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First Morphological Description of a Larval Sleek Unicornfish *Naso hexacanthus* (Acanthuridae, Perciformes) Identified by COI Barcoding in the East China Sea

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ABSTRACT Here, we present the first morphological description of a larval *Naso hexacanthus* (5.2 mm in body length) from the East China Sea identified by cytochrome c oxidase subunit I (COI) barcoding. The larva had a kite-shaped body with long serrated first spine of dorsal and anal fins. There were four melanophores on the base of the anal fin, dense melanophores on the caudal peduncle, and scattered melanophores on the surface of the brain. There was one small spine on the snout and behind each eye, with serrations on the head, top of the eye, inner- and outer-preopercle, and on the lower part and side of the opercle. The morphological characteristics of larval *N. hexacanthus* identified by COI barcoding will be useful for species identification of larval fish.

Key words: DNA barcoding, East China Sea, larval fish, morphological description, *Naso hexacanthus*

INTRODUCTION

The Acanthuridae comprise six genera and 85 species (Fricke *et al*., 2022) that inhabit coral reefs in subtropical and tropical areas (Frank, 1971; Allen and Robertson, 1994). They have a long, deep, and compressed body and one or more spines on the side of the caudal peduncle (Desoutter, 1986; Smith and Heemstra, 2012). Unlike adult fish, Acanthuridae larvae have a transparent, diamond-shaped body and are referred to as acronurus, a term that originated from misidentification of the larva as its own family, Acronuridae, due to the large morphological differences between the larval and adult fish (Leis and Richards, 1984; Richards, 2005).

The morphological features of larval fish take the shape of the adult fish during growth, making it difficult to identify the species (Powels and Markle, 1984). This is because of the large differences in morphology between the larval and adult fish, and the paucity of morphological characteristics available for species identification. Morphological descriptions of larvae of similar developmental stages are traditionally used to determine the species. Recently, molecular identification based on mitochondrial DNA sequences has been introduced (Ko *et al*., 2013; Kimmerling *et al*., 2017; Hou *et al*., 2021). Unlike morphology, DNA is consistent throughout life. Comparisons of intra- and inter-genetic distances enable species identification (Avis, 1994; Hebert *et al*., 2003).

In this study, a larval fish (5.2 mm) collected from the East China Sea was identified as *Naso hexacanthus* based on the cytochrome c oxidase subunit I sequence. The genus *Naso* belongs to the Acanthuridae and includes 20 species s (Guala, 2019; Fricke *et al*., 2022), of which three have been recorded in Korean waters (Lee *et al*., 2000; Kim *et al*., 2008; Kwun and Jung, 2018) and 13 in Japanese waters (Nakabo, 2013). For the morphological description of larvae, two species *N. brevirostris* and *N. lituratus* were recorded in the waters of Japan (Manabe and Ozawa, 2014) and *N. unicornis* was reported in the Indian Ocean (Leis and Richards, 1984), the genus level of which was based on

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specimens collected in the waters of Australia (Leis and Rennis, 2000) and Kenya (Mwaluma *et al.*, 2014). Here, we report the morphology of a larval *N. hexacanthus* collected in the East China Sea.

MATERIALS AND METHODS

1. Larval fish collection

A larval fish was collected from the East China Sea (32°30′00″N, 127°05′14″E; Fig. 1) during the R/V Tamgu 3 survey (August 27, 2021). Sampling was performed by obliquely towing a ichthyoplankton net (diameter, 80 cm; mesh size, 330 μm) from 10 m above the sea bottom to the surface. A larval fish was obtained from the sample, and photographed under a stereomicroscope. The larval fish was preserved in 95% ethanol under name 2108ECS 31513L. The specimen was deposited in the National Institute of Fisheries Science. The morphology of the larval fish was compared with the descriptions of Manabe and Ozawa (2014) and Leis and Richards (1984).

