Evolution, 59(7), 2005, pp. 1479–1491

POPULATION DIFFERENTIATION DECREASES WITH DEPTH IN DEEP-SEA BIVALVES

RON J. ETTER,[1,2] MICHAEL A. REX,[1] MICHAEL R. CHASE,[1] AND JOSEPH M. QUATTRO[3]
[1]*Biology Department, University of Massachusetts, Boston, Massachusetts 02125*
[2]*E-mail: ron.etter@umb.edu*
[3]*Department of Biological Sciences, Program in Marine Sciences, University of South Carolina, Columbia, South Carolina 29208*

Abstract.—The deep sea is the largest ecosystem on Earth. Recent exploration has revealed that it supports a highly diverse and endemic benthic invertebrate fauna, yet the evolutionary processes that generate this remarkable species richness are virtually unknown. Environmental heterogeneity, topographic complexity, and morphological divergence all tend to decrease with depth, suggesting that the potential for population differentiation may decrease with depth. To test this hypothesis, we use mitochondrial DNA (16S rRNA gene) to examine patterns of population differentiation in four species of protobranch bivalves (*Nuculoma similis*, *Deminucula atacellana*, *Malletia abyssorum*, and *Ledella ultima*) distributed along a depth gradient in the western North Atlantic. We sequenced 268 individuals from formalin-fixed samples and found 45 haplotypes. The level of sequence divergence among haplotypes within species was similar, but shifted from between populations at bathyal depths to within populations at abyssal depths. Levels of population structure as measured by Φ_{ST} were considerably greater in the upper bathyal species (*N. similis* = 0.755 and *D. atacellana* = 0.931; 530–3834 m) than in the lower bathyal/abyssal species (*M. abyssorum* = 0.071 and *L. ultima* = 0.045; 2864–4970 m). Pairwise genetic distances among the samples within each species also decreased with depth. Population trees (UPGMA) based on modified coancestry coefficients and nested clade analysis both indicated strong population-level divergence in the two upper bathyal species but little for the deeper species. The population genetic structure in these protobranch bivalves parallels depth-related morphological divergence observed in deep-sea gastropods. The higher level of genetic and morphological divergence, coupled with the strong biotic and abiotic heterogeneity at bathyal depths, suggests this region may be an active area of species formation. We suggest that the steep, topographically complex, and dynamic bathyal zone, which stretches as a narrow band along continental margins, plays a more important role in the evolutionary radiation of the deep-sea fauna than the much more extensive abyss.

Key words.—Deep sea, depth gradient, mollusks, phylogeography, population divergence, population structure.

Received September 7, 2004. Accepted May 11, 2005.

The deep ocean (below 200 m) is an enormous and complex ecosystem covering two-thirds of the planet. The fauna inhabiting this remote environment is highly endemic and surprisingly rich (Hessler and Sanders 1967). Although numerous studies have explored evolutionary processes in shallow-water systems (e.g., Vermeij 1987, 1991; Palumbi 1994, 1999; Burton 1996, 1998; Barber et al. 2000; Grosberg and Cunningham 2001; Palumbi and Warner 2003) and in the unusual and highly constrained deep-water chemosynthetic environments (e.g., Vrijenhoek 1997; Baco et al. 1999; Van Dover et al. 2002; Goffredi et al. 2003), virtually nothing is known about how evolution has unfolded in the vast stretches of the deep ocean. We lack even the most basic information about what processes might be important or the geographic and bathymetric scales over which they operate.

Many aspects about the deep-sea environment and fauna seem to defy conventional theories on diversification and subsequent coexistence of species. There are few obvious barriers to gene flow, and a large proportion of species are now known to have pelagic larval dispersal (Young 2004). Local diversity can exceed 300 macrofaunal species m^{-2} (Etter and Mullineaux 2001), but most species exploit the same detrital food resources (Gage and Tyler 1991), and the structural complexity typical of other highly diverse systems such as coral reefs and rainforests is lacking. The deep sea also appears to be a relatively young environment for such an extensive evolutionary radiation since there was a major mass extinction event caused by widespread anoxia during the Paleocene (Jacobs and Lindberg 1998) and potentially as recently as the Pleistocene (Hayward 2001).

Several mechanisms have been identified that promote divergence in marine organisms with good dispersal, including isolation by distance, extrinsic barriers to gene flow, selection, and various historical processes (e.g., Palumbi 1994; Grosberg and Cunningham 2001). There is good evidence for these operating in shallow-water systems but none for the deep sea. To begin to unravel the evolutionary processes operating in this vast, complex, and remote ecosystem, we need to quantify genetic variation within and among species—the primary evidence for inferring patterns of evolution in other environments.

Recent evidence suggests that the potential for population differentiation and speciation may vary with depth (Etter and Rex 1990; France and Kocher 1996; Rogers 2003). The deep sea is not a uniform environment, pronounced bathymetric gradients in both biotic and abiotic factors exist that may influence the geography of population divergence. The bathyal region is considerably more heterogeneous than the abyss. It descends, often steeply, from 200 to 4000 m; is topographically more complex (Mellor and Paull 1994), carved into segments by numerous submarine canyons; and the sediments are more heterogeneous (Cooper et al. 1987; Etter and Grassle 1992). This region also is impacted by oxygen-minimum zones that might isolate gene pools and facilitate population differentiation and speciation (White 1987; Wilson and Hessler 1987; Wilson 1998; Rogers 2000; Helly and Levin 2004). Species diversity (Rex 1981; Etter and Grassle 1992), intraspecific morphological divergence (Etter and Rex 1990), and zonation (Grassle et al. 1979; Rex 1981; Carney et al. 1983; Etter and Rex 1990) all peak at bathyal depths, suggesting communities are more heterogeneous. In contrast, the much more extensive abyss descends very gradually from 4000 to

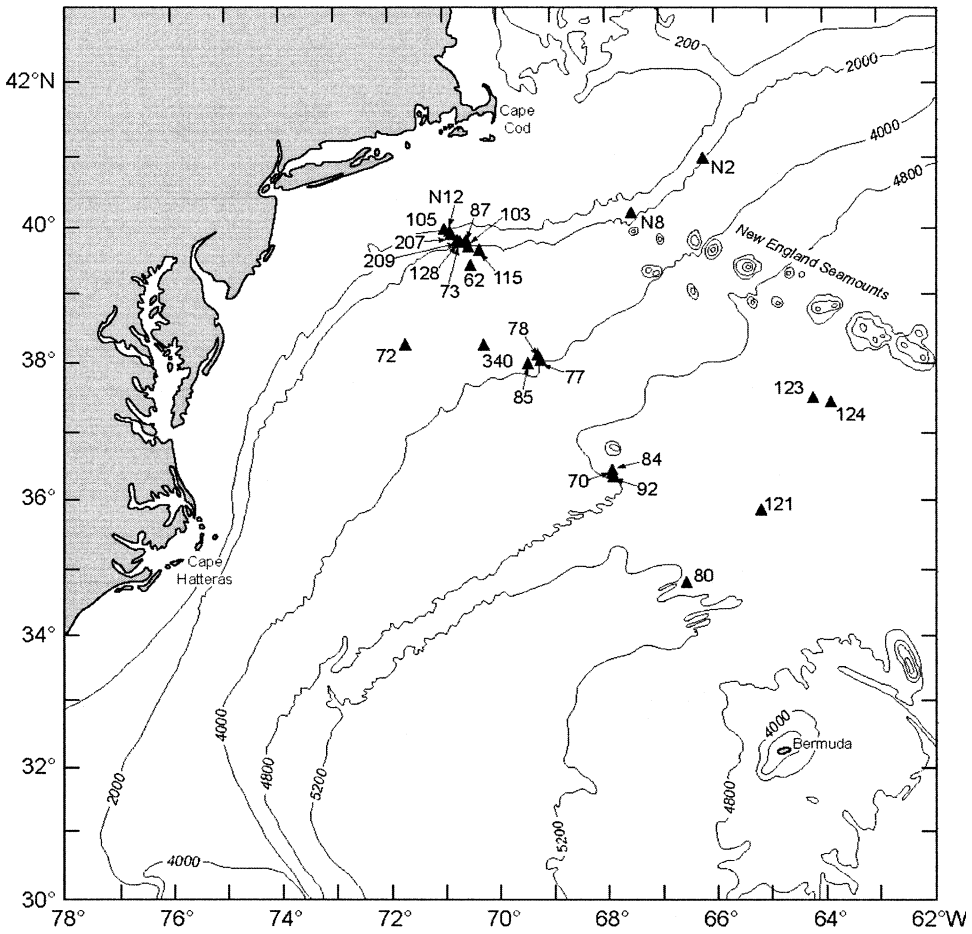

FIG. 1. A map of the North American basin showing the location of the samples used in this study. The samples were collected as part of the Woods Hole Oceanographic Institution's Benthic Sampling Program (Sanders 1977) and the Atlantic Continental Slope and Rise Study (Maciolek et al. 1987).

