Molecular Phylogeny and Node Age Estimation of Bioluminescent Lantern Sharks (Neoselachii: Etmopteridae)

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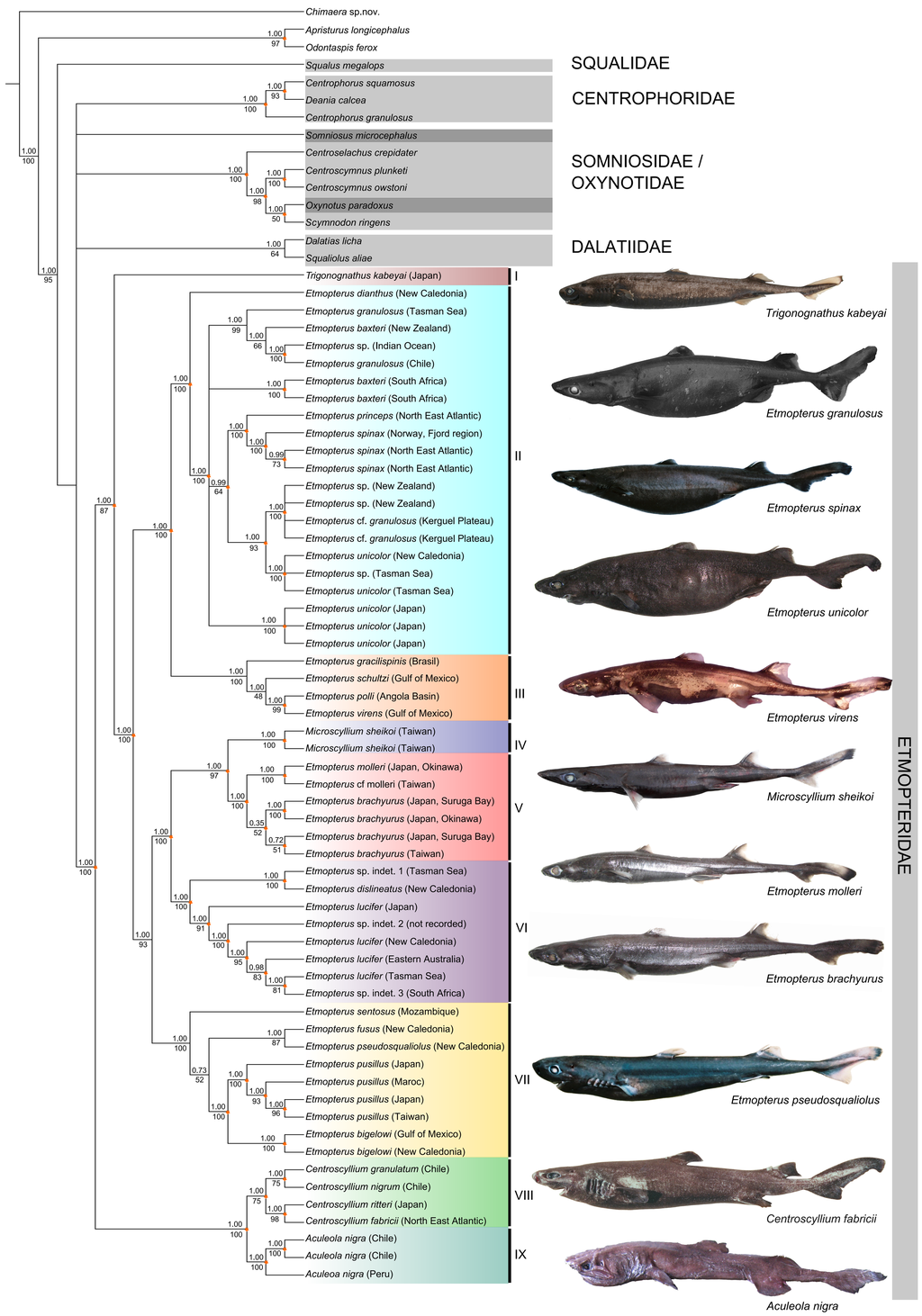

Figure 1: dendrogram displaying phylogenetic relationships of Etmopteridae, reconstructed with Bayesian inference. Widely congruent topologies were attained with ML and MP analyses. Numbers above internal nodes indicate posterior probabilities (PPs) from Bayesian analyses, numbers below branches bootstrap scores attained from ML search strategies. Orange asterisks refer to nodes found in MP analysis with a bootstrap support > 50%. Nodes displaying PPs and bootstrap scores < 0.95 (PP) and < 50% (bootstrap support) were collapsed. Roman numerals refer to nine major clades resulting from phylogenetic analyses. Among the speciose genus *Etmopterus*, four species groups can be identified, partially morphologically characterizable: *E. spinax* species group (clade II), *E. gracilispinis* species group (clade III), *E. lucifer* species group (clades V & VI), and *E. pusillus* species group (clade VII): *Etmopterus* sp. indet. 1: preliminary identified as *Etmopterus* cf *molleri*; *E.* sp. indet. 2: preliminary identified as *E. lucifer*; *E.* sp. indet. 3: preliminary identified as *Etmopterus* cf *brachyurus*. Dark grey colors mark taxa differing from traditional Squaliform families (light gray).

