PROC. N.S. INST. SCI. (1978)
Volume 28, pp. 191-199

A CULTURE AND CYTOLOGICAL STUDY OF THE LIFE HISTORY OF *NEMALION HELMINTHOIDES* (RHODOPHYTA, NEMALIALES)[1]

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The life history of Nemalion helminthoides (Vell. in With.) Batt. is described based on results from culture and cytology. This consists of morphologically dissimilar gametophytic and tetrasporophytic generations. The former is haploid (n=10) and the latter is diploid (2n=20), with meiosis presumably occurring in the tetrasporangium. Tetrasporogenesis was induced by increasing the temperature from 5° to 20°C. Gametophytic plants developed only under 15°C and a long duration of light, 16:8 h. Spermatia and carpogonia occurred on the same thallus.

Introduction

Aspects of the life history of species of *Nemalion* have been reported by Fries (1967), Martin (1967, 1969), Umezaki (1967a,b, 1972), and Masuda and Umezaki (1977). In common with other Nemaliales, species of *Nemalion* appear to have a heteromorphic life history, although culture studies (Fries 1967; Umezaki 1967b, 1972) have failed to re-establish the characteristic macroscopic gametophyte. Germination of tetraspores resulted only in microscopic, filamentous sporelings, but there is justification for assuming that these sporelings give rise directly to the characteristic macroscopic gametophyte thallus. Both Ramus (1969) and von Stosch (1965) found that the filamentous sporeling derived from tetraspores of *Pseudogloiophloea confusa* and *Liagora farinosa* respectively (Nemaliales) formed "buds" which initiated the macroscopic gamtophyte. Further, Masuda and Umezaki (1977) in Japan have reported on the gametophytic thallus in culture for *N. vermiculare*.

Nemalion helminthoides has been investigated cytologically by Magne (1961) who reported that the haploid number of chromosomes in the gametophyte was n=8. It was assumed that meiosis occurred during tetrasporogenesis.

On the basis of culture and cytological observation of *N. helminthoides*, we now describe the developmental sequence of the life history and chromosome counts in nuclear phases.

Materials and Methods

Gametophytic plants with carpospores (Fig 1) were collected at Martinique Beach, Halifax, Co. in 1969, 1970 and Bras d'Or Lake, Victoria Co. (McLachlan &

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Table 1 Conditions of incubation for induction of tetraspore formation and development of upright gametophyte. Tetrasporic filaments were maintained at 5°C, 8:16 h light prior to incubations under conditions indicated.

Temp (°C)	Light* (L:D)	Days incubation 7	14	21	28	Remarks‡
			Tetraspore release†			
5	16:8	0	0	0	0	Few monospores only
10	12:12	0	++	+++	+++	Tetraspores germinated slowly
10	16:8	+	++	++++	++++	Tetraspores germinated slowly
13	10:14	+	++	++++	++++	Tetraspores germinated slowly
15	10:14	+	+++	++++	++++	Tetraspores germinated
15	16:8	+++	+++++	+++++	+++++	Tetraspores germinated giving rise to characteristic upright fronds after 6 weeks**
20	12:12	++	++++	++++	++++	Tetraspores germinated
20	16:8	+++	+++++	+++++	+++++	Tetraspores germinated giving rise to characteristic upright fronds after 6 weeks**

* Illuminance 40± 15μE $m^{-2}s^{-1}$
† Visual estimation
‡ Monospores formed under all conditions
**Upright gametophytic fronds maintained growth above 10°C; 10-16-h light periods were satisfactory

Edelstein 1970-71) in 1976, both in Nova Scotia. Segments with carposporangia were brushed and cleaned in sterile seawater, and incubated at 15°C, 16:8 h (L:D) and 13°C, 10:14 h. In both cases illumination of about 20μE $m^{-2}s^{-1}$ was provided by 40-W cool-white, fluorescent lamps. After 12 h incubation, carpospores were discharged and these were transferred by finely-drawn pipettes to small (60 x 20 mm) disposable, plastic petri dishes containing SWM-3 medium (McLachlan 1973). Some sporelings were cultured in medium containing 5-10 mg $^{-1}$ of GeO_2 to suppress diatom contaminants. Individual sporelings were, at an early stage (Fig 2), re-isolated by pipette, and unialgal cultures were thus established.

