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Molecular Phylogenetic and Morphological Analyses of Prostrate *Codium* (Chlorophyta) in Japan

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The morphology of utricles was critically studied in order to elucidate the species composition of prostrate *Codium* in Japan. Utricle morphology, such as length, diameter, shape of apical portions, presence or absence of hairs (or hair-scars) and alveolate ornamentation, are shown to characterize the clades recognized in our previous molecular analysis. These molecular and morphological congruences indicate that these morphological features reflect the phylogeny of *Codium*. The prostrate *Codium* in Japan was classified into the following five species: *C. arabicum* Kützing, *C. capitulatum* Silva & Womersley, *C. dimorphum* Svedelius, *C. hubbsii* Dawson, *C. lucasii* Setchell.

Key words: Chlorophyta, *Codium*, molecular phylogeny, morphology, *rbcL*.

The green macroalgal genus *Codium* is distributed from tropical to cold temperate waters of both hemispheres (Jones and Kraft 1984). Chacana et al. (1997) reported four prostrate species of *Codium*; *C. arabicum* Kützing, *C. hubbsii* Dawson, *C. lucasii* Setchell and an undescribed species in Japanese waters. In contrast Yoshinaga et al. (1999) suggested that only one prostrate species occurs in Japan, although the species name was not cited in their report. However, three prostrate species of *Codium*; *C. hubbsii*, *C. lucasii* and *C. arabicum* were then recognized in Yoshida et al. (2000).

In a preliminary report (Shimada et al. 2004), we determined the sequences of the plastid encoded large subunit of ribulose-1,5-bisphosphate carboxylase/oxgenase (*rbcL*) genes of samples of *Codium* from various parts of Japan. The phylogenetic tree indicated that Japanese prostrate *Codium* was separated into five clades (Shimada et al. 2004). Consequently, Yoshida et. al. (2005) preliminary reported *C. hubbsii*, *C. lucasii*, *C. arabicum*, *C. capitulatum* Silva & Womersley and *C. dimorphum* Svedelius as prostrate *Codium* in Japan. Of these, *C. capitulatum* and *C. dimorphum* were newly reported from Japan (Yoshida et. al. 2005). However, detailed morphological studies of utricles have not been undertaken for prostrate *Codium* in Japan.

In this study, morphology of utricles was critically studied from the samples belonging to the five clades recognized in our molecular analysis to determine whether the samples belonging to each clade share stable morphological characteristics. We also

investigated the type specimen of *C. arabicum* deposited in the Universiteit Leiden branch of the National Herbarium Nederland, NHN (L 937.277.29) for microscopic observation. The clades were then evaluated whether these fit any of the described species of prostrate *Codium*.

Materials and Methods

Materials

Thalli of prostrate *Codium* collected from around Japan (34 specimens) are listed in Table 1. Voucher specimens are deposited in the Herbarium of the Graduate School of Science, Hokkaido University, Sapporo (SAP 095329–095362). The type specimen of *C. arabicum* was also used for observation of utricles. A small part of the type specimen of *C. arabicum* was rehydrated, and made slide-glasse specimens. These slide-grasses were preserved in the National Herbarium Nederland.

Molecular analysis

Thirty-four sequences of prostrate *Codium* and eleven of other species of *Codium* from GenBank were used for reconstruction of the molecular phylogenetic tree of *Codium*. The *rbcL* sequences (804 bp) were aligned manually because no deletion/insertion mutations were detected, and correspond to positions 1-804 of the *rbcL* sequence of *Codium fragile* (M67453). The alignment is available from the first author upon request. *Ulva pertusa* Kjellman and *Bryopsis maxima* Okamura were used as outgroups, because these two species represent a sister group to *Codium* in the *rbcL* tree (Sherwood et al. 2000).

The maximum likelihood (ML) analysis was implemented in PAUP* 4.0b10 (Swofford 2002). The ML parameters were estimated using the likelihood ratio test. The program MODELTEST version 3.7 (Posada and Crandall 1998) was used to find the model of sequence evolution that best fit each data set by a hierarchical likelihood ratio test ($\alpha = 0.01$). When the best sequence evolution model was determined, an ML tree search was performed using the estimated parameters with the following options: heuristic search, starting tree option = obtained by neighbor joining and branch swapping algorithm = TBR. Bootstrap analysis (Felsenstein 1985) based on 100 re-samplings of the dataset was calculated (TBR, full heuristic search option).

Morphological analysis

To seek morphological features that are critical for species discrimination of prostrate *Codium*, all source thalli used in the DNA analysis were examined for their utricle morphology. We separately measured means and standard deviations of the length and diameter of 20 primary and 20 secondary utricles in both marginal and central portions of the thallus. We also observed the shape of utricles, the presence or absence of hairs (or hair-scars), and the alveolate ornamentation of utricular apices. The utricles of the type specimen of *C. arabicum* could be observed only marginal portion of thallus.

Results

Molecular analysis

Pairwise intra-specific divergence ranged from 0% to 1.1%, and inter-specific divergence ranged between 1.8% and 12.3% [calculated by Kimura's (1980) two-parameter method]. The phylogenetic tree obtained from ML analysis is presented in Fig. 1. Identical sequences were excluded from the alignment for the phylogenetic analysis. For the ML method, likelihood settings from best-fit model (F81+I+G) were selected: assumed nucleotide frequencies A = 0.31450, C = 0.14800, G = 0.19280, and T = 0.34470; proportion of invariable sites = 0.4807; gamma distribution with shape parameter = 0.3706. With these settings, the heuristic search was performed and the ML

Table 1. List of species used in our molecular analysis and GenBank accession number

