

Four newly reported ophichthid leptocephali species revealed by mitochondrial 12S rDNA, with implications of their occurrence in Korea

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Four species of ophichthid leptocephali were identified using 12S rDNA sequences, and their morphological descriptions were first provided based on six individuals (S1–S3, M1, and E1–E2) collected from the East Sea and the Korea Strait between September 2008 and October 2010. Mitochondrial 12S rDNA 859–861 base pairs of ophichthid leptocephali were compared with those of 16 ophichthids adult and 2 outgroups (*Anguilla japonica* and *Conger myriaster*). Leptocephali of S1 and E1 were very closely clustered with adult of *Scolecenchelys borealis* ($D = 0.002$) and *Echelus uropterus* ($D = 0.000$), respectively. However, leptocephali of S2–S3 and M1 were slightly far clustered with leptocephalus of S1 ($D = 0.006$) and adult of *Muraenichthys gymnopterus* (0.034), respectively. We believe that S1 and E1 are *S. borealis* and *E. uropterus*, respectively, in which the former is unrecorded species in Korea. However, S2–S3 and M1 may be undescribed species belonging to genus *Scolecenchelys* and *Muraenichthys*, respectively, because total numbers of myomeres for S2–S3 (148–158) and M1 (151) were not consistent with total numbers of vertebrae or distribution for any adult of *Scolecenchelys* spp. and *Muraenichthys* spp. in the world. We propose the new Korean name ‘Dong-hae-mul-baem’ for *S. borealis*.

Keywords: *Scolecenchelys borealis*; *Scolecenchelys* sp.; *Muraenichthys* sp.; *Echelus uropterus*; leptocephalus; 12S rDNA; undescribed species; Ophichthidae

Introduction

Leptocephalus of the family Ophichthidae shows the most striking features: its deeply compressed, transparent body, fang-like teeth, well-developed eyes, and large size of 50–100 mm in total length (Tabeta and Mochioka 1988). The families Anguillidae, Muraenidae, Synphobranchidae, Congridae, Elopidae, Megalopidae, and Albulidae also have leptocephalus during larval stage. Although the morphologies of many of those in the Atlantic Ocean have been documented (Leiby 1982, 1984), most have not been identified to the species level due to morphological similarities and/or deficiency of morphological information. The Japanese ophichthid leptocephali have been identified to the subfamily level (i.e., Ophichthinae or Myrophinae) based on morphological characters (Tabeta and Mochioka 1988). The Korean ophichthid leptocephali were also identified to the subfamily level, in which seven types were reported from Jeju Island (Kim et al. 2004). However, Richardson and Cowen (2004) identified many kinds of ophichthid leptocephali to the species level using the following morphological combinations: distribution of melanophores, shape of head, and numbers of myomeres and gut swellings, in which

the number of myomeres is regarded as the most important taxonomic character, although the ranges of this character in different species overlap (Michael and Obenchain 1978; Tabeta and Mochioka 1988). Notwithstanding, larval identification using morphological method can have much uncertainty because of morphological similarities and ontogenetic change during larval stage (Leiby 1984; Taylor and Watson 2004). Therefore, in recent years, studies on larval identification using molecular method have been abruptly increased (Paine et al. 2008; Kwun et al. 2010), but only few studies on leptocephalus were conducted (Ji and Kim 2010; Ji et al. 2011).

The family Ophichthidae, comprising 52 genera and about 260 species, has been recorded worldwide (McCosker 2010), but only 23 genera and 59 species are known in East Asia (Hatooka 2002; Tang and Zhang 2004), 9 genera and 18 species in Taiwan (Shao and Chen 1982), 19 genera and 38 species in Japan (Hatooka 2002), and 6 genera and 11 species in Korea (Ji and Kim 2011a, 2011b). Ophichthid fishes distribute from temperate to tropical area, mainly in tropical area (McCosker 1977; McCosker 2010). During the fisheries resources investigation around the Dokdo between

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2005 and 2010, four ophichthids leptocephali species have been found since 2008, of which two are revealed as undescribed species and one as unrecorded species. We herein provide their molecular identification results and morphological descriptions.

Materials and methods

Sample collection

Two individuals of ophichthid leptocephali (S1, 59.2 mm TL; E1, 47.4 mm TL) were collected from the Dokdo (St. A, 37°14'N; 131°51'E) in the East Sea and one individual of ophichthid leptocephalus (E2, 19.6 mm TL) from the Korea Strait (St. D, 32°22'N; 128°43'E) between September and November 2008, using a Bongo net with 0.33 mm mesh. Also, two individuals of ophichthid leptocephali (S2 and S3, 22.2 mm and 27.2 mm TL, respectively) from the East Sea (St. B, 35°49'N; 129°49'E) and one individual of leptocephalus (M1 52.4 mm TL) were collected from the East Sea (St. C, 35°25'N; 129°45'E) in October 2010, using a RD80 net with 0.33 mm mesh (Figure 1). All leptocephali samples were preserved in 99% EtOH, except E2, which was preserved in 5% formalin.

