

## One unusual species, *Coilia* sp. (Engraulidae, Pisces) from the Yellow Sea

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Four specimens of unknown *Coilia* sp. were collected for the first time from the Yellow Sea in 2008 and compared with *Coilia mystus* and *Coilia nasus*. *Coilia* sp. showed similar morphology to *C. mystus* and *C. nasus*, but differed in that its tail was considerably shorter. We conducted an analysis of the morphological and genetic characteristics in an effort to clarify the taxonomic position of *Coilia* sp. In counts and measurements, *Coilia* sp. were well distinguished from *C. nasus* by the number of scutes (42–44 in *Coilia* sp. vs. 40–45 in *C. mystus* vs. 45–55 in *C. nasus*), ratio of dorsal base length to head length (43.4–47.6 vs. 37.9–47.6 vs. 33.0–41.0), and eye length to head length (19.2–20.8 vs. 17.0–22.4 vs. 13.8–18.2). In caudal skeleton of *Coilia* sp., urostyle, hypural and epural bones were not observed; instead of them, caudal fin rays were supported by the last vertebra, neural and haemal spines' extension. The molecular phylogenetic relationship was analyzed using 414 base-pair 12S rRNA mitochondrial DNA sequences. The Kimura-2-parameter distance between *Coilia* sp. and *C. mystus* was 0.3%, but was 1.3% between *Coilia* sp. and *C. nasus*. Both the neighbor-joining tree and maximum-likelihood tree showed that *Coilia* sp. are closely clustered with *C. mystus*. Therefore, our results suggest that the *Coilia* sp. may be a deformed fish of *C. mystus*.

**Keywords:** *Coilia* sp.; *Coilia nasus*; *Coilia mystus*; morphology; mtDNA 12S rRNA; Yellow Sea

### Introduction

Genus *Coilia* Gray, 1831 belongs to the family Engraulidae under the order Clupeiformes. This genus has a widespread distribution, including Korea (Youn and Kim 1996), Japan (Takita 1978; Aonuma 2002; Yamada et al. 2007), China (Cheng et al. 2005), Vietnam, Indonesia and India (Whitehead et al. 1988; Nelson 2006). A total of 13 species in the genus *Coilia* have been identified worldwide (Whitehead et al. 1988) and four species (*Coilia brachygnathus*, *Coilia ectenes*, *Coilia nasus*, *Coilia mystus*) have been recognized in the Yellow and East China Seas (Cheng et al. 2005; Yamada et al. 2007). However, only two species (*C. nasus*, *C. mystus*) have been identified in Korea so far (Chyung 1977; Youn and Kim 1996; Kim et al. 2005).

There have been many taxonomic studies on the clupeoid fishes, including the genus *Coilia* (Jordan and Seale 1926; Wongratana 1980; Whitehead et al. 1988). In recent years, morphological differences between populations of two *Coilia* spp. (Cheng et al. 2005), molecular phylogeny of Clupeiformes (Li and Orti 2007), and mitochondrial DNA diversity of *C. mystus* (Cheng et al. 2008) have been conducted. In Korea, a study of the reproductive cycle of *C. nasus* (Lee et al. 2003) and a taxonomic review of the family

Engraulidae (Youn and Kim 1996) were performed after the recognition of *C. nasus* and *C. mystus* by Jordan and Metz (1913) and Mori (1952), respectively. Since Takita (1978) first regarded *C. mystus* as a junior synonym of *C. nasus*, Youn and Kim (1996) and Kim et al. (2005) have insisted that there were no differences in the number of anal fin rays between *C. mystus* and *C. nasus*, suggesting the topic warrants further attention.

While investigating Korean *Coilia* spp. from the Yellow Sea, an unusual species was found in the southern Yellow Sea, which differed substantially from *C. nasus* and *C. mystus* in terms of the shape of its tail. It was decided that, given this shorter tail, our specimens could be placed within the genus *Demicoilia*, which was established by Jordan and Seale (1926) on the basis of one specimen from Sri Lanka. Subsequently, however, Menon (1951) and Jones and Menon (1952) identified the genus *Demicoilia* as a deformed fish with a damaged tail, perhaps inflicted by a predator's attack. We need to investigate whether our short-tailed *Coilia* specimens are merely deformed fishes or indeed a new species.

When morphology-based identification does not yield conclusive answers, the next approach is to employ DNA-based identification. This methodology has gained great prominence in recent years,

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Table 1. List of specimens of the present study.

