

# Morphogenesis of Male Sporangiphores of *Zamia amblyphyllidia* D. W. Stev.

M. Mundry and T. Stützel

Ruhr-Universität Bochum, Lehrstuhl für Spezielle Botanik, Bochum, Germany

Received: June 6, 2002; Accepted: April 9, 2003

**Abstract:** Morphology, development and anatomy of the male cones of *Zamia amblyphyllidia* are studied here to amass additional information to develop a more convincing concept of the evolution of seed plants. The male sporangiphores of the cycads are usually regarded as simple sporangiphores with synangia on the abaxial side. Our developmental study of the male sporangiphores of *Zamia amblyphyllidia*, by scanning and light microscopy, suggests that the sporangiphores are pinnate, with synangia on reduced leaflets. This indicates that a significant difference between female and male sporangiphores in cycads, as previously stated, does not exist. Comparisons of male sporangiphores of cycads with those of conifers show that they are not homologous. Rather, the synangia of the cycads may better be homologised with the radial synangia groups in some pteridosperm taxa. Although several authors assume that the cycad precursor displayed entire, fertile and sterile leaves, our interpretations of the male sporangiphores indicate that cycads originated from a pinnate pteridospermous ancestor with radial synangia groups. These hypotheses may point to the Medullosaceae, although this remains to be substantiated.

**Key words:** Gymnosperms, Cycadales, *Zamia*, morphology, male cone.

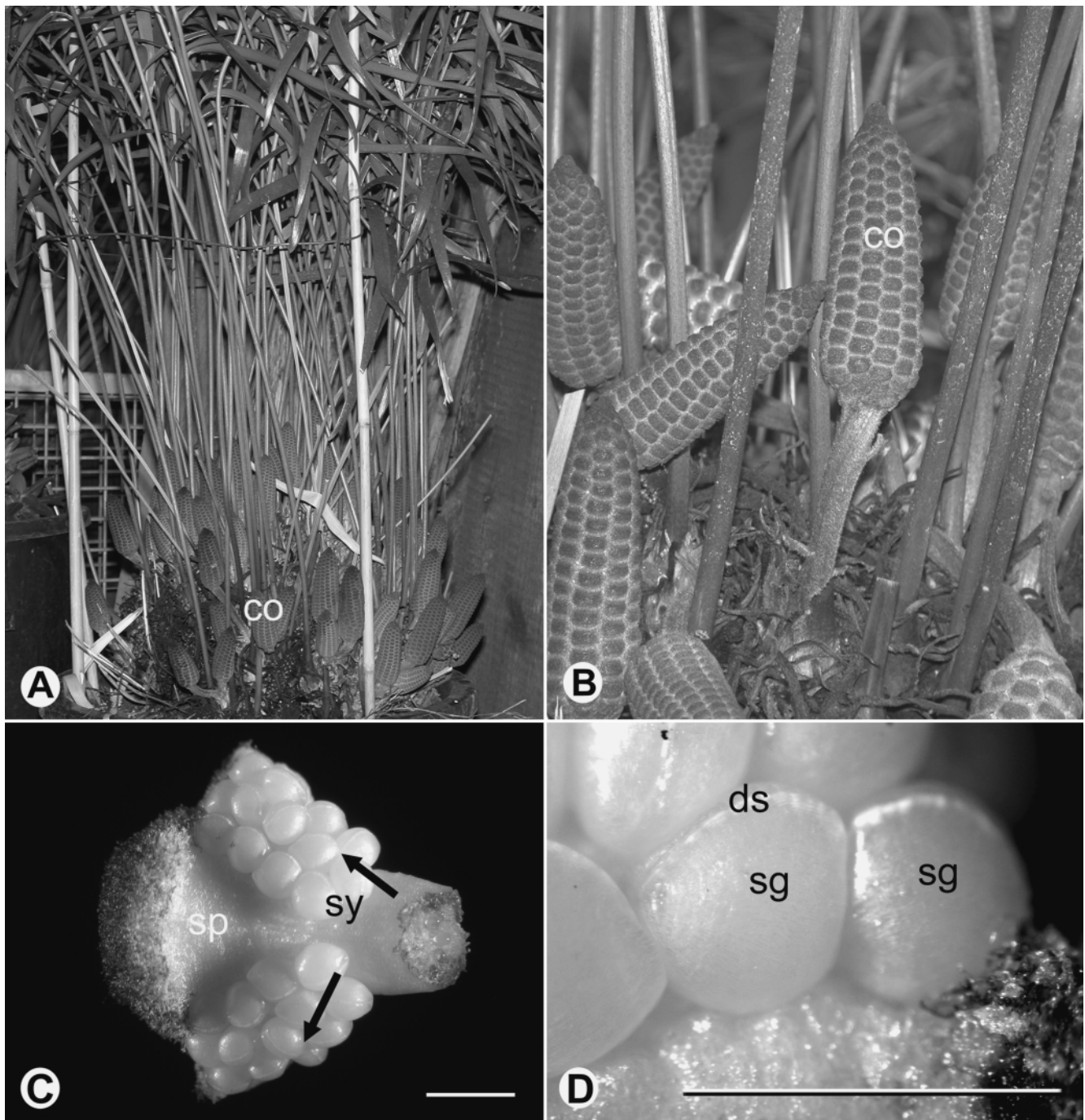
## Introduction

The phylogeny of seed plants is still controversial. During the last 20 years, several morphological and molecular studies have attempted to elucidate the relationships between the various groups of seed plants. There, the focus has been on the relationship between the gnetids and angiosperms. While the morphological data (reviewed in Doyle, 1998) support the hypothesis that the gnetids are a sister group to the angiosperms (Anthophyte Theory), recent molecular studies seem to contradict the Anthophyte Theory (Chaw et al., 2000; Donoghue and Doyle, 2000; Bowe et al., 2000; Frohlich and Parker, 2000; Winter et al., 1999). Not only the systematic position of the angiosperms, but also the relationships among the extant

seed plants seem equivocal. In morphological studies, the cycads are mostly placed at the base of the gymnosperms, or they are grouped as a sister group to ginkgo. If the fossil record is considered, however, the best guess for the systematic position of the cycads is again different to that of morphological and molecular studies. In the past, it was assumed that the ancestors of the cycads displayed frond-like, sterile and fertile leaves, similar to the extant cycads (Delevoryas, 1982; Stewart and Rothwell, 1993). Therefore, the earliest cycads could have originated in Palaeozoic seed ferns like the Medullosaceae (Stewart and Rothwell, 1993; Meyen, 1984). An alternative origin of the cycads was proposed by Mamay (1976) and seems to be widely accepted at present (Norstog and Nicholls, 1997). In his comprehensive study of fossil cycads, Mamay assumed that the precursors of the cycads had simple leaves, making an origin in the Medullosaceae unlikely.

To overcome the great clash over the results of palaeontological, morphological, and molecular studies, a reanalysis of morphological characters and a search for new characters seems to be necessary. In morphological data sets, mainly characters of the female reproductive structures and of the vegetative structures are used. Characters of the male reproductive structures, however, are often limited to features of late sporangia development and of pollen, of the male gametophyte. The bauplans of the male reproductive structures, and of the male sporangiphores, are often regarded as less significant because of "the wide parallelism in the structure of male fructifications" (Meyen, 1984). If male sporangiphores are used in morphological analysis, they are often considered as homologous structures and are normally termed microsporophylls, which implies a leaf-like nature of the male sporangiphore. Due to this uncertain homology of the microsporangia-bearing structures, in this paper the neutral term male sporangiphore is used instead.

The male sporangiphores of cycads are described as simple, with several sporangia on their abaxial side (Bierhorst, 1971; Stewart and Rothwell, 1993). In some genera (e.g. *Cycas*) the entire abaxial side is covered with sporangia, whereas in other genera (e.g. *Zamia*) the sporangia occur in two lateral zones (Coulter and Chamberlain, 1917; Bierhorst, 1971; Smith, 1907). Many report that the sporangia are arranged in groups (or synangia) of 2–5 (Bierhorst, 1971; Stevenson, 1990) and, sometimes, these groups are termed a "soral cluster" (Gifford and Foster, 1989; Smith, 1907). However, Taylor (1988) pointed



**Fig. 1** *Zamia amblyphyllidia*. (A) Male plant with several male cones growing in the Botanical Garden of the University of Bochum. (B) Male cones in detail with vertical rows of sporangiophores. (C) Male sporangiophore from the abaxial side with several synangia composed of two

sporangia, exceptionally of three (arrows). (D) Synangium with two sporangia, lateral view. Scale bar: C, D 2 mm. co, cone; sg, sporangium; sp, sporangiophore; sy, synangium; ds, dehiscence slits.

