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Morphology and Taxonomy of the Japanese Representative of Batrachospermales, Rhodophyta: Thallus Structure and Reproductive Organs of *Batrachospermum japonicum* Mori

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Abstract Detailed morphological studies have been carried out on the vegetative thallus, gametangia and carposporophytes of *Batrachospermum japonicum* Mori collected from the type locality. The thallus structure is uniaxial construction, 5 to 7 periaxial cells were issued from a centralaxial cell, each pericentral cell issues 3, rarely 4 whorled fascicle branches and rhizoidal filaments. The rhizoidal filaments form a cortex and issue secondary fascicle baranches. Monoecious. The carpogonial branch is 6 to 8 celled and carpogonia conical with obtrullate shaped trichogyne. After fertilization, gonimoblast initials are produced directly from the fertilized carpogonium and a compact carposporophyte is formed. The distal cells of gonimoblast filaments produced a carpospore.

Key words: *Batrachospermum japonicum*, fresh water, Rhodophyta, carposporophyte formation.

Introduction

The order Nemaliales, termed by Schmitz the Nemalionales, was estabished by Schmitz in 1892, on the basis of the lack of an auxiliary cell in the formation of the carposporophyte. The order was accepted by Kylin (1956) in his outstanding monographic work on the Rhodophyceae. He added one more feature, a haplobiontoc life cycle, to characterize the Nemaliales, and recognized 8 families: Acrochaetiaceae, which he called Chantransiaceae, Batrachospermaceae, Lemaneaceae, Thoreaceae, Naccariaceae, Bonnemaisoniaceae, Helminthocladiaceae and Galaxauraceae, which he called Chaetangiaceae. However, an auxiliary cell-like structure has been found in several members of this group of algae (Svedelius, 1915, 1942), and furthermore, the presence of a tetrasporophyte in the life cycle has been demonstrated by culture experiments (Chihara, 1961; von Stosch, 1965; Ramus, 1969; West, 1969). Pueschel and Cole (1982) have done ultrastructural studies of pit connections on the Rhodophyceae. They have suggested that the Nemaliales should be restricted to those members having primary pit connections with two-layered caps. This fact, along with other correlated findings, supports the removal of 5 families from the Nemalionales: Batrachospermaceae, Lemaneaceae and Thoreaceae to the Batrachospermales; and Bonnemaisoniaceae and Naccariaceae to the Bonnemaisoniales. Recent works on the sequence of the rbcL gene and 18S rRNA genes of *Thorea violacea* imply that the Thoreaceae is not closely related to Batrachospermales (Vis *et al.* 1998). Sheath *et al.* (2000) and Hanyuda *et al.* (2001) invalidly proposed the order Thoreales. Saunders & Necchi (2002) considered the Thoreaceae as an affinity of the Batrachospermales rather than the Acrochaetiales–Nemaliales–Palmariales lineage.

The structure of the female sexual organs, and the method of carposporophyte formation are still essential characteristics for the classification

of the Nemaliophyceae at the ordinal, the familial and genus level. Since, the author has been interested in the morphology and taxonomy of the Japanese representative of Batrachospermales, he undertook a morphological study of the reproductive systems, male and female reproductive organs and carposporophyte formation of *Batrachospermum japonicum* Mori.

Materials and Method

The materials used in this study were collected at the type locality. The type locality is mentioned by Mori (1975) as Oomachi Shrine near Ashikaga city in Tochigi Prefecture where the Kobonoike pond is located behind the Imomori shrine, Minamioomachi, Ashikaga city, Tochigi Prefecture.

The materials were collected by the author on 5 December 1993, and 22 March 1997. They were preserved in 5% formalin water. The materials were picked up with tweezers to cut into small pieces and then they were stained on the slide glass with 0.5% Erythrosin in water. For observation, section and squash methods were employed. The sections were cut using a freezing microtome. Slides were made by squashing the stained pieces in Karo Syrup under a cover slip. All figures were drawn with the aid of a lucida camera.

