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A new classification of viviparous brotulas (Bythitidae) – with family status for Dinematichthyidae – based on molecular, morphological and fossil data

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ABSTRACT

The order Ophidiiformes is a large but not very well known group of fishes, unique among teleosts for showing high diversity in both deep sea and shallow reef habitats. The current classification includes more than 500 species, 115 genera and four families, based primarily on mode of reproduction: viviparous Aphyonidae and Bythitidae vs oviparous Carapidae and Ophidiidae. Since 2004 we revised the bythitid tribe Dinematichthyini, described more than 100 new species and noticed that this group has unique morphological characters, perhaps supporting a higher level of classification than the current status. Here we study the viviparous families phylogenetically with partial mitochondrial (*nd4*, *16s*) and nuclear (*Rag1*) DNA sequences (2194 bp). We use a fossil calibration of otolith-based taxa to calibrate the age of the clade comprising bythitid and dinematicththyid representatives, together with fossil calibrations adopted from previous phylogenetic studies. The separation of the order into two major lineages, the viviparous Bythitoidei and the oviparous Ophidioidei is confirmed. At the familial level, however, a new classification is presented for the viviparous clades, placing Aphyonidae as a derived, pedomorphic member of Bythitidae (new diagnosis provided, 33 genera and 118 species). The current subfamily Brosmophycinae is considered polyphyletic and we propose family status for Dinematichthyidae (25 genera, 114 species), supported by unique, morphological synapomorphic characters in the male copulatory apparatus. Previous use of the caudal fin separation or fusion with vertical fins is ambiguous. Age estimates based on calibrated molecular phylogeny agrees with fossil data, giving an origin within the Cretaceous (between 84 and 104 mya) for a common ancestor to Ophidiiformes.

1. Introduction

The order Ophidiiformes consists of about 116 genera and more than 500 species, currently placed in two viviparous (Aphyonidae and Bythitidae) and two oviparous (Carapidae and Ophidiidae) families (Cohen and Nielsen, 1978; Nelson, 2006; Nielsen et al., 1999) (Table 1). They live in a variety of habitats and depths, and the order is probably unique in reaching its greatest diversity in deep-sea habitats (in Ophidiidae, Bythitidae and Aphyonidae) as well as in warm, tropical reefs (mainly Carapidae and Bythitidae). It is one of the dominating deep-sea fish orders, including the deepest known fish *Abyssobrotula galatheae*, caught at 8370 m (Nielsen, 1977). A large number of species (mainly of the tribe Dinematichthyini) inhabit coral reefs and a few live in inland limestone caves. The number of described species has increased significantly in recent years (e.g., Møller et al., 2005; Møller and Schwarzhans, 2006, 2008; Schwarzhans and Møller, 2007, 2011; Schwarzhans et al., 2005). Other recent changes in Bythitidae were the removal of *Thalassobathia* to Ophidiidae (Nielsen et al., 2010) and *Lucifuga* from Dinematicthyini to Brosmophycini (Møller et al., 2004). The bythitids may be important in conservation and biogeographic studies, since the special viviparous mode of reproduction has resulted in many species with a restricted, endemic distribution pattern in tropical seas (e.g. Møller and Schwarzhans, 2008). Surprisingly, certain species seem to be very

Abbreviations: AICc, akaike information criterion; bp, base-pairs; Ma, mega-annus – i.e. one million years; ESS, effective sample size; FAD, First Appearance Dates; LCA, Last Common Ancestors; MCC, Maximum Clade Credibility; mtDNA, mitochondrial DNA; MRCA, most recent common ancestor; NCBI, National Center for Biotechnology Information; nDNA, nuclear DNA; PCR, polymerase chain reaction; SL, standard length.

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Table 1
Catch data, tissue samples and associated museum specimens used in the present study.

