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A correction to: biological and oceanographic insights from larval labrid (Pisces: Labridae) identification using mtDNA sequences

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Abstract Previously published mtDNA cytochrome *b* sequences for *Xyrichtys novacula* (Linnaeus) and two morphologically distinct types of *Xyrichtys* larvae (Pisces: Labridae) included human cytochrome *b* sequence, presumably due to sample contamination and/or poor preservation of sample DNA. Those sequences had been used to identify the two types of *Xyrichtys* larvae as *X. novacula*, but owing to the contamination, the identifications are invalid. Fresh specimens were collected: *X. martinicensis* (Cuvier and Valenciennes), *X. novacula*, *X. splendens* (Castelnau), and *Xyrichtys* sp. larvae of each of the two morphotypes (dorsal-forward eye and ventral-forward eye). The cytochrome *b* fragment was amplified from each specimen using the polymerase chain reaction. Comparison of the sequences with human cytochrome *b* sequence confirmed that the new sequences were not contaminated. The interspecific differences in *Xyrichtys* sequences were less than previously reported, but still greater than observed in many fish genera. Distance and parsimony analyses indicated that *X. novacula* and *X. martinicensis* were more closely related to each other than to *X. splendens*. This conclusion differs from our previous conclusion that *X. martinicensis* and *X. splendens* were more closely related to each other than to *X. novacula*. Distance and parsimony analyses also demonstrated that both larval morphotypes were *X. novacula* and, thus, our previous conclusions regarding larval ecology and oceanographic transport remain unchanged.

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Introduction

We recently reported (Hare et al. 1996) that our previously published (Hare et al. 1994) cytochrome *b* mtDNA sequences for *Xyrichtys novacula* (Pisces: Labridae) and two morphotypes of *Xyrichtys* sp. larvae were 99% similar to human cytochrome *b* sequences and probably resulted from contamination during sample collection or processing. The purpose of our original work was to use mtDNA sequences to link two morphologically distinct types of *Xyrichtys* sp. larvae (based on the orientation of the ovoid eye) to one of three western Atlantic *Xyrichtys* species (Randall 1965), thereby allowing for the specific identification of the larval types. Contamination of the sequences with human mtDNA invalidated our species identification of the two larval types and brought into question our conclusions regarding the ecology of *Xyrichtys* sp. larvae and the evolution of the genus *Xyrichtys*. In the present study, we correct the cytochrome *b* sequences for *X. novacula* and the two larval morphotypes and discuss our prior conclusions in light of the corrected sequence data.

Materials and methods

Eight individuals were used as tissue sources for polymerase chain reaction amplification and subsequent DNA sequencing. Two *Xyrichtys novacula* (Linnaeus) adults were collected off the coast of Georgia in June 1996. One *Xyrichtys splendens* (Castelnau) adult and one *Xyrichtys martinicensis* (Cuvier and Valenciennes) adult

were collected off Barbados, West Indies in May 1996. Two ventral-forward eye type *Xyrichtys* sp. larvae and two dorsal-forward eye type *Xyrichtys* sp. larvae were collected in Raleigh Bay, North Carolina in May 1996. Larvae were identified to genus based on meristics (Randall 1965) and larval morphology (Richards and Leis 1984). All material was preserved in 95% ethanol between collection and DNA extraction.

DNA was extracted from ~5 mg of adult muscle tissue or a whole larva using the procedure of González-Villaseñor et al. (1986). Primers for polymerase chain reaction (PCR) amplification were as previously reported (Hare et al. 1994), and PCR procedures generally followed Kocher et al. (1989). Briefly, a thermocycler was preheated to 94 °C and amplification began with a 1 min incubation at 94 °C followed by 45 cycles of three temperatures: 94 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s. PCR was ended with 10 min at 72 °C followed by 4 °C until samples were removed. The amplified product was sequenced in both directions with the primers used for PCR using an automated Applied Biosystems 373A DNA sequencer. DNA extraction, amplification and sequencing were performed by Biotech Research Labs (Molecular Biology Division, Rockville, Maryland, USA). Sites that differed between the forward and reverse sequences were designated with an N. Sequences have been deposited in GenBank (dorsal-forward eye type larvae: Accession No. U91998, U92000; ventral-forward eye type larvae: U91999, U92001; *Xyrichtys martinicensis*: U92005; *X. novacula*: U92002, U92003; *X. splendens*: U92004).

