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Short communication

A New Record of *Phyllidia varicosa* (Nudibranchia: Phyllidiidae) from Korea

Dae-Wui Jung[1], Chang-Bae Kim[2,*]

[1]Korea Marine-Bio Laboratory, Daejeon 34130, Korea
[2]Department of Biotechnology, Sangmyung University, Seoul 03016, Korea

ABSTRACT

In this article, a phyllidiid nudibranch which is distributed widely in the Indo-Pacific region, *Phyllidia varicosa* Lamarck, 1801, is reported based on a specimen collected from Seopseom Islet, Jeju Island, Korea. This species is characterized by bluish-gray dorsal tubercles with a yellow cap and three distinct ridges consisting of dorsal tubercles going from the anterior to posterior region, the presence of bluish-black pigment between the dorsal ridges, and a characteristic black longitudinal stripe along the midline of the sole. In this study, we provide a key to species belonging to the genus *Phyllidia* discovered in Korea, the morphological descriptions, photographs, and a sequence of partial mitochondrial cytochrome *c* oxidase subunit I of *P. varicosa*. Currently, four species of the genus *Phyllidia* have been reported to be present in Korea, including *P. varicosa*.

Keywords: *Phyllidia varicosa*, new record, DNA barcode, Nudibranchia, Korea

INTRODUCTION

The genus *Phyllidia* Cuvier, 1797 possesses a characteristic prominent dorsal tubercle, often accompanied by longitudinal ridges, distinct oral tentacles, and a large and protruding glandular body on the pharyngeal bulb (Gosliner et al., 2018). This genus was established on the basis of a single specimen without a scientific name, which was assumed to be a taxon close to limpet and chiton (Cuvier, 1797). Lamark (1801) recorded the scientific name of the specimen unnamed by Cuvier (1797) as *Phyllidia varicosa* Lamarck, 1801, and subsequently identified it as a type species of the genus *Phyllidia*. This specimen was later designated as *P. trilineata* by Cuvier (1804a). However, Blainville (1816), considered this specimen to be a junior synonym of *P. varicosa*. Rafinesque (1814) selected *Phyllidia* Cuvier, 1797 as a type genus of a newly proposed family, the Phyllidiidae.

Phyllidia varicosa is one of the most frequently studied phyllidiid nudibranch located in the Indo-Pacific region (Gosliner et al., 2018; Papu et al., 2022). This species is associated with a distinctive odor due to the species-specific chemicals they secrete, which are known to destroy other aquarium creatures (Gosliner et al., 2018). Several biochemical synthetic substances have been isolated from this species (Burreson et al., 1975; Fisch et al., 2017).

Twenty-eight valid species belonging to the genus *Phyllidia* have been recorded globally (MolluscaBase, 2023) until now, and three species among these have been reported from Korea, these species being *Phyllidia babai* Brunckhorst, 1993, *Phyllidia ocellata* Cuvier, 1804 and *Phyllidia picta* Pruvot-Fol, 1957 (Choe and Lee, 1997; Jung et al., 2014; Kil et al., 2020; National Institute of Biological Resources, 2022). We have recently added *P. varicosa* to the Korean fauna with its morphological description and DNA barcode data in this paper. Furthermore, a key to species belonging to the genus *Phyllidia* in Korea is provided.

During the ongoing faunal study of Korean nudibranchs, one organism of *P. varicosa* was collected from depths of 17 m during scuba diving at Seopseom Islet, Seogwipo-si, Jeju Island. Morphological characteristics were observed under a dissecting microscope (SZ-61; Olympus, Tokyo, Japan). The specimen was preserved in 94% ethanol and deposited at the National Institute of Biological Resources, Incheon (NIBR).

Genomic DNA was extracted from the foot tissue using a DNeasy blood and tissue kit (Qiagen, Hilden, Germany) following the protocol described by the manufacturer. DNA con-

*To whom correspondence should be addressed
Tel: 82-2-2287-5288, Fax: 82-2-2287-0070
E-mail: evodevo@smu.ac.kr

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centration and purification were quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, USA). Universal cytochrome *c* oxidase subunit I (COI) primers (LCO1490 and HCO2198) (Folmer et al., 1994) were used for amplification of the mitochondrial COI gene. The PCR analysis was carried out under the following time and temperature specifications: 95°C for 5 min, 95°C for 1 min, 42°C for 45 s, and 72°C for 2 min, followed by 35 cycles, and final elongation was carried out at 72°C for 10 min. The PCR product was purified using a QIAquick PCR Purification Kit (Qiagen). Sequencing of the purified PCR product was carried out by using an ABI Prism 3730XL DNA Analyzer (Applied Biosystems, Foster City, USA). Each primer was removed and translated into protein to avoid nuclear mitochondrial DNA segments (numts) to secure an open reading frame in Geneious Pro R11 (Kearse et al., 2012). Sequences of this species were obtained from GenBank and aligned through the Muscle algorithm (Edgar, 2004). Intra- and interspecific genetic distances were calculated using the Kimura-2 parameter model (Kimura, 1980), including 1,000 bootstrap replications on the MEGA11 program (Tamura et al., 2021).

