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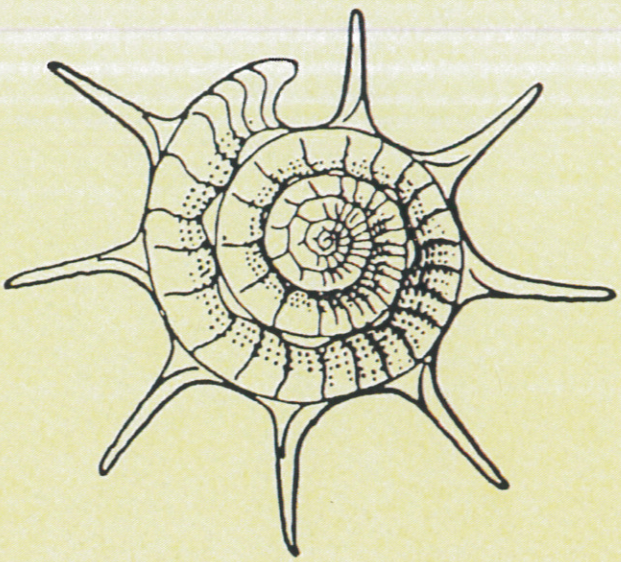

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Another New *Calyptogena* Clam from Deep-Sea Seeps off the Sanriku Coast and in Sagami Bay, Japan (Mollusca: Bivalvia)

Takashi Okutani[1]*, Shigeaki Kojima[2], Masaru Kawato[1], Eriko Seo[3] and Katsunori Fujikura[1]

[1]*Japan Agency for Marine-Earth Science & Technology, 2-15 Natsushima, Yokosuka, Kanagawa 237-0061, Japan*
[2]*Atmosphere and Ocean Research Institute, University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8568, Japan*
[3]*Tokyo University of Marine Science & Technology, 4-5-1 Konan, Minato-ku, Tokyo 108-8477, Japan*

Abstract: Among deep-sea chemosynthesis-based communities with large-scale *Calyptogena soyoaelokutanii* aggregations in the Off Hatsushima Island seep site, another new vesicomyid species, *C. fortunata*, was discovered. The analyzed DNA data of these specimens correspond to those from the Off Sanriku coast site, in spite of the fact that shell characters of specimens from both localities show a slight difference, probably representing phenotypic variability.

Keywords: *Calyptogena*, new species, Hatsushima Island seep site, Sanriku coast site, phenotypes

Introduction

Since a large-scale *Calyptogena*-community was discovered by the HOV *Shinkai-2000* in 1983 at the Off Hatsushima Island seep site in Sagami Bay (Okutani & Egawa, 1985), more species of Vesicomyidae have been discovered from the same site and its surrounding areas, namely *Calyptogena okutanii* Kojima & Ohta, 1997, *Vesicomya kaikoae* Okutani, Fujikura & Kojima, 2000 and an as-yet unidentified species (Sasaki *et al.*, 2007).

During more precise investigations of biota around this site, particularly applying molecular analyses, another vesicomyid clam was discovered among a large lot of *C. soyoaelokutanii* collected by the ROV *Hyper-Dolphin*. It is similar at first glance to a young specimen of *C. soyoae*, but close morphological observation and DNA analysis revealed that this specimen represents a new taxon, which is conspecific with an unnamed species hitherto collected from the Off Sanriku coast site.

Institutional abbreviations: JAMSTEC – Japan Agency for Marine and Earth Science & Technology; NSMT – National Museum of Science & Nature ; ORI – Ocean Research Institute (now Atmosphere & Ocean Research Institute), University of Tokyo.

Materials and Methods

The *Calyptogena* specimens in the present study were collected at the seep sites off the Sanriku coast (Pacific coast of northeastern Honshu) and off Hatsushima Island in Sagami Bay (Fig. 1). All detailed information, including coordinates, depth, vehicles/gear, and date of

