Molecular Phylogenetics and Evolution 67 (2013) 140–155

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

New nuclear markers and exploration of the relationships among Serraniformes (Acanthomorpha, Teleostei): The importance of working at multiple scales

A.-C. Lautredou [a,*], H. Motomura [b], C. Gallut [a], C. Ozouf-Costaz [a], C. Cruaud [c], G. Lecointre [a], A. Dettai [a]

[a] UMR7138 CNRS-UPMC-IRD-MNHN, Département Systématique et Evolution, Muséum National d'Histoire Naturelle, Paris 75005, France
[b] Kagoshima University Museum, 1-21-30 Korimoto, Kagoshima 890-0065, Japan
[c] Genoscope, Centre National de Sequençage, Evry 91057, France

ARTICLE INFO

Article history:
Received 2 August 2012
Revised 30 November 2012
Accepted 28 December 2012
Available online 7 January 2013

Keywords:
Serraniformes
Scorpaenidae
Nuclear genes
Phylogeny
Scorpaeniformes
Perciformes

ABSTRACT

We explore the relationships within Serraniformes (Li et al., 2009) using a dense taxon sampling and seven nuclear markers. Six had already used been for teleost phylogeny (IRBP, MC1R, MLL4, Pkd1, Rhodopsin, and RNF213) at other scales, and one (MLL2) is new. The results corroborate the composition of Serraniformes described in previous publications (some Gasterosteiformes, Perciformes and Scorpaeniformes). Within the clade, Notothenioidei and Zoarcoidei are each monophyletic. Cottoidei was not monophyletic due to placement of the genus *Ebinania* (Psychrolutidae). Our independent data confirm the sister-group relationship of Percophidae and Notothenioidei as well as the division of Platycephaloidei in four different groups (Bembridae, Platycephalidae, Hoplichthyidae and Peristediidae with Triglidae). Within Cottoidei, Liparidae and Cyclopteridae formed a clade associated with Cottidae, the genus *Cottunculus* (Psychrolutidae), and Agonidae. Serranidae and Scorpaenidae are not monophyletic, with the Serranidae divided in two clades (Serraninae and Epinephelinae/Anthiinae) and Scorpaenidae including Caracanthidae and the genus *Ebinania* (Psychrolutidae). We discuss some morphological characters supporting clades within the Scorpaenidae.

1. Introduction

Through molecular phylogenetics studies, the last 15 years provided many surprises for the phylogeny of Percomorpha, the "bush at the top" of Teleosts (Nelson, 1989). Few before would have dared to propose relationships such as for instance the one uniting saithe and cods with john dorys (Wiley et al., 2000; Chen et al., 2003), or a group uniting groupers (Serranidae), perches (Percidae), sticklebacks (Gasterosteidae), searobins (Triglidae), and assemblages like icefishes (Notothenioidei), sculpins, and snailfishes (Cottoidei), eelpouts (Zoarcoidei) and scorpionfishes (Scorpaenoidei) (Wiley et al., 2000; Chen, 2001; Chen et al., 2003; Miya et al., 2003, 2005; Dettai & Lecointre, 2004, 2005, 2008; Smith & Wheeler, 2004, 2006; Smith & Craig, 2007; Li et al., 2009; Matschiner et al., 2011; Near et al., 2012). Yet these groups have been recovered time and time again by multiple studies performed by different teams and with different species and samples. It is now time to start taking seriously the clades that have been repeatedly corroborated, and to explore them in more detail. This has been started already on some groups, and we are here going to explore relationships within Serraniformes (Li et al., 2009) using a denser sampling and additional markers.

For the name and content of the species, families, suborders and orders, we will follow Nelson (2006).

Very reduced versions of the group started to appear in molecular phylogenies in the early 2000 (Wiley et al., 2000; Chen, 2001; Chen et al., 2003; Miya et al., 2003, 2005; Orrell et al., 2006). More complete versions with a better taxonomic coverage were published in Dettai and Lecointre (2004, 2005), Smith and Wheeler (2004, 2006) and Li et al. (2009). In this diverse and particularly interesting assemblage of families, some are important for world fisheries (groupers) or toxin evolution (mail-cheeked fishes); others have been used as developmental and genomic models (sticklebacks) or possess unique evolutionary biochemical and physiological traits (notothenids, channichthyids).

From 8 to 28 families have been included in analyses of the Serraniformes depending on the sampling of the molecular study considered (Miya et al., 2003; Dettai & Lecointre, 2005; Smith & Wheeler, 2006; Li et al., 2009). While there is little contradiction among the studies, this lack of overlap makes assessment of

* Corresponding author.
E-mail addresses: lautredou@mnhn.fr (A.-C. Lautredou), gallut@mnhn.fr (C. Gallut), ozouf@mnhn.fr (C. Ozouf-Costaz), lecointr@mnhn.fr (G. Lecointre), adettai@mnhn.fr (A. Dettai).
URL: http://www.genoscope.fr (C. Cruaud).

1055-7903/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ympev.2012.12.020

corroboration across studies more difficult, and the composition of the clade remains to be checked with a better sampling of acanthomorphs. Serraniformes are an assemblage of some members, but not all, of several previously used Acanthomorph orders (Nelson, 2006). From Gasterosteiformes, they only include sticklebacks (Gasterosteidae) and aulorhynchids (Smith & Wheeler, 2004, 2006; Li et al., 2009), while the other members of the Gasterosteiformes are reliably grouped with other clades. Many Perciform families are members of the Serraniformes: wolffishes (Anarhichadidae), perches (Percidae), gunnels (Pholidae), groupers (Serranidae), weeverfishes (Trachinidae), eelpouts (Zoarcidae), icefishes, Antarctic dragon-fish or plunderfish (Notothenioidei) (Smith & Wheeler, 2004, 2006; Smith & Craig, 2007; Li et al., 2009; Matschiner et al., 2011; Near et al., 2012; Wainwright et al., 2012), while again, all the other tested families from this polyphyletic order are reliably grouped with other clades. All Scorpaeniformes tested to this day appear to belong in Serraniformes, whether they were part of the Cottoidei, the Scorpaenoidei or the Hexagrammoidei: poachers (Agonidae), sculpins (Cottidae), pigfishes (Congiopodidae), lumpfishes (Cyclopteridae), snailfishes (Liparidae), fatheads (Psychrolutidae), scorpionfishes (Scorpaenidae), searobins (Triglidae) (Smith & Wheeler, 2004, 2006; Smith & Craig, 2007; Li et al., 2009; Near et al., 2012), but the combinations are new and the suborders are not all regrouped. Gasterosteiformes, Perciformes, and Scorpaeniformes are therefore para- or polyphyletic, as had already been suggested by morphological studies, albeit not always with the same groupings (Johnson & Patterson, 1993; Imamura & Yabe, 2002). For most of these groups, non-monophyly is not surprising. Perciformes never received a clear definition (Nelson, 2006). Some scorpaeniforms appear to be included in Perciformes (Johnson & Patterson, 1993; Mooi & Gill, 1995; but see review of Shinohara & Imamura, 2007). Inversely, several families belonging to the Perciformes might be included within Scorpaeniformes, like Champsodontidae (Mooi & Johnson, 1997). To explore Serraniformes, a large and representative sampling of both Perciformes and Scorpaeniformes is therefore necessary. At a smaller scale, some families present monophyly issues: Serranidae (Schoelinck et al., in preparation; Smith & Craig, 2007), Nototheniidae (Dettai et al., 2012), and Scorpaenidae (Smith & Wheeler, 2004; Li et al., 2009) are not monophyletic. However, the evaluation at this scale needs a fine-grained taxonomic sampling. By including as many families as possible from these groups, as well as multiple outgroups, we provide a clearer picture of which groups are really included in the Serraniformes, and what the relationships within this group are.

Marker choice has a high importance for the reliability and relevance of phylogenetic studies. Several studies showed that IRBP (*interphotoreceptor retinoid-binding protein*) (Dettai & Lecointre, 2008; Chen & Mayden, 2009), MLL4 (*Mixed-lineage leukemia-like protein 4*) (Dettai & Lecointre, 2005), Rhodopsin retrogene (Chen et al., 2003) and RNF213 (*ring finger protein 213*) (Li et al., 2009) are relevant for the large scale phylogeny of acanthomorphs. However, these markers have not resolved many of the relationships sufficiently or convincingly. While previous studies have provided valuable results on a limited number of markers, new and suitable nuclear markers are still needed to increase accuracy and resolution.

We selected Pkd1 (*polycystic kidney disease*) used previously only at a small scale (Lautredou et al., 2010, 2012). The gene Pkd1 encodes a glycoprotein, but its function is unclear except that it is involved in adhesive cell–cell/matrix interactions (Gluecksmann-Kuis et al., 1995). We also selected MC1R (*melanocortin type 1 receptor*), a protein involved in changes of body pigmentation in many groups (Mundy, 2005; Rosenblum et al., 2004; Healy et al., 2001) including teleosts (Logan et al., 2003b; Gross et al., 2009). It has been used for phylogeny in primates (Mundy & Kelly, 2003) and cichlids (Henning et al., 2010).

Genome sequencing has created a wealth of new information for scientists to analyze, and genome/genome comparison allows to easily define and test new markers (Li et al., 2007, 2009). We included one new nuclear marker, MLL2 (*Mixed-lineage leukemia-like protein 2*), a histone methyltransferases (HMTs) (Yu et al., 1998; Hess, 2004; Ansari et al., 2008). While Pkd1, MC1R, and MLL2 are extensively studied for their function, little is known about their evolution and diversity in most groups. Acquiring sequences for a large diversity of species over an important group like acanthomorphs has an interest not only for the phylogenetic resolution it provides, but is also essential to make hypotheses about the evolution of protein structure and to improve the accuracy of functional classification (Rost, 1999; Sjolander, 2004; Jaroszewski et al., 2009; Godzik, 2011).

The first objective of this study is to assess the variability of the seven markers used in this study by comparing their divergence between chosen pairs of taxa with other nuclear markers used in teleosts (Chen et al., 2003; Smith & Wheeler, 2004, 2006; Li et al., 2007; Smith & Craig, 2007). The second objective is to use these markers (with a focus on Pkd1, MC1R and MLL2, never used for Serraniformes) to test the monophyly of clades within Serraniformes, especially Scorpaenidae and Serranidae, and provide phylogenetic hypotheses within the clade. New insights on the relationships within Serraniformes could change our interpretation of the evolution of Antarctic teleosts (Chen et al., 2003), of venom evolution (Nelson, 2006; Smith & Wheeler, 2006), and could bring a new light on the distribution of morphological characters, especially in Scorpaenidae.

2. Materials and methods

2.1. Gene sampling

Seven protein-coding markers were used in this study. Four have already been used for large scale phylogenies on Acanthomorpha: IRBP, MLL4, Rhodopsin retrogene and RNF213. Pkd1, MLL2 and MC1R have never been used before in Serraniformes. They were developed with a protocol described in Li et al. (2009) and Lautredou et al. (2012).

New primers have been developed here for MLL4, RNF213, MC1R, MLL2 and Pkd1. Some of them were aimed at Serranidae (MLL2F100-MLL2R945, Pkd1F51b-Pkd1R737) or Notothenioidei (MLL2F30-MLL2R950) but they also work for other Serraniformes species (Table 1). The primers with a * in Table 1 are the most efficient.

We compared the position of MLL2 and MC1R in the genome of all teleosts available in the ensembl database (http://www.ensembl.org/).

2.2. Taxon sampling

Our sampling includes 193 specimens representing 148 species from Gasterosteiformes (8 families, 11 species), Perciformes (32 families, 87 species) and Scorpaeniformes (16 families, 50 species). Within Scorpaenidae, 26 species are represented (Table 2).

2.2.1. Choice of families and outgroups

To check the monophyly of each family, at least two specimens from each were included in the sampling when it was possible.

In the light of the acanthomorph phylogeny of Li et al. (2009), multiple outgroups were selected. This included two distant species from every large, recognized acanthomorph clade; therefore, an additional 57 perciforms were included in the sampling to test

Table 1
List of the primers used in this study. Frag. Size is the size of the fragment expected; F = Forward; R = Reverse; total of hyb. is the temperature of hybridization used to amplify every marker.

Gene	Frag. size (bp)	Name	Sens	Primers	Total of hyb. (°C)	Sources
IRBP	≈800	F104	F	ATAGTYNTGGACAANTACTGCTC	52	Dettai and Lecointre (2008)
		F110*	F	TGGACAAYTACTGCTCRCCAGA		
		R869	R	GTNACYTCCCAGGWNGAGCC		
		R936*	R	CACGGAGGYTGAYNATCTTGAT		
MLL4	≈550 to 650	F1499	F	GTCAATCAGCAGTTCCAGC	50	Dettai and Lecointre (2005)
		R2158	R	ARAGTAGTGGGATCYAGRTACAT		
		F11*	F	CAG TTC CAG CCY CTC TA		This study
		F111	F	CTCYATGACNCTYACCAC		
		R645*	R	GGTCTCTTGATAATATTTGGGAC		
Rhodo	≈500 to 800	F193*	F	CNTATGAATAYCCTCAGTACTACC	50	Chen et al. (2003)
		F545	F	GCAAGCCCATCAGCAACTTCCG		
		R1039*	R	TGCTTGTTCATGCAGATGTAGA		
		R1073	R	CCRCAGCACARCGTGGTGATCATG		
RNF 213	≈700 to 1000	F3111	F	GCTGACTGGATTYAAAACCTT	47–60	Li et al. (2009)
		R4111	R	AACTGTCCAAARTCCCACAC		
		F114*	F	TAY ATY TTC TTY AAT GAT GAC CAY	52	This study
		R784	R	GAA ATC ATC TYA TCK GTG TCT TTC		
		R828*	R	CGAACTCTGTACCCARG		
MC1R	≈700	F113	F	CCGCATCCCSCAGGARCTNTTCC	58	This study
		R841	R	CCTGSAGGGTTTTNCGCAGC		
MLL2	≈800 to 1000	F4164	F	GARAGTCAGGTNTGCAGGC	62	This study
		R5171	R	TGGGAYTGKGGRCTKGAGG		
		F30	F	CCTGACTGCGGCCTGGGAGTCA	56	
		F100*	F	GACTGYCTGTAGCCYTCAGA		
		R930*	R	CCCTCCACCTTCAGGCT		
		R945	R	AGTTTACACCTCCAGATCCCT		
		R950	R	CCTCCACCTTCAGGCTCT		
Pkd1	≈650 to 800	F62*	F	CATGAGYGTCTACAGCATCCT	50	Lautredou et al. (2010)
		R952*	R	YCCTCTNCCAAAGTCCCACT		
		F51b	F	TAY GGC ATT CAC TAY GAY TGG	54	This study
		F63b	F	ACT GGC RCT TYG GAG A		
		R697	R	CAY CCA GTT GAA GAA GTT NGC		This study
		R737	R	AGG CTG ATG TTG GTT AAM GC		

the limits of the Serraniformes (listed A in Table 2). For many, the position in the acanthomorph phylogeny was previously unclear, so the potential of their inclusion in the Serraniformes needed to be tested. Further, 11 Gasterosteiformes were also included to assess how many clades that order is divided in (listed B in Table 2).

