# First report of an aeolid nudibranch *Flabellina athadona* and an identification key for the genus *Flabellina* from Korea

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## ABSTRACT

An aeolid nudibranch species *Flabellina athadona* (Bergh, 1875) is newly recorded from Korean waters. This species is distinguished from other congeneric species by the following characteristics: translucent white body, deep orange colored digestive glands, plenty of white specks, and a reversed Y-shaped marking on the head between rhinophores and oral tentacles. In this report, we also provide an identification key for the genus *Flabellina* in Korea, photographs, and diagnostic characteristics of *F. athadona*. Molecular identification using mtDNA *cox1* partial sequences is also provided to help differentiate *F. athadona* from some of its closely related congeneric species.

Keywords: Flabellinidae, Flabellina athadona, mtDNA cox1, identification key, Korea

# INTRODUCTION

The genus *Flabellina* Gray, 1833 is among the largest genera of the family Flabellinidae and is characterized by presence of propodeal tentacles on the foot corners, pleuroproctic position of the anus, and triserrate radula with denticulate rachidian teeth. They occur in a variety of habitats and feed on hydroids (Debelius & Kuiter, 2007; Millen & Hermosillo, 2007). The Indo-Pacific species of the genus *Flabellina* have been reviewed by Gosliner & Willan (1991). In Korea, four *Flabellina* species have been reported but without detailed morphological descriptions (Min *et al.*, 2004; Koh, 2006; Noseworthy *et al.*, 2007): *Flabellina bicolor* (Kelaart, 1858), *F. exoptata* Gosliner & Willan, 1991, *F. rubrolineata* 

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(O'Donoghue, 1929), and F. verrucosa (M. Sars, 1829). In this report, we provide a morphological description of F. athadona and an identification key for the genus *Flabellina* in Korea, along with molecular identification using mtDNA cox1 sequences for some congeneric species.

# MATERIALS AND METHODS

Individuals were collected from the East Sea in Korea by SCUBA diving on 25 March 2015. Samples were relaxed in a 6:4 mixture of seawater and 7% MgCl<sub>2</sub> solution. After anesthetization, specimens were preserved in 10% neutral formalin for morphological examination or fixed in 95% ethyl alcohol for molecular analysis. External features were examined under a stereoscopic microscope (Leica M205C, Wetzlar, Germany).

Genomic DNA was extracted from small pieces of foot tissue using DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). Polymerase chain reaction (PCR) was performed with TaKaRa Ex Taq<sup>TM</sup> (Takara Bio, Shiga, Japan) in a total volume of 50  $\mu$ l containing 31.75  $\mu$ l of distilled water, 5  $\mu$ l 10X Ex Taq<sup>TM</sup> buffer, 4  $\mu$ l of metazoan universal primers (LCO1490 and

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HCO2198; Folmer et al., 1994), 0.25 µl of TaKaRa Ex Taq<sup>TM</sup>, and 5  $\mu$ l template DNA. PCR amplification for mtDNA cox1 partial gene fragment was performed with an initial denaturation at  $94^{\circ}$  for 30 s, followed by 35 cycles of 45 s at 94°C, 30 s at 42°C, 1 min at 7  $2^{\circ}$ C, and 10 min at  $72^{\circ}$ C for a final extension. Sequencing reaction was performed on an ABI PRISM 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) in both directions. cox1 sequences of 7 congeneric species from GenBank were aligned with that of F. athadona using Clustal W (Thompson et al., 1994) in MEGA 6.06 software (Tamura et al., 2013). Genetic distances were calculated using the Kimura-2-parameter model (K2P; Kimura, 1980). The neighbor-joining tree (Saitou & Nei, 1987) was constructed with 1,000 replications of bootstrap analysis using K2P in MEGA.

The specimens examined were deposited in the Marine Mollusk Resource Bank of Korea (MMRBK) in Seoul, Korea and the National Institute of Biological Resources (NIBR) in Incheon, Korea. The mtDNA *cox1* sequence obtained in this study was deposited in GenBank (Accession No. KT724949).

