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Elasmobranch Phylogeny: A Mitochondrial Estimate Based on 595 Species

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2.1 Introduction

2.1.1 Background

Interest in elasmobranch biodiversity and taxonomy has grown in recent years, catalyzed primarily by four influences: (1) the large number of new species that have been described over the past 30 years (e.g., Last and Stevens, 2009); (2) the recognition that many species of elasmobranchs, several of which have not yet been formally described, may be threatened with extinction from fishing pressures and habitat destruction (Stevens et al., 2000); (3) the growing interest in DNA "barcoding" as a tool to augment taxonomic description (e.g., Ward et al., 2007); and (4) an emerging recognition of the important role that elasmobranchs play as top predators in marine ecosystems (Heithaus et al., 2008).

Increasingly, elasmobranch workers across a wide range of fields of science, both pure and applied, are recognizing the importance of an accurate species-level taxonomy. Fisheries scientists are ever more keenly aware of the need for accurate species-level assessments of catches to manage fisheries effectively. Ecologists have become more careful to ensure that the animals to which life history attributes are ascribed constitute distinct species rather than assemblages of closely related congeners with potentially different ecological roles and life history attributes. Finally, conservation biologists are beginning to recognize how critically important it is to have an accurate understanding of species compositions based on careful taxonomy to prioritize and manage units of biodiversity for conservation (Griffiths et al., 2010; Iglésias et al., 2009; White and Kyne, 2010).

2.1.2 Motivation

While interest in the taxonomy of elasmobranchs is probably at an all-time high, efforts to understand their phylogenetic interrelationships have lagged behind (Thomson and Shaffer, 2010). Contributions to our understanding of elasmobranch phylogeny have thus far been restricted to studies focusing on the interrelationships of particular groups, including *Arctoraja* (Spies et al., 2011), Batoidea (Aschliman et al., in press; McEachran and Aschliman, 2004; Rocco et al., 2007), *Carcharhinus* (Dosay-Akbulut, 2008), Carcharhinidae (Naylor, 1992), Carcharhiniformes (Compagno, 1988), Dasyatidae (Sezaki et al., 1999), *Dasyatis* (Rosenberger, 2001), Etmopteridae (Shirai and Nakaya, 1990; Straube et al., 2010), Lamnidae (Dosay-Akbulut, 2007; Martin, 1997), Laminiformes (Martin and Naylor, 1997; Naylor et al., 1997; Shimada, 2005), Myliobatiformes (de Carvalho et al., 2004; Dunn et al., 2003; Gonzalez-Isáis and Dominguez, 2004; Lovejoy, 1996; Nishida, 1990), Orectolobidae (Corrigan and Beheregaray, 2009), Rajiformes (McEachran and Dunn, 1998; McEachran and Miyake, 1990; Turan, 2008), Scyliorhinidae (Human et al., 2006; Iglésias et al., 2005), Sphyrnidae (Cavalcanti, 2007; Lim et al., 2010), Selachii (Vélez-Zuazo and Agnarsson, 2011), *Squatina* (Stelbrink et al., 2009), and Triakidae (López et al., 2006), or studies focusing on the relationships among major lineages using a few carefully chosen exemplars for multiple lineages (Compagno, 1977; de Carvalho, 1996; Douady et al., 2003; Heinicke et al., 2009; Maisey, 1984a,b; Maisey et al., 2004; Mallatt and Winchell, 2007; Naylor et al., 2005; Shirai, 1992, 1996; Winchell et al., 2004). To our knowledge, no phylogenetic studies of elasmobranchs have incorporated dense taxon sampling at the species level across the entire breadth of elasmobranch diversity. This is, in part, because the most immediate concerns have centered on documenting the extant diversity as quickly as possible, before fishing pressure and habitat destruction drive it to extinction. However, it is also a result of the fact that obtaining samples from the broad spectrum of taxa required for a comprehensive phylogenetic analysis is particularly challenging. Nonetheless, a phylogenetic perspective provides a context for understanding the historical forces that have shaped extant biodiversity. This information can be helpful to conservation efforts and effective fisheries management because degree of relatedness can often be a good predictor of life history attributes and sensitivity to environmental change.

2.1.3 Barcodes, GenBank, and Phylogenetic Estimation

We believe that the lag in interest in generating a comprehensive phylogeny for elasmobranchs is unlikely to last long. As CO1 barcode sequences pour into GenBank for a diversity of elasmobranchs (e.g., Holmes et al., 2009; Mariguela et al., 2009; Quattro et al., 2006; Richards et al., 2009; Serra-Pereira et al., 2011; Smith et al., 2008; Spies et al., 2006; Straube et al., 2010, 2011; Toffoli et al., 2008; Ward and Holmes, 2007; Ward et al., 2005, 2007, 2008, 2009; Wong et al., 2009; Wynen et al., 2009; Zemlak et al., 2009), driven in part by the various Barcode of Life initiatives, it will only be a short time before enterprising efforts are made to estimate phylogenetic trees from these barcode sequences. We anticipate that when this happens, there will be a profusion of trees forwarded in the literature that suggest conflicting phylogenetic relationships. If past experience is any guide, trees derived this way will contain a high proportion of accurate and credible relationships interspersed with a few erroneous groupings. Unfortunately, it will be difficult to tell which relationships are erroneous, as the misleading inferences are likely to vary from study to study, depending on the taxon-sampling scheme and individual specimens used. More insidiously, it is also likely that congruent misleading inferences will surface in different studies, unwittingly leading to strong confidence in an erroneous consensus topology. This can occur, for example, in instances of model misspecification exacerbated by missing data (Lemmon et al., 2009) and uneven taxon sampling.

Barcode sequences downloaded from GenBank are especially prone to yielding misleading estimates of phylogeny, in large part because the 650-bp CO1 barcode fragment has become the *de facto* standard for molecular identification of species. It is now routine to remove tissue samples from specimens in the field and send them off to sequencing centers for "barcoding." Unfortunately, some of the specimens from which tissue samples are derived are misidentified when collected, and, because there is no expertly curated reference dataset against which to compare sequences, many are added to GenBank with their original incorrectly assigned identities (Bridge et al., 2003; Vilgalys, 2003; Wesche et al., 2004).

Notwithstanding the potential problems with misidentification, barcode sequences are not well suited to phylogenetic analysis from the outset. Being relatively short (approximately 650 bp in most vertebrate taxa), they do not provide a large number of characters upon which to base phylogenetic inferences; also, being relatively fast evolving, they are generally not useful for estimating relationships among deeply divergent taxa. There are, of course, other sources of error that can lead to incorrect phylogenetic inferences such as labeling errors, sequencing errors, dissonance between gene trees and the species trees that contain them, sampling errors due to stochasticity of the evolutionary process, model violation, and exacerbations of model violation caused

by sparse taxon sampling, and missing data. These problems are not unique to CO1 barcode data, however, and can affect any molecular phylogenetic study.

2.1.4 The Current Study

We take the position that accurate and reliable estimates of phylogeny are best achieved through analysis of congruence among a carefully selected suite of independent single-copy markers whose patterns of evolutionary change can be accommodated with simple i.i.d. (independently and identically distributed) models. However, as an interim measure, our goal for the current chapter is to provide a phylogenetic analysis of a densely taxon-sampled, mitochondrial, protein-coding gene. In an effort to minimize the types of errors referred to above, we have avoided using any sequences derived from GenBank. All of our sequences have been generated *de novo* from samples taken from specimens collected by the authors or identified by taxonomic experts. Our primary motivation for this contribution is to provide a baseline against which future phylogenetic studies can be contrasted and evaluated, particularly those based on datasets compiled from GenBank submissions. As noted above, these potentially include sequences from misidentified specimens or sequences of questionable provenance, and a substantial fraction of missing data that can often yield peculiar results. This is exemplified by the recent paper of Vélez-Zuazo and Agnarsson (2011), whose inferences were based on a dataset with 85% missing entries, a Bayesian analysis that had not fully converged, and a taxon sampling scheme that included several misidentified specimens (evidenced by their untenable placements on the tree presented). The inferred relationships we present doubtlessly depict several relationships that are incorrect; however, we anticipate that any errors are likely to be the consequence of model violation of one type or another or differential fixation of ancestral polymorphism in descendant lineages, rather than those associated with questionable specimen provenance or misidentification, although we are certainly not immune to these type of problems, either.

2.2 Methods

2.2.1 Taxon Sampling

We recently completed a survey of sequence variation in elasmobranchs using the NADH2 mitochondrial gene (Naylor et al., in press). That study was based on an analysis of sequences derived from a total of 4283 specimens of elasmobranchs representing 574 (of approximately 1200 described) species in 157 (of 193 described) genera in 56 (of 57 described) elasmobranch families. Its primary goal was to better understand the taxonomy and species boundaries among elasmobranchs from a genetic perspective based on mitochondrial sequence variation. In that study, we were careful to point out that the summary tree presented, being based on "p" distances and a neighbor-joining cluster analysis, was not to be interpreted as a phylogeny, although many of the clusters among closely related forms likely do reflect phylogenetic groupings.

In contrast, the current study, explicitly sets out to conduct a model-based Bayesian phylogenetic analysis of a representative subset consisting of 585 of those 4283 NADH2 sequences from the Naylor et al. (in press) study. This subset includes single representatives of 570 species as well as two to seven replicates each of four problematic potential species complexes (i.e., *Rhinoptera steindachneri*, *Bathyraja kincaidii/interupta*, *Amblyraja hyperborea/badia/jensenae*, and *Dipturus batis/oxyrinchus*) included in the Naylor et al. (in press) analysis. These sequences have been augmented here to include NADH2 data from an additional two genera (*Miroscyllium* and *Trigonognathus*) and 21 species, one of which (*Etmopterus viator*) (Straube et al., 2011b) was replicated from widely separated parts of its range. The identities assigned to these specimens in the current analysis are given in Table 2.1, along with names assigned in the previous analyses of Straube et al. (2010, 2011a). Exemplars representing each species were selected on the basis of the availability of photographic or voucher material associated with the specimen from which the sequence was derived. In total, 69% of the specimens used in this study are represented by images in our online database (http://tapewormdb.uconn.edu/index.php/hosts/specimen_search/elasmobranch) or their identifications have been verified by taxonomic experts. Of these specimens, 35% have been deposited in museums; several are types. The elasmobranch sample in the current study represents approximately 50% (i.e., 595) of all known species, 83% (i.e., 159) of all genera, and 98% (i.e., 56) of all families; these numbers represent a relatively even spread across sharks and rays. Locality data, voucher information, and GenBank accession numbers are provided in Naylor et al. (in press) for the 585 sequences taken from that study and in Table 2.1 for the specimens new to this study. In addition, four chimaeroid species, representing two genera and two families, were used to represent the outgroup. These are *Hydrolagus bemisi*, *Hydrolagus colliei*, *Hydrolagus novaezealandiae*, and *Rhinochimaera pacifica*.

