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# Flatfishes interrelationships revisited based on anatomical characters

by

Bruno CHANET\*, Jorge MONDÉJAR-FERNÁNDEZ  
& Guillaume LECOINTRE (1)



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**Abstract.** – In 1993, Chapleau proposed the first comprehensive cladistic work on interfamilial relationships of flatfishes (order Pleuronectiformes) based on anatomical data. This work has been, and still remains, a cornerstone for phylogenetic works dedicated to flatfishes. Nevertheless, this valuable work lacked important elements of the current knowledge associated with flatfish outgroups and suffered from scarcity of data for some characters. We propose here the results of a new analysis of Chapleau's matrix with reexamination of some characters (e.g. *recessus orbitalis*) and addition of two fossil genera (*Heteronectes* and *Amphistium*), plus two extant genera as outgroups, chosen on the basis of recent molecular analyses. This reassessment leaves the monophyly of the Pleuronectiformes ambiguous while the interrelationships within flatfishes proposed by Chapleau are not significantly modified. Moreover, the polarity of one character (structure of the first anal-fin pterygiophore) is reviewed and enlightens differently the first steps of the evolution of flatfishes.

**Résumé.** – Réévaluation des relations de parenté entre poissons plats sur la base de caractères anatomiques.

En 1993, Chapleau propose la première analyse cladistique traitant des relations interfamiliales des poissons plats (ordre des Pleuronectiformes) fondées sur des données anatomiques. Cet article a été et demeure un travail de référence pour toute analyse de l'évolution des poissons plats. Néanmoins, cette étude souffrait de l'absence de connaissances sur l'identité d'extra-groupes appropriés et du manque de données pour certains caractères. Nous proposons ici les résultats d'une ré-analyse de la matrice de Chapleau fondée sur la réinterprétation de certains caractères (ex. *recessus orbitalis*), l'addition de deux genres fossiles (*Heteronectes* et *Amphistium*) et l'ajout de deux extra-groupes, fondé sur les résultats des travaux moléculaires récents. Cette réévaluation ne soutient pas la monophylie des poissons plats – laquelle reste ambiguë – tandis que les relations de parenté à l'intérieur du groupe proposées par Chapleau (1993) ne sont pas modifiées dans leur ensemble. Par ailleurs, la polarité d'un caractère (structure du premier ptérygiophore de la nageoire anale) est reconsidérée et éclaire différemment les premiers pas de l'évolution des poissons plats.

## INTRODUCTION

In 1993, François Chapleau provided the first genuine (i.e. comprehensive) cladistic analysis of flatfishes (order Pleuronectiformes) interrelationships. Prior to this work, the only cladistic analysis dedicated to taxa of this order was by Lauder and Liem (1983). But these authors acknowledged that it was “a tentative phylogenetic hypothesis” (Lauder and Liem, 1983: 185) as they used limited data for both characters and taxa. Shortly after their publication, Hensley and Ahlstrom (1984) examined characters, questioned homologies and reassessed flatfish interrelationships, providing an important synthesis on the knowledge of flatfish anatomy. Nevertheless, their work was more a list of data, and was eclectic in its phylogenetic approach with no hypothesis in terms of primitive or derived character states. Consequently, the work of Chapleau (1993), because of its cladistic approach, became rapidly a seminal reference in the field of flatfish phylogeny (see for example Munroe, 2015) and the

phylogeny from his work (Fig. 1) was used as a comparative landmark by every further work dedicated to the understanding of flatfish interrelationships whenever molecular or anatomical. Nevertheless, in light of new knowledge regarding flatfish extra-relationships, homology of characters and the discovery of fossils, a re-analysis has become necessary. Indeed, beside new characters, the inclusion of fossil taxa and a sampling relevant in the light of new phylogenetic consensus have been shown to significantly impact the robustness of phylogenies based on morphological characters and highly increase their congruence with molecular ones notably in the case of acanthomorph clades (Davesne *et al.*, 2014, 2016).

The existence of the Pleuronectiformes as a natural group has seemed obvious to ancient naturalists. For instance, Linnaeus (1758) gathered all of the known flatfish species of his time into a single genus: *Pleuronectes* (Linnaeus, 1758). Most scientists since Linnaeus (1758) did not question the monophyly of the order (Cuvier, 1817; Agassiz, 1834-1843;

(1) Institut de Systématique, Évolution et Biodiversité (ISYEB), UMR 7205 CNRS-MNHN-SU-EPHE, Département Origines et Évolution, Muséum national d'Histoire naturelle, Sorbonne Université, CP 30, 57 rue Cuvier, 75005 Paris, France.  
[bruno.chanet@mnhn.fr] [jorge.mondejar-fernandez@mnhn.fr] [guillaume.lecointre@mnhn.fr]

\* Corresponding author

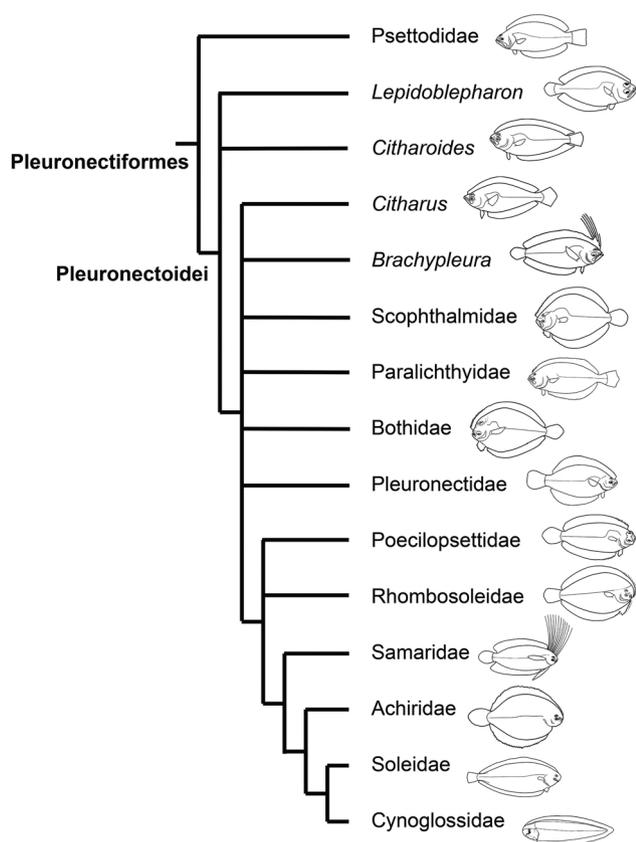


Figure 1. – Consensus tree of flatfish interrelationships proposed by Chapleau (1993), illustration modified from Chapleau (1993: 533, fig. 7).

Cope, 1871; Jordan and Evermann, 1898; Cunningham, 1892; Holt, 1894; Boulenger, 1902; Thilo, 1902; Regan, 1910; Norman, 1934; Blot, 1970, among others). Over time, only a few authors (Kyle, 1923; Chabanaud, 1949; Amaoka, 1969) have argued for their polyphyly. *Psettodes* was considered as the most basal flatfish genus or at least as “a good potential sister group” to the other flatfishes (T.A. Munroe, pers. comm. to BC). Chapleau (1993: 517-518) logically used *Psettodes*, considered as the most plesiomorphic extant flatfish, as the primary outgroup to define interrelationships within the Pleuronectoidei. He also incorporated in the analysis as secondary outgroup percoids (*sensu* Johnson, 1980, 1984) and Beryciformes plus he proposed a hypothetical ancestor with plesiomorphic states for all characters to include in his analysis. However, the distribution of character-states was not shown in the data matrix for any of these secondary outgroups. Moreover, based on recent molecular studies (Chen, 2001, Chen *et al.*, 2003; Miya *et al.*, 2003, 2005; Dettaï and Lecointre, 2005; Smith and Wheeler, 2006; Li *et al.*, 2009) both these percoids (*sensu* Johnson, 1980, 1984) and the Beryciformes are presently known as too distantly related to flatfishes to be used as outgroups. Of course, Chapleau used knowledge of his time and one cannot expect

the choice of outgroups made in 1993 to take into account results from some 25 years later. But these recent results stimulate a renewed interest to Chapleau’s data matrix.

