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Independent Origins of Filter-Feeding in Megamouth and Basking Sharks (Order Lamniformes) Inferred from Phylogenetic Analysis of Cytochrome b Gene Sequences

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Abstract There are conflicting hypotheses with regard to the origins of filter-feeding in lamniform sharks. Maisey (1985) proposed that the megamouth (*Megachasma pelagios*) and the basking shark (*Cetorhinus maximus*) are sister taxa, implying a single origin of filter-feeding within the Lamniformes. By contrast, Compagno (1990) hypothesized that adaptations for filter-feeding evolved independently in the two lineages from different ancestral conditions. Phylogenetic analysis of cytochrome b gene sequences from representatives of all genera of lamnoid sharks refutes the single-origin of filter-feeding hypothesis, implying independent origins. Moreover, the data permit confident refutation of published phylogenetic hypotheses of the group. However, for many lineages there is little hierarchical signal in the molecular data, prohibiting promulgation of a single, "best" supported alternative hypothesis for the group. Lack of confident resolution of the phylogenetic relationships among species of lamnoid sharks most likely reflects divergence of ancient lineages in a brief period of time. Fossils indicate a Middle to Late Cretaceous origination for most extant genera of lamnoid sharks.

Filter-feeding has evolved independently in a variety of marine and freshwater animals. In elasmobranchs filter-feeders are classified in four families representing three orders (Myliobatiformes, Mobulidae [manta rays]; Lamniformes, Cetorhinidae [basking shark] and Megachasmidae [megamouth]; and Orectolobiformes, Rhincodontidae [whale shark]). Three of these families are sharks, and all of the filter-feeding sharks resemble baleen whales in size and life style. Two of the three species (the basking shark [*Cetorhinus maximus*] and megamouth [*Megachasma pelagios*]) are thought to be close relatives in the order Lamniformes (Maisey, 1985). The other species, the whale shark (*Rhincodon typus*), is a distant relative of the lamnoid filter-feeders, being classified in the Orectolobiformes, an order of sharks consisting mostly of benthic species. As in the baleen whales, specialization by sharks on small, planktonic organisms is associated with modification of a suite of morphological and behavioral traits. The chondrocranium is modified, there is a loss of dental differentiation and simplification of tooth cusp and root morphology, and the gill rakers are greatly enlarged (Compagno, 1984; Maisey, 1985). Moreover, with few exceptions, vertebrate filter-feeders are strong swimmers and are constrained to live near the surface of nutrient-rich waters where plankton concentrations reach their zenith. For example, the basking shark, which is the largest of the lamniform sharks, attaining a total length of up to 30 feet, sports a rigid and streamlined body, firm muscles and connective tissue, features that aid feeding by straining plankton through highly modified gillrakers as it moves through the water column with its huge mouth agape (similar to some baleen whales). The whale shark is also most commonly encountered in surface waters, and like the basking shark, has a streamlined body, and firm muscles and connective tissue. Unlike

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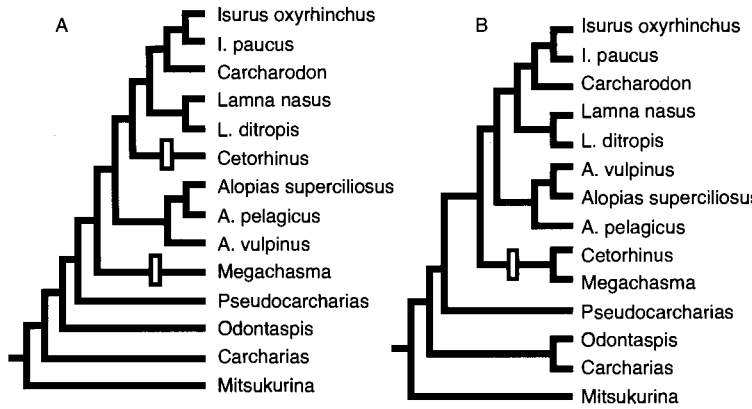

Fig. 1. Alternative phylogenetic hypotheses for the relatioships among species of Lamniformes. In A, the hypothesis of Compagno (1990) is reproduced exactly. The topology in B is based on the hypothesis of Maisey (1985), but differs in that it is the minimum length tree for the cytochrome b data subject to the constraints specified in Maisey's hypothesis; namely, that the Lamnidae and Alopiidae are monophyletic and sister groups, that *Cetorhinus* and *Megachasma* are sister taxa, and that these two taxa comprise the sister group to the Lamnidae + Alopiidae. Open rectangles mark the origination of filter-feeding.

the basking shark, however, the whale shark is a suction-feeder with a broad head and a very wide mouth. The suction capacity allows the whale shark to feed on a greater variety and larger size of prey than the basking shark; moreover, whale sharks are not dependent on forward motion to feed (Compagno, 1984). Compagno (1984) notes, however, that whale sharks can filter a much smaller volume of water than basking sharks and may therefore be less efficient at foraging on abundant but diffuse zooplankton. Like the whale shark, megamouth may also be a suction-feeder. Unlike the whale and basking sharks, megamouth probably is a sluggish, slow-swimming, deep-water shark. Inspection and dissection of the type specimen led Compagno (1990) to suggest a unique feeding strategy. Megamouth is thought to have bioluminscent lips. In addition, the back and roof of the mouth probably are reflective. Thus, megamouth may act like an enormous flashlight in the dark meso- and epipelagic realm. Small shrimp and invertebrates may be attracted near its enormous mouth, and the prey may then be sucked in by the combined action of jaw protusion and depression of its large tongue (Diamond, 1985; Compagno, 1990).

