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SHORT NOTE

Connectivity in solitary ascidians: Is a 24-h propagule duration sufficient to maintain large-scale genetic homogeneity?

Peter R. Teske

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Abstract Ascidians are considered to have lower dispersal potential than most other sessile marine invertebrates with planktonic propagules by virtue of a very brief propagule duration. The larvae of colonial forms remain in the water column for only a few minutes, whereas most solitary forms settle in less than 24 h. This difference in propagule duration has been used to explain why allozyme data from colonial ascidians on the Australian east coast were genetically distinct at different sampling sites, whereas a solitary species exhibited no genetic structure. Spatial homogeneity in solitary species is surprising because genetic structure of species with much higher dispersal potential can be characterised by isolation by geographic distance, suggesting that these disperse by means of a stepping-stone pattern of dispersal. I reassessed the dispersal potential of solitary ascidians using DNA sequence data from the mitochondrial cytochrome oxidase subunit 1 gene and the intron of the nuclear adenine nucleotide transporter gene of a common south-eastern Australian solitary ascidian, *Pyura praeputialis*, using samples that span the species' distribution range. Congruent with earlier findings, there was no evidence for stepping-stone dispersal, but it must be conceded that these results could be strongly affected by frequent adult dispersal, particularly by means of anthropogenic vectors, as well as insufficient marker resolution.

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P. R. Teske (✉)
Department of Zoology, University of Johannesburg,
Auckland Park 2006, Johannesburg, South Africa
e-mail: pteske101@gmail.com

Introduction

Assessing genetic connectivity of marine organisms over their distribution ranges is important for the sustainable management of exploited species, identification of management units for conservation, and the design of marine reserves (Stobutzki 2000; Shanks et al. 2003). In many sessile or sedentary organisms whose life history includes free-swimming propagules such as gametes or larvae, connectivity is considered to depend primarily on aspects that affect propagule dispersal, including time spent in the plankton (propagule duration, PD), larval behaviour, and the direction and velocity of ocean currents (Ayre et al. 1997; Bell and Okamura 2005). The idea that PD is a good predictor of connectivity, and that species with long PD have high levels of connectivity that manifest themselves in low levels of genetic structure (Siegel et al. 2003; Kinlan et al. 2005), is largely discredited (Weersing and Toonen 2009). Some species with long PD that could potentially disperse over vast distances employ behavioural mechanisms that allow them to recruit close to the parent habitat, making it problematic to predict connectivity on the basis of PD alone (Taylor and Hellberg 2003; Bowen et al. 2006). Nonetheless, in species with direct development, and in those whose propagules settle within a short period of time (<12 h), the prediction that recruitment is mostly local has been confirmed on the grounds of short observed dispersal distances and high levels of genetic structure (Ayre et al. 1997; Teske et al. 2007; Shanks 2009).

Connectivity in species with a slightly longer PD seems to be difficult to predict. This group includes the solitary ascidians (Urochordata:Tunicata:Ascidiacea), whose planktonic tadpole larvae remain in the water column for up to 24 h, although they often settle earlier (Svane and Young 1989; Clark et al. 1999). Their dispersal capabilities can be

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expected to be greater than in social or compound ascidians, which settle within minutes (Svane and Young 1989) and for which dispersal distances of <10 to <200 m have been reported (Bingham and Young 1991; van Duyl et al. 1981). However, it is questionable whether the levels of gene flow remain sufficiently high to counteract the diversifying effects of genetic drift, and maintain large-scale genetic homogeneity, as previously reported on the basis of allozyme data (e.g. Ayre et al. 1997; Nobrega et al. 2004), because direct observations suggest that recruitment can be highly localised (Petersen and Svane 1995). Given that the degree of population structure (estimated using *F*-statistics such as F_{ST} or Φ_{ST}) is generally higher for mitochondrial DNA (mtDNA) than for allozymes (Weersing and Toonen 2009), it is possible that mtDNA sequence data are more suitable to reject the idea that solitary ascidians are panmictic throughout their ranges.

