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Thallus Structure and Reproductive Organs of *Nemalionopsis tortuosa* (Rhodophyta)

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Abstract Detailed morphological studies have been carried out on the vegetative thallus, gametangia and carposporophytes of *Nemalionopsis tortuosa* Yagi et Yoneda collected from southern Japan. The carpogonium is borne laterally or terminally on a cell of an assimilatory filament. The gonimoblast initials are produced directly from the fertilized carpogonium, dividing soon afterwards to form several filaments. The gonimoblast filaments produce branches which grow among the assimilatory filamets, resulting in the formation of a diffuse carposporophyte. On the basis of these and other observation, *Nemalionopsis* is mainteined in the Thoreaceae, Thoreales.
Key words : *Nemalionopsis tortuosa*, fresh water algae, carposporphyte formation, Batrachospermales, Thoreales, Rhodophyta

Introduction

Nemalionopsis was estabished by Skuja 1934 with *Nemalionopsis shawii* Skuja as the type species. Since monosporangia are located at the tips of assimilatory filaments.

The genus includes two species at present, and has been reported from only seven localities worldwide in Asia and North America (Sheath, Vis and Cole 1993). *Nemalionopsis tortuosa* was esatabished by Segi and Yoneda 1949 from Okichi-izumi, Ehime Prefecture, Japan. The habitat, thallus structure and monosporangia formation have been described for the species, but gametangia have not been recorded for the genus. Since the taxonomy of the Florideophyceae is based on the structure of the carpogonia, the systematic position of the genus has been uncertain. Recent collections of *Nemalionopsis tortuosa* from southern Japan included male and female plants with carposporophytes, and provided an opportunity to carry out a detailed study of the gametangial and carposporophyte formation. The systematic position of the genus is also discussed on the basis of the results obtained.

Materials and Methods

Used materials were collected in the irrigation channel of Khojirogawa River at Kunimi Town, Nagasaki Prefecture, on March 10, 2001 by myself and May 19, 2001 by Dr. Masafumi Iima, and were preserved in 10% formalin in water. After staining with 1% erythrosin, the marerials were mounted in 50% Karo syrup. Satisfactory thin preparation were eventually obtained by using a Bright OT/FAS cryostat microtome. Drawing were made with a camera lucida.

Examind specimens: The type locality, Okichi-izumi, Matsuyama, Ehime Prefecture on March 19, l942, collecter Y. Okada, and Identified by Y. Okada, in TNS-Al no. 30809. The irrigation channel of Khojirogawa River at Kunimi Town, Nagasaki Prefecture, on March 25, 2001, collecter M. Yoshizaki and identified by M. Yoshizaki, in TNS-Al no 156879 and 156880, and on May 19, 2001, collecter Dr. Masafumi Iima and identified by M. Yoshizaki.

Thallus morphology

Plants of *Nemalionopsis tortuosa* Yagi et

Yoneda (Fig. 1) grow on rocks or stone walls attaching by means of a small discoidal holdfast, 2–3 mm in diam. Usually one, or sometimes two or three erect fronds are producing from a holdfast. They are terete, soft, gelatinous in texture, grow to a height of 2.3 m, and are up to 2 mm in diam, with two to 10 times iregularly dichotomously branches. Sometimes older plants produce several short buranches at the lower basal parts of the thallus. Thallus structure is multiaxial, consisting of medullary filaments and assimilatory cortical filaments (Fig. 2a). The medullary filaments are 7–10 μm in diam. and interlace with one another, forming a core. The assimilatory filaments usually branch dichotomously one to three times, but only in the upper parts. The lower parts of assimilatory filaments are terete, 4–8 μm in diam, and the basal parts of the assimilatory filaments produce rhizoidal filaments which contribute the structure of the medulla (Fig. 2f). The upper parts of the assimilatory filaments are composed of barrel-shaped cells. Sometimes the cells of the assimilatory filaments are barrel-shaped continuously form base to the tip (Fig. 2f).

Reproduction

Plants of *Nemalionopsis tortuosa* are unisexual. Spermatangia (spn) are borne terminally or in twos or threes in short small clusters. (Fig. 2b, c). The spermatangia are ovoid 8–10 μm long and 6–9 μm in diam. Liberated spermatia are spherical, measuring 10 m in diam.

Carpogonia (cp) are formed in abundance in the younger parts of the thallus. The carpogonium is borne laterally or terminally on a cell of an assimilatory filament. They are one-celled (Fig. 2e, d), urceolate in shape, 12–15 μm long and 6–7 μm wide at the base, each having a stright and cylindrical trichogyne (tr) that is up to 1200 μm long and 3–4 μm in diam.

