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New record of *Neoclinus lacunicola* (Actinopterygii: Perciformes: Chaenopsidae) from Ulleung Island, Korea revealed by body morphometry and mitochondrial DNA barcoding

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Abstract

One specimen (38.3 mm SL) of *Neoclinus lacunicola* Fukao, 1980, belonging to the family Chaenopsidae, was first recorded from Ulleung Island, Korea (East Sea, otherwise known as the Sea of Japan) on 5 January 2021. This species was characterized by paired external pores of incomplete lateral line running from the upper margin of the opercle, seven pairs of supraorbital cirri arranged in two rows, occipital region with a pair of cirri, and 13 rays of pectoral fin. This species is morphologically similar to the *Neoclinus toshimaensis* Fukao, 1980, but differs in the number of cirri on the supraorbital (6–7 versus 9–11 cirri). This study documents the first report of *N. lacunicola* in Korean waters and proposes the new Korean name of 'eol-lug-bi-neul-be-do-la-chi' for the species. For the confirmation of the identity of the species, a partial gene sequence of the mt COI (570 bp) of *N. lacunicola* was obtained for the first time.

Keywords

Neoclinus species, tube blenny, new record, rocky shore, East Sea, Sea of Japan

Introduction

The family Chaenopsidae comprises 96 species belonging to 14 genera distributed worldwide, with one genus and eight species occurring in Japanese waters (Aizawa and Doiuchi 2013; Nelson et al. 2016), but only two species representing a single genus have recently been recorded in Korean waters (Kim and Kang 1991; Myoung et al. 2021). The tube blennies (Chaenopsidae) are reef-associated marine fishes, mainly distributed in warm North and South American waters (Nelson et al. 2016). Generally, small-sized, usually elongated and compressed, with a large mouth. The supraorbital and nasal cirri are present or absent (Murase et al. 2015). They exhibit a hiding behavior in crevices between rocky matrices, but occasionally inhabit empty gastropod shells (empty barnacles and empty worm shells) (Stephens and Springer 1971; Fukao 1980; Fukao and Okazaki 1987). Such behavior is advantageous to protect their bodies from potential predators and to care for their spawned eggs within the habitats (Murase and Sunobe 2011; Froese and Pauly 2020).

Neoclinus lacunicola Fukao, 1980 was firstly reported as a new species in 1980 (Fukao 1980) and later, several studies have reported on the taxonomic checklists, phylogeny, and encyclopedic information (Patzner et al. 2009; Aizawa and Doiuchi 2013; Lin and Hastings 2013;

Soniyama et al. 2020). However, there is still a lack of information on its biology and ecology.

To date, one species of tube blennies, i.e., *Neoclinus bryope* (Jordan et Snyder, 1902) has firstly been reported in Korean waters (Kim and Kang 1991) and, more recently, Myoung et al. (2021) documented the first record of *Neoclinus chihiroe* Fukao, 1987 in Dokdo, Korea; consequently, a total of two species have been recorded in Korean waters. This study provides the first record of an additional chaenopsid species (*N. lacunicola*) collected from Ulleung Island, describes its morphological characteristics, and records its mitochondrial DNA cytochrome c oxidase subunit I (mt COI) sequences.

Methods

One specimen of *N. lacunicola* was collected from Tonggumi (37°27′33.50″N, 130°51′28.84″E) in the coastal waters of Ulleung Island, Gyeongsangbuk-do, Korea (East Sea, otherwise known as the Sea of Japan), by a SCUBA diver on 5 January 2021 (Fig. 1). Sampling was conducted at a depth of approximately 22 m, close to the shoreline during the day and the sample was transported to the laboratory immediately after capture in a living condition, using a small fishbowl with seawater. In the laboratory, an image of the specimen was taken, it was euthanized and then meristic counts and body morphometrics were recorded following the method of Hubbs and Lagler (1958). The specimen was measured to the nearest 0.1 mm using digital Vernier calipers and a microscope. The specimen was then preserved in 5% formalin for 24 h and later transferred to 70% ethanol for depositing at the Marine Biodiversity Institute of Korea (MABIK).

