

# DNA Barcoding Results for Some Southern African Elephantfish, Guitarfish, Rattails, Rays, Sharks and Skates

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## Short Communication

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## Abstract

Sharks and their fins have been targeted increasingly. Fishermen can sell fins for a much higher price than they are able to sell shark meat; shark fin soup is an expensive delicacy. This results in a wasteful and inhumane practice of finning (fins are cut off sharks that are often still alive). Countries such as South Africa have banned finning. This study of two out group species (bony fishes), species from the subclasses Elasmobranchii (rays, sharks and skates) and Holocephali (elephantfish and rattails) indicate the individuals' phylogenetic relationships for the first time. Twenty species from 17 genera and 13 families were analysed and all the individuals have never been DNA barcoded in the HVDBE project. It reveals most of the groups of fishes to be monophyletic (as also reported in previous studies, but for different individuals and mostly other species). However, the Elasmobranchii are polyphyletic. Average K2P intraspecies distances between the fishes range from zero to 14.65%. The focus of this study is on the correct identification of the individuals, contributing to the DNA sequence library for use in species identification; it can be used for law enforcement (i.e. even for individuals lacking diagnostic body parts).

**Keywords:** Cytochrome oxidase 1 gene; Divergence; Chondrichthyes; Genetic; Mitochondrial data

**Abbreviations:** MP: Maximum Parsimony; CCDB: Canadian Centre for DNA Barcoding; K2P: Kimura 2-parameter; TBR: Tree Bisection Reconnection.

## Introduction

The Chondrichthyes (cartilaginous fishes) comprising chimaeras, rays, sharks, and skates are the oldest living group of jawed vertebrates that diverged in the early

Silurian about 420 Ma from a common ancestor of bony fishes (Osteichthyes: coelacanths, lung-, ray-finned fishes and tetrapods) [1,2]. Representatives from all 13 orders of cartilaginous fishes (with 49 families and 111 genera) are included in the southern African chondrichthyofauna [3]. "Approximately 204 species occur in southern Africa, representing 20% of all known chondrichthyans with 117 shark, 79 batoid and 8 chimaera species and 13% of those endemic to the region" [3]. The Holocephali share many

features with the Elasmobranchii, but elephantfish have only a single gill opening on each side as opposed to 5-7 gill slits in rays and sharks (without gill covers), and four gill slits in rattails (with gill covers). The floppy proboscis on its snout is characteristic for elephantfish [4]. DNA barcoding is the sequencing of a region of the mitochondrial cytochrome c oxidase 1 gene (CO1). It has many advantages including life stage, product and species identification, and even system level phylogeographic resolution, with very few disadvantages [e.g. see reviews in 5-15]. The aim of this study is to compile a reference library for the identification of species (e.g. from body parts such as shark fins confiscated in the illegal trade).

## Material and Methods

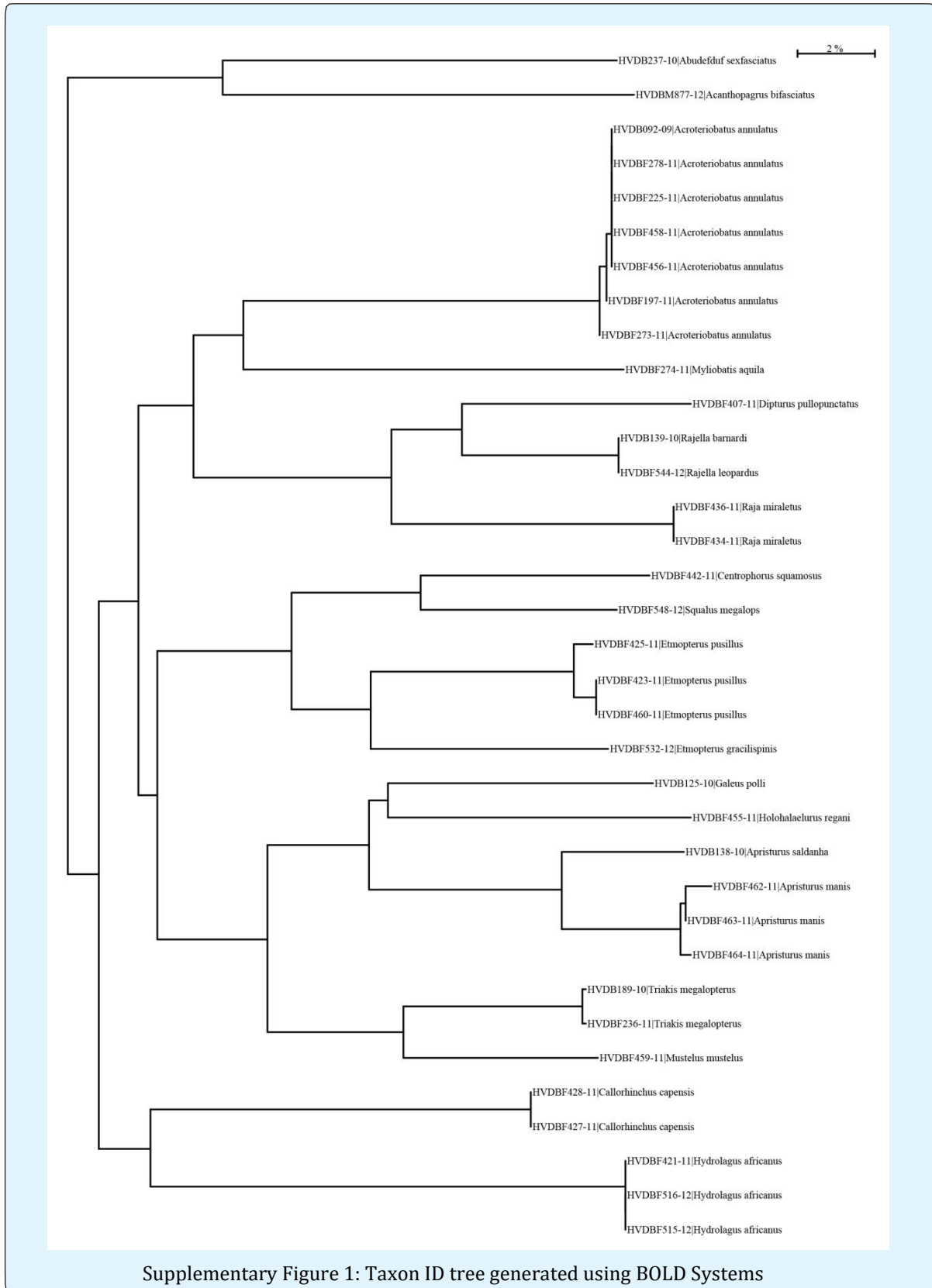
Authorities, localities, GenBank accession and process, dates of collections, localities, museum voucher numbers, images, measurements, barcodes, percent nucleotide composition, and detailed laboratory protocols for the specimens processed will be freely accessible from the BOLD system ([www.barcodinglife.org](http://www.barcodinglife.org)) project code 'HVDBE' after publication. BOLD system analyses were used throughout this study.

