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Complex genetic patterns and a phylogeographic disjunction among New Zealand mud snails *Zeacumantus subcarinatus* and *Z. lutulentus*

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Abstract The comparative analysis of genetic structure and phylogeography of marine species can reveal the relative importance of common biogeographic barriers and species-specific evolutionary histories. In the present study, mitochondrial COI sequences from a total of 724 individuals collected from 38 localities throughout New Zealand during 2006 and 2007 were used to examine the genetic structure and demographic histories of the intertidal gastropods *Zeacumantus subcarinatus* and *Zeacumantus lutulentus*. For both species, results revealed isolation of populations located along the northern South Island and southern and western North Island, and extensive genetic structure throughout the remainder of their ranges. Despite this, long-distance dispersal and secondary admixture of divergent haplotypes was evident, especially for the widespread *Z. subcarinatus*. These findings reveal the importance of common barriers to gene flow and highlight how the interaction of inherent dispersal limitations of direct-developing marine organisms and periodic long-distance movements can produce complex genetic patterns.

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Introduction

Comparing the genetic population structure and demographic histories of co-distributed species can reveal the importance of common barriers to gene flow that have shaped the evolution of a region's organisms (Avise 1992; Arbogast and Kenagy 2001; Plouviez et al. 2009) and unique genetic patterns resulting from species-specific dispersal events and responses to environmental changes (Bowen and Avise 1990; Wares and Cunningham 2001). One advantage of using a comparative approach is the ability to distinguish differing genetic patterns and demographic histories among closely related species. These species often share similar life history traits and therefore appear likely to display congruent patterns when co-distributed. However, studies from diverse taxa have revealed distinct genetic patterns and/or evolutionary histories among phylogenetically related or ecologically similar species (Michaux et al. 2005; Lejeusne et al. 2011; Santos et al. 2011; Albaina et al. 2012).

In marine environments, life history traits such as larval dispersal and habitat requirements often shape a species' genetic structure and evolutionary history in response to dispersal barriers such as ocean currents and environmental gradients (Galarza et al. 2009; Pelc et al. 2009). Marine gastropods may utilize either direct (eggs hatching into crawl away juveniles) or planktonic larval development (Geiger 2006). While the duration of pelagic larvae does not always correlate well with population connectivity in marine species, direct development typically increases population structure in marine organisms (Weersing and Toonen 2009). In gastropods, an inverse relationship

between larval dispersal potential and the degree of genetic structure among populations has been observed in several species (Janson 1987; Hoskin 1997; Wilke and Davis 2000; Collin 2001), with factors such as environmental tolerance and microhabitat requirements also playing a role (Wilke and Davis 2000; Albaina et al. 2012). However, larval mortality and diffusion can make populations of apparently dispersive marine species reliant on local larval retention (Cowen et al. 2000; Cowen and Sponaugle 2009), and highly dispersive gastropods have displayed genetic differentiation over relatively small geographic scales (Waters et al. 2005, 2007). In addition, species that appear to have limited dispersal capabilities can be successful colonizers over a range of geographic distances (Johannesson 1988; Johannesson and Warmoes 1990; Donald et al. 2011).

New Zealand is home to two endemic species of coastal marine mud snails belonging to the genus *Zeacumantus* (Batillariidae), *Zeacumantus subcarinatus* and *Zeacumantus lutulentus*. *Zeacumantus lutulentus* appears to be restricted to New Zealand, while *Z. subcarinatus* has established populations in New South Wales, Australia (Powell 1979; Andrews et al. 2010). *Zeacumantus subcarinatus* occurs throughout most of New Zealand in intertidal bays, mudflats, rock pools and other protected areas, where it grazes on macro- and microalgae (Morton and Miller 1968; McClatchie 1979; Jones and Marsden 2005). This species produces thick gelatinous egg strings, often laid under rocks, with eggs hatching into crawling larvae (Morton and Miller 1968; Pilkington 1974; Fredensborg and Poulin 2006). This form of direct development without an extended pelagic larval stage can make populations reliant on local reproduction (Fredensborg and Poulin 2006), a prediction supported by the species' genetic population structure along the southern two-thirds of the South Island (Keeney et al. 2009). *Zeacumantus lutulentus* is common on mid-tidal mud flats throughout the North Island and northern portion of the South Island (Powell 1979). This species prefers silty sand and muddy environments and is often upshore of *Z. subcarinatus* in more sheltered areas (Morton 2004). It also produces thick gelatinous egg strings (Morton and Miller 1968), but the dispersal capability of larvae is unknown.

In this study, we use cytochrome *c* oxidase subunit I gene (*COI*) sequences to examine the genetic structure and phylogeography of *Z. subcarinatus* and *Z. lutulentus* throughout New Zealand to test the hypotheses that (1) both species will display extensive genetic structure throughout their ranges characterized by an isolation by distance pattern with geographic isolation of divergent haplotypes, and (2) they will share common genetic disjunctions and population histories where their ranges overlap. These predictions are based on the reproductive mode and genetic differentiation previously detected throughout the southern South Island for *Z. subcarinatus*, and *Z. lutulentus*' phylogenetic relatedness and use of similar egg strings.

Materials and methods

Snail collection, PCR amplification and DNA sequencing

Approximately 50–300 snails were collected from each sample site during low tides. Within sites, snails were collected from as large a geographic area as possible in an effort to represent the genetic diversity present, as limited dispersal could lead to patchiness of related offspring within sites. Snails were collected from a total of 38 coastal sites along the North and South Islands of New Zealand from December 2006 to November 2007 (Fig. 1a, b). Snails were placed in 2 l containers with water from the site of collection until dissection. A small portion of the foot (approximately 4 mm^3) was removed from each snail; DNA was then extracted by digesting foot tissue in 800 µl of 5 % chelex containing 0.1 mg/ml proteinase K, incubating at 60 °C overnight and heating at 100 °C for 8 min. An approximately 1,100-bp region of the cytochrome *c* oxidase subunit I gene (*COI*) was amplified using the primers LCO1490: 5′-GGTCAACAAATCATAAAGAT ATTGG-3′ (Folmer et al. 1994) and H7005: 5′-CCG GATCCACNACRTARTANGTRTCRTG-3′ (Hafner et al. 1994) as described in Keeney et al. (2009). PCR products were purified using PureLink (Invitrogen) and QIAquick (Qiagen) PCR Purification Kits. DNA from purified PCR products was sequenced using PCR primers at the Allan Wilson Centre Genome Sequencing Service at Massey University with an ABI3730 Genetic Analyzer and the SUNY Upstate Medical University DNA core facility with an ABI3100 Genetic Analyzer.

