

A taxonomic and karyological study of the *Codium geppiorum* complex (Chlorophyta) in southern Taiwan, including the description of *Codium nanwanense* sp. nov.

Jui-Sheng Chang¹, Chang-Feng Dai^{1,*}, and Jeng Chang²

¹Institute of Oceanography, National Taiwan University, Taipei 106, Taiwan

²Institute of Marine Biology, National Taiwan Ocean University, Keelung 202, Taiwan

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Abstract. Morphological variations of the *Codium geppiorum* complex from southern Taiwan were examined, and their karyotypes were studied by microspectrophotometry. Morphological analysis distinguished three prostrate *Codium* species, including *C. edule* P.C. Silva, *C. geppiorum* O.C. Schmidt, and a new taxon, *C. nanwanense*. Although these species show an anatomical continuum as the length of their utricles increases with the thickness of the branches, there are some apparent differences of thallus type and utricle form. The utricles of *C. edule* are about 1000 μm or more in length, and with abundant hair scars on the utricle tip. *Codium geppiorum* has a typical pyriform utricle, which is often less than 500 μm in length. The utricles of *C. nanwanense* are cylindrical and medium-sized, between *C. edule* and *C. geppiorum*. Karyological study on the three *Codium* species also showed an obvious divergence in nuclear size and relative fluorescent unit (RFU). *Codium edule* has the largest nucleus (8.90 μm in average length) and expectedly the highest RFU among the three species. The ratio of nuclear long axis vs. width is about 3.01, 1.46 and 1.00 for *C. edule*, *C. geppiorum*, and *C. nanwanense*. This paper summarizes the taxonomic characters of these species and provides microfluorescence data useful in distinguishing members of the *Codium geppiorum* complex.

Keywords: *Codium geppiorum* complex; *Codium nanwanense* sp. nov.; Karyology; Microfluorescence; Morphology.

Introduction

The coenocytic, siphonous green alga *Codium* Stackhouse (Bryopsidales, Codiaceae) is one of the most common and widespread seaweeds in the world (Silva, 1951, 1952). There are about 100 described species, which frequently grow on rocky shores in tropical and temperate areas (Silva, 1962, 1992). *Codium* is characterized by its spongy thallus, composed of a colorless medulla of densely intertwined filaments and a green palisade-like cortex composed of vesicles called utricles. Thallus type varies from flattened crusts to globular and decumbent or erect systems, consisting of cylindrical branches or unbranched laminate blades (Bold and Wynn, 1985). The coenocytic utricles have discoid chloroplasts devoid of pyrenoids. Dark-green female and yellowish-green male gametangia are commonly borne on the distal half of the utricles. *Codium* exhibits marked anisogamy and possesses biflagellate gametes (Silva, 1952, 1982; Van den Hoek et al., 1995; Lee, 1999).

The taxonomy of *Codium* is complicated by the high degree of anatomical and habit variability within and be-

tween populations (Silva, 1951). Most of the *Codium* species are distinguished on the basis of gross morphological features combined with anatomical details, such as size and form of utricles and reproductive organs, structure of the apex of the utricles, position of utricle hairs and septa, and ontogenetic patterns (Silva, 1982). Morphological variations of thallus type and utricle form present taxonomic difficulties for some closely related *Codium* species because an anatomical continuum appears as the length of their utricles increases with thickness of the branches. *Codium fragile* (Suringar) Hariot with several subspecies is one such group within this diverse genus and is an interesting taxonomic case study because of its widespread distribution and invasive characteristics (Trowbridge, 1998). Other groups, such as the *C. arabicum* complex and the *C. geppiorum* complex, are still poorly delineated and often confused with each other due to the utricle size continuum in similar morphological characters. Although some molecular studies have been performed, they remain focused on the species *C. fragile* and its subspecies (Manhart et al., 1989; Goff et al., 1992).

Codium geppiorum O.C. Schmidt is a substitute name for *C. divaricatum* A. Gepp & E.S. Gepp, which is a later homonym of *C. divaricatum* (C. Agardh) Biasoletto (Schmidt, 1923). *Codium geppiorum* is a complex of repent, procumbent, or decumbent anastomosing types

*Corresponding author. Tel: 02-23916693; Fax: 02-23916693; E-mail: corallab@ccms.ntu.edu.tw

found throughout the Indo-Pacific region and is the ecological and taxonomic counterpart of *C. repens* P.L. Crouan & H.M. Crouan *ex* Vickers in the Atlantic (Silva, 1960; Jones and Kraft, 1984, Silva et al., 1987, 1996). Both species encompass a wide range of morphological variations. Silva (1960) treated the bewildering variability within *Codium geppiorum* as elements of a species complex rather than representatives of discrete species and considered the repent *Codium* species as a whole to represent "several species or complexes of microspecies" (Silva, 1962). All members of the so-called *C. geppiorum* complex are procumbent with regularly or irregularly dichotomous branches, including *C. geppiorum* O.C. Schmidt (Gepp and Gepp, 1911; Durairatnam, 1961; Egerod, 1975; Tseng, 1983), *C. edule* Silva (Silva, 1952; Silva et al., 1987), *C. bulbopilum* Setchell (Jones and Kraft, 1984; South, 1993), and *C. taitense* Setchell (Setchell, 1926). Any confusion they cause is a result of the variations of thallus type and utricle form in the anatomical continuum created as the length of their utricles increases with the thickness of the branches (Setchell, 1926; Silva, 1960; Jones and Kraft, 1984; Silva et al., 1987, 1996).

