Proc. Natl. Sci. Counc. ROC(B)
Vol. 23, No. 2, 1999. pp. 85-98

Development of Staminate Cone and Microsporogenesis in *Cephalotaxus wilsoniana* Hay.

HANN-CHUNG LO AND YU-FEN WANG

Department of Forestry
National Taiwan University
Taipei, Taiwan, R.O.C.

(Received December 7, 1998; Accepted February 4, 1999)

ABSTRACT

Cephalotaxus wilsoniana Hay. is a species of gymnosperms endemic to Taiwan. This study examined morphologically and anatomically the development of staminate cones and microsporogenesis of the species for the purpose of conservation. The duration from formation of staminate buds to shedding of pollen grains in *C. wilsoniana* is more than six months. Staminate buds appear in early October. The archesporium originates in microsporophyll primordium in late October. The sporogenous tissue proliferates and the tapetum layer becomes distinct in the middle of November. Pollen mother cells develop at late December and rest in January. Meiosis takes place in pollen mother cells in mid-February. Tetrad microspores separate from each other between late February and early March. A generative cell and a tube cell appear in each pollen grain in late March. Pollen grains are shed between late March and early April. A mature compound head consists of 8~9 (5 basal, 2~3 lateral and 1 terminal) staminate cones. Each male cone has a cone axis, on which 4~15 microsporophylls are spirally arranged. There are two forms of microsporophyll which bear 2~4 abaxial hyposporangia or 4~6 whorled perisporangia on a stalk.

Key Words: *Cephalotaxux wilsoniana* Hay., staminate cone development, microporogenesis

I. Introduction

The genus *Cephalotaxus* earlier included six species (Pilger and Melchior, 1954) but now includes nine species (Fu, 1984), of which seven species are distributed in mainland China and one species in Taiwan. *Cephalotaxus wilsoniana* Hay. is the only species found endemic to Taiwan (Li and Keng, 1994).

Earlier phenological observations on staminate cones in *Cephalotaxus* were described by Lawson (1907). He noticed that the shedding of pollen grains of *C. drupacea* begins in March and extends for three weeks. According to Coker (1907), pollen grains of *C. fortunei* are two-celled at the time of shedding. Gong and Chiang (1971) reported briefly that pollen grains of *C. wilsoniana* mature between January and March and are shed in March.

Singh (1961) studied the life history and systematic position in *C. drupacea*. Wilde (1975) gave a new interpretation of microsporangiate cones in Cephalotaxaceae and Taxaceae, with reference to morphological and anatomical significances.

The staminate cone structure of *C. wilsoniana* was described by Li and Keng (1954), Gong and Chiang (1971), Liu and Liao (1980) and Liu *et al*. (1988), but their observations were limited, and there were differences among them. It is the purpose of this study to inventory the development of staminate cones and microsporogenesis.

II. Materials and Methods

Reproductive buds of *Cephalotaxus wilsoniana* Hay. were collected during the stages of their development from Dec. 23, 1994 to Nov. 10, 1995 in Nan-tou County, in the central part of Taiwan at Chi-tou, the Experimental Forest of National Taiwan University and at Tsuei-fuen, the Natural Protection Area of Ruei-yen Brooke (Table 1).

Microtechniques for sectioning materials followed Johansen (1940) and Chiang (1975). Buds with stalk were collected and immediately fixed in FAA (formalin:acetic acid:50% ethanol = 1:1:18 in volume) after collection. Materials were then dehydrated in TBA-series and embedded in paraffin for sectioning. Sections were obtained on a sliding microtome with a thickness of 12-15 μm. After double staining with safranin-O and fast green or methyl green and dehydration, sections were mounted in Canada balsam resin for observation and photography under a light microscope. Morphological photography was performed under a stereo microscope.

III. Results and Discussion

After serial morphological and anatomical observations on head buds of staminate cones, the development and microsporogenesis included the following stages:

Table 1. Number of Staminate Heads Collected on indicated Dates (yy/mm/dd) in Different Development Stages (i-xii) from the Locality "Chi-Tou" (CT) or "Tsuei-Fen" (TF) and then Sectioned

date(locality)	no. of staminate heads		stage of development
	collected	sectioned	
94/12/23(CT)	12	4	(iv) development of pollen mother cells
95/01/17(CT)	15	3	(v) resting phase of pollen mother cells
95/01/27(CT)	27	6	
95/01/31(CT)	19	2	
95/02/08(CT)	10	4	(vi) degeneration of the middle layers
95/02/18(CT)	10	4	(vii) meiosis of pollen mother cells
95/02/28(CT)	14	4	(viii) development of microspore tetrads
95/03/10(CT)	11	3	(ix) development of free microspores
95/03/16(CT)	8	1	
95/03/24(CT)	6	2	(x) completion of head development
95/03/28(CT)	12	3	(xi) formation of 2-celled pollens
95/04/03(CT)	5	2	(xii) pollen grain shedding
95/04/15(CT)	6	1	
95/10/05(CT)	16	3	(i) origination of microsporophyll
95/10/11(TF)	10	2	
95/10/23(CT)	15	4	(ii) differentiation in microsporangium
95/11/10(CT)	19	4	(iii) proliferation of sporogenous tissue

1. Origination of Microsporophyll (1995/10/05)

The compound nature of the so-called male "capitulum" in *Cephalotaxus* has been long recognized by various authors: Thibout (1896), Worsdell (1901), Dluhosch (1937), Singh (1961) and Wilde (1975). A bud of a staminate cone is a swelling body at the end of a vegetative twig end, and there is no conspicuous constriction at the bud base (Fig. 1(a)). Wilde (1975) defined this kind of short, determinate vegetative branch as a peduncle, which is axillary to leaves of the current season's growth in *C. harringtonia* var. *harringtonia*.

Buds are mainly covered with scale leaves, which are spirally arranged as on the twig. Scale leaves, on the upper part of the bud, are acute at the apex and on the lower part, and are acuminate at the apex. A few young cone bracts are present at the apex of the bud and over the scale leaves (Fig. 1(b)). Within these bracts, there are many primordia of microsporophyll around a main stele. That is the so-called fertile branch, which is borne on the peduncle (Wilde, 1975). Figure 1(c) shows a primordium which extrudes toward one side in a central longitudinal section. Along the convex edge of the primordium, the outer layer of cells, which neighbor each other closely with anticlinal cell walls, is the protoderm. Immediately beneath the protoderm, a few cells are in anticlinal-orientated files and appear to be formed after periclinal cell division. They are hypodermal cells of archesporium, which may give rise to microsporangium. This is similar to the origin of archesporium in *C. drupacea* (Singh, 1961). A plate of hypodermal archesporial cells, five to seven cells wide, is present on the abaxial surface near the base of the microsporophyll.

2. Differentiation in Microsporangium (1995/10/23)

Globose buds are bigger than 1.5 mm in diameter and well stalked with a stem. Cone bracts cover the bud body above leaf scales (Fig. 2(a)). In the CS, a fertile branch appears at the center (Fig. 2(b)), around which there are 5 basal cone axes. Each basal cone axis bears a few microsporophylls, each of which gives rise to 2-6 microsporangia. Outside each basal cone axis, there is an opposite bract. Some cells of microsporangium protoderm undergo anticlinal cell division (Fig. 2(c)). Beneath the protoderm, there are 2-3(4) layers of cells of more or less periclinally flatted form. They will develop to become the wall layer. Cells at the core center are large, with a large nucleus, and are densely compacted together. This is the sporogenous tissue with meristem characteristics. Similarly, in *C. drupacea*, archesporial cells divide periclinally to form the primary parietal layer, which is organized in a four-layered wall, and the primary sporogenous layer (Singh, 1961).

3. Proliferation of Sporogenous Tissue (1995/11/10)

Buds become bigger, over 3.0 mm thick, and are in compressed globose form. The length of the bracts is about two times that of the scale leaves. Inside the bracts, there are clusters of microsporophylls. A microsporophyll consists of an apex, microsporangia and a stalk. The apex is toward the bud axis and contains a resin cyst. It will develop the so-called "lamina" (Singh, 1961). The distal part of the microsporangia is abaxially adnate to the stalk.

