



## NEW RECORDS OF THREE DEEP-SEA BATHYMODIOLUS MUSSELS (BIVALVIA: MYTILIDA: MYTILIDAE) FROM HYDROTHERMAL VENT AND COLD SEEPS IN TAIWAN

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# NEW RECORDS OF THREE DEEP-SEA BATHYMODIOLUS MUSSELS (BIVALVIA: MYTILIDA: MYTILIDAE) FROM HYDROTHERMAL VENT AND COLD SEEPS IN TAIWAN

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Key words: new record, *Bathymodiolus*, deep-sea, hydrothermal vent, cold seep, Taiwan.

## ABSTRACT

The deep sea mussel genus, *Bathymodiolus* Kenk & Wilson, 1985, contains 31 species, worldwide. Of which, one endemic species (*Bathymodiolus taiwanesis*) was reported from Taiwan (MolluscaBase, 2018). Herein, based on the mitochondrial COI results, we present 3 new records of the *Bathymodiolus* species from Taiwan, namely *Bathymodiolus platifrons*, *Bathymodiolus securiformis*, and Sissano *Bathymodiolus* sp.1 which were collected from vent or seep environments at depth ranges of 1080-1380 m. Therefore, a total of four species in the genus of *Bathymodiolus* are now known from Taiwan.

## I. INTRODUCTION

The genus of *Bathymodiolus* (Family: Mytilidae) consists of species entirely living at hydrothermal vent or cold seep environments. This genus, *Bathymodiolus*, has 31 deep-sea species worldwide (MolluscaBase, 2018). To date, *Bathymodiolus*

*taiwanesis* (von Cosel, 2008) is the only reported species of this genus from Taiwan. It was collected from hydrothermal vents near Kueishan Islet off the northeast coast of Taiwan at depths of 200-355 m.

Along with traditional morphological classification, molecular techniques are commonly used to study the taxonomy and phylogenetic relationships of deep sea mussels. Recently, the complete mitochondrial genomes have been sequenced from mussels of *Bathymodiolus japonicus*, *B. platifrons* and *B. septemdierum* (Ozawa et al., 2017). Even more, the whole genome of *B. platifrons* was reported with sequence length of 1.64 Gb nucleotides (Sun et al., 2017).

Since 2013, under the Phase II National energy program of "Gas hydrate investigation and survey", deep sea cold seep environments offshore of Taiwan were explored. A series of deep-sea cruises were conducted and *Bathymodiolus* mussels were collected. Here, three new records were reported based on the sequenced results of COI gene.

## II. MATERIALS AND METHODS

Specimens of *Bathymodiolus platifrons*, *Bathymodiolus securiformis*, and *Bathymodiolus* sp. were collected from hydrothermal vent (Okinawa Trough) or cold seeps (Formosa Ridge, Four Way Closure Ridge) at depths of 1000-1300 m (Fig. 1). Voucher specimens were preserved in 70% ethanol and deposited in the National Museum of Natural Science (NMNS), Taichung, Taiwan (see Table 1).

Crude DNA was extracted using the Tissue & Cell Genomic DNA Purification Kit (GeneMark, DP021-150), following the manufacturer's instructions. Part of adductor muscles or mantle tissues (5-25 mg) were homogenized with extraction solution and proteinase K, incubated at 60°C, about 2-3 hours. Samples were then lysed and centrifuged several times to precipitate nuclei and debris. Finally, 50 µl double-distilled water was added