We then use the matrix built by Chapleau (1993) modified in respect to these three points. (1) While Chapleau used *Psettodes* as primary outgroup and basal percoids (*sensu* Johnson, 1980, 1984) and Beryciformes (Zehren, 1979; Keene and Tighe, 1984) as secondary outgroups, we used *Caranx* (Carangidae) and *Lates* (Latidae), since each of these taxa have been alternatively recovered in sister taxa position of Pleuronectiformes by several molecular analyses (Li *et al.*, 2009; 2011; Wainwright *et al.*, 2012; Harrington *et al.*, 2016; Betancur-R *et al.* 2017, among others), while Beryciformes are now considered as basal acantomorphs not related to flatfishes (Betancur-R *et al.*, 2013a, b). (2) We include two fossil taxa: *Heteronectes chaneti* Friedman, 2008 and *Amphistium paradoxum* Agassiz, 1835, both from the Eocene of the Monte Bolca (Italy) (Chanet, 1997; Friedman 2008, 2012), which have been considered as basal to Pleuronectiformes (Friedman, 2008, 2012). (3) At last, the coding of one character has been modified: *Psettodes* is coded “0” (= absence of *recessus orbitalis*) for character 3 “*recessus orbitalis*” (see discussion).

## MATERIAL AND METHODS

### Nomenclature

Chapleau (1993) used the subfamilial status for the following taxa: Pleuronectidae, Poecilopsettidae, Rhombosoleidae, Samaridae. In the present work, following Chapleau and Keast (1988) and Cooper and Chapleau (1998), we consider each of these groups as a valid family. Latin species names have been checked using Eschmeyer and Fricke (2010).

### Choice of outgroups

The monophyly of flatfishes has been questioned in many studies, for instance with *Lates* (Latidae) appearing as included in Pleuronectiformes (Dettaï and Lecointre, 2005; Li *et al.*, 2009) and *Psettodes* being sister group of *Toxotes* (Toxotidae) outside of the Pleuronectiformes. In their global review of phylogenetic classification of bony fishes based on molecular data, Betancur-R *et al.* (2017) found *Psettodes* as the sister-group of the Pleuronectoidei, retrieving a monophyletic Pleuronectiformes, with centropomids (among them *Lates*) as their sister-group, but with very short branch lengths. Indeed, molecular studies in recent years (Chen, 2001, Chen *et al.*, 2003; Miya *et al.*, 2003, 2005; Dettaï and Lecointre, 2005; Smith and Wheeler, 2006; Li *et al.*, 2009) have tried to decipher the best candidates to be considered the closest relatives of flatfishes and, in doing so, discarded some of the classical groups, for instance Epinephelinae

(Norman, 1934), viewed as relatives to flatfishes. Among these new hypotheses, Li *et al.* (2009) gathered flatfishes with centropomids, carangids, echeneids, coryphaenids, menids, xiphids, polynemids and sphyraenids. Depending on the taxonomical sampling, the completeness of this assemblage was variable; as a result, flatfishes appeared to be related only to carangids (Miya *et al.*, 2003, 2005; Mabuchi *et al.*, 2007; Smith and Craig, 2007; Yagishita *et al.*, 2009), or xiphids (Little *et al.*, 2010), or sphyraenids (Meynard *et al.*, 2012), or billfishes and carangids (Near *et al.*, 2012), or centropomids (Wainwright *et al.*, 2012). This assemblage was called “clade L” by Chen *et al.* (2003), then later “Carangimorpha” by Li *et al.* (2009), a term that was conserved in the synthesis of Betancur-R *et al.* (2017). Based on these evidence, we have chosen *Lates* and *Caranx* as secure outgroups.

**New information on character states**

Except for the taxa newly included alongside with those used by Chapleau (1993), we use the character states he proposed. One exception is to be made for a single character in *Psettodes*. Among the skeletal characters, Chapleau used a soft anatomy character in his analysis, the presence of a peculiar structure called the *recessus orbitalis*. The *recessus orbitalis* is an accessory organ associated with the eyes allowing flatfishes to protrude their eyes while buried in the substrate (Chapleau, 1993: 521). Chapleau (1993: 521) considered that this organ was present in all flatfishes, including *Psettodes*. This organ was mentioned in earlier studies by Holt (1894), Cole and Johnstone (1902) and Norman (1934)

but it has been studied only in a few species: *Hippoglossus vulgaris* (now valid as *Hippoglossus hippoglossus* (L., 1758) (Pleuronectidae), *Hippoglossoides platessoides* (Fabricius, 1780) (Pleuronectidae), *Pleuronectes platessa* L., 1758 (Pleuronectidae), *Pleuronectes flesus* (now valid as *Platichthys flesus* (L., 1758) (Pleuronectidae), *Limanda limanda* (L., 1758) (Pleuronectidae), *Microstomus kitt* (Walbaum, 1792) (Pleuronectidae), *Solea solea* (L., 1758) (Soleidae) and *Scophthalmus rhombus* (L., 1758) (Scophthalmidae) (Holt, 1894; Cole and Johnstone, 1902 for *Pleuronectes platessa*). Furthermore, Chabanaud (1937) wrote that the eyes of *Psettodes* did not protrude as opposed to all other flatfish species, meaning that the *recessus orbitalis* was probably absent in *Psettodes*. Bürgin (1986) detailed the muscular anatomy of the orbital region of *Psettodes erume*, but did not mention the existence of a *recessus orbitalis*. One of us (BC) could not see this structure in dissecting a frozen *Psettodes*. We then chose to code absence of *recessus orbitalis* in *Psettodes*.

The character states for *Lates* and *Caranx* are based on the anatomical descriptions made respectively by Holden (1967), Greenwood (1976), Smith-Vaniz and Carpenter (2007), and Springer and Smith-Vaniz (2008) and on our examination of one specimen of *Caranx caninus* Günther, 1867, MNHN-6500 (SL: 49.0 cm) (Terцерie *et al.*, 2016), and one specimen of *C. melampygus* Cuvier, 1833, CAS-SU 2845 (SL: 11.4 cm); the character states for fossils are based on Blot (1969) and Friedman (2008) description for *Amphistium*, and on Friedman (2012) for *Heteronectes*. See Appendix 1 for a detailed description of characters.

Table I. – Data matrix. Changes to Chapleau (1993) are indicated in bold.

Taxon	1-10	11-20	21-30	31-39
<i>Lates</i>	<b>00?0000000</b>	<b>0000001000</b>	<b>0100000000</b>	<b>000000000</b>
<i>Caranx</i>	<b>00?0000000</b>	<b>0000001000</b>	<b>0110000000</b>	<b>000000000</b>
<i>Heteronectes</i>	<b>1??0000000</b>	<b>00??000000</b>	<b>?010?00000</b>	<b>?0000?00?</b>
Psettodidae	1100000000	0000001000	0100000000	0000000000
<i>Lepidoblepharon</i>	1111111111	1010000000	0010000000	0000000000
<i>Citharus</i>	1111111111	1000000000	0010000000	000110000
<i>Citharoides</i>	1111111111	1010000000	0010000000	000010000
<i>Brachypleura</i>	1111111111	1000001100	0000011100	000110000
Scophthalmidae	1111111111	1000010000	0111011100	000110000
Paralichthyidae	1111111111	1010010000	0011011100	000111000
Bothidae	1111111111	1010010100	0011111100	000111000
Pleuronectidae	1111111111	1010010000	0111011100	000111000
Poecilopsettidae	1111111111	1010001100	0111200000	000111000
Rhombosoleidae	1111111111	1110100100	0111200000	000111000
Samaridae	1111111111	1011111111	1011200000	000111000
Achiridae	1111111111	1110101111	1111200000	000011111
Soleidae	1111111111	1111101111	2111200011	111111121
Cynoglossidae	1111111111	1111001111	2111200011	111111121
<i>Amphistium</i>	<b>10?000100</b>	<b>00??000000</b>	<b>?010?00000</b>	<b>?0000?00?</b>

**Cladistic analysis**

The data matrix including 19 taxa and 39 characters (Tab. I) was analyzed with PAUP 3.1.1. using the branch-and-bound search for the parsimonious trees. Trees were rooted on the two outgroups as defined above and character optimization was set for accelerated transformation (ACCTRAN).

