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Testing morphologically based phylogenetic theories within the cartilaginous fishes with molecular data, with special reference to the catshark family (Chondrichthyes; Scyliorhinidae) and the interrelationships within them

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Abstract

A molecular phylogenetic investigation was conducted to examine phylogenetic relationships between various members of the catsharks (Chondrichthyes; Carcharhiniformes; Scyliorhinidae), and is the largest chondrichthyan data set yet analysed, consisting of nearly 130,000 nucleotides. Three mitochondrial DNA genes were used to construct the phylogenies, cytochrome *b*, NADH-2, and NADH-4, with 41 sequences from 18 taxa being novel. These sequences were either used separately or combined into a single data set, and phylogenies were constructed using various methods, however, only the Bayesian inference tree derived from the cytochrome *b* data set was resolved sufficiently for phylogenetic inferences to be made. Interestingly, the family Scyliorhinidae was not supported by the results and was found to be paraphyletic. The Scyliorhininae and Pentanchinae were supported, whereas the Pentanchini clade was present, but not well supported. The Halaelurini hypothesis was supported with *Holohalaelurus* identified as the basal genus of that clade, and *Haploblepharus edwardsii* identified as the basal taxon for that genus. Elsewhere within the Chondrichthyes, the Carcharhiniformes and the Lamniformes were found to be monophyletic, and the Heterodontiformes was placed within the Squalimorphs. The placement of the skates and rays in these analyses support the Batoidea as being sister to the Elasmobranchii.

Keywords: Cytochrome *b*; NADH-2; NADH-4; Chondrichthyes; Elasmobranchii; Scyliorhinidae; Catsharks; Southern Africa; Phylogeny

1. Introduction

Sharks belonging to the family Scyliorhinidae Gill, 1862 are colloquially known as catsharks. They are members of the order Carcharhiniformes Compagno, 1973 and have a global distribution from tropical to cold temperate latitudes, and can be found from the intertidal zone down to at least 2000 m (Compagno, 1984b). The present number of recognised scyliorhinid taxa includes approximately 151 species, accounting for 13% of all extant cartilaginous fishes (Class, Chondrichthyes), which is second only to the family Rajidae (skates) in terms of number of species (Compagno, 2005 and unpublished data).

Many species of scyliorhinids in southern Africa are common to abundant, including sharks from the genera *Halaelurus*, *Haploblepharus*, *Holohalaelurus*, *Poroderma* and *Scyliorhinus*. Southern Africa has an unusually high percentage of scyliorhinid endemics with a total of 2 endemic genera, 13 endemic species and a number of near endemics (Compagno, 1999; Compagno and Human, 2003; Human, 2003). Many of these genera display a high degree of morphological conservatism within the genus (Compagno, 1988; Human, 2003).

The phylogeny based on the morphological examination by Compagno (1988), of the scyliorhinid taxa available for

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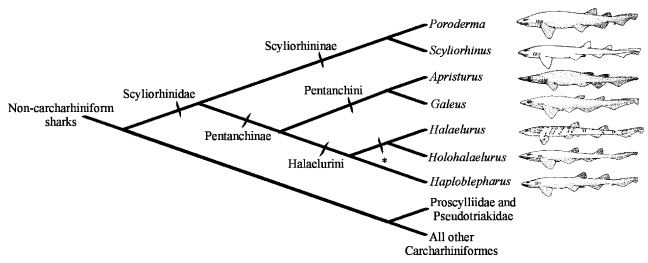

Fig. 1. Phylogenetic interrelationships of the scyliorhinid taxa used in this study, and relationship to immediate families within the Carcharhiniformes, as determined by morphological studies (based on Compagno, 1988), see text for details. (Branch lengths are not significant, (*) probable subdivision of the tribe Halaelurini.)

this study, is summarised in Fig. 1. The taxa in this study belong to two main groups of catsharks, the subfamilies Scyliorhininae Gill 1862 and Pentanchinae Smith & Radcliffe 1912. Within the Pentanchinae are the tribes Pentanchini Smith & Radcliffe 1912 and Halaelurini Compagno, 1988. Compagnos' study highlighted a clade of catsharks that included genera that are well represented within southern Africa. This tribe, the Halaelurini, consists of the genera *Halaelurus*, *Haploblepharus*, and *Holohalaelurus*. However, the interrelationships within the Halaelurini could not be resolved.

