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

J. Andrew Cooper

National Marine Fisheries Service, Systematics Laboratory
Museum of Natural History, Washington, D.C. 20560-0153

E-mail address: cooper.andrew@nmnh.si.edu

François Chapleau

Ottawa-Carleton Institute of Biology, Faculty of Science
University of Ottawa, P.O. Box 450, Station A., Ottawa, Ontario, K1N 6N5

Abstract.—A cladistic analysis of interrelationships for 53 (of 59) pleuronectid species was performed by using 106 morphological and osteological characters. The analysis resulted in 128 equally parsimonious cladograms (heuristic search, 403 steps, consistency index=0.33, retention index=0.79). A 50% majority-rule consensus cladogram indicated that 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 is monophyletic according to ten synapomorphies. In addition, five subfamilies were defined: Hippoglossinae, Eopsettinae, Lyopsettinae, 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 at the genus-group level. The following genera are reclassified: *Atherestes* and *Reinhardtius* to *Reinhardtius*; *Errex*, *Glyptocephalus*, and *Tanakius* to *Glyptocephalus*; *Embassichthys*, and *Microstomus* to *Microstomus*; *Hypsopsetta* and *Pleuronichthys* to *Pleuronichthys*; and *Kareius* and *Platichthy* to *Platichthys*. To preserve the monophyletic status of *Eopsetta*, *E. exilis* was reassigned to the genus *Lyopsetta* (Lyopsettinae). The genus *Pleuronectes* (as defined by Sakamoto in 1984) was found to be polyphyletic. Monophyly of this genus is established by revising it to include only five species; *Pleuronectes glacialis*, *P. pinnifasciatus*, *P. platessus*, *P. putnami*, and *P. quadrituberculatus*. Other species, formerly placed in *Pleuronectes*, are now reclassified to *Isopsetta*, *Limanda*, *Parophrys*, *Psettichthys*, and *Pseudopleuronectes*. The monophyletic status of *Limanda* (six species) is uncertain because of unresolved relationships between these species and other taxa in the tribe Pleuronectini.

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The Pleuronectidae (*sensu* Chapleau and Keast, 1988) contains 59 nominal species of right-eyed flatfishes distributed in marine waters of the Northern Hemisphere. As presently composed, this family excludes the subfamilies Poecilopsettinae, Samarinae, Rhombosoleinae, and Paralichthodinae, formerly included in Norman (1934). It contains many commercial species that have long been harvested in coastal seas off Europe, North America, and Asia. Species such as the Petrale sole (*Eopsetta jordani*), 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). In total, there are over 36 species of flatfish monitored by the Food and Agricultural Organization of the United Nations, with a total annual catch of 256,353 metric tons (t) in 1995 (FAO, 1997). Nearly one half (18) of these species are classified within the Pleuronectidae (FAO, 1997). Total annual commerical harvest of Pacific halibut (*Hippoglossus stenolepis*) was estimated at 25,968 t in 1995 a decrease from 40,584 t in 1985 (FAO, 1997). A similar situation has been observed in witch flounder (*Glyptocephalus cynoglossus*) for which 19,537 t was harvested in 1995 as compared to 30,074 t in 1985 (FAO, 1997). With few exceptions, this decline in annual harvest is observed for most pleuronectid species (FAO, 1997). The commercial popularity of pleuronectid species, coupled with a need to manage these renewable resources, strongly emphasizes the necessity for a better understanding of relationships within this group, as well as an informative taxonomic nomenclature that will provide a framework for management policy. Variation in life history traits are observed at many phylogenetic levels. Populations of the American plaice (*Hippoglossoides platessoides*) are recorded to have age of maturity ranging from 3 to 15 years (Roff, 1981). Within the family, variation in maximum length ranges from 220 mm in *Dexistes rikuzenius* (Sakamoto, 1984b) to over 2500 mm in *Hippoglossus stenolepis* (Hart, 1973). An hypothesis of species interrelationships can be used as a framework to assess phylogenetic constraint on life history traits versus a species' ability to respond to changing environmental conditions (Brooks and McLennan, 1991). An assessment of

phylogenetic constraint versus environmental influence could provide a more informed understanding of observed changes in life history traits.

The objectives of this study are to clarify the monophyletic status of the Pleuronectidae, to offer an hypothesis of relationships within the group based on adult morphology, and to establish a phylogenetically informative nomenclature. To attain these objectives, a reassessment of morphological evidence in the literature and new morphological characters were compiled in a matrix analyzed within a cladistic framework. A new classification based on the phylogenetic information is offered.

Historical classification and diagnosis of Pleuronectidae

The Pleuronectidae was regarded by early ichthyologists to represent all known flatfishes (Norman, 1934). For example, Cuvier (1816) subdivided the Pleuronectidae into five subfamilies: Hippoglossinae, Pleuronectinae, Platessinae, Soleinae, and Cynoglossinae; as did Jordan and Goss (1889) who also added the subfamilies: Samarinae and Oncopterinae. Changes to the early classification in flatfish focused on revisions that accommodated new species without a complete revision of the entire scheme. 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, the Pleuronectidae became a "garbage" group.

Jordan and Evermann (1898) raised flatfishes to the suborder Heterosomata with two distinct families: Pleuronectidae and Soleidae. The Pleuronectidae, with three subfamilies: Hippoglossinae, Pleuronectinae, and Psettinae, were characterized 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 were subdivided into two subfamilies, Soleinae and Cynoglossinae, and were characterized 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 characterized 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, at this point containing five subfamilies (Pleuronectinae, Samarinae, Rhombosoleinae, Poecilopsettinae, and Paralichthodinae) were characterized 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 the fin-rays articulated; pelvic of from 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; on each side a single post-cleithrum; ribs present; egg without an oil-globule in the yolk." Later classifications removed the genera *Brachypleura* and *Lepidoblepharon* from the Pleuronectidae and placed them in the Citharidae (Hubbs, 1945) but essentially agreed with the classification proposed by Norman (1934).

Nelson (1984) listed the Poecilopsettinae, Rhombosoleinae, Samarinae, and Pleuronectinae as subfamilies in Pleuronectidae on the basis of two characteristics: eyes almost always dextral and no oil globule in yolk of egg. Sakamoto's (1984a) hypothesis of pleuronectid intrarelationships assumed that the Pleuronectinae, Samarinae, Rhombosoleinae, Poecilopsettinae, and Paralichthodinae were monophyletic because both eyes were on right side of the body, optic nerve of the left eye was always dorsal, preopercle had a free margin and fin rays were without spines. 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 of major taxa within the order supported the hypothesis that the 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). This new interpretation of taxonomic ranks in right-eyed flounders was recognized

by Hensley (1993) and in part by Nelson (1994). Species in Samarinae of Nelson (1984) and *Paralichthodes algoensis* were classified to Samaridae (Nelson, 1994). The subfamilies Pleuronectinae, Rhombosoleinae, and Poecilopsettinae, remained in Pleuronectidae. Nelson (1994) argued that without a comprehensive understanding of monophyly for some major groups it is difficult to provide an accurate revision of the nomenclature within the order. Although this approach is an obvious attempt to minimize unnecessary changes to the nomenclature, it does not reflect the understanding that only species in Pleuronectinae possess a bothoid caudal-fin complex that clearly distinguishes these 59 north temperate species as being closely related to Bothidae, Paralichthyidae, Scophthalmidae, and *Brachypleura* (Hensley and Ahlstrom, 1984; Chapleau, 1993). The Poecilopsettinae, Rhombosoleinae, and Samaridae do not have this caudal-fin complex and are phylogenetically related to Achiridae, Soleidae, and Cynoglossidae (Chapleau, 1993). Given that Pleuronectinae is the nominotypical subgroup, it is correct to reclassify it to Pleuronectidae. For the species of Poecilopsettinae, and Rhombosoleinae, it is a misrepresentation of the present cladistic framework to classify them in Pleuronectidae when the order level phylogeny (Chapleau, 1993) suggests a relationship with Samaridae, Achiridae, Soleidae, and Cynoglossidae. Therefore, only the 59 nominal species of Pleuronectinae are considered in this study and classified as Pleuronectidae (*sensu* Chapleau and Keast, 1988).

Two tribes in Pleuronectinae (*sensu* Norman, 1934), the Hippoglossini and Pleuronectini, were classified on the basis of jaw morphological characters (Nelson, 1984). 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 were 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 characters defining Hippoglossini were all plesiomorphic for the order and the characters defining the Pleuronectini were also observed in many lineages closely related to the Pleuronectinae (*sensu* Norman, 1934).

The 59 nominal species in this group had been historically classified in as many as 28 genera, many of which were monotypic. This 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. However, the alpha taxonomy for this group has been well established, and only one new species of Pleuronectidae, *Microstomus shuntovi* Borets, 1983, has been described in the latter half of this century. Intuitively, a simplified and more informative nomenclature with fewer monotypic genera would seem appropriate.

There are few published studies that have dealt with phylogenetic relationships among pleuronectid taxa. The most extensive examination of interrelationships and classification within Pleuronectidae (*sensu* Norman, 1934) was established by Sakamoto (1984a). This phenetic hypothesis of interrelationships among 77 species was not aimed at defining relationships within an evolutionary framework; nor was it aimed at determining taxonomic structure on the basis of natural groups. Sakamoto (1984a) concluded his study with a reclassification of several genera within the Pleuronectinae (*sensu* Norman, 1934). In revision, the species of *Eopsetta* and *Lyopsetta*, as well as *Cleisthenes* and *Hippoglossoides*, were reclassified into *Eopsetta* and *Hippoglossoides*. *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*, previously classified in its own subfamily, Paralichthodinae (Nelson, 1994), was placed within the Pleuronectinae on the basis of overall similarity and the presence of the first neural arch, a symplesiomorphy for the order (Hensley and Ahlstrom, 1984).

Chiu (1990) examined the relationships among four glyptocephaline species (formerly classified in *Glyptocephalus* and *Tanakius*). The results of this phenetic analysis of body shape were similar to Sakamoto's (1984a) results with respect to the relationships between *Glyptocephalus cynoglossus, G. stelleri*, and *G. zachirus*. The limited scope of this analysis does not provide adequate information to infer relationships beyond these three species.

Sakamoto's (1984a) nomenclatural changes and classification were adopted in the American Fisheries Society checklist for flatfish species (Robins et

al., 1991). The adoption of this reclassification was a recognition of the first and only study that attempted to define intrarelationships in the Pleuronectidae (*sensu* Norman, 1934). This new classification has not been widely accepted (Wheeler, 1992; Rass, 1996). It is argued here that because the Pleuronectidae used by Sakamoto (1984a) was determined to be polyphyletic (Chapleau, 1993) and given the phenetic nature of the analysis, it is unlikely that the nomenclatorial revisions summarized in that work represent natural groups.

The dubious nature of this 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 require that a more comprehensive examination of pleuronectid intrarelationships be based on natural groups. A cladistic analysis based on structural variation within Pleuronectidae, in contrast with characters observed in closely related outgroups will establish an hypothesis of genealogical descent (Wiley, 1981).

Outgroup hypothesis for Pleuronectidae

Relationships within the order reveal that only the Pleuronectidae (*sensu* Chapleau and Keast, 1988) have a caudal skeletal complex, synapomorphic with taxa belonging to the Paralichthyidae, Scophthalmidae, *Brachypleura*, and Bothidae (Chapleau, 1993). 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 other taxa formerly in Pleuronectidae (Samaridae, Rhombosoleidae, and Poecilopsettidae) were placed in a clade that included the soles Achiridae, Soleidae, and Cynoglossidae (Chapleau, 1993). *Paralichthodes algoensis* was not included in Chapleau's (1993) cladistic revision but has recently been determined to be the sister lineage to these other taxa formerly in Pleuronectidae (Cooper and Chapleau, 1998). 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 for which the intrarelationships 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 structure can be used to determine the most likely candidates. It is assumed that the outgroup for the Pleuronectidae should have large symmetrical jaws and pointed teeth. Within Pleuronectiformes, the evolutionary trend for jaw structure and feeding strategy may be considered unidirectional. Symmetry of jaw and dentition found in piscivorous flatfishes, like Psettodidae and Citharidae (de Groot, 1971), is considered to be the plesiomorphic condition. The osteological characters observed in these two taxa are most similar to the generalized acanthopterygian structure (Yazdani, 1969). Taxa with symmetrical jaw structure are hypothesized to have given rise to groups with more specialized dentition types and jaw asymmetry, as observed in the Achiridae, Soleidae, and Cynoglossidae (Yazdani, 1969; Chapleau, 1993), but the reverse situation is not indicated in any study of relationships.

Within subgroups of Pleuronectiformes, the same evolutionary trend is assumed to occur. Left-eyed flounders within the bothoid lineage have large, nearly symmetrical jaws for piscivory, or a more specialized, asymmetrical jaw structure that accommodates capture of benthic prey (Yazdani, 1969; de Groot, 1971). Likewise, the Pleuronectidae contains both piscivores with nearly symmetrical jaws, *Hippoglossus stenolepis* and *Reinhardtius hippoglossoides,* as well as more specialized predators with asymmetrical jaws, such as *Glyptocephalus stelleri* (de Groot, 1971). Assuming that evolutionary trends in pleuronectid jaw structure are consistent with trends observed in the order, the ancestral pleuronectid would have near symmetrically developed jaws. The bothoid family, Paralichthyidae, appears to be one of the most plesiomorphic groups of left-eyed flounders and is chosen as the outgroup for the Pleuronectidae. In addition, *Psettodes* and *Lepidoblepharon* are also chosen as secondary outgroups. These assumptions are only valid if the Pleuronectidae is monophyletic. If Pleuronectidae is not monophyletic, then multiple outgroup taxa with either symmetrical or asymmetrical jaw structures may account for the variation observed within the Pleuronectidae.

Materials and methods

Fifty-three of 59 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 cleared and stained specimens were dissected and examined for osteological characters. Nomenclature follows the conclusions of this analysis with the previous classification of Sakamoto (1984a) indicated in

parentheses. Changes in species-group nomenclature recognize 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). Length, in mm, is standard length (Hubbs and Lagler, 1970). Radiographs for all specimens, as well as 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. *Glyptocephalus cynoglossus* (Linnæus); NMC 77-1087, 89, 114 mm. *Glyptocephalus kitaharai* (Jordan and Starks); UMMZ 141741, 139 mm. *Glyptocephalus stelleri* (Schmidt); UMMZ 159566, 125 mm. *Glyptocephalus* (=*Errex*) *zachirus* Lockington; NMC 65-0211, 95 mm; NMC 81-1027, 133 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* (Linnæus); 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* (Linnæus); 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 kitt* (Walbaum); FMNH 35527, 135 mm. *Microstomus pacificus* (Lockington). NMC 81-1027, 109 mm. *Microstomus shuntovi* Borets; not examined. *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* (Linnæus); 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* (Linnæus); 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 the Appendix. 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 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. Morphological states obtained from the literature are indicated by the citation immediately following the italic character description (Appendix). Morphological states based on meristic counts represented the modal value of the sample population. Characters at each node of the cladogram are described and numbered in order of presentation in the text.

Given the unresolved nature of the interrelationships for Pleuronectidae and other bothoid taxa within the order (Chapleau, 1993), the establishment of character polarity by 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 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 if both secondary outgroup taxa *Lepidoblepharon ophthalmolepis* and *Psettodes* sp. possessed the alternative state. One exception to this rule is stated in character 82 (Appendix). Character states hypothesized as plesiomorphic for the family are coded as zero (0).

Heuristic search methods

The matrix was analyzed with all combinations of heuristic search parameters available in PAUP 3.1.1.[1] An exhaustive search of the most parsimonious tree with this many taxa (53) would have required the analysis of an estimated 2.84×10^{82} bifurcating trees (Felsenstein, 1978) and is not possible within the current standard of computational time. For these same reasons, a branch and bound search technique proved inadequate to resolve relationships for more than 25 taxa (Forey et al., 1992). A two-step procedure (a sequential addition of taxa to produce a cladogram that minimizes homoplasy followed by the subsequent branch-swapping of this addition tree to search for more parsimonious cladograms) was used to search for the most parsimonious result. The random addition sequence in combination with all of the branch-swapping algorithms was a nonrigorous means of assessing the efficiency of the heuristic methods (Forey et al., 1992). If 50 random 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).

To minimize confounding effects of local minima, the "keep" option was used on successive searches to allow swapping on nonminimal trees (Forey et. al., 1992). Both the "MULPARS" and "swap on all trees" options were employed during each heuristic search to minimize the effect of plateau (Forey et. al., 1992). All searches assumed that the ingroup was monophyletic, and all uninformative characters were ignored. Character optimization 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 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 with 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 nonminimal trees up to 410 steps but did not resolve cladograms shorter than 403 steps. The "simple" and "closest" addition sequences were biased by taxa with large numbers of unknown character states. 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, *Reinhardtius evermanni*, *Clidoderma asperrimum*, *Hippoglossoides dubius*, *Microstomus shuntovi*, *Pleuronichthys coenosus*, and *Pseudopleuronectes obscurus* were not included in the heuristic searches. The "as is" and "simple" addition se-

[1] Swofford, D. L. 1991. PAUP Version 3.1.1. Unpublished software documentation.