**RESULTS**

The parsimony analysis yielded 12 equally parsimonious trees (length: 63 steps). The consistency index (CI) is 0.67, the homoplasy index (HI) is 0.33 and the retention index is 0.84, showing a moderate level of homoplasy. The Majority-rule consensus of these 12 trees is shown in Fig. 2; the two nodes showing a frequency of 67% (therefore collapsing in the strict consensus tree) are marked with a star, all remaining nodes having a frequency of 100% are present in the strict consensus tree.

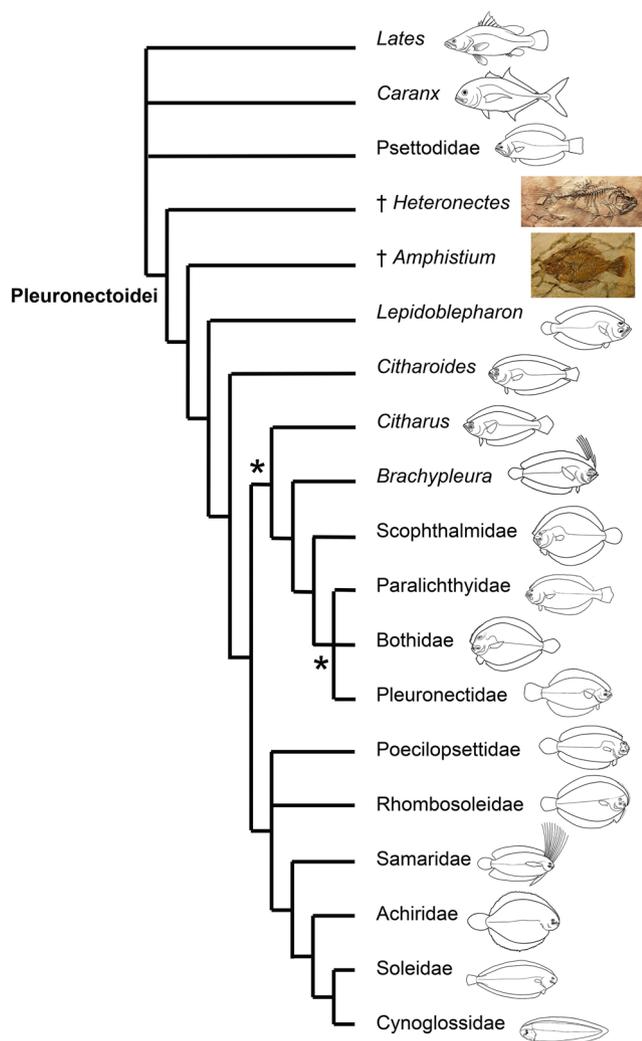


Figure 2. – Majority-rule consensus tree of the 12 equiparsimonious trees of 63 steps obtained from a branch-and-bound search (CI = 0.67, RI = 0.84). Nodes with a star have a frequency of 67%, all others 100%. Illustrations for Psettodidae, Bothidae, Paralichthyidae, Pleuronectidae, Poecilopsettidae, Rhombosoleidae, Samaridae, Achiridae and Cynoglossidae modified after Chanet *et al.* (2004) and original work by J.F. Dejouanet.

Results of the analysis show that the monophyly of flatfishes – in its classic view, i.e. including *Psettodes* – is neither supported nor contradicted by the present dataset. Six of the parsimonious trees show *Lates* and *Caranx* outside the ingroup (Fig. 3A), making flatfishes monophyletic, while the six other parsimonious trees show *Caranx* more closely related to flatfishes than *Psettodes* (Fig. 3B), disrupting the split between outgroups and ingroup, and making flatfishes non-monophyletic.

Regarding the results, it is then difficult to claim that the present dataset either contradicts or supports flatfish monophyly. The more accurate interpretation of these results is considering an unresolved monophyly of flatfishes. Concerning the fossils, *Heteronectes* appears as the most basal pleu-

ronectiform fish and *Amphistium* immediately crownward to the latter. The global intrarelations of extant flatfishes remain basically unchanged with the notable exception of the monophyly of the bothoid group, which herein gathers the genus *Brachypleura*, the Scophthalmidae, the Paralichthyidae, the Bothidae and the Pleuronectidae in a clade.

In the present study, the skull asymmetry is resolved as a synapomorphy of Pleuronectoidei + *Amphistium* and *Heteronectes* and appears to have convergently evolved in *Psettodes*. This apparent parallelism is due to the choice of presenting the result under the form of a consensus, i.e. due to the ambiguity of our data with regard to flatfish monophyly. In six of the twelve most parsimonious trees skull asymmetry is the synapomorphy of flatfishes (Fig. 3A); while in the six others it appears in parallel in *Psettodes* and in the flatfishes (Fig. 3B). Skull asymmetry could thus still be a pleuronectiform synapomorphy but the present matrix needs more data to confirm it. At the present state of knowledge, the *recessus orbitalis* seems to be present only in the flatfishes, with the exception of *Psettodes*. A robust and elongate anteriormost proximal pterygiophore of the anal fin (character 23) is present in *Caranx*, *Heteronectes* (Friedman, 2012), *Amphistium* (Friedman, 2008) (Fig. 4) and all flatfishes except *Psettodes* and *Brachypleura*. This character (coded 0 in *Lates* and 1 in *Caranx*) is the one that, in six of the most parsimonious trees, makes *Caranx* (coded 1) more closely related to the flatfishes (coded 1), whereas *Psettodes* (coded 0) is more distantly placed, and, given a reversion in *Brachypleura* (coded 0), this taxon is well nested within the flatfishes. If flatfishes are monophyletic (Fig. 3A), a robust and elongate anteriormost proximal pterygiophore of the anal fin is a synapomorphy of the Pleuronectoidei, as stated by Chapleau (1993), given a convergence in the outgroup *Caranx* and a reversion in *Brachypleura*. If *Caranx* is more closely related to flatfishes than *Psettodes* is (Fig. 3B), this character state is the synapomorphy of a new clade uniting *Caranx* and the Pleuronectoidei (given a reversion in *Brachypleura*). As six of our most parsimonious trees support the first option and six others support the second hypothesis, it is too early to provide a defining statement on this character optimization. The distributions of the other characters remain the same as those in Chapleau (1993).

## DISCUSSION

The bothoid group, which herein gathers the genus *Brachypleura*, the Scophthalmidae, the Paralichthyidae, the Bothidae and the Pleuronectidae in a clade, was proposed by Hensley and Ahlstrom (1984) based on the distribution of several morphological characters and, interestingly, it is recovered in every tree of the present analysis (Fig. 2) on the basis of the structure of caudal fin endoskeleton (characters