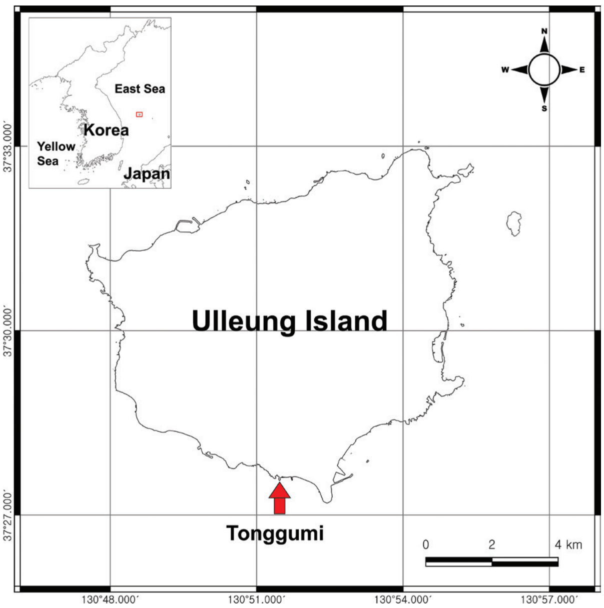

Figure 1. Map showing the sampling area of *Neoclinus lacunicola* from Tonggumi, Ulleung Island, Korea.

To compare molecular data, total genomic DNA was extracted from the muscle tissue using 10% Chelex resin (Bio-Rad, Hercules, CA). A portion of the mitochondrial COI gene was amplified using universal primers (Ward et al. 2005). PCR was performed in a 20 μL reaction tube containing 1 μL genomic DNA, 2 μL 10× PCR buffer, 2 μL 2.5 mM dNTP, 1 μL of each primer, 0.1 μL Ex-Taq DNA polymerase, and 12.9 μL sterile distilled H_2O, using a thermal cycler (MJmini PTC-1148, Bio-Rad USA). The PCR profile consisted of initial denaturation at 95°C for 5 min, followed by 34 cycles of denaturation at 95°C for 1 min, annealing at 50°C, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products were purified using ExoSAP-IT (United States Biochemical Corporation USA) and sequenced, using an ABI PRISM BigDye Terminator v.3.1 Ready Reaction cycle sequencing kit (Applied Biosystems Inc. USA) on an ABI 3730xl DNA analyzer (Applied Biosystems Inc.). We compared our molecular data with those of the mtDNA COI sequences from other *Neoclinus* species obtained from GenBank (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov). Sequences were aligned using ClustalW (Thompson et al. 1994) in BioEdit, version 7 (Hall 1999). The genetic divergences were calculated using the Kimura 2-parameter (K2P) (Kimura 1980) model with Mega 6 (Tamura et al. 2013). Phylogenetic trees were constructed using the neighbor-joining method (Saitou and Nei 1987) in Mega 6 (Tamura et al. 2013), with confidence assessed, based on 1000 bootstrap replications.

Results

Material examined

MABIK PI00049728, 1 specimen, 38.3 mm SL, Tonggumi, Ulleung Island, Gyeongsangbuk-do, Korea (37°27′33.50″N, 130°51′28.84″E).

Description. The body counts, measurements, and proportions of body parts are shown in Table 1. Body elongated and compressed (Fig. 2). Head short and round. Snout short, but mouth relatively large; posterior tip of upper jaw beyond posterior margin of eyes. Distance of interorbital area narrow. Single nasal cirrus on posterior rim of nostril; cirrus long, thin, and slender with four branches. Seven pairs of supraorbital cirri arranged in double rows with four cirri on outer row and three cirri on inner row and thick and short branches on each cirrus. Occipital region with single pair of cirri; each cirrus short, wide, and monotonous (Fig. 2B). Specimen having single dorsal fin and dorsal fin membrane shallow notched; soft part of ray usually higher than spinous part. Origin of dorsal fin located forward to origin of pelvic fin. Origin of soft part of dorsal fin ray located behind anus. Caudal fin separated from both dorsal and anal fins. End of caudal fin rounded. Entire margin of anal fin serrated; origin of anal fin located in front part of central portion of body. Pectoral fin rounded and located behind pelvic fins.