SYSTEMATIC ACCOUNTS

[1]*Phylum Mollusca Linnaeus, 1758
[2]*Class Gastropoda Cuvier, 1797
[3]*Order Nudibranchia Cuvier, 1817
[4]*Family Phyllidiidae Rafinesque, 1814

[5]*Genus *Phyllidia* Cuvier, 1797

Type species. *Phyllidia varicosa* Lamarck, 1801, by subsequent designation (Brunckhorst, 1993).

Key to the species of the genus *Phyllidia* Cuvier, 1797 from Korea

1. Ground color white to yellow ····· 2
− Ground color bluish-gray to black ····· 3
2. 21−24 lamellae on rhinophoral clavus····· *Phyllidia babai*
− 27−30 lamellae on rhinophoral clavus····· *Phyllidia ocellata*
3. Independent dorsal tubercles present in an arrangement of one median and two mediolateral, and dorsal ridges absent ····· *Phyllidia picta*
− Dorsal tubercles arrange in multiple rows, and several longitudinal dorsal ridges present····· *Phyllidia varicosa*

[6]****Phyllidia varicosa*** **Lamarck, 1801 (Fig. 1)**

Phyllidia varicosa Lamarck, 1801: 66; Cuvier, 1804a: 268; Eliot, 1910: 435; Pruvot-Fol, 1957: 105; Brunckhorst, 1993: 26−29, figs. 2, 4−6, 23, 24, pl. 1A−D; Fahrner & Schrödl, 2000: 165−168, figs. 1A−F, 2A, B; Okutani, 2000: 795; Gosliner et al., 2008: 284; 2018: 210.
Phyllidia trilineata Cuvier, 1804a: 268, pl. A, figs. 1−6; 1804b: 277.
Phyllidia borbonica Cuvier, 1804b: 277.
Phyllidia quinquelineata Blainville, 1816: 52; 1826: 99.
Phyllidia arabica Ehrenberg, 1831: pages unnumbered (cited from Fahrner and Schrödl, 2000).
Phyllidia fasciolata Bergh, 1869: 507, 508 (cited from Brunckhorst, 1993).
Phyllidia honloni Risbec, 1956: 22−24, figs. 71−75, 79−81.
Phyllidia sp. Yonow, 1986: 1415, fig. 6b.
non *Phyllidia varicosa* Gosliner, 1987: 90, fig. 152 (= *Phyllidia coelestis* Bergh, 1905; cited from Yonow, 2012).

Material examined. One individual, Korea: Jeju-do, Seogwipo-si, Seopseom Islet (33°13′50″N, 126°36′03″E), 23 May 2021, Jung DW, collected by scuba from the rocky area of the subtidal zone, deposited in NIBR (NIBRIV0000893845).

Description. Body ovate and elongate, body length 59 mm in preserved specimen (Fig. 1A). Ground color bluish gray. Rhinophores in color with yellow, clavus lamellated, and stalk smooth. Various sized and shaped of numerous tubercles present on dorsum; shapes of tubercles single rounded, single angular, conical, and compound; most of prominent tubercles had a yellow cap. Base of tubercles bluish-gray. Tubercles at median dorsal surface generally large in size and size decreased toward edge of mantle. Tubercles on median dorsum form three ridges running from anterior to posterior dorsum. Few tubercles on mantle edge form several arranged in clusters. Black pigment present between dorsal ridges and between these clusters. Anus located five-sixths on posterior body and slightly opened (Fig. 1C). Foot gray in color, with a single black longitudinal marking present along midline of sole (Fig. 1B). Anterior foot bilabiate. Oral tentacles gray and blunt end, tip of oral tentacles pale yellow. Flat and triangular respiratory structures present on hyponotum (Fig. 1D). Oral tube passes through gray pharyngeal bulb, and connects to esophagus, which leads to digestive gland mass (Fig. 2A). Length of digestive gland about three-fifths of body length. Compartments of digestive gland mass indistinct. Nidamental glands round in shape, distinctly yellow, and located below esophagus. Reproductive system triaulic, situated between pharyngeal mass and digestive gland (Fig. 2B). Vas deferens relatively short, connects to prostate gland. Prostate gland relatively narrow and curved several times, connects with ampulla. Ampulla approximately four times as wide as prostate gland. Vagina approximately two times as wide as vas