DNA extraction, PCR, and sequencing

Genomic DNA was extracted from the right eyeballs of leptocephali of S1–S3, M1, and E1 and from the muscle tissue of an adult *Scolecenchelys borealis*, *Muraenichthys gymnopterus*, and *Echelus uropterus* using forceps, and was placed in a 0.2 mL PCR tube containing 150 µL of 10% Chelex 100 resin (Bio-Rad, USA) in deionized water. The mitochondrial 12S rDNA sequence was amplified using primers 12S-F (5'-CAAAGGCCTGGTCCTGACTTTAA-3') and 12S-R (5'-CCTTCCGGTACACTTACCATGTTA-3') (Ji and Kim 2010), which were later used for sequencing. The PCR was performed in a 50 µL reaction consisting of 5 µL of 10 × PCR buffer, 5 µL each of the primer (10 µM), 4 µL of each dNTP (2 mM), 5 µL of genomic DNA, and 0.25 µL of FX *Taq* polymerase, and 25.75 µL sterile distilled H₂O. The PCR conditions consisted of an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 56.5°C for 30 s, and extension at 72°C for 1 min, and a final extension at 72°C for 7 min. The PCR products were purified with ExoSAP-IT (USB, USA) and directly sequenced with the ABI PRISM BigDye™ Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, USA). The nucleotide sequence data reported here

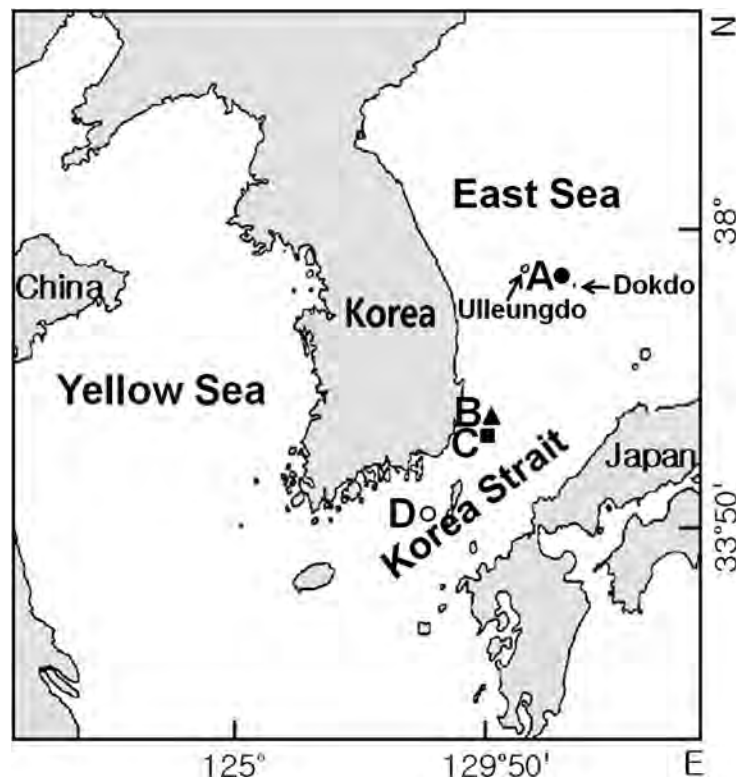


Figure 1. Map showing the sampling areas for ophichthid leptocephali. (A) S1 and E1 were collected near the Dokdo, the East Sea, in November 2008; (B) S2 and S3 were collected from the East Sea in October 2010; (C) M1 was collected from the East Sea in October 2010; (D) E2 was collected from the Korea Strait in September 2008.

appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers as follows: adults of Ophichthidae, *Pisodonophis sanguensis* (HQ185619), *E. uropterus* (HQ185623), *Ophichthus asakusae* (HQ185624), *M. gymnopterus* (HQ185625), *S. borealis* (HQ185626), and adults of Anguillidae and Congridae, *Anguilla japonica* (HQ185628), *Conger myriaster* (HQ185627); leptocephali of Ophichthidae, S1 (JQ178220), S2 (JQ178221), S3 (JQ231225), M1 (JQ178222), E1 (JQ178218).

DNA analysis

The DNA sequences were aligned and edited with Clustal W (Thompson et al. 1994) using BioEdit version 7.0.0 (Hall 1999). The genetic divergences were calculated with the Kimura two-parameter model using Mega version 4 (Tamura et al. 2007). The neighbor-joining (NJ) tree was constructed using the Kimura-2-parameter model (Kimura 1980), where its confidence was assessed via 1000 bootstrap replications. The NJ tree shows the genetic relationships among 21 ophichthid species (5 leptocephali and 16 adults) and 2 outgroups (*A. japonica* and *C. myriaster*).

Morphological analysis

Leptocephali of S1–S3, E1–E2, and M1 were identified based on the criteria of Tabeta and Mochioka (1988). Each body part was measured with digital vernier calipers to the nearest 0.01 mm. Counts and measurements followed those of Tabeta and Mochioka (1988). Measurements were made under a research zoom stereomicroscope (Olympus SZX-16, Japan). The specimens collected in this study are deposited at the Pukyong National University. The taxonomic system followed Michael and Obenchain (1978) and Tabeta and Mochioka (1988).

Results and discussion

Scolecenchelys borealis (Machida and Shioyaki 1990)

(New Korean name: Dong-hae-mul-baem)

(Figures 3A and 4A–C; Table 2)

Muraenichthys borealis Machida and Shioyaki, 1990: 1 (type locality: Mutsu Bay, Japan); Machida and Ohta, 1996: 79 (Japan); Hatooka, 2002: 216 (Japan).

Muraenichthys okamurai Machida and Ohta, 1996: 79 (Mie Prefecture, Japan); Hatooka, 2002: 216 (Japan).

Scolecenchelys okamurai Castle and McCosker, 1999: 121 (revision).

Scolecenchelys borealis Castle and McCosker, 1999: 121 (revision); Hoshino et al., 2011: 184 (synonym).

Materials examined. PKU5997 (S1), one specimen, 59.2 mm TL, Dokdo, East Sea, bongo net, 7 Nov. 2008.

