# How many species of living sharks, skates and rays are there, and how did they arise over the course of evolution?

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## Abstract

There are more than 27,000 described species of bony fishes but only about 1,100 named elasmobranchs (including 420+ sharks and 650+ skates and rays). Several hypotheses have been forwarded to explain why there are so many more kinds of bony fishes than elasmobranchs. Some of the more plausible hypotheses are based on the fact that teleostean ray-finned fishes experienced whole genome duplication early in their evolution. It is argued that these whole genome duplications provided the necessary raw material for evolutionary experimentation and biodiversification in bony fishes, whereas no such opportunity presented itself for elasmobranchs. However, recent work based on DNA sequence comparisons is uncovering new sharks and rays that were previously not thought to be distinct species. Thus at least some of the apparent lack of diversity in elasmobranchs may be a consequence of our inability to recognize physically similar - but in fact different - species, due to the morphologically conservative evolution of the group. In the current work we present a DNA sequence survey across elasmobranchs. We show three distinct applications for DNA sequence comparisons. First, we show that relatively fast-evolving mitochondrial gene sequences can be useful for verifying species identities in the field. Second, we show that these same sequences can reveal geographic sub-structure and evolutionary diversity that has previously gone undetected. Finally, we show how carefully selected gene sequences can be paired with carefully tailored analyses to estimate the evolutionary relationships among distantly related groups of elasmobranchs. This last application can be particularly challenging, as different genes frequently suggest different evolutionary relationships depending on the method of analysis used.

## Introduction

Humans have long been fascinated by sharks and rays. The fascination, fueled by a mixture of curiosity and fear, probably stems from the fact that these ancient animals live in an environment in which humans are poorly adapted and in which they are occasionally attacked. Because few people experience direct interactions with these animals, the accounts of those that do tend to be cherished, recycled, embellished and exaggerated. The number of popular books and film documentaries that repackage old information about sharks with a new “spin” attests to a seemingly insatiable human interest in these animals.

With the cultural shift toward environmentalism, public opinion about sharks has been changing. Popular culture now emphasizes that these animals are not a marine menace, but are instead stewards of the environment that play a critical role at the apex of marine food chains. The public is told in plaintive tones that sharks are misunderstood and that there is a need to study them because fisheries are depleting their numbers to such an extent that there will likely be dire consequences for marine ecosystems. As a result, a wave of instant “shark researchers” has sprung up all over the world. Most seem to be associated with tourist diving operations using protective cages. These businesses often cater to a sense of adventure while offering clients the impression that they are contributing to a scientific understanding of these animals. Unfortunately, despite considerable press coverage and shifting public awareness, this has done little to promote much needed basic research on chondrichthyan diversity and evolution. In spite of the fact that much of the general public is fascinated with “Shark Week,” we actually know surprisingly little about the basic biology of these animals. We don’t know how many extant species exist (current estimates suggest between 1200-1500 [Compagno et al., 2005; P.R. Last, pers. comm.]) .We don’t know the geographic ranges of most of the described species. We know little about their life histories and almost nothing of their evolutionary inter-relationships.

## Background

Sharks and rays collectively comprise Elasmobranchii or “plate-gilled” fishes. Together with their archaic relatives the chimaeras, they constitute the Chondrichthyes, or cartilaginous fishes. While chondrichthyans, in the broad sense, have a fossil record that extends back at least 400 million years to the early Devonian, modern sharks and rays did not arise until much later in the Late Triassic or Early Jurassic, about 200 million years ago (Maisey, 1984; Maisey et al., 2004). Today there are about 1,100 named species of living sharks and rays (Compagno et al., 2005). By contrast there are 27,000 described species of living bony fishes (Nelson, 2006). Why there are so many more species of bony fishes than elasmobranchs is not immediately clear, but several hypotheses have been forwarded to explain the differences. These range from the intrinsic differences in size (the average elasmobranch is generally much larger than the average bony fish) to differences in fecundity (sharks generally have fewer offspring than do bony fishes) and differences in generation time (sharks have much longer generation times than most bony fishes). More recently scientists have been drawn toward explanations that are based on genetic architecture. Comparative genomic studies indicate that bony fishes have had at least one complete genome duplication that was not shared by their chondrichthyan counterparts. The argument is made that the extra genetic material provided by this whole genome duplication may have provided more raw material for evolutionary experimentation in bony fishes than in chondrichthyans (reviewed by Donoghue & Purnell, 2005).

