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Morphology of a Larval Hammerjaw *Omosudis lowii* Günther 1887 (Aulopiformes, Omosudidae) Identified by Partial Mitochondrial 12S rRNA Gene Analysis

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ABSTRACT The morphological characteristics of a larval fish (7.8 mm in body length) collected off Chuuk, Micronesia were highly similar to those of larval *Omosudis* sp., except fin development and body length. It was identified as *Omosudis lowii* by partial mitochondrial 12S rRNA gene analysis. The morphological traits of the larval fish validated by the molecular genetic marker will be informative for species-level identification of larval *Omosudis lowii*.

Key words: Genetic marker, larval fish, morphology, *Omosudis lowii*, species identification

INTRODUCTION

Morphological traits of larval fish are the key to identify species. Many morphological descriptions of various larval fish species have been compiled into illustrated identification guides (Moser *et al*., 1984; Okiyama, 1984, 1988, 2014; Leis and Carson-Ewart, 2004; Richards, 2006; Fahay, 2007). The accuracy of larval fish identification depends on the morphological descriptions in similar size with larval specimen (Powles and Markle, 1984). There are still unrecorded species or developmental stages. Describing serial developmental stages is difficult unless the larvae are raised (Hunter, 1984). Thus, the descriptions on one species have been accumulated by one or more taxonomists. The completeness of the morphological description varies on the taxonomists (Sumida *et al*., 1984). Recently, due to differences on descriptions for the *Omosudis lowii* larvae, its taxonomic status of larval *Omosudis lowii* was changed to *Omosudis* sp. (Okiyama, 2014).

One way improving the accuracy of larval fish identification is to use molecular genetic markers (Kim *et al*., 2008; Ko *et al*., 2013; Puncher *et al*., 2015; Kimmerling *et al*., 2018). Genetic markers, inherent properties of species, are not affected by the morphological changes that occur during growth or in response to physical damage. Based on the genetic marker, fish larvae and also eggs can be identified via comparison with the adult (Shao *et al*., 2002; Valdez-Moreno *et al*., 2010; Tsukamoto *et al*., 2011; Oh and Kim, 2015; Lewis *et al*., 2016; Choi *et al*., 2018). A common genetic marker used to identify fish is mitochondrial DNA (e.g. 12S and 16S rRNAs, and COI). In this study, we identified a larval fish resembling with the larval *Omosudis* sp. using a genetic marker.

MATERIALS AND METHODS

A larval fish was caught off Chuuk (7.575°N; 152.083° E), Federated States of Micronesia using an Isaacs-Kidd midwater trawl net (mesh size: 0.417 mm; mouth area: 8.76 m^2; wire out: 1,000 m) onboard the research vessel Onnuri (September 17, 2006). The larval specimen was preserved in 95% ethanol and photographed using a stereomicroscope (Stemi-2000C; Carl Zeiss, Jena, Germany) with a camera. The morphological characteristics of the larval specimen were compared with the records from Ege (1958)

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cited in Rofen (1966), Belyanina (1982), and Okiyama (1984, 1988, 2014).

Genomic DNA of the larva was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The partial 12S rRNA gene was polymerase chain reaction (PCR)-amplified using the L1091 (5′-AAAAAG-CTTCAAACTGGGATTAGATAC-CCCACTAT-3′) and H1478 (5′-TGACTGCAGAGGGT-GACGGGCGGTGTGT-3′) primers (Kocher *et al*., 1989). The PCR reaction mixture (total volume: 20 μL) comprised 10 μL of 2 × DNA-free Taq Master Mix (CellSafe, Yongin, Korea), 0.4 μL of each primer (10 pM), 7.2 μL of distilled water, and 2 μL of genomic DNA. The thermal cycling conditions were 94°C for 4 min, 53°C for 2 min, and 72°C for 1 min (one cycle); 94°C for 1 min, 53°C for 2 min, and 72°C for 1 min (30 cycles); and 94°C for 1 min, 53°C for 2 min, and 72°C for 5 min (one cycle). The PCR product was sequenced on a 3730xl DNA Sequencer (Applied Biosystems Inc., Foster City, CA, USA). Sequences from both strands were aligned and trimmed in BioEdit (Hall, 1999). The consensus sequence of the larva was compared with all available sequences using the Basic Local Alignment Search Tool (Altschul *et al*., 1990). A partial 12S rRNA phylogenetic neighbor-joining tree (Saitou and Nei, 1987) of the larva and related taxa was constructed based on the Kimura-2-parameter method (Kimura, 1980) in MEGA-X (Kumar *et al*., 2018). The outgroup species were in the order Rajiformes. All sequences used in this study were obtained from the NCBI GenBank (http://www.ncbi.nlm.nih.gov; Benson *et al*., 2013). All species names follow those of the Catalog of Fishes (Fricke *et al*., 2018).

