

**A phylogenetic assessment of flatfish (Order Pleuronectiformes)
intrarelationships based on molecular evidence**

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This work is dedicated to my son Hunter.

I have embodied many qualities while undertaking this project. Perseverance. Resilience. Following a path that you truly believe in. I hope that I am able to cultivate in you some of these values as we navigate your upbringing.

This thesis is also dedicated in loving memory of Uncle Georgie.

Always ready with canoe paddles, a campfire or a homemade fishing rod, and the general advice to go outside and play. Summers at Lac Labelle piqued my biological curiosity at an early age.

My life is much richer for having had the luxury of these experiences.

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Abstract

The fish order Pleuronectiformes (flatfishes) include over 800 fish species, and are generally considered to be monophyletic. Phylogenetic relationships within the order remain largely unresolved, particularly for the Paralichthyidae, a very poorly defined family. In addition to the lack of consensus on intrarelationships within this order, flatfish diversification patterns are still poorly understood. Here we want to test the hypothesis that (i) a densely sampled phylogenetic tree will help to unravel the phylogenetic relationships within flatfish, and (ii) that the formation of the Isthmus of Panama has played a role in the diversification of flatfish, following their distribution in both oceans, Atlantic and Pacific. Maximum likelihood and Bayesian analyzes were performed on a dataset consisting of nine mitochondrial and nuclear loci on a set of 309 flatfish species. The heterogeneity of substitution rates, known to cause phylogenetic errors in large multigene alignments, can be corrected by data partitioning, or by using heterogeneous site models. Very few studies have compared the effects of using these approaches. We present a robust phylogenetic tree with different heterogeneous and well-supported models, and the intraordinal relationships of flatfish are discussed in light of these results. We present evidence for three new suborders within the flatfishes. The monophyletic status of most families is corroborated. The family Paralichthyidae and the family Poecilopsettidae were found to be polyphyletic. Heterogeneous models have not been able to solve the deeper relationships between flatfish. We also show that flatfish diversification occurred in a few families in at least two stages, related to distinct geological events.

Résumé

Les poissons plats, ou Pleuronectiformes, sont un ordre de poissons qui contient plus de 800 espèces, généralement considérés comme monophylétique. Les relations phylogénétiques à l'intérieur de cet ordre restent non résolues, en particulier pour les Paralichthyidae, une famille mal définie. En plus de l'absence de consensus sur les relations au sein de cet ordre, les modèles de diversification des poissons plats sont toujours mal connus. Ici, nous voulons tester l'hypothèse (i) qu'un arbre phylogénétique densément échantillonné aidera à élucider les relations phylogénétiques au sein des poissons plats, et (ii) que la formation de l'Isthme de Panama a joué un rôle dans la diversification des poissons plats, suite à leur distribution dans les deux océans, Atlantique et Pacifique. Des analyses de maximum de vraisemblance et Bayésienne ont été effectuées sur un ensemble de données composé de neuf loci mitochondriaux et nucléaires sur un ensemble de 309 espèces de poissons plats. L'hétérogénéité des taux de substitution, connue pour causer des erreurs phylogénétiques dans les grands alignements multigénétiques, peut être corrigée par le partitionnement des données, ou en utilisant des modèles site hétérogènes. Très peu d'études ont comparé les effets de l'utilisation de ces approches. Nous présentons un arbre phylogénétique robuste à différents modèles hétérogènes et bien supporté, et les relations intraordinales des poissons plats sont discutées à la lumière de ces résultats, ou le statut monophylétique de la plupart des familles est confirmé. La famille Paralichthyidae et la famille Poecilopsettidae sont polyphylétiques. Les modèles hétérogènes n'ont pas été capables de résoudre les relations plus profondes entre les poissons plats. Nous montrons aussi que la diversification des poissons plats s'est produite dans quelques familles en au moins deux étapes, liées à des événements géologiques distincts.

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Chapter 1: General Introduction

The Pleuronectiformes, or flatfishes, are a speciose group of ray-finned fish, containing fourteen families and more than 800 known species (Munroe 2015). Flatfish begin life in the pelagic zone and undergo a larval metamorphosis in which one eye, either left or right, depending on the species, migrates to the other side of the cranium. The adult fish then adopts a mostly benthic lifestyle. Flatfish have asymmetrical, laterally-compressed bodies, and have lost their swim bladders during transformation (Chapleau and Amaoka 1998). With eyes facing upwards, flatfish are also capable of protruding them. Flatfish inhabit marine, freshwater, and brackish habitats (Chapleau and Amaoka 1998). There are many economically important species of flatfishes, including the soles, plaice and halibut (FAO 2012). Despite the attention flatfish have garnered due to their unique morphology and economic importance, little is known about their intrarelationships.

Chapleau (1993) provided the first comprehensive cladistic analysis of the order Pleuronectiformes and reconstructed a cladogram based on 39 morphological (mostly osteological) characters. Since Chapleau's (1993) study, there have been numerous studies that have argued for or against the monophyly of flatfishes based on molecular evidence (Berendzen et al. 2002; Azevedo et al. 2008; Betancur-R and Ortí 2014; Campbell et al. 2014). Chapleau (1993) addressed suprafamilial relationships within the order. No other studies since Hoshino (2001) have specifically attempted an in depth examination of the relationships within the entire order.

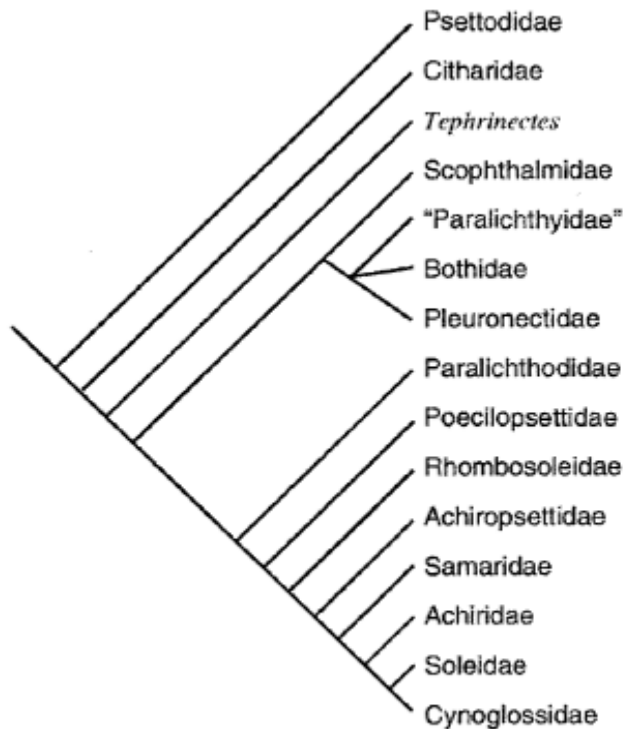


Figure 1. **Hypothesized relationships of the Pleuronectiformes based on a composite interpretation of morphological phylogenetic studies.** Placement of families follow Chapleau (1993), placement of *Tephriinectes* following Hoshino (2001), Paralichthodidae according to Chapleau and Cooper (1998) and placement of Achiropsettidae following Evseenko (2000). Figure taken from Munroe (2015) in Flatfishes: Biology and Exploitation.

Currently, there is support for the monophyly of most families except for the Paralichthyidae, both in molecular (Berendzen et al. 2002; Azevedo et al. 2008; Betancur-R and Ortí 2014) and morphological studies (Hensley and Ahlstrom 1984; Chapleau 1993). A composite cladogram (Fig. 1) of several studies (Munroe 2015), illustrates the most current hypotheses of flatfish intrarelationships. In this work, we will use the flatfish taxon names of Munro (2015), unless

otherwise noted. Taxonomic names used in this work follow those listed in Fishbase (www.fishbase.org).

With expanding technologies and sequences for multiple genes readily available to researchers, an in depth molecular investigation of relationships within this order of fishes is long overdue. Thus, we aim to provide a phylogenetic tree based on molecular evidence that may help guide future morphological works.

Most molecular works studying flatfish relationships have essentially focused on the monophyly of the order, and therefore only required a limited number of sequences from each family (Berendzen et al. 2002; Pardo et al. 2005; Azevedo et al. 2008; Betancur-R et al. 2013; Campbell et al. 2013). Taxonomists usually rely on morphological investigations to discern the intricacies of flatfish relationships at the rank of family or genus.

Previous works have indicated possible synapomorphies for suprafamilial groupings within the Pleuronectiformes, such as the ‘bothoid’ lineage. The “bothoid” lineage, proposed by Hensley and Ahlstrom (1984), consists of the Pleuronectidae, Paralichthyidae (excluding *Tephrinectes* and *Thysanopsetta*), Bothidae, Scophthalmidae, and one of the Citharidae (*Brachypleura*) and are suspected to be closely related based on a unique caudal fin arrangement. Hensley and Ahlstrom summarized previous groups and classifications of the flatfishes. Based mainly on the works of Regan (1910) and Norman (1934), they depicted flatfishes as having three suborders: Psettoidei (consisting of only *Psettodes*), Pleuronectoidei (comprised of Citharidae, Scophthalmidae, Bothidae, Paralichthyidae, Pleuronectidae, Poecilopsettidae, Samaridae and Rhombosoleidae) and Soleoidei (consisting of Achiridae, Soleidae and Cynoglossidae). Chapleau and Keast (1988) provided a cladistic analysis of the Soleidae (sensu

Norman 1934) based on osteological characters, and determined that the Soleidae were not monophyletic, but the subfamilies within the Soleidae are monophyletic. The Pleuronectoidei were found to be monophyletic if the suborder Soleoidei were included. They suggested raising the subfamilies of the Pleuronectoidei to the level of family. The Samaridae were tentatively suggested as the sister group to the Soleidae-Achiridae-Cynoglossidae clade, although this hypothesis has not yet been corroborated with molecular evidence.

At the level of intrarelationships, our priority is therefore to test the monophyletic status of the family Paralichthyidae. The Paralichthyidae, or large-toothed flounders, are an ill-defined group in need of taxonomic and phylogenetic assessment (Hensley and Ahlstrom 1984). The Paralichthyidae are a family of mostly exclusively left eyed flatfishes that are found in the Atlantic, Pacific and Indian Oceans. Many species are of commercial importance, such as the Pacific Sanddab (*Citharichthys sordidus*) (Fishbase: www.fishbase.org). Within the family Paralichthyidae, there are 14 genera and approximately 110 valid species (Catalog of Fishes: <https://www.calacademy.org/scientists/projects/catalog-of-fishes>) The family has been proposed to be somewhat of a taxonomic “garbage”, or catch-all group, with no defining synapomorphies (Hensley and Ahlstrom 1984, Chapleau 1993). The definition of monophyletic units within this family awaits a detailed phylogenetic study (Chapleau 1993; Hoshino 2001). We hypothesize that a densely sampled molecular tree may shed some light on monophyletic units within the larger family. Hensley and Ahlstrom stated that there exist two possible monophyletic groups within the Paralichthyidae. The “*Cyclopsetta*” group, consisting of the genera *Cyclopsetta*, *Syacium*, *Etropus* and *Citharichthys*, are suspected to have a close relationship with the Bothidae, based on shared morphological characters (absence of the first neural spine, and having transverse apophyses on the vertebrae). The “*Cyclopsetta*” group also possess some

morphological characters (position of the urinary papillae, ventral fin morphology) that are likely synapomorphic for the group (Hensley and Ahlstrom 1984). Amaoka (1969) and Hensley and Ahlstrom (1984) also recognized the possibility of another monophyletic entity, the “*Pseudorhombus*” group, consisting of the genera *Pseudorhombus*, *Cephalopsetta*, and *Tarphops*. However, no phylogenetic analysis has been performed to confirm these suggestions. We will assess these hypotheses with molecular evidence, to provide a first phylogenetic analysis of the family Paralichthyidae. Our study will provide insights in to paralichthyid relationships. Additionally, a phylogenetic tree with many sampled taxa across all flatfishes will also allow us to assess the status of other proposed clades within the Pleuronectiformes.

Building a robust phylogenetic tree

We build a robust phylogeny by alleviating several sources of phylogenetic error. As suggested by Xia et al. (2003), the reliability of results from molecular phylogenetic analysis of sequence data depends on how well the analysis deals with the following issues: the correct identification of homology, whether substitution rates vary greatly over sites, whether nucleotide frequencies vary over lineages (non-stationarity), whether long branch attraction has occurred (for a variety of reasons), and whether sequences have lost any phylogenetic information due to substitution saturation.. Heterogeneity exists within an alignment, both in rates of substitution and pattern of substitution. There are different ways to model heterogeneity of the substitution process found within an alignment. The most common methods are a partitioning approach (Nylander et al. 2004), or one of the various types of mixture models, such as the CAT models (Lartillot and Philippe 2004) that rely on mixtures of profiles, or models that incorporate mixtures of amino acid replacement matrixes (Le et al. 2008). Several molecular phylogenies of the flatfishes have been constructed with attempts to mitigate various sources of phylogenetic error, such as

saturation (Roje 2010) at the level of family, and non-stationarity of base composition (Betancur-R et al. 2013) at the level of order. To date, no molecular study of flatfish systematics has made an attempt to mitigate biases that may occur due to heterogeneity of substitution pattern within the data set. We also ask, what are the effects of accounting for heterogeneity within the data? Do the resulting phylogenies differ greatly from previous works? Additionally, we are now in a position to evaluate the performance of partitioning or mixture models using empirical data sets.

We ask what might be the best approach to take with large multigene sequence alignments. In addition to accounting for heterogeneity, appropriate and extensive taxon sampling is one of the most important determinants of accurate phylogenetic estimation (Heath et al. 2008). Careful taxon sampling and evenness is of critical importance, even when working with large multi-gene data sets, to accurately infer a correct topology and overcome systematic error (Parfrey et al. 2010). In fish systematics in particular, sparse taxonomic sampling can lead to conflicting phylogenetic results (Betancur-R and Ortí 2014). By mitigating the effects of multiple sources of phylogenetic error, and using a data set that samples broadly across all families in the order, we will build a robust and up to date phylogenetic tree of the order. With this new information, we will have solid insights into flatfish intrarelationships at the familial and suprafamilial levels, which will guide future taxonomic research.

First insights into flatfish biogeographic patterns

The emergence of the Isthmus of Panama was one of the most important biogeographic events in the Neotropics. The formation of the landmass between North and South America essentially cut off all genetic flow between the Atlantic and Pacific oceans. Rates of molecular evolution can be estimated using sister species that have been separated by the Isthmus of Panama (Lessios 1979, 1988; Knowlton and Weigt 1998). When aquatic connections between the Caribbean Sea and the

Pacific oceans closed approximately 3 to 12 million years ago (Coates et al. 1992; Haug and Tiedemann 1998; ODea et al. 2016), geographical isolation between both oceans was completed. Some molecular work (Knowlton and Weigt 1998; Bacon et al. 2015) estimates that some speciation events are thought to have occurred before the full closure of the land mass. Using the estimated dates for the formation of the Isthmus as a type of calibration in our Bayesian analysis, we can estimate times of separation (speciation events) for pairs of sister species. Estimating divergence times of flatfish speciation events that were influenced by the formation of the Isthmus could help provide information to aid in the development of biogeographical models for the New World Tropics. With divergence dates across a large number of taxonomic groups, hypotheses such as whether vicariance or dispersal plays a greater role in driving allopatric speciation can be tested (Waters 2008). It has also been suggested that allopatric speciation may not be the most important driver of marine biodiversity in the Neotropics, and ecological boundaries could play a greater role than geographic isolation in divergence of marine fauna (Bowen et al. 2013). Additionally, as there are very few phylogenetic studies on flatfishes from the area (Munroe 2015), the resulting species-rich tree would provide new frameworks of reference for taxonomic studies as new species are discovered and described.

The goals of this study are therefore:

- (1) To provide the first reassessment of intraordinal flatfish relationships since Hoshino (2001) by building the most taxon and gene rich molecular phylogenetic tree to date for flatfishes. We examine suprafamilial relationships within the order, verify the monophyletic status and phylogenetic placement of all flatfish families, and provide a first attempt at a phylogenetic analysis of the family Paralichthyidae.

- (2) To account for sources of phylogenetic error within the tree that can arise from heterogeneity within the data set. We investigate whether this heterogeneity is best modelled at the level of genes (using a partitioned data set) or at the level of sites (using mixture models).
- (3) To use the resulting phylogeny to answer questions about flatfish biogeography. Specifically, did the formation of the Isthmus of Panama play a role in flatfish speciation patterns?

Chapter 2: How the Central American Seaway and an ancient northern passage affected Flatfish diversification

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Contributions

LB was responsible for the concept of the study, gathered data, prepared the sequence alignments, performed the analyses, prepared the figures and drafted the manuscript. FC was responsible for the design of the study. SAB was responsible for the design of the study, wrote the custom R scripts, performed the analysis, edited the final figures and manuscript. All authors read and approved the final manuscript.

Abstract

The Pleuronectiformes, or flatfishes, are an order of fishes containing 14 families and over 800 species distributed worldwide, with some sister species present in the Atlantic and Pacific oceans. While the monophyletic status of all families but the Paralichthyidae has long been established, little is known about flatfish diversification both in terms of timing and rate. To address these outstanding questions, we assembled the largest molecular data set to date, comprising nine genes for > 300 taxa sampled over the entire Pleuronectiformes. With this, we conducted a series of analyses both under site-heterogeneous and relaxed molecular clock models to (i) assess the ability of these models to resolve the phylogenetic placement of Paralichthyidae, (ii) estimate the mode and tempo of the diversification of the flatfish, and (iii) test a vicariance hypothesis calibrating the divergence of sister taxa on the closure of the Isthmus of Panama, estimated to have taken place between 12-3 million years ago (MYA). Our phylogenetic results are consistent with previous molecular studies, based on smaller data sets, and suggest that Paralichthyidae are polyphyletic, forming two distinct groups that are more closely related to the Pleuronectidae and the Bothidae, respectively. We further show that tropical species diverged when the Isthmus of Panama closed, while Northern species diverged more recently. This suggests that the diversification of flatfish in this region occurred in two bouts, both of them linked to geological events.

Keywords: flatfish, systematics, phylogenetics, molecular, vicariance, Isthmus of Panama

Introduction

The flatfishes are generally considered to be monophyletic, based on both morphological (Chapleau 1993) and molecular evidence (Berendzen et al. 2002; Pardo et al. 2005; Azevedo et al. 2008; Betancur-R et al. 2013; Harrington et al. 2016). All these previous studies also support the monophyletic status of most of the families within the order, to the exception of the Paralichthyidae. Interestingly, all molecular studies to date have been concerned mainly with the monophyletic status of the order, but not with the intra-order relationships among the families and genera. For this reason, previous taxon sampling within the order was sparse. On the other hand, these intra-order relationships have been much debated in the morphological literature (Norman 1933; Hensley and Ahlstrom 1984; Chapleau 1993; Cooper and Chapleau 1998; Hoshino 2001). In particular, the monophyly of the Paralichthyidae, a large family of flatfishes, has always been questioned, as they seem to be an ill-defined group in need of taxonomic and phylogenetic assessment (Hensley and Ahlstrom 1984). Here we present a first attempt to clarify the monophyletic status of this family based on species- and gene-rich evidence, incorporating both paleontological and geological data in the framework of molecular clocks (dos Reis et al. 2015).

While few flatfish fossils are known, making the calibration of a molecular clock challenging, our dense species sampling enabled us to take advantage of a singular feature of flatfishes: extant species are found both in the Pacific and Atlantic oceans. Furthermore, the existence of geminate species pairs of flatfishes, where sister taxa have one member in each ocean, suggests a speciation event pre-dating the closure of the Isthmus of Panama, which occurred approximately 12 to 3 million years ago [MYA] (Haug and Tiedemann 1998; ODea et al. 2016b). Our first hypothesis is then that this information can be used to calibrate molecular

clocks. Relaxed molecular clocks potentially improve phylogenetic inference (Drummond et al. 2006), and will allow us to unravel the timing of flatfish evolution, as how rapidly they diversified remains an unsolved question.

Multigene relaxed molecular clock analyses are not new, and have been used, *e.g.*, to estimate the diversification of the major Eukaryotic lineages (Parfrey et al. 2011). However, concatenating a multigene sequence alignment may lead to some problems. Indeed, each position of a gene may be under its own selective constraint and evolve faster or slower than others. Likewise, some genes may evolve faster than others. At any time, only a few of the possible outcomes for each position (of a nucleic acid or amino acid alignment) are possible, as some substitutions are much more likely to have deleterious consequences. Partitioning a multigene sequence alignment is one way to account for this heterogeneity of the rates of evolution across sites (Nylander et al. 2004). With this, a sequence alignment is divided into sets, which are assumed to evolve under their own rate (Brandley et al. 2005). This partitioning can therefore be done according to codon positions, genes, or multiple genes that are then evolving under similar constraints. Several phylogenetic algorithms exist to determine the best partitioning scheme for an alignment, while simultaneously choosing the best-fit model of evolution. Several phylogenetic algorithms exist to determine the best partitioning scheme for an alignment, while simultaneously choosing the best-fit model of evolution. A hierarchical cluster approach (Li et al. 2008) uses a heuristic algorithm to explore different partitioning schemes based on Bayesian and maximum likelihood criteria. Partitions are usually defined based on the overall similarity among pre-determined data blocks, which can be protein-coding genes, codon positions, *etc.*. One pitfall of the hierarchical method is that decisions about how to determine these relative similarities need to be determined *a priori*. Partitionfinder (Lanfear 2012) works using a greedy

algorithm. The greedy algorithm joins one of the pre-defined data blocks with every other possible subset, and then selects the combination that most improves the AIC or BIC score. Alternatively, k-clustering (Frandsen et al. 2015) is a method that is related to hierarchical clustering, and selects partition schemes based on dividing the alignments into subsets that have similar rates of evolution. The k-clustering method estimates the number of subsets from the data, eliminating the need for determining subsets *a priori*. A downside to the partitioning approach is that one still has to define the genes *a priori*. Partitioning also ignores the heterogeneity that can exist within each partition (Whelan and Halanaych 2017). Among site rate variation has been modeled using a gamma distribution (Yang , 1994). While a gamma distribution can model variation in the rate of evolution along the alignment, nucleotides can have uneven equilibrium frequencies among sites. One heterogeneous model that does account for these differences is the CAT model (Lartillot and Philippe 2004), which can identify equilibrium profiles. Equilibrium profiles represent the overall rate of substitution (at any given nucleotide or amino acid). These profiles are then combined with a set of universal exchange rates, that is the probabilities that one nucleotide will be exchanged for another, which can be set at fixed values (in a CAT-F81 model all nucleotides have an equal exchange rate), or inferred directly from the data (in a CAT-GTR model). The CAT models can further incorporate a Dirichlet process prior to assign the site-specific profiles. A Dirichlet process is a type of random process that will assume that the different lineages in a phylogenetic tree will have different rate classes. The number of these classes, the rate of evolution of each class, and which branches on a tree belong to which class are all treated as random variables.

