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# Geometric morphometric analysis of skeletal shape variation across the pleuronectiformes

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# GEOMETRIC MORPHOMETRIC ANALYSIS OF SKELETAL SHAPE VARIATION ACROSS THE PLEURONECTIFORMES

An Abstract of a Thesis

Submitted

in Partial Fulfillment

of the Requirements for the Degree

Master of Science

Corinthia R. Black

University of Northern Iowa

May 2014

# ABSTRACT

Pleuronectiformes, commonly called flatfishes, is a large order of highly specialized fishes that display two eyes on one side of the head. Comprised of approximately 716 species, flatfishes share many similar characteristics. However, the complex history of the classification of the group reveals the diversity of shape across the order. This study focused on the diversity of shape across the order by examining skeletal elements. Landmark-based geometric morphometrics was used to visualize shape variation across the order of Pleuronectiformes. A total of 457 specimens were radiographed from collections at the University of Kansas Natural History Museum and the Smithsonian National Museum of Natural History. Sixteen landmarks and one curve were digitized and superimposed using a generalized least squares Procrustes superimposition. A multivariate analysis was performed on all individuals of Pleuronectiformes using a principal component analysis (PCA). PCA analyses were performed on each family individually to examine the shape variation among genera. The PCA of all Pleuronectiformes showed a difference in shape among families. The results of the multivariate analysis revealed tight clustering and clear separation for some families, but showed broad scattering and significant overlap in others. Psettodidae was revealed to have tight clustering and clear separation from the other Pleuronectiformes, suggesting Psettodidae is morphologically distinct from other Pleuronectiformes. Morphological analysis suggests that some families with specialized features had a more conserved shape, whereas some families with generalized characters had greater variation in shape. Paralichthyidae, a non-monophyletic family, showed variation between the

three distinct lineages on the PCA of all Pleuronectiformes, suggesting variation in shape across the three lineages. *Tephrinectes*, a genus of Paralichthyidae which has been suggested to be removed and elevated to the family level, showed no distinct variation in shape from other genera in Paralichthyidae. This result suggests that *Tephrinectes* did not vary in shape from other genera within Paralichthyidae. Multivariate analysis showed little variation across most genera of Bothidae; however, four genera showed distinct shape within the morphospace, suggesting Bothidae had a large variation in shape across the genera. Furthermore, genera within Achiridae showed distinct shape variation grouped by habitat type (i.e. freshwater, brackish water, and saltwater species) suggesting convergence of shape based on life history. This study is novel in applying landmark-based geometric morphometric methods to shape variation in skeletal elements across the order of Pleuronectiformes. By focusing on skeletal elements, this study helps to clarify shape variation in relation to phylogenetic hypotheses and illustrates the large morphological diversity that flatfishes represent.

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This Study by: Corinthia R. Black

Entitled: GEOMETRIC MORPHOMETRIC ANALYSIS OF SKELETAL SHAPE VARIATION ACROSS THE PLEURONECTIFORMES

has been approved as meeting the thesis requirement for the

Degree of Master of Science in Biology

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# CHAPTER 1

# PLEURONECTIFORMES RELATIONSHIPS

Commonly called flatfishes, Pleuronectiformes is a highly specialized order of fishes that displays obvious asymmetrical morphology. Asymmetry occurs when one eye migrates over the dorsal median of the head to rest beside the other eye.  The side of the body that contains both eyes is referred to as the eyed side, where as the side with no eyes is called the blind side.

Flatfishes occupy primarily marine habitats, with ten species known to reside in fresh water, and have a worldwide distribution. About twenty species are known to occasionally enter fresh water, but predominantly inhabit marine environments (Nelson 2006). Approximately 678 species are currently recognized in fifteen families containing 134 genera (Nelson 2006). The great diversity in shape, size, trophic level, and habitat type across the phylogeny of Pleuronectiformes is what makes this order of considerable interest to evolutionary biologists.

The Pleuronectiformes is currently recognized as containing two major lineages; the suborders Psettoidei and Pleuronectoidei. Psettoidei is comprised of one family, Psettodidae, whereas Pleuronectoidei is comprised of the remaining families; Citharidae, Scophthalmaidae, Bothidae, Paralichthyidae, Pleuronectidae, Paralichthodidae, Poecilopsettidae, Rhombosoleidae, Achiropsettidae, Samaridae, Achiridae, Soleidae, and Cynoglossidae. Until recently, Psettoidei had been considered to be the sister taxa to Pleuronectoidei, but recent phylogenetic analyses have suggested excluding Psettoidei entirely from Pleuronectiformes (Munroe 2005; Campbell et al. 2013).

Many hypotheses on phylogenetic relationships within Pleuronectiformes have been proposed. Although flatfishes have been extensively studied since they were first described, there are many questions that remain regarding their relationships. There have been many major works that have examined classification and relationships of Pleuronectiformes, which will be discussed herein. These studies include Jordan and Evermann (1898), Kyle (1900, 1921), Regan (1910, 1929), Norman (1934), Hubbs (1945), Lauder and Liem (1983), Hensley and Ahlstrom (1984), Chapleau (1993), Verneau et al. (1994), Berendzen and Dimmick (2002), Pardo et al. (2005), Azevedo et al. (2008), and Campbell et al. (2013).

## History of Classification

Historically, classification of flatfishes was based on placement of the eyes. Jordan and Evermann (1898) divided Pleuronectiformes into right-eyed (dextral) and left-eyed (sinistral) flounders and right-eyed and left-eyed soles. They recognized the suborder Heterosomata, the flatfishes, within the order Acanthopteri. The suborder was further divided into two families, Pleuronectidae and Soleidae. The Pleuronectidae, the founders, was comprised of the subfamilies Hippoglossinae, Pleuronectinae, Psettinae, Pelecanichthyinae, Samarinae, and Oncopterinae. The family was united by a preopercular margin that is more or less distinct and not hidden by skin and scales of the head, large and well separated eyes, moderately large mouths, and the presence of teeth. The Soleidae, the soles, was comprised of the subfamilies Soleinae, Achirinae, and Cynoglossinae. Support for this family included a preopercular margin that is hidden by skin and scales of the head, small and closely positioned eyes, a small twisted mouth, and rudimentary or absent teeth (Jordan and Evermann 1898).

Kyle (1900) studied the metamorphosis and anatomy of flatfishes to examine phylogenetic relationships of Pleuronectiformes. He deemed the following characters particularly important: 1) condition of the preopercular margin, 2) condition of the olfactory laminae, 3) position of the pelvic fins, 4) position of the nasal organs in relation to the dorsal fin, 5) size of the mouth and dentition, and 6) position of the eyes in relation to each other. The first, fifth, and six characters are consistent with Jordan and Evermann (1898).

Kyle (1900) recognized Jordan and Evermann’s (1898) families within Heterosomata based on the state of the preopercular margin. Within Pleuronectidae, Kyle (1900) divided the group into four subfamilies, Hippoglossinae, Pleuronectinae, Hippoglosso-rhombinae, and Rhombinae. Hippoglossinae was comprised of eleven genera distributed in the arctic and northern temperate zone. He considered Hippoglossinae to have primitive characteristics based on position of the blind-side eye on the dorsal ridge of head, which was observed in two of the eleven genera. Pleuronectinae was comprised of nine genera distributed just south of the distribution of Hippoglossinae in the northern temperate zone. Pleuronectinae was hypothesized to be closely related to Hippoglossinae, and was united based on similar olfactory laminae, position of the pelvic fins, the dextral position of the eyes, position of the nasal organ in relation to the dorsal fin, and the size of the mouth and dentition. Given similar characteristics shared by Soleidae and Pleuronectinae, Kyle (1900) hypothesized that Soleidae was derived from Pleuronectinae. Hippoglosso-rhombinae was comprised of eighteen genera with a distribution in tropical and sub-temperate zones, both north and south of the hemisphere. This subfamily was supported by a large symmetrical mouth and dentition, shared by Hippoglossinae, and the position of the nasal organ in relation to the dorsal fin, which is similar to Pleuronectinae. Kyle (1900) considered this subfamily to be weakly supported and based off "hazardous generalizations." Rhombinae is considered the equivalent to Psettinae of Jordan and Evermann (1898), and is comprised of eleven genera distributed in sub-temperate and tropical zones. They share the similar olfactory laminae and sinistral eyes with Hippoglosso-rhombinae. The size of the mouth and dentition is similar to that of Hippoglossine. Rhombinae is united by the position of the pelvic fin, and the position of the nasal organ in relation to the dorsal fin.

Kyle (1900) retained the Soleidae from Jordan and Evermann (1898), but stated the classification may obscure the natural relationships of subfamilies. Cynoglossidae was described as more specialized than other subfamilies of Soleidae, with a tropical distribution and sinistral eye migration. Soleinae was described as dextral with a distribution in temperate waters in the northern hemisphere. Achirinae was united by the extended base of the right pelvic fin that is confluent with the anal fin, with a distribution in temperate waters of the northern hemisphere. Kyle (1900) erected the Solei-Pleuronectinae subfamily consisting of three genera and equivalent to the Jordan and Evermann's (1898) Oncopterinae. Solei-Pleuronectinae shared characteristics of the preopercular margin state, the olfactory laminae state, position of the nasal organ to the dorsal fin, and the size of the mouth and dentition with Pleuronectidae. The character uniting the subfamily was asymmetrical pelvic fins. Kyle (1900) could not classify several genera, *Brachypleura*, *Samaris*, and *Lepidopsetta*, due to the uncertainty of character states or inability to collect characters.

Regan (1910) followed the historical classification by placing importance on dextral versus sinistral forms. He recognized Heterosomata as an order with two suborders, Psettodidae and Soleiformes. In Psettodoidae an equal number of sinistral and dextral individuals with a dimorphic optic chiasma, the placement of the right optic nerve above the left as frequently as the placement of the left optic nerve above the right, were observed. Regan (1910) suggested these traits supported the basal relationship of Psettodoidae to other Pleuronectiformes. Soleioformes, the soles, and Pleuronectiformes, the plaice, form a clade that is sister to Psettodoidae. Within Soleiformes and Pleuronectiformes, dextral and sinistral versions were grouped. Soleidae, a dextral group, and Cynoglossidae, a sinistral group, form two clades within Soleiformes. These families were united by one character which is the left or right orientation of the eyes. Bothidae and Pleuronectidae form two clades within the Pleuronectiformes. These families were defined by orientation of the eye, structure of the olfactory organs, a monomorphic optic nerve chiasma, and presence or absence of oil globules on the surface of the yolk. Bothidae was comprised of the sinistral subfamilies Bothinae, Platophrinae, and Paralichthyinae. Pleuronectidae was comprised of the dextral subfamilies Pleuronectinae, Samarinae, and Rhombosoleinae. Furthermore, Regan (1929) removed *Paralichthodes* from Samarinae and elevated the group to a monotypic subfamily of Pleuronectidae. He additionally removed suborder classifications.

Following the work of Regan (1929), Norman (1934) completed an extensive monograph of Heterosomata. He recognized Regan's (1929) families and subfamilies, but made modifications in the relationships between them. Norman (1934) added the subfamily Poecilopsettinae to Pleuronectidae, and rearranged Bothidae to contain the subfamilies Bothinae, Paralichthinae, and Scophthalminae. He agreed with previous hypotheses (Kyle 1900; Regan 1910; Regan 1929) that *Psettodes* was the most generalized flatfish, and listed similar characters to distinguish *Psettodes* as a basal group. Norman (1934) placed importance on dextral and sinistral forms for classification, but stated the characters shared by Soleidae, a dextral group, and Cynoglossidae, a sinistral group, did not form a distinct lineage. He suggested soles did not diverge from Bothidae or Pleuronectidae as previously proposed by Jordan and Evermann (1898) and Kyle (1900), but that they diverged independently from a *Psettodes*-like ancestor. Although Norman (1934) recognized the separation of flounders into Bothidae and Pleuronectidae, he stated that the characters supporting Bothidae and Pleuronectidae were of "generic importance" and should not be relied heavily upon in classification of relationships.

Hubbs (1945) erected the family Citharidae, and reviewed relationships of the flatfishes using the classification of Norman (1934) with some modification. Citharidae was comprised of two subfamilies. The subfamily Citharinae was erected from genera of the Bothidae, *Citharus*, *Citharoidae*, and *Paracitharus*. The subfamily Brachypleurinae was erected from genera of Pleuronectidae, *Brachypleura*, and *Lepidoblepharon*. Furthermore, Hubbs (1945) elevated Scophthalminae to the family level. He suggested Heterosomata may be polyphyletic and was supported by one character, both eyes located on one side of the body. Hubbs (1945) recognized three suborders, Psettodoidae, Pleuronectoidae, and Soleoidae. The basal group for flatfishes was classified as the family Psettodidae within Psettodoidae. Pleuronectoidae comprised four families, Citharidae, Scophthalmidae, Bothidae, and Pleuronectidae. Citharidae was considered to be transitional between Psettodidae and Pleuronectoidae. Scophthalmidae was stated to share many characters with Citharidae, which supported Hubbs's (1945) hypothesis that Scophthalmidae was derived from the subfamily Citharinae. The monomorphic optic chiasma and specialization of branchiostegal structures supported Hubbs's (1945) hypothesis that Bothidae and Pleuronectidae were sister taxa. Hubbs (1945) defined the suborder of Soleoidae to be comprised of the families Cynoglossidae and Soleidae. He also hypothesized that Pleuronectoidae and Soleoidae were sister taxa supported by the characteristics in brain structures and structure of anterior cranial nerves. Hubbs (1945) concluded that sinistral and dextral body forms arose three times independently within flatfishes, once in each family Citharidae, Pleuronectoidae, and Soleoidae.

All previous analyses were based solely on morphological characteristics. Lauder and Liem (1983) completed the first cladistic analysis over many actinopterygian relationships, including the Pleuronectiformes, by reviewing known characteristics and analyzing relationships using character mapping methods. They recognized eight families, Psettodidae, Citharidae, Scophthalmidae, Pleuronectidae, Bothidae, Rhombosoleidae, Soleidae, and Cynoglossidae. Lauder and Liem (1983) stated that Pleuronectiformes are monophyletic based on the placement of the eyes, and agreed with others that there are many problems with relationships within the order. They believed Psettodidae to be the sister taxa of the remaining Pleuronectiform families based on primitive characteristics of the dorsal fin not extending onto the head. The remaining families shared four characterisitics: 1) presence of palatine teeth, 2) presence of basihyal teeth, 3) presence of dorsal and anal fin spines, and 4) extension of the dorsal fin onto the head. They believed Citharidae was the sister taxa to the remaining seven families, based on the deflection of the anus onto the eyed side. Lauder and Liem (1983) did not recognize the three suborders, and their hypothesis stated that Cynoglossidae and Soleidae did not arise independently from a *Psettodes* like ancestor.

Hensley and Ahlstrom (1984) reviewed character states to analyze relationships within the Pleuronectiformes. They generated the Regan-Norman model, using a combination of phyletic and morphological methods based off hypotheses by Regan (1910) and Norman (1934), with modifications from Hubbs (1945), Amaoka (1969), Futch (1977), and Hensley (1977). Using the Regan-Norman model, Hensley and Ahlstrom (1984) examined adult, larval, and egg characteristics to evaluate the support of these relationships. Many hypotheses were concluded to be incorrect, but further information to amend the phylogeny was not available. With the information Hensley and Ahlstrom (1984) gathered they provided tentative relationships.

The characters supporting Soleoidei were deemed to be plesiomorphic for the order, and Hensley and Ahlstrom (1984) could provide only two possible synapomorphies to unite the group: 1) the skin that covers the dentary and interopercular bones is continuous across the chin and hides the isthmus and branchiostegal rays, and 2) the absence of the pleural ribs. Their data supported the subfamilies Achirinae and Soleinae by dextrality, and suggested Soleinae was not monophyletic. Hensley and Ahlstrom suggested Soleinae was more closely related to Cynoglossidae than Achirinae. Pleuronectoidei was united by one synapomorphy, the loss of a dimorphic optic chiasma. Hensley and Ahlstrom (1984) determined this synapomorphy to not be reliable as it is hard to determine what state is expressed and only a few species had been observed within Pleuronectoidei. After a thorough examination of the caudal osteology, six different hypural patterns were observed leading Hensley and Ahlstrom (1984) to question the monophyly of Pleuronectoidei and construct the bothoid group. The bothoid group was identified based on unique hypural patterns, and contained Pleuronectidae, Paralichthyidae, Scophthalmidae, Botidae, and Citharidae. Subfamilies Poecilopsettinae and Paralichthodinae, within the Pleuronectidae, were not included with the bothoid group based on a primitive hypural pattern. A monophyletic Samarinae was supported by a unique hyplural pattern and was deemed distinct from the bothoid group. The subfamily of Rhombosoleinae displayed two different hyplural patterns from other bothoids leading Hensley and Ahlstrom (1984) to determine relationships were unresolved.

By reviewing previous studies of relationships, Chapleau (1993) created a matrix of morphological characters for a cladistics analysis of family and subfamily relationships. Chapleau (1993) listed three synapomorphies that supported the Pleuronectiformes: 1) ontology characterized by the migration of one eye, 2) anterior position of the origin of the dorsal fin, and 3) presence of the *recessive orbitalis*. Although he questioned the monophyly of Paralichthodinae, Poecilopsettinae, and Rhomobosoleinae, Chapleau (1993) included them in the phylogenetic study. All four genera, *Brachypleura*, *Lepidoblepharon*, *Citharus*, and *Citharoidas* were included for Citharidae. Chapleau's (1993) analysis included thirty-nine morphological characters. *Psettodes* was used as a primary outgroup with percoids and beryciforms as secondary outgroups. The consensus of eighteen most parsimonious trees resulted in seven resolved nodes that Chapleau (1993) named lineage I though VII (Fig. 1). Lineage I contained the suborder of Psettodoidei with Psettodidae, which was the primary outgroup. Lineage II contained the suborder of Pleuronectoidei with all the remaining flatfishes and soleoid taxa. Chapleau (1993) reviewed Hensley and Ahlstrom's (1984) bothoid group and concluded monophyly was not supported. Only one of the eighteen most parsimonious trees resolved the bothoid group; however, there were too many conflicts with other characters. Chapleau (1993) concluded that the characters supporting this group were three characters that were assumed independent of each other, and to consider bothoids as monophyletic required the inclusion of soleoid taxa. Lineage III consisted of an unresolved polytomy of the Citharidae genera *Citharoidae* and *Lepidoblepharon* with the remaining pleuronectoids and soleoid taxa. Lineage IV contained Poecilopsettinae, Rhombosoleinae, and the clade containing Samearinae and soleoid taxa in an unresolved polytomy. Samarinae was resolved to be sister to soleoid taxa in Lineage V. In lineage VI

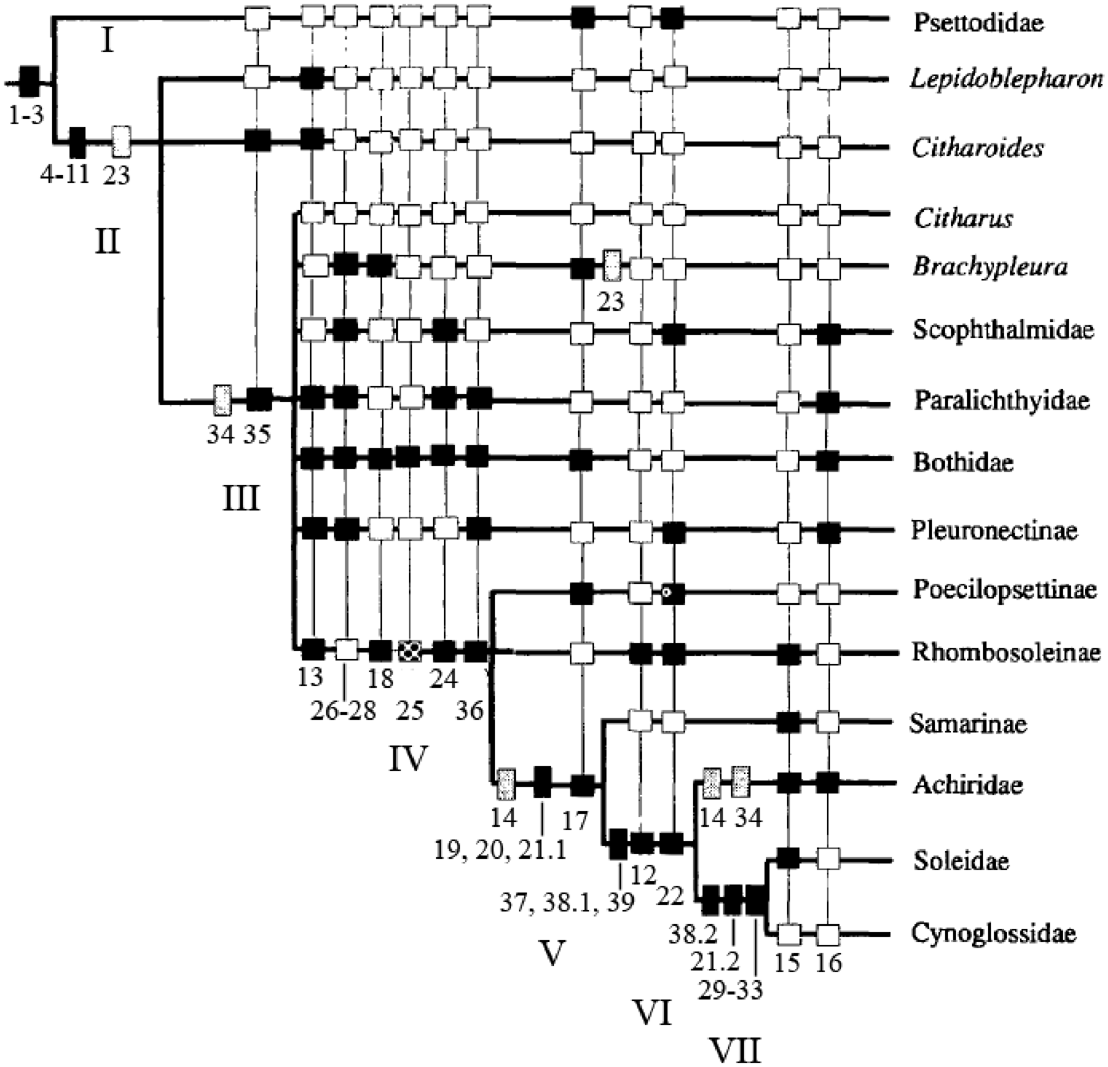

Figure 1. Chapleau (1993) consensus tree representing interrelationships of Pleuronectiformes calculated from a matrix of 39 character states. Characters used to define branching points are represented by rectangles: black rectangles represent uniquely derived character states, shaded rectangles represent derived character states with one reversal. Squares represent several reversals or convergences of traits: empty squares are plesiomorphic states, black squares are first apomorphic states, dotted squares are second apomorphic states. Roman numerals indicate lineages and decimal numbers indicate order of apomorphic states.

Achiridae was sister to the clade containing Soleidae and Cynoglossidae. The last lineage, lineage VII, contained Soleidae and Cynoglossidae. Chapleau (1993) made many modifications to historical classification. He elevated the subfamilies Achirinae, Soleidae, Pleuronectinae, Samarinae, Rhombosoleinae, and Poecilopsettinae to family level, and suggested all families to be included in Pleuronectoidei with the exception of Psettodidae.

Given that prior work exclusively utilized morphological techniques, Verneau et al. (1994) used isoenzyme electrophoresis and DNA hybridization methods to further examine the phylogeny of flatfishes. This study was limited to a few Mediterranean and Atlantic species, the genera *Scophthalmus*, *Psetta*, and *Lepidorhombus* in Scophthalmidae, the genera *Platichthys*, *Limandam*, and *Pleuronectes* in Pleuronectidae, *Arnoglossus* in Bothidae, *Citharus* in Citharidae, and *Solea* and *Microchirus* in Soleidae. Verneau et al. (1994) used two analyses to examine the isoenzyme and DNA hybridization data. A DOLLOP analysis was used to generate a phylogeny that allowed ancestral polymorphisms, and a CLIQUE analysis was used to generate a phylogeny that excluded homoplastic events. An outgroup was not included for the analyses. Verneau et al. (1994) were unable to find common characters that united the Soleidae with the other taxa included in the study, resulting in Soleidae as sister taxa to all other taxa.

