

## Laboratory Culture and Taxonomy of *Bryopsis* (Class Ulvophyceae) in Japan I. *Bryopsis triploramosa*, sp. nov.

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*Bryopsis triploramosa* Kobara et Chihara is described on the basis of the specimens collected from Okinawa and Amami Oshima Islands, southern Japan. The thalli are generally found growing singly, being composed of a main axis, primary branches, secondary branches and ramuli. These branches and ramuli are originating polystichously and densely. Ramuli are frequently bifurcated or trifurcated. The species is dioecious. Zygotes germinate into prostrate filaments which produce stephanokont zoospores holocarpically. The zoospores develop into a new *Bryopsis* thallus on germination.

### Introduction

For more than a decade we have studied the taxonomy and the life history of Japanese representatives of the *Bryopsis-Derbesia* complex. We have already published some of the results: for *Trichosolen* (= *Pseudobryopsis*) (Kobara and Chihara 1978a, 1978b); *Halicystis* (Kobara and Chihara 1980); *Derbesia* (Kobara and Chihara 1981) and *Pedobesia* (Kobara and Chihara 1984).

Ten species of *Bryopsis* have been reported in Japan (Okamura 1902, 1916, 1936; Yendo 1915, 1917; Yamada 1934) and summarized by Okamura (1936) as follows: *B. caespitosa* Suhr, *B. muscosa* Lamouroux, *B. corymbosa* J. Agardh, *B. harveyana* J. Agardh, *B. hypnoides* Lamouroux, *B. ryukyuensis* Yamada, *B. maxima* Okamura, *B. indica* A. et E. S. Gepp, *B. corticulans* Setchell, and *B. plumosa* (Hudson) C. Agardh. In his studies on European species of *Bryopsis*, Rietema (1969, 1970, 1975) showed that the type of life history and the developmental se-

quence of the germinating zygotes or zoospores are important diagnostic characteristics. In contrast, very few studies have been made for the Japanese representatives from this point of view. Tatewaki (1973, 1977) is the only phycologist to have undertaken laboratory culture studies on *Bryopsis* in Japan. He studied three species, *Bryopsis plumosa*, *B. maxima* and *B. ryukyuensis*, and showed that the life history of all the three was fundamentally identical with the so-called "Roscoff-type" of *B. plumosa* described by Rietema (1969, 1975). In order to have a better understanding on the genus *Bryopsis* in Japan, and to establish a more workable classification, we have made many collections. They have been studied with respect to thallus morphology, method of reproduction and the type of life history exhibited in laboratory culture. In this paper, a new species, *Bryopsis triploramosa* Kobara et Chihara, collected from southern Japan, including Okinawa and Amami Oshima Islands is described.

### Materials and Methods

Specimens used in the laboratory culture were collected at Kyan, Itoman-shi, Okinawa-ken (Okinawa Island), February 28, 1977, and Setta, Kasari-machi, Oshima-gun (Amami Oshima Island), Kagoshima-ken, April 11, 1979. The specimens were washed and brushed, and then cultured in an incubator at 23°C and under a light regime of 14:10 (light:dark cycle), with light intensity of about 3,000–5,000 lux from cool white fluorescent lamps. Under these conditions, the algae matured within a few days. The filamentous germlings resulting from the zygotes were cultured in the same conditions. In order to advance the germlings to the subsequent developmental stage, we have adopted Tatewaki's method (1973, 1977) as follows. When the germlings had grown to 10–30 mm long, they were transferred into a fresh medium, and the light regime altered to 10:14 (light:dark cycle). All the cultures were grown in Schreiber solution. Specimens of *Bryopsis hypnoides* which were used for the comparison with the present new species, were collected

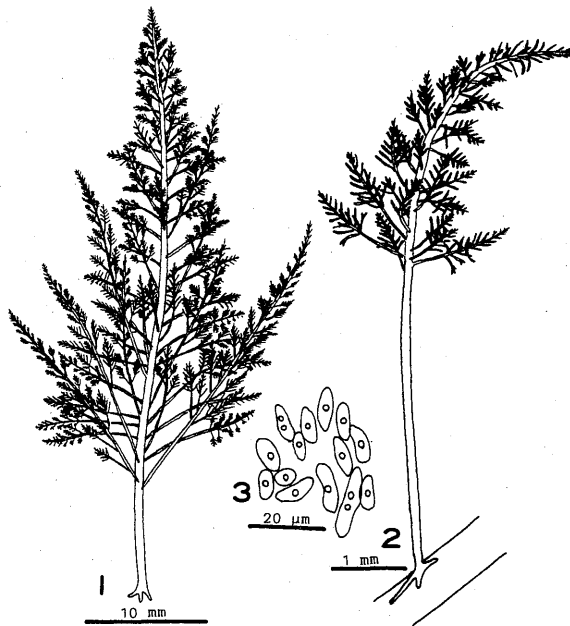
at Zeeland, in the southern part of the Netherlands, on May 25, 1993.

