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Genetic connectivity in the Florida reef system: comparative phylogeography of commensal invertebrates with contrasting reproductive strategies

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Abstract

Effective spatial management of coral reefs including design of marine protected areas requires an understanding of interpopulation genetic connectivity. We assessed gene flow along 355 km of the Florida reef system and between Florida and Belize in three commensal invertebrates occupying the same host sponge (*Callyspongia vaginalis*) but displaying contrasting reproductive dispersal strategies: the broadcast-spawning brittle star *Ophiothrix lineata* and two brooding amphipods *Leucothoe kensleyi* and *Leucothoe ashleyae*. Multiple analytical approaches to sequence variation in the mitochondrial COI gene demonstrated a high degree of overall connectivity for all three species along the Florida reef system. *Ophiothrix lineata* showed significant genetic structuring between Florida and Belize, and a pattern of isolation by distance but no significant genetic structuring along the Florida coastline. Bayesian estimates of migration detected a strong southerly dispersal bias for *O. lineata* along the Florida reef system, contrary to the general assumption of northerly gene flow in this region based on the direction of the Florida Current. Both amphipods, despite direct development, also showed high gene flow along the Florida reef system. Multiple inferences of long-distance dispersal from a nested clade analysis support the hypothesis that amphipod transport, possibly in detached sponge fragments, could generate the high levels of overall gene flow observed. However, this transport mechanism appears much less effective across deep water as connectivity between Florida and Belize (1072 km) is highly restricted.

Keywords: brittle star, commensal, coral reef connectivity, Leucothoid amphipods, life history, nested clade analysis

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Introduction

There is broad consensus that management and conservation efforts needed to stem the global decline of coral reefs will benefit substantially from improved understanding of coral reef ecosystem dynamics (Hughes *et al*. 2003; Bellwood *et al*. 2004). For example, the extent of genetic connectivity within and among coral reefs is important information to aid in ecologically effective sizing and placement of marine protected areas, a management strategy advocated for the conservation of reef communities (NRC 2001; Thorrold *et al*. 2002; Palumbi 2003).

Recent ecological theory has highlighted the fundamental influence of facilitation (i.e. positive species interactions such as commensalism and mutualism) on structure and function of aquatic communities (Bruno *et al*. 2003). Consequently, attempts to derive general principles about genetic connectivity patterns in coral reef ecosystems should include examination of the numerous reef species involved in

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facilitation. However, this area of study remains largely unexplored (for exception see Duffy 1993). Furthermore, robust assessment of reef connectivity should encompass organisms displaying diverse reproductive strategies typical in these complex ecosystems (Hughes *et al*. 2003).

In general, it is expected that brooding species with direct development will exhibit limited dispersal capabilities compared to broadcast spawning species, and will therefore show lower genetic connectivity over similar geographical scales. Although this expectation has been supported by numerous comparative studies (e.g. Hunt 1993; Hellberg 1996; Ayre *et al*. 1997; Arndt & Smith 1998; Collin 2001), including the only study to date on sponge commensal species (Duffy 1993), several studies have also revealed high gene flow for brooders (e.g. Grant & da Silva-Tatley 1997; Ayre & Hughes 2000; Sponer & Roy 2002; Le Gac *et al*. 2004) indicating that some species with direct development are able to disperse over wide geographical distances.

The majority of continental US coral reefs are located in Florida, and there is considerable concern about their state of advanced impairment (Harvell *et al*. 1999; Causey *et al*. 2002; Pandolfi *et al*. 2005). To address these concerns, but cognizant of significant socio-political limitations, only 6% of the reef system has been zoned as no-take areas. There are, however, increasing calls for strategically located expansion of these areas to reduce and potentially reverse reef degradation (Pandolfi *et al*. 2005). Despite the socio-economic importance of the Florida reefs, there are few data on genetic connectivity in this system to aid managers in ecologically effective expansion of protected areas. To provide these data we assessed genetic connectivity within three invertebrate species displaying contrasting reproductive development. These species, the brittle star *Ophiothrix lineata* and two amphipods *Leucothoe kensleyi* and *Leucothoe ashleyae* occupy the same microhabitat as commensal inhabitants of the branching vase sponge *Callyspongia vaginalis*.

Prior to this study, the exact mode of development for *Ophiothrix lineata* was unknown. Laboratory rearing experiments with *O. lineata* that we collected from one of the Florida study sites during February 2004 were conducted at 23 °C to match the 17-year temperature average for February at the collection site [National Data Buoy Center: National Oceanic and Atmospheric Administration (NOAA)]. Embryo development occurred entirely within the fertilization membrane, and individuals escaped as miniature crawl away juveniles after 6–8 days (V.P.R., unpublished data). This form of development appears rare in ophiuroids with only one example reported (*Amphioplus abditus*; Hendler 1977). The developing *O. lineata* embryo did not appear to pass through an abbreviated ophiopluteus stage, and approximately 20 h after fertilization five rudimentary arms had developed. *Amphioplus abditus* embryos have been collected 0.5 m off the bottom suggesting that they could be dispersed via water currents (Hendler 1977). It is likely that *O. lineata* with its similar development, is also subject to transport via currents over its 6–8 day embryonic stage, facilitating enhanced dispersal compared to brooding species.

In contrast, female *L. kensleyi* and *L. ashleyae* brood fertilized eggs, which undergo direct development in their marsupium until the fully formed young are released as crawl-away juveniles. This brooding and commensal life history strategy coupled with the often-patchy distribution of available sponge hosts leads to expectations of highly restricted gene flow among reefs and possibly even among local host sponges for these amphipods. Previous studies of habitat-limited, brooding crustaceans have provided support for these expectations. Duffy (1993), for example, showed significant genetic population structure among sponge dwelling snapping shrimp separated by less than 3 km and Lessios *et al*. (1994) showed significant population structure among intertidal isopods separated by less than 1 km.

Our mitochondrial DNA analyses indicate considerable genetic connectivity between northern and southern portions of the Florida reef system, but a high degree of phylogeographical disjunction between Florida and Belize reefs independent of reproductive strategy. We report on potential causes of the observed phylogeography and suggest possible transport mechanisms influencing dispersal patterns along the Florida reef system.

Materials and methods

Sampling sites and collections

A total of 446 individuals were collected from their host sponge habitat. With one exception, all sponges were sampled nearshore (< 10 m depth) along 355 km of coastline encompassing the northern and southern ends of the Florida reef system (Fig. 1, Table 1). *Leucothoe kensleyi* (n = 182) were collected from 13 individual sponges distributed among seven sites from four major Florida locations: Palm Beach, Fort Lauderdale, Long Key and Key West. One of the collection sites was a shipwreck, the Donal G. McAllister, sunk in 1998 at 23 m depth, 2.3 km from the closest shallow water collection site (Johnson Reef). *Leucothoe ashleyae* (n = 136) were collected from 16 individual sponges distributed among five sites from the same four major Florida locations. *Ophiothrix lineata* (n = 128) were collected from 23 individual sponges distributed among six sites from the same four Florida locations. To gain a comparative perspective of species population structure over larger geographical scales, we also collected *L. ashleyae* and *O. lineata* from an unrecorded number of sponges at Glover's Reef Atoll in Belize (Fig. 1, Table 1). We were unable to find *L. kensleyi* at Glover's Reef. All individuals were preserved in 95% ethanol at 4 °C.

