Korean J. Malacol. 35(3): 243-248 2019
https://doi.org/10.9710/kjm.2019.35.3.243

[단보, Short communication]

New Record of the red-mouth frog shell, *Tutufa bufo* (Röding, 1798) (Gastropoda: Littorinimorpha: Bursidae) from Korea

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ABSTRACT

A single specimen belonging to the family Bursidae was collected during a scuba diving survey in Jeju Island in Korea and was identified as the red-mouth frog shell, *Tutufa bufo* (Röding, 1798). To date, two bursid species, *Bufonaria rana* (Linnaeus, 1758) and *Bursa bufonia* (Gmelin, 1791), have been recorded in Korean waters. In this study, we describe *Tutufa bufo*, including coloration and radula shape based on a newly collected specimen. In addition, the partial gene sequence of mitochondrial cytochrome c oxidase subunit I (mt COI) is determined for molecular analysis.

Keywords: *Tutufa bufo*, Bursidae, red-mouth frog shell, Korea

INTRODUCTION

The species of the family Bursidae (Thiele, 1925) are widely distributed in tropical and subtropical warm waters, including the Indo-West Pacific Ocean, Atlantic Ocean, Caribbean Sea, and Mediterranean Sea, and are found on coral reefs and rocky shores from the intertidal zone to the shallow waters (Beu, 1981; Zhongyan, 2004; Thach, 2007; Kil *et al*., 2012). They are carnivores and feed on other invertebrates such as polychaete worms using well-developed hunting organs. The bursid species are commonly called "frog shells" or "frog snails" because their external shell surface resembles the dorsal surface of toads (Thach, 2007; Kil *et al*., 2012). Among the Bursidae, *Tutufa* (Jousseaume, 1881) species are easily distinguished from other bursid species by their markedly large size (Beu, 1981).

Received: July 17, 2019; Revised: September 15, 2019; Accepted: September 25, 2019
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1225-3480/24736

A total of 55 bursid species categorized in six genera has been recorded in the world to date (Abbott & Boss, 1989; Kil *et al*., 2012), and two frog shells, *Bufonaria rana* (Linnaeus, 1758) and *Bursa bufonia* (Gmelin, 1791), have been reported in Korean waters (Kil *et al*., 2012; Lee, 2016).

A single individual of red-mouth frog shell, *Tutufa bufo* (Röding, 1798) was collected on the southern coast of Jeju Island in Korea. In this study, we describe *T. bufo* as a new record from Korean waters. We also provide a partial gene sequence of the mitochondrial cytochrome c oxidase subunit I (mt COI) for molecular analysis.

MATERIALS AND METHODS

1. Morphological observation.

A single specimen was collected from the Jeju-do subtidal area near Bumseom Islet by scuba diving and immediately fixed in 70% ethanol. The radula was extracted from the buccal mass for observation using a scanning electron microscopy (SEM, JSM-6390). All organic matters were cleaned out around the radula mechanically and by using a NaOCl solution. The cleaned radula was dried on an aluminum stub

attached with carbon tape and coated with pure gold.

2. DNA sequencing.

A small part of the soft tissue was used for total genomic DNA extraction by using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the instructions supplied by the company. For PCR amplification, the TaKaRa Ex Taq™ polymerase (Takara Bio, Shiga, Japan) was used in combination with the universal mt COI primer set [LCO1490 forward primer (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 reverse primer (5'-TAAACTTCAGGGTGACCAAAAAATCA-3')] (Folmer *et al*., 1994). The amplified PCR product was sequenced using the ABI 3700 sequencer (Applied Biosystems, Foster City, CA, USA) for both directions. The 658 base pairs partial sequence of mt COI (GenBank accession number: MF538533) was obtained and compared with the sequences of 15 other bursid species mined from GenBank (Table 1). Mt COI sequences were automatically aligned using MUSCLE (Edgar, 2004) and multiple alignments were implemented using default parameters in Geneious v. 9 (Biomatters Ltd.). An unrooted phylogenetic tree was reconstructed using the neighbor-joining (NJ) method with the Kimura's two-parameter model in MEGA 7 (Kumar *et al*., 2016).

The specimen was housed at the National Institute of Biological Resources (NIBR), Incheon, Republic of Korea.