Tetrasporophytic filaments were incubated under various conditions of

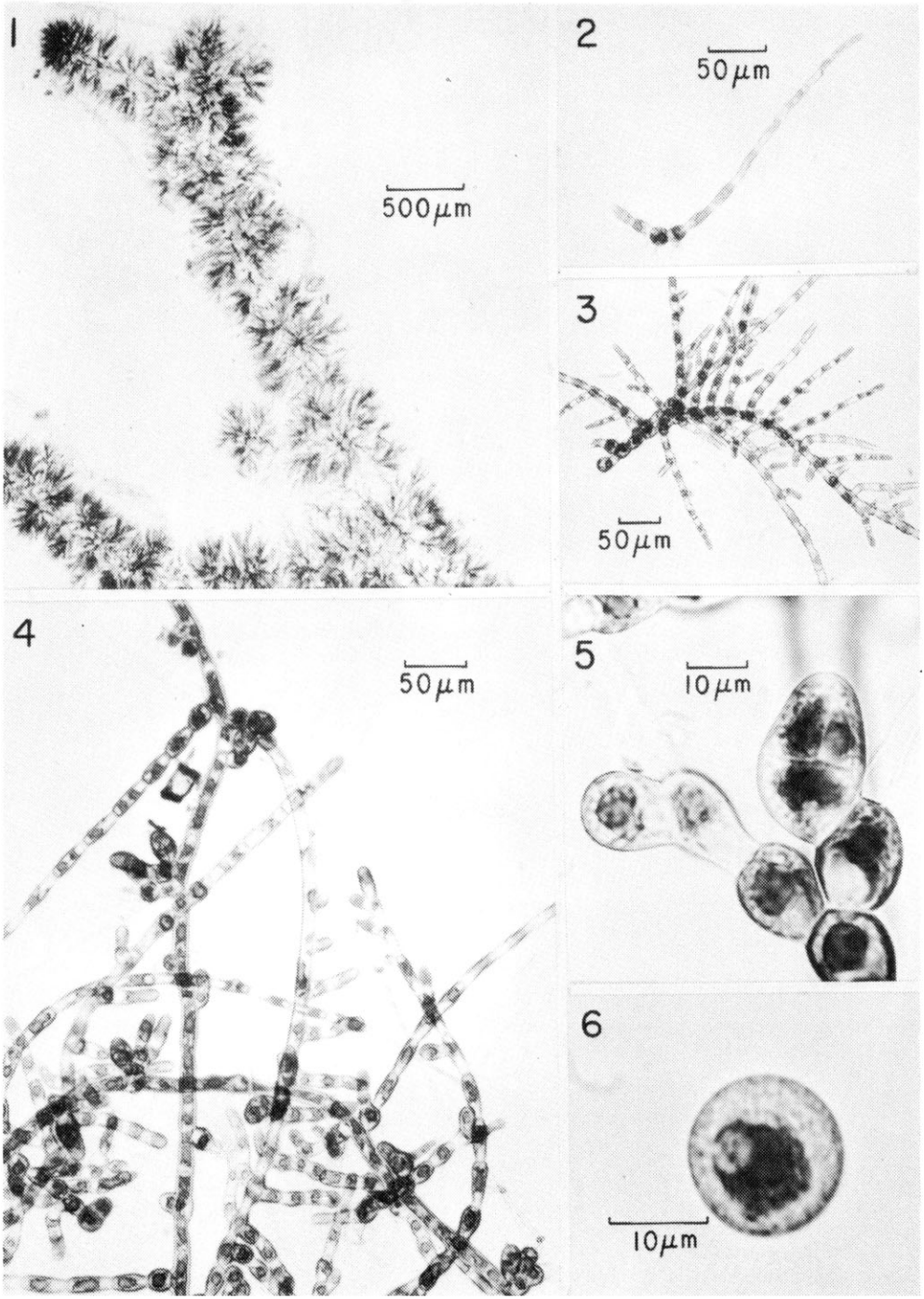
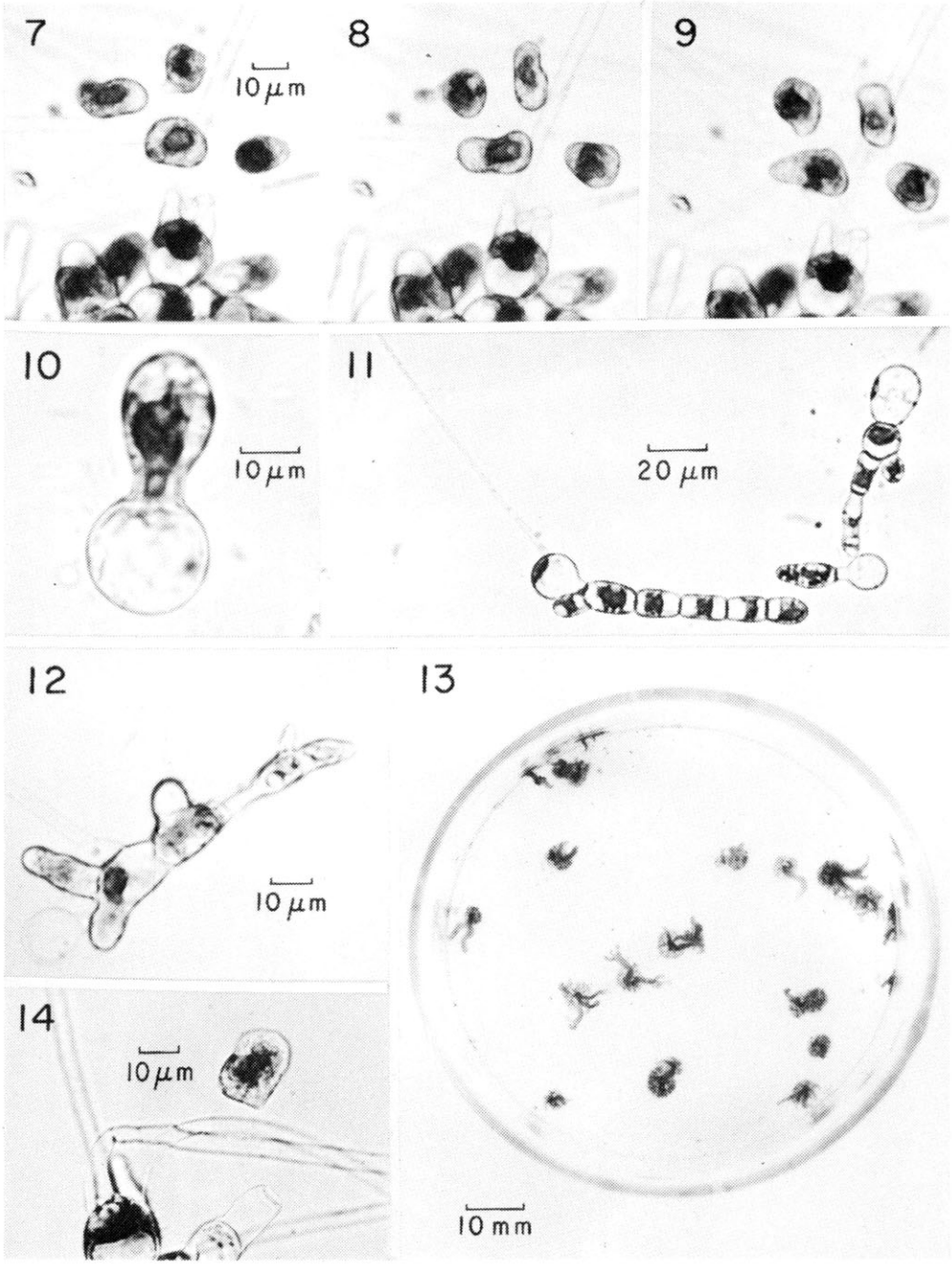