While a genome duplication would “free up” components of an organism’s genetic architecture to explore new configurations, the associated constraint release would also foster faster rates of evolution in the component parts of the architecture. Such speed-ups would cause bony fishes to diverge away from their ancestral condition at a faster rate than chondrichthyans. This has important implications for comparative vertebrate biology. If we are interested in identifying the attributes that made vertebrates so successful, it is important to identify the ancestral vertebrate condition. This is much more easily done when there has been little evolutionary change from the starting point. Because elasmobranchs have not undergone the accelerated rates of evolution that are seen in the bony fishes, they are likely much closer to those of the ancestral vertebrate condition. Indeed recent work by Venkatesh's group has shown that the elephant shark genome is architecturally more similar to that of mammals than is the genome of bony fishes, even though bony fishes are more closely related to mammals (Venkatesh et al., 2007). It is likely that chondrichthyans will play an increasingly important role in comparative genomic studies in the near future.

## Diversity within elasmobranchs and the use of sequence comparisons

Elasmobranchs are not merely ciphers to shed light on vertebrate origins. They have attributes that make them useful for understanding multiple aspects of the evolutionary process. Because they are a relatively old group, we can use them as to explore the effects of plate tectonics on the distribution of marine organisms (e.g. Long, 1994); because they have a relatively dense fossil record we can use them to measure rates of molecular and morphological evolution; and because they exhibit such tremendous variation in life history traits we can use them to explore life history trait evolution (e.g. Musick & Ellis, 2005).

Somewhat surprisingly, there has been relatively little interest in understanding relationships within elasmobranchs, until Drs. Nakaya and Compagno brought attention to their diversity in the 1970's (Compagno 1973, 1977). In our own work, inspired by the work of Drs. Nakaya and Compagno, we have set out to explore the diversity and evolutionary relationships among the lineages of elasmobranchs guided by patterns seen in DNA sequence data. Over the past 25 years, we have amassed more than 5000 tissue samples from approximately 400 different species of elasmobranchs. We have employed DNA sequences as markers in multiple contexts. At a very basic and practical level we have used the fast evolving mitochondrial sequence ND2 to validate our field identifications, something that is especially important when dealing with morphologically conservative groups such as elasmobranchs. Second, we have used the very same ND2 sequences as markers to shed light on population substructure within species. Third, we have used a combination of single copy genes from both the nuclear (RAG1) and mitochondrial (cytochrome B, ND2 and ND4) genomes to estimate the phylogenetic relationships among the major groups of extant elasmobranchs. Our phylogenetic work has focused on representation at the generic level. We have taken steps to ensure a balanced sampling of taxa to represent all of the major morphological groups. Finally, because the current symposium is focused on understanding reproduction in aquatic animals, we have taken this opportunity to use the phylogenetic tree estimated from both nuclear and mitochondrial markers to shed light on the evolution of different modes of reproduction in elasmobranchs.

In what follows, we present empirical examples employing sequence comparisons in the three levels of analysis to give a sense of the breadth of application and utility of comparative sequence data.

## Field ID validation

In a recent survey of the elamobranch diversity, we sequenced every animal in our tissue database for the fast-evolving mitochondrial gene ND2. The sequences were aligned and subjected to Neighbor Joining analysis. In several instances, we quickly discovered that animals identified as one species in the field were genetically identical to specimens that were originally identified as different species. In Figure 1 we present one such example in which an animal identified as Dasyatis was actually a specimen of Himantura gerrardi. In some scientific circles, it is common practice to base assessments from a single individual to represent a species. If a mistake is made in identifying the species, then the reported results may be invalid. Inaccurate species identification likely impacts many online databases such as GenBank, because there are no species validation requirements that must be met before submitting sequencing information. People using these databases should therefore be cautious about drawing strong conclusions unless they have independent means of validating their claims.

