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Incongruent patterns of genetic connectivity among four ophiuroid species with differing coral host specificity on North Atlantic seamounts

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Abstract

Seamounts are considered to play a defining role in the evolution and diversity of marine fauna, acting as 'stepping-stones' for dispersal, regional centers of genetic isolation and speciation, and refugia for deep-sea populations. This study focused on the patterns of dispersal and genetic connectivity of four seamount ophiuroid species (*Asteroschema clavigera*, *Ophiocreas oedipus*, *Ophioplinthaca abyssalis*, and *Ophioplinthaca chelys*) displaying differing levels of associative (epifaunal) specificity to cold-water coral hosts inhabiting the New England and Corner Rise Seamount chains, and Muir Seamount in the North-western Atlantic. Analyses of mt16S and mtCOI revealed evidence for recent population expansion and high gene flow for all four species. However, species-specific genetic differentiation was significant based on seamount region and depth. Significant differences were found among regional seamount groups for *A. clavigera*, within seamount regions and seamounts for *O. chelys*, among 250-m depth intervals for *A. clavigera*, among 100-m depth intervals for *O. oedipus*, and there were indications of isolation by distance for *A. clavigera* and *O. oedipus*. In addition, *A. clavigera* and *O. oedipus*, broadcast spawners with high fidelity to specific coral hosts, displayed predominantly westward historical migration, whereas the ophioplinthacids, with lower host-specificity, displayed predominantly eastward migration. No congruent patterns of historical migration were evident among species and seamounts, yet these patterns can be correlated with species-specific host specificity, specific depth strata, and dispersal strategies. Conservation efforts to protect seamount ecosystems should promote multi-species approaches to genetic connectivity, and consider the impact of the 'dependence' of biodiversity on host fauna in these vulnerable marine ecosystems.

Introduction

The mechanisms of faunal dispersal are key unknowns in understanding the connective processes that shape biogeography, regional endemism, biodiversity, and approaches to protect vulnerable marine ecosystems in the deep sea. Seamounts, mountains that rise more than 1000 m from the surrounding sea floor but are not emergent above the sea surface (Staudigal *et al.* 2010), can be considered

dynamic agents where these processes are pronounced. Often formed by mantle plumes associated with mid-plate hot spots, mid-ocean ridge volcanism, and plate tectonic shifts (Fryer & Fryer 1987; Epp & Smoot 1989; Batiza 2001), the number of seamounts has been conservatively estimated at more than 800 in the Atlantic, 30,000–50,000 in the Pacific, and more than 100,000 (>1 km elevation) throughout the world's oceans (Epp & Smoot 1989; Rogers 1994, 2004; Wessel *et al.* 2010), collectively covering

an area more than 28 million km² (Etnoyer *et al.* 2010). As such, they may fundamentally impact the evolutionary processes of life in the deep ocean.

Functionally, seamounts provide elevated hard-substrate habitats above a typically sedimented deep sea, and often in the form of chains perpendicular to coastlines and mid-ocean ridges. Thus, they possess the potential to play a significant role in facilitating the dispersal and maintenance of faunal species, isolating populations, promoting speciation, and driving the biogeographic structure of the deep ocean (*e.g.* Hamilton 1956; Hubbs 1959; Rogers 1994; Shank 2010a). Several studies have found high levels of seamount endemism, suggesting that seamounts act as foci of genetic isolation, speciation, and refugia for relict populations (Rogers 2004; Clark *et al.* 2010). Wilson & Kaufmann (1987) estimated that 15% of the invertebrate fauna on seamounts were endemic. Higher endemism levels of 16–33% were found among invertebrate species on the seamounts off Tasmania (Koslow *et al.* 2001), while 29–34% of the macro- and megafauna of seamounts in the Tasman Sea and Southeast Coral Sea were potential seamount endemics (Richer de Forges *et al.* 2000). Even higher rates were found on the Nasca and Sala-y-Gomez seamount chains off the coast of Chile, which had apparent invertebrate endemism levels of 52% (Parin *et al.* 1997). It is widely noted that results of such endemism studies are highly sensitive to regional sampling efforts and methods, with difficulty distinguishing between true local endemism and sampling biases, particularly those arising from the occasional collection of rare but widespread species (O'Hara 2007; McClain *et al.* 2009; Clark *et al.* 2010).

In contrast to the aforementioned morphological studies, genetic studies of bamboo coral species on seamounts in the Southwest and Northwest Pacific Ocean (Smith *et al.* 2004a) and of octocoral and antipatharian seamount populations in the Northwest Atlantic (Thoma *et al.* 2009) have revealed a notable lack of endemism. Endemism infers the isolation of populations and species on evolutionary and ecological timescales. In this regard, seamounts can be considered 'isolated' only if significant historical or modern day genetic exchange can be shown to be significantly restricted (and not simply geographically distant) (Shank 2010a). To infer the 'isolating' processes responsible for structuring seamount populations, genetic studies have examined the intra-specific distribution and connectivity of populations on different seamounts with varying results. The majority of studies targeting fish and larger mobile fauna have revealed little to no genetic or geographical isolation between seamounts. For example, investigations of polymorphic allozymes in the fish *Hyperoglyphe antarctica* and three mitochondrial gene regions in the fish

Pseudopentaceros wheeleri revealed a lack of differentiation between different populations inhabiting neighboring seamounts and adjacent non-seamount areas (Martin *et al.* 1992; Bolch *et al.* 1993). In contrast, other population genetic studies of several invertebrates have shown genetic differentiation between even closely spaced (*e.g.* less than 50-km) seamount populations, such as chemosynthetic mussels inhabiting seamounts near New Zealand (Smith *et al.* 2004b) and populations of the coral *Corallium lauuense* among several seamounts and islands in the Hawaiian Archipelago (Baco & Shank 2005). A study of invertebrates from the Norfolk Ridge seamounts in the Southwest Pacific revealed variable results with no genetic structure for species with planktotrophic larvae: two galatheid squat lobsters, *Munida thoe* and *Munida zebra*, two chirostylid squat lobsters, *Eumunida* species and the gastropod *Sassia remensa*, but significant structure was detected for the non-planktotrophic gastropod *Nassaria problematica* (Samadi *et al.* 2006). The variable results of these and other studies (Shank 2010a) suggest both high levels of genetic connectivity as well as genetic isolation of seamount populations depending on the taxa examined, molecular markers employed, and the particular seamount region studied. Differing results of genetic connectivity among seamount populations have been attributed to a consistent variety of explanations, largely the distinct differences in larval dispersal modes (*e.g.* Samadi *et al.* 2006), depth differences of available habitat, and inference of physical oceanographic circulation, including both long-distance transport driven by global circulation patterns as well as theoretical local hydrodynamics that act as barriers or agents of retention to dispersal (*e.g.* Smith *et al.* 2004b). An additional potential explanation that has yet to be investigated is the marked differences in the habitat requirements of invertebrate species in association with biological hosts.

To assess the specificity and abundance of these coral host-epifaunal invertebrate relationships on seamounts, the distribution and host specificity of 22 ophiuroid morphospecies on the Northwestern Atlantic seamounts were surveyed via high-definition video (Cho 2008). Markedly varying degrees of ophiuroid association with their coral hosts were identified. In particular, four ophiuroid species were identified as having distinctive habitat requirements or host-associate patterns (Cho 2008). The ophiuroid *Asteroschema clavigera* Verrill (1894) displayed a high fidelity in association with two gorgonian corals *Paramuricea* sp. and *Paragorgia* sp. All observations (726 total occurrences) consistently revealed that this ophiuroid occurred with either one to two individuals with arms wrapped amongst the branches of *Paramuricea* sp. or one to many individuals on *Paragorgia* sp. The ophiuroid

Ophiocreas oedipus Lyman (1879) was associated with the coral *Metallogorgia melanotrichos* with only a single individual occurring on the central axial stalk of a single host coral (Mosher & Watling 2009). *Ophioplinthaca abyssalis* Cherbouet & Sibouet (1972) predominantly occurred as tens of individuals on the coral host *Candidella imbricata* (1285 total occurrences), but this species was also observed on other hosts and substrate. *Ophioplinthaca chelys* Wyville Thomson (1878) was observed predominantly on hard sea floor habitats, lacking an association with a particular coral host.

Understanding the role of seamounts in structuring the regional biogeography and biodiversity has reached a critical level given the current threats of fisheries and mining activities that target seamount ecosystems and mineral resources (Clark *et al.* 2010). For example, deep-sea fishing techniques such as trawling and long-line fishing have been shown to destroy benthic habitat, in particular the coral habitat upon which many invertebrates live (Probert *et al.* 1997; Kaiser 1998; Thrush *et al.* 1998; Probert 1999; Waller *et al.* 2007). With expanding deep-sea fisheries and the concurrent disturbance of seamount habitats, particularly those occupied by slow-growing cold-water corals, conservation efforts for these areas have increased (Clark *et al.* 2010). Relevant to the present study is the precautionary closure of four seamount areas in the North Atlantic to deep-sea fisheries activities, including 25 peaks on 13 fishable seamounts in the Corner and New England seamount chains (Waller *et al.* 2007; Shank 2010b). Understanding the extent of genetic connectivity between seamounts and seamount regions provides strategic information for the creation and design of marine protected areas, conservation strategies and efforts, information similarly utilized for the conservation of shallow coral reef communities (Palumbi 2003; Richards *et al.* 2007).

To assess (and inform managers with insights into) the extent of genetic connectivity among North Atlantic seamounts and the hypothesis that these seamounts act either as isolating mechanisms or stepping-stones for invertebrate dispersal, we examined mt16S and mt COI gene sequence data from populations of four ophiuroid species inhabiting the New England and Corner Rise Seamounts. In addition to examining the fundamental question of gene flow and isolation among seamounts in different regions of the North Atlantic, correlations of host-specificity with patterns of gene flow were investigated to assess the potential effect host or habitat specificity may have on genetic structure. The varying fidelity to their coral hosts (Cho 2008; Shank 2010a) and current knowledge of species-specific reproductive modes (Tyler 1980; Hendler 1991; McEdward & Miner 2001; Mosher & Watling 2009) in the four target ophiuroid species were

used to provide *a priori* expectations of relative connectivity based on these ecological differences. As such, we predicted that *A. clavigera* and *O. oedipus* would have similar patterns of dispersal and rates of gene flow as both are considered to be broadcast spawners and maintain highly specific associations with coral hosts through which genetic connectivity may be influenced by similar intrinsic and extrinsic co-evolutionary forces (*e.g.* smaller effective population sizes and selection for specific hosts). Distinctive (or contrasting) patterns were expected between these broadcast spawning ophiuroid species and the two (potentially brooding) ophioplinthacid species, most notably *O. chelys*, as this ophiuroid displays no association with any faunal host.

