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GENETIC ANALYSIS OF BAMBOO CORALS (CNIDARIA: OCTOCORALLIA: ISIDIDAE): DOES LACK OF COLONY BRANCHING DISTINGUISH *LEPIDISIS* FROM *KERATOISIS*?

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ABSTRACT

Bamboo corals (family Isididae) are among the most easily recognized deep-water octocorals due to their articulated skeleton comprised of non-sclerite calcareous internodes alternating with proteinaceous nodes. Most commonly encountered in the deep-sea are species in the subfamily Keratoisidinae, including the genera *Acanella* Gray, 1870, *Isidella* Gray, 1857, *Keratoisis* Wright, 1869, and *Lepidisis* Verrill, 1883. Systematists have debated whether *Lepidisis* and *Keratoisis* should be defined on the basis of "colony branching." Although recent taxonomic keys use "colonies unbranched" to distinguish *Lepidisis*, the original description of the genus included both branched and unbranched morphologies, with both forms also classified in *Keratoisis*. This study analyzed mitochondrial DNA sequence variation from isidids collected between 500–2250 m depth to address the following question: are unbranched, whip-like bamboo corals in the subfamily Keratoisidinae monophyletic? DNA sequences of the *msh1* gene (1426 nucleotides) from 32 isidids were used to construct a phylogeny. Coding of gaps provided additional informative characters for taxon discrimination. The results show five well-supported clades, all grouping both branched and unbranched colony morphologies; there was no single monophyletic clade of unbranched Keratoisidinae. The *msh1* phylogeny suggests that the distinction between the genera *Lepidisis* and *Keratoisis* should not be based on whether or not colonies branch.

Bamboo corals (Family Isididae) are common in deep-sea habitats. The family is characterized by its axial skeleton, which is composed of calcareous internodes alternating with proteinaceous, sclerite-free nodes (Fig. 1D)(Grant, 1976). Many members of the subfamily Keratoisidinae grow to large size and stand out in visual surveys of deep-sea hard substrates. Because the solid calcareous internodes may grow to a relatively large diameter and preserve well, and colonies live for long periods, they have come under increased study by geochemists interested in analyzing isotopic signatures as recorders of paleo-oceanographic events (e.g., Neil et al., 2005; Roark et al., 2005; Noé and Dullo, 2006; Thresher et al., 2007) and biologists interested in aging deep-sea coral populations (Mills and Mullineaux, 2005). The focus of these studies has been on the genera *Keratoisis* Wright, 1869 and *Lepidisis* Verrill, 1883, whose species are among the largest and most common of the deep-sea Isididae.

Classification of the Subfamily Keratoisidinae.—The most recent taxonomic treatment (Bayer, 1990) of the Keratoisidinae lists six genera in the subfamily: *Keratoisis* Wright, 1869; *Isidella* Gray, 1857; *Acanella* Gray, 1870; *Lepidisis* Verrill, 1883; *Tenuisis* Bayer and Stefani, 1987; and *Orstomisis* Bayer, 1990. Bayer and Stefani (1987a) outline the six morphological characteristics that have been used to classify and identify bamboo corals: (1) polyp retractability, (2) colony branching, (3) structure of the axial skeleton, (4) sclerite morphology, (5) sclerite arrangement, and (6) presence or absence of an operculum on the polyps. Of particular interest for the current study is the branching growth form of the colonies, which most observers use to distinguish *Lepidisis* from *Keratoisis*. In practice, most deep-sea coral biolo-

