The Zoological Society of Japan

ZOOLOGICAL SCIENCE **23**: 835–842 (2006)

© 2006 Zoological Society of Japan

# Synonymy of *Calyptogena solidissima* with *Calyptogena kawamurai* (Bivalvia: Vesicomyidae) and Its Population Structure Revealed by Mitochondrial DNA Sequences

**Shigeaki Kojima[1]*, Eiji Tsuchida[1], Hideki Numanami[2], Katsunori Fujikura[3] and Takashi Okutani[3]**

[1]*Ocean Research Institute, University of Tokyo, Tokyo 164-8639, Japan*
[2]*Tokyo Kasei Gakuin University, Tokyo 194-0292, Japan*
[3]*Japan Agency for Marine-Earth Science and Technology, Kanagawa 237-0061, Japan*

**Nucleotide sequences of part (1,101 bp) of the mitochondrial cytochrome oxidase *c* subunit I (COI) gene were determined for two specimens of *Calyptogena kawamurai* collected in Kashima Nada and Suruga Bay, respectively. These sequences were identical to each other and to those from many individuals of *Calyptogena solidissima*, i.e., 11 of 12 specimens from a seep area in Nankai Trough, two of 20 from hydrothermal-vent fields in Okinawa Trough, and one of 14 from a seep area on Kuroshima Knoll. The nucleotide sequences of the 5' part (about 700 bp) of the first internal transcribed spacer (ITS-1) also showed a close relationship between *C. kawamurai* and *C. solidissima*. The radiating threads on the shell surface that were emphasized in describing *C. solidissima* are not consistent throughout these local populations. Variation in cardinal dentition was confirmed to be intraspecific by observations of a series of specimens. The shell length-height and shell length-width relationships of both species all fit a single regression line. These results suggest that *C. solidissima* is a junior synonym of *C. kawamurai*. The populations of Nankai Trough, Okinawa Trough, and Kuroshima Knoll were shown to be diverging genetically from each other. Populations of Okinawa Trough and Kuroshima Knoll are suggested to have derived independently from the most common haplotype of Nankai Trough.**

**Key words:** *Calyptogena*, synonymy, mitochondrial DNA, ITS-1, population structure

## INTRODUCTION

Bivalves in the family Vesicomyidae are one of the primary members of deep-sea chemosynthesis-based communities in the northwestern Pacific as well as the eastern Pacific (Kojima, 2002). To date, more than 50 species have been described worldwide. Most species were described on the basis of specimens collected by submersibles or remotely operated vessels (ROVs), while some were based on those collected by trawls or dredges from research vessels or fishing boats. For example, Okutani (1957) described *Calyptogena soyoae* from two dead shells collected by a deep-sea trawl during a cruise of the research vessel *Soyo-Maru* of the Tokai Regional Fisheries Research Laboratory in 1955. About 30 years later, Okutani and Egawa (1985) discovered a colony of this species during a dive of the submersible *Shinkai 2000* of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC).

Kuroda (1943) described *Akebiconcha kawamurai* as the type species of the genus *Akebiconcha* on the basis of a specimen collected at a depth of about 180 m off Odawara, Sagami Bay, Central Honshu, the Japanese mainland. Kuroda (1943) stated that it appeared to belong to the family Arcticidae (= Cyprinidae). This species has also been collected from depths of 250–420 m off Choshi, Kashima-Nada (Ozaki, 1958; Shikama, 1961; Horikoshi, 1987). In addition, dead shells of this species were collected in trawls from depths of 196–413 m off the Kii Channel (Tsuchida, 1986). A subsequent examination of specimens reported by Okutani (1962) from 700–850 m off Jogashima Islet, Sagami Bay, revealed them to be not *A. kawamurai*, but young *C. soyoae*.

In the original description of *C. soyoae*, Okutani (1957) pointed out an alliance with *A. kawamurai*. He also compared these two species with two vesicomyid species from the northeastern Pacific and transferred *C. soyoae* to the genus *Akebiconcha* (Okutani, 1966). Bernard (1974) transferred *A. kawamurai* and *A. soyoae* to the genus *Calyptogena*. Although Boss and Turner (1980) questioned the distinction between these two species, Horikoshi (1987) showed clear characters in hinge structure that separate them. Recently, more than a dozen *Calyptogena* species have been described from Japanese waters, and at present they are classified into three subgenera (Okutani *et al.*, 2000, 2002).

* Corresponding author. Phone: +81-3-5351-6469;
Fax : +81-3-5351-6470;
E-mail: kojima@ori.u-tokyo.ac.jp
doi:10.2108/zsj.23.835

NII-Electronic Library Service

The Zoological Society of Japan



Though *C. kawamurai* had been thought to be a shelf species and not to occur in bathyal depths, the collection depths reported by fishermen are often approximate or inaccurate. Field observation using submersibles has not yet been successful. Because it is very difficult to collect living specimens of *C. kawamurai*, its phylogenetic position has not been determined using molecular data. Recently, single specimens of *C. kawamurai* were obtained from off Choshi, Kashima-Nada, and Suruga Bay. Our preliminary analysis using molecular phylogenetic methodology has shown a close relationship between *Calyptogena kawamurai* and *C. solidissima*, as reported here.

*Calyptogena solidissima* was described on the basis of specimens collected in hydrothermal-vent fields at depths of 670–710 m on Minami-Ensei Knoll in the Mid-Okinawa Trough (Okutani *et al.*, 1992). Okutani *et al.* (1992) stated that *C. solidissima* is separated from *C. kawamurai* by having very fine radial threads on the shell surface and a less oblique arrangement of cardinal teeth compared to *C. kawamurai* specimens of the same size. In Okutani *et al.* (1992), however, the number of specimens available was insufficient to determine intraspecific variation, and subsequent examination of additional specimens found individuals without radial threads, as well as large variation in the arrangement of cardinal teeth, even within the Minami-Ensei Knoll population (Okutani, unpublished data). *Calyptogena solidissima* has also been reported from three seep areas at depths of 594–650 m in Nankai Trough, Daini (second) Tenryu Knoll, Muroto Knoll, and Ashizuri Knoll, as well as from a seep area at a depth of 682 m on Kuroshima Knoll in the southwestern part of the Ryukyu Arc (Fujikura *et al.*, 2000). Living specimens from seep areas have been collected only on Daini Tenryu and Kuroshima Knolls.

