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Redescription of a samlid and three dorid nudibranchs (Nudibranchia: Gastropoda) from South Korea and their COI genetic variation

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ABSTRACT

In this study, four nudibranch species including a samlid and three dorids from Korean waters were redescribed, which were discovered while conducting a domestic nudibranch biodiversity survey from 2021 to 2023: *Samla takashigei* Korshunova, Martynov, Bakken, Evertsen, Fletcher, Mudianta, Saito, Lundin, Schrödl and Picton, 2017, *Diaphorodoris mitsuii* (Baba, 1938), *Atagema intecta* (Kelaart, 1858), and *Martadoris amakusana* (Baba, 1987). In addition, here we provided photographs of living animals and DNA barcode data based on partial sequences of the mitochondrial COI gene. We also analyzed pairwise genetic distances of the COI gene between each species and their congeners, with which we reconstructed neighbor-joining trees to show their phylogenetic relationships and evolutionary distances.

Keywords: *Samla takashigei*, *Diaphorodoris mitsuii*, *Atagema intecta*, *Martadoris amakusana*, Nudibranchia, Korea, COI

INTRODUCTION

Nudibranchs are carnivorous mollusks, represented by 'naked gills', that lose their shells during the developmental stage and are known to inhabit a wide range from the temperate and tropical regions to polar regions (Wägele and Klussmann-Kolb, 2005; Kiko *et al*., 2008; Gosliner *et al*., 2018). More than 3,000 species of nudibranchs have been recorded worldwide (Valdés, 2004; Goodheart, 2017), and 86 species have been recorded in South Korea (National Institute of Biological Resources, 2022). The order Nudibranchia is classified into two suborders, Cladobranchia and Doridina, according to the shape and location of the respiratory structures on their body (Bouchet *et al*., 2017; Gosliner *et al*., 2018). First, Cladobranchia is characterized by the respiratory structures produced by integumentary protrusions and the anus being located on the right side of the body (Willan and Morton, 1984; Goodheart, 2017). Secondly, Doridina is possessed that the gill and anus are located at a postero-median of the body (Thomson and Brown, 1984; Wägele and Willan, 2000).

Nudibranchian species tend to have abounding body colors and unique patterns, which is essential to verify the species-specific colors and patterns on the mantle to identify nudibranch species. However, since these

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characteristics disappear when fixed in ethanol or formalin for a long time (Fahrner and Schrödl, 2000; Jung and Kim, 2023), it is crucial to secure underwater photographs of living individuals. Recently, DNA barcode analyses using the mitochondrial COI gene in mollusks, including nudibranchs, have been widely used (Pola *et al*., 2007, 2012; Elejalde *et al*., 2008; Padula *et al*., 2014; Hirose *et al*., 2015; Sørensen *et al*., 2020).

In this study, we redescribed four nudibranchs including a samlid cladobranch and three dorid nudibranchs with underwater photographs of living animals and DNA barcode data using partial mitochondrial COI gene sequences: *Samla takashigei* Korshunova, Martynov, Bakken, Evertsen, Fletcher, Mudianta, Saito, Lundin, Schrödl and Picton, 2017, *Diaphorodoris mitsuii* (Baba, 1938), *Atagema intecta* (Kelaart, 1858), and *Martadoris amakusana* (Baba, 1987).

MATERIALS AND METHODS

Specimens of four nudibranch species were collected by SCUBA diving while surveying South Korean nudibranch biodiversity from the Southern part of the Korean Peninsula and Jeju Island from Jun 2021 to Oct 2023. The living individuals were photographed underwater (Tg-6; Olympus, Tokyo, Japan) to record their original color patterns, and observed by the unaided eyes and under a stereoscopic microscope (SZ-61; Olympus, Tokyo, Japan). The length of the samlid cladobranch was measured from between the bases of the oral tentacles on the head to the end of the metapodium, and for Dorid, from the anterior mantle to the metapodium. The individuals were fixed in 95% ethyl alcohol. The specimens were deposited at the Honam National Institute of Biological Resources (HNIBR) and the Korea Marine-Bio Laboratory (KOMBI).

