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# 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)

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# ABSTRACT

The interphotoreceptor retinoid-binding protein (IRBP) coding gene has been used with success for the large-scale phylogeny of mammals. However, its phylogenetic worth had not been explored in Actinopterygians. We explored the evolution of the structure of the gene and compared the structure predicted from known sequences with that of a basal vertebrate lineage, the sea lamprey Petromyzon marinus. This sequence is described here for the first time. The structure made up of four tandem repeats (or modules) arranged in a single gene, as present in Chondrichthyes (sharks and rays) and tetrapods, is also present in sea lamprey. In teleosts, one to two paralogous copies of IRBP gene have been identified depending on the genomes. When the sequences from all modules for a wide sampling of vertebrates are compared and analyzed, all sequences previously assigned to a particular module appear to be clustered together, suggesting that the divergence among modules is older than the split between lampreys and other vertebrates. Finally, 92 acanthomorph teleosts were sequenced for the partial module 1 of the gene 2 (713 bp) to assess for the first time the use of this marker for the systematic studies of the Teleostei. The partial sequence is slightly more variable than other markers currently used for this group, and the resulting trees from our sequences recover most of the clades described in the recent molecular multi-marker studies of the Acanthomorpha. We recommend the use of partial sequences from the IRBP gene 2 as a marker for phylogenetic inference in teleosts.

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### 1. Introduction

Teleosts represent approximately one-half of extant vertebrate species. Sixty percent of them, representing 16,908 species, (Froese and Pauly, 2006) are acanthomorphs. This clade is supported by both morphological (Rosen, 1973; Johnson and Patterson, 1993; Stiassny, 1986) and molecular studies (Miya et al., 2003), and includes all fishes with true spines in the dorsal and anal fins. To date, many parts of the interrelationships of the 309 families (Nelson, 2006) remain poorly known. Recent molecular results, while presenting support for some groups defined with morphological characters (for example Tetraodontiformes and Atherinomorpha), also severely challenged several of the accepted hypotheses of acanthomorph relationships (Johnson and Patterson, 1993). Some larger groups where monophyly was poorly supported by morphological data and generally controversial (Stiassny et al., 2004) were not recovered. For instance, the Paracanthopterygii (Greenwood et al., 1966, see for instance the critics by Rosen, 1985), the extanded Zeiformes (Rosen, 1973, 1984; Johnson and Patterson, 1993; Tyler and Santini, 2005), the Scorpaeniformes (see Imamura and Shinohara, 1998 among others), and the Smegmamorpha (Johnson and Patterson, 1993) were absent in the trees inferred in the various molecular studies. In these studies, independent teams working on different markers and with different taxonomic samplings repeatedly recovered several new clades (Chen et al., 2003, 2007; Wiley et al., 2000; Miya et al., 2001, 2003, 2005; Dettaï and Lecointre, 2004, 2005; Holcroft, 2004; Smith and Wheeler, 2004, 2006; Smith and Craig, 2007). Nonetheless, additional work is needed for many parts of the acanthomorph tree, where currently available markers have not permitted the resolution of several nodes.

The power of multi-locus approaches for the resolution of troublesome phylogenetic inferences has been demonstrated on several large-scale groups in the last few years, including mammals (for instance Springer et al., 2001; Madsen et al., 2001), squamates (Vidal and Hedges, 2005) and ray-finned fishes (Chen et al., 2004; Kikugawa et al., 2004; Hurley et al., 2006). While mitochondrial genomes provide valuable information when analyzed as a whole

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(see for instance Inoue et al., 2003; Lavoue et al., 2005; Miya et al., 2003 or Jondeung et al., 2007), mitochondrial genes taken separately might perform poorly for inferring ancient divergences because of a marked saturation at least at the third codon position of protein-coding genes (Orti and Meyer, 1997; Springer et al., 2001). Moreover, mitochondrial genes cannot be considered to evolve completely independently from one another, and additional nuclear datasets are much needed to provide an independent data source.

The partial sequence of the nuclear gene encoding the interphotoreceptor retinoid-binding protein (IRBP) has been used with success for the phylogeny of mammals, alongside numerous other markers, and within families as well as among orders. Stanhope et al. (1992) were the first to use these sequences for phylogenetic inference.

In tetrapods, the gene contains four tandem repeats (called modules hereafter), each approximately 900 bp long. The first three modules and part of the fourth form one large exon, while the last part of the fourth module contains three introns (Fig. 1). The coding sequence for teleost IRBP was first described in *Carassius auratus* (Wagenhorst et al., 1995), followed closely by the expression assays and description in another cyprinid, the zebrafish *Danio rerio* (Rajendran et al., 1996). This hypothesized structure, based on genomic sequences and mRNAs, was composed of only two modules, as opposed to the four modules present in mammals (*Danio rerio* gene 2 in Fig. 1). The second of these modules contains three introns (Rajendran et al., 1996). But very recently, sequence data mining of complete genome databases for a number of model fishes (cyprinid *Danio rerio* and acanthomorphs *Tetraodon nigroviridis, Takifugu rubripes*,

Gasterosteus aculeatus and Oryzias latipes) led to question this description (Nickerson et al., 2006). A second IRBP locus was detected at a location preceding the first identified locus in some of the species for which complete genomes were available. In G. aculeatus and O. latipes, no copy of the new locus was found, and in T. nigroviridis, only a very partial sequence was present. The new locus was named IRBP gene 1, and the previously identified locus was named gene 2. These names are the ones used in the present study. But the study of Nickerson et al. (2006) was based on a small number of teleost taxa that were available in GenBank and/or complete genomic databases. Here, we explored more thoroughly the presence of this additional copy of the IRBP gene in other species of acanthomorphs, especially focusing on groups that are closely related to the species where no copy or only a partial sequence of gene 1 could be found in the available complete genomes. We also performed a search for the IRBP coding gene in taxa where it had not been described previously, and could identify IRBP-like sequences in the lamprey Petromyzon marinus. The new sequences allowed us to reconstruct a tree comparing the modules with one another in a wider range of taxa to study the evolution of the structure of the gene.

In parallel, we examined the phylogenetic usefulness of the IRBP gene 2 partial first module sequences for a wide sampling of acanthomorph species (Table 1). As will be discussed, the inferred clades are largely congruent with previously published molecular but also morphological results for the group. This module can bring valuable information for the reconstruction of the phylogeny of acanthomorph fishes, even with sequences as short as 713 bp.



**Fig. 1.** Representation of the alignment of the full-length tetrapod coding sequence with the coding sequences found in Teleostei and *Petromyzon marinus*. Deletions are represented by grey areas in the affected sequence. The oligonucleotides primers used in the present study are represented by arrows. Sequences of the sarcopterygian single gene, and the teleost gene one are represented by white rectangles, sequences of teleost gene 2 by light-grey rectangles. The position of the sequences obtained for this study is indicated at the bottom of the figure. The module 1 of the actinopterygian gene 2 is the one tested here for acanthomorph phylogeny.

# Table 1

Taxonomic sampling, GenBank CoreSequence and trace archive accession numbers, and Ensembl scaffold numbers of the sequences

