Molecular Phylogenetics and Evolution
Vol. 13, No. 1, October, pp. 77–81, 1999
Article ID mpev.1999.0629, available online at http://www.idealibrary.com on IDEAL®

Molecular Phylogeny and Speciation of the Surfperches (Embiotocidae, Perciformes)

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Received July 15, 1998; revised November 6, 1998

Labroid fishes include a variety of families, such as wrasses (Labridae), odacids (Odacidae), damselfishes (Pomacentridae), parrotfishes (Scaridae), cichlids (Cichlidae), and surfperches (Embiotocidae). With only 23 species, the small embiotocid family exhibits a remarkably low species diversity compared to the large species diversity of the Cichlidae. Using mitochondrial DNA sequences of all 14 extant embiotocid genera, we established a molecular phylogeny of the family and compared it with a previously proposed morphological phylogeny. Genetic differentiation among embiotocids was compared to that among cichlids. Although species numbers are extremely different between these two families, the degrees of genetic differentiation within each family was found to be very similar. © 1999 Academic Press

Key Words: **Embiotocidae; surfperches; Cichlidae; mtDNA; speciation; sexual selection.**

INTRODUCTION

The surfperches (Embiotocidae) are northern Pacific fishes that belong to the perciform suborder, Labroidei. Embiotocids, along with other members of the labroid suborder, such as cichlids (Cichlidae), parrotfishes (Scaridae), damselfishes (Pomacentridae), odacids (Odacidae), and wrasses (Labridae), have pharyngeal jaws that allowed them to develop complex feeding strategies (Liem, 1986; see Streelman and Karl, 1997, for a discussion on Labroid monophyly). Surfperch feeding adaptations and behavior are reminiscent of cichlids. Embiotocids display elaborate courtship behavior (M. Cummings, pers. com.) and have a unique mode of reproduction, as they are viviparous and give birth to fully developed young. While considerable work has focused on embiotocids, their phylogenetic relationships have been addressed with morphological data (Tarp, 1952) but not previously with molecular data, as opposed to their labroid relatives, the cichlids. Indeed, over the past few years, cichlids have been the focus of several studies in molecular evolution and are now becoming an influential paradigm in general evolutionary theory. The ecological success of cichlids in Central America and in the African great lakes has been attributed to unique characteristics, such as breeding behavior, niche partitioning, color-based sexual selection, and feeding adaptations (Fryer and Iles, 1972; Greenwood, 1984). Yet the recent explosive speciation of cichlids resulted in only modest genetic differentiation (e.g. Meyer, 1993a).

Embiotocids include 14 genera divided into 23 species. The most species-rich genera comprise only 3 species, and 7 of 14 surfperch genera are monotypic. One possible explanation for the low number of embiotocid species would be the incorrect taxonomic assignment of this group of species to family level. If this were the case, we would expect to find unusually low levels of genetic differentiation between surfperch genera.

Keeping in mind the vast difference in evolutionary success between cichlids and embiotocids, our goal was first to establish a molecular phylogeny of the surfperch family and then test the hypothesis that the genetic differentiation between surfperch genera is not as large as would be expected for genera that group together at the family level.

MATERIALS AND METHODS

Collections and DNA Extraction

All embiotocid genera were sampled and analyzed. *Ditrema temmincki* and *Neoditrema ransonneti* were obtained from Dr. N. Okada (Tokyo, Japan). The freshwater *Hysterocarpus traski* was collected using a beach seine in Lake El Estero, Monterey, California (introduced population). All other specimens were collected in the Monterey Bay, California. *Zalembius rosaceus* was collected using an otter trawl. *Brachyistus frenatus, Damalichthys vacca, Embiotoca jacksoni, Rhacochilus toxotes,* and *Hypsurus caryi* were collected by scuba diving using a pole spear. *Amphisticus argenteus, Cymatogaster aggregata, Hyperprosopon argenteum, Micrometrus minimus,* and *Phanerodon furcatus* were collected

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1055-7903/99 $30.00

using a beach seine. Two pomacentrid species (Pomacentridae) were used as outgroups: *Dascyllus trimaculatus* and *Chromis chromis,* as they have been shown to be the closest relatives to Embiotocidae (Streelman and Karl, 1997). Both species were collected by free-diving using a pole spear in Rangiroa Atoll, Tuamotu, French Polynesia, and in Orbetello, Italy, respectively. Liver tissue was extracted and preserved at ambient temperature in ethanol until DNA extraction. Tissues were digested overnight at 55°C in 500 µl of extraction buffer (10 mM Tris, 400 mM NaCl, 2 mM EDTA, 2% SDS, Proteinase K). The DNA was then purified by chloroform extraction and ethanol precipitation.