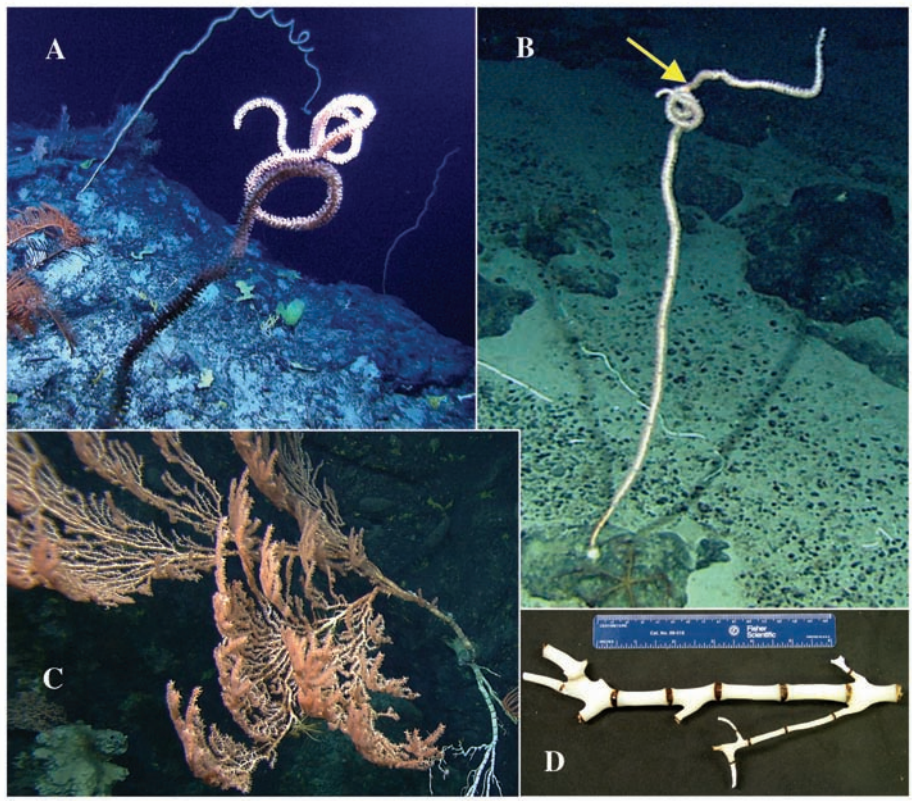

Figure 1. Examples of bamboo corals in the subfamily Keratoisidinae. (A) Unbranched whip-like colonies ("*Lepidisis*") > 2 m in height, with characteristic spiraling, distal ends, on Manning Seamount; (B) distally-forked colony on Balanus Seamount, 1560 m depth; the branching point, indicated by the arrow, is > 1 m above bottom and is obscured by the coiled end of one branch pointing out of the plane of the page; (C) internodal-branching colony ("*Keratoisis*") on Bear Seamount, 1478 m depth; (D) skeleton fragment from colony in 1C, showing dark proteinaceous nodes, white calcareous internodes, and internodal branching (ruler = 15 cm). (Photo credits for A, B, C: Mountains in the Sea research group–Institute for Exploration (IFE)–NOAA)

gists label as *Lepidisis* unbranched, whip-like bamboo corals that often spiral as they get taller (Fig. 1A). Colonies that branch from the internodes are classified as *Keratoisis* (Fig. 1C).

There has been much debate in the systematic literature on the topic of branching as related to definitions of the genera *Lepidisis* and *Keratoisis* (see Grant [1976] and Bayer [1990] for a complete discussion). Verrill (1883) originally erected the genus *Lepidisis* for three West Indian species, but he did not designate a holotype for the genus, which has led to subsequent confusion. One of the three species branched at the nodes, one was unbranched, and the other had an unknown branching pattern. Grant (1976) argued that original descriptions for *Lepidisis* and *Keratoisis* allowed for both branched and unbranched species in each genus, and he further amended Verrill's original description of *Lepidisis* to include species that branched at the node or the internode. He distinguished *Lepidisis* from *Keratoisis* on the basis of sclerites, not branching pattern. Muzik (1978) wrote that sclerites were unreliable characters in distinguishing among isidid genera in the subfamily Keratoisidinae, and proposed distinguishing these genera on the basis of colony branching pattern. Unbranched,

whip-like Keratoisidinae were assigned to *Lepidisis* (to conform with Kükenthal's (1915) designated type for the genus, the unbranched *Lepidisis caryophyllia* Verrill, 1883), and branched colonies were assigned to *Keratoisis* if branching occurred at the internodes, or *Isidella* if branching occurred at the nodes. Muzik (1978) synonomized *Acanella* Gray 1869 with *Isidella*, but Bayer (1990) noted that the "verticillate branching" of *Acanella* was sufficiently distinctive from the planar growth form of *Isidella* that the two genera should be retained pending a complete revision of the subfamily. These distinctions have been followed in the most recently published keys to the Isididae (Bayer and Stefani, 1987a,b; Bayer, 1990).

