

**Monophyly and intrarelationships of the family Pleuronectidae
(Pleuronectiformes), with a revised classification**

J. Andrew Cooper

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"The best fish swim near the bottom." - What is most valuable is not to be found near the surface, nor is anything really worth having to be obtained without effort and trouble.

Brewer's Dictionary of Phrase and Fable

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Abstract

A cladistic analysis of interrelationships for 53 (of 58) pleuronectid (Order Pleuronectiformes) species was performed using 106 morphological and osteological characters. The analysis resulted in a 50% majority-rule consensus tree of 128 equally parsimonious cladograms (heuristic search, 403 steps, $ci = 0.33$, $ri = 0.79$). Only five of 47 resolved nodes were observed in less than 100% of the cladograms. These five nodes are restricted to interrelationships within one subfamily. The Pleuronectidae (*sensu* Chapleau and Keast, 1988) are monophyletic based on ten synapomorphies. In addition, five subfamilies were defined: Hippoglossinae, Eopsettiniae, Lyopsettiniae, Hippoglossoidinae and Pleuronectinae. The largest subfamily, the Pleuronectinae, was further subdivided into four tribes: Psettichthyini, Isopsettini, Microstomini and Pleuronectini. The interrelationships established within Pleuronectidae provide a strong foundation for a simplified yet phylogenetically informative taxonomic nomenclature. The genera *Atherestes* and *Reinhardtius*, *Embassichthys*, *Errex*, *Glyptocephalus*, *Tanakius* and *Microstomus*; *Hypsopsetta* and *Pleuronichthys* are regrouped in *Reinhardtius*, *Microstomus* and *Pleuronichthys*, respectively. To preserve the monophyletic status of *Eopsetta*, *E. exilis* was reassigned to the genus *Lyopsetta* (Lyopsettiniae). The genus *Pleuronectes* (as defined by Sakamoto, 1984a) is polyphyletic. It now includes only five species; *Pleuronectes glacialis*, *P. pinnifasciatus*, *P. platessa*, *P. putnami* and *P. quadrituberculatus*. Other *Pleuronectes* species are reclassified in *Isopsetta*, *Limanda*, *Parophrys*, *Platichthys*, *Psettichthys*, and *Pseudopleuronectes*. The monophyletic status of the genus *Limanda* (six species) is uncertain based on unresolved relationships between these species and other taxa in the tribe Pleuronectini.

Résumé

Une analyse cladistique servant à définir les interrelations phylogénétiques de 53 des 58 espèces de Pleuronectidae (Ordre Pleuronectiformes) a été faite sur une matrice de 106 caractères morphologiques et ostéologiques. L'analyse a résulté en un arbre de consensus majoritaire (50%) construit à partir de 128 cladogrammes équiparcimonieux (recherche heuristique, 404 pas, i.c. = 0.33, i.r. = 0.79). Seulement cinq des 47 noeuds résolus ont été observés dans moins de 100% des cladogrammes. Ces noeuds litigieux sont restreints aux interrelations entre espèces à l'intérieur d'une seule sous-famille. La famille Pleuronectidae (*sensu* Chapleau et Keast, 1988) est définie comme étant monophylétique par la présence de dix synapomorphies. De plus, cinq sous-familles sont définies: Hippoglossinae, Eopsettinæ, Lyopsettinæ, Hippoglossoidinae and Pleuronectinae. La plus grande sous-famille est subdivisée en quatre tribus: Psettichthyini, Isopsettini, Microstomini and Pleuronectini. Les interrelations trouvées à l'intérieur des Pleuronectidae donnent une base solide pour la création d'une nomenclature taxinomique à la fois simple et informative au niveau phylogénétique. Les genres *Atherestes* et *Reinhardtius*, *Embassichthys*, *Errex*, *Glyptocephalus*, *Tanakius* et *Microstomus*, *Hypsopsetta* et *Pleuronichthys* sont regroupés dans *Reinhardtius*, *Microstomus* et *Pleuronichthys*, respectivement. Dans le but de préserver le monophylétisme de *Eopsetta*, l'espèce *E. exilis* est transférée dans le genre *Lyopsetta* (Lyopsettinæ). Le genre *Pleuronectes* (*sensu* Sakamoto, 1984a) est polyphylétique. Il ne comprend maintenant que cinq espèces: *Pleuronectes glacialis*, *P. pinnifasciatus*, *P. platessa*, *P. putnami* et *P. quadrituberculatus*. Les autres espèces de *Pleuronectes* sont reclassifiées dans les genres *Isopsetta*, *Limanda*, *Parophrys*, *Platichthys*, *Psettichthys*, and *Pseudopleuronectes*. Le statut monophylétique de *Limanda* (six espèces) demeure incertain.

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Introduction

The family Pleuronectidae (*sensu* Chapleau and Keast, 1988) contains 58 extant species of right-eyed flatfish distributed in marine waters of the Northern Hemisphere. This family excludes the subfamilies Poecilopsetinae, Samarinae, Rhombosoleinae and Paralichthodinae as outlined in Norman (1934). This family contains many commercial species that have long been harvested in Europe, North America and Asia. Species such as the Petrale sole (*Eopsetta jordanii*), Pacific halibut (*Hippoglossus stenolepis*), American plaice (*Hippoglossoides platessoides*) and Dover sole (*Microstomus pacificus*), to name but a few, are valued for their large size and excellent meat (Hart, 1973; Scott and Scott, 1988). Flatfish aquaculture has been a more recent commercial endeavour. European countries such as the Netherlands have invested large amounts of resources in the research and development of flatfish in aquaculture, while Canadian research is growing in this field. The commercial popularity of pleuronectid species coupled with a need to manage this renewable resource strongly emphasises the necessity for a better understanding of the relationships within this group, as well as an informative taxonomic nomenclature that will provide a framework for management policy.

The objective of this study is to clarify the monophyletic status of the Pleuronectidae, to offer an hypothesis of relationships of species within the group and to establish a phylogenetically informative nomenclature. To attain this objective, a cladistic reassessment of all morphological evidence in the literature will be compiled. New morphological characters will be added to the resulting matrix and analysed within a cladistic framework. A new classification based on the phylogenetic information will then be offered.

Historical classification and diagnosis of Pleuronectidae

The Pleuronectidae was regarded by early ichthyologists to represent all known flatfish (Norman, 1934). This classification was adopted by Cuvier (1816) who subdivided the Pleuronectidae into five subfamilies: Hippoglossinae, Pleuronectinae, Platessinae, Soleinae and Cynoglossinae and later by Jordan and Goss (1889) with the addition of two more subfamilies: Samarinae and Oncopterinae. The trend in early flatfish classification was to accommodate new groups of species by revising the existing classification. This involved raising taxonomic rank and the addition of subgroups. Newly discovered species, thought to represent distinct morphological groups, were classified into new subgroups; but original species, and those that did not have special morphologies, were to remain within the Pleuronectidae. Thus Pleuronectidae became a "garbage" group.

Jordan and Evermann (1898) raised flatfish to the suborder Heterosomata with two distinct families Pleuronectidae and Soleidae. The Pleuronectidae, with three subfamilies Hippoglossinae, Pleuronectinae and Psettiniae, were characterised by "a more or less distinct

preopercular margin (i.e. not hidden by the skin and scales of the head), eyes large, well separated; mouth moderate or large; teeth present" (Jordan and Evermann, 1898). The Soleidae was subdivided into two subfamilies Soleinae and Cynoglossinae and characterised by "an adnate preopercular margin, hidden by the skin and scales of the head; eyes small, situated close together; mouth very small, much twisted; teeth rudimentary or wanting" (Jordan and Evermann, 1898).

Regan (1910) proposed a new classification that raised the Heterosomata to the level of order with two suborders Psettodoidea and Pleuronectoidea. Within the second suborder, the Pleuronectidae now contained three subfamilies; Pleuronectinae, Samarinae and Rhombosoleinae. The family was characterised by "having eyes on right side of head, nerve of left eye always dorsal, olfactory lamellae slightly raised, parallel without central rachis and eggs without oil globules" (Regan, 1910).

This classification was adopted by Norman (1934), who incorporated minor revisions from Regan (1920, 1929) and Jordan (1923). The Pleuronectidae now containing five subfamilies: Pleuronectinae, Samarinae, Rhombosoleinae, Poecilopsettinae and Paralichthodinae was characterised by Norman (1934) as "having eyes on the right side; optic chiasma monomorphic, the nerve of the left eye always dorsal; dorsal fin extending forward on the head at least to above the eye; all fin-rays articulated; pelvic with 3 to 13 rays; mouth usually terminal, with the lower jaw more or less prominent; maxillary without a supplemental bone; palatines toothless; lower edge of urohyal deeply emarginate, so that the bone appears forked; preoperculum with free margin; nasal organ of blind side usually near edge of head, but sometimes

nearly opposite that of ocular side; vertebrae never fewer than 30; a single post-cleithrum on each side; ribs present; and an egg without an oil-globule in the yolk". Later classifications removed the genera *Brachypleura* and *Lepidoblepharon* from Pleuronectidae and placed them in the Citharidae (Hubbs, 1945).

Two tribes were classified within the Pleuronectinae (*sensu* Norman, 1934), the Hippoglossini and Pleuronectini (*sensu* Nelson, 1984). This classification was entirely based on jaw morphology. The Hippoglossini identified by "mouth large and symmetrical; maxillae extending to or behind pupil of eyes; teeth well developed on both sides of jaws, contained ten genera (e.g., *Atherestes*, *Eopsetta*, *Hippoglossoides*, *Hippoglossus*, *Lyopsetta*, *Psettichthys* and *Reinhardtius*)". The Pleuronectini was identified by "mouth small and asymmetrical; maxillae usually not extending to pupil of eye; teeth chiefly on blind side of jaw and contained 16 genera (e.g., *Embassichthys*, *Glyptocephalus*, *Hypsopsetta*, *Isopsetta*, *Lepidopsetta*, *Limanda*, *Liopsetta*, *Microstomus*, *Parophrys*, *Platichthys*, *Pleuronectes*, *Pleuronichthys* and *Pseudopleuronectes*)" (Nelson, 1984). Although this classification was effective in identifying two morphological types within Pleuronectinae (*sensu* Norman, 1934), it was not based on an examination of interrelationships within the group, nor did it accurately identify natural groups. The morphologies defining Hippoglossini were all plesiomorphic for the order and the morphologies defining the Pleuronectini are also observed in many lineages closely related to Pleuronectinae (*sensu* Norman, 1934).

Hensley and Ahlstrom (1984), in a review of flatfish classification, indicated that the evidence for monophyly of Pleuronectidae (*sensu* Norman, 1934) was not convincing. The diagnostic characters reviewed in Norman (1934) were found to be plesiomorphic for the order or had distributions that were unknown for many pleuronectiform taxa (Hensley and Ahlstrom, 1984). Subsequent cladistic analysis for major taxa within the order supported the hypothesis that Pleuronectidae was not monophyletic and suggested that the subfamilies: Pleuronectinae, Samarinae, Rhombosoleinae and Poecilopsettinae should be elevated to the family level (Chapleau and Keast, 1988; Chapleau, 1993). Elevating the taxonomic rank of Pleuronectinae to family Pleuronectidae, required reclassification within the family. To simply elevate the two tribes Hippoglossini and Pleuronectini (Nelson, 1984) to the level of subfamily would be inconsistent with a new classification based on cladistic evidence. A cladistic analysis of interrelationships within the Pleuronectidae (*sensu* Chapleau and Keast, 1988) was necessary to establish a revised classification.

The interrelationships and classification of 77 species within Pleuronectidae (*sensu* Norman, 1934) was examined by Sakamoto (1984a) using 78 morphological characters (Appendix A). This was the first detailed morphological study to address the intrarelationships of Pleuronectidae (*sensu* Norman, 1934). This analysis was phenetic by nature and, as such, was not aimed at defining relationships within an evolutionary framework; nor was it aimed at determining taxonomic structure based on natural groups. Sakamoto (1984a) concluded with a reclassification of several genera within the Pleuronectinae (*sensu* Norman, 1934). The species of *Eopsetta* and *Lyopsetta*, *Cleisthenes* and *Hippoglossoides*, *Kareius* and *Platichthys* were

reclassified as *Lyopsetta*, *Hippoglossoides* and *Platichthys* respectively. *Glyptocephalus zachirus* became *Errex zachirus*. All species of *Isopsetta*, *Parophrys*, *Lepidopsetta*, *Limanda*, *Pseudopleuronectes*, *Pleuronectes* and *Liopsetta* were regrouped under the genus *Pleuronectes*. Finally *Paralichthodes algoensis*, originally classified in its own subfamily, Paralichthodinae, was placed within the Pleuronectinae based on overall similarity and the presence of the first neural arch, a symplesiomorphy for the order (Hensley and Ahlstrom, 1984). Because the Pleuronectidae used by Sakamoto (1984a) has since been determined as being polyphyletic (Chapleau, 1993) and the phenetic nature of the analysis, it is unlikely that this revised nomenclature represents natural groups.

Sakamoto's (1984a) nomenclatorial changes were acknowledged by the American Fisheries Society and instituted into the classification of North American flatfish species (Robins et al., 1991). Adopting this reclassification was a recognition of the first and only study that attempted to define intrarelationships in the Pleuronectidae. The synonymization of pleuronectid genera simplified a previously complex and uninformative nomenclature. The 53 species under examination have been historically classified in as many as 28 genera, many of which were monotypic. This previous nomenclature was established prior to any understanding of phylogeny and reflected the morphological diversity within Pleuronectidae. The number of genera used in identifying pleuronectid species would presumably be used to accommodate new species as they were discovered. The alpha taxonomy for this group has been well established and new extant species of Pleuronectidae have not been described since *Limanda sakhalinensis*, Hubbs 1915. Intuitively, a simplified and more informative nomenclature with fewer monotypic genera would

seem appropriate. However, the dubious nature of the most recent reclassification (i.e. Sakamoto, 1984a), the uninformative nature of the previous classification (i.e. Norman, 1934) and the commercial importance of this group requires a more comprehensive examination of pleuronectid intrarelationships based on natural groups. To do this, a cladistic analysis using morphological variation within Pleuronectidae must be compared with morphology observed in closely related outgroups. This will identify morphologies that are uniquely inherited within the Pleuronectidae (apomorphy) as opposed to morphologies retained from ancestral lineages outside the group of interest (plesiomorphy). The cladistic analysis will only determine groups based on apomorphic features; in this manner an hypothesis of genealogical descent will be determined (Wiley, 1981).

Outgroup hypothesis for Pleuronectidae

A comparison with taxa closely related to Pleuronectidae is necessary to determine which character states are apomorphic. This is known as an outgroup comparison (Maddison et al., 1984). The pleuronectid outgroup is not found within the other four families Samaridae, Rhombosoleidae, Poecilopsettidae and Paralichthodinae previously united as Pleuronectidae. Analysis of relationships within the order reveal that only the Pleuronectidae have a caudal skeleton complex that is synapomorphic with taxa belonging to the Paralichthyidae, Scopthalmidae, *Brachypleura*, and Bothidae. These taxa have been identified as the bothoid lineage within Pleuronectiformes (Hensley and Ahlstrom, 1984). This lineage is supported in one of 18 equally parsimonious trees observed in a cladistic analysis for the order (Chapleau, 1993). The Paralichthodinae, with a single species *Paralichthodes algoensis*, was not included in

Chapleau's (1993) cladistic revision. However this species does not have the bothoid caudal fin complex and for this reason, excluded as an outgroup candidate. The other taxa: Samaridae, Rhombosoleidae and Poecilopsettidae were placed in a clade that included the soles: Achiridae, Soleidae and Cynoglossidae (Chapleau, 1993). Consequently, the most likely outgroup for Pleuronectidae, would be represented by species within the bothoid lineage.

There is a wide range of morphological types within the bothoid lineage. It is necessary to determine which bothoid taxon is the sister group to Pleuronectidae and therefore the best candidate for outgroup comparison. The intrarelationships of the bothoid lineage are not resolved (Chapleau, 1993). In the absence of synapomorphies to determine the sister relationship of Pleuronectidae with other bothoid taxa, a comparison of jaw morphology can help to determine the most likely candidates. It is assumed that the outgroup should have large symmetrical jaws and pointed teeth. Within the order Pleuronectiformes the evolutionary trend for jaw morphology and feeding strategy may be considered unidirectional. Symmetry of jaw and dentition found in piscivorous flatfish, like Psettodidae and Citharidae (Groot, 1971), is considered to be the plesiomorphic condition. The morphology observed in these two taxa are most similar to the generalised acanthopterygian morphology (Yazdani, 1969). Taxa with symmetrical jaw morphology give rise to groups with more specialised dentition types and jaw asymmetry, as observed in the families Achiridae, Soleidae and Cynoglossidae (Yazdani, 1969; Chapleau, 1993), but the reverse has not been observed.

The same evolutionary trend is assumed to occur within subgroups of Pleuronectiformes. Left-eyed flounders within the bothoid lineage have a piscivorous type of feeding habit, which requires larger and near symmetrical jaws or a more specialised, asymmetrical jaw structure to accommodate prey capture of a benthic nature (Groot, 1971; Yazdani, 1969). Likewise the, Pleuronectidae contains both piscivorous fish like *Hippoglossus stenolepis* and *Reinhardtius hippoglossoides* as well as more specialised predators like *Microstomus stelleri* (Groot, 1971). Assuming that the evolutionary trend in jaw morphology is consistent, the ancestral pleuronectid would have been a piscivorous feeder. The bothoid family, Paralichthyidae, is one of the most plesiomorphic groups of left-eyed flounders and is chosen as the outgroup for Pleuronectidae. In addition, *Psettodes* and *Lepidoblepharon* are also chosen as secondary outgroups to help determine character polarity. These assumptions are only valid if the Pleuronectidae are monophyletic. If Pleuronectidae are not monophyletic, then multiple outgroup taxa with either symmetrical or asymmetrical jaw morphologies may account for the variation observed within Pleuronectidae.

Establishing a framework for the analysis

It is apparent that the examination of a large number of taxa is necessary to establish the interrelationships within large taxonomic units, but macroscopic analyses are not common in systematics for three reasons. First, to fully resolve relationships within large groups there must be at least as many character transformations as there are taxa, so such analyses are time consuming and difficult. Second, there is an inherent problem of character homoplasy in large analyses. As divergence time increases the probability that a character state will reoccur also

increases. Sanderson and Donoghue (1989) state that "a positive correlation between character homoplasy and number of taxa is consistent with a model of character evolution in which the probability that a character will change somewhere on a tree is monotonically related to the total number of internodes (branch segments) in the tree and to the number of taxa". If the number of possible states for any character is limited and there is an average non-zero probability that a character-state will change along internodes as branches are added, the number of state changes will tend to increase, and this will result in increased homoplasy (Sanderson and Donoghue, 1989). It therefore becomes increasingly difficult to assess phylogeny for large groups due to an increasing amount of expected homoplasy. Third, as the number of taxa increases linearly the number of possible tree topologies increases geometrically (Felsenstein, 1978). An analysis of three taxa only has three possible bifurcating trees, an analysis of four taxa has 15 possible results and an analysis of 20 taxa has greater than 8.2×10^{21} bifurcating trees (Felsenstein, 1978). It becomes difficult to effectively explore all possible resolutions for analyses that have 25 taxa or more because of increased computer time (Forey et al., 1992). This leads to the use of heuristic search techniques. Heuristic search can only examine a fraction of the possible cladograms. If the data contain a large amount homoplasy or do not decisively resolve relationships within the ingroup, it is likely that heuristic search will not find the most parsimonious cladograms, or that many equally parsimonious cladograms will be found (Forey et al., 1992).

Although the problems with large analysis cannot be avoided, these confounding factors can be minimised and the value in assessing interrelationships and establishing nomenclature for large numbers of taxa is no less significant. This is strongly evident in other phylogenetic

analyses. For example, a cladistic examination of the lepidopteran subfamily Plusiinae (Kitching, 1987) used 216 morphological characters to establish the monophyly and interrelationships of 47 genera. This analysis produced at least 50 equally parsimonious trees of 648 steps with a consistency index (ci) of 0.45. These results were used to reclassify the taxa within Plusiinae at various taxonomic levels. Kitching (1987) established one new tribe, 3 subtribes, reclassified many genera and recognised a new species based on this analysis, indicating that the results from an analysis of this size could form the basis of a revised nomenclature. The monophyly and interrelationships of the stomiid fishes (Teleostei: Stomiiformes) was established by examining 70 characters for 25 stomiiform genera (Fink, 1985). One cladogram of 496 steps, ci = 0.49, represented the interrelationships of these taxa. These results were used to reclassify six stomiiform subfamilies within an expanded Stomiidae that was monophyletic based on six synapomorphies. A detailed character analysis of the other morphologies established an hypothesis of interrelationships and monophyly for these 25 stomiid genera. The phylogeny for the subfamily Ostraciinae (Tetraodontiformes: Ostraciidae) was examined using 108 morphologies for 19 species (Klassen, 1995). Two equally parsimonious trees of 158 steps, ci = 0.80 were used to hypothesise the monophyly of ostraciines as well as to reclassify genera.

Data sets that use large numbers of taxa attempt to fully represent the morphological diversity inherent in large taxonomic groups. In many cases, large studies do not examine all species of a particular group. Instead, a single species can represent a larger taxonomic unit. This dimension reducing technique is evident in the above examples and in many other studies. The monophyly and phylogenetic relationships of the Pelicaniformes (Cracraft, 1985) was determined

by examining 52 morphologies in only 13 taxa representing the morphological diversity for the entire order. Chapleau (1993) used a similar approach to examine the intrarelationships of Pleuronectiformes. In his study, 39 morphological characters were examined in 15 taxa used to represent over 530 species. It is noted that in many instances more than one species was examined to establish a representative set of character states for each of the 15 taxa. This has proven to be an effective means of reducing the number of taxa for analysis if there is *a priori* knowledge of subgroup monophyly and interrelationships based on synapomorphies. This cannot not be done for this analysis, since the cladistic interrelationships of Pleuronectidae have not been previously studied, and the monophyletic status of genera within this family is central to this study. Therefore, it is necessary to examine as many species as possible and all 58 known extant species of Pleuronectidae should be used for the analysis.

In summary, a large scale analysis of Pleuronectidae is necessary to address the important issues of interrelationships, monophyletic status of genera and nomenclature. The problems associated with an analysis of this magnitude can be minimised. A large proportion of morphological information was already available in the literature. It was also evident that some morphological regions had not been exploited in previous examinations, providing areas that could be concentrated on, in search for additional characters. This would facilitate the compilation of a sufficiently large matrix of characters necessary to establish the interrelationships for all 58 pleuronectid species. Most species were easily obtained from museums and only five were not cleared and stained for a complete examination. Although it is not feasible to examine all possible trees for a data matrix containing 58 taxa (Graham and Foulds, 1982), heuristic search

methods can be effective at finding the most parsimonious cladograms given that different combinations of search algorithms are used and steps are taken to minimise problems encountered by search algorithms in the light of homoplasy (Forey et al., 1992). A preliminary analysis of intrarelationships of Pleuronectidae was performed to determine the decisiveness of the data already in the literature. Although homoplasy is expected to be high for an analysis of this size, the preliminary analysis suggested that the data were decisive enough to resolve large subgroups.

Materials and Methods

Fifty three of the 58 pleuronectid species were examined. Five outgroup taxa, chosen from the families Psettodidae (*Psettodes* sp.), Citharidae (*Lepidoblepharon ophthalmolepis*) and Paralichthyidae (*Citharichthys arenaceus*, *Paralichthys lethostigmus* and *P. squamilentus*) were also examined. The following is a list of cleared and stained specimens that were dissected and examined for osteological characters. Nomenclature follows Norman (1934) and Sakamoto (1984a). Nomenclatorial changes established by the conclusion in this analysis are incorporated with the previous most recent generic classification (Sakamoto, 1984a) indicated in parentheses (). Changes in the nomenclature recognise gender status of the genus (Eschmeyer, 1990) as specified by the International code of zoological nomenclature, article 31 (Ride et al., 1985). Institutional abbreviations follow Leviton et al. (1985). Specimen size is expressed in millimetres (mm) of standard length (Hubbs and Lagler, 1970). Radiographs from specimens for all collections and radiographs of the specimens listed in Leipertz (1987), were also examined.