Three kinds of tissues are differentiated in the microsporangium (Fig. 3). Epidermal cells are rectangular in form and have a nucleus. Beneath the epidermis, the wall layer is constructed with 2-3(4) layers of flat, thin cells, of which the innermost layer will develop into the tapetum. Cells of sporogenous tissue are compacted together in the core center. They are isodiametric in form and have a large, dark-stained nucleus. In *C. drupacea*, the cells of the innermost wall

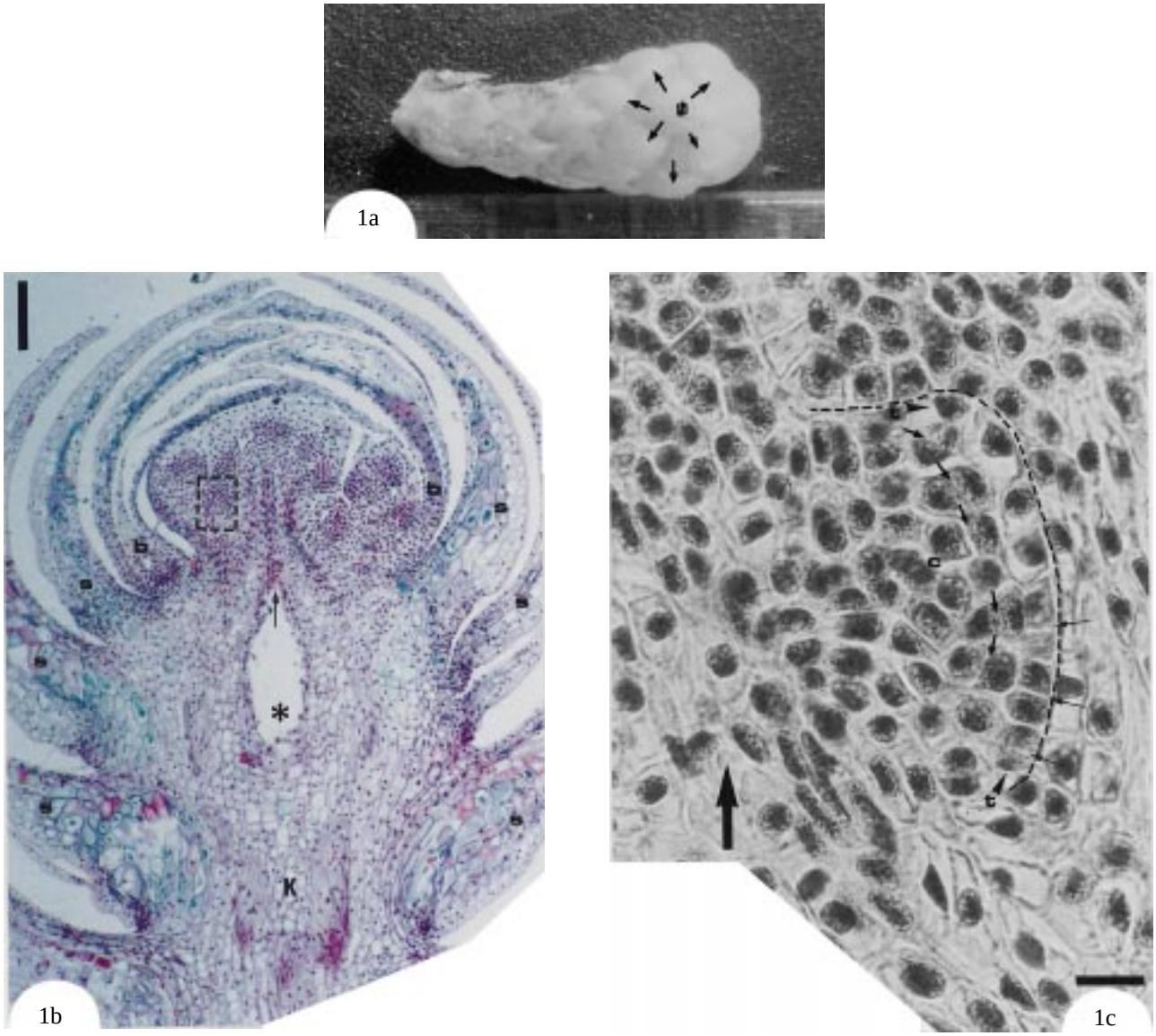
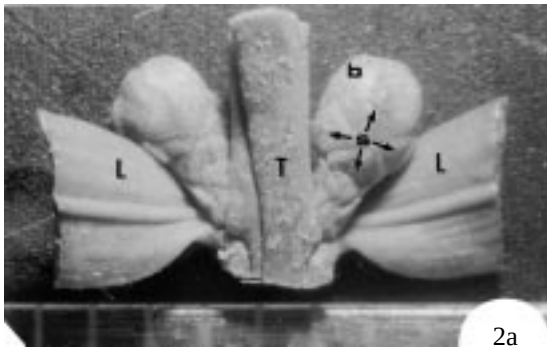
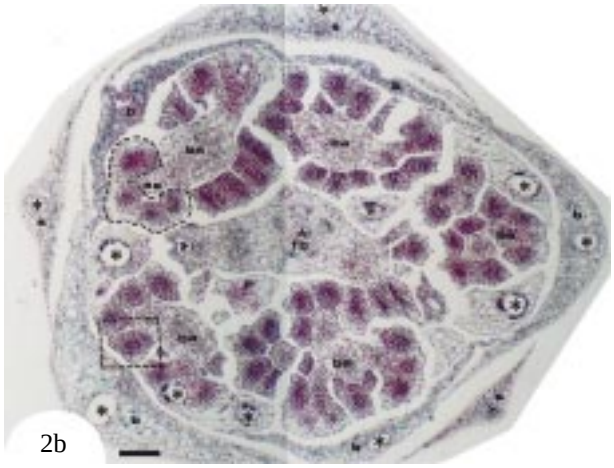


Fig. 1. Origination of microsporophyll (1995/10/05) (Chi-tou). (a) (SV) A male bud on the end of a twig. The whole organ covered with scale leaves (s), which are arranged in a spiral manner. (b) (LS) (225 μm) A bud consisting of a fertile branch (arrow) on a stalk (K) and groups of meristematic tissues. A few bracts (b) envelope the bud body. Resin duct (asterisk). (c) (LS) (22.5 μm) Enlargement from the frame area in Fig. 1(b). Along the border (dotted line) of a convex meristem body (c), there is a layer of protoderm (t), which is divided by anticlinal cell walls (long arrows), and beneath the protoderm periphery cells neighboring each other by periclinal cell walls (short arrows). The inner tissue is irregularly organized. Axial direction (big arrow).

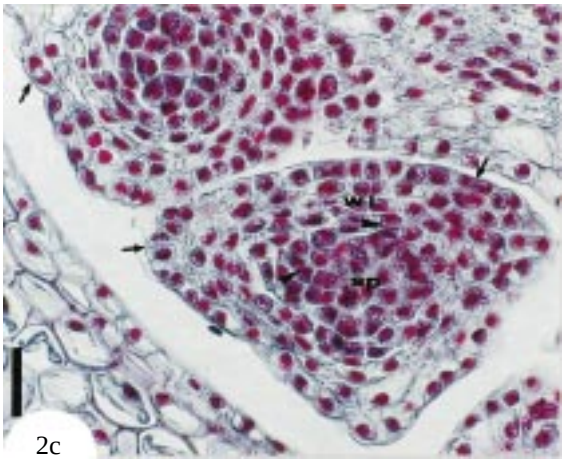
Notes: (1) materials collected on the date(yy/mm/dd) and at the location.
(2) views of observations or orientations of sections:
(TV) = top view
(SV) = side view
(CS) = cross section
(LS) = longitudinal section
(OLS) = oblique longitudinal section
(3) ratio of enlargement: scale unit of linear = 1 mm under stereomicroscopy or bar in μm under light microscopy



2a



2b



2c

Fig. 2. Differentiation of microsporangium (1995/10/23) (Chi-tou). (a) (SV) Globose buds are covered with bracts (b), and each bud is stalked with a twig. Twigs are covered with scale leaves (s), and each twig is axillary to a vegetative leaf (L) on the shoot (T). (b) (225 μm) A CS is near the base of a bud. There is a fertile branch (fb) at the center, 5 basal cone axes (ba), 5 bracts(b) and a few scale leaves (s) outside. A sporophyll stalk (ss) diverts from a basal cone axis, and bears, e.g., 4 microsporangia (frame area with dotted line). Resin ducts (asterisk) are present in different organs. (c) (CS) (45 μm) Enlargement of the rectangular frame area in Fig. 2(b). A microsporangium is organized from outside inwards: a layer of protodermal cells, a few of which are undergoing division (arrow), 2-(3) layers of wall layer cells (wl) and the sporogenous tissue (sp) at the core center with division activity (arrow head).

layer also undergo frequent anticlinal division and differentiate into tapetum (Singh, 1961). Furthermore, some cells in the wall layers contain darkly staining substances in *C. drupacea*, and a few others show tannin. However, this feature was not found in the present study.