2.2.2. Identification

Morphological identification of 168 specimens was followed by molecular identification using COI (Table 2).

A distance tree of all COI sequences was made to check whether all sequences belonging to the same species clustered together (data not shown). If the position of a sequence was doubtful, it was compared using the blast tool of BOLD to the identified sequences available in the Barcode of Life Database (http://www.boldsystems.org/) for corroboration. We performed the same operations for the rhodopsin retrogene, searching in the NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/). For the other markers, there are not enough sequences available in the databases to make this worthwhile.

2.3. Molecular data

For each sample, a small piece of muscle tissue was stored at –24 °C or preserved in 70% ethanol at 3 °C. All DNA extractions followed a classical CTAB protocol with a chloroform isoamylalcohol step (Winnpennminck et al., 1993).

For the seven markers, DNA amplifications were performed by PCR in a final 21 µl volume containing 1 µl of DMSO, 1 µl of BSA, 0.80 µl of dNTP 6.6 mM, 0.12 µl of Taq DNA polymerase (MP Biomedicals or Qiagen), using 2 µl of the buffer provided by the manufacturer, 0.32 µl of each of the two primers at 10 pM (primer sequences in Table 1); 1 µl of DNA extract was added. After denaturation for 2 min, the PCR was run for 50 cycles of (20 s, 94 °C; 20 s, see Table 2 for hybridization temperatures; 50 s to 1 min 10 s, 72 °C) using a Biometra trioblock cycler (T3000). The results were visualised on ethidium-bromide stained agarose gels. Sequencing was performed by the National Center for Sequencing (Génoscope) at Evry using the same primers.

All markers were sequenced in both directions and sequence chromatograms were checked manually using Sequencher 4.8 (Gene Codes Corporation). The sequences were aligned by eye using BioEdit (Hall 1999), which was straightforward for IRBP, Rhodopsin, RNF213, Pkd1 and MC1R due to the rarity of gaps and because the markers are protein coding. The MLL2 and MLL4 amplicons exhibit more insertions-deletions events, but the alignment was easily performed when taking into account the reading frame.

2.3.1. Contaminations

Distance analyses were performed for each marker and the position of the specimens in the seven distance trees were compared to one another to check whether sequences were identical when they should not. Only doubtless sequences were kept for the analyses.

The sequences were deposited in GenBank (accession numbers in Table 2).

2.4. Analyses of the datasets

Mean pairwise differences among three pairs of taxa (a gasterosteid: *Gasterosteus aculeatus*, several species of zoarcid: *Lycodapus*

Table 2
Specimens included in this study. Specimen information, GenBank Accession numbers are listed. Sequences in bold have been produced for the present study. The two underlined sequences came from the study of Venkatesh et al. (1999) so the species is the same than for our study but not the specimen.

	Family	Species	COI	RNF213	RH	MLL4	IRBP	Pkdt	MLL2	MC1R	Sample number	Voucher number
Scorpaeniformes												
Cottoidei	Agonidae	*Agonus cataphractus*	**AP115-12**	**JX628498**	**AP115-12**	**JX628247**		**JX628377**	**JX628115**	**JX628001**	BPS1034	
	Cottidae	*Cottus gobio*	**AP113-12**	**JX628497**	**AP113-12**	**JX628246**	**JX627885**	**JX627885JX6**	**JX62824**	**JX628000**	BPS1011	
	Cyclop teridae	*Cyclopterus lumpus*	**AP041-12**	**JX628484**	**AP041-12**	**JX628232**		**JX628359**	**JX628359JX6**	**JX627987**	BPS0248	MNHN 2004-1506
	Liparidae	*Liparis montagui*	**AP112-12**	**JX628496**	**AP112-12**		**JX627884**	**JX628375**	**JX628113**	**JX627999**	BPS1001	
		Careproctus longipectoralis	EATF268	**JX628542**	EATF268		**JX627928**		**JX628162**	**JX628036**	si273n2117	MNHN 2008-2593
		Edentoliparis terraenovae	EATF471	**JX628545**	EATF471		**JX627931**	**JX628426**	**JX628165**	**JX628038**	si478n3092	MNHN 2008-2626
		Paraliparis mawsoni	EATF089	**JX628537**	EATF089		**JX627924**	**JX628421**	**JX628158**		si89n460	MNHN-2008-2605
	Psychrolutidae	*Ebinania costaecanariae*	**AP105-12**			**JX628239**	**JX627878**	**JX628239 JX6278**	**JX628106**	**JX627994**	BPS0601	
		Cottunculus thomsonii	**AP116-12**			**JX628248**		**JX628378**	**JX628116**	**JX628002**	BPS1064	
Dactylopteroidei	Dactylopteridae	*Dactylopterus volitans*	**AP118-12**				**JX627887**				BPS1219	
		Dactyloptena orientalis	JQ431672	**JX628523**	**KC222256**	**JX628279**	**JX627909**	**JX6280**	**JX628145**	**JX628408JX62**	MBIO1136	
Hexagrammoidei	Hexagrammidae	*Hexagrammos decagrammus*				**JX628293**	**JX627922**		**JX628166**		T636	KU 23723
		Oxylebius pictus	**AP168-12**				**JX627921**	**JX628420**			T56	KU 30409
		Pleurogrammus monopterygius			**AP048-12**						T2027	KU 28382
Platycephaloidei	Bembridae	*Bembradium sp.*		**JX628471**	**AP167-12**	**JX628221**	**JX627856**	**JX628349**	**JX628083**	**JX627975**	ASIZP0913782	
		Bembradium sp.		**JX628472**	**AP166-12**	**JX628222**	**JX627857**	**JX628350**	**JX628084**	**JX627976**	ASIZP0913789	
	Hoplichthyidae	*Hoplichthys langsdorfii*	**AP063-12**	**JX628461**	**AP063-12**	**JX628212**	**JX627850**	**JX628341**	**JX628077**	**JX627966**	ASIZP0913580	
		Hoplichthys regani	**AP062-12**	**JX628460**	**AP062-12**		**JX627849**				ASIZP0913579	
	Platycephalidae	*Onigocia bimaculata*	JQ431939	**JX628511**	**KC222255**	**JX628264**	**JX627899**				MBIO185	MNHN 2008-0277
		Onigocia bimaculata	JQ431940	**JX628515**	**KC222254**	**JX628272**	**JX627904**	**JX628400**	**JX628400**	**JX628016**	MBIO479	MNHN 2008-0479
	Peristediidae	*Peristedion orientale*	**AP071-12**	**JX628465**	**AP071-12**	**JX628216**	**JX627854**	**JX628345**		**JX627970**	ASIZP0913625	
		Satyrichthys hians	**AP069-12**	**JX628463**	**AP069-12**	**JX628214**	**JX627852**	**JX628343**		**JX627968**	ASIZP0913618	
		Satyrichthys hians	**AP082-12**				**JX627862**				ASIZP0913915	
		Satyrichthys murrayi	**AP070-12**	**JX628464**		**JX628215**	**JX627853**	**JX628344**	**JX628078**	**JX627969**	ASIZP0913619	
		Satyrichthys welchi	**AP076-12**	**JX628469**							ASIZP0913704	
		Satyrichthys sp.	**AP077-12**	**JX628470**	**AP077-12**	**JX628220**	**JX627855**	**JX62808**	**JX627855JX62**	**JX627974**	ASIZP0913720	
		Satyrichthys sp.	**AP064-12**	**JX628462**	**AP064-12**	**JX628213**	**JX627851**	**JX628342**		**JX627967**	ASIZP0913583	
	Triglidae	*Lepidotrigla sp.*	**AP085-12**	**JX628477**	**AP085-12**	**JX628227**	**JX627866**	**JX628354**	**JX628089**	**JX627981**	ASIZP0913968	
		Trigla lyra	**AP013-11**		**AP013-11**						30	MNHN 2011-780
		Trigloporus lastoviza	**AP098-12**	**JX628487**	**AP098-12**	**JX628234**	**JX627873**	**JX628234JX62**	**JX62809**	**JX62809**	BPS0400	
Scorpaenoidei	Aploactinidae	*Erisphex pottii*		**JX628474**	**AP165-12**		**JX627865**	**JX628353**	**JX628088**	**JX627979**	ASIZP0913965	
	Caracanthidae	*Caracanthus unipinna*	JQ431536	**JX628527**	**KC222253**	**JX628283**	**JX627912**	**JX628412**	**JX628149**	**JX628026**	MBIO1357	MNHN 2008-0986
	Congiopodidae	*Zanclorhynchus spinifer*	**AP139-12**	**JX628548**	EU638021	**JX628304**	EU638165	**JX628429**	**JX628170**	**JX628045**	111	
	Scorpaenidae											
	Scorpaeninae	*Dendrochirus biocellatus*	JQ431683	**JX628529**	**KC222252**	**JX628286**	**JX627915**	**JX628415**	**JX628152**	**JX628029**	MBIO1580	MNHN 2008-1067
		Dendrochirus sp.	**AP090-12**				**JX627868**			**JX627984**	ASIZP0914018	
		Ebosia bleekeri	**AP088-12**	**JX628478**	**AP088-12**	**JX628228**			**JX628090**	**JX627982**	ASIZP0914001	
		Neomerinthe folgori	**AP099-12**	**JX628488**	**AP099-12**	**JX628235**	**JX627874**	**JX6283**	**JX628100**	**JX627990**	BPS0473	
		Parascorpaena mossambica	JQ431973	**JX628530**	**KC222251**	**JX628287**	**JX627916**	**JX628416**	**JX628153**	**JX6280**	MBIO1582	MNHN 2008-1068
		Pontinus macrocephalus	JX093922	**JX628475**	JX093947	**JX628225**	**JX627863**	JX093923	**JX628086**	**JX627980**	ASIZP0913936	
		Pontinus sp.	**AP134-12**	**JX628533**	**AP134-12**	**JX628290**		JX628418	**JX628155**	**JX628032**	MB110	
		Pterois antennata	JQ432074	**JX628519**	**KC222250**	**JX628275**	**JX627906**	JX628404	**JX628141**	**JX628019**	MBIO662	
		Scorpaena loppei	**AP017-11**	**JX628551**		**JX628309**	**JX627939**	**JX628435**	**JX628178**	**JX628049**	247	MNHN 2011-802
		Scorpaena onaria		EU638257	AY141288	AY362236	**DQ168114**					
		Scorpaena scrofa	**AP101-12**	**JX628489**	**AP101-12**		**JX627875**	**JX628364**	**JX628102**		BPS0514	
		Scorpaenodes corallinus	JQ432121	**JX628528**	**KC222249**	**JX628285**	**JX627914**	**JX627914**	**JX628151**	**JX628028**	MBIO1539	MNHN 2008-1053

(*continued on next page*)

Table 2 (*continued*)