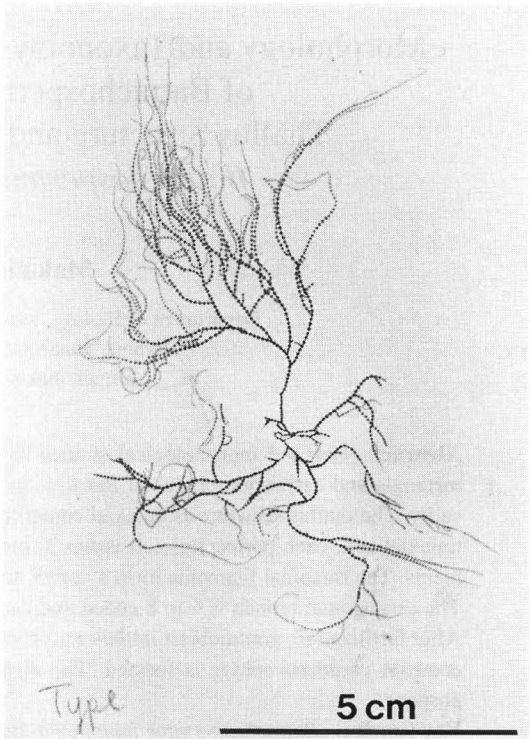


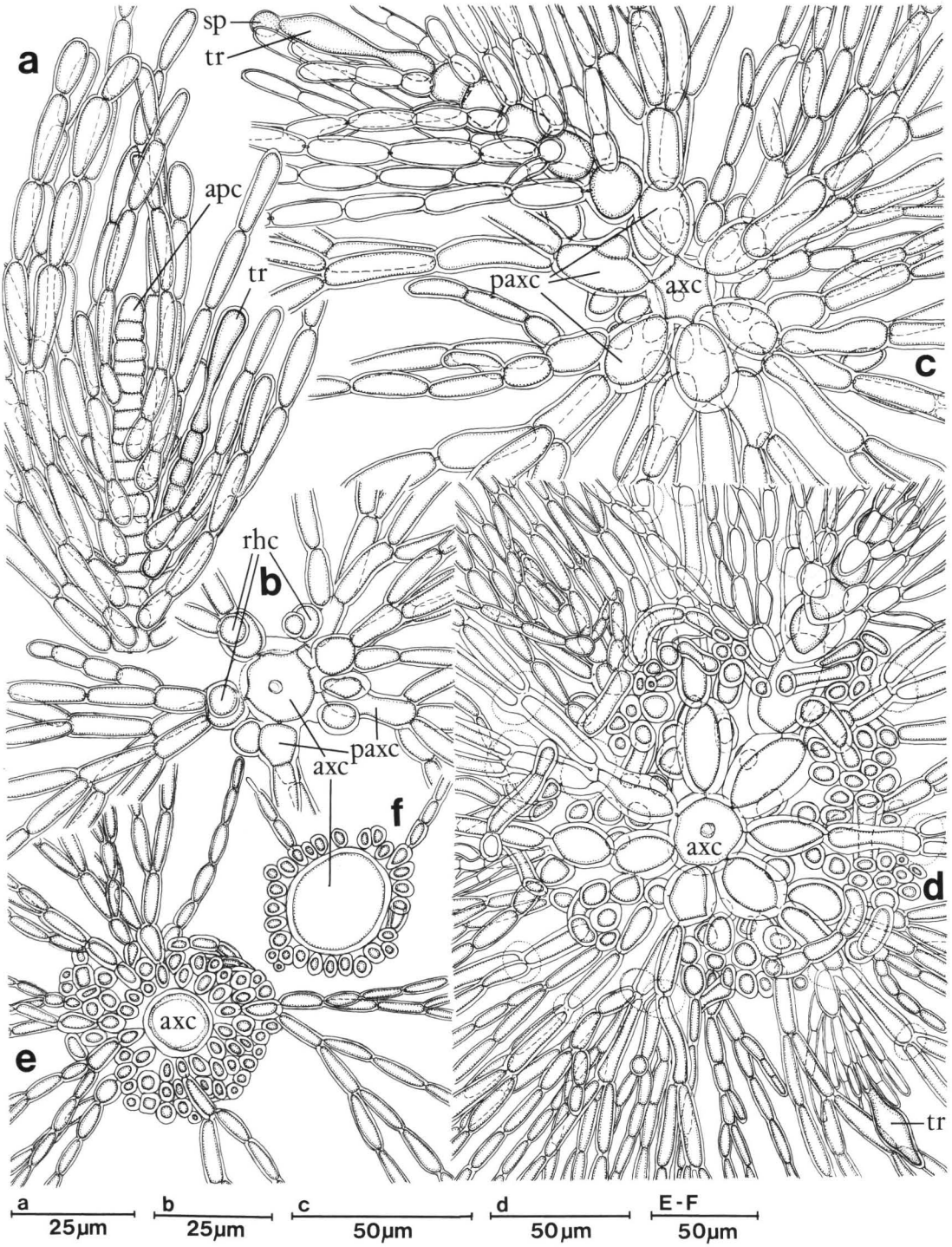
Fig. 1. Holotype specimen of *Batrachospermum japonicum* Mori.
Oomachi Shrine near Ashikaga city in Tochigi Prefecture on Mar. 1970, TNS-Al no. 35555, collector M. Mori, identified by M. Mori.

