Phylogenetic analysis of molecular and morphological data highlights uncertainty in the
relationships of fossil and living species of Elopomorpha (Actinopterygii: Teleostei)

RUNNING HEAD: ELOPOMORPH PHYLOGENY

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Abstract

Elopomorpha is one of the three main clades of living teleost fishes and includes a range of disparate lineages including eels, tarpons, bonefishes, and halosaurs. Elopomorphs were among the first groups of fishes investigated using Hennigian phylogenetic methods and continue to be the object of intense phylogenetic scrutiny due to their economic significance, diversity, and crucial evolutionary status as the sister group of all other teleosts. While portions of the phylogenetic backbone for Elopomorpha are consistent between studies, the relationships among *Albula*, *Pterothrissus*, Notacanthiformes, and Anguilliformes remain contentious and difficult to evaluate. This lack of phylogenetic resolution is problematic as fossil lineages are often described and placed taxonomically based on an assumed sister group relationship between *Albula* and *Pterothrissus*. In addition, phylogenetic studies using morphological data that sample elopomorph fossil lineages often do not include notacanthiform or anguilliform lineages, potentially introducing a bias toward interpreting fossils as members of the common stem of *Pterothrissus* and *Albula*. Here we provide a phylogenetic analysis of DNA sequences sampled from multiple nuclear genes that include representative taxa from *Albula*, *Pterothrissus*, Notacanthiformes and Anguilliformes. We integrate our molecular dataset with a morphological character matrix that spans both living and fossil elopomorph lineages. Our results reveal substantial uncertainty in the placement of *Pterothrissus* as well as all sampled fossil lineages, questioning the stability of the taxonomy of fossil Elopomorpha. However, despite topological uncertainty, our integration of fossil lineages into a Bayesian time calibrated framework provides divergence time estimates for the clade that are consistent with previously published age estimates based on the elopomorph fossil record and molecular estimates resulting from traditional node-dating methods.

Keywords: Bayesian, Fossil Tip Dating, Eels, Tarpon, Gissu

Elopomorph fishes contain a broad range of teleost lineages including Anguilliformes (eels); Saccopharyngiformes (gulper eels); Notacanthiformes (halosaurs and spiny eels); Elopiformes (tarpons and ladyfishes); and Albulidae (bonefishes). Despite substantial ecological and morphological diversity, elopomorphs have long been interpreted as a clade (Greenwood et al., 1966; Nelson, 1973; Patterson and Rosen, 1977; Hulet and Robins, 1989). While this evolutionary hypothesis was not without controversy (Gosline, 1971; Nybelin, 1971; Filleul and Lavoue, 2001), several morphological synapomorphies (Forey, 1973; Arratia, 1996; Forey et al., 1996; Arratia, 1997; Wiley and Johnson, 2010), including the unique leptocephalus larval stage (Greenwood et al., 1966; Pfeiler, 1986; Arratia, 1997), as well as continual support from molecular and morphological analyses (Forey et al., 1996; Arratia, 1997; Inoue et al., 2004; Arratia, 2008; Near et al., 2012; Betancur-R et al., 2013; Faircloth et al., 2013; Chen et al., 2014) provide evidence for elopomorph monophyly. Many elopomorph species, such as eels and tarpons, are economically important (Briand et al., 2003; Dekker, 2003; Cooke and Philipp, 2004; Ault, 2010; Adams et al., 2014), and investigations of elopomorph species have yielded significant insights into our understanding of the diversity of teleost feeding strategies (Mehta and Wainwright, 2007, 2008; Mehta et al., 2010), respiratory physiology (Geiger et al., 2000; Seymour et al., 2004; Farrell et al., 2007), and genomic architecture (Inoue et al., 2001; Robinson-Rechavi et al., 2001; Guo et al., 2010). Given the socioeconomic and scientific importance of Elopomorpha, it is not surprising that substantial work has been conducted to determine its evolutionary intrarelationships.

Elopomorphs were subjects of early applications of Hennigian phylogenetic systematics to teleosts in the late 1960s and early 1970s (e.g., Nelson, 1969; Forey, 1973). The systematic history of elopomorphs prior to the mid 1990s is thoroughly reviewed by Forey et al. (1996), who also presented a phylogenetic analysis of 56 discretely coded morphological characters

relating almost exclusively to skeletal structure (e.g., braincase, sensory canals, and pectoral and pelvic girdle). A majority of recent molecular analyses investigating higher-level relationships of elopomorphs have resulted in phylogenies broadly similar to the tree inferred using morphology presented in Forey et al. (1996). For example, most studies resolve *Elops* and *Megalops* as sister lineages that form the earliest diverging clade of living elopomorphs (Near et al., 2012; Chen et al., 2014). This corroboration of major features of elopomorph phylogeny is complemented by major advances in the resolution of lower-level relationships within the major elopomorph groups (Johnson et al., 2012; Santini et al., 2013). However, despite over 50 years of phylogenetic studies, questions concerning higher-level relationships within Elopomorpha remain (Chen et al., 2014).

The evolutionary relationships of the albulid and notacanthiform lineages are particularly contentious. While notacanthiform monophyly is supported by several synapomorphies, including modifications of the maxilla and intercalated connective tissue between the pterygoid arch and maxilla (Forey et al., 1996; Wiley and Johnson, 2010; Datovo and Vari, 2014), their phylogenetic relationships with *Albula*, *Pterothrissus*, and anguilliforms remains unclear (Kanehira et al., 2012). Based on over a dozen synapomorphies, Forey et al. (1996) resolved Anguilliformes as the sister lineage of Notacanthiformes. This contrasts with a clade comprising *Albula*, *Pterothrissus*, and Notacanthiformes to the exclusion of eels hypothesized by other authors (Nelson, 1973; Greenwood, 1977; Patterson and Rosen, 1977; Datovo and Vari, 2014). Instead, Forey et al. (1996) resolved *Albula* and *Pterothrissus* as the sister lineage to their notacanthiform/anguiliform clade. Forey et al. (1996) identified the clade containing *Albula*, *Pterothrissus*, and their close fossil relatives like †*Brannerion* as Albuliformes. However, a series of phylogenetic studies using mtDNA sequences contradict this result, and support a reconstituted albuliform clade comprising *Albula*, *Pterothrissus*, and Notacanthiformes, with the

latter two representing sister lineages (Obermiller and Pfeiler, 2003; Wang et al., 2003; Inoue et al., 2004). Throughout this paper, we use the term Albuliformes as defined by Inoue et al. (2004): the clade comprising *Albula*, *Pterothirssus*, and Notacanthiformes (i.e., non-elopiform, non-anguilliform elopomorphs). This is not an endorsement of the status of this putative clade, but rather a convention to circumvent confusion arising from alternative concepts that have applied the same taxonomic name (e.g., Forey et al., 1996).

The monophyly of Albuliformes has been challenged by a recent phylogenetic analysis of two mitochondrial genes. This resolves a notacanthiform-anguilliform clade, which is sister to a clade containing *Albula* and *Pterothrissus*, although this result was not strongly supported (Tang and Fielitz, 2013). Molecular studies based on multiple nuclear genes have also consistently indicated paraphyly of Albuliformes (Broughton et al., 2013; Chen et al., 2014), though this is not always the case (Near et al., 2012). However, to date no multi-locus nuclear gene dataset has sampled representative taxa from all albuliform lineages, rendering development of hypotheses regarding the interrelationships of *Albula*, *Pterothrissus* and Notacanthiformes—and comparisons between studies—difficult. Some of our principal goals center on testing whether this collection of lineages represents a clade or grade, and if *Albula* and *Pterothrissus* are united in a monophyletic Albulidae *sensu* Nelson (2006).

