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Identification of *Trichiurus* (Pisces: Trichiuridae) Eggs and Larvae from Korea, with a Taxonomic Note

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Abstract

The hairtail (currently recognized as *Trichiurus lepturus* in Korea) is one of the most important commercial fish species in Korea, Japan, China, and Taiwan. Because the amount of catches has been steadily declining, we must determine the early life stages of the hairtail from the viewpoint of resource management. Furthermore, the taxonomic status of the hairtail is unclear among ichthyologists, potentially creating management difficulties. Therefore, the purpose of this study was to compare morphological and molecular information on eggs, larvae, and adults of hairtail from Korea with that of *T. lepturus* from the Atlantic Ocean, and to review the taxonomic status of the hairtail. A total of 510 base pairs of the mitochondrial DNA cytochrome oxidase subunit I sequences of 12 eggs, 2 larvae, and 11 adults of the hairtail from the Korean waters clearly matched those of *Trichiurus japonicus* adults (d = 0.000-0.014) from the East China Sea rather than those of *T. lepturus* (d = 0.100-0.110) from the Atlantic Ocean. Our results also showed that larvae of the Korean hairtail are different than those in the Atlantic Ocean in having no melanophores along the ventral edge of the lower jaw. Therefore, our findings suggest that the hairtail in the Korean waters may not be *T. lepturus*, but *T. japonicus*.

Keywords: *Trichiurus japonicus, Trichiurus lepturus*, Egg, Larvae, MtDNA COI, Korea

Introduction

The hairtail (currently recognized as *Trichiurus lepturus* in Korea) is a widely distributed fish in temperate and tropical seas, and one of the most important commercial fish species in Korea, Japan, China, and Taiwan (Yamada, 1964; Park et al., 1998). In recent years, the number of hairtail catches in Korea has been steadily declining with overfishing, loss of fishing grounds, and/or climate change (Kim et al., 2011b). Therefore, appropriate resource management should urgently be applied to prevent population decline. However, the taxonomic uncertainty of the *Trichiurus* species complex (Chakraborty et al., 2006a, 2006b) has caused great confusion in hairtail management.

Species in the genus *Trichiurus* are easily confused because they have similar body shape and coloration (Chakraborty et al., 2006a). Since the original description of *T. lepturus* (Linnaeus, 1758) (type locality: America and China), many new species in the genus have been reported. However, most have been named synonyms of *T. lepturus* (Tucker, 1956; Nakamura and Parin, 1993). More recently, Burhanuddin et al. (2002) suggested that not all individuals classified as *T. lepturus* are of the same species because counts of different characters (e.g., dorsal fin rays) have been found to differ among four syntypes collected from America and China. Some ichthyologists, using morphological and molecular methods, have also suggested that along with *T. lepturus*, different *Trichiurus* species such as *Trichiurus* sp. 2 (sensu Nakabo, 2002) and *Trichiurus japonicus* exist in the northwestern Pacific (Nakabo, 2002; Hsu et al., 2009; Tzeng and Chiu, 2012). Ya-

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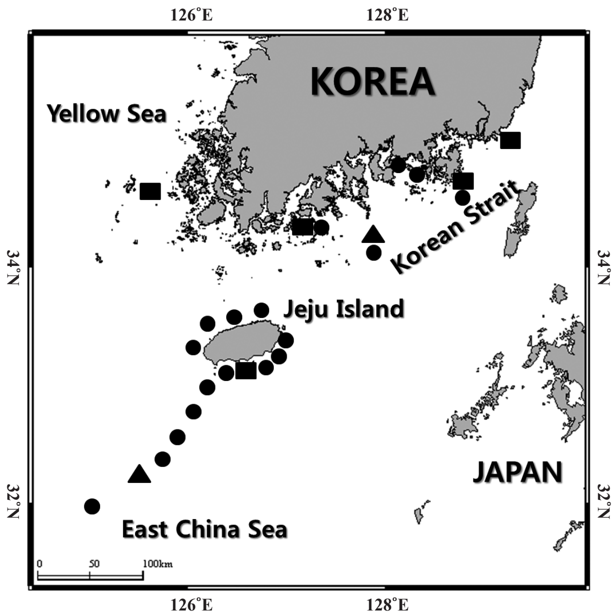

