

Morphological description and DNA barcode information of seven newly reported nudibranch species from Korea

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The order Nudibranchia Cuvier, 1817 is among the most colorful gastropod group that lacks an external shell and mantle cavity at their adult stage. They are widely found in the intertidal to subtidal zones of marine habitats worldwide. To date, a total of 58 species are known from Korean waters. In this study, we report seven nudibranch species that are newly reported from Korean waters and provide their color images of external body, morphological description, taxonomic remarks, and mtDNA *cox1* barcode sequence information: *Dendronotus primorjensis* Martynov, Sanamyan & Korshunova, 2015; *Doto japonica* Odhner, 1936; *Trinchesia ornata* (Baba, 1937); *Antiopella fusca* (O'Donoghue, 1924); *Cadlina paninae* Korshunova, Fletcher, Picton, Lundin, Kashio, N. Sanamyan, K. Sanamyan, Padula, Schrödi & Martynov, 2020; *Rostanga bifurcata* Rudman & Avern, 1989; and *Goniodoridella savignyi* Pruvot-Fol, 1933.

Keywords: *Antiopella*, *Cadlina*, *Dendronotus*, *Doto*, *Goniodoridella*, mtDNA *cox1*, Nudibranchia, *Rostanga*, *Trinchesia*

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INTRODUCTION

Nudibranchia Cuvier, 1817 (often called sea slug) is among the most colorful gastropod groups and widely found in the intertidal to subtidal zones of marine habitats worldwide. Unlike most other gastropod species, they are bilaterally symmetrical with a soft body lacking an external shell and mantle cavity in their adult stage. To protect themselves from predators, nudibranch species have evolved various protective strategies using behavioral (camouflage) and chemical defenses (Faulkner and Ghiselin, 1983; Wollscheid-Lengeling *et al.*, 2001; Yonow, 2008). About 2,500 nudibranch species have been reported worldwide (WoRMS, 2021) from the tropic to polar seas, but relatively little is known about nudibranch species diversity in Korea: only 58 species are currently recorded in Korea, according to the National Species List of Korea (NIBR, 2020). In an attempt to explore species diversity and their distribution, a survey of Korean nudibranch fauna was conducted. As a result of this survey, we found seven nudibranch species. Although occurrence of these species from Korean waters was previously listed in pictorial at-

lases (Koh, 2006; Kil *et al.*, 2020), this study provides further information on color images of their external body, morphological description, taxonomic remarks and mitochondrial *cox1* sequences as their DNA barcode information.

MATERIALS AND METHODS

Sample collection and species identification

Samples were collected from the subtidal zone of South Korea by SCUBA diving and preserved in 95% ethyl alcohol. For species identification, morphological characters were examined under a stereoscopic dissecting microscope (Leica M205C; Wetzlar, Germany). The voucher specimens were deposited in the Marine Mollusk Resource Bank of Korea (MMRBK), the National Marine Biodiversity Institute of Korea (MABIK), and the National Institute of Biological Resources (NIBR). The color images of nudibranch samples were taken underwater or at collection sites when animals were alive.

Molecular techniques for mitochondria *cox1* sequence

Genomic DNA was extracted from foot tissue using the E.Z.N.A. Mollusc DNA kit (OMEGA Omega Bio-tek, Norcross, USA) following the manufacturer's instructions. The PCR amplification of mitochondrial *cox1* partial sequence was performed with the LCO1490 and HCO2198 primer set (Folmer *et al.*, 1994) in a 50 µL of reaction mixture containing 2 µL of template DNA, 36.75 µL of D.W., 5 µL of 10× Ex Taq Buffer, 4 µL of dNTP, 2 µL of primer set, and 0.25 µL of TaKaRa Ex Taq (TaKaRa Bio, Japan). The PCR condition consisted of an initial denaturation at 95°C for 2 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 45°C for 30 seconds, elongation at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. The PCR-amplified target fragment was purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, USA) and sequenced using an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, USA). The *cox1* sequence of seven nudibranch species were deposited in the GenBank.

RESULTS AND DISCUSSION

Systematic accounts

Phylum Mollusca Linnaeus, 1758
 Class Gastropoda Cuvier, 1795
 Order Nudibranchia Cuvier, 1817
 Family Dendronotidae Allman, 1845
 Genus *Dendronotus* Alder & Hancock, 1845

1. *Dendronotus primorjensis*

Martynov, Sanamyan & Korshunova, 2015 (Fig. 1A)
 갈색긴수지갯민숭이

Dendronotus primorjensis Martynov, Sanamyan & Korshunova, 2015a: 60, fig. 5A–G; Martynov *et al.*, 2015b: 75, 76; Korshunova *et al.*, 2016: 15–28, figs. 1, 2; Nakano, 2018: 386; Korshunova *et al.*, 2019: 6, fig. 3A–F; Kil *et al.*, 2020: 98, 99.