Total genomic DNA was extracted from the foot tissue of the fixed specimen following the manufacturer's protocol of the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). Their partial fragments of the mitochondrial COI gene were amplified with a primer set of LCO1490 and HCO2198 (Folmer *et al*., 1994), and deposited into the NCBI GenBank database (OR800617-OR800622). Uncorrected pairwise genetic distances (*p*-distance) were calculated and the neighbor-joining phylogenetic trees (1000 replications of bootstrap; Saitou & Nei, 1987) were constructed using Kimura-2-parameter (Kimura, 1980) distance with MEGA 11 program (Tamura *et al*., 2021).

SYSTEMATIC ACCOUNTS

Phylum Mollusca Linnaeus, 1758 연체동물문
Class Gastropoda Cuvier, 1797 복족강
Order Nudibranchia Cuvier, 1817 나새목
Suborder Cladobranchia Willan and Morton, 1984 갯민숭이아목
Family Samlidae Korshunova, Martynov, Bakken, Evertsen, Fletcher, Mudianta, Saito, Lundin, Schrödl and Picton, 2017 고리꼭지도롱이갯민숭이과
Genus *Samla* Bergh, 1900 고리꼭지갯민숭이속

***Samla takashigei* Korshunova, Martynov, Bakken, Evertsen, Fletcher, Mudianta, Saito, Lundin, Schrödl and Picton, 2017 주홍고리도롱이갯민숭이(신칭) (Figs. 1A, 2A)**

Samla takashigei Korshunova *et al*., 2017: 62-63, fig. 47; Ota *et al*., 2021: 32, fig. 16A.

Type locality. Osezaki, Japan.

Distribution. Korea and Japan.

Specimens examined. South Korea: 1 specimen, Jeju-do, Seogwipo-si, Seogwi-dong, Munseom Islet, 17 XI 2021, D-W Jung (HNIBRIV2430); 3 specimens, Jeollanam-do, Yeosu-si, Samsan-myeon, Geomundo Island, 25 X 2023, D-W Jung (KOMBI-KM117, 118, 119).

Measurement. Body length 8-42 mm in living specimens.

Diagnosis. Body elongated and narrow (Fig 1A). Ground color pale bluish translucent white. Rhinophoral clavus perfoliate, pale orange; tip of each clavus deep orange, stalk smooth and translucent white. Seven to eight rows of ceratal clusters present on dorso-lateral body, except for mid-dorsal surface; anterior first to third or fourth rows with eight, next two rows with four, and last two rows with two cerata on each row. Each ceras finger-shaped, pointed or

Fig. 1. Photographs of four living nudibranchs. **A**. Dorsal view of *Samla takashigei*; **B**. Dorsal view of *Diaphorodoris mitsuii*; **C**. Dorsal view of *Atagema intecta*; **D**. Dorso-lateral view of *Martadoris amakusana*.

blunt end, and elevated; tip of each ceras pale orange with deep orange sub-apical ringed marking; digestive glands on each ceras yellowish white to brown. Notal edge present below the cerata. Oral tentacles elongated and smooth, four to five times longer than rhinophores. Foot corners relatively long, similar to rhinophores in length. Oral tentacles and foot corners opaque white. Gonophore located between first and second ceratal clusters.

GenBank Accession No. OR800620

Remark. Pairwise genetic distances (*p*-distances) were calculated and an unrooted phylogenetic tree was reconstructed by constructing a sequence alignment based on 658 bp of partial COI gene sequences from a total of eight individuals including four species of the genus *Samla* (Table 1; Fig. 2A). The *p*-distances within the genus *Samla* ranged from 0.3% to 22.4%. The intraspecific *p*-distance of *S. takashigei* was 0.8% (between the individuals from South Korea and Japan), and the *p*-distances to congeners ranged from 17.4% (between *S. takashigei* and *S. bilas*) to 22.4% (between *S. takashigei* from Korea and *S. riwo*).