Methods

Study region

The seamounts of the Northwest Atlantic include three regional seamount groups, the New England Seamounts, the Corner Rise Seamounts, and Muir Seamount (Fig. 1). The New England Seamounts comprise the longest seamount chain in the North Atlantic consisting of more than 35 major peaks with elevations ranging from 400 to 4000 m (Shank 2010b). The seamount chain extends 1200 km in a northwest-southeast direction, with Bear Seamount on the continental slope south of the Georges Bank and Nashville Seamount at the end of the Bermuda Rise (Zheng & Arkani-Hamed 2002). The Corner Rise Seamounts are a cluster of seamounts between the Mid-Atlantic Ridge and the New England Seamount chain. The Corner Rise Seamounts consist of ~50 seamounts (Cairns 2007) whose western-most end is ~300 km east of the New England Seamounts. Muir Seamount, an elongate seamount located north of Bermuda, is separated by ~420 and ~950 km at its closest point to the New England and Corner Rise Seamounts, respectively.

The physical oceanographic circulation in the region is dominated by the dynamics of the Gulf Stream flow and eddy system. The mean directional flow of the Gulf Stream bisects the New England Seamounts near Manning Seamount (Fig. 1) and continues northeast well above the Corner Rise Seamounts to at least 2000 m depth (Qiu 1994). This interaction is believed to form gyres to depths of 2000–3000 m, with circulation occurring in opposite directions (*e.g.* to the east in the north and to the west in the south) (Ezer 1994; Qiu 1994). Circulation models based on empirical current data suggest a predominant 'gyre-like' circulatory pattern that encompasses almost the entirety of the Corner Rise Seamounts to depths greater than 2000 m (see Fig. 7, Qiu 1994).

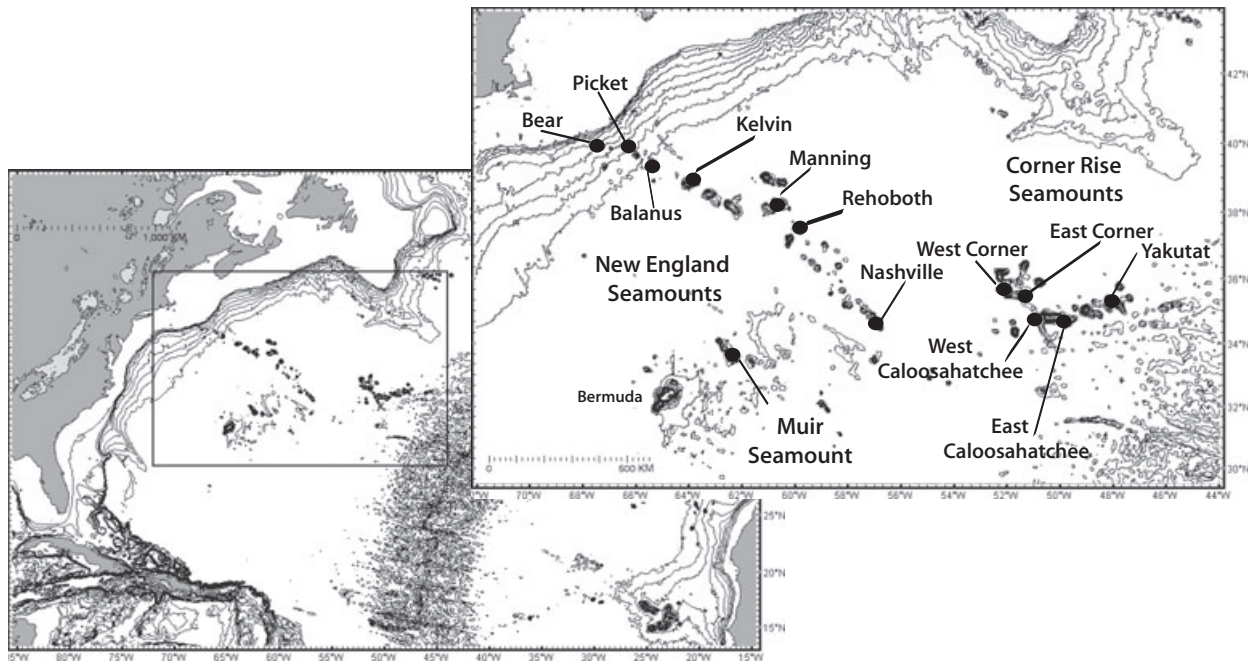


Fig. 1. Regional map of the North Atlantic with inset indicating the seamounts of the New England Seamount chain, the Corner Rise Seamounts, and Muir Seamount from which samples were obtained for this study.

Sample collection

Individuals of the four target ophiuroid species, *Asteroschema clavigera* Verrill (1894), *Ophiocreas oedipus*, Lyman (1879), *Ophioplinthaca abyssalis* Cherbonnier & Sibouet (1972), and *Ophioplinthaca chelys* Wyville Thomson (1878) were collected from 12 seamount peaks in 2005 during the 2005 Deep Atlantic Stepping Stones research expedition aboard the R/V *Ronald H. Brown* using the ROV *Hercules* (Table 1). In 2003, specimens from Manning and Muir Seamount were collected using the DSV *Alvin* and the R/V *Atlantis*. Both *Alvin* and *Hercules* utilized Kraft Predator force-reflecting manipulator arms for the collection of individual host corals and ophiuroid associates, insulated bioboxes for *in situ* sample storage, and two suction vacuum samplers for additional specimen collection. The manipulator claw was modified with a custom-designed cutting blade to aid in the collection of host corals and attached ophiuroid associates. Individuals were processed immediately after the recovery of the vehicles, and initially sorted and identified on board ship to preliminary morphospecies. Arms of individuals identified as *A. clavigera* and *O. oedipus* were subsampled and frozen for shore-based genetic analyses and the remaining whole individuals were preserved in 4% formalin, and transferred after 24 h to 70% ethanol. Whole individuals of *O. abyssalis* and *O. chelys* were frozen for subsequent genetic analysis. Museum voucher specimens were pro-

vided to the Yale-Peabody Museum (New Haven, CT) and the National Museum of Natural History (Washington, DC) for morphological confirmation of species identification. Additional ophiuroid specimens from Bear Seamount were collected on research cruise DE 02-06 in 2002 aboard the R/V *Delaware II* and preserved in 70% ethanol. Samples from Picket Seamount were processed and frozen following collection by DSV *Alvin* in 2005 during research cruise AT12-1.

DNA extraction, PCR, and sequencing

Genomic ophiuroid DNA was extracted using a 5% Chelex extraction protocol described previously (Walsh *et al.* 1991; Sponer & Roy 2002). A 1–3-mm piece of ophiuroid arm tissue was minced, added to 500 μ L of 5% Chelex solution (Bio-Rad, Hercules, CA), and heated for 3 h at 65 °C with the tissue completely submerged within the Chelex beads. The solution was then vortexed, heated to 95–100 °C for 10 min, and then centrifuged at 13,500 *g* for 10 min. Ethanol-preserved samples were processed in the same way with the addition of an initial rinse of the tissue in deionized water. The genomic extracts were stored at 4 °C for short-term storage (weeks) and –20 °C for long-term storage (months).

Both mitochondrial 16S and COI genes have been used in genetic studies of ophiuroids including the identification of cryptic species in ophiuroids (Baric & Sturmbauer

Table 1. Summary of the gene region and number of individuals examined by seamount and geographic regions, in this study. The depth distribution (in 50-m depth bins) of these individuals is provided for each species and gene region dataset.

Geographic region	Seamount peak	16S				COI		16S+COI		
		<i>Asteroschema clavigera</i>	<i>Ophiocreas oedipus</i>	<i>Ophioplithaca abyssalis</i>	<i>Ophioplithaca chelys</i>	<i>Asteroschema clavigera</i>	<i>Ophiocreas oedipus</i>	<i>Asteroschema clavigera</i>	<i>Ophiocreas oedipus</i>	
New England	Bear	6	–	–	–	–	–	–	–	
Seamounts	Picket	5	–	–	1	–	–	–	–	
	Balanus	1	2	16	1	1	2	1	2	
	Kelvin	9	2	25	15	5	2	5	2	
	Rehoboth	10	5	23	13	7	5	7	5	
	Manning	39	–	1	5	14	–	13	–	
	Nashville	5	5	13	–	3	5	3	5	
Corner Rise	W. Corner	1	1	8	–	1	1	1	1	
	Seamounts	E. Corner	3	4	27	9	2	4	2	4
		E. Caloosahatchee	2	2	–	14	2	2	2	2
		W. Caloosahatchee	–	2	–	–	–	1	–	1
Yakutat	2	1	–	10	2	1	1	1		
Muir	Muir	8	1	–	–	–	2	–	1	
Seamount	Depth bin									
	1300–1350	36	–	–	1	14	–	13	–	
	1350–1400	–	1	–	–	–	1	–	1	
	1400–1450	–	1	–	–	–	1	–	1	
	1450–1550	–	–	–	1	–	–	–	–	
	1450–1500	–	–	–	2	–	–	–	–	
	1500–1550	1	1	–	14	–	1	1	1	
	1550–1600	4	1	–	9	1	1	–	1	
	1600–1650	4	1	–	–	–	–	–	–	
	1650–1700	1	1	2	–	1	1	1	1	
	1700–1750	2	1	1	–	–	1	–	1	
	1800–1850	9	3	8	13	7	3	7	3	
	1850–1900	–	–	8	–	–	–	–	–	
	1900–1950	6	1	55	7	2	1	2	1	
	1950–2000	4	–	–	9	–	–	–	–	
	2000–2050	1	1	–	–	–	1	–	1	
	2050–2100	1	3	4	–	1	3	1	3	
	2100–2150	10	6	20	–	5	6	5	6	
	2150–2200	3	1	–	4	4	1	3	1	
	2200–2250	1	3	12	–	–	3	–	3	
2250–2300	–	–	–	–	–	1	–	–		

1999; Sponer *et al.* 2001; Roy & Sponer 2002; Sponer & Roy 2002; Hunter & Halanych 2008). However, COI has been noted as a difficult gene to use reliably with specific ophiuroid species (Sponer *et al.* 2001). To identify informative population genetic markers in ophiuroids, several molecular markers were examined for their utility to detect genetic variation, including mitochondrial 16S, COI, COX2, NAD5, ATP6, nuclear ITS, and internally developed microsatellites (W. Cho, unpublished observations). NAD5, ATP6, nuclear ITS, and the microsatellite markers were largely invariable, whereas COX2 and ATP6 were too inconsistent in amplification for reliable use. Two mitochondrial DNA markers consistently amplified gene fragments from the four species of ophiuroids.

Specifically, one fragment from the 16S rRNA mitochondrial gene was sequenced for all four species and one fragment from the mtCOI was sequenced for *A. clavigera* and *O. oedipus*.