Berendzen and Dimmick (2002) were the first to analyze relationships among Pleuronectiformes using nucleotide sequence data of 12S and 16S mitochondrial ribosomal genes. Samples were obtained from Achiridae, Bothidae, Citharidae, Cynoglossidae, Paralichthyidae, Pleuronectidae, Poecilopsettidae, Psettodidae, Samaridae, Scophthalmidae, and Soleidae. Individuals from the genera *Perca*, *Pterois*, *Melichthys*, *Scopeloberyx*, *Beryx*, and *Zeus* were used as outgroups. Unweighted parsimony resulted in a monophyletic Pleuronectiformes, consisting of three parts, with *Psettodes* as sister to all other flatfish taxa (Fig. 2). In part I, Bothidae and Paralichthyidae was monophyletic in all analyses (Fig. 2). Part II resulted in a monophyletic clade of *Trinectes*, Citharidae, Cynoglossidae, Poecilopsettidae, Samaridae, and Soleidae in some analyses (Fig. 2). Pleuronectidae and the remaining Paralichthyidae, part III, were monophyletic in all analyses. *Scopthalmus* formed a tricotomy with parts II and III (Fig. 2).

Weighted parsimony analyses resulted in relationships similar to unweighted parsimony with a few discrepancies. Pleuronectid genera, *Isopsetta*, *Lepidosetta*, and *Plathichthy*, showed different relationships among each other than were shown in weighted parsimony. Pleuronectiformes were monophyletic and sister to *Psettodes*, like in the unweighted parsimony analyses. *Scophthalmus* resolved as sister to part II, and part III did not form a monophyletic group. Bayesian analyses resulted in a monophyletic Pleuronectiformes with *Psettodes* as sister to other taxa, with part I consisting of Bothidae and Paralichthyidae, and part III consisting of Pleuronectidae and the remaining Paralichthyidae. Part II was not monophyletic in the Bayesian analyses.

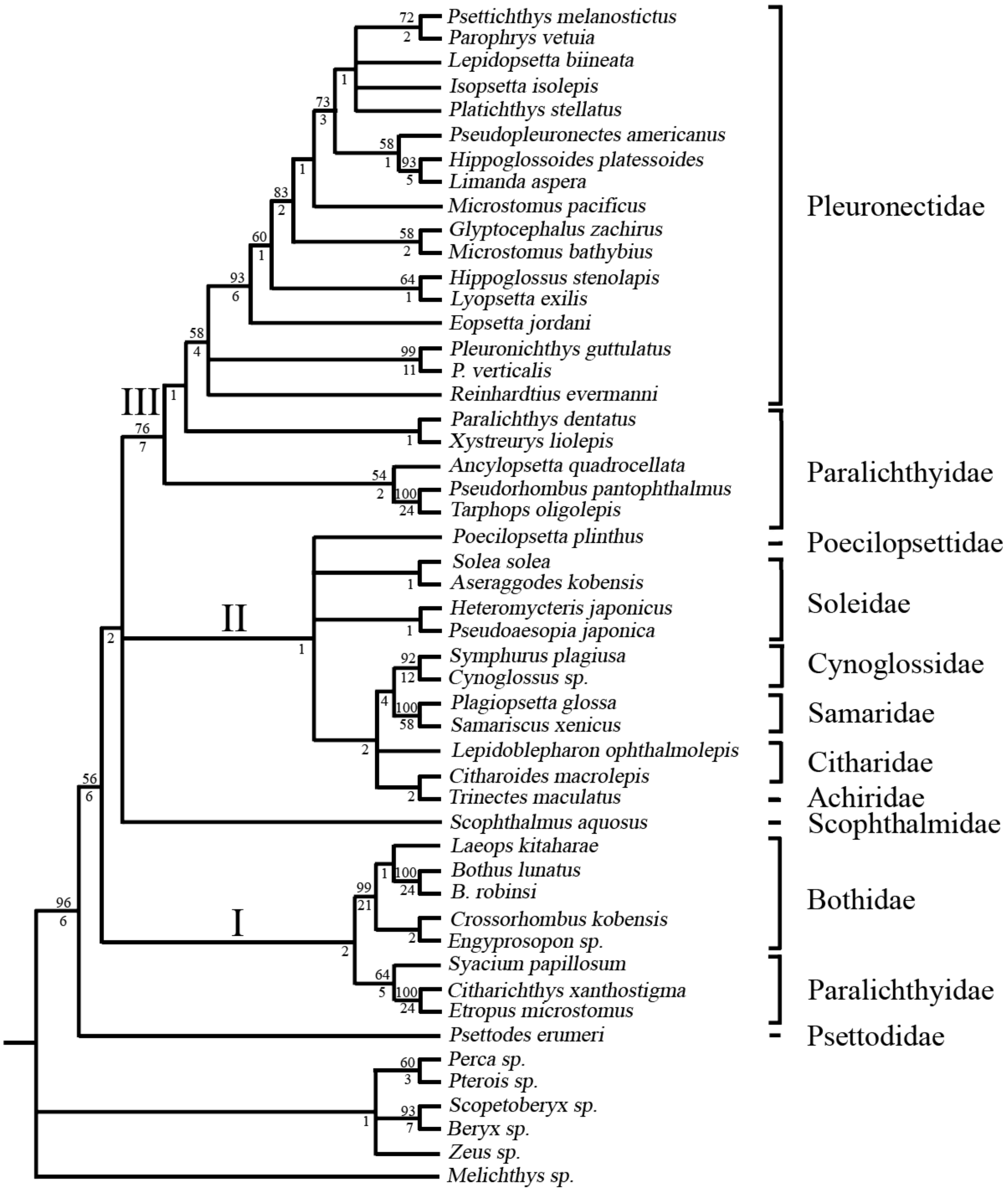

Figure 2. Berendzen and Dimmick (2002) strict consensus tree resulting from 32 equally most-parsimonious trees representing relationships of Pleuronectiformes. All nucleotides were weighted equally. Roman numerals represent distinct parts. Numbers above the node indicate bootstrap values, and numbers below indicate Bremer decay indices.

Following Berendzen and Dimmick (2002), Pardo et al. (2005) utilized 16S rRNA genes to generate a phylogeny of Scophthalmidae, Pleuronectidae, Paralichthyidae, Cynoglossidae, Soleidae, Bothidae, and Achiridae. The objective of this study was to analyze relationships of Pleuronectiformes as well as outgroups that may result in better support for relationships within the Pleuronectiformes. *Aulopus purpurissatus* and *Hyporhamphus* showed the best “overall effect” on tree topology, reducing the number of polytomies and increasing the consistency values in Pleuronectiformes. Using the genera *Aulopus* and *Hyporhamphus*, Pardo et al. (2005) generated phylogenies using Bayesian, maximum parsimony, maximum likelihood, and neighbor-joining methods. The results supported prior findings of the monophyletic origin of Pleuronectiformes, as well as a polyphyletic Paralichthyidae (Chapleau 1993; Berendzen and Dimmick 2002). Their phylogenies resulted in two groups of Paralichthyidae. Paralichthyidae I contained *Citharichthys*, *Etropus*, and *Syacium*, which were related to members of Bothidae and Achiridae and supported previous hypotheses (Hensley and Ahlstrom 1984; Berendzen and Dimmick 2002). Paralichthyidae II was a clade containing *Paralichthys* and *Pseudorhombus*, and was related to members of Pleuronectidae. Given this data, Pardo et al. (2005) suggested including the genera *Paralichthys* and *Pseudorhombus* in the Pleuronectidae. Unlike Berendzen and Dimmick (2002), whose data supported a close relationship between Achiridae and Soleidae, Pardo et al. (2005) was unable to find those relationships, but instead supported a close relationship between a clade containing Achiridae and Bothidae and *Cycolpsetta*. When combining their data with Berendzen and Dimmick (2002), the main clades were supported, and Paralichthyidae still showed two distinct groups. A joint analysis could not resolve relationships between Pleuronectidae and Paralichthyidae II, nor between Achiridae and Poecilopsettidae.

Azevedo et al. (2008) continued Berendzen and Dimmick’s (2002) and Pardo’s et al. (2005) work by collecting 12S and 16S mitochondrial rRNA sequences from nineteen species from seven families of flatfish, Achiridae, Bothidae, Cynoglossidae, Paralichthyidae, Pleuronectidae, Scophthalmidae, and Soleidae, and combining this data with forty-two additional sequences from GenBank. Maximum parsimony, maximum likelihood, and Bayesian inference were performed using a single species of Psettodidae. As in previous studies, all families of Pleuronectiformes were monophyletic with the exception of Paralichthyidae (Chapleau 1993; Berendzen and Dimmick 2002; Pardo et al. 2005). The first group, Paralichthyidae I, was found to be related to Bothidae in all analyses, and was composed of *Cyclopsetta*, *Syacium*, *Citharichthys*, and *Etropus*. *Etropus* was paraphyletic with the species *E. microstomus* being closely related to *Citharichthys xanthostigma*. The second group, Paralichthyidae II, was polyphyletic and composed of two clades. The first clade was composed of the genera *Pseudorhombus* and *Tarphops*, with the second monophyletic group comprised of the genera *Paralichthys* and *Xystreurys*. Azevedo et al. (2008) concluded that their data corroborated the monophyletic status of most Pleuronectiformes, but the order needed further work.

Previous studies assumed the sister group to all other Pleuronectiformes was Psettodidae, but the most recent phylogenetic hypothesis has concluded the order of Pleuronectiformes is polyphyletic. The monophyletic suborder Pleuronectoidei was shown to be sister to Centropomidae, a family of Perciformes which include the common snook, *Centropomus undecimalis*, excluding Psettodidae entirely (Campbell et al. 2013). Campbell et al. (2013) analyzed six independent, single copy, protein-coding nuclear genes of ninety taxa, including twenty-five Pleuronectiformes. All maximum likelihood analyses supported a non-monophyletic Pleuronectiformes, with Bayesian tree inference and divergent estimates suggesting the origin of Psettodidae at 77.4 million years ago (Ma) and the split of Pleuronectoidei and Centropomidae at 75.3 Ma. Campbell et al. (2013) mentioned that targeting Centropomidae as the sister taxa to Pleuronectiformes over other groups could have potential bias. This is the first study to support the hypothesis of a polyphyletic Pleuronectiformes, but the hypothesis has a long standing history. Amaoka (1969), Hubbs (1945), Kyle (1921), Norman (1934), and Regan (1910, 1929) have defined similarities of Psettodidae to percoids. This discovery provides further evidence of convergent evolution of eye migration, although further evidence will be needed to validate the hypothesis of a non-monophyletic Pleuronectiformes.

# CHAPTER 2

# FAMILIES OF PLEURONECTIFORMES

The order of Pleuronectiformes is highly diverse, consisting of fifteen recognized families. This chapter will provide a detailed background of each family, including unique characteristics, habitat preference, and the historical taxonomy of each family.

## Psettodidae

The Psettodidae, known as the toothed or spiny flatfishes, are characterized by their plesiomorphic characteristics as the position of the migrating eye on the dorsal midline of the skull and less asymmetry of the eyed and blind sides. This family consists of one genus, *Psettodes*, and three species, *P. belcheri*, *P. bennetti* and *P. erumei* ranging from western Africa to the Indo-West Pacific (Nelson 2006). Psettodidae can be distinguished externally by the posterior location of the dorsal fin, spines in the dorsal and anal fin, a large mouth with specialized teeth, nearly rounded bodies, and no obvious asymmetry in the lateral musculature. They are large in size and display dextral and sinistral individuals within populations (Chapleau 1993; Munroe 2005). Internally, the location of the pseudomesial bar, located between the blind side lateral ethomoid and blind side frontal, is extended anteriorly past the lateral ethomoid, which is a synapomorphy for Psettodidae (Chabanaud 1934; Gibson 2005). This family has been widely recognized a member of Pleuronectiformes (Chapleau 1993; Berendzen and Dimmick 2002; Pardo et al. 2005; Azevedo et al. 2008), but recent evidence suggests Psettodidae is excluded from Pleuronectiformes entirely (Campbell et al. 2013).

Campbell et al. (2013) provides evidence supporting convergent evolution of eye migration of Psettodidae and the rest of Pleuronectiformes (see Chapter 1).

## Citharidae

The Citharidae is comprised of five genera and six species distributed in the Mediterranean and Indo-West Pacific (Nelson 2006). Also known as the large-scale flounder, relationships within this family have been highly controversial (Munroe 2005). Hubbs (1945) erected this family by regrouping two opposite ocular asymmetrical genera from Bothidae (sinistral asymmetry) and Pleuronectidae (dextral asymmetry). Support for union of this family includes the deflection of the vent to the eyed-side, and a strong bilateral asymmetry of the pectoral rays (Hubbs, 1945). Hensley and Ahlstrom (1984) disputed the monophyly of the family as many of the synapomorphies defined by Hubbs (1945) are plesiomorphic for Pleuronectiformes: 1) retention of the pelvic spines; 2) retention of the supramaxillae; 3) close location of the urinary papilla to the anus; 4) separated branchiostegals; 5) retention of vomerine teeth; and 6) retention of short-based ventral fins. They stated that the only character that could be interpreted as a synapomorphy for the family was the position of the vent on the ocular side. However, Hensley and Ahlstrom (1984) questioned whether or not Hubbs (1945) classified the character state correctly because it can be hard to determine where the vent is located if it is close to the midventral line. Furthermore, Hensley and Ahlstrom identified different hypural patterns for every genus. Based on these patterns, they suggested removing *Brachypleura* from Citharidae, placing it in Bothidae. A cladistic analysis including all four genera of Citharidae performed by Chapleau (1993) did not support monophyly of the family. Chapleau (1993) determined that vent deflection varied greatly within the group and therefore should not be used as a synapomorphy. Hoshino’s (2001) phylogenetic examination of forty-five osteological, mycological, and external characters supported the monophyly of Citharidae. Hoshino (2001) was able to unite the citharids by three synapomorphies; 1) the exoccipitals form the ventral margin of the foramen magnum, 2) there are teeth present on epibranchial three, and 3) the arterial canal perforates the anterior ceratohyal. Berendzen and Dimmick (2002) concluded that Citharidae was not monophyletic. Their phylogeny was based on nucleotide sequence data for 12S and 16S mitochondrial ribosomal genes and included one representative for each subfamily. In contrast, the phylogenetic analysis by Azevedo et al. (2008) based on partial sequences of the 12S and 16S mitochondrial ribosomal genes supported a monophyletic Citharidae. However this may be misleading because two individuals within the same subfamily were used to support a monophyly of the group.

## *Tephrinectes*

Although not currently classified as a family, *Tephrinectes* has been suggested for removal from its current classification in Paralichthyidae. The genus is thought to be a distinct lineage. Also known as the flower flounder, this genus contains one species, *Tephrinectes sinensis*. Populations consist of sinistral and dextral individuals found in coastal seas of China. (Munroe 2005; Nelson 2006)

The removal of the monotypic genus from Paralichthyidae was first suggested by Hensley and Ahlstrom (1984) based on osteological observations. Hensley and Ahlstrom (1984) recommended the genus be recognized as a distinct lineage because the caudal skeleton does not share “bothid” characteristic. Hoshino and Amaoka (1998) agreed with the reclassification of *Tephrinectes* and hypothesized that *Tephrinectes* is the sister group to Chapleau’s (1993) clade of Poecilopsettidae, Rhombosoleidae, Samaridae, Achiridae, Soleidae, and Cynoglossidae. Hoshino (2001) suggested the relationship of *Tephrinectes* was more basal, being the sister group to the remaining Pleuronectoidei excluding Citharidae.

### Scophthalmidae

Scophthalmidae, known as the turbots, consists of four genera with approximately eight species distributed in the North Atlantic, Baltic, Mediterranean, and Black seas (Nelson 2006). They range from small to large in size, and populations consist of only sinistral individuals. They are characterized by a relatively large mouth and eyes (Munroe 2005).

Kyle (1900) was the first to recognize the similarity of these fishes and assembled them into a turbot-like group in Rhombinae. Regan (1910) reclassified these species as the subfamily Bothinae of Bothidae based on the sinistral eye migration. Upon further morphological investigation, Norman (1934) reclassified the scophthalmid genera, uniting *Scophthalmus*, *Lepidorhombus*, *Phrynorhombus*, and *Zeugopterus*, as a subfamily within Bothidae based on anterior extension of two pelvic fins, sinistral migration of the eye, and presence of vomerine teeth. Scophthalmidae was elevated by Hubbs (1945) to family level. Supplementary support for monophyly of Scophthalmidae was added by Hensley and Ahlstrom (1984) and Chapleau (1993). Chapleau (1993) included an elongated supraoccipital process forming a bridge with the dorsal margin of the blind side frontal bone to the synapomorphies of this family. He concluded that several species displayed this morphology, but this characteristic is hard to observe and further observations must be made before considering this synapomorphy. Chanet (2003) supported monophyly of the group and provided five additional synapomorphies that unite the family: 1) anterior extension of both pelvic fins to the isthmus, 2) a bridge formed by the supraoccipital with the dorsal margin of the right frontal, 3) asymmetric lateral expansions of both pelvic bones, 4) asymmetrical transverse apophyses on the caudal vertebrae, and 5) bent contact of the first neural spine to the dorsal margin of the cranium. Genetic evidence, based on 12S and 16S mitochondrial data of four species within Scophthalmidae, also supported monophyly of the family (Pardo et al. 2005; Azevedo et al. 2008).

### Paralichthyidae

Paralichthyidae is a generalized group of mostly sinistral flatfishes that is currently recognized as a paraphyletic group. Commonly called the large toothed flounders, the family is comprised of sixteen genera and 105 species. They can be found in the Atlantic, Indian, and Pacific oceans, and are considered a family until further study demonstrates otherwise (Munroe 2005, Nelson 2006).

Norman (1934) recognized Paralichthyinae as one of three subfamilies of the Bothidae based on pelvic fin morphology and vertebral structure. Amaoka (1969) elevated Norman’s subfamily to family level based on nine morphological characters. Based on bothid like characteristics, the position of the ocular ventral fin on the midventral line and anteriorly extended base, Hensley and Ahlstrom (1984) removed

Norman’s (1934) paralichthyid genera, consisting of *Trichopsetta*, *Engyophrys*, *Taeniopsetta*, *Monolene*, and *Perissia*, and placed them in the bothids. Hensley and Ahlstrom also determined Amaoka’s (1969) characters, defined by hypural pattern six, were plesiomorphic for the bothids. Two genera, *Thysanopsetta* and *Tephrinectes*, were determined by Hensley and Ahlstrom (1984) to have a primitive hypural pattern and were thus removed from the bothiods. Hensley and Ahlstrom determined the *Cyclopsetta* group, consisting of *Cyclopsetta*, *Syacium*, *Citharichthy*, and *Etropus*, was monophyletic based on pelvic fin morphology, position of the urinary papilla, and the arrangement of the caudal fin rays. The *Pseudorhombus* group, consisting of *Pseudorhombus*, *Tarphops*, and *Cephalopsetta*, was determined to have a possible monophyletic status, but Hensley and Ahlstrom were unable to define synapomorphies to support the group. Hensley and Ahlstrom (1984) suggested that the *Pseudorhombus* group was more specialized than other genera. The remaining genera, *Ancylopsetta*, *Gastropsetta*, *Hippoglossina*, *Lioglossina*, *Paralichthys, Verecundum,* and *Xystreurys*, were grouped into *Paralichthys* by Hensley and Ahlstrom (1984). This group is defined by plesiomorphic characteristics for the order of the bothoid group, and is recognized as paraphyletic (Hensley and Ahlstrom 1984). Chapleau (1993) provided further support for the monophyly of *Cyclopsetta* group with five synapomorphies; 1) position of the urinary papilla oriented toward the blind side, 2) the ocular pelvic fin positioned on the midventeral line of body, 3) the blind side pelvic-fin base anteriorly located to the ocular side, 4) the caudal fin with seventeen rays not supported by preural, neural or hemal spince, and 5) the hypural five fused with epural. Chapleau (1993) could not provide evidence for the monophyly of

*Paralichthys*, and he was unable to find unique characters that defined *Pseudorhombus*. Hoshino (2000, 2001) did not find support for monophyly of the bothoid group based on morphological evidence. Hoshino (2000, 2001) discovered a more basal position of *Tephrinectes* to the remaining families, following Citharidae. Furthermore, genetic evidence has been unable to support the monophyly of Paralichthyidae. In Berendzen and Dimmick's (2002) phylogeny, based on 12S and 16S mitochondrial genes of eight genera, two distinct groups were found. One clade, consisting of *Citharichtys, Etropus,* and *Syacium*, was closely related to Bothidae. The other clade, consisting of *Pseudorhombus, Tarphops, Ancylopsetta,* was closely related to Pleuronectidae. Two genera fell within Pleuronectidae: *Xystreurys* and *Paralichthys*. The relationships within Paralichthyidae have been supported by further genetic work based on 12S and 16S mitochondrial genes (Pardo et al. 2005; Azevedo et al. 2008). Azevedo et al. defined three independent lineages. The first lineage is related to Bothidae, and consists of *Cyclopsetta, Syacium, Citharichthys,* and *Etropus*. The second lineage is composed of *Pseudorhombus* and *Tarphops* and is related to Pleuronectidae. The third lineage contains *Paralichthys* and *Xystreurys* and falls within Pleuronectidae.

### Pleuronectidae

Pleuronectidae is a large family of mostly dextral fishes commonly called the right-eye flounders. Found primarily in marine waters in the Arctic, Atlantic, Indian, and Pacific oceans, a few reside in brackish and fresh water. This family contains many commercially important fishes and is divided into five subfamilies, four tribes, twenty-three genera, and sixty species. The subfamily Hippoglossinae contains five genera, *Atheresthes, Clidoderma, Hippoglossus, Reinhardtius,* and *Verasper* with eight species. The subfamily Eopsettinae contains one genus, *Eopsetta*, with two species. The subfamily Lyopsettinae contains one monotypic genus, *Lyopsetta*. The subfamily Hippoglossoidinae contains three genera, *Acanthopsetta*, *Cleisthenes*, and *Hippoglossoides*, with seven species. The last subfamily, Pleuronectinae, is currently divided into four tribes. The tribe Psettichthyini contains one monotypic genus, *Psettichthys*. The tribe Isopsettini contains one monotypic genus, *Isopsetta*. The tribe Microstomini contains six genera, *Dexistes, Embassichthys, Glyptocephalus, Lepidopsetta, Microstomus,* and *Pleuronichthys,* with twenty species. The tribe Pleuronectini contains five genera, *Limanda*, *Parophrys*, *Platichthys*, *Pleuronectes,* and *Pseudopleuronectes,* with twenty species (Nelson 2006). Monophyly of Pleuronectidae has been further supported by mitochondrial evidence (Berendzen and Dimmick 2002; Pardo et al. 2005; Azevedo et. al 2008).

Jordan and Evermann (1898) first recognized Pleuronectidae, grouping all flounder like fishes, with six subfamilies, Hippoglossinae, Psettinae, Samarinae, Pleuronectinae, Oncopterinae, and Pelecanichthinae. Kyle (1900) revised Jordan and Evermann's (1989) classification to include four subfamilies, Hippoglossinae, Pleuronectinae, Hippoglosso-rhombinae, and Rhombinae. Regan (1910) restricted the family to right-eyed flounders, reorganizing genera into three subfamilies, Pleronectinae, Samarinae, and Rhombosoleinae. Furthermore, Regan (1929) removed *Paralichthodes* from Samarinae and elevated the genus to the subfamily Paralichthodinae. Norman (1934) recognized Regan's (1929) subfamilies, erecting a fifth subfamily, Poecilopsettinae, containing the genera *Poecilopsetta*, *Nematops*, and *Marleyella*. Regan (1910, 1929) and Norman (1934) defined Pleuronectidae based on dextrality and the absence of oil globules in the egg. Nelson (1984) grouped Pleuronectinae into two tribes, Hippoglossini and Pleuronectini. Sakamoto (1984) recognized four subfamilies, Pleuronectinae, Rhombosoleinae, Samrinae, and Poecilopsettinae, in a phenetic study of seventy-seven species based on twelve skeletal elements and a few external and other internal characteristics. He defined the family based on several plesiomorphic characters, including dextrality, monomorphism of the optic chiasma, the free margin of one preopercle, and the absence of spines in fins (Chapleau 1993). Sakamoto (1984) defined Pleuronectinae, including the genus of *Paralichthodes*, by the presence of a neural arch on the first precaudal vertebrae. Sakamoto (1984) grouped a number of genera without the recognition of Nelson's (1984) tribes. Hensley and Ahlstrom (1984) concluded that many synapomorphies used to unite the family were plesiomorphic for the order or the bothids. Chapleau (1993) agreed with Hensley and Ahlstrom (1984) that Sakamoto's (1984) characters were plesiomorphic, stating that a dextral body is not restricted to the group and the absence of an oil globule in the egg was too variable. Chapleau (1993) also concluded that the distribution and homology of Norman's (1934) characteristics of the olfactory laminae was not well known.