6000 m, and has a much simpler topography of gently rolling hills and plains, with more uniform sediments and fauna. The greater levels of environmental heterogeneity at bathyal depths may lead to increased levels of population divergence and ultimately speciation through either selective or nonselective processes.

In fact, the peak in diversity at upper bathyal depths, in part, may reflect a higher probability of population differentiation and speciation in this region of the deep ocean. Large-scale gradients in biodiversity (e.g., latitude) can be produced by geographic variation in ecological and/or evolutionary mechanisms (Rohde 1992; Willig et al. 2003). Although the relative importance of various forces in shaping these macroecological patterns remain controversial (e.g., Rohde 1992; Rosenzweig 1995; Gaston 2000; Hawkins et al. 2003; Willig et al. 2003), recent evidence from paleontology (Jablonski 1993; Flessa and Jablonski 1996; Sepkoski 1998; Buzas et al. 2002), comparative phylogenetics (Cardillo 1999; Davies et al. 2004), and molecular evolution (Bromham and Cardillo 2003; Wright et al. 2003; Martin and McKay 2004; Williams and Reid 2004; Xiang et al. 2004; Gillooly et al. 2005; although see Brown and Pauly 2005) suggest that geographic variation in evolutionary rates may play an important role in producing large-scale gradients in diversity. Similarly, depth-related variation in evolutionary rates might help explain why species diversity is greatest at intermediate depths.

Here we test the hypothesis that population differentiation decreases with depth by quantifying geographic and bathymetric patterns of mitochondrial DNA (mtDNA) variation in four species of protobranch bivalves arrayed along a depth gradient (530–4970 m) in the western North Atlantic. All four species have the same mode of feeding and larval development. Genetic divergence in these protobranch bivalves decrease with depth, suggesting that the bathyal zone may play a key role in the evolution of the deep-sea fauna.

MATERIALS AND METHODS

The four species analyzed, *Nuculoma similis* (Rhind and Allen 1992), *Deminucula atacellana* (Schenck 1939), *Malletia abyssorum* Verrill and Bush 1898, and *Ledella ultima* (Smith 1885), were collected from south of New England as part of the Woods Hole Oceanographic Institution's Benthic Sampling Program (Sanders 1977) and the Atlantic Continental Slope and Rise Study (Maciolek et al. 1987). All belong to the order Nuculida. Sampling stations are shown in Figure 1. The four species are common within their depth

ranges and become the numerically dominant bivalves at upper bathyal, mid bathyal, lower bathyal, and lower bathyal to abyssal depths, respectively (Allen and Sanders 1996). They are infaunal deposit feeders, and have nonfeeding swimming demersal pericalymma larvae (Zardus 2002).

The main obstacle to understanding population differentiation in the deep-sea has been the paucity of data on geographic variation at the genetic level. Most deep-sea samples are fixed in formalin, which degrades the DNA, making it difficult to quantify genetic variation. We recently developed protocols to extract, amplify (with polymerase chain reaction, PCR), and sequence mtDNA from small macrofaunal metazoans that have been preserved in formalin (described in Chase et al. 1998b) and use these techniques to sequence a 166- to 198-bp fragment of the variable region of the 16S rRNA mitochondrial gene. The same region was amplified in each species, although species-specific differences (e.g., deletions, additions) result in sequences of slightly different lengths.

DNA Extraction and Sequencing

To extract DNA, whole individuals were placed in microfuge tubes with 200 μl of tissue lysis buffer ATL from the QIAam tissue extraction kit (Qiagen, Chatsworth, CA) and incubated for 24 h at 55°C. Then 5 μl of a 50 mg ml^{-1} solution of proteinase K and an additional 95 μl of lysis buffer were added and incubation continued at 55°C for an additional 72 h. The extraction then followed the manufacturer's instructions, except that buffer ATL and ethanol were increased from 200 μl to 300 μl. DNA was eluted with one 200 μl aliquot of 10 mM Tris pH 8.0.

Species-specific primers were developed for each species using the general procedures described previously (Chase et al. 1998b). Extracted DNA was amplified in 50-μl reaction volumes consisting of 10 μl template (no dilution of stock DNA eluted from column), 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.2 μM each dNTP, 20 *p*m each primer, 1.0 unit *Taq* (Promega, Madison, WI), an equal volume of Taqstart Antibody (Clonetech, Palo Alto, CA) and H_2O to 50 μl. Reactions were layered with mineral oil and heated to 95°C for 2 min prior to five cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min, then 35–40 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec.

The size of the PCR products were confirmed on 1.5% agarose gels, and the remaining PCR products purified with a QIAquick PCR purification kit (Qiagen), and run out on a 1.5% agarose gel against standards to quantify template. The purified PCR products were sequenced with a *Taq* Dye Deoxy Termination cycle sequencing kit (PE Applied Biosystems, Foster City, CA) or Big Dye kit (PE Applied Biosystems), ethanol precipitated, resuspended in formamide loading buffer, and run on an Applied Biosystems Model 373 Automated DNA sequencer or with conventional isotopic methods. DNA sequences were aligned and edited with Sequencher, version 3.1 (Gene Codes Corp., Ann Arbor, MI) for the Macintosh.

Analysis

Arlequin 2.0 (Schneider et al. 2000) was used to calculate haplotype (*h*) and nucleotide (π) diversity and to estimate levels of population structure within species from analyses of molecular variance (AMOVA). Levels of intraspecific divergence were estimated from pairwise genetic distances (Φ_{ST} and modified coancestry coefficients) among samples. Population (sample) trees (UPGMA) for each species were created from the pairwise modified coancestry coefficients (Reynolds et al. 1983) using MEGA (Sudhir et al. 2001). The population trees depict the relative degree of genetic divergence among the samples for each species and allow us to compare levels of population divergence among species.

Phylogenetic relationships among the haplotypes were inferred using statistical parsimony (Templeton et al. 1992) as implemented in TCS (Clement et al. 2000). Ambiguities in the cladograms were resolved using predictions from coalescent theory (Crandall and Templeton 1993; Crandall et al. 1994; Posada and Crandall 2000). As summarized in Pfenninger and Posada (2002), we used three criteria to resolve ambiguities (loops): (1) frequency: haplotypes are more likely to be connected to haplotypes with higher frequencies than to singletons; (2) topology: haplotypes are more likely to be connected to interior haplotypes than to tip haplotypes; and (3) geography: haplotypes are more likely to be connected to haplotypes from the same population or region than to haplotypes occurring in distant populations. Each criterion has been verified in empirical data (Crandall and Templeton 1993).

Nested clade analysis (NCA) was used to test for significant associations between haplotype variation and geography and to determine whether any significant geographic structure might represent contemporary or historical processes (Templeton et al. 1995; Templeton 1998, 2004). We used GeoDis to calculate the NCA (Posada et al. 2000). NCA uses the temporal information in a haplotype phylogeny along with the spatial distribution of the haplotypes to statistically test for phylogeographic associations and to infer whether any significant associations reflect recurrent but restricted gene flow or historical processes such as fragmentation or range expansion. Although the inferences lack estimates of statistical error (Knowles and Maddison 2002), we use them to identify potential processes that can be tested subsequently with more specific a priori statistical phylogeographic methods (e.g., Barber et al. 2002; Knowles and Maddison 2002).

RESULTS

Although we tried to sequence a similar number of individuals (i.e., 20) from each station, the numbers varied because samples differed in the number of individuals collected and because our ability to obtain amplifiable DNA from the fixed tissues varied (Boyle et al. 2004). Because of the inherent difficulties of estimating genetic composition and divergence from small samples, results for stations with $n < 5$ should be interpreted cautiously. In total, we sequenced 268 individuals and found 45 haplotypes across all four species (Table 1; Genbank accession: *N. similis* AY762137–AY762142, *D. atacellana* AF29093–AY29104, *M. abyssorum* AY762143–AY762150, *L. ultima* AY762117–AY762136).

All four species possess similarly divergent haplotypes, with eight to 19 substitutions separating the most distant forms (Fig. 2). In the two upper bathyal species, the most

TABLE 1. Station-specific information for each species. Depth, latitude, longitude, the number of individuals sequenced (*n*), the number of haplotypes, and the identity of haplotypes (letters indicate distinct haplotypes) at each station. Station numbers are Woods Hole Oceanographic designations. Stations N2, N8, and N12 are from Maciolek et al. (1987).