A fragment of the mitochondrial 16S rRNA (16S) gene was amplified with the universal primers 16SarL (5'-CGCCTGTTTATCAAAAACAT-3') and 16SbrH (5'-CCGGTCTGAACCTCAGATCACGT-3') (Palumbi *et al.* 1991). These primers performed consistently for all four species and were used for sequencing 426 bp of the 16S gene for *A. clavigera*, 466 bp for *O. oedipus*, 432 bp for *O. abyssalis*, and 421 bp for *O. chelys*. Most fragments were amplified in 25 μ L PCR reactions containing 1 \times PCR buffer (Promega), 2.5 mM MgCl₂, 1.0 mM of each

dNTP, 0.5 μM of each primer, 0.5 M betaine, 1–2 μL of extracted template, and 0.2 U of Taq polymerase (Promega). Some fragments were amplified using 1 \times PCR Buffer (Eppendorf), 2.5 mM MgCl_2 , 1.0 mM of each dNTP, 0.5 μM of each primer, 1 \times TaqMaster PCR Enhancer (Eppendorf), 1–2 μL of extracted template, and 0.2 U of Taq polymerase (Eppendorf). PCR was performed under the following conditions: initial denaturation for 2 min at 94 °C, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 1 min, and extension at 72 °C for 45 s, followed by a final extension phase of 72 °C for 7 min.

A fragment of the mitochondrial COI gene was amplified for analyses from *A. clavigera* and *O. oedipus* samples using the primers mCOIF100 for (5'-GCCGGAAGTGTAGGAACAG-3') and COIR1340a rev (5'-GAGTATCGTCGGGCAT-3') developed by Dr. Paula Cisternas (personal communication). These primers amplified 924 bp of mtCOI for *A. clavigera* and 1161 bp for *O. oedipus* in 25 μL PCR reactions under the same PCR conditions as described above except for modifications to the annealing temperature. Genomic DNA was amplified with an annealing temperature of 56 °C for *A. clavigera* and 50 °C for *O. oedipus*.

Amplicons of 16S were purified directly using either the QIAquick PCR Purification kit (Qiagen) or the SV Gel and PCR Cleanup Kit (Promega) and eluted into a final 30 μL volume. PCR products of COI were gel purified using the QIAquick Gel Extraction kit (Qiagen) and the SV Gel and PCR Cleanup Kit (Promega) and eluted into a final 30 μL volume. Purified PCR products were then used in one-eighth format sequencing reactions using Big Dye terminators (version 3, Perkin-Elmer) in 96-well plates and purified by isopropanol precipitation before sequencing on an ABI 3730XL capillary sequencer. PCR products were sequenced bi-directionally for all ophiuroids except for three individuals (one *A. clavigera* individual from Manning Seamount, one *O. abyssalis* individual and one *O. chelys* individual from Kelvin Seamount) from which one direction was sequenced.

Genetic analyses

Mitochondrial COI and 16S sequences were edited using SEQUENCHER 4.8 (Gene Codes Corporation Inc.) and aligned using MAFFT v6.527 (Katoh *et al.* 2002). Alignments were viewed by eye in MACCLADE 4.08 (Maddison & Maddison 2000) and COI sequences were translated to amino acids to ensure that stop codons were not present. Representative haplotypes are available in GenBank (*A. clavigera* 16S: HM587828–HM587849, COI: HM587850–HM587877; *O. oedipus* 16S: HM587797–HM587799, COI: HM587878–HM587887; *O. abyssalis*

16S: HM587813–HM587827; *O. chelys* 16S: HM587800–HM587812). Intra-specific genetic diversity was evaluated by computing the number of haplotypes, the number of polymorphic sites, haplotype diversity (h), and nucleotide diversity (π) for all populations greater than one individual. Pairwise Φ_{ST} were computed between the seamount populations creating a genetic distance matrix in ARLEQUIN (Excoffier *et al.* 2005). To examine for congruent patterns of genetic differentiation and migration, a series of analyses (below) were performed for each species and each molecular marker separately (*i.e.* the four 16S datasets for *A. clavigera*, *O. oedipus*, *O. abyssalis*, and *O. chelys*; the two COI datasets for *A. clavigera* and *O. oedipus*; the two concatenated 16S+COI datasets for *A. clavigera* and *O. oedipus*).

Analyses of molecular variance

Analyses of molecular variance (AMOVA) were conducted using the eight datasets described above using ARLEQUIN v3.11 (Excoffier *et al.* 2005). The pairwise Φ_{ST} values were used in a series of analyses of molecular variance (AMOVA) implemented with ARLEQUIN to test for the geographic isolation of populations and regional groups based on seamounts. The AMOVA was performed with three hierarchical levels: within each seamount, among seamounts but within regional groups, and among regional groups where the regional groups were defined as the New England Seamounts, the Corner Rise Seamounts, and Muir Seamount. To detect and examine genetic structure on the scale of 'adjacent seamounts,' a series of AMOVA were also performed with a sliding window (Plouviez *et al.* 2009), grouping three 'adjacent'-sampled seamount populations together at a time, moving from west to east. The pairwise Φ_{ST} and AMOVA were each performed with 10,000 permutations using the Kimura-2-parameter model (Kimura 1980).

A series of AMOVA was performed to test for genetic structure at depth intervals over which the populations were sampled (Table 1). Samples were grouped into 50-m depth bins except for a single *O. chelys* individual whose sampling depth could only be resolved to a 100-m depth bin. Pairwise Φ_{ST} were computed between the depth bins and used in ARLEQUIN to test for genetic differentiation structured by depth. The AMOVA was performed with three hierarchical levels: within each 50-m depth bin, among depth bins but within depth intervals, and among depth intervals. To identify potentially broader patterns of genetic structure based on depth, the 50-m depth bins were grouped into 100-, 250-, and 500-m depth intervals to systematically test for genetic differentiation at different scales within the 1300–2300 m range of the samples available. For those species

and markers where significant differentiation was detected based on depth, a series of sliding window AMOVA was also performed. For the COI and 16S+COI datasets of *A. clavigera*, four 250-m depth intervals were examined to identify depth intervals containing genetic structure. For the COI and 16S+COI datasets of *O. oedipus*, three 'adjacently'-sampled 100-m depth intervals were grouped, proceeding from shallow to deep. The pairwise Φ_{ST} and AMOVA were each performed with 10,000 permutations using the Kimura-2-parameter model (Kimura 1980).

The Mantel test (Mantel 1967) of matrix comparison was used (in ARLEQUIN) to test for isolation-by-distance, comparing the matrix of Kimura-2-parameter genetic distances between seamount populations and a matrix of direct (over water) geographical distances between seamounts. Tajima's *D* (Tajima 1989) test statistic for neutral selection was calculated and the goodness-of-fit test was performed within ARLEQUIN to determine whether the mismatch distributions of the observed data fit a model of rapid population expansion for each species.

Nested clade analysis

Parsimony networks were constructed with mtDNA haplotypes from each species, and for eight datasets (*Asteroschema clavigera*: 16S, COI, 16S+COI; *Ophiocreas oedipus*: 16S, COI, 16S+COI; *Ophioplinthaca abyssalis*: 16S, *Ophioplinthaca chelys*: 16S) in TCS 1.21 (Clement *et al.* 2000), using a 95% connection limit between haplotypes and treating gaps as a fifth state. Network ambiguities were resolved using criteria derived from coalescent theory wherein haplotypes are more likely to be connected to other haplotypes that occur with higher frequency, are located interior of the cladogram, and are from populations that are geographically close (Crandall & Templeton 1993; Pfenninger & Posada 2002). Nested clade analysis was then performed using ANECA v1.2 (automated nested clade analysis) (Panchal 2006; Panchal & Beaumont 2007) which uses GEODIS 2.5 (Posada *et al.* 2000) and automates the inference process. Although there has been debate over the effectiveness of nested clade approaches, including the high frequency of false positives (Panchal & Beaumont 2007; Petit 2008a,b), when used as part of a 'battery of analyses' to validate its results (Garrick *et al.* 2008) it can be a practical and appropriate method to identify evolutionary processes and geographical patterns in population genetic data (Templeton 1998, 2004, 2008; Templeton *et al.* 2000; Panchal & Beaumont 2007; Beaumont & Panchal 2008; Garrick *et al.* 2008; Petit 2008a,b). Nested clade analysis was used in this way in this study, as it is one of a series of analyses among which support and congruence are sought in the results. The

haplotype networks were also transformed into geographic maps of haplotype distributions (Figs 2–4).

Bayesian estimation of migration rates

Historic levels of migration between seamount regions were estimated via the software package MIGRATE version 3.0.3 using the Bayesian framework, which provides estimates for the posterior probability density of the model parameters, given the data (Beerli & Felsenstein 2001). For these analyses, seamounts were grouped by two regions, the New England Seamounts and the Corner Rise Seamounts for all four species (mirroring the AMOVA analyses). Based on results of the sliding AMOVA, analysis of *Asteroschema clavigera* was performed by grouping Nashville Seamount with the Corner Rise Seamounts and an additional analysis of *Ophioplinthaca chelys* seamount populations was performed grouping into three regions, the western New England Seamounts, eastern New England Seamounts, and the Corner Rise Seamounts. Muir Seamount was excluded due to lack of COI data from this location. Estimates of population genetic parameters were obtained from a random sample of 10 individuals from each location for each marker for each species, except for *Ophiocreas oedipus*, which had a total of nine samples from the Corner Rise Seamounts. Given our use of mitochondrial genes, the HKY model was used with back mutation (Won & Hey 2005; Johnson *et al.* 2006). Preliminary runs were conducted to establish the uniform prior distributions for the parameters θ (minimum = 0.005, maximum = 10) and M (minimum = 0, maximum = 7500). One long chain was used with one replicate, 5,000,000 total steps sampled, with a burn-in of 50,000 and a heating scheme with start temperatures of 1.0, 1.5, 3.0, and 1000. We assessed convergence by the comparison of results from multiple runs.

