REPRODUCTIVE BIOLOGY OF *CHAMAECYPARIS*

II. Pollen development and pollination mechanism[1]

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Abstract: Staminate buds of *Chamaecyparis* collected on Alishan were studied. A time scale for the microsporogenesis has been worked out.

INTRODUCTION

Of the seven species of *Chamaecyparis*, two are native to Taiwan, *C. formosensis* Mats. and *C. taiwanensis* Masam. & Susuki (Li, 1953; Liu, 1966), all species produce lumber of high quality. So far very few reports have been made regarding the reproductive biology of this genus. Pistillate cones of the Taiwan cypresses have been investigated by the author (Li, 1972). The objective of this paper is to study the development of the microsporangia, microspores and pollen grains. Based on the pollen grains it is possible to postulate the method of pollination.

LITERATURE REVIEW

Coker (1904) reported that the nucleus of the microspores of *Chamaecyparis* had divided before anthesis, forming pollen grain with two cells (tube cell and generative cell). On the other hand, he also reported that the pollen grains of *Cupressus* and *Juniperus* have only one nucleus. The materials he used included *C. lawsoniana*, *C. sphaeroides* (*C. thyoides* BSP), *C. obtusa* and *C. pisifera*.

Erspamer (1952) reported that the microsporangial initials of *C. lawsoniana* are "strictly hypodermal in origin". This means that the microsporangium originates from the division of a series of hypodermal cells under the epidermis, abaxial to the base of young sporphyll. The Microsporangium is of the eusporangiate type. The wall of the microsporangium consists of two layers of cells. The inner layer is developed into a tapetum. At maturity, these two layers of the sporangial wall become crushed or obliterated. The outermost layer of the matured pollen sac is actually the epidermis of the sporophyll.

Courtot and Bailland (1955) observed the position of the strobilus primordia on the shoot of *Chamaecyparis* and reported that the primordia on the lower twigs of the shoot nearly always become staminate strobili while the primordia on the upper twigs more often become pistillate strobili or vegetative buds.

Hashizume (1963) investigated the bud development of strobili in *C. obtusa*. He wrote that in staminate strobili, the sporogenous tissue started to differentiate during mid August, and during early October the formation of anthers was observed. Meiosis of the pollen mother cells (microsporocytes) took place in early March and the pollen grains contained two cells through mitosis of the microspore.

Kung (1972) studied the development of flower buds of *Chamaecyparis formosensis* by the external morphology and tabulated a time scale of the flower bud development.

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MATERIALS AND METHODS

The leaf buds and staminate buds of adult trees of *C. formosensis* and *C. taiwanensis* (mostly the former) were collected on Alishan (2200 m) bimonthly from August 1973 through February 1974 and were fixed in Carnoy's Fluid 2 (absolute ethyl alcohol 6: glacial acetic acid 1: chloroform 3) (Johansen, 1940). After twenty four hours the specimens were transfered to 75% ethyl alcohol for storage. For the large buds an aspirator was used to make the penetration more rapid. The paraffins used has a melting point of 52°C for winter and 58°C for summer. Sections of 10–12 μ thick were double stained with safranin 0 and fast green (Johanse, 1940).

All the photos were taken with a trinocular research microscope by means of an Asashi Pentax adaptor.