Species	Station	Depth (m)	Latitude °N	Longitude °W	*n*	Haplotypes	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	U
Nuculoma similis																										
	105	530	39°56.6′	71°03.6′	6	2	4	2																		
	N12	530	39°54.3′	70°55.1′	9	3	4	4				1														
	207	808	39°51.3′	70°54.3′	5	2	1	4																		
	128	1254	39°46.5′	70°45.2′	8	1			8																	
	73	1400	39°46.5′	70°43.3′	5	2	2	3																		
	N2	2100	40°57.2′	66°13.8′	10	4	1	7		1	1															
	N8	2180	40°10.3′	67°37.4′	10	2	3		7																	
Deminucula atacellana																										
	87	1102	39°48.7′	70°40.8′	11	2	10											1								
	73	1400	39°46.5′	70°43.3′	6	2	5	1																		
	209	1600	39°47.6′	70°49.9′	14	4	10							2	1	1										
	103	2022	39°43.6′	70°37.4′	17	3	15	1	1																	
	115	2040	39°39.2′	70°24.5′	6	2	5	1																		
	62	2496	39°26.0′	70°33.0′	8	4	5			1	1						1									
	340	3305	38°14.4′	70°20.3′	4	1						4														
	77	3806	38°00.7′	69°16.0′	17	1						17														
	85	3834	37°59.2′	69°26.2′	8	2						7	1													
Malletia abyssorum																										
	72	2864	38°16.0′	71°47.0′	2	1	2																			
	77	3806	38°00.7′	69°16.0′	2	1	2																			
	85	3834	37°59.2′	69°26.2′	19	5	14	2	1	1	1															
	84	4794	36°24.4′	67°56.0′	14	2	13					1														
	123	4870	37°29.0′	64°14.0′	3	2	1							2												
	80	4970	34°49.8′	66°34.0′	3	3					1	1	1													
Ledella ultima																										
	77	3806	38°00.7′	69°16.0′	13	6	3	1	6	1	1	1														
	78	3828	38°00.8′	69°18.7′	8	5	2		3								1					1	1			
	334	4400	40°42.6′	46°13.8′	8	5	1		3							2	1				1					
	70	4680	36°23.0′	67°58.0′	11	3	3		7				1													
	92	4694	36°20.0′	67°56.0′	4	3			2							1								1		
	84	4749	36°24.4′	67°56.0′	15	5	3	1	9					1	1											
	121	4800	35°50.0′	65°11.0′	16	8	3	1	5							3	1	1	1	1						
	123	4853	37°29.0′	64°14.0′	3	2	2		1																	
	124	4862	37°26.0′	63°59.5′	3	3			1																1	1

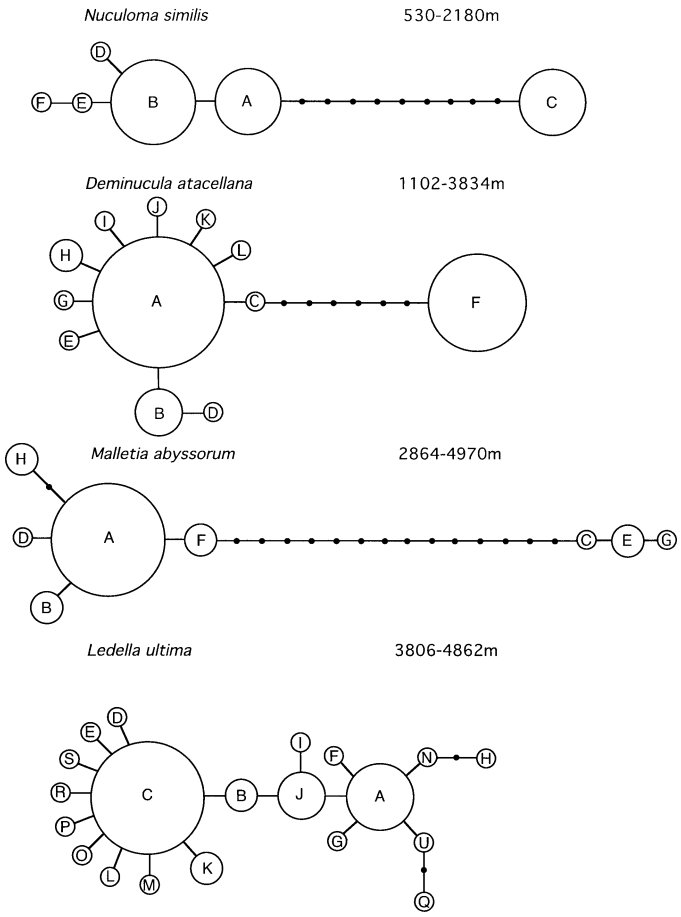

FIG. 2. Statistical parsimony network indicating interrelationships among haplotypes for the 16S mitochondrial DNA for each species. Letters represent different haplotypes within species. Each haplotype is represented by a circle. The area of the circle is directly proportional to the number of individuals possessing that haplotype. Each line connecting haplotypes represents a single mutational step. Haplotypes that were not found but are necessary intermediates are shown as small solid circles.

TABLE 2. Estimates of haplotype (h) and nucleotide (π) diversity for each station. Average for each species is also shown.

Species	Station	h	π
Nuculoma similis			
	105	0.533	0.0024
	N12	0.667	0.0045
	207	0.400	0.0018
	128	0.000	0.0000
	73	0.600	0.0027
	N2	0.520	0.0027
	N8	0.467	0.0239
	Average	0.455	0.0054
Deminucula atacellana			
	87	0.182	0.0009
	73	0.333	0.0017
	209	0.494	0.0028
	103	0.257	0.0014
	115	0.333	0.0017
	62	0.643	0.0052
	340	0.000	0.0000
	77	0.000	0.0000
	85	0.250	0.0120
	Average	0.277	0.0029
Malletia abyssorum			
	72	0.000	0.0000
	77	0.000	0.0000
	85	0.462	0.0189
	84	0.143	0.0009
	123	0.667	0.0000
	80	1.000	0.0849
	Average	0.379	0.0175
Ledella ultima			
	77	0.769	0.0175
	78	0.857	0.0120
	334	0.857	0.0080
	70	0.564	0.0073
	92	0.833	0.0091
	84	0.629	0.0084
	121	0.867	0.0088
	123	0.667	0.0081
	124	1.000	0.0164
	Average	0.783	0.0106

divergent haplotypes are numerous and are often geographically or bathymetrically separated from the most common haplotype. For instance, haplotype F in *D. atacellana*, which is eight substitutions from the most common haplotype A, is found in 29 individuals that only occur below 3000 m. In *N. similis*, the common haplotypes A and C both occur at N8, but are separate at all other stations. In contrast, in the lower bathyal/abyssal species, the most divergent haplotypes are rare (represented by only one or two individuals) and coexist with the most common haplotype (Table 1).

Haplotype diversity varied among species (ANOVA df = 3,27, $P = 0.0012$), with the highest diversity found in the deepest species, *L. ultima* (Table 2). However, there were no clear bathymetric trends in haplotype diversity among samples within a species (least-squares regressions, all $P > 0.19$). Across species, haplotype diversity increased slightly with depth ($R^2 = 0.12$, df = 1,29, $P = 0.0488$), but this was largely a reflection of the influence of the high haplotype diversities for *L. ultima*. Nucleotide diversity (Table 2) did not differ among species (ANOVA df = 3,27, $P = 0.317$) and showed no clear pattern within or among species with depth (least-squares regressions, all $P > 0.23$). Although there appear to be similar levels of haplotype divergence within species (Table 3), there is a shift from between-population divergence at upper bathyal depths to within populations at lower bathyal to abyssal depths (Table 4). Population structure, estimated from an AMOVA, was much greater for the two shallower species *N. similis* and *D. atacellana* than for the deeper-dwelling *M. abyssorum* and *L. ultima* (Table 4).

Population Structure

Population trees (UPGMA) based on modified coancestry coefficients (MCCs) indicate strong population-level divergence in the two bathyal species *N. similis* and *D. atacellana* (Fig. 3). *Nuculoma similis* samples separated by as little as 3 km and 146 m in depth (stations 73 and 128) are highly divergent with a Φ_{ST} of 0.983 (Table 5). The maximum Φ_{ST} in this species was 0.989 for samples separated by 16 km and 446 m in depth. The strong divergence for station 128 reflects the presence of the divergent haplotype C and the lack of the most abundant and widespread haplotype A. Note that station N8 also has haplotype C, but is less divergent

TABLE 3. Summary of uncorrected pairwise distances among haplotypes for each species. The minimum is zero in some cases because the mutations identifying haplotypes occur at gaps in others.

	Average	Maximum	Minimum
Nuculoma similis	0.024	0.062	0.004
Deminucula atacellana	0.016	0.062	0.005
Malletia abyssorum	0.048	0.094	0.000
Ledella ultima	0.015	0.030	0.000

from other stations because of the presence of the common haplotype A. The largest Φ_{ST} recorded in this study (0.991) was for the upper bathyal species *D. atacellana*, but the distance and depth separating these samples were somewhat larger (234 km and 2704 m, respectively). Nevertheless, *D. atacellana* exhibits strong divergence among samples on surprisingly small geographic and bathymetric scales. Samples 62 and 340 separated by only 134 km and 809 m in depth are sufficiently divergent to be placed in different clades (Fig. 3). No haplotypes are shared between the upper bathyal (stations 62, 73, 87, 103, 115, and 209) and lower bathyal clades (stations 77, 85, and 340) of *D. atacellana*. In contrast, the lower bathyal/abyssal species *M. abyssorum* and *L. ultima* exhibit much less divergence. The deepest-dwelling species, *L. ultima*, shows no significant divergence among any of the samples despite some being separated by 1056 m in depth and 500 km in distance. The largest Φ_{ST} for *L. ultima* was 0.1741, and only three of 36 pairwise Φ_{ST} values were greater than zero (Table 5).