**Fig. 2** *Zamia amblyphyllidia* – SEM micrographs of the morphogenesis of a male cone at the beginning of July. (A) Lateral view of the apex of a fertile branch with two male cones in different developmental stages. (B) The young cone dome and the apex in detail. (C) The apex of the branch covered by bases of leaves. (D) Overview of a dissected older cone with several young sporangiophores. (E) Primordium of a young

sporangiphore, abaxial view. The distal end is flattened and hexagonal, at the base a stalk and two lobes have formed. (F) Primordium of a sporangiophore, lateral view; the lobes are dorsiventrally flattened. Scale bar: A, B, D 1 mm, C, E 500  $\mu$ m, F 250  $\mu$ m. ca, cone axis; co, cone; pr, primordium; rp, reproductive apex; va, vegetative apex; br, bract; le, leaf; sp, sporangiophore; lo, lobe.

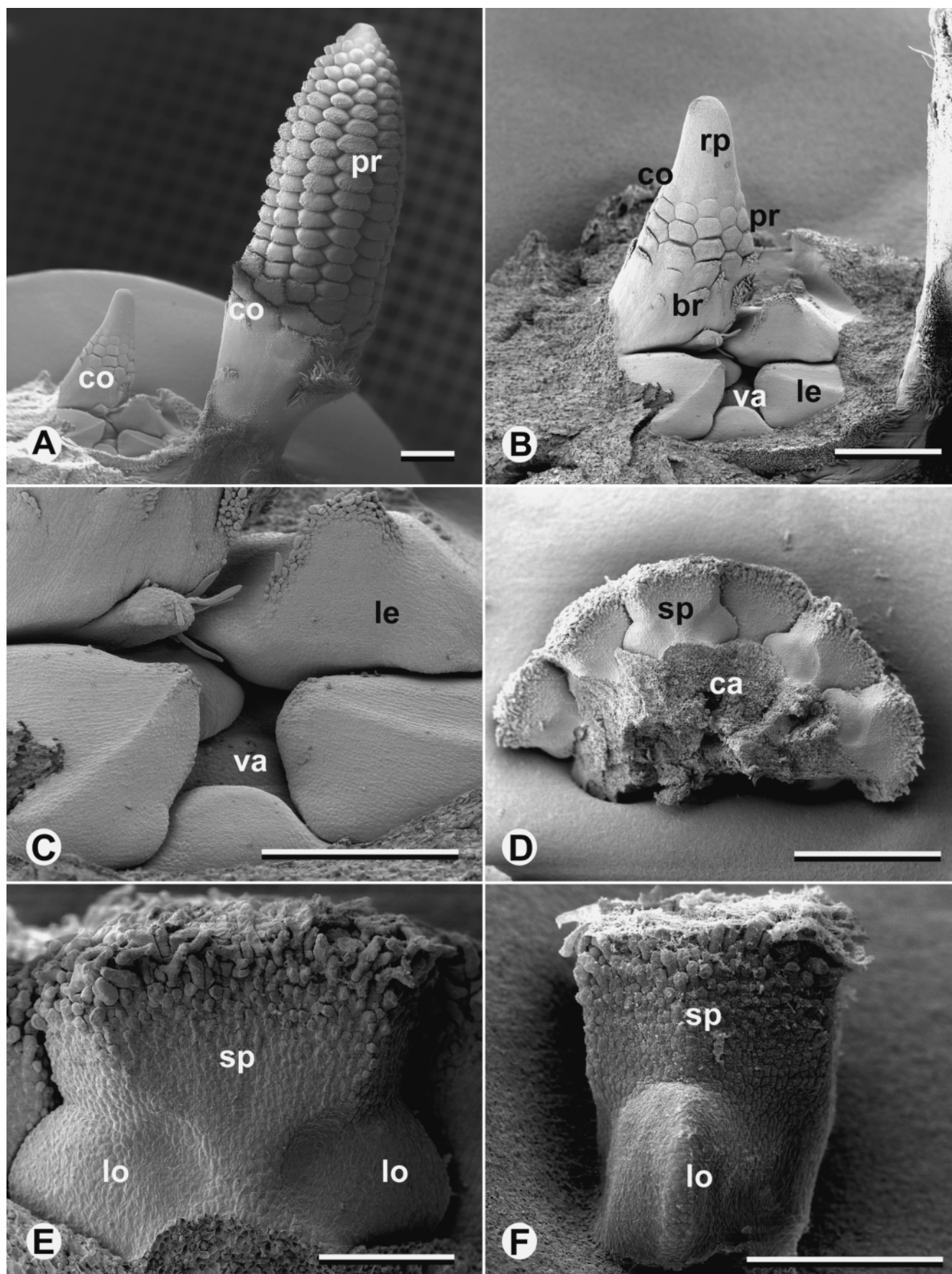


Fig. 2

out that in cycads the male sporangiophore is characterised by the loss of "syngiate organization". The male sporangiophores of the conifers, on the other hand, are also regarded as simple, with sporangia on the abaxial side, but a "soral cluster" is not present.

Nonetheless, the homology of male sporangiophores among the various gymnosperm groups seemed to be generally accepted. On the other hand, studies of Taxaceae show that the sporangiophores of different genera (e.g. *Torreya* and *Cephalotaxus*) are not homologous, based on morphogenesis, although they display striking similarities when they are mature (Mundry and Mundry, 2001). Therefore, in this present investigation, we studied the morphogenesis of male sporangiophores of *Zamia amblyphyllidia* by SEM and light microscopy for comparison with conifers and with hypothetical ancestors. Special attention was paid to the development of the vascular system. A further purpose of this paper was to clarify the arrangement of the synangia, particularly with regard to their morphogenesis.

## Materials and Methods

*Zamia amblyphyllidia* D. W. Stev. was studied continuously throughout one year in the Botanical Garden of the University of Bochum. The first reproductive branches, with two young cones, were present at the beginning of July. Without dissection it is not possible to distinguish reproductive and vegetative branches at this time because the apices are densely covered with cataphylls and are deeply sunken into the trunk (Stevenson, 1988). In July and August, more fertile branches and a total of about 5 older cones had developed. From the end of July until February, the cones could be removed from the plant without dissection of the branches. Immediately after the cones were collected, they were dissected under a stereomicroscope and fixed in FAA (formalin, acetic acid, ethanol 70%; 5:5:90). The fixed material was dehydrated in 70% ethanol and immersed in formaldehyde dimethyl acetal (FDA) for at least 24 h (Gerstberger and Leins, 1978). After critical point drying, the specimens were examined with a DSM 950 SEM (Zeiss), and the results documented using Digital Image Processing Software 2.2 (DIPS Leipzig). Transverse and longitudinal serial sections of male sporangiophores of different developmental stages were made using a classical paraffin technique and Astrablue-safranin staining (Gerlach, 1984). The microtome sections, of 10 µm thickness, were examined using a Zeiss Axioplan light microscope, and the results documented using the software AnalySIS (Soft Imaging Systems). The sporangiophores were cleared with 12–14% sodium hypochloride and examined using a M400 light microscope (Wild) with transmitted light. The results were documented using a Nikon Coolpix 990 digital camera.

## Results

### Morphology of *Zamia amblyphyllidia*

*Zamia amblyphyllidia* is a small cycad, naturally distributed in Jamaica, western Cuba and northern Puerto Rico (Jones, 1993). The trunks are much branched and can reach diameters up to 4 cm. The leaves are up to 1.5 m long and consist of about 20 leaflets. Each crown consists of 4 to 10 trophophylls. Each series of trophophylls is followed by a series of small brownish

cataphylls. If fertile branches are formed, the male cones arise after a series of cataphylls. Each branch forms 2 to 4 male cones. The plant studied in the Botanical Garden of the Ruhr University of Bochum is about 25 years old (Fig. 1A). The male cones are about 6 cm long and about 2 cm wide (Fig. 1B). The peduncles of the cones are 4–8 cm long. The cones are reddish brown and villose. Each cone consists of up to 200 sporangiophores. The sporangiophores are 1 cm long (Fig. 1C). They consist of a stalk, flanked by two dorsiventrally flattened lobes, and a distal hexagonal tip. The abaxial sides of the lobes bear the sporangia. Two sporangia form one synangium with a common stalk (Fig. 1D). Usually, about 12 synangia are formed, 6 per lobe. Synangia with 3 sporangia instead of 2 were found in some cases (arrow, Fig. 1D). The cones are mature in February.