RESULTS

The larval specimen was 7.8 mm in body length (BL), and one fish (BL, ~5.0 mm) was found in the stomach (Fig. 1). The larval specimen had 41 myomeres. Pre- and post-anal myomere counts were not available because the anus location was ambiguous due to an expanded abdomen. The head of the specimen reached 49% of the BL. Snout and mouth lengths were 43% and 80% of head length. Three pairs of distinct serrated ridges lined both sides of the head. There were small spines on preopercle. Both jaws had a pair of large, well-developed canines and small teeth. Melanophore pigments were distributed around the mouth, between the nostrils, behind the eyes, and on the preopercular, parietal, dorsal side, and bases of the dorsal and anal fins. The peritoneal melanophores were dense in the dorsal area and widely distributed over the abdomen, which were not segmented. None of the fins, except the caudal fin, was fully developed. Most morphological characteristics, except fin development and BL, were highly similar to those of the *Omosudis* sp. larvae described by Belyanina (1982; 11.5 mm BL) and Okiyama (1984 and 2014; 22.5 mm BL; 1988, 13.0 mm BL).

The partial 12S rRNA gene sequence of the larval specimen (388 bp; GenBank accession number MK689244) was 100% identical to that of *Omosudis lowii* (AP004210) (Fig. 2). In 12S rRNA gene neighbor-joining tree, a clade of *O. lowii* including our larval fish was within the Aulopiformes and was distinct from *Alepisaurus ferox* (AP004211) in the Alepisauridae. Based on the 12S rRNA gene analysis, we identified the larval specimen as *O. lowii*.

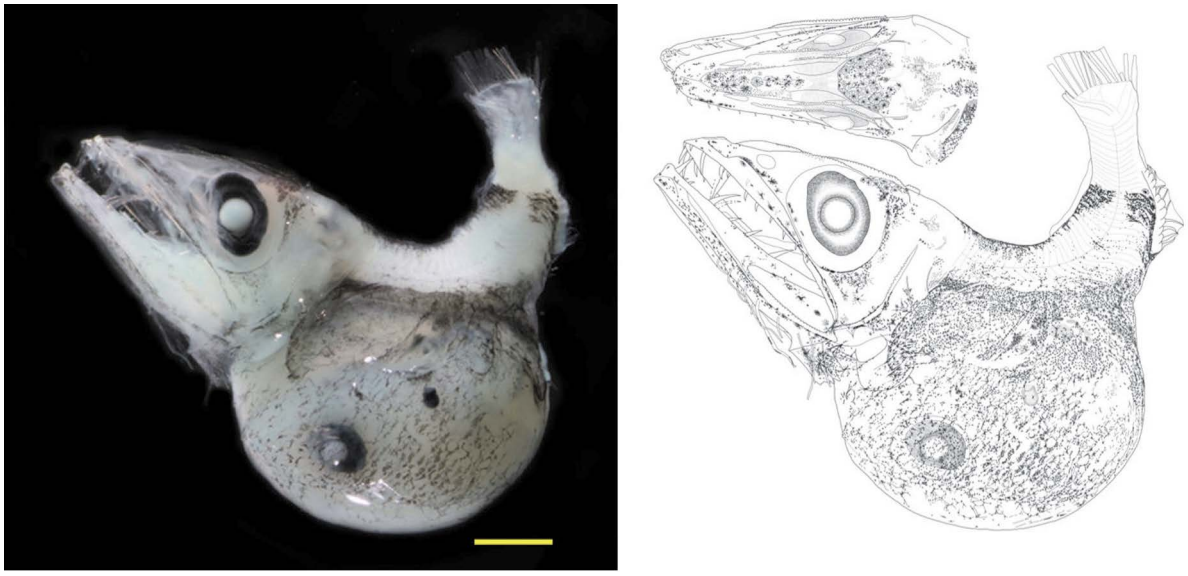

Fig. 1. Photo and illustration of a larva collected off Chuuk, Micronesia, 2006. Scale bar, 1 mm.