| Changebox     Partner     Subset     Case of the construction of   | Outgroups            | Order/sub-order   | Family           | Genus and<br>species        | Gene 2<br>module 1 | Gene 1       | Single IRBP-coding gene                                |  |  |
|--|----------------------|-------------------|------------------|-----------------------------|--------------------|--------------|--|--|--|
| Compatibility     Contrast entrange     Non-second entrange     Performation entrange       Compatibility     Replaction entrange     Non-second entrange     Non-second entrange       Performation     Non-second entrange     Non-second entrange     Non-second entrange       Performation     Replaction entrange     Non-second entrange     Non-second entrange       Performation     Restancial entrange     Non-second entrange     Non-second entrange       Performation     Restancial entrange     Non-second entrange     Non-second entrange       Performation     Non-second entrange     Non-second entrange     Non-second entrange       Non-second entrange     Non-second entrange     Non-second entrange     Non-second entrange       Performation     Non-second entrange     Non-second entrange     Non-second entrange       Non-second entrange     Non-second entrange     Non-second entrange     Non-second entrange       Performation     Non-second entrange     Non-second entrange     Non-second entrange       Non-second entrange     Non-second entrange     Non-second entrange     Non-second entrange       Second entrange     Restancial entrange     Non-second  | Cypriniformes        |                   | Cyprinidae       | Danio rerio                 | X85957             | CR450691     | Accession numb<br>to reconstruct th<br>Petromyzon mari | ccession numbers of the trace files used<br>reconstruct the IRBP coding gene of<br>etromyzon marinus |  |
| Charophalamoles ( papelies ( papelies ( papelies ( papelies)) ( papelies)) ( papelies) ( p |                      |                   |                  | Carassius auratus           | X80802             |              | Petromyzon mari  | inus   |  |
| Lampsfilder     Ragines (simulation solution of all soluti                                     | Chlorophthalmoidei   |                   | Ipnopidae        | Bathypterois dubius         | DQ168042           |              | Contig-containin                                       | ng exon1   |  |
| Physical interaction     Registical action     Dijota interaction     1311130     PMAC-ary20000-11       Paracenthoprorygi     Paracenthoprorygi     Cadilor     1311130     PMAC-ary20000-11       Cadilor     Cadilor     Cadilor     12134580     PMAC-ary2000-11       Cadinor     Cadilor     Cadilor     12134580     PMAC-ary2000-11       Cadinor     Cadilor     Cadilor     Cadilor     12134580     PMAC-ary2000-11       Cadinor     Cadilor     Cadilor     Cadilor     12134580     PMAC-ary2000-11       Cadior     Cadior     Cadior     Cadior     12134180     PMAC-ary2000-11       Cadior     Cadior     Cadior     Cadior     12134180     PMAC-ary2000-11       Cadior     Cadior  | Lampridiformes       |                   | Lampridae        | Lampris immaculatus         | DQ168077           |              | 1229775045   | PMAC-aqw52f02.b1   |  |
| Dolymacinitionnes     Polymeinidia     Dolymeinidia     Dolymeinidia     Dolymeinidia     Dolymeinidia       Presentifyery     Bareholdson     Bareholdson     Balakamen (Bareholdson)     113013901     PMCA-catr270(BA)       Catiformes     Bareholdson     Catiformes     0(168067     11232480     PMCA-catr270(BA)       Catiformes     Precopionnes     Antocaterizate     Antocaterizate     0(16809     120124233     PMCA-catr200(BA)       Catiformes     Precopionnes     Antocaterizate     Antocaterizate     0(168127     120124233     PMCA-catr200(BA)       Catiformes     Techicher     Zengeriz conthir     0(168128     120124233     PMCA-catr200(BA)       Reyrotion     Antonalizate     Antonalizate     Catiformes     12551036     PMCA-catr200(BA)       Reyrotion     Daramalog     Daramalog     Daramalog     PMCA-catr200(BA)     PMCA-catr200(BA)       Reyrotion     Daramalog     PMCA-catr200(BA)     PMCA-catr200(BA)     PMCA-catr200(BA)       Reyrotion     Daramalog     Entransida     Matcattar200(BA)     PMCA-catr200(BA)       Reyrotion     Daramalog   |                      |                   | Regalecidae      | Regalecus glesne            | DQ168109           |              | 1431135039   | PMAC-ayu28b06.b1   |  |
| Paracathopeygil     Barta-biolitoms     Barta-biolitoms     Barta-biolitoms     Barta-biolitoms     Barta-biolitoms     Barta-biolitoms       Barta-biolitoms     Barta-biolitoms     Barta-biolitoms     Contropannes sp.     100108018     118072425     WhAC-bartSr0551       Percopiorum     Adarounde     Adarounde     Name     122702304     WhAC-bartSr0551       Percopiorum     Aphendoletike     Apirodiorus syouns     DQ168087     12291321     WhAC-bartSr0551       Colomes     Zaciaki     Aphendoletike     Apirodiorus syouns     DQ16817     12951321     WhAC-bartSr0551       Brycuforume     Caciaki     Anonalogica     Marounde     DQ16807     12951321     WhAC-bartSr0551       Brycuforume     Data-biolitopica     Anonalogica     Marounde     DQ16807     12951321     WhAC-bartSr0551       Brycuforume     Data-biolitopica     Marounde     DQ16807     11825245     WhAC-bartSr0551       Brycuforume     Radocacita     Marounde     Marounde     DQ16807     11825345     WAC-bartSr0551       Brycuforume     Radacacita/biota     Marounde/biota <td< td=""><td>Polymixiiformes</td><td></td><td>Polymixiidae</td><td>Polymixia nobilis</td><td>DQ168104</td><td></td><td>1293713991</td><td>PMAC-atn37c06.b1</td></td<>  | Polymixiiformes      |                   | Polymixiidae     | Polymixia nobilis           | DQ168104           |              | 1293713991   | PMAC-atn37c06.b1   |  |
| Barneholdirom     Gadiers     Auto-Analogio Science     11827428     MAC-Analogio Science       Gadiers     Gadiers     Cadae     12129480     MAC-Analogio Science       Moria     Moria     Moria     12129480     MAC-Analogio Science       Moria     Moria     Moria     12129480     MAC-Analogio Science       Depiniforme     Auto-Cartelido     12129480     MAC-Analogio Science       Depiniforme     Cartalido     Mac-Cartelido     12129480     MAC-Analogio Science       Depiniforme     Cartalido     Cartalido     120142323     MAC-Analogio Science       Depiniforme     Cartalido     Cartalido     Cartalido     122531036     MAC-Analogio Science       Berycläforme     Deresida     Cartalido     Cartalido     Cartalido     12353103     MAC-Analogio Science       Berycläforme     Deresida     Cartalido     Cartalido     Cartalido     12354131     MAC-Analogio Science       Berglaforme     Macience     Cartalido     Cartalido     Cartalido     12354131     MAC-Analogio Science       Berglaforme     Macience   | Paracanthopterygii   |                   |                  |                             |                    |              | 1180179031   | PMAC-adz75g08.b1   |  |
| Galdromes     Galdromes     Galdromes     Galdromes     PMAC     P   |                      | Batrachoidiformes | Batrachoidae     | Halobatrachus didactylus    | DQ168069           |              | 1188732452   | PMAC-ahu86e05.b1   |  |
| Automate in a section of the                        |                      | Gadiformes        | Gadidae          | Gadus morhua                | DQ168066           |              | 1212949869   | PMAC-amk23b03.b1   |  |
| Macroandae     More and mome     Op/168/18     Unitable     MMAC-aim 200551       Peroperiforme     Peroperiforme     Annean 2016     Peroperiforme     Peroperiforme       Annean 2016     Annean 2016     Peroperiforme     Peroperiforme     Peroperiforme       Berydformes     Tranhichthysiol     Annean 2016     Peroperiforme  |                      |                   |                  | Gaidropsarus sp.            | DQ168067           |              | 1484755358   | PMAC-bri37g11.b1   |  |
| Moria de monitale     Mora mono     DQ188188     P(2128)232     P(MAC-mar)400.51       Percepsiforme     Aptrosofication and the monitoria strinure     DQ18817     P(MAC-mar)400.51       Zeinder     Zeinder     Zeinder     Zeinder     P(MAC-mar)400.51       Zeinder     Zeinder     Zeinder     DQ18817     P(MAC-mar)400.51       Berycolor     P(218)     P(218)     P(218)     P(218)       Berycolor     P(218)     P(218)     P(218)     P(218)       Sephanophysiche     P(218)  |                      |                   | Macrouridae      | Trachyrincus murrayi        | DQ168124           |              | 1277028634   | PMAC-arg55c05.b1   |  |
| Percogniforms     Aphrediaderiska     Opulis0039     122891321     PMAC-arg24101.51       Zeilor     Ceratifica     Carlaition     DQ180307     128891320     PMAC-arg24107.51       Zeilor     Zeilor     Zeilor     2001     2001     128891320     PMAC-arg24107.51       Berycrifermes     Tachichhydid     Anomalopitate     PmothAphoron platebran     DQ188101     128897530     PMAC-arg2407.51       Berycrifermes     Berycrifermes     Dertermidas     PmothAphoron platebran     DQ188101     144227147     PMAC-arg2407.31       Sophanobnycfformes     Holocentrificas     Marpatrifis batche     DQ18804     11383548     PMAC-arg2407.31       Mugliolei     Marpatrifis batche     DQ18804     1223847832     PMAC-arg2407.31       Atherinomorpha     Bedoniade     Bedoniade argyri     DQ18804     1223847823     PMAC-arg2407.31       Atherinomorpha     Bedoniade     Bedoniade argyri     DQ18804     120374502     PMAC-arg120.11       Atherinomorpha     Bedoniade     Bedoniade argyri     DQ18804     120374502     PMAC-arg120.11       Atherinomorpha <t< td=""><td></td><td></td><td>Moridae</td><td>Mora moro</td><td>DQ168089</td><td></td><td>1201242823</td><td>PMAC-alm40b05.b1</td></t<>   |                      |                   | Moridae          | Mora moro                   | DQ168089           |              | 1201242823   | PMAC-alm40b05.b1   |  |
| Lop Mathemse     Caratitáce     Amtenanísáce     Amtenanísáce     Amtenanísáce     Decisión     143683262     PMAC-ami261251       Zaiformes     Zaiola     Zais fabr     DO (1813)     14368126     PMAC-ami261256331       Beryceiformes     Tachichthyside     Amtenanization     PMAC-ami26126331     PMAC-ami26126331       Beryceiformes     Beryceiformes     DO (18010     1442221743     PMAC-ami26126331       Beryceiformes     Bordoceitidae     Minipristis hatche     DQ (18060     1183594531     PMAC-ami2612031       Magliodei     Bordoceitidae     Rondecletida sp.     DQ (18060     1183594531     PMAC-adi/391051       Magliodei     Bordoceitidae     Rondecletida sp.     DQ (180811     1183594541     PMAC-adi/391054       Magliodei     Belonidei     Bordoceitidae     Rondecletida sp.     DQ (180811     112329461     PMAC-adi/321054       Attenanichtylaz     Carasitaritylaz     Paratitaritylaz     Paratitaritylaz     PMAC-adi/3210541       Attenanichtylaz     Attenanichtylaz     Paratitaritylaz     PMAC-adi/3210541     PMAC-adi/3210541       Attenanichylaz     A  |                      | Percopsiformes    | Aphredoderidae   | Aphredoderus sayanus        | DQ168038           |              | 1212919321   | PMAC-amg34b01.b1   |  |
| Antennarius     Antennarius     Matennarius     DQ168123     H4888352     PMAC-bmg11607.1       Beryciformes     Trachichthysiel     Anomalopida     Zeinges cont/life     DQ168127     H372100.1     PMAC-au8500.62       Beryciformes     Trachichthysiel     Normalopida     Depression control of the second co  |                      | Lophiiformes      | Ceratiidae       | Ceratias holboelli          | DQ168049           |              | 1267811279   | PMAC-arn35e12.b1   |  |
| Zelores     Zeloke     Zous fabr     DQL68123     137710471     PMAC-aut8908.81       Beryctionnes     Trachichthyoted     Amanapukae     Photolefphron pdpetrats     DQL68128     1258100.00     PMAC-aut702.00.1       Beryctionnes     Beryctionnes     Beryctionnes     Photolefphron pdpetrats     DQL68010     12883795.00     PMAC-aut70.00.1       Beryctionnes     Barbouristian andra     Photolefphron pdpetrats     DQL68011     118373457.00     PMAC-aut70.00.1       Sphanoberyctionnes     Barbouristian andra     PDQL68011     11837457.00     PMAC-aut70.00.1       Maglioide     Barbouristian andra     PDQL68011     11837457.00     PMAC-aut70.00.1       Alterinnompha     Bedotidae     Rondeletida sp.     DQL68043     122384780.2     PMAC-aut70.00.1       Alterinnompha     Bedotidae     Rondeletida sp.     DQL68043     122384780.2     PMAC-aut70.00.1       Gatrosterinnes     Sympanobici     Bedotidae     Rondeletida sp.     DQL68043     12237550.2     PMAC-aut70.00.1       Gatrosterinnes     Sympanobici     Macroamphosite     Macroamphosite     Macroamphosite     Macroamphosite </td <td></td> <td></td> <td>Antennariidae</td> <td>Antennarius striatus</td> <td>DQ168037</td> <td></td> <td>1468863562</td> <td>PMAC-bmg41h07.b1</td>   |                      |                   | Antennariidae    | Antennarius striatus        | DQ168037           |              | 1468863562   | PMAC-bmg41h07.b1   |  |
| Bergetiformes     Trachichthyside     Anonalpiae     Perophise conchier     DQ188107     1255810.366     PMAC-ait8705.51       Srephanobryciformes     Dirtmidae sp.     DQ188108     EU625596     1452217.82     PMAC-ait8705.51       Srephanobryciformes     Barbourisite sp.     DQ18800     EU625596     118735564     PMAC-ait8705.51       Srephanobryciformes     Barbourisite shorther     DQ18801     1173755648     PMAC-ait8707.51       Atherinomorpha     Bedorisite and Barbourisite shorther     DQ18802     123384.63     PMAC-ait8707.51       Atherinomorpha     Bedorisite and Barbourisite and Streps     DQ18808     123384.58     PMAC-ait877.21       Atherinomorpha     Bedorisite and Barbourisite and Streps     DQ18808     123387.38     PMAC-ait877.21       Atherinomorpha     Synganathica     Arranishter bysine and Streps     DQ18808     12307.11     PMAC-ait8705.51       Synganathica     Synganathica     Synganathica     Synganathica     DQ18807     113939538     PMAC-ait8705.51       Synganathica     Songarenitis typine     DQ18907     11393937.53     PMAC-ait8705.51       Synganathica  | Zeiformes            | Zeioidei          | Zeidae           | Zeus faber                  | DQ168128           |              | 1377120471   | PMAC-aut89b08.g1   |  |
| Beryciformes   Tachchthysidel   Anomalopidae   Photoblepharon palgebraus   DQ168101   1434632667   PMAC-sr22603.h1     Brycnidei   Berycidae   Berycidae   Berycidae   Percentride   Percentride <td></td> <td></td> <td></td> <td>Zenopsis conchifer</td> <td>DQ168127</td> <td></td> <td>1255810366</td> <td>PMAC-air87c05.b1</td>   |                      |                   |                  | Zenopsis conchifer          | DQ168127           |              | 1255810366   | PMAC-air87c05.b1   |  |
| Diretmidae     Diretmidae     Diretmidae     Diretmidae     Diretmidae     Part Mail       Berycoide1     Berycoide1     Berycoide1     Holocentroide     Myriprisis brothe     QUI88091     1188594531     PMAC-hels/C0231       Stephanoberyciforms     Bendreitrice     Bendreitrice </td <td>Beryciformes</td> <td>Trachichthyoidei</td> <td>Anomalopidae</td> <td>Photoblepharon palpebratus</td> <td>DQ168101</td> <td></td> <td>1434632667</td> <td>PMAC-azz26c03.b1</td>   | Beryciformes         | Trachichthyoidei  | Anomalopidae     | Photoblepharon palpebratus  | DQ168101           |              | 1434632667   | PMAC-azz26c03.b1   |  |
| Bergy cale     Bergy c  |                      |                   | Diretmidae       | Diretmoides sp.             | DQ168060           |              | 1442721743   | PMAC-bek61c09.g1   |  |
| Inducentroide     Myripristis barker     DQ168091     1188594531     PMAC-acg12013g1       Sephanoberycines     Barbourisian dia Non-<br>Mugliolei     Nagliolei     1179789762     PMAC-acg12013g1       Mugliolei     Mugliolei     Lis p.     DQ168003     1129789762     PMAC-acg12013g1       Atherinomorpha     Bedonidac     Bedonidac     Bedonidac     Bedonidac     PMAC-acg12012g1     PMAC-acg12012g1       Castrosterilormes     Synganaboide     Advasanithriydac     Procinidac     PMAC-acg12012g1     PMAC-acg12012g1       Synganaboide     Advasanithriydac     Procinidac     PMAC-acg12012g1     PMAC-acg12012g1       Synganaboide     Macroarambosida     Macroarambosida     Macroarambosida     PMAC-acg12012g1       Synganaboide     Synganachidac     Macroarambosida     Macroarambosida     PMAC-acg12012g1 </td <td></td> <td>Berycoidei</td> <td>Berycidae</td> <td>Beryx splendens</td> <td>DQ168045</td> <td>EU625596</td> <td>1218957550</td> <td>PMAC-aoh60g03.b1</td>  |                      | Berycoidei        | Berycidae        | Beryx splendens             | DQ168045           | EU625596     | 1218957550   | PMAC-aoh60g03.b1   |  |
| Stephanobergeformes     Barbourisitide     Barbourisitide     Polle8041     119735562     PMAC-ady303051       Muglioidei     Muglidae     Liza sp.     DQ168082     1423496401     PMAC-ady303051       Metrinomorpha     Belonoidei     Belonidae     Belonicae     DQ168082     123847863     PMAC-ady303051       Gasterosteriformes     Synganthidae     Adriant-thiydad     Adriant-thiydae     Practicitae     PMAC-ady31307       Gasterosteriformes     Synganthidae     Practicitar chineruis     DQ168084     137761023     PMAC-ady32107       Synganthidae     Synganthidae     Practicitar chineruis     DQ168083     Centig containing exon 2     PMAC-ady32107       Synganthidae     Synganthidae     Synganthidae     DQ168084     Centig containing exon 2     PMAC-ady32107       Synganchiformes     Synganchiforme     DQ168089     Centig containing exon 2     PMAC-ady32107       Sorpaenider     Sorpaenide     Sorpaenide     Cortig containing exon 2     PMAC-ady32107       Cottoidei     Exotoider exoto protein oara     DQ168079     181182534     PMAC-ady32101       Dactylopteriformes <td< td=""><td></td><td>Holocentroidei</td><td>Holocentridae</td><td>Myripristis botche</td><td>DQ168091</td><td></td><td>1188594531</td><td>PMAC-aeg12c03.g1</td></td<>  |                      | Holocentroidei    | Holocentridae    | Myripristis botche          | DQ168091           |              | 1188594531   | PMAC-aeg12c03.g1   |  |
| Rondeletiidae     Rondeletiinage     Rondeletiinage     Rondeletiinage     POI[8110     117978970     PMAC-ax09130051       Atherinomorpha     Bedotiidae     Bedotiidae     Bedotiidae     Bedotiidae     Rondeletiinage     Poilas03     -     123384763     PMAC-ax0140551       Gasterosteriformes     Syngnathiota     Adrianichthydiae     Adrianichthydiae     Poilas International Society     -     1485092142     PMAC-adv772L21       Gasterosteriformes     Syngnathiota     Aduistomus colinearis     DQ168004     -     1377461023     PMAC-adv72L21       Syngnathiota     Maxterombolitae     Syngnathiotae     Maxterombolitae     Syngnathiotae     Poilas003     -     1275405518     PMAC-adv72L21       Davlopteriforme     Masterombolitae     Masterombolitae     Masterombolitae     Poilas01     -     1255405518     PMAC-adv72L21       Strandominitae     Masterombolitae     Gorpaenaidae     PMAC-adv72L21     144245179     PMAC-adv72L21     144245179     PMAC-adv72L21     144245179     PMAC-adv72L21     144245179     PMAC-adv72L21     144245170     PMAC-adv72L21     144245170     PMAC-a  | Stephanoberyciformes |                   | Barbourisiidae   | Barbourisia rufa            | DQ168041           |              | 1197355648   | PMAC-aih29h11.b1   |  |
| Muglialer Muglialee Lize sp. DQ168082 142349640 PMAC-sav81805.11   Alberinnompha Belonidei Belonikale Belonizae Belonizae DQ168043 1226387763 PMAC-sm14Q731   Adrianchridyide Arianchridyide Orizaistairee DQ168043 120533224 PMAC-sm14Q731   Casterosterinformes Syngarthoidyide Neccilia reciularia DQ16803 1180171 PMAC-sm14Q631   Synbranchider Necromphososte DQ16803 12377461023 PMAC-sm14Q6321   Synbranchider Synbranchide Macromphosots scolopax DQ168083 12377461023 PMAC-sm14Q6451   Synbranchider Synbranchide Macromphosots scolopax DQ168083 1297754012 PMAC-sm14Q6521   Synbranchider Synbranchide Mastacembelidei Mastacembelidei Mastacembelidei Mastacembelidei Mastacembelidei Macromphosots DQ168071 1182284 PMAC-sm14Q6521   Soropaenidormes Soropaenidormes Soropaenidorme DQ168074 121250406 PMAC-sm14Q6521   Soropaenidormes Tetraodontide Tetraodontide Tetraodontila DQ168075 1243245705 PMAC-sm14Q6405   Soropaenidormes Tetraodontide Tetraodontide Tetraodontila PMAC-sm142140141 </td <td></td> <td></td> <td>Rondeletiidae</td> <td>Rondeletia sp.</td> <td>DQ168110</td> <td></td> <td>1179789762</td> <td>PMAC-ady93d05.g1</td>   |                      |                   | Rondeletiidae    | Rondeletia sp.              | DQ168110           |              | 1179789762   | PMAC-ady93d05.g1   |  |
| Atherinomorpha   Bedotioidei   Bedotinide   Bedotinide   Bedotinide   Bedotinide   Patiente   Dittematie   | Mugiloidei           |                   | Mugilidae        | Liza sp.                    | DQ168082           |              | 1423496401   | PMAC-axv81d05.b1   |  |
