Mar Biol (2015) 162:1319–1327
DOI 10.1007/s00227-015-2671-3

ORIGINAL PAPER

Molecular evidence supports coastal dispersal among estuaries for two benthic marine worm (Nephtyidae) species in southeastern Australia

Laura M. Smith[1] · Pat Hutchings[2] · Ceridwen I. Fraser[1]

Received: 3 December 2014 / Accepted: 20 April 2015 / Published online: 5 May 2015

Abstract Understanding patterns of dispersal of marine organisms among estuaries is important for the conservation of biodiversity and the design of marine park networks. Whereas numerous studies have recently assessed dispersal potential among key marine vertebrates and habitat-forming macroalgae, relatively few have assessed the potential for dispersal in ecologically important benthic polychaete worms. Here, we used phylogeographic analyses to test for evidence of genetic disjunctions among populations of polychaete worms from different estuaries in southeastern Australia. Our study focused on two species from the family Nephtyidae (*Aglaophamus australiensis* and *Nephtys longipes*) that are found intertidally in soft sediments in estuaries. Both species have planktonic larvae, but little is known about the survival times of the larvae, or their potential to disperse to other estuaries rather than settling locally. Genetic analyses of two mitochondrial (cytochrome c oxidase subunit I and 16S rDNA) markers in both species and a nuclear marker (28S rDNA) in *A. australiensis* were carried out to assess whether geographically distinct populations show genetic differences. Little evidence of genetic differentiation among populations was found, despite a high level of genetic diversity within each species. Although some significant population pairwise F_{ST} differences were detected for both species via AMOVA, these appeared largely driven by singleton haplotype diversity, whereas several common haplotypes were shared among all populations. Our results suggest that sedentary, benthic estuarine organisms with planktonic larvae can disperse to distant estuaries with the aid of tidal flushing and coastal ocean currents.

Communicated by C. Riginos.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-015-2671-3) contains supplementary material, which is available to authorized users.

✉ Ceridwen I. Fraser
ceridwen.fraser@gmail.com

[1] Fenner School of Environment and Society, Australian National University, Acton, ACT 2601, Australia

[2] Australian Museum Research Institute, Australian Museum, 6 College Street, Sydney, NSW 2010, Australia

Introduction

Predicting dispersal among marine populations is an important aspect of modelling population dynamics and levels and patterns of genetic diversity (Coleman et al. 2011). Such information is of particular importance for marine ecosystems such as estuaries due to major declines in fishery stocks and rapid degradation of natural coastal habitat (Cowen et al. 2006). Studies measuring metapopulation dynamics and connectivity are common for many terrestrial organisms but are comparatively rare for marine environments (Bradbury et al. 2008a), particularly for benthic invertebrates. Identifying patterns of connectivity in the marine realm can assist in the identification of sources and sinks of larval dispersal (Coleman et al. 2011), and is crucial for optimising the design of effective marine protected areas (MPAs). There are, however, long-standing assumptions about long-distance dispersal ability for species with planktonic larval stages (Bradbury et al. 2008a, b) that can lead to the overestimation of dispersal potential (Cowen and Sponaugle 2009) and can thus misinform the design of protected areas.

Estuaries provide a number of ecosystem services including water treatment such as filtering by suspension

feeders and detoxification by submerged vegetation and wetlands (Barbier et al. 2010). Estuaries are also important nursery habitats for many organisms and a source of fishery stocks (Bradbury et al. 2008a). Estuarine health can suffer from harbour and coastal development, pollution, overfishing and overharvesting, freshwater diversions and dredging (Kennish 2002). Projected sea level rise with a changing climate will cause the structure and composition of estuarine communities to change as species disperse or disappear (Jenkins et al. 2011). Understanding the factors influencing the connectivity of estuarine species—especially those towards the base of the trophic chain—can help in mitigating anthropogenic damage to estuaries by identifying whether specific areas should be protected.

In this study, we used phylogeographic approaches to test for evidence of restricted gene flow in two species of polychaete worms from the family Nephtyidae (*Aglaophamus australiensis* and *Nephtys longipes*) commonly found in estuaries along the coast of New South Wales (NSW), Australia. These species are found only in estuaries and are absent from the NSW open coast. Dispersal of polychaetes can be influenced by abiotic and biotic factors such as ocean currents, larval survival (Sherman et al. 2008) and bathymetry (Piggott et al. 2008; Bors et al. 2012). Polychaetes with planktonic larvae are dependent on environmental factors for dispersal and have been variously found to show both high (Jolly et al. 2004; Barroso et al. 2010) and low (Kesäniemi et al. 2012) levels of connectivity among populations. Although both of our study species have a planktonic larval phase, they also have discontinuous distributions and might not readily disperse among populations, particularly as the predominantly southward flow of the East Australian Current (EAC) is often interrupted by eddies (Coleman et al. 2013) that could isolate some populations (Fig. 1). Estuaries can also represent ecophysiological boundaries to marine organisms due to their somewhat restricted connection to the ocean (Bilton et al. 2002; Kennish 2002); this intermittent connection to the sea could promote population differentiation. We hypothesised that populations of the same species might therefore show strong genetic differences, indicating limited connectivity for these benthic invertebrates among estuaries along the southeastern coast of Australia.