### Observations

#### *Bryopsis triploramosa* Kobara et Chihara, sp. nov.

Thallus solitarius, ad 60 mm longum, ex axe principale, ramis primariis, ramis secundariis, et ramulis constans; rami primarii et secundarii radiatim dispositi; ramuli 0.6–2.0  $\mu\text{m}$  longi, 50–80  $\mu\text{m}$  diam., saepe bi-furcati vel trifurcati, radiatim dispositi.

Thalli conical to tuft-like (Figs. 1, 4–6), dark green or sometimes light green, to 60 mm high, growing solitary. Thalli generally composed of four parts: main axis, primary branches (first order's branches), secondary branches (second order's branches) and ramuli (third order's branches). Primary branch bearing secondary branches and ramuli also conical as a whole (Figs. 2, 7). Main axis 400–600  $\mu\text{m}$  wide, with many interwoven rhizoids at the base. Primary branches 20–45 mm long, 250–300  $\mu\text{m}$  wide,



Figs. 1–3. *Bryopsis triploramosa*. 1: Habit sketch. 2: Primary branch with secondary branches and ramuli. 3: Chloroplasts with a pyrenoid.

with 1–3 basal rhizoids growing down the main axis. Secondary branches 2.5–9.0 mm long, 90–140  $\mu\text{m}$  wide. Ramuli 0.6–2.0 mm long, 50–80  $\mu\text{m}$  wide, frequently bifurcated or trifurcated, often forming subsequent ramuli. Chloroplasts elliptical, 7–20  $\mu\text{m}$  long, 5–7  $\mu\text{m}$  wide, with one or sometimes two pyrenoids (Fig. 3). A blue fluorescent substance is often present in the vacuoles of the main axis and branches.

*Holotype.* TNS-AL-41393 collected at Kyan, Itoman-shi, Okinawa Pref. (Okinawa Island), February 28, 1977.

*Habitat.* Growing solitarily on coral rock from mid to lower tide mark.

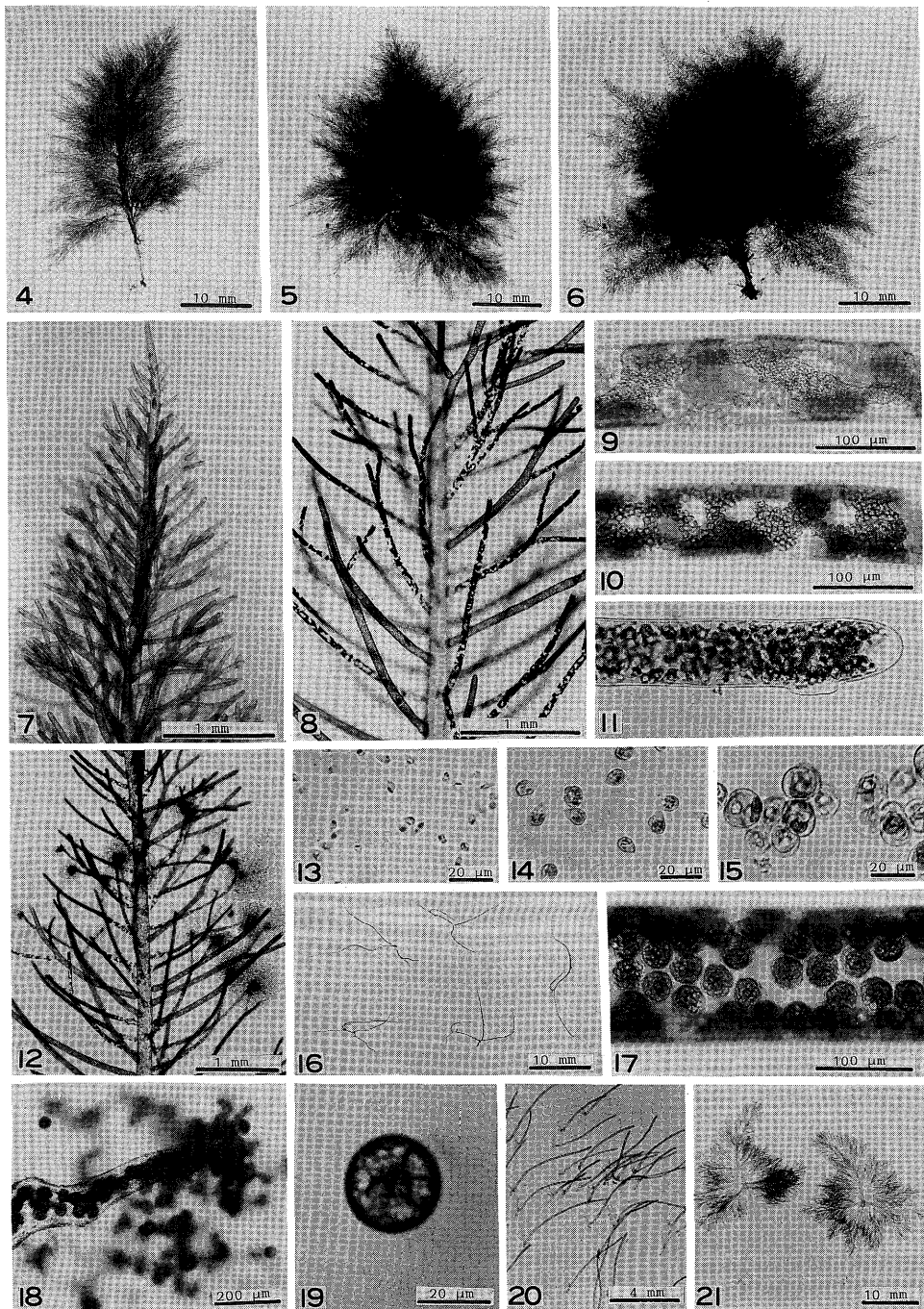
*Geographical distribution.* Okinawa Island, Amami Island and Miyako Island. Probably distributed widely in subtropical regions in Japan.