The classification of whale sharks in a separate order from the basking shark and megamouth implies independent origination of filter-feeding in the two groups. Within the lamniformes, the existence of similarities and differences between the basking shark and megamouth suggests that filter-feeding may have evolved once and diverged under selection in the two lineages (Maisey, 1985; Fig. 1); or, alternatively, filter-feeding may have evolved independently twice from different ancestral conditions (Compagno, 1990; Fig. 1). We have subjected the order Lamniformes to molecular phylogenetic analysis to evaluate the alternative hypotheses of single versus multiple origins of filter-feeding.

Materials and Methods

Table 1 lists the species included in this study, collecting localities, and the person who collected the tissue. DNA was extracted from ethanol or DMSO-EDTA-salt preserved tissue by incubation in SDS/proteinase K buffer at 65°C overnight. Standard phenol:chloroform extraction and ethanol precipitation were used to extract and recover the DNA. DNA was

Table 1. List of species, DNA identification number, collection locality, and collector's name for all samples for which cytochrome b gene sequences were determined

Species	ID	Locality	Collector
Alopias pelagicus	AlsuBJ133	Baja California, Mexico	J. Caira
A. pelagicus	AlpeTai81	Taiwan	Chen
A. vulpinus	Alvu429	Long Island, NY, USA	G. Naylor
A. vulpinus	AlvuMM13	Japan	M. Miya
A. superciliosus	Alsu622	Florida, USA	G. Naylor
A. superciliosus	AlsuTai	Taiwan	Chen
Carcharodon carcharias	CacaNA	East Coast, USA	G. Naylor
C. carcharias	CacaCA1	Farallon Is., CA, USA	K. Goldman
Cetorhinus maximus	1058	Plymouth, UK	D. Sims
C. maximus	CemaJS1	Tasmania, Australia	J. Stevens
Carcharias taurus	627	Georgia, USA	G. Naylor
C. taurus	CataSA1	South Africa	G. Cliff
Isurus oxyrinchus	412		G. Naylor
I. paucus	614	Florida Keyes, USA	D. de Maria
Lamna ditropis	LadiMM12	Japan	M. Miya
L. nasus	633	Gulf of Maine	G. Naylor
Mitsukurina owstoni	1057	Australia	G. Naylor
M. owstoni	MiowMM1	Japan	M. Miya
Megachasma pelagios	Mepe1	Japan	K. Yano/M. Miya
Odontaspis ferox	Odfe		J. Castro
Pseudocarcharias kamoharai	Pska	Taiwan	S. Young
Outgroups			
Galeocerdo cuvier	Gacu553	Hawaii, USA	G. Naylor
Heterodontus franscii	Hefr	California, USA	A. Martin
Urolophus concentricus	Urco	Baja California, Mexico	A. Martin

stored at −20°C in 0.1 × TE (pH = 8.0) buffer.

The cytochrome b gene was amplified in a 25-μl reaction using 12.5 picomoles of the primers GluDG and Cb1211H (Fig. 2). For all amplifications we used the Perkin-Elmer buffer, 200 μM of each nucleotide, and 1 U of Perkin-Elmer Taq polymerase. Following one round of amplification for 30 cycles of 94°C for 30 sec, 52°C for 15 sec, and 72°C for 60 sec, 1 μl of the amplified product was used to seed a 150 μl reaction containing 75 picomoles of the GluDG primer and 7.5 picomoles of biotin-labeled Cb1211H. Following 35 cycles, the DNA was precipitated with ammonium acetate and 50% ethanol, pelleted by centrifugation at high speed for 10 min, washed once with ethanol, air dried, and resuspended in 40 μl of water.

For each sample, 20 μl of Dynal streptavadin beads were washed with 50 μl of binding and washing (BW) buffer (4 M NaCl, 10 mM Tris, 1 mM EDTA, 0.1% NP-40). The beads were resuspended in 40 μl of BW buffer, combined with the DNA, and the solution incubated for 1 hour with slow rotation at 45°C to allow the biotin-labeled DNA to bind to the streptavadin beads. The beads were washed once with 50 μl of BW buffer, twice with 50 μl of sterile, distilled water, and resuspended in 12 μl of water. The sample was boiled for 15 sec and quickly put on a magnet to remove the beads from solution. The solution containing the non-biotin-labeled DNA was collected, 28 μl of water added, the tube was labeled, and stored at 4°C. Following heat denaturation, the beads were incubated at room temperature for 10 min in 0.1 N NaOH, washed twice with 50 μl of sterile, distilled water, and resuspended in 40 μl of water. Both strands were sequenced using a battery of primers (Fig. 2) and the Sequenase (US Biochemicals) protocol. Fragments were separated on two 7 M urea, 6% Long Ranger gels (AT Biochem): a short run (≈2 hr) to resolve the first 200-300 base pairs, and a long run (≈5-7 hr) to resolve the identity of nucleotides between 250-500 base pairs from the primer.