SYSTEMATIC ACCOUNTS

Class Gastropoda Cuvier, 1795 복족강
Subclass Caenogastropoda Cox, 1960 신생복족아강
Order Littorinimorpha Golikov & Starobogatov, 1975 총알고둥목
Superfamily Tonnoidea Suter, 1913 위고둥상과
Family Bursidae Thiele, 1925 두꺼비고둥과
[1*]Genus *Tutufa* Jousseaume, 1881 넓은입두꺼비고둥속(신칭)
[2*]*Tutufa bufo* (Röding, 1798) (Figs. 1, 2) 붉은입두꺼비고둥(신칭)

Restricted synonymy

Tritonium bufo Röding, 1798: 128 (refers to Martini, 1780, 4, pl.129, Fig. 1238).
Bursa (*Tutufa*) *bufo*: Kira, 1955: 43, pl. 21, Fig. 20; 1961: 55, pl. 21, Fig. 20.
Bursa (*Tutufa*) *lissostoma*: Kira, 1962: 57, pl. 22, Fig. 20.
Tutufa bufo: Oyama and Takemura, 1963: Tutufa p1. 2, Figs 2-4; Okutani, 2000: 271, pl. 134, Fig. 18; Thach, 2007: 78, 280, pl. 16, Fig. 275.
Tutufa lissostoma: Okutani, 1970: 124, pl. 8, Fig. 1;

Table 1. List of 16 bursid species with voucher and GenBank registration numbers used for phylogenetic analysis in this study.

Name of the species	Voucher no.	NCBI accession no.
Bufonaria perelegans	IM-2007-43057	JX241364
Bufonaria rana	-	MF693400
Bursa awatii	IM-2007-43058	JX241365
Bursa bufonia	IM-2007-43041	JX241350
Bursa fosteri	IM-2007-43048	JX241356
Bursa granularis	Br-NT-856	MF124223
Bursa lamarckii	IM-2007-43045	JX241354
Bursa latitudo	IM-2007-40255	JX241227
Bursa nobilis	IM-2007-43052	JX241360
Bursa quirihorai	IM-2007-43590	JX241415
Bursa ranelloides	IM-2009-5150	JX241429
Bursa rhodostoma	IM-2007-43068	JX241373
Bursina fijiensis	IM-2007-40279	JX241251
Tutufa bufo	NIBRIV0000849407	MF538533 (Present study)
Tutufa rubeta	IM-2007-43036	JX241346
Tutufa tenuigranosa	IM-2007-43061	JX241368