To date, three species of the genus *Calyptogena*, *C. solidissima*, *Calyptogena okutanii*, and *Calyptogena nankaiensis*, have been reported from both seep areas and hydrothermal-vent fields around Japan (Fujikura *et al.*, 2000). Significant genetic divergence between populations in hydrothermal-vent fields in Okinawa Trough and those in seep areas off the Pacific coast of central Japan was shown for both *C. okutanii* and *C. nankaiensis* (Kojima *et al.*, 2005). Our previous study of *C. solidissima* (Kojima *et al.*, 2004) showed that no haplotype was shared between populations in seep areas off Honshu (the Japanese mainland) and vent fields in Okinawa Trough, although the analysis was carried out using only three specimens for each local population and relatively short nucleotide sequences (501 bp). Among these three species, only *C. solidissima* inhabits a cold seep site around the Ryukyu Islands, Kuroshima Knoll, which is about 600 km from the only habitat of this species among the vent fields in Okinawa Trough (Minami-Ensei Knoll). Thus, whether individuals inhabiting the seep area on Kuroshima Knoll are genetically related to those from the geographically close vent fields in Okinawa Trough or to those from the distant seep area in Nankai Trough is an interesting issue for understanding the processes of dispersal and evolution in vesicomyid bivalves. Unfortunately, no molecular data have been available for specimens from Kuroshima Knoll.

In the present study, we investigated the phylogenetic relationship between *C. kawamurai* and *C. solidissima* using mitochondrial DNA sequences and also compared shell morphology, and concluded that *C. solidissima* is a junior synonym for *C. kawamurai*. In addition, we analyzed the genetic population structure of this species using longer mitochondrial DNA sequences and as many specimens as possible.

## MATERIALS AND METHODS

A specimen of *C. kawamurai* was incidentally collected at a depth of about 100 m off Choshi, Kashima-Nada, in a commercial trawl by a local fisherman (Fig. 1). Its shell was reportedly sold to a shell collector and only soft tissues were available, which were stored in a freezer (–80°C). Unfortunately, therefore, the shell morphology of this specimen could not be examined. An additional specimen was collected at a depth of about 300 m on Seno-umi Bank, Suruga Bay, also in a commercial trawl for the giant spider crab (Figs. 1 and 2), and fixed and preserved in 100% ethyl alcohol by one of the present authors (H.N.).

During dives of the submersible *Shinkai 2000*, the ROV *Dolphin 3K*, and the ROV *Hyper-Dolphin* of JAMSTEC, 20, 12, and 14 live specimens of *C. solidissima* were collected at depths of 640–710 m in the hydrothermal-vent field on Minami-Ensei Knoll, Central Okinawa Trough; a seep area at depths of 590–610 m on Daini Tenryu Knoll, Nankai Trough; and the seep area at depths of 640–670 m on Kuroshima Knoll, respectively. The soft tissues of each specimen were stored in a freezer (–80°C) or in liquid nitrogen, with the exception of the five specimens from Kuroshima Knoll, which were fixed and preserved in 100% ethyl alcohol.

Mitochondrial DNA samples of all specimens from Minami-Ensei Knoll were obtained in a previous study (Kojima *et al.*, 1995), and total DNA samples of three specimens from Daini Tenryu Knoll were obtained from Kojima *et al.* (2004). Total DNA was obtained from the foot or adductor muscles of other specimens by grinding, digestion with sodium dodecyl sulfate, and extraction with phenol and chloroform, with the exception of a single specimen collected during dive no.1351 of the *Shinkai 2000*, from which total DNA was extracted using a DNeasy Tissue Kit (QIAGEN Inc., Valencia, CA, USA), as this specimen had been badly damaged.

Part (approximately 1,300 bp) of the mitochondrial gene for cytochrome oxidase *c* subunit I (COI) was amplified by polymerase chain reaction (PCR) with total DNA as a template and primers LCO1490 (Folmer *et al.*, 1994) and COI-6 (Shimayama *et al.*, 1990). The conditions for PCR were: incubation at 94°C for 60 s, followed by 30 to 40 cycles of 92°C for 40 s, 50°C for 60 s, and 72°C for 90 s. Genereleaser (BioVenture Inc., Murfreesboro, TN, USA) was used to sequester products of cell lysis that might have inhibited the polymerase.

Nucleotide sequences of 1,101 bp of the amplified fragment, which correspond to amino acid residues 41–407 of COI of *Katharina tunicata* (Boore and Brown, 1994), were determined with an automated sequencer (ABI3100; Applied Biosystems Inc., Foster City, CA, USA) using primers CA-12 and CA-17 (Kojima *et al.*, 2005). When appropriate, nucleotide sequences were confirmed using primers CA-14 (Kojima *et al.*, 2005) and/or COI-3 (Shimayama *et al.*, 1990). Nucleotide sequences of the upper part (513 bp) of the fragments were reported in our previous study (Kojima *et al.*, 2004) for three individuals of *C. solidissima* from Minami-Ensei Knoll and three individuals from Daini Tenryu Knoll.