	Family	Species	COI	RNF213	RH	MLL4	IRBP	Pkdt	MLL2	MC1R	Sample number	Voucher number
		Scorpaenodes guamensis	JQ432122	**JX628514**	**KC222248**	**JX628269**	**JX628397JX62**	**JX628397**	**JX628136**	**JX628013**	MBIO344	MNHN 2008-0385
		Scorpaenodes guamensis	JQ432125			**JX628270**	**JX627903**	**JX628398**		**JX628014**	MBIO348	
			AFG18072	**JX628526**	**KC222247**	**JX6282**		**JX628411**	**JX628148**	**JX628025**	MBIO1319	
		Scorpaenopsis diabolus	JQ432132	**JX628521**	**KC222246**	**JX628277**	**JX627907**	**JX628406**	**JX628143**	**JX628420**	MBIO1003	MNHN 2008-0821
			AFG18078	**JX628524**	**KC222245**	**JX628280**	**JX627910**	**JX628409**	**JX628146**	**JX628023**	MBIO1183	MNHN 2008-0904
		Scorpaenopsis possi	JQ432137	**JX628518**	**KC222244**	**JX628274**	**JX627905**	**JX628403**	**JX6288140**	**JX628018**	MBIO604	MNHN 2008-0568
		Sebastapistes tinkhami	JQ432147	**JX628513**	**KC222243**	**JX628267**		**JX628395**	**JX628134**	**JX628011**	MBIO303	MNHN 2008-0359
	Sebastinae	*Helicolenus dactylopterus*	**AP027-11**	**JX628547**	**AP027-11**	**JX628302**	**JX627933**	**JX628428**	**JX628169**	**JX628042**	58	MNHN 2011-781
		Helicolenus dactylopterus	**AP121-12**		**AP121-12**		**JX627891**	**JX628383**	**JX628121**	**JX628003**	BPS1260	
		Sebastes mentella	**AP122-12**	**JX628504**	**AP122-12**	**JX628253**	**JX627892**	**JX628384**	**JX628124**	**JX628004**	BPS1301	
		Trachyscorpia cristulata	**AP111-12**		**AP111-12**	**JX628245**	**JX627883**	**JX628374**	**JX628112**	**JX627998**	BPS0998	
	Synanceinae	*Synanceia verrucosa*		EU638267	**EU638011**	**JX628310**	EU638156	**JX628436**	**JX628179**		255	
		Synanceia verrucosa	JQ432179	**JX628520**	KC222242	**JX628276**		**JX628405**	**JX628142**		MBIO833	MNHN 2008-0706
Perciformes												
Notothenioidei	Artedidraconidae	*Dolloidraco longedorsalis*	EATF179	**JX628539**	EATF179	**JX628295**	**JX627926**	JQ688777	**JX628160**		si182n1370	MNHN-2009-0955
	Bathydraconidae	*Cygnodraco mawsoni*	EATFR018		EATFR018		**JX627940**	JQ688767		**JX628053**	559	
		Racovitzia glacialis	EATF219	**JX628541**	EATF219	**JX628297**	**JX627927**	**JX628424**			si224n1697	MNHN-2009-1066
		Racovitzia glacialis	EATF220		EATF220			JQ688778			si225n1698	MNHN-2009-1067
	Bovichtidae	*Bovichtus diacanthus*	FKCI042	**JX628555**	FKCI042	**JX628317**	**JX627942**	JQ688741	**JX628184**		1205	MNHN2005-0102
		Cottoperca gobio	EATFR003	**JX628556**	EATFR003	**JX628319**		JQ688745	**JX628186**		1233	
	Channichthyidae	*Champsocephalus gunnari*	**AP145-12**	**JX628557**	**AP145-12**	**JX628321**	**JX627944**	**JX628441**	**JX628188**	**JX628056**	1272	
	Eleginopsidae	*Eleginops maclovinus*	FKCI050	EU638199	FKCI050	EU638047	EU638121	JQ688746	**JX628210**		1234	MNHN2005-0093
	Harpagiferidae	*Harpagifer kerguelenensis*	EATF605	**JX628552**	EATF605	**JX628312**		JQ688766			412	MNHN2000-0269
	Nototheniidae	*Lepidonotothen squamifrons*	FKCI057	**JX628559**	JQ063278	**JX628320**	**JX627943**	JQ063252	**JX628187**		1251	
		Trematomus eulepidotus	**EATF325**	**JX628543**	GU997285	**JX628298**	**JX627929**	**JX628425**	**JX628163**	**JX628037**	si330n2440	MNHN 2009-1298
		Trematomus tokarevi	**EATF442**	**JX628544**	GU997387	**JX628299**	**JX627930**	GU997593	**JX628164**		si447n3011	MNHN-2009-1345
Percoidei	Acropomatidae	*Acropoma sp.*	A			**AP164-12**	**JX628224**	**JX627861**			**JX627978**	ASIZP0913886	
		Malakichthys griseus	A	**AP080-12**		**AP080-12**		**JX627858**				ASIZP0913880	
		Malakichthys griseus	A	**AP081-12**		**AP081-12**		**JX627860**				ASIZP0913881	
		Malakichthys sp.	A	**AP061-12**	**JX628459**	**AP061-12**	**JX628211**	**JX627848**	**JX628340**	**JX628076**	**JX627965**	ASIZP0913578	
		Malakichthys sp.	A			**AP163-12**						ASIZP0913898	
		Neoscombrops pacificus	A	**AP131-12**		**AP131-12**	**JX628263**	**JX627898**			**JX628035**	JNC2653	
	Caristiidae	*Caristius macropus*	A	**AP107-12**			**JX628241**	**JX627879**	**JX628370**	**JX628108**		BPS0626	
	Centracanthidae	*Spicara alta*	A	**AP144-12**	**JX628553**		**JX628315**		**JX628439**	**JX628182**		923	
		Spicara maena	A		**JX628486**	**AP123-12**	**JX628254**	**JX627893**	**JX62825**	**JX628122**	**JX628005**	BPS1329	
	Cirrhitidae	*Amblycirrhitus bimacula*	A	JQ431404		**KC222241**		**JX627913**	**JX628413**	**JX628150**	**JX628027**	MBIO1407	MNHN 2008-0994
			A	AFG17584		**KC222240**	**JX628268**	**JX627900**	**JX628396**	**JX628135**	**JX628012**	MBIO311	MNHN 2008-0365
		Paracirrhites forsteri	A	JQ431959		**KC222239**						MBIO61	
		Paracirrhites hemistictus	A	AFG17905		**KC222238**	**JX628266**	**JX627900**	**JX628394**	**JX628133**	**JX628010**	MBIO261	MNHN 2008-0332
	Emmelichthyidae	*Erythrocles monodi*	A	**AP046-12**		**AP046-12**						T8371	MCZ 167694
	Gerreidae	*Diapterus peruvianus*	*A*	**AP047-12**		**AP047-12**						T8533	KU 40323
		Eucinostomus argenteus	*A*	**AP045-12**		**AP045-12**						T3838	USNM 351280
		Gerres cinereus	*A*	**AP042-12**		**AP042-12**						T62	USNM 343870
	Kyphosidae	*Kyphosus sectator*	*A*	**AP103-12**	**JX628491**	**AP103-12**		**JX627876**	**JX627876**	**JX628104**	**JX627993**	BPS0572	
		Kyphosus vaigiensis	*A*	JQ431874	**JX628512**	**KC222237**	**JX628265**		**JX628393**	**JX628132**	**JX628009**	MBIO207	MNHN 2008-0293
	Lethrinidae	*Gnathodentex aureolineatus*	*A*	JQ431755	**JX628516**	**KC222236**	**JX628273**		**JX628401**	**JX628138**		MBIO487	MNHN 2008-0485
		Lethrinus olivaceus	*A*	JQ431885	**JX628525**	**KC222235**	**JX628281**	**JX627911**	**JX628410**	**JX628147**	**JX62814**	MBIO1269	
		Monotaxis grandoculis	*A*	JQ431910		**KC222234**	**JX628271**		**JX628399**	**JX628137**	**JX628015**	MBIO468	MNHN 2008-0471
	Percichthyidae	*Howella parini*	*A*	**AP079-12**	**JX628473**	**AP079-12**	**JX628223**	**JX627859**	**JX628351**	**JX628085**	**JX627977**	ASIZP0913816	
		Howella zina	*A*	**AP078-12**		**AP078-12**						ASIZP0913761	

	Percidae	*Pe rcafluvia ti lis*		**AP040-12**	**JX628485**	**AP040-12**		**JX627871**	**JX628360**	**JX628097**		BPS0265	MNHN 2004-1522
		Stizostedion lucioperca		**AP117-12**	**JX628499**	**AP117-12**	**JX628249**	**JX627886**	**JX628379**	**JX628117**		BPS1154	
	Priacanthidae	*Heteropriacanthus cruentatus*	A	JQ431859	**JX628522**	**KC222233**	**JX628278**	**JX627908**	**JX628407**	**JX628144**	**JX628021**	MBIO1028	MNHN 2008-0834
		Priacanthus blochii	A	**AP089-12**		**AP089-12**						ASIZP0914013	
		Priacanthus hamrur	A	JQ432031	**JX628531**	**KC222232**	**JX62828**	**JX627917**	**JX628417**	**JX628154**	**JX628031**	MBIO1613	MNHN 2008-1084
		Pri acanthus macracanthus	A	**AP091-12**		**AP091-12**						ASIZP0914061	
		Pri acanthus sp.	A	**AP060-12**		**AP060-12**		**JX627847**			**JX627964**	ASIZP0913565	
		Priacanthidae sp.	A	**AP055-12**		**AP055-12**						ASIZP0913544	
		Pristigenys meyeri	A	**AP073-12**		**AP073-12**						ASIZP0913647	
		Pristigenys niphonia	A			**AP056-12**					**JX627963**	ASIZP0913552	
		Pristigenys niphonia	A	**AP087-12**		**AP087-12**						ASIZP0914000	
		Pristigenys sp.	A	**AP074-12**	**JX628467**	**AP074-12**	**JX628218**		**JX628346**	**JX628080**	**JX627972**	ASIZP0913677	
	Serranidae												
	Anthiinae	*Holanthias chrysostictus*			EU638206	AY141290	AY362209	DQ168073					
		Pseudanthias sp.			**JX628466**	AP072-12	**JX628217**			**JX628079**	**JX627971**	ASIZP0913637	
		Pseudanthias sp.			**JX628476**	AP084-12	**JX628226**	**JX627864**	**JX628352**	**JX628087**		ASIZP0913962	
	Epinephelinae	*Aporops bilinearis*		AFG17400		KC222230	**JX628308**	**JX627938**	KC222260	**JX628176**	**JX628050**	MBIO226	MNHN 2008-0307
		Belonoperca chabanaudi		AFG17427	**JX628532**	KC222231	**JX628289**	**JX627918**	**KC222259**	**JX628192**	**JX628059**	MBIO1850	MNHN 2008-1159
		Cephalopholis sonnerati		JQ431575	**JX628562**	KC222229	**JX628324**	**JX627945**	**JX628443**	**JX628193**	**JX628060**	MBIO1869	
		Dermatolepis sp.		**AP137-12**	**JX628546**	AP137-12	**JX628300**	**JX627932**	**JX628427**	**JX628168**	**JX628040**	24	
		Epinephelus aeneus		**AP127-12**	**JX628507**	AP127-12	**JX628258**	**JX627896**	**JX628389**	**JX628127**		BPS1414	
		Epinephelus altivelis		**AP148-12**		AP148-12	**JX628328**	**JX627946**	**JX628446**	**JX628195**	**JX628064**	3164	
		Epinephelus leucogrammicus		**AP133-12**		AP133-12	**JX628325**		**JX628444**		**JX628061**	1929	
		Epinephelus sp.		BFS285-11		EU637934						DHS353	
		Grammistops ocellatus		JQ431777	**JX628554**	KC222228	**JX628316**	**JX627941**	**JX628440**	**JX628183**	**JX628054**	MBIO975	
		Liopropoma lunulatum		JQ431889	**JX628561**	KC222227	**JX628323**	**JX627947**	**JX628442**	**JX628191**	**JX628058**	MBIO1710	
		Mycteroperca costae		**AP126-12**	**JX628506**	AP126-12	**JX628257**	**JX627895**	**JX628388**	**JX628126**	**JX628008**	BPS1396	
		Mycteroperca marginata		**AP102-12**	**JX628490**	AP102-12	**JX628237**		**JX628365**	**JX628103**	**JX627992**	BPS0522	
		Plectropomus leopardus		**AP147-12**		AP147-12	**JX628326**		**JX628445**		**JX628062**	2555	
		Pogonoperca punctata		AFG17966	**JX628558**	JX093951	**JX628318**	**JX62818**	**JX093927**	**JX628185**	**JX628055**	MBIO1217	
		Pseudogramma polyacantha		AFG18006			**JX628314**		**KC222258**			MBIO509	
		Rypticus saponaceus			EU638253	AY368329	AY362257	DQ168111					
		Saloptia powelli		AFG18033	**JX628560**	JX093954	**JX62832**	**JX627949**	**JX093930**	**JX628189**	**JX628057**	MBIO1460	MNHN 2008-1014
		Variola louti		EPINBO016	**JX628563**	EPINBO016	**JX628327**	**JX627950**	EPINBO016	**JX628194**	**JX628063**	3069	MNHN-icti-2964
	Serraninae	*Chelidoperca sp.*			**JX628468**		**JX628219**		**JX628347**	**JX628081**	**JX627973**	ASIZP0913683	
		Chelidoperca sp.			**JX628479**		**JX628229**	**JX627867**	**JX628355**	**JX628091**	**JX627983**	ASIZP0914008	
		Serranus atricauda		**AP095-12**	**JX628482**	**AP095-12**		**JX627869**	**JX628357**	**JX628094**	**JX627985**	BPS0127	
		Serranus cabrilla		**AP110-12**	**JX628495**		**JX628244**	**JX627882**	**JX628373**	**JX628111**	**JX627997**	BPS0985	
		Serranus cabrilla		**AP100-12**			**JX628236**			**JX628101**	**JX627991**	BPS0482	
		Serranus scriba		**AP109-12**	**JX628494**	**AP109-12**	**JX628243**	**JX627881**	**JX628372**	**JX628110**	**JX627996**	BPS0980	
		Serranus sp.		**AP136-12**	**JX628535**	**AP136-12**	**JX628292**	**JX627920**	**JX628419**	**JX628157**	**JX628034**	MB123	
	Terapontidae	*Teraponjarbua*	A	**AP129-12**	**JX628509**	**AP129-12**	**JX628261**			**JX628130**		JNC1990	
		Teraponjarbua	A	**AP130-12**	**JX628510**	**AP130-12**	**JX628262**	**JX627897**	**JX628392**	**JX628131**		JNC1991	
Trachinoidei	Ammodytidae	*Gymnammodytes semisquamatus*	A		**JX628500**		**JX628250**	**JX627888**	**JX628380**	**JX628118**		BPS1229	
		Hyperoplus lanceolatus	A	**AP119-12**	**JX628501**		**JX628251**	**JX627889**	**JX628381**	**JX628120**		BPS1230	
		Ammodytes marinus	A	**AP120-12**	**JX628502**		**JX628252**	**JX627890**	**JX628382**	**JX628119**		BPS1232	
	Cheimarrhichthyidae	*Cheimarrichthys fosteri*	A	**AP142-12**	EU638185	AY141307	**JX628311**	DQ168052	**JX628437**	**JX628180**	**JX628051**	281	
	Champsodontidae	*Champsodon snyderi*	A	**AP140-12**	EU637949	EU638182	EU638038		**KC222227**			205	
		Champsodontidae sp.	A	**AP094-12**	**JX628480**		**JX628230**		**JX628356**	**JX628092**		BOA052	
	Chiasmodontidae	*Pseudoscopelus altipinnis*	A	**AP097-12**			**JX628233**	**JX627872**	**JX628361**	**JX628098**	**JX627988**	BPS0391	
		Chiasmodon niger	A	**AP106-12**	**JX628493**	**AP106-12**	**JX628240**		**JX628369**	**JX628107**		BPS0625	
	Creediidae	*Chalixodytes chameleontoculis*	A	JQ431598			**JX628284**					MBIO1388	MNHN 2008-0991
	Percophidae	*Bembrops sp.*		BFS310-11	**JX628508**		**JX628259**		**JX628390**	**JX628128**		DHS380	MNHN-2010-804
		Bembrops sp.		BFS311-11			**JX628260**		**JX628391**	**JX628129**		DHS381	MNHN-2010-805