2. Genomic DNA extraction, PCR, sequencing

Genomic DNA (gDNA) was extracted from the right eye of the larval fish using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. A partial region of the COI gene was amplified using the primers VF2_t1, FishF2_t1, FishR2_t1, and FR1d_t1 (Ivanova *et al.*, 2007). PCR was performed in a total volume of 20 μL composed of 10 μL 2× master mix (CellSafe, Yongin, Korea), 0.2 μL each of the four primers, 2 μL gDNA, and 7.2 μL distilled water. PCR conditions consisted of an initial denaturation step at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 40 s, extension at 72°C for 1 min, and a final extension step at 72°C for 7 min. The PCR product was sequenced using a 3730xl DNA analyzer (Applied Biosystems, CA, USA). The COI sequence of the larval fish was submitted to NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

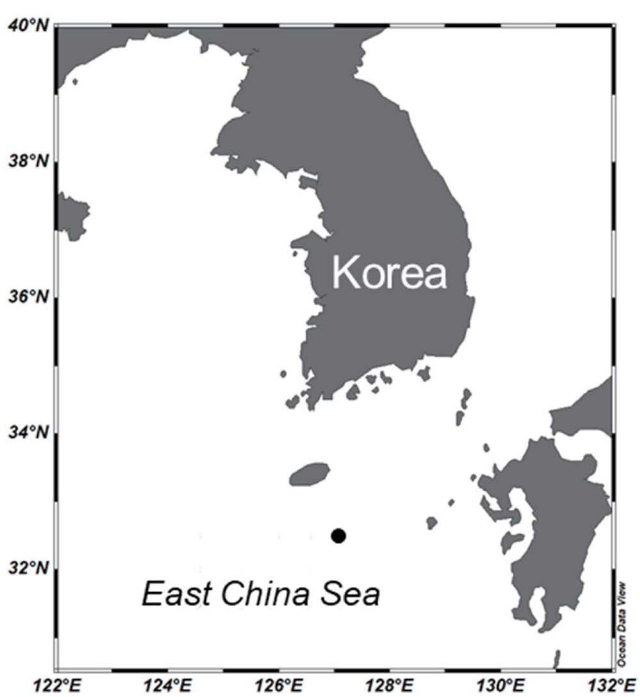

Fig. 1. Sampling station (●) for a larval fish in the East China Sea.

3. Data analysis

The COI sequence of the larval fish was used for a BLAST search to identify sequences of related taxa. Sequences of the larval fish, related taxa and outgroups were aligned using Clustal Omega (Sievers *et al.*, 2011) in Geneious Prime (ver. 2021.2.2; Kearse *et al.*, 2012). The neighbor-joining tree (Saitou and Nei, 1987) and Kimura 2-parameter distance (Kimura, 1980) were analyzed in MEGA X (ver. 11.0.10; Kumar *et al.*, 2018).

RESULTS

***Naso hexacanthus* (Bleeker, 1855)**
(Fig. 3; Table 1)

Priodon hexacanthus Bleeker, 1855: 421 (type locality: Ambon Island, Molucca Islands, Indonesia).
Callicanthus hexacanthus: Chen *et al.*, 1997: 155 (Nansha Islands, China).
Naso hexacanthus: Schultz and Woods in Schultz *et al.*, 1953: 644 (Bikini Atoll, Marshall); Kishimoto in Masuda *et al.*, 1984: 225 (Japan); Randall in Randall and Lim, 2000: 642 (South China Sea); Shimada in Nakabo, 2002: 1322 (Ryukyu Islands, Japan); Kim *et al.*, 2008: 66 (Jeju Island, Korea).

COI barcoding of larval fish. The partial COI sequence of the larval fish (652 bp; GenBank Accession number: OM 033462) was analyzed to identify the species. The sequence of the larval fish formed a clade with that of *Naso hexacanthus* in the neighbor-joining tree (Fig. 2). The genetic distance of clade of the *N. hexacanthus* was very close (average ± standard error, 0.004 ± 0.001). This distance was smaller than distances between them and other species of *Naso* (0.082 ± 0.003; min, 0.060; max, 0.100) (Table 1).