Species	Collection data	Sample no.	Clade	Accession no.
Ulva pertusa				AB097624
Bryopsis maxima				X55877
Codium fragile				M67453
C. minus	Tateyama, Chiba Pref., Japan (31 May 2000)			AB102958
C. spongiosum	Tosashimizu, Kochi Pref., Japan (5 May 2000)			AB102978
C. intricatum	Kashinoura, Kochi Pref., Japan (4 May 2000)			AB102990
C. contractum	Tsuyazaki, Fukuoka Pref., Japan (13 Mar. 2000)			AB102995
C. subtubulosum	Nemoto, Chiba Pref., Japan (30 May 2000)			AB102998
C. repens	Hachijo Is. Tokyo, Japan (19 Jul. 2001)			AB103001
C. latum	Shimoda, Shizuoka Pref., Japan (24 Jul. 2001)			AB103005
C. barbatum	Susaki, Kochi Pref., Japan (21 Jun. 2000)			AB103007
C. yezoense	Akkeshi, Hakkaido, Japan (20 Jun. 2000)			AB103024
C. cylindricum	Tateyama, Chiba Pref., Japan (2 May 2001)			AB103026
C. capitulatum	Kashinoura, Kochi Pref., Japan (5 May 2000)	No. 26	clade A	AB102961
	Susaki, Kochi Pref., Japan (21 Jun. 2000)	No. 58	clade A	AB102962
	Kagoshima, Kagoshima Pref., Japan (19 May 2001)	No. 132	clade A	AB102963
	Kagoshima, Kagoshima Pref., Japan (19 May 2001)	No. 133	clade A	AB102964
C. hubbsii	Hakata, Fukuoka Pref., Japan (18 Apr. 2000)	No. 23	clade B	AB102965
	Tateyama, Chiba Pref., Japan (31 May 2000)	No. 27	clade B	AB102966
	Cape of Sata, Kagoshima Pref., Japan (6 Jun. 2000)	No. 44	clade B	AB102967
	Tateyama, Chiba Pref., Japan (30 May 2000)	No. 75	clade B	AB102968
	Tateyama, Chiba Pref., Japan (30 May 2000)	No. 78	clade B	AB102969
	Esumi, Wakayama Pref., Japan (29 Mar. 2001)	No. 124	clade B	AB102970
	Himi, Toyama Pref., Japan (4 Jul. 2001)	No. 143	clade B	AB102971
	Shimoda, Shizuoka Pref., Japan (22 Jul. 2001)	No. 169	clade B	AB102972
	Shimoda, Shizuoka Pref., Japan (24 Jul. 2001)	No. 173	clade B	AB102973
	Shimoda, Shizuoka Pref., Japan (24 Jul. 2001)	No. 175	clade B	AB102975
	Miura, Kanagawa Pref., Japan (29 Apr. 2002)	No. 212	clade B	AB102976
	Tappi, Aomori Pref., Japan (29 May 2002)	No. 213	clade B	AB102977
C. lucasii	Kushimoto, Wakayama Pref., Japan (28 Mar. 2001)	No. 122	clade C	AB102980
	Kushimoto, Wakayama Pref., Japan (28 Mar. 2001)	No. 123	clade C	AB102981
	Tanega Is., Kagoshima Pref., Japan (5 Mar. 2001)	No. 199	clade C	AB102982
	Ogasawara Is., Tokyo, Japan (16 May 2002)	No. 220	clade C	AB102983
C. arabicum	Ishigaki Is., Okinawa Pref., Japan (25 Mar. 2001)	No. 121	clade D	AB102984
	Tokuno Is., Kagoshima Pref., Japan (19 Jul. 2001)	No. 146	clade D	AB102985
	Amami Is., Kagoshima Pref., Japan (15 Mar. 2002)	No. 200	clade D	AB102986
	Amami Is., Kagoshima Pref., Japan (15 Mar. 2002)	No. 201	clade D	AB102987
	Ogasawara Is., Tokyo, Japan (12 May 2002)	No. 217	clade D	AB102989
C. dimorphum	Tateyama, Chiba Pref., Japan (31 May 2000)	No. 29	clade E	AB103009
	Susaki, Kochi Pref., Japan (21 Jun. 2000)	No. 66	clade E	AB103010
	Susaki, Kochi Pref., Japan (21 Jun. 2000)	No. 67	clade E	AB103011
	Tateyama, Chiba Pref., Japan (30 May 2000)	No. 74	clade E	AB103012
	Tateyama, Chiba Pref., Japan (30 May 2000)	No. 76	clade E	AB103013
	Himi, Toyama Pref., Japan (4 Jul. 2001)	No. 142	clade E	AB103015
	Shimoda, Shizuoka Pref., Japan (23 Jul. 2001)	No. 151	clade E	AB103016
	Shimoda, Shizuoka Pref., Japan (24 Jul. 2001)	No. 172	clade E	AB103017
	Shimoda, Shizuoka Pref., Japan (24 Jul. 2001)	No. 176	clade E	AB103018

tree (-ln L = 3610.35696) (Fig. 1) was obtained after 11815 rearrangements. The tree showed four major clades. Clade 1 included *C. spongiosum* Harvey and four subclades of prostrate *Codium* (clades A, B, C and D); clade 2 contained only *C. minus* (O. C. Schmidt) P. C. Silva; clade 3 encompassed *C. latum* Suringar, *C. barbatum* Okamura, *C. repens* Crouan frat., *C. subtubulosum* Okamura, *C. contractum*