Data analyses

DNA sequences were aligned using Clustal W (Thompson et al. 1994) as implemented in MEGA5 (Tamura et al. 2011). All *Zeacumantus subcarinatus COI* data from Keeney et al. (2009) have been included in the present study. A 95 % statistical haplotype network was constructed using TCS 1.21 (Clement et al. 2000) for each species. The number of unique haplotypes, number of polymorphic sites, haplotype diversity (h) and nucleotide diversity (π) within each sample site and over all sample sites were estimated with Arlequin 3.5 (Excoffier et al. 2005).

To identify the genetic population structure of each species, we initially used the simulated annealing approach of SAMOVA (spatial analysis of molecular variance) 1.0 (Dupanloup et al. 2002). SAMOVA utilizes geographic

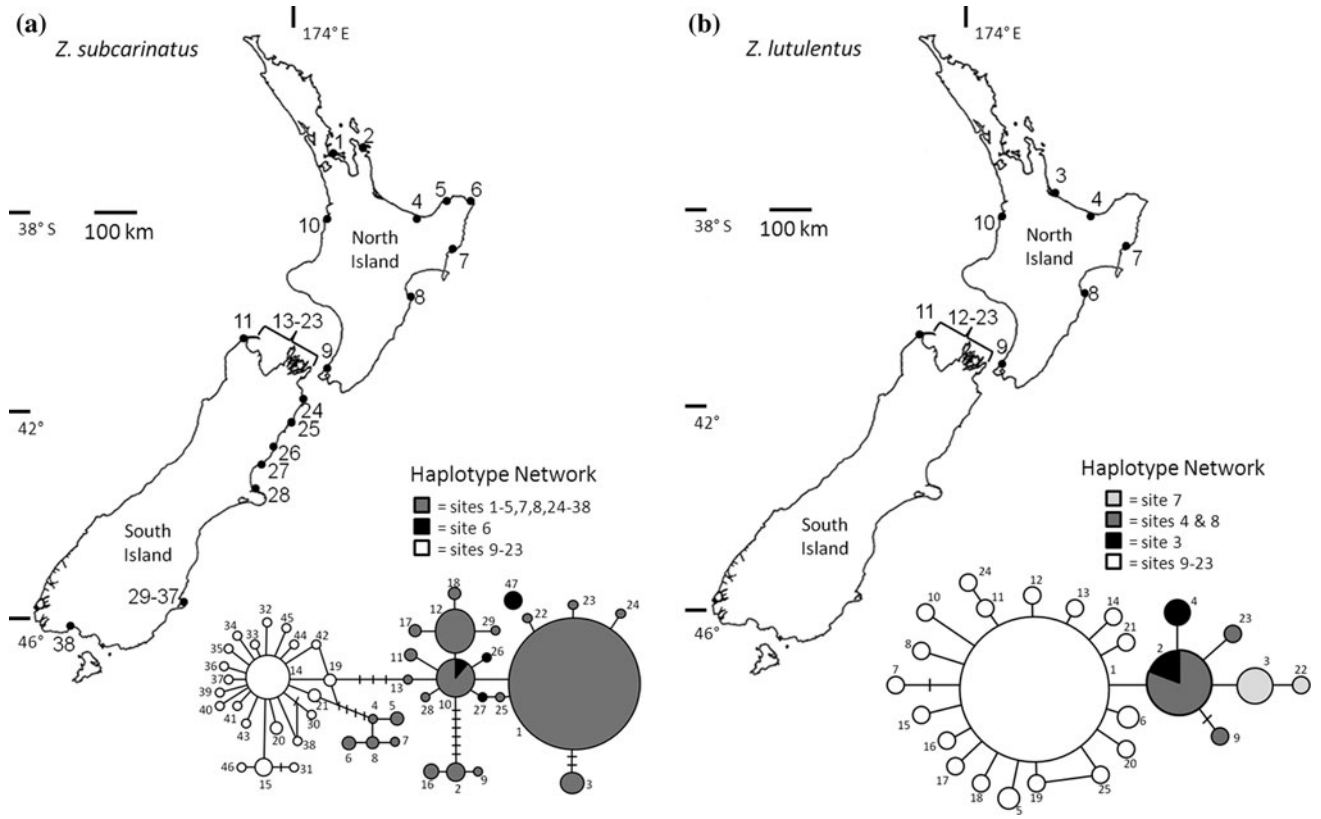

Fig. 1 Sample site locations with statistical parsimony haplotype networks for (**a**) *Zeacumantus subcarinatus* and (**b**) *Zeacumantus lutulentus.* Site numbers follow Table 1. For haplotype networks, haplotype frequencies are proportional to the area of associated *circles* within each network. *Dashes* represent inferred intermediate haplotypes. All connections between haplotypes represent one mutation. The proportion of each haplotype recovered from each SAMOVA region is indicated by *shading*: Group 1 = *white*, Group 2 = *dark gray*, Group 3 = *black*, Group 4 = *light gray* (*Z. lutulentus* only). Haplotype numbering follows Appendix S1

information and empirically identifies groups of populations that are maximally genetically differentiated. Optimum population groupings were determined by repeatedly running the analyses while increasing the number of possible groups (k) by one each run until it equaled the total number of sample sites, with 100 permutations of each run. This method identifies the major genetic discontinuities within each species, but does not reveal all of the genetic differences among collection sites as genetic structure can exist within SAMOVA groups.

