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Laminarionema elsbetiae gen. et sp. nov. (Ectocarpales, Phaeophyceae), a new endophyte in *Laminaria* sporophytes

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SUMMARY

Laminarionema elsbetiae gen. et sp. nov. (Ectocarpaceae, Ectocarpales, Phaeophyceae), a new endophyte of *Laminaria japonica* Areschoug (Laminariales, Phaeophyceae), is described from Muroran, Hokkaido, Japan. *Laminarionema elsbetiae* grows in the host tissues forming networks in the epidermal and subcortical layers as well as penetrating into the cortical and medullary layers. Only phaeophycean hairs emerge from the surface of the host tissue. No reproductive cells were found in field material. However, under host-free culture the species formed three morphologically different reproductive structures. Macrosporangia containing a single large motile spore were formed under long and short day conditions below 20°C, transformed from vegetative cells, conical to elongated in shape, 50–75 μm in length and ca. 10 μm in diameter. Microsporangia were linear to lanceolate, sometimes branched, formed under long and short day conditions below 15°C. Unilocular sporangia were more or less irregular in shape, formed under short day conditions of 5–15°C, 60–75 μm in length and 40–45 μm in diameter. Sexual fusion between macro- and microspores was not seen. In mixed cultures of *L. elsbetiae* with young sporophytes of *L. japonica* Areschoug as well as *Saccorhiza dermatodea* (de la Pylaie) J. Agardh, *L. elsbetiae* infected both hosts, grew in the same manner as in natural hosts, and formed macrosporangia between host epidermal cells.

Key words: Ectocarpales, endophyte, infection, *Laminaria*, *Laminarionema elsbetiae* gen. et sp. nov., life history, Phaeophyceae.

INTRODUCTION

Sporophytes of Laminariales (so-called kelps) are considered to be good hosts for epiphytic and endophytic algae, because of their more or less solid thallus constructions and generally long life span. Although most older laminarialean sporophytes are infected by various epiphytes and endophytes, the taxonomy of these minute algae has not been well studied, especially that of endophytes. This seems to be due to the difficulties in examining the morphology of endophytes from field-collected material, thus it is sometimes rather difficult to obtain sufficient information for their identification. Therefore, when we started a taxonomic survey of endophytic brown algae in various laminarialean species, we examined field-collected material as well as cultured material derived from the endophytes found in the tissues of laminarialean sporophytes, in order to study their morphology and life history in detail. In the course of this survey, we noticed an unknown brown alga that forms a very distinctive reproductive structure, and which occurs rather commonly in older *Laminaria* tissues. Here we report the morphology, life history and taxonomy of this new phaeophycean endophyte.

MATERIALS AND METHODS

Sporophytes of various laminarialean species (e.g. *Laminaria japonica* Areschoug, *Costaria costata* (C. Agardh) Saunders and *Undaria pinnatifida* (Harvey) Suringar) were collected at Muroran (42°21′ N, 140°59′ E), Pacific coast of Hokkaido, Japan, in April, May, June, August, October and December 1992. Small fragments of the cortical layer of the kelps including phaeophycean endophytes were excised and precultured in plastic Petri dishes containing PESI medium (Tatewaki 1966) at 10°C for 3–4 weeks. Clonal cultures of the endophytes were established from those cultures by excising several-celled filaments of the endophytes. The culture conditions used were 5°C SD (short day; 8 h light and 16 h dark), 5°C LD (long day; 16 h light and 8 h dark), 10°C SD, 10°C LD, 15°C SD, 15°C LD, 20°C SD, 20°C LD and 25°C LD, under a photon flux of 30 μmol $m^{-2}s^{-1}$ (5°C) or 50 μmol $m^{-2}s^{-1}$ (10°C, 15°C, 20°C, 25°C) illuminated with day-light type white fluorescent tubes. Mixed cultures of the fertile endophyte filaments bearing macrosporangia and young sporophytes of *L. japonica* and *Sacchorhiza dermatodea* (de la Pylaie) J. Agardh were grown at 10°C LD, and the infected sporo-

