

Origins and relationships of the Pleuronectoidei: Molecular and morphological analysis of living and fossil taxa

Matthew A. Campbell¹  | Bruno Chanet² | Jhen-Nien Chen¹ | Mao-Ying Lee¹  | Wei-Jen Chen¹ 

¹Institute of Oceanography, National Taiwan University, Taipei, Taiwan

²Département Origines et Évolution, Muséum National d'Histoire Naturelle, Paris, France

Correspondence

Wei-Jen Chen, Institute of Oceanography, National Taiwan University, Room 301, No.1 Sec. 4 Roosevelt Rd., Taipei 10617, Taiwan.

Email: wjchen.actinops@gmail.com

Funding information

Ministry of Science and Technology, Taiwan, Grant/Award Number: 102-2923-B-002 -001 and 107-2611-M-002-007; Fulbright Taiwan; Agence Nationale de la Recherche, Grant/Award Number: 12-ISV7-0005-01

Abstract

Flatfishes (Pleuronectiformes) are a species-rich and distinct group of fishes characterized by cranial asymmetry. Flatfishes occupy a wide diversity of habitats, including the tropical deep-sea and freshwaters, and often are small-bodied fishes. Most scientific effort, however, has been focused on large-bodied temperate marine species important in fisheries. Phylogenetic study of flatfishes has also long been limited in scope and focused on the placement and monophyly of flatfishes. As a result, several questions in systematic biology have persisted that molecular phylogenetic study can answer. We examine the Pleuronectoidei, the largest suborder of Pleuronectiformes with >99% of species diversity of the order, in detail with a multilocus nuclear and mitochondrial data set of 57 pleuronectoids from 13 families covering a wide range of habitats. We combine the molecular data with a morphological matrix to construct a total evidence phylogeny that places fossil flatfishes among extant lineages. Utilizing a time-calibrated phylogeny, we examine the timing of diversification, area of origin and ancestral temperature preference of Pleuronectoidei. We find polyphyly or paraphyly of two flatfish families, the Paralichthyidae and the Rhombosoleidae, and support the creation of two additional families—Cyclopsettidae and Oncopteridae—to resolve their non-monophyletic status. Our findings also support the distinctiveness of Paralichthodidae and refine the placement of that lineage. Despite a core fossil record in Europe, the observed recent diversity of pleuronectoids in the Indo-West Pacific is most likely a result of the Indo-West Pacific being the area of origin for pleuronectoids and the ancestral temperature preference of flatfishes is most likely tropical.

KEYWORDS

Ancestral Range Reconstruction, biogeography, Carangimorpharia, Indo-West Pacific, Pleuronectiformes, systematics

1 | INTRODUCTION

Flatfishes (Pleuronectiformes) are a very diverse order placed within a phenotypically varied assemblage of percomorph fishes known as Clade L, Carangimorphia or

Carangimorpharia (Betancur-R., Broughton, et al., 2013; Campbell, Chen, & López, 2013; Chen, Bonillo, & Lecointre, 2003; Dettai & Lecointre, 2005; Li et al., 2009; Near et al., 2012). More than 800 described species of flatfishes are currently classified in 14 taxonomic families centred in the

marine tropics (Appendix S1) (Hensley, 1997; Munroe, 2015a, 2015b). Scientific research has focused on temperate shallow water and commercially important flatfishes such as the Pleuronectidae, overlooking the bulk of diversity in this group (Gibson et al., 2015; Hensley, 1997; Munroe, 2015a). Due in part to this research bias, several questions have arisen and persisted regarding inter-relationships and a comprehensive hypothesis of the evolutionary origins and biogeography of the order has not been put forth. Hensley (1997) raised the following key questions which 20 years later still have not been satisfactorily answered: What are the intergeneric relationships of monophyletic families of flatfishes? What are the sister groups of genera excluded from redefined families? What is the phylogenetic placement of Achiropsettidae? What are the relationships of the traditionally recognized Citharidae? Hensley (1997) indicated the biogeography and evolutionary origins of the group also remain poorly characterized, and, in light of the core diversity of flatfishes being in the Indo-West Pacific (IWP) (Appendix S1), does that indicate the area of origin for the group? Thus, where did flatfish originate? When and how did they diversify? Molecular phylogenetic study should be an avenue to resolve some of the persistent questions regarding flatfishes.

Recent molecular study has instead focused on the placement and monophyly of the group, not on relationships within the flatfishes or large-scale biogeography (Betancur-R., Li, Munroe, Ballesteros, & Ortí, 2013; Betancur-R. & Ortí, 2014; Campbell et al., 2013; Campbell, Chen, & López, 2014; Campbell, López, Satoh, Chen, & Miya, 2014; Harrington et al., 2016; Shi et al., 2018). Flatfishes have been clearly identified as carangimorph fishes, for example Clade L of Chen et al. (2003), and while the monophyly of Pleuronectoidei has been consistently supported in molecular study, the monophyly of Pleuronectiformes (Psettoidei + Pleuronectoidei) has not, for example (Campbell et al., 2013; Campbell, Chen, et al., 2014; Campbell, López, et al., 2014). Thus, substantial scientific effort has been directed along this theme. For further discussion on the alternatives to monophyly of Pleuronectoidei or Pleuronectiformes from both anatomical and molecular perspectives see Campbell et al. (2013).

From an anatomical perspective, the monophyly of Pleuronectiformes was defined based on synapomorphies by Chapleau (1993). More recently, Chanet, Mondejar-Fernandez, and Lecointre (personal communication) reanalysed the data from Chapleau (1993) and concluded that the monophyly of the order was still ambiguous based on morphological study. While over time molecular studies have moved to widely incorporate diverse species of flatfishes, morphological efforts have focused on the reassessment of family-level classification schemes, for example (Chapleau, 1993; Cooper & Chapleau, 1998a; Hoshino, 2001). Similarly to molecular studies, effort has also been directed by anatomists to seek the sister group of flatfishes, for example (Friedman, 2008). The focus on

either family-level classification or identifying the sister group of flatfishes is understandable as substantial effort would be needed to identify characteristics required to refine intrafamilial relationships and to document these characteristics from numerous pleuronectiform taxa. As indicated by Hensley (1997), important lineages may be omitted from morphological studies of flatfishes due to lack of data, such as Achiropsettidae and Paralichthodidae from Chapleau (1993), leading to ambiguity in the recognition of these taxonomic units and their phylogenetic affinities. Molecular studies have provided several advances in flatfish taxonomy in part due to the wide sampling of lineages possible with molecular methods with comparatively lower effort. The main focus of flatfish phylogenetics overall—from both anatomical and molecular perspectives—has been the phylogenetic placement and monophyly of pleuronectiform fishes. By and large as a consequence, discussion of intraordinal relationships has been omitted and molecular data sets have not been leveraged to answer key questions of flatfish systematic biology despite the wide-ranging importance of flatfishes (Hensley, 1997; Munroe, 2015a).

We investigate the relationships of the Pleuronectoidei including sequences from previously unrepresented key lineages to more wholly address the inter-relationships of pleuronectoids and characterize their expansion into different habitats (freshwater, deep-sea). We combine morphological and molecular data at the family level with key fossil flatfishes to refine placement of fossils and provide a family-level taxonomy of the lineage. Utilizing fossil placement indicated by the total evidence approach we generate a time-calibrated phylogeny capturing the broad diversity of pleuronectoids and discuss the evolutionary origins and biogeography of the Pleuronectoidei.

2 | METHODS

2.1 | Sampling

Key lineages representing maximal diversity within pleuronectoids were targeted for sequencing or selected from existing data sets such as Campbell et al. (2013) (Appendix S2). Previously unrepresented lineages in phylogenetic study of pleuronectiforms inter-relationships included *Brachypleura novaezeelandiae* (Citharidae), *Paralichthodes algoensis* (Paralichthodidae) and *Pardachirus pavoninus* (Soleidae). Outgroups selected for rooting of Pleuronectoidei were *Psettodes erumei* (Pleuronectiformes: Psettoidei) and *Lates calcarifer* and *Centropomus undecimalis* (Centropomidae), taxa identified to be the potential sister group of flatfishes (Campbell et al., 2013). For each species of pleuronectoid examined, we noted if it occupies the deep-sea or freshwaters based on the information given in the FishBase (<http://www.fishbase.org/>) or the sample collection data from our biodiversity exploratory surveys entitled “*Tropical Deep-Sea*

Benthos" (<https://expeditions.mnhn.fr/>; Appendix S2). A species was referred to be deep-sea when the species bathymetric distribution range extends to 200 meter depth and below.

2.2 | Molecular phylogenetic data generation and alignment

Nine loci were targeted for retrieval from GenBank or sequencing. The loci included six nuclear protein-coding genes and three mitochondrial gene regions. The nuclear protein-coding genes were early growth response protein genes 1, 2B and 3 (*EGR1*, *EGR2B* and *EGR3*), mixed-lineage leukaemia (*MLL*), recombination activating gene 1 (*RAG1*) and rhodopsin (*RHI*). The experimental conditions followed Campbell et al. (2013) with primers described previously in appendix B of Campbell et al. (2013) (Chen et al., 2003; Chen, Lavoué, & Mayden, 2013; Chen, Miya, Saitoh, & Mayden, 2008; Chen, Ruiz-Carus, & Ortí, 2007; Dettai & Lecointre, 2005; López, Chen, & Ortí, 2004). Sequences from three mitochondrial gene regions were also obtained: 12S rRNA (12S), 16S rRNA (16S), and the first subunit of cytochrome c oxidase I (*COI*). Conditions and primers for mitochondrial loci amplification are described in Chen, López, Lavoué, Miya, and Chen (2014).

2.3 | Maximum-likelihood molecular phylogenetic data analysis

Maximum-likelihood (ML) inference was conducted with Randomized Axelerated Maximum-likelihood (RAxML) v. 7.4.2 (Stamatakis, 2006). Each of the seven protein-coding genes was partitioned by codon position (1,2,3), and 12S and 16S genes were partitioned independently but not subdivided. Nucleotide evolution in the resulting twenty-three partitions was modelled with a general time reversible (GTR) model with both a gamma-distributed rate variation (Γ) and a proportion of invariant sites (I). Confidence was assessed through 100 rapid bootstraps.

2.4 | Family-Level Relationships from Combined Evidence and Placement of Fossil Pleuronectoids

The classification of flatfishes has long been produced at a family level from morphological data with the most recent and relevant work following suit (Chanet, Chapleau, & Desoutter, 2004; Chapleau, 1993; Cooper & Chapleau, 1998a; Hoshino, 2001). Consequences include combining a family widely recognized as paraphyletic, Paralichthyidae, and a lack of representation of families due to missing data (i.e., Achirosettidae, Paralichthodidae). Developments in molecular phylogenetics have led to the incorporation of fossil data to allow divergence times to be estimated with a

relaxed-clock (Drummond, Ho, Phillips, & Rambaut, 2006; Ronquist et al., 2012). Calibration with fossils in these cases, however, requires that constraints be placed on the tree a priori. Therefore, the placement of fossils in trees in these cases requires assumptions about relationships. To clarify family-level relationships incorporating morphological data and to place key fossil taxa we produced a combined morphological and molecular data set at the family level for flatfishes.