4. Development of Pollen Mother Cells (1994/12/23)

The male compound heads of *C. harringtonia* var. *harringtonia* at early developmental stage are small and globose in shape and compact in structure (Wilde, 1975). In the present study, head-like staminate buds are compressed globose, ca. 3.5 mm in diameter. Five bracts and microsporophylls with microsporangia spread out slightly (Fig. 4(a)). At a level near the top end of the bud, there is a fertile branch at the center, from which a sterile bract diverts. A few microsporophylls with several microsporangia are present, with bracts and scale leaves further outside. A microsporophyll constructs a stalk at the center and a few, e. g. 4, microsporangia around it. Epidermal cells are similar in form and size to one another (Fig. 4(b)). The wall layer is intact. Sporogenous cells appear to retain meristematic or just-after-division activities in morphology. They develop into pollen mother cells.

5. Resting Phase of Pollen Mother Cells (1995/01/17)

Head-like bud is about 3.5 mm in thickness, and its 5 bracts and microsporophylls containing microsporangia spread out gradually (Fig. 5(a)). Each basal cone axis diverts from the fertile branch and fuses abaxially with a bract at its base. Fusion of the cone axis with a bract is also commonly observed in other species (Wilde, 1975). In the oblique longitudinal section of the microsporangium, the epidermal cells at the ab-stalk-axial surface of the microsporangium are obviously thicker than the others (Fig. 5(b)). Its nucleus is small, and the outer cell walls are thickened. Some of 1-2 layered middle layers cells collapse. Consequently, the tapetum is partially, loosely separate from the outer or inner tissues. Some of its cells are binucleate. This is the same description given by Singh (1961), who noted that some of tapetal cells become binucleate at the mother cell stage of the sporangium of *C. drupacea*. Pollen mother cells with relatively large and dark-stained

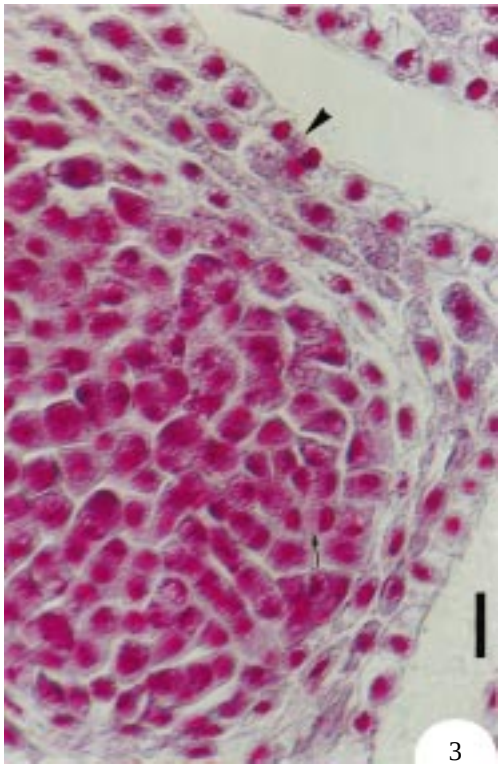


Fig. 3. Proliferation of sporogenous tissue (1995/11/10) (Chi-tou). (OLS) (25 μm) Three parts of the microsporangium seen from outside inwards: the protoderm, in which a cell is undergoing anticlinal division (arrowhead), the wall layer of 2-(3) layers of cells and the sporogenous tissue with cell division (arrow).

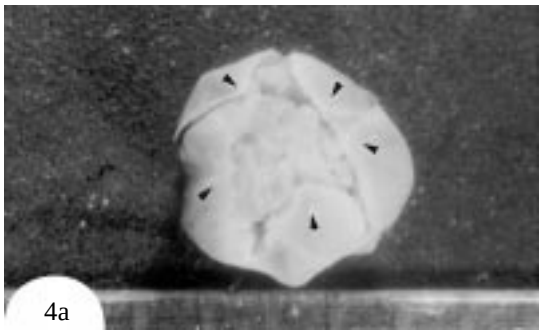
nucleus appear to be static and contain no secondary substances, such as starch grains. However, Singh (1961) noted that starch grains were present in the pollen mother cells of *C. drupacea*.

6. Degeneration of the Middle Layers (1995/02/08)

Figure 6(a) shows a head bud of staminate cones. It is less than 4.0 mm in diameter. Five bracts are present at the base, to each of which a basal cone is axillary. Two smaller bracts with lateral cones are above these basal cones. At the center it is the terminal cone. The epidermis and the pollen mother cells in microsporangium are similar in morphology to those of the cones in the last developmental stage (Fig. 6(b)). The tapetal cells are flat. Cells in the middle layers, which lies outside the tapetum, are either collapsed without a nucleus or have a lightly stained nucleus. The intercellular spaces, therefore, are wider than before. This degeneration of the middle layers was not mentioned in other previous studies. However, Singh (1961) noted that the tapetum of *C. drupacea* is of the glandular type, and degenerates simultaneously with the reduction division in the mother cells. According to this timing of meiosis in pollen mother cells, it seems that the tapetum of *C. drupacea* was misidentified as the middle layers by Singh (1961).

7. Meiosis of Pollen Mother Cells (1995/02/18)

Heads of staminate cones grow further, to over 5.0 mm in dia. (Fig. 7(a)). The construction of each part, 5 basal cones, 2 lateral cones and the terminal cone, is clearly identified in top view. Microsporangia of a head develop independently and not synchronously. The epidermis and the tapetum of the microsporangium remain intact in their appearance (Fig. 7(b)), while some pollen mothers are ready to undergo meiosis. This is the premeiotic interphase. In another microsporangium, there is group of bivalent chromosomes in each pollen mother cell during the meiosis stage prophase I (Fig. 7(c)). At diakinesis, 12 bivalents were counted for this genus in earlier papers (Sax and Sax, 1933; Sugihara, 1940; Mehra and Khoshoo, 1956; Singh, 1961). Then, each group of 12 bivalent chromosomes divide into two sets of monovalent chromosomes during the meiosis



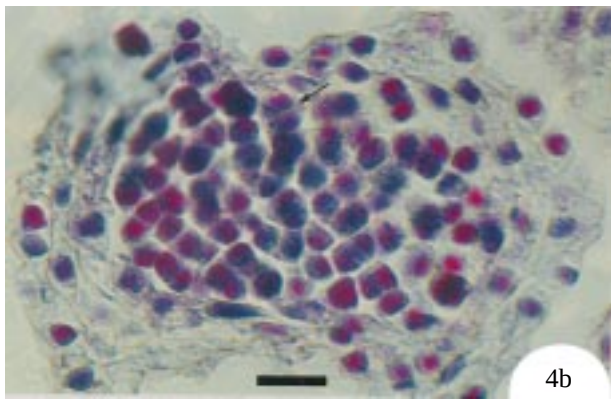
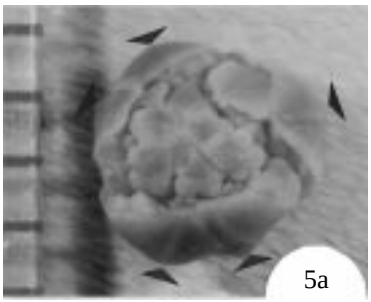


Fig. 4. Development of pollen mother cells (1994/12/23) (Chi-tou). (a) (TV) A head-like bud with 5 bracts (arrow heads) slightly spread out, so that microsporophylls with microsporangia are visible. (b) (CS) (25 μm) A microsporangium consisting of three tissues parts, from outside inwards: a layer of epidermis and (1)-2 wall layers and pollen mother cells. Epidermal cells are similar in form and size. Pollen mother cells appear to have undergone division activity (arrow).



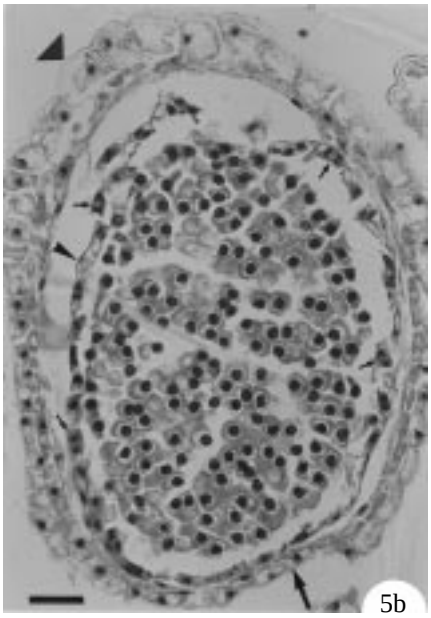
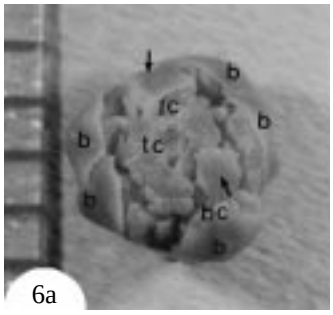


Fig. 5. Resting phase of pollen mother cells (1995/01/17) (Chi-tou). (a) (TV) 5 bracts (broad arrowhead) and microsporophylls with microsporangia of a head-like bud gradually spreading further out. (b) (OLS) (45 μm) The epidermal cells (broad arrowhead) at the ab-stalk-axial surface of microsporangium are thickest of all while the others on the opposite side (big arrow) are thinnest. In the layer of tapetum (narrow arrowhead), some cells are binucleate (arrows). Most of the cells in the middle layers, between the epidermis and the tapetum, lack a visible nucleus or are collapsed. Pollen mother cells are inside with a large, dark-stained nucleus.

stage anaphase I (Fig. 7(d)). Finally, four groups of monovalent chromosomes are distributed separately within a cell. However, no cell wall formation is detected between the grouped chromosomes (Fig. 7(e)). This is the stage of tetrads, or the end of meiosis II. Singh (1961) also found that wall formation took place after the stage meiosis II in *C. drupacea*.