In the face of all these diverse approaches to modeling among-site rate heterogeneity in a multigene alignment, one question that arises is whether this heterogeneity should be modeled at

the gene level or at the level of individual sites. There have been a limited number of studies to date where the performance of these CAT models have been compared to other methods of accounting for heterogeneity in the substitution process, such as partitioning (*e.g.*, (Whelan and Halanych 2017). Here we investigate the impact of these two classes of approaches (genes vs. sites) on flatfish phylogeny using a large empirical data set.

Methods and Materials

Data retrieval and alignment

Prior to retrieving sequence data, GenBank was surveyed to identify all the genes belonging to species of Pleuronectiform families (as of August 2016), based on GenBank's taxonomy browser. DNA sequences for a total of 332 flatfish species (out of over 800 species in the order) were identified and downloaded from GenBank for five nuclear genes (KIAA1239, MYH6, RIPK4, RAG1, SH3PX3), and four mitochondrial genes (12S, 16S, COX1 and CYTB). These represented all the taxa having at least one sequence in GenBank; see Table S1 for the corresponding accession numbers. Diversity was richly sampled and distributed over 13 of the 14 families in the order Pleuronectiformes. The family Psettodidae was chosen as the outgroup to all other taxa (Chapleau 1993).

These sequences were aligned using MUSCLE ver. 3.8.31 (Edgar, 2004) on a gene-by-gene basis. Each alignment was visually inspected with AliView ver. 1.18 (Larsson 2014), and was manually edited where necessary. In particular, large indels (> 10 bp) were removed prior to all phylogenetic analyses. The 5' and 3' ends of sequences were also trimmed. The aligned sequences were then concatenated using a custom R script. Alignments and scripts are available from <https://github.com/sarisbro>.

Data pre-processing In order to gauge the phylogenetic content of our data set and identify sister taxa to later test our vicariance hypothesis, we performed a first series of molecular clock analyses on the concatenated data matrix, with all the nine gene sequences obtained above (12S, 16S, COI, Cyt-b, KIAA1239, MYH6, RIPK4, SH3PX3 and RAG1), and all the 332 taxa representing all families of flatfish. Partitions were first identified with PartitionFinder 2 (Lanfear 2012), selecting the best-fit model of nucleotide substitution based on AIC.

This optimal partition scheme was then employed in a first Bayesian phylogenetic analysis, conducted with BEAST ver. 1.8.3 (Drummond and Rambaut 2007), that implements a Markov chain Monte Carlo algorithm (MCMC) sampler, and which co-estimates both tree topology and divergence times. A GTR+I+ Γ model was applied to each data partition, as determined by PartitionFinder. Partitions were unlinked, while both clock model and tree model partitions were linked. An uncorrelated relaxed clock was assumed with a lognormal distribution prior on rates, and a Yule speciation prior (Drummond et al. 2006). Due to the paucity of flatfish fossils, a unique calibration point was placed on the most recent common ancestor (MRCA) of the ingroup; a mean-one exponential prior was defined, with an offset of 40 million years reflecting the age of 47.8-42.1 MYA (Chanet 1997). To stabilize the estimate, a lognormal $\ln(0, 1.5)$ prior with a 40 MYA was also placed on the root of the tree (root height). As the only useable fossil calibration is the first appearance of flatfish in the fossil record, we incorporated additional (outgroup) species to have both a calibration prior and a root height prior. The outgroup was defined as the genus *Psettodidae*, for which corresponding sequences were downloaded (Table S1). Two separate MCMC samplers were run, each for 10,000,000 generations. Trees were sampled every 5,000 generations, and convergence was checked visually using Tracer ver. 1.6. (Rambaut et al 2014). Tree log files from each run were combined in

LogCombiner, after conservatively removing 10% of each run as burn-in. The resulting maximum a posteriori (MAP) tree was generated with TreeAnnotator (Drummond and Rambaut 2007).

As the topology of this resulting MAP tree was unconventional, we suspected the presence of rogue taxa, *i.e.* species evolving either much faster or much slower than the majority, which can contribute negatively to consensus tree support (Aberer et al. 2013). Rogue taxa were identified using the RogueNaRok (Aberer et al. 2013) webserver (<http://rnr.h-its.org>). The consensus trees from the preliminary analysis using 10,000 iterations were used as the tree set for rogue taxon analysis. A total of 23 rogues were identified and pruned from the analysis, leaving 309 species.

Phylogenetic coherence with site heterogeneous models

Given the heterogeneous nature of the retrieved genes, two independent approaches were employed, one based on gene-by-gene heterogeneous model, and one based on a site-by-site heterogeneous model. First, to assess the effect of a gene-by-gene partitioning scheme on our phylogenetic reconstructions while accounting for within-site rate variation, maximum likelihood analyses were performed using IQTREE ver. 1.4.4 (Nguyen et al. 2015). The partitioning scheme was determined by IQTREE. A partitioned analysis was run where each partition had its own set of branch lengths.

Second, to assess the effect of partitioning on a gene-by-gene basis vs. on a site-by-site basis, Bayesian analyses were carried out with Phylobayes MPI ver. 1.7 (Lartillot et al. 2013). Because this approach has convergence issues with very large number of taxa, we performed the analyses on a reduced data set. For this, 100 taxa were removed from the families Achiridae, Soleidae and Cynoglossidae, as they were shown in our previous analysis to belong to a separate

clade than the Paralichthyidae, our group of interest. The analyses of the reduced data set were performed using three types of mixture models, the CAT model (also known as CAT Poisson or CAT-F81), CAT-GTR, and a CAT-GTR with a Dirichelet process (CAT-BP). The CAT model accounts for site-specific nucleotide preferences (Lartillot and Philippe 2004). The CAT and CAT-GTR runs used a pre-specified number of profiles (four), while the CAT model with a Dirichelet process infers the number of profiles from the data. Two identical MCMC samplers were run for each model. Convergence was assessed visually in Tracer.

Molecular Dating and vicariance testing

To further assess the phylogenetic reconstruction of flatfish, and test the vicariance hypothesis with respect to the closure of the Central American Seaway (forming the Isthmus of Panama), another set of partitioned relaxed molecular clock analyses were performed on the same partitioned concatenated matrix (without the rogue). The timing of the closure of the Central American Seaway is estimated to have occurred between 12 and 3 MYA (Duque-Caro 1990; Coates et al. 1992; Haug and Tiedemann 1998; ODea et al. 2016b), and we used this time window as a prior to inform the relaxed molecular clock-based phylogenetic reconstructions. The analyses were performed on the same concatenated data set, with the same single fossil calibration, but we also placed a lognormal prior $\ln(3,1.5)$, that has most of its mass on the 12-3 MYA window, on the MRCA of each pair of sister taxa. From the initial BEAST analyses, these were the following 14 pairs: *Hippoglossina oblonga* and *H. stomata*, *Poecilopsetta hawaiiensis* and *P. natalensis*, *Citharichthys arctifrons* and *C. platophrys*, *C. darwini* and *C. minutus*, *Pleuronectes platessa* and *P. quadrituberculatus*, *Limanda ferruginea* and *L. proboscidea*, *L. aspera* and *L. limanda*, *Hippoglossoides dubius* and *H. platessoides*, *Hippoglossus stenolepis* and *H. hippoglossus*, *Glyptocephalus stelleri* and *G. cynoglossus*, *Syacium maculiferum* and *S.*

micrurum, *Cyclopsetta chittendeni* and *C. querna*. Again, two independent MCMC samplers were run each for 100 million iterations, with samples taken every 5000 step.

Because these pairs of sister species show a contrasted geographic distribution, having either a southern (equatorial) or a northern range, two additional sets of analyses were performed. In a first set, priors were placed only sister taxa that had a geographic range in the southern hemisphere were calibrated, while in a second set priors were placed only on sister species with a northern range. Finally, a set of analyses was performed using no sister taxa calibrations at all. For each analysis, results from the two MCMC runs were combined using LogCombiner after removing a conservative burn-in of 50%. The final MAP tree was generated with TreeAnnotator.

In an attempt to rank these different models (priors on all sister taxa; only on southern taxa; only on southern taxa; no “Panama” priors), a modified Akaike’s Information Criterion (AICM) that accounts for uncertainty in the MCMC sampling (Raftery et al. 2007) was computed for each model (Baele et al. 2013). These computations were performed in Tracer for each of the four different calibration models, based on 100 replications.

Results and Discussion

Site-heterogeneous models still leave deep flatfish relationships unresolved

In a first attempt to reconstruct the phylogeny of the flatfish, a maximum likelihood tree from the full and partitioned data set was obtained (309 species; IQTREE: gene-by-gene), as well as Bayesian trees using three types of mixture models but from a reduced data set to help convergence (100 species; Phylobayes: site-by-site). All four approaches recovered the main known families, placed the Bothidae and the “Cyclopsetta” group as sister clades, and most

estimated that the Bothidae/Cyclopsetta, the Cynoglossidae and the Soleidae are the most divergent families. However, the deepest relationships among these families were either not consistent among themselves, not highly supported, or inconsistent with the literature (Hensley and Ahlstrom 1984; Chapleau 1993; Hoshino 2001; Berendzen et al. 2002; Betancur-R and Ortí 2014). In particular, the tree reconstructed with IQTREE did not recover the Citharidae as a clade (Figure 1A). The Bayesian analyses using the CAT models resulted in trees that were also not well resolved above the family level (Figure 1B-D). However, the CAT-F81 was the only model to have achieved convergence with Phylobayes, as the MCMC runs under each of the CAT-GTR and CAT-BP models displayed a maxdiff value of 1, even after over a month of analysis run time. Such a high value indicates that at least one of the chains was trapped in a local maximum and / or that mixing was poor. Phylogenetic trees inferred from these analyses are therefore problematic. In spite of this, some consistencies across the three site-profile models were found, such as placing Citharidae as the most basal family, splitting the Poecilopsettidae into two distinct clades, but also failing to resolve the Paralichthyidae, which, however, are considered to be polyphyletic (Hensley and Ahlstrom 1984; Chapleau 1993). It has been suggested that the Paralichthyidae could be interpreted as monophyletic with the removal of all species within this “Cyclopsetta” group clade (Hensley and Ahlstrom 1984). While our study was still missing sufficient sampling of all genera in the remaining Paralichthyids to determine monophyly, we do have stronger molecular support for the elevation of the “Cyclopsetta” clade to the rank of family. The CAT-F81 model did recover some relationships that do not make sense in light of what is known about flatfish systematics. In particular, the family Soleidae was split into two clades, with one of them being more related to the Rhombosoleidae, a grouping that has not been suggested in any molecular or morphological works to date. The CAT-F81

model was also unable to resolve relationships among morphologically specialized taxa, such as the Cynoglossidae, which placement has been consistent in previous molecular studies (Berendzen et al. 2002; Azevedo et al. 2008; Betancur-R and Ortí 2014).

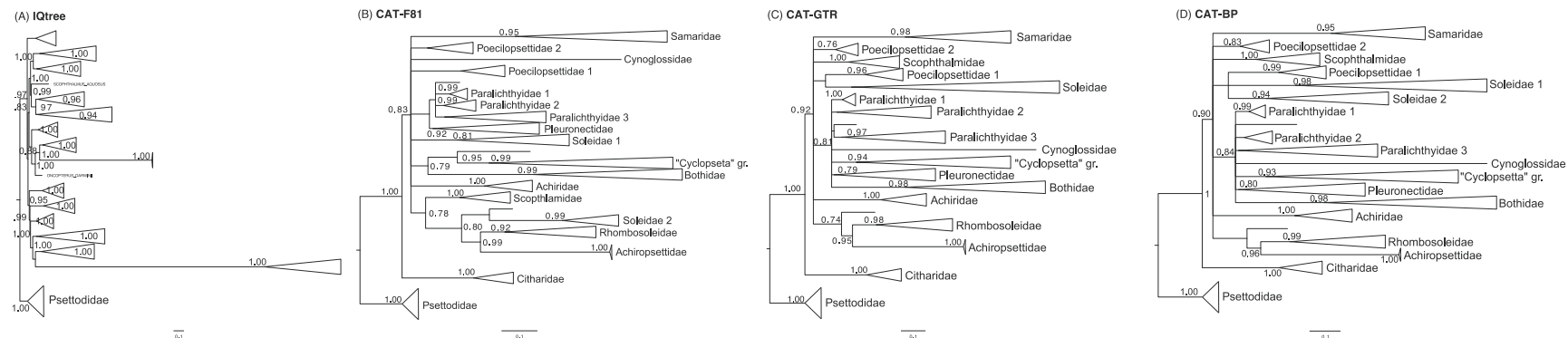


Figure 2: Phylogenetic trees reconstructed based on site-heterogeneous models. (A) Maximum likelihood tree estimated from the full alignment based on a gene-by-gene partitioning scheme (IQTREE). The other trees were estimated from a subset of the full alignment by a Bayesian approach based on site profiles (Phylobayes) under the CAT-F81 (B), CAT-GTR (C) or CAT-BP (D) model. Node values show bootstrap proportions (A) or posterior probabilities (B-D). Scale bars are in expected number of substitutions per site. Unlabelled branches represent potential additional rogue taxa not identified by RogueNaRok.

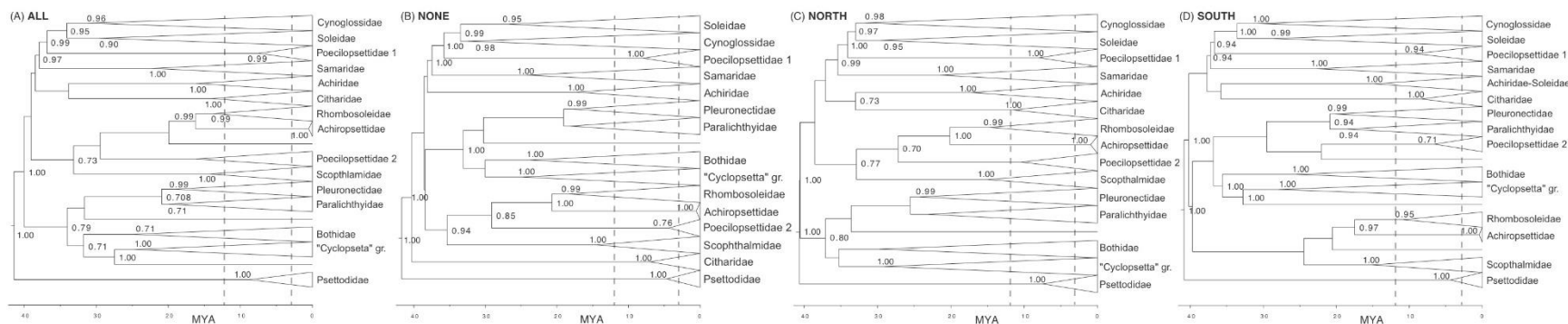


Figure 3: Phylogenetic trees reconstructed based on relaxed clock models. Four models were employed, representing different specifications of prior distributions set on sister taxa. (A) Priors were set on all pairs of taxa. (B) No priors were set on sister taxa. (C) Priors were set only on sister taxa with a current northern range. (D) Priors were set only on sister taxa with a current southern range. Horizontal scale is in million years ago (MYA). The closure of the Central American Seaway (12-3 MYA) is shown between vertical gray broken lines.

Clock models reveal the co-divergence of northern and of southern species

Given this lack of resolution among flatfish families, can the use of relaxed molecular clock shed light on the history of this group – especially in the context of a poor fossil record? To address this question, we assessed how the use of a vicariance hypothesis, based on the distribution of sister taxa on each side of the Isthmus of Panama, could help us obtain more robust date estimates, and also a more highly resolved phylogeny. Bayesian phylogenetic trees from the concatenated data matrix were therefore obtained under four relaxed molecular clock models, each one of them being based on a different calibration scheme (Figure 1). Under the first model, no priors were placed on internal nodes. The resulting tree (Figure 1A) was used to identify pairs of sister taxa that are split between the two oceans, with one species in the Atlantic and the other in the Pacific Ocean. This led us to single out 12 pairs of such species, on which we placed priors corresponding to the closure of the Central American Seaway (Figure 1B). The major difference between these trees is that when using all calibrations are used, the family Citharidae, usually considered to be a basal family considered as a transitional group between the Psettodidae and the rest of the flatfishes, is recovered as the sister group to the Achiridae. While examining the posterior distributions of divergence times for these pairs of sister taxa, it appeared that some species had a very narrow speciation window where all the mass of the posterior distribution was between 5-3 MYA, while others had a wider distribution (Figure 4A). Closer inspection of the results further revealed that most of the species with the narrow posterior distributions have a northern range (Figure 4A, in blue), while those with the wider posterior distributions occupy

a southern range, closer to the Isthmus of Panama (Figure 4A, in red). To further assess this observation, we first went back to the original clock model, with no priors on these sister taxa, and were able to validate that even in this case, northern and southern species showed, to one exception each (*Hippoglossus hippoglossus* and *H. stenolepis* in the north, and *Poecilopsetta natalensis* and *P. hawaiiensis* in the south), shifted posterior distributions. Models with priors placed only on northern (Figure 4C) or southern (Figure 4D) species also showed a similar temporal shift. This shift suggested that southern species diverged early, before the complete closing of the seaway, while northern species diverged later, at or possibly after the isthmus was completed. Averaging these posterior distributions for the northern and southern species, to the exception of the two outliers noted above, showed these results more clearly (Figure 4 E-H).

In an attempt to tease out these models and their predictions about the exact timing of divergence between northern and southern species, we assessed model fit by means of AICM. Even if model ranking based on this measure is known to be unstable (Baele et al. 2012), it is clear that the models with priors only on the northern or on the southern species perform significantly more poorly than the two other models, which may be difficult to tease apart. Their predictions suggest that *Hippoglossus hippoglossus* and *H. stenolepis*, both northern species, consistently diverged before the complete closure of the seaway, in tandem with the average southern species, and that the average northern species diverged in tandem with *Poecilopsetta natalensis* and *P. hawaiiensis* (Figure 4E-F).

Table 1: AICM values for the phylogenetic analyses using four different calibration schemes. SE: standard error.

Calibration Model	AICM value	SE
All	374,082.199	+/-1.579
None	374,110.488	+/-3.789
South	374,367.749	+/-0.696
North	374,746.628	+/-3.789

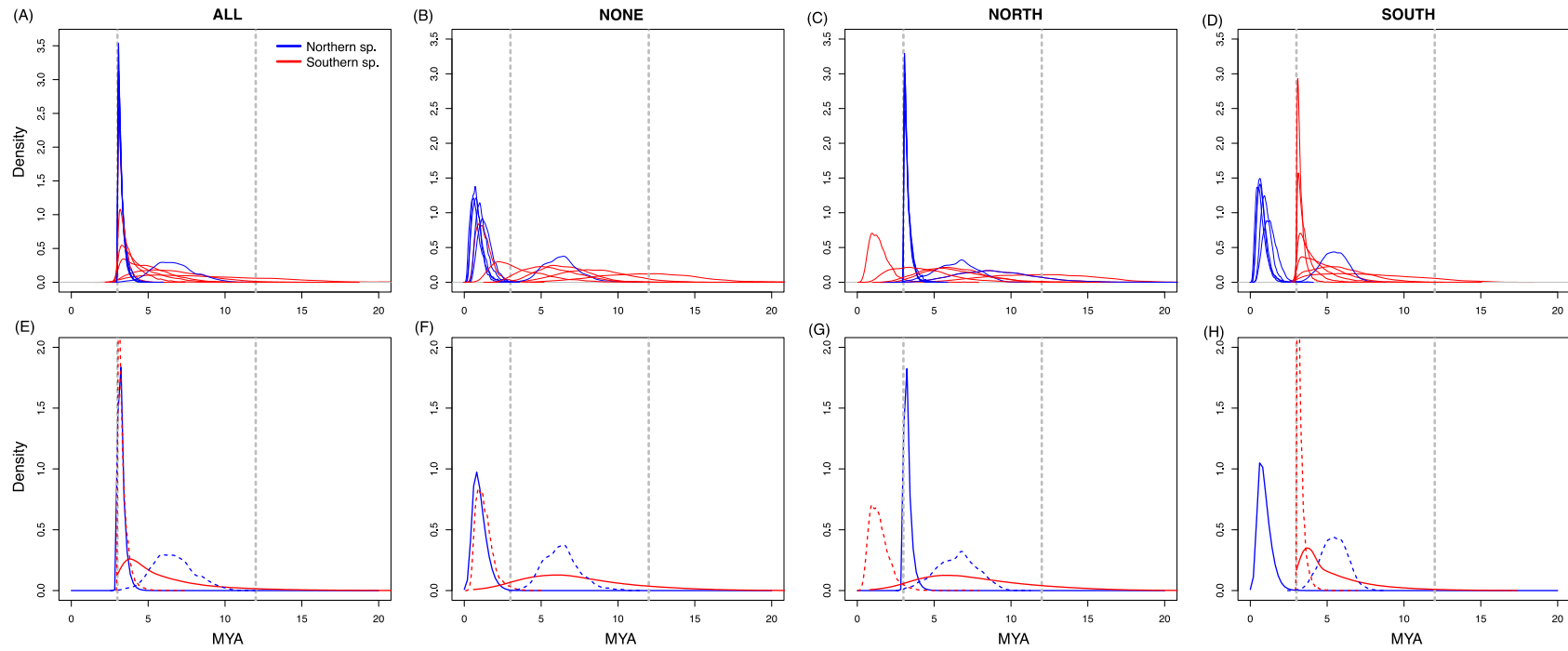


Figure 4: Posterior densities of divergence times for sister taxa used as calibration points in the relaxed molecular clock analyses, under four different calibration schemes. ALL: priors were placed on all pairs of sister taxa; NONE: no priors were placed on sister taxa; NORTH: priors were placed on only pairs of sister taxa with a northern distribution; SOUTH: priors were placed on only pairs of sister taxa with a southern distribution. Top row (A-D) shows all 17 distributions, while bottom row (E-H) shows range-averaged distributions (solid) to the exception of outlier pairs (dashed lines). Densities are color-coded for species with northern (blue) and southern (red) range. Dashed vertical gray lines indicate the closure of the Central American Seaway (21-3 MYA).

