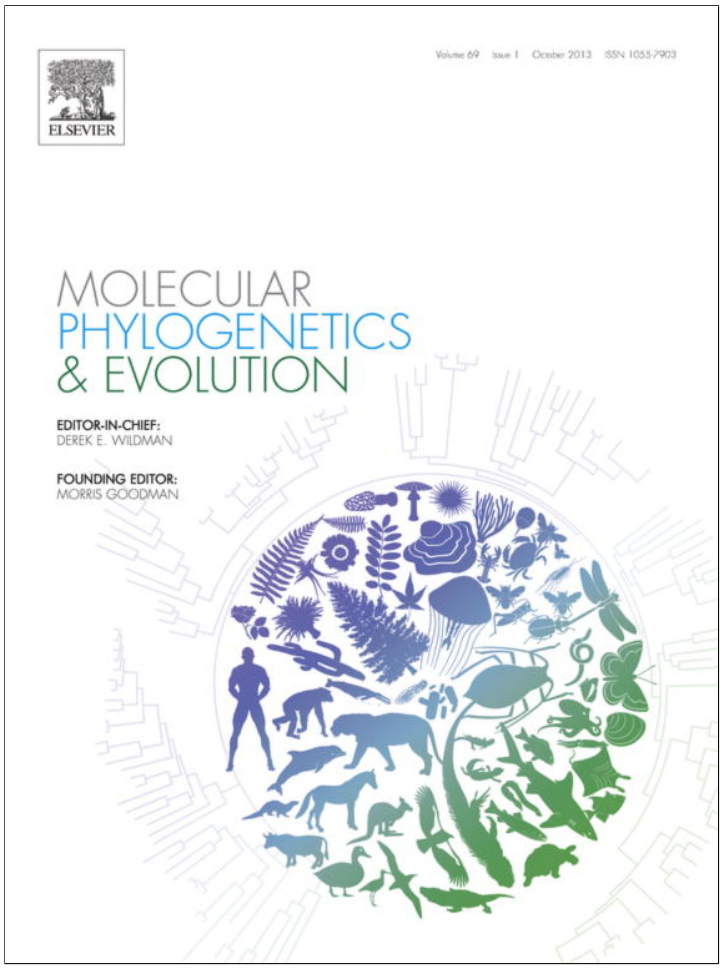

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A new multi-locus timescale reveals the evolutionary basis of diversity patterns in triggerfishes and filefishes (Balistidae, Monacanthidae; Tetraodontiformes)

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ABSTRACT

Balistoid fishes (triggerfishes, filefishes, leatherjackets) represent one of the most successful radiations of tetraodontiform fishes across the world's oceans. Balistids (triggerfishes) are largely circumtropical in coral reef environments while most monacanthids (filefishes, leatherjackets) are distributed across reef and non-reef habitats in the Indo-western Pacific. Although members of these clades share a distinctive mode of locomotion that relies upon coordinated oscillation or undulation of enlarged dorsal and anal fins, species richness as well as morphologial and ecological diversity are generally considered to be higher in monacanthids than in triggerfishes. Explicit evolutionary comparisons of diversity patterns between these sister clades have been hampered by the paucity of systematic studies of filefishes relative to triggerfishes. Furthermore, a well-sampled molecular timescale for balistoids is lacking, hindering our understanding of the evolutionary history of these fishes. Here, we produce the largest balistoid molecular dataset to date, based on two mitochondrial and three nuclear loci, for a total of 86 species, and we time-calibrate it using three tetraodontiform fossils. We show that several of the traditional monacanthid genera are not monophyletic and that the balistid *Xenobalistes tumidipectoris* is nested within the genus *Xanthichthys*, and suggest that the generic name *Xenobalistes* be dissolved. Our timetree reveals a Late Miocene origin of balistids, in accordance with previous studies, but a Late Eocene age for the crown monacanthids, which experienced significant diversification during the Late Oligocene and Early Miocene. Comparative analyses reveal no significant family-level differences in rates of speciation or body size evolution, suggesting that the greater diversity of filefishes can be attributed to their more ancient crown age compared to triggerfishes.

1. Introduction

With about 148 extant species distributed across all temperate and tropical seas (Froese and Pauly, 2012), balistoid fishes (triggerfishes, filefishes, leatherjackets), represent an important radiation of marine percomorph fishes. Currently divided into two families, the Balistidae (triggerfishes) and the Monacanthidae (filefishes, leatherjackets), balistoid fishes are characterized by a number of morphological reductions such as loss of the pelvic spines; miniaturization of the pelvic fin rays, which become encased within scale plates; and reduction in the number of dorsal spines and their associated pterygiophores (Tyler, 1980; Santini and Tyler, 2003). Balistoids are also characterized by a unique swimming style in which coupled oscillations or undulations of the soft dorsal and anal fins are used to obtain forward thrust (Blake, 1978; Dornburg et al., 2011).

In spite of their close evolutionary relationship, triggerfishes and filefishes exhibit strongly contrasting patterns of species richness and morphological diversity. Balistids comprise 42 species while monacanthids include at least 106 species (Froese and Pauly, 2012). Body shape diversity and osteology in balistids appear relatively conserved (e.g., Matsuura, 1979; Tyler, 1980; Dornburg et al., 2008). In contrast monacanthids, which span from just 2 cm in body length in *Rudarius minutus* to over 110 cm in *Aluterus scriptus*, range from oblong (e.g., *Monacanthus*) to almost circular (e.g., *Brachaluteres*) to eel-like (e.g., *Anacanthus*) in body shape. Sexual dimorphism in triggerfishes occurs in only a few species (e.g. *Xanthichthys*) but occurs in many genera of filefishes (Hutchins, 1992). Distributional patterns also differ signficantly between triggerfishes and filefishes. Whereas almost all triggerfishes are found circumtropically in association with coral reefs, filefishes are

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largely restricted to the Indo-West Pacific, with only a few species in the Atlantic and Red Sea. However, filefishes occur in a much greater diversity of habitats across their range, including coral reefs, rocky reefs, seagrass beds and sandy bottom environments up to a depth of about 200 m (Froese and Pauly, 2012).

A lack of a robust balistoid-wide phylogeny has hindered quantitative study of the underlying causes of differential diversification in triggerfishes and filefishes. Filefishes have been especially understudied, with the pre-cladistic morphological phylogenies only including one representative per each known genus (Matsuura, 1979; Tyler, 1980). Although several recently published molecular phylogenies included some monacanthid species (Holcroft, 2005; Alfaro et al., 2007; Yamanoue et al., 2009), the largest study published to date (Yamanoue et al., 2009) included only 21 species, about 20% of the extant diversity. Furthermore, these studies have typically included only a single representative species per genus, thus limiting phylogenetic tests of the current taxonomy. Testing the monophyly of currently recognized filefish lineages is important to understanding whether the high morphological variability found in many of the currently recognized monacanthid genera (Hutchins and Swainston, 1985; Hutchins, 1992, 1997) is real or an artifact of errors in taxonomic assignment driven by the pervasive patterns of sexual dimorphism and cryptic coloration across the group.

The phylogenetic and evolutionary history of triggerfishes is better understood due to morphological (Matsuura, 1979; Tyler, 1980) and molecular phylogenies (Yamanoue et al., 2009, Dornburg et al., 2008, 2011), some of which have been time-calibrated (e.g. Dornburg et al., 2008, 2011) with the help of fossils (Santini and Tyler, 2003, 2004). Triggerfishes diverged from filefishes in the Middle Eocene, but the crown radiation did not begin until the Late Miocene, suggesting that the modern fauna may have diversified in response to the Late Miocene/Early Pliocene reorganization of coral reef communities (Wood, 1999). Currently available timescales for monacanthids are extremly limited. Alfaro et al. (2007) estimated a Late Oligocene origin for crown filefishes based upon 10% of the extant diversity. In contrast, Yamanoue et al. estimate a much more ancient divergence in the Mesozoic between triggerfishes and filefishes based upon mitogenomic data (Yamanoue et al., 2006).

