

## The Phylogenetic Position of *Megachasma pelagios* Inferred from mtDNA Sequence Data

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**Abstract** Studies on the relationship of *Megachasma* to other lamnoids have been infrequent and results have been contradictory. The objective of this study was to infer the phylogenetic position of *Megachasma* within order Lamniformes using molecular data. DNA was extracted from seven of ten lamniform genera and three outgroup taxa. A portion of the 12S rRNA mitochondrial gene was amplified, sequenced, and analyzed phylogenetically using parsimony. These analyses yielded a single most-parsimonious tree with a length of 104 steps and a consistency index of 0.779. The topology of this tree was not affected by a variety of weighting schemes nor by deletion of outgroup taxa. The results suggest that *Megachasma* is a) the most primitive living lamniform shark and b) not the sister-group of *Cetorhinus*, suggesting independent evolution of filter feeding within order Lamniformes.

The first specimen of *Megachasma pelagios* was captured on 15 November 1976 by the naval research vessel AFB-14. Its discovery was surprising for two reasons. First, its size (446 cm) should not have allowed this species to remain undiscovered for so long. Second, it represents only the fifth genus of filter-feeding elasmobranch (the others, *Cetorhinus*, *Manta*, *Elasmobranch* known to be a deep-sea planktivore (Diamond, 1985), and the first (Taylor et al., 1983) suggested that *Megachasma* is a lamniform galeomorph shark (sensu Compagno, 1973, 1977). Taylor et al. (1983) stated that its galeomorph affinity is revealed by chondrocranium, and vertebral column. They went on to list phenetic characters of its nostrils, mouth, eyes, teeth, claspers, dorsal fin skeleton, chondrocranium, pectoral fin skeleton, jaw al (1983) listed an impressive array of phenetic characters as evidence for the separation of *Megachasma* from all other lamniform families, including the other filter feeder, *Cetorhinus*. Finally, they tentatively suggested that *Megachasma* may be the primitive sister-group of all extant lamnoid sharks, but stated that “a detailed account of lamnoid interrelationships is beyond the scope” of their paper.

Two studies have broached the question of megamouth's phylogenetic position, in particular, and lamnoid interrelationships, in general, since the formal description of *Megachasma*. The first was Mansey (1985), which acknowledged the many differences between *Cetorhinus* and *Megachasma* tallied by Taylor et al. (1983), but still suggested that *Cetorhinus* and *Megachasma* form a confamilial group of lamniforms specialized for filter feeding. He speculated that his Cetorhinidae (i.e. *Cetorhinus* + *Megachasma*) may be the sister-group of

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other advanced lamnoid families that possess plesiodic pectoral skeletons (i.e., Lamnidae and Alopiidae), and that *Megachasma* is not the primitive sister to all living lamnoid sharks. Implicit in Maisey's (1985) phylogenetic hypothesis is the contention that the filter-feeding mechanism of *Megachasma* and *Cetorhinus* is autapomorphic for his family Cetorhinidae. Hence, filter feeding evolved only once among the lamnoids.

Compagno (1990) rejected Maisey's (1985) confamilial classification of *Cetorhinus* and *Megachasma* by summarizing a great deal of phenetic evidence of their morphological dissimilarity and by contradicting the five sets of synapomorphies of Maisey's (1985) Cetorhinidae. Compagno (1990) went on to present a cladistic analysis of all living lamnoid sharks. His phylogenetic hypothesis suggested that a) *Megachasma* is not the primitive sister-group of all other lamniforms, b) *Megachasma* + *Cetorhinus* do not form a monophyletic group and acquired their filtering mechanisms independently), and c) lamniforms with plesiodic pectoral skeletons (i.e., Megachasmidae, Alopiidae, Cetorhinidae, and Lamnidae) are most derived. Nevertheless, Compagno (1990) admitted that "there are problems with the cladogram...and supporting arguments." Therefore, the present study was undertaken to infer the phylogenetic position of *Megachasma* within the order Lamniformes using molecular data.