| Belonoidei     Belonoide     Belonoide     Belone iden     DQ168044     1268034     1285031228     PMAC-alm14-11.b1       Adrian intrihuyida     Prociii idae     Pecciii retualuta     DQ168102     1180171166     PMAC-abr1712.b1       Sontomidae     Autoramphosis scolopa     DQ168003     1287746102.5     PMAC-abr12056.31       Synbranchiformes     Synbranchide     Synbranchide     Macroamphosis scolopa     DQ168081     Contig containing exon 2       Synbranchiformes     Synbranchide     Mastacembelide     Mastacembelide     Mastacembelide     Mastacembelide     DQ168081     Contig containing exon 2       Soropaenider     Soropaenide     Soropaenide     Soropaenide     DQ168071     DQ16803     EU62594     PMAC-alm2406 g11       Soropaenider     Soropaenide     Soropaenide     DQ168071     118118254     PMAC-alm2406 g11       Soropaenider     Cottidae     Taurnis bindis     DQ168073     EU62594     121949600     PMAC-alm2406 g11       Soropaenider     Tetraodontoide     Tetraodontoide     Cottidae     DQ168075     121949607     PMAC-alm24026       Tetraodontoide </td <td>Atherinomorpha</td> <td>Bedotioidei</td> <td>Bedotiidae</td> <td>Bedotia geayi</td> <td>DQ168043</td> <td></td> <td>1263847863</td> <td>PMAC-arl14g07.g1</td>   | Atherinomorpha       | Bedotioidei       | Bedotiidae       | Bedotia geayi               | DQ168043           |              | 1263847863   | PMAC-arl14g07.g1   |  |
| AdrianichthyideAdrianichthyideOryzis IafresDQ168094148092142PMAC-ad0791213GasterosteriformesSyngnathideiAulostomidaeRecilia retriautes chinensisDQ1680401377461023PMAC-ad07904b13SyngnathideiAulostomidaeKaroramphossasNaroramphossasDQ168033122975026PMAC-adv25022.11SynbranchideiSynbranchideiSynbranchideiSynbranchideiNaroramphossasDQ1680711178693400PMAC-adv2505.g1SynbranchideiSynbranchideiSobranchideaMonopterus voltansDQ168093118182584PMAC-adv2505.g1DactylopteridormesScorpaenidieScorpaenidieScorpaenidieDactylopteridormesDQ1680931181141201200825PMAC-adv2506.g1CottoideiCottoideiTriglidaeChelidointhylis lueranaDQ16809311812584PMAC-adv2506.g1PMAC-adv2506.g1LiparidaeLiparidaeLiparidaeLiparis fibriciiDQ168093118432584PMAC-adv2506.g1CottoideiTetraodontideiTetraodontingiorisCA40100779yes148638370PMAC-adv2506.g1PleuronectidaeNarcino sp.DQ168095119941313PMAC-adv25040.11YesYesPleuronectidaePetrudoindeiPetrudoingeCA40100779yes14868370PMAC-adv25040.11PleuronectidaePetrudoindeiPetrudoingeCA40100779yes14868370PMAC-adv25040.11PleuronectidaePetrudoindeiPetrudoingeCA401000719yes1422318370PMA  |                      | Belonoidei        | Belonidae        | Belone belone               | DQ168044           |              | 1205833228   | PMAC-alm14c11.b1   |  |
| Cyprindomotedie<br>GasterosteriformesCyprindomotedie<br>Nucleasional<br>Macronamphoside<br>Macronamphoside<br>SympanthicsPeerlinitar<br>Macronamphoside<br>Sympanthics<br>Macronamphoside<br>Sympanthics<br>Macronamphoside<br>Sympanthics<br>Sympanthics<br>Sympanthics<br>Sympanthics<br>Sympanthics<br>Sympanthics<br>Sympanthics<br>Sympanthics<br>Sympanthics<br>Sympanthics<br>Sympanthics<br>   |                      | Adrianichthyidae  | Adrianichthyidae | Oryzias latipes             | DQ168094           |              | 1485092142   | PMAC-brd77e12.b1   |  |
| GasterosteriformesSyngnathiodieAulostomidaeAulostomidaeD0168081377461023PMAC-ava13c0621SynbranchiderSyngnathidaeMacroramphosidaMacroramphosidaNerophits ophileD16812012497545PMAC-ava72005.g1SynbranchiderSynbranchiderSynbranchiderMonogerus albusD16808712927503PMAC-av12205.g1SynbranchiderSynbranchiderMastacembelidaeMastacembelidaeMastacembelidaeNastacembelidae  |                      | Cyprinodontoidei  | Poeciliidae      | Poecilia reticulata         | DQ168102           |              | 1180171186   | PMAC-ado79e04.b1   |  |
| MacroamphosideMacroamphosideSourgandius scologueD0168021229775045PMAC-aqv52002.11SymbranchiderSymbranchideSymbranchideSymbranchidePMAC-aqv5101.11PMAC-aqc51201.11SymbranchiderMastacembelideMastacembelideMastacembelideContig containing even 2DactylopteridormesScorpaenideDactylopteridormeiD168005Contig containing even 2ScorpaeniformesScorpaenideScorpaeni onariaD0168015UE2554PMAC-ait87.05.g1ScorpaeniformesScorpaenideScorpaeni onariaD0168015UE25541211494050PMAC-ait82.05.g1ScorpaeniformesScorpaenideCottidaeTeurulos bublisD0168015UE2554121145000PMAC-ait82.05.g1TetradontiformesTetradontideTetradontideTeurulos bublisD0168016UE2554121494305PMAC-ait82.05.g1TetradontiformesTetradontideTetradon nigroviridisScAF10211Very partialH42361700PMAC-ach28.06.g1Tetradon nigroviridisScAF10211Very partialH42436170PMAC-ach28.06.g1NotAch28.07.61PeuronectiformesPetrodoideNotaratidaeOstracidaeScAF10211Very partialH4438370PMAC-ach28.06.g1PeuronectiformesPetrodoideiTetradon nigroviridisScAF10211Very partialH44236170PMAC-ach28.06.g1PeuronectiformesPetrodoideiTetradontideeScorpaenideeNotAch28.07.61NotAch28.07.61NotAch28.07.61PeuronectiformesPetr   | Gasterosteriformes   | Syngnathoidei     | Aulostomidae     | Aulostomus chinensis        | DQ168040           |              | 1377461023   | PMAC-ava13e06.g1   |  |
| Synparthic yphie<br>Nerophis ophiodomDQ1680711424824779PMAC-ay2205.g1<br>PMAC-ay2205.g1<br>NARC-ay2205.g1<br>NARC-ay2205.g1<br>NARC-ay2205.g1<br>NARC-ay2205.g1Synbranchider<br>Mastacembelide<br>Mastacembelide<br>Mastacembelide<br>Mastacembelide<br>Mastacembelide<br>Mastacembelide<br>Mastacembelide<br>Mastacembelide<br>Dactylopterirs volitans<br>Corpaenidae<br>Doctylopterirs volitans<br>DQ1680441424824779<br>TRARC-ay2205.g1<br>TRARC-ay2205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1MARC-ay2205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-ay205.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>TRARC-Ay105.g1<br>T   |                      |                   | Macroramphosidae | Macroramphosus scolopax     | DQ168083           |              | 1229775045   | PMAC-aqw52f02.b1   |  |
| NorparachidorSynbranchidesSynbranchidesSynbranchidesMonopterus albusOQ168088Contig containing exon 2DactylopteriformesScorpaenideScorpaenidesScorpaenidesScorpaenides1255405530PMAC-air2806.g1ScorpaeniformesScorpaenidesScorpaenidesScorpaenidesD0168053EU6255411211948050PMAC-air2806.g1ScorpaeniformesScorpaenidesScorpaenidesScorpaenidesD0168053EU6255411211948050PMAC-air2806.g1TetraodontiformesTetraodontideiTetraodontidesIparis fabriciiD01680571464380256PMAC-bert8110.b1TetraodontiformesTetraodontideTetraodontidesSCAF10211Very partia147057826PMAC-bert8110.b1TetraodontiformesOstracidaeOstracidaeNaticaentholdesD01680571200373563PMAC-bert810.b1TracantholdideTracantholdesPO1680571200373563PMAC-aid75404.g1PleuronectiformesPsettodideiPsettodidesPratochirus variegatusD01680551203373563PMAC-aid75404.g1PleuronectiformesPsettodidesPsettodidesPsettodicesCithariaeD01680551203373563PMAC-aid75404.g1PleuronectiformesPsettodidesPsettodiceCithariaeCithariaeD01680551203373563PMAC-aid75404.g1PleuronectiformesPsettodidesPsettodiceSecarum sicrumD0168171LissamplibiaXeopta.g203.b1PleuronectiformesPsettodidesCaproidaeCaproidae <td< td=""><td></td><td></td><td>Syngnathidae</td><td>Syngnathus typhle</td><td>DQ168120</td><td></td><td>1424824779</td><td>PMAC-ayt22f05.g1</td></td<>   |                      |                   | Syngnathidae     | Syngnathus typhle           | DQ168120           |              | 1424824779   | PMAC-ayt22f05.g1   |  |
| Synbranchider     Matacambelide     Mastacambelides     Mastacambelide   |                      |                   |                  | Nerophis ophiodon           | DQ168071           |              | 1179693405   | PMAC-acq51g01.b1   |  |
| MastacembelideMastacembelideMastacembelideMastacembelideMastacembelideDissolutionDissolutionPMAC-arit8705.g1DactylopterindeScorpaenoideScorpaenoideScorpaenoideScorpaenoideDissolutionDissolutionPMAC-arit8705.g1ScorpaenoideScorpaenoideCortidaeCheldointichtys luceranDQ168033EU525594PMAC-arit8705.g1TetraodontideiEparidaeIparidaeDQ168031EU525594PMAC-arit8705.g1PMAC-arit8705.g1TetraodontideiTetraodontidaeIgacephalus laevigatusDQ168071442361700PMAC-arit870201.b1TetraodontideiTetraodon mbuErroadon mbuPMAC-arit870201.b1PMAC-brit850201.b1TetraodontideiTetraodon sp.DQ168097Yest148968370PMAC-arit87044.g1PleuronectiformesPsettodoideiPistodaeSettodes belcheriDQ168105EU525911213523859PMAC-arit8504.b1PleuronectiformesPsettodoideiPistodaeRiccohirus variegatusDQ168105PMAC-arit8704.g1PMAC-arit8704.g1PleuronectiformesPsettodoideiCaproidaeCararitades sp.DQ168105PMAC-arit8704.g1PMAC-arit8704.g1PreciderPsettodoideiPistodiaeRiccohirus variegatusDQ168105PMAC-arit8704.g1PMAC-arit8704.g1PreciderPsettodoideiCaroridaeCararitades sp.DQ168105PMAC-arit8704.g1PMAC-arit8704.g1PreciderPsettodoideiPistodaeCararitadesDQ16807PMAC-arit8704.g1  | Synbranchiformes     | Synbranchoidei    | Synbranchidae    | Monopterus albus            | DQ168088           |              | Contig containin                                       | ig exon 2  |  |
| Dactylopteridormes   Dactylopteridormes   Scorpaenidei   PRAC-alm40005.g1     Cottoide   Cattoide   Tetraadontidei   Tetraadontidei   Tetraadontidei   Tetraadontidei   Tetraadontidei   PRAC-sent-Storpa     Tetraadontidei   Tetraadontidei   Tetraadontidei   Tetraadontidei   Scorpaenidei   Scorpaenidei   Scorpaenidei   PRAC-beitStorpa     Nature   Tetraadontidei   Tetraadontidei   Tetraadontidei   Scorpaenidei   PRAC-beitStorpa     Nature   Nature   Tetraadontidei   Scorpaenidei   Scorpaenidei   PRAC-beitStorpa     Nature   Nature   Nature   CAB0100779   yes   149968370   PMAC-beitScorpa     Nature   Nature   Nature   Nature   Nature   PRAC-beitScorpaenidei   PRAC-beitSco   |                      | Mastacembeloidei  | Mastacembelidae  | Mastacembelus erythrotaenia | DQ168084           |              | 1255405503   | PMAC-air87c05.g1   |  |
| Scorpaenide<br>corpaenide<br>rigildaeScorpaenidae<br>frigildaeScorpaenidae<br>chelionithtys luceni<br>pariadeScorpaenidae<br>pariadeDecoma pariad<br>pariadeDeliaso<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40065.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g1<br>pariadePundC-alm40067.g2<br>pariadePundC-al   | Dactylopteriformes   |                   | Dactylopteridae  | Dactylopterus volitans      | DQ168059           |              | 1181182584   | PMAC-aff28g06.g1   |  |
| TrigildaeChelidonichthys lucernaDQ1681211948050PMAC-arng34b01.g1CottoideiCottidaeTarrulus bubdisDQ16801142261700PMAC-beck10e.0b.1TetraodontideiTetraodontidaeLiparis fabriciiDQ1680761423261700PMAC-beck10e.0b.1TetraodontideiTetraodontidaeLiparis fabriciiDQ1680761442361700PMAC-barn29608.b1Tetraodon nigrovirdisSCAF10211Veroy partial1470057826PMAC-barn2201.b1Tafifigur urbrigesCAAB01007779yes1489683570PMAC-barn2201.b1MolidaeMolidaeMolidaeMolidaeDQ168095119941313PMAC-ado79e04.g1MolidaeMolidaePricaonthodes sp.DQ168075120373558PMAC-ahof9609.g1PleuronectidoriPestrodidaePestrodiaePestrodiaeDQ1680551184647640PMAC-aeg12c03.b1PleuronectidoeiCitharidaeCitharus linguatulaDQ1680551184647640PMAC-aeg12c03.b1PleuronectideiElassomatidaeCaproidaeCaproidaeCaproidaeAvesAvesPercidireeCaproidaeCaproidaeCaprositcusDQ168073Mas musculusMM_015745PercidireeCaproidaeLatesolaruitisDQ168073Mas musculusMM_015745PercidieCaproidaeLatesolaruitisDQ168073Mas musculusMM_015745PercidieCaproidaeCaleodontidaeLatesolaruitisDQ168073Mas austulusNM_015745PercidieCateodontidaeLateolaruitis<   | Scorpaeniformes      | Scorpaenoidei     | Scorpaenidae     | Scorpaena onaria            | DQ168114           |              | 1201200882   | PMAC-alm40b05.g1   |  |
| Cottoidei Cottide Taurulus bubaiis DQ168121 142361700 PMAC-bek61c09.b1   Tetraodontiformes Tetraodontide Liparis fabrici DQ16807 177550940 PMAC-birl B10.b1   Tetraodon ingrovirdis SCAF10211 Very parial 1470657826 PMAC-birl B10.b1   Tetraodon ingrovirdis SCAF10211 Very parial 1470057826 PMAC-birl B10.b1   Tetraodon ingrovirdis SCAF10211 Very parial 1470057826 PMAC-birl B207.b1   Tratanthodia Mola main DQ168075 117994131 PMAC-ado7904.g1   Pleuronectidei Psettodidae Partonodos sp. DQ168075 14223183767 PMAC-ado7904.g1   Pleuronectidei Psettodidae Psettodiche belcheri DQ168055 14223183767 PMAC-ado60903.g1   Pleuronectidei Psettodidae Ostracina DQ168055 1423183787 PMAC-ado60903.g1   Soleidae Microchrius variegatis DQ168055 1423183787 PMAC-ado80903.g1   Pleuronectidei Soleidae Microchrius variegatis DQ168055 1423183787 PMAC-ado80903.g1   Pleuronectidei Soleidae Microchrius variegatis DQ168055 1423183787 PMAC-ado809.91   Pleuronectidei Soleidae Microchrius variegatis <td></td> <td></td> <td>Triglidae</td> <td>Chelidonichthys lucerna</td> <td>DQ168053</td> <td>EU625594</td> <td>1211948050</td> <td>PMAC-amg34b01.g1</td>  |                      |                   | Triglidae        | Chelidonichthys lucerna     | DQ168053           | EU625594     | 1211948050   | PMAC-amg34b01.g1   |  |
| Liparia<br>TetraodontidoLiparia<br>fabriciD0[1680811175509040PMAC-aem2908.b1TetraodontidoTetraodon mbuEU625595Contig containuexon 3Tetraodon mbuTetraodon mbuEU625595Contig containuexon 3Tetraodon mbuTetraodon mbuVery partial1470057826PMAC-bbr/2810.1b1Tetraodon mbuCAAB01007779yes148068370PMAC-bbr/28070.1b1MolidaeOstracion sp.D0[1680951170941313PMAC-adr9904.9g1MolidaeMolidaeMolidaeTriacanthodise sp.D0[1681251422318367PMAC-adr9904.1b2.1b1TriacanthodidaeTriacanthodise sp.D0[168108EU625593121323599PMAC-adr9904.2b1PleuronectidoeCitharidaeCitharidaeD0[168108EU6255931184647640PMAC-age1203.b1SoleidaeMicrochirus varegatusD0[168119Kenopus leevisX5473ElassomatideiPercoideiSerraniaD0[168073MusNu_015745PerciformesCaproideCaproidaeCapros aperD0[168074MusNu_015745PercideSerraniaScarariferD0[168073MusNu_015745S03844PercideLates calariferD0[168073MusNu_015745S039844PercidaeLates calariferD0[168074Huon sapiensS039844PercidaeLates calariferD0[168074Huon sapiensS039844PercidaeLates calariferD0[168074Huon sapiensS039844 <t< td=""><td></td><td>Cottoidei</td><td>Cottidae</td><td>Taurulus bubalis</td><td>DQ168121</td><td></td><td>1442361700</td><td>PMAC-bek61c09.b1</td></t<>   |                      | Cottoidei         | Cottidae         | Taurulus bubalis            | DQ168121           |              | 1442361700   | PMAC-bek61c09.b1   |  |
| Tetraodontiformes   Tetraodontide   Lagocephalus leavigatus   DQ168076   1464380256   PMAC-bkr18h10.b1     Tetraodon miar   FUE   SCAF10211   Very partial   1470057826   PMAC-bkr18h10.b1     Tetraodon migroviridis   SCAF10211   Very partial   1470057826   PMAC-bkr28h10.b1     Traicarthodiae   Ostraciidae   Molidae   Mola nola   DQ168075   117994131   PMAC-ado7904.g1     Pleuronectiformes   Psettodiodei   Psettodidae   Psettodis Psettodis belcheri   DQ168075   142231867   PMAC-aqve540A1b1     Soleidae   Microtinus varigatus   DQ168075   1184647640   PMAC-agve130Ab1     Pleuronectidei   Soleidae   Microtinus varigatus   DQ168175   1184647640   PMAC-agve130Ab1     Elassomatoidei   Pleuronectidae   Syacium micrurum   DQ16817   Very partial   X994153   X9473     Elassomatoidei   Caproidae   Caproidae   Capros aper   DQ168105   Maus musculus   X05473     Perciformes   Caproidei   Seranidae   Elassomatoicus   DQ168103   Very partial   XMAC-abc1900406     Perciformes   Caproide   Caproidae <td></td> <td></td> <td>Liparidae</td> <td>Liparis fabricii</td> <td>DQ168081</td> <td></td> <td>1175509040</td> <td>PMAC-aem29g08.b1</td>  |                      |                   | Liparidae        | Liparis fabricii            | DQ168081           |              | 1175509040   | PMAC-aem29g08.b1   |  |
| Pleuronectiformes     Psettodoidei     Contig containing exon 3       Pleuronectiformes     Network     CAAB01007779     yes     1489683570     PMAC-bm22011.b1       Takfugu rubripes     CAAB01007779     yes     1489683570     PMAC-bm2201.b1       Molidae     Ostracion sp.     DQ168095     1779941313     PMAC-abo/204.g1       Molidae     Triacanthodidae     Triacanthodidae     Psettodiole     PMAC-bm2203.91       Pleuronectidie     Psettodiolei     Pietrodicebe belcheri<  | Tetraodontiformes    | Tetraodontoidei   | Tetraodontidae   | Lagocephalus laevigatus     | DQ168076           |              | 1464380256   | PMAC-bkr18h10.b1   |  |
| Tetradon nigrovindisSCAF102.11Very partal1470057826PMAC-bms0201.b1Nakifugu rubripesCAR01007779yes1439683570PMAC-brs0207.b1Nakifugu rubripesOstraciidaeOstracion sp.DQ1680551179941313PMAC-ado79e04.g1PleuronectidiciTriacanthodiaeTriacanthodies sp.DQ1680551202373563PMAC-adv54504.b1PleuronectidiciCithariaeCitharus lingualuaDQ168055121523859PMAC-adv54504.b1PleuronectidiciCitharus lingualuaDQ168055118464760PMAC-adv54304.b1SoleidaeMicrochirus variegatusDQ168065118464760PMAC-adv5120.b1PleuronectidieSoleidaeMicrochirus variegatusDQ168065118464760PMAC-adv5120.b1PleuronectidieSoleidaeRegroum microrumDQ168068118464760PMAC-adv5120.b1PleuronectidieSeramitaeElassoma zonatusDQ1680684XesX95473PercidieCaproidaeCaproidaeCaproidaeDQ1680481400ms apiensN015745PercidieSeramitaeCaproidaeDQ168075MammaliaMus musculusNMU15745PercidieSeramitaePogonoperca punctataDQ168078Mas musculusNMU15745Pogonoperca punctataDQ168078VertaeKM_546201KM_546201Pogonoperca punctataDQ168078VertaeKM_546201KM_546201Pogonoperca punctataDQ168078VertaeKM_546201KM_546201Pogonoperca punctataDQ168076   |                      |                   |                  | Tetraodon mbu               |                    | EU625595     | Contig containin                                       | ig exon 3  |  |
| Takifugu rubripesCAAB01007779yes148968370PMAC-brk25a07.b1Ostracion sp.Ogtracion sp.Ogt68095120037366PMAC-adr39604.g1MolidaeMolaDQ1680871200373650PMAC-adr39604.g1TiacanthodidaePsettodiseliOgt68108EU62599121523859PleuronectideiPsettodiseliPettodes belcheriDQ168108EU625991184647640PleuronectideiCitharidaeCitharus linguatulaDQ1680151184647640PMAC-agr45a04.b1PleuronectideiPettodes belcheriDQ168016EU625931184647640PMAC-agr45a04.b1PerciomesCaproideCaproideSolici vugriegausDQ168016Kenopus leevis95473ElassomatideiElassomatia contusDQ168017Kenopus leevis95473PerciomesCaproideCaproideCapros aperDQ168013Mas musculusMQ015745PercioleeiSeranuis accraensisDQ168016Mas musculusMU_015745B039844PercioleeiPercoideiLites calcariferDQ168075Mas musculusMU_015745PercioleeiCentropomidaeLates calcariferDQ168076Mas musculusMU_015745PercideaPerca flyricus saponaceusDQ168075Macara mulatuiSNSMUT0000016682PorpanidaeDerpane africanDQ168076Homo sapiensSNSMUT0000016682PorpanidaeCatecdon scruusDQ168076Homo sapiensSNSMUT0000016682PorpanidaePerca flyricus saponaceusDQ168075<  |                      |                   |                  | Tetraodon nigroviridis      | SCAF10211          | Very partial | 1470057826   | PMAC-bms02f01.b1   |  |
| PleuronectiformesPsettodioideOstraciidaeOstraciion sp.DQ1680951179941313PMAC-ado7904.g1MoliadeMola molaDQ168125120037365PMAC-adb6912.b1TriacanthodidaeTriacanthodes sp.DQ1681251422318367PMAC-ab69012.b1PleuronectoideiCitharidaeCitharus linguatulaDQ168108EU6255331213523859PMAC-aoh6003.g1SoleidaeNicrochirus variegatusDQ168015LissamphibiaPMAC-aeg12c03.b1SoleidaeSoleidavSolea vulgarisDQ168117LissamphibiaPleuronectidaeSyacium micrurumDQ168063AvesSolea vulgarisPerciformesCaproideiCapro agerDQ168063AvesPercoideiSerranus accraensisDQ168063MammaliaMo15745PercoideiSerranus accraensisDQ168064Homo sagientSole3844PercoideiSerranus accraensisDQ168073Mus musculusNM_015745PercoideiCentropomidaeLates calariferDQ168073Mus musculusSMMUT0000016682PercidaePerca fluviatilisDQ168073Macaca mulataENSMUT00000016682CentropomidaeLates calariferDQ168065SerranusSMUT00000016682PercidaePerca fluviatilisDQ168076SerranusSMUT00000016682CentropomidaeLates calariferDQ168076SerranusSMUT00000016682CentropomidaeCheatodon senilarvatusDQ168066SerranusSMUT00000016682PoponacanthidaeDrepa   |                      |                   |                  | Takifugu rubripes           | CAAB01007779       | yes          | 1489683570   | PMAC-brk26a07.b1   |  |
| MolidaeMolidaeMoliaDQ168087120373563PMAC-alb69h12.b1TriacanthodidaeTriacanthodiss pp.DQ168108EU625593112323859PMAC-alb69h12.b1PleuronectoideiPsettodidaePsettodes belcheriDQ168108EU6255931213523859PMAC-aohG0g3.g1PleuronectoideiCitharidaeCitharis linguatulaDQ1680051134647640PMAC-aeg12c03.b1SoleidaeSoleidaeSoleidauDQ168119Xenopus leavisX95473ElassomatideiElassomatidaeElassomatonatusDQ168016AvesPerciformesCaproideiCaproidaeCapros aperDQ168015MurmaliaeXenopus leavisX994153PercoideiSerranus accraensisDQ168013Mus musculusNM_015745BC039844PercoideiSerranus accraensisDQ168016Hono sapiensBC039844PercoideiCentropomidaeLates clacariferDQ168063Hono sapiensBC039844PercoideiCentropomidaeLatesolarigaponicusDQ168075Mus musculusNM_015745PercidaePerca fluviatilisDQ168068SSSSPercidaePerca fluviatilisDQ168063SSSSPercidaeCapronocephalus cernusDQ168063SSSSPercidaePerca fluviatilisDQ168063SSSSPercidaeCapronocephalus cernusDQ168063SSSSPomacanthidaeHolacanthus c   |                      |                   | Ostraciidae      | Ostracion sp.               | DQ168095           |              | 1179941313   | PMAC-ado79e04.g1   |  |