More than one spermatium (sp) may attach to a trichogyne (Fig. 2e). After fertilization, the trichogyne withere or cut off at the base. The fertilized carpogonium produces a protuberance on the upper side (Fig. 2f) from which a gonimoblast initials is cut off by a cell division (Fig. 2g). Two to five gonimoblast initials are produced from the same zygote (Figs. 3, 4) which divide to form gonimoblast filaments that develope in one of two ways (Fig. 4). One develops upperwardly parallel to the assimilatory filaments. The other develops downwardly toward the medulla, but does not mix with the medullary filaments. Instind they run out parallel to the surface of the thallus, extending among the assimilatory filaments and give rise to a diffuse carposporophyte. The tips of gonimoblast filaments produce many short, erect branches (Fig. 4). The short erect branches branch again resultng in another order of branches grow among the assimilatory filaments. The carposporangia are formed on the tips of these branches of the gonimoblast filaments (Fig. 4). The mature carposporangia are ovoid 12-14 μm long and 8–10 μm in diam. Only the terminal cells produce carposporangia, and the gonimoblast filaments remain after carpospore have been released.

Discussion

Pueschel et Cole (1982) have proposed the establishment of the order Batrachospermales on the basis of thallus construction, pattern of life cycle, chloroplast structure and their presence in fresh water, and including three families: Batrachospermaceae, Lemaneaceae and Thoreaceae. Vis, Saunders, Sheath, Dunse and Entwisleet (1998) circumscribed the Batrachospermales as a having heterotricous life history; absence of tetraspore formation; a two-layered pit plug, the outer layer of which is dome-shaped; and a strictly fresh water habitat, the include three families: Batrachospermaceae, Lemaneaceae and Psilosiphonaceae. On their studies, the family Thoreaceae does not appear to be a natural grouping within the Batrachospermales.

Multiaxial thallus construction in found in the Nemaliales, including the Helminthocladiaceae, Dermonemataceae and Galaxauraceae among marine families, and the Thoreaceae among