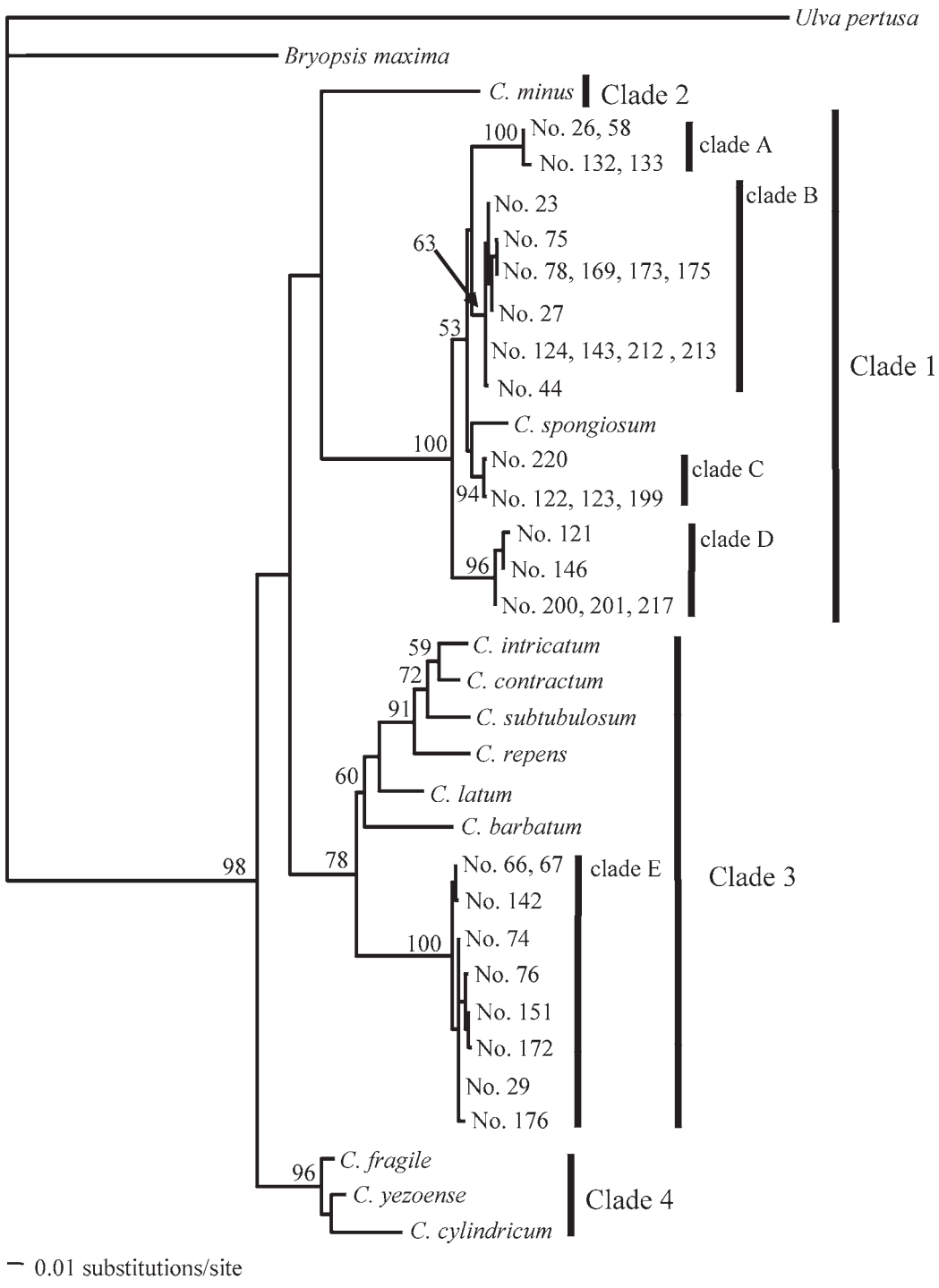
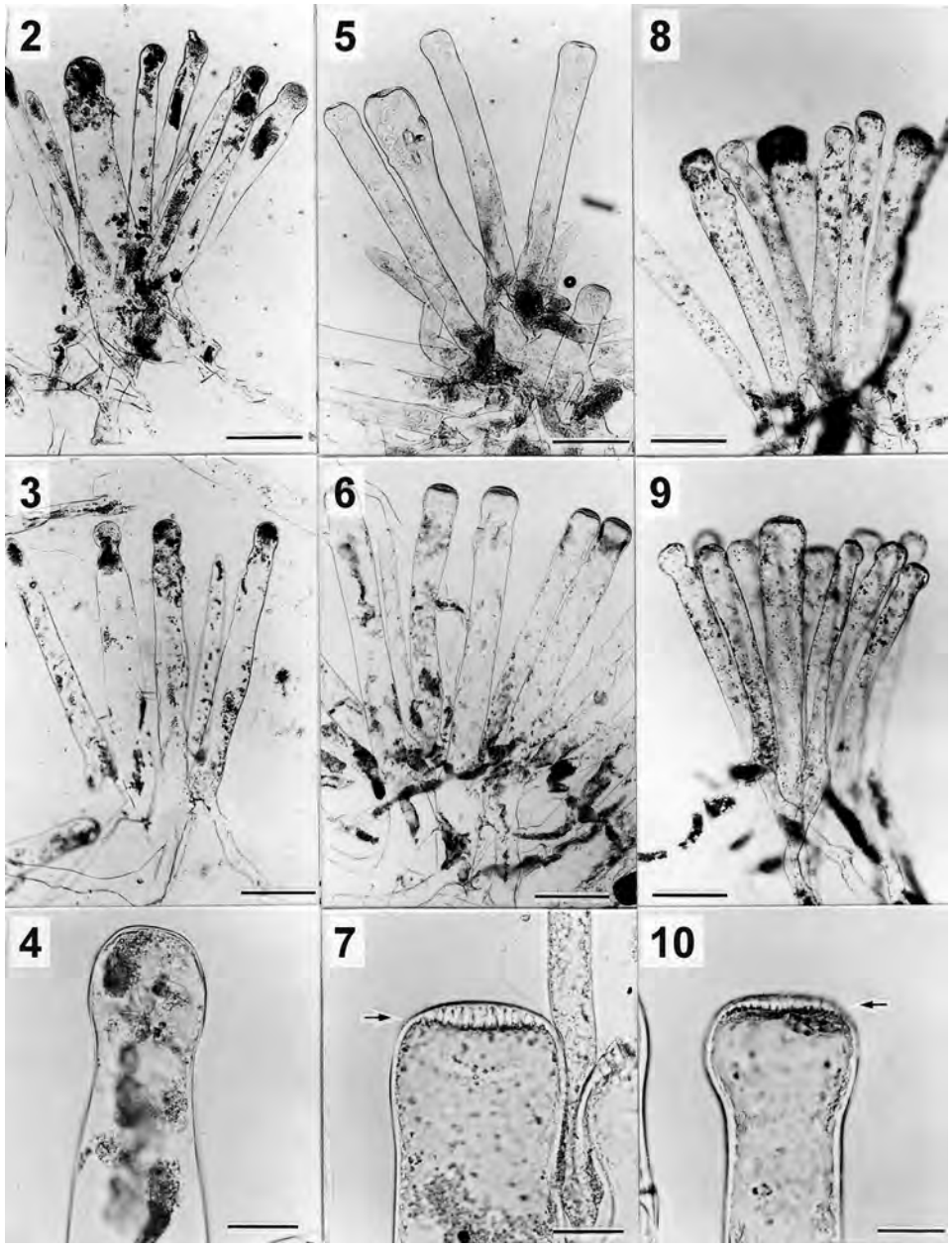