Chapleau (1993) suggested the elevation of Pleuronectinae from subfamily to the family level, supported by well-developed lateral lines on both sides of the body and olfactory laminae that are nearly always parallel without a rachis (Norman 1934). Cooper and Chapleau (1998) used characters from Sakamoto (1984) plus their own characters to examine intrarelationships of the family Pleuronectidae. Based on a resolved species-level cladogram, Cooper and Chapleau (1998) suggested the elevation of the subfamily Pleuronectinae to the family level of Pleuronectidae. They provided evidence for the monophyly of the group based on ten synapomorphies; 1) the ocular-side frontal is articulated with the mesethmoid, 2) the ocular side preorbital sensory canal is absent, 3) the ventral margin of metapterygoid is flattened, 4) the first and second basibranchials are loosely joined by cartilage, 5) the second and third badibranchial are loosely joined by cartilage, 6) the most posterior abdominal vertebrae lacks a haemapophysis, 7) the accessory processes on caudal vertebrae are absent, 8) the ocular-side infraorbital bones are present, 9) oil globules in eggs are absent, and 10) the olfactory laminae are parallel without a central rachis. Furthermore, genetic evidence based on 12S and 16S mitochondrial DNA supported monophyly of the family (Berendzen and Dimmick 2002; Pardo et al. 2005; Azevedo et al. 2008). Azevedo et al. (2008) suggested the subfamilies needed to be re-evaluated because Eopsettinae and Isopsettini were the only subfamilies that were monophyletic.

## Bothidae

Bothidae, also known as the left-eyed flounders, is a large and diverse family consisting of two subfamilies, twenty genera, and 140 species of mostly sinistral fishes (Nelson 2006). Bothidae displays sexual dimorphism with characteristics that differ between female and male specimens including scales, rostral and orbital spines, interorbital width, fin shapes, coloration, and teeth (Norman, 1934). Fishes of this family can be found in the Atlantic, Indian, and Pacific oceans (Amaoka and Mihara 2001; Nelson 2006).

Bothidae was erected by Regan (1910) based on five morphological synapomorphies: 1) sinistral location of the eye, 2) dorsal location of the optic nerve of the right eye, 3) transversal arrangement of the olfactory laminae to or branching from a central rachis, 4) eggs have a single oil-globule in the yolk, and 5) the presence of pectoral radials. Within the family, Regan (1910) recommended three subfamilies: Paralichthyinae, Platophrinae, and Bothinae. Norman (1934) recognized Regan's (1910) Bothidae, but substituted Regan's subfamilies with Paralichthyinae, Bothinae, and Scophthalinae based on the high degree of ventral-fin asymmetry and the presence of vertebral transverse apophyses. Amaoka (1969) analyzed relationships of sinistral flounders, including bothids, off the coast of Japan based on morphological characteristics. He removed paralichthyines and elevated Bothinae to the family level. Amaoka (1969) redefined Bothidae to have two subfamilies: Taeniopsettinae, removed from Norman's (1934) Paralichthyidae, and Bothinae. Amaoka (1969) recognized eighteen characteristics that defined Bothidae from Psettodidae, Citharidae, and Paralichthyidae. Hensley and Ahlstrom (1984) collected data on larval and adult morphology and concluded that Amaoka's (1969) hypothesis was monophyletic and definable by adult synapomorphies. They recognized eight synapomorphies in total, three adult and five larval, and reviewed many of the remaining bothids. They concluded that the remaining bothids displayed all synapomorphies defining Bothidae except *Mancopsetta*, which showed a primitive hypural pattern. *Mancopsetta* was removed from the family and placed in Rhombosolediae. Hensley and Ahlstrom (1984) also added *Perissias* to the Bothidae based on morphological characteristics. Evseenko (1984, 2000) removed several genera from Bothidae to Achiropsettidae based on morphological characteristics. Chapleau (1993) concluded that the monophyly of Taeniopsettinae was questionable based on plesiomorphic characteristics used as synapomorphies for the family. He also discredited two characteristics believed by Hensley and Ahlstrom (1984) to be unique to bothids: an elongated eyed-side pelvic fin base on the mid-ventral line, and the absence of the blind-side preorbital. Genetic evidence based on 12S and 16S mitochondrial data supported monophyly of the family, although low sampling numbers did not allow for comment on interrelationships (Berendzen and Dimmick 2002; Pardo et al. 2005; Azevedo et al. 2008).

### Paralichthodidae

Paralichthodidae is a monotypic family endemic to the inner continental shelf of South Africa. Commonly called measles or peppered flounder, *Paralichthodes algoensis* is a dextral medium sized fish (Munroe 2005; Nelson 2006).

Regan (1910) originally placed *P. algoensis* in Samarinae based on the absence of a distinct caudal peduncle, the extension of the dorsal fin to the end of the snout, and asymmetrically placed pelvic fins. Regan (1910, 1929) later revised this classification, erecting the subfamily Paralichthodinae within Pleuronectidae, based on dextrality, nerve of left eye always dorsal, a terminal mouth, and a prominent lower jaw. Norman (1934) and Hubbs (1945) accepted Regan's (1929) subfamily Paralichthodinae. Nelson (1984) revised Regan's (1929) classification, regrouping Paralichthodinae with Samarinae based on the origin of dorsal fin, a well-developed lateral line, and the symmetry of the pelvic fins. Hensley and Ahlstrom (1984) indicated that Paralichthodinae was not part of their bothoid group based on hypural pattern. They also stated the description of the optic nerve morphology, as used by Regan (1929), was not well understood and should not be included as a synapomorphy. Sakamoto (1984) removed *P. algoensis* from Nelson's (1984) revision, placing it within Pleuronectinea (Pleuronectidae) based on overall osteological similarity.

Chapleau (1993) recognized Nelson's (1984) character, anterior origin of the dorsal fin, as present in all Pleuronectiformes. Chapleau (1993) also stated that the remaining two characters, the terminal mouth and prominent lower jaw, are plesiomorphic. He stated that the osteological similarities used by Sakamoto (1984) were plesiomorphic. Nelson (1984) reclassified *P. algoensis* as a monotypic subfamily Paralichthodinae within Pleuronectidae, but indicated his uncertainty of this classification. Cooper and Chapleau (1998) were able to establish the monophyly of Paralichthodinae with two synapomorphies, the horizontal location of the first pterygiophore over the orbital region, and the absence of teeth on the third epibranchial. Cooper and Chapleau (1998) suggested the re-elevation of Paralichthodinae to Regan's family of Paralichthodiae, and determined the phylogenetic position as the sister group to a clade containing Poecilopsettidae, Rhombosoleidae, Samaridae, Achiropsettidae, Soleidae, and Cynoglossidae.

## Poecilopsettidae

The Poecilopsettidae, also known as the bigeye flounders, is a group of small sized, dextral fish that reside in deep waters of the Atlantic, Indian, and Pacific oceans. Poecilopsettidae consists of three genera, *Marleyella*, *Nematops*, and *Poecilopsetta*, with twenty species (Munroe 2005; Nelson 2006).

Norman (1934) united the genera of *Marleyella*, *Nematops*, and *Poecilopsetta* based on two characters: a rudimentary lateral line on the blind side, and the structure of the olfactory laminae. The united genera were raised to the subfamily level in Poecilopsettinae within Pleuronectidae (Norman 1934). Hensley and Ahlstrom (1984) stated the characters used to define the group were inadequately investigated, and the subfamily was poorly known. Sakamoto (1984) redefined Poecilopsettinae by two characteristics: the absence of the lachrymal, and the attachment of both lateral ethmoids to each other on the lower anterior portion of the frontal and ocular side. Chapleau (1993) recognized Sakamoto's (1984) character, regarding the attachment of the lateral ethmoids, as the only synapomorphy uniting the Poecilopsettinae. Chapleau (1993) disregarded Norman's (1934) characters because they were understudied, and Sakamoto's (1984) lachrymal character because it was not unique to the Poecilopsettinae. Chapleau (1993) further suggested the elevation of Poecilopsettinae from subfamily level to the family level of Poecilopsettidae based on an extensive examination of Pleuronectidae.

## Rhombosoleidae

The Rhombosoleidae is a group that closely resembles Soleidae. They can be found in relatively shallow water around Australia and New Zealand, with one species found in the south-western Atlantic. Two species of rhombosolea are known to enter fresh water in New Zealand. This family comprises nine dextral genera, *Ammotretis, Azgopus*, *Colistium*, *Oncopterus*, *Pelotretis*, *Peltorhamphus*, *Psammodiscus*, *Rhombosolea*, and *Taratretis*, with nineteen species (Munroe 2005; Nelson 2006).

Regan (1910) and Sakamoto (1984) characterized the subfamily Rhombosoleinae by pelvic fin asymmetry; the ocular side fin is unusually long and situated along the mid-ventral line. Norman (1934) classified Rhombosoleinae as a subfamily of Pleuronectidae, and defined this group by six characters: 1) there are no radials associated with the pectoral fins, 2) the position of the dorsal fin is anterior, 3) the hemapophyses on the precaudal vertebrae is absent, 4) the reduced size of the coracoids, 5) equally developed lateral line on the ocular and blind sides, and 6) absence or presence of a rachis on the olfactory lamina. Both Hensley and Ahlstrom (1984) and Chapleau (1993) concluded that Norman’s (1934) characters were too variable or found in other Pleuronectiformes. Hensley and Ahlstrom (1984) suggested the Rhombosoleinae may be monophyletic, but could not provide evidence. Based on an examination of Pleuronectidae, the removal of Rhombosoleinae and subsequent elevation of the group to family level was suggested by Chapleau and Keast (1988). Chapleau (1993) suggested the placement of pelvic fin bases may be a synapomorphy for the family, but asymmetry of pelvic fins was not unique to the group. Chapleau (1993) discussed the need for more research to define relationships of Rhombosoleidae.

### Achiropsettidae

The Achiropsettidae, commonly known as the armless or southern flounders, is a group of sinistral-bodied fish defined by the absence of pectoral fins in adults. Distributed in the Southern Ocean, this family contains four genera, *Achiropsetta*, *Mancopsetta*, *Neoachiropsetta*, and *Pseudomancopsetta*, with five to six species (Munroe 2005; Nelson 2006).

Evseenko (1984) erected the family Achiropsettidae by grouping three genera, formally classified in Bothidae, with his *Pseudomancopsetta*. Grouped by absence of pectoral fins and a few other traits, Evseenko (1984) claimed Achiropsettidae represented an intermediate group between Citharidae and Paralichthyidae, and Bothidae. Hensley and Ahlstrom (1984) suggested the genera previously removed from Bothidae, *Achiropsetta*, *Mancopsetta*, and *Neoachiropsetta*, should be united with Rhombosoleidae. They also stated that these genera, with more evidence, may comprise a monophyletic group. Evseenko (1996) listed twenty-one shared characters, and suggested a closer relationship of his Achiropsettidae to *Branchypleura* of Citharidae. Furthermore, Evseenko (2000) provided more evidence for the monophyly of Achiropsettidae, and hypothesized the family as a sister group to a clade containing Samaridae, Achiridae, Soleidae, and Cynoglossidae. Evseenko (2000) was able to show evidence of two clades within Achiropsettidae, *Pseudomancopsetta* and *Mancopsetta*, and *Neoachiropsetta* and *Achiropsetta* based on osteological characters and external morphology.

## Samaridae

The Samaridae, commonly called crested flounders, are dextral fishes that reside in deep water. Located in tropical and subtropical waters of the Indo-Pacific, this group consists of three genera, *Plagiopsetta*, *Samaris*, and *Samariscus*, with twenty species. The type specimen of *Samaris* is characterized by elongated and filamentous anterior dorsal rays, ocular pectoral fins, and ocular pelvic fins (Munroe 2005; Nelson 2006).

Historically, Regan (1910) grouped three genera, *Paralichthodes*, *Brachypleura*, and *Samaris*, in the subfamily Samarinae within Pleuronectidae. Norman (1934) removed *Paralichthodes* and added the genera *Lepidoblepharon* and *Samariscus* to Samarinae. Hubbs (1945) removed *Brachypleura* and *Lepidoblepharon* and placed them in Citharidae. Hensley and Ahlstrom (1984) examined four characters and concluded that a unique hypural pattern was the lone character supporting monophyly of Samarinae. Sakamoto (1984) defined ten characteristics that validated monophyly of Samarinae based on osteological characters. Chapleau (1993) determined that all of Norman's (1934) characteristics were plesiomorphic, except the absence of the blind-side pectoral fin. Chapleau (1993) reviewed Sakamoto's (1984) characters and determined four could be used to support Samarinae: 1) the lateral ethmoids are attached on the dorsal part of the anterior portion of eyed-side frontal, 2) the blind-side lateral ethmoid is attached to the eyed-side frontal in the middle portion of the dorsal cavity of the migrated eye, 3) the eyed-side frontal is broadly attached to the parasphenoid in the inter-orbital region, and the metapterygoid is small. The last two characteristics were determined autapomorphies for the group. Lastly, Chapleau and Keast (1988) and Chapleau (1993) suggested the elevation of Samarinae to Samaridae. Furthermore, 12S and 16S mitochondrial DNA supported the monophyly of Samaridae, although this was based on a limited number of species, *Plagiopsetta glossa* and *Samariscus xenicus* (Berendzen and Dimmick 2002; Azevedo et al. 2008).

### Achiridae

The Achiridae is a dextral group of fishes that reside in waters from the United States to Argentina. This is a diverse family that can be found in temperate and tropical fresh, estuarine, and coastal marine water. Commonly called American soles, this family consists of seven genera, *Achirus*, *Apionichthys*, *Baiostoma*, *Catathyridium*, *Gymnachirus*, *Hypoclinemus*, and *Trinectes*, with thirty-three species (Munroe 2005; Nelson 2006).

Historically, Achiridae was a subfamily of Soleidae, but was elevated to family status based on six characters (Chapleau and Keast 1988). Ramos's (1998) data further supported monophyly of Achiridae and proposed a phylogenetic hypothesis of familial relationships. Based on 12S and 16S mitochondrial DNA of five species in four genera, *Achirus*, *Catathyridium*, *Hypoclinemus*, and *Trinectes*, Azevedo's et al. (2008) data was able to support a monophyletic status of the family. Azevedo et al. (2008) concluded that *Hypoclinemus* and *Catathyridium*, both freshwater genera, formed a sister group to other Achiridae and most likely derived directly and independently from saltwater ancestors.

## Soleidae

The Soleidae, commonly known as the true soles, are a dextral group of fishes that are found worldwide. They have been described as diverse and specialized (Munroe 2005), with one species, *Pardachirus marmoatus*, that is known to use a chemical defense against predation. Soleidae reside in fresh water, estuarine, and marine habitats in tropical to temperate seas from Europe to Australia and Japan. Soleidae contains thirty-five genera with 130 species (Munroe 2005; Nelson 2006).

Jordan and Evermann (1898) were the first to recognize Soleidae, and divided the family into three subfamilies, Achirinae, Saleinae, Cynoglossinae. Kyle (1900) followed the classifications of Jordan and Evermann (1898), but suggested their organization my hide the actual relationships of the group. Regan (1910) recognized the family of Soleidae, but did not recognize the subfamilies and elevated Cynoglossinae to family level. Hubbs (1945) and Norman (1934, 1966) agreed with the removal of Cynoglossinae by Regan (1910), but recognized the two subfamilies, Achirinae and Soleinae. Hensley and Ahlstrom (1984) noted large differences between the two subfamilies, stating the main uniting character was dextrality. They concluded that Soleinae and Cynoglossidae may be more closely related than Soleinae is to Achirinae. Chapleau and Keast's (1988) osteological study refuted Hensley and Ahlstrom's (1984) monophyletic status for the family based exclusively on dextrality. Chapleau and Keast (1988) found that Soleinae was more closely related to Cynoglossidae than to Achirinae based on seven characteristics: 1) the edge of preopercle is completely concealed by scales and skin, 2) the absence of the eyed-side mesopterygoid, 3) the opercular series is deeply fimbriated,

4) convex shape of the blindside dentary, 5) the long anterior process of the first proximal pterygiophore of the dorsal fin 6) the proximal tip of hypural plates fussed to the PU1, and 7) the formation of the entire margin of the opper orbit by the blind-side lateral ethmoid. Based on these features, Chapleau and Keast (1988) suggested the elevation of Soleinae and Achirinae to family level. Furthermore, Chapleau and Keast (1988) were able to identify support for the monophyly of Achiridae and Soleidae based on five and six characters respectively.

Desoutter and Chapleau's (1997) discoveries made progress in establishing monophyly of Soleidae, by uniting *Bathysolea* by two apomorphic characters: the filamentous structure of the pectoral fins and dark pigmentation inside the abdominal and branchial cavities. Pardo et al. (2005) and Azevedo et al. (2008) were able to support the monophyly of *Bathysolea* with mitochondrial evidence. Berendzen and Dimmick (2002) found conflicting data between Bayesian analysis and parsimony. Bayesian analysis supported the monophyly of Soleidae, whereas parsimony resulted in paraphyletic relationships. Azevedo et al. (2008) concluded that the genera of *Solea* and *Microchirus* may not be monophyletic within Soleidae, but their results did support monophyly of the family.

### Cynoglossidae

The Cynoglossidae, commonly called tonguefishes, are a group of sinistral fishes found in marine, estuarine, and fresh water environments. They can be found in tropical to subtropical seas, and have been divided into two subfamilies. The subfamily of Symphurinae contains one genus, *Symphurus,* with seventy-seven species. These fishes have a snout without a hook shape, and most of them are found in deep water on both sides of the Americas, including Hawaii. The subfamily of Cynoglossinae contains two genera, *Cynoglossus* with fifty species and *Paraplagusia* with three species. Cynoglossinae are characterized by a hooked snout, and can be found in shallow water from the eastern Atlantic to the western Pacific. Five species of this family are known to enter fresh water, and three species may only reside in fresh water (Munroe 2005, Nelson 2006).

Jordan and Evermann (1898) classified these fishes as a subfamily of Solidae, Cynoglossinae. Regan (1910) elevated the subfamily to the family status. Hensley and Ahlstrom (1984) supported the monophyletic status of these fishes based on the orientation of pelvic fin of the blind side along midventral line and placement of the pelvic fin on the eyed side more dorsally or missing. Chapleau’s (1988) research supported the monophyly based on twenty-seven characters for the family. Chapleau’s (1988) data was able to support monophyly of Cynoglossidae subfamilies, Symphurinae with six characters and Cynoglossinae with nine characters. Munroe (2005) stated that at the species level the taxonomy remains problematic. Mitochondrial evidence helped support the monophyly of two species within *Cynoglossus* and *Symphurus* (Berendzen and Dimmick 2002; Pardo et al. 2005, Azevedo et al. 2008). Azevendo et al. (2008) concluded that more species must be examined before stating all genera within Cynoglossidae are monophyletic.

# CHAPTER 3

# GEOMETRIC MORPHOMETRICS

To analyze the shape variation across the phylogeny of Pleuronectiformes on the important characteristics listed above, this study utilizes geometric morphometrics. The analysis of shape in organisms is important to understanding the processes of growth, morphogenesis, functional roles, and responses to selective pressures. Shape analyses are also important in understanding the differences in the descriptions mentioned above. Understanding shape variation can be the gateway to understanding what causes morphological variation (Zeldich et al. 2012).

General shape (i.e. circular, square, etc.) has been historically used as a way to describe individuals from one another, but these methods are vague, inaccurate, and misleading. Morphometrics is a quantitative way to address shape variation by utilizing mathematical shape analysis. Modern geometric morphometrics arose as a way to answer questions regarding the alignment of megalithic ‘standing stones’, such as Stonehenge (Kendall and Kendall 1980; Zeldich et al. 2012). Geometric morphometrics illustrates and explains shape differences that have been mathematically analyzed to allow for visualization of complex shapes that may not be seen by the human eye. Morphometrics utilizes both morphology and statistics to quantify shape variation. It utilizes two mathematical areas, general linear models to assess statistical power, and algebraic models to calculate principal component analyses.

Traditional methods of analyzing shape in fishes utilized length, depth, and width measurements, but these methods contained many redundancies and overestimated the amount of shape information that is actually collected (Lagler et al. 1962; Zeldich et al. 2012). Improvements in the traditional method came with the advent of box trusses (Strauss and Bookstein 1982; Bookstein et al. 1985). The box truss, or a truss network, reconstructs form by a series of measurements based on homologous landmarks. These measurements can be standardized to a common reference size and form can be reconstructed from the measurements. The box truss method samples more dimensions of the organism as compared to previous methods.

Both of these older methods, traditional and the truss network, share problems. They fail to collect all the information available from endpoints of measurements, and convey no information about the geometry of the structure. All measurements gathered using the traditional and box truss methods are variants of size. This makes it difficult to extract shape data from the size of the measurements. In addition, users of these methods discarded principal component one data as size information, but in reality all principal components contain information about shape and size (Zeldich et al. 2012).

Current methods of analyzing shape involve landmark coordinates. X-Y coordinates of landmarks contain all positional information, including the ability to reconstruct box truss units. Landmark points are anatomical loci that are homologous in all individuals in the analysis. Finding homologous landmarks can be difficult when analyzing a morphologically variable group of specimens as it is necessary to include the same number of landmarks per individual. Landmarks can be estimated when dealing with damaged fossils or degraded specimens, but this is not suggested as estimation of landmarks can produce misleading data. When homologues landmarks are not possible to determine, semilandmarks may be used. Semilandmarks are not individually homologous, but sample points along a homologous curve (Zeldich et al. 2012).

A simple algebraic manipulation, called Procrustes superimposition, allows the partition of data into size and shape, while removing irrelevant information like position and orientation. The manipulation works by removing translation and rotation, or the placement of the specimens along a plane, and uniformly scales the specimens. Procrustes superimposition is named after a Damastes innkeeper in Greek mythology who stretched or chopped off limbs of his travelers to fit his bed exactly (Andrade et al. 2004). This method contains all information about the geometric structure of the landmarks.

To view and compare data in a graphic format, a principal component analysis (PCA) can be implemented. A PCA reduces size and shape data into a graph by projecting the maximum amount of variation on the fewest dimensions across an axis. A PCA graph may contain many axes representing an eigenvector of the covariance matrix of shape variability and can be viewed in two-dimensional and three-dimensional graphs. The axes can be analyzed statistically by a chi-squared test (Morrison 1967). Clustering of data points on a PCA graph can reflect similarities and differences in shapes that may indicate phylogenetic relationships.

Additionally, current methods of analyzing shape draw informative pictures to illustrate results. A thin plate spline produces a deformation grid that reflects the deflection of a landmark from one end of a PCA axis to the landmark of the other end of the axis. The grid looks stretched in regions where shape is elongated, and compressed where the shape is shortened. Another way to visualize shape variation is by vector deformation. The vector deformations show the magnitude and direction that the landmark is displaced from one end of the PCA axis to the other (Zeldich et al. 2012).

