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New Finding on Range Expansion and Geographic Variation of *Eumicrotremus jindoensis* (Cyclopteridae) Collected from Boryeong in the Western Coast of Korea

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ABSTRACT Since the original description of new species, *Eumicrotremus jindoensis*, we confirmed the first occurrence of *E. jindoensis* based on a single specimen (22.3 mm SL) caught by inshore stow net at the coastal waters of Boryeong of Korea. However, our specimen slightly differed from type specimens in having more vertebrae (26 vs. 21~24), longer snout (17.4% vs. 8.1~9.1%), longer preanus length (67.5% vs. 58.0~58.3%) and shorter second dorsal fin base (15.3% vs. 20.2~20.8%). Comparing with mtDNA COI and Cyt*b* sequences, we could not find any differences in mtDNA Cyt*b* sequences between our specimen and type specimens, which suggest that those morphological differences may belong to local variation by habitat and environmental condition between off Jindo Island and off Boryeong in Korea. *Eumicrotremus uenoi* is known from the southern sea of Korea narrowly (Busan, Tongyeong, and Jeju Island), the other congeneric species (*E. asperrimus*, *E. pacificus*, and *E. taranetzi*) from only the eastern sea of Korea, but *E. jindoensis* from the central coast to southern coast of western Korea.

Key words: *Eumicrotremus jindoensis*, distribution, morphology, variation, Korea

INTRODUCTION

The smallest lumpsucker (Family Cyclopteridae), which inhabits the benthic substrates throughout Arctic, northern regions of the North Pacific and Atlantic (Mecklenburg and Sheiko, 2003), comprise approximately 29 species in 8 genera in the world (Froese and Pauly, 2020) and two genera six species have been reported from Korean waters (MABIK, 2019). Especially, the genus *Eumicrotremus* Gilbert, 1896 is the most charged of lumpsucker, comprising five species (MABIK, 2019). Interestingly, in a recent morphological and molecular taxonomic review of *Eumicrotremus* species from the coastal waters of Korea, Lee *et al*. (2017) revealed that several specimens collected from Busan and Tongyeong in Korea were reported as new species, *Eumicrotremus uenoi* Kai, Ikeguchi and Nakabo, 2017 based on the presence of interorbital and supraorbital pores and long caudal fin is discordant with original description of *Eumicrotremus awae* (Jordan and Snyder, 1902). The morphological character of *E. uenoi* was well matched with first record and description of *E. awae* of the northern Jeju Island in Korea (sensu Kim, 2015). In addition, *Eumicrotremus jindoensis* Lee and Kim, 2017 was originally described based on two specimens (19.7~24.8 mm SL) from Jindo Island (west-southern sea of Korea) according to the taxonomic review that the specimens previously misidentified as *Lethotremus awae*. This species is well distinguished from congeneric species by having interorbital and suborbital pore, triangular opercular flap, no papillae on ventral disk, and long caudal fin (32.7~42.1% of SL) (Lee *et al*., 2017). In present study, since the original description

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of the new species, we therefore report the first occurrence of *E. jindoensis* based on single specimen caught at the coastal waters of Boryeong of Korea, and discuss the range expansion and the morphological variation of *E. jindoensis* in Korea.

MATERIALS AND METHODS

We collected a single specimen (22.3 mm in standard length, SL) of *Eumicrotremus jindoensis* from the coastal waters of Boryeong of Korea, caught by inshore stow net in 22 February, 2020 (Fig. 1). The specimen has been deposited in the Marine Fish Resource Bank of Korea (MFRBK) at Pukyong National University (PKU), Busan-si, Korea. To investigate and compare the morphological characters, we examined the type specimens of *E. jindoensis*. We performed morphological analyses to compare with a total of 5 counts and 24 measurements. Counting, measuring, and terminology were based on the previous studies of Ueno (1970) and Lee *et al.* (2017). Each body part was measured to the nearest 0.1 mm using digital Vernier calipers, and data were converted to percentages of the standard length (SL). All fin elements and vertebrae were counted and observed from X-radiographs (SOFTEX M60, Japan), utilizing a stereomicroscope (Olympus SZX-16, Japan). Ventral disk shape was also observed using a same stereomicroscope. Images were analyzed using an image analyzer (Shinhan Scientific Optics, Korea), and features were sketched using a camera lucida (Olympus SZX-16, Japan).