Psettodidae

Psettodes sp., Bennet. ANSP 145394, 65 mm

Citharidae

Lepidoblepharon ophthalmolepis, Weber. AMS I.20118-012, 122 mm

Paralichthyidae

Citharichthys arenaceus, Evermann and Marsh. USNM 00203510, 69 mm

Paralichthys lethostigmus (Jordan and Gilbert). ANSP 143209, 90 mm

Paralichthys squamilentus, Jordan and Gilbert. ANSP 150694, 50 mm

Pleuronectidae

Acanthopsetta nadeshnyi, Schmidt. USNM 77122, 89 mm; UW 22792, 224 mm

Cleisthenes (= *Hippoglossoides*) *herzensteini* (Schmidt). USNM 051441, 85, 89 mm

Cleisthenes (= *Hippoglossoides*) *pinetorum*, Jordan and Starks. UMMZ 159566, 76 mm

Clidoderma asperrimum (Temminck and Schlegel). not examined

Dexistes rikuzenius, Jordan and Starks. UMMZ 159662, 122 mm

Eopsetta grigorjewi (Herzenstein). UMMZ 159590, 66, 88 mm

Eopsetta jordani (Lockington). NMC 81-1015, 68 mm

Hippoglossoides dubius, Schmidt. not examined

Hippoglossoides elassodon, Jordan and Gilbert. NMC 61-0117, 71, 82 mm

Hippoglossoides platessoides (Fabricius). NMC 80-0601, 31, 61, 63, 77 mm ; ROM 504CS, 73 mm, ROM 786CS, 70, 83, 87, 90 mm

Hippoglossoides robustus, Gill and Townsend. ANSP 105133, 110 mm

Hippoglossus hippoglossus (Linæus). ARC 8808487, 148 mm

Hippoglossus stenolepis, Schmidt. NMC 61-0072, 100 mm; UW 22743, 54, 56, 67 mm

Isopsetta (= *Pleuronectes*) *isolepis* (Lockington). UMMZ 63214, 119, 123 mm

Lepidopsetta (= *Pleuronectes*) *bilineata* (Ayres). NMC 61-0050, 51 mm, NMC 81-1027, 56 mm

Lepidopsetta (= *Pleuronectes*) *mochigarei*, Snyder. UMMZ 159575, 113 mm

Limanda (= *Pleuronectes*) *aspera* (Pallas). NMC 66-0016, 156 mm

Limanda (= *Pleuronectes*) *ferruginea* (Storer). NMC 80-0217, 102, 107 mm; ROM 560CS, 49 mm

Limanda (= *Pleuronectes*) *limanda* (Linnaeus). MNHN 1959-560, 119 mm

Limanda (= *Pleuronectes*) *proboscidea*, Gilbert. USNM 268496, 141 mm; UW 22742, 98, 115 mm

Limanda (= *Pleuronectes*) *punctatissima* (Steindachner). HUMZ 93958, 135 mm

Limanda (= *Pleuronectes*) *sakhalinensis*, Hubbs. HUMZ 60455, 138 mm

Lyopsetta (= *Eopsetta*) *exilis* (Jordan and Gilbert). NMC 60-0501, 111, 115 mm

Microstomus *achne* (Jordan and Starks). UMMZ 159434, 145 mm

Microstomus (= *Embassichthys*) *bathybius* (Gilbert). UW 22791, 167 mm

Microstomus (= *Glyptocephalus*) *cynoglossus* (Linnaeus). NMC 77-1087, 89, 114 mm

Microstomus (= *Tanakius*) *kitaharai*, Jordan and Starks. UMMZ 141741, 139 mm

Microstomus *kitt* (Walbaum). FMNH 35527, 135 mm

Microstomus *pacificus* (Lockington). NMC 81-1027, 109 mm

Microstomus (= *Glyptocephalus*) *stelleri* (Schmidt). UMMZ 159566, 125 mm

Microstomus (= *Errex*) *zachirus* (Lockington). NMC 65-0211, 95 mm; NMC 81-1027, 133 mm

Parophrys (= *Pleuronectes*) *vetula*, Girard. FMNH 97128, 97, 128 mm; NMC 81-1121, 39, 61, 66, 68 mm; NMC 85-0025, 53, 67 mm

Platichthys (= *Kareius*) *bicoloratus* (Basilewsky). UMMZ 159667, 117 mm

Platichthys *flesus* (Linnaeus). ANSP 93141, 78 mm

Platichthys *stellatus* (Pallas). NMC 61-0044, 53, 85, 90 mm

Pleuronectes *glacialis* (Pallas). NMC 62-0352, 72 mm

Pleuronectes *pinnifasciatus*, [Kner] Steindachner and Kner. HUMZ 75681, 150 mm

Pleuronectes *platessus* (Linnaeus). ANSP 93145, 66, 88 mm

Pleuronectes putnami (Gill). ROM 23214, 28 mm, ROM 556CS, 104, 110 mm

Pleuronectes quadrituberculatus, Bean. USNM 064042, 85 mm

Pleuronichthys coenosus, Girard. not examined

Pleuronichthys cornutus (Temminck and Schlegel). UMMZ 159618, 91 mm

Pleuronichthys decurrens, Jordan and Gilbert. CAS 23703, 58 mm

Pleuronichthys (= *Hypsopsetta*) *guttulatus*, Girard. NMC 74-0242, 64 mm

Pleuronichthys ocellatus, Starks and Thompson. CAS 82189, 105 mm

Pleuronichthys ritteri, Starks and Morris. CAS 11403, 46 mm

Pleuronichthys verticalis, Jordan and Gilbert. CAS 34728, 83 mm

Psettichthys melanostictus, Girard. NMC 62-2158, 58, 63 mm

Pseudopleuronectes (= *Pleuronectes*) *americanus* (Walbaum). ANSP 105133, 43, 66 mm; NMC 82-0016, 61, 73 mm; ROM 670CS, 27, 35, 46, 49, 54, 60 mm

Pseudopleuronectes (= *Pleuronectes*) *herzensteini* (Jordan and Snyder). UMMZ 159631, 88 mm

Pseudopleuronectes (= *Pleuronectes*) *obscurus* (Herzenstein). not examined

Pseudopleuronectes (= *Pleuronectes*) *schrenki* (Schmidt). HUMZ 75697, 123 mm

Pseudopleuronectes (= *Pleuronectes*) *yokohamae*, Günther. UMMZ 159548, 58, 83 mm; UMMZ 220249, 74 mm; USNM 056359 86 mm

Reinhardtius (= *Atheresthes*) *evermanni* (Jordan and Starks). not examined

Reinhardtius hippoglossoides (Walbaum). NMC 64-0756, 103, 119 mm

Reinhardtius (= *Atheresthes*) *stomias* (Jordan and Gilbert). NMC 65-0262, 97 mm; NMC 66-0022, 39 mm; NMC 80-0073, 80, 117 mm; NMC 80-1024, 119 mm

Verasper moseri, [Jordan and Gilbert] Jordan and Evermann. USNM 056385, 78 mm

Verasper variegatus (Temminck and Schlegel). USNM 056375, 91 mm

All characters used in the study are described in Appendix B. This list also includes the number of steps and character consistency index (cci) for each character in the analysis. The character matrix (Table 1) illustrating the distribution of 106 characters for 58 taxa, combines all available information from the literature (Norman, 1934; Batts, 1964; Amaoka, 1969; Ahlstrom et al., 1984; Hensley and Ahlstrom 1984; Sakamoto, 1984a) as well as 67 new morphological features observed through examination of cleared and stained material, whole preserved specimens and radiographs. Morphologies obtained from the literature are indicated by the citation immediately following the italicised character description (Appendix B). Characters at each node of the cladogram are described and numbered in the order of presentation in the text.

Table 1: Matrix of 106 morphological characters for 5 outgroup taxa, representing the Psettodidae, Citharidae and Paralichthyidae and 53 ingroup taxa representing the Pleuronectidae. Numbers in the matrix represent character state for each morphology as described in Appendix B. Blanks represent the hypothesised ancestral state (state 0). Question marks (?), indicate an unknown state due to poor specimen quality. Character states deemed not applicable due to extreme variation in morphology are coded (na).

	83	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106
1					1		?											
2	2				1		1											
3							?											
4										1	1							
5															?			
6									1						1			
7															1			
8															1			
9										1					1			
10															1			
11									?						1			
12										1					1			
13										1					1			
14															1			
15										1					1			
16															1			
17															1			
18															1			
19															1			
20									1						1			
21															1			
22									1						1			
23										1					1			
24															1			
25															1			
26															1			
27	1		1	1	1										1			
28	2								?						1			
29	2														1			
30	2														1			
31	1		1	1	1										1			
32			1	1	1										1			
33	2			?	1	1	1	1	1	1	1	1	1	1	1	1	1	
34	2				1	1	1	1	1	1	1	1	1	1	1	1	1	
35															1			
36															1			
37															1			
38															1			
39															1			
40															1			
41															1			
42															1			
43															1			
44															1			
45	2														1			
46															1			
47															1			
48	2														1			
49															1			
50															1			
51															1			
52															1			
53															1			
54	1														1			
55	1														1			
56	1														1			
57	1														1			
58	1														1			

Given the unresolved nature of the interrelationships for Pleuronectidae and other bothoid taxa within the order (Chapleau, 1993), establishing character polarity using outgroup comparison as outlined by Watrous and Wheeler (1981) and Maddison et al. (1984) was not possible. Character polarity was determined through a direct examination of states observed in the outgroup taxa (Table 1). For each character, the majority state observed in the three bothoid taxa *Citharichthys arenaceus*, *Paralichthys lethostigmus* and *P. squamilentus* was assumed to represent the plesiomorphic condition. This decision was only overruled if there was heterogeneity in the distribution of states within these three taxa and both secondary outgroup taxa *Lepidoblepharon ophthalmolepis* and *Psettodes* sp. possessed the alternative state. One exception to this rule is stated in character 82 (Appendix B). Character states hypothesised as plesiomorphic for the family are coded as zero (0).

Heuristic search methodology

The matrix was analysed with all heuristic search parameters available in PAUP 3.1.1 (Swofford, 1991). This was necessary to reliably interpret the relationships among all 53 ingroup taxa. An exhaustive search for this many taxa would have required the analysis of an estimated 2.84×10^{82} bifurcating trees (Felsenstein, 1978) and would not have provided a solution within a realistic computation time. For these same reasons, a branch and bound search technique has proved inadequate to resolve relationships for more than 25 taxa (Forey et al., 1992). Because heuristic search techniques sample only a small fraction of the possible trees, it is necessary to ensure that heuristic search is effective in searching for the most parsimonious tree.

The most efficient heuristic search methods sample a portion of the possible tree space using a two-step procedure. This involves the sequential addition of taxa to produce a cladogram that minimises homoplasy; and the subsequent branch-swapping of this addition tree to search for more parsimonious cladograms. In the addition sequence, an initial tree is constructed with three taxa. Subsequent taxa are positioned one at a time to this initial tree. The parsimony criterion for the placement of each taxon is that which results in the smallest increase in evolutionary steps for each subsequent addition.

This stepwise addition procedure attempts to produce a single tree that is close to the most parsimonious result (Swofford and Olsen, 1990). Different addition algorithms alter the criteria for building the initial three taxon tree and to establishing the order of addition of remaining taxa. It is assumed that by using different addition sequence algorithms, a cladogram representing the most parsimonious tree will be constructed. A potential problem with stepwise addition algorithms is that they assess optimality only with respect to the addition of the next taxon, rather than with the tree as a whole. This purely local approach tends to produce cladograms that represent only local minima (Swofford and Olsen, 1990). Unless the data are nearly free of homoplasy and the path to finding the most parsimonious tree descends uninterrupted, it is unlikely that the addition sequence will converge to a global minimum (ie. maximally parsimonious tree) (Forey et al., 1992). Consequently a second procedure which swaps internal branches is often used. The branch-swapping rearranges the topology of the initial tree in search for a more parsimonious result. If a shorter tree is found, another round of

branch-swapping will be performed on the new tree and so on, until no further improvements are found.

Using combinations of addition sequence with subsequent branch-swapping, it is assumed that a globally parsimonious result may be found (Forey et al., 1992). Searches were performed using four addition sequences: "as is", "closest", "simple" and "random" in combination with the three branch-swapping algorithms available in PAUP; "tree bisection regrafting" (tbr), "sub-tree re pruning" (spr) and "nearest neighbour interchange" (nni) (Swofford, 1991). Using the random addition sequence in combination with all of the branch-swapping algorithms was a non-rigorous means of assessing the efficiency of the heuristic methods. The random addition sequence randomly orders the taxa in the data set. An addition tree is then constructed using the "as is" algorithm (Forey et al., 1992). If 50 replicates give the same set of tree topologies, then it is likely that the maximally parsimonious trees have been found. However, if after 100 replicates shorter cladograms are still being found, then it is likely that more trees remain (Forey et al., 1992).

Heuristic search algorithms are constrained by two problems, local minima and plateaux, both of which prevent branch-swapping from finding the most parsimonious result (Swofford and Olsen, 1990). Searches may converge to local minima, unless branch-swapping is performed on non-minimal trees. Swapping on non-minimal trees allows the search algorithm to "climb up", out of a local minima and continue on the path to a global minimum (Forey et al., 1992). However determining how far the search must "climb up" is a somewhat arbitrary decision. If the

parameters are set too low, the search may not "climb" high enough to escape the local minimum. If the parameters are set too high, the analysis may not be able to examine all of the possible trees in a reasonable amount of computational time. To test if branch-swapping was entrapped in local minima an *a posteriori* search is performed to allow branch-swapping on non-minimal trees.

Using the "keep" option in PAUP (Swofford, 1991), a limit for swapping on non-minimal trees, that is at least one or more steps higher than the most parsimonious cladogram produced from the initial search, is established and the search is rerun using the same parameters. Determining the optimal number of steps above the most parsimonious, is dependent on how many additional trees the search must examine based on the data.

A plateau is a region in the search space for which all nearest-neighbour trees have the same number of steps (Forey et al., 1992). If only one cladogram is kept at each branch-swapping step then it is likely that the search will stop at the plateau. Plateaux can be circumvented if all equally parsimonious trees are kept for subsequent branch-swapping (Forey et al., 1992) using the "MULPARS" and "swap on all trees" options available in PAUP (Swofford, 1991).

All searches assumed that the ingroup was monophyletic, with all uninformative characters (those that did not have variation in Pleuronectidae or those with a state in only one species) were ignored during the heuristic searches. Character optimisation was set for accelerated transformation (ACCTRAN). The five outgroup taxa were not included in the analysis. Instead, ancestral states for all characters were set as zero according to established character polarity to

represent a hypothetical outgroup. All off the most parsimonious trees were saved from each search and combined (without duplication) to establish a 50% majority-rule consensus of the equally parsimonious results. Character analysis, character consistency index (cci), tree statistics and tree presentations were generated using MacClade version 3.04 (Maddison and Maddison, 1992).

Results and Discussion

Phylogenetic analysis

The heuristic searches found multiple trees of equal length (Table 2). The most parsimonious trees were found to be 403 steps from a minimum of 131 steps, with a consistency index (ci) of 0.33, excluding uninformative characters, and a retention index (ri) of 0.79. Additional rounds of heuristic search allowed swapping on non-minimal trees up to 410 steps, but did not resolve cladograms shorter than 403 steps. The "simple" and "closest" addition sequences were affected by taxa with a large number of unknown character states. The criteria for establishing the initial tree and the addition of taxa are strongly influenced by unknown character states in these two algorithms. As a result the species *Reinhardtius evermanni*, *Clidoderma asperrimum*, *Hippoglossoides dubius*, *Pleuronichthys coenosus* and *Pseudopleuronectes obscurus* were not included in the heuristic searches. The "as is" and "simple" addition sequence using the "nearest neighbour interchange" branch-swapping were the only combinations that did not find trees with 403 steps (Table 2). The results using 100 "random" addition replicates were not different from those observed by the other three methods (Table 2). This non-rigorous test suggests that the heuristic search combinations were effective in finding all of the most parsimonious cladograms.

A quantitative estimate of data decisiveness (Goloboff, 1991) is not possible for an analysis of 53 taxa, which requires the calculation of mean length of all possible cladograms. However a generalised statement of data decisiveness, suggests that the data for this analysis is

decisive. "Data are strongly decisive if one or more cladograms explaining them is very much shorter than others, and only weakly decisive if all possible cladograms are not very different for each other in length" (Forey et al., 1992). In total only 128 unique cladograms trees were observed (Table 2), which represents a minute fraction of possible trees, and only a slightly smaller fraction of those actually examined during the search. Assuming that all trees of 403 steps are found, there must be many trees that have more than 403 steps. Although the frequency distribution of trees with number of steps cannot be determined, it is assumed that the number of trees with more than 403 steps must increase dramatically given that the maximum number of steps is 1384.

Table 2: Heuristic search results. * Trees found by this method were not of minimum length and not added to the total number of trees observed. ** One hundred replicates for each random addition sequence.

Addition sequence	Branch-swapping algorithm	Tree length (steps)	Number of trees found	Cumulative total of unique trees
as is	tbr	403	112	112
as is	spr	403	80	112
as is	nni*	405	16	128
closest	tbr	403	16	128
closest	spr	403	16	128
closest	nni	403	16	128
simple	tbr	403	112	128
simple	spr	403	16	128
simple	nni*	409	48	128
random**	tbr	403	128	128
random**	spr	403	128	128
random**	nni	403	128	128

The majority-rule consensus of these trees (Figure 1) illustrates clades observed in 50% or more of the 128 results. Clades found in less than 100% of the trees are indicated in parentheses as the percent of trees observed with this node. Only 5 of 47 resolved nodes were observed in less than 100% of the trees and only 2 were observed in less than 75% of the trees. Examination of character distribution in the tree reveals the homoplastic nature of many of the characters used. This is reflected by the low consistency index ($ci = 0.33$) observed in the 128 most parsimonious cladograms (Rohlf, 1982).

Low consistency index is usual for in studies that examine interrelationships among large numbers of taxa (Sanderson and Donoghue, 1989). The expected consistency index for a study of 53 taxa would be 0.14 using an equation of linear regression derived from 60 previous cladistic studies (Sanderson and Donoghue, 1989). This suggests that there is less homoplasy describing the interrelationships of Pleuronectidae than in other studies of this size. The retention index ($ri = 0.79$) indicates that homoplasy is mostly occurring at terminal nodes and not internal nodes. This suggests that relationships at the level of subfamily, tribe and genera are not purely based on homoplastic morphologies (Forey et al., 1992), and that the strength of this analysis can be used to determine relationships at this level. The retention index also suggests that this analysis is not effective at determining relationships near the terminal ends such as interrelationships among species within a genus. Consequently, the character analysis is restricted to the level of subfamily and tribe, and intra-genera analysis will only be explored for relationships well corroborated by uniquely derived morphologies.

Figure 1. 50% majority-rule consensus tree from 128 equally parsimonious cladograms (404 steps, $ci = 0.33$, $ri = 0.79$) in an analysis of 106 characters, for 53 ingroup taxa and one hypothetical outgroup. All nodes were observed in 100% of the trees unless indicated in parentheses ().

Monophyly of the Pleuronectidae

Ten synapomorphies define the Pleuronectidae. The distributions of these states were also surveyed in the literature for taxa within the bothoid lineage (Hensley and Ahlstrom, 1984) and basal lineages within Pleuronectiformes (Chapleau, 1993).

1. The frontal of the ocular side is articulated with the mesethmoid. The outgroup taxa have the prefrontal of the ocular side separating the frontal from the mesethmoid. The distribution of this morphology within other pleuronectiform taxa reveals that the frontal on the ocular side is not articulated with the mesethmoid in Psettodidae, Citharidae, Paralichthyidae, the subfamily Taeniopsettinae, and some genera of Bothinae; *Arnoglossus*, *Psettina*, *Asterorhombus*, *Japonolaeops*, *Laeops*, *Komoharaia*, *Neolaeops* and *Chascanopsetta* and is only observed to be in contact with the mesethmoid in the Bothinae genera, *Parabothus*, *Engyprosopon*, *Tosarhombus*, *Crossorhombus* and *Bothus* (Amaoka, 1969).
2. The sensory canal on the ocular side preorbital is absent, with two exceptions in *Reinhardtius hippoglossoides* and *Acanthopsetta nadeshnyi*. In members of the bothoid group, as well as in the Citharidae and Psettodidae, this sensory canal is present (Amaoka 1969).
3. The ventral margin of the metapterygoid is flattened (Figure 2B), with only one exception in *Reinhardtius stomias*, which has a distinct curvature along the ventral margin of the metapterygoid. The outgroup taxa also possess this ventral curvature of the metapterygoid (Figure 2A).

4. First and second basibranchials loosely joined by cartilage with exceptions observed in *Reinhardtius stomias*, *Eopsetta grigorjewi*, *Psettichthys melanostictus*, *Isopsetta isolepis*, *Parophrys vetula*, *Limanda punctatissima* and *L. ferruginea*, in which the basibranchials are sutured. Outgroup taxa also have a suture between first and second basibranchials (Figure 3A).
5. Second and third basibranchial loosely joined by cartilage (Figure 3B, C, D) with exceptions in *Psettichthys melanostictus* and *Limanda punctatissima*. Outgroup taxa have a suture between second and third basibranchials (Figure 3A).
6. Posterior most abdominal vertebrae lack haemapophysis (Figure 4B). Exceptions are observed in *Reinhardtius stomias*, *Eopsetta grigorjewi*, *Microstomus pacificus*, *M. kitt* and *M. achne* in which the haemapophysis are present. Outgroups have a fusion of the parapophysis to form a haemal arch on the posterior-most abdominal vertebrae (Figure 4A).
7. Absence of accessory processes on the caudal vertebrae (Figure 5B, C). Exceptions (*Microstomus bathybius*, *Hippoglossus hippoglossus*, *H. stenolepis*, *Pleuronichthys guttulatus*, *P. decurrens* and *P. ritteri*) and outgroups have accessory processes on the ventral surface of the centrum for all caudal vertebrae (Figure 5A).
8. Presence of infraorbital bones on the ocular side, except in *Microstomus bathybius*. All outgroup taxa, except *Psettodes*, do not have infraorbital bones on the ocular side. *Psettodes* has

four infraorbital bones on the ocular side. The presence of infraorbital bones in Pleuronectidae, although not unique when compared to all outgroup taxa is probably a reversal in the bothoid lineage and synapomorphic for the family.

9. Absence of oil globules in the egg, with the exception of one oil globule found in *Pleuronichthys cornutus*, *P. guttulatus* and *P. ritteri* (Ahlstrom et al., 1984). All members of the outgroup have at least one oil globule (Ahlstrom et al., 1984). The distribution of this character has not been confirmed in the specimens used for this analysis, however the source (Ahlstrom et al., 1984) confirms this distribution in 46 of the 58 pleuronectid species.

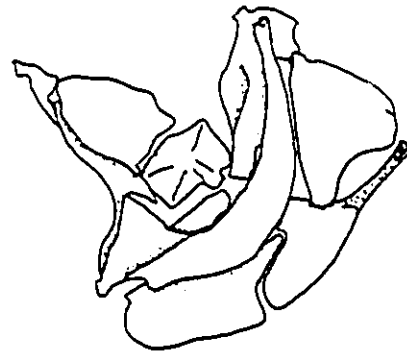
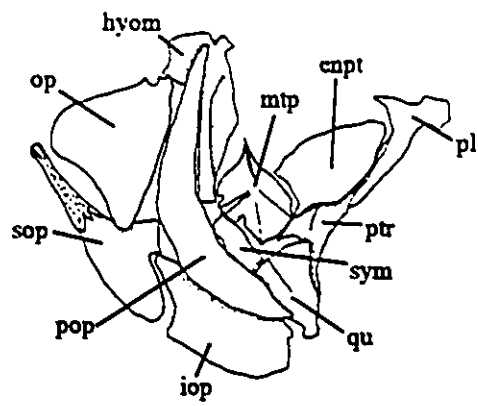
10. Olfactory laminae are parallel without a central rachis, with one exception in *Reinhardtius evermanni* and *R. stomias*. As in the outgroup, these two species of *Reinhardtius* have laminae that radiate from a central rachis (Norman, 1934). The distribution of this morphology was not confirmed in this analysis. Historically this morphology has been used to diagnose the Pleuronectinae (*sensu* Norman, 1934) and appears to be unique in flatfish. There has not been any evidence to suggest that this morphology is observed in any other flatfish species.