Morphological description of larval fish. The larval fish (5.2 mm in body length; dorsal fin rays, VI, 28; anal fin rays, II, 28; pectoral fin rays, 14; caudal fin rays, 20) identified by the COI sequence as *N. hexacanthus* had a kite-shaped compressed body (Fig. 3). The body length of the specimen was reduced by 11.5% after preservation in 95% ethanol. The abdomen protruded and its tip was pointed. The specimen had a long and recessed snout. The nostrils had a rounded triangular shape. Twelve small spines were present at the base of each dorsal and anal fin. One small spine was present on the snout and behind each eye. Minute serrated spines were present on the head (number of spines, 19), above the eyes (4), inner (11) and outer (8) preopercle, and lower part (9) and side (4) of the opercle. Melanophores were distributed across the surface of the brain. The abdomen had melanophores on the side and near the base of the pectoral fin. At the middle of the base of the anal fin, there were three melanophores located side by side with one melanophore located a little way off. Dense melanophores were present on the caudal peduncle.

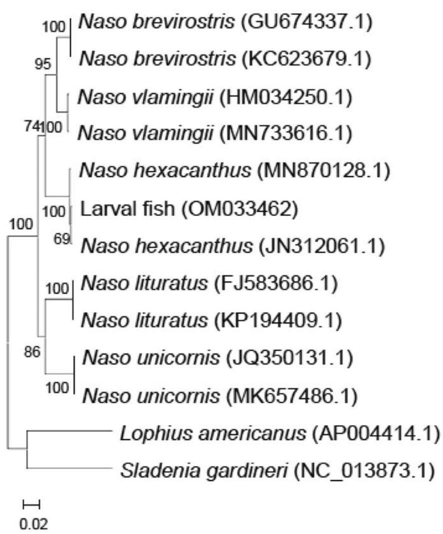

Fig. 2. Neighbor-joining tree based on COI sequences from the larval fish specimen described in this study, related taxa, and outgroups. Bootstrap values (1,000 replicates) are shown on the branches.

Ecological notes. The larval fish was collected from a depth of 120 m to the surface. The sea surface and bottom temperatures were 27.7°C and 18.0°C, respectively. And, sea surface and bottom salinities were 31.3 psu and 34.6 psu, respectively.

Distribution. *Naso hexacanthus* is distributed in the Red

Table 1. Genetic COI distances between a larval fish, *Naso*, and outgroups

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*Naso hexacanthus* (MN870128.1)													
2	Larval fish (OM033462)	0.003												
3	*Naso hexacanthus* (JN312061.1)	0.006	0.003											
4	*Naso brevirostris* (GU674337.1)	0.060	0.063	0.063										
5	*Naso brevirostris* (KC623679.1)	0.060	0.063	0.063	0.000									
6	*Naso vlamingii* (HM034250.1)	0.070	0.074	0.074	0.035	0.035								
7	*Naso vlamingii* (MN733616.1)	0.067	0.071	0.071	0.032	0.032	0.003							
8	*Naso lituratus* (FJ583686.1)	0.091	0.091	0.095	0.090	0.090	0.085	0.087						
9	*Naso lituratus* (KP194409.1)	0.091	0.091	0.095	0.090	0.090	0.085	0.087	0.000					
10	*Naso unicornis* (JQ350131.1)	0.100	0.100	0.100	0.088	0.088	0.074	0.078	0.075	0.075				
11	*Naso unicornis* (MK657486.1)	0.100	0.100	0.100	0.088	0.088	0.074	0.078	0.075	0.075	0.000			
12	*Lophius americanus* (AP004414.1)	0.220	0.217	0.215	0.220	0.220	0.222	0.224	0.224	0.224	0.229	0.231		
13	*Sladenia gardineri* (NC_013873.1)	0.217	0.215	0.215	0.232	0.232	0.217	0.217	0.223	0.223	0.222	0.223	0.226	