As part of a larger scale investigation into the molecular phylogeny and taxonomy of southern African scyliorhinids (Human, 2003), a molecular dataset was assembled using the mitochondrial genes cytochrome *b*, NADH-2, and NADH-4, consisting of 129,128 base pairs in total. The dataset is well represented by taxa from the Halaelurini, and other catsharks, as well as numerous other Chondrichthyan taxa representing deeper phylogenetic relationships, and is used here to explore the interrelationships within the Scyliorhinidae, as well as to test the monophylicity of the Halaelurini. Insights into other interrelationships within the Scyliorhinidae and other Chondrichthyan taxa can be made due to the size of the data set.

2. Methods and materials

2.1. Sample collection and DNA extraction

Taxa novel to this study were either collected by recreational and commercial fishers, or collected by hand by the author (BAH) on SCUBA or snorkel. Sharks collected by fishers were caught with various types of gear including line fishing and trawling. These sharks were either biopsied and released, or fresh frozen and biopsied at a later date. White muscle tissue was targeted for the biopsy and either immediately prepared for DNA extraction or stored in 70–100% ethanol for later extraction.

When one of the authors (BAH) was present during the capture, approximately 200–300 μL of blood was taken with a 0.9 mm × 40 mm hypodermic needle and 5 mL syringe from the anterior cardinal sinus, located mesodorsally above the gill slits (Daniel, 1922) and immediately transferred into 1 mL of lysis buffer (1 mM EDTA, 10 mM Tris, 1% SDS, and 750 mM NaCl, Proteinase K).

For biopsy material, approximately 200 mg of muscle tissue was ground with mortar and pestle into a paste and placed into 5 mL of lysis buffer. Hundred micrograms per millilitre freshly made Proteinase K (Amersham) was added to the mix and incubated overnight at 56 °C. Protein was removed using three phenol/chloroform extractions. DNA was precipitated using 10 mol L^{-1} ammonium acetate and ice cold 100% ethanol, suspended in 1× TE (1 mM EDTA, 10 mM Tris, pH 7.5) and quantified by scanning the sample over the range 240–360 nm using a diode array spectrophotometer.

For DNA preparation from blood, the nucleated erythrocytes were transferred immediately after collection into lysis buffer. Hundred micrograms per millilitre freshly made Proteinase K (Amersham) was added and the mix incubated overnight at 56 °C. After incubation, protein was removed using phenol/chloroform. DNA was precipitated using 10 mol L^{-1} ammonium acetate and ice cold 100% ethanol, suspended in 1× TE, and quantified as above. Blood samples generally provided a much greater yield of DNA than the muscle samples.

2.2. Gene amplification and sequencing

Three mitochondrial genes, cytochrome *b* (cytb), nicotinamide adenine dehydrogenase subunit 2 (NADH-2), and nicotinamide adenine dehydrogenase subunit 4 (NADH-4), were used in the present study and were amplified, and sequenced, using the same basic PCR protocol (Palumbi et al., 1991). Primers were either generously provided or designed from existing sequences (for details, see Table 1). Each PCR had a total volume of 12 μL using 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.1% gelatin, 5 pmol each of forward and reverse primer, 0.25 U *Taq* polymerase and 50 ng of DNA template. PCR comprised one denaturation cycle of 94 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing for 15 s, elongation at 72 °C for 2 min, and a final elongation cycle at 72 °C for 10 min. Annealing temperatures used were 58 °C for cytb and NADH-2, and 48 °C for NADH-4. Sequencing of the genes was carried out using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit ver 2.0 and PCR conditions were as per manufacturers instructions. The products of the cycle sequencing were run on an ABI Prism 3100 genetic analyser (Central Analytical Facility, University of Stellenbosch).

Previously published sequences were used in addition to the sequences obtained here and came from sources acknowledged in Table 2.