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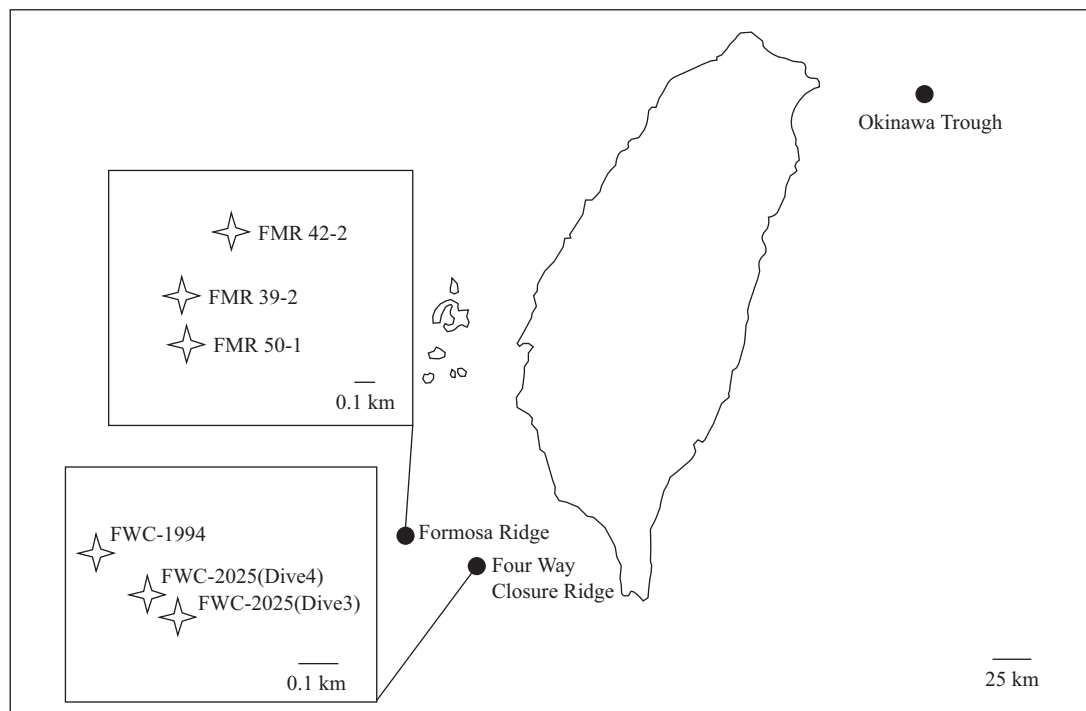
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**Table 1. List of sampling dates and sites of *Bathymodiolus* mussels. N: sample size.**

Species	Sampling site	Cruise	Latitude, Longitude	Depth (m)	Date	N	
<i>Bathymodiolus platifrons</i>	Formosa Ridge	39-2	SO 227	22°06.94'N; 119°17.12'E	1079	2013.04.27	2
		42-2	SO 227	22°06.97'N; 119°17.13'E	1150	2013.04.29	1
		50-1	SO 227	22°06.93'N; 119°17.12'E	1122	2013.05.01	2
	Four Way Closure Ridge	-	OR3-1994	22°03.51'N; 119°48.02'E	1300	2017.05.03	1
		Dive 3	OR3-2025	20°03.50'N; 119°48.03'E	1350	2017.09.27	1
		Dive 4	OR3-2025	20°03.50'N; 119°48.02'E	1347	2017.09.28	2
	Okinawa Trough	-	OR1-1139	24°50.74'N; 122°41.99'E	1383	2016.06.12	1
-		OR1-1202	-	-	2018.07	2	
<i>Bathymodiolus securiformis</i>	Four Way Closure Ridge	-	OR3-1994	22°03.51'N; 119°48.02'E	1300	2017.05.03	2
Sissano	Formosa Ridge	42-2	SO 227	22°06.97'N; 119°17.13'E	1150	2013.04.29	2
<i>Bathymodiolus</i> sp.1							

**Fig. 1. Map showing collection sites of *Bathymodiolus* mussels.**

to the resulting DNA pellets, stored at  $-20^{\circ}\text{C}$  for later use.

The sequences of mitochondrial COI were amplified by PCR using the sense primer LCO1490: 5'-ggtaacaatacataagatattgg-3' and the antisense primer HCO2198: 5'-taaacttcagggtgacacaaaaatca-3' (Folmer, 1994). Amplification was conducted in a thermal cycler (Applied Biosystems 2720), the PCR condition was as follows: initial denaturation step at  $94^{\circ}\text{C}$  for 5 min, followed by another 40 cycles: denaturation at  $94^{\circ}\text{C}$  for 30 s, annealing at  $53^{\circ}\text{C}$  for 30 s and extension at  $72^{\circ}\text{C}$  for 20 s. Then  $72^{\circ}\text{C}$  for 7 min for the final extension step. The amplified DNA was directly sequenced on an automated DNA sequencer (Applied Biosystems 3730xl DNA Analyzer). Additional sequences from NCBI data-

base were also used, and the sequence of *Modiolus nipponicus* (accession number: AB076912.1) was used as the outgroup taxa.