The first internal transcribed spacer (ITS-1; approximately 880 bp) was amplified using PCR with total DNA as a template and primers 5.8SR and either 18S-5 or 18S-9, which were designed on the basis of nucleotide sequences of various organisms (Kodama *et al.*, unpublished data). Samples of total DNA from two closely related species, *C. okutanii* and *C. nankaiensis* (Kojima *et al.*, 2004), were obtained from Kojima *et al.* (2005). PCR conditions were the same as for COI. Nucleotide sequences of the 5' part of

NII-Electronic Library Service

The Zoological Society of Japan



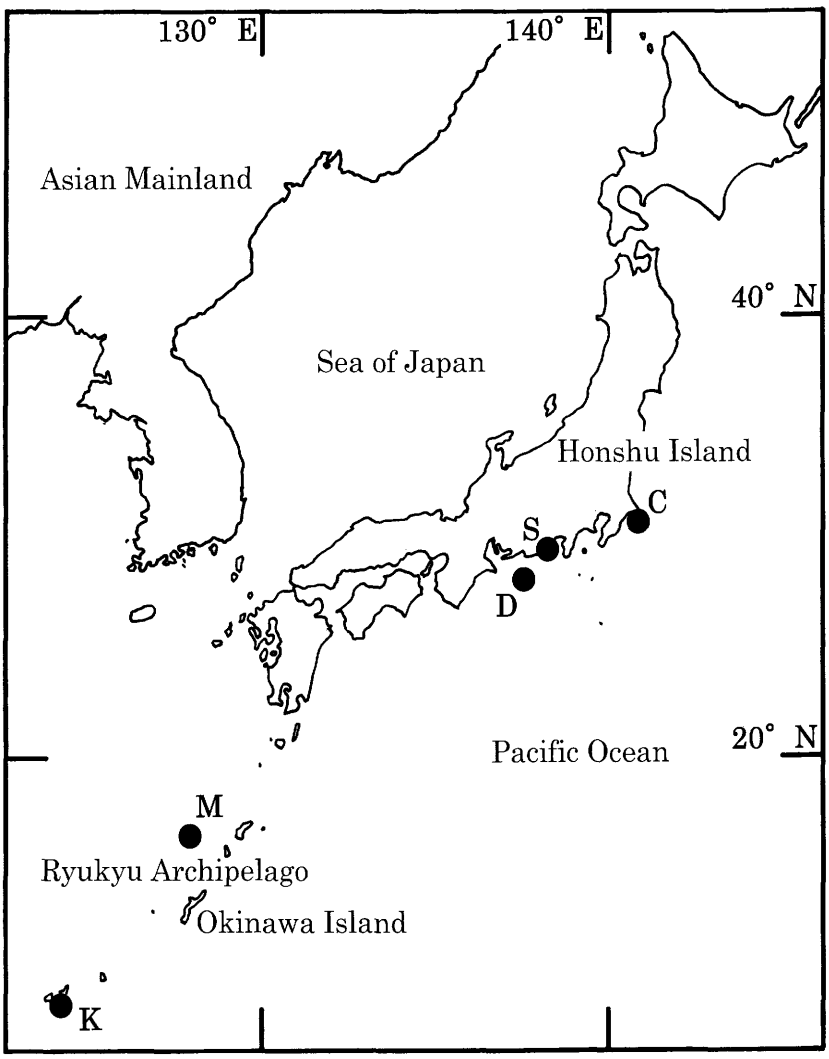


**Fig. 1.** Sampling sites for *Calyptogena kawamurai* and *Calyptogena solidissima*: **C**, off Choshi; **S**, Seno-umi Bank; **D**, Daini Tenryu Knoll; **M**, Minami-Ensei Knoll; **K**, Kuroshima Knoll.

ITS-1 (about 700 bp) were determined with an automated sequencer (ABI3130; Applied Biosystems Inc.) using primers 18S-9, CalTS1-1, CalTS1-2, and 5.8SR. Primers CalTS1-1 and CalTS1-2 were designed from nucleotide sequences determined using primers 18S-9 and 5.8SR.

The nucleotide sequences of all the primers used in the present study are shown in Table 1. The nucleotide sequences reported in the present study will appear in the GSDB, DDBJ, EMBL, and NCBI databases under accession numbers AB191397–191403 (COI) and AB208745–208750 and AB252629–252632 (ITS-1).

A phylogenetic tree was constructed by the maximum-parsimony (MP) method, using the multiple equally parsimonious exhaustive search option with tree bisection-reconnection and 1,000 random addition sequence replicates, implemented in PAUP*, Version 4.0b10 (Swofford, 2002). Alignment gaps were treated as a fifth character state.

Differences in haplotype frequencies between populations were examined by the exact test of population differentiation (Raymond and Rousset, 1995) using Arlequin software (Schneider *et al.*, 2000). An unbiased fixation index, $F_{ST}$ (Weir and Cockerham, 1984), was estimated and the significance of the indices was tested using a nonparametric permutation approach with Arlequin.

For morphological assessment, we reinvestigated the type specimens of *A. kawamurai* (NSMT-Mo 60915) and *C. solidissima* (NSMT-Mo 69675 and 69676) in the National Science Museum, Tokyo; specimens deposited in JAMSTEC, including 21 specimens of *C. solidissima* collected live on Minami-Ensei, Kuroshima, and Daini Tenryu Knolls by either the *Shinkai 2000* (dive nos. 357, 428, 1059, 1355, 1357, 1360, 1364, and 1377) or the ROV *Hyper-Dolphin* (dive no. 300); and 19 dead shells of *C. kawamurai* taken incidentally in a catch of giant spider crabs in Suruga Bay. We placed special emphasis on variability in hinge structure and external shell sculpture, among other morphological characters. Shell length, height, and width were measured in specimens preserved in good condition to obtain allometric data.

## RESULTS

For two individuals of *C. kawamurai*, nucleotide sequences of part (501 bp) of the COI gene, which had been reported for 26 described and 15 undescribed vesicomyid species (Peek *et al.*, 1997, 1998, 2000; Goffredi *et al.*, 2003; Van Dover *et al.*, 2003; Kojima *et al.*, 2004), were determined. The sequences obtained were identical to each other and to those from three of six individuals of *C. solidissima* reported in Kojima *et al.* (2004).

