Species Composition of Genus *Calyptogena* in Sagami Bay: A Morphology-based Study

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Abstract: The species composition of two species in genus *Calyptogena*, *C. soyoae* and *C. okutanii*, was examined in three areas in Sagami Bay. Species were identified based on shell morphology. The dominant species in cold-seepage sites Off Hatsushima Island (OHI) and at the Okinoyama Bank (OB) was *C. okutanii* (74.4 and 60.5%, respectively). However, most *Calyptogena* at the Sagami Knoll (SK) were *C. soyoae* (90.7%). The proportion of these two *Calyptogena* species differed significantly among the three seepage sites. Given that most ecological studies of *Calyptogena* have been conducted at the OHI cold-seepage site, these results suggest that most of the ecological information on *Calyptogena* in Sagami Bay is derived not from *C. soyoae* but from *C. okutanii*.

Key words: *Calyptogena okutanii*, *Calyptogena soyoae*, chemosynthetic community, species composition

INTRODUCTION

Bivalves of the genus *Calyptogena* are one of the dominant groups within chemosynthetic communities (Lutz 1993). Many researchers have conducted ecological and physiological studies of these species in various hydrothermal vents and cold-seepage areas (Turekian & Cochran 1981; Endow & Ohta 1990; Barry et al. 1997; Kojima & Ohta 1997a; Lisin et al. 1997). In deep waters off Japan, numerous *Calyptogena* species have been described and studied (Okutani et al. 2000; Kojima 2002). The first population of *Calyptogena* species was described from Sagami Bay in Japan; a large population of *Calyptogena* was found at a depth around 1,100 m at a cold-seepage site off Hatsushima Island (OHI) in Sagami Bay during submersible dives in 1984 (Okutani & Egawa 1985). Since that discovery, smaller *Calyptogena* populations have been discovered in other locations in Sagami Bay, such as the Okinoyama Bank (OB; Hashimoto et al. 1988) and the Sagami Knoll (SK; Hattori et al. 1993).

In population biology, correct species identification is indispensable; when the classification criteria of a

species are re-examined, ecological findings of the species may also need to be re-examined. Ecological studies have been conducted on the *Calyptogena* populations in Sagami Bay (e.g., Hashimoto et al. 1995; Fujiwara et al. 1998). In these studies, it was presumed that the populations consisted of a single species, *C. soyoae*, which had been described based on the morphological findings of dead shells (Okutani 1957). However, Kojima and coworkers discovered that the *Calyptogena* populations of Sagami Bay included two mitochondrial DNA haplotypes (Kojima et al. 1995a, 1995b). Kojima & Ohta (1997b) revealed that the two haplotypes were discriminated also by a morphological characteristic (the length-height ratio of the shell), and described one haplotype as a new species, *C. okutanii*. When re-examining the result of this earlier study of *Calyptogena* in Sagami Bay, it is important to measure the species ratio of the two *Calyptogena* species. However, there is little information about the coexistence of the two species.

The main aim of this study was to examine the species composition of the two *Calyptogena* species in three local populations in Sagami Bay (OHI, SK, and OB), based on the shell morphology described by Kojima & Ohta (1997b). Based on the results, we discussed whether the given information on *Calyptogena* in Sagami Bay was derived from *C. soyoae* or *C. okutanii*. In addi-

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tion, growth-related changes in shell morphology were also examined. Kojima & Ohta (1997b) assume that the key characteristic of the morphological classification (the length-height ratio of the shell) does not change with shell growth; however, this assumption has not been examined yet.

MATERIALS AND METHODS

The shells of *C. okutanii* in Sagami Bay are narrower than those of *C. soyoae*; the length-height ratios are 2.19 ± 0.10 and 1.98 ± 0.11 , respectively (Kojima & Ohta 1997b). Based on that previous morphological description, we assumed that specimens with a length-height ratio greater than 2.09 were *C. okutanii*.

Specimens of *Calyptogena* were collected from three sites (OHI, SK, and OB) in Sagami Bay from 1987 to 1997 using submersible and ROV surveys conducted by the Japan Marine Science and Technology Center (Fig. 1). All *Calyptogena* specimens were alive when collected. Most of these specimens were fixed with formalin or ethanol, and some were preserved as dried shells. Kojima & Ohta (1997b) did not use small specimens (shell length <55 mm) in their description, and the

smaller shells of *Calyptogena* in Sagami Bay had a rounder shape than that of the larger shells (see Results). Therefore, we selected and analyzed specimens with shell lengths greater than 55 mm, for a total of 253 specimens: 172 specimens from OHI, 43 specimens from SK, and 38 specimens from OB. Sampling location and date, number of samples, and dive number are presented in Table 1.