Results

From 12 seamount populations, mitochondrial sequence data was obtained from a total of 91 individuals of *Asteroschema clavigera*, 25 individuals of *Ophiocreas oedipus*, 113 individuals of *Ophioplinthaca abyssalis*, and 68 individuals of *Ophioplinthaca chelys* for 16S. For mtCOI, a total of 37 sequences were obtained from *A. clavigera*, and 25 sequences from *O. oedipus* (Table 1). Overall haplotypic diversity for all populations greater than one individual for COI was higher than that for 16S in *A. clavigera* and *O. oedipus*. In the 16S dataset of *A. clavigera*, haplotype number ranged from 2 to 13, the number of polymorphic sites ranged from 0 to 14, haplotype diversity ranged from 0.33 to 1.00, and nucleotide diversity ranged from 0 to 0.0024. In the COI dataset of

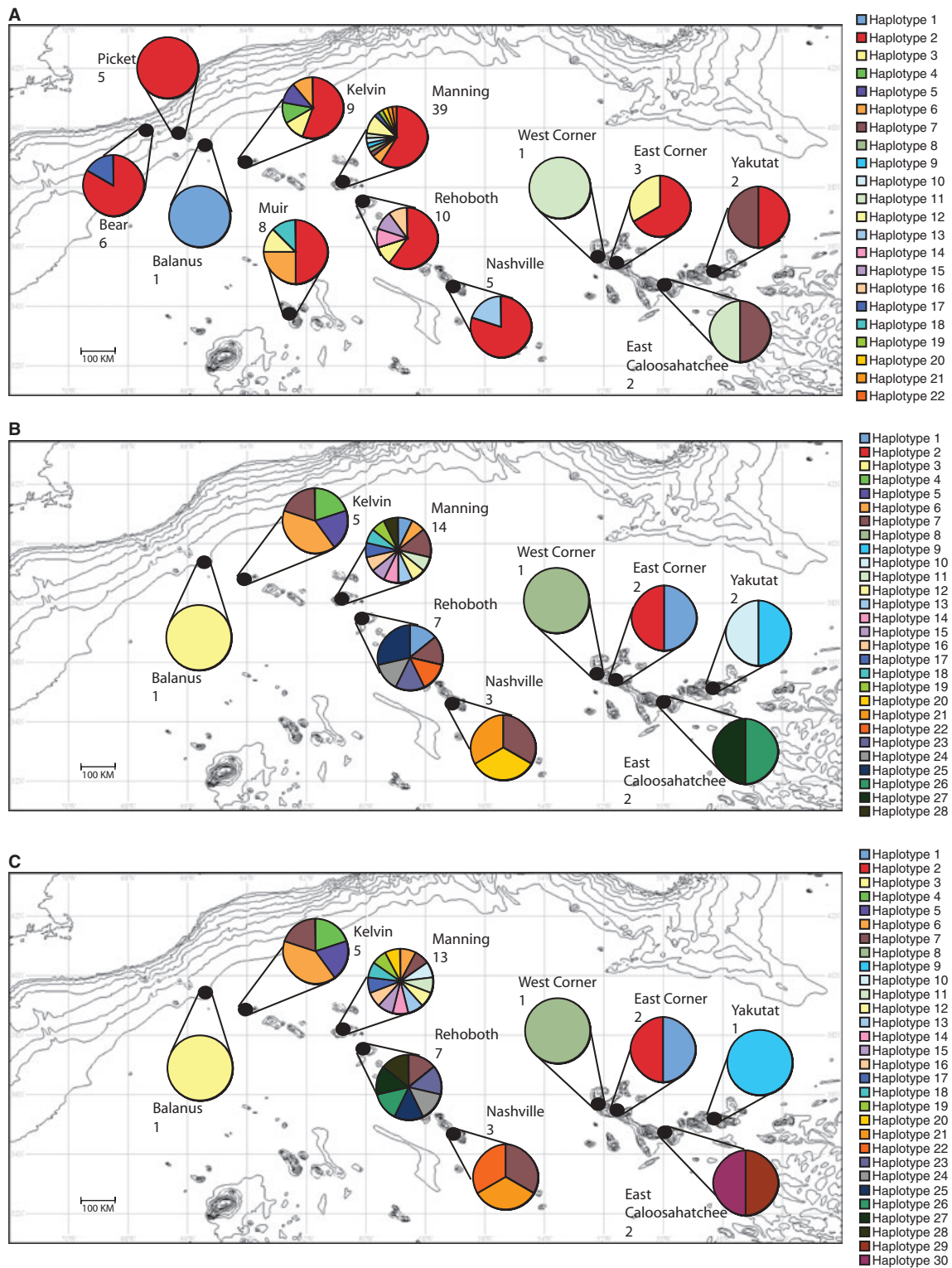


Fig. 2. Haplotype map for *Asteroschema clavigera* (A) 16S sequences, (B) COI sequences, and (C) 16S+COI sequences. Pie graphs indicate haplotypic composition of each location and numbers indicate total number of samples from each location.

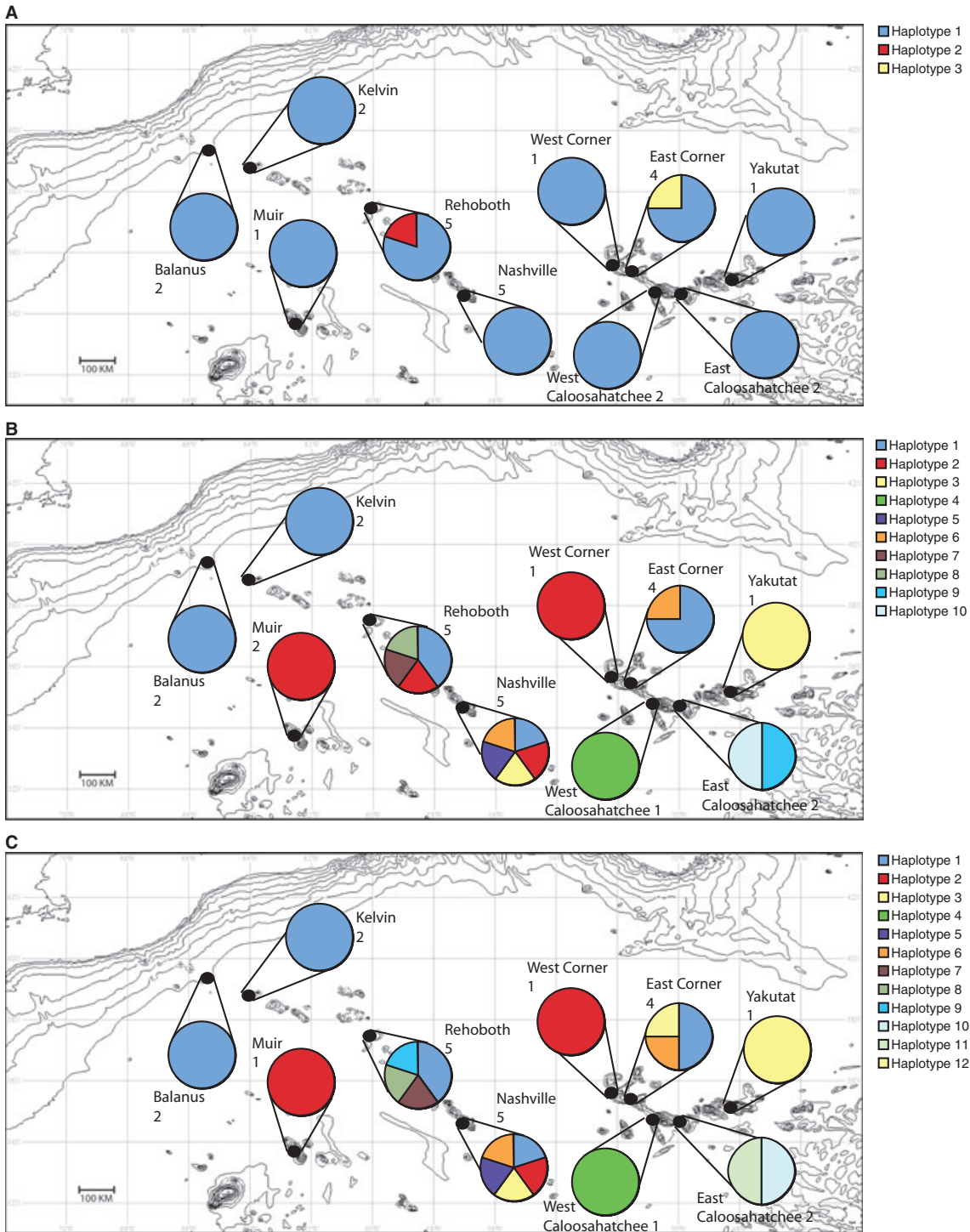


Fig. 3. Haplotype map for *Ophiocreas oedipus* (A) 16S sequences, (B) COI sequences, and (C) 16S+COI sequences. Pie graphs indicate haplotypic composition of each location and numbers indicate total number of samples from each location.

A. clavigera, haplotype number ranged from 2 to 13, the number of polymorphic sites ranged from 4 to 23, haplotype diversity ranged from 0.90 to 1.00, and nucleotide

diversity ranged from 0.0037 to 0.0098. In the 16S+COI dataset of *A. clavigera*, haplotype number ranged from 2 to 13, the number of polymorphic sites ranged from 5 to

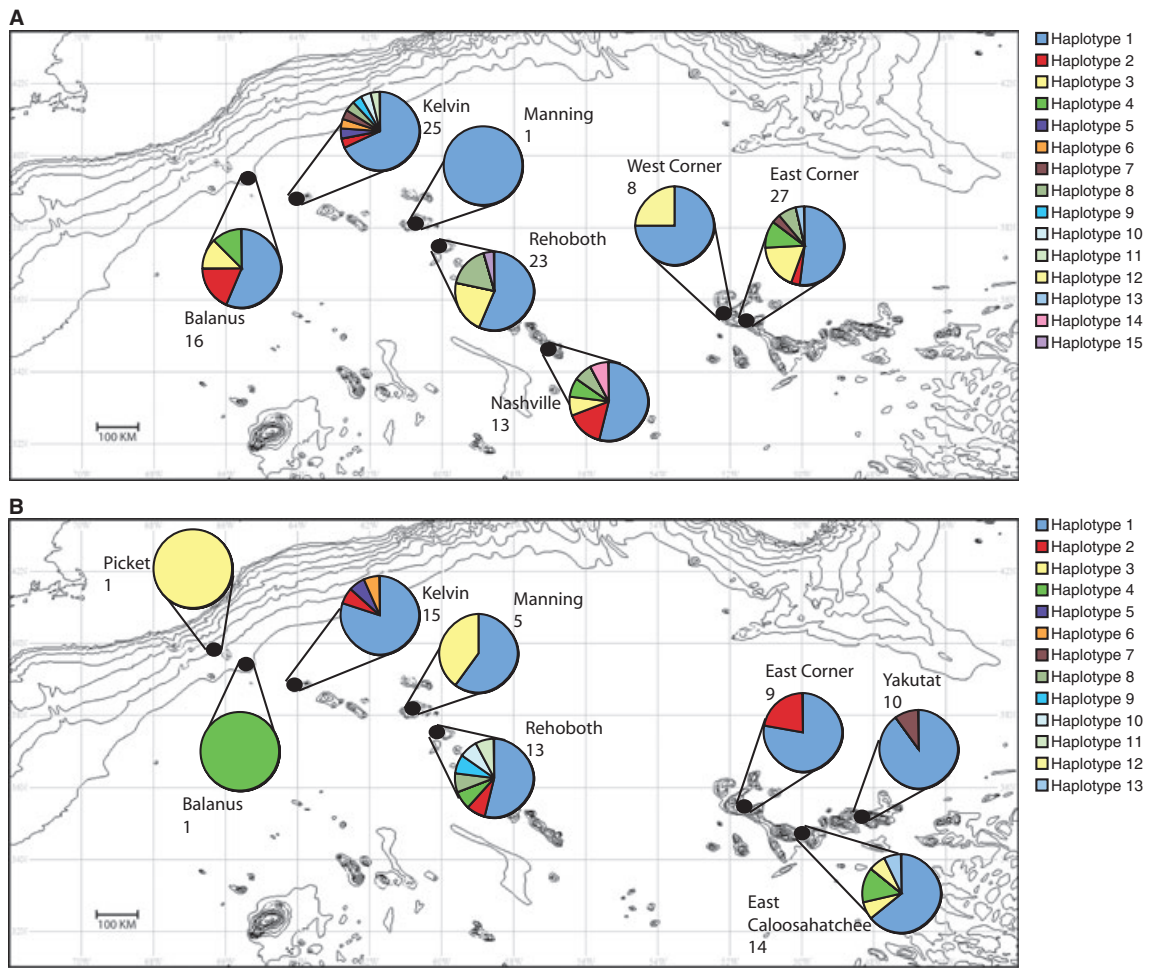


Fig. 4. Haplotype map for (A) 16S sequences of *Ophioplathaca abyssalis* and (B) 16S sequences of *Ophioplathaca chelys*. Pie graphs indicate haplotypic composition of each location and numbers indicate total number of samples from each location.

