

## Some new aspects about the evolution of pollen cones and perisporangiate microsporangiophores in Taxaceae

### Abstract

Pollen cones of several Taxaceae (*Cephalotaxus*, *Pseudotaxus*, *Taxus* and *Torreya*) and especially their microsporangiophores were investigated with paraffin technique and SEM. A lot of the investigated pollen cones of *Torreya* and *Cephalotaxus* formed several hyposporangiate microsporangiophores in the distal part of the cone axis instead of the typical terminal perisporangiate one. These hyposporangiate microsporangiophores are always supplied by only one collateral vascular bundle strand. Investigations on the vasculature of the terminal perisporangiate microsporangiophores have shown that they are always supplied with several, mostly 2-4 collateral vascular bundle strands, which enter the cone axis in separate strands. This leads to the idea that the terminal perisporangiate microsporangiophore reflects a radial synangium consisting of several fused hyposporangiate microsporangiophores. Furthermore it could be shown that the lateral perisporangiate microsporangiophores of *Pseudotaxus* and *Taxus* also represent radial synangia consisting of minimum 2 fused hyposporangiate microsporangiophores. The lateral hyposporangiate microsporangiophores of *Torreya* and *Cephalotaxus* are supplied by only a single collateral vascular bundle. Due to the number and the position of microsporangiophores, and also the vasculature within a single pollen cone, *Torreya* is quite similar to the situation within a single lateral pollen cone within the branched *Cephalotaxus* pollen cone. However, it remains open, if the *Torreya* pollen cone represents a strongly reduced *Cephalotaxus* pollen cone or if the branched *Cephalotaxus* pollen cone represents a cluster of several *Torreya*-like pollen cones.

### 1 Introduction

Among extant conifers the seed cones are characterized by the bract-/seed-scale-complex, which is, however, strongly modified in the different systematic groups. Thus, seed cones in conifers can be generalized as branched structures fulfilling the definition given for an “inflorescence” in angiosperms (SCHUHMAN 1902; HERZFELD 1914; PILGER 1926; FLORIN 1951, 1954; SCHWEITZER 1963; KRÜSSMANN 1983; FARJON 1984, 2005, 2010; PAGE 1990; STÜTZEL & RÖWEKAMP 1997, 1999; MUNDRY 2000; FARJON & ORTIZ GARCIA 2003; ECKENWALDER 2009; DÖRKEN 2011).

In contrast to this, the situation for the coniferous pollen cones is not as homogenous. Among the different systematic groups of extant conifers, pollen cones have different structures. The majority are unbranched, “flower”-like. Only in a few extant Taxaceae, e.g. in *Cephalotaxus* and *Pseudotaxus* (WILDE 1975; MUNDRY & MUNDRY 2001; DÖRKEN *et al.* 2011), and in Cupressaceae *s. str.* only in *Juniperus drupacea* (e.g. LEMOINE-SEBASTIAN 1967; FARJON 2005; ECKENWALDER 2009), and a few Podocarpaceae like some species of *Podocarpus*, *Prumnopitys* and *Retrophyllum* (SCHULZ *et al.* 2014) branched pollen cones are formed.

In pollen cones of extant conifers, two different types of microsporangiophores are developed: (1) hyposporangiate (dorsiventral) microsporangiophores, with microsporangia formed only on the abaxial side of the central stalk and an adaxial scutellum (fig 1A); (2) perisporangiate (radial) microsporangiophores, with microsporangia all around the central stalk (fig 1B). The majority of extant conifers are hyposporangiate. Only some extant Taxaceae (*Pseudotaxus*

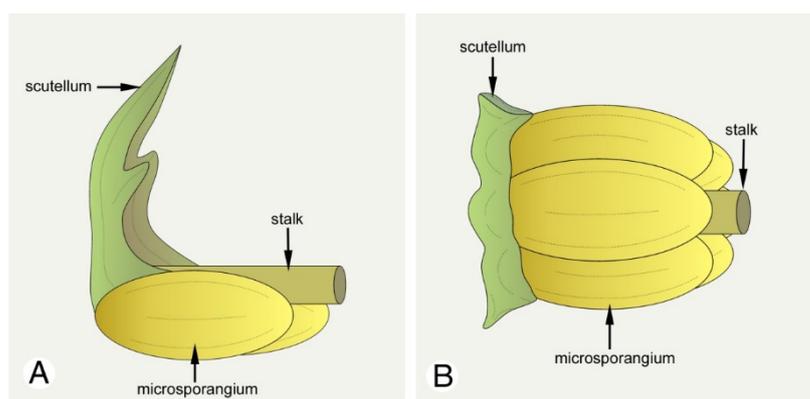


Fig. 1: Microsporangiophores, A: hyposporangiate; B: perisporangiate.

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and *Taxus*) are exclusively perisporangiate. Among other Taxaceae, *Cephalotaxus* and *Torreya* hypo- as well as perisporangiate microsporangiophores are developed within the same cone.

Two contrary major evolutionary concepts about the coniferous microsporangiophores exist. Some authors regard all coniferous microsporangiophores as homologous structures and hyposporangiate (dorsiventral) microsporangiophores are derived from a perisporangiate ancestral condition (e.g. WORDSELL 1901; DUPLER 1919; DLUHOSCH 1937). Other authors (e.g. THOMSON 1940; WILDE 1975; MUNDRY & MUNDRY 2001; DÖRKEN *et al.* 2011), however, suggest perisporangiate microsporangiophores are derived from a radial synangium consisting of several dorsiventral reduced microsporangiophores. However, both scenarios are still discussed controversially.

In contrast to the quite heterogenous pollen cone structure in Taxaceae (branched vs. unbranched; perisporangiate vs. hyposporangiate), the situation among nearly all other conifers is quite homogenous, and mostly one type of pollen cones is developed: unbranched, hyposporangiate and without a terminal microsporangiophore. Thus, suggesting evolutionary scenarios about the coniferous pollen cones and their microsporangiophores is quite difficult. We found pollen cones and microsporangiophores in different Taxaceae showing several types of anomalies, which were also included in our investigations. Our morpho-anatomical investigations should help towards a better understanding of the evolution of the taxaceous pollen cones and especially their microsporangiophores.

## 2 Material & Methods

### 2.1 Material

Typical and anomalous pollen cones of *Torreya californica* TORR. were collected in the Botanic Garden of the Ruhr-University Bochum, Germany. *Pseudotaxus chienii* (W.C.CHENG) W.C.CHENG was collected in the private living collection of HUBERTUS NIMSCH, Bollschweil, St. Ulrich, Germany where the specimens are cultivated as potted plants in a temperate house. *Cephalotaxus harringtonii* (KNIGHT ex J.FORBES) K. KOCH was collected in a private garden in Verbania, Italy, in the Botanic Garden of the Ruhr-University Bochum (Germany), and on the Isle of Mainau (Konstanz, Germany). Pollen cones of *Taxus baccata* L. were collected from several trees growing on the campus of the University of Konstanz (Germany). From each taxon material we collected from several individuals, and within an individual also from different branches within the crown.

### 2.2 Methods

Freshly collected material was photographed and then fixed in FAA (100 ml FAA = 90 ml 70% ethanol + 5 ml acetic acid 96% + 5 ml formaldehyde solution 37%) before being stored in 70% ethanol. The cone anatomy was studied from sections using the classical paraffin technique and subsequent astrablue/safranin staining (GERLACH 1984). For SEM-analysis the FAA-material was dehydrated in formaldehyde dimethyl acetal (FDA) for at least 24 hours (GERSTBERGER & LEINS 1978) and critical point dried. Sputter coating was done with a sputter coater SCD 50 BAL-TEC (BALZERS). The specimens were examined with an AURIGA ZEISS TM. Macrophotography was accomplished using a digital camera (CANON POWERSHOT IS2) and microphotography with a digital microscope (KEYENCE VHX 500F) equipped with a high-precision VH mounting stand with X-Y stage and bright field illumination (KEYENCE VH-S5).

### 2.3 Special terms

Most authors regard the microsporangia bearing structure in conifers as “microsporophylls”. We avoid using the term “sporophyll” or “microsporophyll” for the microsporangia bearing structure. Otherwise we would introduce *a priori* a homology that is applied to it. Thus we are calling them microsporangiophores. The green, adaxial scale-like structure in hyposporangiate microsporangiophores will be termed a scutellum, and not “phyllid rest” as is frequently done. “Inflorescence”-like pollen cones consisting of several lateral cones, each of them inserted in the axil of a bract, are called branched.

## 3 Results

### 3.1 Anomalous hyposporangiate microsporangiophores

#### 3.1.1 *Pseudotaxus chienii* (W.C.CHENG) W.C.CHENG

Pollen cones of *Pseudotaxus chienii* are inserted at the lower side of the branches (figs 2A, C, D). Typical pollen cones are branched and consist of exclusively perisporangiate microsporangiophores,

each inserted in the axil of a scale-like bract (figs 2B, E, F), that cover the microsporangiophores before anthesis (figs 2A, B, E). In distal parts of the pollen cones bracts are sometimes lacking. In all, 19% of the 300 investigated pollen cones differed from the majority which have perisporangiate microsporangiophores, by having some hyposporangiate ones with a different number of abaxial microsporangia (2–4) and a green phyllo- like scutellum developed (fig. 3). Within some perisporangiate microsporangiophores a central green column-like structure is developed (fig. 4).

### **3.2 Hyposporangiate microsporangiophores in the distal part of the cone axis instead of the typical terminal perisporangiate microsporangiophores**

#### **3.2.1 *Torreya californica* TORR.**

Pollen cones of *Torreya californica* are located mostly at the lower side of a branch, although some can also be developed on the upper side, but not terminal at the shoot axis. Typical shaped pollen cones consist of several spirally arranged lateral hyposporangiate microsporangiophores, each of them bearing 2–5 microsporangia and a terminal perisporangiate microsporangiophore with 2–8 microsporangia. The microsporangia developed at the perisporangiate terminal microsporangiophore have the same dimensions as these of the lateral hyposporangiate ones (fig. 5). The terminal perisporangiate microsporangiophore is supplied with a varying number of collateral vascular bundle strands. In the investigated material 2 (figs 6 A–C), 3 and 4 (figs 6 D–F) collateral vascular bundles are developed within the central stalk of the microsporangiophore. The bundle strands do not fuse. They enter the cone axis in separate strands. In 38% of the 300 investigated pollen cones a terminal perisporangiate microsporangiophore was absent. Here 2 (figs 7 A & B), 3 (figs 7 C & D) or 4 (figs 7 E & F) hyposporangiate microsporangiophores are arranged in distal parts of the cone axis in the position where usually the terminal perisporangiate microsporangiophore is located. The dimensions of these anomalous distal hyposporangiate microsporangiophores together conform to the dimensions and the number of microsporangia in typical perisporangiate microsporangiophores (fig. 5). Each of the hyposporangiate microsporangiophores is supplied with a single collateral vascular bundle (fig. 8).