| Species              | Area           | Location          | Number of specimens | Voucher number |
|----------------------|----------------|-------------------|---------------------|----------------|
| <i>Coilia</i> sp.    | Jin-do(Is.)    | 35°75'N, 125°75'E | 1                   | PKU 417        |
| <i>Coilia</i> sp.    | Jin-do(Is.)    | 34°75'N, 125°75'E | 3                   | PKU 418–420    |
| <i>Coilia nasus</i>  | Jin-do(Is.)    | 34°75'N, 126°25'E | 10                  | PKU 935–944    |
| <i>Coilia nasus</i>  | Gunsan         | 35°75'N, 126°25'E | 1                   | KS01           |
| <i>Coilia nasus</i>  | Ganghwa        | 37°25'N, 126°25'E | 1                   | KH06           |
| <i>Coilia mystus</i> | Pyeongannam-do | 38°40'N, 125°00'E | 20                  | NFRDI 105      |

specifically because mtDNA has been shown to be the most appropriate DNA region for species identification and phylogenetic reconstruction (Magoulas 2005). As 12S rRNA is highly conserved among mtDNA regions, it has been applied to a variety of molecular phylogenetic studies in animals, including the order Clupeiformes (Nam et al. 1997; Wang et al. 2001, 2003; Wang and Lee 2002; Kim et al. 2004; Lavoue et al. 2007).

Based on the four specimens of unusual *Coilia* sp. collected from the Yellow Sea, we hypothesized that they represented new species. In order to prove our hypothesis, their morphological, osteological and molecular characteristics were evaluated and compared with those of *C. nasus* collected from the same and adjacent waters. We also added the *C. mystus* collected from North Korea in order to clarify the taxonomic status between *C. nasus* and *C. mystus*.

## Materials and methods

### Sampling

The four specimens of *Coilia* sp. were collected from the southern Yellow Sea (35°25'N, 125°75'E; 34°75'N, 125°75'E) by a bottom trawl in April 2008, whereas ten specimens of *C. nasus* were collected via the same method from the southern and central Yellow Sea in September 2008. In contrast, we could not collect any specimens of *C. mystus* during our survey. Therefore, we borrowed and analyzed 20 specimens of *C. mystus* from the National Fisheries Research and Development Institute (NFRDI) collection. Those specimens were collected from Pyeongannam-do, North Korea in April 1939 (Table 1, Figure 1). *Coilia* sp. were fixed in 99% ethanol and *C. mystus* were fixed in 10% formalin as a whole body, whereas the *C. nasus* specimens were fixed in 10% formalin solution after the extraction of muscle tissues for DNA analysis.

### Morphological analysis

Counts and measurements followed the method of Whitehead (1985) except for the prepelvic length, which was measured as the distance from the tip of the snout to the origin of the pelvic fin. Each body part

was measured with digital vernier calipers to the nearest 0.1 mm. Skeletal observations and illustrations were made on Alizarin Red-stained specimens under a Research zoom stereo microscope (Olympus SZX-16, Japan). The vertebrae were counted from radiographs using soft X-ray equipment (Hitex Co., Japan). *Coilia* sp. and *C. nasus* were deposited at the Pukyong National University (PKU) and *C. mystus* were deposited at the National Fisheries Research and Development Institute (NFRDI).

### Genetic analysis

Genomic DNA was extracted from ethanol-preserved muscle tissues using the G-spin for Cell/Tissue genomic DNA extraction Kit (iNtRON Biotechnology Inc., Korea). Fragments of mtDNA 12S rRNA were amplified using the primers of Li and Orti (2007) [12S229F (5'-GYCGGTAAAAYTCGTGCCAG-3') and 12S954R (5'-YCCAAGYGCACCTTCCGGTA-3')] with a Thermal cycler (ABI 2720 Thermal cycler, USA). The polymerase chain reaction (PCR) fluid was 25 µl in volume, and the fluid mixture consisted of 7 µl DNA template, 2 µl dNTP, 2.5 µl 10X buffer, 1 µl of each

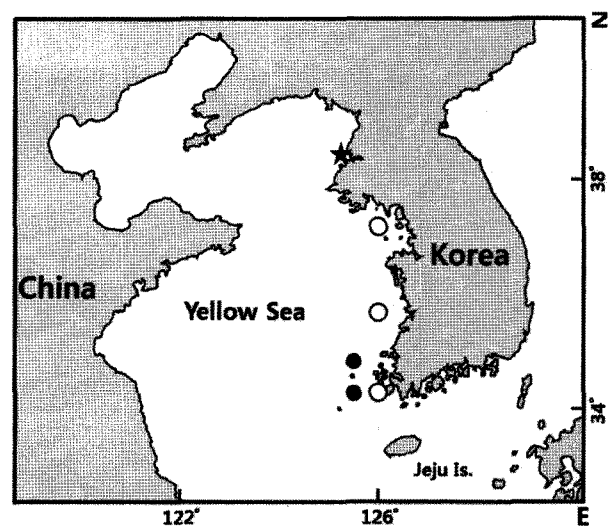


Figure 1. Sampling sites of *Coilia* sp. (●), *Coilia mystus* (\*) and *Coilia nasus* (○).

primer, 0.25 µl of Taq polymerase, with 11.25 µl of distilled water added. The PCR proceeded under the following conditions: initial denaturation at 95°C for 3 min, 31 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products were purified with a Core-One™ PCR Purification Kit (Core Bio System Co. LTD., Korea) and used for direct sequencing with an ABI PRISM BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Inc., USA). The sequences were acquired using an ABI 3730XL sequencer (Applied Biosystems Inc., USA). Nucleotide sequence data reported here have been supplied to the DDBJ/EMBL/GenBank nucleotide sequence databases (accession numbers, GU382852–GU382855).