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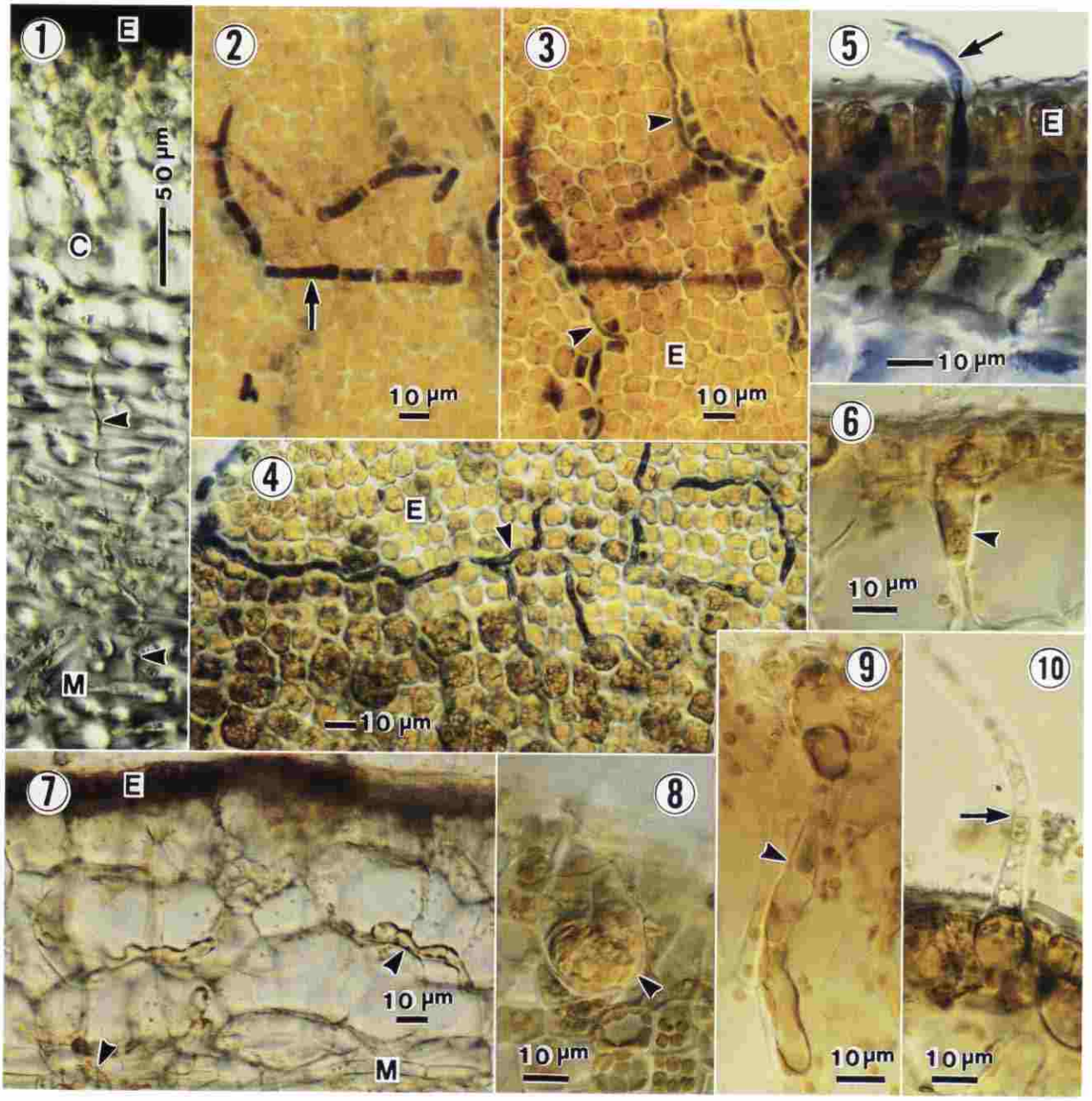
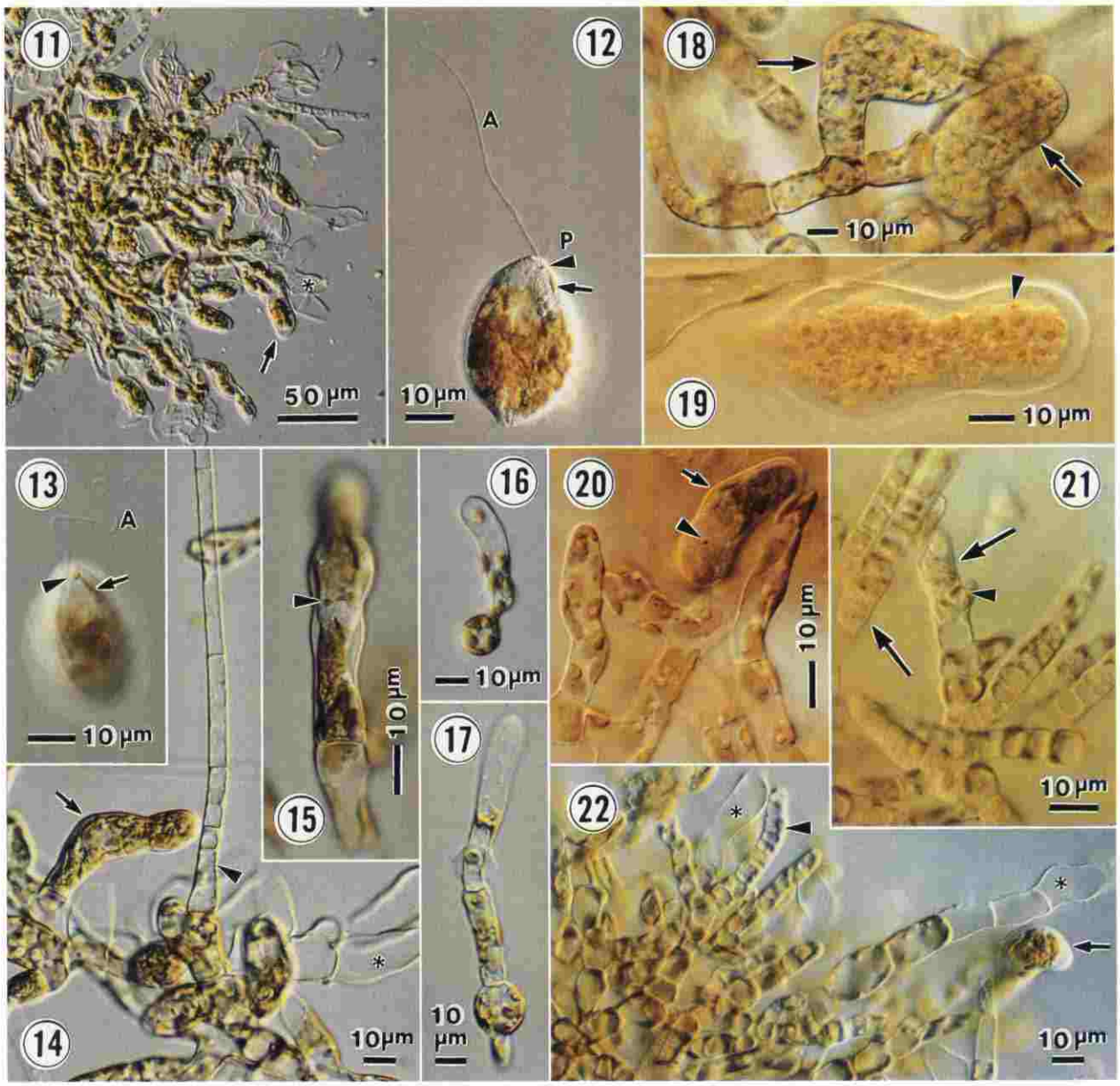