Our results demonstrate a first speciation bout across the Central American Seaway that led to the species that today, have an equatorial range, and that the formation of the Isthmus of Panama resulted in a barrier to gene flow leading to their speciation. Thus, species that currently have a northern range either emerged at the closure of the seaway, or after its closure. If the latter is correct, these results imply that this second bout of speciation was not caused by gene flow impeded by the closure of the seaway, but demand an interpretation involving a northern route, where gene flow was interrupted by a climatic event. The geological evidence is strikingly in line with our date estimates. The fossil record suggests that the first aquatic connection between the Pacific and Arctic (and Atlantic) oceans through the Bering Strait occurred approximately 5.5-5.4 MYA (Gladenkov et al. 2002) due to a rise in sea levels, linked to tectonic activity (Marincovich and Gladenkov 2001). This would have permitted the migration of populations ancestral to today's northern species from one ocean to the other through this ancient "northern passage." This global warming event, between the late Miocene to early Pleiocene, was then followed by a significant period of cooling during the Pleiocene into the Pleistocene (Zachos 2001), leading to periods of repeated glaciations and a subsequent ice age. These cold events would have resulted in the closure of this ancient "northern passage", hereby stopping gene flow between the two oceans, and leading to the recent speciation of the northern taxa.

For approximately a million years after the first opening of the Bering Strait, water flowed in a southern direction, until the formation of the Isthmus of Panama occurred close to the equator (Berta 2012). With the formation of the Isthmus, and the closing of the Central American Seaway,

the ocean currents reversed due to a change in global ocean circulation (Haug and Tiedemann 1998; De Schepper et al. 2015) and have since flowed from the Pacific to the Arctic (Marincovich, 2000). Dispersal or migration from the Pacific into the Atlantic in a northern direction through this strait is known as the trans-Arctic interchange (Vermeij 1991). Fossil data also show that the Bering land bridge has been exposed and submerged on multiple occasions since the Pleistocene (Gladenkov and Gladenkov 2004). These openings and closings of the Bering Strait could have provided a mechanism for divergence and the evolution of sister taxa (Taylor and Dodson 1994; Väinölä 2003).

Our vicariance hypothesis also has implication at the family level of flatfish. Further significant global cooling during the Pleistocene resulted in major glaciation events (Zachos et al. 2008) that could be responsible for creating barriers that isolated populations. All of the remaining sister taxa in our analysis, who have divergence estimates of less than 2 million years in our study belong to the family Pleuronectidae. The Pleuronectidae are the predominant flatfish family found in cold temperature seas of the northern hemisphere (Norman 1934; Cooper and Chapleau 1998) There are far more Pleuronectidae species in the Pacific Ocean, most of them endemic to the North Pacific Ocean off of north America and Asia in the region extending from the Bering straight to the gulf of California (Norman 1934). None of the arctic species are restricted solely to arctic waters (Munroe 2015). Munroe also noted that Cooper (in an unpublished manuscript) identified areas of endemism among the current distribution of the Pleuronectidae. It has been shown that during the trans-Arctic interchange, there was a far higher number of species (up to eight times higher) migrating to the Atlantic than to the Pacific (Vermeij 1991). Fossil and phylogenetic evidence suggest the Pacific

Ocean as the origin for diversification of the Pleuronectidae (Munroe 2015) and our phylogenetic results are highly congruent with this hypothesis.

It is possible that the outliers, the Atlantic and Pacific halibut, diverged during one of the first openings of the Bering Strait. The estimated dates from the molecular clock analysis are approximately 5.5 MYA, in accordance with the hypothesized dates for the first aquatic connection (Marincovich and Gladenkov 2001). The remaining sister taxa have a younger age estimate of ~1-2 MYA, corresponding with global cooling during the Pleistocene and repeated glaciations (Zachos 2001) which likely formed more barriers to genetic flow. In the second pair of outliers, *Poecilopsetta hawaiiensis* inhabits waters of the Eastern Central Pacific to the Hawaiian Islands, while *P. natalensis* inhabits coastal waters of Eastern Africa. Owing to the far reaching range of *P. natalensis*, and the relatively younger age estimates for divergence (1-2 MYA), these results beg for future research into other vicariant hypotheses, or dispersal routes, as they speciated long after the Isthmus had closed. Taken altogether, our results give first insights into flatfish speciation patterns, support hypothesized timelines of geological events, and confirm that the paleogeography of the Isthmus of Panama and of the Bering Strait are linked to cladogenesis in at least some marine organisms.

Conclusions

Based on the most extensive multigene sequence alignment available to date across all flatfish species, we have showed that we were not able to resolve the methodological debate about the relative merit and utility of gene-by-gene vs. site-by-site heterogeneous models. As a result, the actual history of this group is still unresolved. However, known families could be recovered, and that

the evolutionary dynamics of sister species that are distributed across the two oceans strongly supported the existence of two bouts of speciation: one triggered by the closure of the Central American Seaway 12-3 MYA, and a second one due to the closure of an ancient northern passage 5-0.01 MYA. The most intriguing implication of our results is that other marine organisms than the flatfish should have been affected by the two same geological processes, the closure of the Central American Seaway and of the ancient northern passage, so that future studies should be able to extend our findings to other living forms inhabiting these two oceans.

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Chapter 3: Flatfish Intrarelationships: New Insights using Dense Taxon Sampling

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Contributions

LB was responsible for the concept and design of the study, gathered data, prepared the sequence alignments, performed the analyses, prepared the figures and drafted the manuscript. FC was responsible for the concept of the study and edited the manuscript. All authors read and approved the final manuscript.

Keywords: Pleuronectiformes, flatfish, taxonomy, systematics, molecular

Abstract

The Pleuronectiformes, or flatfishes, are a large order of fishes (> 800 species), generally considered to be monophyletic. However, flatfish intrarelationships are much debated, and there remain some very poorly defined families, such as the Paralichthyidae. We used a dense taxonomic sampling approach to address these remaining issues in flatfish systematics. Bayesian relaxed clock analyses were performed to estimate the phylogeny of 310 species of flatfish based on sequences from five nuclear (KIAA1239, RIPK4, MYH6, RAG1, SH3PX3) and four mitochondrial (COX-1, CYT-B, 12S, 16S) genes. The results provide the most detailed molecular phylogeny of the flatfishes to date. The monophyletic status of most families is confirmed. We present novel evidence for three new suborders. We have provided a first phylogenetic analysis of the Paralichthyidae, a family that we confirm to be polyphyletic. We also find first evidence that the Poecilopsettidae may be polyphyletic. We cannot confirm the presence of previous taxonomic groupings such as the “bothoid” lineage of Hensley and Ahlstrom (1984). Four genera within the Paralichthyidae have been found to be a monophyletic group and we propose raising this group to the level of family.

Keywords: flatfish, phylogenetics, molecular, cladogram, systematics

Introduction

Cope (1871) determined that flatfishes formed an order, which he named Heterosomata. The name was replaced by Berg (1940) to Pleuronectiformes. Flatfish have greatly intrigued evolutionary biologists due to the apparently sudden appearance of a novel body plan. Even Darwin (1872) commented on their unique cranial asymmetry, which requires the migration of one eye from one side of the head over to the other during larval development. However, the evolution of this asymmetry remained unknown until recently, due to a lack of transitional forms. The discovery of the intermediate fossil forms *Amphistium* and *Heteronectes* indicated that the transition to this extreme cranial morphology was gradual in nature (Friedman 2008, 2012).

Sister-group of flatfishes

Previous morphological studies did not agree on the possible sister-group to the flatfishes. (Chapleau 1993, Hensley 1997, Norman 1934, Regan 1910). Regan (1910), Norman (1934) and Hubbs (1945) described characters that suggest a relationship between the most basal taxa of the Pleuronectiformes, *Psettodes*, and the Perciformes. However, Chapleau (1993) noted that there was a lack of traits common to all families of Perciformes, which makes it difficult to infer if a close relationship with *Psettodes*. In more recent molecular revisions (Betancur-R et al. 2017), the Perciformes were for the first time defined as a monophyletic unit, and the Pleuronectiformes have been recovered as a possible suborder, with either the Centropomidae (“snooks”) or the Canrangiformes (jacks, marlins and spearfishes) as the closest candidate for the sister group.

Intrarelationships of flatfishes: the bothoid controversy

Hensley and Ahlstrom (1984) provided a critical review of flatfish classification and phylogeny, based on earlier works of Regan (1910), Norman (1934) and Hubbs (1945). They re-examined the homology of morphological characters that were used to classify taxa within the order. While they did not conduct any phylogenetic analysis to substantiate their views, they employed new characters, and suggested a possible monophyletic lineage within the Pleuronectoidei consisting of the genera of the Pleuronectidae (*sensu* Norman 1934), Paralichthyidae (except *Tephrinectes* and *Thysanopsetta*), Scophthalmidae, Bothidae, and one genus from Citharidae (*Brachypleura*), which they termed the “bothoid” group. They based this hypothesis on the presence of a shared caudal fin complex pattern: hypural pattern 6: hypurals 1 and 2 fused, hypurals 3 and 4 fused to the terminal half centrum.

Chapleau (1993) presented the first cladogram of flatfish relationships, and also provided a review of flatfish studies since Hensley and Ahlstrom (1984). In Chapleau’s study, monophyly of flatfishes was based on three synapomorphies; cranial asymmetry resulting from ocular migration, an advanced position of the dorsal fin over the cranium, and the presence of a *recessus orbitalis*, a muscle used in the protruding process of the eyes. This analysis, based on an extensive data matrix of mostly osteological characters, did not provide unambiguous support for the monophyly of a bothoid group. Following this, Hoshino (2001) provided a new phylogenetic analysis based on morphological characters. He used Chapleau’s (1993) data matrix, and expanded it with 17 new characters. In this analysis, the monophyly of a ‘bothoid’ lineage could still not be confirmed, as *Brachypleura* (included by Hensley and Ahlstrom in this assemblage) was more closely related to other citharids. Excluding the genus *Brachypleura* from the analysis did, however, result in a

monophyletic bothoid clade. Betancur-R et al (2013) investigated evidence for the monophyly of flatfishes. Although their work did not address flatfish intrarelationships, their phylogenetic analyses showed a clade corresponding to the bothoids, minus *Brachypleura* (which was not included in their analysis). In the first phylogenetic analysis attempted for the order, Chapleau (1993) identified a number of other possible monophyletic lineages.

Flatfish intrarelationships: the Paralichthyidae

In 1934, Norman defined the Paralichthyinae based on pelvic fin morphology (ventral fins nearly symmetrical), and the lack of transverse apophyses on the vertebrae; Norman considered the group to be a subfamily of the Bothidae. Amaoka (1969) first proposed raising the Paralichthyinae to the level of family. Munroe (2003) listed the diagnostic characters uniting the group as follows: having no spines present in the fins, the mouth being protractile and asymmetrical, lower jaw being prominent, and having no teeth in the vomer, the preopercle is exposed (the margin is visible and not covered by scales or skin), both pelvic fins are present and have 6 rays, the dorsal and anal fins are not attached to the caudal fin, the caudal fin having 17-18 rays with 10-13 rays branched, and having a lateral line that is present on both sides of the body. Munroe also suggested that the Paralichthyidae are probably not monophyletic, as no synapomorphies have been formally described for the family.

Within the Paralichthyidae, Amaoka (1969) further indicated that *Pseudorhombus*, *Tarphops* and *Cephalopsetta* formed a recognizable group that was most likely monophyletic based on the presence of highly specialized structures (epural fused to the fifth hypural, caudal ray count of more than 17, no splinter ray on the most ventral caudal fin ray, and urogenital papillae on the ocular side (Munroe 2015). Hensley and Ahlstrom (1984) also confirmed the presence of this paralichthyid

group, which they named the “Pseudorhombus” group. They suspected that these genera form a monophyletic group as they all have a caudal fin-ray count of 17, the epural fused to the fifth hypural, and lack of a splinter ray on the ventral most caudal fin-ray. Hensley and Ahlstrom (1977) recognized another distinct group consisting of the genera *Cyclopsetta*, *Syacium*, *Etropus* and *Citharichthys*. It was hypothesized that this group is most likely monophyletic, based on a unique arrangement of caudal fin rays. Hensley and Ahlstrom (1977) also indicated that they also share other characters associated with the lateral line, ventral fins and urogenital papillae but these characters are symplesiomorphic for the group. Hensley and Ahlstrom also suggested a close relationship of this “*Cyclopsetta*” group with the bothids based on the observation that both groups have no neural spine on the first precaudal centrum of the vertebral column and have transverse vertebral apophyses.

Within the *Cyclopsetta* group, three of the four genera have undergone recent taxonomic revisions: *Etropus* (Leslie et al. 1986), *Syacium* (Murakami and Amaoka 1992) and *Cyclopsetta* (Khidir et al. 2004). No such taxonomic revision has been done for *Citharichthys*. The most recent reviews of the genus *Citharichthys* are from Parr (1931), and Norman (1934). Norman (1934) noted that although *Citharichthys* is a very morphologically heterogeneous genus, he was unable to find valid reasons for its subdivision. Some species of *Citharichthys* display sexual dimorphism. Several species differ greatly in their interorbital space, as well as having dimorphic features of the urogenital papilla (Munroe 2005), a trait unique to these flatfishes among the Pleuronectiformes. Ahlstrom (1984) divided the four genera into two groups, the clade *Citharichthys*-*Etropus*, and the clade *Cyclopsetta*-*Syacium*, based on larval characters. Khidir (2005) found the *Cyclopsetta*-

Syacium clade to be monophyletic, supported by six synapomorphies, Cyclopsetta being defined by ten synapomorphies and Syacium by five. This clade was also found to be monophyletic in molecular studies using a limited number of taxa (Berendzen et al. 2002; Betancur-R et al. 2013). The Citharichthys-Etropus clade has yet to undergo a phylogenetic assessment.

One species of Paralichthyidae, *Tephrinectes sinensis*, has a contentious position within the flatfishes. *Tephrinectes sinensis* is the single member of the genus *Tephrinectes*. Hoshino and Amaoka (1998) suggested that *Tephrinectes* was a sister group to the Poecilopsettidae-Rhombosoleidae-Samaridae-Achiridae-Soleidae-Cynoglossidae clade (known as Lineage IV) proposed by Chapleau (1993). In a later work, Hoshino (2001) hypothesized that *Tephrinectes* was actually a transitional clade between the Citharidae and the rest of the Pleuronectiformes. Hensley and Ahlstrom (1984) showed that *Tephrinectes* (along with *Thysanopsetta*) possessed the most primitive form of caudal fin arrangement of all the Paralichthyidae, and proposed that they definitely did not belong to their “bothoid” lineage.

The remaining Paralichthyidae (*Ancyclopsetta*, *Gastropsetta*, *Hippoglossina*, *Lioglossina*, *Paralichthys*, and *Xystreus*) were regrouped by Hensley and Ahlstrom (1984) under the term “Paralichthys” group. This group is most likely not monophyletic as its species do not share any synapomorphies. However, no phylogenetic analysis has been attempted for the family. Previous molecular investigations have been plagued by either poor taxon sampling, and / or limited number of genes used. By using the dense taxonomic sampling from multiple nuclear and mitochondrial genes assembled in the previous chapter, where we reconstructed a more detailed phylogenetic tree of the flatfishes, we here aim at using these results to revisit the intrarelationships within the group.

Phylogenetic reassessment: objectives

This present chapter reviews studies on flatfish phylogenetic studies since the reassessment of Chapleau (1993) and addresses the following issues: What is the monophyletic status of each family within the order and what do we know about their intrarelationshiPs? What is the status of the “bothoid” lineage of Hensley and Ahlstrom (1984)? What is the monophyletic status of the Paralichthyidae? We will provide the first formal phylogenetic analysis of the family Paralichthyidae. Are monophyletic subunits, such as the proposed “Cyclopsetta” group, recovered in the analysis, and what is their status? Finally, we will pinpoint some issues that should be addressed in future phylogenetic studies of flatfishes. .

Reinterpretation of flatfish intrarelationshiPs

The cladogram previously obtained (Figure 3A) contains three major lineages, summarized in Figure 5.

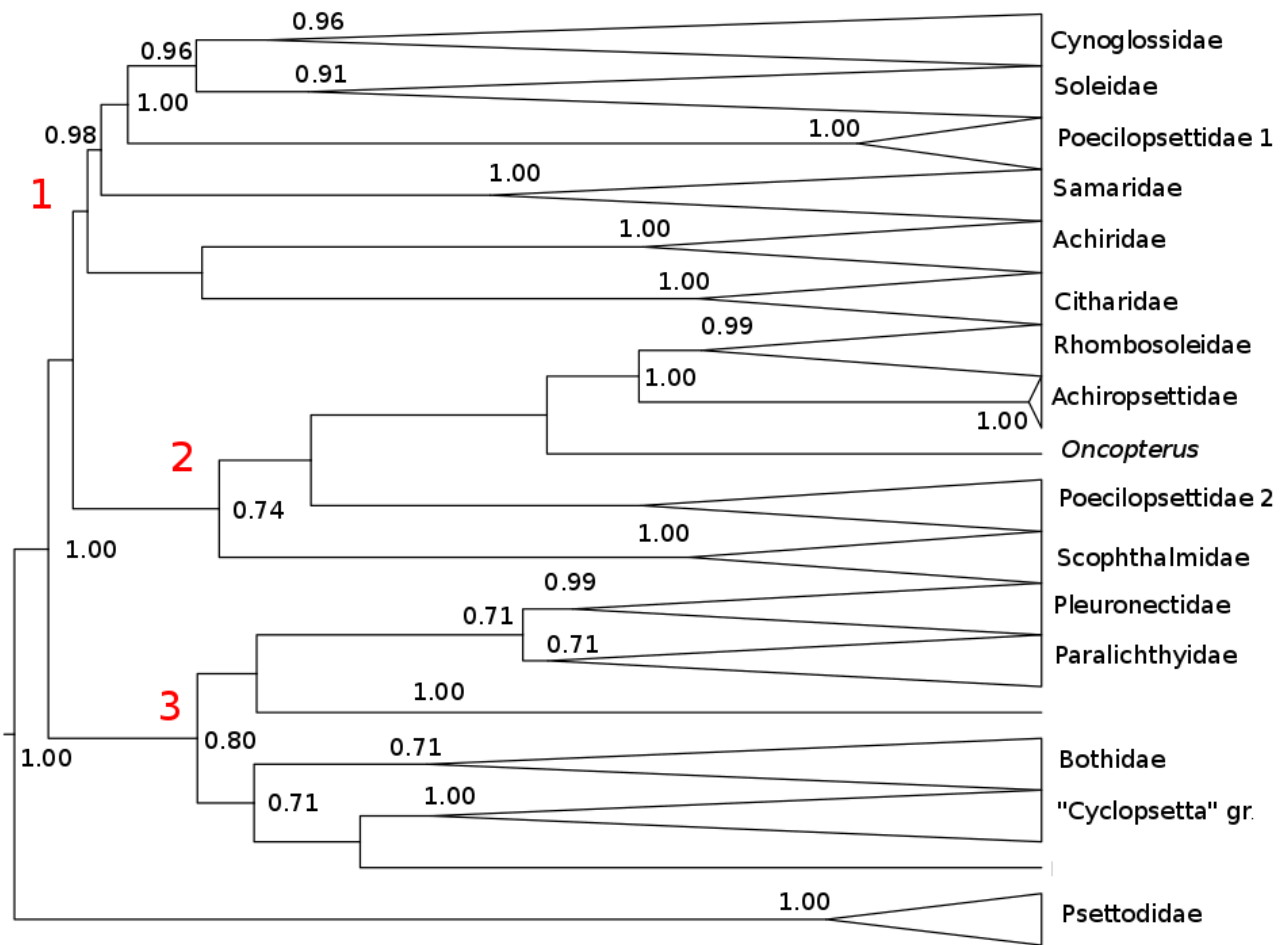


Figure 5: **Time calibrated Bayesian phylogenetic analysis of 9 mitochondrial and nuclear loci from 309 species of flatfish.** Priors were set on all sister taxa as detailed in Figure 3A. Posterior probabilities indicated on node labels. Three main lineages are labelled as 1, 2, 3.

Lineage 1

In this rooted tree, the most derived of the three clades, here named Lineage 1, contains the families Cynoglossidae, Soleidae, some of the Poecilopsettidae (*Poecilopsetta plinthus* and *P. beanii*), Samaridae, Achiridae and the Citharidae (PP=0.60).

The monophyly of Cynoglossidae is highly supported (posterior probability (PP)=0.99) by our results. This monophyly has been suggested by many authors (Hensley and Ahlstrom 1984; Chapleau 1993; Berendzen et al. 2002; Pardo et al. 2005; Azevedo et al. 2008; Betancur-R et al. 2013), Chapleau (1988) found the Cynoglossidae to be monophyletic, and also suggested the genus *Symphurus* was monophyletic and the sister group of a monophyletic Cynoglossus-Paraplagusia lineage. Our molecular study supports this hypothesis, but we cannot confirm the monophyly of the genus *Paraplagusia* suggested by Chapleau, as the species were observed to be dispersed within the cynoglossid clade.

Our results also suggest that the Soleidae are monophyletic (PP = 0.91). However, nodal supports are too low within the family to be able to discern any relationships among the genera.