DNA extraction, PCR and sequencing were done at the Canadian Centre for DNA Barcoding (CCDB) for CO1 using the primer pair dgLCD-1490/dgHCO-2198. The following parameters were selected: DNA alignment using MUSCLE [16], exclude contaminants, records with stop codons and flagged as misidentified or with errors. The kimura 2-parameter (K2P) model [17] was selected to calculate sequence divergence as implemented in the 'Sequence analysis module of BOLD. DNA sequences, diagnostic characters, pair-wise genetic distances, DNA barcode gap and many other analytical data can be extracted from BOLD. See Figure S1 for arguably a similar tree as Figure 1, which was generated by more advanced techniques as explained below.

A phylogenetic tree (Figure 1) was also generated using maximum parsimony (MP) [18] in PAUP\* v. 4.0b1 [19]. *Abudefduf sexasciatus* and *Acanthopagrus bifasciatus* were used as outgroups. Tree searches were conducted using 1 000 replicates of random taxon addition, retaining 10 trees at each step, with tree-bisection-reconnection (TBR) branch swapping and MulTrees in effect. Support for clades was estimated using bootstrap analysis [20] with 1 000 replicates. Seven additional sequences were mined from GenBank (not HVDB numbers) to improve bootstrap support values.

Title	: Tree Result - HVDBE (62 records selected)
Date	: 17-Feb-2019
Data Type	: Nucleotide
Distance Model	: Kimura 2 Parameter
Marker	: COI-5P
Colourization	: [blue]=Stop Codons [red]=Contamination or misidentification
Label	: Process ID
Label	: Species
Filter	: length > 200bp only
Filter	: exclude records flagged as misidentifications
Filter	: exclude records with stop codons
Filter	: exclude contaminants
Sequence Count:	35
Species count:	20
Genus count:	17
Family count:	13
Unidentified:	0
BIN Count:	18