Diagnosis. Total number of myomeres 135; the number of gut swelling 10; the number of supraorbital pores 3; the origin of dorsal fin located a little in front of anus.

Morphological description. The counts and measurements of leptocephalus of S1 (59.2 mm TL) are presented in Table 2. The body was transparent, compressed, and relatively elongated. The head was small and had fang-like teeth on both jaws. The anus was located somewhat posteriorly (Figure 3A). The origin of dorsal fin located a little in front of anus. The pectoral and caudal fins were present (Figure 4A,C). Two nostrils were present in front of eyes and three supraorbital pores were present near eye (Figure 4A). The anterior end of both jaws coincided well. The body depth was somewhat high at the back of head and slightly lower in front of the caudal fin. The melanophores were distributed on the 10 gut swellings, on both jaws and on partial anal fin and caudal fin base.

Molecular identification. Kimura's genetic distances were smallest between leptocephalus of S1 and *S. borealis* (0.002), with S2–S3 (0.006), and finally with *Scolecenchelys breviceps* (0.011) (Table 1). On the NJ tree, S1 was very closely clustered with *S. borealis*, and then with S2–S3; and this cluster was grouped into a clade with *S. breviceps*, showing a high bootstrap value (99) (Figure 2). S1 was thus believed to be a leptocephalus of *S. borealis*, with the closest genetic distance among the individuals surveyed.

Distribution. East Sea of Korea (present study), Japan (Hatooka 2002).

Remarks. Among leptocephali species reported from the world, the S1 (leptocephalus of *S. borealis*) is most similar to the leptocephalus of *Scolecenchelys gymnota* (sensu Castle and McCosker 1999) in their total numbers of myomeres, the location of the vertical vessels, and the distribution of the melanophores (Tabeta and Mochioka 1988; Table 2, Figure 3). Although our study could not compare 12S rDNA of S1 with that of adult of *S. gymnota*, our molecular results strongly support that S1 is leptocephalus of *S. borealis*. The other reason is because

Table 2. Comparison of the measurements and counts for leptocephali of S1–S3 and *Scolecenchelys gymnota*.

	S1 (present study)	S2 (present study)	S3 (present study)	<i>Scolecenchelys gymnota</i> (Tabeta and Mochioka 1988)
Measurements (mm)				
Total length	59.2	22.2	27.2	60.5
In % of total length				
Head length	7.4	9.5	8.1	–
Predorsal length	53.5	–	–	–
Preanal length	57.7	68.0	65.4	–
Body depth	10.1	7.2	7.7	–
In % of head length				
Eye diameters	11.4	19.0	18.2	–
Snout length	25.0	42.9	45.5	–
Counts				
TM	135	158	148	131–140
PDM	64	–	–	–
PAM	69	74	73	66–70
VBV 1 st	16	9	11	16–20
VBV last	63	69	69	58–65
PAP	–	8	–	–
Nostril	2	2	2	2
Gut swelling	10	10	10	9

TM, total number of myomeres; PDM, predorsal myomeres; PAM, preanal myomeres; PAP, postanal pigment; VBV 1st, first vertical blood vessel; VBV last, last vertical blood vessel.

S. gymnota inhabit only in the Pacific coast of southern Japan (Hatooka 2002); however, *S. borealis* inhabit in the East Sea (Aomori Pref. and Wakasa Bay) (Hatooka 2002). This species was revealed to be the unrecorded species in Korea. Therefore, we propose the new Korean name ‘Dong-hae-mul-baem’ for *S. borealis*.

Scolecenchelys sp.

(Figures 3B and 4D–F; Table 2)

Materials examined. PKU5999–6000 (S2–S3), two specimens, 22.2 and 27.2 mm TL, East Sea, RN80 net, 28 Oct. 2010.

Diagnosis. Total number of myomeres 148–158; the number of gut swelling, 10; the number of horizontal deep pigmented bands, 8, behind the anus and along the notochord.

Morphological description. The counts and measurements of leptocephali of S2–S3 (22.2 and 27.2 mm TL) are presented in Table 2. The anus was located somewhat posteriorly (Figure 3B). Two nostrils were present

in front of eyes (Figure 4D). Lower jaw was longer than upper jaw. The melanophores were distributed on the 10 gut swellings. The eight horizontal deep pigmented bands were present behind the anus and along the notochord.

Molecular identification. Kimura’s genetic distances were smallest between leptocephali of S2–S3 and S1 (0.006) or *S. borealis* (0.006), and then *S. breviceps* (0.014) (Table 1). On the NJ tree, S2–S3 were slightly closely clustered with S1 or *S. borealis*, and this cluster was grouped into a clade with *S. breviceps*, showing a high bootstrap value (97) (Figure 2). Despite the close genetic distance between S2–S3 and S1 (0.006), we identified S2–S3 as *Scolecenchelys* sp. because they showed additional molecular differences in mtDNA cytochrome c oxidase subunit I (data not shown) and also distinctive morphological characteristics, differing from those of S1.

Distribution. East Sea of Korea (present study).