Shell length and height were measured using a slide caliper to the nearest 0.01 mm (Fig. 2). Based on the shell-ratio criterion for the classification of *C. soyoae* and *C. okutanii*, the proportion of each species was estimated at each site. To evaluate the difference in species composition of *Calyptogena* among the sites, we used the chi-squared test with Tukey-type multiple comparisons (Zar 1999).

To examine the effect of body growth on the key characteristic of the morphological classification, we compared the shell length-height ratios between the young and old growth stages in the same individual. We selected 15 subsamples from the specimens collected from OHI and SK, respectively. There were many growth rings on the shell surface in each specimen. We selected two external rings on the shell surface (shell length: small ring, 36.1–62.5 mm; large ring, 72.6–111.7 mm)

Fig. 1. Locations where samples were collected in Sagami Bay: (OHI) Off Hatsushima Island; (OB) Okinoyama Bank; (SK) Sagami Knoll. Depths are in meters.

Table 1. List of samples used in the present study.

Location	Sampling Date	Submersible	Dive No.	No. of samples
Off Hatsushima	1989.10.21	<i>Shinkai 2000</i>	450	22
	1989.10.22	<i>Shinkai 2000</i>	451	67
	1990.7.13	<i>Dolphin 3K</i>	78	15
	1990.11.11	<i>Shinkai 2000</i>	518	5
	1990.11.12	<i>Shinkai 2000</i>	519	9
	1993.4.9	<i>Shinkai 2000</i>	667	3
	1995.4.20	<i>Dolphin 3K</i>	211	5
	1997.5.27	<i>Dolphin 3K</i>	321	42
	1997.5.29	<i>Dolphin 3K</i>	324	4
Okinoyama Bank	1987.5.17	<i>Dolphin 3K</i>	45	17
	1987.5.22	<i>Shinkai 2000</i>	278	7
	1988.6.11	<i>Dolphin 3K</i>	29	1
	1988.10.11	<i>Dolphin 3K</i>	38	8
	1990.7.12	<i>Dolphin 3K</i>	75	5
Sagami Knoll	1991.11.15	<i>Shinkai 2000</i>	583	5
	1993.11.6	<i>Dolphin 3K</i>	166	38

Fig. 2. Measurement of (A) shell length and (B) shell height of *Calypptogena*.

and measured the length and height of the rings individually. For each individual, we compared the ratio of length and height between the small and the large rings using Wilcoxon's signed-rank test.

All statistical tests were performed at a $p < 0.05$ significance level.

RESULTS

The frequency distributions of the length-height ratio of the shells were different among the sites (Fig. 3). Specimens from OHI and SK had a single peak in the 2.20–2.25 and 2.0–2.05 classes, respectively, and specimens from OB had two peaks, one at the 2.05–2.10 class and one at the 2.25–2.30 class. In this study, we assumed that specimens with a length-height ratio greater than 2.09 were *C. okutanii* (see Materials and Methods). Table 2 shows species ratio of two *Calypptogena* species based on this criterion. The ratios of the two *Calypptogena* species were significantly different at SK compared to the other two sites. The dominant species of *Calypptogena* at OHI and OB was *C. okutanii*, whereas the majority of

Fig. 3. Frequency distribution of shell length-height ratio of *Calypptogena* in three seep sites in Sagami Bay. Black arrows denote the criteria of classification between *C. soyoae* and *C. okutanii* (shell length-height ratio=2.09, see Materials and Methods).

Fig. 4. Comparison of length-height ratio of larger and smaller rings of *Calyptogena* in Sagami Bay. Horizontal lines denote the criteria of classification between *C. soyoe* and *C. okutanii* (shell length-height ratio=2.09, see Materials and Methods). The value of large ring is significantly higher than small ring (Wilcoxon's sign rank test, $p<0.001$).

Calyptogena at SK were *C. soyoe*.

In the analysis above, we assume, following Kojima & Ohta (1997b), that the key characteristic of the morphological classification (the length-height ratio of the shell) does not change through life. To examine the stability of morphological-classification criterion in this study, the length-height ratios were compared between small and large rings (Fig. 4). At SK and OHI, the length-height ratios of the large rings (i.e., older stages) were significantly higher than those of the small rings (younger stages; Fig. 4). Some of the length-height ratio of large rings (>72.6 mm; five of 15 samples from OHI and two of 15 samples from SK) was more than 2.09. However, all of the length-height ratios of small rings (<62.3 mm shell length) were less than 2.09.