Examined specimens

The holotype specimen: Oomachi Shrine near Ashikaga city in Tochigi Prefecture, Mar. 1970, TNS-Al no. 35555, collecter M. Mori, identified by M. Mori.

The type locatity, April 29, 1993, in TNS-AL no. 151437; June 1983, in TNS-AL no. 151436; January 2, 1984 TNS-AL no. 151439 & TNS-AL no. 151440; July 8, 1990, in TNS-AL no. 151435; December 5, 1993, TNS-AL no. 151433; March 2, 1996, TNS-AL no. 151438 collector M. Yoshizaki, identified by the collector. The type locality, December 3rd, 1983, TNS-AL no. 151434 collector K. Iura, identified by M. Yoshizaki. Hitomaru, Konakachyo, Sano City, Tochigi Prefecture, TNS-AL 151442, collector M. Yoshizaki, identified by the collector. Yadai-jinnuma, Nittamachi. Nitta County, Gunma Pre-

Fig. 2. Vegetative structure of thallus of *Batrachospermum japonicum*.
a. Portion of growing tip, showing an apical cell. b. Cross section of younger part of thallus at nodal part, showing six periaxial cells arising from an axial cell, and rhizoidal cells arising from the periaxial cells. c. Cross section of younger part of thallus at nodal part, showing six periaxial cells arising from an axial cell, and a carpogonial branch arising from a periaxial cell. d. Cross section of older part of thallus at nodal part, showing seven periaxial cells arising from an axial cell, and many rhizoidal filaments. e. & f. Cross sections of older part of thallus at internodal part, showing many rhizoidal filaments making cortical tissue, and arising secondary fascicles from rhizoidal cells.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fecture, July 9, 1993, TNS-AL no. 151441, collecter M. Yoshizaki, identified by the collector.

Observations

Thallus structure

Thallus usually (3–)5–8(–12) cm, up to 12 cm in spring, 5–10 times dichotomously branched to form a globose mass, mucilaginous, brown to dark brown color while fresh, bright purple after drying. The whorls are globosal moniliforme-shaped in the upper portion, and barrel to cylindrical-shaped in the lower-basal portion. In the mature thallus, whorls are (400–)500–600 (–800) μm in diameter, and central axial cells are 500–700 μm long and 80–100 μm in diameter.

