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Dendrodoris guttata (Nudibranchia: Dendrodorididae) from Korean Waters

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ABSTRACT

The genus *Dendrodoris* Ehrenberg, 1831 includes about 46 valid species worldwide, and is found in relatively shallow waters in the Indo-Pacific, Atlantic, and Australian regions. To date, five *Dendrodoris* species have been reported from Korea. In this study, we report *D. guttata* (Odhner, 1917) collected from Jeju Island. Morphology is distinguished from other *Dendrodoris* species by the shape of the black spots on the dorsal mantle and coloration of the gills. We determined mitochondrial cytochrome *c* oxidase I (*cox1*) partial sequences and combined them with publically available sequences of closely related congeneric species to examine its phylogenetic position among *Dendrodoris* species.

Keywords: Dendrodorididae, *Dendrodoris*, radula-less dorid, mtDNA *cox1*, Korea

INTRODUCTION

The nudibranch genus *Dendrodoris* Ehrenberg, 1831 is the type genus of the family Dendrodorididae and includes about 46 species (Bouchet, 2018) distributed across the Indo-Pacific region, Atlantic Ocean, and Australia (Valdés et al., 1996; Helmut and Rudie, 2007). Species in this genus display a variety of body colorations and lack a radula, instead having a highly modified foregut adapted for suctorial feeding on sponges (Alder and Hancock, 1864; Young, 1969; Valdés and Gosliner, 1999; Brodie, 2001). Five species of *Dendrodoris* have previously been reported from Korean waters: *D. arborescens* (Collingwood, 1881), *D. fumata* (Rüppell and Leuckart, 1830), *D. krusensternii* (Gray, 1850), *D. nigra* (Stimpson, 1855), and *D. tuberculosa* (Quoy & Gaimard, 1832) (see Choe and Lee, 1994; Lee and Min, 2002; Choi, 2003; Min et al., 2004; Koh, 2006). Here, we provide a morphological description of *D. guttata* (Odhner, 1917), newly reported from Korea, and determined partial sequences of mitochondrial *cox1* sequences as DNA barcode references.

MATERIALS AND METHODS

Samples were collected from the underside of rocks in the intertidal zone from Jeju Island, Korea and preserved in 95% ethyl alcohol or in 5% neutral formalin. Morphological characters of external feature were observed using a stereoscopic microscope (Leica M205C; Wetzlar, Germany).

In order to obtain a partial sequence of the mitochondrial cytochrome *c* oxidase subunit I gene, genomic DNA was extracted from foot tissue using an E.Z.N.Z. mollusc DNA kit (OMEGA Bio-tek, Norcross, GA, USA). Polymerase chain reaction (PCR) was performed with TaKaRa Ex Taq (Takara Bio, Shiga, Japan) in reactions containing 31.75 μL distilled water, 5 μL 10 × Ex Taq buffer, 4 μL dNTP mixture (2.5 mM each), 2 μL of each universal primer (LCO1490, HCO2198) (Folmer et al., 1994), 0.25 μL of TaKaRa Ex Taq, and 5 μL of template DNA to total 50 μL. PCR amplification conditions were an initial denaturation at 95°C for 1 min, followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 48°C for 30 sec, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The amplified PCR products were purified using a QIAquick gel extraction kit (Qiagen, Valencia, CA, USA). Sequencing in both directions was performed using an ABI PRISM 3730xl DNA analyzer (Applied Biosys-