2.2.2 Sequence Generation

Although most of the sequences for this study were taken directly from those used in the Naylor et al. (in press) study, sequences for 22 specimens were generated

TABLE 2.1

Voucher Information for the 22 Specimens Added in This Study

Order and Family	Species	Database ID	GenBank ID	Museum Voucher No.	Locality	Tissue Sample No.
Carcharhiniformes						
Carcharhinidae	*Carcharhinus leiodon*[a]	GN5013	JQ400110	BMNH 2010.2.8.1 (jaws only)	Kuwait City, Kuwait, Persian (Arabian) Gulf	BW-A6072
Scyliorhinidae	*Bythaelurus canescens*	GN7459	JQ400111	ZSM-33566	Chile, Pacific Ocean	ZSM-P-CH_0290
Squaliformes						
Centrophoridae	*Centrophorus acus*	GN7425	JQ400112	Photo voucher	Suruga Bay, Japan, Pacific Ocean	ZSM-P-CH_0076
Centrophoridae	*Deania profundorum*	GN7456	JQ400113	OCA-P-20061202.3C; photo voucher	Okinawa, Japan, Pacific Ocean	ZSM-P-CH_0257
Echinorhinidae	*Echinorhinus* sp. 1[b]	GN7438	JQ400114	No voucher specimen	Oman, Indian Ocean	ZSM-P-CH_0149
Etmopteridae	*Centroscyllium ritteri*	GN7428	JQ400115	No voucher specimen	Suruga Bay, Japan, Pacific Ocean	ZSM-P-CH_0082
Etmopteridae	*Centroscyllium nigrum*	GN7443	JQ400116	No voucher specimen	Chile, Pacific Ocean	ZSM-P-CH_0210
Etmopteridae	*Centroscyllium granulatum*[c]	GN7445	JQ400117	No voucher specimen	Chile, Pacific Ocean	ZSM-P-CH_0212
Etmopteridae	*Etmopterus* sp. B[d]	GN7398	JQ400118	No voucher specimen	Norfolk Ridge,Tasman Sea, Pacific Ocean	ZSM-P-CH_0017
Etmopteridae	*Etmopterus granulosus*[c]	GN7399	JQ400119	NMV A25150-016	Norfolk Ridge,Tasman Sea, Pacific Ocean	ZSM-P-CH_0022
Etmopteridae	*Etmopterus sentosus*[c]	GN7402	JQ400120	SAIAB 82362	Mozambique, Indian Ocean	ZSM-P-CH_0040
Etmopteridae	*Etmopterus* sp. 1	GN7406	JQ400121	No voucher specimen	South Africa, Atlantic Ocean	ZSM-P-CH_0045
Etmopteridae	*Etmopterus* sp. 2[e]	GN7409	JQ400122	No voucher specimen	South Africa, Atlantic Ocean	ZSM-P-CH_0050
Etmopteridae	*Etmopterus viator*[f]	GN7412	JQ400123	NMNZ P.42742	Chatham Rise, New Zealand, Pacific Ocean	ZSM-P-CH_0053
Etmopteridae	*Etmopterus viator*	GN7415	JQ400124	MNHN 20071666	Kerguel Plateau, Indian Ocean	ZSM-P-CH_0059
Etmopteridae	*Etmopterus schultzi*[c]	GN7418	JQ400125	Photo voucher	USA, Gulf of Mexico	ZSM-P-CH_0065
Etmopteridae	*Etmopterus polli*	GN7420	JQ400126	No voucher specimen	Angola Basin, Western Africa, Atlantic Ocean	ZSM-P-CH_0070
Etmopteridae	*Etmopterus brachyurus*	GN7423	JQ400127	Photo voucher	Suruga Bay, Japan, Pacific Ocean	ZSM-P-CH_0074
Etmopteridae	*Etmopterus unicolor*[c]	GN7434	JQ400128	Photo voucher	Suruga Bay, Japan, Pacific Ocean	ZSM-P-CH_0097
Etmopteridae	*Miroscyllium sheikoi*[c]	GN7440	JQ400129	No voucher specimen	Tashi Fish market, Illan, Taiwan, Pacific Ocean	ZSM-P-CH_0151
Etmopteridae	*Trigonognathus kabeyai*[c]	GN7431	JQ400130	HMD 2003-18	Japan Pacific Ocean	ZSM-P-CH_0093
Rajiformes						
Rajidae	*Dipturus trachyderma*	GN7449	JQ400131	Photo voucher	Huinay Fjord, Chile, Pacific Ocean	ZSM-P-CH_0246

Note: Specimen information for the remaining 585 specimens can be found in Naylor et al. (in press).

Abbreviations: BMNH, Natural History Museum (formerly British Museum [Natural History]), London, UK; HMD, Hekinan Seaside Aquarium, Hekinan City, Aichi Prefecture, Japan; MNHN, Muséum National d'Histoire Naturelle, Paris, France; NMNZ, Museum of New Zealand, Te Papa Tongarewa, Wellington, New Zealand; NMV, Museum Victoria (formerly National Museum of Victoria), Melbourne, Victoria, Australia; OCA, Okinawa Churaumi Aquarium, Okinawa, Japan; SAIAB, South African Institute for Aquatic Biodiversity, Grahamstown; ZSM, Zoologische Staatssammlung München, Munich, Germany (tissue collection).

[a] Specimen included in Moore et al. (2011).

[b] Specimen included in Straube et al. (2010) (as *Echinorhinus brucus*).

[c] Specimen included in Straube et al. (2010).

[d] *Sensu* Last and Stevens (1994).

[e] Specimen included in Straube et al. (2011a) (as *Etmopterus baxteri*).

[f] Specimen included in Straube et al. (2010, 2011a) (as *Etmopterus* cf. *granulosus*).

de novo using the same primer sets and amplification conditions described in Naylor et al. (in press). All sequences used in the current study have been entered in GenBank. In an effort to minimize problems associated with missing data (see Lemmon et al., 2009), only specimens for which close to the full sequence complement of NADH2 (see below) was available were included. The proportion of missing data in the final full matrix was less than 0.25%.

2.2.3 Sequence Alignment

Electropherogram trace files were assessed for quality and base assignments were made using the software package Phred (Ewing et al., 1998), and fragments were subsequently assembled using Phrap (Ewing et al., 1998). A script was written (by CL) to translate the assembled nucleotide sequences to amino acids, subject the amino acid sequences to alignment using ClustalW (Thompson et al., 1994), and to back translate the aligned amino acids to their original nucleotide sequences. The final alignment across all 607 elasmobranch sequences was 1044 bp long.

2.2.4 Phylogenetic Analysis

2.2.4.1 Model Choice

There is a trade-off between the number of model parameters used to estimate a quantity of interest and the statistical power underlying the estimate. The Akaike Information Criterion (AIC) (Akaike, 1973, 1983), or its Bayesian equivalent, provides a general framework to assess the trade-off between the accuracy gained by adding parameters and the attendant loss in statistical power across both nested and non-nested models. For molecular phylogenetic datasets with few taxa, or datasets with patterns of low complexity, AIC will favor models with fewer parameters. Datasets with more complex patterns often require a larger number of parameters, which, in turn, compromises their statistical power. When estimating phylogenetic trees from parameter-rich models, it is important that the dataset to which the model is applied can meaningfully inform the additional parameters of the model. In general, analyses of large, taxon-rich datasets that sample the evolutionary process in an even and balanced way are more likely to benefit from parameter-rich models. It should be pointed out, however, that complex models often yield a better fit to datasets with sparse or biased sampling than do simpler models, but they do not necessarily guarantee a better tree. The critical issue is that parameterizations should be tailored to capture the salient aspects of the process rather than to simply account for variance.

One of the more commonly used parameter-rich models in phylogenetic analyses is the GTR+I+Γ model. If the six substitution rates among the different nucleotides are constrained to sum to one, the substitution rate component of the model has ten free parameters, the values of which must be estimated: five parameters for the relative substitution rates, three for the base frequencies, and two to capture patterns of rate variation over sites (the proportion of invariant sites and the shape parameter of the distribution) (Gu et al., 1995; Waddell and Penny, 1996; Yang, 1994). Nevertheless, this model (GTR+I+Γ) does not accommodate the fact that patterns of nucleotide change are generally different among codon positions due to the architectural constraints of the genetic code (differences in patterns of change among the three codon positions due to redundancy of the code at third positions and hydrophobicity constraints at second positions). We used AIC and its small-sample-size corrected counterpart, AICc (Hurvich, and Tsai, 1989) to determine if modeling each codon position separately or pooling the information across codon positions was warranted for the assembled NADH2 dataset. Although results from AIC indicated that a separate GTR+I+Γ model for each codon position was warranted, the (approximate) AICc measure indicated that the model pooled over codon positions had the better score (Table 2.2).

Both models yielded very similar, although not identical, tree topologies. We take this to indicate that the hierarchical signal in the dataset is not highly sensitive to model choice, at least not between the two models we tested. We speculate that this is probably due to the dense and evenly balanced taxon sampling scheme used (595 distinct elasmobranch species sampled across the diversity of the class). Given the AICc scores, the tree presented here was generated using the GTR+I+Γ model pooled across codon positions. Instances in which the topologies of the trees resulting from the two models differed with respect to monophyly or placement of taxa are indicated in the relevant sections below.

2.2.4.2 Bayesian Analysis

The Bayesian phylogenetic analysis was performed with the parallel implementation of MrBayes (Altekar et al., 2004; Huelsenbeck and Ronquist, 2001; Ronquist and

TABLE 2.2

Model Comparison (K = Number of Free Parameters Including Branch Lengths)

Model	K	AIC	AICc
GTR+I+Γ (pooled across codons)	1227	289248.7	272870.9
GTR+I+Γ (each codon position)	3681	288611.8	278336.2

Huelsenbeck, 2003), version 3.2 (http://sourceforge.net/projects/mrbayes/). For each model, four independent analyses were run, each with one cold chain and three heated chains, using the default heating and chain-swapping parameter settings. The proposal mechanism autotuning feature was used for substitution-model parameters, and chains were sampled every 500 generations. Topological convergence was assessed by comparing the standard deviations of split frequencies (SDSF) between the tree samples of the runs. For each of the other parameters, the potential scale reduction factor (PSRF) (Gelman and Rubin, 1992) is also given.

2.3 Results

After discarding the first 25% of samples as burn-in, comparing the samples of the cold chains strongly indicated that all of the independent runs had converged to the same stationary distribution. The MrBayes runs that estimated parameters separately for each codon position were stopped after 28,771,000 generations to yield a largely resolved tree. The average (maximum) SDSF across runs at the time the analysis was stopped was <0.0076 (maximum, <0.06). The average (maximum) PSRF for the substitution model parameters was 1.0006 (maximum, 1.003), and for the branch length parameters it was 1.001 (maximum, 1.067). The runs for which the model parameters were shared across codon positions were stopped after 22,813,500 generations at an average (maximum) SDSF of 0.0085 (maximum, 0.126). The average (maximum) PSRF for the substitution model parameters was 1.0015 (maximum, 1.004), and for the branch length parameters it was 1.001 (maximum, 1.017).

The fact that the NADH2 dataset yielded a tree that was well resolved (see Figures 2.1 to 2.11) was unexpected. NADH2 was selected for the original Naylor et al. (in press) study to distinguish among closely related species because it ranks as one of the fastest evolving protein-coding genes in the mitochondrial genome. The decision to subject a representative subset of these sequences to phylogenetic analysis was expected to yield a tree poorly resolved at its base with a few well-resolved subsets toward the tips. The tree resulting from the analysis was not only resolved at multiple levels of divergence but was also largely consistent with existing elasmobranch taxonomy and classification. We speculate that much of the unanticipated phylogenetic signal in this dataset may be due to the dense and balanced taxon sampling scheme employed. We discuss the topology obtained from the analysis in light of current taxonomy and classification below.

2.3.1 Assessing Monophyly of Previously Recognized Groups

2.3.1.1 Monophyly of Genera

Of the 159 genera sampled, 43 are known to be monotypic. An additional 30 are represented by only a single species in this analysis and thus are not amenable to tests of monophyly here. Assessments of the monophyly of the 86 genera for which two or more species were included in the study are addressed below. Our results suggest that 54 of these 86 are monophyletic. Most of these are supported by robust posterior probabilities. Caution is advised in interpretation because we do not regard robust posterior probabilities as definitive evidence of the monophyly of a group because they can vary tremendously across models and taxon-sampling schemes. The remaining 32 genera are inferred not to be monophyletic in this study. They include several in which the monophyly is compromised by the inclusion of either a single monotypic genus or a closely related subset of taxa or by the exclusion of a single species (see Section 2.3.1.1.1), as well as a number of more problematic cases in which genera are rendered non-monophyletic through the inclusion of species collectively assigned to several different genera or in which multiple species ostensibly assigned to the same genus fall in disparate parts of the tree (see Section 2.3.1.1.2).