Genetic population structure among all sample sites, within and among population groups identified by SAMOVA, and between all pairs of sample sites was analyzed using analysis of molecular variance (AMOVA) with Arlequin 3.5 (Excoffier et al. 2005). AMOVA analyses incorporated the most appropriate models of sequence evolution available as determined using the Akaike information criterion (AIC) of jModelTest (Posada 2008). *Zeacumantus subcarinatus* analyses incorporated Tamura's (1992) model of sequence evolution and gamma-distributed substitution rate variation with shape parameter $\alpha = 0.139$. *Zeacumantus lutulentus* analyses incorporated Tamura and Nei's (1993) model of sequence evolution. To ensure that potential long-distance dispersal of divergent haplotypes was not biasing estimates, overall and pairwise Φ_{ST} estimates were repeated utilizing conventional F-statistics with Arlequin 3.5.

The divergence time of COI lineages separated by the major disjunction identified was estimated for both species using unique haplotypes in BEAST v1.7.2 (Drummond and Rambaut 2007), with a random local molecular clock and Bayesian Skyline coalescent tree prior. As a fossil calibration point was not available, we applied a prior on the COI substitution rate consisting of a normal distribution with mean value = 0.01425 substitutions/my (SD = 0.00113), based on marine gastropod COI divergence rates of 2.4–3.3 % per million years (Lessios 2008; Miura et al. 2010). Since these rates were based on Kimura's two-parameter model of sequence evolution (Kimura 1980), we utilized the HKY option in BEAST with equal nucleotide frequencies. The analyses were run for 20,000,000 generations sampled every 1,000 generations. Sufficiency of estimated sample sizes and convergence statistics were analyzed with Tracer v1.5 with the first 10 % of samples discarded as burn-in (Rambaut and

Drummond 2009). Results were analyzed and visualized with TreeAnnotator v1.7.2 using the maximum clade credibility tree and mean node heights, and with FigTree v1.3.1 (Rambaut 2009).

To determine whether the degree of genetic differentiation among populations was related to their geographic distance, Mantel tests for isolation by distance (Mantel 1967) were performed using pairwise genetic distances (Φ_{ST}) and geographic coastal distances. Raw Φ_{ST} values were used instead of linearized values ($\Phi_{ST}/1-\Phi_{ST}$) as several pairwise estimates were 1.00. Significance was determined with 10,000 randomizations using Arlequin 3.5.

To compare the demographic histories of the two species and examine regional differences among intraspecific populations, F_S (Fu 1997) and R_2 (Ramos-Onsins and Rozas 2002) test statistics were calculated for individual sample sites using DnaSP v5.10 (Librado and Rozas 2009). While both tests can detect deviations from selective neutrality and demographic expansions, R_2 has more power with small sample sizes, while F_S has increased power with larger sample sizes (Ramos-Onsins and Rozas 2002). Here, utilizing both provides a more robust assessment of demographic histories as sample sizes varied. The significance of both statistics was determined using 10,000 coalescent simulations.

Results

A 900-bp fragment of the *COI* gene was analyzed from a total of 551 *Zeacumantus subcarinatus* from 32 localities and 173 *Zeacumantus lutulentus* from 16 localities. Seventy-five polymorphic sites producing 47 haplotypes and 25 polymorphic sites producing 25 haplotypes were identified for *Z. subcarinatus* and *Z. lutulentus*, respectively (GenBank accession numbers GQ868066-GQ868078 and KC669629-KC669687). We did not detect strong trends in haplotype diversity for either species, as areas of high and low diversity were spread throughout New Zealand. The major exception was northeastern South Island localities (sites 24–27) consistently lacking haplotype diversity (Table 1). Haplotype diversity often differed between species within the same sample site. For example, sites 7, 8 and 18 all possessed relatively high *Z. subcarinatus* diversity and low *Z. lutulentus* diversity. Nucleotide diversity was relatively low for both species across all sites, with the exception of the site 6 *Z. subcarinatus* population (Table 1).

Four relatively common haplotypes ($n > 20$) were recovered from *Z. subcarinatus* (Fig. 1a). Each haplotype was dominant in different regions of the species range: haplotype 1 in the southern South Island and sites 1 and 8 on the North Island, haplotype 10 in site 38 and the eastern and northern North Island, haplotype 12 along the central and northeastern South Island, and haplotype 14 in northern South Island and southern and western North Island populations (Fig. 1a, Appendix S1a in Supporting Information). For *Z. lutulentus,* four haplotypes were common ($n \geq 5$), with haplotype 1 restricted to northern South Island and southern and western North Island populations and haplotypes 2–4 common in different eastern and northern North Island sites (Fig. 1b, Appendix S1b).

For *Z. subcarinatus*, a highly divergent haplotype ($n = 7$) was recovered from site 6 that did not join the 95 % statistical haplotype network and differed from all other haplotypes by at least 30 mutations. To ensure that individuals with this haplotype were *Z. subcarinatus*, a 487-bp portion of the 16S rRNA gene was amplified and sequenced for four individuals using the primers 16Sar: 5′-CGCCTGTTTAACAAAAACAT-3′ and 16Sbr 5′-CCG GTCTGAACTCAGATCACGT-3′ (Simon et al. 1994) with an annealing temperature of 46 °C. This gene was used in a phylogenetic analysis of all extant Batillariidae, providing an ideal database for species identification (Ozawa et al. 2009). All four individuals possessed an identical 16S rRNA haplotype that a NCBI BLASTN search (Altschul et al. 1997) revealed most closely matched the 16S rRNA gene of *Z. subcarinatus* (GenBank Accession #s FJ606948.1 and FJ 606947.1) with 100 % query coverage, 98 % maximum identity and 481/487 matching nucleotides. After these two haplotypes, there was a decrease in haplotype similarities, with maximum identities of 89–91 %, supporting our identification of these snails as *Z. subcarinatus*.