A more robust phylogenetic framework would permit the first tests of general hypotheses that could explain differences in balistoid species richness, and phenotypic and ecological diversification. If crown monacanthids did originate in the Oligocene, the greater diversity of filefishes could simply reflect the greater length of time this group has had to diversify relative to triggerfishes, rather than any intrinsic differences in the rate of speciation or character evolution between these two clades (Collar et al., 2005). Alternatively, monacanthid diversity might be due to higher intrinsic rates of evolution within this group. Habitat may also explain some aspects of biodiversity. For example, most triggerfishes and many filefishes are found on coral reefs, which have been shown to increase diversification rates in other fishes (Alfaro et al., 2007; Price et al., 2011; Cowman and Bellwood, 2011; Santini et al., 2013a).

To answer these questions, and to shed further light on the evolutionary history of this group, we assembled the largest molecular dataset to date for balistoid fishes by sequencing two mitochondrial and three nuclear loci for 86 balistoid species (about 60% of the extant diversity) and three outgroups. We time calibrated the new molecular phylogeny with three fossil constraints, and used the resulting timetree to investigate the pattern of lineage diversification of balistoid fishes, as well as the evolution of body size among trigger- and filefishes. We also used this new phylogeny to revise the classification of the balistids by showing that *Xenobalistes tumidipectoris*, known from only a small specimen of about 60 mm found in the stomach of a tuna (Matsuura, 1981), is likely a juvenile *Xanthichthys*, and suggest changing its name to *Xanthichthys tumidipectoris*.

2. Materials and methods

2.1. Sampling

We secured tissue samples for 32 species of balistids (76% of extant species; 11 of 12 described genera), 43 species of monacanthids (41% of extant species; 21 of 31 described genera), plus three outgroups through loans from university or museum collections, or purchases through the pet trade (Table 1). We also obtained sequence data for the mitochondrial genes used in this study for *Xenobalistes tumidipectoris*, the only representative of the one balistid genus missing from our sampling, and an additional 10 species of monacanthids from GenBank, bringing our final sampling to 33 balistids and 53 monacanthids. To verify the identification of our tissue samples all available voucher specimens were compared to fish identification keys, and all extractions were barcoded for cytochrome oxidase subunit I; sequences were compared to those in the barcode of life database, with only those having 99% or higher similarity to corresponding sequences in the database being retained. (http://www.barcodinglife.com/index.php/IDS_OpenIdEngine).

The sister lineage to the balistoids is not known with confidence due to uncertainty among higher-level tetraodontiform relationships (Winterbottom, 1974; Tyler, 1980; Santini and Tyler, 2003, 2004; Holcroft, 2005; Alfaro et al., 2007; Yamanoue et al., 2008). We included two molids (*Mola mola*, *Ranzania laevis*) and a diodontid (*Diodon hystrix*) as outgroups. These lineages have been suggested to be closely related to balistoids in other recent molecular studies (Holcroft, 2005; Alfaro et al., 2007; Yamanoue et al., 2008).

2.2. DNA extraction, PCR amplification, and sequencing

DNA was extracted from muscle tissue samples or fin clips stored in 70% ethanol using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the protocol suggested by the manufacturer. Two mitochondrial genes, cytochrome oxidase subunit I (*cox1*) and cytochrome b (*Cytb*), and three nuclear genes, cardiac muscle myosin heavy chain 6 alpha (*myh6*), recombination activating gene 1 (*Rag1*), and rhodopsin (*Rh*), were amplified using the polymerase chain reaction (PCR). Primers and PCR conditions are as described in Santini et al. (2013a, 2013b) and Sorenson et al. (2013). We used ExoSap (Amersham Biosciences) to remove the excess dNTPs and unincorporated primers from the PCR products. Purified products were cycle-sequenced using the BigDye Terminator v.3.1 cycle sequencing kit (1/8th reaction) (Applied Biosystems) with each gene's original or additional internal primers used for amplification. The cycle sequencing protocol consisted of 25 cycles with a 10-s 94 °C denaturation, 5-s of 50 °C annealing, and a 4-min 60 °C extension stage. Sequencing was conducted at the Yale University DNA Analysis Facility using an ABI 3730xl DNA Genetic Analyzer (Applied Biosystems).

2.3. Phylogenetic analysis

Chromatograms were visually assessed and assembled into contigs using Geneious 5.4 (Drummond et al., 2011). The consensus sequences for each individual gene were aligned in Geneious using the MUSCLE software (Edgar, 2004), and the alignments subsequently checked by eye for accuracy. The sequences were trimmed to minimize missing characters, and our final data matrix consisted of 651 bp for *cox1*, 1107 bp for *Cytb*, 774 bp for *myh6*, 1392 bp for

Table 1
List of taxa included in this study with tissue voucher and GenBank numbers. Tissues were from the personal collections of Michael E. Alfaro (MEA), Brian Victor Alfaro (VRA), Sean Kong (SK) and Francesco Santini (FS) at UCLA, and Peter Wainwright (PW) at UC Davis, as well as from the Australian Museum (EBU), Natural History Museum in Victoria (NMV), South African Institute of Aquatic Biology (SAIAB), California Academy of Sciences (CAS), Natural History Museum of the University of Kansas (KU), and Scripps Institute of Oceanography (SIO).

