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Redescriptions of five nudibranch species from Korea, including the first record of the family Cuthonellidae from Korea (Gastropoda: Nudibranchia)

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ABSTRACT

This study documents the first records of the family Cuthonellidae and the genera *Cuthonella* and *Tenellia* from Korean waters. Five nudibranch species previously unrecorded in Korea were identified: *Cuthonella georgstelleri*, *Cuthona futairo*, *Tenellia melanobrachia*, *Tenellia zvezda*, and *Tambja morosa*. Each species was redescribed based on distinctive morphological characteristics, and underwater photographs of living individuals were provided. To evaluate genetic divergence, mitochondrial cytochrome c oxidase subunit I (COI) sequences were analyzed for four of the five species. The calculated intraspecific *p*-distances were 0.6% in *C. futairo*, 0-1.5% in *T. melanobrachia*, and 0.6-1.7% in *T. zvezda*, while *T. morosa* exhibited considerably higher intraspecific variation (0.3-11.7%). These results offer a useful molecular reference framework for future integrative taxonomic investigations of nudibranchs in the Northwest Pacific.

Keywords: Nudibranchia, Taxonomy, *Cuthona futairo*, *Cuthonella georgstelleri*, *Tambja morosa*, *Tenellia melanobrachia*, *Tenellia zvezda*, Korea, COI

INTRODUCTION

Nudibranchs are a diverse group of shell-less marine gastropods recognized for their vivid coloration and variable morphology (Gosliner *et al.*, 2018). These characteristics are closely associated with ecological adaptations, such as dietary specialization and chemical defense mechanisms (Gosliner *et al.*, 2018; Wägele and Klussmann-Kolb, 2005). Nudibranchs inhabit a wide range of marine environments, from tropical coral reefs to polar and deep-sea habitats. Some nudibranch species tolerate high water temperatures of up to 32.2°C (Armstrong *et al.*, 2019), while others, such as *Bathydevius caudactylus*, occur in cold waters as low as 1.6-2.8°C (Robison *et al.*, 2024). This thermal tolerance likely contributes to their broad ecological distribution and evolutionary success (Valdés, 2002; Wägele and Klussmann-Kolb, 2005). Despite their broad distribution and high species richness, the nudibranch fauna of Korea remains poorly characterized, especially from a molecular and integrative taxonomic perspective. In this study, subtidal surveys were conducted along the Korean coast at depths of 6-55 m, where seawater temperatures ranged seasonally from 4°C to 23°C. Five species previously unrecorded from Korea were identified: *Cuthonella georgstelleri*, *Cuthona futairo*, *Tenellia melanobrachia*, *Tenellia zvezda*, and *Tambja morosa*. Among these, *Cuthonella* and *Tenellia* represent newly recorded genera, and the family Cuthonellidae is reported from Korea for the first time.

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Each species is redescribed based on diagnostic morphological characteristics, and underwater photographs of living individuals are provided. COI sequences were obtained for four species to assess intra- and interspecific genetic divergence and support species delimitation. These results contribute new molecular and distributional data that refine our knowledge of nudibranch diversity in the Korean region of the Northwest Pacific.

MATERIALS AND METHODS

Five nudibranch species were collected from Korean coastal waters at depths ranging from 6 to 55 meters between January 2022 and June 2024. Underwater photographs were taken using either a Canon EOS 5D Mark IV (Canon Inc., Tokyo, Japan) or an Olympus Tough TG-6 (Olympus Corp., Tokyo, Japan). Collected specimens were examined under a stereomicroscope (Olympus SZ61; Olympus Corp., Tokyo, Japan). Body length was measured from the anterior head to the posterior end of the metapodium. Prior to fixation, individuals were anesthetized using a 7-10% $MgCl_2$ solution and then initially preserved in 70% ethanol. Samples were subsequently transferred to 95% ethanol. All examined specimens have been deposited in the National Marine Biodiversity Institute of Korea (MABIK MO00187883-7) and Korea Marine-Bio Laboratory (KOMBI KM122-5).