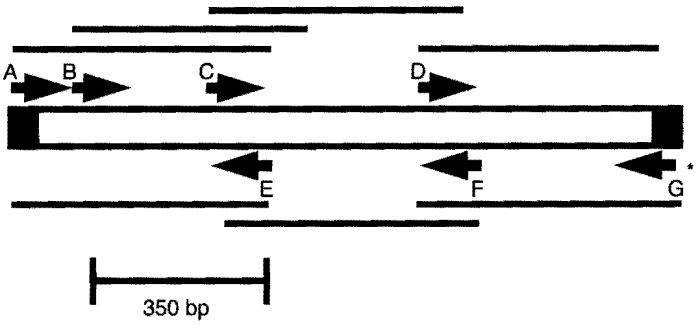

Fig. 2. Schematic of the cytochrome b gene and flanking tRNA genes showing the approximate relative location of primers (arrows) and the sequencing strategy (lines). Primer sequences are as follows: A GluDG TGA CTT GAA RAA CCA YCG TTG; B Cb22 ATG GCC ATA AAY ATY CGA AAA A; C Cb2L CCC TGA GGA CAA ATA TCC TTY TG; D Cb702L CCC CAT ATT AAA CCC GAR TGR TA; E Cb2H CTA AAG GAT ATT TGT CCT CAG GG; F Cb702.H AAG TAT CAT TCR GGT TTR AT; G Cyb1211.H TAG TTA AGG CTG AGG ATT TTR TTT TC. Primer sequences are based on cytochrome b sequences from sharks and rays (see Martin 1995). L and H refer to the light and heavy strand respectively. The asterisk on primer G denotes biotin. Y = C, T; R = A, G.

Patterns of accumulation of transitions and transversions were investigated by determining the minimum length tree using maximum parsimony (i.e. PAUP; Swofford, 1993) and counting the numbers of transitions and transversions per phylogenetically-informative site. This provided the basis to evaluate the relative amount of homoplasy for the two classes of substitutions. Two methods of phylogenetic inference were employed: maximum parsimony using the branch and bound algorithm (implemented using PAUP [Swofford, 1993]) and neighbor-joining cluster analysis on corrected genetic distances between taxa (implemented using PHYLIP [Felsenstein, 1994]). Genetic distances were corrected for multiple-hits using a one-parameter model (the Jukes-Cantor option in PHYLIP; Felsenstein, 1994). In both cases, only transversion substitutions were included because of evidence for extreme homoplasy of transitions relative to transversions. Tests of alternative phylogenetic hypotheses were accomplished using the constraint option in PAUP (Swofford, 1992) and testing for differences in the distribution of character state changes per site using nonparametric Wilcoxon sign-rank tests (Templeton, 1983). Two methods were used to evaluate the support for groups of taxa: bootstrap analysis using maximum parsimony, and comparison of internal and terminal branch lengths for a specified topology.

Results and Discussion

Complete (or nearly complete) sequences of the mitochondrial protein-coding gene cytochrome b were determined for 25 individuals, representing all but two of the 16 species and all genera of lamnoid sharks, and for three outgroup species. An alignment of phylogenetically-informative sites is provided for the species of lamnoid sharks surveyed (Fig. 3). (See also Martin, 1995 for GenBank accession numbers of outgroup taxa.) In cases where multiple individuals of a given species were sampled, levels of within species divergence were small relative to between species divergence; therefore, phylogenetic analyses focused on representatives of the species for which the greatest amount of sequence data was available.

For most comparisons, transitions (purine-purine or pyrimidine-pyrimidine) are saturated. On average, there are about three transitions per phylogenetically-informative site (Fig. 4). Considering that transitions flip back and forth between two states (i.e. between C and T or G and A), such a large number of changes per site suggests that little phylogenetic information can be extracted from patterns of shared transitions. There is also a signature of homoplasy for transversions, although there are far fewer total changes and fewer average numbers of changes per site (Fig. 4). Moreover, because there are twice as many possible transversion than transition substitutions, phylogenetic signal is probably still evident for transversions. Studies