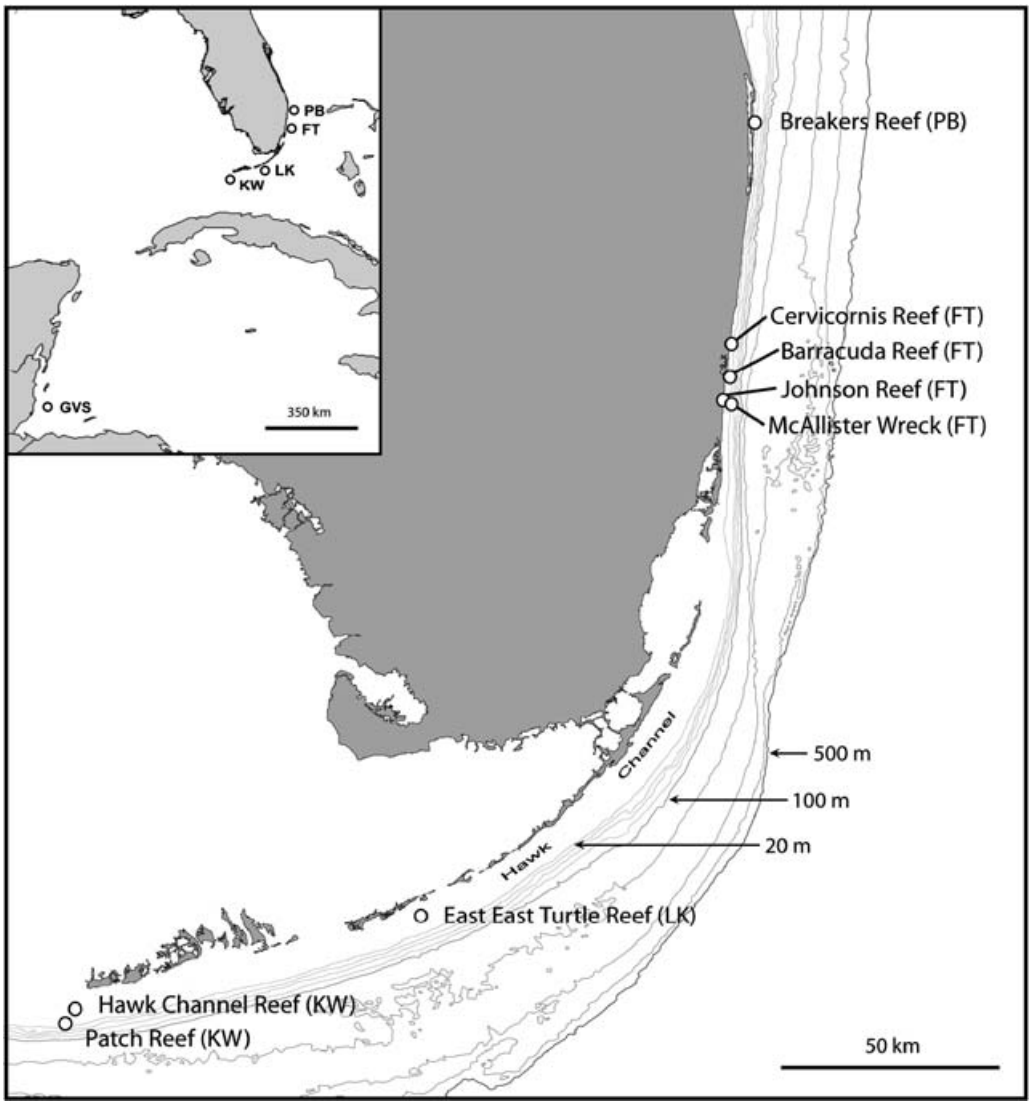

Fig. 1 Map showing individual sponge sampling sites along the Florida reef system. (Depth contour data from http://www.ngdc.noaa.gov/mgg/ibcca). Inset shows the five major reef sampling locations: PB, Palm Beach; FT, Ft Lauderdale; LK, Long Key; KW, Key West; GVS, Glover's Reef.

Polymerase chain reaction (PCR) and sequencing

Genomic DNA was extracted from 25 mg of *O. lineata* tissue and whole individual amphipods using the DNeasy Tissue Kit (QIAGEN Inc.). For the amphipods, the primer pair LCO1490 and HCO2198 (Folmer *et al*. 1994) was used to initially amplify approximately 665 bp of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. Because these primers did not sequence well, we designed the following amphipod specific internal primers: Ls(4)COI-F2 (5′-ATTATTCGAACAGAATTATCAACCCC-3′), Ls(4)COI-R2 (5′-TGTAATGGCTCCCGCTAAAACTGG-3′) and Ls(3)COI(FL)-F1 (5′-AACAGAATTATCCACCCCGGGAAATTTAAT-3′). The Ls(4) primer pair was used to amplify and sequence a 422-bp fragment of the *L. kensleyi* COI gene. The same primer pair was used to amplify and sequence a 414-bp fragment of the *L. ashleyae* COI gene. However, the forward primer Ls(4)COI-F2 would occasionally give poor results and in these instances it was replaced with Ls(3)COI(FL)-F1. The primer pair Olin(COI)-F1 (5′-TTTGGCGCTTGAGCAGGAACCGTA-3′) and Olin(COI)-R4 (5′-CTGTTGGGATAGCTATTATCATTGTGGC-3′) was designed and used to amplify and sequence a 718-bp fragment at the five prime end of the *O. lineata* COI gene. Total PCR volumes were 50 μL and contained 1 μL of the extracted genomic DNA, 5 μL 10× PCR buffer, 50 μM of each dNTP, 0.25 μM of each primer, and 0.75–1.75 U of HotStar *Taq* DNA Polymerase (QIAGEN Inc.). PCR was performed in a Mastercycler

Table 1 Number of individuals of each species sampled from each host sponge at the five major sampling locations in Florida and Belize

Location	Sampling site	GPS coordinates	Species	Sponge ID number followed by number of individuals collected from the sponge								Total	Total sponges
Palm Beach	Breakers Reef	26 43.077 N 80 01.774 W	*L. kensleyi*			BK3: 17	BK4: 6	BK5: 13				36	3
	Breakers Reef		*L. ashleyae*			BK3: 7	BK4: 5	BK5: 9	BK6: 9			30	4
	Breakers Reef		*O. lineata*	BK1: 3	BK2: 4	BK3: 6	BK4: 3		BK6: 4		BK8: 8	28	6
Ft Lauderdale	Cervicornis Reef	26 09.792 N 80 05.492 W	*L. kensleyi*	CR1: 20		CR3: 13						33	2
	Cervicornis Reef		*L. ashleyae*	CR1: 11	CR2: 10	CR3: 10	CR4: 6					37	4
	Cervicornis Reef		*O. lineata*	CR1: 11	CR2: 3	CR3: 3						17	3
	Barracuda Reef	26 04.720 N 80 05.710 W	*O. lineata*	CU1: 2	CU2: 4	CU3: 3	CU4: 2					11	4
	McAllister wreck	26 00.548 N 80 05.565 W	*L. kensleyi*	MC1: 26								26	1
	Johnson Reef	26 01.140 N 80 06.827 W	*L. kensleyi*	JR1: 23								23	1
Long Key	East East Turtle Reef	24 43.498 N 80 55.128 W	*L. kensleyi*		ET2: 13	ET3: 12	ET4: 6					31	3
	East East Turtle Reef		*L. ashleyae*		ET2: 7	ET3: 5	ET4: 7			ET7: 4		23	4
	East East Turtle Reef		*O. lineata*	ET1: 9	ET2: 7	ET3: 3	ET4: 4	ET5: 3	ET6: 3			29	6
Key West	Patch Reef	24 27.274 N 81 52.090 W	*L. kensleyi*	PR1: 5						PR7: 15		20	2
	Patch Reef		*L. ashleyae*	PR1: 6				PR5: 7		PR7: 8		21	3
	Patch Reef		*O. lineata*	PR1: 5		PR3: 2						7	2
	Hawk Channel Reef	24 29.399 N 81 50.497 W	*L. kensleyi*	HK1: 13								13	1
	Hawk Channel Reef		*L. ashleyae*	HK1: 8								8	1
	Hawk Channel Reef		*O. lineata*	HK1: 3	HK2: 3							6	2
Belize	Glover's Reef	16 44.000 N	*L. ashleyae*									17	
	Glover's Reef	87 42.500 W	*O. lineata*									30	
All Locations			*L. kensleyi*									182	13
			L. ashleyae									136	16
			O. lineata									128	23
			TOTAL									446	

BK, Breakers Reef; CR, Cervicornis Reef; CU, Barracuda Reef; MC, McAllister Reef; JR, Johnson Reef; ET, East East Turtle Reef; PR, Patch Reef; HK, Hawk Channel Reef.

Gradient (Eppendorf Inc.) thermal cycler as follows: 95 °C initial heating for 15 min to activate the hot start DNA polymerase, followed by 35–45 cycles of 94 °C for 1 min, 40–50 °C for 1–2 min, 72 °C for 1–2 min, and a 5-min final extension step at 72 °C. Because *L. ashleyae* juveniles (< 2 mm length) yielded very low amounts of genomic DNA, the PCR thermal profile and *Taq* polymerase were empirically adjusted (within the above parameters) to increase amplification efficiency. A negative control (no genomic DNA) was included in each PCR set to check for reagent contamination. PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN Inc.) and sequenced in both directions on an ABI 3730xl genetic analyser. Individual haplotype sequences are available from GenBank (Accession nos EF053456–EF053503 for *L. kensleyi*, EF053411–EF053423 for *L. ashleyae*, EF053424–EF053455 for *O. lineata*).

Data analysis

Individual COI sequences were aligned, edited and translated in GENEDOC version 2.6.02 (Nicholas *et al*. 1997). To confirm protein functionality as a check for amplification of nuclear pseudogenes, codons were checked for correct coding of invertebrate mtDNA amino acids and aberrant start/stop codons. For *L. ashleyae* and *O. lineata*, average pairwise nucleotide distances between Florida and Glover's Reef populations were calculated using Kimura's two-parameter model (Kimura 1980) in MEGA3 (Kumar *et al*. 2004). The program DNASP version 4.0 (Rozas *et al*. 2003) was used to estimate molecular diversity indices, Tajima's *D* test statistic, and calculate mismatch distributions for each species.

Genetic population structure was examined by an analysis of molecular variance (AMOVA) as implemented in ARLEQUIN version 2.000 (Schneider *et al*. 2000). With the commensal lifestyle of these species in mind, we explored partitioning of genetic variation within and among different sponges by estimating hierarchical variance components for *L. kensleyi*, *L. ashleyae* and *O. lineata* in the following manner: variance among haplotypes within a sponge, variance among sponges within each of the four major geographical locations, and variance among the four geographical locations. All Florida populations of *L. ashleyae* and *O. lineata* were then grouped by species and compared to Glover's Reef via simple pairwise AMOVA. Genetic isolation by distance (IBD) was tested by comparing geographical distances to pairwise Φ_{ST} values among the collection sites and significance of the results determined using the Mantel Test (MANTEL version 1.01, Bohonak 2002). The shortest geographical distances by sea between collection sites were calculated in ARCVIEW 3.0 (ESRI). Intraspecific evolutionary relationships were estimated for each species by constructing unrooted parsimony haplotype networks using the Templeton *et al*. (1992) method as implemented in the software package TCS version 1.13 (Clement *et al*. 2000). Ambiguous loops in the networks were resolved using criteria based on coalescent theory (Crandall & Templeton 1993), summarized by Pfenninger & Posada (2002) as follows: (i) Frequency criterion: haplotypes are more likely to be connected to haplotypes with higher frequency than to singletons; (ii) Topological criterion: haplotypes are more likely to be connected to interior haplotypes than to tip haplotypes; and (iii) Geographical criterion: haplotypes are more likely to be connected to haplotypes from the same population or region than to haplotypes occurring in distant populations.