Scientific name	Tissue #	*Cox1*	*Cytb*	*Myh6*	*Rag1*	*Rh*
Diodon hystrix	FS 05	KF025664	KF025730	KF025800	KF025868	KF025906
Mola mola	EBU 38997	KF025665	KF025731	KF025801	KF025869	KF025907
Ranzania laevis	SIO 340531	KF025666	KF025732		KF025870	KF025908
Balistidae						
Abalistes stellaris	PW 1324	KF025667	KF025733	KF025802	KF025871	
Abalistes stellatus	SAIAB 81914	KF025668	AP009202	KF025803	AY700318	EU108845
Balistapus undulatus	MEA 144	KF025669	KF025734	KF025804	EU108869	KF025909
Balistes capriscus	MEA 584	KF025670	KF025735	KF025805	AY700308	DQ874818
Balistes polylepis	MEA 360	KF025673	KF025738		AY700309	KF025912
Balistes punctatus	MEA 283	KF025674	KF025739	KF025808	EU108868	EU108848
Balistes vetula	PW 1181	KF025671	KF025736	KF025806	AY700310	KF025910
Balistoides conspicillum	FS 2	KF025672	KF025737	KF025807	AY700311	KF025911
Balistoides viridescens	KU 4556	KF025675	KF025740	KF025809	AY700320	KF025913
Canthidermis maculata	MEA 251	KF025676	KF025741	KF025810	AY700312	EU108851
Canthidermis sufflamen		JQ841088				
Melichthys indicus	KU 7163	KF025677	KF025742	KF025811	KF025872	KF025914
Melichthys niger	MEA 562	KF025678	KF025743		AY700313	EU108852
Melichthys vidua	MEA 168	KF025679	KF025744	KF025812	EU108870	EU108853
Odonus niger	MEA 147	KF025680	AP009208	KF025813	EU108871	EU108854
Pseudobalistes flavimarginatus	PW 313	AP009209	AP009209	KF025814	EU108872	EU108855
Pseudobalistes fuscus	MEA 158	KF025681	KF025745	KF025815	AY700314	EU108856
Pseudobalistes naufragium	MEA 347	KF025682	KF025746	KF025816	GU014460	KF025915
Rhinecanthus aculeatus	MEA 194	KF025683	KF025747	KF025817	AY308790	
Rhinecanthus assasi	MEA 167	KF025684	KF025748	KF025818	AY700315	EU108858
Rhinecanthus lunula	MEA 142	KF025685	KF025749	KF025819	EU108873	EU108859
Rhinecanthus rectangulus	MEA 110	KF025686	KF025750	KF025820	KF025873	EU108860
Rhinecanthus verrucosus	PW 1323	KF025687	KF025751	KF025821	EU108875	EU108861
Sufflamen albicaudatum	MEA 100		KF025798	KF025866	KF025904	KF025944
Sufflamen bursa	MEA 160	FJ584129	KF025752	KF025822	AY700319	EU108863
Sufflamen chrysopterum	PW 1325	JQ350381	KF025753	KF025823	AY700321	EU108864
Sufflamen fraenatum	KU 7210	AP004456	KF025754	KF025824	KF025874	KF025916
Sufflamen verres	MEA 345	KF025688	KF025755	KF025825	KF025875	KF025917
Xanthichthys auromarginatus	MEA 164	KF025689	AP009211	KF025826	AY700316	EU108865
Xanthichthys lineopunctatus	SAIAB 74689	KF025690	KF025756	KF025827	KF025876	KF025918
Xanthichthys mento	MEA 149	KF025691	KF025757	KF025828	EU108877	EU108866
Xanthichthys ringens	MEA 174	KF025692	KF025758	KF025829	EU108878	EU108867
Xenobalistes tumidipectoris		AP009182	AP009182			
Monacanthidae						
Acanthaluteres brownii		AP009212	AP009212			
Acanthaluteres spilomelanurus	NMV Z 10832	KF025693	KF025759	KF025830	KF025877	
Acanthaluteres vittiger	NMV Z 10906	KF025694	KF025760		KF025878	
Acreichthys tormentosus	MEA 277	KF025695	KF025761	KF025831	KF025879	KF025919
Aluterus heudeloti	KU 5218	KF025696	KF025762		KF025880	KF025920
Aluterus monoceros	CAS 123	KF025697	KF025763	KF025832	KF025881	KF025921
Aluterus schoepfi	KU 5120	KF025698	KF025764	KF025833	KF025882	KF025922
Aluterus scriptus	MEA 170	KF025699	KF025765	KF025834	AY700331	KF025923
Amanses scopas	KU 4457	KF025700	KF025766	KF025835	AY308793	
Brachaluteres jacksonianus	EBU 20482	KF025701	KF025767	KF025836	AY700337	KF025924
Brachaluteres ulvarum		AP009216	AP009215			
Cantherhines dumerilii	MEA 188	EU791285	KF025768		AY700332	
Cantherhines macroceros		JQ842801				
Cantherhines pardalis	SIO 340451	KF025702	KF025769	KF025837	KF025883	KF025925
Cantherhines pullus	MEA 246	KF025703	KF025770	KF025838	AY700333	KF025926
Cantherhines sandwichiensis	MEA 912	JQ411523	KF025771	KF025839	KF025884	
Cantherhines verecundus		DQ521021				
Chaetodermis penicilligerus	MEA322	KF025704	KF025772	KF025840	KF025885	KF025927
Eubalichthys bucephalus	NMV Z 7896	KF025705	KF025773	KF025841	KF025886	
Eubalichthys mosaicus	NMV Z 10836	KF025706	KF025774		KF025887	
Meuschenia freycineti	NMV Z 10819	KF025707	KF025775	KF025842		
Meuschenia hippocrepis	NMV Z 7894	KF025708	KF025776	KF025843		
Meuschenia trachylepis	EBU 30878	KF025709	KF025777	KF025844	AY700338	KF025928
Monacanthus chinensis	MEA 512	KF025710	KF025778	KF025845	KF025888	KF025929
Monacanthus ciliatus	PW 1183	KF025711	KF025779	KF025846		KF025930
Monacanthus tuckeri	MEA 254	JQ840165	KF025780	KF025847	KF025889	
Nelusetta ayraudi	NMV Z 10823	KF025712	KF025781	KF025848	AY700340	
Oxymonacanthus longirostris	MEA 134	KF025713	KF025782	KF025849	AY700339	KF025931
Paraluteres prionurus	PW 1268	KF025714	KF025783	KF025850	AY700336	KF025932
Paramonacanthus choirocephalus	VRA 011	KF025715	AP009223	KF025851	KF025890	KF025933
Paramonacanthus filicauda	EBU 22126	KF025716	KF025784	KF025852	KF025891	KF025934
Paramonacanthus oblongus	EBU 40666	KF025717	KF025785	KF025853		

(*continued on next page*)

Table 1 (*continued*)

Scientific name	Tissue #	*Cox1*	*Cytb*	*Myh6*	*Rag1*	*Rh*
Paramonacanthus pusillus	SAIAB 82334	KF025718	KF025786	KF025854	KF025892	
Paramonacanthus sulcatus		EF607471				
Pervagor janthinosoma	PW 1322	KF025719	KF025787	KF025855	KF025893	KF025935
Pervagor melanocephalus	SIO 340458	KF025720	KF025788	KF025856	KF025894	KF025936
Pervagor nigrolineatus	MEA 273	KF025721	KF025789	KF025857	KF025895	KF025937
Pseudolutarius nasicornis	MEA 196	KF025722	KF025790	KF025858		
Pervagor aspricaudatus		JQ431991				
Pervagor spilosoma		DQ521020				
Pseudomonacanthus macrurus	MEA 503	KF025723	KF025791	KF025859	KF025896	KF025938
Pseudomonacanthus peroni	MEA 319	KF025724	KF025792	KF025860	KF025897	KF025939
Rudarius ercodes	MEA 501	KF025725	AP009227	KF025861	KF025898	KF025940
Scobinichthys granulatus	EBU 40838-032	KF025726	KF025793		KF025899	
Stephanolepis auratus	SAIAB 82011	KF025727		KF025862	KF025900	KF025941
Stephanolepis cirrhifer	KU 8688	KF025728	KF025794	KF025863	KF025901	KF025942
Stephanolepis hispidus	PW 1209	KF025729	KF025795	KF025864	AY700335	DQ197910
Stepanolepis setifer	MEA 227	JQ840719	KF025796		KF025902	KF025943
Thamnaconus arenaceous		JF494680				
Thamnaconus fajardoi	SAIAB 82037-1		KF025799	KF025867	KF025905	
Thamnaconus modestus	SK 4	EF607583	KF025797	KF025865	KF025903	
Thamnacosus septentrionalis		EF607583				
Thamnacosus tessellatus		JQ681349				

Rag1, and 741 bp for *Rh*, for a total of 4665 nucleotides used in the concatenated analyses. All sequences generated for this study were deposited in GenBank (Table 1).

We used jModelTest (Posada, 2008) to select the best fitting model of sequence evolution from the candidate pool of models that can be utilized in MrBayes 3.2 (Ronquist et al., 2012) using AICc (Akaike, 1973). We did not include the proportion of invariant sites parameter in the candidate pool, as this parameter is already taken into consideration by the gamma parameter (Yang, 2006). The GTR + G model was selected as the most appropriate for *myh6* and *Rag1*, while HKY + G was selected as the best model for *cox1*, *Cytb*, and *Rh*.

Individual gene datasets were subjected to maximum likelihood analyses using RAxML (Stamatakis, 2006) to test for incongruence between gene histories of different loci and to identify potentially contaminated or mislabeled sequences. We assigned a GTR + G model to each individual gene partition, implementing the RAxML model closest to the jModelTest results, and ran 100 fast bootstrap replicates using the GTR + CAT model. The five individual gene datasets were then concatenated in Mesquite 2.75 (Maddison and Maddison, 2011), and the full dataset was partitioned by gene and subjected to maximum likelihood analyses with RAxML (Stamatakis, 2006). Each partition was again assigned its own GTR + G model, and 1000 fast bootstrap replicates were generated using the GTR + CAT model.

We used MrBayes 3.2 (Ronquist et al., 2012) to perform Bayesian analyses. We partitioned the concatenated dataset by locus and assigned the model selected by jModelTest. We ran multiple replicates with two analyses of 20 million generations each, with four chains (one cold, three heated) sampling every 1000 generations. The trace files were checked in Tracer 1.5 (Drummond and Rambaut, 2007) to ensure that the chains had reached convergence, and the first 25% of trees was discarded as burnin. Post-burnin trees were combined to obtain a 50% majority rule consensus tree. The topologies of the different replicates were then compared to each other to assess support for the results of the analyses.

2.4. Divergence time estimation

The concatenated alignment was analyzed as five unlinked gene partitions, with each locus being assigned the model selected by jModelTest, with uncorrelated lognormal priors in BEAST 1.7.2 (Drummond and Rambaut, 2007). A birth-death prior was assigned to rates of cladogenesis. We ran two analyses of 50 million generations each, with sampling every 5000 generations, and used Tracer 1.5 (Rambaut and Drummond, 2009) to inspect the trace files, ensuring that the chains had reached convergence and the ESS values for all parameters were greater than 200. We removed the first 20% of the trees from each analysis as burnin and used LogCombiner to merge the files with the remaining trees, and TreeAnnotator (Drummond and Rambaut, 2007) to obtain a timetree.