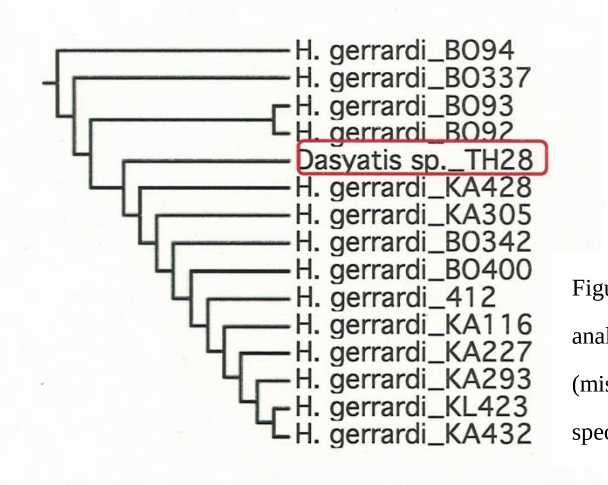

Figure 1. Example showing how neighbor-joining analysis of aligned ND2 sequences reveals that a specimen (mis) identified in the field as *Dasyatis* sp. is actually a specimen of *Himatura gerardi*.

### Geographic structure of populations

When subjecting mitochondrial DNA sequence data to Neighbor Joining analysis we observed several instances in which distinct geographic substructure is evident within species. In some instances the divergence between populations is larger than the divergence seen between recognized, distinct species, suggesting that revision to the current taxonomy may be warranted.

For the purposes of this contribution we bring attention to geographic substructuring seen in three different species of sharks: the black tip shark Carcharhinus limbatus, the Sand-bar shark Carcharhinus plumbeus, and the silky shark Carcharhinus falciformis. In all three cases there is a clear distinction between populations from the Indo-West Pacific and from the North Atlantic (Figure 2). It is intriguing that in the case of C. limbatus, the Indo-West Pacific population appears more closely related to a different species (the graceful shark Carcharhinus amblyrhnchoides) than it does to the North Atlantic population of C. limbatus. Similarly, the Indo-West Pacific population of C. plumbeus appears more similar to the big-nose shark (Carcharhinus altimus) than it does to North Atlantic C. plumbeus. This may indicate that the Indo-West Pacific populations of both C. plumbeus and C. limbatus are different species from their North Atlantic counterparts. However, this needs to be confirmed with independent nuclear markers as it is also possible that it is a reflection of lineage sorting of ancestral haplotypes.