SAMOVA results partitioned populations of *Z. subcarinatus* into three groups: Group 1 = all northern South Island sites (sites 11, 13–15,18, 21–23) + all southern and western North Island sites (sites 9 and 10), Group 2 = all southern and eastern South Island sites (sites 24–38) + all eastern and northern North Island sites except site 6 (sites 1, 2, 4, 5, 7, 8), and Group 3 = site 6 on the northeastern tip of the North Island. Populations of *Z. lutulentus* were partitioned into four groups: Group 1 = all northern South Island sites (sites 11, 12, 15–20, 22, 23) + all southern and western North Island sites (sites 9 and 10), Group 2 = sites 4 and 8, Group 3 = site 7, and Group 4 = site 3. Therefore, Group 1 for both species was geographically consistent, and *Z. lutulentus* Groups 2–4 were consistent with the North Island portion of *Z. subcarinatus* Group 2. For both species, each group was characterized by different dominant haplotypes (Fig. 1a, b). Also for both species, Group 1 consisted of sites not sharing any haplotypes with other groups and possessing a star-shaped phylogeographic pattern (Fig. 1a, b). The estimated divergence times of the lineages in the isolated Group 1 populations were 282,100 years ago (95 % HPD = 155, 200–430, 100 years

Table 1 Summary statistics for two species of the snail genus *Zeacumantus* from 38 localities in New Zealand

Location	*Z. subcarinatus*					*Z. lutulentus*				
	n	Nh	Np	*h*	π	*n*	Nh	Np	*h*	π
1. Takapuna Beach	10	4	3	0.533 ± 0.180	0.0007 ± 0.0007	–	–	–	–	–
2. Kuaotunu	10	1	0	0.000 ± 0.000	0.0000 ± 0.0000	–	–	–	–	–
3. Waihi	–	–	–	–	–	10	2	1	0.556 ± 0.075	0.0006 ± 0.0006
4. Ohiwa	10	2	1	0.200 ± 0.154	0.0002 ± 0.0003	12	3	3	0.318 ± 0.164	0.0006 ± 0.0006
5. Waihau	10	1	0	0.000 ± 0.000	0.0000 ± 0.0000	–	–	–	–	–
6. Hicks Bay	16	4	33	0.650 ± 0.075	0.0248 ± 0.0129	–	–	–	–	–
7. Gisborne	14	5	4	0.802 ± 0.069	0.0013 ± 0.0010	12	2	1	0.167 ± 0.134	0.0002 ± 0.0003
8. Napier	10	3	10	0.644 ± 0.101	0.0060 ± 0.0036	11	1	0	0.000 ± 0.000	0.0000 ± 0.0000
9. Porirua	10	4	2	0.644 ± 0.152	0.0008 ± 0.0007	10	2	1	0.200 ± 0.154	0.0002 ± 0.0003
10. Raglan	9	3	3	0.417 ± 0.191	0.0008 ± 0.0007	12	2	1	0.167 ± 0.134	0.0002 ± 0.0003
11. Whanganui Inl	11	5	5	0.618 ± 0.164	0.0010 ± 0.0009	10	4	4	0.533 ± 0.000	0.0009 ± 0.0008
12. Port Puponga	–	–	–	–	–	11	1	0	0.000 ± 0.000	0.0000 ± 0.0000
13. Parapara	10	3	2	0.378 ± 0.181	0.0004 ± 0.0005	–	–	–	–	–
14. Port Motueka	10	3	3	0.600 ± 0.131	0.0010 ± 0.0008	–	–	–	–	–
15. Kina Beach	11	2	1	0.182 ± 0.144	0.0002 ± 0.0003	10	2	1	0.200 ± 0.154	0.0002 ± 0.0003
16. Mapua	–	–	–	–	–	12	4	3	0.455 ± 0.170	0.0007 ± 0.0007
17. Cable Bay	–	–	–	–	–	12	3	2	0.318 ± 0.164	0.0004 ± 0.0004
18. MP Beach	10	4	3	0.533 ± 0.180	0.0007 ± 0.0007	11	1	0	0.000 ± 0.000	0.0000 ± 0.0000
19 Okiwi Bay	–	–	–	–	–	10	3	2	0.378 ± 0.181	0.0004 ± 0.0005
20. Havelock	–	–	–	–	–	11	4	3	0.491 ± 0.175	0.0006 ± 0.0006
21. Clova Bay	11	3	2	0.346 ± 0.172	0.0004 ± 0.0005	–	–	–	–	–
22. Ngakuta Bay	8	4	3	0.643 ± 0.184	0.0008 ± 0.0008	10	3	2	0.378 ± 0.181	0.0004 ± 0.0005
23. Waikawa Bay	9	2	1	0.389 ± 0.164	0.0004 ± 0.0005	9	2	1	0.389 ± 0.164	0.0004 ± 0.0005
24. CC Lighthouse	10	1	0	0.000 ± 0.000	0.0000 ± 0.0000	–	–	–	–	–
25. Paparoa Point	10	1	0	0.000 ± 0.000	0.0000 ± 0.0000	–	–	–	–	–
26. South Bay	10	1	0	0.000 ± 0.000	0.0000 ± 0.0000	–	–	–	–	–
27. Omihi	12	1	0	0.000 ± 0.000	0.0000 ± 0.0000	–	–	–	–	–
28. Christchurch	30	4	3	0.251 ± 0.102	0.0005 ± 0.0005	–	–	–	–	–
29. Blueskin Bay	30	2	8	0.370 ± 0.084	0.0033 ± 0.0020	–	–	–	–	–
30. Aramoana	30	3	16	0.131 ± 0.082	0.0013 ± 0.0009	–	–	–	–	–
31. Deborah Bay	30	4	17	0.356 ± 0.106	0.0024 ± 0.0015	–	–	–	–	–
32. Sawyers Bay	30	3	10	0.191 ± 0.093	0.0010 ± 0.0008	–	–	–	–	–
33. Andy Bay	30	1	0	0.000 ± 0.000	0.0000 ± 0.0000	–	–	–	–	–
34. Company Bay	30	1	0	0.000 ± 0.000	0.0000 ± 0.0000	–	–	–	–	–
35. LPB	30	6	11	0.460 ± 0.109	0.0037 ± 0.0022	–	–	–	–	–
36.Otakau	30	3	10	0.536 ± 0.048	0.0022 ± 0.0014	–	–	–	–	–
37. Papanui Inlet	30	1	0	0.000 ± 0.000	0.0000 ± 0.0000	–	–	–	–	–
38. Bluff Harbour	30	4	3	0.481 ± 0.094	0.0006 ± 0.0006	–	–	–	–	–
Total	551	47	75	0.746 ± 0.016	0.0047 ± 0.0026	173	25	25	0.585 ± 0.041	0.0010 ± 0.0008

Localities 28–38 were included in Keeney et al. (2009)

Parameters are abbreviated as follows: *n* number of individuals analyzed, *Nh* number of different haplotypes observed, *Np* number of polymorphic sites observed, *h* haplotype diversity ± SD and π nucleotide diversity ± SD

ago) for *Z. subcarinatus* and 121,100 years ago (95 % HPD = 63, 100–196, 200 years ago) for *Z. lutulentus*.