In this study I analyzed mitochondrial DNA sequence variation from isidids collected from the deep North Atlantic and North Pacific. Compared to other invertebrates, octocorals show low rates of mitochondrial DNA sequence evolution (France and Hoover, 2002; Shearer et al., 2002). Nonetheless, mitochondrial genes, and in particular, the gene *msh1* (France and Hoover, 2001), can provide phylogenetic information at the genus (Sánchez et al., 2003) and intrageneric level (Lepard, 2003; Wirshing et al., 2005) of octocorals. I have sequenced two regions of the *msh1* gene from internodal branching, nodal branching, and unbranched species of Keratoisidinae, and ask the following question: are unbranched, whip-like bamboo corals in the subfamily Keratoisidinae monophyletic? If so, this would support the position of Muzik, Bayer and others that unbranched Keratoisidinae belong to the genus *Lepidisis*, and the common practice among deep-sea coral biologists to refer to all these bamboo whips as *Lepidisis*.

Methods

Collections.—Tissue samples from bamboo corals were collected on a series of expeditions to the New England Seamounts (North Atlantic) from 2003–2005, the Aleutian Ridge (2004) and Hawaiian seamounts and islands (1993, 1996). Colonies, or portions thereof, were sampled using a manipulator claw of an ROV (Hercules, Jason II) or HOV (Alvin, Pisces V) and stored in a biobox until recovery of the vehicle on the surface. Samples remained in chilled seawater until they were processed in a shipboard lab. All colonies were first photographed, after which fragments for genetics were removed and stored in 100% or 95% ethanol. Voucher samples were stored in 70% ethanol. Additional ethanol-preserved samples were obtained from the National Museum of Natural History, Smithsonian Institution, and outgroup taxa from the isidid subfamilies Circinisidinae [*Zignisis phorinema* Alderslade, 1998 and *Zignisis repens* (Briggs, 1915)] and Mopseinae [*Pteronisis whiteleggei* (Thomson and Mackinnon, 1911) and *Pteronisis incerta* Alderslade, 1998] from the Museum and Art Gallery of the Northern Territory, Darwin, Australia. Table 1 provides the list of the Keratoisidinae specimens whose sequences are reported in this study, including localities and depths of collections, and museum voucher catalog numbers (collections from 2005 have not yet been assigned catalog numbers but vouchers are available). Preliminary identifications of specimens from the North Atlantic and North Pacific were made by the author and L. Watling (University of Maine); museum voucher specimens were identified by T. Bayer and S. Cairns (NMNH) or P. Alderslade (NTM, Australia). For the most part in this paper, species names are not given as identifications were preliminary and some species are new to science. Four additional species, from the families Primnoidae (*Narella dichotoma* Versluys, 1906 and *Calyptrophora* cf. *versluyi* Nutting, 1908) and Coralliidae (*Corallium niobe* Bayer, 1964 and *Corallium ducale* Bayer, 1955), were included as outgroups to root the phylogenetic tree generated using the combined data (see below).

DNA Extraction, Amplification, and Sequencing.—DNA was extracted using a CTAB protocol (Berntson and France, 2001). Prior to macerating and digesting tissue, coral fragments were washed for several hours in CTAB buffer to remove ethanol. France and Hoover (2001) were unable to PCR amplify bamboo coral DNA with *msh1* primers that worked with other octocorals. We have since discovered that mitochondrial gene order differs in isidids compared to other octocorals (S. France and M. Brugler, University of Louisiana at Lafayette, unpubl. data), and have designed new PCR primers to amplify the 5′ end of the *msh1* gene. Two separate regions of the *msh1* gene were amplified. Primers *msh5p8f* (5′-TAGAATCGCCATGGGGAA-3′) and *msh3458r* (5′-TSGAGCAAAAGCCACTCC-3′) amplify a fragment of ≈850 bp at the 5′ end of the gene (primer *msh5p8f* spans the start codon); primers *MutS4759f* (5′-TGTAGCTCATGATATTAG-3′) and *16s5pr* (5′-TCACGTCCTTACCGATAG-3′) amplify a fragment of ≈950 bp at the 3′ end of the gene (primer *16s5pr* sits at the start of the large subunit rRNA gene). Prior to designing *16s5pr,* PCR reactions paired primer *16s140r* (5′-TCTCSGTTGATGTATTCC-3′) with *MutS4759f,* which incorporates a longer stretch of the large subunit rRNA gene in the amplified product.