I tested this prediction using sequence data from a mitochondrial gene of the solitary ascidian *Pyura praeputialis* (cunjevoi), a species that is common along the coast of south-eastern Australia, i.e. in a region that includes the sampling area previously studied by Ayre et al. (1997). For comparison, I also generated sequence data from a nuclear intron. The life history of *P. praeputialis* is particularly well understood. The species' non-feeding planktonic larvae usually settle within 2 h and cannot delay settlement by more than 24 h (Clark et al. 1999). In addition, this species uses biofoam to retain gametes and larvae in the vicinity of the adults (Castilla et al. 2007). This suggests that if dispersal was primarily by means of early developmental stages, then *P. praeputialis* is unlikely to maintain high levels of connectivity over large distances, and there should be a positive correlation between genetic differentiation and geographic distance.

Materials and methods

Study species and sampling

Samples of *Pyura praeputialis* were collected during low tide at ten localities (Table 1) that span most the species' distribution range, which comprises southern Queensland, New South Wales and Victoria. This region includes a well-documented biogeographic disjunction near Wilson's Promontory, across which *P. praeputialis* exhibits shallow genetic structure (Teske et al. 2011). To exclude the effects of this barrier on the estimates of genetic connectivity, samples from either side of Wilson's Promontory were analysed separately.

The majority of sequences used in this study were published previously (Teske et al. 2011; Rius and Teske 2011, 2013). These previous studies primarily focused on species delimitations using phylogenetic methods. Although some analyses of genetic structure were performed that identified Wilson's Promontory as a phylogeographic break, geographic cover was insufficient to study genetic connectivity in this species. To improve geographic cover, I generated additional sequences from some sites (shown in brackets in Table 1). A small piece of tissue from the mantle (<1 cm^3) was preserved in 1.5 ml of absolute ethanol, which was replaced on a daily basis until it no longer changed colour and until the tissue had become completely white. DNA was then extracted using a salting-out protocol (Sunnucks and Hale 1996).

The cytochrome oxidase subunit 1 gene (*COI*) was amplified using primers developed for the genus *Pyura* (PyCOI-F 5′-GAA TTG TCT CAA GTA RGG CAG GT-3′ and PyCOI-R, 5′-GAC CCY AGC TAA ATG CAA AG-3′; Rius and Teske 2013). This primer combination amplifies

Table 1 Sampling sites of *Pyura praeputialis* in eastern and south-eastern Australia, number of mitochondrial *COI* gene and nuclear *ANT* intron sequences generated, F_{IS} values per site (*ANT* data) and *P* values indicating the proportion of randomisations that had a larger (P_L) or smaller (P_S) F_{IS} value than the one observed

Region	Site name	Site no.	GPS coordinates	*COI*	*ANT*	F_{IS}	P_L	P_S
East of Wilson's promontory	Fingal Head	1	28°11′S 153°34′E	20	38	0.18	0.10	0.97
	Port Macquarie	2	31°25′S 152°55′E	21	38	0.46	<0.01	1.00
	Black Head	3	32°04′S 152°32′E	20	42	−0.08	0.86	0.39
	Newcastle	4	32°55′S 151°47′E	16 (16)	24 (24)	0.24	0.18	0.97
	Mallacoota	5	37°34′S 149°45′E	18	24	−0.01	0.72	0.73
	Cape Conran	6	37°48′S 148°43′E	22	28	0.23	0.25	0.96
West of Wilson's promontory	Kilcunda	7	38°33′S 145°28′E	30	30	−0.07	0.85	0.48
	Cowes	8	38°26′S 145°14′E	21 (13)	40 (24)	0.20	0.07	0.99
	Portsea	9	38°19′S 144°42′E	16	24 (10)	−0.28	0.99	0.12
	Marengo Bay	10	38°46′S 143°39′E	13 (2)	18 (2)	0.14	0.44	0.91
			Total	197	306			