2.3. Phylogenetic analysis

Sequences were first viewed with Chromas ver 1.43 (McCarthy, 1997) and then manually aligned in BioEdit ver

Table 1
PCR amplification and sequencing primers used in this study detailing the target gene, primer name, sequencing direction, the primer sequence, and the source of the primer

Gene	Primer name	Direction	Sequence	Source
cytb	Glu14314L	Forward	5′ ccaat aactt gaaaa actat cg 3′	Andrew Martin (pers. commun.)
	Thr15546H	Reverse	5′ tcttc gactt acaag gtc 3′	Andrew Martin (pers. commun.)
NADH-2	ILE	Forward	5′ ccgga tcact ttgat agagt 3′	Gavin Naylor (pers. commun.)
	NADH-2-Scyl	Forward internal	5′ ggtgt aacat caaca attg 3′	B. Human; designed against *A. saldanha*, *P. africanum*, *P. pantherinum*, *S. capensis* and *H. edwardsii* from current study, and *S. canicula* from Delarbre et al. (1998)
	ASN	Reverse	5′ cgcgt ttagc tgtta actaa 3′	Naylor et al. (1997) universal primer from Kocher et al. (1995)
NADH-4	NADH-4-L	Forward	5′ tgact accaa aagct catgt agaag c 3′	Gavin Naylor (pers. commun.)
	LEU-H	Reverse	5′ catta ctttt acttg gattt gcacc a 3′	Gavin Naylor (pers. commun.)

5.0.9 (Hall, 1999). Aligned sequences were transferred from BioEdit into the program DAPSA ver 6.31 (Harley, 2003) where the aligned sequences were translated and raw nexus files were then generated for each gene alignment, in which all nucleotides were included.

The data sets for each gene were analysed separately to maximise the number of taxa in each analysis and to examine if any of the data sets converge on different trees. Also, a combined (total evidence) data set, achieved by concatenating the three genes, was used to average the phylogenetic signal and noise from each data set, and increase the number of nucleotides in the analysis to maximise the possibility of finding the optimal topology. The concatenated gene order of the combined dataset was 5′ NADH-4-NADH-2-cytb 3′ for all analyses.

Maximum parsimony (MP) with bootstrap pseudoreplicates, and maximum likelihood (ML) analyses were all performed using the PC version of PAUP* ver 4.0b10 (Swofford, 2002), and Bayesian inference (BI) analysis was carried out using the program Mr. Bayes (Huelsenbeck and Ronquist, in press). All computations were performed on a Pentium III 1.7 GHz PC with 96 Mb RAM. The MP analysis was performed heuristically using the TBR option in PAUP to search the tree space for the most parsimonious tree, which was then bootstrapped to assess tree support. For the ML analysis, the quartet puzzling (QP) heuristic search was employed (Strimmer and von Haeseler, 1996) using 100,000 puzzling operations and exact likelihood values were calculated. The MP and ML methodologies are detailed more extensively in Human (2003).

To avoid potential problems such as autocorrelation and slow mixing in BI analysis, it is necessary to perform numerous Metropolis coupled Markov chain Monte Carlo (MCMCMC) generations, and in the current study 3 million MCMCMC generations were performed for each of the data sets. The general time reversible model (GTR) was opted for with the following parameters: 1st, 2nd, and 3rd codon positions are defined, among site variation is codon specific, trees were sampled every 100 MCMC generations with one cold chain and three heated chains (variably heated using Mr. Bayes defaults), and the consensus tree is a 50% majority rule consensus tree with the first 50 trees (5000 MCMC) generations discarded as burn in. The log likelihood scores for each data set were obtained by averaging the log likelihood scores of each tree at stationarity (after burn in). The burn in is discarded because it is not representative of the posterior probability of the given data (Huelsenbeck and Ronquist, in press; Huelsenbeck et al., 2002; Lewis, 2001).

3. Results

A total of 41 sequences from 18 taxa are novel to this study (see Table 2). Table 2 also provides a list of the taxa included in the current study, with classification and genes amplified and sequenced, to illustrate the representation of the various orders. The only major lineage of sharks not represented here are the carpet sharks (Orectolobiformes), which are rare in southern Africa, and to the authors' knowledge are yet to be sequenced for the genes used in the current study. The data set analysed constitutes the largest purely chondrichthyan data set analysed to date, with a total of 129,128 characters.

