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Laboratory culture and taxonomy of two species of *Halicystis* (Class Chlorophyceae) in Japan[1),2)]

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Two species of *Halicystis* (Class Chlorophyceae), collected from Aomori-ken, and Ohiragata, Hachijo Island, respectively, have been studied to obtain a better understanding of their systematic significance, using living specimens as well as laboratory cultures. The life histories of both algae, starting with zygotes and zoospores, were completed in the laboratory. The life history patterns were fundamentally identical to those previously demonstrated for the genus, zygotes giving rise on germination to a *Derbesia*-phase, while zoospores grow into a *Halicystis*-phase. The thallus of the *Derbesia*-phase derived from zygotes of *Halicystis* collected at Shiriya-zaki agrees with description of *D. marina*, whereas that derived from *Halicystis* collected from Hachijo Island agrees with *D. tenuissima*. From the results of the present study, it is determined that the *Halicystis* collected from Shiriya-zaki is *H. ovalis* while the *Halicystis* from Hachijo Island is *H. parvula*.

Key Index Words: *Chlorophyceae*; Derbesia marina; Derbesia tenuissima; Halicystis ovalis; Halicystis parvula; *life history*; *taxonomy*.

The genus *Halicystis* was established by ARESCHOUG (1850) on the basis of specimens collected in Faeroes Island, with *H. ovalis* as the type species. It is a siphonous green alga characterized by having a thallus consisting of an erect vesicular part, and a rhizoidal part penetrating into crustose coralline red algae. At present the following five species of *Halicystis* have been described: *H. ovalis* (LYNGBYE) ARESCHOUG, *H. parvula* SCHMITZ, *H. osterhoutii* BLINKS et BLINKS, *H. boergesenii* IYENGAR et RAMANATHAN, and *H. pyriformis* LEVRING. Of these five species, all except *H. pyriformis* have been shown through cultural studies to produce gametes whose zygotes give rise to *Derbesia*-thalli as an asexual phase (KORNMANN 1938, FELDMANN 1950, PAGE 1970, MAYHOUB 1974). Consequently, it is now generally accepted that *Halicystis* is only a sexual phase in the life history of the genus *Derbesia* established by SOLIER in 1847.

Specimens assignable to *Halicystis* have been collected in Japan at several localities: at Hachijo Island by YAMADA (1952); at Susaki, Izu Peninsula, by CHIHARA (1954); at Horiuchi, Iwate-ken, by CHIHARA and YOSHIZAKI (1968) and at Enrumu, Hokkaido, by CHIHARA (1972). However, only one of the Japanese specimens collected have been determined as to species. The material collected at Enrumu was identified as *H. ovalis* (CHIHARA 1972). A major difficulty in identifying *Halicystis* species is the simplicity of the thallus organization. Since the discovery of the life history in which *Halicystis* alternates with *Derbesia* as an asexual phase, the thallus of *Derbesia* has become critical in determining the taxono-

1) Dedicated to the memory of the late Professor Tomoo MIWA.

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mic status of *Halicystis*. The *Derbesia*-thallus has more features recognized as diagnostic characteristics than the thallus of *Halicystis*-phase.

Concerning the life history of *Halicystis*, studies have been made on the species occurring in the North Sea, the Mediterranean Sea and the Atlantic Ocean, but the specimens in the Pacific Ocean, including the coast of Japan, have not yet been investigated. It is therefore worthwhile to study the life history of Japanese *Halicystis* in order to understand their systematic position. With this problem in mind, we carried out cultural studies of the Japanese specimens of *Halicystis* and examined their morphological details.

Materials and Methods

Specimens collected at Shiriya-zaki, Aomori-ken, on August 8, 1979, were in the drift. Specimens collected at Ohiragata, Hachijo Island, on May 10, 1979, were growing on crustose coralline red algae in shaded tide pools in the lower intertidal zone.