Larval and adult cytochrome *b* sequences were compared to identify larval morphotypes to species. All sequences generated herein were included, as were three previously generated sequences: a *Xyrichtys martinicensis* adult (Hare et al. 1994; GenBank L16905), a *X. splendens* adult (Hare et al. 1994; GenBank L16910) and a *Gomphosus varius* adult (Normark et al. 1991; GenBank M64896). Intraspecific percent differences were calculated based on the sequences of the two adult specimens of each *Xyrichtys* species. A consensus sequence for each species was then determined; sites were designated with an N in the consensus sequence if they were variable between individuals or were not resolved in the sequences of both individuals. Percent differences were calculated between human cytochrome *b* sequence (Kocher et al. 1989) and the consensus sequence for each *Xyrichtys* species, as well as the sequences from the individual *Xyrichtys* sp. larvae to evaluate the possibility of contamination. All pairwise percent differences were also calculated between the consensus sequences, the larval sequences and the *G. varius* sequence.

Distance and parsimony analyses were conducted using the sequences from the six individual adult *Xyrichtys*, the four *Xyrichtys* sp. larvae and *Gomphosus varius*. Distance analyses were performed using TREECON software (Van de Peer and De Wachter 1994). Data were bootstrapped 100 times, and a Jukes Cantor distance correction and UPMGA clustering (unweighted pair-group method using arithmetic averages) were used to develop distance trees. *G. varius* was used as a reference species. Parsimony analyses were performed using the DNAPARS and CONSENSE programs of PHYLIP (Felsenstein 1995). Data were bootstrapped 100 times and the order of the input sequences was jumbled. Each base pair was treated as an independent, equally weighted character, and *G. varius* was designated as an outgroup.

Results

The two larval types and *Xyrichtys novacula* were genetically very similar (Fig. 1). There was substantial difference between all *Xyrichtys* sequences and human cytochrome *b* sequences (~28%), indicating that the sequences reported here did not result from contamination with human DNA. Intraspecific variation was minimal (*X. martinicensis* 2.7% different, *X. novacula* 1.4% different, *X. splendens* 2.2% different), and interspecific variation was much greater than intraspecific variation (Table 1). Larval sequences were very similar to each other and to *X. novacula* sequences, but were substantially different than *X. martinicensis* and *X. splendens* sequences (Table 1). Of the base pair differences identified, transitions (A-G or C-T) outnumbered transversions (A-C, A-T, G-C or G-T) within the genus *Xyrichtys* but between *Xyrichtys* species and *G. varius* the ratio was closer to 50% (Table 2).

Distance analysis indicated that both morphological types were larvae of *Xyrichtys novacula* (Fig. 2). The node combining the larvae and *X. novacula* was supported by all 100 bootstrap replications. The node combining *X. novacula* and *X. martinicensis* was supported by 68 of the 100 bootstrap replications (Fig. 2).

Parsimony analysis mirrored the results of the distance analysis. The node combining the larvae and *Xyrichtys novacula* was supported by all 100 bootstrap replicates and the node combining *X. novacula* and *X. martinicensis* was supported by 84 of 100 bootstraps.

Discussion

Western Atlantic *Xyrichtys* species can be distinguished using cytochrome *b* sequences. Intraspecific variation ranged from 1 to 3%, whereas interspecific variation ranged from 11 to 15%. Interspecific variation is somewhat higher (Table 1) than that reported for other fish genera, but the transition-to-transversion ratios are consistent with other studies (Table 2). The sequence data suggest, based on molecular clock arguments, that the genus *Xyrichtys* within the western North Atlantic is relatively old, at least 15 million years (see Fig. 1 in Moritz et al. 1987). Confirmation of this conclusion will require calibration of the molecular clock for the genus *Xyrichtys*, possibly by generating comparable sequences for eastern Pacific *Xyrichtys* species. The distance and parsimony analyses indicated that *X. novacula* and *X. martinicensis* are more closely related to each other than either is to *X. splendens*, which differs from our prior results.

Both larval morphotypes can be identified as larvae of *Xyrichtys novacula* based on the cytochrome *b* sequence data. There is about a 28% difference between human sequences and the sequences presented herein and thus, these sequences are not affected by contamination with human mtDNA. *X. novacula* adults are found as far north as Cape Hatteras, North Carolina but larvae of both eye types are commonly collected on the continental shelf north of Cape Hatteras (Hare and Cowen 1991; Cowen et al. 1993; Hare et al. 1994). During 1988 on the New York Bight continental shelf, larvae of the two different eye types had different birthdates and arrived on the shelf at different times (Hare et al. 1994), indicating an ecological difference that is not represented in the genetic data analyzed here. Inability to genetically differentiate between the two