To infer the processes that led to the phylogeographical patterns of the three species, we complemented other statistical approaches with nested clade analysis (NCA) (Templeton *et al*. 1995). Although Knowles & Maddison (2002) have criticized NCA for it's ability to distinguish among alternative biological inferences to explain phylogeographical patterns, Templeton (2004) has addressed this issue by highlighting that adequate sampling and correct use of the inference key can minimize false positives. NCA offers the advantage over more descriptive approaches of being able to test these inferences within a rigorous statistical framework (Avise 2000). For NCA, haplotypes were nested into hierarchal clades according to the standard rules in Templeton *et al*. (1987). Three distance measures were calculated: (i) clade distance (D_c), which is a measure of how geographically widespread a particular haplotype or clade is; (ii) nested clade distance (D_n), which is a measure of how geographically widespread a particular haplotype or clade is relative to the clade it is nested within; and (iii) tip-interior distance (I-T), which is a comparison between average D_c and D_n for tip and interior haplotypes or clades (Templeton *et al*. 1995). Haplotype–geographical association was tested by an exact permutation contingency analysis (10 000 random permutations) using the software GEODIS version 2.2 (Posada *et al*. 2000). The revised inference key of Templeton (2004) was used to interpret significant haplotype–geographical associations and make the appropriate biological inference. User-defined geographical distances were calculated using ARCVIEW.

Migration rates among locations were estimated using the program MIGRATE version 2.1.3 (Beerli & Felsenstein 2001; Beerli 2004). Rates were also calculated between Glover's Reef and a grouping of all Florida locations for both *L. ashleyae* and *O. lineata*. The maximum-likelihood (ML) approach implemented in MIGRATE can be problematic due to lack of run convergence or in providing poor estimates of migration in cases of data that are sparse or show very high or low levels of variation (Abdo *et al*. 2004; Beerli 2006). However, the Bayesian framework recently incorporated into MIGRATE, which allows for the establishment of prior distributions, can resolve these problems and produce more reliable results (Beerli 2006). Indeed, initial runs on our data using the ML implementation showed nonconvergence; however, switching to the Bayesian method eliminated this problem.

Parameters (Θ and m/μ) from preliminary runs of MIGRATE with uniform prior distributions (three long chains, 300 000 steps sampled, with a burn in of 10 000) were averaged and used to establish the boundaries for exponential prior distributions on a second run. We compared two approaches to the second run: the first (two long chains, 1 000 000 steps sampled, with a burn in of 10 000) used an adaptive heating scheme (start temperatures: 1.0, 1.2, 1.5, 3.0), combined over three replicate runs. The second used only one replicate, but the steps sampled and burn in were increased to 3 000 000 and 30 000, respectively. The second procedure improved results by narrowing the 2.5% and 97.5% confidence intervals. Given impractical computational demands associated with analysing all sampling locations in the same run, we minimized the number of parameters estimated by restricting each run to pairwise location comparisons.

Table 2 Genetic diversity indices for each species in Florida and Belize

	Leucothoe kensleyi					*Leucothoe ashleyae*					*Ophiothrix lineata*				
Location	*n*	*H*	*S*	*h*	π	*n*	*H*	*S*	*h*	π	*n*	*H*	*S*	*h*	π
Palm Beach	36	13	16	0.908	0.0075	30	3	5	0.191	0.0017	28	11	17	0.868	0.0036
Ft Lauderdale	82	25	28	0.914	0.0083	37	7	9	0.608	0.0065	28	11	19	0.783	0.0036
Long Key	31	10	11	0.763	0.0044	23	3	6	0.170	0.0013	29	12	20	0.874	0.0065
Key West	33	16	25	0.938	0.0091	29	2	1	0.069	0.0002	13	8	16	0.808	0.0065
Glover's Reef						17	3	2	0.324	0.0008	30	5	14	0.632	0.0046
Total	182	48	50			136	13	80			128	32	45		
Avg diversity				0.881	0.0073				0.272	0.0021				0.793	0.0050

n, sample size; *H*, number of haplotypes; *S*, number of segregating sites; *h*, haplotype diversity; π, nucleotide diversity.

Results

Diversity indices and population expansion

Genetic diversity indices are shown in Table 2. Overall, there were 48 haplotypes for *Leucothoe kensleyi* (*n* = 182, 422 bp), 13 for *Leucothoe ashleyae* (*n* = 136, 414 bp), and 32 for *Ophiothrix lineata* (*n* = 128, 718 bp). Average haplotype and nucleotide diversity across all three species ranged from 0.272 to 0.881 and from 0.0021 to 0.0073, respectively. *Leucothoe kensleyi* had the highest haplotype and nucleotide diversity. θ_w estimates for Florida were: *L. kensleyi* = 8.83, *L. ashleyae* = 2.06, and *O. lineata* = 5.40. θ_w estimates for Glover's Reef were: *L. ashleyae* = 0.59, and *O. lineata* = 3.79. The mismatch distribution for *L. kensleyi* was smooth and unimodal indicating a population expansion, whereas the distributions for *L. ashleyae* and *O. lineata* were bimodal and ragged indicating stable population sizes (Harpending *et al*. 1998) (Fig. 2). Tajima's *D* for each species corroborated the interpretation of the mismatch distributions, as the statistic was significantly negative for *L. kensleyi* indicating increasing population size, whereas the statistic was not significantly different from zero for *L. ashleyae* and *O. lineata* indicating stable population size (Tajima 1989) (Fig. 2).

Spatial patterns of population structure

AMOVA results are summarized in Table 3 and pairwise sponge comparisons provided in Appendices I, II and III. Hierarchal analysis of population differentiation among the four Florida reef system locations produced nonsignificant Φ_{CT} values for *L. kensleyi* and *O. lineata*. However, the result for *L. ashleyae* was highly significant (0.49) due to a divergent haplotype (five mutational steps; see Fig. 3b) that dominated (60%) the Fort Lauderdale location and was rare in the remaining Florida locations. When the Fort Lauderdale location was excluded from the AMOVA, the Φ_{CT} became nonsignificant (0.01). Overall, most of the genetic variation was found within individual host sponges: *L. kensleyi* ~ 80%,

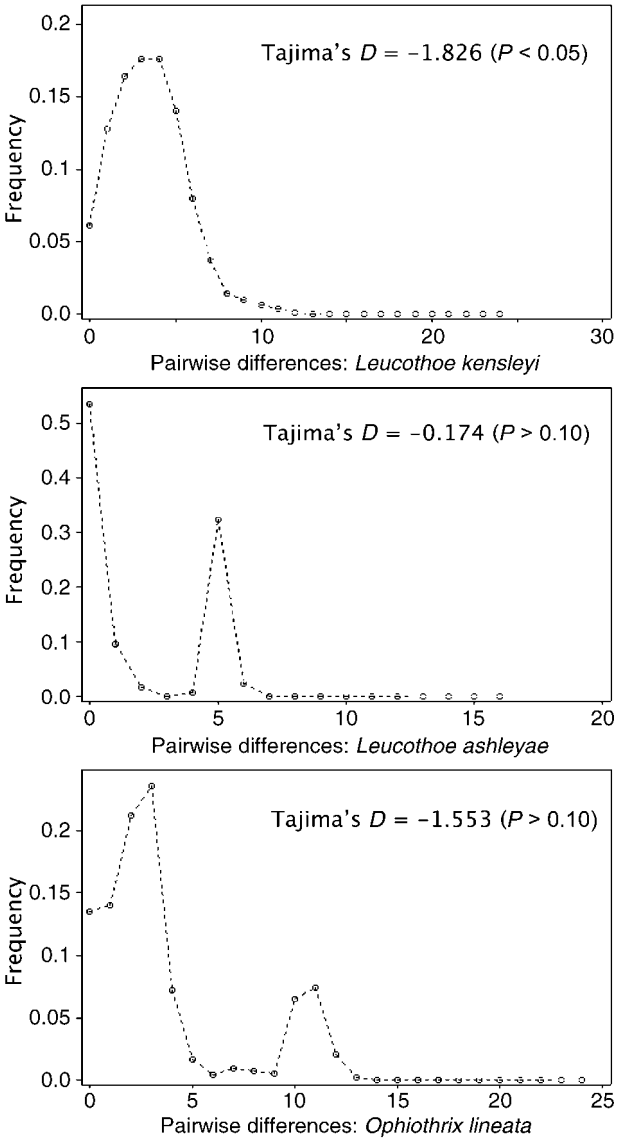

Fig. 2 Mismatch distributions and Tajima's D statistic for *Leucothoe kensleyi*, *Leucothoe ashleyae* and *Ophiothrix lineata* within the Florida reef system.