Table 1

Matrix of 106 morphological characters for 5 outgroup taxa, representing the Psettodidae, Citharidae, and Paralichthyidae and 53 ingroup
Blanks represent the hypothesized ancestral state (state 0). Question marks (?) indicate an unknown state. Character states deemed not

Character number	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49
Psettodidae	
Psettodes sp.	? 1 1 na 1 na na na 1
Citharidae	
Lepidoblepharon ophthalmolepis	1 na 1 1 na na na 1 1 1
Paralichthyidae	
Citharichthys arenaceus	1 1 2 1 1 1 1 2 1 1 1
Paralichthys lethostigmus	1 1 1 1 1 1
P. squamilentus	1 1 1 1
Pleuronectidae	
Acanthopsetta nadeshnyi	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 2 1 1 1 1 1
Cleisthenes herzensteini	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 2 1 1 1 1 1 1 1 1 1 1 1
C. pinetorum	1 1 ? 1 1 1 1 1 1 1 1 ? 1 1 1 1 2 1 1 1 ? 1 1 ? ? ? 1 1 1 1
Dexistes rikuzenius	1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 2 1 1 1 1 1 1 1 1
Eopsetta grigorjewi	1 1 1 1 1 1 1 1 1 1 1 1
E. jordani	1 1 1 1 1 1 1 1 1 1 ? 1 ? ? ? ? ? ? ? ? ? 1 1 ? ? ? 1 1
Glyptocephalus cynoglossus	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1 1 1
G. kitaharai	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
G. stelleri	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1 1
G. zachirus	1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 1 1 1 1 1
Hippoglossoides elassodon	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 2 1 1 1 1 1 1 1
H. platessoides	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 2 1 1 1 1 1 1
H. robustus	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 2 1 1 1 1 1 1
Hippoglossus hippoglossus	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
H. stenolepis	1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1 1
Isopsetta isolepis	1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 2 1 1 1 1 1 1
Lepidopsetta bilineata	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 ? 1 1 1 1 1 ? 1
L. mochigarei	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 2 1 1 1 1 1 1 1
Limanda aspera	1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 2 1 1 1 1 1 1 1 2
L. ferruginea	1 1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 2 1 1 1 1 1 1 2
L. limanda	1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 2 1 1 1 1 1 1 1 2
L. proboscidea	1 1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 2 1 1 1 1 1 1 2
L. punctatissima	1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 1 1 1 1 1 1 1 2
L. sakhalinensis	1 1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 2 1 1 1 1 1 1 2
Lyopsetta exillis	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Microstomus achne	1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 1 1 1 1
M. bathybius	1 1 1 1 1 1 1 1 ? 1 ? 2 1 1 1 2 1 1 2 1 1 1 1 ? 1 1 1 1 1
M. kitt	1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1
M. pacificus	1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1
Parophrys vetula	1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 2 1 1 1 1 1 1 2
Platichthys bicoloratus	1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 1 2 2 1 1 1 1 2 1 1 3
P. flesus	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 2 1 1 1 2 2 1 1 2 1 1 3
P. stellatus	1 1 1 1 1 1 1 1 1 1 1 2 1 1 2 1 1 1 2 1 2 2 1 1 1 2 1 1 3
Pleuronectes glacialis	1 1 1 1 1 1 1 1 1 1 1 1 2 1 2 2 1 1 1 2 1 1 1 2 1 1 3
P. pinnifasciatus	1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 2 1 1 1 2 1 1 1 1 2 1 1 3
P. platessus	1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 2 2 1 1 1 2 1 1 1 2 1 1 1 3
P. putnami	1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 2 2 1 1 1 2 1 1 1 2 1 1 1 3
P. quadrituberculatus	1 1 1 1 1 1 1 1 1 1 1 1 1 1 ? 1 2 2 1 1 1 ? 1 1 ? ? ? ? 1 ? 1 1 2 ? 1 ? ? 1 1 2
Pleuronichthys cornutus	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
P. decurrens	1 1 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1
P. guttulatus	1 1 1 1 1 1 1 1 1 ? 1 1 1 1 1 1 2 1 1 1
P. ocellatus	1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1
P. ritteri	1 1 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1
P. verticalis	1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1
Psettichthys melanostictus	1 1 1 1 1 1 1 1 1 1 1 1 2 1 2 1 1 1 2 1 1 1 1 1
Pseudopleuronectes americanus	1 1 1 1 1 1 1 1 1 1 1 1 1 2 2 1 1 1 2 1 1 1 1 2 1 1 2
P. herzensteini	1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 2 1 1 1 2 1 1 1 1 1 1 2
P. schrenki	1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 2 2 1 1 1 2 1 1 1 1 2 1 1 2
P. yokohamae	1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 2 1 1 1 2 1 1 1 1 2 1 1 2
Reinhardtius hippoglossoides	1 1 1 1 1 1 1 1 1 1 1 ? 2 1 1 2 1 1 1 1 1 1
R. stomias	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Verasper moseri	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1
V. variegatus	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1 1

taxa representing the Pleuronectidae. Numbers in the matrix represent character state for each morphology as described in the Appendix. applicable due to extreme variation in morphology are coded "na."

50	51	52	53	54	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106
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Table 2

Heuristic search results for an analysis of 106 characters for 53 ingroup taxa.

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[1]	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[1]	409	48	128
random[2]	tbr	403	128	128
random[2]	spr	403	128	128
random[2]	nni	403	128	128

[1] Trees found by this method were not of minimum length and not added to the total number of trees observed.
[2] One hundred replicates for each random addition sequence.

quences using the "nearest neighbour interchange" branch-swapping were the only combinations that did not find trees with 403 steps (Table 2). The results with 100 "random" addition replicates were not different from those observed with the three other methods (Table 2). This nonrigorous test suggests that 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 calculation of mean length for all possible cladograms. However, a generalized statement of data decisiveness suggests that data for this analysis are 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 larger fraction of those actually examined during the search. Assuming that all trees of 403 steps were 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.

The majority-rule consensus of these trees (Fig. 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 at 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 characters used in the analysis. This is reflected by the low consistency index (ci=0.33) observed in the 128 most parsimonious cladograms (Rohlf, 1982).

Low consistency index is expected in studies of interrelationships for large numbers of taxa (Sanderson and Donoghue, 1989). The expected consistency index for a study of 53 taxa would be 0.14 with an equation of linear regression derived from 60 previous cladistic studies (Sanderson and Donoghue, 1989). This consistency index suggests that there is less homoplasy describing the interrelationships of the Pleuronectidae than in other studies of this size. The retention index (ri=0.79) indicates that homoplasy is occurring at terminal nodes and not internal nodes, which, in turn, suggests that relationships at the level of subfamily, tribe, and genera are not based purely 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 terminal ends, such as interrelationships among species within a genus. Consequently, the character analysis is restricted to the level of subfamily and tribe, and intragenera analysis will be explored only for relationships well corroborated by uniquely derived character states.

Monophyly of the Pleuronectidae

Ten synapomorphies define the Pleuronectidae. 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 Ocular-side frontal articulated with mesethmoid. Outgroup taxa with ocular-side prefrontal separating frontal from mesethmoid. Distribution of this structure within other pleuronectiform taxa reveals that the frontal on the ocular side is not articulated with the mesethmoid in Psettodidae, Citharidae, Paralichthyidae, Taeniopsettinae, and some genera of Bothinae (*Arnoglossus, Psettina, Asterorhombus, Japonolaeops, Laeops, Komoharaia, Neolaeops,* and *Chascanopsetta)* and is observed to be in contact with the mesethmoid only in the Bothinae genera (*Parabothus,*

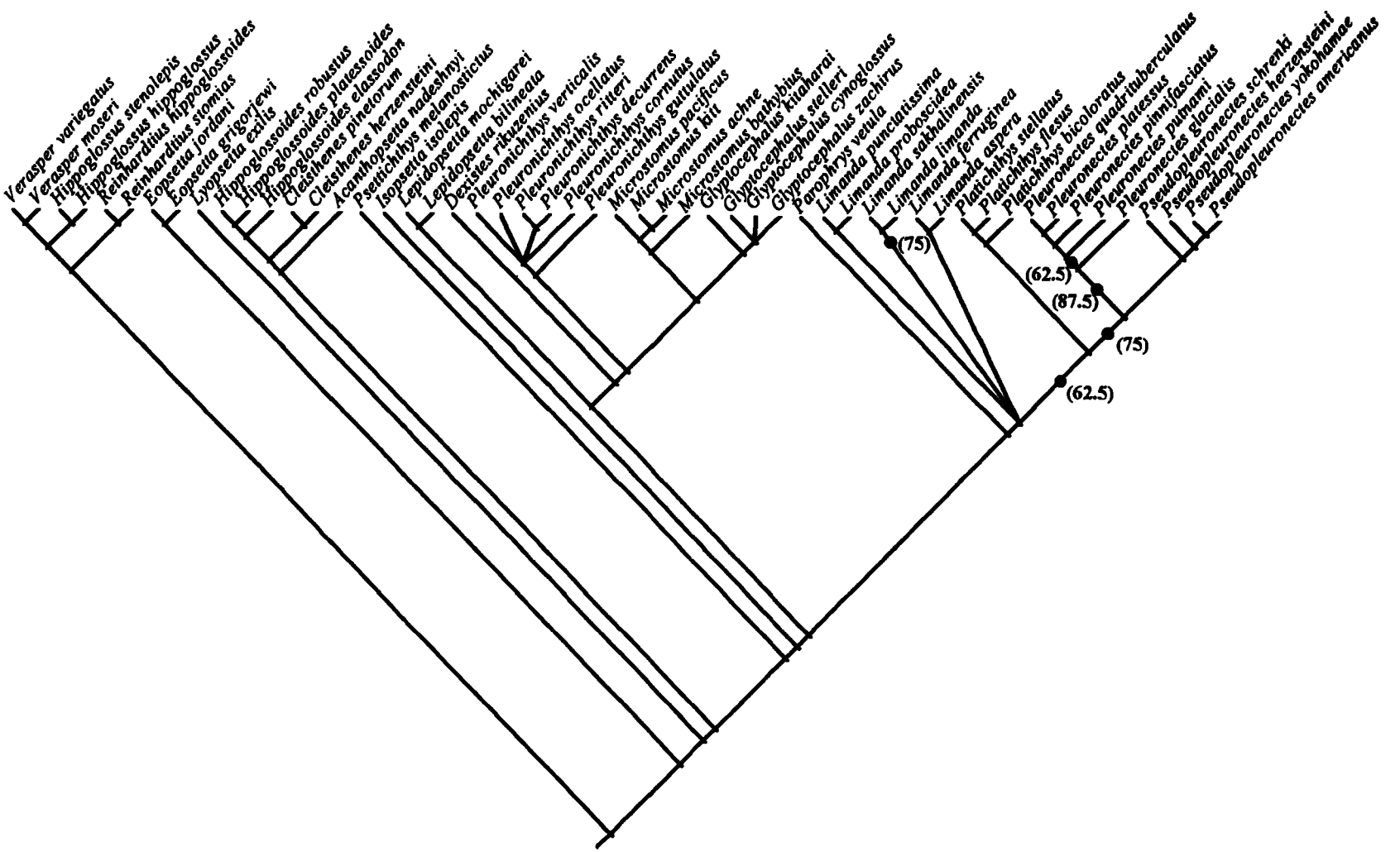

Figure 1

Fifty-percent 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.

Engyprosopon, Tosarhombus, Crossorhombus, and *Bothus* [Amaoka, 1969]).

2 Ocular-side preorbital sensory canal absent, with exceptions only in *Reinhardtius hippoglossoides* and *Acanthopsetta nadeshnyi*. In the bothoid group, as well as in the Citharidae and Psettodidae, this sensory canal is present (Amaoka, 1969).

3 Ventral margin of metapterygoid flattened (Fig. 2, B–D), with exception in *Reinhardtius stomias*, which has a distinct curvature along the ventral margin of this bone. Outgroup taxa also possess this ventral curvature of the metapterygoid (Fig. 2A).

4 First and second basibranchials loosely joined by cartilage with exceptions observed in *Eopsetta grigorjewi, Isopsetta isolepis, Limanda ferruginea, L. punctatissima, Parophrys vetula, Psettichthys melanostictus,* and *Reinhardtius stomias* in which basibranchials are sutured. Outgroup taxa also have a suture between first and second basibranchials (Fig. 3A).

5 Second and third basibranchial loosely joined by cartilage (Fig. 3, B–D) with exceptions in *Limanda punctatissima* and *Psettichthys melanostictus*. Outgroup taxa have a suture between second and third basibranchials (Fig. 3A).

6 Posteriormost abdominal vertebrae lack haemapophysis (Fig. 4C), with exceptions in *Eopsetta grigorjewi, Microstomus achne, M. kitt, M. pacificus,* and *Reinhardtius stomias* in which haemapophysis are present (Fig. 4D). Outgroups have fused parapophysis forming a haemal arch on the posteriormost abdominal vertebrae (Fig. 4, A and B).

7 Accessory processes on caudal vertebrae absent (Fig. 5, B and C), with exceptions in *Hippoglossus hippoglossus, H. stenolepis, Microstomus bathybius, Pleuronichthys decurrens, P. guttulatus,* and *P. ritteri*. Outgroups have accessory processes on ventral surface of centrum for all caudal vertebrae (Fig. 5A).

8 Ocular-side infraorbital bones present with exception in *Microstomus bathybius*. Outgroup taxa, except *Psettodes*, do not have infraorbital bones on the ocular side. *Psettodes* has four infraorbital bones on the ocular side. Presence of infraorbital bones in Pleuronectidae, although not unique in comparison with all outgroup taxa, is likely a reversal in the bothoid lineage and synapomorphic for the family.

9 Oil globules in egg absent, with exceptions of one oil globule found in *Pleuronichthys cornutus, P. guttulatus,* and *P. ritteri* (Ahlstrom et al., 1984). Outgroups have at least one oil globule (Ahlstrom et al., 1984). Distribution of this character was not confirmed in the specimens used for this analysis; however the source for this information (Ahlstrom et al., 1984) confirms this distribution in 46 of the 59 pleuronectid species.

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

Exceptions in distribution of these ten synapomorphies do not have a common phylogenetic pattern and do not corroborate exclusion of any of the 53 species examined in this analysis. The exceptions suggest independent cases of reversed characters (reversals) for either the species or their immediate ancestors, and these 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. The most parsimonious explanation places this species in the Hippoglossinae on the basis of seven additional synapomorphies. Other characters (48, 77, 83, and 104) appear to be synapomorphies for the Pleuronectidae. However, distribution of these characters was examined in only 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 character states 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 morphological characters.

Intrarelationships of the Pleuronectidae

The phylogenetic analysis reveals various monophyletic lineages within the Pleuronectidae, four of which illustrate the interrelationships of five newly defined subfamilies: Hippoglossinae, Eopsettinae, Lyopsettinae, Hippoglossoidinae, and Pleuronectinae (Fig. 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

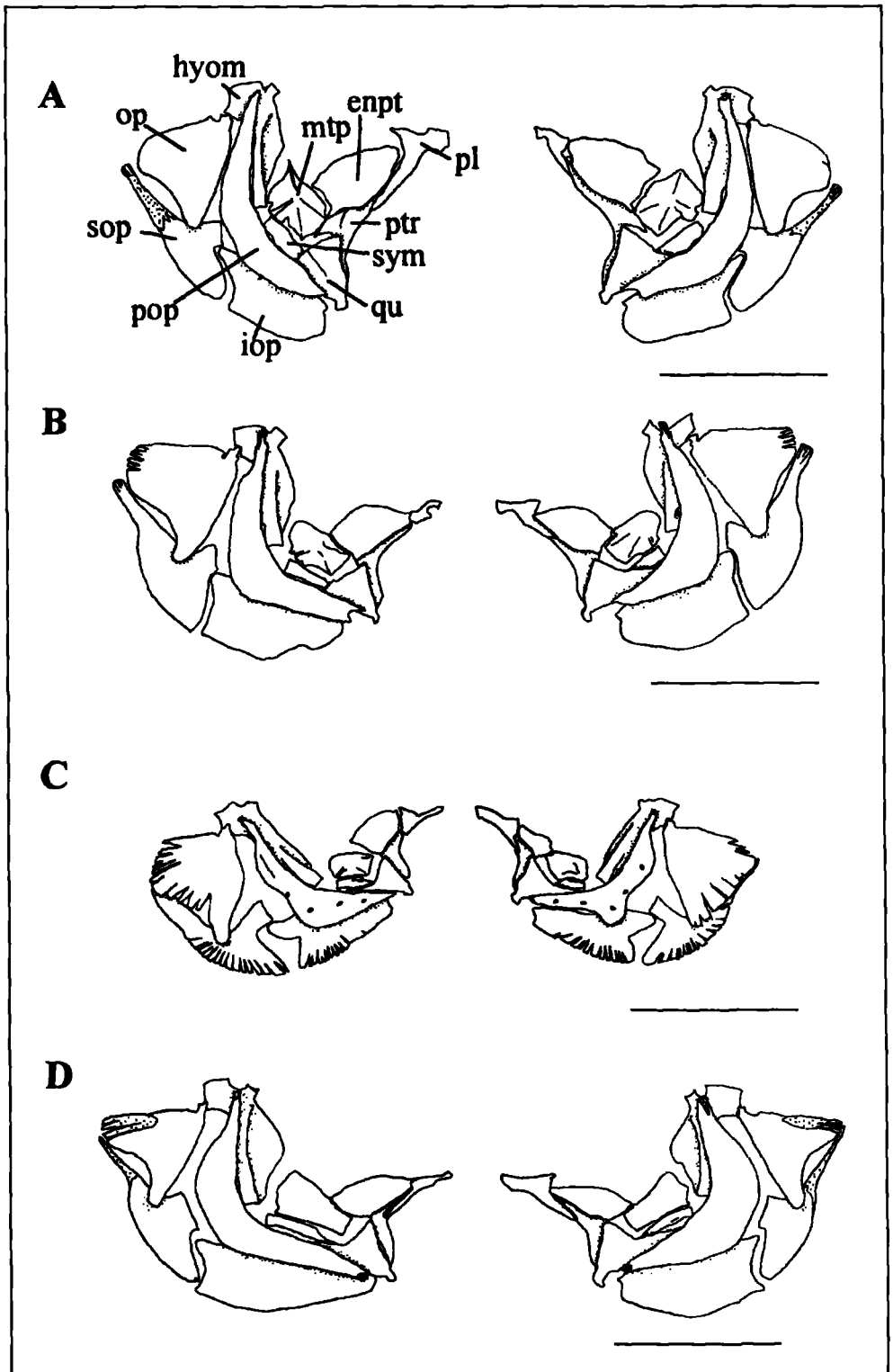

Figure 2

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 = subopercular; sym = symplectic; qu = quadrate. Scale bars are 10 mm.

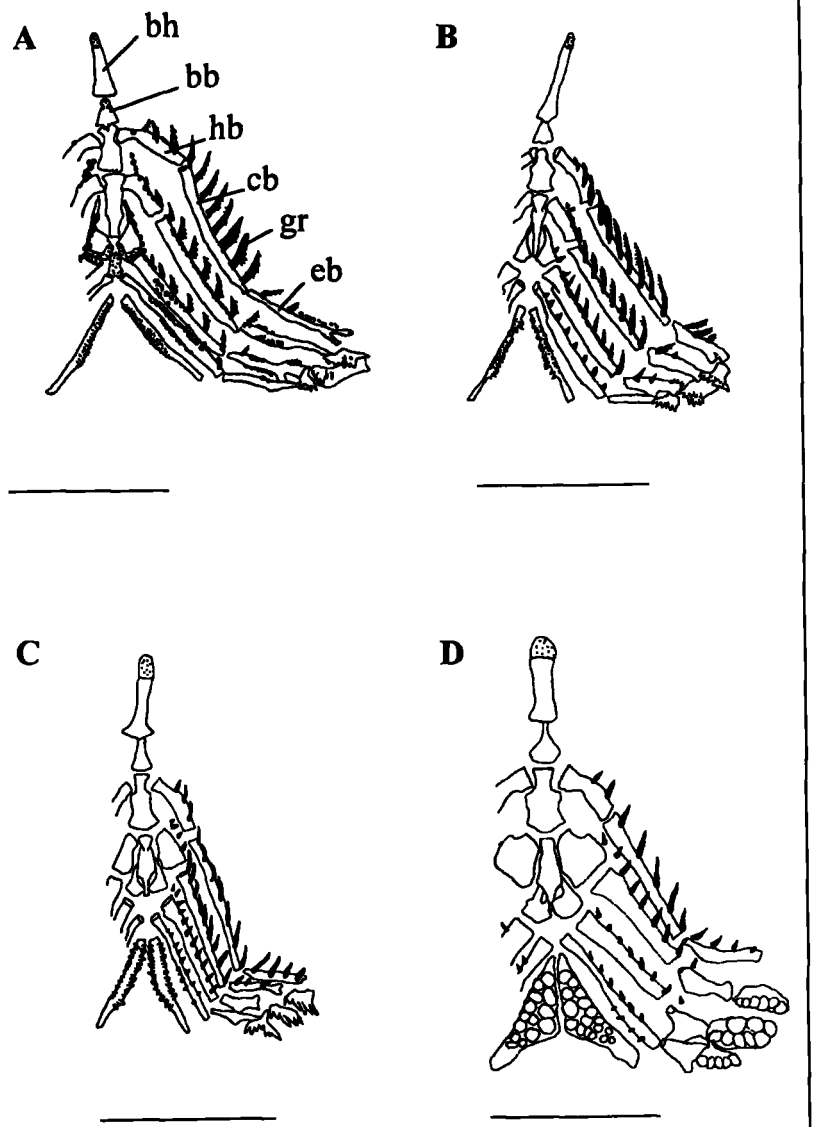

Figure 3

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; eb = epibranchial; gr = gill raker; hp = hypobranchial. The fourth basibranchial is cartilagenous and illustrated in only Fig. 3A. Scale bars are 10 mm.

character states. As a result, interrelationships within these groups are supported by few synapomorphies. Serving as successive outgroups (Stiassny and de Pinna, 1994), the position of these three subfamilies determines the polarity and relationships within the Hippoglossoidinae and the diversified Pleuronectinae.