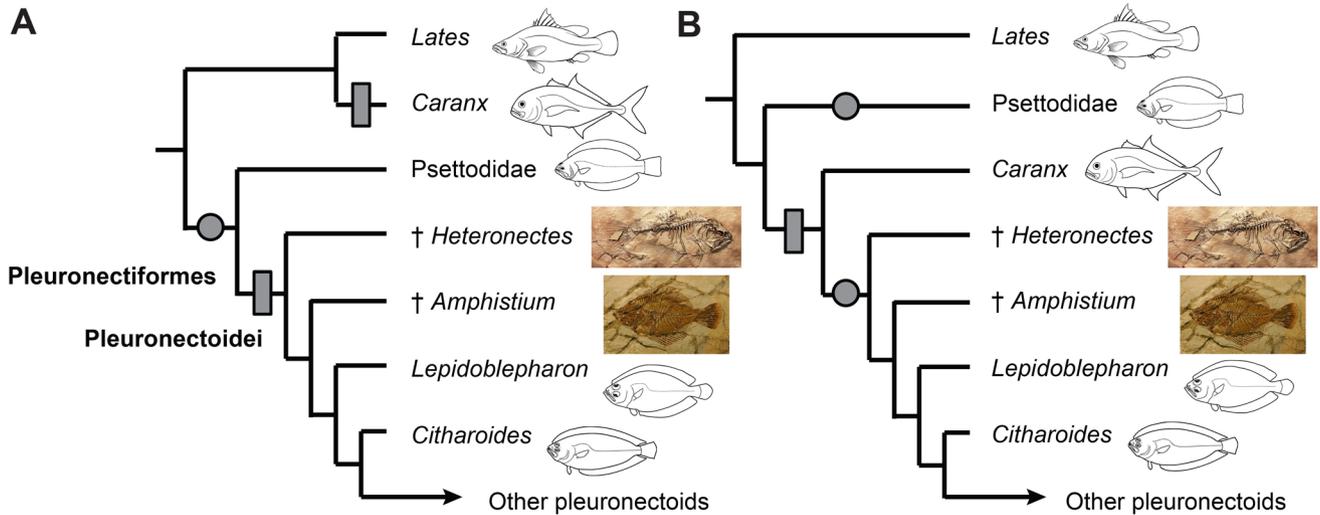


Figure 3. – Among twelve equiparsimonious trees, six of them show a monophyletic Pleuronectiformes including *Psettodes* (A), while six of them show *Psettodes* outside of the Pleuronectiformes and separating members of the outgroup (*Lates* and *Caranx*, B). Circles indicate the rise of the asymmetric skull (character 1); squares indicate a robust and elongate anteriormost proximal pterygiophore of the anal fin (character 23).

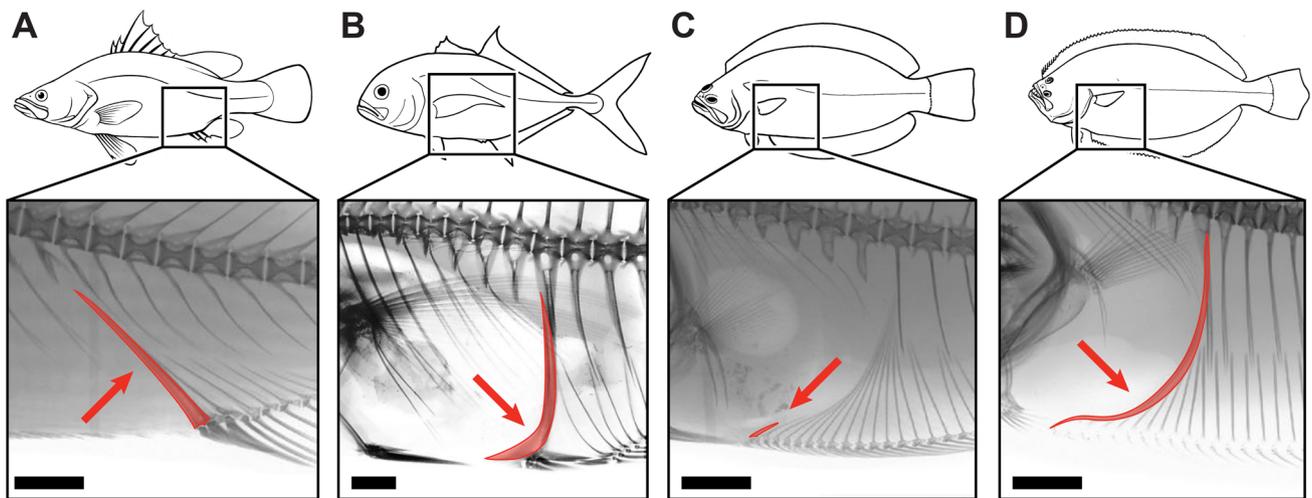


Figure 4. – Skeletal anatomy of the abdominal region of four carangimorph genera illustrating the variation of the first anal pterygiophore. A: *Lates calcarifer* (Centropomidae); B: *Caranx melampygus* (Carangidae); C: *Psettodes erumei* (Psettodidae); D: *Paralichthys albigutta* (Paralichthyidae). Each red arrow shows the first anal-fin pterygiophore (enhanced in red). Scale bars = 10 mm. Figures A, C and D from Harrington *et al.* (2016: 10, fig. 4).

26, 27 and 28). Chapleau (1993) found only unresolved relationships among its five components. He observed a bothoid clade in only “one of the 18 equally parsimonious trees” (p. 535-536) and concluded (p. 536) that “more congruent derived characters are needed to corroborate the monophyletic status of this taxon”. The existence of the bothoid group has been criticized by Hoshino (2001) based on the study of other morphological characters (especially myological), who proposed the monophyly of the citharid family, gathering the genera *Citharus*, *Citharoides*, *Brachypleura*, *Lepidoblepharon* and *Paracitharus*. This gathering was not present in Chapleau (1993), although Chapleau did not use

the genus *Paracitharus* in his analysis. This taxon has often been placed in the genus *Citharoides* (see Hoshino, 2001: 402).

Friedman (2008, 2012) reanalysed fossils once considered to be related to flatfishes and found evidence that two fossil species, *Amphistium paradoxum* and *Heteronectes chaneti*, possess an asymmetrical skull and thus should belong to the pleuronectiform order. Moreover, Friedman (2012) conducted a phylogenetic analysis including flatfish species, recent and fossil, some carangids, one ehippid species, one scorpidid species, one monodactylid species, and some zeiforms. In this work, flatfishes appeared as closely

related to a clade gathering ehippids, scorpidids and monodactylids, this clade being related to a clade formed by the two carangid species (Friedman, 2008: 211, fig. 2a). Surprisingly, in the simplified cladogram of the figure 2c (p. 211), the sister-group of the Pleuronectiformes is *Trachinotus*, a carangid species. However, a close relationship between flatfishes and ehippids and monodactylids is contradicted by several molecular works (Dettaï and Lecointre, 2005; Li *et al.*, 2009). In their synthetic phylogenetic classification of bony fishes, Betancur-R. *et al.* (2017) considered monodactylids and ehippids as members of the Eupercaria, far from the Carangimorpha. Friedman's (2008) work provided outstanding data to reconstruct the evolutionary process leading to flatfishes, but he did not take into consideration the information brought by recent, especially molecular, works to define his sampling of species for the analysis.

In 2012, Friedman considered the results of molecular works to elucidate the problem of the relationships of *Heteronectes chaneti* and flatfishes within acanthomorphs. Similarities between *Heteronectes* and latids were considered ambiguous and no phylogenetic analysis was provided in this work to test the validity of these statements (Friedman, 2012). Campbell *et al.* (2013) did not recover the monophyly of Pleuronectiformes, *Psettodes* being resolved as far from the other flatfishes. This point was controversial and several authors (Smith and Wheeler, 2006; Li *et al.*, 2009, 2011; Near *et al.*, 2013; Betancur-R. *et al.*, 2013a, b; Campbell *et al.*, 2013; Betancur-R and Ortí, 2014) tried to solve this issue thanks to molecular data with diverse results. Some plead for the monophyly of flatfishes, including *Psettodes* (Li *et al.*, 2009; Campbell *et al.*, 2013; Betancur-R and Ortí, 2014), while others supported the exclusion of *Psettodes* from flatfishes (Li *et al.*, 2011; Near *et al.*, 2013; Betancur-R. *et al.*, 2013a, b; Campbell *et al.*, 2013).