* Corresponding author: okutani@jamstec.go.jp

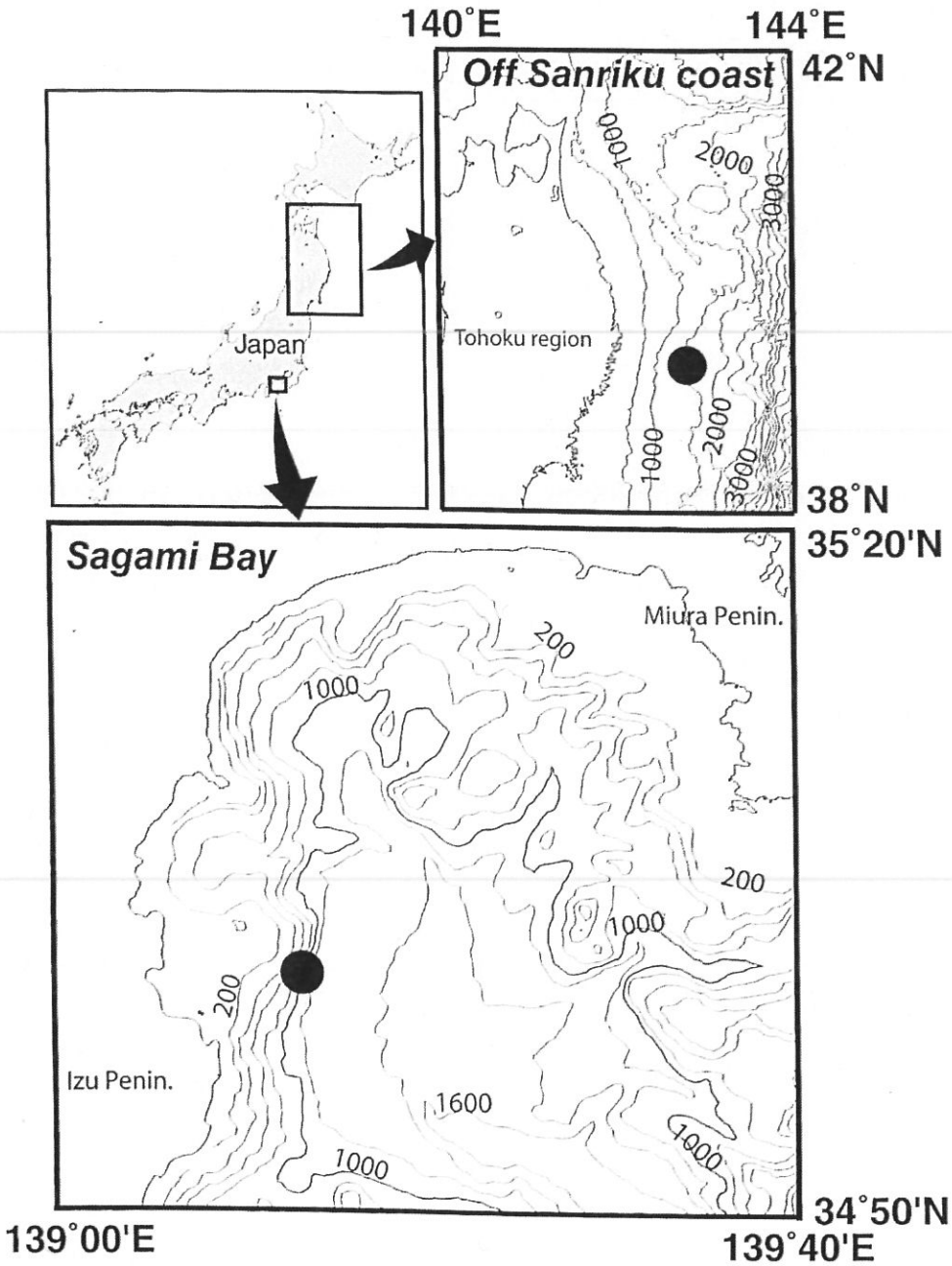

Fig. 1. Sampling sites of *Calyptogena* specimens in the Off Sanriku coast site (Pacific coast of northeastern Honshu) and the Off Hatsushima Island seep site, Sagami Bay.

dives/station of collection, of samples of the new taxon as well as those used for comparison is listed in Table 1. Shell length and width of each species were measured using calipers. Differences of shell proportions, *i.e.* length-width, of each species were analyzed by ANCOVA.

Live-taken specimens for DNA analysis: NSMT-Mo 77368 (holotype) and 77369 (paratype #1), R/V *Tansei-Maru* KT-90-08, St. SR127, Off Sanriku coast site, 1700 m depth; NSMT-Mo 77370 (paratype #2; ex-JAMSTEC 076221), ROV *Hyper-Dolphin* Dive 846, Off Hatsushima Island seep site, Sagami Bay, 1172 m depth; JAMSTEC 083641 (paratype #3), ROV *Hyper-Dolphin* Dive 978, Off Hatsushima Island seep site, Sagami Bay, 1171 m depth.

Additional empty shells: NSMT-Mo 77387, ROV *Dolphin-3K* Dive 79, Off Hatsushima Island seep site, Sagami Bay, 1184 m depth; JAMSTEC 083707, R/V *Tansei-Maru* KT-90-08, St.

Table 1. Source of samples under the present study.