We find a polyphyletic Poecilopsettidae, split into two groups, with one of them being more related to the Soleidae (PP=100). To date, molecular works have not included many species from Poecilopsettidae, so their placement within the flatfishes remains to be determined. A detailed revision of this family (Guibord 2003, unpublished thesis) could not find any morphological synapomorphies to unite the 19 species in the group, and they are assumed to be polyphyletic. Betancur-R et al (2017) validated the Poecilopsettidae as a monophyletic unit, however, their study included only two species, both from the same genus. In our work, the citharid species *Citharoides*

macrolepidotus consistently grouped with two species of *Poecilopsetta* (*Poecilopsetta beanii* and *Poecilopsetta plinthus*) in a clade that was observed as sister to the Soleidae-Cynoglossidae clade. This positioning of Poecilopsettidae was also observed in Betancur-R et al. (2013), but without the inclusion of *C. macrolepidotus*, as they did not include this species in their analysis. The second Poecilopsettidae clade includes other species of *Poecilopsetta* and *Marleyella*, and are positioned as the sister lineage to the Rhombosoleidae-Achiropsettidae clade. This placement however, has low support (PP=0.62). The remaining genus of Poecilopsettidae, *Nematops*, was not included in our analysis, as none were currently available in GenBank. We recommend that these groups be treated as Poecilopsettidae 1 and 2 until further studies including more species, especially from the genus *Nematops* are conducted.

We find that the Samaridae are monophyletic (PP=100). The monophyly of the family was proposed by Sakamoto (1984) and confirmed by Chapleau (1993), who also found the Samaridae to be the sister group to the Cynoglossidae-Soleidae-Achiridae clade. Excluding the uncertain placement of Achiridae in our analysis, we also find Samaridae as the family most closely related to the Soleidae-Cynoglossidae-Poecilopsettidae clade (PP=0.97).

We estimated a monophyletic Achiridae (PP=100), as the sister lineage to a Soleidae-Cynoglossidae-Samaridae clade (with part of a polyphyletic Poecilopsettidae). However, this positioning has very weak support (PP = .60). Monophyly of the Achiridae was proposed by Chapleau and Keast (1988) and corroborated again by Ramos (1998). Chapleau (1993) hypothesized that the Achiridae formed the sister-group of the clade Soleidae-Cynoglossidae; the Samaridae being the sister group of these three taxa. This configuration has not been observed in many molecular

studies (Berendzen et al. 2002; Pardo et al. 2005; Betancur-R et al. 2013; Harrington et al. 2016). Internal branches do not have high enough nodal support to discern relationships among the genera.

We did not find any support for the monophyly of the Citharidae in our analysis due to the placement of *C. macrolepidotus* with the Poecilopsettidae. We find the remaining Citharidae as the sister lineage to Achiridae, albeit with very weak support (PP= 0.60). The family has been considered polyphyletic in many previous works (Hensley and Ahlstrom 1984, Chapleau 1993, Hoshino and Amaoka 1998, Berendzen and Dimmick 2002). Our study did not find Citharidae to be a transitional monophyletic family between Psettodidae and the rest of the flatfishes as hypothesized by Hoshino (2001).

Lineage 2

The second major lineage is a clade consisting of the Rhombosoleidae, Achiropsettidae, a clade containing some of the Poecilopsettidae (*Poecilopsetta hawaiiensis*, *P. natalensis*, *P. praelonga*, and *Marleyella bicolorata*), and the Scopthalmidae (PP=0.74).

The Rhombosoleidae seem to be monophyletic (PP=0.99), with caution, as only sequences from four of the nine genera (*Ammotretis*, *Pelotretis*, *Peltorhampus* and *Rhombosolea*) were available in GenBank to include in our work. Our study also places *Oncopterus darwinii*, a tentative genus in the Rhombosoleidae, as a monotypic family that is sister to the Rhombosoleidae. The family has been defined as monophyletic based on eight morphological synapomorphies (Guibord, 2003, unpublished PhD thesis). In previous molecular phylogenies of flatfishes, some works did not

include the Rhombosoleidae in the analysis (Pardo et al. 2005; Harrington et al. 2016), and others included only one genus (*Rhombosolea*) (Betancur-R et al. 2013).

We find the family Achiropsettidae placed as a subfamily of the Rhombosoleidae (PP=100). Evseenko (1985, 1996) considered the Achiropsettidae at one time to be a transitional group between the Citharidae and a Paralichthyidae-Bothidae clade. In later works, Evseenko (2000) suggested that the group is in fact the sister lineage to the Samaridae-Achiridae-Soleidae-Cynoglossidae clade of Chapleau (1993). The group was observed to be the sister group of the Achiridae in one molecular investigation (Harrington et al. 2016), but they did not include sequences from the Rhombosoleidae in the analysis. Other molecular works contradict this hypothesis (Betancur-R et al. 2013) by placing the Achiropsettidae within the Rhombosoleidae, and are currently considered to be part of the Rhombosoleidae clade (Betancur-R et al. 2017). The results from our study are in agreement with these previous works (Hensley and Ahlstrom 1984; Betancur-R et al. 2013; Betancur-R et al. 2017) placing the group in the Rhombosoleidae.

The Scopththalmidae are monophyletic (PP=100). This family has been recognized as monophyletic by several authors (Hensley and Ahlstrom, 1984; Chapleau, 1993; Hoshino and Amaoka, 1998; Berendzen and Dimmick, 2002). Chanet 2003 confirmed the family as monophyletic based on four synapomorphies. Most of the interrelationships proposed by Chanet (2003) are corroborated in our analysis. *Scopththalmus aquosus*, was placed as a sister lineage to the Pleuronectidae-Paralichthyidae clade. It is possible that this is a rogue sequence not identified by the RogueNaRok algorithm. The placement of the Scopththalmidae within the flatfishes remains elusive.

Berendzen (2002) observed alternate placements of the Scophthalmidae in their multiple analyses, as did Azevedo (2008). Betancur-R et al. (2013) and Harrington et al. (2016) observed a topology that agrees of that of Chapleau (1993), with Scophthalmidae as the sister lineage to a Pleuronectidae-Paralichthyidae-Bothidae clade. Our work places the Scophthalmidae in a much more derived position than previously hypothesized, which would disintegrate the ‘bothoid’ group.

Lineage 3

The most basal of the lineages, Group 3, contains the Pleuronectidae, the Paralichthyidae (minus the “Cyclopsetta” group), the Bothidae, and a new clade consisting of species from the “Cyclopsetta” group of Hensley and Ahlstrom (1984) (PP=0.79).

The Pleuronectidae (sensu Chapleau, 1993) are monophyletic (PP=0.99). Although our molecular results are congruent with some previous molecular works examining relationships within the Pleuronectidae (Berendzen et al. 2002), more insight is needed to confirm these results. Roje (2010) and Betancur-R et al. 2013 relied on sparse taxon sampling. Although the study presented in Roje (2010) did include larval morphology, they failed to include all available morphological characters.

Our results presented here do not find evidence for the proposed Paralichthyid clades, the ‘Pseudorhombus’ group and the ‘Paralichthys’ group. However, the nodal support uniting the Paralichthyidae is low (PP=0.71), and we lack sequences for five genera. All remaining genera form a clade which is the sister-group of the Pleuronectidae. This clade also contains one species of Bothidae, *Psettina hainanensis*. The presence of this species in the Paralichthyidae is possibly due to

error associated with data retrieval, such as identification or sequencing errors. In our study, *Tephrinectes* was recovered with the remaining paralichthyids, possibly having a close relationship with the genus *Pseudorhombus*. This position contradicts the one that was hypothesized by Hoshino (2001), where *Tephrinectes* is considered as a possible monotypic family.

The Bothidae are monophyletic (PP=0.71). Monophyly of the Bothidae was proposed by Hensley and Ahlstrom (1984) and again by Chapleau (1993). Fukui (1997) corroborated monophyly in a study based on larval characters. Here the family is found to be monophyletic but intra-relationships do not have strong enough support to make statements regarding the validity of clades.

Some molecular investigations have found Paralichthyidae to be monophyletic if the taxa belonging to the “Cyclopsetta” group were removed and included as their own family (Betancur-R. et al. 2013; Harrington et al. 2016; Betancur-R et al. 2017). Our work is in agreement with this hypothesis. We find support for the *Cyclopsetta* group to be the sister-group of the Bothidae. Betancur-R et al. (2017) suggested the need for a formal description of a new family for the Cyclopsetta group, in compliance with the International Code of Zoological Nomenclature. Following the regulations set out in the code, and following the Principle of Priority, if raised to the level of family, the name should follow that of the first named genus in the grouping. Here, *Syacium* (Ranzani 1842) precedes the other named genera in the group, namely *Citharichthys* (Bleeker, 1862), *Cyclopsetta* (Gill, 1899) and *Etropus* (Jordan and Gilbert, 1882). Thus, the new family name should be the Syaciumidae.

Status of the ‘Bothoid’ lineage

Hensley and Ahlstrom (1984) suggested a monophyletic lineage made of the Scopthalmidae, Paralichthyidae (except *Tephrinectes* and *Thysanopsetta*), Bothidae, Pleuronectidae and the Citharidae genus *Brachypleura*. based on an apomorphic caudal skeleton pattern. We do not find evidence for a monophyletic “bothoid” lineage. Chapleau (1993) and Hoshino (2001) did not find evidence for the “bothoid” lineage, but suggested that it could be considered as a monophyletic entity if *Brachypleura* is excluded from the taxa. In our analysis, Paralichthyidae, Bothidae and Pleuronectidae are included in Lineage 3. Scopthalmidae is found to be the sister group to the other members of Lineage 2 (Rhombosoleidae, Achiropsettidae and one of the Poecilopsettidae clades).

Table 2: Classification of the Pleuronectiformes according to our study (Figure 5), compared to the most recent flatfish classification (Munro 2015) for comparison.

Munroe (2015)	Present Study (2017)
Order Pleuronectiformes	Order Pleuronectiformes
Suborder Psettodoidei	Suborder Psettodoidei
Family Psettodidae	Family Psettodidae
Suborder Pleuronectoidei	Suborder Pleuronectoidei
Family Citharidae	Superfamily Bothoidea
Family Tephripectes	Family Syaciumidae
Family Scophthalmidae	Family Bothidae
Family Paralichthyidae	Superfamily Pleuronectoidea
Family Bothidae	Family Paralichthyidae
Family Pleuronectidae	Family Pleuronectidae
Family Paralichthoides	Suborder Scophthalmoidei
Family Poecilopsettidae	Family Scophthalmidae
Family Rhombosoleidae	Family Poecilopsettidae 1
Family Achiropsettidae	<i>Oncopterus darwinii</i>
Family Samaridae	Family Rhombosoleidae
Family Achiridae	Subfamily Rhombosoleinae
Family Soleidae	Subfamily Achiropsettinae
Family Cynoglossidae	Suborder Soleoidei
	Family Samaridae

Superfamily Citharidoidea

Family Citharidae

Family Achiridae

Superfamily Soleiodea

Family Samaridae

Family Poecilopsettidae 1

Family Soleidae

Family Cynoglossidae

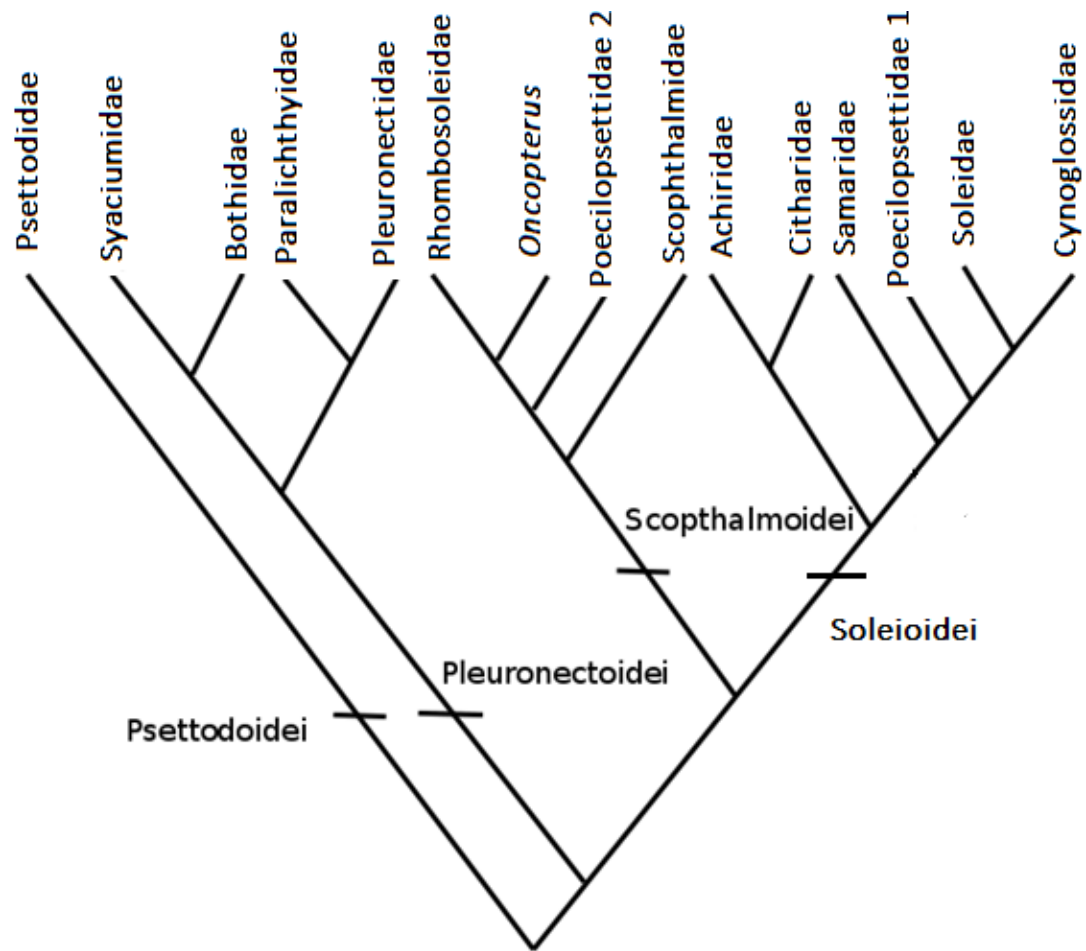


Figure 6: Cladogram of new classification obtained from the results of our Bayesian analysis.

Chapter 4: General Conclusions

In this first phylogenetic reassessment of flatfish intrarelationships since 1993, we have tested, within a molecular framework, the monophyly of families and suprafamilial relationships within the order. Our results, based on the largest molecular body of evidence to date, show that the flatfishes are organized into fifteen families. Indeed, we find critical differences in classification with previous work (Chapleau 1993; Berendzen et al. 2002; Pardo et al. 2005; Azevedo et al. 2008; Betancur-R et al. 2013, 2017) . At the supra familial level, we do not find evidence for the proposed “bothoid” lineage of Hensley and Ahlstrom. We find evidence for three new suborders within the flatfishes. At the level of intrarelationships, we can confirm that the Paralichthyidae are polyphyletic, which was expected based on past interpretations (Berendzen et al. 2002; Pardo et al. 2005; Azevedo et al. 2008; Betancur-R et al. 2013). We propose that a monophyletic unit within the Paralichthyidae, consisting of the genera *Citharichthys*, *Cyclopsetta*, *Etropus* and *Syacium* be raised to the level of family under the name Syaciumidae. *Tephrinectes* is found to be a monotypic genus within the Paralichthyidae, not as a monotypic family as suggested by Hoshino (2001). For the first time, we also present molecular evidence that the Poecilopsettidae are polyphyletic. More research, using more taxa sampled from each family is needed to confirm the polyphyletic status of Poecilopsettidae. Likewise, a heavier taxon sampling could be used to further investigate the phylogenetic placements of Achiridae, Citharidae and Scopthalmidae within the order. Both Achiridae and Citharidae are found here in unusual placements, with very low support values (PP=0.60, placing them as sister groups, and PP=0.60 placing these two families as sister group to the Cynoglossidae-Soleidae-Poecilopsettidae clade). In maximum likelihood estimates, bootstrap

values are interpreted as the probability that the node represents the true phylogeny. Hillis and Bull (1993) performed a simulation analysis to investigate the accuracy of bootstrap results in a parsimony analysis. They found that, bootstrap proportions usually underestimated the probability that a clade is correct, and should not be interpreted as estimates of accuracy. Alternatively, in a Bayesian framework, Huelsenbeck and Rannala (2004) have shown that the posterior probability assessing node support represent the probability that the node is correct, given that the model is also correct. These authors also suggested that only the Bayesian approach directly addresses the probability that a clade is correct, because in frequentist statistics (with the bootstrap), parameters are not treated as random variables, and therefore cannot be assigned a probability. They found that Bayesian estimates are sensitive to model misspecification, and therefore should be used only with the most complex models. As our work attempted to address some prevalent biological complexity by modeling the heterogeneous nature of sequence evolution, our results may not be too affected by model misspecification, and the posterior probabilities that we present may be quite reliable.

Large multi-gene data sets are subject to variability in heterogeneity within the data. We have attempted to account for the error that can arise from such heterogeneity by using heterogeneous models at the level of genes and at the level of sites. However, attempts to model this heterogeneity using site-heterogeneous models has not proven advantageous in discerning deeper node flatfish relationships.

Confirmation of Achiropsettidae as a subfamily of the Rhombosoleidae could be corroborated by attempting to find morphological synapomorphies to unite the group. Although our

data sampling efforts have provided new insights into relationships between the families, relationships within each family lacked strong nodal support. We suggest the possibility of incorporating a total evidence approach, and adding morphological data matrices to the molecular information (Huelsenbeck et al. 1996; Lewis 2001; Wiens 2004) to attempt to elucidate some of the relationships within each family that remain problematic. Detailed molecular studies at the level of family are also needed to attempt discerning monophyletic units within the largest and most diverse flatfish families, such as the Bothidae and the Soleidae.

Our results are a first investigation into flatfish speciation and diversification patterns, and we have shown that at least two geological events can be linked to their evolutionary history. There remain some speciation patterns, in *Poecilopsetta* for example, that are not explained by these events, and this opens path to testing other hypotheses of vicariance. The formation of an ancient northern passage across the Bering Strait occurred as a series of glaciations and melting. Here we showed that global warming periods have contributed to cladogenesis in some marine fishes.

In addition to providing this first look into flatfish speciation patterns, some of the results obtained here also raise new interesting questions regarding the phylogeography of the other flatfish families. For example, the Rhombosoleidae inhabit waters surrounding Australia and New Zealand, while the Achiropsettidae are known only in Antarctic waters. Until about 30 MYA, Australia and Antarctica were part of the same land mass, which suggests that a geological event may be a driving force of the divergence between species of Rhombosoleidae and species of the proposed subfamily Achiropsettinae.

Future Directions

Although we have here attempted to model the heterogeneity in substitution pattern, there are other types of heterogeneity that can exist within a data set. Our analysis accounted for variation in substitution across sites, but not for variation in substitution across lineages. The time-heterogeneous model (Blanquart and Lartillot 2008) implemented in another version of Phylobayes, *nh_Phylobayes*, can test for the effect of rate variation in time. If performing another in depth analysis of the Pleuronectiformes, it may be interesting to see if the robustness of the phylogeny also holds up under these models.

Additionally, there are fossil flatfish specimens that have been used as calibrations in previous molecular works (Near et al. 2012; Harrington et al. 2016), that we have chosen to not employ in our analysis due to uncertainty of their phylogenetic placement. It may be worthwhile to test the effect of adding these constraints to our time-calibrated analysis versus using geological calibrations or minimal calibration points.

The findings presented in this work provide novel frameworks for examining relationships and testing new evolutionary hypotheses within the Pleuronectiformes. The continuation of building phylogenies at the level of family using this approach will help determine relationships within families that have been determined as monophyletic. Future work should focus on family level analyses in the more speciose monophyletic families, such as the Bothidae and Soleidae. The tenuous placement of the families Achiridae and Citharidae within the order also call for further investigations. For the first time, we now have a phylogenetic tree for the Pleuronectiformes containing a wealth of species sampled across all families. A densely sampled tree has here proven

to give valuable insight into phylogeographic hypotheses. From the resulting tree of this work alone, many more biogeographic hypotheses can be tested.

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Appendix 1: Glossary of Terms

Apomorphy: a derived character state

Bootstrap: A statistical procedure used to assess whether the majority of sites in an alignment support the tree. Method based on repeated random sampling with replacement from an original sample to provide a collection of new pseudoreplicate samples, from which sampling variance can be estimated.

Character: A variable feature that in any given taxon or sequence takes one out of a set of two or more different states

Clade: A monophyletic taxon; a group of organisms which includes the most recent common ancestor of all of its members and all of the descendants of that most recent common ancestor.

Cladogram: a cladistic representation of a phylogeny, whereby only the branching order is displayed

Indel: an insertion or deletion of bases in the genome of an organism

Molecular Clock: a measure of evolutionary change over time at the molecular level that is based on the theory that specific DNA sequences or the proteins they encode spontaneously mutate at constant rates and that is used chiefly for estimating how long ago two related organisms diverged from a common ancestor

Monophyletic: A group of taxa that contains an ancestor and all of its descendants.

Paraphyletic: descended from a common evolutionary ancestor or ancestral group, but not including all the descendant groups.

Phylogeography: The study of biogeography as revealed by a comparison of estimated phylogenies of populations or species with their geographic distributions

Robustness: A measure of how sensitive a particular method is to violation of its assumptions

Sister group: The two clades resulting from the splitting of a single lineage.

Synapomorphy: A shared derived character state

Symplesiomorphy: A shared ancestral character state.