Recently, Harrington *et al.* (2016) tackled this phylogenetic problem by analysing more than 1000 ultraconserved DNA element (UCE) loci covering 45 carangimorph species. They concluded that Pleuronectiformes, including *Psettodes*, form a clade and that the origin of ocular asymmetry occurred only once. Moreover, they found that this event took place rapidly during the Paleogene (66.0-23.03 Ma) and that flatfish asymmetry evolved over an interval of no more than 2,97 million years. They concluded that this longstanding uncertainty in phylogenetic hypotheses for flatfishes and their carangimorph relatives was the result of successive and rapid divergences. A point that is reflected in Betancur-R *et al.* (2017) synthesis with short branch lengths linking the Centropomidae, the Psettodidae and the Pleuronectoidei, and the following comment in their classification: "although contentious (e.g. Campbell *et al.*, 2013), the monophyly of Pleuronectiformes is resolved by several molecular studies (Betancur-R *et al.* 2013a, b; Sanciangco *et al.*, 2015; Harrington *et al.*, 2016)". Considering these recent results from

molecular studies, we consider that (1) pleuronectiforms are monophyletic; (2) *Psettodes* is a pleuronectiform and the sister-group of the Pleuronectoidei. In such a context of rapid lineage divergences at the origin of the Pleuronectiformes, it seems not surprising that the present anatomical data fails to clearly recover monophyletic flatfishes. This is partly due to the complexity of both definition and distribution of several characters like the presence of a *recessus orbitalis* or the structure of the first anal-fin pterygiophore. The first one was considered as a synapomorphy of flatfishes while the later was a synapomorphy of the Pleuronectoidei. A detailed anatomical survey of the *recessus orbitalis* is still needed and, until then, this character should not be used to define any group. A strong and robust first anal-fin pterygiophore is not only present in Pleuronectoidei, but, at least, in some carangid species among other members of the Carangimorpha (Fig. 4). In order to identify the sister group of flatfishes, keeping in mind the conclusion of Harrington *et al.* (2016) that flatfishes arose quickly, the distribution of this character, among others, has to be surveyed in carangids, coryphaenids, menids, lactariids, polynemids, rachycentrids, echeneids, sphyraenids, leptobramids, toxotids, xiphiids, istiophorids, nemastids, and some fossil taxa among others.

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## REFERENCES

- AGASSIZ L., 1834-1843. – Recherches sur les Poissons fossiles. Petitpierre, Neuchâtel.
- AMAOKA K., 1969. – Studies on the sinistral flounders found in the waters around Japan. Taxonomy, anatomy and phylogeny. *J. Shimonoseki Univ. Fish.*, 18(2): 65-340.
- AMAOKA, K. 1972. – Osteology and relationships of the citharid flatfish *Brachypleura novaezeeland. Jpn. J. Ichthyol.*, 19(4): 263-273.
- BETANCUR-R R. & ORTI G., 2014. – Molecular evidence for the monophyly of flatfishes (Carangimorpharia: Pleuronectiformes). *Mol. Phylogenet. Evol.*, 73: 18-22. DOI: 10.1016/j.ympev.2014.01.006
- BETANCUR-R R., BROUGHTON R.E., WILEY E.O., CARPENTER K., LÓPEZ J.A., LI C., HOLCROFT N.I., ARCILA D., SANCIANGCO M., CURETON LI J.C., ZHANG F., BUSER T., CAMPBELL M.A., BALLESTEROS J.A., ROA-VARON A., WILLIS S., BORDEN W.C., ROWLEY T., RENEAU P.C., HOUGH D.J., LU G., GRANDE T., ARRATIA G. & ORTÍ G., 2013a. – The tree of life and a new classification of bony fishes. *PLoS Curr.*, 5.

- BETANCUR-R R., LI C., MUNROE T.A., BALLESTEROS J.A. & ORTÍ G., 2013b. – Addressing gene-tree discordance and non-stationarity to resolve a multi-locus phylogeny of the flatfishes (Teleostei: Pleuronectiformes). *Syst Biol.*, 62(5): 763-85.
- BETANCUR-R R., WILEY E.O., ARRATIA G., ACERO A., BAILLY N., MIYA M., LECOINTRE G. & ORTÍ G., 2017. – Phylogenetic classification of bony fishes. *BMC Evol. Biol.*, 17: 162. DOI: 10.1186/s12862-017-0958-3
- BLOT J., 1969. – Les Poissons fossiles du Monte Bolca. Classés jusqu'ici dans les Familles des Carangidae – Menidae – Ehippididae – Scatophagidae. *Stud. Ric. Giacimenti Terziari Bolca* 1: 525 p.
- BLOT J., 1970. – Les origines des poissons plats. *Sci. Avenir*, 284: 836-843.
- BOULENGER G.A., 1902. – LIX. – Descriptions of new fishes from the collection made by Mr. E. Degen in Abyssinia. *Ann. Mag. Nat. Hist.*, 10(60): 421-439. DOI: 10.1080/00222930208678700
- BÜRGIN T., 1986. – The oral jaw apparatus of the Indian halibut, *Psettodes erumei* (Bloch & Schneider, 1801) (Teleostei; Pleuronectiformes) – a formal description with functional considerations. *Prog. Zool.*, 35: 463-466.
- CAMPBELL M.A., CHEN W.J. & LOPEZ J.A., 2013. – Are flatfishes (Pleuronectiformes) monophyletic? *Mol. Phylogenet. Evol.*, 69(3): 664-673. DOI: 10.1016/j.ympev.2013.07.011
- CHABANAUD P., 1937. – Téléostéens dissymétriques du Mokkatam inférieur de Tourah. *Mém. Inst. Egypte*, 32: 1-121.
- CHABANAUD P., 1949. – Le problème de la phylogénèse des Heterosomata. *Bull. Inst. Océanogr.*, 950: 1-24.
- CHANET B., 1997. – A cladistic reappraisal of the fossil flatfishes record consequences on the phylogeny of the Pleuronectiformes (Osteichthyes: Teleostei). *Ann. Sci. Nat. Zool.*, 18: 105-117.
- 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 cladistics reassessment. *Bull. Mar. Sci.*, 52(1): 516-540.
- CHAPLEAU F. & KEAST A., 1988. – A phylogenetic reassessment of monophyletic status of the family Soleidae, with comments on the suborder Soleoidei (Pisces, Pleuronectiformes). *Can. J. Zool.*, 66: 2797-2810. DOI: 10.1139/z88-408
- CHEN W.-J., 2001. – La répétitivité des clades comme critère de fiabilité: application à la phylogénie de Acanthomorpha (Teleostei) et des Notothenioidei (acanthomorphes antarctiques). Ph.D. Thesis, University of Paris VI.
- CHEN W.J., BONILLO C. & LECOINTRE G., 2003. – Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. *Mol. Phylogenet. Evol.*, 26: 262-288. DOI: 10.1016/S1055-7903(02)00371-8
- COLE F.J. & JOHNSTONE J., 1902. – Scientific books: Pleuronectes (The Plaice). *Science*, 15: 465-466.
- COOPER J.A. & CHAPLEAU F., 1998. – Phylogenetic status of *Paralichthodes algoensis* (Pleuronectiformes: Paralichthodidae). *Copeia*, (2): 477-481. DOI: 10.2307/1447445
- COPE E.D., 1871. – Observations on the systematic relations of the fishes. *Am. Nat.*, 5(8/9): 579-593.
- CUNNINGHAM J.T., 1892. – Report on the probable ages of young fish collected by Mr. Holt in the North Sea. *J. Mar. Biol. Ass. U.K.*, 2(4): 344-362. DOI: 10.1017/S0025315400072301
- 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. Édition 1, Vol. 2, 532 p. Paris.
- DAVESNE D., FRIEDMAN M., BARRIEL V., LECOINTRE G., JANVIER P., GALLUT C. & OTERO O., 2014. – Early fossils illuminate character evolution and interrelationships of Lampridiformes (Teleostei, Acanthomorpha). *Zool. J. Linn. Soc.*, 172: 475-498. DOI: 10.1111/zoj.12166
- DAVESNE D., GALLUT C., BARRIEL V., JANVIER P., LECOINTRE G. & OTERO O., 2016. – The phylogenetic intrarelationships of spiny-rayed fishes (Acanthomorpha, Teleostei, Actinopterygii): fossil taxa increase the congruence of morphology with molecular data. *Front. Ecol. Evol.*, 4, 129. DOI: 10.3389/fevo.2016.00129
- DETTAÏ A. & LECOINTRE G., 2005. – Further support for the clades obtained by multiple molecular phylogenies in the acanthomorph bush. *C. R. Biol.*, 328: 674-689. DOI: 10.1016/j.crv.2005.04.002
- ESCHMEYER W.N. & FRICKE R., 2010. – Catalog of Fishes. World Wide Web electronic publication. Online Version, Updated 2 Aug. 2018. Available at <http://research.calacademy.org/ichthyology/catalog/fishcatmain.asp>.
- FRIEDMAN M., 2008. – The evolutionary origin of flatfish asymmetry. *Nature*, 454: 209-212. DOI: 10.1038/nature07108
- FRIEDMAN M., 2012. – Osteology of †*Heteronectes chaneti* (Acanthomorpha, Pleuronectiformes), an Eocene stem flatfish, with a discussion of flatfish sister-group relationships. *J. Vertebr. Paleontol.*, 32: 735-756. DOI: 10.1080/02724634.2012.661352
- GREENWOOD P.H., 1976. – A review of the Family Centropomidae (Pisces, Perciformes). *Bull. Br. Mus. (Nat. Hist.) Zool.*, 29(1): 1-81.
- 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 Evol. Biol.*, 16: 224. DOI: 10.1186/s12862-016-0786-x
- HENSLEY D.A. & AHLSTROM E.H., 1984. – Pleuronectiforms: Relationships. In: *Ontogeny and Systematics of Fishes* (Moser H.G., Richard W.J., Cohen D.M., Fahay M.P., Kendall A.W. & Richardson S.L., eds), *Am. Soc. Ichthyol. Herpetol.*, Spec. Publ. 1: 670-687.
- HOLDEN M.J., 1967. – The systematics of the genus *Lates* (Teleostei: Centropomidae) in lake Albert, East Hist. *Zoology*, 29(1): 1-81.
- HOLT E.W., 1894. – Studies in teleostean morphology from the marine laboratory at Cleethorpes. *Proc. Zool. Soc. Lond.*, 1894: 413-446.
- HOSHINO K., 2001. – Monophyly of the Citharidae (Pleuronectoidei: Pleuronectiformes: Teleostei) with considerations of pleuronectoid phylogeny. *Ichthyol. Res.*, 48(4): 391-404. DOI: 10.1007/s10228-001-8163-0
- JOHNSON D.G., 1980. – The limits and relationships of the Lutjanidae and associated families. *Bull. Scripps Inst. Oceanogr.*, Univ. Calif. Press, 24: 114 p.
- JOHNSON D.G., 1984. – Percoidei: development and relationships. In: *Ontogeny and Systematics of Fishes* (Moser H.G., Richard W.J., Cohen D.M., Fahay M.P., Kendall A.W. & Richardson S.L., eds), *Am. Soc. Ichthyol. Herpetol.*, Spec. Publ. 1: 464-478.