Exceptions in the distribution of these ten synapomorphies do not have a common phylogenetic pattern and do not corroborate the exclusion of any of the 53 species examined in this analysis. The exceptions suggest independent cases of reversals for either the species or their immediate ancestors that will be discussed in the context of species interrelationships within the Pleuronectidae. It is noted that *Reinhardtius stomias* has four exceptions to the ten

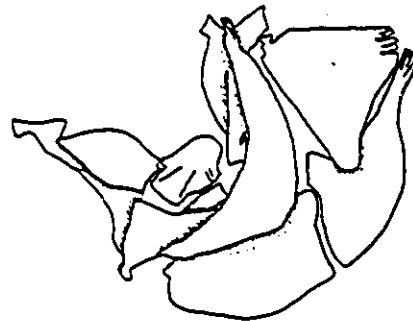
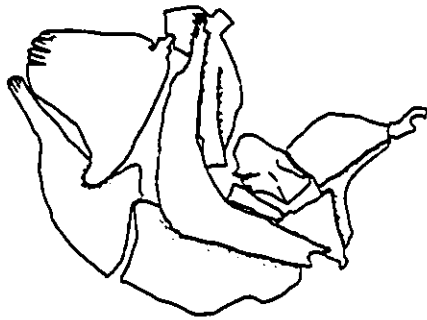
synapomorphies in Pleuronectidae. However the most parsimonious explanation places this species in the Hippoglossinae based on seven additional synapomorphies. Other characters (48, 77, 83, and 104) appear to be synapomorphies for Pleuronectidae. However, the distribution of these morphologies was only examined for the 53 ingroup taxa and in the five outgroup taxa used for this analysis. This limited survey is not sufficient to provide a full understanding of the distribution of these character states within the order. Therefore these morphologies are not presented as synapomorphies for the family and are only presented in a phylogenetic context within Pleuronectidae. Future analysis examining higher-level relationships within Pleuronectiformes should include these morphologies.

Figure 2. Lateral aspect of suspensorium. Left, ocular side; right, blind side. A) *Paralichthys lethostigmus*, ANSP 143209; B) *Verasper moseri*, USNM 056385; C) *Microstomus pacificus*, NMC 81-1027; D) *Limanda ferruginea*, NMC 80-0217. enpt, entopterygoid; hyom, hyomandibular; iop, interoperculum; mtp, metapterygoid; op, operculum; pl, palatine; pop, preoperculum; ptr, pterygoid; sop, suboperculum; sym, symplectic; qu, quadrate. Scale bars are 10 mm.

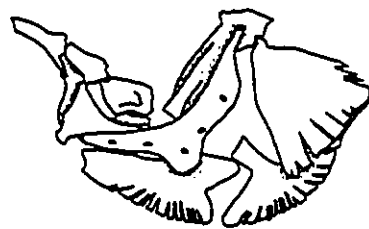
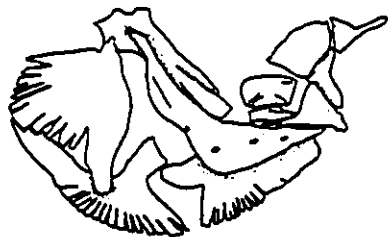
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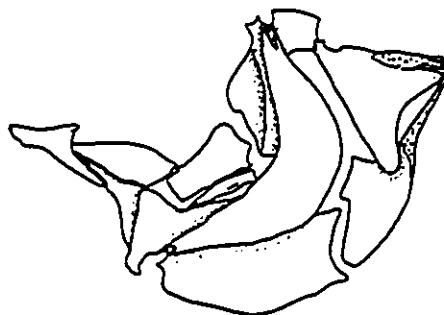
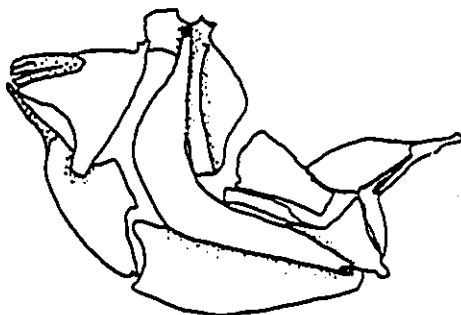
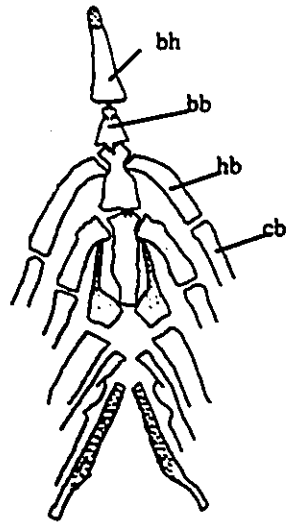
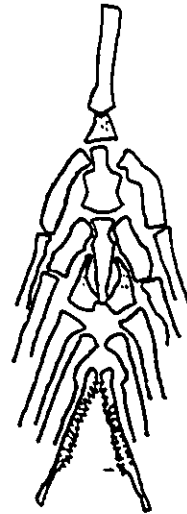


Figure 3. Dorsal aspect of branchial apparatus. A) *Paralichthys lethostigmus*, ANSP 143209; B) *Lyopsetta exilis*, NMC 60-0501; C) *Limanda ferruginea*, NMC 80-0217; D) *Pleuronectes putnami*, ROM 556. bb, basibranchial; bh, basihyal; cb, ceratobranchial; hp, hypobranchial. Scale bars are 10 mm.

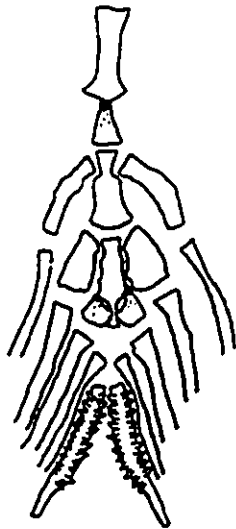
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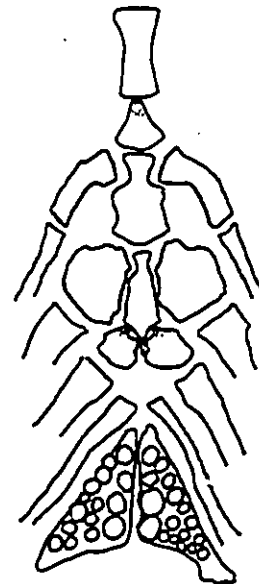
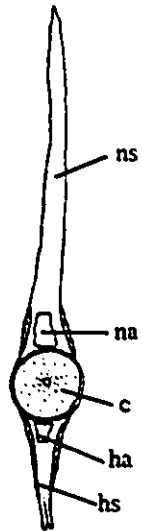
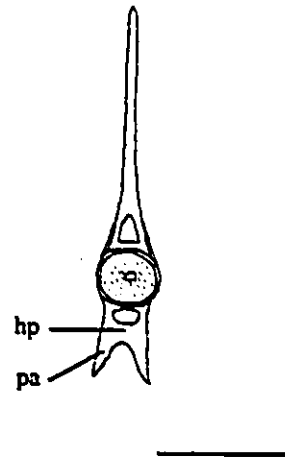


Figure 4. Cranial aspect of abdominal vertebrae. A) *Lepidoblepharon ophthalmolepis*, AMS I.20118-012; B) *Paralichthys lethostigmus*, ANSP 143209; C) *Hippoglossoides robustus*, ANSP 105133; D) *Microstomus kitt*, FMNH 355327. c, centrum; ha, haemal arch; hp, haemapophysis; hs, haemal spine; na, neural arch; ns, neural spine; pa, parapophysis. Scale bars are 5 mm.

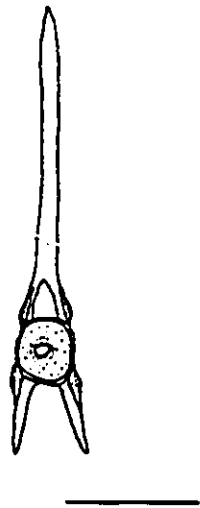
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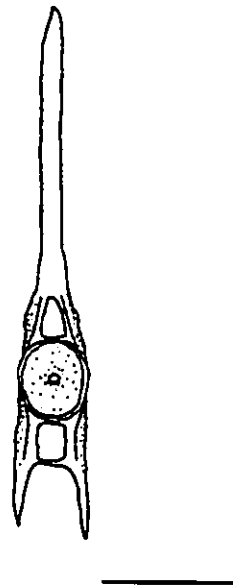
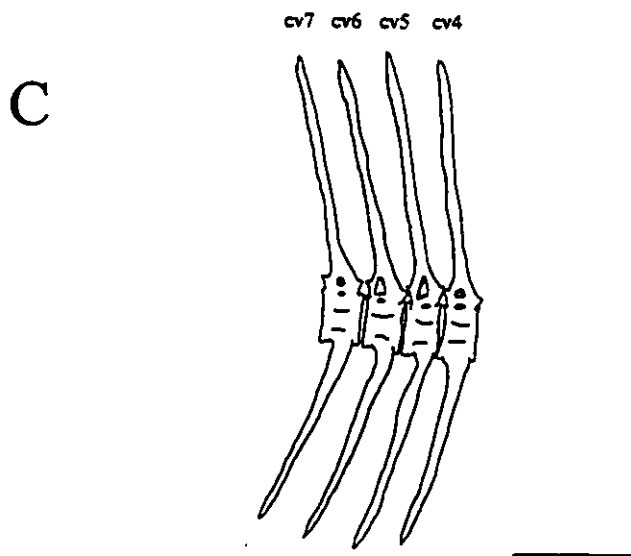
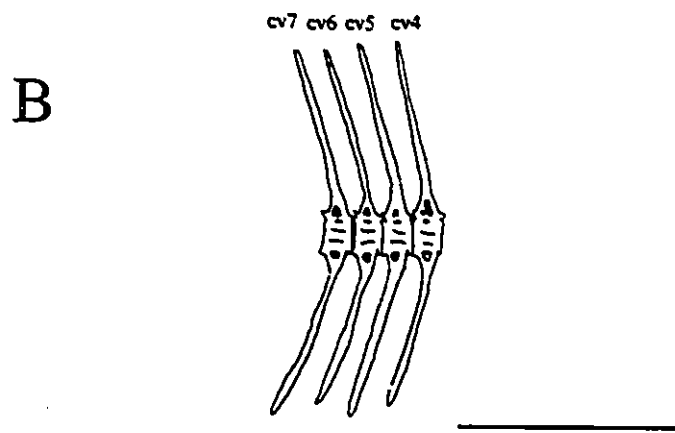
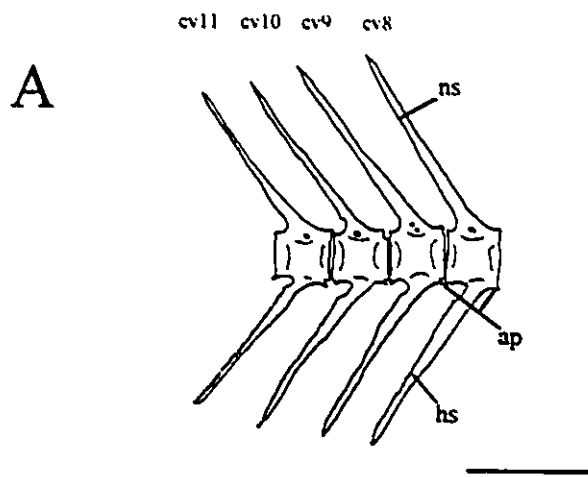


Figure 5. Lateral aspect of caudal vertebrae. A) *Lepidoblepharon ophthalmolepis*, AMS I.20118-012; B) *Pleuronectes platessus*, ANSP 93145; C) *Parophrys vetula*, FMNH 97128. ap, accessory process; hs, haemal spine; ns, neural spine. Number represents position of vertebrae from cranial end. Scale bars are 5 mm.



Intrarelationships of Pleuronectidae

The phylogenetic analysis reveals four monophyletic lineages within the Pleuronectidae illustrating the interrelationships of five newly defined subfamilies: Hippoglossinae, Eopsettinae, Lyopsettinae, Hippoglossoidinae and Pleuronectinae (Figure 6). These subfamilies are separated by a gradation of characters (11 to 22) such that the first three; Hippoglossinae, Eopsettinae and Lyopsettinae contain species with a large proportion of plesiomorphic character states. As a result, the interrelationships within these groups are not supported by many synapomorphies. Serving as successive outgroups, the position of these three subfamilies determine the polarity and relationships within the Hippoglossoidinae and the diversified Pleuronectinae (Stiassny and de Pinna, 1994).

The first lineage distinguishes the subfamily Hippoglossinae from all other pleuronectid taxa. The second lineage contains all species classified in the new subfamilies Eopsettinae, Lyopsettinae, Hippoglossoidinae and Pleuronectinae. This second lineage is supported by two synapomorphies (Figure 6). The pterosphenoid and prootic join to form the dorsal margin of the anterior prootic foramen on the ocular side (11, Figure 7B, C) and the first epibranchial is bifurcated at its distal end (12).

Exceptions to the distribution of these two characters are observed in Pleuronectinae and *Verasper* (Hippoglossinae). The pterosphenoid and prootic do not unite to form the dorsal margin of the anterior prootic foramen (Figure 7A) in the following pleuronectine species:

Figure 6. Intrarelationship for subfamilies and tribes in Pleuronectidae. Numbers correspond to characters in text. Asterisk * represent character state reversal.

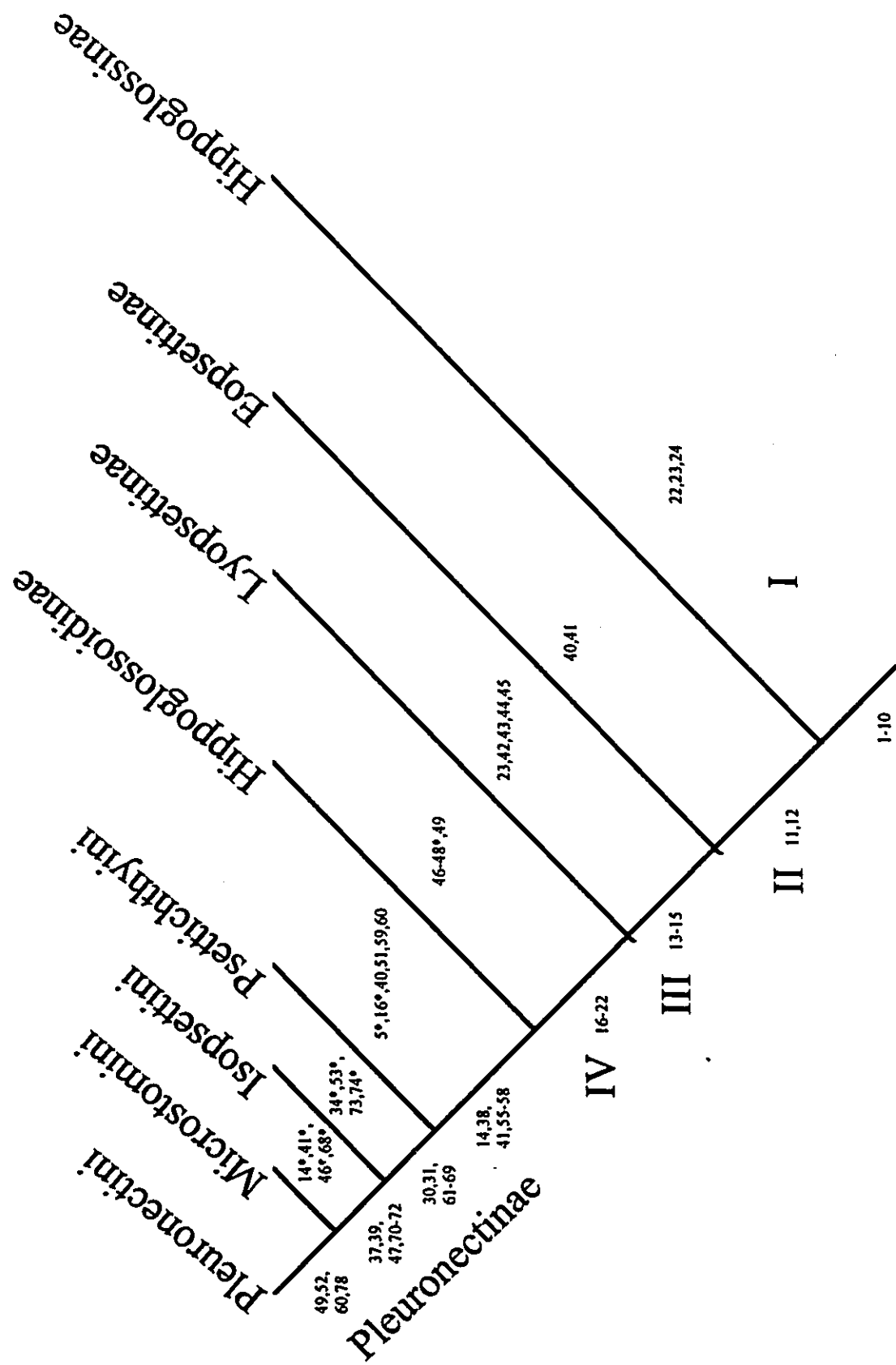
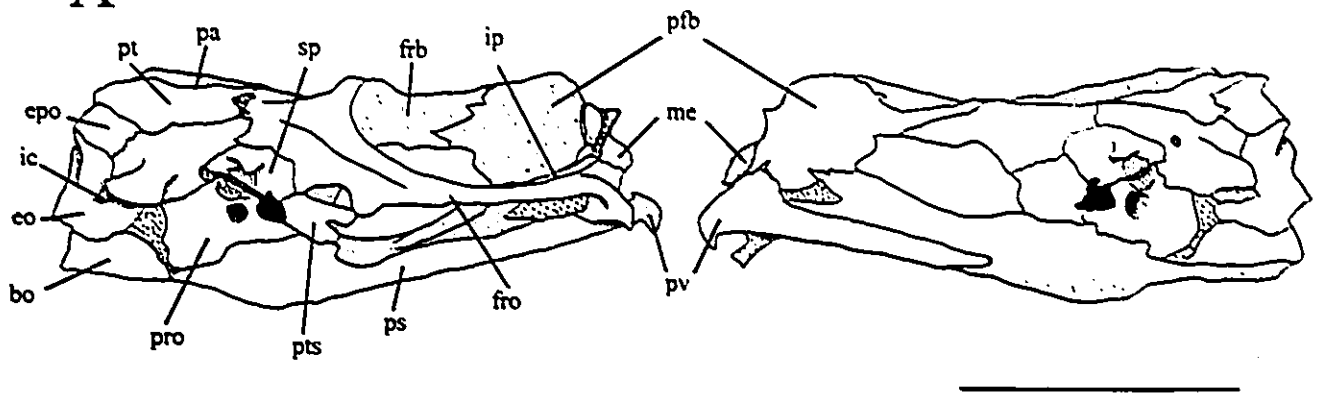
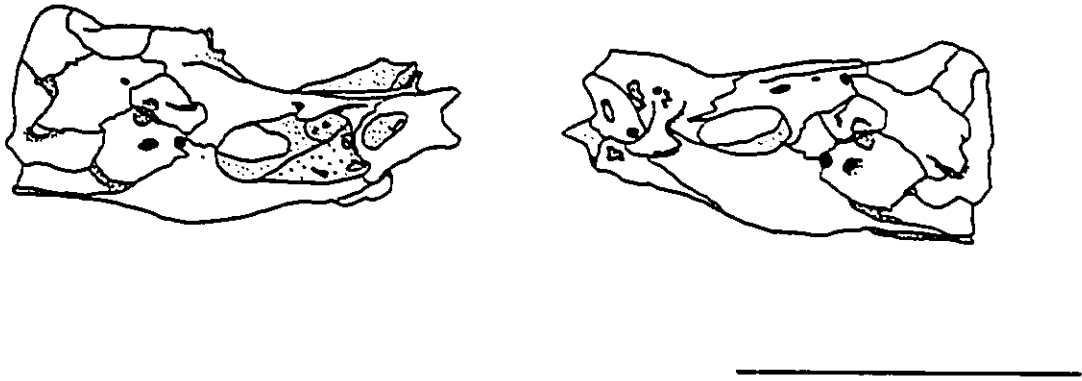


Figure 7. Lateral aspect of crania. A) *Hippoglossus hippoglossus*, ARC 8808487; B) *Pleuronichthys cornutus*, UMMZ 159618; C) *Limanda limanda*, MNHN 1959-560. Left, ocular side; right, blind side. bo, basioccipital; eo, exoccipital; epo, epiotic; frb, frontal (blind side); fro, frontal (ocular side); ic, intercalar; ip, interorbital process; me, mesethmoid; pa, parietal; pfb, prefrontal (blind side); pro, prootic; ps, parasphenoid; pt, pterotic; pts, pterosphenoid; pv, prevomer; sp, sphenotic. Scale bars are 10 mm.

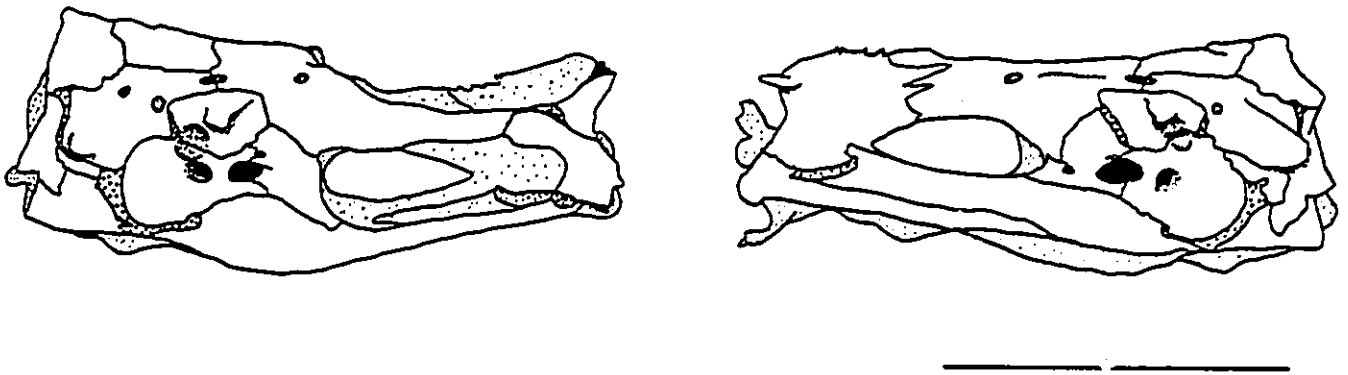
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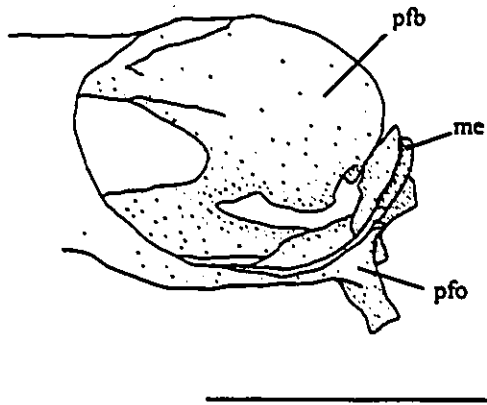
Pleuronichthys guttulatus, *Microstomus zachirus*, *M. cynoglossus*, *M. stelleri*, *Limanda proboscidea*, *L. punctatissima*, *L. ferruginea*, *Pseudopleuronectes americanus* and *Platichthys stellatus*. *Verasper variegatus*, not classified in this second lineage, has the dorsal margin of this foramen formed by the pterosphenoid and prootic on both ocular and blind side. The first epibranchial is not bifurcated in the pleuronectine species *Limanda aspera*, *L. limanda*, *L. sakhalinensis*, *Dexistes rikuzenius*, *Microstomus* and *Pleuronichthys*. Both species of *Verasper* were observed to have a bifurcated first epibranchial. The pattern of these exceptions is similar in these two characters, but the analysis did not indicate an alternative topology that would exclude any of the previously mentioned species, or include *Verasper* within the second lineage. It does suggest that these exceptions are instances of reversal or convergence and may determine phylogeny within these other two groups.

The third pleuronectid lineage, indicates a common ancestor for the subfamilies Lyopsettinæ, Hippoglossoidinæ and Pleuronectinæ. Three synapomorphies define this lineage (Figure 6). The spines on the gill rakers are absent (13); anterior margin of the upper orbit is complete with an overlap between mesethmoid and prefrontal of the blind side (14, Figure 8C, D); and the first anal pterygiophore is broadly thickened (15).