Species	Locality	Lat. (N)	Long. (E)	Depth (m)	Fixiation	Facility	Dive/Cruise#	Date	N	Remarks
Calyptogena fortunata n. sp.	Off Sanriku coast site (Pacific coast of northeastern Honshu)	39°18.73′	142°50.30′	1700	–80° frozen	Beam-trawl / RV *Tansei-Maru*	KT-90-08 St. SR127	17 June 1999	2	
		39°18.73′	142°50.30′	1700					1	Empty shell
		39°19.31′	142°50.28′	1703		ROV *Kaiko*/ RV *Kairei*	243	15 June 2002	1	Empty shell
	Off Hatsushima Island seep site, Sagami Bay	35°00.15′	139°13.55′	1184			79	14 July 1990	1	Empty shell
		35°00.07′	139°13.49′	1175	10% buffered formalin	ROV *Dolphin-3K*/ RV *Natsushima*	846	1 June 2008	1	
		35°00.07′	139°13.50′	1171	99.5% ethanol		978	28 Apr 2009	1	
								Subtotal	7	
Calyptogena soyoae	Off Hatsushima Island seep site, Sagami Bay	35°00.07′	139°13.49′	1175	10% buffered formalin	ROV *Hyper-Dolphin*/ RV *Natsushima*	846	1 June 2008	11	
		35°00.06′	139°13.48′	1172			920	6 Dec. 2008	6	
		35°00.10′	139°13.51′	1178	–80° frozen or bouin's solution		974	25 Apr 2009	8	
		35°00.07′	139°13.50′	1171			978	28 Apr 2009	4	
		35°00.09′	139°13.52′	1176	99.5% ethanol		979		2	
		35°00.07′	139°13.50′	1171			990	5 May 2009	1	
								Subtotal	32	
Calyptogena okutanii	Off Hatsushima Island seep site, Sagami Bay	35°00.07′	139°13.49′	1175	10% buffered formalin	ROV *Hyper-Dolphin*/ RV *Natsushima*	846	1 June 2008	7	
		35°00.10′	139°13.47′	1172			904	27 Sept. 2008	2	
		35°00.06′	139°13.48′	1172			920	6 Dec. 2008	1	
		35°00.10′	139°13.51′	1178	–80° frozen or bouin's solution		974	25 Apr 2009	6	
		35°00.07′	139°13.50′	1171	99.5% ethanol		978	28 Apr 2009	1	
		35°00.09′	139°13.52′	1176			979		1	
								Subtotal	18	
								Total	57	

SR127, Sanriku coast site, 1700 m depth; JAMSTEC 083642, ROV *Kaiko* (10K) Dive 243, Sanriku coast site, 1703 m depth.

DNA analysis: DNA was extracted from the mantle muscle of two *Calyptogena* specimens collected at the Off Hatsushima Island seep site using a DNeasy Tissue and Blood Kit (Qiagen Japan, Tokyo, Japan). Extracted DNA was used as the template for PCR reactions. The mitochondrial cytochrome c oxidase subunit I (mtCOI) gene was amplified using an Ex Taq Kit (TaKaRa, Kyoto, Japan). Two oligonucleotide primers, LCO1490 and HCO2198 (Folmer *et al.*, 1994) were used for mtCOI gene. An amplified mtCOI gene was used for the sequencing reaction with a Big Dye Terminator Cycle Sequencing Ready Reaction Kit ver. 3.1 (PE Applied Biosystems, Foster City, CA, USA), using LCO1490 and HCO2198 primers. Sequencing was accomplished with an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems Japan Ltd., Tokyo, Japan).

Partial sequences of the mtCOI gene obtained from two specimens were identical and were deposited in the DDBJ database under accession number AB634285. This sequence was analyzed using the gapped-BLAST search algorithm to estimate the degree of similarity to other relative sequences deposited in GenBank. Using the CLUSTAL X software package (Thompson *et al.*, 1997), mtCOI sequences were aligned and then manually edited. Phylogenetic analysis was restricted to nucleotide positions that were unambiguously alignable in all sequences. Calculation of the distance matrix and neighbor-joining (NJ) analysis were accomplished using CLUSTAL X.

Taxonomy

Family Vesicomyidae Dall & Simpson, 1901

Calptogena (*Archivesica*) *fortunata* n. sp.

(Figs. 2, 3)

Description: Shell elongate veneriform, length 63–65% of height. Surface off-white, ornamented by densely fine growth lines; lines somewhat lamellate near margin. Shell covered by thin filmy periostracum, becoming thick, straw-colored, partially but weakly wrinkled in posterior region. Umbo prosogyrous. No lunule present. Escutcheon weakly demarcated. Trace of flexure running from umbo to posterior margin. Antero-dorsal margin straightly descending. Anterior margin smoothly round. Ventral margin smoothly convex. Postero-dorsal margin almost straight, creating obtuse angle with slanting posterior margin. Posterior tip rather acutely curved. (Right valve in paratype #2, NSMT-Mo 77370, with accidental radial scar in middle.)

Inner surface pure white, porcellaneous. Hinge plate moderate. Ligament parvincular, with thick calcareous fibrous layer within. In right valve, ventral cardinal tooth (1) thick, roundly trigonoid in shape; anterior ramus of subumbonal tooth (3a) thin, eave-like, bridged with horizontal posterior ramus (3b) forming angle of about 120° overhanging ventral cardinal tooth; socket between 1 and 3a narrow, deep, but that between 1 and 3b broad; subumbonal pit trace. Nymph rather short, smooth, followed by shallow excavation at posterior extremity of ligament. In left valve, subumbonal cardinal teeth (2a and 2b) disposed in inverted V-shape, with narrow, deep socket between both rami. Postero-dorsal cardinal tooth (4b) short, oblique, rather lunate; subumbonal pit trace. No sign of either anterior or posterior lateral tooth on both valves.