A morphological data matrix of 49 characters was composed for families of flatfishes and the outgroup taxa in this study. The origins of characters are described and states presented in Appendix S3. Four fossil flatfishes were included, *Eobothus mimimus*[†] (Agassiz, 1833), *Oligobothus pristinus*[†] (Baciu & Chanet, 2002), *Numidiopleura enigmatica*[†] (Gaudant & Gaudant, 1969) and *Eobuglossus eocenicus*[†] (Woodward, 1910). Data on the osteology of these species were drawn from several sources (Baciu & Chanet, 2002; Chanet, 1994, 1999; Gaudant & Gaudant, 1969). We created a family-level combined data set by identifying families from our ML analysis previously described (Figure 1) and duplicating morphological data if needed with paraphyletic families (i.e., Paralichthyidae, Rhombosoleidae). Molecular sequences for families composed of two or more representatives in our data set were generated by computing marginal ancestral states (-f A) with RAxML version 8.0.19 (Stamatakis, 2014). This function in RAxML required a rooted reference tree (-t), and we provided the tree from Figure 1 with a partition of data based on each gene with a GTR+ Γ model of nucleotide evolution (-m GTRGAMMA). If a family had a single representative in our data set, for example Paralichthodidae, we used that sequence data to represent the family.

The combined morphological and molecular (total evidence) data set at the family level was then analysed in MrBayes version 3.2.6 (Ronquist et al., 2012). The morphological partition was assigned gamma-distributed rate variation (Γ , rates = gamma) and the coding indicated to be only variable states (coding = variable). Gene data were partitioned by protein-coding genes (seven partitions) and 12S and 16S combined (one partition) for a total of eight DNA sequence data partitions in the analysis that were unlinked. Nucleotide evolution was modelled under the GTR+ Γ model of sequence evolution with two separate runs with four chains with a length of 25,000,000 generations sampled every 1,000. A 25% burn-in was applied, and effective sample size (ESS) of each parameter was verified to be »200.

2.5 | Time-calibrated phylogenetic tree

We conducted relaxed-clock dating with MrBayes version 3.2.6 (Ronquist et al., 2012). Following the MrBayes version 3.2 manual, we constrained the ingroup, Pleuronectoidei, to be monophyletic. We then set the underlying strict clock

model (prset brlenspr = clock:uniform) and the independent gamma rates relaxed clock model (prset clockvarpr = igr). Four fossil calibrations were used with prior probability distributions given by exponential distributions described in Appendix S4. The combined data analysis (Figure 2) indicated that *Eobothus mimimus*[†], discovered in the Upper Eocene (50 mya) of Monte Bolca (Italy), dates the time to most recent common ancestor (TMRCA) of Bothidae, Pleuronectidae and Paralichthyidae. *Oligobothus pristinus*[†], from the Oligocene (30 mya) of Piatra Neamt, Romania, was used to date TMRCA of Bothidae and *Eobuglossus eocenicus*[†], discovered in the Upper Lutetian (45 mya) of Egypt, for Soleidae. The stratigraphy of *Numidiopleura enigmatica* is highly uncertain as the fossil was isolated and is lost; therefore, we did not include it as a calibration point though it may be considered to belong to the Eocene strata (34–56 mya) of Tunisia (Chanet, 1997; Gaudant & Gaudant, 1969). A fossil calibration in the outgroup taxa for Centropomidae (*Lates calcarifer* + *Centropomus undecimalis*) was also included (described in Appendix S4).

Initial MrBayes tree searches were partitioned and modelled following the ML analysis in the section “*Maximum-Likelihood Molecular Phylogenetic Data Analysis*.” Despite long run times, these analyses demonstrated low ESS values for the TH and TL parameters of MrBayes indicating over-parameterization. We reduced parameterization by generating an optimal partitioning strategy with PartitionFinder version 2.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012) by providing partitions by gene to PartitionFinder, except for 12S and 16S which were combined. Branch lengths were linked, the GTR+ Γ model was specified, RAxML indicated, a greedy search heuristic applied and model selection by the Bayesian information criterion (BIC) (Schwarz, 1978). The resulting “best scheme” was then specified in MrBayes. Subsequently, to decrease convergence time, we supplied the tree from Figure 1 as a starting tree. Sufficient ESS of parameters was reached through combining three separate runs of four chains of 40,000,000 generations sampled every 2,000 and applying a 10% burn-in.

2.6 | Biogeographic inference and ancestral temperature preference

By combining a time-calibrated hypothesis of relationships with current distribution data, it is possible to model the evolutionary history such as origin and historical biogeography of a group of organisms (Clark et al., 2008). We applied the Dispersal–Extinction–Cladogenesis (DEC) model with the program Lagrange C++ version 0.1 (Ree & Smith, 2008). For this analysis, we removed the two centropomid species from the time-calibrated tree and coded the distribution of flatfish species with eight different geographic regions. We defined biogeographic units as in Lavoué, Miya,

Musikasinthorn, Chen, and Nishida (2013) except we combined South Africa, Southern South America, South Australia and New Zealand regions into a single “Southern Ocean” biogeographic unit and did not make a separate Ponto–Caspian biogeographic unit from the Northeast Atlantic (including the Mediterranean). Species in our data set were coded as occurring in one or more of the eight biogeographic regions (Appendix S5). We did not constrain the number of ancestral states nor did we limit connectivity between biogeographic units when implementing the DEC model. We additionally instructed Lagrange to infer the ancestral states at all nodes in the tree.

To infer ancestral temperature preference, we first assigned a binary state of 0 for warm ($t > 25^{\circ}\text{C}$) or 1 for cool ($t < 25^{\circ}\text{C}$) to each species of pleuronectiform fish in our data set based on their distribution inside or outside the tropics, or if in tropics, if they are deep-sea species or not (Appendix S5). We consulted FishBase (www.fishbase.org) for information about species ranges and temperature preferences as well as the United Nations Food and Agriculture Organization FishFinder (FAO, 2019). We calculated ancestral temperature preferences utilizing the same time-calibrated tree as the DEC analysis with the Analyses of Phylogenetics and Evolution (ape) package version 5.2 in R version 3.4.1 (R Development Core Team, 2017). Three scenarios were evaluated with the ape package function, ancestral character estimation (ace). First, we computed ML estimations with a (a) symmetrical equal rates model (ER), and an (b) all rates different model (ARD) and conducted a likelihood test to see which was a better fit and compared standard errors of model output. Finally, we evaluated squared-change parsimony, setting all branch lengths to one and applying an ER ML model.

2.7 | Summary of pleuronectoid family diversity

We compiled a list of families, number of species, and number of species described in the last 10 years from the Catalog of Fishes (Fricke, Eschmeyer, & Fong, 2018). The Paralichthyidae was split along genera as indicated by Chapleau (1993), and the Rhombosoleidae II was defined to be monotypic containing only *Oncopterus darwinii*. We computed net diversification rates of species per million years (species/myr) by dividing the number of extant species per family by age of the family.

3 | RESULTS

3.1 | Maximum-likelihood phylogeny

The alignment of the data set combining nine genes is 7,122 characters long with 12.48% gaps or missing data and 4,138 distinct alignment patterns. The inferred phylogeny from

the data set is shown in Figure 1 with three monophyletic pleuronectoid superfamilies (tree with all nodes labelled is included in the Data Supplement). Support for monophyly of Citharoidea is not high, bootstrap support (BS) = 56%. Soleoidea and Pleuronectoidea receive more support, BS = 82% and 98%, respectively. Placement of the three superfamilies is high, BS ≥ 97%.

The earliest-branching lineage of Pleuronectoidea is Scopthalmidae, and Paralichthyidae is inferred as polyphyletic with strong support. Paralichthyidae I consists of the genera *Xystreurus*, *Pseudorhombus* and *Paralichthys*. Paralichthyidae I forms a sister clade to the Pleuronectidae and has strong support for placement and monophyly (BS = 100%). Paralichthyidae II is formed from the genera

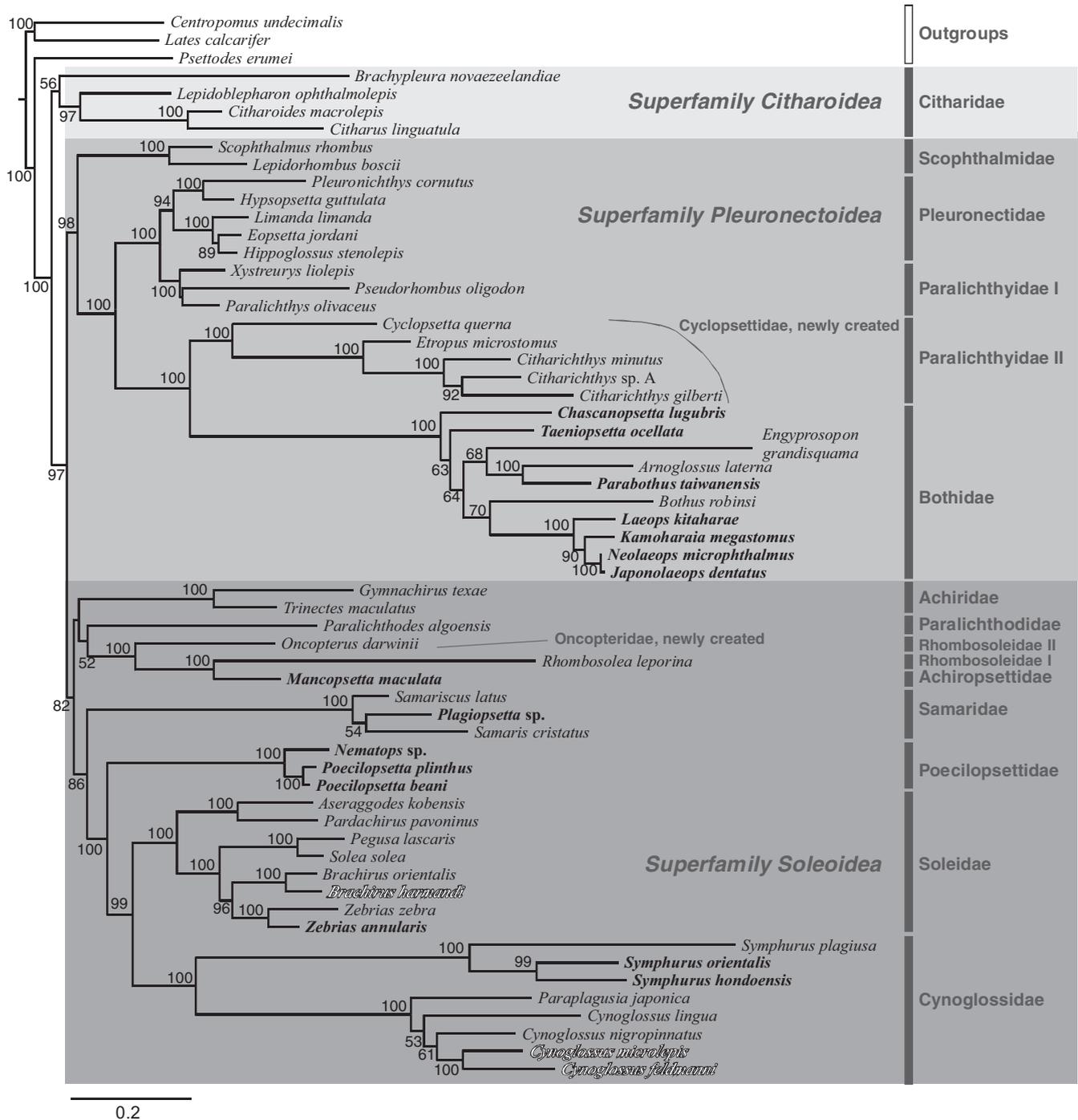


FIGURE 1 Maximum-likelihood (ML) phylogenetic tree of 57 pleuronectoids and three outgroup species. The three superfamilies of Citharoidea, Pleuronectoidea and Soleoidea are indicated by background shading. Recognized flatfish families are indicated by vertical bars and are labelled. Within non-monophyletic lineages, the monophyletic groupings containing the type genus of the family are indicated and labelled I, with the other lineage labelled II with the new name suggested. Bootstrap support values are indicated at nodes, with values less than 50 not shown