2.3.1.1.1 Simple Cases of Generic Non-Monophyly

Simple cases in which monophyly of a genus is compromised by the inclusion of a single monotypic genus or monophyletic subset of taxa in the clades resulting from the analysis are treated below in alphabetical order:

- *Aetomylaeus* (Figure 2.11)—Our analysis included all four species of *Aetomylaeus* and one of the two described species of *Pteromylaeus*. *Pteromylaeus bovinus* is deeply nested within the otherwise monophyletic genus *Aetomylaeus*. This result questions the wisdom of recognizing *Pteromylaeus* as a distinct genus.
- *Aetoplatea* (Figure 2.11)—*Aetoplatea zonura* groups deeply among the eight species of *Gymnura* included here. The second species of *Aetoplatea* recognized by Compagno (2005), *A. tentaculata*, was not included in our sampling. The current analysis supports the reassignment of *A. zonura* to *Gymnura* following Naylor et al. (in press), as suggested, for example, by Smith et al. (2009) and Jacobson and Bennet (2009).
- *Centrophorus* (Figure 2.7)—The monophyly of the genus *Centrophorus* is potentially compromised by its inclusion of *Deania* species that group in a polytomy with two lineages of

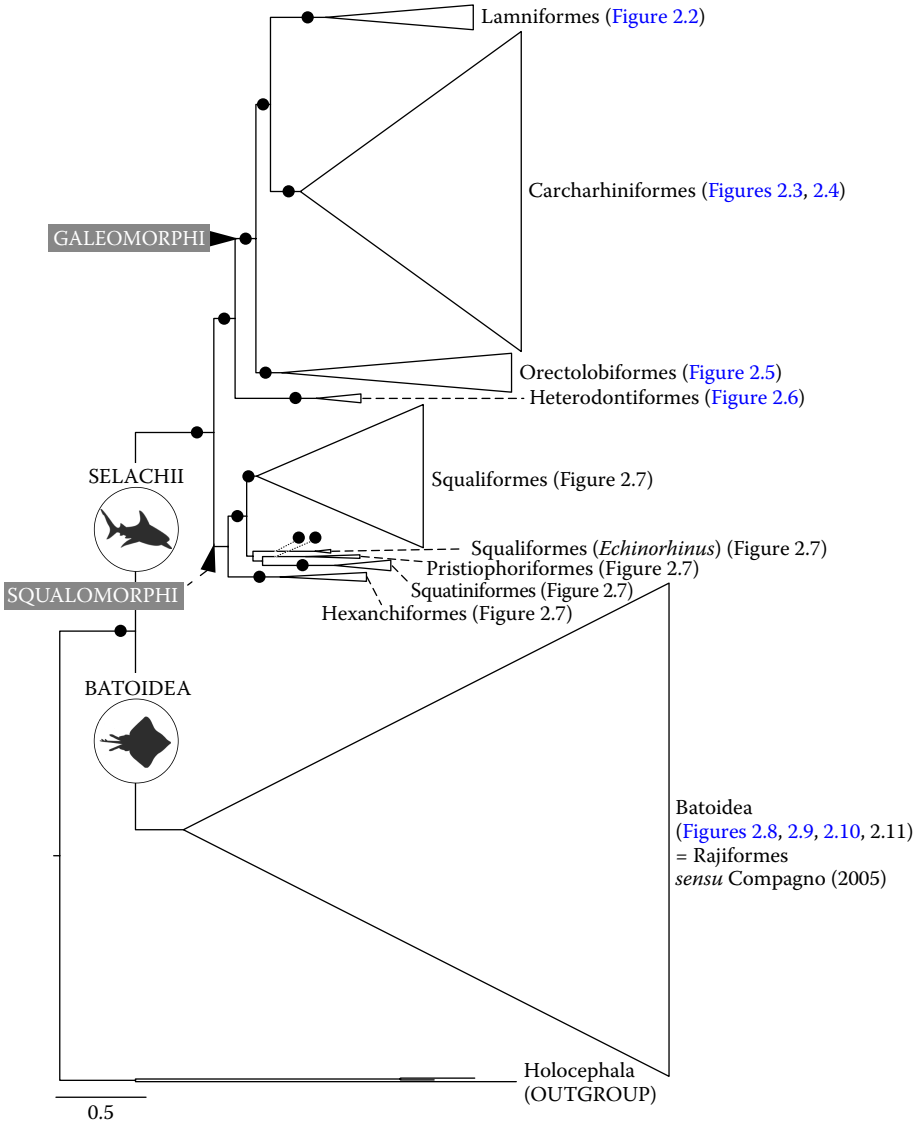

FIGURE 2.1
Summary of the phylogenetic relationships among elasmobranch orders based on NADH2 sequence data (1044 bp) of 595 elasmobranch species inferred from a Bayesian analysis using a separate GTR+I+Γ model pooled over codon positions. Black dots indicate posterior probabilities of >95%.

Centrophorus. We have included 12 of the 14 recognized species of *Centrophorus* and all but one of the species of *Deania*. Interestingly, a similar nesting of *Deania* within *Centrophorus* was obtained by Straube et al. (2010), although with a different suite of molecular markers. This polytomy clearly requires additional investigation.

- *Chiloscyllium* (Figure 2.5)—The analysis included six of the eight known species of *Chiloscyllium* and one of the nine known species of *Hemiscyllium*. Although poorly supported, the species of *Hemiscyllium* grouped among the species of *Chiloscyllium*; however, the phylogenetic placement of the specimen of *Hemiscyllium ocellatum* was found to be model dependent, and this grouping may merely reflect the close relationships between these two genera.
- *Dipturus* (Figure 2.9)—Our analysis suggests that *Dipturus* is monophyletic only if the species currently placed in *Zearaja* are included. This is a relatively robust result given how deeply the *Zearaja* species, which include three of the four known members of this genus, are nested among *Dipturus* species; the position of *Spiniraja whitleyi* further potentially compromises the monophyly of *Dipturus*. In addition, our results support Compagno's (2005) suggestion that the generic placement of *Dipturus linteus*, which

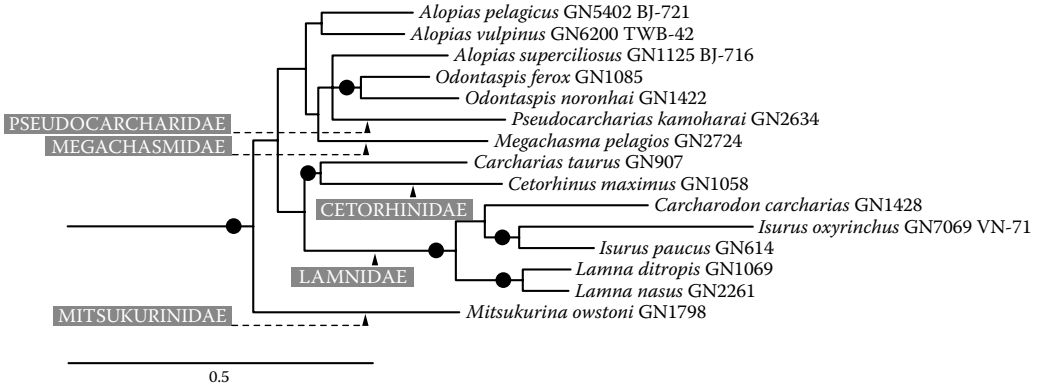

FIGURE 2.2
Hypothesis of the phylogenetic relationships of Lamniformes based on NADH2 sequence data (1044 bp) inferred from a Bayesian analysis using a separate GTR+I+Γ model pooled over codon positions. Black dots indicate posterior probabilities of >95%.

was represented by five specimens in the analysis of Naylor et al. (in press), requires further investigation.

- *Etmopterus* (Figure 2.7)—The analysis included approximately 50% of the greater than 40 species currently described from this genus, as well as the monotypic *Miroscyllium*. *Miroscyllium sheikoi* was found to fall squarely within an otherwise monophyletic *Etmopterus.* This placement was also seen in the multi-gene study of the phylogenetic relationships among etmopterids by Straube et al. (2010).
- *Galeus* (Figure 2.3)—The monophyly of this genus is called into question by the placement of *Galeus sauteri* in a clade outside of that containing its four congeners. This result is inconsistent with the work of Iglésias et al. (2005) based on 16S rDNA sequences; their analysis suggested that *Galeus* forms a monophyletic group including *G. sauteri.*
- *Halaelurus* (Figure 2.3)—Our results suggest that the monophyly of *Halaelurus* is compromised by the inclusion of *Haploblepharus* among *Halaelurus* species. The South African species *Haploblepharus edwardsii* grouped with the two South African species of *Halaelurus* (*H. lineatus* and *H. natalensis*) to the exclusion of the Australian and Southeast Asian representatives of *Halaelurus* (*H. sellus* and *H. buergeri*). The relationships among these two genera warrant further exploration with additional taxon sampling and markers.
- *Hexanchus* (Figure 2.7)—The monotypic *Heptranchias* clusters as sister to *Hexanchus griseus*, potentially compromising the monophyly of the genus *Hexanchus*; however, the topology presented is weakly supported. Further exploration of this question with nuclear markers is recommended before any taxonomic reassignments are made.
- *Mobula* (Figure 2.11)—The analysis included five of the nine species of *Mobula* and one of the two described species of *Manta*. The latter species was deeply nested within the otherwise monophyletic genus *Mobula* in the current analysis.
- *Mustelus* (Figure 2.3)—The genus *Mustelus* is monophyletic only if the clade containing *Scylliogaleus quecketti* and *Triakis megalopterus* is included. The grouping of these two taxa within *Mustelus* was also found by López et al. (2006).
- *Okamejei* (Figure 2.9)—The genus *Okamejei* is monophyletic but for the exclusion of *Okamejei jensenae*, which groups outside the three other species of *Okamejei* with a cluster of *Rostroraja* and *Raja* and species. The membership of *O. jensenae* within the genus *Okamejei* should be reassessed.
- *Pristiophorus* (Figure 2.7)—The analysis included two of the eight known species of *Pristiophorus*, as well as the monotypic *Pliotrema*. In the presented analysis, *Pliotrema warreni* grouped as the sister of *Pristiophorus japonicus*, rendering the genus *Pristiophorus* non-monophyletic. This result was model dependent. When a GTR+I+Γ model was run for each codon separately, *Pliotrema* was inferred to be the sister to a monophyletic *Pristiophorus.*
- *Sphyrna* (Figure 2.4)—Our study included all but one of the described species of *Sphyrna*, as well as *Eusphyra blochii* (*S. media* was not included). The current analysis implies that recognition of the monotypic genus *Eusphyra* may be unwarranted; this result is consistent

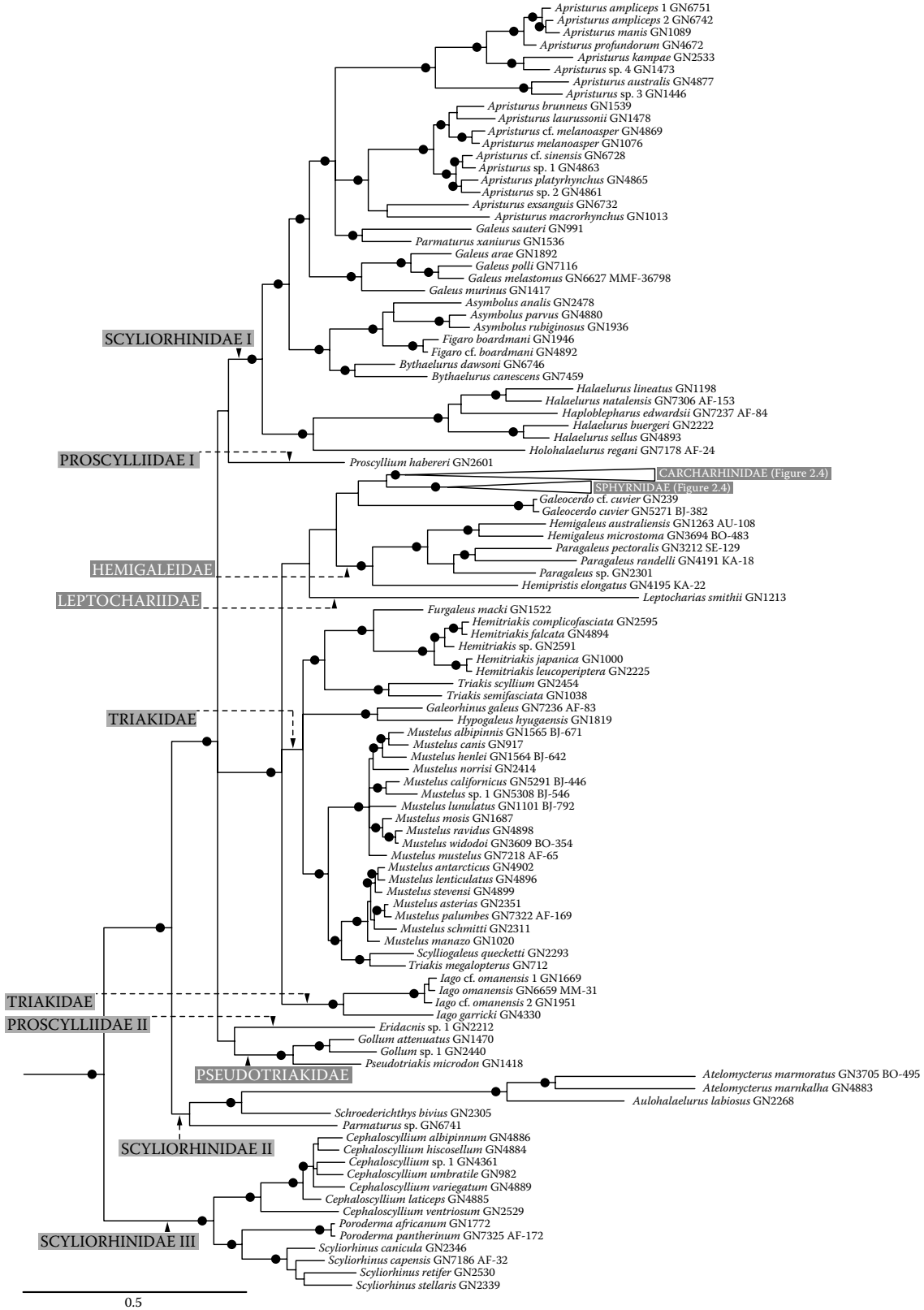

FIGURE 2.3
Hypothesis of the phylogenetic relationships of Carcharhiniformes based on NADH2 sequence data (1044 bp) inferred from a Bayesian analysis using a separate GTR+I+Γ model pooled over codon positions. Carcharhinid and sphyrnid relationships are shown in detail in Figure 2.4. Paraphyletic families are indicated with black text in family label boxes. Black dots indicate posterior probabilities of >95%.