Polymerase Chain Reaction (PCR) Amplification

Amplification of the mitochondrial cytochrome *b* and 16S ribosomal gene regions was accomplished using primers from the literature (Kocher *et al.,* 1989; Meyer, 1993b). The amplifications (25 µl) contained 10 to 100 ng of DNA, 10 mM Tris–HCL (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 units of Taq DNA Polymerase (Perkin–Elmer, Norwalk, CT), 150 mM each dNTP, and 0.3 mM each primer, and used a cycling profile of 45 s at 94°C, 45 s at 48°C, 1 min at 72°C, for 35 cycles. Automated sequencing was performed in both directions with the amplification primers using an ABI 373 automated sequencer (Applied Biosystems, Foster City, CA).

Sequence Analysis and Phylogenetic Reconstructions

Sequences were aligned with the aid of the computer program Clustal V in Sequence Navigator (Applied Biosystems). In order to make our data comparable with the cichlid literature, genetic distances were calculated from all observed substitutions (Jukes and Cantor, 1969). Phylogenetic relationships were assessed using the maximum parsimony (MP, using branch and bound) method and the neighbor-joining (NJ) method implemented by the software package PAUP (version 4.0.d63; Swofford, 1998). Statistical confidence was evaluated using 2000 bootstrap replicates (Felsenstein, 1985; Hedges, 1992). Homogeneity tests, alternative tree topology tests using the topology-dependent tail permutation test (with 100 replicates; Faith, 1991; Trueman, 1996; Faith and Trueman, 1996) and the KH test (Kishino and Hasegawa, 1989) were performed using PAUP.

RESULTS AND DISCUSSION

Sequences

The mitochondrial cytochrome *b* and 16S ribosomal genes were partially sequenced for one representative of each embiotocid genus and two outgroups (GenBank Accession nos. AF 159327–AF 159355). Of the 1170 aligned base pairs (687 for the cytochrome *b* region, 483 for the 16S region), 19 positions from the 16S portion of the dataset were ambiguously aligned and were therefore excluded from the analysis. Among the remaining aligned positions, 405 were variable, and 316 were phylogenetically informative. Tree topologies were found to be identical when 16S and cytochrome *b* datasets were used independently. Furthermore, a Homogeneity Test was performed, and the two datasets were not found to be significantly different (not shown); thus we considered it legitimate to combine the datasets for further analysis.

A single 998-step (Consistency Index 0.53) most parsimonious tree was obtained as shown in Fig. 1. This tree was topologically identical to the tree obtained using the neighbor-joining method. Transitions were more frequent than transversions; the ratio was 3.9, indicating that we were not within the saturation zone (Meyer, 1993b). Several weighting schemes (transitions vs tranversions) were also used to evaluate alternative topological hypotheses. The topology of the tree (Fig. 1) remained unchanged when using any of the weighting schemes (4:1, 3:1, 2:1, 1:2, 1:3, 1:4) or when only third codon position transversions of the cytochrome *b* were used (Kocher and Carleton, 1997).

Phylogenetic Relationships

The phylogenetic relationships based on molecular data (Fig. 1) and morphological data (Fig. 2) were found to be different. The difference between the two topologies was statistically significant (Kishino and Hasegawa test, $P < 0.0001$). However, while the morphological topology was found to be incompatible with our data as a whole, some groupings were common to both analyses.