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Ispa     CCCCACTGACACCCCCGCAGCCCATTACCATTACCCGTACCCAACCCACCCTCCATCTCCCCCT
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Lana     ATCTTCTAACGTCCCTGCAACCCACTTTCGTTGACCATTCCTGACCTACTATCTGCTTTTCCCC
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Cema     ????????CGCCCCCATAACTCACCCTTATTCTCCATATCTAATCCGCCCCCCGCTTTTCTCC
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Miow     ATTCACCGACGTCTTCACCCCTAATTCTCATTATCCCTATTCAATTCACCACCCACCTCTTCCT
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Mepe     CTCCCCCCCATCCATCACATTATTTTTTTAATATGCTTTGCACACCATTATAAGGCGTTTTCCC
Alpe     CTCTTCTCCACCTACCGTCCCATTTCCCCAATATACTTCATACACCACTTCATAATGCTCTTCC
Alvu     CCCTTTCCCATCTACCGTACTATTTCTTTAATGTACCTTGTACACCACTACAAACTCCTCTCCC
Alsu     TTCTTTCCCATCTACCGTTCTATCTCCTTGATATACTCTATACACCACTACGAAACGCCCCCCC
Miow     CTCTTCTCCATCCGCTGCACCACTTCTCCAATGGACCTTATATACCAATATACAACGCCCTCCC
Odfe     CTCTTTTTTATTTACCATATTATTTCCTTAATATATCTCTTACACCACTTTAAAACGCCTTCTT
Pska     TCCTTTTCCACCCATTGTACTATTATTTTAATATACTTCATACACCACTATAAGACGTCTTCTC
Cata     CTTTCTCTCACCCGCTGTATTTCCCCTACAACATATTCTACATACTAATCTATAACGGTTCCCC

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Lana     CTCCCCTATAGCCACCCGCCTCCCTCGTCTGCCTTTACTTTACCTCCCTTACTCCCTTCCCTCT
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Cema     CCCCTTCATAGTCATCCCCCCCCACCACCCCCCACTATCCTGTCCTCTCCATATTCTTCCCTTT
Mepe     TA?CCATATAATTGTCTTTCTCCACCGTCCACCATAATTTTATTTTTTCCAATTTTTTTCCTC?
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Alvu     TACCTCTATAGTTACATTCTCCCACCGTTC?TCATAACTCTACCTTTCCCAATTTCTCTCCACA
Alsu     CACCTTTATAGTTACCTACCTCCACCGCCTATTACAGCGCCACCCTCCTTACTCTCTTCTCTCT
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Ispa            CCGCTACTGCCCCCTACCTTGGCCGTCTTACCCCTTCGTTCCCCTCCCCTCGCCTCCCCTCTTC
Caca            CCGCAGTCAGTCCCTATCCCAATCGCCTTCCCCCTGTACTCCCCTCCACTAACCTCCCCCTTTT
Lana            CCGCTACTCTCCCCCCCCCCAATCACCTACCCCTCACGTTCCCTTCCTTTCACCTTCCCCCCTC
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Alsu            TCGCTCTCCCCCACCAACCCAGTCGCCTTCTTCATACGCACCACTTCCCTTACCTTCTTCCTTC
Miow            CCACCTTTTTTAAATGTTCCATCCGCCCTCTTTATACGTTTTACTTCCTTCACCCCTCCTCCCC
Odfe            TTACCCTTCCCAAATAAACCAGTCACTCCTTTCATATGTTTTTCTTCCTTTATCCTCCCACTTT
Pska            CCGCCCCTTTCAAAT????TAGTTGTTTCTTTCTCATGTTTCCTTTTCTTCACCTCCCCACCCT
Cata            CCCTCCCTTCCTATTATTCCAATCGCCTCCCCCCCACGTTTCTTTCTATTCACCTTTCCCCCCC

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Node            ---------------------------------------------------------------------
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Caca            AACTCTCCCCCTTTGCCTTCTCCCCGCCCTGCCATTTAGCCAGTCTCACATTTCCTTCTCCCCT
Lana            AACCCTCTCCCCCTGCCCTCCCTCCGCCCTATCGCCTTGTTAATTCTGTATTTTTTTTCACCT
Ladi            AACCCACCCCCCCTGCCCCCCCTCCGCCCTACCGCCCTATTAATTCTACACTTTTTTCTCACCT
Cema            AGCTTGCCTCCCCCGTCCCCCCTTCGTTTCATCATCAAGTCAACCCCGAACCCACTTTACCCCT
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Alsu            AACCCCCCTCCCTTGTCCTCACCTCGCTCCGCCACCCAATCAACTCTAAATCTTTTTCACCCTC
Miow            ATCCCCCCCTTCCCGTGCTCCCCTTACTTCATCATTTAACCTACTCTGAATTCCCCCCACCCTC
Odfe            AAACCTCCCCTTTTATACACATCTTGCTCTATTATCTAATCAACTCTAAATTTTCTTTACCTTC
Pska            AACCCCTCCTCTTTGCATACTTCTCGTTCCATTATCTAGCTAATTCTACATTCCTTCTACTCCC
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Mepe            TATTTGTATAAA???
Alpe            TATTTATCTGAAAC?
Alvu            TATTTGTGCAAAAC?
Alsu            ????????????????
Miow            TACTTATACCATACA
Odfe            TATTTGTACAAAGC?
Pska            CATTTGTATAAAACG
Cata            CATTTGTATCAA???
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Fig. 3. Alignment of phylogenetically-informative sites in the cytochrome b gene for single representatives of each species of lamnoid sharks surveyed. Isox = *Isurus oxyrinchus*; Ispa = *I. paucus*; Caca = *Carcharodon*; Lana = *Lamna nasus*; Ladi = *L. ditropis*; Cema = *Cetorhinus*; Mepe = *Megachasma*; Alpe = *Alopias pelagicus*; Alvu = *A. vulpinus*; Alsu = *A. superciliosus*; Miow = *Mitsukurina*; Odfe = *Odontaspis*; Pska = *Pseudocarcharias*; Cata = *Carcharias*.