The obtained sequences were aligned using the CLUSTAL W in MEGA v. 6. (Tamura et al., 2013), the Kimura 2-parameter model, and maximum-likelihood (ML) method (Kimura, 1980). Bootstrap probability (BS) estimates (1,000 replicates) (Felsenstein, 1985) were also made to indicate robustness of nodes in neighbour-joining trees.

### III. TAXONOMY

The resulting sequence length of COI for *Bathymodiolus*

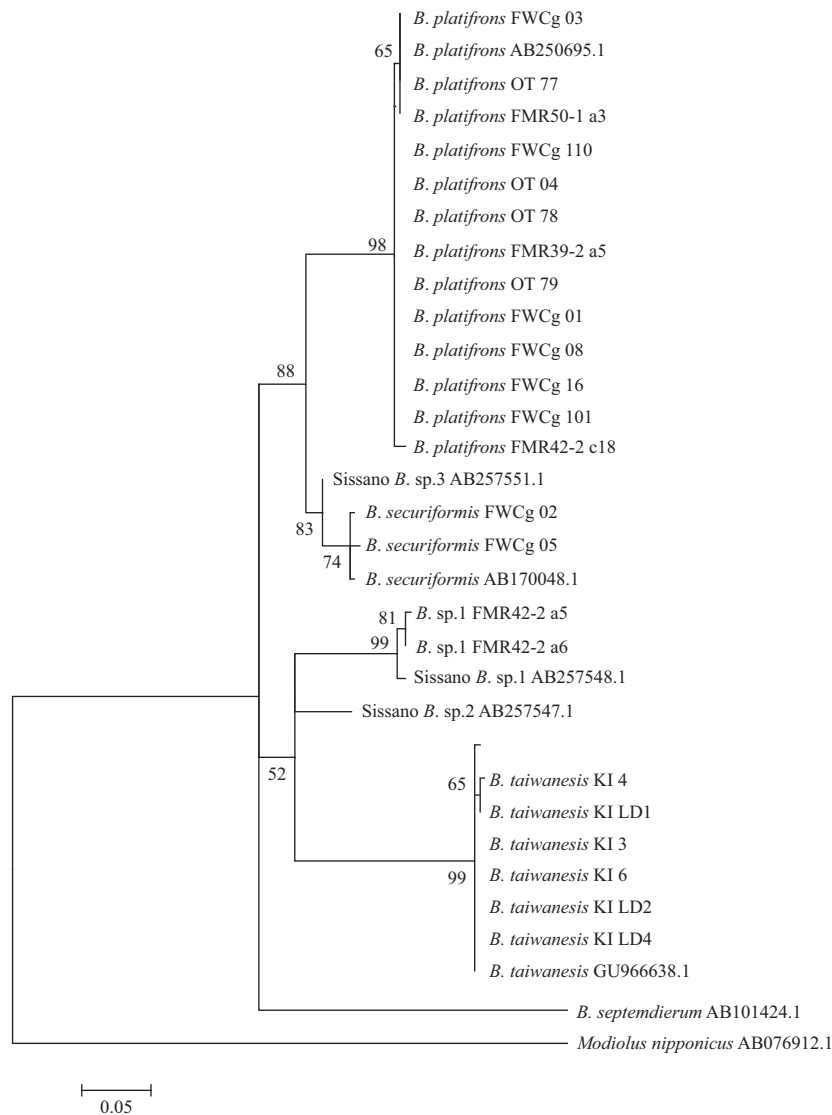


Fig. 2. The maximum-likelihood tree based on mitochondrial COI sequences obtained from *Bathymodiolus* mussel specimens from Taiwan, and combined with additional sequences from NCBI database. FMR: Formosa Ridge; FCWg: Four Way Closure Ridge; KI: nearby Kueishan Islet; OT: Okinawa Trough.

mussels was approximately 710 bp nucleotides. And, the COI sequences of examined species are provided in Appendix 1. Neighbour-Joining (NJ) analysis on COI gene from seven *Bathymodiolus* species grouped the same species into one sub-clade with bootstrap support of 74-99% (Fig. 2). The unknown *Bathymodiolus* sp. collected from Formosa Ridge was grouped to the clade of Sissano *Bathymodiolus* sp.1. Based on the results, a total of three *Bathymodiolus* new records were obtained. The descriptions of the three *Bathymodiolus* mussels are as follows.