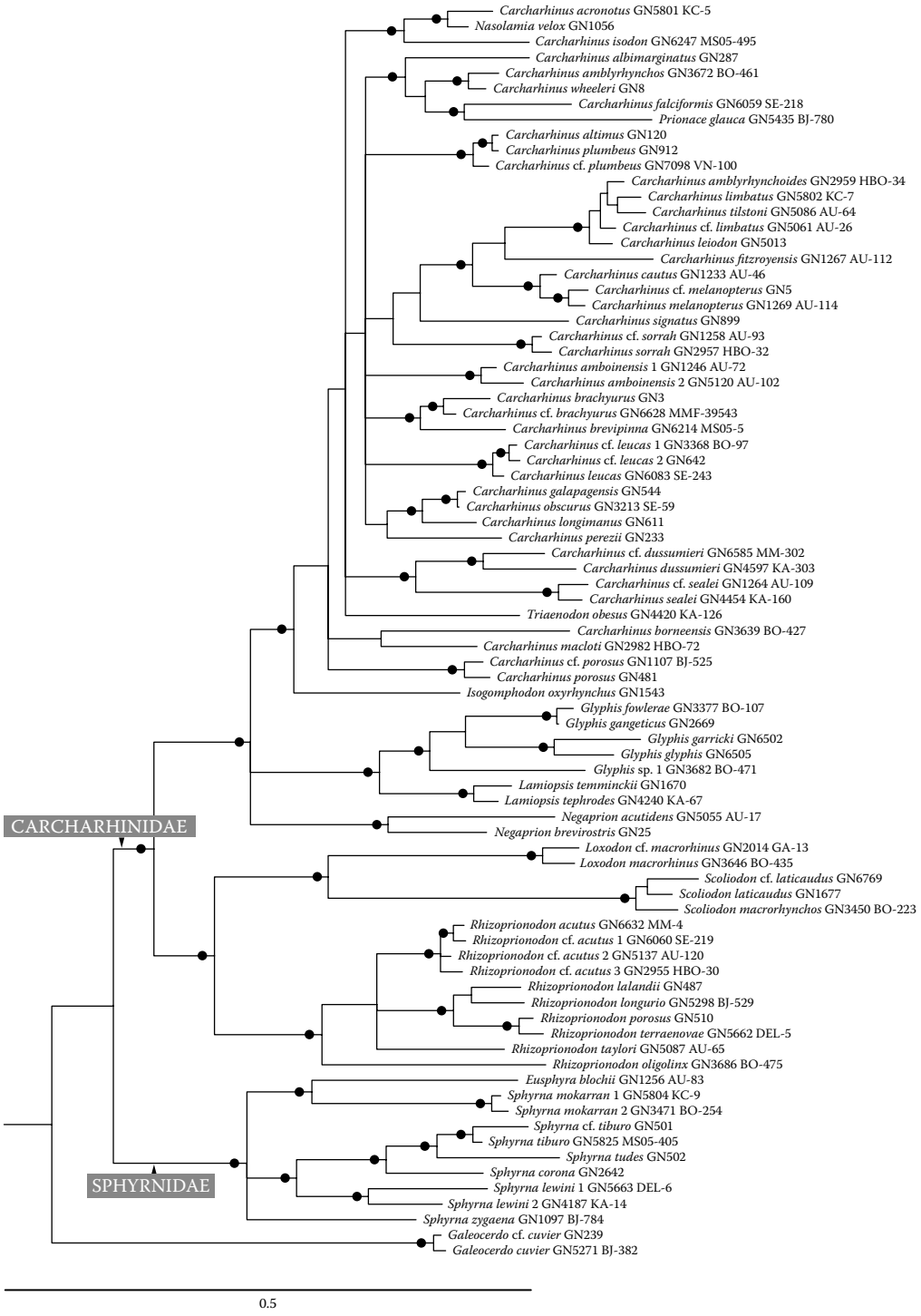

FIGURE 2.4
Hypothesis of the phylogenetic relationships of Carcharhinidae and Sphyrnidae based on NADH2 sequence data (1044 bp) inferred from a Bayesian analysis using a separate GTR+I+Γ model pooled over codon positions. Black dots indicate posterior probabilities of >95%.

with Compagno (1988). Nonetheless, a recent multi-gene study (Lim et al., 2010) focusing on the relationships among hammerhead sharks revealed variation in inferred relationships across genes. Lim et al. (2010) concluded that the signal among all of the genes they analyzed was most consistent with a basal placement of the monotypic *Eusphyra*, which is consistent with the current taxonomy and different from our findings with NADH2.

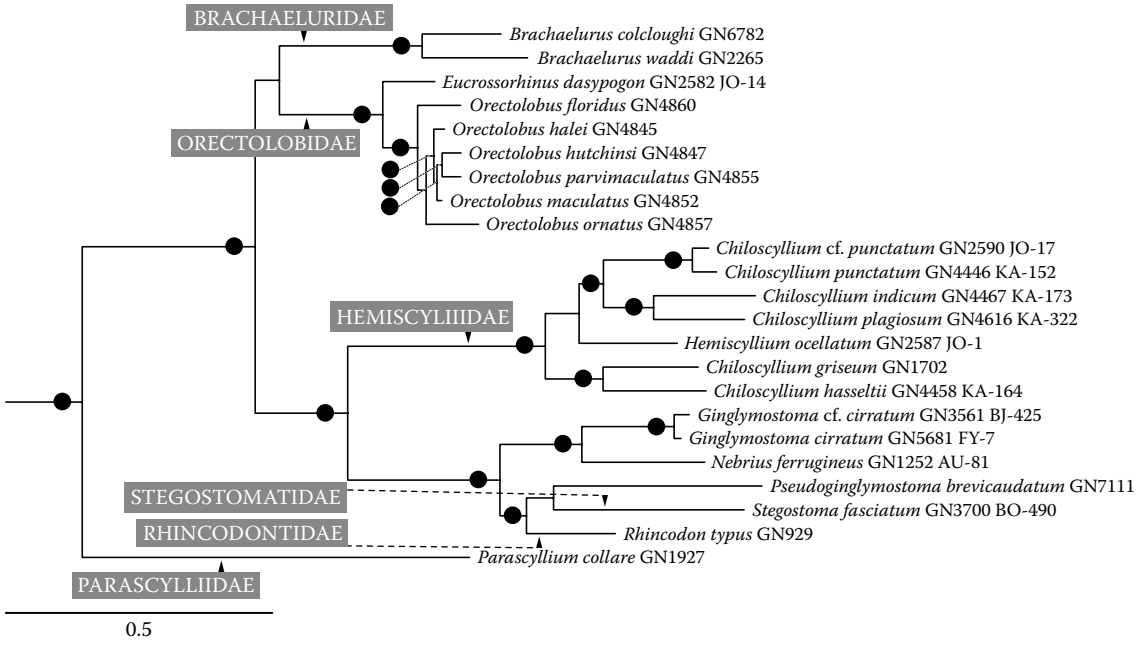

FIGURE 2.5
Hypothesis of the phylogenetic relationships of Orectolobiformes based on NADH2 sequence data (1044 bp) inferred from a Bayesian analysis using a separate GTR+I+Γ model pooled over codon positions. Black dots indicate posterior probabilities of >95%.

- *Squaliolus* (Figure 2.7)—The analysis included both described species of *Squaliolus* as well as the monotypic *Euprotomicrus*. The latter taxon grouped as sister to *S. aliae* with strong support. The justification for recognizing *Euprotomicrus* as a distinct genus warrants closer scrutiny and further analysis with additional markers.
- *Squalus* (Figure 2.7)—The 17 species of *Squalus* included in the analysis comprise a monophyletic group only if the genus also includes the two (of a total of three) species of *Cirrhigaleus*. *Squalus acanthias* and *S. suckleyi* were found to group with the two *Cirrhigaleus* species, albeit with relatively weak support and a short branch, as sister to the remaining *Squalus* species. If the close relationship between *Cirrhigaleus* and these two species of *Squalus* is borne out with further data, consideration should be given to expanding *Squalus* to include the three known species of *Cirrhigaleus*, especially given that *S. acanthias* is the type species of the genus.
- *Taeniura* (Figure 2.11)—*Taeniura grabata* groups with *Taeniurops meyeni*, well away from the two other putative forms of *Taeniura lymma*. Our analysis supports the transfer of *Taeniura grabata* to *Taeniurops*.
- *Triakis* (Figure 2.3)—Although *Triakis scyllium* groups with *Triakis semifasciata*, their congener, *Triakis megalopterus*, groups with *Scylliogaleus quecketti*. This same result was seen in the multi-gene analysis conducted by López et al. (2006). We suggest that the generic assignment of *T. megalopterus* be re-examined.
- *Urobatis* (Figure 2.11)—Our analysis included three species of *Urobatis* from the Gulf of California and one from the Caribbean Sea.

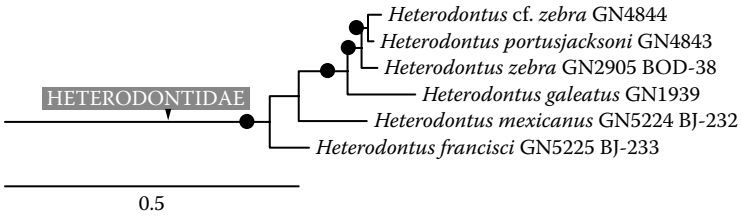

FIGURE 2.6
Hypothesis of the phylogenetic relationships of Heterodontiformes based on NADH2 sequence data (1044 bp) inferred from a Bayesian analysis using a separate GTR+I+Γ model pooled over codon positions. Black dots indicate posterior probabilities of >95%.