The first lineage (I) distinguishes Hippoglossinae from all other pleuronectid taxa. The second lineage (II) contains all species classified in Eopsettinae, Lyopsettinae, Hippoglossoidinae, and Pleuronectinae. This second lineage is supported by two synapomorphies (Fig. 6): ocular-side pterosphenoid and prootic join to form the dorsal margin of anterior prootic foramen (11, Fig. 7, B and C); and first epibranchial not bifurcated at its distal end (12, Fig. 3, C and D).

Exceptions to the distribution of these two characters are observed in Pleuronectinae and *Verasper*

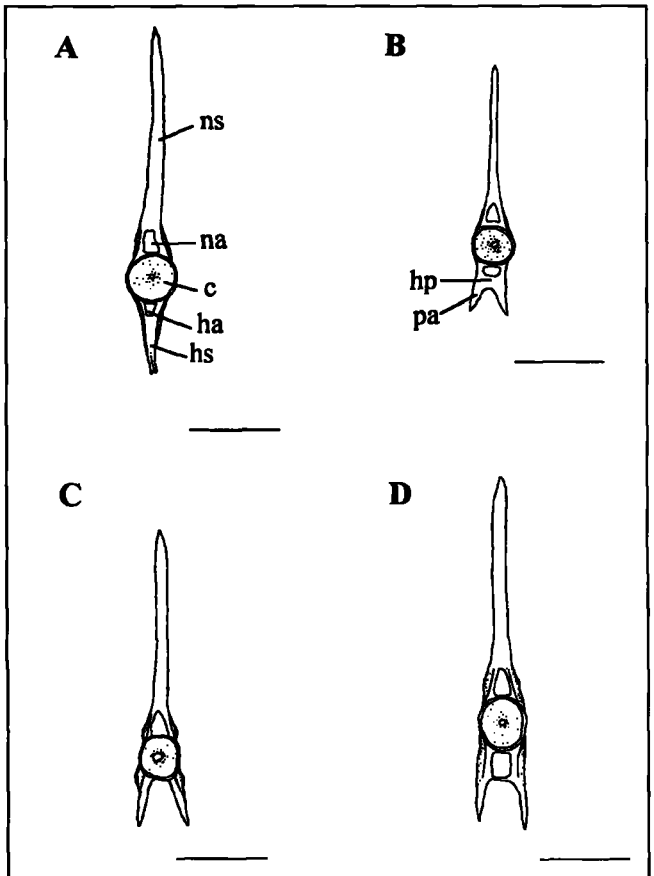

Figure 4

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.

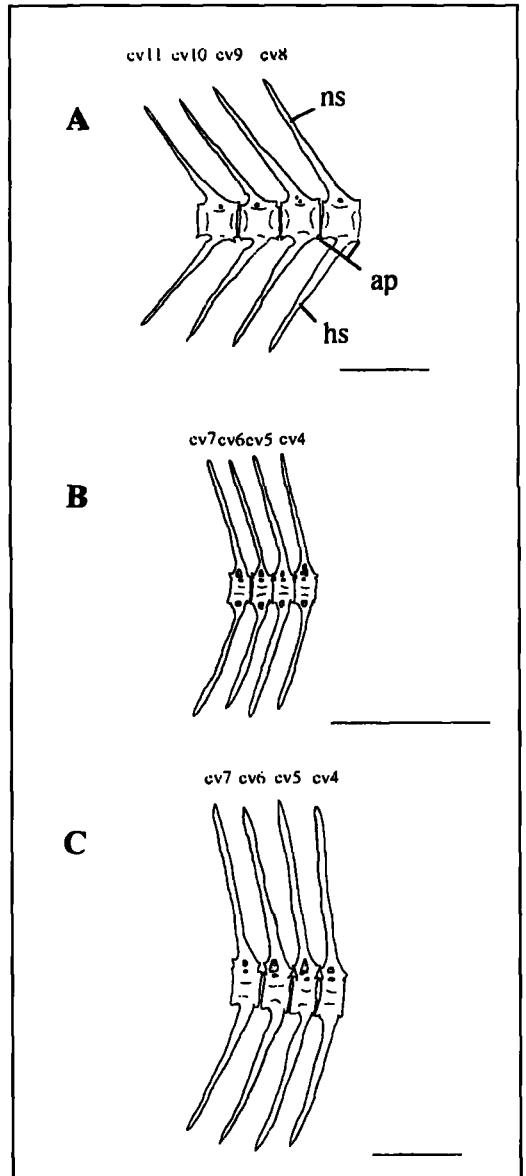

Figure 5

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.

(Hippoglossinae). The pterosphenoid and prootic do not unite to form the dorsal margin of the anterior prootic foramen (Fig. 7A) in the following pleuronectine species: *Glyptocephalus cynoglossus*, *G. stelleri*, *G. zachirus*, *Limanda ferruginea*, *L. proboscidea*, *L. punctatissima*, *Platichthys stellatus*, *Pleuronichthys guttulatus*, and *Pseudopleuronectes americanus*. *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 bifurcated in the pleuronectine species *Dexistes rikuzenius*, *Limanda aspera*, *L. limanda*, *L. sakhalinensis*, *Glyptocephalus*, *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. The pattern does suggest that these exceptions are instances of reversal or convergence and may determine phylogeny within these other groups.

The third pleuronectid lineage (III) indicates a common ancestor for Lyopsettinae, Hippoglossoidinae,

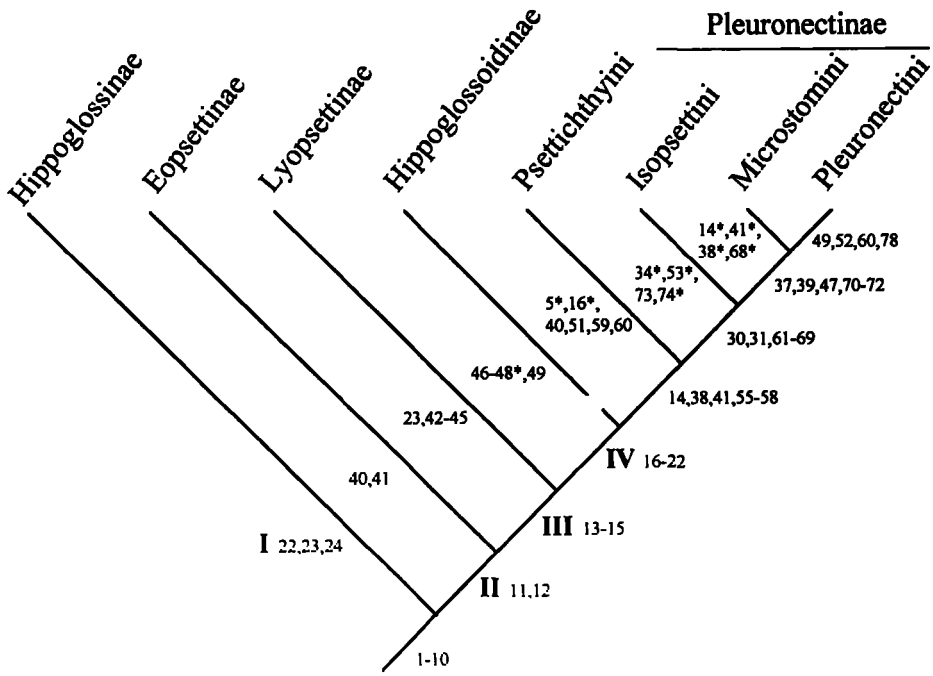

Figure 6

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

and Pleuronectinae. Three synapomorphies defining this lineage (Fig. 6) are spines absent on gill rakers (13, Fig. 3, C and D); anterior margin of upper orbit complete with an overlap between mesethmoid and prefrontal of the blind side (14, Fig. 8, C and D); and first anal pterygiophore broadly thickened (15).

Exceptions to the distribution in these synapomorphies are found in Pleuronectinae and Hippoglossinae. The anterior margin of the upper orbit is incomplete in *Microstomus achne, M. bathybius, M. kitt, M. pacificus, Pleuronectes pinnifasciatus, Pseudopleuronectes americanus, P. herzensteini*, and *P. yokohamae. Reinhardtius stomias,* which also has the derived state for this character, is excluded from this third lineage. The first anal pterygiophore is not thickened in *Microstomus achne, M. bathybius, M. kitt*, and *M. pacificus*, whereas in *Hippoglossus* and *Verasper* (Hippoglossinae) the first anal pterygiophore is broadly thickened.

The fourth lineage (IV) includes all species of Hippoglossoidinae and Pleuronectinae. The sister relationship between these two subfamilies is determined by seven synapomorphies (Fig. 6): dentition of uniform size (16, Fig. 9, B–D); interorbital process reduced or completely absent (17, Fig. 10, B, D, and E); hyomandibula broadened anteriorly (18, Fig. 2D); dentition on third epibranchial absent (19, Fig. 3, C and D); bony plates absent on branchial arches (20, Fig. 3, C and D); two rows of teeth present on fourth ceratobranchial (21, Fig. 3, C and D); and dorsoposterior margin of operculum fimbriated (22, Fig. 2, B–D).

Exceptions to these character distributions are found in only two species of *Hippoglossoides* and in Microstomini. *Hippoglossoides platessoides* and *H. robustus* have larger anterior teeth (16) that were historically termed as "canines" (Norman, 1934). *Glyptocephalus kitaharai, G. zachirus, Microstomus achne, M. kitt, M. pacificus*, and all species in *Pleuronichthys* have an interorbital process (17, Fig. 10C). The anterior margin of the hyomandibular (18) is not broadened in *Glyptocephalus* and *Microstomus* (Fig. 2C). Dentition on the third epibranchial and bony plates on branchial arches are observed in *Pleuronichthys guttulatus*. The number of rows of teeth on the fourth ceratobranchial are reduced to only one in *Glyptocephalus, Platichthys bicoloratus*, and *Pleuronichthys* and are absent in *Limanda punctatissima*. Fimbriation of the operculum (22) is also observed in Hippoglossinae (Fig. 2B) but is absent in the Eopsettinae and Lyopsettinae. This last exception suggests that fimbriation of the operculum may be synapomorphic for Pleuronectidae because only *Eopsetta grigorjewi, E. jordani*, and *Lyopsetta*

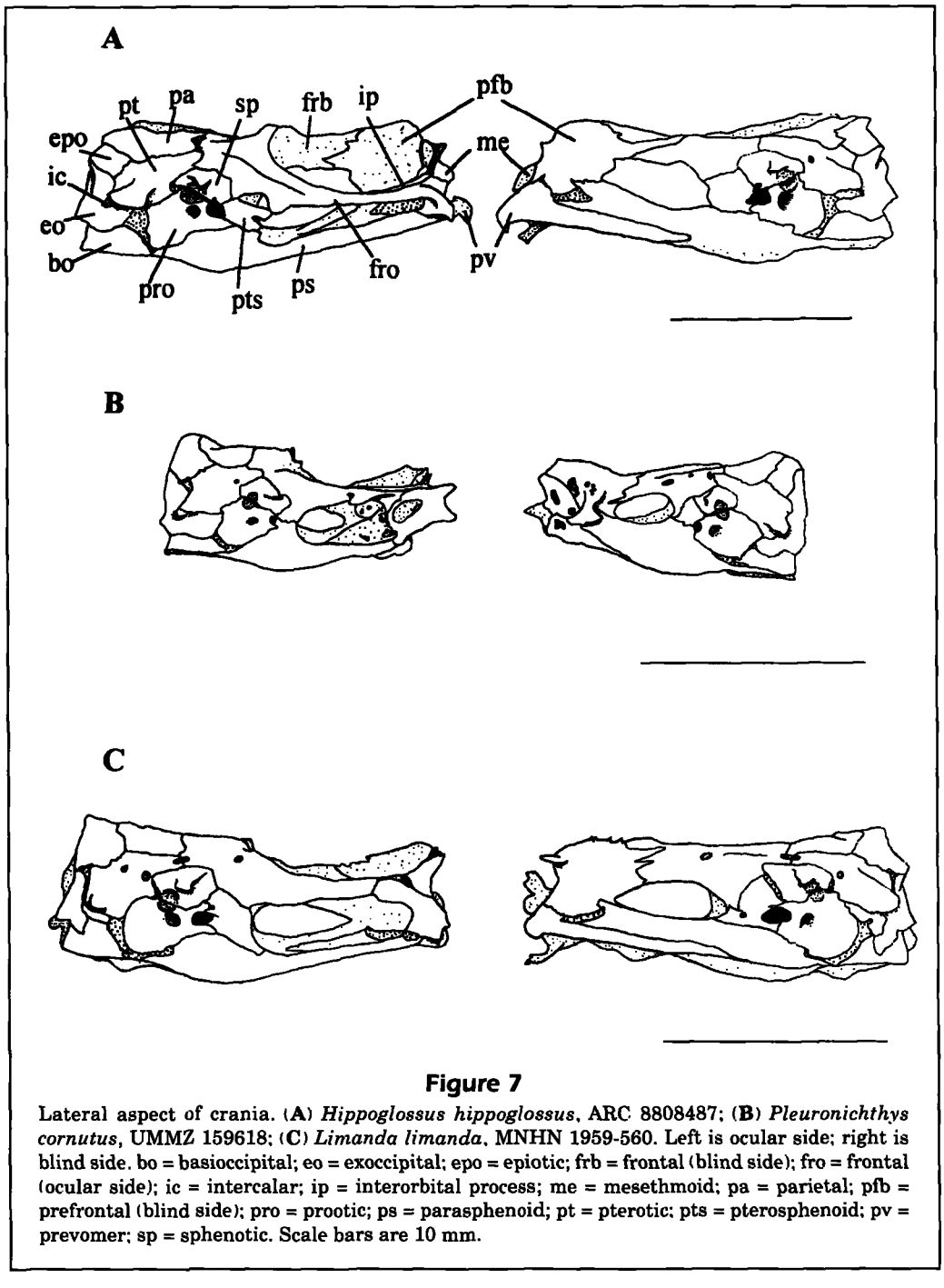

Figure 7

Lateral aspect of crania. (**A**) *Hippoglossus hippoglossus*, ARC 8808487; (**B**) *Pleuronichthys cornutus*, UMMZ 159618; (**C**) *Limanda limanda*, MNHN 1959-560. Left is ocular side; right is 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.

exilis do not show this condition. This alternative hypothesis would require three evolutionary steps, one more than is presently hypothesized. 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 Hippoglossinae, with eight species (6 examined) in four genera: *Clidoderma* (*incertae sedis*), *Hippoglossus*, *Reinhardtius*, and *Verasper* (Fig. 11). This subfamily, as well as the intrarelationships of its species, were observed in all of the most parsimonious cladograms. The Hippoglossinae are hypothesized to be monophyletic ac-

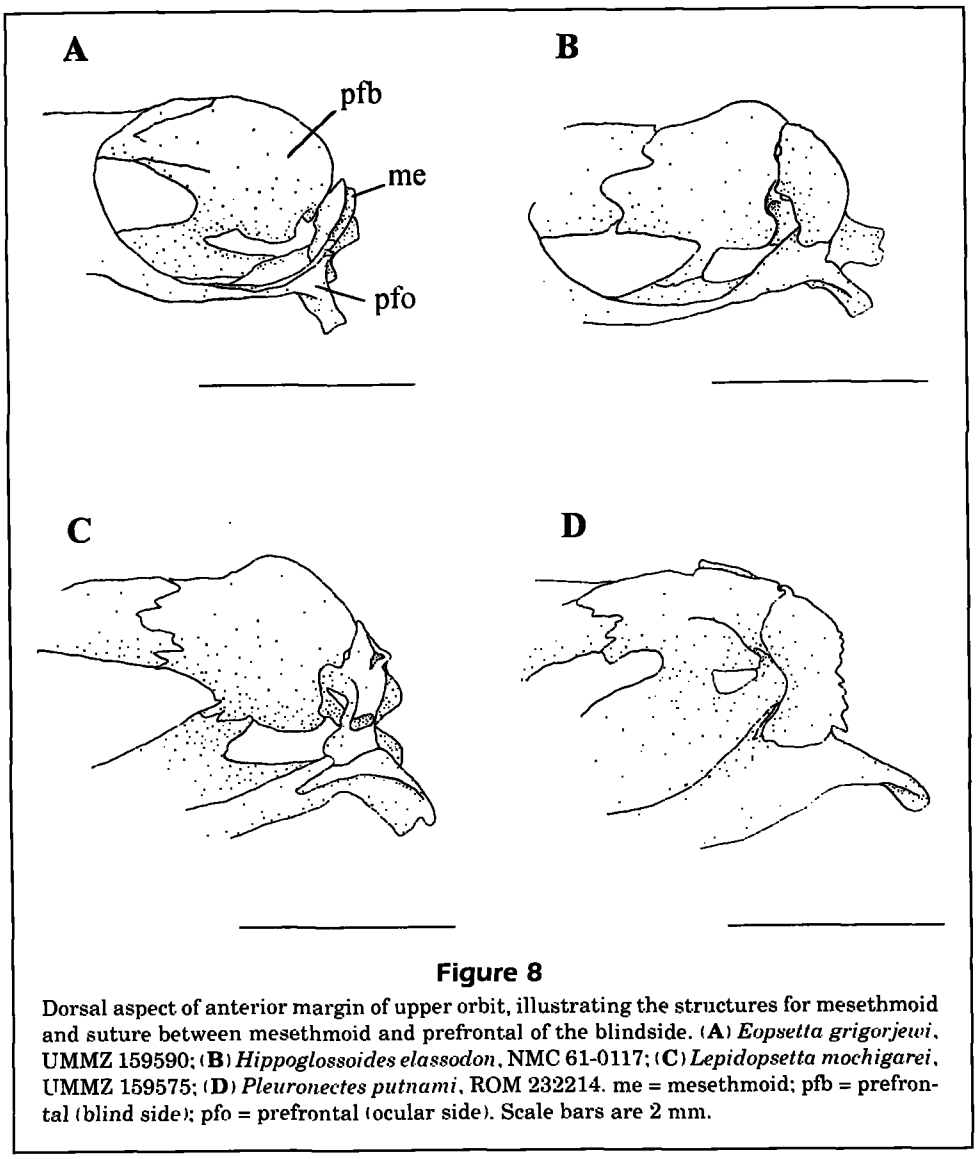

Figure 8

Dorsal aspect of anterior margin of upper orbit, illustrating the structures for mesethmoid and suture between mesethmoid and prefrontal of the blindside. (**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). Scale bars are 2 mm.

cording to three synapomorphies (Fig. 6): increase in number of abdominal vertebrae to more than 12 (23); lunate-shaped caudal fin (24); and presence of fimbriation on dorsoposterior opercular margin (22, Fig. 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 in *Eopsetta* and *Lyopsetta* that comprise 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 hypothesized for *Verasper*. Monophyletic status of this clade is suspect. The low proportion of derived character states inherent for basal lineages provides little support for monophyly and intrarelationships for these taxa. Analysis of morphological characters 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 intrarelationships.

