

# A New Record of Juvenile *Chromis mirationis* (Perciformes: Pomacentridae) from Korea, Revealed by Molecular Analysis, with a Comparison to Juvenile *Chromis notata*

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## Abstract

A single juvenile pomacentrid specimen (5.9 mm standard length) was collected from the Korea Strait in October 2010. The specimen is characterized by punctate-stellate melanophores scattered on the operculum and dorso-ventral region in front of the caudal peduncle, the lack of melanophores on the posterior end of the anal fin base, the presence of 14 spines and 14 soft rays on the dorsal fin, and the presence of 2 spines and 12 soft rays on the anal fin. A molecular analysis based on mitochondrial DNA 16S rRNA sequences showed that this specimen is closely related to adult *Chromis mirationis* ( $d = 0.002$ ), but that it differs from *Chromis notata* ( $d = 0.017$ ). Juvenile *C. mirationis* differ from juvenile *C. notata* in having no melanophores on the posterior end of the anal fin base. We propose a new Korean name, “tti-ja-ri-dom” for *C. mirationis*.

**Key words:** *Chromis mirationis*, *Chromis notata*, Juvenile, New record, MtDNA 16S rRNA, Korea

## Introduction

The family Pomacentridae (Perciformes) is one of the most diverse groups of reef fishes, and is distributed mainly in the tropical and temperate seas of the Indo-Pacific (Allen, 1991; Nelson, 2006; Allen and Erdmann, 2012). The Pomacentridae is comprised of approximately 350 species worldwide (Nelson, 2006), including 105 species in Japan (Aonuma et al., 2013), but only 17 species in Korea (Kim et al., 2005; Kim, 2011; Song et al., 2013). Although numerous studies have been conducted on the early life histories of pomacentrid species (Okiyama, 1988; Leis and Carson-Ewart, 2000; Kim et al., 2001; Richards, 2006; Murphy et al., 2007), identification of congeneric species remains difficult because of the extreme color variations that exist at all life stages (Neal, 1993; Song et al., 2013), and because research on only 15% of species has been conducted (Murphy et al., 2007). Because external mor-

phological traits of larval and adult stages are very different, the characters used for adult stages cannot be used for larval stages (Blaxter, 1984; Miller and Kendall, 2009). Thus, molecular methods have been used widely in recent taxonomic studies of this group (Kim et al., 2008; Vandersea et al., 2008; Victor et al., 2009; Kwun and Kim, 2010; Ji et al., 2012; Kwun et al., 2012; Ko et al., 2013; Kwun et al., 2013; Lee and Kim, 2013).

In October 2010, a single juvenile pomacentrid specimen was collected from the Korea Strait (south of Tong-young), and based on molecular analyses, we identified it as a juvenile *Chromis mirationis*. Our study is the first to report the morphology of juvenile *C. mirationis*, and the first to report an occurrence of this species in Korean waters. We also compared the characteristics of juvenile *C. mirationis* with those of

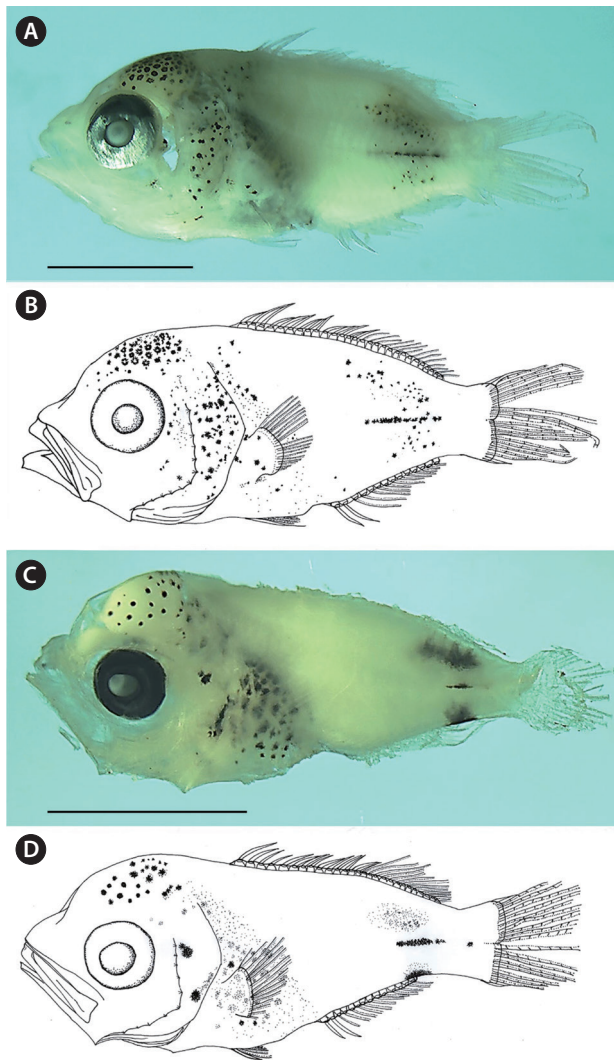
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**Fig. 1.** Photo (A) and illustration (B) of *Chromis mirationis*, NIBR-P22498, 5.9 mm standard length (SL); photo (C) and illustration (D) of *Chromis notata*, PKUI 96, 5.1 mm SL. Scale Bars = 2 mm.

juvenile *Chromis notata*, because the two species were found to be closely related by molecular analyses.