Codium species are mainly classified on their morphological characters as there are seldom other reliable methods to identify them. The recent application of epifluorescence microscopy and DNA-fluorochrome studies reveals that macroalgae are exceedingly diverse with respect to their nuclear features (Goff and Coleman, 1990; Kapraun, 1993). The number, size, and position of nuclei in cells, as well as their DNA content, vary considerably in different taxa (Wik-Sjostedt, 1970; Kapraun et al., 1988; Goff and Coleman, 1990). Karyological studies of marine green algae have continued to find use in providing additional criteria for distinguishing morphologically similar species (Wik-Sjostedt, 1970). Although cytophotometric estimation of nuclear content has been used to study some *Codium* species found in the Caribbean region (Kapraun and Martin, 1987; Kapraun et al., 1988; Kapraun, 1994), it has not been used to resolve the taxonomic problems within *Codium* that occur in the Indo-Pacific region.

The mass-production of *Codium* thallus occurs in Nanwan Bay, southern Taiwan every spring, and most species are prostrate and dichotomous (Dai, 1996, 1997). Phycologists investigating the marine flora of southern Taiwan have recorded thus far only *Codium intricatum*, which belongs to the prostrate *Codium* (Chiang and Wang, 1987; reviewed in Lewis and Norris, 1987). Some interesting variations in other prostrate, dichotomous *Codium* were observed from Nanwan Bay that these species are easily and conveniently relegated to the *C. geppiorum* complex, not to the *C. intricatum*. The aim of the present study is to identify morphological differences among these *Codium* species. Differences in morphological description between these prostrate *Codium* species and *C. intricatum* were compared. Microfluorescence is also used with the goal of discovering reliable cytological characters for distinguishing these morphologically similar species.

Materials and Methods

Prostrate *Codium* plants were collected by scuba and skin diving from fringing reefs along the coast of Nanwan Bay (21° 57' N, 120° 46' E) in southern Taiwan from May to August 2000. Samples were preserved in 10% formalin-seawater solution immediately after collection, then transferred to 5% formalin-seawater solution or processed as dry herbarium specimens. Liquid-preserved and dried herbarium specimens were used in this study. Several liquid-preserved samples collected prior to 1996 were also used to compare morphological characters.

Utricles were excised from the plant body using needles and fine forceps while being observed under the dissecting microscope. Tissues were placed in a bottle filled with water and agitated vigorously to inflate and separate the utricles. The separated utricles were mounted in glycerin water solution (1:1) for microscopic observations. Anatomical details were photographed using a Nikon digital camera (Coolpix 990) and/or traced using a Nikon microscope fitted with a camera lucida. All voucher specimens examined are deposited in the Institute of Oceanography, National Taiwan University, Taipei and the Herbarium of the National Museum of Natural Science, Taichung, Taiwan (TNM).

For fluorescence microscopy studies, freshly collected thalli were fixed immediately in 0.5% glutaraldehyde seawater solution for 24h and then transferred into 100% methanol for preservation at 4°C. The methanol was allowed to evaporate, and utricles were separated from the preserved thalli before staining. The separated utricles were stained with 0.5 $\mu\text{g}\cdot\text{mL}^{-1}$ DAPI (4', 6-diamidino-2-phenylindole) in McIlvaine buffer (pH 4.4) mixed solution for 30 min, and examined with a Nikon fluorescence microscope (Chang and Carpenter, 1988). Photographs were taken using a Nikon digital camera (Coolpix 990) with a shutter setting at 0.5 sec and 2.7 in halo to avoid possible interference by a different exposure time on the RFU. The digitized photographs were then analyzed with image analysis software (Image-Pro Plus, Media Cybernetics). The images of DAPI-stained nuclei were separated from the background with a threshold light intensity. Next, morphological characteristics, including area, long axis, short axis, and integrated light intensity, were measured for each nucleus.

Results

Morphological Studies

Based on the morphological data, four species can be distinguished among the prostrate *Codium* specimens from Nanwan Bay, southern Taiwan. Among them, three species, including *Codium edule*, *C. geppiorum*, and an undescribed taxon, *Codium nanwanense* sp. nov. are known members of the *Codium geppiorum* complex based on a cylindrical thallus and utricle shape (Figure 1). The fourth species is *C. intricatum*, distinguished from the other three species by its tightly combined and flattened

thallus (Figure 1F). A key to all known species of *C. geppiorum* complex and *C. intricatum* was given.

Key to species of Codium geppiorum complex and C. intricatum

- 1. Branch compressed and connected tightly *C. intricatum*
- 1. Branch terete and connected loosely 2

- 2. Utricles are ovate or pyriform 3
- 2. Utricles are clavate, cylindrical or subcylindrical 5
- 3. Tip of utricles without thickening 4
- 3. Tip of utricles thickening *C. taitense*
- 4. Thallus not forming prominent hummocks, utricule size shorter than 500 µm in length *C. geppiorum*