(*continued on next page*)

Table 2 (*continued*)

	Family	Species		COI	RNF213	RH	MLL4	IRBP	Pkdt	MLL2	MC1R	Sample number	Voucher number
	Pinguipedidae	*Parapercis millepunctata*	A	IQ431972	**JX628517**				**JX628402**	**JX628139**	**JX628017**	MBIO546	MNHN 2008-0526
	Trachinidae	*Echiichthys vipera*		**AP002-11**	**JX628549**		**JX628306**	**JX627936**	**JX628432**	**JX628173**	**JX628147**	176	MNHN 2011-793
		Trachinus draco		**AP006-11**	**JX628550**	AP006-11	**JX628307**	**JX627937**	**JX628433**	**JX628174**	**JX628048**	177	MNHN 2011-794
		Trachinus draco		**AP104-12**	**JX628492**	**AP104-12**	**JX628238**	**JX627877**	**JX628367**	**JX628105**		BPS0592	
	Uranoscopidae	*Uranoscopus scaber*	A	**AP125-12**	**JX628505**	**AP125-12**	**JX628256**	**JX627894**	**JX628387**	**JX628125**	**JX628007**	BPS1386	
Zoarcoidei	Anarhichadidae	*Anarhichas lupus*		**AP039-12**	**JX628483**	**AP039-12**	**JX628231**	**JX627870**	**JX628358**	**JX628095**	**JX627986**	BPS0141	MNHN 2003-1599
	Pholidae	*Pholis gunnellus*		**AP093-12**	EU638241	**AP093-12**	AY362285	DQ168100	**JX628430**	**JX628093**		B114	
	Zoarcidae	*Lycodapus pachysoma*		EATF164	**JX628538**		**JX628294**	**JX627925**	**JX628422**	**JX628159**		si166n1250	MNHN-2009-1396
		Pachycara brachycephalum		EATF181	**JX628540**	EATF181	**JX628296**		**JX628423**	**JX628161**		si184n1389	MNHN-2009-0029
Gasterosteiformes													
Gasterosteoidei	Indostomidae	*Indostomus paradoxus*	B	**AP156-12**	**JX628578**	EU637967	EU638057					1063	
		Indostomus sp.	B	**AP138-12**		**AP138-12**	**JX628301**					36	
	Gasterosteidae	*Gasterosteus aculeatus*		**AP108-12**		**AP108-12**	**JX628242**	**JX627880**	**JX628371**	**JX628109**	**JX627995**	BPS0807	
		Spinachia spinachia		**AP124-12**	**JX628503**	**AP124-12**	**JX628255**		**JX628386**	**JX628123**	**JX628006**	BPS1348	
Syngnathoidei	Aulostomidae	*Aulostomus chinensis*	B	**AP143-12**		**AP143-12**	**JX628313**		**JX628438**	**JX628181**	**JX628052**	414	
		Aulostomus chinensis	B	**AP149-12**		AY141279	AY362226	DQ168040					
		Aulostomus chinensis	B	**AP169-12**	**JX628536**	**AP169-12**		**JX627923**		**JX628167**	**JX628039**	T6839	SAIAB 77945
	Centriscidae	*Aeoliscus strigatus*	B	**AP146-12**		EU637931		EU638100		**JX628190**		1658	
	Fistulariidae	*Fistularia petimba*	B	**AP141-12**	EU638202	AY141324				**JX628177**		234	
	Macroramphosidae	*Macroramphosus scolopax*	B	**AP012-11**		**AP012-11**	JX628303	**JX627934**			**JX628043**	59	MNHN 2011-782
		Macroramphosus sp.	B	**AP135-12**	**JX628534**	**AP135-12**	JX628291	**JX627919**		**JX628156**	**JX628033**	MB112	
	Pegasidae	*Pegasus volitans*	B	**AP157-12**	**JX628579**				**JX628457**	**JX628209**		1656	
Outgroup	Syngnathidae	*Hippocampus hippocampus*	B	**AP014-11**							**JX628044**	96	MNHN 2011-785
	Berycidae	*Beryx splendens*		**AP154-12**	**EU638174**	AY141265	**JX628339**	DQ168045	**JX628456**	**JX628208**		1031	
	Blenniidae	*Blennius ocellaris*		**AP001-11**		**AP001-11**						152	MNHN 2011-791
	Bothidae	*Arnoglossus laterna*		**AP032-11**	**JX628571**	**AP032-11**	**JX628332**	**JX627953**		**JX628200**		161	MNHN 2011-790
		Arnoglossus imperialis		**AP009-11**		**AP009-11**				**JX628197**		28	MNHN 2011-779
	Callionymidae	*Callionymus maculatus*			**JX628566**		**JX628329**	**JX627952**			**JX628041**	25	MNHN 2011-777
	Carangidae	*Trachurus trachurus*		**AP023-11**	**JX628574**	**AP023-11**		**JX627958**	**JX628452**	**JX628203**	**JX628070**	203 204	MNHN 2011-796
		Trachurus mediterraneus			**JX628575**	**AP031-11**				**JX628175**			MNHN 2011-797
	Cepolidae	*Cepola macrophthalma*		**AP005-11**	**JX628564**			**JX627951**		**JX628196**	**JX628065**	9	MNHN 2011-773
	Gadidae	*Gadus morhua*			**JX628570**	AF137211	EU638050	**JX627960**	**JX628454**	**JX628205**	**JX628072**	125	
		Dicentrarchus labrax				**AP018-11**	**JX628336**					218	MNHN 2011-801
	Labridae	*Labrus bergylta*		**AP029-11**	**JX628576**		**JX628337**	**JX627961**	**JX628455**	**JX628206**		277	MNHN 2011-805
	Merlucciidae	*Merluccius merluccius*		**AP026-11**		**AP026-11**						27	MNHN 2011-778
	Molidae	*Mola mola*		**AP150-12**	**JX628565**	**AF137215**	AY362251	AY362251	**JX628447**		**JX628066**	18	
	Moroni dae	*Merlangius merlangus*		**AP024-11**		**AP024-11**						217	MNHN 2011-800
	Mugilidae	*Liza ramada*		**AP016-11**		**AP016-11**	**JX628335**	**JX627959**	**JX628453**	**JX628204**	**JX628071**	205	MNHN 2011-798
	Mullidae	*Mullus surmuletus*			**JX628573**	**AP028-11**	**JX628333**	**JX627957**	**JX628451**	**JX628202**		178	MNHN 2011-795
	Myctophidae	*Lampanyctus crocodilus*		**AP010-11**	**JX628568**	**AP010-11**	**JX628330**			**JX628199**		72	MNHN 2011-784
	Phycidae	*Phycis blennoides*		**AP033-11**		**AP033-11**	**JX628331**					123	
	Pomacentridae	*Dascyllus trimaculatus*		**AP152-12**		EU637953	EU638043	EU638117			**JX628075**	66	
	Scophthalmidae	*Scophthalmus rhombus*			**JX628569**	**AP003-11**	**JX628334**	**JX627955**	**JX628449**	**JX628172**	**JX628068**	151	MNHN 2011-789
	Siganidae	*Siganus vulpinus*		**AP151-12**	**JX628567**	EU638007	EU638090	**JX627954**	**JX628448**	**JX628198**	**JX628067**	54	
	Sparidae	*Boops boops*		**AP007-11**	**JX628572**	**AP007-11**		**JX627956**	**JX628450**	**JX628201**	**JX628069**	175	MNHN 2011-792
	Synbranchidae	*Monopterus albus*		**AP155-12**	**JX628577**	AY141276	AY362252	AY362252	**JX628458**		**JX628074**	1016	
	Zeidae	*Zeusfaber*		**AP153-12**		EU638023	AY362287	DQ168128		**JX628207**	**JX628073**	358	

pachysoma, *Zoarces viviparous*, *Lycodes diapterus,* and a scorpaenid: *Sebastes* spp.) were calculated using MEGA 4 (Tamura et al., 2007) for the seven markers of this study, four markers of Li et al. (2007): myh6, ENC1, Ptr and Tbr1 and the nuclear markers H3, 28S rDNA and TMO-4c4 used in different studies including the Serraniformes (Chen et al., 2003; Smith & Wheeler, 2004, 2006; Smith & Craig, 2007).

Gaps were treated as missing data in all analyses. First, each of the seven markers was treated as a unique dataset and analyzed separately. Then, IRBP, Rhodopsin, RNF213 and MLL4 were concatenated to make dataset 1 for comparison purposes with the separate analyses of the new markers. Dataset 2 is the concatenation of all the seven markers.

For the datasets 1 and 2, only the specimens for which at least three out of seven sequences were available were kept. For this study, ML and BI analyses of the seven markers and the two combined datasets were performed. Each marker was partitioned by codon position in all analyses, yielding 12 partitions for datasets 1 and 21 for dataset 2.

2.4.1. Maximum Likelihood analyses (ML)

The ML analyses were run with RaxML 7.2.7 on the Cipres Science Gateway online web server (Miller et al., 2010) with the GTR+G model implemented. The robustness of the nodes of the cladograms was estimated by the bootstrap method (Felsenstein, 1985) with 1000 replicates for each analysis.

2.4.2. Bayesian phylogenetic inference (BI)

Using jModelTest (Posada, 2008), we found the recommended model for partition is GTR+I+G under the AIC criterion. All the parameters were estimated during the analyses. The BI analyses were conducted with Mr. Bayes v. 3.1.2 (Huelsenbeck & Ronquist, 2005) on each dataset. Four chains (three heated and one cold) were run for 20 000 000 generations for each of the two independent runs for each dataset (one tree sampled out of 100). All the trees were pooled after a burn-in of 5%, after checking it was sufficient for convergence.

Reliability of the clades was assessed by comparing congruence of different trees inferred from independent markers. Then, reliability is considered to increase for a given clade if it is also recovered by an independent research team using different data.

We compared our results to the studies of Smith and Wheeler (2004, 2006) and Smith and Craig (2007). These used different markers and have different sampling, therefore providing independent results from ours. Only clades repeatedly recovered are going to be discussed.

2.5. Comparison of topologies

We evaluated the congruence between the topology of each new marker and the topology of the concatenated tree of IRBP, Rhodopsin, RNF213, and MLL4 using TOPD–FMTS version 3.3 (Puigbo et al., 2007).

The TOPD–FMTS analysis was performed under default conditions, with 100 simulated trees as a null model. The terminals not included in both trees are pruned during the analysis. To assess congruence, the nodal distance is calculated between the trees using the root-mean squared distance (RMSD) of the distance matrices. It is entirely based on the branching pattern and hence does not account for evolutionary rate variation across the phylogeny. The RMSD is 0 for identical trees and increases as the trees become more dissimilar. This analyse was estimated using ML trees because RMSD method is restricted to binary trees (Puigbo et al., 2007).

3. Results and discussion

Except for Pkd1, the new primers outperformed the older ones (see * in Table 1). In total, 130 specimens could be sequenced for IRBP (66%), 142 for MLL4 (72%), 160 for Rhodopsin (81%), and 131 for RNF213 (67%). Concerning the new markers, 133 specimens were sequenced for Pkd1 (67%), 114 for MC1R (58%), and 134 for MLL2 (68%). Among our specimens, MC1R had the lowest percentage of successful sequencing; however, no sucessful new primers were designed for this marker. Even without new primers, Rhodopsin is the easiest marker to amplify for all specimens, explaining its success as a first go-to nuclear marker in acanthomorph phylogeny (Venkatesh et al., 1999; Chen et al., 2003; Chen & Mayden, 2009; Lecointre et al., 2011).

3.1. Structure and location of MC1R and MLL2 in Teleostei

The Pkd1 gene is single-copy in acanthomorphs. It is composed of 58 exons in *Gasterosteus aculeatus*. The primers were designed on the exon 20 of the gene which is 2615 bp long, to amplify a 650–800 bp fragment (Fig. 1c).

From six to four members (MC1R, MC2R, MC3R, MC4R, and to orthologues of MC5R) of the melanocortin receptor family have been identified in pufferfish and zebrafish (Logan et al., 2003a,b), but the divergence across paralogues is ancient, and they can be steadily recognized (Selz et al., 2007). The MC1R gene is single-copy and approximately 1000 bp long (Logan et al., 2003b; Selz et al., 2007). It is composed of one or two exons, depending on the species. The primers were designed in the larger exon (Fig. 1a).

The MLL family includes seven paralogues in teleosts (MLL1, MLL2, MLL3a, MLL3b, MLL4a, MLL4b, MLL5) (Nagasawa et al., 2012). The MLL2 and MLL4a and MLL4b paralogues diverged before the diversification of vertebrates (Nagasawa et al., 2012). They are located on different chromosomes (see Table 3), and they are not providing the same functions, so they can be considered as totally independent markers for phylogenetics in fishes. Even if the separation between MLL4a and MLL4b is recent compared to MLL2 versus MLL4, their divergence is also sufficient to reliably differentiate, identify, and amplify the two copies reliably across species by PCR (see Fig. 3 of Nagasawa et al., 2012).

The fragment amplified in this study is part of MLL4b. The fragment of MLL2 amplified is 1000 bp long and is located in the exon 28 in *Gaterosteus aculeatus* (Fig. 1b). In *Oreochromis niloticus*, but no taxon included in our study, the amplified fragment is split in two exons according to the gene description in Ensembl.

Neither MC1R nor MLL2 could be found in the Ensembl *Gadus morhua* genome; perhaps, this isbecause the genome of this species is new and incomplete.

We have verified if one or more of the seven markers under study are located on the same chromosome pair for each of the species having genetic maps available in the Ensembl database (Table 3). Partial information is provided: the markers are located on different chromosomes in *Tetraodon nigroviridis* (six markers) and *Danio rerio* (three markers). No data are available for *Gadus morhua* and *Oreochromis niloticus*. Rhodopsin and MLL2 both map on chromosome seven of *Oryzias latipes* and on chromosome 12 of *Gasterosteus aculeatus*, suggesting the synteny of at least one chromosome segment between these two species.