Methods

Sampling

In February and March, 2013 and 2014, specimens of *A. australiensis* and *N. longipes* were collected from estuaries along the coast of NSW, southeastern Australia. For each species, samples were collected from estuaries separated by at least 30 km (and up to 590 km). Samples of *N. longipes* were collected from four estuaries, and *A. australiensis* from ten estuaries (Table 1; Figs. 2 and 3). To allow assessment of fine scale, within-estuary structure, samples of *A. australiensis* were also collected from four sites within the connected Pittwater/Hawkesbury estuaries (Table 1; Fig. 4). Intertidal benthic sediment was collected at low tide using a spade and then gently sieved using a 0.5-mm mesh. All samples were collected towards the mouth of estuaries in full marine salinities. *N. longipes* was generally found closer to the ocean than *A. australiensis*, and only in sites with cleaner sand, whereas *A. australiensis* was found in muddier sand usually associated with *Zostera* seagrasses. *N. longipes* was only found in the four estuaries listed for that species in Table 1. Polychaetes were identified to species level and preserved in 95 % ethanol within approximately an hour of collecting, and alcohol was changed at least twice in the 24 h following collection.

DNA amplification

We sequenced parts of the mitochondrial cytochrome c oxidase subunit I (COI), mitochondrial ribosomal (16S) and nuclear ribosomal (28S) genes. Although more slowly

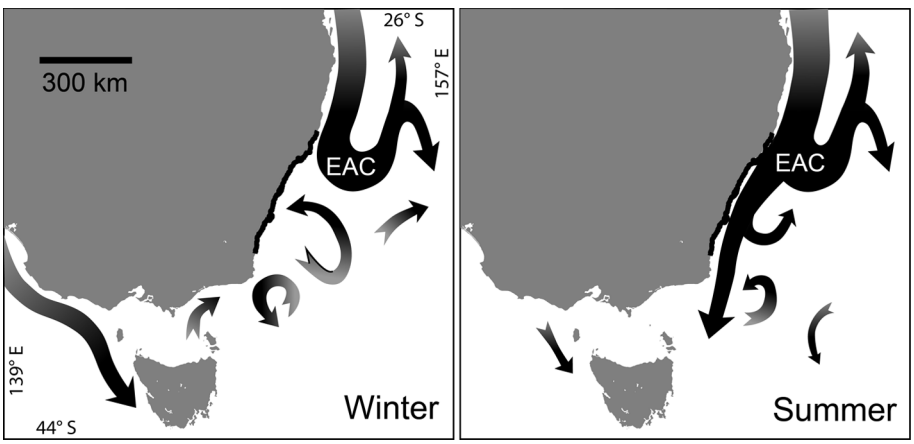

Fig. 1 Schematic of flow directions of major currents in southeastern Australia in winter (*left*) and summer (*right*) (modified and generalised from Figure 1 in Coleman et al. 2013). The EAC is stronger and extends further south in summer, whereas in winter, coastal flows in southern NSW can be dominated by eddies. The spatial extent of sites used in this study is indicated by a *thick black line* along part of the southeastern Australian coast

Table 1 List of field sites with species collected and GPS coordinates, and numbers of individuals sequenced for each marker

Site name	Species	Latitude	Longitude	COI	16S	28S
Wallis Lake	*A. australiensis*	32°10′52.09″S	152°30′4.53″E	20	11	0
Lemon Tree Passage	*A. australiensis*	32°43′51.48″S	152°2′22.27″E	23	16	0
Stockton Bridge	*A. australiensis*	32°52′39.62″S	151°47′36.94″E	21	9	0
Careel Bay, Pittwater	*A. australiensis*	33°37′8.86″S	151°19′38.15″E	26	13	0
Patonga, Hawkesbury	*A. australiensis*	33°33′3.64″S	151°16′5.03″E	24	13	0
Bayview, Pittwater	*A. australiensis*	33°39′39.30″S	151°18′12.23″	14	11	0
Dangar Island, Hawkesbury	*A. australiensis*	33°32′24.93″S	151°18′12.23″	20	12	0
Fig Tree Bridge	*A. australiensis*	33°49′45.03″S	151°8′43.21″E	24	11	0
Taren Point	*A. australiensis*	34°0′39.94″S	151°7′39.35″E	21	9	0
Lake Illawarra entrance	*A. australiensis*	34°32′16.20″S	150°52′3.75″E	7	5	0
Lake Illawarra jetties	*A. australiensis*	34°31′37″S	150°51′53″E	7	6	0
Tuross Heads	*A. australiensis*	36°03′59.08″S	150°07′40.58″E	7	3	1
Wallaga Lake	*A. australiensis*	36°22′12.68″S	150°04′6.14″E	14	6	2
Shadracks Creek	*A. australiensis*	37°04′37.59″S	149°52′39.21″E	25	10	8
Dolls Point	*N. longipes*	34°00′20.89″S	151°07′49.07″E	17	10	0
Lake Illawarra	*N. longipes*	34°32′16.20″S	150°52′3.75″E	14	4	0
Cuttagee Beach	*N. longipes*	36°31′37.08″S	150°03′22.31″E	20	8	0
Pambula	*N. longipes*	36°56′48.65″S	149°54′55.48″E	12	13	0