Fig. 1. Sampling area of eggs, larvae and adults of the hairtail from Korea (circles: eggs; triangles: larvae; squares: adults).

mada et al. (1995) reported that *Trichiurus* specimens from Ryukyu Island and the East China Sea have a yellowish dorsal fin and a light oral cavity. Subsequently, Nakabo (2002) classified specimens of Yamada et al. (1995) as *Trichiurus* sp. 2, and *T. japonicus* (Temminck and Schlegel, 1844) (type locality Nagasaki, Japan) was considered a synonym of *T. lepturus* by Nakamura and Parin (1993). However, some ichthyologists have recently treated *T. japonicus* as a valid species (Nakabo, 2002; Chakraborty et al., 2006a, 2006b; Hsu et al., 2009; Zemnukhov and Epur, 2011; Tzeng and Chiu, 2012).

In Korea, the hairtail was first reported by Mori (1928) as *T. japonicus*, but Chyung (1977) amended the species to be *T. lepturus*; thereafter, Korean ichthyologists have used *T. lepturus* and no taxonomic review of this species has been performed (Kim et al., 2005; Kim, 2011). Therefore, the taxonomic status of the hairtail in Korea must be clarified using morphological and molecular methods. Here we compared the mitochondrial DNA (mtDNA) cytochrome oxidase subunit I (COI) sequences of hairtail eggs, larvae, and adults from Korea with those of two *Trichiurus* species (sensu Ribeiro et al., 2012; Tzeng and Chiu, 2012), and compared morphological traits of Korean hairtail larvae with those of *T. lepturus* larvae (sensu Richards, 2006).

Materials and Methods

Sampling

Hairtail eggs and larvae were collected mainly on Jeju Island, including the Korean Strait, between 2009 and 2012, using an ichthyoplankton net (mouth opening diameter of 0.8 m, mesh size of 330 μm) (Fig. 1). Samples were immediately preserved in 99% EtOH and RCL2 solution (RCL2-CS 1000; Alphelys, Plasir, France).

Morphological identification

Measurements followed the methods of Okiyama (1988) and terminology followed those of Russell (1976). Each body part was measured to the nearest 0.1 mm using the Image program (Active measure program, Korea). Morphological identification followed Okiyama (1988), Richards (2006), and Kim et al. (2011a). We used a camera (Moticam Pro 205A;

Table 1. List of specimens of *Trichiurus* species including outgroup used for molecular analyses in the present study.

Sample	Locality	Accession No.	Voucher No.
All *Trichiurus* eggs	Jeju Island	KF788271-KF788282	PKUI 97-PKUI 108
Trichiurus larva	Jeju Island	KF788269	PKUI 92
Trichiurus larva	Southern sea of Korea	KF788270	PKUI 93
Trichiurus adult	Tongyeong	KF788258	PKU 923
Trichiurus adult	Deukryangman	KF788260	PKU 2556
Trichiurus adult	Deukryangman	KF788261	PKU 2558
Trichiurus adult	Deukryangman	KF788262	PKU 2559
Trichiurus adult	Jeju Island	KF788264-KF788268	PKU 8976-PKU 8980
Trichiurus adult	Heuksando	KF788263	PKU 7694
Trichiurus adult	Busan	KF788264	PKU 1483
Trichiurus japonicus	Japan, China, Hong Kong, Taiwan, Vietnam	JN990867-JN990871	TJB371, 371, 374, 377, 379
Trichiurus lepturus	Brazil	GU702467	LBP-35029
Trichiurus lepturus	Brazil	JX124916	LBPV48587
Trichiurus sp. 2	Taiwan	FM998058.1	-
Trichiurus sp. 2	Taiwan	FM998059.1	-
Scomber japonicus	-	JQ354333	WTU:UW048864