| PleuronectiformesPictodoidePricatanthodidePricatanthodides sp.DQ1681251422318367PMAC-aye45a04.b1PleuronectidieCitharidaeCitharus inguatulaDQ168108EU625531213523859PMAC-aye45a04.b1PleuronectidieCitharidaeCitharus variegatusDQ1680861184647640PMAC-aye12c03.b1Soleid au ulgarisDQ168117LissamphibiaX95473ElassomatiodeiPleuronectidaeElassoma zonatusDQ168063AvesPerciformesCaproideiCaproidaeCapros aperDQ168115MammaliaPercoideiSeranidaeCapros aperDQ168073Mus musculusNM_015745PercoideiSeranidaeIateolaristicusDQ168063Mus musculusNM_015745PercoideiSeranidaeLateolarix japonicusDQ168073Mus musculusNM_015745PercoideiPercoideiLateolarix japonicusDQ168073Mus musculusNM_015745PercidaePercoideiCentropomidaeLateolarix japonicusDQ168073KM_546201PercidaePercidaePerca fluviatilisDQ168065KM_546201KM_546201PercidaePerca fluviatilisDQ168051KM_546201KM_546201PercidaeChaetodon semilarvatusDQ168051KM_546201KM_546201PercidaePerca fluviatilisDQ168051KM_546201KM_546201PercidaePerca fluviatilisDQ168051KM_546201KM_546201PercidaePerca fluviatilisDQ168051KM_5462   |                      |                   | Molidae          | Mola mola                   | DQ168087           |              | 1200373563   | PMAC-alb69h12.b1   |  |
| Pleuronectiformes   Psettodidei   Psettodide   Psettodide   Psettodis belcheri   DQ168108   EU625593   1213523859   PMAC-aoh60g03,g1     Pleuronectidio   Citharidae   Citharus linguatula   DQ168005   1184647640   PMAC-ade0203,b1     Elassomatidei   Peuronectidae   Solei vulgaris   DQ168117   Lissamphibia   Xeropus laevis   X95473     Elassomatidei   Elassomatidae   Elassoma zonatus   DQ168063   Aves   X994153     Perciformes   Caproidei   Caproidae   Capros aper   DQ168015   Mamanlia     Percoidei   Serranus accraensis   DQ168013   Camois familiaris   NM_015745     Percoidei   Serranus accraensis   DQ168013   Canis familiaris   XM_54201     Rypticus saponaceus   DQ168078   KM_54201   KM_54201   KM_54201     Rypticus saponaceus   DQ168078   KM_54201   KM_54201   KM_54201     Percidae   Letcolabraix japonicus   DQ168050   KM_54201   KM_54201     Rypticus saponaceus   DQ168078   KM_54201   KM_54201   KM_54201     Rypticus saponaceus   DQ168078   KM_54201  |                      |                   | Triacanthodidae  | Triacanthodes sp.           | DQ168125           |              | 1422318367   | PMAC-aye45a04.b1   |  |
| Pleuronectoidei   Citharuis   Citharuis   Iingualua   DQ168055   1184647640   PMAC-aeg12c03.b1     Soleidae   Soleidae   Microchirus variegatus   DQ168017   Lissamphibia   Lissamphibia     Elassomatoidei   Pleuronectidae   Syacium micrurum   DQ168013   Aves   X95473     Elassomatoidei   Caproide   Caproidae   Capros aper   DQ168048   Gallus gallus   AY994153     Percideri   Serranus accreansis   DQ168064   Mammalia   Monospienos   MQ15745     Epinephelus aeneus   DQ168064   Homo sapienos   BO39844     Pogonoperca punctata   DQ168064   Homo sapienos   BO39844     Pogonoperca punctata   DQ168073   Maacca mulata   ENSMMUT0000001685     Percidae   Lates calcarifer   DQ168078   NM_546201   NM_546201     Pognooperca punctata   DQ168078   Porcidae   Enversidentas citarijes   DQ168078   NM_546201     Percidae   Centropomidae   Lates calcarifer   DQ168078   Porcidae   Seites   Fundotio1685     Centropomidae   Lates calcarifer   DQ168075   Fundoticae   Fundoticae <td>Pleuronectiformes</td> <td>Psettodoidei</td> <td>Psettodidae</td> <td>Psettodes belcheri</td> <td>DQ168108</td> <td>EU625593</td> <td>1213523859</td> <td>PMAC-aoh60g03.g1</td>  | Pleuronectiformes    | Psettodoidei      | Psettodidae      | Psettodes belcheri          | DQ168108           | EU625593     | 1213523859   | PMAC-aoh60g03.g1   |  |
| Soleidae Microchirus variegatus<br>Solea vulgaris DQ168086   Elassomatoidei Pleuronectidae Syacium micrurum<br>DQ168013 Xenopus laevis X95473   Elassomatoidei Elassomatidae Elassomatoaus DQ168013 Aves   Perciformes Caproidei Caproidae Capros aper DQ168015 Mammalia   Percoidei Serranias accraensis DQ168015 Mus musculus NM_015745   Percoidei Serranias accraensis DQ168064 Homo sapiens BC039844   Pogonoperca punctata DQ168013 Canis familiaris XM_546201   Rypticus saponaceus DQ168013 Macaca mulatu ENSMMUT00000016682   Rypticus saponaceus DQ168075 KM_546201 KM_546201   Porena filter Perca filteriary setting aternatintary setting aternatintary setting aternation aternation a   |                      | Pleuronectoidei   | Citharidae       | Citharus linguatula         | DQ168055           |              | 1184647640   | PMAC-aeg12c03.b1   |  |
| Elassomatoidei Pleuronectidae Syacium micrurum Dq168119 Xenopus lavis X95473   Elassomatidae Elassomatidae Elassomatoidei Caproidei Caproidei Caproidei Caproidei Caproidei Caproidei Caproidei Serranus accraensis Dq168015 Mammalia   Percoidei Serranidae Berranisa Dq168073 Mus muscus NM_015745   Epinephelus aeneus Dq168013 Canis familiaris XM_546201   Pogonoperca punctata Dq168013 Canis familiaris XM_546201   Percide Lateolabrax ignoricus Dq168075 ENSMMUT00000016682   Centropomidae Lateolabrax ignoricus Dq168061 Hartification   Percidae Perca fluviatilis Dq168068 File File   Opinocephalus cernuus Dq168061 File File File   Precidae Chaetodontake Dq168061 File File   Pomacanthidae Holadarhus ciliaris Dq168061 File File   Pomacanthidae Pomadarhus ciliaris Dq168061 File File   Pomacanthidae Pomadarhus ciliaris Dq168061 File File   Pomacanthidae Pomadarhus ciliaris Dq168061 File   |                      |                   | Soleidae         | Microchirus variegatus      | DQ168086           |              |  |  |  |
| PleuronectidaeSyacium micrurumDQ168119Xenopus laevisX95473ElassomatoideiElassomatidaeElassomatonatusDQ168063AvesPerciformesCaproideiSerranidaeCapros aperDQ168015Gallus gallusAV994153PercoideiSerranidaeSerranus accraensisDQ168073Mus musculusNM_015745PercoideiSerranidaePonoporera punctataDQ168113Mus musculusNM_015745Pognoperca punctataDQ168103Canis familiarisXM_546201NoronidaeLates calcariferDQ168075ENSMMUT00000016682PercidaePerca fluviatilisDQ168078FercidaeENSMMUT00000016682PercidaeChaetodontidaeLateolabrax japonicusDQ168076FercidaeFercidaePercidaePerca fluviatilisDQ168078FercidaeFercidaeFercidaePercidaePerca fluviatilisDQ168078FercidaeFercidaeFercidaePercidaePerca fluviatilisDQ168078FercidaeFercidaeFercidaePercidaePrepane africanaDQ168076FercidaeFercidaeFercidaePomacanthidaeHolacanthus ciliarisDQ168075FercidaeFercidaeFercidaePomacanthidaeHolacanthus ciliarisDQ168076FercidaeFercidaeFercidaePomacanthidaeHolacanthus ciliarisDQ168076FercidaeFercidaeFercidaePomacanthidaeHolacanthus ciliarisDQ168076FercidaeFercidaeFercida  |                      |                   |                  | Solea vulgaris              | DQ168117           |              | Lissamphibia   |  |  |
| Elassomatiolei   Elassomatidae   Elassoma zonatus   DQ168063   Aves     Perciformes   Caproidei   Caproide   Capros aper   DQ168048   Callus gallus   AY994153     Percoidei   Serranidae   Serranus accraensis   DQ168073   Mus musculus   NM_015745     Percoidei   Serranidae   Serranidae   DQ168064   Homo sapiens   BC039844     Pogonoperca punctata   DQ168003   Mus musculus   NM_015745     Percoidei   Lateo alcarifer   DQ168073   Macaca mulata   ENSMMUT00000016682     Centropomidae   Lateolabrax japonicus   DQ168075   ENSMMUT00000016682   ENSMMUT00000016682     Percidae   Perca fluviatilis   DQ168075   ENSMMUT00000016682   ENSMMUT00000016682     Percidae   Perca fluviatilis   DQ168075   ENSMMUT00000016682   ENSMMUT00000016682     Chaetodontidae   Chaetodon semilarvatus   DQ168068   ENSMMUT00000016682   ENSMMUT0000016682     Pomacanthidae   Holacanthus ciliaris   DQ168068   ENS   ENSMMUT0000016682     Mullidae   Mulus surmuletus   DQ168068   ENS   ENSMMUT0000016682     Mullidae <td></td> <td></td> <td>Pleuronectidae</td> <td>Syacium micrurum</td> <td>DQ168119</td> <td></td> <td>Xenopus laevis</td> <td>X95473</td>   |                      |                   | Pleuronectidae   | Syacium micrurum            | DQ168119           |              | Xenopus laevis   | X95473   |  |
| Perciformes   Caproidei   Caproidae   Caprois aper   DQ168048   Gallus gallus   AY994153     Percoidei   Serranis accraensis   DQ168115   Marmalia   NM_015745     Percoidei   Serranis accraensis   DQ168064   Homo sapiens   BC039844     Pogonoperca punctata   DQ168064   Homo sapiens   BC039844     Pogonoperca punctata   DQ168075   XM_546201     Rypticus saponaceus   DQ168075   Serranis destoras   DQ168075     Moronidae   Lateolabrax japonicus   DQ168075   Serranis destoras   Serranis destoras     Percidae   Perca fluviatilis   DQ168075   Serranis destoras   Serranis destoras   Serranis destoras     Chaetodontidae   Chaetodon semilarvatus   DQ168075   Serranis destoras   Serranis destoras     Chaetodontidae   Chaetodon semilarvatus   DQ168075   Serranis destoras   Serranis destoras     Depenidae   Drepan darican   DQ168075   Serranis destoras   Serranis destoras     Chaetodontidae   Chaetodon semilarvatus   DQ168050   Serranis destoras   Serranis destoras     Drepanidae   Pomacanthis ciliaris   DQ168075 </td <td>Elassomatoidei</td> <td></td> <td>Elassomatidae</td> <td>Elassoma zonatus</td> <td>DQ168063</td> <td></td> <td>Aves</td> <td></td>   | Elassomatoidei       |                   | Elassomatidae    | Elassoma zonatus            | DQ168063           |              | Aves   |  |  |
| PercoideiSerranidaeSerranus accraensisDQ168115MammaliaHolanthilas chrysosticusDQ168073Mus musculusNM_015745Epinephelus aeneusDQ168073Mus musculusNM_015745Pogonoperca punctataDQ168103Canis familiarisXM_546201Rypticus saponaceusDQ168111Macaca mulataENSMMUT00000016683CentropomidaeLates calcariferDQ168075SouthornidaeEnterclaberaPercidaePerca fluviatilisDQ168068SouthornidaeSouthornidaeSouthornidaeChaetodontidaeChaetodon semilarvatusDQ168068SouthornidaeSouthornidaeSouthornidaePrepanidaeDrepan de dricanaDQ168061SouthornidaeSouthornidaeSouthornidaeSouthornidaeHaemulidaePomadasys perotaeiDQ168061SouthornidaeSouthornidaeSouthornidaeSouthornidaeMulidaeMullus surmuletusDQ168061SouthornidaeSouthornidaeSouthornidaeSouthornidaeMulidaeMullus surmuletusDQ168061SouthornidaeSouthornidaeSouthornidaeSouthornidaeMulidaeMullus surmuletusDQ168065SouthornidaeSouthornidaeSouthornidaeSouthornidaePolynemidaePentancus duriguariusDQ168054SouthornidaeSouthornidaeSouthornidaePolynemidaePentancus duriguariusDQ168054SouthornidaeSouthornidaeSouthornidaePolynemidaePentancus duriguariusDQ168054SouthornidaeSouthorn   | Perciformes          | Caproidei         | Caproidae        | Capros aper                 | DQ168048           |              | Gallus gallus  | AY994153   |  |
| Holanthias chrysostictusDQ168073Mus musculusNM_015745Epinephelus aeneusDQ168064Homo sapiensBC039844Pogonoperca punctataDQ168103Canis familiarisXM_546201Rypticus saponaceusDQ168111Macaca mulattaENSMMUT00000016682CentropomidaeLateo calcariferDQ168075ENSMMUT00000016682MoronidaeLateolabrax japonicusDQ168078ENSMMUT00000016682PercidaePerca fluviatilisDQ168068ENSMMUT00000016682Opmocephalus cernuusDQ168068ENSMMUT00000016682ENSMMUT00000016682Opmocephalus cernusDQ168068ENSMMUT00000016682ENSMMUT00000016682Opmocephalus cernusDQ168068ENSMMUT00000016682ENSMMUT00000016682Opmocephalus cernusDQ168068ENSMMUT00000016682ENSMMUT00000016682Opmocephalus cernusDQ168068ENSMMUT00000016682ENSMMUT00000016682OpmacanthidaeAbacanthus ciliarisDQ168061ENSMMUT0000016682PomacanthidaePomadasys perotaeiDQ168072ENSMMUT0000016682MullidaeMullus surmuletusDQ168090ENSMMUT0000016682MullidaeMenemaculataDQ168085ENSMMUT001900016682PolynemidaePentanemus quinquariusDQ168098ENSMPolynemidaeChroscombrus chrysurusDQ168098ENSMPolynemidaeCarangidaeChloroscombrus chrysurusDQ168054Trachinotus ovatusDQ168054ENSMENSMTrachinotus ovatusDQ168056ENSM<  |                      | Percoidei         | Serranidae       | Serranus accraensis         | DQ168115           |              | Mammalia   |  |  |
| Epinephelus aeneusDQ168064Homo sapiensBC039844Pogonoperca punctataDQ168103Canis familiarisXM_546201Rypticus saponaceusDQ168111Macaca mulattaENSMMUT0000001668:CentropomidaeLates calcariferDQ168075ENSMMUT0000001668:MoronidaeLateolabrax japonicusDQ168078ENSMMUT0000001668:PercidaePerca fluviatilisDQ168078ENSMMUT0000001668:ChaetodontidaeChaetodon semilarvatusDQ168068ENSMMUT0000001668:DrepanidaePrepane africanaDQ168050ENSMMUT0000001668:DrepanidaePrepane africanaDQ168061ENSMMUT0000001668:PomacanthidaeHolacanthus ciliarisDQ168072ENSMMUT000001668:MullidaeMullus surmuletusDQ168050ENSMMUT000001668:MullidaeMullus surmuletusDQ168090ENSMMUT000001668:MonidaePomadasys perotaeiDQ168090ENSMMUT000001668:MullidaeMullus surmuletusDQ168095ENSMMUT000001668:MullidaeMullus surmuletusDQ168095ENSMMUT000001668:CarangoideiCarangidaeChloroscombrus chrysurusDQ168095CarangidaeChloroscombrus chrysurusDQ168095ENSMENTCarangidaeChloroscombrus chrysurusDQ168095ENSMENTCarangidaeChloroscombrus chrysurusDQ168095ENSMENTCarangidaeChloroscombrus chrysurusDQ168095ENSMENTCoryphenidaeCoryphena hippurusDQ168056ENSMENT <td></td> <td></td> <td></td> <td>Holanthias chrysostictus</td> <td>DQ168073</td> <td></td> <td>Mus musculus</td> <td>NM_015745</td>  |                      |                   |                  | Holanthias chrysostictus    | DQ168073           |              | Mus musculus   | NM_015745  |  |
| Pogonoperca punctata<br>Rypticus saponaceusDQ168103Canis familiarisXM_546201Rypticus saponaceusDQ168111Macaca mulattaENSMMUT0000001668:CentropomidaeLates calcariferDQ168075MoronidaeLateolabrax japonicusDQ168078PercidaePerca fluviatilisDQ168068ChaetodontidaeChaetodon semilarvatusDQ168068DrepanidaeDrepane africanaDQ168061PomacanthidaeHolacanthus ciliarisDQ168072HaemulidaePomadasys perotaeiDQ168072MullidaeMulus surmuletusDQ168085PolynemidaePentanemus quinquariusDQ168085PolynemidaeCharoscombrus chrysurusDQ168098CarangoideiCarangidaeChoroscombrus chrysurusCoryphaenidaeCoryphaenidaeDQ168054CoryphaenidaeCoryphaeni hippurusDQ168056  |                      |                   |                  | Epinephelus aeneus          | DQ168064           |              | Homo sapiens   | BC039844   |  |
| Rypticus saponaceusDQ168111Macaca mulattaENSMMUT0000001668:CentropomidaeLates calcariferDQ168075MoronidaeLateolabrax japonicusDQ168078PercialaePerca fluviatilisDQ168099ChaetodontidaeChaetodon semilarvatusDQ168050DrepanidaeDrepane africanaDQ168072PomacanthidaeHolacanthus ciliarisDQ168072HaemulidaePomadasys perotaeiDQ168050MullidaeMullus surmuletusDQ168050MullidaeMullus surmuletusDQ168050PolynemidaePentanemus quinquariusDQ168054PolynemidaeCharangoideiCarangidaeCarangidaeChorscombrus chrysurusDQ168054CoryphaenidaeCoryphaenidaeDQ168054CoryphaenidaeCoryphaenidaeDQ168056CoryphaenidaeCoryphaeni hippurusDQ168056  |                      |                   |                  | Pogonoperca punctata        | DQ168103           |              | Canis familiaris                                       | XM_546201  |  |
| CentropomidaeLates calcariferDQ168075MoronidaeLateolabrax japonicusDQ168078PercidaePerca fluviatilisDQ168099ChaetodontidaeChaetodon semilarvatusDQ168068DrepanidaeDrepane africanaDQ168061PomacanthidaeHolacanthus ciliarisDQ168072HaemulidaePomadasys perotaeiDQ168050MullidaeMullus surmuletusDQ168061MullidaeMullus surmuletusDQ168072PolynemidaePotanemuletusDQ168050CarangoideiCharoscombrus chrysurusDQ168054<br>Trachinotus ovatusCarangidaeChoryphaen dippurusDQ168054<br>Trachinotus ovatusCoryphaenidaeCoryphaen hippurusDQ168054<br>Trachinotus ovatus  |                      |                   |                  | Rypticus saponaceus         | DQ168111           |              | Macaca mulatta   | ENSMMUT00000016682   |  |
| MoronidaeLateolabrax japonicusDQ168078PercidaePerca fluviatilisDQ168099Cymnocephalus cernuusDQ168068ChaetodontidaeChaetodon semilarvatusDQ168061DrepanidaeDrepane africanaDQ168061PomacanthidaeHolacanthus ciliarisDQ168072HaemulidaePomadasys perotaeiDQ168050MullidaeMullus surmuletusDQ168072PolynemidaePomacanthidaeDQ168050CarangoideiCarangidaePentanemus quinquariusCoryphaenidaeCoryphaenidaeDQ168054CoryphaenidaeCoryphaenidaeDQ168054Lateolos corresCoryphaenidaeDQ168054Lateolos corresCoryphaenidaeDQ168054Lateolos corresCoryphaenidaeDQ168054Lateolos corresCoryphaenidaeDQ168054Lateolos corresCoryphaenidaeDQ168054Lateolos corresCoryphaenidaeDQ168054Lateolos corresCoryphaenidaeDQ168056Lateolos corres   |                      |                   | Centropomidae    | Lates calcarifer            | DQ168075           |              |  |  |  |
| PercidaePerca fluviatilisDQ168099Gymnocephalus cernuusDQ168068ChaetodontidaeChaetodon semilarvatusDQ168050DrepanidaeDrepane africanaDQ168061PomacanthidaeHolacanthus ciliarisDQ168072HaemulidaePomadasys perotaeiDQ168090MullidaeMullus surmuletusDQ168085PolynemidaePentanemus quinquariusDQ168085PolynemidaeChioroscombrus chrysurusDQ168094CarangoideiCarangidaeChioroscombrus chrysurusDQ168054CoryphaenidaeCoryphena hippurusDQ168054CoryphaenidaeCoryphaeni hippurusDQ168056   |                      |                   | Moronidae        | Lateolabrax japonicus       | DQ168078           |              |  |  |  |
| Cymnocephalus cernuusDQ168068ChaetodontidaeChaetodon semilarvatusDQ168050DrepanidaeDrepane africanaDQ168061PomacanthidaeHolacanthus ciliarisDQ168072HaemulidaePomadasys perotaeiDQ168105MullidaeMullus surmuletusDQ168090MenidaeMene maculataDQ168098PolynemidaePentanemus quinquariusDQ168098CarangoideiCarangidaeChloroscombrus chrysurusDQ168094Trachinotus ovatusDQ168120CoryphaenidaeCoryphaen hippurusDQ168056   |                      |                   | Percidae         | Perca fluviatilis           | DQ168099           |              |  |  |  |
| ChaetodontidaeChaetodon semilarvatusDQ168050DrepanidaeDrepane africanaDQ168061PomacanthidaeHolacanthus ciliarisDQ168072HaemulidaePomadasys perotaeiDQ168105MullidaeMullus surmuletusDQ168090MenidaeMene maculataDQ168098CarangoideiCarangidaeChloroscombrus chrysurusCarangoideiCoryphaenidaeCoryphaenidaeCoryphaenidaeCoryphaenidaeDQ168056   |                      |                   |                  | Gymnocephalus cernuus       | DQ168068           |              |  |  |  |
| Drepanidae Drepane africana DQ168061<br>Pomacanthidae Holacanthus ciliaris DQ168072<br>Haemulidae Pomadasys perotaei DQ168105<br>Mullidae Mullus surmuletus DQ168090<br>Menidae Mene maculata DQ168085<br>Polynemidae Pentanemus quinquarius DQ168098<br>Carangoidei Carangidae Chloroscombrus chrysurus DQ168054<br>Trachinotus ovatus DQ168120<br>Coryphaenidae Coryphaena hippurus DQ168056   |                      |                   | Chaetodontidae   | Chaetodon semilarvatus      | DQ168050           |              |  |  |  |
| PomacanthidaeHolacanthus ciliarisDQ168072HaemulidaePomadasys perotaeiDQ168105MullidaeMullus surmuletusDQ168090MenidaeMene maculataDQ168085PolynemidaePentanemus quinquariusDQ168098CarangoideiCarangidaeChloroscombrus chrysurusDQ168054Trachinotus ovatusDQ168120*CoryphaenidaeCoryphaena hippurusDQ168056  |                      |                   | Drepanidae       | Drepane africana            | DQ168061           |              |  |  |  |
| HaemulidaePomadasys perotaeiDQ168105MullidaeMullus surmuletusDQ168090MenidaeMene maculataDQ168085PolynemidaePentanemus quinquariusDQ168098CarangoideiCarangidaeChloroscombrus chrysurusDQ168054Trachinotus ovatusDQ168120CoryphaenidaeCoryphaena hippurusDQ168056  |                      |                   | Pomacanthidae    | Holacanthus ciliaris        | DQ168072           |              |  |  |  |
| MullidaeMullus surmuletusDQ168090MenidaeMene maculataDQ168085PolynemidaePentanemus quinquariusDQ168098CarangoideiCarangidaeChloroscombrus chrysurusDQ168054Trachinotus ovatusDQ168120CoryphaenidaeCoryphaena hippurusDQ168056  |                      |                   | Haemulidae       | Pomadasys perotaei          | DQ168105           |              |  |  |  |
| MenidaeMene maculataDQ168085PolynemidaePentanemus quinquariusDQ168098CarangoideiCarangidaeChloroscombrus chrysurusDQ168054Trachinotus ovatusDQ168120CoryphaenidaeCoryphaena hippurusDQ168056   |                      |                   | Mullidae         | Mullus surmuletus           | DQ168090           |              |  |  |  |
| Polynemidae Pentanemus quinquarius DQ168098<br>Carangoidei Carangidae Chloroscombrus chrysurus DQ168054<br>Trachinotus ovatus DQ168120<br>Coryphaenidae Coryphaena hippurus DQ168056   |                      |                   | Menidae          | Mene maculata               | DQ168085           |              |  |  |  |
| Carangoidei Carangidae Chloroscombrus chrysurus DQ168054<br>Trachinotus ovatus DQ168120<br>Coryphaenidae Coryphaena hippurus DQ168056  |                      |                   | Polynemidae      | Pentanemus quinquarius      | DO168098           |              |  |  |  |
| Trachinotus ovatus DQ168120<br>Coryphaenidae Coryphaena hippurus DQ168056  |                      | Carangoidei       | Carangidae       | Chloroscombrus chrysurus    | D0168054           |              |  |  |  |
| Coryphaenidae Coryphaena hippurus DQ168056   |                      | - and - golder    | Landingrade      | Trachinotus ovatus          | D0168120*          |              |  |  |  |
| corypracticate corypractical inppartas Derotobolo  |                      |                   | Corvnhaenidae    | Coryphaena hinnurus         | D0168056           |              |  |  |  |
| Echeneidae Echeneis naucrates D0168062   |                      |                   | Echeneidae       | Echeneis naucrates          | DQ168062           |              |  |  |  |

