover, Hill-Spanik et al. (2015) reported *B. exitiosa* gene flow between Australia, New Zealand, and Argentina, which is putatively facilitated by the dispersal of *B. exitiosa*-infected oysters by surface currents. Although not yet empirically demonstrated, it is inferred that *O. chilensis* can disperse by rafting (Foighil et al. 1999), which has been demonstrated for other marine fauna: marine invertebrates colonising pieces of pumice rafts could disperse > 5000 km in 7–8 months (Bryan et al. 2012), and successful colonisation of ten New Zealand intertidal species occurred after dispersing 400–600 km via kelp rafts for a minimum duration of 20–65 days (Fraser et al. 2011). Considering this as well as that *Bonamia* sp.-induced mortalities of naïve pre-recruit *O. chilensis* exposed to environmental levels of *B. exitiosa* are likely to occur after 3–4 months (Diggles and Hine 2002), it is conceivable that *Bonamia* sp.-infected *O. chilensis* could disperse to the Chatham Islands. Rafting is presumably a low-frequency event and given that *B. exitiosa* is at a low prevalence and that only a few successful migrants per generation is sufficient to maintain neutral genetic homogeneity, dispersal of *B. exitiosa*-infected *O. chilensis* could be rare. On the other hand, *B. ostreae* has been reported at a much higher prevalence in the Marlborough Sounds (Lane et al. 2016), which would increase its probability of dispersal. Sampling for this study took place concurrently during the *B. ostreae* epizootic in the Marlborough Sounds and there may not have been sufficient time for *Bonamia* sp.-infected *O. chilensis* dispersal to occur. Future testing of *O. chilensis* in the Chatham Islands with the same assay used in Michael et al. (2015) as well as a *B. ostreae* real-time PCR (Ramilo et al. 2013) would enable greater detection sensitivity to elucidate the dispersal of *Bonamia* parasites around New Zealand. Detecting *B. exitiosa* from the Chatham Islands would support Hill-Spanik et al.'s (2015) postulation, and provide evidence that *B. exitiosa* can co-disperse with *O. chilensis* or other oyster hosts over longer distances, which would alter the conclusion of this study. Until such data become available, we cannot conclude that *B. exitiosa* and, as likely, *B. ostreae,* are co-dispersing with *O. chilensis* over longer distances around New Zealand.

The means in which *B. exitiosa* populations are sharing haplotypes despite the high genetic structure of corresponding *O. chilensis* populations is uncertain. This is confounded

by the frequent translocations of *O. chilensis* from Foveaux Strait around the country to establish new oyster beds (K. Michael pers. comm.). The concomitant translocation of *B. exitiosa* with these movements may have influenced the findings of this study to a greater or lesser extent, but this is not reflected in the genetic structuring of *O. chilensis*. With the absence of data on alternate hosts for *Bonamia*, the influence of anthropogenic factors could explain this; although whether this is through intentional translocation of infected oyster stock, unintentional dispersal via ballast water or fouled ship hulls, or a combination of these is difficult to ascertain. If the former is true, then the CAN is likely to be effective in restricting the dispersal of *B. ostreae* to other New Zealand oyster beds. Ballast water and fouled ship hulls are often proposed as vectors for *Bonamia* spp., however, this hypothesis remains to be explicitly tested. Indeed, the reports of *Bonamia* sp. to new areas have occurred near ports of entry (e.g., Kroeck and Montes 2005), but this evidence is correlative and empirical data are lacking. Therefore, in addition to the development of higher resolution genomic markers for *O. chilensis* and *B. exitiosa* to augment the data presented in this study, testing the hypothesis that ballast water and ship hull fouling can act as vectors for *Bonamia* is vital to our understanding of how these parasites disperse and the effective management of them.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflicts of interests.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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