#### **3.2.2 *Cephalotaxus harringtonii* (KNIGHT ex J. FORBES) K.KOCH**

Pollen cones of *Cephalotaxus* are developed mostly on the lower side of a branch, and are inserted in the axil of a typical needle-leaf. The pollen cones are branched and consist of 6–11 lateral units (cones), each inserted in the axil of a small scale-like bract. Within the branched cone bud scales for the lateral cones are lacking. Each cone consists of several lateral spirally hyposporangiate microsporangiophores carrying between 2–4 microsporangia. In most cases the cone axis terminates with a perisporangiate microsporangiophore with a varying number of microsporangia (figs 9 A–E). Microsporangia in both, the peri- and in the hyposporangiate microsporangiophores as well have the same dimensions. Within the terminal perisporangiate microsporangiophores 2–3 collateral vascular bundle strands are developed, which do not fuse when entering the stem bundle of the cone axis (fig. 9F). In 37% of the 300 investigated pollen cones, instead of the typical terminal perisporangiate microsporangiophore 2 (figs 10 A & B), 3 (figs 10 C & D) or rarely 4 (fig. 10E) hyposporangiate microsporangiophores are developed in the distal part of the cone axis, each supplied with a single collateral vascular bundle strand (fig. 10F). Taking the size and also the number of microsporangia of all anomalous distal hyposporangiate microsporangiophores together, they fit quite well to the dimensions and also to the number of microsporangia developed within a perisporangiate microsporangiophore (figs 9 A–E).

### **3.3 Vasculature in lateral microsporangiophores**

The results of the morpho-anatomical studies on the vasculature within the lateral microsporangiophores show significant differences between the hypo- and the perisporangiate type. The lateral hyposporangiate microsporangiophores of *Torreya californica* (fig. 11A) and *Cephalotaxus harringtonii* (fig. 11B) are always supplied by a single collateral vascular bundle strand. Within the vascular bundle, xylem is pointing towards the adaxial scutellum, the phloem towards the abaxial microsporangia. The lateral perisporangiate microsporangiophores of *Pseudotaxus chienii* (fig. 11C) and *Taxus baccata* (figs 11D–E) are mostly supplied with 2 collateral vascular bundle strands, as is also the case for anomalous hyposporangiate microsporangiophore found in *Taxus baccata* (fig. 11F). Especially in *Taxus baccata* it can be clearly observed that the xylem parts of both bundle strands are orientated towards the centre of the stalk so that they are facing each other. The phloem parts are pointing towards the microsporangia.

### 3.4. Proliferated pollen cones

On a c. 30 year old specimen of *Cephalotaxus harringtonii* growing on the Isle of Mainau (Konstanz, Germany), several pollen cones were found showing proliferation in different intensities (fig. 12). In all proliferated pollen cones a terminal pollen cone is absent and the apical meristem is still active and develops a well expanding shoot axis (figs 12A, B, D). The basal part of this shoot axis is surrounded by persisting bud scales. The outer bud scales are all sterile (figs 12C, F). Several of the inner bud scales are fertile and carry a pollen cone in their axil (figs 12C, E, F). If the internodes developed between the inner bud scales are small, the pollen cones remain densely packed closely to each other in the basal part (figs 12A, B, C), as is also the case for typical shaped pollen cones. In some of the proliferated pollen cones the internodes between the inner bud scales are strongly expanded and the inner bud scales are arranged spirally and distantly from each other on the shoot axis (figs 12D, E, F). In distal parts of the shoot, typical shaped needle leaves are developed (fig. 12A, B, D). Such proliferated pollen cones were not found in the investigated individuals of *Torreya californica*, *Taxus baccata* and *Pseudotaxus chienii*.

## 4 Discussion

The systematic relationships within Taxaceae are still controversial. Morpho-anatomical studies of vegetative structures e.g. leaves have shown that *Taxus* + *Pseudotaxus* and *Cephalotaxus* + *Torreya* each form a closely related complex (e.g. GHIMIRE & HEO 2014). The close relationship of *Pseudotaxus* + *Taxus* is also proved by molecular data. Here it could be shown that the *Pseudotaxus*-*Taxus*-*Austrotaxus*-complex (Taxeae) represents the sister clade of the *Torreya*-*Amentotaxus*-complex (Torreyae), however, *Cephalotaxus* is placed basal to all other Taxad genera (e.g. CHENG *et al.* 2000). Apart from this, the evolutionary pathway of taxaceous pollen cones and their microsporangiophores are still controversial. Features such as branched vs. unbranched cones and perisporangiate vs. hyposporangiate microsporangiophores are of great interest within this discussion. The results gained in this study seem to be quite helpful to deliver new insights in the evolution of taxaceous pollen cones and their microsporangiophores.

The terminal position of the perisporangiate microsporangiophore in *Cephalotaxus* and *Torreya* clearly indicates that the microsporangia bearing structure cannot represent just a simple peltate microsporophyll, because a leaf is always developed lateral at the apex and can therefore never be inserted terminal. However, it should be considered that leaves can be shifted by subsequent growing processes out of their original positions into a secondary ones. Within the investigated anomalous pollen cones of *Torreya* and *Cephalotaxus*, several circle-like arranged hyposporangiate microsporangiophores (figs 7, 8, 10) were formed in the distal part of the cone axis instead of the usual single perisporangiate microsporangiophore (figs 5, 6, 9). If these anomalous distal hyposporangiate microsporangiophores were to fuse, a radial structure would be formed. Its position, the size dimensions and the number of microsporangia would correspond to a typical terminal perisporangiate microsporangiophore. Depending on the number of fused hyposporangiate microsporangiophores the number of microsporangia within the perisporangiate microsporangiophores varies greatly. During the formation of perisporangiate microsporangiophores the scutellum gets nearly completely reduced. However, within *Torreya* and *Pseudotaxus*, some perisporangiate microsporangiophores were found still showing a conspicuous green central column-like structure (fig. 4). It seems that this represents rudiments of the scutella of the fused hyposporangiate microsporangiophores as it might also be the case that the green central region developed within typical shaped microsporangiophores (fig. 2F).

Taking these anomalies together, they support the idea that the perisporangiate type of microsporangiophores represents a radial synangium consisting of several fused hyposporangiate microsporangiophores as was suggested earlier by e.g. THOMSON (1940), NOZERAN (1955), MUNDRY & MUNDRY (2001), DÖRKEN *et al.* (2011) and SCHULZ *et al.* (2014). When regarding the perisporangiate microsporangiophores within Taxaceae as a radial synangium consisting of several fused hyposporangiate microsporangiophores, it seems that they are developed exclusively terminally at the cone axis due to a lack of space in this region. This would mean, if the apex of the cone axis is small in diameter, the distal hyposporangiate microsporangiophores would fuse to a radial structure, and if the diameter of the apex is unusually broad the microsporangiophores in the distal part would not fuse, as can be observed in several pollen cones of *Torreya* and *Cephalotaxus* (figs 7, 8, 10). This could explain why within these taxa the perisporangiate type of microsporangiophores is exclusively formed

terminally at the cone axis. This idea is supported by the fact that the number of vascular bundle strands developed within the central stalk of the terminal perisporangiate microsporangiophore varies between 2 and 4. Each vascular bundle strand belongs to one of the microsporangiophores that are involved in forming the perisporangiate structure.

It could be shown that the lateral perisporangiate microsporangiophores in *Taxus baccata* (figs 11D–F) and *Pseudotaxus chienii* (fig. 11C) are mostly supplied with two vascular bundle strands. Within the central stalk of the microsporangiophores the xylem parts are facing each other, while the phloem parts are pointing towards the microsporangia. This fact is a strong argument supporting the idea that the lateral perisporangiate microsporangiophores in both taxa are also representing radial synangia consisting in this case of 2 fused microsporangia. It seems that the former hyposporangiate microsporangiophores were fused with their adaxial sides, where the scutella were developed. This would explain why the xylem parts of both strands are facing each other. The lateral hyposporangiate microsporangiophores of *Torreya californica* (fig 11A) and *Cephalotaxus harringtonii* (fig 11B) are supplied by a single collateral bundle strand with xylem pointing towards the scutellum and phloem pointing towards the microsporangia. If two hyposporangiate microsporangiophores were to fuse along their adaxial sides an arrangement of xylem and phloem would occur as is developed within the stalk of *Pseudotaxus chienii* and *Taxus baccata*.

Different evolutionary scenarios exist about the pollen cones in Taxaceae. In one of the latest scenarios (e.g. MUNDY & MUNDY 2001; DÖRKEN *et al.* 2011; SCHULZ *et al.* 2014) the unbranched pollen cones of e.g. *Taxus* and *Torreya* are regarded as derived from an ancestral branched pollen cone similar to these developed in extant *Cephalotaxus*. In this transitional line pollen cones of *Pseudotaxus* are derived from a *Cephalotaxus*-like ancestor by the reduction of all lateral hyposporangiate microsporangiophores and also the lateral cone axes. If the bracts within the *Pseudotaxus* pollen cones become reduced the *Taxus* pollen cone would be formed. By the reduction of adaxial microsporangia within the lateral perisporangiate microsporangiophores of *Taxus*, the *Torreya* pollen cone is formed (fig. 13A). In this scenario the lateral hyposporangiate microsporangiophores in *Torreya* are homologous to lateral strongly reduced perisporangiate microsporangiophore of *Pseudotaxus* and *Taxus*. Thus, pollen cones of *Torreya* might reflect the most derived ones within Taxaceae. In this case the pollen cones of *Pseudotaxus* (figs 1B & F) with partly reduced bracts would represent an intermediary stage. The pollen cones of *Taxus* differ from *Pseudotaxus* only in lacking bracts. Within this scenario the perisporangiate microsporangiophores of *Pseudotaxus*, *Taxus* and the terminal synangium of *Cephalotaxus* would be homologous and the perisporangiate microsporangiophores of *Pseudotaxus* and *Taxus* would comply with an entire, however, strongly reduced lateral unit within the branched *Cephalotaxus* pollen cone. It seems that in *Pseudotaxus* and *Taxus* the cone axis is so strongly reduced that only some cells remain, which can no longer be recognised as shoot tissue. Such a strong reduction of shoot axis is nothing unusual in conifers and can also be found in vegetative parts e.g. in cladodes of *Sciadopitys verticillata*, *Sciadopityaceae* (DÖRKEN & STÜTZEL 2011a, b). Thus, the evolutionary scenario for taxaceous pollen cones as described above can demonstrate quite well how sporophyll-like perisporangiate microsporangiophores can be developed by the reduction of lateral cones. This idea is supported by two further facts:

1. The number of perisporangiate microsporangiophores in *Pseudotaxus* and *Taxus* corresponds quite well with the number of lateral units within the branched *Cephalotaxus* pollen cones.
2. The pollen cones of *Pseudotaxus* and *Taxus* are exclusively perisporangiate. As it could be shown the perisporangiate microsporangiophores developed in *Cephalotaxus* and *Torreya* represent radial synangia resulting from a fusion of hyposporangiate microsporangiophores, which can only take place when the apex of the cone axis is so small that in earliest ontogenetic stages the primordia of the microsporangiophores get in contact and fuse to each other.