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Table 1 (continued)

| Outgroups | Order/sub-order | Family                    | Genus and species                         | Gene 2<br>module 1   | Gene 1                    | Single IRBP-coding gene |
|-----------|-----------------|---------------------------|---|----------------------|---------------------------|-------------------------|
|           | Acanthuroidei   | Acanthuridae<br>Siganidae | Ctenochaetus striatus<br>Siganus vulpinus | DQ168057<br>DQ168116 |                           |                         |
|           | Labroidei       | Labridae                  | Labrus bergylta                           | DQ168075             |                           |                         |
|           |                 | Scaridae                  | Scarus hoefleri                           | DQ168112             |                           |                         |
|           |                 | Cichlidae                 | Haplochromis nubilus                      | DQ168070             | EU625591                  |                         |
|           |                 |                           | Labeotropheus fuellebornii                |                      | gnl ti 1368495966         |                         |
|           |                 |                           |   |                      | BSYA18863.x1 <sup>*</sup> |                         |
|           |                 |                           | Melanochromis auratus                     |                      | gnl ti 1370326225         |                         |
|           |                 |                           |   |                      | BSYG62496.y1              |                         |
|           | Zoarcoidei      | Pholidae                  | Pholis gunnellus                          | DQ168100             |                           |                         |
|           | Notothenioidei  | Bovichtidae               | Bovichtus variegatus                      | DQ168046             | *                         |                         |
|           |                 | Nototheniidae             | Notothenia coriiceps                      | DQ168093             | EU625597                  |                         |
|           |                 | CI                        | Trematomus bernacchu                      | D010000              |                           |                         |
|           | The shine is a  | Channichthyidae           | Neopagetopsis ionah                       | DQ168092             |                           |                         |
|           | Trachinoidei    | Irachinidae               | Irachinus draco                           | DQ168123             | FU COSSOO                 |                         |
|           |                 | Uranoscopidae             | Uranoscopus albesca                       | DQ168126             | EU625592                  |                         |
|           |                 | Chinamartichthyldae       | Cheimarrichtnys Josteri                   | DQ168052             |                           |                         |
|           | Diamaiaidai     | Planniidae                | Kall macrura                              | DQ168074             |                           |                         |
|           | Biennioidei     | Triptorugiidaa            | Forstorygion lanillym                     | DQ168097             |                           |                         |
|           | Cobiososoidoi   | Cobiososidae              | Longdoggstor longdoggstor                 | DQ168080             |                           |                         |
|           | GODIESOCOIDEI   | Goblesocidae              | Anletodon dentatus                        | DQ108080             |                           |                         |
|           | Callionymoidei  | Callionymidae             | Callionymus lyra                          | DQ108055             |                           |                         |
|           | Cobioidei       | Cobiidae                  | Pomatoschistus sp                         | DQ168106             |                           |                         |
|           | Scombroidei     | Sphyraenidae              | Sphyraena sphyraena                       | DQ168118             |                           |                         |
|           | Scombronder     | Scombridae                | Scomber ianonicus                         | DQ168113             |                           |                         |
|           | Stromateoidei   | Stromateidae              | Pampus argenteus                          | DQ168096             |                           |                         |
|           |                 | Centrolophidae            | Psenopsis anomala                         | DO168107             |                           |                         |
|           | Channoidei      | Channidae                 | Channa striata                            | DO168051             |                           |                         |
|           | Anabantoidei    | Anabantidae               | Ctenopoma sp.                             | DQ168058             |                           |                         |
|           |                 |                           | , r.                                      | <b>-</b>             |                           |                         |