Material examined. 2 individuals (MO00178868, MMR BK7450): Munamjin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea, 38°18'04.04"N, 128°34'10.08"E, 27 April 2021; 1 individual (MMRBK7454): Munamjin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea, 38°18'04.04"N, 128°34'10.08"E, 05 May 2021; 1 individual (NIBRIV0000876678): Gonghyeonjin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea, 38°21'15.21"N, 128°31'00.13"E, 22 February 2019.

Diagnosis. Body elongate (length 11 mm, width 3 mm), laterally compressed, ground color opaque white or brown. Small whitish and yellowish dots and light brown

and brown tuberculate scattered in mantle. Rhinophores lamellate, rhinophoral stalks long, with five appendages. Four to six pairs of highly branched dorsolateral appendages and decreasing in size towards tail. Foot narrow, rounded in front, narrowed towards tail.

GenBank accession number. OK143204.

Distribution. Korea, Japan, Russia.

Remarks. From morphological and molecular analysis, *Dendronotus dudkai* was synonymized with *D. primorjensis* by Korshunova *et al.* (2016). The examined specimens of the present study match the morphological features of *D. primorjensis* described by Korshunova *et al.* (2016).

Family Dotidae Gray, 1853

Genus *Doto* Oken, 1815

2. *Doto japonica* Odhner, 1936 (Fig. 1B)

솔방울갯민숭이

Doto japonica Odhner, 1936: 1121, 1122, pl. 1, figs. 11–16, text-fig. 47; Kil *et al.*, 2020: 102, 103.

Doto (Doto) japonica: Baba, 1949: 94, 171, pl. 39, fig. 137, text-fig. 117; Baba, 1971: 75; Okutani, 2000: 799, pl. 398, fig. 1.

Material examined. 2 individuals (MO00178867, MMR BK7448): Munamjin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea, 38°18'04.04"N, 128°34'10.08"E, 27 April 2021; 2 individuals (NIBRIV0000882622, NIBRIV0000882626): Munamjin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea, 38°18'04.04"N, 128°34'10.08"E, 16 July 2020.

Diagnosis. Body slender, elongated (length 5 mm, width 1 mm), ground color opaque white to brown or black. Rhinophores slender, smooth with blunted, white-colored terminal ends and extruded from slightly flared sheaths. Six to seven pairs of dorsal cerata decrease gradually in size towards posterior end of body, each covered with rounded tubercles spotted in white at their terminal ends. Digestive gland in cerata pale yellow or yellowish green in color. Foot narrow and extends from under mantle.

GenBank accession number. OK143203.

Distribution. Korea, Japan.

Remarks. This species is similar to *D. bella* in external morphology, but distinguished by the color pattern of rhinophores and terminal end of ceratal tubercles. *D. japonica* shows rhinophores and the ceratal tubercles have white-colored terminal spots, while *D. bella* possesses black-colored rhinophores and dorsal ceratal tubercles that are terminated with a large, rounded, black spot.