Regarding the perisporangiate microsporangiophores in *Pseudotaxus* and *Taxus* as complying with an entire lateral cone than it is not astonishing, that here exclusively perisporangiate microsporangiophores are developed, due to lateral cone axis that are reduced to some cells that can no longer be recognized as such ones. Thus, microsporangiophores developed at such a strongly reduced lateral cone axis have to fuse consequently to a radial synangium due to the reduced apex.

That the lateral hyposporangiate microsporangiophores in *Torreya* might be derived from a perisporangiate ancestor is supported by the anomalous hyposporangiate microsporangiophores found in

*Pseudotaxus* (figs 3, 4) which are quite similar to these of *Torreya*. For the anomalous hyposporangiate *Pseudotaxus* microsporangiophores it could be shown that the adaxial scutellum is formed by the reduction of one or more adaxial microsporangia which became sterile and scale-like.

However, two of the largest barriers in regarding the hyposporangiate pollen cones of *Torreya* as being derived from a *Taxus*-like ancestor is the different number of microsporangiophores between both taxa, and more importantly the number of vascular bundles supplying the microsporangiophores. The number of lateral hyposporangiate microsporangiophores within *Torreya* significantly exceeds the number of perisporangiate microsporangiophores in *Pseudotaxus* and *Taxus* and also the number of the lateral units (cones) within the branched *Cephalotaxus* pollen cone. Furthermore the lateral hyposporangiate microsporangiophores in *Torreya* are always supplied by only a single vascular bundle strand as is also the case for the lateral hyposporangiate microsporangiophores in *Cephalotaxus*. Within both taxa the orientation of xylem and phloem within the bundle strand is exactly the same. Thus, it would be very unlikely that the hyposporangiate *Torreya* microsporangiophore is derived from a radial synangium as developed within *Pseudotaxus* and *Taxus*. This incongruity is strongly conflicting with the evolutionary scenario described above and leads to another hypothesis about the evolution of taxaceous pollen cones. Pollen cones of *Torreya* could be regarded as being derived directly from a branched *Cephalotaxus*-like ancestor. The pollen cones of *Torreya* show great similarities with a lateral unit within the branched *Cephalotaxus* pollen cone, especially in respect to the number, the position and the vasculature within the microsporangiophores. Thus, a single pollen cone of *Torreya* fits significantly better to a complete lateral unit within the branched *Cephalotaxus* pollen cone than to a single *Pseudotaxus* and *Taxus* pollen cone. Within such an evolutionary scenario the pollen cones of *Pseudotaxus* and *Taxus* do not represent intermediate forms between *Cephalotaxus* and *Torreya* (fig. 13B). Thus it might be possible that within taxaceous pollen cones two lineages have evolved independently from each other:

- (1) *Cephalotaxus* → *Torreya*;
- (2) *Cephalotaxus* → *Pseudotaxus* → *Taxus*.

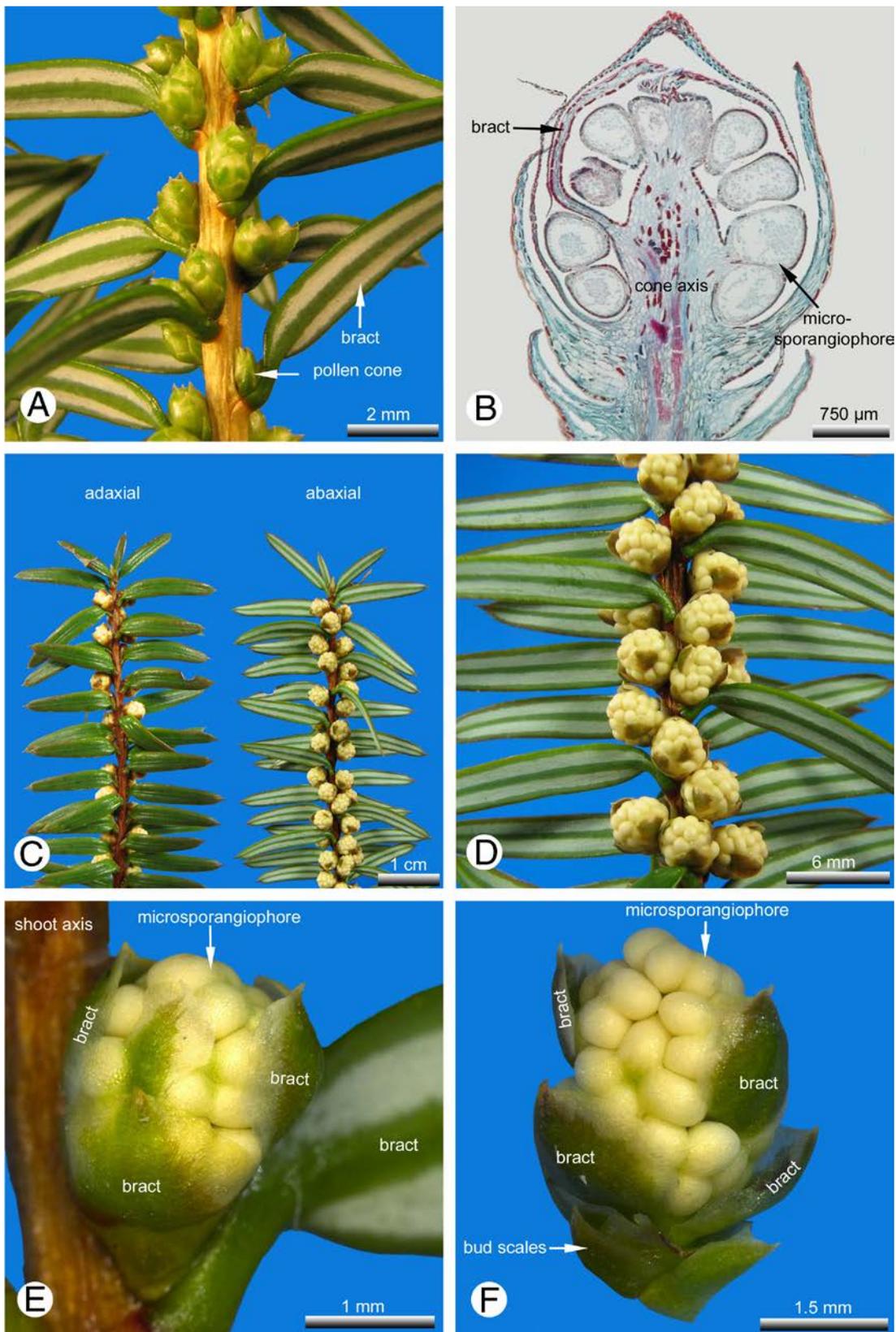
In this case pollen cones of *Torreya* and *Taxus* would reflect the most derived cones within each lineage. However, the situation could be also completely different from that. Pollen cones of *Torreya* might also reflect the most primitive condition. If the inner bud scales that are developed at the stalk of the *Torreya* pollen cone would become fertile and carry an axillary pollen cone, a structure similar to the *Cephalotaxus* pollen cone is formed. In this case the *Cephalotaxus* pollen cone could represent a cluster of *Torreya*-like pollen cones (fig. 13C). This idea is supported by the anomalous proliferated pollen cones found in *Cephalotaxus harringtonii* (fig. 12). Here it could be clearly shown that the pollen cones are inserted in the axil of the inner bud scales. In typical *Cephalotaxus* pollen cones the cone axis is terminated by a pollen cone and the apex is completely consumed and a further development of the cone is excluded. If the terminal pollen cone is absent the apex can continue growing and forming a typical shaped shoot axis with bud scales in the basal and green needle leaves in the distal part. If the internodes between the inner bud scales become expanded the pollen cones are also arranged distantly from each other at the shoot axis (figs 12D, F). Thus the branching pattern in typical shaped *Cephalotaxus* pollen cones corresponds exactly to the phyllotaxis of the inner bud scales. Thus it would be not so unlikely that the branched pollen cone of *Cephalotaxus* represents a cluster of several *Torreya* like pollen cones. The number of lateral units within a *Cephalotaxus* pollen cone is corresponding to the number of fertile inner bud scales. In this scenario the pollen cones of *Pseudotaxus* and *Taxus* can be derived from a *Cephalotaxus*-like ancestor in the way as described in the first scenario above (fig. 13A). In this case pollen cones of *Taxus* reflect the most derived condition. Unfortunately pollen cones of *Austrotaxus* and *Amentotaxus* were not available for this study. However, there is an urgent need to investigate the pollen cones of these taxa to see how they fit in the evolutionary scenarios as described above.