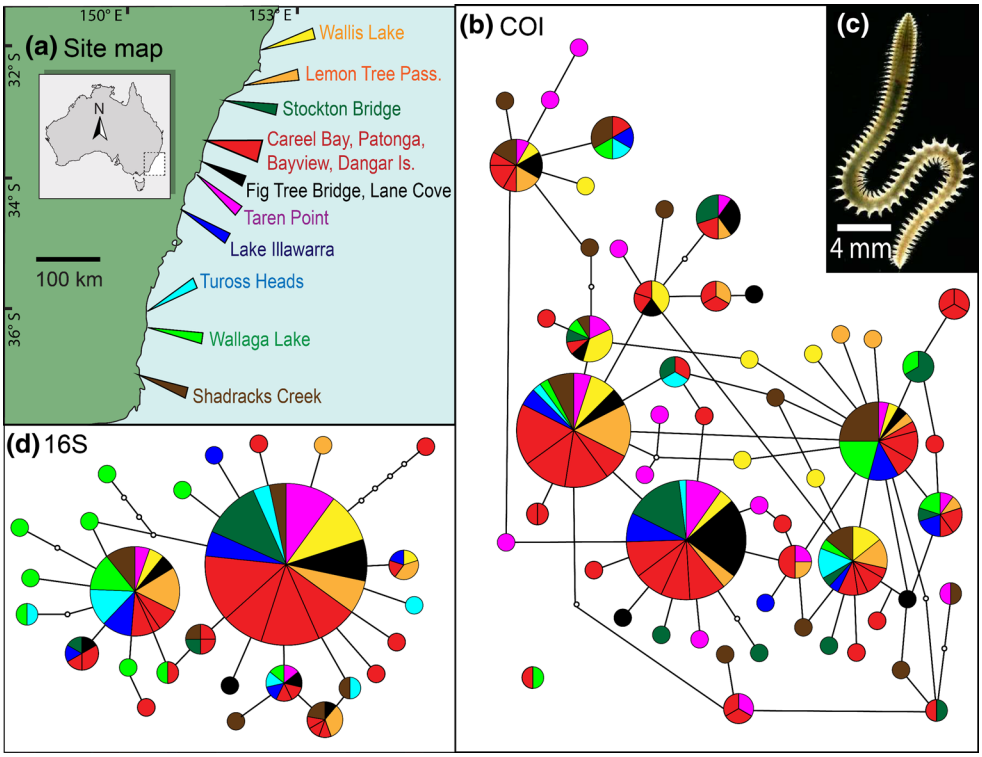

Fig. 2 Sites (**a**) and haplotype networks for COI, (**b**) and 16S (**d**) for *Aglaophamus australiensis* (pictured in **c** [Photo by K. Atkinson])

evolving than microsatellites, these markers can nonetheless shed light on phylogeographic structure (and hence on dispersal capacity over long time frames) in polychaetes. For example, COI was used by Carr et al. (2011) to determine phylogeographic structure in polychaetes in the Pacific, Arctic and Atlantic Oceans, and by Meissner et al. (2014) to infer dispersal among polychaetes North Atlantic seamounts. COI and 16S have also been used to infer past connectivity for polychaetes in Europe (Jolly et al. 2006) and the New Zealand region (Bors et al. 2012), and Schüller and Hutchings (2012) used 16S to infer dispersal for a trichobranchid polychaete in the Southern Atlantic.

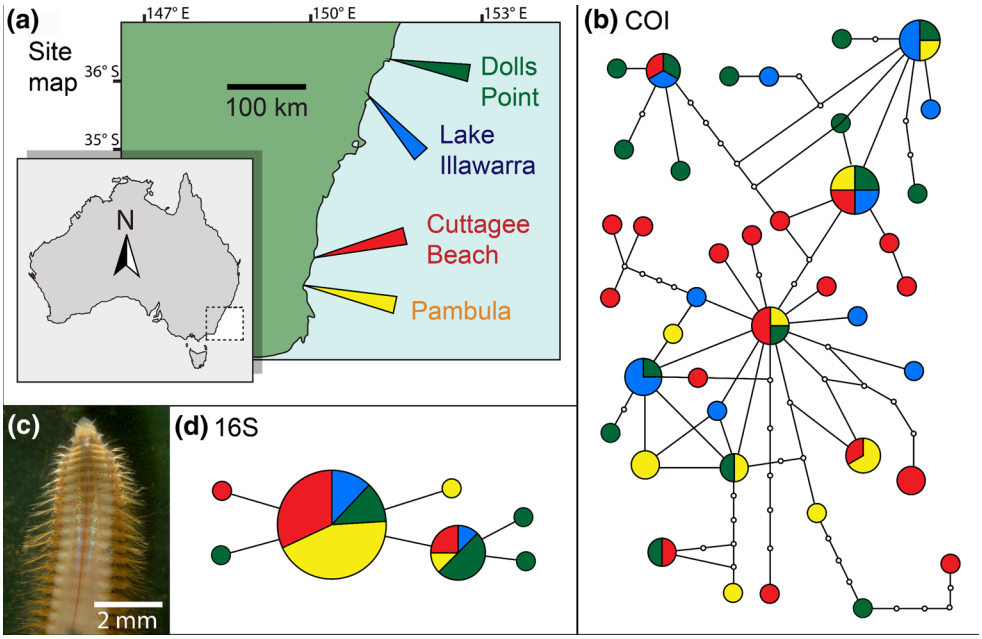

Fig. 3 Sites (**a**) and haplotype networks for COI, (**b**) and 16S (**d**) for *Nephtys longipes* (pictured in **c** [photo by S. Lindsey]. Note the much longer anterior chaetae than for *A. australiensis*)