27, haplotype diversity ranged from 0.90 to 1.00, and nucleotide diversity ranged from 0.0025 to 0.0075. In the 16S dataset of *O. oedipus*, haplotype number ranged from 1 to 2, the number of polymorphic sites ranged from 0 to 1, haplotype diversity ranged from 0 to 0.50, and nucleotide diversity ranged from 0 to 0.0011. In the COI dataset of *O. oedipus*, haplotype number ranged from 1 to 5, the number of polymorphic sites ranged from 0 to 5, haplotype diversity ranged from 0 to 1.00, and nucleotide diversity ranged from 0 to 0.0043. In the 16S+COI dataset of *O. oedipus*, haplotype number ranged from 1 to 4, the number of polymorphic sites ranged from 0 to 5, haplotype diversity ranged from 0 to 1.00, and nucleotide diversity ranged from 0.00093 to 0.0031. In the 16S dataset of *O. abyssalis*, haplotype number ranged from 2 to 9, the number of polymorphic sites ranged from 2 to 11, haplotype diversity ranged from 0.43 to 0.72, and nucleotide diversity ranged from 0.0017 to 0.0039. In the 16S dataset of *O. chelys*, haplotype number ranged from 2 to

7, the number of polymorphic sites ranged from 1 to 7, haplotype diversity ranged from 0.20 to 0.73, and nucleotide diversity ranged from 0.00093 to 0.0026.

Haplotype distribution

The geographical distributions of the ophiuroid haplotypes in each species are presented in Figs 2–4.

Asteroschema clavigera

There were a total of 16 unique haplotypes in the New England Seamount chain, many found in only a single individual (Fig. 2A). Each of the *Asteroschema clavigera* haplotypes from the Corner Rise Seamounts was shared by populations within the New England Seamount chain. Haplotype 2 was the dominant 16S haplotype and was distributed across both the New England and Corner Rise Seamounts. Most haplotypes of COI for *A. clavigera* were unique and occurred singly, except for Haplotypes 1, 6, 7,

and 25. Haplotype 1 was shared across the New England and Corner Rise Seamount chains, six haplotypes were restricted to the Corner Rise Seamounts, and the remaining 21 haplotypes were restricted to the New England Seamounts (Fig. 2B). The 16S+COI haplotype network showed a similar pattern, with six haplotypes restricted to the Corner Rise Seamounts and 24 haplotypes restricted to the New England Seamount chain and most haplotypes occurring only once except for haplotypes 6 and 7 (Fig. 2C).

Ophiocreas oedipus

A single dominant 16S haplotype for *Ophiocreas oedipus* (Haplotype 1) was observed across the New England, Corner Rise, and Muir Seamounts (Fig. 3A). Haplotype 2 was observed only on Rehoboth Seamount and Haplotype 3 only on East Corner Seamount. COI was more variable than 16S in *O. oedipus*. One haplotype was shared across the New England, Corner Rise, and Muir Seamounts, three haplotypes were shared across the New England and Corner Rise seamounts, and three haplotypes were restricted to the New England and Corner Rise Seamounts each (Fig. 3B). A similar pattern was observed for the 16S+COI haplotype network with one haplotype shared across the three seamount regions, three haplotypes shared across the New England and Corner Rise

Seamounts, and four haplotypes restricted to the New England and Corner Rise Seamounts (Fig. 3C).

Ophioplinthaca abyssalis

Six 16S haplotypes of *Ophioplinthaca abyssalis* were shared across the New England and Corner Rise Seamounts, seven haplotypes were restricted to the New England Seamounts, and two haplotypes were restricted to the Corner Rise Seamounts (Fig. 4A).

Ophioplinthaca chelys

Four 16S haplotypes of *Ophioplinthaca chelys* were distributed across the New England and Corner Rise seamounts, six haplotypes were restricted to the New England Seamounts, and three haplotypes were restricted to the Corner Rise Seamounts (Fig. 4B).

Analyses of molecular variance

The AMOVA of *Asteroschema clavigera* populations revealed significant differentiation among the geographic regional groups (New England Seamount chain, Corner Rise Seamounts, and Muir Seamount) for the COI and 16S+COI datasets ($P < 0.05$, Table 2). The AMOVA for *Ophioplinthaca chelys* yielded significant differences within populations, which explained the largest amount

Table 2. Hierarchical AMOVA results of 16S, COI, and 16S+COI for the four ophiuroid species.

Species	Gene region	Source of variation	df	SS	Variance components	% variation	P value
<i>Asteroschema clavigera</i>	16S	among regional groups	2	0.964	0.01045	2.97	0.09109 ± 0.00258
		among populations within regional groups	9	3.034	-0.00079	-0.22	0.43287 ± 0.00505
		within populations	79	27.022	0.34205	97.25	0.37901 ± 0.00471
	COI	among regional groups	1	4.278	0.16105	6.8	0.02485 ± 0.00157
		among populations within regional groups	7	17.457	0.10699	4.52	0.34822 ± 0.00474
		within populations	28	58.781	2.09931	88.68	0.05218 ± 0.00249
	16S+COI	among regional groups	1	4.658	0.19376	7.05	0.01426 ± 0.00128
		among populations within regional groups	7	19.543	0.09372	3.45	0.45109 ± 0.00522
		within populations	26	63.931	2.45887	89.5	0.11079 ± 0.00342
<i>Ophiocreas oedipus</i>	16S	among regional groups	2	0.092	-0.00025	-0.32	0.79842 ± 0.00372
		among populations within regional groups	7	0.279	-0.2582	-33.34	1.00000 ± 0.00000
		within populations	15	1.553	0.10353	133.65	0.84545 ± 0.00360
	COI	among regional groups	2	1.531	-0.00088	-0.12	0.36693 ± 0.00502
		among populations within regional groups	7	5.358	0.01886	2.55	0.71515 ± 0.00387
		within populations	15	10.823	0.72155	97.57	0.40663 ± 0.00480
	16S+COI	among regional groups	2	1.116	-0.04058	-5.02	0.80653 ± 0.00418
		among populations within regional groups	7	5.624	-0.03441	-4.26	0.83475 ± 0.00425
		within populations	14	12.369	0.88353	109.27	0.71950 ± 0.00476
<i>Ophioplinthaca abyssalis</i>	16S	among regional groups	1	0.572	-0.00085	-0.13	0.62208 ± 0.00528
		among populations within regional groups	5	3.135	-0.00182	-0.28	0.40584 ± 0.00499
		within populations	106	69.157	0.65242	100.41	0.46020 ± 0.00480
<i>Ophioplinthaca chelys</i>	16S	among regional groups	1	0.171	-0.0175	-5.51	0.87822 ± 0.00341
		among populations within regional groups	6	3.539	0.0421	12.67	0.04832 ± 0.00183
		within populations	59	17.386	0.29468	92.84	0.03158 ± 0.00194

Significant values of $P < 0.05$ are in bold.

of total variance for 16S (92.84%, $P < 0.05$), followed by differences among populations within regional groups (12.67%, $P < 0.05$). No significant differentiation was detected for *Ophiocreas oedipus* and *Ophioplinthaca abyssalis* (Table 2).

The series of sliding window AMOVA (of adjacent seamounts) resulted in significant differentiation within *A. clavigera* seamount populations when Nashville Seamount and the Corner Rise Seamounts were grouped together. For *O. chelys*, the sliding window series of AMOVA found significant differentiation in all possible seamount groupings. No significant genetic differentiation was observed in the sliding window analysis of *O. oedipus* or *O. abyssalis*.

The AMOVA structured by depth intervals, with taxa grouped into 50-m depth bins for each species and molecular marker had a sampled depth range of 1300–2300 m (Table 1). Significant genetic differentiation among 250-m depth intervals in both the COI and 16S+COI datasets for *A. clavigera* ($P < 0.005$ and $P < 0.01$, respectively) was observed. Significant differentiation was also found among both the 100- and 500-m depth intervals for *O. oedipus* in both the COI and 16S+COI datasets ($P < 0.05$).

Mantel test of isolation by distance

The Mantel test revealed a significant correlation between genetic and geographic distances for both *Asteroschema clavigera* (16S: $P < 0.05$; 16S+COI: $P < 0.005$) and *Ophiocreas oedipus* (COI: $P < 0.05$; 16S+COI: $P < 0.05$) supporting isolation by distance for these species. The sliding window AMOVA detected significant differentiation at the 1550–1800 m depth region for *A. clavigera* and at the 1600 m depth horizon for *O. oedipus*.

Tajima's *D* test of neutrality and mismatch distribution

Tajima's *D* was found to be negative and significant in four populations of three ophiuroid species. The Rehoboth populations of both *Asteroschema clavigera* and *Ophioplinthaca chelys* had significant negative values of Tajima's *D* in 16S ($P < 0.05$ and $P < 0.01$, respectively). Similarly, the East Caloosahatchee Seamount population of *O. chelys* ($P < 0.05$), the Manning Seamount population of *A. clavigera* (16S: $P < 0.05$, COI: $P < 0.05$, 16S+COI: $P < 0.01$), and the Kelvin Seamount population of *Ophioplinthaca abyssalis* were also significantly negative ($P < 0.05$). The goodness-of-fit test of the model of rapid expansion could not be rejected for those seamount populations with negative Tajima's *D* values (data not shown). Such negative values typically signify an excess of low frequency polymorphisms, indicating population size expansion and/or positive selection.

Nested clade analysis

Parsimony network analysis of the 16S datasets for *Asteroschema clavigera*, *Ophiocreas oedipus*, and *Ophioplinthaca chelys* resulted in star-like phylogenies with a single central haplotype, identified as the 'ancestral' or 'root haplotype' (Crandall & Templeton 1993; Castelleo & Templeton 1994). The root haplotype was numerically dominant in each of these cases (Figs 5A and 6A,E). The haplotype network obtained from 16S sequences of *O. abyssalis* comprised 15 haplotypes, the two most common were centrally located within the network (Fig. 6D). This pattern is similar to the one observed for the COI and 16S+COI dataset of *O. oedipus* (Fig. 6B). The haplotype networks obtained from the COI and 16S+COI datasets of *A. clavigera* were highly reticulated and did not show the star-phylogeny found in the other datasets (Fig. 5B,C). Nested clade analysis revealed several events of contiguous range expansion, restricted gene flow with isolation by distance, and allopatric fragmentation for *A. clavigera*, *O. oedipus*, and *O. abyssalis* (Table 3).