Family, subfamily and tribe Species	Museum catalog number	Tissue number	Catch locality	Catch position	Catch depth (m)	Catch date
Ophidiinae						
Chilara taylori	KU28277	KU2201	NE Pacific, off California	34°32′N; 120°47′W	unkn.	25Oct1997
Lepophidium profundorum	KU27197	KU1571	NW Atlantic, Mid Atlantic Bight	35°58′N; 074°45′W	unkn.	11Mar1995
Neobythitinae						
Acanthonus armatus	MNHN 2004-1311	MNHN 2 of 5	E Atlantic, Guinea Gulf	05°48′S; 009°43′E	3156	30Dec2003
Apagesoma australis	ZMUC P771525	#518	S Indian Ocean, Crozet Isl.	49°01′S; 051°04′E	4187	29Dec2005
Bassogigas walkeri	SIO 08-109	SIO 08-109	SW Pacific	13°35′N; 144°32′E	2590	20Apr2008
Dannevigia tusca	none-discarded	CSIRO uncat.	SW Pacific, Tasman Sea			
Dicrolene intronigra	MCZ158834[a]	KU3677	NW Atlantic, off Delaware	39°57′N; 067°30′W	2100	03Dec2000
Holcomycteronus brucei	ZMUC P771528	#515	S Indian Ocean, Crozet Isl.	49°01′S; 051°04′E	4187	29Dec2005
Leucicorus atlanticus	ZMUC P771571	7364	SW Pacific, Solomon Sea	07°49′S; 156°03′E	4350	02Dec2006
Luciobrotula bartschi	ZMUC P771572	7355	SW Pacific, Solomon Sea	13°45′S; 156°41′E	2255	19Dec2005
Monomitopus garmani	ZMUC P771573	7353	SW Pacific, Solomon Sea	07°25′S; 155°45′E	1012	27Dec2006
Porogadus miles	ZMUB/MAR 2661	22601	N Atlantic, Mid-Atlantic Ridge	42°46′N; 029°16′W	3036	11Jul2004
Ventichthys biospeedoi	MNHN 2004-2038	–	SE Pacific, South East Pacific Rise	17°25′S; 113°12′W	2586	29Apr2004
Bythitidae						
Bythitinae						
Bellotia galatheae	ZMUC P771582	7338	SW Pacific, Solomon Sea	07°26′S; 155°30′E	440	25Dec2006
Cataetyx messieri	ZMUC P771617	7867	SE Pacific, Chile	50°21′S; 074°51′W	450	03Feb2007
Cataetyx niki	NMNZ P.41257	TS1294	SW Pacific, New Zealand			
Diplacanthopoma brachysoma	MCZ155341[a]	KU3292	NW Atlantic, off New England	35°08′N; 075°08′W	unkn.	13Jan1999
Tuamotuichthys schwarzhansi	ZMUC P771583	7342	SW Pacific, Solomon Sea	07°26′S; 155°30′E	440	25Dec2006
Brosmophycinae						
Brosmophycini						
Bidenichthys capensis	SAIAB uncat.[a]	KU6466	SE Indian Ocean, South Africa			
Dinematichthyini						
Dinematichthys iluocoeteoides	USNM 334123	KU674	SE Pacific, Tonga, Malinoa Isl.	21°02′S; 175°12′E	unkn.	27Oct1993
Diancistrus jeffjohnsoni	NT#159 (1 of 2)	HL02-01	E. indian Ocean, Australia, Darwin	12°32′S; 130°51′E	0–1	28Mar2002
Diancistrus tongaensis	USNM 374200[b]	KU676	SE Pacific, Tonga, Malinoa Isl.	21°02′S; 175°12′E	unkn.	27Oct1993
Diancistrus typhlops	uncat.	SeN427	E Indian Ocean, Sulawesi, Cave on Muna Isl.	05°00′S; 122°30′E	unkn.	13Sep2007
Ogilbia cayorum	ZMUC P771463	2	NW Atlantic, Bermuda		1	27Apr2005
Ogilbia sp.	ZMUC P771733	8985	NW Atlantic, Mores Isl., Bahamas		unkn.	07Mar2007
Ogilbichthys sp. n.	USNM 349073	KU168	NW Atlantic, Caribbean Sea	16°47′N; 088°04′W	unkn.	16Jul1991
Typhliasina pearsei	ZMUC P771455	P771455	W Atlantic, Mexico, Yucatan		0–1	26Jul2003
Aphyonidae						
Sciadonus galatheae	ZMUC P771588	7310	SW Pacific, Solomon Sea	13°45′S; 156°41′E	2255	19Dec2006
Barathronus diaphanus	ZMUC P771586	7352	SW Pacific, Solomon Sea	07°25′S; 155°45′E	1012	27Dec2006
Aphyonus gelatinosus	ZMUB/MAR 3880	22573	N Atlantic, Mid-Atlantic Ridge			

Classification follows Nielsen et al. (1999).
[a] Specimen not examined.
[b] Previously cataloged as USNM334123.

widespread, and the explanation for this discrepancy in distribution modes requires further studies, for instance of the reproductive details in the group.

Except for the pearlfishes (Carapidae), which were analyzed phylogenetically by Markle and Olney (1990) and Parmentier et al. (2004), little phylogenetic work has been done on the families and genera of Ophidiiformes. Fossil data are rich in otoliths (Schwarzhans, 1981, 2012), but poor in articulated skeletons (Carnevale and Johnson, 2015). The early evolutionary history of the order is generally poorly known phylogenetically, but is likely to get more attention in the future, due to the sister group to the order in the "crown group of teleosts" (Percomorpha) (Miya et al., 2003; Near et al., 2012a,b, 2013; Broughton et al., 2013; Miya and Nishida, 2015; Sanciangco et al., 2016) or Eupercaria – as introduced by Betancur-R et al. (2013, 2014). This is in contrast to earlier consistent placement in Paracanthopterygii (Nelson, 1994, 2006; Patterson and Rosen, 1989). Betancur-R et al. (2013) introduced 'Ophidaria' as to make the suffix for the name for the order consistent with the other orders presented in their study (Betancur-R et al., 2013), but we have decided to use Ophidiiformes throughout our study, as Ophidaria *sensu* Betancur-R et al. (2013) can be regarded equivalent to Ophidiiformes, and since Ophidiiformes is an established name.

Howes (1992) did a study based on osteology, but molecular methods have not yet been employed, except for a few studies with different scopes, where a few ophidiiform taxa were included (Betancur-R et al., 2013, 2014; Miya et al., 2003; Near et al., 2012b; Sanciangco et al., 2016). The order was found to be monophyletic in all but one (Sparks et al., 2005) molecular study. Cohen and Nielsen (1978) used the position of the anterior nostril and the mode of reproduction to separate ophidiiform fishes into Ophidioidei (oviparous, nostril high) and Bythitoidei (viviparous, nostril low). In Nielsen et al. (1999), this overall splitting of the order was adopted, without mentioning the nostril character. Nevertheless all recent studies confirm the overall separation of the order into a viviparous and an oviparous clade (Miya et al., 2003; Near et al., 2012b; Betancur-R et al., 2013, 2014; Sanciangco et al., 2016).

The present study focuses on the interrelationship of the viviparous genera in Bythitidae (currently 59 genera and 209 species)