Next, longer sequences of the COI gene (1,101 bp) were determined and compared among the two individuals of *C. kawamurai* and a total of 46 individuals of *C. solidissima*. Seven haplotypes were obtained from the 48

NII-Electronic Library Service

The Zoological Society of Japan



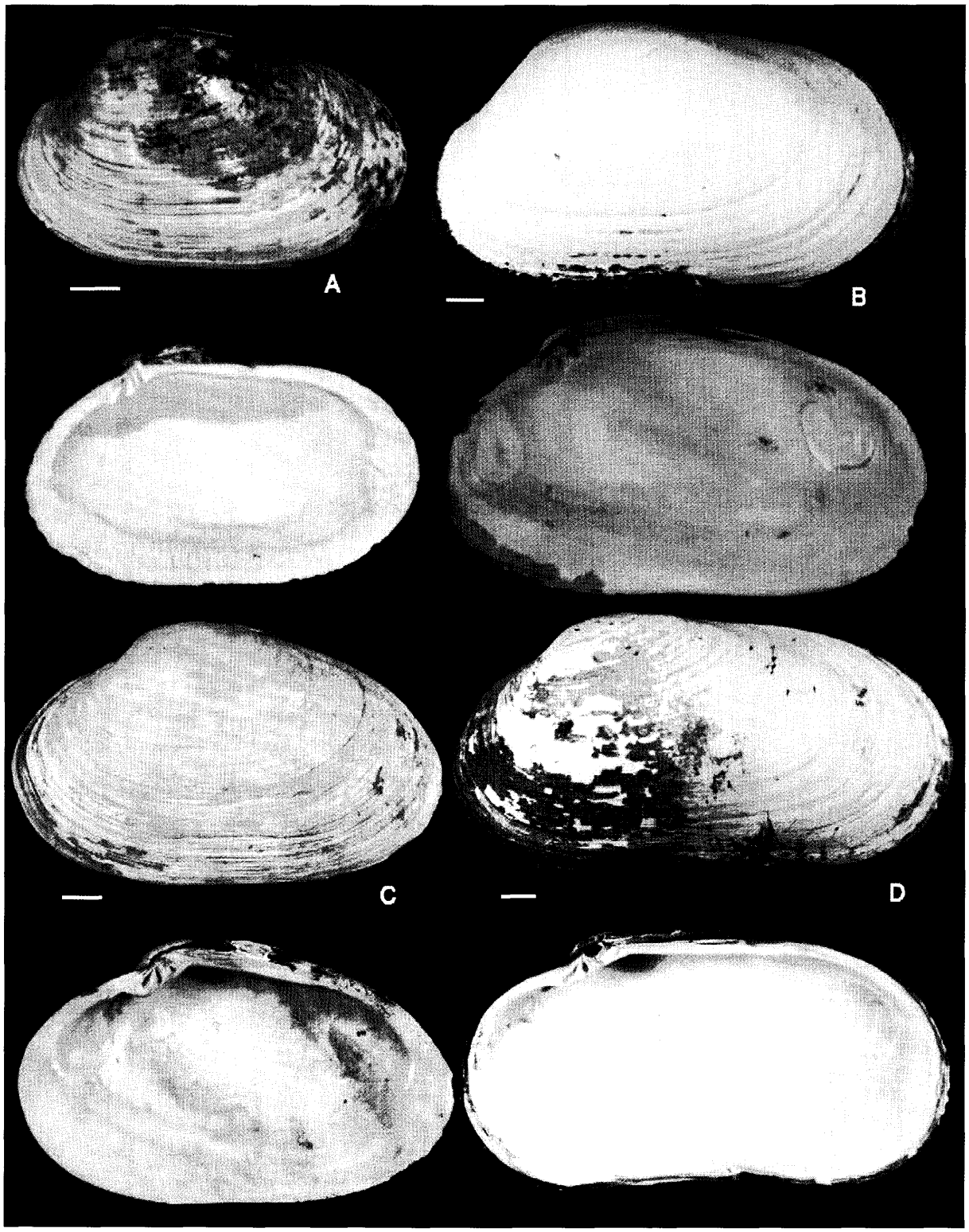


**Fig. 2.** *Calyptogena kawamurai* and *Calyptogena solidissima*. **A)** Holotype of *Akebiconcha kawamurai* (National Science Museum, Tokyo, NSMT-Mo 60915); shell length=76.4 mm. **B)** Holotype of *C. solidissima* from Minami-Ensei Knoll (National Science Museum, Tokyo, NSMT-Mo 69675); shell length=1287.5 mm. **C)** Specimen from Seno-umi Bank, Suruga Bay, depth 300 m; shell length=103.9 mm. **D)** Specimen from Kuroshima Knoll, depth 642 m (JAMSTEC: HDP Dive 300); shell length=103.9 mm. Scale bar=10 mm.

individuals, as shown in Table 2. An identical sequence was obtained from both individuals of *C. kawamurai,* and this was also identical to the most common haplotype from *C. solidissima* from a seep area on Daini Tenryu Knoll (haplotype 1 in Table 2), obtained from 11 of 12 individuals from this site. In addition, the identical sequence was obtained from two of 20 individuals from hydrothermal-vent fields on Minami-Ensei Knoll and one of 14 individuals from a seep area on Kuroshima Knoll (Table 2). Each of the other haplotypes differed from this haplotype by one or two nucleotide substitutions, of which only one (haplotype 5 in Table 2) was nonsynonymous. Phylogenetic relationships among