### Morphogenesis of male sporangiophores of *Zamia amblyphyllidia*

The fertile branches initiate the 2–4 male cones at the beginning of July (Figs. 2A,B). The vegetative apex of the branch is located close to the base of the youngest cone and is covered by bases of leaves (Fig. 2C). An axillary branching pattern of the male cones is not detectable. The older cone is about 1 cm long and still has about 150 initiated primordia of sporangiophores (Fig. 2A). The base of the cone forms a peduncle, which has a length of about 3 mm and a diameter of about 2 mm. At the top, the distal end of the cone apex continues to form further primordia. The younger cone dome is about 150 µm long and has just initiated about 30 primordia of sporangiophores (Fig. 2B). The primordia are initiated spirally in 10–12 orthostichies. The base of the young cone has started to elongate the peduncle, which shows more or less rudimentarily developed cataphylls. The top of this cone dome still shows an undifferentiated apex, about 500 µm high. The youngest primordia of the sporangiophores are initially elliptical, with a length of about 200 µm, but later become hexagonal. During July, the buds of the fertile branches increase and the cones become visible without dissection of the branch. At this time, the distal end of the oldest primordium of the sporangiophores alters to a flattened, hexagonal shape (Figs. 2D,E). The hexagonal, distal end is about 400 µm high, about 800 µm long (Fig. 2E), and densely covered with hairs. Near the base of the primordium, two dorsiventrally flattened lobes are initiated (Fig. 2F). Between the two lobes, a stalk is formed. In this stage, the sporangiophore contains only a single vascular bundle (Fig. 4A). The differentiation of this bundle starts from the cone axis and continues towards the distal end of the sporangiophore.

**Fig. 3** *Zamia amblyphyllidia* – SEM micrographs showing the morphogenesis of a male cone in August. (A) Sporangiophore from abaxial at the beginning of August, the formation of primordia of the synangia has started. (B) Lobe in abaxial view, with the initiation of primordia of the synangia starting near the stalk. (C) Dissected cone from the middle of August, abaxial view; 4 primordia of the synangia are still initiated per lobe. (D) Sporangiophore from the same developmental stage in detail. (E) Lobe of the sporangiophore in detail, the initiation of the primordia starts near the stalk and continues in a zigzag fashion to the lateral margin of the lobe (arrow). (F) The innermost primordium has taken on an elliptical shape and is starting to bifurcate. Scale bar: A, D 500 µm; B, E, F 200 µm; C 1 mm. sp, sporangiophore; st, stalk; lo, lobe; pr, primordium; ca, cone axis.



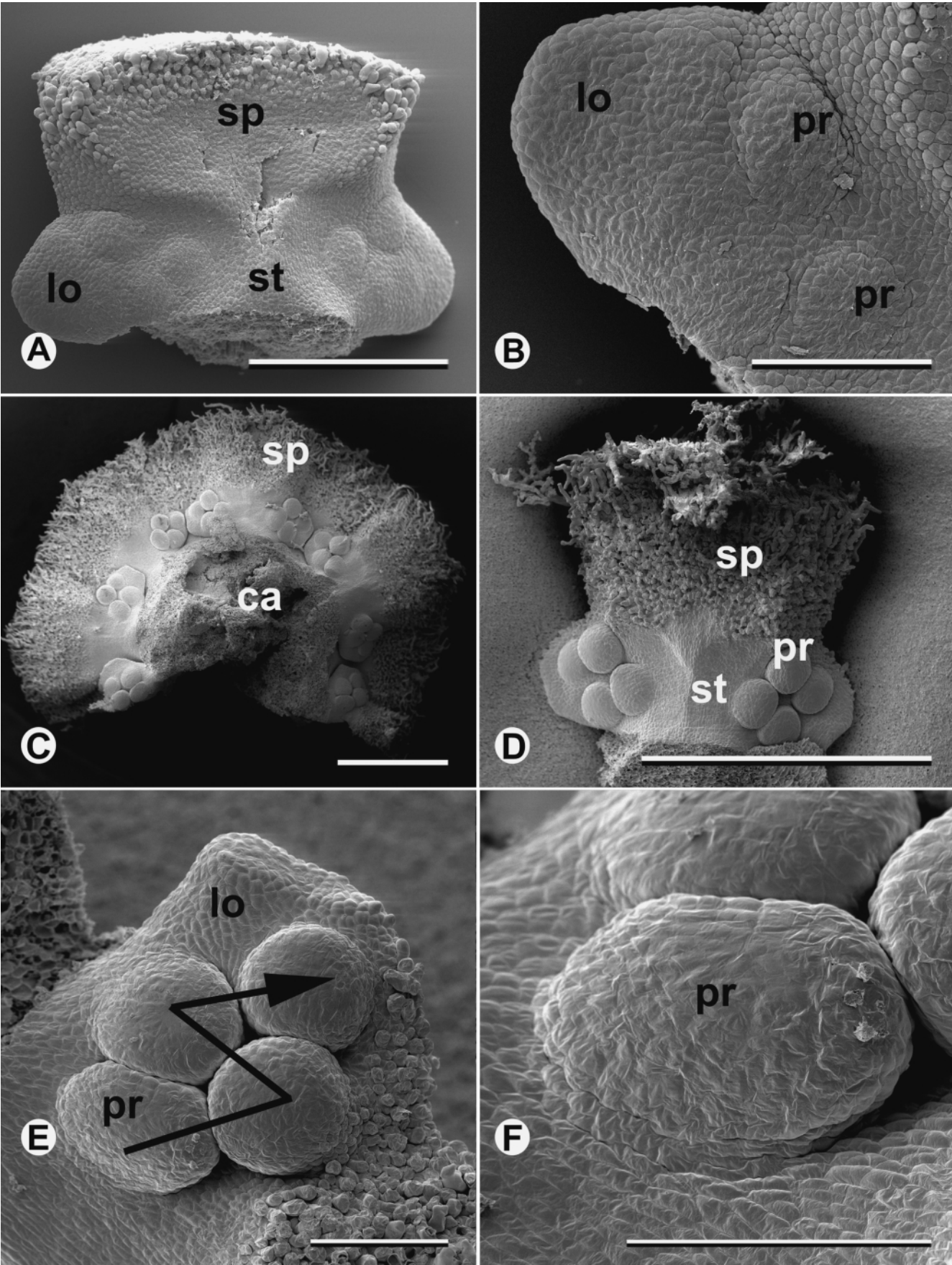


Fig. 3

On the abaxial side of both lobes the primordia of the synangia develop at the beginning of August (Fig. 3A). At this stage, the distal end of the sporangiophore is about 500 µm high and 1 mm long. The initiation of the primordia of the synangia starts near the stalk of the sporangiophores (Fig. 3B). Serial sections of a sporangiophore at the same developmental stage demonstrate that the cells of the lobes remain meristematic (Fig. 4B) and that the formation of the synangia starts from hypodermal cells (Fig. 4C). Longitudinal sections of a lobe show two areas with a higher number of mitoses (Fig. 4C, arrows). Each area forms one primordium; this process is also revealed by the slightly bulging surface. Two weeks later, the sporangiophores are about 600 µm long and the lobes show 4 well developed primordia on the abaxial side (Figs. 3C,D). The first 3 to 4 primordia may show early simultaneous development, whereas the last 1 or 2 primordia develop with considerable delay. The primordia initiate in a zigzag mode from near the stalk and proceed towards the lateral margin (Fig. 3E). At first, the primordia are circular with a diameter of about 100 µm, but later they become more elliptical with a major diameter of about 200 µm (Fig. 3F). At this time, the first vascular bundle starts to dichotomise (Fig. 4D). This is the main bundle, which supplies the stalk and the distal part of the sporangiophore. At the border between sporangiophore and cone axis, two lateral vascular bundles start to differentiate. These lateral bundles vascularise the lobes. Longitudinal microtome sections of a sporangiophore at the same stage show two primordia of the synangia (Fig. 4E). The flattened, distal part of the sporangiophore no longer displays meristematic tissue. Only the tissue close to the primordium remains meristematic. The primordial tissue has no further differentiation (Fig. 4F). All cells are meristematic and have intensively stained cytoplasm. The epidermis shows only anticlinal divisions.

At the end of August, the sporangiophores are about 2 mm long. The hexagonal, distal tip is densely covered with long hairs (Fig. 5A). Towards the lateral margins of the lobes, further more or less circular primordia were initiated (Fig. 5B). The older, elliptical primordia start to bifurcate (Fig. 5C). Each part develops into a sporangium and, as a result, each synangium consists of two sporangia. The connective between the two sporangia of a synangium develops into a short stalk. At the same time, each branch of the main vascular bundle has, once again, bifurcated to supply the distal end of the sporangiophore (Fig. 4G). Both lateral vascular bundles at the base of the sporangiophore start to divide for the first time. Microtome sections of a sporangiophore during this stage of development show that the cells of the distal hexagonal end of the sporangiophore are now nearly completely vacuolated (Fig. 4H), and no further cell divisions occur in this tissue. Only the epidermal cells remain meristematic and follow the growth of the inner cells by anticlinal divisions. Longitudinal sections of the bifurcating primordia show two developing sporangia (Fig. 4I). The tissues of the sporangia consist of an intensely stained cytoplasm with several mitoses. The cytoplasm of the cells between both sporangia is less dense. The cells in this area start to differentiate into the stalk of the synangia.