The specimens collected at Shiriya-zaki were washed with sterilized seawater and then cultured in the laboratory to obtain their gametes, while the specimens collected at Ohiragata were cultured starting from protoplast fragments obtained by the destruction of the thallus by needle, in the manner described by TATEWAKI and NAGATA (1970) with *Bryopsis* and RIETEMA (1973) with *Halicystis*. The material was placed in 200 m*l* petri dishes containing about 100 m*l* culture medium, and the culture dishes were kept in an incubator at 20°C, and 14 : 10 hr (light : dark cycle), and 2,500–3,000 lux from 30 W cool white fluorescent lamps. The culture medium used in this study was PROVASOLI's ES (PROVASOLI 1966).

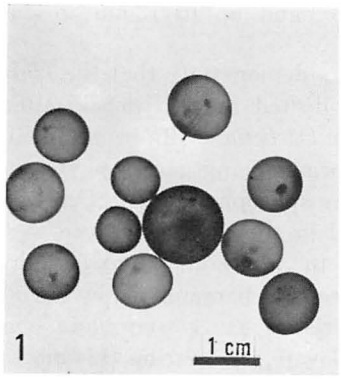
Observations and Results

Halicystis collected at Shiriya-zaki, Aomori-ken

More than a dozen *Halicystis*-thalli drifting ashore were collected at Shiriya-zaki, Aomori-ken, in the northern part of Honshu, in August, 1979. It appeared that the *Halicystis* had been growing on crustose red algae, because pieces of coralline algae were attached to the rhizoidal part of the thallus. The *Halicystis*-thalli were spherical or obovate, measuring 5–12 mm in diameter, with a short and slender rhizoidal base (Fig. 1). Chloroplasts were lenticular and relatively small, measuring 3–6 μm in length and 2–3 μm in width (Fig. 2). No pyrenoid was observed. Most of thalli collected were sterile, but a few individuals possessed colored portions, resulting from the accumulation of protoplasm, a feature characteristic of the initiation of gametogenesis. Two days after the culture started, gametangia were produced in the upper and the lateral portions of the vesicular parts of the thalli (Figs. 3, 4). The gametangial region was clearly differentiated, and one to several papillae, seen as white dots, were formed on the cell wall. The Shiriya-zaki material was dioecious, the male and female thalli being distinguishable by their colors. The male gametangia were yellow green, whereas the female were dark green. After three days, the liberation of gametes took place at the beginning of the light regime. Gametes were discharged forcibly through a pore formed in the papilla. Both male and female gametes were pyriform, having two

Figs. 1–13. *Derbesia marina*.

