Jpn. J. Phycol. (Sôrui) 42: 377–384, December 10, 1994

Studies on *Dictyopteris longifolia* (Dictyotales, Phaeophyta) from South Africa. I. Production and morphogenesis of tetraspores

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Blackmore, N. L., Critchley, A. T. and Pienaar, R. N. 1994. Studies on *Dictyopteris longifolia* (Dictyotales, Phaeophyta) from South Africa. I. Production and morphogenesis of tetraspores. Jpn. J. Phycol. 42: 377–384.

The production and germination of tetraspores of *Dictyopteris longifolia* Papenfuss (in ed.), were promoted at 20°C and 22 μmol·m^{-2}·s^{-1} irradiance. Growth rates of the thalli and primary rhizoids of germinating tetraspores were compared at different temperatures; 20°C was found to be optimal. Production of tetrasporangia began within the cortex, followed by the elevation of a "cuticle" by the enlarging spore mother cell, prior to meiotic division. Germination of the tetraspores was initiated by elongation and division of a single primary rhizoid which established attachment, before production of a secondary rhizoid or thallus development. The production of unusual tip morphology of rhizoids, not reported in the genus before, is described.

Key Index Words: brown algal reproduction—Dictyopteris longifolia—*tetrasporogenesis and tetraspore morphogenesis—South African brown algae*

Members of the genus *Dictyopteris* (Dictyotales, Phaeophyceae) are found world-wide in tropical and temperate regions (Allender and Kraft 1983). This study investigates *Dictyopteris longifolia* Papenfuss (in ed.), which is found locally abundant along the sub-tropical east coast of South Africa (Stephenson and Stephenson 1972). The mature thallus (Fig. 1) consists of a fibrous holdfast and flat, dichotomously branching laminae which may reach a width of 2 cm and attain a length of 70 cm. The plants occur in clumped stands in areas of varying exposure to waves and sand inundation.

Tetrasporogenesis in *Dictyopteris divaricata* was examined by Inoh (1936) and Ishii *et al.* (1959). Germination of the tetraspores of the latter species was compared to two other members of the Dictyotales, namely *Padina japonica* Yamada and *Dictyota dichotoma* (Hudson) Lamouroux (Nishibayashi and Inoh 1959). Various studies have been performed to determine the effects of environmental parameters on the germination process in members of the order. Few reports, however, report the effect of temperature on the production and morphogenesis of tetraspores in members of the Dictyotales.

This investigation examines tetraspore formation on the surface of the lamina, morphogenesis following release and the effect of temperature on these features in *D. longifolia*.

Materials and Methods

Dictyopteris longifolia was collected at Palm Beach on the Natal coast of eastern South Africa (Fig. 2) in mid-May (autumn) 1989. Entire plants were removed from shallow pools and exposed reefs, at low water of spring tides. The material was collected in plastic bags and transferred to the laboratory in cooled, insulated boxes. After being cleaned of superficial epiphytes, individual

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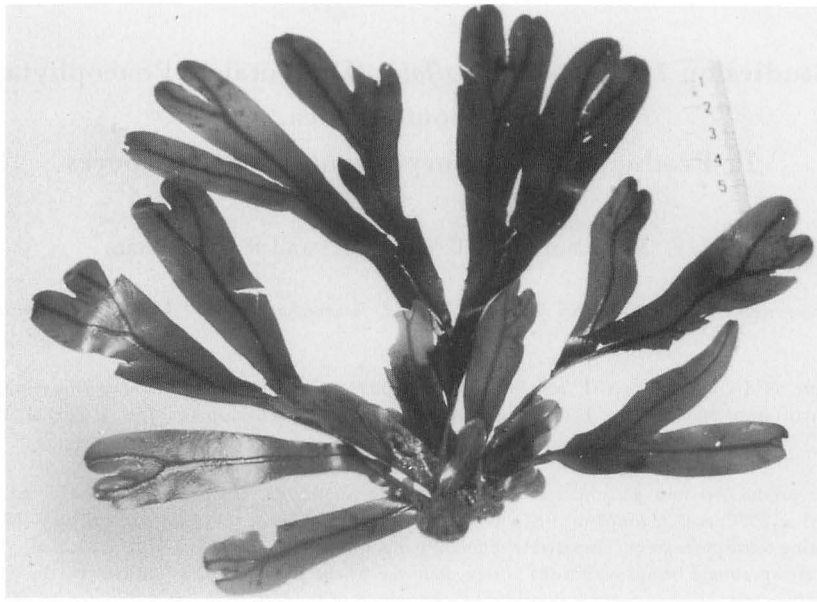