Genomic DNA was extracted from ceras or foot tissue of each individual using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. Polymerase chain reaction (PCR) was conducted to amplify the mitochondrial cytochrome c oxidase subunit I (COI) gene regions using the universal primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). Each PCR reaction was performed in a 20 μL volume containing 10 μL of 2 × PCR premix (TOPsimple™ DyeMIX-nTaq, Enzynomics), 1 μL of each primer (10 pmol), 1-2 μL of DNA template, and distilled water to volume. The thermal cycling conditions included an initial denaturation at 95°C for 3 minutes; followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 46-48°C for 30 seconds, and extension at 72°C for 1 minute; with a final extension at 72°C for 5 minutes. The newly obtained sequences were submitted to the NCBI GenBank database (OR800616, PV792013-PV792017).

For molecular identification and phylogenetic inference, related sequences were retrieved from GenBank, and uncorrected pairwise genetic distances were calculated using the Kimura 2-Parameter (K2P) model in MEGA 11 (Kimura, 1980; Tamura *et al.*, 2021). Neighbor-joining (NJ) phylogenetic trees were constructed using COI sequences, with 1,000 bootstrap replicates (Saitou and Nei, 1987).

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 Cuthonellidae M. C. Miller, 1977
매끈촉각갯민숭이과 (신칭)

Type genus. *Cuthonella* Bergh, 1884.

Diagnosis. Body moderately broad. Lateral notal edge absent. Cerata not elevated, arranged in several rows. Rhinophores smooth or wrinkled, with indistinct lamellae. Anus located on anterior mid-lateral side of body, either acleioproctic or cleioproctic. Radula formula 0.1.0. Rachidian teeth with a strong central cusp, not laterally compressed by denticles. Distal receptaculum seminis present. Vas deferens of moderate length, with an indistinct prostate and an associated supplementary gland. Penis unarmed (Korshunova *et al.*, 2020).

Genus *Cuthonella* Bergh, 1884 둥근족편갯민숭이속 (신칭)

Type species. *Cuthonella abyssicola* Bergh, 1884.

Diagnosis. Body broad, with numerous cerata arranged in continuous rows. Rhinophores smooth or wrinkled, equal in length to or longer than oral tentacles. Foot corners generally rounded, not tentaculate. Anus usually acleioproctic. Rachidian

teeth with a distinct central cusp and lateral denticles. Vas deferens long, lacking a separate prostate. Penial gland opens into vas deferens. Penis unarmed and conical in shape (Korshunova *et al.*, 2020).

***Cuthonella georgstelleri* Korshunova, N. P. Sanamyan, K. E. Sanamyan, Bakken, Lundin, Fletcher and Martynov, 2020 까미갯민숭이 (신칭) (Fig. 1A)**

Cuthonella georgstelleri Korshunova *et al.*, 2020: 243-247, Figs. 1, 2, 11, 20B, 21.

Type locality. Matua Island, Middle Kuril Islands, Russia.

Distribution. Russia and Korea (Korshunova *et al.*, 2020; This study).

Specimens examined. SOUTH KOREA: 5 specimens, Gangwon-do, Goseong-gun, Toseong-myeon, Gyoam-ri, 6 III 2023, DW Jung and YM Choi (MABIK MO00187884; KOMBI-KM124).

Measurement. Body length 8-13 mm (preserved specimens).

Diagnosis. Body aeolid, moderately broad, translucent white with pale ground color (Fig. 1A). Rhinophores and oral tentacles cylindrical, smooth, of moderate length, opaque white with translucent white specks; Rhinophores nearly equal in length to oral tentacles, though slightly shorter. Cerata digitiform, arranged in continuous rows, with digestive gland dark brown to bluish-black, and white hue around cnidosac area. Anal opening cleioproctic. Foot corners indistinct and rounded.

Remark. According to Korshunova *et al.* (2020), most species of the genus *Cuthonella* are associated with cold-water environments. The Korean specimens examined in this study were collected at depths of 50 meters, where temperatures ranged around 4°C, in line with the known ecological preferences of the genus.