1. *Halicystis*-thalli collected at Shiriya-zaki, Aomori-ken, on August 8, 1979; 2. Portion of thallus, showing chloroplasts possessing no pyrenoid; 3. Male thallus, producing gametangium; 4. Female thallus, producing gametangium; 5. Male gametes; 6. Female gametes; 7. Germinating zygote; 8. *Derbesia*-thalli derived from zygotes; 9. Portion of filament of *Derbesia*-thallus, showing chloroplasts possessing no pyrenoid; 10. Sporangium; 11. Stephanokont zoospore; 12. Prostrate filaments derived from zoospores on germination; 13. *Halicystis*-thalli derived from zoospores.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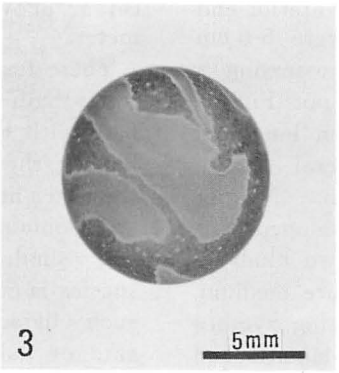
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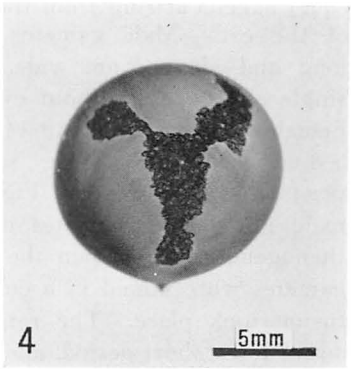
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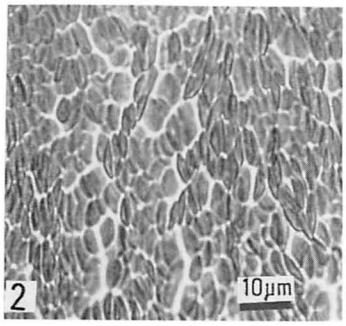
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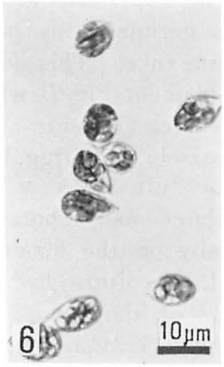
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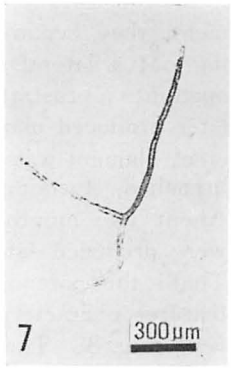
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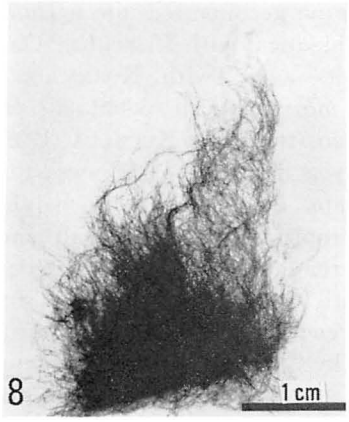
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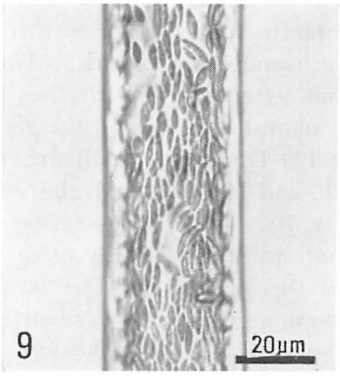
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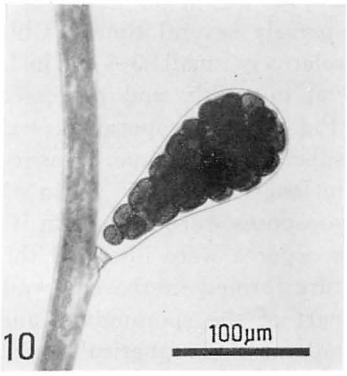
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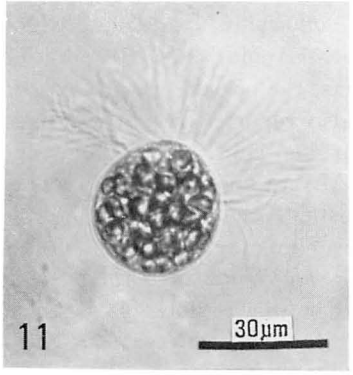
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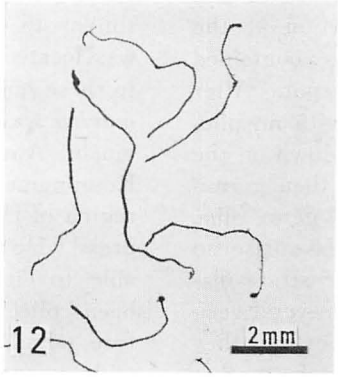
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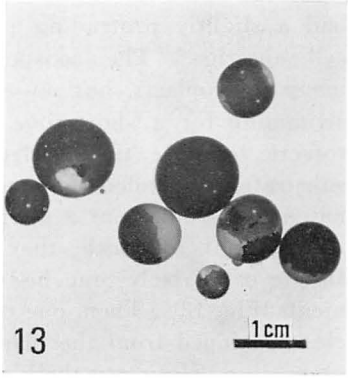
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1cm