Uncertain relationships between albuliform lineages and anguilliformes also present a challenge to understanding the phylogenetic diversity of elopomorphs in the fossil record. (e.g., de Figueiredo et al. 2012). Key albuliform taxa like †*Baugeichthys*, †*Brannerion*, and †*Bullichthys* appear in the Early Cretaceous (Filleul, 2000; Mayrinck et al., 2010; de Figueiredo et al., 2012; Bartholomai, 2013; Sweetman, 2013), but their significance is obscured by two interrelated factors. First, the exclusion of notacanthiforms and anguilliforms from many paleontological analyses (Forey and Maisey, 2010; de Figueiredo et al., 2012) means that the

relationships of putative fossil albuliforms to several modern elopomorph lineages remain largely untested. Instead, paleontological studies generally interpret these fossils as stem members of the clade comprising *Albula* and pterothrissids (e.g. Forey et al, 1996). However, available paleontological trees cannot exclude a wide variety of alternative interpretations for these fossils because they do not sample notacanthiforms and eels. Second, the albuliform hypothesis of relationships implies very different patterns of morphological character distribution than that inferred by Forey et al. (1996), which could have profound implications for the phylogenetic placement of fossil taxa. Clarification of these issues requires analyses of combined multi-locus sequence data with a morphological matrix of fossil and living taxa.

In this study we present an investigation of higher-level elopomorph relationships that samples representatives of all elopomorph lineages, including examples of all putative albuliform lineages, using a multi-locus nuclear gene dataset. Phylogenies inferred using Bayesian and maximum likelihood methods, include a clade containing notacanthiforms and anguilliforms to the exclusion of other living lineages, and differ from phylogenetic hypotheses regarding the placement of *Pterothrissus* (Obermiller and Pfeiler, 2003; Wang et al., 2003; Inoue et al., 2004). We integrate our molecular data with published morphological data matrices spanning both contemporary and extinct lineages in a Bayesian approach to divergence time estimation that allows for the inclusion of species known only from the fossil record as tip taxa (Pyron, 2011; Ronquist et al., 2012). The phylogenetic trees inferred from these molecular and morphological data matrices provide a new basis for investigations into the evolutionary history of this fascinating group of vertebrates.

2. Methods

2.1 Sequence Data Acquisition, Alignment, and Model Selection

We sampled seventeen species for DNA sequencing that span all major elopomorph lineages (Table 1). Two species of Osteoglossomorpha, *Gymnarchus niloticus* and *Hiodon tergisus*, were sampled to serve as outgroups for the phylogenetic analyses. DNA was extracted from muscle tissue biopsies using Qiagen DNAeasy Tissue Extraction Kits (Qiagen, Valencia, CA). We used polymerase chain reaction (PCR) to amplify 6 nuclear genes: *myh6* (~729 bp), *Ptr* (~699 bp), *plagl2* (~630 bp), *rag1* (~1386 bp), *zic1* (~835 bp), and *Glyt* (~834 bp) using the primers and the protocols described by Li et al. (2007) and Lopez et al. (2004). Amplified products resulting from PCR were purified using Qiagen Qiaquick PCR Purification Kits and sequenced using Applied Biosystems Big Dye chemistry and 3730xL DNA analyzers (Applied Biosystems) at the Keck Biotechnology Resource Laboratory (Yale University).

Our data matrix was 78% complete with coverage for individual genes varying between 63 and 90% (Table 1). Alignment of all genes was conducted using MUSCLE v3.7 (Edgar, 2004), then refined by eye using the translated amino acid sequences. Sequences were trimmed to the size of the smallest fragment for each gene to minimize missing characters in the data matrix. Our aligned nucleotide data matrix consisted of 5133 base pairs, and all sequences were archived in GenBank (Table 1: accession numbers XXXXX-XXX) with corresponding alignments archived in Zenodo (DOI: 10.5281/zenodo.15936). We used the heuristic search algorithms in PartitionFinder (Lanfear et al., 2012) with a Bayesian Information Criterion (BIC) to simultaneously choose the best-fit models of nucleotide substitution and partitioning strategies (Table 2). All three combinations of codon positions were considered as candidate partitions within each gene of the alignment, with the candidate pool of potential partitioning strategies spanning the range from a single partition for the entire alignment, to each gene and codon position treated as a partition.

2.2 Bayesian and Maximum Likelihood Inference

Mixed partitioned analyses were performed for each of the sampled genes and on the concatenated matrices in MrBayes 3.2 (Ronquist and Huelsenbeck, 2003). All analyses were conditioned on the best-fitting nucleotide substitution model and partitioning strategies identified by PartitionFinder (Lanfear et al. 2012). For each analysis set we assigned default priors to all model parameters and ran all analyses using three heated and one cold Markov Chain. In each instance we ran the Markov Chain Monte Carlo (MCMC) sampler for 30 million generations, sampling every 1,000, for a total of 30,000 trees. To ensure that our samples came from the target distribution, convergence of the chains was assessed by visualizations of the state likelihoods, potential scale reduction factors, and the average deviation of the split frequencies using the MrBayes output and AWTY (Nylander et al., 2008). Majority rule consensus trees were constructed from the remaining post-burn in samples of each data set.

We used the Randomized Accelerated Maximum Likelihood (RAxML) software v. 7.3.0 as an alternate mode of tree inference for the concatenated gene datasets (Stamatakis, 2006). As RAxML has a restricted selection of nucleotide substitution models, we used the GTR+ Γ model to model each partition identified using PartitionFinder (Lanfear et al., 2012). While this approach potentially adds extra parameters that do not substantially increase model-fit, Dornburg et al. (2008) demonstrated that over-parameterization of nucleotide substitution models has a negligible effect on topological estimation as well as the calculation of both bootstrap support values and Bayesian posterior probabilities. In all analysis iterations, we conducted 200 maximum likelihood searches using a random starting tree (-d setting) with a random search convergence criterion based on Robinson-Foulds distances (-D setting). One thousand bootstrap replicates were conducted using the thorough bootstrap search (option -f i) for each dataset.

Bootstrap support values were mapped onto the tree topology with the highest maximum likelihood inferred from the random searches.

2.3 Divergence Time Analysis

We used a Bayesian relaxed molecular clock approach to generate a posterior distribution of time-calibrated phylogenies of Elopomorpha in the BEAST version 1.8 software package (Drummond et al., 2006; Drummond and Rambaut, 2007). Trees were generated under an uncorrelated model molecular evolutionary rate heterogeneity that assumes a lognormal distribution of molecular rates, with a birth-death speciation prior was used for branching rates in the phylogeny. The nuclear gene dataset was partitioned according to the optimal partitioning strategy identified by PartitionFinder, with the UCLN clock and nucleotide substitution models unlinked across partitions. Clock models were partitioned by morphology and identified partitions of the molecular data. We used the modifications of Pyron (2011) to include fossil taxa as noncontemporaneous sampled taxa. Our molecular data were combined with a dataset of 74 discretely coded morphological characters, 67 binary and 7 three-state, taken from de Figueiredo et al. (2012). The Lewis (2001) Markov model (Mk_v) was applied to the morphological characters and ascertained site patterns were applied to account for the sampling of only variable characters (Alekseyenko et al., 2008). The morphological matrix includes representatives of several living elopomorph lineages; however, eels were not scored in the morphological studies (de Figueiredo et al., 2012). As such we restricted the divergence time analyses to reflect anguilliform monophyly, which has been well established on the basis of morphological and molecular analyses (Inoue et al., 2004; Wiley and Johnson, 2010; Johnson et al., 2012; Chen et al., 2014). Uniform priors were used to accommodate uncertainty in the ages of specific tip-based calibrations. Details on the geological record of the sampled fossil taxa and justification

for prior ages are outlined in Appendix A. To ascertain whether morphological data were unduly influencing the divergence time estimates, we conducted a second set of analyses using identical conditions for the molecular data outlined above and omitted the fossil taxa and morphological data. For these analyses, node age priors were conditioned on the two node based calibrations. To assess the influence of the prior on our posterior divergence time inferences, we replicated two analyses without sequence data that sampled solely from the prior distribution of parameter estimates (Drummond et al., 2006).