To compare molecular characters, total genomic DNA was extracted from the muscle tissues using 10% Chelex 100 resin (Bio-Rad, Hercules, CA) and PCR was then performed for mitochondrial DNA cytochrome b gene (Cyt*b*) and cytochrome c oxidase subunit I (COI), using an MJ Mini Thermal Cycler PTC-1148 (Bio-Rad) in mixtures consisting 1 mL of genomic DNA, 2 mL of 10 × PCR buffer, 1.6 mL of 2.5 mm dNTPs, 0.5 mL of each primer, 0.1 mL of TAKARA EX-*Taq* polymerase (TAKARA Bio Inc., Japan), and distilled water to bring the final volume to 20 mL. PCR products were amplified using universal primers: VF2-F (5′-TCA ACC AAC CAC AAA GAC ATT GGC AC-3′) and FishR2-R (5′-ACT TCA GGG TGA CCG AAG AAT CAG AA-3′) designed by Ward *et al.* (2005) and GluDG-L (5′-TGA CTT GAA RAA CCA YCG TTG-3′) and CB3-H (5′-GGC AAA TAG GAA RTA TCA TTC-3′) designed by Palumbi *et al.* (1996). The PCR profile for the Cyt*b* and COI region consisted of initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min (at 52°C in COI), extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products were purified using a Davinch™ PCR Purification Kit (Davinch-K Co., Korea). The DNA was sequenced with an Applied Biosystems ABI 3730XL sequencer (Applied Biosystems, Foster City, CA) using an ABI PRISM Bigdye™ Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems). We compared our molecular data with those of the mtDNA Cyt*b* and COI sequences from other *Eumicrotremus* species obtained from the National Center for Biotechnology Information (NCBI). 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 (NJ) method (Saitou and Nei, 1987) in Mega 6 (Tamura *et al.*, 2013), with confidence assessed based on 1000 bootstrap replications.

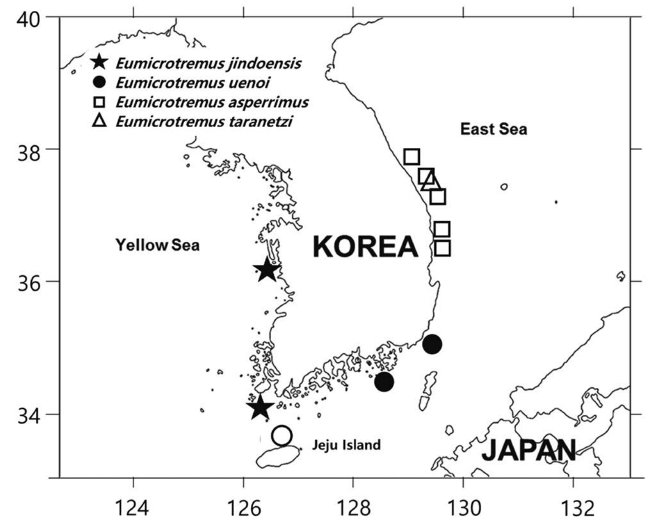

Fig. 1. Collection cites of *Eumicrotremus jindoensis* and other *Eumicrotremus* species in the coasts of Korea. Closed symbols - based on specimens examined in this study; open symbols - based on literature records.

RESULTS AND DISCUSSION

The specimen collected in this study matches the original description of *Eumicrotremus jindoensis* (Lee *et al.*, 2017) well; notably, it has a triangular opercular flap, no papillae on ventral disk (Figs. 2, 3). However, our specimen slightly differed from type specimens in having more vertebrae (26 vs. 21∼24), longer snout (17.4% vs. 8.1∼9.1%), longer