Significant genetic differentiation was detected over all populations ($\Phi_{ST} = 0.661, P < 0.0001$) and among SAMOVA groups ($\Phi_{CT} = 0.740$, $P < 0.0001$) for *Z. subcarinatus* (Table 2). Significant genetic differentiation was also detected among sites within SAMOVA groups ($\Phi_{SC} = 0.318$, $P < 0.0001$). Within Group 1, all pairwise comparisons involving site 14 were significant ($\Phi_{ST} = 0.286$–0.438, $P < 0.007$) (see Appendix S2a). No

additional pairwise comparison was significant within this group ($\Phi_{ST} = -0.039$–0.112, $P \geq 0.061$). Separation of site 14 as a fourth group was the second-best supported SAMOVA scenario, and decreased variation among groups only slightly ($\Phi_{CT} = 0.736$, $P < 0.0001$). Within Group 2, 174 out of 210 pairwise comparisons were significant ($P < 0.05$) (see Appendix S2b). Ten of the nonsignificant comparisons involved sites 24–28 along the northeastern coast of the South Island, four of which contained only a single haplotype. Several additional nonsignificant comparisons involved some of the southernmost and northernmost samples included in the study, for example, site 1 versus sites 30–32. Analyses utilizing conventional F-statistics were consistent with these results with the exception that 171 out of 210 comparisons within Group 2 were significant (Appendix S2b). For *Z. lutulentus*, significant genetic differentiation was also detected over all populations ($\Phi_{ST} = 0.639$, $P < 0.0001$) and among SAMOVA groups ($\Phi_{CT} = 0.791$, $P < 0.0001$) (Table 2). Significant genetic differentiation was detected among sites within SAMOVA groups ($\Phi_{SC} = 0.018$, $P = 0.0070$), but no pairwise comparison within *Z. lutulentus* groups was significant ($\Phi_{ST} = -0.035$–0.156, $P \geq 0.086$; see Appendix S3), suggesting that moderate genetic differences may exist among some of these populations. Analyses utilizing conventional F-statistics were consistent with these results.

Zeacumantus subcarinatus displayed an extremely weak but statistically significant isolation by distance pattern ($r^2 = 0.081$, $P = 0.0001$). Several of the most geographically distant populations were genetically similar while several of the most genetically divergent populations were geographically close (Fig. 2a). While a stronger relationship between genetic and geographic distances was observed for *Z. lutulentus* ($r^2 = 0.496$, $P = 0.0013$), the relationship was driven by the presence of two distinct sets of points, one consisting of population comparisons displaying moderate to weak genetic differentiation and the other consisting of comparisons displaying strong genetic differentiation, not continuous variation (Fig. 2b). The strongly differentiated set consisted of population comparisons from different SAMOVA groups, while the other set consisted of comparisons within groups. Within each set, isolation by distance was not apparent. In addition, several populations isolated by up to 1,000 km were genetically similar, while more proximate sites were genetically differentiated (Fig. 2b).

Demographic analyses revealed evidence of population size expansions for both *Z. subcarinatus* (4 sites) and *Z. lutulentus* (2 sites) (Table 3). For both species, significant population expansion events were restricted to the northern South Island and southern/western North Island (SAMOVA Group 1 for both species), with the exception of *Z. subcarinatus* site 1 ($F_S = -1.964$, $P = 0.011$; $R_2 = 0.153$, $P = 0.035$).

Discussion

Genetic structure throughout New Zealand

Both species displayed strong genetic structure throughout their distributions with extremely similar Φ_{ST} values (0.661

Table 2 Analysis of molecular variance for SAMOVA groupings for two species of the snail genus *Zeacumantus* from 38 localities in New Zealand

Species and geographic analysis	Source of variation	*df*	Variance components	Percent of variation	Fixation indices	*P* value
Z. subcarinatus						
All sites ungrouped	Among sites	31	1.438	66.07	$\Phi_{ST} = 0.661$	<0.0001
	Within sites	519	0.739	33.93		
	Total	550	2.177	100.00		
3 population groups	Among groups	2	3.082	74.01	$\Phi_{CT} = 0.740$	<0.0001
	Among sites within groups	29	0.344	8.25	$\Phi_{SC} = 0.318$	<0.0001
	Within sites	519	0.739	17.74		
	Total	550	4.165	100.00	$\Phi_{ST} = 0.823$	<0.0001
Z. lutulentus						
All sites ungrouped	Among sites	15	0.291	63.94	$\Phi_{ST} = 0.639$	<0.0001
	Within sites	157	0.164	36.06		
	Total		0.455	100.00		
4 population groups	Among groups	3	0.632	79.08	$\Phi_{CT} = 0.791$	<0.0001
	Among sites within groups	12	0.003	0.39	$\Phi_{SC} = 0.018$	0.0070
	Within sites	157	0.164	20.53		
	Total	172	0.799	100.00	$\Phi_{ST} = 0.795$	<0.0001