*Representative specimens examined.* Kyan, Itoman-shi, Okinawa Pref., February 28, 1977; March 28, 1979; March 15, 1986; December 26, 1990. Setta, Kasari-machi, Oshima-gun, Kagoshima Pref., March 17, 1978; April 11, 1979; March 14, 1987. Shimosato, Hirara-shi (Miyako Island), Okinawa Pref., January 5, 1992.

*Japanese name.* Nankai-hanemo.

*Laboratory culture.* At the type locality, Kyan, on south eastern coast of Okinawa Island, the alga grew abundantly in December to March and began to disappear in April. At Setta, located at the north western part of Amami Oshima Island, about 320 km north of Kyan, it was still abundant in April. Specimens from the type locality and Setta were cultured. No differences were observed between cultures from the two localities. Within three days, gametangia developed. In the formation of gametangia the protoplasmic content became aggregated and gave a reticular appearance (Fig. 8). The algae were dioecious, with male and female gametes produced in separate thalli. The gametangia of opposite sexes were distinguishable from each other by color and the size of gametes;

the male gametangia were orange and the gametes were small (Fig. 9), while the female ones were dark green and the gametes were relatively larger (Fig. 10). Even the bifurcated or trifurcated ramuli were capable of transforming into gametangia (Fig. 8). At maturity, the gametangia produced a papilla near the tip (Fig. 11). At the beginning of the light period, gametes were liberated through an aperture formed at the tip of the papilla (Fig. 12). Both male and female gametes were pear-shaped, but differed in size, the presence or absence of an eyespot, and the color of their chloroplasts. Male gametes were 4–5  $\mu\text{m}$  long and about 2  $\mu\text{m}$  wide (Fig. 13), and possessed a light green chloroplast and no eyespot. Female gametes were 8–11  $\mu\text{m}$  long and 4–6  $\mu\text{m}$  wide (Fig. 14), and possessed one or two darker green chloroplasts and a red eyespot. Both male and female gametes possessed two equal flagella at their anterior ends, and exhibited a positive phototactic response. Gametes from opposite sexes fused with each other when mixed. Zygotes were motile for a few hours and showed negative phototaxis. After attachment to the substratum, the zygotes increased in volume, with the formation of a vacuole in the cells (Fig. 15). They then germinated and developed into prostrate filaments with a few branches. At about three months old, the germinating filaments grew to 15–30 mm long and 80–180  $\mu\text{m}$  wide (Fig. 16). They were then transferred into a fresh medium and the light conditions changed. After two weeks, some of the germlings had matured. The protoplasmic content of the germlings moved towards the centre and accumulated, so that the distal part became transparent. At this stage, the chloroplasts divided in a similar fashion to that in gametogenesis. After 24 hours, the protoplasmic content divided into numerous spherical protoplasts which later produced stephanokontic flagella. In this way, zoospores were produced holocarpically (Fig. 17). At the beginning of the light period, zoospores were liberated through an aperture formed at the terminal end of the filament