Anterior adductor scar kidney-shaped. Posterior adductor scar round, fused with posterior retractor scar. Pallial line entire, not sinuous behind, except with obtuse ventral angle. No secondary pallial scar present. Ventral margin entirely smooth. Ctenidia with both inner and outer demibranchs. Foot elongate with pointed tip.

Type locality: Off Sanriku coast site, 39°18.73′N, 142°50.30′E–39°18.73′N, 142°50.30′E,

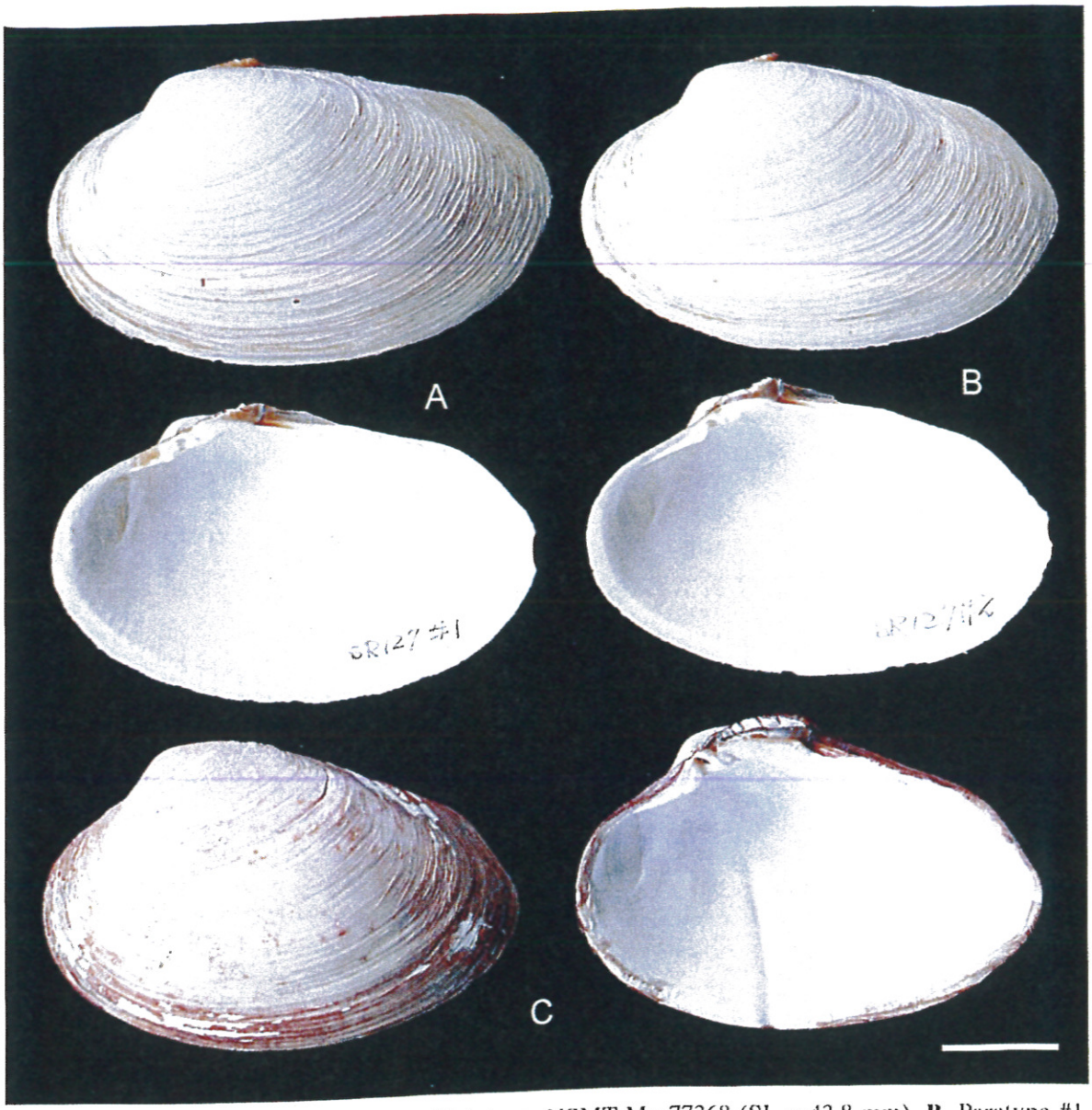

Fig. 2. *Calyptogena fortunata* n. sp. **A**. Holotype, NSMT-Mo 77368 (SL = 43.8 mm). **B**. Paratype #1, NSMT-Mo 77369, Off Sanriku coast site (SL = 42.0 mm). **C**. Paratype #2, NSMT-Mo 77370, Off Hatsushima Island seep site, Sagami Bay (SL = 41.4 mm).

1700 m depth (R/V *Tansei-Maru*, KT-90-08, St. SR127).