df degrees of freedom

and 0.639 for *Zeacumantus subcarinatus* and *Zeacumantus lutulentus*, respectively). These results are not surprising for *Z. subcarinatus*, given that it utilizes direct development (Pilkington 1974; Fredensborg and Poulin 2006). For *Z. lutulentus*, our findings provide genetic evidence that this species likely utilizes direct development as even short planktonic dispersal abilities will decrease population structure among marine populations (Weersing and Toonen 2009). While phylogenetic affinity and egg string morphology suggest that both species possess similar dispersal capabilities, this could not be assumed since congeneric marine gastropods can vary in their developmental strategies and dispersal capabilities (Johannesson 1988; Furota et al. 2002; Miura et al. 2011a). Both species often displayed limited dispersal as even relatively proximate sites were often genetically distinct (e.g., site 7 vs. 8 $\Phi_{ST} = 0.920$, $P < 0.0001$, and site 3 vs. 4 $\Phi_{ST} = 0.298$, $P = 0.0090$ for *Z. lutulentus* and numerous comparisons among sites 29–37 for *Z. subcarinatus*). The accumulation of genetic differences among proximate sites could be facilitated by genetic drift/founder effects as many sites possessed a single haplotype, indicating a lack of separate colonizations by genetically distinct individuals and insufficient time for mutations to accumulate (Keeney et al. 2009). Low genetic diversity of founding individuals can rapidly differentiate gastropod populations, and direct development can assist in the establishment of successful populations from small founding numbers as potential mates will not disperse (Knight et al. 1987; Johannesson 1988; Hellberg 2009).

While isolation by distance characterized the overall genetic differentiation within both *Z. subcarinatus* and *Z. lutulentus*, the pattern of increasing genetic differentiation with geographic distance was inconsistent within either species as some of the most distant populations were genetically similar and relatively proximate populations were differentiated. This was especially true for *Z. subcarinatus* in which populations separated by up to 2300 km were genetically similar (see site 1 vs. sites 30–32, see Appendix S2b), while some sites separated by <500 km were genetically distinct (Fig. 2a). Marine organisms can lack clear geographic patterns of genetic differentiation from larval cohorts remaining together and/or temporal genetic variation within sites producing "chaotic genetic patchiness" (Johnson and Black 1982; Arnaud-Haond et al. 2008; Hellberg 2009; Selkoe et al. 2010). The previously mentioned founder events and low postcolonization dispersal could create geographic patchiness in the extent of genetic differentiation among proximate *Zeacumantus* populations. In addition, some *Z. subcarinatus* populations may be temporally unstable as the relatively recent invader maintains small, ephemeral populations in Sydney, Australia, with successful populations occasionally reaching large sizes (Andrews et al. 2010). While we did not quantify snail densities, both species were typically common in our New Zealand sites, suggesting relatively large, stable populations.

Shared haplotypes were previously detected over the southern half of the South Island for *Z. subcarinatus* (Keeney et al. 2009), and the present study greatly expands these findings as haplotypes were shared over the entire length of New Zealand. For example, the same haplotype was recovered from site 1 on the North Island and site 38 on the South Island (see Appendix S1a), suggesting sufficient time has not occurred for haplotypes to diverge between these distant areas. While shared haplotypes can reflect remnants of a once widespread lineage, dispersal is likely given the genetic differences detected among many populations throughout New Zealand. Marine gastropods can disperse by a diversity of mechanisms, including floating (Anderson 1971; Adachi and Wada 1999), rafting (Highsmith 1985; Helmuth et al. 1994; Donald et al. 2005, 2011), via migratory birds (Wilke and Davis 2000; Miura et al. 2011b), and shipping (Miura et al. 2006). These mechanisms can overcome the inherent limited dispersal abilities of gastropods, and direct development may actually assist in the establishment of populations after rare, long-distance dispersal events, allowing them to be found over wide geographic ranges (e.g., Johannesson 1988; Parker and Tunnicliffe 1994) and decreasing isolation by distance patterns (Colson and Hughes 2007). As previously mentioned, one reason for this is that potential mates will remain in close proximity, especially if drifting egg masses are dispersed (Johannesson 1988; Hellberg 2009).

Both *Zeacumantus* species have been observed entwining their egg coils around *Corallina* algal tufts (Morton and Miller 1968). Rafting of adult *Z. subcarinatus* or their eggs on detached algae or other flotsam has been proposed as a possible dispersal mechanism that has enabled *Z. subcarinatus* to become established in the four major estuarine systems in the Sydney region of Australia since its invasion (Andrews et al. 2010). Shipping may also have been an important dispersal means for *Zeacumantus* species throughout New Zealand as it is the proposed mechanism that allowed *Z. subcarinatus* to spread from New Zealand to eastern Australia (Powell 1979). As haplotypes are shared among northernmost and southernmost populations, shipping may be likely as other passive dispersal mechanisms, such as rafting, would go against prevailing currents for at least half the distance in either direction (Sponer and Roy 2002).

Isolation of northern South Island and southern/western North Island populations

Populations of both species along the northern South Island and southern/western North Island (sites 9–23) were