Family Mytilidae Rafinesque, 1815

Genus *Bathymodiolus* Kenk & Wilson, 1985

*Bathymodiolus platifrons* Hashimoto & Okutani, 1994 (Fig. 3)

*Bathymodiolus platifrons* Hashimoto & Okutani, 1994: 64, fig. 2;

pl. 1, figs. 1-2; pl. 2, fig. 3; pl. 3, fig. 1; Sasaki, Okutani and Fujikura 2005: 87, fig. 5D; Kurozumi, 2017: 1172, pl. 476, fig. 5

#### Type depository

Holotype, NSMT (National Science Museum, Tokyo)-Mo 70026

#### Type locality

Okinomiya Bank, Sagami Bay, Japan, 1180 m, seep

#### Material examined.

2 specimens from Okinawa Trough (24° 50.7408'N, 122° 41.9991'E), depth 1383 m (OR1-1139, June 12, 2016); 4 specimens from Four Way Closure Ridge (22°03.507'N, 119° 48.016'E), depth 1300 m (OR3-1994, May 3, 2017); 2 specimens from Formosa Ridge 39-2 (22° 6.94'N, 119° 17.12'E), depth 1079 m (RV SONNE cruise SO227, April 27, 2013), 1

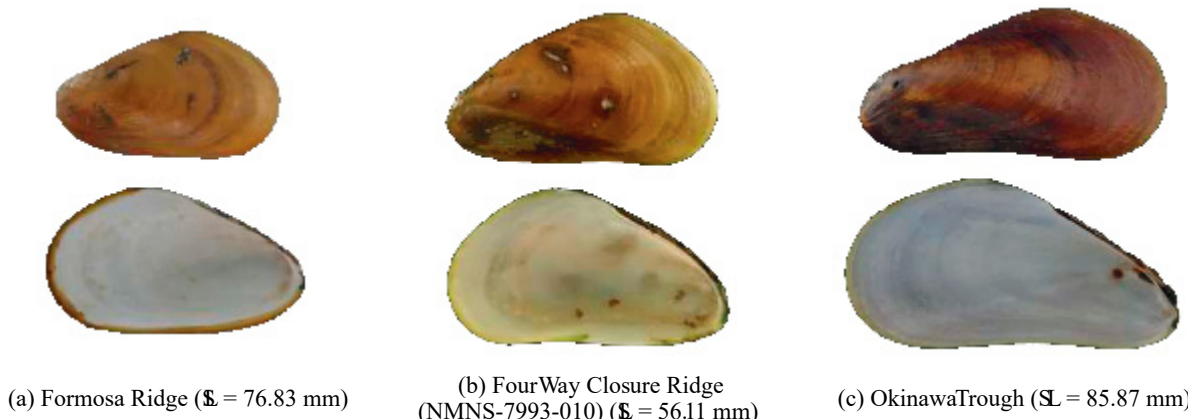


Fig. 3. *Bathymodiolus platifrons*, exterior and interior view of left valve. Scale bar 5 mm.

specimens from Formosa Ridge 42-2 (22° 06.971'N, 119° 17.133'E), depth 1150 m (RV SONNE cruise SO227, April 29, 2013), 2 specimens from Formosa Ridge 50-1 (22° 06.930'N, 119° 17.119'E), depth 1122 m (RV SONNE cruise SO227, May 1, 2013).