Fig. 1. Habit of *Dictyopteris longifolia*.

laminae were wiped with 100% alcohol and dipped in a germanium dioxide solution ($1\ mg \cdot l^{-1}$) in order to reduce subsequent diatom contamination (Lewin 1966). Each lamina was cut into segments (approximately 1.5 cm by 1.0 cm) and placed in "Sterilin" repli-dishes, in 5 ml of unfiltered seawater. The dishes were kept in a Labex (model L.T.G.C.) growth chamber at a temperature of 21.5°C (±2°C) and an irradiance of 22 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 14 : 10 light : dark cycle, for 30 days.

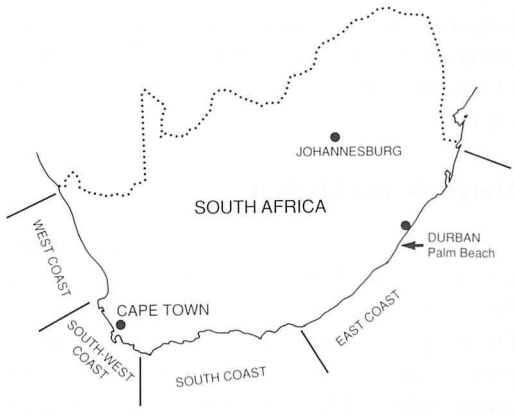

Fig. 2. Map showing the southern African coastline and the position of Palm Beach on the Natal coast (biogeographic zones after Stephenson and Stephenson, 1972).

Following release, tetraspores of *D. longifolia* were transferred to 50 ml, pre-sterilized Erlemeyer flasks, using Pasteur pipettes. The flasks were maintained in thermostatically controlled water-baths at an irradiance of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$, supplied by "cool" white fluorescent tubes. Cultures were kept at temperatures of 10, 15, 20, 25, 30 and 35°C (±2°C maximum variation). Tetraspore growth was measured on a Wild Inverted Microscope with a calibrated, graduated eyepiece. The mean of 30 measurements of the length of both the thallus and longest rhizoid were taken and graphed.

Sections (50 μm thick) of the spore producing thallus were cut using a freezing microtome, in a supporting medium of Tissue-Tek (Miles Laboratories, USA). Sections were stained with Toluidine Blue and mounted in glycerol for viewing under a Zeiss Photomicroscope. Drawings and photographs were recorded.

Results

Tetrasporogenesis was found to occur after 28 days in material maintained at 21.5°C