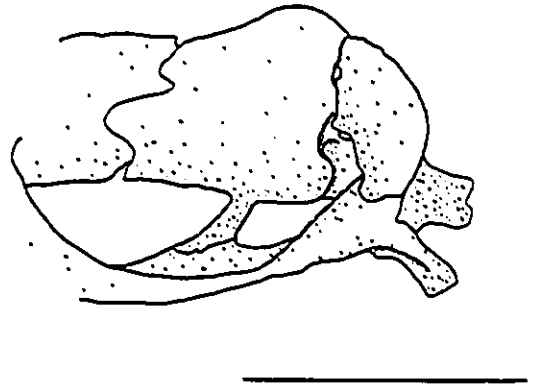
Exceptions to the distribution in these synapomorphies are found in Pleuronectinæ and Hippoglossinæ. The anterior margin of the upper orbit is incomplete in *Microstomus bathybius*, *M. pacificus*, *M. kitt*, *M. achne*, *Pleuronectes pinnifasciatus*, *Pseudopleuronectes americanus*, *P. herzensteini* and *P. yokohamae* while *Reinhardtius stomias* is the only species excluded from

Figure 8. Dorsal aspect of anterior margin of upper orbit, illustrating morphologies for mesethmoid and suture between mesethmoid and prefrontal of the blind side. A) *Eopsetta grigorjewi*, UMMZ 159590; B) *Hippoglossoides elassodon*, NMC 61-0117; C) *Lepidopsetta mochigarei*, UMMZ 159575; D) *Pleuronectes putnami*, ROM 232214. me, mesethmoid; pfb, prefrontal blind side; pfo, prefrontal ocular side.

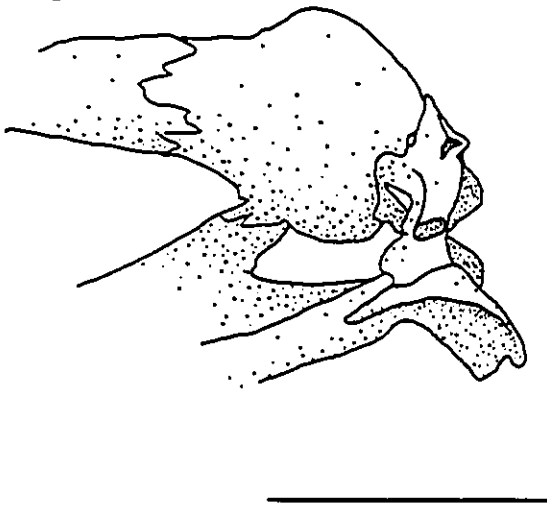
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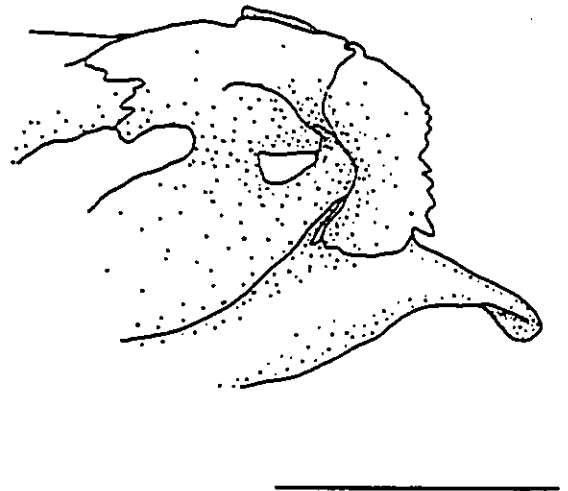
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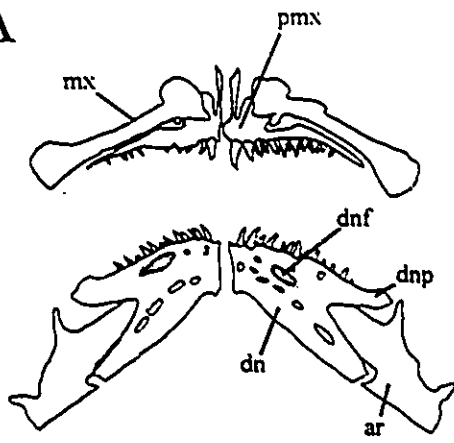
the third lineage that also has the derived state for this morphology. The first anal pterygiophore is not thickened in *Microstomus bathybius*, *M. achne*, *M. kitt* and *M. pacificus* while *Hippoglossus* and *Verasper* (Hippoglossinae) have a first anal pterygiophore that is broadly thickened.

The fourth lineage includes all species of Hippoglossoidinae and Pleuronectinae. The sister relationship between these two subfamilies is determined by seven synapomorphies (Figure 6). The dentition is a uniform length (16, Figure 9B, C, D); interorbital process is reduced or completely absent (17, Figure 10B, D, E); hyomandibular is broadened anteriorly (18, Figure 2D); dentition on the third epibranchial is absent (19); bony plates on the branchial arches are absent (20); two rows of teeth are present on the fourth ceratobranchial (21); and the dorso-posterior margin of the operculum is fimbriated (22, Figure 2B, C, D).

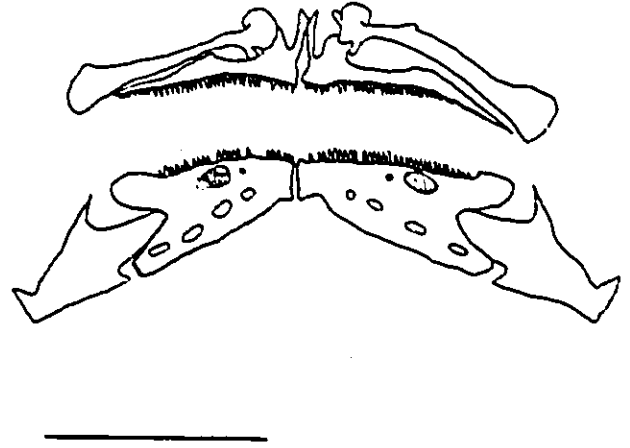
Exceptions to these character distributions are found in only two species of Hippoglossoides and in the tribe Microstomini. *Hippoglossoides platessoides* and *H. robustus* have larger anterior teeth (16) historically interpreted as the presence of canines (Norman, 1934). The genus *Pleuronichthys*, *Microstomus achne*, *M. kitaharai*, *M. kitt*, *M. pacificus* and *M. zachirus* show the presence of an interorbital process (17, Figure 10C). The anterior margin of the hyomandibular (18) is not broadened in *Microstomus* (Figure 2C). Dentition on the third epibranchial and bony plates on the branchial arches are observed in *Pleuronichthys guttulatus*. The rows of teeth on the fourth ceratobranchial are reduced to only one row in species of *Pleuronichthys* and *Microstomus*, and absent in *Limanda punctatissima*. The fimbriation of the

Figure 9. Frontal aspect of jaw apparatus. A) *Hippoglossus hippoglossus*, ARC 8808487; B) *Cleisthenes herzensteini*, UMMZ 159566; C) *Isopsetta isolepis*, UMMZ 63214; D) *Pseudopleuronectes schrenki*, HUMZ 75697. ar, articular; dn, dentary; dnf, dentary foramen; dnp, dentary process; mx, maxilla; pmx, premaxillae. Scale bars are 10 mm.

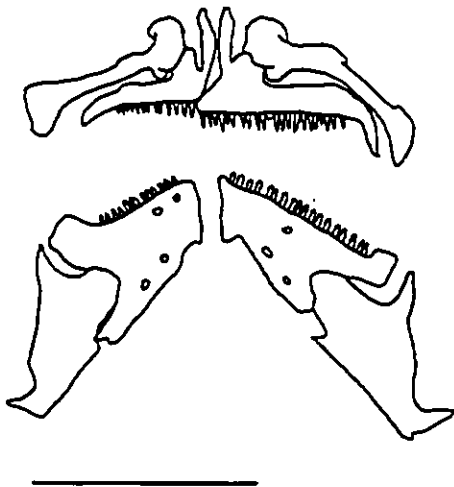
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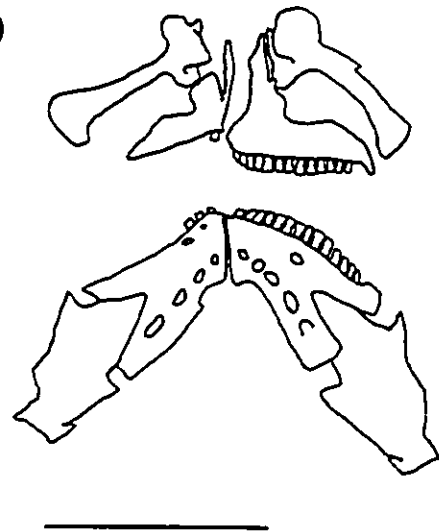
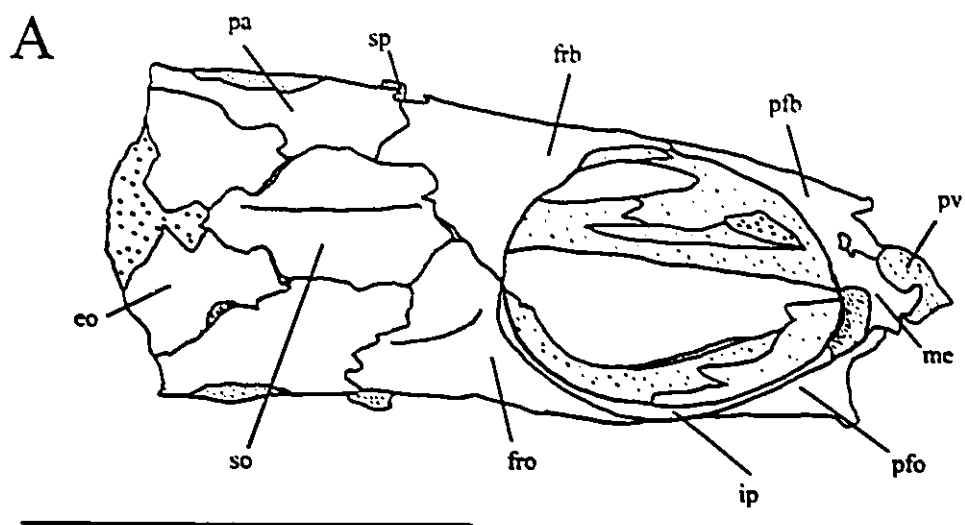
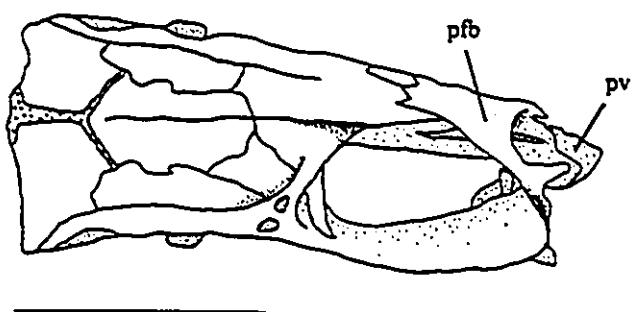


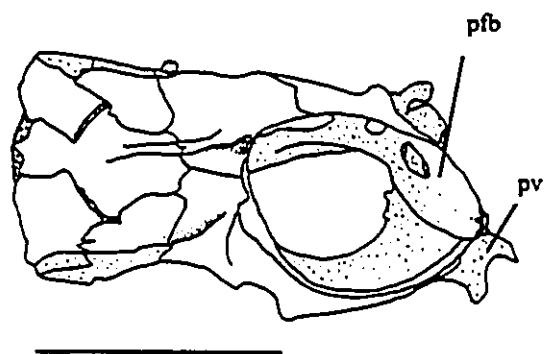
Figure 10. Dorsal aspect of crania. A) *Reinhardtius stomias*, NMC 81-1024; B) *Hippoglossoides robustus*, ANSP 105133; C) *Pleuronichthys ocellatus*, CAS 82189; D) *Parophrys vetula*, FMNH 97128; E) *Limanda punctatissima*, FUMZ 93958. eo, exoccipital; frb, frontal (blind side); fro, frontal (ocular side); ip, interorbital process; me, mesethmoid; pa, parietal; pfb, prefrontal (blind side); pfo, prefrontal (ocular side); pv, prevomer; so, supraoccipital; sp, sphenotic. Scale bars are 10 mm.



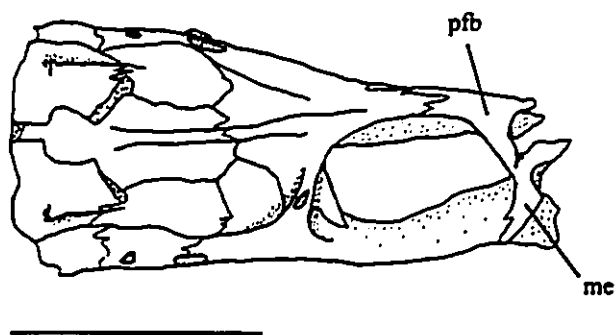
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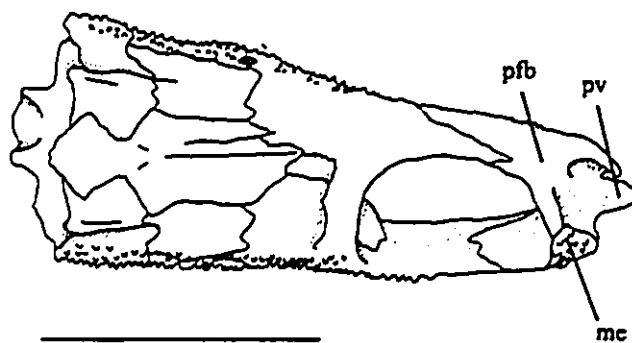
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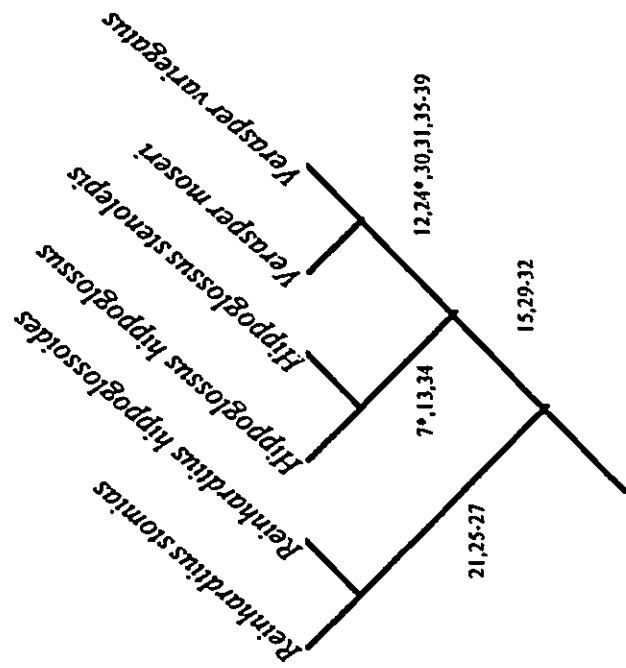
operculum (22) is also observed in Hippoglossinae (Figure 2B), but is absent in the Eopsettinæ and Lyopsettinæ. This last exception suggests that fimbriation of the operculum may be synapomorphic for Pleuronectidae, since only three species *Eopsetta jordani*, *E. grigorjewi* and *Lyopsetta exilis* do not show this fimbriation. This alternative hypothesis would require three evolutionary steps, one more than is presently hypothesised. An equivocal alternative (two steps) would require a single reversal in character 22, to define a monophyletic group of *Eopsetta* and *Lyopsetta*. However this topology was not observed in any of the 128 most parsimonious trees.

Subfamily Hippoglossinae

The first pleuronectid lineage is classified as the subfamily Hippoglossinae containing eight species (6 examined) in four genera: *Clidoderma* (*incertae sedis*), *Hippoglossus*, *Reinhardtius*, and *Verasper* (Figure 11). This subfamily as well as the intrarelationships of its species were observed in all of the most parsimonious cladograms. The Hippoglossinae are hypothesised monophyletic by three synapomorphies (Figure 6); an increase in the number of abdominal vertebrae to more than 12 (23); a lunate shaped caudal fin (24); and the presence of fimbriation along the dorso-posterior margin of the operculum (22, Figure 2B). These characters are found to have a high degree of homoplasy within the Pleuronectidae. The fimbriation pattern of the opercular margin (22) appears in all other species of Pleuronectidae except the genera *Eopsetta* and *Lyopsetta* that make up the next two lineages. An increase in the number of abdominal vertebrae (23) is also observed in other subfamilies and the lunate shaped caudal fin (24) is not observed in all members of this lineage; a reversal is hypothesised for the genus *Verasper*. The monophyletic status of this clade is suspect. The low proportion of derived character states

Figure 11. Intrarelations of Hippoglossinae. Numbers correspond to characters in text.

Asterisk * represents character state reversal.



inherent for basal lineages has the tendency to provide little support for monophyly and intrarelations for these taxa. An analysis of morphologies that are homoplastic at the familial level but not so at lower levels of universality, might clarify both the monophyletic status of this subfamily and its intrarelations.

Intrarelations of Hippoglossinae

Genus *Reinhardtius*: This genus contains three species: *Reinhardtius hippoglossoides*, *R. evermanni* (not examined) and *R. stomias*, characterised by four synapomorphies (Figure 11). The gill rakers on the fourth ceratobranchial are absent (21); the eye of the blind side is situated near the dorsal midline of the cranium, making it visible from the blind side (25); there are more than 35 caudal vertebrae (26); and the dentary foramen is absent on the blind side (27).

Homoplasy observed for these characters is observed in unrelated pleuronectid taxa and does not corroborate an alternative hypothesis. The absence of gill raker is observed in only one other species *Limanda punctatissima* (Pleuronectinae). The position of the eye in relation to the dorsal midline is a reversal that is also observed in *Cleisthenes pinetorum* (Hippoglossoidinae). An increase in the number of caudal vertebrae is observed in *Microstomus* a genus with eight species whose monophyletic status is strongly supported within the Pleuronectinae. The absence of dentary foramen on the blind side is only observed in four other pleuronectid species including *Hippoglossus hippoglossus*.

Reinhardtius evermanni was not examined, but morphologies found in the literature indicate monophyly for *R. stomias* and *R. evermanni*. These two species share two morphologies; olfactory lamellae that radiate from a central rachi; and jaw and pharyngeal teeth with barbed tips (Norman 1934). If these morphologies are considered synapomorphic for the genus, then *Reinhardtius stomias* and *R. evermanni* are sister species with *R. hippoglossoides* immediately basal to them.

The sister relationship between *Hippoglossus* and *Verasper* is supported by six synapomorphies (Figure 11). The first anal pterygiophore is broadly thickened (15); palatine on the ocular side is reduced and not articulated with the pterygoid (28, Figure 2B); gill rakers on the first epibranchial are absent (29) with exception in *Hippoglossus stenolepis*; gill rakers on the second and third epibranchials are reduced (genus *Hippoglossus*) or absent (genus *Verasper*); and gill rakers on the first hypobranchial are reduced (32).

These states are homoplastic in other lineages of the Pleuronectidae. The thickening of the first anal pterygiophore (15) unites taxa in the third pleuronectid lineage. A reduction of the palatine on the ocular side also (28, Figure 2B) defines the sister relationship of *Microstomus* and *Pleuronichthys* within Pleuronectinae. The absence or reduction of gill rakers on the first, second and third epibranchials (29, 30, 31) is found to define basal lineages within the Pleuronectinae, while the reduction of gill rakers on the first hypobranchial is only observed in some species of *Pleuronichthys* and *Platichthys bicoloratus*.

Genus *Hippoglossus*: This genus contains two species *Hippoglossus hippoglossus* and *H. stenolepis* and is monophyletic with three synapomorphies (Figure 11). The presence of subdivisions of the hypural and parahypural plates in the caudal fin, is autapomorphic for the genus (33); accessory processes on the ventral margin of the caudal vertebrae are present (7, Figure 5A), a reversal for the family; and the metapterygoid is articulated with the entopterygoid on the blind side (34, Figure 2C). Only three species of *Pleuronichthys*, and *Microstomus bathybius* have accessory processes on the caudal vertebrae. The metapterygoid is also articulated with the entopterygoid of the blind side in *Reinhardtius hippoglossoides* and species within Pleuronectinae.

Genus *Verasper*: This genus containing the *Verasper moseri* and *V. variegatus* is monophyletic with nine synapomorphies (Figure 11). The presence of a large foramen between the mesethmoid and the prefrontal of the blind side (35, Figure 8), is autapomorphic for *Verasper*; the first epibranchial is bifurcated (12); the caudal fin is rounded (24), a reversal in this subfamily; the gill rakers on both second and third epibranchials (30, 31) are absent; gill rakers are reduced on the second hypobranchial (36); sphenotic process does not form the dorsal margin of the hyomandibular socket (37, Figure 7B, C); a groove along the supraoccipital crest for the insertion of pterygiophores is present (38, Figure 10C, D, E); and the cardiac apophysis of the urohyal is bifurcated (39, Figure 12C).

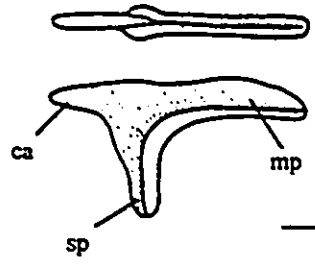
Many of the character states found within *Verasper* are also observed in the Pleuronectinae, but the strength of the hypothesis placing *Verasper* within the Hippoglossinae

exceeds the characters mentioned above and illustrates convergent evolution of these morphologies.

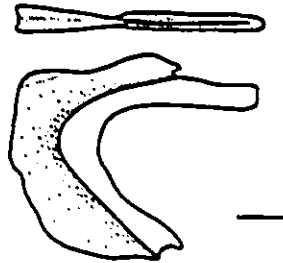
Genus *Clidoderma*: *Clidoderma asperrimum* was not included in the analysis. This species has modified scales on the ocular side to form distinct bony tubercles very similar to those observed in *Platichthys*. However, this unique species is more closely related to *Verasper* than to *Platichthys* (Norman, 1934). This species has subsymmetrical jaws, a mix of pointed and bluntly conical teeth that are not uniform in length. The teeth are set in multiple rows on both upper and lower jaw. These morphologies are plesiomorphic for the family and only exclude *Clidoderma* from the fourth pleuronectid lineage. This species has 14 abdominal vertebrae, determined synapomorphic for Hippoglossinae, it also has a thickened first anal pterygiophore suggesting common ancestry with *Hippoglossus* and *Verasper*. A rounded caudal fin, a reversal in *Verasper* is also observed in *Clidoderma*. The phylogenetic position of this species could be further determined with an examination of accessory processes on the caudal vertebrae, the morphology of the palatine, and the morphology of gill rakers on the first, second and third epibranchials.

Figure 12. Dorsal (above) and lateral (below) aspects of urohyal (modified from Sakamoto, 1984a). A) *Reinhardtius hippoglossoides* B) *Hippoglossus stenolepis* C) *Verasper variegatus* D) *Dexistes rikuzenius* E) *Pseudopleuronectes herzensteini* F) *Microstomus kitaharai*. ca, cardiac apophysis; mp, main part; sciatic part. Scale bars are 2 mm.

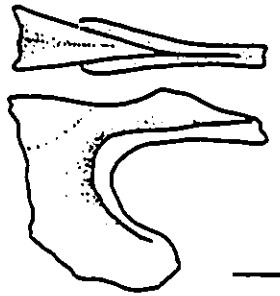
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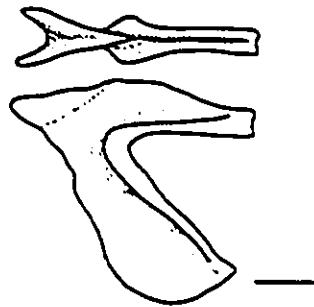
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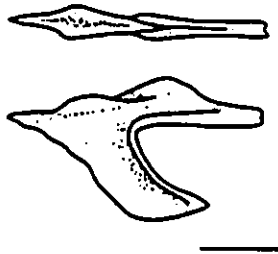
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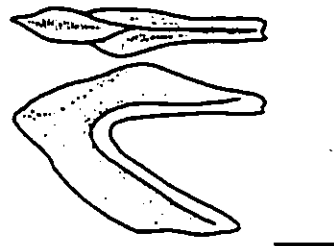
D



E



F



Subfamily Eopsettinae

The subfamily Eopsettinae consists of two species, *Eopsetta grigorjewi* and *E. jordani*. This analysis defines this subfamily with two synapomorphies (Figure 6). The presence of gill rakers on the fourth epibranchial (40); and a single row of teeth on the lower jaw (41). Gill rakers on the fourth epibranchial are only observed in three other pleuronectid species, *Cleisthenes herzensteini*, *C. pinetorum* (Hippoglossoidinae) and *Psettichthys melanostictus* (Psettichthyini). The rows of teeth on the lower jaw were found to be much more homoplastic. This morphology was also observed in the *Verasper moseri*, *V. variegatus* and *Reinhardtius hippoglossoides* (Hippoglossinae) as well as in many species of the subfamily Pleuronectinae.