2.4 Bayesian species tree inference

As incongruent gene histories can mislead inference of evolutionary relationships over deep timescales (Oliver, 2013), we simultaneously inferred gene trees and the underlying species tree using the *BEAST package (Heled and Drummond, 2010) within BEAST v.1.8. (Drummond et al., 2006; Drummond and Rambaut, 2007). Trees were unlinked by gene region and each gene was partitioned using the best fit partitioning strategy nucleotide substitution model identified in PartitionFinder (Lanfear et al., 2012). We ran three independent MCMC analyses for 200 million generations each, sampling every 5,000 generations. Convergence of the chains was assessed by visualizations of the state likelihoods in TRACER v. 1.5 (Rambaut and Drummond 2012) and effective sample sizes (ESS) for all model parameters were calculated to ensure adequate mixing of each chain (ESS > 200).

2.5 Bayesian analysis of amino acid sequences

Bayesian analyses of the amino acid translations were conducted using the site heterogeneous CAT-GTR+ Γ 4 (Lartillot and Philippe, 2004) model in Phylobayes MPI 1.5 (Lartillot et al., 2013). As the CAT model assumes heterogeneous site patterns, we removed

constant sites to prevent biasing our inferences using the `-dc` option (Lartillot et al., 2013). We conducted four replicate analyses, running each chain for 20,000 cycles while sampling posterior parameter estimates every ten cycles. We discarded 10% of each run as burn-in and assessed convergence between replicate runs and effective sampling of the posterior distribution of individual runs using the *tracecomp* and *bpcomp* packages of Phylobayes.

3. Results

The phylogenetic trees estimated from each gene demonstrated substantial uncertainty in the resolution of higher-level elopomorph relationships (Figure 1). The tree inferred using *plag12* was the least resolved, with Bayesian posterior probabilities (BPP) greater than 0.95 restricted to Notacanthiformes (Figure 1). However, other gene trees show consistent resolutions. Elopiformes is supported as the sister lineage to all other elopomorphs with BPPs between 0.91 and 1.0 by all genes trees except that inferred from *zic1*. The *zic1* gene tree resolves Saccopharyngiformes as the sister lineage to all other elopomorphs, but this conflicts with the other gene trees that strongly support gulper eels as nested within the Anguilliformes (Figure 1). Examination of the *zic1* sequence data revealed several indels unique to saccopharyngiforms, which may explain the peculiar phylogenetic resolution arising from this gene. While relationships among albuliform lineages vary by gene, no individual gene tree supports albuliform monophyly (Figure 1). The *ptr* gene tree strongly supports *Pterothrissus* as the sister lineage of Anguilliformes (BPP > 0.95), while the gene tree for *myh6* strongly supports a clade containing *Pterothrissus*, Notacanthidae, and Anguilliformes (BPP > 0.95 Figure 1). Gene trees inferred from *rag1* and *glyt* also resolve a clade containing Notacanthidae and Anguilliformes with strong support (BPP = 1.00). The phylogeny inferred from the morphological dataset lacked

resolution, but did not support albuliform monophyly and instead provided only moderate support for a clade containing †*Istieus* and *Pterothrissus* (BPP = 0.90, Figure 1).

Phylogenetic analyses of the concatenated nuclear gene dataset results in identical topologies between Bayesian and maximum likelihood inference methods (Figures 2A & 2B). In both analyses, elopiform monophyly is supported with bootstrap support (BSS) of 100 or a BPP of 1.0, and strongly supported as the sister lineage of all other elopomorphs (BSS = 100, BPP = 1.0; Figure 2). Anguilliform monophyly, inclusive of Saccopharyngiformes, is strongly supported (BSS = 100, BPP = 1.0). However, there is little support for albuliform monophyly, as notacanthids are strongly supported as the sister lineage to Anguilliformes (BSS = 0.99, BPP = 1.0 Figure 2). *Pterothrissus* is resolved as the sister lineage of the clade containing notacanthids and anguilliforms; however, this relationship is not strongly supported (BSS = 59, BPP = 0.81; Figures 2A & 2B). The addition of the phenotypic data lowers support values globally, reflecting the uncertainty in the placement of the sampled fossil taxa. There is strong support for a clade containing †*Istieus* and *Pterothrissus* (BPP = 0.99; Figure 2C); however, no other clades containing both living and extinct elopomorphs are resolved with BPP > 0.74.

The maximum clade credibility tree estimated from the posterior distribution of the fossil tip-dated Bayesian relaxed clock analyses demonstrated substantial uncertainty in the placement of the sampled fossil elopomorph taxa (Figure 3). This uncertainty is similar to the low topological support in the MrBayes inferred phylogeny from the combined morphological and molecular datasets (Figure 2C). However, even when accounting for uncertainty in the evolutionary relationships of fossil taxa, the inferred timeline of elopomorph diversification is remarkably consistent with previous relaxed molecular clock estimates. As there was broad overlap between divergence times estimated with and without fossil tip taxa we focus the results on those estimated using fossil tip taxa (Figure 3). The estimated 95% highest posterior density

interval (HPD) for the crown age of Elopomorpha spans 157 [Late Jurassic] to 200 [Late Triassic] million years ago (Ma; Figure 3). This estimate overlaps with most recently published age estimates (Alfaro et al., 2009; Near et al., 2012; Broughton et al., 2013; Santini et al., 2013), with the notable exception of the mitogenomic based estimates of Johnson et al. (2012) that fall largely within the Triassic and Permian (Figure 3). The age estimates resulting from our tip-dated relaxed molecular clock analysis indicates that both crown lineage anguilliforms (95% HPD: 94-140 Ma) and notacanthiforms (95% HPD: 67-129 Ma) diversified primarily in the Cretaceous, a result that is also consistent with previous studies (Figure 3) and the presence of anatomically modern fossil examples of both groups in the Late Cretaceous (Siegfried, 1954; Taverne, 2002; Taverne and Capasso, 2014).

The phylogeny of elopomorphs inferred using the *BEAST multispecies coalescent species tree analysis resulted in monophyly of Elopiformes with strong support (BPP = 1.0, Figure 4A), and the resolution of this clade as the sister lineage of all other Elopomorpha (BPP = 1.00, Figure 4A). A clade containing Notacanthiformes and Anguilliformes is resolved with moderate support (BPP = 0.84). There is substantial uncertainty regarding the relationships of *Pterothrissus*, which is resolved as the sister lineage of the clade containing Notacanthiformes and Anguilliformes with poor support (BPP = 0.56; Figure 4A).

Bayesian analyses of the concatenated amino acid dataset under a CAT-GTR+ Γ 4 model resulted in similar strongly supported relationships and areas of uncertainty as all of the other analyses (Figure 4B). There is strong support for the monophyly of Elopomorpha (BPP=1.0; Figure 4B), Elopiformes (BPP=1.0; Figure 4B), and a clade containing all remaining elopomorphs (BPP=1.0; Figure 4B). The monophyly of Notacanthiformes is strongly supported (BPP=0.96; Figure 4B) and there is strong support for a clade comprising Notacanthiformes and Anguilliformes (BPP=1.0; Figure 4B). *Pterothrissus* and *Albula* are resolved as a clade with

weak support (BPP = 0.58). Relationships among major anguilliform clades and Notacanthiformes were unresolved and represented as a polytomy in the 50% majority-rule consensus tree. Saccopharyngiformes are strongly supported as the sister lineage to a clade containing Serrivomeridae (sawtooth eels) and Muraenidae (moray eels).