In the constructed phylogenetic tree, the members of *S. bicolor* are not monophyletic and split into three different clades (Fig. 2A). The intraspecific *p*-distances within *S. bicolor* ranged from 0.3% (between the individuals of *S. bicolor* from Hawaii; MW278644 and MW278850) to 19.9% (between those of *S. bicolor* from Vietnam and the Philippines; KY129050 and MF523383). Previously, Korshunova *et al.* (2017) suggested that *S. bicolor* is a species complex, and reported *S. takashigei* as a new species isolated from *S. bicolor* species complex. The specimen examined in this study was clustered with *S. takashigei* (MF523384) with a 100% bootstrap value, and this *S. takashigei* group was located in a separate group from other congeners. Considering that the relatively high intraspecific variation within *S. bicolor* is somewhat greater than the genetic divergence of previous studies on nudibranchs and mollusks based on the COI gene (Palomar *et al.*, 2014; Hirose *et al.*, 2015; Sørensen *et al.*, 2020), there is still a possibility that *S. bicolor* is a

Table 1. Uncorrected *p*-distances (%) of the partial mitochondrial COI gene (658bp) among the four *Samla* species

No.	Species	Locality	1	2	3	4	5	6	7	8	GenBank accession No.	References
1	*Samla takashigei*	Korea									OR800620	This study
2	〃	Japan	0.8								MF523384	Korshunova *et al.*, 2017
3	*Samla bilas*	Philippines	17.4	17.4							KY129051	Cella *et al.*, 2016
4	*Samla bicolor*	Hawaii	20.0	19.4	19.6						MW278850	Unpublished
5	〃	〃	20.0	19.4	19.4	0.3					MW278644	〃
6	〃	Vietnam	20.2	20.4	21.0	12.2	12.2				MF523383	Korshunova *et al.*, 2017
7	〃	Philippines	20.3	20.3	17.3	17.9	17.9	19.9			KY129050	Cella *et al.*, 2016
8	*Samla riwo*	Maldives	22.4	22.1	22.0	22.1	22.1	22.4	22.3		OQ207010	Cunha *et al.*, 2023