Table 3 Hierarchical analysis of molecular variance for Florida and Belize populations

Species	Location grouping	Variance component	% variance	Φ statistic	*P* value
L. kensleyi	PB, FT, LK, KW	Among locations	4.5	$\Phi_{CT} = 0.05$	0.155
		Among sponges	15.9	$\Phi_{SC} = 0.17$	*
		Within sponges	79.6	$\Phi_{ST} = 0.20$	*
L. ashleyae	PB, FT, LK, KW	Among locations	48.6	$\Phi_{CT} = 0.49$	*
		Among sponges	2.0	$\Phi_{SC} = 0.04$	0.158
		Within sponges	49.4	$\Phi_{ST} = 0.51$	*
	PB, LK, KW (FT excluded)	Among locations	1.4	$\Phi_{CT} = 0.01$	0.246
		Among sponges	–3.7	$\Phi_{SC} = -0.04$	0.740
		Within sponges	102.3	$\Phi_{ST} = -0.02$	0.651
	FL (FT included), GVS	Between locations	97.6	$\Phi_{ST} = 0.98$	*
		Within locations	2.4		
O. lineata	PB, FT, LK, KW	Among locations	2.7	$\Phi_{CT} = 0.03$	0.159
		Among sponges	10.6	$\Phi_{SC} = 0.11$	0.061
		Within sponges	86.7	$\Phi_{ST} = 0.13$	0.017
	FL, GVS	Between locations	62.1	$\Phi_{ST} = 0.62$	*
		Within locations	37.9		

PB, Palm Beach; FT, Fort Lauderdale; LK, Long Key; KW, Key West; FL, Florida; GVS, Glover's Reef. * $P < 0.00001$.

L. ashleyae ~ 49% (102.3% with Fort Lauderdale excluded), *O. lineata* ~ 87%. Genetic differentiation among sponges in the same location was significant only for *L. kensleyi*. Pairwise comparisons between the grouped Florida locations and Glover's Reef produced highly significant Φ_{ST} values for *L. ashleyae* (0.98) and *O. lineata* (0.62), and corrected average pairwise nucleotide distances of 20.3% for *L. ashleyae* and 1.1% for *O. lineata*.

In Florida reefs, the Mantel test detected significant association between Φ_{ST} and geographical distance only for *O. lineata* (Table 4). When Glover's Reef was included in the analysis, both *L. ashleyae* and *O. lineata* showed significant IBD.

Table 4 Mantel test results for genetic isolation by geographic distance

Species	Sampling sites	*r*	*P* value
L. kensleyi	SE Florida (BK, CR, JR, MC, ET, HC, PR)	0.27	0.109
L. ashleyae	SE Florida (BK, CR, ET, HC, PR)	0.12	0.205
	SE Florida & GVS	0.86	0.032
O. lineata	SE Florida (BK, CR, CU, ET, HC, PR)	0.52	0.029
	SE Florida & GVS	0.95	0.004

Palm Beach site: BK, Breakers Reef. Ft Lauderdale sites: CR, Cervicornis Reef; CU, Barracuda Reef; JR, Johnson Reef; MC, McAllister wreck. Long Key site: ET, East East Turtle Reef. Key West sites: HC, Hawk Channel Reef; PR, Patch Reef. Belize site: GVS, Glover's Reef.

Haplotype network estimation and nested clade analysis

The TCS analysis joined all *L. kensleyi* haplotypes into a single 8-step network at the 95% probability level (Fig. 3a). With the exception of two haplotypes (clade 1-1), all Florida Keys haplotypes (Long Key and Key West) are restricted to clade 3-2. TCS produced an 8-step network for *L. ashleyae* where Florida and Glover's Reef haplotypes were separated by 79 mutational steps and therefore not connected at the 95% probability level (Fig. 3b). *Leucothoe ashleyae* showed far less diversity of haplotypes than the other two species and its network was dominated by a single haplotype (HC121) that constituted 71% of the Florida populations. *Ophiothrix lineata* haplotypes were joined into an 11-step network at the 95% probability level with Glover's Reef haplotypes segregated from Florida haplotypes (Fig. 3c). All three species networks contained a small number of alternate connections between some haplotypes.

NCA detected significant associations between haplotypes and geography for all three species at various temporal scales (Table 5). However, the sum of the outgroup weights produced by TCS using the method of Castelloe & Templeton (1994) at the total cladogram nesting level for all three species was not greater than or equal to 0.95; therefore, all clades at the total cladogram level of nesting were regarded as tips in accordance with Templeton's (2004) revised inference key. Consequently, tip-interior clade status could not be determined, producing inconclusive outcomes for each species at the total cladogram level.

A useful property of NCA is that the temporal polarity of clades within the network can be combined with the distance measures associated with patterns of long-distance dispersal, contiguous range expansion, and long distance

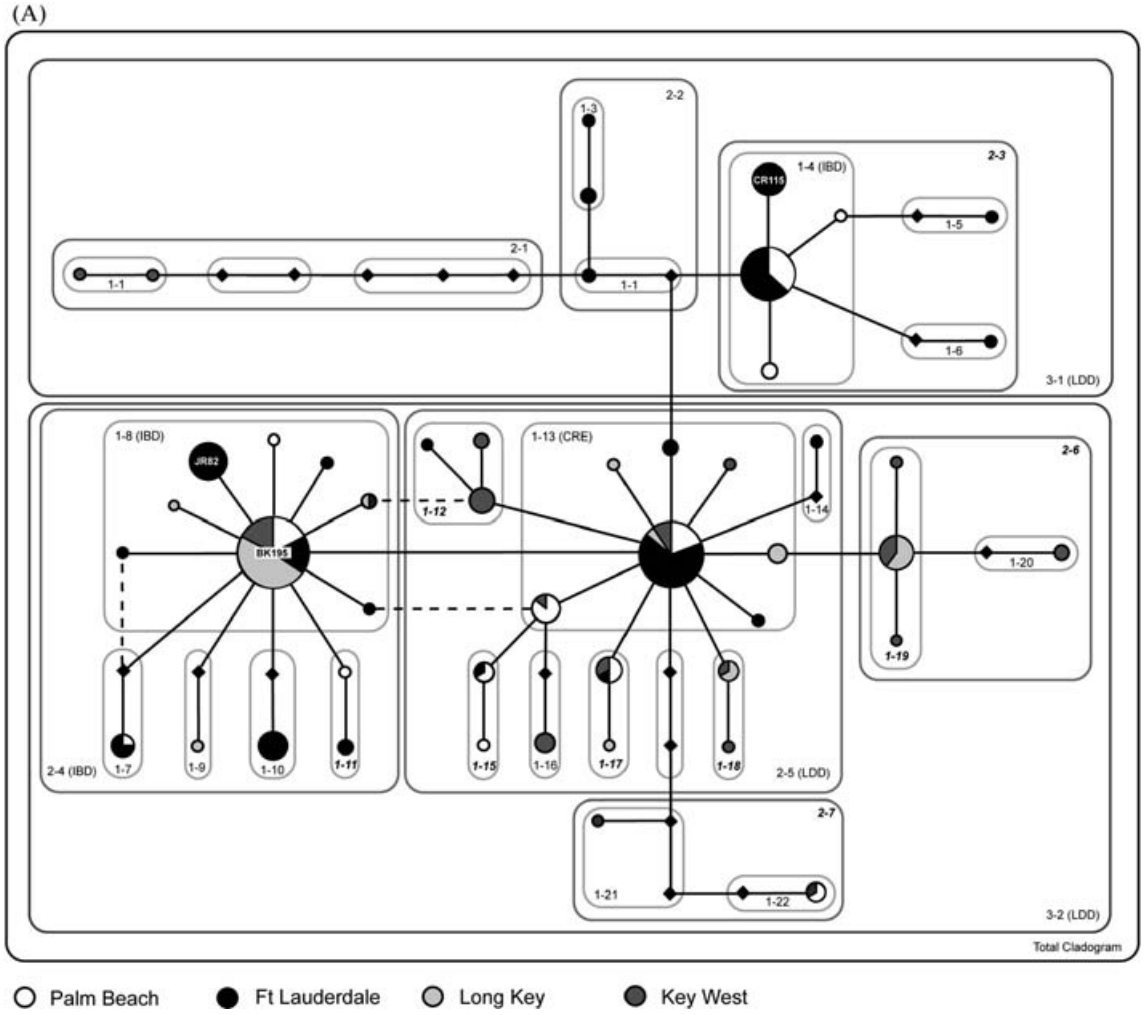

Fig. 3 Unrooted 95% probability haplotype network and nesting design for (A) *Leucothoe kensleyi* (B) *Leucothoe ashleyae*, and (C) *Ophiothrix lineata*. Circles represent individual haplotypes with circle size proportional to frequency of occurrence. Circle shading indicates the proportional distribution of each haplotype among the major sampling locations. Solid black diamonds indicate hypothetical missing haplotypes that were not sampled and connecting lines are equivalent to one mutational step. Dashed lines show alternative connections considered less likely (see text). The bold vertical line in Fig. 3(B) partitions two subnetworks separated by 79 mutational steps. Clades with significant NCA inferences are annotated as follows: IBD, restricted gene flow with isolation by distance; LDD, restricted gene flow with some long-distance dispersal; CRE, contiguous range expansion. Individual haplotypes with significant NCA distances are also labelled (also see Table 5). Nonsignificant clades are numbered in bold italics.

colonization to infer the direction of these movements (Templeton 2002). For example, haplotypes expanding out of an ancestral population will be more derived (younger) and are therefore likely to form tip haplotypes or clades, an expectation supported by coalescent theory (Castelloe & Templeton 1994). For *L. kensleyi*, inferences for clades 3-2, 3-1 and 2-5 were restricted gene flow with some long-distance dispersal. This pattern of gene flow is inferred when older haplotypes are widespread producing large interior clade distances (D_c), most younger haplotypes are less widespread producing small tip clade distances (D_c), and the few young dispersing haplotypes are very widespread producing large tip nested clade distances (D_n) (clades 1-12, 1-16, 2-1 and 2-6) (Templeton 1998). With the exception of one haplotype from Fort Lauderdale in clade 1-12, these four clades only contained haplotypes from Long Key and Key West indicating that the dispersal of *L. kensleyi* was from the northern portion of the reef system south into the Florida Keys (see Fig. 3a).