At the beginning of September, the sporangiophores are about 2.5 mm long (Fig. 5D). All 6 synangia per lobe are developed. A synangium is about 400 µm long (Fig. 5E). At this time, the stalk between both sporangia is clearly visible (Fig. 5F) and

the sporangia are about 200 µm in diameter. At this developmental stage, the main vascular bundle is subdivided several times and forms a branched system of vascular strands that supplies the distal part of the sporangiophore (Fig. 6A). The lateral vascular bundle is divided only once. The inner strand of each lateral bundle is more differentiated than the outer. Longitudinal sections of the lobe of a sporangiophore at this developmental stage show two synangia, consisting of two sporangia. The cells of the lobes are vacuolated, except for the cells of the differentiating, lateral vascular system (Fig. 6B). Each synangium is supplied by its own developing vascular strand. At this time, differentiation of the sporangia begins. In the centre of the sporangia, the sporogenous tissue is formed. A 3–4-layered sporangial wall, whose cells are less stained, surrounds it. The epidermis of the sporangia follows the growth of the inner cells by anticlinal divisions (Fig. 6D).

From a basal intercalary meristem, the stalk of the sporangiophore enlarges further. At the same time, the lateral vascular bundles are formed. Each synangium is served by a branch of the lateral vascular system. The branching pattern of both lateral systems is often not symmetric. The first dichotomy of both lateral vascular bundles is often shifted asymmetrically due to intercalary growth. (Fig. 6C, arrows). The lateral vascular systems divide into 2–3 sub-bundles. Usually, the synangia numbers 1, 3 and 5 (series of initiation) are connected to the outermost sub-bundles. Synangium number 6 is either supplied by a median bundle of its own (left lobe Fig. 6C) or, together with synangia number 4 and 5, it is connected to the innermost bundle (right lobe Fig. 6C). If synangium number 6 is supplied by a bundle of its own, synangia number 2 and 4 are connected to an innermost, third bundle of the lateral vascular strand. At the proximal part of the stalk, both lateral vascular bundles and the main vascular bundle are single-stranded (Fig. 6E). Cross sections show that these 3 equal vascular bundles are united simultaneously (Fig. 6F), and that one common vascular bundle enters the cone axis (Fig. 6G). Towards anthesis, the length of the sporangiophore reaches about 1 cm (Fig. 1C). The sporangia are 1 mm in diameter. They open by a longitudinal slit (Fig. 1D) which originates from the stalk of the synangium and continues to the distal part of the sporangium.

**Fig. 4** *Zamia amblyphyllidia* – light micrographs of sporangiophores. (A) Cleared sporangiophore in July; one vascular bundle is differentiated between both lobes and is connected to the cone axis. (B) Longitudinal section of a sporangiophore through one of the two lobes in July. (C) Longitudinal section of a lobe with two primordia of synangia. (D) Cleared sporangiophore at the beginning of August with one main vascular bundle and two lateral vascular bundles. (E) Longitudinal section of a sporangiophore through one or both lobes at the beginning of August. (F) Longitudinal section of a primordium of a synangium in detail. (G) Cleared entire sporangiophore that was harvested in August; the main vascular bundle has dichotomised several times, the lateral vascular bundles are starting their first division. (H) Longitudinal section of a sporangiophore through one of the two lobes with two primordia starting to bifurcate. (I) Primordium is bifurcated into two sporangia connected by a short stalk. Scale bar: B,E 500 µm; C,F 50 µm; H 200 µm, I 100 µm. lo, lobe; sp, sporangiophore; vb, vascular bundle; ca, cone axis; pr, primordium; mvb, main vascular bundle; lvb, lateral vascular bundle; ep, epidermis; st, stalk; sg, sporangium.