In our analysis, the genera *Amphisticus* and *Hyperprosopon* grouped together and were found to be sister taxa of the remaining embiotocids (Fig. 1). This grouping was well supported by bootstrap analysis (100%) and is in agreement with phylogenetic relationships of the family based on morphological characters (Fig. 2) (Tarp, 1952). These two genera have previously been grouped in the subfamily Amphistichinae, which was placed as the sister group of the subfamily Embiotocinae, which includes all the remaining embiotocids.

Within the subfamily Embiotocinae, three genera, *Cymatogaster, Micrometrus,* and *Hysterocarpus,* formed a basal well-supported monophyletic assemblage (bootstraps were 99% with NJ and 92% with MP). The tree based on morphological characters also places these three genera close together (along with *Brachyistius*). Within this group, *Hysterocarpus* was found to be the sister clade of *Cymatogaster* (100% bootstraps). These two species have been shown to exhibit ecological affinities, as *Hysterocarpus traski* is the only freshwater embiotocid and *Cymatogaster* is the only member of the family to occasionally venture to brackish water, suggesting that *Hysterocarpus* may have first invaded brackish waters and then moved into freshwater habitats.

The three genera described above were found to be

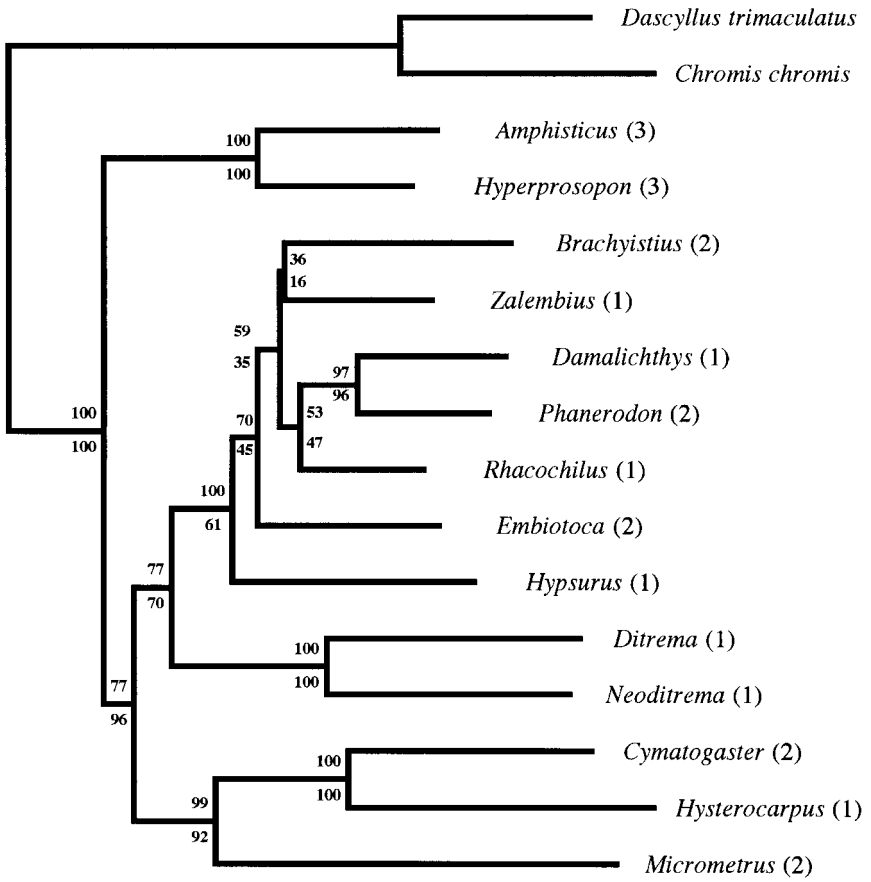

FIG. 1. Phylogenetic tree based on the cytochrome *b* and 16S ribosomal regions (identical topology was obtained using the maximum parsimony and neighbor-joining methods). Pomacentridae (*Dascyllus trimaculatus* and *Chromis chromis*) were used as outgroups. All genera of the family are represented; number of species within each genus is in parentheses. Numbers above and below the nodes represent bootstrap support using neighbor-joining (above) and maximum parsimony (below). Branches are drawn according to the number of inferred substitutions.