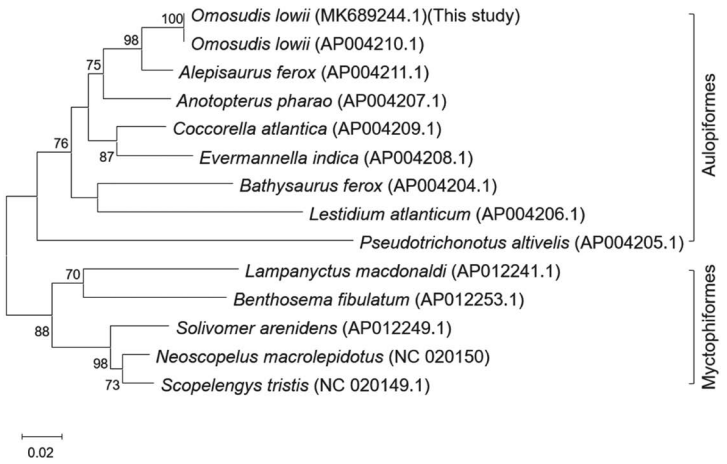

Fig. 2. Neighbor-joining tree based on 12S rRNA sequences from a larva of this study and related taxa. Bootstrap values (1,000 replicates) greater than 70% support are shown at nodes.

DISCUSSION

The first step to identify a larval fish is to compare it against records of species having similar morphological characteristics and developmental stages (Powles and Markle, 1984). Our larval specimen (7.8 mm BL) was very similar to descriptions of larval *Omosudis* sp. (Okiyama, 2014; 11.5 and 22.5 mm BL). These descriptions were initially identified as *O. lowii* (valid name of *O. lowei*) from the Pacific by Belyanina (1982; 11.5 mm BL) and Okiyama (1984; 22.5 mm BL). However, there were discrepancies in morphological descriptions of larval *O. lowii* from the Pacific (Belyanina, 1982; Okiyama, 1984, 2014) versus Atlantic (Ege, 1958; 5.1~44.1 mm BL). Okiyama (1984) and Richards (2006) noted that the preopercular spines and spinous ridges were missing in the records of Atlantic specimens. On the other hand, Fahay (2007) reported the possibility that the genus *Omosudis* contains more than one species. Subsequently, Okiyama (2014), who investigated the records of Ege (1958), Belyanina (1982), and Okiyama (1984), changed the taxonomic hierarchy of *O. lowii* larvae to *Omosudis* sp. based on the records of Okiyama (1984) (22.5 mm BL) and Belyanina (1982) (11.5 mm BL). These two morphological descriptions (11.5 mm and 22.5 mm BL) were more similar to a related species *Alepisaurus ferox* (Okiyama, 1984; 10.0 mm BL) than the *O. lowii* larvae of Ege (1958). *A. ferox* and *Omosudis* sp. have preopercular spines and the serrated ridges on head in common. But, differences between the two species were size of pectoral fin, location of dorsal and pectoral fins, and distribution of melanophores. Additionally, the description of the larval specimen (22.5 mm BL) of Okiyama (1984) was described as 13.0 mm BL by Okiyama (1988), and then modified to 22.5 mm BL in Okiyama (2014). Given these disputes, it was difficult to determine the species of our larva based on morphological characteristics alone.

Genetic markers are useful for identification of fish eggs and larvae as species level (Shao *et al*., 2002; Ko *et al*., 2013; Choi *et al*., 2018). According to the taxonomic hierarchy and genetic distances of the 12S rRNA gene sequences, we determined the larval specimen highly resembled *Omosudis* sp. larvae as *Omosudis lowii*. In the 12S tree, the close sequence to the *O. lowii* clade formed with the larval sequence within the Aulopiformes was that of *Alepisaurus ferox* (AP004211). This relationship was similar in phylogenetic tree with morphological characters (Johnson, 1982; Baldwin and Johnson, 1996; Sato and Nakabo, 2002) and in multi-gene tree (Davis, 2010). The morphological traits of our larval *O. lowii* validated by the genetic marker will be informative for species level identification for larval fish.

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12S rRNA로 동정한 홍메치목 *Omosudis lowii* 치어의 형태적 특징

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요 약 : 마이크로네시아의 축 주변해에서 채집한 치어(체장 7.8 mm)의 형태적 특징은 체장과 지느러미의 발달 단계를 제외하면 *Omosudis* sp.와 매우 유사하였다. 이 표본을 12S rRNA 마커를 이용하여 홍메치목의 *Omosudis lowii*로 동정하였다. 분자마커로 동정된 *Omosudis lowii* 치어의 형태적 특징은 이 종의 자치어 동정에 매우 유용할 것이다.

찾아보기 낱말 : 분자마커, 자치어, 형태, *Omosudis lowii*, 종동정