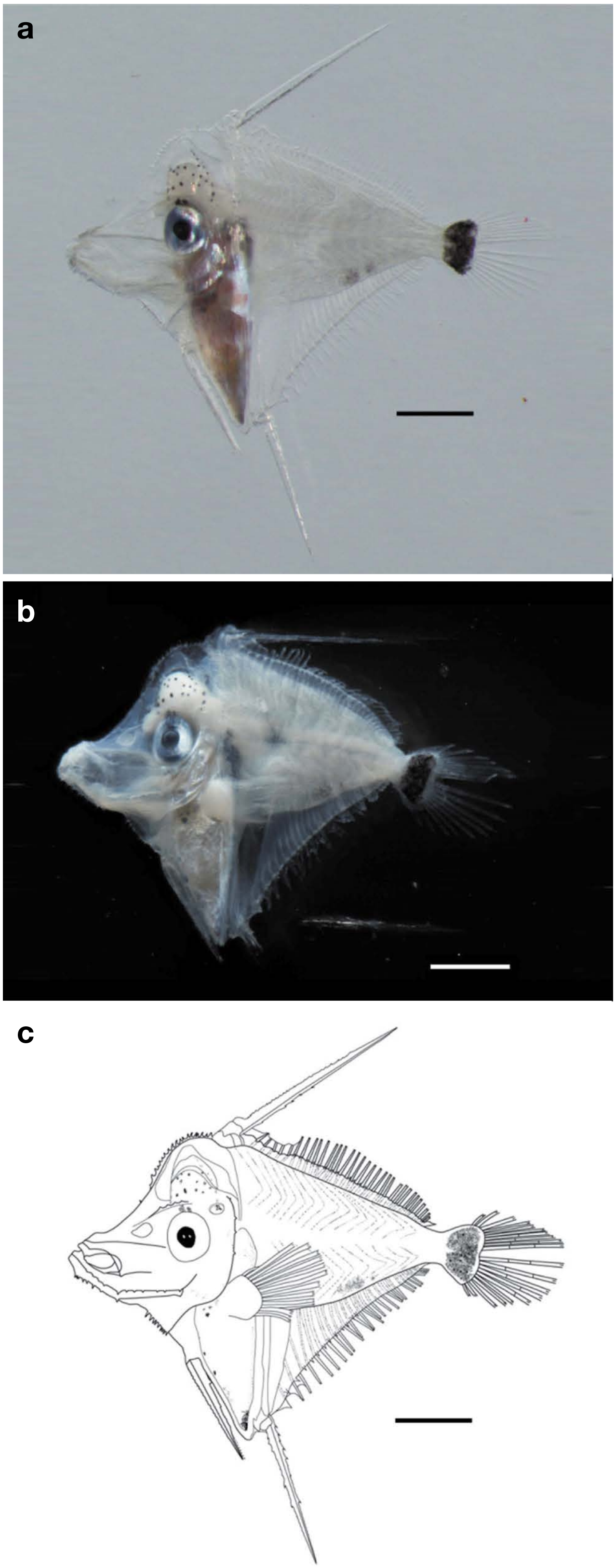

Fig. 3. Morphology of larval *Naso hexacanthus*. Scale bar, 1 mm. a. Photograph of the fresh specimen. b. Specimen preserved in 95% ethanol. c. Illustration based on the fresh specimen.

Sea (Golani and Fricke, 2018) and Indo-Pacific Oceans: around Madagascar Islands (Fricke *et al*., 2018; Durville *et al*., 2021), Pakistan (Psomadakis *et al*., 2015), Christmas Island (Allen, 2000), Taiwan (Ho *et al*., 2011), southern Korea (Kim *et al*., 2008; this study) and Japan (Akaike *et al*., 2021), Australia (Hutchins, 2001), Hawaiian Islands (Mundy, 2005).

DISCUSSION

Species identification is a key step in investigating the ecology of larval fish. Misidentification or difficulties in identification lead to errors in interpretation (Powels and Markle, 1984). These problems occur with morphology-based identification due to the absence of morphological descriptions, the condition of specimens, researcher inexperience, and other factors. Identification of larval fish can be validated by DNA barcoding (Ko *et al*., 2013), which enables species with similar morphologies to be distinguished but is of limited use for species that are not distinguishable by DNA region (Choi *et al*., 2018) or for which comparable DNA sequences do not exist. Therefore, the accuracy of species identification can be increased by using both morphological and DNA sequence information (Leis, 2014).