## Materials and Methods

A single juvenile pomacentrid specimen was collected from the Korea Strait (south of Tong-young) at a depth of 100 m by an RN80 net (Sanga, Busan, Korea) on 29 October 2010. The specimen was fixed in 99% ethanol immediately following collection. Counts and measurements were performed under an Olympus SZX-16 stereomicroscope (Olympus, Tokyo, Japan) following Leis and Carson-Ewart (2000). Measurements were recorded to the nearest 0.1 mm using an Active Measure

image analyzer (Shinhan Scientific Optics, Seoul, Korea). Sketches of the external shape of the juvenile were created using a Olympus SZX-DA camera lucida (Olympus) attached to a microscope. Analyses of melanophore shapes and distributions followed Russell (1976). The specimen was deposited in the National Institute of Biological Resources (Voucher number, NIBR-P22498; GenBank accession number, JQ178234), Korea; other specimens are registered in Pukyong National University (PKU and PKUI), and were deposited in the collections of the Kanagawa Prefectural Museum of Natural History (KPM-NI).

Genomic DNA extraction and PCR analyses for molecular identification were performed according to Kwun and Kim (2010) and Kwun et al. (2012). The nucleotide sequences were deposited in the DNA Data Bank of Japan (DDBJ), in the European Molecular Biology Laboratory (EMBL), and in GenBank. Sequences were aligned using ClustalW (Thompson et al., 1994) in BioEdit version 7 (Hall, 1999). Sequence data were obtained for an adult *C. mirationis* (KPM-NI 30479, KF957467; Voucher number, NCBI registration number, respectively), and a juvenile *C. notata* (PKUI 96, KF957468) specimen. Sequence data for *C. albicauda* (PKU 5802, KF957469) and three *Chromis* species which were *C. analis* (no voucher number, FJ616423), *C. fumea* (PKU 5535, KC767733), and *C. notata* (PKU 5566, KC767732) were obtained from the National Center for Biotechnology Information (NCBI) and were used for molecular comparisons. A *Thalassoma lunare* specimen (PKU 6501, JQ178236) was selected as an outgroup. Genetic distances were calculated using the Kimura two-parameter method (Kimura, 1980) in MEGA 5 (Tamura et al., 2011). A neighbor-joining (NJ) tree was constructed using the Kimura two-parameter method (Kimura, 1980) and 10,000 bootstrap replications in MEGA 5 (Tamura et al., 2011).

## Results and Discussion

### *Chromis mirationis* Tanaka, 1917

(New Korean name: Tti-ja-ri-dom; Fig. 1A and 1B)

*Chromis mirationis* Tanaka, 1917: 8 (type locality: Off Goto Island, Japan); Randall et al., 1981: 230 (Japan); Masuda et al., 1984: 192 (Japan); Allen, 1991: 75 (Japan); Yamada et al., 2009: 512 (Japan); Aonuma et al., 2013: 1037 (Japan and Taiwan).

*Chromis* sp.: Kim et al., 2011: 200 (Korea).

### Material examined

NIBR P-22498, 5.9 mm standard length (SL), Korea Strait (south of Tong-young, 34°20' N, 128°21' E), 29 October 2010 (Fig. 1A and 1B).



**Fig. 2.** Comparative specimens of *Chromis mirationis* examined. (A) 24.7 mm standard length (SL), KPM-NI 23617, (B) 52.8 mm SL, KPM-NI 30479, and (C) 100.1 mm SL, KPM-NI 18916.

**Comparative material examined**

*Chromis notata*: PKUI 96, 5.1 mm SL, Jeju Island, August 2012 (Fig. 1C and 1D). *Chromis mirationis*: KPM-NI 30479, 52.8 mm SL, Sagami Bay, Japan, 15 March 2012; KPM-NI 23617, 24.7 mm SL, Shizuoka Bay, Japan, 23 March 2009; KPM-NI 18916, 100.1 mm SL, Shizuoka Bay, Japan, 30 April 2007 (Fig. 2).

**Description**

Counts are shown in Table 1. The following body measurements are expressed in terms of percent standard length (% SL): body depth, 44.2; head length, 46.6; snout length, 14.5; eye diameter, 16.9; upper jaw length, 26.0; predorsal length, 53.6; preanal length, 65.0; caudal peduncle depth, 16.0; caudal peduncle length, 11.7; dorsal fin base length, 49.2; anal fin base length, 33.7; pelvic fin length, 16.9; second anal fin spine length, 11.0.

The body is short and slightly deep; the head and eyes are large, the snout is slightly pointed; the anterior margin of the head is slightly concave; the mouth is terminal, the posterior tip of the maxilla is located beyond the anterior margin of the eye; the teeth are small and conical on both jaws; the poste-

rior margin of the preopercle is weakly serrated; the origin of the dorsal fin is located above the posterior margin of the opercle; the caudal peduncle is short; the anus is located behind the middle of the body; and the posterior tip of the pelvic fin reaches the anus.