8. Development of Microspore Tetrads (1995/02/28)

Heads of staminate cones grow relatively slowly and are less than 5.5 mm in diameter (Fig. 8(a)). A head may be composed of 5 basal cones, 3 lateral cones and a terminal cone. Each axis of a basal cone or lateral cone is connate with a bract abaxially. Microsporangia are elliptic in form in longitudinal sections. It is obvious that the outer walls of epidermal cells, which lie at the ab-stalk-axial surface of microsporangia, are thick (Fig. 8(b)). The tapetum consists of one layer of cells after degeneration of the middle layers. Tapetal cells are regularly flat-formed. Some of them are binucleate, and some may have a vacuole. Tetrads of microspores are formed after meiosis of pollen mother cells and formation of cell walls between them. There are two arrangements of microspore tetrads: mostly tetrahedral and a few isobilateral (Fig. 8(c)). The microspore tetrads of *C. drupacea* may also be tetrahedral or isobilateral (Singh, 1961). Microspores of each tetrad gradually undergo separation later (Fig. 8(d)).



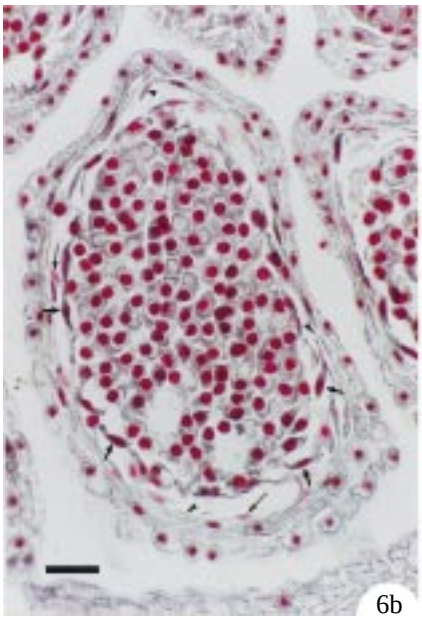
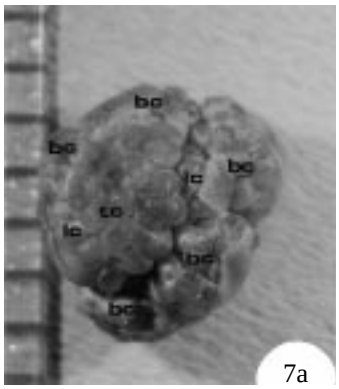
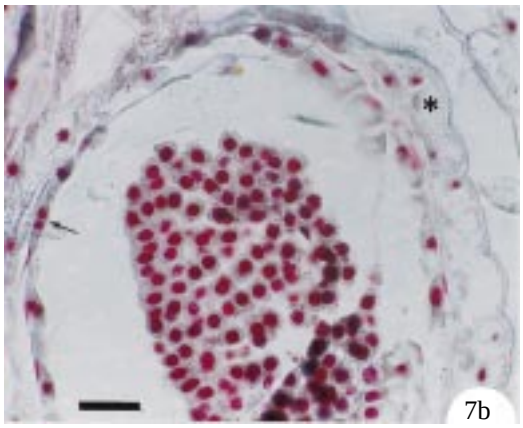


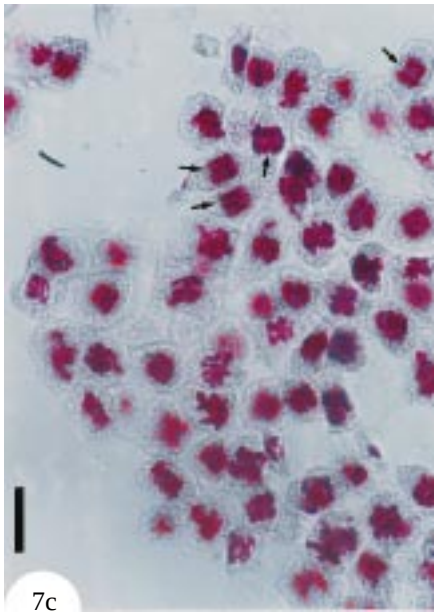
Fig. 6. Degeneration of the middle layers (1995/02/08) (Chi-tou). (a) (TV) 5 basal cones (bc) with 5 bracts (b), 2 lateral cones (lc) with 2 smaller bracts (arrows) and a terminal cone (tc). (b) (CS) (45 μm) Tapetal cells are flat (short arrows). Some cells in the middle layers, which are outside the tapetum, are either collapsed without a nucleus (arrowheads) or have a light-stained nucleus (long arrows).



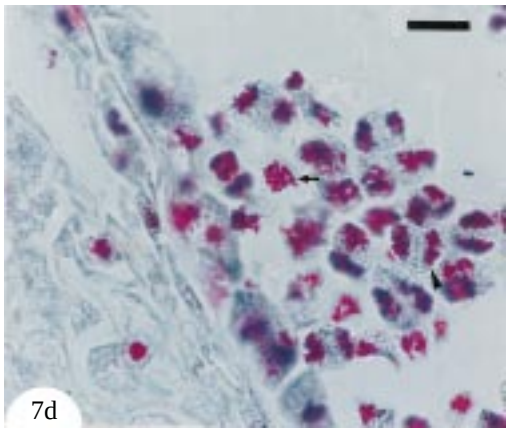
7a



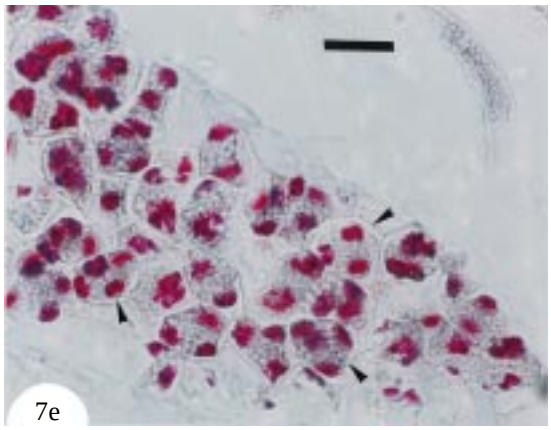
7b



7c



7d



7e

Fig. 7. Meiosis of pollen mother cells (1995/02/18) (Chi-tou). (a) (TV) 5 basal cones (bc), 2 lateral cones (lc) and a terminal cone (tc). (b) (45 μm) Epidermal cells are thick (asterisk), and a few tapetal cells are binucleate (arrow). Much intercellular space is around the pollen mother cells, which is in the premeiotic interphase. (c) (22.5 μm) A group of bivalent chromosomes in each pollen mother cell (arrows) during meiosis stage prophase I. (d) (22.5 μm) Side view of two sets (arrowhead) and polar view of one set of bivalent chromosomes (arrow) during meiosis stage anaphase I. (e) (22.5 μm) Four sets of monovalent chromosomes distributed separately in a cell (arrowheads) during meiosis stage tetrad.

9. Development of Free Microspores (1995/03/10)

A head becomes ca. 6.0 mm thick (Fig. 9(a)). The anatomical features of the epidermis and the tapetum remain almost the same as before (Fig. 9(b)). Microspores are free inside the tapetum. Some of them are round (16-18 μm in dia.) while some are wrinkled (Fig. 9(c)). Microspores have a nucleus and thick wall (ca. 1.5 μm). They appear to have an aperture or a concave wrinkle while mature pollens are inaperturate in *C. wilsoniana* as found by Huang (1972). The cytoplasm of a microspore is full of light reflective particles, which are probably starch grains.

Singh (1961) observed several abnormalities during microsporogenesis of *C. drupacea*, such as in the behavior of the chromosomes, nuclei separation due to wall formation and exine development of pollen grains. However, no significant abnormalities were found during microsporogenesis in this study although advanced cytological techniques were not performed here.