of another group of sharks revealed that transitions saturate quickly and can mislead inferences of evolutionary history (Martin, 1995). The rapid onset of saturation causes the accumulation of transitions over time to become non-linear, and for deep divergences, transitions become fewer in number as they are replaced by transversions (Martin, 1995). Rapid saturation and non-linear

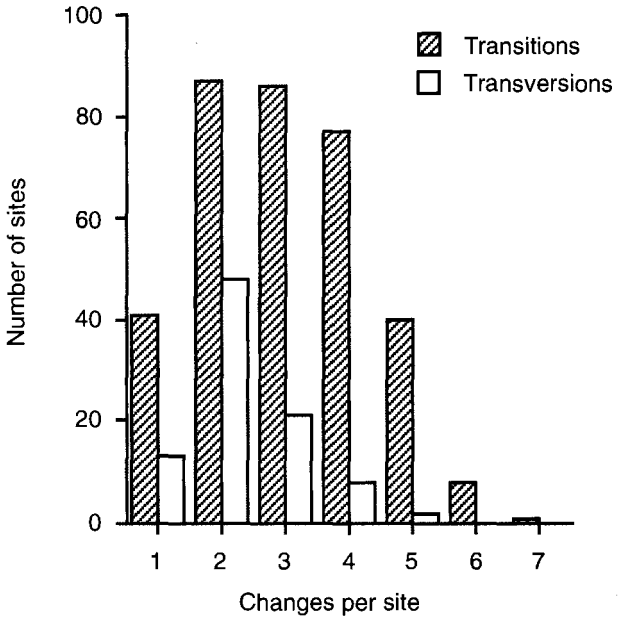

Fig. 4. Estimate of the number of transitions and transversions at phylogenetically-informative sites based on the minimum-length tree for all substitutions. Both transitions and transversions exhibit a strong indication of multiple substitutions at a site (homoplasy); nevertheless, given the greater number of possible transversion changes relative to transitions, this graph indicates that there is considerably less homoplasy for transversions than for transitions. Other measures (consistency and retention indices) show similar results.

accumulation of transitions argues in favor of transforming the data into purines and pyrimidines when investigating levels of divergences encompassed by the lamniform sharks (Martin, 1995). Thus, most of the analyses focused on transversion substitutions.

Maximum parsimony analysis of all individuals surveyed using only transversion substitutions yielded two minimum-length trees (Tree length [TL] = 348, Retention Index [RI] = 0.564). The genus *Alopias* (thresher sharks) was paraphyletic (Fig. 5) in both the minimum length trees. Although it is possible the thresher sharks are not monophyletic, their distinctive, shared, derived morphology strongly argues in favor of thresher shark monophyly. When the maximum parsimony analysis was repeated with *Alopias* constrained to be monophyletic, one minimum length tree was found that differed from the maximum parsimony trees (without constraints) by a single mutation, a difference that was not significant (Table 2).

Alternative phylogenetic hypotheses were tested against the minimum length tree with threshers constrained to be monophyletic using nonparametric tests (Table 2). The hypotheses of Compagno (1990) and Maisey (1985) were refuted (Table 2). Similarly, the single-origin of filter-feeding hypothesis proposed by Maisey (1985) was also rejected ($p = 0.022$; Table 2). Thus, based on analysis of distributions of character state changes per site, previously proposed hypotheses for lamnoid sharks can be refuted (see also Naylor et al., 1997).

Different methods of phylogenetic analysis yielded different topologies. The topology determined using neighbor-joining cluster analysis of transformed genetic distances (i.e.

Table 2. Summary of tree-length statistics and results of Wilcoxon sign-rank tests of alternative phylogenetic hypotheses in relation to the minimum length tree with Alopiids constrained to be monophyletic. Only single representatives of each species were included. *Galeocerdo* and *Heterodontus* were included as outgroups. TL = tree length; RI = retention index; Z = Wilcoxon sign-rank test statistic