DNA sequences were edited, checked, and aligned using Clustal W (Thompson et al. 1994) using BioEdit version 7 (Hall 1999). Genetic divergences were calculated using the Kimura-2-parameter model (Kimura 1980) using Mega version 4 (Tamura et al. 2007). Phylogenetic trees were constructed via the distance method using Mega version 4 (Tamura et al. 2007) and the maximum-likelihood method using PAUP version 4 (Swofford 2002). The neighbor-joining tree was constructed using the Kimura-2-parameter model (Kimura 1980), where its confidence was assessed via 1000 bootstrap replications. The maximum-likelihood tree was constructed using the GTR + I model (base frequencies: A = 0.3075, C = 0.2849, G = 0.1248, T = 0.3002; rate matrix: R(a) [A–C] = 4.8718, R(b) [A–G] = 17.1279, R(c) [A–T] = 6.2338, R(d) [C–G] = 0.0000, R(e) [C–T] = 45.6248, R(f) [G–T] = 1.0000; proportion of invariable sites = 0.6723; variable sites rates = equal), which was selected in accordance with the Akaike Information Criterion using modeltest 3.5 (Posada and Crandall 1998).

Furthermore, mtDNA 12S rRNA sequences of *Coilia* sp. were compared with those of the other *Coilia* spp. deposited in the GenBank database (*C. nasus*, NC009579; *C. ectenes*, DQ315675; *C. brachygnathus*, DQ912054; *C. grayii*, DQ315680; *C. mystus*, DQ912057), and *C. nasus* collected from the Yellow Sea (KS01, KH06) (Table 2). In addition, *Setipinna taty* (DQ912056) and *Clupea pallasii* (EU552682) were selected as outgroups and compared (Table 2).

## Results

### Morphological characteristics

Dorsal fin rays 13–14; anal fin rays 23–29; pectoral fin rays 6 + 10–12; pelvic fin rays 7; caudal fin rays 20–24; scutes 15–16 + 26–28 = 42–44; lower gill rakers 22–25; vertebrae 36–39. Counts and measurements are shown in Table 3.

The body was highly compressed and short, and the caudal fin was well developed (Figure 2A). The mouth was inferior, and the posterior tip of the maxillary reached the origin of pectoral fin or beyond (Figure 3). The percentage of maxillary length to head length (115.0–127.7%) was greater than the head length (Table 3). A row of small canine-like teeth presented on both jaws. Very sharp scutes were present in a mid-ventral body line from isthmus to anterior margin of anus. The pectoral fin had six upper filaments and its longest ray reached the anus or beyond. The origin of the pelvic fin corresponds to the origin of the dorsal fin or beyond. The percentage of prepelvic length to head length (154.5–158.8%) was equal to or greater than the percentage of predorsal length to head length. The caudal fin was well developed and the caudal peduncle was deep.

In caudal skeleton, urostyle, hypural and epural bones were not observed, and caudal fin rays were

Table 2. List of nucleotide samples of genus *Coilia* and outgroup.

| Species                     | Voucher number | Location                | Accession number | References         |
|-----------------------------|----------------|-------------------------|------------------|--------------------|
| Genus <i>Coilia</i>         |                |                         |                  |                    |
| <i>Coilia</i> sp.           | PKU 418        | 34°75'N, 125°75'E       | GU382852         | Present study      |
| <i>Coilia</i> sp.           | PKU 420        | 34°75'N, 125°75'E       | GU382853         | Present study      |
| <i>Coilia nasus</i>         | KS01           | 35°75'N, 126°25'E       | GU382854         | Present study      |
| <i>Coilia nasus</i>         | KH06           | 37°25'N, 126°25'E       | GU382855         | Present study      |
| <i>Coilia nasus</i>         | –              | –                       | NC009579         | Lavoue et al. 2007 |
| <i>Coilia brachygnathus</i> | –              | Pudong, Shanghai, China | DQ912054         | Li and Orti 2007   |
| <i>Coilia ectenes</i>       | –              | –                       | DQ315675         | NCBI Genebank      |
| <i>Coilia grayii</i>        | –              | –                       | DQ315680         | NCBI Genebank      |
| <i>Coilia mystus</i>        | –              | Pudong, Shanghai, China | DQ912057         | Li and Orti 2007   |
| Outgroup                    |                |                         |                  |                    |
| <i>Setipinna taty</i>       | –              | Pudong, Shanghai, China | DQ912056         | Li and Orti 2007   |
| <i>Clupea pallasii</i>      | –              | –                       | EU552682         | Lavoue et al. 2007 |

Table 3. Comparison of meristic and morphometric characters of *Coilia* sp., *Coilia nasus* and *Coilia mystus*.