equal flagella arising from the anterior end of the cell. Male gametes were 5–6 μm long and about 2 μm wide, possessing a single chloroplast without eyespot (Fig. 5). Female gametes were 10–14 μm long and 5–8 μm wide, possessing several chloroplasts and no eyespot (Fig. 6). Neither male nor female gametes developed parthenogenetically. When the two kinds of gametes were mixed in a culture medium, fusion took place. The resulting zygotes swam for a short period, and then attached to the substratum. Three days after attachment, they began to germinate by pushing out a lateral germ tube. This developed into a prostrate filament (Fig. 7), which later produced many erect filaments. The erect filament was sparsely and irregularly branched, forming a tuft as a whole. About two months later many sporangia were produced laterally on the filaments. Thalli thus obtained in culture had the features characteristic of the genus *Derbesia* (Fig. 8). They were 1–2 cm in height and 20–45 μm in diameter and branched sparsely several times. Chloroplasts were relatively small, 3–6 μm in length and 2–3 μm in width, and possessed no pyrenoid (Fig. 9). The sporangia were obovate to subclavate in shape, measuring 120–175 μm in length and 60–75 μm in width, and 24–32 zoospores were formed in it (Fig. 10). The zoospores were liberated through an aperture formed in the cell wall at the apical part of the sporangium, and were stephanokont and spherical in shape (Fig. 11). They measured 28–33 μm in diameter and had a slightly protruding portion at the cell anterior. The zoospores contained many chloroplasts, but no eyespot. After swimming for a short time, with no phototactic response, they settled down on the substratum, rounded up, and then germinated by pushing out a lateral germ tube. After about a month, they gave rise to simple or sparsely branched prostrate filaments (Fig. 12). Then, one or several vesicles developed from the filament and they grew into *Halicystis*-thalli after another month (Fig. 13). These thalli were spherical or obovate, and up to 12 μm in diameter.

These results demonstrate that the *Halicystis*-thalli collected at Shiriya-zaki alternate with the *Derbesia*-thalli in their life history, the former being gametophyte and the latter being sporophyte. The *Derbesia*-thalli obtained in the present culture were very similar to *Derbesia marina*. This species is currently characterized by having such characteristics as: 1) sporangia obovate or subclavate, measuring 164 μm in length and 60 μm in width (SEARS and WILCE 1970), 2) filaments 15–(38–) 48 μm in diameter (SEARS and WILCE 1970) and 3) chloroplasts with no pyrenoid (KORNMANN 1938). These values and features characterize the specimens we obtained in culture.

Regarding the life history, KORNMANN (1938) has demonstrated through culture study that *Derbesia marina* in Helgoland, Europe, produces stephanokont zoospores which develop on germination into a *Halicystis*-thallus identical with *H. ovalis*. Our present results agree with KORNMANN's work. The morphological detail of *H. ovalis* has been studied by KUCKUCK (1907) and the alga was described as follows: the thalli are ovate, up to 12 mm in height and the chloroplasts are very small and possess no pyrenoid. These characteristics also agree with our results.