the sister clade to the remaining embiotocids, which were divided into two clades. The first clade includes the only Japanese representatives of the family, *Ditrema* and *Neoditrema* (100% bootstrap support for this clade). While it has been proposed that the origin of diversification of the family was in California, two species are found in Japan but are absent from the Aleutian Islands. As such, embiotocids display a characteristic amphipacific distribution (Andriashev, 1939). It has been suggested that the divergence of the Japanese species from other embiotocids resulted from migration during a warm early Pleistocene interglacial period (Tarp, 1952; DeMartini, 1969). However, our data do not support this suggestion. Sequence divergence between Japanese species and their sister clade is 10.5%, which, when using the generally accepted mitochondrial substitution rate (2% per My), dates the approximate time of divergence at approximately 5 million years ago. Although molecular clocks should be used with caution, our results point toward a more ancient migration of surfperches across the northern Pacific than previously proposed.

The second clade includes all the remaining genera.

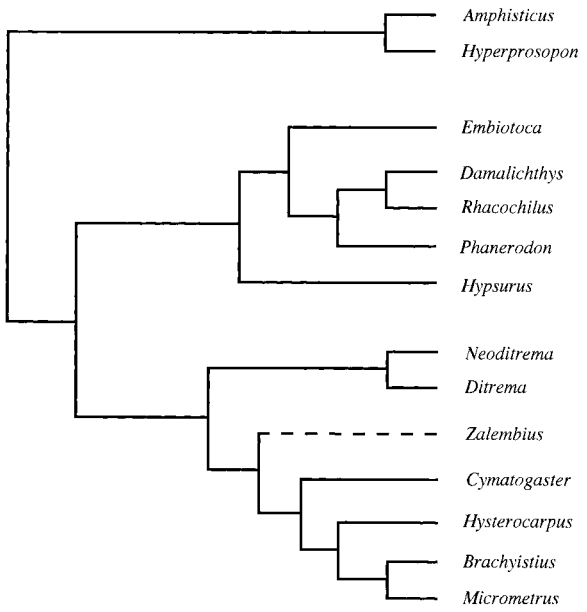

FIG. 2. Phylogenetic relationships of the surfperch genera based on morphological characters (Tarp, 1952). Tarp (1952) described an uncertain relationship for *Zalembius* which is represented here with a dashed branch.

Although some of the relationships within this group are only weakly supported (Fig. 1), results are mostly consistent with the morphology-based phylogeny. Among the few differences between the molecular and the morphological phylogenies are the positions of the genera *Brachyistius* and *Damalichthys.* These two genera were synonymized by Tarp (1952) with the genera *Micrometrus* and *Rhacochilus,* respectively (Fig. 2). In both cases our results rejected such groupings (T-PTP tests, $P < 0.01$ in both cases). In contrast, our results are in agreement with later studies that give full taxonomic ranking to *Brachyistius* (Hubbs and Hubbs, 1953) and *Damalichthys* (Morris, 1982). Our data also placed the pink surfperch, *Zalembius rosaceus,* in this clade, while Tarp had tentatively placed this species as a close relative of *Cymatogaster.*

In conclusion, the molecular phylogeny based on 16SrRNA and cytochrome *b* sequences divides embiotocids into two major subfamilies, Amphistichinae and Embiotocinae. Amphistichinae includes two genera, *Amphistichus* and *Hyperprosopon.* Within the subfamily Embiotocinae, three groups can be distinguished: an ancestral clade consisting of three genera of smaller species (*Cymatogaster, Hysterocarpus, Micrometrus*); a monophyletic Japanese clade that includes the species found in the western Pacific, which may have diverged approximately 5 million years ago; and a clade comprising all the remaining species. These three clades are all well supported by molecular data.

Molecular Divergences

Cytochrome *b* sequences have been widely used to study molecular divergence in cichlids (e.g., Meyer *et al.,* 1990; Meyer, 1993a; Sturmbauer and Meyer, 1992) but little has been done on cichlid 16S ribosomal regions. Thus, we decided to restrict this part of the analysis to the cytochrome *b* dataset. Molecular divergence has been shown to be very variable in cichlids. Indeed, some genera, such as the African *Tropheus* or the Neotropical *Cichlasoma* complex, have been shown to exhibit more cytochrome *b* sequence divergence (5 and 11%, respectively, Fig. 3) than whole species flocks, such as those encountered in Lake Malawi or Lake Victoria (Meyer, 1993a).