Figs 1–10. *Laminarionema elsbetiae* gen. et sp. nov. in host (*Laminaria japonica*) tissues in nature (Figs 1–5) and in culture (Figs 6–10). C, cortex; E, epidermis; M, medulla. Figs 2–5 stained with aniline blue. 1. Vegetative filaments (arrowheads) penetrating cortical and medullary layer of the host in cross section. 2–3. Emerging phaeophycean hair (arrow) and vegetative filaments (arrowheads) in host epidermal tissue (surface views of the same portion in different focal planes). 4. Network of vegetative filaments (arrowhead) between host epidermal and subcortical cells in tangential section. 5. Emerging phaeophycean hair (arrow) between host epidermal cells in cross section. 6. Germling of macrospore (arrowhead) penetrating between host epidermal cells in cross section. 7. Vegetative filaments (arrowheads) in cortical and medullary layer of host tissue. 8. Macrosporangium (arrowhead) borne among host epidermal cells in surface view. 9. Several-celled germling (arrowhead) of macrospore penetrating between host cortical cells. 10. Emerging phaeophycean hair (arrow) between host epidermal cells in cross section.

phytes were examined in cross and tangential sections. For specific staining of the endophyte filaments, infected tissues were preserved in 5% formaldehyde-seawater and stained with dilute aniline blue for 20–30 min. Infected tissues were then hand-sectioned in the planes parallel and perpendicular to the host surface.

RESULTS

Laminarionema elsbetiae was found in older laminae of *Laminaria japonica* collected in April, June, October and December. It was not found in the sporophytes of *Costaria costata* and *Undaria pinnatifida*. *Laminarionema elsbetiae*



Figs 11–22. *Laminarionema elsbetiae* gen. et sp. nov. in host-free culture. 11. Filaments bearing mature (arrow) and empty (asterisk) macrosporangia. 12–13. Released macrospores. A, anterior flagellum; P, posterior flagellum; arrowhead, stigma; arrow, chloroplast associated with stigma. 14. Phaeophycean hair (arrowhead), mature macrosporangium (arrow) and emptied macrosporangium (asterisk). 15. Mature macrosporangium; arrowhead shows stigma. 16–17. Germlings of macrospores corresponding to Figs 6 and 9 in host tissues. 18. Young unilocular sporangia (arrows). 19. Mature unilocular sporangium containing unispores with stigma (arrowhead). 20. Vegetative filaments containing chloroplasts with prominent pyrenoids and mature macrosporangium (arrow). Arrowhead shows stigma. 21. Plurilocular microsporangia (arrows). Arrowhead shows stigma of plurispore. 22. Micro- (arrowhead) and mature (arrow) and emptied (asterisk) macrosporangia borne on the same thallus.

formed undulate, branched, uniseriate filaments that grew between host cells, forming networks in the epidermal and subcortical layers (Figs 2–4) as well as penetrating into the cortical and medullary layers (Fig. 1). Infection of *L. elsbetiae* was not obvious to the naked eye from the surface view, and sometimes may be overlooked even using dissection microscopy. Cells of the vegetative filaments measured 40–50 µm in length and 8–15 µm in diameter, and contained several discoid or irregularly elongated chloroplasts having an obvious protruding pyrenoid. Only phaeophycean hairs emerged from the host surface (Figs 2, 5). No reproductive organs were found in field material.