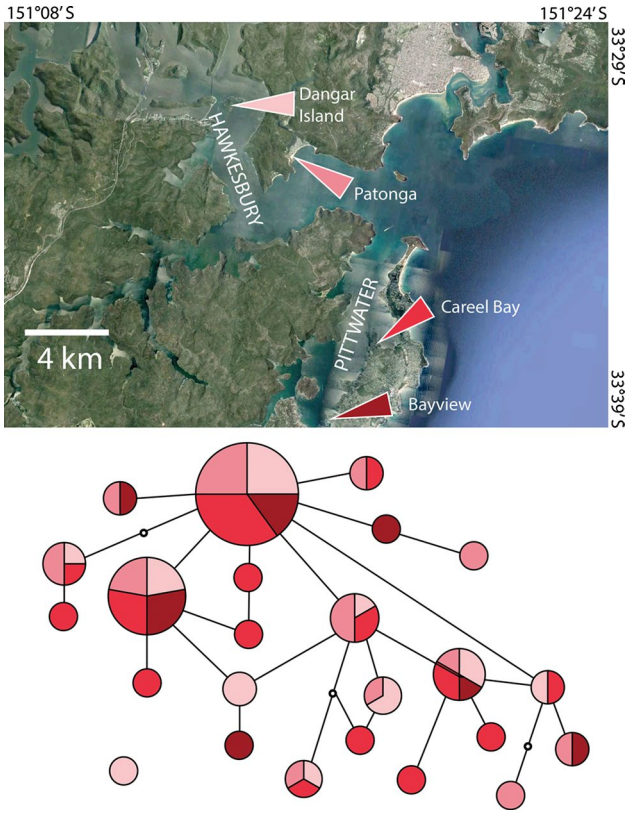

Fig. 4 Sites (*top*) and COI haplotype network (*bottom*) for *Aglaophamus australiensis* samples from four sites in the Pittwater/Hawkesbury estuaries, north of Sydney

DNA extraction of a small section of worm (approximately 1 mm) was carried out using a standard Chelex extraction procedure (Walsh et al. 1991). All samples used were required to have a head to avoid sampling the same individual twice. PCR amplification of the three markers was achieved using published primers (Table 2). Various other primer pairs were tested for each marker, but the greatest success was achieved with those shown in Table 2. PCRs were carried out using an Eppendorf Flexlid Mastercycler Nexus thermocycler in 20 µl volumes containing 1.0 µl of DNA, 1.0 µl of 10 µM of each the forward and reverse primer, 2.0 µl buffer, 2.0 µl of 8 mM dNTPS, 1.0 µl of 25 mM $MgCl_2$, 0.2 µl of 5 U/µl Taq and 11.6 µl water, using the following program: 94 °C for 120 s, then 40 cycles of 94 °C for 15 s, 48 °C for 30 s and 72 °C for 60 s, finishing with 72 °C for 240 s. Amplified PCR product was purified using IllustraTM ExoProStar 'enzymatic PCR and sequencing clean-up' kit and sequenced by Macrogen (Korea).

Genetic analyses

Sequences were aligned using Geneious 6.1.6 (Biomatters), and any ambiguities were assessed by eye. Haplotype networks were constructed using TCS (Clement et al. 2000). Isolation by Distance (IBD) analyses were carried out for each species for COI and 16S by paired Mantel tests using 999 permutations in GenAlEx (Peakall and Smouse 2012). For the Mantel test, the geographic distances among sites were calculated using a Coordinate Distance Calculator (http://boulter.com/gps/distance/), and mean uncorrected pairwise distances between sites were calculated using MEGA (Tamura et al. 2011). AMOVA analyses were used to compare within versus between population diversity for each species and marker, implemented in Arlequin version 3.5.1.3

Table 2 List of primers used for amplifying COI, 16S and 28S markers in *A. australiensis* and *N. longipes*

Gene	Name	Direction	Primer Sequence (5′–3′)	Source
COI	LCO1490	Forward	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
COI	HCO2198	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
16S	16Sar	Forward	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)
16S	16Sbr	Reverse	CCGGTCTGACTCAGATCACGT	Palumbi et al. (1991)
28S	LSUD1,D2,Fw1	Forward	AGCGGAGGAAAAGAAACTA	Sonnenberg et al. (2007)
28S	LSUD1,D2,rev2	Reverse	ACGATCGATTTGCACGTCAG	Sonnenberg et al. (2007)

(Excoffier and Lischer 2010). Directionality in gene flow between populations of the most widespread taxon, *A. australiensis,* was investigated using COI and 16S data via maximum likelihood in Migrate-n 3.6.6 (Beerli 1998; Beerli and Felsenstein 2001). The transition/transversion ratios were set to 16.70 for COI and 1.88 for 16S as calculated by Arlequin. The inheritance scalar was set to 0.25 for both loci. The four populations from the Pittwater/Hawkesbury estuaries were combined. The DNA sequence model was run with 10 short chains, each sampling 100,000 genealogies, and three long chains, each sampling 1000,000 genealogies, with a burn-in of 10,000 per chain. Three runs for both a full migration (asymmetric dispersal) model and a symmetric migration model were carried out, and a likelihood-ratio test (LRT) was used to assess which model best fit the data [LRT = 2*(lnL(asymmetric model) − lnL(symmetric model)], where lnL is the natural log of the likelihood).

Results

A fragment of COI 594 bp long was successfully amplified for 63 samples of *N. longipes* (yielding 39 haplotypes), and a fragment 340 bp long was amplified for 258 samples of *A. australiensis* (yielding 56 haplotypes). A fragment of 16S 397 bp long was amplified for 38 samples of *N. longipes* (yielding seven haplotypes), and a fragment 423 bp long was amplified for 149 samples of *A. australiensis* (yielding 26 haplotypes). The differences in lengths of the COI and 16S fragments amplified for each species were the result of differences in sequencing success; unreadable ends due to poor sequence quality in some samples were trimmed from all alignments, resulting in shorter fragments for some data sets. 28S only amplified for eleven samples in *A. australiensis*, and none in *N. longipes*. The 11 sequences obtained for 28S (eight from Shadracks Creek, one from Tuross Heads and two from Wallaga Lake), each with a length of 372 base pairs after trimming of ambiguous ends, showed no variation. All unique sequences from this study have been deposited with GenBank (accessions: *A. australiensis* COI: KP836357-412; 16S: KP836413-438; 28S: KP860235-245; and *N. longipes* COI: KP792237-275: 16S: KP836439-445).