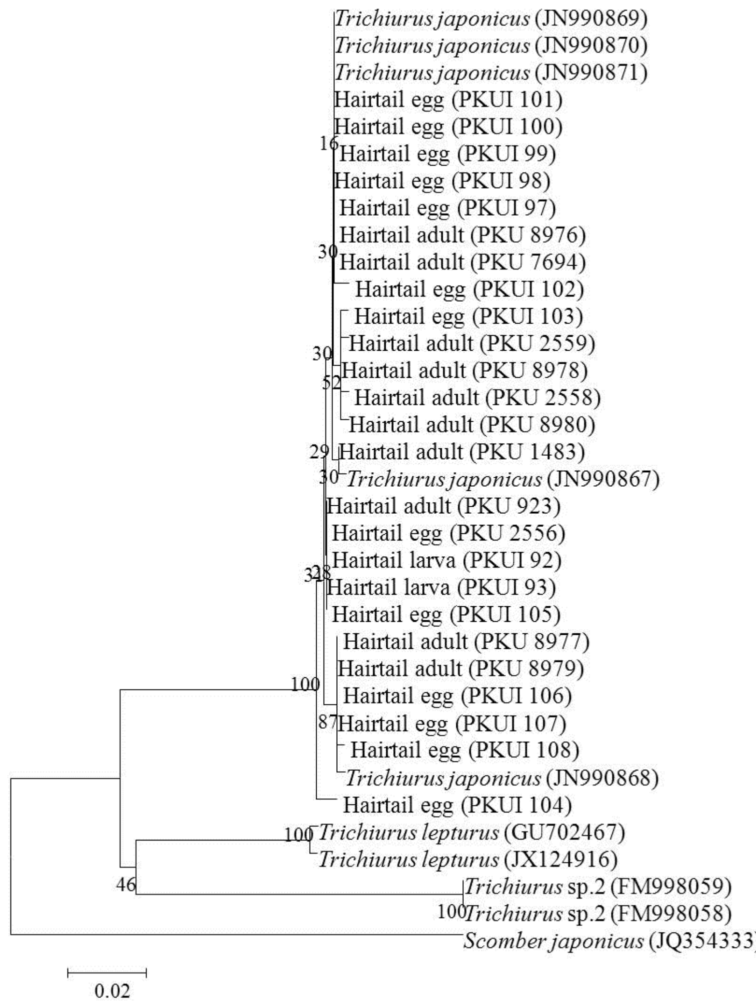

Fig. 2. Neighbor joining tree based on partial mitochondrial DNA cytochrome oxidase subunit I sequences, showing the relationships among eggs, larvae and adults of hairtail from Korea, and three *Trichiurus* species with one outgroup (*Scomber japonicus*). The tree was constructed using the Kimura-2 parameter model (Kimura, 1980) and 1,000 bootstrap replications. The bar indicates a genetic distance of 0.02.

Motic, Xiamen, China) attached to a stereomicroscope (SZX-16; Olympus, Tokyo, Japan) to take photographs of eggs and larvae. Hairtail adults were collected on Jeju Island and the Korean Strait between 2008 and 2013, using a long-line and set-net method. Measurements of adults followed the methods of Nakabo (2002). Adult specimens were deposited at the Pukyong National University (PKU), and eggs and larvae were deposited at the Ichthyoplankton Laboratory of PKU (PKUI).