### Materials and Methods

Skeletal muscle samples were obtained from the following seven lamniform species (which represent seven of ten extant genera): *Alopias vulpinus*, *Carcharodon carcharias*, *Cetorhinus maximus*, *Isurus oxyrinchus*, *Lamna nasus*, *Megachasma pelagios*, and *Odontaspis ferrox*. Skeletal muscle samples also were obtained from three outgroup genera (*Heptranchias perlo*, *Hydrolagus collei*, and *Ginglymostoma cirratum*). The skeletal muscle was digested and the DNA was extracted following Dunn and Morrissey (1995).

A portion of the 12S rRNA mitochondrial gene was amplified with the primers of Kocher et al. (1989) via the polymerase chain reaction (PCR). Successful amplification was confirmed by electrophoresis of the PCR product on a 1.4% agarose gel and staining with ethidium bromide. Excess primers were removed enzymatically, the PCR product was sequenced manually (Sequense version 2.0; US Biochemical), and the reaction was visualized on a 6% acrylamide gel (Dunn and Morrissey, 1995).

The resulting nucleotide sequences were edited with SeqApp (version 1.9a169; Gilbert, 1992), aligned with Clustal (version V; Higgins et al., 1992), and analyzed with Phylogenetic Analysis Using Parsimony (PAUP; version 3.1; Swofford, 1990). All topologies under a variety of weighting schemes were examined using the EXHAUSTIVE SEARCH option with ACCTRAN optimization of the unordered (FITCH) characters. Confidence in the resulting topology was estimated using the BOOTSTRAP (Felsenstein, 1985) and BRANCH AND BOUND (Hendy and Penny, 1982) options of PAUP.

### Results

The nucleotide sequences of a homologous 203-base-pair region of the 12S rRNA mitochondrial gene were obtained for the seven ingroup and three outgroup taxa. By weighting transitions and transversions equally, a single most-parsimonious tree was obtained (Fig. 1) with a length of 104 steps and a consistency index (CI; Kluge and Farris, 1969) of 0.779. Eight one-step-off trees were found and the distribution of lengths of all possible tree topologies was skewed significantly ( $g1 = -0.98$ ;  $t = 6.43$ ;  $P < 0.01$ ) to the left. Next, the possibility of saturation of transitions, a common occurrence in sharks (Martin, 1995), was evaluated by weighting transversions two and ten times more than transitions. A tree identical to Figure 1

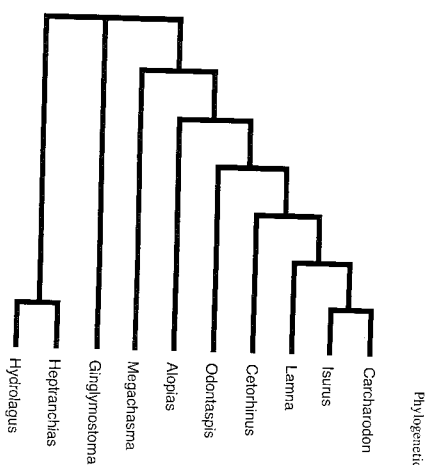


Fig. 1. Single most-parsimonious tree obtained using the EXHAUSTIVE SEARCH option and ACCTRAN optimization in PAUP. Length = 104 steps, CI = 0.779.

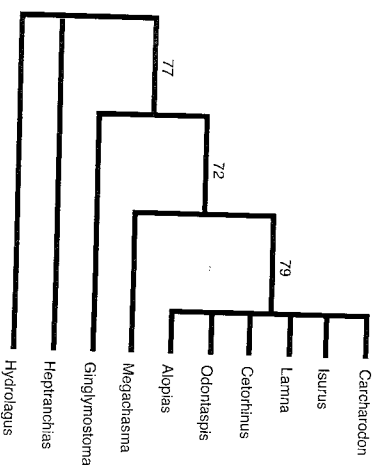


Fig. 2. Majority rule (50%) consensus tree derived from 1000 bootstrap replications. Values at each node represent the percentage of bootstrap replicates possessing that node.

was obtained in both cases. Moreover, an identical resolution of the ingroup taxa was obtained using only *Heptranchias* as the outgroup (65 steps; CI = 0.867), or by excluding *Ginglymostoma* from the analysis (87 steps; CI = 0.828). One thousand replications of a 50% majority-rule bootstrap analysis resulted in Figure 2. All fully resolved nodes were found in at least 72% of the trees (i.e., there is a probability of  $\geq 95\%$  that those clades are real; Hillis and Bull, 1993).