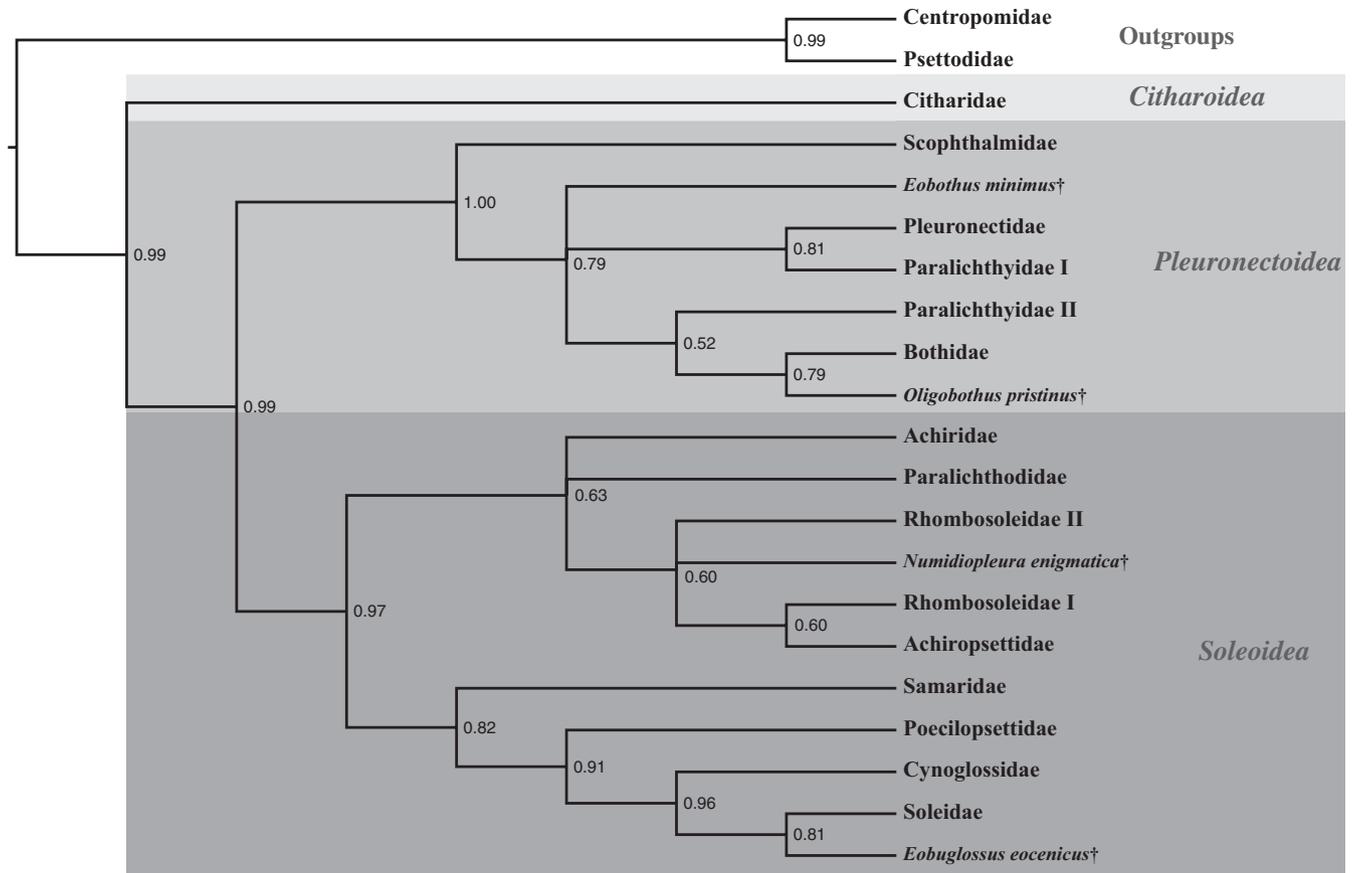


FIGURE 2 Bayesian consensus tree of combined morphological and molecular data for pleuronectoid families and four fossils (indicated by dagger, †) presented as a cladogram. Superfamilies are indicated by background shading. Poly- or paraphyletic recognized families are divided into I & II based on the inclusion of the type genera into I (i.e., *Paralichthys* in Paralichthyidae I, *Rhombosolea* in Rhombosoleidae I). Posterior probability values are shown at nodes

Cyclopsetta, *Etropus* and *Citharichthys* and receives strong support for monophyly (BS = 100%). Bothidae and Paralichthyidae II are sister clades with BS = 100%.

There is low support for two major divisions within Soleoidea. The first division consists of Achiridae, Paralichthodidae, Rhombosoleidae and Achiropsettidae. Support for monophyly of these four families is low (BS < 50%). Sampling two rhombosoleid lineages, *Oncopterus* and *Rhombosolea*, with the achiropsettoid *Mancopsetta maculata* again demonstrated non-monophyly of a pleuronectoid family. The rhombosoleid lineage *Rhombosolea leporina* is more closely related to *Mancopsetta maculata* than *Oncopterus darwini*, the other rhombosoleid lineage in this study, with support for the relevant nodes being maximal (BS = 100%). All other soleoid families form a single clade (BS = 86%).

3.2 | Family-level phylogeny and fossil placement

We generated a family-level alignment with 21 taxa and 7,270 total characters, 7,221 as DNA sequence data and 49

morphological characters. The family-level phylogeny resolved three superfamilies within Pleuronectoidei (Figure 2, nexus file provided to MrBayes and consensus tree file are included in the Data Supplement). The Citharoidea (Citharidae) is sister to Pleuronectoidea + Soleoidea with high support, posterior probability (PP) = 0.99. The monophyly of the Pleuronectoidea and Soleoidea are highly supported, PP = 1.00 and PP = 0.97, respectively (Figure 2).

Within Pleuronectoidea, the Scophthalmidae is sister to the remaining Pleuronectoidea, with strong support (PP = 1.00). *Eobothus mimimus*[†] is indicated to form part of a three-branch polytomy with, 1, (Pleuronectidae + Paralichthyidae I) and, 2, ((Bothidae + *Oligobothus pristinus*[†]) + Paralichthyidae II). The support for this arrangement is moderate (PP = 0.79).

Within Soleoidea, two major clades are indicated. One clade is comprised of Achiridae, Paralichthodidae, Rhombosoleidae (I & II), Achiropsettidae and *Numidiopleura enigmatica*[†] (PP = 0.63). The relationships between Achiridae, Paralichthodidae and (Rhombosoleidae + Achiropsettidae + *Numidiopleura enigmatica*[†]) are resolved as a polytomy. *Numidiopleura enigmatica*[†] is indicated to be

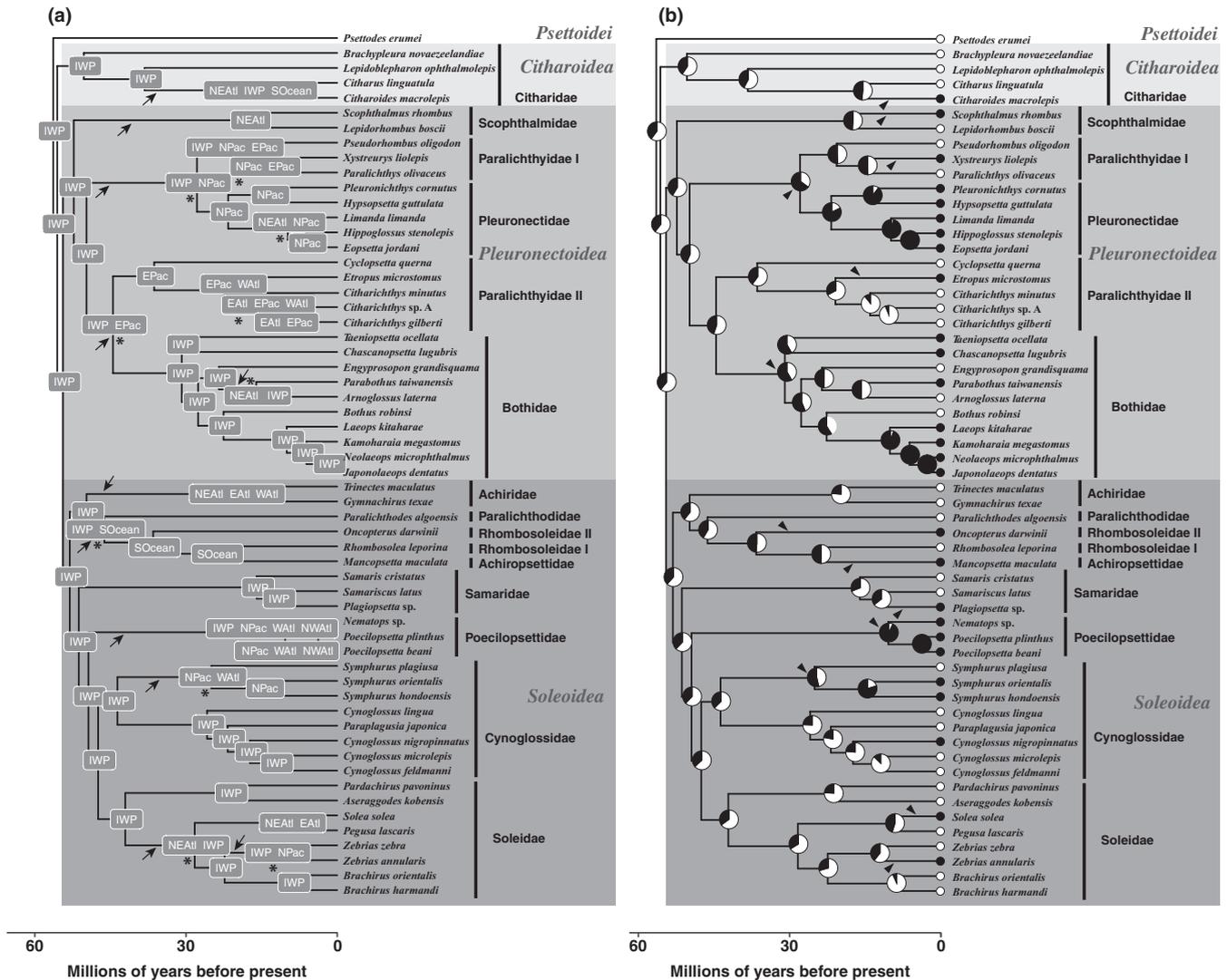


FIGURE 3 Time-calibrated phylogenetic tree with inferred ancestral ranges (a) or temperature preference (b) presented at nodes. The three superfamilies are shown through shading of the tree. Geographic ranges follow the following abbreviations: NEAtl = Northeast Atlantic; EAAtl = East Atlantic; IWP = Indo-West Pacific; NPac = North Pacific; EPac = East Pacific; WAAtl = West Atlantic; NWAtl = Northwest Atlantic; SOcean = Southern Ocean. Details on geographic region character states for each taxon are given in Supplementary Document S5. Black arrows indicate the dispersal events, and asterisks indicate subsequent allopatric cladogenesis. Temperature preference for warm ($>25^{\circ}\text{C}$) is coded white, cool ($<25^{\circ}\text{C}$) is coded black. Tip states are shown as filled circles of a single colour, with nodes as pie charts with the area proportional to the support for a particular state. Black triangles indicate transitions from tropical to temperate or deep-sea environments

closely related to Rhombosoleidae and Achirosettidae, again, as a polytomy (PP = 0.60). A second clade, composed of Samaridae, Poecilopsettidae, Cynoglossidae and (Soleidae + *Eobuglossus eocenicus*[†]) receives higher support (PP = 0.82) and has no internal polytomies. Soleidae and *Eobuglossus eocenicus*[†] are most closely related to each other with moderate support (PP = 0.81).