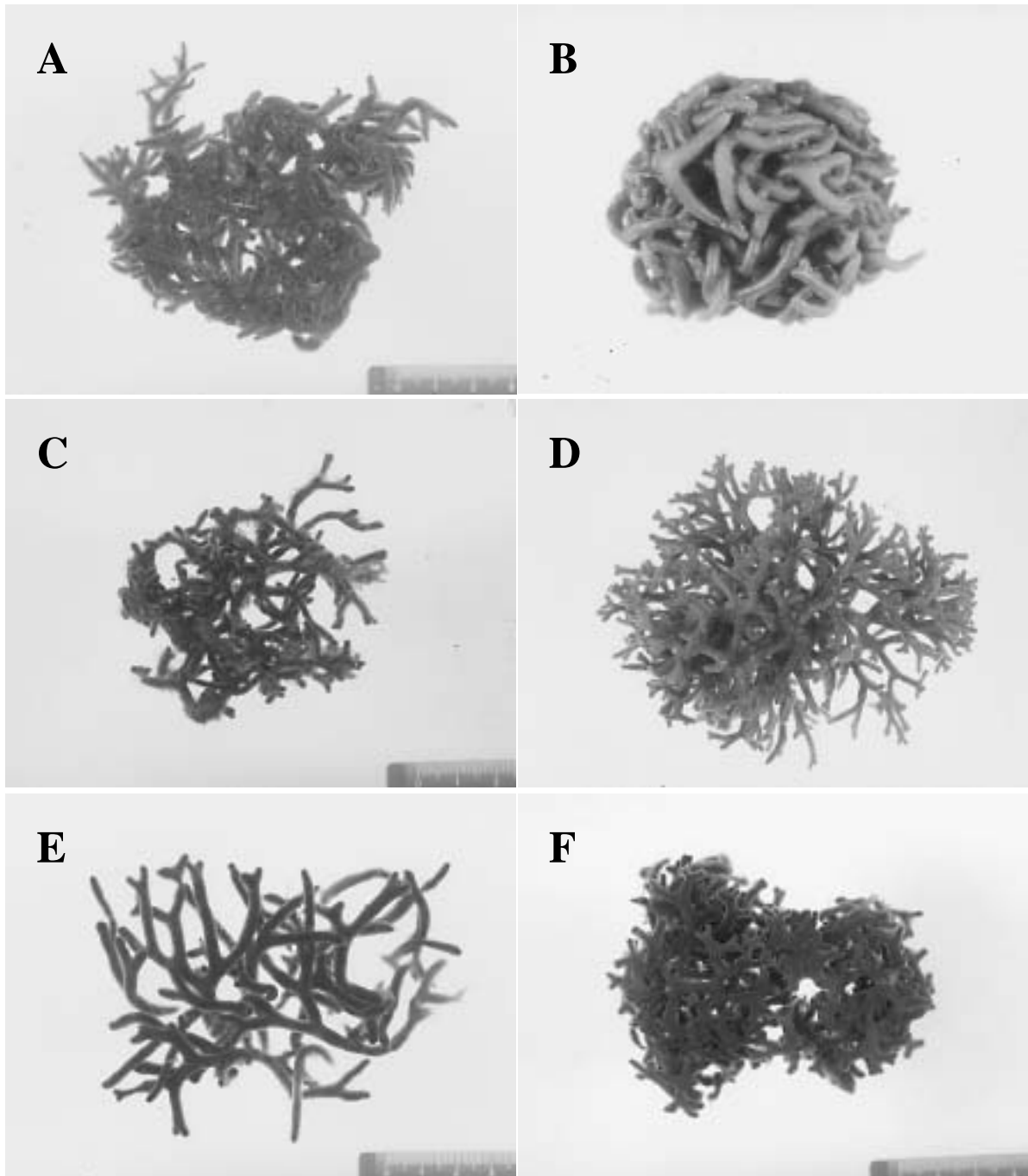


Figure 1. A-B, The habit of *Codium edule*. A, Chang 89072602, Houbihu; B, Chang 83041405. Scale bar = 1 cm; C-D, The habit of *Codium geppiorum*; C, Chang 89072604; D, Chang 83102208. Scale bar = 1 cm; E, The habit of *Codium nanwanense*, Chang 89072606; F, The habit of *Codium intricatum*, Chang 89072601.

4. Thallus often forms imbricating hummocks, utricle length exceeding 500 μm *C. bulbopilum*
5. Thallus light green with soft texture, tips of branches tapering, utricle length often exceeding 1000 μm *C. edule*
5. Thallus dark green with rigid texture, tips of branches rounded, utricle size seldom reaching 1000 μm *C. nanwanense*

Codium edule P.C. Silva 1952: 392—Silva 1952: 392-395, fig. 18, pl. 35b (type locality: Waikiki, Oahu, Hawaiian Islands)—Silva et al. 1987: 112—Trono & Ganzon-Fortes 1988: 47-48, figs. 28A, 28B—Silva 1996: 854—Trono 1997: 48-49, fig. 30. Figures 1A, B, 2

The species forms spongy masses of intertwined light green to grass green branches, with the alga often growing to more than 1 meter in diameter. The dichotomous to trichotomous branches are terete and tapering to the tip, 3-5 mm in diameter, and attached to each other or to the substrate by small, cushion-like rhizoidal structures. The