	TL	RI	Z	p
Minimum length (fig. 5)	253	0.53		
Alopiids monophyletic (fig. 5)	254	0.53	-0.58	0.564
Hypothesis				
Compagno (fig. 1A)	266	0.49	-2.82	0.005
Maisey (fig. 1B)	272	0.47	-3.05	0.002
Single-origin of filter-feeding	265	0.49	-2.29	0.022
NJ (fig. 6)	257	0.52	-0.83	0.405

corrected numbers of transversions per site) (Fig. 6) differed from the maximum parsimony tree, although the difference was not significant based on nonparametric tests (Table 2). Topological incongruency reflects differences in the pattern of relationships for *Alopias pelagicus, A. superciliosus, A. vulpinus, Megachasma, Pseudocarcharias*, and *Odontaspis*. An important observation is that virtually any arrangement of these taxa yields a tree that is not significantly different in length from the minimum length tree! For instance, if the analysis is restricted to the Alopidae, *Megachasma, Odontaspis, Carcharias, Pseudocarcharias*, and *Mitsukurina*, the distribution of tree lengths is unimodal, exhibits slight (but significant) left skew ($g1 = -0.44$) (Hillis and Huelsenbeck, 1992), and therefore provides little support for choosing minimum length trees as the "best" hypothesis over alternative, less parsimonious trees (Fig. 7). If we use the Wilcoxon sign-rank test to define the length at which trees become significantly longer, on average, than the minimum length tree (Fig. 7), roughly one-half of the 10,395 alternative bifurcating topologies are not statistically "worse" than the minimum length tree(s). Lack of strong hierarchical signal for these taxa is reflected by the complete collapse of

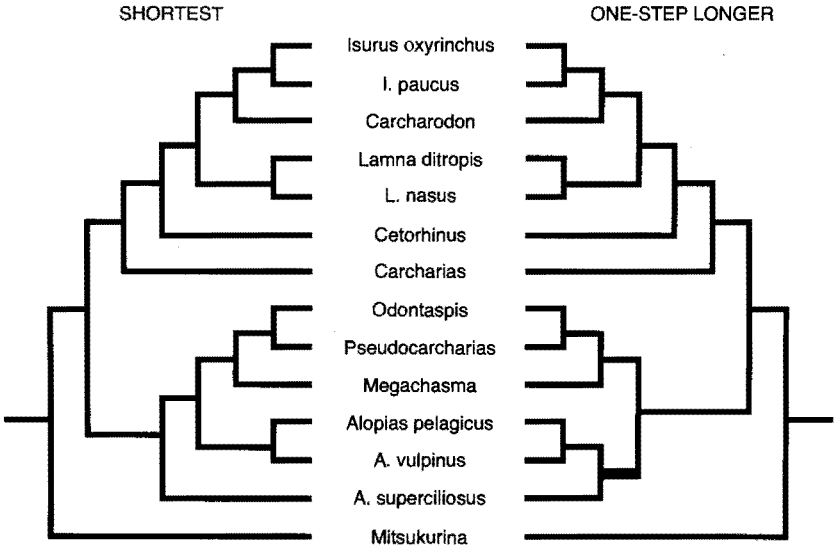

Fig. 5. One of the two minimum length trees based on analysis of transversions and a tree that is one step longer in which the thresher sharks (*Alopias*) are constrained to be monophyletic. These two trees are not significantly different (Table 2). Trees were rooted with *Galeocerdo, Heterodontus*, and *Urolophus*.

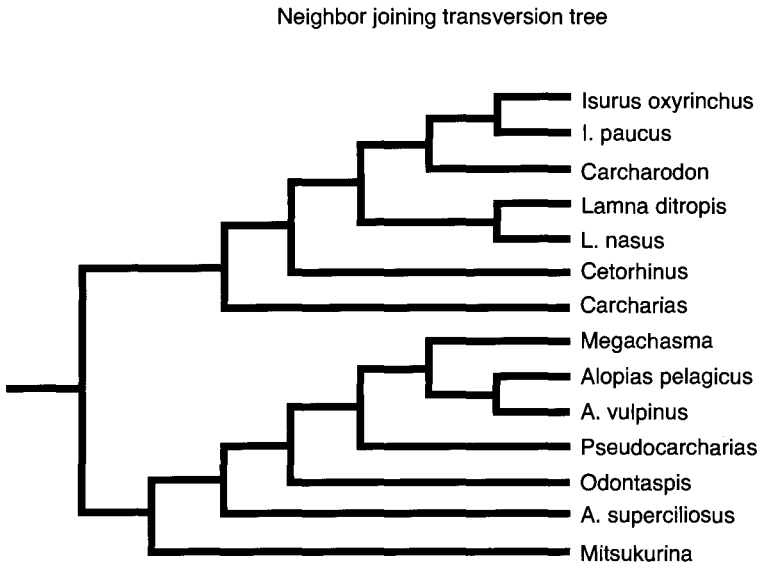

Fig. 6. Neighbor-joining tree determined from a matrix of corrected transversions per site. Tree was rooted with *Galeocerdo, Heterodontus*, and *Urolophus*.