NII-Electronic Library Service

The Zoological Society of Japan



**Table 1.** Nucleotide sequences of primers used in the present study. Y, R, W and N denote T or C; A or G; A or T; and G, A, T or C, respectively. Positions refer to the corresponding amino acid residues encoded by the gene for mitochondrial cytochrome *c* oxidase subunit I from *Katharina tunicata* (Boore and Brown, 1994), for 18S and 5.8S ribosomal DNAs of *Xenopus laevis* (GenBank, Accession No. X02995), or ITS-1 of *Calyptogena solidissima* from the Daini Tenryu Knoll.

| Name | Sequence | Position | Direction |
|---|---|---|---|
| LCO1490 | 5'-GGTCAACAAATCATAAAGATATTGG-3' | 5–13 | Forward |
| CA-14 | 5'-ACAAARTCYACAGARGGRCCRGC-3' | 137–144 | Reverse |
| CA-12 | 5'-GCTATACCNGTYTTRGCNGG-3' | 196–202 | Forward |
| COI-3 | 5'-GTNTGRGCNCAYCAYATRTTYACNGT-3' | 284–292 | Forward |
| CA-17 | 5'-ACCGTAAATATGTGGTGCCCYCARAC-3' | 284–292 | Reverse |
| COI-6 | 5'-GGRTARTCNSWRTANCGNCGNGGYAT-3' | 433–441 | Reverse |
| 18S-5 | 5'-GCGAAAGCATTTGCCAA-3' | 1983–1999 | Forward |
| 18S-9 | 5'-GTACACACCGCCCGTC-3' | 2679–2694 | Forward |
| CalTS1-1 | 5'-CGGTGCGCTGTAGGG-3' | 383–397 | Forward |
| CalTS1-2 | 5'-CCGGAATCGCCGACACC-3' | 505–521 | Reverse |
| 5.8SR | 5'-CGAAGTGTCGATGATCAATGTG-3' | 3495–3516 | Reverse |

**Table 2.** Haplotype compositions of *Calyptogena kawamurai* collected off Choshi (C) and at the Seno-umi Bank (S), and of *Calyptogena solidissima* from the Daini Tenryu Knoll (D), Minami-Ensei Knoll (M) and Kuroshima Knoll (K).

| | Sampling site | | | | |
|---|---|---|---|---|---|
| Haplotype | C | S | D | M | K |
| 1 | 1 | 1 | 11 | 2 | 1 |
| 2 | | | | | 13 |
| 3 | | | | 13 | |
| 4 | | | | 3 | |
| 5 | | | 1 | | |
| 6 | | | | 1 | |
| 7 | | | | 1 | |

haplotypes are shown as a network in Fig. 3.

The genetic divergences among these seven haplotypes calculated using Kimura's two-parameter model (Kimura, 1980) were 0.001–0.004, much less than the gen-

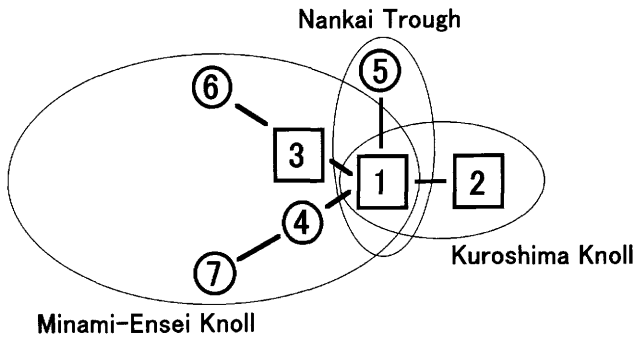


**Fig. 3.** Network of mitochondrial haplotypes of *Calyptogena solidissima*. Numbers are the same as in Table 2. Squares and circles denote common and rare haplotypes, respectively. Haplotypes obtained from each sampling site are enclosed by an ellipse. Lines connecting haplotypes denote single nucleotide substitutions between them.

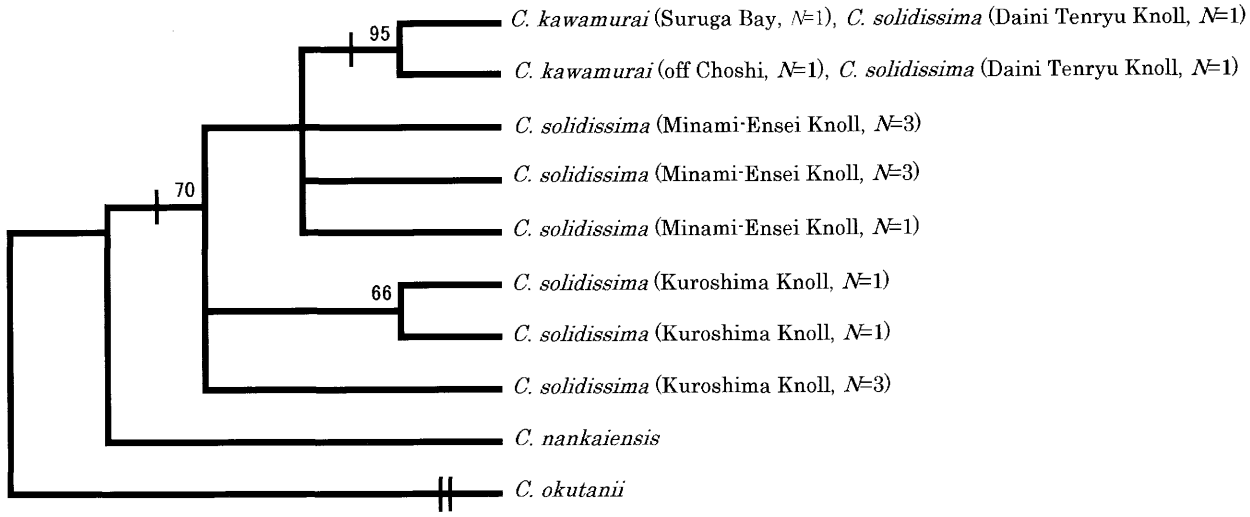


**Fig. 4.** The most parsimonious tree based on nucleotide sequences of the 5' part of the first internal transcribed spacer for *Calyptogena kawamurai* and *Calyptogena solidissima* (37 steps; CI=0.7895, excluding non-informative characters; RI=0.7500). *Calyptogena okutanii* and *Calyptogena nankaiensis* were included as outgroup taxa. Bootstrap values greater than 50% are shown above nodes. Each bar on a branch denotes an insertion of a repeat unit of CA or CG.