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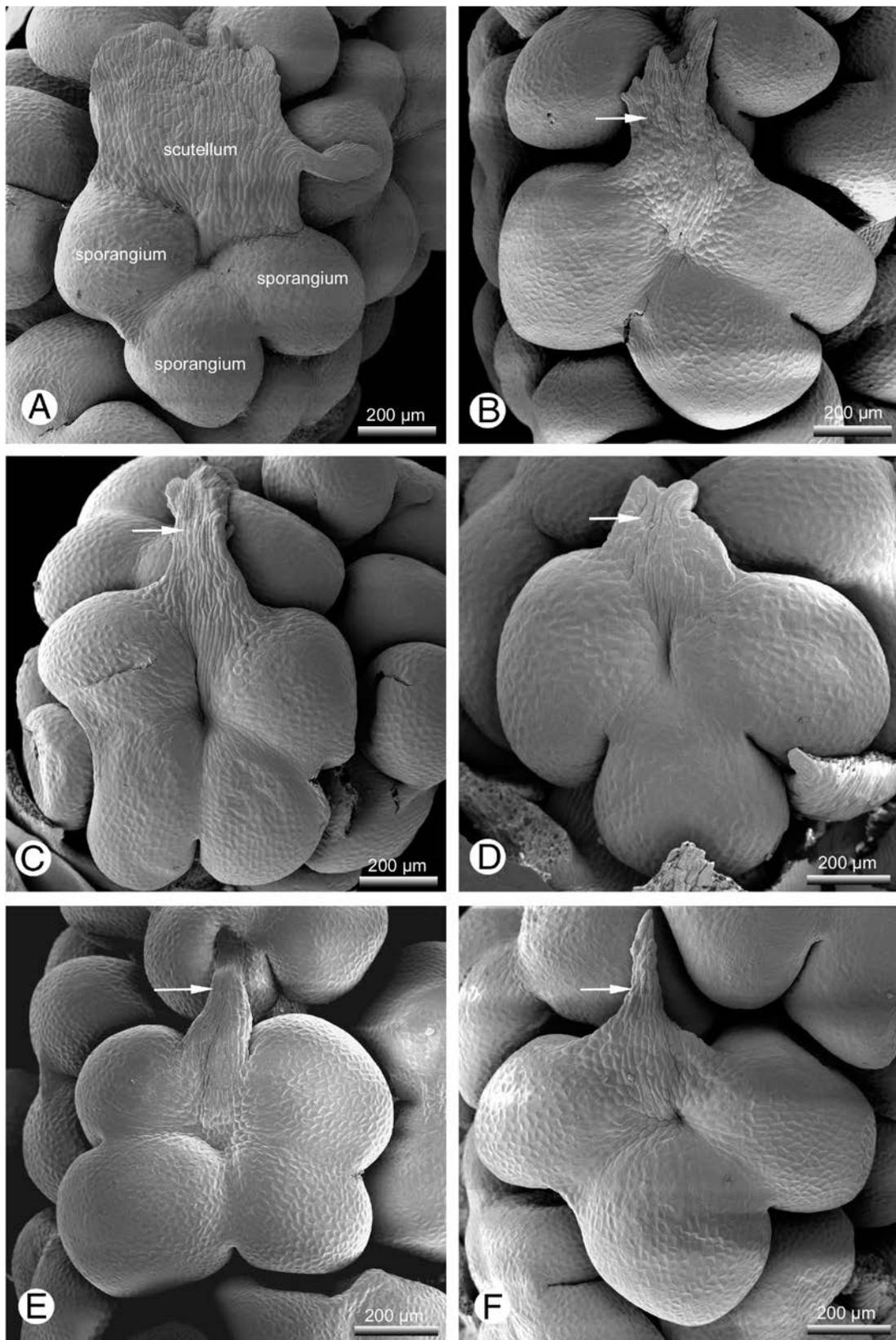
## References

- CHENG, Y., NICOLSON, R. G., TRIPP, K. & CHAW, S. M. (2000). Phylogeny of Taxaceae and Cephalotaxaceae genera inferred from Chloroplast *matK* gene and nuclear rDNA ITS region. *Mol. Phyl. & Evol.* 14 (3): 353-365.
- DLUHOSCH, H. (1937). Entwicklungsgeschichtliche Untersuchungen über die Mikrosporophylle der Koniferen. *Bibliot. Bot.* 114 (3): 1-24.
- DÖRKEN, V. M. (2011). Proliferating seed cones in *Metasequoia glyptostroboides* HU & CHENG (Cupressaceae s.l., Coniferales) elucidate the evolution of seed cones and ovules in Cupressaceae s.l. *Feddes Repert.* 122: 1-12.
- DÖRKEN, V. M. & STÜTZEL TH. (2011a). Morphology and anatomy of anomalous cladodes in *Sciadopitys verticillata* Siebold & Zucc. (Sciadopityaceae). *Trees* 25: 199-213.
- DÖRKEN, V. M. & STÜTZEL, TH. (2011b). Pflanzliche Missbildungen und deren Interpretation am Beispiel von *Sciadopitys verticillata* Siebold & Zucc. (Sciadopityaceae) mit intermediär gestalteten Kladodien. *Mitt. Dtsch. Dendrol. Ges.* 96: 125-149.
- DÖRKEN, V. M., ZHANG, Z. X., MUNDY, I. B. & STÜTZEL, TH. (2011). Morphology and anatomy of male reproductive structures in *Pseudotaxus chienii* (W.C. CHENG) W.C. CHENG (Taxaceae). *Flora* 206 (5): 444-450.
- DUPLER, A. W. (1919). Staminate strobilus of *Taxus canadensis*. *Bot. Gaz.* 68: 345-366.
- ECKENWALDER, J. E. (2009). *Conifers of the World*. Timber Press, Portland.
- FARJON, A. (1984). *Pines, drawings and descriptions of the genus Pinus*. Brill, Leiden.
- FARJON, A. (2005). *A monograph of Cupressaceae and Sciadopitys*. Royal Botanic Gardens, Kew.
- FARJON, A. (2010). *A handbook of the world's conifers*. Vol. I & II. Brill, Leiden.
- FARJON, A. & ORTIZ GARCIA, S. (2003). Cone and ovule development in *Cunninghamia* and *Taiwania* (Cupressaceae sensu lato) and its significance for conifer evolution. *Am. J. Bot.* 90 (1): 8-16.
- FLORIN, R. (1951). Evolution in cordaites and conifers. *Acta Horti Berg.* 17: 7-37.
- FLORIN, R. (1954). The female reproductive organs of conifers and taxads. *Biol. Rev. Cambridge Philos. Soc.* 29: 367-389.
- GHIMIRE, B. & HEO, K. (2014). Cladistic analysis of Taxaceae s.l. *Plant. Syst. Evol.* 300: 217-223.
- GERLACH, D. (1984). *Botanische Mikrotomtechnik, eine Einführung*. 2<sup>nd</sup> ed., Thieme, Stuttgart.
- GERSTBERGER, P. & LEINS, P. (1978). Rasterelektronenmikroskopische Untersuchungen an Blütenknospen von *Physalis philadelphia* (Solanaceae). *Ber. Deutsch. Bot. Ges.* 91: 381-387.
- HERZFELD, S. (1914). Die weibliche Koniferenblüte. *Österr. Botan. Z.* 64 (8): 321-358.
- KRÜSSMANN, G. (1983). *Handbuch der Nadelgehölze*. 2<sup>nd</sup> ed. Parey, Berlin, Hamburg.
- LEMOINE-SEBASTIAN, C. (1967). Appareil reproducteur male de *Juniperus*. *Travaux du laboratoire forestier de Toulouse*. 1 (6): 1-35.
- MUNDY, I. (2000). Morphologische und morphogenetische Untersuchungen zur Evolution der Gymnospermen. *Bibliot. Bot.* 152.
- MUNDY, I., & MUNDY, M. (2001). Male cones in Taxaceae s.l. - an example of WETTSTEIN's pseudanthium concept. *Plant.Biol.* 3: 405-416.
- NOZERAN, R. (1955). Remarques sur l'appareil reproducteur de certaines préphanérogames et gymnospermes. *Ann. des Sc. Nat. Bot.* 11: 1-224.
- PAGE, C. N. (1990). Gymnosperms. In KUBITZKY, K. (ed.): *The Families and Genera of Vascular Plants*. Vol. 1. Berlin, Heidelberg. u.a.: Springer: 279-391.
- PILGER, R. (1926). Coniferae. In: ENGLER, A.: *Die natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten insbesondere der Nutzpflanzen*. Leipzig: Engelmann.
- SCHUHMANN, K. (1902). Ueber die weiblichen Blüten der Koniferen. *Verhandl. Botan. Ver. Prov. Brandenburg* 44: 5-79.
- SCHULZ, C., KLAUS, K. V., KNOPF, P., MUNDY, M., DÖRKEN, V. M. & STÜTZEL, TH. (2014). Male Cone Evolution in Conifers: Not All That Simple. *Am. J. Plant Sciences* 2014 (5): 2842-2857.
- SCHWEITZER, H. J. (1963). Der weibliche Zapfen von *Pseudovoltzia liebeana* und seine Bedeutung für die Phylogenie der Koniferen. *Palaeontographica Abt. B.* 113 (1-4): 1-29.
- STÜTZEL, TH. & RÖWEKAMP, I. (1999). Bestäubungsbiologie bei Nacktsamern (Gymnospermen). In: ZIZKA, G. & SCHNECKENBURGER, S.: *Blütenbiologie – faszinierendes Miteinander von Pflanzen und Tieren. Kleine Senckenberg-Reihe* 33: 107-117.
- STÜTZEL, TH. & RÖWEKAMP, I. (1997). Bestäubungsbiologie bei Nacktsamern. *Palmengarten* 61 (2): 100-109.
- THOMSON, R. B. (1940). The structure of the cone in the Coniferae. *Bot. Rev.* 6 (2): 73-84.
- WILDE, M. H. (1975). A new interpretation of microsporangiate cones in Cephalotaxaceae and Taxaceae. *Phytomorphology* 25: 434-450.
- WORDSELL, W. C. (1901). The morphology of the "flower" of *Cephalotaxus*. *Ann. Bot.* 15(60): 637-652.