3.2. Variability of the markers

Previous studies (Li et al., 2007) have proposed new markers, but many of them have little variability at smaller scales. The variability of IRBP, Rhodopsin, RNF213, and MLL4 is taxon dependent,

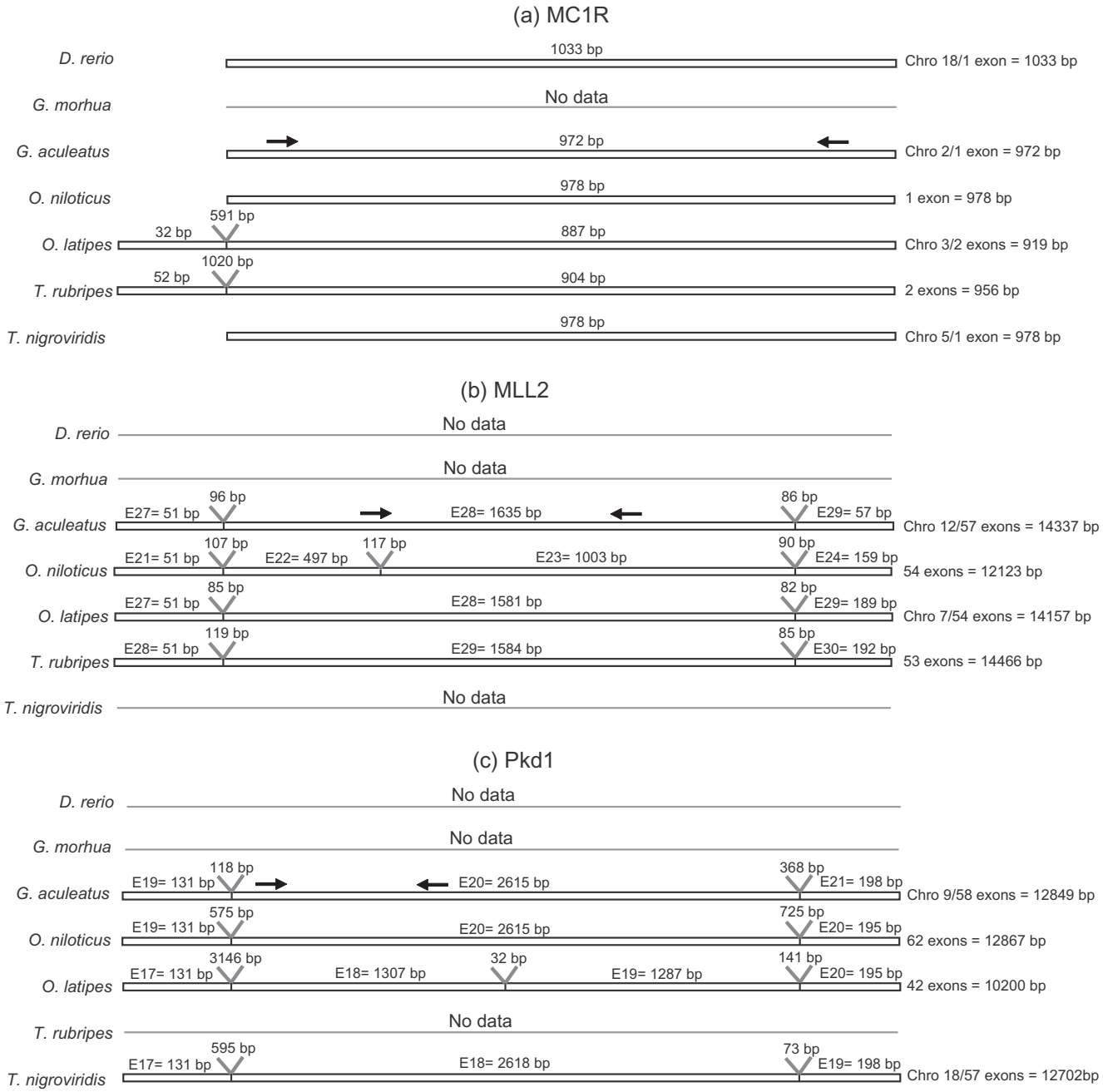

Fig. 1. Structure of (a) MC1R, (b) MLL2 and (c) Pkd1 and location of the amplified fragment for the seven species available in the Ensembl database. Introns are represented by gray areas. The Ex = y bp represente the number of the exons. The size of each exon is indicated after the signe equal. At the right of the figure are indicated: the localization of each gene on the chromosome of each species (Chro X) as well as the total number of exons of the gene and is total length. For several species, the localization of the genes on the chromosomes is unknown. Oligonucleotides primers positions are indicated by arrows.

but it has greater variability than ENC1 (Li et al., 2007) or H3 and 28S rDNA (Chen et al., 2003; Smith & Wheeler, 2004, 2006; Smith & Craig, 2007) (Fig. 2).

The exceptions to this increased variability are tbr1 for the Gasterosteidae/Zoarcidae and Pkd1, TMO4C4, and myh6 for the Cottidae/Gasterosteidae (Fig. 2). However, the markers of Li et al. (2007) were difficult to amplify for the Serraniformes.

Our seven markers have different variability depending on the taxa considered. This is of interest because some markers might be more interesting for reconstructing deeper divergences (MLL4, Rhodopsin, or RNF213) while others might present enough variability for more recent events like Pkd1 already used at smaller scale. Among the three new markers, Pkd1 has the highest mean pairwise divergences for all the three pairs of taxa. It is in the same order of magnitude as the four previously used markers (IRBP, Rhodopsin, RNF213; and MLL4) (Fig. 2). Those four markers have proved very efficient at recovering relationships within teleosts (Dettai et al., 2012; Chen et al., 2003; Dettai & Lecointre, 2005, 2008; Chen & Mayden, 2009; Li et al., 2009). The MC1R gene has the lowest variability of the five others markers of this study; MLL2 has slightly more variability. The TMO4C4 and the 28S genes have variability that is similar to MC1R, MLL2, and Pkd1; TMO4C4 is the most variable marker for the comparison between Cottidae and Gasterosteidae. However, 28S and TMO4C4 must be used more

Table 3
Chromosome location of the seven nuclear markers under study in the genetic maps of acanthomorph species available in the Ensembl database.

	IRBP	Rhodopsin	RNF213	MLL4	MC1R	MLL2	Pkd1
Tetraodon nigroviridis	chro 2	chro 9	chro 3	chro 10	chro 5	_–	chro 18
Takifugu rubripes	–	–	–	–	–	–	–
Danio rerio	–	chro 8	chro 3	_	chro 18	_	_
Gadus morhua	–	–	–	–	–	–	–
Oreochromis niloticus	–	–	–	–	–	–	–
Oryzias latipes	–	**chro 7**	chro 8	chro 13	chro 3	**chro 7**	_–
Gasterosteus aculeatus	chro 5	**chro 12**	chro 11	chro 1	chro 2	**chro 12**	chro 9

cautiously when comparing acanthomorphs (Li et al., 2009). The tbr1 gene is the most variable marker for Gasterosteidae and Zoarcidae, the variability of myh6 is in the same order of magnitude of MLL4 and MLL2. This can explain why myh6, ptr, and tbr1 are the three mostly used markers of (Li et al., 2007) in phylogenetic studies on acanthomorphs (Holcroft & Wiley, 2008;

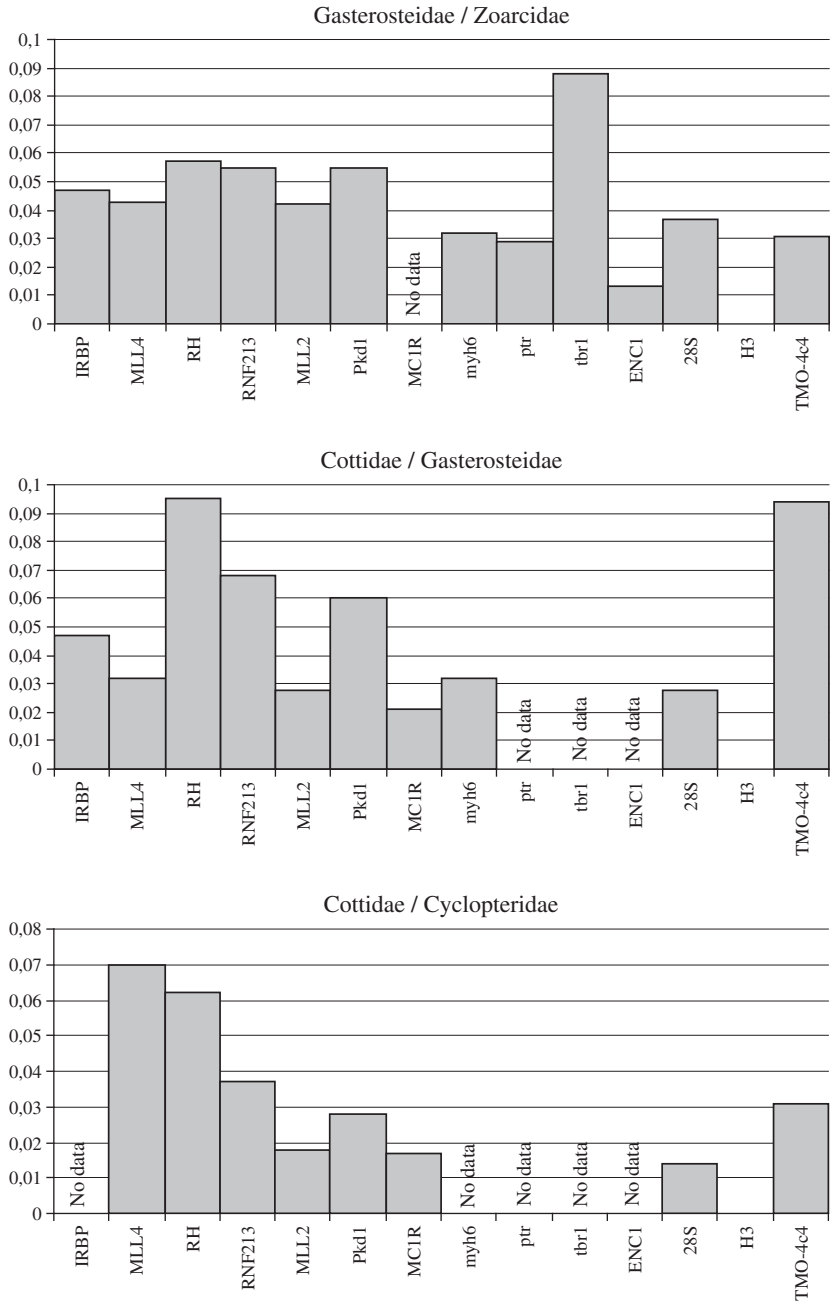

Fig. 2. Comparison of the mean pairwise differences for three pairs of taxa for the seven nuclear markers used in this study and for seven other nuclear markers commonly used for teleosts (myh6, ENC1, Ptr and Tbr1: Li et al., 2007; H3, 28S rDNA and TMO-4c4: Chen et al., 2003; Smith and Wheeler, 2004, 2006; Smith and Craig, 2007). The values were calculated with MEGA 4.

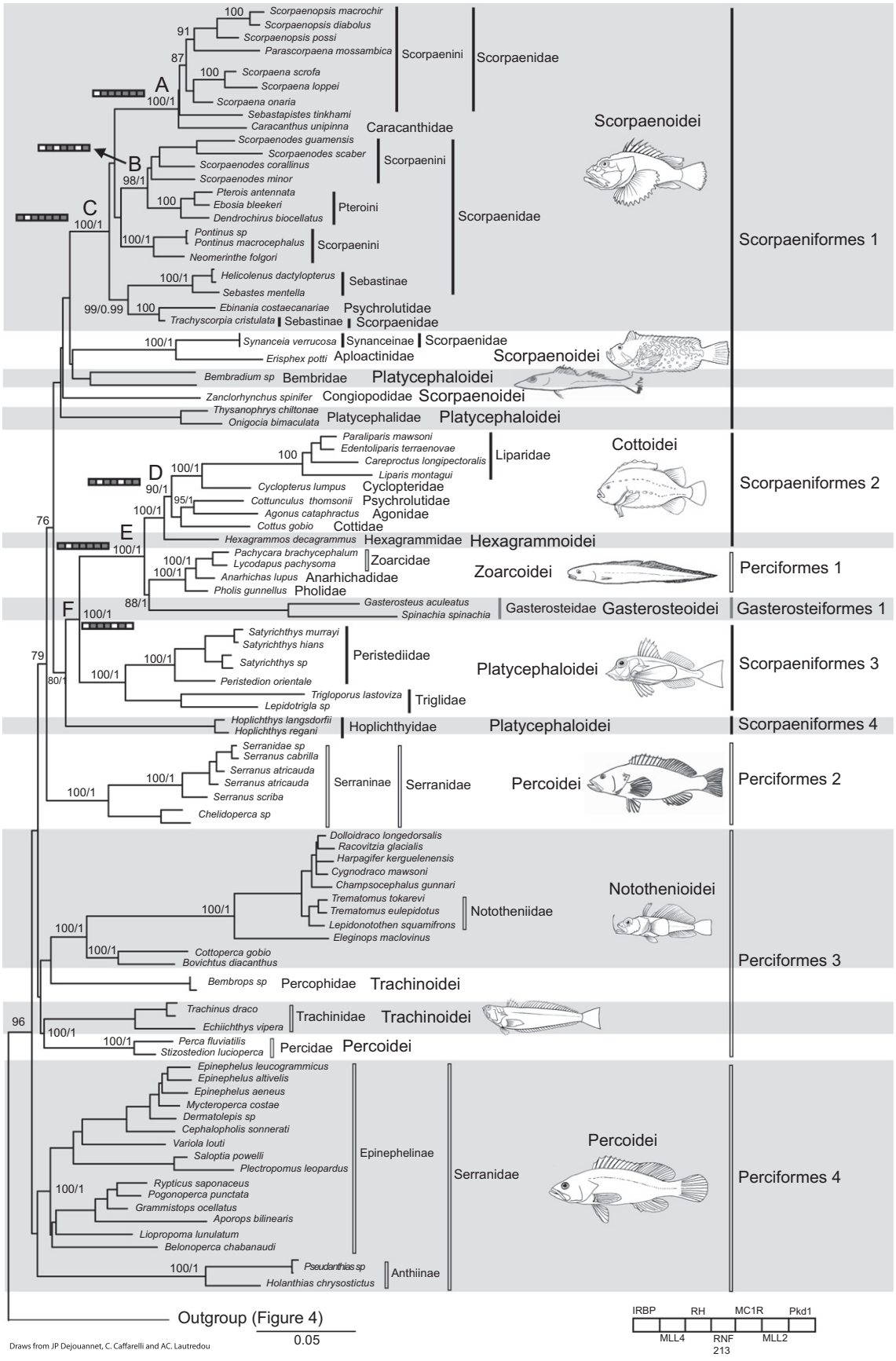

Fig. 3. Bayesian and Maximum likelihood trees of the ingroup (complete concatenation, dataset 2). Nodes with no indication have bootstrap values or posterior probabilities below 70 (ML analysis) or 0.90 (Bayesian analysis). The seven squares represent the seven markers. IRBP, MLL4, Rhodopsin, RNF213, MC1R, MLL2 and Pkd1. Nodes not recovered in the separate analyse of a marker are indicated with a white square, nodes recovered in the separate analyse of a marker are indicated by a gray square.