3.3 | Time-calibrated phylogenetic tree

Within the Pleuronectoidei, the three subfamilies of Citharoidea, Pleuronectoidea and Soleoidea are resolved with high support for monophyly and placement

(PP \geq 0.99, tree file and alternative presentations of values at nodes are provided in the Data Supplement). The time to most recent common ancestor (TMRCA) of Pleuronectoidei, dating the split between Citharoidea and (Pleuronectoidea + Soleoidea) is estimated to be 55.54 million years ago (MYA) with a 95% highest posterior density, 95% HPD, of (52.21–59.53). The TMRCA of Citharoidea in our data set is 50.27 MYA (42.7–56.63). Pleuronectoidea and Soleoidea diverge at 54.41 MYA (51.34–58.21), with the TMRCA of each of those lineages being 52.26 MYA (49.69–55.48) and 53.06 MYA (49.57–57.03), respectively. The topology of Pleuronectoidea with Scopthalmidae as the earliest-branching lineage, a sister relationship between

TABLE 1 Summary of pleuronectoid family higher classification (superfamily assignment), divergence time of families from sister lineage, time to most recent common ancestor (TMRCA) of families, total species, species described in the last 10 years and net diversification rate for each family (species/million years). For time estimates, we provide the 95% highest posterior density (95% HPD) and use those ranges in calculation of net diversification rate. The number of species for each family and number of species described in the last 10 years was taken from the California Academy of Sciences Catalog of Fishes (Fricke et al., 2018). Non-monophyletic families were split along genera described in the text

| Superfamily | Family | Divergence from sister lineage | 95% HPD range | TMRCA for species in dataset | 95% HPD range | Total species | Species described in last 10 years | Species/million years | 95% HPD high | 95% HPD low |
|-----------------|-------------------------------------|--------------------------------|---------------|------------------------------|---------------|---------------|------------------------------------|-----------------------|--------------|-------------|
| Citharoidea | Citharidae | 55.54 | 52.21–59.53 | 50.27 | 42.70–56.63 | 6 | 0 | 0.11 | 0.10 | 0.11 |
| Pleuronectoidea | Scophthalmidae | 52.26 | 49.59–55.48 | 17.44 | 9.53–26.92 | 9 | 0 | 0.17 | 0.16 | 0.18 |
| | Pleuronectidae | 27.83 | 20.52–34.68 | 21.84 | 15.03–28.54 | 65 | 2 | 2.34 | 1.87 | 3.17 |
| | Paralichthyidae I | 27.83 | 20.52–34.68 | 20.56 | 13.24–27.50 | 64 | 0 | 2.30 | 1.85 | 3.12 |
| | Paralichthyidae II (Cyclopsettidae) | 44.48 | 38.98–49.34 | 36.32 | 27.88–43.89 | 45 | 1 | 1.01 | 0.91 | 1.15 |
| | Bothidae | 44.48 | 38.98–49.34 | 30.85 | 29.62–34.32 | 172 | 5 | 3.87 | 3.49 | 4.41 |
| Soleoidea | Achiridae | 49.73 | 44.22–54.97 | 20.14 | 11.19–29.13 | 35 | 2 | 0.70 | 0.64 | 0.79 |
| | Paralichthodidae | 46.19 | 39.18–52.49 | - | - | 1 | 0 | 0.02 | 0.02 | 0.03 |
| | Rhombosoleidae I | 24.06 | 14.15–33.55 | - | - | 18 | 0 | 0.75 | 0.54 | 1.27 |
| | Rhombosoleidae II (Oncopteridae) | 36.51 | 27.57–45.02 | - | - | 1 | 0 | 0.03 | 0.02 | 0.04 |
| | Achirosettidae | 24.06 | 14.15–33.55 | - | - | 4 | 0 | 0.17 | 0.12 | 0.28 |
| | Samaridae | 51.26 | 47.37–55.49 | 15.98 | 9.02–23.41 | 29 | 4 | 0.57 | 0.52 | 0.61 |
| | Poecilopsettidae | 49.36 | 45.35–53.58 | 10.33 | 4.65–16.27 | 21 | 1 | 0.43 | 0.39 | 0.46 |
| | Soleidae | 47.44 | 43.41–51.67 | 42.06 | 41.20–44.53 | 182 | 4 | 3.84 | 3.52 | 4.19 |
| | Cynoglossidae | 47.44 | 43.41–51.67 | 43.61 | 37.88–48.78 | 159 | 8 | 3.35 | 3.08 | 3.66 |

Pleuronectidae and Paralichthyidae I, and Bothidae and Paralichthyidae receive maximal support (PP = 1.00). The monophyly of Achiridae, Paralichthodidae, Rhombosoleidae (I & II) and Achirosetidae is well supported (PP = 0.84). Support for the arrangement of (Samaridae (Poecilopsettidae (Soleidae + Cynoglossidae))) is found with relevant posterior probabilities maximal (PP = 1.00).

3.4 | Biogeographic inference and ancestral temperature preference

The DEC model supported an origin of the Pleuronectiformes and Pleuronectoidei in the IWP (Figure 3a). Furthermore, the ancestral state for all three superfamilies (Citharoidea, Pleuronectoidea, and Soleoidea) and five families (Citharidae, Bothidae, Samaridae, Cynoglossidae, and Soleidae) of pleuronectoids was also inferred to be an IWP distribution. Based on our analysis, a minimum of 11 “out of the IWP” dispersal or range expansion events occurred during pleuronectoid evolutionary history, and most of them predated the end of the Eocene period (around 34 MYA) when the global climate was still warm (Figure 3a). The ancestral temperature preference estimation found the ER model to be preferred over the ARD model, with a small standard error (0.01). Squared-change parsimony produced large standard error (0.16) but is in agreement (ER estimation at root, tropical 0.60, squared-change parsimony 0.77). Ancestral temperature preference of pleuronectiforms and pleuronectoids are most likely both tropical, with ER model results presented in Figure 3b. Here, we inferred at least 13 transitions from tropical to temperate or deep-sea environments within all three superfamilies. These events are not synchronized with biogeographic transitions and predominantly occurred during the Eocene–Oligocene transition or later (Figure 3b). The global temperature decline during and after the Eocene–Oligocene transition might play an important role shaping the present pattern of pleuronectoid diversity with later diversifications through a local (cold) adaptation or allopatric speciation due to climate change (Figure 3).

3.5 | Summary of pleuronectoid family diversity

A summary of pleuronectoid family diversity and relative diversification rates is presented in Table 1 and graphically in Appendix S6. Net diversification rates range from 0.02 species/myr (Paralichthodidae) to 3.87 species/myr (Bothidae). The lowest net diversification rates are exhibited by monotypic families (Paralichthodidae, Rhombosoleidae II) and the Citharoidea. Of the 857 pleuronectoidei species described to date, ~63% (513) are from the Bothidae, Soleidae and

Cynoglossidae with those three families exhibiting the highest net diversification rates (Table 1; Appendix S6).

4 | DISCUSSION

4.1 | Pleuronectoidei taxonomy

Flatfish taxonomy has long overlooked the family-level diversity present in Pleuronectoidei. The earliest flatfish classification simply placed all fifteen known species into a single genus, *Pleuronectes*, within the order Thoracici without any family divisions present (Linnaeus, 1758). Later, Pleuronectidae of Cuvier contained five subfamilies, the Cynoglossinae, Hippoglossinae, Platessinae, Pleuronectinae and Soleinae (Cuvier, 1817). Additional complexity with flatfish taxonomy was advanced with the concept of flatfishes as a suborder, the Heterosamata, and two families—the Pleuronectidae and Soleidae (Jordan & Evermann, 1896). Subsequently, Regan (1910) proposed two suborders of the Psettodoidea with the single family of Psettodidae and the Pleuronectoidea with two divisions (Pleuronectiformes and Soleiformes). The Pleuronectiformes of Regan (1910) had two families, the Bothidae and Pleuronectidae, and a Soleiformes with two families, the Soleidae and Cynoglossidae. Since then, the largest divisions within the suborder Pleuronectoidei have followed a division between pleuronectids and their relatives (superfamily Pleuronectoidea; Nelson (2006)) and soleids and their relatives (superfamily Soleoidea; Nelson (2006)) such as Norman (1934) proposing three families for the flatfish lineage, Psettodidae, Bothidae and Pleuronectidae. From the two divisions with Pleuronectoidei, the distinction of Citharidae became evident in further investigations. Hubbs (1945) defined a Citharidae (Citharinae + Brachypleurinae) closely related to Scopthalmidae, and these being most closely related to all other Pleuronectoidea indicating another major division within the flatfish lineage and a concept that has been reflected in subsequent classifications (Chapleau, 1993; Hensley & Ahlstrom, 1984).

The citharids composed of the genera *Brachypleura*, *Citharoides*, *Citharus*, *Lepidoblepharon*, and *Paracitharus* were demonstrated by Hoshino (2001) to be monophyletic—contradicting Chapleau (1993) who indicated they were paraphyletic—and the earliest-branching lineage of Pleuronectoidei. The current taxonomy of the Pleuronectoidei may be characterized by three superfamilies: Citharoidea, Pleuronectoidea and Soleoidea (Chanet et al., 2004; Hoshino, 2001; Nelson, 2006). In this study, we find support for these three superfamilies.

4.2 | Citharoidea

The monophyly of Citharoidea (Citharidae) receives low support in (BS% = 56) in the concatenated ML analysis.

Sampling of this family in previous multilocus studies, for example (Betancur-R., Broughton, et al., 2013; Betancur-R., Li, et al., 2013; Campbell et al., 2013; Harrington et al., 2016) have found high support for monophyly of Citharidae. Low support for the monophyly of this family in molecular phylogenetic study was previously shown in mitogenomic phylogenies (Campbell, López, et al., 2014), sampling to date, however, has not included more than three genera of this lineage. This study includes *Brachypleura*, representing four of the five genera in the family, only leaving *Paracitharus* unsampled. The earliest-branching lineage of the family based on these four genera and the molecular data presented here is *Brachypleura*. Consequently, the low support for monophyly of this family here may be a result of the short internodes in the tree in this region. Numerous studies have doubted the monophyly of Citharidae, for example (Chapleau, 1993; Cooper & Chapleau, 1998b; Hensley, 1997; Hensley & Ahlstrom, 1984); however, Hoshino (2001) established synapomorphies for Citharidae and indicated them to be monophyletic.