PCR amplifications used Ex Taq™ polymerase (TaKaRa Mirus Bio) and followed manufacturer's protocols, except that half-volume reactions were set up. All reactions were run concurrent with a negative control (no DNA). PCR products were purified using low-melting point agarose, and the resultant gel slices digested with 5–10 units of agarase enzyme (Sigma Chemical Co.) at 37–40 °C for at least 1 hr to overnight. A portion of this digestion was used in a cycle sequencing reaction using fluorescently-labeled dideoxy terminators according to the manufacturer's recommended conditions (ABI PRISM BigDye Terminator v.1.1 or Beckman Coulter CEQ DTCS kit). The products were run on an ABI3100 (Applied Biosystems) or CEQ8000 (Beckman Coulter) automated DNA sequencer. Most samples were sequenced in both directions with the same primers used in the PCR amplification. The sequences have been deposited to GenBank (accession numbers in Table 1).

Data Analysis.—Sequence traces were edited using Beckman's CEQuence Investigator or GeneCodes Sequencher software. Nucleotide sequences were translated to amino acids using the cnidarian mitochondrial genetic code (NCBI translation table 4; National Center for Biotechnology Information at http://www.ncbi.nlm.nih.gov) and aligned by eye using the software SeqApp (Gilbert, 1992). Phylogenetic trees were constructed using corresponding nucleotide alignments for each gene region separately as well as for the combined dataset (only results from the combined dataset are shown). Insertion/deletion mutations (indels) were common among groups of isidids and appeared to have useful phylogenetic information, and thus were included as characters in the distance-based and maximum parsimony analyses. Indels were uniquely coded using the "simple indel coding" method, where each indel with a different start and/or end position is considered a separate character (Simmons and Ochoterena, 2000; Young and Healy, 2003). Bayesian phylogenetic analyses were conducted using MrBayes v.3.04 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), with a GTR model and site-specific rates set to vary by codon position, run for 6.4×10^6 generations (burnin = 10,000 generations). PAUP* v. 4.0b10 (Swofford, 2002) was used for both distance-based and maximum parsimony analyses. For maximum parsimony, I conducted a heuristic search with TBR branch-swapping, and ran 500 bootstrap replicates each with two random addition sequences.

Results

In total, 37 and 57 isidids were amplified and sequenced for the *msh1* 5′ and 3′ regions, respectively. I present data herein from 35 specimens (Table 1) for which sufficient sequence data were available to combine the two regions into a single alignment totaling 1426 bp (including indels)(819 bp from the 5′ region + 607 bp from the 3′ region). No conclusions relevant to the current study are lost using the dataset

Table 1. Collection data for Keratoisidinae used in this study. No species names have been assigned to taxa pending further taxonomic study. "Nominal genus" refers to the identification prior to genetic analysis. Where available, a museum catalog number for the voucher specimen has been included below the specimen number (USNM–US National Museum, Smithsonian Institution; YPM–Peabody Museum, Yale University). "*msh1* haplotype" refers to the unique DNA sequences observed. DNA sequences for the 5' and 3' regions of *msh1* were combined and submitted to GenBank. Three of the specimens used in this study were also sequenced for the mitochondrial non-coding region (ncr) or 16S rDNA by Smith et al. (2004) and are indicated by an *.