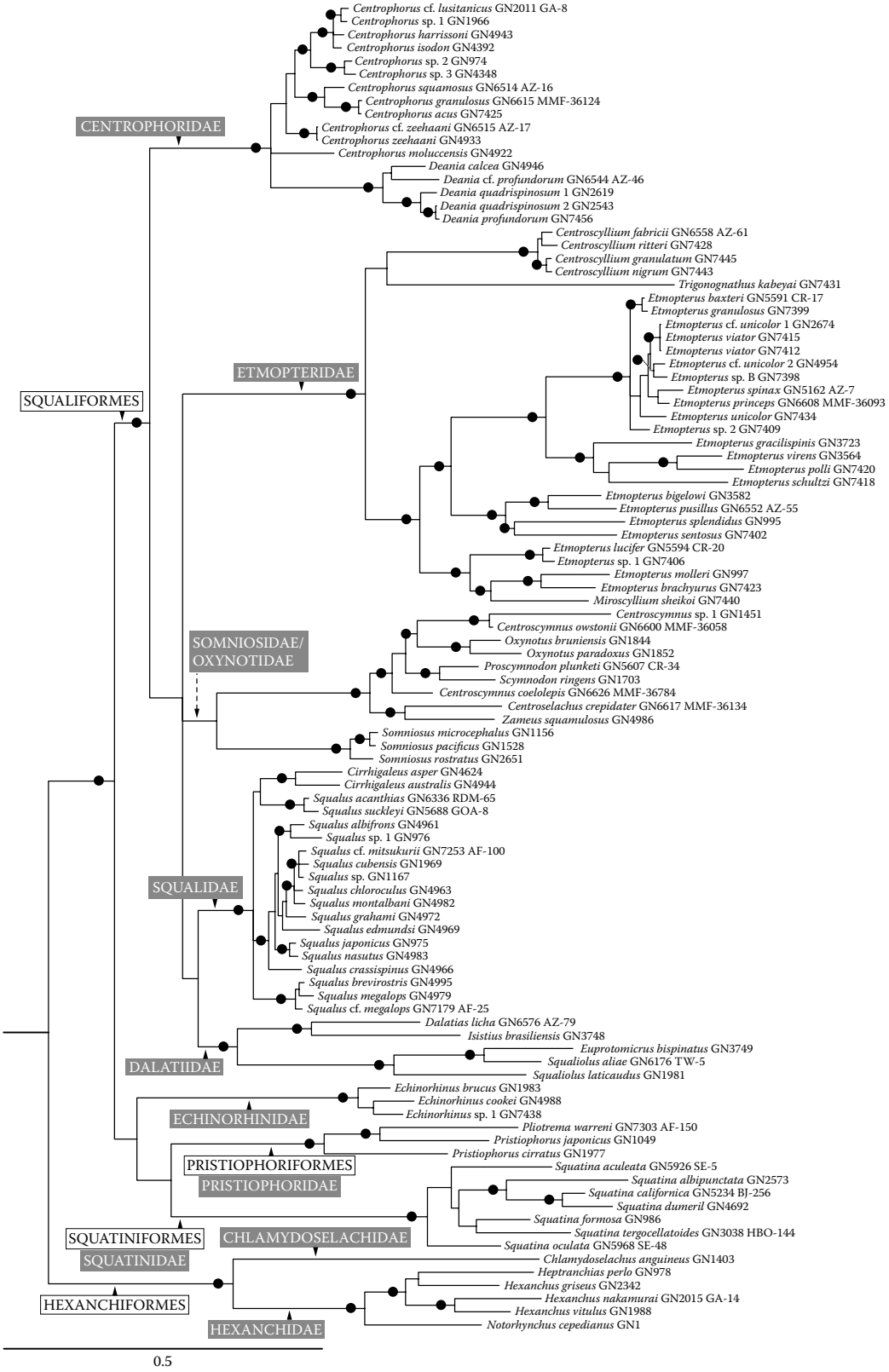

FIGURE 2.7
Hypothesis of the phylogenetic relationships of Squaliformes, Pristiophoriformes, Squatiniformes, and Hexanchiformes based on NADH2 sequence data (1044 bp) inferred from a Bayesian analysis using a separate GTR+I+Γ model pooled over codon positions. Black dots indicate posterior probabilities of >95%.

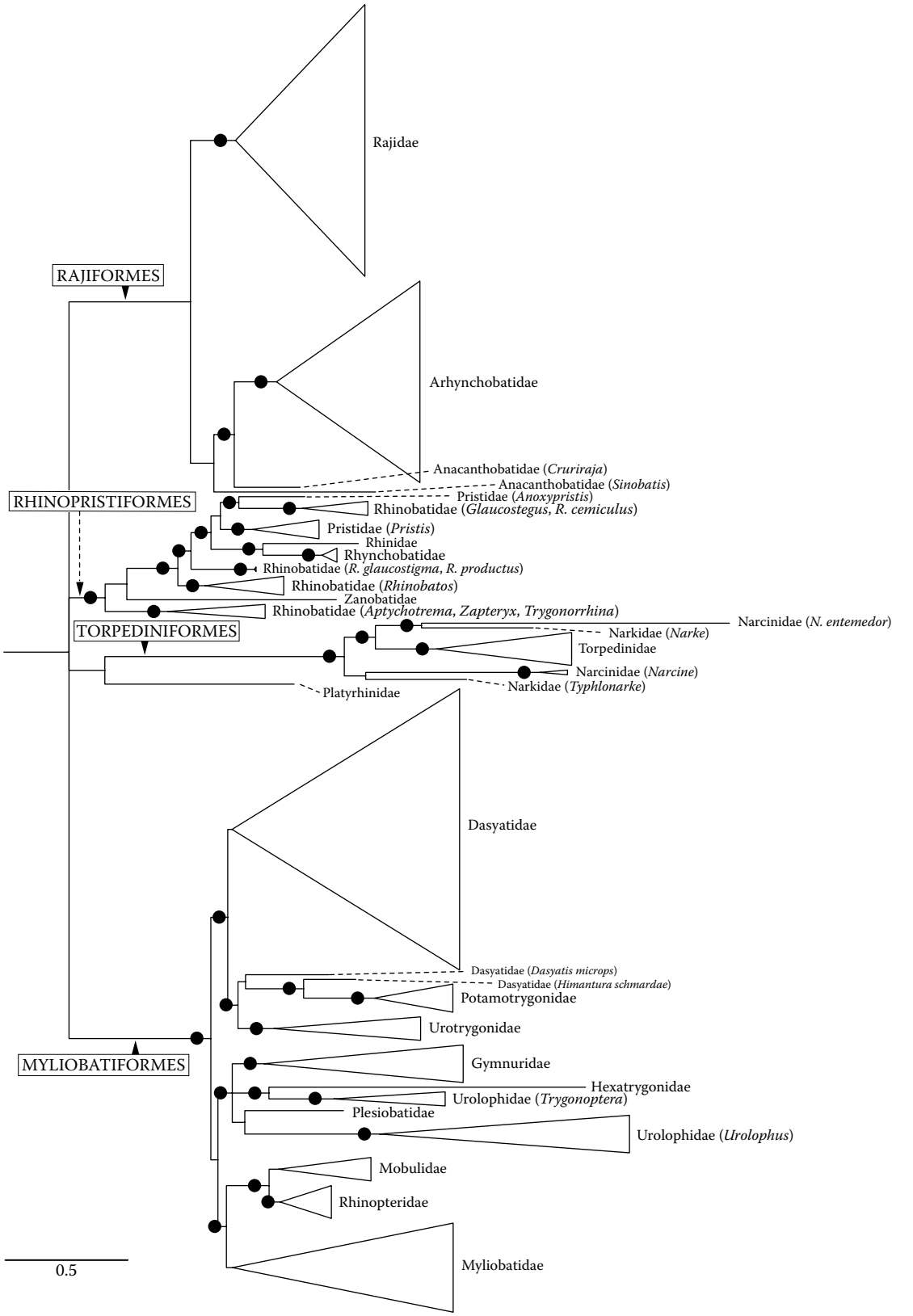

FIGURE 2.8
Summary of the phylogenetic relationships among the families and orders of Batoidea (i.e., Rajiformes *sensu* Compagno, 2005) based on NADH2 sequence data (1044 bp) inferred from a Bayesian analysis using a separate GTR+I+Γ model pooled over codon positions. Details of relationships for individual families are shown in Figures 2.9, 2.10, and 2.11. Black dots indicate posterior probabilities of >95%.

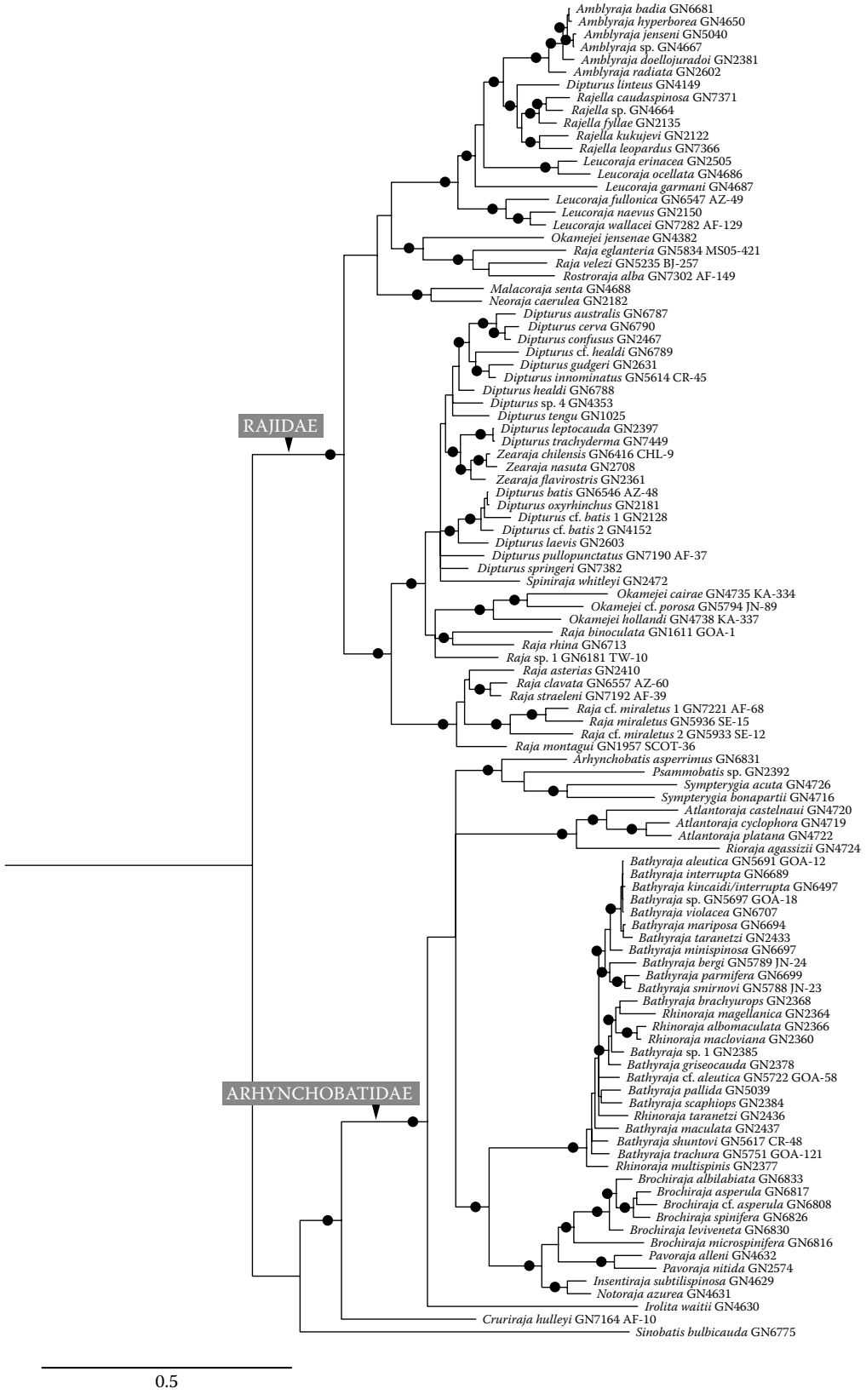

FIGURE 2.9
Hypothesis of the phylogenetic relationships of Rajiformes *sensu stricto* based on NADH2 sequence data (1044 bp) inferred from a Bayesian analysis using a separate GTR+I+Γ model pooled over codon positions. Black dots indicate posterior probabilities of >95%.