New sequences obtained in this study are indicated in bold. Classification follows Nelson (1994) except for the Caproidei (Johnson and Patterson, 1993). A star (\*) indicates sequences that are not full-length.

### 2. Material and methods

### 2.1. PCR and sequencing

2.1.1. Amplification and sequencing of the partial teleost IRBP gene 1 The RNA sequences for the IRBP gene 2 described in Wagenhorst et al. (1995) and Rajendran et al. (1996) were retrieved from GenBank using the NCBI portal (http://www.ncbi.nlm.nih.gov/) and used to BLAST-search (Altschul et al., 1997) the complete and nearly complete genomes of D. rerio, T. nigroviridis, T. rubripes, G. aculeatus and O. latipes through the ENSEMBL (Hubbard et al., 2005) MultiBlastview Tool (http://www.ensembl.org/Multi/blastview, releases 40-42). We then used the sequence of gene 1 in the D. rerio complete genome to query all the other available teleost complete genomes using the ENSEMBL portal, and also the trace archives for all the available actinopterygians, as well as for available non-sarcopterygian and non-actinopterygian sequences using the Discontiguous Megablast tool of the NCBI portal. Sequences producing significant hits were retained, and used in turn to query the sequence databases. We assembled the trace sequences for P. marinus using SEQUENCHER (Gene Codes Corporation). All these sequences were aligned by hand using BioEdit (Hall, 1999), and used to design primers for the partial gene 1 for teleosts (see Table 2), using Primer3 (Rozen and Skaletsky, 2000; http://frodo.wi.mit.edu/cgi-bin/primer3/primer3\_www.cgi). Samples listed in Table 1 were conserved in 70% ethanol and extracted following the protocol of Winnpennminck et al. (1993). Sequencespecific amplifications were performed by PCR in a final 50- $\mu$ L volume containing 5% DMSO, 300 µM of each dNTP, 0.3 µM of Taq DNA polymerase (Qiagen) and 5  $\mu$ L of 10× CoralLoad buffer (Qiagen) and 1.7 pM of each of the two primers (see Table 2 for the primers), with 0.1-0.3 µg of DNA added depending on species. After denaturation for 2 min at 94 °C, the PCR ran for 45 cycles of (20 s, 94 °C; 30 s, 58 °C; 5 min, 72 °C), with a terminal elongation of 10 min. The result was visualized on ethidium bromide-stained agarose gels. The PCR products were sequenced through commercial contract (Genoscreen, Lille, France). Each sequence was obtained at least twice and checked against its chromatograms in BioEdit (Hall, 1999). We controled for potential contaminations and mix-ups by pairwise sequence comparison. All new sequences were deposited in GenBank (accession numbers listed in Table 1).

# 2.1.2. Amplification and sequencing of the partial teleost IRBP gene 2

The sequences of IRBP gene 2 recovered in the genomic databases of D. rerio, T. nigroviridis, T. rubripes, G. aculeatus and O. latipes were used as references to design oligonucleotide primers (Table 2) using the same computer programs as for gene 1 for a fragment of approximately 800 bp of the first module of the gene 2. We amplified the partial gene 2 from genomic DNAs extracted with the same protocol as for gene 1, but for a much wider and more representative sampling within Acanthomorpha (see Table 1). Sequence-specific amplifications were performed by PCR in a final 25-µL volume containing the same products as before except for the polymerase and buffer: 0.3 µM of Qbiotaq (QBioGen), 2.5  $\mu$ L of 10× buffer (QBioGen); 0.01–0.1  $\mu$ g of DNA were added depending on species. After denaturation for 2 min, the PCR ran for 40 cycles of (30 s, 94 °C ; 30 s, 50 or 54 °C; 1 min, 72 °C), with a terminal elongation of 3 min. The result was visualized on ethidium bromide-stained agarose gels, and purified with the MinElute PCR Purification kit or the MinElute Gel purification kit (Qiagen). Sequencing was performed on a CEO2000 sequencer (Beckman Coulter), version 4.3.9, with the products and according to the instructions of the manufacturers' kit. Sequence assembly and checking was as before (accession numbers in Table 1).

### 2.1.3. Alignment and analyses

Alignment was performed by hand under BioEdit (Hall, 1999). The partial gene 2 IRBP contains almost no gaps (a single 3-bp deletion for *Pentanemus quinquarius*) and therefore presented no alignment ambiguities. The alignment of the modules of gene 1 and 2 with one another, as well as with the modules of other vertebrates, was done using the sequences translated in amino-acids and aligned as guideline. We analyzed several datasets: partial nucleic acids sequence of the teleost IRBP gene 2 (this dataset will be referred to hereafter as partial gene 2), and the almost complete sequence of the modules for a large sampling of vertebrates, under both nucleic acids and amino acids form. Because there was an ambiguously aligned region for the two vertebrate module datasets, analyses were performed with and without this region for the amino acids dataset.

We evaluated saturation (Philippe and Douzery, 1994; Hassanin et al., 1998) for the partial gene 2 separately for transitions and transversions, and also separately for each codon position (Fig. 2). Mean pairwise differences among three pairs of taxa (*D. re-rio* and *T. rubripes*, *T. rubripes* and *T. nigroviridis*, and *T. rubripes* and a cichlid) were calculated using PAUP<sup>\*</sup>4.0b10 (Swofford, 2002) for all modules of the IRBP-coding genes, MLL4 (Dettaï and Lecointre, 2005), rhodopsin retrogene (Chen et al., 2003), RAG1 and eight markers described by Li et al. (2007).

Gene 2 was analyzed by maximum likelihood (ML) and Bayesian phylogenetic inference method (BA). Both AIC and BIC approaches as implemented in Modeltest 3.7 (Posada and Crandall, 1998; Posada and Buckley, 2004) were used to identify the level of complexity of the model of nucleotide substitution that best fit the gene 2 dataset. We tested the dataset first as a whole, and then tested 1st, 2nd and 3rd positions separately (Table 3). BA was used as implemented in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). GTR + I +  $\Gamma$  were set as model, with the defaults settings for the priors for the proportion of invariable sites and for the gamma shape parameter. The analyses were run, using the 1st, 2nd and 3rd codon positions as different partitions. Eight analyses were run with the following parameters: four chains, 8 million generations, sampling of every 80th tree and discarding of the first 40,000 trees after checking the burn-in zone was included in this interval. After checking convergence had been reached, the trees and parameters resulting from the eight analyses were pooled and combined in a consensus. The ML analysis was run with PhyML (Guindon and Gascuel, 2003) on the PhyML online web server (Guindon et al., 2005) with a GTR + I +  $\Gamma$  model and estimation of all the parameters.

#### Table 2

Primers used for the amplification and sequencing of the partial first module of the IRBP-coding gene 2, and partial gene 1 in various teleosts

|   | Primer na | ame   | 5'-3' sequences                   |  |  |  |  |  |
|---|-----------|-------|-----------------------------------|--|--|--|--|--|
| Oligonucleotide primers for the 1st module of the IRBP coding gene 2      |           |       |                                   |  |  |  |  |  |
| Forward primers   | IRBP      | U104  | ATA GTY NTG GAC AAN TAC TGC TC    |  |  |  |  |  |
|   | IRBP      | U110  | TGG ACA AYT ACT GCT CRC CAG A     |  |  |  |  |  |
| Reverse primers   | IRBP      | L953  | CNG GAA YYT GAR CAC GGA GG        |  |  |  |  |  |
|   | IRBP      | L936  | CAC GGA GGY TGA YNA TCT TGA T     |  |  |  |  |  |
|   | IRBP      | L922  | TGA TNN CRG TKG CRA GGG CAT Y     |  |  |  |  |  |
|   | IRBP      | L916  | GTG KCA AGG GCA TCT TCT GC        |  |  |  |  |  |
| Oligonucleotide primers for the partial 1st, 2nd and 3rd module of gene 1 |           |       |                                   |  |  |  |  |  |
| Forward primers   | IRBP      | F147  | AAA CTA CTG CWT CCC KGA GAA       |  |  |  |  |  |
|   | IRBP      | F1325 | ATA YCT GCG YTT KGA CAG GTT       |  |  |  |  |  |
|   | IRBP      | F1798 | CAA CAA CGG DGA RTD CTG GCT       |  |  |  |  |  |
| Reverse primers   | IRBP      | R543  | AVA GYY CTC CMG CTG TGC TG        |  |  |  |  |  |
| •   | IRBP      | R1638 | GAC TGB ATY ARG TAA GCA AAC TC    |  |  |  |  |  |
|   | IRBP      | R2300 | TGT TGT ACC TCA TRT CRA TTA TGA C |  |  |  |  |  |

All primers were designed for this study. See the position of the primers on the gene on Fig. 1.

We analyzed the module datasets using maximum parsimony only. Heuristic searches (TBR search, 1000 replicates, gaps considered as missing) were conducted with PAUP<sup>\*</sup>4.0b10 (Swofford, 2002), and a 1000 bootstrap replicates were performed on the dataset.

# 3. Results and discussion

### 3.1. New IRBP sequences

While the size of the IRBP protein of the skate (Chondrichthyes) has been published (Sun and Ripps, 1992), its sequence is not known yet. Searches for chondrichthyan sequences using the whole gene 1 and gene 2 sequences for *D. rerio*, as well as with the sequences of the IRBP gene of *Macaca mulatta* and the *Xenopus laevis*, yielded no result, neither in the CoreSequence database nor in the trace archives of GenBank. Searches in the two available complete genomes of tunicates (*Ciona savignyi* and *Ciona intestinalis*), and in the currently available trace archives for *Oikopleura dioika* and *Branchiostoma floridae* were also unfruitful, regardless of the Blast-search settings. The absence of IRBP-like sequences in the available *Ciona* genomes had already been described by Nickerson et al. (2006).

#### 3.2. Petromyzon marinus

However, similar searches in the *Petromyzon marinus* whole genome shotgun sequences (WGS) produced positive hits that could be assembled in three contigs. Using the available whole genome shotgun sequences recovered by Blast-search, it was possible to reconstruct a partial sequence of the IRBP gene for *P. marinus*. The first of these contigs is 4371 bp long, and can be aligned unambiguously with the first exon of the tetrapod IRBP gene from its beginning to its end (see Fig. 2). Comparisons of each of the modules of *Petromyzon* with all the modules of *Danio, Xenopus* and *Homo*, using Blast2Sequences (Tatusova and Madden, 1999) and the phylogeny of the modules (Fig. 3) corroborated this homology hypothesis. It has no stop codons along this aligned part, but the 9th codon after what corresponds to the end of the exon in tetrapods and teleosts is a stop codon. It also shares with all the tetrapods (except *Gallus*) a conserved six base pairs (ATGCAG) next to where the exon ends in these.

The second contig is 997 bp, and can be aligned unambiguously with the complete exon 2 of the single gene present in sarcopterygians, and the exon 2 of gene 2 of Teleostei (201 bp). There are stop codons 58 codons before the beginning of the alignment and 19 codons after its end.

The third contig is 1602 bp long, and can be aligned unambiguously with the complete exon 3 of the single gene present in Sarcopterygians, and the exon 3 of gene 2 of Teleostei (141 bp). There are stop codons 37 codons before the beginning of the alignment and 51 codons after its end.

Unfortunately, the contigs could not be assembled to one another, even using as query for the search the ends of the contigs. It can not yet be excluded that they are not located close to one another, or even are part of different genes in the *Petromyzon* genome. Blast searches on the newly available assembly of the *Petromyzon* genome brought no answer, as the coding sequence for exon 1 is surrounded by unknown sequences (Ns). The sequence corresponding to the fourth and last exon could not be recovered in *P. marinus*. But if they are indeed all part of a single gene, then the structure of the IRBP gene in *P. marinus* is similar to the one in Sarcopterygii and the one hypothesized in sharks and rays (see Fig. 2). The two gene structure detected in teleosts is derived, as already proposed in Rajendran et al. (1996) and in Nickerson et al. (2006).

The high conservation in length of the predicted exons of the IRBP gene, and of the position of the potential introns, led us to



Fig. 2. Saturation plots for the partial module 4 of the IRBP coding gene. The observed pairwise differences were calculated with PAUP<sup>\*</sup>, the inferred pairwise differences were obtained using the maximum parsimony tree.

concatenate the "coding" part of the three contigs in a single sequence for the phylogenetic analysis of the modules.