Thallus structure of the gametophyte is uniaxial. The apical cell of an indeterminate filament is a domed cylinder that cuts off short, discoid segments basally (Fig. 2a). Apical cells are 4–6 μm in diameter. Each discoidal segment cell becomes a nodal axial cell, which produces 5 to 7 periaxial cells (Fig. 2b, c, d.). It consists of branched indeterminate axes, each segment of which bears a whorl of determinate lateral filament a so-called fascicle branch. Each periaxial cell issues 3, rarely 4 whorled fascicle branches. Each fascicle branches dichotomously, pseudodichotomously and unilaterally secund. From the lower side of the periaxial cells descending rhizoidal filaments initiate proximally. Descending rhizoidal filaments of successive segments may overlap, enveloping the axis, to form the cortex around the axial cells. The rhizoidal filaments give rise to secondary fascicles. On the distal cell of the fascicle, sometimes, hyalin hair cells are formed (Fig. 2a).

Spermatangia

The plants of *Batrachospermum japonicum* Mori are monoecious. Spermatangia in dense clusters form in terminal parts of the assimilatory fascicles (Fig. 3a). The spermatangia are spherical 5–6 μm in diameter, and yellowish white in color.

Carpogonia

The carpogonial branches are formed in the younger parts of the thallus (Fig. 2a). The carpogonial branches are formed on pericentral axial cell, fascicle cells, secondary fascicle cells and occasionally in cells of the carpogonial branch. The carpogonial branches consist of 5 to 7 cells. The distal cell is a carpogonium, conical in shape, 12–15 μm long and 6–7 μm wide at the base, each with a trichogyne (Fig. 3b, c). The trichogyne is obtrullate shaped, and up to 30 μm long and 10–12 μm wide at its wider part.

Before fertilization, the cells of the carpogonial branch produce involucral filaments. Usually, one to three involucral filaments are produced from each cell of the carpogonial branch except the carpogonium. The involucral filaments are branched and grow upwardly; their shape and form are simillar to the fascicles.

Carposporophyte

One or many spermatium may attach to a terminal part of a trichogyne (Fig. 3d–g). After fertilization, protoplasm between the carpogonium and trichogyne separates at the trichogyne base (Fig. 3d). Later, in many cases, the trichogyne not broken down, remains on the well developed carposporophytes. The fertilized carpogonium produces a protuberance on the side which froms a gonimoblast initial (Fig. 3d). Three to five gonimoblast initials are formed from the same zygote (Fig. 3e–g); these initials divide to produce gonimoblast filaments, which develop downward toward the hypogynous cell (Fig. 3d–g). The gonimoblast filaments branch out and compactly surround the hypogynous cell to form a tight globose mass (Fig. 3g–j). The involucral filaments grow straight, and extend through the compactly developed gonimoblast mass (Fig. 3j–j & Fig 4a–d).

As the gonimoblast filaments develop further, the cells of the carpogonial branch become broader at their pit connection, and fuse with one another making a large fused area (Fig. 4c, d).

The gonimoblast filaments ultimately produce many short, erect branches (Fig. 4b, c); car-