No convincing pattern of IBD was found for either species ($P > 0.05$); for *N. longipes*, neither marker showed a significant IBD trend (COI: $P = 0.479$; 16S: $P = 0.095$), and although a slight IBD trend was found for *A. australiensis* for 16S ($R^2 = 0.192$, $P = 0.031$), this pattern was not observed for the more informative COI marker ($P = 0.273$). IBD plots are shown in supplementary figures S1-S4. Network analysis (Figs. 2, 3, 4) also did not indicate any clustering of haplotypes according to geography; indeed, several haplotypes were shared between multiple sites in each species. A single haplotype shared by one individual of *A. australiensis* from Wallaga Lake, and one from Dangar Island, could not be joined to the COI network at the 95 % confidence limit (Fig. 2). Network analysis indicated considerable diversity within sites; for example, 19 distinct COI haplotypes were recovered from 20 samples of *N. longipes* from Cuttagee Beach (Fig. 3). Haplotype diversity values were between 0.78 and 0.99 for both species and all sites (supplementary tables S1–S2). No evidence of directional dispersal was found, with Migrate-n analysis of *A. australiensis* sequences indicating in all three runs that a symmetric dispersal model fits the data better than an asymmetric model ($P < 0.001$).

AMOVA analyses showed that within population variation was far greater than between population variation (COI: *A. australiensis:* within 99.05 %, between 0.95 %, $P > 0.05$; *N. longipes*: within 96.24 %, between 3.76 %, $P = 0.025$; 16S: *A. australiensis*: within 93.34 %, between 2.92 %, $P = 0.047$; *N. longipes*: within 88.29 %, between 11.71 %, $P = 0.045$). Significant population differentiation was detected for both species for both markers. For *N. longipes*, population differentiation was entirely driven by the Dolls Point population differing from the other three estuaries. This population did, however, share haplotypes for COI and 16S with all of the other estuaries sampled for this species (Fig. 3). For *A. australiensis*, most population pairwise differences in the 16S marker were between Wallaga Lake and other sites (Table 3), but more pairwise differences were detected for COI (Table 4). For example, the two most southern sites, Wallaga Lake and Shadracks Creek, were different to most sites from Taren Point northward. Nonetheless, as for *N. longipes*, several of the most common haplotypes for *A. australiensis* were found across

Table 3 Population pairwise F_{ST} values for *A. australiensis* for 16S. Pairwise differences that were significant ($P < 0.05$) are in bold and underlined

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Wallis Lake	0.00												
2. Lemon Tree Passage	0.00	0.00											
3. Stockton Bridge	−0.01	0.06	0.00										
4. Dangar Island	0.01	−0.03	0.04	0.00									
5. Patonga	−0.02	−0.01	−0.03	−0.02	0.00								
6. Careel Bay	0.01	0.02	−0.03	0.03	0.01	0.00							
7. Bayview	−0.04	0.04	−0.06	0.04	−0.03	0.00	0.00						
8. Fig Tree Bridge	−0.02	−0.04	−0.01	−0.06	−0.03	−0.03	−0.01	0.00					
9. Taren Point	−0.06	−0.02	−0.04	−0.02	−0.04	−0.04	−0.05	−0.07	0.00				
10. Lake Illawarra	−0.03	−0.03	0.05	−0.06	−0.02	0.06	0.03	−0.03	−0.02	0.00			
11. Tuross Heads	0.02	−0.02	0.11	−0.03	0.00	0.12	0.07	0.00	0.03	−0.04	0.00		
12. Wallaga Lake	**0.15**	**0.11**	**0.23**	0.08	**0.10**	**0.26**	**0.19**	**0.13**	**0.17**	0.05	0.01	0.00	
13. Shadracks Creek	0.05	−0.05	0.08	−0.03	−0.02	0.04	0.06	−0.04	0.01	0.01	0.00	0.11	0.00

Table 4 Population pairwise F_{ST} values for *A. australiensis* for COI. Pairwise differences that were significant ($P < 0.05$) are in bold and underlined

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Wallis Lake	0.00												
2. Lemon Tree Passage	0.00	0.00											
3. Stockton Bridge	0.06	0.03	0.00										
4. Dangar Island	0.02	0.00	0.00	0.00									
5. Patonga	0.00	−0.02	0.01	−0.01	0.00								
6. Careel Bay	0.02	−0.01	0.00	−0.02	−0.01	0.00							
7. Bayview	0.05	0.02	−0.01	0.00	0.00	0.00	0.00						
8. Fig Tree Bridge	**0.09**	**0.05**	−0.01	0.04	0.03	0.03	−0.02	0.00					
9. Taren Point	**0.05**	0.03	0.00	0.00	0.00	0.01	−0.04	0.00	0.00				
10. Lake Illawarra	**0.05**	0.00	0.01	−0.04	0.00	−0.01	0.02	**0.07**	0.01	0.00			
11. Tuross Heads	−0.04	−0.06	0.01	−0.02	−0.02	−0.02	0.02	0.07	0.04	0.00	0.00		
12. Wallaga Lake	**0.06**	**0.06**	**0.11**	0.01	**0.06**	**0.08**	**0.13**	**0.19**	**0.11**	0.02	0.01	0.00	
13. Shadracks Creek	0.02	0.01	**0.10**	**0.04**	0.02	**0.05**	**0.08**	**0.14**	**0.07**	0.04	−0.04	0.02	0.00

almost all sites, including the most northern and most southern sites (Fig. 2), and differences therefore appear to be largely the result of diversity in rare (e.g. singleton) haplotypes at each site. Within the connected Pittwater and Hawkesbury estuaries, no fine-scale genetic structure was observed among sites, with several of the same haplotypes found at all four sites (Fig. 4) and no significant population differentiation found by AMOVA ($P \gg 0.05$).