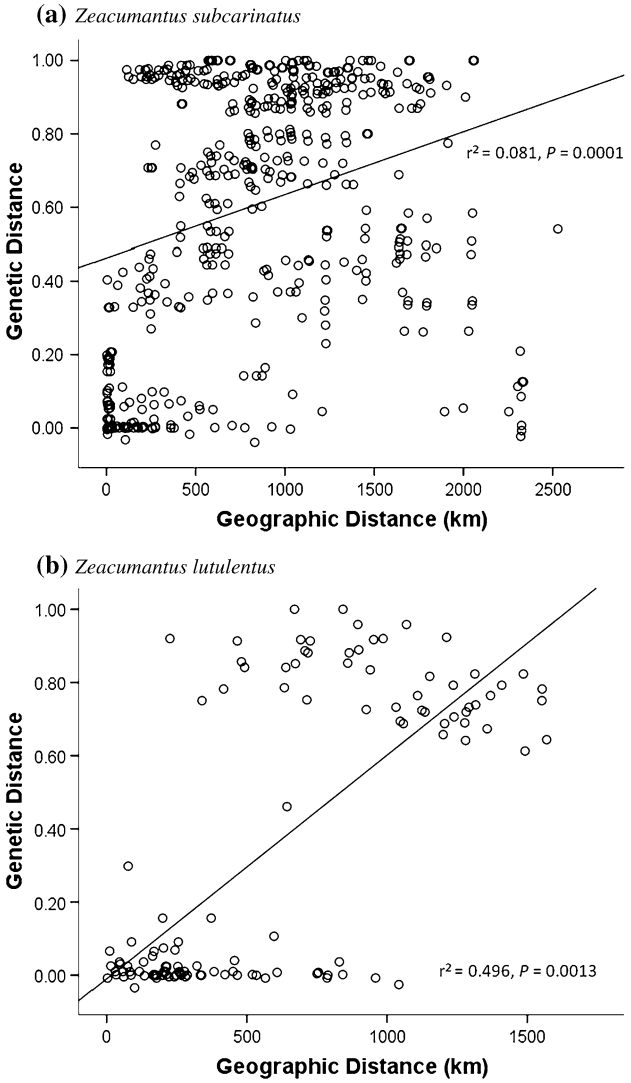

Fig. 2 Relationships between pairwise genetic distances (Φ_{ST}) and geographic (coastal) distances for all populations of two *Zeacumantus* species collected in New Zealand

genetically similar and distinct from all remaining populations with haplotype networks and demographic analyses indicating population expansions in these areas. The North and South Islands have been connected in this region to various degrees during Pleistocene glaciation cycles, including the formation of a land bridge between them as recent as ~18,000 years ago (Lewis et al. 1994; Trewick and Bland 2012). These periodic land connections could have served two functions consistent with our observed data: (1) increased connectivity of populations now located along the northern South Island and southern/western North Island allowing for range expansions/shifts and (2) separated populations along eastern and western coasts of both islands. While the estimates of COI lineage divergence for both species predate the most recent land connection, they are consistent with Pleistocene isolations,

Table 3 Demographic parameters for two species of the snail genus *Zeacumantus* in New Zealand

Site	*Z. subcarinatus*		*Z. lutulentus*	
	F_S/P	R_2/P	F_S/P	R_2/P
1	−1.964/0.011	0.153/0.035	–	–
2	–	–	–	–
3	–	–	1.096/0.835	0.278/0.799
4	−0.339/0.313	0.300/0.698	−0.614/0.149	0.198/0.461
5	–	–	–	–
6	12.654/1.000	0.247/1.000	–	–
7	−1.250/0.140	0.143/0.232	−0.476/0.326	0.276/0.710
8	4.877/0.979	0.242/0.965	–	–
9	−1.596/0.022	0.215/0.538	−0.339/0.385	0.300/0.691
10	−0.380/0.205	0.222/0.495	−0.476/0.317	0.276/0.699
11	−2.310/0.007	0.131/0.027	−1.345/0.048	0.166/0.123
12	–	–	–	–
13	−1.164/0.055	0.200/0.342	–	–
14	0.206/0.500	0.215/0.601	–	–
15	−0.410/0.352	0.288/1.000	−0.339/0.355	0.300/0.693
16	–	–	−1.590/0.029	0.134/0.020
17	–	–	−1.325/0.042	0.186/0.324
18	−1.964/0.012	0.153/0.031	–	–
19	–	–	−1.164/0.053	0.200/0.315
20	–	–	−2.042/0.008	0.149/0.054
21	−1.246/0.045	0.193/0.291	–	–
22	−1.832/0.016	0.161/0.033	−1.164/0.058	0.200/0.307
23	0.477/0.667	0.194/0.116	0.477/0.647	0.194/0.110
24	–	–	–	–
25	–	–	–	–
26	–	–	–	–
27	–	–	–	–
28	−1.530/0.087	0.086/0.097	–	–
29	8.422/0.995	0.185/0.942	–	–
30	1.949/0.892	0.117/0.397	–	–
31	2.668/0.912	0.089/0.169	–	–
32	1.378/0.821	0.128/0.480	–	–
33	–	–	–	–
34	–	–	–	–
35	2.113/0.836	0.148/0.793	–	–
36	3.866/0.958	0.146/0.719	–	–
37	–	–	–	–
38	−1.093/0.226	0.097/0.193	–	–

Values for Fu's F_S and Ramos-Onsins and Rozas R_2 test statistics are shown above their corresponding P values for each sample site. Sample site numbering follows Table 1

For Fu's F_S test, P values <0.020 are significant (Fu 1997)

with isolation occurring earlier in *Z. subcarinatus*. As no haplotypes were shared, these populations have remained isolated from the remainder of sampled areas; including *Z. subcarinatus* populations sampled just outside of this

region (site 24 for example). Interestingly, this sharp genetic disjunction coincides with a coastal upwelling zone south of the Cook Strait between the North and South Islands at approximately latitude 42°S (Bowman et al. 1983) that has been implicated in decreasing gene flow to various degrees in marine organisms (Apte and Gardner 2002; Sponer and Roy 2002; Star et al. 2003; Waters and Roy 2004; Ayers and Waters 2005). The upwelled water may deter juvenile and adult *Zeacumantus* dispersal and also disrupt the means by which these snails are dispersed over long distances, such as rafting or floating.

Further isolation of these populations could be facilitated by strong upwelling at the western tip of the South Island (Harris 1990; Apte and Gardner 2002) and lack of suitable estuarine habitat along the western coast of the South Island (Donald et al. 2011), which is characterized by exposed shoreline, potentially prohibiting colonization by *Zeacumantus*. Historically, neither species has been identified along this coast (Powell 1979). For this study, ten sites were visited along the western coast of the South Island from Jackson Bay (~S43°58′24″, E168°36′52″) to Karamea (~S41°14′52″, E172°6′22″) without encountering either *Zeacumantus* species. Both species also demonstrated isolation of west versus east North Island populations, although sampling on the west was limited, indicating that there is a biogeographic barrier to gene flow in the northern tip of the North Island. While our sampling did not extend into the northernmost area of the North Island, we have identified a disjunction between sites 10 and 1 for *Z. subcarinatus* and between sites 10 and 3 for *Z. lutulentus*. Although speculative for direct-developing species, the southern flowing West and East Auckland currents may facilitate the separation of the northernmost populations as they flow along either side of the northern North Island. This genetic disjunction was not detected in the highly dispersive gastropods *Nerita atramentosa*, *Austrolittorina antipodum* and *Scutus breviculus* (Waters et al. 2005, 2007), but significant genetic differentiation was detected between eastern and western North Island populations of sea stars, potentially resulting from these current patterns (Waters and Roy 2004).