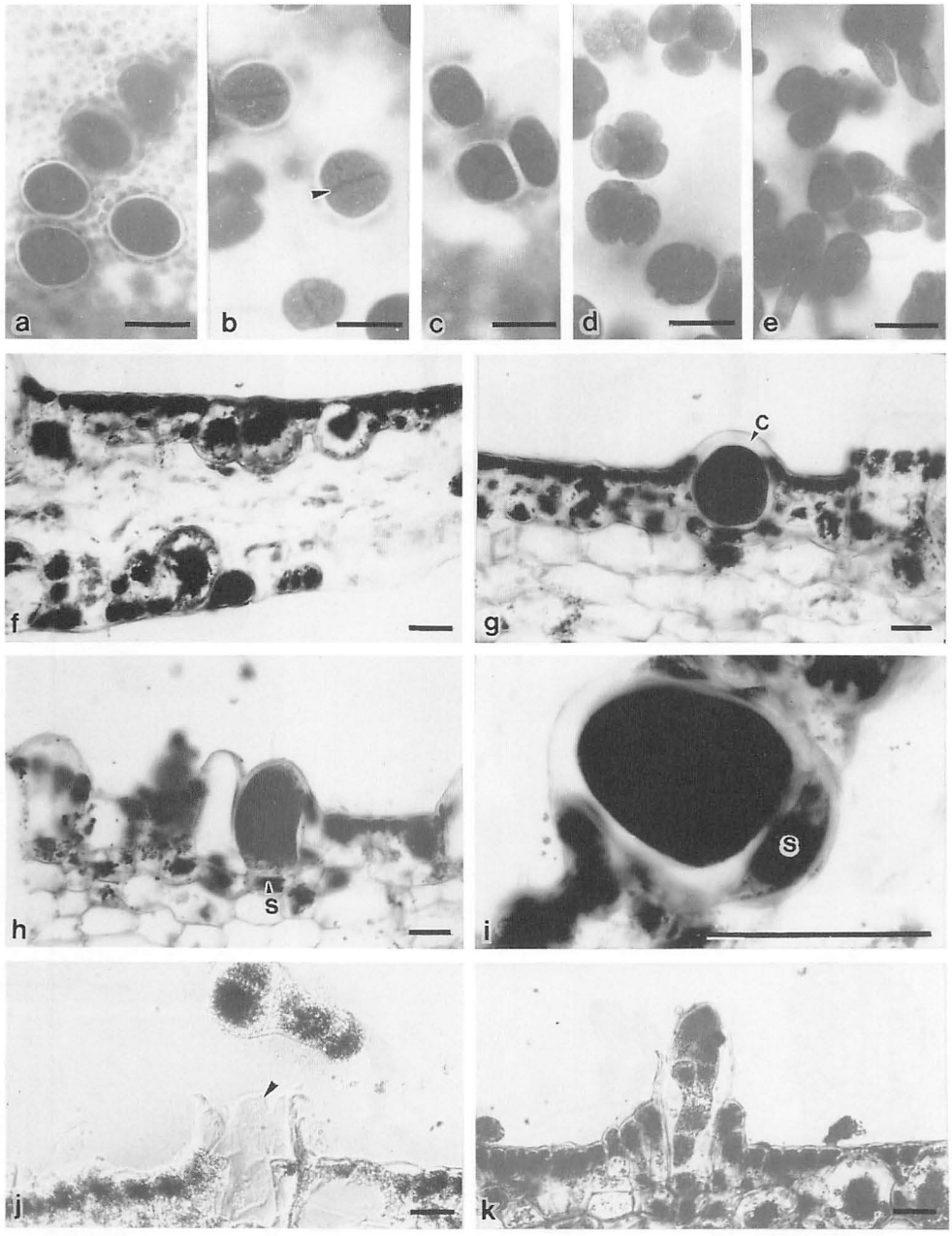

Fig. 3. a) Early spore mother cell development prior to division; b) Spore mother cell division (arrow); c, d) Formation of the individual tetraspores; e) Initiation of tetraspore germination whilst still in a clump on the lamina surface; f) Formation of the tetrasporangium within the cortex of the lamina; g) Early stages of spore mother cell protrusion, showing the raised cuticle (c); h, i) Mature spore mother cell, prior to meiotic division, partially embedded with the cortex, showing the stalk cell (s); j) Vacated tetrasporangium (arrow) following release of the tetraspores; k) Unreleased tetraspore germinating within the tetrasporangium. Scale bar=1000 μm.

(± 2°C), at an irradiance of 22 μmol·m^{-2}·s^{-1}. Tetrasporangia developed within sori at the surface of the laminae (Fig. 3a). The sporangia (i.e., site of meiosis) each produced four tetrahedrally arranged tetraspores (Figs. 3b, c, d). The sporangium began develop-