Figs. 4-21. *Bryopsis triploramosa*. 4-6: Pyramidal or tuft-like thalli collected at Kyan, Okinawa Island. 7: Apex of a primary branch, showing tertiary branches or ramuli. 8: Portion of the thallus, showing simple or furcated gametangia. 9: Male gametangium. 10: Female gametangium. 11: Tip of a female gametangium, showing papilla. 12: Liberation of gametes from distal end of gametangia. 13: Male gametes. 14: Female gametes. 15: Germinating zygotes. 16: Filamentous germling, derived from zygotes. 17: Portion of sporangia of the germling. 18: Liberation of zoospores. 19: Stephanokont zoospore. 20: Germlings developed from zoospores. 21: *Bryopsis*-thalli developed from zoospores.

(Fig. 18). The zoospores had a spherical body (Fig. 19), 28–32  $\mu\text{m}$  in diameter with a hemi-spherical anterior part, and many flagella forming a subapical ring. The zoospores contained many chloroplasts except in the anterior portions, and possessed no eyespots. After swimming for up to one hour, zoospores attached to the substratum by the anterior part of the cells. Within 2–3 days, the attached zoospores increased in volume, and a vacuole formed in the cells. Within another 2–3 days they began to germinate and had developed into erect unbranched siphonous filaments after a week (Fig. 20). About two weeks later, they produced branches polystichously on their upper parts. After a further two weeks, they developed in the thallus whose morphology was typical of *B. triploramosa* found in nature (Fig. 21).

*Taxonomic remarks.* The species has a life history in which the gametophytic macrothallus (*Bryopsis* phase) alternates with the sporophytic microthallus (filamentous phase) which produces stephanokont zoospores holocarpically. This is fundamentally identical with the so-called “Roscoff-type of *Bryopsis plumosa*” (Rietema 1975). On the basis of its life history the species clearly belongs in the genus *Bryopsis* and in morphology it differs from any described species. This new species is characterized by: 1) solitary thalli with pyramidal or tuft-like gross morphology, 2) repeated branching which occurs three (sometimes four) times, 3) branches which are originating very densely from all sides of the axis, and 4) final order branchlets which are often bifurcate or trifurcated. The new species most closely resembles a figure of *Bryopsis hypnoides* Lamouroux in Vickers (1908, pl. LIII-2), in having branches that are polystichous and bifurcate or trifurcate. However it differs in gross morphology: her alga collected at Barbados, Lesser Antilles of West Indies, is not densely tufted and does not exhibit a pyramidal shape. Setchell and Gardner (1920) pointed out that the Vickers’ alga could not be referred to *B. hypnoides* and the matter

remains unsolved. Observations on *B. hypnoides* specimens collected at Zeeland, the Netherlands, show that there are some morphological differences between *B. triploramosa* and *B. hypnoides*. In *B. hypnoides*, several main axes originate from a common rhizoidal base, the thallus is relatively larger, up to 120 mm, and the ramuli are not so frequently furcated. In any case, *B. hypnoides* is reported to be mostly monoecious (Feldmann 1957, Neumann 1969, Rietema 1970), while all *B. triploramosa* observed were dioecious. Where dioecious thalli of *B. hypnoides* are reported (Rietema 1975, Kornmann and Sahling 1977), the dioecious thalli grew mixed with monoecious thalli.

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高原隆明, 千原光雄: 日本産ハネモ属 (アオサ藻綱) の培養と分類 I. ナンカイハネモ

沖縄本島南部の喜屋武および奄美大島の笠利町節田で採集したハネモ属の1種を新種とみなし, *Bryopsis triploramosa*, sp. nov. (ナンカイハネモ) として記載した。本種は潮間帯中部から下部にかけての珊瑚礁の岩上に生育する。単独で生育することが特徴の一つで、他の多くのハネモ属の種のように、共通の仮根部から多数の藻体が生じることはない。藻体の形状は円錐状または房状を呈する。いずれの場合も、主軸から生じる第一番目の

枝は円錐状を呈する。3回の分枝を行っているものがほとんどで、枝および小羽枝は各方面に、しかも密に生じる。小羽枝は2又、あるいは3又に分かれることが多い。本種は雌雄異株で、雌雄の配偶子の接合子は葡萄糸状体に発達する。糸状体は短日条件下で、全実的に遊走子を形成する。遊走子は直立して伸長し、細胞糸の下部に仮根を、上部に枝と小羽枝を形成して、天然で見られるハネモ世代の藻体に発達する。