4. Discussion

4.1 Trends and uncertainty in the classification of living and fossil Elopiformes

In the almost 50 years following the Hennigian phylogenetic revolution, proposed relationships between living elopid and megalopid taxa, currently recognized as Elopiformes (Nelson, 2006), and other elopomorph fishes have remained remarkably consistent. Our results are congruent with other phylogenetic studies that have resolved Elopiformes as the earliest diverging lineage of Elopomorpha (Forey et al., 1996; Obermiller and Pfeiler, 2003; Wang et al., 2003; Inoue et al., 2004; Alfaro et al., 2009; Near et al., 2012; Betancur-R et al., 2013; Chen et al., 2014). This finding is consistent with the observation that elopiforms possess patterns of larval development that differ substantially from remaining elopomorphs, as they maintain some of the “whitebait” larval features characteristic of other teleosts in early stages of development (Tsukamoto and Okiyama, 1997; Inoue et al., 2004). There is also little controversy surrounding the sister-group relationship between Elopidae and Megalopidae (Patterson and Rosen, 1977; Wang et al., 2003; Inoue et al., 2004; Near et al., 2012; Chen et al., 2014), which is also strongly supported in our phylogenetic analyses (Figures 1, 2, 3, & 4). The affinity between these clades has been recognized for over a century, with taxonomic debate instead often centering on whether Elopiformes should be recognized as containing one or two distinct families (Boulenger, 1904; Jordan, 1923; Fowler, 1936; Hildebrand and Bigelow, 1963; Romer, 1966). Forey (1973) discussed the merit of recognizing two major clades, suggesting that this delimitation highlights

not only the anatomical differences of living representatives, but also provides the necessary framework for resolving the relationships of fossil elopomorph taxa.

While the phylogenetic affinity between living elopids and megalopids is fairly uncontroversial, our analyses demonstrate that substantial uncertainty surrounds the phylogenetic placement of extinct elopiform fishes. Based on phylogenetic analyses of morphological characters, de Figueiredo et al. (2012) revised the classification of fossil elopiform fishes, placing †*Ichthyemidion* within crown Elopiformes and †*Paraelops* as stem Elopiform. However, our analyses do not provide strong phylogenetic support for this classification. †*Ichthyemidion* is resolved as either on the stem of Elopiformes (Figure 3) or within its crown (Figure 2), but both resolutions are poorly supported. Our analyses also fail to provide a clear placement for †*Paraelops*, resolving this taxon as either a stem elopiform (Figure 3) or stem elopomorph (Figure 2). Our analyses suggest that †*Bullichthys* may also represent a stem elopiform or even a stem elopomorph (Figures 2 & 3), substantially deviating from the current interpretation of this taxon on the common stem of *Albula* and *Pterothrissus* (Mayrinck et al., 2010; de Figueiredo et al., 2012). †*Bullichthys* was first described as a stem albuliform based on its large otic bulla and ectopterygoid process (Mayrinck et al., 2010) and resolved as the earliest diverging lineage on the common stem of *Pterothrissus* and *Albula* based on a maximum parsimony analysis of morphology (de Figueiredo et al., 2012). As de Figueiredo et al. (2012) did not include notacanthiforms or anguilliformes in their analyses, it is difficult to evaluate the significance of this placement, although their classification indicates that these authors favor a placement of †*Bullichthys* these taxa on the common stem of *Pterothrissus* and *Albula*.

4.2 What does it mean to be a bonefish?

In contrast to Elopiformes, hypotheses of the evolutionary relationships among Albuliformes have remained incongruent between and among molecular and morphological based phylogenetic analyses. Nelson (1973) proposed a clade containing *Pterothrissus*, *Albula*, Halosauridae, and Notacanthidae based on structure of the lower jaw. This arrangement is supported in subsequent morphological investigations by Greenwood (1977), and molecular studies using mtDNA 12S rRNA sequences (Wang et al., 2003) and whole mitochondrial genomes (Inoue et al., 2004; Johnson et al., 2012). However, molecular studies utilizing different combinations of mitochondrial and nuclear genes strongly support a clade containing Anguilliformes and Notacanthiformes (Forey et al., 1996; Tang and Fielitz, 2013; Chen et al., 2014), a result that is also supported by 14 morphological apomorphies that span muscular, cranial, and caudal skeletal elements (Forey et al., 1996; Wiley and Johnson, 2010). Surprisingly, the designation of *Pterothrissus* as an albulid was not questioned in these studies, despite the placement of *Pterothrissus* as sister to a clade comprising Notacanthidae and Halosauridae to the exclusion of *Albula* in mitogenomic studies (Inoue et al. 2004). Both our concatenated nucleotide and amino acid based analyses support the emerging consensus that Anguilliformes and Notacanthiformes represent sister lineages in (Figures 2 & 4). This raises the question of what factors underlie topological incongruence observed between molecular datasets and what this means for the placement of *Pterothrissus*.

While conflicting gene histories often offer a potential explanation for observed instances of topological discordance (Oliver, 2013), there are several reasons to believe that gene-tree species-tree discordance does not represent the full explanation. First, these cases of incongruence are based on multiple mitogenomic studies or studies sampling overlapping nuclear genes. For example, our study utilized six of the nine genes sampled in Near et al. (2012), but provides a different phylogenetic result. Furthermore, none of the individual gene

trees strongly support a clade containing all living “albuliform” lineages, sensu Inoue et al. (2004) (Figure 1). It is instead likely that our taxon sampling strategy, which sampled primarily deeply diverging elopomorph lineages, is responsible for the discordance between the phylogenies generated in our study and those presented in Near et al. (2012). Notacanthiformes and Anguilliformes represent a Cretaceous divergence (Figure 3) and investigations of the substitution rates for both the nuclear and mitochondrial genes used in this and previous investigations of elopomorph systematics suggest a high potential for chance convergences in nucleotide state to have accrued (Dornburg et al., 2014b). Even minor substitution saturation can mislead phylogenetic inference at deeper time scales when the time between divergences is short (Townsend et al., 2012); however, increased taxon sampling may enable substitution patterns to be correctly polarized (Heath et al., 2008; Townsend and Lopez-Giraldez, 2010). This is not to say that random taxon sampling or adding more tipward lineages will automatically overcome substitution saturation, but targeted sampling that attempts to add taxa as close as possible to a focal node may greatly improve phylogenetic inference (Townsend and Lopez-Giraldez, 2010). Taxon sampling appears to be a potential explanation for this case of discordance given our sampling of *Pterothrissus*, representatives of deeply diverging anguilliform lineages, and both notacanthid and halosaurid species. Similarly, taxon sampling may also explain incongruence between studies that support a clade containing Notacanthiformes and Anguilliformes relative to those that use similar loci, as the former are characterized by sampling more deeply diverging lineages (Tang and Fielitz, 2013; Chen et al., 2014). Although outside the scope of this study, obtaining a phylogenomic scale dataset would permit a test of this hypothesis as greatly increasing the number of characters can mitigate the influence of saturation on phylogenetic inferences of topology (Townsend and Lopez-Giraldez, 2010; Townsend et al., 2012), or provide

enough data to prevent loss of power when using filtration metrics that attempt to remove saturated sites (Goremykin et al., 2009).