Nominal genus	Specimen number	Date collected	Location	Latitude	Longitude	Depth (m)	*msh1* haplotype	GenBank acc #
Acanella	MIL101-1	Aug 2005	Caloosahatchee Smt, Atlantic	34°49.07′N	50°30.37′W	1,688	A1a	EF060014
Acanella	RET106-1, YPM 35389	May 2004	Retriever Smt, Atlantic	39°48.45′N	66°15.00′W	2,035	A1a	EF060013
Isidella	J2095-2-7-5	July 2004	Aleutian Ridge, Pacific	51°48.69′N	173°49.96′W	843 ~~2,095~~	I1c	EF060020
Isidella	KEL601-2	Aug 2005	Kelvin Smt, Atlantic	38°45.45′N	64°05.46′W	2,593	I2a	EF060018
Isidella	KEL604-2	Aug 2005	Kelvin Smt, Atlantic	38°45.50′N	64°05.49′W	2,554	I3a	EF060022
Isidella	MAN804-1, YPM 35372	May 2004	Manning Smt, Atlantic	38°08.73′N	61°05.14′W	1,550	I1a	EF060015
Isidella	PN101-1, USNM 94449	Aug 1993	Pensacola Smt, Pacific	18°20.50′N	157°20.00′W	1,500	I3a	EF060021
Isidella	VER210-1	Aug 2005	Caloosahatchee Smt, Atlantic	34°32.41′N	49°47.64′W	1,642	I2a	EF060016
Keratoisis	CR206-4*, USNM 94445	Aug 1993	Cross Smt, Pacific	18°38.70′N	158°16.80′W	1,040	B1d	EF060040
Keratoisis	HAS-25, USNM 98807	Sept 1996	Hawaiian Islands, Pacific	19°37.46′N	156°02.06′W	507	D2b	EF060044
Keratoisis	J2105-5-1	Aug 2004	Aleutian Ridge, Pacific	51°52.95′N	178°20.90′W	2,031	B1c	EF060028
Keratoisis	J2105-6-1	Aug 2004	Aleutian Ridge, Pacific	51°52.94′N	178°20.87′W	2,006	B1c	EF060027
Keratoisis	KEL612-2	Aug 2005	Kelvin Smt, Atlantic	38°45.93′N	64°05.43′W	2,130	D2c	EF060046
Keratoisis	MAN101-4, YPM 37154	July 2003	Manning Smt, Atlantic	38°15.79′N	60°33.01′W	1,735	B1a	EF060037
Keratoisis	MAN707-2	May 2004	Manning Smt, Atlantic	38°08.92′N	61°06.11′W	1,826	D1a	EF060023
Keratoisis	MAN807-1, YPM 35376	May 2004	Manning Smt, Atlantic	38°08.61′N	61°05.54′W	1,469	D1a	EF060025
Keratoisis	RET114-1	May 2004	Retriever Smt, Atlantic	39°48.59′N	66°14.97′W	1,982	D2d	EF060045

Table 1. Continued.