# CHAPTER 4

# OBJECTIVES

Outwardly all flatfishes seem to have very similar characteristics related to their sidedness. However, the complex history of trying to classify the groups within flatfishes reveals just how diverse shape is across the order (e.g. Jordan and Evermann 1898; Kyle 1900, 1921; Regan 1910, 1929; Norman 1934; Hubbs 1945; Lauder and Liem 1983; Hensley and Ahlstrom 1984; Chapleau 1993). In fact, shape has posed the main obstacle in classification of these organisms. Geometric morphometrics is a useful tool that allows for visualization of complex shapes that may not be apparent to the human eye, lending itself nicely to analyzing shape variation across the order Pleuronectiformes. Until this study, landmark-based geometric morphometric analyses have only been utilized to quantify sexual dimorphism and differences in habitat preference within a single species of flatfish (Cadrin and Silva 2005; Russo et al. 2008). This study is novel in the way landmark-based geometric morphometric methods will be applied to examine shape variation in skeletal elements across the entire order Pleuronectiformes. The objective of this study is to determine if the morphometric variation observed within flatfishes is consistent with current phylogenetic hypotheses and classification within the group.

Recent phylogenetic studies of the Pleuronectiformes based on DNA sequence data have determined the relationships among flatfishes independent of morphology and shape (e.g. Berendzen and Dimmick 2002; Pardo et al. 2005; Azevedo et al. 2008). These phylogenetic hypotheses allow the possibility to answer questions regarding shape variation across the order. In this study, geometric morphometric methods will be used to assess the morphological variation of skeletal shape within the order Pleuronectiformes utilizing radiographic images. An effort was made to choose landmarks that represent skeletal elements that have the most drastic change during late development resulting from eye migration. These landmarks focus on shape characteristics that may be unique to flatfishes and have the potential to show variation within the order.

The specific questions this study will address are:

1. Do families within the Pleuronectiformes exhibit differences in shape? Given the current classification and phylogenetic hypotheses, I hypothesize that shape differences will be observed among families across the phylogeny while more closely related groups being more similar in shape.

2. Is Psettodidae different in shape than all other flatfishes? The recent study by Campbell et al. (2013) hypothesized that the Pleuronectiformes are not a monophyletic group. They suggested that Psettodidae is more closely related to the family Centropomidae in the order Perciformes than the Pleuronectiformes. I hypothesize that the family of Psettodidae will differ in shape from the other families of Pleuronectiformes.
3. Do families with highly specialized morphologies exhibit less shape variation within the group than families with more general features? Highly specialized families have strong support for their monophyly that have rarely been questioned, whereas generalized families have less support for monophyly, leading to the continual reorganization and splitting of these groups. This suggests that specialized families

have a more conserved shape, whereas generalized families display a larger variety of shapes. I hypothesize that families with highly specialized features will have less variation in shape within the family compared to more generalized families.

4. Do families and genera that are hypothesized to be polyphyletic show a great amount of shape variation? There is strong evidence supporting polyphyletic relationships for several groups within the Pleuronectiformes. I hypothesize that families with polyphyletic relationships will show a greater diversity of shape given the separate divergence of clades. The following questions fall under this hypothesis:
    a. Are there differences in shape among the three hypothesized lineages (Azevedo et al. 2008) within the paraphyletic Paralichthyidae? Paralichthyidae has been considered polyphyletic based on morphological and genetic analyses, and the most current phylogeny suggested three separate lineages within the group (Norman 1934; Hensley and Ahlstrom 1984; Chapleau 1993; Azevedo et al. 2008). I hypothesize that the three lineages of Paralichthyidae will have different shapes.
    b. Does the genus *Tephrinectes* differ in shape from all the other genera currently recognized in the family Paralichthyidae? There is considerable support for the removal of the genus *Tephrinectes* from Paralichthyidae and elevating it to the family level (Hensley and Ahlstrom 1984; Hoshino and Amaoka 1998; Hoshino 2001). The suggestion to remove *Tephrinectes* from Paralichthyidae has been supported by osteological morphology. I hypothesize that *Tephrinectes* has a different shape than the rest of Paralichthyidae.

    c. Given the diversity and size of Bothidae, is there variation in shape within the family, relative to other families? Previous studies are able to find support for a monophyletic Bothidae, but these are based on limited data and taxon sampling. Only five species out of 130 species total are included in these studies (Berendzen and Dimmick 2002; Pardo et al. 2005; Azevedo et al. 2008). Given the size of the family and limited evidence supporting monophyly I hypothesize that Bothidae will show a great diversity in shape.

5. Are freshwater lineages different in shape from brackish water and marine water lineages within the family of Achiridae? Based on a phylogeny of four genera, *Achirus*, *Catathyridium*, *Hypoclinemus*, and *Trinectes*, it was hypothesized that two freshwater lineages derived from brackish water genera independently from one another (Azevedo et al. 2008). *Achirus*, a brackish water genus, and *Hypoclinemus*, a freshwater genus, form a sister group to a clade consisting of *Catathyridum*, a freshwater genus, and *Trinectes*, a brackish water genus. I hypothesize that freshwater genera will have a different shape from brackish water and marine water lineages.

# CHAPTER 5

# METHODS

## Specimens Examined

Individuals were chosen based on quality and availability of the specimen. A total of 457 specimens were radiographed from collections at the University of Kansas Natural History Museum and Smithsonian National Museum of Natural History. When possible, representatives with minimal visible damage and the youngest collection dates were chosen to reduce chances of bone degradation. Approximately three individuals of every species were captured with radiographic imagery. Less than three individuals were included when availability of the specimen was limited. Specimens identified to the species level were given preference during collection. To reduce distortion of the body caused during the preservation process, each individual was flattened using a sheet of acrylic glass. In cases of severe distortion, fabric hook-and-loop fastener straps were used to flatten individuals to the acrylic glass. Individuals collected from the University of Kansas Natural History Museum were radiographed using medical x-ray film. The film was developed manually and scanned on to a computer at a high resolution. Individuals from the Smithsonian National Museum of Natural History were collected digitally and manipulated in Photoshop.

An examination of the radiographs resulted in reducing the total number used in analyses to 392 individuals based on quality of the images. Eleven families were represented by a number of species as follows; Achiridae, n=10, Achiropsettidae, n=1, Bothidae, n=26, Citharidae, n=4, Cynoglossidae, n= 7, Paralichthyidae, n= 26, Pleuronectidae, n=42, Psettodidae, n=2, Samaridae, n=2, Scophthalmidae, n=1, and Soleidae, n=25 (Appendix: Table 1).

## Morphometric Analysis

Landmarks were chosen based on traditional methods of geometric morphometrics in fishes with consideration given to unique characteristics displayed by flatfishes. Traditional landmarks included fin insertion points and jaw elements. To quantify the height of the specimen, the insertion point of the dorsal and anal fins between the interneural spines of the first caudal vertebrae were marked. The curvature of the spine, which is related to changes during metamorphosis, was also captured using a series of landmarks and semi-landmarks. Semilandmarks were used when homologous landmarks were not possible to determine. The semilandmarks utilized in this study are not individually homologous along the spine, but sample points along a homologous curve of the spinal column (Zeldich et al. 2012). The diversity of Pleuronectiformes severely limited the number of landmarks, as homologous points could not be found across all flatfishes. An attempt to include the frontal bones and other head features in the shape analyses was made. These skeletal elements are particularly interesting in flatfishes because they are uniquely shaped by eye migration. Unfortunately these elements exhibit huge variation across the Pleuronectiformes and it was impossible to determine homology.

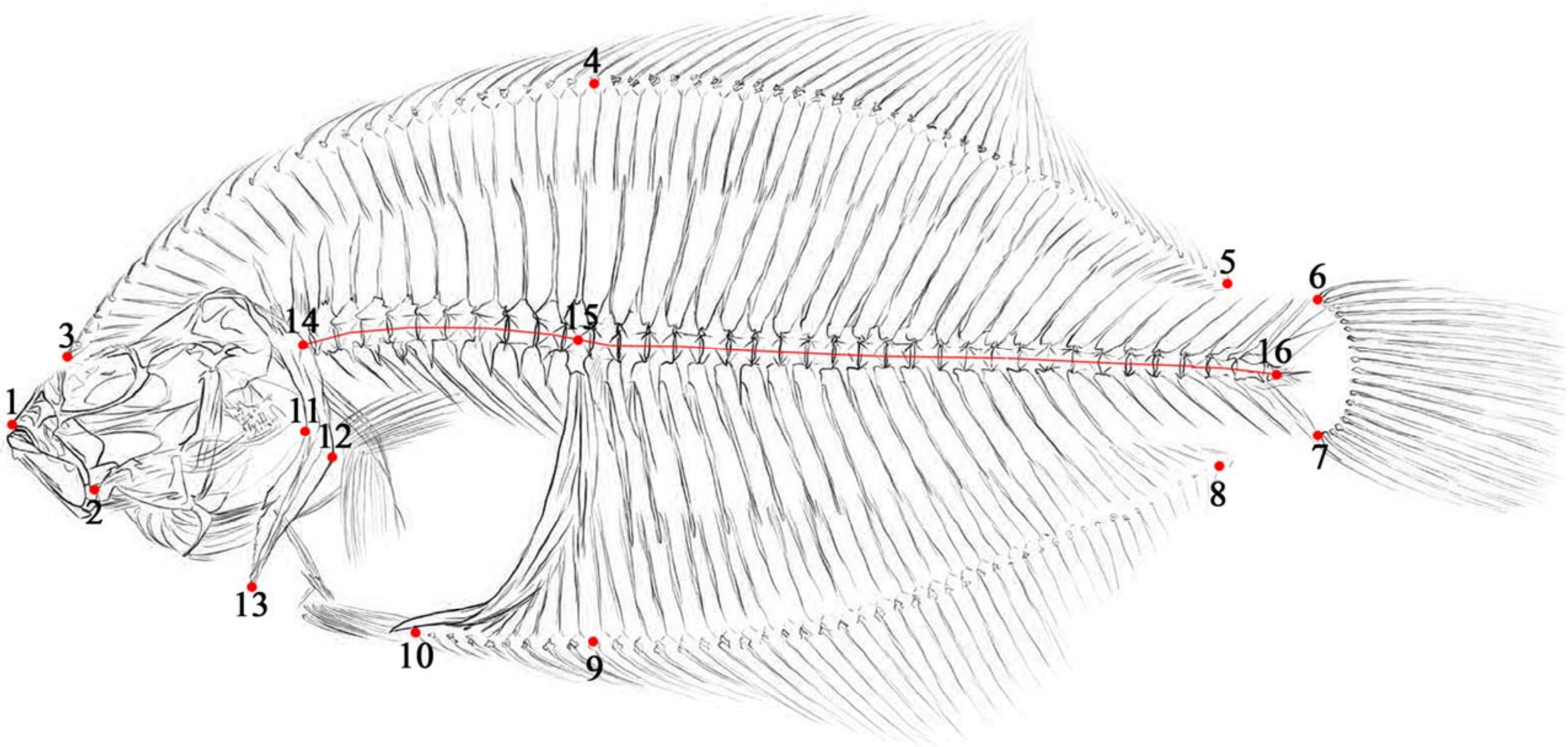

Figure 3: Morphometric landmarks used in this study: 1 = anterior tip of the premaxilla, 2 = junction of quadrate and articular, 3 = insertion of anterior portion of the dorsal fin, 4 = basal bone of dorsal fin between interneural spines of the first caudal vertebrae, 5 = insertion of the posterior portion of the dorsal fin, 6 = dorsal insertion of the caudal fin, 7 = ventral insertion of the caudal fin, 8 = insertion of posterior anal fin, 9 = basal bone of anal fin between interneural spines of the first caudal vertebrae, 10 = insertion of anterior anal fin, 11 = inflection point of the anterior cleithrum, 12 = inflection point of the posterior cleithrum, 13 = ventral point of the cleithrum, 14 = mid-point of first abdominal vertebrae, 15 = mid-point of first caudal vertebrae, and 16 = mid-point of the urostyle. The curve follows the spinal column, connecting landmarks 14, 15, and 16, and containing twenty-five semi-landmarks. The species pictured above is *Pseudopleuronectes americanus*. Outline courtesy of Megan Merner.

Landmarks and the curve were digitized using the software TPSdig ver. 2.16 (Rohlf 2010). All landmarks were digitized by one person to ensure consistency. Specimens were superimposed using a generalized least squares Procrustes superimposition to remove non-shape related information using the program CoordGen7a (Sheets 2011). Landmark data was not standardized to eliminate allometric growth as the age of the specimen was unknown at death, the sampling size was highly diverse, and there were not enough specimens per species to estimate a growth trajectory. There is limited evidence describing allometry within Pleuronectiformes and the data focuses on larval growth of hatchery-reared species (Klingenberg and Froese 1991; Gisbert et al. 2002). However, all specimens chosen for this study were individuals with complete ossification of the body indicating a fully mature individual. Unfortunately, little is known about allometric growth in adults, which, if present, could bias results.

A multivariate analysis was performed on all individuals of Pleuronectiformes and Psettodidae using a principal component analysis (PCA) in PCAGen7a (Sheets 2011). For multivariate analysis of Pleuronectiformes and Psettodidae, eleven families were represented. PCA analyses were also performed on each family individually to look at shape variation across genera. Within families, groups consisted of the genera included in the study, with the exception of Pleuronectidae which was grouped by subfamily. Pleuronectidae was grouped by subfamily because of restrictions in PCAGen7 which only allowed identification of twenty-four groups (Sheets 2011). Eigenvalues and chi-square values were calculated in PCAGen7. Eigenvalues represent the amount of variability associated with the principal component. When two or more eigenvalues have similar variances they may be linked. PCAGen7 calculates a modified chi-squared statistic using an expression given by Morrison (1967). This analysis is used to determine how many distinct eigenvalues there are by doing a series of pairwise comparisons (Sheets 2001; Zeldich et al. 2012). Eigenvalues are considered distinct when the modified chi-squared value is over 5.99 (Sheets 2001). Vector deformation grids were generated using PCAGen7 for distinct principal components. Vector deformation grids indicate the direction and relative magnitude of displacements at each landmark with the negative end of the PC represented by a dot and the positive end of the PC represented an arrow (Zeldich et al. 2012). Individuals and catalog numbers used in these analyses are listed in Appendix 1.

# CHAPTER 6

# RESULTS

For the principal component analysis (PCA) representing shape variation for all Pleuronectiformes and Psettodidae, the three distinct principal components were supported by chi-squared values of 56.02, 99.43, 27.47, representing 46.62%, 21.72%, and 7.77% of the variation, respectively.

PC1 (Fig. 4) was loaded by variables that represented the height of the body, size of the head, and curvature of the spinal column. Individuals with negative values had small heads, shorter bodies, and less curvature to the spinal column (Fig. 5). Families with negative means included Cynoglossidae, Soleidae, and Samaridae (Fig. 4). Individuals with positive values had larger heads, taller bodies, and greater curvature to the spinal column (Fig. 5). Families with positive means included Pleuronectidae, Bothidae, Achiridae, Paralichthyidae, Achiropsettidae, Citharidae, Psettodidae, and Scophthalmidae (Fig. 4). Although the means of these families fell to one side of the graph or the other, some families had individuals with positive and negative values. These families included Pleuronectidae, Paralichthyidae, Bothidae, Soleidae, Scophthalmidae, and Achiridae.

PC2 (Fig. 4) was loaded by variables that represented the overall body shape and fin insertion positions in relation to the pre-maxilla. Individuals with negative values had slimmer, more fusiform shaped bodies, with anterior dorsal fin insertion points posterior to the tip of the pre-maxilla (Fig. 6). Families with negative means included Psettodidae, Citharidae, Pleuronectidae, Cynoglossidae, and Paralichthyidae (Fig. 4). Individuals with positive values had more disk-like shaped bodies, anterior dorsal fin insertion points anterior to the tip of the pre-maxilla, and caudal fin insertion points inset to the ventral insertion points of the dorsal and anal fins (Fig. 6). Families with positive means included Achiropsettidae, Samaridae, Soleidae, Bothidae, Scophthalmidae, and Achiridae (Fig. 4). Although the means of these families fell to one side of the graph or the other, some families had individuals with positive and negative values. These families included Pleuronectidae, Paralichthyidae, Bothidae, Soleidae, Scophthalmidae, Cynoglossidae, Samaridae, Achiropsettidae, and Achiridae.

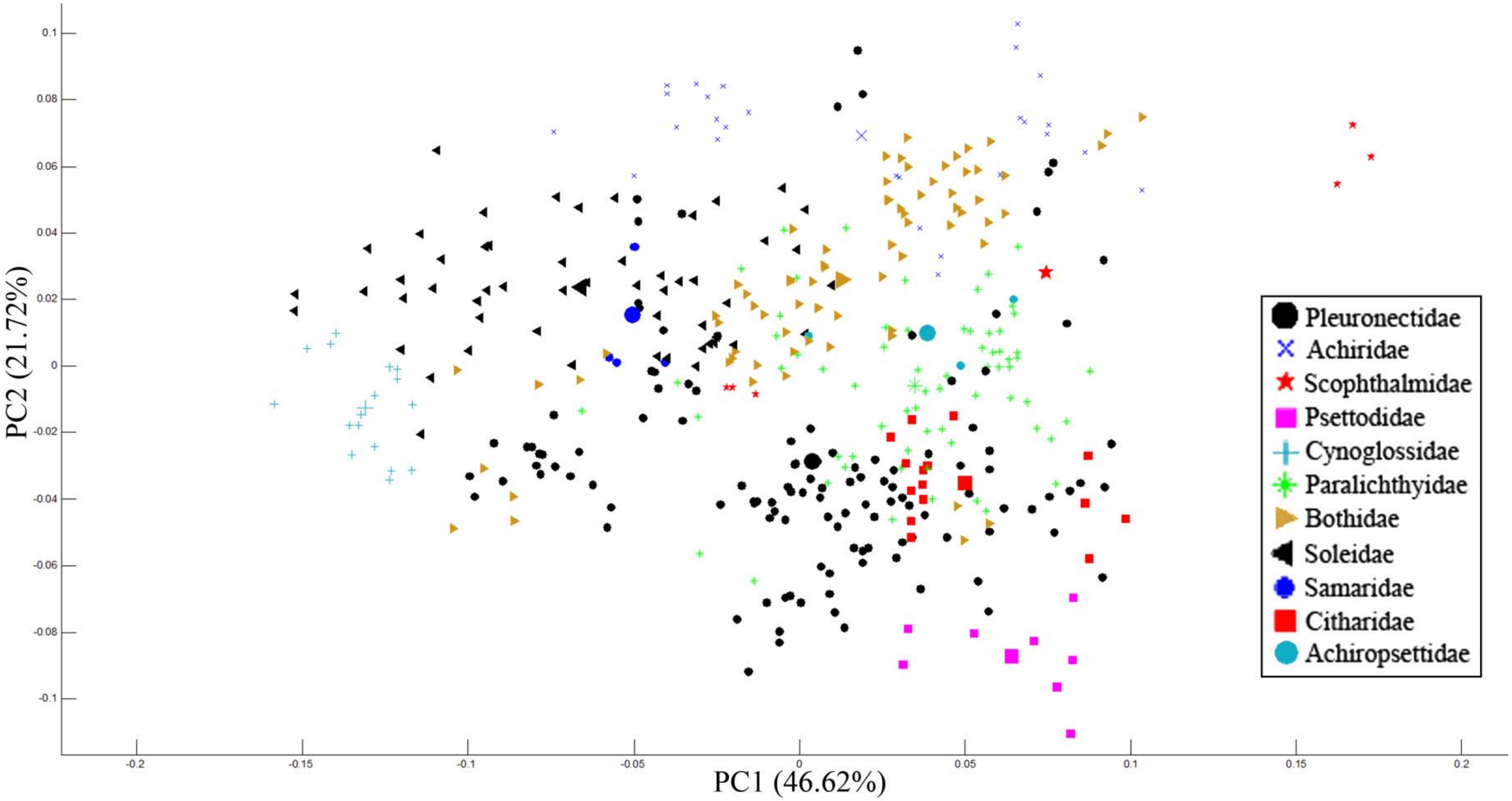

Figure 4: Principal component analysis of PC1 and PC2 of pleuronectiform data with PC1 represented on the x-axis and PC2 represented on the y-axis. Families are depicted by the symbols displayed in the key. Larger symbols represent the median for representative families.

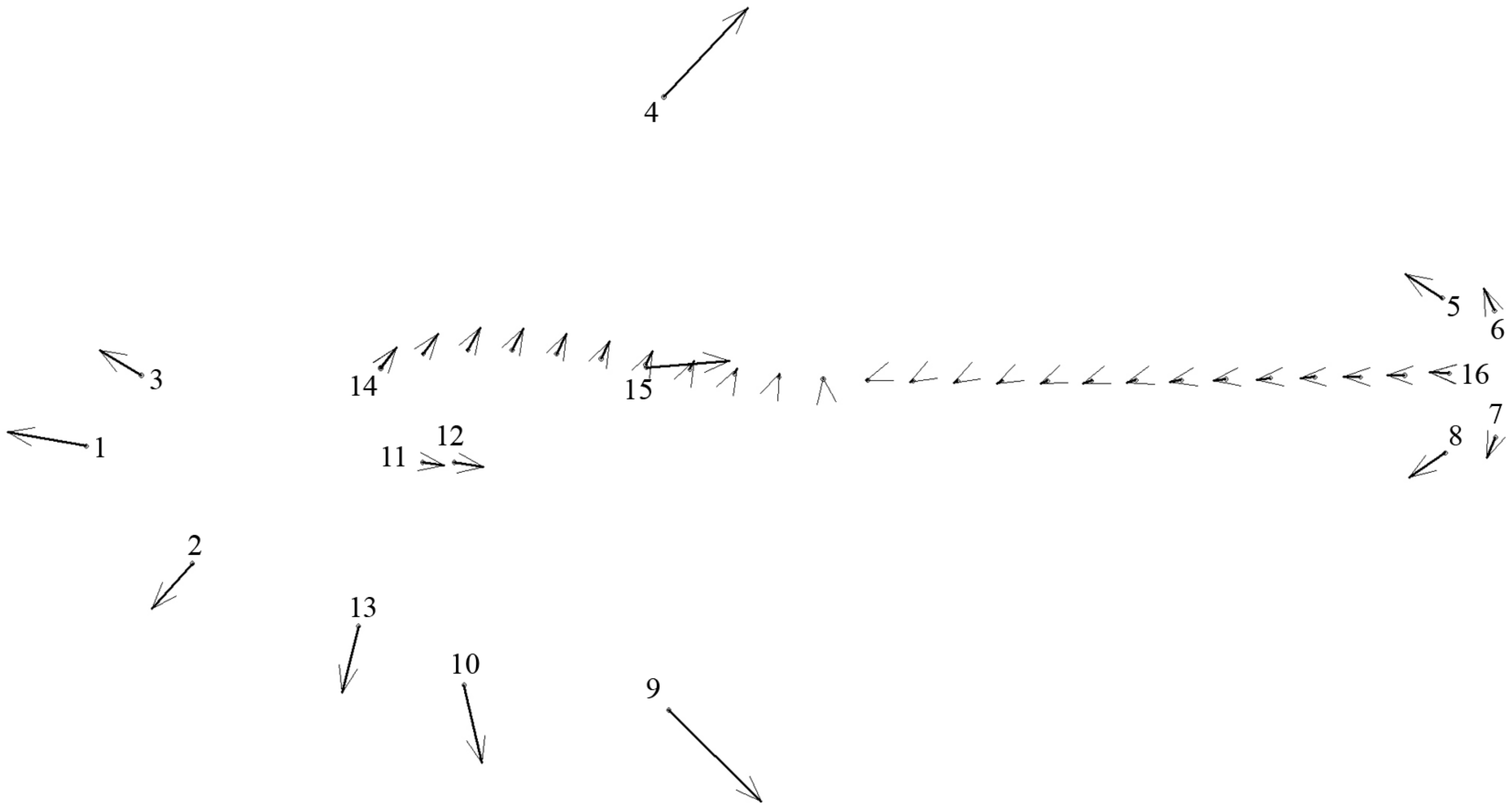

Figure 5. Vector deformation grid representing PC1 of pleuronectiform data (on the x axis of Fig. 4). Numbers correlate to landmarks represented in Figure 3. Vectors point from landmark configuration found at the negative values of PC1 and towards landmark configuration found at positive values of PC1.