OBSERVATION AND DISCUSSION

1. Morphology of staminate buds

A. Position of staminate buds on the shoot.

The staminate buds of these two species are produced on the branches in the middle and lower parts of the crown. The arrangement of branches in *Chamaecyparis* is very regular. There are many lateral branches on the main axis making a tree of pyramidal appearance. The secondary branches are alternately spaced on opposite sides at every second node of a lateral branch (Figs. 1 & 2, A). The difference between staminate and pistillate branches is very distinct. The internodes of the pistillate branch are much longer than those of the staminate. On the secondary branches of both there are teriary branches arranged in the same way (Figs. 1 & 2, B). The fourth branches (the finest twigs) are also alternatively arranged on the tertiary branches but mostly on one side of the reproductive braches (Figs. 1 & 2, C). The staminate and pistillate buds are always the terminal bud of these fourth branches (Figs. 1 & 2, D). Usually the staminate and pistillate strobili are produced on different lateral branches. The terminal buds of the main axis and lateral branches. whether young or old, are always in the vegetative condition. The pistillate strobili are often produced on the upper branches and the staminate strobili on the lower ones. Occasinally there are a few pistillate strobili borne on the vigorous twigs of the staminate branch. This arrangement of strobili has also been reported in other plants of the Cupressaceae, such as: *Cupressus* (Owens & Pharis, 1967), *Thuja* (Pharis, Morf, & Qwens, 1969) and *Libocedrus* (Lawson, 1907). But in the Pinaceae both kinds of strobili are often produced on the same twig, the staminate cones being borne on the basal part and the pistillate ones on the distal end, such as: *Pinus* (Foster & Gifford, 1954; Wang, 1971) and *Pseudotsuga* (Owens, 1969). The results reported by Courtot and Bailland (1955) are confirmed by our observations.

B. Anatomy of vegetative and staminate buds

a. Branching buds

The terminal bud of the lateral and secondary branches can produce new branches so they may be termed 'branching buds'. In Figure 3 the branch meristems were observed to be arranged alternately in the leaf axils of every second node on the tortuous axis. This pattern of branching system was reported in *Cupressus arizonica* by Owens and Pharis (1967).

b. Leaf buds

Another type of terminal bud is borne on the tertiary branches and produces no axillary bud primordium (Fig. 1, C). The leaf primordia arise from opposite sides of the meristem symmetrically (Fig. 4). The meristematic apex is long and conical in shape like that of the branching bud.

c. Staminate buds

Staminate buds are mostly produced on the apices of the fourth branches (Fig. 1, D). Sometimes the terminal buds of the tertiary branches are differentiated into staminate buds. The staminate bud of *C. formosensis* in October is much different in anatomy from the branching bud or leaf bund. The apex of the staminate bud is about 400 μ or more in diameter (Fig. 5) while the diameters of the branching bud and leaf bud are about 90 μ and 100 μ, respectively (Figs. 3 & 4). The apex of staminate bud is larger and has ceased elongation. The microsporophylls have thicker bases and a bulge on their undersides (Fig. 6). On the other hand, the pistillate bud in October is of the same size as the staminate bud but has a very different structure. The ovule primordia originate from the stem tissue in the axils of the megasporophylls.

2. Development of the microsporangia

The microsporangia begin their differentiation as soon as the newly formed microsporophylls are about 150 μ in length just as in *Cupressus arizonica* (Doak, 1932; Owens & Pharis 1967). The hypodermal and deeper cells at the base on the abaxial side of the sporophyll initiates a bulge. This bulge of tissue then elongates continuously in a downward direction and takes on somewhat the appearance of an inverted apex (Fig. 6). The sporangial initial cells divide anticlinally and periclinally. The epidermal cells around the sporangial initials divide only anticlinally and elongate parallel to the enlargement of the sporangium except the epidermal cells circled the whole bulge. The extension of the sporangium including the epidermal cells makes a furrow between the developing sporangium and sporophyll. The upper portion of sporophyll grows rapidly but the basal part much slower, so that the mature sporangium is not covered by the lower part of the sporophyll. This is not the same as in *Cupressus arizonica* (Owens & Pharis, 1967).

As in *C. lawsoniana* the sporangial initials in the young stage are only a mass of cells with the same appearance (Erspamer, 1952). The epidermal cells of the sporophyll are not involved in sporangial initiation but form a separate layer outside the sporangium (Fig. 7).

In December the microsporangial wall and tapetum are differentiated from the sporogenous tissue (Figs. 7 & 8). These two layers are just beneath the epidermis while the sporogenous cells are derived from deeper cells. This eusporangiate pattern of development is similar to that found in *C. lawsoniana* (Erspamer, 1952), *Cupressus arizonica* (Owen & Pharis, 1967), *Taxodium* (Vasil & Sahni, 1964), *Podocarpus* (Boyle & Doyle, 1953) and *Pinus* (Konar & Oberoi, 1969).