Nominal genus	Specimen number	Date collected	Location	Latitude	Longitude	Depth (m)	*msh1* haplotype	GenBank acc #
Lepidisis	BAL111-1	May 2004	Balanus Smt, Atlantic	39°22.30′N	65°22.31′W	1,560	C1a	EF060035
Lepidisis	BAL208-3	Aug 2005	Balanus Smt, Atlantic	39°24.88′N	65°24.65′W	1,815	C1a	EF060030
Lepidisis	BAL211-3	Aug 2005	Balanus Smt, Atlantic	39°24.76′N	65°24.48′W	1,689	C1a	EF060033
Lepidisis	NAS103-1	Aug 2005	Nashville Smt, Atlantic	34°34.98′N	56°50.59′W	2,252	D1b	EU100103
Lepidisis	DE0409-23-2	June 2004	Bear Smt, Atlantic	39°52.43′N	67°26.19′W	1,431–1,464	C1a	EF060029
Lepidisis	GIL101-1	Oct 2005	Gilbert Canyon, Atlantic	40°06.41′N	67°52.92′W	2,152	I3a	EF060017
Lepidisis	GOO102-1	Aug 2005	Corner Smt, Atlantic	35°23.61′N	51°15.91′W	2,137	D1b	EF060024
Lepidisis	KEL111-1	July 2003	Kelvin Smt, Atlantic	38°47.25′N	64°07.66′W	1,800	C1a	EF060034
Lepidisis	KEL209-1	July 2003	Kelvin Smt, Atlantic	38°50.99′N	63°55.57′W	1,859	D2e	EF060047
Lepidisis	KEL408-1	May 2004	Kelvin Smt, Atlantic	38°46.98′N	63°57.76′W	2,252	B1b	EF060041
Lepidisis	KEL508-1, YPM 35407	May 2004	Kelvin Smt, Atlantic	38°51.32′N	63°46.17′W	1,990	B1b	EF060042
Lepidisis	LAD-25*	Sept 1996	Hawaiian Islands, Pacific	20°46.96′N	157°08.93′W	1,067	I1b	EF060019
Lepidisis	MAN707-1	May 2004	Manning Smt, Atlantic	38°08.92′N	61°06.11′W	1,826	C1a	EF060036
Lepidisis	PIC102-1	Oct 2005	Picket Smt, Atlantic	39°39.12′N	65°56.50′W	2,060	B1b	EF060038
Lepidisis	PIC107-2	Oct 2005	Picket Smt, Atlantic	39°39.24′N	65°56.91′W	1,946	B1b	EF060039
Lepidisis	REH110-1	Aug 2005	Rehoboth Smt, Atlantic	37°27.62′N	59°57.00′W	1,827	C1a	EF060031
Lepidisis	REH112-1	Aug 2005	Rehoboth Smt, Atlantic	37°27.60′N	59°56.98′W	1,821	C1a	EF060032
Lepidisis	USNM 100897*	Dec 2000	Bear Smt, Atlantic	39°58.00′N	67°30.82′W	2,000	D2a	EF060043