NII-Electronic Library Service

The Zoological Society of Japan



eral uppervalue of 0.018 for intraspecific divergences in the COI gene in vesicomyids (Baco *et al.*, 1999).

Differences in frequencies of mitochondrial haplotypes among the three populations of *C. solidissima* were examined on the basis of pairwise $F_{ST}$ values and the exact test of population differentiation. The populations of Daini Tenryu, Minami-Ensei, and Kuroshima Knolls were shown to differ significantly from one another ($p<0.001$).

For the phylogenetic analysis based on ITS-1, only specimens were used for which single nucleotide sequences could be determined by direct sequencing (two individuals of *C. kawamurai*, and two, seven and five individuals of *C. solidissima* from Daini Tenryu, Minami-Ensei, and Kuroshima Knolls, respectively). Nucleotide sequences of the 5' part (692–698 bp) of ITS-1 were determined for these specimens and for one individual each of *C. okutanii* and *C. nankaiensis*. The nucleotide sequence from each individual of *C. kawamurai* was identical to one of two sequences obtained from *C. solidissima* from Daini Tenryu Knoll. These four individuals shared a unique additional unit of a CA repeat (Fig. 4). Phylogenetic analyses using the MP method showed that *C. kawamurai* forms a monophyletic group with *C. solidissima* from Daini Tenryu Knoll, with high bootstrap support (95%).

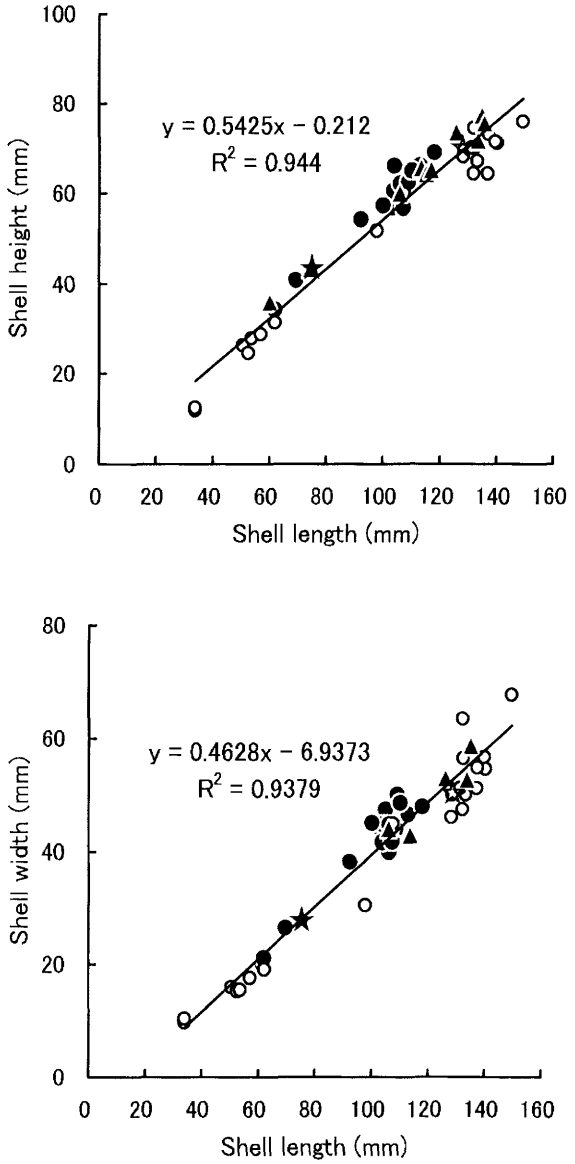


**Fig. 5.** Relationships of shell height and shell width to shell length for *Calyptogena kawamurai* and *Calyptogena solidissima*. Measurements were made on the type specimens of *C. kawamurai* (solid stars) and *C. solidissima* (open stars); 21 specimens of *C. solidissima* collected alive on Minami-Ensei, Kuroshima, and Daini Tenryu Knolls (open circles); and 19 shells of dead *C. kawamurai* taken incidental to a catch of giant spider crabs in Suruga Bay (solid circles). Reliable measurement data (solid triangles) from reports in the literature by Ozaki (1958), Shikama (1961), Tsuchida (1986), and Horikoshi (1987) were also included.

Our examination revealed that the specimens collected from Daini Tenryu and Kuroshima Knolls lack the radial threads that were reported in specimens from Minami-Ensei Knoll and thought to be the basis for separating *C. solidissima* from *A. kawamurai* (Okutani *et al.*, 1992). The minor differences in dental configuration appear to be intraspecific and attributable to the growth stage or individual, as confirmed in our recent investigations of a large series of specimens of *Calyptogena* species. The allometric measurements also confirmed the conspecificity of *C. kawamurai* and *C. solidissima*, as both the shell length-height and shell length-width relationships fit the regression line well (Fig. 5).