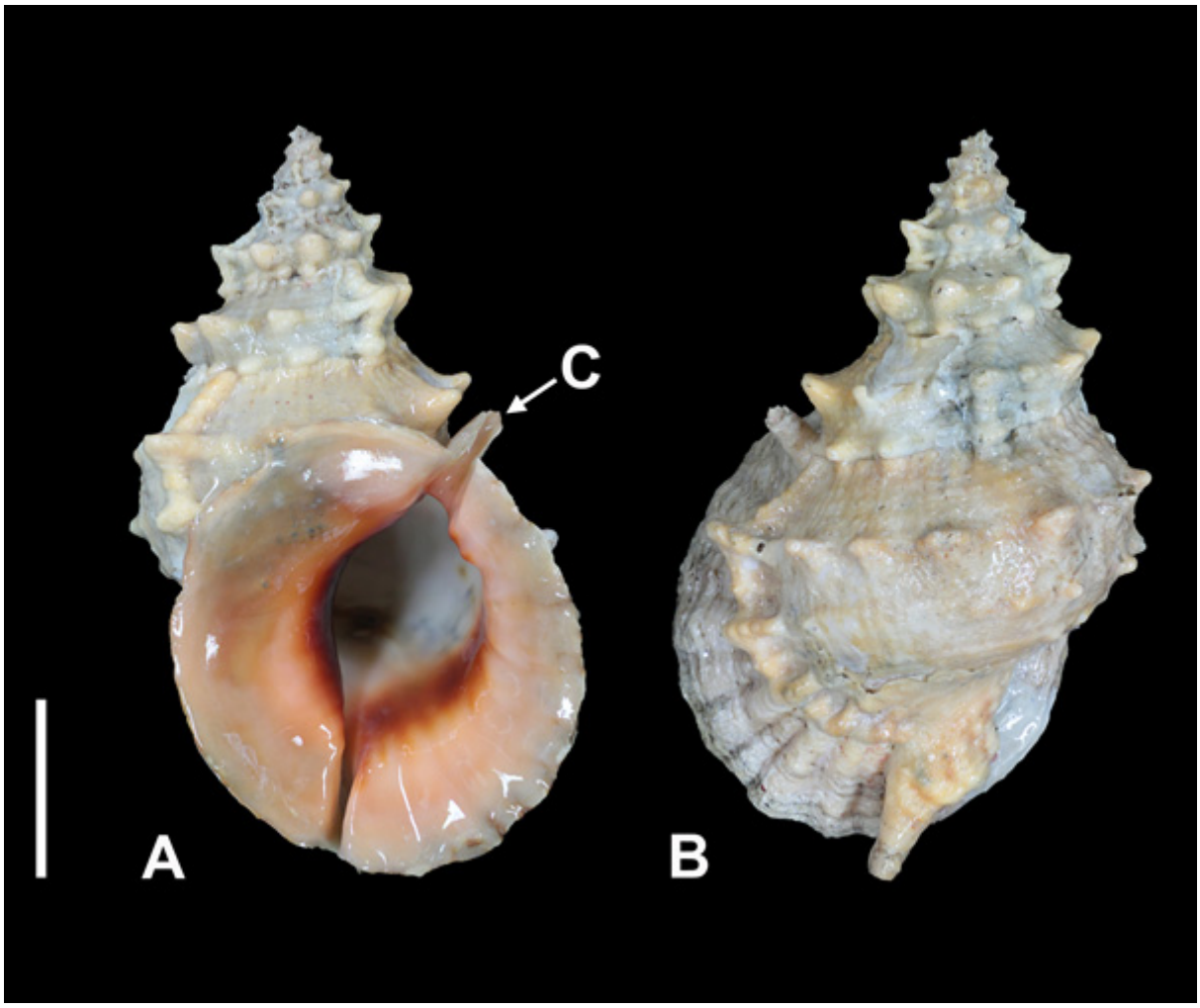

Fig. 1. *Tutufa bufo*. **A**. ventral view; **B**. dorsal view; **C**. anal canal. Scale bar: 50 mm.

Kuroda, Habe & Oyama, 1971: 134, pl. 34.
Tutufa (*Tutufa*) *bufo*: Beu, 1981: 272-277, Figs 1d, g, p; 2c; 5b, c, f; 11a, c-f; 12a, g; 1999: 52, Fig. 99; Higo, Callomon & Goto, 1999: 162.
Tutufa (*Tutufa*) *lissostoma*: Higo & Goto, 1993: 163.

Type locality: Madagascar (Kuroda *et al*., 1971; Beu, 1999).
Habitat: Sand, coral reefs, and rocky shores from intertidal zone to subtidal zone.
Distribution in East Asia: Japan, Korea (Jeju Island, present study) (Fig. 3).
Material examined: Single individual (NIBRIV0000849407), Beomseom (islet), Seogwipo-si, Jeju-do, depth 29 m, rocky area, 30 July 2015, S-H. Lee and T. Park.
Measurement: Shell height of 210 mm; shell width of 130 mm.
Description: Shell large, thick, and heavy, with spinous tubercles on external sculpture and large aperture (Fig. 1). Whole body composed of total 8-9 whorls and mixed colors, ivory and pale pink. Acute large spiral row of ventral and dorsum compressed nodules in the middle of spire whorls, small and rounded nodules row on sutures. Two-thirds varices on the whorl. Aperture large oval, internal ring area dark brownish, glossy flesh pink toward outer lip. Inner lip widely flared, protruding hemi-tubular anal siphon (Fig. 1C). Outer lip flared with 10-12 denticles internally, sharp edge.

