



Identification of *Odontamblyopus lacepedii* via morphology and DNA barcoding



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ABSTRACT

To evaluate taxonomic and genetic variation in *Odontamblyopus lacepedii* collected in East Asia, morphometric characters and molecular analysis were described. The body was ribbon-like. The body and head were grayish-purple with no embedded scales. Eyes were rudimentary, but distinct, covered by skin. The dorsal and anal fins were continuous with the caudal fin. The edges of the dorsal, anal, and caudal fins were dark chocolate-brown. Genetic analysis of four species revealed higher mean A + T (55.4%) than G + C (44.6%). Twenty-two haplotype sequences were identified, containing 22 haplotypes, 131 variable sites and 122 parsimony-informative sites, with haplotype and nucleotide diversity differing among species. The intraspecific genetic distances ranged from 0.2% to 0.5% (average 0.3%) and interspecific differences from 1.1% to 17.6% (average 14.6%), respectively, indicating that these four species of Amblyopinae can be accurately identified. The NJ tree constructed using COI fragments based on 22 haplotypes was composed of two main branches: the upper branch comprising *O. lacepedii* and *O. sp.* showing a close relationship, with *Trypauchen vagina* and *Ctenotrypauchen microcephalus* clustering on the second branch. Thus, the results validated the identification of *O. lacepedii* at the morphological and genetic level.

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1. Introduction

The eel goby, *Odontamblyopus lacepedii* (Temminck and Schlegel) (Gobiidae: Amblyopinae) is an air-breathing burrow-inhabiting fish of coastal waters and intertidal zones of East Asia (Gonzales et al., 2006). It is widely distributed along the coasts of China water (Murdy and Shibukawa, 2001), and in Ariake Bay of Japan (Gonzales et al., 2008a, 2008b). Tang et al. (2010) classified *O. lacepedii* on the basis of the pectoral-fin rays, anal-fin rays and caudal vertebrae, which were not sufficient for species identification. Murdy and Shibukawa (2001) provided systematic taxonomic and ecological descriptions of the species, but some information are outdated and inaccurate. Identification of *O. lacepedii* still remains a challenge, and a more detailed description of its morphological characters is needed. This, combined with DNA barcoding, would offer a more reliable approach to identification.

Advanced DNA technologies and sequencing have become reliable tools for investigation of evolutionary and phylogenetic relationships (Hajibabaei et al., 2007). DNA barcoding, using the mitochondrial cytochrome oxidase (COI) gene as a tag to identify species, is recognized as an effective tool for the species identification (Puckridge et al., 2013). The COI gene present in all animals (Barrett and Hebert, 2005) appears to exhibit sequence divergence sufficient to discriminate a wide range of animal taxa (Pyle et al., 2008; Ratnasingham and Hebert, 2007). It facilitates species identification from fragments at any life-history stage, allowing direct comparison of specimens to a global reference database.

The goal of this study was to describe the morphometric and meristic characters of *O. lacepedii* and to obtain partial COI sequences adding three additional species within Amblyopinae, which can help provide the information for *O. lacepedii* identification and assess the phylogenetic relationship.

2. Materials and methods

2.1. Sample collection

Odontamblyopus lacepedii specimens were collected from Huanghua and Dongying in China and from Ariake Bay in Japan (Table 1). All specimens were identified based on morphometric and meristic characteristics (Murdy and Shibukawa, 2001, 2003; Wu and Zhong, 2008). Muscle tissues were preserved in 95% ethanol or directly used to extract DNA. Specimens were frozen and preserved at the Fishery Ecology Laboratory, Fisheries College, Ocean University of China in Qingdao. Relevant COI sequences were downloaded from GenBank (Table 1).

2.2. Morphological investigation

We compared taxonomic data and meristic counts with previous records of *O. lacepedii*. The investigated meristic characters included dorsal fin spines (DFS), dorsal fin rays (DFR), pectoral fin rays (PF), anal fin rays (AFR), pelvic fin rays (PFR), gill rakers (GR) and caudal vertebrae (CV). Morphological measurements included total length (TL), standard length (SL), head length (HL), head height (HH), snout length (SNL), postorbital length (POL), interorbital width (IW), body height (BH), dorsal fin length (DFL), pectoral fin length (PFL), and anal fin length (AFL). Measurements were made with calipers to the nearest

Table 1
Four species of Amblyopinae used in the study.

Species	Sampling location	Sampling date	Sampling number	GenBank sequences
<i>Odontamblyopus lacepedii</i>	Dongying (DY)	5/2010	1	HM180729 JX679044
	Ariake Bay (AB)	2/2014	24	
	Huanghua (HH)	9/2015	24	
<i>Odontamblyopus</i> sp.	Zhoushan (ZS)	9/2014	6	KP260455
	Rizhao (RZ)	3/2015	7	
	Dongying (DY)	5/2010	3	
<i>Trypauchen vagina</i>	Fujian (FJ)	8/2009	6	KP260458
	Beihai (BH)	8/2014	6	
<i>Ctenotrypauchen microcephalus</i>	Rizhao (RZ)	3/2015	3	

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