Apart from clade 1-13 (contiguous range expansion), the inference for the remaining *L. kensleyi* 2 and 1-step clades (2-4, 1-8 and 1-4) was one of restricted gene flow with IBD. Clade 1-13 was difficult to interpret as the only significant distance was the I-T D_c and the haplotypes were distributed evenly among populations. NCA also supported the inference of high gene flow for *L. kensleyi* along the Florida reef

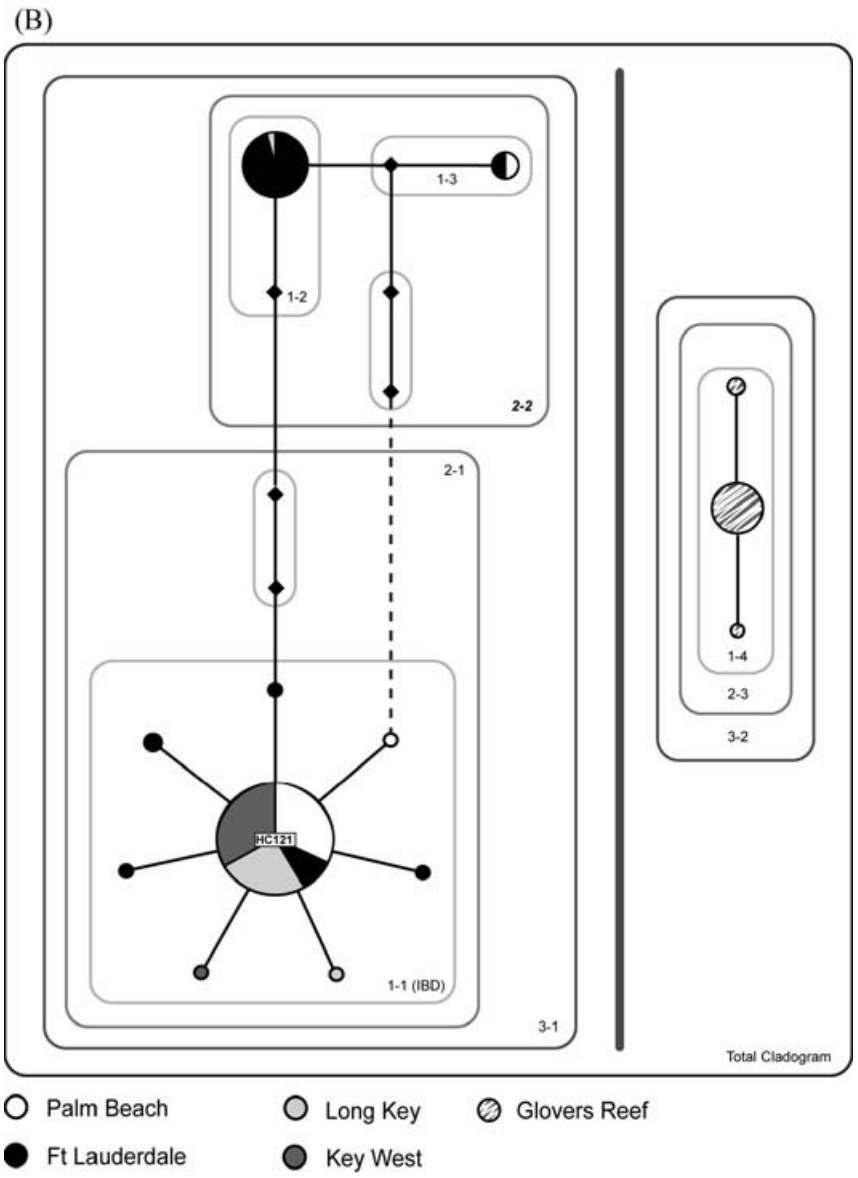

Fig. 3 *Continued*

system obtained from the AMOVA results, as six of the eight significant clades (TC and 1-13 excepted, Table 5) showed patterns of recurrent gene flow and the remaining nine non-significant clades were unable to reject the null hypothesis of a random association between haplotypes and geography (not shown).

Due to low *L. ashleyae* haplotype diversity, nesting resulted in only four clades, with three producing statistically significant results (Table 5). Analysis at the total cladogram and 3-step levels produced inconclusive outcomes due to indeterminate tip-interior status. The 1-step clade (1-1), containing the dominant Florida haplotype, gave an inference of restricted gene flow with IBD. Clade 1-2 contained the frequent ($n = 23$) and divergent haplotype (five steps from the dominant Florida haplotype HC121) that was responsible for creating the high level of population structure at Fort Lauderdale.

For *O. lineata*, NCA (Table 5) at the total cladogram level was again inconclusive due to indeterminate tip-interior status. For clade 3-3, it was not possible to discriminate between IBD vs. long-distance dispersal due to lack of intermediate geographical samples. The result for clade 3-1 was contiguous range expansion involving clades 2-1 and 2-2. Both clades contained a distribution of haplotypes from all Florida populations making it difficult to determine the geographical direction of this range expansion. Restricted gene flow with IBD was the inference for both 2-step clades.

NCA again supported the inferences of high gene flow along the Florida reef system obtained from the AMOVA results as two (2-2 and 2-1) of the five significant clades

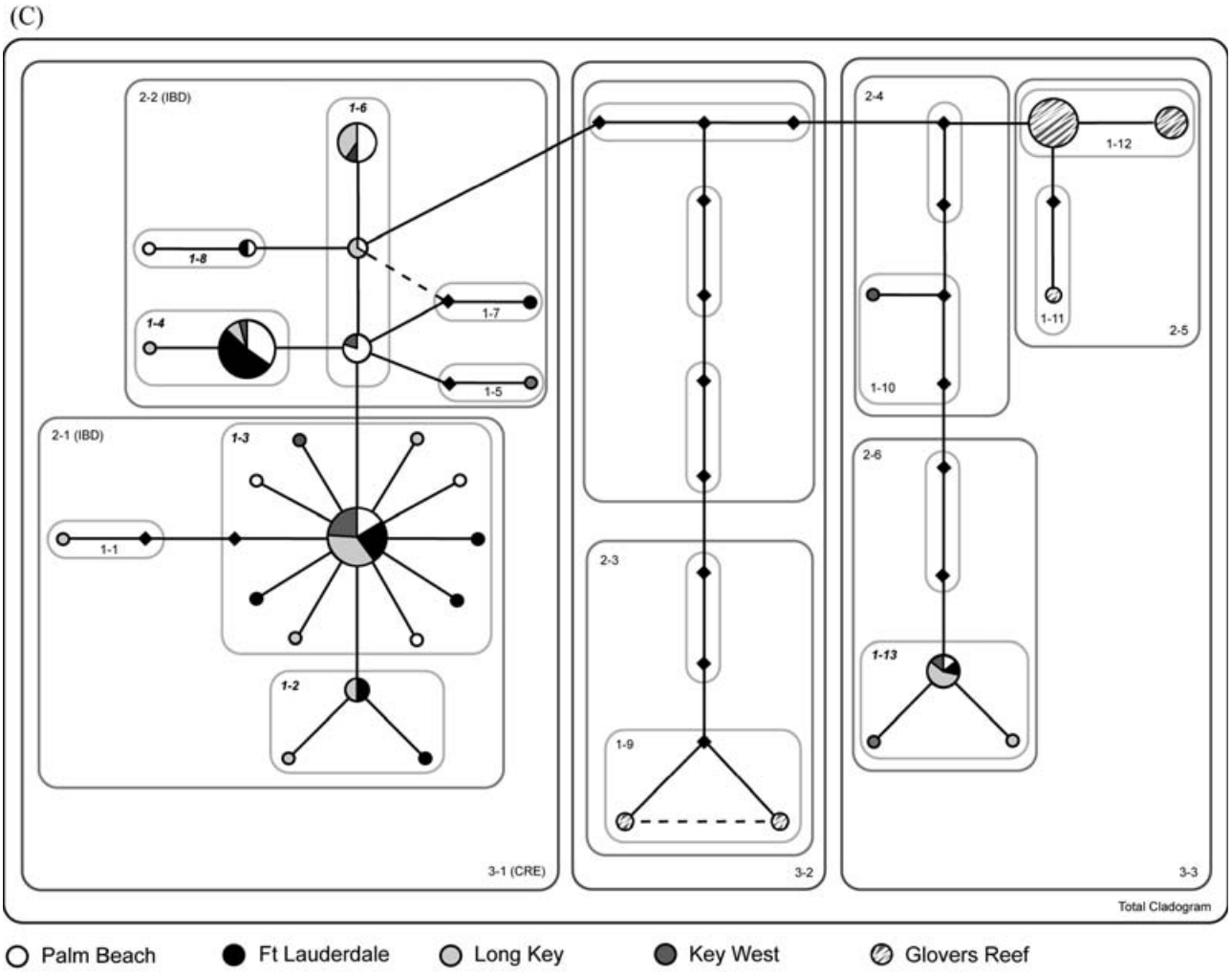

Fig. 3 *Continued*

showed patterns of recurrent gene flow and the remaining six nonsignificant clades were unable to reject the null hypothesis of a random association between haplotypes and geography (not shown).