In later development the sporangial wall cells gradually disintegrate and the tapetal cells become irregular and difficult to distinguish during the microspore formation in February (Fig. 9). In the mature stage (Fig. 10) the epidermal cells are the only cells enclosing the microsporangium just as in many other conifers (Boyle & Doyle, 1953; Erspamer, 1952; Foster & Gifford, 1959; Konar & Oberoi, 1969; Owens & Pharis, 1967; Vasil & Sahni, 1964).

3. Development of microspores

The sporogenous cells divide and differentiate from October through December (Figs. 7 & 8) while the microsporangial wall cells differentiate into two layers (wall and tapetum). The microsporocytes collected in December showed a pachytene stage of the first meiotic division (Fig. 8). From staminate strobili collected in February in the following spring it was observed that the microsporangial wall cells had disintegrated and that meiosis of the microsporocytes was taking place (Fig. 9). Comparing these two facts it is probable that the meiosis of pollen mother cells begins at an early time and rests in a prolonged pachytene stage until the next spring, similar to that in *Thuja plicata* and *Tsuga heterophylla* (Owens & Molder, 1971). It has been suggested that the prolonged pachytene and the diffuse diplotene in other conifers during severe winter months might reduce the percentage of pollen that would be damaged and so affect seed-set (Ekberg *et al.*, 1968; Owens & Molder, 1971). But it has been reported that in *Pinus taiwanensis* growing at low elevation (600 m) the first meiotic divison occurs slowly but continuously during the fall and winter, and the pollen viability does not seem to be affected (Wang, 1972). The materials used in the present investigation were collected from the trees growing at higher elevation (2,200 m) where the temperature in winter may drop to 0°C. So that the prolonged pachytene stage in meiosis may be an adaptation for protecting the pollen mother cells from the severe winter climate as stated by Ekberg *et al.* (1968).

Although the prolonged pachytene stage was discovered in the present study, it can not be said the pollen mother cells being in dormancy. Owens and Molder (1971) reported that the pollen mother cells of Douglas-fir are quite active during this stage. Kung (1972) tabulated a time scale for the flower bud development in *C. formosensis* in which there is a "dormant stage" of staminate flower bud from late September through early January. But he did not give any detailed description or photograph to support his conclusion. On the contrary, the data he recorded reveals that the length and width of staminate buds grow continuously in these months. The description in Kung's report is too simple and evidently not reliable.

In Figure 9 the first and second meiotic divisions can be observed in the same sporangium collected in February 1974. This means that probably these two successive divisions take place after the pachytene stage and form microspores in the spring.

Like most conifers (Konar, 1962; Wang, 1948) the young microspores in the sporocyte are arranged in the two ways: tetrahedral and bilateral (Fig. 9). Soon after meiosis, cell walls appear between the microspores while they are still within the wall of the sporocyte. The average diameter of the tetrads is about 15 μ. In another staminate strobilus on the same tree the microspores had simultaneously escaped from the wall of sporocyte and developed into young pollen which were larger (averaging about 21 μ in diameter) and had a thickened wall (Fig. 10).

4. Development of pollen grains

After the spores fromed, the young pollen grains enlarge very rapidly to their maximum diameter of about 32 μ (Figs. 11 & 12) while the nucleus divides into two. The two nuclei are cutted off by a cell wall making a large tube cell and a small generative cell. In fact the mature pollen grains contain more than one cell and are young male gametophytes. The pollen grains of the species investigated, rest in the two-cell stage until germination after pollination. When pollen was

examined in July after being stored at 5°C in a refrigerator for five months the grains still in the two-cell stage (Figs. 11 & 12). This result confirms Coker's (1904) and Hashizume's (1963) observation on *Chamaecyparis* and is similar to many reports for other conifers (Foster & Gifford, 1959; Hashizume, 1963; Konar, 1962; Lawson, 1907; Vasil & Sahni, 1964). The mitotic division of the nucleus in pollen grains is not uncommon in other conifers but in them there are more than two cells in the matured grains, such as: four cells in *Pinus* and *Pseudotsuga* (Konar & Oberoi, 1969), five cells in *Abies*, *Cedrus*, *Picea*, *Larix* and *Pseudolarix* (Konar & Oberoi, 1969; Wang, 1948), ten in *Podocarpus* (Looby & Doyle, 1944) and fifteen to forty in *Araucaria* (Konar & Oberoi, 1969).

