



Estimating divergence times of lizardfishes and their allies (Euteleostei: Aulopiformes) and the timing of deep-sea adaptations

Matthew P. Davis^{a,*}, Christopher Fielitz^b

^a Museum of Natural Science, Louisiana State University, 119 Foster Hall, Baton Rouge, LA 70803, USA

^b Department of Biology, Emory & Henry College, Emory, VA 24327, USA

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ABSTRACT

The divergence times of lizardfishes (Euteleostei: Aulopiformes) are estimated utilizing a Bayesian approach in combination with knowledge of the fossil record of teleosts and a taxonomic review of fossil aulopiform taxa. These results are integrated with a study of character evolution regarding deep-sea evolutionary adaptations in the clade, including simultaneous hermaphroditism and tubular eyes. Divergence time estimations recover that the stem species of the lizardfishes arose during the Early Cretaceous/Late Jurassic in a marine environment with separate sexes, and laterally directed, round eyes. Tubular eyes have arisen independently at different times in three deep-sea pelagic predatory aulopiform lineages. Simultaneous hermaphroditism evolved a single time in the stem species of the suborder Alepisauridae, the clade of deep-sea aulopiforms during the Early Cretaceous. This result indicates the oldest known evolutionary event of simultaneous hermaphroditism in vertebrates, with the Alepisauridae being the largest vertebrate clade with this reproductive strategy.

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1. Introduction

The order Aulopiformes (Euteleostei: Cyclosquamata) includes 44 extant genera with approximately 236 species of lizardfishes and their allies (Nelson, 2006). Taxa within the order include predatory marine fishes that range in habitat from inshore coastal systems to the deep sea. Many aulopiform fishes have evolved fascinating deep-sea evolutionary adaptations including a number of highly specialized anatomical eye modifications and a reproductive strategy of simultaneous hermaphroditism, one of the rarest methods of reproduction among vertebrate taxa.

Aulopiformes have been recovered as monophyletic with both morphological (e.g., Rosen, 1973; Baldwin and Johnson, 1996; Sato and Nakabo, 2002) and molecular data (Davis, 2010). Previous studies have recovered them as the sister group to the crown euteleostean clade Ctenosquamata (e.g., Rosen 1973; Davis, 2010), which includes the lanternfishes (Myctophiformes) and the spiny-ray fishes (Acanthomorpha). The fossil record for aulopiform fishes is robust with extinct taxa described from two of the three suborders, the Aulopoidei and the Alepisauridae. The majority of fossil taxa are associated with the crown aulopiform clade of alepisaurids (Lancetfishes) from Late Cretaceous deposits.

* Corresponding author.

E-mail addresses: matthewdavis@lsu.edu (M.P. Davis), cfielitz@ehc.edu, mpdavis@ku.edu (C. Fielitz).

The focus of this work is to explore the divergence times of aulopiform fishes and the character evolution of deep-sea adaptations within a robust molecular phylogenetic framework. Additionally we provide a taxonomic review and synthesis of fossil aulopiform diversity to serve as a resource for future phylogenetic and divergence time studies within this group. In this study we investigate the divergence times of (1) the common ancestor of aulopiforms, (2) the major aulopiform lineages, and (3) the evolutionary history of two aulopiform deep-sea adaptations, eye specializations and simultaneous hermaphroditism. Estimating the divergence times of aulopiform fishes is important to our understanding of the evolutionary history of one of the most diverse deep-sea vertebrate lineages, including the timing and character evolution of deep-sea adaptations.

1.1. Overview of fossil aulopiform taxa and relationships

A listing of the fossil aulopiform fishes is found on Table 1. The oldest complete aulopiform fossil is †*Atolvorator longipectoralis*¹ from the Sergipe-Alagoas basin in northeastern Brazil (Gallo and Coelho, 2008). This formation is dated to the Barremian of the Lower Cretaceous and is estimated to be 125 million years old. Gallo and Coelho (2008) did not conduct a phylogenetic study to explore the relationship of †*A. longipectoralis* to other aulopiform taxa, but hypothesized that the taxon was closely aligned to other extinct

¹ Throughout this manuscript a † denotes an extinct lineage

Table 1

Time ranges of fossil aulopiform genera. Subordinal classifications follow Goody (1969) and Davis (2010). A † denotes an extinct lineage.

Taxa	Species	Time range (Ma)	Geologic range (stage age)
†Enchodontoidei			
† <i>Enchodus</i>	17	60.5–99.6	Lower Cenomanian to Paleocene (Danian)
† <i>Eurypholis</i>	3	88.5–94.6	Upper Cenomanian to Turonian
† <i>Palaeolycus</i>	1	74–83.5	Campanian
† <i>Parenchodus</i>	1	93.5–94.6	Upper Cenomanian
† <i>Rharbichthys</i>	1	93.5–99.6	Cenomanian
† <i>Saurorhamphus</i>	3	93.5–94.6	Upper Cenomanian
†Cimolichthyoidei			
† <i>Apulidercetis</i>	1	65.5–83.5	Coniacian to Early Campanian
† <i>Brazilodercetis</i>	1	88.5–90.5	Campanian to Maastrichtian
† <i>Cimolichthys</i>	2	65.5–99.6	Turonian
† <i>Cyranichthys</i>	1	93.5–99.6	Cenomanian to Maastrichtian
† <i>Dercetis</i>	4	70.6–99.6	Cenomanian
† <i>Dercetooides</i>	1	96–99.6	Lower Cenomanian to Upper Campanian
† <i>Hastichthys</i>	1	94.8–99.6	Lower Cenomanian
† <i>Leptecodon</i>	1	65.5–88.6	Lower to mid Cenomanian
† <i>Nardodercetis</i>	1	65.5–83.5	Coniacian to early Campanian
† <i>Nardorex</i>	1	65.5–83.5	Campanian to Maastrichtian
† <i>Pelargorhynchus</i>	1	70.6–76.3	Upper Campanian
† <i>Prionolepis</i>	2	94.6–95	Middle Cenomanian
† <i>Ophidercetis</i>	1	83.5–65.5	Campanian to Maastrichtian
† <i>Rhynchodercetis</i>	6	88.5–99.6	Cenomanian to Turonian
† <i>Robertichthys</i>	1	92.1–93.5	Lower Turonian
† <i>Stratodus</i>	1	80.6–88.6	Coniacian to Early Campanian
†Ichthyotringoidei			
† <i>Apateodus</i>	2	65.5–106.4	Albian to Maastrichtian
† <i>Apateopholis</i>	1	93.5–94.8	Upper Cenomanian
† <i>Ichthyotringa</i>	3	70.6–99.6	Lower Cenomanian to Campanian
†Halecoidei			
† <i>Halec</i>	1	83.5–99.6	Cenomanian to Santonian
† <i>Hemisaurida</i>	1	93.5–99.6	Cenomanian
† <i>Phylactocephalus</i>	1	93.5–94.6	Upper Cenomanian
† <i>Serrilepis</i>	3	93.5–99.6	Lower Cenomanian
Alepisauroidae			
† <i>Acrognathus</i>	1	128–130	Hauterivian
† <i>Drimys</i>	1	2.5–7.5	Placenzian to Messinian
† <i>Holosteus</i>	3	28.4–33.9	Rupelian to Lower Chattian
† <i>Polymerichthys</i>	1	5.3–23	Zanclian to Aquitanian
Aulopoidei			
† <i>Nematonotus</i>	2	93–96	Upper Cenomanian
<i>Aulopiformes in. sed.</i>			
† <i>Atolvorator</i>	1	125–130 (128)	Hauterivian
† <i>Telepholis</i>	2	70.6–94.6	Middle Cenomanian to Upper Campanian
† <i>Yabrudichthys</i>	1	96–99.6	Lower Cenomanian

alepisauroids (e.g., †Cimolichthyidae, †Serrilepidae). Additionally, isolated tooth elements were suggested to belong to an unidentified alepisauroid taxon which has been described from Barremian deposits of Alcañe in northeastern Spain (Kriwet, 2003). The time range for most of the fossil aulopiforms can be placed between the Lowest Cenomanian to the Maastrichtian.

While there have been many studies focused on the evolutionary relationships of extant aulopiforms (e.g., Rosen, 1973; Johnson, 1982; Baldwin and Johnson, 1996; Sato and Nakabo, 2002; Davis, 2010), relationships within the group including extinct aulopiforms are unclear with the exception of the family †Enchodontidae. Currently, the only phylogenetic study of aulopiform fishes to include both extant and extinct taxa is that of Fielitz (2004), which examined the interrelationships of the family †Enchodontidae. Fielitz (2004) recovered a clade consisting of the extant family Alepisauridae (*Alepisaurus* and *Omosudis*) and the extinct families †Cimolichthyidae and †Enchodontidae, classified under the superfamily Alepisauroidae (Fig. 1; Fielitz, 2004). The oldest specimen analyzed in this study was from the Lower Cenomanian Stage of the Late Cretaceous, approximately 100 million years ago. All other studies examining aulopiform fossils have assigned taxa to extant families based on morphological characteristics with no systematic

analysis (e.g., Rosen, 1973), or have left the taxa *incertae sedis* within the order (e.g., Taverne, 2004, 2005).

Hypotheses of aulopiform divergence times have never been explored with molecular data from a robust dataset with comprehensive aulopiform taxonomic sampling. Alfaro et al. (2009) included two aulopiform taxa (*Synodus intermedius* and *Chlorophthalmus* sp.) in their analysis of divergence and diversification rates among vertebrates, and recovered a mean divergence time for the aulopiform clade of 102 Ma (95% HPD 96–138 Ma). Overall, the young mean age recovered for the divergence of the entire clade resulted from their calibration of the aulopiform node. Alfaro et al. (2009) placed a minimum age for the aulopiform clade at 96 Ma, based on fossil representatives †*Nematonotus* spp. (Aulopidae) and †*Acrognathus dodgei* (Chlorophthalmidae), and a soft maximum age of 128–130 Ma based on teeth from an undetermined fossil taxon (Kriwet, 2003). Their calibration scheme for aulopiforms is problematic as the minimum age imposed for the clade is nearly 30 Ma younger than the oldest complete aulopiform fossil †*Atolvorator longipectoralis* (Gallo and Coelho, 2008) and they imposed a soft maximum clade age based on fossil teeth elements from an undetermined taxon that was hypothesized to be closely related to the crown aulopiform lineage of alepisauroids (Kriwet, 2003).