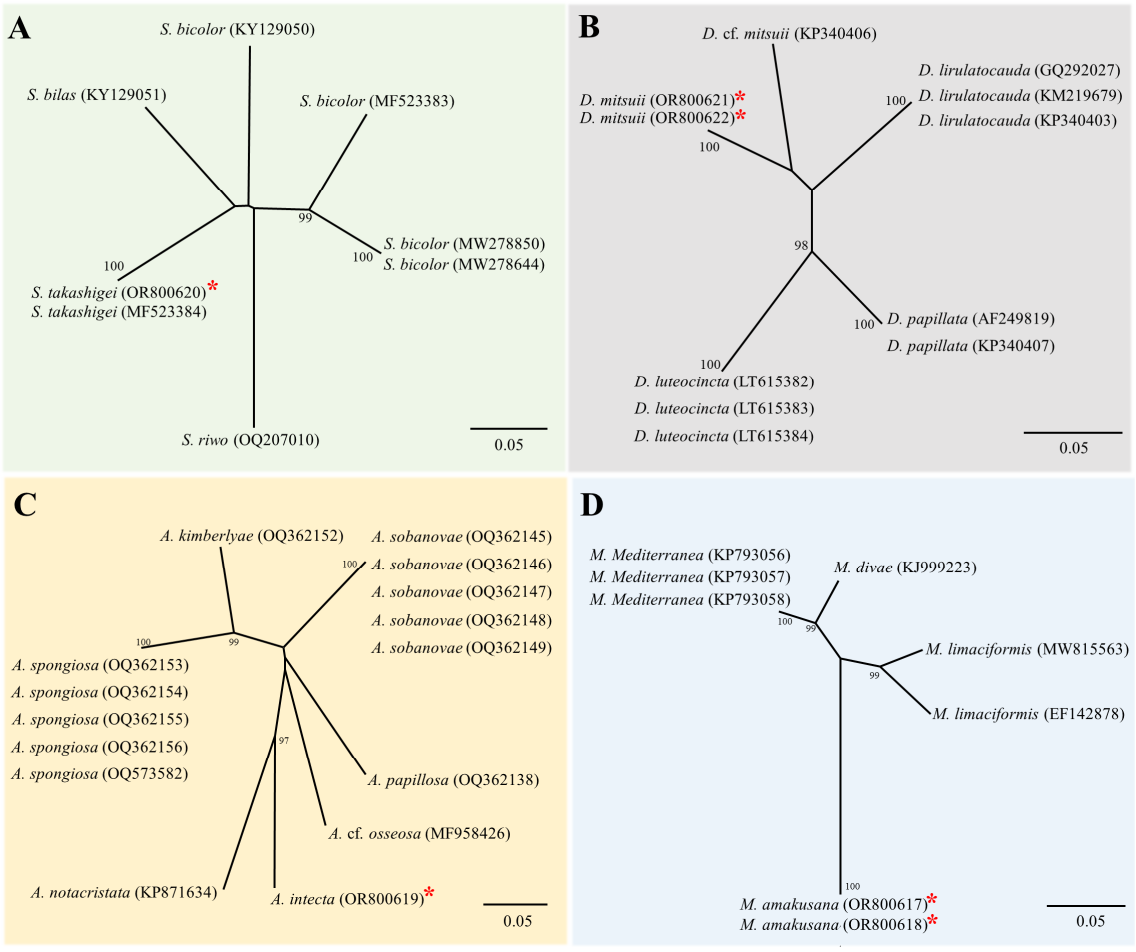

Fig. 2. Neighbor-joining phylogenetic trees for each genus using partial COI sequences. Bootstrap values of ≥ 75% are indicated above or below the branches and GenBank accession numbers are shown in parentheses after species. The sequences examined in this study are denoted by a red asterisk. **A**. Genus *Samla*; **B**. Genus *Diaphorodoris*; **C**. Genus *Atagema*; **D**. Genus *Martadoris*.

species complex though *S. takashigei* was isolated.

Suborder Doridina Odhner, 1934 갯민숭달팽이아목

Family Calycidorididae Roginskaya, 1972 테고무신갯민숭달팽이과

Genus *Diaphorodoris* Iredale and O'Donoghue, 1923 테고무신갯민숭달팽이속

***Diaphorodoris mitsuii* (Baba, 1938) 테고무신갯민숭달팽이 (Figs. 1B, 2B)**

Lamellidoris (*Lamellidoridella*) *mitsuii* Baba, 1938: 130-131, fig. 1; 1949: 137-138, pl. 14, fig. 51, text-fig. 44. *Diaphorodoris mitsuii* Millen, 1985: 90-91; Baba, 1988: 83-87, figs. 1-5; Okutani, 2000: 783; Gosliner *et al*., 2008: 140; 2015: 146; 2018: 64; Kil *et al*., 2020: 212-213; Chow *et al*., 2022: 17, fig. 6I.

Type locality. Shizuoka, Japan.

Distribution. Taiwan, China, Korea, Japan, Papua New Guinea, and Australia (Chow *et al*., 2022).

Specimens examined. South Korea: 2 specimens, Gyeongsangbuk-do, Pohang-si, Nam-gu, Guryongpo-eup, Jangil-ri, 9 V 2022, D-W Jung (HNIBRIV2428, KOMBI-KM120).

Measurement. Body length 5-8 mm in living specimens.