Intrarelationships of Hippoglossinae

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

Homoplasy observed for these characters is observed in unrelated pleuronectid taxa and does not

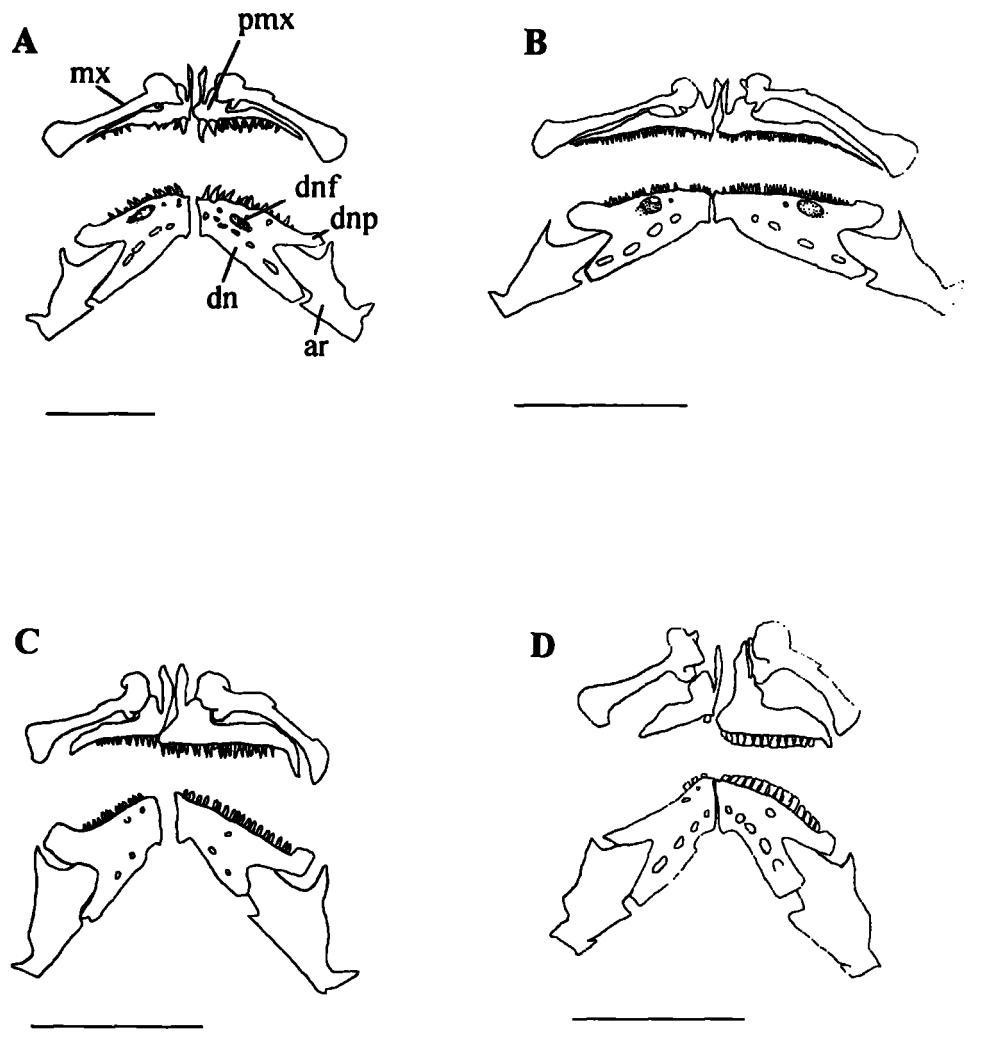

Figure 9

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.

corroborate an alternative hypothesis. The absence of gill rakers on fourth ceratobranchial is observed in *Limanda punctatissima* (Pleuronectinae). The position of the migrated eye in relation to the dorsal midline is a reversal also observed in *Cleisthenes pinetorum* (Hippoglossoidinae). An increase in the number of caudal vertebrae is also observed in *Glyptocephalus* and *Microstomus*, a clade of nine species whose monophyletic status is strongly supported within the Pleuronectinae. Absence of the dentary foramen on the blind side is only observed in four other pleuronectid species including *Hippoglossus hippoglossus*.

Reinhardtius evermanni was not examined, but morphological characters reported in the literature suggest monophyly for *R. evermanni*, *R. hippoglossoides*, and *R. stomias*. *Reinhardtius evermanni* also has an increased number of caudal vertebrae, ranging from 37 to 40 (Sakamoto, 1984a). *Reinhardtius evermanni* and *R. stomias* share two unique structures: olfactory lamellae that radiate from a central rachis; and jaw and pharyngeal teeth with barbed tips (Norman, 1934). If these structures are considered synapomorphic within the genus, then *Reinhardtius evermanni* and *R. stomias* are sister species, with *R. hippoglossoides* immediately basal to them.

The sister relationship between *Hippoglossus* and *Verasper* is supported by six synapomorphies (Fig. 11):

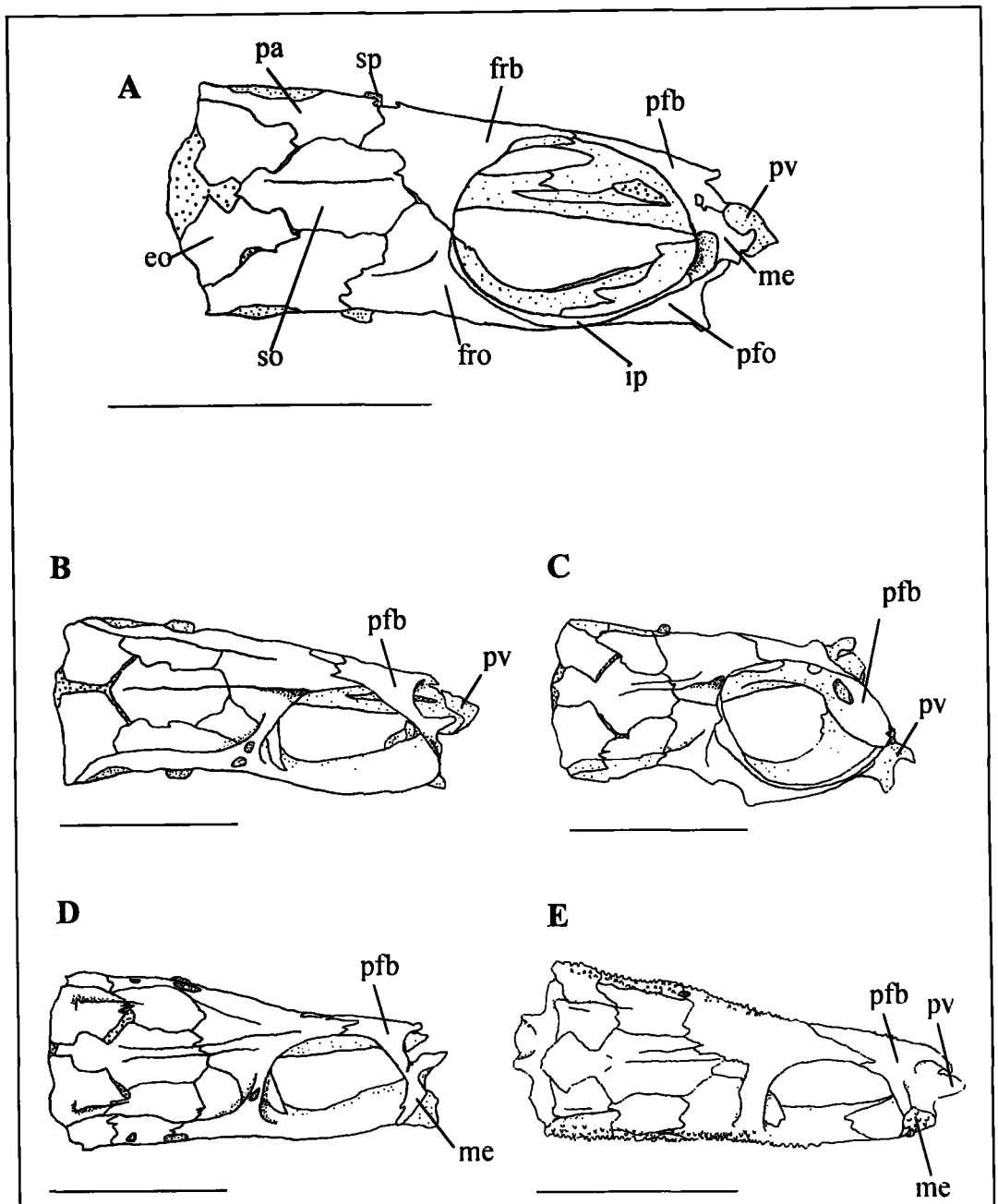

Figure 10

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*, HUMZ 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.

first anal pterygiophore broadly thickened (15); ocular-side palatine reduced and not articulated with pterygoid (28, Fig. 2B); gill rakers on first epibranchial absent (29) with exception in *Hippoglossus stenolepis*; gill rakers on second and third epibranchials (30, 31) reduced (*Hippoglossus*) or absent (*Verasper*); and gill rakers on first hypobranchial reduced (32).

These states are homoplastic in other lineages of the Pleuronectidae. Thickening of the first anal pterygiophore (15) unites taxa in the third pleuronectid lineage. Reduction of the palatine on the ocu-

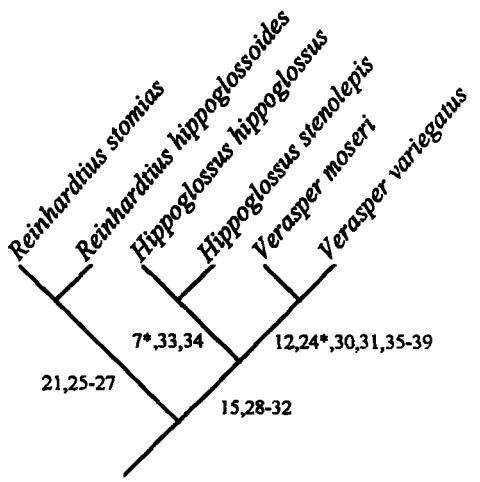

Figure 11
Intrarelationships of Hippoglossinae. Numbers correspond to characters in text. Asterisk represents character state reversal.

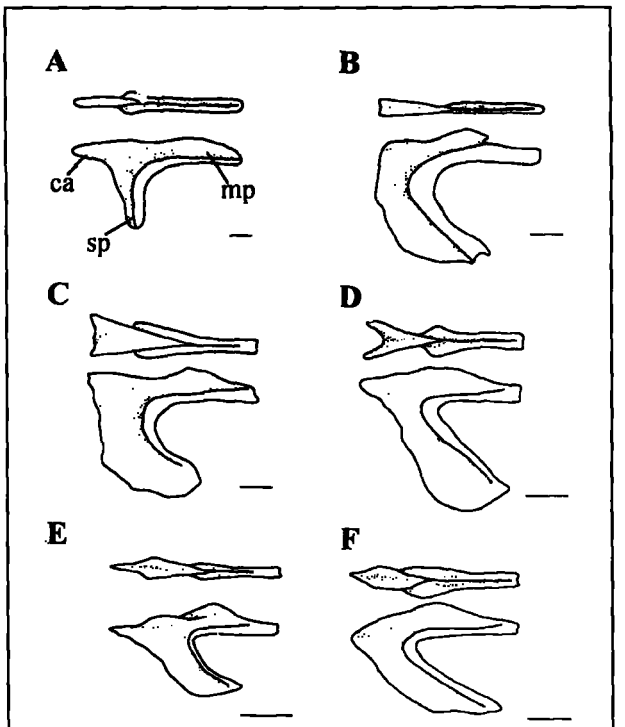

Figure 12
Dorsal (above) and lateral (below) aspects of urohyal (modified from Sakamoto, 1984a). (**A**) *Reinhardtius hippoglossoides* (**B**) *Hippoglossus stenolepis* (**C**) *Verasper variegatus* (**D**) *Pseudopleuronectes herzensteini* (**E**) *Glyptocephalus kitaharai* (**F**) *Dexistes rikuzenius*.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

lar side (28, Fig. 2, B and C) also defines the lineage uniting *Glyptocephalus*, *Microstomus*, and *Pleuronichthys* within the Pleuronectinae. Absence or reduction of gill rakers on first, second, and third epibranchials (29, 30, 31) is found to define basal lineages within the Pleuronectinae, whereas reduction of gill rakers on the first hypobranchial is observed in only some species of *Pleuronichthys* and *Platichthys bicoloratus*.

Genus *Hippoglossus* This genus contains two species: *H. hippoglossus* and *H. stenolepis* and is monophyletic with three synapomorphies (Fig. 11): presence of subdivisions in hypural and parahypural plates, an autapomorphy for the genus (33); presence of accessory processes on ventral margin of caudal vertebrae (7, Fig. 5A), a reversal for the family; and metapterygoid articulated with the blind-side entopterygoid (34, Fig. 2C). Only *Microstomus bathybius* and three species of *Pleuronichthys* 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 *V. moseri* and *V. variegatus* is monophyletic with nine synapomorphies (Fig. 11): presence of a large foramen between mesethmoid and blind-side prefrontal (35), autapomorphic for *Verasper*; first epibranchial bifurcated (12); caudal fin is rounded (24), a reversal in this subfamily; gill rakers absent on both second and third epibranchials (30, 31); gill rakers reduced on second hypobranchial (36); sphenotic process not forming dorsal margin of hyomandibular socket (37, Fig. 7, B and C); groove present along supraoccipital crest for insertion of pterygiophores (38, Fig. 10, C–E); and cardiac apophysis of urohyal bifurcated (39, Fig. 12C).

Many character states found within *Verasper* are also observed in the Pleuronectinae, but the strength of the hypothesis placing *Verasper* within Hippoglossinae exceeds the characters mentioned above and illustrates the convergent evolution of these structures.

Genus *Clidoderma* *C. asperrimum* was not available for 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 appears to be more closely related to *Verasper* than to *Platichthys* (Norman, 1934). This species has subsymmetrical jaws and a mix of pointed and bluntly conical teeth, not uniform in length, set in multiple rows on both upper and lower jaws. These features are plesiomorphic for the fam-

ily and only exclude *Clidoderma* from the fourth pleuronectid lineage. This species has 14 abdominal vertebrae found to be synapomorphic for Hippoglossinae; it also has a thickened first anal pterygiophore suggesting a common ancestry with *Hippoglossus* and *Verasper*. A rounded caudal fin, observed in *Verasper* is also observed in *Clidoderma*. Phylogenetic position of this species could be further ascertained by an examination of the accessory processes on caudal vertebrae, the palatine structure, and the structure of gill rakers on first, second, and third epibranchials.

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

Genus *Eopsetta* This genus was described in Norman (1934) by a number of plesiomorphic characters. The presence of distinct canines on the upper jaw was suggested in Norman (1934) as diagnostic for *Eopsetta*. However, distribution of this character in the Pleuronectidae is not well defined. Members of the Hippoglossinae also have teeth of irregular lengths (16, Fig. 9A). The longer teeth can also be interpreted as canines in these species. From this analysis, data supporting monophyly of *Eopsetta* are not conclusive, but no other interpretation is available owing to insufficient information.

Subfamily Lyopsettinae

Genus *Lyopsetta* This lineage contains only *L. exilis*. Its position as a monotypic lineage within the Pleuronectidae is determined by five derived character states (Fig. 6): 12 to 14 abdominal vertebrae (23); barbed teeth present on dentaries and premaxillae (42); supratemporals on both ocular and blind sides are jointed at anterior ends of their bifurcation (43, 44, Fig. 13B); and presence of scales on eye surfaces (45).

These structures 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*. Bifurcation of the supratemporals is also observed in *Reinhardtius hippoglossoides*, *Cleisthenes*, *Hippoglossoides*, *Glyptocephalus cynoglossus*, *G. stelleri*, *G. zachirus*, *Microstomus pacificus*, *Limanda aspera*, and *Pleuronectes platessus*. Presence of scales on the eye surfaces is also found in *Reinhardtius stomias*, *Acanthopsetta nadeshnyi*, *Dexistes rikuzenius*, *Glyptocephalus kitaharai*, and *Microstomus*. The distribution of these character states within the family does not indicate an alternative hypothesis of relationships between *Lyopsetta exilis* and other pleuronectid taxa.