The trees constructed using parsimony and maximum likelihood analysis were poorly resolved, as were the trees constructed using the genes NADH-2, NADH-4, and the combined datasets (results not shown). The tree with the greatest resolution was produced by the cytb dataset using Bayesian inference and is used here to draw the phylogenetic conclusions discussed below. For a detailed discussion of all analytical and data set combinations, readers are referred to Human (2003).

The 50% majority rule consensus phylogram, showing relative branch lengths, from the Bayesian analysis of cytb is shown in Fig. 2, however only branches with support values of 80% or greater are labelled. Stationarity was observed at approximately 3000 generations for cytb and the burn in was set at 5000 generations (see Section 2), therefore stationarity had been reached by this point. The log likelihood of the posterior probability for the cytb data set was −20593.18.

Only six branches had less than 80% support for them in the cytb data set (Fig. 2). Non-rajiform and non-chimaeroid taxa formed a monophyletic group (100%) and the Heterodontiformes were placed sister to the squalimorph sharks (95%). *Mitsukurina* is the basal taxon of the Lamniformes

Table 2
Taxa used in the current study indicating classification, common name, and in which dataset(s) the taxon was present

Order	Family	Species	Common name	Cytb	NADH-2	NADH-4	Comb
Chimaeriformes	Callorhinchidae	*Callorhinchus capensis*+	St. Joseph		*	*	
	Chimaeridae	*Chimaera monstrosa*	Rabbitfish	*	*	*	*
		Hydrolagus africanus+	African chimaera			*	
Rajiformes	Rhinobatidae	*Rhinobatos hynnicephalus*	Ringstraked guitarfish	*			
	Pristidae	*Pristis perotteti*	Largetooth sawfish	*			
	Rajidae	*Amblyraja radiata*	Thorny skate	*	*	*	*
		Dipturus pullopunctatus+	Slime skate			*	*
		Rajella caudaspinosa+	Munchkin skate			*	
	Narkidae	*Narke capensis*+	Cape numbfish		*	*	
	Urotrygonidae	*Urobatis concentricus*	Bullseye stingray	*			
	Dasyatidae	*Dasyatis akajei*	Red stingray	*			
	Gymnuridae	*Gymnura japonica*	Japanese butterfly ray	*			
		Gymnura marmorata	California butterfly ray	*			
		Gymnura natalensis+	Diamond ray			*	
	Myliobatidae	*Myliobatis tobijei*	Kite ray	*			
Hexanchiformes	Chlamydoselachidae	*Chlamydoselachus anguineus*	Frilled shark	*			
Heterodontiformes	Heterodontidae	*Heterodontus francisci*	Horn shark	*	*	*	*
Pristiophoriformes	Pristiophoridae	*Pliotrema warreni*+	Sixgill sawshark	*	*		
		Pristiophorus japonicus	Japanese sawshark	*			
Squaliformes	Squalidae	*Squalus acanthias*	Piked dogfish	*	*	*	*
Squatiniformes	Squatinidae	*Squatina nebulosa*	Clouded angelshark	*			
Lamniformes	Alopiidae	*Alopias pelagicus*	Pelagic thresher	*	*		
		Alopias superciliosus	Bigeye thresher	*	*		
		Alopias vulpinus	Thresher	*	*		
	Carchariidae	*Carcharias taurus*	Spotted raggedtooth	*	*		
	Odontaspididae	*Odontaspis ferox*	Bumpytail raggedtooth	*	*		
	Lamnidae	*Carcharodon carcharias*	White shark	*	*		
		Isurus oxyrinchus	Mako	*	*		
		Isurus paucus	Longfin mako	*	*		
		Lamna ditropis	Salmon shark	*	*		
		Lamna nasus	Porbeagle	*	*		
	Megachasmidae	*Megachasma pelagios*	Megamouth shark	*	*		
	Mitsukurinidae	*Mitsukurina owstoni*	Goblin shark	*	*		
	Cetorhinidae	*Cetorhinus maximus*	Basking shark	*	*		
	Pseudocarchariidae	*Pseudocarcharias kamoharai*	Crocodile shark	*	*		
Carcharhiniformes	Scyliorhinidae	*Apristurus microps*+	Smalleye catshark			*	
		Apristurus saldanha+	Saldanha catshark	*	*	*	*
		Galeus polli+	African sawtail catshark	*		*	
		Halaelurus natalensis+	Tiger catshark	*	*	*	*
		Haploblepharus edwardsii+	Happy eddie	*	*	*	*
		Haploblepharus fuscus+	Plain happy	*	*	*	*
		Haploblepharus pictus+	Pretty happy	*	*	*	*
		Holohalaelurus regani+	Izak	*		*	
		Poroderma africanum+	Pyjama shark	*	*	*	*
		Poroderma pantherinum+	Leopard catshark	*	*	*	*
		Scyliorhinus canicula	Smallspotted catshark	*	*	*	*
		Scyliorhinus capensis+	Yellowspotted catshark	*	*	*	*
	Triakidae	*Mustelus asterias*	Starry smoothhound	*			
		Mustelus manazo	Starspotted smoothhound	*	*		
		Mustelus mustelus	Smoothhound	*			
		Mustelus punctulatus	Blackspot smoothhound	*			
	Carcharhinidae	*Carcharhinus plumbeus*	Sandbar shark	*	*		
		Carcharhinus porosus	Smalltail shark	*	*		
		Galeocerdo cuvier	Tiger shark	*	*		
		Negaprion brevirostris	Lemon shark	*	*		
		Prionace glauca	Blue shark	*	*		
	Sphyrnidae	*Sphyrna lewini*	Scalloped hammerhead	*	*		
		Sphyrna tiburo tiburo	Atlantic bonnethead	*			
		Sphyrna tiburo vespertina	Pacific bonnethead	*			