Genus *Eopsetta*: The genus *Eopsetta* is described in Norman (1934) by a number of characters that are plesiomorphic with respect to this analysis. The presence of distinct canines on the upper jaw is suggested in Norman (1934) as an indication of monophyly for *Eopsetta*. However, the distribution of this character in Pleuronectidae is not well defined. Members of the subfamily Hippoglossinae also have teeth of irregular lengths (16, Figure 9A). The longer teeth can be interpreted as canines present in these species as well. From this analysis, it would appear that the data supporting the monophyly of *Eopsetta* is not conclusive but no other interpretation is warranted due the lack of information to contradict this classification.

Subfamily Lyopsettinae

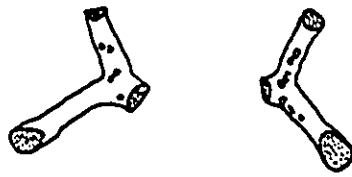
Genus *Lyopsetta*: This lineage contains the single species *Lyopsetta exilis*. Although the monophyletic status of this subfamily cannot be questioned its position as a monotypic lineage

within Pleuronectidae is determined by five derived morphologies (Figure 6). There are between 12 and 14 abdominal vertebrae (23); barbed teeth are present on dentaries and premaxillae (42); supratemporals on both ocular and blind side (43, 44) are jointed at the anterior ends of their bifurcation (Figure 13B); and scales are present on the surface of each eye (45).

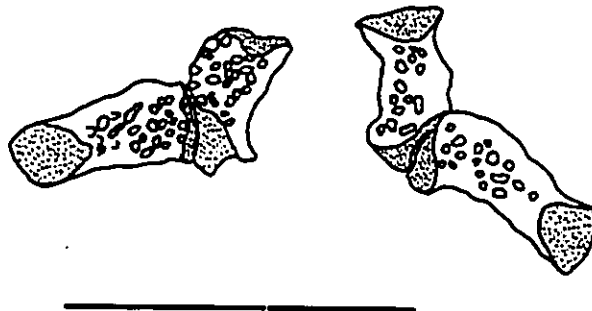
These morphologies are also distributed within other pleuronectid taxa. An increase in abdominal vertebrae is found in Hippoglossinae, some species of Hippoglossoidinae and in two separate lineages of Pleuronectinae. Barbed teeth are also observed in *Reinhardtius*. The bifurcation of the supratemporals is also observed in *Reinhardtius hippoglossoides*, *Cleisthenes*, *Hippoglossoides*, species of *Microstomus*, *Limanda aspera* and *Pleuronectes platessus*. The presence of scales on the surface of each eye can be also found in *Reinhardtius stomias*, *Acanthopsetta nadeshnyi*, *Dexistes rikuzenius*, and most species of *Microstomus*. The distribution of these character states within the family fails to indicate an alternative hypothesis of relationships between *Lyopsetta exilis* and other pleuronectid taxa.

Figure 13. Lateral aspect of supratemporal. A) *Verasper moseri*, USNM 056385; B) *Limanda aspera*, NMC 66-0016. Left, ocular side; right, blind side. Scale bars are 5 mm.

A



B



Subfamily Hippoglossoidinae

This subfamily contains seven species (6 examined) in three genera: *Acanthopsetta*, *Cleisthenes*, and *Hippoglossoides*. This group is characterised by four synapomorphies (Figure 6). The absence of a supraoccipital plate extending postero-ventrally between the epiotics (46, Figure 10B); pterosphenoid and prootic of the blind side join to form the dorsal margin of the anterior prootic foramen (47, Figure 7A); pterosphenoid of the blind side is reduced so as not to form the posterior margin of the orbit (48, Figure 7B), except in *Cleisthenes*; and two uniform rows of teeth are present on the fifth ceratobranchial (49).

The distribution of these states within other pleuronectid taxa indicate a degree of homoplasy for these characters but does not refute the monophyly of Hippoglossoidinae. The supraoccipital plate is also absent in *Microstomus bathybius*, *M. pacificus*, *M. kitt* and *M. achne* (Microstomini). The pterosphenoid and prootic of the blind side are also united to form the dorsal margin of the anterior prootic foramen in *Verasper variegatus* and in most species of the Pleuronectinae. The reduction of the blind side pterosphenoid may be a reversal in Pleuronectidae that is also observed in *Pleuronichthys* (Microstomini). Two rows of uniform teeth are also present in *Dexistes rikuzenius* (Microstomini) and Pleuronectini.

Intrarelationships of Hippoglossoidinae

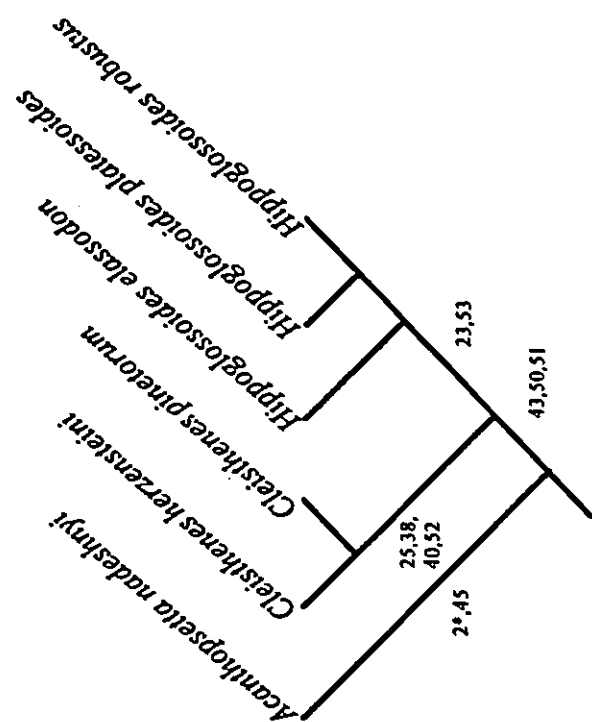
Genus *Acanthopsetta*: *Acanthopsetta nadeshnyi* is the sister species to *Cleisthenes* and *Hippoglossoides* (Figure 14). Its placement as a distinct lineage within Hippoglossoidinae is supported by two morphologies not present in other hippoglossoidine taxa. The presence of a

sensory canal on the ocular side preorbital (2) and the presence of scales on the surface of each eye (45). The presence of a sensory canal on the ocular side preorbital is a reversal for the family that also occurs in *Reinhardtius hippoglossoides*. Scales present on the surface of each eye has a more homoplastic distribution as it is found in *Reinhardtius stomias*, *Lyopsetta exilis*, *Dexistes rikuzenius* and five species of *Microstomus*.

The sister relationship between *Cleisthenes* and *Hippoglossoides* is supported by three synapomorphies (Figure 14). The supratemporal on the ocular side is jointed at the anterior end of its bifurcation that extends to supratemporal and infraorbital canals (43, Figure 13B); nasal bones of the blind side are absent (50); and greater than seven infraorbital bones on the ocular side (51).

These morphological states are not unique within the family. The bifurcation of the supratemporal on the ocular side is also observed in *Lyopsetta exilis* on both ocular and blind sides, but not all species of *Cleisthenes* and *Hippoglossoides* have the supratemporal bifurcation on the blind side. The absence of nasal bones on the blind side and number of infraorbitals are shared with species in Pleuronectinae, the latter is also observed in *Hippoglossus stenolepis*.

Figure 14. Intrarelationships of Hippoglossoidinae. Numbers correspond to characters in text.
Asterisk * represents character state reversal.



Genus *Cleisthenes*: The genus *Cleisthenes* contains two species, *C. herzensteini* and *C. pinetorum* and is diagnosed by four synapomorphies (Figure 14). The eye of the blind side is near the dorsal midline so that it is visible on the blind side (25); gill rakers on the fourth epibranchial are present (40); a double crest or groove is present on the supraoccipital and blind side frontal (38, Figure 10C, D, E); and the crest extending from supraoccipital to the frontal of the blind side is reduced (52, Figure 10D, E).

These morphologies are also distributed within other pleuronectid taxa but are not observed in any other Hippoglossoidinae. The position of the eye on the blind side is also observed in *Reinhardtius*. The presence of gill rakers on the fourth epibranchial also has a limited distribution in *Eopsetta grigorjewi*, *E. jordani* and *Psettichthys melanostictus*. The double crest on the supraoccipital and blind side frontal is recurrent throughout the family but unique within Hippoglossoidinae. The reduced crest on the blind side frontal is also observed in *Reinhardtius stomias*, *Pleuronichthys verticalis* and the tribe Pleuronectini.

Genus *Hippoglossoides*: The genus *Hippoglossoides* contains 4 species: *Hippoglossoides dubius* (not examined), *H. elassodon*, *H. platessoides* and *H. robustus*. It is defined by two synapomorphies (Figure 14). The morphology of the anterior margin of the mesethmoid is complex but consistent in *Hippoglossoides*. In this genus the "thin plate" morphology of the mesethmoid (53, Figure 8B) is different from other members of Hippoglossoidinae who have thickened triangular shaped mesethmoid (Figure 8C), or the plesiomorphic morphology of an open canal found in *Cleisthenes pinetorum* (Figure 8A). In addition, there are between 12 and 14

abdominal vertebrae (23), an increase from 11 or fewer. The interrelationships within Hippoglossoides are not fully resolved by this analysis. *Hippoglossoides dubius* has 13 abdominal vertebrae (Norman, 1934; Sakamoto 1984a) and is assumed to have common ancestry with other species of *Hippoglossoides*.

Subfamily Pleuronectinae

The subfamily Pleuronectinae is the largest within Pleuronectidae containing 40 species, 38 examined in this analysis. Seven synapomorphies define this group as monophyletic (Figure 6). The absence of a dentary fossa (55, Figure 9C, D); and the absence of a ceratohyal foramen (56) are autapomorphic for Pleuronectinae. The mesethmoid and the prefrontal of the blind side are sutured but the foramen between these two bones is now absent (14, Figure 8D); a double supraoccipital crest that forms a groove for the insertion of the anterior pterygiophores for the dorsal fin (38, Figure 10C, D, E); a single row of teeth is observed on the lower jaw (41); the intercalar is in contact with the basioccipital (57, Figure 7C); and the posterior extension of the supratemporal branch is present (58, Figure 10C, D, E).

Some of these morphologies are not found in all pleuronectines, but the occurrence of these transformations at basal lineages and their predominance within the Pleuronectinae indicates that the absence of these synapomorphies within the subfamily are instances of evolutionary reversal. For example the presence of a supratemporal branch (58) is observed in 11 pleuronectine taxa and is hypothesised to arise at this node with a secondary loss observed in the more advanced lineages within Microstomini and Pleuronectini.

Intrarelationships of Pleuronectinae

The Pleuronectinae is classified into 4 tribes: Psettichthyini, Isopsettini, Microstomini and Pleuronectini (Figure 6). The evolution of branchial morphology and jaw asymmetry determine the interrelationships of these tribes.

Tribe Psettichthyini

Genus *Psettichthys*: The first lineage in Pleuronectinae contains only one species *Psettichthys melanostictus*. This monotypic lineage is unique within Pleuronectinae having six distinct morphologies (Figure 6). The dorsal fins are prolonged beyond the dorsal fin membrane, an autapomorphy for the species (59); the second and third basibranchials are sutured (5, Figure 3A), a reversal in Pleuronectidae; teeth are not uniform in length (16); there are greater than seven infraorbital bones (51); gill rakers are present on the fourth epibranchial (40); and the dentition of the upper jaw is in a single row (60).

These morphological states are shared with taxa both within and outside Pleuronectinae. This evidence clearly position Psettichthyini as a basal tribe of Pleuronectinae. A suture between second and third basibranchial is only observed in *Limanda punctatissima*. The absence of a uniform dentition length is not found in any other pleuronectine taxa. An increase in the number of infraorbitals is observed in two other pleuronectine species *Lepidopsetta bilineata* and *Pleuronichthys decurrens* as well as *Cleisthenes*, *Hippoglossoides* and *Hippoglossus stenolepis*. The presence of gill rakers on the fourth epibranchial is a morphology found only in *Cleisthenes*

and *Eopsetta*. Whereas a single row of teeth on the upper jaw is found in all species of Pleuronectini and the genus *Microstomus*.

The second lineage contains the newly classified tribes Isopsettini, Microstomini and Pleuronectini. Taxa within this lineage are characterised by 11 synapomorphies (Figure 6). There is one gill raker observed at the proximal base of the second and third epibranchials (30, 31); the blind side premaxillae protrudes past the sagittal axis at its symphysis with the ocular side (61, Figure 9C, D); ocular side premaxillae is much longer than blind side (62, Figure 9C, D); ventral posterior curvature on the blind side premaxillae is present (63, Figure 9C, D); asymmetry in the space between the dentary and articular such that the blind side space is larger than the ocular side (64, Figure 9C, D); dorso-posterior process of the ocular side dentary is also larger than its blind side counterpart (65, Figure 9C); teeth on both premaxilla and dentary on the ocular side are reduced (66, 67, Figure 9C, D); epiotic processes are present (68, Figure 10D, E); and the entopterygoid is larger on the ocular side than on the blind side (69, Figure 2C, D).

The distribution of these character states is not without exceptions or homoplasies. The reduction of gill rakers on the second epibranchial was not found in *Limanda* and a reduction of gill rakers on the third epibranchial was not observed for *Pleuronectes quadrituberculatus*. These reductions are homoplastic in *Hippoglossus* and *Verasper* (Hippoglossinae).

The third lineage indicating a sister relationship between the tribes Microstomini and Pleuronectini, is based on six synapomorphies (Figure 6). There is an evolution of dentition, from

pointed or bluntly conical teeth to incisor-like or even molariform teeth with uniform cutting edges (70); the sphenotic process is positioned high on the sphenotic (37, Figure 7B, C); the urohyal has a strongly bifurcate cardiac apophysis (39, Figure 12D, E, F); pterosphenoid and prootic of the blind side form the dorsal margin of the anterior prootic foramen (47, Figure 7B, C); medial margin of the fifth ceratobranchial is slightly curved (71, Figure 3C); and the teeth on the fifth ceratobranchial are bluntly pointed (72, Figure 3C).

Exceptions to the distribution of character states within the third lineage are observed in few species and appear to be cases of reversal. They do not contradict the sister relationship between Microstomini and Pleuronectini. The sphenotic process is not positioned high on the sphenotic in *Microstomus cynoglossus*, *M. stelleri*, *M. zachirus* and *M. kitaharai*. A strongly bifurcated cardiac apophysis on the urohyal was not found in *Limanda punctatissima*, *Pleuronichthys ritteri*, *P. ocellatus* and *Parophrys vetula*. These two morphologies are homoplastic in *Verasper* (Hippoglossinae). The sphenotic forms the dorsal margin of the anterior prootic foramen on the blind side (47, Figure 7A) in *Limanda ferruginea*, *L. proboscidea*, *Microstomus achne*, *M. kitt*, *M. zachirus*, *M. stelleri*, *M. cynoglossus* and *Pleuronichthys guttulatus*. However the blind side pterosphenoid and prootic uniting to form the dorsal margin of the anterior prootic foramen is homoplastic in Hippoglossoidinae and *Verasper variegatus* (Hippoglossinae). The medial curvature of the fifth ceratobranchial is not observed in *Microstomus* and *Pleuronichthys* and the teeth on the fifth ceratobranchial are sharply pointed in *Microstomus bathybius*, *Pleuronichthys* and *Lepidopsetta*.

Tribe Isopsettini

Genus *Isopsetta*: The tribe Isopsettini is monotypic with *Isopsetta isolepis*. Four character transformations identify the lineage (Figure 6). The metapterygoid of the blind side is not articulated with the entopterygoid (34, Figure 2A); anterior margin of the mesethmoid forms an open canal (53, Figure 8A); haemal spines of the anterior most caudal vertebrae are broadly attached to the centrum (73, Figure 5B); and the epiotics are sutured along the dorsal posterior margin of the skull (74).

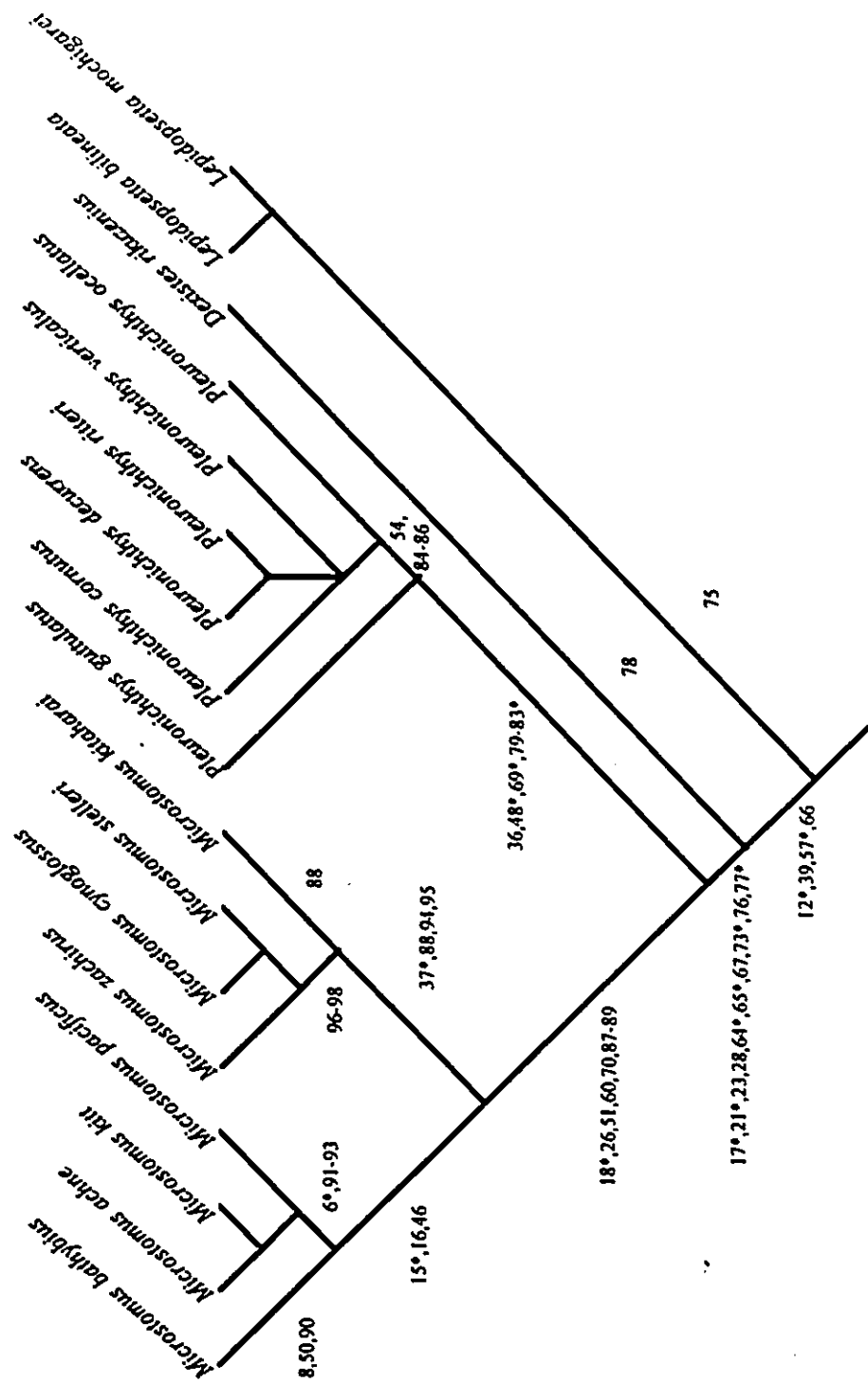
Three of these four character states are reversals within Pleuronectinae. The absence of an articulation between the blind side metapterygoid and entopterygoid is a reversal of the morphology observed in Psettichthyini and in most species of Microstomini and Pleuronectini except *Lepidopsetta*, *Pleuronichthys guttulatus*, *Limanda aspera* and *L. ferruginea*. The open canal on the anterior margin of the mesethmoid is a reversal that is also observed in the pleuronectines, *Microstomus* and *Pleuronichthys* (except *P. verticalis*) and in *Cleisthenes pinetorum* (Hippoglossoidinae). Epiotics sutured along the dorsal posterior margin is only observed in one other pleuronectid species, *Microstomus pacificus* and the broad attachment of haemal spines to the anterior most caudal vertebrae is a reversal that is recurrent throughout the family indicating a homoplastic morphology with a complex distribution. Despite the reversals noted for this lineage the placement of Isopsettini within Pleuronectinae is supported by the eight synapomorphies for Pleuronectinae and the 11 derived morphologies for the second lineage in Pleuronectinae.

Tribe Microstomini

The tribe Microstomini contains 17 species (16 examined) classified in four genera: *Lepidopsetta*, *Dexistes*, *Pleuronichthys* and *Microstomus* (Figure 15). Although the placement of this tribe within the Pleuronectinae is supported by the 25 character transformations presented for the subfamily, the status of this tribe as well as its intrarelationships is defined by many character reversals. The tribe is characterised by four character transformations, all are reversals within Pleuronectinae (Figure 6). The suture between the mesethmoid and prefrontal of the blind side is either incomplete or complete with a small foramen present (14, Figure 8A, B, C); a single crest on the supraoccipital is present (46, Figure 10A, B); the lower jaw has multiple rows of teeth (41); and the process on the dorso-posterior edge of the epiotics is reduced or absent (68, Figure 10A, B, C).

The few exceptions to the distribution of these states within Microstomini and the occurrence of these same reversals outside of Microstomini indicate the homoplastic nature of these morphologies. The presence of multiple rows of teeth on the lower jaw was not observed in *Microstomus* and is homoplastic within Hippoglossinae and is recurrent for basal lineages of the family but only has this one instance of reversal in Pleuronectinae. The absence or reduction of a crest on the epiotics is a reversal that is also found in *Pseudopleuronectes herzensteini* and *P. yokohamae*.

Figure 15. Intrarelations of Microstomini. Numbers correspond to characters in text. Asterisk
* represents character state reversal.



Intrarelationships of Microstomini

Three lineages of Microstomini are defined by 16 character transformations, ten of these are reversals (Figure 15).

Genus Lepidopsetta: The first lineage of Microstomini, the genus *Lepidopsetta* contains two species *L. bilineata* and *L. mochigarei* (Figure 15). This genus is diagnosed by the presence of a demersal egg (75) a morphology that is only observed in three other pleuronectid species *Pseudopleuronectes americanus*, *P. schrenki* and *P. yokohamae*.

The second lineage indicates common descent for *Dexistes*, *Pleuronichthys* and *Microstomus*. Four morphologies unite these genera (Figure 15). Bifurcation of the first epibranchial is absent (12); cardiac apophysis is simple at the tip with a bifurcation positioned anteriorly (39, Figure 12E, F), except in *Pleuronichthys ocellatus* and *P. ritteri*; intercalar is not in contact with the basioccipital (57, Figure 7B), a reversal for Pleuronectinae; and there are less than seven teeth on the ocular side premaxillae (66).

Exceptions to character states within the second lineage of Microstomini were not congruent and did not provide alternative topologies that either excluded taxa observed to be exceptions to this distribution nor included taxa that were homoplastic with these morphologies. The bifurcation of the first epibranchial is a reversal in Pleuronectidae. The shape of the cardiac apophysis of the urohyal was only observed in one other pleuronectid, *Limanda aspera*. The intercalar is in contact with the basioccipital in *Microstomus cynoglossus* (57, Figure 7C). The

reduction of teeth on the ocular side premaxillae (less than 7) is not observed in *Pleuronichthys guttulatus*, *Microstomus zachirus*, *M. cynoglossus*, *M. stelleri* and *M. kitaharai*. However, these species have a smaller proportion of teeth on the ocular side premaxillae than what was observed in previous lineages of Pleuronectinae. *Microstomus stelleri* and *M. cynoglossus* have as few as seven and eight teeth on the ocular side premaxillae, respectively, while *M. zachirus* has 12 to 16 teeth (Norman, 1934).