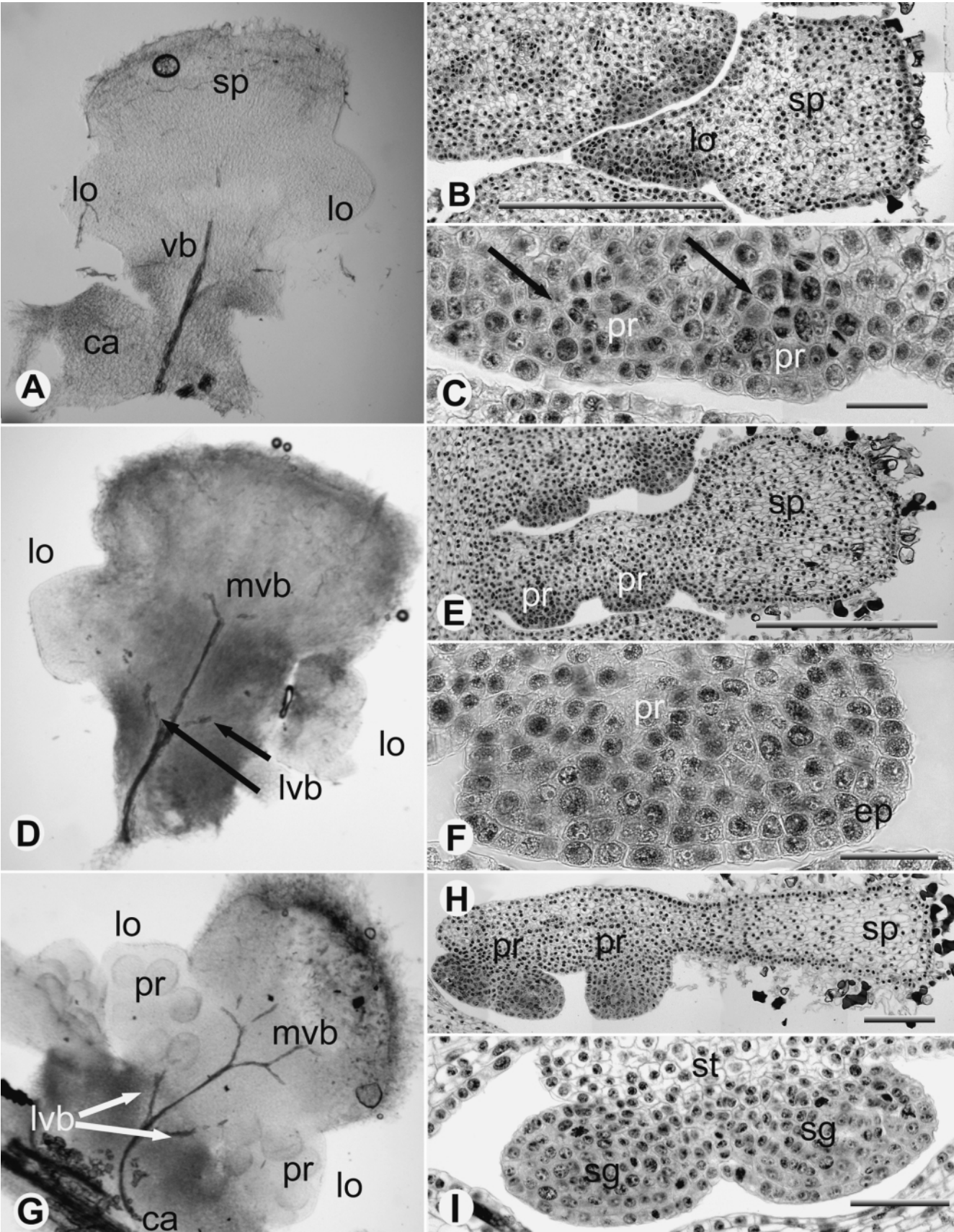


Fig. 4



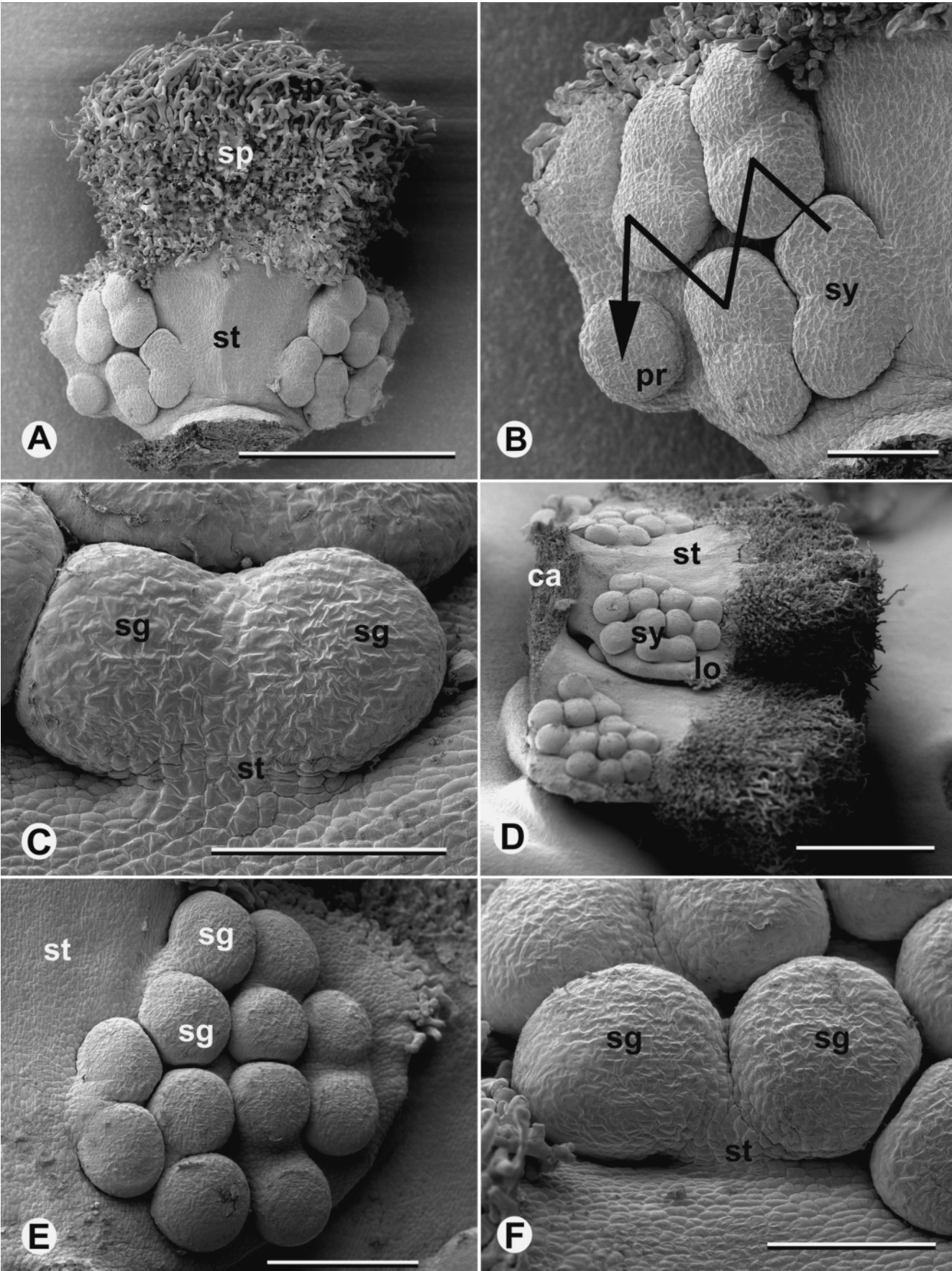


Fig. 5



## Discussion

### Morphology of the male sporangiphores

The results presented here demonstrate that the sporangiphores of *Zamia amblyphyllidia* are not simple, displaying instead a pinnate organisation. The sporangiphores consist of a distal sterile part and a stalk that is flanked by two dorsiventrally flattened lobes, which we interpret as reduced leaflets. On the abaxial side of each lobe 6 synangia are usually formed. Each synangium consists of two sporangia on a minute common stalk. This is contradictory to the interpretation of cycad male sporangiphores given by many authors (Bierhorst, 1971; Chamberlain, 1935; Gifford and Foster, 1989; Smith, 1907; Stewart and Rothwell, 1993). They regard the male sporangiphore as non-pinnate, and simple. In their description, the sporangia cover the entire abaxial surface (e.g. in *Cycas*), or they are divided into two distinct groups separated by a "median sterile line" (e.g. in *Zamia*). The two groups of sporangia are termed "flanks" (Smith, 1907) or "separate zones" (Bierhorst, 1971). In contrast to the cited authors, we submit that these fertile zones may be called lobes, to emphasize their homology with reduced leaflets of a pinnate sporangiphore. The "median sterile line" is thus homologous to the rachis of a pinnate leaf and the position of the synangia is on the abaxial side of reduced leaflets.

The developmental similarities between a pinnate leaf with a basipetal sequence of leaflet formation and the male sporangiphore of *Zamia amblyphyllidia* are striking. At first, the terminal, unpaired segment of a pinnate leaf develops. This corresponds in *Zamia* to the hexagonal tip with the stalk. Thereafter, lateral leaflets are formed that correspond in *Zamia* to the lateral lobes (Figs. 2E,F). The stalk or rachis represents the "sterile line", as mentioned by Coulter and Chamberlain (1917) (or the median, bare region of the sporangiphore according to Smith, 1907).

The vascular system and its development are also in line with the interpretation of a basipetal, pinnate leaf. Each lobe is supplied by its own vascular strand (Fig. 6C). Each of these lateral vascular strands is divided into 2–3 sub-branches which supply the 6 synangia. This pattern of the vascular system is similar to that found in the female sporangiphores of some cycads (e.g. *Ceratozamia mexicana*) as described by Coulter and Chamberlain (1917) referring to studies of Matte (1904). In the case of female sporangiphores (with the exception of *Cycas*), two lateral (outer) vascular systems supply the ovules. Stevenson (1990) emphasized that, in general form, the male

sporangiphores of each genus and species closely resemble (*Stangeria*) or are identical to the female sporangiphores. The female sporangiphores are usually regarded as homologous to a reduced pinnate leaf with ovules in the position of leaflets. Based on these considerations, a similar interpretation for the male sporangiphore is likely.

As mentioned above, in some genera (e.g. *Cycas*, *Encephalartos* and *Stangeria*), the entire "abaxial side" is covered with synangia. This seems to be in contradiction to our interpretation. However, the development of the synangia in these genera also starts in two lateral groups, and the entire covering of the abaxial side is due to further growth of the sporangia (Smith, 1907; Lang, 1897; Warming, 1877). Moreover, in *Encephalartos altensteinii*, only the sporangiphores from the middle part of the cone show an entirely covered abaxial side, but sporangia of the sporangiphores at the tip and at the base of the cone are grouped in two lateral portions (Smith, 1907). Unfortunately, we have no detailed information on the morphogenesis of the sporangiphores of *Cycas*, but the vascular system of the male sporangiphore displays two laterally major bundles that supply the sporangia (Stevenson, 1990). It is noteworthy that the vascular system of *Cycas* displays the same pattern of two lateral branches, similar to *Zamia amblyphyllidia*. This also indicates that the situation with an entirely covered abaxial surface is derived from a situation with two lateral groups that then could be cogently interpreted as leaflets of pinnate sporangiphores.

### Comparison with conifers

The morphology and the morphogenesis of the male sporangiphores of *Zamia amblyphyllidia* as an example of the cycads are different from those of all other recent conifers (Figs. 7A,B). The male sporangiphore of *Cephalotaxus harringtonii*, which we regard as a primitive type of conifer (Mundry and Mundry, 2001), consists of a stalk, a phylloid tip, and normally 2–4 sporangia inserted between the stalk and phylloid tip. The initiation of the sporangiphore starts with the formation of a phylloid primordium. The sporangia develop at the base of the abaxial side of the primordium. They are fused to the phylloid tip and stalk.

The entire sporangiphore of *Cephalotaxus harringtonii* is supported by a single vascular bundle and the sporangia do not possess their own vascular bundles. A structure comparable to the lobes of *Zamia amblyphyllidia* does not exist. Furthermore, the sporangiphore of *Zamia amblyphyllidia* is supplied by a complex vascular system, consisting of one main vascular system and two lateral vascular systems which support the lobes and the synangia.

In *Cephalotaxus*, the sporangia are fused to the phylloid tip and the stalk. Although the sporangia of *Cephalotaxus* are not free, they are not combined into an aggregate, as in the stalked synangia of *Zamia amblyphyllidia*. In the latter case, usually two, exceptionally three, sporangia are fused to form a synangium which is connected to the abaxial side of the lobe. In other genera of the cycads, up to five sporangia may be grouped together. Although these synangia are not explicitly described as stalked, the sporangia arise "from a cushion of meristematic tissue" (Smith, 1907) which can be interpreted as a short stalk.

◀ **Fig. 5** *Zamia amblyphyllidia* – SEM micrographs of the morphogenesis of a male cone harvested at the end of August to mid-September. (A) Sporangiphore from abaxial; further primordia are initiated at the lateral margin of the lobes at the end of August (B) Lobe in detail, the young primordia are circular, the older ones elliptical in shape. (C) The sporangium develops by bifurcation of the primordium. (D) Dissected cone from the lateral abaxial side in mid-September. (E) Lobe from abaxial in detail, all 6 synangia of a lobe are developed. (F) Synangia with two sporangia connected by a short stalk. Scale bar: A, D 1 mm; B, C, F 200 µm; E 400 µm. sp, sporangiphore, st, stalk; sy, synangium; pr, primordium; sg, sporangium; co, cone; lo, lobe.