- JORDAN D.S. & EVERMANN B.W., 1898. – The fishes of North and Middle America: a descriptive catalogue of the species of fish-like vertebrates found in the waters of North America, north of the Isthmus of Panama. Bulletin (United States National Museum) no. 47. Washington: Smithsonian Institution. DOI: 10.5962/bhl.title.46755
- KEENE M.J. & TIGHE K.A., 1984. – Beryciformes: development and relationships. In: Ontogeny and Systematics of Fishes (Moser H.G., Richards W.J., Cohen D.M., Fahay M.P., Kendall, Jr. A.W. & Richardson S.L., eds), pp. 383-398. Am. Soc. Ichthyol. Herpetol. Spec Publ no 1.
- KYLE H.M., 1923. – The asymmetry, metamorphosis, and origin of flat-fishes. *Philos. Trans. R. Soc. Lond. B*, 211: 75-129. DOI: 10.1098/rstb.1923.0002
- LAUDER G.V. & LIEM K.F., 1983. – The evolution and interrelationships of the actinopterygian fishes. *Bull. Mus. Comp. Zool. Harvard*, 150: 95-197.
- LI B., DETTAI A., CRUAUD C., COULOUX A., DESOUTTER-MENIGER M. & LECOINTRE G., 2009. – RNF213, a new nuclear marker for acanthomorph phylogeny. *Mol. Phylogenet. Evol.*, 50: 345-363. DOI: 10.1016/j.ympev.2008.11.013
- LI C., BETANCUR-R R., SMITH W.L. & ORTI G., 2011. – Monophyly and interrelationships of snook and barramundi (Centropomidae sensu Greenwood) and five new markers for fish phylogenetics. *Mol. Phylogenet. Evol.*, 60: 463-471. DOI: 10.1016/j.ympev.2011.05.004
- LITTLE A.G., LOUGHEED S.C. & MOYES C.D., 2010. – Evolutionary affinity of billfishes (Xiphiidae and Istiophoridae) and flatfishes (Pleuronectiformes): independent and trans-subordinal origins of endothermy in teleost fishes. *Mol. Phylogenet. Evol.*, 56: 897-904. DOI: 10.1016/j.ympev.2010.04.022
- LINNAEUS C., 1758. – Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Ed. 10, Tomus 1. L. Salvii, Stockholm, Sweden, 823 p.
- MABUCHI K., MIYA M., AZUMA Y. & NISHIDA M., 2007. – Independent evolution of the specialized pharyngeal jaw apparatus in cichlid and labrid fishes. *BMC Evol. Biol.*, 7(1): 10. DOI: 10.1186/1471-2148-7-10
- MIYA M., TAKESHIMA H., ENDO H., ISHIGURO N., INOUE J., MUKAI T., SATOH T., YAMAGUCHI M., KAWAGUCHI A., MABUCHI K., SHIRAI S. & NISHIDA M., 2003. – Major patterns of higher Teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. *Mol. Phylogenet. Evol.*, 26: 121-138. DOI: 10.1016/s1055-7903(02)00332-9
- MIYA M., SATOH T. & NISHIDA M., 2005. – The phylogenetic position of toadfishes (order Batrachoidiformes) in the higher ray-finned fish as inferred from partitioned Bayesian analysis of 102 whole mitochondrial genome sequences. *Biol. J. Linn. Soc.*, 85(3): 289-306. DOI: 10.1111/j.1095-8312.2005.00483.x
- MEYNARD C.N., MOUILLOT D., MOUQUET N. & DOUZERY E.J., 2012. – A phylogenetic perspective on the evolution of Mediterranean teleost fishes. *PLoS ONE*, 7(5): e36443. DOI: 10.1371/journal.pone.0036443
- MUNROE T.A., 2015. – Systematic diversity of the Pleuronectiformes. Chapter 2. In: Flatfishes: Biology and Exploitation (Gibson R.N., ed.), pp. 10-41. Oxford: Blackwell Science Ltd. DOI: 10.1002/9780470995259.ch2
- NEAR T.J., EYTAN R.I., DORNBURG A., KUHN K.L., MOORE J.A., DAVIS M.P., WAINWRIGHT P.C., FRIEDMAN M. & SMITH W.L., 2012. – Resolution of ray-finned fish phylogeny and timing of diversification. *Proc. Nat. Acad. Sci. USA*, 109(34): 13698-13703. DOI: 10.1073/pnas.1206625109
- NEAR T.J., DORNBURG A., EYTAN R.I., KECK B.P., SMITH W.L., KUHN K.L., MOORE J.A., PRICE S.A., BURBRINK F.T., FRIEDMAN M. & WAINWRIGHT P.C., 2013. – Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. *Proc. Nat. Acad. Sci. USA*, 110(31): 12738-12743. DOI: 10.1073/pnas.1304661110
- NORMAN J.R., 1934. – A Systematic Monograph of the Flatfishes (Heterosomata). London: British Museum of Natural History.
- REGAN C.T., 1910. – XLVIII. – The caudal fin of the Elopidae and of some other Teleostean fishes. *J. Nat. Hist.*, 5(28): 354-358. DOI: 10.1080/00222931008692779
- SANCIANGCO M.D., CARPENTER K.E., BETANCUR-R. R., 2015. – Phylogenetic placement of enigmatic percomorph families (Teleostei: Percomorphaceae). *Mol. Phylogenet. Evol.*, 94: 565-576. DOI: 10.1016/j.ympev.2015.10.006
- SMITH W.L. & CRAIG M.T., 2007. – Casting the percomorph net widely: the importance of broad taxonomic sampling in the search for the placement of serranid and percid fishes. *Copeia*, (1): 35-55. DOI: 10.1643/0045-8511(2007)7[35:CTPNWT]2.0.CO;2
- SMITH W.L. & WHEELER W.C., 2006. – Venom evolution widespread in fishes: a phylogenetic road map for the bioprospecting of piscine venoms. *J. Hered.*, 97: 206-217. DOI: 10.1093/jhered/esj034
- SMITH-VANIZ W.F. & CARPENTER K.E., 2007. – Review of the crevalle jacks, *Caranx hippos* complex (Teleostei: Carangidae), with a description of a new species from West Africa. *Fish. Bull.*, 105: 207-233.
- SPRINGER V.G. & SMITH-VANIZ W.F., 2008. – Supraneural and pterygiophore insertion patterns in carangid fishes, with description of a new Eocene carangid tribe, †*Paratrachinotini*, and a survey of anterior anal-fin pterygiophore insertion patterns in Acanthomorpha. *Bull. Biol. Soc. Washington*, 1: 1-73. DOI: 10.2988/0097-0298(2008)16[1:SAPIPI]2.0.CO;2
- TERCERIE S., BEAREZ P., PRUVOST P., BAILLY N. & VIGNES-LEBBE R., 2016. – Osteobase. World Wide Web electronic publication. osteobase.mnhn.fr, version Sep. 2016.
- THILO O., 1902. – Die Vorfahren der Schollen. *Bull. Sci. Acad. Imp. Sci. St.-Petersbourg*, 14(3): 315-350.
- WAINWRIGHT P.C., SMITH W.L., PRICE S.A., TANG K.L., SPARKS J.S., FERRY L.A., KUHN K.L., EYTAN R.I. & NEAR T.J., 2012. – The evolution of pharyngognath: a phylogenetic and functional appraisal of the pharyngeal jaw key innovation in labroid fishes and beyond. *Syst. Biol.* 61(6): 1001-1027. DOI: 10.1093/sysbio/sys060
- YAGISHITA N., MIYA M., YAMANOUE Y., SHIRAI S.M., NAKAYAMA K., SUZUKI N., SATOH T.P., MABUCHI K., NISHIDA M. & NAKABO T., 2009. – Mitogenomic evaluation of the unique facial nerve pattern as a phylogenetic marker within the perciform fishes (Teleostei: Percomorpha). *Mol. Phylogenet. Evol.*, 53(1): 258-266. DOI: 10.1016/j.ympev.2009.06.009
- ZEHREN S.J., 1979. – The comparative osteology and phylogeny of the Beryciformes (Pisces: Teleostei). *Evolutionary Monographs*, Univ. Chicago, 1. 389 p.