Remarks. The S2–S3 (leptocephali of *Scolecenchelys* sp.) differed to those of *S. gymnota* and *Myrophinae* sp. in total myomeres (148–158 in *Scolecenchelys* sp. vs. 131–140 in *S. gymnota* vs. 125–146 in *Myrophinae* sp.), preanal myomeres (73–74 in *Scolecenchelys* sp. vs. 66–70 in *S. gymnota* vs. 46–62 in *Myrophinae* sp.), and location of last vertical blood vessel (69 in *Scolecenchelys* sp. vs. 58–65 in *S. gymnota* vs. 48–63 in *Myrophinae* sp.) (Tabeta and Mochioka 1988). The total numbers of myomeres of S2–S3 (148–158) lies within the range of the number of vertebrae in *M. gymnopterus* (155–156) (Ji and Kim 2011a), but their genetic distance was considerably large (0.086). The S2 and S3 are most similar to *Scolecenchelys australis* and *Scolecenchelys chilensis* in their total numbers of myomeres (148–158 in S2–S3 vs. 152 in *S. australis* vs. 146–159 in *S. chilensis*) (McCosker 1970, 1977), but their distribution areas are different (northeast Asia in S2–S3 vs. southwest Pacific in *S. australis* vs. southeast Pacific in *S. chilensis*). The number of vertebrae of the remaining 10 *Scolecenchelys* species differed from the number of myomeres of S2–S3 (148–158 in S2–S3; 131–137 in *S. borealis*; 130 in *Scolecenchelys cookei*; 137 in *Scolecenchelys japonica*; 127–132 in *Scolecenchelys macroptera*; 161–167 in *S. breviceps*; 129–135 in *S. gymnota*; 162 in *Scolecenchelys profundorum*; 180–186 in *Scolecenchelys castlei*; 126–139 in *Scolecenchelys laticaudata*; 139–144 in *Scolecenchelys xorae*) (McCosker 1977; McCosker and Castle 1988; McCosker and Parin 1995; Hatooka 2002; McCosker 2006). Accordingly, the S2–S3 may be undescribed species, but further research, such as comparing adult specimens of the same species,

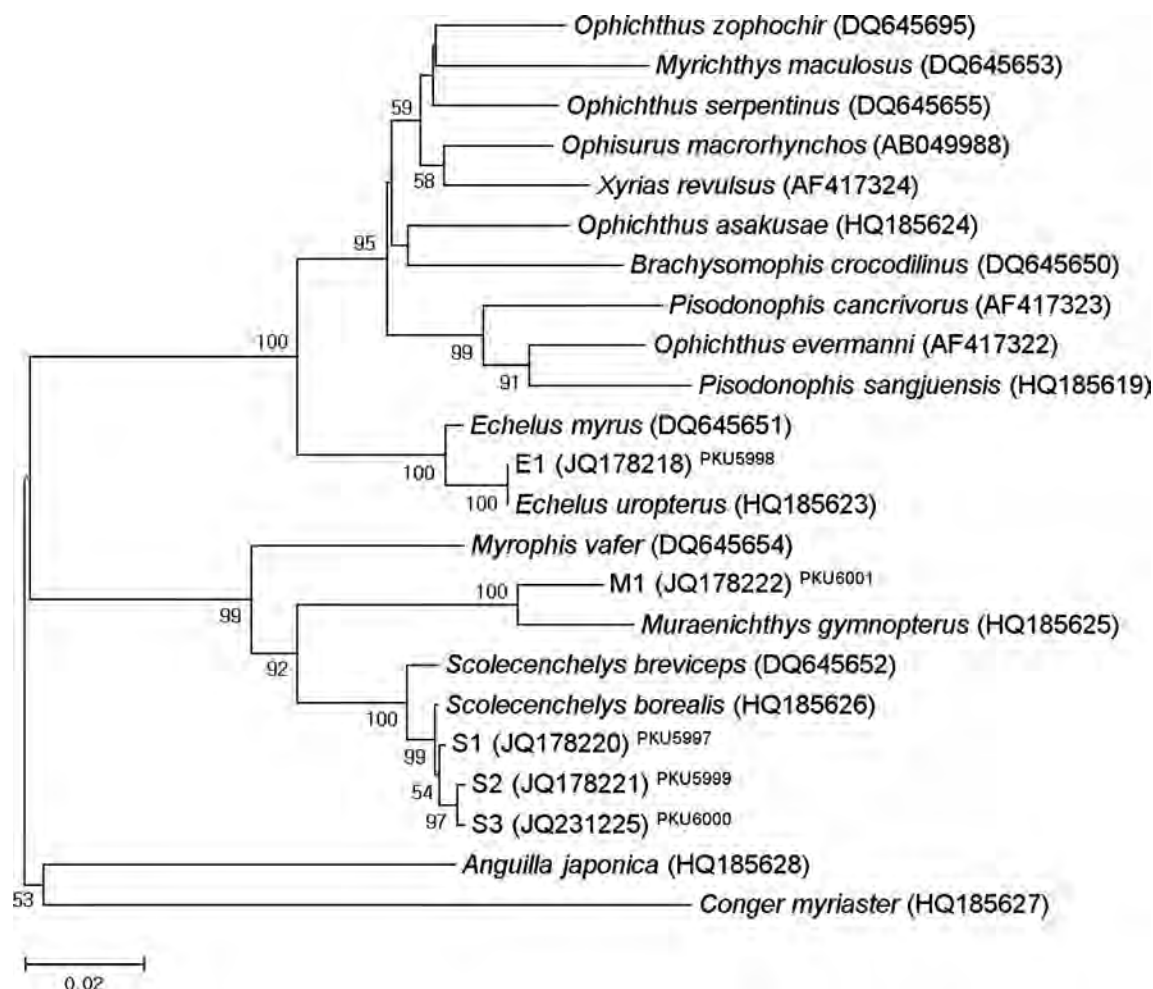


Figure 2. Neighbor-joining tree based on mtDNA 12S rDNA sequences showing the relationships between five leptocephali specimens collected in the present study and other ophichthid members. The numbers on branches are bootstrap values ($> 50\%$) obtained from 1000 replications. The bar indicates a genetic distance of 0.02. Superscripts indicate the registration number of voucher specimens.

is needed for clarifying their taxonomic status confidently.