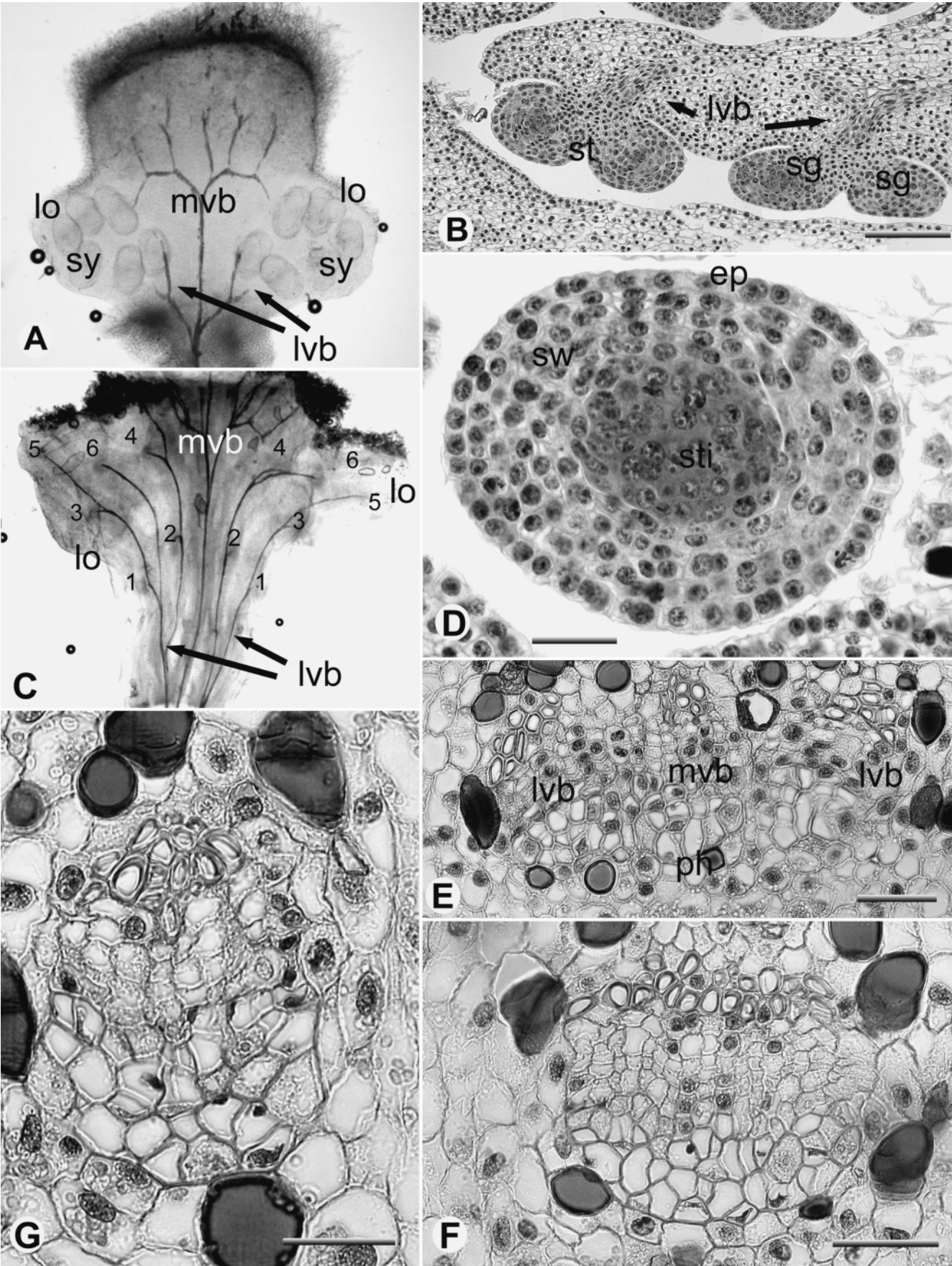


Fig. 6

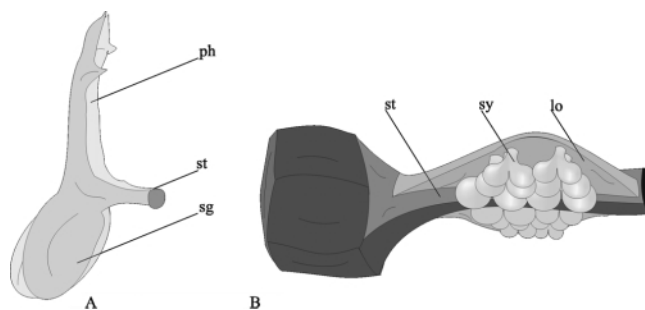
Furthermore, not only the morphology is different, but also the initiation of the sporangia in *Zamia amblyphyllidia* differs from that found in *Cephalotaxus harringtonii*. In the latter species, the sporangia develop on the abaxial side, and each sporangium develops independently (Mundry and Mundry, 2001). In *Zamia*, on the other hand, the sporangia are initiated at the abaxial side of the two lateral lobes (leaflets), and thus the position of the sporangia is actually lateral. Both sporangia of one synangium develop together by bifurcation of a single common primordium and not each independently as in *Cephalotaxus*.

With the exception of *Taxus* and *Torreya* (Mundry and Mundry, 2001), the early morphogenesis of sporangiphores of other conifer genera is quite similar to *Cephalotaxus*. In *Podocarpus macrophyllus*, as an example for the Podocarpaceae, the development also starts with the formation of a distinct phylloid primordium, and the sporangia are also initiated on the abaxial side of a simple sporangiphore (Mundry, 2000). The initiation of the sporangiphores of *Cryptomeria* and *Chamaecyparis* starts in the same way (Erspamer, 1952), although the mature peltate sporangiphores are quite different from those of *Cephalotaxus*. In the Pinaceae, the morphogenesis of the sporangiphores of *Cedrus* is also quite similar to what is seen in *Cephalotaxus* and *Podocarpus*. The morphogenesis of the sporangiphores of *Abies* and *Picea* seems to be ambiguous, because the primordia of the sporangiphores lack a distinct phylloid shape (Mundry, 2000). The sporangia of all mentioned genera do not form any kind of synangia.

Even though conifer sporangiphores differ in some characters, e.g. the number of sporangia or the size of the phylloid tip, all extant conifer sporangiphores display a simple arrangement, with sporangia singularly attached to the abaxial side. Based on these differences, we propose that the sporangiphores of the cycads and the conifers are not homologous structures.

#### Possible ancestor of the cycads

Since the male sporangiphores of the extant gymnosperms display considerable differences, it is necessary to study the fossil record. Although some authors (e.g. Meyen, 1984) con-



**Fig. 7** Drawing of different types of sporangiphores. (A) Sporangiphore of *Cephalotaxus harringtonii* with 4 sporangia connected to a stalk and a phylloid tip. (B) Drawing of a sporangiphore of *Zamia amblyphyllidia* from the lateral side. st, stalk; sg, sporangium; sy, synangium; ph, phylloid tip; lo, lobe.

sider the male sporangiphores as “less significant”, we want to discuss hypothetical ancestors of the cycads.

The discussion about the origin of the cycads is dominated by the question of the primitive appearance of the cycads. In the past, it was assumed by several authors that the primitive cycads displayed frond-like, sterile and fertile leaves, similar to the extant member of this group. Therefore, a close relationship between cycads and Palaeozoic pteridosperms seemed likely. Delevoryas (1955) postulated a possible ancestor of the cycads in the Palaeozoic medullosans. The “single, largest roadblock” in such a derivation of the cycads seemed to be the origin of the cycad male sporangiphore (Stewart and Rothwell, 1993).

However, Mamay (1976) proposed that the female sporangiphore of the cycads derived from the Carboniferous genus *Spermopteris*. The genus *Spermopteris* display fertile, entire leaves, with two rows of seeds on each side of the mid-vein. Forms like *Archaeocycas* and *Phasmatocycas*, with an entire distal lamina of the female sporangiphore, are transitions towards the cycad-like female sporangiphore with incisions of the distal lamina. Furthermore, Mamay assumed that a similar process occurred for the sterile leaves, so that the primitive cycads would display sterile leaves with entire margins. Later, Gillespie and Pfefferkorn (1986) confirmed the organic attachment of the entire distal lamina (*Taeniopteris coriacea*) to *Phasmatocycas*. These assumptions make an origin of the cycads from the medullosans equivocal.