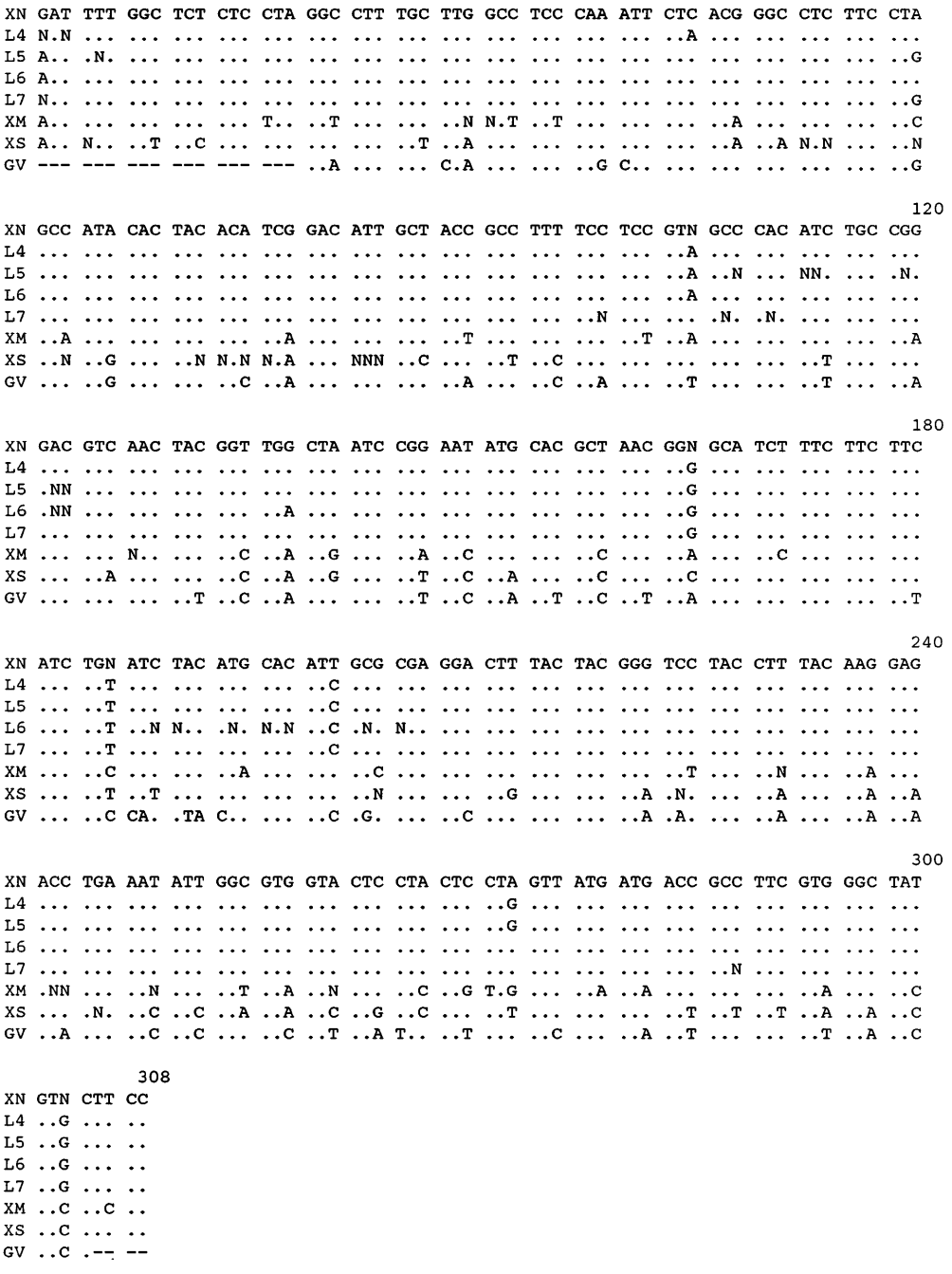

Fig. 1 *Xyrichtys martinicensis*, *X. novacula*, *X. splendens* and *Gomphosus varius*. Mitchondrial DNA sequences of a 308 bp fragment of the cytochrome *b* gene. Consensus sequences are presented for the three western Atlantic *Xyrichtys* species: *X. martinicensis* (XM), *X. novacula* (XN), and *X. splendens* (XS). Sequences from individual larvae are also presented. Larvae 4 and 6 (L4 and L6) were dorsal-forward eye type and Larvae 5 and 7 (L5 and L7) were ventral-forward eye type. *G. varius* (GV) sequence data were obtained from GenBank (M64896) as reported by Normark et al. (1991). Identical matches to the *X. novacula* (XN) consensus sequence are indicated by dots (*N* base position was variable between individuals of a given species or was not resolved through sequencing)