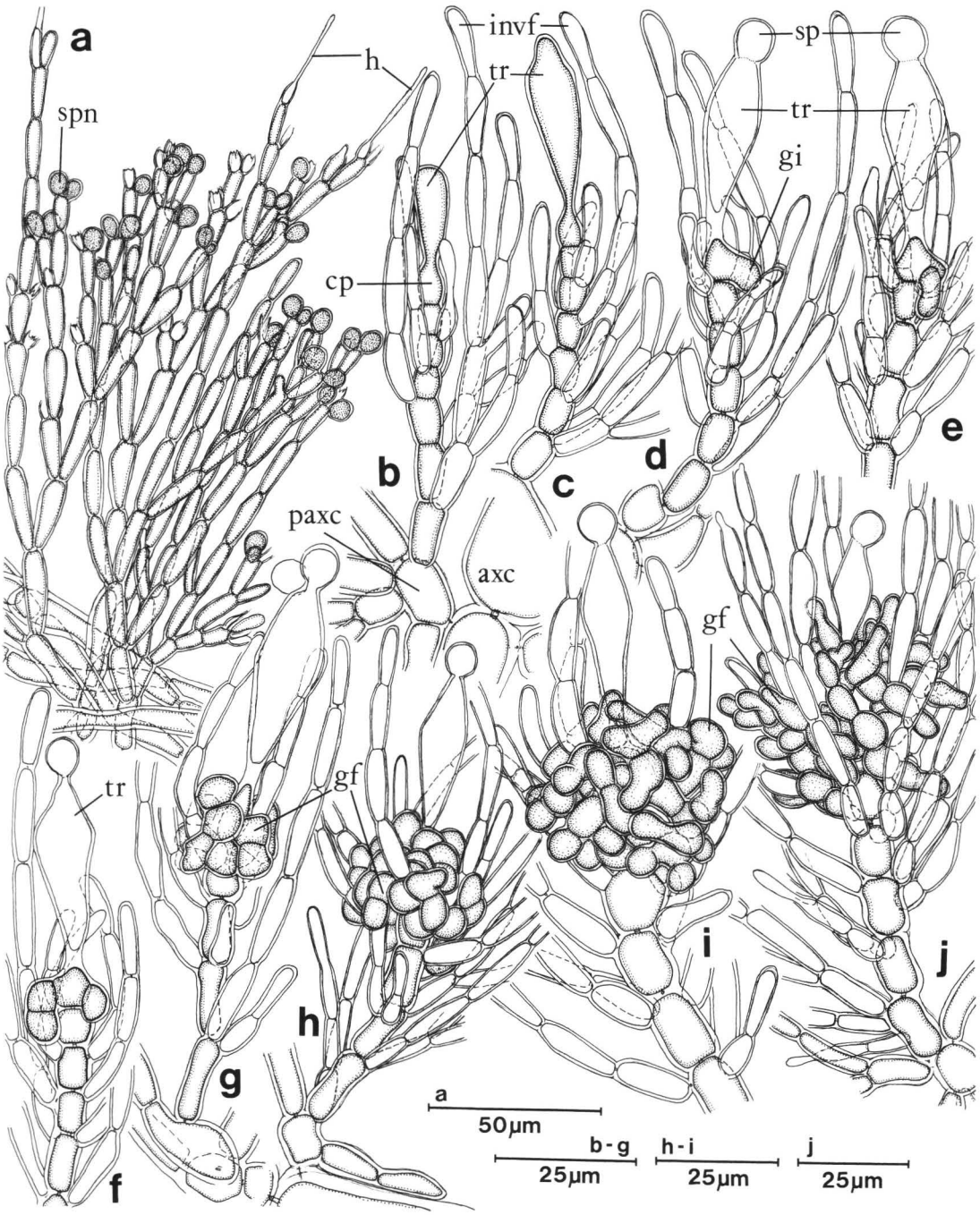


Fig. 3. Male and female sexual organs and carposporophyte development of *Batrachospermum japonicum*. a. Spermatangia terminal on fascicle and arising the terminal hyalin hairs. b. Young carpogonial branch arising periaxial cell, showing involucral filaments arising from cells of carpogonial branch. c. Young carpogonial branch before fertilization, showing ovate carpogonium and obtrullate trichogyne. d–j. Post fertilization stages, showing gonimoblast development. d. Post fertilization stage, showing swelling protuberance a primary gonimoblast initiated from the zygote. e–f. Post fertilization stages, showing gonimoblast initiation from the zygote.

posporangia are formed on the tips of these branches of the gonimoblast filaments (Fig. 4b, c). The mature carposporangia are dome-, obovate- or spherical-shaped, measuring 12–15 μm long and 8–10 μm wide. Mature carposporophytes are spherical, and 100–150 μm in diameter. One to five carposporophytes form within the whorl at various distances from the axis, and rarely a carposporophyte exerted from the globose whorl. Only terminal cells of gonimoblast filaments become carposporangia. In the old withered carposporophyte, empty carposorangia remain on the gonimoblast filaments (Fig. 4d).