Vicariance : Speciation which occurs as a result of the separation and subsequent isolation of portions of an original population. i.e allopatric speciation

Appendix 2

Ascension

Number

Description

LC037080.1	Symphurus strictus mitochondrial gene for 12S rRNA
LC145953.1	Asterorhombus cocosensis mitochondrial gene for 12S rRNA
LC069805.1	Cynoglossus robustus mitochondrial gene for 12S rRNA
AB974673.1	Cynoglossus robustus mitochondrial gene for 12S rRNA
LC049621.1	Cynoglossus joyneri mitochondrial gene for 12S rRNA
LC037079.1	Paraplagusia japonica mitochondrial gene for 12S rRNA
AB972088.1	Paraplagusia japonica mitochondrial gene for 12S rRNA
LC037076.1	Cynoglossus ochiaii mitochondrial gene for 12S rRNA
AB972086.1	Cynoglossus interruptus mitochondrial gene for 12S rRNA
AB972224.1	Cynoglossus nigropinnatus mitochondrial gene for 12S rRNA
FJ786630.1	Cynoglossus semilaevis 12S ribosomal RNA gene
AB972089.1	Cynoglossus abbreviatus mitochondrial gene for 12S rRNA
LC026791.1	Bothus myriaster mitochondrial gene for 12S rRNA
AY998037.1	Symphurus tessellatus voucher LBP513 12S ribosomal RNA gene
AF488497.1	Symphurus plagusia 12S ribosomal RNA gene
LC026782.1	Psettina iijimae mitochondrial gene for 12S rRNA
LC026783.1	Psettina tosana mitochondrial gene for 12S rRNA
LC026781.1	Psettina gigantea mitochondrial gene for 12S rRNA
LC026789.1	Arnoglossus maculophus mitochondrial gene for 12S rRNA
LC026784.1	Arnoglossus aspidos mitochondrial gene for 12S rRNA
AF488514.1	Lepidoblepharon ophthalmolepis 12S ribosomal RNA gene
AB972105.1	Citharoides macrolepidotus mitochondrial gene for 12S rRNA
AF488513.1	Citharoides macrolepis 12S ribosomal RNA gene
AF542220.1	Citharus linguatula 12S small subunit ribosomal RNA gene
AF542210.1	Arnoglossus laterna 12S small subunit ribosomal RNA gene
LC145954.1	Engyophrys senta mitochondrial gene for 12S rRNA
LC026773.1	Trichopsetta ventralis mitochondrial gene for 12S rRNA
LC026780.1	Engyproson longipelvis mitochondrial gene for 12S rRNA
AB972226.1	Engyproson grandisquama mitochondrial gene for 12S rRNA
LC026779.1	Tosarhombus brevis mitochondrial gene for 12S rRNA
AF488503.1	Syacium papillosum 12S ribosomal RNA gene
AB974641.1	Poecilopsetta plinthus mitochondrial gene for 12S rRNA
AY998036.1	Trinectes paulistanus voucher LBP720 12S ribosomal RNA gene
AY430282.1	Trinectes maculatus 12S ribosomal RNA gene
AY998041.1	Achirus declivis voucher LBP1397 12S ribosomal RNA gene
AY998035.1	Hypoclinemus mentalis voucher LBP1385 12S ribosomal RNA gene
AB125239.1	Microchirus boscanion mitochondrial gene for 12S rRNA
AF542217.1	Microchirus boscanion 12S small subunit ribosomal RNA gene
AB125240.1	Monochirus hispidus mitochondrial gene for 12S rRNA
AF542219.1	Monochirus hispidus 12S small subunit ribosomal RNA gene
AY141359.1	Microchirus variegatus 12S ribosomal RNA gene
AF542218.1	Microchirus ocelatus 12S small subunit ribosomal RNA gene
AB125242.1	Dicologlossa hexophthalma mitochondrial gene for 12S rRNA
AB125238.1	Microchirus azevia mitochondrial gene for 12S rRNA
AF542216.1	Microchirus azevia 12S small subunit ribosomal RNA gene
AB125234.1	Solea lascaris mitochondrial gene for 12S rRNA

AB125241.1	Dicologlossa cuneata mitochondrial gene for 12S rRNA
AB125237.1	Solea kleinii mitochondrial gene for 12S rRNA
AB125235.1	Solea senegalensis mitochondrial gene for 12S rRNA
EF095556.1	Solea solea 12S ribosomal RNA gene
AF542204.1	Solea vulgaris 12S small subunit ribosomal RNA gene
AY998032.1	Etropus crossotus voucher LBP506 12S ribosomal RNA gene
AY998033.1	Etropus longimanus voucher LBP886 12S ribosomal RNA gene
AY998031.1	Cyclopsetta chittendeni voucher LBP884 12S ribosomal RNA gene
HQ641687.1	Austroglossus microlepis voucher 09051 12S ribosomal RNA gene
GU946480.1	Austroglossus microlepis voucher US<ZAF>:09023 12S ribosomal RNA gene
GU946487.1	Austroglossus pectoralis voucher US<ZAF>:RET30 12S ribosomal RNA gene
AF488512.1	Scophthalmus aquosus 12S ribosomal RNA gene
DQ874685.1	Paralichthys dentatus isolate JJ13:5 12S ribosomal RNA gene
AY998040.1	Paralichthys patagonicus voucher LBP831 12S ribosomal RNA gene
AY998044.1	Scophthalmus rhombus 12S ribosomal RNA gene
AF542214.1	Psetta maxima 12S small subunit ribosomal RNA gene
AF517557.1	Scophthalmus maximus 12S ribosomal RNA gene
AM931031.1	Lepidorhombus boscii mitochondrial partial 12S rRNA gene
AF517556.1	Lepidorhombus whiffiagonis 12S ribosomal RNA gene
AY368282.1	Psettodes belcheri 12S ribosomal RNA gene
AF488518.1	Psettodes erumei 12S ribosomal RNA gene
AF488506.1	Crossorhombus kobensis 12S ribosomal RNA gene
AF542209.1	Arnoglossus imperialis 12S small subunit ribosomal RNA gene
AF542208.1	Arnoglossus thori 12S small subunit ribosomal RNA gene
LC026785.1	Parabothus kiensis mitochondrial gene for 12S rRNA
AF488511.1	Laeops kitaharae 12S ribosomal RNA gene
LC026790.1	Arnoglossus yamanakai mitochondrial gene for 12S rRNA
LC026787.1	Japonolaeops dentatus mitochondrial gene for 12S rRNA
AF488509.1	Bothus robinsi 12S ribosomal RNA gene
LC026778.1	Bothus mancus mitochondrial gene for 12S rRNA
AF488508.1	Bothus lunatus 12S ribosomal RNA gene
FJ711006.1	Rhombosolea tapirina 12S ribosomal RNA gene
AF542221.1	Bothus podas 12S small subunit ribosomal RNA gene
LC049682.1	Pseudorhombus levisquamis mitochondrial gene for 12S rRNA
LC049674.1	Paralichthys olivaceus mitochondrial gene for 12S rRNA
AB125243.1	Synaptura lusitanica mitochondrial gene for 12S rRNA
AF542212.1	Synaptura lusitanica 12S small subunit ribosomal RNA gene
HQ615464.1	Ammotretis rostratus 12S ribosomal RNA gene
LC049641.1	Atheresthes evermanni mitochondrial gene for 12S rRNA
LC049643.1	Atheresthes stomias mitochondrial gene for 12S rRNA
AF488475.1	Reinhardtius evermanni 12S ribosomal RNA gene
LC145935.1	Pleuronichthys decurrens mitochondrial gene for 12S rRNA
AF488504.1	Xystreurus liolepis 12S ribosomal RNA gene
AF488487.1	Pleuronichthys guttulatus 12S ribosomal RNA gene
LC069802.1	Pleuronichthys japonicus mitochondrial gene for 12S rRNA
AB972097.1	Pleuronichthys cornutus mitochondrial gene for 12S rRNA
LC145919.1	Pleuronichthys coenosus mitochondrial gene for 12S rRNA
AF488489.1	Pleuronichthys verticalis 12S ribosomal RNA gene
AF488476.1	Eopsetta jordani 12S ribosomal RNA gene
AB972102.1	Eopsetta grigorjewi mitochondrial gene for 12S rRNA
AB974640.1	Dexistes rikuzenius mitochondrial gene for 12S rRNA

AB972098.1	Limanda punctatissima mitochondrial gene for 12S rRNA
AB972095.1	Tanakius kitaharae mitochondrial gene for 12S rRNA
DQ834442.2	Verasper moseri 12S ribosomal RNA gene
AF488488.1	Parophrys vetula 12S ribosomal RNA gene
AF488490.1	Microstomus bathybius 12S ribosomal RNA gene
LC049659.1	Clidoderma asperrimum mitochondrial gene for 12S rRNA
AF488484.1	Lyopsetta exilis 12S ribosomal RNA gene
AF488486.1	Glyptocephalus zachirus 12S ribosomal RNA gene
LC049638.1	Pseudopleuronectes schrenki mitochondrial gene for 12S rRNA
LC049622.1	Pseudopleuronectes yokohamae mitochondrial gene for 12S rRNA
LC049624.1	Pseudopleuronectes obscurus mitochondrial gene for 12S rRNA
LC049628.1	Pseudopleuronectes herzensteini mitochondrial gene for 12S rRNA
AF488483.1	Hippoglossus stenolepis 12S ribosomal RNA gene
LC049631.1	Glyptocephalus stelleri mitochondrial gene for 12S rRNA
LC049637.1	Microstomus achne mitochondrial gene for 12S rRNA
AF488480.1	Microstomus pacificus 12S ribosomal RNA gene
AF517555.1	Reinhardtius hippoglossoides 12S ribosomal RNA gene
AM931024.1	Hippoglossus hippoglossus mitochondrial partial 12S rRNA gene
LC049652.1	Pleuronectes quadrituberculatus mitochondrial gene for 12S rRNA
AF488491.1	Limanda aspera 12S ribosomal RNA gene
LC049630.1	Kareius bicoloratus mitochondrial gene for 12S rRNA
AF488477.1	Hippoglossoides platessoides 12S ribosomal RNA gene
AF488478.1	Pseudopleuronectes americanus 12S ribosomal RNA gene
LC049654.1	Hippoglossoides robustus mitochondrial gene for 12S rRNA
AB972228.1	Hippoglossoides dubius mitochondrial gene for 12S rRNA
LC049648.1	Hippoglossoides elassodon mitochondrial gene for 12S rRNA
LC049657.1	Limanda sakhalinensis mitochondrial gene for 12S rRNA
LC049625.1	Acanthopsetta nadeshnyi mitochondrial gene for 12S rRNA
AB972227.1	Cleisthenes pinetorum mitochondrial gene for 12S rRNA
LC049632.1	Liopsetta pinnifasciata mitochondrial gene for 12S rRNA
AF488482.1	Platichthys stellatus 12S ribosomal RNA gene
AB125244.1	Platichthys flesus mitochondrial gene for 12S rRNA
AF542207.1	Pleuronectes platessa 12S small subunit ribosomal RNA gene
AF488485.1	Psettichthys melanostictus 12S ribosomal RNA gene
AF488481.1	Isopsetta isolepis 12S ribosomal RNA gene
LC049639.1	Lepidopsetta mochigarei mitochondrial gene for 12S rRNA
LC092091.1	Lepidopsetta polyxystra mitochondrial gene for 12S rRNA
AF488479.1	Lepidopsetta bilineata 12S ribosomal RNA gene
LC146327.1	Chascanopsetta lugubris lugubris mitochondrial gene for 12S rRNA
LC026775.1	Chascanopsetta lugubris mitochondrial gene for 12S rRNA
LC026788.1	Grammatobothus polyophthalmus mitochondrial gene for 12S rRNA
LC026805.1	Aesopia cornuta mitochondrial gene for 12S rRNA
AB972090.1	Aesopia cornuta mitochondrial gene for 12S rRNA
LC026808.1	Soleichthys heterorhinos mitochondrial gene for 12S rRNA
AB972092.1	Pseudaesopia japonica mitochondrial gene for 12S rRNA
AF488495.1	Pseudaesopia japonica 12S ribosomal RNA gene
LC049677.1	Tarphops elegans mitochondrial gene for 12S rRNA
LC049678.1	Tarphops oligolepis mitochondrial gene for 12S rRNA
LC026804.1	Gymnachirus melas mitochondrial gene for 12S rRNA
LC026802.1	Gymnachirus texae mitochondrial gene for 12S rRNA
AB972094.1	Aseraggodes kobensis mitochondrial gene for 12S rRNA

AB972087.1	Cynoglossus itinus mitochondrial gene for 12S rRNA
AB974674.1	Zebrias zebra mitochondrial gene for 12S rRNA
LC026803.1	Achirus lineatus mitochondrial gene for 12S rRNA
LC026806.1	Pardachirus pavoninus mitochondrial gene for 12S rRNA
AB972225.1	Pardachirus pavoninus mitochondrial gene for 12S rRNA
LC049681.1	Pseudorhombus duplisciellatus mitochondrial gene for 12S rRNA
AF488502.1	Etropus microstomus 12S ribosomal RNA gene
LC092080.1	Citharichthys stigmaeus mitochondrial gene for 12S rRNA
LC145918.1	Pseudorhombus pentophthalmus mitochondrial gene for 12S rRNA
LC049679.1	Pseudorhombus arsius mitochondrial gene for 12S rRNA
LC026810.1	Solea ovata mitochondrial gene for 12S rRNA
AB972093.1	Heteromycteris japonicus mitochondrial gene for 12S rRNA
LC026809.1	Heteromycteris matsubarae mitochondrial gene for 12S rRNA
AY998034.1	Catathyridium jenynsi voucher LBP989 12S ribosomal RNA gene
AF488499.1	Citharichthys xanthostigma 12S ribosomal RNA gene
LC145943.1	Citharichthys sordidus mitochondrial gene for 12S rRNA
JQ939120.1	Psettodes belcheri voucher FMNH119721 16S ribosomal RNA gene
JQ939119.1	Psettodes erumei voucher CL-P167 16S ribosomal RNA gene
JQ970472.1	Psettodes bennettii isolate R1646 16S ribosomal RNA gene
KT323973.1	Dagetichthys commersonnii voucher COFMNG-FRM-05 16S ribosomal RNA gene
GU946581.1	Austroglossus pectoralis voucher RET30 16S ribosomal RNA gene
GU946577.1	Austroglossus microlepis voucher 09052 16S ribosomal RNA gene
AY359663.1	Buglossidium luteum 16S large subunit ribosomal RNA gene
AY359661.1	Solea senegalensis 16S large subunit ribosomal RNA gene
AY359660.1	Dicologlossa cuneata 16S large subunit ribosomal RNA gene
AY359659.1	Bathysolea profundicola 16S large subunit ribosomal RNA gene
AB125254.1	Synaptura lusitanica mitochondrial gene for 16S rRNA
AB125253.1	Dicologlossa hexophthalma mitochondrial gene for 16S rRNA
AB125252.1	Dicologlossa cuneata mitochondrial gene for 16S rRNA
AB125251.1	Monochirus hispidus mitochondrial gene for 16S rRNA
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AB125248.1	Solea kleinii mitochondrial gene for 16S rRNA
AB125247.1	Solea solea mitochondrial gene for 16S rRNA
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EU848450.1	Ammotretis rostratus 16S ribosomal RNA gene
JQ939117.1	Neoachirosetta milfordi voucher LS715 16S ribosomal RNA gene
JQ939116.1	Mancopsetta maculata voucher LS714 16S ribosomal RNA gene
JQ939107.1	Rhombosolea plebeia voucher CL-P145 16S ribosomal RNA gene
JQ939106.1	Rhombosolea leporina voucher CL-P144 16S ribosomal RNA gene
JQ939090.1	Poecilopsetta plinthus voucher KU2473 16S ribosomal RNA gene
FJ870406.1	Pleuronectes quadrituberculatus voucher UW 118094 16S ribosomal RNA gene
AF488427.1	Hippoglossoides platessoides 16S ribosomal RNA gene
EF119320.1	Atheresthes stomias voucher WTU:047693 16S ribosomal RNA gene
DQ983931.1	Hippoglossus hippoglossus 16S ribosomal RNA gene
DQ242489.1	Verasper moseri 16S ribosomal RNA gene
FJ870410.1	Hippoglossoides robustus voucher UW 117291 16S ribosomal RNA gene
AF488431.1	Isopsetta isolepis 16S ribosomal RNA gene
AY958634.1	Pleuronichthys coenosus 16S ribosomal RNA gene
AY368897.1	Limanda limanda 16S ribosomal RNA gene
EF458382.1	Pleuronichthys decurrens voucher UW:048834 16S ribosomal RNA gene
AF488430.1	Microstomus pacificus 16S ribosomal RNA gene
AF488440.1	Microstomus bathybius 16S ribosomal RNA gene
FJ870385.1	Embassichthys bathybius voucher UW 111523 16S ribosomal RNA gene
EF119245.1	Parophrys vetulus voucher WTU:047297 16S ribosomal RNA gene
AF113180.1	AF113180 Platichthys flesus 16S large subunit ribosomal RNA gene
AY157328.1	Pleuronectes platessa 16S large subunit ribosomal RNA gene
EF119261.1	Hippoglossoides elassodon voucher WTU:047315 16S ribosomal RNA gene
FJ870422.1	Reinhardtius hippoglossoides voucher UW 114782 16S ribosomal RNA gene
AF488439.1	Pleuronichthys verticalis 16S ribosomal RNA gene
AF488437.1	Pleuronichthys guttulatus 16S ribosomal RNA gene
FJ870391.1	Atheresthes evermanni voucher KU 2075 16S ribosomal RNA gene
KJ128830.1	Microstomus kitt voucher NRM:47494 16S ribosomal RNA gene
AY368899.1	Pseudopleuronectes herzensteini 16S ribosomal RNA gene
FJ870409.1	Limanda proboscidea voucher UW 117296 16S ribosomal RNA gene
JQ178239.1	Pseudopleuronectes yokohamae isolate PKU 6514 16S ribosomal RNA gene
EF458354.1	Lepidopsetta polyxystra voucher UW:048789 16S ribosomal RNA gene
AF488433.1	Hippoglossus stenolepis 16S ribosomal RNA gene
AF488435.1	Psettichthys melanostictus 16S ribosomal RNA gene
AF488426.1	Eopsetta jordani 16S ribosomal RNA gene
AF488436.1	Glyptocephalus zachirus 16S ribosomal RNA gene
AY368903.1	Pleuronichthys cornutus 16S ribosomal RNA gene
FJ870419.1	Dexistes rikuzenius voucher FAKU 131266 16S ribosomal RNA gene
AF488429.1	Lepidopsetta bilineata 16S ribosomal RNA gene

AY952505.2	Clidoderma asperimum 16S ribosomal RNA gene
FJ870408.1	Limanda sakhalinensis voucher UW 117279 16S ribosomal RNA gene
AY368896.1	Eopsetta grigorjewi 16S ribosomal RNA gene
AF488434.1	Lyopsetta exilis 16S ribosomal RNA gene
AY368898.1	Microstomus achne 16S ribosomal RNA gene
AY368895.1	Cleisthenes herzensteini 16S ribosomal RNA gene
AY368901.1	Verasper variegatus 16S ribosomal RNA gene
AY958639.2	Pleuronichthys ritteri 16S ribosomal RNA gene
AF488432.1	Platichthys stellatus 16S ribosomal RNA gene
GU248349.1	Kareius bicoloratus voucher IOCASFY-Kbch02 16S ribosomal RNA gene
FJ870412.1	Pseudopleuronectes americanus voucher KU 5419 16S ribosomal RNA gene
AF420447.1	Glyptocephalus cynoglossus 16S ribosomal RNA gene
AF488441.1	Limanda aspera 16S ribosomal RNA gene
AF420452.1	Limanda ferruginea 16S ribosomal RNA gene
AF420448.1	Paralichthys oblongus 16S ribosomal RNA gene
KJ010672.1	Hippoglossina stomata voucher UW:119910 16S ribosomal RNA gene
HM211198.1	Paralichthys adspersus 16S ribosomal RNA gene
JQ939111.1	Paralichthys albigutta voucher LS172 16S ribosomal RNA gene
DQ874717.1	Paralichthys dentatus isolate JJ13:5 16S ribosomal RNA gene
AB441031.1	Paralichthys olivaceus mitochondrial gene for 16S ribosomal RNA
GU324157.1	Paralichthys patagonicus voucher DAAPV F28 16S ribosomal RNA gene
AY359658.1	Pseudorhombus arsius 16S large subunit ribosomal RNA gene
AY046950.1	Pseudorhombus cinnamomeus 16S ribosomal RNA gene
AM182422.1	Pseudorhombus duplionicellatus mitochondrial 16S rRNA gene
KR153521.1	Pseudorhombus jenynsii isolate 94618 16S ribosomal RNA gene
AM182425.1	Pseudorhombus levisquamis mitochondrial 16S rRNA gene
AM182423.1	Pseudorhombus oligodon mitochondrial 16S rRNA gene
AB441037.1	Pseudorhombus pentophthalmus mitochondrial gene for 16S ribosomal RNA
AF488457.1	Tarphops oligolepis 16S ribosomal RNA gene
AM182424.1	Tephrinectes sinensis mitochondrial 16S rRNA gene
JQ939073.1	Xystreurus liolepis voucher KU465 16S ribosomal RNA gene
GU324145.1	Xystreurus rasile voucher DAAPV F13 16S ribosomal RNA gene
KU170682.1	Symphurus longirostris voucher FAKU:135487 16S ribosomal RNA gene
KT323971.1	Cynoglossus arel voucher COFMNG-FRM-04 16S ribosomal RNA gene
KC900872.1	Symphurus leucochilus voucher NMMBP:17767 16S ribosomal RNA gene
KC900864.1	Symphurus microrhynchus voucher ASIZP:72371 16S ribosomal RNA gene
JN678765.1	Symphurus strictus isolate AI 16S ribosomal RNA gene
JN678763.1	Symphurus orientalis isolate AG 16S ribosomal RNA gene
JN678741.1	Symphurus megasomus isolate J 16S ribosomal RNA gene
JN678733.1	Symphurus hondoensis isolate B 16S ribosomal RNA gene
AY359668.1	Symphurus tessellatus 16S large subunit ribosomal RNA gene
AY359669.1	Cynoglossus cynoglossus 16S large subunit ribosomal RNA gene
HQ003913.1	Cynoglossus robustus isolate PKU_2307 16S ribosomal RNA gene
HQ003910.1	Cynoglossus joyneri isolate PKU_1870 16S ribosomal RNA gene
DQ112685.1	Paraplagusia japonica 16S ribosomal RNA gene
DQ112684.1	Cynoglossus sinicus 16S ribosomal RNA gene
DQ112683.1	Cynoglossus lighti 16S ribosomal RNA gene
DQ112682.1	Cynoglossus semilaevis 16S ribosomal RNA gene
DQ112681.1	Cynoglossus abbreviatus 16S ribosomal RNA gene
DQ112680.1	Cynoglossus purpureomaculatus 16S ribosomal RNA gene
AY998029.1	Symphurus plagusia voucher LBP717 16S ribosomal RNA gene