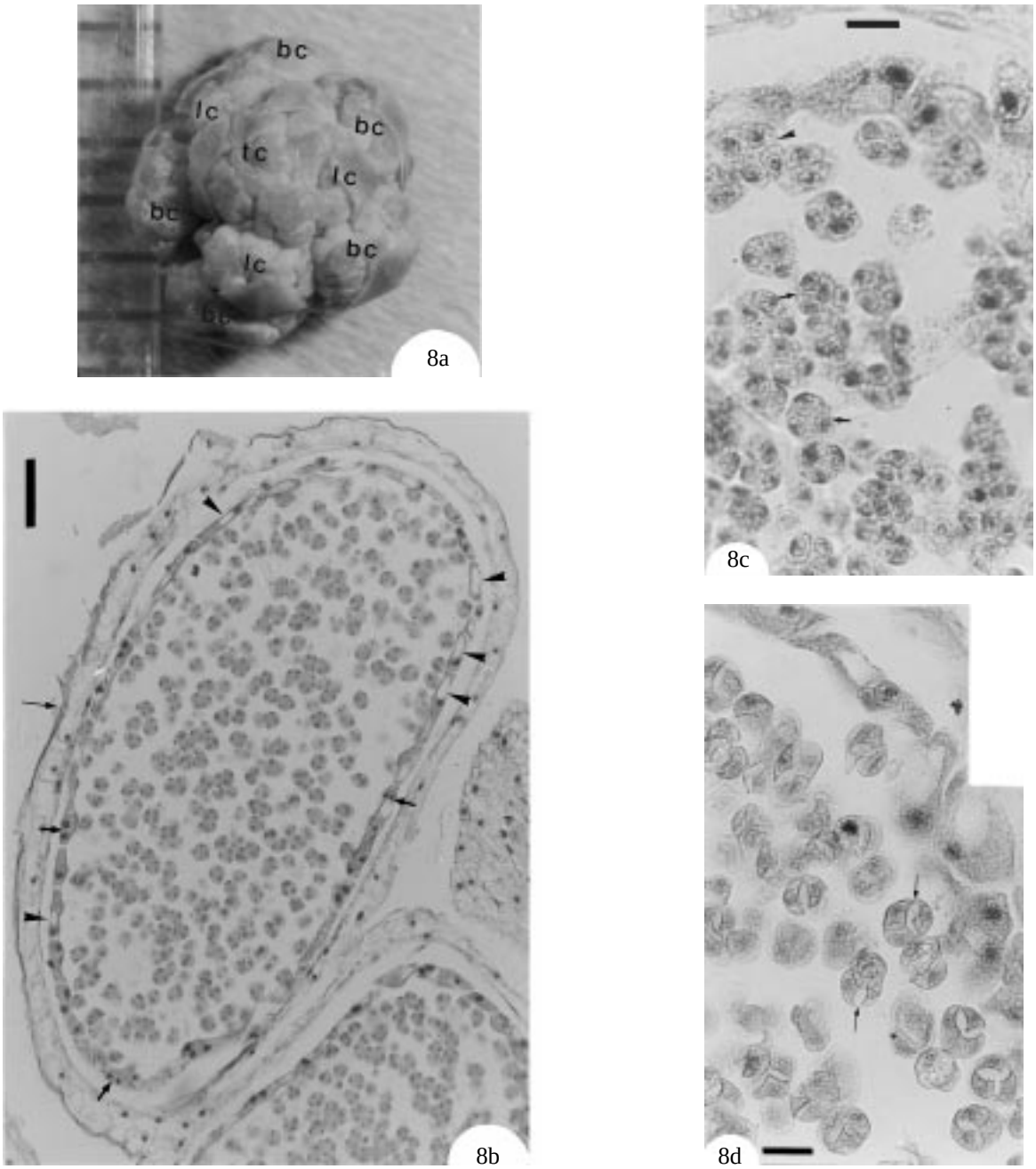
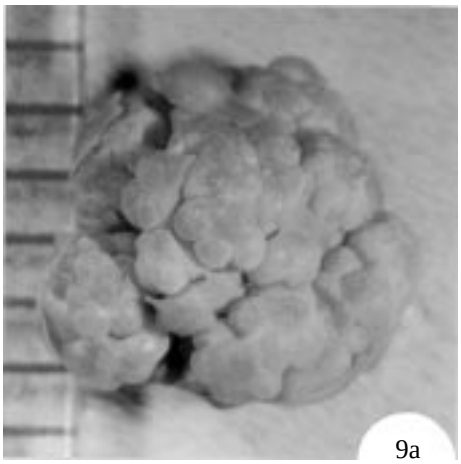


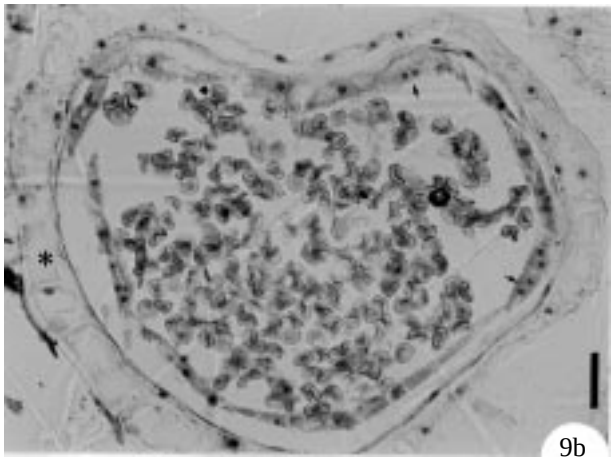
Fig. 8. Development of microspore tetrads (1995/02/28) (Chi-tou). (a) (TV) A head composed of 5 basal cones (bc), 3 lateral cones (lc) and a terminal cone (tc). (b) (LS) (90 μm) The outer wall of the epidermal cells is thick (long arrow). Tapetal cells are flat, a few of them may have vacuoles (arrowheads) and a few of the others are binucleate (short arrows). (c) (LS) (22.5 μm) Two arrangements of microspore tetrads: tetrahedral (arrows) and isobilateral (arrowhead). (d) (LS) (22.5 μm) Microspores of tetrads undergoing separation (arrows).

10. Completion of Heads of Staminate Cones (1995/03/24)

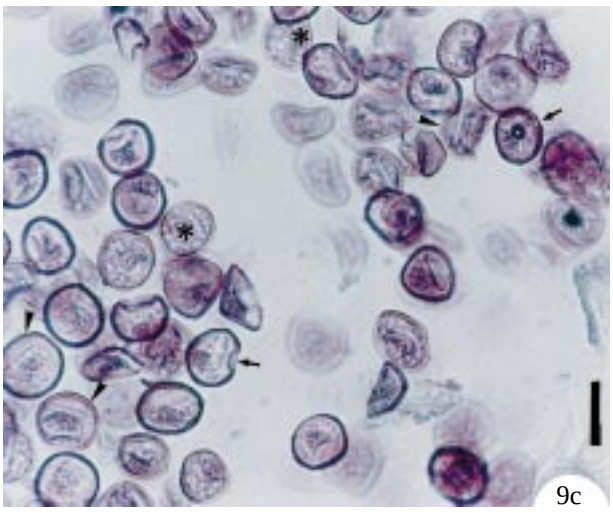
Heads of staminate cones can reach about 8.0 mm in diameter, and every part of the head spreads out completely at this stage so that the microsporangia are visible (Fig. 10(a) and (b)). The morphological structure of the heads of staminate cones approaching maturity is schemed in Fig. 10(c). Basically, there are 8(-9) cones on a fertile branch in three levels: a terminal cone, 5 basal ones and 2(-3) smaller lateral cones in the middle between them. The terminal cone and each basal cone (Fig. 10(d)) is composed of 7-8 microsporophylls while the lateral cones have 4-5. Each basal and lateral cones is axillary to a bract, which is



9a



9b



9c

Fig. 9. Development of free microspores (1995/03/10) (Chi-tou). (a) (TV) A head. (b) (CS) (45 μm) Anatomical features of the tapetum (arrows) and the epidermis (asterisk) remain almost the same as before in Fig. 8(b). (c) (CS) (22.5 μm) Free microspores with a nucleus, thickened wall (arrowheads) and monoporate (short arrow). The cytoplasm is full of light-reflective particles, which possibly are starch grains (asterisk).

abaxially connate with the cone axis while only the terminal one lacks such a fertile bract (Fig. 10(c)). However, 1-2 smaller, steno-formed sterile bracts are present below the terminal cone on the fertile branch. Similar structures of other species were reported by Wilde (1975). Also below the terminal cone on the fertile branch of *C. harringtonia* var. *harringtonia* are usually 1 or 2 small bracts without axillary cones while 2-5 sterile bracts may or may not occur under the terminal cone in *C. harringtonia* var. *drupacea*. However, midway on the fertile branch of *C. oliveri*, 1 or 2 bracts with or without small axillary cones are usually present, together with a terminal cone.