The shape of the dry pollen at anthesis in February is irregular which is caused by dehydration. The spheroidal to subspheroidal grains are swollen as illustrated in Figures 11 and 12, and as described by Huang (1972). In these figures the tube cell and the generative cell are clearly seen. When the pollen grains are soaked in water most of the grains will rupture or throw off the exine (Fig. 12). This phynomenon had been observed in many other conifers: in *Pseudotsuga* by Barner and Christiansen (1962), and in *Cryptomeria* by Li (1967).

5. Specific gravity of pollen grains

The specific gravity of dry pollen was tested by soaking in salt solutions of different concentrations. The grains of these two Taiwan species all sank immediately in salt solution until the concentration was raised to 1.15 specific gravity. This means that a moderate wind is necessary to carry the pollen grains for pollination. If the air is quite calm or if the pollen is wet at the blooming season, pollination will be retarded or inhibited.

6. Proposed time scale of pollen development in *Chamaecyparis*

Before Augrst:	Staminate strobili initiated.
August-September:	(1) Microsporophylls initiated.
	(2) Microsporangial inital differentiated.
October-November:	(1) Microsporangia formed.
	(2) Microsporangial wall and tapetum differentiated.
	(3) Sporogenous cells divided and differentiated.
December-January:	(1) Microsporocytes formed.
	(2) Micorsporangial wall and tapetum formed.
	(3) Meiosis started and resting in pachytene stage.
February:	(1) Microsporangial wall disintegrated.
	(2) Tapetum reduced and disrupted.
	(3) Meiosis completed.
	(4) Microspores formed.
	(5) Pollen grains formed.
March:	Pollen grains shedded.

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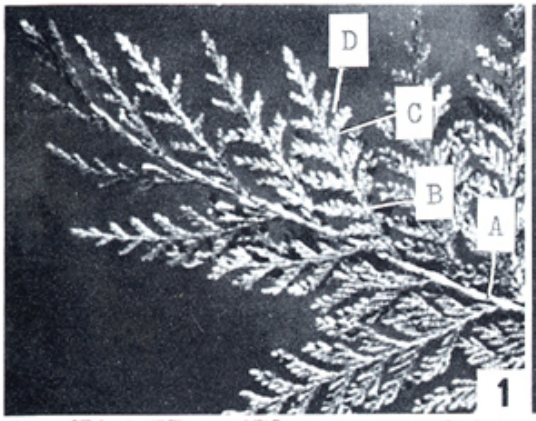
The material illustrated in all the figures is of *Chamaecyparis formosensis* except indicated in particular figure.

Figures 1. A staminate branch; 2. A pistillate branch; 3. Long section of apex of a branching bud in October (280×); 4. Long section of apex of a leaf bud in October (280×); 5. Long section of apex of a staminate bud in October (100×); 6. Long section of the lower part of the same staminate bud in fig. 5 (150×). *A.* lateral branch; *B.* secondary branch; *C.* tertiary branch; *D.* fourth branch; *SI.* sporangial initial; *SP.* sporophyll.



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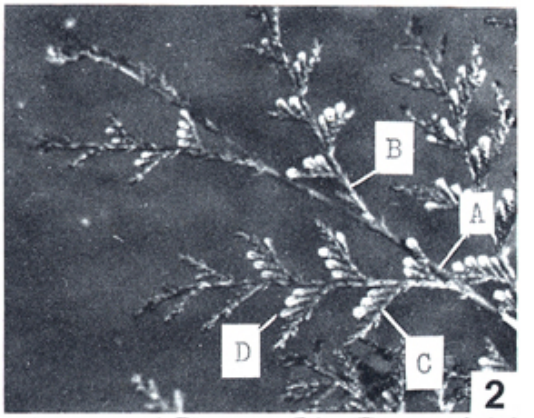