AY998024.1	<i>Symphurus tessellatus</i> voucher LBP513 16S ribosomal RNA gene
KR153527.1	<i>Cynoglossus broadhursti</i> isolate 94687 16S ribosomal RNA gene
JQ939063.1	<i>Symphurus civitatum</i> voucher KU5106 16S ribosomal RNA gene
JQ939062.1	<i>Symphurus atricaudus</i> voucher KU504 16S ribosomal RNA gene
JQ939061.1	<i>Cynoglossus interruptus</i> voucher KU2478 16S ribosomal RNA gene
FJ859011.1	<i>Symphurus thermophilus</i> isolate Volcano_1_R1053_R154_14_05_07 16S ribosomal RNA gene
AY157319.1	<i>Symphurus nigrescens</i> 16S large subunit ribosomal RNA gene
AM182039.1	<i>Cynoglossus oligolepis</i> mitochondrial 16S rRNA gene
AM182038.1	<i>Symphurus strictus</i> mitochondrial 16S rRNA gene
AM182037.1	<i>Symphurus rafinesque</i> mitochondrial 16S rRNA gene
AM181779.1	<i>Paraplagusia blochi</i> mitochondrial 16S rRNA gene
AY835657.1	<i>Symphurus atricaudus</i> 16S ribosomal RNA gene
AY157325.1	<i>Citharus linguatula</i> 16S large subunit ribosomal RNA gene
AF488464.1	<i>Lepidoblepharon ophthalmolepis</i> 16S ribosomal RNA gene
JQ939060.1	<i>Citharoides macrolepis</i> voucher KU2468 16S ribosomal RNA gene
JQ939054.1	<i>Asterorhombus fijiensis</i> voucher KU7102 16S ribosomal RNA gene
AM181786.1	<i>Crossorhombus azureus</i> mitochondrial 16S rRNA gene
EU848459.1	<i>Lophonectes gallus</i> 16S ribosomal RNA gene
AM181783.1	<i>Arnoglossus tapeinosoma</i> mitochondrial 16S rRNA gene
AM181762.1	<i>Arnoglossus tenuis</i> mitochondrial 16S rRNA gene
AM181769.1	<i>Engyproson maldivensis</i> mitochondrial 16S rRNA gene
AM181767.1	<i>Engyproson multisquama</i> mitochondrial 16S rRNA gene
AY359652.1	<i>Bothus ocellatus</i> 16S large subunit ribosomal RNA gene
AY157322.1	<i>Arnoglossus imperialis</i> 16S large subunit ribosomal RNA gene
AM181766.1	<i>Bothus myriaster</i> mitochondrial 16S rRNA gene
AM181788.1	<i>Japonolaeops dentatus</i> mitochondrial 16S rRNA gene
AM181776.1	<i>Kamoharaia megastoma</i> mitochondrial 16S rRNA gene
AF488456.1	<i>Crossorhombus kobensis</i> 16S ribosomal RNA gene
AF488461.1	<i>Laeops kitaharae</i> 16S ribosomal RNA gene
AM181781.1	<i>Bothus pantherinus</i> mitochondrial 16S rRNA gene
AM181765.1	<i>Arnoglossus polyspilus</i> mitochondrial 16S rRNA gene
AM181761.1	<i>Neolaeops microphthalmus</i> mitochondrial 16S rRNA gene
AM181773.1	<i>Parabothus chlorospilus</i> mitochondrial 16S rRNA gene
JQ939121.1	<i>Chascanopsetta lugubris</i> voucher FMNH119729 16S ribosomal RNA gene
JQ939055.1	<i>Bothus lunatus</i> voucher KU154 16S ribosomal RNA gene
AM181772.1	<i>Psettina iijimae</i> mitochondrial 16S rRNA gene
JQ939058.1	<i>Psettina tosana</i> voucher KU2511 16S ribosomal RNA gene
JQ939059.1	<i>Trichopsetta ventralis</i> voucher KU5085 16S ribosomal RNA gene
AY359653.1	<i>Arnoglossus laterna</i> 16S large subunit ribosomal RNA gene
AY157329.1	<i>Arnoglossus thori</i> 16S large subunit ribosomal RNA gene
JQ939056.1	<i>Bothus robinsi</i> voucher KU1177 16S ribosomal RNA gene
AY157326.1	<i>Bothus podas</i> 16S large subunit ribosomal RNA gene
KP213881.1	<i>Trinectes microphthalmus</i> voucher PNT UERJ 362 16S ribosomal RNA gene
KP213880.1	<i>Trinectes paulistanus</i> voucher PNT UERJ 335 16S ribosomal RNA gene
KP213876.1	<i>Hypoclinemus mentalis</i> voucher PNT UERJ 380 16S ribosomal RNA gene
KP213872.1	<i>Gymnachirus nudus</i> voucher PNT UERJ 471 16S ribosomal RNA gene
KP213870.1	<i>Apionichthys dumerili</i> voucher GEA ICT 01706 16S ribosomal RNA gene
KP213868.1	<i>Achirus lineatus</i> voucher PNT UERJ 364 16S ribosomal RNA gene
KP213865.1	<i>Achirus declivis</i> voucher PNT UERJ 353 16S ribosomal RNA gene
KP213862.1	<i>Achirus achirus</i> voucher GEA ICT 01695 16S ribosomal RNA gene
AY998022.1	<i>Catathyridium jenynsi</i> voucher LBP989 16S ribosomal RNA gene

JQ939051.1	Gymnachirus texae voucher KU5187 16S ribosomal RNA gene
JQ939050.1	Gymnachirus melas voucher KU5123 16S ribosomal RNA gene
AY359656.1	Citharichthys macrops 16S large subunit ribosomal RNA gene
JQ939072.1	Syacium micrurum voucher KU5200 16S ribosomal RNA gene
AF488453.1	Syacium papillosum 16S ribosomal RNA gene
JQ939068.1	Etropus crossotus voucher KU5244 16S ribosomal RNA gene
AY998020.1	Etropus longimanus voucher LBP886 16S ribosomal RNA gene
JQ939069.1	Etropus microstomus voucher KU1506 16S ribosomal RNA gene
JQ939065.1	Citharichthys arctifrons voucher KU1468 16S ribosomal RNA gene
AF488449.1	Citharichthys xanthostigma 16S ribosomal RNA gene
JQ939067.1	Cyclopsetta chittendeni voucher KU5080 16S ribosomal RNA gene
JQ939066.1	Citharichthys sordidus voucher KU3255 16S ribosomal RNA gene
AY952497.1	Citharichthys stigmaeus 16S ribosomal RNA gene
JQ939070.1	Paralichthys californicus voucher KU456 16S ribosomal RNA gene
KF009651.1	Psettodes erumei voucher ARO 49 cytochrome oxidase subunit 1 (COI) gene
EU513688.1	Psettodes bennettii isolate PbenII2 cytochrome oxidase subunit I (COI) gene
JF952754.1	Heteromycteris japonicus voucher SSU9 cytochrome oxidase subunit 1 (COI) gene
JF494801.1	Zebrias regani voucher ADC262.16-1 cytochrome oxidase subunit 1 (COI) gene
JF494536.1	Pegusa nasuta voucher ADC262.12-2 cytochrome oxidase subunit 1 (COI) gene
JF494095.1	Pardachirus morrowi voucher ADC262.10-1 cytochrome oxidase subunit 1 (COI) gene
JF494093.1	Pardachirus marmoratus voucher ADC08 Smith 262.9 #1 cytochrome oxidase subunit 1 (COI) gene
JF492934.1	Austroglossus pectoralis voucher Smith 262.3 #3 cytochrome oxidase subunit 1 (COI) gene
HQ945820.1	Synaptura marginata voucher ADC10_262.14 #2 cytochrome oxidase subunit 1 (COI) gene
KF809410.1	Pardachirus pavoninus cytochrome oxidase subunit 1 (COI) gene
KP975836.1	Solea solea voucher FLID019 cytochrome oxidase subunit 1 (COI) gene
KJ709633.1	Solea aegyptiaca voucher CSFOM-190 cytochrome oxidase subunit 1 (COI) gene
KJ709575.1	Pegusa impar voucher CSFOM-068 cytochrome oxidase subunit 1 (COI) gene
KJ709561.1	Microchirus ocellatus voucher CSFOM-129 cytochrome oxidase subunit 1 (COI) gene
KJ768310.1	Solea senegalensis voucher MLFPI83 cytochrome oxidase subunit 1 (COI) gene
KJ768259.1	Microchirus variegatus voucher MLFPI274 cytochrome oxidase subunit 1 (COI) gene
KJ768257.1	Microchirus azevia voucher MLFPI64 cytochrome oxidase subunit 1 (COI) gene
KJ768215.1	Bathysolea profundicola voucher MLFPI33 cytochrome oxidase subunit 1 (COI) gene
JN312474.1	Buglossidium luteum voucher MT-0613 cytochrome oxidase subunit 1 (COI) gene
KJ205042.1	Microchirus variegatus voucher MT02875 cytochrome oxidase subunit 1 (COI) gene
KJ205298.1	Pegusa lascaris voucher DWCS06-058 cytochrome oxidase subunit 1 (COI) gene
KF930447.1	Soleichthys heterorhinos voucher KUT 7229 cytochrome oxidase subunit 1 (COI) gene
KF930327.1	Pseudaesopia japonica voucher KUT 2504 cytochrome oxidase subunit 1 (COI) gene
KF929630.1	Aseraggodes heemstrai voucher KUT 4996 cytochrome oxidase subunit 1 (COI) gene
JQ775008.1	Dicologlossa cuneata voucher FCFOPB088-18 cytochrome oxidase subunit 1 (COI) gene
JQ774673.1	Microchirus boscanion voucher FCFOPS171 cytochrome oxidase subunit 1 (COI) gene
JQ774672.1	Microchirus azevia voucher FCFOPS199 cytochrome oxidase subunit 1 (COI) gene
JQ431466.1	Aseraggodes melanostictus voucher MBIO998.4 cytochrome oxidase subunit 1 (COI) gene
JQ350178.1	Pardachirus morrowi voucher ECOMAR<FRA>:NBE1039 cytochrome oxidase subunit 1 (COI) gene
JQ350177.1	Pardachirus marmoratus voucher ECOMAR<FRA>:NBE1291 cytochrome oxidase subunit 1 (COI) gene
EU600148.1	Solea ovata voucher SOL-LT1 cytochrome oxidase subunit I (COI) gene
KP267606.1	Aseraggodes kaianus isolate 114_RBZLT cytochrome oxidase subunit I (COI) gene
KT951837.1	Brachirus orientalis voucher BCO-LSJ cytochrome oxidase subunit I (COI) gene
KT323972.1	Dagetichthys commersonnii voucher COFMNG-FRM-05 cytochrome oxidase subunit 1 (COI) gene
EU513755.1	Synaptura lusitanica isolate SlusII3 cytochrome oxidase subunit I (COI) gene
EU513752.1	Synapturichthys kleinii isolate SkleII1 cytochrome oxidase subunit I (COI) gene
EU513738.1	Pegusa impar isolate PimII2 cytochrome oxidase subunit I (COI) gene

EU513720.1	Bathysolea profundicola isolate Bpro11 cytochrome oxidase subunit I (COI) gene
FJ347913.1	Zebrias synapturoides voucher NBFGR:Cyz01-A cytochrome c oxidase subunit I (COI) gene
EU595351.1	Zebrias quagga voucher MBCSC:Z711182 cytochrome oxidase subunit I (COI) gene
EF607337.1	Brachirus orientalis isolate FSCS009-06 cytochrome oxidase subunit I (COI) gene
JX501465.1	Zebrias crossolepis isolate YL1 cytochrome oxidase subunit 1 (COI) gene
JX501464.1	Zebrias zebrinus isolate ST cytochrome oxidase subunit 1 (COI) gene
KF268187.1	Zebrias regani voucher CIFE:FGB-ZR-001 cytochrome oxidase subunit I (COI) gene
HM180939.1	Zebrias fasciatus voucher NSMK:PI-000121 cytochrome oxidase subunit I (COI) gene
EU513697.1	Lepidorhombus boscii isolate Boscii1 cytochrome oxidase subunit I (COI) gene
EU513700.1	Lepidorhombus whiffiagonis isolate Lw11 cytochrome oxidase subunit I (COI) gene
EU513703.1	Phrynorhombus norvegicus isolate Pnl1 cytochrome oxidase subunit I (COI) gene
EU513712.1	Zeugopterus punctatus isolate Zpl1 cytochrome oxidase subunit I (COI) gene
EU513706.1	Psetta maxima isolate Smax11 cytochrome oxidase subunit I (COI) gene
KC501373.1	Scophthalmus maximus voucher TR1414EK cytochrome oxidase subunit 1 (COI) gene
EU752184.1	Scophthalmus aquosus voucher SCOPAQUO-H94-002 CERT cytochrome oxidase subunit 1 (COI) gene
KF930263.1	Plagiopsetta glossa voucher KUT 2475 cytochrome oxidase subunit 1 (COI) gene
JQ432092.1	Samariscus triocellatus voucher MBIO1413.4 cytochrome oxidase subunit 1 (COI) gene
KP267651.1	Samariscus longimanus isolate 35_CBSD cytochrome oxidase subunit I (COI) gene
JN640723.1	Neoachirosetta milfordi voucher BW-A2251 cytochrome oxidase subunit 1 (COI) gene
JN640696.1	Mancopsetta maculata voucher BW-A2243 cytochrome oxidase subunit 1 (COI) gene
JN640575.1	Achirosetta tricholepis voucher BW-A4491 cytochrome oxidase subunit 1 (COI) gene
EU513696.1	Peltorhamphus novaezeelandiae isolate Pnov12 cytochrome oxidase subunit I (COI) gene
EU513693.1	Pelotretis flavilatus isolate Pfla11 cytochrome oxidase subunit I (COI) gene
EF609448.1	Rhombosolea tapirina voucher BW-2104 cytochrome oxidase subunit 1 (COI) gene
KJ669383.1	Ammotretis rostratus isolate CES-240 cytochrome oxidase subunit 1 (COI) gene
HQ945903.1	Marleyella bicolorata voucher ADC10_260.1 #1 cytochrome oxidase subunit 1 (COI) gene
HQ945815.1	Poecilopsetta natalensis voucher ADC10_260.3 #5 cytochrome oxidase subunit 1 (COI) gene
KP267665.1	Poecilopsetta praelonga isolate 74_CTWD cytochrome oxidase subunit I (COI) gene
DQ521023.1	Poecilopsetta hawaiiensis voucher BPBM:FR 347 cytochrome c oxidase subunit I (COI) gene
KF930279.1	Pleuronectes quadrituberculatus voucher KUT 383 cytochrome oxidase subunit 1 (COI) gene
FN688944.1	Hippoglossoides platessoides mitochondrial partial COI gene for cytochrome oxidase subunit I
KT247652.1	Atheresthes stomias voucher ATS-117 cytochrome oxidase subunit 1 (COI) gene
KX164003.1	Hippoglossus hippoglossus cytochrome oxidase subunit I (COI) gene
DQ242491.1	Verasper moseri cytochrome oxidase subunit I (COI) gene
GU804856.1	Hippoglossoides robustus voucher RUSALCA09-04 cytochrome oxidase subunit 1 (COI) gene
KP835306.1	Glyptocephalus stelleri isolate PKU_5922 cytochrome oxidase subunit I (COI) gene
JQ354149.1	Isopsetta isolepis voucher UW:110228 cytochrome oxidase subunit 1 (COI) gene
GU936488.1	Pleuronichthys coenosus voucher SIO 04-103 cytochrome oxidase subunit I (COI) gene
KF930052.1	Limanda limanda voucher KUT 5418 cytochrome oxidase subunit 1 (COI) gene
EU522920.1	Pleuronichthys decurrens cytochrome c oxidase subunit I (COI) gene
EU522918.1	Microstomus pacificus cytochrome c oxidase subunit I (COI) gene
KF929832.1	Embassichthys bathybius voucher KUT 2269 cytochrome oxidase subunit 1 (COI) gene
EU522919.1	Parophrys vetulus cytochrome c oxidase subunit I (COI) gene
EU524279.1	Platichthys flesus voucher ROM:ICH:BCF-0522-1 cytochrome oxidase subunit I (COI) gene
KJ128581.1	Pleuronectes platessa voucher NRM:49437 cytochrome oxidase subunit I (COI) gene
KF386376.1	Liopsetta pinnifasciata isolate 2K cytochrome c oxidase subunit 1 (COI) gene
EU513641.1	Hippoglossoides dubius isolate Hdub11 cytochrome oxidase subunit I (COI) gene
KJ450892.1	Hippoglossoides elassodon voucher ATS-79 cytochrome oxidase subunit 1 (COI) gene
EU513683.1	Reinhardtius hippoglossoides isolate Rh1111 cytochrome oxidase subunit I (COI) gene
JQ354287.1	Pleuronichthys verticalis voucher UW:119903 cytochrome oxidase subunit 1 (COI) gene
KM019221.1	Atheresthes evermanni voucher ATS-45 cytochrome oxidase subunit 1 (COI) gene

KJ128549.1	Microstomus kitt voucher NRM:47494 cytochrome oxidase subunit I (COI) gene
JF952817.1	Pseudopleuronectes herzensteini voucher MGR2 cytochrome oxidase subunit 1 (COI) gene
HM421795.1	Limanda proboscidea voucher RUSALCA09-173 cytochrome oxidase subunit 1 (COI) gene
KT920020.1	Pseudopleuronectes yokohamae isolate PYKEI24 cytochrome oxidase subunit I (COI) gene
FJ164711.1	Lepidopsetta polyxystra voucher TZ05-FROSTI-191 cytochrome oxidase subunit 1 (COI) gene
GU440343.1	Hippoglossus stenolepis voucher MFC345 cytochrome oxidase subunit 1 (COI) gene
EU752079.1	Eopsetta jordani voucher EOPSJORD-I94-002 CERT cytochrome oxidase subunit 1 (COI) gene
KF930326.1	Psettichthys melanostictus voucher KUT 430 cytochrome oxidase subunit 1 (COI) gene
JN204303.1	Pleuronichthys japonicus isolate P.sp.4 cytochrome oxidase subunit I (COI) gene
KF386358.1	Pseudopleuronectes obscurus isolate PO1-09 cytochrome c oxidase subunit 1 (COI) gene
EU752093.1	Glyptocephalus zachirus voucher ERREZACH 001 CERT cytochrome oxidase subunit 1 (COI) gene
HM180796.1	Pleuronichthys cornutus voucher NSMK:PI-000113 cytochrome oxidase subunit I (COI) gene
JF952718.1	Dexistes rikuzenius voucher MIGG1 cytochrome oxidase subunit 1 (COI) gene
EU513658.1	Lepidopsetta bilineata isolate Lbil cytochrome oxidase subunit I (COI) gene
EU522913.1	Clidoderma asperrimum cytochrome c oxidase subunit I (COI) gene
HM421779.1	Limanda sakhalinensis voucher RUSALCA09-157 cytochrome oxidase subunit 1 (COI) gene
KR052265.1	Limanda punctatissima isolate PKU 10862 cytochrome oxidase subunit I (COI) gene
KP835299.1	Eopsetta grigorjewi isolate PKU_4570 cytochrome oxidase subunit I (COI) gene
FJ164801.1	Lyopsetta exilis voucher TZ-06-RICKER-525 cytochrome oxidase subunit 1 (COI) gene
KR052261.1	Microstomus achne isolate PKUI 340 cytochrome oxidase subunit I (COI) gene
GU357849.1	Cleisthenes herzensteini voucher IOCASFY_Ch_001 cytochrome oxidase subunit I (COI) gene
JF952703.1	Cleisthenes pinetorum voucher SOHH1 cytochrome oxidase subunit 1 (COI) gene
DQ242490.1	Verasper variegatus cytochrome oxidase subunit I (COI) gene
GU440469.1	Pleuronichthys ritteri voucher MFC159 cytochrome oxidase subunit 1 (COI) gene
EU752153.1	Platichthys stellatus voucher PLATSTEL-H95-005 CERT cytochrome oxidase subunit 1 (COI) gene
KP835307.1	Tanakius kitaharae isolate PKU_6062 cytochrome oxidase subunit I (COI) gene
EU752157.1	Pseudopleuronectes americanus voucher PLEUAMER-H94-003 CERT cytochrome oxidase subunit 1 (COI) gene
EU752091.1	Glyptocephalus cynoglossus voucher GLYPCYNO-H94-003 CERT cytochrome oxidase subunit 1 (COI) gene
EU513661.1	Limanda aspera isolate Laspl1 cytochrome oxidase subunit I (COI) gene
EU752109.1	Limanda ferruginea voucher PLEUFERR-H94-003 CERT cytochrome oxidase subunit 1 (COI) gene
KT075304.1	Hippoglossina oblonga voucher USNM:FISH:433072 cytochrome oxidase subunit I (COI) gene
JQ354125.1	Hippoglossina stomata voucher UW:119910 cytochrome oxidase subunit 1 (COI) gene
KF461215.1	Paralichthys albigutta voucher FDA 103 cytochrome oxidase subunit 1 (COI) gene
KM077536.1	Paralichthys californicus voucher JUPA-822 cytochrome oxidase subunit 1 (COI) gene
KF930226.1	Paralichthys dentatus voucher KUT 1446 cytochrome oxidase subunit 1 (COI) gene
KF965291.1	Paralichthys isosceles isolate 2 cytochrome oxidase subunit I gene
KU236833.1	Paralichthys olivaceus voucher IOCASFY-RCB09-Po7 cytochrome oxidase subunit I (COI) gene
EU074520.1	Paralichthys orbignyanus isolate FARG286-07 cytochrome oxidase subunit I (COI) gene
JX124847.1	Paralichthys patagonicus voucher LBPV53033 cytochrome oxidase subunit 1 (COI) gene
KF930230.1	Paralichthys squamilentus voucher KUT 5205 cytochrome oxidase subunit 1 (COI) gene
KF809414.1	Pseudorhombus arsius cytochrome oxidase subunit 1 (COI) gene
KF965432.1	Pseudorhombus cinnamoneus isolate PKU 9390 cytochrome oxidase subunit I gene
JF494314.1	Pseudorhombus elevatus voucher ADC259.18#10 cytochrome oxidase subunit 1 (COI) gene
EU541312.1	Pseudorhombus malayanus isolate INAPKID-SIFT-93 cytochrome oxidase subunit I (COI) gene
KF489723.1	Pseudorhombus natalensis voucher ADC11_259.19 #10 cytochrome oxidase subunit 1 (COI) gene
KF965433.1	Pseudorhombus pentophthalmus isolate PKU 8682 cytochrome oxidase subunit I gene
GU440568.1	Xystreurus liolepis voucher MFC145 cytochrome oxidase subunit 1 (COI) gene
GU324179.1	Xystreurus rasile voucher DAAPV F13 cytochrome oxidase subunit I (COI) gene
JX983282.1	Cynoglossus cynoglossus voucher NF554 cytochrome oxidase subunit 1 (COI) gene
JF952714.1	Cynoglossus interruptus voucher GEK8 cytochrome oxidase subunit 1 (COI) gene
JF493328.1	Cynoglossus zanzibarensis voucher ADC09_261.9#9 cytochrome oxidase subunit 1 (COI) gene