Number of newly generated sequences is shown in brackets

a PCR product that is considerably shorter than that amplified with the universal primers used previously (e.g. Teske et al. 2011). However, it amplifies much more reliably and for that reason was more suitable to generate data from a large number of individuals, which is crucial for analyses that are based on allele frequencies. Given that the amplified product in *P. praeputialis* contains a comparatively large number of variable sites (see Results), I considered this approach preferable to generate longer DNA sequences from fewer individuals. The adenine nucleotide transporter gene (*ANT*) was amplified using an intron-spanning primer combination of a forward primer designed for ascidians of the Order Stolidobranchia (Stolido-ANTf: 5′-CAG GGT ATC ATT GTR TAC MGA G-3′) (Teske et al. 2011) in conjunction with universal reverse primer ANTr1 (5′-CCA GAC TGC ATC ATC ATK CGR CGD C-3′) (Jarman et al. 2002). PCRs were performed in 20-µl reaction volumes that contained 3 µl of 10× reaction buffer (Promega), 1.5 mM of $MgCl_2$, 6 µl of dNTP mixture containing 125 mM of each dNTP, 0.3 µl of each primer (10 mM dilutions), 1 U of Taq DNA polymerase (Promega) and 5 µl of DNA template. PCRs consisted of an initial denaturation step (94 °C for 3 min), 35 cycles of denaturation (94 °C for 30 s), annealing (55 °C for 45 s) and extension (72 °C for 1 min), followed by a final extension step at 72 °C for 20 min. PCR products were purified using the Qiaquick kit (Qiagen), sequenced in both directions using Big Dye terminator version 3.1 (Applied Biosystems) and run on a 3130xl Genetic Analyser (Applied Biosystems) according to the manufacturer's instructions. *COI* and *ANT* sequences were aligned by eye and trimmed to a consensus sequence length of 208 bp (*COI*) and 131 bp (*ANT*). *ANT* sequences were phased with the PHASE algorithm (Stephens et al. 2001; Stephens and Donelly 2003) in DnaSP version 5 (Librado and Rozas 2009) using default parameters. The *ANT* sequences contained no length differences, and only two polymorphic sites contained more than two variant bases (in both cases, a third base was identified in a single individual).

Genealogical relationships between alleles were presented by constructing median-joining haplotype networks in Network 4516 (2009 version) for each locus (Bandelt et al. 1999). The inbreeding coefficient F_{IS} (Weir and Cockerham 1984) of the *ANT* sequences from each site, and its departure from the expectations of random mating (based on the proportion of 4,600 randomisations that produced either larger or smaller values of F_{IS} than the ones observed), was estimated using FSTAT version 2.9.3.2 (Goudet 2001). Genetic structure was assessed by performing an analysis of molecular variance (AMOVA; Excoffier et al. 1992), which estimates the hierarchical distribution of variation (e.g. among sites and among individuals within sites), and by calculating F_{ST} values between pairs of sampling sites. These statistics were calculated in Arlequin version 3.5.1.2 (Excoffier and Lischer 2010) for the *COI* data and in Genalex version 6.5 (Peakall and Smouse 2012) for the *ANT* data. Tests for significant departures from the assumption of genetic homogeneity were based on 10,000 permutations. The program Genalex was also used to test for any effect of geographic distance on genetic structure that would indicate that regional populations of *P. praeputialis* are not panmictic. First, I performed simple Mantel tests (Mantel 1967) to test for correlations between *F*-statistics among pairs of individuals, and the corresponding geographic distances, specifying 999 random permutations to test for significance. Second, to assess the importance of local recruitment, I calculated mean pairwise relatedness among the samples from individual sites using the relatedness coefficient *r* (Queller and Goodnight 1989) in Genalex. Sequences from all sites within a particular region were permuted 999 times to obtain 95 % confidence intervals for the null distribution under conditions of panmixia. Values of *r* that are significantly greater than the upper confidence bound support the hypothesis that recruitment is mostly local.

Results and discussion

The *COI* sequences had 34 variable sites and *ANT* sequences 24. All samples of *P. praeputialis* in the sampling area were part of the same evolutionary lineage, and no distinct regional clusters were identified using median-joining haplotype networks (Fig. 1).