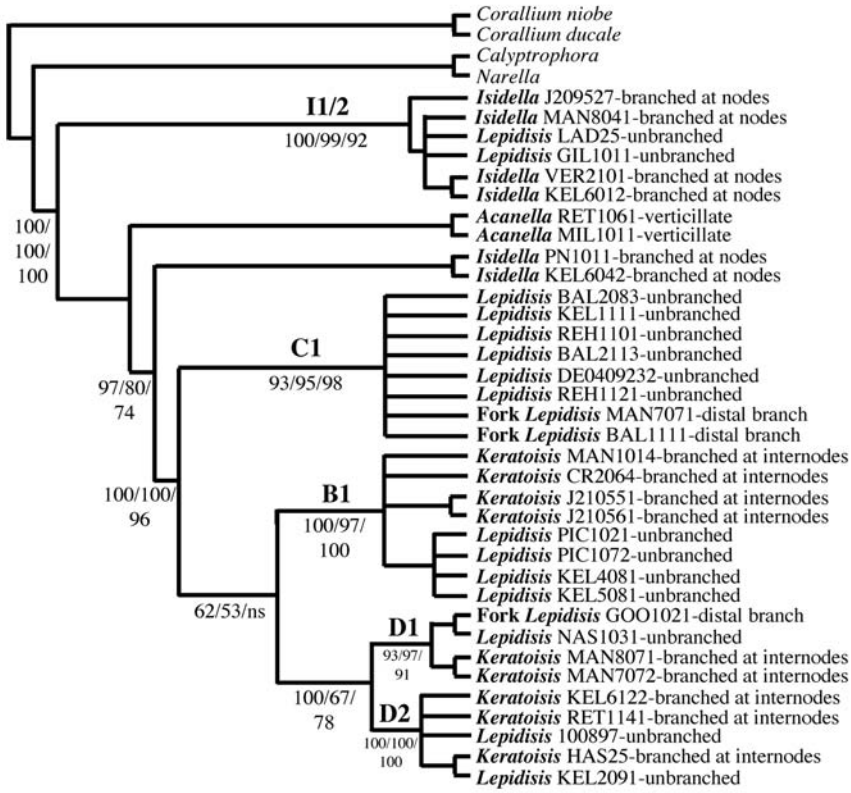

Figure 2. Bayesian-likelihood tree of phylogenetic relationships among Keratoisidinae (branch lengths not shown). Values at nodes are Bayesian posterior probabilities (10^6 generations, 15,002 trees, indels not coded)/bootstrap percentages for maximum parsimony (500 replicates, indels coded)/bootstrap percentages for neighbor-joining (2000 replicates, HKY, indels coded); ns indicates that a branch was not supported. Alphanumeric labels identify clades that include unbranched *"Lepidisis"* as discussed in text, e.g., D1, C1, etc. Specimen numbers are as in Table 1. Outgroup taxa are *Narella dichotoma* (Hawaii; GenBank Acc #EF060048), *Calyptrophora* cf. *versluyi* (Hawaii; GenBank Acc #EF060049), *Corallium ducale* (Cross Seamount, Pacific; GenBank Acc #EF060050), and *Corallium niobe* (Kelvin Seamount, Atlantic; GenBank Acc #EF060051).

with fewer taxa, but support for nodes is stronger with the greater number of characters used in the combined analysis. There are 13 unique indels in the combined alignment (not including outgroup taxa), with a range of lengths from 3–48 bp (1–16 amino acids), such that the total lengths of the *msh1* fragments range from 696–765 bp (232–255 aa) for the 5′ region, and 558–600 bp (186–200 aa) for the 3′ region. Two areas are particularly variable with respect to indel structure, one in the 5′ region and one in the 3′ region. Coding of gaps using the "simple indel coding" method added an additional 24 characters. Of the total 1449 nucleotides and coded gaps, 1161 characters were invariant, and 58 of 230 variable sites were parsimony-informative.

Phylogenetic analyses of the 3′ region of *msh1* used bamboo corals from the subfamilies Circinisidinae and Mopseinae as outgroups. However these species would not amplify at the 5′ region, possibly because these are museum specimens not specifically preserved for genetics (Berntson and France, 2001), and therefore they

could not be used as outgroups in the combined-data analysis. Species in the families Primnoidae and Coralliidae were thus used as outgroups. The major clades did not differ when using either set of outgroups for the 3′ region sequences.

Bayesian, maximum parsimony, and distance analyses on the combined 5′ and 3′ datasets all recovered similar topologies (Fig. 2). These trees show five well-supported clades (B1, C1, D1, D2, I1/2), each of which includes at least one nominal *Lepidisis*. As has been demonstrated in previous studies (e.g., Suzuki et al., 2002; Simmons et al., 2004), Bayesian posterior probabilities tend to show higher clade support than other methods such as boostrap. Sequences varied among taxa within these well-supported clades, but all haplotypes (unique mitochondrial sequences) within the clades were of the same length (except Aleutian Ridge *Keratoisis* have seven additional amino acids relative to other B1 haplotypes in the 3′ region). However, sequence length differed among all clades (*Acanella* shares the same indel structure as clade I1/2). Strong support for these clades could result from the coding of indels as separate characters, i.e., the gaps are overweighted. However, the Bayesian analysis excluded gaps as characters and recovered the same well-supported clades (see posterior probabilities in Fig. 2), indicating that the clusters are not simply a reflection of indel structure. There is less support in the tree for the branching order between *Acanella* and *Isidella*, but those genera are not the focus of this study and will not be considered here.

Unique haplotypes were assigned a code that identified their position in the tree, i.e., as part of clade B1, C1, etc. (Table 1). Clade B1 groups unbranched "*Lepidisis*" from the New England Seamounts (haplotype B1b) with branched "*Keratoisis*" from Alaska (B1c) and Hawaii (B1d). Clade C1 combines unbranched "*Lepidisis*" with colonies that are distally forked (e.g., BAL111-1 and MAN707-1; see Fig. 1B), and is the only one of the five well-supported clades that does not include more complexly branched colonies. Clade D1 includes a distally-forked colony (D1b), branched "*Keratoisis*" (D1a) and unbranched "*Lepidisis*" (D1b), all from North Atlantic seamounts. Clade D2 groups branched "*Keratoisis*" from Hawaii (D2b) with branched "*Keratoisis*" (D2c, D2d) and unbranched "*Lepidisis*" from the New England Seamounts (D2a, D2e). The fifth clade (I1/2) combines unbranched "*Lepidisis*" from Hawaii (I1b) and Gilbert Canyon (I2b) with Atlantic seamount taxa whose colonies branch from the node, e.g., *Isidella* (I1a, I2a). This clade includes *L. caryophyllia*, the designated type species of the genus *Lepidisis*.