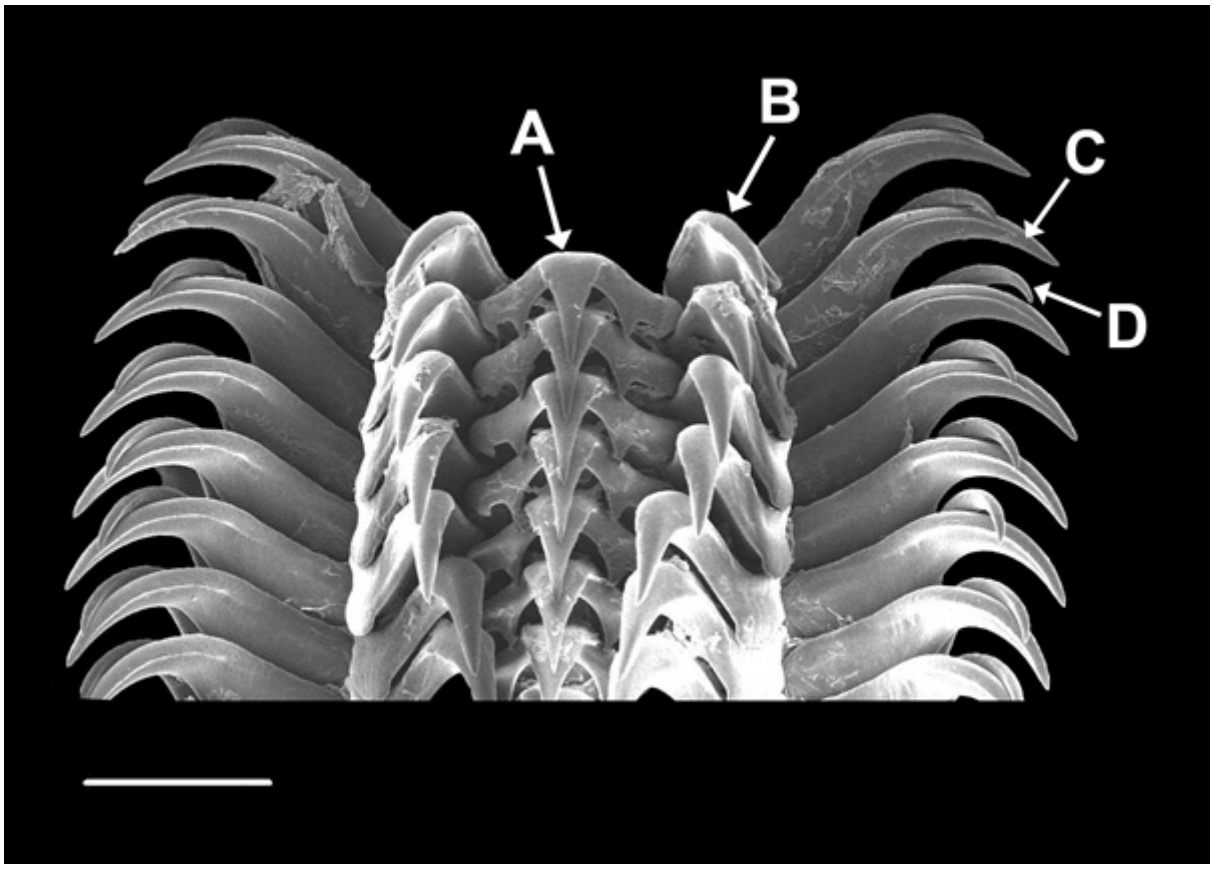

Fig. 2. Radula of *Tutufa bufo*. **A**. central tooth; **B**. lateral tooth; **C**. inner marginal tooth; **D**. outer marginal tooth. Scale bar = 500 μm (37X).

Columellar smooth, expanded, glazy. Anterior siphonal canal short, hemi-tubular.

Radula: Central tooth comparatively low, with an embowed base, and two small hooked cusps on each side of a long main triangular cusp (Fig. 2A). Lateral tooth with long base and relatively short cusp (Fig. 2B). Each of inner and outer marginal tooth curved and crossed terminally, claw-shaped cusp diminishing outward (Fig. 2C, D).