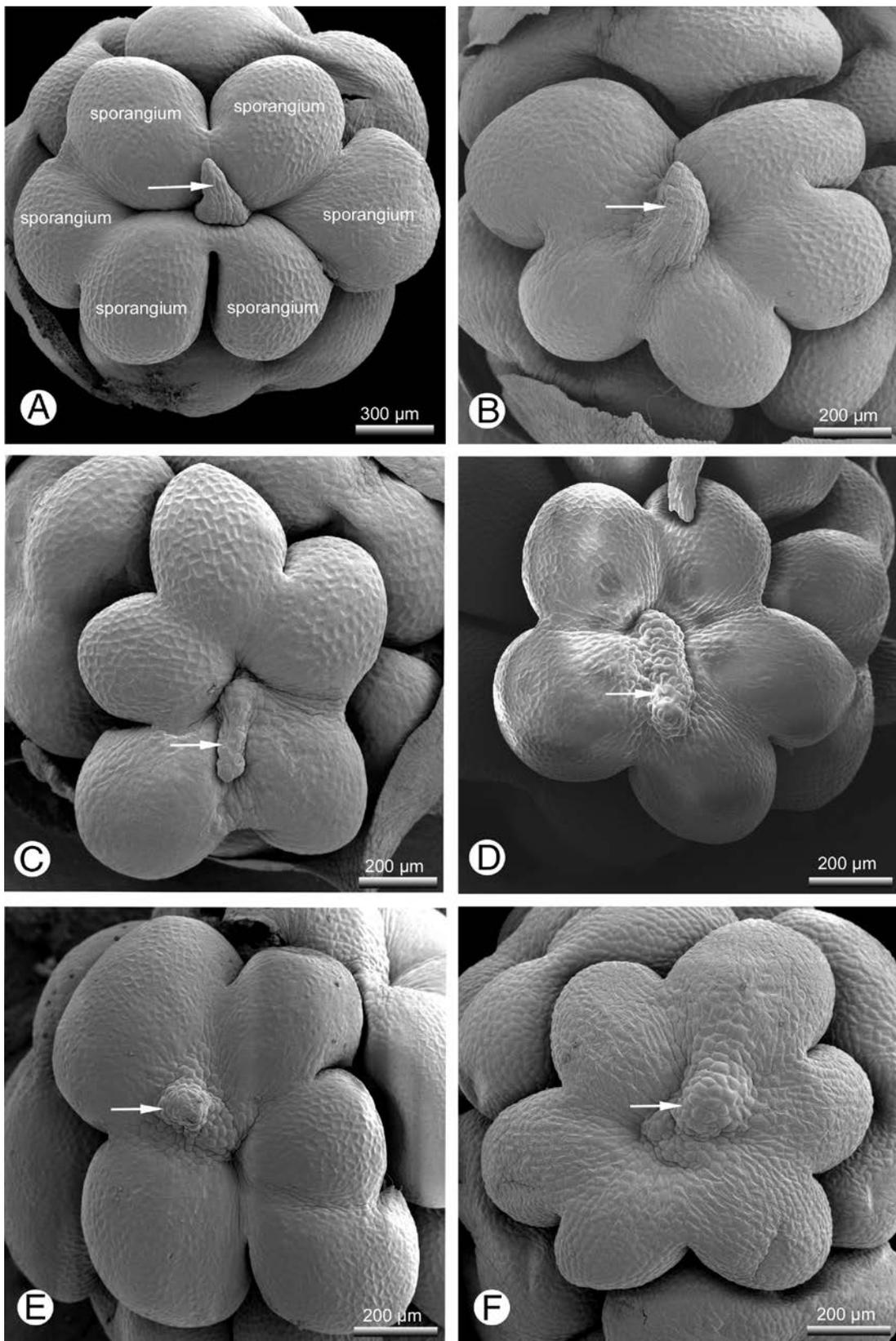
**Fig. 2:** *Pseudotaxus chienii*, typical shaped pollen cones.

**A:** Young cones at the shoot axis. **B:** Longitudinal section of a young cone; the exclusively perisporangiate microsporangiphores are covered by bud scales and bracts. **C:** Ripe pollen cones. **D:** Detail of C. **E:** Pollen cone shortly before anthesis; bracts within the cone still cover the microsporangiphores. **F:** Detail of a ripe pollen cone; the bracts are spreading distantly out of the cone.



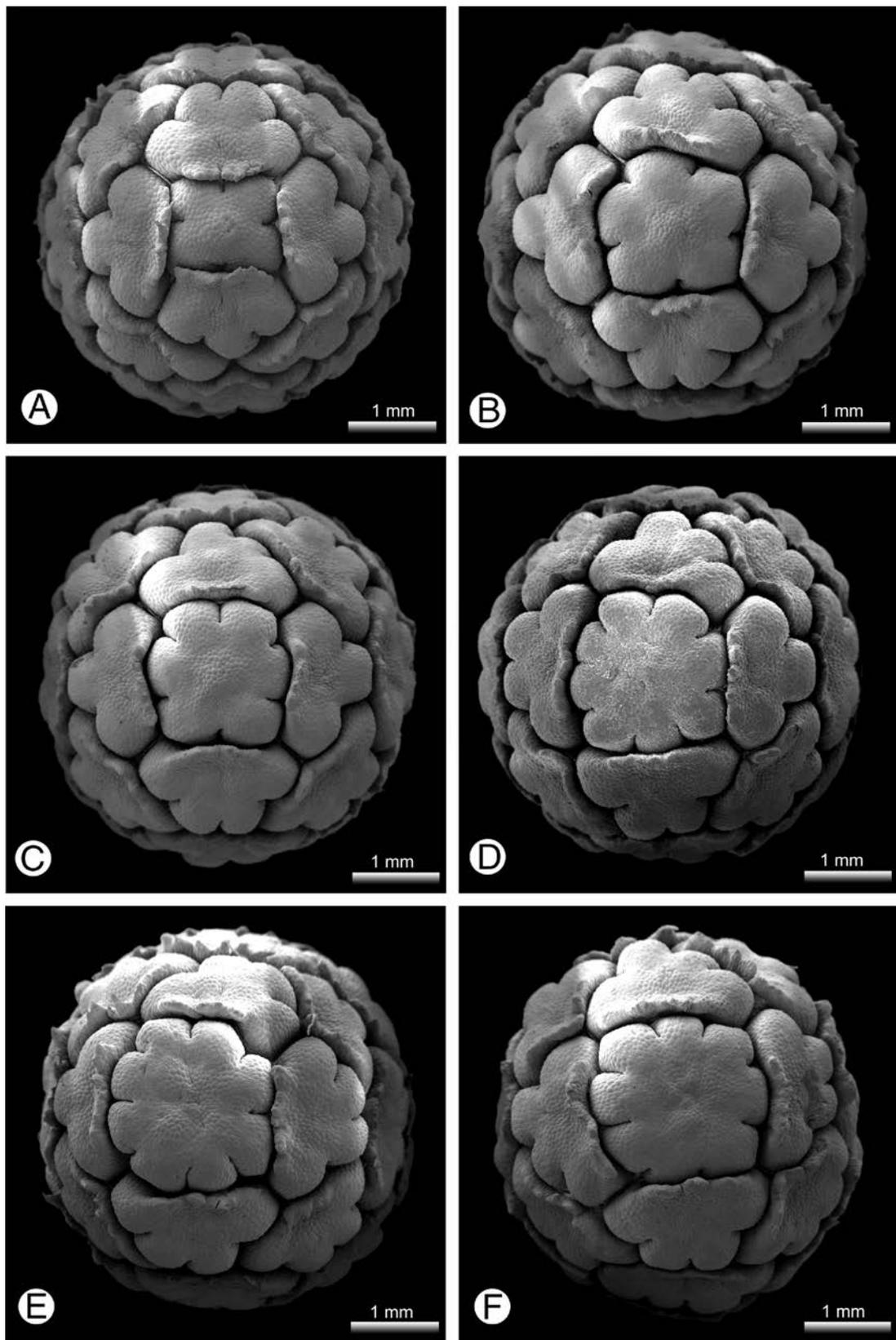
**Fig. 3:** *Pseudotaxus chienii*, anomalous shaped microsporangiophores.

Instead of typical perisporangiate microsporangiophores several hyposporangiate microsporangiophores showing a distinct adaxial scutellum are formed (arrow).



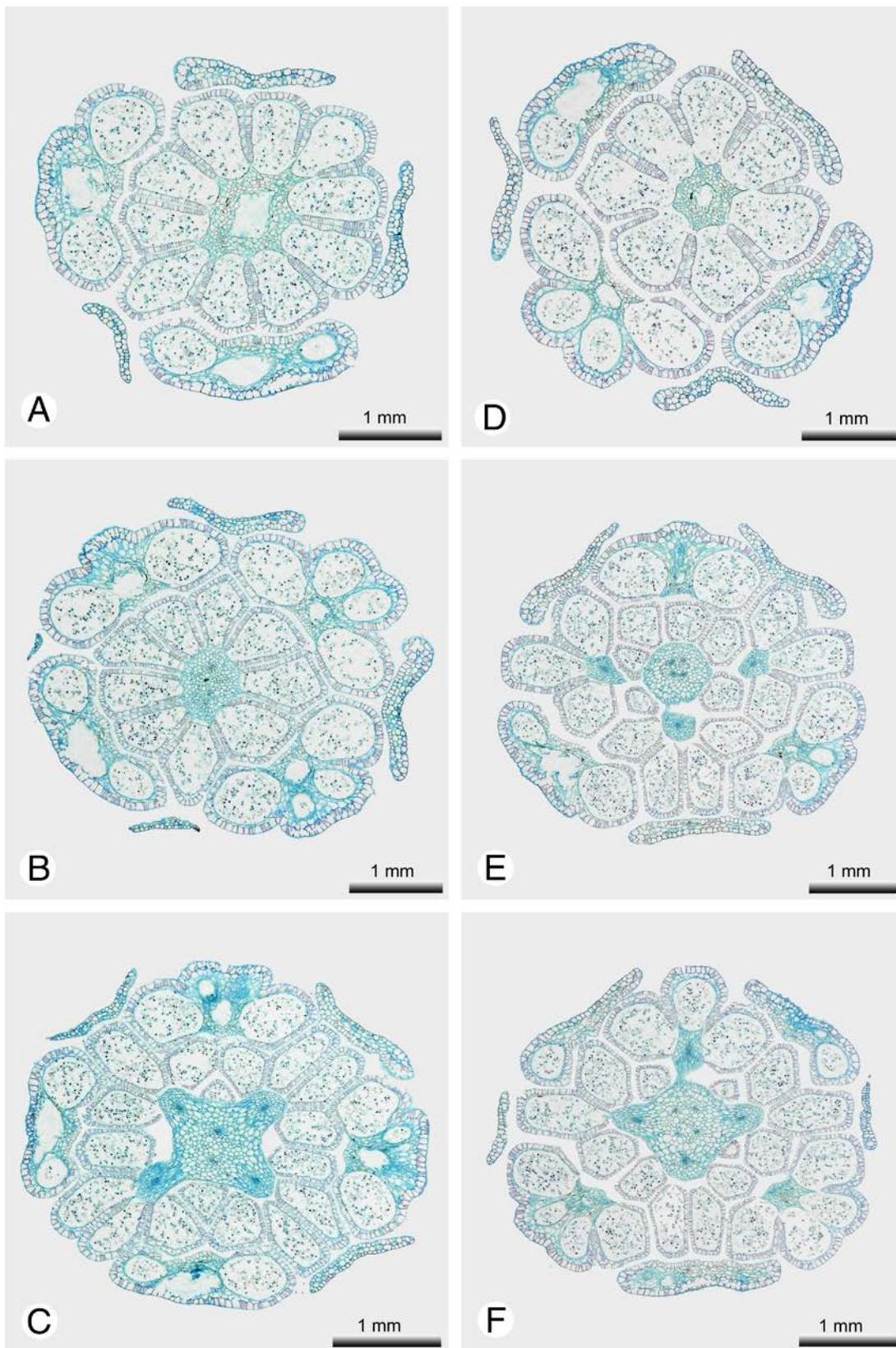
**Fig. 4:** *Pseudotaxus chienii*.