Measurements in mm (shell length × shell height × shell width): Holotype (NSMT-Mo 77368), 43.8 × 26.5 × 15.4; Paratype #1 (NSMT-Mo 77369), 42.0 × 25.5 × 15.4; Paratype #2 (NSMT-Mo 77370), 41.4 × 27.1 × 14.0; paratype #3 (JAMSTEC 083641), 29.2 × 19.0 × 10.5; (NSMT-Mo 77387, empty shell), 42.6 × 29.4 × 19.1; (JAMSTEC 083707, empty shell), 55.0 × 31.5 × 22.9; (JAMSTEC 083642, empty shell), 69.5 × 44.2 × 32.4.

Etymology: Species name means "fortunate". A specimen was fortunately segregated out of a large mass of *Calyptogena soyoae/okutanii* from the Off Hatsushima Island seep site in Sagami Bay. Another piece of luck was the agreement of DNA with that of the "hitherto unnamed" species from the Off Sanriku coast site known for more than ten years previously.

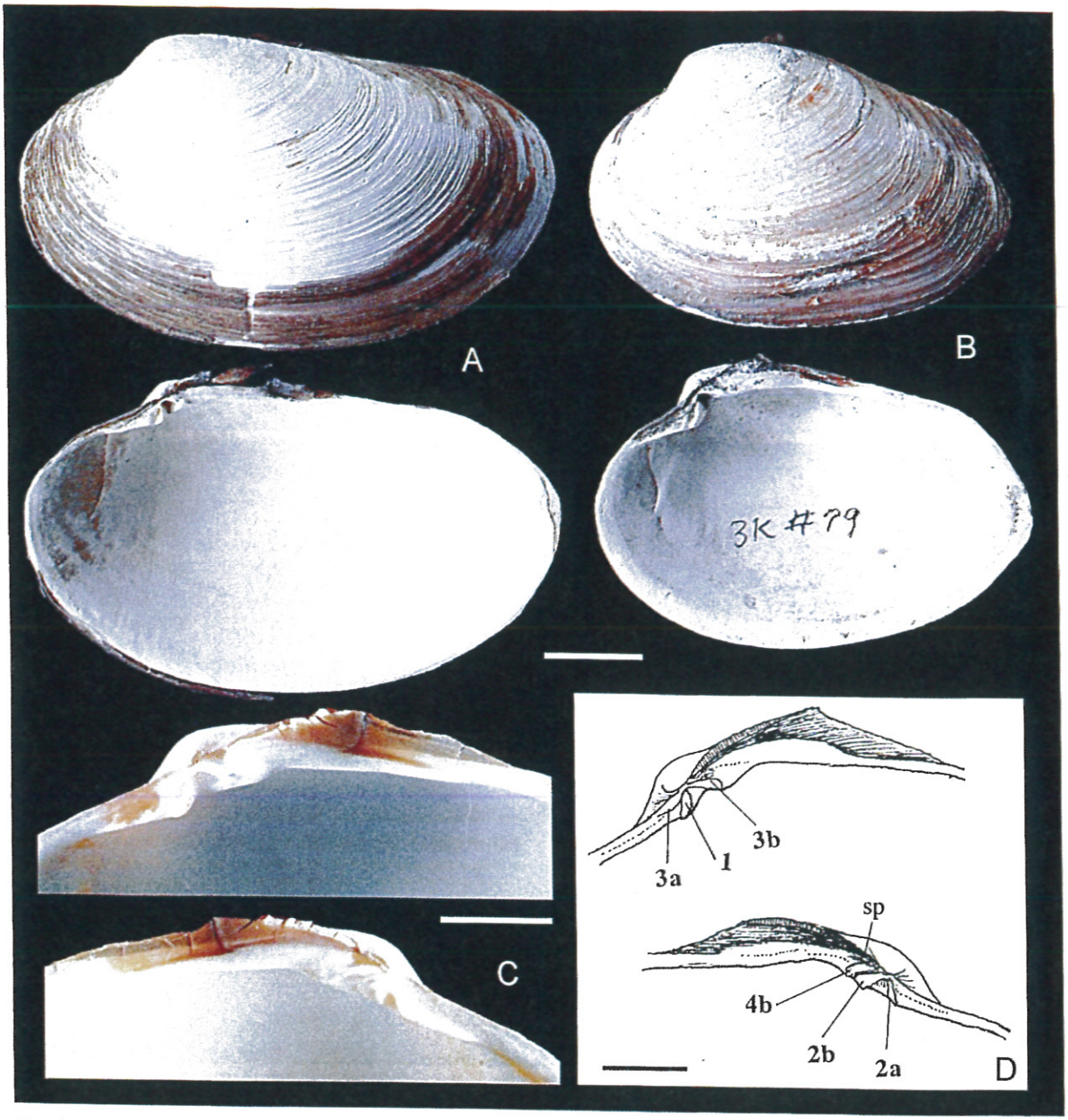

Fig. 3. *Calyptogena fortunata* n. sp. **A**. Empty shell, JAMSTEC 083707 (SL = 55.0 mm), Off Sanriku coast site. **B**. Empty shell, NSMT-Mo 77387, Off Hatsushima Island seep site, Sagami Bay (SL = 42.6 mm). **C**. Hinge of holotype, NSMT-Mo 77368. **D**. Hinge of paratype #2, NSMT-Mo 77370, Off Hatsushima Island seep site, Sagami Bay (Rf. Fig. 2C: sp denotes trace of subumbonal pit.).