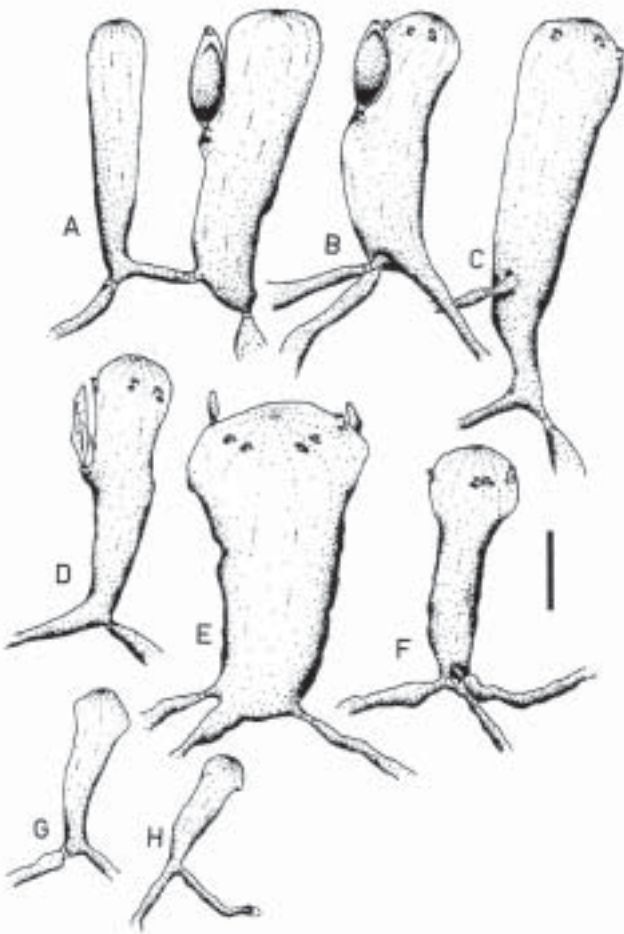


Figure 2. *Codium edule*. Utricle morphology from various collections. A-D, Chang 89072602, Houbihu; E-H, Chang 83041405, Nanwan Bay; G-H, the utricle from the tip of branch. Scale bar = 300 μm

branches under the imbricating hummocky thallus are always arched. Filaments of the medulla are 30-80 μm broad, separated from the utricles by deep constrictions. The utricles at mid branches are clavate or subcylindrical, slightly tapering towards the base and with rounded tips. Some utricles have slight swellings just below the apex. The utricles are 100-360 μm (some up to 600 μm) in diameter, 830-1200 μm long. The utricles at branch tips are obconical or obpyriform, 70-145 μm in diameter, 275-450 μm in length. Gametangia ellipsoidal to elongate-ovate, 65-85 μm diameter, 270-330 μm long, 1 or 2 per utricle, issued 275-360 μm below apex, not extending to apex of utricle. Hairs or hair scars usually present, 2 to 6 per utricle and located 55-75 μm below apex.

Geographic distribution. Hawaii, Philippines, Taiwan and Maldives.

Specimens examined. Moupito: 29 Jun 1991, Chang 80062907; 29 Jun 1991, Wang 80062908—Nanwan Bay: 14 Apr 1994, Chang 83041405; 22 Oct 1994, 83102207—Houbihu: 26 Jul 2000, Chang 89072602; 26 Jul 2000, 89072603; 12 Aug 2000, 89081201.

Remarks. *Codium edule* was previously identified as *Codium intricatum* in southern Taiwan due to their close morphological similarities, and for lack of detailed anatomical studies. However, differences between the two species are clear. The branches of *Codium edule* are terete and tapering to the tip, while *C. intricatum* has compressed to cylindrical branches which are truncate or slightly rounded at the tip. In addition, the branches of *C. intricatum* tend to anastomose more tightly than in *C. edule*, with the former showing shorter and closer intersecting points. Chiang (1973) rightly proposed that the great mass of prostrate *Codium* thalli (regarded by previous workers as *C. intricatum*) found in April each year in Nanwan Bay should be regarded as *C. edule*. The species occurs on rock, coral rubbles, or sand in Nanwan Bay, from the littoral zone to the edge of the fringing reef about 15 m deep, and sometimes the thallus can grow like a ball if detached from the substrate (Figure 1B). *Codium edule* is an abundant species, spreading over the coral reef in Nanwan Bay from March to June every year. The species has a specialized reproductive structure which may sprout out the siphons directly while attached to utricles with a pedicel (Figure 3). This structure then separates from the parent utricle and may act as a "propagule" to perform reproduction. The mode of reproduction of *C. edule* is possibly the reason for the species blooming in Nanwan Bay. *Codium edule* is perhaps most closely related to *C. bulbopilum* Setchell (type specimen from Samoa), the utricles of which, however, are obovate or pyriform (Jones and Kraft, 1984; South, 1993).

Codium geppiorum O.C. Schmidt 1923: 50—Schmidt 1923: 50, fig. 33 (as *geppii*, syntype localities: Kai Island and Celebes (now Sulawesi), Indonesia).—Børgesen 1946: 49-52, figs. 19-22—Børgesen 1948: 38-39, fig. 18—Durairatnam 1961: 23, pl. IV: fig. 4, pl. XX: fig. 2—

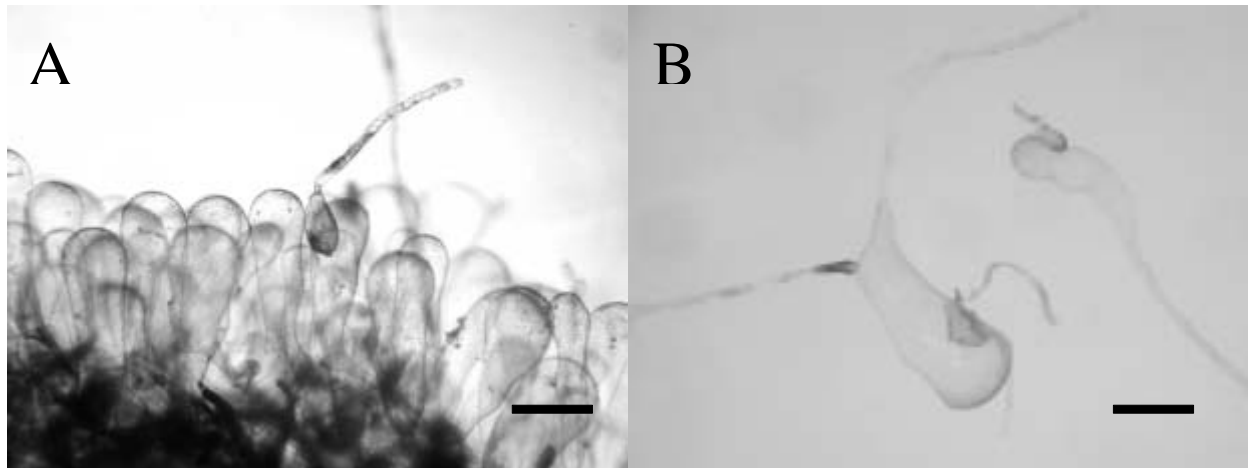


Figure 3. Sprouting filaments on the specialized reproductive organs of *C. edule* (arrow). Scale bar = 500 μ m