**Pigmentation**

Stellate melanophores are densely distributed on the occipital; punctate and punctate-stellate melanophores are scattered on the operculum, shoulder, abdomen, and pectoral fin base; rod-like stellate melanophores are sparsely distributed on the mediolateral; small punctate-stellate melanophores are scattered on the dorso- and ventro-laterals. Stellate melanophores are also densely distributed on the peritoneum. No melanophores exit on the snout, the middle region of the body, the dorsal and ventral contours, the caudal peduncle, the posterior end of the anal fin base, or the fin membranes (Fig. 1).

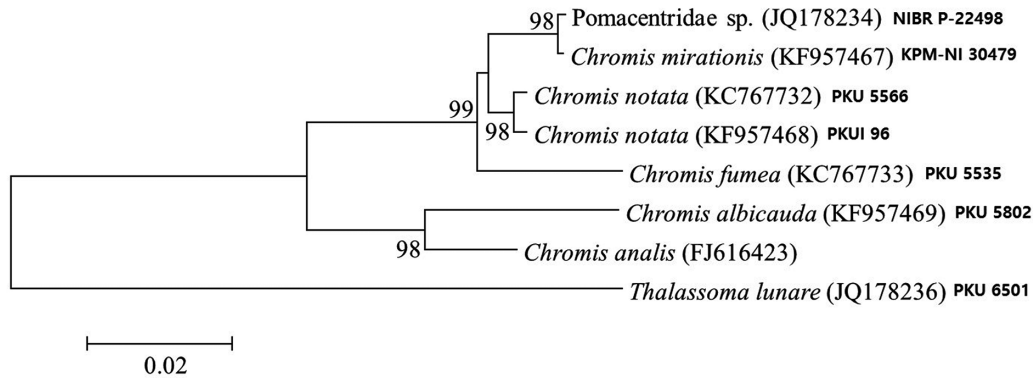
**Distribution**

*Chromis mirationis* is reported from Japan (Randall et al., 1981; Yamada et al., 2009; Aonuma et al., 2013), Taiwan (Aonuma et al., 2013), and Korea (Korea Strait, present study).

**Table 1.** Comparison of meristic characters between *Chromis mirationis* and four species of genus *Chromis*

	<i>Chromis mirationis</i>		Tanaka (1917)	<i>C. notata</i> Koh et al. (1997)	<i>C. fumea</i>	<i>C. albicauda</i>	<i>C. analis</i>
	Present study						
	Juvenile	Adult					
Number of specimens	1	3	-	-	-	-	-
Standard length (mm)	5.9	24.7-100.1	-	-	-	-	-
Dorsal fin rays	XIV, 14	XIV, 14	XIV, 14	XIII-XIV, 12-14	XIII-XIV, 10-12	XIII, 12	XIII, 11-13
Anal fin rays	II, 12	II, 12	II, 11	II, 10-12	II, 9-10	II, 12	II, 12-13
Pectoral fin rays	-	18-20	19	18-20	18-20	19	17-18

'-' represents no data.



**Fig. 3.** Neighbor-joining tree constructed by the mitochondrial DNA 16S rRNA sequences for juvenile Pomacentridae sp. and five *Chromis* species, with one outgroup (*Thalassoma lunare*). Numbers at branches indicate bootstrap probabilities in 10,000 bootstrap replications. Bar indicates genetic distance of 0.02. Parenthesis and superscripts indicate the NCBI registration number and voucher number, respectively.

### Mitochondrial DNA sequence analysis

Analysis of 566 base pairs of mitochondrial DNA 16S rRNA sequences shows that our juvenile specimen is closely related to adult *C. mirationis* ( $d = 0.002$ ;  $d$  is genetic distance), but that it differs from *C. notata* ( $d = 0.017$ ), *C. fumea* ( $d = 0.032$ ), *C. analis* ( $d = 0.063$ ), and *C. albicauda* ( $d = 0.077$ ). In the NJ tree, the juvenile specimen clusters closely to adult *C. mirationis*, supported by a 98% bootstrap value (Fig. 3).

### Remarks

The present juvenile specimen can be classified into the genus *Chromis*, based on the following morphological traits: short and deep body, large head, and similar fin counts (Okuyama, 1988; Leis and Carson-Ewart, 2000). Among *Chromis* species, our juvenile specimen is most similar to *C. notata* in that it has a dorsal fin with 14 spines (Table 1). However, it differs from *C. notata* because it has melanophores distributed on the operculum (numerous melanophores on juvenile *C. mirationis* vs. a few melanophores on juvenile *C. notata*) and on the posterior end of the anal fin base (absent vs. present). Morphologically, our juvenile *C. mirationis* specimen matches the original description of the species (Tanaka, 1917) in having a dorsal fin with 14 spines (Table 1), and is clearly distinguished from *C. analis* and *C. albicauda* by the number of dorsal fin spines (13 spines; Song et al., 2013). We propose the new Korean name “Tti-ja-ri-dom” for *C. mirationis*, following Yamada et al. (2009).

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