Anomalous shaped microsporangioophores with a central, green column-like structure (arrow).



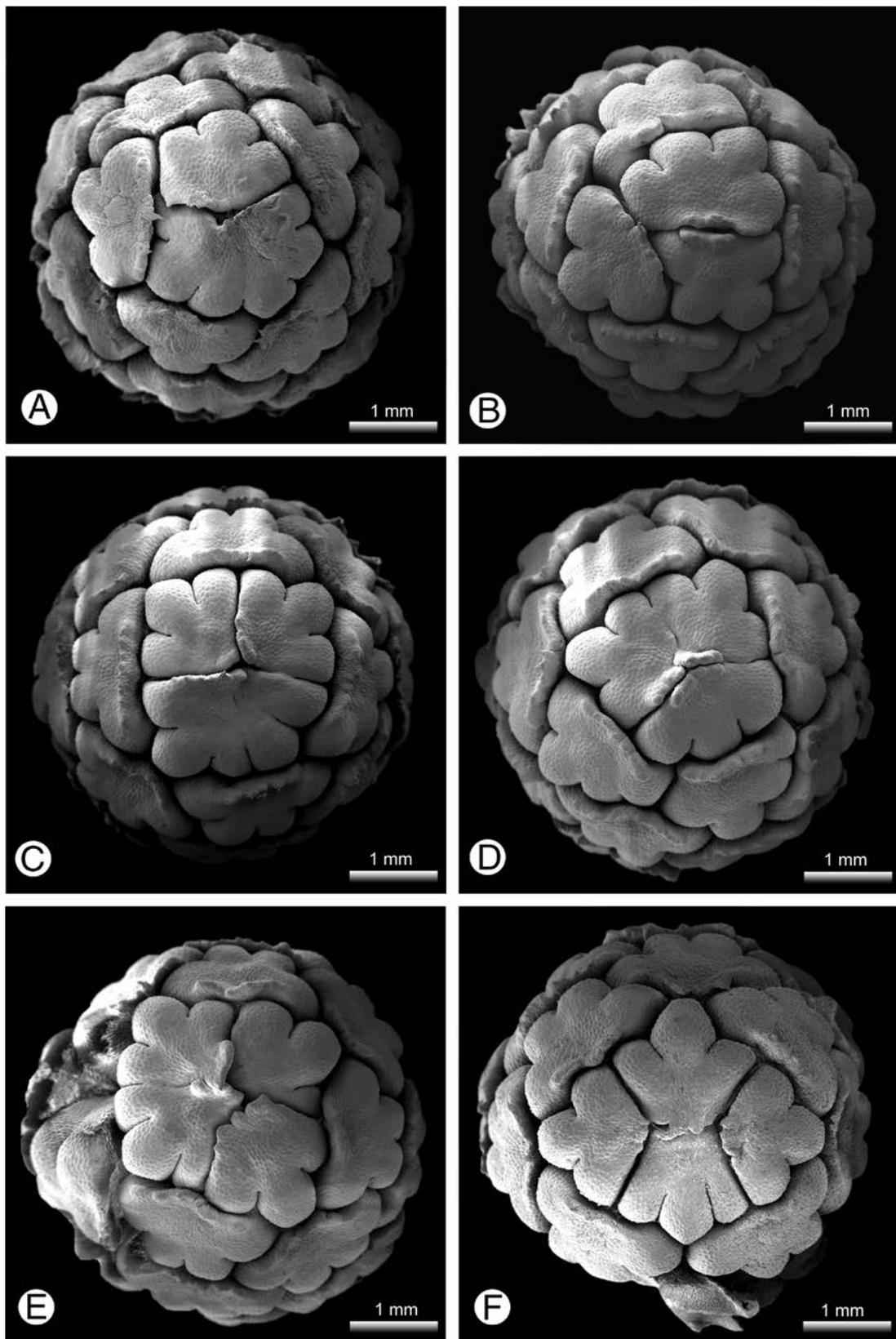
**Fig. 5:** *Torreyya californica*.

Top view of typical shaped pollen cones with a perisporangiate terminal microsporangiochore bearing four (A), five (B), six (C), eight (D & E) or nine (F) microsporangia.



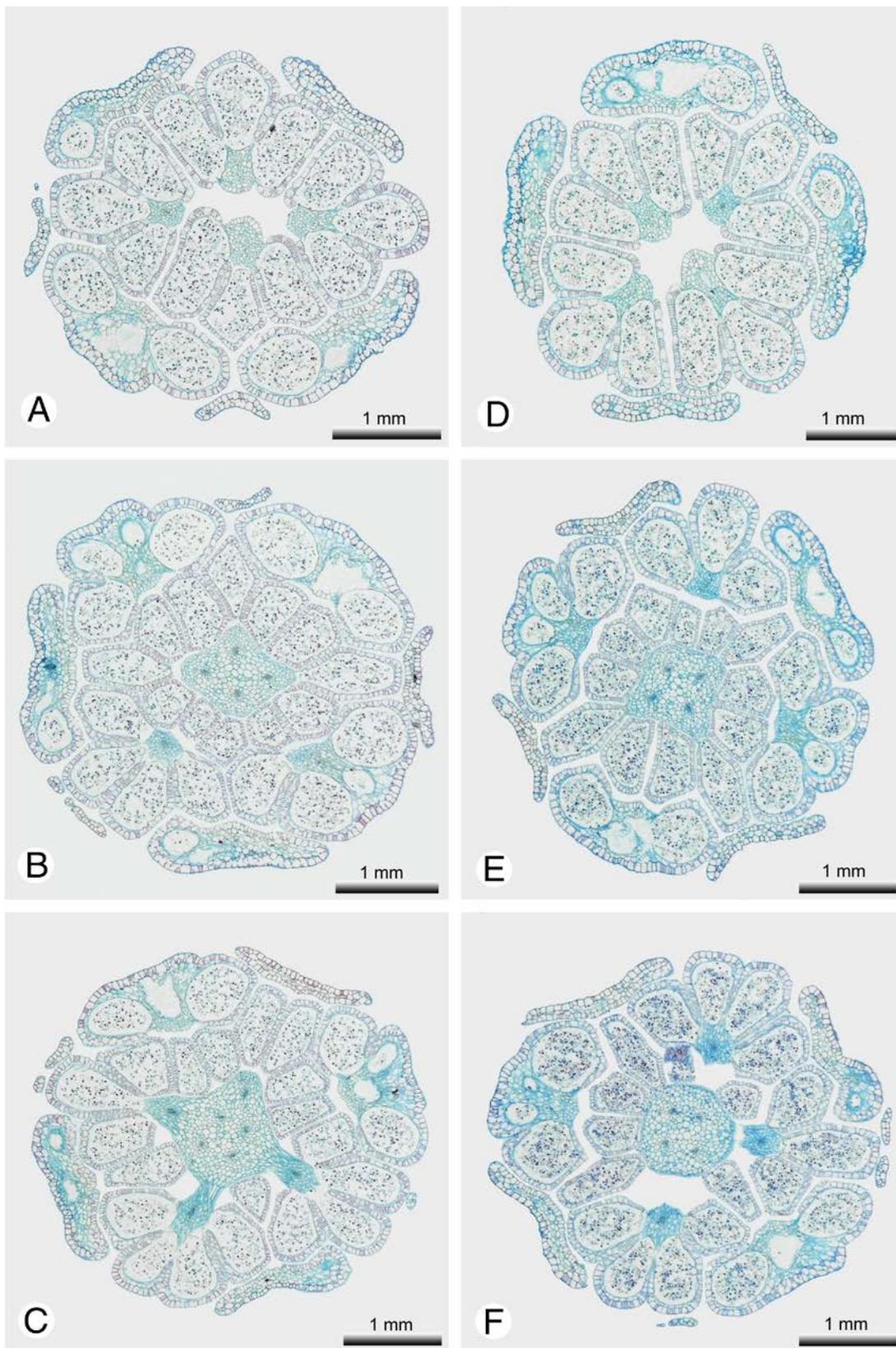
**Fig. 6:** *Torreyia californica*.

Cross section of the distal part of two (A-C & D-F) typical shaped pollen cones; the terminal perisporangiate microsporangiochore is supplied with two (A-C) or four (D-F) collateral vascular bundle strands, each enters the cone axis in separate strands; a huge resin duct is developed in the central distal part of the perisporangiate microsporangiochore as is also developed in the scutellum of the lower hyposporangiate ones.