A larval fish collected from the East China Sea was identified as belonging to the genus *Naso* based on its morphological characteristics. This larval specimen had in common with *Naso* species (*N. brevirostris*, *N. unicornis*, *N. lituratus*) a kite-shaped body, pointed abdomen, dense melanophores on the caudal peduncle, scattered melanophores on the surface of the brain, and melanophores behind the opercle and the abdomen (Table 2). The larval specimen and *N. brevirostris* had melanophores on the base of the pectoral fin. The number of pectoral fin rays was the least in *N. hexacanthus* (5.2 mm BL) and the most in *N. unicornis* (5.9 mm BL). The maximum number of pectoral fin rays in adult fish was 17 (*N. brevirostris*, *N. lituratus*), 18 (*N. hexacanthus*, *N. unicornis*) (Nakabo, 2013). The distinct features of the larval specimen were four melanophores on the middle of the base of the anal fin, melanophores on the anterior abdomen, and one minute spine on the snout and behind each eye.

Although there was no morphological description in common with our larval fish, DNA barcode analysis made it possible to identify the larval specimen at the species level. The COI sequence of the larval fish was 99.6% identical to that of *N. hexacanthus*, forming a clade with *N. hexacanthus*. Based on the phylogenetic tree and genetic

Table 2. Comparisons of measurements and counts of the larvae of four *Naso* species

Characters	*N. hexacanthus*[1]	*N. brevirostris*[2]	*N. unicornis*[3]	*N. lituratus*[2]
Number of specimens	1	1	1	1
Body length (mm)	5.2	7.8	5.9	8.1
Counts				
Dorsal fin rays	VI, 28	VI, 29	VI, 28	VI, 30
Anal fin rays	II, 28	II, 25	II, 26	II, 30
Pectoral fin rays	14	16	17	15
In % of the body length				
Head length	40.4	38.8	42.0	36.8
Snout length	23.1	21.2	24.7	17.4
Eye diameter	9.6	12.4	12.3	12.1
Body depth	76.9	82.4	86.4	63.2
Other				
Melanophore distribution	Surface of brain, caudal peduncle, base of anal fin, base of pectoral fin under epidermis, behind opercle, surround of abdomen	Surface of the brain, caudal peduncle, base of anal fin, base of pectoral fin under epidermis, behind opercle, posterior abdomen	Surface of the brain, caudal peduncle, base of anal fin, behind opercle, posterior abdomen	Surface of the brain, caudal peduncle, base of anal fin, behind opercle, posterior abdomen
Number of melanophores on the base of anal fin	4	3	8	9

[1], This study; [2], Manabe and Ozawa (2014); [3], Leis and Richards (1984)

distance, the larval fish was determined to *N. hexacanthus*. Therefore, we report the first morphological description of larval *N. hexacanthus* identified by COI barcoding from the East China Sea. The morphological description and distribution of larval *N. hexacanthus* will be useful for identification of the species as well as study of larval fish ecology.

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COI 바코딩으로 동정한 남방표문쥐치(*Naso hexacanthus*) 치어의 첫 형태 기재

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요 약 : 본 연구는 동중국해에서 수집한 치어(체장 5.2 mm)의 종을 COI 바코딩을 통해 남방표문쥐치(*Naso hexacanthus*)로 동정하고, 형태를 처음으로 보고한다. 치어는 마름모꼴의 몸통으로 등지느러미와 뒷지느러미의 첫 극조는 길고 톱니가 나 있었다. 뒷지느러미 기저부에 네 개의 흑색소포, 미병부에 밀집한 흑색소포, 뇌 표면에 퍼져 있는 흑색소포가 있었다. 작은 가시 한 개가 각각 콧등과 눈 뒤에 있었고, 톱니 모양의 돌기가 머리, 눈 위, 전새개골 내부와 외부, 새개골 아래와 옆 부분에 발달하였다. COI 바코딩으로 동정한 남방표문쥐치 치어의 형태적 특징은 치어의 종 동정에 유용할 것이다.

찾아보기 낱말 : 남방표문쥐치, 동중국해, DNA 바코딩, 자치어, 형태 기재