Family Trinchessiidae F. Nordsieck, 1972

Genus *Trinchesia* Ihering, 1879

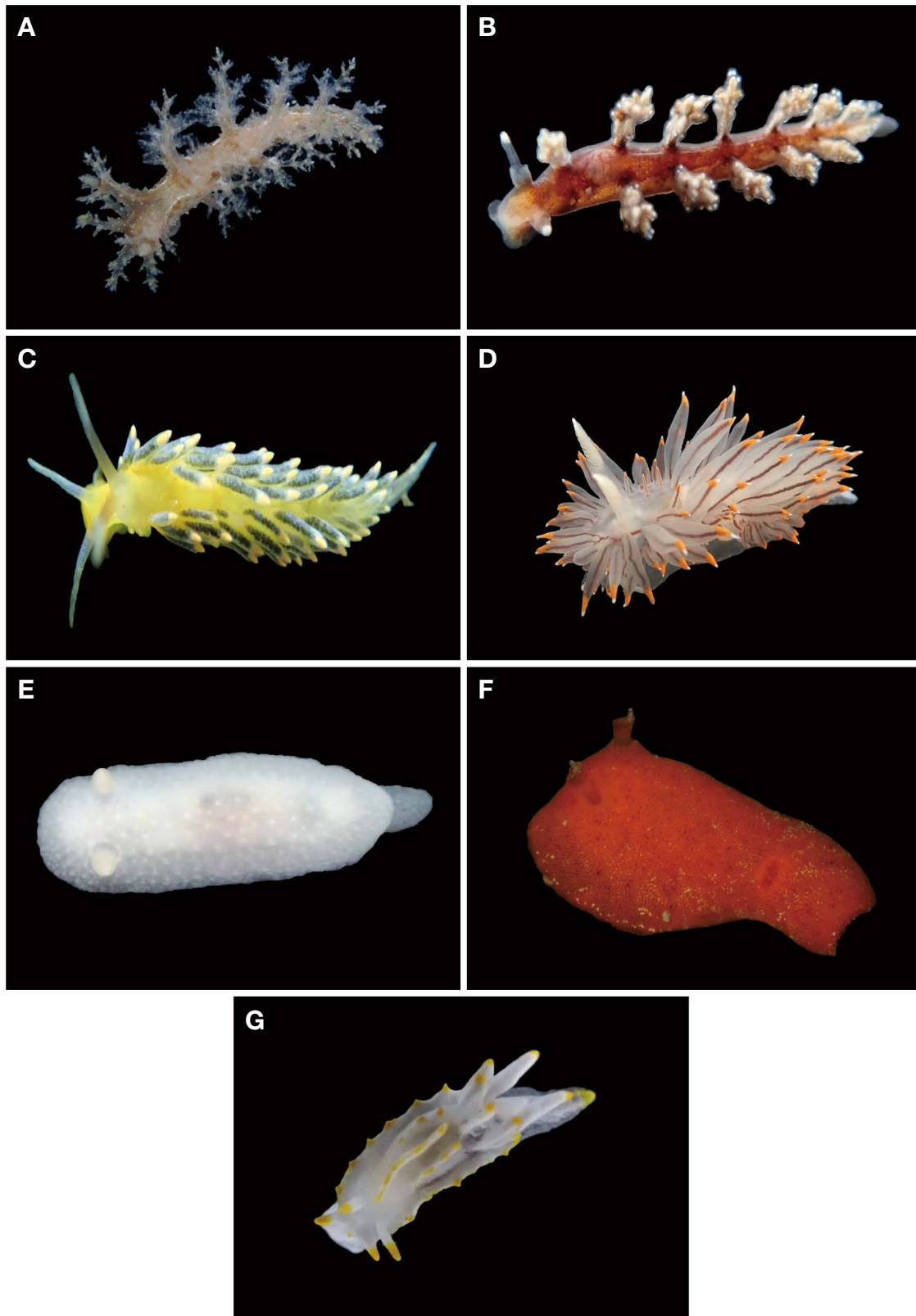


Fig. 1. Images of seven Nudibranchia species in Korea. A, *Dendronotus primorjensis* Martynov, Sanamyan & Korshunova, 2015; B, *Doto japonica* Odhner, 1936; C, *Trinchesia ornata* (Baba, 1937); D, E, *Antiopella fusca* (O'Donoghue, 1924); F, *Cadlina paninae* Korshunova, Fletcher, Picton, Lundin, Kashio, N. Sanamyan, K. Sanamyan, Padula, Schrödi & Martynov, 2020; G, *Rostanga bifurcata* Rudman & Avern, 1989; and H, *Goniodoridella savignyi* Pruvot-Fol, 1933.

3. *Trinchesia ornata* (Baba, 1937) (Fig. 1C)

장식도롱이갯민숭이

Cuthona (Hervia) ornata Baba, 1937: 331–333, pl. 2, fig. 4, text-fig. 17.

Cuthona ornata: Baba, 1961: 367; Okutani, 2000: 805, pl. 400, fig. 3.

Trinchesia ornata: Debelius and Kuitert, 2007: 351; Chichvarkhin, 2016: 27, 28, fig. 9J; Kil *et al.*, 2020: 126, 127.

Material examined. 3 individuals (MO00178664, MMR BK7444, MMRBK7445): Donji-ri, Saryang-myeon, Tongyeong-si, Gyeongsangnam-do, Korea, 34°50'10.97" N, 128°09'28.64"E, 07 April 2021; 1 individual (NIBRIV 0000882623): Gisamun-ri, Hyeonbuk-myeon, Yangyang-gun, Gangwon-do, Korea, 38°01'19.87"N, 128°44'22.46" E, 27 May 2020.