Diagnosis. Body elongated ovate (Fig. 1B). Ground color translucent to opaque white or yellowish green. Rhinophores relatively long; rhinophoral clavus perfoliate, tip of each clavus opaque white, stalk cylindrical. Minute and conical nodules cover entire dorsum. Continuous marginal band present along

Table 2. Uncorrected *p*-distances (%) of the partial mitochondrial COI gene (560bp) among the five *Diaphorodoris* species

No.	Species	Locality	1	2	3	4	5	6	7	8	9	10	11	GenBank accession No.	References
1	*D. mitsuii*	Korea												OR800621	This study
2	〃	〃	0.9											OR800622	〃
3	*D.* cf. *mitsuii*	Philippines	11.0	11.4										KP340406	Hallas & Gosliner, 2015
4	*D. lirulatocauda*	USA	13.3	13.3	15.2									KP340403	〃
5	〃	〃	13.5	13.5	14.9	1.6								GQ292027	Unpublished
6	〃	〃	13.7	13.7	14.8	0.6	1.0							KM219679	Hallas & Gosliner, 2015
7	*D. papilata*	Spain	14.8	15.5	13.1	15.4	15.7	15.4						KP340407	〃
8	〃	〃	15.0	15.7	14.1	15.1	15.7	15.4						AF249819	Wollscheid-Lengeling *et al.*, 2001
9	*D. luteocincta*	Italy	17.9	18.2	16.9	17.5	16.4	17.5	12.8	12.1				LT615382	Furfaro *et al.*, 2016
10	〃	〃	18.2	18.4	17.1	17.7	16.4	17.7	12.6	11.9	0.2			LT615383	〃
11	〃	〃	18.2	18.4	17.1	17.7	16.4	17.7	12.6	11.9	0.2	0.0		LT615384	〃

mantle margin and foot margin, fluorescent yellow in color. Sub-marginal band orange in color. Gill with five to six gill plumes, simply pinnated. Rhinophoral sheath and gill sheath indistinct. Metapodium relatively long with distinct mid-dorsal ridge.

GenBank Accession No. OR800621, OR800622

Remark. This species is found on the rock surface covered with fine sediment. The specimens examined in this study are consistent in all characteristics with the original description and other previous records (Baba, 1938; 1949; 1988; Millen, 1985; Okutani, 2000; Goliner *et al*., 2008; 2015; 2018; Kil *et al*., 2020; Chow *et al*., 2022).

The *p*-distances were calculated and an unrooted phylogenetic tree was reconstructed by constructing a sequence alignment based on 560 bp of partial COI gene sequences from a total of eleven individuals including five species of the genus *Diaphorodoris* (Table 2, Fig. 2B). The *p*-distances within the genus *Diaphorodoris* ranged from 0.0% to 19.2%. The intraspecific *p*-distance of *D. mitsuii* was 0.9% (between two individuals from South Korea; OR800621-2), and the *p*-distances to congeners ranged from 11.0% (between *D. mitsuii* and *D.* cf. *mitsuii*; OR800621-2 and KP340406) to 18.2% (between *D. mitsuii* and *D. luteocincta*; OR800621-2 and LT615382-4). The phylogenetic tree shows that *D. mitsuii* is placed in a distinct group with their congeners with 100% bootstrap support value (Fig 2B).

Family Discodorididae Bergh, 1891 구름갯민숭달팽이과

Genus *Atagema* Gray, 1850 해면갯민숭달팽이속

***Atagema intecta* (Kelaart, 1858) 갈색해면갯민숭달팽이 (Figs. 1C, 2C)**

Doris intecta Kelaart, 1858: 302.

Trippa ornata Bergh, 1877: 543-546, taf. 58, figs. 3-8.

Trippa intecta: Baba, 1949: 64, 151, pl. 24, fig. 89, text-fig. 78; Okutani, 2000: 795.

Atagema intecta: Gosliner *et al*., 2008: 184; 2015: 191; 2018: 114; Chow *et al*., 2022: 14, fig. 5L.

Type locality. Sri Lanka.

Distribution. East Africa, Korea, Japan, Vanuatu, New Caledonia, and Fiji (Chow *et al*., 2022).