# 3.3. New actinopterygian gene 1 sequences

All available actinopterygian sequences, whether in the CoreSequence database or in the trace archives were Blast-searched for gene 1. Very few new sequences were recovered: only two trace files for *Labeotropheus fuellebornii* and *Melanochromis auratus*. We recovered the same results as Nickerson et al. (2006) as to the presence of gene 1 in the available complete genomes of teleosts: no sequences for *G. aculeatus* and *O. latipes*, and a very partial sequence for *T. nigroviridis* (see Fig. 2). Yet, *T. rubripes*, where a full-length gene 1 is present, is much more closely related to *Tetraodon* than *Gasterosteus* or *Oryzias*, raising some questions about the distribution of gene 1 in acanthomorphs. To explore this, a number of

Table 3

| Estimated with Modeltest              | AIC            |          |            | BIC     |                  |            |        |
|---------------------------------------|----------------|----------|------------|---------|------------------|------------|--------|
|                                       | Model          | Alpha p  | Value of I | Model   | Alpha <i>p</i> . | Value of I |        |
| Confidence level 0.01                 |                |          |            |         |                  |            |        |
| complete seq.                         | GTR+I+G        | 0.927    | 0.201      | TrN+I+G | 0.326            | 0.203      |        |
| 1st codon pos.                        | TrN+G          | 0.543    |            | TrN+G   | 0.543            |            |        |
| 2nd codon pos.                        | GTR+G          | 0.369    |            | GTR+G   | 0.369            |            |        |
| 3rd codon pos.                        | GTR+G          | 2.939    |            | TVM+G   | 2.937            |            |        |
|                                       | Selected model | Alpha p. | Value of I | Freq. A | Freq. C          | Freq. G    | Freq T |
| Means estimated through Mr. Bayes 3.1 |                |          |            |         |                  |            |        |
| 1st codon pos.                        | GTR + I + G    | 0.732    | 0.117      | 0.374   | 0.218            | 0.266      | 0.266  |
| 2nd codon pos.                        | GTR + I + G    | 0.583    | 0.264      | 0.291   | 0.309            | 0.161      | 0.239  |
| 3rd codon pos.                        | GTR + I + G    | 2.897    | 0.007      | 0.166   | 0.359            | 0.257      | 0.217  |

| Parameters and | models as selected | d by Modeltest 3.7 | and as inferred | in the BA with | MrBaves 3.1 |
|----------------|--------------------|--------------------|-----------------|----------------|-------------|

Complete results of the analyses are available upon request.

species which are representative of the main clades of acanthomorphs as established in several molecular studies (Chen et al., 2003; Dettaï and Lecointre, 2004, 2005; Miya et al., 2003, 2005; Smith and Wheeler, 2006) were selected, especially focusing on species closely related to the species where no gene 1 could be detected in the available complete and almost complete genomic sequences. Amplification and sequencing of a large part of gene 1 was possible for specimens representing most clades (see Table 1) published in previous molecular studies (Chen et al., 2003; Miya et al., 2003, 2005; Dettaï and Lecointre, 2004, 2005; Smith and Wheeler, 2006; Smith and Craig, 2007; Chen et al., 2007). The hypothesis that the different structure of the IRBP coding genes in teleosts is specific to this group has been put forward by Nickerson et al. (2006). This remains to be tested by the exploration of the gene or genes in basal actinopterygians, where it has not been studied yet.

### 3.4. Analysis of the modules

Previous publications have identified the three dimensional structure of IRBP modules in mammals (Loew and Gonzalez-Fernandez, 2002). Comparison of structure and sequences helped identify a larger family of proteins with similarities to the modules of the gene. Among them are Tsp, a tail-specific protease degrading selectively proteins with nonpolar C termini (Silber et al., 1992), enoyl-coenzyme A hydratase (Engel et al., 1996), dienoyl coenzyme A isomerase (Modis et al., 1998), 4-chlorobenzoyl coenzyme A dehalogenase (Benning et al., 1996) and C-terminal processing protease (Liao et al., 2000). Unfortunately, the sequences identified as having similarities with IRBP were so distant that alignment was highly unreliable and therefore they were of limited use as outgroups. The trees were thus left unrooted.



**Fig. 3.** Strict consensus of eight equiparsimonious trees of 3441 steps obtained through maximum parsimony analysis with PAUP<sup>\*</sup> of the amino acid sequences of the modules of the IRBP coding genes for *Petromyzon marinus*, teleosts and tetrapods. The dataset comprises 68 sequences and is 324-aa-long. Values associated with the branches are bootstrap proportions obtained with 1000 bootstrap replicates. The tree is unrooted. The module names are given indicated according to the position of the sequence in the gene(s).

The unrooted tree depicting module interrelationships of vertebrate IRBP genes is shown in Fig. 3. The trees have the same general structure whether inferred with the amino-acid sequences or the nucleic acid sequences. All sequences from a given module cluster together in both cases (considering a hypothetic root on one of the five central branches), and tetrapods are separated from teleosts within each module except for the module 2 with inference from the nucleic acid sequences where Gallus and Xenopus are sister groups of the teleost clade. Very deep divergences are generally difficult to recover from a single molecular marker using unweighted parsimony, which is why multiple markers are generally used for this type of studies. In the present case, the success of our analyses in recovering basal gnathostome interrelationships within each module using no model corrections suggests that these sequences have suitable properties for deep phylogeny. For the trees inferred from amino acid sequences, there are high bootstrap supports for the basal branches of each module. Petromvzon module sequences are basal in all module clades except for the module 4/module 2 gene 2 clade, where it is a sister group of tetrapods, but with a very low bootstrap proportion. In all the other clades, the basal position is much better supported. This and the high conservation of the limits of the exons, leads to the conclusion that the four module structure of the IRBP gene is older than the lampreys/ gnathostomes split. Beside in Osteichthyes, previous studies had only found IRBP in Chondrichthyes, and the gene was not sequenced. The molecular weight of the protein in this group pointed to a single, four-module protein (Sun and Ripps, 1992). The present result gives a considerably older age for the formation of the gene with its four-module structure.

The pattern within teleosts and tetrapods in each module/cluster is more variable, but mostly compatible with known relationships among vertebrates. Bootstrap supports are low except for the groupings of closely related taxa (Tetraodontidae, Primates, and Cichlidae).

### 3.5. Properties and structure of the IRBP-coding gene

Fig. 1 summarizes the previously published results (Rajendran et al., 1996; Nickerson et al., 2006), the results of the alignment and of the modules analyses. Module 1 of teleost IRBP gene 2 is



Fig. 4. Comparison of the mean pairwise differences for three pairs of taxa for the five modules of the IRBP coding gene and for 11 other nuclear markers used for (MLL, rhodopsin retrogene, RAG1) or proposed (Li et al., 2007) for use in acanthomorph phylogeny. The values were calculated with PAUP<sup>\*</sup>.

closest to the clade which groups module 1 teleost IRBP gene 1 and module 1 of other vertebrate IRBP gene, while module 2 of the teleost IRBP gene 2 is closest to module 4 of other vertebrate IRBP gene. This corroborates the findings of Nickerson et al. (2006), and supports the hypothesis of a single-ancestral IRBP-coding gene made of four modules.

It is easy to distinguish the sequences belonging to each module in the alignment, no matter how deep the divergence, as there are module-specific patterns and deletions shared by all included taxa. The clear cut difference between module sequences, and the clustering of all the sequences of each module in clades makes their use as phylogenetic markers very interesting. Indeed, it is not possible to confuse the modules with one another, and no gene even remotely similar to IRBP was found in all the genomes searched.

It is especially noteworthy that it was possible to amplify and sequence partial IRBP gene 1 for samples selected from most of the main clades of acanthomorphs, as highlighted on Fig. 5 (Chen et al., 2003; Miya et al., 2003, 2005; Dettaï and Lecointre, 2004, 2005; Smith and Wheeler, 2006). This gene 1 might also be useful as a phylogenetic marker in the acanthomorph groups where it is present. But for larger scale studies, it would be necessary to further investigate the exact distribution among teleosts of the complete IRBP gene 1. In the absence of further data, it is not possible to say if unsuccessful amplification in a group is due to technical problems (non-adapted primers) or to the absence of the gene 1. It is therefore better to use, at least provisionally, the first module of gene 2 for phylogenetic purposes, as this module is present in all the groups tested in this study.

Comparison of mean pairwise differences among pairs of sequences within modules (Fig. 4) showed some variability among the modules. The differences were the smallest within module 1 and 2 of gene 2, and greatest within module 2 of gene 1 for most pairs of taxa. This is of interest if the gene is to be used for phylogenetic reconstruction, as some modules might be more interesting for reconstructing deeper divergences (module 1 of gene 2 for instance), while others might present enough variability for more recent events.

### 3.6. Properties of the gene 2 dataset for teleosts

The Acanthomorpha dataset for the first module of gene 2 is composed of 96 terminals, 713 positions, 471 positions informative for parsimony. Partial results for the model search performed with Modeltest 3.7 and *a posteriori* values of the model parameters estimated during the BA are given in Table 3. The models and parameters recommended by Modeltest are different for each codon position in this part of the gene. The BA also gave different end-values for the parameters and nucleotide frequencies for the three codon positions. This might explain the better recovery of previously defined clades by the BA than by the ML analysis. MrBayes 3.1 allows definition of different codon partitions with different models and parameters, but PhyML does not. In this case, the different properties of the three codon partitions call for a different treatment of each of these partitions, and therefore MrBayes 3.1 is probably better suited to the analysis.

The IRBP dataset only shows a beginning of saturation for the 3rd codon position transitions (see Fig. 2). A  $\chi^2$  heterogeneity test performed with PAUP on the nucleotidic composition heterogeneity shows a highly significant heterogeneity for 3rd codon positions, but not for 1st and 2nd. The GC content varies from 47% (for *Apleto-don dentatus*) to 89.7% for *Gaidropsarus*, with an average of 65%.

But more significantly, the inferred trees, even from sequences as short as 713 bp, show a very high congruence with groups published on other markers on this group (Chen et al., 2003, 2007; Miya et al., 2003, 2005; Dettaï and Lecointre, 2004, 2005; Smith and Wheeler, 2006; Smith and Craig, 2007). Table 4 compares the clades present in three previous studies (Dettaï and Lecointre, 2005; Miya et al., 2005; Smith and Wheeler, 2006) using combined analyses of multiple genes and the clades present in the trees inferred from the first module of gene 2 of IRBP with BA and ML.

Table 4

Clades within the Acanthomorpha recovered by previous multigene combined analyses (Dettaï and Lecointre, 2005; Miya et al., 2005; Smith and Wheeler, 2006) and with the partial 1st module of the IRBP coding gene 2

|  | Dettaï and Lecointre<br>(2005) Partial MLL,<br>rhodopsin, 12S, 16S<br>and 28S rDNA |        | Complete mitochondrion<br>Miya et al. (2005) | Present study |          |
|--|--|--------|--|---------------|----------|
|  | Presence   | Name   |  | IRBP BA       | IRBP ML  |
| Zeioidei, Gadiformes   | Yes  | А      | Yes  | Yes           | Yes      |
| Mugiloidei, Atherinomorpha   | Yes - 1  | С      | No   | ?             | Yes      |
| Blennioidei, Gobiesocoidei   | Yes  | D      | Yes  | Yes + 1       | No       |
| Cichlidae, Mugiloidei, Atherinomorpha, Blennioidei,Gobiesocoidei   | Yes  | Q      | Yes*   | Yes + 1       | No       |
| Syngnathoidei, Callionymoidei, Mullidae  | Yes  | E'     | •  | ?             | No       |
| Aulostomidae, Macrorhamphosidae, Dactylopteridae   | Yes  | E      | •  | ?             | No       |
| Stromateoidei, Scombridae, Chiasmodontidae   | Yes  | Н      | •  | Yes           | Yes – 1  |
| Syngnathoidei, Callionymoidei, Mullidae, Stromateoidei, Scombridae, Chiasmodontidae  | Yes  | E+E'+H | *  | ?             | Yes - E' |
| Channidae, Anabantoidei  | Yes  | f1     | •  | Yes           | Yes      |
| Channidae, Anabantoidei, Symbranchiformes  | Yes  | F      | Yes*   | Yes           | Yes      |
| Uranoscopidae, Ammodytidae, Cheimarrichthyidae   | Yes  | G      | •  | Yes           | No       |
| Cottoidei, Zoarcoidei  | ?  | I      | Yes + 1                                      | Yes           | Yes      |
| Cottoidei, Zoarcoidei, Gasterosteiformes, Triglidae  | Yes  | Isc    | Yes + 1                                      | Yes +1        | No       |
| Notothenioidei, Percidae   | Yes  | К      | *  | No            | No       |
| Pleuronectiformes, Centropomidae, Carangidae, Menidae, Sphyraenidae, Polynemidae,<br>Echeneidae                                  | Yes  | L      | Yes*   | Yes           | Yes – 1  |
| Tetraodontiformes, Lophiiformes, Caproidei, Elassomatidae, Acanthuridae, Siganidae,<br>Pomacanthidae, Drepanidae, Chaetodontidae | Yes  | Ν      | Yes <sup>*</sup>                             | ?             | No       |
| Labridae. Scaridae   | Yes  | М      | *  | Yes           | Yes      |
| Polymixiiformes, Zeioidei, Gadiformes  | Yes  | 0      | Yes + 2                                      | Yes           | Yes      |
| Notothenioidei, Percidae, Triglidae, Trachinidae, Scorpaenidae, Serranidae   | ?  | Х      | Yes  | $Yes^{*} - 2$ | Yes + 3  |
| Zeioidei, Gadiformes, Percopsiformes, Lampridiformes, Polymixiiformes  | Yes - 1  |        | No   | Yes           | No       |

Presence of a clade is indicated by yes, irresolution in the tree by ?, incomplete answer due to incomplete taxonomic sampling by a . Clades present, but with intruder taxon or missing taxon are indicated by Yes + 1 or Yes – 1. Clades M and Q are also recovered with the mitogenome in the more complete sampling of Mabuchi et al. (2007).

The BA analysis yields a tree that is considerably more congruent with other studies than the ML analysis. Out of 20 scored nodes, 10 are perfectly recovered (vs. 6 for ML), four are imperfectly recovered (vs. 4), and only one is contradicted (vs. 9). With BA, some groups that have proven hard to recover using other molecular datasets, like Tetraodontiformes, for instance, are recovered with high posterior probability. But overall, the posterior probabilities of larger recovered clades are low (Fig. 5). The analyses of IRBP recover several clades that had been first proposed based on morphological characters like the Atherinomorpha (Parenti, 1993) and their relationship with mugilids (Stiassny, 1993), or the relationship of Tetraodontiformes with siganids, acanthurids and chaetodontids (Winterbottom, 1974), although other groups also insert in this clade (Caproidei, Lophiiformes). Other, less consensual morphologically defined groups are not recovered here, as they were also not in previous molecular studies (Miya et al., 2003, 2005; Dettaï and Lecointre, 2004, 2005; Smith and Wheeler, 2006; Smith and Craig, 2007; Mabuchi et al., 2007): Scorpaeniformes, Smegmamorpha, Perciformes or



**Fig. 5.** Bayesian analysis majority rule consensus of the analysis of the partial module 1 of the second teleost IRBP coding gene for a wide sampling of Acanthomorpha. Values indicated next to the nodes are posterior probabilities. Nodes with no indication have a posterior probability of 1. Letters refer to the clades recovered by other studies (see Table 4). + Indicates groups where an almost complete first gene could be identified, O points species where the first gene could not be found in the complete genome available through Ensembl nor in the WGS sequences.

Paracanthopterygii, all already criticized in morphological studies (Imamura and Shinohara, 1998; Rosen, 1985). Labroids sensu Stiassny and Jensen (1987), an another doubtful group because of a possible high level of homoplasy in its proposed synapomorphies, is also polyphyletic as already proposed by Dettaï and Lecointre (2005) and Mabuchi et al. (2007). The cichlids group with the Atherinomorpha in clade Q, while the labrids and scarids form their own distinct clade. Finally, the sometimes surprising new clades proposed solely based on molecular studies (Chen et al., 2003, 2007; Miya et al., 2003, 2005; Dettaï and Lecointre, 2004, 2005; Smith and Wheeler, 2006) are recovered here (see Table 4).