Discussion

Probable phenotypes: Specimens from the Off Hatsushima Island seep site in Sagami Bay, and those from the Sanriku coast site exhibit slight differences in shell characters. The specimens from the former have a solid shell with a smooth surface and weak postero-dorsal angle. The cardinal teeth are arranged rather radially, as the bridge between 3a and 3b is very weak, representing a senescent phase. On the other hand, the shells from the Off Sanriku coast site are thinner with distinct growth lines, a gentle postero-dorsal corner, and a slightly more convex ventral margin. The bridge between 3a and 3b is thicker and overlies trigonoid 1, suggesting an

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New Additions of Luminous Bobtail Squids to the Japanese Cephalopod Fauna (Sepiolida: Sepiolidae: Heteroteuthinae)

Tsunemi Kubodera[1]* and Takashi Okutani[2]

[1]*Department of Zoology, National Museum of Nature and Science, 3-23-1 Hyakunin-cho, Shinjuku-ku, Tokyo 169-0073, Japan*
[2]*Japan Agency for Marine-Earth Science & Technology, 2-15 Natsushima-cho, Yokosuka 237-006, Japan*

Abstract: Three lots of Pacific sepiolid specimens in the collection of National Museum of Nature and Science, Tokyo, were found to contain three new species: *Heteroteuthis nordopacifica* n. sp., *Sepiolina petasus* n. sp. and *Stoloteuthis japonica* n. sp. The second-mentioned taxon is the second species in the genus *Sepiolina*, and the third species represents the first occurrence of the genus *Stoloteuthis* in the Pacific Ocean. The descriptions of each species and comparison with related species are given herewith.

Keywords: Sepiolidae, taxonomy, new species, Japanese fauna

Introduction

The bobtail squids of the family Sepiolidae are quite diverse in the subtidal to slope and bathyal zones in temperate and tropical seas. Among the cephalopod fauna in Japanese waters, twelve species in six genera of this family have hitherto been recorded (Sasaki, 1929; Kubodera, 2000; Kubodera & Okutani, 2002; Okutani, 2005; Kubodera *et al.*, 2009).

Within this family, the subfamily Heteroteuthinae contains bioluminescent species. The single luminescent organ in the mantle cavity is oval in shape. In Japanese waters, *Sepiolina nipponensis* (Berry, 1911), the sole species of the genus, commonly occurs in catches from deep tows of both biological nets and commercial trawls. Since Kubodera (1996) first reported *Heteroteuthis* sp. from off Sanriku (Pacific coast of northeastern Honshu), *H. hawaiiensis* (Berry, 1909) was discovered by submersible at a depth of 920 m near the Ogasawara Islands (Okutani & Tsuchida, 2005). *Heteroteuthis ryukyuensis* Kubodera, Okutani & Kosuge, 2009 has recently been described from fish stomachs in Okinawan waters.

The extensive cephalopod collection in the National Museum of Nature and Science, Tokyo (= formerly the National Science Museum, Tokyo) contained three lots of unidentified sepioloids, including the *Heteroteuthis* sp. depicted by Kubodera (1996, 2000). In this paper we resolve and fully describe these specimens that are certainly new additions to the Japanese cephalopod fauna.

Materials and Methods

The specimens treated here are all in the collection of the National Museum of Nature and Science. The registration number, name of ship, station number of the cruises, date, position, depth, gear, and other relevant data are all given under "*Material examined*" for each specimen. Collection localities of the specimens are shown in Fig. 1.

* Corresponding author: kubodera@kahaku.go.jp

immature stage in cardinal development.

Such slight disparities between similarly sized shells may suggest the existence of two phenotypes at geographically distant localities. Another explanation may be that there are separate populations with different growth patterns, with the population at the Off Hatsushima Island seep site reaching maturity at a smaller size than that at the Off Sanriku coast site, probably because of oceanographic and geochemical conditions.

Similar phenotypic differences have been observed among *Calyptogena nautilei* populations from the Nankai Trough and Mariana Trench (Okutani *et al.*, unpublished), and *C. laubieri* from the Nankai Trough and the Kurile Trench (Okutani *et al.*, 2009). Krylova & Sahling (2006) demonstrated pronounced infraspecific variation within various *Calyptogena* species.

Comparison: A congeneric species with a superficial resemblance to *C. fortunata* is *Calyptogena nanshaensis* Xu & Shen, 1991 from the Nansha Islands (off the northwest coast of Borneo) at 2626 m depth (Xu & Shen, 1991). However, *C. nanshaensis* is separable from the present species in having a thin, lamellated 4b, and distinct subumbonal pit. To clarify the relationship between two species, nucleoid sequence data for *C. nanshaensis* are badly needed.

The new species resembles the young stage of *C. soyoae*, but can be readily distinguished by the different configuration of the cardinal teeth and its feeble postero-dorsal angulation. The ANCOVA results of shell length-width regression equations between *C. fortunata* n. sp. vs. *C. okutanii*, and *C. fortunata* n. sp. vs. *C. soyoae* are both < 0.001, significantly low. However, that of *C. okutanii* and *C. soyoae* is > 0.001. This means shell length-width proportions of *C. fortunata* n. sp. are significantly different in comparison with the other two sympatric species (Fig. 4).