|                      | <i>Coilia</i> sp. |               | <i>Coilia mystus</i> |               | <i>Coilia nasus</i> |              | <i>Coilia mystus</i> <sup>a</sup> | <i>Coilia nasus</i> <sup>a</sup> | <i>Coilia mystus</i> <sup>b</sup> | <i>Coilia nasus</i> <sup>b</sup> | <i>Coilia ectenes</i> <sup>b</sup> |
|----------------------|-------------------|---------------|----------------------|---------------|---------------------|--------------|-----------------------------------|----------------------------------|-----------------------------------|----------------------------------|------------------------------------|
|                      | PKU 417-420       | NFRDI 105     | NFRDI 105            | PKU 935 ~944  | PKU 935 ~944        | PKU 935 ~944 |                                   |                                  |                                   |                                  |                                    |
| Number of specimens  | 4                 | 20            |                      |               |                     |              |                                   |                                  |                                   |                                  |                                    |
| Standard length (mm) | 61.4-79.7         | 120.0-171.8   |                      | 187.1-256.3   |                     |              |                                   |                                  |                                   |                                  |                                    |
| Head length (mm)     | 16.8-21.3         | 20.6-27.7     |                      | 26.0-39.9     |                     |              |                                   |                                  |                                   |                                  |                                    |
| Count                |                   |               |                      |               |                     |              |                                   |                                  |                                   |                                  |                                    |
| Dorsal fin           | 13-14             | 12-14         |                      | 12-13         |                     |              |                                   |                                  | 14-15                             | 14                               | 13-15                              |
| Pectoral fin         | 6 + 10-12         | 6 + 10-12     |                      | 6 + 11-12     |                     |              | 6 + 10-14                         | 6 + 11-12                        | 6 + 11                            | 6 + 11-12                        | 6 + 11-13                          |
| Pelvic fin           | 7                 | 7             |                      | 7             |                     |              |                                   |                                  |                                   |                                  |                                    |
| Anal fin             | 23-29             | 77-86         |                      | 92-102        |                     |              |                                   |                                  | II, 80-84                         | II, 90-95                        | II, 97-115                         |
| Caudal fin           | 21-24             | 18-20         |                      | 18-20         |                     |              |                                   |                                  |                                   |                                  |                                    |
| Scutes               | 15-16 + 26-28     | 15-18 + 24-29 |                      | 16-21 + 29-34 |                     |              | 16-19 + 24-32                     | 16-26 + 25-                      | 15-19 + 24-26                     | 18 + 29-30                       | 19-23 + 30-35                      |
|                      | =42-44            | =40-45        |                      | =47-52        |                     |              | =41-55                            | 26 = 43-61                       | =41-43                            | =47-48                           | =49-55                             |
| Lower gill rakers    | 22-25             | 23-25         |                      | 23-24         |                     |              | 25-31                             | 23-26                            | 24-26                             | 24                               | 24-25                              |
| Vertebrae            | 36-39             | 63-69         |                      | 75-78         |                     |              | 65-69                             | 75-82                            | 67-69                             | 76-78                            | 79-82                              |
| Measurements (%HL)   |                   |               |                      |               |                     |              |                                   |                                  |                                   |                                  |                                    |
| Body depth           | 88.3-95.8         | 78.3-107.5    |                      | 87.2-107.9    |                     |              |                                   |                                  |                                   |                                  |                                    |
| Predorsal length     | 146.0-153.0       | 146.1-163.7   |                      | 157.6-181.4   |                     |              |                                   |                                  |                                   |                                  |                                    |
| Prepelvic length     | 154.5-158.8       | 140.3-159.2   |                      | 154.8-168.1   |                     |              |                                   |                                  |                                   |                                  |                                    |
|                      |                   |               |                      | (n = 9)       |                     |              |                                   |                                  |                                   |                                  |                                    |
| Dorsal base length   | 43.4-47.6         | 37.9-47.6     |                      | 33.0-41.0     |                     |              |                                   |                                  |                                   |                                  |                                    |
| Pelvic fin length    | 46.9-54.2         | 38.8-53.9     |                      | 44.9-53.1     |                     |              |                                   |                                  |                                   |                                  |                                    |
| Snout length         | 22.0-27.7         | 19.7-26.1     |                      | 19.6-24.1     |                     |              |                                   |                                  |                                   |                                  |                                    |
| Postorbital length   | 54.8-61.9         | 56.3-62.1     |                      | 59.4-65.2     |                     |              |                                   |                                  | 58.8-62.5                         | 62.5-66.7                        | 62.5-71.4                          |
| Eye length           | 19.2-20.8         | 17.0-22.4     |                      | 13.8-18.2     |                     |              |                                   |                                  |                                   |                                  |                                    |
| Upper jaw length     | 115.0-127.7       | 118.3-132.3   |                      | 108.5-132.8   |                     |              |                                   |                                  |                                   |                                  |                                    |
|                      |                   |               |                      | (n = 9)       |                     |              |                                   |                                  |                                   |                                  |                                    |

<sup>a</sup>Whitehead et al. 1988.<sup>b</sup>Yamada et al. 2007.