Head red and body uniformly white with black patterns (Fig. 2). Upper part of head red and white spots scattered under eyes and on chin. Red part of head appearing before fourth spine of dorsal fin and color gradually becoming faded to fourth spine of dorsal fin. Jaw and pelvic fins light yellow. Cirri in nostrils almost transparent. Lower part of supraorbital cirri red, but tip of cirri transparent. Background color of body white, with eight large black bands going down from dorsal to lower middle body and starting points of eight black patterns irregularly appear under the basement of dorsal fin. Black blotch surrounded by red between second and third dorsal spines. Spine and soft rays of dorsal fin having irregular red color and transparent membrane. Upper part of opercular membrane having no black blotch. Overall, pectoral fin is pale red. Basement of pectoral fins with no black dot. Pelvic fins covered in yellow. Anal and caudal fins light red but almost transparent.

Based on an analysis of the mitochondrial cytochrome oxidase subunit I gene (COI) sequence (570 bp), the presently reported specimen was different from other six species of the genus *Neoclinus* with genetic distances of 0.141–0.220 (Fig. 3).

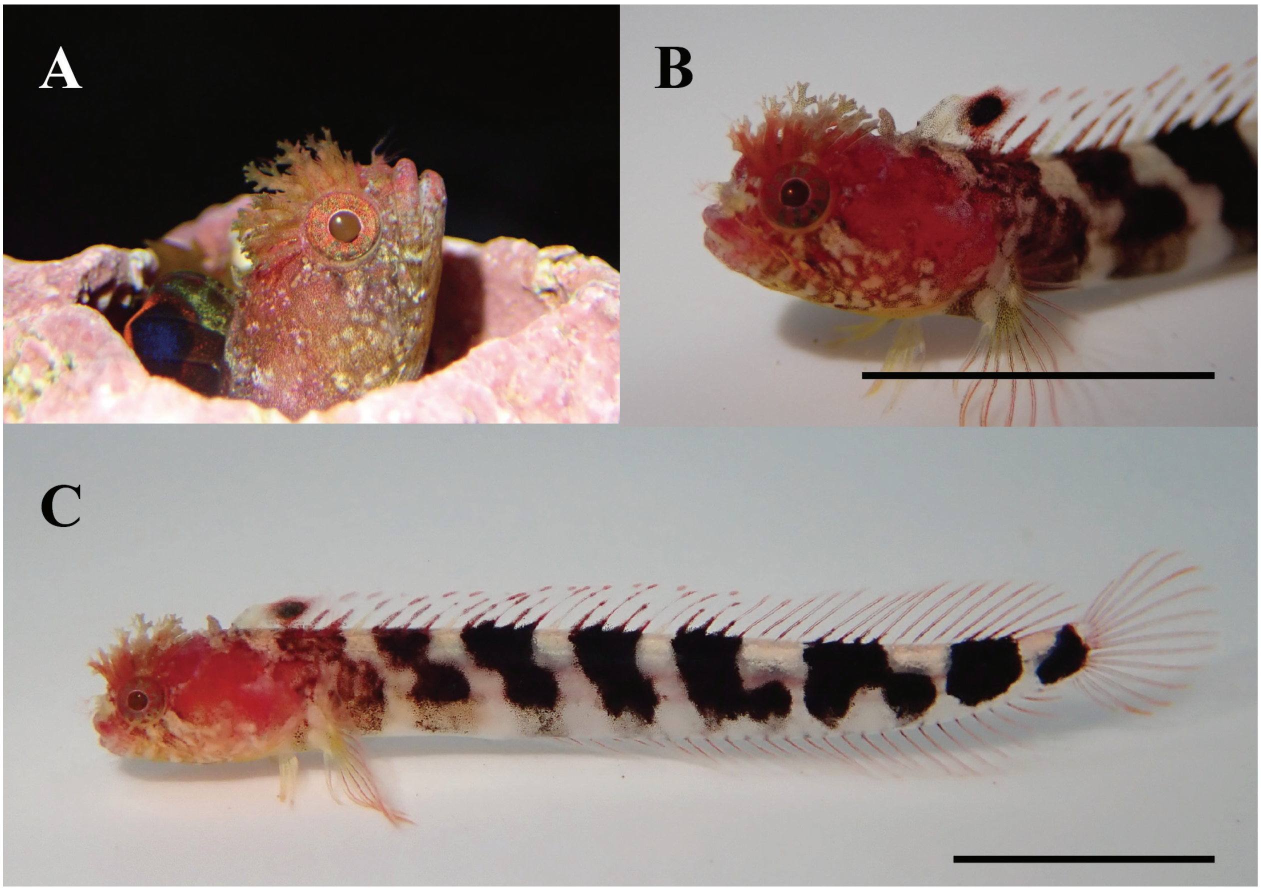

Figure 2. *Neoclinus lacunicola*, living condition, MABIK PI00049728, 38.3 mm SL, Tonggumi, Ulleung Island, Korea. **A.** An underwater photo; **B** and **C.** Images taken in the laboratory. Scale bar: 1 cm.