Bayesian estimation of migration rates

The estimates of the parameters θ and m/μ converged when samples were grouped into seamount regions, the New England Seamounts and the Corner Rise Seamounts, for *Ophiocreas oedipus*, *Ophioplinthaca abyssalis*, and *Ophioplinthaca chelys*. For *Asteroschema clavigera*, estimates converged when Nashville Seamount was grouped with the Corner Rise Seamounts and compared to the remaining New England Seamounts (Table 4). Asymmetrical gene flow was strongly evident in the 16S+COI datasets of *A. clavigera* and *O. oedipus* and the 16S datasets of *O. abyssalis* and *O. chelys*. Estimates of historical migration indicated a predominant westward movement in both *A. clavigera* and *O. oedipus*, suggesting migrants from the Corner Rise Seamounts (including Nashville Seamount for *A. clavigera*) being an order of magnitude greater than the historical number of migrants traveling in the reverse direction. For *O. abyssalis* and *O. chelys*, the pattern was reversed, with the historical number of migrants from the New England Seamounts going to the Corner Rise Seamounts being two orders of magnitude greater than those traveling in the reverse direction (Fig. 7).

Discussion

Study limitations

This study is a multi-species approach to understanding the extent of genetic connectivity and isolation among the

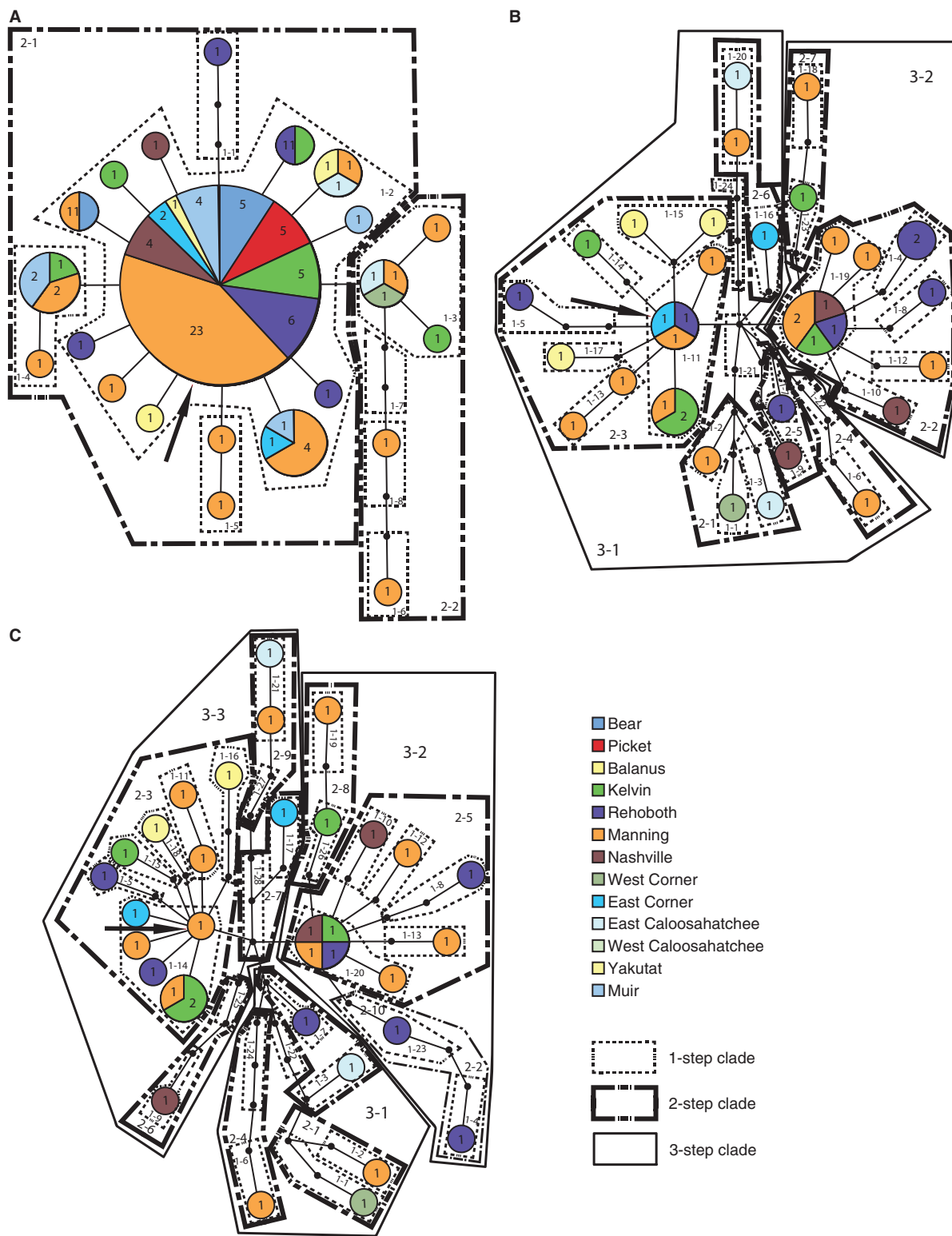


Fig. 5. TCS haplotype network for *Asteroschema claviger* (A) 16S sequences, (B) COI sequences, and (C) 16S+COI sequences. Circles represent the different haplotypes observed, with circle areas proportional to the number of individuals possessing that haplotype. Haplotypes shared among geographic locations are further broken down into pie graphs. Connecting lines represent 1 bp difference and small black circles represent interior node haplotypes not present in the samples. The ancestral haplotype is marked with an arrow.

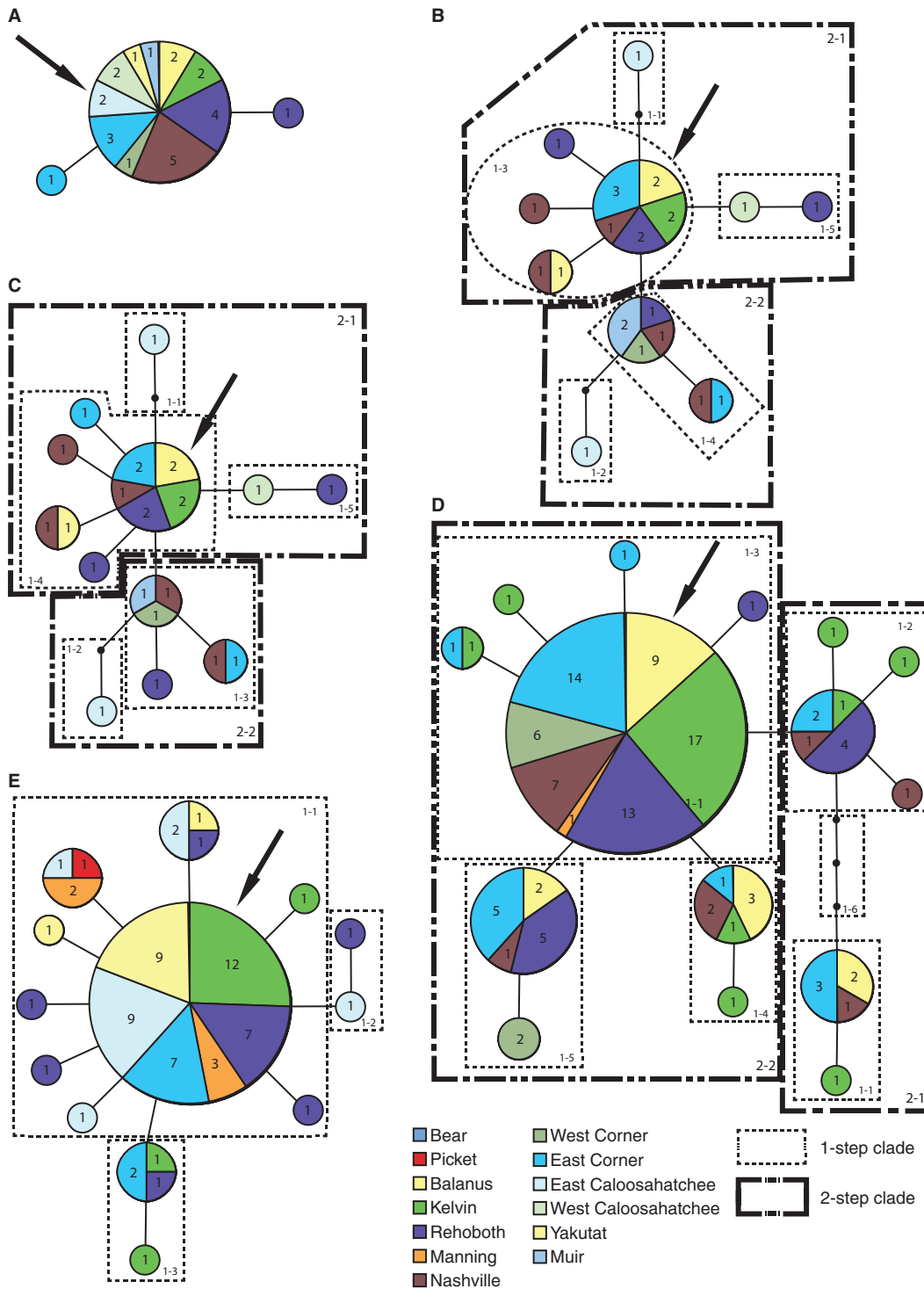


Fig. 6. TCS haplotype network for (A) *Ophiocreas oedipus* 16S sequences, (B) *O. oedipus* COI sequences, (C) *O. oedipus* 16S+COI sequences, (D) *Ophioplithaca abyssalis* 16S sequences, and (E) *Ophioplithaca chelys* 16S sequences. Circles represent the different haplotypes observed, with circle areas proportional to the number of individuals possessing that haplotype. Haplotypes shared among geographic locations are further broken down into pie graphs. Connecting lines represent 1 bp difference and small black circles represent interior node haplotypes not present in the samples. The ancestral haplotype is marked with an arrow.

Table 3. Nested clad analysis results from three species of ophiuroids displaying regionally significant genetic patterns.

Species	Gene region	Clade	Chain of inference	Demographic event inferred
<i>Asteroschema clavigera</i>	16S	Total cladogram	1-2-3-4 no	restricted gene flow with isolation by distance (restricted dispersal by distance in non-sexual species)
	COI	2-3	1-2-11-12 no	contiguous range expansion
		3-1	1-2-11-12 no	contiguous range expansion
	16S+COI	Total Cladogram	1-2-3-4 no	restricted gene flow with isolation by distance (restricted dispersal by distance in non-sexual species)
<i>Ophiocreas oedipus</i>	COI	2-1	1-2-11-12 no	contiguous range expansion
	16S+COI	Total cladogram	1-19 no	allopatric fragmentation
<i>Ophioplithaca abyssalis</i>	16S	2-1	1-2-11-12 no	contiguous range expansion

Table 4. Estimated rates of gene flow as migrants per generation, $M = \theta m / \mu$ for the 16S+COI datasets of *Asteroschema clavigera* and *Ophiocreas oedipus* and the 16S datasets of *Ophioplithaca abyssalis* and *Ophioplithaca chelys*.