### Discussion

The phylogenetic position of *Megachasma* has been considered infrequently. Only two

phylogenetic hypotheses regarding the relationship of megamouth to other lamnoids have been published since the discovery of the species in 1976 (Taylor et al., 1983). Maisey (1985) suggested that megamouth sharks are rather derived (based on their possession of a plesiodic pectoral fin skeleton) and form a monophyletic family with basking sharks, *Cetorhinus*. Compagno (1990) disagreed with the idea that *Megachasma* is the most primitive lamnoid, attacked the suggestion that megamouth and basking sharks are confamilial, and suggested that *Megachasma* is relatively derived and sister to a clade containing Alopiidae, *Cetorhinus*, and the lamnids.

The phylogenetic hypothesis presented in this study (Fig. 1) may clear up some of the confusion surrounding the relationships of *Megachasma* to other lamnoid sharks. Figure 1 suggests that *Megachasma* is, in fact, the primitive sister-group of all living lamniforms (as originally suggested by Taylor et al. [1983] on the basis of primitive characters of the teeth and palatognathate). Recognition by Maisey (1985) and Compagno (1990) of *Megachasma* as the most primitive lamnoid was prevented by its possession of a supposedly derived character, a plesiodic skeleton in its pectoral fins. Plesiodic pectoral fins (wherein the pectoral radials extend far into the fin to augment the ceratotrichia) are variously displayed by some oreoichthiforms (*Segastoma*, *Ginglymostoma*, *Nebrius*, and *Rhinodon*), some lamniforms (*Megachasma*, *Alopius*, *Cetorhinus*, and all lamnids), and some carcharhiniforms (hemigaleids, carcharhinids, and sphyriids) (Compagno, 1988). However, Taylor et al. (1983) clearly stated that megamouth possessed a plesiodic skeleton in its pectoral fin, yet still considered *Megachasma* to be primitive to all living lamniform sharks. Perhaps they were skeptical of the polarity of this character due to its homoplasious distribution among extant sharks and its well-known presence in many Paleozoic elasmobranchs (Compagno, 1973; Zangerl, 1973; Bendix-Almgreen, 1975).

Although the interrelationships within the lamnoids were made dubious by a bootstrap analysis (Fig. 2), two things are clear. First, *Megachasma* is primitive to and distinct from all other lamnoids. This notion, that *Megachasma* represents a very distinct taxon that is well separated from all other lamnoid families, is not unique to the present study (see Taylor et al., 1983; Compagno, 1990). Second, the ancient appearance of many lamnoid lineages (e.g., see Cappetta, 1987; Ward and West, 1990; Swenson, 1992, 1995) must have occurred concurrently with rapid speciation events, phenomena which are manifested in the lack of resolution of these lamnoid species. This combination of long branch lengths (i.e., ancient lineages) and short internodes (i.e., rapid speciation) has been theorized to camouflage the phylogenetic signal within a data set (Huelsenbeck and Hillis, 1993). Finally, Figure 1 also supports several other well-documented relationships within order Lamniformes (see Compagno, 1977, 1990). Specifically, it confirms the idea a) that lamnid sharks form a single clade, b) that lamnids are the most derived lamniform family, and c) that *Lamna* is the sister-group to *Isurus* + *Carcharodon*. Clearly, the accumulation of additional molecular data is necessary (Hillis, 1995; Miyamoto and Fitch, 1995) before the interrelationships of lamniform taxa can be resolved fully with confidence.

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