Discussion

Our results suggest that dispersal of planktonic polychaete larvae can readily occur among many estuaries in southeastern Australia. Although AMOVA analyses indicated differentiation among a few sites, these differences were apparently driven by relatively rare haplotypes in a few individuals, whereas the finding that several common haplotypes were shared among most sites for both species and markers suggests widespread inter-estuarine dispersal. Our hypothesis that populations might be poorly connected was thus not supported by our results.

Although several other molecular studies on polychaete worms have identified the presence of unrecognised species (e.g. Barroso et al. 2010; Nygren and Pleijel 2011; Borda et al. 2013; Glasby et al. 2013), the close relationships of haplotypes from each marker for each species in this study suggest their taxonomy is well resolved across the study region. Indeed, although Nephtyidae worms in southeastern Australia have recently been the focus of taxonomic

revision, the two species used in this study appear valid (Dixon-Bridges et al. 2014).

Polychaetes are abundant in marine and estuarine environments (Hutchings 2004), and the widespread distributions of some polychaete species are probably due to their long pelagic larval stages. There is no literature on the larval duration of *N. longipes* and *A. australiensis*; however, the duration of larval stages in other members of the Nephtyidae is estimated to be 11–42 days (Caron et al. 1995). This is a relatively long planktonic phase, consistent with the potential for widespread dispersal suggested by our study. The average distance among estuaries in NSW (south of Sydney) is approximately 10 km (estimated from Google Earth path measurements), and the EAC can have coastal flows of ~1 ms^{-1} (Roughan and Middleton 2004). Larval dispersal among adjacent estuaries could therefore potentially occur in a matter of a few hours or days, once the larvae have been flushed by tides into the sea.

Several other studies have likewise found evidence of high dispersal capacity in polychaete worms. Barroso et al. (2010) found that populations of *Eurythoe complanata* appeared to be well connected along 2500 km coast of the Caribbean and Brazil and proposed that the teleplanic larvae of *E. complanata* were being transported by the westward South Equatorial Current and the eastward South Equatorial Countercurrent (Brown 1990). Widespread larval dispersal facilitating population connectivity was also inferred for the polychaete *Pectinaria koreni* along the north coast of France (Jolly et al. 2004), with evidence for past divergence due to historical processes but contemporary dispersal with ocean currents. Polychaetes with pelagic larvae do not, however, always show evidence of connectivity among populations. Kesäniemi et al. (2012) found, for example, that many European populations of the spionid polychaete *Pygospio elegans* were genetically distinct. Life history alone is inadequate to predict dispersal capacity (Johannesson 1988), and in the case of our study, tidal flushing, ocean currents and anthropogenic influences are likely to play a major role in the transport of larvae.

Despite estuaries potentially acting as eco-physiological barriers (Bilton et al. 2002; Kennish 2002), ballast water from ships can transport marine organisms long distances, particularly among ports (Hutchings 1992; Ruiz et al. 1997). At least one of the sites in our study was close to a busy industrial port (Dolls Point, near Botany Bay), and all other sites, while not major ports, are estuaries that are open to the sea and experience considerable tidal influences. Coleman (2013) found that estuarine and nearby open-ocean populations of the kelp *Ecklonia radiata* in southeastern Australia were not genetically differentiated, suggesting that well-flushed estuaries are not isolated environments for benthic marine organisms. Indeed, for many of the estuaries studied, the environment is almost entirely marine, with relatively little freshwater input from streams and rivers, and strong tidal flushing via fast-flowing currents around sand banks. The entrance to the Pittwater/Hawkesbury River estuaries (location of sites Dangar Island, Patonga, Careel Bay and Bayview), Sydney Harbour (Fig Tree Bridge site) and Botany Bay (Taren Point site) are all well-flushed estuaries with wide, deep river mouths. However, sites such as Wallis Lake and Wallaga Lake have shallow entrances, partially blocked by sand bars that limit flushing which varies between neap and spring tides (Roy et al. 2001). Future research could assess whether less well-flushed estuaries, such as those that are often completely closed by sand movements at the river mouth, or by anthropogenic sea walls, house more differentiated polychaete populations than 'open' estuaries.

The high dispersal potential of these two estuarine worm species has positive implications for their resilience in the face of ongoing anthropogenic environmental change, as damaged populations may be able to be 'rescued' by colonists from elsewhere (Bradbury et al. 2008a). Which populations would be most likely to act as sources of such rescues could not, however, be determined by this research. Although the EAC flows southward along the eastern coast of Australia, and thus could be expected to connect from north to south only, bidirectional dispersal may also be facilitated by inshore, north-flowing counter currents and eddies due to seasonal variation of strength and positioning of the EAC (Coleman et al. 2011, 2013; see Fig. 1). Indeed, our Migrate-n analyses suggest that bidirectional, rather than asymmetric, dispersal of polychaete larvae is occurring along the NSW coast. Furthermore, if north–south dispersal were the norm, genetic diversity could be expected to decrease towards the south, but this pattern was not supported by haplotype diversity values (see supplementary tables S1, S2).