Bayesian estimation of migration rates

Estimates of the number of migrants per generation among Florida locations for all species were generally high (Table 6). Conversely, rates between Florida and Glover's Reef were very low for *O. lineata* and *L. ashleyae*. Direction of migration along the Florida coastline for both amphipods was complex, with all pairwise location comparisons showing migration biased either to the north or south. Conversely, with the exception of the Palm Beach–Fort Lauderdale comparison, all the *O. lineata* comparisons showed a southerly migration bias with the rates progressively increasing southwards, becoming very large between locations in the Keys.

Discussion

Elucidating the roles of biological and/or physical factors in generating phylogeographical patterns in the marine realm is a topic of considerable interest for understanding the evolution of marine biodiversity. The precarious state and degenerating trajectory of coral reefs has lent additional urgency to understanding these roles for informed coral reef conservation efforts. Although the Florida coastline has been the focus of multiple phylogeographical studies (see Lee & Ó Foighil 2004 and references therein), none have focused specifically within Florida's coral reef system. Here, we have provided a reasonably detailed, multispecies view of the extent of genetic connectivity within the main Florida reef system. We also include a comparison between Florida reefs as a whole and a comparatively healthy Caribbean reef ecosystem (Belize). The main findings of this study are addressed below.

Table 5 Summary of nested clade analysis results for (A) *Leucothoe kensleyi*: 17 clades, (B) *Leucothoe ashleyae*: 4 clades, and (C) *Ophiothrix lineata*: 11 clades. Only clades with significant clade distances are shown ($P < 0.05$)

Clade	Subclade	D_c	D_n	Chain of inference	Inference
(A) *Leucothoe kensleyi*					
TC	3-1 (T)	S	S	1-2	Inconclusive outcome
	3-2 (T)	L	L		
3-2	2-4 (T)	S	S	1-2-3-5-6-7-YES	Restricted gene flow with long-distance dispersal
	2-5 (I)	L	L		
	2-6 (T)	S	L		
	I-T	L	L		
3-1	2-1 (T)	—	L	1-2-3-5-6-7-YES	Restricted gene flow with long-distance dispersal
	2-3 (T)	S	S		
2-5	1-12 (T)	S	L	1-2-3-5-6-7-YES	Restricted gene flow with long-distance dispersal
	1-13 (I)	S	S		
	1-16 (T)	—	L		
	I-T	—	S		
2-4	1-8 (I)	L	L	1-2-3-4-NO	Restricted gene flow with isolation by distance
	1-10 (T)	S	S		
	I-T	L	L		
1-13	I-T	S	—	1-2-11-12-NO	Contiguous range expansion
1-8	JR82 (T)	S	S	1-2-3-4-NO	Restricted gene flow with isolation by distance
	BK195 (I)	—	L		
	I-T	L	L		
1-4	CR115 (T)	S	S	1-2-3-4-NO	Restricted gene flow with isolation by distance
	I-T	L	—		
(B) *Leucothoe ashleyae*					
TC	3-1 (T)	S	S	1-2	Inconclusive outcome
	3-2 (T)	S	L		
3-1	2-1 (T)	L	L	1-2	Inconclusive outcome
	2-2 (T)	S	S		
1-1	HC121 (I)	L	L	1-2-3-4-NO	Restricted gene flow with isolation by distance
	I-T	L	—		
(C) *Ophiothrix lineata*					
TC	3-1 (T)	S	S	1-2	Inconclusive outcome
	3-2 (T)	S	L		
	3-3 (T)	—	L		
3-3	2-5 (T)	S	S	1-2-3-5-6-7-8-NO	Inadequate geographical sampling to discriminate between isolation by distance and long-distance dispersal
	2-6 (T)	S	L		
	I-T	L	—		
3-1	2-1 (T)	—	L	1-2-11-12-NO	Contiguous range expansion
	2-2 (I)	S	—		
	I-T	—	S		
2-2	1-6 (I)	L	L	1-2-3-4-NO	Restricted gene flow with isolation by distance
	I-T	L	—		
2-1	1-3 (I)	L	L	1-2-3-4-NO	Restricted gene flow with isolation by distance
	I-T	—	L		

TC, Total Cladogram; (T), tip clade; (I), interior clade; S, significantly small clade distance; L, significantly large clade distance. Dash indicates a nonsignificant distance. Chain of inference was according to Templeton's revised key (2004).

Amphipod genetic diversity

An interesting finding of this comparative study was the stark contrast in genetic diversity between the two amphipod congeners. In Florida, the nucleotide and haplotype diversity for *Leucothoe kensleyi* was over three times that of *Leucothoe ashleyae* and the θ_w estimate was over four times higher. Assuming equal mutation rates and selective neutrality of the COI gene, this result indicates that the effective female population size for *L. kensleyi* is approximately four times larger than that of *L. ashleyae*. This result is in concordance with the average ratio of approximately four *L. kensleyi* individuals to one *L. ashleyae* individual observed in a typical sponge sampled in Florida (J.D.

Table 6 Pairwise estimates of migration

		Number of immigrants/generation into receiving population			
Species	Comparison	2.5% percentile	Mean	97.5% percentile	Directional bias
L. kensleyi	PB into FT	6.5	27.3	68.3	PB & FT = South
	FT into PB	0.1	2.9	9.9	
	PB into LK	0.01	1.5	6.9	PB & LK = North
	LK into PB	0.5	7.2	23.4	
	PB into KW	0.6	9.6	32.7	PB & KW = South
	KW into PB	0.03	3.4	13.2	
	FT into LK	0.02	1.1	5.3	FT & LK = North
	LK into FT	3.9	17.9	45.2	
	FT into KW	0.02	1.3	6.0	FT & KW = North
	KW into FT	1.6	9.1	23.5	
	LK into KW	3.0	28.7	92.5	LK & KW = South
	KW into LK	0.2	4.9	16.3	
L. ashleyae	PB into FT	0.4	4.1	13.6	PB & FT = South
	FT into PB	0.1	1.8	7.3	
	PB into LK	0.01	4.1	21.2	PB & LK = South
	LK into PB	0.03	2.8	13.1	
	PB into KW	0.01	1.0	6.1	PB & KW = North
	KW into PB	0.3	6.2	28.0	
	FT into LK	0.1	2.8	13.7	FT & LK = North
	LK into FT	0.4	7.4	27.1	
	FT into KW	0.01	0.7	3.6	FT & KW = North
	KW into FT	0.1	3.2	11.7	
	LK into KW	0.01	1.0	6.4	LK & KW = North
	KW into LK	0.4	14.4	77.4	
	FL into GVS	0.001	0.02	0.1	None
	GVS into FL	0.003	0.04	0.2	
O. lineata	PB into FT	0.6	8.0	29.4	PB & FT = North
	FT into PB	0.9	17.7	60.0	
	PB into LK	1.1	12.1	36.8	PB & LK = South
	LK into PB	0.8	8.2	24.5	
	PB into KW	0.4	15.0	73.2	PB & KW = South
	KW into PB	0.01	3.9	16.3	
	FT into LK	1.7	24.1	83.4	FT & LK = South
	LK into FT	0.6	7.8	27.5	
	FT into KW	2.6	58.9	173.4	FT & KW = South
	KW into FT	0.1	3.5	12.7	
	LK into KW	5.9	60.9	161.5	LK & KW = South
	KW into LK	0.5	4.9	12.7	
	FL into GVS	0.004	0.5	1.9	None
	GVS into FL	0.02	0.4	1.6	

PB, Palm Beach; FT, Fort Lauderdale; LK, Long Key; KW, Key West; FL, Florida; GVS, Glover's Reef.

Thomas, personal observation). Interestingly, this result contrasts with our observations in numerous other Caribbean locations where *L. kenseyi* is rare but *L. ashleyae* common. A population expansion for *L. kensleyi* in Florida could explain these observations. This hypothesis is supported by the mismatch distributions and Tajima's D indicating a stable population size for *L. ashleyae* and a population expansion for *L. kensleyi*. Similarly, population expansions have been recorded for other crustaceans along the southeast United States coastline (McMillen-Jackson & Bert 2003, 2004a, 2004b).

Connectivity within the Florida reef ecosystem

As expected for a broadcast spawner, the brittle star *Ophiothrix lineata* showed high levels of gene flow along the Florida reef system. However, contrary to expectations of restricted dispersal based on brooding development, the amphipods *L. kensleyi* and *L. ashleyae* showed a surprising absence of population structure along the same stretch of coastline, with the exception of *L. ashleyae* off Fort Lauderdale (see next paragraph).