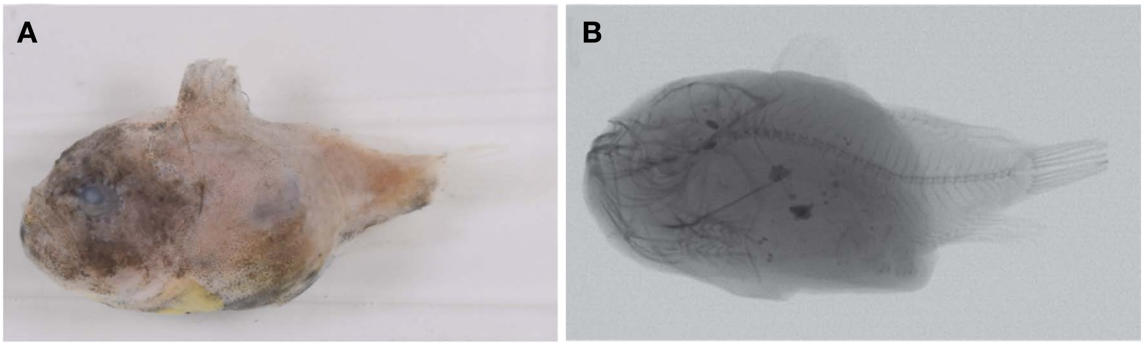

Fig. 2. The photographs of *Eumicrotremus jindoensis* from Boryeong in the western coast of Korea, PKU 62149, Boryeong, 22.3 mm SL. (A) fresh condition; (B) X-ray.

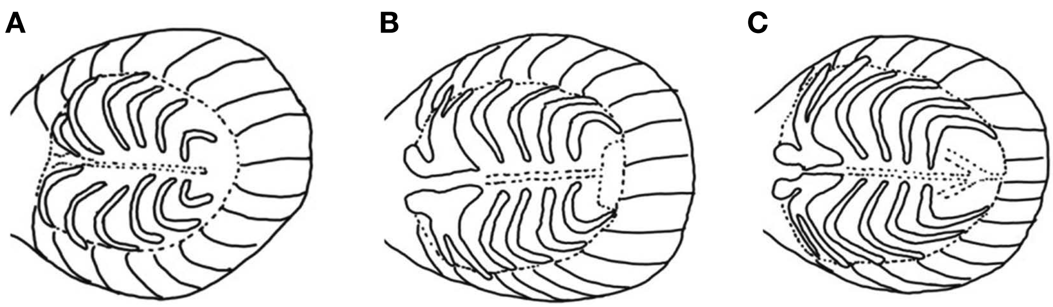

Fig. 3. Illustrations of ventral disk of *Eumicrotremus jindoensis*. (A) PKU 62149, Boryeong, nontype, 22.3 mm SL; (B) PKU 10232, Jindo Island, holotype, 19.7 mm SL; (C) PKU 10233, Jindo Island, paratype, 24.8 mm SL.

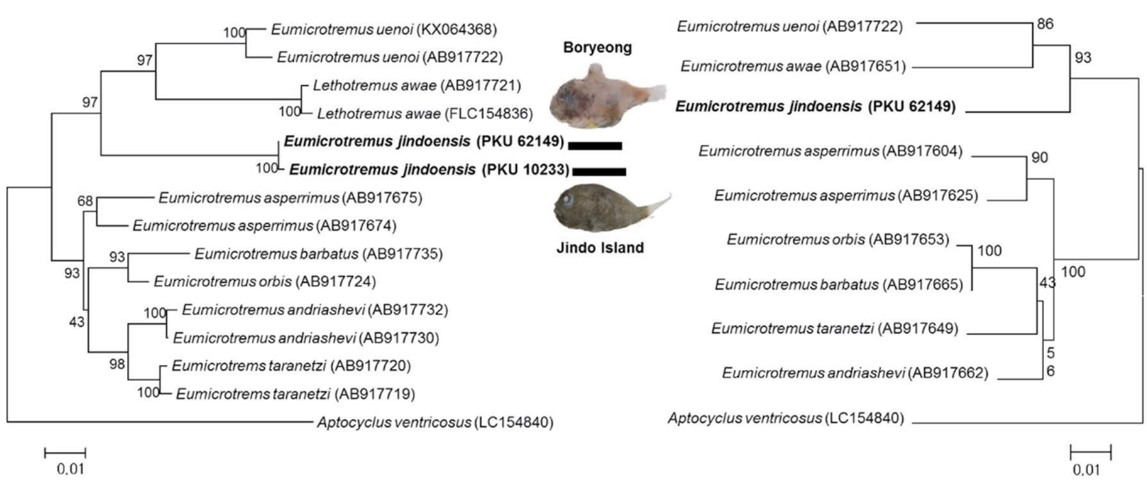

Fig. 4. A neighbor-joining tree based on partial mtDNA Cyt*b* (left) and COI (right) sequences using *Eumicrotremus jindoensis* and other species of *Eumicrotremus*, rooted by an outgroup taxa, *Aptocyclus ventricosus*. Numbers at branches indicate bootstrap probabilities in 1,000 bootstrap replications. Scale bar equals 0.01 of Tamura and Nei's distance (1993) with K2 parameter model.