Fig 1. Portion of field-collected gametophyte. Fig 2. Filamentous tetrasporophyte from carpospore. Fig 3. Further development tetrasporophyte showing pronounced branching. Fig 4. Portion of a mature tetrasporophyte showing cruciate tetraspores within sporangia. Fig 5. Tetrasporangium showing formation of tetraspores. Fig 6. Released tetraspore.



Figs 7-9. Chronological sequence of 4 tetraspores after discharge from the sporangium (10-min intervals); note amoeboid shapes. Fig 10. Germinated tetraspore with a protuberance. Fig 11. Various stages of tetraspore germination, with formation of a transverse wall and hyaline hair. Fig 12. Later stage of tetraspore germination; note the empty original cell and the intercalary branching. Fig 13. Upright gametophytic plants; from tetraspores. Fig 14. Monospore released from sporangium.

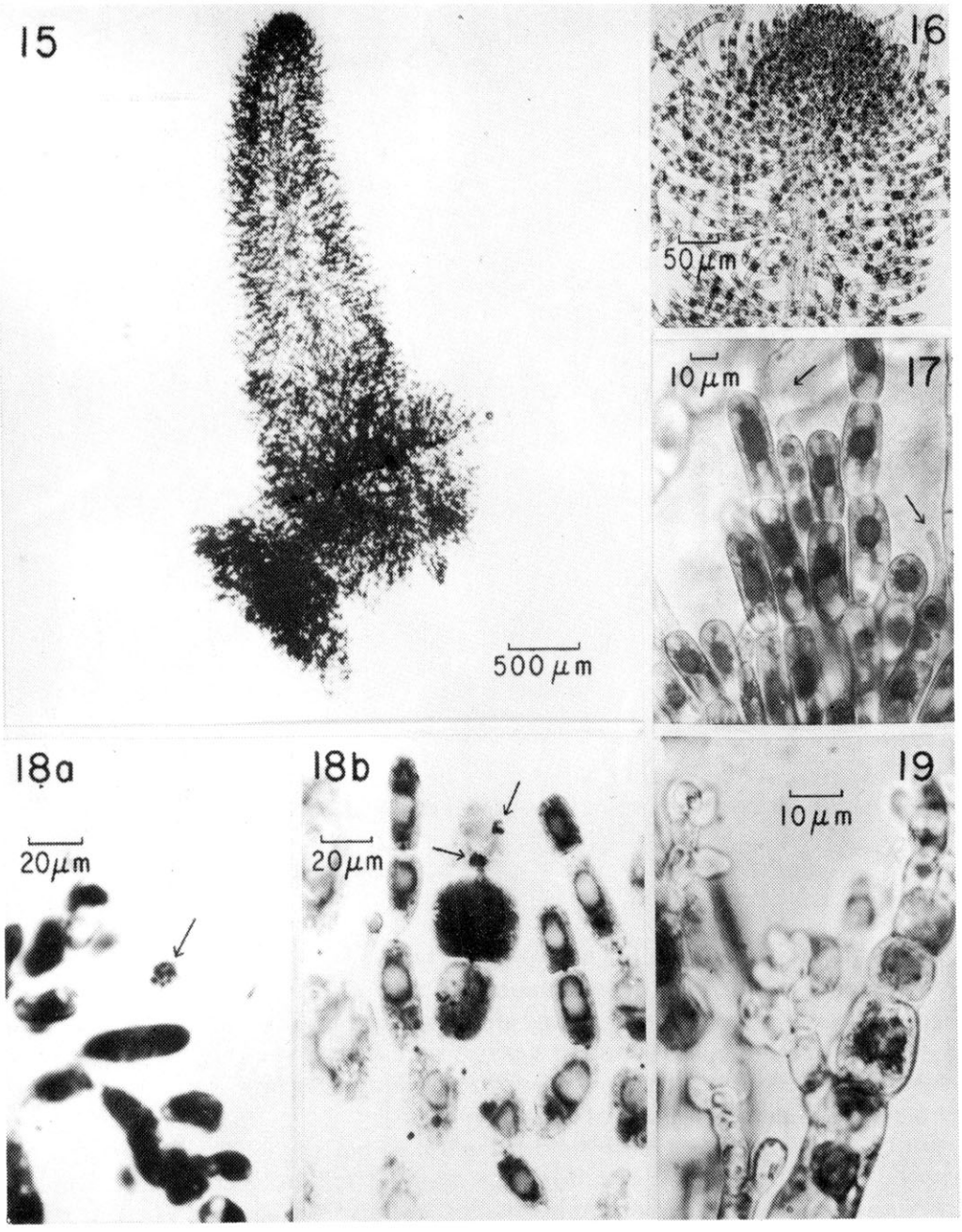