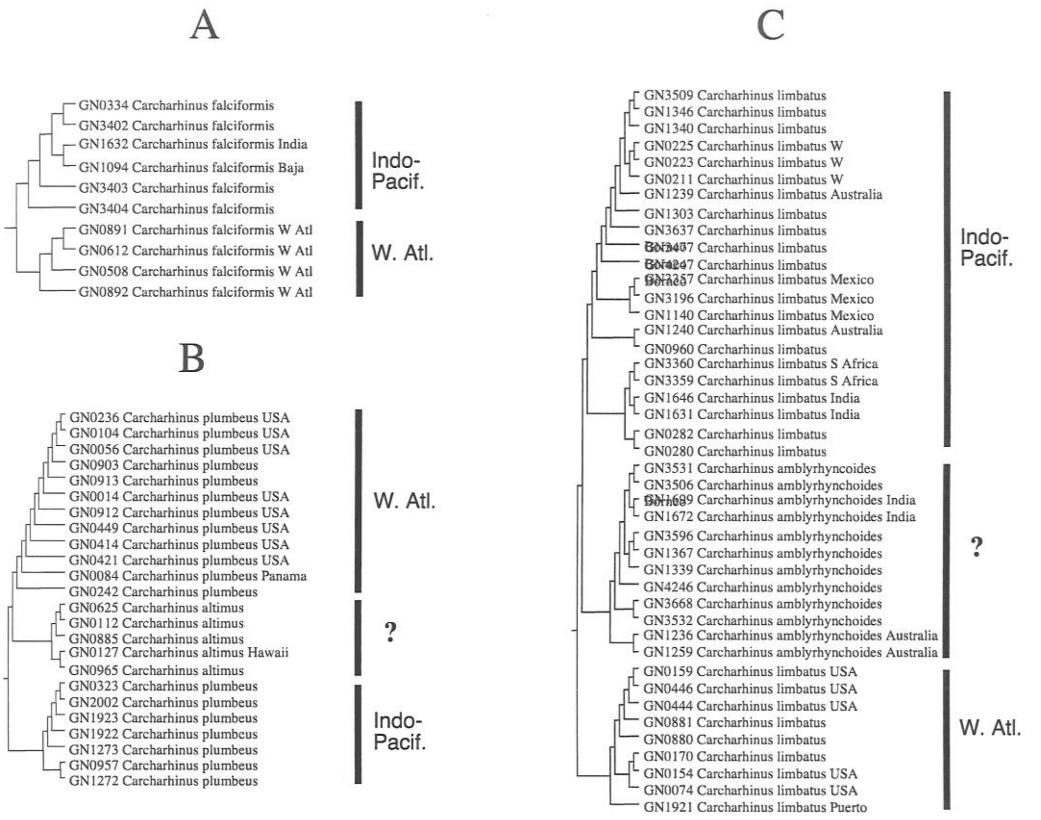

Figure 2. Neighbor-joining trees depicting within-species relationships for three different species (*C. falciformis*, *C. limbatus* and *C plumbeus*). In all three cases there is a clear distinction between North Atlantic and Indo-Pacific populations. In the case of *C. limbatus* it appears that the Indo-Pacific population of *C. limbatus* is more closely related to the graceful shark *C. amblyrhynchoides* than it is to the North Atlantic population of *C. limbatus*. In a similar vein the Western Atlantic population of *C. plumbeus* appears closer to the Big-nose shark *C. altimus* than it does to the Indo-Pacific population of *C. plumbeus*.

## Phylogeny

While clustering fast-evolving mitochondrial sequences with Neighbor Joining can be useful for assessing sequence similarity among closely related organisms, the efficacy of the approach diminishes for comparisons among more distant taxa. This is partly because multiple hits in fast evolving sequences erase phylogenetic signal, and partly because of shortcomings intrinsic to applying distance-based approaches to phylogenetic estmation. In order to estimate phylogeny, we need to collect data with rates of change better suited to estimating deeper divergences. Furthermore, steps must be taken to ensure that the comparisons made are among orthologous rather than paralogous copies of the target marker gene. In general this is done by amplifying a gene that is only present in a single copy among the organisms being compared. Finally, when attempting to estimate phylogenetic relationships among distantly related taxa, it is important that the sampling of taxa across the evolutionary process be as even as possible.

For the tree that we present in this symposium (Figure 3), we used the mitochondrial genes ND2, cytB and ND4, and the single-copy nuclear gene RAG1. Our analyses were carried out at the amino acid level (as opposed to the nucleotide level) using a mixture model in a Bayesian framework (MRBAYES: Ronquist & Huelsenbeck, 2003).