## APPENDIX

**List of Characters.** – This section includes the list of characters used in this work and by Chapleau (1993). The distribution of character states for each character amongst taxonomic units is summarized in Table I.

1. **Adult cranium.** Symmetrical cranium with one eye on each side of head (0); symmetrical with both eyes on one side of head (1).  
Present in all flatfishes, *Psettodes* included and also in *Heteronectes* and *Amphistium*.
2. **Epicranial section of dorsal fin.** Dorsal fin without epicranial section (0); presence of an epicranial section (1).  
Present in all flatfishes, *Psettodes* included and uncertain in *Heteronectes*, absent in *Amphistium* (see text for discussion).
3. **Recessus orbitalis.** Absent (0); present (1).  
Present in all flatfishes, very uncertain (considered as absent) in *Psettodes*, unknown in *Amphistium* and *Heteronectes*.
4. **Palatine teeth.** Present (0); absent (1).  
Absent in all flatfishes, except for *Psettodes*, unknown in *Amphistium* and *Heteronectes*.
5. **Toothed plates on basihyal.** Present (0); absent (1).  
Absent in all flatfishes, except for *Psettodes*.
6. **Basisphenoid.** Present (0); absent (1).  
Present in all flatfishes, except for *Psettodes*.
7. **Spines in median fins.** Present (0); absent (1).  
Absent in all flatfishes, except for *Psettodes*.
8. **Sciatic portion of urohyal.** Absent or not well developed (0); distinct and often elongated sciatic portion of urohyal (1).  
Derived in all flatfishes, except for *Psettodes*; present in *Amphistium*.
9. **Uroneural 1.** Present (0); reduction and losses of uroneurals (1).  
Present in all flatfishes, except for *Psettodes*.
10. **Shape of second infrapharyngobranchial.** Elongate second infrapharyngobranchial (0); elliptical or rounded infrapharyngobranchial of various sizes (sometimes very small) (1).  
Derived condition in all flatfishes, except for *Psettodes*. The absence of a second infrapharyngobranchial is an autapomorphy of Cynoglossidae.
11. **Supramaxilla.** Large supramaxilla (0); vestigial or absent (1).  
Absent in all flatfishes, except for *Psettodes*.
12. **Teeth on eyed side premaxilla.** Present (0); absent (1).  
Absent in Rhombosoleidae (except *Oncopterus*, *Psammodycus*, *Azygopus*), Achiridae, Soleidae, and Cynoglossidae. Noted but not coded for pleuronectine *Hypsopsetta*, *Pleuronichthys*, two species of *Pleuronectes* and *Microstomus* and in the bothid *Laeops*.
13. **Vomerine teeth.** Present (0); absent (1).  
Absent in *Lepidoblepharon*, *Citharoides*, Paralichthyidae, Bothidae, Pleuronectinae, Poecilopsettinae, Rhombosoleinae, Samarinae, Achiridae, Soleidae and Cynoglossidae, uncertain in *Heteronectes* and *Amphistium*.
14. **Pterosphenoid.** Present (0); absent (1).  
Present in Samarinae, Soleidae, Cynoglossidae. Absent, but not coded for rhombosoleine *Azygopus* and two species of bothid *Laeops*, unknown in *Heteronectes* and *Amphistium*.
15. **Epioccipitals and foramen magnum.** Exoccipitals forming dorsal margin of foramen magnum (0); epioccipitals forming dorsal margin of foramen magnum (1).  
Derived condition in Rhombosoleinae (except *Psammodycus*), Samarinae, Achiridae and Soleidae, unknown in *Heteronectes* and *Amphistium*.
16. **Ventral margin of foramen magnum.** Exoccipitals forming ventral margin of foramen magnum (0). Basioccipital forming part of ventral margin of foramen magnum (1). Scopthalmidae, Paralichthyidae, Bothidae, Pleuronectinae and Samarinae unknown in *Heteronectes* and *Amphistium*.
17. **Blind side infraorbitals (including lachrymal).** Present (0); reduction to one bone or absent (1).  
Derived condition in *Psettodes*, *Brachypleura*, Poecilopsettinae, Samarinae, Achiridae, Soleidae and Cynoglossidae unknown in *Heteronectes* and *Amphistium*.
18. **Eyed-side infraorbitals.** Present (0); infraorbitals reduced to one or two small bones (1).  
Derived condition in *Brachypleura*, Bothidae, Poecilopsettinae, Rhombosoleinae, Samarinae, Achiridae, Soleidae and Cynoglossidae. Observed but not coded in Pleuronectine *Embassichthys* (Sakamoto, 1984) and *Cyclopsetta* group of Hensley and Ahlstrom (1984), unknown in *Heteronectes* and *Amphistium*.
19. **Articulation of hyomandibula to cranium.** Hyomandibula articulates with cranium along its posterodorsal margin (sometimes in the form of a short process) into an articulatory facet formed by prootic and pterotic (0); long posterodorsal process (definitively longer than anterodorsal one) articulating with pterotic and sometimes with intercalar (prootic not involved in articulation) (1).  
Derived condition in Samarinae, Soleidae, Achiridae and Cynoglossidae.
20. **Postcleithra and coraco-scapular complex on blind-side pectoral fin.** Large coraco-scapular complex with elongate coracoid, some radials and postcleithra (0); postcleithra missing and entire coracoscapular complex sometimes absent or reduced to small cartilaginous plates (1).  
Derived condition in Samarinae, Achiridae, Soleidae and Cynoglossidae.
21. **Shape and orientation of first proximal pterygiophore of dorsal fin.** First proximal pterygiophore of dorsal fin without long anterior process (0); first proximal pterygiophore oriented almost parallel to the orbital region (1); first proximal pterygiophore with long anterior process extending anteriorly relative to point of attachment of anteriormost fin rays or extending well in front of orbital and rostral regions (2).  
Derived state (1) was observed in Samarinae and Achiridae. Derived state (2) was observed in Soleidae and Cynoglossidae.
22. **Teeth on third epibranchial.** Present (0); absent (1).  
Absent in Psettodidae, Scopthalmidae, Pleuronectinae, Poecilopsettinae, Rhombosoleinae, Achiridae, Soleidae and Cynoglossidae. Plesiomorphic condition noted (in Sakamoto, 1984) but not encoded for pleuronectine *Atherestes*, *Reinhardtius* and *Hippoglossus stenolepis* Schmidt 1904, unknown in *Heteronectes* and *Amphistium*.