Fig. 1. Maximum likelihood tree constructed from an analysis of *rbcL* sequences of *Codium*. The tree was rooted with *Ulva pertusa* and *Bryopsis maxima*. The numbers at each node represent full heuristic bootstrap values (100 replicates) greater than 50%. The sample numbers correspond to those in Table 1.



Figs. 2–10. Utricle morphology of clade A (*Codium capitulatum*), clade B (*C. hubbsii*) and clade C (*C. lucasii*). Scar bars = 150 μm (Figs. 2, 3, 5, 6, 8, 9); 30 μm (Figs. 4, 7, 10). Fig. 2. Utricles at the margin of thallus of clade A (*C. capitulatum*). Fig. 3. Utricles in the center of thallus of clade A (*C. capitulatum*). Fig. 4. Apex of utricle, appearing capitate of clade A (*C. capitulatum*). Fig. 5. Utricles at the margin of thallus of clade B (*C. hubbsii*). Fig. 6. Utricles in the center of thallus of clade B (*C. hubbsii*). Fig. 7. Apex of utricle, showing alveolate ornamentation (arrow) of clade B (*C. hubbsii*). Fig. 8. Utricles at the margin of thallus of clade C (*C. lucasii*). Fig. 9. Utricles in the center of thallus of clade C (*C. lucasii*). Fig. 10. Apex of utricle, showing alveolate ornamentation (arrow) of clade C (*C. lucasii*).

Kjellman, *C. intricatum* Okamura and one sub-clade of prostrate *Codium* (clade E), and clade 4 contained *C. fragile* (Suringar) Hariot, *C. yezoense* (Tokida) Vinogradova and *C. cylindricum* Holmes, all cylindrical, upright species. The four principal clades were supported by moderate to high bootstrap values in both trees (63–100%).

In the *rbcL* tree, the 34 samples of prostrate *Codium* were divided into five subclades (clades A, B, C, D and E) with 63–100% bootstrap values. One of them (clade E) occurred distantly from the other four (Fig. 1).

Morphological analysis

Morphological data are shown in Table 2, Figs. 2–24. Length and diameter of utricles were variable in size within individuals. Primary utricles were larger than secondary utricles. Utricles in the central portion were longer and thinner than those at the margin. Although length and diameter of utricles overlapped between the five prostrate clades, they appeared to be clade specific, e. g., thalli of clade D possessed the widest utricles at the margin (Fig. 11), and thalli of clade E had the longest utricles in the center (Fig. 15). Presence or absence of hairs (or hair-scars) and alveolate ornamentation at the apex of utricles was also found to be clade specific. All nine thalli of clade E lacked hairs (Figs. 14, 15), while all thalli of the other four prostrate clade possessed hairs (or hair-scars). All twelve thalli of clade B, four thalli of clade C and three of five (Nos. 200, 201, 217) thalli of clade D possessed alveolate ornamentation at the apex of utricles, while the two thalli (Nos. 121, 146) of clade D and all thalli of clade A and clade E lacked alveolate ornamentation.

Type specimen of *C. arabicum* possessed utricles that a few secondary ones develop from the lower portion of larger utricles (primary utricles) (Figs. 18, 21). Primary utricles of marginal portion of thallus were relatively stout, clavate to pyriform, 350–664 μm in length and 139–333 μm in diameter (Table 2). Primary utricles of marginal portion of thallus were cylindrical, 272–450 μm in length and 77–130 μm in diameter (Table 2). The type specimen of *C. arabicum* possessed hairs (or hair-scars), but no alveolate ornamentation at the apex of utricles were observed.

The following five species have been identified by the comparisons with the type specimen of *C. arabicum* and previous descriptions (Abbott and Hollenberg 1976, Adams 1994, Børgesen 1940, 1946, Burrows 1991, Dawson 1950, Jones and Kraft 1984, Kraft 2000, Kützing 1856, Lucas 1935, 1936, Schmidt 1923, Setchell 1937, Silva 1951, 1959, Silva and Womersley 1956, Svedelius 1900, Van den Heede and Coppejans 1996).

Clade A (Figs. 2–4)

Codium capitulatum Silva & Womersley in Aust. J. Bot. **4**: 263 (1956). Type locality: Elliston, southern Australia.