#### Systematic accounts

Phylum Mollusca Linnaeus, 1758 연체동물문 Class Gastropoda Cuvier, 1795 복족강 Order Nudibranchia Cuvier, 1817 나새목 Family Flabellinidae Bergh, 1889 꼭지도롱이갯민숭이과 <sup>1\*</sup>Genus *Flabellina* Gray, 1833 꼭지도롱이갯민숭이속 (신칭)

# Key to the species of the genus *Flabellina* Gray, 1833 from Korea

- 3. Short papilla present on each rhinophoral apex ..... F. bicolor
- Elongate papilla present on each rhinophoral apex
- 4. Three longitudinal red lines present on dorsum and both side of body .....F. rubrolineata
- Longitudinal line absent on body ..... F. exoptata

# <sup>2\*</sup>Flabellina athadona (Bergh, 1875) (Fig. 1)

### 2\*흰점박이도롱이갯민숭이(신칭)

Coryphella athadona Bergh, 1875: 635-638, pl. 13, figs. 1-13; Baba, 1935: 123, 124, pl. 8, figs. 6-8; 1940: 107, 108, text-fig. 7; Abe, 1964: 61, pl. 30, fig. 106; Baba, 1987: 151-156, figs. 1-3; Okutani, 2000: 803, pl. 399, fig. 1.

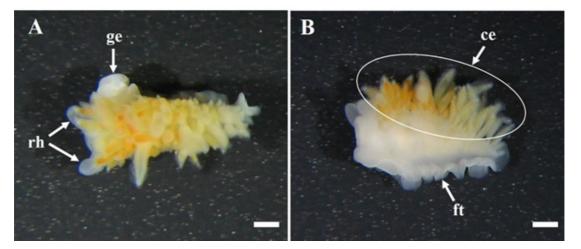
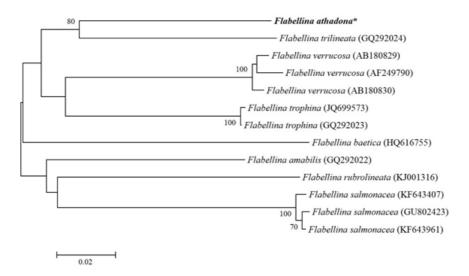


Fig. 1. Flabellina athadona (Bergh, 1875), preserved specimen. A. Dorsal view; B. Lateral view. ge = genital orifices; rh = rhinophore; ce = cerata; ft = foot. Scale bar: 1 mm.



**Fig. 2**. Unrooted phylogenetic tree from neighbor-joining method using K2P parameters for the mtDNA *cox1* partial sequences of 8 *Flabellina* species. Bootstrap values of  $\geq$  70% are indicated on the branches and GenBank accession numbers are shown in parentheses after the species names. \*the *cox1* sequence obtained in this study.

*Flabellina athadona*: Rudman, 1999; Martynov, 2013: 117, table 1.

Material examined. Korea: 15 individuals, Gangwon-do, Yangyang-gun, Hyeonnam-myeon, Namae-ri, 25 March 2015.

Description. Body aeolidiform, elongate and narrow in width (length 3-7 mm, width 1-3 mm; preserved specimens) (Fig 1A, B). Ground color opaque white. Oral tentacles and rhinophores simple, with smoothed surface, pointed end, and translucent white in color. Length of rhinophores shorter than oral tentacles. Numerous cerata clustered on dorsolateral side of the body, generally fusiform, but rarely conical shaped. Digestive glands of each ceras deep orange in color. Cnidosacs on tip of each ceras, opaque white in color. Genital orifices situated one-fifth of body length from anterior end on the dextral side of the body. Anus located behind the genital opening. Foot narrowed, metapodium long and blunted, propodeal tentacles present on foot corners. White specks irregularly scattered on whole body. White lines from tips of each oral tentacle converging between basal oral tentacles and base of rhinophores, converged line running to the metapodium end, reversed Y-shaped line present, the line sometimes disconnected or unclear.

Distribution. Korea and Japan.

Remarks. Flabellina athadona is very similar to its congener F. amabilis Hirano & Kuzirian, 1991. It is distinguished by white markings on the body and the shape of the foot corners. F. athadona has a white Y-shaped marking between the rhinophores and oral tentacles and a foot with rounded corners. In contrast, F. amabilis has no head markings and has small and pointed foot corners (Hirano & Kuzirian, 1991). Otherwise these two species are morphologically very similar, but they can be clearly distinguished by molecular analysis of cox1 partial sequences. Genetic divergence between F. athadona and F. amabilis (GQ292022) measured using the Kimura-2-parameter model was 16.5%. The unrooted phylogenetic tree using the neighbor-joining method showed that F. athadona was clustered with F. trilineata (GQ292024) with 80% of bootstrap branch support, and separated from F. amabilis (GQ292022) (Fig. 2).

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