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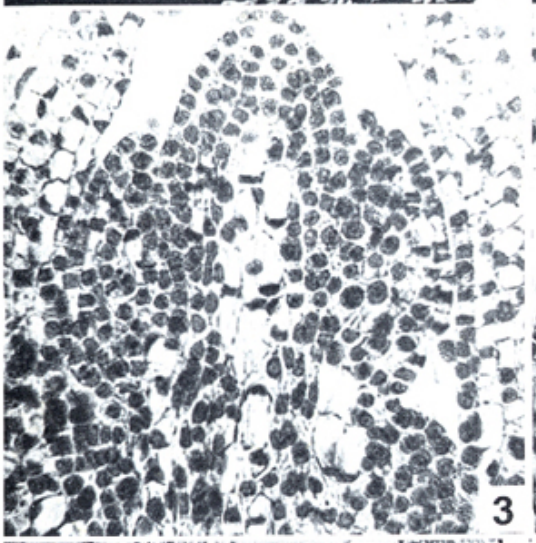

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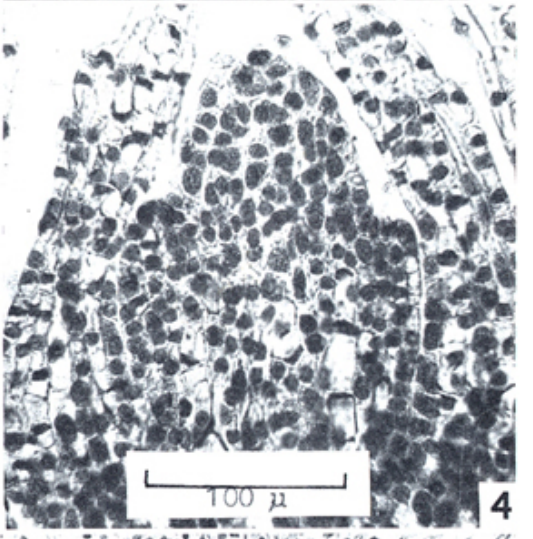

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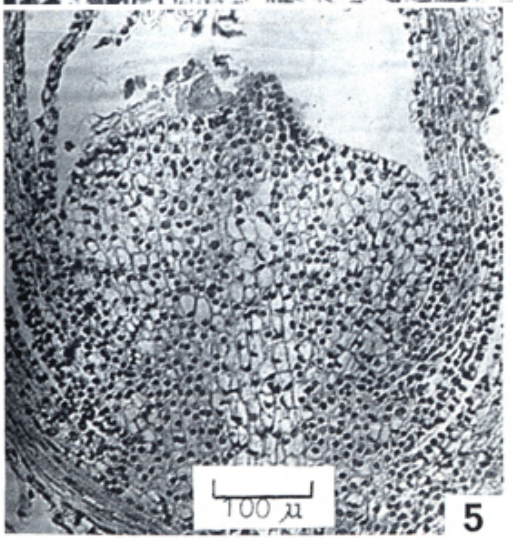

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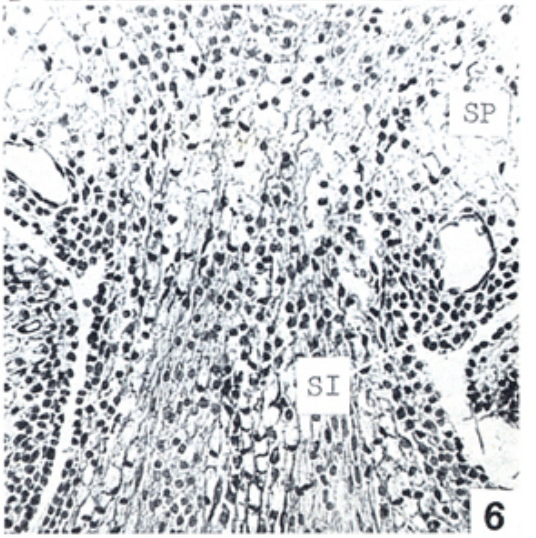