Muraenichthys sp.

(Figures 3C and 4G–I; Table 3)

Materials examined. PKU6001 (M1), one specimen, 52.4 mm TL, East Sea, RN80 net, 31 Oct. 2010.

Diagnosis. Total number of myomeres, 151; the number of gut swelling, 4; the origin of dorsal fin located a little in front of anus; caudal fin present; melanophores were present on opercle and four gut swellings.

Morphological description. The counts and measurements of leptocephalus of M1 (52.4 mm TL) are presented in Table 3. The anus was located somewhat anteriorly than the middle of the body (Figure 3C). The

origin of dorsal fin located a little in front of anus. The pectoral and caudal fins were present (Figure 4G,I). Two nostrils were present in front of eyes (Figure 4G). The anterior end of both jaws coincided well. The melanophores were distributed on opercle and 4 gut swellings.

Molecular identification. Kimura's genetic distances were smallest between leptocephalus of M1 and *M. gymnopterus* (0.034) and then *S. borealis* (0.072) (Table 1). On the NJ tree, M1 was clustered with *M. gymnopterus*, showing a high bootstrap value (100) (Figure 2). These results suggest M1 belong to the genus *Muraenichthys*.

Distribution. East Sea of Korea (present study).

Remarks. Although the M1 (leptocephalus of *Muraenichthys* sp.) is similar to that of Myrophinae

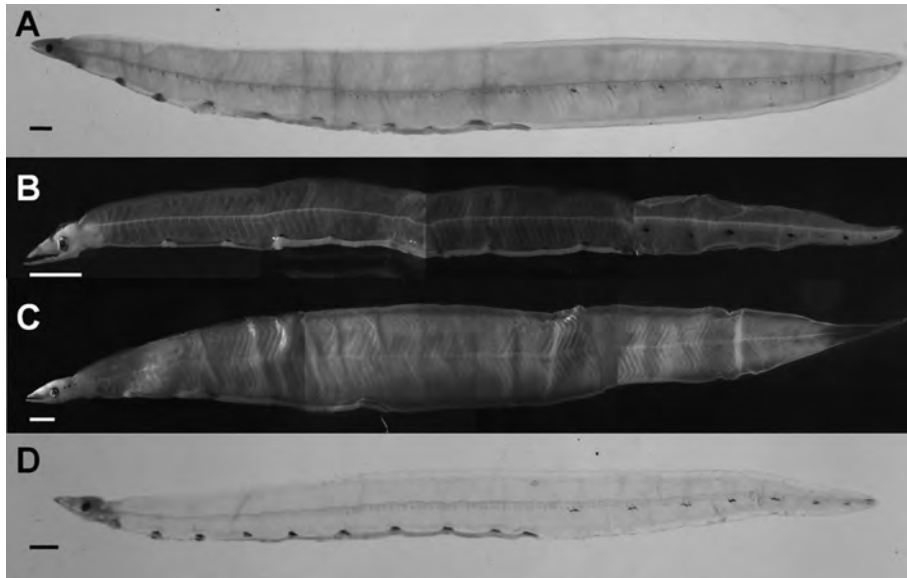


Figure 3. (A) Leptocephalus of *Scolecenchelys borealis* (S1), 59.2 mm TL. (B) Leptocephalus of *Scolecenchelys* sp. (S2), 22.2 mm TL. (C) Leptocephalus of *Muraenichthys* sp. (M1), 52.4 mm TL. (D) Leptocephalus of *Echelus uropterus* (E1), 47.4 mm TL. Scale bars = 1.5 mm.

sp. 1 (sensu Tabeta and Mochioka 1988), they are well differentiated in the following morphological characteristics: postanal number of pigment, the distribution of the melanophores, and the number of total and preanal myomeres (Table 3). The M1 was identified undescribed *Muraenichthys* sp. because the total number of myomeres of M1 (151) was not consistent with the number of vertebrae of the three *Muraenichthys* species from the northeast Asia (155–156 in *M. gymnopterus*; 122–132 in

Muraenichthys schultzei; 128–133 in *Muraenichthys thompsoni*) (McCosker 1977; Hatooka 2002; Ji and Kim 2011a). The remaining four *Muraenichthys* species are in question for the number of vertebrae. But, their distribution areas have been known to be different from each other (northeast Asia in M1 vs. Philippine or Indonesia, western central Pacific in *Muraenichthys elerae*, *Muraenichthys philippinensis*, *Muraenichthys macrostomus*, and *Muraenichthys sibogae*) (Schultz and

