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The name of the father: conflict between Louis and Alexander Agassiz and the *Embiotoca* surfperch radiation

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The surfperch genus *Embiotoca* currently comprises two species, *Embiotoca jacksoni*, the black surfperch, and *Embiotoca lateralis*, the striped surfperch. Originally, however, Louis Agassiz described a third species in the genus *Embiotoca*, the rainbow surfperch, *Embiotoca caryi*. This latter name was changed by Louis' son, Alexander, to *Hypsurus caryi*, a name that remains valid. In this study, new molecular data (3545 bp of DNA from four mitochondrial and two nuclear DNA regions) indicated that the rainbow surfperch should be retained within the genus *Embiotoca*, a result consistent with recent morphological data. Adaptive radiation combined with sexual selection resulting in rapid morphological changes in the rainbow surfperch may have contributed to the conflicting position of this species.

Key words: Agassiz; *Embiotoca*; Embiotocidae; *Hypsurus*; marine fish; Pacific Ocean.

INTRODUCTION

Surfperches (family Embiotocidae) are livebearing fishes that underwent an ecological radiation in the North Pacific Ocean (Tarp, 1952; DeMartini, 1969; Ebeling & Laur, 1986; Bernardi & Bucciarelli, 1999). Their radiation resulted in several genera (13) but surprisingly few species (22). Two genera (with three species) are found in the northwestern Pacific (*Ditrema* and *Neoditrema*), one freshwater genus (*Hysterocarpus*) is found in California (one species), and the remaining 10 genera are marine reef fishes found in the north-eastern Pacific (18 species).

In 1852, A. C. Jackson caught a gravid black surfperch in Sausalito, CA, which he sent to Louis Agassiz. Louis Agassiz later received from his brother-in-law, T. G. Cary, specimens of rainbow surfperch. For these remarkable fishes, Louis Agassiz erected a new family (called Holconoti or Embiotocidae), a new genus (*Embiotoca*) and two species *Embiotoca jacksoni* Agassiz, 1853, and *Embiotoca caryi* Agassiz, 1853. The following year, Louis Agassiz

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described more surfperch species, including the congeneric striped surfperch, *Embiotoca lateralis* Agassiz, 1854. For the most part, the names assigned by Louis Agassiz remained valid. The rainbow surfperch, however, was renamed by Louis Agassiz's son, Alexander, *Hypsurus caryi* (Agassiz, 1853), a name that remains valid. Thus, currently the genus *Embiotoca* includes only two species, the black surfperch, *E. jacksoni*, and the striped surfperch, *E. lateralis*, and the genus *Hypsurus* includes a single species *H. caryi* (Agassiz, 1853).

The radiation of surfperches was, at least in part, triggered by resource partitioning (Ebeling & Laur, 1986), which resulted in the colonization of several ecological niches. For sympatric species, several factors contributed to successful partitioning, including the capability, by some species, of sorting food within their mouth (winnowing) (Drucker & Jensen, 1991). For the two sympatric congeneric species *E. jacksoni* and *E. lateralis* that are known to compete for food resources (Hixon, 1980; Holbrook *et al.*, 1985), the capability of winnowing in *E. jacksoni* and the absence of winnowing in *E. lateralis* have been suggested as a contributing factor in the outcome of the competition (Bernardi, 2005). Interestingly, the rainbow surfperch, *H. caryi*, is also capable of winnowing (Drucker & Jensen, 1991), raising the question of a possible close phylogenetic relationship between *H. caryi* and *E. jacksoni*, thus potentially bringing back *H. caryi* into the genus *Embiotoca*.

Phylogenetic relationships of surfperches have been proposed based on morphological (Tarp, 1952; Cassano, 2000) and molecular (Bernardi & Bucciarelli, 1999; Cassano, 2000) data. Yet in all cases, the relationship between the two *Embiotoca* species and *Hypsurus* was unresolved. Indeed, morphological analysis resulted in unresolved polytomies (Cassano, 2000), while molecular analyses either included a single *Embiotoca* species (*E. jacksoni*) (Bernardi & Bucciarelli, 1999) or lacked resolution because of the type of molecular marker used (16S rRNA; Cassano, 2000), thus preventing a proper assessment of the issue. Therefore, the goal of this study was to reassess the position of the three taxa, *E. jacksoni*, *E. lateralis*, and *H. caryi*, by using additional mtDNA and nuclear DNA markers and critically re-examining morphological characters for those three species.