## DISCUSSION

Out results strongly support the view that *C. solidissima* is a junior synonym of *C. kawamurai*. In describing *C. solidissima* from bathyal depths (670–710 m) on Minami-Ensei Knoll, Okutani *et al.* (1992) mentioned the similarity of the dental arrangement to that of *A. kawamurai*. However, the holotype specimen of *C. solidissima* was ornamented by fine but apparent radial threads all over the shell. This shell sculpturing had not been observed in any other member of the genus *Calyptogena*, and thus it was emphasized as the basis for separation of the two species. However, further examination using additional specimens found individuals without radial threads, even among those from Minami-Ensei Knoll (Okutani, unpublished data). No specimens subsequently collected from Nankai Trough and Kuroshima Knoll exhibited this external structure, and thus the radial threads may be a characteristic restricted to some individuals of the Minami-Ensei Knoll population.

*Calyptogena kawamurai* has been considered to be a rare species, and its habitats were unknown for a long time. To date, *C. kawamurai* has been collected at depths of 180–420 m off the Pacific coast of central Honshu, while all known localities for *C. solidissima* are reducing environments at depths of 590–710 m. It is probable that some seep areas remain undiscovered off Choshi, in Sagami Bay, in Suruga Bay, and off the Kii Channel. According to information from fishermen (T. Watanabe, personal communication, 21 January 2003), *C. kawamurai* appears to inhabit depths of 400–600 m in Katagai Submarine Canyon (fishing grounds for *Beryx splendens*), where bubbling from the sea bottom has been observed. This hearsay evidence suggests the existence of a gas seep, but no confirmatory investigation has yet been carried out.

Populations of *C. kawamurai* of Daini Tenryu, Minami-Ensei, and Kuroshima Knolls were shown to differ significantly from one another. In addition to this species, two vesi-

NII-Electronic Library Service

The Zoological Society of Japan



comyid species, *C. okutanii* and *C. nankaiensis*, have been reported from both seep and vent sites in the western Pacific (Fujikura *et al.*, 2000). Kojima *et al.* (2005) reported significant genetic differentiation in COI between populations of *C. okutanii* and *C. nankaiensis* in seep areas off the Pacific coast of central Japan and hydrothermal-vent fields in Okinawa Trough. For *C. okutanii*, for which sufficient specimens were available, the most common haplotype of the populations off central Japan was shown to occur at low frequency in two localities in Okinawa Trough, whereas the most common haplotype in Okinawa Trough was not detected in the sea area off central Honshu. This asymmetric distribution of haplotypes of *C. okutanii* corresponds to the present results for *C. solidissima*. Thus, genetic divergence between seep areas off Honshu and vent fields in Okinawa Trough appears to be a common phenomenon among Japanese vesicomyid clams.

Kuroshima Knoll offers an interesting opportunity for evolutionary research on deep-sea chemosynthesis-based communities, as it is the only known seep area around the Ryukyu Islands where numerous vent fields have been discovered (Kojima, 2002). The present analysis showed that the population of vesicomyid bivalves on Kuroshima Knoll has a haplotype composition different from those of both the Nankai Trough and Okinawa Trough populations. The most common haplotype of the population in Nankai Trough (haplotype 1 in Table 2) occurred in the other two localities at low frequency, while those in Okinawa Trough and on Kuroshima Knoll (haplotypes 3 and 2, respectively, in Table 2) were not detected in Nankai Trough. This suggests that haplotype 1 is older than haplotypes 2 and 3. If so, haplotypes endemic to Okinawa Trough and Kuroshima Knoll were independently derived from haplotype 1 (Fig. 3). Kojima *et al.* (1997, 2005) noted that *C. okutanii* probably migrated from seep areas off Honshu to vent fields in Okinawa Trough, as subduction of the Pacific plate off Honshu has continued for a much longer time than hydrothermal activities in Okinawa Trough. Although the detailed history of the Ryukyu Arc has not yet been elucidated, it is thought to be a relatively young system (Machiyama *et al.*, 2004). The present results may indicate a more recent origin of the seepage on Kuroshima Knoll than that in Nankai Trough.

## ACKNOWLEDGMENTS

We thank Drs. Suguru Ohta, University of Tokyo; Tomoyuki Miura, Miyazaki University; Shin-ichi Kuramoto, Japan Agency for Marine-Earth Science and Technology; and Kenji Okoshi, Ishinomaki Senshu University for providing specimens of *C. solidissima*. Drs. Chitoshi Mizota, Iwate University, and Kenji Okoshi, Ishinomaki Senshu University, kindly provided us with measurement data for specimens in their possession. This study was supported in part by a grant from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (No. 12NP0201).

## REFERENCES

Baco AR, Smith CR, Peek AR, Roderick GK, Vrijenhoek RC (1999) The phylogenetic relationships of whale-fall vesicomyid clams based on mitochondrial COI DNA sequences. Mar Ecol Prog Ser 182: 137–147

Bernard FR (1974) The genus *Calyptogena* in British Columbia with a description of a new species (Bivalvia: Vesicomyidae). Venus (Jpn J Malac) 33: 11–22

Boore JL, Brown WM (1994) Complete DNA sequence of the mitochondrial genome of black chiton, *Katharina tunicata*. Genetics 138: 423–443

Boss KJ, Turner RD (1980) The giant clam from the Galapagos Rift, *Calyptogena magnifica* species novum. Malacologia 20: 161–194

Folmer O, Black M, Hoeh W, Lutz RA, Vrijenhoek RC (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3: 294–299

Fujikura K, Kojima S, Fujiwara Y, Hashimoto J, Okutani T (2000) New distribution records of vesicomyid bivalves from deep-sea chemosynthesis-based communities in Japanese waters. Venus (Jpn J Malac) 59: 103–121

Goffredi SK, Hurtado LA, Hallam S, Vrijenhoek RC (2003) Evolutionary relationships of deep-sea vent and cold seep clams (Mollusca: Vesicomyidae) of the "*pacifica/lepta*" species complex. Mar Biol 142: 311–320

Horikoshi M (1987) Distinctions in the hinge structure between *Akebiconcha* and *Calyptogena*, with special reference to "subumbonal pit" and "cardinal ligament". Venus (Jpn J Malac) 45: 245–257