The specimens examined in this study were entirely consistent with the original description in all morphological characteristics. This species is morphologically similar to *Cuthonella anastasia*, but can be distinguished by several key characteristics. *C. georgstelleri* possesses an elongate body reaching up to 14 mm in length, with orange-brown to bluish black cerata, lacking any additional pigmentation except for white apical bands. The body is whitish, occasionally with faintly dispersed white pigment. In contrast, *C. anastasia* exhibits a pale orange body, brown cerata densely covered with opaque white specks, and a considerably smaller body size, reaching only about 5 mm in length (Ekimova *et al.*, 2024).

For the molecular identification of this species, we attempted to amplify the COI gene using the primer set, LCO1490 and HCO2198 (Folmer *et al.*, 1994) under various PCR conditions. However, all attempts were unsuccessful.

Fig. 1. Photographs of living animals. **A.** Dorsal view of *Cuthonella georgstelleri*; **B.** Dorsal view of *Cuthona futairo*; **C.** Dorso-lateral view of *Tenellia melanobrachia*; **D.** Dorso-lateral view of *Tenellia zvezda*; **E.** Dorsal view of *Tambja morosa*.

Family Cuthonidae Odhner, 1934 촘촘도롱이갯민숭이과
Genus *Cuthona* Alder and Hancock, 1855
촘촘도롱이갯민숭이속

Cuthona futairo **Baba, 1963 흰도롱이갯민숭이 (Figs. 1B, 2)**

Cuthona futairo Baba, 1963: 109-114, pl. 5, figs. 1-16, pl. 6, figs. 1-18.

Cuthona bicolor: Baba, 1933: 279-280, text-fig. 6, misid.

Cuthona (*Cuthona*) *bicolor*: Baba, 1935: 353-354, pl. 7, fig. 5, misid.

Cratena bicolor: Baba, 1949: 97-98, 174, pl. 45, fig. 154, text-figs. 123-124, misid.

Catriona bicolor: Baba, 1955: 56; Hamatani, 1960: 68-69, text-fig. 8, misid.

Tenellia futairo: Ono and Kato, 2020: 348.

Type locality. Mukaishima Island, Hiroshima, Japan.

Distribution. Korea and Japan.

Specimens examined. SOUTH KOREA: 2 specimens, Gyeongsangbuk-do, Pohang-si, Nam-gu, Guryongpo-eup, Jangil-ri, 13 VI 2024, DW Jung (MABIK MO00187887; KOMBI-KM123).

Measurement. Body length 13-21 mm (preserved specimens).

Diagnosis. Body slender, aeolid form, translucent yellowish green to yellowish orange (Fig. 1B). Rhinophores and oral tentacles smooth, elongate, concolorous with body; both rhinophores and oral tentacles translucent yellow basally and orange distally, occasionally with a white hue around their bases. Rhinophores slightly longer than oral tentacles. Cerata digitiform, numerous, and arranged in irregular rows; digestive gland bright yellow-green to reddish brown, with fine white specks scattered along surface of each ceras. Tip of each ceras white. Anal opening cleioproctic. Foot narrow, translucent white; foot corners indistinct.

GenBank Accession Nos. PV792016 and PV792017.

Remark. *Cuthona futairo* and *Trinchesia bicolor* share several external similarities, including a similar body shape, translucent ground color, and pigmented cerata. However, these two species can be distinguished by the following morphological characteristics: *C. futairo* has more numerous and slightly irregular ceratal rows, with pigmentation confined mainly to the ceratal tips and lacking distinct banding. Its radular teeth are laterally compressed and smooth-edged. In contrast, *T. bicolor* exhibits fewer, more orderly ceratal rows, distinct pigment banding on the cerata, and robust, serrated radular teeth (Baba, 1963).