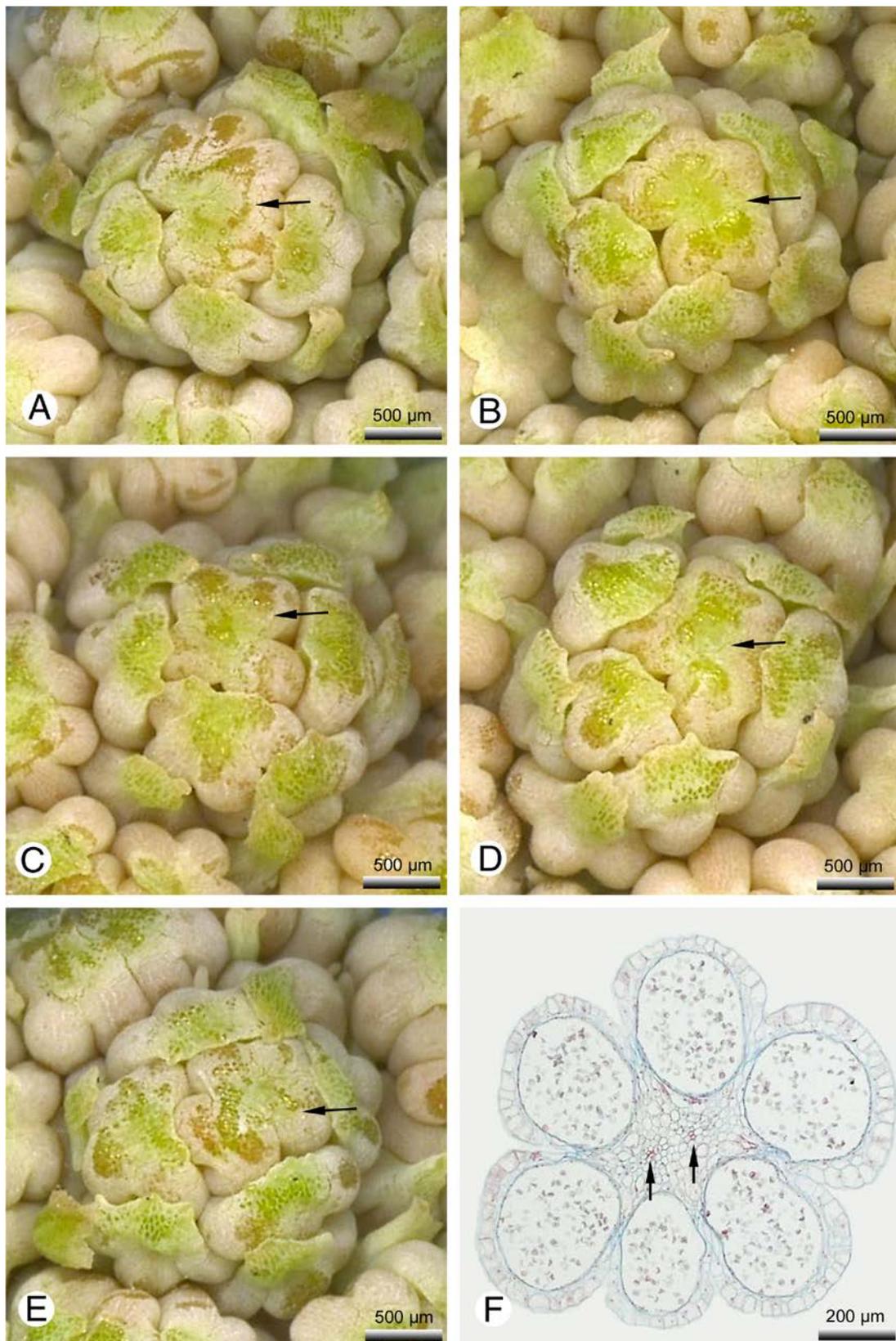
**Fig. 7:** *Torreyya californica*.

Top view of anomalous pollen cones showing two (A & B), three (C & D) or four (E & F) hyposporangiate microsporangiophores instead of the typical perisporangiate terminal microsporangiophore.



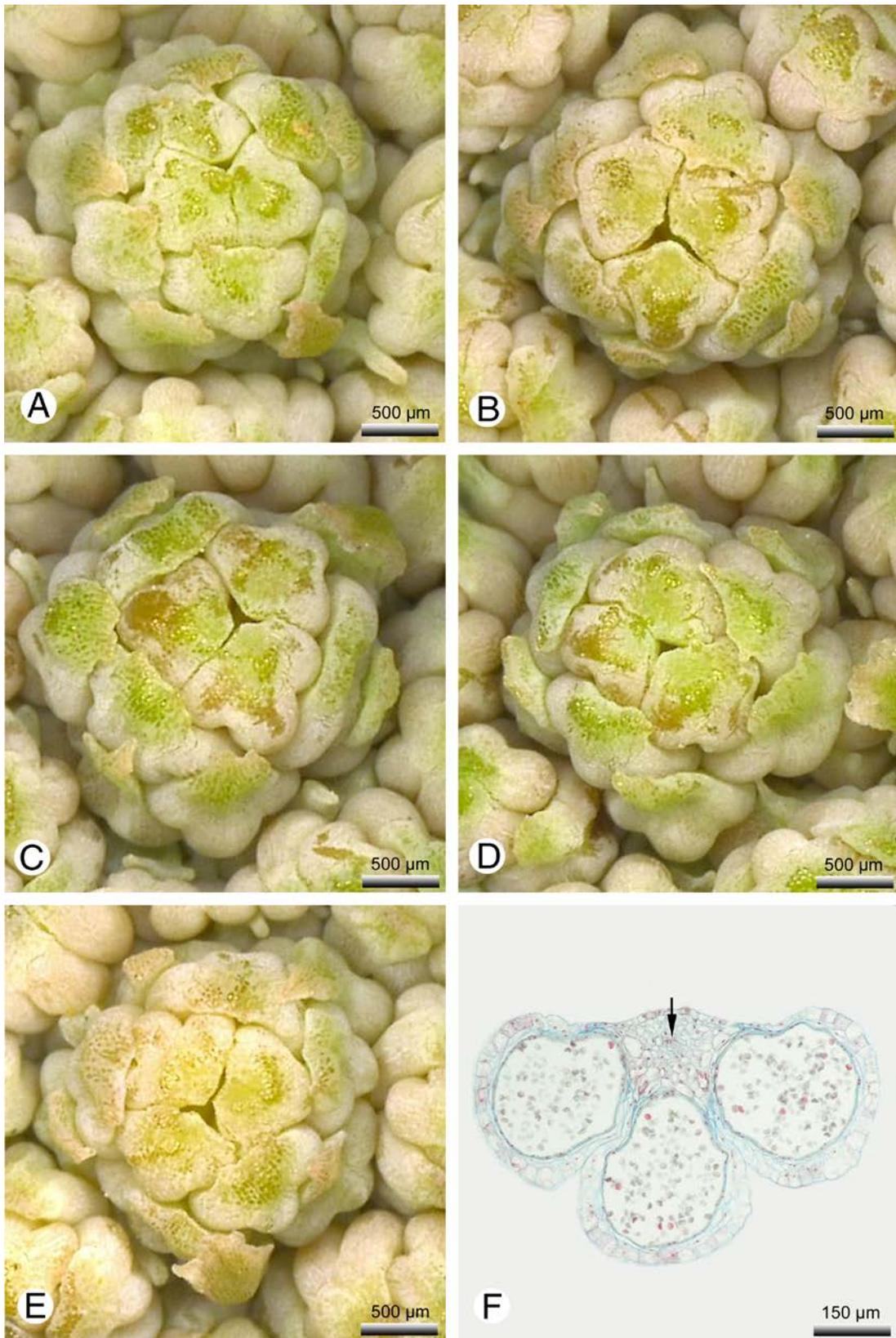
**Fig. 8:** *Torreyia californica*,

Cross section of the distal part of two (A-C & D-F) anomalous shaped pollen cones; instead of the usually developed perisporangiate microsporangiophore four hyposporangiate ones are formed; each microsporangiophore is supplied with one collateral vascular bundle strand.



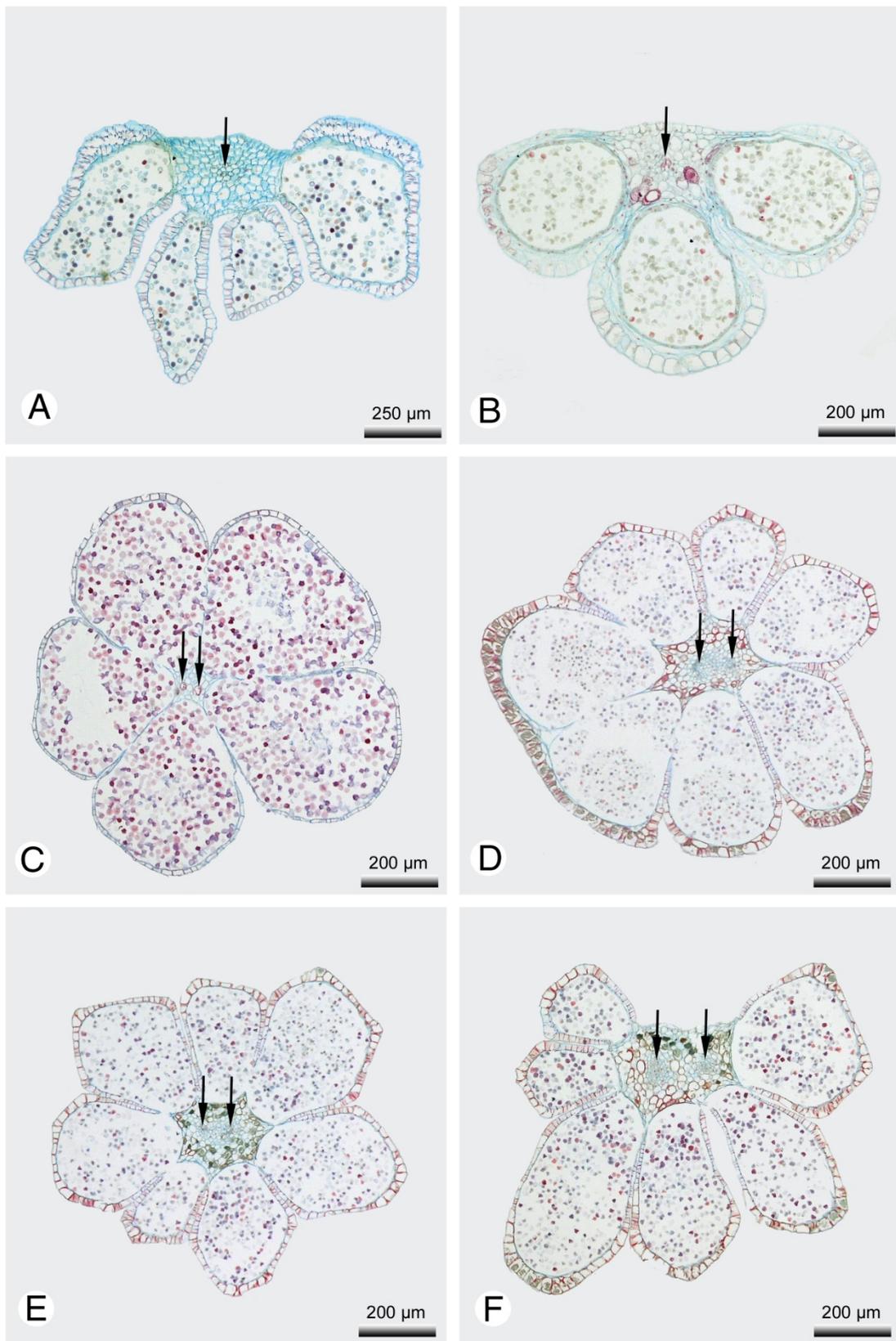
**Fig. 9:** *Cephalotaxus harringtonii*.