Pairwise modified coancestry coefficients (MCCs, Reynolds et al. 1983) between conspecific samples decrease with depth (Fig. 4). MCCs of zero are found at all depths indicating that some samples can be genetically identical in both bathyal and abyssal zones. However, large genetic distances occur only in the upper bathyal species *N. similis* and *D. atacellana*. Populations of the lower bathyal/abyssal species *L. ultima* and *M. abyssorum* do not exhibit large genetic distances

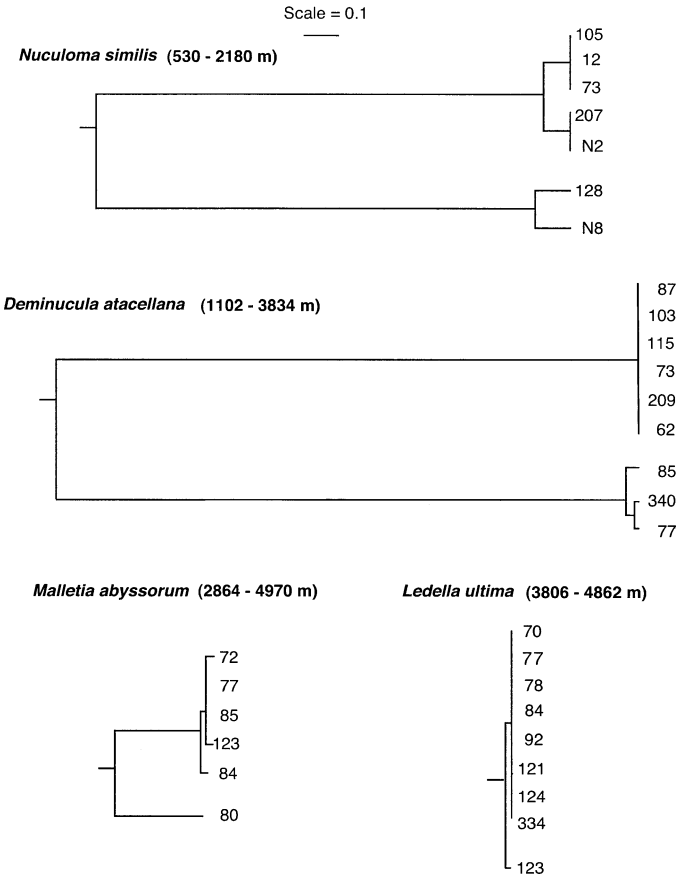

FIG. 3. Distance trees (UPGMA) based on pairwise modified coancestry coefficients (Reynolds et al. 1983) among stations for each species showing the relative genetic distances among samples. Station numbers are shown at branch tips.

TABLE 4. Estimates of Φ_{ST} and the amount of variation within and among samples (populations) calculated from an AMOVA for each species. The *P*-levels were derived from 1000 permutations.

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Nuculoma similis				
Among populations	6	107.457	2.30	75.48
Within populations	46	34.300	0.75	24.52
Total	52	141.756	3.04	
Fixation index	$\phi_{ST} = 0.755$		$P < 0.0001$	
Deminucula atacellana				
Among populations	8	421.284	5.41	93.02
Within populations	80	32.514	0.41	6.98
Total	88	453.798	5.82	
Fixation index	$\phi_{ST} = 0.930$		$P < 0.0001$	
Malletia abyssorum				
Among populations	3	43.237	0.68	7.14
Within populations	35	311.122	8.89	92.86
Total	38	354.359	9.57	
Fixation index	$\phi_{ST} = 0.071$		$P = 0.185$	
Ledella ultima				
Among populations	8	8.513	−0.07	−4.53
Within populations	72	122.845	1.71	104.53
Total	80	131.358	1.63	
Fixation index	$\phi_{ST} = -0.045$		$P = 0.934$	

TABLE 5. Pairwise Φ_{ST} among stations for each species. Station number, depth, and number of specimens (*N*) are shown for each sample. Station numbers are as in Table 1.

Nuculoma similis

	Station	Depth (m)	*N*	1	2	3	4	5	6
1	105	530	6						
2	N12	550	9	0.000					
3	207	808	5	0.216	0.000				
4	128	1254	8	0.982	0.967	0.989			
5	73	1400	5	0.000	0.000	0.000	0.983		
6	N2	2100	10	0.314	0.103	0.000	0.974	0.032	
7	N8	2180	10	0.558	0.602	0.551	0.182	0.540	0.632

Deminucula atacellana

	Station	Depth (m)	*N*	1	2	3	4	5	6	7	8
1	87	1102	11								
2	73	1400	8	0.026							
3	209	1600	14	0.018	0.000						
4	103	2022	17	0.000	0.000	0.028					
5	115	2040	6	0.026	0.000	0.000	0.000				
6	62	2496	8	0.028	0.000	0.032	0.011	0.000			
7	340	3300	4	0.982	0.975	0.947	0.973	0.975	0.919		
8	77	3806	17	0.991	0.990	0.970	0.984	0.990	0.964	0.000	
9	85	3834	8	0.850	0.804	0.841	0.865	0.804	0.787	0.000	0.102

Malletia abyssorum

	Station	Depth (m)	*N*	1	2	3	4	5
1	72	2864	2					
2	77	3806	2	0.000				
3	85	3834	19	0.000	0.000			
4	84	4749	14	0.000	0.000	0.022		
5	123	4853	3	0.000	0.000	0.000	0.000	
6	80	4970	3	0.414	0.414	0.542	0.827	0.514

Ledella ultima

	Station	Depth (m)	*N*	1	2	3	4	5	6	7	8
1	77	3806	13								
2	78	3828	8	0.000							
3	334	4400	8	0.000	0.000						
4	70	4680	11	0.000	0.000	0.000					
5	92	4694	4	0.000	0.000	0.000	0.000				
6	84	4749	15	0.000	0.000	0.000	0.000	0.000			
7	121	4800	16	0.000	0.000	0.000	0.000	0.000	0.000		
8	123	4853	3	0.024	0.000	0.035	0.000	0.174	0.000	0.000	
9	124	4862	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

among any samples (Fig. 4a). Because genetic distance can be influenced by both the geographic distance and depth separating samples, comparisons of divergence among samples in bathyal/abyssal regions should be controlled for differences in the distribution of samples. The continental rise and abyssal samples are actually more widespread, reinforcing the interpretation that divergence there is diminished. Still, to eliminate this potentially confounding influence, we used least-squares regression to remove the effects of distance and depth separating samples. The residuals of genetic distance after the depth and distance separating samples have been statistically held constant exhibit basically the same pattern as the uncorrected genetic distances—population divergence decreases with depth (Fig. 4b). This is not surprising because pairwise MCCs were not correlated with the depth or distance separating the samples for any of the species except *D. atacellana* (Chase et al. 1998a).

Nested Clade Analysis

The nested clade analysis revealed significant geographic structure for both upper to mid-bathyal species, *N. similis* and *D. atacellana* (Table 6). Geographic structure was identified at the total clade and clade 3.1 levels in *N. similis* (clade nesting available from the authors upon request), but due to the lack of significant clade distances, it was not possible to distinguish between contemporary or historical processes. *Deminucula atacellana* also exhibited strong geographic structure at the total clade level. The pattern of clade distances suggested fragmentation (Table 6), however, this inference is dependent on whether *D. atacellana* occurs between stations 62 (2496 m) and 340 (3309 m) and whether this region has been adequately sampled (Templeton 2004). Because no samples have been taken between these two stations, it is not known whether *D. atacellana* is part of the deep-sea community in this region. The gap in sampling makes it difficult to distinguish between fragmentation or isolation by distance. We suggest fragmentation because the distance is relatively small (134 km) and the dispersal window of *D. atacellana* is likely to be fairly large (Chase et al. 1998a), thus we would expect little isolation by distance at these scales. This is also clear from the upper bathyal populations that exhibit little