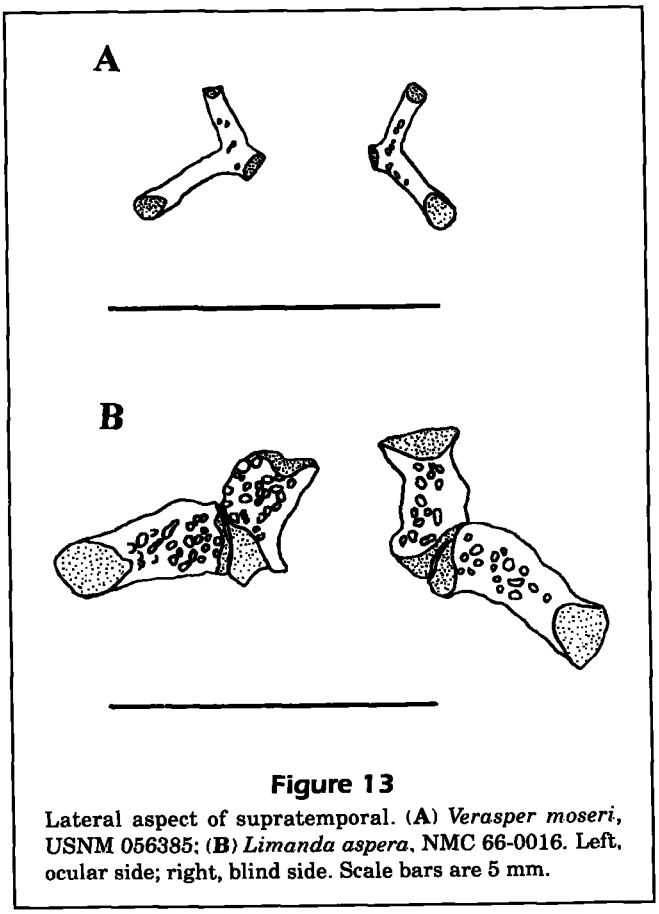

Figure 13
Lateral aspect of supratemporal. (**A**) *Verasper moseri*, USNM 056385; (**B**) *Limanda aspera*, NMC 66-0016. Left, ocular side; right, blind side. Scale bars are 5 mm.

Subfamily Hippoglossoidinae This subfamily contains seven species (6 examined) in three genera: *Acanthopsetta, Cleisthenes,* and *Hippoglossoides*. This group is characterized by four synapomorphies (Fig. 6): absence of supraoccipital plate extending posteroventrally between epiotics (46, Fig. 10B); pterosphenoid and prootic of blind side join to form dorsal margin of anterior prootic foramen (47, Fig. 7, B and C); pterosphenoid of blind side is reduced and does not form posterior margin of orbit (48, Fig. 7B), a reversal, except in *Cleisthenes;* and two uniform rows of teeth present on fifth ceratobranchial (49).

Distribution of these states within other pleuronectid taxa indicates a degree of homoplasy for these characters but does not refute the monophyly of Hippoglossoidinae. The supraoccipital plate is also absent in *Microstomus* (Microstomini). 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. Reduction of the blind-side pterosphenoid is also observed in *Pleuronichthys* (Microstomini) and may be a reversal within the Pleuronectidae. Two rows of uniform teeth are also present in *Dexistes rikuzenius* (Microstomini) and the Pleuronectini.

Intrarelationships of Hippoglossoidinae

Genus *Acanthopsetta* *A. nadeshnyi* is the sister species to *Cleisthenes* and *Hippoglossoides* (Fig. 14). Placement of *A. nadeshnyi* as a distinct lineage within Hippoglossoidinae is supported by two structures not present in other Hippoglossoidinae: sensory canal on ocular-side preorbital present (2); and presence of scales on eye surfaces (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 have a more homoplastic distribution as they are found in *Reinhardtius stomias, Lyopsetta exilis, Dexistes rikuzenius, Glyptocephalus kitaharai*, and *Microstomus*.

The sister relationship between *Cleisthenes* and *Hippoglossoides* is supported by three synapomorphies (Fig. 14): ocular-side supratemporal jointed at anterior end of bifurcation (43, Fig. 13B); nasal bones of the blind side are absent (50); and more than seven infraorbital bones on ocular side (51).

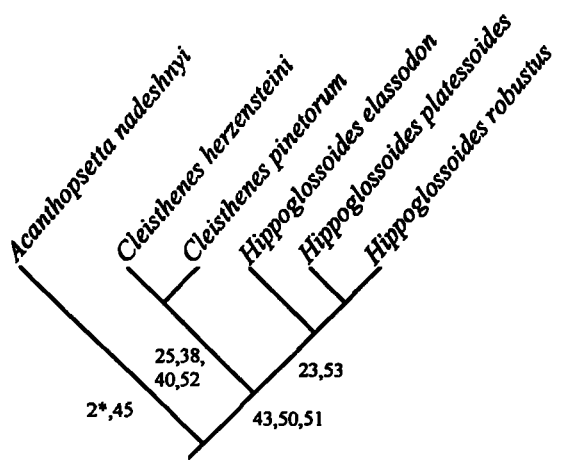

Figure 14

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

These morphological states are not unique within the family. The bifurcation of the supratemporal is observed in *Lyopsetta exilis* on both ocular and blind side, 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 some species in the Pleuronectinae, the latter being also observed in *Hippoglossus stenolepis*.

Genus *Cleisthenes* This genus contains two species, *C. herzensteini* and *C. pinetorum*, and is diagnosed by four synapomorphies (Fig. 14): migrated eye is near dorsal midline (25); gill rakers on fourth epibranchial present (40); a double crest or groove present on supraoccipital and blind-side frontal (38, Fig. 10, C–E); and crest extending from supraoccipital to blind side reduced (52, Fig. 10, D and E).

These morphological characters are distributed within other pleuronectid taxa but are not observed in any other Hippoglossoidinae. The position of the migrated eye is also observed in *Reinhardtius*. Presence of gill rakers on the fourth epibranchial 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 Pleuronectini.

Genus *Hippoglossoides* This genus contains 4 species: *H. dubius* (not examined), *H. elassodon, H. platessoides*, and *H. robustus*, defined by two synapomorphies (Fig. 14). The structure of the anterior margin of the mesethmoid is complex but consistent in *Hippoglossoides*. In this genus, the "thin plate" structure of the mesethmoid (53, Fig. 8B) is distinct in relation to other members of Hippoglossoidinae who have a thickened triangular-shaped mesethmoid (Fig. 8C), or the plesiomorphic structure of an open canal found in *Cleisthenes pinetorum* (Fig. 8A). In addition, there are 12 to 14 abdominal vertebrae (23), an increase from 11 or fewer in other taxa. 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 Pleuronectinae is the largest subfamily within the Pleuronectidae with 40 species (38 examined in

this analysis). Seven synapomorphies define this group as monophyletic (Fig. 6): absence of a dentary fossa (55, Fig. 9, C and D); and absence of ceratohyal foramen (56) are both autapomorphic for Pleuronectinae; mesethmoid and blind-side prefrontal are sutured but without a foramen between these bones (14, Fig. 8D); a double supraoccipital crest forms a groove for insertion of anterior dorsal-fin pterygiophores (38, Fig. 10, C–E); a single row of teeth on lower jaw (41); intercalar in contact with basioccipital (57, Fig. 7C); and presence of a posterior extension of supratemporal branch of the lateral line (58).

Some of these character states are not found in all pleuronectines, but the occurrence of these states 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 hypothesized to arise at this node with two secondary losses observed in more advanced lineages within Microstomini and Pleuronectini.

Intrarelationships of Pleuronectinae The Pleuronectinae is classified into 4 tribes: Psettichthyini, Isopsettini, Microstomini, and Pleuronectini (Fig. 6). Branchial structure and characters associated with jaw asymmetry determine the interrelationships of these tribes.

Tribe Psettichthyini Genus *Psettichthys* Monotypic with only *P. melanostictus*, this lineage is unique within the Pleuronectinae, having six distinct characters (Fig. 6): dorsal fin rays are elongated beyond dorsal-fin membrane, an autapomorphy for the species (59); second and third basibranchials are sutured (5, Fig. 3A), a reversal within Pleuronectidae; teeth are not uniform in length (16); greater than seven infraorbital bones (51); gill rakers on fourth epibranchial present (40); and one row of teeth on upper jaw (60).

These morphological states are shared with taxa both within and prior to the Pleuronectinae. This evidence clearly positions Psettichthyini as a basal tribe of the Pleuronectinae. A suture between the second and third basibranchials is observed elsewhere only in *Limanda punctatissima*. Tooth length is uniform in other pleuronectine taxa. An increase in infraorbital number is observed in two other pleuronectine species, *Lepidopsetta bilineata* and *Pleuronichthys decurrens*, as well as in *Cleisthenes, Hippoglossoides*, and *Hippoglossus stenolepis*. The presence of gill rakers on the fourth epibranchial is found elsewhere only in *Cleisthenes* and *Eopsetta*. A single row of teeth on the upper jaw is observed in all species of the Pleuronectini, and in *Glyptocephalus* and *Microstomus*.

The second lineage contains the newly defined tribes Isopsettini, Microstomini, and Pleuronectini. Taxa within this lineage are characterized by 11 synapomorphies (Fig. 6): one gill raker at proximal base of second and third epibranchials (30, 31, Fig. 3D); blind-side premaxilla protruding past the sagittal axis at its symphysis with that of ocular side (61, Fig. 9, C and D); ocular-side premaxilla much longer than that of blind side (62, Fig. 9, C and D); ventral posterior curvature on blind-side premaxilla is present (63, Fig. 9, C and D); asymmetry in space between dentary and articular such that blind-side space is larger than on ocular side (64, Fig. 9, C and D); dorsoposterior process of ocular-side dentary larger than its blind-side counterpart (65, Fig. 9C); teeth on both ocular-side premaxilla and dentary reduced (66, 67, Fig. 9, C and D); epiotic processes present (68, Fig. 10, D and E); and ocular-side entopterygoid larger than that of blind side (69, Fig. 2, C and D).

Distribution of these character states is not without exceptions or homoplasies. Reduction of gill rakers on the second epibranchial was not observed in *Limanda* (Fig. 3C), 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 (Fig. 6): within this lineage there is an evolution of dentition, from pointed or bluntly conical teeth to incisorlike or even molariform teeth with uniform cutting edges (70); sphenotic process positioned high on sphenotic (37, Fig. 7, B and C); urohyal with strongly bifurcate cardiac apophysis (39, Fig. 12, D–F); blind-side pterosphenoid and prootic form dorsal margin of anterior prootic foramen (47, Fig. 7, B and C); medial margin of fifth ceratobranchial slightly curved (71, Fig. 3C); and teeth on fifth ceratobranchial bluntly pointed (72, Fig. 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 *Glyptocephalus*. A strongly bifurcated cardiac apophysis on the urohyal was not found in *Limanda punctatissima, Pleuronichthys ritteri, P. ocellatus*, and *Parophrys vetula*. These two morphological characters are homoplastic in *Verasper* (Hippoglossinae). The sphenotic forms the dorsal margin of the anterior prootic foramen on the blind side (47, Fig. 7A) in *Limanda ferruginea, L. proboscidea, Microstomus achne, M. kitt, Glypto-*

cephalus cynoglossus, G. stelleri, G. zachirus, 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, Glyptocephalus*, and *Pleuronichthys,* and 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 (Fig. 6): blind-side metapterygoid is not articulated with entopterygoid (34, Fig. 2A); anterior margin of mesethmoid forming an open canal (53, Fig. 8A); haemal spines of anteriormost caudal vertebrae broadly attached to centrum (73, Fig. 5B); and epiotics are sutured along dorsal posterior margin of skull (74).

Three of these four character states are reversals within the Pleuronectinae. The absence of an articulation between the blind-side metapterygoid and entopterygoid is a reversal of the structure observed in the 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, *Glyptocephalus, Microstomus*, and *Pleuronichthys* (except *P. verticalis*), and in *Cleisthenes pinetorum* (Hippoglossoidinae). Epiotics sutured along the dorsal posterior margin are observed only in one other pleuronectid species, *Microstomus pacificus*, and the broad attachment of haemal spines to the anteriormost caudal vertebrae is a reversal recurrent throughout the family, indicating a homoplastic structure with a complex distribution. Despite the reversals noted for this lineage, placement of Isopsettini within Pleuronectinae is supported by the eight synapomorphies for Pleuronectinae and the 11 derived morphological characters for the second lineage in Pleuronectinae.

Tribe Microstomini The tribe Microstomini contains 19 species (17 examined) classified in five genera: *Lepidopsetta, Dexistes, Pleuronichthys, Glyptocephalus*, and *Microstomus* (Fig. 15). Although the placement of this tribe within the Pleuronectinae is supported by 25 character transformations presented for monophyly and intrarelationships of the subfamily, the status of this tribe, as well as its intrarelationships, are determined mostly by character reversals. The monophyly of this tribe is characterized by four character transformations. All are reversals within the Pleuronectinae (Fig. 6): suture between mesethmoid and blind-side prefrontal is either incomplete or complete with a small foramen present (14, Fig. 8, A–C); single crest on supraoccipital (38, Fig. 10, A and B); lower jaw with multiple rows of teeth (41); and reduced or absent process on dorsoposterior edge of epiotics (68, Fig. 10, A–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 structures. Multiple rows of teeth on the lower jaw were not observed in *Microstomus* and *Glyptocephalus,* which is homoplastic within Hippoglossinae and recurrent for basal lineages within the family but which has only this one instance of reversal in Pleuronectinae. The absence or reduction of an epiotic crest is a reversal also found in *Pseudopleuronectes herzensteini* and *P. yokohamae.*

Intrarelationships of Microstomini Three lineages of Microstomini are defined by 16 character transformations; ten are reversals (Fig. 15).

Genus *Lepidopsetta* The first lineage of Microstomini, consists of *Lepidopsetta* containing two species, *L. bilineata* and *L. mochigarei* (Fig. 15). This genus is diagnosed by the presence of demersal eggs (75), a feature observed in only four other pleuronectid species: *Pseudopleuronectes americanus, P. schrenki, P. yokohamae*, and *P. obscurus* (not examined).

The second lineage indicates common descent for *Dexistes, Pleuronichthys, Microstomus*, and *Glyptocephalus*. Four character states unite these genera (Fig. 15): first epibranchial bifurcated at distal end (12, Fig. 3A); cardiac apophysis simple at tip with a bifurcation positioned anteriorly (39, Fig. 12, E and F), except in *Pleuronichthys ocellatus* and *P. ritteri;* intercalar not in contact with basioccipital (57, Fig. 7B), a reversal in Pleuronectinae; and less than seven teeth on ocular-side premaxilla (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 or included taxa that were homoplastic with these structures. Bifurcation of the first epibranchial is a reversal in the Pleuronectidae. Shape of the cardiac apophysis of the urohyal was observed only in one other pleuronectid, *Limanda aspera*. The intercalar is in contact with the basioccipital in *Glyptocephalus cynoglossus* (57, Fig. 7C). The reduction of teeth on the ocular-side premaxilla (less than 7) is not observed in *Pleuronichthys guttulatus* and *Glyptocephalus*. In *Glyptocephalus*, variation in tooth number on the ocular-side premaxilla ranged from 8 to 16 in *G. zachirus*, but in all species there is a

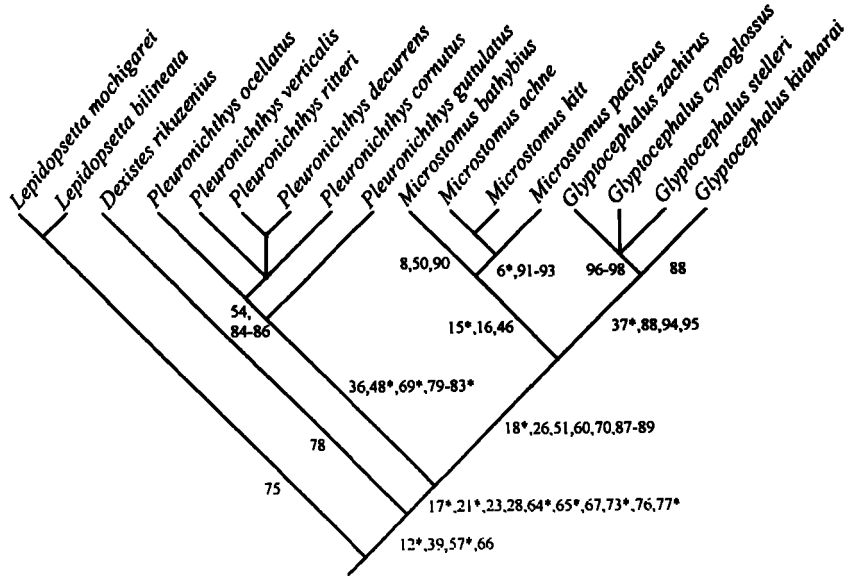

Figure 15

Intrarelationships of Microstomini. Numbers correspond to characters in text. Asterisk represents character state reversal.

smaller number of teeth on the ocular-side premaxilla than the number observed in previous lineages of Pleuronectinae (Norman, 1934). *Glyptocephalus cynoglossus* is reported to have 8 to 15 teeth on the ocular-side premaxilla and 17 to 26 on the associated blind-side premaxilla. *G. stelleri* has only seven teeth on the ocular-side premaxilla and 20 to 27 teeth on the blind side. *G. tanakius* has 12 to 14 teeth on the ocular-side premaxilla and 14 to 16 on the blind side, and *G. zachirus* has 12 to 16 teeth on the ocular-side premaxilla and 20 to 27 teeth on the blind side (Norman, 1934).

The third lineage of Microstomini indicates monophyly for *Pleuronichthys, Microstomus*, and *Glyptocephalus*. It is based on ten character transformations, four synapomorphies, and six reversals (Fig. 15). Synapomorphies include increased number of abdominal vertebrae to between 12 and 14 (23); lips thickened or fleshy (76); palatine of ocular side reduced and not attached to pterygoid (28, Fig. 2C); and teeth on ocular-side dentary reduced to fewer than six (67). The reversals are exoccipital and prootic in contact with each other (77, Fig. 7B); presence of an interorbital process (17, Fig. 10C); one row of gill rakers on fourth ceratobranchial (21, Fig. 3, A and B); haemal spine broadly attached to centrum (73, Fig. 5B); space between both ocular- and blind-side dentary and articular equal in size (64, Fig. 9D); and dorsoposterior process of similar size on ocular- and blind-side dentaries (65, Fig. 9D).

Exceptions and homoplasy in this distribution do not support an alternative hypothesis. *Glyptocephalus kitaharai* has only 11 abdominal vertebrae (Sakamoto, 1984a). Reduction of the ocular-side palatine was also observed in *Hippoglossus* and *Verasper* (Hippoglossinae). The exoccipital and prootic are not joined, and secondary reduction of teeth on the dentary of the ocular side are not observed in *Glyptocephalus*. These species all have proportionally fewer teeth on the ocular-side dentary, but the exact number ranges from 11 to 15 in *Glyptocephalus kitaharai* to 10 to 18 in *G. zachirus* (Norman, 1934).

Reversals in this lineage reproduce plesiomorphic states observed prior to the fourth lineage of Pleuronectidae or states that are plesiomorphic within the Pleuronectinae. The contact between exoccipital and prootic, may be a reversal for the family. The lost interorbital process in the fourth pleuronectid lineage is observed (at least partially) in these two pleuronectine genera. The single row of gill rakers on the fourth ceratobranchial is a reversal of the two rows that define the fourth pleuronectid lineage. The broad attachment of the haemal spine to the centrum is a reversal of a narrower attachment defining the fourth pleuronectid lineage. Sym-

metry in the space between dentary and articular, as well as the dentary process, are reversals for Pleuronectinae. These reversals are also observed in some species of the Pleuronectini (Table 1).