Molecular identification

Genomic DNA was extracted from 12 eggs according to a method of modified Aranishi (2006). Genomic DNA was also extracted from eyeballs of two hairtail larvae, and from muscle tissues of 11 hairtail adults using 10% Chelex 100 resin (Table 1). A polymerase chain reaction (PCR) was used to amplify the mtDNA COI region using previously designed primers (Ivanova et al., 2007). The PCR conditions were as follows: initial denaturation at 94°C for 2 min; 34 cycles of denaturation at 94°C for 45 s, annealing at 53°C for 1 min, and extension at 72°C for 1 min 40 s; and a final extension at 72°C for 7 min. The DNA was sequenced using an ABI 3730XL sequencer (Applied Biosystems, Foster City, CA, USA) and the ABI PRISM BigDye Terminator v 3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems). For comparison, we obtained mtDNA COI sequences of *Trichiurus* species and one outgroup (*Somber japonicus*) from the National Center for Biological Information (NCBI) database (Table 1). The mtDNA COI sequences were aligned using BioEdit version 7 (Hall, 1999). Genetic distances were calculated with the Kimura 2-parameter model (Kimura, 1980) using MEGA version 5 software (Tamura et al., 2011). A neighbor joining tree was constructed using MEGA, and its confidence was assessed via 1,000 bootstrap replications.

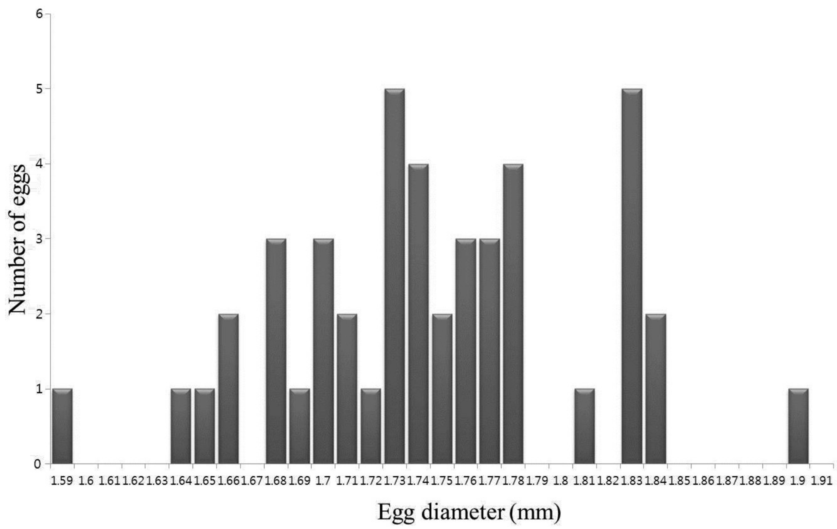

Fig. 3. Egg diameter frequency of the hairtail from Korea.

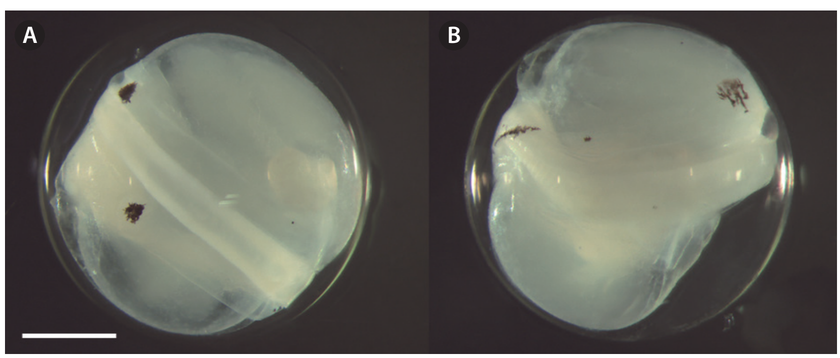

Fig. 4. Egg development of the hairtail from Korea. (A) Middle formation stage of embryo, stellate shaped melanophores were diagonally distributed on the fin folds, 1.74 mm ED. (B) Fully formation stage of embryo, stellate shaped melanophores were changed to branch shaped melanophores, 1.79 mm ED. Scale bar = 0.5 mm.