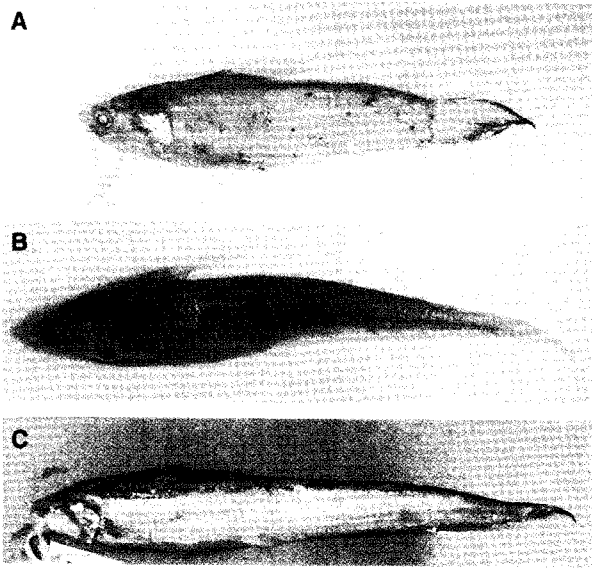


Figure 2. *Coilia* sp., PKU 420 (A), *Coilia mystus*, NFRDI 105 (B) and *Coilia nasus*, PKU 935 (C).

connected to the posterior end of the last vertebra. The posterior extension of the last neural and haemal spines supported caudal fin rays (Figure 4B).

Coloration of alcohol-preserved specimens showed bright white on the whole, dorsally darkish, the body without dots and marks. The tip of the caudal fin was darkish, other fins transparently white.

#### Molecular characteristics

A total of 414 base-pairs of mtDNA 12S rRNA were compared among two specimens of *Coilia* sp. and two specimens of *C. nasus* with the other *Coilia* spp. obtained from NCBI. Two specimens of *Coilia* sp. were closely clustered with *C. mystus* with the comparison of *C. nasus* from the NJ tree. Two specimens of *C. nasus* showed no difference to cluster with *C. ectenes* and *C. branchygnathus* (Figure 5A). *Coilia* sp. closely clustered with *C. mystus*, with comparison of *C. nasus*

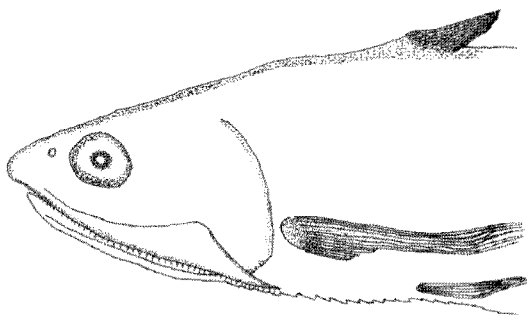


Figure 3. Illustration of head of *Coilia* sp. PKU 417.

from the ML tree (Figure 5B). Genetic divergence of *Coilia* sp. was closest to *C. mystus* by 0.3%, but was 1.3% distant from *C. nasus*, *C. branchygnathus*, *C. grayii* and *C. ectenes*. Genetic divergence of *Coilia* sp. was considerably distant from outgroups; *S. taty* by 7.6% and *C. pallasii* by 18.1% (Table 4). In the sequence analysis, *Coilia* sp. showed almost the same variable patterns as *C. mystus* (Table 5).

#### Discussion

##### Morphological characteristics

Wongratana (1980) divided the genus *Coilia* group into two main subgroups by two morphological characteristics; the number of scutes (11–23 in the first subgroup vs. 34–61 in the second subgroup) and location of posterior tip of maxillary (not reaching to edge of gill cover vs. reaching or beyond). Our unidentified *Coilia* specimens belonged to the second subgroup of Wongratana (1980), and were very similar to *C. nasus* as well as *C. mystus* in location of posterior tip of the maxillary and the number of pectoral filaments. According to Youn and Kim (1996) and Kim et al. (2005), *C. mystus* needs to be reviewed because the *C. mystus* and *C. nasus* are very similar in morphology. In this study, we compared *C. nasus* with *C. mystus* in Korea for the first time. As a result, *C. mystus* and *C. nasus* were well distinguished by the number of anal fin rays (77–86 in *C. mystus* vs. 92–102 in *C. nasus*), scutes (40–45 vs. 47–52) and vertebrae (63–69 vs. 75–78) (Table 6). Comparing *Coilia* sp. with two *Coilia* species, *Coilia* sp. were closer to *C. mystus* than *C. nasus* in the number of scutes (42–44 in *Coilia* sp. vs. 40–45 in *C. mystus* vs. 45–55 in *C. nasus*), ratio of dorsal base length to head length (43.4–47.6 vs. 37.9–47.6 vs. 33.0–41.0), and eye length to head length (19.2–20.8 vs. 17.0–22.4 vs. 13.8–18.2) (Figure 6; Table 6). However, *Coilia* sp. differed from *C. mystus* and *C. nasus* in having a shorter tail, deep caudal peduncle and well-developed caudal fin. *Coilia* sp. also differed from *C. mystus* and *C. nasus* in meristic characters: anal fin rays 23–29 and vertebrae 36–39 (Table 6).