Thalli are firm, dark green, applanate and lobed, up to 9 cm across. At the margin, primary utricles are 530–1300 μm in length and 40–170 μm in diameter; secondary utricles are 470–900 μm in length and 30–150 μm in diameter (Fig. 2). In the center of the thallus, primary utricles are 600–1420 μm in length and 40–160 μm in diameter; secondary utricles are 500–1180 μm in length and 30–100 μm in diameter (Fig. 3). Utricles are cylindrical with rounded apices that constricted below, thus appearing capitate (Fig. 4). Hairs (or hair-scars) are abundant (Fig. 2). Alveolate (cribrose) ornamentation is absent (Fig. 4).

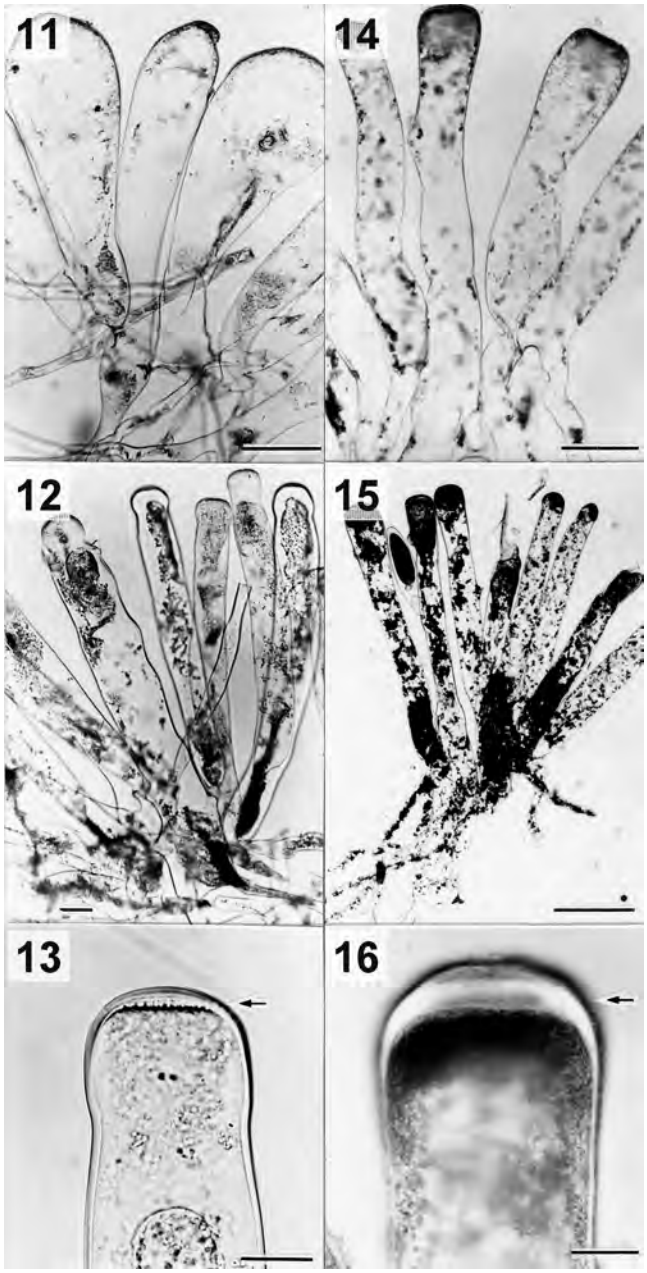
Clade B (Figs. 5–7)

Codium hubbsii Dawson in Am. J. Bot. **37**: 151 (1950). Type locality: Islas San Benito, Mexico.

Thalli are firm, dark green, applanate,

Table 2. Anatomical details of prostrate *Codium* distributed in Japan. Length and diameter of primary and secondary utricles in both marginal and central portion of thallus are indicated as minimum–average (±SD)–maximum

Sample	Clade A	Clade B	Clade C	Clade D	Clade E	Type specimen of *C. arabicum*
Marginal portion						
Primary utricles						
length (μm)	530–879.9(±198.6)–1300	670–998.8(±175.4)–1600	420–610.3(±103.3)–810	480–570(±67.3)–670	710–1088.3(±225.4)–1820	350–527.1(±86.2)–664
diameter (μm)	40–104.7(±34.7)–170	60–116.7(±32.2)–240	40–75.3(±21.4)–140	130–209.3(±58.9)–310	100–167.4(±37.6)–280	139–248.2(±58.7)–333
ratio of length to diameter	8.4	8.6	8.1	2.7	6.5	2.2
Secondary utricles						
length (μm)	470–668.7(±118.2)–900	500–773.7(±134.9)–1170	350–429.3(±62.7)–670	350–419.6(±104.0)–780	520–775.8(±160.6)–1480	272–340.1(±47.8)–450
diameter (μm)	30–74.3(±32.3)–150	50–89.8(±24.2)–160	40–65.3(±18.5)–120	60–121.2(±45.0)–250	70–134(±30.6)–220	77–99.4(±14.9)–130
Central portion						
Primary utricles						
length (μm)	600–984.4(±198.8)–1420	840–1123.4(±146.2)–1480	440–692.3(±127.4)–960	500–749.4(±120.1)–1030	820–1183.3(±238.2)–1690	
diameter (μm)	40–79(±30.8)–160	50–95.6(±24.3)–180	50–81.5(±22.7)–150	70–111.8(±34.9)–220	90–153.7(±34.1)–240	
Secondary utricles						
length (μm)	500–748.4(±164.2)–1180	520–855.6(±132.3)–1200	320–551.5(±118.3)–850	380–589.2(±10.5.9)–820	510–878(±204.8)–1400	
diameter (μm)	30–58.4(±18.6)–100	50–79.6(±19.9)–130	30–65.8(±20.2)–130	50–85.1(±19.2)–130	70–124.2(±26.6)–200	
Shape of utricles	cylindrical with rounded apices that constricted below, appearing capitate	cylindrical with depressed to subtruncate apices	cylindrical with rounded apices that constricted below, appearing capitate	relatively stout, clavate to pyriform at the margin of thallus; cylindrical to capitate with rounded apices in the center of thallus	cylindrical wth rounded to subtruncate apices that slightly to markedly thickened	relatively stout, clavate to pyriform at the margin of thallus
Hairs (or hairs scars)	abundant	present	present	present	absent	present
Alveolate ornamentation	absent	present	present	rarely present	absent	absent