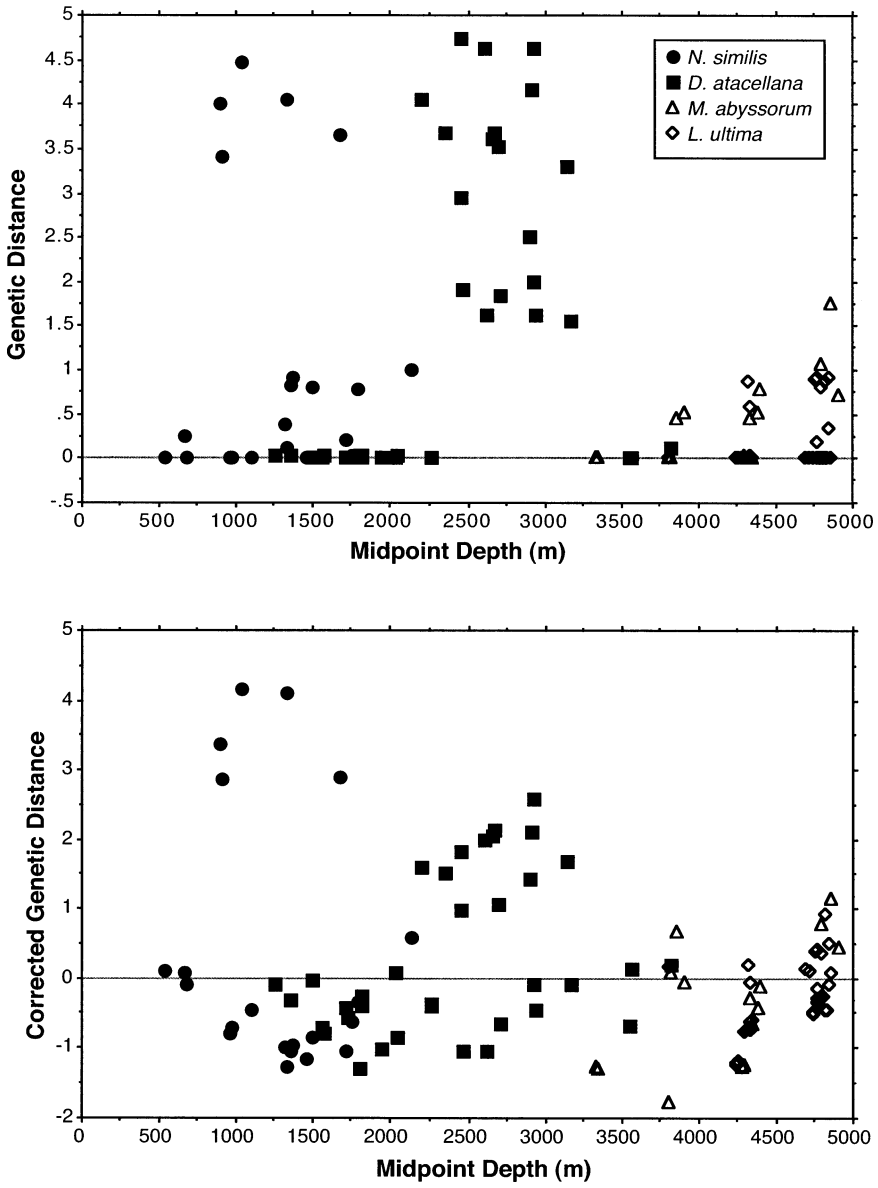

FIG. 4. (Top) Intraspecific pairwise genetic distances (modified coancestry coefficients) as a function of the midpoint in depth between pairs of stations. (Bottom) Residual genetic distances after the depth and distance separating stations have been removed statistically using least-squares regression.

TABLE 6. Chi-square tests of the geographical association of clades and biological inference chain from a nested clade analysis. Inferences were derived from the key cited in Templeton (2004).

Clades	χ^2	P	Inference chain	Inference
Nuculoma similis				
Clade 3.1	10.98	0.037	No significant clade distances	
Total clade	38.95	< 0.001	No significant clade distances	
Deminucula atacellana				
Total clade	86.41	< 0.001	2-3-5-15	fragmentation or inadequate sampling
Malletia abyssorum				
Clade 2.5	23.96	0.007	2-11-12	continuous range expansion
Clade 3.2	19.91	0.03	2-3-4-9-10	inadequate sampling
Ledella ultima				
All levels	ns			

differentiation over similar depths and geographic distances (Fig. 3).

In contrast, the lower bathyal/abyssal species exhibit much less geographic structure. The NCA was unable to detect any significant geographic structure in *L. ultima*, similar to the results for the Φ_{ST} analysis (Tables 4, 5). *Malletia abyssorum* exhibits structure at two hierarchical levels (clades 2.5 and 3.2), but both are due to the occurrence of two individuals of halpotype H at station 123. The pattern of clade distances for clade 2.5 suggested continuous range expansion, whereas sampling was inadequate to infer a process for clade 3.2.

Discussion

Population trees, AMOVA, modified coancestry coefficients, and nested clade analyses of the haplotype networks all clearly indicate that the upper to mid bathyal species *N. similis* and *D. atacellana* show more pronounced geographic variation than the lower bathyal and abyssal species *M. abyssorum* and *L. ultima*. The results generally corroborate the earlier study of phenotypic variation in gastropods (Etter and Rex 1990), despite major differences in natural history between the two taxa. Genetic population structure in deep-sea gastropods is much less well documented, but also appears to decrease with depth. The upper bathyal *Frigidoalvania brychia* shows extreme divergence in mtDNA on small spatial scales (Quattro et al. 2001), whereas preliminary work with two abyssal prosobranch gastropods, *Benthonella tenella* ($n = 56$, seven stations) and *Xyloskenea naticiformis* ($n = 26$, four stations), found virtually no variation in 16S mtDNA in the western North Atlantic (R. J. Etter et al. unpubl. ms.). Although data on other taxa are limited, this pattern does not appear to be restricted to mollusks. Stronger genetic differentiation in bathyal as compared to abyssal populations has been observed in the amphipod *Eurythenes gryllus* (France and Kocher 1996).

Why do bathyal mollusks appear to show more population divergence than their abyssal counterparts? Selective gradients, barriers to gene flow, and historical events have been implicated in producing population differentiation and speciation in shallow-water marine organisms (e.g., Palumbi 1994; Grosberg and Cunningham 2001). It is likely that bathymetric differences in all three mechanisms play roles in explaining greater divergence in bathyal species.

The rate of environmental change in the deep-sea ecosystem is a function of the rate of change in depth and proximity to coastal production. In the more steeply descending bathyal zone, depth parallels gradients of decreasing temperature, decreasing metabolic rates, and increasing pressure (Gage and Tyler 1991). Perhaps the single most important gradient influencing ecological and evolutionary opportunity in the deep sea is the rate of nutrient input from sinking phytodetritus, which decreases exponentially with depth. Consequently, benthic standing stock decreases two to three orders of magnitude with depth across the bathyal zone. It reaches very low levels of around 10–100 individuals m^{-2} and ≤ 1 g m^{-2} at the base of the bathyal zone and then continues to gradually decline with increased distance seaward in the abyss (Rex et al. 2005).

The bathyal zone is also a heterogeneous environment on large and small spatial scales. The continental margin is cut deeply by submarine canyons. Canyons are very large (kilometers across and several hundred meters deep) V-shaped valleys that extend from the shelf-slope transition to the base of the continental slope. In this region, canyons occur at intervals of about 20–40 km along the slope face. The biological and physical features of these canyons have been described (Hecker et al. 1983; Cooper et al. 1987) and are quite distinct from the slope face. The slope face is dominated by fine-grained sediments that are more resistant to erosion and typically less affected by bottom currents (MacIlvaine and Ross 1979). In contrast, canyons are more physically energetic and contain a broader range of habitats including massive rock outcrops, boulder fields, and gravel pavements as well as sand and muddy deposits. Strong descending currents scour the canyon floor, and their V-shaped topography can accelerate oscillatory tidal flows to resuspend sediments (Gardner 1989). The high-energy environment, different substrate type, and distinct communities of canyons might constitute formidable selective barriers that effectively isolate sections of slope-face habitat for small macrofaunal animals like protobranchs that rely on fine-grained sediments for food and have very low adult mobility. At smaller scales, the bathyal environment has a more heterogeneous sedimentary regime (MacIlvaine and Ross 1979; Etter and Grassle 1992) than the abyss. Not surprisingly, with strong vertical environmental gradients and a fragmented heterogeneous landscape, the bathyal zone supports high α- and β-species diversity (Grassle et al. 1979; Rex 1981, 1983; Etter and Rex 1990). The strong gradients and greater biotic and abiotic heterogeneity at bathyal depths might impose different selective regimes that increase the probability of population differentiation and speciation.

Gene flow might also be more restricted at bathyal depths. Protobranchs have pelagic demersal larvae that disperse in the bottom currents possibly for several days (Allen and Sanders 1996; Zardus 2002). In this area of the western North Atlantic, large-scale deep-water currents appear to be well mixed (Hogg 1983; Schmitz and McCartney 1993) and there are no obvious oceanographic or topographic features that would limit dispersal among these populations. However, mesoscale current patterns may be redirected by the rugged topography of the upper bathyal zone. Larvae with near-bottom dispersal are likely to be entrained in small-scale topographically driven currents. Populations living on or within certain topographic features (e.g. gulleys, canyons, valleys, leeward cliffs perpendicular to flow) might become geographically isolated allowing population differentiation to occur on much smaller scales than previously thought. In contrast, the gentle topography of the abyss affords less opportunity for isolation.

The bathyal zone, because of its close proximity to land and coastal systems, must have been strongly impacted by global climatic and oceanographic changes in the past. The deep sea experienced significant cooling during the Pliocene and Pleistocene as high-amplitude glaciation intensified in the Northern Hemisphere (Zachos et al. 2001). At glacial maxima, sea level was depressed by as much as 120 m (Lambeck and Chappell 2001) and the thermohaline circulation shoaled and weakened (Adkins et al. 1988; Raymo et al.