JF493321.1	Cynoglossus lida voucher Smith 261.7 #2_05 cytochrome oxidase subunit 1 (COI) gene
JF493319.1	Cynoglossus capensis voucher ADC09_261.3#3 cytochrome oxidase subunit 1 (COI) gene
JF493318.1	Cynoglossus attenuatus voucher Smith 261.2 #5 cytochrome oxidase subunit 1 (COI) gene
JF493316.1	Cynoglossus attenuatus voucher Smith 261.2 #6_05 cytochrome oxidase subunit 1 (COI) gene
JF493312.1	Cynoglossus acaudatus voucher ADC08 Smith 261.1 #2 cytochrome oxidase subunit 1 (COI) gene
HQ945839.1	Cynoglossus marleyi voucher ADC10_261.8 #2 cytochrome oxidase subunit 1 (COI) gene
GU440541.1	Symphurus atricaudus voucher MFC045 cytochrome oxidase subunit 1 (COI) gene
GU225494.1	Symphurus civitatum voucher MX759 cytochrome oxidase subunit 1 (COI) gene
KP112241.1	Cynoglossus itinus voucher ihb201306666 cytochrome oxidase subunit 1 (COI) gene
KM538311.1	Cynoglossus sinusarabici voucher CySi200 cytochrome oxidase subunit 1 (COI) gene
KP975542.1	Cynoglossus senegalensis voucher FLID089 cytochrome oxidase subunit 1 (COI) gene
JX124904.1	Symphurus ginsburgi voucher LBPV51336 cytochrome oxidase subunit 1 (COI) gene
JX124905.1	Symphurus tessellatus voucher LBPV48594 cytochrome oxidase subunit 1 (COI) gene
JX260856.1	Cynoglossus elongatus voucher 7v cytochrome oxidase subunit 1 (COI) gene
KC015938.1	Symphurus diomedeanus voucher 06-455 cytochrome oxidase subunit 1 (COI) gene
JQ842337.1	Symphurus ommaspilus voucher CURA8170 cytochrome oxidase subunit 1 (COI) gene
JQ841019.1	Symphurus arawak voucher BZLW6287 cytochrome oxidase subunit 1 (COI) gene
JQ774555.1	Symphurus nigrescens voucher FCFPI043-18 cytochrome oxidase subunit 1 (COI) gene
KU170680.1	Symphurus longirostris voucher FAKU:135487 cytochrome oxidase subunit I (COI) gene
EU541318.1	Cynoglossus puncticeps isolate INAPKKD-SIFT-99 cytochrome oxidase subunit I (COI) gene
EU541317.1	Cynoglossus lingua isolate INAPKKD-SIFT-98 cytochrome oxidase subunit I (COI) gene
KT951838.1	Paraplagusia bilineata voucher PCB-GQD cytochrome oxidase subunit I (COI) gene
KT323970.1	Cynoglossus arel voucher COFMNG-FRM-04 cytochrome oxidase subunit 1 (COI) gene
KC900885.1	Symphurus leucochilus voucher NMMBP:17767 cytochrome c oxidase subunit 1 (COI) gene
KC900878.1	Symphurus microrhynchus voucher ASIZP:67658 cytochrome c oxidase subunit 1 (COI) gene
JN678775.1	Symphurus megasomus isolate I cytochrome oxidase subunit I (COI) gene
JQ842726.1	Symphurus plagiusa voucher SMSA7552 cytochrome oxidase subunit 1 (COI) gene
EU513629.1	Cynoglossus canariensis isolate Ccal2 cytochrome oxidase subunit I (COI) gene
EU513627.1	Cynoglossus browni isolate Cbroll1 cytochrome oxidase subunit I (COI) gene
EU595089.1	Cynoglossus puncticeps voucher MBCSC:Z711156 cytochrome oxidase subunit I (COI) gene
EU595088.1	Cynoglossus itinus voucher MBCSC:Z711035 cytochrome oxidase subunit I (COI) gene
KP244531.1	Cynoglossus carpenteri isolate KN52 cytochrome c oxidase subunit I (COI) gene
KP641367.1	Cynoglossus monodi isolate PKU_10423 cytochrome oxidase subunit I (COI) gene
KJ713179.1	Cynoglossus oligolepis voucher CO-01 cytochrome c oxidase subunit I (COI) gene
KF979127.1	Cynoglossus joyneri isolate PKU_1802 cytochrome oxidase subunit I (COI) gene
HQ711865.1	Cynoglossus lighti cytochrome oxidase subunit I (COI) gene
KF564301.1	Cynoglossus sinusarabici cytochrome oxidase subunit I (COI) gene
JQ738570.1	Cynoglossus purpureomaculatus isolate F00037 cytochrome oxidase subunit I (COI) gene
JN242743.1	Cynoglossus semilaevis voucher MBCSC:Fish:ZP1141202 cytochrome oxidase subunit I (COI) gene
FJ347908.1	Cynoglossus dubius voucher NBFGR:CD181 cytochrome c oxidase subunit I-like (COI) gene
KP266744.1	Cynoglossus macrolepidotus isolate 21_JL cytochrome oxidase subunit I
GU702347.1	Citharichthys spilopterus voucher LBP-41593 cytochrome oxidase subunit 1 (COI) gene
KC170027.1	Citharichthys gilberti voucher KU8493 cytochrome oxidase subunit 1 (COI) gene
KF930479.1	Syacium micrurum voucher KUT 5200 cytochrome oxidase subunit 1 (COI) gene
JX516097.1	Citharichthys darwini voucher galgwicth cytochrome oxidase subunit 1 (COI) gene
JX516100.1	Citharichthys minutus voucher n7528ac200 cytochrome oxidase subunit 1 (COI) gene
JX887478.1	Syacium maculiferum voucher gv85310sm60 cytochrome oxidase subunit 1 (COI) gene
KC170029.1	Syacium papillosum voucher KUT 5095 cytochrome oxidase subunit 1 (COI) gene
KF929880.1	Etropus crossotus voucher KUT 5244 cytochrome oxidase subunit 1 (COI) gene
JX516090.1	Etropus microstomus voucher KUT 1505 cytochrome oxidase subunit 1 (COI) gene
JX887475.1	Cyclosetta panamensis voucher gv85310cp160 cytochrome oxidase subunit 1 (COI) gene

JX516098.1	Citharichthys arctifrons voucher KUT 1468 cytochrome oxidase subunit 1 (COI) gene
KC170026.1	Citharichthys cornutus voucher KUT 5196 cytochrome oxidase subunit 1 (COI) gene
KF929768.1	Citharichthys xanthostigma voucher KUT 450 cytochrome oxidase subunit 1 (COI) gene
JX516094.1	Cyclopsetta chittendeni voucher KUT 5080 cytochrome oxidase subunit 1 (COI) gene
KF929766.1	Citharichthys sordidus voucher KUT 569 cytochrome oxidase subunit 1 (COI) gene
HQ987852.1	Citharichthys arenaceus voucher n7529af124 cytochrome oxidase subunit 1 (COI) gene
JX516095.1	Citharichthys arenaceus voucher n762bc130 cytochrome oxidase subunit 1 (COI) gene
JX887477.1	Syacium gunteri voucher n761f190 cytochrome oxidase subunit 1 (COI) gene
JX516089.1	Citharichthys platophrys voucher gv85310cp90 cytochrome oxidase subunit 1 (COI) gene
KF929767.1	Citharichthys stigmaeus voucher KUT 2844 cytochrome oxidase subunit 1 (COI) gene
GU702495.1	Citharichthys macrops voucher LBP-35209 cytochrome oxidase subunit 1 (COI) gene
JX887476.1	Cyclopsetta querna voucher JHLOW00205 cytochrome oxidase subunit 1 (COI) gene
KF930225.1	Paralichthys californicus voucher KUT 456 cytochrome oxidase subunit 1 (COI) gene
HM180528.1	Citharoides macrolepidotus voucher NSMK:PI-000059 cytochrome oxidase subunit I (COI) gene
GU804949.1	Citharoides macrolepis voucher ADC09_258.1#4 cytochrome oxidase subunit 1 (COI) gene
JF493719.1	Laeops nigromaculatus voucher ADC09_259.12#1 cytochrome oxidase subunit 1 (COI) gene
JF493287.1	Crossorhombus valderostratus voucher ADC259.7-2 cytochrome oxidase subunit 1 (COI) gene
JF493117.1	Chascanopsetta lugubris voucher Smith 259.6 #4_05 cytochrome oxidase subunit 1 (COI) gene
JF492977.1	Bothus pantherinus voucher ADC 259.5-2 cytochrome oxidase subunit 1 (COI) gene
JF492900.1	Arnoglossus capensis voucher Smith 259.1 #1_05 cytochrome oxidase subunit 1 (COI) gene
HQ945827.1	Crossorhombus valderostratus voucher ADC10_259.7 #4 cytochrome oxidase subunit 1 (COI) gene
HM421813.1	Laeops pectoralis voucher ADC09_259.13#1 cytochrome oxidase subunit 1 (COI) gene
GU224729.1	Bothus ocellatus voucher MFL862 cytochrome oxidase subunit 1 (COI) gene
GU225159.1	Bothus lunatus voucher MX193 cytochrome oxidase subunit 1 (COI) gene
KU176364.1	Psettina brevirectis voucher ADC2013 259.16 #1 cytochrome oxidase subunit 1 (COI) gene
KM538256.1	Bothus podas voucher BoPo21C cytochrome oxidase subunit 1 (COI) gene
KJ709702.1	Arnoglossus thori voucher MCF507042 cytochrome oxidase subunit 1 (COI) gene
KJ709697.1	Arnoglossus laterna voucher MCF507143 cytochrome oxidase subunit 1 (COI) gene
KF489624.1	Laeops pectoralis voucher ADC11_259.13 #3 cytochrome oxidase subunit 1 (COI) gene
KF489622.1	Laeops nigromaculatus voucher ADC11_259.12 #5 cytochrome oxidase subunit 1 (COI) gene
KF930512.1	Trichopsetta ventralis voucher KUT 5085 cytochrome oxidase subunit 1 (COI) gene
KF929672.1	Bothus robinsi voucher KUT 1169 cytochrome oxidase subunit 1 (COI) gene
KC015698.1	Monolene sessilicauda voucher 06-330 cytochrome oxidase subunit 1 (COI) gene
JQ840775.1	Bothus maculiferus voucher BZLW6106 cytochrome oxidase subunit 1 (COI) gene
JQ774973.1	Arnoglossus rueppelii voucher FCFOPB064-06 cytochrome oxidase subunit 1 (COI) gene
JQ774775.1	Arnoglossus imperialis voucher FCFOP69-06 cytochrome oxidase subunit 1 (COI) gene
JQ431491.1	Bothus mancus voucher MBIO1387.4 cytochrome oxidase subunit 1 (COI) gene
KP267582.1	Crossorhombus azureus isolate 734_QYP cytochrome oxidase subunit I (COI) gene
KP267575.1	Parabothus kiensis isolate 1026_SLNP cytochrome oxidase subunit I (COI) gene
KP266862.1	Psettina hainanensis isolate 2_HNJP cytochrome oxidase subunit I (COI) gene
EU513618.1	Bothus leopardinus isolate BleoII1 cytochrome oxidase subunit I (COI) gene
EU513603.1	Arnoglossus capensis isolate AcapII2 cytochrome oxidase subunit I (COI) gene
EF607331.1	Arnoglossus polyspilus isolate FSCS116-06 cytochrome oxidase subunit I (COI) gene
DQ521032.1	Parabothus chlorospilus voucher BPBM:FR 360 cytochrome c oxidase subunit I (COI) gene
DQ521030.1	Engyproson xenandrus voucher BPBM:FR 359 cytochrome c oxidase subunit I (COI) gene
DQ521029.1	Chascanopsetta prorigera voucher BPBM:FR 358 cytochrome c oxidase subunit I (COI) gene
DQ521028.1	Taeniopsetta radula voucher BPBM:FR 357 cytochrome c oxidase subunit I (COI) gene
KP244578.1	Laeops macrophthalmus isolate PK52 cytochrome c oxidase subunit I (COI) gene
KP244518.1	Chascanopsetta lugubris isolate AK115 cytochrome c oxidase subunit I (COI) gene
KP266859.1	Psettina iijimae isolate 1404_DBJP cytochrome oxidase subunit I
KP266855.1	Arnoglossus scapha isolate 205_DY cytochrome oxidase subunit I

KP266819.1	Crossorhombus kanekonis isolate 2089_DCYP cytochrome oxidase subunit I
KP266783.1	Arnoglossus tenuis isolate 2_XYSP cytochrome oxidase subunit I
KP266767.1	Engyprosonon macrolepis isolate 724_DL cytochrome oxidase subunit I
JN028438.1	Trinectes maculatus voucher NAFF 3077 cytochrome oxidase subunit 1 (COI) gene
GU225115.1	Achirus lineatus voucher ECO-CH-P5510E cytochrome oxidase subunit 1 (COI) gene
KF929924.1	Gymnachirus texae voucher KUT 5187 cytochrome oxidase subunit 1 (COI) gene
JN988777.1	Catathyridium jenynsii voucher LBPV-9849 cytochrome oxidase subunit 1 (COI) gene
JQ843095.1	Trinectes inscriptus voucher TOB9370 cytochrome oxidase subunit 1 (COI) gene
JQ841979.1	Trinectes maculatus voucher FCC8071 cytochrome oxidase subunit 1 (COI) gene
KT310067.1	Trinectes paulistanus voucher GEA.ICT 01710 cytochrome oxidase subunit I (COI) gene
KT310064.1	Trinectes microphthalmus voucher PNT.UERJ 362 cytochrome oxidase subunit I (COI) gene
KT310062.1	Hypoclinemus mentalis voucher PNT.UERJ 377 cytochrome oxidase subunit I (COI) gene
KT310058.1	Gymnachirus nudus voucher PNT.UERJ 470 cytochrome oxidase subunit I (COI) gene
KT310056.1	Soleonassus finis voucher MPEG 23849.2 cytochrome oxidase subunit I (COI) gene
KT310054.1	Apionichthys dumerili voucher GEA.ICT 01705 cytochrome oxidase subunit I (COI) gene
AF113206.1	Buglossidium luteum cytochrome b (cytb) gene
AF113205.1	Monochirus Hispidus cytochrome b (cytb) gene
AF113202.1	Microchirus variegatus cytochrome b (cytb) gene
AF113199.1	Microchirus ocellatus isolate 2 cytochrome b (cytb) gene
AF113196.1	Solea kleini cytochrome b (cytb) gene
AF113195.1	Solea lascaris cytochrome b (cytb) gene
AF113183.1	Solea solea cytochrome b (cytb) gene
AB125335.1	Monochirus hispidus mitochondrial cytb gene for cytochrome b
AB125333.1	Synaptura lusitanica mitochondrial cytb gene for cytochrome b
AB125332.1	Dicologlossa hexophthalma mitochondrial cytb gene for cytochrome b
AB125331.1	Dicologlossa cuneata mitochondrial cytb gene for cytochrome b
AB125330.1	Microchirus boscanion mitochondrial cytb gene for cytochrome b
AB125329.1	Microchirus azevia mitochondrial cytb gene for cytochrome b
AB125328.1	Solea kleinii mitochondrial cytb gene for cytochrome b
AB125327.1	Solea solea mitochondrial cytb gene for cytochrome b
AB125326.1	Solea senegalensis mitochondrial cytb gene for cytochrome b
AB125325.1	Solea lascaris mitochondrial cytb gene for cytochrome b
EU492127.1	Buglossidium luteum voucher MNHN 2005-1526 cytochrome b (cytb) gene
EU224070.1	Solea lascaris isolate PegLas-CB-01 cytochrome b (cytb) gene
DQ198002.1	Solea senegalensis cytochrome b (cytb) gene
DQ197963.1	Microchirus azevia cytochrome b (cytb) gene
AY164468.1	Synaptura lusitanica cytochrome b (cytb) gene
AY164467.1	Monochirus hispidus cytochrome b (cytb) gene
AY164465.1	Dicologlossa cuneata cytochrome b (cytb) gene
AY164464.1	Microchirus azevia cytochrome b (cytb) gene
AY170842.1	Microchirus boscanion cytochrome b (cytb) gene
EF456015.1	Pegusa cadenati voucher BMVP/0726 cytochrome b (cytb) gene
EF392624.1	Synapturichthys kleinii voucher BMVP/0602 cytochrome b (cytb) gene
EF439605.1	Dagetichthys lusitanica lusitanica voucher BMVP/1253 cytochrome b (cytb) gene
AF113194.1	Solea impar cytochrome b (cytb) gene
AF38430.2	Synaptura lusitanica cytochrome b (cytb) gene
JN225432.1	Solea aegyptiaca voucher 5029 cytochrome b (cytb) gene
AY164470.1	Scophthalmus rhombus cytochrome b (cytb) gene
AY164469.1	Lepidorhombus boscii cytochrome b (cytb) gene
EU224009.1	Lepidorhombus whiffiagonis isolate LepWhi-CB-01 cytochrome b (cytb) gene
FN688411.1	Phrynorhombus norvegicus mitochondrial partial cytb gene for cytochrome b

FJ515663.1	<i>Zeugopterus punctatus</i> cytochrome b (cytb) gene
AY164471.1	<i>Scophthalmus maximus</i> cytochrome b (cytb) gene
EU492273.1	<i>Psetta maxima</i> voucher NRM 52878 cytochrome b (cytb) gene
KF445174.1	<i>Pleuronectes quadrituberculatus</i> isolate 271 cytochrome b (cytb) gene
EU492285.1	<i>Hippoglossoides platessoides</i> voucher NRM 53129 cytochrome b (cytb) gene
HE964779.1	<i>Atheresthes stomias</i> partial cytb gene for cytochrome b
EU492256.1	<i>Hippoglossus hippoglossus</i> voucher NRM 53139 cytochrome b (cytb) gene
AB326991.2	<i>Verasper moseri</i> mitochondrial cytb gene for cytochrome b
DQ464121.1	<i>Hippoglossoides robustus</i> isolate 12 cytochrome b (cytb) gene
KF445207.1	<i>Glyptocephalus stelleri</i> isolate 46-08 cytochrome b (cytb) gene
EU224010.1	<i>Limanda limanda</i> isolate LimLim-CB-01 cytochrome b (cytb) gene
DQ464117.1	<i>Pseudopleuronectes schrenki</i> isolate 1 cytochrome b (cytb) gene
EU513812.1	<i>Microstomus pacificus</i> isolate Mpl3 tRNA-Glu gene
AB326983.2	<i>Lepidopsetta mochigarei</i> mitochondrial cytb gene for cytochrome b
AF113179.1	<i>Platichthys flesus</i> cytochrome b (cytb) gene
AY164472.1	<i>Pleuronectes platessa</i> cytochrome b (cytb) gene
KF445184.1	<i>Liopsetta pinnifasciata</i> isolate 13 cytochrome b (cytb) gene
AB326972.2	<i>Hippoglossoides dubius</i> mitochondrial cytb gene for cytochrome b
KF445223.1	<i>Hippoglossoides elassodon</i> isolate 34 cytochrome b (cytb) gene
KF445156.1	<i>Reinhardtius hippoglossoides</i> isolate 256 cytochrome b (cytb) gene
HE964777.1	<i>Atheresthes evermanni</i> partial cytb gene for cytochrome b
EU513809.1	<i>Microstomus kitt</i> isolate Mkl2 tRNA-Glu gene
AB326985.2	<i>Pseudopleuronectes herzensteini</i> mitochondrial cytb gene for cytochrome b
AB326984.2	<i>Pseudopleuronectes yokohamae</i> mitochondrial cytb gene for cytochrome b
KF007183.1	<i>Lepidopsetta polyxystra</i> isolate 13RS-185-16 cytochrome b (cytb) gene
JN204289.1	<i>Pleuronichthys japonicus</i> isolate 4 cytochrome b (cytb) gene
AB326986.2	<i>Pseudopleuronectes obscurus</i> mitochondrial cytb gene for cytochrome b
KF445209.1	<i>Glyptocephalus zachirus</i> isolate 112 cytochrome b (cytb) gene
AB326989.2	<i>Pleuronichthys cornutus</i> mitochondrial cytb gene for cytochrome b
AB126393.1	<i>Clidoderma asperimum</i> mitochondrial cytb gene for cytochrome b
KF445188.1	<i>Limanda sakhalinensis</i> isolate 71 cytochrome b (cytb) gene
KF445195.1	<i>Limanda punctatissima</i> isolate 86-07 cytochrome b (cytb) gene
KU315049.1	<i>Eopsetta grigorjewi</i> voucher JNU 0903 cytochrome b (Cytb) gene
AB326987.2	<i>Microstomus achne</i> mitochondrial cytb gene for cytochrome b
AB522946.1	<i>Verasper variegatus</i> mitochondrial Cytb gene for cytochrome b
KF445178.1	<i>Platichthys stellatus</i> isolate 4K cytochrome b (cytb) gene
KT920137.1	<i>Kareius bicoloratus</i> isolate KBTS2 cytochrome b (cytb) gene
AB326988.2	<i>Tanakius kitaharae</i> mitochondrial cytb gene for cytochrome b
KJ701367.1	<i>Glyptocephalus cynoglossus</i> cytochrome b (cytb) gene
KF386570.1	<i>Limanda aspera</i> isolate LA24VSL cytochrome b (CYTB) gene
KR422572.1	<i>Hippoglossina stomata</i> voucher UW:119910 cytochrome b gene
JQ182398.1	<i>Paralichthys californicus</i> haplotype Hap92 cytochrome b (cytb) gene
AB000667.1	<i>Paralichthys olivaceus</i> mitochondrial Cyt-b gene for cytochrome b
FJ264271.1	<i>Citharichthys sordidus</i> voucher UW:047316 cytochrome b gene
FJ264272.1	<i>Citharichthys sordidus</i> voucher UW:047317 cytochrome b gene
FJ264300.1	<i>Citharichthys sordidus</i> voucher UW:047667 cytochrome b gene
FJ264301.1	<i>Citharichthys sordidus</i> voucher UW:047668 cytochrome b gene
FJ264358.1	<i>Citharichthys stigmaeus</i> voucher UW:048784 cytochrome b gene
FJ264380.1	<i>Citharichthys stigmaeus</i> voucher UW:048811 cytochrome b gene
FJ786631.1	<i>Cynoglossus semilaevis</i> cytochrome b (cytb) gene
DQ197938.1	<i>Cynoglossus senegalensis</i> cytochrome b (cytb) gene