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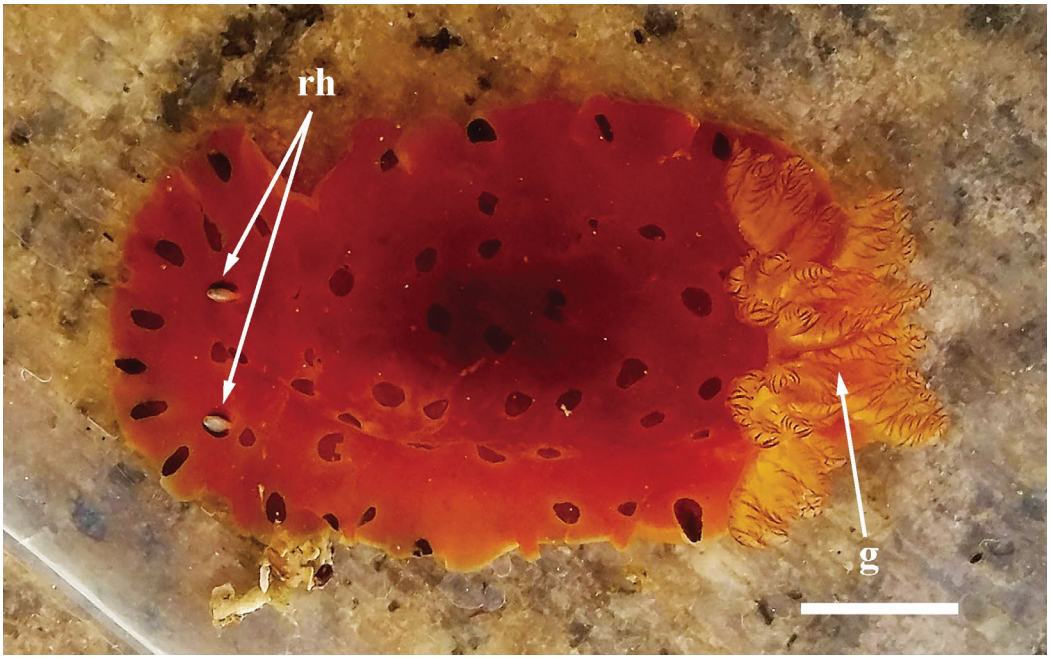

Fig. 1. *Dendrodoris guttata* (Odhner, 1917). rh, rhinophore; g, gill. Scale bar = 5 mm.

tems, Foster City, CA, USA). The *cox1* sequences of 8 congeneric species and 1 previously sequenced *D. guttata* individual from GenBank (accession Nos. AB917441, AB917443, AB917446, AB917447, AB917456, GQ292043, KJ001303, KT833268, and MF958434) were aligned with sequences from *D. guttata* specimens determined in the present study (accession Nos. MG948855, and MG948856) using Geneious version 11.0.4 (Geneious) (Kearse et al., 2012). A phylogenetic tree was reconstructed in MEGA 7 (Kumar et al., 2016) using the neighbor-joining method with 1,000 bootstrap replications. The specimens were deposited in the Marine Mollusk Resource Bank of Korea (MMRBK) in Seoul, Korea (MMRBK Nos. 00006361 and 00006362) and the National Institute of Biological Resources (NIBR) in Incheon, Korea (NIBR No. VQUMIV0000006457).

SYSTEMATIC ACCOUNTS

Phylum Mollusca Linnaeus, 1758
Class Gastropoda Cuvier, 1795
Order Nudibranchia Cuvier, 1817
Family Dendrodorididae O'Donoghue, 1924 (1864)
Genus *Dendrodoris* Ehrenberg, 1831

[1]**Dendrodoris guttata* (Odhner, 1917) (Fig. 1)

Doridopsis guttata Odhner, 1917: 62, Pl. 2, fig. 71.
Dendrodoris guttata Baba, 1937: 309, Pl. 1, fig. 13; Okutani, 2000: 796–797; Helmut and Rudie, 2007: 260; Nimbs and Smith, 2014: 8, 9; Dixit et al., 2017: 49, fig. 3i.