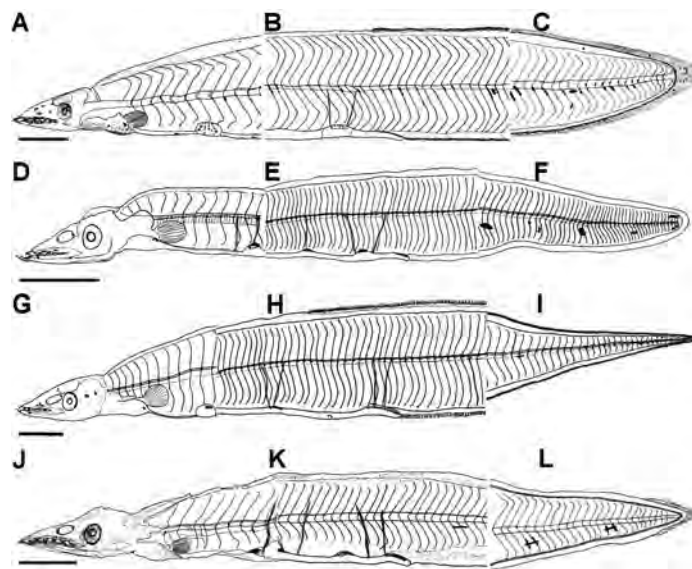


Figure 4. (A) Magnification of head part; (B) anal part; (C) caudal part for leptocephalus of *Scolecenchelys borealis* (S1), 59.2 mm TL; (D) magnification of head part; (E) anal part; (F) caudal part for leptocephalus of *Scolecenchelys* sp. (S2), 22.2 mm TL; (G) magnification of head part; (H) anal part; (I) caudal part for *Muraenichthys* sp. (M1), 52.4 mm TL; (J) magnification of head part; (K) anal part; (L) caudal part for *Echelus uropterus* (E1), 47.4 mm TL. Scale bars = 1.5 mm.

Table 3. Comparison of the measurements and counts for leptocephali of M1 and Myrophinae sp. 1.

	M1 (present study)	Myrophinae sp. 1 (Tabeta and Mochioka 1988)
Measurements (mm)		
Total length	52.4	42.0–65.0
In % of total length		
Head length	5.7	–
Predorsal length	32.9	–
Preanal length	41.7	–
Body depth	12.4	–
In % of head length		
Eye diameters	19.4	–
Snout length	48.5	–
Counts		
TM	151	137–146
PDM	37	38–43
PAM	51	46–48
PAP	5	–
VBV 1st	21	–
VBV last	48	48–51
Nostril	2	2
Gut swelling	4	4

TM, total number of myomeres; PDM, predorsal myomeres; PAM, preanal myomeres; PAP, postanal pigment; VBV 1st, first vertical blood vessel; VBV last, last vertical blood vessel.

Woods 1949; McAllister 1990). Accordingly, the *Muraenichthys* sp. may be undescribed species, but further research, such as comparing adult specimens of the same species, is needed for clarifying their taxonomic status confidently.

Echelus uropterus (Temminck and Schlegel 1846)

(Korean name: Nal-bung-jang-eo)

(Figures 3D and 4J–L; Table 4)

Conger uropterus Temminck and Schlegel, 1846: 261 (type locality: Nagasaki, Japan).

Myrophis uropterus Jordan and Snyder, 1901: 861 (Japan).

Echelus uropterus Jordan et al., 1913: 83 (Japan); Hatooka, 2002: 224 (Japan); Ji and Kim, 2011a: 48 (Review).

Materials examined. PKU5998 (E1), one specimen, 47.4 mm TL, Dokdo, East Sea, bongo net, 7 Nov. 2008. PKU6517 (E2), one specimen, 19.6 mm TL, Korea Strait, RN80 net, 29 Sep. 2008.

Table 4. Comparison of the measurements and counts for leptocephali of E1–E2, and Ophichthinae sp. 1–3.

	E1 (present study)	E2 (present study)	Ophichthinae sp. 1 (Tabeta and Mochioka 1988)	Ophichthinae sp. 2 (Tabeta and Mochioka 1988)	Ophichthinae sp. 3 (Tabeta and Mochioka 1988)
Measurements (mm)					
Total length	47.4	19.6	41.0–54.0	69.0–107.0	15.0–72.0
In % of total length					
Head length	9.0	6.2	–	–	–
Predorsal length	–	–	–	–	–
Preanal length	69.2	60.2	–	–	–
Body depth	8.5	8.6	–	–	–
In % of head length					
Eye diameters	18.7	14.3	–	–	–
Snout length	56.2	57.1	–	–	–
Counts					
TM	153	150	144–149	148–157	149–160
PDM	–	–	72–73	135–144	38–45
PAM	71	66	70–73	51–54	70–75
PAP	8	8	6	10	8
VBV 1st	10	9	–	–	–
VBV last	66	62	68–69	51–55	57–60
Nostril	2	2	2	2	2
Gut swelling	9	9	9	7	8

TM, total number of myomeres; PDM, predorsal myomeres; PAM, preanal myomeres; PAP, postanal pigment; VBV 1st, first vertical blood vessel; VBV last, last vertical blood vessel.

Diagnosis. Total number of myomeres, 150–153; the number of gut swelling, 9; caudal fin present; head was small and narrow; the number of horizontal deep pigmented bands, 8, behind the anus and along the notochord.

Morphological description. The counts and measurements of leptocephali of E1–E2 (19.6 and 47.4 mm TL) are presented in Table 4. The body was transparent, very compressed, and relatively elongated. The head was small and narrow. The anus was located somewhat posteriorly (Figure 3K). The dorsal fin was restricted but caudal fin was well-developed (Figure 4L). Two nostrils were present in front of eyes (Figure 4J). The anterior end of both jaws coincided well. The body depth was somewhat high at the back of head and slightly lower in front of the caudal fin. The melanophores were distributed on the nine gut swellings. The eight horizontal deep pigmented bands were present behind the anus and along the notochord.

Molecular identification. Kimura's genetic distances were smallest between leptocephalus of E1 and *E. uropterus* (0.000), and then *Echelus myrus* (0.013) (Table 1). On the NJ tree, E1 was clustered with *E. uropterus*, and then with *E. myrus*, showing a high bootstrap value (100) (Figure 2). E1 was identified as *E. uropterus*, at the closest in genetic distance observed among the individuals surveyed.