DQ197937.1	<i>Cynoglossus browni</i> cytochrome b (cytb) gene
DQ082908.1	<i>Symphurus atricaudus</i> cytochrome b (cytb) gene
KJ531265.1	<i>Cynoglossus senegalensis</i> cytochrome b (cytb) gene
JQ937271.1	<i>Cynoglossus roulei</i> cytochrome b (Cytb) gene
AY164466.1	<i>Citharus linguatula</i> cytochrome b (cytb) gene
EU513764.1	<i>Bothus podas</i> isolate BpodII1 tRNA-Glu gene
EU513761.1	<i>Arnoglossus rueppelii</i> isolate ArulI1 tRNA-Glu gene
EU513758.1	<i>Arnoglossus imperialis</i> isolate ArimIII2 tRNA-Glu gene
AY029189.1	<i>Arnoglossus thori</i> cytochrome b (cytb) gene
JQ939636.1	<i>Neoachirosetta milfordi</i> voucher LS715 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939635.1	<i>Mancopsetta maculata</i> voucher LS714 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939630.1	<i>Rhombosolea tapirina</i> voucher CL-P146 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939629.1	<i>Rhombosolea plebeia</i> voucher CL-P145 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939627.1	<i>Symphurus plagiusa</i> voucher GO610 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939591.1	<i>Symphurus civitatum</i> voucher KU5106 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939622.1	<i>Aseraggodes heemstrai</i> voucher KU4996 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939620.1	<i>Aseraggodes kobensis</i> voucher KU2476 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939621.1	<i>Heteromycteris japonicus</i> voucher KU2493 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939642.1	<i>Pegusa lascaris</i> voucher FMNH119725 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939641.1	<i>Brachirus annularis</i> voucher FMNH119730 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939624.1	<i>Soleichthys heterorhinos</i> voucher KU7229 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939623.1	<i>Pseudaesopia japonica</i> voucher KU2504 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939579.1	<i>Gymnachirus texae</i> voucher KU5187 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939578.1	<i>Gymnachirus melas</i> voucher KU5123 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939580.1	<i>Trinectes maculatus</i> voucher KU1501 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939577.1	<i>Achirus lineatus</i> voucher KU5115 cardiac muscle myosin heavy chain 6 (MYH6) gene
KF139971.1	<i>Citharoides macrolepidotus</i> isolate CmroA cardiac muscle myosin heavy chain 6 alpha (myh6) gene
JQ939619.1	<i>Scophthalmus aquosus</i> voucher KU1253 cardiac muscle myosin heavy chain 6 (MYH6) gene
JX189770.1	<i>Paralichthys dentatus</i> cardiac muscle myosin heavy chain 6 alpha (myh6) gene
JQ939638.1	<i>Psettodes erumei</i> voucher CL-P167 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939639.1	<i>Psettodes belcheri</i> voucher FMNH119721 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939617.1	<i>Poecilopsetta plinthus</i> voucher KU2473 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939618.1	<i>Samariscus xenicus</i> voucher KU2484 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939598.1	<i>Pseudorhombus pentophthalmus</i> voucher KU2481 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939590.1	<i>Cynoglossus interruptus</i> voucher KU2478 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939634.1	<i>Citharus linguatula</i> voucher LS596 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939588.1	<i>Citharoides macrolepis</i> voucher KU2468 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939601.1	<i>Atheresthes evermanni</i> voucher KU2075 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939616.1	<i>Poecilopsetta beani</i> voucher KU3271 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939600.1	<i>Xystreurus liolepis</i> voucher KU465 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939609.1	<i>Limanda limanda</i> voucher KU5418 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939606.1	<i>Hypsopsetta guttulata</i> voucher KU484 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939597.1	<i>Paralichthys californicus</i> voucher KU456 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939632.1	<i>Paralichthys albigutta</i> voucher LS172 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939612.1	<i>Platichthys stellatus</i> voucher KU637 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939605.1	<i>Hippoglossoides elassodon</i> voucher KU3175 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939611.1	<i>Parophrys vetulus</i> voucher KU3254 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939607.1	<i>Isopsetta isolepis</i> voucher KU431 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939615.1	<i>Psettichthys melanostictus</i> voucher KU583 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939608.1	<i>Lepidopsetta bilineata</i> voucher KU3231 cardiac muscle myosin heavy chain 6 (MYH6) gene

JQ939610.1	Microstomus pacificus voucher KU3210 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939602.1	Embassichthys bathybius voucher KU2269 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939603.1	Eopsetta jordani voucher KU3251 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939633.1	Lyopsetta exilis voucher LS301 cardiac muscle myosin heavy chain 6 (MYH6) gene
JX189771.1	Pseudopleuronectes americanus cardiac muscle myosin heavy chain 6 alpha (myh6) gene
EU001930.1	Pleuronectes platessa isolate R43 cardiac muscle myosin heavy chain 6 alpha (myh6) gene
JQ939582.1	Asterorhombus fijiensis voucher KU7102 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939626.1	Arnoglossus imperialis voucher GO358 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939585.1	Laeops kitaharae voucher KU2506 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939586.1	Psettina tosana voucher KU2511 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939640.1	Chascanopsetta lugubris voucher FMNH119729 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939587.1	Trichopsetta ventralis voucher KU5085 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939584.1	Bothus robinsi voucher KU1177 cardiac muscle myosin heavy chain 6 (MYH6) gene
JQ939583.1	Bothus lunatus voucher KU154 cardiac muscle myosin heavy chain 6 (MYH6) gene
JX190501.1	Bothus lunatus isolate BlunA cardiac muscle myosin heavy chain 6 alpha (myh6) gene
KC442209.1	Psettodes erumei recombination activating protein 1 (RAG1) gene
EF095644.1	Solea solea recombination-activating protein 1 (RAG1) gene
JQ938326.1	Pegusa lascaris voucher FMNH119725 recombination activating protein 1 (RAG1) gene
JQ938319.1	Soleichthys heterorhinos voucher KU7229 recombination activating protein 1 (RAG1) gene
JQ938318.1	Pseudaesopia japonica voucher KU2504 recombination activating protein 1 (RAG1) gene
JQ938317.1	Aseraggodes heemstrai voucher KU4996 recombination activating protein 1 (RAG1) gene
JQ938316.1	Heteromycteris japonicus voucher KU2493 recombination activating protein 1 (RAG1) gene
KF141177.1	Aseraggodes kobensis isolate AkobA recombination activating protein 1 (rag1) gene
KF312005.1	Scophthalmus rhombus recombination activating protein 1 (RAG1) gene
JX189932.1	Scophthalmus aquosus recombinase activating protein 1 (RAG1) gene
KF312004.1	Samariscus latus recombination activating protein 1 (RAG1) gene
KF312003.1	Samaris cristatus recombination activating protein 1 (RAG1) gene
JQ938315.1	Samariscus xenicus voucher KU2484 recombination activating protein 1 (RAG1) gene
JQ938314.1	Samariscus japonicus voucher KU2469 recombination activating protein 1 (RAG1) gene
JQ938313.1	Plagiopsetta glossa voucher KU2475 recombination activating protein 1 (RAG1) gene
JX190894.1	Samariscus latus isolate SlatA recombinase activating protein 1 (rag1) gene
JQ938327.1	Oncopsetta darwinii voucher INIDEP_T514-GO919 recombination activating protein 1 (RAG1) gene
KF311986.1	Mancopsetta maculata recombination activating protein 1 (RAG1) gene
KF141208.1	Citharichthys sordidus isolate CsorB recombination activating protein 1 (rag1) gene
KF141207.1	Citharichthys sordidus isolate CsorA recombination activating protein 1 (rag1) gene
JQ938312.1	Poecilopsetta plinthus voucher KU2473 recombination activating protein 1 (RAG1) gene
KF312001.1	Poecilopsetta beanii recombination activating protein 1 (RAG1) gene
FJ769824.1	Hippoglossus hippoglossus recombination activation protein 1 (RAG1) mRNA
KF312000.1	Limanda limanda recombination activating protein 1 (RAG1) gene
JQ938306.1	Embassichthys bathybius voucher KU2269 recombination activating protein 1 (RAG1) gene
JQ938311.1	Pleuronectes platessa voucher KU1845 recombination activating protein 1 (RAG1) gene
JQ938309.1	Hippoglossoides elassodon voucher KU3175 recombination activating protein 1 (RAG1) gene
JQ938310.1	Hypsopsetta guttulata voucher KU484 recombination activating protein 1 (RAG1) gene
JQ938305.1	Atheresthes evermanni voucher KU2075 recombination activating protein 1 (RAG1) gene
KF311999.1	Hippoglossus stenolepis recombination activating protein 1 (RAG1) gene
JQ938307.1	Eopsetta jordani voucher KU3251 recombination activating protein 1 (RAG1) gene
JQ938308.1	Glyptocephalus zachirus voucher KU3142 recombination activating protein 1 (RAG1) gene
KF141270.1	Lepidopsetta bilineata isolate LbilA recombination activating protein 1 (rag1) gene
KF312002.1	Poecilopsetta plinthus recombination activating protein 1 (RAG1) gene
AF137182.1	Pleuronectes americanus RAG1 protein (RAG1) gene
AF369067.1	Pseudopleuronectes americanus RAG1 (RAG1) gene

JX189930.1	<i>Paralichthys dentatus</i> recombinase activating protein 1 (RAG1) gene
KC442210.1	<i>Paralichthys olivaceus</i> recombination activating protein 1 (RAG1) gene
KJ551379.1	<i>Paralichthys orbignyanus</i> RAG1 gene
KF311996.1	<i>Pseudorhombus oligodon</i> recombination activating protein 1 (RAG1) gene
JQ938303.1	<i>Pseudorhombus pentophthalmus</i> voucher KU2481 recombination activating protein 1 (RAG1) gene
KF311997.1	<i>Xysteureys ioilepis</i> recombination activating protein 1 (RAG1) gene
KF311995.1	<i>Symphurus orientalis</i> recombination activating protein 1 (RAG1) gene
KF311994.1	<i>Paraplagusia japonica</i> recombination activating protein 1 (RAG1) gene
KF311993.1	<i>Cynoglossus lingua</i> recombination activating protein 1 (RAG1) gene
JQ938322.1	<i>Symphurus plagiusa</i> voucher GO610 recombination activating protein 1 (RAG1) gene
JQ938300.1	<i>Symphurus civitatum</i> voucher KU5106 recombination activating protein 1 (RAG1) gene
JQ938299.1	<i>Symphurus atricaudus</i> voucher KU504 recombination activating protein 1 (RAG1) gene
JQ938298.1	<i>Cynoglossus interruptus</i> voucher KU2478 recombination activating protein 1 (RAG1) gene
JX190892.1	<i>Symphurus atricaudus</i> isolate SartA recombinase activating protein 1 (rag1) gene
JQ938324.1	<i>Citharus linguatula</i> voucher LS596 recombination activating protein 1 (RAG1) gene
JQ938297.1	<i>Citharoides macrolepis</i> voucher KU2468 recombination activating protein 1 (RAG1) gene
KF141209.1	<i>Citharoides macrolepidotus</i> isolate CmroA recombination activating protein 1 (rag1) gene
JQ938292.1	<i>Asterorhombus fijiensis</i> voucher KU7102 recombination activating protein 1 (RAG1) gene
JQ938291.1	<i>Arnoglossus imperialis</i> voucher GO374 recombination activating protein 1 (RAG1) gene
JQ938294.1	<i>Laeops kitaharae</i> voucher KU2506 recombination activating protein 1 (RAG1) gene
KF311989.1	<i>Engyproson grandisquama</i> recombination activating protein 1 (RAG1) gene
KF311988.1	<i>Chascanopsetta lugubris</i> recombination activating protein 1 (RAG1) gene
AY308769.1	<i>Bothus lunatus</i> recombinase activating gene-1 (RAG1) gene
JQ938295.1	<i>Psettina tosana</i> voucher KU2511 recombination activating protein 1 (RAG1) gene
JQ938296.1	<i>Trichopsetta ventralis</i> voucher KU5085 recombination activating protein 1 (RAG1) gene
KF311987.1	<i>Arnoglossus laterna</i> recombination activating protein 1 (RAG1) gene
JQ938293.1	<i>Bothus robinsi</i> voucher KU1177 recombination activating protein 1 (RAG1) gene
JQ938290.1	<i>Gymnachirus texae</i> voucher KU5187 recombination activating protein 1 (RAG1) gene
JQ938289.1	<i>Gymnachirus melas</i> voucher KU5123 recombination activating protein 1 (RAG1) gene
JQ938288.1	<i>Achirus lineatus</i> voucher KU5115 recombination activating protein 1 (RAG1) gene
AY430224.1	<i>Trinectes maculatus</i> recombination activating protein 1 (RAG1) gene
JQ940106.1	<i>Psettodes belcheri</i> voucher FMNH119721 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940105.1	<i>Psettodes erumei</i> voucher CL-P167 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940108.1	<i>Pegusa lascaris</i> voucher FMNH119725 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940107.1	<i>Brachirus annularis</i> voucher FMNH119730 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940101.1	<i>Microchirus frechkopi</i> voucher LS619 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940089.1	<i>Soleichthys heterorhinos</i> voucher KU7229 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940088.1	<i>Solea solea</i> voucher KU1846 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940086.1	<i>Aseraggodes heemstrai</i> voucher KU4996 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940087.1	<i>Pseudaesopia japonica</i> voucher KU2504 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940085.1	<i>Heteromycteris japonicus</i> voucher KU2493 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940084.1	<i>Aseraggodes kobensis</i> voucher KU2476 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940082.1	<i>Lepidorhombus boscii</i> voucher KU3496 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940083.1	<i>Scophthalmus aquosus</i> voucher KU1253 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940081.1	<i>Samariscus xenicus</i> voucher KU2484 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940080.1	<i>Samariscus japonicus</i> voucher KU2469 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940079.1	<i>Plagiopsetta glossa</i> voucher KU2475 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940061.1	<i>Syacium micrurum</i> voucher KU5200 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940058.1	<i>Etropus crossotus</i> voucher KU5244 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
KC828827.1	<i>Citharichthys arcifrons</i> receptor-interacting serine-threonine kinase 4 (RIPK4) gene
JQ940057.1	<i>Cyclosetta chittendeni</i> voucher KU5080 receptor-interacting serine-threonine kinase 4 (RIPK4) gene

JQ940056.1 Citharichthys sordidus voucher KU3255 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940109.1 Oncopterus darwinii voucher INIDEP_T514-GO919 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940103.1 Neoachirosetta milfordi voucher LS715 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940102.1 Mancopsetta maculata voucher LS714 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940095.1 Rhombosolea plebeia voucher CL-P145 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940094.1 Rhombosolea leporina voucher CL-P144 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940078.1 Poecilopsetta plinthus voucher KU2473 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940077.1 Poecilopsetta beani voucher KU3271 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940066.1 Hippoglossus hippoglossus voucher KU5417 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940068.1 Isopsetta isolepis voucher KU431 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940070.1 Limanda limanda voucher KU5418 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940071.1 Microstomus pacificus voucher KU3210 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940062.1 Embassichthys bathybius voucher KU2269 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940072.1 Parophrys vetulus voucher KU3254 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940075.1 Pleuronectes platessa voucher KU1845 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940065.1 Hippoglossoides elassodon voucher KU3175 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940067.1 Hypsopsetta guttulata voucher KU484 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940076.1 Psettichthys melanostictus voucher KU583 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940063.1 Eopsetta jordani voucher KU3251 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940064.1 Glyptocephalus zachirus voucher KU3142 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940069.1 Lepidopsetta bilineata voucher KU3231 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940099.1 Lyopsetta exilis voucher LS301 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940073.1 Platicthys stellatus voucher KU637 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 Pseudopleuronectes americanus voucher KU1037-KU5419 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940074.1 Paralichthys albigutta voucher LS172 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940098.1 Paralichthys californicus voucher KU456 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940059.1 Pseudorhombus pentophthalmus voucher KU2481 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940092.1 Symphurus plagiusa voucher GO610 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940054.1 Symphurus civitatum voucher KU5106 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940053.1 Cynoglossus interruptus voucher KU2478 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 KC829006.1 Symphurus atricaudus receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940100.1 Citharus linguatula voucher LS596 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940052.1 Lepidoblepharon ophthalmolepis voucher KU2495 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940051.1 Citharoides macrolepis voucher KU2468 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940091.1 Arnoglossus imperialis voucher GO358 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940050.1 Psettina tosana voucher KU2511 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940049.1 Asterorhombus fijiensis voucher KU7102 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940048.1 Gymnarchus texae voucher KU5187 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940047.1 Gymnarchus melas voucher KU5123 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940046.1 Achirus lineatus voucher KU5115 receptor-interacting serine-threonine kinase 4 (RIPK4) gene
 JQ940224.1 Psettodes belcheri voucher FMNH119721 SH3 and PX domain-containing 3 protein (SH3PX3) gene
 JQ940223.1 Psettodes erumei voucher CL-P167 SH3 and PX domain-containing 3 protein (SH3PX3) gene
 JQ940199.1 Syacium micrurum voucher KU5200 SH3 and PX domain-containing 3 protein (SH3PX3) gene
 JQ940197.1 Etropus crossotus voucher KU5244 SH3 and PX domain-containing 3 protein (SH3PX3) gene
 JQ940194.1 Citharichthys arcifrons voucher KU1468 SH3 and PX domain-containing 3 protein (SH3PX3) gene
 JQ940196.1 Cyclopsetta chittendeni voucher KU5080 SH3 and PX domain-containing 3 protein (SH3PX3) gene
 JQ940195.1 Citharichthys sordidus voucher KU3255 SH3 and PX domain-containing 3 protein (SH3PX3) gene
 JQ940209.1 Lepidorhombus boscii voucher KU3496 SH3 and PX domain-containing 3 protein (SH3PX3) gene
 JX189524.1 Scopthalmus aquosus SH3 and PX3 domain-containing 3-like protein (SH3PX3) gene
 JQ940221.1 Neoachirosetta milfordi voucher LS715 SH3 and PX domain-containing 3 protein (SH3PX3) gene

JQ940220.1	Mancopsetta maculata voucher LS714 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940204.1	Hippoglossus hippoglossus voucher KU5417 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940207.1	Microstomus pacificus voucher KU3210 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940208.1	Parophrys vetulus voucher KU3254 SH3 and PX domain-containing 3 protein (SH3PX3) gene
EU002091.1	Pleuronectes platessa isolate R43 SH3 and PX domain-containing 3-like protein (SH3PX3) gene
JQ940203.1	Hippoglossoides elassodon voucher KU3175 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940205.1	Hypsopsetta guttulata voucher KU484 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940201.1	Eopsetta jordani voucher KU3251 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940202.1	Glyptocephalus zachirus voucher KU3142 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940206.1	Lepidopsetta bilineata voucher KU3231 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JX189523.1	Pseudopleuronectes americanus SH3 and PX3 domain-containing 3-like protein (SH3PX3) gene
JQ940217.1	Paralichthys albigutta voucher LS172 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JX189522.1	Paralichthys dentatus SH3 and PX3 domain-containing 3-like protein (SH3PX3) gene
JQ940198.1	Pseudorhombus pentophthalmus voucher KU2481 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940200.1	Xystreuryx liolepis voucher KU465 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JX191027.1	Symphurus atricaudus isolate SartA SH3 and PX3 domain-containing 3-like protein (SH3PX3) gene
JQ940218.1	Citharus linguatula voucher LS596 SH3 and PX domain-containing 3 protein (SH3PX3) gene
KF141445.1	Citharoides macrolepidotus isolate CmroA SH3 and PX3 domain-containing 3-like protein (SH3PX3) gene
JQ940225.1	Chascanopsetta lugubris voucher FMNH119729 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940213.1	Arnoglossus imperialis voucher GO358 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940193.1	Trichopsetta ventralis voucher KU5085 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940192.1	Psettina tosana voucher KU2511 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940191.1	Bothus lunatus voucher KU154 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940190.1	Asterorhombus fijiensis voucher KU7102 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940188.1	Trinectes maculatus voucher KU1501 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940187.1	Gymnarchus texae voucher KU5187 SH3 and PX domain-containing 3 protein (SH3PX3) gene
JQ940186.1	Achirus lineatus voucher KU5115 SH3 and PX domain-containing 3 protein (SH3PX3) gene
KF141478.1	Gymnarchus melas isolate GmelA SH3 and PX3 domain-containing 3-like protein (SH3PX3) gene

Appendix 3: Detail of Figure 3-A