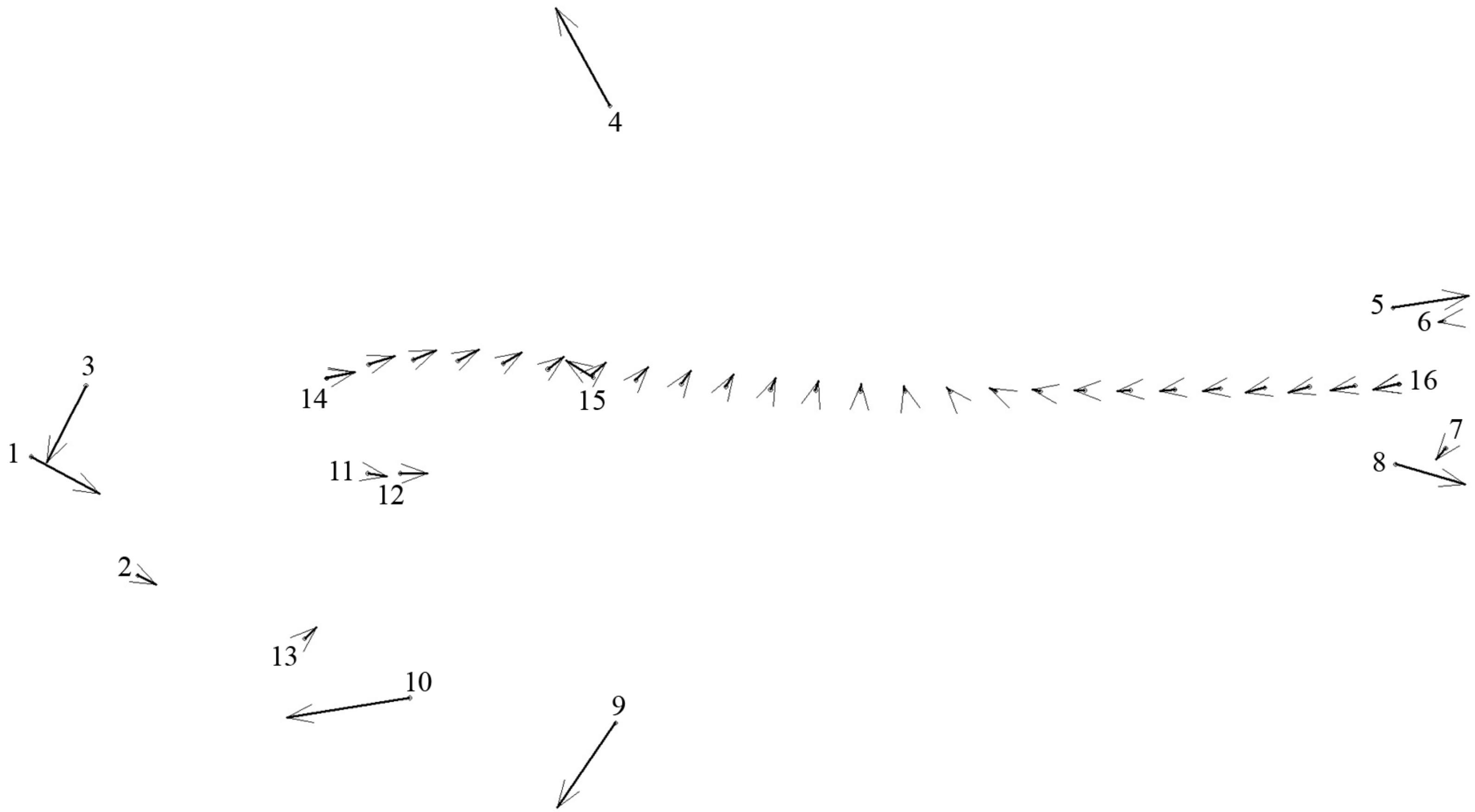

Figure 6. Vector deformation grid representing PC2 of pleuronectiform data (on the y axis of Fig. 4). Numbers correlate to landmarks represented in Figure 3. Vectors point from landmark configuration found at the negative values of PC2 and towards landmark configuration found at positive values of PC2.

PC3 (Fig. 7) was loaded by variables that represented the ventral shape of the body, position of the spinal column in relation to the dorsal portion of the body, and the position of the tip of the pre-maxilla to the junction of the articular and quadrate (Fig. 8). Individuals with negative values had rounded ventral sides, a spinal column positioned away from the dorsal side of the body, and a shorter distance from the tip of the pre-maxilla and the junction of the articular and quadrate (Fig. 7). Individuals with positive values had flatter ventral sides, a spinal column positioned closer toward the dorsal side of the body, and a longer distance from the tip of the pre-maxilla to the junction of the articular and quadrate (Fig. 8). All families overlapped each other and had individuals with negative and positive values, with the exception of Psettodidae which had positive values (Fig. 7).

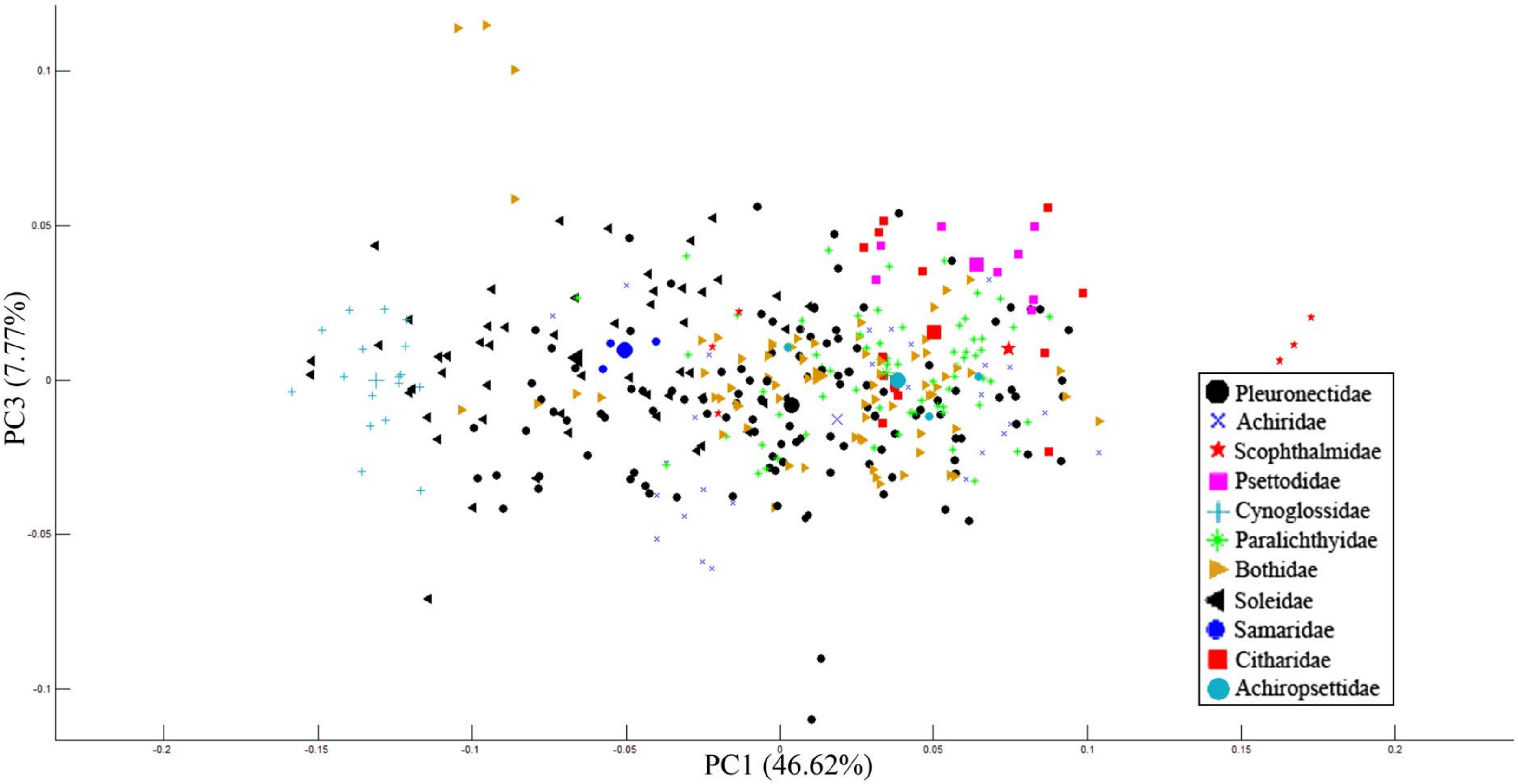

Figure 7: Principal component analysis of PC1 and PC3 of pleuronectiform data with PC1 represented on the x-axis and PC3 represented on the y-axis. Families are depicted by the symbols displayed in the key. Larger symbols represent the median for representative families.

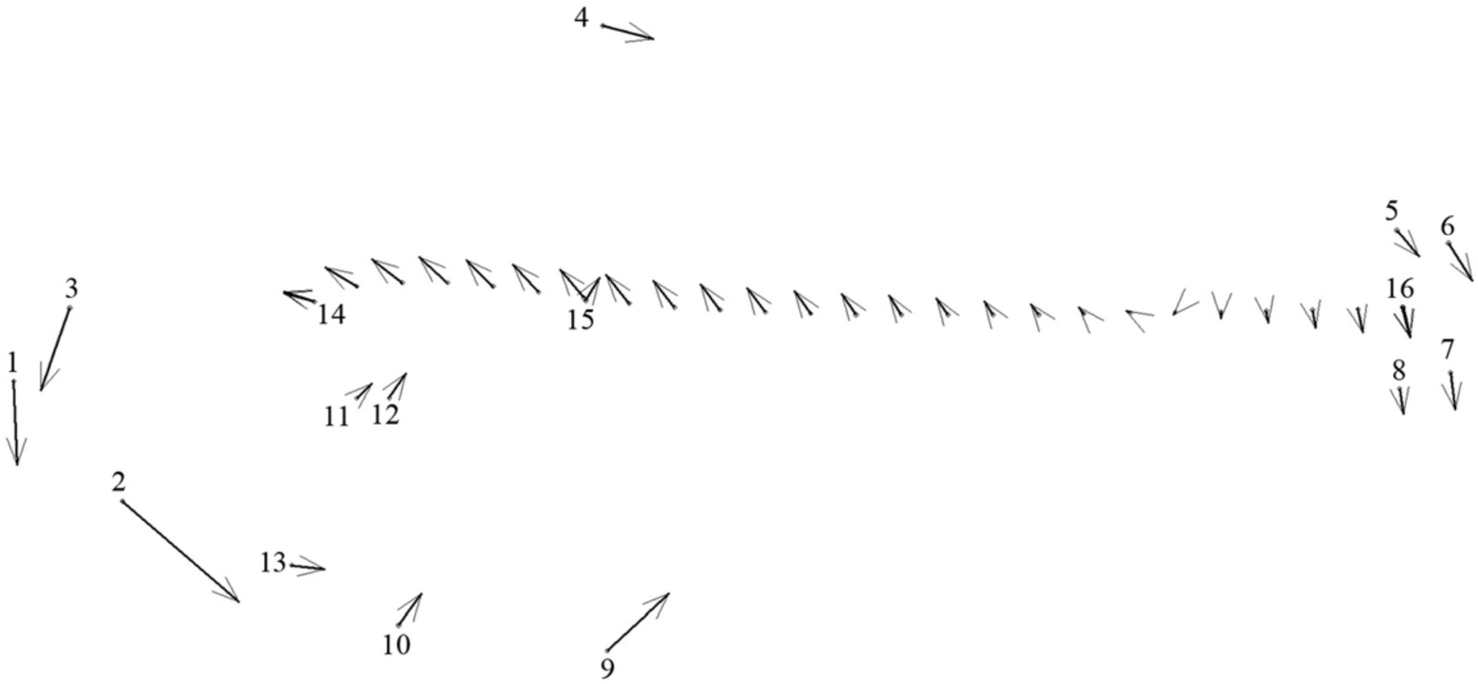

Figure 8. Vector deformation grid representing PC3 of pleuronectiform data (on the y axis of Fig. 7). Numbers correlate to landmarks represented in Figure 3. Vectors point from landmark configuration found at negative values of PC3 and towards landmark configuration found at positive values of PC3.

## Family Level Analyses

To look at shape variation within the families of Pleuronectiformes and Psettodidae, additional PCA analyses were executed. Of the eleven families analyzed for shape variation, only four families, Achiridae, Bothidae, Pleuronectidae, and Soleidae, exhibited shape variation with at least one distinct principal component.

For the PCA representing shape variation within the family Achiridae, one distinct principal component explained 61.68% of the variation (Fig. 9). The distinct eigenvalue was supported by a chi-squared of 11.70. The PC1 axis is loaded by variables that represented the height of the body, size of the head, and length of the cleithrum from the inflection point (Fig. 9). Individuals with negative values had a small head, shorter body, and shorter cleithrum (Fig. 10). Genera with negative means included *Apionichthys* and *Gymnachirus* (Fig. 9). Individuals with positive values had larger heads, taller bodies, and longer cleithrums (Fig. 10). Genera with positive means included *Achirus*, *Trinectes, Catathyridium*, and *Hypoclinemus* (Fig. 9).

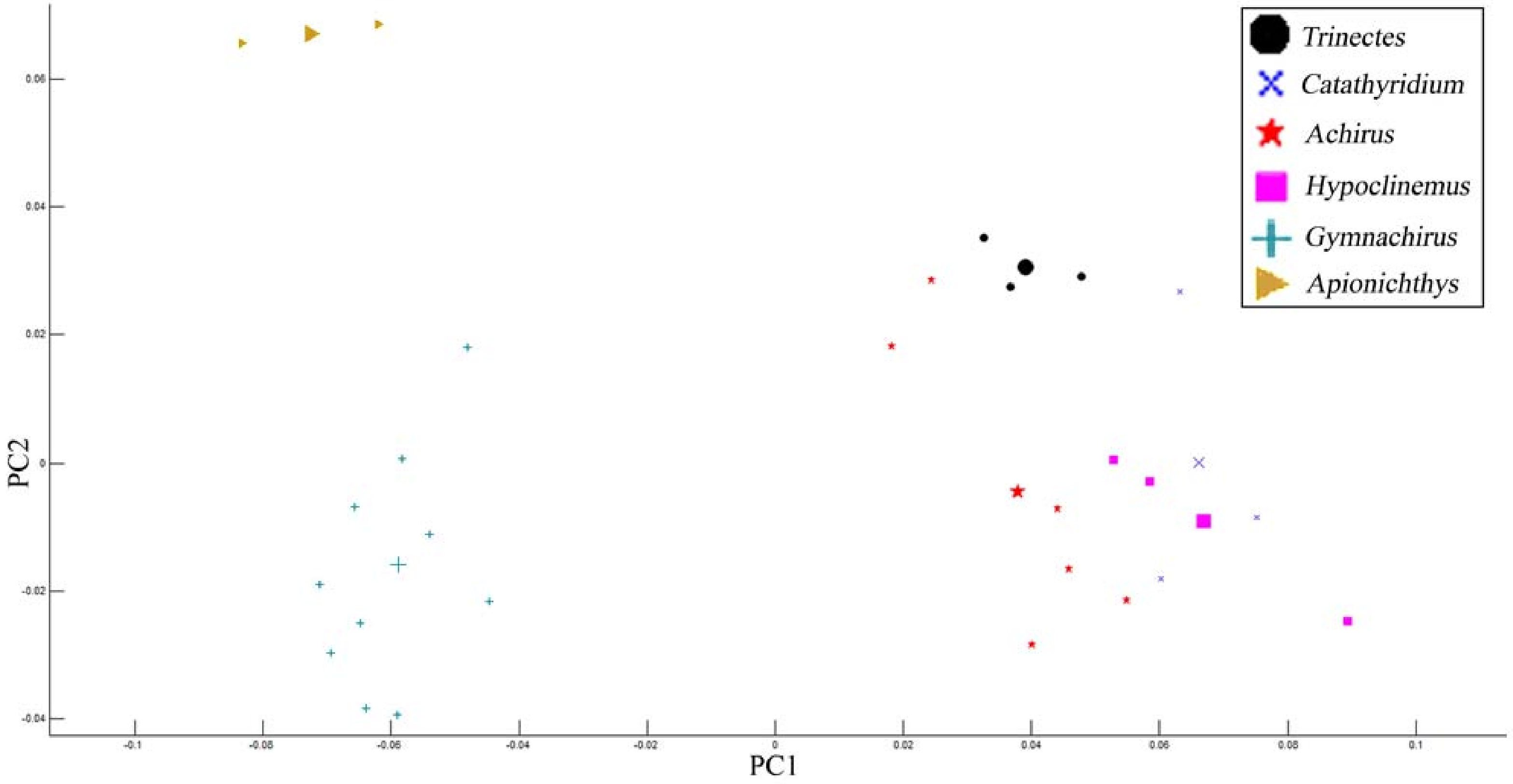

Figure 9: Principal component analysis of superimposed Achiridae data with PC1 (61.68%) represented on the x-axis and PC2 (15.32%) represented on the y-axis. Genera are depicted by the symbols displayed in the key. Larger symbols represent the median for representative genera.

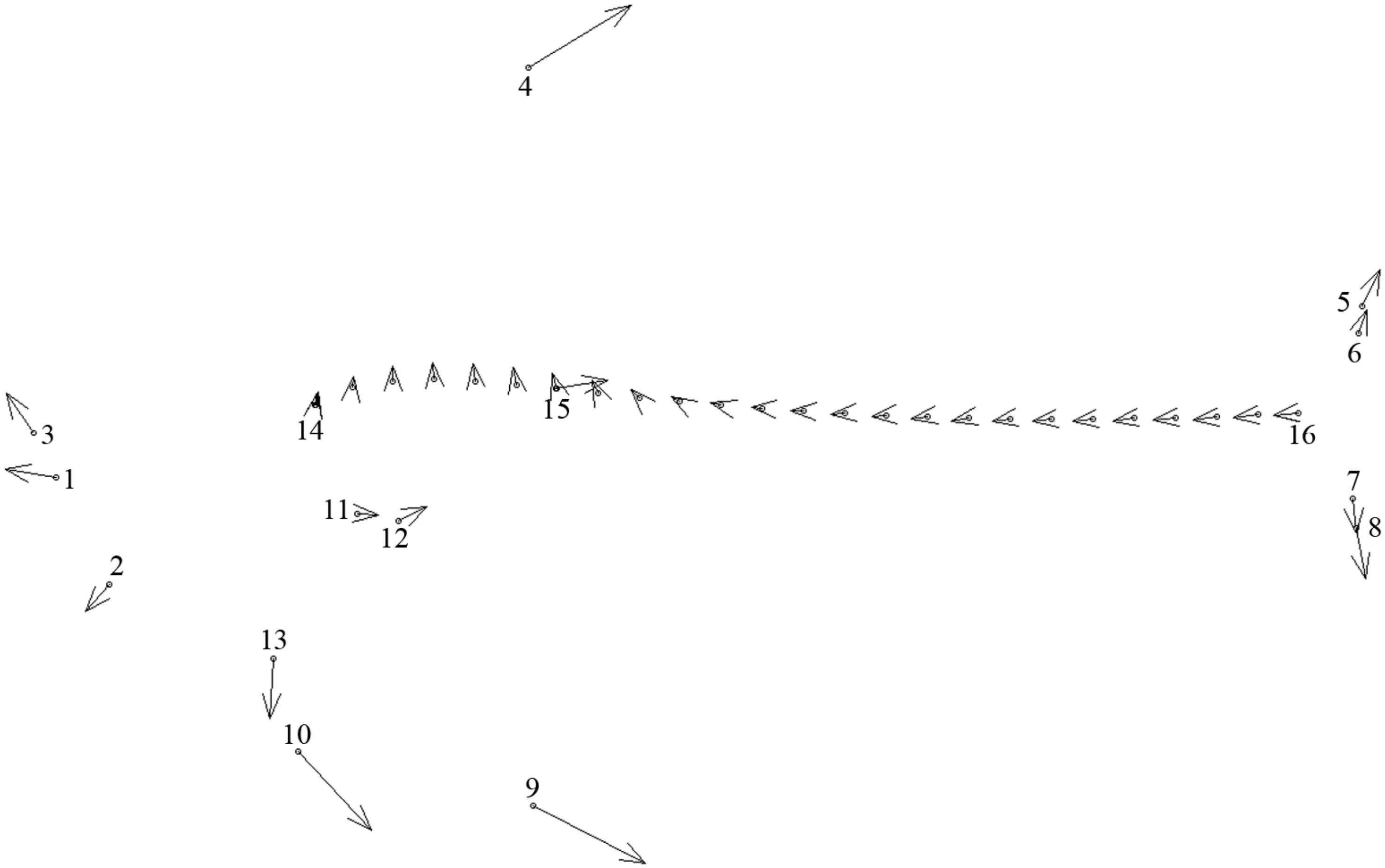

Figure 10. Vector deformation grid representing PC1 of Achiridae data (on the x axis of Fig. 9). Numbers correlate to landmarks represented in Figure 3. Vectors point from landmark configuration found at negative values of PC1 and towards landmark configuration found at positive values of PC1.

For the PCA representing shape variation within the family Bothidae, one distinct principal component explained 60.52% of the variation (Fig. 11). The distinct eigenvalue was supported by a chi-squared value of 37.59. The PC1 axis was loaded by variables that represented the height of the body, size of the head, and point of flexure of the spine (Fig. 11). Individuals with negative values had small heads, shorter bodies, and more flexion of the spine toward the brain case (Fig. 12). Genera with negative means included *Pelecanichthys, Chascanopsetta*, *Monolene, Arnoglossus*, *Psettina*, *Parabothus*, *Trichopsetta*, and *Perissias* (Fig. 11). Individuals with positive values had larger heads, taller bodies, and more flexion toward the first caudal vertebrae (Fig. 12). Genera with positive means included *Engyophrys*, *Crossrhombus*, *Grammatobothus*, *Taeniopsetta*, *Platophrys*, *Engyprosopon*, *Bothus*, *Asterorhombus*, and *Scophthalmus* (Fig. 12).