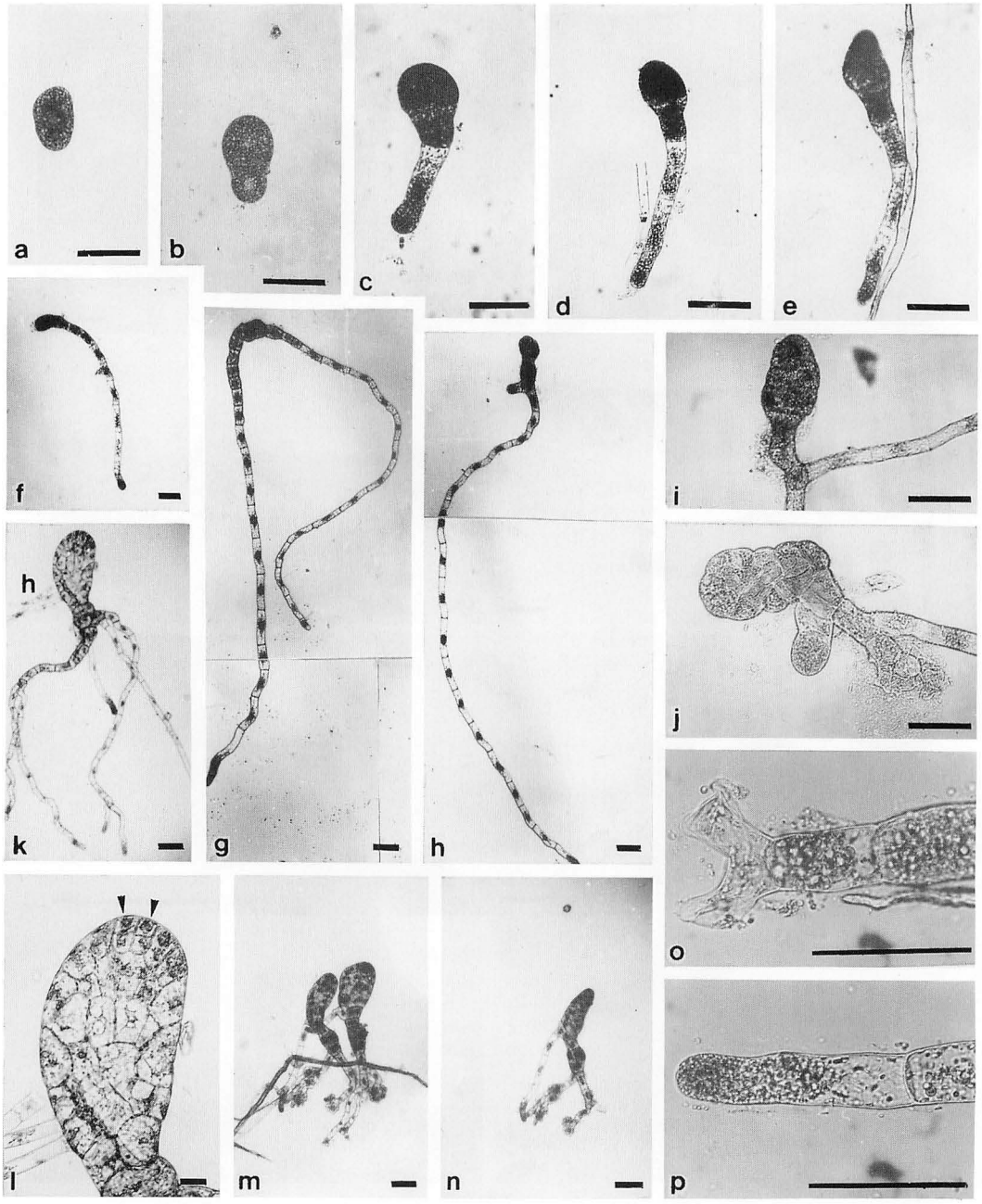

Fig. 4. a–f) Tetraspore germination (juvenile gametophyte), primary rhizoid elongation prior to development of the thallus or secondary rhizoid; g) Tetraspore with two well-developed rhizoids; h) Tetraspore showing early development of secondary rhizoid and thallus; i, j) Multicellular thallus in early stages of development; k) Juvenile gametophyte with multiple rhizoids and hairs (h); l) Apex of juvenile gametophyte showing dividing meristematic cells (arrow); m–o) Enlarging gametophyte and "jigsaw puzzle", aseptate, terminal outgrowths of rhizoids grown at 20°C; p) Normal rhizoid apex. Scale bar = 1000 μm.

ment from a sub-epidermal cell (Fig. 3f), which divided unequally to give rise to a stalk (basal) cell and sporangial initial. The sporangium increased in size and secreted a bi-layered cuticle (Figs. 3g, h, i) before dividing and releasing the tetraspores (Fig. 3j).