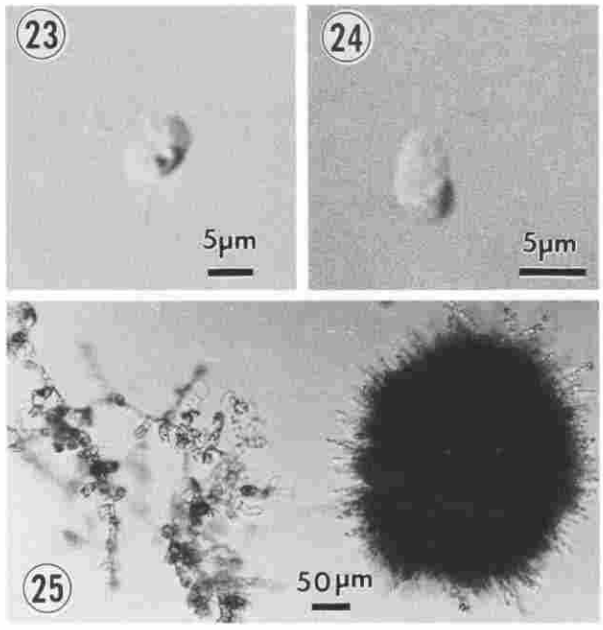
Cultures of *L. elsbetiae* that originated from isolated vegetative filaments and were grown under host-free conditions developed into branched filaments with phaeophycean hairs (Figs 11, 14). Each vegetative cell of the filaments contained

several irregularly disc-shaped chloroplasts with protruding pyrenoids (Fig. 20). They formed large macrosporangia transformed from lateral branches, sometimes in series, containing a single large motile spore in each sporangium under long and short day conditions below 20°C. The sporangia were conical to elongated in shape, 50–75 μm in length and *ca* 10–15 μm in diameter. The sporangia were easily distinguished from vegetative cells by their condensed cytoplasm containing well-separated chloroplasts, and the presence of an obvious stigma (Figs 11, 14, 15, 20, 22). In mature macrosporangia, the stigma was usually located near the central part of the sporangium where the chloroplasts were sparse. Released macrospores (swarmers) were pear-shaped with two heterokont flagella emerging almost at the tip of the cell. They contained 40–45 disc-shaped chloroplasts and were 25–30 μm in length (Figs 12, 13). The shape of the macrospores was very variable and tended to become roundish after a time. The extended anterior flagellum measured *ca* 70 μm and the posterior flagellum measured *ca* 20 μm. The posterior flagellum showed green flagellar autofluorescence upon excitation with blue light. Macrospores swam relatively slowly but essentially in the same manner as ordinary phaeophycean swarmers, rotating while swimming by undulating beats of the anterior flagellum. Chloroplasts were absent near the tip of the cell except for the one bearing the stigma and associated with the posterior flagellum. Unlike typical phaeophycean stigmata, these appeared to be independent from the chloroplast, but connected by a narrow bridge (Figs 12, 13). Released macrospores showed weak positive phototaxis. After settlement, they became rounded, 18–20 μm in diameter, and then germinated unipolarly (Figs 16, 17) to develop into branched filaments.

In mixed cultures of fertile *L. elsbetiae* forming macrosporangia and young sporophytes of *L. japonica* and *S. dermatodea, Laminarionema* macrospores attached and infected the host tissues by elongating germination tubes within host tissues (Figs 6, 9), and formed networks of vegetative filaments among cortical and medullary layers within 2 weeks (Fig. 7). Only phaeophycean hairs emerged from the host surface (Fig. 10). Macrosporangia formed among cortical cells of the host tissue (Fig. 8) and released macrospores.

Unilocular sporangia were formed in a similar manner as macrosporangia, transforming from vegetative cells in host-free cultures under short day conditions (Figs 18, 19). They were sessile, ovoid or pyriform but sometimes irregular in shape, 60–90 μm in length and 30–45 μm in diameter. Unispores released from unilocular sporangia were *ca* 8 μm in length, having two heterokont flagella with a longer anterior and a shorter posterior flagellum, containing a chloroplast with a stigma (Figs 19, 24).