Several fossil calibration points were used to convert the molecular phylogeny into a timetree. The oldest known balistoid fossil is *Gornylistes prodigiosus*, a likely stem balistid from the Middle Eocene of northern Caucasus (Late Lutetian, Early Bartonian, 41–42 Ma) (Bannikov and Tyler, 2008). Several additional stem balistids are known from Oligocene deposits of Caucasus and Switzerland (Tyler and Santini, 2002; Santini and Tyler 2003, 2004), while a number of crown balistids are known from Miocene deposits in both Europe and northern Africa (Santini and Tyler, 2003; Schultz, 2004). Unfortunately, the uncertain phylogenetic placement of these crown fossils, partially due to their highly incomplete state (Schultz, 2004) and partly due to their lack of inclusion in morphological phylogenetic studies, prevents us from considering them as reliable calibration points. The fossil record of monacanthids is much less well known. Currently, only two species have been described, both from the Middle-Upper Pliocene and Lower Pleistocene of Italy and Greece (Sorbini and Tyler, 2004). Both species have been assigned to the *Aluterus*-like extinct genus *Frigocanthus*, and appear to be too young to be assigned to any split in our tree.

We thus used *Gornylistes prodigiosus* to date the minimum age for the crown balistoid clade, and the Middle Eocene stem balistoid *Bolcabalistes vari* (Tyler and Santini, 2002; Santini and Tyler, 2003, 2004) to put a soft upper boundary (marking the 95% of the prior probability for that split) of 50 Ma on this node. We used the Early Miocene *Austromola* (Gregorova et al., 2009) to provide a minimum age of 22 Ma for the split between *Mola* and *Ranzania* among the outgroups, and used the Eocene *Eomola* to set the soft upper bound for this node (Tyler and Santini, 2002). We also used the Paleocene *Moclaybalistes danekrus*, a stem balistoid from the Late Paleogene of Denmark (58–59 Ma) (Tyler and Santini, 2002), to put a minimum age prior on the root of the tree. The Santonian *Protriacanthus gortani* (~85 Ma) (Santini and Tyler, 2003) was used to set the soft upper bound for the root age (crown balistoids: offset = 41,

mean = 3.0; crown molids: offset = 22, mean = 6.5; root: offset = 59, mean = 6.5).

2.5. Comparative analyses

All analyses were performed using the software package R version 2.15.2 (R Development Core Team, 2012), using functions in the packages Geiger (Harmon et al., 2008), Laser (Rabosky, 2006), and APE (Paradis et al., 2004).

To test whether rates of speciation differed between triggerfishes and filefishes, we used the BiSSE model as implemented in Diversitree (Maddison et al., 2007; FitzJohn, 2012). We compared a model where speciation rates were allowed to vary by family to a model where a single speciation rate was held constant across the tree. We fixed the extinction rates in both models to be 0 since estimates of extinction from molecular phylogenies are often unreliable (Rabosky, 2010).

To identify exceptionally radiating lineages of balistoids we used MEDUSA, an AIC-based method that can use phylogenetic and taxonomic richness data to estimate rate shifts on a chronogram with incomplete taxon sampling (Alfaro et al., 2009). We collapsed the timetree to a backbone tree where most of the balistoid diversity was assigned to 30 lineages. Birth-death models of increasing complexity were used to estimate the rates of speciation and extinction, and an improvement in AIC scores of four units or greater were used as the threshold for retaining rate shifts (Burnham and Anderson, 2002).

To determine the ancestral habitat for monacanthids, and investigate the number of transitions between coral reefs and non-reef environments, we used habitat information found in Fishbase (Froese and Pauly, 2012), as well as published literature (e.g., FAO identification sheets) to assign a reef/non-reef status to each species in our balistoid tree. As we were interested in the evolutionary consequences of the ability to colonize reef habitats, species that are found in both reef and non-reef habitats were coded as reef following the example of Santini et al. (2013a) for habitat types in pufferfishes. We then used a maximum likelihood approach to reconstruct ancestral habitats on the timetree using the MK1 model in Geiger (Harmon et al., 2008).

To examine size diversity in balistoids we obtained size data for 33 species of triggerfish and 53 species of filefish from Fishbase (Froese and Pauly, 2012). Most records were for total length (TL) (Table 2). Measures of standard length were converted to total length using a regression based upon multispecies measurements (Gaygusuz et al., 2006). We compared log-transformed size diversity between monacanthids and balistids using an *F* test. We reconstructed the ancestral size at each node on the tree using the MK1 model as implemented in Geiger (Harmon et al., 2008). To compare rates of size evolution between monacanthids and balistids we used BrownieLite (O'Meara et al., 2006) as implemented in PhyTools (Revell, 2012). We used a *Chi*-squared test to determine whether rates of log-transformed size evolution differed significantly between families.

3. Results

3.1. Phylogenetic analyses

Both maximum likelihood and Bayesian analyses of the concatenated dataset inferred similar topologies with high support values present for most inter-specific and generic relationships. The monophyly of both families is supported with bootstrap proportions (BSP) of 100% in the maximum likelihood phylogeny (Fig. 1), and posterior probabilities (PP) greater than 0.98 in the Bayesian phylogeny (Fig. S1).

Table 2
List of taxa with information about total length (in cm) and habitat association (data from http://www.fishbase.org).

Species	Length	Habitat
Abalistes stellatus	60	Reef
Abalistes stellaris	60	Non-reef
Balistapus undulatus	30	Reef
Balistes capriscus	60	Reef
Balistes polylepis	76	Reef
Balistes punctatus	60	Non-reef
Balistes vetula	60	Reef
Balistoides conspicillum	50	Reef
Balistoides viridescens	75	Reef
Canthidermis maculata	50	Reef
Canthidermis sufflamen	65	Reef
Melichthys indicus	25	Reef
Melichthys niger	50	Reef
Melichthys vidua	40	Reef
Odonus niger	50	Reef
Pseudobalistes flavimarginatus	60	Reef
Pseudobalistes fuscus	55	Reef
Pseudobalistes naufragium	100	Reef
Rhinecanthus aculeatus	30	Reef
Rhinecanthus assasi	30	Reef
Rhinecanthus lunula	28	Reef
Rhinecanthus rectangulus	30	Reef
Rhinecanthus verrucosus	23	Reef
Sufflamen albicaudatum	22	Reef
Sufflamen bursa	25	Reef
Sufflamen chrysopterum	30	Reef
Sufflamen fraenatum	38	Reef
Sufflamen verres	40	Reef
Xanthichthys auromarginatus	30	Reef
Xanthichthys lineopunctatus	30	Reef
Xanthichthys mento	30	Reef
Xanthichthys ringens	25	Reef
Xenobalistes tumidipectoris	7	Non-reef
Acanthaluteres brownii	55	Reef
Acanthaluteres spilomelanurus	14	Non-reef
Acanthaluteres vittiger	35	Non-reef
Acreichthys tomentosus	12	Reef
Aluterus heudelotii	45	Non-reef
Aluterus monoceros	76	Reef
Aluterus schoepfii	61	Reef
Aluterus scriptus	110	Reef
Amanses scopas	20	Reef
Brachaluteres jacksonianus	10	Reef
Brachaluteres ulvarum	8	Non-reef
Cantherhines dumerilii	38	Reef
Cantherhines macrocerus	46	Reef
Cantherhines pardalis	25	Reef
Cantherhines pullus	20	Reef
Cantherhines sandwichiensis	19	Reef
Cantherhines verecundus	13	Non-reef
Chaetodermis penicilligerus	31	Reef
Eubalichthys bucephalus	50	Non-reef
Eubalichthys mosaicus	60	Non-reef
Meuschenia trachylepis	40	Reef
Meuschenia freycineti	60	Non-reef
Meuschenia hippocrepis	51	Non-reef
Monacanthus ciliatus	20	Reef
Monacanthus chinensis	38	Reef
Monacanthus tuckeri	10	Reef
Nelusetta ayraud	100	Non-reef
Oxymonacanthus longirostris	12	Reef
Paraluteres prionurus	11	Reef
Paramonacanthus choirocephalus	13	Non-reef
Paramonacanthus filicauda	22	Non-reef
Paramonacanthus oblongus	8	Non-reef
Paramonacanthus pusillus	18	Non-reef
Paramonacanthus sulcatus	24	Non-reef
Pervagor janthinosoma	16	Reef
Pervagor melanocephalus	16	Reef
Pervagor nigrolineatus	10	Reef
Pseudalutarius nasicornis	19	Reef
Pervagor aspricaudus	13	Reef
Pervagor spilosoma	18	Reef