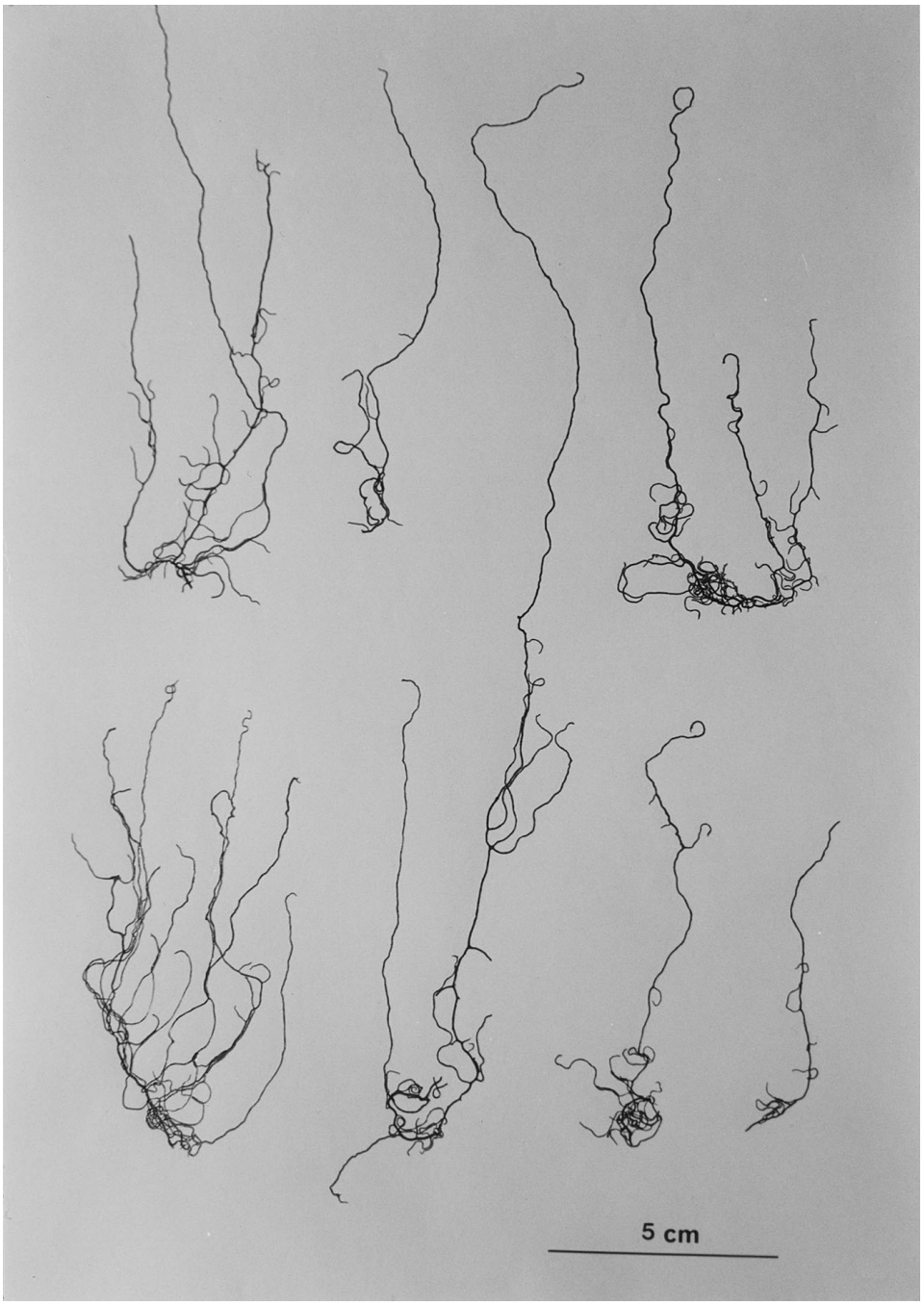

Fig. 1. Specimen of *Nemalionopsis tortuosa* Yagi et Yoneda.
The irrigation channel of Khojirogawa River at Kunimi Town, Nagasaki Prefecture, on March 25, 2001, TNS-Al no 156879.