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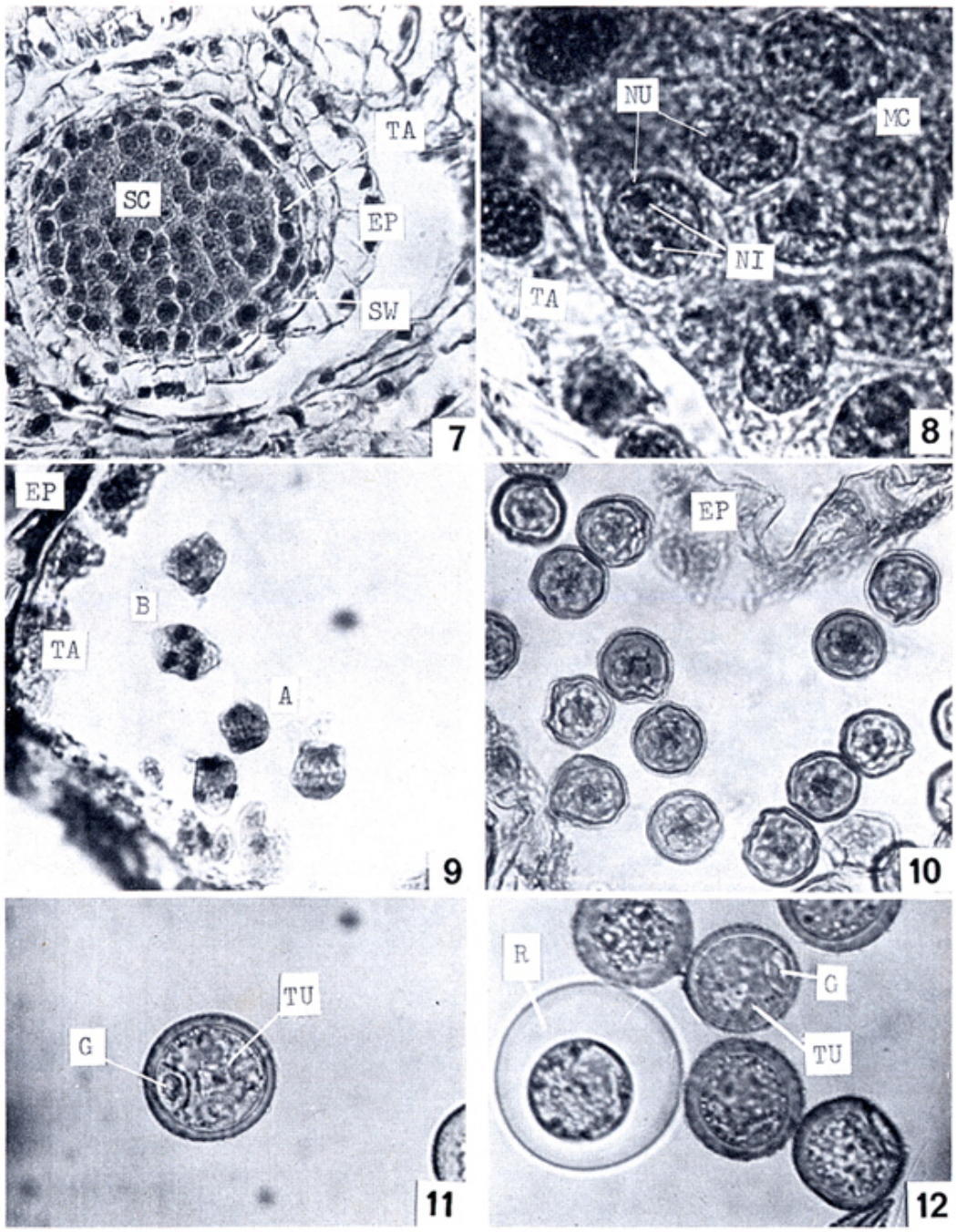

Figures 7. A microsporangium in December (280×); 8. A portion of microsporangium in December (1500×); 9. A portion of microsporangium in February (600×); 10. A portion of another microsporangium in February (600×); 11. Pollen grain of *C. taiwanensis* (1500×); 12. Pollen grains of *C. formosensis* (1500×). *A.* first meiotic division; *B.* second meiotic division; *EP.* epidermis; *G.* generative cell; *MC.* microsporocytes; *NI.* nucleoli; *NU.* nuclei; *SW.* sporangial wall; *TA.* tapetum; *TU.* tube cell; *R.* pollen grain ruptured off the exine.