Egerod 1975: 59-60, figs. 25, 26—Tseng 1983: 300, pl. 149: fig. 1—Silva et al. 1987: 112—Wang & Chiang 1993: 87—Silva et al. 1996: 856—Van den heede & Coppejans 1996: 400, fig. 11, 16. Figures 1C, D, 4

Codium divaricatum A. Gepp & E.S. Gepp 1911: 136, 145, pl. XXII: figs. 195-199.

Thallus repent, branching dense, regular or irregularly dichotomous; branches terete, about 0.8-2.5 mm in diameter, attached to the substrate by means of irregularly disposed rhizoids, light to olive green. Thallus composed of individual utricles that are clavate, ovate or elongate obpyriform. Mature utricles are 83-310 μ m in diameter and 330-570 μ m long, apices rounded or truncate, utricular wall 2 μ m thick, and slightly thinner at the apices. Hairs or hair scars in small number: 0-3 per utricle, borne 70-80 μ m below the apex. Gametangia fusiform to elliptical, 50-75 μ m

in diameter, 165-190 μ m long, generally borne singly on a pedicel produced on a protuberance around 255-375 μ m below the apex. Medullary filaments mostly 20-35 μ m in diameter.

Geographic distribution. Common in the tropical Indian and Pacific Oceans.

Specimens examined. Nanwan Bay: 14 Apr 1994, Chang 83041420; 23 Oct 1994, 83102208—Houbihu: 26 Jul 2000, Chang 89072604; 26 Jul 2000, 89072605.

Remarks. *Codium geppiorum* is a complex of repent, procumbent or decumbent, anastomosing forms found throughout the Indo-Pacific region. It is the counterpart of *C. repens* P.L. Crouan & H.M. Crouan ex Vickers in the Atlantic (Silva, 1960). Both *C. geppiorum* and *C. repens* form a continuous anatomical spectrum as the length of

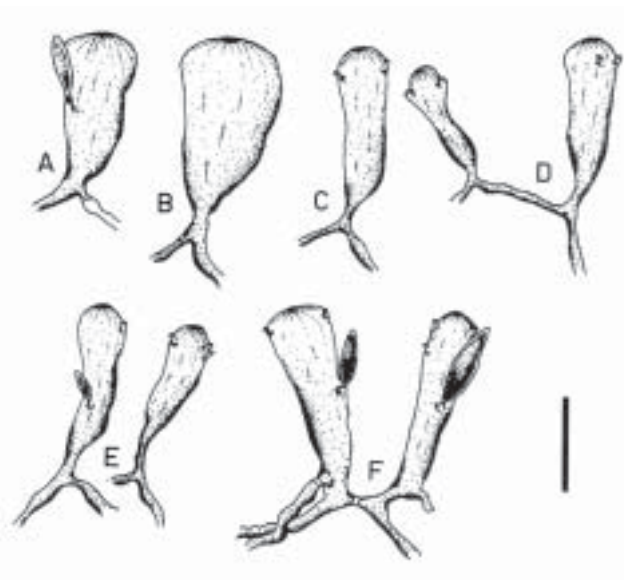


Figure 4. *Codium geppiorum*. Utricle morphology from various collections. A-E, Chang 89072604, Houbihu; F, Chang 83102208, Nanwan Bay. Scale bar = 200 μ m.

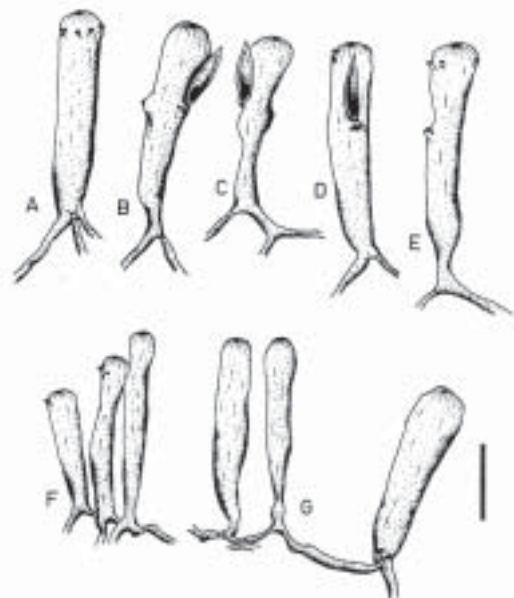


Figure 5. *Codium nanwanense* Utricle morphology from various collections. A-D, Chang 89072606, Houbihu; E-G, Chang 89072607, Houbihu. Scale bar = 300 μ m.

their utricles increases with the thickness of their branches (Silva, 1960). *Codium repens* has been recorded in Taiwan by some phycologists (Yamada, 1925; Okamura, 1931, 1936; Shen and Fan, 1950; Tseng, 1983), but the name should be regarded as misapplied. *Codium geppiorum* is not common in Nanwan Bay, but it is easy to distinguish in the field by its more slender branches. The densely disposed branches, unlike those found in *C. edule* or *C. intricatum*, extend to all directions and seldom anastomose with one another. The obovate or pyriform utricles are also distinctive and are the smallest among those in the *C. geppiorum* complex.