**A-E:** Top view of a typical shaped pollen cones terminating with a perisporangiate microsporangiophore that is varying strongly in size, shape and in the number of microsporangia. **F:** Cross section of a terminal perisporangiate microsporangiophore; this microsporangiophore is supplied with two collateral vascular bundles (arrow).



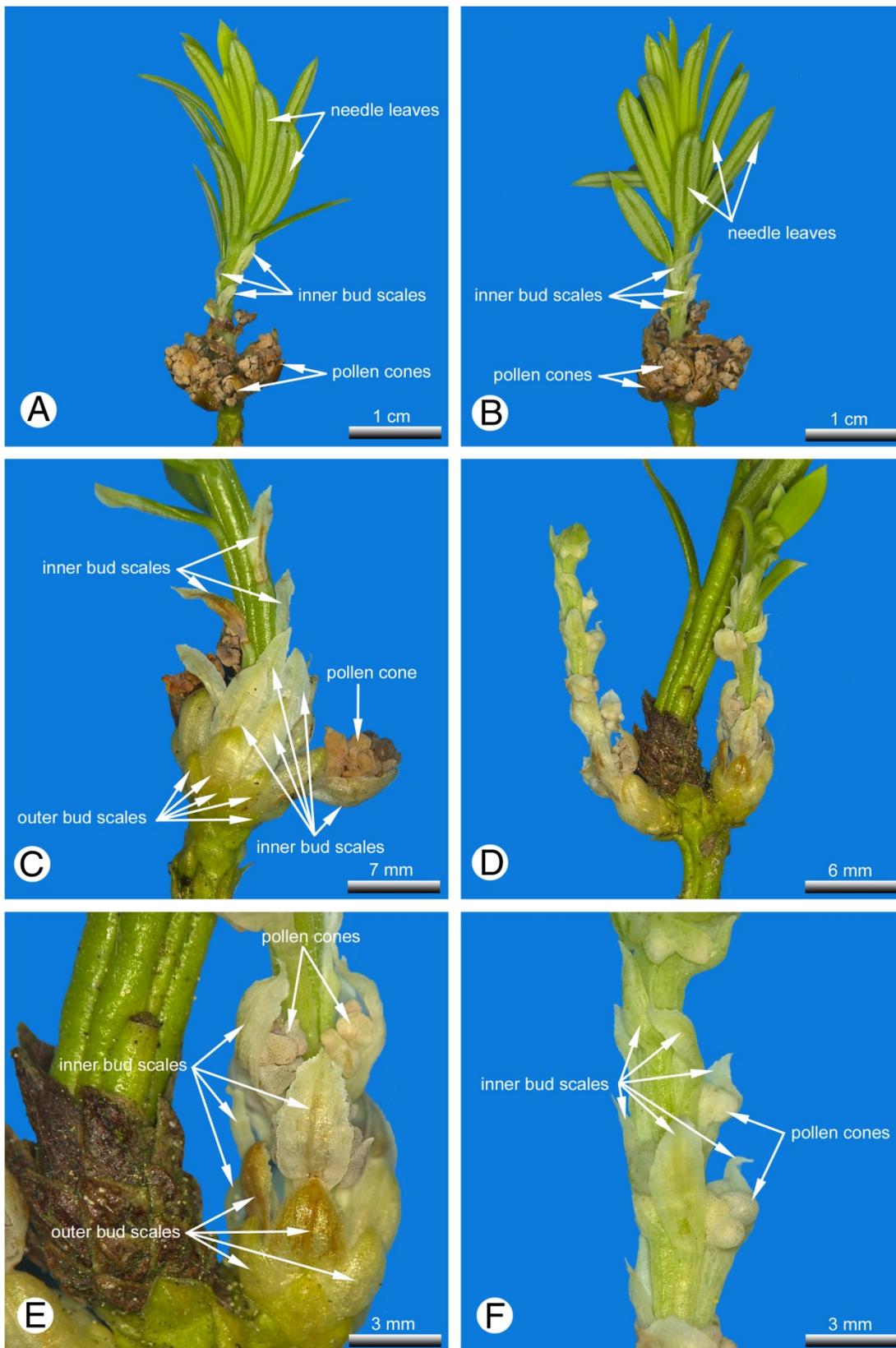
**Fig. 10:** *Cephalotaxus harringtonii*.

**A-E:** Top view of anomalous pollen cones showing two (A-B), three (C & D) or four (E) hyposporangiate microsporangiophores instead of the typical perisporangiate terminal microsporangiophore. **F:** Cross section of a terminal hyposporangiate microsporangiophore; the microsporangiophore is supplied with a single collateral vascular bundle (arrow).



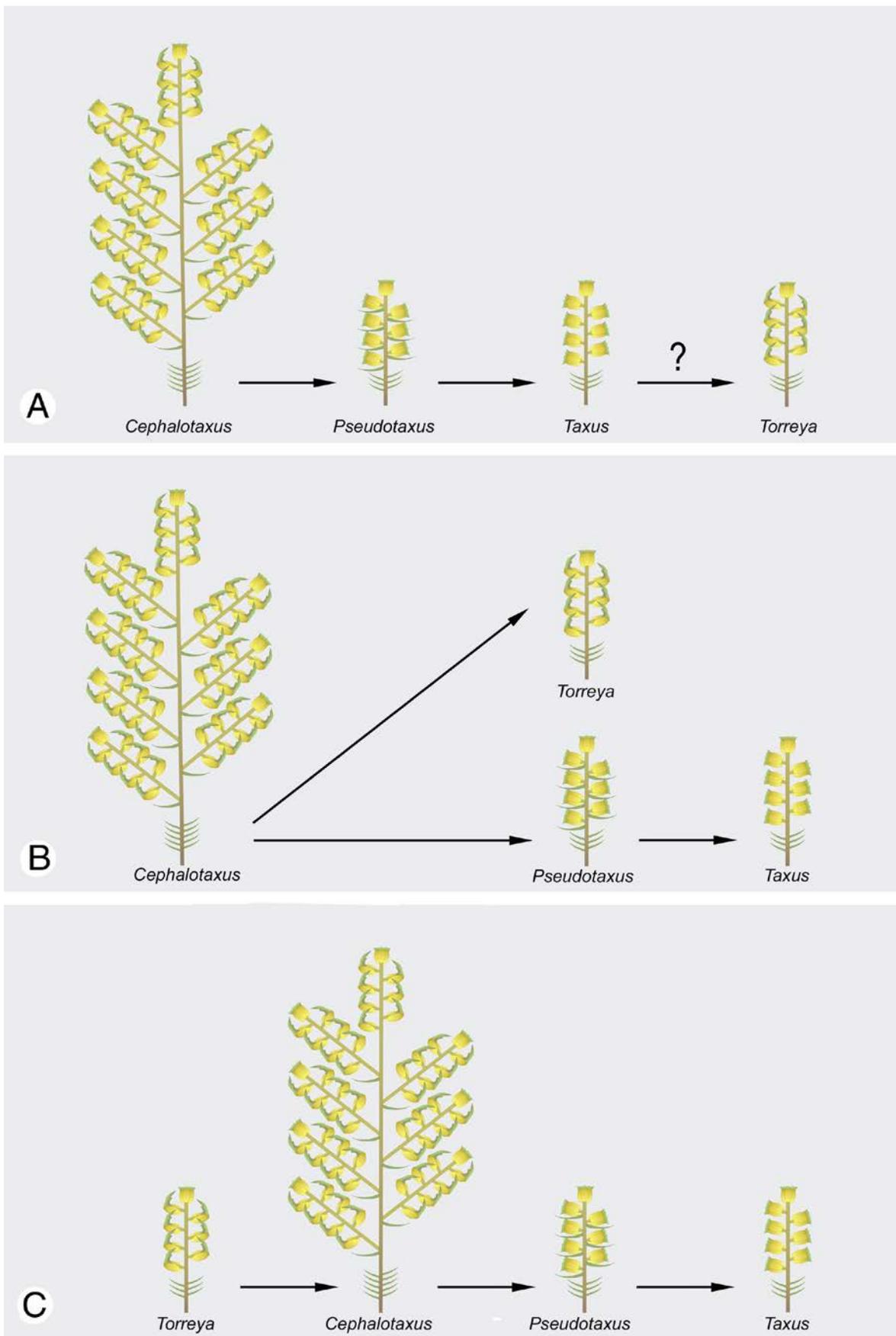
**Fig. 11:** Cross sections of lateral microsporangioophores in different Taxaceae.

The hyposporangiate microsporangioophores of *Torreyia californica* (A) and *Cephalotaxus harringtonii* (B) are supplied with a single collateral vascular bundle (arrow); the perisporangiate microsporangioophores of *Pseudotaxus chienii* (C) and *Taxus baccata* (D & E) are supplied with 2 vascular bundles (arrows). F: *Taxus baccata*, anomalous hyposporangiate microsporangioophore.



**Fig. 12:** *Cephalotaxus harringtonii*, proliferated pollen cones.

Due to the lack of a terminal pollen cone the cone axis can continue growing. Pollen cones are inserted in the axils of the inner bud scales. **A-C:** If the internodes of the fertile inner bud scales are not expanded the pollen cones are arranged closely to each other. **D-F:** If the internodes of the fertile inner bud scales are expanded the pollen cones are arranged distantly from each other at the shoot axis. **E & F:** Detail of D. **E:** Basal part of a pollen cone. **F:** Fertile inner bud scales with well developed internodes.



**Fig. 13:** Possible evolutionary scenarios for microsporangiophores in the investigated Taxaceae.

For a better overview the true dimensions among the different cones were not respected in this scheme.