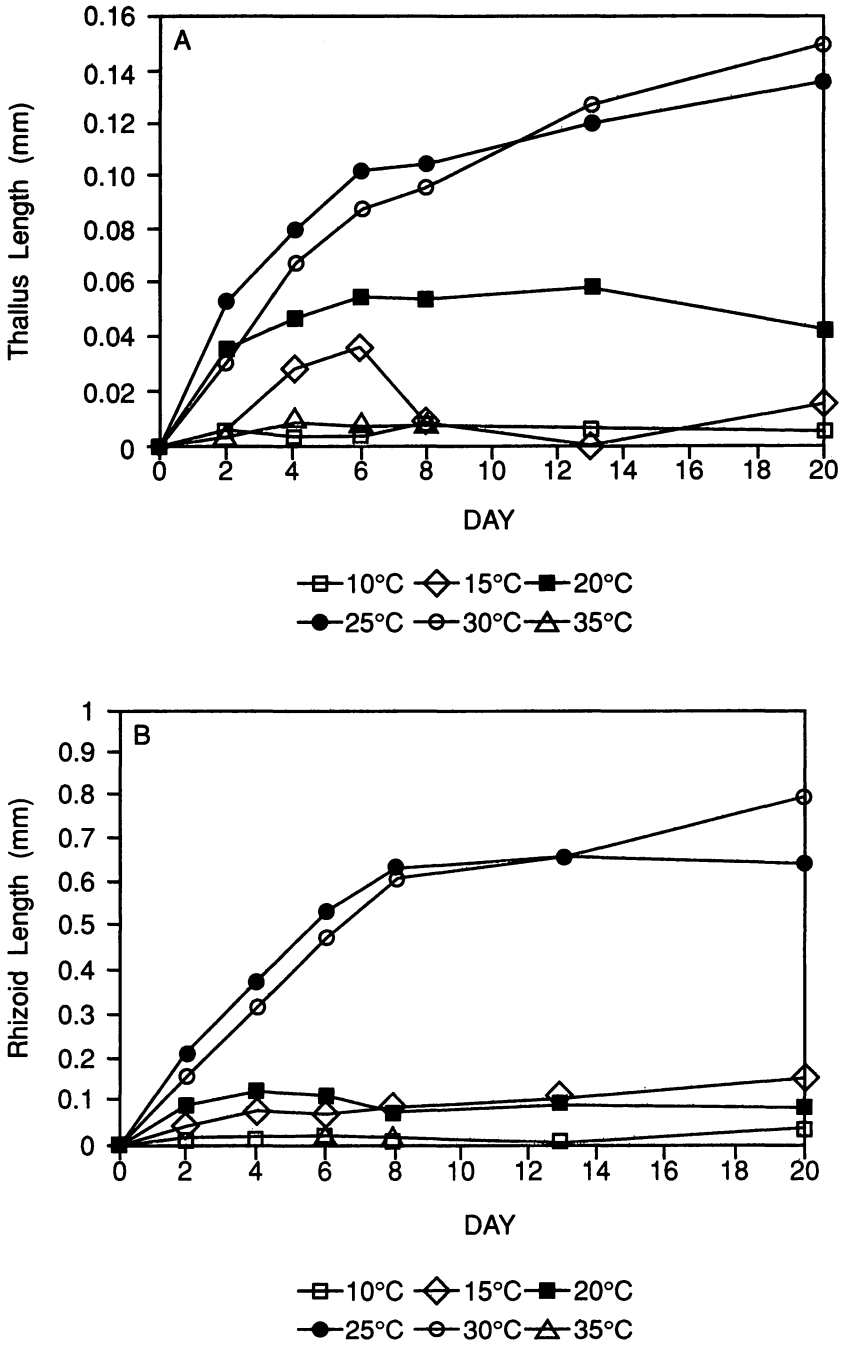

Fig. 5. Thallus (A) and rhizoid (B) elongation rates with respect to temperature.

Observations indicated that the stalk cell did not divide further (Figs. 3g, h, i). In some cases the four spores produced by meiosis, did not separate before germination began (Fig. 3e) and occasionally were not released from the sporangium at all before germination was initiated (Fig. 3k). Lack of water movement around the spores under experimental conditions may account for the *in situ* developmental patterns observed. Germinating tetra-

spores on the blades of *D. longifolia* have not been observed in the field.