Korean name: [1]*연체동물문, [2]*복족강, [3]*나새목, [4]*혹갯민숭이과, [5]*혹갯민숭이속, [6]*노랑혹갯민숭달팽이 (신칭)

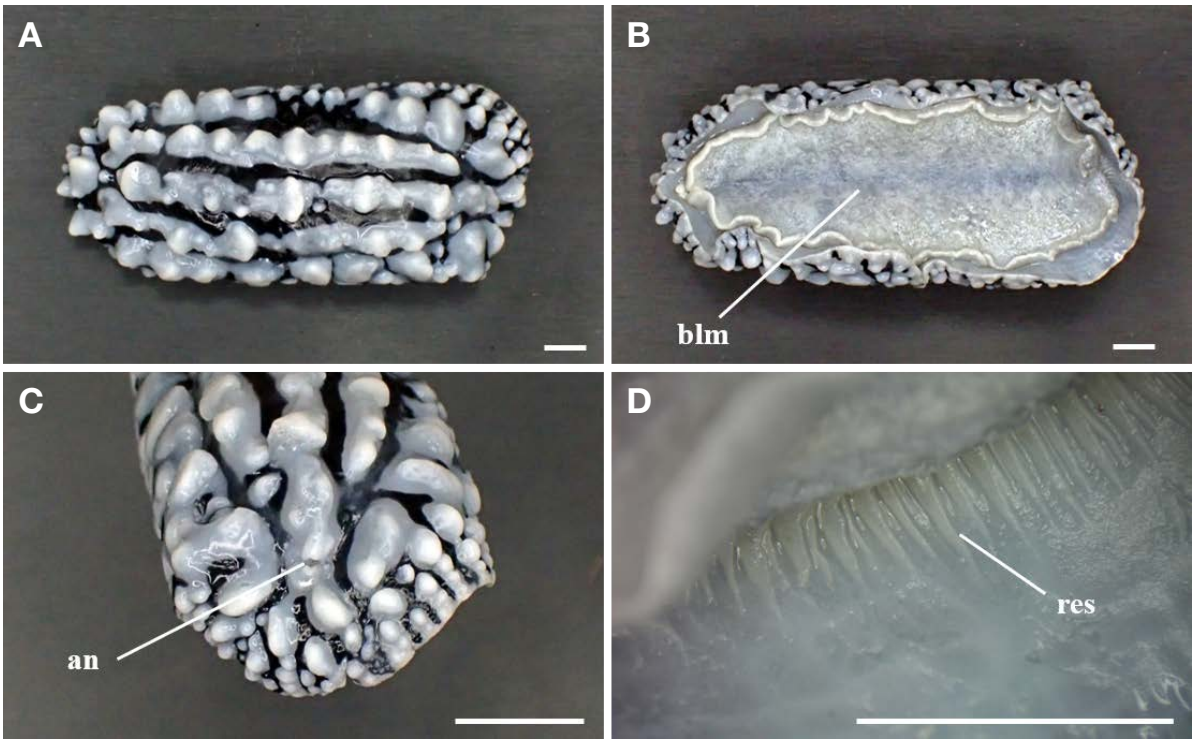

Fig. 1. Photographs of *Phyllidia varicosa* Lamarck, 1801, preserved specimen (body length, 59 mm). A, Dorsal view; B, Ventral view; C, Posterior view; D, Secondary respiratory structures. Scale bars: A–D = 5 mm. an, anus; blm, black longitudinal marking; res, respiratory structure.

deferens, thin and convoluted. Vagina bifurcates into bursa copulatrix and receptaculum seminis.

GenBank accession number. OR237803.

Distribution. Korea, Japan, Philippines, Vietnam, Thailand, Malaysia, Indonesia, Papua New Guinea, Solomon Islands, Australia, Palau, Guam, Hawaii, Sri Lanka, Egypt, Tanzania, Madagascar, La Réunion, and South Africa (Brunckhorst, 1993; Fahrner and Schrödl, 2000; Gosliner et al., 2008; this study).

Remarks. *Phyllidia varicosa* is easily distinguished from its congeneric species by having several distinct ridges composed of dorsal tubercles from anterior to posterior, with yellow caps on the dorsal tubercles. This species is also characterized by a bluish-black pigment between the dorsal ridges and a longitudinal black marking running on the midline of its sole. However, sometimes the yellow cap on the dorsal tubercles and the black markings on the sole are faint or difficult to examine in fixed specimens (Fahrner and Schrödl, 2000; this study). The yellow cap on the dorsal tubercles and black stripe on the sole of the specimen examined in this study also faded after being stored in 94% ethanol for four months (Fig. 1A, B). It has been controversial whether the presence or absence of a black stripe on the sole of the species (Yonow, 1986; Brunckhorst, 1993; Fahrner and Schrödl, 2000). Neither Cuvier (1797) nor Lamark (1801) had documented a black longitudinal stripe marking on the sole of *P. varicosa*. Yonow (1986) resurrected the species name with black stripes on the sole as *Phyllidia arabica* Erenberg, 1831, and considered the species without black stripes on the sole as *P. varicosa*. Since the holotype of *P. varicosa* was missing, the presence or absence of this black stripe could not be confirmed (Yonow, 1986). However, Willan et al. (1998) identified a holotype specimen of *P. varicosa*, with a confirmation of the presence of the black stripe in the original drawing of *P. trilineata* Cuvier, 1804. Based on this, *P. arabica* was synonymized to *P. varicosa* by Brunckhorst (1993). Later, this controversy was resolved through experiments by Fahrner and Schrödl (2000) which proved that the black stripe could be eliminated from the specimen regardless of preservation conditions.