preanus length (67.5% vs. 58.0∼58.3%) and shorter second dorsal fin base (15.3% vs. 20.2∼20.8%) (Table 1). Generally, the number of vertebrae of lumpsuckers is ranged from 21∼29 (Froese and Pauly, 2020). When comparison with congeneric species, there is ranged from 21∼26 in *E. uenoi* (Lee *et al*., 2017), 26∼28 in *E. pacificus* (Voskoboinikova and Balanov, 2019), and *E. asperrimus* 23∼29 (Mecklenburge and Sheiko, 2003). In addition, in the NJ tree inferred from the sequences of mtDNA Cyt*b* region (695 bp) based on a molecular approach, we confirmed that

Table 1. Counts and measurements of *Eumicrotremus jindoensis*

	Present study n = 1	Type specimens* n = 2
Standard length (mm)	22.3	19.7～24.8
Counts		
First dorsal fin	VI	VI
Second dorsal fin	9	9
Anal fin	8	8
Pectoral fin	21	21
Vertebrae	26	21～24
Measurements (in % of SL)		
Head length	45.1	41.9～50.3
Body depth	40.8	42.3～46.2
Body width	43.9	37.1～38.7
Snout length	17.4	8.1～9.1
Orbital diameter	10.2	14.1～17.3
Interorbital width	20.1	21.4～24.4
Postorbital length	19.5	19.8～24.4
Upper jaw length	21.9	18.3～20.6
Gill slit length	9.1	–
Preanal fin length	77.3	78.1～79.4
Preanus length	67.4	58.0～58.3
Pectoral fin length	26.9	20.2
Inner disc length	13.2	11.3～17.3
Inner disc width	11.9	11.7～17.3
Disc length	21.5	22.6～32.0
Disc width	21.8	18.1～25.4
Length of first dorsal fin base	19.7	18.8～19.4
Length of second dorsal fin base	15.3	20.2～20.8
Length of anal fin base	21.0	11.7～21.3
Caudal fin length	20.4 + (damaged)	32.7～42.1
Upper peduncle length	10.9	8.1～13.7
Lower peduncle length	12.1	14.9～15.2
Caudal peduncle depth	11.5	11.7～14.9

*Lee *et al.* (2017)

Boryeong specimen was very well corresponded with type specimen of *E. jindoensis* with high bootstrap probabilities (100%). The seven dwarf species of *Eumicrotremus* species were also clearly separated by the molecular analysis (Fig. 4). Because we could not find any differences in mtDNA Cyt*b* sequences between our specimen and type specimens of *E. jindoensis*, which suggest that morphological differences between Boryeong and Jindo Island specimens may result from the intraspecific and geographical variation of a single species. Besides, *Eumicrotremus* species also has a considerable ranges in the snout length within lumpsucker species (*E. awae*, 8.4～17.8% and *E. uenoi*, 12.6～20.1% in, Lee *et al.*, 2017; *E. pacificus*, 9.7～18.9% in Voskoboinikova and Balanov, 2019). Interestingly, the length of snout is closely related to the diets and feeding behavior in marine fishes (Manning *et al.*, 2019). The shape of mouthpart could be determined by feeding conditions during ontogenetic development (Endo and Watanabe, 2020). Especially, Endo and Watanabe (2020) suggested that the divergence of mouth width within- and among-population could reflect the habitat adaptation to some specific environmental condition (e.g., grain size of the substrate) in benthic fish. Therefore, it would be good to reveal the relationship between snout and diets thorough analysis of the stomach composition for *Eumicrotremus* species in future study. In case of geographical variation, *E. asper-*

rimus have also the morphological variation such as first dorsal fin spine, pectoral fin rays, first- and second dorsal fin length between eastern and western North Pacific specimens (Kai *et al*., 2014).

In lumpsuckers (Cyclopteridae), the pelvic fins and girdle have been transformed into a pelvic disk that exerts forces stronger than those of any other fishes (Nachtigall, 1974). A transformation as single ventral sucker enables to survival in environments where wave surge by attaching to substrata in marine areas of high turbulence, and even to climb strong tidal currents (Nachtigall, 1974; Schoenfuss and Blob, 2003; Budney and Hall, 2010). When compared with the ventral disk of *Eumicrotremus* species, the presence or absence of papillae is considered as meaningful character for environmental condition. Papillae to increase friction in the cyclopterids and liparids might function to help prevent shear (Green and Barber, 1988). The ventral disk with papillae is thought to play an important role in increasing the adhesion to the substrate (Budney and Hall, 2010). The amount and size of papillae might strengthen the adhesion capability between body and substrate. In Korean *Eumicrotremus* species, only *E. jindoensis* with no papillae on the ventral disk might be adapted in different habitat condition (ex., water depth, temperature, substrate, tidal current etc.) from other *Eumicrotremus* species with papillae. Interestingly, *E. jindoensis* has a relatively shallow water depth (20∼30 m) including the western coast of Korea (off Boryeong and Jindo Island) but there is a difference in substrate composition. Boryeong coast only consists of sand substrate while Jindo Island coast consists of muddy sand substrate (Koh and Khim, 2014).