Given that a single mitochondrial gene was utilized, we cannot rule out the possibility of genetic incompatibilities maintaining the degree of genetic differentiation observed among the major *Zeacumantus* groups, especially for *Z. subcarinatus*. Genetic incompatibilities among genomes can produce hybrid inviability or sterility and are a post-zygotic isolation mechanism responsible for maintaining species integrity (Dobzhansky 1937; Presgraves 2010). Within species, asymmetrical fertility arising from incompatibilities can influence observed genetic patterns among diverged populations after secondary contact (Hale and Hoffmann 1990). Inviability can arise from mutations influencing the interaction between nuclear and mitochondrial genomes, prohibiting the introgression of mitochondria into a population (Hale and Hoffmann 1990; Ellison and Burton 2008; Lee et al. 2008). If genetic incompatibilities arose in the mitochondria of isolated *Z. subcarinatus* or *Z. lutulentus* populations, later genetic exchange would not be detected among their populations as individuals receiving the divergent mtDNA would not persist.

Additional phylogeographic patterns

Compared to other regions, northeastern South Island *Z. subcarinatus* sites (24–27) lacked genetic diversity, with only a single haplotype (haplotype 12) recovered (Table 1, Appendix S1a). These sites were exposed rocky areas separated by sandy shoreline which dominates this region. Ayers and Waters (2005) detected low haplotype diversity in this region for the sea star *Patiriella regularis* and proposed that its location at the boundary between the D'Urville and Southland currents may have facilitated local recruitment of pelagic larvae. While currents may serve to isolate populations of *Z. subcarinatus*, it is also likely that the lack of large estuaries in this region has resulted in the patchy distribution of relatively small, genetically impoverished populations. Much of this area also experienced heavy Pleistocene glaciations and erosion, and species with limited dispersal abilities may be less able to colonize and diversify in these areas (see Wallis and Trewick 2009). The haplotype found at these sites was dominant in McCormacks Bay in the Avon-Heathcote Estuary outside site 28, with 26 out of 30 snails possessing it. This is a large estuary system and *Z. subcarinatus* attains densities of up to 18,000 per m^2 in McCormacks Bay (Jones and Marsden 2005). It is likely that snails from this estuary colonized regions just north of it. This haplotype was rarely encountered elsewhere, with four snails from site 7 on the North Island and one from site 38 possessing it (see Appendix S1a), potentially revealing additional long-distance dispersal.

While our expectation of divergent *Z. subcarinatus* haplotypes was met, the expectation of their consistent geographic isolation was not. Several populations consisted of snails possessing highly divergent haplotypes, suggesting secondary admixture of previously isolated lineages. On the South Island, this pattern was previously identified in Otago Harbour (sites 29–37) (Keeney et al. 2009) and increased sample coverage has not significantly altered those findings, as intermediate haplotypes were not detected. Five divergent haplotypes that were restricted to sites 29–37 (haplotypes 4–8) showed closer relatedness to haplotypes from the isolated northern South Island and southern/western North Island region than to any other

haplotypes in New Zealand, but were still relatively divergent from these (Fig. 1a). These haplotypes continued to show a closer genetic association with a *Z. subcarinatus* haplotype in Sydney, Australia (GenBank Accession # AY296834; Colgan et al. 2000, 2003), which differs by 1–2 out of 864 nucleotides (Keeney et al. 2009), despite the larger geographic coverage of the present study. We were not able to include additional Australian *Z. subcarinatus* in the present study.

On the North Island, snails in site 8 possessed either the dominant haplotype in sites 29-37 (haplotype 1), a related haplotype (23), or the divergent haplotype 16 which was also related to those restricted to sites 29–37, suggesting insufficient time for haplotype divergence since genetic exchange between these areas. The only additional North Island site possessing haplotype 1 was site 1. This haplotype was common there (7 out of 10 snails), and all remaining snails possessed unique haplotypes, a single mutation different from haplotype 1. This suggests colonization by individuals possessing the common South Island haplotype and subsequent population expansion and production of new haplotypes, a scenario supported by demographic analyses.

Site 6 included one group of snails possessing haplotype 10, a widespread haplotype in the North Island also dominant in site 38, and its closely related haplotypes which were restricted to site 6. Seven additional snails possessed the extremely divergent haplotype 47. This haplotype did not show affinities to any lineage identified throughout New Zealand, differing equally from Group 1 and Group 2 haplotypes by $\geq$30 mutations (model-corrected minimum sequence divergence $\sim$4.5 %), suggesting an estimated divergence time from all other *Z. subcarinatus* haplotypes of 1,330,500 years ago (95 % HPD = 886,700–1,823,800 years ago) or revealing exceptionally rapid diversification of COI in this lineage. While its lack of association to other haplotypes prevents identification of the possible source of the divergent haplotype, it may have diverged during earlier Pleistocene sea level oscillations or evolved on offshore islands as these have served as sources of introgression of divergent haplotypes into mainland populations (Wallis and Trewick 2009).

Conclusion

Due to its isolation, geologic history and high endemism, many population genetic and phylogeographic studies have been conducted in New Zealand. However, while several genetic patterns have been identified, general patterns have been elusive with resolution requiring data from additional species (Wallis and Trewick 2009). In the present study, we detected a consistent phylogeographic disjunction for both species, with increased divergence of *Z. subcarinatus* lineages. Both species possessed strong genetic structure throughout New Zealand with evidence of long-distance dispersal and secondary admixture, especially for the more wide-spread *Z. subcarinatus*. Our results highlight the complexity of the historical and contemporary factors influencing the genetic structure and geographic lineage distributions of marine organisms.

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