Specimen examined. South Korea: 1 specimen, Gyeongsangnam-do, Tongyeong-si, Sanyang-eup, Youngun-ri, 20 VI 2021, D-W Jung (HNIBRIV2429).

Measurement. Body length 39 mm in a living specimen.

Diagnosis. Body ovate. Ground color reddish brown (Fig. 1C). Rhinophores lamellate, clavus reddish brown, tip of each clavus and stalk translucent brown. Spiculated tubercles present on dorsum; round, lobulated, and ragged in shape, tubercles covered by caryophyllidia, decrease in size and increase in

Table 3. Uncorrected *p*-distances (%) of the partial mitochondrial COI gene (658bp) among the six *Atagema* species

No.	Species	Locality	1	2	3	4	5	6	7	8	9	10	11	12	13	14	GenBank accession No.	References
1	*A. intecta*	Korea															OR800619	This study
2	*A. notacristata*	Panama	17.4														KP871634	Mahguib and Valdés, 2015.
3	*A. sobanovae*	New Caledonia	20.2	22.6													OQ362148	Innabi *et al.*, 2023
4	〃	〃	20.2	22.6	0.0												OQ362149	〃
5	〃	〃	20.4	22.8	0.2	0.2											OQ362145	〃
6	〃	〃	20.7	23.0	0.3	0.3	0.2										OQ362146	〃
7	〃	〃	20.7	23.0	0.3	0.3	0.2	0.0									OQ362147	〃
8	*A. papillosa*	〃	22.7	20.4	17.6	17.6	17.8	17.8	17.8								OQ362138	〃
9	*A. kimberlyae*	〃	23.6	22.9	16.3	16.3	16.1	15.9	15.9	20.0							OQ362152	〃
10	*A. spongiosa*	〃	23.5	25.9	20.8	20.8	21.0	21.2	21.2	20.3	12.7						OQ362154	〃
11	〃	〃	23.5	25.9	21.2	21.2	21.4	21.6	21.6	20.3	12.7	0.6					OQ573582	〃
12	〃	〃	23.5	25.9	21.2	21.2	21.4	21.6	21.6	20.3	12.7	0.6	0.0				OQ362156	〃
13	〃	〃	23.7	25.2	20.9	20.9	21.2	21.4	21.4	19.8	12.9	1.1	0.8	0.8			OQ362153	〃
14	〃	〃	23.7	25.7	20.7	20.7	20.9	21.2	21.2	20.0	12.3	0.9	0.3	0.3	1.1		OQ362155	〃

number toward mantle margin. A distinct white stripe on mid-dorsal ridge; from between rhinophores to gill. Gill with six tripinnated gill plumes. Rhinophoral sheath and gill sheath distinct and tuberculated. Metapodium short with blunt end.

GenBank Accession No. OR800619

Remark. This species can be easily distinguished with congeners based on a mid-dorsal white stripe marking from between the rhinophores to the gills, and distinct caryophillidia on dorsal tubercles. The specimen examined in this study are consistent in all characteristics with the original description and other previous records (Kelaart, 1858; Bergh, 1877; Baba, 1949; Okutani, 2000; Goliner *et al*., 2008; 2015; 2018; Chow *et al*., 2022).

The *p*-distances were calculated and an unrooted phylogenetic tree was reconstructed by constructing a sequence alignment based on 658 bp of partial COI gene sequences from a total of fourteen individuals including six species of the genus *Atagema* (Table 3, Fig. 2C). The *p*-distances within the genus *Atagema* ranged from 0.0% to 25.9%. The calculated interspecific *p*-distance of the specimen examined ranged from 17.4% (*A. notacristata*; KP871634) to 23.7% (*A. spongiosa*; OQ362153 and OQ362155). The phylogenetic tree shows that *A. intecta* is placed in a distinct group with long branches from their congeners (Fig. 2C).