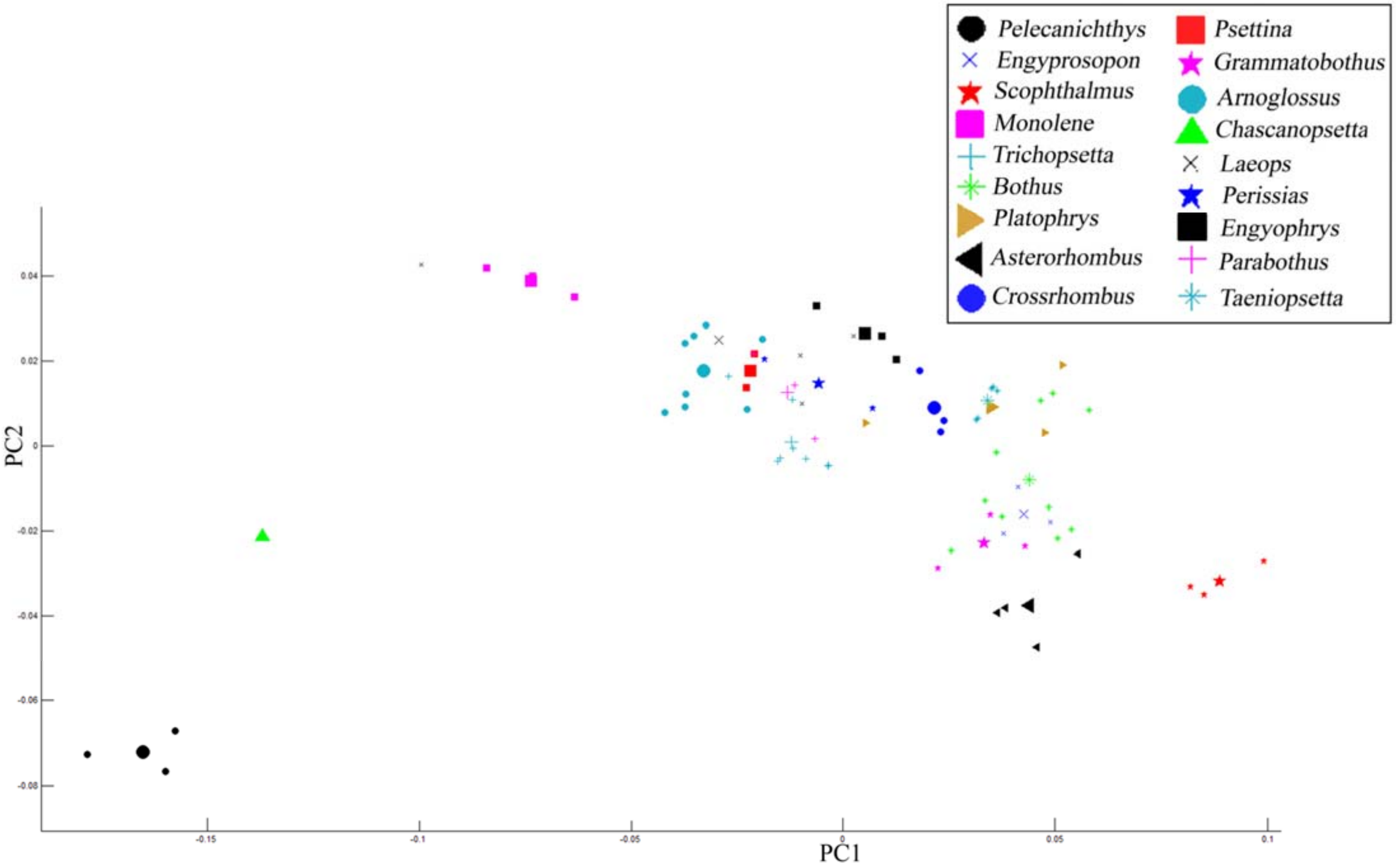

Figure 11: Principal component analysis graph of superimposed Bothidae data with PC1 (60.52%) represented on the x-axis and PC2 (13.23%) represented on the y-axis. Genera are depicted by the symbols displayed in the key. Larger symbols represent the median for representative genera.

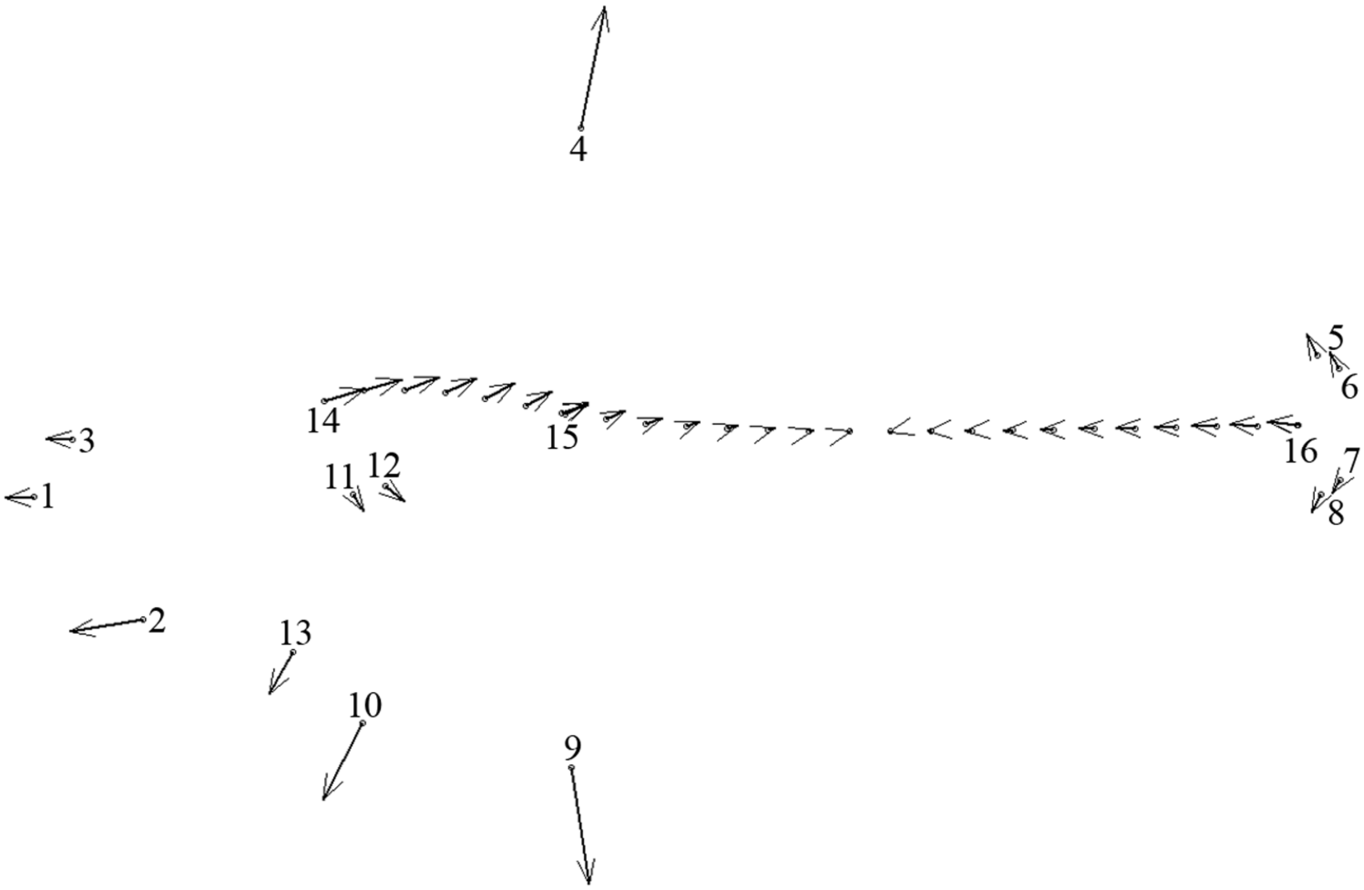

Figure 12. Vector deformation grid representing PC1 of Bothidae data (on the x axis of Fig. 11). Numbers correlate to landmarks represented in Figure 3. Vectors point from landmark configuration found at negative values of PC1 and towards landmark configuration found at positive values of PC1.

For the PCA representing shape variation within the family Pleuronectidae, two distinct principal components explained 39.98% and 2.47% of the variation respectively (Fig. 13). The two distinct eigenvalues were supported by chi-squared values of 6.80 and 22.76. The PC1 axis is loaded by variables that represented the height of the body, placement of the cleithrum, and point of flexure of the spinal column (Fig. 13). Individuals with negative values had shorter bodies, a cleithrum located closer to the head, and more flexion of the spine closer to the brain case (Fig. 14). Subfamilies with negative means included Poecilopsettinae, Lyopsettinae, and Pleuronectinae (Fig. 13). Individuals with positive values had a taller body, cleithrum located further from the head, and more flexion of the spine closer to the first caudal vertebrae (Fig. 14). Subfamilies with positive means included Hippoglossoidinae, Rhombosoleinae, and Hippoglossinae (Fig. 13).

The PC2 axis is loaded by variables that represented the dorsal body shape (Fig. 13). Individuals with negative values had taller bodies that are more rounded at the dorsal side (Fig. 15). Subfamilies with negative means included Rhombosoleinae and Poecilopsettinae (Fig. 13). Individuals with positive values had shorter bodies with little curvature at the dorsal side (Fig. 15). Subfamilies with positive means included Eopsettinae, Pleuronectinae, Hippoglossoidinae, Hippoglossinae, and Lyopsettinae (Fig. 13).

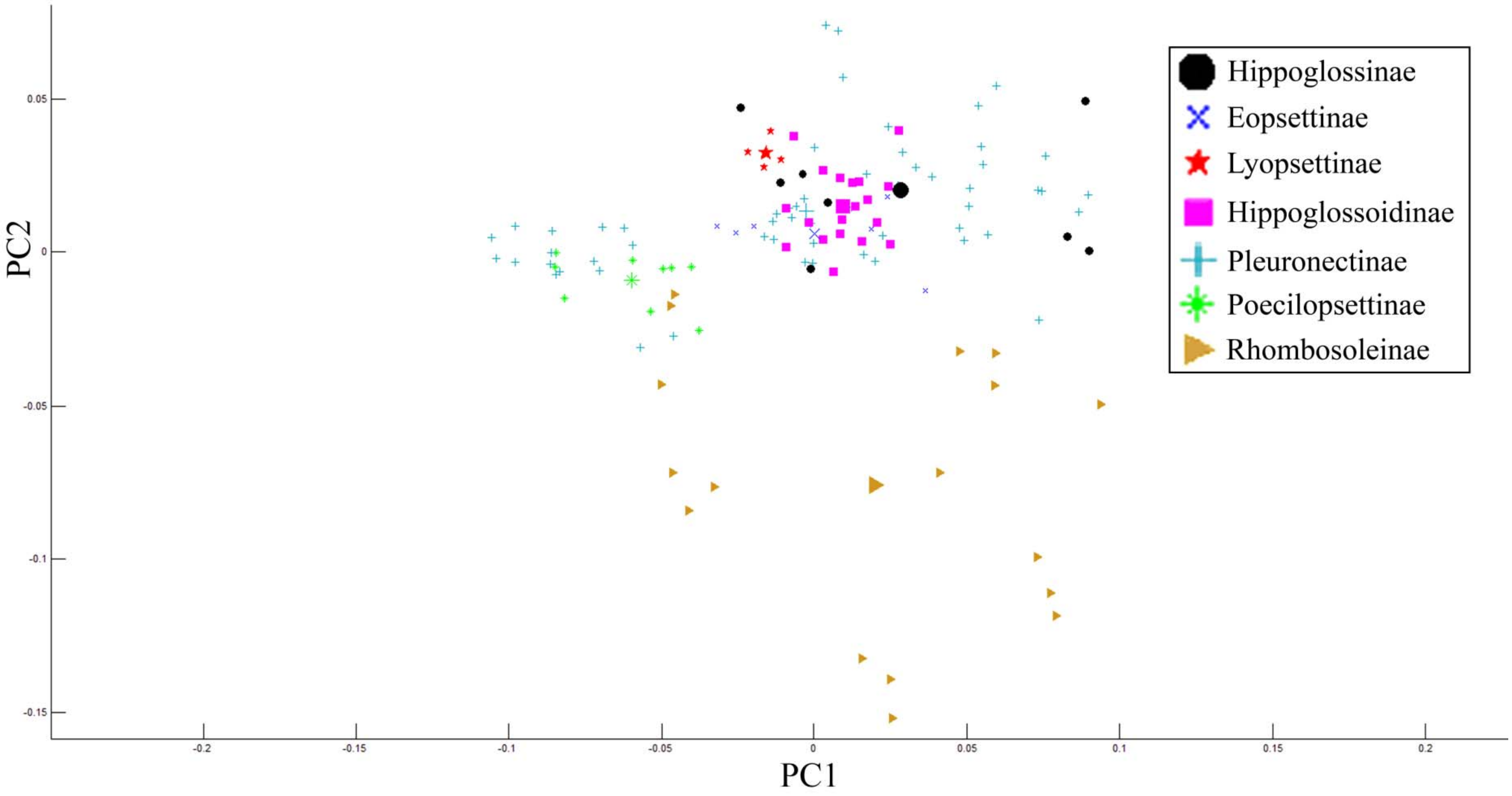

Figure 13: Principal component analysis of superimposed Pleuronectidae data with PC1 (39.98%) represented on the x-axis and PC2 (2.47%) represented on the y-axis. Subfamilies are depicted by the symbols displayed in the key. Larger symbols represent the median for representative subfamilies.

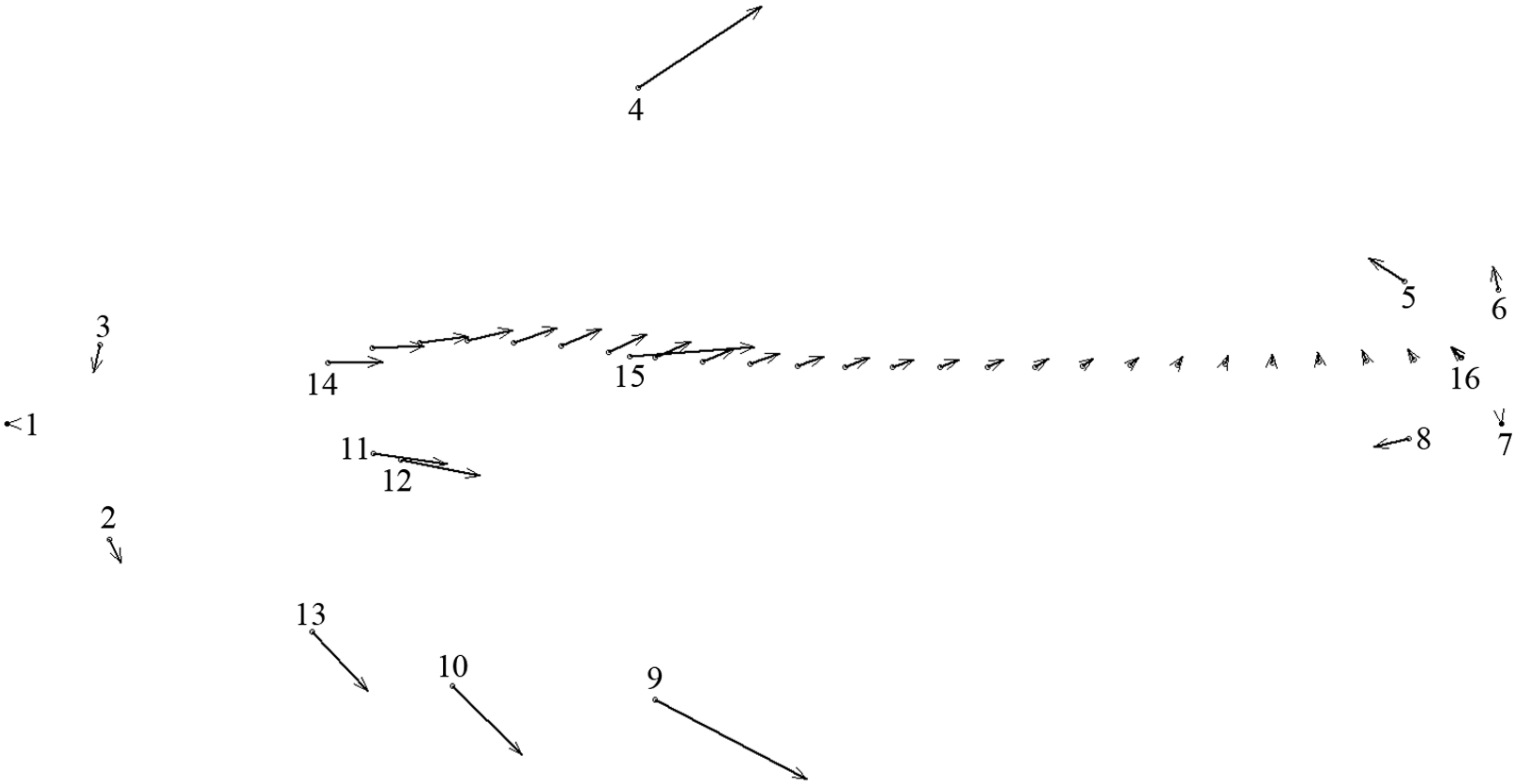

Figure 14. Vector deformation grid representing PC1 of Pleuronectidae data (the x axis of Fig. 13). Numbers correlate to landmarks represented in Figure 3. Vectors point from landmark configuration found at negative values of PC1 and towards landmark configuration found at positive values of PC1.

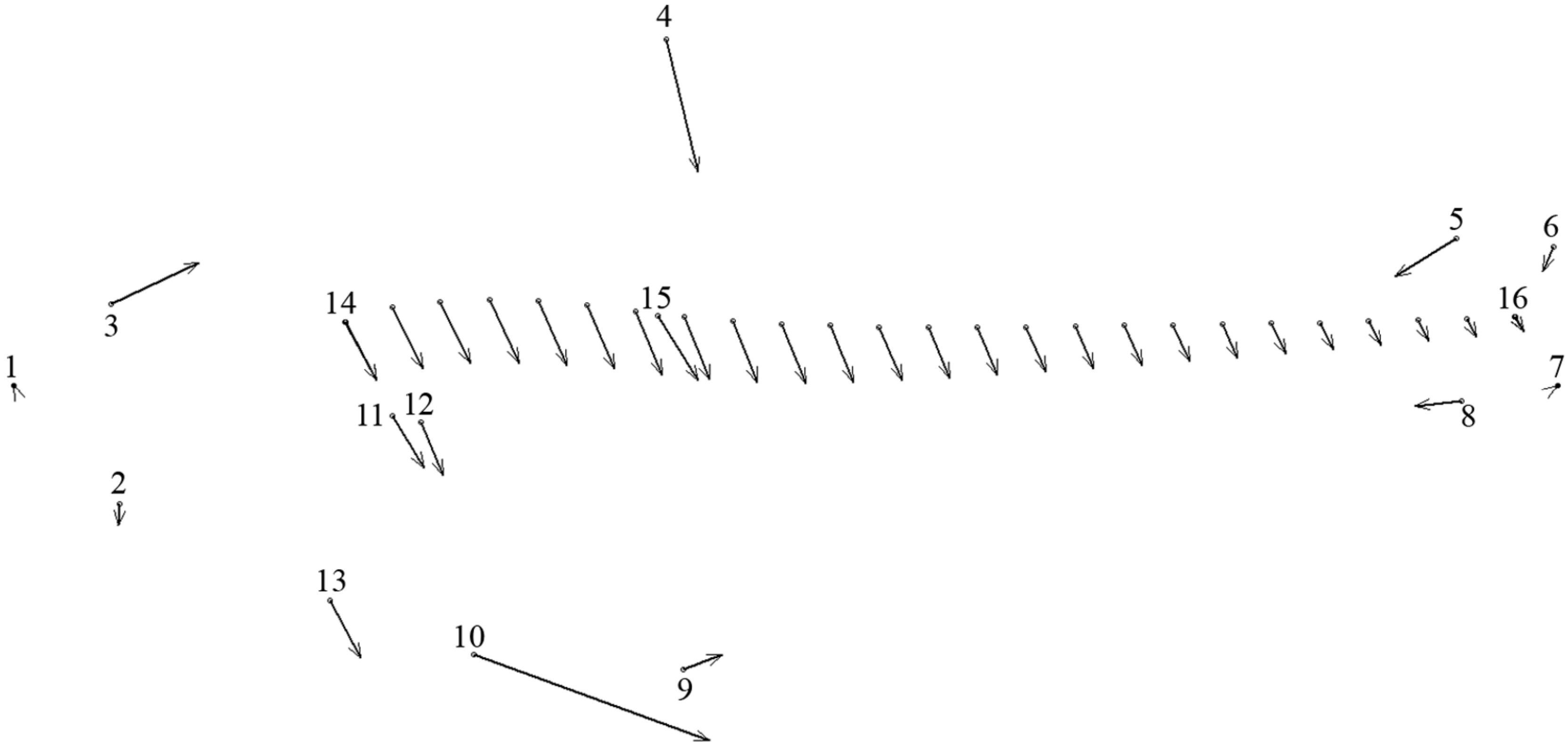

Figure 15. Vector deformation grid representing PC2 of Pleuronectidae data (the y axis of Fig. 13). Numbers correlate to landmarks represented in Figure 3. Vectors point from landmark configuration found at negative values of PC2 and towards landmark configuration found at positive values of PC2.

For the PCA representing shape variation within the family Soleidae, two distinct principal components explained 50.4% and 22.15% of the variation, respectively (Fig. 16). The distinct eigenvalue was supported by a chi-squared value of 8.72 and 9.77. The PC1 axis is loaded by variables that represented the height of the body and size of the head (Fig. 16). With negative values had small heads and shorter bodies (Fig. 17). Genera with negative means included *Phyllichthys*, *Zebrias*, *Soleichthys*, *Bathysolea*, *Syanapturichthy*, *Pegusa*, *Pardachirus*, *Vanstraelenia*, *Strandichthys*, *Euryglossa*, *Dicologoglossa*, and *Solea* (Fig. 16). Individuals with positive values had larger heads, and taller bodies (Fig. 17). Genera with positive means included *Amate*, *Microchirus*, *Monochirus*, *Brachirus*, *Aseraggodes*, *Soleidae*, *Liachirus*, *Achiroides*, and *Parachirus* (Fig. 16).

The PC2 axis was loaded by variables that represented the curvature of the spinal column and location of the anterior dorsal fin insertion point (Fig. 16). Individuals with negative values had less flexure of the spine and anterior dorsal fin insertion points located dorsal to the tip of the pre-maxilla (Fig. 18). Genera with negative means included *Vanstraelenia*, *Bathysolea*, *Solea*, *Pardachirus*, *Liachirus*, *Dicologoglossa*, *Zebrias*, *Aseraggodes*, *Soleidae*, *Parachirus*, and *Euryglossa* (Fig. 16). Individuals with positive means had more curvature to the spine and anterior dorsal fin insertion points located anterior to the tip of the pre-maxilla (Fig. 18). Genera with positive means included *Microchirus*, *Monochirus*, *Achiroides*, *Brachirus*, *Pegusa*, *Soleichthys*, *Phyllichthys*, *Strandichthys*, *Syanapturichthy*, and *Amate* (Fig. 16).

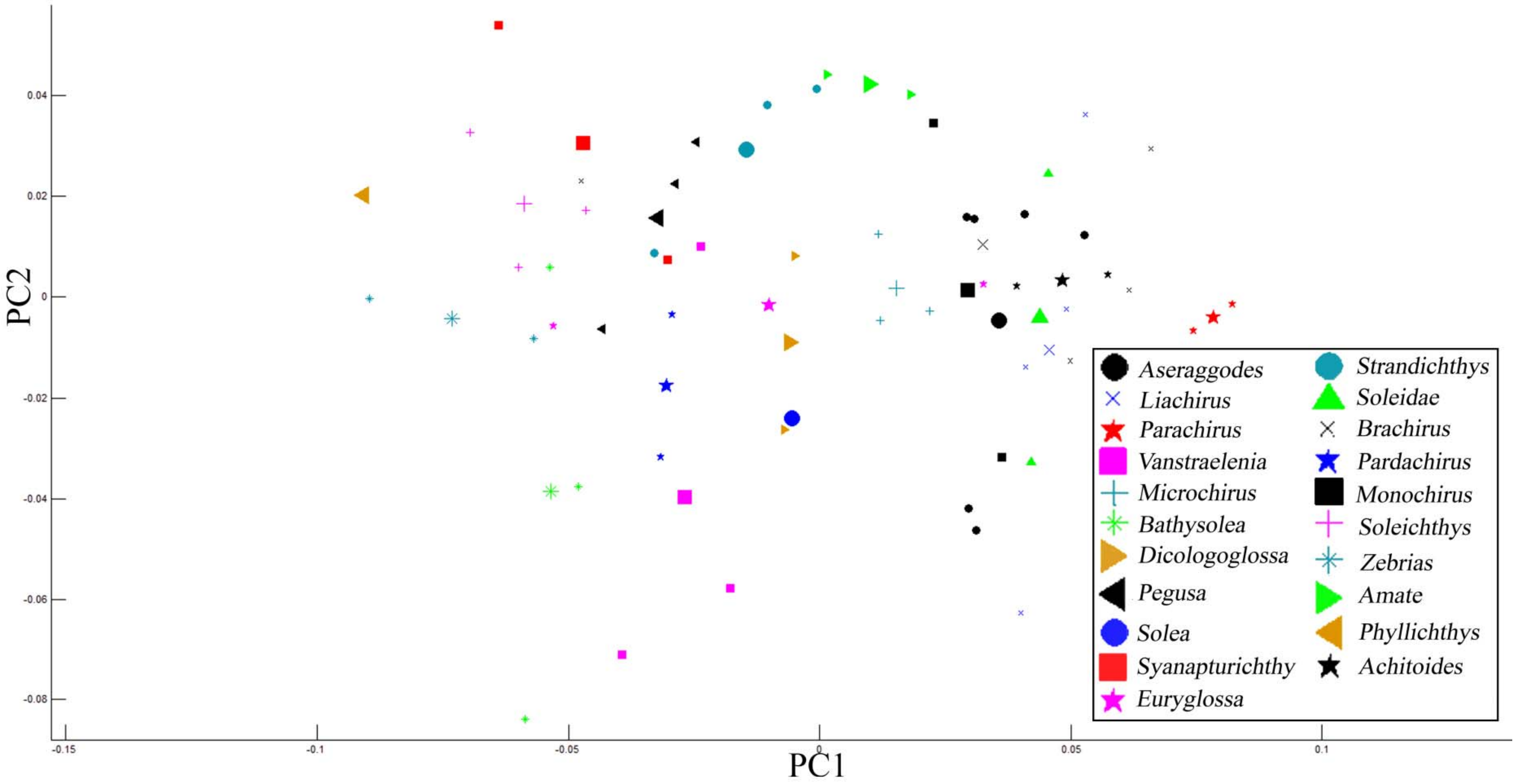

Figure 16: Principal component analysis of superimposed Soleidae data with PC1 (50.4%) represented on the x-axis and PC2 (22.15%) represented on the y-axis. Genera are depicted by the symbols displayed in the key. Larger symbols represent the median for representative genera.