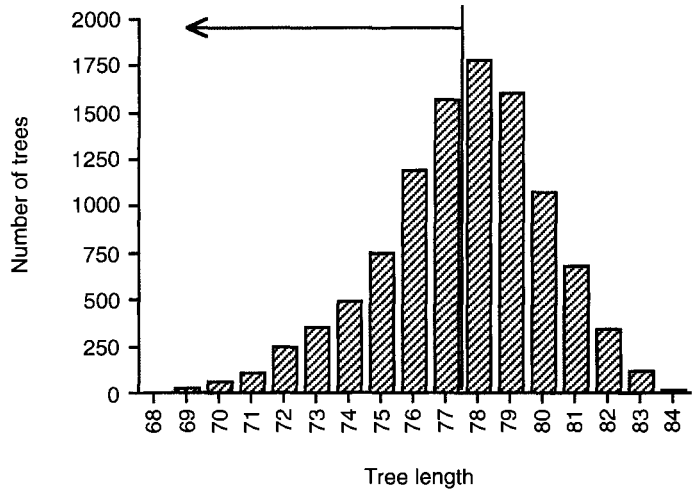

Fig. 7. Histogram of all possible trees (N = 10,395, g1 = -0.44) for the relationships among eight species (the three species of *Alopias, Megachasma, Pseudocarcharias, Carcharias, Mitsukurina*, and *Odontaspis*). The vertical line and arrow pointing to the ordinate defines the set of trees that are not significantly different (p values > 0.05), based on mean scores of Wilcoxon sign-rank tests. In other words, topologies with lengths less than 78 are not significantly "worse" than the minimum length trees.

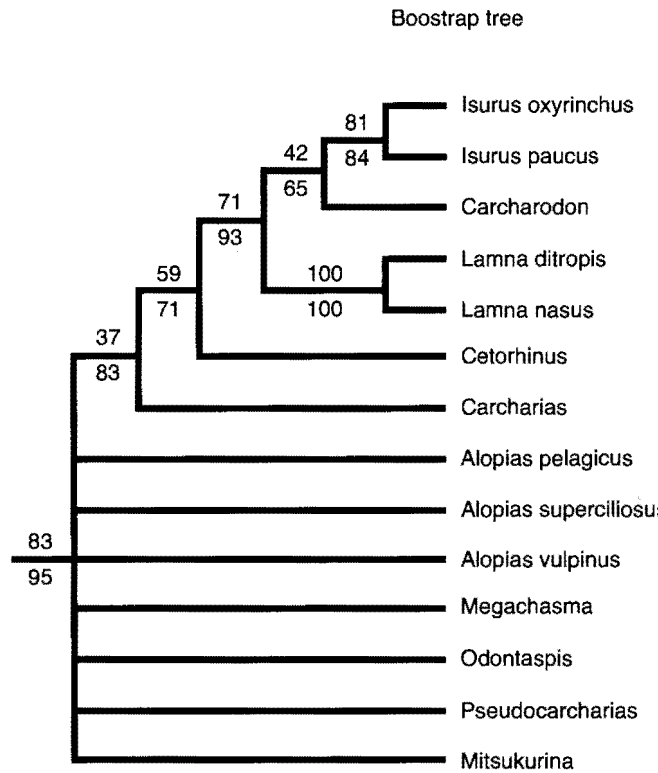

Fig. 8. Groups compatible with the 50% majority rule consensus bootstrap tree (200 replications) for all substitutions (values above branches) and only transversions (values below branches). Nodes in which less than 50% of the trees were compatible for both all substitutions and transversions only were collapsed. Topology was rooted with *Galeocerdo*, *Heterodontus*, and *Urolophus*.

internodes for this group of taxa in the 50% majority rule bootstrap consensus tree (Fig. 8). Thus, while the molecular data can refute some alternative hypotheses (i.e. the hypotheses of Compagno [1990], Maisey [1985], and the single-origin of filter-feeding), it is impossible to refute an enormous number of alternative phylogenetic hypotheses in favor of one single inference of evolutionary relationships.

Theoretical studies indicate that long terminal branch lengths coupled with short internodes can seriously compromise phylogenetic inference from molecular data (Huelsenbeck and Hillis, 1993). Analysis of the cytochrome b sequence data provides clear evidence of long terminal branch lengths and short internodes (Table 3) and suggests that many of the extant lineages are

Table 3. Mean branch lengths for internal and terminal branches. Values are the percent change in sequence due to transversions. Branch lengths were estimated using least-squares (implemented using the FITCH algorithm of PHYLIP [Felsenstein 1992]) for the topology in Figure 5 (with *Alopias* constrained to be monophyletic)

Taxa included	Internal	Terminal
All	0.29	1.14
Lamnidae + *Cetorhinus*	0.46	0.93
Unresolved group	0.11	1.25

ancient and originated in a relatively brief period of time. The published fossil record also suggests that many extant lineages first appeared in a relatively brief period of time long ago, during the Late Middle to Late Cretaceous (Cappetta, 1987; Ward and Wiest, 1990; Kemp, 1991; Siverson, 1992, 1995; Purdy, 1993; Case, 1994). Thus, lack of resolution among many of the species most likely reflects ancient origination of lineages coupled with speciation events that happened over a relatively brief period of time. This situation means that for lamnoid sharks, robust tests of phylogenetic hypotheses will have to rely on corroboration by sampling additional genes (Miyamoto and Fitch, 1995; Hillis, 1995; Naylor et al., 1997).