There are two morphological forms of microsporophylls in *C. wilsoniana*, i.e., either hyposporangiate or perisporangiate. Hyposporangiate sporophylls bear 2-4 microsporangia under the apex abaxially on the stalk (Fig. 10(e)). This kind of sporophyll always appears below the terminal perisporangiate sporophylls on the same cone axis (Fig. 10(c) and (d)). Wilde (1975) described microsporangia of *C. harringtonia* var. *harringtonia* similarly. They are attached to the distal, under portion of the stalk, the upper portion of which is expanded into a small lamina, sometimes pointed or variously toothed, or even reduced to a ridge or a knob. Perisporangiate sporophylls of *C. wilsoniana* bear 4 to 6 microsporangia distributed radially and symmetrically at the distal part of the stalk and retain a wart-like apex (Fig. 10(e)). These microsporophylls are present only at the terminal end of a cone axis (Fig. 10(c) and (d)). Wilde (1975) gave an evolutionary interpretation to this construction in *C. harringtonia* var. *harringtonia* and *C. harringtonia* var. *drupacea*, that 2-(3) hyposporangiate sporophylls tended to become whorled at the cone apex, and fused partially or completely with 4-7 perisporangia and 1-2 central knobs.

The present descriptions on the head organization of staminate cones are more or less different from those of previous studies on *C. wilsoniana*. A comparison of all these works is given in Table 2.

In previous studies, the observations on staminate heads made by Gong and Chiang (1971) are more detailed than any of the others. Their observation on the number of microsporophylls per cone and the number of microsporangia per microsporophyll are very close to those of the present work. Only the range of the number of cones per head (4-12) is wider than that of the present study (8-9). This inconsistency in observation might be caused by the amount of materials sampled from different individuals at different sites.

In order to summarize the fine construction of the staminate cones of *Cephalotaxus* as a whole, the present results in comparison with those of other studies in different species are reviewed in Table 3.

According to the revision on the genus *Cephalotaxus* by Fu (1984), the three taxa (I) *C. drupacea* Sieb. *et* Zucc.,

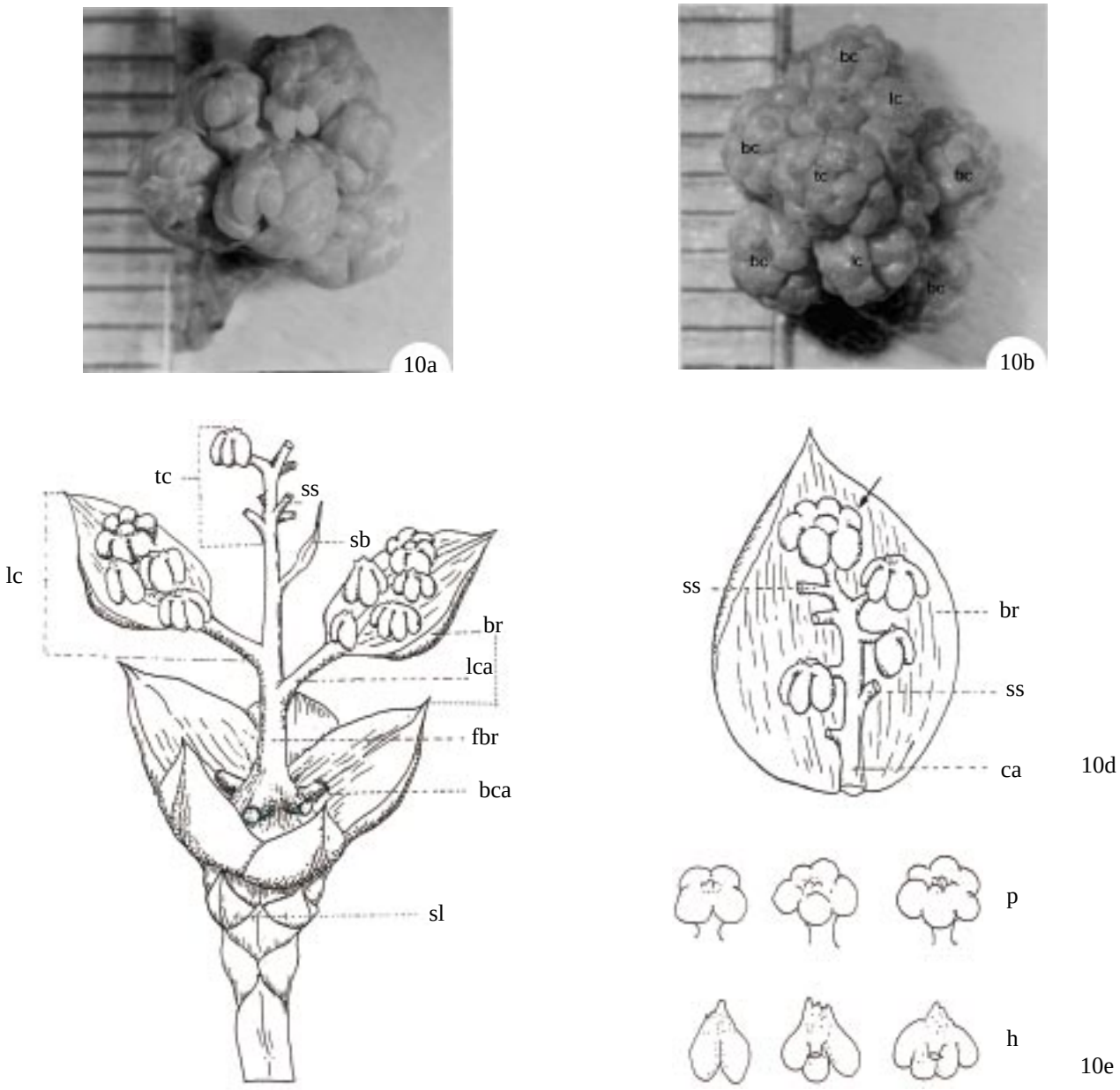


Fig. 10. Completion of heads of Sstaminate cones (1995/03/24) (Chi-tou). (a) (SV) A head. (b) (TV) A terminal cone (tc), 5 basal cones (bc) and, among them, 2 smaller lateral cones (lc). (c) (SV) Schematic construction of a mature head with staminate cones. Basal cone axis (bca), bract (br), fertile branch (fbr), lateral cone (lc), lateral cone axis (lca), sterile bract (sb), scale leaf (sl), sporophyll stalk (ss), and terminal cone (tc). (d) A basal cone with a bract (br), cone axis (ca) and sporophyll stalk (ss). Only the terminal microsporophyll (arrow) is perisporangiate, under which other ones are hyposporangiate. (e) Perisporangiate microsporophyll (p) with 4, 5 or 6 microsporangia; hyposporangiate microsporophyll (h) with 2, 3 or 4 microsporangia.

Table 2. Comparison among Different Descriptions on Head Organization of Staminate Cones in *C. wilsoniana*

organization			citation
cones no. per head	microsporophylls no. per cone	microsporangia no. per microsporophyll	
7-8	5-8	3	Li and Keng (1954)
4-12	4-15	3-6	Gong and Chiang (1971)
—	4-12	3	Liu and Liao (1980)
5-11	7-12	3	Liu *et al*. (1988)
8-9	4-15	2-6	(present)

Table 3. Fine Construction of Staminate Cones of *C. wilsoniana* in Comparison with those of Other Species of *Cephalotaxus*

taxon construction	(I)	(II)	(III)	(IV)	(V)
basal cones no./head		8-10	4-5	5-6	5
lateral cones no./head		0[a]	3-4	(0)1-2	2-3
terminal cones no./head		1	1	1	1
total cones no./head	6-8	9-11	8-10	(6)7-9	8-9
hyposporangia no./ sporophyll		2-3	2-3	2-3	2-4
perisporangia no./ sporophyll		4-7	4-7	4	4-6
sporangia no./sporophyll	(2)3-4	2-7	2-7	2-4	2-6

Notes: taxon
(I): *C. drupacea* (Singh, 1961)
(II): *C. harringtonia* var. *drupacea (C. drupacea)* (Wilde, 1975)
(III): *C. harringtonia* var. *harringtonia (C. pedunculata)* (Wilde, 1975)
(IV): *C. oliveri* (Wilde, 1975)
(V): *C. wilsoniana* (present)
[a]great reduction in the central part of the fertile branch

(II) *C. harringtonia* var. *drupacea* (*C. drupacea* Sieb. *et* Zucc.) and (III) *C. harringtonia* var. *harringtonia* (*C. pedunculata* Sieb. *et* Zucc.) (Table 3) are all synonyms of the species *C. harringtonia* (Forbes) Koch. The surveys on the fine constructions of the staminate cones of both taxa (II) and (III) are very similar, except that the lateral cones of the head could not be identified in taxon (II). Wilde (1975) noted that the variety *C. harringtonia* var. *drupacea* had a basal cluster of 8-10 male cones, and that lateral cones along the distal part of the fertile branch were rare because of the reduction in its central fertile branch. It seems that it was a young, male head with the fertile branch not completely elongated at the time of collection. The data on taxon (I) are limited and deviate more or less from those of taxa (II) and (III). Basically, there are few differences in the fine constructions of the staminate cones among three species, *C. harringtonia*, *C. oliveri*, and *C. wilsoniana*.