(*continued on next page*)

Table 2 (*continued*)

Species	Length	Habitat
Pseudomonacanthus macrurus	25	Reef
Pseudomonacanthus peroni	48	Reef
Rudarius ercodes	8	Non-reef
Scobinichthys granulatus	30	Non-reef
Stephanolepis auratus	28	Non-reef
Stephanolepis cirrhifer	30	Non-reef
Stephanolepis hispidus	28	Reef
Stephanolepis setifer	20	Reef
Thamnaconus arenaceus	23	Non-reef
Thamnaconus fajardoi	22	Non-reef
Thamnaconus modestus	36	Reef
Thamnaconus septentrionalis	26	Non-reef
Thamnaconus tessellatus	25	Non-reef

Within balistids, several major clades are identified (Fig. 1): the first contains a lineage formed by *Melichthys*, *Balistapus* and *Balistoides conspicillum*, which represents the sister group to the remaining balistids. The monotypic *Odonus niger* appears to be the next lineage to branch off the balistid tree, followed by a clade formed by *Balistoides viridescens* and *Pseudobalistes flavimarginatus*, plus a *Xanthichthys* and *Xenobalistes* clade (with *Xenobalistes* nested deeply within *Xanthichthys*). The last balistid clade is composed of two main lineages: the first includes *Balistes*, *Pseudobalistes fuscus* and *P. naufragium*; the second lineage includes *Canthidermis*, *Abalistes*, *Sufflamen* and *Rhinecanthus*. Monophyly of most genera is strongly supported, although *Pseudobalistes* and *Balistoides* are shown to be polyphyletic.

Within monacanthids, both Bayesian and maximum likelihood trees reveal the existence of three well-supported clades. The first contains a lineage, formed by *Pseudolutarius* + *Oxymonacanthus*, which is sister to all other monacanthids. The second clade includes a subclade formed by *Brachaluteres*, *Paraluteres*, and *Rudarius*; a *Chaetodermis* + *Acreichthys* + *Paramonacanthus* + *Monacanthus chinensis* subclade and a *Pervagor* + *Stephanolepis* + *Paramonacanthus pusillus* lineage. The third clade includes all species of *Aluterus* in our dataset, as well as two of the three species of *Monacanthus* (*M. ciliatus* and *M. tuckeri*); a *Cantherhines* + *Amanses scopas* group sister to *Pseudomonacanthus*; and a group containing *Eubalichthys*, *Thamnaconus*, *Nelusetta*, *Meuschenia*, *Acanthaluteres* and *Scobinichthys*. Unlike the case with balistids, the monophyly of many of the traditional genera (*Acanthaluteres*, *Aluterus*, *Cantherhines*, *Monacanthus*, *Paramonacanthus*, *Stephanolepis*) was not supported: *Monacanthus ciliatus* and *tuckeri* appear to be nested within *Aluterus*, while *M. chinensis* belongs within *Paramonacanthus*; *Paramonacanthus pusillus* appears to nest within *Stephanolepis*; *Cantherhines* is paraphyletic without the inclusion of *Amanses scopas*, as is *Acanthaluteres* without *Scobinichthys*; and the two species of *Eubalichthys* in our dataset never appear as sister taxa.

3.2. Divergence time estimation

The topology of the BEAST analysis (Fig. 2) is largely congruent with the RAxML and MrBayes trees. Within balistids two subclades are recovered: the first includes *Melichthys*, *Balistapus* and *Balistoides conspicillum*, as well as *Odonus*, and is the sister group to *Balistoides viridescens* + *Pseudobalistes flavimarginatus* plus *Xanthichthys* and *Xenobalistes*. All taxa that are hypothesized in the BEAST tree that belong to different lineages than in the MrBayes or RAxML analyses are supported by nodes with very weak PP or BSP (Figs. 1 and S1). The second subclade includes *Canthidermis*, *Pseudobalistes fuscus* and *P. naufragium*, and *Balistes*, as well as *Abalistes*, *Sufflamen* and *Rhinecanthus*. Relationships among the species are identical to the RaxML and MrBayes trees. Within the monacanthids only a few minor differences exist between the BEAST and the MrBayes/RAxML topologies: *Monacanthus ciliatus* and *M. tuckeri* appear to be the sister group to the largest monacanthid subclade, instead of being nested within *Aluterus* (itself a member of this subclade), while *Pseudomonacanthus* is not the sister taxon to *Cantherhines* + *Amanses*, but is sister to a group containing *Cantherhines* + *Amanses* as well as several additional genera (*Eubalichthys*, *Thamnaconus*, *Nelusetta*, *Meuschenia*, *Acanthaluteres* and *Scobinichthys*).

Our timetree suggests an Early to Middle Eocene age for the split between monacanthids and balistids, with a mean age of 52 Ma and a 95% confidence interval for the highest posterior density (HPD) of 42–60 Ma. The crown balistids are 20 Ma (95% HPD: 16–25 Ma), while the two main balistid subclades are about 18 Ma each (95% HPD: 13–22 Ma and 14–22 Ma, respectively). The diversity of several balistid genera appears to be the product of fairly recent radiations, with ages of 6 Ma (95% HPD: 4–8 Ma 95% HPD) for *Rhinecanthus*, 8 Ma (95% HPD: 6–10 Ma) for *Sufflamen*, 3 Ma (95% HPD: 2–4 Ma) for *Xanthichthys* and 1 Ma (95% HPD: 0.6–1.7) for *Melichthys*.

The monacanthids appear to have a Late Eocene origin, with a crown age of 38 Ma (95% HPD: 31–44 Ma). The *Pseudolutarius* + *Oxymonacanthus* group originated 15 Ma (95% HPD: 10–21 Ma), while the two other main subclades both originated within a fairly short interval in the Early Oligocene: 28 Ma (95% HPD: 23–33 Ma) and 32 Ma (95% HPD: 26–39 Ma), respectively. Most filefish genera are paraphyletic and also appear to have originated by the Miocene, with only *Thamnaconus* having originated within the Pliocene (95% HPD: 4 Ma, 2–5 Ma).

3.3. Comparative analyses

BiSSE results revealed that triggerfishes diversified somewhat faster than filefishes: $\lambda_{\text{triggerfishes}} = 0.127$, $\lambda_{\text{filefishes}} = 0.090$. However, allowing family-dependent diversification rates did not produce a significant improvement in model fit (*Chi*-squared P = 0.129). MEDUSA (Fig. 3) reveals no rate shifts within balistids, while a major increase in diversification rate ($r_1 = 0.094$; $r_2 = 0.249$) is found within monacanthids in the lineage leading to the clade formed by *Eubalichthys*, *Thamnaconus*, *Nelusetta*, *Meuschenia*, *Acanthaluteres* and *Scobinichthys*. This clade has a very young age (12 Ma, 10–15 Ma 95% HPD) and accounts for roughly 30% of the filefish diversity (32 species out of 106).

Maximum likelihood reconstruction of the habitat type on the molecular timetree (Fig. S2) shows that while both balistids and monacanthids are ancestrally reef-associated, only a handful of triggerfish species have colonized non-reef environments. Within filefishes, at least seven lineages have independently invaded non-reef environments (this is likely to be an underestimate due to our sampling only including 50% of living filefish species). In spite of this multiple invasion of non-reef environments, only one of these lineages appears to have achieved a significant diversity (the clade formed by *Eubalichthys*, *Thamnaconus*, *Nelusetta*, *Meuschenia*, *Acanthaluteres* and *Scobinichthys*). Most lineages of non-reef monacanthids have also subsequently re-invaded reefs (Fig. S2).