In concordance with our results, Piggott et al. (2008) did not find a significant IBD relationship in abalones (*Haliotis coccoradiata*) along the south coast of NSW. There was, however, additional evidence of fine-scale genetic structure, provided by microsatellite analysis, which suggested some degree of local larval retention and local recruitment (Piggott et al. 2008). The authors were able to conclude that the most likely scenario was that abalones have the ability to infrequently disperse over long distances, although recruitment occurs primarily on a small spatial scale. Comparable results were found using microsatellite loci for populations of the kelp *Ecklonia radiata* along the eastern coast of Australia, with no IBD pattern but mosaic genetic differentiation apparently driven by eccentricities in ocean current flow (Coleman et al. 2011). Rapidly evolving molecular markers such as microsatellites are ideal for analysing patterns of contemporary connectivity among marine populations (Sherman et al. 2008; Chust

et al. 2013). Unfortunately, polymorphic microsatellite loci have not yet been identified for our study species (nor for any species in the polychaete family Nephtyidae), and our research therefore relied on the more slowly evolving, but nonetheless phylogeographically informative, mitochondrial markers COI and 16S. Although we were able to infer connectivity among estuaries, we cannot be sure whether this connectivity is ongoing or is simply a reflection of processes in the recent past. Future research using microsatellite markers could shed light on the finer-scale population structure and connectivity of Nephtyidae worms in eastern Australia. Research on the life history of these polychaetes, such as their larval duration and length of spawning, would also help us to understand the likely extent of their dispersal capacity. Nonetheless, our results indicate that benthic polychaetes with planktonic larvae may have little trouble dispersing among estuaries connected by strong ocean currents. For less dispersive estuarine species, such as those without a planktonic stage, and for organisms in poorly flushed estuaries, dispersal is likely to be more of a challenge. Research on a wide range of taxa with differing dispersal mechanisms is critical if the design of protected areas such as marine park networks is to be effective.

Acknowledgments We thank C. McGrath and J. Tan for laboratory assistance, and J. Pierson for assistance with some analyses. P. Rodgers, A. Murray and S. Keable helped with collecting, and S. Lindsey with photography. Funding was provided by the Australian Museum and by research funds for CIF from the Fenner School of Environment and Society at the Australian National University.

References

Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2010) The value of estuarine and coastal ecosystem services. Ecol Monogr 81:169–193. doi:10.1890/10-1510.1

Barroso R, Klautau M, Solé-Cava AM, Paiva PC (2010) *Eurythoe complanata* (Polychaeta: Amphinomidae), the 'cosmopolitan' fireworm, consists of at least three cryptic species. Mar Biol 157:69–80. doi:10.1007/s00227-009-1296-9

Beerli P (1998) Estimation of migration rates and population sizes in geographically structured populations. In: Carvalho G (ed) Advances in Molecular Ecology., NATO-ASI workshop seriesIOS Press, Amsterdam, pp 39–53

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc Natl Acad Sci USA 98:4563–4568

Bilton DT, Paula J, Bishop JDD (2002) Dispersal, genetic differentiation and speciation in estuarine organisms. Estuar Coast Shelf Sci 55:937–952. doi:10.1006/ecss.2002.1037

Borda E, Kudenov JD, Chevaldonné P, Blake JA, Desbruyères D, Fabri M-C, Hourdez S, Pleijel F, Shank TM, Wilson NG, Schulze A, Rouse GW (2013) Cryptic species of *Archinome* (Annelida: Amphinomida) from vents and seeps. Proc R Soc Lond B Biol Sci. doi:10.1098/rspb.2013.1876

Bors EK, Rowden AA, Maas EW, Clark MR, Shank TM (2012) Patterns of deep-sea genetic connectivity in the New Zealand region: implications for management of benthic ecosystems. PLoS One 7:e49474. doi:10.1371/journal.pone.0049474

Bradbury IR, Campana SE, Bentzen P (2008a) Low genetic connectivity in an estuarine fish with pelagic larvae. Can J Fish Aquat Sci 65:147–158. doi:10.1139/f07-154

Bradbury IR, Laurel B, Snelgrove PVR, Bentzen P, Campana SE (2008b) Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. Proc R Soc Biol Sci Ser B 275:1803–1809. doi:10.1098/rspb.2008.0216

Brown CW (1990) The significance of the South Atlantic Equatorial countercurrent to the ecology of the green turtle breeding population of Ascension Island. J Herpetol 24:81–84. doi:10.2307/1564294

Caron A, Boucher L, Desrosiers G, Retiere C (1995) Population dynamics of the polychaete *Nephtys caeca* in an intertidal estuarine environment. J Mar Biol Assoc UK 75:871–884. doi:10.1017/S0025315400038212 **(Quebec, Canada)**

Carr CM, Hardy SM, Brown TM, Macdonald TA, Hebert PDN (2011) A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. PLoS One 6:e22232. doi:10.1371/journal.pone.0022232

Chust G, Albaina A, Aranburu A, Borja Á, Diekmann OE, Estonba A, Franco J, Garmendia JM, Iriondo M, Muxika I, Rendo F, Rodríguez JG, Ruiz-Larrañaga O, Serrão EA, Valle M (2013) Connectivity, neutral theories and the assessment of species vulnerability to global change in temperate estuaries. Estuar Coast Shelf Sci 131:52–63. doi:10.1016/j.ecss.2013.08.005

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659. doi:10.1046/j.1365-294x.2000.01020.x

Coleman MA (2013) Connectivity of the habitat-forming kelp, *Ecklonia radiata* within and among estuaries and open coast. PLoS One 8:e64667. doi:10.1371/journal.pone.0064667