#### Measurements of selected specimens.

Cruise	Museum code	Sampling code	Shell length (mm)	Shell height (mm)	Shell width (mm)
SO227	NMNS-7993-002	50-1 a14	53.27	30.32	24.40
SO227	NMNS-7993-003	39-2 a15	62.48	34.99	27.04
SO227	NMNS-7993-004	39-2 a5	63.06	31.13	24.38
SO227	NMNS-7993-005	42-2 c18	50.89	30.30	22.44
SO227	NMNS-7993-006	50-1 a3	107.18	51.97	44.33
OR3-1994	NMNS-7993-007	FWCg 01	83.60	40.16	36.61
OR3-2025	NMNS-7993-008	FWCg 08	69.85	36.60	27.88
OR3-2025	NMNS-7993-009	FWCg 16	81.17	39.99	30.86
OR3-2025	NMNS-7993-010	FWCg 101	56.11	33.05	22.39
OR1-1139	NMNS-7993-013	OT 04	51.11	28.84	22.71
OR1-1202	NMNS-7993-014	OT 78	53.66	30.28	23.48
OR1-1202	NMNS-7993-015	OT 79	16.79	9.75	7.04

#### Distribution

Distribution in Northwest Pacific. In methane seep environments: Sagami Bay, Myojin Knoll, Four Way Closure Ridge and Formosa Ridge; in hydrothermal vent: Okinawa Trough. Depth ranges 1000-1500 m (Kyuno et al., 2009; Kurozumi, 2017; Sasaki et al., 2005).

#### Diagnosis

Shell inflated, modioliform, rounded-triangular. Umbones proogyrate, nearly subterminal. Umbonal ridge prominent. Anterior portion of shell low and narrow. Posterior margin broadly rounded, ventral margin slightly concave or nearly straight. Postero-dorsal corner weakly angulated. Priostracum smooth and brown.

#### Description

Shell large, up to 107.2 mm, height/length ratios 0.48-0.60; and width/length ratios 0.38-0.46.

#### Remarks

The occurrence of *Bathymodiolus platifrons* has been reported by Lin et al., (2007) from Formosa Ridge. The present material was deposited in the NMNS [see page 2], Taichung, Taiwan.

#### *Bathymodiolus securiformis*

Okutani, Fujikura & Sasaki, 2004 (Fig. 4)

*Bathymodiolus securiformis* - Okutani, Fujikura and Sasaki, 2004: 105 (Okutani et al., 2003), figs. 4C, D, 7C, D, 8, 9.; Sasaki, Okutani and Fujikura 2005: 87 (Sasaki et al., 2005), Fig. 5E; Kurozumi, 2017: 1172 (Kurozumi, 2017), pl. 477, fig. 2.

#### Type depository.

Holotype, UMUT (University Museum of the University of Tokyo)-RM 28478

#### Type locality

Kuroshima Knoll off the Yaeyama Islands, Okinawa, 644 m, seep

#### Material examined

2 specimens, Four Way Closure Ridge (22°03.507'N, 119° 48.016'E), depth 1300 m (OR3-1994, May 3, 2017).

#### Measurements of selected specimens.

Cruise	Museum code	Sampling code	Shell length (mm)	Shell height (mm)	Shell width (mm)
OR3-1994	NMNS-7993-011	FWCg 02	37.91	15.86	11.84
OR3-1994	NMNS-7993-012	FWCg 05	71.11	29.28	21.91

#### Distribution

Presently known from methane seep sites at Kuroshima Knoll and Nankai Trough in Japan, Four Way Closure Ridge in Taiwan. Depth ranges 624 m and 1300 m (Kurozumi, 2017; Okutani et al., 2003; Sasaki et al., 2005).

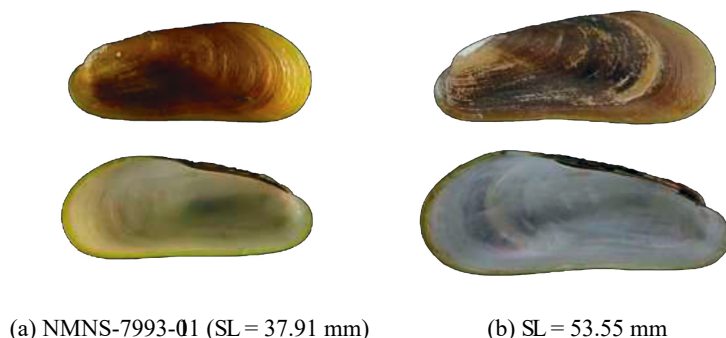


Fig. 4. *Bathymodiolus securiformis*, exterior and interior view of left valve, from Four Way Closure Ridge. Scale bar 5 mm.