Results and Discussion

Molecular identification of eggs and larvae

Based on morphological characters, 291 eggs, 3 larvae, and 11 adults of the hairtail were identified as belonging to the genus *Trichiurus*. In total, 510 base pairs of the mtDNA COI sequence were successfully obtained from 12 eggs, 2 larvae and 11 adults. The genetic distances (d) between eggs and larvae ranged from 0.000 to 0.008, indicating that they belonged to the same species. The mtDNA COI sequences of eggs and larvae were consistent with those of hairtail adults from Korea (d = 0.000-0.014). All eggs, larvae, and adults showed sequences identical to those of *T. japonicus* (d = 0.000-0.014), but very different from those of *T. lepturus* (d = 0.100-0.110). Therefore, our molecular results suggest that the Korean hairtail eggs, larvae, and adults that we examined may belong to the species *T. japonicus* rather than *T. lepturus* (Fig. 2).

Morphological description of eggs and larvae

The eggs were spherical, with a narrow perivitelline space. The surfaces of the egg membrane and yolk sac were smooth and lacked structure. The mean egg diameter was 1.75 mm (range was usually 1.66-1.84 mm; *n* = 33) (Fig. 3). A single oil globule was located at the posterior end of the yolk sac. At the early stage of embryo formation in the eggs, two melanophores began to form diagonally on the dorsal and ventral fin folds (Fig. 4A); they were still present by the prelarval stage, but disappeared thereafter (Fig. 5). The shape of the melanophores on the fin folds changed from branched to stellate with development (Fig. 4B).

The proportional measurements of larvae and adults of the Korean hairtail are presented in Table 2. The larva (5.5 mm in total length [TL]) has a compressed and elongated body, and the anus was located on the anterior. Snout length and eye diameter were similar. Melanophores were faintly present,

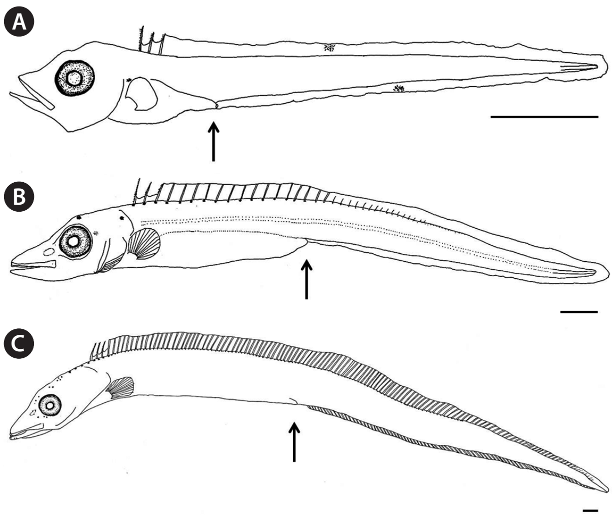

Fig. 5. Larval development of the hairtail from Korea. (A) Lateral view of larva of 5.5 mm total length (TL). (B) lateral view of larva of 14.9 mm TL. (C) lateral view of larva of 26.7 mm TL. The arrows indicate the location of the anus. Scale bars = 1.0 mm.

distributed on the dorsal body and on the dorsal and ventral fin folds. Three serrated dorsal spines were present on the anterior dorsal fin (Fig. 5A). The postlarva (14.9 mm TL) had a compressed and elongated body, and the anus was located around the middle of the body. The snout was slightly longer than the eye. Stellate-shaped melanophores first occurred on the dorsal head and opercle, and punctate- and stellate-shaped melanophores were found along the dorsal fin base. Silver-white skin occurred slightly on the anterior body and was detached from the body after preservation (Fig. 5B). The postlarva (26.7 mm TL) resembled hairtail adult. The snout was slightly longer than the eye. Punctate-shaped melanophores also were observed on the dorsal head, and the tips of the jaws. And silver-white skin entirely covered the body. The caudal fin was absent (Fig. 5C). The adults (294-668 mm TL; *n* = 11) had a ribbonlike silver body. The lower jaw protruded and the teeth on both jaws were very sharp and strong. The dorsal fin base was extremely long. The anal fins were hidden under the skin. Finally, the pectoral fins were located low on body and the color of the mouth cavity was dark.