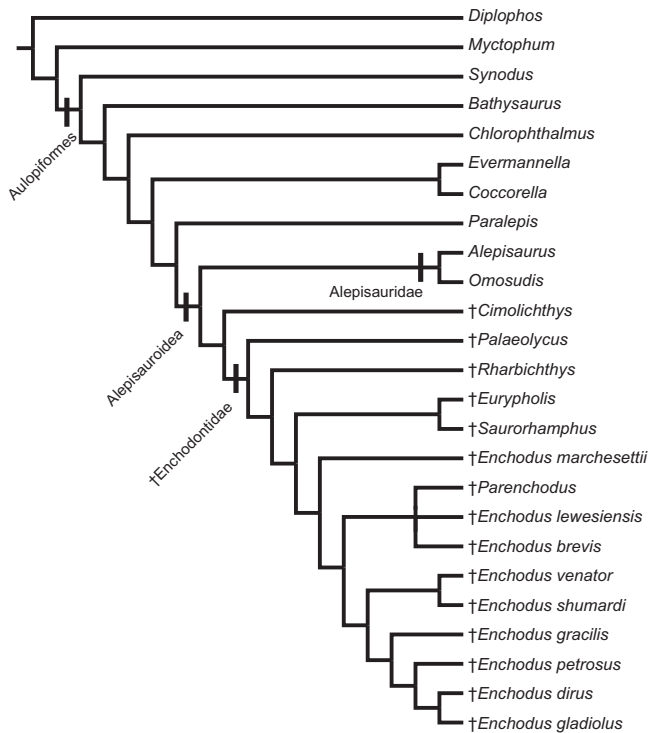


Fig. 1. Evolutionary relationships of the †Enchodontidae. Reproduced from Fielitz (2004). Consensus of three equally parsimonious trees.

1.2. Taxonomic review of fossil aulopiform taxa

Currently there are over 31 genera of recognized fossil aulopiforms (Table 1), with specimens found on every continent except Antarctica. Goody (1969) recognized four suborders of extinct aulopiform taxa that he placed within the order Salmoniformes (†Enchodontoidei, †Ichthyotringoidei, †Cimolichthyoidei, and †Halecoidei), however these taxa were recognized by Rosen (1973) as members of his newly erected order Aulopiformes and assigned to his suborder Alepisauridae. Nelson (1994) ranked all of Goody's (1969) suborders as super families and placed them within a single suborder, the †Enchodontoidei in the Aulopiformes. Described below is a brief review of fossil aulopiform taxa from each major lineage.

†Enchodontoidei (*sensu* Goody, 1969) – Six genera comprise this group (Fielitz 2004). Arambourg (1954) added †Rharbichthys to the family. Re-examining Woodward's (1901) composition of the †Enchodontidae, Goody (1969) removed all but †Enchodus, †Eurypholis, †Saurorhamphus, and †Palaeolycus. He separated †Saurorhamphus and †Eurypholis in their own family, the †Eurypholidae. The †Enchodontidae and †Eurypholidae were then placed within the suborder †Enchodontoidei. Raab and Chalifa (1987) placed †Parenchodus within the †Enchodontidae. The phylogenetic analysis of Fielitz (2004) recovered †Rharbichthys at the base of the †Enchodontoidei followed by †Palaeolycus. †Enchodus was not monophyletic because it contained †Parenchodus (Raab and Chalifa, 1987). †Eurypholis and †Saurorhamphus formed a monophyletic group, and this clade was sister to the †Enchodus + †Parenchodus clade. †Cimolichthys, which was an out-group taxon, was sister to the †Enchodontoidei (Fielitz 2004). †Enchodus is thought to extend into the Paleocene, however, there is evidence that this is reworked Cretaceous material (Goody 1976).

†Cimolichthyoidei (*sensu* Goody, 1969) – This is arguably the largest group of fossil aulopiform fishes. It consists of 16 genera and numerous species. Goody (1969) separated this suborder into

to the †Cimolichthyidae, the †Nardorexidae, the †Prionolepididae, and the †Dercetidae. The †Cimolichthyidae and the †Prionolepididae and †Nardorexidae consists of †Cimolichthys, †Prionolepis, and †Nardorex, respectively. The largest of the four, the †Dercetidae, consists of 13 genera. Many contain only one species, and are restricted in their stratigraphic range. There has been much debate on the taxonomy and phylogenetic interrelationships among members of the †Dercetidae (e.g., Chalifa 1989a; Taverne 1987, 1991, 2005; Gallo et al. 2005). Of the various genera, †Cimolichthys has the longest stratigraphic range, extending from the Cenomanian to the Maastrichtian.

Ichthyotringoidei (*sensu* Goody, 1969) – This group is currently composed of †Ichthyotringa, †Apteopholis, and †Apteodus (Goody 1969). †Apteodus is the oldest of the group with a range from the Albian to Maastrichtian. †Ichthyotringa and †Apteopholis have younger and more restricted ranges. †Ichthyotringa is known from Lower Cenomanian to Campanian, whereas †Apteopholis is restricted to the Upper Cenomanian. Prior to Goody's (1969) classification, †Ichthyotringa was in the Scopelidae (Woodward, 1901) whereas †Apteopholis, and †Apteodus were members of the Enchodontidae Woodward, 1902). Taverne (2006) questioned the inclusion of †Apteodus within this suborder. A phylogenetic analysis by Fielitz and González Rodríguez (2008) recovered a topology where †Apteodus was nested within the †Ichthyotringa clade. Fielitz and González Rodríguez (2008), however, questioned this relationship due to the lack of post-cranial characters in †Apteodus. Recently, a new species of †Apteodus has been described that has a post-cranial skeleton preserved, but its phylogenetic relationship within the suborder was not assessed (Fielitz and Shimada 2009).

Halecoidei (*sensu* Goody, 1969) – This group consists of †Halec, †Hemisauroidea, †Phylactcephalus and †Serrilepis (Goody 1969; Forey et al. 2003). †Halec originally was in the †Enchodontidae (Woodward, 1901) until Goody (1969) placed it within the new suborder †Halecoidei. Woodward (1901) synonymised †Phylactcephalus with Halec, but Goody (1969) stated that there were enough differences to maintain it as a separate genus. Forey et al. (2003) placed †Serrilepis within the Halecoidei. †Halec is from the Cenomanian to the Santonian, whereas †Hemisauroidea, †Serrilepis, and †Phylactcephalus are restricted to the Cenomanian.

Aulopiformes *incertae sedis* – There are several species that have not been assigned to a particular aulopiform lineage, or their taxonomic position is unclear. Chalifa (1989b) did not assign †Yabrudichthys to any fossil aulopiform suborders is of Lower Cenomanian in age. The phylogenetic analysis of the ichthyotringoids by Fielitz and González Rodríguez (2008) supported evidence that †Yabrudichthys may be a member of that suborder. †Telepholis was originally placed in the Chirothricidae by Woodward (1901). Although it is cited as a fossil aulopiform, it is not assigned to any specific suborder. A phylogenetic analysis by Dietz (2008) placed †Telepholis as a sister to the Stomiiformes, however this study included very few other fossil aulopiforms in the analysis. It is found in the Middle Cenomanian deposits of Lebanon and from Upper Campanian beds of Sendenhorst, Germany. As described earlier, the oldest known complete aulopiform fossil †Atolvorator longipectoralis (Gallo and Coelho, 2008) phylogenetic position within Aulopiformes is unknown.

Extinct species assigned to extant aulopiform groups – Although rare, there are fossil taxa that have been assigned within living aulopiform families. As discussed earlier, teeth have been recovered from the early Cretaceous of Spain and have been attributed to that of an unknown alepisaurid (Kriwet, 2003). If correct, then this is one of the oldest records for the clade. Uyeno (1967) described a new alepisaurid from the Miocene of Japan, †Polymerichthys nagurai, and placed it within its own family, the †Polymerichthyidae. The Miocene †Drimys defensor (Jordan,

1925), and the Lower Oligocene †*Holosteus mariae* (Menner, 1948) were both placed in the Paralepididae (Jordan 1925; Prokofiev 2005).

1.3. Aulopiform deep-sea evolutionary adaptations

Many deep-sea fishes are subject to similar selective pressures as a result of the extreme habitat; thus, convergent adaptations, such as bioluminescence, thin bones, tubular or greatly reduced eyes, hermaphroditism, and large mouths with daggerlike teeth, are extremely common (Marshall, 1954; Helfman et al., 1997). The eye modifications that are a common evolutionary adaptation in many deep-sea teleost lineages can be attributed to the two main sources of illumination in the deep sea – residual sunlight and bioluminescence (Douglas et al., 1998). At depths greater than 1000 m, teleosts cannot detect residual sunlight; hence, the fish depend solely on bioluminescence for any visual functions, such as identifying predators and prey, and finding mates (Denton, 1990). While most

deep-sea fishes possess large eyes with a large pupils (Fig. 2A) that aid in detecting distinct sources of residual or bioluminescent light, numerous lineages have evolved highly modified morphological specializations of the eyes (e.g., Stomiiformes, Osmeriformes, Lampridiformes, Lophiiformes) (Land, 1981, 1990).