Linear or lanceolate plurilocular sporangia (microsporangia) were occasionally formed under long and short day conditions below 15°C (Fig. 21). They were simple or branched, and each locule contained a swarmer (microspore). Released microspores were *ca* 9 μm in length resembling the



Figs 23–25. *Laminarionema elsbetiae* gen. et sp. nov. 23. Microspore released from plurilocular microsporangium. 24. Unispore released from unilocular sporangium. 25. Comparison of the thalli forming macrosporangia (left) and microsporangia (right) under the same magnification.

unispores (Fig. 23). Macrosporangia and microsporangia were usually borne on independent thallus (Fig. 25), but were sometimes observed on the same thalli (Fig. 22). Unispores and microspores germinated to develop into branched filaments similar to those from macrospores. Sexual attraction and gamete fusion were not found between the macrospores and microspores derived from different strains.

Laminarionema elsbetiae gen. et sp. nov.

Thalli endophytici intra *Laminariae* sporophyton, microscopici, filamentosi, ramosi, 8–15 μm diametro, cum pilis, macrosporangiis, microsporangiis, sporangiis unilocularibus. Nihil praeter pilos ex superficie hospitis prominens. Cellulae thalli cum chloroplastis aliquot discoideis ad elongatis cum pyrenoidibus. Macrosporangia 50–75 μm longa et 10–15 μm diametro, cum sporis unicis magnis flagelligeris 25–30 μm longis. Microsporangia plurilocularia 1–2 seriata, simplicia ad ramosa, cum sporis flagelligeris *ca* 9 μm longis. Sporangia unilocularia ovoidea ad pyriformia, 60–90 μm longa et 30–45 μm diametro, cum sporis flagelligeris *ca* 8 μm longis.

Thallus endophytic in *Laminaria* sporophyte, microscopic, filamentous, branched, 8–15 μm in diameter, with phaeophycean hairs, macrosporangia, microsporangia and unilocular sporangia. Only phaeophyceaen hairs emergent from host surface. Cells of the thallus contain several chloroplasts with pyrenoids. Macrosporangia, 50–75 μm in length and 10–15 μm in diameter, containing one large flagellated

spore with many chloroplasts and a stigma, 25–30 μm in length. Microsporangia, plurilocular, 1–2 seriate, simple or branched, containing flagellated spores, *ca* 9 μm in length. Unilocular sporangia ovoid or pyriform, 60–90 μm in length and 30–45 μm in diameter, containing flagellated spores *ca* 8 μm in length.

Etymology: The generic name originates from filaments (-nema) in *Laminaria*. The specific epithet commemorates the late Elsbet Fölster, University of Konstanz, for her contributions to phycology.

Type species: *Laminarionema elsbetiae* sp. nov.

Holotype: Herb. SAP Slide, June 1992, in *Laminaria japonica* Areschoug sporophyte, Muroran, Hokkaido, Japan.

DISCUSSION

The chloroplast morphology (i.e. several disc-shaped or elongated chloroplasts with a protruding pyrenoid in each cell) and the branched filamentous nature of the vegetative cells, lacking any discoid basal system or large hyaline medullary cells show that the taxon belongs to the family Ectocarpaceae of the order Ectocarpales, according to the current taxonomic system of the brown algae (Fletcher 1987; Womersley 1987). Regarding its generic and specific taxonomy, *Streblonema*, *Laminariocolax*, and *Gononema* are commonly known as more or less endophytic brown algae (Womersley 1987; Peters 1991). Taxa with vegetative filaments that penetrate deeply into the host tissues and only a small part of the thallus emerging from the host surface have been placed in *Streblonema* (Loiseaux 1970; Yoshida and Akiyama 1979; Fletcher 1983; Apt 1988; Peters 1991). However, the present endophyte of *Laminaria* considerably differs from any known species of *Streblonema* in the following characteristics: (i) Strictly endophytic nature of the thallus in which only phaeophycean hairs emerge from the host tissue surface; and (ii) occurrence of characteristic large macrosporangia forming a single very large macrospore with unique morphology. Some ectocarpacean species form large macrosporangia (e.g. *Hincksia* = *Giffordia*, Cardinal 1964), but they are more or less ectocarpoid and plurilocular. Therefore, we propose the new genus *Laminarionema* to accommodate the present taxon.