While our study contributes to a growing consensus that Notacanthiformes represent the sister lineage to Anguilliformes (Forey et al., 1996; Belouze, 2001; Wiley and Johnson, 2010; Santini et al., 2013; Tang and Fielitz, 2013; Chen et al., 2014), the phylogenetic placement of *Pterothrissus* remains unresolved. Examination of the posterior distribution of species trees estimated from the individual genes (Figure 3) provides poor support for the phylogeny that places *Pterothrissus* as the sister lineage of the clade containing Notacanthiformes and Anguilliformes (PP = 0.56). This result is identical to the placement inferred by the concatenated Bayesian nucleotide (PP = 0.81) and maximum likelihood analyses (BSS = 59). Although the placement of *Pterothrissus* within a clade containing *Albula* and Notacanthiformes has been strongly supported by mitogenomic based phylogenetic analyses, these studies resolve *Pterothrissus* as either the sister lineage of Notacanthiformes or a clade containing Notacanthiformes and *Albula* (Inoue et al., 2004; Johnson et al., 2012). While lacking strong support, these studies also do not resolve a clade containing *Pterothrissus* with *Albula*, but both taxa are currently classified in Albulidae (Nelson 2006: 112).

Morphological investigations of *Pterothrissus* have also revealed several puzzling features (Greenwood, 1977). *Pterothrissus* shares several traits with *Albula* including reductions of the intercalar bone and caudal fin ray bases (Greenwood, 1977). *Pterothrissus* and *Albula* also possess a conus arteriosus that contains two rows of valves, a feature also found in *Elops* and *Megalops*. This feature was once considered unique among *Elops*, *Megalops*, and *Pterothrissus* (Gosline, 1965), but both prior and subsequent investigations revealed a conus arteriosus that contains two rows of valves in *Albula* (Smith, 1918; Icardo, 2006). In contrast to *Albula*, *Pterothrissus* also possesses a divided levator arcus palatini muscle and scale modifications of

the lateral line, features that are shared with Notacanthidae but not observed in any other elopomorphs including halosaurids (Forey, 1973; Greenwood, 1977). As there are several characters uniting Notacanthidae and Halosauridae (Figure 5), *Pterothrissus* was placed within bonefishes as this arrangement minimized the instances of convergent character states across the elopomorph tree (Figure 5; Greenwood 1977). When combined, the evidence for an affinity between *Pterothrissus* and *Albula* seems minimal at best, with the hypothesis that *Pterothrissus* represents the sister lineage to Notacanthiformes appearing increasingly more credible. While there is a general lack of phylogenetic resolution with regard to *Pterothrissus*, our analyses and consideration of previous morphological characters cast doubt on both the monophyly of Albuliformes and Albulidae as currently defined (Inoue et al., 2004; Nelson, 2006).

The sister relationship between *Pterothrissus* and †*Istieus* represents the only strongly supported relationship between living and fossil elopomorphs in our analyses (Figure 2C & Figure 3). This result is concordant with other phylogenetic investigations of fossil elopomorphs beginning with Woodward (1901), who proposed a close affinity between †*Istieus* and living *Pterothrissus*. This hypothesis has been continually supported based on various aspects of cranial and caudal anatomy (Greenwood et al., 1966; Forey, 1973; Forey and Maisey, 2010; de Figueiredo et al., 2012). Indeed, the close correspondence between †*Istieus* and *Pterothrissus* led Robins (1989) to suggest that the latter should be a junior synonym of the former, with naming of the fossil genus predating the living form by several decades. With regard to the remaining fossil taxa, our maximum clade credibility tree suggests a very different set of relationships compared to those presented by de Figueiredo et al. (2012), though lacking strong node support (Figure 3). This uncertainty is driven not only by lack of phylogenetic resolution offered by the morphological character matrix (Figure 1), but also reflects the uncertain relationships of *Pterothrissus*. Fossil taxa are often described based on the assumption that *Pterothrissus* and

Albula are a clade, and that †*Farinichthys*, †*Osmeroides*, †*Bullichthys*, †*Brannerion*, †*Baugeichthys*, and †*Lebonichthys* are early diverging members of their common stem lineage (de Figueiredo et al., 2012). However, these interpretations are based on analyses that did not sample Notacanthiformes or Anguilliformes. If the group comprising *Pterothrissus* and *Albula* is in fact not monophyletic, this will require a reevaluation of character evolution that could substantially transform the classification of these fossil taxa. Clearly the affinity of *Pterothrissus* warrants further investigation. In addition, it is possible that further anatomical information from unsampled but potentially critical fossil taxa like the Late Cretaceous †*Enchelurus*, which is interpreted as the immediate sister lineage to the clade comprising Notacanthiformes and Anguilliformes by Belouze (2002), might help resolve conflicting morphological signals among “albuliform” taxa.

4.3 Anguilliform phylogenetic relationships

In an effort to assess elopomorph inter-ordinal relationships, our study minimized sampling of all but the deeply divergent anguilliform lineages. Despite the low taxon sampling of Anguilliformes, our results are largely congruent with an emerging consensus regarding their phylogenetic relationships. The monophyly of *Conger*, *Heteroconger*, and Ophichthidae (Figures 2, 3, and 4) has also been suggested by several other recent studies (Johnson et al., 2012; Santini et al., 2013; Chen et al., 2014). Likewise, our resolution of Saccopharyngiformes (deep sea gulper eels) as nested within Anguilliformes (Figures 2, 3, and 4) is also in agreement with most phylogenetic studies (Forey et al., 1996; Belouze, 2001; Inoue et al., 2003; Wiley and Johnson, 2010; Johnson et al., 2012; Near et al., 2012; Santini et al., 2013; Chen et al., 2014). While the phylogenies resulting from analyses of concatenated datasets are unconventional in strongly supporting monophyly of gulper eels and a clade comprised of Muraenidae and Serrivomeridae

(Figures 2 & 4B), this result is not supported in the species tree analysis, instead resolving gulper eels as the sister lineages of a clade containing Anguillidae and Serrivomeridae (Figure 4A) that is congruent with most molecular studies of Anguilliform relationships that posit a deep sea origin of freshwater eels (Inoue et al., 2010b). Our analyses also offer little insight into the phylogenetic relationship of *Protanguilla* (Figures 2, 3, and 4), which was described as the earliest diverging lineage of Anguilliformes based on a suite of character states found only in Late Cretaceous fossil lineages (Johnson et al., 2012). However, subsequent molecular investigations have resolved *Protanguilla* in a clade with Synphobranchidae (Santini et al., 2013; Chen et al., 2014), thereby questioning whether this species represents a “living fossil” or highly unusual synphobranchid (Santini et al., 2013). As our study does not sample synphobranchids we provide little insight into this question and suggest that a phylogenetic investigation of living and fossil anguilliformes, including *Protanguilla*, represents an important next step towards resolution of the elopomorph phylogeny.

4.4 The timeline of elopomorph cladogenesis

Our relaxed molecular clock age estimates for the timing of diversification among the major lineages of elopomorphs is congruent with the majority of published molecular age estimates whether or not we included fossils as tip taxa. While node based age estimates were largely younger than fossil tip based estimates, the 95% HPD intervals between these approaches broadly overlapped (Figure 3). This pattern of younger ages for analyses conditioned on node based calibrations has been found in several other studies (Wood et al., 2013; Arcila et al., 2014; Dornburg et al., 2014a), and suggests that further evaluations of the potential pitfalls that are encountered when conducting divergence time estimates using morphological characters represents an exciting and much needed area of exploration in systematic biology. However,

given the broad overlap between ages inferred for this study, this suggests any bias introduced through clade-specific heterogeneity of character evolution is minimal (Soltis et al., 2002; Dornburg et al., 2012).

While divergence time estimates for early diverging ray-finned fishes as well as more derived acanthomorph lineages often differ by over one hundred million years between studies (Inoue et al., 2005; Yamanoue et al., 2006; Dornburg et al., 2011b; Near et al., 2013; Near et al., 2014b; Price et al., 2014), instances of extremely discordant age estimates are not prevalent among different elopomorph molecular divergence time estimates (Broughton, 2010; Johnson et al., 2012; Near et al., 2012; Santini et al., 2013). Despite sampling different genes and using different calibration strategies, most studies including ours estimate that crown-lineage elopomorphs originated in the Jurassic (Figure 3). Although Johnson et al. (2011) estimate a Triassic origin of Elopomorpha, this age estimate was reconciled with nuclear gene based estimates in a recent study of nucleotide saturation in the mitochondrial genomes of ray-finned fishes (Dornburg et al., 2014b). The congruence in molecular age estimates from multiple studies is encouraging and suggests the establishment of a consistent timeline from which to investigate the mode and tempo of evolutionary diversification among living elopomorph fishes.