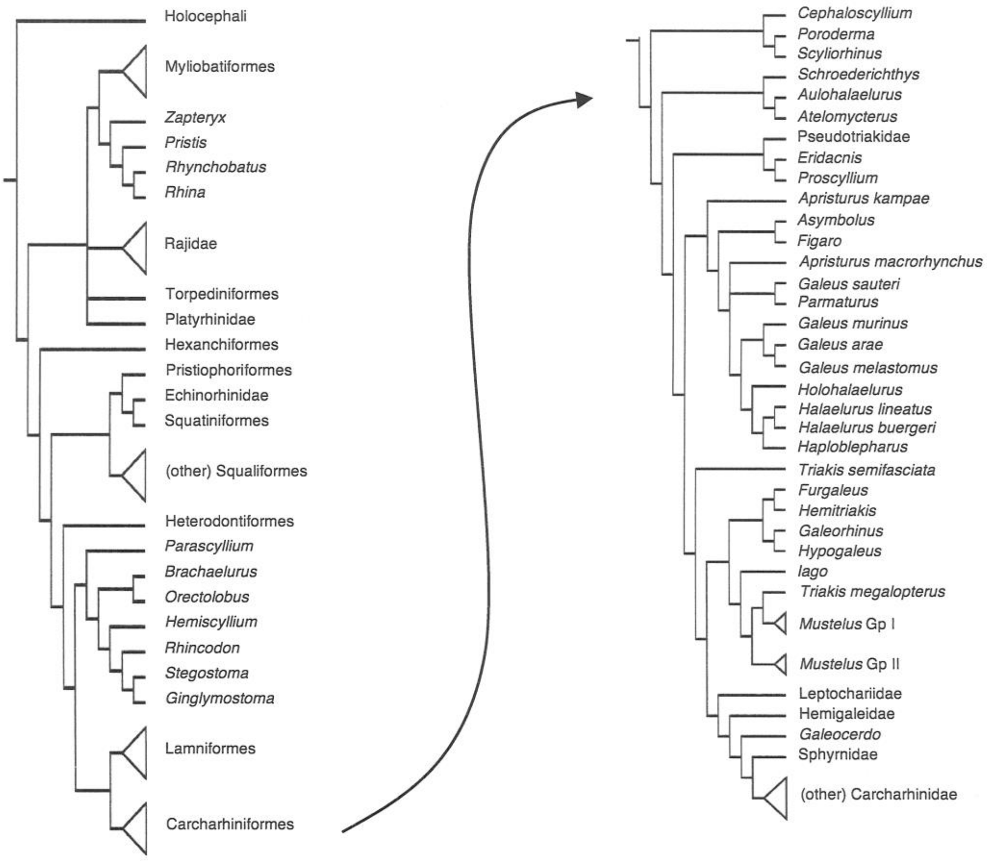

Figure 3. Estimated tree of relationships among the major groups of elasmobranchs based on Bayesian analysis of mitochondrial and nuclear protein coding sequence data.

**Using phylogenies to understand trait evolution and make predictions.**

We have used the phylogeny shown in figure 3 as a framework on which to map the distribution of reproductive traits in elasmobranchs. We assigned states for one of five modes of reproduction to each taxon, based on the coding scheme of Dulvy and Reynolds (1997). The categories were (1) oviparity; (2) yolk sac ovoviviparity; (3) ovoviviparity with maternal input through histotroph; (4) oophagy/cannibalism; (5) placental viviparity.

When plotted onto the tree it is possible to deduce the number of times different strategies arose over the course of evolution. Several things are striking from the distribution shown in Figure 4. However herein we focus on two that are especially revealing. The first is that there are both ovoviviparous and species with placental viviparity within the genus *Mustelus*. This suggests that changing strategies over the course of evolution is not especially difficult, which can also be deduced from the fact that there have clearly been numerous changes in strategy over the entire tree. The second observation is controversial. *Pristis* is the sole member of a clade of organisms that is reported in the literature to exhibit yolk sac only ovoviparity. Except for the oviparous skates, all of its immediate relatives (the rays) employ maternal nutrition in the form of histotroph to nourish their young. This can be in the form of lipid-rich histotroph exhibited by stingrays, or limited histotrophy suspected in most other batoid fishes. The embryos of some species of *Pristis* are born at greater than a meter in length. It would seem highly unlikely that an animal of this size could be supported by yolk reserves alone. We conjecture that this is an error in the literature and that pristids, like the closely related rays, employ a form of maternal supplementing of the yolk sac reserves. This is a prediction based on the phylogenetic position of *Pristis* in the tree, but one that needs to be checked empirically.