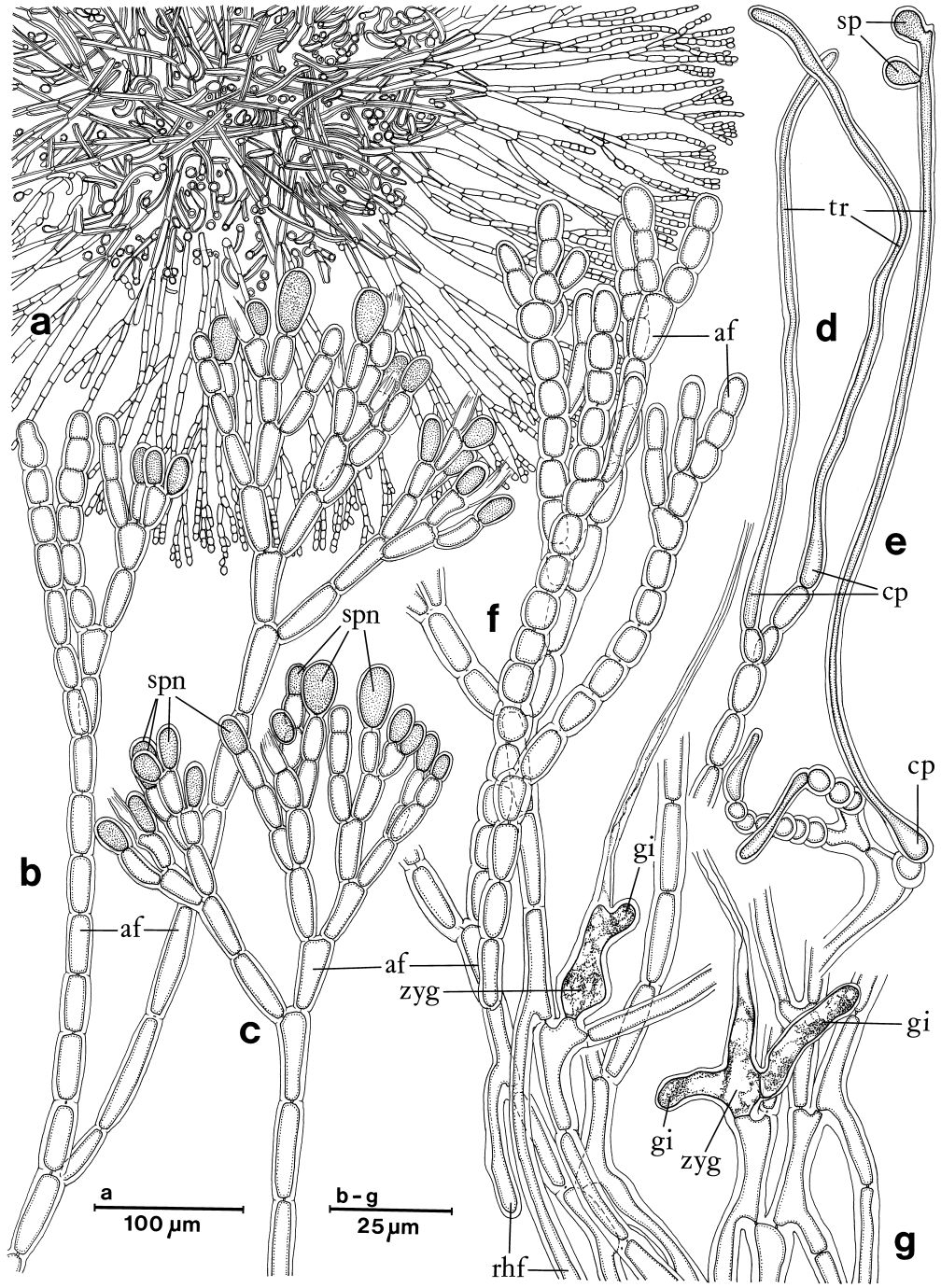

Fig. 2. The vegetative structure of thallus, and male and female sexual organs and gonimoblast initiations of *Nemalionopsis tortuosa*. a. Cross section of the younger part of thallus, Showing cortical assimilatory filaments and filamentous medulla; b, c. Spermatangia are borne terminally or in twos or threes in short small clusters. d. two carpogonia (cp) are produced terminally on the assimilatory filaments; e. Spermatia (sp) attached on the tip of trichogyne. f. Postfertilization stage, showing the gonimoblast initial (gi) developing from the upper part of the zygote (zyg); g. Gonimoblast initials (gi) developing, one of then is already cut off from the zygote, and other one is arising from other side.