Fig 15. Gametophytic plant showing the arrangement of filaments and the discoid basal portion. Fig 16. Portion of gametophytic frond showing the apex and central axis. Fig 17. Portion of a gametophytic frond showing carpogonia. Fig 18. Mature gametophytic plant with discoid base and upright branched frond. Fig 19. Portion of a gametophytic frond showing spermatia.

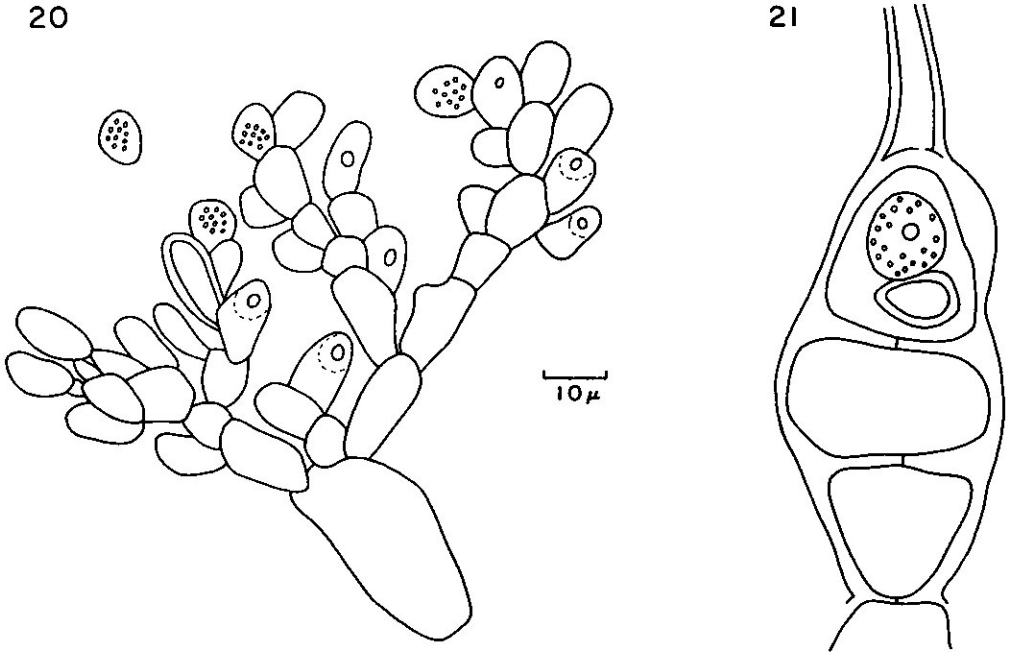


Fig 20. Chromosomes in spermatium; n=10. Fig 21. Carpogonial branch; diploid (2n=20) number of chromosomes in nucleus of carpogonium.

temperature and duration of light (Table 1). The culture medium was changed weekly. Filaments which had been maintained at 5°C, 8:16 h for 18 months were segregated into about equal portions and inoculated into small plastic dishes. Sets of 4 dishes were incubated under various conditions (Table I) for a period of 4 weeks. The medium was changed weekly and plants were observed for formation of tetrasporangia and for release of tetraspores.