Several more recent studies from the Permian in China provide more evidence for the Palaeozoic origin of the modern cycads (Zhu and Du, 1981; Liu and Yao, 2002; Hu et al., 1999). Of some interest are the cycad-like female sporangiphores in *Crossozamia* from the Lower Permian in China (Gao and Thomas, 1989). On the one hand, *Crossozamia* displays the next step in the evolution from an entire distal lamina to a dissected lamina but, on the other hand, the species of *Crossozamia* “are of the same geological age, if not slightly older, than those of *Archaeocycas* and *Phasmatocycas* from Kansas” (Gao and Thomas, 1989). Moreover, Gao and Thomas assume that *Yuania chinensis* belongs to the *Crossozamia*. This leaf species is pinnate, quite similar to the trophophylls of the modern cycads. If *Crossozamia* with pinnate leaves is really older than *Phasmato-*

◀ **Fig. 6** *Zamia amblyphyllidia* – light micrographs of sporangiphores. (A) Cleared sporangiphore, harvested at the end of August; lateral vascular bundles have divided once, the inner strand is more differentiated than the outer. (B) Longitudinal section of a sporangiphore through one of the two lobes with two synangia. (C) Cleared, well matured sporangiphore, the lateral vascular bundles have divided several times. Each lateral vascular system supplies 6 synangia. (D) Longitudinal section of a sporangium at the beginning of September showing a multilayered sporangial wall and the sporogenous tissue. The sporogenous tissue has intensely stained cytoplasm. (E) Cross section of both (single strand) lateral vascular strands and the main vascular strand at the proximal part of the stalk of the sporangiphore. (F) All three vascular strands fuse to form one strand. (G) Cross section of the common vascular bundle which is connected to the cone vascular system at the proximal part of the stalk. Scale bar: B 200 µm; D–F 50 µm; G 30 µm. lo, lobe; sy, synangium; mvb, main vascular bundle; lvb, lateral vascular bundle; sg, sporangium; st, stalk; ep, epidermis; sw, sporangium wall; sti, sporogenous tissue.

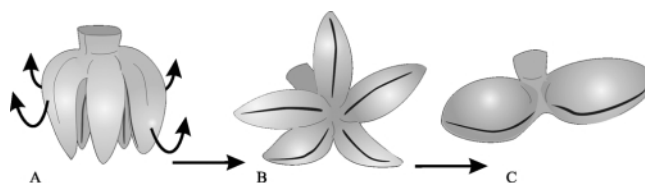
cycas and *Archaeocycas*, one could infer that the pinnate situation is primitive in the cycads.

The fossil record of the male reproductive structures seems to be less important for the discussion of the origin of the cycads. Nevertheless, there are several well-preserved Jurassic and Triassic male cones belonging to the form genus *Androstrobus* (Harris, 1941, 1961; Hu et al., 1999). The Jurassic male sporangiophores of *Androstrobus prisma* display a striking similarity to modern cycads like *Encephalartos* or *Zamia* (Harris, 1961). The whole abaxial surface of the male sporangiophore of these species is covered with sporangia which are grouped in sori of two to three with apertures facing one another. Although in other species of *Androstrobus* a soral arrangement could not be identified with certainty, the aperture of the sporangia facing one another make a soral arrangement quite likely.

Although the ultrastructure of the pollen of *A. balmei* and *A. wonnacotti* shows characters of both extant families (Zamiaceae and Cycadaceae), Hill (1990) assumed that these species closely resembled the Zamiaceae and that the characters which are more typical of Cycadaceae are heterobathmic. A further Jurassic cycad male cone is *Cycandra*, described by Krassilov (1996). This species is quite similar to *Androstrobus prisma*, although the sporangia seemed not to be grouped in soral clusters, rather being adnate up to the shoulder line. Another Triassic male cone of *Leptocycas gracilis* is described by Delevoryas and Hope (1982), but unfortunately the structure of the male sporangiophores seems not to be preserved.

There are few Palaeozoic male cones that might belong to the cycads. The oldest known male cone with cycadalean affinity is described by Taylor (1968) from the late Carboniferous. Later, Taylor (1970) gave a description of this male cone under the name *Lasioistrobus* and assumed, in spite of his early idea, that *Lasioistrobus* is probably a coniferalean male cone. Further species are described from the Permian of China. One is the male cone *Liulinia lacunculata* consisting of about 10–15 spirally arranged sporangiophores with a broadly pinnate dissected distal part (Wang, 1986). The sporangia probably cover the basal part on the abaxial side. The other male cone from the Permian in China is *Cycadostrobus paleozoicus* (Zhu et al., 1994). The form of the male cone is described as similar to *Androstrobus*. The sporangia are attached at the abaxial side and seem to be arranged in radial groups of 3–4 with a longitudinal dehiscent line.

Our results and our interpretation of the male sporangiophores of *Zamia amblyphyllidia* provide a new insight into the evolution of the cycads. In *Zamia amblyphyllidia* the synangia consist of two sporangia, in some cases three sporangia. In several other genera of the cycads the synangia are composed of up to five sporangia that form radial synangia groups. This property of radial synangia groups seemed to join the extant male cones with fossils like the Jurassic *Androstrobus prisma* and the Permian male cone *Cycadostrobus paleozoicus*. This implies that the arrangement of the sporangia in synangia is a plesiomorphic character, having evolved before the Permian. Unfortunately, no details about the sporangia arrangement of the other Permian male cone *Liulinia lacunculata* seem to be preserved. The problematic cone of *Lasioistrobus* lacks any kind of radial synangia groups or radial arrangement. Although con-



**Fig. 8** Hypothetical transformation series of the synangia of *Zamia amblyphyllidia*. (A) Radial synangium of some pteridosperms, e.g. *Cordaites* surrounding a central hollow (redrawn after Stewart and Rothwell, 1993). (B) Derivation of the radial synangium of e.g. *Cycas* by tipping over of the sporangia, (C) Reduction of the number of sporangia leads to the synangium of *Zamia amblyphyllidia*.

siderably older, this might support the assumption that *Lasioistrobus* has no cycadalean affinity.

We obtained no direct support from the fossil record for our interpretation that the male sporangiophores display a pinnate arrangement. The sporangia of the fossil male cones of the cycads cover the entire abaxial surface of the sporangiophores. Nevertheless, our interpretation of the pinnate arrangement of the sporangiophores still remains possibly correct. As mentioned above, in several extant genera the entire abaxial surface is also covered with sporangia, although the morphogenesis of the sporangia starts in two distinct groups. Moreover, the sporangia of the genera *Cycas* are supplied by two lateral vascular systems, similar to the vascular system of *Zamia amblyphyllidia*. Unfortunately, the morphogenesis and the arrangement of the vascular system of the fossil male sporangiophores are unknown. Hence, it is still possible that the male sporangiophores of the fossil cycads are also reduced pinnate structures.

Following this argument, the hypothetical ancestor of the cycads displayed pinnate male sporangiophores with clusters of sporangia or with radial synangia groups. This pattern is present in primitive pteridosperm pollen organs (Stewart and Rothwell, 1993). According to Millay and Taylor (1979), radial synangia groups occur in the Lyginopteridaceae of the Mississippian and later in several Medullosaceae of the Pennsylvanian. In both groups, the sporangia are fused and form a simple ring "that usually surrounds a hollow central area" (Stewart and Rothwell, 1993). In this central area, the pollen is shed by longitudinal dehiscent slits.

Although cycad synangia are prostrate at the abaxial side of the lobes, Stewart and Rothwell (1993) regard the condition in the pteridosperms as the evolutionary origin of the cycads ("although highly speculative"). The radial synangia groups of the cycads could be easily derived from the ring-like structure of the pteridosperms by tipping over the sporangia. In this case, the inward-facing side of the ring would become the distal side of the synangia (Figs. 8A–C). This corresponds with the position of the dehiscent slits of cycads (Fig. 1D). A reduction of the number of sporangia leads to the synangia of *Zamia amblyphyllidia*.

We obtain further support for a pteridospermous origin of the cycads in that the development of the sporangiophores of the extant cycads is similar to the development of a pinnate leaf with synangia on the abaxial side of the leaflets. In the Medullosaceae and Callistophytaceae the radial synangia groups are



also located on pinnules or pinnae. Millay and Taylor (1979) pointed out that "abaxial synangia on pinnules are presumably the most advanced condition" in the pteridosperms. These inferences contradict the opinion that the ancestor of the cycads displayed entire sterile and fertile leaves, based mainly on the studies of Mamay (1976).

Our suggestions with respect to the male cones of the cycads give new arguments for a cycad precursor with a pinnate arrangement of the reproductive structures, at least for the male sporangiophores. The radial synangia groups may eventually point to the Medullosaceae, although this is quite hypothetical for now. To answer this question, more morphogenetic studies on the sporangiophores of the extant cycads and more detailed analysis of the Palaeozoic cycads are necessary.