4.3 | Pleuronectoidea

Pleuronectoidea is identified to contain the families Scopthalmidae, Pleuronectidae, Paralichthyidae and Bothidae. Scopthalmidae is the earliest-branching lineage of the Pleuronectoidea and sister to the remainder of this clade with high support (Figures 1 and 2). This placement receives support from several studies (Betancur-R., Li, et al., 2013; Campbell et al., 2013; Harrington et al., 2016; Near et al., 2012), but is not widely supported across multilocus and mitochondrial DNA-based studies. Alternative placements of Scopthalmidae closely related to Rhombosoleidae and Achiropsettidae are found in other data sets, not exclusively those based on mitochondrial genomes (Betancur-R., Broughton, et al., 2013; Campbell, Chen, et al., 2014; Shi et al., 2018). Some morphological classifications have indicated that Scopthalmidae is closely related to Citharidae, for example (Chapleau, 1993; Hubbs, 1945); however, the most recent studies provide additional evidence that Scopthalmidae is a lineage within Pleuronectoidea (Chanet et al., 2004; Hoshino, 2001).

Early flatfish classification schemes placed all species in Pleuronectidae and it continued to contain many fishes of uncertain affinity. More recent efforts have advanced the understanding of composition and inter-relationships within this family (Cooper & Chapleau, 1998a; Vinnikov, Thomson, & Munroe, 2018). The placement of Pleuronectidae as the sister lineage to one lineage of the polyphyletic Paralichthyidae (Paralichthyidae I) and Bothidae to another (Paralichthyidae II) in this study was demonstrated in early molecular phylogenetic studies and has continued to be apparent with larger samplings of lineages and characters, for example

(Berendzen & Dimmick, 2002; Campbell, López, et al., 2014; Harrington et al., 2016). Non-monophyly of paralichthyids is well known from both anatomical and molecular studies (Azevedo, Oliveira, Pardo, Martínez, & Foresti, 2008; Berendzen & Dimmick, 2002; Chanet et al., 2004; Chapleau, 1993; Khidir, Chapleau, & Renaud, 2005; Pardo et al., 2005). The affinities of some paralichthyids with pleuronectids and some with bothids have long been indicated by classifications, with all or some of these fishes being considered most closely related to one or both of these other families, for example (Hensley, Amaoka, Hensley, Moser, & Sumida, 1984; Hubbs, 1945; Norman, 1934). We identify a clade including *Paralichthys* and relatives (*Paralichthys*, *Pseudorhombus*, *Xystreureys*)—Paralichthyidae I in this study—as sister to the Pleuronectidae. Paralichthyidae II is composed of *Cyclopsetta*, *Etropus* and *Citharichthys* in this study and is sister to the Bothidae (Figures 1 and 2).

A polyphyletic Paralichthyidae may be resolved through the creation of two families, Paralichthyidae and Cyclopsettidae. Previously, Hensley and Ahlstrom (1984) indicated a *Cyclopsetta* group and a *Pseudorhombus* group. Based on our analyses and others, Paralichthyidae presumably contains 10 genera including the *Pseudorhombus* group: *Ancyllopsetta*, *Cephalopsetta*, *Gastropsetta*, *Hippoglossina*, *Lioglossina*, *Paralichthys*, *Pseudorhombus*, *Tarphops*, *Verecundum* and *Xystreureys* (Chapleau, 1993). Cyclopsettidae contains at least four genera, *Cyclopsetta*, *Etropus* and *Citharichthys*, which were examined in this study, and *Syacium*. *Syacium*, while not examined here, based on previous anatomical and molecular work should be considered a member of Cyclopsettidae (Chapleau, 1993; Hensley & Ahlstrom, 1984; Pardo et al., 2005). Within the Cyclopsettidae, the monophyly of genera requires further evaluation, for example (Azevedo et al., 2008; Betancur-R., Broughton, et al., 2013; Betancur-R., Li, et al., 2013). Morphological support for a sister relationship between Cyclopsettidae and Bothidae, as shown in this study, is present as both clades have vertebral apophyses and lack a first neural spine (Hensley & Ahlstrom, 1984).

We included two fossils in our total evidence analysis that belong in the Pleuronectoidea (Figure 2). The relationships of *Eobothus minimus*[†] have long been uncertain, and the most-resolved placement of *Eobothus minimus*[†] to date has been as a member of the Pleuronectoidea. *Eobothus minimus*[†] had previously been indicated to be a bothid species (Berg, 1940, 1941; Blot, 1980; Norman, 1934; Patterson, 1993b), a scopthalmid species (Chabanaud, 1936, 1940, 1949) and later a crown pleuronectoid (Chanet, 1997). *Eobothus minimus*[†] may not be considered a member of an extant family, as it shares derived characteristics with Bothidae, Pleuronectidae, Paralichthyidae (I & II), Scopthalmidae and *Brachypleura* (Chanet, 1999). Chanet (1999) considered *Eobothus minimus*[†] to be *incertae sedis* within a “bothoid” lineage of *Brachypleura*, Scopthalmidae, Paralichthyidae (I & II),

Bothidae and Pleuronectidae. Here, we refine the placement so that *Eobothus minimus*[†] forms part of a three-branch polytomy (*Eobothus minimus*[†], (Pleuronectidae, Paralichthyidae I), (Bothidae, Paralichthyidae II)). *Eobothus minimus*[†] is more closely related to Bothidae, Paralichthyidae (I & II) and Pleuronectidae than Scophthalmidae or *Brachypleura*. The results of our total evidence analysis indicate that *E. minimus*[†] is most likely a member of an extinct family-level lineage within Pleuronectoidea. *Oligobothus pristinus*[†] has previously been clearly placed in the Bothidae and we recover that relationship (Baciu & Chanet, 2002).

4.4 | Soleoidea

The third superfamily in Pleuronectoidei is composed of eight families in this study and its monophyly is well supported (Figure 1, BS = 82%, Figure 2, PP = 0.97). There is weak support in the ML analysis (Figure 1, BS = 35%) and moderate support in the Bayesian combined approach (Figure 2, PP = 0.63) for a monophyletic group of Achiridae, Paralichthodidae, Rhombosoleidae and Achiropsettidae and *Numidiopleura enigmatica*[†]. This study includes representation of Achiridae, Paralichthodidae, the two divergent lineages of Rhombosoleidae, and Achiropsettidae for the first time to our knowledge in a phylogenetic study and indicates these families in total may form a monophyletic assemblage and Rhombosoleidae is paraphyletic.

Support for arrangements among the families Achiridae, Paralichthodidae, Rhombosoleidae and Achiropsettidae is low, indicated by support values in the ML analysis and a polytomy in the Bayesian combined analysis. The placement of Achiridae as most closely related to Soleidae + Cynoglossidae is well supported from a morphological perspective (Chanet et al., 2004; Chapleau, 1993; Hoshino, 2001). Nonetheless, here and other studies Achiridae is shown to be closely related to Paralichthodidae, Rhombosoleidae and Achiropsettidae (Betancur-R., Li, et al., 2013; Harrington et al., 2016), though see Shi et al. (2018) for another placement. Chapleau and Keast (1988) indicate a relationship of (Samaridae (Achiridae (Soleidae + Cynoglossidae))). It may be possible that this disagreement between placements of Achiridae may result from the particular species examined in anatomical works. Paralichthodidae has held different placements in alternative classification schemes, with integration into Chapleau (1993)'s data set placing it as a family and earliest-branching member of Soleoidea (Cooper & Chapleau, 1998b). Our analysis refines placement of the lineage to indicate near relatives of Achiridae, Rhombosoleidae and Achiropsettidae.

Rhombosoleidae is demonstrated to be paraphyletic, with *Rhombosolea* most closely related to *Mancopsetta* with maximal support values in the ML analysis (BS = 100%) and moderate support in the Bayesian combined analysis (PP = 0.60). Sampling in mitogenomic studies have not

included *Oncopterus*, but show very high support for a sister relationship between Rhombosoleidae and Achiropsettidae (Campbell, López, et al., 2014; Shi et al., 2018). *Oncopterus darwinii* is unlike other rhombosoleids as it is found in the Southwest Atlantic and all other rhombosoleids are distributed mainly around Australia and New Zealand (Nelson, 2006). In our time-calibrated tree, this monotypic genus is indicated to diverge as an independent lineage 36.51 mya as well. As a resolution for the paraphyly of Rhombosoleidae, we suggest that the monotypic family Oncopteridae be created containing the single species *Oncopterus darwinii*. Rhombosoleidae then includes nine genera and 18 species: *Ammotretis*, *Azygopus*, *Colistium*, *Pelotretis*, *Peltorhamphus*, *Psammodiscus*, *Rhombosolea* and *Taratretis* (Nelson, 2006). Our findings support a sister relationship between Rhombosoleidae as defined above and Achiropsettidae.

One of the fossils in our analysis, *Numidiopleura enigmatica*[†], is placed with Achiridae, Paralichthodidae and (Oncopteridae, (Rhombosoleidae + Achiropsettidae)). When *Numidiopleura enigmatica*[†] was first described, it was thought to be a missing link between *Psettodes* and Pleuronectoidei (Gaudant & Gaudant, 1969). However, cladistic analysis of this fossil by Chanet (1997) resolved it as Pleuronectoidei *incertae sedis*. Here, we find that *N. enigmatica*[†] represents extinct family diversity within the Soleoidea, with close affinities to Rhombosoleidae and Achiropsettidae. *Numidiopleura enigmatica*[†] is the oldest representative of this putative clade. Reconciling the position of *N. enigmatica*[†] from a biogeographic standpoint is problematic as the fossil is from the Mediterranean and the distribution of inferred relatives is in the Southern Hemisphere. A cautious interpretation is necessary as the fossil of *N. enigmatica*[†] is lost and of uncertain age.

Within Soleoidea, four other families form a monophyletic assemblage—Samaridae, Poecilopsettidae, Soleidae and Cynoglossidae—with well-supported monophyly (BS = 86%, PP = 0.82, Figures 1 and 2). The monophyly and same branching arrangements of these four families have been documented in three previous studies utilizing independent data sets (Campbell et al., 2013; Campbell, López, et al., 2014; Harrington et al., 2016). Note that in Figure 2 of Harrington et al. (2016), *Aseraggodes xenicus* is incorrectly labelled as a cynoglossid, although it is a soleid. Alternative branching arrangements of Samaridae, Poecilopsettidae, Soleidae and Cynoglossidae are presented in a recent mitogenomic study, but the families are monophyletic (Shi et al., 2018). The fossil *Eobuglossus eocenicus*[†] while recently not considered a soleid by (Near et al., 2012) is demonstrated to be a soleid, in line with previous hypotheses (Chanet, 1994; Chapleau & Keast, 1988).

Research in flatfish alpha taxonomy is active and room for continued development of the beta taxonomy of the Pleuronectoidei is present. Two notable genera were not

examined in this study, *Tephrinectes* and *Thysanopsetta*, suggested by Hensley and Ahlstrom (1984) to be removed from Paralichthyidae. Detailed anatomical investigation of *Tephrinectes* indicates it is a distinct lineage from Paralichthyidae (Hoshino, 2001; Hoshino & Amaoka, 1998). Further investigations may reveal more family-level diversity within Pleuronectoidei either through the identification of new distinct lineages or refinement of known genera.