The third lineage of Microstomini represents the sister relationship between *Pleuronichthys* and *Microstomus*. It is based on ten character transformations, four synapomorphies and six reversals (Figure 15). The number of abdominal vertebrae is increased to between 12 and 14 (23); lips are thickened or fleshy (76); palatine of the ocular side is reduced and not attached to the pterygoid (28, Figure 2C); and the teeth on the ocular side dentary are reduced to fewer than six (67). The reversals are: exoccipital and prootic are in contact with each other (77, Figure 7B); the presence of an interorbital process (17, Figure 10C); one row of gill rakers on the fourth ceratobranchial (21); haemal spine is broadly attached to the centrum (73, Figure 5B); the space between the dentary and articular is the same size for both ocular and blind side (64), Figure 9 D); and the dorso-posterior process of the dentary is also the same size between ocular and blind side (65, Figure 9D).

Exceptions and homoplasy in this distribution do not support an alternative hypothesis. *Microstomus kitaharai* only has 11 abdominal vertebrae (Sakamoto, 1984a). The reduction of the palatine on the ocular side was also observed in the tribe Hippoglossini (Hippoglossoidinae).

The exoccipital and prootic are not joined, and the secondary reduction of teeth on the dentary of the ocular side are not found, in *Microstomus kitaharai*, *M. cynoglossus*, *M. stelleri* and *M. zachirus*. These species all have proportionally fewer teeth on the ocular side dentary but the exact number ranges from 11-15 in *Microstomus kitaharai* to 10-18 in *M. zachirus* (Norman, 1934).

The reversals in this lineage reproduce plesiomorphic states observed prior to the fourth lineage of Pleuronectidae or states plesiomorphic within Pleuronectinae. The exoccipital and prootic in contact with each other, may be a reversal for the family. The interorbital process lost in the fourth lineage of Pleuronectidae, is observed (at least partially) in these two pleuronectine genera. The single row of gill rakers on the fourth ceratobranchial is a reversal from the two rows that also define the fourth lineage of Pleuronectidae. The broad attachment of haemal spine to centrum is a reversal from a narrow attachment that defines the fourth lineage of Pleuronectidae. The symmetry in the space between the dentary and articular as well as the dentary process is a reversal for Pleuronectinae. These last two reversals were also observed in *Pseudopleuronectes americanus* and *P. herzensteini* and in *Pleuronectes glacialis*, *P. pinnifasciatus*, *P. putnami*, *Pseudopleuronectes americanus*, *P. herzensteini* and *P. schrenki* respectively.

Genus *Dexistes*: The genus *Dexistes* is monotypic with *D. rikuzenius* (Figure 15). The singular status of this species is based on the morphologies examined for intrarelationships of Microstomini and one unique morphology. *Dexistes rikuzenius* is the only species in Microstomini with a post-ocular ridge on the eyed side (78). This morphology also has one other

evolutionary step within the sister tribe Pleuronectini, identifying the clade of *Limanda*,

Platichthys, *Pleuronectes* and *Pseudopleuronectes* (Figure 16).

Genus *Pleuronichthys*: The genus *Pleuronichthys* contains seven species: *Pleuronichthys coenosus* (not examined), *P. cornutus*, *P. decurrens*, *Pleuronichthys guttulatus*, *P. ritteri*, *P. ocellatus* and *P. verticalis*. This genus is identified by eight character transformations (Figure 15). The presence of villiform teeth, is autapomorphic for *Pleuronichthys* (79); large foramen is present in the prefrontal of the blind side (80); gill rakers on the second hypobranchial are reduced to just one at the proximal base (36); lateral process on the frontal of the ocular side is present (81, Figure 7B, 10C); 25 or less caudal vertebrae (82); pterosphenoid on the ocular side is reduced and is not part of the posterior part of the orbit (83, Figure 7B); pterosphenoid on the blind side is also reduced (48, Figure 7B); and the entopterygoid on the ocular side is the same size as the blind side (69, Figure 2A). *Pleuronichthys coenosus* is assumed to be a member of this genus based on the presence of villiform teeth, the reduced number of caudal vertebrae (24-25) and the presence of a lateral process on the ocular side frontal (Sakamoto, 1984a).

There are few exceptions to the distribution of character states within *Pleuronichthys* and instances of homoplasy do not indicate an alternative hypothesis. The large foramen in the blind side prefrontal was only observed in one other species, *Microstomus stelleri*. The reduction of gill rakers on the second hypobranchial was not observed in *Pleuronichthys ocellatus*. In this species there are at least two gill rakers on the second hypobranchial. The presence of a lateral process on the ocular side frontal is also observed in *Microstomus achne*, *M. kitt* and *M.*

pacificus. However, it is not nearly as distinct as in *Pleuronichthys*. Only in *Platichthys flesus*, *P. stellatus* and *Pleuronectes putnami* (Pleuronectini) are there also fewer than 25 caudal vertebrae. The reduction of the pterospheneid on the ocular side is not observed in *Pleuronichthys ritteri*, where the pterospheneid separates frontal and parasphenoid to form the posterior margin of the orbit. The reduction of the blind side pterospheneid is also observed in *Acanthopsetta* and *Hippoglossoides* (Hippoglossoidinae). These two morphologies are reversals in Pleuronectidae. The symmetry between ocular and blind side entopterygoids is a reversal from the asymmetrical morphology observed prior to Isopsettini. This reversal only occurs in *Pleuronichthys* and *Microstomus kitaharai*.

The intrarelationships of *Pleuronichthys* are not fully resolved. *Pleuronichthys guttulatus* is the sister species to all other species of *Pleuronichthys* (Figure 15). Four synapomorphies unite *Pleuronichthys cornutus*, *P. coenosus*, *P. decurrens*, *P. ocellatus*, *P. ritteri* and *P. verticalis*. The dorsal fin originates on the blind side of the head (84); the cartilaginous interspace between the prefrontal and parasphenoid of the blind side is reduced or absent (85, Figure 7B); the mesethmoid forms only part of the anterior margin of the upper orbit (86); and the metapterygoid of the ocular side is articulated with the entopterygoid (54). This last morphology is homoplastic in *Reinhardtius hippoglossoides* and *Limanda punctatissima*.

Genus *Microstomus*: The genus *Microstomus* contains eight species. *Microstomus achne*, *M. bathybius*, *M. cynoglossus*, *M. kitaharai*, *M. kitt*, *M. pacificus*, *M. stelleri* and *M. zachirus*. Eight synapomorphies define *Microstomus* (Figure 15). The intestine extends posteriorly into the

body cavity (87), and is autapomorphic for *Microstomus*; there are more than 20 caudal fin rays, an increase from 18 or 19 (88); more than six branched caudal fin rays, an increase from the relative number of six (89); 36 to 41 caudal vertebrae, an increase from 25 to 35 (26); less than five infraorbital bones, a reduction from between five and seven (51); the teeth are incisor-like (70); there is a single row of teeth on the upper jaw (60); and the hyomandibular does not have a broad antero-ventral margin (18, Figure 2C).

Exceptions to the distribution of these synapomorphies do not indicate exclusion of any species defined in *Microstomus* nor does homoplasy suggest the inclusion of additional taxa. An increase in caudal fin rays is only observed in one other pleuronectid, *Pleuronectes platessus* which usually has 20 caudal fin rays. An increase in the relative number of branched caudal fin rays is also observed in *Pleuronichthys decurrens*, *P. ritteri* and *Pseudopleuronectes yokohamae*. An increase in the number of caudal vertebrae is homoplastic in *Reinhardtius* (Hippoglossinae). A reduction in the number of infraorbital bones is not observed in *Microstomus kitaharai* and *M. pacificus* but is reduced in *Pseudopleuronectes americanus*. The incisor-like morphology of the teeth is unique within Microstomini but is observed in *Parophrys vetula*, *Pleuronectes* and *Pseudopleuronectes*. A single row of teeth on the upper jaw is also unique within Microstomini but is also observed in *Psettichthys melanostictus* (Psettichthyini) and Pleuronectini. The absence of a broadened hyomandibular is a reversal unique to *Microstomus*. All other taxa within the fourth lineage of Pleuronectidae have a hyomandibular with a broadened anterior margin (Figure 2D).

The species within *Microstomus* are classified in two subgenera, *Microstomus* and *Glyptocephalus*. The subgenera *Microstomus* contains four species: *Microstomus achne*, *M. kitt* and *M. pacificus* and *M. bathybius* and is defined with three synapomorphies (Figure 15). The posterior extension of the supraoccipital is absent (46, Figure 10B); first anal pterygiophore is thin (15); teeth in both upper and lower jaws are uniform in length and form a continuous cutting edge (16, Figure 9D).

The absence of the posterior extension of the supraoccipital crest has evolved independently in the Hippoglossoidinae. The thin morphology of the first anal pterygiophore is a reversal from a thickened morphology observed to define the third pleuronectid lineage. The continuous cutting edge of the teeth is an advanced state, also present in *M. zachirus*, *Pseudopleuronectes schrenki*, *Pleuronectes glacialis*, *P. pinnifasciatus* and *P. putnami*.

Microstomus bathybius is the sister species to *M. achne*, *M. kitt* and *M. pacificus* (Figure 15). This deep-sea species is unique, being the only pleuronectid with a series of infraorbital bones on the ocular side (8). It is the only species of *Microstomus* in which the blind side nasal bone is absent (50) and it is the only pleuronectid in which the antero-ventral tip of the pelvis is anterior to the cleithrum (90).

The other three members of the subgenus *Microstomus* share four character transformations (Figure 15). Scales on the ocular side of the body have radii completely surrounding the focus (91); margins of the interoperculum and suboperculum are fimbriated (92,

93, Figure 2C); haemapophysis on the posterior most abdominal vertebrae is present (6, Figure 4A). This last morphology is a reversal for Pleuronectidae, and only *M. zachirus* has a subopercular margin that is also fimbriated.

The subgenus *Glyptocephalus* contains four species: *Microstomus cynoglossus*, *M. stelleri*, *M. zachirus* and *M. kitaharai*. Three character transformations are hypothesised at this node (Figure 15). There are greater than 21 caudal fin rays (88); the cleithra is inserted by the tip of the urohyal (94); two to four pyloric appendages plus two or three on upper intestine, an increase from two to three plus one on upper intestine (95); the sphenotic process is positioned low on the sphenotic to form the dorsal roof of the hyomandibular socket (37, Figure 7A). This last character is a reversal from the morphology observed in all other Microstomini and Pleuronectini.

Microstomus kitaharai is the sister species to *M. cynoglossus*, *M. stelleri* and *M. zachirus*. It is unique within this clade and all other pleuronectids in having 23 caudal fin rays, the highest number observed in the family (88). The other three species are united by three morphologies (Figure 15). The blind side nasal bones are larger than those of the ocular side (96) and the development of large mucous cavities on the blind side (97) are both unique for these three species. The presence of an interpterosphenoid bar (98), is not observed in any other species of *Microstomus* or immediate lineages, but it is homoplastic in *Hippoglossoides*, *Acanthopsetta*, *Psettichthys*, *Limanda aspera*, *L. ferruginea*, *L. proboscidea*, *L. punctatissima*, *Pseudopleuronectes americanus*, *P. yokohamae* and *Pleuronectes*.

Tribe Pleuronectini

The tribe Pleuronectini contains 20 species (19 examined). Four synapomorphies define this tribe (Figure 6). There are at least two regular rows of teeth on the fifth ceratobranchial (49), a post-ocular ridge is present on the ocular side (78), the teeth of the upper jaw are in a single row (60), and the dorsal crest extending anteriorly from the supraoccipital to the blind side frontal is reduced or absent (52, Figure 10D, E).

Exceptions and homoplasy did not indicate an alternative hypothesis for Pleuronectini, all 19 species examined were grouped in all 128 of the equally parsimonious trees. A post-ocular ridge is only observed in one other pleuronectid taxa, *Dexistes rikuzenius*. A single row of teeth in the upper jaw was also observed in *Psetichthys melanostictus* and *Microstomus*. The morphology of the dorsal crest, extending along the supraoccipital and blind side frontal, was homoplastic within Pleuronectini and in other pleuronectid lineages. *Limanda aspera*, *Pleuronectes quadrituberculatus*, *P. glacialis*, *P. pinnifasciatus* and *P. putnami* retain a prominent crest. *Pleuronichthys verticalis*, *Cleisthenes* and *Reinhardtius stomias* share the reduced morphology with Pleuronectini.

Intrarelationships of Pleuronectini

Genus *Parophrys*: The first lineage in Pleuronectini has only one species, *Parophrys venula*, which is distinct with five character transformations (Figure 16). A suture between the first and second basibranchial (4, Figure 3A) and haemal spines broadly attached to the ventral

surface of the centrum (73) are both reversals within Pleuronectidae. Other morphologies like the absence of dentary foramen on the ocular side (99); a reduction of teeth on the ocular side (67); and incisor-like teeth (70) are shared with higher lineages within Pleuronectini but are homoplastic, and observed in Microstomini and species of Hippoglossinae.

Limanda, *Platichthys*, *Pleuronectes* and *Pseudopleuronectes* represent the second lineage in Pleuronectini and are hypothesised to share an immediate common ancestor based on three derived morphologies (Figure 16). The presence of a post-ocular ridge on the blind side of the cranium (100); the presence of bony prominences on the post-ocular ridge of the ocular side (101, Figure 10E); and presence of an interpterosphenoid bar (98). The blind side post-ocular ridge and bony prominences on the ocular side post-ocular ridge are unique within Pleuronectidae. The latter was not observed in observed in *Limanda limanda*, *L. sakhalinensis*, *L. aspera*, *Pseudopleuronectes herzensteini*, *P. yokohamae* and *P. americanus*. The presence of an interpterosphenoid bar was not observed in *Limanda limanda*, *L. sakhalinensis*, *Pseudopleuronectes schrenki* and *P. herzensteini* and is homoplastic in *Microstomus cynoglossus*, *M. stelleri*, *M. zachirus*, *Psettichthys*, *Acanthopsetta* and *Hippoglossoides*.

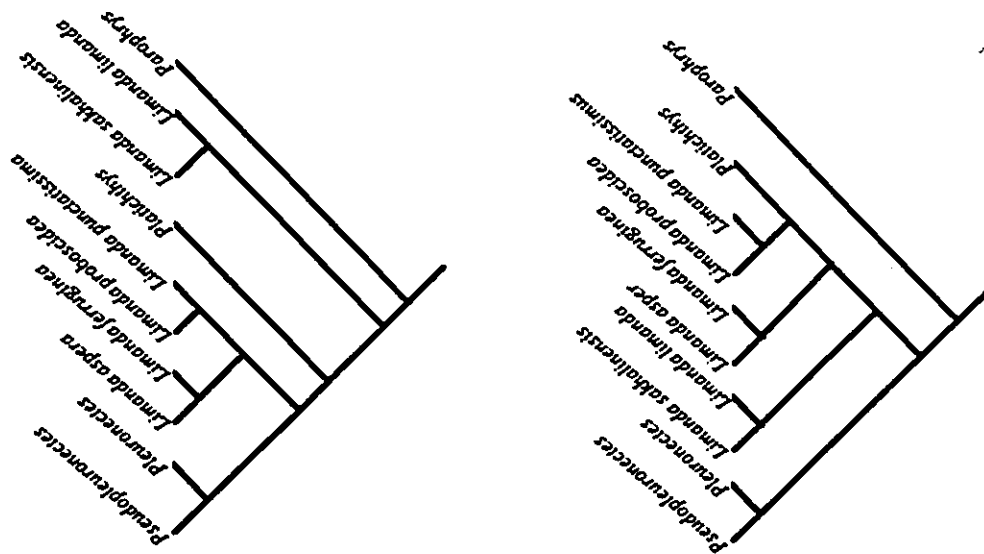
Figure 16. Intrarelationships of Pleuronectini. Numbers correspond to characters in text. Asterisk
* represents character state reversal.

The third lineage of Pleuronectini is a clade uniting *Platichthys*, *Pleuronectes* and *Pseudopleuronectes*. This result was observed in 80 of 128 (62.5%) trees found by heuristic search and is supported by four morphologies (Figure 16). Bony prominences on post-ocular ridge of the blind side (102, Figure 10E); a strong medial curvature on the fifth ceratobranchial resulting in the close approximation or union of ceratobranchials (71, Figure 3D); the rows of teeth on the fifth ceratobranchial have increased from two regular rows to multiple rows that are regular in length (49, Figure 3D); and the anterior margin of the mesethmoid (53) is either a thin plate (Figure 8B) or has a thickened triangular edge (Figure 8C).

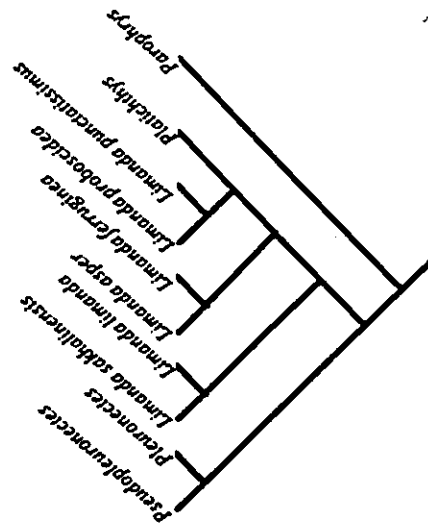
Exceptions and homoplasy observed in these morphologies contributed to three alternative topologies for the intrarelationships of Pleuronectini (Figure 17A, B, C). Bony prominences on the blind side post-ocular ridge were not observed in *Pseudopleuronectes*, *Pleuronectes platessus*, *P. quadrituberculatus* and homoplastic in *Limanda proboscidea* and *L. punctatissima*. Both morphologies of the fifth ceratobranchial, close approximation of the medial margins and multiple rows of teeth, are not observed in *Pseudopleuronectes*. The thin plate morphology of the mesethmoid (Figure 8B), observed in *Pseudopleuronectes* and *Pleuronectes platessus* is homoplastic in *Dexistes rikuzenius*, *Pleuronichthys verticalis* (Microstomini) and *Hippoglossoides* (Hippoglossoidinae). The thickened triangular edge of the mesethmoid (Figure 8C) is unique within Pleuronectidae, but it is not observed in *Pseudopleuronectes*, *Pleuronectes platessus* and *P. quadrituberculatus*. The three alternatives are equally represented in 48 (37.5%) of the most parsimonious results, but are not illustrated in the 50% majority-rule consensus tree

Figure 17. Alternative topologies for intrarelationships of Pleuronectini. A, B and C) Alternative topologies observed for the six species in *Limanda*. Each topology was resolved 16 of the 128 equally parsimonious cladograms. D) Alternative topology for interrelationships in *Pleuronectes* observed in 16 of 128 equally parsimonious cladograms. Numbers correspond to characters in text.

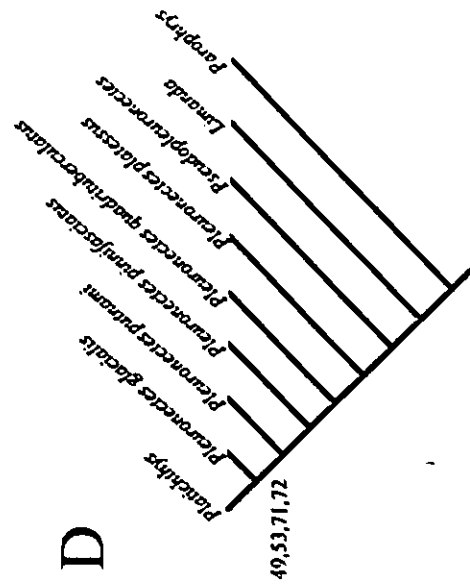
A



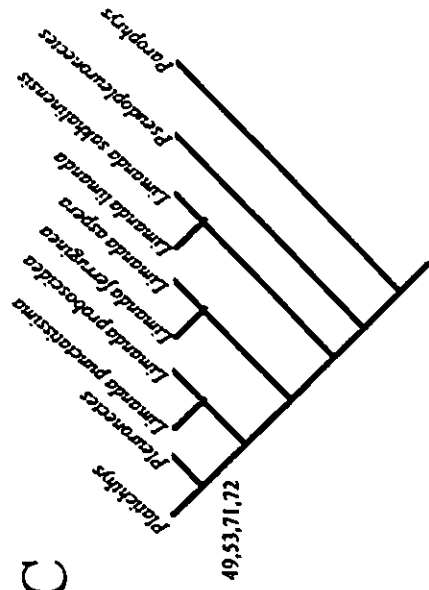
B



D



C



(Figure 1). All three maintain monophyly for *Platichthys*, *Pleuronectes* and *Pseudopleuronectes*, individually. These alternatives, place some or all species of *Limanda* as paraphyletic within a *Platichthys*, *Pleuronectes*, *Pseudopleuronectes* clade but do not exclude any species of *Platichthys*, *Pleuronectes* and *Pseudopleuronectes*. Morphologies in support of these conflicting nodes illustrate homoplasy observed in *Limanda* (Figure 17A, B, C). Some or all species of *Limanda* are observed to share these morphologies with the other three genera. However, most of the exceptions and homoplasy observed in these morphologies fail to illustrate an evolutionary hypothesis that is more convincingly corroborated than the topology presented in the consensus tree (Figure 1).

The fourth lineage unites *Pleuronectes* and *Pseudopleuronectes* (Figure 16). This clade was observed in 75% of the equally parsimonious trees (Figure 1) and is supported by four synapomorphies (Figure 16). There are less than six teeth on the ocular side maxilla (66); the teeth are incisor-like (70); the teeth form a continuous cutting edge (16, Figure 9D); and the dorso-posterior process of the dentary is the same size on both ocular and blind sides (65, Figure 9D), a reversal from the second lineage of Pleuronectinae.

Homoplasy observed in these morphologies reveal a pattern of parallel evolution between this lineage and in *Microstomus* and *Pleuronichthys* (Microstomini). Less than six teeth on the ocular side maxilla is also observed in *Microstomus* and *Pleuronichthys*. The presence of incisor-like teeth is observed in *Parophrys vetula* as well as *Microstomus*. The teeth forming a continuous cutting edge has independently evolved in *Microstomus*. The symmetrically sized

dorso-posterior process of the dentary is not observed in *Pseudopleuronectes herzensteini*, *Pleuronectes platessus* and *P. quadrituberculatus*, and is a reversal that is paralleled in *Microstomus*.

Alternative topologies observed in 32 of 128 (25%) trees unite either a monophyletic or paraphyletic *Pleuronectes* with *Platichthys* (Figure 17C, D). In *Pleuronectes* and *Platichthys* the mesethmoid has a thickened, triangular anterior margin (53, Figure 8C). The fifth ceratobranchial has a strong medial curve so that they form a triangular plate (71, Figure 3D). The teeth of the fifth ceratobranchial are in multiple rows (49, Figure 3D) and these teeth are generally rounded or molariform (72, Figure 3D). Three of these morphologies (49, 53, 71) are used to define the third lineage of Pleuronectini but notably exclude *Pseudopleuronectes*. The molariform teeth on the fifth ceratobranchial (72, Figure 3D) is observed in *Pleuronectes* and only in *Platichthys bicoloratus*. The alternative hypothesis indicating a sister relationships between *Pleuronectes* and *Platichthys* has as much character support as the hypothesis presented in the consensus tree. However this topology is only parsimonious in 32 trees and only in conjunction with one of the two alternatives that either position a paraphyletic *Limanda* after *Pseudopleuronectes* (Figure 17C) or hypothesise a paraphyletic *Pleuronectes*. The support for either of these topologies is not well corroborated morphologically. Therefore, the alternatives to the consensus tree are not as robust to the homoplasy observed in the analysis.

Genus *Limanda*: Monophyly and intrarelationships of *Limanda* are unresolved by this analysis due to homoplasy observed in these six species (Figure 16). *Limanda punctatissima* and

L. proboscidea are the only two pleuronectid species with the bony prominences of the post-ocular ridge that extend anteriorly onto the interorbital bar (103, Figure 10E). *Limanda aspera* and *L. ferruginea* are united by three morphologies. The supraoccipital crest does not form a groove for the dorsal fin pterygiophores (38, Figure 10A, B); the blind side metapterygoid is not articulated with the entopterygoid (34, Figure 2A), both reversals in Pleuronectini; and the supratemporal of the blind side is jointed at its anterior bifurcation (44, Figure 13B). This morphology has evolved independently five times in Pleuronectidae as it is also observed in *Cleisthenes herzensteini*, *Hippoglossoides elassodon*, *Lyopsetia exilis* and *Reinhardtius hippoglossoides*. *Limanda limanda* and *L. sakhalinensis* are monophyletic in 75% of the trees (Figure 1). Two morphologies unite these species. The first epibranchial is not bifurcated (12) and there are no bony prominences on the post-ocular ridge of the ocular side (101). Both of these morphologies are reversals and also observed in *Limanda aspera*. Alternative topologies observed in 32 (25%) other trees, place *Limanda sakhalinensis* and *L. limanda* as paraphyletic taxa in the tribe or paraphyletic within a clade uniting these two species with *Limanda punctatissima* and *L. proboscidea*.