1. Introduction

Lanternsharks (Etmopteridae) are a highly diverse family of poorly known, bioluminescent deep-sea elasmobranchs with 42 species in five genera (Compagno et al. 2005). Although they represent the largest family of Squaliformes very few data on their biology, life history conservation and phylogenetics have been gathered. The large and growing species number within Etmopteridae, one of the largest among Chondrichthyans, as well as a number of unresolved questions around their radiation provoked us to apply DNA based molecular phylogenetics to a new and extensive worldwide sampling of etmopterid sharks to provide 20 years after Shirai & Nakaya's (1990) first phylogenetic approach new insights into the taxonomy and evolution of this still poorly known family of sharks. Specifically, we compiled an extensive DNA dataset to (1) identify the sistergroup of Etmopteridae among Squaliformes, to (2) test for the monophyly of Etmopteridae and for the (3) independent development of bioluminescene within Squaliformes, to (4) test for the monophyly of the twp polytypic etmopterid genera, to (5) test for a Lower Eocene origin of Etmopteridae as indicated by the fossil record, and to (6) analyse sequential versus rapid speciation in the course of the speciose etmopterid radiation.

2. Material & Methods

Samples were either taken from museum collections, from commercially deep sea fishery or from fisheries monitoring programs and represent 26 of the extant 42 etmopterid species plus ten samples being either unidentified or identification is preliminary. Species missing for a complete taxon sampling of extant Etmopteridae were too difficult to attain, since they are partially only known from remote locations. However, our sampling includes all five genera of Etmopteridae and all previously identified species groups are well represented. In additon, representatives of the remaining five squaliform Centrophoridae, Oxynotidae, Somniosidae, Dalatiidae, and Squalidae families were included. *Odontaspis ferox*, *Apristurus longicephalus* and *Chimaera* sp. nov. were chosen as distantly related nested outgroups. Sequences were attained from a portion of nuclear RAG1 (1437 bp) and mitochondrial 12s rRNA, full valine tRNA, 16s rRNA (altogether 2594 bp), as well as cytochrome oxidase I (654 bp). Phylogenetic analyses were carried out on single loci and the concatenated alignment comprising 4685 bp with different analysing approaches such as Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inferences. For reconstructing node ages, Penalized Likelihood and Bayesian statistics were applied, the relaxed molecular clock approach was calibrated with five fossil calibration points.

3. Results

- Within Squaliformes only the basal split of *Squalus* (Squalidae) from the rest of Squaliformes is strongly supported.
- Somniosidae sensu Compagno (2005) render to be paraphyletic with respect to Oxynotidae and *Somniosus*.
- Etmopterid intrafamilial relationships identify nine major clades, each supported with 99%-100 % bootstrap support in ML and MP analyses or 1.00 PPs (Fig. 1)
  - I : *Trigonognathus* (North Pacific)
  - II : *Etmopterus spinax* species group (panoceanic)
  - III : *Etmopterus gracilispinis* species group (subtropical and tropical Atlantic)
  - IV : *Microscyllium sheikoi* (North Pacific)
  - V : northern hemissphere part of the *Etmopterus lucifer* species group (North Pacific)
  - VI : southern hemissphere part of *Etmopterus lucifer* species group and *E. lucifer* as widespread species
  - VII : *Etmopterus pusillus* species group (panoceanic)
  - VIII : *Centroscyllium* (panoceanic)
  - IX : *Aculeola* (Eastern Pacific)
- *Etmopterus* is paraphyletic with regard to *Microscyllium* and is split into two major sister clades (clades II and III vs clades IV, V, and VI).
- A summary of node age estimations is given in Figure 2 below.

node #	node description	BEAST node age	BEAST height 95% HPD	r8s node age
1	Root age	367.7	366.33 – 370.4	369.51
2	Split Squaliformes	170.23	133.37 – 218.42	337.1
3	Split *Squalus*	128.15	127.27 – 129.94	129.14
4	Split Centrophoridae	71.26	69.28 – 74.18	70.6
5	Split Etmopteridae & *Somniosus* from Somniosidae & Dalatiidae	68.78	67.06 – 70.89	-
6	Split Somniosidae / Dalatiidae	67.42	66.87 – 68.49	70.6
7	Split *Somniosus* / Etmopteridae	61.38	52.79 – 68.71	59.85
8	Split clades *Aculeola* & *Centroscyllium* from *Trigonognathus* and *Etmopterus*	43.89	41.26 – 48.46	56.81
9	Split *Aculeola* / *Centroscyllium*	22.7	12.48 – 38.78	40.78
10	Split *Trigonognathus* / *Etmopterus*	40.67	35.70 – 46.02	44.25
11	Split of clades II & III from IV, V & VI	36.48	31.55 – 41.36	34.24
12	Split clades II & III	29.65	21.67 – 36.29	30.34
13	Split clades IV, V & VI from VII	32.88	27.10 – 38.72	32
14	Split of *E. lucifer* species group in clades IV, V and VI	24.26	17.18 – 31.52	19.08
15	Split *Microscyllium*	19.06	11.36 – 26.59	15.02