11. Formation of 2-Celled Pollens (1995/03/28)

Each part of a head diverges. The color of a few microsporangia begins to change from greenish to yellowish-brown (Fig. 11(a) and (b)). Anticlinal and inner periclinal walls of epidermal cells are thicker than the outer surface wall, which is smooth or more or less concave (Fig. 11(c) and (d)). The epidermis develops similarly at maturity of the sporangium in *C. drupacea* (Singh, 1961): the epidermal cells become highly cutinized, their radial and inner tangential walls become thick, and fibrous thickenings develop as the microspores undergo their first division. The tapetum of *C. wilsoniana* has now disappeared (Fig. 11(d)). Therefore, the epidermis alone forms the wall of the sporangium. Free pollen grains are distributed loosely within the microsporangium. A few pollens undergo cell division (Fig. 11(e)) while most of the pollen grains are already 2-celled with a cell wall between them (Fig. 11(f)). The bigger cell, which is located at the pollen core center, is the tube cell. The other, smaller one is the generative cell, which is attached to the inside of the wall.

12. Pollens Shedding (1995/04/03)

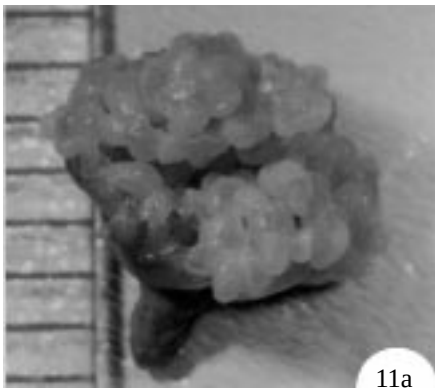
Almost all microsporangia are dehiscent and empty (Fig. 12(a)). Splits of microsporangia always open towards their own sporophyll stalk, where the layer of the epidermis is thin (Fig. 12(b)). The outer surface walls of the epidermal cells of these dehiscent microsporangia are more or less wrinkled while those of intact microsporangia remain smooth. Singh (1961) described this development in *C. drupacea*, too. A few cells of the epidermis of each sporangium facing the stalk of the microsporophyll do not have any thickenings. Dehiscence occurs along these unthickened cells. The partition walls between the sporangia, where they are united with each other, collapse.

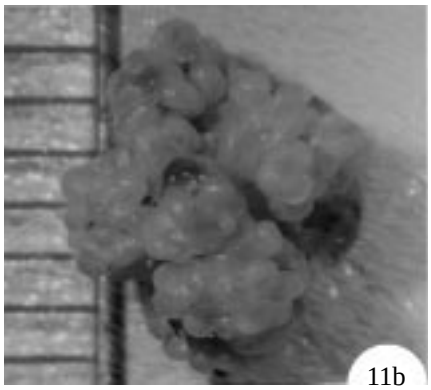
Based on the continuous observations (Dec. 1994—Nov. 1995) described above, the entire process including different stages of microsporogenesis are given in Table 1. In order to compare the phenology of microsporogenesis in *C. wilsoniana* with that of other *Cephalotaxus* species, results of two studies are summarized in Table 4.

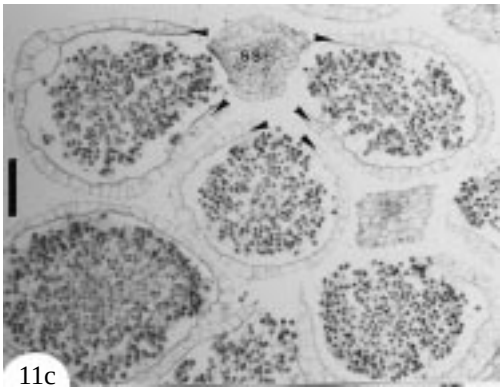
It can be concluded that the microsporogenesis of various species adapted to specific habitat proceeds at different developmental rates during the seasons most suitable for their reproductive growth (Table 4). However, the phenologies of the microsporogenesis in *C. drupacea* and *C. wilsoniana* are more or less similar. It seems that the latitude of the habitat may play a primary role in the phenology if we compare that of both species (30.5 degree N vs. 23 degree N). Singh (1961) noted that the phenology of microsporogenesis in *C. drupacea* varied between Dehra Dun (India) and Paris (Favre-Duchartre, 1957). According to Favre-Duchartre (1957), meiosis of the microspore mother cells started in early December, while microspore formation and pollen maturation were completed by mid-February and mid-March, respectively. The mother cells underwent reduction division during the resting phase between December and February in a cooler place (Paris) (Favre-Duchartre, 1957). It also happened on a temperate species, *C. oliveri*, in mainland China that meiosis took place during the season from late December to mid-February(Li *et al*., 1986) (Table 4).

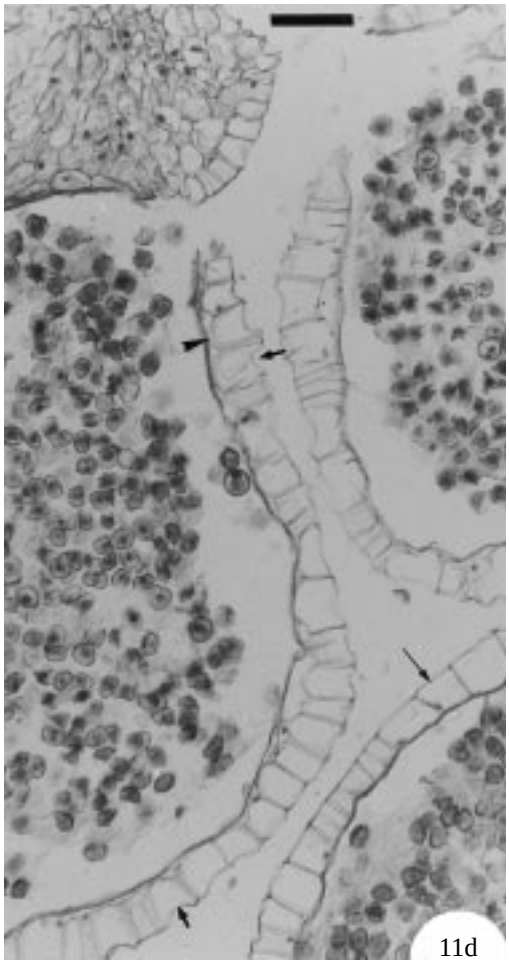
Acknowledgment

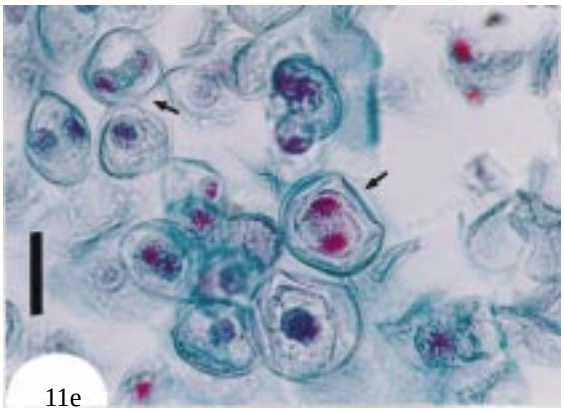
This research was supported under a grant (NSC 86-2313-B-002-120) from the National Science Council of the Republic of China. The authors are grateful to Dr. N. J. Chung, associate researcher, Experimental Forest, National Taiwan University, for collecting materials of staminate cones at Chi-Tou.











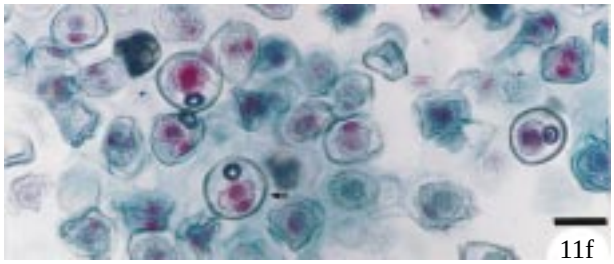


Fig. 11. Formation of 2-celled pollens (1995/03/28) (Chi-tou). (a) (SV) A head. (b) (TV) A head.(c) (CS) (225 μm) Microsporangia adnate (arrow heads) to sporophyll stalk (ss). (d) (CS) (90 μm) Enlargement of Fig. 11(c). Anticlinal and inner periclinal walls (arrowhead) of epidermal cells are thicker than the outer surface walls, which are either smooth (long arrow) or more or less concave (short arrows). (e) (CS) (22.5 μm) A few microspores undergoing division (arrows). (f) (CS) (22.5 μm) Most of the pollens are 2-celled (arrow).