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111–120

Kojima S (2002) Deep-sea chemoautosynthesis-based communities in the northwestern Pacific. J Oceanogr 58: 343–363

Kojima S, Kobayashi T, Hashimoto J, Ohta S (1995) RFLP analysis of a mitochondrial gene, cytochrome oxidase I (COI) of three species of the genus *Calyptogena* around Japan. J Oceanogr 51: 257–259

Kojima S, Fujikura K, Hashimoto J, Ohta S (1997) Distribution and genetic structure of *Calyptogena soyoae* complex. JAMSTEC Deep Sea Res 13: 691–696 (in Japanese with English abstract)

Kojima S, Fujikura K, Okutani T (2004) Multiple trans-Pacific migrations of deep-sea vent/seep-endemic bivalves in the family Vesicomyidae. Mol Phylogenet Evol 32: 396–406

Kojima S, Fujikura K, Okutani T (2005) Genetic differentiation of two vesicomyid bivalves, *Calyptogena okutanii* and *Calyptogena nankaiensis* between seep areas off the central Honshu and hydrothermal vent fields in the Okinawa Trough. Venus 64: 45–53

Kuroda T (1943) *Akebiconcha*, a new pelecypod genus. Venus (Jpn J Malac) 13: 14–18 (partly in Japanese)

Machiyama H, Akimoto K, Komatsubara J, Jenkins RG, Takeuchi R, Fujino K, Yamazaki A, Ookubo K, Yamaoka K, Yamano M (2004) Outline of R/V Kaiyo KY03-09 Cruise (piston coring and heat flow surveys) in the Kuroshima Knoll area, southwestern part of the Ryukyu Arc. JAMSTEC Deep Sea Res 24: 85–98 (in Japanese with English abstract)

Okutani T (1957) Two new species of bivalves from the deep water in Sagami Bay collected by the R.V. Soyo-Maru. Bull Tokai Reg Fish Res Lab 17: 27–30, pl. 1

Okutani T (1962) Report on the archibenthyal and abyssal lamellibranchiate mollusca mainly collected from Sagami Bay and adjacent waters by the R.V. *Soyo-Maru* during the years 1955–1960. Bull Tokai Reg Fish Res Lab 32: 1–40, pls. 1–5

Okutani T (1966) Identity of *Calyptogena* and *Akebiconcha* (Bivalvia, Cyprinidae). Venus (Jpn J Malac) 24: 297–303 (in Japanese with English abstract)

Okutani T, Egawa K (1985) The first underwater observation on living habit and thanatocenoses of *Calyptogena soyoae* in bathyal depth of Sagami Bay. Venus (Jpn J Malac) 44: 285–289

Okutani T, Hashimoto J, Fujikura K (1992) A new species of vesicomyid bivalve associated with hydrothermal vents near Amami-Oshima Island, Japan. Venus (Jpn J Malac) 51: 225–

NII-Electronic Library Service

The Zoological Society of Japan



233

Okutani T, Fujikura K, Kojima S (2000) New taxa and review of vesicomyid bivalves collected from the Northwest Pacific by deep sea research systems of Japan Marine Science & Technology Center. Venus (Jpn J Malac) 59: 267–275

Okutani T, Iwasaki N, Kojima S (2002) New and known vesicomyid bivalves recently collected from the western and central Nankai Trough off Shikoku-Honshu, Japan by deep sea research systems of Japan Marine Science and Technology Center. Venus 61: 129–140

Ozaki H (1958) Stratigraphical and paleontological studies on the Neogene and Pleistocene formations of the Tyoshi District. Bull Natl Sci Mus (Tokyo) 42: 1–182, pls. 1–24

Peek AS, Gustafson RG, Lutz RA, Vrijenhoek RC (1997) Evolutionary relationships of deep-sea hydrothermal vent and cold-water seep clams (Bivalvia: Vesicomyidae): results from mitochondrial cytochrome oxidase subunit I. Mar Biol 130: 151–161

Peek AS, Feldman RA, Lutz RA, Vrijenhoek RC (1998) Cospeciation of chemoautotrophic bacteria and deep sea clams. Proc Natl Acad Sci USA 95: 9962–9966

Peek AS, Grant BS, Feldman RA, Barry JP, Kochevar RE, Lutz RA, Vrijenhoek RC (2000) Neutral and nonneutral mitochondrial genetic variation in deep-sea clams from the family Vesicomyidae. J Mol Evol 50: 141–153

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49: 1280–1283

Schneider S, Roessli D, Excoffier L (2000) Arlequin: A software for population genetic data analysis. Ver 2.000, Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva

Shikama T (1961) On some noteworthy shells from off Choshi, Chiba Prefecture. Sci Rep Yokohama Natl Univ Sec II 8: 29–59, pls. 1–3

Shimayama T, Himeno H, Sasuga J, Yokobori S, Ueda T, Watanabe K (1990) The genetic code of a squid mitochondrial gene. Nucleic Acids Symp Ser 22: 77–78

Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (* and other methods). Ver 4.0b10, Sinauer Associates, Sunderland, Massachusetts

Tsuchida E (1986) *Akebiconcha kawamurai* collected from off Kii Channel. The Chiribotan 17: 29–31 (in Japanese)

Van Dover CL, Aharon P, Bernhard JM, Caylor E, Doerries M, Flickinger W, Gilhooly W, Goffredi SK, Knick KE, Macko SA *et al.* (2003) Blake Ridge methane seeps: characterization of a soft-sediment, chemosynthetically based ecosystem. Deep Sea Res Part I Oceanogr Res Pap 50: 281–300

Weir BS, Cockerham CC (1984) Estimation of *F*-statistics for the analysis of population structure. Evolution 38: 1358–1370

(Received April 20, 2005 / Accepted May 5, 2006)

NII-Electronic Library Service