The eyes of deep-sea aulopiform fishes possess some of the most bizarre modifications of any teleost lineage, making them ideal candidates for studying the character evolution of various eye morphologies (Fig. 2). Three families (Giganturidae, Evermannellidae, and Scopelarchidae) have taxa with tubular eyes – a highly specialized type of eye usually characterized by a large spherical lens, large pupil, a thick main retina, and often an accessory retina (Fig. 2D and E). Species of *Gigantura* have rostrally directed and elongated tubular/telescopic eyes (Fig. 2E), whereas species of *Evermannella*, *Coccorella*, *Benthalbella*, *Rosenblattichthys*, *Scopelarchoides*, and *Scopelarchus* have dorsally directed tubular eyes (Fig. 2D).

Two genera within the family Ipnopidae have greatly reduced (*Bathytyphlops*, *Bathypterois*) eyes, and one (*Bathymicrops*) lacks

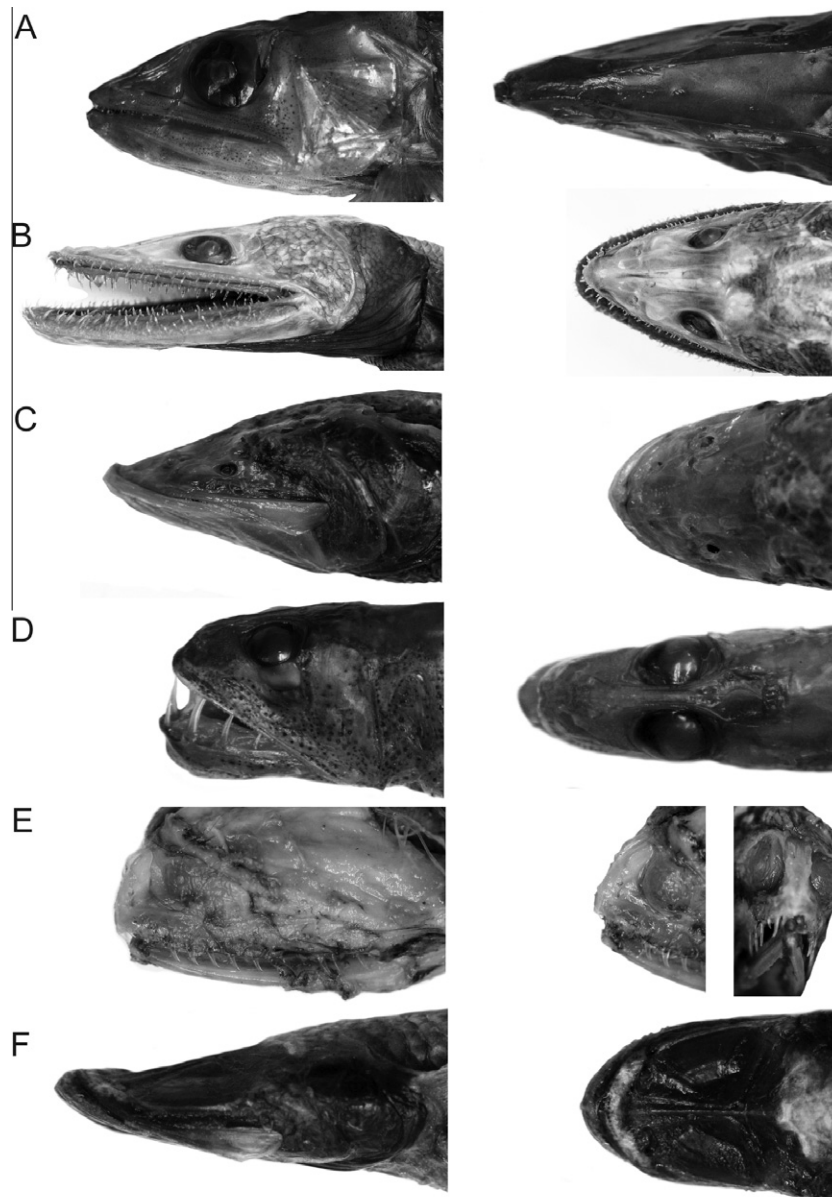


Fig. 2. Aulopiform eye specializations. A – Round and laterally directed (0), *Alepisaurus brevirostris*, MCZ 43134. B – Slightly flattened to elliptical (1), *Bathysaurus ferox*, MCZ 165208. C – Minute or reduced (2), *Bathypterois longipes*, MCZ 36634. D – Dorsally directed tubular/semiteubular (3), *Evermannella balbo*, MCZ 101362. E – Anteriorly directed tubular/telescopic (4), *Gigantura chuni*, MCZ 59485. F – Broad lensless plates on dorsal surface of head (5), *Ipnops murrayi*, KU CI-159. Scale bar denotes 10 mm.

superficial eyes (Fig. 2C); another genus, *Ipnops*, has one of the most bizarre eye adaptations among fishes. Prior to the work of Munk (1959), members of *Ipnops* had been reported to be the only vertebrate that lacked every trace of an eye (e.g., optic nerve, rods, cones, muscle attachments). Munk (1959) documented that *Ipnops* possessed highly modified eyes in the form of a flattened, upward-directed cephalic organ that was innervated by optic nerves, and a retinal layer with typical rods. This modified eye is covered by transparent, fused frontal bones (Fig. 2F).

In addition to modified eye structures, many deep-sea aulopiform fishes also are hermaphroditic. Aulopiforms are one of only four teleostean clades that have evolved simultaneous hermaphroditism (Mank et al., 2006) and are the only deep-sea fish lineage in which this strategy has evolved. Simultaneous hermaphrodites are capable of producing functional male and female gametes at the same time; however, there is currently no evidence that any aulopiform taxa are capable of self-fertilization. Of the other three lineages, two are coral reef predators (Muraenidae: Elopiformes; Serranidae: Perciformes) and one is found in neotropical freshwaters (Rivulidae: Cyprinodontiformes). Because of their diverse habitat and reproductive strategies, aulopiform fishes offer a unique opportunity to study the evolutionary timing of this rare adaptation within a phylogenetic context.

2. Materials and methods

2.1. Phylogenetic analyses

Molecular data included portions of four nuclear genes (*Rag1*, 1498 bp; *Zic1*, 916 bp; *Enc1*, 845 bp; *Plagl2*, 858 bp) and one mitochondrial gene (*COI*, 781 bp), for a total of 4898 base pairs as used in Davis's study of aulopiform interrelationships (2010). The alignment used was identical to the alignment of Davis (2010). Taxonomic sampling included 43 aulopiform species representing 32 of 44 aulopiform genera (Table 2) and every family with the exception of the recently elevated Bathysauropsidae and Bathysauroididae (Sato and Nakabo 2002). Outgroup sampling included tissue samples for 15 species representing 13 actinopterygian orders (Table 2). Outgroups were chosen to maintain a broad sampling of groups hypothesized to be basal to or closely related to Aulopiformes (e.g., Rosen, 1973; Johnson, 1992; Arratia, 2004) including members of the following groups (Nelson, 2006): Neopterygii, Osteoglossomorpha, Otocephala, Protacanthopterygii, Sternopterygii, Ateleopodomorpha, Ctenosquamata, and Acanthomorpha.

A Bayesian phylogenetic analysis was performed in BEAST v. 1.47 (Drummond and Rambaut, 2007), which simultaneously estimates topology and divergence times. Each codon position was assigned a separate GTR + I + G model. Mean substitution rates were estimated under a relaxed uncorrelated lognormal clock that allows for independent rates to vary on different branches in the topology (Drummond et al., 2006). Four separate analyses were performed with 100 million generations each, with a burn-in of 10 million generations for each analysis. Parameters and trees were sampled every 1000 iterations for a total of 400,000 trees, 360,000 post-burnin.

The program Tracer v 1.41 (Rambaut and Drummond, 2007) was used to inspect the effective sample size (ESS) of all parameters in each analysis and check for parameter stationarity. All parameters appeared to converge on a stationary distribution, and possessed ESS's greater than 200, indicating that all analyses sampled the posterior distributions of each parameter satisfactorily. Two clades were constrained in the BEAST analysis, including a monophyletic suborder Aulopoidei and a monophyletic family Scopelarchidae (Table 3). A monophyletic Aulopoidei was recovered with weak (DNA only) and strong support (Total Evidence)

in Davis's (2010) analysis. The family Scopelarchidae was not recovered with DNA evidence alone in Davis's (2010) analysis, but was recovered with strong statistical support in the total evidence analysis when morphological data was considered in combination with DNA. Topology tests (Shimodaira-Hasegawa; Shimodaira and Hasegawa, 1999) based on DNA evidence alone identify that topologies with the forced constraints (monophyletic Aulopoidei, monophyletic Scopelarchidae) are not statistically different from topologies without the constraints (Davis, 2010, Table 3).

2.2. Fossil calibrations

Fossil calibrations were implemented using a lognormal prior because this prior choice allows for hard minimum ages of clades to be set *a priori*, resulting in a distribution of estimated divergence ages that are never younger than the hard minimum age. Minimum dates were based on the oldest known representative of each of the teleost clades discussed below (Fig. 3, Table 3). In order to be conservative with calibrations, dates were based on taxa attributed to the following nodes in previous phylogenetic analyses.

Teleostei – The fossil taxon used to date the clade Teleostei was †*Pholidophorus bechei*, recovered as the basal teleost lineage in Arratia's (2000b, 2001) phylogenetic study of lower teleost relationships. The taxon †*Pholidophorus bechei* is known from the Early Jurassic, with the fossil dated at approximately 220 Ma (Arratia, 2000a). Thus, 220 Ma was set as the minimum age for the most recent common ancestor (MRCA) of the clade Teleostei.

Ostarioclupeomorpha – The systematic placement of genus †*Tischlingerichthys* (Arratia, 1997, 1999, 2000b) as the stem ostariophysan was used to date the MRCA of the clade Ostarioclupeomorpha at 146 Ma. Specimens of †*Tischlingerichthys* examined by Arratia (1997, 1999, 2000b) are from the Late Jurassic, Upper Tithonian (Malm Z3) of Mühlheim, Bavaria, Germany.