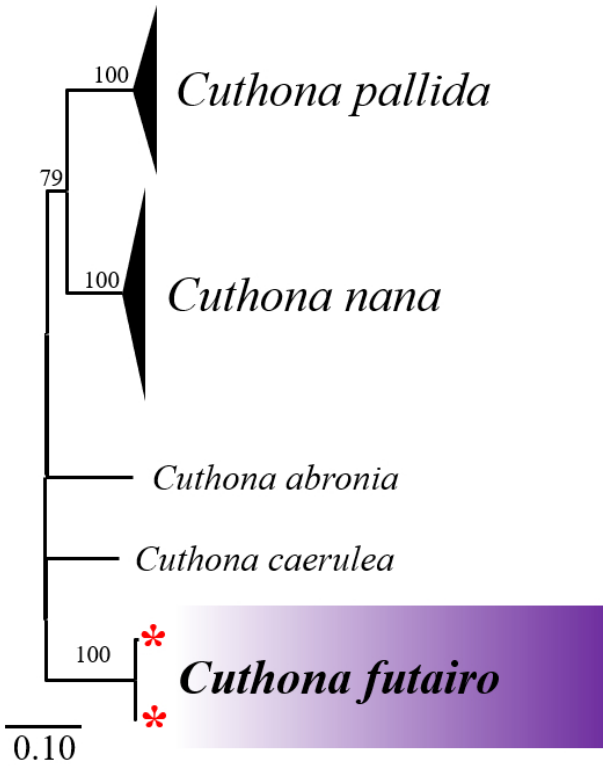

Fig. 2. Neighbor-joining (NJ) phylogenetic tree of *Cuthona* species inferred from mitochondrial COI sequences obtained from GenBank. Bootstrap values ≥ 80% from 1,000 replicates are shown above the corresponding nodes. Sequences newly generated in this study are marked with a red asterisk (*). Focal species are shown in bold and highlighted with colored shading.

In the neighbor-joining (NJ) phylogenetic analysis, sequences of *Cuthona futairo* obtained in this study clustered into a distinct monophyletic clade with bootstrap value of 100, clearly differentiated from other congeners (Fig. 2). However, no reference sequences of *C. futairo* are currently available in GenBank, preventing direct intraspecific comparison.

Family Trinchesiidae Nordsieck, 1972 도롱이갯민숭이과

Genus *Tenellia* Costa, 1866 날씬도롱이갯민숭이속 (신칭)

Type species. *Tenellia mediterranea* Costa, 1866 (= *Tergipes adspersus* Nordmann, 1845), by monotypy (Gosliner and Bertsch, 2017).

Diagnosis. Body relatively small and slender, often transparent or semi-transparent. Oral tentacles long and narrow, generally equal in length to rhinophores. Rhinophores smooth or slightly wrinkled, lacking lamellae. Cerata arranged in multiple rows, typically elongated and cylindrical, cnidosac present. Radula formula 0.1.0. Rachidian teeth possess a single central cusp without lateral denticles. Penis unarmed. Prostate indistinct. Vas deferens relatively short (Korshunova *et al.*, 2017).

***Tenellia melanobrachia* (Bergh, 1874) 컵산호갯민숭이 (신칭) (Figs. 1C, 3)**

Phestilla melanobrachia Bergh, 1874: 1-5, taf. 2, figs. 1-14; Okutani, 2000: 805; Gosliner *et al.*, 2008: 372; 2015: 357.

Tenellia melanobrachia: Cella *et al.*, 2016: 14; Gosliner *et al.*, 2018: 300.

Phestilla melanobranchia [sic], Baba, 1984: 241-246, figs. 2-5 (incorrect original spelling)

Type locality. Philippines.

Distribution. Indo-Pacific and Eastern Pacific (Gosliner *et al.*, 2018).

Specimen examined. SOUTH KOREA: 1 specimen, Jeju-do, Seogwipo-si, Seogwi-dong, Museom islet, 2 X 2023, CY Park (MABIK MO00187885).

Measurement. Body length 9 mm (preserved specimen).