The dental structures of the Vesicomyidae have been the subject of recent detailed studies including those by Cosel & Salas (2001), Krylova & Sahling (2006, 2010), Amano & Kiel (2007) and Cosel & Olu (2008) among others. There are some discrepancies among researchers in the generic definition, which is mostly based on dental configuration, but the common concept leads us to place the present new species in the genus *Calyptogena* Dall, 1891, and the subgenus *Archivesica* Dall, 1908 in the sense of Okutani *et al.* (2000), particularly considering the

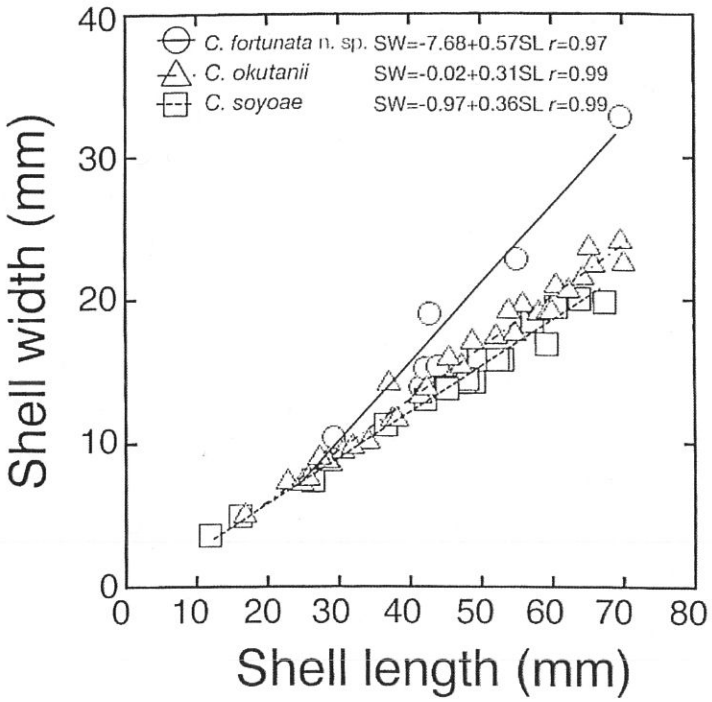

Fig. 4. Shell length-width relationship of *Calyptogena fortunata* n. sp., *C. okutanii* and *C. soyoae*.

elongated outline, more or less radially disposed cardinal teeth, lack of lunule, trace of subumbonal pit, shallow pallial sinus, and absence of secondary pallial scar.

Among the vesicomyid genera, *Vesicomya*, *Waisiuconcha* and *Pliocardia* usually have a tumid shell with markedly incised lunule. The genera *Callogonia* and *Isorropodon* include species with a somewhat elongated shell with an indistinct or absent lunule, but the members of these two genera have a rather weak hinge plate that carries the teeth more or less in a line parallel to the dorsal margin (Cosel & Salas, 2001). The shell profile of the present new species is similar to the genus *Laubiericoncha* recently established by Cosel & Olu (2008), but is separable in having a thick and almost posteriorly directed 3b, and a shallower pallial sinus. Krylova & Janssen (2006) and Krylova & Sahling (2006) maintained that *Archvesica*, *Akebiconcha* and *Phreagena* are all independent genera separable from *Calyptogena* (s.s.), but we still prefer to follow our view (Okutani *et al.*, 2000) until the subgeneric or generic systematics of the family Vesicomyidae become reasonably stable in future.

Phylogeny: Similarity analysis showed that the partial sequence of the mtCOI gene (513 bp) of *C. fortunata* n. sp from Sagami Bay was identical to that of an unidentified *Calyptogena* clam collected the Sanriku coast site (Kojima *et al.*, 2004) and different from the mtCOI gene sequences of other vesicomyid clams reported so far (Fig. 5). Molecular phylogenetic analysis (NJ) demonstrated the monophyly of *C. fortunata* n. sp and *Calyptogena laubieri*, supported by a bootstrap value of 99%. *C. laubieri* has occurred from methane seep sites from 3761 to 3835 m