MATERIALS AND METHODS

COLLECTIONS AND DNA SAMPLES

Two individuals for each of the three study species *E. jacksoni*, *E. lateralis* and *H. caryi* were collected by spear in Monterey, CA, and La Bufadora, Mexico, but for one *H. caryi* was obtained from a fish market in Ensenada, Mexico. Surfperches are divided into two subfamilies, Amphistichinae and Embiotocinae (Tarp, 1952; Bernardi & Bucciarelli, 1999). The three focal species belong to the Embiotocinae, thus an amphisticine was used as an outgroup, the barred surfperch, *Amphistichus argenteus* Agassiz, 1854, which is collected with hook and line at La Selva Beach, CA ($n = 2$). After collection, samples were immediately placed in 95% ethanol and stored at ambient temperature in the field and then at 4° C in the laboratory. Muscle or liver tissue was later dissected from these samples. Total genomic DNA was prepared from 75 to 150 mg of muscle or liver tissue by proteinase K digestion in lysis buffer [10 mM Tris, 400 mM NaCl, 2 mM EDTA and 1% sodium dodecyl sulphate (SDS)] overnight at 55° C. This was followed by purification using chloroform extractions and alcohol precipitation (Sambrook *et al.*, 1989).

POLYMERASE CHAIN REACTION AMPLIFICATION AND SEQUENCING

Three mtDNA [cytochrome *b* (cyt *b*), cytochrome oxidase 1 (COI) and adenosine triphosphatase (ATPase)] and two nuclear DNA [S7 protein, recombination activating gene 1 (RAG1)] regions were used in this study. Amplification of the cyt *b* region was carried out according to Bernardi *et al.* (2003). Amplification of COI was carried out according to Palumbi (1996). Amplification of the first intron of the nuclear S7 ribosomal protein (S7) used the primers and protocols of Chow & Hazama (1998), and amplifications of the RAG1 and ATPases 6 and 8 used the primers and protocols of Quenouille *et al.* (2004). After purification following the manufacturer's protocol [ABI; Perkin-Elmer, www.perkinelmer.com], sequencing was performed in both directions with the primers used in the polymerase chain reaction amplification on an ABI 3100 automated sequencer (Applied Biosystems; www.appliedbiosystems.com).

PHYLOGENETIC ANALYSES

Morphological characters investigated by Cassano (2000) were reanalysed using PAUP* 4.0 (phylogenetic analyses using parsimony; Swofford, 2003). For molecular characters, Clustal V implemented by Sequence Navigator (Applied Biosystems) was used to align the DNA sequences. Phylogenetic relationships were assessed by maximum likelihood (ML) implemented in GARLI (Zwickl, 2006), maximum parsimony (MP, PAUP*; Swofford, 2003) and neighbour-joining (NJ, PAUP*) methods. For ML topologies, 10 independent runs were conducted in GARLI, using default settings and the automated stopping criterion, terminating the search when the ln score remained constant for 20 000 consecutive generations. The best likelihood of those runs was retained and is presented in this study. MP searches included 100 random addition replicates and TBR branch swapping with the MulTrees option. NJ reconstructions used distances based on substitution model obtained with Modeltest (HKY+G). Statistical confidence in nodes was evaluated using 2000 non-parametric bootstrap replicates (Felsenstein, 1985) (100 replicates for ML in GARLI, using the automated stopping criterion set at 10 000 generations). Topological differences were tested using a Shimodaira & Hasegawa (1999) (SH) test implemented in PAUP, based on resampling of estimated log-likelihood tests (1000 replicates).

RESULTS

At first glance, the main morphological difference between *H. caryi* and the two *Embiotoca* species is a 'belly extremely elongate and straight (between the pelvics and the origin of the anal fin)' (Tarp, 1952) in *Hypsurus*. This difference seems to be the main reason for the erection of a new genus by Agassiz (1861). An analysis of the morphological characters originally scored by Cassano (2000) for the entire family, but restricted here to the pertinent taxa, is shown in Table I. Of 80 scored morphological characters, which comprised 66 morphometric and 14 meristic characters, 13 were phylogenetically informative. Five of these characters were shared by *E. jacksoni* and *E. lateralis* (all morphometric), four were shared by *H. caryi* and *E. lateralis* (three morphometric and one meristic) and also four were shared by *H. caryi* and *E. jacksoni* (three morphometric and one meristic). The resulting phylogenetic tree is therefore a sister relationship between the two currently recognized *Embiotoca* species (not shown). However, because of the small difference in shared characters between the alternative groupings (4 *v.* 5), alternative hypotheses of a sister relationship