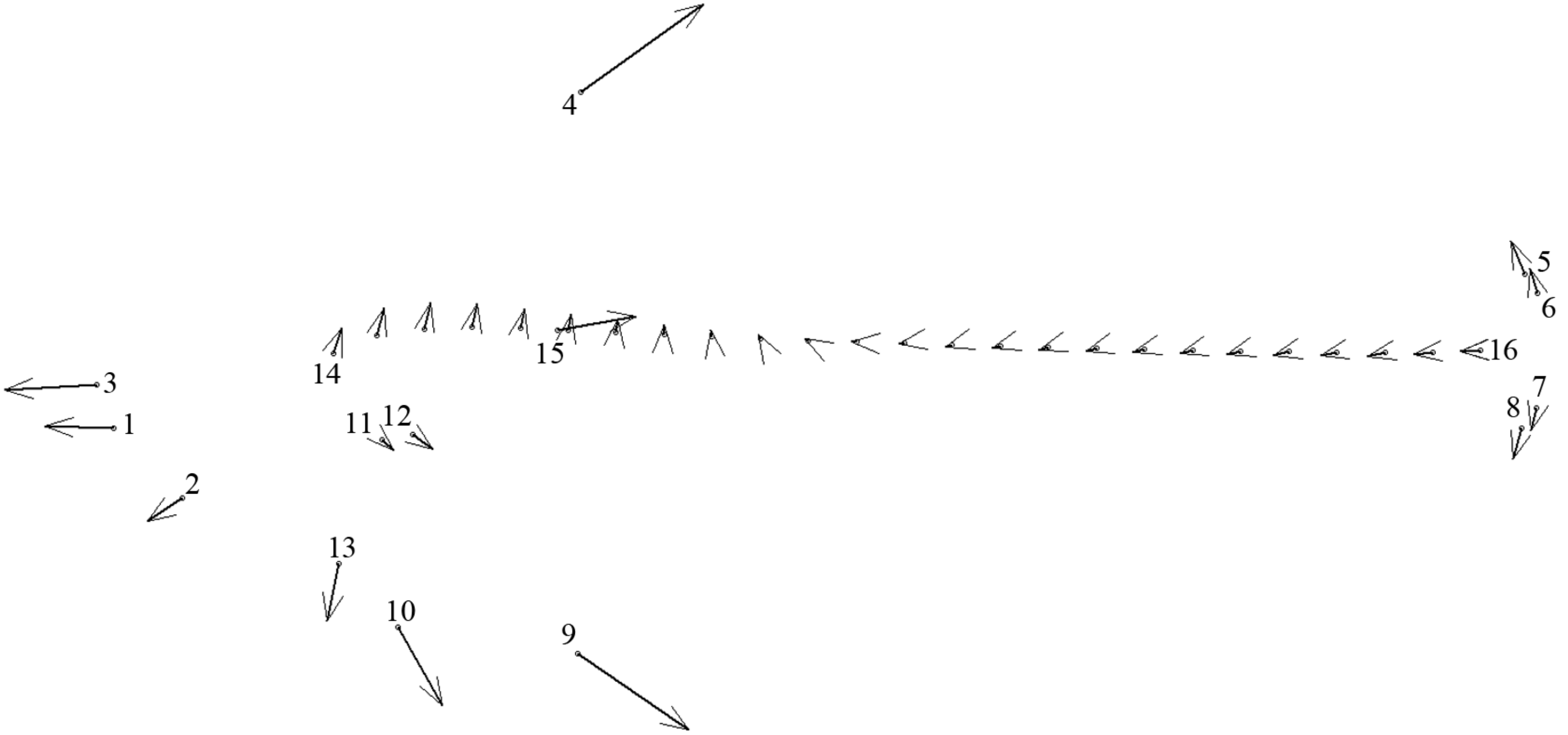

Figure 17. Vector deformation grid representing PC1 of Soleidae data (the x axis of Fig. 16). Numbers correlate to landmarks represented in Figure 3. Vectors point from landmark configuration found at negative values of PC1 and towards landmark configuration found at positive values of PC1.

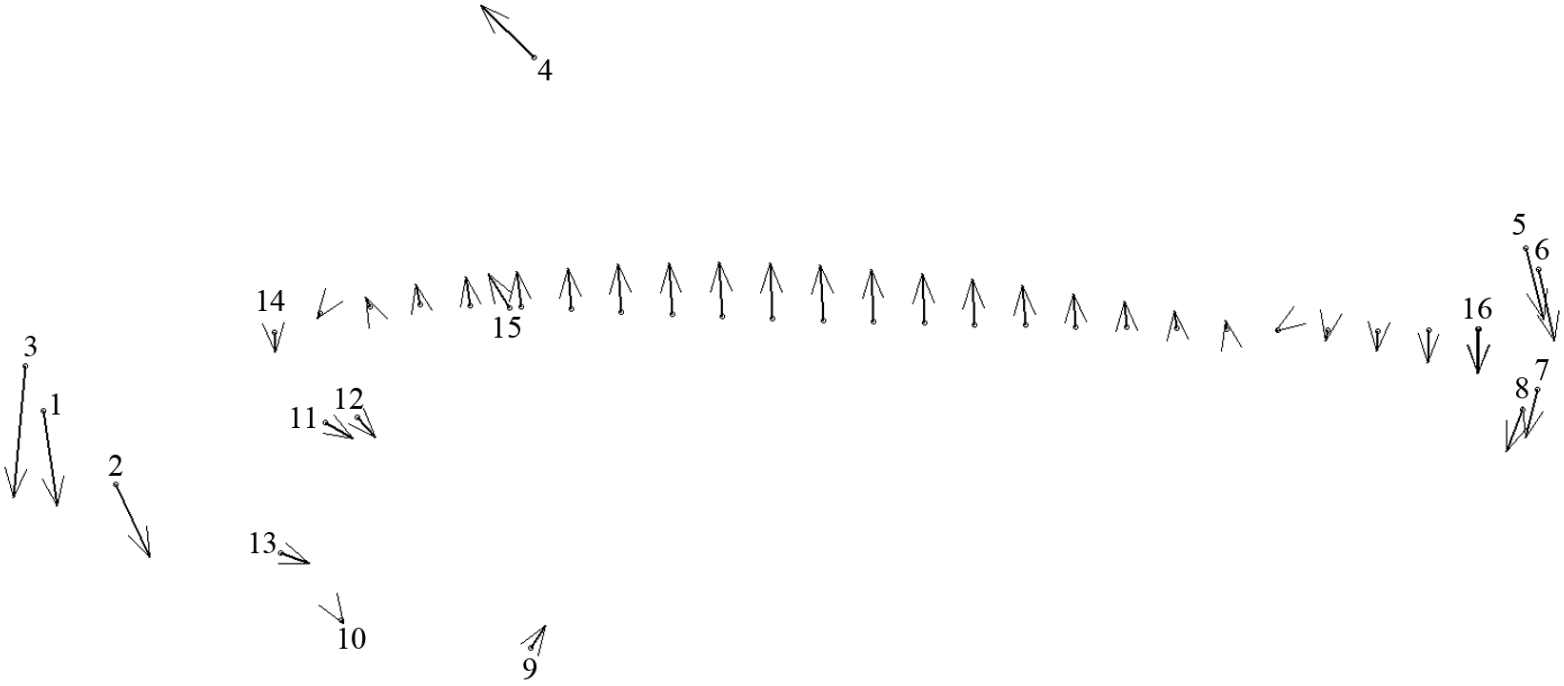

Figure 18. Vector deformation grid representing PC2 of Soleidae data (the y axis of Fig. 16). Numbers correlate to landmarks represented in Figure 3. Vectors point from landmark configuration found at negative values of PC2 and towards landmark configuration found at positive values of PC2.

# CHAPTER 7

# DISCUSSION

A long and complex history of pleuronectiform classification illuminates the morphological diversity of these fishes. Recent phylogenetic studies utilizing DNA sequence data have resolved problematic clades by determining relationships among flatfishes independent of morphology and shape. The focus of this study was to examine morphological diversity using geometric morphometrics in relation to the most current phylogenetic hypotheses and classification of Pleuronectiformes.

The first objective of this study was to determine if families within Pleuronectiformes exhibited differences in shape. The hypothesis that families would display distinct differences in shape was partially supported. The results of the morphometric analyses revealed tight clustering and clear separation for some families, but showed broad scattering and significant overlap in others. The plot of PC1 and PC2 scores showed clear separation and tight clustering of Psettodidae, Cynoglossidae, Samaridae, Citharidae, and Achiropsettidae (Fig. 4). This suggests these families have distinct shapes that differ from one another and that variation in shape within each family is conserved. Psettodidae and Citharidae shared similar traits of larger heads, taller and more fusiform bodies, and a greater curvature to the spinal column, but they clustered separately across on the PC2 axis (Fig. 4). Achiropsettidae was similar to Psettodidae and Citharidae, but showed more disk-like bodies (Fig. 4). Cynoglossidae and Samaridae shared traits of a small head with less curvature to the spinal column, but Cynoglossidae had slimmer more fusiform bodies, whereas Samaridae had disk-like bodies (Fig. 4).

There is strong evidence based on molecular and morphological data that these families are monophyletic (Hensley and Ahlstrom 1984; Berendzen and Dimmick 2002; Azevedo et al. 2008), which is further supported by the geometric morphometric analyses.

The remaining families within the Pleuronectiformes had an expansive distribution across the PCA plot (Fig. 4), including Pleuronectidae, Scophthalmidae, Soleidae, Bothidae, Paralichthyidae, and Achiridae. At least one individual from each of these families fell on both the negative and positive ends of both PC1 and PC2, with the exception of Achiridae which showed less variation in PC2 (Fig. 4). This broad scattering indicated that shape is highly diverse within these families. Interestingly, many of the families that displayed highly diverse shape morphology have a complex taxonomic history. Achiridae was originally classified as a subfamily of Soleidae, and Scophthalmidae and Paralichthyidae were considered subfamilies of Bothidae (Jordan and Evermann 1898; Kyle 1900; Hubbs 1945; Norman 1934, 1966; Hensley and Ahlstrom 1984). Furthermore, the diversity in shape observed in these families may be a result of their large, worldwide distribution and presence in a wide variety of habitats (Munroe 2005; Nelson 2006; Froese and Pauly 2011). Achiridae is endemic to the Atlantic and Pacific around the Americas, but consists of species that live in fresh, brackish, and marine water environments. Pleuronectidae, Soleidae, Bothidae, and Paralichthyidae can all be found throughout the Atlantic, Pacific, and Indian oceans; Pleuronectidae has the widest distribution expanding into the Arctic Circle (Munroe 2005; Nelson 2006). Scophthalmidae is the only exception to this observation. This group has a relatively small distribution from the North Atlantic Ocean to the Baltic, Mediterranean, and Black seas.

Patterns observed among families in the PCA analysis was compared to their phylogenetic relationships to make inferences on the evolutionary history of shape variation. The phylogenetic hypothesis proposed by Azevedo et al. (2008) was primarily used in these comparisons (Fig. 19). This phylogeny was chosen, because it is the most extensive molecular phylogeny published to date; however, many of the deep nodes in this tree are poorly supported. Phylogenetic hypothesis will also be considered from two additional published molecular studies (Berendzen and Dimmick 2002; Pardo et al. 2005). A pattern of shape and relationships were found for few clades.

Inferences on the evolutionary history of shape variation were made for families revealing similarity in shape due to common ancestry and the convergence of shape in distantly related groups. The phylogeny by Azevedo et al. (2008) revealed a sister group relationship between Soleidae and Samaridae although there was weak support for this relationship (Fig. 19). Interestingly, these families showed high levels of overlap in the morphospace, with Samaridae completely nested within Soleidae (Fig. 4). This pattern indicated a similarity in shape between these families with species having small heads and short bodies. Furthermore, the genus *Lepidorhombus* in the family of Scophthalmidae overlapped with Bothidae, Soleidae, Samaridae, Paralichthyidae, and Pleuronectidae. Historically, Scophthalmidae was considered a subfamily of Bothidae based on morphological evidence (Regan 1910; Norman 1934). The phylogenetic

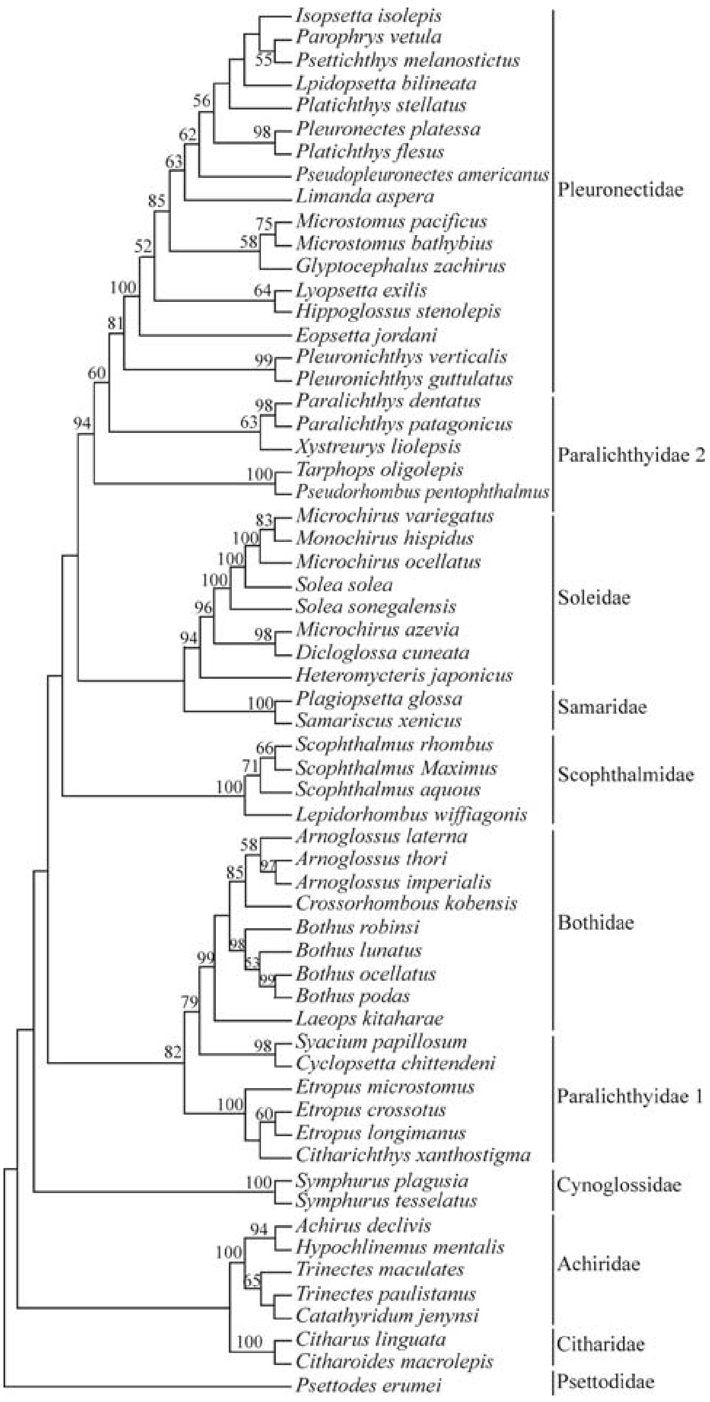

Figure 19. Azevedo et al. (2008) consensus tree representing relationships of Pleuronectiformes based on 12s and 16s rRNA. Numbers above branches represent bootstrap values.

hypothesis by Azevedo et al. (2008) revealed Scophthalmidae as the sister taxon to a clade consisting of Soleidae, Samaridae, Paralichthyidae, and Pleuronectidae. This suggests that Scophthalmidae may share similar morphologies with Soleidae, Samaridae, Paralichthyidae, and Pleuronectidae based on common ancestry.

Morphological hypotheses have suggested Cynoglossidae and Soleidae share a close relationship with one another (Regan 1910; Norman 1934; Hubbs 1945; Lauder and Liem 1983; Hensley and Ahlstrom 1984; Chapleau 1993). Interestingly, the relationship between these two families is not consistent among the molecular hypotheses. Azevedo et al. (2008) suggested these families are distantly related (Fig. 19), but there is little support for this relationship. However, the hypothesis by Berendzen and Dimmick (2002) did support a close relationship between Cynoglossidae and Soleidae (Fig. 2) consistent with the morphological data. The morphological and Berendzen and Dimmick (2002) hypotheses suggest the similarities in shape observed between Cynoglossidae and Soleidae could be due to common ancestry (Fig. 4).

The second objective of this study asked if Psettodidae differed in shape from all other flatfishes. A recent study by Campbell et al. (2013) concluded that Pleuronectiformes are not a monophyletic group, suggesting convergence of morphogenesis and characteristics related to sidedness. It was hypothesized herein that Psettodidae is different in shape from the rest of the Pleuronectiformes. The results of the geometric morphometric analyses supported this hypothesis. The plot of PC1 and PC2 revealed a tight clustering and clear separation of Psettodidae from the other

Pleuronectiformes, suggesting Psettodidae is morphologically distinct from other Pleuronectiformes.

Interestingly shape variation across the morphospace of Psettodidae from Pleuronectiformes was not distinguished when PC1 and PC2 were examined on an individual bases. Psettodidae overlapped with Pleuronectidae, Citharidae, Paralichthyidae, Achiropsettidae, Scophthalmidae, Bothidae, and Achiridae on PC1, and with Pleuronectidae on PC2 (Fig. 4). This suggests some characteristics common to Psettodidae and all other Pleuronectiformes may be examples of convergent evolution. Psettodidae shared the traits of a large head, taller body, and more curvature to the spinal column with Pleuronectidae, Citharidae, Paralichthyidae, Achiropsettidae, Scophthalmidae, Bothidae, and Achiridae, and shared a fusiform body with Pleuronectidae (Fig. 4). These results, in correlation with the newly hypothesized phylogeny, could provide a base to understanding convergence of morphogenesis in these fishes, leading to further studies (Campbell et al. 2013).

The third objective of this study asked if families with highly specialized morphologies exhibited less shape variation within the group than families with more general features. It was hypothesized that families with highly specialized features would have less variation in shape as compared to more generalized families that would show more variation in shape. Some families with specialized features had less variation in shape, whereas other families with specialized features had great variation in shape. Furthermore, some families with generalized features had more variation in shape, where as other families with generalized features had less variation in shape. This result presented conflicting evidence that did and did not support the hypothesis. Families that were identified as having a specialized morphology (i.e. reduction, or loss of paired fins, confluent dorsal, caudal, and anal fins, and more asymmetry between the eyes and blind sides) include Cynoglossidae, Samaridae, Citharidae, Soleidae, and Achiridae. The observed pattern of shape variation in Cynoglossidae, Samaridae, and Citharidae supported the hypothesis. These groups were each tightly clustered revealing little variation in shape across the PC1 and PC2 axes (Fig. 4).  This result suggests that specialized families have a more conserved shape. However, the diverse yet specialized families of Soleidae (Fig. 9) and Achiridae (Fig. 16) had large variation in shape across the morphospace. Interestingly, Achiridae has historically been identified as a subfamily of Soleidae, which could explain the large variation in shape shown by both families (Jordan and Evermann 1989; Kyle 1900; Hubbs 1945; Norman 1934, 1966; Hensley and Ahlstrom 1984). The monophyly of all the specialized families is strongly supported based on both morphological and molecular data which is further supported by the observed pattern of shape variation in this study.

Families with more generalized characteristics (i.e. paired fins present, separate dorsal, caudal, and anal fins, and more symmetry between the eyed and blind sides) included Bothidae, Paralichthyidae, and Pleuronectidae. Pleuronectidae (Fig. 13) and Bothidae (Fig. 11) contained large variation in shape within their family which supported the hypothesis that families with more generalized characters will have greater variation in shape. The historical taxonomy of these groups is complex. Pleuronectidae and

Bothidae have historically contained a large number of subfamilies that have been rearranged or removed based on morphological evidence (Jordan and Evermann 1898; Kyle 1900; Regan 1910; Norman 1934; Hubbs 1945; Lauder and Liem 1983; Hensley and Ahlstrom 1984; Chapleau 1993). These families are currently recognized as monophyletic, although detailed phylogenetic analyses of the groups are wanting (Berendzen and Dimmick 2002; Pardo et al. 2005; Azevedo et al. 2008).

Although the hypothesis that families with highly specialized features would have less variation in shape as compared to more generalized families that would show more variation in shape was largely supported, sampling size may have imposed bias in the outcome of shape variation. For example, families that showed little variation in shape (Cynoglossidae, Samaridae, and Citharidae) had smaller numbers of species included in the analyses. Whereas, families with great variation in shape (Soleidae, Achiridae, Pleuronectidae, and Bothidae) had a larger number of species included.

The fourth objective of this study asked if families and genera that are hypothesized to be polyphyletic show great amounts of shape variation. It was hypothesized in this study that families with polyphyletic relationships would show a greater diversity of shape given the evidence for divergence of lineages. Recent phylogenetic hypotheses show strong evidence supporting polyphyletic relationships within Paralichthyidae (Berendzen and Dimmick 2002; Pardo et al. 2005; Azevedo et al. 2008). The first part of the fourth objective focused on if there were differences in shape among three hypothesized lineages within Paralichthyidae (Azevedo et al. 2008). The results of the geometric morphometric analyses both supported and rejected the hypothesis. No variation in shape was found based on the PCA of the family, which suggested there was no difference between the three hypothesized lineages. However, there was visual evidence for shape variation between the three distinct lineages on the PCA (Fig. 4) including all flatfishes, suggesting there may be morphological differences across the proposed lineages. The first lineage is closely related to Bothidae and showed overlap with Pleuronectidae and Bothidae (Fig. 4), suggesting this lineage and Bothidae share similar characteristics. Interestingly, Paralichthyidae was historically recognized as a subfamily of Bothidae based on morphology, which further supports the evidence in this study (Norman 1934). The second and third lineages of Paralichthyidae are closely related to Pleuronectidae and showed great overlap in the PCA (Fig. 4). The concordance of overlap in the morphospace (Fig. 4) with the molecular phylogeny suggests that the observed variation in shape is consistent with the molecular hypotheses.

The second part of the fourth objective asked if the genus *Tephrinectes* differed in shape from all the other genera currently recognized in the family Paralichthyidae. Considerable osteological evidence has suggested the removal and elevation to the family level of the genus *Tephrinectes* (Hensley and Ahlstrom 1984; Hoshino and Amaoka 1998; Hoshino 2001). It was hypothesized that *Tephrinectes* would have a different shape than the rest of Paralichthyidae. The results of the geometric morphometric analysis rejected this hypothesis showing great overlap of *Tephrinectes* with Paralichthyidae. A lack of variation in shape could have led to the misclassification of *Tephrinectes*; however, there is bias in this study as the results may be due to low sampling numbers of *Tephrinectes*.