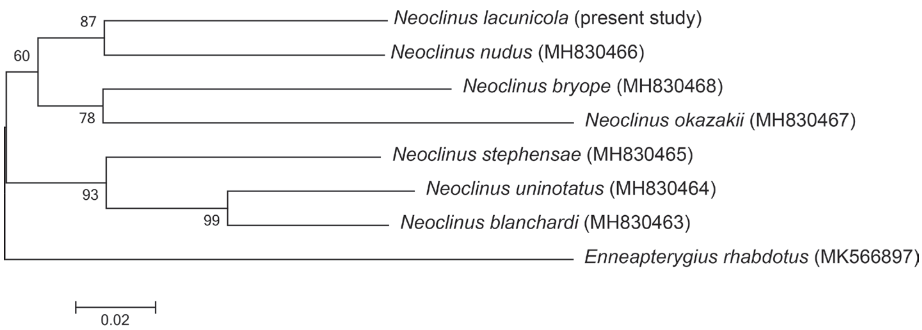

Figure 3. A neighbor-joining tree, based on partial mtDNA COI region using *Neoclinus lacunicola* (MABIK PI00049728) and other species of *Neoclinus*. Numbers at branches indicate bootstrap probabilities in 10 000 bootstrap replications. Scale bar equals 0.02 of Tamura and Nei’s distance (1993) with K2 parameter model.

Table 1. Morphometric measurements of the *Neoclinus lacunicola* in comparison with previous records.

Morphological characters	Presently reported specimen	Fukao 1980	Murase et al. 2015	
Sex (number of specimens)	Female (1)	Male (1)	Female (2)	Male (2)
Counts				
Dorsal rays	XXII, 18	XXIII, 19	XXII–XXIII, 17–19	
Anal rays	II, 27	II, 27	II, 27	
Pectoral rays	13	13	12 or 13	
Pelvic rays	I, 3	I, 3	—	
Principal caudal rays	7 + 6 = 13	7 + 6 = 13	7 + 6 = 13	
Measurements				
Standard length [mm]	38.3	48.8	38.9–49.4	38.0–42.8
% of standard length				
Total length	113.8	112.9	113.8–114.7	114.7–115.5
Body depth	12.3	14.5	12.6–13.6	12.9
Head length	20.4	20.5	21.5–24.2	23.1
Head width	13.8	14.1	—	—
Head depth	13.3	—	13.6–15.4	14.5
Orbit diameter	4.3	4.3	5.9–6.9	6.1
Postorbital length	13.1	13.3	—	—
Interorbital width	1.3	—	1.0	0.9
Snout length	4.0	3.9	4.4–4.5	5.1
Upper jaw length	9.9	10.2	10.3–11.1	11.2
Pre-dorsal length	15.9	15.6	15.4–17.5	17.3
Pre-anal length	40.9	41.0	38.7–42.7	40.4
Length of dorsal fin base	82.0	83.8	81.7–87.0	82.9
Length of soft dorsal fin base	34.5	34.8	—	—
Length of anal fin base	53.5	56.1	54.0–56.7	55.4
Anal origin to pelvic insertion	20.1	23.3	—	—
Depth of caudal peduncle	6.1	6.4	5.1–5.4	5.3–5.6
Length of caudal peduncle	5.0	5.7	6.1–6.9	6.3–7.1
Length of 1st dorsal-fin spine	5.5	6.4	5.9	7.0
Longest dorsal spine length	9.0 (13th)	8.4 (13th)	—	—
Longest dorsal soft ray length	11.8 (11th)	10.2 (11th)	—	—
Longest anal ray length	9.1 (24th)	8.2 (24th)	—	—
Length of last dorsal-fin spine	8.1	—	6.5–8.2	7.7
Length of 1st dorsal-fin soft ray	9.7	—	8.7–10.5	9.3
Length of 1st anal-fin spine	5.0	3.9	2.6–4.4	4.0
Length of 1st anal-fin soft ray	7.6	6.4	5.7–6.9	7.9
Longest pectoral ray length	16.4 (9th)	12.5 (9th)	—	—
Longest pelvic ray length	9.4 (2nd)	9.1 (2nd)	—	—