Remarks: Most species of the genus *Tutufa* have very similar morphological features, such as shell size, surface sculpture, and color. Each species also has highly intraspecific morphological variations (Beu, 1981). Therefore, it is difficult to distinguish between species, especially *T. bufo*, *T. bubo* (Linnaeus, 1758), *T. rubeta* (Linnaeus, 1758), *T. tenuigranosa* (E. A. Smith, 1914), and *T. bardeyi* (Jousseaume, 1894). However, *T. bufo* is the only *Tutufa* species among above species

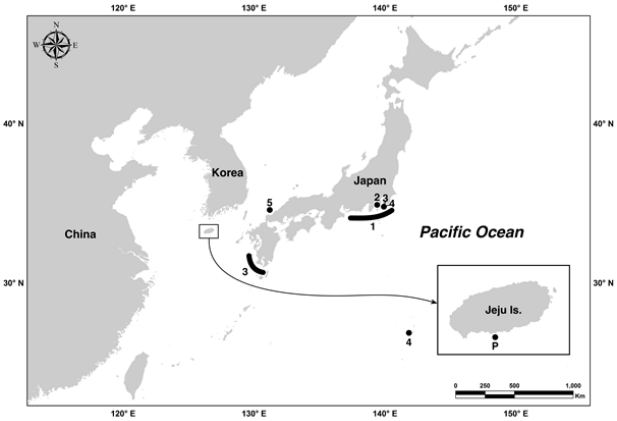

Fig. 3. Distribution of *Tutufa bufo* in East Asian waters based on the present study (P) and literature (1-5). **1**: Kira (1955, 1961, 1962; South of central Honshu), **2**: Kuroda *et al*. (1971; Sagami Bay), **3**: Higo and Goto (1993; Boso Peninsula, Southwest of Kyushu), **4**: Higo *et al*. (1999; Boso Peninsula, Ogasawara Islands, **5**: Beu (1999; Yamaguchi Prefecture).

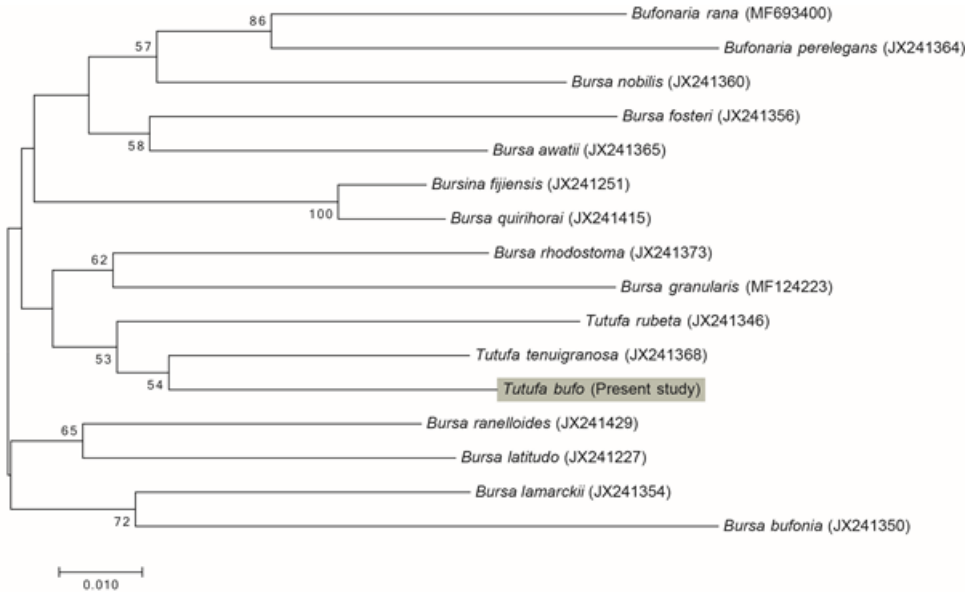

Fig. 4. Neighbor-joining (NJ) analysis based on mitochondrial cytochrome c oxidase subunit I (mt COI) sequences of bursid species.

with a long projected anal canal (Fig. 1C) and a red ring (Fig. 1D) in the aperture which are distinguishing features. In the present study, a partial sequence of mt COI from *T. bufo* was determined for the first time. As a result of the mt COI sequence comparison with its congeners, *T. bufo* formed a distinct clade, closely related to *T. tenuigranosa* and *T. rubeta* (Fig. 4).

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR No. 201501111). It was also partially supported by the National Marine Biodiversity Institute Research Program (MABIK No. 2019M00200).

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