The third part of the fourth objective of this study asked if there was variation in shape within Bothidae. The monophyletic status of Bothidae is supported by the molecular hypotheses; however, these studies are based on limited data (Berendzen and Dimmick 2002; Pardo et al. 2005; Azevedo et al. 2008). In this study it was hypothesized that Bothidae would show a large diversity in shape, as the family contains a large number of species. Geometric morphometric analyses could not accept nor rejected the hypothesis. Little to no difference in shape between most genera was found; however, four genera had a distinct shape within the morphospace (Fig. 11). *Pelecanichthys*, *Chascanopsetta*, and *Monolene* shared a similar shape having small heads, shorter bodies, and more flexion of the spine toward the brain case, whereas *Scophthalmus* had larger heads, taller bodies, and more flexion toward the first caudal vertebrae. Further genetic evidence is needed to determine whether the variation in shape is due to the great diversity within the family, or if variation is due to non-monophyletic relationships within Bothidae.

The fifth objective of this study asked if freshwater lineages were different in shape from saltwater lineages within the family of Achiridae. Achiridae is a diverse and highly specialized family that can be found in fresh, brackish and marine water (Nelson 2006). It was hypothesized that freshwater genera would be different in shape from brackish water and marine water genera. Geometric morphometric analysis supported the hypothesis as it showed three distinct shape groups which correlated to freshwater, brackish water, and marine water genera (Fig. 9). Marine water genera had small heads, shorter bodies, and shorter cleithrums, and were very different in shape compared to freshwater and brackish water genera. The group that contained freshwater genera was very similar to the group containing brackish water genera. Both groups had larger heads, taller bodies, and longer cleithrums. Phylogenetic hypothesis suggests freshwater genera derived from brackish water ancestors, which may suggest that freshwater and brackish water genera would be similar in shape.

## Conclusion

Flatfishes have an incredible morphological diversity and complex evolutionary history, making them one of the most interesting groups of fishes to study. Historical classification relied on morphological observations and measurements, which limited accurate classification of species in regards to evolutionary relationships. Phylogenetic hypothesis based on molecular evidence has provided a clearer picture of relationships, while supporting the hypothesis that morphological evidence is not congruent with phylogenetic relationships (Chapleau 1998). In fact, most current evidence points to a non-monophyletic Pleuronectiformes and a convergence of sidedness and characters related to sidedness between Psettodidae and Pleuronectiformes (Campbell et al. 2013).

By focusing on skeletal elements associated with developmental changes and general geometric morphometrics, this study was able to examine shape variation in relation to the most current phylogenetic hypotheses and taxonomy. Morphological variation in flatfishes was generally concordant with known monophyletic relationships. Although the number of specimens and landmark points were relatively low, this was the first study to use geometric morphometrics to analyze difference in shape across the whole of Pleuronectiformes and Psettodidae by examining skeletal elements. Additional statistical analyses that will be able to determine whether or not groups are significantly different from one another will be performed. These include tests such as Goodall's F and misclassification tests. This study illustrates the large morphological diversity that flatfishes represent, and provides insight into what factors may affect morphological differences.

# REFERENCES

Amaoka K. 1969. Studies on the sinistral flounders found in the waters around Japan. Taxonomy, anatomy and phylogeny. J. Shimonoseki Univ. Fish. 18(2): 65-340.

Amaoka K, Mihara E. 2001. *Asterorhombus annulatus* (Weber, 1913), a valid species distinct from *Asterorhombus intermedius* (Bleeker, 1865) (Pleuronectiformes: Bothidae). Ichthyological Research. 48(2): 192-96.

Andrade JM, Gómez-Carracedo MP, Krzanowski W, Kubista M. 2004. Procrustes rotation in analytical chemistry, a tutorial. Chemometrics and Intelligent Laboratory Systems. 72(2): 123-32.

Azevedo MFC, Oliveira C, Pardo BG, Martínez P, Foresti1 F. 2008. Phylogenetic analysis of the order Pleuronectiformes (Teleostei) based on sequences of 12S and 16S mitochondrial genes. Genetics and Molecular Biology. 31(1). 284-92.

Berendzen PB, Dimmick WW. 2002. Phylogenetic Relationships of Pleuronectiformes Based on Molecular Evidence. Copeia. 2002(3): 642-52.

Bookstein FL, Chernoff B, Elder RL, Humphries JM, Smith GR, Strauss RE. 1985. Morphometrics in evolutionary biology: the geometry of size and shape change, with examples from fishes. Philadelphia: Academy of Natural Sciences of Philadelphia.

Cadrin, SX, Silva, VM. 2005. Morphometric variation of yellowtail flounder. ICES Journal of Marine Science: Journal du Conseil. 62(4): 683-94.

Campbell MA, Chen W-J, Andrés López J. 2013. Are Flatfishes (Pleuronectiformes) Monophyletic. Molecular Phylogenetics and Evolution. 69(3): 664-73.

Chabanaud P. 1934. Le complexe basisphenoi'dien et Ie septum orbitaire des Poissons heterosomes. C. R. Acad. Sci. 198: 1875-77.

Chanet B. 2003. Interrelationships of scophthalmid fishes (Pleuronectiformes: Scophthalmidae). Cybium. 27(4): 275-86.

Chapleau F. 1993. Pleuronectiform Relationships: A Cladistic Reassessment. Bulletin of Marine Science. 52(1): 516-40.

Chapleau F, Keast A. 1988. A phylogenetic reassessment of the monophyletic status of the family Soleidae, with comments on the suborder Soleoidei (Pisces; Pleuronectiformes). Canadian Journal of Zoology. 66(12): 2797-810.

Cooper JA, Chapleau F. 1998. Monophyly and intrarelationships of the family Pleuronectidae (Pleuronectiformes), with a revised classification. Fishery Bulletin-National Oceanic and Atmospheric Administration. 96: 686-726.

Desoutter M, Chapleau F. 1997. Taxonomic status of *Bathysolea profundicola* and *B. polli* (Soleidae; Pleuronectiformes) with notes on the genus. Ichthyological Research. 44(4): 399-412.

Evseenko SA. 1984. New genus and species of 'Armless' flounders, *Pseudomancopsetta andriahevi* gen. et sp. Nova (Pisces, Pleuronectoidei) and its position in the suborder Pleuronectoidei. Journal of Ichthyology. 25: 1-10.

Evseenko SA. 1996. Ontogeny and relationships of the flatfishes of the Southern Ocean (Achiropsettidae, Pleuronectoidei). Journal of Ichthyology. 36(9): 687-712.

Evseenko SA. 2000. Family Achiropsettidae and Its Position in the Taxonomic and Ecological Classification of the Pleuronectiformes. Journal of Ichthyology. 40(1): S110.

Froese R, Pauly D. 2011. FishBase. World Wide Web electronic publication. www.fishbase.org

Futch CR. 1977. Larvae of *Trichopsetta ventralis* (Pisces: Bothidae), with comments on intergeneric relationships within the Bothidae. Bull. Mar. Sci. 27: 740–57.

Gibson RN. 2005. Flatfishes: biology and exploitation. 1st ed. Wiley-Blackwell. p 1-9.

Gisbert E, Merino G, Muguet JB, Bush D, Piedrahita RH, Conklin DE. 2002. Morphological development and allometric growth patterns in hatchery-reared California halibut larvae. Journal of Fish Biology. 61(5): 1217-29.

Hensley DA, 1977. Larval development of *Engyophrys senta* (Bothidae), with comments on intermuscular bones in flatfishes. Bulletin of Marine Science. 27(4): 681-703.

Hensley DA, and Ahlstrom EH. 1984. Pleuronectiformes: relationships. Ontogeny and systematics of fishes. 670-87.

Hoshino K. 2000. Citharoides orbitalis, a new sinistral citharid flounder from Western Australia (Pleuronectoidei, Pleuronectiformes). Ichthyol. Res. 47: 321–26.

Hoshino K. 2001. Monophyly of the Citharidae (Pleuronectoidei: Pleuronectiformes: Teleostei) with considerations of pleuronectoid phylogeny. Ichthyol. Res. 48: 391-404.

Hoshino K, Amaoka K. 1998. Osteology of the flounder, *Tephrinectes sinensis* (Lacepède) (Teleostei: Pleuronectiformes), with comments on its relationships. Ichthyol. Res. 45: 69–77.

Hubbs CL. 1945. Phylogenetic position of the Citharidae, a family of flatfishes. Ann Arbor (MI): Univ. of Mich. Press. 63: 1-40.

Jordan DS, Evermann BW. 1898. The fishes of North and Middle America: a descriptive catalogue of the species of fish-like vertebrates found in the waters of North America, north of the Isthmus of Panama. No. 47. US Government Printing Office.

Kendall DG, Kendall WS. 1980. Alignments in two-dimensional random sets of points. Advances in Applied Probability. 12: 380-424.

Klingenberg CP, Froese R. 1991. A multivariate comparison of allometric growth patterns. Systematic Biology. 40(4): 410-19.

Kyle HM. 1900. On the Presence of Nasal Secretory Sacs and a Naso-pharyngeal Communication in Teleostei, with especial reference to Cynoglossus semilævis. Gthr. Journal of the Linnean Society of London, Zoology. 27(178): 541-56.

Kyle HM. 1921. The asymmetry, metamorphosis and origin of flat-fishes. Phil. Trans. Roy. Soc. London, Zoology. 211: 75-128.

Lagler KF, Bardach JE, Miller RR. 1962. Ichthyology. New York: John Wiley and Sons.

Lauder GV, Liem KF. 1983. The evolution and interrelationships of the actinopterygian fishes. Bull. Mus. Compo. Zool. 150: 95-197.

Morrison DF. 1967. Multivariate Statistical Methods. New York: McGraw-Hill Book Company.

Munroe TA. 2005. Systematic diversity of the Pleuronectiformes. In: Gibson RN. Flatfishes: biology and exploitation. 1st ed. Hoboken (NJ): Wiley-Blackwell. p 10-41.

Nelson JS. 1984. Fishes of the world. 2nd ed. New York: John Wiley and Sons. p 523.

Nelson JS. 2006. Fishes of the world. 4th ed. New York: John Wiley and Sons. p 523.

Norman JR. 1934. A systematic monograph of the flatfishes (heterosomata). Vol. I Psettodidae, Bothidae, Pleuronectidae.

Pardo BG, Machordom A, Foresti F, Porto-Foresti F, Azevedo MF, Banon R, Sanchez L, Martinez P. 2005. Phylogenetic analysis of flatfish (Order Pleuronectiformes) based on mitochondrial 16s rDNA sequences. Scientia Marina. 69(4): 531-43.

Regan CT. 1910. The origin and evolution of the Teleostean fishes of the order Heterosomata. The Annals and Magazine of Natural History. 6(35): 484-496.

Regan CT. 1929. Fishes. Encyclopaedia Britannica. 14th ed. IX. Heterosomata. p 324-25.

Rohlf J. 2010. tpsDig, version 2.12. Stony Brook (NY): Department of Ecology and Evolution, State University of New York at Stony Brook.

Russo T, Pulcini D, O'Leary Á, Cataudella S, Mariani S. 2008. Relationship between body shape and trophic niche segregation in two closely related sympatric fishes. Journal of Fish Biology. 73(4): 809-828.

Sakamoto K. 1984. Interrelationships of the family Pleuronectidae (Pisces: Pleuronectiformes) [dissertation]. [Yamagochi, Japan]: Hokkaido University.

Sheets HD. 2001. PCAGen Manual. Buffalo (NY): Canisius College. http://www3.canisius.edu/~sheets/PCAGenManual.pdf

Sheets HD. 2011. PCAGen7. Buffalo (NY): Canisius College. http://www3.canisius.edu/~sheets/imp7.html

Strauss RE, Bookstein FL. 1982. The truss: body form reconstructions in morphometrics. Systematic Biology. 31(2): 113-135.

Verneau O, Moreau C, Catzeflis FM, Renaud F. 1994. Phylogeny of flatfishes (Pleuronectiformes): comparisons and contradictions of molecular and morpho-anatomical data. Journal of fish biology. 45(4): 685-696.

Zeldich ML, Swiderski DL, Sheets HD. 2012. Geometric Morphometrics for Biologists, Second Edition: A Primer. 2nd ed. Waltham (MA): Academic Press.

# APPENDIX

Table 1: Taxonomy and catalog numbers of specimens used in analyses.

| Taxon | Catalog Number (Count) |
|---|---|
| Pleuronectiformes | |
| Achiridae | |
| *Achirus declivis* | USNM 286840 (2) |
| *Achirus lineatus* | USNM 156403 (2) |
| *Achirus mazatlanus* | KU 22694 (2) |
| *Apionichthys dumerili* | USNM 233556 (1), USNM 233588 (1) |
| *Catathuridium jenunsii* | USNM 55583 (2), USNM 181499 (1) |
| *Gymnachirus melas* | KU 30098 (1), KU 30120 (1), USNM 291088 (3) |
| *Gymnachirus texae* | KU 29675 (1), KU 29675 (1), USNM 158296 (1), USNM 358229 (1) |
| *Hypoclinemus mentalis* | USNM 167720 (1), USNM 191555 (2) |
| *Nodogymnus fasciatus* | USNM 152033 (1) |
| *Trinectes maculatus* | USNM 15091 (1), USNM 34837 (2) |
| Achiropsettidae | |
| *Mancopsetta maculata* | USNM 362523 (1), USNM 362528 (2) |
| Bothidae | |
| *Arnoglossus blachei* | USNM 282031 (3) |
| *Arnoglossus conspersus* | USNM 282245 (3) |
| *Arnoglossus imperialis* | USNM 357926 (3) |
| *Asterorhombus fijiensis* | USNM 260366 (3), USNM 362478 (1) |
| *Bothus lunatus* | USNM 282590 (1), USNM 349048 (1), USNM 359466 (1) |
| *Bothus pantherinus* | USNM 375617 (3) |
| *Bothus poda* | KU 19935 (1) |
| *Bothus robinsi* | USNM 159614 (3) |
| *Chascanopsetta lugubris* | USNM 282744 (1) |
| *Crossorhombus azureus* | USNM 260395 (3) |
| *Engyophrys sanctilaurentii* | USNM 375570 (3) |
| *Engyprosopon grandisquama* | USNM 56384 (3) |
| *Grammatobothus polyophthalmus* | USNM 260448 (1), USNM 260449 (1), USNM 260481 (1) |
| *Laeops kitaharae* | USNM 362498 (1) |
| *Laeops nigromaculatus* | USNM 307566 (3) |
| *Monolene atrimana* | USNM 159442 (3) |
| *Parabothus chlorospilus* | USNM 394618 (3) |
| *Pelecanichthys crumenalis* | USNM 55256 (3) |

| Taxon | Catalog Number (Count) |
|---|---|
| *Perissias taeniopterus* | USNM 362514 (1), USNM 362515 (1) |
| *Platophrys* | USNM 169911 (2) |
| *Psettina gigantea* | USNM 260446 (1), USNM 260482 (1) |
| *Scophthalmus aquosus* | USNM 91255 (3) |
| *Scophthalmus maximus* | USNM 22996 (1), USNM 25963 (2) |
| *Taeniopsetta radula* | USNM 394619 (3) |
| *Trichopsetta caribbaea* | USNM 159579 (3) |
| *Trichopsetta ventralis* | USNM 159510 (1), USNM 395224 (2) |
| Citharidae | |
| *Brachypleura novaezeelandiae* | USNM 261526 (3) |
| *Citharoides macrolepis* | KU 27264 (2), USNM 308017 (3) |
| *Citharus linguatula* | USNM 362482 (1), USNM 362485 (2), USNM 397277 (3) |
| *Lepidoblepharon ophthalmolepis* | USNM 127409 (1) |
| Cynoglossidae | |
| *Arelia bilineata* | USNM 203758 (3) |
| *Cynoglossus arel* | USNM 203995 (1) |
| *Cynoglossus interruptus* | KU 27260 (1) |
| *Symphurus atricaudus* | USNM 38018 (3) |
| *Symphurus bathyspilus* | USNM 138062 (3) |
| *Symphurus civitatium* | USNM 157694 (2), USNM 158278 (1) |
| *Symphurus plagiusa* | USNM 316767 (3) |
| Paralichthyidae | |
| *Ancylopsetta cycloidea* | USNM 282409 (1), USNM 282411 (1), USNM 282412 (1) |
| *Ancylopsetta dilecta* | KU 30118 (1) |
| *Ancylopsetta quadrocellata* | USNM 93598 (2), USNM 125387 (1), USNM 156077 (1) |
| *Azevia panamensis* | USNM 81038 (1) |
| *Citharichthys arctifrons* | USNM 29064 (1) |
| *Citharichthys gilberti* | KU 40338 (2) |
| *Citharichthys macrops* | KU 5112 (1) |
| *Citharichthys stigmaeus* | KU 23709 (1) |
| *Cyclopsetta chittendeni* | USNM 155724 (1), USNM 156026 (1), USNM 156028 (1) |
| *Etropus crossotus* | USNM 93611 (1), USNM 300513 (2) |
| *Etropus microstomus* | USNM 119050 (3) |
| *Gastropsetta frontalis* | USNM 286092 (1), USNM 286096 (2) |
| *Hippoglossina bollmani* | USNM 362262 (1), USNM 362276 (2) |
| *Lioglossina tetrophthalmus* | USNM 362503 (1), USNM 375893 (1) |

| Taxon | Catalog Number (Count) |
|---|---|
| *Paralichthys adspersus* | USNM 362302 (3) |
| *Paralichthys albigutta* | USNM 157642 (3) |
| *Paralichthys californicus* | USNM 54775 (3) |
| *Paralichthys lethostigma* | KU 20072 (1) |
| *Pseudorhombus arsius* | USNM 375500 (3) |
| *Pseudorhombus pentophthalmus* | USNM 71465 (3) |
| *Syacium gunteri* | USNM 118643 (3) |
| *Syacium micrurum* | USNM 286626 (3) |
| *Tarphops oligolepis* | KU 27269 (1), USNM 77071 (1), USNM 152478 (2) |
| *Tephrinectes sinensis* | USNM 86372 (3), USNM 87056 (1) |
| *Thysanopsetta naresi* | USNM 77392 (1), USNM 103793 (1) |
| *Xystreurys liolepis* | USNM 41906 (1), USNM 46317 (1) |
| Pleuronectidae | |
| *Acanthopsetta nadeshnyi* | USNM 77114 (1), USNM 77118 (1), USNM 77123 (1) |
| *Ammotretis rostratus* | USNM 282708 (3) |
| *Atheresthes stomias* | USNM 125529 (1) |
| *Cleisthenes herzensteini* | USNM 77093 (1), USNM 77095 (1), USNM 77097 (1) |
| *Cleisthenes pinetorum* | USNM 77089 (1), USNM 150375 (3) |
| *Drepanopsetta platessoides* | USNM 197612 (3) |
| *Embassichthys bathybius* | USNM 150190 (1), USNM 187656 (1) |
| *Eopsetta grigorjewi* | USNM 71960 (1), USNM 77081 (1), USNM 77083 (1) |
| *Eopsetta jordani* | USNM 27499 (1), USNM 46429 (1), USNM 365701 (1) |
| *Glyptocephalus cynoglossus* | USNM 261360 (2), USNM 261527 (3) |
| *Glyptocephalus zachirus* | USNM 306352 (3) |
| *Hippoglossoides dubius* | USNM 77059 (1), USNM 77061 (2) |
| *Hippoglossoides elassodon* | USNM 60659 (3) |
| *Hippoglossus hippoglossus* | USNM 39743 (1), USNM 54300 (1), USNM 163652 (1) |
| *Hypsopsetta guttulata* | USNM 286147 (1) |
| *Isopsetta isolepis* | USNM 54037 (3) |
| *Kareius bicoloratus* | USNM 56373 (1), USNM 71997 (2) |
| *Lepidopsetta bilineata* | USNM 76430 (3) |
| *Limanda angustirostris* | USNM 77181 (1), USNM 77182 (3) |
| *Limanda limanda* | USNM 261534 (3) |
| *Liopsetta glacialis* | USNM 29928 (1), USNM 48630 (2) |
| *Lyopsetta exilis* | USNM 60632 (1), USNM 63562 (3) |

| Taxon | Catalog Number (Count) |
|---|---|
| *Microstomus pacificus* | USNM 46411 (2), USNM 63573 (1) |
| *Oncopterus darwini* | USNM 86732 (1) |
| *Parophrys vetulus* | USNM 46435 (3), USNM 127075 (2) |
| *Pelotretis flavilatus* | USNM 176808 (3) |
| *Peltorhamphus novaezeelandiae* | USNM 320592 (3) |
| *Platichthys flesus* | USNM 10031 (3) |
| *Platichthys stellatus* | USNM 54485 (3) |
| *Pleuronectes platessa* | USNM 197577 (3) |
| *Poecilopsetta albomarginata* | USNM 159446 (3) |
| *Poecilopsetta beanii* | USNM 164146 (3) |
| *Poecilopsetta plinthus* | USNM 77186 (2), USNM 150688 (1) |
| *Protopsetta herzensteini* | USNM 71961 (1), USNM 71996 (1) |
| *Psettichthys melanostictus* | USNM 36894 (1), USNM 67272 (1) |
| *Pseudopleuronectes americanus* | USNM 48972 (3) |
| *Reinhardtius hippoglossoides* | USNM 286576 (1) |
| *Rhombosolea leporina* | USNM 304937 (1) |
| *Rhombosolea plebeia* | USNM 176810 (3) |
| *Rhombosolea tapirina* | USNM 286578 (3) |
| *Tanakius kitaharae* | USNM 77162 (1), USNM 77165 (2) |
| *Verasper moseri* | USNM 49456 (3) |
| Psettodidae | |
| *Psettodes belcheri* | USNM 286357 (1), USNM 286358 (1), USNM 286359 (1) |
| *Psettodes erumei* | USNM 36896 (1), USNM 122016 (1), USNM 122017 (1), USNM 345415 (1), USNM 361608 (1) |
| Samaridae | |
| *Plagiopsetta glossa* | USNM 396096 (1) |
| *Samariscus longimanus* | USNM 137384 (1), USNM 137385 (2) |
| Scophthalmidae | |
| *Lepidorhombus boscii* | USNM 286177 (3) |
| Soleidae | |
| *Achiroides melanorhynchus* | USNM 230355 (2) |
| *Amate japonica* | USNM 71608 (1), USNM 72090 (1) |
| *Aseraggodes cyaneus* | USNM 137676 (1), USNM 137677 (1) |
| *Aseraggodes kobensis* | USNM 71464 (3), USNM 286826 (1) |
| *Bathysolea polli* | USNM 286834 (1), USNM 286835 (2) |
| *Brachirus aenea* | USNM 305762 (3) |
| *Brachirus aspilos* | USNM 137679 (1) |
| *Dicologoglossa hexophthalma* | USNM 290983 (1), USNM 290985 (2) |

| Taxon | Catalog Number (Count) |
|---|---|
| *Euryglossa orientalis* | USNM 291012 (1) |
| *Euryglossa sorsogonensis* | USNM 340538 (1) |
| *Liachirus melanospilos* | USNM 76657 (1), USNM 236108 (3) |
| *Microchirus frechkopi* | USNM 274752 (2), USNM 274759 (1) |
| *Monochirus monochir* | USNM 34359 (2) |
| *Parachirus xenicus* | USNM 218768 (3) |
| *Pardachirus balius* | USNM 306429 (2) |
| *Pegusa impar* | USNM 291006 (1), USNM 291008 (2) |
| *Phyllichthys sclerolepis* | USNM 174031 (1) |
| *Solea impar* | USNM 291007 (1) |
| *Soleichthys microcephalus* | USNM 47886 (2), USNM 59956 (1) |
| *Soleidae* | USNM 291140 (2) |
| *Strandichthys muelleri* | USNM 22853 (1), USNM 291084 (1), USNM 291085 (1) |
| *Synapturichthys kleini* | USNM 291009 (1), USNM 291101 (1) |
| *Vanstraelenia chiropthalmus* | USNM 274741 (3) |
| *Zebrias fasciatus* | USNM 191154 (1) |
| *Zebrias japonicus* | USNM 56372 (1) |