Triggerfishes are significantly larger than filefishes in a non-phylogenetic comparison ($r^2 = 0.115$, $F = 12.1$, $p < 0001$; Fig. 4) and show a smaller range of body sizes. However this difference in size diversity is not statistically significant ($F = 0.5961$; $p < 0.1205$). Brownie-estimated rates of body size evolution between families were also not statistically significantly different (*Chi*-squared P = 0.328). Maximum likelihood reconstruction of the log transformed TL (Fig. S3) reveals several lineages which may have experienced directional evolution towards large or small body size. Within triggerfishes, the *Balistoides viridescens* + *Pseudo-*

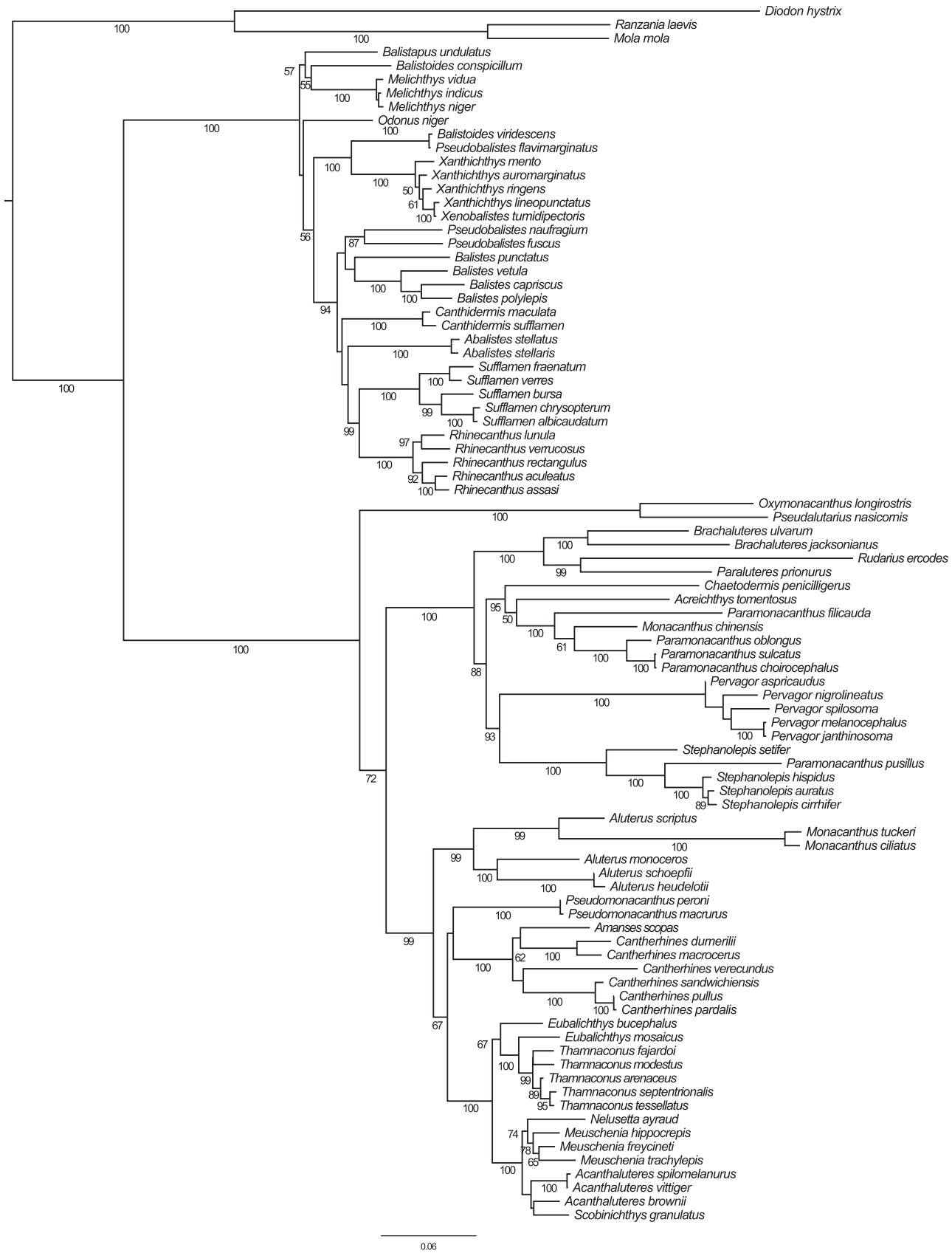

Fig. 1. Maximum likelihood phylogenetic hypothesis of balistoid relationships based on analysis of the concatenated dataset using RAxML. Bootstrap proportions over 50% indicated below branches.

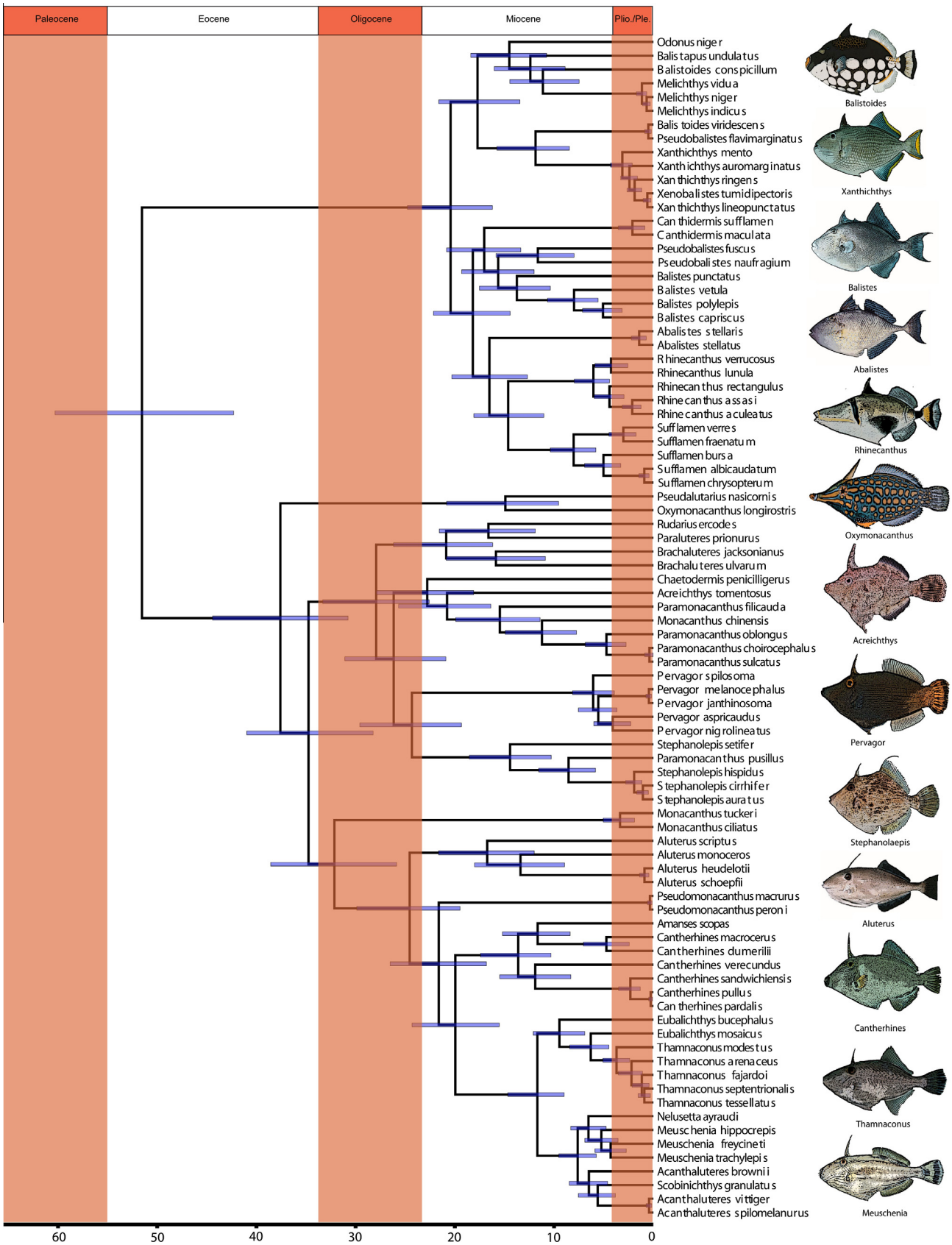

Fig. 2. Balistoid timetree based on a Bayesian relaxed clock approach implemented in BEAST 1.7.2. Horizontal bars on each node indicate 95% HPD confidence intervals. Timescale at bottom of figure is in million years before present. Fish images modified under Creative Commons license from original photographs by J.E. Randall (retrieved from http://www.fishbase.org).