1998). Major climatic transitions attending glaciation were often abrupt, occurring on decadal to millennial time scales (Severinghaus et al. 1998). As in coastal (Hellberg et al. 2001) and terrestrial (Davis and Shaw 2001) environments, glacial cycles resulted in geographic and bathymetric range shifts in species living at bathyal depths (Cronin and Raymo 1997; Kurihara and Kennett 1988). Because benthic standing stock decreases exponentially with depth (Gage and Tyler 1991), population size must have fluctuated with bathymetric range displacement and the variation in surface production caused by glacial cycles (Slowey and Curry 1995). Consistent with this notion, historical estimates of effective population size inferred from generalized skyline plots (Strimmer and Pybus 2001) using maximum likelihood phylogenies of the mtDNA sequences indicate that the two bathyal species experienced more dramatic fluctuations in population size in recent times (Fig. 5, note the difference in effective population size from $t = 0.03$ forward). Sea level lowstands were also times of active canyon formation and catastrophic slope failure (Cooper et al. 1987; Mellor and Paull 1994), which partitioned the bathyal zone. The combination of paleoenvironmental change, fluctuations in populations size, and the isolating effects of slope erosion during glaciation might have promoted population differentiation in the bathyal zone by both selective and nonselective mechanisms. The deeper, more remote, and topographically simpler environment of the abyssal plain is probably considerably less conducive to evolutionary divergence.

Recent biogeographic evidence suggests that many abyssal organisms might exist as sink populations from nearby bathyal environments and thus have little potential for evolutionary divergence (Rex et al. 2005). The majority of abyssal populations represent deeper range extensions for a subset of bathyal species that have pelagic larval dispersal and abyssal endemism appears to be low. The extraordinarily low density of many abyssal populations suggests that they could not be reproductively viable and are vulnerable to chronic local extinction from inverse density dependence. Bathyal and abyssal habitats may act as a source-sink system in which much of the abyssal fauna is maintained as a balance between extinction from Allee effects and immigration from bathyal sources. Nonreproducing sink populations could not differentiate along selective gradients in the abyss.

So far we have considered the observed levels of genetic variation for each species to be intraspecific. Another possible interpretation is that the strongly divergent haplotypes in *N. similis*, *D. atacellana*, and *M. abyssorum* represent cryptic species, potentially eliminating any depth-related difference in population differentiation. The maximum pairwise difference between the haplotypes within each species (Table 3) is in the lower range of what has been found for 16S among congeners in other bivalves (e.g., Etter et al. 1999; *Tridacna*, Schneider and Ó Foighil 1999; *Lasea*, Jozefowicz and Ó Foighil 1998; *Crassostrea*, Lapegue 2002; *Dreissena*, Therriault 2004). This comparison needs to be interpreted cautiously because estimates of interspecific divergence in the nonprotobranch bivalves were based on a much larger fragment of the 16S gene. Our fragment is smaller and centered in a highly variable region of the gene, which may inflate estimates of haplotype distance.

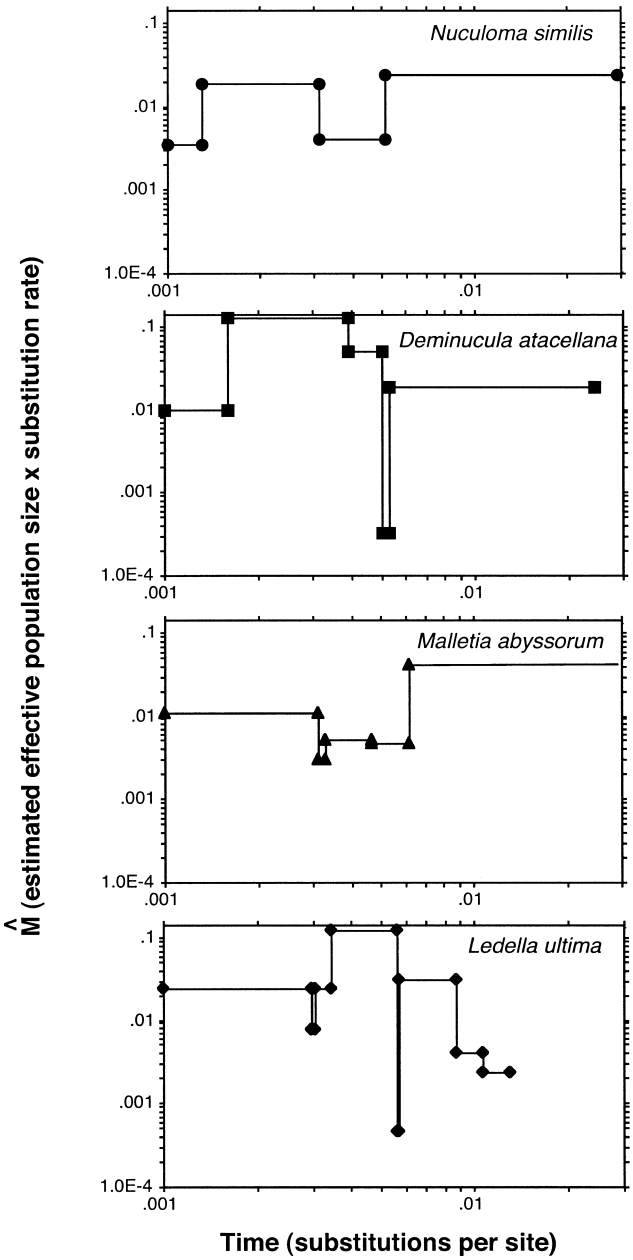

FIG. 5. Generalized skyline plots for each species depicting estimated population sizes through time derived from variation in the 16S mitochondrial DNA fragment. The estimates were obtained from GENIE 2 (http://evolve.zoo.ox.ac.uk/software/Genie/Genie.html) based on Strimmer and Pybus (2001).

More importantly though, we lack a basic understanding of the levels of intraspecific genetic variation in deep-sea organisms, and it may be naive to assume that they will mirror shallow-water analogs. There are at least two reasons why levels of intraspecific variation might be higher in deep-sea bivalves. First, many species, including those considered here, may have enormous populations—they have pan-Atlantic distributions and are numerically abundant across a wide bathymetric range (Allen and Sanders 1996). Given the areal extent of the deep Atlantic, the continuity of soft-sediment habitats and the relatively high abundance of these

species, population size is likely to be much larger than is typical for shallow-water analogs. Second, because the deep sea is generally more stable than shallow water, populations may be more stable, limiting the loss of genetic diversity due to population fluctuations and environmentally induced bottlenecks. Both the large population size and the greater population stability would allow organisms to retain higher levels of ancestral polymorphism. In addition, extreme intraspecific genetic divergence is not unprecedented in mollusks (Thomaz et al. 1996; Quattro et al. 2001; Pfenninger and Posada 2002) or other invertebrates (Burton and Lee 1994; Romano and Palumbi 1997). Unfortunately, we lack a phylogenetic framework to clearly arbitrate between high levels of intraspecific polymorphism or the presence of cryptic species.

However, even if the divergence is sufficient to represent sibling cryptic species, it is not inconsistent with our inference that bathyal depths are more conducive to population differentiation and speciation. In fact, the logical outcome of any enhanced divergence at bathyal depths would be the formation of sibling species. The divergence we observe at bathyal depths may simply reflect the culmination (or near culmination) of this process.

The remarkable diversity of the deep-sea fauna is difficult to explain and continues to challenge contemporary ecological and evolutionary theory. We have made great strides in understanding the ecological mechanisms that allow coexistence and shape geographic patterns of diversity in this remote environment (Etter and Mullineaux 2001; Levin et al. 2001; Snelgrove and Smith 2002), but little is known about how this highly endemic fauna evolved. Results presented here suggest that the narrow bathyal zone may play a more important role in generating deep-sea biodiversity than the more extensive abyss. These results represent a first step in understanding the geographic and bathymetric scales of population differentiation in the largest ecosystem on Earth and may provide important insights into why diversity varies geographically and bathymetrically.

Acknowledgments

We would like to especially thank H. Sanders and J. Allen for generously providing the samples used in this study. Insightful comments from E. Bermingham, D. Ó Foighil, and two anonymous reviewers significantly improved the manuscript. Support for this research was provided by National Science Foundation grants OCE-9402855 and OCE-9811925. This work was influenced by a series of workshops on deep-sea biodiversity conducted at the National Center for Ecological Analysis and Synthesis, a center funded by the National Science Foundation (grant no. DEB-94-21535), the University of California/Santa Barbara, and the State of California.

Literature Cited

Adkins, J. F., H. Cheng, E. A. Boyle, E. R. M. Druffel, and R. L. Edwards. 1988. Deep-sea coral evidence for rapid change in ventilation of the deep North Atlantic 15,400 years ago. Science 280:725–728.

Allen, J. A., and H. L. Sanders. 1996. The zoogeography, diversity and origin of the deep-sea protobranch bivalves of the Atlantic: the epilogue. Prog. Oceanogr. 38:95–153.