Species	Gene	Comparison	Number of migrants/ generation	Directional bias
<i>Asteroschema clavigera</i>	16S+COI	NESC into CR+Nashville Seamount	160.78	West
		CR+Nashville Seamount into NESC	1376.8	
<i>Ophiocreas oedipus</i>	16S+COI	NESC into CR	117.7	West
		CR into NESC	1644.1	
<i>Ophioplithaca abyssalis</i>	16S	NESC into CR	2351.2	East
		CR into NESC	84.8	
<i>Ophioplithaca chelys</i>	16S	NESC into CR	24878.7	East
		CR into NESC	98.9	

NESC, New England Seamount chain; CR, Corner Rise.

North Atlantic seamounts, identifying congruent and incongruent patterns of connectivity and dispersal among four ophiuroid species, of which three are intimately associated (with varying specificity) to their host coral. Before discussing the findings of this study, it is important to note that the sample sizes for several populations inhabiting different seamounts were low, and may have limited the full recovery of genetic diversity within populations. For example, the smallest samples sizes were in *Ophiocreas oedipus* with less than six individuals for each of 10 seamounts. Sufficient non-destructive sampling (*i.e.* discrete submersible collections rather than bottom trawling-type practices) for population genetic studies in the deep sea is commonly limited by the inaccessibility to the abundance of individuals from any given population. This restriction is particularly acute when advancing studies of species with small census population sizes and sea floor encounter rates (and submersible collection capacity). This limitation of sufficient or abundant samples has previously been identified as a pervasive factor that hinders the accurate estimation of endemism, population genetics, and faunal isolation on seamounts (McClain 2007; Thoma *et al.* 2009). Small populations are inherently those in greatest need of conservation genetic approaches (Frank-

ham 1995) and critical to understand for the effective protection management of vulnerable seamount ecosystems, such as cold-water corals and their associates, from the destructive threats of fisheries activities and resource mining (Clark *et al.* 2010). Therefore, attempts were made to minimize the biases of small sample sizes through conservative analytical approaches and interpretations of patterns, recognizing that additional sampling of these understudied taxonomic groups, ecological relationships, and vastly underexplored New England and Corner Rise Seamounts (and adjacent regions) will allow further elucidation of the observed patterns described below.

Patterns of genetic connectivity

Several congruent patterns of connectivity and dispersal among ophiuroid species were revealed. In general, gene flow was evident for all four ophiuroid species across the three seamount regions. Similar levels of gene flow, with little to no evidence of seamount population structure, have been observed between populations of fish (*e.g.* Martin *et al.* 1992; Bolch *et al.* 1993), corals (*e.g.* Smith *et al.* 2004a; Thoma *et al.* 2009), and squat lobsters and a planktotrophic gastropod (*e.g.* Samadi *et al.* 2006).

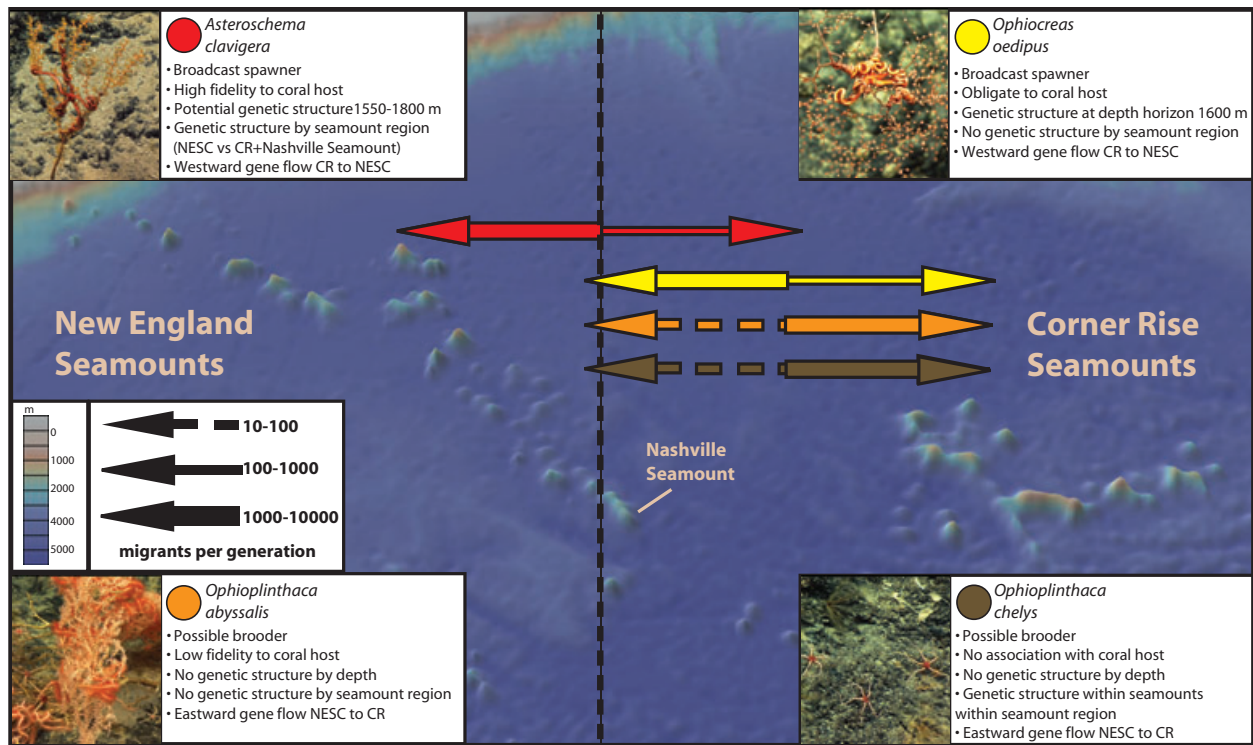


Fig. 7. Asymmetrical gene flow estimated as migrants per generation for the 16S+COI datasets of *Asteroschema clavigera* and *Ophiocreas oedipus* and the 16S datasets of *Ophioplinthaca abyssalis* and *Ophioplinthaca chelys*. Arrows indicate the asymmetrical direction of inferred historical gene flow from one region to another and magnitudes are indicated by arrow thickness. Colors correspond to species. NESC, New England Seamount Chain; CR, Corner Rise.

However, unlike the majority of these studies, each of the 16S haplotype networks for *Asteroschema clavigera*, *Ophiocreas oedipus*, and *Ophioplinthaca chelys* revealed star-like phylogenies and an abundant common haplotype with many closely associated rare haplotypes, indicative of rapid population expansion (Slatkin & Hudson 1991; Teske *et al.* 2005). Expansion (and non-equilibrium dynamics) was further supported by the (i) statistically significant negative Tajima’s *D* values for several seamount populations of *A. clavigera*, *O. abyssalis*, and *O. chelys*; (ii) inability of the goodness-of-fit test of mismatch distributions to reject the null hypothesis of a model of expansion for these populations (Aris-Brosou & Excoffier 1996; Tajima 1996); (iii) nested clade analysis results in support of contiguous range expansion for several clades of the COI haplotype networks of *A. clavigera* and *O. oedipus*, and the 16S haplotype network of *O. abyssalis*; and the (iv) widespread distribution of haplotypes in each of the four ophiuroid species, where every species hosted haplotypes shared across all seamount regions.

In contrast to these congruent patterns of gene flow observed in all four ophiuroid species, several incongruent patterns emerge in the connectivity and population

structure for the different species. Population structure was detected through the AMOVA based on seamount regions, which identified significant structure between Nashville and the Corner Rise Seamounts and the rest of the New England Seamounts in *A. clavigera*. A dissimilar pattern of genetic differentiation was detected for populations of *O. chelys*, where significant genetic structure was detected between seamounts within seamount regional groups as well as within seamount populations. This would indicate that the genetic structure within this species occurs on a smaller geographic scale in contrast to the scale of seamount regional groups observed in *A. clavigera*. In addition, several haplotypes were restricted to either the Corner Rise or New England Seamount chains with a greater abundance of unique haplotypes occurring within the New England Seamount than in the Corner Rise Seamounts. These results suggest that while genetic connectivity among regional seamount groups has occurred in the past, there are clear indications of genetic structure between the New England Seamount region and the Corner Rise Seamounts.

Although incongruent patterns of connectivity are apparent when each species is examined separately, there is a striking congruence among species within the same

family (Asterocheimataceae, *A. clavigera* and *O. oedipus* versus Ophiacanthidae, *O. abyssalis* and *O. chelys*). As seamounts are often distributed in linear chains and provide discrete hard substrate habitat in the abyssal plains, it has been hypothesized that seamount fauna follow a stepping-stone model of dispersal, which would result in patterns of genetic isolation by distance (Wright 1931, 1951; Hubbs 1959; Kimura & Weiss 1964; Rogers 1994). Nested clade analysis supports a stepping stone model of dispersal and isolation by distance for *A. clavigera* as did the Mantel test for *A. clavigera* and *O. oedipus*. Both *A. clavigera* and *O. oedipus* displayed genetic structure based on depth while *O. abyssalis* and *O. chelys* did not. In addition, both *A. clavigera* and *O. oedipus* strongly favor westward historical migration with migrants from the Corner Rise Seamounts (including Nashville Seamount for *A. clavigera*) being an order of magnitude greater than the number of migrants traveling in the reverse direction. In *O. abyssalis* and *O. chelys*, this directional migration is reversed, with strongly favored eastward historical migration (Fig. 7). Interestingly, this striking asymmetry of migration correlates with species dispersal modes and fidelity to host corals, *i.e.* 'broadcast spawners' with high fidelity to the west versus 'brooders' with low fidelity to the east.

These congruent and incongruent patterns of gene flow and genetic structure may be due to a variety of factors previously regarded as inherently important in the connectivity of seamount populations (*e.g.* depth, dispersal strategies, and physical oceanographic processes; Rogers 1994; Clark *et al.* 2010; Shank 2010a). Indeed, the observed patterns may be best explained by the complex interaction of biological and physical factors, which include life history strategies, depth and environmental gradients, the temporal and spatial dynamics of hydrographic circulation, as well as the specificity of host-associate relationships.

Life history and dispersal strategies

As observed in seamount gastropod and squat lobster species (Samadi *et al.* 2006), potential differences in reproductive strategies may relate to the differences in observed patterns of gene flow and genetic structure. Deep-sea ophiuroids are known to have a wide-range of reproductive strategies (Tyler 1980; Hendler 1991). Reproductive studies of *O. oedipus* have characterized this species as a broadcast spawner with large yolky eggs (Mosher & Watling 2009). A similar larval mode of dispersal has been hypothesized in the closely related *A. clavigera* (C. Mosher pers. comm.). Although no reproductive studies have determined empirically the reproductive strategies or mode of larval dispersal for *O. abyssalis* or *O. chelys*, studies of species from closely related species

within the same family, Ophiacanthidae, found benthic, lecithotrophic larvae characterized by either brooding, viviparity, or direct development (Hendler 1991; McEdward & Miner 2001). These markedly different strategies of dispersal- 'broadcast spawners' versus 'brooders' in the examined ophiuroids correspond directly with the observed 'incongruent' patterns of inferred dispersal and asymmetrical gene flow between the New England and Corner Rise Seamounts (Fig. 7). Detailed examination of the reproductive biology in these species would be highly informative in assessing the importance of reproductive strategy in generating the observed patterns of dispersal and genetic connectivity.