TABLE I. Shared derived morphological characters among pairs of species based on Cassano's (2000) data set. Numbers in parentheses correspond to Cassano's annotations (characters were numbered from 1 to 80). Asterisks correspond to Meristic characters

Embiotoca jacksoni *Embiotoca lateralis*	*Embiotoca jacksoni* *Hypsurus caryi*	*Embiotoca lateralis* *Hypsurus caryi*
Dorsal base length (6)	Prepectoral length (36)	Dorsal origin to pectoral insertion (14)
First dorsal ray to anal fin (17)	Ethmoid–frontal joint to prefrontal–frontal notch (44)	Isthmus to origin of dorsal fin (19)
First dorsal ray to pelvic insertion (18)	Prefrontal–frontal notch to pectoral insertion (59)	Left opercular cleft to right pectoral insertion (49)
Length of caudal peduncle (21)	Number of dorsal fin rays (71)*	Number of rakers on epibranchial of first brachial arch (75)*
Length of third anal spine (31)		

between *Hypsurus* and either species of *Embiotoca* could not be rejected (SH test, $P = 0{\cdot}74$).

Aligned portions of mtDNA cyt *b* (687 bp), 16S rRNA (469 bp), COI (764 bp), ATPases 6 and 8 (814 bp), and nuclear RAG1 (570 bp) and S7 (181 bp) were used. Phylogenetic relationships obtained with separate data sets differed slightly from one another; however, none resulted in the grouping of *E. jacksoni* + *E. lateralis* (Fig. 1). Specifically, the cyt *b* data set grouped *E. jacksoni* with *H. caryi*; the nuclear data sets resulted in an unresolved trichotomy; all other data sets resulted in a *E. lateralis* + *H. caryi* grouping, a topology that is statistically consistent with all molecular data sets. Therefore, the molecular data were pooled into a data set including 3545 bp. Of the 3545 aligned nucleotides, 532 were variable and 98 were phylogenetically informative. A single most parsimonious tree (631 steps, consistency index 0·92) was obtained and was identical in topology to both the NJ and ML trees (Fig. 2). In this tree, *H. caryi* and *E. lateralis* grouped together in a well-supported clade (bootstrap: 100% ML, 98% MP and 99% NJ). The two alternative groupings *H. caryi* + *E. jacksoni* (662 steps) and *E. jacksoni* + *E. lateralis* (640 steps) were both rejected by the SH test ($P < 0{\cdot}001$ and $< 0{\cdot}05$).

DISCUSSION

Adaptive radiations, as exemplified by Galapagos finches, African Great Lakes cichlids and Hawaiian silverswords, present formidable and interesting challenges to the evolutionary biologist (Schluter, 2000). Adaptive radiations occur rapidly and result in the occupation of many adjacent ecological niches. As such, embiotocids present their own unique challenges. In the case of the three focal species of this study, both morphological (Cassano, 2000) and molecular data are consistent with the inclusion of *H. caryi* in the genus