Diagnosis. Body elongate (length 9 mm, width 2 mm), dorso-ventrally flattened. Background body color opaque yellow. Oral tentacles and rhinophores smooth, tapering with an ivory white tip. Dorsal cerata finger-shaped, relatively long, slender, with ivory white or yellow terminal tip. Cerata arranged in nine transverse rows. Digestive gland blue to purple in color. Foot narrow. Metapodium elongate, with a sharply pointed end.

GenBank accession number. OK143200.

Distribution. Korea, Australia, Hong Kong, Indo Pacific, Japan, Philippines, Russia (Primorsky).

Remarks. This species is found in the northwestern Pacific and Indo-Pacific, including Australia, and the Peter the Great Bay, Russia (Primorsky). The specimens examined in this study show blue-colored digestive glands, giving their cerata a blue look with a yellow terminal tip (or ivory white tip in some individuals), the most distinctive coloration of this species.

Family Janolidae Pruvot-Fol, 1933

Genus *Antiopella* Hoyle, 1902

4. *Antiopella fusca* (O'Donoghue, 1924) (Figs. 1D)

갈색버들잎갯민숭이

Janolus fuscus O'Donoghue, 1924; Chichvarkhin *et al.*, 2016: 24, figs. 2, 3.

Antiopella fusca: Pola *et al.*, 2018: 363, 364, fig. 10; Kil *et al.*, 2020: 130, 131.

Material examined. 1 individual (MO00178871): Munamjin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea, 38°18'04.04"N, 128°34'10.08"E, 05 May 2021; 1 individual (NIBRIV0000811823): Gangmun-dong, Gangneung-si, Gangwon-do, Korea, 38°49'22.35"N, 128°57'46.55"E, 27 April 2017.

Diagnosis. Body elongate, ovate (length 30 mm, width 16 mm), ground color translucent white to light yellow.

Rhinophoral clubs lamellate, stalks smooth. Oral tentacles short, triangular in shape. Dorsal cerata fusiform, with an orange or yellow band and a white pointed tip. Digestive gland in cerata straight, brown to black in color. Metapodium elongate, pointed posteriorly.

GenBank accession number. OK143206.

Distribution. Korea, North America (Alaska to central California), Japan, Russia.

Remarks. The genus *Antiopella* includes eight taxonomically valid species (WoRMS, 2021). This species is similar to *A. barborensis* in external morphology, but distinguished by the color pattern of ceratal tip: In *A. fusca*, the cerata have an orange or yellow band with a white terminal tip, whereas *A. barborensis* have cerata with sky blue-colored tips. The *Antiopella* species are found in inter- and shallow subtidal waters feeding on bryozoans. The occurrence of this species from Korean waters (East Sea) was firstly reported in 2006 (Koh, 2006), but this study provides detailed information on external morphology, and molecular data of DNA barcode sequences.

Family Cadlinidae Bergh, 1891

Genus *Cadlina* Bergh, 1879

5. *Cadlina paninae* Korshunova, Fletcher, Picton, Lundin, Kashio, N. Sanamyan, K. Sanamyan, Padula, Schrödi & Martynov, 2020 (Fig. 1E)

수수갯민숭달팽이

Cadlina paninae Korshunova, Fletcher, Picton, Lundin, Kashio, N. Sanamyan, K. Sanamyan, Padula, Schrödi & Martynov, 2020: 792–794, fig. 9; Kil *et al.*, 2020: 154, 155.

Material examined. 1 individual (MO00178870): Munamjin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea, 38°18'04.04"N, 128°34'10.08"E, 06 May 2021; 1 individual (NIBRIV0000811855): Munamjin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea, 38°18'04.04" N, 128°34'10.08"E, 26 April 2017.

Diagnosis. Body ovate, swollen (length 10 mm, width 4 mm), ground color opaque white. Rhinophoral clubs lamellate, stalks smooth. Each rhinophoral sheath slightly protrude, covered with small tubercles. Mantle wide and covers whole foot. Small and yellow or white tubercles cover dorsal surface. Gills six to seven in number, multipinnate, united, forming a gill circle surrounding anus. Foot broad, anteriorly rounded, slightly thicken. Metapodium rounded.

GenBank accession number. OK143205.

Distribution. Korea, Japan, Russia.

Remarks. *Cadlina paninae* is easily distinguished from other congeneric species by the absence of yellow marginal band or yellow (or white) spots.