When the divergence between chosen pairs of taxa is compared with other nuclear markers used in teleosts, the first module of the IRBP gene 2 (Fig. 4) has one of the highest mean of pairwise differences, well above the Rag1 and rhodopsin sequences, or most markers proposed by Li et al. (2007). It scores slightly below MLL, another marker (Dettaï and Lecointre, 2005) very efficient at recovering clades found by analyses of much larger datasets (Dettaï and Lecointre, 2005; Miya et al., 2003, 2005; Smith and Wheeler, 2004, 2006). In both IRBP and MLL, the differences are spread evenly along the sequences, a desirable property for a phylogenetic marker (Collins et al., 2005). Only one-third of all the codon positions of the IRBP module 1 of gene 2 dataset is conserved across our whole taxonomic sampling.

The exons of the IRBP coding gene are long, so it is not necessary to include an intron to have a sequence long enough for phylogenetic purposes. This limits the risk of amplification problems on nuclear DNA due to unexpected intron size variation in some species. Moreover, the gene does not belong to a multigenic family. The modules are also clearly distinct from one another in sequence, so specific primers for one or the other module can be designed. In the worst case scenario, where amplification of an unwanted module still occur, it can be detected at the alignment stage or by using a phylogenetic tree of the modules, as all sequences of a module cluster together in analyses.

## 4. Conclusions

By showing the presence of a single four-module IRBP gene in the genome of the lamprey *Petromyzon marinus*, our study has corroborated that the two-gene condition observed in Teleostei is derived compared to the one-gene condition, and increased considerably the known age of the apparition of the gene. The good support of each of the branches of the modules hints at an even older age, and the basal branches of the module clades show a noticeable divergence.

IRBP could not be detected in the genomes of available *Ciona* species nor in other available non-vertebrates. It would be of the highest interest to test for its presence in hagfishes.

The exploration of the absence or presence of the first three modules of IRBP in Teleostei must be extended, and assessed using other methods like high stringency Northern Blot for instance. If this first gene was really lost in some groups, this has happened repeatedly in the teleost tree. Yet both gene 1 and gene 2 are transcriptionally active in zebrafish adult eyes as well as in whole larval bodies, with different timing and pattern of the expression (Nickerson et al., 2006) hinting at possible subfunctionalization. Corroborating the absence of this module in some species might lead to new insights about the possible subfunctionalization of the two genes in teleosts. The timing of the acquisition of the derived structure of the genes in teleosts also needs to be investigated by studying the sequence of the IRBP coding gene or genes in basal Actinopterygians.

The current problems with the phylogeny of acanthomorph fishes will probably require new nuclear markers to supplement those most often used at present: almost complete mitogenomes, RAG1 and RAG2, Tmo4c4, 28S rDNA, rhodopsin retrogene, MLL4, and, very recently, the markers proposed by Li et al. (2007). While no single gene is sufficient for phylogenetic reconstruction, the partial IRPB sequences provide an interesting marker, less saturated than the mitogenome but more variable than most markers used for teleosts, and nonetheless recovering efficiently the clades proposed by other studies. The modules are different enough from one another to avoid confusion. The interrelationship tree of the modules shows that each of the IRBP modules is an interesting candidate for use with a wider sampling in the very diverse Acanthomorpha without risk of confusion between modules and genes. Even short sequences (713 bp) give a satisfactory result, comparable to those derived from multiple datasets combination. We therefore recommend this marker for large-scale phylogenies with wide taxonomic sampling in Acanthomorpha and Teleostei.

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### References

- Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.
- Benning, M.M., Taylor, K.L., Liu, R.-Q., Yang, G., Xiang, H., Wesenberg, G., Dunaway-Mariano, D., Holden, H.M., 1996. Structure of 4-chlorobenzoyl coenzyme A dehalogenase determined to 1.8 Å resolution: an enzyme catalyst generated via adaptive mutation. Biochemistry 35, 8103–8109.
- Chen, W.J., Bonillo, C., Lecointre, G., 2003. Repeatability as a criterion of reliability of new clades in the acanthomorph (Teleostei) radiation. Mol. Phylogenet. Evol. 26 (2), 262–288.
- Chen, W.-J., Ortí, G., Meyer, A., 2004. Novel evolutionary relationships among four fish model systems. Trends Genet. 20 (9), 424–431.
- Chen, W.-J., Ruiz-Carus, R., Ortí, G., 2007. Relationships among four genera of mojarras (Teleostei: Perciformes: Gerreidae) from the western Atlantic and their tentative placement among percomorph fishes. J. Fish Biol. 70 (sB), 202– 218.
- Collins, T.M., Fedrigo, O., Naylor, G.J., 2005. Choosing the best genes for the job: the case for stationary genes in genome-scale phylogenetics. Syst. Biol. 54 (3), 493–500.
- Dettaï, A., Lecointre, G., 2004. In search of the Notothenioid (Teleostei) relatives. Antarctic Sci. 16, 71–85.
- Dettaï, A., Lecointre, G., 2005. New clades in the acanthomorph bush inferred by the study of taxonomic congruence. C.R. Biol. 328 (7), 674–689.
- Engel, C.K., Mathieu, M., Zeelen, J.P., Hiltunen, J.K., Wierenga, R.K., 1996. Crystal structure of enoyl-coenzyme A (CoA) hydratase at 2.5 angstroms resolution: a spiral fold defines the CoA-binding pocket. EMBO J. 15, 5135–5145.
- Froese, R., Pauly, D., 2006. FishBase. World Wide Web electronic publication (accessed in 2006).
- Greenwood, P.H., Rosen, D.E., Weitzman, S.H., Mayers, G.S., 1966. Phyletic studies of teleostean fishes, with a provisional classification of living forms. Bull. Am. Mus. Nat. Hist. 131, 339–455.
- Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52 (5), 696–704.
- Guindon, S., Lethiec, F., Duroux, P., Gascuel, O., 2005. PHYML online—a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Res. 33 (Web Server issue), W557–W559.
- Hall, T.A., 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Res. Symp. 41, 95–98.
- Hassanin, A., Lecointre, G., Tillier, S., 1998. The 'evolutionary signal' of homoplasy in protein-coding gene sequences and its consequences for a priori weighting in phylogeny. C.R. Biol. 321 (7), 611–620.

- Holcroft, N.I., 2004. A molecular test of alternative hypotheses of tetraodontiform (Acanthomorpha: Tetraodontiformes) sister group relationships using data from the RAG1 gene. Mol. Phylogenet. Evol. 32, 749–760.
- Hubbard, T., Andrews, D., Caccamo, M., Cameron, G., Chen, Y., Clamp, M., Clarke, L., Coates, G., Cox, T., Cunningham, F., Curwen, V., Cutts, T., Down, T., Durbin, R., Fernandez-Suarez, X.M., Gilbert, J., Hammond, M., Herrero, J., Hotz, H., Howe, K., Iyer, V., Jekosch, K., Kahari, A., Kasprzyk, A., Keefe, D., Keenan, S., Kokocinsci, F., London, D., Longden, I., McVicker, G., Melsopp, C., Meidl, P., Potter, D., Proctor, G., Rae, M., Rios, D., Schuster, M., Searle, S., Severin, J., Slater, G., Smedley, D., Smith, J., Spooner, W., Stabenau, A., Stalker, J., Storey, R., Trevanion, S., Ureta-Vidal, A., Vogel, J., White, S., Woodwark, C., Birney, E., 2005. Ensembl 2005. Nucleic Acids Res. 33 (Database issue), D447–D453.
- Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.
- Hurley, H.a., Lockridge Mueller, R., Dunn, K.A., Schmidt, E.J., Friedman, M., Ho, R.K., Prince, V.E., Yang, Z., Thomas, M.G., Coates, M.I., 2006. A new time-scale for rayfinned fish evolution. Proc. R. Soc. B. 274, 489–498.
- Imamura, H., Shinohara, G., 1998. Scorpaeniform fish phylogeny: an overview. Bull. Nat. Sci. Mus., Tokyo, Ser. A 24, 185–212.
- Inoue, J.G., Miya, M., Tsukamoto, K., Nishida, M., 2003. Basal actinopterygian relationships: a mitogenomic perspective on the phylogeny of the "ancient fish". Mol. Phylogenet. Evol. 26 (1), 110–120.
- Johnson, G.D., Patterson, C., 1993. Percomorph phylogeny: a survey of acanthomorphs and a new proposal. Bull. Mar. Sci. 52, 554-626.
- Jondeung, A., Sangthong, P., Zardoya, R., 2007. The complete mitochondrial DNA sequence of the Mekong giant catfish (*Pangasianodon gigas*), and the phylogenetic relationships among Siluriformes. Gene 387 (1–2), 49–57.
- Kikugawa, K., Katoh, K., Kuraku, S., Sakurai, H., Ishida, O., Iwabe, N., Miyata, T., 2004. Basal jawed vertebrate phylogeny inferred from multiple nuclear DNA-coded genes. BMC Biol. 2, 3.
- Lavoue, S., Miya, M., Inoue, J.G., Saitoh, K., Ishiguro, N.B., Nishida, M., 2005. Molecular systematics of the gonorynchiform fishes (Teleostei) based on whole mitogenome sequences: implications for higher-level relationships within the Otocephala. Mol. Phylogenet. Evol. 37 (1), 165–177.
- 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. 20, 7–44.
- Liao, D.I., Qian, J., Chisholm, D.A., Jordan, D.B., Diner, B.A., 2000. Crystal structures of the photosystem II D1 C-terminal processing protease. Nat. Struct. Biol. 7, 749– 753.
- Loew, A., Gonzalez-Fernandez, F., 2002. Crystal structure of the functional unit of interphotoreceptor retinoid binding protein. Structure 10, 43–49.
- Mabuchi, K., Miya, M., Azuma, Y., Nishida, M., 2007. Independent evolution of the specialized pharyngeal jaw apparatus in cichlid and labrid fishes. BMC Evol. Biol. 7, 10.
- Madsen, O., Scally, M., Douady, C.J., Kao, D.J., DeBry, R.W., Adkins, R., Amrine, H.M., Stanhope, M.J., deJong, W.W., Springer, M.S., 2001. Parallel adaptive radiations in two major clades of placental mammals. Nature 409 (6820), 610–614.
- Miya, M., Kawaguchi, A., Nishida, M., 2001. Mitogenomic exploration of higher teleostean phylogenies: a case study for moderate-scale evolutionary genomics with 38 newly determined complete mitochondrial DNA sequences. Mol. Biol. Evol. 18, 1993–2009.
- Miya, M., Takeshima, H., Endo, H., Ishiguro, N.B., Inoue, J.G., Mukai, T., Satoh, T.P., Yamaguchi, M., Kawaguchi, A., Mabuchi, K., Shirai, S.M., Nishida, M., 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. Mol. Phyl. Evol. 26, 121–138.
- Miya, M., Satoh, T., 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.
- Modis, Y., Filppula, S.A., Novikov, D.K., Norledge, B., Hiltunen, J.K., Wierenga, R.K., 1998. The crystal structure of dienoyl-CoA isomerase at 1.5 Å resolution reveals the importance of aspartate and glutamate sidechains for catalysis. Structure 6, 957–970.
- Nelson, J.S., 1994. Fishes of the World, third ed. John Wiley and Sons, Inc., Hoboken, New Jersey.
- Nelson, J.S., 2006. Fishes of the World, fourth ed. John Wiley and Sons, Inc., Hoboken, New Jersey.
- Nickerson, J.M., Frey, R.A., Ciavatta, V.T., Stenkamp, D.L., 2006. Interphotoreceptor retinoid-binding protein gene structure in tetrapods and teleost fish. Mol. Vision 12, 1565–1585.
- Orti, G., Meyer, A., 1997. The radiation of characiform fishes and the limits of resolution of mitochondrial ribosomal DNA sequences. Syst. Biol. 46, 75–100.

- Parenti, L.R., 1993. Relationships of atherinomorph fishes (Teleostei). Bull. Mar. Sci. 52, 170–196.
- Philippe, H., Douzery, E., 1994. The pitfalls of molecular phylogeny based on four species, as illustrated by the Cetacea/Artiodactyla relationships. J. Mamm. Evol. 2, 133–152.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14 (9), 817–818.
- Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. Syst. Biol. 53, 793–808.
- Rajendran, R.R., Van Niel, E.E., Stenkamp, D.L., Cunningham, L.L., Raymond, P.A., Gonzalez-Fernandez, F., 1996. Zebrafish interphotoreceptor retinoid-binding protein: differential circadian expression among cone subtypes. J. Exp. Biol. 199, 2775–2787.
- Rosen, D.E., 1973. Interrelationships of higher euteleostean fishes. In: Greenwood, P.H., Miles, S., Patterson, C. (Eds.), Interrelationships of Fishes. J. Linn. Soc. (London) 53 Suppl. 1. Academic Press, New York, pp. 397–513.
- Rosen, D.E., 1984. Zeiformes as primitive plectognath fishes. Am. Mus. Novit. 2782, 1-45.
- Rosen, D.E., 1985. An essay on euteleostean classification. Am. Mus. Novit. 2827, 1–57.Rozen, S., Skaletsky, H., 2000. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz, S., Misener, S. (Eds.), Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ, pp. 365–386.
- Silber, K.R., Keiler, K.C., Sauer, R.T., 1992. Tsp: a tail-specific protease that selectively degrades proteins with nonpolar C termini. Proc. Natl. Acad. Sci. USA 89, 295–299.
- 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 1, 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 (3), 206–217.
- Springer, M.S., DeBry, R.W., Douady, C., Amrine, H.M., Madsen, O., de Jong, W.W., Stanhope, M.J., 2001. Mitochondrial versus nuclear gene sequences in deeplevel mammalian phylogeny reconstruction. Mol. Biol. Evol. 18 (2), 132–143.
- Stanhope, M.J., Czelusniak, J., Si, J.S., Mickerson, J., Goodman, M., 1992. A molecular perspective on mammalian evolution from the gene encoding interphotoreceptor retinoid binding protein, with convincing evidence for bat monophyly. Mol. Phylogenet. Evol. 1, 148–160.
- Stiassny, M.L.J., 1986. The limits and relationships of the acanthomorph teleosts. J. Zool. (Lond.) (B) 1, 411-460.
- Stiassny, M.L.J., 1993. What are grey mullets? Bull. Mar. Sci. 52, 197–219.
- Stiassny, M.L.J., Jensen, J.S., 1987. Labroid interrelationships revisited: morphological complexity, key innovations, and the study of comparative diversity. Bull. Mus. Comp. Zool. 151, 261–319.
- Stiassny, M.L.J., Wiley, E.O., Johnson, G.D., de Carvalho, M.R., 2004. Gnathostome fishes. In: Donaghue, M.J., Cracraft, J. (Eds.), Assembling the Tree of Life. Oxford University Press, New York.
- Sun, Y., Ripps, H., 1992. Rhodopsin regeneration in the normal and in the detached/ replaced retina of the skate. Exp. Eye Res. 55 (5), 679–689.
- Swofford, D.L., 2002. PAUP<sup>\*</sup>. Phylogenetic Analysis Using Parsimony (\*and Other Methods), version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- Tatusova, T.A., Madden, T.L., 1999. Blast 2 sequences—a new tool for comparing protein and nucleotide sequences. FEMS Microbiol. Lett. 174, 247–250.
- Tyler, J.C., Santini, F., 2005. A phylogeny of the fossil and extant zeiform-like fishes, Upper Cretaceous to Recent, with comments on the putative zeomorph clade (Acanthomorpha). Zool. Scripta 34 (2), 157–175.
- Vidal, N., Hedges, S.B., 2005. The phylogeny of squamate reptiles (lizards, snakes, and amphisbaenians) inferred from nine nuclear protein-coding genes. C.R. Biol. 328 (10–11), 1000–1008.
- Wagenhorst, B.B., Rajendran, R.R., Van Niel, E.E., Hessler, R.B., Bukelman, A., Gonzalez-Fernandez, F., 1995. Goldfish cones secrete a two-repeat interphotoreceptor retinoid-binding protein. J. Mol. Evol. 41, 646–656.
- 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., Backeljau, T., Wachter, R.D., 1993. Extraction of high molecular weight DNA from molluscs. Trends Genet. 9, 407.
- Winterbottom, R., 1974. A descriptive synonymy of the striated muscles of the Teleostei. Proc. Acad. Nat. Sci. Philadelphia 125 (12), 225–317.