While our age estimates are consistent with other molecular studies, our incorporation of fossil lineages as tip taxa extends the potential geologic range of several elopomorph lineages (Figure 3). †*Istieus* and †*Lebonichthys* are known from the Late Cretaceous (Mayrinck et al. 2010); however, our analyses suggest lineages related to these fossil taxa diverged in the Early Cretaceous (Figure 3). Likewise, the lineage that gave rise to the Paleocene †*Farinichthys* may also potentially extend back into the Early Cretaceous. It is likely that the lack of precision in the divergence time estimates is driven by a combination of topological uncertainty and by the limited number of characters in the morphological matrix (Rannala and Yang, 2007). However

this branch length estimation bias should be somewhat mitigated by the large numbers of calibration age priors (Inoue et al., 2010a; Dornburg et al., 2011a). Additionally, branch length estimation biases resulting from the number of characters sampled should lead to a global loss of precision (Rannala and Yang 2007); however, the divergence times for the Early Cretaceous lineages †*Ichthyemidion*, †*Baugeichthys*, and †*Paraelops* are closer to the expectations from the geological record (Gomez et al. 2002; Mayrinck et al. 2010; Appendix A). These results mirror recent fossil discoveries, which are leading to a reinterpretation of the evolutionary history of elopomorph fishes (Mayrinck et al., 2010; de Figueiredo et al., 2012; Sweetman, 2013). Combining our results with other molecular clock studies suggests that we are honing in on a timeline of elopomorph diversification (Broughton, 2010; Johnson et al., 2012; Near et al., 2012; Santini et al., 2013).

5 Conclusions

Since the groundbreaking assessment of relationships among major lineages of teleost fishes by Greenwood et al. (1966), there has been a continual effort to resolve not only the placement of Elopomorpha among teleosts (Arratia, 1996, 1998; Near et al., 2012; Faircloth et al., 2013), but also their inter-ordinal and species level relationships (Forey et al., 1996; Filleul and Lavoue, 2001; Inoue et al., 2003; Obermiller and Pfeiler, 2003; Wang et al., 2003; Inoue et al., 2010b; Santini et al., 2013; Tang and Fielitz, 2013; Chen et al., 2014). Our study represents a novel perspective on the phylogenetic relationships of elopomorph fishes through the integration of neontological and paleontological data in a time calibrated framework. We highlight several areas of congruence among phylogenetic investigations, such as the sister relationship between Elopiformes and all other elopomorphs and the nested placement of Saccopharyngiformes with Anguilliformes (Figures 2, 3, and 4). While this congruence among different studies provides

confidence in these particular inferences, our study also highlights that the phylogenetic relationships among albuliform fishes remains largely unresolved. While we provide a set of strongly supported phylogenetic inferences that Notacanthiformes is the sister lineage of Anguilliformes, we fail to provide a well-supported resolution of the phylogenetic relationships of *Pterothrissus*.

Our study highlights the critical role of resolving the placement of *Pterothrissus* in the context of early albuliform fossil lineages, making this a clear target for future studies of elopomorph relationships using phylogenomic approaches (Faircloth et al., 2012; Lemmon et al., 2012). The taxonomy and interpretation of morphological character states in fossil Albuliformes is based on the assumption that *Albula* and *Pterothrissus* represent sister lineages (de Figueiredo et al., 2012); however, the results of our analyses suggest little support for this clade, and indicate there is little support for the phylogenetic resolution of most extinct elopomorph lineages. Future analyses of elopomorph fossil lineages should aim to include notacanthiform taxa. Despite the substantial uncertainty result observed in these phylogenetic hypotheses, the explicit integration of fossil lineages into a Bayesian molecular clock calibrated framework reveals clear concordance with other published molecular age estimates and the fossil record, suggesting we are indeed resolving a time calibrated Elopomorph Tree of Life.

Acknowledgements

Tissue specimens for this work were graciously provided by K.-T. Shao of the Biodiversity Research Museum, M. Miya, J. A. Moore, W. L. Smith, G. Wagner, and P. C. Wainwright. G. Watkins-Colwell assisted with museum collections. We thank the two anonymous referees for providing valuable feedback to an earlier version of this manuscript. This research was supported by the Peabody Museum of Natural History and research grants from the National Science Foundation (DEB-1061806, DEB-1110552, and ANT-1341661)

Appendix A

Appendix A.1: Age evidence for node-based calibrations

Node: MRCA Elopomorpha.

First: †*Anaethalion zapporum* from the Rögling Formation, village of Schamhaupten, near Eichstätt, Bavaria, Germany (Arratia, 2000).

Cladistic justification: †*Anaethalion* is resolved as member of crown-group Elopomorpha, and more specifically crown-group Elopiformes, in a maximum parsimony analysis of 41 taxa and 167 morphological characters (Arratia, 2013).

Stratigraphy: The Rögling Formation at Schamhaupten can be constrained to the †*Neochetoceras rebouletianum* Horizon within the †*Lithacoceras ulmense* Subzone of the †*Hybonoticeras beckeri* Ammonite Zone, indicating a latest Kimmeridgian age (Schweigert, 2007; Ogg and Hinnov, 2012b). The top of the Kimmeridgian is dated to 152.1 ± 0.9 Ma (Ogg and Hinnov, 2012b), from which we derive a minimum age of 151.2 Ma.

Calibration: Minimum age of 151.2 Ma with a mean of 167.1 Ma and 95% confidence interval of 189.3 Ma. This probabilistic bound has been calculated based on the sequence of successive outgroups to Elopomorpha with stratigraphically consistent first appearances (i.e., more distant outgroups show earlier first appearances) (Hedman 2010). We based our sequence of outgroups on Arratia (2013). Stratigraphically consistent successive outgroups to the calibrated clade are: †Ichthyodectiformes (166.1 Ma, based on †*Occithrissops willsoni*; see argumentation in Friedman et al. 2013); †*Leptolepis coryphaenoides* (181.7 Ma; see argumentation in Friedman et al. 2013); †*Dorsetichthys bechei* (193.81 Ma; see argumentation for †*Pholidophorus bechei* in Friedman et al. 2013); †Pholidophoridae (221.0 Ma, based on †*Knerichthys bronni*; approximate age estimate for the top of the Carnian given by Ogg 2012); †*Prohalecites porroi* (236.0; based

on age estimate and associated uncertainty for the top of the Norian given by Ogg (2012));
 Holostei (247.1 Ma, based on †*Watsonulus eugnathoides*; see argumentation in Friedman et al.
 2013). As in Friedman et al. (2013), we specify a hard upper bound for our calculations of 322.8
 Ma based on the stem neopterygian †*Discoserra*.

Node: MRCA Anguilliformes and Notacanthiformes.

First: †*Angullavis bathshebae*, †*A. quadripinnis*, †*Libanenchelys bultyncki*, †*Hayenchelys*
germanus (Belouze, 2001; Belouze et al., 2003; Forey et al., 2003; Taverne, 2004), Hadjoula,
 Lebanon.

Cladistic justification: †*Angullavis* and †*Hayenchelys* are resolved as stem anguilliforms in a
 maximum parsimony analysis of 60 taxa and 123 morphological characters (Belouze, 2001).
 †*Libanenchelys* has not been included in a formal phylogenetic analysis, but shows numerous
 anguilliform synapomorphies including absence of the pelvic girdle (Taverne, 2004).