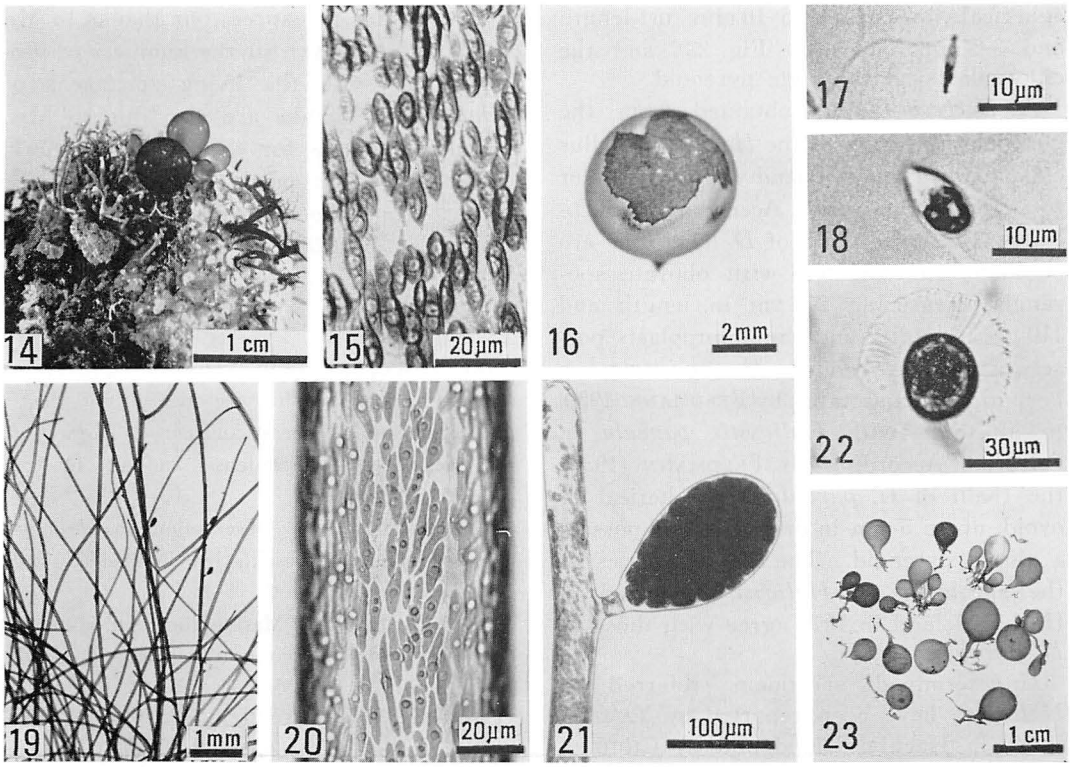
The occurrence of *H. ovalis* in Japan was reported by CHIHARA (1972) at Enrumu, Hokkaido. CHIHARA and YOSHIZAKI (1968) also reported the occurrence of a *Halicystis* similar to *H. ovalis* in Iwate-ken, which was located near Aomori-ken. In addition to these reports, the occurrence of *Derbesia marina* was also reported recently at Asamushi, Aomori-ken and at Shiogama and Kesennuma, Miyagi-ken, in the northern region of Honshu (KOBARA and CHIHARA, in press). However, no other specimens assignable to either *Halicystis* or *Debesia* have been collected in this region. It is therefore possible that *Halicystis ovalis* has a wide geographic distribution in the subboreal regions, including the northern parts

of Japan, alternating with *Derbesia marina*.

Halicystis collected at Ohiragata, Hachijo Island

Eight specimens of *Halicystis*-thalli growing on crustose red algae were collected at Ohiragata, Hachijo Island, in May, 1979. Thalli were obovate, pyriform or spherical and were up to 5 mm in length (Fig. 14). Chloroplasts were lenticular and relatively large, measuring 8–15 μm in length and 3–5 μm in width (Fig. 15). They possessed a single pyrenoid. All specimens collected in nature were sterile.

Cultures were started from protoplast fragments obtained by the destruction of the thallus by needle. These protoplasts synthesized cell walls within a few days and then developed into spherical *Halicystis*-thalli after about two months. They were obovate to spherical when matured, measuring 5–7 mm in diameter, with a short rhizoidal base. Then gametangia were produced appearing as irregular patches on the upper part of the sphere (Fig. 16). The alga was dioecious, and the gamentangia of two sexes were easily distinguished from each other, the male gametangia being yellow green whereas female ones being dark green. The zygotes resulting from the fusion of male and female gametes immediately germinated and developed into filamentous thalli (Figs. 17, 18). After two months, the filaments branched sparsely and irregularly several times, and became 1–3 cm in length and



Figs. 14–23. *Derbesia tenuissima*.

14. *Halicystis*-thalli growing on crustose coralline red alga, collected at Ohiragata, Hachijo Island on May 10, 1979; 15. Portion of thallus, showing chloroplasts possessing pyrenoids; 16. *Halicystis*-thallus, producing gametangium; 17. Male gametes; 18. Female gametes; 19. *Derbesia*-thalli derived from zygotes; 20. Portion of filament, showing chloroplasts possessing pyrenoids; 21. Sporangium; 22. Stephanokont zoospore; 23. *Halicystis*-thalli derived from zoospores.