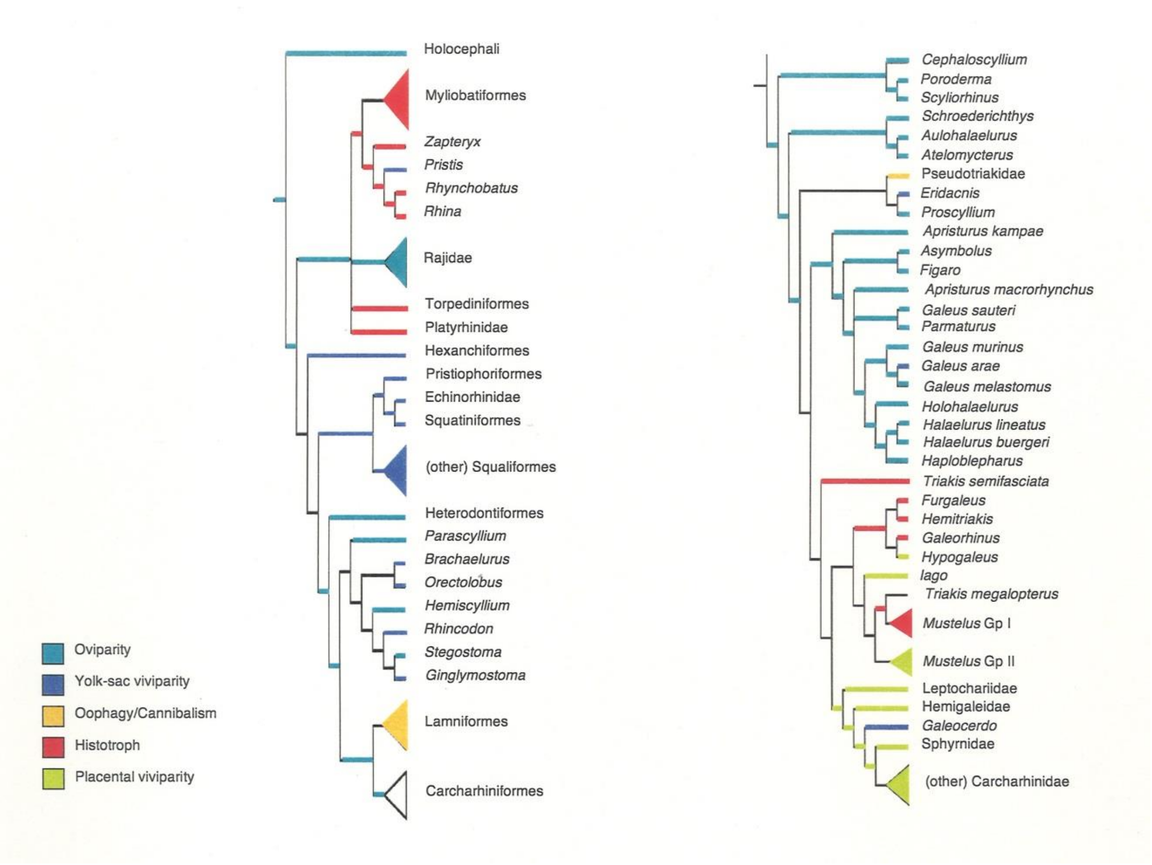

Figure 4. Different modes of reproduction, taken from Dulvy and Reynolds (1997) plotted on to the phylogenetic tree of relationships seen in Fig. 3.

## Conclusions

While there is considerable diversity within modern elasmobranchs, much of it remains poorly known. We are attempting to characterize extant diversity using molecular techniques and hope to provide a rigorous evolutionary framework that subsequent researchers can use to explore character evolution within the group. We hope that our documenting the extant diversity in the group will catalyze much needed further exploration .

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