In morphology, the hairtail larvae from Korea are very similar to those of *T. lepturus* (sensu Richards, 2006), but the two show distinct differences. For example, at 14.9 mm TL, no melanophores occurred on the lower jaw of hairtail larva from Korea, whereas we observed obvious melanophores on the lower jaw of *T. lepturus* larva from the Atlantic Ocean at 17.0 mm TL (sensu Richards, 2006). Moreover, the ratio of snout length to eye diameter differed between the two during the larval stage (see Table 2). This measurement has been considered the differentiating taxonomic character between *T. japonicus* and *T. lepturus* during the adult stage (Zemnukhov and Epur, 2011). The Korean hairtail postlarvae (14.9-26.7 mm TL) had a smaller snout length to eye diameter ratio (1.7) than the postlarva (17.0 mm TL) of *T. lepturus* (2.1). Therefore, the two are easily distinguishable by the melanophores on their lower jaw and the ratios of snout length to eye diameter during the larval stage. Nevertheless, further study is necessary, because melanophores may be contracted or have disappeared after fixing.

Taxonomic note on the hairtail in Korea

We compared the morphological characters of hairtail adults from Korea with reference data for *T. lepturus* adults. Our hairtail adults seemed to be very closely related to *T. japonicus* but quite different from *T. lepturus*. The ratio of TL to head length in the hairtail adults from Korea was 8.5-10.0, the ratio of snout length to eye diameter was 1.8-2.2, and the caudal peduncle length was 52-64% of the preanal length. According to some references, *T. japonicus* has a larger head

Table 2. Proportional measurements of larvae and adults between *Trichiurus japonicus* and *Trichiurus lepturus*

	Larva of *T. japonicus*	Postlarvae of *T. japonicus*		Postlarva of *T. lepturus*[*]	Adults of *T. japonicus*	Adults of *T. lepturus*
Total length (mm)	5.5	14.9	26.7	17.0	294-668 (n=11)	-
Total length (%)						
Preanus length	36.0	51.7	51.0	55.0	-	-
Preanal length	-	-	-	-	25.9-30.8 (29.4)	-
Head length	26.0	26.2	16.0	26.3	10.0-11.8 (10.9)	13.3-14.2[†]
Head length (%)						
Snout length (SnL)	36.0	36.7	32.0	39.7	30.8-35.3 (33.1)	-
Eye diameter (ED)	32.0	21.3	20.0	19.0	15.3-17.7 (16.6)	-
Preanal length (%)						
Caudal peduncle length	-	-	-		52.2-63.8 (56.3)	40.0[‡]
SnL/ED	1.1	1.7	1.6	2.1	1.8-2.2 (2.0)	3.0[§]

[*]Richards (2006), [†]Boeseman (1947) and Tucker (1956), [‡]Li (1992), [§]Günther (1860).

length to TL ratio (>8.0 in *T. japonicus* vs. 7.0-7.5 in *T. lepturus*) (Boeseman, 1947; Tucker, 1956; Zemnukhov and Epur, 2011), a smaller snout length to eye diameter ratio (2.2 in *T. japonicus* vs. 3.0 in *T. lepturus*) (Günther, 1860), and a greater caudal peduncle length relative to preanal length (52% in *T. japonicus* vs. 40% in *T. lepturus*) (Li, 1992). Furthermore, *Trichiurus* sp. 2 (sensu Nakabo, 2002) is very similar to *T. japonicus*, but they have different dorsal fin and oral cavity colors (white dorsal fin and dark oral cavity in *T. japonicus* vs. yellowish dorsal fin and light oral cavity in *Trichiurus* sp. 2).

In the northwestern Pacific, *T. lepturus* and *T. japonicus* are still often regarded as synonymous (Tucker, 1956; Nakamura and Parin, 1993; Froese and Pauly, 2013). The species *T. lepturus* is still questionable (Zemnukhov and Epur, 2011) because four syntypes (type localities America and China) show different numbers of some characteristics and only a single syntype remains today (Burhanuddin et al., 2002). Therefore, confirming *T. lepturus* as a species is very difficult, and considerable taxonomic work is necessary.

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