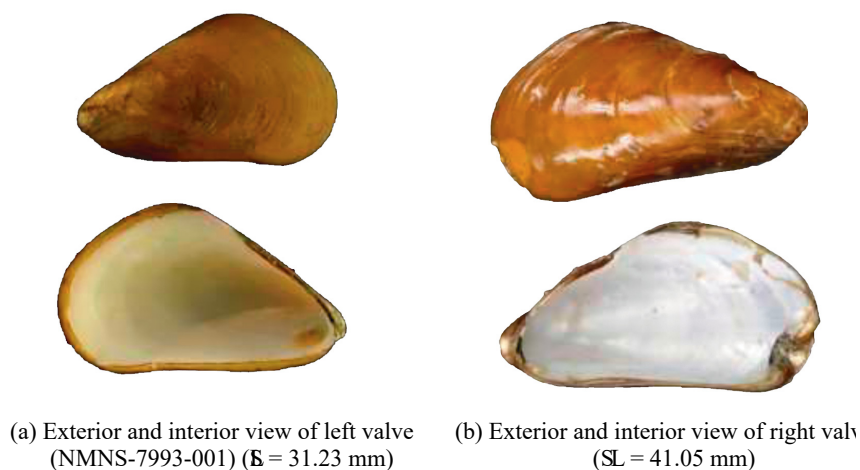


Fig. 5. Sissano *Bathymodiolus* sp.1, from Formosa Ridge. Scale bar 5 mm.

### Diagnosis

Shell long, inflated, modioliform, oblong. Anterior portion of shell low and narrow, posterior higher and wider. Anterior margin rounded. Postero-dorsal angle rounded. Priostracum smooth, dark brown but olive brown along the ventral margin.

### Description

Shell long but narrow, height/length ratios 0.41 & 0.42; width/length ratio 0.31.

Sissano *Bathymodiolus* sp.1 (Fig. 5)

Sissano *Bathymodiolus* sp.1 - Fujita et al., 2009: 123 (Fujita et al., 2009) [unidentified and undescribed mussel, sequence data only]

**Holotype.** Not-assigned

**First record locality.** Sissano, Papua New Guinea, 1646 & 1881 m, seep

### Material examined

2 specimens from Formosa Ridge 42-2 (22°06.971'N, 119°17.133'E) at a depth of 1150 m (RV SONNE cruise SO227, April 29, 2013).

### Measurements of selected specimens.

Cruise	Museum code	Sampling code	Shell length (mm)	Shell height (mm)	Shell width (mm)
SO227		42-2 a5	41.05	22.90	18.44
SO227	NMNS-7993-001	42-2 a6	31.23	18.32	24.40

### Distribution

Known from methane seep sites Papua New Guinea and Formosa Ridge in Taiwan. Depth range 1150-1881 m (Fujita et al., 2009; Kyuno et al., 2009).

### Diagnosis

Shell inflated, modioliform, rounded-triangular. Umbones prosogyrate, nearly subterminal. Umbonal ridge significantly prominent. Dorsal margin nearly straight, posterior margin rounded and ventral margin slightly concave. Postero-dorsal corner weakly angulated. Priostracum smooth and yellow.

Description

Shell short but wide, height/length ratios 0.56 & 0.59; width/length ratio 0.45 & 0.78.

Remarks

This unidentified species was firstly collected from Sissano, Papua New Guinea and examined by the sequence of COI gene (Fujita et al., 2009).

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APPENDIX 1. MITOCHONDRIAL COI SEQUENCES FROM BATHYMODIOLUS MUSSELS FROM TAIWAN

Table with 4 columns: Species/Accession, Position (1-700), and DNA Sequence. Rows include B. taiwanesis KI 6, B. taiwanesis KI LD4, B. platifrons FMR39-2 a5, B. platifrons OT 78, B. platifrons FWCg 08, B. platifrons FWCg 110, B. sp.1 FMR42-2 a5, B. securiformis FWCg 02, and B. securiformis FWCg 05.



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