DISCUSSION

Our morphological data suggest that the *Calyptogena* populations at OB and OHI were composed mainly

Table 2. Number of *C. soyoe* and *C. okutanii* in samples of *Calyptogena* from sites in Sagami Bay. There were significant differences in species ratio among the sites ($\chi^2=61.474$, $df=2$, $p<0.001$). Off Hatsushima Island (OHI)=Okinoyama Bank (OB)≠Sagami Knoll (SK).

	Sampling sites		
	OHI	OB	SK
<i>C. soyoe</i>	44	15	39
<i>C. okutanii</i>	128	23	4

of *C. okutanii*, and that most *Calyptogena* collected at SK were *C. soyoe* (Table 2). This trend supports the data in a report by Kojima & Ohta (1997b) based on molecular analysis: 13 of 22 samples collected from OHI and 11 of 13 samples collected from OB were classified as *C. okutanii*, and all three samples collected from SK were classified as *C. soyoe*. This concordance between morphological and molecular data implies that the true species composition is reflected in the frequency distribution of shell length-height ratios (Fig. 3). To better understand the difference in *Calyptogena* species composition among the sites, it may be effective to compare SK and the other cold-seepage areas in terms of the environment of the *Calyptogena* population. However, habitat description and ecological studies of *Calyptogena* have not been fully conducted at SK.

Calyptogena okutanii has been found in a wide area around Japan (seepage areas of Sagami Bay, the Ryuyo Canyon, the Nankai Trough and around hydrothermal vents of Iheya Ridge, the Okinawa Trough), whereas *C. soyoe* has been collected only in Sagami Bay (Kojima 2002). In Sagami Bay, *C. soyoe* is dominant only in a small local population of SK (Table 2). These results imply that, compared with *C. okutanii*, the environmental conditions in which *C. soyoe* can survive are restricted.

Shell shape of *Calyptogena* species in Sagami Bay tends to become slender with growth (Fig. 4), a pattern reported in Sagami Bay by Horikoshi & Hashimoto (1992) and Hattori et al. (1993). It is likely that this morphological change occurs in both *C. okutanii* and *C. soyoe* because this growth pattern was observed in samples taken from SK and OHI, where the dominant species of *Calyptogena* are *C. soyoe* and *C. okutanii*, respectively (Table 2).

The shell length-height ratio of *Calyptogena* with shell length less than 62.3 mm was below 2.09 (Fig. 4). Therefore, based on the length-height ratio, small *C. okutanii* tends to be classified as *C. soyoe*. Kojima & Ohta (1997b) stated that it is impossible to discriminate accu-

rately between *C. soyoae* and *C. okutanii* based solely on the shell length-height ratio because the ratios of the species overlap. The overlap may be due to growth-related changes in shell shape. To detect the species composition accurately, molecular analysis (or more stable key characteristics for classification) will be needed.

Most ecological studies of the genus *Calyptogena* in Sagami Bay have been conducted at the OHI cold-seepage site; reports have provided descriptions of the habitat and colonies (Okutani & Egawa 1985; Hashimoto et al. 1989, 1995) and lifehistory characteristics such as growth, metamorphosis (Horikoshi & Hashimoto 1992; Hattori et al. 1993), and reproduction (Momma et al. 1995; Fujiwara et al. 1998). In these studies, all *Calyptogena* species from OHI were treated as *C. soyoae*. According to our data, however, 74.4% of the *Calyptogena* species at OHI are *C. okutanii* (Table 2). Therefore, most of the ecological information on the *Calyptogena* species in Sagami Bay has been derived almost exclusively from *C. okutanii*. In contrast, ecological information on *Calyptogena* from SK has been derived predominantly from *C. soyoae*, given that more than 90% of the *Calyptogena* at this site are *C. soyoae* (Hattori et al. 1993).

In conclusion, the species composition of *Calyptogena* in Sagami Bay differed from that in the local cold-seepage areas; the dominant species of *Calyptogena* at OHI and OB was *C. okutanii*, and that at SK was *C. soyoae*. Ecological information on *Calyptogena* in Sagami Bay is derived predominantly from *C. okutanii*, given that most ecological studies have been conducted at OHI. To determine the causes of differences in species composition among cold-seepage areas, additional field surveys are needed, especially at SK.

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