Diagnosis. Body elongate, aeolid form, pale orange background color (Fig. 1C). Rhinophores and oral tentacles smooth, elongate, translucent white basally with pale orange pigmentation distally; rhinophores slightly longer than oral tentacles. Cerata numerous, digitiform, arranged in up to seven discrete rows per side, digestive gland yellow, tip of each ceras translucent white to orange. Anal opening cleioproctic. Foot wide, translucent white with numerous white specks, foot margin undulated, foot corners well-developed.

GenBank Accession No. PV792015.

Remark. The specimen examined is collected from a rocky substrate at a depth of 6 m, where the water temperature was 23°C. A total of 43 COI sequences of *Tenellia* species (564 bp) were analyzed. The

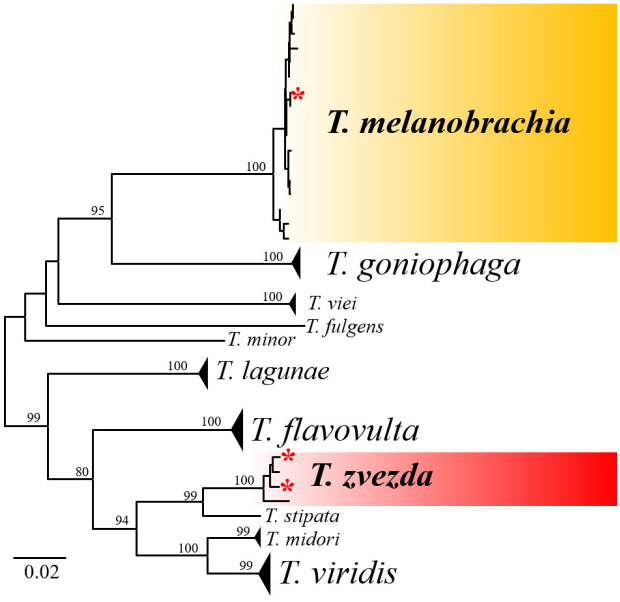

Fig. 3. NJ phylogenetic tree of *Tenellia* species inferred from mitochondrial COI sequences obtained from GenBank. Bootstrap values ≥ 80% from 1,000 replicates are shown above the corresponding nodes. Sequences newly generated in this study are marked with a red asterisk (*). Focal species are shown in bold and highlighted with colored shading.

intraspecific genetic distance within *T. melanobrachia* ranged from 0 to 1.5%. Notably, the Korean specimen showed 0.0% genetic divergence from a specimen collected in Guam, USA (GenBank accession no. DQ417277). The minimum interspecific distance was observed between *T. melanobrachia* and *T. goniophaga* (GenBank accession nos. DQ417277 and MW148915), with a divergence of 13.1%. In the NJ tree, *T. melanobrachia* clade was strongly supported with a bootstrap value of 100 (Fig. 3).

***Tenellia zvezda* (Korshunova, Fletcher, Bakken and Martynov, 2023) 별무리갯민숭이 (신칭) (Figs. 1D, 3)**

Diaphoreolis zvezda Korshunova *et al.*, 2023: 650-652, figs. 1, 2, 4F, 9.

Tenellia zvezda: Kim *et al.*, 2024: Table 1.

Type locality. Urup Island, Russia.

Distribution. Russia and Korea (Korshunova *et al.*, 2023; This study).

Specimens examined. SOUTH KOREA: 2 specimens, Gangwon-do, Goseong-gun, Toseong-myeon, Gyoam-ri,

Table 1. Uncorrected *p*-distances (%) among *Tambja* species based on partial COI sequences (658 bp)