Morphogenesis of the spores began with an initial elongation of one side of the cell (Fig. 4a), before the first division occurred (Fig. 4b). The latter polarised the spore into rhizoidal and thalloid poles (Figs. 4b, c). Further growth was observed to occur with rapid elongation and division of the cells of the rhizoid (Figs. 4c, e, f). In some spores a secondary rhizoid was observed to develop prior to, or during, thallus development (Figs. 4g, h, respectively). Once the thallus became multicellular (Figs. 4h, i, j, k, l) and well established, the apical meristematic cells and pit became visible; hairs were also produced (Figs. 4k, l). It was observed that a large number of the individuals maintained at 20°C developed a highly branched "jigsaw puzzle" terminal rhizoid system (Figs. 4m, n, o), as opposed to the normal rounded tip (Fig. 4p). This phenomenon was rare in cultures kept at other temperatures.

Growth and development of the tetraspores differed under the various temperatures (Fig. 5). The optimum temperature range for development of both thallus and rhizoid was between 20°C and 25°C, at 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The increase in length of the longest (primary) rhizoid at 20°C and 25°C, and to a lesser extent at 15°C and 30°C, was considerable up until day 8. After this the rate of elongation decreased in all treatments. No elongation of the primary rhizoid occurred at 10°C or 35°C. The thalli elongated at a much slower rate than the rhizoids, but the 20°C and 25°C individuals still had the greatest growth rate. There was either a slight initial elongation (15°C and 30°C) or no elongation at all (10°C and 35°C) of the thalli at the other temperatures. At 35°C there was no development of either the thallus or the rhizoid.

Discussion

Development of the tetrasporangium in *D. longifolia* differs from that described for *D. divaricata* (Ishii *et al.* 1959; Fig. 6). The latter is reported to have a supporting stalk cell which divides into two to four cells. The tetraspore mother cell is initiated and develops superficially and there was no elevated cuticle reported for the field collected material. *D longifolia*, however, has a single stalk cell (Figs. 3h, i) and the sporangium elevates a bi-layered cuticle (Figs. 3g, h, i; 6). The spore mother cell remains partially embedded in the cortex of the thallus. Meiosis results in the production of four tetraspores on the surface of the thallus. This pattern of tetrasporogenesis is similar to that described for *Padina japonica* (Ishii *et al.* 1959) which similarly has one stalk cell and an elevated cuticle. However, the tetrasporangium in *P. japonica* develops externally, which is more similar to *D. divaricata*.

The lack of separation of released spores, or lack of release of spores from the sporangium altogether, prior to germination (Figs. 3e, k) was not observed to occur in the field where plants are exposed to wave activity. All the aggregated spores appeared to be equally viable in culture and germinated in the same way and at the same rate as separately released spores.

Development of the tetraspores of *D. divaricata* was described as first producing a "multicellular, oval body" that developed one or several protruding cells, which later became the meristematic, apical cell of the juvenile gametophyte plant (Nishibayashi and Inoh 1959). Inoh (1936) described the division and elongation of the rhizoid, prior to the development of the protrusion from which the thallus developed. A tetraspore may produce more than one of these protruding cells. Although *D. longifolia* was not observed to produce more than one protrusion, the process of the initial elongation of the rhizoid followed by the development of the thallus, was similar to the previous author's descriptions.

Initial morphogenesis of *D. longifolia* tetraspores was similar at all temperatures investigated. However, at the stage when a secondary rhizoid emerged or the thallus began development, there was considerable variation and a large number of peculiarities were

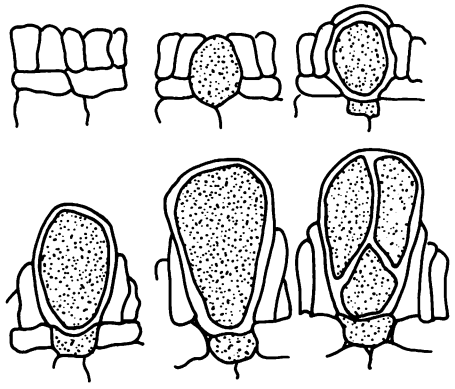

Fig. 6. Tetrasporogenesis in *Dictyopteris longifolia*.

observed. The "jigsaw puzzle" terminal rhizoid observed in *D. longifolia* (Figs. 4 m, n, o; 7), has been reported for the developing tetraspore rhizoids in *Dictyota dichotoma* (Nishibayashi and Inoh 1959; Gaillard 1977; Gaillard *et al.* 1986). The structures consist of lobed proliferations of the terminal cell of the rhizoid, lacking any cross-walls. This undivided proliferation of the rhizoid has not previously been reported for other species of *Dictyopteris*. Other than this peculiarity, morphogenesis was similar to that described by Nishibayashi and Inoh (1959) and Inoh (1936), with the primary rhizoid emerging and developing prior to the development of the thallus from a protrusion of the original spore (Figs. 4a-h). Taking similarities of spore production, germination and juvenile gametophyte development into consideration, there seems to be a high degree of consistency in the Order Dictyotales.