Sexual reproduction was not observed in Japanese *L. elsbetiae* and its life history is not yet fully clarified. In contrast, Peters and Ellertsdottir (1996) reported the occurrence of the same species at Helgoland, North Atlantic Ocean and confirmed the occurrence of sexual reproduction in their material. According to them, *L. elsbetiae* from Helgoland alternates between sporophytes forming unilocular sporangia and macrosporangia and gametophytes forming plurilocular gametangia. Sporophytes and gametophytes were slightly heteromorphic and male and female gametangia were isomorphic.

At Muroran, *L. elsbetiae* is not uncommon in older *Laminaria* sporophytes, although they were not found in *Costaria* and *Undaria*, which also grow in the locality. However, since *L. elsbetiae* in culture also infected sporophytes of *Saccorhiza*, which is systematically more distant from *Laminaria* than *Costaria* and *Undaria*, it is probable that *Laminarionema* does not show high host specificity and is potentially able to infect many other laminarialean species. The finding of *L. elsbetiae* on *Laminaria saccarina* (L.) Lamouroux (Peters and Ellertsdottir 1996) also support that its host specificity is not very strict. Therefore, although the ability of *L. elsbetiae* to infect *Costaria* and *Undaria* sporophytes was not examined in the present study, it is probable that *L. elsbetiae* can infect them. The absence of *L. elsbetiae* in those species in the field may be explained by the shorter life-span of *Costaria* and *Undaria* sporophytes, or that they were simply overlooked in the present study. For a more detailed survey of the presence of such endophytes, random sampling and examination of the host tissues in culture might be necessary.

The macrospore of *L. elsbetiae* is apparently one of the largest flagellated cells in the brown algae. Recently, the flagellated spore of *Homoeostrichus olsenii* Womersley was reported to be the largest motile spore in marine algae (Phillips and Clayton 1994). *Laminaria angustata* Kjellman also forms a large flagellated (but non-motile) egg (Motomura and Sakai 1988), which is as large as the *Laminarionema* macrospore. However, the flagella of those two flagellated cells are more or less remnant and atypical of brown algal flagella. They are considered to be successive stages in the evolution to non-motile spores or female gametes, which are more common in those orders (i.e. tetraspores in Dictyotales and eggs in Laminariales). In contrast, *Laminarionema* macrospores have heterokont flagella that are typical of the Phaeophyceae and swim in a similar manner to ordinary phaeophycean swarmers showing phototaxis. Therefore, the large flagellated cells in brown algae comparable to *Laminarionema* macrospores are the female gametes of *Cutleria* spp. (Yamanouchi 1912; Kitayama *et al.* 1992) and *Giffordia michelliae* (Harvey) Hamel (Cardinal 1964). Compared with them, the *Laminarionema* macrospore is unique in the position of the flagellar insertion and the characteristic stigma morphology.

Stigmata of the Phaeophyceae are believed to be involved in the photoreception of phototaxis, although their actual function has not been clarified. Recently, Kawai *et al.* (1990) suggested their reflective nature and Kreimer *et al.* (1991) experimentally confirmed their reflective function of focusing the stimulation light onto the flagellar swelling, which is considered to be the photoreceptive site in phototaxis (Kawai 1992). Based on these results, Kawai *et al.* (1990) postulated that phaeophycean phototactic swarmers use the periodic illumination signal caused by the cell rotation while swimming, the bending motion of the posterior flagellum responding to those signals. However, the possibility remained that the chloroplast behind the stigma and the flagellar swelling could act as a shading device for the photoreceptor. Therefore, the unique stigma of *Laminarionema*, which is almost independent of the chloroplast, strengthens the postulated reflective function of the stigma in the Phaeophyceae. The occurrence of green flagellar autofluorescence in

the posterior flagellum associated with the stigma in *Laminarionema*, that is considered to be involved in the photoreception further supports this postulation (Kawai 1988, 1992).

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