Taxonomic remarks

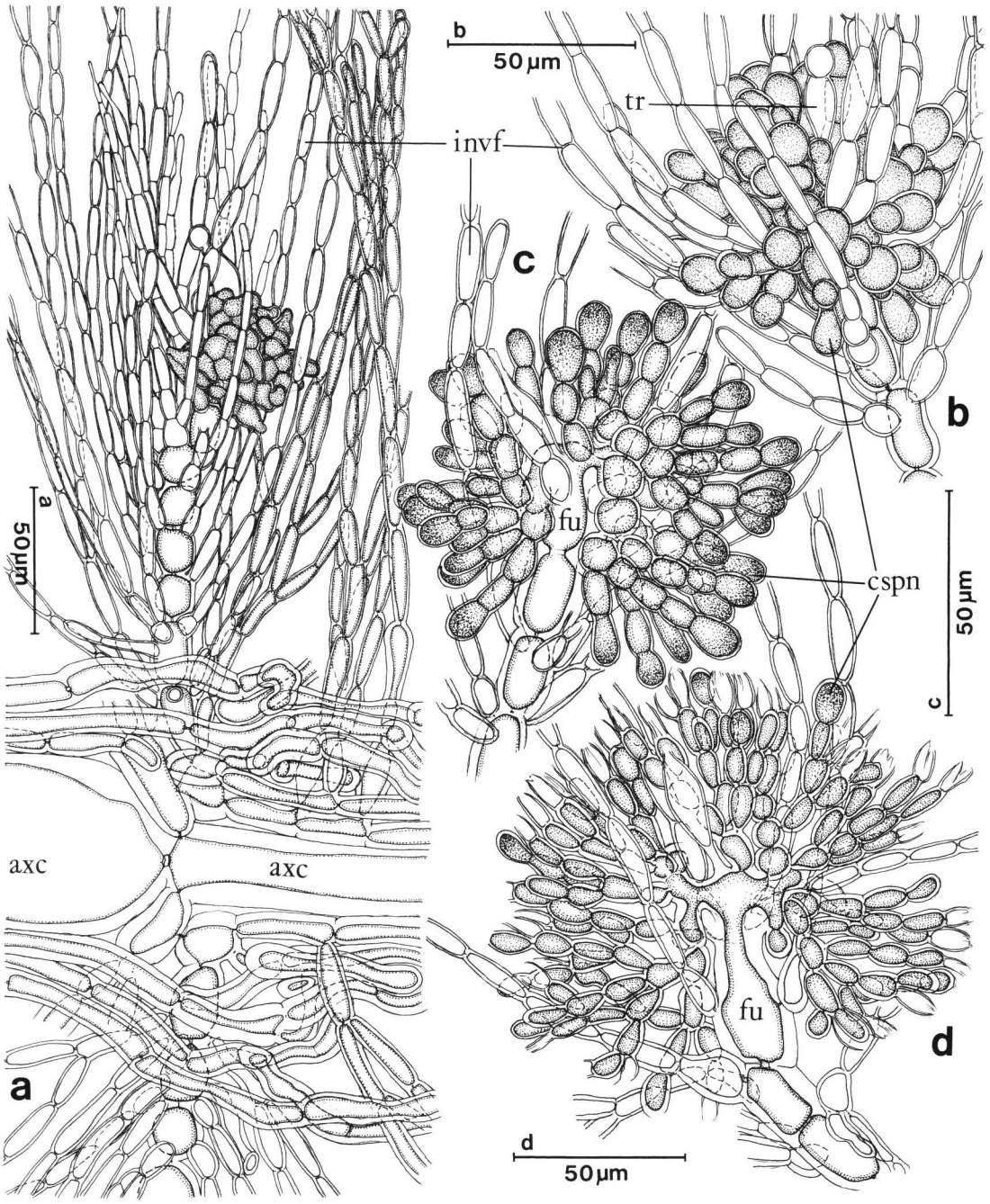
The life history of the order Batrachospermales is characterized by a macroscopic gametophyte that alternates with a diminutive audonielloid sporophyte which is called the chantransia stage (Pueschel and Cole 1982). On the pebbles in the pond of type locality, the chantransia stage of *Batrachospermum japonicum* is growing more abundantly than the gametophyte. The habit, the structure of sexual organs and the method of carposporophyte development of this species were studied by Mori (1975). The author's morphological observations described above fundamentally agree with the results obtained by Mori (1975).