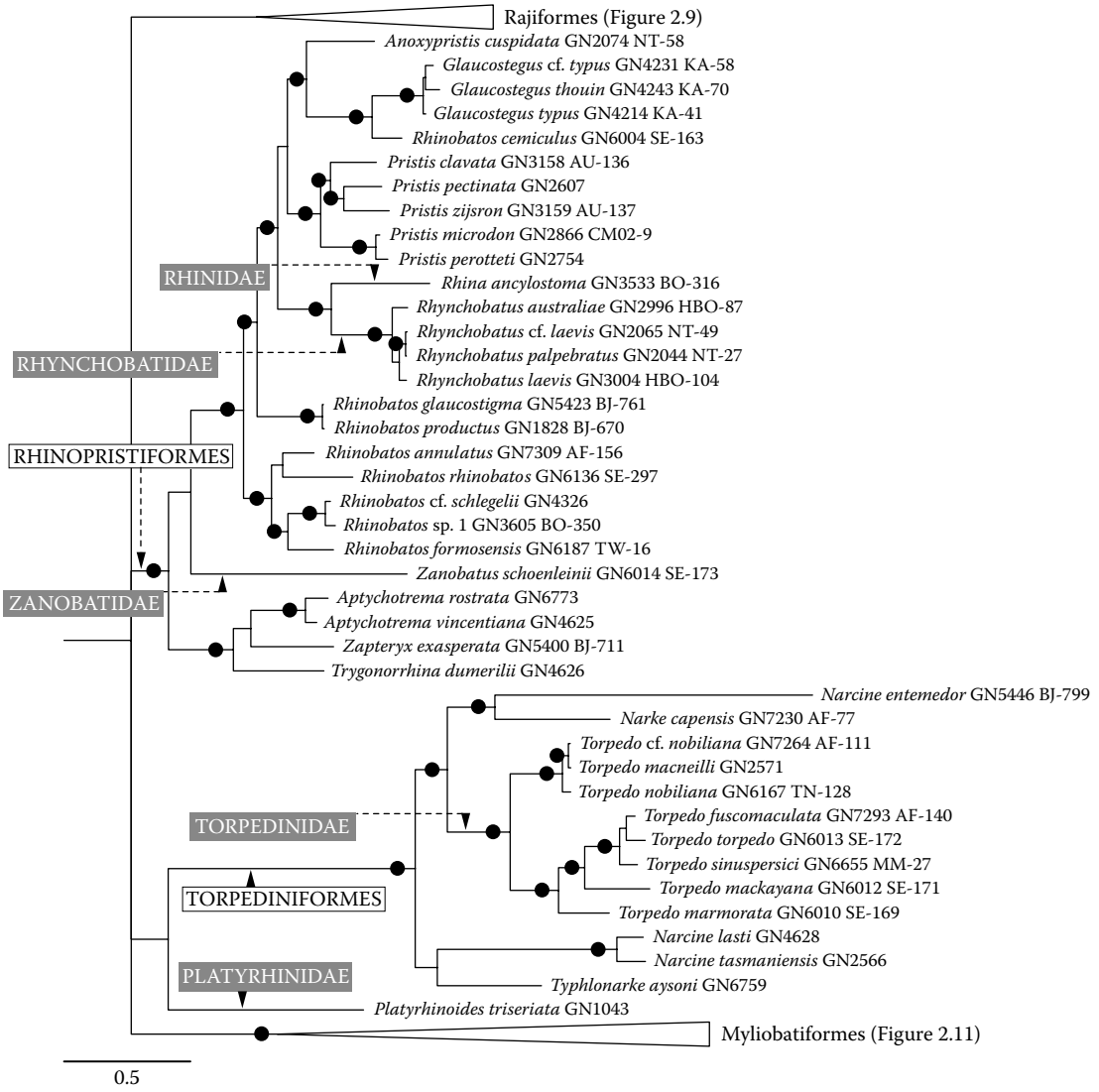

FIGURE 2.10
Hypothesis of the phylogenetic relationships of Rajiformes, Rhinopristiformes, Torpediniformes, *Platyrhinoides*, and Myliobatiformes based on NADH2 sequence data (1044 bp) inferred from a Bayesian analysis using a separate GTR+I+Γ model pooled over codon positions. Myliobatiform relationships are shown in detail in Figure 2.11. Black dots indicate posterior probabilities of >95%.

Our current results suggest that the genus is monophyletic only if either the two species of *Urotrygon* included in the analysis are also considered members of the genus or *Urobatis jamaicensis* is transferred to *Urotrygon*.

2.3.1.1.2 Complex Cases of Generic Non-Monophyly

The monophyly of the following genera is more problematic. These genera were rendered non-monophyletic in the trees resulting from our analysis, either because species collectively assigned to two or more genera are intermingled within a single clade or because multiple species ostensibly assigned to the same genus were found to fall in disparate parts of the tree. These genera are treated below in alphabetical order:

- *Alopias* (Figure 2.2)—Results of the current analysis suggest that the genus *Alopias* is non-monophyletic, although with a low posterior probability. The lack of monophyly for the genus, while highly surprising and seemingly unlikely, is consistent with analyses based on whole mitochondrial genomes (Ferrara, unpublished master's thesis) and also with preliminary analyses based on nuclear markers (GJPN, unpublished). Nonetheless, previous analyses of cytochrome *b* (Martin and Naylor, 1997) and cytochrome *b*/NADH2 (Naylor et al., 1997) data indicate that the genus *Alopias* is monophyletic, as did the morphological analysis of Shimada (2005). It is important to note that the

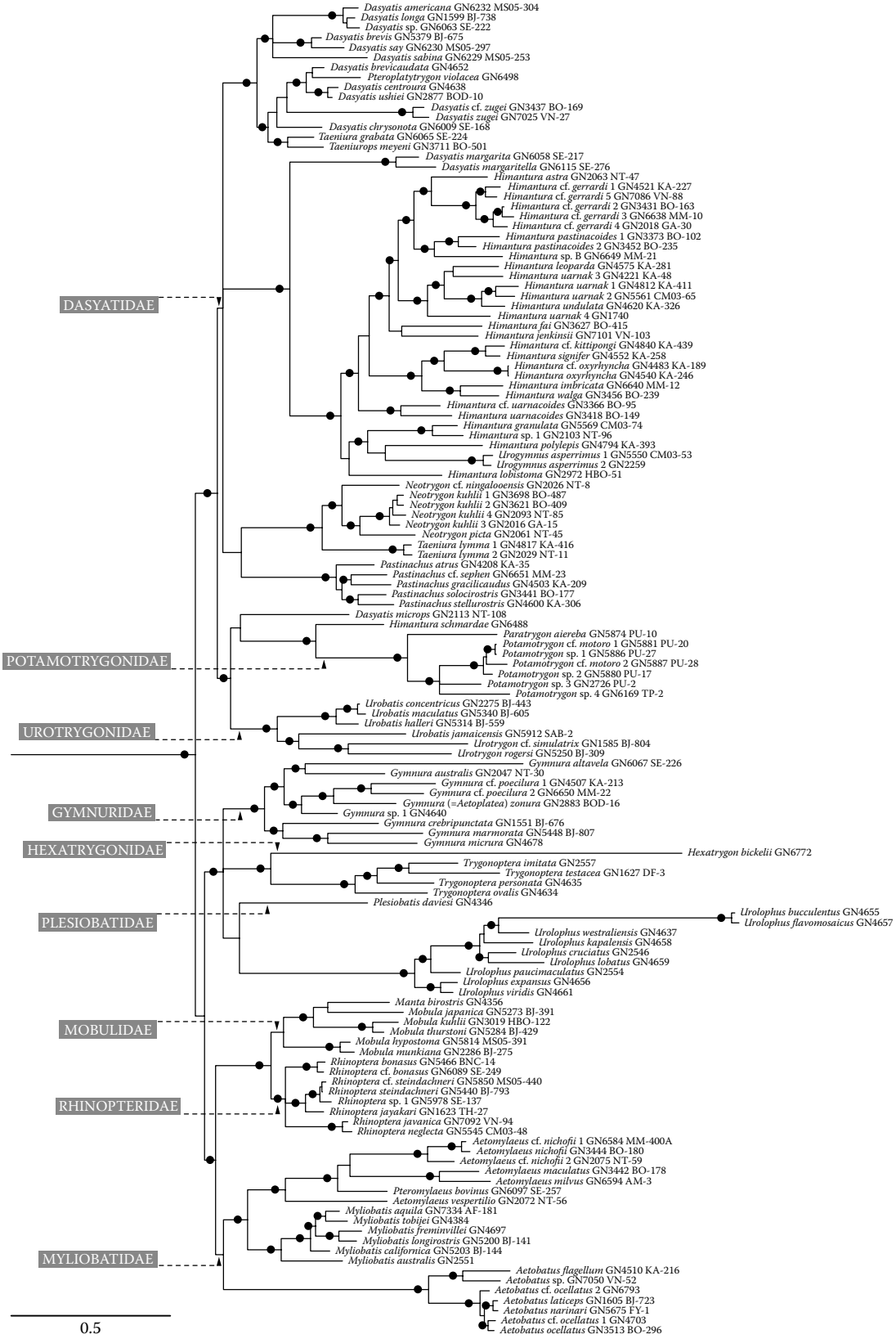

FIGURE 2.11
Hypothesis of the phylogenetic relationships of Myliobatiformes based on NADH2 sequence data (1044 bp) inferred from a Bayesian analysis using a separate GTR+I+Γ model pooled over codon positions. Black dots indicate posterior probabilities of >95%.

Lamniformes, as a group, seem to be especially recalcitrant to phylogenetic analysis. Inferred topologies for this group yield trees with long pendant edges separated by short internodal branches for most taxa, and the topology fluctuates from gene to gene. The order is clearly old and spans considerable morphological diversity, but it contains relatively few species. It is comprised of seven families, four of which are monotypic. Given these patterns of diversification, it is not surprising that conclusive branching patterns are difficult to estimate and that inferences vary across genes and models.

- *Apristurus* (Figure 2.3)—The monophyly of *Apristurus* is potentially compromised by the placement of one of the five species of *Galeus* (*G. sauteri*) and one of the two species of *Parmaturus* (*P. xaniurus*) included in this study as part of a polytomy with the *Apristurus* species. Assuming our specimen identifications are correct, these results call into question not only the monophyly of *Apristurus* but also the monophyly of both *Galeus* and *Parmaturus* as currently circumscribed. Interestingly, a molecular analysis based on 16S rDNA sequences by Iglésias et al. (2005) yielded a result in which *G. sauteri* formed a monophyletic group with the other *Galeus* species in their study. Clearly, further work is needed before any conclusions can be drawn.
- *Bathyraja* and *Rhinoraja* (Figure 2.9)—These two genera are among the most problematic in the current study. All five species of *Rhinoraja* included here were found to be interspersed among the 20 species of *Bathyraja*. This result is consistent with the analysis of Spies et al. (2011), in which the species of *Rhinoraja* nested among the *Bathyraja* species. These results suggest that these two genera are unlikely to be reciprocally monophyletic; however, as noted in Naylor et al. (in press), several of the species in these genera are difficult to identify. Clarification of relationships will require additional samples and careful specimen identity validation.
- *Carcharhinus* (Figure 2.4)—The monophyly of *Carcharhinus* is challenged by the inclusion of the three monotypic genera: *Nasolamia*, *Prionace*, and *Triaenodon*. The relationships among members of the genus *Carcharhinus* and their immediate close relatives are highly unstable, even when based on whole mitochondrial genome analyses (GJPN, unpublished). Accordingly, we do not recommend any taxonomic reassignment until a comprehensive study is undertaken that includes sampling of geographic variants within species across multiple nuclear markers. That said, although the relationships among the different species of *Carcharhinus* are collectively unclear, a consistently close relationship is seen between *Nasolamia* and *Carcharhinus acronotus* across datasets. These relationships were suggested previously by Compagno (1984, 1988), based on morphological data. Similarly, the blue shark, *Prionace glauca*, is almost always inferred to be deeply nested within the genus *Carcharhinus*, and most often allied with the silky shark, *Carcharhinus falciformis*, as is the case in the current analysis. The nesting of *P. glauca* among *Carcharhinus* species has been observed in previous studies (e.g., Compagno, 1988; Dosay-Akbulut, 2008; Naylor, 1992), but in each case with different specific affinities than recovered here. In contrast, the phylogenetic placement of the whitetip reef shark, *Triaenodon obesus*, was unresolved with respect to species in the genus *Carcharhinus*.
- *Centroscymnus* (Figure 2.7)—Although *Centroscymnus owstoni* and *Centroscymnus* sp. 1 (*sensu* Naylor et al., in press) cluster together, *Centroscymnus coelolepis* was placed at the base of a clade including these two species, as well as *Oxynotus*, *Proscymnodon*, and *Scymnodon*. This result is independently supported by nuclear gene data (GJPN, unpublished).
- *Dasyatis* (Figure 2.11)—Our analysis raises several issues with the current composition of *Dasyatis*. First, it seems likely that *Dasyatis microps* does not belong in the genus because it grouped well away from its 14 putative congeners, most closely with *Himantura schmardae* and the potamotrygonids. Second, the species from Senegal (*D. margarita* and *D. margaritella*) appear to be more closely allied with *Himantura* species than with the majority of the *Dasyatis* species; this result is generally consistent with Rosenberger (2001). Third, the monotypic *Pteroplatytrygon* groups among the main group of 14 species of *Dasyatis*; some consideration should be given to whether a unique genus designation is appropriate for *P. violacea*. Perhaps the biggest issue raised by our analysis is that *Taeniura grabata* and *Taeniurops meyeni* group among *Dasyatis* species. This arrangement has been suggested previously by Lovejoy (1996) based on morphological data. It is interesting that the relationships among *Dasyatis* species seen here generally differed from those seen by Rosenberger (2001).