Matschiner et al., 2011). The H3 gene is not variable at all for the three taxa chosen.

3.3. Promises and advantages of new markers and new samples

While variability is important to evaluate the interest of markers at smaller scales, topology comparison allows evaluating their relevance for deeper divergences. We compared the topologies of the separate analyses of MC1R, MLL2, and Pkd1 versus the topology of the tree obtained from the dataset 1 (see Figs. 1a and 1b in the Supplementary material) (Mueller, 2006).

The RMSD of MC1R is 4.01 within the 95% confidence interval for random trees (5.33 ± 0.37); the RMSD of MLL2 is 3.62 (5.48 ± 0.36), the RMSD of Pkd1 is 3.27 (5.44 ± 0.35).

The RMSD values of each of the three new genes are close to each other, so there is no marker-specific bias affecting one of them and strong enough to provoke aberrant isolated topologies. In light of both its variability and the RMSD value, Pkd1 would appear to be the most adapted marker for phylogenetic work on this group. It had already been used for phylogeny at small scale on Acanthomorpha, but it appears to be a good marker in general.

3.4. Relationships among the Serraniformes

The separate ML and BI analyses of the seven markers were not well resolved but are congruent with each other (data not shown). In these trees, the Serraniformes are monophyletic only in the ML and BI trees of MLL2. The BI trees of the datasets 1 and 2 (Figs. 1b and 2 of the Supplementary material) were less resolved than the ML trees (Figs. 3 and 4 and Fig. 1a of the Supplementary material) but were not in contradiction with them. Serraniformes are monophyletic only in the ML trees.

3.4.1. A mix of Gasterosteiformes, Perciformes, and Scorpaeniformes

In this study, nodes are considered supported when the bootstrap is above 70 and the posterior probability (pp) is above 0.90. We will discuss here only the clades that have been recovered by several independent loci or combinations of loci.

All the Perciformes that we tested for inclusion in the Serraniformes (listed under A in the Table 2) were positioned outside the clade. As shown by several previous molecular studies, members of three different orders are present within this clade: Gasterosteiformes, Perciformes, and Scorpaeniformes (Chen et al., 2003; Miya et al., 2003; Smith & Wheeler, 2004, 2006; Dettai & Lecointre, 2005, 2008; Smith & Craig, 2007; Kawahara et al., 2008; Li et al., 2009; Matschiner et al., 2011). Morphological studies have suggested that sticklebacks could be divided in several different clades but the composition of these clades is not the same depending on the study (Nelson, 1984; Springer & Orrell, 2004). In our sampling Gasterosteiformes are well represented with eight of 11 families. In the topologies resulting from datasets 1 and 2 (Figs. 3 and 4), Gasterosteiformes are divided in four groups: (1) a strongly supported clade composed of Fistularidae, Aulostomidae, Centriscidae, and Macroramphosidae that grouped with Dactylopteridae (Scorpaeniformes) and Mullidae (Perciformes), as in Chen et al. (2003), Dettai and Lecointre (2005) and Li et al. (2009), (2) Pegasidae, (3)

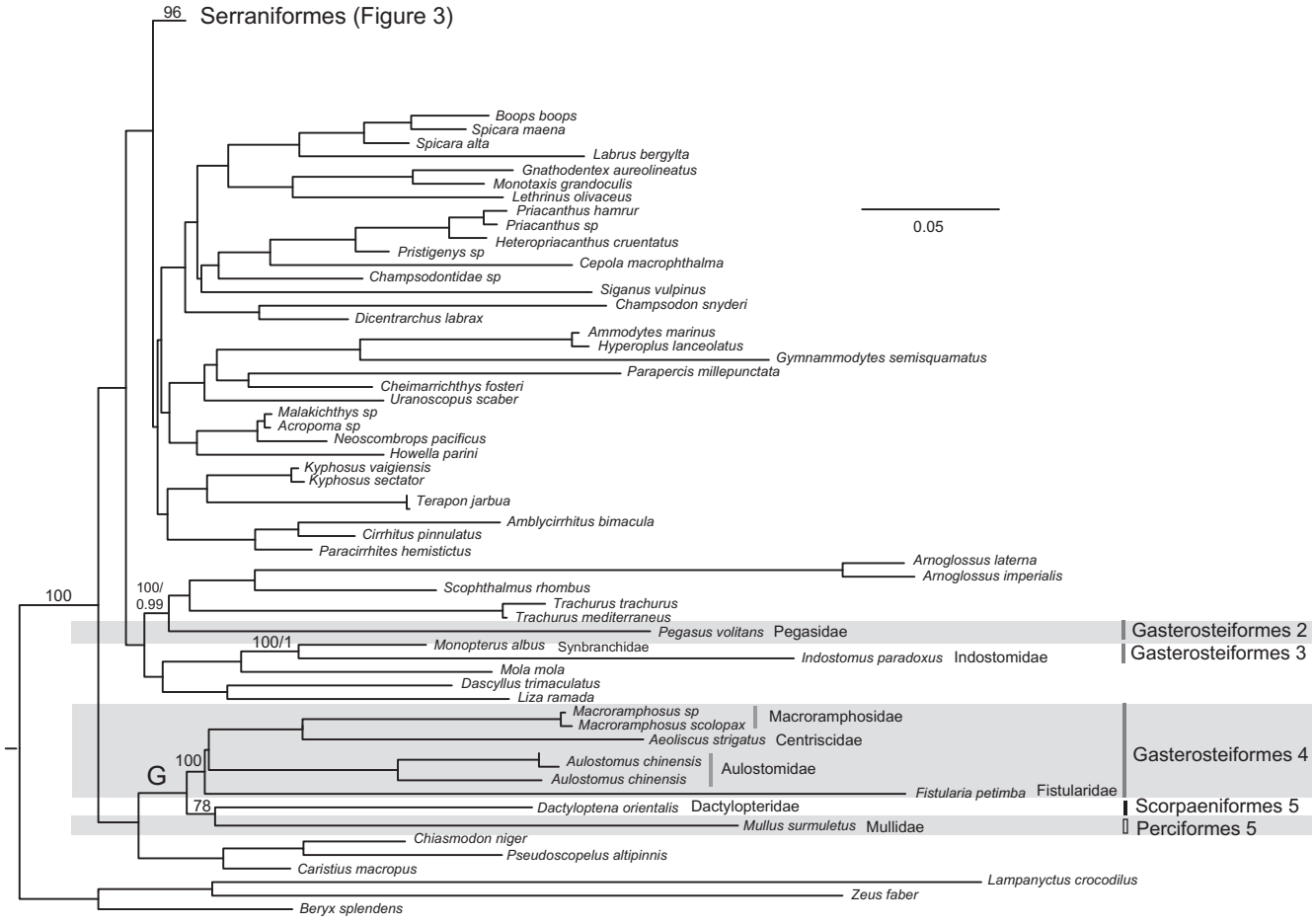

Fig. 4. Bayesian and Maximum likelihood trees of the outgroups (complete concatenation, dataset 2). Nodes with no indication have bootstrap values or posterior probabilities below 70 (ML analysis) or 0.90 (Bayesian analysis).

Indostomidae grouped with Synbranchidae as in Kawahara et al. (2008) and Li et al. (2009), (4) Gasterosteidae, the only one included within the Serraniformes in the present study as in Smith and Wheeler (2004, 2006), Kawahara et al. (2008). Both Smith and Wheeler (2004, 2006) and Kawahara et al. (2008) found that Aulorhynchidae grouped with Gasterosteidae (and therefore belong to Serraniformes), but we have no representative of the family in the present study.

Perciformes and Scorpaeniformes are divided in four distinct clades. The para- or polyphyly of these groups has been discussed for a long time using either morphological or molecular data (Johnson & Patterson, 1993; Imamura & Yabe, 2002; Chen et al., 2003; Miya et al., 2003; Smith & Wheeler, 2004, 2006; Matschiner et al., 2011).

3.4.2. Confirmation of the monophyly of several groups

Within Serraniformes, some clades described based on morphological data are repeatedly recovered using independent molecular data. They can therefore be considered reliable, within the obvious limitations of the molecular taxonomic sampling.

As in other several studies, the three sub-orders Notothenioidei and Zoarcoidei are monophyletic (Dettai et al., 2012; Dettai & Lecointre, 2004, 2005; Li et al., 2009; Matschiner et al., 2011). Cottoidei is monophyletic to the exclusion of *Ebinania* (Psychrolutidae) that groups with the genus *Trachyscorpia*. This will be discussed in Section 3.4.3. Trachinoidei are not monophyletic as suggested by many molecular and morphological studies (see Imamura & Odani, 2012 for a review). The question of the origin of Notothenioidei is still standing, and a number of hypotheses have been made. Based on morphological characters, multiple authors have suggested that Notothenioidei could be placed among trachinoids. Trichonotidae (sanddivers: Gosline, 1968; Hastings, 1993), Pinguipedidae (sandperches: Gosline, 1968; Pietsch, 1989; Hastings, 1993; Balushkin, 2000), and Cheimarrichthyidae (torrentfishes: Gosline, 1968) have been proposed as a sister-group of Notothenioidei, but the two last families are sister-group to each other outside the Serraniformes.

The sister-group of Notothenioidei is Percophidae, with Trachinidae and Percidae as a sister-group to both (Fig. 3). In previous molecular studies, Notothenioidei has grouped with Trachinidae (Li et al., 2009), with Percidae (Dettai & Lecointre, 2004) or with *Serranus* and Percidae (Matschiner et al., 2011), but there were no percophids in these samplings. Smith and Wheeler (2006) and Smith and Craig (2007) were the only ones to include both Percophidae (Bembropinae) and Notothenioidei, and recover the same result. But this clade is not well supported in our tree and is not recovered in any of the separate analyses, so it would still need to be corroborated with additional data. Smith and Craig (2007) suggested recognizing the superfamily Notothenioidea (Notothenioidei and Bembropinae) as they share a number of morphological features: the loss of one pectoral radial, i.e. with three distinct radials present instead of four, and the rostral displacement of the pelvic fins such that they originate anteriorly to the pectoral fins (Smith, 2005).

Within Cottoidei, Liparidae and Cyclopteridae formed a clade associated with Cottidae, Psychrolutidae, and Agonidae. Hexagrammidae are the sister-group of this set (clade D, Fig. 3). The sister-group relationship of Cyclopteroidea and Cottoidea is supported by molecular (Smith & Wheeler, 2004) and morphological studies (Imamura et al., 2005). Here, the monophyly of the lumpfish-snailfish group is supported in five of the seven separate analyses. It is congruent with morphological characters shared by these two groups such as pelvic fins modified into a sucking disc (when present); lateral line usually absent (Nelson, 2006).

Zoarcoidei and Gasterosteoidei are the sister-group of clade D plus Hexagrammidae (clade E), supported in six of the seven separate analyses. Each of these relationships is very well supported. Clades D and E are recovered in every molecular study of Serraniformes (Chen et al., 2003; Miya et al., 2003; Dettai & Lecointre, 2004, 2005, 2008; Smith & Wheeler, 2006; Smith & Craig, 2007; Li et al., 2009). According to Imamura and Yabe (2002), the relationship between Cottoidei and Zoarcoidei is supported by 13 synapomorphies (including a parietal sensory canal without spines, and the lack of supraneurals).

3.4.3. New clades

2.4.3.1. Platycephaloidei. In the present study (Fig. 3), Platycephaloidei are divided in four different clades, Bembridae, Platycephalidae, Hoplichthyidae, and a clade composed of Triglidae and Peristediidae. Peristediids and triglids are separated from the others by well supported nodes. This very interesting result is also recovered by Smith and Wheeler (2004, 2006) and Smith and Craig (2007). The Triglidae–Peristediidae are the sister-group of the clade E and form the clade F, very well supported by both bootstrap and pp. Li et al. (2009) also found Peristediidae in this position, but it was the only platycephaloid included in their sampling. For the first time, the position of Hoplichthyidae is very well supported in the Fig. 3 as the sister-group of the clade F (clade Isc of Dettai and Lecointre (2005) and Li et al. (2009)).

The suborder has been supported by morphological features for a long time. Imamura (1996) provided evidence that platycephaloids are monophyletic and are characterized by a posterior pelvic fossa. In the light of these results, this character needs to be reconsidered carefully, as it might be the result of a convergence.