Coleman MA, Chambers J, Knott NA, Malcolm HA, Harasti D, Jordan A, Kelaher BP (2011) Connectivity within and among a network of temperate marine reserves. PLoS One 6:e20168. doi:10.1371/journal.pone.0020168

Coleman MA, Feng M, Roughan M, Cetina-Heredia P, Connell SD (2013) Temperate shelf water dispersal by Australian boundary currents: implications for population connectivity. Limnol Oceanogr Fluids Environ 3:295–309. doi:10.1215/21573689-2409306

Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. Ann Rev Mar Sci 1:443–466. doi:10.1146/annurev.marine.010908.163757

Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science 311:522–527. doi:10.1126/science.1122039

Dixon-Bridges K, Gladstone W, Hutchings P (2014) Two new species of *Micronephthys* Friedrich, 1939 and one new species of *Nephtys* Cuvier, 1817 (Polychaeta: Phyllodocida: Nephtyidae) from eastern Australia with notes on *Aglaophamus australiensis* (Fauchald, 1965) and a key to all Australian species. Zootaxa 3872:513–540. doi:10.11646/zootaxa.3872.5.5

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567. doi:10.1111/j.1755-0998.2010.02847.x

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3:294–299

Glasby CJ, Wei N-WV, Gibb KS (2013) Cryptic species of Nereididae (Annelida: Polychaeta) on Australian coral reefs. Invertebr Syst 27:245–264. doi:10.1071/IS12031

Hutchings P (1992) Ballast water introductions of exotic marine organisms into Australia: current status and management options. Mar Pollut Bull 25:196–199. doi:10.1016/0025-326X(92)90225-U

Hutchings P (2004) Polychaetes — their biological diversity. Rec S Aust Mus 7:39–49 **(Adel)**

Jenkins KM, Kingsford RT, Closs GP, Wolfenden BJ, Matthaei CD, Hay SE (2011) Climate change and freshwater ecosystems in Oceania: an assessment of vulnerability and adaptation opportunities. Pac Conserv Biol 17:201–219

Johannesson K (1988) The paradox of Rockall—why is a brooding gastropod (*Littorina saxatilis*) more widespread than one having a planktonic larval dispersal stage (*L. littorea*)? Mar Biol 99:507–513

Jolly MT, Jollivet D, Gentil F, Thiebaut E, Viard F (2004) Sharp genetic break between Atlantic and English channel populations of the polychaete *Pectinaria koreni,* along the North coast of France. Heredity 94:23–32. doi:10.1038/sj.hdy.6800543

Jolly MT, Viard F, Gentil F, Thiebaut E, Jollivet D (2006) Comparative phylogeography of two coastal polychaete tubeworms in the Northeast Atlantic supports shared history and vicariant events. Mol Ecol 15:1841–1855. doi:10.1111/j.1365-294X.2006.02910.x

Kennish MJ (2002) Environmental threats and environmental future of estuaries. Environ Conserv 29:78–107. doi:10.1017/S0376892902000061

Kesäniemi JE, Geuverink E, Knott KE (2012) Polymorphism in developmental mode and its effect on population genetic structure of a spionid polychaete, *Pygospio elegans*. Integ Comp Biol 52:181–196. doi:10.1093/icb/ics064

Meissner K, Bick A, Guggolz T, Götting M (2014) Spionidae (Polychaeta: Canalipalpata: Spionida) from seamounts in the NE Atlantic. Zootaxa 3786:201–245. doi:10.11646/zootaxa.3786.3.1

Nygren A, Pleijel F (2011) From one to ten in a single stroke—resolving the European *Eumida sanguinea* (Phyllodocidae, Annelida) species complex. Mol Phylogenet Evol 58:132–141. doi:10.1016/j.ympev.2010.10.010

Palumbi SR, Martin AP, Romano S, McMillan WO, Stice L, Grabowski G (1991) The simple fool's guide to PCR. Department of Zoology, University of Hawaii, Honolulu

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. doi:10.1093/bioinformatics/bts460

Piggott MP, Banks SC, Tung P, Beheregaray LB (2008) Genetic evidence for different scales of connectivity in a marine mollusc. Mar Ecol Prog Ser 365:127–136. doi:10.3354/Meps07478

Roughan M, Middleton JH (2004) On the East Australian current: variability, encroachment, and upwelling. J Geophys Res Ocean 109:C07003. doi:10.1029/2003JC001833

Roy PS, Williams RJ, Jones AR, Yassini I, Gibbs PJ, Coates B, West RJ, Scanes PR, Hudson JP, Nichol S (2001) Structure and function of south-east Australian estuaries. Estuar Coast Shelf Sci 53:351–384. doi:10.1006/ecss.2001.0796

Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. Am Zool 37:621–632. doi:10.1093/icb/37.6.621

Schüller M, Hutchings PA (2012) New species of *Terebellides* (Polychaeta: Trichobranchidae) indicate long-distance dispersal between western South Atlantic deep-sea basins. Zootaxa: 1–31

Sherman CDH, Hunt A, Ayre DJ (2008) Is life history a barrier to dispersal? Contrasting patterns of genetic differentiation along an oceanographically complex coast. Biol J Linn Soc 95:106–116. doi:10.1111/j.1095-8312.2008.01044.x

Sonnenberg R, Nolte AW, Tautz D (2007) An evaluation of LSU rDNA D1-D2 sequences for their use in species identification. Front Zool 4:6. doi:10.1186/1742-9994-4-6

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739. doi:10.1093/molbev/msr121

Walsh PS, Metzger DA, Higuchi R (1991) Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTech 10:506–513