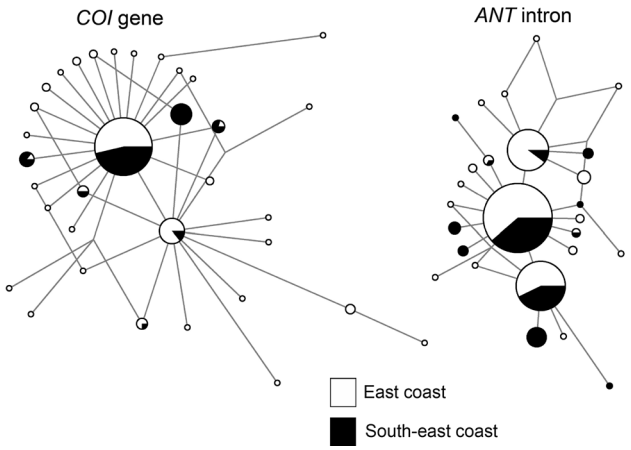

Fig. 1 Median-joining haplotype networks reconstructed using DNA sequence data of the *COI* gene and *ANT* intron of *Pyura praeputialis* from the Australian east and south-east coasts. Each circle represents a distinct haplotype, and the size of each haplotype is proportional to its frequency. The smallest circles represent one individual, and the shortest connections between haplotypes correspond to one nucleotide substitution

A heterozygote deficit was only found for the *ANT* data from site 2 ($P_L = 0.0004$, Table 1), and there was no heterozygote excess at any of the 10 sites. AMOVA analyses did not identify genetic structure among sites in eastern (*COI*: $F_{ST} = 0.003$, $P = 0.356$; *ANT*: $F_{ST} = -0.003$, $P = 0.541$) and south-eastern (*COI*: $F_{ST} = 0.029$, $P = 0.120$; *ANT*: $F_{ST} = 0.011$, $P = 0.230$) Australia. However, significant pairwise F_{ST} values were found between some individual sites (Table 2).

Mantel tests for correlations between matrices of genetic versus geographic distances were non-significant on the east coast (*COI*: $Rxy = 0.025$, $P = 0.234$; *ANT*: $Rxy = -0.060$, $P = 0.099$). On the south-east coast, no significant correlation was found for the *ANT* data ($Rxy = 0.002$, $P = 0.435$), while the *COI* data showed a negative correlation between genetic and geographic distance ($Rxy = -0.109$, $P < 0.01$). Mean pairwise relatedness estimates within sites indicated that in most cases, individuals from the same site were not more closely related to each other than they were to the individuals from other sites within a particular region. Exceptions were site 2 (*ANT* data, Fig. 2), whose individuals were more closely related to each other than individuals from other site on the basis of a significant positive relatedness coefficient r ($P < 0.05$), and site 10 (*COI* data, Fig. 2), whose individuals were less closely related ($P < 0.01$).

Even though *P. praeputialis* pursues a strategy of larval retention, using biofoam to prevent the dispersal of propagules away from the parent habitat, I found no genetic evidence that this limits connectivity throughout the environmentally continuous coastlines of eastern and south-eastern Australia. Regional F_{ST} values were not significant (although some pairwise values were), a departure from random mating based on the inbreeding coefficient F_{IS} was only found in a single population, there were no positive correlations between genetic differentiation and geographic distance, and there was little evidence that individuals from the same site were more closely related to each other than they were to individuals from different sites.

These results are surprising given that species with much higher theoretical dispersal potential can be characterised by a pattern of isolation by distance (e.g. Pogson et al. 2001; Coleman et al. 2011; Crandall et al. 2012). While the results of the present study are congruent with the findings of earlier allozyme-based studies, there are two reasons why I consider them to be tentative, and insufficient to state conclusively that the PD of solitary ascidians is sufficient

Table 2 Pairwise F_{ST} values for *Pyura praeputialis* among sampling sites in south-eastern Australia

Site	1	2	3	4	5	6
COI						
1		0.473	0.908	0.647	0.880	0.187
2	0.000		0.218	0.168	0.375	0.034*
3	0.000	0.013		0.589	0.780	0.483
4	0.000	0.026	0.020		0.505	0.264
5	0.000	0.000	0.000	0.000		0.274
6	0.013	0.073	0.000	0.012	0.007	
	7	8	9	10		
7		0.668	0.568	0.045*		
8	0.000		0.709	0.012*		
9	0.000	0.000		0.110		
10	0.108	0.167	0.077			
ANT						
	1	2	3	4	5	6
1		0.350	0.326	0.374	0.363	0.356
2	0.000		0.374	0.383	0.365	0.399
3	0.004	0.000		0.348	0.048*	0.033*
4	0.000	0.000	0.000		0.337	0.346
5	0.000	0.000	0.054	0.000		0.403
6	0.000	0.000	0.052	0.000	0.000	
	7	8	9	10		
7		0.220	0.379	0.343		
8	0.008		0.036*	0.364		
9	0.000	0.064		0.372		
10	0.000	0.000	0.000			