Euteleostei – The age of †*Leptolepides sprattiformis*, the oldest member of a stem extinct euteleostean clade recovered as the sister group to extant eutelosts in Arratia's (1997, 1999) phylogenetic study on the relationships of lower teleosts was used to date the MRCA of euteleosts at a minimum age of 150 Ma. Specimens of †*Leptolepides sprattiformis* are known from Solnhofen, Germany, in Late Jurassic, Early Tithonian (Malm Z2) deposits (Arratia, 1997).

Acanthomorpha – The node representing the MRCA of acanthomorphs was given a minimum age of 94 Ma, following the recommendations of Hurley et al. (2007). Fossil taxa attributed to extant stem acanthomorph lineages (e.g., *Polymixia*) are known from Cenomanian deposits dated to approximately 94 Ma (Patterson 1993; Hurley et al., 2007).

Order Myctophiformes – The oldest representatives of Myctophiformes are known from the Campanian in the Early Cretaceous from the extinct genus †*Sardinioides*, which has been recovered as the stem myctophid taxon (Rosen, 1973; Prokofiev, 2006). The minimum age for the MRCA of Myctophiformes was dated to 72 Ma.

Family Alepisauridae – The systematic placement of a clade including the families †Enchodontidae and †Cimolichthyidae sister to Alepisauridae *sensu* Fielitz (2004) (Fig. 1) was used to date a minimum age for the MRCA of the *Alepisaurus* + *Omosudis* clade at 100 Ma (Fig. 3, Table 3), the approximate age of the oldest taxa in that systematic analysis, †*Enchodus brevis* and †*Saurorhamphus freyeri* (Fielitz, 2004). Although the oldest aulopiform fossil is dated at approximately 125 Ma (Gallo and Coelho, 2008), its current systematic position among extant taxa is unknown, therefore the age of †*A. longipectoralis* was

Table 2

List of species examined in this study. Classification follows Nelson (2006) with GenBank accession numbers.

Taxon	Catalog number	Accession Nos.				
		<i>Rag1</i>	<i>Zic1</i>	<i>Enc1</i>	<i>Plagl2</i>	<i>COI</i>
Order Amiiformes						
Family Amiidae						
<i>Amia calva</i>	Various	AY430199	EF032909	EF032974	EF033013	AB042952
Order Hiodontiformes						
Family Hiodontidae						
<i>Hiodon alosoides</i>	Various	AY430200	EU366766	–	–	AP004356
Order Clupeiformes						
Family Clupeidae						
<i>Dorosoma cepedianum</i>	KU T7841	DQ912099	EU366767	–	–	EU366583
Order Cypriniformes						
Family Cyprinidae						
<i>Danio rerio</i>	Various	U71093	EF032910	EF032975	EF033014	NC002333
Order Argentiniformes						
Family Argentinidae						
<i>Argentina sialis</i>	KU T519	AY430228	EU366773	EU366634	EU366680	–
Order Osmeriformes						
Family Osmeridae						
<i>Thaleichthys pacificus</i>	KU T3135	AY380537	EU366774	EU366635	EU366681	–
Order Salmoniformes						
Family Salmonidae						
<i>Oncorhynchus mykiss</i>	U15663	EF032911	EF032976	EF033015	NC001717	
Order Stomiiformes						
Family Gonostomatidae						
<i>Diplophos taenia</i>	KU T3781	EU366724	EU366768	EU366630	EU366676	EU366584
Order Ateleopodiformes						
Family Ateleopodidae						
<i>Ijimaia antillarum</i>	KU T5411	EU366725	EU366769	EU366631	EU366677	EU366585
Order Aulopiformes						
Suborder Synodontoidei						
Family Paraulopidae						
<i>Paraulopus oblongus</i>	CBM-ZF T99-109	EU366709	EU366752	EU366615	EU366664	EU366568
Family Aulopidae						
<i>Aulopus filamentosus</i>	USNM T3816	EU366688	EU366733	EU366593	EU366642	EU366546
<i>Aulopus japonicus</i>	CBM-ZF T99-124	EU366687	EU366732	EU366592	EU366641	EU366545
<i>Hime sp.</i>	SIO T02-68	EU366701	EU366746	EU366606	EU366654	EU366559
Family Pseudotriconotidae						
<i>Pseudotriconotus altivelis</i>	CBM-ZF T99-156	EU366711	EU366754	EU366617	–	EU366570
Family Synodontidae						
<i>Synodus kaianus</i>	CBM-ZF T99-128	EU366719	EU366761	EU366625	EU366672	EU366578
<i>Synodus variegatus</i>	KU T6901	EU366720	EU366762	EU366626	EU366673	EU366579
<i>Synodus intermedius</i>	KU T5219	EU366721	EU366763	EU366627	EU366674	EU366580
<i>Trachinocephalus myops</i>	KU T5225	EU366723	EU366765	EU366629	–	EU366582
<i>Saurida undosquamis</i>	CBM-ZF T99-162	EU366712	EU366755	EU366618	EU366665	EU366571
<i>Harpadon microchir</i>	CBM-ZF T99-148	EU366700	EU366745	EU366605	EU366653	EU366558
Suborder Chlorophthalmoidei						
Family Chlorophthalmidae						
<i>Chlorophthalmus agassizi</i>	KU T3759	EU366695	EU366740	EU366600	–	EU366553
<i>Parasudis truculenta</i>	KU T959	EU366710	EU366753	EU366616	–	EU366569
Family Notosudidae						
<i>Ahliesaurus berryi</i>	KU T5285	EU366685	EU366731	EU366590	EU366639	EU366544
<i>Scopelosaurus harryi</i>	KU T3244	EU366713	EU366756	EU366619	EU366666	EU366572
<i>Scopelosaurus lepidus</i>	KU T3641	EU366714	EU366757	EU366620	EU366667	EU366573
Family Ipnopidae						
<i>Bathypterois grallator</i>	KU T5935	EU366690	EU366735	EU366595	EU366644	EU366548
<i>Bathypterois mediterraneus</i>	CBM-ZF T99-139	EU366691	EU366736	EU366596	EU366645	EU366549
<i>Bathypterois phenax</i>	KU T3625	EU366692	EU366737	EU366597	EU366646	EU366550
<i>Ipnops sp.</i>	CBM-ZF T99-144	EU366702	EU366747	EU366607	EU366655	EU366560
Suborder Alepisauroidi						
Family Scopelarchidae						
<i>Benthalbella dentata</i>	KU T3239	EU366693	EU366738	EU366598	EU366647	EU366552
<i>Benthalbella macropinna</i>	KU T926	EU366694	EU366739	EU366599	EU366648	EU366552
<i>Scopelarchus sp.</i>	KU T3783	EU366715	EU366758	EU366621	EU366668	EU366574
Family Evermannellidae						
<i>Coccorella atlantica</i>	KU T5314	EU366696	EU366741	EU366601	EU366649	EU366554
<i>Evermannella indica</i>	KU T3790	EU366697	EU366742	EU366602	EU366650	EU366555
<i>Odontostomops sp.</i>	CBM-ZF T99-129	EU366706	EU366749	EU366612	EU366661	EU366565
Family Alepisauridae						
<i>Alepisaurus brevirostris</i>	KU T5258	EU366684	EU366730	EU366589	EU366638	EU366543
<i>Alepisaurus ferox</i>	KU T5395	EU366683	EU366729	–	EU366637	EU366542
<i>Omosudis lowei</i>	KU T5909	EU366707	EU366750	EU366613	EU366662	EU366566
Family Paralepididae						
<i>Anotopterus pharao</i>	KU T2305	EU366686	–	EU366591	EU366640	–
<i>Lestidiops jayakari</i>	KU T3792	EU366705	–	EU366610	EU366658	EU366562

(continued on next page)

Table 2 (continued)

Taxon	Catalog number	Accession Nos.				
		<i>Rag1</i>	<i>Zic1</i>	<i>Enc1</i>	<i>Plagl2</i>	<i>COI</i>
<i>Lestidiops ringens</i>	SIO T93-297	–	–	–	EU366659	EU366563
<i>Lestidium atlanticum</i>	KU T3544	EU366703	–	EU366608	EU366656	EU366561
<i>Lestrolepis intermedia</i>	KU T3557	EU366704	–	EU366609	EU366657	–
<i>Macroparalepis johnfitchi</i>	SIO T94-266	EU366722	EU366764	EU366628	EU366675	EU366581
<i>Magnisudis atlantica</i>	KU T5928	–	EU366748	EU366611	EU366660	EU366564
<i>Paralepis coregonoides</i>	KU T3719	EU366708	EU366751	EU366614	EU366663	EU366567
<i>Stemonosudis macrurus</i>	KU T93-238	EU366716	–	EU366622	EU366669	EU366575
<i>Sudis atrox</i>	KU T3107	EU366717	EU366759	EU366623	EU366670	EU366576
<i>Sudis sp.</i>	KU T3798	EU366718	EU366760	EU366624	EU366671	EU366577
Suborder Giganturoidei						
Family Bathysauridae						
<i>Bathysaurus ferox</i>	KU T5934	EU366689	EU366734	EU366594	EU366643	EU366547
Family Giganturidae						
<i>Gigantura chuni</i>	KU T6533	EU366698	EU366743	EU366603	EU366651	EU366556
<i>Gigantura indica</i>	KU T5270	EU366699	EU366744	EU366604	EU366652	EU366557
Order Myctophiformes						
Family Neoscopelidae						
<i>Neoscopelus macrolepidotus</i>	KU T3297	EU366727	EU366771	EU366632	EU366678	EU366587
Family Myctophidae						
<i>Benthoema glaciale</i>	KU T3734	EU366728	EU366775	–	–	–
<i>Nannobranchium lineatum</i>	KU T3634	EU366726	EU366770	–	–	EU366586
Order Polymixiiformes						
Family Polymixiidae						
<i>Polymixia japonicus</i>	KU T258	AY308765	EU366776	EU366636	EU366682	AB034826
Order Lampriformes						
Family Veliferidae						
<i>Metavelifer multiradiatus</i>	KU T1252	EF094949	EU366772	EU366633	EU366679	EU366588
Order Perciformes						
<i>Morone chrysops</i>	Various	AY308767	EF032917	EF032982	EF033021	–

not utilized for dating any nodes within Aulopiformes in an effort to have the most accurate calibrations possible.