Codium nanwanense J.S. Chang sp. nov. Figures 1E, 5

Thallus repens, rami teretes et sparsi, 2.7-4.6 mm diametro, dichotomi sive subdichotomi, apicem rotundus, thallus substrato adhaerens, per fila rhizoidea irregulariter efferens, rami vivens tumidi, color viridis obscurus. Utriculi cylindrici et gracilis, 600-960 µm longitudine, 90-160 µm diametro, apices teretes sive truncati, ad apicem utriculi perspicue tenuis. Fila medullosa 20-42 µm diametro, saepe duo fila basi utriculorum orientia. Cicartrices semper autem prope apices utriculorum. Gametangia ellipsoidea, 230-310 µm longitudine, 50-85 µm diametro, 1-aliquot per utriculum, omnia in pediculis conspicuis, supra mediam utriculorum positis.

Thallus repent, branches terete and sparse, 2.7-4.6 mm in diameter, dichotomous or subdichotomous, with rounded branch tips, attached at irregular intervals to the substratum and anastomosing by rhizoidal filaments, branches turgid when living, deep dark green in color. Utricles slender, cylindrical, 600-960 µm in length, 90-160 µm in diameter, apices rounded or truncate, thin at the apices. Medullary filaments 20-42 µm in diameter, often arising in pairs from the base of each utricle, with a plug formed close to point of departure. Hair scars occasionally issued from portions near apex of utricles. Gametangia ellipsoidal, 230-310 µm long, 50-85 µm wide, borne on a short pedicel in the middle portion of utricles, one per utricle.

Holotype. Chang 89072606, reef in front of Houbihu, Hengchun, Pingtung County, Taiwan, 22 Jul 2000 (A4393 in TNM); *Isotypes:* Chang 89072607: (A4394 in TNM), 89072608: (A4395 in TNM).

Geographic distribution. Known only from Nanwan Bay, southern Taiwan.

Remarks. *Codium nanwanense* is morphologically similar to *C. edule*. However, the new species is easily distinguished by its dark green color and sparse branches of constant diameter. Interwoven branches seldom connect with each other, and the branch end is rounded, not tapering as in *C. edule*. The cylindrical utricles are also seldom variable within the uniform branches. This new species is also closely related to *C. bulbopilum*, which was described from the south Pacific (Jones and Kraft, 1984; South, 1993). Unlike *C. bulbopilum*, the branches of *C.*

Table 1. Morphological characters of five known species of *Codium geppiorum* complex and *C. intricatum*.

Species	Thallus		Utricle		Gametangia			Siphon diam.		Hair or hair scar	
	Form	Branch diam. (mm)	Form	Length (µm)	Width (µm)	Form	Length (µm)	Width (µm)	Site to utricle tip (µm)	(µm)	hair scar
<i>C. edule</i>	Terete, di-to trichotomous	3.0-5.0	Subcylindrical, clavate	830-1300	300-600	Elongate-ovate	270-330	120-150	380-450	30-80	3-6
<i>C. geppiorum</i>	Terete, irregular dichotomous	0.8-2.5	Obvoid, pyriform, clavate	330-570	85-310	Fusiform	165-190	50-75	220-250	20-35	1-3
<i>C. nanwanense</i>	Terete, dichotomous	2.7-4.6	Cylindrical	600-960	90-160	Ellipsoidal	230-310	50-85	320-400	30-40	0-2
<i>C. bulbopilum</i> ¹	Terete, dichotomous	2.0-4.0	Obvoid, pyriform	240-950	50-310	Pyriform, ellipsoidal	220-320	60-100	-	20-42	-
<i>C. taitense</i> ²	Terete, irregular dichotomous	1.0-3.0	Obvoid, pyriform, with thickening tip	400-630	105-365	Pyriform, ellipsoidal	180-250	65-105	-	-	2-6
<i>C. intricatum</i> ³	Compressed, irregular dichotomous	1.5-8.0	Clavate	520-750	150-250	Ovate	275-300	70-80	280-300	30-35	1-2

¹Morphological characters were based on Jones and Kraft (1984) and South (1993).

²Morphological characters were based on Setchell (1926) and Chang (1995).

³Morphological characters were based on Okamura (1913) and Chang (1995).

nanwanense do not form prominent hummocks and always extend on the substrate, and the utricles are never pyriform. The utricles of *C. nanwanense* also resemble those of *C. intricatum* in their shape, but the former are longer and more slender. On the other hand, the thallus of *C. intricatum* is often flattened, and the interutricular filaments are markedly shortened. The dark green color of this species is mainly caused by red filament epiphyte, which fills the space between the utricles and is seldom found in *C. edule* and *C. geppiorum*. A comparison of major morphological characters of the three prostrate *Codium* species is shown in Table 1. The morphological characters of *C. intricatum* and others in the *C. geppiorum* complex were also compared using past studies (Jones and Kraft, 1984; South, 1993; Chang, 1995).