Table 1 *Xyrichtys martinicensis*, *X. novacula*, *X. splendens*, and *Gomphosus varius*. *Above diagonal* number of nucleotide differences and *below diagonal* percent nucleotide differences in a 308 base pair cytochrome *b* fragment of the western Atlantic species of *Xyrichtys*: *X. martinicensis* (XM), *X. novacula* (XN) and *X. splendens* (XS); specimens of the two larval types of *Xyrichtys*; ventral-forward eye type (L5 and L7) and dorsal-forward eye type (L4 and L6); and of *Gomphosus varius* (GV), a Pacific labrid. Percent base pair differences based on the number of comparable positions between each pair of sequences

Species	XM	XN	XS	L4	L5	L6	L7	GV
X. martinicensis		34	41	37	36	36	37	53
X. novacula	11.5%		41	3	4	3	2	51
X. splendens	14.4%	14.2%		44	43	41	42	44
Larva 4	12.4%	1.0%	15.2%		2	3	3	56
Larva 5	12.3%	1.4%	15.1%	0.7%		3	1	54
Larva 6	12.4%	1.0%	14.5%	1.0%	1.0%		2	52
Larva 7	12.6%	0.7%	14.6%	1.0%	0.3%	0.7%		51
G. varius	19.0%	18.1%	16.2%	19.6%	19.3%	18.8%	18.2%	

Table 2 *Xyrichtys martinicensis*, *X. novacula*, *X. splendens* and *Gomphosus varius*. *Above diagonal* number of transitions (*left hand number*) and transversions (*right hand number*) and *below diagonal* the percent transitions of all base substitutions observed in consensus cytochrome *b* sequences of western Atlantic species of *Xyrichtys*: *X. martinicensis* (XM), *X. novacula* (XN) and *X. splendens* (XS); and the Pacific labrid, *Gomphosus varius* (GV)

Species	XM	XN	XS	GV
X. martinicensis		29/5	31/10	29/24
X. novacula	85.3%		30/11	30/21
X. splendens	75.6%	73.2%		23/21
G. varius	54.7%	58.8%	52.3%	

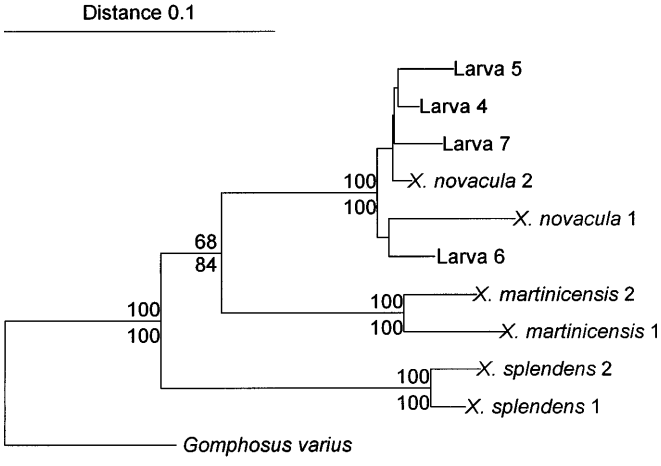

Fig. 2 *Xyrichtys martinicensis*, *X. novacula*, *X. splendens* and *Gomphosus varius*. UPMGA clustering of Jukes–Cantor distance correction calculated using cyctochrome *b* sequences of six adult *Xyrichtys* (representing the three western Atlantic *Xyrichtys* species), four larval *Xyrichtys* (representing two larval morphotypes; Larvae 4 and 6 were dorsal-forward eye type, Larvae 5 and 7 were ventral-forward eye type larvae) and *G. varius*. Values above nodes indicate the number of times the node was supported in 100 bootstrapped replicates. Values below nodes indicate the number of times the node was supported in 100 bootstrapped replicates of a parsimony analysis of the same sequences. Numbers are provided for all nodes where one of the bootstrapped values was >80. The phylogeny resulting from the parsimony analysis was the same as that shown for the distance analysis except that the *X. novacula* 1 sequence was the sister group to all other *X. novacula* and larval sequences

morphologically distinct larval forms could be a function of the conservative nature of the cytochrome *b* gene (see Meyer 1994). Other, less conservative regions of DNA (e.g. the control region of mtDNA) should be analyzed to determine if there are genetic differences between the two larval types of *X. novacula*. The physical processes responsible for the differential supply of the two larval morphotypes to the New York Bight shelf edge also deserve further study and may be linked to the Gulf Stream entrainment of water from different regions of the continental shelf south of Cape Hatteras.

Identification of larvae using molecular techniques provides a powerful tool, especially when applied in families that have traditionally presented problems to larval fish taxonomists (e.g. serranids, labrids, pomacentrids). Once larvae of these families are identifiable to species, a variety of questions regarding larval ecology and larval transport can finally be addressed.

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