A curious finding was that *L. ashleyae* collected from multiple sponges at Fort Lauderdale exhibited high frequency of a haplotype that was rare elsewhere in the Florida reef system (only one other observation in Long Key), providing the only indication of population differentiation along the SE Florida coastline. A plausible explanation for this geographically restricted haplotype in the midst of otherwise high gene flow may be a dramatic reduction of local haplotypes with subsequent recolonization by a rare haplotype (Wade & McCauley 1988; Whitlock & McCauley 1990; Lessios *et al*. 1994). For example, interspecies competition among amphipods (e.g. Thiel 2000) could cause a decline or extinction of haplotypes locally, especially with a species like *L. ashleyae* that is less common than its cohabitating congener *L. kensleyi* in Florida reefs.

The high level of amphipod gene flow observed in Florida raises the question of how both species disperse so effectively along the whole Florida reef system when they lack a pelagic dispersal phase. Both amphipods inhabit the inner sponge canals where they filter feed (Thomas & Klebba 2006). The AMOVA results showed that the vast majority of genetic variation for the amphipods was within sponges, indicating that these commensals leave their hosts at some stage in their life cycle. Thiel (2000), for example, showed a seasonal shift in abundance for a leucothoid species inhabiting sponges along the Atlantic coast of Florida.

Precisely when and how amphipods leave their host is unknown. If the amphipods leave their hosts and crawl along the reef, given their commensal habit it is likely that they would only crawl relatively short distances until a suitable new host was found. Furthermore, the physical bounds of the reef structure would probably constrain the extent of dispersal by crawling. Therefore, if this were the only dispersal mechanism, and assuming equilibrium between gene flow and genetic drift, a pattern of fine scale population structure with IBD would be expected (Slatkin 1993). Interestingly, significant structure among sponges within locations was evident for *L. kensleyi* (Table 3; Appendix I); however, the overall result among locations showed high levels of gene flow with no signal of IBD. This finding implies that another mechanism (such as long-distance dispersal) is operating to homogenize overall haplotype frequencies and swamp any signal of IBD. A similar pattern was reported for corophiid amphipods along the New Zealand coastline where ocean currents were suggested as possible dispersal agents (Stevens & Hogg 2004) and for a species of talitrid amphipod in the Mediterranean Sea where ocean currents were shown to control dispersal via drifting wrack (De Matthaeis *et al.* 2000).

The NCA results provided statistical support for long-distance dispersal of *L. kensleyi* (clades 2-5, 3-1, 3-2) along the Florida coastline. A second major NCA inference for this species was that of restricted gene flow with IBD (clades 1-4, 1-8, 2-4). The combination of both inferences are congruent with the premise of fine-scale population structure (with IBD) among sponge/reef patches, overlaid by long-distance dispersal along the entire reef tract (as derived from the AMOVA results discussed above). Transport inside detached sponge fragments (see below) may provide a mechanism for such long-distance dispersal, also allowing for dispersal through the (presumably) unsuitable habitat surrounding patch reefs. Low genetic diversity (only 10 Florida haplotypes) is possibly why a long-distance dispersal pattern was not also detected for *L. ashleyae* by NCA (Templeton 1998), and could also explain why AMOVA failed to detect significant differentiation among sponges in this species.

Transport via sponges seems possible as asexual fragmentation is an important mode of dispersal for many species of branching sponge (Wulff 1991), and strong storms and hurricanes are able to detach and transport numerous sponge species (including *Callyspongia vaginalis*) from their place of origin (Wulff 1985, 1995a, 1995b). Furthermore, inspection of numerous *C. vaginalis* tubes drifting along the sediment often revealed the presence of live leucothoid amphipods (V. P. Richards, unpublished observations).

In contrast to the amphipod results, *O. lineata* exhibited a significant pattern of IBD (supported by NCA) along the same stretch of reef and there was no significant population structure evident among sponges. NCA results for the brittle star also contrasted with the amphipod results in that there was no inference of long-distance dispersal within Florida. Consequently, long-distance dispersal of the brittle star in detached sponge fragments appears uncommon, and although a few individuals may be transported in a drifting fragment, considerably more individuals will be dispersed via spawning (females can produce approximately 10 000 eggs; V.P.R., unpublished data). Reproductive strategy may therefore play a more important role in *O. lineata* dispersal dynamics.

Gene flow and migration patterns

Migration rates observed among Florida locations for each species indicate that levels of gene flow should be sufficient to override diversification due to genetic drift (Birky *et al*. 1983), and the lack of overall significant population structure detected by AMOVA and the high levels of gene flow inferred by NCA confirm this expectation.

For *L. kensleyi*, north to south dispersal was inferred by NCA in 18% of the clades, with 53% indicating panmixia. Interestingly MIGRATE also indicated north to south migration bias in 50% of the pairwise location comparisons, with the balance showing migration bias in the opposite direction. These results indicate gene flow in *L. kensleyi* is occurring in both directions, and not just south to north as expected if the Florida Current were the dominant dispersal agent.

The complex directionality of gene flow and migration detected for both amphipod species along the Florida coastline may result from sponge fragmentation and random

transport mediated by storms and hurricanes. In contrast, the strong north to south migration bias evident for *O. lineata* suggests that prevailing current patterns (see below) may have a more direct influence on the dispersal of its embryos, which likely act as passive propagules. Currents have been implicated in the high gene flow observed for several planktotrophic coastal species such as sea stars, crabs and barnacles (Hunt 1993; Bunch *et al*. 1998; Sotka *et al*. 2004). For the spiny lobster (*Panulirus argus*), Silberman *et al*. (1994) showed absence of genetic structuring along the SE Florida coastline and the strong northerly flow of the Florida Current was suggested as a probable cause. Similarly, Reeb & Avise (1990) hypothesized that the Florida Current was transporting American oyster (*Crassostrea virginica*) larvae northwards along the SE Florida coastline.

The strong north to south gene flow bias detected for *O. lineata* is contrary to the general assumption that the north-flowing Florida Current is the dominant transport mechanism in this region. The opposite directionality of the *O. lineata* gene flow might be explained in part by the well-characterized counter current that runs south along a large section of the Florida reef system through Hawk Channel (Lee & Williams 1999; Yeung & Lee 2002; see Fig. 1). The migration patterns in the north, which included a single northerly bias for the Palm Beach and Fort Lauderdale comparison (Table 6), possibly result from the complex pattern of counter currents and eddies which exist inshore of the Florida Current north of Miami (Lee & Mayer 1977; Shay *et al*. 2002; Soloviev *et al*. 2003).

The high levels of genetic connectivity between the northern and southern portions of the Florida reef system have important implications for the management and conservation of the Florida Keys reefs. The northern portions of the Florida reef system are situated immediately adjacent to areas undergoing extensive human population growth and urban development, which are likely to be adversely impacting coastal ocean habitat (Lapointe 1997; Finkl & Charlier 2003). Continued deterioration of the northern reefs with concomitant loss of migrants from the north could disrupt food webs and depress genetic diversity in the southern reefs, rendering their populations less able to respond to environmental stressors. Consequently, conservation efforts may also have to focus on Florida's northern reefs, which receive relatively much less management attention (Causey *et al*. 2002).

Florida and Belize population structure

Both *L. ashleyae* and *O. lineata* exhibited highly restricted levels of gene flow between the Florida reefs and Glover's Reef, Belize. The negligible migration rate (< 0.05), and very large Φ_{ST} value (0.98) and genetic distance (79 mutational steps; 20.3%) between the Florida and Glover's Reef populations for *L. ashleyae* indicate a substantial barrier to gene flow, likely resulting from the wide expanse of deep, open water separating these locations. Sponge transport is unlikely to provide an effective mode of long-distance dispersal across such a barrier because detached branching vase sponge tubes are negatively buoyant and if driven over the edge of the continental or insular slope and into deep water (e.g. the depth immediately surrounding Glover's Reef ranges from 400 m to 1000 m) (Gibson 2003), neither the sponge nor its commensal amphipods would likely survive.

High levels of intraspecific genetic distance for the COI gene have been observed in other amphipod species, e.g. *Gammarus pulex* = 8.2% (Meyran *et al*. 1997); *Hyalella azteca* = 8.7–27.6% (Witt & Hebert 2000), and were used to infer the presence of multiple cryptic species for *H. azteca*. The extremely high genetic divergence observed between the *L. ashleyae* of Florida reefs and Glover's Reef is also suggestive of cryptic speciation, warranting further investigation.

Several studies on species with planktonic dispersal, such as the spiny lobster (*P. argus*) and queen conch (*Strombus gigas*), within the Caribbean show a general lack of population structure (Mitton *et al*. 1989; Silberman *et al*. 1994). More specifically, comparisons between Florida and Central America for both tarpon (*Megalops atlanticus*) and elkhorn coral (*Acropora palmata*) have also revealed no genetic differentiation (Blandon *et al*. 2002; Baums *et al*. 2005). Here we have shown considerable population structure for the spawning brittle star *O. lineata* in this region. However, restricted dispersal has been detected elsewhere in the Caribbean for spawning fish species whose larvae have the ability for long-range dispersal (Shulman & Bermingham 1995; Taylor & Hellberg 2003), and both larvae behaviour and physical oceanographic factors have been suggested as probable causes. The nature of *O. lineata* dispersal is passive, thus eliminating behavioural restriction to dispersal and implicating physical oceanographic factors. Hence, deep water and possible entrapment in eddy currents over the Meso–American Barrier Reef System (Sheng & Tang 2003, 2004) could be factors. However, detection of a strong IBD signal within Florida and between Florida and Belize indicates that geographical distance may be the most important factor restricting gene flow in this species.