Microfluorescence Study

Abundant nuclei were observed in cytoplasm from the utricles and siphons of the three prostrate *Codium* species prepared for DAPI staining. That the nuclear size and relative fluorescence unit (RFU) differed in the three species studied was obvious, with most nuclei being spherical or ovoid, except in *C. edule* (Figure 6). The average nuclear area, long axis, short axis, and RFU of nuclei in

the three species are shown in Table 2. *Codium edule* has the largest nuclear area and the highest RFU among the three prostrate species examined. The nuclear shape in *C. edule* is elliptic to elongate, and the long axis may reach 12.75 μm with an average of $8.98 \pm 2.07 \mu\text{m}$. The long axes to width ratios of the three species are about 3.01, 1.46, and 1.00 for *C. edule*, *C. geppiorum* and *C. nanwanense*, respectively. The intensity of the RFU seen in the three species also shows the same decreasing pattern corresponding to the ratios and species ranking listed above. The analysis of variance also showed a significant difference among the three prostrate *Codium* species, based on area, long axis, short axis, and RFU (Table 2). The nuclei of *C. intricatum* was also compared with the *C. geppiorum* complex. The results showed that microspectrophotometry is a convenient method of distinguishing closely similar *Codium* species, especially within the *C. geppiorum* complex.

Discussion

Among the three species included in this study, both *C. edule* and *C. geppiorum* are new locality records, while *C. nanwanense* is proposed as a taxon new to science.

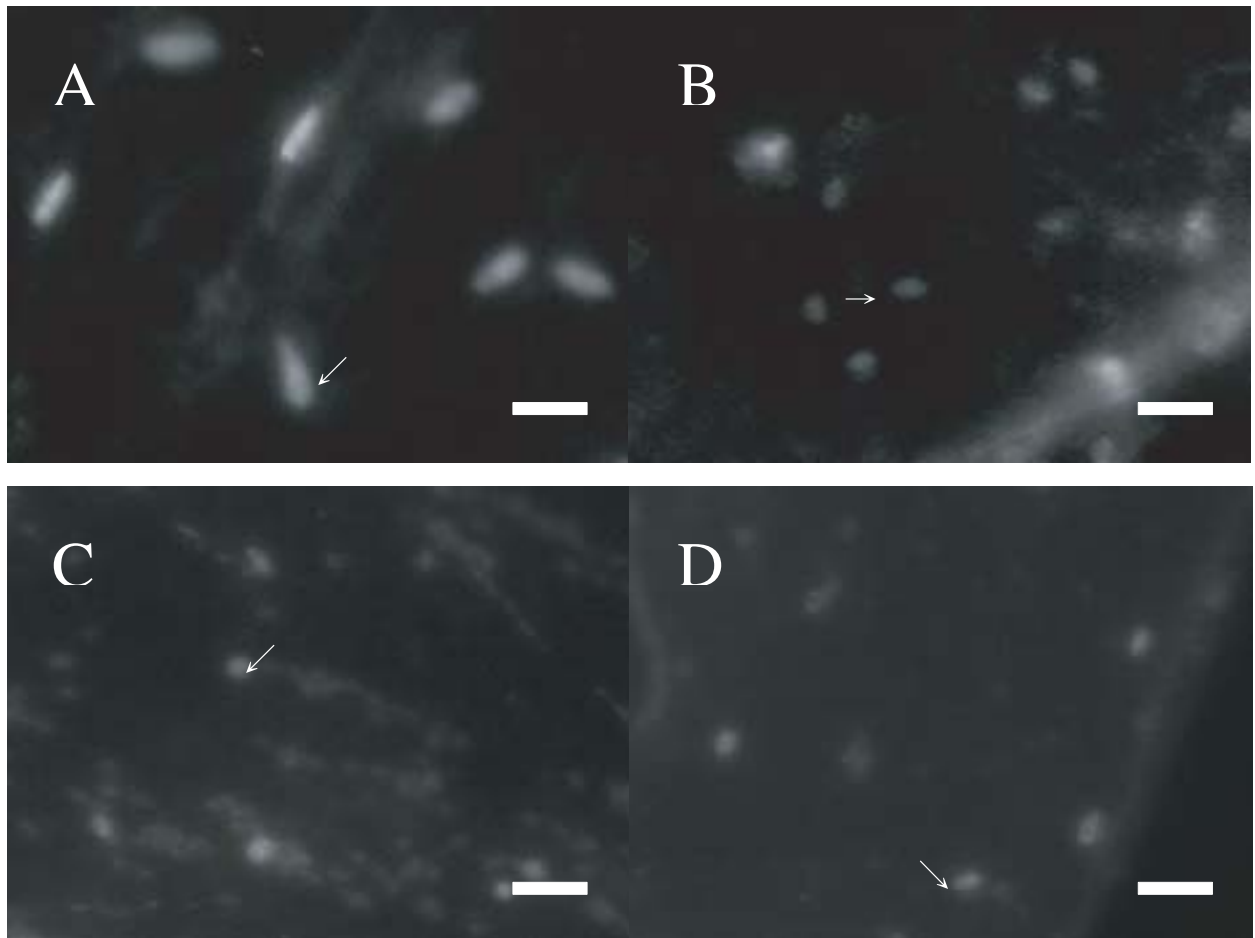


Figure 6. The nuclei (arrows) of four prostrate *Codium* species, A, *C. edule*; B, *C. geppiorum*; C, *C. nanwanense*; D, *C. intricatum*. Scale bar = 10 μm .

Table 2. Mean \pm standard error for area, long axis, short axis, and relative fluorescence unit (RFU) of nuclei for four *Codium* species estimated by cytofluorometry. Values in the same column with different superscripts (^{a, b, c, d}) are significantly different ($P < 0.05$, Duncan's multiple range test).