Family Discodorididae Bergh, 1891
Genus *Rostanga* Bergh, 1879

6. *Rostanga bifurcata* Rudman & Avern, 1989 (Fig. 1F)
붉은응단갯민숭달팽이

Rostanga bifurcata Rudman and Avern, 1989: 293–300, figs. 1B, C, 2B, 3B, 7C, 8–11, 28; Debelius and Kuitner, 2007: 247; Kil *et al.*, 2020: 204, 205.

Material examined. 1 individual (MO00178866): Donjiri, Saryang-myeon, Tongyeong-si, Gyeongsangnam-do, Korea, 34°50'10.97"N, 128°09'28.64"E, 07 April 2021; 1 individuals (NIBRIV0000882547): Gageodo-ri, Heuksan-myeon, Sinan-gun, Jeollanam-do, Korea, 38°03'46.06"N, 128°05'40.55"E, 30 July 2020.

Diagnosis. Body ovate, swollen (length 12 mm, width 5 mm), ground color orange to red. Rhinophore stalks smooth, with white tip, horizontally arranged lamellae on clubs. Mantle wide, covering an entire foot. Dorsal surface of mantle covered by caryophyllidia and ornamented by minute black or white specks. White specks arranged along the mantle edge. Gills seven to ten in number, tripinnate, arranged in a gill circle surrounding anus on dorsal surface of mantle.

GenBank accession number. OK143201.

Distribution. Korea, Hong Kong, Japan, Australia, Hawaii, Malaysia, Philippines (Indo-Pacific), Tanzania (Africa).

Remarks. *Rostanga* species are characterized by having reddish-orange body color and caryophyllidia, specialized sensory tubercles on the dorsal surface of mantle. *R. bifurcata* and *R. orientalis* are commonly found in the northwestern Pacific and morphologically very similar, but distinguished by the lamellae in rhinophores. *R. bifurcata* has horizontally arranged lamellae in rhinophoral clubs, while *R. orientalis* has rhinophoral lamellae that are vertically arranged.

Family Goniodorididae H. Adams & A. Adams, 1854
Genus *Goniodoridella* Pruvot-Fol, 1933

7. *Goniodoridella savignyi* Pruvot-Fol, 1933 (Fig. 1G)
별사탕갯민숭달팽이

Goniodoridella savignyi Pruvot-Fol, 1933: 117, 118, pl. 2, figs. 23–26; Baba, 1960: 81, pl. 8, figs. 1A–1F; Debelius and Kuitner, 2007: 28; Kil *et al.*, 2020: 214, 215.

Material examined. 1 individual (MO00178869): Munamjin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea, 38°18'04.04"N, 128°34'10.08"E, 06 May 2021; 1 individual (NIBRIV0000882618): Munamjin-ri, Jugwang-myeon, Goseong-gun, Gangwon-do, Korea, 38°18'04.04"N, 128°34'10.08"E, 16 July 2020; 1 individual (NIBRIV0000882375): Geojin-ri, Geojin-eup, Goseong-

gun, Gangwon-do, Korea, 38°27'09.01"N, 128°28'03.01"E, 23 February 2019.

Diagnosis. Body limaciform (length 5 mm, width 1.2 mm), ground color opaque, creamy white. Middorsal longitudinal crests of bright yellow specked-tubercles run from rhinophores to gill. Lateral papillae with yellow tip seven in number present on each side of marginal mantle edge. Two elongated, yellow tipped papillae in front of rhinophores. Rhinophores slender, long, cylindrical, tipped with yellow. Gills three, bipinnate, finger-shaped, tipped in yellow. Metapodium elongate, pointed, tipped in yellow.

GenBank accession number. OK143202.

Distribution. Korea, Australia, Japan, Philippines, Russia, Singapore, the Red Sea, South Africa.

Remarks. This species is widely distributed throughout the world from the tropical to subtropical seas, including Indo-Pacific waters. The color patterns of external body and the presence of middorsal longitudinal crests tipped with yellow examined from our specimens are distinct morphological features of this species. The occurrence of this species from Korean waters (Yellow Sea) was firstly reported in 2006 (Koh, 2006), but this study provides morphological description and molecular data of DNA barcode sequences.

CONCLUSIONS

Nudibranchs are a group of soft-bodied, marine gastropods lacking an external shell and mantle cavity at their adult stage, comprising about 2,500 species reported worldwide. However, relatively little is known about their species diversity in Korea: a total of 58 species have been reported from Korean waters to date, according to the National Species List of Korea (NIBR, 2020). From a comprehensive survey of Korean nudibranch fauna, here we provide detailed information on color images of external body, morphological description, taxonomic remarks, and mtDNA *coxI* barcode sequences of the seven nudibranch species.

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