Discussion

In this study, one specimen of chaenopsid fishes has been collected from Ulleung Island with body elongated and compressed, no scales, supraorbital and nasal cirri present (Williams 2003; Nelson et al. 2016). The body morphometrics of the specimen were well matched with the original description of *N. lacunicola* with paired external pores of the incomplete lateral line, seven pairs of supraorbital cirri, occipital region with a pair of cirri, and 13 rays of pectoral fin (Fukao 1980; Aizawa and Doiuchi 2013). There are some differences between the presently reported specimen and that described by Fukao (1980); the differences were mainly the anal origin to pelvic insertion and slightly shorter length of caudal peduncle and longest pectoral ray length. Nonetheless, it is consistent with original descriptions with the main features of no black spot between first and second dorsal spines, occipital region with a pair of cirri, and seven pairs of supraorbital cirri (Fukao 1980; Aizawa and Doiuchi 2013; Murase et al. 2015). The body morphology of *N. lacunicola* is almost similar to *Neoclinus toshimaensis* Fukao, 1980, but distinctly different in the number of supraorbital cirri (6~7 in *N. lacunicola* vs. 9~11 in *N. toshimaensis*) (Fukao 1980; Aizawa 2002; Aizawa and Doiuchi 2013). The findings of two chaenopsids of the genus *Neoclinus*, namely, *N. bryope* and *N. chihiroe* were recently reported from Korean waters (MABIK 2020; Myoung et al. 2021). *Neoclinus lacunicola* is easily distinguished from the other two by lacking the black spot between first and second dorsal spines (without a black spot in *N. lacunicola* vs. with black spot in *N. bryope* and *N. chihiroe*) (Fig. 2B) (Aizawa 2002; Aizawa and Doiuchi 2013). The specimen collected in this survey had a black spot in the membrane of the second and third dorsal spines. This morphological character i.e., the presence or absence of a black spot between first and second dorsal spines, is one of the things that distinguish between males and females of *Neoclinus monogrammus* Murase, Aizawa et Sunobe, 2010 (see Murase et al. 2010) and it cannot be classified as *Neoclinus* species according to the taxonomic key suggested by Aizawa and Doiuchi (2013). For the presently reported specimen, yellow pelvic fins are key to distinguishing it as a female, despite the presence of black spots in the second and third dorsal spines membrane (Murase et al. 2015). Therefore, the presence or absence of the black spot is thought to be more variable in the same species than previously considered. In addition, our specimen was well distinguished from *Neoclinus nudus* Stephens et Springer, 1971 and *N. bryope* was well separated from other congeners by genetic distances (*d*) of range 0.141 and 0.191 when a comparison of mitochondrial DNA COI sequences is made (Fig. 3). Therefore, this study documents the first record of *N. lacunicola* in Korean waters and suggests the new Korean name of 'eol-lug-bi-neul-be-do-la-chi' for the species.

Chaenopsid fishes, occurring in Korean waters, have previously been known only as two species (*N. bryope* and *N. chihiroe*), but an additional species has now been reported through this study. The ecological characteristics of chaenopsid fishes are known that they do not have large movements and inhabit small caves between rocks or in empty gastropod tubes (Stephens and Springer 1971; Fukao and Okazaki 1987; Fukao 1990). Due to this characteristic, their distribution ranges are expected to be considerably narrow (Hongjamrassilp et al. 2020). However, although the mobile abilities are limited within the habitat ranges, their larvae have potential dispersal possibilities through the oceanographic processes, including currents (Murase et al. 2015). According to Murase et al. (2015), the distribution range of *N. lacunicola* has been extended northwards along with the Tsushima Current and this study confirmed such northern distribution of the species. Although this species has firstly been reported to Korean waters, it is also expected that a number of individuals live in eastern Korean waters as well as Dokdo. In addition, because of the scarcity and the narrow habitat range of the species, there is a need for more ecological information, including their genetic group structure, geographic distribution range, and larval dispersals.

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