Stratigraphy: The Hajoula *Lagerstätte* is located near the top of subdivision IVd in the local
 stratigraphic for Lebanese Cenomanian deposits (Hückel, 1970: fig. 3), and lies stratigraphically
 below the Hakel *Lagerstätte* (see details below for *Lebonichthys*). Ammonites attributed to
 †*Mantilliceras mantelli* are known from the overlying Cenomanian subdivision Va (Zummofen,
 1926; Dalla Vecchia et al., 2002). †*M. mantelli* defines the earliest complete ammonite zone of
 the Cenomanian. The top of the †*Mantelliceras mantelli* Zone can be dated as approximately
 98.0 Ma (Ogg and Hinnov, 2012a), which we apply as our minimum age estimate for the MRCA
 of Anguilliformes and Notacanthiformes.

Calibration: Minimum age of 98.0 Ma with a mean of 123.5 Ma and 95% confidence interval of
 152.7 Ma. This probabilistic bound has been calculated based on the sequence of successive

outgroups to Notacanthiformes + Anguilliformes with stratigraphically consistent first appearances (Hedman, 2010). Our sequence of outgroups includes those given above for Elopomorpha plus immediate outgroups to Notacanthiformes + Anguilliformes in the phylogenetic hypothesis presented by Forey et al. (1996). Stratigraphically consistent successive outgroups to the calibrated clade are: Albuliformes (130.3 Ma, based on †*Baugeichthys caeruleus*; see argumentation below); Elopiformes (151.2 Ma, based on †*Anaethalion zapporum*; see argumentation above and in Friedman et al. 2013); outgroup sequence to Elopomorpha given above, with the same hard upper bound of 322.8 Ma.

Appendix A.2: Age evidence for non-contemporaneous terminal taxa

†*Ichthyemidion* (131.3-125.9 Ma). †*Ichthyemidion* is known from the deposits of El Montsec, Lérida Province, Spain (Poyato-Ariza, 1995). The lithographic limestones of El Montsec are often reported as late Berriasian-early Valanginian in age (De Gibert et al., 2000; Brocklehurst et al., 2012) and this date is widely applied by molecular clock analyses using fossil-based calibrations from the site (Peng et al., 2006; Lavoué et al., 2012; Near et al., 2012). However, clear evidence in support of this estimate is lacking, and a younger age for at least some portions of this unit seems likely. The arguments presented here represent a synthesis of those presented by Gomez et al. (2002) in their review of the geological setting of El Montsec. The marine unit underlying this horizon yields benthic foraminifera (†*Trocholina alpina*) and dasycladales indicating a Berriasian age (Gomez et al., 2002). The freshwater lithographic limestones that bear specimens of †*Ichthyemidion* are estimated to be of Berriasian-early Barremian age on the basis of ostracodes and charophyte algae (Brenner et al., 1974; Martín-Closas and López-Morón, 1995). These deposits are overlain by marine limestones dated to the late Barremian-early Aptian

on the basis of orbitolinid foraminifera (Peybernès, 1976; Schroeder et al., 1982), which constrain the minimum age of †*Ichthyemidion*. The Barremian is dated as 130.8 ± 0.5 to 126.3 ± 0.4 Ma (Ogg and Hinnov, 2012a), from which we derive an age range estimate of 131.3-125.9 Ma that has also been used in a previous study dating Barremian gonorynchiform fossil fishes (Near et al., 2014a).

†***Lebonichthys* (100.9-83.3 Ma)**. †*Lebonichthys* is known from three sites in the Lebanese Cretaceous: Hakel, Namoura and Sahel Alma (Forey et al., 2003). The Hakel Lagerstätte is placed within subdivision Va in the scheme used to divide the Lebanese Cenomanian (Hückel, 1970; Dalla Vecchia F. M. et al., 2002). Ammonites attributed to †*Mantilliceras mantelli* are known from this subdivision (Zumoffen, 1926; Dalla Vecchia F. M. et al., 2002); this species defines the earliest complete ammonite zone of the Cenomanian. The top of the †*Mantilliceras mantelli* Zone can be dated as approximately 98.0 Ma, while the base of the Cenomanian is dated as 100.5 ± 0.4 Ma (Ogg and Hinnov, 2012a). The exact stratigraphic relationship of Namoura to Hakel is unclear. Foraminifera collected above the Namoura fish bed indicate a middle Cenomanian or older age for this horizon (Dalla Vecchia F. M. et al., 2002). The fish beds of Sahel Alma are interpreted as late Santonian in age (Ejel and Dubertret, 1966). The Santonian is dated as 86.3 ± 0.5 to 83.6 ± 0.3 Ma (Ogg and Hinnov, 2012a). We apply an age range estimate of 100.9-83.3 Ma for †*Lebonichthys*.

†***Osmeroides* (110.22-87.86 Ma)**. †*Osmeroides* is represented by several species. †*Osmeroides lewesiensis* appears to be the basis of codes provided by de Figueiredo et al. (2012), and is the subject of the most detailed description of any species of †*Osmeroides* by Forey (1973). This species is known the Albion Gault Clay and several levels within the English Chalk. Woodward (1902-1912) lists specimens from ‘Turonian zones’ and the Zone of †*Holaster planus*

(†*Micraster cortestudinarium* Zone of later usage; Mortimore et al., 2001). This correlates the youngest occurrences of †*Osmeroides* with the †*Scaphites ventricosus* Zone of the Western Interior. The top of this zone is estimated as 87.86 Ma (Ogg and Hinnov, 2012a). The Gault Clay Formation spans the †*Hoplites dentatus* through †*Mortoniceras inflatus* zones of the Albian (Gale and Owen, 2010). The base of the †*Hoplites dentatus* Zone is estimated as 110.22 Ma (Ogg and Hinnov, 2012a). We therefore propose an age-range estimate of 110.22-87.86 Ma for †*Osmeroides*.

†***Istieus* (81.28-80.67 Ma)**. †*Istieus* is known from the Coesfeld Member of Westphalia, Germany (Riegraf, 1990). The presence of the ammonite †*Trachyschaphites spiniger* permits correlation with sequences in the Western Interior of the United States, and indicates an age younger than the †*Scaphites hippocrepis* III Zone but younger than the †*Baculites mclearnii* Zone (Cobban and Kennedy, 1992). The top of the †*Scaphites hippocrepis* III Zone is estimated as 81.28 Ma, while the base of the †*Baculites mclearnii* Zone is estimated as 80.67 Ma (Ogg and Hinnov, 2012a). On this basis, we propose an age-range estimate of 81.28-80.67 Ma for †*Istieus*.

†***Brannerion* (113.4-110 Ma)**. This genus is best known from the Romualdo Member of the Santana Formation (Blum, 1991), which is assigned an early Albian age based on palynological studies (Heimhofer and Hochuli, 2010). The end of the early Albian is placed approximately at 110 Ma (Ogg and Hinnov, 2012a), while the base of the stage is placed at 113 ± 0.4 Ma. We therefore propose an age range estimate of 113.4-110 Ma for †*Brannerion*.

†***Baugeichthys* (134.5-130.3 Ma)**. †*Baugeichthys* is known exclusively from limestones in the Massif Des Bauges, France. This unit has been interpreted as Hauterivian in age (Filleul, 2000). The Hauterivian is dated from as 133.9 ± 0.6 to 130.8 ± 0.5 Ma (Ogg and Hinnov, 2012a). We therefore propose an age-range estimate of 134.5-130.3 Ma for †*Baugeichthys*.

†*Paraelops* (113.4-110 Ma). This genus is best known from the Romualdo Member of the Santana Formation (Maisey and Blum, 1991). We therefore apply the same age range estimate given above for †*Brannerion*.