Species	Specimens	Nuclei	Area (μm^2)	Long axis (μm)	Short axis (μm)	RFU
<i>C. edule</i>	4	116	30.04 ± 9.98^a	8.98 ± 2.07^a	4.30 ± 0.88^a	32622 ^a
<i>C. geppiorum</i>	3	58	9.97 ± 4.13^b	4.36 ± 1.02^b	2.96 ± 0.68^b	8234 ^b
<i>C. nanwanense</i>	3	163	4.65 ± 1.78^c	2.98 ± 0.63^c	2.09 ± 0.45^c	3980 ^c
<i>C. intricatum</i>	3	51	6.41 ± 2.65^c	3.38 ± 0.79^c	2.41 ± 0.53^d	5025 ^c

This study showed that the prostrate *Codium* specimens exhibit distinguishable morphological variations and are highly diverse in Nanwan Bay. Although these species show an anatomical continuum as the length of their utricles increases with the thickness of the branches, and their branch width and utricle size may show some overlaps, there are some apparent differences of thallus type and utricle form (Table 1). The lengths of utricle from small to large were *C. geppiorum*, *C. nanwanense*, and *C. edule*, respectively. It was also obvious that the utricle length would follow the variant branch width of the three species. Another prostrate species, *Codium intricatum*, is not common in Nanwan Bay, but can easily be distinguished from the other three species by its cylindrically compressed and tightly combined branches. It forms a group together with *C. carolinianum* Searles and *C. prostratum* Levring (Silva et al., 1997), not with *C. geppiorum*.

Previous phytogeographical reports of *Codium* examined in connection with this study showed that the species belonging to the *C. geppiorum* complex seldom show distributional overlaps, except in the Philippines (Silva et al., 1987). All specimens of *Codium geppiorum* in the Indo-Pacific region can be divided into two groups according to their utricle size. One group, with longer utricles ($> 500 \mu\text{m}$), is distributed in the Indian Ocean (Egerod, 1975; Van den Heede and Coppejans, 1996), and another one, with shorter utricles ($< 500 \mu\text{m}$), occurs in the Pacific Ocean (Tseng, 1983). Most of the *C. geppiorum* specimens found from Nanwan Bay agree well with those given by Tseng (1983) although some utricles were longer and fatter. Silva (1960) and Silva et al. (1987) claimed that *C. geppiorum* is a morphological and ecological counterpart in the tropical Pacific Ocean of *C. repens* in the Atlantic. Although *C. repens* has also been recorded in the South China Sea (Tseng, 1983), Taiwan (cited in Lewis and Norris, 1987), and the Philippines (cited in Silva et al., 1987), it should be regarded as mis-identified based on biogeographical affinity (Silva et al., 1987).

Cytofluorometry has been used to determine the site of meiosis and to elucidate the changes in nuclear DNA content associated with reproduction in multinucleate green algae (reviewed in Kapraun, 1993; Kapraun, 1994). In addition, cytofluorometry has been employed to quantify nuclear genomes in members of the genus *Codium* (Kapraun and Martin, 1987; Kapraun et al., 1988; Kapraun, 1994). DAPI is the preferred fluorochrome and has replaced mithramycin and hydroethidine as a useful tool for

most algae in the past decade, including the multinucleate green algae and red algae. It is more stable than mithramycin and has a relatively rapid and simple staining schedule (Goff and Coleman, 1990). Karyological studies have revealed that *Codium* species are exceedingly diverse with respect to nuclear cytology (Kapraun and Martin, 1987; Kapraun et al., 1988; Kapraun, 1994). Our study also showed that nuclear types varied in different species of the *Codium geppiorum* complex. Our results suggest that different cytological characters exist in different *Codium* species and can be used as a diagnostic character. When used in conjunction with more traditional anatomical methods, microspectrophotometry can be a useful, supplemental approach in the taxonomy of marine macroalgae, especially in the *C. geppiorum* complex.

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南灣海域杰氏松藻種群形態與核型之研究，含一新種的描述

張睿昇¹ 戴昌鳳¹ 張正²

¹國立台灣大學海洋研究所

²國立台灣海洋大學海洋生物研究所

本研究針對南灣海域數種外型疑似杰氏松藻種群的松藻，分析其外部形態及內部構造等特徵作為分類的依據，並且以細胞核的螢光染色法，比較其細胞核的大小及所反應的相對螢光值。形態分析的結果顯示，採自南灣海域的松藻標本中，共有三種屬於杰氏松藻種群的種類，包括食用松藻 (*C. edule* Silva)，杰氏松藻 (*C. geppiorum* Schmidt) 及一新種南灣松藻 (*C. nanwanese* sp. nov.)。雖然此三種松藻的囊胞長度可能隨著分枝直徑的增加而呈現出連續性，但從藻體外觀和囊胞型式仍可明顯區分。其中，食用松藻的囊胞為棍棒形，長度通常大於 1000 μm 以上，為三種松藻中最大者；杰氏松藻的囊胞屬亞梨形，長度很少超過 500 μm ；南灣松藻的囊胞則為瘦長圓柱狀，長度介於食用松藻與杰氏松藻之間。細胞核螢光染色的研究結果也顯示，三種松藻細胞核的面積、長徑、短徑及其反應 DNA 含量的相對螢光值 (RFU) 均有顯著差異。其中，食用松藻的細胞核最大 (平均長徑約 8.90 μm)，並有最強的相對螢光值。而三種松藻細胞核的長度比分別為 3.01 (食用松藻) : 1.46 (杰氏松藻) : 1.00 (南灣松藻)。綜合形態與核型的研究結果顯示，分布於南灣海域的杰氏松藻種群，可區分成三個分類單元，而細胞核的螢光染色法能輔助鑑別杰氏松藻種群。

關鍵詞：食用松藻；杰氏松藻；南灣松藻；核型學；螢光染色；形態學。