Yamada et al. (2007) insisted that the genus *Coilia* comprised three species in the Yellow and East China Seas, which were distinguished by the number of anal fin rays (80–84 in *C. mystus* vs. 90–95 in *C. nasus* vs. 97–115 in *C. ectenes*), vertebrae (67–69 vs. 76–78 vs. 79–82), scutes (41–43 vs. 47–48 vs. 49–55) and branchiostegal rays (9 vs. 9–10 vs. 10–11). According to the taxonomic criteria of Yamada et al. (2007), *Coilia* sp. is identical to *C. mystus* in the number of scutes, but differs in the number of anal fin rays and vertebrae.

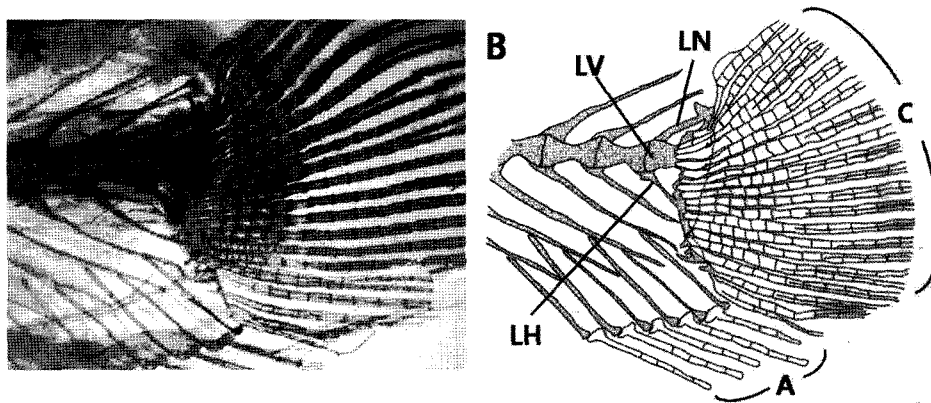


Figure 4. Photograph (A) and illustration (B) of caudal skeleton of *Coilia* sp., PKU 417. LV, last vertebrae; LN, last neural spine; LH, last hemal spine; A, Anal fin; C, caudal fin.

Jordan and Seale (1926) established the genus *Demicoilia* for the first time based on short-tailed specimens; subsequently, Menon (1951) and Jones and Menon (1952) reported that the caudal fins of the genus *Demicoilia* regenerated around the last vertebra. Comparative osteological analysis suggested that the caudal skeleton of *Coilia* sp. differs from that of normal fish species. Unlike the caudal skeleton of *C. nasus* (Fujita 1990; Youn 1996), *Coilia* sp. has caudal fin rays that are well-developed behind the last vertebra, and has the last haemal and neural spines' extension supporting the caudal fin rays.

Therefore, in terms of caudal skeleton, *Coilia* sp. must be a deformed fish which has a regenerated caudal fin after a tail breakage.

**Molecular characteristics**

The result of mtDNA 12S rRNA sequences analysis showed that the genetic divergence of *Coilia* sp. with

*C. mystus* was as close as 0.3% and with *C. nasus* as distant as 1.3% (Table 4). *Coilia* sp. was clustered with *C. mystus* but *C. nasus* was clustered with *C. brachygnathus* and *C. ectenes* by NJ tree and ML tree. Therefore, in terms of DNA, *Coilia* sp. must be *C. mystus*. Interestingly, *C. nasus* was closer to *C. brachygnathus* than *C. mystus*, as demonstrated previously by Li and Orti (2007). Wongratana (1980) thought *C. brachygnathus* was a junior synonym of *C. nasus*, but the former has maxillary that do not reach the edge of the gill-cover; as a result, *C. brachygnathus* is often regarded as a distinct species (Whitehead et al. 1988; Yamada et al. 2007). However, in this study, no genetic differences were found between *C. brachygnathus* and *C. nasus*, based on a genetic distance of 0%. Even though mtDNA 12S rRNA information allows for the comparison of conservable regions with other regions (Scheffler 1999; Hwang and Kim 1999; Wang and Lee 2002), genetic intraspecific divergence has been noted in 0.2–4.1% of *Engraulis*