The fourth lineage in Microstomini represents the sister relationship between *Microstomus* and *Glyptocephalus*. Eight synapomorphies support this hypothesis (Fig. 15): intestine extending posteriorly into body cavity (87), unique for this lineage; more than 20 caudal-fin rays, an increase from 18 or 19 (88); more than six branched caudal-fin rays (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); teeth incisorlike (70); single row of teeth on upper jaw (60); and hyomandibula without a broad anteroventral margin (18, Fig. 2C).

Exceptions to the distribution of these synapomorphies do not indicate exclusion of any species defined in *Glyptocephalus* or *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 *Glyptocephalus kitaharai* and *Microstomus pacificus* but is reduced in *Pseudopleuronectes americanus*. The incisorlike tooth structure 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 hyomandibula is a reversal unique within this lineage of *Glyptocephalus* and *Microstomus*. All other taxa within the fourth lineage of Pleuronectidae have a hyomandibular with a broadened anterior margin (Fig. 2D).

Genus *Dexistes* The monotypic genus contains *D. rikuzenius* (Fig. 15). The monotypic status of this species is based on the morphologlical characters examined for intrarelationships of Microstomini and the presence of an ocular-side postocular ridge (78). The latter character has an additional evolutionary step within the sister tribe Pleuronectini, uniting a clade comprising *Limanda*, *Platichthys*, *Pleuronectes*, and *Pseudopleuronectes*.

Genus *Pleuronichthys* This genus contains seven species: *Pleuronichthys coenosus* (not examined), *P. cornutus*, *P. decurrens*, *P. guttulatus*, *P. ritteri*, *P. ocellatus*, and *P. verticalis*. This genus is identified by eight character transformations (Fig. 15): presence of villiform teeth, autapomorphic for *Pleuronichthys* (79); large foramen in blind-side prefrontal (80); gill rakers on second hypobranchial reduced to one at proximal base (36); lateral process present on ocular-side frontal (81, Fig. 7B, 10C); 25 or less caudal vertebrae (82); ocular-side pterosphenoid reduced and not forming posterior margin of orbit (83, Fig. 7B); blind-side pterosphenoid is similarily reduced (48, Fig. 7B); and ocular-side entopterygoid similar in size to that of blind side (69, Fig. 2, A and B). *Pleuronichthys coenosus* is assumed to be a member of this genus on the basis of presence of villiform teeth, reduced number of caudal vertebrae (24 to 25), and presence of a lateral process on 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 observed in only one other species, *Glyptocephalus stelleri*. Reduction of gill rakers on the second hypobranchial was not observed in *Pleuronichthys ocellatus*, which has 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 that in *Pleuronichthys*. Only *Platichthys flesus*, *P. stellatus*, and *Pleuronectes putnami* (Pleuronectini) have also fewer than 25 caudal vertebrae. Reduction of the ocular-side pterosphenoid is not observed in *Pleuronichthys ritteri*, where the pterosphenoid separates frontal and parasphenoid to form the posterior margin of the orbit. Reduction of the blind-side pterosphenoid is also observed in *Acanthopsetta* and *Hippoglossoides* (Hippoglossoidinae). These two character states are reversals in the Pleuronectidae. The symmetry between ocular-side and blind-side entopterygoids is a reversal of the asymmetrical structure observed prior to the Isopsettini. This reversal only occurs in *Pleuronichthys* and *Glyptocephalus kitaharai*.

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

Genus *Microstomus* This genus contains five species: *M. achne, M. bathybius, M kitt., M. pacificus,* and *M. shuntovi* (not examined) and is defined by three synapomorphies (Fig. 15): posterior extension of supraoccipital absent (46, Fig. 10B); first anal pterygiophore thin (15); teeth in both upper and lower jaws uniform in length forming a continuous cutting edge (16, Fig. 9D). Descriptions of *Microstomus shuntovi* indicate that this species is very similar to *M. kitt* (Borets, 1983). The teeth are described as "chisel-like" (Borets, 1983), a term that similarly describes teeth in other species of *Microstomus*. An examination of the supraoccipital and the first anal pterygiophore would verify this classification.

Absence of the posterior extension on the supraoccipital has evolved independently in the Hippoglossoidinae. The thin structure of the first anal pterygiophore is a reversal of a thickened structure defining the third lineage. The continuous cutting edge of the teeth is an advanced state, also present in *Glyptocephalus zachirus, Pleuronectes glacialis, P. pinnifasciatus, P. putnami,* and *Pseudopleuronectes schrenki.*

Microstomus bathybius is the sister species to *M. achne, M. kitt,* and *M. pacificus* (Fig. 15). This deep-sea species is unique in 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 is the only pleuronectid in which the anteroventral tip of the pelvis is anterior to the cleithrum (90). The other species of *Microstomus* share four character states (Fig. 15): ocular-side scales with radii completely surrounding the focus (91); margins of interoperculum and subopercular fimbriated (92, 93, Fig. 2C); and haemapophysis present on most posterior abdominal vertebrae (6, Fig. 4D). This last feature is a reversal for Pleuronectidae, and only *Glyptocephalus zachirus* has also a fimbriated subopercular margin.

Genus *Glyptocephalus* This genus contains four species: *G. cynoglossus, G. kitaharai, G. stelleri,* and *G. zachirus*. Four synapomorphies are hypothesized at this node (Fig. 15): more than 21 caudal-fin rays (88); cleithra inserted by tip of urohyal (94); presence of two to four pyloric appendages and two or three on upper intestine, an increase from two to three and one on upper intestine (95); sphenotic process positioned low on sphenotic and forming dorsal roof of hyomandibular socket (37, Fig. 7A). This last character is a reversal of that observed in all other Microstomini and Pleuronectini.

Glyptocephalus kitaharai is the sister species to *G. cynoglossus, G. stelleri,* and *G. zachirus*. It is unique within this clade and with all other pleuronectids in having 23 caudal-fin rays, the highest number observed in the family (88). The other species are united by three character states (Fig. 15): blind-side nasal bones larger than those of ocular side (96); development of large mucous cavities on blind-side head (97), both characters unique for these three species; and presence of an interpterosphenoid bar (98, Fig. 16), not observed in any other species of *Glyptocephalus* or immediate lineages, but homoplastic in *Hippoglossoides, Acanthopsetta, Psettichthys, Limanda aspera, L. ferruginea, L. proboscidea, L. punctatissima, Pseudopleuronectes americanus, P. yokohamae,* and *Pleuronectes*. The monophyletic status of these three species is in agreement with relationships inferred through an analysis of body shape (Chiu, 1990).

Tribe Pleuronectini The tribe Pleuronectini contains 20 species (19 examined). Four synapomorphies define this tribe (Fig. 6): at least two regular rows of teeth on fifth ceratobranchial (49, Fig. 3, C and D); postocular ridge present on ocular side (78); upper jaw teeth in single row (60); and dorsal crest extending anteriorly from supraoccipital to blind-side frontal reduced or absent (52, Fig. 10, D and E).

Exceptions and homoplasy did not indicate an alternative hypothesis for Pleuronectini; all 19 species examined were grouped in all 128 of the equally par-

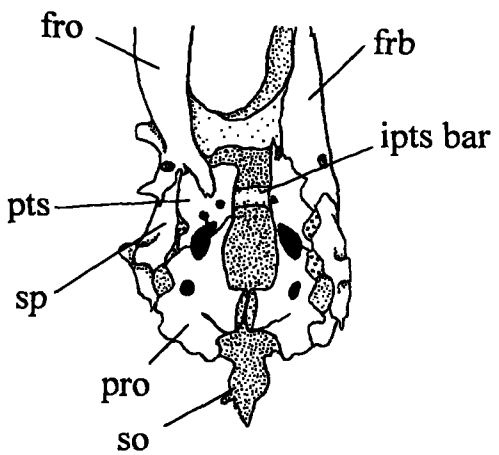

Figure 16

Ventral aspect of cranium, *Pleuronectes quadrituberculatus* USNM 064042, with parasphenoid removed to reveal interpteroshenoid bar. frb= frontal (blind side); fro, frontal (ocular side); ipts bar = interpterosphenoid bar; pro = prootic; pts = pteroshenoid; so = supraoccipital; sp = sphenotic. Anterior region of orbit and occipital region have been removed. Scale bar is 10 mm.

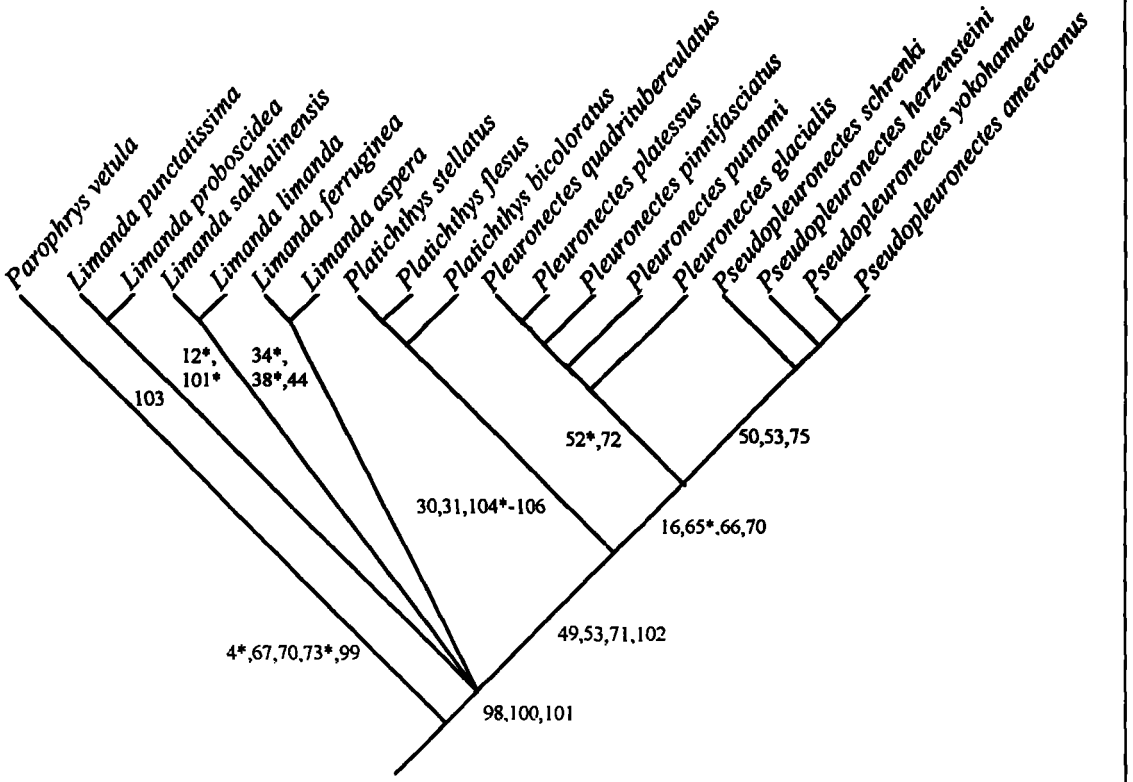

Figure 17

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

simonious trees. A postocular ridge is observed in only one other pleuronectid taxon, *Dexistes rikuzenius*. A single row of teeth in the upper jaw was also observed in *Psettichthys melanostictus*, *Glyptocephalus*, and *Microstomus*. The structure 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 morphological character with Pleuronectini.

Intrarelationships of Pleuronectini

Genus *Parophrys* The first lineage in Pleuronectini has only one species, *Parophrys vetula*, which is distinct with five character transformations (Fig. 17): suture between first and second basibranchial (4, Fig. 3A); haemal spines broadly attached to ventral surface of centrum (73), are both reversals within Pleuronectidae; absence of dentary foramen on ocular side (99); reduction of teeth on ocular-side dentary (67) and incisorlike teeth (70, Fig. 9D) 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 hypothesized to share an immediate common ancestor on the basis of three synapomorphies (Fig. 17): presence of a blind-side postocular ridge (100); presence of bony prominences on ocular-side postocular ridge (101, Fig. 10E); and presence of an interpterosphenoid bar (98, Fig. 16). The blind-side postocular ridge and bony prominences on the ocular-side postocular ridge are unique within Pleuronectidae. The latter was not 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 *Glyptocephalus cynoglossus, G. stelleri, G. zachirus, Psettichthys, Acanthopsetta*, and *Hippoglossoides*.

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 (Fig. 17): bony prominences on blind-side postocular ridge (102, Fig. 10E); a strong medial curvature on fifth ceratobranchial resulting in close approximation or union of ceratobranchials (71, Fig. 3D); an increase of rows of teeth on fifth ceratobranchial from two regular rows to multiple rows of regular length (49, Fig. 3D); and anterior margin of mesethmoid (53) either a thin plate (Fig. 8B) or thickened triangular edge (Fig. 8C).

Exceptions and homoplasy observed in these morphological characters contributed to three alternative topologies for intrarelationships of Pleuronectini (Fig. 18, A–C). Bony prominences on the blind-side postocular ridge were not observed in *Pseudopleuronectes, Pleuronectes platessus*, and *P. quadrituberculatus* and are homoplastic in *Limanda proboscidea* and *L. punctatissima*. Both attributes of the fifth ceratobranchial, the close approximation of the medial margins and the multiple rows of teeth, are not observed in *Pseudopleuronectes*. The thin plate structure of the mesethmoid (Fig. 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 (Fig. 8C) is unique within

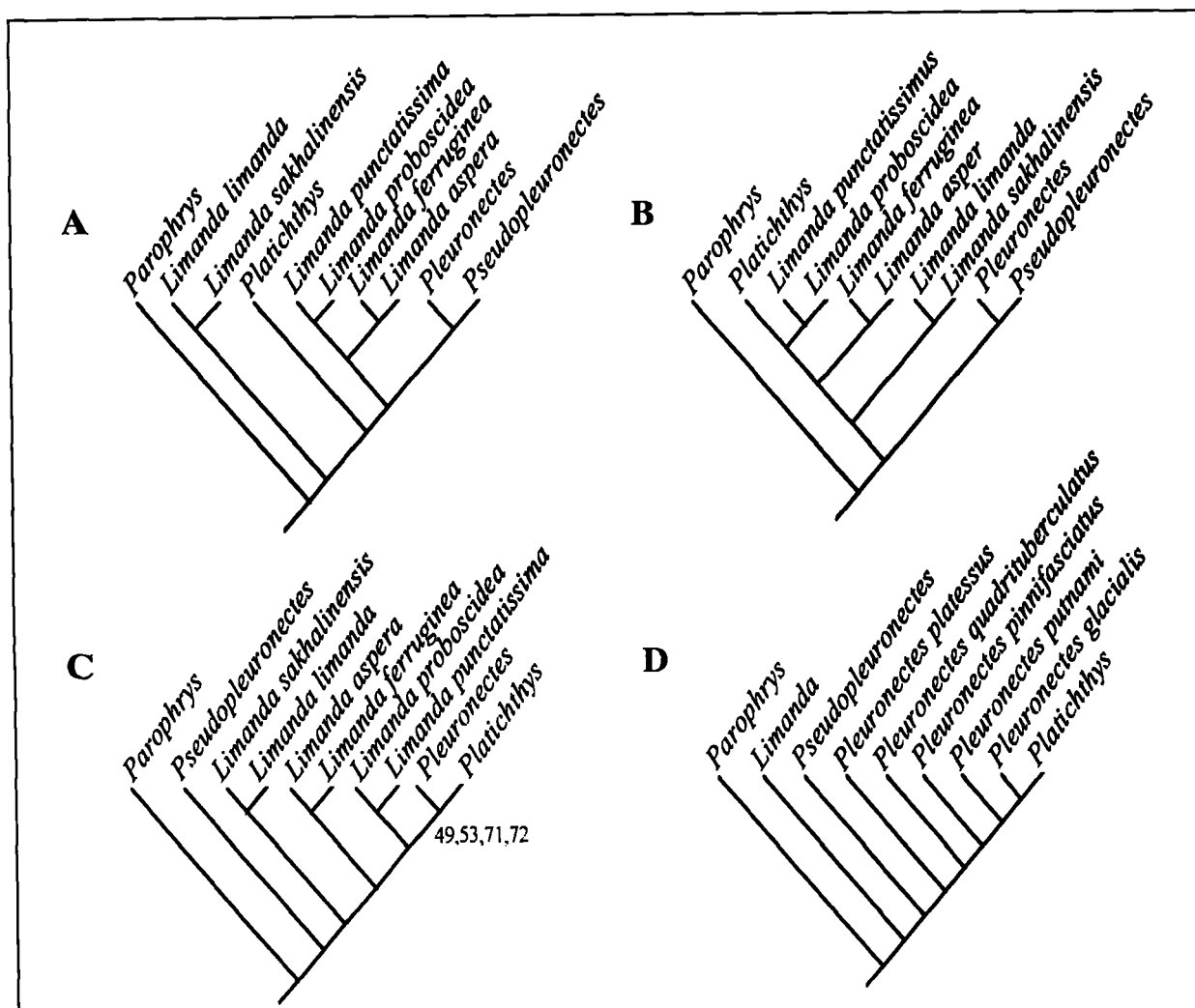

Figure 18

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.

Pleuronectidae but 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 (Fig. 1). All three alternatives (Fig. 18) 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*. Character states in support of these conflicting nodes illustrate homoplasy observed in *Limanda* (Fig. 18, A–C). Some or all species of *Limanda* are observed to share these character states with the other three genera. However, most of the exceptions and homoplasy observed in these structures fail to illustrate an evolutionary hypothesis that is more convincingly corroborated than the topology presented in the consensus tree (Fig. 1).

The fourth lineage unites *Pleuronectes* and *Pseudopleuronectes*. This clade was observed in 75% of the equally parsimonious trees (Fig. 1) and is supported by four synapomorphies (Fig. 17): less than six teeth on ocular-side maxilla (66, Fig. 9D); teeth incisorlike (70, Fig. 9D); teeth forming a continuous cutting edge (16, Fig. 9D); and dorsoposterior process of dentary similar in size on both ocular and blind sides (65, Fig. 9D), a reversal of the second lineage of Pleuronectinae.

Homoplasy observed in these morphological characters reveals a pattern of parallel evolution between this lineage and the clade formed by *Glyptocephalus*, *Microstomus*, and *Pleuronichthys* (Microstomini). Less than six teeth on the ocular-side premaxilla are also present in these three genera of Microstomini. The presence of incisorlike teeth is also observed in *Parophrys vetula* as well as *Glyptocephalus* and *Microstomus*. A continuous cutting edge found on the teeth has independently evolved in *Glyptocephalus* and *Microstomus*. The symmetrically sized dorsoposterior process of the dentary is not observed in *Pseudopleuronectes herzensteini*, *Pleuronectes platessus*, and *P. quadrituberculatus* and is a reversal that is paralleled in *Glyptocephalus* and *Microstomus*.