Sites east (1–6) and west (7–10) of Wilson's Promontory were analysed separately

Site numbers correspond to those in Table 1 and Fig. 1. F_{ST} values shown are below the diagonal, and P values (the probability that random F_{ST} is greater than observed F_{ST}) are above the diagonal (* $P < 0.05$, ** $P < 0.01$). Negative F_{ST} values were converted to zero

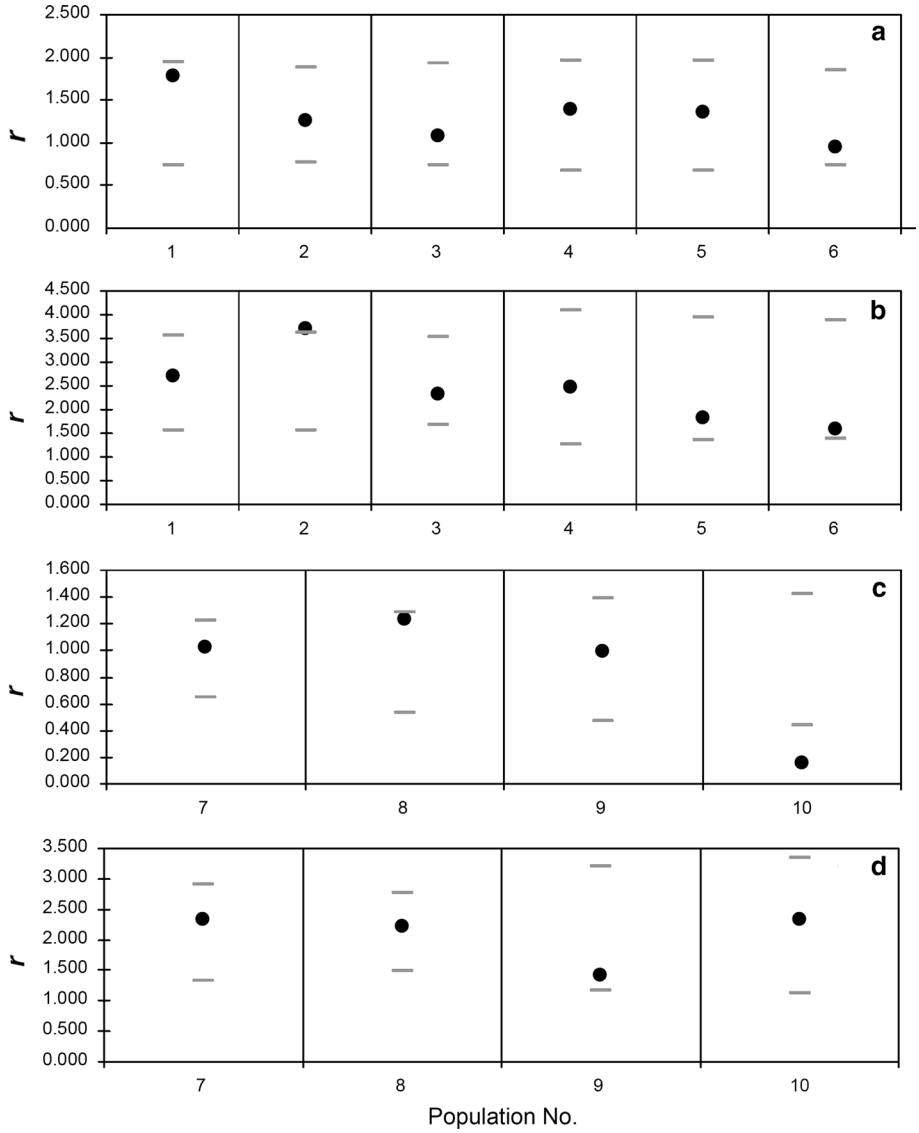

Fig. 2 Mean within-population pairwise relatedness at ten sites at which *Pyura praeputialis* was sampled. *Black circles* represent the relatedness statistic *r*, and *grey bars* are 95 % confidence intervals beyond which the expectations for panmixia are rejected; **a** *COI* data, east coast; **b** *ANT* data, east coast; **c** *COI* data, south-east coast; **d** *ANT* data, south-east coast

to maintain conditions of genetic homogeneity over large geographic ranges. These are discussed below.