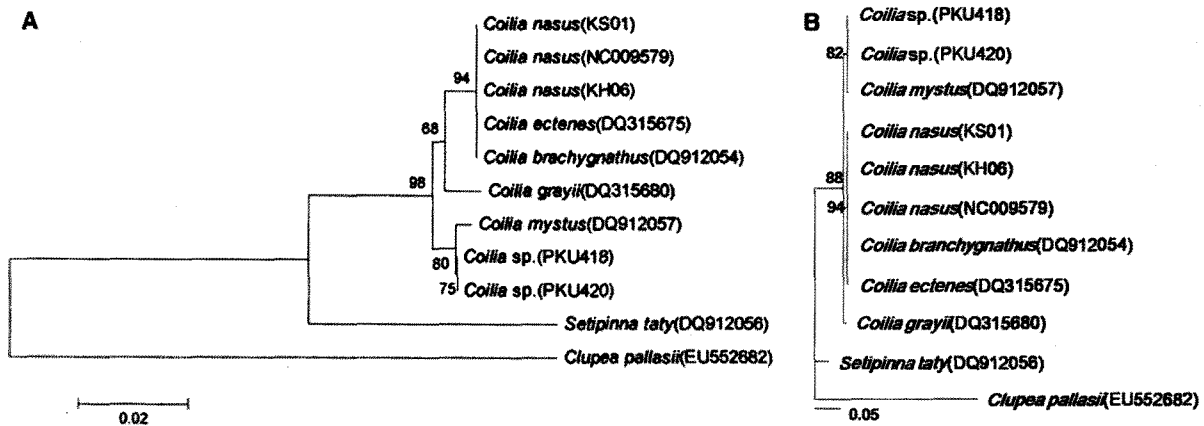


Figure 5. Neighbor-joining (A) and maximum-likelihood (B) trees constructed by mtDNA 12S rRNA sequences of six *Coilia* species and two outgroups. The numbers on banches are NJ bootstrap values (>50%) and ML bootstrap values (>50%) obtained from 1000 replications.

Table 4. Genetic divergence among six *Coilia* species including unusual *Coilia* sp. with two outgroups.

| Species                        | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    |
|--------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 <i>Coilia</i> sp. (PKU 418)  |       |       |       |       |       |       |       |       |       |       |
| 2 <i>Coilia</i> sp. (PKU 420)  | 0.000 |       |       |       |       |       |       |       |       |       |
| 3 <i>Coilia nasus</i> (KS01)   | 0.013 | 0.013 |       |       |       |       |       |       |       |       |
| 4 <i>Coilia nasus</i> (KH06)   | 0.013 | 0.013 | 0.000 |       |       |       |       |       |       |       |
| 5 <i>Coilia nasus</i>          | 0.013 | 0.013 | 0.000 | 0.000 |       |       |       |       |       |       |
| 6 <i>Coilia branchygnathus</i> | 0.013 | 0.013 | 0.000 | 0.000 | 0.000 |       |       |       |       |       |
| 7 <i>Coilia ectenes</i>        | 0.013 | 0.013 | 0.000 | 0.000 | 0.000 | 0.000 |       |       |       |       |
| 8 <i>Coilia grayii</i>         | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 |       |       |       |
| 9 <i>Coilia mystus</i>         | 0.003 | 0.003 | 0.016 | 0.016 | 0.016 | 0.016 | 0.016 | 0.016 |       |       |
| 10 <i>Setipinna taty</i>       | 0.076 | 0.076 | 0.076 | 0.076 | 0.076 | 0.076 | 0.076 | 0.076 | 0.076 |       |
| 11 <i>Clupea Pallasii</i>      | 0.181 | 0.181 | 0.185 | 0.185 | 0.185 | 0.185 | 0.185 | 0.189 | 0.181 | 0.200 |

Table 5. Nucleotide variable position in consensus sequences of the 12S rRNA.

| Species                      | Position |   |   |   |   |   |   |
|------------------------------|----------|---|---|---|---|---|---|
|                              | 9        | 2 | 1 | 1 | 1 | 3 | 3 |
| <i>Coilia</i> sp. (PKU 418)  | A        | G | C | C | C | T | T |
| <i>Coilia</i> sp. (PKU 420)  | ●        | ● | ● | ● | ● | ● | ● |
| <i>Coilia mystus</i>         | ●        | ● | ● | T | ● | ● | ● |
| <i>Coilia nasus</i> (KS01)   | T        | A | T | C | T | C | C |
| <i>Coilia nasus</i> (KH06)   | ●        | ● | ● | ● | ● | ● | ● |
| <i>Coilia nasus</i>          | ●        | ● | ● | ● | ● | ● | ● |
| <i>Coilia branchygnathus</i> | ●        | ● | ● | ● | ● | ● | ● |
| <i>Coilia ectenes</i>        | ●        | ● | ● | ● | ● | ● | ● |
| <i>Coilia grayii</i>         | ●        | ● | C | ● | C | ● | ● |

Dots (●) represent identical nucleotide sequences relative to the species directly above.

*japonicus* (Kim et al. 2004) and 2.0–5.5% of *Cyprinus carpio* (Nam et al. 1997). Our result indicates that there are no genetic differences among *C. nasus*, *C. branchygnathus* and *C. ectenes*, thus leading us to the conclusion that they may be the same species (Table 4). Interestingly, the latter two species were in

consideration for *C. nasus* in the past (Whitehead et al. 1988). Nevertheless, Yamada et al. (2007) distinguished three species (*C. nasus*, *C. branchygnathus*, *C. ectenes*) based on their morphological characteristics; therefore, a more detailed taxonomic review among the three *Coilia* species is needed.