Alternative topologies observed in 32 of 128 (25%) trees unite either a monophyletic or paraphyletic *Pleuronectes* with *Platichthys* (Fig. 18, C and D). In *Pleuronectes* and *Platichthys* the mesethmoid has a thickened, triangular anterior margin (53, Fig. 8C). The fifth ceratobranchial has a strong medial curve forming a triangular plate (71, Fig. 3D). The teeth of the fifth ceratobranchial are in multiple rows (49, Fig. 3D), and these teeth are generally rounded or molariform (72, Fig. 3D). Three of these characters (49, 53, 71) are used to define the third lineage of Pleuronectini but notably exclude *Pseudopleuronectes*. The molariform teeth on the fifth ceratobranchial (72, Fig. 3D) are observed in *Pleuronectes* and only in *Platichthys bicoloratus*. The alternative hypothesis, indicating a sister relationship between *Pleuronectes* and *Platichthys*, has the same character support as the hypothesis presented in the consensus tree. However, this topology is parsimonious in only 32 trees and only in conjunction with one of the two alternatives that either position a paraphyletic *Limanda* after *Pseudopleuronectes* (Fig. 18C) or hypothesize a paraphyletic *Pleuronectes* (Fig. 18D). Support for either of these topologies is not well corroborated. Therefore, 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 owing to homoplasy observed in these six species (Fig. 17). *Limanda punctatissima* and *L. proboscidea* are the only two pleuronectid species with bony prominences of the postocular ridge extending anteriorly onto the interorbital bar (103, Fig. 10E). *Limanda aspera* and *L. ferruginea* are united by three morphological characters: the supraoccipital crest not forming a groove for the dorsal-fin pterygiophores (38, Fig. 10, A and B); blind-side metapterygoid not articulated with entopterygoid (34, Fig. 2A), both reversals in Pleuronectini; and supratemporal of the blind side jointed at its anterior bifurcation (44, Fig. 13B). This character state has evolved independently five times in Pleuronectidae as it is also observed in *Cleisthenes herzensteini*, *Hippoglossoides elassodon*, *Lyopsetta exilis*, and *Reinhardtius hippoglossoides*. *Limanda limanda* and *L. sakhalinensis* are monophyletic in 75% of the trees (Fig. 1). Two character states unite these species: first epibranchial is bifurcated (12, Fig. 3A); and bony prominences on ocular-side postocular ridge absent (101). Both of these characters are reversals 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 as paraphyletic within a clade that unites 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 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, whereas all other members of this tribe have com-

pressed teeth with an incisorlike shape (Norman, 1934; Sakamoto, 1984a). Both of these tooth structures in *Limanda* are interpreted as synapomorphies for the Pleuronectini. There is little evidence in support for the monophyly of *Limanda*. Only one character state is shared by all six species: presence of gill rakers on second epibranchial (30, Fig. 3C). This character is a reversal of a reduced number of gill rakers defining the second lineage of Pleuronectinae (Fig. 6). There were 16 trees of 403 steps that hypothesized a monophyletic *Limanda* based on three reversals (Fig. 18D), including the presence of gills rakers on the second epibranchial. All 16 topologies were correlated with a hypothetical alternative to the consensus tree (Fig. 18D) 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 morphological characters mentioned above suggest 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* This genus contains three species: *P. bicoloratus*, *P. flesus*, and *P. stellatus*. It is monophyletic with five synapomorphies (Fig. 17): scales along median fins absent (104), a possible reversal in Pleuronectidae; scales on the body modified to form bony tubercles (105); gill rakers on second and third epibranchial absent (30, 31); and supratemporals on ocular and blind sides are fused to the cranium (106). Absence of gill rakers on the second epibranchial is observed only in the distant lineage *Verasper*. The absence of gill rakers on the third epibranchial also occurs in *Verasper* and *Pleuronectes platessus*. The ocular-side supratemporal fused to the cranium is homoplastic in *Limanda punctatissima*.

Genus *Pleuronectes* This genus contains five species: *P. 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 (Fig. 17): prominent dorsal crest extending from the supraoccipital to the blind-side frontal (52, Fig. 10, B and C); and presence of molariform teeth on fifth ceratobranchial (72, Fig. 3D). A prominent crest extending from the 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 species of *Pleuronectes* as paraphyletic with *Platichthys stellatus*, *P. flesus*, and *P. bicoloratus* at the terminal end (Fig. 18D). 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* is correlated and observed in the same 16 trees indicating monophyly of *Limanda* (Fig. 18D).

Genus *Pseudopleuronectes* This genus 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 three synapomorphies (Fig. 17): anterior margin of mesethmoid forming a thin plate (53, Fig. 8B); blind-side nasal bone absent (50); and demersal eggs (75). *Pseudopleuronectes obscurus* has character states synapomorphic for the fourth lineage of Pleuronectinae, uniting *Pleuronectes* and *Pseudopleuronectes*. These are the presence of incisorlike teeth forming a continuous cutting edge and close approximation of the fifth ceratobranchials (Norman, 1934). Although teeth on the fifth ceratobranchial are bluntly conical (Norman, 1934), as in *Pseudopleuronectes*, this character state is plesiomorphic at this phylogenetic level. However, presence of a demersal egg in *P. obscurus* (Hensley and Ahlstrom, 1984), is synapomorphic for *Pseudopleuronectes*.

Homoplasy and two exceptions observed in these morphological characters do not corroborate an alternative hypothesis. A clade uniting the four species of *Pseudopleuronectes* examined here, is observed in all 128 equally parsimonious cladograms. The thin plate structure of the mesethmoid is also observed in *Pleuronectes platessus*, and in more distant 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*. A demersal egg is not observed in *P. herzensteini* and is homoplastic in *Lepidopsetta*.

Summary of intrarelationships

The species examined in this analysis (53 of 59), represent the complete range of morphological variation found in the family. The character analysis outlines the characters synapomorphic for clades revealed in the consensus tree (Fig. 1).

The Pleuronectidae can be summarized as comprising large piscivorous species at the basal lineages,

whose jaws and dentition are nearly symmetrical, and species possessing more specialized dentition, jaw structure, and diverse feeding habits at subsequent lineages. The monophyly and intrarelationships of basal lineages in the Pleuronectidae are not supported by a large number of synapomorphies. This is inherent for taxa associated with 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 (Fig. 18) were supported by 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 summarized by the consensus tree. Instead, the limited occurrence of these alternatives indicates that these topologies are not as robust to homoplasy as those summarized in the consensus tree. The taxonomic nomenclature established by the consensus tree is not in contradiction with alternatives. Based on the cladogram, this classification represents the most conservative nomenclature for such a diverse group of pleuronectids.

The interrelationships within most genera cannot be confidently supported, either because of a lack of observed morphological variation (as in *Glyptocephalus, Hippoglossoides*, and *Pleuronichthys*) or because of 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 description based on synapomorphies from this study. Additional morphological characters, such as the number of dorsal- and anal-fin rays, homoplastic at higher levels of universality may prove informative for species-level relationships. The developmental information summarized in Ahlstrom et al. (1984) has been expanding owing to the commercial popularity of many pleuronectid species (for example Fukuhara, 1988; Markle et. al., 1992). Yet there are still many pleuronectid species for which information of this kind is not yet available. Despite this lack of information across the family, it is certain that these developmental data can also be used in the future to help resolve natural groups among well-documented pleuronectid species.

The phylogenetic limitations of jaw and dental morphological characters is clearly illustrated in the convergent evolution of jaw structures observed between the tribes Microstomini and Pleuronectini. This is most evident in the dentition of *Glyptocephalus*, and *Microstomus,* compared with *Platichthys, Pleuronectes*, and *Pseudopleuronectes,* both of which have single row of incisorlike teeth that sometimes form a continuous cutting edge. However, monophyly for these taxa, based on jaw morphology and dentition, is not supported by other character states observed in the cranial bones and branchial apparatus. These other characters clearly resolve the monophyletic status and intrarelationships of these two groups within each of the two tribes, Microstomini and Pleuronectini. The trend of character reversal, 19 in total (Figs. 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. This analysis clearly places Microstomini as a lineage within the Pleuronectinae on the basis of 28 character states that were not reversals.

Six pleuronectid species were not examined in this analysis. They are *Clidoderma asperrimum*, *Hippoglossoides dubius*, *Microtomus shuntovi*, *Pseudopleuronectes obscurus*, *Pleuronichthys coenosus*, and *Reinhardtius evermanni*. These species are classified as members of Pleuronectidae on the basis of morphological characters obtained from the literature. In addition, their phylogenetic position is based on examination of external characters, radiographs, and literature. The *a posteriori* classification of these six species lends support for the functionality of this analysis, such that an individual species can be classified on the basis of uniquely derived features.

Classification

The objectives for a formal classification of the Pleuronectidae were to recognize large groups within the family and to ensure that only natural groups are represented in the classification. This implied a revision of the established nomenclature. However, changes were minimal and aimed mainly at simplifying the existing nomenclature with its many monotypic genera.

Our results indicate that the Pleuronectidae is monophyletic and can be subdivided 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 (Fig. 1). This is the first classification based on a complete phylogenetic analysis of the group. It

provides a simplified yet phylogenetically informative framework for future studies involving this family.

Taxonomic list of the Pleuronectidae

Subfamily **Hippoglossinae**
Genus *Reinhardtius* (*R. evermanni*,[2] *R. hippoglossoides*, *R. stomias*)
Genus *Hippoglossus* (*H. hippoglossus*, *H. stenolepis*)
Genus *Verasper* (*V. moseri*, *V. variegatus*)
Genus *Clidoderma* (*C. asperrimum*[2])
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. dubius*,[2] *H. elassodon*, *H. platessoides*, *H. robustus*)
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*,[2] *P. cornutus*, *P. decurrens*, *P. guttulatus*, *P. ocellatus*, *P. ritteri*, *P. verticalis*)
Genus *Microstomus* (*M. achne*, *M. bathybius*, *M. kitt*, *M. pacificus*, *M. shuntovi*[2])
Genus *Glyptocephalus* (*G. cynoglossus*, *G. kitaharai*, *G. stelleri*, *G. zachirus*)
Tribe **Pleuronectini**
Genus *Parophrys* (*P. vetula*)
Genus *Limanda*[3] (*L. aspera*, *L. ferruginea*, *L. limanda*, *L. proboscidea*, *L. punctatissima*, *L. sakhalinensis*)
Genus *Platichthys* (*P. bicoloratus*, *P. flesus*, *P. stellatus*)
Genus *Pleuronectes* (*P. glacialis*, *P. pinnifasciatus*, *P. platessus*, *P. putnami*, *P. quadrituberculatus*)
Genus *Pseudopleuronectes* (*P. americanus*, *P. herzensteini*, *P. obscurus*,[2] *P. schrenki*, *P. yokohamae*)

[2] Species not included in analysis, phylogenetic position and classification determined *a posteriori*.
[3] Monophyletic status is uncertain.

The reclassification of several species is necessary to incorporate the new phylogenetic information. The changes to the classification are as follows:

1 *Atherestes evermanni*, *A. stomias*, and *Reinhardtius hippoglossoides* are united within *Reinhardtius* which is the oldest valid genus-group name. *Reinhardtius* (=*Atherestes*) *evermanni* was not examined and its position within the subfamily Hippoglossinae was determined *a posteriori*. Placement of *R. evermanni* within the clade uniting these three species is based on examination of external morphological characters, number of abdominal vertebrae, and the description provided in Norman (1934).
2 The genus *Eopsetta* (*sensu* Sakamoto, 1984a) with *E. exilis*, *E. grigorjewi*, and *E. jordani* is paraphyletic. The revised *Eopsetta* contains *E. grigorjewi* and *E. jordani* whereas *E. exilis*, is reassigned to the genus *Lyopsetta* as in Norman (1934).
3 *Hippoglossoides herzensteini* and *H. pinetorum* (*sensu* Sakamoto, 1984a) are reclassified as *Cleisthenes*, as in Norman (1934). This is a subjective reclassification because 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 hierarchically consistent nomenclature within the family.
4 A number of revisions were made to produce a simplified nomenclature that is hierarchically consistent with the phylogenetic interrelationships. Species of *Hypsopsetta* and *Pleuronichthys* (*sensu* Sakamoto, 1984a) are regrouped under *Pleuronichthys*. The species-group name (*guttulata*) is revised to agree with gender in the genus (i.e. *Pleuronichthys guttulatus*). The species of *Embassichthys* and *Microstomus* (*sensu* Sakamoto, 1984a) are regrouped under *Microstomus*. The species of *Errex*, *Glyptocephalus*, and *Tanakius* (*sensu* Sakamoto, 1984a) are regrouped under *Glyptocephalus*. The species of *Kareius* and *Platichthys* (*sensu* Sakamoto, 1984a) are regrouped under *Platichthys*.
5 The genus *Pleuronectes* (*sensu* Sakamoto, 1984a), with 19 species, is not monophyletic. According to our results *Pleuronectes* comprises only five species: *P. glacialis*, *P. pinnifasciatus*, *P. platessus*, *P. putnami*, and *P. quadrituberculatus*. The species-group name (*platessa*) is revised to correspond with gender in the genus (i.e. *Pleuronectes platessus*). Other species of *Pleuronectes* (*sensu* Sakamoto, 1984a) are reclassified as *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* on the basis of the oldest valid genus-group name for each species, with the exception of *Pseudopleuronectes schrenki*, and *P. obscurus*, previously classified as *Limanda schrenki* (Sakamoto, 1984b; Hensley and Ahlstrom, 1984) and *Liopsetta obscura* (Norman, 1934), respectively. Sakamoto's (1984a) classification was based on a 100% similarity observed among these 19 species. The new phylogeny is based on 67 additional morphological features that clearly differentiate and classify these 19 species.

6 The reclassification of *Pseudopleuronectes schrenki* is based on its position in all 128 most parsimonious trees, none of which united *P. schrenki* with other taxa exclusive of the three other species in *Pseudopleuronectes*. In addition to the three synapomorphies uniting *Pseudopleuronectes*, the most notable characteristic of *P. schrenki* in observation and in the literature is the presence of incisorlike teeth that form a continuous cutting edge (character 70, Fig. 9D, see also Sakamoto, 1984b). This character 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, Fig. 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 characteristics also seem to synonymize *Pseudopleuronectes schrenki* with *P. yokohamae* (Yusa, 1960; Pertseva-Ostroumova, 1961). Recent studies (Sakamoto, 1984b) 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 also have similar dental structure. However, *P. schrenki* has a distinct pattern of bars on the dorsal- and anal-fin rays (Sakamoto, 1984b). Uncertainty surrounding the status of *Pseudopleuronectes schrenki* is also the 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 on the basis of uniquely derived features. Although a further analysis is required to clearly assess the taxonomic status of *Pseudopleuronectes schrenki* and *P. yokohamae*, this analysis clearly indicates that neither should be classified as *Limanda*.

7 The reclassification of *Pseudopleuronectes obscurus* is based solely on information available from the literature. This species is confidently placed in the fourth lineage of Pleuronectini and its placement as a species of *Pseudopleuronectes* is supported by one synapomorphy, the presence of a demersal egg. A complete osteological examination of this species may test the validity of this classification.

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Literature cited

Ahlstrom, E. H., K. Amaoka, D. A. Hensley, H. G. Moser, and B. Y. Sumida.
1984. Pleuronectiformes: development. *In* H. G. Moser, W. J. Richard, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richardson, (eds.), Ontogeny and systematics of fishes, p. 640–670. Am. Soc. Ichthyol. Herpetol. Spec. Publ. 1.

Amaoka, K.
1969. Studies on the sinistral flounders found in the waters around Japan: taxonomy anatomy and phylogeny. J. Shimonoseki Univ. Fish. 18:65–40.

Batts, B. S.
1964. Lepidology of the adult pleuronectiform fishes of the Puget Sound, Washington. Copeia. 1964(4):666–673.

Borets, L. A.
1983. A new species of flounder, *Microstomus shuntovi* sp. n. (Pleuronectidae), and two rare flounder species (Bothidae) from seamounts of the Northwestern and Hawaiian ridges. J. Ichthyol. 23(5):1–6.

Brooks, D. R., and D. A. McLennan.
1991. Phylogeny, ecology, and behavior: a research program in comparative biology. Univ. Chicago Press, Chicago, IL, 434 p.

Chapleau, F.
1993. Pleuronectiform relationships: a cladistic reassessment. Bull. Mar. Sci. 52(1):516–540.

Chapleau, F., and A. Keast.
1988. A phylogenetic reassessment of the monophyletic status of the family Soleidae, with comments on the suborder Soleoidei (Pisces; Pleuronectiformes). Can. J. Zool. 66: 2797–2810.

Chiu, T.-S.
1990. Inference on the phylogenetic relationships of the genus *Glyptocephalus* (Pleuronectiformes: Pleuronectidae) by shape. Bull. Inst. Zool., Academia Sinica 29(2):95–104.

Cooper, J. A., and F. Chapleau.
1998. Phylogenetic status of *Paralichthodes algoensis* (Paralichthodidae). Copeia 1998(2):477–481.

Cuvier, G.
1816. Le règne animal distribué d'après son organisation pour servir de base a l'histoire naturelle des animaux et d'introduction à l'anatomie comparée. Les reptiles, les poissons, les mollusques et les annelides, edition 1, vol. 2, 532 p.

de Groot, S. J.
1971. On the interrelationships between morphology of the alimentary tract, food and feeding behaviour in flatfishes (Pisces: Pleuronectiformes). Neth. J. Sea Res. 5(2):121–196.

Eschmeyer, W. N.
1990. Catolog of the genera of the recent fishes. California Academy of Sciences, San Francisco, CA, 697 p.

FAO (Food and Agriculture Organization).
1997. FAO yearbook, fishery statistics-catches and landings 1995, vol. 80. FAO, Rome, 714 p.

Felsenstein, J.
1978. The number of evolutionary trees. Syst. Zool. 27: 27–33.

Forey, P. L., C. J. Humphries, I. J. Kitching, R. W. Scotland, D. J. Siebert, and D. M. William.
1992. Cladistics: a practical course in systematics. Clarendon Press, Oxford, 191 p.

Fukuhara, O.
1988. Morphological and functional development of larval and juvenile *Limanda yokohamae* (Pisces: Pleuronectidae) reared in the laboratory. Mar. Biol. 99:271–281.

Goloboff, P. A.
1991. Homoplasy and the choice among cladograms. Cladistics 1991(7):215–232.

Hart, J. L.
1973. Pacific fishes of Canada. Fish. Res. Board Can., Bulletin 180, Ottawa, 740 p.

Hensley, D. A.
1993. Two new flatfish records from the Red Sea, an Indo-Pacific samarid (*Samariscus inornatus*) and the European plaice (*Pleuronectes platessa*). Israel J. Zool. 39:371–379.