No.	Species	Locality	1	2	3	4	5	6	7	8	9	10	GenBank accession No.	References
1	*T. morosa*	Korea											OR800616	This study
2	"	Philippines	0.3										DQ230997	Pola *et al.*, 2006
3	"	Japan	6.5	6.5									EF142867	Pola *et al.*, 2007
4	"	Philippines	6.8	6.8	0.3								EF142868	Pola *et al.*, 2006
5	"	Mauritius	11.5	11.7	10.1	10.5							OK501283	Ah Shee Tee *et al.*, 2023
6	*T. affinis*	France	10.0	10.0	10.9	10.9	13.1						EF142866	Pola *et al.*, 2007
7	*T. sagamiana*	Japan	12.1	12.2	11.4	11.4	9.8	11.2					EF142870	"
8	*T. olivaria*	Vanuatu	7.0	7.0	1.7	1.7	10.8	11.1	12.0				KJ999225	Pola *et al.*, 2014
9	*T. verconis*	Australia	12.7	13.1	12.9	12.9	11.8	11.6	10.8	13.1			EF142869	Pola *et al.*, 2007
10	*T. victoriae*	Vanuatu	7.3	7.3	5.8	5.8	13.7	12.1	13.2	6.1	14.5		KJ999228	Pola *et al.*, 2014

7 III 2023, DW Jung (MABIK MO00187886; KOMBI-KM122).

Measurement. Body length 12-17 mm in living specimens.

Diagnosis. Body aeolid form, moderately broad, translucent white ground color (Fig. 1D). Rhinophores and oral tentacles, moderate length, smooth, opaque white with translucent white tips, both approximately equal in length. Cerata digitiform, arranged in continuous rows, digestive gland brown to reddish brown, numerous white specks present on each ceras irregularly, apices of cerata translucent brown. Anal opening acleioproctic. Foot corners present.

GenBank Accession Nos. PV792013 and PV792014.

Remark. The morphological characteristics of the present specimens are consistent with the original description by Korshunova *et al.* (2023), with no significant differences observed. However, a difference was noted in the depth of occurrence. The Korean specimens were collected from deeper waters at depths of 48-55 meters with water temperatures ranging from 4°C, whereas the Russian specimens described in the original description were recorded from shallower waters at depths of 15-20 meters. This difference in depth likely reflects regional ecological adaptation, although further research is needed to confirm this.

A total of 43 COI sequences of *Tenellia* species (564 bp) were analyzed. The intraspecific genetic distance within *T. zvezda* ranged from 0.61% to 1.7%. The specimen examined in this study exhibited the lowest intraspecific distance (0.61%) to a specimen from Russia (GenBank accession nos. PV792013 and OQ779524). The minimum interspecific distance was 4.92%, observed between *T. zvezda* and *T. stipata* (GenBank accession nos. PV792013 and OQ779521). In the NJ phylogenetic tree, the *T. zvezda* clade was strongly supported with a bootstrap value of 100 (Fig. 3).

Suborder Doridina Odhner, 1934 갯민숭달팽이아목

Family Polyceridae Alder and Hancock, 1845

Genus *Tambja* Burn, 1962 능선갯민숭달팽이속

***Tambja morosa* (Bergh, 1877) 용궁능선갯민숭달팽이**

(Table 1; Figs. 1E, 4)

Trevelyana morosa Bergh, 1873: pl. XXV, fig. 9.

Nembrotha morosa: Bergh, 1877: 457-458, taf. 25, fig. 9, taf. 33, fig. 7.

Nembrotha amitina Bergh, 1905: 201, taf. 18, figs. 23-28.

Nembrotha capensis Bergh, 1907: 68-70, pl. 12, figs. 8-17.
Tambja kushimotoensis Baba, 1987: 13-14, figs. 1-2.
Tambja morosa: Okutani, 2000: 781, Pola *et al.*, 2006: 493-497, figs. 1A, B, I, 2A-F, 3A: table. 1; Gosliner *et al.*, 2008: 118; 2018: 43; Nimbs *et al.*, 2020: 19.

Type locality. The Philippines.

Distribution. Tropical Indo-Pacific Ocean (Pola *et al.*, 2006; Gosliner *et al.*, 2018)

Specimens examined. SOUTH KOREA: 2 specimens, Jeju-do, Seogwipo-si, Seogwi-dong, Munseom Islet, 22 VI 2022, DW Jung (MABIK MO00187883; KOMBI-KM125).