Classification and common names follows Compagno (2005); Compagno and Human (2003); Human (2003). (*) presence of the taxon in the data set. (+) sequence data used in the phylogenetic analysis from taxa that are novel to this study, sequence data for other taxa come from Martin et al. (1992), Martin (1995), Kitamura et al. (1996), Martin and Naylor (1997), Naylor et al. (1997), Cao et al. (1998), Delarbre et al. (1998), Rasmussen and Arnason (1999a), Rasmussen and Arnason (1999b), and Arnason et al. (2001). *Abbreviations:* Cytb, cytochrome *b*; NADH-2, nicotinamide adenine dehydrogenase subunit 2; NADH-4, nicotinamide adenine dehydrogenase subunit 4, Comb, combined dataset.

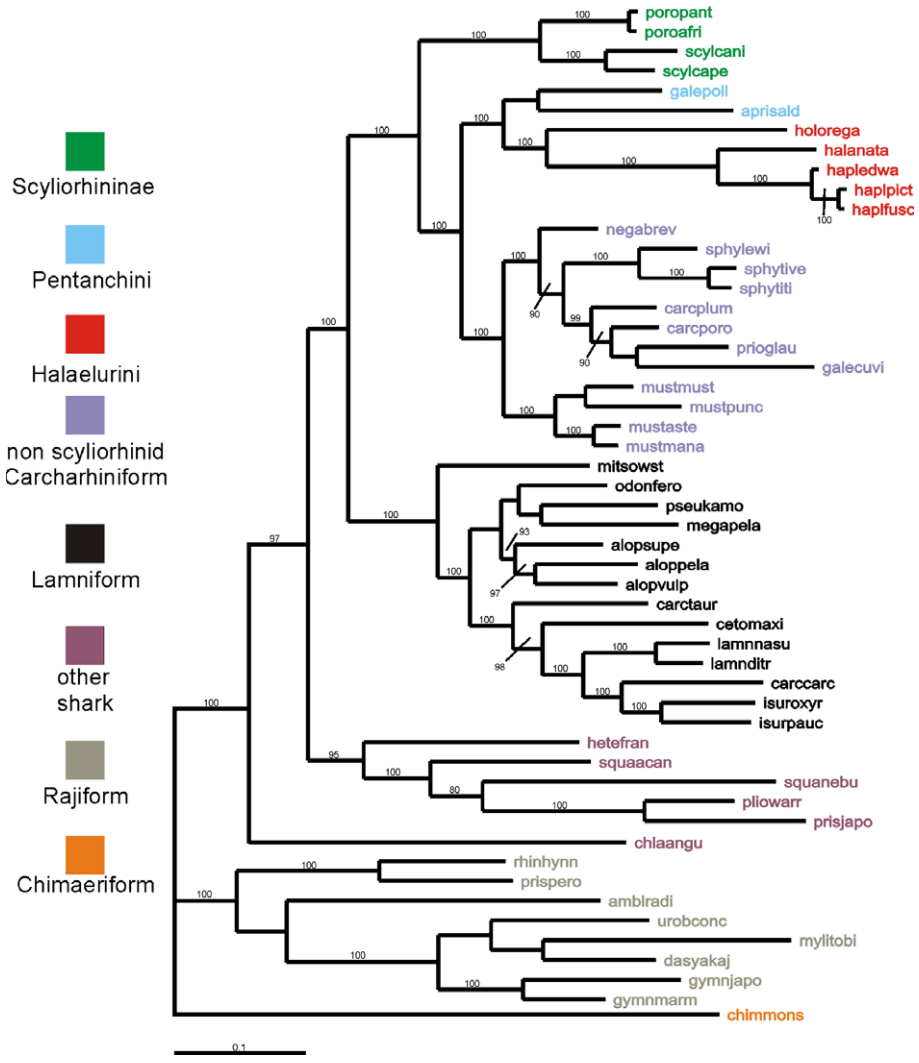

Fig. 2. 50% majority rule consensus phylogram from the Bayesian inference analysis of the cytochrome *b* dataset. Percentage support values, 80% or better, are shown above the branches. Branches with less than 80% support are considered unresolved and collapse. Scale bar indicates relative branch lengths.

(100%) and *Alopias superciliosus* is the basal taxon of the Alopiidae (93%). Within the Carcharhiniformes, which proved to be monophyletic in this analysis, the Scyliorhinidae are diphyletic (100%) with the Scyliorhininae forming a clade basal to the rest of the Carcharhiniformes (100%). Pentanchini + Halaelurini form a clade sister to the remainder of the Carcharhiniformes (100%), with *Apristurus* and *Galeus* resolved but not well supported, whereas the Halaelurini clade is well supported (100%). Within the Halaelurini, *H. edwardsii* is the basal taxon within *Haploblepharus* (100%), *Halaelurus* is basal to *Haploblepharus* (100%) and *Holohalaelurus* is basal to *Halaelurus* + *Haploblepharus* (100%). *Negaprion* is placed as the basal taxon of the Sphyrnidae + Carcharhinidae clade (100%), and *Sphyrna lewini* is the basal sphyrnid taxon (100%). In the carcharhinid clade, *Carcharhinus* is paraphyletic (99 and 90%), and *Galeocerdo* and *Prionace* are unsupported. The results of the Bayesian analysis on the cytb data set supports the Halaelurini hypothesis and suggests that *Holohalaelurus* is sister to *Halaelurus* + *Haploblepharus*.

4. Discussion

The most surprising result from this study is that the Scyliorhinidae was determined to be paraphyletic. This contrasts with the morphological conservation displayed by members of this family, and also the relative recency of the family, which is first recorded from the Tithonian, at the end of the Jurassic (Cappetta, 1987). A summary diagram of the phylogeny of the scyliorhinids based on the current molecular analysis is shown in Fig. 3. Whereas the position of the Scyliorhinidae within the Carcharhiniformes has been problematic, the Scyliorhinidae have always been considered a monophyletic clade, albeit without a well-defined autapomorphy for the group (Compagno, 1988). Posteriorly placed dorsal fins (origin of first dorsal fin above or posterior to the pelvic fin base), rounded dorsal and pectoral fins, enlarged anterior nasal flaps, clasper morphology and other finer scale skeletal and anatomical details unite the Scyliorhinid taxa morphologically (see Compagno (1988) for detailed discussion of the morphology of the