2.3. Ancestral character state reconstruction

Ancestral character states were reconstructed using likelihood and parsimony methods in Mesquite 2.7 (Maddison and Maddison, 2009). The Mk1 model (Lewis, 2001), was used to identify the state at each node that maximizes the probability of the states observed in the terminal taxa under the likelihood framework. All character states were unordered for the parsimony analysis. Character states for eye morphologies and reproductive strategies were taken from Baldwin and Johnson (1996), and modified by Davis (2010). These include eyes that are round and laterally directed (0, Fig. 2A), slightly flattened to elliptical (1, Fig. 2B), minute or reduced (2, Fig. 2C), dorsally directed tubular/semitubular (3, Fig. 2D), anteriorly directed tubular/telescopic (4, Fig. 2E), broad lensless plates on dorsal surface of head (5, Fig. 2F). Character states for reproductive strategies include taxa with: separate sexes (0), and simultaneous hermaphrodites (1). Character states were reconstructed on the total evidence Bayesian phylogeny presented by Davis (2010) in an effort to include a representative from nearly every aulopiform genera, as well as the fifty percent consensus maximum clade credibility tree (mean heights) from the BEAST analysis.

3. Results

3.1. Divergence time estimation

The time tree based on Bayesian divergence time analysis from portions of four nuclear (*Rag1*, *Zic1*, *Enc1*, *Plagl2*) and one mitochondrial (*COI*) gene is shown in Fig. 3. Information on lineage divergences including posterior probabilities, mean clade age, and 95% highest posterior densities can be found in Table 3. High-

est posterior densities (HPD) include the interval of age ranges from which 95% of all sampled ages were found during the divergence analysis. The reconstructed phylogeny in BEAST was identical to the topology recovered by Mr. Bayes analysis of DNA alone in Davis (2010), with the exception of the constraints enforced and the movement of *Bathysaurus* as the sister group of the family Ipnopidae rather than Giganturidae. A relationship between *Bathysaurus* and *Gigantura* had low statistical support in Davis (2010), and it is unsurprising that its systematic position changed within the well-supported superfamily Ipnopoidea clade that includes these taxa.

Teleostei is recovered as monophyletic, with a mean clade age of 222 Ma (95% HPD 220–226), suggesting a Late Triassic origin. The divergence date for a lineage split between Ostarioclupeomorpha and Euteleostei is 193 Ma (95% HPD 171–212; Early Jurassic–Late Triassic). Dates for Ostarioclupeomorpha include a mean age of 148 Ma in the Late Jurassic (95% HPD 146–151) and Euteleostei with a mean age of 165 Ma and a Middle Jurassic origin with a range of possible origin from the Early to Late Jurassic (95% HPD 150–186). The estimated age of Protacanthopterygii is 138 Ma (95% HPD 99–174), with a lineage split between Stomiiformes and Osmeriformes at 82 Ma (95% HPD 35–126). The mean date of divergence for Neoteleostei is 155 Ma (95% HPD 139–176), with the divergence of Eurypterygii at 148 Ma (95% HPD 133–166). The divergence date of Ctenosquamata is estimated at 124 Ma in the Early Cretaceous (95% HPD 101–147), with Myctophiformes diverging at 74 Ma in the Late Cretaceous (95% HPD 72–77) and Acanthomorpha diverging at 96 Ma, also in the Late Cretaceous (95% HPD 94–100).

The origin of the Aulopiformes clade is estimated at 140 Ma in the Early Cretaceous, with a possible range into the Late Jurassic (95% HPD 127–156). The suborder Aulopoidei has a divergence date of 133 Ma (95% HPD 115–152), with the origin of the common ancestor of Paraulopoidei and Alepisauridae occurring at 135 Ma (95% HPD 123–149). Suborder Alepisauridae has an estimated origin at 128 Ma (95% HPD 118–140) in the Early Cretaceous. The

Table 3

Divergence times of Aulopiformes. Clades with (C#) were constrained to a minimum age; see Fig. 2.3. An * indicates minimum age constrained. Bold posterior probabilities (PP) indicate the clade was constrained as monophyletic.

Clade/node	Posterior probability	Mean age (Ma)	95% HPD age
1 Neopterygii	1.00	264	220–337
2 Teleostei (C1)	1.00	222	220–226
3	1.00	193	171–212
4 Ostarioclupeomorpha (C2)	1.00	148	146–151
5 Euteleostei (C3)	1.00	165	150–186
6 Protacanthopterygii	1.00	138	99–174
7	0.88	120	79–161
8 Stomiiformes + Osmeriformes	1.00	82	35–126
9 Neotelostei	1.00	155	139–176
10 Eurypterygii	1.00	148	133–166
11 Ctenosquamata	1.00	124	101–147
12 Order Myctophiformes (C4)	1.00	74	72–77
13 Family Myctophidae	1.00	41	19–62
14 Acanthomorpha (C5)	1.00	96	94–100
15	1.00	75	52–94
16 Order Aulopiformes	1.00	140	127–156
17 Suborder Aulopoidei	1.00	133	115–152
18 <i>Synodus</i> + <i>Trachinocephalus</i>	1.00	85	55–115
19	1.00	56	27–88
20	1.00	56	25–86
21	1.00	118	93–143
22	0.61	104	74–134
23 <i>Harpadon</i> + <i>Saurida</i>	1.00	60	26–97
24 Family Aulopidae	0.95	95	60–127
25	1.00	53	20–87
26 Paraulopoidei + Alepisauroidi	0.98	135	123–149
27 Suborder Alepisauroidi	1.00	128	118–140
28 Superfamily Ipnopoidea	1.00	102	72–129
29 Family Giganturidae	1.00	35	10–65
30	0.74	91	62–120
31 Family Ipnopidae	0.90	80	50–112
32 <i>Bathypterois</i>	1.00	49	23–78
33	1.00	26	7–46
34	1.00	121	113–131
35 Family Chlorophthalmidae	1.00	101	65–127
36	1.00	120	112–130
37 Family Notosudidae	1.00	67	31–106
38 <i>Scopelosaurus</i>	1.00	24	6–47
39 Superfamily Alepisauroidi	1.00	119	111–129
40 Family Scopelarchidae	1.00	88	50–119
41 <i>Benthalbella</i>	1.00	67	26–107
42	1.00	115	108–123
43 Family Evermannellidae	1.00	56	27–88
44	1.00	34	12–60
45	1.00	113	106–120
46 Family Sudidae	1.00	61	26–95
47	1.00	109	104–116
48 Family Alepisauridae	1.00	105	101–109
49	1.00	33	7–67
50 <i>Omosudis</i> + <i>Alepisaurus</i> + family †Enchodontidae (C6)	1.00	101	100–102
51 <i>Alepisaurus</i>	1.00	41	12–74
52 Family Paralepididae	1.00	95	75–110
53	1.00	63	33–91
54	1.00	74	47–98
55	1.00	3	1–8
56	1.00	55	30–81
57	1.00	27	7–47

superfamily Ipnopoidea has an origin at 102 Ma (95% HPD 72–129), with a possible range from the Late to Early Cretaceous. The superfamily Chlorophthalmoidea has an estimated divergence date of 101 Ma (95% HPD 65–127). This is followed by a series of divergences, including the lineage split between the superfamilies Notosudoidea and Alepisauroidi, at 120 Ma (95% HPD 112–130), and the origin of Alepisauroidi at 119 MY (95% HPD 111–129). Notosudoidea is found to have an origin at 67 MY (95% HPD 31–106).

Within Alepisauroidi, the family Scopelarchidae has a mean age of divergence of 88 Ma (95% HPD 50–119), while the family Evermannellidae has a younger estimated divergence of 56 Ma (95% HPD 27–88). The origin of the family Sudidae was estimated

at 61 Ma (95% HPD 26–95), with the family Alepisauridae having an older estimated divergence date in the Early Cretaceous at 105 Ma (95% HPD 101–109). The crown aulopiform family Paralepididae has a mean origin of 95 Ma in the Late Cretaceous with its range extending into the Early Cretaceous (95% HPD 75–110).

3.2. Character evolution: eye morphology

Ancestral character state reconstructions of aulopiform eye morphological specializations in the likelihood analysis from the total evidence topology are shown in Fig. 4 and discussed below. Character state reconstruction results from the BEAST maximum