4.5 | Biogeography and distribution of diversity

Marine species richness is not equally distributed across the globe and follows two general trends. Firstly, species diversity is centred in the tropics, and declines moving towards temperate and then the Polar Regions. This trend is the latitudinal gradient in species richness and was apparent to early naturalists (Hillebrand, 2004; Humboldt, 1828). Secondly, the diversity of marine organisms is located in the IWP, especially in the central IWP (Bellwood & Wainwright, 2002; Briggs, 1999), and declines heading towards other regions (Atlantic and East Pacific). This second trend is known as the longitudinal gradient in species richness. Both the latitudinal and longitudinal gradients in species richness have strongly intensified in the last 65 million years (during the Cenozoic) (Crame, 2003). These patterns of diversity may be explained by either the “Region of Origin” or “Region of Accumulation” hypotheses (Briggs, 2007; Jokiel & Martinelli, 1992; Lavoué et al., 2013; Rocha, Rocha, Robertson, & Bowen, 2008; Rosen, 1988). That is, diversity centred in the IWP in general may be a result of either the region producing many lineages or collecting lineages.

Flatfishes are no exception to either the latitudinal or longitudinal gradients in species richness (Appendix S1). The localization of the flatfish diversity centre in the IWP does appear strange when considering the flatfish fossil record. The oldest flatfish fossils have been discovered in the Lutetian of Europe and crown flatfish lineages appear suddenly in the fossil record (Chanet, 1997, 1999; Munroe, 2005; Patterson, 1993a; Schwarzhans, 1999). As such, the fossils examined in this study are all from the Mediterranean region and have ages that are near the inferred ages of Pleuronectoidei and its superfamilies (Appendix S4). But, as Chanet (1997, 1999) indicated, fossil pleuronectiforms are true rarities and may await discovery or identification from the rocks of the IWP. Our results indicate that the major lineages of flatfishes, the Pleuronectiformes, Pleuronectoidei, Citharoidea, Pleuronectoidea and Soleidea all originated and diversified in the IWP (Figure 3a). The present-day distribution of extant pleuronectoid fishes outside of the IWP may be explained by multiple events of range expansion or long distance dispersals between oceans during the early evolutionary history of the fishes. Furthermore, ancestral habitat preference in these fishes is towards tropical waters

(Figure 3b). Thus, the diversity of flatfishes centred in the IWP fits with a “Region of Origin” hypothesis.

Within flatfishes, known species diversity is highest in Pleuronectidae, Paralichthyidae I (=Paralichthyidae, newly defined), Bothidae, Soleidae and Cynoglossidae. Largely, diversity is again, tropical among these families. These families all have net diversification rates greater than the mean for the pleuronectoidei (1.31), and the differences in net diversification rate are striking (Table 1, Appendix S6). Pleuronectidae, Paralichthyidae, Bothidae, Soleidae and Cynoglossidae also show continued activity in species descriptions as noted by the species in the last 10 years column of Table 1. Whether diversification is actually higher in particular flatfish lineages over others, and why that would be so, is difficult to address with our current phylogeny and taxonomic sampling. Nonetheless, further description of new flatfish lineages should be expected from the tropics and the deep sea.

4.6 | Transitions to freshwaters and the deep sea

Flatfishes are largely a marine group of nearly global distribution (Munroe, 2015b). The common ancestor of flatfishes and pleuronectoids was most likely tropical and distributed in the marine IWP. Pleuronectoids, however, have moved to occupy estuarine, freshwater, and deep-sea environments.

From the perspective of freshwater transitions, the existence of multiple events of colonization of freshwaters and estuarine environments by flatfish species is a well-documented phenomenon. Several families inhabit freshwaters and more than one colonization by a family is possible. Families with members that may be found in freshwater are the Achiridae (Lovejoy et al., 2006; Munroe, 2015b), the Soleidae (Chapleau & Desoutter, 1996; Evseenko & Bolshakov, 2018; Munroe, 2015b), the Cynoglossidae (Munroe, 2015b), the Scopthalmidae (Bailly & Chanet, 2010; Chanet & Branellec, 2008), the Pleuronectidae (Munroe, 2015b), the Paralichthyidae (Díaz de Astarloa, 1997; Carnikián, Acuña, & Viana, 2006; Cortez, Balbontín, & Landaeta, 2015) and the Rhombosoleidae (McDowall, 2010; Munroe, 2015b). In this study, we had representatives of three families that provide the greatest number of species that inhabit estuary and freshwater environments (Munroe, 2005): Achiridae, Soleidae and Cynoglossidae (Appendix S2). Chapleau (1993) found Achiridae, Soleidae and Cynoglossidae to be most closely related to each other. Soleidae and Cynoglossidae are sister lineages in our study, with Achiridae distantly related to the other two families (Figure 1).

Regarding deep-sea transitions, our analysis with a sampling of 14 deep-sea taxa (Appendix S2) indicates at least five transitions which are widespread phylogenetically within Pleuronectoidei (Figures 1 and 3b). In the Bothidae, we identify two independent deep-sea lineages with fishes that share an elongate body form (*Chascanopsetta lugubris*,

Japonolaeops dentatus, *Kamohaira megastomus*, *Laeops kitarahae* and *Neolaeops microphthalmus*). Our results suggest that elongate body form is present as an adaptive homoplasy, as there is an apparent trend that longer and more elongate fish are found with increasing depth (Priede, 2017). In Poecilopsettidae, all fishes are distributed in the deep sea and derive from a common ancestor that most likely lived in the deep sea (Figures 1 and 3b). Samaridae as a family is generally distributed in near shore coastal regions, from the coral reef area to 150 m depth. Only a few samarid species belonging to the genera *Plagiopsetta* and *Samariscus* evolved secondarily in the deep sea. As with freshwater transitions, deep-sea transitions may define a family (i.e., Poecilopsettidae) or may exhibit several transitions within a family perhaps based on exadaptations (e.g., elongate body shape in some bothids). However, it should be noted that the event of the transitions remains incompletely examined by our data set.

5 | CONCLUSIONS

Our findings support a three-superfamily classification scheme as put forth by Nelson (2006) and provide additional evidence for refining the relationships among and within the pleuronectoid superfamilies. Our sampling for molecular data covers all 13 previously recognized families and we support the creation of two additional families (Cyclopsettidae and Oncopteridae) to resolve paraphyletic or polyphyletic flatfish families for a total of 15 flatfish families. Beyond molecular data, we also find that there has been unrecognized family-level diversity in the flatfish fossil record. Alpha and beta flatfish taxonomy are still in progress and ongoing research in flatfish systematics is very likely to produce new species and perhaps new families that may be recognized from integrated taxonomic study of new species—especially from the deep sea—known morphologically distinct genera (e.g., *Tephrinectes*), or, the application of molecular and total evidence phylogenetics to known species. These additions are unlikely to significantly alter our findings regarding when major flatfish lineages originated or flatfish ancestral ranges and temperature preferences. Additional sampling of flatfishes will, however, improve our understanding of flatfish taxonomy and diversity in this species-rich group that has long been characterized by over simplistic taxonomy and unrecognized diversity.

5.1 | Cyclopsettidae, new family

5.1.1 | Diagnosis

Diagnostic characters are compiled following the information from Hensley and Ahlstrom (1984) and Chapleau (1993). Cyclopsettidae is recognized from other families by this combination of features: Eyes on the left side; the urinary

papilla relatively close the blind side; ocular-side pelvic fin located at the mid-ventral line of the body; blind-side pelvic fin base is more anterior than that of the ocular side; 17 caudal-fin rays, and all fin rays are supported by hypurals, not located on preural, neural nor haemal spines; five hypurals and hypural 5 fused with the epural; first neural spine absent; and vertebral apophyses present.

5.1.2 | Composition

This family includes four genera, *Citharichthys*, *Cyclopsetta* (type genus), *Etropus* and *Syacium* evidenced from anatomical and molecular data sources and currently 45 recognized species (Chapleau, 1993; Fricke, Eschmeyer, & Fong, 2018; Hensley & Ahlstrom, 1984; Pardo et al., 2005). The monophyly of all genera in this family has not been shown in molecular studies (Azevedo et al., 2008; Betancur-R., Broughton, et al., 2013; Betancur-R., Li, et al., 2013).

5.1.3 | Distribution

Eastern (Senegal to Angola) and western (U.S.A to Brazil) Atlantic, Eastern Pacific (Baja California to Peru), distributed in brackish waters and marine coastal waters into the deep sea (2000 m).

5.2 | Oncopteridae, new family

5.2.1 | Diagnosis

The diagnostic characters follow the information from Norman (1934) and Chapleau (1993). Oncopteridae is recognized by this combination of features: Eyes on the right side; large foramen on branchial septum between lower pharyngeals and urohyal; origin of dorsal fin anterior of eyes above blind-side nostrils; first dorsal-fin ray specialized, enlarged, hard, curved, and movable, and connects with first strongly developed basal bone of the fin, contained in a deep groove on blind-side head; pelvic fins asymmetric and separated by anal fin, ocular-side pelvic fin located much more anterior than that of the blind side; six pelvic-fin rays; lateral line with distinctive semi-circular curve above the pectoral fin and several transverse supratemporal accessory branches from main lateral line to dorsal edge of body.

5.2.2 | Composition

This family includes a single genus, *Oncopterus*, with a single species *Oncopterus darwinii* Steindachner, 1874.

5.2.3 | Distribution

Southwestern Atlantic (Brazil to Argentina), distributed in shallow coastal waters (20–80 m).

ACKNOWLEDGEMENTS

Our gratitude goes to Drs. R.L. Mayden, G. Duhamel, S. Lavoué, A. Connell, J. Díaz de Astarloa, L. Jawad and other organizations including the KU Biodiversity Institute & Natural History Museum, and the Cryobanking Program for Wildlife Genetic Material in Taiwan (Academic Sinica) for sharing important tissue samples. We would like to thank the participants of expedition cruises from BIOPAPUA, PAPUA NIUGINI, KAVIENG 2014, KAVIENG 2014, MADEEP (see details at: <https://expeditions.mnhn.fr>) and crews of the research vessel ALIS for the assistance of sampling. We also thank Dr. L. Bachmann and two anonymous reviewers for providing helpful comments on this article and the members from the Marine Biodiversity and Phylogenomics Laboratory (WJC, IONTU), especially H-C. Lin and P. Tongboonkua, for their assistance in the laboratory and sample management. This work was supported by the Ministry of Science and Technology, Taiwan (MOST 102-2923-B-002-001-MY3 and MOST 107-2611-M-002-007 to WJC) and the French National Research Agency (ANR 12-ISV7-0005-01 to S. Samadi). MAC would like to acknowledge the Fulbright Foundation for Scholarly Exchange for supporting his visit to National Taiwan University.