Baco, A. R., C. R. Smith, A. S. Peek, G. K. Roderick, and R. C. Vrijenhoek. 1999. The phylogenetic relationships of whale-fall vesicomyid clams based on mitochondrial COI DNA sequences. Mar. Ecol. Prog. Ser. 182:137–147.

Barber, P. H., S. R. Palumbi, M. V. Erdmann, and M. K. Moosa. 2000. A marine Wallace's line? Nature 406:692–693.

———. 2002. Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. Mol. Ecol. 11:659–674.

Boyle, E. E., J. D. Zardus, M. R. Chase, R. J. Etter, and M. A. Rex. 2004. Strategies for molecular genetic studies of preserved deep-sea macrofauna. Deep-Sea Res. 51:1319–1336.

Bromham, L., and M. Cardillo. 2003. Testing the link between the latitudinal gradient in species richness and rates of molecular evolution. J. Evol. Biol. 16:200–207.

Brown, J. M., and G. B. Pauly. 2005. Increased rates of molecular evolution in an equatorial plant clade: an effect of environment or phylogenetic nonindependence? Evolution 59:238–242.

Burton, R. S. 1996. Molecular tools in marine ecology. J. Exp. Mar. Biol. Ecol. 200:85–101.

———. 1998. Intraspecific phylogeography across the Point Conception biogeographic boundary. Evolution 52:734–745.

Burton, R. S., and B. N. Lee. 1994. Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogenetic break in the copepod *Tigriopus californicus*. Proc. Natl. Acad. Sci. USA 91:5197–5201.

Buzas, M. A., L. A. Collins, and S. J. Culver. 2002. Latitudinal differences in biodiversity caused by higher tropical rate of increase. Proc. Natl. Acad. Sci. USA 99:7841–7843.

Cardillo, M. 1999. Latitude and rates of diversification in birds and butterflies. Proc. R. Soc. Lond. B 266:1221–1225.

Carney, R. S., R. L. Haedrich, and G. T. Rowe. 1983. Zonation of fauna in the deep sea. Pp. 371–398 *in* G. T. Rowe, ed. The sea. Vol. 8. Wiley, New York.

Chase, M., R. J. Etter, M. A. Rex, and J. Quattro. 1998a. Bathymetric patterns of genetic variation in a deep-sea protobranch bivalve. Mar. Biol. 131:301–308.

———. 1998b. Extraction and amplification of mitochondrial DNA from formalin-fixed tissue from deep-sea mollusks. Biotechniques 24:243–247.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9:1657–1660.

Cooper, R. A., P. Valentine, J. R. Uzmann, and R. A. Slater. 1987. Submarine Canyons. Pp. 52–63 *in* R. H. Backus, ed., Georges Bank. MIT Press, Cambridge, Ma.

Crandall, K. A., and A. R. Templeton. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. Genetics 134:959–969.

Crandall, K. A., A. R. Templeton, and C. F. Sing. 1994. Intraspecific phylogenetics: problems and solutions. Pp. 273–297 *in* R. W. Scotland, D. J. Siebert, and D. M. Williams, eds. Models in phylogeny reconstruction. Clarendon Press, Oxford U.K.

Cronin, T. M., and M. E. Raymo. 1997. Orbital forcing of deep-sea benthic species diversity. Nature 385:624–627.

Davies, T. J., V. Savolainen, M. W. Chase, J. Moat, and T. G. Barraclough. 2004. Environmental energy and evolutionary rates in flowering plants. Proc. R. Soc. Lond. B 271:2195–2200.

Davis, M. B., and R. G. Shaw. 2001. Range shifts and adaptive responses to Quaternary climate change. Science 292:673–679.

Etter, R. J., and J. F. Grassle. 1992. Patterns of species diversity in the deep sea as a function of sediment particle size diversity. Nature 360:576–578.

Etter, R. J., and L. Mullineaux. 2001. Deep-sea communities. Pp. 367–393 *in* M. D. Bertness, S. Gaines, and M. Hay, eds. Marine community ecology. Sinauer, Sunderland, MA.

Etter, R. J., and M. A. Rex. 1990. Population differentiation decreases with depth in deep-sea gastropods. Deep-Sea Res. 37: 1251–1261.

Etter, R. J., M. A. Rex, M. Chase, and J. Quattro. 1999. A genetic dimension to deep-sea biodiversity. Deep-Sea Res. 46: 1095–1099.

Flessa, K. W., and D. Jablonski. 1996. The geography of evolutionary turnover: a global analysis of extant bivalves. Pp. 376–