- *Himantura* (Figure 2.11)—There are two issues associated with the monophyly of *Himantura*. First, as noted above, the North American species *H. schmardae* groups well outside of the other *Himantura* species, as the sister to the South American freshwater stingrays as observed by Lovejoy (1996). Second, the two putative *Urogymnus* species (*sensu* Naylor et al., in press) included here fall among the Indo–Pacific *Himantura* species in this analysis. This result suggests that *Himantura* is monophyletic only if it includes the *Urogymnus* species. Interestingly, the squamation pattern at the base of the tail in both *Himantura granulata* and *Urogymnus* are similar, supporting the placement seen in the current analysis (Last, pers. comm.).
- *Leucoraja* (Figure 2.9)—The analysis included 6 of the 14 recognized species of *Leucoraja*. The genus overall was conspicuously paraphyletic in that three of the included species grouped in a strongly supported clade with *Rajella* and *Amblyraja* species, away from the three other *Leucoraja* species.
- *Narcine* (Figure 2.10)—*Narcine lasti* and *Narcine tasmaniensis* group together in this analysis, but the third included species, *Narcine entemedor*, grouped with *Narke capensis*. This result is supported with a strong posterior probability and appears robust across models for this dataset, suggesting that the generic placements among the various species of *Narcine* and *Narke* warrant further exploration with a denser taxon sampling across the 25 currently recognized species of *Narcine*.
- *Parmaturus* (Figure 2.3)—The two species identified as *Parmaturus* (*P. xaniurus* and *Paramaturus* sp.) appear in different parts of the tree. The specimen identified as *P. xaniurus* falls out as the sister taxon to *Galeus sauteri* (see above). This clade, in turn, appears as the sister group to the genus *Apristurus*. The specimen identified as *Parmaturus* sp. is sister to a clade containing *Atelomycterus*, *Aulohalaelurus*, and *Schroederichthys*. Given these patterns, it is possible, indeed likely, that the specimen identified as *Parmaturus* sp. is an as of yet undescribed genus, rather than a different species of *Parmaturus*.
- *Raja* (Figure 2.9)—Our analysis validates much of the comparative anatomical work conducted over the last decade on the substructure within the Rajidae. Many of the proposed new genera are supported as monophyletic in the current molecular analysis. Several issues remain, however. Species recognized in the genus *Raja* by Compango (2005) appear in three different places on the tree. The clade most appropriately considered to represent *Raja*, because it contains the three variants of the type species for the genus (i.e., *Raja miraletus*), also includes *R. clavata*, *R. straeleni*, *R. asterias*, and *R. montagui*; however, other species typically assigned to *Raja* group outside this clade: *Raja binoculata*, *R. rhina*, and *Raja* sp. 1 group with the *Dipturus*, *Zearaja*, *Spiniraja,* and most of the *Okamejei* species. These are assigned to the "new genus 1" by McEachran and Dunn (1998); our results support the recognition of these as a distinct genus. Finally, *Raja eglanteria* and *R. velezi* group together with *Rostroraja alba* and *Okamejei jensenae*. Both *R. eglanteria* and *R. velezi* were assigned to the "new genus 2" by McEachran and Dunn (1998). The results presented herein support the recognition of this grouping as members of *Rostroraja*.
- *Rhinobatos* (Figure 2.10)—Species currently placed in *Rhinobatos* appear at three different points on the tree, suggesting that the genus is not monophyletic as currently configured. The West African *R. cemiculus* is strongly allied with the three included species of *Glaucostegus.* The two species of *Rhinobatos* from the Gulf of California (*R. productus* and *R. glaucostigma*) group robustly together as the sister taxon to a clade consisting of *Rhynchobatus*, *Pristis*, *Anoxypristis*, and *Glaucostegus*, as well as *R. cemiculus*. The remaining five species, which include the type, *R. rhinobatos*, comprise a clade that is sister to the previous clade.

The following observations regarding the monophyly of the higher level groups (i.e., orders and families) are made in the context of the classification of elasmobranchs presented in Compagno (2005).

2.3.1.2 Monophyly of Families

Our analysis included representation of 56 of the 57 families recognized by Compagno (2005), with only the Hypnidae missing from consideration. Ten of these families are monotypic: Cetorhinidae, Hexatrygonidae, Leptochariidae, Megachasmidae, Mitsukurinidae, Plesiobatidae, Pseudocarchariidae, Rhincodontidae, Rhinidae, and Stegostomatidae. An additional ten families are monogeneric: Alopiidae, Chlamydoselachidae, Echinorhinidae, Heterodontidae, Oxynotidae, Rhinopteridae, Rhynchobatidae, Squatinidae, Torpedinidae, and Zanobatidae. Finally, three families (Narcinidae, Parascylliidae, Platyrhinidae), although not monogeneric, were

represented by species belonging to only a single genus in our sampling scheme, providing little to explore with respect to family-level monophyly. The monophyly of each of the 33 families that were represented by two or more genera in our analysis is addressed below.

Our results support the monophyly of 17 of the 33 elasmobranch families for which this issue can be addressed here; these are treated below in alphabetical order. In each case, the relative representation for the sample is given. The monophyletic families are as follows: Arhynchobatidae (43 species representing 10 of 12 genera) (Figures 2.8 and 2.9); Centrophoridae (17 species representing both genera) (Figure 2.7); Dalatiidae (5 species representing 4 of 7 genera) (Figure 2.7); Etmopteridae (29 species representing 4 of 5 genera) (Figure 2.7); Gymnuridae (9 species representing both genera) (Figures 2.8 and 2.11); Hemiscylliidae (7 species representing both genera) (Figure 2.5); Hexanchidae (5 species representing all 3 genera) (Figure 2.7); Lamnidae (all 5 species in all 3 genera represented) (Figure 2.2); Myliobatidae (20 species representing all 4 genera) (Figures 2.8 and 2.11); Orectolobidae (7 species representing 2 of 3 genera) (Figure 2.5); Potamotrygonidae (7 species in 2 of 4 recognized genera, including that of Ishihara and Taniuchi, 1995) (Figures 2.8 and 2.11); Pristiophoridae (3 species representing both genera) (Figure 2.7); Pseudotriakidae (3 species in 2 of 3 genera, including an undescribed species of *Gollum*) (Figure 2.3); Rajidae (60 species representing 11 of 17 families) (Figures 2.8 and 2.9); Sphyrnidae (7 of 8 species in both genera) (Figures 2.3 and 2.4); Squalidae (19 species representing both genera) (Figure 2.7); and Urotrygonidae (6 species representing both genera) (Figures 2.8 and 2.11).

Our results call into question the monophyly of 13 families as currently circumscribed (e.g., Compagno 2005). These are treated below in alphabetical order.

- Anacanthobatidae (Figure 2.9)—Two of the three genera in this family, each represented by a single species, were included in the analysis. Results for the current study fail to provide support for the monophyly of this family. Whereas *Cruriraja hulleyi* grouped as the sister to the Arhynchobatidae, *Sinobatis bulbicauda* grouped as the sister to that larger group. It is possible, however, that the groupings observed here are the consequence of model misspecification associated with long branch taxa. Nonetheless, a similar pattern was seen in the recent work on batoid phylogeny by Aschliman et al. (in press). In their morphological analysis, however, McEachran and Dunn (1998) found the two genera of anacanthobatids to group together, but in a clade comprised essentially of the Rajidae as recovered here.
- Carcharhinidae (Figure 2.4)—All 12 genera of carcharhinids were represented in the analysis. This family was well supported as monophyletic, with the exception of a clade comprising the two putative species of *Galeocerdo* (*sensu* Naylor et al., in press). The *Galeocerdo* clade was grouped as the sister to a clade comprising the Carcharhinidae and Sphyrnidae. Although this node was poorly supported in the current analysis, similar results were obtained by López et al. (2006). Furthermore, independent nuclear gene evidence (GJPN, unpublished) and its unique reproductive biology suggest that *Galeocerdo* does not belong in the Carcharhinidae.
- Dasyatidae (Figure 2.11)—Our analysis included representation of all eight dasyatid genera. The analysis yielded a clade consisting of 61 of the 63 species included in this study. The two exceptions were *Dasyatis microps* and *Himantura schmardae*, both of which grouped outside of the dasyatids, along with the potamotrygonids.
- Ginglymostomatidae (Figure 2.5)—The monophyly of this family was not supported. Our analysis included representation of all four species in the three genera recognized in this family by Compagno (2005). Although both *Ginglymostoma* species (*sensu* Naylor et al., in press) grouped with the monotypic *Nebrius*, the monotypic *Pseudoginglymostoma* grouped in a clade with the monotypic Stegostomatidae and Rhincodontidae.
- Narkidae (Figure 2.10)—The two species representing two of the five genera included in the analysis grouped well away from one another. *Narke capensis* grouped with *Narcine entemedor*, while *Typhlonarke aysoni* grouped, albeit with relatively low support, with the two other species of *Narcine* included in the analysis. These results, although preliminary, suggest that the monophyly of the families of Torpediformes requires further study.
- Odontaspidae (Figure 2.2)—All three species in both genera were included. The analysis suggests that the family is not monophyletic, an observation consistent with previous analyses (Human et al., 2006; Martin and Naylor, 1997; Naylor et al., 1997). Although the two species of *Odontaspis* grouped together, they clustered well away from *Carcharias taurus*, which grouped, with strong support, as the sister to the basking shark.
- Pristidae (Figure 2.10)—The analysis included six of the seven species in both genera. Our results suggest that the family may not be monophyletic

as currently configured. Curiously, although the six species of *Pristis* comprise a robust clade, they do so to the exclusion of the monotypic *Anoxypristis cuspidatus*. The latter species grouped in a clade comprised of three species of *Glaucostegus,* and one of the seven included species of *Rhinobatos* (i.e., *R. cemiculus* from Senegal). These results are surprising and at odds with the work of Faria (unpublished doctoral dissertation), who found the Pristidae to be strongly monophyletic but who notably did not include any specimens of *Rhinobatos* in his study. The NADH2 results are also at odds with an analysis based on combined nuclear and mitochondrial genes (Aschliman, pers. comm.). Clearly, these relationships require further work before firm conclusions can be drawn.

- Proscylliidae (Figure 2.3)—The two included species, representing two of the three genera, did not form a monophyletic group in the current analysis. *Proscyllium habereri* (Proscylliidae I) was placed at the base of one of the three clades of scyliorhinids (Scyliorhinidae I), whereas the specimen identified as *Eridacnis* sp. 1 (Proscyliidae II) grouped as sister to the clade consisting of the Pseudotriakidae; however, the placement of neither species is strongly supported. In contrast, independent data from the more slowly evolving RAG1 gene places these taxa together, thus rendering the Proscyllidae a monophyletic group (GJPN, unpublished).
- Rhinobatidae (Figure 2.10)—Four of the five genera (including the newly resurrected *Glaucostegus*) were represented by a total of 15 species in the analysis. Our results suggest that this group is monophyletic only if it also includes the Pristidae, Rhinidae, Rhynchobatidae, and Zanobatidae; however, the inclusion of *Zanobatus* in the group was found to be model dependent for the current dataset.
- Scyliorhinidae (Figure 2.3)—The analysis included 55 species representing 15 of the 17 families of catsharks. Our results indicate that the family is not monophyletic; rather, there exist three distinct, paraphyletic lineages, two of which are relatively well supported. The first, and largest, group (Scyliorhinidae I) consists of *Apristurus*, *Galeus*, *Asymbolus*, *Figaro*, *Bythaelurus*, *Halaelurus*, *Haploblepharus*, and *Holohalaelurus* species, and *Parmaturus xaniurus*; the second group (Scyliorhinidae II) consists of *Atelomycterus*, *Aulohalaelurus*, and *Schroederichthys* and *Parmaturus* species; and the third group (Scyliorhinidae III), consists of *Cephaloscyllium*, *Poroderma*, and *Scyliorhinus* species. The primary issue is that the Proscylliidae, Carcharhinidae, Sphyrnidae, Hemigaleidae, Leptochariidae, Triakidae, and Pseudotriakidae are interspersed among these three catshark clades, thereby compromising their collective monophyly. The non-monophyly of the Scyliorhinidae has been previously documented by Iglésias et al. (2005) and Human et al. (2006), although in both cases based on a much less representative sample of taxa.
- Somniosidae (Figure 2.7)—The ten species in this family included in the current study represent six of the seven genera. Our analysis suggests that the family is monophyletic only if the Oxynotidae are included. This result appears to be robust; the two species of *Oxynotus* included grouped deeply within the Somniosidae. These results corroborate the similar placement for *Oxynotus* seen in the recent work by Straube et al. (2010) using a different suite of molecular markers.
- Triakidae (Figure 2.3)—A total of 34 species, representing eight of the nine triakid genera, were included in this study. Analysis indicates that all but *Iago* form a monophyletic group, albeit with weak support. The four putative species of *Iago* grouped as part of a larger polytomy that also contained the Hemigaleidae, Carcharhinidae, and Sphyrnidae, as well as several monotypic families and problematic groups treated above. These results are consistent with the patterns observed by López et al. (2006).
- Urolophidae (Figure 2.11)—The current analysis, based on 13 species representing both genera of this family, failed to support the monophyly of this family. Not only did the species in its two constituent genera not group with one another, but also the clade containing the *Trygonoptera* species was placed as the sister group to *Hexatrygon bickelii*, and the clade of *Urolophus* species grouped as sister to *Plesiobatis daviesi*, albeit with relatively weak support. Further work with nuclear markers will be required before any firm conclusions can be drawn.