### Acknowledgements

We thank Michael Krings, Thomas Speck, and an anonymous reviewer for their critical and helpful comments on the manuscript. Randy Cassada has made substantial, linguistic improvements. The Botanical Garden of the Ruhr University of Bochum allowed us to dissect entire plants of *Zamia amblyphyllidia*. We are grateful to I. Mundry for many helpful discussions and improvements of the manuscript.

### References

- Bierhorst, D. W. (1971) Morphology of Vascular Plants. New York: Macmillan.
- Bowe, L. M., Coat, G., and dePamphilis, C. W. (2000) Phylogeny of seed plants based on all three genomic compartments: Extant gymnosperms are monophyletic and a close Gnetales-conifer relationship. *Proc. Natl. Acad. Sci.* 97, 4092–4097.
- Chamberlain, C. J. (1935) *Gymnosperms Structure and Evolution*. Chicago: University of Chicago Press.
- Chaw, S.-M., Parkinson, P. C., Cheng, Y., Vincent, T. M., and Palmer, J. D. (2000) Seed plant phylogeny inferred from all three plant genomes: Monophyly of extant gymnosperms and origin of Gnetales from conifers. *Proc. Natl. Acad. Sci.* 97, 4086–4091.
- Coulter, J. M. and Chamberlain, C. J. (1917) *Morphology of Gymnosperms*. 2nd ed. Chicago (Reprint 1982): University of Chicago Press.
- Delevoryas, T. and Hope, R. (1982) A new Triassic cycad and its phylogenetic implications. *Postilla* 150, 1–14.
- Delevoryas, T. (1955) The Medullosae structure and relationship. *Palaeontographica B* 97, 114–167.
- Delevoryas, T. (1982) Perspectives on the origin of cycads and cycadeoids. *Review of Palaeobotany and Palynology* 37, 115–132.
- Donoghue, M. J. and Doyle, J. A. (2000) Seed plant phylogeny: Demise of the anthophyte hypothesis? *Curr. Biol.* 10, R106–109.
- Doyle, J. A. (1998) Phylogeny of vascular plants. *Annu. Rev. Ecol. Syst.* 29, 567–599.
- Ersamer, J. L. (1952) Ontogeny and morphology of the microsporangium in certain genera of the Coniferales – Ph. D. Diss. Berkeley: University of California.
- Frohlich, M. W. and Parker, D. S. (2000) The mostly male theory of flower evolutionary origins: from genes to fossils. *Syst. Bot.* 25, 155–170.
- Gao, Z. and Thomas, B. A. (1989) A review of fossil cycad megasporophylls, with new evidence of *Crossozamia* Pomel and its associated leaves from the Lower Permian of Taiyuan, China. *Rev. Palaeobot. Palynol.* 60, 205–223.
- Gerlach, D. (1984) *Botanische Mikrotechnik*. 3rd ed. Stuttgart: Thieme.
- Gerstberger, P. and Leins, P. (1978) Rasterelektronenmikroskopische Untersuchungen an Blütenknospen von *Physalis philadelphia* (Solanaceae). *Ber. Deutsch. Bot. Ges.* 91, 381–387.
- Gifford, E. M. and Foster, A. S. (1989) *Morphology and evolution of vascular plants*. 3rd ed. New York: Freeman and Company.
- Gillespie, W. H. and Pfefferkorn, H. W. (1986) Taeniopterid lamina on *Phasmatocycas megasporophylls* (Cycadales) from the Lower Permian of Kansas, U.S.A. *Rev. Palaeobot. Palynol.* 49, 99–116.
- Harris, T. M. (1941) Cones of extinct Cycadales from the Jurassic rocks of Yorkshire. *Philos. Trans. R. Soc. Lond. B* 231, 75–98.
- Harris, T. M. (1961) The fossil cycads. *Palaeontology* 4, 313–323.
- Hill, C. R. (1990) Ultrastructure of *in situ* fossil cycad pollen from the English Jurassic, with a description of the male cone *Androstrobus balmei* sp. nov. *Rev. Palaeobot. Palynol.* 65, 165–173.
- Hu, Y.-F., Chen, Z.-D., and Geng, B.-Y. (1999) Discoveries of some fossils of cycad reproductive organs from China and their significance to the origin of cycads. In *Biology and Conservation of Cycads – Proceedings of the Fourth International Conference on Cycad Biology* (Chen, C. J., ed.), Beijing: International Academic Publishers, pp. 135–141.
- Jones, D. L. (1993) *Cycads of the world*. Australia: Reed Books.
- Krassilov, V. A., Delle, G. V., and Vladimirova, H. V. (1996) A new Jurassic pollen cone from Georgia and its bearing on cycad phylogeny. *Palaeontographica B* 238, 71–75.
- Lang, W. H. (1897) Studies in the development and morphology of cycadean sporangia I. The microsporangia of *Stangeria paradoxa*. *Ann. Bot. (London)* 11, 421–438.
- Liu, L. J. and Yao, Z. Q. (2002) Lepingia: A new genus of probable cycadalean affinity with taeniopterid lamina from the Permian of South China. *Int. J. Plant Sci.* 163, 175–183.
- Mamay, S. H. (1976) Paleozoic origin of the cycads. U. S. Geological Survey Professional Paper.
- Matte, H. (1904) Recherches sur l'appareil libero-ligneux des Cycadees. *Mémoires de la Société Linnéenne de Normandie* 22, 1–223.
- Meyen, S. V. (1984) Basic features of gymnosperm systematics and phylogeny as evidenced by the fossil record. *Bot. Rev.* 50, 1–111.
- Millay, M. A. and Taylor, T. N. (1979) Paleozoic seed fern pollen organs. *Bot. Rev.* 45, 301–375.
- Mundry, I. and Mundry, M. (2001) Male cones in Taxaceae sl. – an example of Wettstein's *Pseudanthium* concept. *Plant. Biol.* 3, 405–416.
- Mundry, I. (2000) Morphologische und morphogenetische Untersuchungen zur Evolution der Gymnospermen. *Bibl. Bot.* 152, 1–90.
- Norstog, K. J. and Nicholls, T. J. (1997) *The biology of the cycads*. New York: Cornell University Press.
- Smith, F. G. (1907) Morphology of the trunk and development of the microsporangium of cycads. *Bot. Gaz.* 43, 187–204.
- Stevenson, D. W. (1988) Strobilar ontogeny in the Cycadales. In *Aspects of Floral Development* (Leins, P., Tucker, S. C., and Endress, P., eds.), Berlin: Cramer, pp. 205–224.
- Stevenson, D. W. (1990) Morphology and systematics of the Cycadales. *Memoirs of the New York Botanical Garden* 57, 8–55.
- Stewart, W. N. and Rothwell, G. W. (1993) *Paleobotany and the evolution of plants*. 2nd ed. Cambridge: University Press.
- Taylor, T. N. (1988) Pollen and pollen organs of fossil Gymnosperms. Phylogeny and reproductive biology. In *Origin and Evolution of Gymnosperms* (Beck, C. B., ed.), New York: Columbia University Press, pp. 177–217.
- Taylor, T. N. (1968) Cycads: evidence from the Upper Pennsylvanian. *Science* 164, 294–295.
- Taylor, T. N. (1970) *Lasiostrobus* gen. nov., a staminate strobilus of gymnosperm affinity from the Pennsylvanian of North America. *Amer. J. Bot.* 57, 670–690.
- Wang, Z. (1986) *Luilinia*, a new male cycad cone from the uppermost Permian of Shansi. *Acta Palaeontologica Sinica* 25, 610–616.

- Warming, E. (1877) Undersogelser og Betragtninger over Cycadeerne. Overs. Kongel. Danske Vidensk. Selsk. Forh. Medlemmiers Arbejder., 88 – 144.
- Winter, K. U., Becker, A., Münster, T., Kim, J. T., Saedler, H., and Theissen, G. (1999) MADS-box genes reveal that gnetophytes are more closely related to conifers than to flowering plants. Proc. Natl. Acad. Sci. 96, 7342 – 7347.
- Zhu, J., Zhang, X., and Jie, M. (1994) A new genus and species – *Cycadostrobus palaeozoicus* Zhu of Cycadaceae from the Permian of China. Acta Phytotaxonomica Sinica 32, 340 – 344.
- Zhu, J.-N. and Du, X.-N. (1981) A new cycadale, *Primocycas chinensis* gen. et sp. nov. discovered in the Lower Permian of Shanxi, China, and its significance. Acta Botanica Sinica 23, 401 – 404.

M. Mundry  
Ruhr-Universität Bochum  
Spezielle Botanik  
NDEF 05/776  
44780 Bochum  
Germany  
E-mail: marcus.mundry@ruhr-uni-bochum.de

Section Editor: G. Gottsberger