Figs. 11–16. Utricle morphology of clade D (*Codium arabicum*) and clade E (*C. dimorphum*). Scar bars = 150 μm (Figs. 11, 12, 14); 30 μm (Figs. 13, 16); 300 μm (Fig. 15). Fig. 11. Utricles at the margin of thallus of clade D (*C. arabicum*). Fig. 12. Utricles in the center of thallus of clade D (*C. arabicum*). Fig. 13. Apex of utricle, showing alveolate ornamentation (arrow) of clade D (*C. arabicum*). Fig. 14. Utricles at the margin of thallus of clade E (*C. dimorphum*). Fig. 15. Utricles in the center of thallus of clade E (*C. dimorphum*). Fig. 16. Apex of utricle, showing thickened utricular wall (arrow) of clade E (*C. dimorphum*).

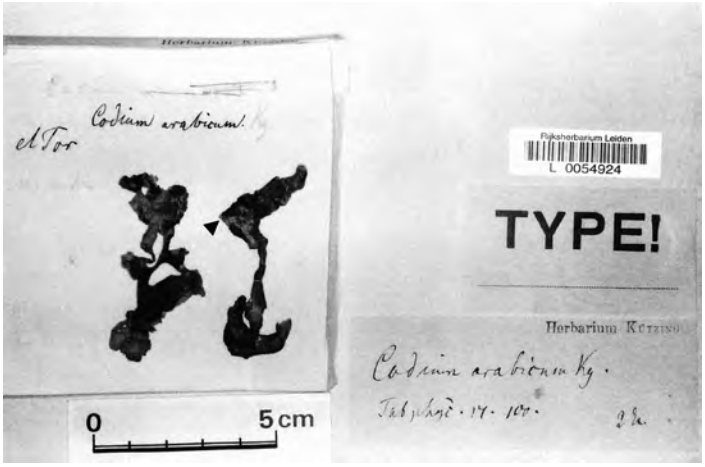


Fig. 17. Type specimen of *Codium arabicum* (L 937.277.29). Arrowhead indicates the portion for the present microscopic observation.

more or less lobed, up to 8 cm across. At the margin, primary utricles are 670–1600 µm in length and 60–240 µm in diameter; secondary utricles are 520–1170 µm in length and 50–160 µm in diameter (Fig. 5). In the center of the thallus, primary utricles are 870–1480 µm in length and 50–180 µm in diameter; secondary utricles are 520–1200 µm in length and 50–130 µm in diameter (Fig. 6). Utricles are cylindrical with depressed to subtruncate apices (Fig. 7). Hairs (hair-scars) are present. Alveolate (cribrose) ornamentation is also present (Fig. 7).

Clade C (Figs. 8–10)

Codium lucasii Setchell in Proc. Linn. Soc. N.S.W. **60**: 200 (1935). Type locality: Bondi, New South Wales, Australia.

Thalli are firm, dark green, applanate, lobed, up to 7 cm across. At the margin, primary utricles are 420–810 µm in length and 40–140 µm in diameter; secondary utricles are 350–670 µm in length and 40–120 µm in diameter (Fig. 8). In the center of the thallus, primary utricles are 440–960 µm in length and 50–150 µm in diameter; secondary utricles are 320–850 µm in length and 30–130 µm in diameter (Fig. 9). Utricles are cylindrical with rounded apices that constricted below, thus appearing capitate (Fig. 10). Hairs (hair-scars) are present. Alveolate (cribrose) ornamentation is also present (Fig. 10).

Clade D (Figs. 11–13)

Codium arabicum Kützing in Tabulae Phycologicae **6**: 100 (1856). Type locality: El Tor, Egypt.

Thalli are soft to firm, dark green, applanate, lobed many times, up to 10 cm across. At the margin, primary utricles are 420–990 µm in length and 80–300 µm in diameter; secondary utricles are 350–780 µm in length and 60–250 µm in diameter (Fig. 11). In the center of the thallus, primary utricles are 500–1030 µm in length and 70–220 µm in diameter: secondary utricles are 380–820 µm in length and 50–130 µm in diameter (Fig. 12). The shape of the utricles differs according to position in the thalli: relatively stout, clavate to pyriform at the margin; cylindrical to capitate with rounded apices constricted below in the center (Figs. 11, 12). Hairs (hair-scars) are present.

Alveolate (cribrose) ornamentation was present only in three of five (Nos. 200, 201, 217) thalli (Fig. 13).

Clade E (Figs. 14–16)

Codium dimorphum Svedelius in Svenska Expeditionen Till Magellandländer na **3**: 300 (1900). Type locality: Guaitecas Island, Chile.

Thalli are soft to firm, medium to dark green, applanate and rounded with marginal lobes, up to 6 cm in diameter. At the margin, primary utricles are 710–1820 µm in length and 100–280 µm in diameter; secondary utricles are 520–1480 µm in length and 70–220 µm in diameter (Fig. 14). In the center of the thallus, primary utricles are 820–1690 µm in length and 90–240 µm in diameter; secondary utricles are 520–1400 µm in length and 70–200 µm in diameter (Fig. 15). Utricles are cylindrical with rounded to subtruncate apices that slightly to markedly thickened (Fig. 16). Hairs (hair-scars) are absent. Alveolate (cribrose) ornamentation is also absent (Fig. 16).