- 23. Shape of anteriormost proximal pterygiophore of anal fin.** Short proximal pterygiophores with one fin ray (0); anteriormost pterygiophore is elongated (reaches hemal spine of corresponding vertebrae and supports two anal fin rays (1). Derived condition in *Caranx*, *Heteronectes*, *Amphistium* and all flatfishes, except for *Psettodes* and *Brachypleura* (figured in Amaoka, 1972).
- 24. Spine in pelvic fin.** Present (0); absent (1). Absent in Scophthalmidae, Paralichthyidae, Bothidae, Pleuronectinae, Poecilopsettinae, Rhombosoleinae, Samarinae, Achiridae, Soleidae and Cynoglossidae.
- 25. Neural arch and spine on first precaudal vertebra.** Neural arch and neural spine on first precaudal centrum (0); absence of neural spine on neural arch (1); incomplete (or absent) neural arch (2). State (1) found in Bothidae (also found in *Cyclopsetta* group (see Chapleau (1993) discussion on Paralichthyidae) not encoded). State (2) observed in Poecilopsettidae, Rhombosoleidae, Samaridae, Achiridae and Soleidae. The first precaudal centrum missing (lost or fused to second precaudal vertebrae) is an autapomorphy of Cynoglossidae (Chapleau and Keast, 1988).
- 26. Fusion of hypural plates.** No fusion of hypural plates (0); hypurals 3 and 4 fused and hypurals I and 2 fused (1). Derived condition in *Brachypleura*, Scophthalmidae, Paralichthyidae (except *Thysanopsetta* and *Tephrinectes*), Bothidae (except *Mancopsetta*), and Pleuronectidae. Other apomorphic state noted only for Samaridae (see Chapleau (1993)) is hypurals 2-3-4 fused. Achirides *Trinectes fimbriata* (Hensley and Ahlstrom, 1984) and *Apionichthys unicolor* (Chapleau (1993), unpubl. data) and rhombosoleid *Psammodydiscus* show samarid type of caudal skeleton (not coded in Chapleau, 1993). Ontogenetic data and outgroup comparisons indicate that apomorphic states were acquired independently. Data corroborating independent origin of apomorphic character states are in Hensley and Ahlstrom (1984) for bothid *Engyophrys senta* Ginsburg 1933 and Achiridae (Chapleau, 1993). In latter case, state within plesiomorphic achirids (*Achirus*) is presence of 5 distinct hypural plates.
- 27. Articulation of hypurals 1 and 2 to PUI.** Hypural 1 and 2 in (or without) contact with PU I but without a large articulatory surface with PU I (0); tight and wide articulatory surface between hypural plate and PUI, resembling a ball-and-socket arrangement (1). Derived condition in *Brachypleura*, Scophthalmidae, Paralichthyidae, Bothidae, Pleuronectidae.
- 28. Relation of hypural plates with PUI.** Absence of fusion (0); fusion of hypurals 3 and 4 to the centrum of PUI (1). Derived condition in *Brachypleura*, Scophthalmidae, Paralichthyidae (except *Tephrinectes* and *Thysanopsetta*), Bothidae (except *Mancopsetta*) and Pleuronectidae. Hensley and Ahlstrom (1984) indicated that *Citharoides* had hypurals 3 and 4 fused to centrum of PU I but Chapleau and Keast (1988) observed closely adjoined but unfused hypurals 3 and 4 to centrum of PUI.
- 29. Fusion of proximal tip of hypurals to PU 1.** Four hypurals articulating but not fused to PU 1 (0); fusion of the proximal tip of hypural plates to PUI (1). Derived condition found in Soleidae and Cynoglossidae (except *Symphurus australis*) and observed but not encoded for two rhombosoleine genera (*Peltorhamphus*, *Rhombosolea*).
- 30. Edge of preoperculum.** Posterior and ventral edge of preopercle partly visible (0); posterior and ventral edge of preopercle entirely concealed by scales and skin (1). Derived condition in Soleidae and Cynoglossidae (see the text of Chapleau (1993) for discussion of this character).
- 31. Eyed-side mesopterygoid.** Present (0); absent (1). Absent in Soleidae (except *Pardachirus*) and Cynoglossidae.
- 32. Indentation of opercular series.** Absent (0); deep fimbriation pattern of opercular bones (except preopercle) (1). Derived condition in Soleidae and Cynoglossidae.
- 33. Shape of blind-side dentary.** Portion of blind-side dentary anterior to anguloarticular not markedly convex and nearly equal in length and deepness on both sides of the head (0); portion of blind side dentary anterior to anguloarticular is convex and is shorter and deeper than the eyed-side dentary (1). Derived condition in Soleidae and Cynoglossidae.
- 34. Hemal arch on PU2.** Hemal arch not fused to centrum of PU2 (0); hemal arch fused to the centrum (1). Derived in all flatfishes, except for *Psettodes*, *Lepidoblepharon*, *Citharoides* and Achiridae.
- 35. Parhypural and centrum of PUI.** Parhypural articulating with centrum of PUI (0); parhypural forms a plate totally free from PUI (1). Derived state in all flatfishes, except *Psettodes* and *Lepidoblepharon*.
- 36. Branchiostegal membranes.** Branchiostegal membranes separate (0); membranes fused medially, with anteriormost branchiostegals attached to each other (1). Derived state in Paralichthyidae, Bothidae, Pleuronectidae, Poecilopsettidae, Rhombosoleidae, Samaridae, Achiridae, Soleidae and Cynoglossidae.
- 37. Blind-side preopercular canal.** Preopercular canal opening at anteroventral tip of preopercle (0); opening (where present) along ventral margin of bone (1). Derived condition in Achiridae, Soleidae (except *Heteromycteris*) and Cynoglossidae. The achirid *Gymnachirus* and the cynoglossid *Symphurus* lack a preopercular canal.
- 38. Contribution of blind-side lateral ethmoid and blind-side frontal to margin of upper orbit.** Important contribution (40% or more) of blind-side frontal forming blind side bony orbit (0); blind side lateral ethmoid forming most of the blind side margin of the upper orbit (blind side frontal contribution being minimal (1); nil (2). State (1) observed in Achiridae. State (2) is present in Soleidae (except *Heteromycteris* and *Typhlachirus*) and Cynoglossidae.
- 39. Skin on lower jaw and interopercle.** Skin covering not continuous ventrally (0); skin is continuous and covering isthmus and branchiostegals (1). Derived condition in Soleidae, Achiridae and Cynoglossidae.