30–60 μm in diameter (Fig. 19). Chloroplats were relatively large, measuring 7–18 μm in length and 3–6 μm in width and each possessed a single pyrenoid (Fig. 20). Later, the filaments produced sporangia on the lateral side. The sporangia were obovate, measuring 135–220 μm in length and 85–135 μm in width, and each contained about 30 zoospores (Fig. 21). The liberated zoospores were stephanokont and spherical, measuring 26–32 μm in diameter (Fig. 22). They swam slowly for a short period, showing no phototaxis, and then attached to the substratum. They germinated directly and developed into *Halicystis*-thalli within about two months. The manner of their development was fundamentally the same as that of *D. marina* described above. This *Halicystis*-thallus was pyriform or spherical, measuring 5–10 mm in length and 3–7 mm in width (Fig. 23), and the chloroplasts had a single pyrenoid.

A *Derbesia*-thallus obtained from the germinating zygote of the *Halicystis*-thallus collected at Hachijo Island was very similar to *Derbesia tenuissima*. According to FELDMANN (1937), the thalli of *D. tenuissima* are 30–50 μm in diameter, with obovate sporangia, measuring 210 μm in length and 110 μm in width, and the chloroplasts possess a single pyrenoid. *D. tenuissima* has been also demonstrated by FELDMANN (1950) to alternate with *Halicystis parvula* in culture. According to FELDMANN (1937), the thalli of *H. parvula* are spherical or ovoid, up to 5 mm in diameter, and possess a single pyrenoid. The characteristics of the specimens of *Halicystis* collected in Hachijo Island in 1979 agree with those of *H. parvula*.

Undetermined specimens referred to *Halicystis* have been reported by YAMADA (1952) in Hachijo Island and by CHIHARA (1954) at Susaki, Izu Peninsula, both sites located in the temparate to subtropical regions. The occurrences of *Derbesia tenuissima* have been recognized recently by us in both localities (KOBARA and CHIHARA, in press). *D. tenuissima* was also reported to occur in Misaki, Miura Peninsula, which is near the localities cited above, by YENDO (1914). From this series of observations, it seems that *H. parvula* is widely distributed in the temperate and subtropical regions of Japan, alternating with *D. tenuissima*, as the gametophyte in the latter's life history.

In connection with the life history of these algae, it should be pointed out here that, according our examination, both species of *Halichystis* have xylan as the main constituent of cell wall, whereas the two species of *Derbesia* have mannan as that of cell wall (CHIHARA, KOBARA and IRIKI unpublished data). This result will be described later.

Acknowledgements

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高原隆明・千原光雄： 邦産管状緑藻ウミノタマ属2種の培養と分類

管状緑藻ウミノタマ属 *Halicystis* の藻類は体制が単純であるために種の同定がむずかしく，邦産のものには正確な種名が与えられていない。著者等は青森県尻屋崎と八丈島大平潟から得たウミノタマ属の藻体について培養により生活史を明らかにし，配偶体世代と胞子体世代の体を調べて種の同定を試みた。両藻とも雌雄異株で2本の鞭毛をもつ異型の配偶子は接合して糸状のツユノイト相 *Derbesia*-phase に発達したが，尻屋崎産のものは *D. marina* (ホソツユノイト) に，また大平潟産のものは *D. tenuissima* (ツユノイトケバ) にそれぞれ形態的に同一の体となった。さらに両藻ともツユノイト相の体に stephanokont zoospore をつくり，それらは発芽して再びもとのウミノタマ相に生長した。それぞれの藻の両世代の藻体を精査した結果から，尻屋崎産の藻は *Halicystis ovalis* (*D. marina* の配偶体) に，大平潟産の藻は *H. parvula* (*D. tenuissima* の配偶体) にそれぞれ同定した。(305 茨城県新治郡桜村天王台 1-1-1 筑波大学生物科学系)