Adult dispersal

The dispersal of adults in sessile or sedentary marine invertebrates with planktonic propagules is considered to be rare (Shanks 2009) and thus of minor importance in maintaining connectivity among populations. However, in ascidians, it cannot be ignored. The larvae of solitary ascidians may attach themselves to floating objects that serve as rafts (Worcester 1994), and these may facilitate dispersal at a larger geographic scale than what is expected on the basis of assumptions about the dispersal capabilities of their early life history stages alone (Grosberg 1991; Bell and Okamura 2005). Adult dispersal may be infrequent under natural conditions, but ascidians often disperse by means of anthropogenic vectors, and numerous species are important marine invaders (Lambert 2007). Human-mediated transport could have completely eroded a natural pattern of isolation by distance in *P. praeputialis*, suggesting that ascidians are unsuitable as model organisms for studying the effects of short PD on connectivity.

Insufficient marker resolution

A prerequisite for identifying genetic structure by means of neutral genetic markers is that populations have reached mutation-drift equilibrium. Given the clear signature of a demographic expansion in both the *COI* and *ANT* data sets by virtue of star phylogenies, it is possible that both markers retain ancestral polymorphism, and that regional diversification may not yet be reflected. MtDNA is often considered to be more sensitive to detect genetic structure than

nuclear markers because of its smaller effective population size and correspondingly faster genetic drift (Bentzen 1998), suggesting that it should be more informative than allozymes or nuclear introns. In a meta-analysis examining the relationship between PD and connectivity, Weersing and Toonen (2009) found that microsatellites, which are much more polymorphic than mtDNA, were no more informative to detect genetic structure than were allozymes. However, it was considered possible that this is an artefact of most studies not standardising F_{ST} to account for marker heterozygosity (Hedrick 2005), and the use of polymorphic microsatellites may be required to settle the issue whether or not a 24-h PD is sufficient to maintain genetic homogeneity.

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References

Ayre DJ, Davis AR, Billingham M, Llorens T, Styan C (1997) Genetic evidence for contrasting patterns of dispersal in solitary and colonial ascidians. Mar Biol 130:51–61

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Bell JJ, Okamura B (2005) Low genetic diversity in marine nature reserve: re-evaluating diversity criteria in reserve design. Proc R Soc Lond B Biol Sci 272:1067–1074

Bentzen P (1998) Seeking evidence of local stock structure using molecular genetic methods. In: Hunt von Herbing I, Kornfield I, Tupper M, Wilson J (eds) The implications of localized fishery stocks. Regional Agricultural Engineering Service, New York, pp 20–30

Bingham RL, Young CM (1991) Larval behaviour of the ascidian Ecteinascidia turbinata Herdman: an in situ experimental study of the effects of swimming on dispersal. J Exp Mar Biol Ecol 145:189–204

Bowen BW, Bass AL, Muss A, Carlin J, Robertson DR (2006) Phylogeography of two Atlantic squirrel fishes (family Holocentridae): exploring links between pelagic larval duration and population connectivity. Mar Biol 149:899–913

Castilla JC, Manríquez PH, Delgado AP, Gargallo L, Leiva A, Radic D (2007) Bio-foam enhances larval retention in a free-spawning marine tunicate. Proc Natl Acad Sci USA 104:18120–18122

Clark M, Ortiz V, Castilla JC (1999) Does early development of the Chilean tunicate *Pyura praeputialis* (Heller, 1878) explain the restricted distribution of the species? Bull Mar Sci 65:745–754

Coleman MA, Roughan M, Macdonald HS, Connell SD, Gillanders BM, Kelaher BP, Steinberg PD (2011) Variation in the strength of continental boundary currents determines continent-wide connectivity in kelp. J Ecol 99:1026–1032