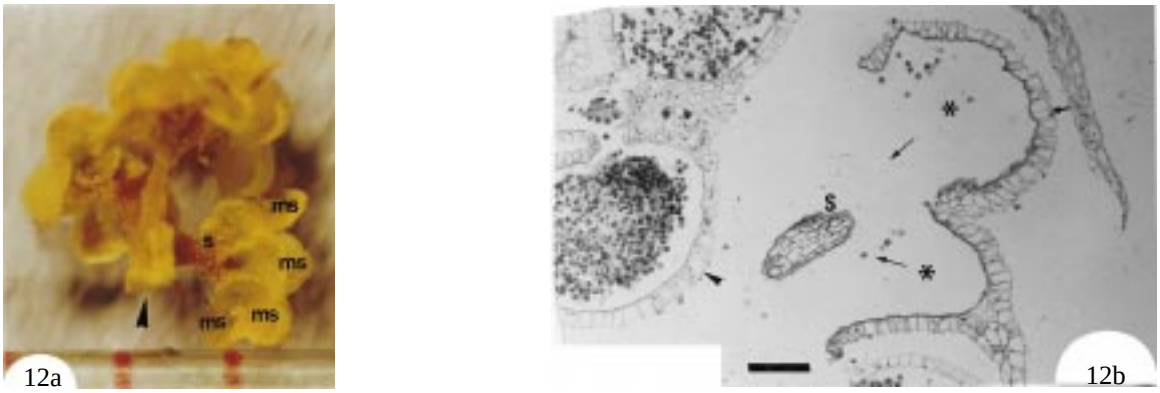


Fig. 12. Pollen shedding (1995/04/03) (Chi-tou). (a) (SV) A sporophyll stalk (s) with 4 microsporangia (ms), which are dehiscent with no pollen grains within. Cone axis (arrowhead). (b) (LS) (225 μm) Splits of microsporangia (asterisks) open towards (long arrows) their sporophyll stalk (s). Outer surface walls of the epidermis are more or less wrinkled (short arrow) while those of intact microsporangia are still smooth (arrowhead).

Table 4. Phenology[a] of Microsporogenesis in the Present Study in Comparison with that of other *Cephalotaxus* Species

stage of development \ species	*C. drupacea*	*C. oliveri*	*C. wilsoniana*
1. origination of microsporophyll	early Sept.		early Oct.
2. differentiation of microsporangium	mid. Oct.		late Oct.
3. proliferation of sporogenous tissue			mid. Nov.
4. development of pollen mother cells	early Dec.	late Oct.	late Dec.
5. resting phase of pollen mother cells	Dec.—Feb.		Jan.
6. degeneration of the middle layers			early Feb.
7. meiosis of pollen mother cell	mid. Feb.	late Dec.	mid. Feb.
8. development of microspore tetrads	late Feb.	mid. Feb.	late Feb.
9. development of free microspores	early Mar.	late Feb.	early Mar.
10. shedding of 2- celled pollen grains	late Mar.—early Apr.	late Mar.—early Apr.	late Mar.—early Apr.
citation	Singh (1961)	Li *et al*. (1986)	(present)

[a] each month is divided into three sections: early, mid. and late; 10 days per section

References

Chiang, T.S.H. (1975) *Outline of Plant Microtechnique* (in Chinese), pp. 11-12, 30-46, 48-49. Maw-Chang, Taipei, Taiwan, R.O.C.

Coker, W.C. (1907) Fertilization and embryogeny in *Cephalotaxus fortunei*. *Bot. Gaz*., **43**:1-10.

Dluhosch, H. (1937) Die Blüten der Coniferen. III. Entwicklungsgeschichtliche Untersuchungen über die Mikrosporophyllgestaltung der Coniferen. *Biblthca Bot*., Stuttgart, **114**(3):1-20.

Favre-Duchartre, M. (1957) Contribution à l'ètude de la reproduction chez *Cephalotaxus drupacea*. *Rev. Cytol. Biol. Veg*., Paris, **18**:305-343.

Fu, L.K. (1984) A study on the genus *Cephalotaxus* Sieb. *et* Zucc. (in Chinese). *Acta Phytotaxonomica Sinica*, **22**(4):277-288.

Gong, C.M. and Chiang, T. (1971) A study on flower buds in Taiwan cow's-tail pine (in Chinese). *Bull. Exper. Forest*, NTU, 95, 20.

Huang, T.C. (1972) *Pollen Flora of Taiwan*, p. 43. Botany Dept. Press, National Taiwan University, Taipei, Taiwan, R.O.C.

Johansen, D.A. (1940) *Plant Microtechnigue*, pp. 80-82, 126-155. McGraw-Hill, New York, NY, U.S.A.

Lawson, A.A. (1907) The gametophytes, fertilization and embryo of *Cephalotaxus drupacea*. *Ann. Bot.* (Lond.), **21**:1-23.

Li, H.L. and Keng, H. (1954) Icones gymnospermum formosanarum (in Chinese). *Taiwania*, **5**:25-84.

Li, H.L. and Keng, H. (1994) Cephalotaxaceae. In: *Flora of Taiwan*, 2nd Ed. Vol. **I**, pp. 555-556. Editorial Committee of the Flora of Taiwan, 2nd Ed. National Taiwan University, Taipei, Taiwan, R.O.C.

Li, Y., Wang, F.H. and Chen, Z.K. (1986) An embryological investigation and systematic position of *Cephalotaxus oliveri* Mast. (in Chinese). *Acta Phytotaxonomica Sinica*, **24**(6):411-422.

Liu, T.S. and Liao, J.J. (1980) *Dendrology* (in Chinese), p.158. Shang-Wu, Taipei, Taiwan, R.O.C.

Liu, Y.C., Lu, F.Y. and Ou, C.H. (1988) *Trees of Taiwan* (in Chinese), pp. 105-106. Monogr. Publ. No.7, Coll. Agr., NCSU, Taichung, Taiwan, R.O.C.

Mehra, P. N. and Khoshoo, T. N. (1956) Cytology of conifers. II. *J. Genetics*, **54**:181-185.

Pilger, R. and Melchior, H. (1954) Gymnospermeae. In: *Syllabus der Pflanzenfamilien*, **I**, pp. 312-344 (Engler, A., Ed.), Gebr. Borntraeger, Berlin, Germany.

Sax, K. and Sax, H. J. (1933) Chromosome number and morphology in conifers. *J. Arnold Arbor*., **14**:356-375.

Singh, H. (1961) The life history and systematic position of *Cephalotaxus drupacea* Sieb. *et* Zucc. *Phytomorphology*, **11**:153-197.

Sugihara, Y. (1940) On the multipartite chromosome-ring in *Cephalotaxus drupacea* Sieb. *et* Zucc. *Sci. Rep. Tohoku Univ. (Biol.)*, **15**:13-18.

Thibout, E. (1896) *Researches sur l'appareil male des gymnosperms*, Lille.

Wilde, N.H. (1975) A new interpretation of microsporangiate cones in Cephalotaxaceae and Taxaceae. *Phytomorphology*, **25**:434-450.

Worsdell, W.C. (1901) The morphology of the 'flower' of *Cephalotaxus*. *Ann. Bot.* (Lond.), **15**:637-652.

H.C. Lo and Y.F. Wang

臺灣粗榧之雄毬花發育及小孢子形成

羅漢強　王玉芬

國立台灣大學森林學系

摘　要

臺灣粗榧(*Cephalotaxus wilsoniana* Hay.)為臺灣固有之裸子植物種，本文即以形態解剖學方法研究其雄毬花發育及小孢子形成，以利保育。臺灣粗榧從形成雄毬花芽至其花粉粒散放，約需時六個月餘。雄毬花芽十月上旬即形成，十月下旬小孢子葉原體已具有孢子原始組織，十一月中造孢組織增殖且絨氈層明顯。十二月下旬小孢子母細胞發育，一月份已進入休眠期。二月中小孢子母細胞開始減數分裂。二月下旬至三月上旬，小孢子四分體逐漸互相分離。三月下旬，花粉粒內具生殖細胞和管細胞。三月底至四月上旬花粉粒散放。頭狀花序成熟時，由8~9個（5基生，2~3側生及1頂生）雄毬花組成。各雄毬花具一軸，著生4~15枚螺旋狀排列之小孢子葉。孢子葉具二型：柄端著生2~4枚背軸之下位孢子囊，或4~6枚輪狀之周位者。