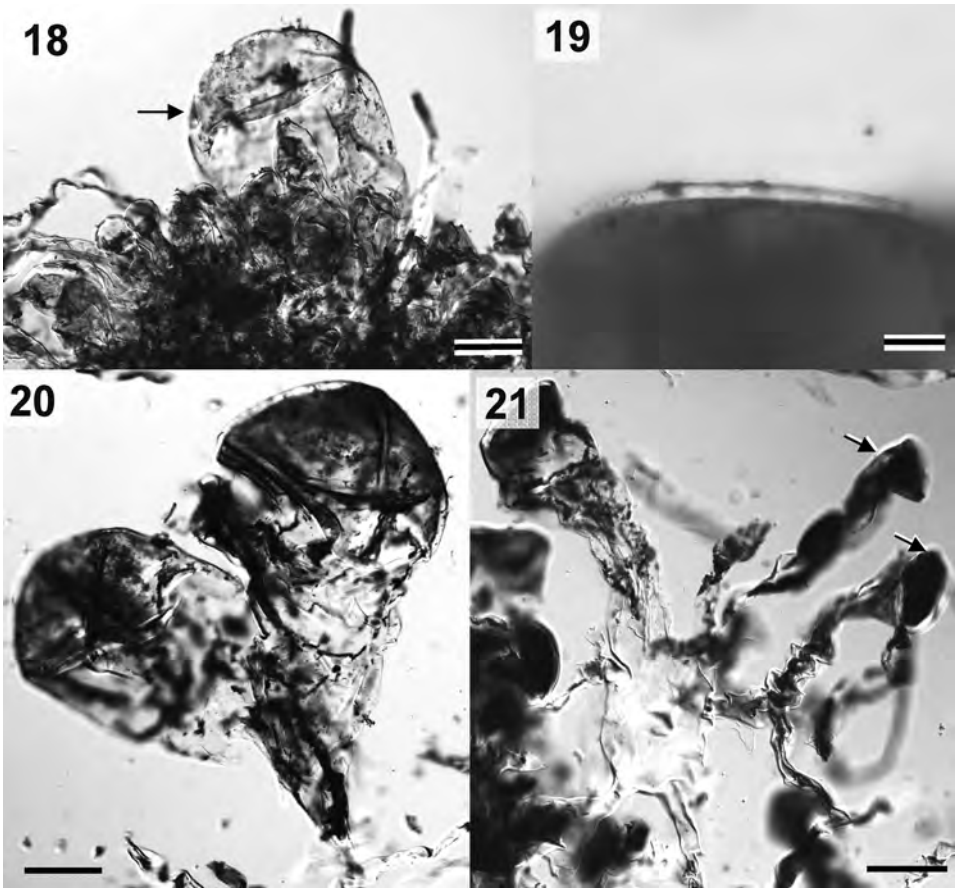
Discussion

We were able to demonstrate that there are five species of prostrate *Codium* in Japan, and they can be distinguished not only by the molecular evidence, but also by distinctive morphological characters. Molecular and morphological analyses of prostrate *Codium* in Japan revealed that five species, *C. capitulatum*, *C. hubbsii*, *C. lucasii*, *C. arabicum* and *C. dimorphum* are distributed in Japanese waters. We could distinguish them by using utricle morphology, including shape of apical portions, presence or absence of hairs (or hair-scars) and alveolate (cribrose) ornamentation. Length and diameter of utricles are highly variable within individuals, however, they are also shown to be species specific as mentioned above, if we separately measure primary and secondary utricles from different parts of the thallus (margin and center). These molecular and morphological congruencies indicate that these morphological features reflect the phylogeny of *Codium*, and a combination of such features can be used for identification of these species.

Codium capitulatum was originally described by Silva and Womersley (1956) from Southern Australia. This species is characterized by capitate utricles. *Codium lucasii* and *C. arabicum* also possess capitate utricles, however, the utricles of *C. lucasii* are shorter than those of *C. capitulatum*, and those of *C. arabicum* are wider than those of *C. capitulatum* at the margin (Womersley 1984). Utricles of *C. setchellii* Gardner are similar to those of *C. capitulatum* in size, however, they lack hairs (or hair-scars) and are not capitate (Silva 1951) (Table 3). Distribution of *C. capitulatum* is restricted to Australia and the Pacific coast of southern Japan (Womersley 1984, this study).

Codium hubbsii was described from Mexico by Dawson (1950), and subsequently reported from Japan (Yoshizaki 1974). This species is characterized by large cylindrical utricles with alveolate ornamentation. Utricles of *C. adhaerens* are similar to those of this species (Burrows 1991), however, those of the former species lack alveolate ornamentation (Table 3). Size and other features of utricles of Japanese *C. hubbsii* agree with those of previous reports (Dawson 1950, Silva 1951, Abbott and Hollenberg 1976). This species is distributed in Baja California and all around the coasts of Japan (Silva 1962, this study). This is the most common prostrate *Codium* species in Japan.

Codium lucasii was originally described from Australia by Setchell (Lucas 1935) and subsequently reported from the Hachijo Island, southern Japan (Yamada and Segawa 1953). Utricles of this species are the shortest among the five prostrate species in Japan (Table 3). *Codium capitulatum* possesses



Figs. 18–21. Utricles of the type specimen of *Codium arabicum*. Scar bars = 100 μm. Fig. 18. Utricles at the margin of thallus, showing big, stout, clavate to pyriform utricle (arrow). Fig. 19. Apex of utricle, showing thin utricular wall. Fig. 20. Utricles at the margin of thallus, showing big, stout, clavate to pyriform utricles. Fig. 21. A few secondary utricles (arrows) develop from the lower portion of larger utricles.

similar utricles to those of this species such as the capitate apex, however, *C. lucasii* can be discriminated from *C. capitulatum* by the presence (*C. lucasii*) or absence (*C. capitulatum*) of alveolate ornamentation (Table 3). This species is distributed in South Africa, Mozambique, Tanzania, Kenya Australia and southern Japan (Van den heede and Coppejans 1996; this study).