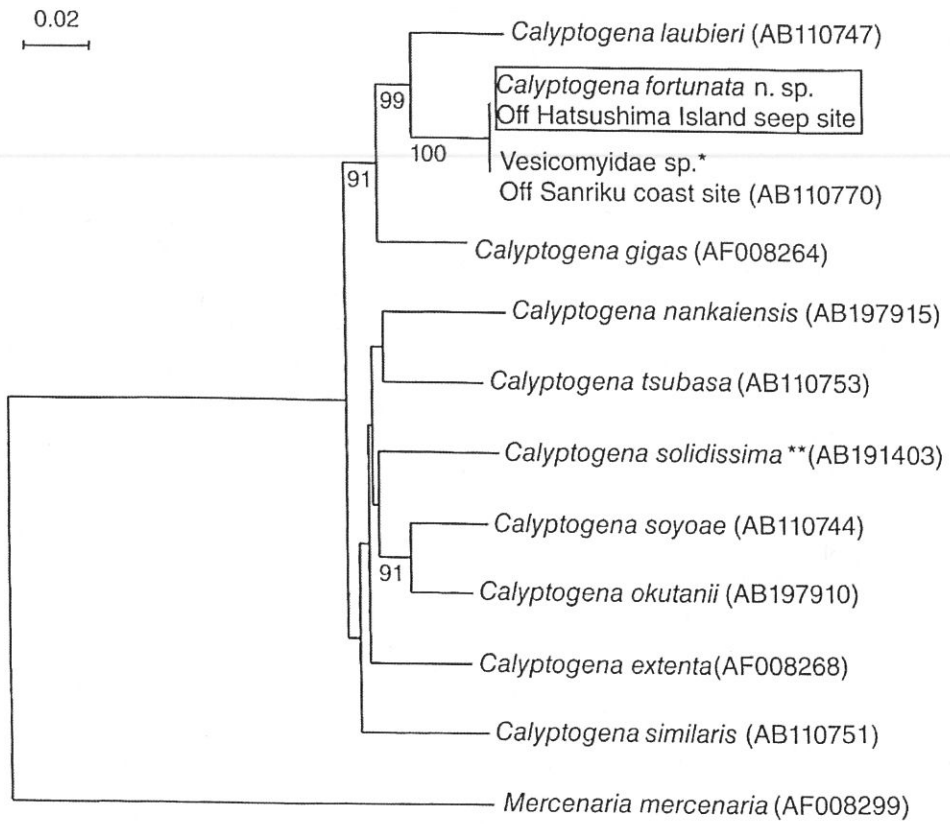

Fig. 5. Phylogenetic placement of *C. fortunata* n. sp. based on the mitochondrial cytochrome c oxidase subunit I (mtCOI) gene sequences. Neighbor-joining (NJ) tree are shown. Scale bar represents 0.02 nucleotide substitution per sequence position. Bootstrap values greater than 60% are shown for each branch. The *C. fortunata* n. sp. examined in this study is highlighted. The accession numbers used for this study are shown in parentheses following the operational taxonomic unit names. (* Vesicomyidae sp. is now the present new species described herein; ** *Calyptogena solidissima* was proved to be conspecific with *C. kawamurai* (Kojima *et al.*, 2006).

depth in the Nankai Trough (Okutani & Métivier 1986, Fujikura *et al.*, 2000).

This fact may suggest the presence of lineage and differentiation of the *C. laubieri*-complex along the Pacific coast of Japan, namely, *C. lauberi laubieri* in the Nankai Trough – *C. fortunata* in Sagami Bay to Sanriku coast – *C. laubieri kurilensis* in the Kurile Trench.

Acknowledgments

We acknowledge the operation teams of the ROVs *Hyper-Dolphin*, *Dolphin-3K* and *Kaiko*, and the crews of the R/Vs *Natsushima* and *Tansei-maru* for their support in collecting the specimens under study. We also thank Drs. T. Maruyama and H. Watanabe (JAMSTEC) for their support for advancing this study, and Drs. H. Saito and K. Hasegawa (NSMT), for taking photographs of specimens and arranged the color plates, respectively. Prof. K. Amano, Joetsu University of Education, and an anonymous reviewer gave useful advice that contributed greatly to the improvement of the manuscript.

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三陸沖と相模湾の深海底湧水域からさらなるシロウリガイ属の新種

奥谷喬司・小島茂明・河戸　勝・瀬尾絵理子・藤倉克則

要　約

相模湾の初島沖の水深 1100 m 付近におけるシロウリガイ *Calyptogena soyoae* とシマイシロウリガイ *C. okutaniit* が混棲する湧水生物群集の中から，形態およびミトコンドリア DNA の塩基配列で識別される種が見つかった。

Calyptogena (*Archivesica*) *fortunata* n. sp. サイワイシロウリガイ（新種・新称）

殻長 43.8 mm 殻高 26.5 mm 殻幅 15.4 mm（ホロタイプ）。

殻は長卵型で同種は形態的にはシロウリガイの若齢個体と紛らわしい。右殻の中央主歯は太い三角錐状で殻頂下前主歯は鹿状で，後主歯と約 120° をなす。左殻の前後殻頂下主歯は逆 V 字型で後背主歯は斜位でやや半月型。側歯はなく，殻頂下洞は痕跡的で小月面もない。外套湾入は殆ど認められない。

初島の標本はかって 1999 年淡青丸が三陸沖の水深 1100～1700 m 付近で採集していた未記載の種とミトコンドリア COI 領域の塩基配列が一致した。相模湾の個体は三陸のものに比し，同サイズでも殻が厚く鉸歯も成長段階が進んでいるが如くで，やや異なった表現型を示す。塩基配列（COI）から見ると，本種はテンリュウシロウリガイ *C. laubieri* と単系統をなすことが判った。