Cytochrome *b* sequence divergence for the family Embiotocidae (average 11.8%, maximum 17.2%) was found to be higher than the average sequence divergence of African lakes cichlid species flocks (Lake Tanganika, 9.6%; Lake Malawi, 4.5%; Lake Victoria, 0%) (Fig. 3) and slightly lower than the average divergence found between Neotropical and African cichlids (17.1%). Therefore, the idea that the low number of embiotocid species may be associated with a low level of genetic differentiation and possibly an incorrect ranking of these species at the family level is not supported by our data.

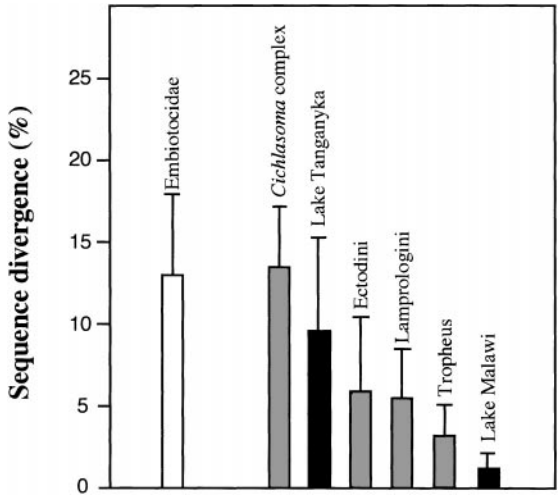

FIG. 3. Comparison of sequence divergences between embiotocid and cichlid fishes. Sequence divergences were based on a 400-bp portion of the cytochrome *b* gene. Histograms represent average sequence divergence; bars represent maximum values of sequence divergence. The white histogram is for Embiotocidae; black histograms are for African Great Lakes cichlid species flocks; grey histograms are for cichlid genera or tribes. Cichlid data are from Sturmbauer and Meyer, 1993; Sturmbauer *et al.,* 1994; and Roe *et al.,* 1997.

Why Are There So Many Cichlids and So Few Embiotocids?

The explosive divergence of cichlids, particularly in the African Great Lakes, has traditionally been explained by the geographic isolation of the various species flocks. Recently, however, geological evidence has shown that Lake Victoria is a much younger lake than previously thought, and simple geographic isolation cannot be the unique cause of speciation in cichlids. Seehausen *et al.* (1997) have shown that sexual selection plays an important role in creating and maintaining separate species in the African Great Lakes. Evidence was provided by the high incidence of hybridization between formerly distinct species of cichlids in Lake Victoria, when turbid water due to eutrophication did not allow females to select appropriately colored males (Seehausen *et al.,* 1997). Similar conclusions were also reached for Lake Malawi cichlids in which sexual selection on male coloration was found to be the most likely cause of speciation (Deutsch, 1997). Galis and Metz (1998) discuss these results in a more general setting and propose that sexual selection, rather than isolation or other factors, may be the main driving force behind rapid speciation in cichlids.

The low number of species in the family Embiotocidae opposes the generally accepted idea that families with low dispersal capabilities or reduced larval life, such as the surfperches, would tend to exhibit a high speciation rate (e.g., Hansen, 1980, 1982; Futuyma, 1998). Further studies will determine if ecological

factors, such as sexual selection, or evolutionary factors have played important roles in determining the speciation rate in this family.

ACKNOWLEDGMENTS

We thank N. L. Crane, K. Clifton, J. Coyer, and D. Canestro for help in collecting samples, P. G. Stipa for providing the *Chromis* sample, and N. Okada for providing Japanese specimens. We thank M. Cummings and V. Cassano for discussion. We thank D. Swofford for the use of the beta version of PAUP. This research was partly supported by faculty research funds granted by the University of California at Santa Cruz, Division of Natural Sciences.

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