D. longifolia is found in sub-tropical to temperate waters with a seawater temperature range of 15-25°C. Temperatures lower or higher than these are rarely experienced. It could thus be expected that the optimal temperature range for spore germination and growth would occur in this range. The growth study performed on the spores of *D. longifolia* showed that maximum growth of both the rhizoid and the thallus occurred at 20°C to 25°C. There was slight initial growth at temperatures lower than 20°C but this ceased after the first five days. At temperatures greater than 25°C, there was an initial

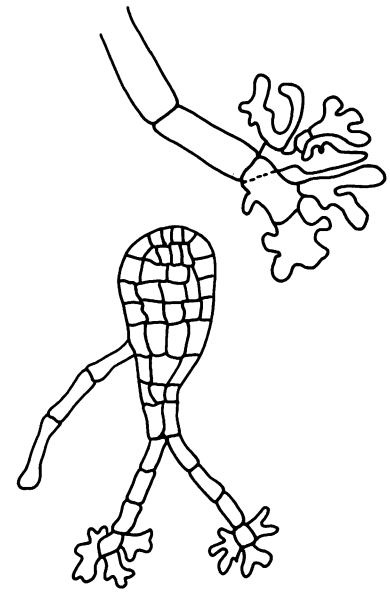

Fig. 7. "Jigsaw puzzle" terminal rhizoid proliferations in *Dictyopteris longifolia*.

increase and then subsequent decrease in length due to necrosis (Fig. 5).

Large numbers of tetraspores are produced by a single lamina and, although many do not germinate, prolific production may explain the clumped appearance of patches of *D. longifolia* in the field. Tetraspores may get caught in the mats of rhizoids and laminae of adult plants and germinate without dispersal. Alternatively, the unreleased or unseparated spores may increase clump size. A further possible explanation for clumping has been reported in *D. membranacea* (Katsaros and Galatis 1988), in which it was observed that cells of the germinating spore were capable of vegetatively producing plantules. This may account for the very localised distribution of the plants. *D. longifolia* was not observed to produce more than one plantule from each tetraspore. However, isolated rhizoids from the holdfast of *D. longifolia* are capable of producing plantules vegetatively (own observation), as has been reported for *D. divaricata* (Tokida *et al.* 1953); this may be another contributing factor to the clumped distribution of individuals in natural stands.

Acknowledgements

We thank Dr T. Horiguchi and Dr I. Mine for kindly providing Japanese articles and translations, Dr M. Aken for commenting on the script and Mr P. Tshabalala for photographic assistance. Mrs M. Marshall is thanked for her patience and retyping of the manuscript. The computer assistance rendered by Mr A. Blackmore is greatly appreciated.

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Blackmore[1], N. L. · Critchley[2], A. T. · Pienaar[2], R. N.：南アフリカ産褐藻 *Dictyopteris longifolia*（アミジグサ目）の研究 I．四分胞子の生殖と形態形成

褐藻 *Dictyopteris longifolia* Papenfuss の四分胞子嚢形成と四分胞子の発芽は 20°C，光強度 22 μmol·m^{-2}·s^{-1} の条件下でおこった。葉状体と四分胞子の発芽体の一次仮根の成長速度を様々な温度条件下で比較したところ 20°C で最も成長が良かった。四分胞子嚢の形成は皮層内で始まり，続いて減数分裂に先立つ胞子嚢母細胞の増大によるクチクラの上昇がおこった。四分胞子の発芽は一本の一次仮根の成長と分裂により始まり，これにより基物に付着した。続いて二次仮根の発達と葉状体の発達がおこった。この属でこれまでに報告されていない特異な形態の仮根末端部の形態につき報告した。([1]Natal Parks Board, St. Lucia, Natal, South Africa, [2]Department of Botany, University of the Witwatersrand, Johannesburg, P O WITS 2050, South Africa)

(*Received July 17, 1994. Accepted September 25, 1994*)