†*Farinichthys* (65.94-62.22 Ma). †*Farinichthys* is known from a single specimen from the Maria Farinha Formation of the Pernambuco-Paraíba Basin, Brazil (Gallo and De Figueiredo, 2002). The Maria Farinha Formation spans planktonic foraminiferal zones P α -P2 (Fauth et al., 2005). The estimated age of the base of P α is 65.94 Ma, while the top of P2 is estimated as 62.22 Ma (Anthonissen and Ogg, 2012). We therefore propose an age-range estimate of 65.94-62.22 Ma for †*Farinichthys*.

†*Bullichthys* (113.4-110 Ma). †*Bullichthys* is known from the Romualdo Member of the Santana Formation (Mayrinck et al., 2010). We therefore apply the same age range estimate given above for †*Brannerion*.

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1066 ***Figure Legends***

1067
 1068 Figure 1: Fifty percent majority-rule consensus trees resulting from a Bayesian analysis of the
 1069 individual nuclear gene datasets and the morphological character matrix. Posterior probability
 1070 (PP) estimates of 1.00 are indicated by solid black circles, while PP between 0.95 and 0.99 are
 1071 represented by open circles. All branch lengths are in substitution units.

1072
 1073 Figure 2: Phylogenetic relationships of Elopomorpha based on concatenated datasets. A.
 1074 Phylogeny inferred using maximum likelihood. Solid black circles at nodes represent bootstrap

support values (BS) of 100, open circles represent BS between 90 and 99, and gray circles represent BS of 70 to 90. B. Fifty percent majority-rule consensus tree resulting from Bayesian analysis of the concatenated the nuclear gene dataset. Posterior probability (PP) estimates of 1.00 are indicated by solid black circles, while PP between 0.95 and 0.99 are represented by open circles. C. Fifty percent majority-rule consensus tree resulting from a combined Bayesian analysis of the nuclear genes and morphological character matrix. Solid black circles represent PP estimates of 1.00 and open circles represent PP estimates of 0.99-0.95. All branch lengths are in substitution units.

Figure 3: Maximum clade credibility chronogram of major living and fossil elopomorph lineages. Bars indicate 95% HPD intervals of age estimates. Gray bars indicate posterior probabilities greater than 0.95. Light bars indicate posterior probabilities below 0.95. Dotted 95% HPD intervals above nodes indicate divergence time estimates obtained using node based calibrations. Insert: Comparison of ages estimated for three major clades in this study to recent hypotheses from the literature.

Figure 4: Phylogenetic relationships of major elopomorph lineages based on coalescent and amino acid analyses. A. Maximum clade credibility tree tree resulting from the multi-species coalescent species tree analysis (*BEAST) using the nuclear genes. B. Maximum clade credibility tree of the posterior distribution of phylogenies based on amino acid analysis using PhyloBayes. Solid black circles represent PP estimates of 1.00, open circles represent PP estimates of 0.95-0.99. All branch lengths are in substitution units.

1098 Figure 5: Two competing hypotheses on the placement of Pterothrissus with resulting
1099 apomorphies presented in Greenwood 1977: (A) Shared character states between Pterothrissus
1100 and Notacanthidae and (B) shared character states between Notacanthidae and Halosauride.

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Figure 1

Figure 2

Figure 3

A.

A.

B. Bayesian, amino acid

Figure 5

A.

B.

Table 1: List of Taxa examined in this study, voucher numbers, and NCBI Genbank accession numbers. Tissue samples were obtained from the Yale Peabody Museum of Natural History (YFTC), the Taiwan Research Center for Biodiversity (ASIZP), the Illinois Natural History Survey (INHS), and the research collections of William Leo Smith (WLS) and Masaki Miya (M. Miya).

Taxon	Tissue source	Genbank gi numbers					
		<i>Glyt</i>	<i>myh6</i>	<i>Ptr</i>	<i>Plag12</i>	<i>rag1</i>	<i>zic1</i>
<i>Gymnarchus niloticus</i>	YPM 17285	JX190256	JX190403	JX190669	JX190542	JX190806	JX191256
<i>Hiodon tergisus</i>	INHS 40058	pending	pending	pending	pending	pending	pending
<i>Albula vulpes</i>	YFTC 11262	---	JX190390	JX190653	---	JX190803	JX191247
<i>Albula glossodonta</i>	ASIZP 0912129	pending	JX190391	JX190654	JX190532	pending	JX191248
<i>Pterothrissus gissu</i>	ASIZP 0912103	pending	pending	pending	---	---	pending
<i>Aldrovandia affinis</i>	ASIZP 0911257	---	JX190392	JX190655	JX190533	pending	JX191249
<i>Halosauropsis macrochir</i>	ASIZP 0911269	JX190246	JX190393	JX190656	JX190534	pending	pending
<i>Notacanthus chemnitzii</i>	genbank	---	---	---	---	FJ896411	FJ906631
<i>Elops saurus</i>	YFTC 11542	JX190247	JX190394	JX190657	JX190535	FJ896408	JX191250
<i>Megalops atlanticus</i>	YFTC 11191	---	pending	JX190658	JX190536	AY430204	JX191251
<i>Anguilla rostrata</i>	YFTC 11192	JX190248	JX190395	JX190659	---	---	JX191252
<i>Protanguilla palau</i>	M. Miya	pending	pending	---	---	KF681948	---
<i>Conger oceanicus</i>	YFTC 17352	JX190249	JX190396	JX190660	JX190537	---	JX191253
<i>Heteroconger hassi</i>	YPM 20545	JX190250	---	JX190661	---	---	JX191254
<i>Echidna rhodochilus</i>	YFTC 11534	JX190251	JX190397	JX190662	JX190538	JX190805	---
<i>Myrichthys maculosus</i>	YFTC 11520	JX190252	JX190399	JX190664	JX190539	---	---
<i>Serrivomer beanii</i>	YFTC 14532	---	JX190400	JX190665	JX190540	FJ896415	pending
<i>Saccopharynx ampullaceus</i>	WLS 1164	JX190253	JX190401	JX190666	JX190541	---	pending
<i>Eurypharynx pelecanoides</i>	YFTC 13692	JX190254	JX190402	JX190667	---	pending	JX191255

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Table 2: Nucleotide substitution models and partitions selected by PartitionFinder. Numbers following the gene name correspond with codon position.

Subset	Partitions	Sites in Alignment	Model
1	<i>Glyt_1, RAG1_1</i>	1-834\3 2983-4278\3	GTR+G
2	<i>Glyt_2, myh6_1, plag12_1, PTR_1, RAG1_2, Zic1_1</i>	2-834\3 835-1563\3 1564-2193\3 2194-2982\3 2984-4278\3 4279-5133\3	HKY+I+G
3	<i>Glyt_3, myh6_3, PTR_3</i>	3-834\3 837-1563\3 2196-2982\3	GTR+G
4	<i>myh6_2, PTR_2</i>	836-1563\3 2195-2982\3	HKY
5	<i>plag12_2, Zic1_2</i>	1565-2193\3 4280-5133\3	JC
6	<i>plag12_3</i>	1566-2193\3	GTR+G
7	<i>RAG1_3</i>	2985-4278\3	HKY+G
8	<i>Zic1_3</i>	4281-5133\3	HKY+I+G

1114 Elopomorphs (eels, tarpons, and bonefish) represent the sister lineage of all other teleost
1115 fishes

1116 Several higher level relationships among fossil and living lineages have been difficult to
1117 evaluate

1118 We integrate nDNA and morphological data from living and extinct taxa to investigate
1119 higher-level elopomorph relationships

1120 We reveal substantial phylogenetic uncertainty, questioning the stability of the taxonomy of
1121 fossil Elopomorpha.

1122 We provide a time-calibrated phylogeny for investigations of both living and extinct
1123 lineages of elopomorph fishes.

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