According to Vis *et al.* (1995) moniliform outer appearance, monopodial thallus construction, globose shaped whorls, carpogonial branches arising from periaxial cell and or cells of both primary and secondary fascicles, symmetrical carpogonia with elongate conical or club-shaped trichogyne, and numerous carposporophyte developed at various distances from the center of the whorls are considered to be characteristic features of the section *Batrachospermum* of the genus *Batarachospermum*.

In a compartive study used for morphometric analysis of *Batrachospermum* section *Batarachospermum*, Vis *et al.* (1995) revised the following description for the species *Batrachospermmum gelatinosum* (Linnaeus) De Candle: monoecious, whorls distant to confluent, sometimes appressed, globose or barrel-shaped, 257–972 μm in diameter with 1–11 carposporophytes exerted or within the whorl at various distances from the axis; main axis with cortication consisting of cylindrical cells only; carposporophytes spherical, pedicellate, 40–139 μm in diameter, gonimoblast filaments of 2–4 cylindrical cells; carpogonia 20–68 μm long with clavate or lanceolate trichogyne 5–17 μm in diameter; carpogonial branch undifferentiated, 3–10 cells long; carposporangia obovoid, 6–16 μm long and 6–12 μm in diameter. Kumano (2002) confirmed this revision. On these characteristic data, *Batrachospermum japonicum* was included in the species of *Batrachospermmum gelatinosum* (Linnaeus) De Candle by Vis *et al.* (1995). However, their size and morphology on the female sexual organ, give carposporangia delicate shades of meaning with other synonymized species of *Batrachospermmum gelatinosum*.

Based on rbcL gene and 18S rRNA gene sequences, Vis *et al.* (1998) stated as follows. "The genus *Batrachospermum* appears to comprise many morphologically similar but distantly related taxa, which will need further investigation to resolve their taxonomic status. The structures of the thallus, sexual organs, and the method of carposporophyte development are still essential characteristics for the classification of Nemaliophycidae at the ordinal, the familial, the genus

Fig. 4. Carposporophyte development of *Batrachospermum japonicum*.
a. cross section of the thallus, showing relationship to axial cell and carpogonial system. b. surface view of carposporophyte, showing carposporangium and extended involucral filament of the carposporophyte. c. cross section of the mature carposporophyte, showing large fused area and gonimoblast branching. d. Cross section of an old carposporophyte, showing large fused cell of gonimoblast base, zygote, hypogynous cells, and only terminal cells becoming carposporangia.
Abbreviations used in the figures: apc–apical cell, axc–axial cell, cp–carpogonium, fu–fused cell, gi–gonimoblast initial, gf–gonimoblast filament, h–hair cell, invf–involucral filament, paxc–periaxial cell, rhc–rhizoidal cell, sp–spermatium, spn–permatangium, tr–trichogyne.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and the species level. Especially, classifications and comparative studies on both species and section levels of the genus *Batrachospermum* are needed.

The following features are considered to be characteristics of *Batrachospermum japonicum* Mori. 1) the moniliform thallus is embedded by a mucilage matrix and of uniaxial construction, 2) 5 to 7 periaxial cells, each pericentral cell issues 3, rarely 4 whorled fascicle branches and rhizoidal filaments, 3) cortex are formed by the rhizoidal filaments, and issue secondary fascicle branches, 4) monoecious, 5) globose antheridia formed terminally on the fascicles, 6) 5 to 7 celled carpogonial branch, each cell of the carpogonial branch issue involucral filaments, 7) carpogonia conical with obtrullate shaped trichogyne, 8) fertilized carpogonium produces a protuberance on the side from which a gonimoblast initial cell is formed, 9) 3 to 4 gonimoblast initial cells are produced from a zygote, 10) tightly branched gonimoblast, and a compact carposporophyte are formed, 11) involucral filaments grow straight and extend between the compactly developed gonimoblast mass, 12) distal cells of gonimoblast filaments produce a carpospore.

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