Hensley, D. A., and E. H. Ahlstrom.
1984. Pleuronectiformes: relationships. *In* H. G. Moser, W. J. Richard, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richardson, (eds.) Ontogeny and systematics of fishes, p. 670–687. Am. Soc. Ichthyol. Herpetol. Spec. Publ. 1.

Hubbs, C. L.
1945. Phylogenetic position of the Citharidae, a family of flatfishes. Misc. Pub. Museum Zool. Univ. Mich. 63:1–38.

Hubbs, C. L., and K. F. Lagler.
1970. Fishes of the Great Lakes region. Univ. Michigan Press, Ann Arbor, MI, 213 p.

Jordan, D. S.
1923. A classification of fishes, including families and genera as far as known. Stanford Univ. Publ., Biol. Sci. 3(2):79–243.

Jordan, D. S., and D. K. Goss.
1889. A review of the flounder and soles (Pleuronectidae) of America and Europe. Rep. U.S. Com. Fish. Washington 14(1886):225–342.

Jordan, D. S., and B. W. Evermann.
1898. The fishes of North and Middle America III. Bull. U.S. Nat. Mus. 47(3): 2602–2712.

Jordan, D. S., and E. C. Starks.
1906. A review of the flounders and soles of Japan. Proc. of the USNM. 31:161–246.

Leipertz, S. L.
1987. Morphometrics and the evolutionary history of fishes of the teleost subfamily Pleuronectinae. Ph.D. diss., Univ. Washington, Seattle, WA, University Microfilm International Microfilm No. 8802282.

Leviton, A. E., R. H. Gibbs Jr., E. Heal, and C. E. Dawson.
1985. Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985(3):802–832.

Maddison, W. P., M. J. Donoghue, and D. R. Maddison.
1984. Outgroup analysis and parsimony. Syst. Zool. 33:83–103.

Maddison, W. P., and D. R. Maddison.
1992. MacClade: analysis of phylogeny and character evolution, version 3.0. Sinauer Sunderland, 398 p.

Markle, D. F., P. M. Harris and C. L. Toole.
1992. Metamorphois and an overview of early-life-history stages in Dover sole *Microstomus pacificus*. Fish. Bull. 90:285–301.

Nelson, J. S.
1984. Fishes of the world, 2nd ed. John Wiley & Sons, New York, NY, 523 p.
1994. Fishes of the world, 3rd ed. John Wiley & Sons, New York, NY, 600 p.

Norman, J. R.
1934. A systematic monograph of the flatfishes (Heterosomata), vol. 1: Psettodidae, Bothidae, Pleuronectidae. British Museum (Natural History), London, 459 p.

Pertseva-Ostroumova, T. A.
1961. The reproduction and development of far eastern flounders (Pleuronectidae). T. Inst. Okeanol. Akak. Nauk. SSSR. [In Russian; transl. avail. Fish. Res. Board Canada, Transl. Serv. No. 856. 1967.]

Rass, T. S.
1996. On taxonomy of Pleuronectini (Pleuronectidae). J. Ichthyol. 36(7):546–548.

Regan, C. T.
1910. The origin and evolution of the teleostean fishes of the order Heterosomata. Ann. Mag. Nat. Hist. 8(6):484–496.
1920. A revision of the flat-fishes (Heterosomata) of Natal. Ann. Durban Mus. 2:205–222.
1929. Fishes. Article *in* Encyclopaedia Britannica, 14th ed., vol. 19, p. 324–325.

Ride, W. D. L., C. W. Sabrosky, G. Bernardi, and R. V. Melville.
1985. International code of zoological nomenclature. International Trust for Zoological Nomenclature, British Museum of Natural History, London, 338 p.

Robins, C. R., R. M. Bailey, C. E. Bond, J. R. Brooker, E. A. Lachner, R. N. Lea, and W. B. Scott.
1991. Common and scientific names of fishes from the United States and Canada, fifth ed., 1991. Am. Fish. Society Spec. Publ. 20, 183 p.

Roff, D. A.
1981. Reproductive uncertainty and the evolution of iteroparity: why don't flatfish put all their eggs in one basket? Can. J. Fish. Aquat. Sci. 38:968–977.

Rohlf, F. J.
1982. Consensus indices for comparing classifications. Math. Biosci. 59:131–144.

Sakamoto, K.
1984a. Interrelationships of the family Pleuronectidae (Pisces: Pleuronectiformes). Mem. Fac. Fish. Hokkaido Univ. 31:95–215.
1984b. Family Pleuronectidae. *In* H. Masuda, K. Amaoka, C. Arai, T. Uyeno, and T. Yoshino (eds.), The fishes of the Japanese Archipelago, p. 351–354. Tokai Univ. Press, Tokyo.

Sanderson, M. J., and M. J. Donoghue.
1989. Patterns of variations in levels of homoplasy. Evolution 43(8):1781–1795.

Scott, W. B., and M. G. Scott.
1988. Atlantic fishes of Canada. Can. Bull. Fisheries and Aquatic Sciences 219. Univ. Toronto Press, Canada, 731 p.

Stiassny, M. L. J., and M. C. C. de Pinna.
1994. Basal taxa and the role of cladistic patterns on the evaluation of conservation priorities: view from freshwater. *In* P. L. Forey, C. J. Humphries, and R. I. Vane-Wright (eds.), Systematics and conservation evaluation, Systematics Association special vol. 50, p. 235–249. Clarendon Press, Oxford.

Watrous, L. E., and Q. D. Wheeler.
1981. The outgroup comparison method of character analysis. Syst. Zool. 30:1–11.

Wheeler, A.
1992 A list of the common and scientific names of fishes of the British Isles. J. Fish Biol. 41(suppl. A), p. 16, 22.

Wiley, E. O.
1981. Phylogenetics: the theory and practice of phylogenetic systematics. Wiley-Interscience Publications, John Wiley and Sons, Toronto, 439 p.

Yazdani, G. M.
1969. Adaptation in the jaws of flatfish (Pleuronectiformes). J. Zool. (Lond.) 159:181–222.

Yusa, T.
1960. Differences of structures of eggs and larvae between *Limanda yokohamae* Günther and *Limanda schrenki* Schmidt. Bull. Mar. Biol. Stn. Asamushi 10(2):127–131.

Appendix

List of characters

1 *Attachment of ocular-side frontal with mesethmoid.* 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 Fig. 104, p. 184 in Amaoka, 1969).

2 *Sensory canal of ocular-side preorbital (Sakamoto, 1984a).* 2 states, 3 steps, cci=0.33: present (state 0), absence (state 1). The presence or 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 Fig. 105, p. 187, in Amaoka, 1969).

3 *Morphology of metapterygoid.* 2 states, 2 steps, cci=0.50: ventral margin concave (state 0, Fig. 2A), observed in *Reinhardtius stomias* and all outgroup taxa. Ventral margin not concave nearly straight (state 1, Fig. 2, B–D).

4 *Articulation between first and second basibranchials.* 2 states, 8 steps, cci=0.13: wedgelike articulation between first and second basibranchial (state 0, Fig. 3A). No wedgelike articulation, first basibranchial loosely attached to second basibranchial by cartilage (state 1, Fig. 3, B–D).

5 *Articulation suture between second and third basibranchials.* 2 states, 3 steps, cci=0.33: wedgelike articulation between second and third basibranchial (state 0, Fig. 3A). No wedgelike articulation, second basibranchial loosely attached to third basibranchial by cartilage (state 1, Fig. 3, B–D).

6 *Haemapophysis through fusion of parapophysis of posterior most abdominal vertebrae (Sakamoto, 1984a).* 2 states, 4 steps, cci=0.25: present (state 0, Fig. 4, A, B, and D), absent (state 1, Fig. 4C).

7 *Accessory processes on ventral side of centrum.* 2 states, 5 steps, cci=0.20: present (state 0, Fig. 5A), absent (state 1, Fig. 5, B and 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 other outgroup taxa examined (*Lepidoblepharon ophthalmolepis*, *Citharichthys arenaceus*, and *Paralichthys squamilentus*), and most taxa within the bothoid lineage, (see Table 6, p. 193 in Amaoka, 1969).

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 rachis (state 0). Olfactory lamellae parallel without a central rachis (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, Fig. 7A). Pterosphenoid and prootic join to form dorsal margin of foramen (state 1, Fig. 7, B and 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, Fig. 3, A and B), distal end of first epibranchial simple (state 1, Fig. 3, C and D).

13 *Spines or teeth on gill rakers (Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: present (state 0, Fig. 3, A and B), absent (state 1, Fig. 3, C and D).

14 *Structure of suture between mesethmoid and blind-side prefrontal.* 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, Fig. 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, Fig. 8, B and C). Same as state 1 but foramen absent (state 2, Fig. 8D). The character state for the mesethmoid and prefrontal is not applicable (na) for the outgroup taxa *Psettodes* sp. and *Lepidoblepharon ophthalmolepis* owing to the morphological differences in this region with respect to the ingroup taxa.

15 *Structure of first anal ptergyiophore.* 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, Fig. 9A). Dentition of near equal or equal length (state 1, Fig. 9, B and C). Uniform dentition as in state 1 but teeth forming a continuous cutting edge (state 2, Fig. 9D).

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

18 *Structure of hyomandibular of both ocular and blind sides.* 2 states, 2 steps, cci=0.50: anterior margin of hyomandibular is not broad (state 0, Fig. 2, A–C). Anterior margin of hyomandibular is flatly broadened (state 1, Fig. 2D).

19 *Dentition of third epibranchial (Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: present (state 0, Fig. 3, A and B), absent (state 1, Fig. 3, C and D).

20 *Bony plates of the branchial arch (Sakamoto, 1984a).* 2 states, 2 steps, cci=0.50: present (state 0, Fig. 3, A and B), absent (state 1, Fig. 3, C and D).

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, Fig. 3, A and B). Zero rows (state 1). Two rows (state 2, Fig. 3, C and D). *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, Fig. 2A), present (state 1, Fig. 2, B–D).

23 *Number of abdominal vertebrae.* 3 states, ordered, 9 steps, cci=0.22: counts for Pleuronectidae taken from Sakamoto (1984a, Table 13). 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 migrated 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: counts for Pleuronectidae taken from Sakamoto (1984a, Table 13). Less than or equal to 35 (state 0). From 36 to 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 ocular-side pterygoid.* 2 states, 2 steps, cci=0.50: palatine and pterygoid attached (state 0, Fig. 2, A and D). Palatine and pterygoid not attached due to a reduction of the posterior ventral arm of the palatine (state 1, Fig. 2, B and C).

29 *Gill rakers on first epibranchial.* 2 states, 2 steps, cci=0.50: present (state 0, Fig. 3, A–D), absent (state 1).

30 *Gill rakers on second epibranchial.* 3 states, ordered, 5 steps, cci=0.40: present (state 0, Fig. 3, B and C). Reduced to just one gill raker at proximal base of epibranchial (state 1, Fig. 3, A and D). Absent (state 2).

31 Gill rakers on third epibranchial. 3 states, ordered, 5 steps, cci=0.40: present (state 0, Fig. 3B). Reduced to just one gill raker at proximal

base of epibranchial (state 1, Fig. 3D). Absence (state 2, Fig. 3C).

32 *Gill rakers on first hypobranchial.* 2 states, 4 steps, cci=0.25: present (state 0, Fig. 3, A–D). Reduced to just one gill raker at distal 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* owing to the morphological differences in this region with respect to the ingroup taxa.

34 *Articulation of blind-side metapterygiod with entopterygoid.* 2 states, 7 steps, cci=0.14: absent (state 0, Fig. 2, A and B), present (state 1, Fig. 2, C and D). The metapterygoid is articulated to the pterygoid in outgroup taxa (Fig. 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 formamen 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* owing 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, Fig. 3, A and C). Reduced to just one gill raker at distal base of hypobranchial (state 1, Fig. 3, B and D).

37 *Sphenotic process in relation to hyomandibular socket.* 2 states, 3 steps, cci=0.33: process position low on sphenotic to form dorsal roof of socket between sphenotic and prootic (state 0, Fig. 7A). Process is positioned higher on sphenotic and more anteriorly so as not to be associated with socket (state 1, Fig. 7, B and C).

38 *Supraoccipital crest.* 2 states, 7 steps, cci=0.14: single crest (state 0, Fig. 10, A and B). Double crest to form a groove for the insertion of the basal ends of pterygiophores for the dorsal fin (state 1, Fig. 10, C–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, Fig. 12, A and B) Cardiac apophysis is simple at tip with bifurcation positioned anteriorly on dorsal edge (state 1, Fig. 12, E and F). Cardiac apophysis is strongly bifurcate at the posterior portion (state 2, Fig. 12, C and D).

40 *Gill rakers on fourth epibranchial.* 2 states, 3 steps, cci=0.33: absent (state 0, Fig. 3, A–D), 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 from published accounts of this character was reexamined in the specimens used for this analysis. The state that was observed in these specimens was used if the number of rows was in conflict with the published accounts in 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, Fig. 13A). Supratemporal in two pieces, jointed at anterior bifurcation point (state 1, Fig. 13B).

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

45 *Scales on the surface eyes (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, Fig. 10, A, and C–E). Absence of posterior extension of supraoccipital (state 1, Fig. 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, Fig. 7, B and C). 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, Fig. 7B). Pterosphenoid large forming posterior margin of orbit (state 1, Fig. 7, A and C).

49 *Rows of teeth on fifth ceratobranchial.* 3 states, ordered 4 steps, cci=0.75: irregular single or multiple rows (state 0, Fig. 3A). Regular 2 rows (state 1, Fig. 3, B and C). Regular multiple rows or clustered (state 2, Fig. 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: from five to 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 ?) owing to the poor preservation of infraorbital bones for this specimen.

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

53 *Anterior margin of mesethmoid.* 3 states, unordered, 10 steps, cci=0.30: open canal extending from frontal on ocular side (state 0, Fig. 8A). Thin plate (state 1, Fig. 8B). Closed canal extending from frontal on ocular side or from interorbital process if present. Process extending anteriorly with a triangular shape to make anterior edge thicker (state 2, Fig. 8C). Similar to state 2 but anterior edge reduced to form an irregular edge (state 3, Fig. 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 ocular-side entopterygoid.* 2 states, 3 steps, cci=0.33: absent (state 0, Fig. 2, A–C), present (state 1, Fig. 2D). The metapterygoid is articulated to the pterygoid in outgroup taxa (Fig. 2A).

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

56 *Ceratohyal foramen (Sakomoto, 1984a).* 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, Fig. 7, A and B). Intercalar and basioccipital in contact (state 1, Fig. 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 mantained 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, Fig. 9, A and B). Blind-side premaxilla overlaps or protrudes past sagittal axis (state 1, Fig. 9, C and 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, Fig. 9, A and B). Ocular-side premaxilla shorter than blind-side premaxilla (state 1, Fig. 9, C and D).

63 *Ventral posterior curvature of blind-side premaxilla.* 2 states, 2 steps, cci=0.50: absent (state 0, Fig. 9, A and B), present (state 1, Fig. 9, C and 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, Fig. 9, A, B, and D). Asymmetrical space, blind side greater than ocular side (state 1, Fig. 9C).

65 *Asymmetry in size of dorsal posterior process of dentary.* 2 states, 5 steps, cci=0.20: symmetrical (state 0, Fig. 9, B and D). Asymmetrical, ocular side greater than blind side (state 1, Fig. 9, A and C).

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

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

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

70 *Shape of teeth (Norman, 1934).* 4 states, ordered 6 steps, cci=0.50: sharply pointed (state 0, Fig. 9, A and B). Bluntly conical or with truncated tips (state 1, Fig. 9C). Incisorlike (state 2, Fig. 9D). Molariform (state 3).

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

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

73 *Structure of haemal spines in anteriormost caudal vertebrae.* 2 states, 6 steps, cci=0.17: broadly attached both anteriorly and posteriorly to centrum, lateral foramen in haemal arch present (state 0, Fig. 5B). Narrow at base, attached anteriorly to centrum, lateral foramen in haemal arch absent (state 1, Fig. 5, A and C).

74 *Structure 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 *Structure of exoccipital and prootic.* 2 states, 2 steps, cci=0.50: exoccipital and prootic joined (state 0, Fig. 7B). Exoccipital and prootic not joined (state 1, Fig. 7, A and C).

78 *Postocular 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 blind-side prefrontal.* 2 states, 2 steps, cci=0.50: absent (state 0), present (state 1).

81 *Lateral process on ocular-side frontal.* 2 states, 3 steps, cci=0.33: absent (state 0, Fig. 7, A and C, Fig. 10, A, B, D, and E), present (state 1, Fig. 7B, Fig. 10C).

82 *Decrease in the number of caudal vertebrae.* 2 states, 3 steps, cci=0.33: counts for Pleuronectidae taken from Sakamoto (1984a, Table 13). 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, Fig. 7B). Pterosphenoid large and forms posterior margin of orbit (state 1, Fig. 7, A and 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 blind-side prefrontal and parasphenoid.* 2 states, 1 step, cci=1.00: present (state 0, Fig. 7, A and C), reduced or absent (state 1, Fig. 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 anterior margin in 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: the number of caudal-fin rays was taken as the modal value for counts in Sakamoto (1984a, Table 18). 18 or 19 (state 0), 20 (state 1), 21 (state 2), 22 (state 3), 23 (state 4).

89 *Degree of caudal-fin ray branching.* 3 states, unordered, 8 steps, cci=0.25: expressed as the number of caudal-fin rays minus the number of branched caudal-fin rays. Six unbranched rays (state 0). Less than 6 unbranched rays (state 1). Greater than 6 unbranched rays (state 2).

90 *Position of ventral anterior tip of pelvis of ocular side in relation 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 *Structure 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 posterior ventral margin of the interoperculum.* 2 states, 1 step, cci=1.00: absent (state 0, Fig. 2, A, B, and D), present (state 1, Fig. 2C).

93 *Fimbriation along posterior margin of the suboperculum.* 2 states, 2 steps, cci=0.50: absent (state 0, Fig. 2, A, B, and D), present (state 1, Fig. 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* owing to the poor condition of the specimens around the dorsal 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 morphological character can be observed only from a lateral view, through the pterosphenoid if it is thin, or from a ventral view if the parasphenoid is removed (Fig. 16).

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 ocular-side postocular ridge.* 3 states, unordered, 4 steps, cci=0.50: bony prominences absent (state 0, Fig. 10D). Many small bony prominences (state 1, Fig. 10E). A series of enlarged prominences on postocular ridge in *Pleuronectes platessus* and *P. quadrituberculatus* (state 2).

102 *Bony prominences along blind-side postocular ridge.* 2 states, 3 steps, cci=0.33: bony prominences absent (state 0, Fig. 10D). Many small bony prominences present (state 1, Fig. 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 of the ocular-side frontal (state 1, Fig. 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 ?), because 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).