Material examined. Korea: 4 individuals, Jeju-do, Seogwipo-si, Gangjeong-dong, Gangjeong port, 33°13′29.29″N, 126°28′20.27″E, 30 Jun 2017.
Description. Body soft and elongate-ovate (L = 23.8 mm, W = 21.1 mm) (Fig. 1). Mantle surface smooth; mantle margin thin and wavy. Background color yellowish orange to bright brick color with black spots scattered over entire mantle surface; spots increase in number from margin toward dorsum. Rhinophore club lamellate, black in color with white-colored tip; stalks orange. Oral pore ventral, anterior to foot. Radula absent. Oral tentacle is expanded narrowly to both sides of the oral pore and continuous to oral veil. Gills six and trippinate, forming a circle surrounding anus. Gill colors translucent orange with black axes. Notum somewhat high, and dark red. Mantle entirely covers foot. Sole broad and opaque orange. Genital orifice locates on the right side of the body at one-third of anterior end.
Habitat. Under rocks in the intertidal zone.
Distribution. Australia, Indo-West Pacific, Japan, Korea
Remarks. Like other congeneric species, *D. guttata* lacks radula, but it has a softer, more delicate mantle than other

Korean name: [1]*검정반점갯민숭달팽이(신칭)

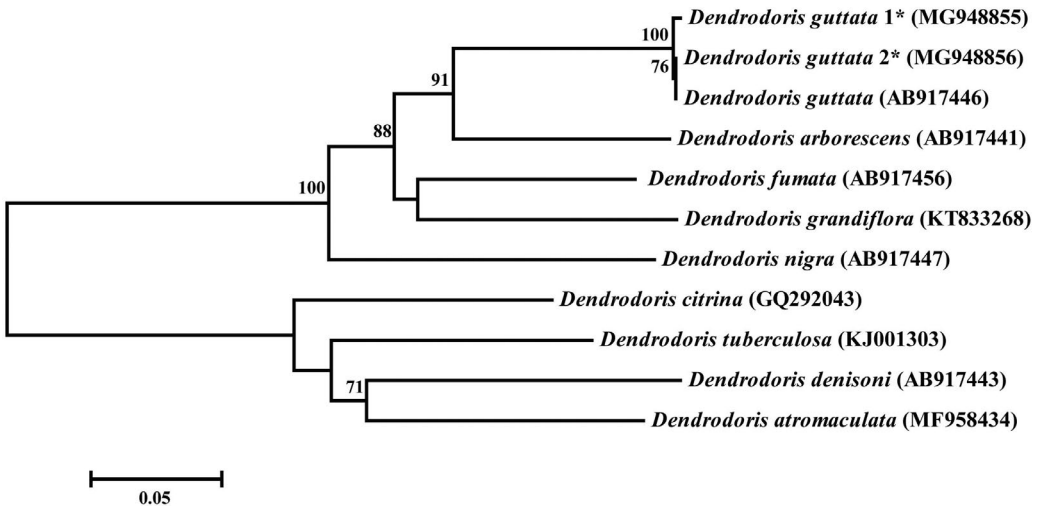

Fig. 2. Unrooted phylogenetic tree for mtDNA *cox1* sequences of *Dendrodoris* species using neighbor-joining method. The tree is drawn to scale with branch lengths, proportional to genetic distances used for phylogenetic analysis. Bootstrap values of ≥70% are indicated on the branches and GenBank accession numbers are shown in brackets after the species name. Asterisks (*) indicate *cox1* sequences obtained in this study.

Dendrodoris species. *Dendrodoris guttata* and *D. fumata* (Ruppell and Leuckart, 1830) are morphologically similar to each other in having a mantle with undulatory margin, well-developed rhinophore club, and trippinate gills (Brodie et al., 1997). However, *D. fumata* has only simple black spots on the dorsal part of the mantle, while *D. guttata* has black spots with a white halo scattered over the entire mantle. In addition, in gill coloration, *D. guttata* is translucent orange with black axes, while the gills of *D. fumata* are the same orange color as their body (Helmut and Rudie, 2007). Although both species are morphologically quite similar, they can be clearly distinguished by molecular analysis using mtDNA *cox1* partial sequences. Genetic divergence between *D. guttata* and *D. fumata* using the Kimura-2-parameter model ranges from 19.4% to 20.4%. The unrooted phylogenetic tree using the neighbor-joining method shows that *D. guttata* is sister to *D. arborescence*, separated from a group where *D. fumata* forms a sister taxa with *D. grandiflora* (Fig. 2).

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