Discussion

Phylogenies constructed using DNA sequences of the mitochondrial *msh1* gene do not recover a single monophyletic clade of unbranched Keratoisidinae. Unbranched colonies were mixed with branched colonies in various parts of the phylogeny, including both nodal- and internodal-branching morphologies. We collected and analyzed branched colonies that were small (< 30 cm height) and unbranched colonies that were large (> 4 m [S. Mills, Woods Hole Oceanographic Institution, pers. comm.]), and therefore reject the idea that all of our unbranched colonies may simply have been sampled before they grew to a sufficient size to branch. Additionally, the phylogenies are not grouping taxa based solely on geographic patterns of divergence. Several clades include representatives from both the Pacific and Atlantic, which suggests we are looking at long-established lineages and not local geographic variants.

Which of the clades includes the "real" *Lepidisis*? Kükenthal (1915) designated *L. caryophyllia* as the type species for the genus. In the present analysis, a representative colony of *L. caryophyllia* groups with *Isidella* species on clade I1/2 and thus supports Kükenthal's description of *Lepidisis* as branching—if it branches—from the nodes. We found the unbranched *L. caryophyllia* growing on soft sediment deep in a canyon cutting into the continental slope of Georges Bank. In contrast, all of the "typical" whip-like, spiraling colonies from seamount hard substrates that we analyzed clustered on clades that included *Keratoisis*-like colonies, i.e., branching from the internodes.

If we assume that all unique *msh1* haplotypes represent separate species (see below), then even this limited sampling of 35 Keratoisidinae suggests 14 species distributed among five or more genera, all of which include both branched and unbranched colonies. Previous studies of octocorals have shown low rates of mitochondrial DNA sequence evolution (France and Hoover, 2002; Shearer et al., 2002), and in some cases mitochondrial DNA sequences have failed to distinguish octocoral colonies identified as different species using morphological characters (Lepard, 2003; Cairns and Bayer, 2005). We have observed no previous instance of intraspecific variation in the DNA sequence of a mitochondrial gene of an octocoral (France and Hoover, 2001, 2002; Shearer et al., 2002; Lepard, 2003; S. France, University of Louisiana at Lafayette, unpubl. data), which suggests that unique haplotypes represent taxa higher than the population level. We have examples where a single nucleotide substitution in an *msh1* haplotype of length 1367 bp can distinguish accepted morpho-species (e.g., *Paramuricea* Kölliker, 1865 from the New England Seamounts [S. France, University of Louisiana at Lafayette, unpubl. data; morphological identifications L. Watling and A. Simpson, pers. comm.]). Conversely, morphological examination of sclerites (the primary character used in octocorallian taxonomy) failed to separate some *Paramuricea* colonies that had *msh1* haplotypes diverging up to 1.6%. This suggests that there may be morphologically-cryptic species variation among the seamount octocorals and reinforces the observation that octocoral taxonomy and systematics cannot rely solely on morphology of skeletal characters (e.g., Sánchez et al., 2003; Wirshing et al., 2005), but will require combined studies of characters from the skeleton, soft tissue, and genetics.

This analysis of mitochondrial *msh1* DNA sequences shows that from an evolutionary perspective, the distinction between the genera *Keratoisis* and *Lepidisis* should not be based on whether or not colonies branch. The "colonies unbranched" character state is not a synapomorphy of any clade, but rather is distributed throughout the tree alongside branching colonies. Bayer and Stefani (1987a) noted that as deep-sea collections led to a proliferation of new isidid taxa without a systematic revision of the family, the distinctions and relationships among genera and subfamilies have become blurred. A thorough analysis of the subfamily Keratoisidinae is required to sort out these taxonomic issues.

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