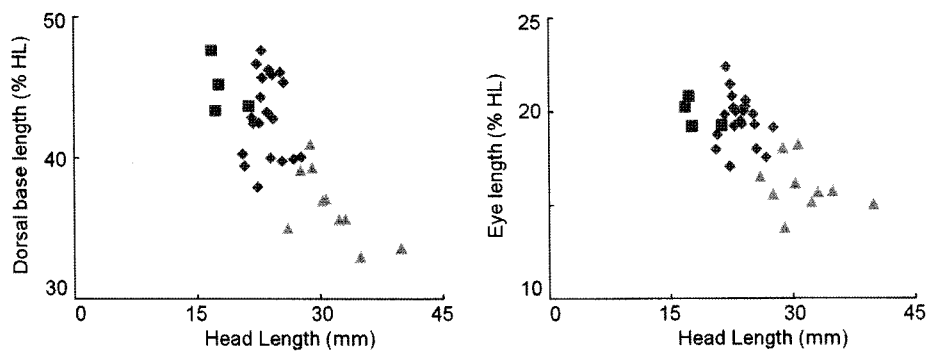


Figure 6. Relationship of dorsal base length to head length, and eye length to head length to head length in *Coilia* sp. (■), *C. mystus* (◆) and *C. nasus* (▲).

Table 6. Comparison of three counts (the number of anal fin, vertebrae and scutes) among three *Coilia* species.

| Species              | HL (mm)   | Anal fin |    |    |     |    |     |    |    |    |    |    |    |    |    |    |    |     |    |    |    |    |    |     |     |     |     |   |
|----------------------|-----------|----------|----|----|-----|----|-----|----|----|----|----|----|----|----|----|----|----|-----|----|----|----|----|----|-----|-----|-----|-----|---|
|                      |           | 23       | 24 | 25 | ... | 29 | ... | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | ... | 92 | 93 | 94 | 95 | 96 | ... | 100 | 101 | 102 |   |
| <i>Coilia</i> sp.    | 16.8–21.3 | 1        | 1  | 1  |     | 1  |     |    |    |    |    |    |    |    |    |    |    |     |    |    |    |    |    |     |     |     |     |   |
| <i>Coilia mystus</i> | 20.6–27.7 |          |    |    |     |    |     | 2  | 3  | 1  | 2  | 5  | 1  | 2  | 3  | 1  |    |     |    |    |    |    |    |     |     |     |     |   |
| <i>Coilia nasus</i>  | 26.0–39.9 |          |    |    |     |    |     |    |    |    |    |    |    |    |    |    |    |     | 2  |    | 1  | 2  | 4  |     |     | 1   |     | 1 |

| Species              | HL (mm)   | Vertebrae |    |    |    |     |    |    |    |    |    |    |    |     |    |    |    |    |  |  |  |  |  |   |   |   |  |   |  |
|----------------------|-----------|-----------|----|----|----|-----|----|----|----|----|----|----|----|-----|----|----|----|----|--|--|--|--|--|---|---|---|--|---|--|
|                      |           | 36        | 37 | 38 | 39 | ... | 63 | 64 | 65 | 66 | 67 | 68 | 69 | ... | 75 | 76 | 77 | 78 |  |  |  |  |  |   |   |   |  |   |  |
| <i>Coilia</i> sp.    | 16.8–21.3 | 2         | 1  |    | 1  |     |    |    |    |    |    |    |    |     |    |    |    |    |  |  |  |  |  |   |   |   |  |   |  |
| <i>Coilia mystus</i> | 20.6–27.7 |           |    |    |    |     |    | 2  | 1  |    | 3  | 8  | 2  | 1   |    |    |    |    |  |  |  |  |  |   |   |   |  |   |  |
| <i>Coilia nasus</i>  | 26.0–39.9 |           |    |    |    |     |    |    |    |    |    |    |    |     |    |    |    |    |  |  |  |  |  | 1 | 2 | 5 |  | 1 |  |

| Species              | HL (mm)   | Scutes |    |    |    |    |    |    |    |    |    |    |    |    |   |  |  |  |  |  |  |  |  |  |  |  |  |   |
|----------------------|-----------|--------|----|----|----|----|----|----|----|----|----|----|----|----|---|--|--|--|--|--|--|--|--|--|--|--|--|---|
|                      |           | 40     | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 |   |  |  |  |  |  |  |  |  |  |  |  |  |   |
| <i>Coilia</i> sp.    | 16.8–21.3 |        |    |    | 2  | 1  | 1  |    |    |    |    |    |    |    |   |  |  |  |  |  |  |  |  |  |  |  |  |   |
| <i>Coilia mystus</i> | 20.6–27.7 | 2      | 3  | 4  | 5  | 3  | 3  |    |    |    |    |    |    |    |   |  |  |  |  |  |  |  |  |  |  |  |  |   |
| <i>Coilia nasus</i>  | 26.0–39.9 |        |    |    |    |    |    |    |    |    |    | 3  | 1  | 2  | 1 |  |  |  |  |  |  |  |  |  |  |  |  | 3 |

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