Although the species of *Limanda* are not resolved as a monophyletic group, they are distinguishable from other members of Pleuronectini. They have a reduced dentition on the ocular-side but maintain more than six teeth, where as other species of Pleuronectini generally have six or fewer (Norman, 1934). The teeth are bluntly conical or pointed with truncated tips, while all other members of this tribe have teeth that are compressed and have an incisor-like shape (Norman, 1934, Sakamoto 1984a). Both of the morphologies observed in *Limanda* are

interpreted as synapomorphies for the tribe. There is little evidence in support for the monophyly of *Limanda*. Only one morphology is shared by all six species. The presence of gill rakers on the second epibranchial (30). This morphology is a reversal from a reduced number of gill rakers defining the second lineage of Pleuronectinae. There were 16 trees of 403 steps that hypothesised a monophyletic *Limanda* based on three reversals (Figure 17D), including the presence of gills rakers on the second epibranchial. All 16 topologies were correlated with an hypothesis alternative to the consensus tree (Figure 17D) such that a monophyletic hypothesis for *Limanda* is not resolved by consensus and is therefore not robust to the homoplasy observed in the analysis. However, the placement of these six species near the base of the second lineage in Pleuronectini and the shared morphologies mentioned above, suggests that a conservative approach to their nomenclature should be considered, and that these six species should remain classified as *Limanda* until a more focused phylogenetic analysis is performed.

Genus *Platichthys*: The genus *Platichthys* contains three species: *Platichthys bicoloratus*, *P. flesus* and *P. stellatus*. It is monophyletic with five synapomorphies (Figure 16) The scales along the median fins are absent (104), a possible reversal in Pleuronectidae; the scales on the body are modified to form bony tubercles (105); gill rakers on the second and third epibranchial are absent (30, 31); and supratemporals on the ocular and blind side are fused to the cranium (106). The absence of gill rakers on the second epibranchial is only observed in the distant lineage *Verasper*. The absence of gill rakers on the third epibranchial is also observed in *Verasper* and *Pleuronectes platessus*. The ocular side supratemporal fused to the cranium is homoplastic in *Limanda punctatissima*.

Genus *Pleuronectes*: The genus *Pleuronectes* contains five species: *Pleuronectes glacialis*, *P. pinnifasciatus*, *P. platessus*, *P. putnami* and *P. quadrituberculatus*. The monophyly of these five species was observed in 112 of 128 trees (87.5%) and is supported by two synapomorphies (Figure 16). A prominent dorsal crest extends from the supraoccipital to the frontal of the blind side (52, Figure 10B, C); and molariform teeth on the fifth ceratobranchial are present (72, Figure 3D). A prominent crest extending from supraoccipital to blind side frontal is a reversal within Pleuronectini, that is also observed in *Limanda aspera*. The presence of molariform teeth on the fifth ceratobranchial is only shared with *Platichthys bicoloratus*.

Alternative topologies observed in 16 of 128 trees (12.5%) place the species of *Pleuronectes* as paraphyletic with *Platichthys stellatus*, *P. flesus* and *P. bicoloratus* at the terminal end (Figure 17, D). This topology does not contradict the synonymy of *Liopsetta* (*sensu* Norman) within *Pleuronectes*. It does contradict the classification of *Platichthys*. However, as previously illustrated the alternative topologies for the intrarelationships within Pleuronectini are based on homoplasy observed in *Limanda* such that the paraphyletic origin in *Pleuronectes* are correlated and observed in the same 16 trees indicating monophyly of *Limanda* (Figure 17D).

Genus *Pseudopleuronectes*: The genus *Pseudopleuronectes* contains five species: *P. americanus*, *P. herzensteini*, *P. obscurus* (not examined), *P. yokohamae* and *P. schrenki*. The monophyletic status of this group is supported by four synapomorphies (Figure 16). The anterior margin of the mesethmoid is a thin plate (53, Figure 8); the nasal bone on the blind side is absent

(50); the dorso-posterior process of the dentaries are symmetrical in size (67, Figure 9D); and the eggs are demersal (75). *Pseudopleuronectes obscurus* has morphologies synapomorphic for the fourth lineage of Pleuronectinae, uniting *Pleuronectes* and *Pseudopleuronectes*. These are the presence of incisor-like teeth forming a continuous cutting edge and close approximation of the fifth ceratobranchials (Norman, 1934). The teeth on the fifth ceratobranchial are bluntly conical (Norman, 1934), as in *Pseudopleuronectes*, but this character state is plesiomorphic at this phylogenetic level. However, the presence of a demersal egg in *P. obscurus* (Hensley and Ahlstrom, 1984), is synapomorphic for *Pseudopleuronectes*.

Homoplasy and two exceptions observed in these morphologies do not corroborate an alternative hypothesis. A clade uniting the four species of *Pseudopleuronectes* that are examined, is observed in all 128 equally parsimonious cladograms. The thin plate morphology of the mesethmoid is also observed in *Pleuronectes platessus* and in more distance taxa, *Pleuronichthys verticalis*, *Dexistes rikuzenius* and *Hippoglossoides*. The absence of a blind side nasal bone is homoplastic in *Pleuronectes platessus* and *Limanda* (except *L. sakhalinensis*). It is also observed outside of Pleuronectini in *Microstomus bathybius*, *Pleuronichthys* (except *P. guttulatus*), *Cleisthenes* and *Hippoglossoides*. The same sized dorso-posterior process of the dentary is a reversal hypothesised for *Pseudopleuronectes* (excluding *P. yokohamae*) but is homoplastic in *Pleuronectes glacialis*, *P. pinnifasciatus*, *P. putnami*, *Microstomus* and *Pleuronichthys*. A demersal type egg is not observed in *P. herzensteini* and is homoplastic in *Lepidopsetta*.

Summary and conclusions

Summary of analysis

The species examined in this analysis (53 of 58), describe the entire range of morphological variation found in the family. The character analysis outlines the morphologies considered synapomorphic for clades revealed in the consensus tree (Figure 1). This opens the cladistic analysis for a general assessment of its validity and functionality as a tool in establishing a useful classification. This character analysis reveals the general trend in morphological types observed in Pleuronectidae; the amount of morphological support for each node in the consensus; the interpretation of alternative hypothesis; the limitations of the analysis to clearly resolve interrelationships; the phylogenetic limitations of some morphologies in defining natural groups; and the identification of unknown species or the classification of species based on uniquely derived features.

The Pleuronectidae can be summarised as having large piscivorous species whose jaws and dentition are nearly symmetrical at the basal lineages with species possessing more specialised dentition, jaw structure and diverse feeding habits found in subsequent lineages. The monophyly and intrarelationships of basal lineages in Pleuronectidae are not supported by a large number of synapomorphies. This is inherent for taxa at basal lineages in which the majority of morphological features are plesiomorphic with respect to the ingroup under examination (Stiassny and de Pinna, 1994). However, the position of these basal lineages is key in establishing the polarity of character states for the more advanced taxa in Pleuronectidae.

Homoplasy observed in most of the 106 morphological characters was expected in an analysis containing this many taxa. The alternative topologies within Pleuronectini (Figure 17), were supported by this homoplasy but do not have better character support than the intrarelationships determined through consensus. These alternative topologies are not any less parsimonious than the topologies summarised by the consensus tree. Instead, the limited occurrence (16 trees out of 128) for these alternatives, indicates that these topologies are not as robust to homoplasy as those summarised in the consensus tree. The taxonomic nomenclature established by the consensus tree is not in contradiction with alternatives. Based on the perspective of relationships within the family, it represents the most conservative nomenclature for such a diverse group of pleuronectids.

The interrelationships within most genera cannot be confidently determined, either due to the lack of observed morphological variation as in *Hippoglossoides* and *Pleuronichthys*, or due to the large amount of homoplasy occurring at terminal nodes indicated by the low consistency index ($ci = 0.33$), and a high retention index ($ri = 0.79$), as in *Limanda*, *Pleuronectes*, *Pseudopleuronectes* and *Platichthys*. In either instance, species interrelationships for these taxa do not warrant a formal analysis based on synapomorphies from this study. Additional morphologies, such as the number of dorsal and anal fin rays, homoplastic at higher levels of universality, could be used to identify natural groups within these genera.

The phylogenetic limitations of the jaw and dental morphologies is clearly illustrated in the convergent evolution of jaw morphologies observed between the tribes Microstomini and Pleuronectini. This is most evident in the dentition of *Microstomus* compared to *Platichthys*, *Pleuronectes* and *Pseudopleuronectes*, both of which have single row of incisor-like teeth that sometimes form a continuous cutting edge. However, monophyly for these taxa based on jaw morphology, is not supported by other morphologies observed in the cranial bones and branchial apparatus. These characters clearly resolve the monophyletic status and intrarelationships of these two groups within each of the two tribes. The trend of character reversal, 19 in total (Figures 6 and 15), observed in Microstomini suggests that some members of this taxon may be positioned at a more basal node in Pleuronectidae prior to the fourth lineage. However, this alternative is not supported in any of the 128 most parsimonious trees. The analysis clearly places Microstomini as a lineage within Pleuronectinae based on the 28 morphologies that were not reversals.

Five pleuronectid species were not examined in this analysis. They are: *Clidoderma asperrimum*, *Hippoglossoides dubius*, *Pseudopleuronectes obscurus*, *Pleuronichthys coenosus* and *Reinhardtius evermanni*. All five are classified as members of Pleuronectidae based on morphologies obtained in the literature. Similarly, their phylogenetic position is determined through the examination of external morphologies, radiographs and literature. The *a posteriori* classification of these five species lends support to the functionality of this analysis, such that an individual species can be classified based on uniquely derived features.

Summary of classification

The objectives for classification in Pleuronectidae were to formally classify large groups within the family and to revise the established nomenclature to accurately represent natural groups. This was administered within a framework that minimized nomenclatorial change yet simplified the existing nomenclature with its many monotypic genera.

The Pleuronectidae is monophyletic with 58 species classified into 5 new subfamilies: Hippoglossinae, Eopsettinae, Lyopsettinae, Hippoglossoidinae and Pleuronectinae. This classification is based on the 50% majority-rule consensus of 128 parsimony trees, obtained through heuristic search (Figure 1). This is the first classification based on a complete phylogenetic analysis of the group and provides a simplified yet phylogenetically informative framework for future study involving this family.

family **Pleuronectidae**

subfamily **Hippoglossinae**

genus *Reinhardtius* (*R. evermanni**, *R. hippoglossoides*, *R. stomias*)

genus *Hippoglossus* (*H. hippoglossus*, *H. stenolepis*)

genus *Verasper* (*V. variegatus*, *V. moseri*)

genus *Clidoderma* (*C. asperrimum**)

subfamily **Eopsettinae**

genus *Eopsetta* (*E. grigorjewi*, *E. jordani*)

subfamily **Lyopsettinae**

genus *Lyopsetta* (*L. exilis*)

subfamily **Hippoglossoidinae**

genus *Acanthopsetta* (*A. nadeshnyi*)

genus *Cleisthenes* (*C. herzensteini*, *C. pinetorum*)

genus *Hippoglossoides* (*H. elassodon*, *H. platessoides*, *H. robustus*, *H. dubius**)
subfamily **Pleuronectinae**

tribe **Psettichthyini**

genus *Psettichthys* (*P. melanostictus*)

tribe **Isopsettini**

genus *Isopsetta* (*I. isolepis*)

tribe **Microstomini**

genus *Lepidopsetta* (*L. bilineata*, *L. mochigarei*)

genus *Dexistes* (*D. rikuzenius*)

genus *Pleuronichthys* (*P. coenosus**, *P. cornutus*, *P. decurrens*, *P. guttulatus*,
P. ocellatus, *P. ritteri*, *P. verticalis*)

genus *Microstomus* (*M. achne*, *M. bathybius*, *M. cynoglossus*, *M. kitaharai*,
M. kitt, *M. pacificus*, *M. stelleri*, *M. zachirus*)

tribe **Pleuronectini**

genus *Parophrys* (*P. vetula*)

genus *Limanda*** (*L. aspera*, *L. limanda*, *L. ferruginea*, *L. proboscidea*,
L. punctatissima, *L. sakhalinensis*)

genus *Pseudopleuronectes* (*P. americanus*, *P. obscurus**, *P. herzensteini*,
P. schrenki, *P. yokohamae*)

genus *Pleuronectes* (*P. glacialis*, *P. putnami*, *P. pinnifasciatus*, *P. platessus*,
P. quadrituberculatus)

genus *Platichthys* (*P. bicoloratus*, *P. flesus*, *P. stellatus*)

* species not included in analysis, phylogenetic position and classification determined *a posteriori*.

** monophyletic status is uncertain.

The reclassification of several species is necessary to incorporate this new phylogenetic information. The changes relative to Sakamoto's (1984a) classification are:

1. The species of *Atheresthes evermanni*, *A. stomias* and *Reinhardtius hippoglossoides* (Norman 1934) are united as *Reinhardtius* which is the oldest valid genus name. This classification forms a hierarchically consistent nomenclature within Pleuronectidae.
2. The species *Eopsetta exilis*, *E. grigorjewi* and *E. jordani* (Sakamoto, 1984a) are paraphyletic and revised as *Lyopsetta exilis*, *Eopsetta grigorjewi* and *E. jordani* (Norman 1934) to indicate this relationship.
3. The species *Hippoglossoides herzensteini* and *H. pinetorum* (*sensu* Sakamoto 1984a) are reclassified as *Cleisthenes* (as in Norman, 1934). This is a subjective reclassification because the interrelationships of these two species within Hippoglossoidinae does not contradict the previous classification. It is based on the monophyletic status of these two species and maintains a hierarchical consistent nomenclature within the family.
4. The genus *Microstomus* is revised to include all species of *Embassichthys*, *Errex*, *Glyptocephalus*, *Microstomus* and *Tanakius*. This greatly simplifies the nomenclature for this monophyletic group within Pleuronectinae.
5. The species of *Hypsopsetta* and *Pleuronichthys* are regrouped under *Pleuronichthys*. Reclassifying the species *Hypsopsetta guttulata* in *Pleuronichthys*, results in a simplified nomenclature that is hierarchically consistent in representing the phylogenetic relationships within

the tribe Microstomini. The species-group name (*guttulata*) is revised to agree with gender in the genus (i.e. *Pleuronichthys guttulatus*).

6. The genus *Pleuronectes* (*sensu* Sakamoto, 1984a), with 19 species, is not monophyletic. Based on this phylogenetic analysis only five species are classified in *Pleuronectes*: *P. glacialis*, *P. pinnifasciatus*, *P. platessus*, *P. putnami* and *P. quadrituberculatus*. Other species of *Pleuronectes* (*sensu* Sakamoto, 1984a) are polyphyletic and reclassified based on the phylogeny determined in this analysis. The previous classification was based on a 100% similarity observed for these 19 species (Sakamoto, 1984a). The new phylogeny is based on the addition of 67 new morphological features that clearly differentiate and classify these 19 species. The nomenclatorial revision classifies: *Lepidopsetta bilineata*, *L. mochigarei*, *Parophrys vetula*, *Limanda aspera*, *L. ferruginea*, *L. limanda*, *L. sakhalinensis*, *L. proboscidea*, *L. punctatissima*, *Pseudopleuronectes americanus*, *P. herzensteini*, *P. obscurus* (not examined), *P. schrenki* and *P. yokohamae* based on the oldest valid generic name for each species, with the exception of *Pseudopleuronectes schrenki* and *P. obscurus*, previously classified as *Limanda schrenki* (in Sakamoto 1984b; Hensley and Ahlstrom, 1984) and *Liopsetta obscurus* (in Norman, 1934) respectively.

7. The reclassification of *Pseudopleuronectes schrenki* (Schmidt) is based on its position in all 128 most parsimonious trees, none of which united *P. schrenki* with other taxa exclusive of the other three species in *Pseudopleuronectes*. In addition to the three synapomorphies uniting *Pseudopleuronectes*, the most notable characteristic of *P. schrenki* in observation and in the literature (Sakamoto, 1984b) is the presence of incisor-like teeth that form a continuous cutting

edge (70, Figure 9D). This morphology defines the fourth lineage of Pleuronectini uniting *Pseudopleuronectes* and *Pleuronectes* and excludes the other species of *Limanda* that have teeth with truncated tips or are bluntly conical (70, Figure 9C). A review of literature describing *Pseudopleuronectes schrenki* (= *Limanda schrenki*) indicates a close relationship with *P. yokohamae* (= *Limanda yokohamae*) (Jordan and Starks, 1906). Norman (1934) synonymized *Limanda schrenki* under *Pseudopleuronectes yokohamae*. Studies dealing with egg morphologies also seem to synonymize *Pseudopleuronectes schrenki* with *P. yokohamae* (Yusa, 1960; Pertseva-Ostroumova, 1961). Recent studies indicate that *P. schrenki* (= *Limanda schrenki*) is a valid species with similar characteristics to *P. yokohamae* (= *Limanda yokohamae*). Meristic counts of vertebrae, dorsal and anal fin rays are overlapping (Sakamoto, 1984a, 1984b). They have similar dental morphology but *P. schrenki* has a distinct pattern of bars on the dorsal and anal fin rays (Sakamoto, 1984b). Uncertainty surrounding the identity of *Pseudopleuronectes schrenki* is also a result of the ineffective use of nomenclature. As illustrated in the above references the classification of *Pseudopleuronectes yokohamae* as *Limanda yokohamae* has led to a degree of confusion, further supporting the need to clearly identify natural groups based on uniquely derived features. Although further analysis is required to clearly assess the synonymy of *Pseudopleuronectes schrenki* with *P. yokohamae*, this analysis clearly indicates that neither should be classified as *Limanda*.

8. The reclassification of *Pseudopleuronectes obscurus* (Herzenstein) is solely based on information available from the literature. This species is confidently placed in the fourth lineage of Pleuronectini but its placement as a species of *Pseudopleuronectes* is supported by one

synapomorphy, the presence of a demersal egg and the absence of synapomorphies that define *Pleuronectes*. A complete examination of osteological features for this species, may test the validity of this classification.

Conclusions

This cladistic analysis clearly establishes the monophyly and intrarelationships of Pleuronectidae. The intrarelationships and new classification are based on a large amount of morphological support at most of the resolved nodes. Interrelationships, not fully supported in this analysis, are not the foundation for this new classification. The conservative nature of this new classification is intended to establish a stable yet phylogenetically informative framework for the continued study of this commercially important group of flatfish. Using the established phylogeny to predict patterns of life history and behaviour in this family, will provide a useful tool in understanding the basic ecology for species under current management, as well as species that are less understood. Management policy for the sustainable use of these fish will benefit from a nomenclature that is informative by representing genealogy within the family, and less subject to change due to the large amount of morphological support.

With the growing trend for the use of molecular data to resolve phylogeny, it may be asked why molecular data was not used in this study. This calls for an argument, admittedly one-sided, in support of morphological data for this study. Many of the major questions involving the monophyly, intrarelationships and classification of and within Pleuronectidae are historically based on different interpretations of morphological data. It was felt that to fully address and

resolve these questions, a cladistic reanalysis of the morphological data was necessary. By establishing nomenclatorial revisions via reanalysis of the same morphologies, the problem is confronted at its roots, namely the phylogenetic interpretation of these morphologies. The interpretation of these morphologies in a cladistic framework supports the functionality of phylogeny and classification based on morphological evidence. Species that were not included in the original analysis have been classified within the Pleuronectidae based on morphological information already available and using the character analysis for clades within Pleuronectidae. It is understood that a complete examination of all morphological features for the new taxon and a reanalysis of the data would be ideal. However, the *a posteriori* placement of additional taxa based on known morphological evidence, effectively replaces this procedure. Molecular analysis would call for the complete sequencing of the relevant nucleotide strand in the new taxon, a multiple alignment of all previous taxa with the new taxon, and a reanalysis of the complete data set to produce a phylogeny. Finally, the cladistic interpretation of morphologies with Pleuronectidae becomes a framework for the future analysis of other taxa within Pleuronectiformes. Like Pleuronectidae, the historical classification of pleuronectiform is based on morphological evidence. Analysis pertaining to classification within the order will have to address the morphological data, and therefore benefit from a better understanding of the distribution of morphological characters in related groups. The phylogenetic limitations of some morphological features observed in this analysis will also help to establish a framework for the interpreting intrarelationships in other groups.

Future systematic research should consider the use of molecular data, focusing both within Pleuronectidae and its relationship within the bothoid lineage. Although there is a wealth of morphological data available within the Pleuronectiformes it is important to recognise the phylogenetic limitations with respect to level of univesality. This was evident within Pleuronectidae as there were nodes not supported by a large number of morphological characters and intrarelationshiPs not fully resolved. The lack of clear morphological evidence to substantiate or refute the monophyly of *Limanda*, indicates that molecular data may be useful to resolve these questions. Molecular data as well as examination of myology and larval morphology will undoubtedly aid in determining the intrarelationshiPs of taxa not fully resolved by this analysis. The phylogenetic relationship of Pleuronectidae within the bothoid lineage is necessary to clearly understand and identify the diversity of all flatfish taxa, many of which are also commercially important.

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Appendix A

Appendix B

List of characters:

1. *Attachment of frontal with mesethmoid on ocular side.* 2 states, 1 step, cci=1.00: Frontal of ocular side not in contact with mesethmoid, separated by prefrontal (state 0), frontal of ocular side in contact with mesethmoid (state 1), see Amaoka (1969); Figure 104, p. 184.
2. *Sensory canal of preorbital on ocular side* (Sakamoto, 1984b). 2 states, 3 steps, cci=0.33: Present (state 0), absence (state 1). The presence/absence of a sensory canal in the ocular side preorbital in the outgroup taxa *Psettodes* sp. could not be determined (state ?). A sensory canal is common in left-eyed flounders, see Amaoka (1969); Figure 105, p. 187.
3. *Morphology of metapterygoid.* 2 states, 2 steps, cci=0.50: Ventral margin concave (state 0, Figure 2A), observed in *Reinhardtius stomias* and all outgroup taxa. Ventral margin not concave nearly straight (state 1, Figure 2B, C, D).
4. *Articulation between first and second basibranchials.* 2 states, 8 steps, cci=0.13: Wedge-like articulation between first and second basibranchial (state 0, Figure 3A). No wedge-like articulation, first basibranchial loosely attached to second basibranchial by cartilage (state 1, Figure 3B, C, D).

5. *Articulation suture between second and third basibranchials*. 2 states, 3 steps, cci=0.33:

Wedge-like articulation between second and third basibranchial (state 0, Figure 3A). No wedge-like articulation, second basibranchial loosely attached to third basibranchial by cartilage (state 1, Figure 2B, C, D).

6. *Haemapophysis through fusion of parapophysis of posterior most abdominal vertebrae*

(Sakamoto, 1984a). 2 states, 4 steps, cci=0.25: Present (state 0, Figure 4A, B, D), absent (state 1, Figure 4C).

7. *Accessory processes on ventral side of centrum*. 2 states, 5 steps, cci=0.20: Present (state 0, Figure 5A), absent (state 1, Figure 5B, C).

8. *Infraorbitals on ocular side* (Sakamoto, 1984a). 2 states, 2 steps, cci=0.50: Absent (state 0), present (state 1). All pleuronectid taxa (except *Microstomus bathybius*) have infraorbital bones on the ocular side. This character state is shared with *Psettodes* and *Paralichthys lethostigmus*. The sensory canal bones are absent in the other outgroup taxa examined (*Lepidoblepharon ophthalmolepis*, *Citharichthys arenaceus* and *Paralichthys squamilentus*) and most taxa within the bothoid lineage, see Amaoka (1969); Table 6, p. 193.

9. *Oil globules in yolk* (Hensley and Ahlstrom, 1984). 2 states, 3 steps, cci=0.33: Present (state 0), absent (state 1). All outgroup taxa have at least one oil globule (Hensley and Ahlstrom, 1984).