Although considerable doubt remains about the phylogenetic relationships among many of the lineages, some progress has been made towards defining the evolutionary history of lamnoid sharks. For instance, analysis of the cytochrome b data failed to refute the hypothesis that *Cetorhinus* is the sister taxon to the Lamnidae (Compagno, 1990). This result, coupled with refutation of the single-origin of filter-feeding hypothesis, argues strongly in favor of the independent origins of filter-feeding from different ancestral conditions. This makes sense in the light of morphological and behavioral differences between the two divergent taxa. Compagno (1990) notes that *Megachasma* may have evolved its distinctive feeding apparatus from odontaspid-like features by jaw size exaggeration, acquisition of papillose gill rakers, and modification of jaw protrusion for suction feeding. It is therefore probably not coincidental that *Megachasma* clusters together in a clade with *Odontaspis* and *Pseudocarcharias* (Fig. 5). Moreover, similarity in jaw suspension between lamnid sharks and *Cetorhinus* (Compagno, 1990) supports the hypothesis of derivation of filter-feeding in basking sharks from a lamnid-like ancestor.

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Literature Cited

Cappetta, H. 1987. Chondrichthyes II. Mesozoic and Cenozoic Elasmobranchii. In Vol. 3b Handbook of Paleoichthyology. Gustav Fischer Verlag, Stuttfart, West Germany, iv + 193 pp.

Case, G. R. 1994. Fossil fish remains from the late Paleocene Tuscahoma and early Eocene Bashi formations of Meridian, Lauderdal County, Mississippi. Paleontographica A, 230: 97-138.

Compagno, L. J. V. 1984. FAO Species Catalog. Vol. 4, Pts. 1&2, Sharks of the world. FAO fisheries synopsis No. 125.

Compagno, L. J. V. 1990. Relationships of the megamouth shark, *Megachasma pelagios* (Lamniformes: Megachasmidae), with comments on its feeding habits. Pages 357-379 *in* H. L. Pratt, S. H. Gruber, and T. Taniuchi, eds. Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of the Fisheries. NOAA Tech. Rep. NMFS, 90.

Felsenstein, J. 1994. PHYLIP, V. 3.4, University of Washington, Seattle.

Hillis, D. M. 1995. Approaches for assessing phylogenetic accuracy. Syst. Biol., 44: 3-16.

Hillis, D. M. and Huelsenbeck, J. P. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. J. Heredity, 83: 189-195.

Huelsenbeck, J. P. and D. M. Hillis. 1993. Success of phylogenetic methods in the four-taxon case. Syst. Biol., 42: 247-264.

Kemp, N. R. 1991. Chondrichthyans in the Cretaceous and Tertiary of Australia. Pages 497-568 *in* P. Vickers-Rich, J. M. Monaghan, R. F. Baird, and T. H. Rich, eds. Vertebrate Paleontology of Australasia. Pioneer Design Studio Pty Ltd., Lilydale, Monash Univ. Publ., Melbourne.

Maisey, J. G. 1985. Relationship of the megamouth shark, *Megachasma*. Copiea, 1985: 228-231.

Martin, A. P. 1995. Mitochondrial DNA sequence evolution in sharks: Rates, patterns, and phylogenetic

inferences. Mol. Biol. Evol., 12: 1114-1123.
Miyamoto, M. M. and W. M. Fitch. 1995. Testing species phylogenies and phylogenetic methods with congruence. Syst. Biol., 44: 64-76.
Naylor, G. J. P., A. P. Martin, E. Mattison, and W. M. Brown. 1997. The inter-relationships of lamniform sharks: Testing phylogenetic hypotheses with sequence data. In press *in* T. D. Kocher and C. Stepien, eds. Molecular Systematics of Fishes. Academic Press, New York.
Purdy, R. W. 1993. Paleoecology of fossil great white sharks. Symp. on the Biology of the Great White Shark, *Carcharodon carcharias*. Bodega Marine Laboratory, Univ. of Cal., Davis, Abs. p. 24.
Siverson, M. 1992. Biology, dental morphology, and taxonomy of lamniform sharks from the Campanian of the Kristianstad Basin, Sweden. Paleontology, 35: 519-554.
Siverson, M. 1995. Revision of the Danian cow sharks, sand tiger sharks, and goblin sharks (Hexanchidae, Odontaspidae, and Mitsukurinidae) from Southern Sweden. J. Vert. Paleon., 15: 1-12.
Swofford, D. S. 1993. PAUP: phylogenetic analysis using parsimony, v. 3.1. Laboratory of Molecular Systematics, Smithsonian Institution, Washington, D. C.
Templeton, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution, 37: 221-244.
Ward, D. J. and R. L. Wiest, Jr. 1990. A checklist of Paleocene and Eocene sharks and rays (Chondrichthyes) from the Pamunkey Group, Maryland and Virginia, USA. Tertiary Res., 12: 81-88.