For cytological studies, both field-collected gametophytic thalli bearing mature reproductive structures and cultured germlings from both tetraspores and carpospores were fixed with acetic acid ethanol (1:3), and stained with Wittmann's stain (Wittmann 1965).

Observations

Nemalion helminthoides becomes apparent in late summer, and has been reported from Prince Edward Island, Cape Breton Island, and the Atlantic coastal waters of Nova Scotia (*in herb.*). However, this species has not been reported from the Bay of Fundy (Wilson 1978).

The released carpospore was thin-walled, spherical (10-15 μm diam), and contained a single, stellate chromatophore. Soon after release, the plastid became diffuse, and the spore swelled and adhered to the substratum. Subsequently, a protuberance of the cell wall developed, into which the cytoplasm migrated, with formation of a transverse wall cutting off the original spore. In some cases a small portion of the cellular contents remained in the original spore. This type of development of the spore agrees with previous observations (Umezaki 1967a, b, 1972; Masuda & Umezaki 1977). Further development, through apical growth, resulted in a

filamentous sporeling (Fig 2) bearing hyaline hairs. Subsequently, branching occurred from intercalary cells of the filament, and within a month a mat of filamentous sporelings (Fig 3) formed on the substratum. Although we maintained these cultures for a year at various temperatures and durations of light, tetrasporangia failed to develop; monospores, however, formed with release and germination under all conditions. Results were similar with additional cultures established the following year.

Sporelings incubated under conditions in Table I were transferred to 5°C, 8:16 h for 18 months. When transferred to higher temperatures, tetrasporangia formed with 4 weeks (Table I) on lateral branches of the filamentous sporeling (Fig 4). Tetraspores, cruciately arranged (Fig 5), were released under all conditions above 5°C (Table I), with maximum release adjudged to occur above 13°C. Monospores (Fig 14), noted under all conditions of incubation, gave rise once more to tetrasporic filaments. Monospores usually were slightly larger than tetraspores, but subsequent development was the only certain means of distinguishing between these 2 types of spores.

After about a month at a higher temperature, formation of tetrasporangia was reduced considerably. In all cases, tetrasporic filaments grew well, but additional plants were formed either by monospores or by vegetative fragmentation of the filaments. Vigorous tetrasporogenesis could be induced only by returning the filaments to a relatively low (5°C) temperature for about a month, and then transferring these again to a higher temperature.

Before adhering to the substratum, discharged tetraspores (Fig 6) were amoeboid (Figs 7, 8, 9). Early development of filamentous, gametophytic sporelings (Figs 10-12) was similar to that of tetrasporic sporelings, as were the appearance of individual cells and the arrangement of cells within the filaments. Radial growth of these filaments resulted in formation of a loosely compacted base (Fig 13) which eventually became the basal holdfast of the upright gametophyte (Fig 15). A few lateral filaments in the central region of the filamentous base became compacted through outgrowths on a secondary level, thus initiating the upright frond. This, however, occurred only at 15°C to 20°C with a 16-h light period (Table I). These initial filaments became axes of fronds from which numerous, richly branched lateral filaments (Fig 16) of limited growth arose, giving the upright frond a smooth appearance (Figs 15, 16). In plants incubated in a light period of less than 16 h, irregular filamentous growth resulted.