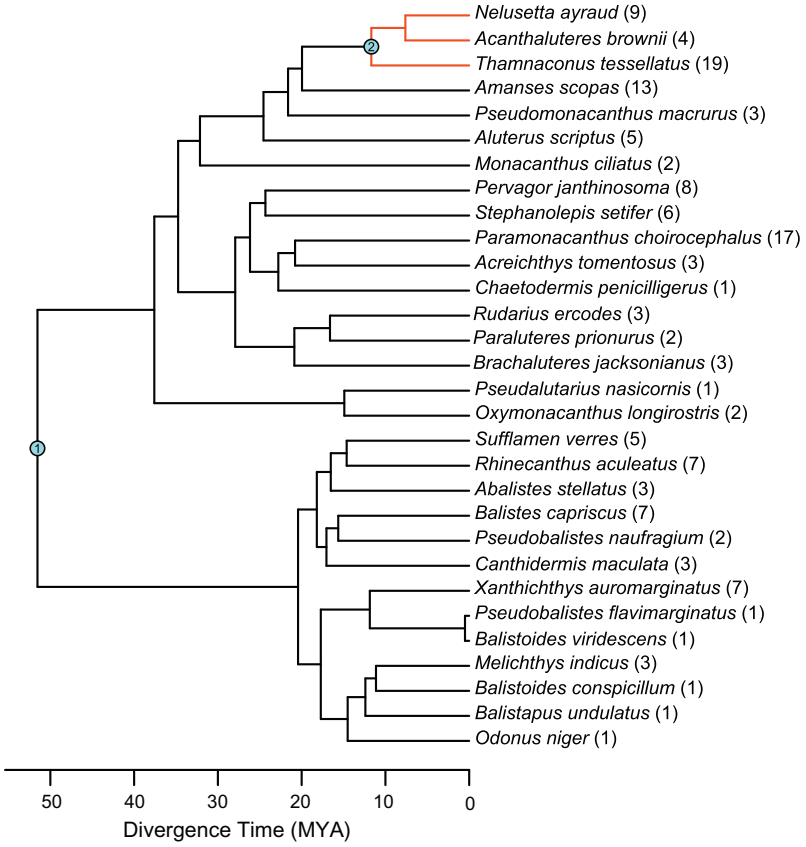

Fig. 3. MEDUSA diversity tree for analyses of lineage diversification in balistoids. Clades from Fig. 2 have been collapsed into 30 representative stem lineages. Each lineage is identified with the name of the species from that taxon that was retained for the MEDUSA analysis, and the total species diversity that was assigned to that lineage. The red branch indicates the diversification rate shift ($r_2 = 0.0249$ vs. $r_1 = 0.094$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

balistes flavimarginatus clade, and *Canthidermis* + *Pseudobalistes* + *Balistes* exhibit large body size (Fig. S3). Within monacanthids, *Aluterus*, *Cantherhines*, and a clade containing *Acanthaluteres* + *Nelusetta* + *Meuschenia* contain mostly large-bodied species. A clade of miniaturized filefishes (i.e., *Rudarius*, *Paraluteres*, *Brachaluteres*) is also recovered.

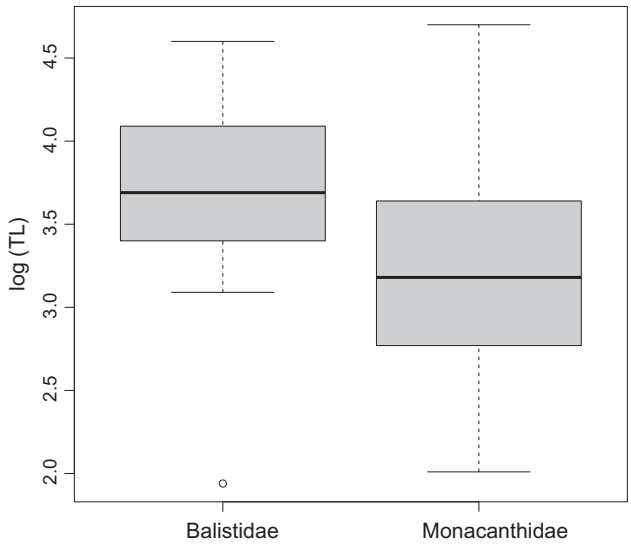

Fig. 4. Plot illustrating range and mean value of body size (expressed as Ln of total length, TL) in balistid and monacanthid fishes.

4. Discussion

4.1. Balistoid relationships

Balistoid fishes are not among the tetraodontiform subclades that have so far been investigated using numerical cladistic methods (e.g., Klassen, 1995; Santini and Tyler 2002a, 2002b). The only detailed phylogenetic treatments of balistoid relationships are found in Matsuura (1979) and the evolutionary taxonomic study of Tyler (1980). However the amount of phylogenetic signal present in these morphological matrices is low, and a Bayesian analysis of a data set of balistid species produced by combining these two earlier studies resulted in trees that are almost entirely unresolved (Dornburg et al., 2008).

This new study supports the monophyly of both Balistidae and Monacanthidae, corroborating all previous studies of tetraodontiform relationships based on both morphological and molecular datasets (Winterbottom, 1974; Matsuura, 1979; Tyler, 1980; Santini and Tyler, 2003, 2004; Holcroft, 2005; Alfaro et al., 2007; Yamanoue et al., 2008, 2009).

Our topology of balistid relationships differs from some of the previous molecular studies (Dornburg et al., 2008; Yamanoue et al., 2009), which identified *Balistes* as the sister group to the other balistids split in two major lineages, and agrees with Dornburg et al. (2011) in recovering *Balistes* nested deeply within triggerfishes. Our study agrees with Dornburg et al. (2008, 2011) in inferring non-monophyly of both *Balistoides* and *Pseudobalistes* (including a sister group relationship of *P. flavimarginatus* and *B. viridescens*), while the relationships among the other genera

inferred in our study are highly congruent to these of Yamanoue et al. (2009).

Within monacanthids, our topology recovers virtually all of the same subclades found in Yamanoue et al. (2009). The only difference involves the placement of *Chaetodermis* and *Acreichthys*, which in our topologies appear as sequential sister taxa to the *Paramonacanthus* + *Monacanthus chinensis* clade, while in Yamanoue et al. (2009) preferred hypothesis appear as each other's sister groups (although Yamanoue et al. (2009) note that their placement differs in the likelihood and Bayesian analyses).

Even though Matsuura (1979) and Tyler (1980) examined multiple species for many genera, both of these morphological studies, as well as Yamanoue et al. (2009), produced generic level phylogenies. By including only one representative species per genus, Yamanoue et al. (2009), Holcroft (2005), and Alfaro et al. (2007) were unable to test the monophyly of the currently recognized genera (the only genus for which Holcroft and Alfaro et al. have more than one species is *Monacanthus*). Our study is the only one to date to broadly sample among and within monacanthid genera, making it difficult to assess if the massive non-monophyly that we infer is the result of major bias in sequence evolution, poor identification of the specimens from which the tissue samples were taken, or highly incorrect existing taxonomy. In order to reduce the risk of misidentification/contamination biasing the results of this study, the *cox1* fragment sequenced from the tissues that we had obtained was blasted against the barcode of life database. Multiple sequences exist in the barcode of life database for many of the species included in this study and offered a similarity of sequence identity greater than 99%, validating the original identification. In some cases, the additional sequences for the barcode of life suggest that the non-monophyly of the traditional genera is warranted. For example, sequences of *Monacanthus chinensis* are over 99% similar to one another, but only show ~92% similarity to sequences of other species of *Monacanthus*, corroborating our finding that *M. chinensis* may not be closely related to other *Monacanthus* species in spite of a very similar external morphology.