BOLD TaxonID Tree

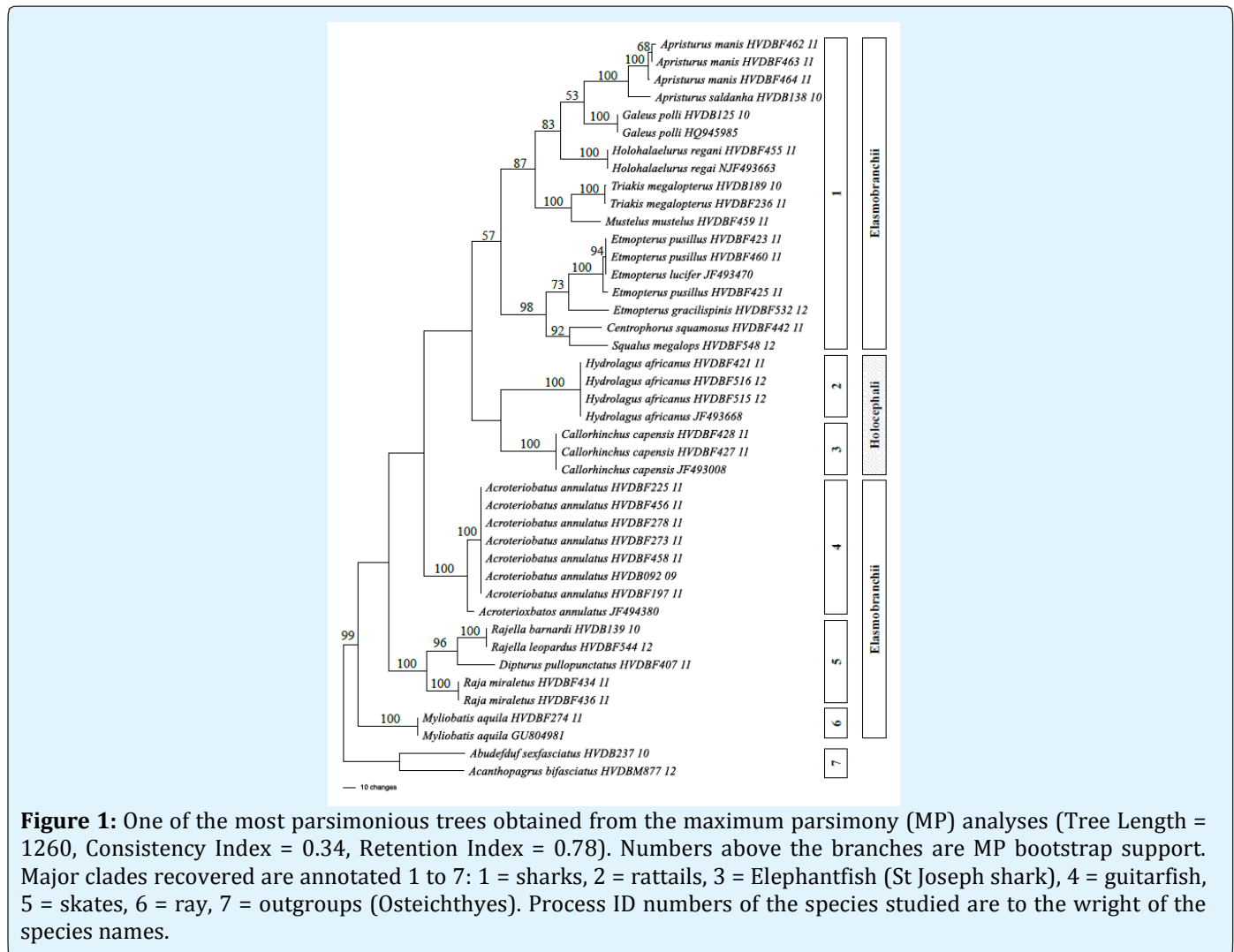


## Results and Discussion

The minimum base pair lengths = 221, maximum = 652 and average = 614. Average K2P intraspecies distances in this fish DNA barcoding study range from 14.65% to zero (e.g. for *A. annulatus* and the skates). As expected, the bony fishes are basal (cartilaginous fishes: class Chondrichthyes). There is a sister-group relationship between the holocephalans and the elasmobranchs (group 1 on Figure 1), but with no support. The trees strongly support (96-100%) a basal divergence between guitarfish, skates, sharks and rays (Figure 1). The groups are monophyletic, as also reported on similar genera, but for mostly other species and different individuals (see introduction). Accurate identification of the sequences was achieved and compared to those available in BOLD and GenBank.

It is not surprising that the elephantfish is grouped

with the ghost sharks (rattails), although not supported – both from the subclass Holocephali. *Rhinobatos annulatus* (mined from GenBank number JF494380) is *Acroteriobatus annulatus* on BOLD. Data for one individual of this species was published, and for *A. manis* [10], with no support. Also, see [2] for similar phylogeny for similar genera (*Callorhinchus*, *Hydrolagus* and *Squalus*). Guitarfish (Order: Rhinopristiformes) are a link between sharks and rays, and are named for their guitar shaped body. Skates (Order: Rajiformes) and rays (Order: Actinopterygii) are cartilaginous fish, like sharks and chimaera, which all make up the class Chondrichthyes. Fishes from other orders are grouped (e.g. *Etmopterus* (Order: Squaliformes), *Galeus* (Order: Carcharhiniformes) and *Triakus* (Order: Carcharhiniformes)). The species studied here should be subjected to detailed taxonomic examination to confirm or refute its taxonomic positions because proper bootstrap support was not achieved at all nodes in this study.



DNA barcoding of specimens, even lacking diagnostic body parts, is a highly effective and fast tool for species identification and can be used for law enforcement. This reference sequence library on BOLD can be used to identify and monitor catches from legal and illegal shark fisheries, because illegal trade of threatened shark species is a worldwide conservation problem [6]. For example, *Callorhynchus capensis* is on the IUCN red list of threatened species. Sharks not only have a spiritual appeal for several Indo-Pacific cultures, but the Chinese believe consuming their fins give them strength and health, and it signals wealth and prosperity. Molecular methods provide a valuable option for the identification of shark meat and body parts such as fins [21].

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