A length of 658 bp of mitochondrial COI sequence was obtained from the specimen examined. The intraspecific pairwise genetic distance (*p*-distance) of *P. varicosa* ranged from 0.00% to 21.45% including the specimen examined and 91 all available data in NCBI. Similarly, Papu et al. (2022) calculated intraspecific *p*-distances for *P. varicosa* ranging from 0.00% to 16.34% based on a total of 130 COI sequences, which was higher than the minimum interspecific *p*-distances between this species and some congeners. The maximum

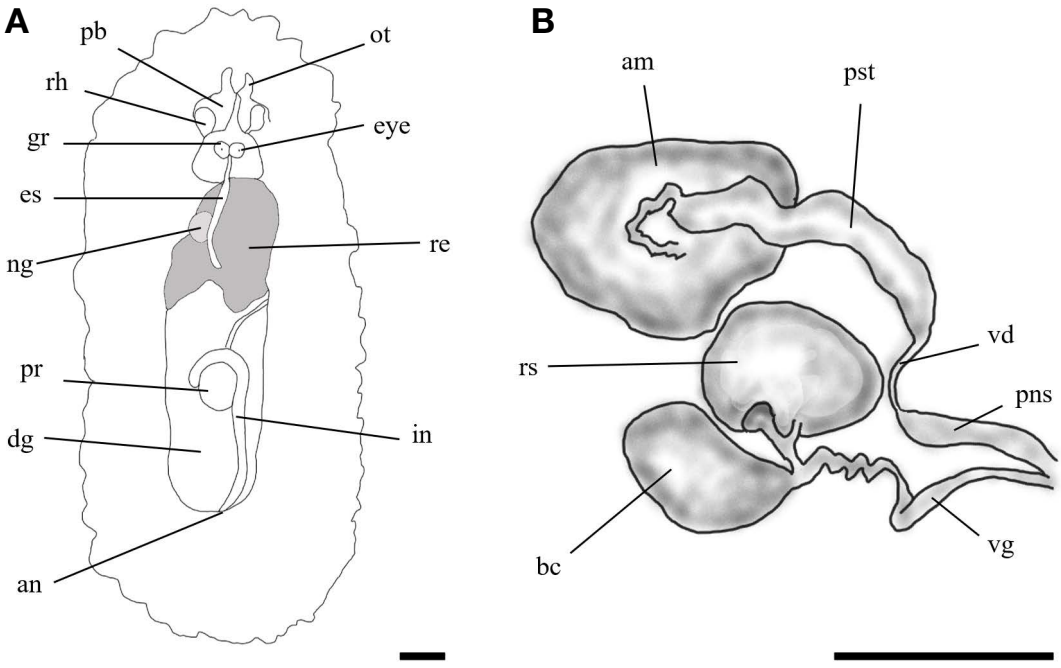

Fig. 2. Internal organ and anatomical diagrams of *Phyllidia varicosa* Lamarck, 1801. A, Diagram of internal organs; B, Diagram of reproductive system. Scale bars: A, B = 5 mm. am, ampula; an, anus; bc, bursa copulatrix; dg, digestive gland; es, esophagus; eye, eye spot; gr, ganglionic ring; in, intestine; ng, nidamental gland; ot, oral tentacle; pb, pharyngeal bulb; pns, penis; pr, pericardium; pst, prostate; re, reproductive system; rh, rhinophore; rs, receptaculum seminis; vd, vas deferens; vg, vagina.

intraspecific *p*-distance of this species is significantly higher than the previous DNA barcoding studies on nudibranch groups and mollusks (Layton et al., 2014; Hirose et al., 2015; Stoffels et al., 2016; Sun et al., 2016). Thus, Additional taxonomic studies integrated morphology and molecular data are needed to elucidate the reason for the wide intraspecific *p*-distances range in this species.

ORCID

Dae-Wui Jung: https://orcid.org/0000-0002-7895-2808
Chang-Bae Kim: https://orcid.org/0000-0002-1040-7600

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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