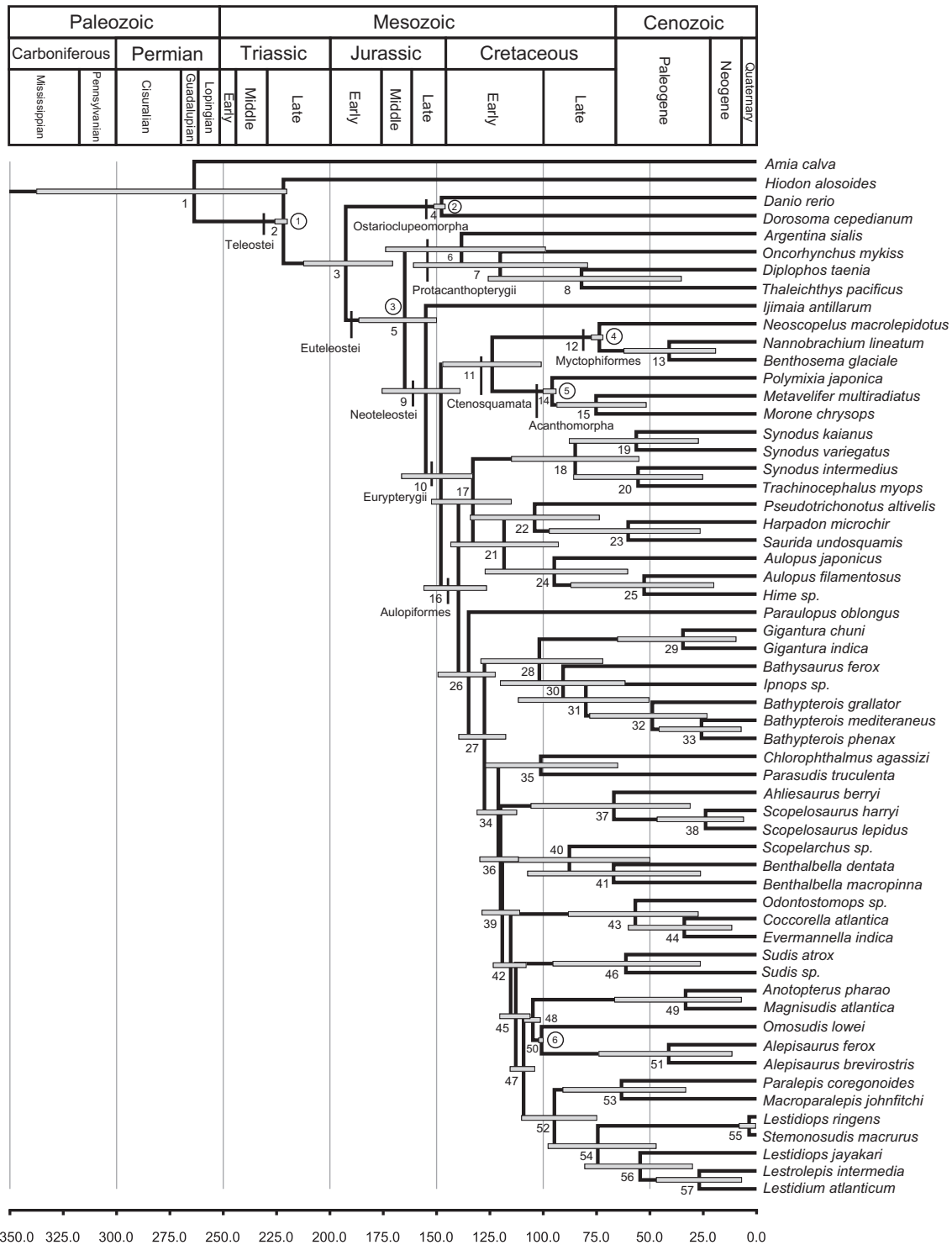


Fig. 3. Divergence time estimations. Bars denote 95% HPD. Numbers at nodes refer to clades in Table 2, which includes information on mean clade age, 95% HPD, and posterior probabilities. Circled numbers refer to calibration points, see materials and methods for discussion on calibrations. Scale is in millions of years.

clade credibility tree were identical and are not shown. The same states identified as most likely are also found to be most parsimonious, with no equivocally parsimonious states found for any node. Of 42 nodes, 37 are found to have a state that was greater than 95% likely for that nodal reconstruction, with the other five nodes having a state greater than 90% likely (Fig. 4A–E). In the following account, “stem species” refers to the inferred ancestor and first member of a particular clade.

Round, laterally directed eyes (State 0) are assigned to the stem species of Aulopiformes, and are common throughout the order. Slightly flattened to elliptical eyes (State 1) arose twice – once in the stem species of Ipnopoidea, and again in the stem species of the Notosudoidea. A single evolutionary event is identified for the origin minute or absent eyes (State 2) in the stem species of Ipnopidae; within Ipnopidae, there is a single evolutionary event of the highly modified broad lensless plates (State 5) in *Ipnops*.

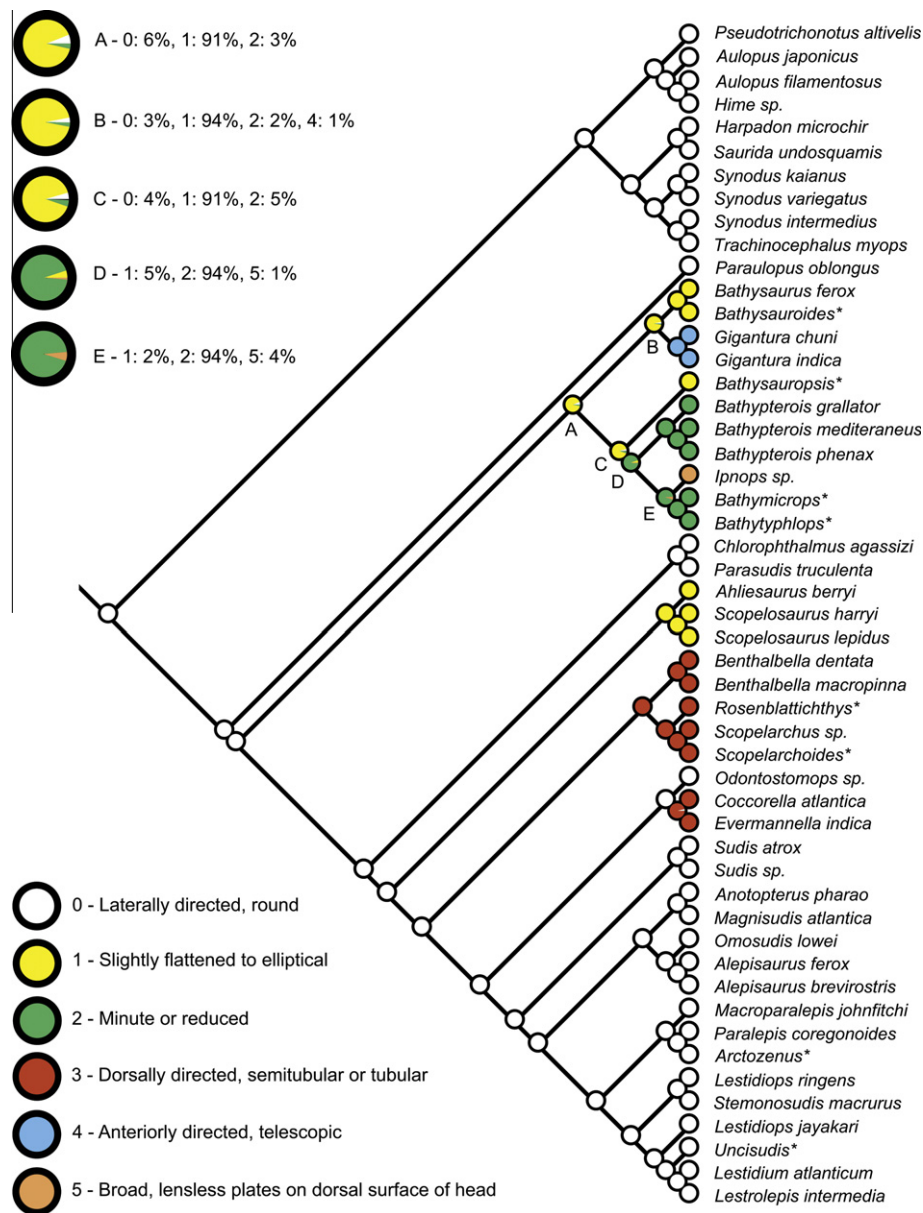


Fig. 4. Likelihood character evolution of aulopiform eye specializations. Tree used for ancestral character state reconstruction taken from Davis's (2010) Bayesian total evidence analysis, with taxa including only morphological data. Circles are pie charts representing probabilities of character state likelihoods. There was no difference between parsimony and likelihood reconstructions. Character states adopted from Baldwin and Johnson (1996).

Anteriorly directed, tubular/telescopic eyes evolved once in the stem species of *Gigantura*. In contrast, dorsally directed tubular eyes have multiple evolutionary origins, once in the stem species of Scopelarchidae, and separately in the stem species of the *Coccorella* + *Evermannella* clade within Evermannellidae.

3.3. Character evolution: reproductive strategies

Reconstruction of ancestral character states for reproductive strategies from the total evidence topology is shown in Fig. 5 and discussed below. Character state reconstruction results from the BEAST maximum clade credibility tree were identical and are not shown. There are no differences between likelihood and parsimony reconstructions, and no equivocal states are identified with parsimony. All nodes showed a likelihood probability for their respective nodes greater than 99% for the reconstructed state.

The evolution of separate sexes (State 0) is reconstructed as the reproductive strategy for the stem species of Aulopiformes, and is

the method of reproduction found in Aulopoidei and Paraulopoidei. A single evolutionary event of simultaneous hermaphroditism occurs in the stem species of Alepisauroides, permeating all members of this clade.

4. Discussion

4.1. Origin of the Aulopiformes

Fielitz (2004) hypothesized that the common ancestor of aulopiforms must have arisen prior to the Late Cretaceous because most aulopiform fossil taxa are derived forms found in Late Cretaceous deposits. This hypothesis is supported by the divergence times recovered (Figs. 3 and 6; Node 16), in which the common ancestor of Aulopiformes is estimated to have an origin in the Early Cretaceous (140 Ma), possibly even the Late Jurassic. Currently, there are no aulopiform fossils known from this time range, with