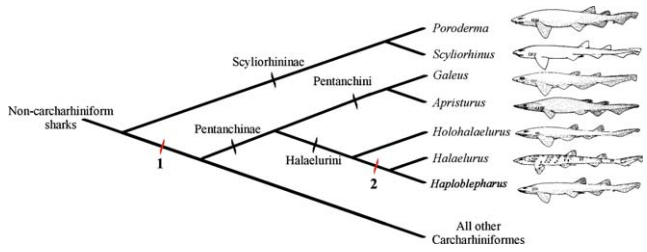

Fig. 3. Schematic diagram of the phylogenetic interrelationships of scyliorhinid taxa as determined by Bayesian inference of the cytochrome *b* dataset. Red lines indicate homoplasic morphological characters inferred from the current molecular analysis: (1) posteriorly placed dorsal fins, rounded dorsal and pectoral fins, enlarged anterior nasal flaps, and clasper morphology; (2) slender body type, and overall similarities in external morphology. (Branch lengths are not significant.)

group). All morphological characters mentioned in the following discussion in reference to the Carcharhiniformes come from Compagno (1988) unless otherwise noted.

The Scyliorhininae is supported by this analysis (Figs. 2 and 3) and is a subfamily that includes the genera *Cephaloscyllium* (swell sharks), *Scyliorhinus*, and *Poroderma* (Compagno, 1988). *Cephaloscyllium* material was not available for this study, however, both *Poroderma* and *Scyliorhinus* proved to be monophyletic. Reductions in the second dorsal fin, clasper, and branchial skeletons are morphological autapomorphies for this group. The Pentanchinae includes ten genera (see Compagno, 1988), represented here by *Apristurus*, *Galeus*, *Halaelurus*, *Haploblepharus*, and *Holohalaelurus*, and was supported by our findings (Figs. 2 and 3). A number of morphological characters separate the Pentanchinae from the Scyliorhininae, including absence, presence or development of a number of cranial structures, second dorsal fin development, clasper skeletal modification, development of nasal barbels, segmentation of pectoral and dorsal fin skeletal elements, development of head musculature, and differences in the number of skeletal elements in the splanchnocranium.

Apristurus and *Galeus* (demon and sawtail catsharks) represent the tribe Pentanchini, which consists of six genera (Compagno, 1988), and although the clade was present in the current analysis, it was not statistically well supported, and was tentatively supported by Compagno (1988). However, the Pentanchini hypothesis is not contradicted. Genetic material is needed for the remaining members of the Pentanchini (*Bythalaelurus*, *Cephalurus*, *Parmaturus*, and *Pentanchus*) to further explore this clade and its' interrelations with the Scyliorhinidae.

The Halaelurini hypothesis was well supported in the current analysis (Figs. 2 and 3), however, *Holohalaelurus* was placed basal to the clade *Halaelurus* + *Haploblepharus*, which contradicts the morphological derived phylogeny. The *Halaelurus* + *Haploblepharus* clade is unlikely due to the morphological similarities of *Halaelurus* and *Holohalaelurus*. There are three autapomorphies of *Haploblepharus* which makes the *Halaelurus* + *Haploblepharus* clade unlikely. In *Haploblepharus* (1) the anterior nasal flaps are fused into a nasal curtain, which serves as an upper lip, whereas there is no fusion of the nasal flaps in either *Halaelurus* or *Holohalaelurus*, (2) there is a novel symphysial (basimandibular) cartilage present in the lower jaw of *Haploblepharus* which is unique amongst extant cartilaginous fishes (Bass et al., 1975; Compagno, 1988; Human, 2003), and (3) *Haploblepharus* is much stockier than *Halaelurus* and *Holohalaelurus*, which are both quite slender (see Human (2003) to compare *Haploblepharus* with *Holohalaelurus*, and Compagno (1988) for comparisons of all taxa).

Haploblepharus edwardsii is the basal taxon for the genus. Compagno (1988) did not address species level relationships, and this is the first examination of the phylogeny within *Haploblepharus*. A fourth species of *Haploblepharus* was recognised only after the analyses presented here had been completed. It is apparently rare, and material suitable for genetic analysis does not exist for this new species (Compagno and Human, in preparation). Morphologically, *Haploblepharus* spA is very similar to *H. edwardsii* and has long been identified as such. A phylogenetic analysis of morphological characters, as well as inclusion of genetic material for the fourth *Haploblepharus* is needed to clarify relationships within this genus.