10. *Morphology of olfactory lamellae* (Norman 1934). 2 states, 2 steps, cci=0.50: Olfactory lamellae radiating around a central rachii (state 0). Olfactory lamellae parallel without a central rachii (state 1).
11. *Morphology of anterior prootic foramen, on ocular side*. 2 states, 8 steps, cci=0.13: Sphenotic forms dorsal margin of foramen (state 0, Figure 7A). Pterosphenoid and prootic join to form dorsal margin of foramen (state 1, Figure 7B, C).
12. *Bifurcation of first epibranchial* (Sakamoto, 1984a). 2 states, 5 steps, cci=0.20: Distal end of first epibranchial divided into two branches (state 0), distal end of first epibranchial simple (state 1), see Sakamoto (1984a); Figures 24, 25, pp. 150-151.
13. *Spines or teeth on gill rakers* (Sakamoto, 1984a). 2 states, 1 step, cci=1.00: Present (state 0), absent (state 1), see Sakamoto (1984a); Figure 26, p. 152.
14. *Morphology of suture between mesethmoid and prefrontal on blind side*. 3 states, ordered, 13 steps, cci=0.15: Anterior margin of upper orbit is incomplete, mesethmoid and prefrontal on blind side not completely overlapping resulting in a space between them (state 0, Figure 8A). Anterior margin on upper orbit is complete, mesethmoid and prefrontal on blind side completely overlapping or sutured with a small foramen present (state 1, Figure 8B, C). Same as state 1 but foramen absent (state 2, Figure 8D). The character state for the mesethmoid and prefrontal is

not applicable (na) for the outgroup taxa *Psettodes* sp. and *Lepidoblepharon ophthalmolepis* due to the morphological differences in this region with respect to the ingroup taxa.

15. *Morphology of first anal pterygiophore*. 2 states, 3 steps, cci=0.33: Thin (state 0). Broadly thickened (state 1).

16. *Uniformity of dentition*. 3 states, ordered, 6 steps, cci=0.33: Dentition of variable lengths and size (state 0, Figure 9A). Dentition of near equal or equal length (state 1, Figure 9B, C).

Uniform dentition as in state 1 but teeth forming a continuous cutting edge (state 2, Figure 9D).

17. *Development of interorbital process* (Sakamoto, 1984a). 3 states, unordered, 4 steps, cci=0.50: Well developed (state 0, Figure 10A, D). Middle part absent (state 1). Not developed (state 2, Figure 10B, D, E).

18. *Morphology of hyomandibular of both ocular and blind side*. 2 states, 2 steps, cci=0.50: Anterior margin of hyomandibular is not broad (state 0, Figure 2A, B, C). Anterior margin of hyomandibular is flatly broadened (state 1, Figure 2D).

19. *Dentition of third epibranchial* (Sakamoto, 1984a). 2 states, 1 step, cci=1.00: Present (state 0), absent (state 1), see Sakamoto (1984a); Figure 24, p. 150.

20. *Bony plates of the branchial arch* (Sakamoto, 1984a). 2 states, 2 steps, cci=0.50: Present (state 0), absent (state 1), see Sakamoto (1984a); Figure 26, 27, pp. 152-153.
21. *Number of rows of gill rakers on fourth ceratobranchial*. 3 states, unordered, 6 steps, cci=0.33: One row, second row absent or very reduced (state 0). Zero rows (state 1). Two rows (state 2). *Reinhardtius* may have 1 row of gill rakers on fourth ceratobranchial, but very modified and difficult to distinguish from bony plates.
22. *Fimbriation along posterior dorsal margin of the operculum*. 2 states, 2 steps, cci=0.50: Absent (state 0, Figure 2A), present (state 1, Figure 2B, C, D).
23. *Number of abdominal vertebrae*. 3 states, ordered, 9 steps, cci=0.22: Less than 12 (state 0). Between 12 and 14 (state 1). Greater than 15 (state 2).
24. *Shape of caudal fin*. 2 states, 2 steps, cci=0.50: Caudal fin truncate, double truncate or rounded (state 0). Caudal fin lunate or emarginate (state 1).
25. *Position of upper eye in relation to dorsal midline* (Sakamoto, 1984a). 2 states, 3 steps, cci=0.33: Located on side of head (state 0). Near dorsal midline, visible on blind side (state 1).
26. *Increase in number of caudal vertebrae*. 3 states, ordered, 5 steps, cci=0.40: Less than or equal to 35 (state 0). Between 36 and 41 (state 1). Greater than 41 (state 2).

27. *Foramen on blind side dentary just below margin of dentition*. 2 states, 5 steps, cci=0.20: Present (state 0), absent (state 1).
28. *Attachment of palatine with pterygoid on ocular side*. 2 states, 2 steps, cci=0.50: Palatine and pterygoid attached (state 0, Figure 2A, D). Palatine and pterygoid not attached due to a reduction of the posterior-ventral arm of the palatine (state 1, Figure 2B, C).
29. *Gill rakers on first epibranchial*. 2 states, 2 steps, cci=0.50: Present (state 0), absent (state 1).
30. *Gill rakers on second epibranchial*. 3 states, ordered, 5 steps, cci=0.40: Present (state 0). Reduced to just one gill raker at proximal base of epibranchial (state 1). Absent (state 2).
31. *Gill rakers on third epibranchial*. 3 states, ordered, 5 steps, cci=0.40: Present (state 0). Reduced to just one gill raker at proximal base of epibranchial (state 1). Absence (state 2).
32. *Gill rakers on first hypobranchial*. 2 states, 4 steps, cci=0.25: Presence (state 0). Reduced to just one gill raker at proximal base of hypobranchial (state 1).
33. *Subdivisions of hypurals or hypurals and parhypurals* (Sakamoto, 1984a). 2 states, 1 step, cci=1.00: Absent (state 0), present (state 1). Character states for the caudal skeleton is not

applicable (na) for the outgroup taxa *Psettodes* sp. and *Lepidoblepharon ophthalmolepis* due to the morphological differences in this region with respect to the ingroup taxa.

34. *Articulation of blind side metapterygoid with entopterygoid*. 2 states, 7 steps, cci=0.14:

Absent (state 0, Figure 2A, B), present (state 1, Figure 2C, D). The metapterygoid is articulated to the pterygoid in outgroup taxa (Figure 2A).

35. *Morphology of mesethmoid and prefrontal on blind side, special type for *Verasper**. 2 states,

1 step, cci=1.00: Large open foramen absent (state 0). Large open foramen formed between mesethmoid and prefrontal on blind side (state 1), autapomorphy for *Verasper*. The character state for the mesethmoid and prefrontal is not applicable (na) for the outgroup taxa *Psettodes* sp. and *Lepidoblepharon ophthalmolepis* due to the morphological differences in this region with respect to the ingroup taxa.

36. *Gill rakers on second hypobranchial*. 2 states, 4 steps, cci=0.25: Present (state 0). Reduced to just one gill raker at proximal base of hypobranchial (state 1).

37. *Sphenotic process relative to hyomandibular socket on blind side*. 2 states, 3 steps,

cci=0.33: Process position low on sphenotic to form dorsal roof of socket between sphenotic and prootic (state 0, Figure 7A). Process is positioned higher on sphenotic and not associated with socket (state 1, Figure 7B, C, D).

38. *Supraoccipital crest*. 2 states, 7 steps, cci=0.14: Single crest (state 0, Figure 10A, B).

Double crest to form a groove for the insertion of the basal ends of pterygiophores for the dorsal fin (state 1, Figure 10C, D, E).

39. *Cardiac apophysis of urohyal* (Sakamoto, 1984a). 3 states, unordered, 8 steps, cci=0.25:

Cardiac apophysis is simple or slightly bifurcate at the posterior portion (state 0, Figure 12A, B)

Cardiac apophysis is simple at tip with bifurcation migrating anteriorly on dorsal edge (state 1, Figure 12E, F).

Cardiac apophysis is strongly bifurcate at the posterior portion (state 2, Figure 12C, D).

40. *Gill rakers on fourth epibranchial*. 2 states, 3 steps, cci=0.33: Absent (state 0), present (state 1).

41. *Rows of teeth in lower jaw* (Norman, 1934). 2 states, 6 steps, cci=0.17: Multiserial or biserial rows of teeth (state 0). Uniserial row of teeth (state 1). The distribution of this characters was examined for all species in the analysis. Character states observed by the author maintained priority in observations conflicting with Norman (1934).

42. *Barbed teeth* (Norman, 1934). 2 states, 2 steps, cci=0.50: Absent (state 0), present (state 1).

43. *Supratemporals on ocular side*. 2 states, 7 steps, cci=0.1: Supratemporal in one piece (state 0, Figure 13A). Supratemporal in two pieces, jointed at anterior bifurcation point (state 1, Figure 13B).

44. *Supratemporals on blind side*. 2 states, 5 steps, cci=0.20: Supratemporal in one piece (state 0, Figure 13A). Supratemporal in two pieces, jointed at anterior bifurcation point (state 1, Figure 13B).

45. *Scales on the surface of each eye* (Sakamoto, 1984a). 2 states, 6 steps, cci=0.17: Absent (state 0), present (state 1).

46. *Posterior region of supraoccipital*. 2 states, 2 steps, cci=0.50: Presence of a flat plate triangular or diamond shaped extending posteriorly from supraoccipital between or overtop epiotics (state 0, Figure 10A, C, D, E). Absence of posterior extension of supraoccipital (state 1, Figure 10B).

47. *Anterior prootic foramen, on blind side*. 2 states, 8 steps, cci=0.13: Sphenotic forms dorsal margin of foramen (state 0, 7A). Pterosphenoid and prootic join to form dorsal margin of foramen (state 1, Figure 7B, C, D). The anterior prootic foramen entirely contained within prootic in outgroup taxa.

48. *Pterosphenoid on blind side*. 2 states, 3 steps, cci=0.33: Pterosphenoid reduced, frontal and parasphenoid join to form posterior margin of orbit (state 0, Figure 7B). Pterosphenoid large and forms posterior margin of orbit (state 1, Figure 7A, C).

49. *Rows of teeth on fifth ceratobranchial*. 3 states, ordered 4 steps, cci=0.75: Irregular single or multiple rows (state 0, Figure 3A). Regular 2 rows (state 1, Figure 3B, C). Regular multiple rows or clustered (state 2, Figure 3D).

50. *Nasal bone on blind side*. 2 states, 7 steps, cci=0.14: Present (state 0), absent (state 1). The presence of a blind side nasal bone could not be determined (state ?) for the outgroup taxa *Citharichthys arenaceus* either through cleared and stained material or through radiograph.

51. *Number of infraorbital bones on blind side* (Sakamoto, 1984a). 3 states, unordered 9 steps, cci=0.22: Between five and seven (state 0). Greater than seven (state 1). Less than five (state 2). The number of infraorbital bones in *Citharichthys arenaceus* could not be determined (state ?) due to the poor preservation of infraorbital bones for this specimen.

52. *Dorsal crest extending anteriorly from supraoccipital to frontal on blind side*. 2 states, 7 steps, cci=0.14: Prominent narrow crest present angled laterally to eyed side (state 0, Figure 10B, C). Reduced or absent crest resulting in a flattened posterior dorsal region on blind side frontal (state 1, Figure 10A, D, E).

53. *Anterior margin of mesethmoid*. 3 states, unordered, 10 steps, cci=0.30: Open canal extending from frontal on ocular side (state 0, Figure 8A). Thin plate (state 1, Figure 8B). Closed canal extending from frontal on ocular side or from interorbital process if present. Process extending anteriorly triangular shaped to make anterior edge thicker (state 2, Figure 8C). Similar to (state 2) but anterior edge reduced to form an irregular edge (state 3, Figure 8D). The morphology of the mesethmoid in the outgroup taxa, *Psettodes* sp. and *Lepidoblepharon ophthalmolepis* is unlike any observed in the other outgroup taxa or within the ingroup and is coded as not applicable (na).

54. *Articulation of metapterygoid with entopterygoid on ocular side*. 2 states, 3 steps, cci=0.33: Absent (state 0, Figure 2A, B, C), present (state 1, Figure 2D). The metapterygoid is articulated to the pterygoid in outgroup taxa (Figure 2A).

55. *Dentary fossa for the insertion of Meckel's cartilage on both ocular and blind side dentary*. 2 states, 1 steps, cci=1.00. Present (state 0, Figure 9A,B), absent (state 1, Figure 9C,D).

56. *Ceratohyal foramen* (Sakamoto, 1984a; Leipertz 1987). 2 states, 1 step, cci=1.00: Present (state 0), absent (state 1).

57. *Intercalar in contact with basioccipital*. 2 states, 4 steps, cci=0.25: Intercalar not joined to basioccipital (state 0, Figure 7A, B). Intercalar and basioccipital in contact (state 1, Figure 7C).

58. *Posterior extension of supratemporal branch of lateral line* (Norman, 1934). 2 states, 4 steps, cci=0.25: Absent (state 0), present (state 1).
59. *Prolongation of anterior dorsal fin rays* (Norman, 1934; Sakamoto, 1984a). 2 states, 1 step, cci=1.00: Absent (state 0), present (state 1).
60. *Rows of teeth in upper jaw* (Norman, 1934). 2 states, 3 steps, cci=0.33: Multiserial or biserial rows of teeth (state 0). Uniserial row of teeth (state 1). Character state based on direct observation of materials maintained priority in observations conflicting with Norman (1934).
61. *Asymmetry in medial symphysis of premaxillae*. 2 states, 1 step, cci=1.00: Blind side premaxilla does not protrude past sagittal axis at anterior symphysis with ocular side premaxilla (state 0, Figure 9A, B). Blind side premaxilla overlaps or protrudes past sagittal axis (state 1, Figure 9C, D).
62. *Asymmetry in length of premaxillae*. 2 states, 2 steps, cci=0.50: Ocular side nearly equal or equal to length of blind side premaxilla (state 0, Figure 9A, B). Ocular side premaxilla shorter than blind side (state 1, Figure 9C, D).
63. *Ventral posterior curvature of blind side premaxilla*. 2 states, 2 steps, cci=0.50: Absent (state 0, Figure 9A, B), present (state 1, Figure 9C, D).

64. *Asymmetry of size of space along ventral margin of dentary and articular*. 2 states, 5 steps, cci=0.20: Space nearly the same size (state 0, Figure 9A, B, D). Asymmetrical space, blind side greater than ocular side (state 1, Figure 9C).

65. *Asymmetry in size of dorso-posterior process of dentary*. 2 states, 5 steps, cci=0.20: Symmetrical (state 0, Figure 9B, D). Asymmetrical, ocular side greater than blind side (state 1, Figure 9A, D).

66. *Reduction of teeth on ocular side maxilla* (Norman, 1934). 3 states, ordered, 5 steps, cci=0.40: Number of teeth on ocular nearly equal or equal to those on blind side (state 0, Figure 9A, B). Ocular side teeth fewer than blind side but greater than 6 (state 1, Figure 9C). Ocular side teeth fewer than blind side less than 6 (state 2, Figure 9D).

67. *Reduction of teeth on ocular side dentary* (Norman, 1934). 3 states, ordered 6 steps, cci=0.33: Number of teeth on ocular side nearly equal or equal to those on blind side (state 0, Figure 9A, B). Ocular side teeth fewer than blind side but greater than 6 (state 1, Figure 9C). Ocular side teeth fewer than blind side less than 6 (state 2, Figure 9D).

68. *Epiotic process on ocular and blind sides*. 2 states, 3 steps, cci=0.33: Process absent *Microstomus pacificus* or barely evident *Hippoglossoides*, *Reinhardtius* (state 0, Figure 10A, B, C). Process is clearly evident extending anteriorly from epiotics onto parietals *Limanda*, *Platichthys* (state 1, Figure 10D, E).

69. *Asymmetry in size of entopterygoid*. 2 states, 3 steps, cci=0.3: Blind side equal in size to ocular side (state 0, Figure 2A, B). Blind side smaller than ocular side (state 1, Figure 2C, D).

70. *Morphology of teeth* (Norman, 1934). 4 states, ordered 6 steps, cci=0.50: Sharply pointed (state 0, Figure 9A, B). Bluntly conical or with truncated tips (state 1, Figure 9C). Incisor-like (state 2, Figure 9D). Molariform (state 3).

71. *Shape of fifth ceratobranchial*. 3 states, ordered 5 steps, cci=0.40: Straight 'rod' shaped (state 0, Figure 3A, B). Slight curve on medial side found in *Limanda*, *Parophrys*, *Psettichthys*, *Isopsetta* etc. (state 1, Figure 3C). Strong curve on medial side, the ceratobranchials forming or almost forming a triangular plate found in *Pleuronectes* and *Platichthys* (state 2, Figure 3D).

72. *Dentition profile on fifth ceratobranchial*. 3 states, ordered, 6 steps, cci=0.33: Pointed (state 0, Figure 3A, B). Bluntly pointed (state 1, Figure 3C). Rounded or molariform (state 2, Figure 3D).

73. *Haemal spines for anterior most caudal vertebrae*. 2 states, 6 steps, cci=0.17: Broadly attached both anteriorly and posteriorly to centrum (state 0, Figure 5B). Narrow at base, attached anteriorly to centrum (state 1, Figure 5A, C).

74. *Morphology of epiotics* (Sakamoto 1984a). 2 states, 2 steps, cci=0.50: Epiotics not joined to each other along dorsal posterior margin of skull (state 0). Epiotics joined (state 1), only observed in *Isopsetta isolepis*, *Microstomus pacificus*.
75. *Egg type* (Hensley and Ahlstrom, 1984). 2 states, 2 steps, cci=0.50: Pelagic egg (state 0). Demersal egg (state 1).
76. *Thickened lips* (Norman, 1934). 2 states, 2 steps, cci=0.50: Absent, thin lips (state 0), present, lips thickened (state 1). Taxa in Pleuronectini are also described as having thickened lips (Norman, 1934) but this is not as evident as in Microstomini.
77. *Morphology of exoccipital and prootic*. 2 states, 2 steps, cci=0.50: Exoccipital and prootic joined (state 0, Figure 7B, D). Exoccipital and prootic not joined (state 1, Figure 7A, C).
78. *Post-ocular ridge on ocular side* (Norman, 1934). 2 states, 2 steps, cci=0.50: Absent (state 0), present (state 1).
79. *Villiform teeth* (Norman, 1934). 2 states, 1 step, cci=1.00: Absent (state 0), present (state 1).
80. *Foramen in prefrontal on blind side*. 2 states, 2 steps, cci=0.50: Absent (state 0), present (state 1).

81. *Lateral process on frontal of ocular side*. 2 states, 3 steps, cci=0.33: Absent (state 0, Figure 7A, C, Figure 10A, B, D), present (state 1, Figure 7B, Figure 10C).
82. *Decrease in the number of caudal vertebrae*. 2 states, 3 steps, cci=0.33: Greater than 25 (state 0). Less than or equal to 25 (state 1). The polarity established for this character is an exception to the general outgroup comparison methodology. It is apparent that a caudal vertebrae count between 25 and 35 is plesiomorphic for Pleuronectidae and for many taxa within the bothoid lineage. Despite the heterogeneity in *Citharichthys arenaceus* and less than 25 vertebrae observed in *Lepidoblepharon ophthalmolepis* and *Psettodes* sp., the reduction of caudal vertebrae observed within Pleuronectidae is considered a derived character state.
83. *Pterosphenoid on ocular side*. 2 states, 2 steps, cci=0.50: Pterosphenoid reduced, frontal and parasphenoid join to form posterior margin of orbit (state 0, Figure 7B). Pterosphenoid large and forms posterior margin of orbit (state 1, Figure 7A, C).
84. *Position and origin of dorsal fin* (Norman, 1934; Sakamoto, 1984a). 2 states, 1 step, cci=1.00: Originating nearly on the median line of head, ancestral (state 0). Originating on blind side (state 1).
85. *Cartilagenous interspace between prefrontal and parasphenoid on blind side*. 2 states, 1 step, cci=1.00: Present (state 0, Figure 7A, C), reduce or absent (state 1, Figure 7B).

86. *Mesethmoid in relation to upper orbit* (Sakamoto, 1984a). 3 states, ordered, 3 steps, cci=1.00: Completely forms anterior margin of orbit (state 0). Forms only part of orbit (state 1). Does not form part of orbit (state 2).
87. *Extension of intestine into body cavity* (Sakamoto, 1984a). 2 states, 1 step, cci=1.00: Absent (state 0), presence (state 1).
88. *Number of caudal fin rays*. 5 states, ordered, 7 steps, cci=0.57: 18 or 19 (state 0), 20 (state 1), 21 (state 2), 22 (state 3), 23 (state 4).
89. *Degree of caudal fin ray branching expressed as the number of caudal fin rays minus the number of branched caudal fin rays*. 3 states, unordered, 8 steps, cci=0.25: Six unbranched rays (state 0). Less than 6 unbranched rays (state 1). Greater than 6 unbranched rays (state 2).
90. *Position of ventro-anterior tip of pelvis of ocular side relative to cleithra* (Sakamoto, 1984a). 2 states, 1 step, cci=1.00: Pelvis posterior to cleithrum ancestral (state 0), observed in majority of outgroup as well as Pleuronectidae. Pelvis anterior to cleithrum autapomorphy for *Microstomus bathybius* (state 1).
91. *Morphology of scales* (Batts, 1964). 2 states, 1 step, cci=1.00: Radii extending anteriorly from focus of each scale (state 0). Radii completely surrounding focus of scale (state 1).

92. *Fimbriation along the postero-ventral margin of the interoperculum.* 2 states, 1 step, cci=1.00: Absent (state 0, Figure 2A, B, D), present (state 1, Figure 2C).

93. *Fimbriation along posterior margin of the suboperculum.* 2 states, 2 steps, cci=0.50: Absent (state 0, Figure 2A, B, D), present (state 1, Figure 2C).

94. *Relation of cleithra with urohyal* (Sakamoto, 1984a). 2 states, 1 step, cci=1.00: Cleithra not inserted by tip of urohyal (state 0), most common state in bothoid group. Cleithra slightly inserted by tip of urohyal (state 1).

95. *Increase in the number of pyloric appendages on upper intestine* (Norman, 1934). 2 states, 1 step, cci=1.00: Two or three pyloric appendages plus one on upper intestine (state 0). Two to four pyloric appendages plus two or three on upper intestine (state 1).

96. *Asymmetry in nasal bones* (Sakamoto, 1984a). 2 states, 1 step, cci=1.00: Ocular side larger than blind side with blind side reduced or absent as recorded in character 50 (state 0). Blind side larger than ocular side (state 1). States could not be observed (state ?) in the outgroup taxa *Psettodes* sp., *Citharichthys arenaceus* and *Paralichthys squamilentus* due to the poor condition of the specimens around the dorso-anterior margin of the upper eye. A blind side nasal bone was not observed in radiographs of these specimens prior to clearing and staining.

97. *Mucous cavities on blind side of head* (Sakamoto, 1984a). 2 states, 1 step, cci=1.00: Absent (state 0), present (state 1).
98. *Interpterosphenoid bar*. 2 states, 7 steps, cci=0.14: Absent (state 0), present (state 1). The interpterosphenoid bar is a medial extension of the pterosphenoid foramen. This morphology can only be observed laterally, through the pterosphenoid if it is thin, or from a ventral perspective if the parasphenoid is removed.
99. *Foramen on ocular side dentary just below margin of dentition*. 2 states, 11 steps, cci=0.09: Present (state 0), absent (state 1).
100. *Post-ocular ridge on blind side*. 2 states, 1 step, cci=1.00: Absent (state 0), present (state 1).
101. *Bony prominences along post-ocular ridge on ocular side*. 3 states, unordered, 4 steps, cci=0.50: Bony prominences absent (state 0, Figure 10D). Many small bony prominences (state 1, Figure 10E). A series of enlarged prominences on post-ocular ridge (state 2).
102. *Bony prominences along post-ocular ridge on blind side*. 2 states, 3 steps, cci=0.33: Bony prominences absent (state 0, Figure 10D). Many small bony prominences present (state 1, Figure 10E).

103. *Bony prominences extending anteriorly onto interorbital bar*. 2 states, 1 step, cci=1.00: Prominences not extended anteriorly (state 0). Prominences extending anteriorly onto interorbital bar (state 1, Figure 10E).

104. *Scales on median fin rays* (Sakamoto, 1984a). 2 states, 1 step, cci=1.00: Absent (state 0), present (state 1). The presence of scales on median fin rays in the outgroup taxa *Paralichthys squamilentus* could not be determined (state ?), as it was apparent that all of the scales on this specimen had been lost during clearing and staining.

105. *Bony plates or tubercles on body* (Sakamoto, 1984a). 2 states, 1 step, cci=1.00: Absent (state 0), present (state 1).

106. *Supratemporals fused to cranium* (Sakamoto, 1984a). 2 states, 2 steps, cci=0.5: Not fused with cranium (state 0). Fused with cranium (state 1).