Depth and environmental gradients

It is well known that benthic community composition on seamounts can be depth-stratified, often reflecting fundamental environmental gradients, such as temperature, oxygen concentration, food availability, and pressure that correlate with depth (O'Hara 2007). Most deep-sea animals have a limited bathymetric range (*e.g.* Rex *et al.* 1999), including cold-water corals that serve as available habitat. Fragmentation and restriction of biogenic habitats over depth horizons may be shared among seamounts and structure the dispersal of larvae at different depth intervals. The 'biological isolation' of seamounts may result from the lack of available and suitable biogenic habitat. Evidence of depth-stratified genetic structure would point to the role of the environmental gradients and the restrictive dependency of associated invertebrates to their biological host as factors regulating genetic diversity and divergence in seamount systems.

Cluster and multivariate analyses of seamount community composition via submersible imaging surveys at the time of sample collection on these seamounts preliminarily revealed compositional changes in faunal community structure at 1300, 2300, and 2600 m (Cho 2008). Similarly, distinct depth patterns for faunal assemblages have been observed on Patton seamount in the Gulf of Alaska (Hoff & Stevens 2005) and several studies have found faunal boundaries associated with depth in the North Atlantic (megabenthic fauna, Haedrich *et al.* 1980; asteroids, Howell *et al.* 2002; cerianthid anemones, Shepard *et al.* 1986). In addition, the maximum depths of deep-water corals often reflect changes in the depths of water masses that have temperatures suitable for the corals, as was observed for deep-water gorgonian corals in the Northeast Channel of Canada (Mortensen & Buhl-Mortensen 2004). The depth distribution of host corals likely has a marked impact on the genetic structure of associated fauna, and as such, attempts were made herein to identify such patterns.

Two ophiuroid species displayed evidence of genetic differentiation significantly structured by depth. Analyses of molecular variance revealed significant genetic differentiation among 250 m depth bins of *A. clavigera* populations (relatively high sample sizes) and 100 m depth bins for *O. oedipus* (relatively low sample sizes). Both of these species have highly restrictive associative relationships with specific coral hosts and their distributions may be limited by the distribution of their coral hosts. As broadcast spawners, their dispersal may be limited hydrographically to specific depth ranges that effectively permit the acquisition of their specific coral host. This may explain why the data available for the two ophioplithacids, which display less host specificity and may be 'brooders', do not suggest genetic structure by depth. In addition, *O. oedipus* has the most restricted relationship of all four ophiuroid species being an obligate symbiont of only one species of coral, while *A. clavigera* has two coral hosts. The combined bathymetric range of *Paramuricea* sp. and *Paragorgia* sp. (host corals of *A. clavigera*) may be greater than the bathymetric range of *M. melanotrichos* (the single host coral of *O. oedipus*) and may therefore account for the smaller scale of genetic structure based on depth for *O. oedipus* (100 m) versus *A. clavigera* (250 m).

These results correspond with other studies that have found genetic structure based on depth. Populations of the deep-sea amphipod *Eurythenes gryllus* were genetically distinct at different depths on the scale of ocean basins (France & Kocher 1996). Genetic analyses of the mitochondrial 16S gene of the deep-sea protobranch bivalve *Deminucula atacellana* detected genetically distinct populations above and below 2500 m (Chase *et al.* 1998) and revealed the greatest genetic divergence among populations at different depths versus geographic distance (Zardus *et al.* 2006). Analyses of three nuclear markers in the deep-water coral *Oculina* sp. revealed a singular deep-water reef as a strong genetic outlier from all other populations (Eytan *et al.* 2009) and genetic analyses of six microsatellites of the symbiotic dinoflagellate *Symbiodinium* sp. revealed strong population differentiation between deep and shallow sites on the same reef (Kirk *et al.* 2009).

As previously recognized, the significant depth results may be a sampling artifact due to the low sample sizes of specific populations. However, the depth interval grouping of populations in the present analyses serves to minimize such biases brought about by small samples sizes and previous studies have shown that limited numbers of haplotypes in sequence-based population genetic studies obtain reliable species genealogies (Hey & Kliman 1993; Kliman & Hey 1993). In addition, the affect of different sample locations relevant to seamount morphology (*i.e.* peak versus slope versus base) were not expressly exam-

ined, as samples were not sufficient to resolve such issues. Nevertheless, the detection of a genetic signal as a function of depth suggests the importance of this factor and warrants further study.

Physical hydrographic dynamics

Additional physical factors such as long-distance transport driven by global circulation patterns and local theoretical hydrodynamics have been proposed as potential barriers or agents of retention to dispersal (*e.g.* Smith *et al.* 2004b). The New England and Corner Rise Seamounts have summits that range from <1 to 3 km depth, and, except for the westernmost of the New England Seamounts that arise from the continental slope, they extend to abyssal depths (4000–5000 m). They lie within oceanographic settings that range from a deep western boundary current (flowing north to south) to the dynamic Gulf Stream, to abyssal quiescence. The Gulf Stream is the major western boundary current in the North Atlantic, initiating in the Caribbean with deep mean flow intersecting the New England Seamount chain near Manning Seamount (Richardson 1980; Hogg *et al.* 1986; Hogg 1992; Qiu 1994). Physical oceanographic studies of North Atlantic circulation have documented the creation of recirculating gyres from the interaction of the Gulf Stream with the New England Seamount chain at depths of 3000 m (Qiu 1994). Such dynamic gyres could serve as complex physical transport mechanisms of dispersing larvae that temporally and spatially facilitate or impede gene flow across the seamounts. If *O. abyssalis* or *O. chelys* have demersal lecithotrophic larvae, their relative potential and realized dispersal may be much lower than that of *A. clavigera* and *O. oedipus*. This may explain why populations of *O. chelys* maintained significant differentiation within seamount regions and within seamount populations, a smaller geographic scale than the regional genetic differentiation observed in *A. clavigera*. Similar results were observed on the Norfolk Ridge seamounts (Samadi *et al.* 2006).

Specificity of ophiuroid associates and coral hosts

In addition to determining the connectivity and genetic structure of seamount populations affected by fisheries activities, a goal of this study was to provide a first assessment of the potential impact that partnered relationships between host coral and the epifaunal invertebrates (from obligate to facultative to indiscriminant habitat requirements) have on the connectivity and gene flow of the associated invertebrate. The connectivity patterns evident for the four species of ophiuroids with varying degrees of association with host corals may provide insight into the

influence these relationships and of the distribution of the host corals have on population genetic structure. As noted previously, *O. oedipus* has an obligate relationship with the coral *M. melanotrichos* with only a single individual occurring on the central axial stalk of a single host coral (Mosher & Watling 2009). *Asteroschema clavigera* displays a high fidelity in association with two gorgonian corals *Paramuricea* sp. and *Paragorgia* sp. occurring with either one to two individuals on a single individual of *Paramuricea* sp. or one to many individuals on *Paragorgia* sp. *O. abyssalis* predominantly occurs as tens of individuals on the primnoid octocoral *Candidella imbricata*, but this species also utilizes other coral hosts and substrates. Based on the genetic data, as *O. abyssalis* does not maintain a similar genetic structure as *O. chelys*, it may be that *O. abyssalis* shares a similar mode of reproduction as *A. clavigera* and *O. oedipus*. *Ophioplinthaca chelys* occurs predominantly on hard sea floor habitats, lacking an association or interaction with any particular coral as a host. As both *A. clavigera* and *O. oedipus* have strong associative relationships with coral hosts, broadcast spawning provides the greatest chance for their offspring to encounter their specific coral hosts. Alternatively, *O. chelys* does not have the habitat requirement of a coral host and therefore lacks the need for such a widespread dispersal strategy.

No genetic differentiation was detected for *O. oedipus*, a similar pattern that coincides over the same region with the genetic variation in *M. melanotrichos* (Thoma *et al.* 2009), the coral host with which *O. oedipus* has an obligate partnership (Mosher & Watling 2009). Genetic analysis of the *msh1* gene of *Paramuricea* sp., a coral host of *A. clavigera*, has revealed three clades across this region, one clade distributed throughout the entire region except for the easternmost Corner Rise Seamount, the second clade distributed throughout the region but absent from Muir Seamount, and the third clade restricted to the westernmost New England seamounts and the continental slope (Thoma *et al.* 2009). Similarly, genetic structure among the three seamount regions in this study was detected for *A. clavigera*. The series of sliding window AMOVA indicated that the genetic discontinuity occurred in the region of Nashville Seamount, the easternmost seamount of the New England Seamount chain, and the Corner Rise Seamounts.

This study is unique in suggesting the potential impact of associative relationships on the genetics of associate invertebrates. Congruent patterns of large-scale gene flow and population expansion were observed among all four species of ophiuroids, while incongruent patterns of dispersal, depth, and genetic structure between the major seamount chains with asymmetrical migration were also observed and species dependent. These differences corre-

late well with both differences in (known and inferred) dispersal strategies and the specificity of associative relationships with coral hosts and perhaps play an important role in the connectivity and genetic structure of these and other seamount populations.

Conservation and management implications

Extensive habitat damage due to intense fishing practices in the 1970s and 1980s (Waller *et al.* 2007) led to the 2007 closure of 13 fishable seamounts, including 25 peaks shallower than 2000 m on the New England and Corner Rise Seamounts to fisheries activity involving demersal fishing gear (Shank 2010b). Continued management efforts are now under consideration as this closure will end on December 31, 2010. In promoting adaptive and effective ecosystem-based management practices to protect and conserve vulnerable marine ecosystems such as those on seamounts, it is important to consider the ecological requirements and interactions of species when trying to understand the connectivity among seamount systems. More than 270 morphospecies have been observed within the Corner Rise and New England Seamounts (Cho 2008), with a diverse array of invertebrates living with 'high' levels of specificity on host coral (Shank 2010b). These include, in addition to the ophiuroid species already noted, the shrimp *Bathypalaemonella serratipalma* inhabiting only specific *Chrysogorgia* and *Iridogorgia* coral species, and the galatheid *Uroptychus* inhabiting only the antipatharian *Parantipathes* sp. The present study has identified that there may be diverse patterns of connectivity and gene flow within and between seamounts and seamount chains which should be taken into account by conservation strategies. For example, the asymmetric historical migration of high host-fidelity species to the west and low host-fidelity species to the east and the specific habitats points to the need for spatial adaptive strategies that both conserve regional sources of larvae that may exist on other seamount chains as well as specific biogenic host habitat to ensure the maintenance of biodiversity in seamount ecosystems. The ecological and evolutionary significance of host-specific relationships on potentially isolated seamounts now presents new and fundamental questions, including the extent to which host specificity plays a role in connectivity and maintenance of the more than 1350 known species associated with cold-water coral ecosystems in the deep sea.

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