2.4.3.2. Serranidae. Serranidae are not monophyletic, neither in our trees nor in previous studies (Dettai & Lecointre, 2004, 2005, 2008; Smith & Wheeler, 2006; Smith & Craig, 2007; Li et al., 2009; Matschiner et al., 2011). They are divided in two non-related clades here, one composed of Epinephelinae and Anthiinae (*Pseudanthias* and *Holanthias*), and the other composed of the Serraninae, like in other studies (Dettai & Lecointre, 2004, 2005; Smith & Wheeler, 2006). The monophyly of Epinephelinae is very well supported by both molecular and morphological data for the present sampling, and they share morphological characters on both larvae and adults. Adults have three opercular spines, and their first dorsal-fin pterygiophore lacks a separate distal radial (Gosline, 1966; Baldwin & Johnson, 1993). This modification might be related to the support of an elongate dorsal-fin spine in larvae (Gosline, 1966; Baldwin & Johnson, 1993). Elongate dorsal-fin spines are uncommon among known larvae of percoids and are lacking in serranines (Gosline, 1966; Baldwin & Johnson, 1993). Serraninae are monophyletic here as in Meisler (1987) who diagnosed this clade by the loss of the supramaxillae and the anterior portion of second infraorbital bone completely lateral to posterior lacrimal.

The position of Anthiinae remains an issue. In Smith and Craig (2007), Anthiinae are mixed with Serraninae, but, in our results, Anthiinae are the sister-group of Epinephelinae. However, their position is supported neither in the separate nor in the combined analyses, and they are the sister-group of Serraninae in ML and BI analyses of dataset 1. The taxonomic changes suggested by Smith and Craig (2007) seem appropriate, but the position of anthiinae as well as their morphological synapomorphies (absence of a toothplate on epibranchial two: (presence of 26 vertebrae: Anderson et al., 1990; and absence of a toothplate on epibranchial two: Baldwin, 1990) have to be explored further.

2.4.3.3. Scorpaenoidei with emphasis on Scorpaenidae. Scorpaenoidei are not monophyletic and are divided in three clades. The position of Congiopodidae is unclear. The genus *Zanclorhynchus* (Congiopodidae) had been proposed as the sister-group of Notothenioidei in Smith and Wheeler (2004), but they are distant from the Antarctic group in this study. However the Congiopodidae are not monophyletic in Smith and Wheeler (2004), and *Congiopodus* is grouped with

Bembridae. The only other well supported scorpaenoid lineage (clade C) groups Scorpaenidae, Caracanthidae, Sebastidae, and Psychrolutidae. It is supported by both bootstrap and pp, and is present in six of the seven separate analyses. In the MLL4 trees, the Psychrolutidae are monophyletic, which is not the case in the other separate analyses where *Ebinania* belongs to the Scorpaenidae. The sequences of the specimen *Ebinania* have been checked twice for each marker so there is no doubt about these sequences. The only molecular study including this family placed them in their expected position (Smith & Wheeler, 2004) in the same clade of Cottidae, and we recover this position for the psychrolutid genus *Cottuncunlus* in our separate analyses and in the combined analyses. In the present study, *Cottunculus* is the sister-group of Agonidae with a bootstrap of 95 and a pp of 1 and these two families form a clade with Cottidae. The genus *Ebinania* is the sister-group of *Trachyscorpia* in Fig. 3 and belongs to a very well supported clade composed of all sebastids in this study. The morphology of *Ebinania* and its position in the phylogeny should be investigated further.

Scorpaenidae are not monophyletic here or in any studies including multiple Scorpaenoidei (Smith & Wheeler, 2004, 2006; Smith & Craig, 2007), and appear to be split in four clades.

The only study where Scorpaenidae are monophyletic is Li et al. (2009) but there were only four specimens from this family included. Here, clade A is well supported, as it is recovered in six of the seven separate analyses. In combined analysis, Caracanthidae are included in Scorpaenidae, and more precisely in tribe Scorpaenini, like they were in previous molecular studies (Smith & Wheeler, 2004, 2006; Smith & Craig, 2007). Several morphological studies supported the close relationship of velvetfishes with Scorpaenidae (Cole & Montgomery, 2003: based on the morphology of the gonads; Shinohara & Imamura, 2005) and more precisely with two genera belonging in the Scorpaenini in a clade with *Taenianotus* and *Pteroidichthys* (Shinohara & Imamura, 2005). It would be very interesting to sample *Taenianotus* and *Pteroidichthys*. For the time being, we propose to include Caracanthidae in Scorpaenidae in agreement with Shinohara and Imamura (2005) and Smith and Craig (2007).

Scorpaena, *Scorpaenopsis*, *Sebastapistes* and *Parascorpaena* are in the same clade, and they are very similar to each other morphologically (Motomura & Buth, 2004; Motomura et al., 2004, 2005a,b, 2009a; Motomura, 2009). There is no morphological definition on a worldwide basis available for each genus (Motomura et al., 2004), but *Sebastapistes* and *Parascorpaena* are sometimes considered a subgenus of *Scorpaena* (Mandrytsa, 2001).

Clade B groups *Scorpaenodes* with Pteroini. *Scorpaenodes* and Pteroini have 13 dorsal-fin spines, a swimbladder, and do not have palatine teeth (Greenfield & Matsuura, 2002; Motomura et al., 2009a,b; Matsunuma & Motomura, 2011). They also shared a distinct morphological character among scorpaenids where the procurrent rays of the caudal fin are unsegmented and spinous. This character could be a synapomorphy of the group.

Scorpaenodes and *Scorpaenopsis* have the same general body shape, live in the same type of habitat, i.e., shallow tropical and subtropical waters, and hidden in rocky and coral reefs (Motomura et al., 2004, 2010), but they are in different clades. The morphology of these two genera appears therefore to be a convergence. Species in the genus *Scorpaenopsis* also lack palatine teeth (Motomura & Causse, 2011), so in the light of this phylogeny, the palatine teeth were lost independently twice.

Pontinus and *Neomerinthe* (Scorpaenini) are separated from the other scorpaenins and form the sister-group of Pteroini plus *Scorpaenodes*. This result is congruent with the study of Smith and Wheeler (2004). Clade B and *Pontinus* plus *Neomerinthe* share a flat occipital region covered with scales, which suggests a close relationship between these species (Motomura, unpublished data).

According to Nelson (2006), Synanceini belong to the Scorpaenidae. Yet *Synanceia* is well separated from the other scorpaenids in several studies, including this one (Smith & Wheeler, 2004, 2006; Smith & Craig, 2007). In these studies, Synanceini are the sister-group of Pataecidae, and both are included in a larger clade comprising also Gnathanacanthidae and Aploactinidae. Here, Synanceini are the sister-group of Aploactinidae (Fig. 3), but neither Pataecidae nor Gnathanacanthidae are present. We propose to recognize Synanceini again as a family Synanceidae. Finally, there is some morphological evidence that groups these families together, both larval (Leis & Rennis, 2000) and adult (presumed fusion of the scapula and uppermost pectoral radial (presumed fusion of the scapula and uppermost pectoral radial, Ishida, 1994).

4. Conclusion

The addition of taxa is important to resolve taxonomic issues. As there are numerous markers in this study as well as a good sampling, the present study can assess the relationships among the families belonging to the Serraniformes, as well as the monophyly or non-monophyly of the different families of this group. We confirm some previous results but also question some others. The clades D and E are very well supported and recovered in several studies. The sister-relationship of Percophidae and Notothenioidei in Smith and Craig (2007) as well as the division of Platycephaloidei in three different groups (Smith and Wheeler, 2004) needs some confirmation. We confirm here that the Platycephaloidei are polyphyletic, peristediids, hoplichthyids, bembrids and platycephalids clearly separated from each other.

For the Serranidae, the taxonomic changes of Smith and Craig (2007) seem appropriate in the light of our results but the monophyly of Anthiinae should be explored.

With 26 species of Scorpaenidae, this study has one of the best sampling after the 29 species (including Sebastidae, Setarchidae and Synanceidae) of Smith and Wheeler (2006). Combined with adapted markers, this sampling brings new light on the intrarelationships on Scorpaenidae: they should include Caracanthidae and perhaps part of the Psychrolutidae.

Acknowledgments

This work was supported by a TOTAL Fundation grant. We also thank Christina Cheng, Samuel Iglesias, Jean-Lou Justine, Charlotte Schoelinck, the Museum Victoria, the University of Kansas, the Moorea Biocode Project, the CEAMARC, SANTO and EVOHE cruises for the tissue samples. We thank the 'Service de systematique moleculaire' (IFR CNRS 101) of the 'Muséum national d'histoire naturelle', Paris, France, for support; the Barcode and the 'Bibliotheque du Vivant' projects for the sequencing. We thank Leo Smith and the 'anonymous reviewer'. We also thank Jean-Francois Dejouannet for the drawings.

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.2012.12.020.

References

Anderson, W.D., Parin, N.V., Randall, J.E., 1990. A new genus and species of anthiine fish (Pisces: Serranidae) from the eastern South Pacific with comments on anthiine relationships. Proc. Biol. Soc. Wash. 103, 922–930.

Ansari, K.I., Mishra, B.P., Mandal, S.S., 2008. Human CpG binding protein interacts with MLL1, MLL2 and hSet1 and regulates Hox gene expression. BBA – Gene Regul. Mech. 1779, 66–73.