Codium arabicum was originally described from Egypt by Kützing (1856), and subsequently reported from Japan (Okamura 1936). This species is characterized by relatively stout, clavate to pyriform utricles at the margins. There is no species possessing such wide utricles among the seven prostrate species (Table 3). Alveolate ornamentation is rarer than in *C. hubbsii* and *C. lucasii*, as described by Van den Heede and Coppejans (1996). This species is distributed in Egypt, Tanzania, Kenya, Mauritius, Seychelles, Sri Lanka, Indonesia, Thailand, Malaysia, Philippines, Taiwan, China, Marshall Island, Hawaii, Australia and southern Japan (Silva 1962, Van den heede and Coppejans 1996, this study). The

Table 3. Comparison of prostrate species of *Codium* in utricles

	C. adhearens[1]	*C. setchellii*[2]	*C. capitulatum*[3]	*C. dimorphum*[3]	*C. hubbsii*[4]	*C. lucasii*[3]	*C. arabicum*[5]
Length of uticles (μm)	500–800	650–1000(–1200)	p: 600–1300 s: 600–1000	550–1000	800–1300	435–800(–1150)	p: (700–)990–1115(–1290) s: (230–)400–530(–780)
Diameter of utricles (μm)	40–100	(45–)65–90(–125)	p: 70–140 s: 40–85	(45–)55–80(–90)	70–80(–250)	(45–)50–100(–130)	p: (90–)185–230(–385) s: (55–)110–140(–175)
Shape of utricles	cylindrical with flatted or rounded apex	cylindrical with slight constriction, truncate or slightly rounded apex	cylindrical, constricted below apex, rounded apex, giving a capitate appearance	cylindrical, rounded to subtruncate apex with marked apical thickening	cylindrical with depressed-globose to inflated apex	cylindrical, markedly constricted below apex, with truncate, depressed or rounded apex	p: subcylindrical to clavate with rounded apex s: cylindrical to catitate, with truncate or rounded apex
Hairs (or hairs scars)	present	almost devoid	present	absent	present	present	present
Alveolate ornamentation	absent	absent	absent	absent	present	present	rarely present

p: primary utricles, s: secondary utricles.

[1] Data are sited from Burrows (1991) except the abcence of the alveolate ornamentation.

[2] Data are sited from Silva (1951).

[3] Data are sited from Silva and Womersley (1956).

[4] Data are sited from Dawson (1950).

[5] Data are sited from V.d.Heede and Coppejans (1996).

ratio of length to diameter of utricles was 2.7 in our materials and 2.2 in the type specimen (Table 2). These values are lower than other species of prostrate *Codium* (Table 2). This is the most distinctive morphological character.

Codium dimorphum was named by Svedelius (1900) because of the existence of two distinct types of utricles, those at the margin being more swollen and with a thickened membrane at the tip, while those in the center of the thallus are more cylindrical with a thin membrane at the tip. However, Setchell (1937), in examining the type material of this species, found that the strict distribution of utricle type on the thallus indicated by Svedelius could not be confirmed. In our materials, swollen utricles with a thickened membrane at the tip were also found in both marginal and central positions. Utricles of this species are similar to those of *C. adhaerens* C. Agardh, which also has cylindrical and flattened to rounded apices (Burrows 1991). However, *C. adhaerens* possesses shorter utricles than those of *C. dimorphum* (Table 3). Although the diameter of utricles of Japanese samples of *C. dimorphum* (70–280 μm) are wider than Australian ones (47–125 μm) (Silva and Womersley 1956) (Table 3), we identified these samples as *C. dimorphum* because of their large-sized utricles, lack of alveolate ornamentation and thickened membrane at the tip of the utricles (Fig. 8). To confirm the identification, molecular analysis, including samples from the type locality of this species is required. Distribution of this species is Chile, Australia, New Zealand and the Pacific coast of central Japan and the Japan Sea (Womersley 1984, this study).

Key to the species of Japanese prostrate *Codium*

1. Utricles devoid of alveolate (cribrose) ornamentation
 2. Primary utricles with hairs (or hair-scars), 600–1400 μm in length in the center of the thallus, apex capitate ....*C. capitulatum*
 2. Primary utricles devoid of hairs (or hair-scars), 800–1700 μm in length in the center of the thallus, apex thickened .... *C. dimorphum*
1. Utricles with alveolate (cribrose) ornamentation
 2. Primary utricles at the margin of the thallus stout, clavate to pyriform, 420–990 μm in length and 80–300 μm in diameter ..... *C. arabicum*
 2. Primary utricles at the margin of the thallus cylindrical
  3. Primary utricles at the margin of the thallus 670–1600 μm in length and 60–240 μm in diameter, apex depressed to subtruncate ..... *C. hubbsii*
  3. Primary utricles at the margin of the thallus 420–810 μm in length and 40–140 μm in diameter, apex rounded, constricted below ..... *C. lucasii*

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嶌田 智[a]，江端弘樹[b]，堀口健雄[c]，栗原 暁[d]，田中次郎[e]：**日本に生育する匍匐性ミル属の分子系統および形態学的解析**

日本産匍匐性ミルの種構成を明らかにすることを目的として，小嚢の形態を光学顕微鏡で詳しく観察した．その結果，長さ，直径，頂端部位の形状，毛（毛跡）や蜂の巣状小孔の有無などの小嚢形態は，予備的に行った分子系統学的解析で認められた5つのクレード毎に特徴的であることが明らかになった．分子と形態のデータが一致したことから，小嚢の形態的特徴は，ミル属の系統を反映した形質であると判断された．この結果に基づいて，日本産匍匐性ミル属は次の5種に分類された；ナンバンハイミル *C. arabicum* Kützing，クビレハイミル *C. capitulatum* Silva & Womersley，オオハイミル *C. dimorphum* Svedelius，ハイミルモドキ *C. hubbsii* Dawson，ハイミル *C. lucasii* Setchell.

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