Measurement. Body length 25-33 mm, preserved specimens

Diagnosis. Body limaciform, ground color greenish-black or bluish-black to black (Fig. 1E). Head slightly rounded. Frontal veil and end of oral tentacles fluorescent blue. Rhinophoral clavus perfoliate, rhinophoral sheath distinct, color of rhinophores and rhinophoral sheath same as ground color, tip of rhinophores fluorescent blue. Several wrinkles on anterior to posterior mantle. Mantle and metapodium with oval or short linear patches of fluorescent blue dorsolaterally. Gill with three branchial leaf rachis, bi- or tripinate, elongated upwards. branchial leaves black. Foot margin fluorescent blue. Metapodium blunt end. Oral tentacles grooved.

GenBank Accession No. OR800616

Remark. The *p*-distances were calculated based on a 658 bp alignment of partial COI sequences from ten individuals representing six *Tambja* species (Table 1). Within the genus, uncorrected *p*-distances ranged from 0.3% to 14.5%. The intraspecific *p*-distances of *T. morosa* varied widely, from 0.3% (between individuals from Korea and the Philippines; OR800616 and DQ230997, and Japan and the Philippines; EF142867-8) to as high as 11.7% (between specimens from the Philippines and Mauritius; DQ230997 and OK501283). The phylogenetic tree also shows that *T. morosa* is a non-monophyletic group (Fig. 4). *T. morosa* from Korea consists of a sister group with *T. olivaria*, *T. victoriae*, and *T. morosa* individuals from Japan and the Philippines (DQ230997, EF142867-8). The calculated interspecific *p*-distances to congeners ranged from 1.7% (between *T. olivaria* and *T. morosa*, from Japan and the Philippines; KJ999225 and EF142867-8) to 13.1% (between *T. morosa* from the Philippines and *T. verconis*; DQ230997 and EF142869). These findings suggest the presence of cryptic diversity within *T. morosa* and highlight the need for further morphological and molecular studies. Similar taxonomic ambiguities have been noted in prior studies (Pola *et al.*, 2006), and future work employing additional markers or multilocus approaches is likely required to resolve these relationships.

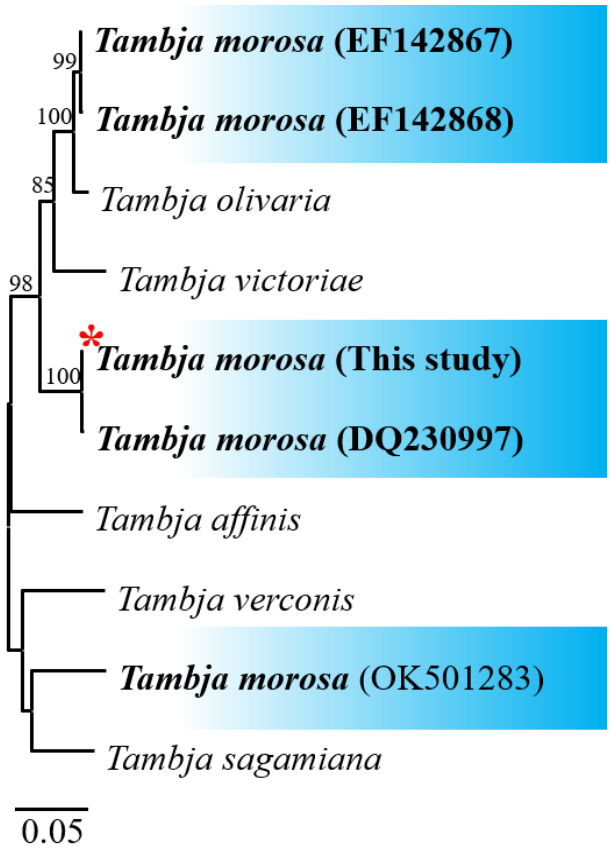

Fig. 4. NJ phylogenetic tree of *Tambja* species inferred from mitochondrial COI sequences obtained from GenBank. Bootstrap values ≥ 80% from 1,000 replicates are shown above the corresponding nodes. Sequences newly generated in this study are marked with a red asterisk (*). Focal species are shown in bold and highlighted with colored shading.

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