4.2. The timing of triggerfish and filefish evolution

Our timetree suggests an Early to Middle Eocene origin for the balistoids, with the two families splitting from one another ~52 Ma. These results are in close agreement with the ages inferred by Alfaro et al. (2007) in their analyses of tetraodontiform family relationships. No other timetree exists for balistoids, or for monacanthids, even though Dornburg et al. (2008, 2011) produced timetrees for the balistids. Their timetrees suggested a Late Miocene age of 11 Ma for the origin of the crown balistids; our timetree suggests older ages (20 Ma), with no overlap of the two 95% HPD intervals (7–14 Ma in Dornburg et al. (2011), 16–25 Ma in this study). This age discrepancy could be due to the inclusion of non-balistoid outgroups in our study, which allowed us to incorporate additional fossil calibration points and obtain a more reliable estimate for the ages of the balistoid taxa. Both our study and Dornburg et al. (2011), however, suggest that the radiation of triggerfishes must have been a fairly recent event, with many genera originating towards the end of the Miocene, and most of the diversity appearing during the Pliocene and Pleistocene. The tempo of diversification appears to have been rather different for monacanthids. Crown filefishes appear in the Late Eocene, and all major clades have stem ages that date back to the Late Eocene or Early Oligocene and crown ages that are no younger than the Early Miocene. Most monacanthid genera appear to be rather old, with many dating back to the Middle Miocene (15 million years or older).

Unlike balistids, which possess a rich fossil record (Tyler and Santini, 2002; Bannikov and Tyler, 2008; Schultz, 2004) suggestive of an intense rate of turnover during the Oligocene and Early Miocene before the origin of the crown taxa, fossil monacanthids are only known from Pliocene and Pleistocene deposits in Italy and Greece, and appear to be already very derived crown taxa. This paucity of fossils might be due to the lack of suitable fish fossil sites in the Indo-western Pacific, where the bulk of the monacanthid diversity is currently found, or could be an indication that for much of their existence, filefishes have been predominantly associated with environments that make fossilization unlikely, such as rocky reefs.

4.3. Comparative analyses

Balistids and monacanthids were shown to be among the fastest diversifying groups of tetraodontiforms (Alfaro et al., 2007); within balistoid fishes only one lineage of predominantly non-reef (Fig. S2), large size (Fig. S3) filefishes is shown to have a significantly higher rate of net diversification than the balistoids as a whole ($r_2 = 0.249$ vs. $r_1 = 0.094$). Both families are derived from coral reef-affiliated ancestors (Fig. S2). Within balistids only a handful of species have subsequently lost reef association, even though a number of reef species have a habitat range that includes non-reef environments (e.g. *Balistes capriscus*, *Canthidermis maculata*). Within monacanthids, multiple lineages have moved away from coral reef environments to occupy either rocky reefs in temperate waters (e.g., New Zealand and southern Australia in the Indo-western Pacific, or the Northwestern Atlantic) or seagrass beds and sandy bottoms in tropical latitudes (Froese and Pauly, 2012). Interestingly, most of these lineages have subsequently given origin to species that have moved back on coral reefs, a pattern not seen in balistids.

Although filefishes are commonly regarded as being more phenotypically diverse than triggerfishes, our comparative analyses reveal that it is the older crown age, rather than an exceptional diversification rate within filesfishes, that underlies this difference (Collar et al., 2005), at least with regard to body size. Filefishes encompass a greater diversity of body sizes, but this difference is not statistically significant (Fig. 4). Furthermore, our evolutionary analysis of body size reveals that the filefishes and triggerfishes have evolved at similar rates. Thus the observed difference in the range of body sizes within filefishes can be explained by the greater age of crown monacanthids (and thus a longer amount of evolutionary time for the accumulation of diversity) relative to balistids. Although we did not find any significant differences in the rate of evolution between these two families, ancestral state reconstruction suggests that some filefish lineages may have experienced directional trends in size evolution (Fig. S3). For example, directional evolution may explain large body size in *Aluterus* and in the clade containing *Acanthaluteres* + *Scobinichthys* + *Nelusetta* + *Meuschenia*. Similarly, directional evolution towards small body size may explain trait diversity in *Monacanthus ciliatus* + *M. tuckeri*, in *Acanthaluteres spilomelanurus* + *A. vittiger*, and in the clade containing the smallest filefish genera, *Rudarius*, *Paraluteres* and *Brachaluteres*. Further comparative tests are needed to determine if evolutionary trends do characterize these and other balistoid lineages, and if there are ecological or geographical correlates to these patterns.

4.4. Taxonomic reclassification

The results of our study suggest that the current taxonomy of monacanthids is in need of major revisions. Due to the current incompleteness of our dataset, which only represents half of the extant 106 described species, we do not feel that we can perform a taxonomic revision of this group. Our study, however, shows convincingly that the balistid *Xenobalistes tumidipectoris* belongs within the genus *Xanthichthys*. The monotypic genus *Xenobalistes* was

described on the basis of a single, partially digested specimen found in the stomach of a tuna (Matsuura, 1981). The specimen was characterized by small size (~60 mm standard length) and the presence of large protuberances, which appear to be lateral expansions of the coracoid bones just below the pectoral fins (Fig. 4 in Matsuura, 1981). We believe that the small size of the *Xenobalistes tumidipectoris* holotype, as well as the large size of the eyes compared to other balistids (Matsuura, 1981; Yamanoue et al., 2009, Fig. 4) indicate that this specimen was likely a juvenile. We examined postlarval and juvenile balistids in the fish collection of a number of museums (LA County Natural History Museum; Museum of Comparative Zoology, Harvard) and observed an enlargement of the region below the pectoral fins in specimens of several balistid species, although the enlargement is not as pronounced as in *Xenobalistes tumidipectoris*. On the basis of both our phylogeny and this information, we suggest renaming *Xenobalistes tumidipectoris* as *Xanthichthys tumidipectoris* and eliminating the generic name *Xenobalistes*.

5. Conclusions

This study, containing 33 of the 42 extant balistid and 53 of the 106 monacanthid species, represents the most comprehensive molecular phylogeny yet produced for balistoid fishes. Our study supports the monophyly of both families, as well as the presence of two robust subclades of balistids and four highly supported subclades of monacanthids. Our study is largely in agreement with the only previous molecular study of generic-level relationships among monacanthids (Yamanoue et al., 2009), while it conflicts with some of the previous studies of balistid relationships (Dornburg et al., 2008, 2011; Yamanoue et al., 2009) in several nodes of the topology. We show that the only known species of the balistid genus *Xenobalistes* was likely described on the basis of a juvenile *Xanthichthys*, and suggest renaming the species as *Xanthichthys tumidipectoris*. We also show that many of the currently recognized monacanthid genera are not monophyletic, and that the taxonomy of this group is in need of major revisions.

The Bayesian chronogram, produced by calibrating the molecular phylogeny with three tetraodontiform fossils, suggests a Miocene age, and a largely Pliocene/Pleistocene radiation for balistids, as well as a Late Eocene origin for monacanthids, with the major filefish lineages having originated between the Late Oligocene and Early Miocene. This pattern is largely similar to that observed in other tetraodontiform clades (e.g., Santini et al., 2013a, 2013b).

The maximum likelihood reconstruction of the ancestral habitat over the molecular chronogram reveals that the common ancestors of both clades were associated with coral reefs. Balistids have remained largely a reef-associated group, but at least five lineages of filefishes have radiated into non-coral reef environments, often with some members of these lineages moving back over scleractinian reefs. Although filefishes exhibit greater species richness and phenotypic diversity, our analyses reveal no significant family-level differences in the rate of diversification or body size evolution. Thus, the greater diversity of filefishes may be simply due to the longer time this family has had to diversify.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.05.015.

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