ORCID

Matthew A. Campbell  <https://orcid.org/0000-0002-5826-0329>

Mao-Ying Lee  <https://orcid.org/0000-0002-4643-3173>

Wei-Jen Chen  <https://orcid.org/0000-0002-2111-589X>

REFERENCES

- Agassiz, L. (1833). *Recherches sur les Poissons fossiles*. Neuchatel, Switzerland: Imprimerie de Petitpierre.
- Azevedo, M. F. C., Oliveira, C., Pardo, B. G., Martínez, P., & Foresti, F. (2008). Phylogenetic analysis of the order Pleuronectiformes (Teleostei) based on sequences of 12S and 16S mitochondrial genes. *Genetics and Molecular Biology*, *31*(1), 284–292. <https://doi.org/10.1590/S1415-47572008000200023>
- Baciu, D. S., & Chanet, B. (2002). Les Poissons plats fossiles (teleostei: Pleuronectiformes) de l'Oligocène de Piatra neamt (Roumanie). *Oryctos*, *4*, 17–38.
- Bailly, N., & Chanet, B. (2010). *Scophthalmus* Rafinesque, 1810: The valid generic name for the turbot, *S. maximus* (Linnaeus, 1758) [Pleuronectiformes: Scophthalmidae]. *Cybius*, *34*, 257–261.
- Bellwood, D. R., & Wainwright, P. C. (2002). The history and biogeography of fishes on coral reefs. In P. F. Sale (Ed.), *Coral reef fishes: Dynamics and diversity in a complex ecosystem*. London, UK: Academic Press. 5–32.
- Berendzen, P. B., & Dimmick, W. W. (2002). Phylogenetic relationships of Pleuronectiformes based on molecular evidence. *Copeia*, *2002*(3), 642–652. [https://doi.org/10.1643/0045-8511\(2002\)002\[0642:PROPOB\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2002)002[0642:PROPOB]2.0.CO;2)
- Berg, L. S. (1940). Classification of fishes, both recent and fossil. *Trudy Zoologicheskogo Instituta*, *5*, 87–517.
- Berg, L. S. (1941). *Eobothus vialovi* n. sp., an Eocene flatfish from the western Tian-Shan. *Bulletin de l'Académie des sciences de l'URSS. Série biologique*, *3*, 480–485.
- Betancur-R., R., Broughton, R. E., Wiley, E. O., Carpenter, K., López, J. A., Li, C., ... Ortí, G. (2013). The tree of life and a new classification of bony fishes. *PLoS Currents Tree of Life*, <https://doi.org/10.1371/currents.tol.53ba26640df0ccaee75bb165c8c26288>
- Betancur-R., R., Li, C., Munroe, T. A., Ballesteros, J. A., & Ortí, G. (2013). Addressing gene tree discordance and non-stationarity to resolve a multi-locus phylogeny of the flatfishes (Teleostei: Pleuronectiformes). *Systematic Biology*, *62*(5), 763–785. <https://doi.org/10.1093/sysbio/syt039>
- Betancur-R., R., & Ortí, G. (2014). Molecular evidence for the monophyly of flatfishes (Carangimorpharia: Pleuronectiformes). *Molecular Phylogenetics and Evolution*, *73*, 18–22. <https://doi.org/10.1016/j.ympev.2014.01.006>
- Blot, J. (1980). La faune ichthyologique des gisements du Monte Bolca (province de Vérone, Italie) - catalogue systématique présentant l'état actuel des recherches concernant cette faune. *Bulletin Du Muséum National D'histoire Naturelle*, *4*, 339–396.
- Briggs, J. C. (1999). Coincident biogeographic patterns: Indo-West Pacific Ocean. *Evolution*, *53*(2), 326–335. <https://doi.org/10.1111/j.1558-5646.1999.tb03769.x>
- Briggs, J. C. (2007). Marine longitudinal biodiversity: Causes and conservation. *Diversity and Distributions*, *13*(5), 544–555. <https://doi.org/10.1111/j.1472-4642.2007.00362.x>
- Campbell, M. A., Chen, W.-J., & López, J. A. (2013). Are flatfishes (Pleuronectiformes) monophyletic? *Molecular Phylogenetics and Evolution*, *69*(3), 664–673. <https://doi.org/10.1016/j.ympev.2013.07.011>
- Campbell, M. A., Chen, W.-J., & López, J. A. (2014). Molecular data do not provide unambiguous support for the monophyly of flatfishes (Pleuronectiformes): A reply to Betancur-R and Ortí. *Molecular Phylogenetics and Evolution*, *75*, 149–153. <https://doi.org/10.1016/j.ympev.2014.02.011>
- Campbell, M. A., López, J. A., Satoh, T. P., Chen, W.-J., & Miya, M. (2014). Mitochondrial genomic investigation of flatfish monophyly. *Gene*, *551*(2), 176–182. <https://doi.org/10.1016/j.gene.2014.08.053>
- Carnikián, A., Acuña, A., & Viana, F. (2006). Ambicolored specimens of the flounder *Paralichthys orbignyanus* (Pleuronectiformes: Paralichthyidae). *Neotropical Ichthyology*, *4*(2), 285–286. <https://doi.org/10.1590/S1679-62252006000200015>
- Chabanaud, P. (1936). *Le neurocrâne des téléostéens dyssymétriques après la métamorphose*. Paris, France: Université de Paris, Masson.
- Chabanaud, P. (1940). Un nouveau téléostéen dyssymétrique fossile, originaire du Lutétien du bassin de Paris. *Archives Du Muséum National D'histoire Naturelle*, *6*(16), 5–31.
- Chabanaud, P. (1949). Le problème de la phylogénèse des Heterosomata. *Bulletin De L'institut Océanographique*, *950*, 1–24.
- Chanet, B. (1994). *Eubuglossus eocenicus* (Woodward 1910) from the Upper Lutetian of Egypt, one of the oldest solejds (Teleostei, Pleuronectiformes). *Neues Jahrbuch Für Geologie Und Paläontologie Abhandlung*, *1994*(7), 391–398.
- Chanet, B. (1997). A cladistic reappraisal of the fossil flatfishes record consequences on the phylogeny of the Pleuronectiformes (Osteichthyes: Teleostei). *Annales De Sciences Naturelles-Zoologie Et Biologie Animale*, *18*, 105–117.

- Chanet, B. (1999). Supposed and true flatfishes [Teleostei: Pleuronectiformes] from the Eocene of Monte Bolca, Italy. *Studi E Ricerche Sui Giacimenti Terziari Di Bolca, Miscellanea Paleontologica*, 8, 220–243.
- Chanet, B., & Branellec, A.-L. (2008). Expliquer la biologie des espèces par leurs relations de parenté. Exemple des Scophthalmidae [Teleostéens : Pleuronectiformes]. *Bulletin De La Société Des Sciences Naturelles De L'ouest De La France*, 30, 81–89.
- Chanet, B., Chapleau, F., & Desoutter, M. (2004). Os et ligaments intermusculaires chez les poissons plats [Teleostei: Pleuronectiformes]: interprétations phylogénétiques. *Cybium*, 28(1), 9–14.
- Chapleau, F. (1993). Pleuronectiform relationships: A cladistic reassessment. *Bulletin of Marine Science*, 52(1), 516–540.
- Chapleau, F., & Desoutter, M. (1996). Position phylogénétique de *Dagetichthys lakdoensis* (Pleuronectiformes). *Cybium*, 20(1), 103–106.
- Chapleau, F., & Keast, A. (1988). A phylogenetic reassessment of the monophyletic status of the family Soleidae, with comments on the suborder Soleoidei (Pisces; Pleuronectiformes). *Canadian Journal of Zoology*, 66(12), 2797–2810. <https://doi.org/10.1139/z88-408>
- Chen, J.-N., López, J. A., Lavoué, S., Miya, M., & Chen, W.-J. (2014). Phylogeny of the Elopomorpha (Teleostei): Evidence from six nuclear and mitochondrial markers. *Molecular Phylogenetics and Evolution*, 70, 152–161. <https://doi.org/10.1016/j.ympev.2013.09.002>
- Chen, W.-J., Bonillo, C., & Lecointre, G. (2003). Repeatability of clades as a criterion of reliability: A case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. *Molecular Phylogenetics and Evolution*, 26(2), 262–288. [https://doi.org/10.1016/S1055-7903\(02\)00371-8](https://doi.org/10.1016/S1055-7903(02)00371-8)
- Chen, W.-J., Lavoué, S., & Mayden, R. L. (2013). Evolutionary origin and early biogeography of otophysan fishes (Ostariophysi: Teleostei). *Evolution*, 67(8), 2218–2239. <https://doi.org/10.1111/evo.12104>
- Chen, W.-J., Miya, M., Saitoh, K., & Mayden, R. L. (2008). Phylogenetic utility of two existing and four novel nuclear gene loci in reconstructing Tree of Life of ray-finned fishes: The order Cypriniformes (Ostariophysi) as a case study. *Gene*, 423(2), 125–134. <https://doi.org/10.1016/j.gene.2008.07.016>
- Chen, W.-J., Ruiz-Carus, R., & Ortí, G. (2007). Relationships among four genera of mojarra (Teleostei: Perciformes: Gerreidae) from the western Atlantic and their tentative placement among percormorph fishes. *Journal of Fish Biology*, 70, 202–218. <https://doi.org/10.1111/j.1095-8649.2007.01395.x>
- Clark, J. R., Ree, R. H., Alfaro, M. E., King, M. G., Wagner, W. L., & Roalson, E. H. (2008). A comparative study in ancestral range reconstruction methods: Retracing the uncertain histories of insular lineages. *Systematic Biology*, 57(5), 693–707. <https://doi.org/10.1080/10635150802426473>
- Cooper, J. A., & Chapleau, F. (1998a). Monophyly and intrarelationships of the family Pleuronectidae (Pleuronectiformes), with a revised classification. *Fisheries Bulletin*, 96(4), 686–726.
- Cooper, J. A., Chapleau, F., & Chapleau, F. (1998b). Phylogenetic status of *Paralichthodes algoensis* (Pleuronectiformes: Paralichthodidae). *Copeia*, 1998(2), 477–481. <https://doi.org/10.2307/1447445>
- Cortez, C., Balbontín, F., & Landaeta, M. F. (2015). Larval development of the southern flounder *Thysanopsetta naresi* from Chilean Patagonia. *Neotropical Ichthyology*, 50(1), 13–23. <https://doi.org/10.4067/S0718-19572015000100002>
- Crame, J. A. (2003). Taxonomic diversity gradients through geological time. *Diversity and Distributions*, 7(4), 175–189. <https://doi.org/10.1111/j.1472-4642.2001.00106.x>
- Cuvier, G. (1817). *Le règne animal distribué d'après son organisation: pour servir de base à l'histoire naturelle des animaux et d'introduction à l'anatomie comparée. Les reptiles, les poissons, les mollusques et les annélides* (1st ed., Vol. 2). Paris, France: Chez Détervill.
- Deltaï, A., & Lecointre, G. (2005). Further support for the clades obtained by multiple molecular phylogenies in the acanthomorph bush. *Comptes Rendus Biologies*, 328(7), 674–689. <https://doi.org/10.1016/j.crvi.2005.04.002>
- Díaz de Astarloa, J. M. (1997). A case of reversal in *Paralichthys orbignyanus* a shallow-water flounder from the south-western Atlantic. *Journal of Fish Biology*, 50(4), 900–902. <https://doi.org/10.1111/j.1095-8649.1997.tb01983.x>
- Drummond, A. J., Ho, S. Y. W., Phillips, M. J., & Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology*, 4(5), e88. <https://doi.org/10.1371/journal.pbio.0040088>
- Evseenko, S. A., & Bolshakov, D. V. (2018). On the morphology of Mekong blind sole *Typhlachirus elongatus* (Soleidae) with notes on the taxonomy of the genus *Typhlachirus*. *Journal of Ichthyology*, 58(5), 688–693. <https://doi.org/10.1134/S0032945218050065>
- FAO (2019). *FAO FishFinder*. FAO Fisheries and Aquaculture Department. Retrieved from <http://www.fao.org/fishery/>
- Fricke, R., Eschmeyer, W., & Fong, J. D. (2018). *Catalog of fishes*. Updated October 2, 2018. Retrieved from <http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp>
- Friedman, M. (2008). The evolutionary origin of flatfish asymmetry. *Nature*, 454(7201), 209–212. <https://doi.org/10.1038/nature07108>
- Gaudant, M., & Gaudant, J. (1969). Note sur un pleuronectiforme nouveau conserve au Service geologique de Tunisie; *Numidiopleura enigmatica* nov. gen., nov. sp. *Bulletin De La Société Géologique De France*, S7-X I(5), 660–665. <https://doi.org/10.2113/gssgfbull.S7-XI.5.660>
- Gibson, R. N., Nash, R. D., Geffen, A. J., & Van der Veer, H. W. (2015). *Flatfishes: Biology and exploitation* (2nd ed.). Chichester, UK: John Wiley & Sons.
- Harrington, R. C., Faircloth, B. C., Eytan, R. I., Smith, W. L., Near, T. J., Alfaro, M. E., & Friedman, M. (2016). Phylogenomic analysis of carangimorph fishes reveals flatfish asymmetry arose in a blink of the evolutionary eye. *BMC Evolutionary Biology*, 16(1), 224. <https://doi.org/10.1186/s12862-016-0786-x>
- Hensley, D. A. (1997). An overview of the systematics and biogeography of the flatfishes. *Journal of Sea Research*, 37(3–4), 187–194. [https://doi.org/10.1016/S1385-1101\(97\)00017-8](https://doi.org/10.1016/S1385-1101(97)00017-8)
- Hensley, D. A., & Ahlstrom, E. H. (1984). Pleuronectiformes: Relationships. In H. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, & S. L. Richardson (Eds.), *Ontogeny and systematics of fishes*. Lawrence, KS: American Society of Ichthyologists and Herpetologists. 670–687.
- Hensley, D. A., Amaoka, K., Hensley, D. A., Moser, H. G., & Sumida, B. Y. (1984). Pleuronectiformes: Development. In H. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, & S. L. Richardson (Eds.), *Ontogeny and systematics of fishes*. Lawrence, KS: American Society of Ichthyologists and Herpetologists. 640–670.
- Hillebrand, H. (2004). Strength, slope and variability of marine latitudinal gradients. *Marine Ecology Progress Series*, 273, 251–267. <https://doi.org/10.3354/meps273251>
- Hoshino, K. (2001). Monophyly of the Citharidae (Pleuronectoidei: Pleuronectiformes: Teleostei) with considerations of pleuronectid