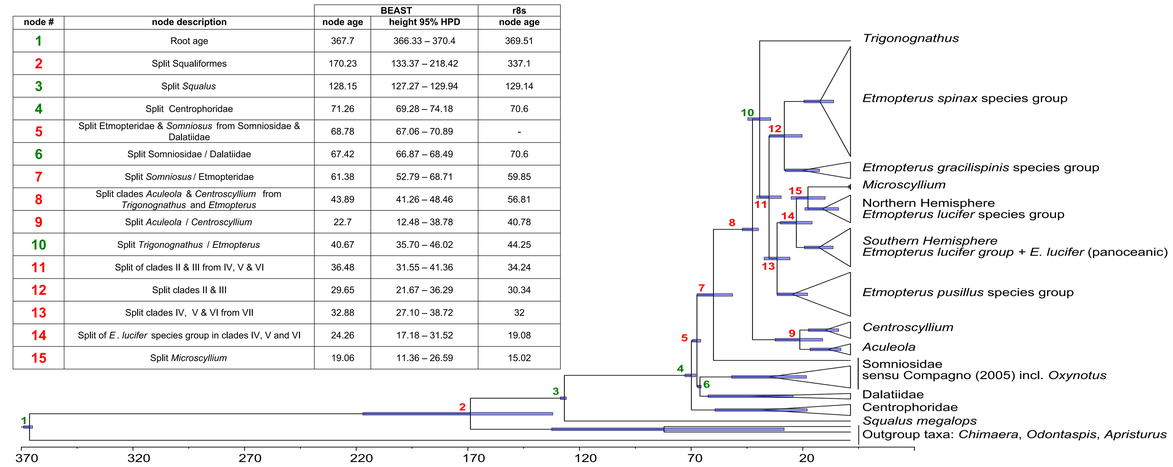

Figure 2: estimated divergence times attained from Bayesian (BEAST) and Penalized Likelihood (r8s) methods. Red numbers refer to node numbers given in the table including node descriptions, mean node ages and confidence intervals of both analysing approaches. Green numbers indicate applied calibration points attained from fossils. Origin of Etmopteridae in between 53 and 69 mya, origin of genus *Etmopterus* in between 36 and 48 mya with further radiation events from 36 to 14 mya.

4. Discussion & Conclusions

(1) Identifying sister-group relationships of Etmopteridae failed since sister-group relationship of Etmopteridae with *Somniosus* is not supported by high confidence in all analyses.

(2) The monophyly of Etmopteridae is strongly supported and four major intrafamilial lineages corresponding largely to the four morphologically well diagnosable genera, i.e. *Aculeola*, *Centroscyllium*, *Trigonognathus* and highly diverse *Etmopterus*.

(3) Our results support the multiple independent evolution of bioluminescene among Squaliformes (Reif 1985) since Dalatiidae are a monophyletic clade among Squaliformes.

(4) Three of five genera display well-supported monophyly, i.e. *Aculeola*, *Centroscyllium*, and *Trigonognathus*. *Etmopterus* renders paraphyletic with respect to *Microscyllium* contradicting Shirai & Nakaya (1990) and Shirai (1992) placing *M. sheikoi* in between *Centroscyllium* and *Etmopterus*.

(5) Age estimates of Etmopteridae, i.e. the split of the (not well supported) Etmopteridae and *Somniosus*, falls at the end of the Cretaceous / beginning of the Paleocene (C/T boundary) and is substantially earlier than the first unambigious etmopterid fossil from deepwater Eocene sediments. The basal etmopterid radiation into four lineages that differ mostly in highly specific dentition characters indicates that trophic specialization played an important role for the early radiation of the group in the Eocene (Fig. 2).

(6) Splitting and evolution of extant genera of Etmopteridae occurred in between 45 and 15 mya (middle Eocene to early Miocene) displaying rather fast radiation of *Etmopterus* species groups at the Oligocene/ Miocene transition.

Results from analyses partially support the flanc markings as synapomorphic characters for identifying species groups, e.g. species nested among the *E. lucifer* species group (clades V & VI) and further deliver evidence for cryptic speciation among *Etmopterus* regarding *E. granulosus* and *E. unicolor* (clade II, *Etmopterus spinax* species group).

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