Baldwin, C.C., 1990. Morphology of the Larvae of American Anthiinae (Teleostei: Serranidae), with Comments on Relationships within the Subfamily. Copeia, pp. 913–955.
Baldwin, C.C., Johnson, D.G., 1993. Phylogeny of the Epinephelinae (Teleostei: Serranidae). Bull. Mar. Sci. 52, 240–283.
Balushkin, A.V., 2000. Morphology, classification, and evolution of notothenioid fishes of the Southern Ocean (Notothenioidei, Perciformes). J. Ichthyol. 40, 74–109.
Chen, W.J., 2001. La répétitivité des clades comme critère de fiabilité: application à la phylogénie des Acanthomorpha (Teleostei) et des Notothenioidei (acanthomorphes antarctiques). Unpubl. Ph.D. diss., Université Paris VI, Paris.
Chen, W.J., Mayden, R.L., 2009. Molecular systematics of the Cyprinoidea (Teleostei: Cypriniformes), the world's largest clade of freshwater fishes: Further evidence from six nuclear genes. Mol. Phylogenet. Evol. 52, 544–549.
Chen, W.J., Bonillo, C., Lecointre, G., 2003. Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. Mol. Phylogenet. Evol. 26, 262–288.
Cole, K.S., Montgomery, W.L., 2003. Hermaphroditic characteristics of gonad morphology and inferences regarding reproductive biology in Caracanthus (Teleostei, Scorpaeniformes). Copeia, 68–80.
Dettai, A., Lecointre, G., 2004. In search of Notothenioid (Teleostei) relatives. Antarct. Sci. 16, 1–14.
Dettai, A., Lecointre, G., 2005. Further support for the clades obtained by multiple molecular phylogenies in the acanthomorph bush. C. R. Biol. 328, 674–689.
Dettai, A., Lecointre, G., 2008. New insights into the organization and evolution of vertebrate IRBP genes and utility of IRBP gene sequences for the phylogenetic study of the Acanthomorpha (Actinopterygii: Teleostei). Mol. Phylogenet. Evol. 48, 258–269.
Dettai, A., Berkani, M., Lautredou, A.C., Couloux, A., Lecointre, G., Ozouf-Costaz, C., Gallut, C., 2012. Tracking the elusive monophyly of nototheniid fishes (Teleostei) with multiple mitochondrial and nuclear markers. Mar. Genom.
Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
Gluecksmann-Kuis, M.A., Tayber, O., Woolf, E.A., Bougueleret, L., Deng, N., Alperin, G.D., et al., 1995. Polycystic kidney disease: the complete structure of the PKD1 gene and its protein. The international polycystic kidney disease consortium. Cell 81, 289–298.
Godzik, A., 2011. Metagenomics and the protein universe. Curr. Opin. Struct. Biol. 21, 398–403.
Gosline, W.A., 1966. The limits of the fish family Serranidae, with notes on other lower percoids. Proc. Calif. Acad. Sci. 33 (6), 91–112.
Gosline, W.A., 1968. The suborders of Perciform fishes. Proc. U.S. Nat. Mus. 124, 1–77.
Greenfield, D.W., Matsuura, K., 2002. Scorpaenodes quadrispinosus: a new Indo-Pacific scorpionfish (Teleostei: Scorpaenidae). Copeia, 973–978.
Gross, J.B., Borowsky, R., Tabin, C.J., 2009. A Novel role for Mc1r in the parallel evolution of depigmentation in independent populations of the cavefish *Astyanax mexicanus*. PLoS Genet. 5 (1), e1000326.
Hastings, P.A., 1993. Relationships of the fishes of the Perciform Suborder Notothenioidei. In: Miller, R.G. (Ed.), History and Atlas of the Fishes of the Antarctic Ocean. Foresta Institute for Ocean and Mountain Studies, Carson City, NV, pp. 99–107.
Healy, E., Jordan, S.A., Budd, P.S., Suffolk, R., Rees, J.L., Jackson, I.J., 2001. Functional variation of MC1R alleles from red-haired individuals. Hum. Mol. Genet. 10, 2397–2402.
Henning, F., Renz, A., Fukamachi, S., Meyer, A., 2010. Genetic, comparative genomic, and expression analyses of the Mc1r locus in the polychromatic midas cichlid fish (Teleostei, Cichlidae *Amphilophus sp.*) species group. J. Mol. Evol. 70, 405–412.
Hess, J.L., 2004. MLL: a histone methyltransferase disrupted in leukemia. Trends Mol. Med. 10, 500–507.
Holcroft, N., Wiley, E., 2008. Acanthuroid relationships revisited: a new nuclear gene-based analysis that incorporates tetraodontiform representatives. Ichthyol. Res. 55, 274–283.
Huelsenbeck, J., Ronquist, F., 2005. Bayesian analysis of molecular evolution using MrBayes. Stat. Method Mol. Evol. 183–226.
Imamura, H., 1996. Phylogeny of the family Platycephalidae and related taxa (Pisces: Scorpaeniformes). Species Divers. 1, 123–233.
Imamura, H., Odani, K., 2012. An overview of the phylogenetic relationships of the suborder Trachinoidei (Acanthomorpha: Perciformes). Ichthyol. Res. 1–15.
Imamura, H., Yabe, M., 2002. Demise of the scorpaeniformes (Actinopterygii: Percomorpha): an alternative phylogenetic hypothesis. Bull. Fish. Sci. Hokkaido Univ. 53, 107–128.
Imamura, H., Shirai, S.M., Yabe, M., 2005. Phylogenetic position of the family Trichodontidae (Teleostei: Perciformes), with a revised classification of the perciform suborder Cottoidei. Ichthyol. Res. 52, 264–274.
Ishida, M., 1994. Phylogeny of the suborder Scorpaenoidei (Pisces: Scorpaeniformes). Bull. Nansei. Natl. Fish. Res. Inst. 27, 1–112.
Jaroszewski, L., Li, Z., Krishna, S.S., Bakolitsa, C., Wooley, J., Deacon, A.M., et al., 2009. Exploration of uncharted regions of the protein universe. PLoS Biol. 7, 1–15.
Johnson, D.G., Patterson, C., 1993. Percomorph phylogeny: a survey of acanthomorphs and a new proposal. Bull. Mar. Sci. 52, 554–626.
Kawahara, R., Miya, M., Mabuchi, K., Lavoue, S., Inoue, J.G., Satoh, T.P., et al., 2008. Interrelationships of the 11 gasterosteiform families (sticklebacks, pipefishes, and their relatives): a new perspective based on whole mitogenome sequences from 75 higher teleosts. Mol. Phylogenet. Evol. 46, 224–236.
Lautredou, A.C., Bonillo, C., Denys, G., Cruaud, C., Ozouf-Costaz, C., Lecointre, G., et al., 2010. Molecular taxonomy and identification within the Antarctic genus *Trematomus* (Notothenioidei, Teleostei): how valuable is barcoding with COI? Polar Sci. 4, 333–352.
Lautredou, A.C., Hinsinger, D.D., Gallut, C., Cheng, C.H.C., Berkani, M., Ozouf-Costaz, C., Cruaud, C., Lecointre, G., Dettai, A., 2012. Phylogenetic footprints of an Antarctic radiation: the Trematominae (Notothenioidei, Teleostei). Mol. Phylogenet. Evol. 65 (1), 87–101.
Lecointre, G., Gallut, C., Bonillo, C., Couloux, A., Ozouf-Costaz, C., Dettaï, A., 2011. The Antarctic fish genus *Artedidraco* is paraphyletic (Teleostei, Notothenioidei, Artedidraconidae). Polar Biol. 34, 1135–1145.
Leis, J.M., Rennis, D.S., 2000. Scorpaenidae (scorpionfishes, stonefishes). In: Leis, J.M., Carson-Ewart, B.M. (Eds.), The Larvae of Indo-Pacific Coastal Fishes: An Identification Guide to Marine Fish Larvae. Brill, Leiden, pp. 226–235.
Li, C., Orti, G., Zhang, G., Lu, G., 2007. A practical approach to phylogenomics: the phylogeny of ray-finned fish (Actinopterygii) as a case study. BMC Evol. Biol. 7, 44–44.
Li, B., Dettaï, A., Cruaud, C., Couloux, A., Desoutter-Meniger, M., Lecointre, G., 2009. RNF213, a new nuclear marker for acanthomorph phylogeny. Mol. Phylogenet. Evol. 50, 345–363.
Logan, D.W., Bryson-Richardson, R.J., Pagan, K.E., Taylor, M.S., Currie, P.D., Jackson, I.J., 2003a. The structure and evolution of the melanocortin and MCH receptors in fish and mammals. Genomics 81, 184–191.
Logan, D.W., Bryson-Richardson, R.J., Taylor, M.S., Currie, P., Jackson, I.J., 2003b. Sequence characterization of teleost fish melanocortin receptors. Ann. N. Y. Acad. Sci. 994, 319–330.
Mandrytsa, S., 2001. Seismosensory System and Classification of Scorpionfishes (Scorpaeniformes: Scorpaenoidei). Perm State University Press, Perm (in Russian).
Matschiner, M., Hanel, R., Salzburger, W., 2011. On the origin and trigger of the notothenioid adaptive radiation. PLoS ONE 6, 1–9.
Matsunuma, M., Motomura, H., 2011. First records of a lionfish, *Pterois mombasae* (Scorpaenidae: Pteroinae), from Japan, and morphological comparisons with *P. antennata*. JNP J. Ichthyol. 58, 27–40.
Meisler, M.R., 1987. Limits and relationships of serranine seabasses, with revisions of Serranus and Mentiperca (Pisces: Serranidae). Unpubl. Ph.D. diss., University of Southern California, Los Angeles, California. In: Smith, L., Craig, M.T. (Eds.), Casting the Percomorph Net Widely: The Importance of Broad Taxonomic Sampling in the Search for the Placement of Serranid and Percid Fishes. Copeia, 35–55.
Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. Gateway Computing Environments Workshop (GCE), New Orleans, pp. 1–8.
Miya, M., Takeshima, H., Endo, H., Ishiguro, N.B., Inoue, J.G., Mukai, T., et al., 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. Mol. Phylogenet. Evol. 26, 121–138.
Miya, M., Satoh, T.P., Nishida, M., 2005. The phylogenetic position of toadfishes (order Batrachoidiformes) in the higher ray-finned fish as inferred from partitioned Bayesian analysis of 102 whole mitochondrial genome sequences. Biol. J. Linn. Soc. 85, 289–306.
Mooi, R.D., Gill, A.C., 1995. Association of epaxial musculature with dorsal-fin pterygiophores in acanthomorph fishes, and its phylogenetic significance. Bull. Nat. Hist. Mus. Zool. 61, 121–137.
Mooi, R., Johnson, G.D., 1997. Dismantling the trachinoidei: evidence of a scorpaenoid relationship for the Champsodontidae. Ichthyol. Res. 44, 143–176.
Motomura, H., 2009. *Sebastapistes taeniophrys* (Fowler, 1943): a valid scorpionfish (Scorpaenidae) from the Philippines. Ichthyol. Res. 56, 62–68.
Motomura, H., Buth, D.G., 2004. New Species of Scorpionfish, *Scorpaena cocosensis* (Scorpaeniformes: Scorpaenidae) from the Cocos Islands, Costa Rica, Eastern Pacific Ocean. Copeia, 818–824.
Motomura, H., Causse, R., 2011. A new deepwater scorpionfish of the genus *Scorpaenopsis* (Scorpaenidae) from Wallis and Futuna Islands, southwestern Pacific Ocean. Bull. Mar. Sci. 87, 45–53.
Motomura, H., Yoshino, T., Takamura, N., 2004. Review of the scorpionfish genus *Scorpaenopsis* (Scorpaeniformes: Scorpaenidae) in Japanese waters with three new records and an assessment of standard Japanese names. JNP J. Ichthyol. 51, 89–115.
Motomura, H., Fricke, R., Eschmeyer, W.N., 2005a. Redescription of a poorly known scorpionfish, *Scorpaena canariensis* (Sauvage), and a first record of *Pontinus leda* (Eschmeyer) from the Northern Hemisphere (Scorpaeniformes: Scorpaenidae). Stuttgarter Beitrage zur Naturkunde, Serie A (Biologie) 674.
Motomura, H., Paulin, C.D., Stewart, A.L., 2005b. First records of *Scorpaena onaria* (Scorpaeniformes: Scorpaenidae) from the southwestern Pacific Ocean, and comparisons with the Northern Hemisphere population. N.Z.J. Mar. Freshw. Res. 39, 865–880.
Motomura, H., Sakurai, Y., Senou, H., Ho, H.C., 2009a. Morphological comparisons of the Indo-West Pacific scorpionfish, *Parascorpaena aurita*, with a closely related species, *P. picta*, with first records of *P. aurita* from East Asia (Scorpaeniformes: Scorpaenidae). Zootaxa 2191, 41–57.
Motomura, H., Sakurai, Y., Shinohara, G., 2009b. First Records of a Scorpionfish, *Scorpaenodes albaiensis*, from East Asia, with a Synopsis of *S. minor* (Actinopterygii: Scorpaeniformes: Scorpaenidae). Species Divers. 14, 75–87.
Motomura, H., Ogihara, G., Hagiwara, K., 2010. Distributional range extension of a scorpionfish, Scorpaenodes quadrispinosus, in the Indo–Pacific, and comments on synonymy of S. parvipinnis(Scorpaeniformes: Scorpaenidae). In: Motomura,

H., Matsuura, K. (Eds.), Fishes of Yaku-shima Island National Museum of Nature and Science, Tokyo, 10 March 2010.
Mueller, R.L., 2006. Evolutionary rates, divergence dates, and the performance of mitochondrial genes in Bayesian phylogenetic analysis. Syst. Biol. 55, 289–300.
Mundy, N.I., 2005. A window on the genetics of evolution: MC1R and plumage colouration in birds. Proc. Roy. Soc. B 272, 1633–1640.
Mundy, N.I., Kelly, J., 2003. Evolution of a pigmentation gene, the melanocortin-1 receptor, in primates. Am. J. Phys. Anthropol. 121, 67–80.
Nagasawa, K., Giannetto, A., Fernandes, J.M.O., 2012. Photoperiod influences growth and mll (Mixed-Lineage Leukaemia) expression in atlantic cod. PLoS ONE, 7.
Near, T.J., Eytan, R.I., Dornburg, A., Kuhn, K.L., Moore, J.A., Davis, M.P., et al., 2012. Resolution of ray-finned fish phylogeny and timing of diversification. PNAS 109 (34), 13698–13703.
Nelson, J.S., 1984. Fishes of the World, second ed. John Wiley and Sons, New York, p. 523.
Nelson, G.J., 1989. Phylogeny of major fish groups. In: Fernholm, B., Bremer, K., Jörnvall, H. (Eds.), Molecules and Morphology in Phylogenetic Analysis. The Hierarchy of Life. International Congress Series 824. Excerpta Medica. Amsterdam, New-York, Oxford.
Nelson, J.S., 2006. Fishes of the World, third ed. John Wiley and Sons, Inc., New York, p. 600.
Orrell, T.M., Collette, B.B., Johnson, G.D., 2006. Molecular data support separate scombroid and xiphioid clades. Bull. Mar. Sci. 79, 505–519.
Pietsch, T.W., 1989. Phylogenetic relationships of trachinoid fishes of the family uranoscopidae. Copeia, 253–303.
Posada, D., 2008. JModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Puigbo, P., Garcia-Vallve, S., McInerney, J.O., 2007. TOPD/FMTS: a new software to compare phylogenetic trees. Bioinformatics 23, 1556–1558.
Rosenblum, E.B., Hoekstra, H.E., Nachman, M.W., 2004. Adaptative reptile color variation and the evolution of the MC1R gene. Evolution 58, 1794–1808.
Rost, B., 1999. Twilight zone of protein sequence alignments. Protein Eng. 12, 85–94.
Schoelinck, C., Hinsinger, D., Dettai, A., Cruaud, C., Justine, J.L., in preparation. Inferring the relationships of groupers (Perciformes, Serranidae, Epinephelinae) based on multiple mitochondrial and nuclear markers.
Selz, Y., Braasch, I., Hoffmann, C., Schmidt, C., Schultheis, C., Schartl, M., et al., 2007. Evolution of melanocortin receptors in teleost fish: the melanocortin type 1 receptor. Gene 401, 114–122.
Shinohara, G., Imamura, H., 2005. Anatomical description and phylogenetic classification of the orbicular velvetfishes (Scorpaenoidea: *Caracanthus*). Ichthyol. Res. 52, 64–76.
Shinohara, G., Imamura, H., 2007. Revisiting recent phylogenetic studies of "Scorpaeniformes". Ichthyol. Res. 54, 92–99.
Sjolander, K., 2004. Phylogenomic inference of protein molecular function: advances and challenges. Bioinformatics 20, 170–179.
Smith, W.L., 2005. The limits and relationships of mail-cheeked fishes (Teleostei: Percomorpha) and the evolution of venom in fishes. Unpubl. Ph.D. diss., Columbia University, New York. In: Smith, L., Craig, M.T. (Eds.), Casting the Percomorph Net Widely: The Importance of Broad Taxonomic Sampling in the Search for the Placement of Serranid and Percid Fishes. Copeia, 35–55.
Smith, W.L., Craig, M.T., 2007. Casting the percomorph net widely: the importance of broad taxonomic sampling in the search for the placement of serranid and percid fishes. Copeia, 35–55.
Smith, W.L., Wheeler, W.C., 2004. Polyphyly of the mail-cheeked fishes (Teleostei: Scorpaeniformes): evidence from mitochondrial and nuclear sequence data. Mol. Phylogenet. Evol. 32, 627–646.
Smith, W.L., Wheeler, W.C., 2006. Venom evolution widespread in fishes: a phylogenetic road map for the bioprospecting of piscine venoms. J. Hered. 97, 206–217.
Springer, V.G., Orrell, T.M., 2004. Phylogenetic analysis of the families of acanthomorph fishes based on dorsal gill-arch muscles and skeleton. Bull. Biol. Soc. Wash. 11, 237–255.
Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.
Venkatesh, B., Ning, Y., Brenner, S., 1999. Late changes in spliceosomal introns define clades in vertebrate evolution. Proc. Natl. Acad. Sci. 96, 10267–10271.
Wainwright, P.C., Smith, W.L., Price, S., Tang, K.L., Sparks, J.S., Ferry, L.A., et al., 2012. The evolution of pharyngognathy: a phylogenetic and functional appraisal of the pharyngeal Jaw key innovation in labroid fishes and beyond. Syst. Biol., 1–27.
Wiley, E.O., Johnson, G.D., Dimmick, W.W., 2000. The interrelationships of Acanthomorph fishes: a total evidence approach using molecular and morphological data. Biochem. Syst. Ecol. 28, 319–350.
Winnpennminck, B., Backeljaut, T., Watcher, R.D., 1993. Extraction of high molecular weight DNA from molluscs. Trends Genet. 9.
Yu, B.D., Hanson, R.D., Hess, J.L., Horning, S.E., Korsmeyer, S.J., 1998. MLL, a mammalian trithorax-group gene, functions as a transcriptional maintenance factor in morphogenesis. Proc. Natl. Acad. Sci. 95, 10632–10636.