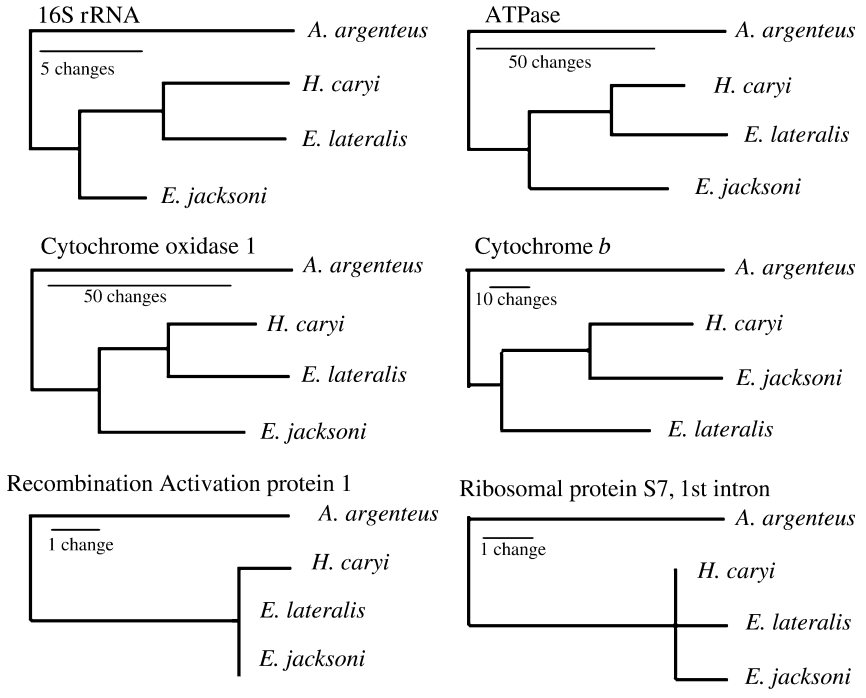

FIG. 1. Phylogenetic trees based on sequences of mitochondrial 16S rRNA (top left), ATPases 6 and 8 (top right), cytochrome oxidase 1 (middle left), cytochrome *b* (middle right), nuclear recombination activating gene 1 (bottom left) and the first intron of the ribosomal protein S7 (bottom right) regions, using the maximum likelihood method (maximum parsimony and neighbour-joining reconstructions resulted in the same topology). *Amphistichus argenteus* was used as an outgroup. Branches drawn according to the number of inferred substitutions; scale bar is presented within the tree.

Embiotoca. Furthermore, the molecular data reject with a high degree of confidence the placement of *Hypsurus* outside the genus *Embiotoca*. Together, these separate data sets prove Louis Agassiz correct in his placement of three

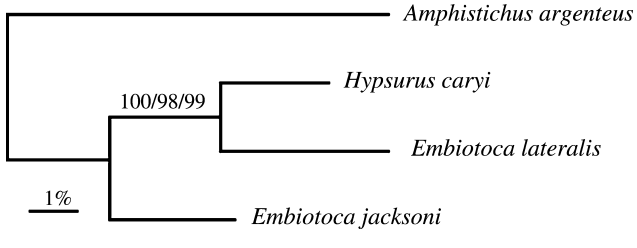

FIG. 2. Phylogenetic tree based on concatenated sequences of mitochondrial 16S rRNA, ATPases 6 and 8, cytochrome oxidase 1, cytochrome *b*, nuclear recombination activating gene 1 and the first intron of the ribosomal protein S7 regions, using the maximum likelihood (ML) method [maximum parsimony (MP) and neighbour-joining (NJ) reconstructions resulted in the same topology]. *Amphistichus argenteus* was used as an outgroup. Bootstrap support is shown above the node for the three methods used (ML, MP and NJ, respectively). Branches drawn according to the number of inferred substitutions; scale bar corresponds to 1% sequence divergence.

species in the genus *Embiotoca*: the black surfperch, *E. jacksoni*, the striped surfperch, *E. lateralis*, and the rainbow surfperch *E. caryi*.

So, why was Louis Agassiz's son, Alexander, stumped by this group of fishes? Very much like East African cichlids, embiotocids underwent a radiation that involved both foraging niche partitioning and rapid colouration pattern changes driven by sexual selection (Fryer & Iles, 1972; Bernardi & Bucciarelli, 1999; Seehausen, 2000). Surfperches inhabit an optically variable environment where males display complex courtship and light-flashing behaviours (Cummings & Partridge, 2001; Cummings, 2004). It is likely that colouration changes, driven by sexual selection, occurred rapidly, and a large display area may have been preferred in rainbow surfperches, resulting in an elongated and almost rectangular body, optimizing an enlarged display area. This shape, which influenced Alexander Agassiz in erecting a new genus, was not, however, the reflection of other morphological or genetic changes, as shown by this study, but was probably an isolated apomorphic change.

The results here have other interesting implications. The apparently independent evolution of winnowing (sorting of food within the mouth) in the genera *Embiotoca* and *Hypsurus* (Drucker & Jensen, 1991) can now be interpreted more parsimoniously. By placing *E. caryi* as the sister species of *E. lateralis*, the data raise the issue of the loss of winnowing in *E. lateralis*, a possible result of ecological competition with *E. jacksoni*. In addition, the three species of *Embiotoca* present a potential case of sympatric speciation in the ocean that raise unique evolutionary questions and warrant further investigation.

This article is dedicated to my father, Giorgio Bernardi, an evolutionary biologist who is seldom wrong.

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