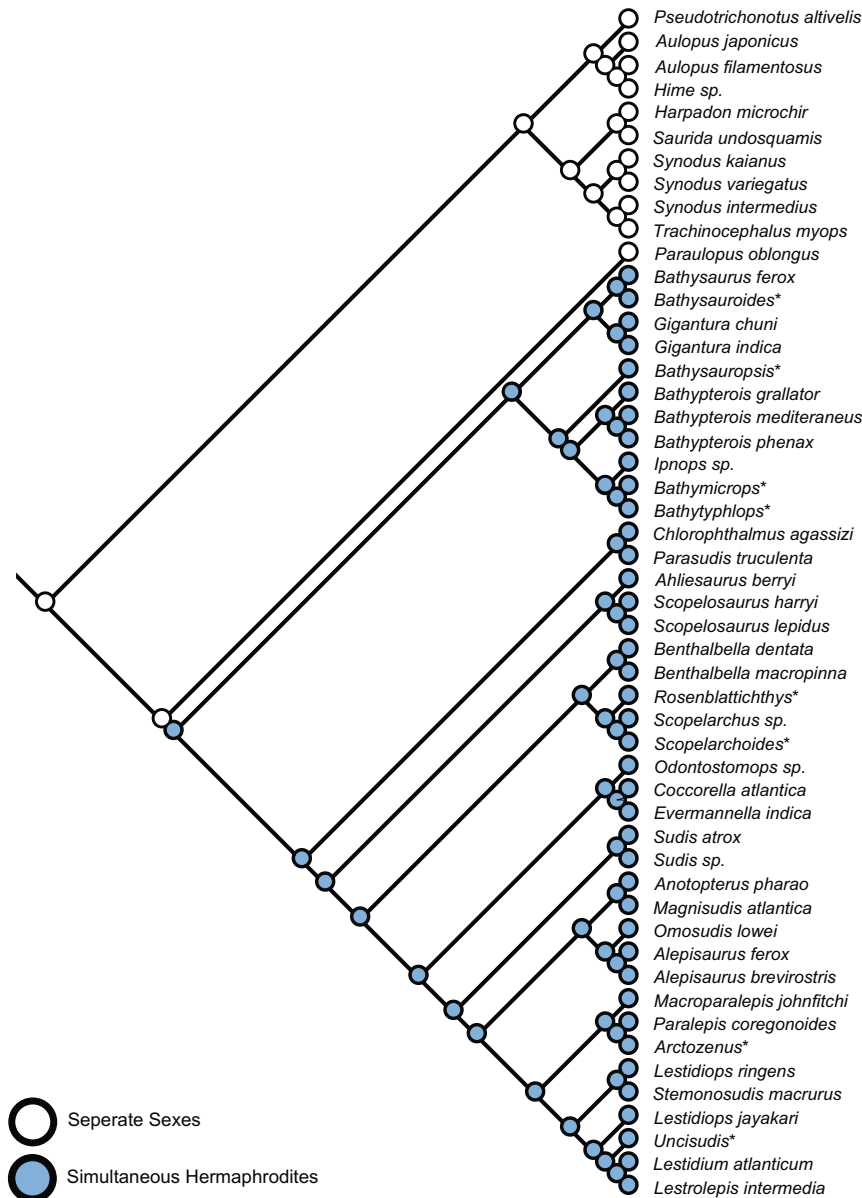


Fig. 5. Likelihood character evolution of aulopiform reproductive strategies. Tree used for ancestral character state reconstruction taken from Davis's (2010) Bayesian total evidence analysis, with taxa including only morphological data. Circles are pie charts representing probabilities of character state likelihoods. There was no difference between parsimony and likelihood reconstructions. Character states adopted from Baldwin and Johnson (1996).

the oldest complete fossil aulopiform †*Atolvorator longipectoralis* having been found in deposits from the Barremian of the Early Cretaceous (Fig. 6) at 125 Ma (Gallo and Coelho, 2008). While the phylogenetic position of †*A. longipectoralis* is unknown, Gallo and Coelho (2008) suggested that the taxon shared some morphological similarities with members of Alepisauoidea and the age of †*A. longipectoralis* falls within the range of possible divergence dates for the origin of Alepisauoidea (95% HPD 111–129). However, a full phylogenetic study is necessary to elucidate further the relationships of †*A. longipectoralis* to the remaining extant and extinct aulopiform taxa.

4.2. Divergence of aulopiform lineages

The roots of all major aulopiform lineages were estimated to have arisen within a span of about 30 Ma in the Early Cretaceous (Fig. 6). Divergence time estimations place the origin of Auloipoidei

into the Early Cretaceous (133 Ma), with a possible origin in the Late Jurassic (Fig. 6; Node 17). Aulopoid fishes consist predominantly of coral reef and continental, shelf-inhabiting benthic fishes, including the lizardfishes (e.g., *Synodus*, *Saurida*) and the flagfin fishes (*Aulopus*). During the Late Jurassic and Early Cretaceous there was tremendous coral reef diversity (Vernon, 1995), and it is likely that the common ancestor of Auloipoidei inhabited coral reef or continental-shelf environments. The oldest fossil taxon attributed to Auloipoidei, †*Nematonotus* spp., was placed in the family Aulopidae by Rosen without a systematic analysis (1973). Fossil specimens of †*Nematonotus* spp. are known from the Cenomanian of the Late Cretaceous (96 Ma), which is near the mean age of Aulopidae estimated by a divergence data at 95 Ma (Fig. 2.6), and falls within the range of possible origin dates (95% HPD 60–127).

There are no known fossil representatives of the suborder Parauloipoidei, although divergence time estimations indicate that the

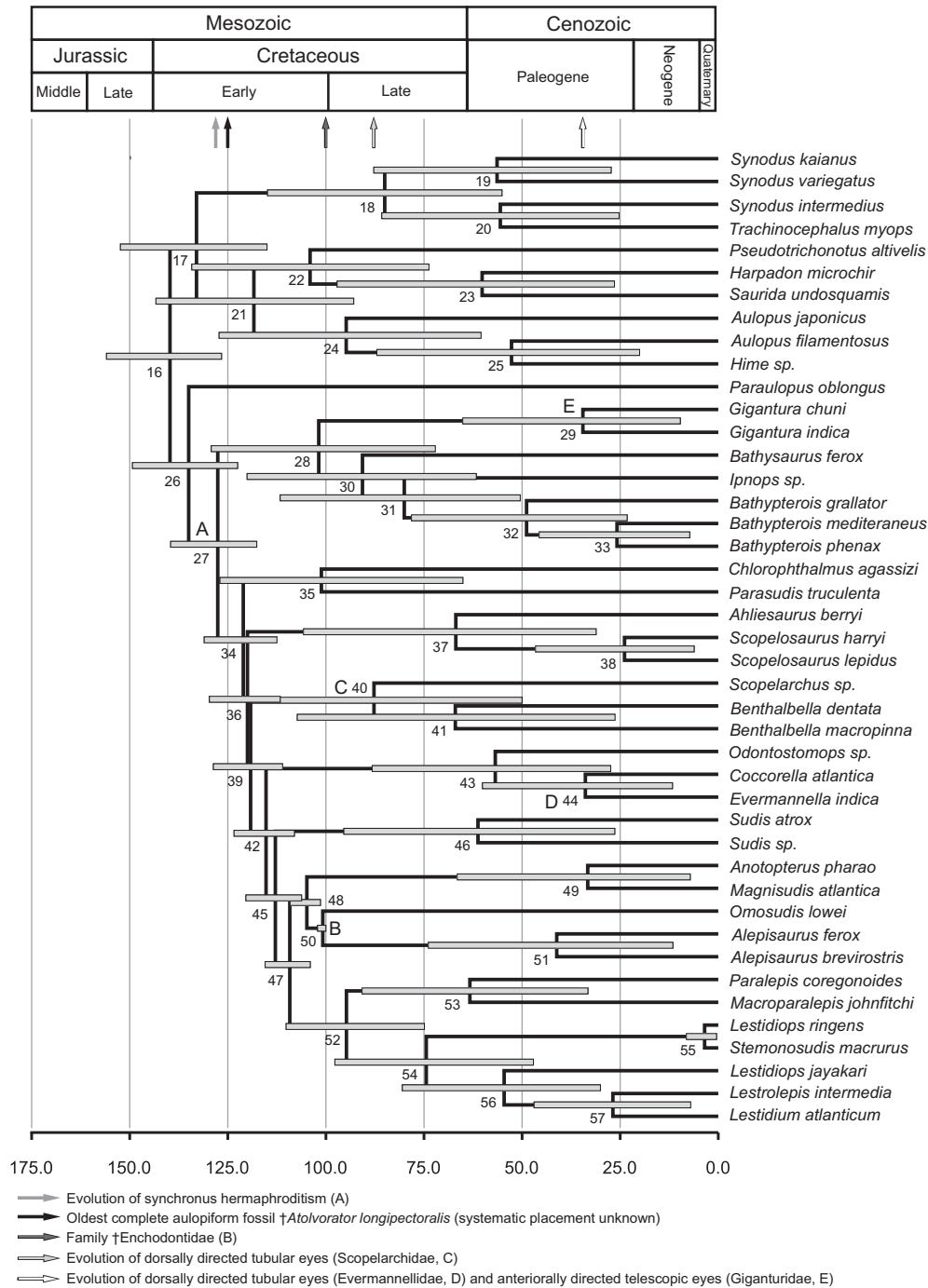


Fig. 6. Aulopiform divergence times. Bars denote 95% HPD. Numbers at nodes refer to clades in Table 2, which includes information on mean clade age, 95% HPD, and posterior probabilities. Scale is in millions of years.

lineage dates to at least the Early Cretaceous, and potentially the Late Jurassic (Fig. 6). Paraulopoidae includes a single genus *Paraulopus*, a benthic group found on the continental shelf in the Indo-Pacific (Sato and Nakabo, 2002).

Most fossil aulopiforms have been attributed to Alepisauridae within the Alepisauridae. Within the superfamily Ipnopoidea, there are no known fossil representatives. Taxa within Ipnopoidea include predominantly benthic-oriented deep-sea fishes, with the exception of the genus *Gigantura* that is bathypelagic. Divergence time estimations recover an Ipnopoidea origin near the end of the Early Cretaceous, with possible ranges extending into the Late Cretaceous (Fig. 6). The family Giganturidae (*Gigantura*)

is estimated to have a date of divergence in the Paleogene of the Cenozoic, with a possible origin in the Neogene. *Gigantura* includes highly specialized deep-sea aulopiform fishes that were a systematic and taxonomic mystery for centuries (e.g., Regan, 1911; Walters, 1961), before being recognized as aulopiforms by Rosen (1973) and Patterson and Johnson (1995). The origin of Ipnopidae is recovered in the middle of the Late Cretaceous (Fig. 6; node 31), but with a broad possible range extending from the Early Cretaceous to the Paleogene. Ipnopids are composed of benthic deep-sea fishes, including the bizarre tripodfishes and the highly specialized *Ipnops*.