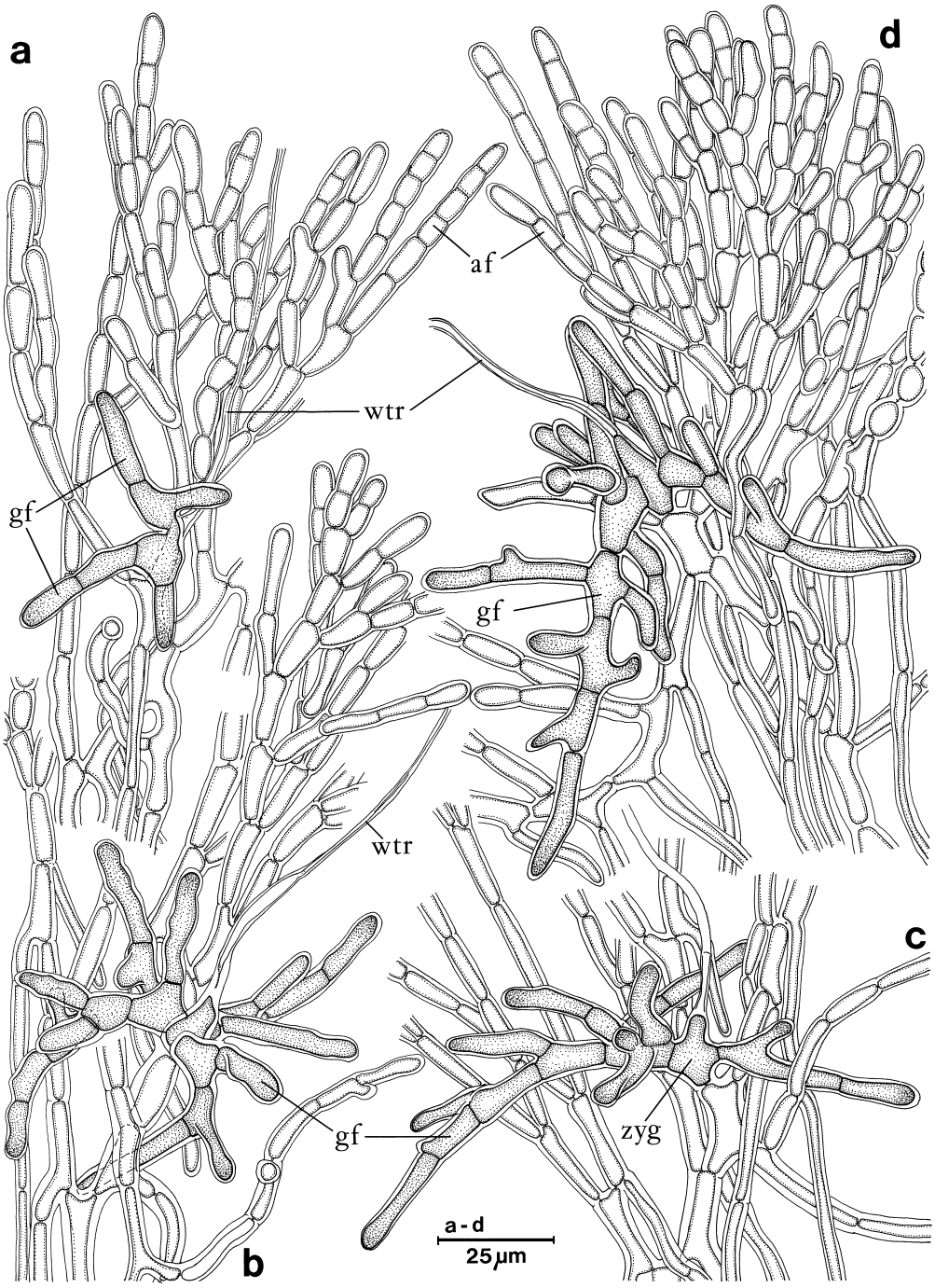

Fig. 3. Carposporophyte development of *Nemalionopsis tortuosa*. Post fertilization stages, showing various stages in the develoed stages of gonimoblast filaments.