- phylogeny. *Ichthyological Research*, 48, 391–404. <https://doi.org/10.1007/s10228-001-8163-0>
- Hoshino, K., & Amaoka, K. (1998). Osteology of the flounder, *Tephrinectes sinensis* (Lacepède) (Teleostei: Pleuronectiformes), with comments on its relationships. *Ichthyological Research*, 45(1), 69–77. <https://doi.org/10.1007/BF02678576>
- Hubbs, C. L. (1945). Phylogenetic position of the Citharidae, a family of flatfishes. *Miscellaneous Publications Museum of Zoology, University of Michigan*, 63, 1–38.
- Jokiel, P., & Martinelli, F. J. (1992). The vortex model of coral reef biogeography. *Journal of Biogeography*, 449–458. <https://doi.org/10.2307/2845572>
- Jordan, D., & Evermann, B. (1896). The fishes of North and Middle America III. *Bulletin of the United States National Museum*, 47(3), 2602–2712.
- Khidir, K. T., Chapleau, F., & Renaud, C. B. (2005). Phylogenetic relationships of *Cyclopsetta* and *Syacium* (Pleuronectiformes: Paralichthyidae). *Cybium*, 29(4), 321–326.
- Lanfear, R., Calcott, B., Ho, S. Y. W., & Guindon, S. (2012). PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, 29(6), 1695–1701. <https://doi.org/10.1093/molbev/mss020>
- Lavoué, S., Miya, M., Musikasinthorn, P., Chen, W.-J., & Nishida, M. (2013). Mitogenomic evidence for an Indo-West Pacific origin of the Clupeoidei (Teleostei: Clupeiformes). *PLoS ONE*, 8(2), e56485. <https://doi.org/10.1371/journal.pone.0056485>
- Li, B., Dettai, A., Cruaud, C., Couloux, A., Desoutter-Meniger, M., & Lecointre, G. (2009). RNF213, a new nuclear marker for acanthomorph phylogeny. *Molecular Phylogenetics and Evolution*, 50(2), 345–363. <https://doi.org/10.1016/j.ympev.2008.11.013>
- Linnaeus, C. (1758). *Caroli Linnaei...Systema naturae per regna tria naturae: secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. (10th ed.). Holmiae (Salvii). Retrieved from <http://resolver.sub.uni-goettingen.de/purl?PPN362053006>
- López, J. A., Chen, W. J., & Ortí, G. (2004). Esociform phylogeny. *Copeia*, 2004(3), 449–464. <https://doi.org/10.1643/CG-03-087R1>
- Lovejoy, N. R., Albert, J. S., & Crampton, W. G. R. (2006). Miocene marine incursions and marine/freshwater transitions: Evidence from Neotropical fishes. *New Contributions on Neogene Geography and Depositional Environments in Amazonia*, 21(1), 5–13. <https://doi.org/10.1016/j.jsames.2005.07.009>
- McDowall, R. M. (2010). Phylogenetic lineages in the fauna and the evolution of diadromy: A broad perspective. In D.L.G. Noakes (Ed.), *New Zealand freshwater fishes* (Vol. 32, pp. 151–168). Dordrecht, The Netherlands: Springer. https://doi.org/10.1007/978-90-481-9271-7_7
- Munroe, T. A. (2005). Systematic diversity of the Pleuronectiformes. In R. N. Gibson (Ed.), *Flatfishes: Biology and exploitation* (pp. 10–41). Oxford, UK: Blackwell.
- Munroe, T. A. (2015a). Distributions and biogeography. In R. N. Gibson, R. D. Nash, A. J. Geffen, & H. W. Van der Veer (Eds.), *Flatfishes: Biology and exploitation* (2nd ed., pp. 52–82). Chichester, UK: John Wiley & Sons, Ltd.
- Munroe, T. A. (2015b). Systematic diversity of the Pleuronectiformes. In R. N. Gibson, R. M. Nash, A. J. Geffen, & H. W. Van der Veer (Eds.), *Flatfishes: Biology and exploitation* (2nd ed., pp. 13–51). Chichester, UK: John Wiley and Sons Ltd.
- Near, T. J., Eytan, R. I., Dornburg, A., Kuhn, K. L., Moore, J. A., Davis, M. P., ... Smith, W. L. (2012). Resolution of ray-finned fish phylogeny and timing of diversification. *Proceedings of the National Academy of Sciences of the United States of America*, 109(34), 13698–13703. <https://doi.org/10.1073/pnas.1206625109>
- Nelson, J. S. (2006). *Fishes of the World* (4th ed.). Hoboken, NJ: John Wiley & Sons Inc.
- Norman, J. R. (1934). *A systematic monograph of the flatfishes (Heterosomata). Vol. 2. Psettodidae, Bothidae, Pleuronectidae*. London, UK: British Museum of Natural History.
- Pardo, B. G., Machordom, A., Foresti, F., Porto-Foresti, F., Azevedo, M. F. C., Bañón, R., ... Martínez, P. (2005). Phylogenetic analysis of flatfish (Order Pleuronectiformes) based on mitochondrial 16s rDNA sequences. *Scientia Marina*, 69(4), 531–543. <https://doi.org/10.3989/scimar.2005.69n4531>
- Patterson, C. (1993a). An overview of the early fossil record of acanthomorphs. *Bulletin of Marine Science*, 52(1), 29–59.
- Patterson, C. (1993b). Osteichthyes: Teleostei. In M. J. Benton (Ed.), *The fossil record 2* (pp. 621–656). London, UK: Chapman and Hall.
- Priede, I. G. (2017). *Deep-sea fishes: Biology, diversity, ecology and fisheries*. Cambridge, UK: Cambridge University Press.
- R Development Core Team (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org/>
- Ree, R. H., & Smith, S. A. (2008). Maximum Likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, 57(1), 4–14. <https://doi.org/10.1080/10635150701883881>
- Regan, C. T. (1910). The origin and evolution of the teleostean fishes of the order Heterosomata. *Annals and Magazine of Natural History*, 8(6), 484–496. <https://doi.org/10.1080/00222931008692879>
- Rocha, L. A., Rocha, C. R., Robertson, D. R., & Bowen, B. W. (2008). Comparative phylogeography of Atlantic reef fishes indicates both origin and accumulation of diversity in the Caribbean. *BMC Evolutionary Biology*, 8(1), 157. <https://doi.org/10.1186/1471-2148-8-157>
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., ... Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61(3), 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Rosen, B. R. (1988). Progress, problems and patterns in the biogeography of reef corals and other tropical marine organisms. *Helgoländer Meeresuntersuchungen*, 42(2), 269–301. <https://doi.org/10.1007/BF02366046>
- Schwarz, G. (1978). Estimating the dimension of a model. *Ann. Statist.*, 2, 461–464. <https://doi.org/10.1214/aos/1176344136>
- Schwarzhan, W. (1999). *Piscium catalogus: Otolithi piscium* (Vol. 2). Munchen, Germany: Verlag Dr. Friedrich Pfeil.
- Shi, W., Chen, S., Kong, X., Si, L., Gong, L., Zhang, Y., & Yu, H. (2018). Flatfish monophyly refuted by the relationship of *Psettodes* in Carangimorphariae. *BMC Genomics*, 19, 400. <https://doi.org/10.1186/s12864-018-4788-5>
- Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22(21), 2688–2690. <https://doi.org/10.1093/bioinformatics/btl446>

- Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9), 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- van Humboldt, A. (1828). *Über das Universum: Die Kosmosvorträge 1827/28 in der Berliner Singakademie* (1993rd ed.). Frankfurt, Germany: Insel Verlag.
- Vinnikov, K. A., Thomson, R. C., & Munroe, T. A. (2018). Revised classification of the righteye flounders (Teleostei: Pleuronectidae) based on multilocus phylogeny with complete taxon sampling. *Molecular Phylogenetics and Evolution*, 125, 147–162. <https://doi.org/10.1016/j.ympev.2018.03.014>
- Woodward, A. S. (1910). On a fossil sole and a fossil eel from the Eocene of Egypt. *Geological Magazine*, 7(9), 402–405. <https://doi.org/10.1017/S0016756800135186>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Campbell MA, Chanet B, Chen J-N, Lee M-Y, Chen W-J. Origins and relationships of the Pleuronectoidei: Molecular and morphological analysis of living and fossil taxa. *Zool Scr.* 2019;48:640–656. <https://doi.org/10.1111/zsc.12372>