2.3.1.3 Monophyly of Orders

All but one of the nine orders of elasmobranchs recognized by Compagno (2005) were strongly supported as monophyletic (Figure 2.1). The exception was the Squaliformes, the monophyly of which was compromised by the placement of the three species of

Echinorhinus (family Echinorhinidae) as the sister taxon to a clade consisting of the Pristiophoriformes plus the Squatiniformes (Figures 2.1 and 2.7). Although support for these groupings was weak, generally similar results were obtained based on the nuclear gene RAG1 by Maisey et al. (2004). These findings are provocative and warrant further exploration with multiple nuclear genes.

Although Compagno (2005) recognized only the single batoid order Rajiformes, our results indicate that the subdivision of the batoids into several orders is warranted. McEachran and Aschliman (2004) and Nelson (2006) recognized the four batoid orders Torpediniformes, Pristiformes, Rajiformes, and Myliobatiformes; Compagno (1999) recognized the Rhiniformes and Rhinobatiformes in addition to these four orders. The constituencies of these orders differed somewhat among the classification schemes presented by these authors; our results support the following blend of their scenarios. Regardless of the circumscription of its families, all three sets of authors recognized the electric rays (Torpediniformes); our results strongly support the monophyly of this order (Figure 2.8). Our analysis included one of the three known species of platyrhinids. This species grouped as the sister taxon to the electric rays. This is an interesting result in light of the fact that the Platyrhinidae was considered to belong among the Rhinobatiformes by Compagno (1999) and among the Myliobatiformes by McEachran and Aschliman (2004) and Nelson (2006). Similarly, regardless of family-level organization, all three sets of authors recognized an order comprised of the skates (i.e., Rajiformes *sensu stricto*); our results provide support for the monophyly of this order (Figure 2.8). Family-level organization aside, the concept of the Myliobatiformes was also remarkably consistent among these authors. At a minimum, the order was considered to include the Dasyatidae, Gymnuridae, Hexatrygonidae, Mobulidae, Myliobatidae, Potamotrygonidae, Rhinopteridae, Urolophidae, and the Urotrygonidae in all three studies. Differences among these studies stem in part from the fact that the Plesiobatidae was included in the order by Compagno (1999) and Nelson (2006) but was not represented in the analysis of McEachran and Aschliman (2004); also, whereas McEachran and Aschliman (2004) and Nelson (2006) considered the Platyrhinidae and Zanobatidae to belong to the Myliobatiformes, Compagno (1999) considered these families to belong to the Rhinobatiformes. Our results provide robust support for the Myliobatiformes as circumscribed by Compagno (1999).

The greatest differences between our results and the classification schemes of these previous authors center around the guitarfish (Rhinobatidae), wedgefish (Rhynchobatidae), sharkrays (Rhinidae), and sawfish (Pristidae). In all three previous studies, the Pristidae was placed in its own order, the Pristiformes. Compagno (1999) also recognized the order Rhiniformes for the monotypic Rhinidae. McEachran and Aschliman (2004) and Nelson (2006) placed the guitarfish, wedgefish, and sharkrays in the Rajiformes along with the skates. But, Compagno (1999) placed the guitarfish and wedgefish in their own order, the Rhinobatiformes (along with the Platyrhinidae and Zanobatidae). Our results suggest a slightly different scenario; there may be some merit to the recognition of an order consisting of the Pristidae, Rhinidae, Rhynchobatidae, Rhinobatidae, and Zanobatidae. The results of the recent analysis of Aschliman et al. (in press) are generally consistent with the latter grouping, except that the group did not include *Zapteryx*, *Trygonorrhina*, or *Zanobatus* species. To our knowledge, no name for the group comprised of the above five families of batoids currently exists. We propose that the name Rhinopristiformes be considered. With respect to the ordinal placement of the Platyrhinidae, our analysis supports the scheme of Compagno (2005) in recognizing this family in its own higher taxonomic category, perhaps at the ordinal level.

2.3.1.4 Interrelationships among Orders

Our results do not support the Hypnosqualea concept of Shirai (1992, 1996), endorsed by de Carvalho (1996); rather, the reciprocal monophyly of sharks (i.e., Selachii) and of the batoids (i.e., Batoidea) was recovered. This result is consistent with the findings of many previous authors working with a diversity of data types (e.g., Arnason et al., 2001; Douady et al., 2003; Human et al., 2006; Maisey et al., 2004; Naylor et al., 2005; Schwartz and Maddock, 2002; Winchell et al., 2004).

Within the Selachii, our results support division of the group into two major subgroups (Figure 2.1). There is support for a subgroup consisting of the four orders Carcharhiniformes, Lamniformes, Orectolobiformes, and Heterodontiformes (Maisey, 1984b). This subgroup is the Galea of Shirai (1992, 1996) and the Galeomorphi of Compagno (2001), reiterated in Nelson (2006); we have followed Compagno (2001) here in referring to this clade as the Galeomorphi. Our results also support the hypothesis of the interrelationships among these four orders obtained by Shirai (1992) and Naylor et al. (2005) in suggesting that the Heterodontiformes (Figure 2.6) are the immediate sister to a group consisting of the Orectolobiformes and the Lamniformes + Carcharhiniformes. These were not, however, the relationships obtained by Douady et al. (2003), Winchell et al. (2004), or Vélez-Zuazo and Agnarsson (2011), each of whom recovered relationships among these orders that differed from our result and also from each other.

The second major subgroup of Selachii recovered in our analysis, albeit with less support, is a clade consisting of the orders Squaliformes, Squatiniformes,

Pristiophoriformes, and Hexanchiformes. This group is the Squalomorphii of Nelson (2006) and is consistent with the concept of the orbitostylic sharks first forwarded by Maisey (1980), based on the type of articulation between the jaw and the brain case. Although the proposed interrelationships among these orders have differed among studies (e.g., de Carvalho, 1996; Shirai, 1996), our results support a clade consisting of the Squaliformes, Pristiophoriformes, and Squatiniformes, to the exclusion of the Hexanchiformes. However, the interrelationships among the Echinorhinidae, Pristiophoriformes, and Squatiniformes remain unclear given the weak support in the current dataset.

We are hesitant to make direct comparisons with the results of Vélez-Zuazo and Agnarsson's (2011) analysis of 229 species of sharks. In a number of respects their results are incongruent with current generic and familial level taxonomy, but they are also incongruent in many respects with ours. As noted by these authors, their analysis suffers from a substantial amount of missing data. Furthermore, the critical step of specimen identity verification was not taken because their data came directly from GenBank. As a consequence, it is unclear which differences to attribute to which issue.

The proposed relationships among the four major lineages of batoids recognized above (i.e., Rajiformes, Torpediniformes [+ Platyrhinidae], Rhinopristiformes, and Myliobatiformes) (Figure 2.8) have also differed among various studies. McEachran et al. (1996) and Rocco et al. (2007) found the Torpediniformes to be basal to a group consisting of the Myliobatiformes and essentially the Rhinopristiformes + Rajiformes *sensu stricto*. Alternatively, Shirai (1996) considered the Rhinopristiformes to be basal to a group consisting of the Torpediniformes and the Rajiformes *sensu stricto* + Myliobatiformes. The current results contribute little to our understanding of these interordinal relationships, for these relationships are unresolved in our analysis.

2.4 Discussion

We have presented a phylogenetic tree depicting inferred relationships among most of the major groups of elasmobranchs. Because the dataset is itself a subset of an even more comprehensive sample of over 4200 specimens that included replicates of most species (Naylor et al., in press), we feel that the results from this survey should provide a good baseline against which to compare subsequent studies. Nonetheless, it is important to remember that the results we present were derived from a single, fast-evolving mitochondrial gene and thus should be regarded as only the first tentative step toward understanding phylogenetic relationships. Our inference will, at best, constitute a single locus gene tree that may or may not reflect species-level relationships at different levels in the hierarchy due to population-level processes differentially affecting patterns of lineage coalescence. At worst, it could be an inaccurate gene tree, depending on how well the model used for inference captures the dynamics of the sequence evolution among the sequences used.

The current dataset was certainly not expected to yield an accurate phylogenetic signal because NADH2 is one of the fastest evolving protein-coding genes in the mitochondrial genome and is thus especially questionable for assessing relationships among deeply diverged groups. However, most of the deep-level relationships retrieved are surprisingly concordant with the current classification. We speculate that this is due to the relative absence of missing data and the density and evenness of the taxon-sampling scheme used. Lakner et al. (2011) have shown explicitly that model inadequacies can be ameliorated by balanced and judicious taxon sampling. Interestingly, convergence occurred much more quickly for our 607 specimen NADH2 dataset than it did for the 229 shark species sequence dataset of Vélez-Zuazo and Agnarsson (2011) which we ran again in an effort to estimate the phylogenetic signal in that dataset (because the published analysis was based on an analysis that had not converged) (Agnarsson, pers. comm.). It would appear that the complexity and shape of the posterior distribution for the larger NADH2 dataset were much less difficult to approximate than was the case for the smaller Vélez-Zuazo and Agnarsson (2011) dataset. We speculate that this may be due to the differences in the amount of missing data in the two datasets—Vélez-Zuazo and Agnarsson (2011) dataset, 85% missing data; current NADH2 dataset, <0.25% missing data.

Although model inadequacies may sometimes be ameliorated by balanced and dense taxon sampling schemes (*sensu* Lakner et al., 2011), such strategies can only be deployed in instances in which taxa actually exist to break up long branches that have accrued extensive evolutionary change. For some groups, the required taxa are simply not available. The lamniform sharks, for example, comprise a sparse collection of highly divergent long branch taxa. Thus, sampling all of the available extant forms in this order does not ameliorate the difficulties associated with estimating an accurate phylogeny. In such instances, it is necessary to use a battery of slowly evolving independent markers in conjunction with carefully parameterized inference models to obtain accurate results, and even under such circumstances accuracy is never guaranteed. These cautions notwithstanding, the taxon sampling in the current study is reasonably good, at least to the extent that the diversity recognized by the current taxonomy is represented.

Finally, it is important to remember that there will likely be a few groups whose phylogenetic relationships will be recalcitrant to analysis even under the best-case scenario (i.e., complete taxon sampling and examination of multiple independent markers). This is because certain patterns of diversification and extinction tend not to leave unambiguous hierarchical traces in DNA sequences or morphological character state distributions. Rapid diversification of multiple lineages over a short period of time followed by subsequent lineage pruning due to extinction can often lead to situations in which ancestral polymorphism in slowly evolving markers is fixed in such a way as to be phylogenetically misleading. Such scenarios can lead to the situation where fast evolving markers are "saturated" and rendered uninformative because they have been overprinted with substitutions while slowly evolving markers leave a clear but misleading pattern of allele fixation.

2.4.1 Closing Remarks

In a number of instances, the results of our analysis call into question the monophyly of recognized genera and families, and even orders, as currently circumscribed. Some of the groupings suggested by the current analysis are clearly questionable as they conflict both with morphological data and assessments based on other genes. Most conspicuously, these include, but are not limited to, the non-monophyly of the following families: Pristidae, Alopiidae, Narkidae, Proscylliidae, and Urolophidae, as well as the non-monophyly of the following genera: *Halaelurus*, *Hexanchus*, and *Urobatis*, and the placement of *Eusphyra* within *Sphyrna*. In such cases, we have described the taxonomic implications revealed by our results, but these discussions are presented solely to inform future taxonomic work. They should not be interpreted as formal taxonomic actions. Instead, we hope they serve to encourage further exploration based on additional nuclear genes and a more thorough sampling of the taxa that were underrepresented in the current study, as well as the morphological work required to fully assess the taxonomic implications of our results.

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