The superfamily Chlorophthalmoidea includes one known extinct aulopiform genus, †*Acrognathus*, which is known from

deposits of 96 Ma and associated with the family Chlorophthalmidae. †*Acrognathus* is recognized as a chlorophthalmid (Patterson, 1993), although there has been no systematic analysis that has placed †*Acrognathus* within the family. Chlorophthalmids have an estimated origin of 101 Ma, with a possible range from 65–127 Ma, which includes the †*Acrognathus* position in the fossil record. The superfamily Notosudidae, which consists of bathy- and mesopelagic wartyfishes, is hypothesized to have originated toward the end of the Late Cretaceous with a broad possible range from the Early Cretaceous to the Paleogene. The oldest fossil representative of the family, †*Scopelosaurus brevirostris*, is known from the Bartonian of the Eocene at 42 MY (Patterson, 1993).

The superfamily Alepisauroidae, includes five extant families – Scopelarchidae, Evermannellidae, Sudidae, Alepisauridae, and Paralepididae. Scopelarchids include bathy-mesopelagic predatory fishes, with the oldest fossil representative †*Scopelarchus nolfi* known from the Chattian of the Oligocene (23–30 Ma, Patterson, 1993). The date of divergence for the scopelarchid lineage is estimated to be in the Late Cretaceous, with a broad range extending from the Early Cretaceous to the Paleogene (Fig. 6: Node 40, Table 3). Evermannellidae (sabertooth fishes), which also includes bathy- to mesopelagic predatory fishes, does not have any fossil representatives. The origin of the evermannellid lineage is estimated in the Ypresian of the Paleogene, with a range extending into the Late Cretaceous (Fig. 6: Node 43, Table 3).

Sudidae has an estimated origin in the Danian of the Paleogene, with a range that extends into the Late Cretaceous. Currently, Sudidae has no fossil record. Alepisauridae include meso- to bathypelagic predators and a rich fossil record. In a systematic study of extant and extinct taxa, Fielitz (2004) recovered the families †Cimolichthyidae + †Enchodontidae as the sister group to his Alepisauridae (*Omosudis* + *Alepisaurus*) in his superfamily Alepisauroidae (Alepisauridae *sensu* Davis, 2010). As discussed previously, this information was used to date the minimum age of an *Alepisaurus* + *Omosudis* clade at 100 Ma (Figs. 3 and 6: Node 50, Table 3). The estimated divergence date for the Alepisauridae is in the Early Cretaceous. The oldest fossil attributed to Paralepididae is †*Lestidiops ypresiensis* from the Ypresian of the Eocene (Patterson, 1993). Paralepididae has an estimated origin in the Late Cretaceous, with a range extending into the Early Cretaceous.

There are also a number of extinct aulopiform families that are presently regarded as *incertae sedis*, including †Ichthyotringidae, †Dercetidae, and †Nardorexidae. All three have taxa that are known from the Late Cretaceous, with †Nardorexidae and †Dercetidae found in Campanian–Maastrichtian deposits (e.g., Taverne 2004, 2005), and †Ichthyotringidae dating to the Albian–Cenomanian (Fielitz and González Rodríguez, 2008). Taxa in these families have been hypothesized to be related to extant taxa within Alepisauroidae, although none has been examined in a phylogenetic study that includes both extant and extinct taxa, so phylogenetic position is unclear (e.g., Chalifa, 1989). Future phylogenetic work on these taxa would increase our knowledge of aulopiform evolutionary relationships and may provide further calibration information.

4.3. Evolution and timing of deep-sea eye adaptations

Laterally directed round eyes (Fig. 2A) were present in the stem species of aulopiforms and permeate the majority of the clades (Fig. 4; State 0). The remaining eye morphologies all evolved in taxa inhabiting the deep-sea in meso- to bathypelagic habitats. The superfamily Ipnopoidea, in particular represents a “hotspot” for eye evolution within the aulopiforms, with ipnopids possessing four of the five deep-sea eye adaptations represented in this study.

Slightly flattened to elliptical eyes (Fig. 2B) have evolved multiple times, once in the stem species of the superfamily Ipnopoidea in the

Early Cretaceous, and again in the stem species of the superfamily Notosudoidea in the Late Cretaceous. The phylogenetic analysis indicates that evolution of slightly flattened to elliptical eyes in the two clades is a result of convergent evolution (Figs. 4 and 6; State 1). A reduction in eyes (Fig. 2C) occurs in the stem species of the family Ipnopidae during the Late Cretaceous (Figs. 4 and 6; State 2), with the further evolution to the flattened, upward directing cephalic organ (Fig. 2F) isolated to the genus *Ipnops* following the reduction in eyes, which is the most likely trait observed in the stem species of a clade *Ipnops* + *Bathymicrops* + *Bathytyphlops* (Fig. 4; State 5). It is difficult to ascertain the timing of the evolutionary appearance of this peculiar feature without molecular data for *Bathytyphlops* and *Bathymicrops*, however, it would most likely trace back to the Late Cretaceous or Paleogene (Fig. 6).

Tubular eyes have evolved multiple times in deep-sea aulopiforms. Dorsally directed tubular eyes (Fig. 2D) has evolved independently in two lineages, one each within the families Scopelarchidae and Evermannellidae, both of which include deep-sea vertically migrating predators found in the meso- to bathypelagic zone (Fig. 4; State 3). Baldwin and Johnson (1996) recovered dorsally directed tubular eyes as a synapomorphy of a Scopelarchidae + Evermannellidae clade, while this study suggests that this trait has independently evolved within these families. Tubular eyes are a common eye specialization among members of teleost lineages inhabiting the deep sea, and convergence of this trait is likely in these two lineages as first suggested by Johnson (1982). Dorsally directed tubular eyes probably arose first in the stem species of Scopelarchidae in the Late Cretaceous, with the trait common among species in this clade (Fig. 4; State 3).

Within Evermannellidae, tubular eyes most likely evolved once in the stem species of the *Evermannella* + *Coccorella* Clade (Figs. 4 and 6; State 3). The genus *Odontostomops* has lateral, round eyes typical of other alepisaurids, and Johnson (1982) hypothesized that *Odontostomops* was the sister group to an *Evermannella* + *Coccorella* Clade, this relationship is corroborated in this study. Baldwin and Johnson (1996) recovered *Coccorella* as the basal evermannellid, and suggested that the lack of tubular eyes was a reversal in *Odontostomops*; this reversal is not supported. Tubular eyes evolved in the Paleogene in evermannellids, whereas they evolved in the Late Cretaceous in scopelarchids. The results of this study indicate that the dorsally tubular eyes of scopelarchids and evermannellids are not homologous structures, and are the result of convergent evolution.

Anteriorly directed tubular/telescopic eyes (Fig. 2E) seem to have evolved once within deep-sea aulopiforms in the stem species of Giganturidae, within the superfamily Ipnopoidea (Figs. 4 and 6; State 4). This eye specialization is estimated to have evolved in the Paleogene. *Gigantura* is the only pelagic member of Ipnopoidea, and is not known to migrate vertically. Among aulopiform lineages, anterior or dorsally directed tubular eyes have only evolved in deep-sea fishes with pelagic lifestyles, and dorsally directed tubular eyes has evolved in predatory taxa that are predominantly vertically migratory.

4.4. Evolution and timing of synchronous hermaphroditism

The stem species of aulopiforms most likely had separate sexes, because this trait is found in the stem lineages of the suborders Aulopoidei and Paraulopoidei (Figs. 5 and 6). The evolution of synchronous hermaphroditism is hypothesized to have evolved in the stem species of the Alepisauroidae, probably during the Early Cretaceous, between the Berriasian and the Barremian stages. This estimate suggests the oldest known date and lineage for the evolution of simultaneous hermaphroditism among vertebrates. Other simultaneous hermaphroditic teleost lineages are younger and are generally known from the Paleogene (Patterson, 1993). Additional synchronous hermaphroditic teleost lineages include a

few species of muraenid eels (e.g., *Sidera grisea*), serranid sea basses (e.g., *Serranus fasciatus*), and killifishes (*Kryptolebias marmoratus*) (Mank et al., 2006). Approximately two thirds of aulopiform fishes (~158 species) are simultaneous hermaphrodites, making the suborder Alepisauroidei the largest vertebrate clade with this reproductive strategy. Determining whether this feature represents a key innovation for aulopiform speciation in the deep sea is beyond the scope of this study and further morphological work is needed to explore the specifics of the reproductive systems across aulopiforms in order to better understand this rare and unique reproductive strategy among vertebrates.

5. Conclusions

The stem species of the aulopiforms arose during the Early Cretaceous, and possibly Late Jurassic in a marine environment that was most likely in an inshore continental shelf habitat, with separate sexes, and laterally directed, round eyes. The major aulopiform lineages originated during the Early Cretaceous, with most extant families appearing by the Late Cretaceous to the Eocene.

There have been multiple independent evolutionary events of flattened elliptical eyes in the stem species of the superfamilies Ipnopoidea and Notosudoidea. Tubular eyes have arisen independently at different times in three deep-sea pelagic predatory aulopiform lineages. Dorsally directed tubular eyes have evolved independently, once in the stem species of Scopelarchidae during the Late Cretaceous, and once within Evermannellidae in the stem species of the *Evermannella* + *Omosudis* clade during the Paleogene. Anteriorly directed tubular eyes evolved a single time in Giganturidae during the Paleogene. Eyes are reduced in the stem species of Ipnopidae during the Late Cretaceous, with the highly specialized, upward-directed cephalic organ evolving in *Ipnops*.

Simultaneous hermaphroditism evolved a single time in the stem species of the suborder Alepisauroidei, the clade of deep-sea aulopiforms. This feature most likely arose in the Early Cretaceous, and is the oldest known simultaneous hermaphroditic strategy among vertebrates. The suborder Alepisauroidei is also the largest vertebrate clade possessing this reproductive strategy with approximately 158 species.

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