Cells of the upright frond were rectangular (Fig 16), with central axial cells more elongated than lateral assimilative cells; hyaline hairs were occasionally formed from the latter. At this stage of development, the morphological features were characteristic of a gametophytic plant (Figs 1, 15, 18). Mature gametophytes, either branched or unbranched (Figs 13, 18), arose from the same parental material. Growth of the upright gametophyte took place under various conditions, although below 15°C filaments soon exhibited disordered growth of the assimilative cells.

After 2 months of incubation at 15°C, 16:8 h and 20°C, 16:8 h, gametophytes became fertile, spermatia (Fig 19) being produced on lateral branchlets of limited growth. Following an additional period of 6 weeks, these same plants formed carpogonial filaments (Fig 17) indicating that they were monoecious. The same results were obtained 3 times during the course of this study, yet the carposporophyte was not formed. During a further 6 weeks in culture, the plants deteriorated. In addition, plants exhibiting irregular filamentous growth developed spermatia after 4 months at 20°C, 12:12 h.

Cytological results showed that the haploid number of chromosomes was $n=10$ in somatic cells of gametophytes, spermatangia (Fig 20), and sporelings started from tetraspores. The diploid number of chromosomes was $2n=20$ as recorded in division of the nucleus within the carpogonium, and in sporelings from carpospores (Fig 21).

Discussion

In culture, the life history of *N. helminthoides* consists of a sequence of carpospore, microscopic filamentous tetrasporophyte, tetraspore, and macroscopic gametophyte which is presumably monoecious. Our cytological evidence indicates that meiosis occurs in the tetrasporangium. Previous investigators working with this species in culture were unsuccessful in obtaining the characteristic gametophytic phase from tetraspores (Fries 1967, 1969). However, Fries (1967) obtained abnormal thalli from tetraspores, and these formed what she believed to be young carpogonia. In our case gametophytes continued normal growth only under relatively high temperature (15° - 20°C) and long photoperiod (≈16h), or essentially summer conditions at our latitude. This could explain why *N. helminthoides* has not been recorded in the Bay of Fundy, where temperatures do not reach 15°C except in muddy areas at the head of the Bay.

The upright gametophyte was initiated from a group of compacted filaments in the center of the loosely formed filamentous base. This mode of development is somewhat different from that of *Liagora farinosa* and *Pseudogloiophloea confusa* in which erect gametophytic thalli formed from buds on the filamentous sporeling.

Both spermatia and carpogonial filaments were formed on the same gametophytic thallus; thus, this species is monoecious. But in culture these structures did not occur simultaneously, and moreover we were unable to obtain formation of the carposporophyte. This may indicate a lack of fertilization of the carpogonium, or possibly conditions of culture were unfavorable for development of the carposporophyte.

Tetrasporangia formed under various conditions of temperature and photoperiod. However, a prior excursion into low (5°C) temperature and short (8 h) photoperiod was necessary to induce sporogenesis and these may be regarded essentially as winter conditions. Quite probably carpospores formed during late summer and autumn give rise to the microscopic tetrasporophyte, which, in turn forms tetrasporangia in spring with increasing temperature and daylength (Söderström 1970). *Nemalion helminthoides* recurs year after year at the same sites, suggesting perennation by the tetrasporophyte. Furthermore, we have experienced no problem in maintaining this phase in culture for many years. Both Fries (1969) and Martin (1969) have reported on the occurrence of the tetrasporophyte in nature. Neither observed tetrasporangia in field-collected material, and Martin noted that the erect gametophyte seemed to arise directly from presumed gametophytic filaments. She further suggested that perennation may occur through the prostrate gametophytic filament.

Monospores commonly were formed on tetrasporic filaments under all conditions of temperature and photoperiod, and in addition to vegetative fragmentation, propagated this phase. Formation of monospores by the tetrasporophyte in culture has been noted previously by both Fries (1967) and Umezaki (1967a, b, 1972). We also observed monospore formation by the filamentous gametophytic sporeling as noted for *Pseudogloiosphlea confusa* (Ramus 1969).

Acknowledgement

We thank A. Barry and M. Greenwell for expert technical assistance.

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