Family Polyceridae Alder and Hancock, 1845 능선갯민숭달팽이과

Genus *Martadoris* Willan and Chang, 2017 작은능선갯민숭달팽이속

***Martadoris amakusana* (Baba, 1987) 작은능선갯민숭달팽이 (Figs. 1D, 2D)**

Tambja amakusana Baba, 1987: 15-17, fig. 3; Gosliner *et al*., 2008: 121; 2015: 128; 2018: 45.

Martadoris amakusana: Willan and Chang, 2017: 18; Kil *et al*., 2020: 260-261; Nimbs *et al*., 2020: 18, fig. 2H.

Type locality. Kumamoto, Japan.

Distribution. Korea, Japan, and Australia.

Specimens examined. South Korea: 2 specimens, Gyeongsangbuk-do, Pohang-si, Nam-gu, Guryongpo-eup, Jangil-ri, 9 V 2022, D-W Jung (HNIBRIV2431, KOMBI-KM121).

Measurement. Body length 11-38 mm in living

Table 4. Uncorrected *p*-distances (%) of the partial mitochondrial COI gene (658bp) among the four *Martadoris* species

No.	Species	Locality	1	2	3	4	5	6	7	8	GenBank accession No.	References
1	*M. amakusana*	Korea									OR800617	This study
2	〃	〃	0.3								OR800618	〃
3	*M. mediterranea*	Malta	18.5	18.5							KP793057	Dominguez *et al.*, 2015
4	〃	〃	18.7	18.7	0.2						KP793058	〃
5	〃	Spain	19.1	19.1	0.8	0.6					KP793056	〃
6	*M. divae*	Brazil	18.9	18.9	5.1	5.3	5.7				KJ999223	Pola *et al.*, 2014
7	*M. limaciformis*	Mauritius	19.5	19.1	10.5	10.7	11.0	10.5			MW815563	Ah Shee Tee *et al.*, 2023
8	〃	Japan	20.5	20.1	11.7	11.9	12.3	11.4	7.0		EF142878	Pola *et al.*, 2007

specimens.

Diagnosis. Body limaciform. Ground color yellow to green (Fig. 1D). Head slightly round. Rhinophoral clavus perfoliate, tip of each rhinophore bluish purple, color of remaining rhinophore ranges from translucent white to dark blue. Mantle with many wrinkles on entire mantle. Gill with five, simple or bipinnated branchial plumes, tip of each branchial plumes purple, rest of gill plumes translucent white to bluish purple. Oral tentacles flat and bluish purple. Occasionally, rhinophoral sheath, wrinkles on mantle, and posterior mantle bluish purple.

GenBank Accession No. OR800617, OR800618

Remark. *Martadoris amakusana* (Baba, 1987), examined in this study, is the type species of the genus *Martadoris* Willan and Chang, 2017. The genus *Martadoris* was proposed as a new genus based on the following characteristics: an elongated sac between the oral tube and the buccal bulb, square rachidian teeth, and a bifid cusp on the inner lateral teeth (Willan and Chang, 2017). The type species of the genus *Martadoris* was initially recorded as *Palio amakusana* Baba, 1960, but this was a mistake by Willan and Chang (2017) and was corrected to *Tambja amakusana* Baba, 1987 as a type species of the genus *Martadoris* (Willan and Chang, 2018).

The *p*-distances were calculated by constructing a sequence alignment based on 658 bp of partial COI gene sequences from a total of eight individuals including four species of the genus *Martadoris* (Table 4). The p-distances within the genus *Martadoris* ranged from 0.2% to 20.5%. The intraspecific *p*-distance of *M. amakusana* was 0.3% (between the individuals from South Korea; OR800617-8). The calculated interspecific *p*-distance of *M. amakusana* ranged from 18.5% (*M. mediterranea*; KP793057) to 20.5% (*M. limaciformis*; EF142878). The phylogenetic tree shows that *M. amakusana* is positioned in a distant group with long branches from their congeners and has a bootstrap support value of 100% (Fig. 2D).

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