Lamniformes were monophyletic in all of the analyses where taxa were present in the data set. The Lamniformes are a highly diverse group, that today represents only fragments of a once speciose taxon (Compagno, 1973, 1977, 1990, 2001; Cappetta, 1987).

Rajoid taxa were never placed within the sharks, which support the Rajoids as a monophyletic assemblage sister to the remainder of the Elasmobranchii, and favours the placement of the skates and rays in the superorder Batoidea. The sister position of the Rajoids relative to the remaining elasmobranchs was a long standing hypothesis that has recently been challenged by Compagno (2001), where the Rajoids are placed in the order Rajiformes within the superorder Squalimorphii, together with the Hexanchiformes, Squaliformes, Pristiophoriformes, and Squatiniformes.

Conflicting results also arose concerning interrelationships proposed for the Heterodontiformes. *Heterodontus* was placed with the squalimorph taxa and not with the galeomorph taxa. Morphologically, the Heterodontiformes have either been placed in a separate taxon (Bigelow and Schroeder, 1948; Garman, 1913; Taylor, 1972), or placed in the superorder Galeomorphii or equivalent (Cappetta, 1987; Compagno, 1973, 1977, 1984a, 2001; Müller and Henle, 1838-1841), however, they have never been placed with the squalimorphs.

In conclusion, most of the scyliorhinid phylogenetic hypotheses put forward by Compagno (1988) are either supported by the cytb Bayesian inference tree, or there is insufficient evidence to argue convincingly against them. The most notable exception is the apparent paraphyly of the family Scyliorhinidae. The phylogeny generated by the molecular data suggests that the tribes Scyliorhininae and Pentanchinae should be elevated to familial status, although the morphological affinities between these

groups conflict with that hypothesis. The other exception is the apparent basal position of *Holohalaelurus* within the Halaelurini, which for the reasons detailed above, is unlikely.

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Appendix A. List of species names and their abbreviations used in figures

aloppela—*Alopias pelagicus*
alopsupe—*Alopias superciliosus*
alopvulp—*Alopias vulpinus*
amblradi—*Amblyraja radiata*
aprisald—*Apristurus saldanha*
carccarc—*Carcharodon carcharias*
carcplum—*Carcharhinus plumbeus*
carcporo—*Carcharhinus porosus*
carctaur—*Carcharias taurus*
cetomaxi—*Cetorhinus maximus*
chimmons—*Chimaera monstrosa*
chlaangu—*Chlamydoselachus anguineus*
dasyakaj—*Dasyatis akajei*
galecuvi—*Galeocerdo cuvier*
galepoll—*Galeus polli*
gymnjapo—*Gymnura japonica*
gymnmarm—*Gymnura marmorata*
halanata—*Halaelurus natalensis*
hapledwa—*Haploblepharus edwardsii*
haplfusc—*Haploblepharus fuscus*
haplpict—*Haploblepharus pictus*
hetefran—*Heterodontus francisci*
holorega—*Holohalaelurus regani*
isuroxyr—*Isurus oxyrinchus*
isurpauc—*Isurus paucus*
lamnditr—*Lamna ditropis*
lamnnasu—*Lamna nasus*
megapela—*Megachasma pelagios*
mitsowst—*Mitsukurina owstoni*
mustast—*Mustelus asterias*
mustmana—*Mustelus manazo*
mustmust—*Mustelus mustelus*
mustpunc—*Mustelus punctulatus*
mylitobi—*Myliobatis tobijei*
negabrev—*Negaprion brevirostris*
odonfero—*Odontaspis ferox*
pliowarr—*Pliotrema warreni*
poroafri—*Poroderma africanum*
poropant—*Poroderma pantherinum*
prioglau—*Prionace glauca*
prisjapo—*Pristiophorus japonicus*
prispero—*Pristis perotteti*
pseukamo—*Pseudocarcharias kamoharai*
rhinhynn—*Rhinobatos hynnicephalus*
scylcani—*Scyliorhinus canicula*
scylcape—*Scyliorhinus capensis*
sphylewi—*Sphyrna lewini*
sphytiti—*Sphyrna tiburo tiburo*
sphytive—*Sphyrna tiburo vespertina*
squaacan—*Squalus acanthias*
squanebu—*Squatina nebulosa*
urobconc—*Urobatis concentricus*

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