397 *in* D. Jablonski, D. H. Erwin, and J. H. Lipps, eds. Evolutionary paleobiology. Univ. of Chicago Press, Chicago.
France, S. C., and T. D. Kocher. 1996. Geographic and bathymetric patterns of mitochondrial 16S rRNA sequence divergence among deep-sea amphipods, *Eurythenes gryllus*. Mar. Biol. 126: 633–644.
Gage, J. D., and P. A. Tyler. 1991. Deep-sea biology. Cambridge Univ. Press, Cambridge, U.K.
Gardner, W. D. 1989. Baltimore Canyon as a modern conduit of sediment to the deep sea. Deep-Sea Res. 36:323–358.
Gaston, K. J. 2000. Global patterns in biodiversity. Nature 405: 220–227.
Gillooly, J. F., A. P. Allen, G. B. West, and J. H. Brown. 2005. The rate of DNA evolution: effects of body size and temperature on the molecular clock. Proc. Natl. Acad. Sci. USA 102: 140–145.
Goffredi, S. K., L. A. Hurtado, S. Hallam, and R. C. Vrijenhoek. 2003. Evolutionary relationships of deep-sea vent and cold seep clams (Mollusca: Vesicomyidae) of the ''*pacifica*/*lepta*'' species complex. Mar. Biol. 142:311–320.
Grassle, J. F., H. L. Sanders, and W. K. Smith. 1979. Faunal changes with depth in the deep sea benthos. Ambio Spec. Rep. 6:47–50.
Grosberg, R. K., and C. W. Cunningham. 2001. Genetic structure in the sea: from populations to communities. Pp. 61–84 in M. D. Bertness, S. Gaines, and M. E. Hay, eds. Marine community ecology. Sinauer Associates, Sunderland, MA.
Hawkins, B. A., R. Field, H. V. Cornell, D. J. Currie, J. F. Guégan, D. M. Kaufman, J. T. Kerr, G. G. Mittelbach, T. Oberdorff, E. M. O'Brien, E. E. Porter, and J. R. G. Turner. 2003. Energy, water and broad-scale geographic patterns of species richness. Ecology 84:3105–3117.
Hayward, B. W. 2001. Global deep-sea extinctions during the Pleistocene ice ages. Geology 29:599–602.
Hecker, B., D. T. Logan, F. E. Gandarillas, and P. R. Gibson. 1983. Megafaunal assemblages in Lydonia Canyon, Baltimore Canyon and selected slope areas. Pp. 1–140 *in* Canyon and slope processes study. Vol. 3. Biological processes. Final report submitted to the U.S. Department of Interior, Minerals Management Service Contract 14-12-001-29178.
Hellberg, M. E., D. P. Balch, and K. Roy. 2001. Climate-driven range expansion and morphological evolution in a marine gastropod. Science 292:1707–1710.
Helly, J., and L. A. Levin. 2004. Global distribution of naturally occurring marine hypoxia on continental margins. Deep-Sea Res. 51:1159–1168.
Hessler, R. R., and H. L. Sanders. 1967. Faunal diversity in the deep sea. Deep-Sea Res. 14:65–78.
Hogg, N. G. 1983. A note on the deep-sea circulation of the western North Atlantic: its nature and causes. Deep-Sea Res 30:945–961.
Jablonski, D. 1993. The tropics as a source of evolutionary novelty through geological time. Nature 364:142–144.
Jacobs, D. K., and D. R. Lindberg. 1998. Oxygen and evoutionary patterns in the sea: onshore/offshore trends and recent recruitment of deep-sea faunas. Proc. Natl. Acad. Sci. USA 95: 9396–9401.
Jozefowicz, C. J., and D. Ó Foighil. 1998. Phylogenetic analysis of Southern Hemisphere flat oysters based on partial mitochondrial 16S rDNA gene sequences. Mol. Phylogenet. Evol. 10: 426–435.
Knowles, L. L., and W. P. Maddison. 2002. Statistical phylogeography. Mol. Ecol. 11:2623–2635.
Kurihara, K., and J. P. Kennett. 1988. Bathymetric migration of deep-sea benthic foraminifera in the southwest Pacific during the Neogene. J. Foraminiferal Res. 18:75–83.
Lambeck, K., and J. Chappell. 2001. Sea level change through the last glacial cycle. Science 292:679–686.
Lapegue, S., I. Boutet, A. Leitao, S. Heurtebise, P. Garcia, C. Thiriot-Quievreux, and P. Boudry. 2002. Trans-Atlantic distribution of a mangrove oyster species revealed by 16S mtDNA and karyological analyses. Biol. Bull. 2023:232–242.
Levin, L. A, R. J. Etter, M. A. Rex, A. J. Gooday, C. R. Smith, J. Pineda, C. T. Stuart, R. R. Hessler, and D. Pawson. 2001. Environmental influences on regional deep-sea species diversity. Annu. Rev. Ecol. Syst. 32:51–93.
MacIlvaine, J. C., and D. A. Ross. 1979. Sedimentary processes on the continental slope of New England. J. Sed. Petrol. 49: 563–574.
Maciolek, N. J., J. F. Grassle, B. Hecker, B. Brown, J. A. Blake, P. D. Boehm, R. Petrecca, S. Duffy, E. Baptiste, and R. E. Ruff. 1987. Study of biological processes on the U.S. North Atlantic slope and rise. Final Report prepared for U.S. Dept. of Interior, Minerals Management Service, Washington, D.C.
Martin, P. R., and J. K. McKay. 2004. Latitudinal variation in genetic divergence of populations and the potential for future speciation. Evolution 58:938–943.
Mellor, C. A., and C. K. Paull. 1994. Sea beam bathymetry of the Manteo 467 Lease Block off Cape Hatteras, North Carolina. Deep-Sea Res. 41:711–718.
Palumbi, S. R. 1994. Genetic divergence, reproductive isolation and speciation in the sea. Ann. Rev. Ecol. Syst. 25:547–572.
———. 1999. All males are not created equal: fertility differences depend on gamete recognition polymorphisms in sea urchins. Proc. Natl. Acad. Sci. USA 96:12632–12637.
Palumbi, S. R., and R. R. Warner. 2003. Why gobies are like hobbits. Science 299:51–52.
Pfenninger, M., and D. Posada. 2002. Phylogeographic history of the land snail *Candidula unifasciata* (Helicellinae, Stylommatophora): fragmentation, corridor migration, and secondary contact. Evolution 56:1776–1788.
Posada, D., K. A. Crandall, and A. R. Templeton. 2000. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Mol. Ecol. 9:487–488.
Quattro, J. M., M. C. Chase, M. A. Rex, and R. J. Etter. 2001. Extreme mitochondrial DNA divergence within populations of the deep-sea gastropod *Frigidoalvania brychia* (Verrill, 1884). Mar. Biol. 139:1107–1113.
Raymo, M. E., K. Ganley, S. Carter, D. W. Oppo, and J. McManus. 1998. Millennial-scale climate instability during the early Pleistocene epoch. Nature 392:699–702.
Rex, M. A. 1981. Community structure in the deep-sea benthos. Annu. Rev. Ecol. Syst 12:331–353.
———. 1983. Geographic patterns of species diversity in the deep-sea benthos. Pp. 453–472 *in* G. T. Rowe, ed. The sea. Wiley, New York.
Rex, M. A., C. R. McClain, N. A. Johnson, R. J. Etter, J. A. Allen, P. Bouchet, and A. Warén. 2005. A source-sink hypothesis for abyssal biodiversity. Am. Nat. 165:163–178.
Reynolds, J., B. S. Weir, and C. C. Cockerham. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics 105:767–779.
Rhind, P. M., and J. A. Allen. 1992. Studies on the deep-sea Protobranchia (Bivalvia): the family Nuculidae. Bull. Br. Mus. Nat. Hist. (Zool) 58:61–93.
Rogers, A. D. 2000. The role of oceanic oxygen minima in generating biodiversity in the deep sea. Deep-Sea Res. 47:119–148.
———. 2003. Molecular ecology and evolution of slope species. Pp. 323–337 *in* G. Wefer, D. Billett, D. Hebbeln, B. Jørgensen, M. Schlüter, and T. Van Weering, eds. Ocean margin systems. Springer-Verlag, Berlin.
Rohde, K. 1992. Latitudinal gradients in species diversity: the search for the primary cause. Oikos 65:514–527.
Romano, S. L., and S. R. Palumbi. 1997. Molecular evolution of a portion of the mitochondrial 16S ribosomal gene region in scleractinian corals. J. Mol. Evol. 45:397–411.
Rosenzweig, M. L. 1995. Species diversity in space and time. Cambridge Univ. Press. Cambridge, U.K.
Sanders, H. L. 1977. Evolutionary ecology and the deep-sea benthos. Pp. 223–243 *in* C. E. Goulden, ed. The changing scenes in natural sciences, 1776–1976. Academy of Natural Sciences Special Publication, Philadelphia.
Schmitz, W. J., and M. S. McCartney. 1993. On the North Atlantic circulation. Rev. Geophys. 31:29–39.
Schneider, J. A., and D. O Foighil. 1999. Phylogeny of giant clams (Cardiidae: Tridacninae) based on partial mitochondrial 16S rDNA gene sequences. Mol. Phylogenet. Evol. 13:59–66.

Sepkoski, J. J. 1998. Rates of speciation in the fossil record. Philos. Trans. R. Soc. Lond. B 353:315–326.
Severinghaus, J. P., T. Sowers, E. J. Brook, R. B. Alley, and M. L. Bender. 1998. Timing of abrupt climate change at the end of the Younger Dryas interval from thermally fractionated gases in polar ice. Nature 391:141–146.
Slowey, N. C., and W. B. Curry. 1995. Glacial-interglacial differences in circulation and carbon cycling within the upper western North Atlantic. Paleoceanography 10:715–732.
Snelgrove, P. V. R., and C. R. Smith. 2002. A riot of species in an environmental calm: the paradox of the species-rich deep sea. Oceanog. Mar. Biol. Annu. Rev. 40:311–342.
Strimmer, K., and O. G. Pybus. 2001. Exploring demographic history of DNA sequences using generalized skyline plot. Mol. Biol. Evol. 18:2298–2305.
Sudhir, K., K. Tamura, I. B. Jakobsen, and M. Nei. 2001. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 12:1244–1245.
Templeton, A. R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. Mol. Ecol. 7:381–397.
———. 2004. Statistical phylogeography: methods of evaluating and minimizing inference errors. Mol. Ecol. 13:789–809.
Templeton, A. R., K. A. Crandall, and C. F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633.
Templeton, A. R., E. Routman, and C. A. Phillips. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics 140: 767–782.
Therriault, T. W., M. F. Docker, M. I. Orlova, D. D. Heath, and H. J. MacIsaaca. 2004. Molecular resolution of the family Dreissenidae (Mollusca: Bivalvia) with emphasis on Ponto-Caspian species, including first report of *Mytilopsis leucophaeata* in the Black Sea basin. Mol. Phylogenet. Evol. 30:479–489.
Thomaz, D., A. Guiller, and B. Clarke. 1996. Extreme divergence of mitochondrial DNA within species of pulmonate land snails. Proc. R. Soc. Lond. B 263:363–368.
Van Dover, C. L., C. R. German, K. G. Speer, L. M. Parson, and R. C. Vrijenhoek. 2002. Evolution and biogeography of deep-sea vent and seep invertebrates. Science 295:1253–1257.
Vermeij, G. 1987. Evolution and escalation: an ecological history of life. Princeton Univ. Press, Princeton, NJ.
———. 1991. Anatomy of an invasion: the trans-Arctic interchange. Paleobiology 17:281–307.
Vrijenhoek, R. C. 1997. Gene flow and genetic diversity in naturally fragmented metapopulations of deep-sea hydrothermal vent animals. J. Hered. 88:285–293.
White, B. N. 1987. Oceanic anoxic events and allopatric speciation in the deep sea. Biol. Oceanogr. 5:243–259.
Williams, S. T., and D. G. Reid. 2004. Speciation and diversity on tropical rocky shores: a global phylogeny of snails of the genus *Echinolittorina*. Evolution 58:2227–2251.
Willig, M. R., D. M. Kaufman, and R. D. Stevens. 2003. Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. Annu. Rev. Ecol. Syst. 34:273–310.
Wilson, G. D. F. 1998. Historical influences on deep-sea isopod diversity in the Atlantic Ocean. Deep-Sea Res. 45:279–301.
Wilson, G. D. F., and R. R. Hessler. 1987. Speciation in the deep sea. Annu. Rev. Ecol. Syst. 18:185–207.
Wright, S. D., R. D. Gray, and R. C. Gardner. 2003. Energy and the rate of evolution: inferences from plant rDNA substitution rates in the western Pacific. Evolution 57:2893–2898.
Xiang, Q., W. H. Zhang, R. E. Rickleffs, H. Qian, Z. D. Chen, J. Wen, and J. H. Li. 2004. Regional differences in rates of plant speciation and molecular evolution: a comparison between eastern Asia and eastern North America. Evolution 58:2175–2184.
Young, C. M. 2004. Reproduction, development and life-history traits. Pp. 381–426 *in* P. A. Tyler, ed. Ecosystems of the deep-oceans. Elsevier, Amsterdam.
Zachos, J., M. Pagani, L. Sloan, E. Thomas, and K. Billups. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292:686–693.
Zardus, J. 2002. Biology of the Protobranchia. Adv. Mar. Biol. 42: 1–65.

Corresponding Editor: D. Ó Foighil