Interestingly, in snailfishes (Family Liparidae), the size of the ventral disk is an important taxonomic characters within their genus (Chernova, 2008). They mentioned that the size of the ventral disk and number of vertebrae is related to the depth of habitation (Song *et al*., 2015). Species living in deeper water might have reduced the functional role of ventral disk than those living in shallow water by more stable environmental condition such as a weak ocean current flow. While species living in shallow water has fewer number of vertebrate in than deeper species, it may be influenced by faster water flow velocity. For these reason, they have inferred intergeneric variation on the size of ventral disk and the number of vertebrae (Song *et al*., 2015).

The number of vertebrae may be related to the strength of tidal current. When comparing the maximum tidal current strength of offshores between Boryeong and Jindo Island, the strength of Boryeong (66.3 cm/s in KHOA, 2020) is interestingly weaker than that of Jindo Island (88.6 cm/s in Choo and Kim, 2013). For this reason, *E. jindoensis* in Boryeong might have relatively more vertebrae than those of Jindo Island. Differentiation in colonization of novel environments may have facilitated ecological speciation along their environmental differences (e.g., substrate, tidal current, temperature, prey, etc.). From the results obtained herein, our finding revealed that *E. jindoensis* occurs more widely distribution range from southern coast of western Korea (Jindo Island; Lee *et al*., 2017) to the central coast of western Korea (Boryeong; present study). *E. uenoi* also previously misidentified as *L. awae*, is known from the southern sea of Korea [Busan, Tongyeong (Lee *et al*., 2017) and Jeju Island (Kim, 2015)], the other congeneric species (*E. asperrimus*, *E. pacificus*, and *E. taranetzi*) from the eastern sea of Korea, but *E. jindoensis* from the central coast to southern coast of western Korea. In the future, it is necessary to collect more specimens of *E. jindoensis* and other *Eumicrotrimus* species, and reveal their geographical distribution, morphological variation, and speciation relationship.

ACKNOWLEDGEMENTS

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한국 서해 보령에서 채집된 긴꼬리엄지도치 (*Eumicrotremus jindoensis*)의 지역 확장 및 지리적 변이에 관한 새로운 발견

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요 약 : 우리나라 진도에서 채집된 도치과(Cyclopteridae)의 긴꼬리엄지도치(*Eumicrotremus jindoensis*)는 2017년에 신종 보고 이후 추가적으로 서해 보령에서 1개체(22.3 mm SL)가 연안개량안강망으로 채집되었다. 그러나, 본 개체는 모식표본보다 더 많은 척추골수(26 vs. 21~24), 긴 주둥이길이(17.4% vs. 8.1~9.1%), 긴 항문장(67.5% vs. 58.0~58.3%), 그리고 짧은 제2등지느러미 앞 길이(15.3% vs. 20.2~20.8%)를 가지는 점에서 약간의 형태적 차이를 나타냈다. 미토콘드리아 DNA COI 영역과 Cyt*b* 영역의 염기서열을 비교했을 때, 본 개체는 모식표본과 유전적인 차이는 발견할 수 없었다. 따라서 이러한 형태적인 차이는 우리나라 진도와 보령 해역의 서로 다른 서식지 및 환경 조건에 따른 지리적 변이로 추정된다. 우리나라 도치과 어류 중 엄지도치(*E. uenoi*)는 우리나라 남해역(부산, 통영 및 제주도)에서만 발견되고 있으며, 동 속의 우릉성치(*E. asperrimus*), 골린어(*E. pacificus*) 그리고 도치(*E. taranetzi*)는 모두 동해역에서만 서식하기 때문에, 긴꼬리엄지도치만이 우리나라 서해의 중부 및 남부해역에서 발견되는 점에서 매우 흥미롭다.

찾아보기 낱말 : 긴꼬리엄지도치, 분포, 형태, 변이, 한국