Crandall EC, Treml EA, Barber PH (2012) Coalescent and biophysical models of stepping-stone gene flow in neritid snails. Mol Ecol 21:5579–5598

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetic analyses under Linux and Windows. Mol Ecol Resour 10:564–567

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). http://www.unil.ch/izea/softwares/fstat.html

Grosberg RK (1991) Sperm-mediated gene flow and the genetic structure of a population of the colonial sea squirt *Botryllus schlosseri*. Evolution 45:130–142

Hedrick PW (2005) A standardized genetic differentiation measure. Evolution 59:1633–1638

Jarman SN, Ward RD, Elliott NG (2002) Oligonucleotide primers for PCR amplification of coelomate introns. Mar Biotech 4:347–355

Kinlan BP, Gaines SD, Lester SE (2005) Propagule dispersal and the scales of marine community process. Divers Distrib 11:139–148

Lambert G (2007) Invasive sea squirts: a growing global problem. J Exp Mar Biol Ecol 342:3–4

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

Nobrega R, Sole-Cava AM, Russo CAM (2004) High genetic homogeneity of an intertidal marine invertebrate along 8000 km of the Atlantic coast of the Americas. J Exp Mar Biol Ecol 303:173–181

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539

Petersen JK, Svane I (1995) Larval dispersal in the ascidian *Ciona intestinalis* (L.). Evidence for a closed population. J Exp Mar Biol Ecol 186:89–102

Pogson GH, Taggart CT, Mesa KA, Boutilier RG (2001) Isolation by distance in the Atlantic cod, Gadus morhua, at large and small geographic scales. Evolution 55:131–146

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258–275

Rius M, Teske PR (2011) A revision of the *Pyura stolonifera* species complex (Tunicata, Ascidiacea), with a description of a new species from Australia. Zootaxa 2754:27–40

Rius M, Teske PR (2013) Cryptic diversity in coastal Australasia: a morphological and mito-nuclear genetic analysis of habitat-forming sibling species. Zool J Linn Soc 168:597–611

Shanks AL (2009) Pelagic larval duration and dispersal distance revisited. Biol Bull 216:373–385

Shanks AL, Grantham BA, Carr MH (2003) Propagule dispersal distance and the size and spacing of marine reserves. Ecol Appl 13:S159–S169

Siegel DA, Kinlan BP, Gaylord B, Gaines SD (2003) Langrangian descriptions of marine larval dispersion. Mar Ecol Prog Ser 260:83–96

Stephens M, Donelly P (2003) A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 73:1162–1169

Stephens M, Smith N, Donelly P (2001) A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68:978–989

Stobutzki IC (2000) Marine reserves and the complexity of larval dispersal. Rev Fish Biol Fisheries 10:515–518

Sunnucks P, Hale DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). Mol Biol Evol 13:510–524

Svane I, Young CM (1989) The ecology and behaviour of ascidian larvae. Oceangr Mar Biol A Rev 27:45–90

Taylor MS, Hellberg ME (2003) Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299:107–109

Teske PR, Papadopoulos I, Zardi GI, McQuaid CD, Edkins MT, Griffiths CL, Barker NP (2007) Implications of life history for genetic structure and migration rates of southern African coastal invertebrates: planktonic, abbreviated and direct development. Mar Biol 152:697–711

Teske PR, Rius M, McQuaid CD, Styan CA, Piggott MP, Benhissoune S, Fuentes-Grunewald C, Walls K, Page M, Attard CRM, Cooke GM, McClusky CF, Banks SC, Barker NP, Beheregaray LB (2011) "Nested" cryptic diversity in a widespread marine ecosystem engineer: a challenge for detecting biological invasions. BMC Evol Biol 11:176

van Duyl FC, Bak RPM, Sybesma J (1981) The ecology of the tropical compound ascidian *Tridemnum solidum*. I. Reproductive strategy and larval behaviour. Mar Ecol Prog Ser 6:35–42

Weersing K, Toonen RJ (2009) Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser 393:1–12

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370

Worcester SE (1994) Adult rafting versus larval swimming: dispersal and recruitment of a botryllid ascidian on eelgrass. Mar Biol 121:309–317