Conclusion

We have combined several analytical approaches to reveal information on genetic connectivity for three commensal species in a coral reef system in strong need of additional management and conservation measures to facilitate recovery (Pandolfi *et al*. 2005). The finding that all three species show substantial connectivity within the Florida reef system regardless of reproductive strategy points to the need for also considering geographical factors such as shallow coastlines and open expanses of deep water in a priori inferences about reef connectivity. The surprising

predominant north to south direction of gene flow in *Ophiothrix lineata* and to some extent in *Leucothoe kensleyi* underscores the importance of expanding our understanding of connectivity across diverse reef inhabitants to effectively inform the spatial management of coral reefs.

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Appendix I

Pairwise Φ_{ST} values between individual sponges for *Leucothoe kensleyi*

	BK3 (PB)	BK5 (PB)	BK4 (PB)	CR1 (FT)	CR3 (FT)	JR1 (FT)	MC1 (FT)	ET2 (LK)	ET3 (LK)	ET4 (LK)	HK1 (KW)	PR1 (KW)
BK5 (PB)	0.022											
BK4 (PB)	**0.165**	0.105										
CR1 (FT)	**0.174**	**0.165**	–0.036									
CR3 (FT)	0.054	**0.159**	**0.524**	**0.404**								
JR1 (FT)	**0.178**	**0.278**	**0.417**	**0.388**	**0.280**							
MC1 (FT)	0.040	0.054	0.023	0.035	**0.162**	**0.225**						
ET2 (LK)	**0.116**	**0.211**	**0.423**	**0.385**	**0.152**	**0.262**	**0.188**					
ET3 (LK)	**0.154**	**0.325**	**0.587**	**0.453**	**0.418**	**0.168**	**0.222**	**0.230**				
ET4 (LK)	0.031	**0.136**	**0.385**	**0.339**	0.086	**0.192**	0.121	–0.058	**0.211**			
HK1 (KW)	**0.090**	**0.164**	**0.307**	**0.314**	**0.119**	**0.218**	**0.141**	–0.013	**0.145**	–0.055		
PR1 (KW)	0.025	0.047	0.157	**0.239**	0.112	**0.228**	0.082	0.027	**0.241**	–0.045	–0.018	
PR7 (KW)	**0.089**	**0.129**	**0.356**	**0.353**	0.071	**0.286**	**0.157**	**0.074**	**0.285**	0.036	**0.072**	0.047

Significant values ($P < 0.05$) are indicated in bold.

Appendix II

Pairwise Φ_{ST} values between individual sponges for *Leucothoe ashleyae*

	BK3 (PB)	BK4 (PB)	BK5 (PB)	BK6 (PB)	CR1 (FT)	CR2 (FT)	CR3 (FT)	CR4 (FT)	ET2 (LK)	ET3 (LK)	ET4 (LK)	ET7 (LK)	PR1 (KW)	HC1 (KW)	PR5 (KW)
BK4 (PB)	–0.016														
BK5 (PB)	0.083	0.000													
BK6 (PB)	–0.120	–0.078	0.000												
CR1 (FT)	**0.711**	**0.861**	**0.890**	**0.764**											
CR2 (FT)	0.109	0.284	**0.379**	0.197	**0.269**										
CR3 (FT)	0.138	0.321	**0.414**	0.227	**0.264**	–0.103									
CR4 (FT)	**0.339**	**0.540**	**0.646**	**0.442**	0.092	–0.049	–0.061								
ET2 (LK)	0.037	–0.055	0.038	–0.022	**0.863**	**0.329**	**0.364**	**0.582**							
ET3 (LK)	–0.016	0.000	0.000	–0.078	**0.861**	0.284	**0.321**	**0.540**	–0.055						
ET4 (LK)	–0.128	–0.055	0.038	–0.117	**0.718**	0.112	0.135	**0.340**	0.000	–0.055					
ET7 (LK)	–0.060	0.000	0.000	–0.116	**0.852**	0.248	0.285	**0.500**	–0.098	0.000	–0.098				
HC1 (KW)	0.064	0.000	0.000	–0.014	**0.884**	0.359	0.395	**0.625**	0.020	0.000	0.020	0.000			
PR1 (KW)	0.016	0.000	0.000	–0.051	**0.870**	**0.313**	**0.349**	**0.573**	–0.024	0.000	–0.024	0.000	0.000		
PR5 (KW)	0.042	0.000	0.000	–0.031	**0.877**	0.337	**0.373**	**0.601**	0.000	0.000	0.000	0.000	0.000	0.000	
PR7 (KW)	0.055	–0.069	0.016	–0.010	**0.870**	**0.350**	**0.384**	**0.606**	0.001	–0.069	0.016	–0.109	–0.040	0.000	–0.018

Significant values ($P < 0.05$) are indicated in bold.

Appendix III

Pairwise Φ_{ST} values between individual sponges for *Ophiothrix lineata*

	BK1 (PB)	BK2 (PB)	BK3 (PB)	BK4 (PB)	BK6 (PB)	BK8 (PB)	CR1 (FT)	CR2 (FT)	CR3 (FT)	CU1 (FT)	CU2 (FT)	CU3 (FT)	CU4 (FT)	ET1 (LK)	ET2 (LK)	ET3 (LK)	ET4 (FT)	ET5 (LK)	ET6 (LK)	HC1 (KW)	HC2 (KW)	PR1 (KW)
BK1 (PB)																						
BK2 (PB)	0.259																					
BK3 (PB)	–0.081	–0.053																				
BK4 (PB)	0.250	–0.197	–0.091																			
BK6 (PB)	–0.119	0.085	0.037	0.034																		
BK8 (PB)	0.111	–0.114	–0.069	–0.033	0.155																	
CR1 (FT)	–0.119	–0.016	–0.054	0.003	–0.069	–0.008																
CR2 (FT)	0.250	0.124	0.133	0.156	0.044	0.145	–0.004															
CR3 (FT)	0.118	0.007	0.019	0.069	–0.014	0.009	–0.101	–0.263														
CU1 (FT)	–0.200	–0.108	–0.235	–0.054	–0.252	–0.186	–0.338	–0.200	–0.435													
CU2 (FT)	–0.030	–0.121	–0.114	–0.138	–0.007	–0.072	–0.041	0.054	–0.044	–0.263												
CU3 (FT)	–0.286	0.128	–0.100	0.160	–0.125	0.017	–0.183	0.053	–0.125	–0.615	–0.079											
CU4 (FT)	–0.200	–0.108	–0.235	–0.054	–0.252	–0.186	–0.338	–0.200	–0.435	–1.000	–0.263	–0.615										
ET1 (LK)	0.209	–0.010	0.087	0.091	**0.200**	0.030	0.032	–0.076	–0.133	–0.160	0.060	0.068	–0.160									
ET2 (LK)	0.251	–0.113	0.067	–0.023	**0.198**	–0.030	0.051	0.102	–0.015	–0.116	0.005	0.116	–0.116	0.010								
ET3 (LK)	0.010	0.012	0.087	–0.042	–0.323	0.156	–0.055	–0.009	–0.064	–0.267	–0.014	–0.050	–0.267	0.156	0.128							
ET4 (LK)	0.293	0.221	0.325	0.149	–0.041	0.380	**0.255**	0.252	0.226	0.111	0.218	0.261	0.111	**0.390**	0.333	–0.278						
ET5 (LK)	–0.059	–0.197	–0.200	–0.200	–0.085	–0.170	–0.128	0.000	–0.125	–0.393	–0.235	–0.167	–0.393	–0.004	–0.080	–0.098	0.165					
ET6 (LK)	0.516	0.459	**0.539**	0.387	0.093	**0.595**	0.430	0.443	0.426	0.338	0.429	0.478	0.338	**0.578**	0.538	–0.196	–0.316	0.394				
HC1 (KW)	0.800	0.134	0.250	0.286	0.163	0.125	–0.001	0.200	0.000	0.250	0.146	0.500	0.250	–0.048	–0.074	0.031	0.272	0.167	0.500			
HC2 (LK)	0.500	–0.053	0.040	0.100	0.064	–0.087	–0.098	0.063	–0.154	–0.200	–0.030	0.182	–0.200	–0.130	–0.137	–0.031	0.238	–0.059	0.471	0.000		
PR1 (LK)	0.298	0.259	**0.344**	0.205	0.003	**0.395**	**0.277**	0.267	0.242	0.141	0.256	0.271	0.141	**0.398**	**0.357**	–0.215	–0.223	0.210	–0.262	0.276	0.247	
PR3 (LK)	0.020	0.033	–0.021	0.020	–0.162	0.054	–0.150	–0.063	–0.200	–0.500	–0.112	–0.200	–0.500	0.047	0.051	–0.200	0.133	–0.200	0.331	0.250	0.020	0.165

Significant values ($P < 0.05$) are indicated in bold.