Distribution. South Sea and East Sea of Korea (present study, Ji and Kim 2011a), Japan (Hatooka 2002), Taiwan (Mok 1993), East Africa, the Society Island (Froese and Pauly 2011).

Remarks. Although E1 and E2 (leptocephali of *E. uropterus*) are most similar to Ophichthinae sp. 3 (sensu Tabeta and Mochioka 1988) in their numbers of total myomeres, the location of the vertical vessels, and the distribution of the melanophores (Table 4), the two species are different in the location of the last vertical vessel; Ophichthinae sp. 3 occurs at the 57–60th myomere, but *E. uropterus* occurs at the 62–66th myomere (Table 4). The total number of myomeres of E1–E2 (150–153) was clearly different from those of the remaining two species (Ophichthinae sp. 1 and Ophichthinae sp. 2; Tabeta and Mochioka 1988). Although the total number of myomeres in Ophichthinae sp. 2 falls within the range of leptocephali of E1 and E2, it has a different number of preanal myomeres (66–71 vs. 51–54, respectively; Tabeta and Mochioka 1988). The total numbers of myomeres of E1–E2 (150–153) are consistent with the number of vertebrae in three *Echelus* spp. (150–153 in *E. uropterus* vs. 149–155 in *E. myrus* vs. 149–157 in *Echelus pachyrhynchus*), but

they differed in their distribution (Indo-Pacific in *E. uropterus* vs. eastern Atlantic in *E. myrus* and *E. pachyrhynchus*) (Leiby 1990; McCosker 1977)

Implication of the occurrence of ophichthid leptocephali

This is the first recorded occurrence of the four ophichthids leptocephali in the East Sea. *S. borealis* is a hitherto unrecorded species in Korea, with a known distribution only in Japan (Aomori Pref., southward to Iwate Pref. and Wakasa Bay; Hatooka 2002). Therefore, our leptocephalus of S1 might have been transported to the Dokdo from the southern East Sea, being resulted from the diversity and complexity of sea current in the East Sea (Chang et al. 2002). According to the buoys experiment in the East Sea (Chang et al. 2002), buoys launched in Sokcho on 1 July 1999 went to northeast direction and changed the direction on 3 September 1999, and then went back to the southwest direction. It is assumed that the reason why the buoys went northward and went back down was influence of the Tsushima warm current and the North Korean cold current, respectively. The other species, *E. uropterus*, distributed in the Indo-Pacific Ocean, including Korea (Kim et al. 2005), Japan (Hatooka 2002), and East Africa to the Society Islands (Froese and Pauly 2011). The smaller leptocephalus of E2 (19.6 mm TL) was collected from the Korea Strait in 29 September 2008, and the larger leptocephalus of E1 (47.4 mm TL) was collected from the Dokdo in 7 November 2008 (about one month later). In the viewpoint of collection date, locality and size of leptocephali, the smaller leptocephalus of E2 might have been transported from the Korea Strait to the Dokdo by the warm Tsushima current during October.

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References

- Castle PHJ, McCosker JE. 1999. A new genus and two new species of Myrophine worm-eels, with comments on *Muraenichthys* and *Scolecenchelys* (Anguilliformes: Ophichthidae). Rec Aust Mus. 51:113–122.
- Chang KI, Kim YB, Suk MS, Byun SK. 2002. Hydrography around Dokdo. Ocean Polar Res. 24:369–389.
- Froese R, Pauly D. 2011. FishBase: World Wide Web electronic publication. [cited 2011 Jun]. Available from: <http://www.fishbase.org/>

- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucl Acids Symp Ser.* 41:95–98.
- Hatooka M. 2002. Ophichthidae. In: Nakabo T, editor *Fishes of Japan with pictorial keys to the species*. English ed. Tokyo: Tokai University Press. p. 215–225.
- Hoshino K, Hibino Y, Kimura S, Machida Y. 2011. The worm eel, *Muraenichthys okamurai* Machida and Ohta 1996, a junior synonym of *Muraenichthys borealis* Machida and Shiogaki 1990. *Ichthyol Res.* 58:184–187.
- Ji HS, Kim JK. 2010. Molecular and morphological identification of ophichthid leptocephali from the South Sea of Korea. *Korean J Ichthyol.* 22:279–284.
- Ji HS, Kim JK. 2011a. Taxonomic review of the snake-eels family Ophichthidae (Anguilliformes) from Korea. *Korean J Ichthyol.* 23:46–60.
- Ji HS, Kim JK. 2011b. A new species of snake eel, *Pisodonophis sanguensis* (Anguilliformes: Ophichthidae) from Korea. *Zootaxa.* 2758:57–68.
- Ji HS, Lee SJ, Kim JK. 2011. Molecular identification, ontogeny and evolutionary note of *Echelus uropterus* leptocephali. *Korean J Ichthyol.* 23:217–224.
- Jordan DS, Snyder JO. 1901. A review of the apodal fishes or eels of Japan, with descriptions of 19 new species. *Proc US Natl Mus.* 23:837–890.
- Jordan DS, Tanaka S, Snyder JO. 1913. A catalogue of the fishes of Japan. *J Coll. Sci., Imp. Univ. Tokyo* 33:1–497.
- Kim IS, Choi Y, Lee CL, Lee YJ, Kim BJ, Kim JH. 2005. Illustrated book of Korean fishes. Seoul: Kyohak-Publishing. p. 1–615.
- Kim BJ, Go YB, Lee SJ. 2004. Morphology of Ophichthidae leptocephalus in coast of Jeju Island. Paper presented at: KOFIS 2004. Proceedings of Biannual Meeting of the Aqua Society of Korea; Jeju, Korea.
- Kimura M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J Mol Evol.* 16:111–120.
- Kwon HJ, Kim YH, Kim JB, Jeong CH, Kim JK. 2010. One undescribed species, *Coilia* sp. from the Yellow Sea. *Anim Cells Syst.* 14:137–145.
- Leiby MM. 1982. Leptocephalus larvae of the tribe Sphagerbranchini (Pisces, Ophichthidae) in the western North Atlantic. *Bull Mar Sci.* 32:220–236.
- Leiby MM. 1984. Leptocephalus larvae of the tribe Callechelyini (Anguilliformes, Ophichthidae, Ophichthinae) in the western North Atlantic. *Bull Mar Sci.* 34:398–432.
- Leiby MM. 1984. In: Ophichthidae: Development and Relationships. Moser HG, Richard WJ, Lohen DM, Fahay MP, Kendall AW, Richard son SL, editors. *Optogeny and Systematics of fishes*. Lawrence: American Society of Ichthyologists and Herpetologists. p. 102–108.
- Leiby MM. 1990. Ophichthidae. In: Quero JC, Hureau JC, Karrer C, Post A, Saldanha L, editors. *Check-list of the fishes of the eastern tropical Atlantic (CLOFETA)*. Paris: UNESCO. p. 176–192.
- Machida Y, Shiogaki M. 1990. A new snake eel, *Muraenichthys borealis*, from Aomori, northern Japan. *Japanese J Ichthyol.* 37:1–5.
- Machida Y, Ohta S. 1996. Description of a new worm eel, *Muraenichthys okamurai*, from western Honshu, Japan (Ophichthidae: Myrophinae). *Mem Fac Sci Kochi Univ.* 16/17:77–81.
- McAllister DE. 1990. A working list of fishes of the world. Ottawa: Canadian Museum of Nature. p. 1–2661.
- McCosker JE. 1970. A review of the eel genera *Leptenchelys* and *Muraenichthys*, with the description of a new genus, *Schismorhynchus*, and a new species, *Muraenichthys chilensis*. *Pac Sci.* 24:506–516.
- McCosker JE. 1977. The osteology, classification, and relationships of the eel family Ophichthidae. *Proc Calif Acad Sci.* 41:1–123.
- McCosker JE. 2006. A new deepwater species of worm-eel, *Scolecenchelys castlei* (Anguilliformes: Ophichthidae), from New Zealand and Australia, with comments on *S. breviceps* and *S. macroptera*. *J Roy Soc N Z.* 36:17–26.
- McCosker JE. 2010. Deepwater Indo-Pacific species of the snake-eel genus *Ophichthus* (Anguilliformes: Ophichthidae), with the description of none new species. *Zootaxa.* 2505:1–139.
- McCosker JE, Castle PHJ. 1988. Ophichthidae. In: McCosker JE, Castle PHJ, editors. *Smiths' sea fishes*. Berlin: Springer-Verlag. p. 176–186.
- McCosker JE, Parin N. 1995. A new species of deepwater worm-eel, *Muraenichthys profundorum* (Anguilliformes: Ophichthidae), from the Nazca Ridge. *Japanese J Ichthyol.* 42:231–235.
- Michael P, Obenchain CL. 1978. Leptocephali of the ophichthid genera *Ahlia*, *Myrophis*, *Ophichthus*, *Pisodonophis*, *Callechelys*, *Letharchus*, and *Apterichtus* on the atlantic continental shelf of the United states. *Bull Mar Sci.* 28:442–486.
- Mok HK. 1993. Ophichthidae. In: Shen SC, editor. *Fishes of Taiwan*. Taipei: Depart Zool Natl Taiwan University. p. 110–114.
- Paine MA, McDowell JR, Graves JE. 2008. Specific identification using COI sequence analysis of scombrid larvae collected off the Kona coast of Hawaii Island. *Ichthyol Res.* 55:7–16.
- Richardson DE, Cowen RK. 2004. New leptocephalus types collected around the island of Barbados (West Indies). *Copeia.* 2004:888–895.
- Schultz LP, Woods LP. 1949. Key to the genera of echelid eels and the species of *Muraenichthys* of the Pacific, with two new species. *J Wash Acad Sci.* 39:169–174.
- Shao KT, Chen HM. 1982. Ophichthidae. In: Shen SC, editor. *Fishes of Taiwan*. Taipei: Department of Zoology, National Taiwan University. p. 110–114.
- Tabeta O, Mochioka N. 1988. Ophichthidae. In: Okiyama M, editor *An Atlas of the early stage fishes in Japan*. Tokyo: Tokai University Press. p. 58–62. (in Japanese)
- Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol.* 24:1596–1599.
- Tang WQ, Zhang CG. 2004. A taxonomic study on snake eel family Ophichthidae in China with the review of Ophichthidae (Pisces, Anguilliformes). *J Shanghai Fish Univ.* 13:16–22.
- Taylor CA, Watson W. 2004. Utility of larval pigmentation to identify nearshore rockfishes of the *Sebastes* subgenus *Pteropodus* from southern California. *CalCOFI Rep.* 45:113–117.
- Temminck CJ, Schlegel H. 1846. Pisces. In: *Fauna Japonica, sive descriptio animalium quae in itinere per Japoniam suscepto annis 1823-30 collegit, notis observationibus et adumbrationibus illustravit P F de Siebold. Lugduni Batavorum: Batavia. Parts 10–14:173–269.*
- Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucl Acids Res.* 22:4673–4680.