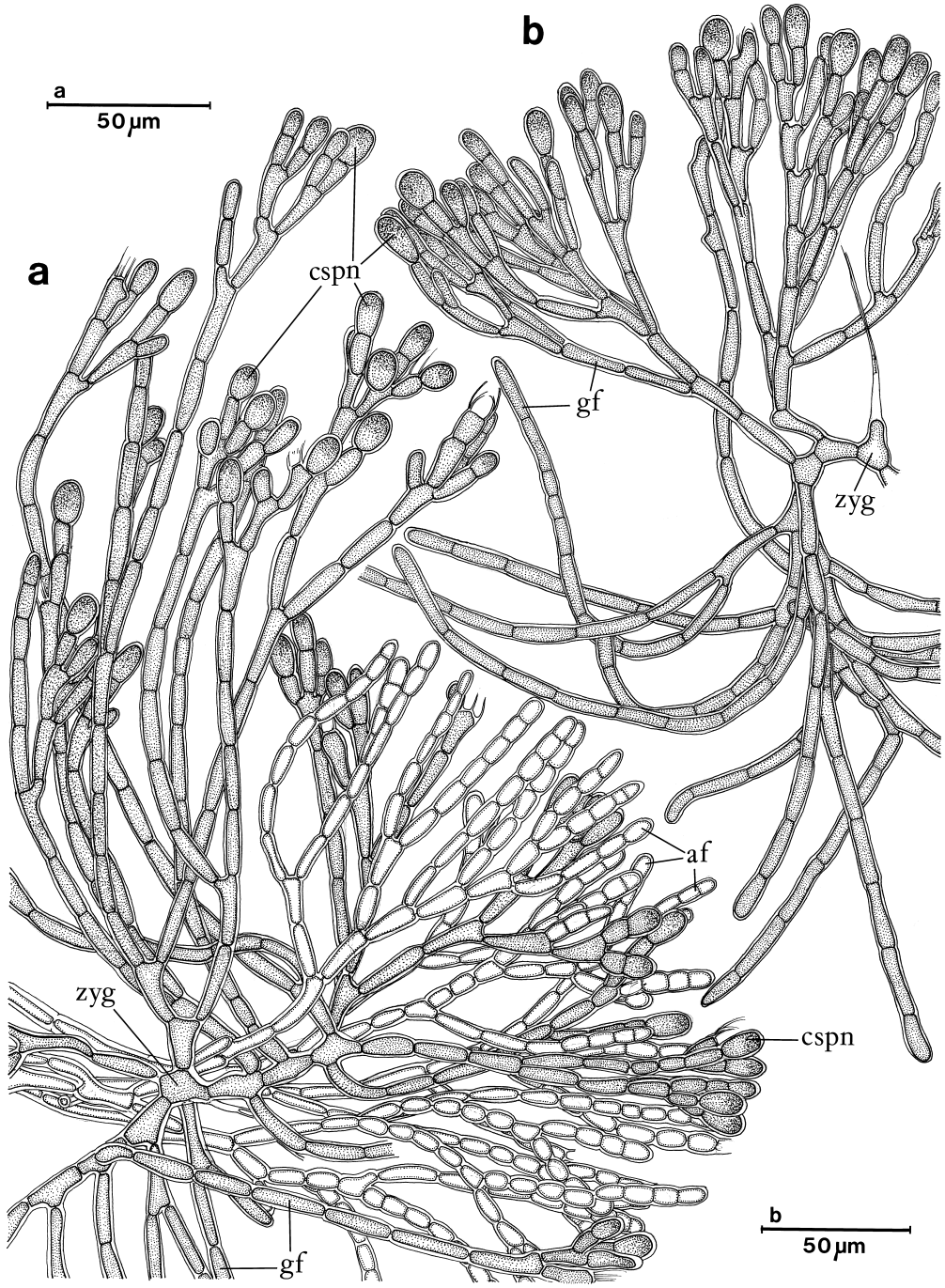

Fig. 4. Mature carposporophyte of *Nemalionopsis tortuosa*. Well developed carposporophyts, showing two stages of diffused gonimoblast filaments and forming clusterts of carposporangia (cspn).

Abbreviations used in the figures: af-assimilatory filament, cp-carpogonium, cspn-carposporangium, gi-gonimoblast initial, gf-gonimoblast filament, rhf-rhizoidal filament, sp-spermatium, spn-spermatangium, tr-trichogyne, wtr-withered trichogyne.

freshwater families. According to Migita (1986), released spores develop into a heterotichous habit composed of prostrate and erect filaments. Hara and Chihara (1974) observed in *N. tortuosa* that many discoid chloroplasts without pyrenoid are present in each cell. Based on these features, it appeares to place the Thoreaceae in the Batrachospermales. In additional feature of the Batarachospermales, pit plugs have two cap layers on either side of the pit plug core and also show a dome-shaped elaboration of the outer layer of the plug cap (Pueschel et Cole 1982). Acording to Mueller, Sherwood, Pueschel, Gutell and Sheath (2002), the pit plugas of the gametophytic and chantransia stages of the Thoreaceae, contein two cap layers, the outer one of which is typically plate-like, though occasionally inflated ones have been seen. No pit plug cap membrane has beeen observed.

Sheath and Mueller (1999) sequenced the rbcL and 18S ribosomal DNA on the members of fresh water rhodophytan *Balbiania* and its relatives. Vis, Saunders, Sheath, Dunse and Entwisle (1998) sequenced the rbcL and 18S ribosomal DNA on the menbers of Batrachospermales, in all trees, *Thorea violacea* was not closely related to the other taxa of the Batrachospermales. Based these data, they considered again that the Thoreaceae does not appear to be a natural grouping within the Batrachospermales. These proposal were supported by Hanyuda, Kumano, Arai, Suzawa, Iima, Ueda (2001) on the sequenced the rbcL and 18S ribosomal DNA on the Japanese menbers of Batrachospermales, especially on the members of Thoreaceae. Both papers of Sheath, Mueller and Sherwood (2000) and Mueller, Sherwood, Pueschel, Gutell and Sheath (2002), proposed the Thoreales to include two genera *Nemalionopsis* and *Thorea*.

Because of their multiaxial thallus construction, pit plugs that lack outer cap layers that are not universally dome-shaped. The phylogenetic relationships of the genera were investigated using a combination of DNA sequence analysis (rbcL and 18S rRNA genes) and transmission electron microscopy. In addition, analysis of the secondary structure of the 18S rRNA gene in *Nemaliopsis* and *Thorea* reveals an additional helix, which is not present in any of the other taxa within the Rhodophyta. The carpogonial branches in Thorea consist of only a single cell. The carpogonial morphology of *Nemalionopsis tortuosa* is similar to that of *Thorea okadai* which is borne on a basal cell of an asimilatory filament and is almost cylindrical in morphology (Yoshizaki 1986). *Nemalionopsis* is distingushed from *Thorea* by the position of the sporangia in the thallus. The monosporangia of *Nemalionopsis* are situated at the apex of the assimilatory filaments, whereas the monosporangia of *Thorea* are located at the base of assimilatory filaments. Among freshwater genera, diffuse gonimoblasts are observed only in Thorea and *Sirodotia*. Sirodotia is classified in the Batrachospermaceae because of its uniaxial thallus construction.

Multiaxial thallus construction, one-celled carpogonia and diffuse gonimoblasts are significant features that can now be employed to characterize the family Thoreaceae in the order Thoreales.

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