Morphology and anatomy of male cones of *Pseudotaxus chienii* (W.C. Cheng) W.C. Cheng (Taxaceae)

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**Abstract**

Results of the present study indicate that male cones of *Pseudotaxus chienii* are representing inflorescences with strongly reduced flowers. The results fit quite well with investigations showing that sporangiophores of *Taxus* and also of *Pseudotaxus* comply with reduced flowers. The only difference between male cones in *Taxus* and *Pseudotaxus* is the absence of pherophylls in *Taxus*. Furthermore our results complete a transition series beginning with *Cephalotaxus* going on to *Pseudotaxus* and ending with *Taxus* and *Torreya*. In this progression *Pseudotaxus* can be regarded as an intermediate link between the inflorescences of *Cephalotaxus* and the simple, unbranched cones of *Taxus*. The entire transition series shows that pherophyll-like sporangiophores can be derived by reduction of lateral cones. There is however no sign that a similar process has occurred in other conifer groups.

**Introduction**

In general male cones of gymnosperms are uniaxial systems with limited growth. Each cone bears several sporangiophores. Thus, most authors regarded sporangiophores as sporophylls and the male cones fulfill the definition of flowers.\(^1\) Some exceptions can be found e.g. in *Cephalotaxus* (*Cephalotaxaceae*) and *Podocarpus* (*Podocarpaceae*). In these genera male cones are branched but not uniaxial systems. Each bud represents a compound cone (inflorescence) and each subunit represents a flower. Usually each subunit (flower) of an inflorescence is inserted in the axil of a fertile bract (pherophyll) (Mundry and Mundry, 2001).

Molecular studies show a close relationship between *Cephalotaxaceae* and *Taxaceae* (Chaw et al., 2000; Cheng et al., 2000; Quinn et al., 2002; Ran et al., 2009). In these studies the genera *Taxus*, *Torreya*, *Amentotaxus*, *Austrotaxus* and *Pseudotaxus* are all placed in the *Taxaceae*. Based on morphological studies from the early 20th century, *Cephalotaxus* and *Amentotaxus* have often been placed in the Coniferosida (e.g., Chamberlain, 1935; Engler, 1954a; Florin, 1948; Krüssmann, 1955, 1983; Pilger, 1926), separated from the other Taxales. Later studies suggest *Taxaceae* and *Cephalotaxaceae* as closely related (Chen and Wang, 1985; Farjon, 1998, 2001, 2010; Keng, 1969; Mundry, 1999; Mundry and Mundry, 2001; Restemeyer, 1999, Stützel et al., 1999). Today the genera of *Cephalotaxaceae* are placed in the *Taxaceae* (Eckenwalder, 2009; Stevens, 2001). For two reasons there exist up to now no morphological and anatomical studies about male cones of *Pseudotaxus*. On one hand the genus has been discovered relatively late (1948). On the other hand it is still today very difficult to get useful material, because *Pseudotaxus* is native to only a small range in China and its habitat is difficult to access.

Two different types of sporangiophores have been described for conifers (Fig. 1). The perisporangiophyte type (Fig. 1C) is bearing sporangia all around a central stalk, and the hyposporangiophyte type is bearing sporangia only on the abaxial side (Fig. 1F). There are two hypotheses about the evolution of the sporangiophores of Taxales. Some authors regard all sporangiophores of conifers as homologous structures (e.g., Dluhosch, 1937; Dupler, 1919; Wordsell, 1901). In this concept the hyposporangiophyte (dorsiventral) sporangiophore of *Cephalotaxus* and *Torreya* is derived from the perisporangiophyte sporangiophore as it can be found in *Taxus*. Assuming this, the adaxial sporangia of the circle-like arranged sporangia would be strongly reduced and present a phyllom-type structure. The sporangiophore gets thus the appearance of dorsiventral scale leaf.

The other hypothesis regards the sporangiophore of *Taxus* as derived from a radial synangium consisting of several dorsiventral reduced sporangiophores (Mundry and Mundry, 2001; Thomson, 1940a,b; Wilde, 1975). Following this, the sporangiophore of *Taxus* corresponds to an entire lateral cone of *Cephalotaxus* and represents an entire male cone, i.e. an inflorescence. Wilde’s theory is based on intermediate forms occurring in *Austrotaxus* and *Pseudo-
Fig. 1. Male cones and sporangiophores in *Taxus baccata* (A–C) and *Torreya californica* (D–F); (A) and (D) male cones are developed only on the last seasonal growth unit; (B) and (E) male cones in detail; (C) and (F) sporangiophores schematically; *Taxus* with perisporangiate sporangiophores and *Torreya* with hyposporangiate sporangiophores (S = sporangium; SC = scutellum; SP = sporangiophore; ST = stalk).

taxus. Her studies are however based for *Austrotaxus* on anatomical slides from Thomson. For her studies about *Pseudotaxus* only two dissected cones (collected by Cheng) were available. She published only some line drawings based on her results. In her drawings the additional pherophylls are evident. Their descriptions are not detailed enough for precise comparisons. Page (1990) described sterile bracts in the male cones of *Pseudotaxus*. Wilde interpreted these bracts as pherophylls, bearing reduced male cones in their axils. Therefore she suggested a new transition series for male reproductive structures of Taxales. Furthermore she suggested the male cone of *Torreya* as homologous to the terminal flower of the inflorescence of *Cephalotaxus*. Mundry and Mundry (2001) could
support Wilde’s results partly. Their results indicate furthermore that the sporangiophore of *Torreya* might be regarded as derived from the sporangiophore of *Taxus* by reduction of the adaxial sporangia. They postulate a phylogenetic transition series of male structures in Taxaceae, beginning with compound cones like they occur in the genus *Cephalotaxus* and ending with the uniaxial cones of e.g. *Taxus* and *Torreya*. Due to this, additional studies especially on *Austrotaxus* and *Pseudotaxus* are important and necessary to find possible intermediate forms. This is undertaken with the present analysis of the male *Pseudotaxus* cones.

Materials and methods

The material (male cones) was collected with special permit and fixed in formalin-acetic acid–alcohol (FAA) in the field by Z.X. Zhang and Th. Stützel (Hangzhou, Zhejiang Province, China) on 14.09.2004. Like in all other gymnosperms cone development is simultaneous for all individuals in the region, so that only one stage could be obtained. The cones were not fully developed, but represented a late developmental stage. The pollen was still very young and not fully developed.

The position of the cones within the branching system was documented in the field using a Nikon Coolpix 995. Small, fertile branchlets were removed and fixed in FAA and later stored in 70% alcohol. Hand sections where made with a razor blade. For serial sections classical paraffin technique and subsequent safranin–astraanblue staining was used (Gerlach, 1984). For SEM-analysis the FAA-material was dehydrated in formaldehyde dimethyl acetal (FDA) for at least 24 h (Gerstberger and Leins, 1978) and critical point dried. Sputter coating was done with a Sputter Coater SCD 50 Bal-tec (Balzers). The specimen were examined in a DSM 950 (Zeiss) and the results were documented with digital photographs.

Microphotographs were made using a dissection microscope Zeiss SV 11 and a light microscope Zeiss Axiosplan. Both microscopes were supplied with an Olympus ColourView II® camera. As record system we used the analySIS® software version 3.2build776. Multiple image alignment was done with the same software package.

Results

*Pseudotaxus* is a monotypic genus (Mabberley, 2008). *Pseudotaxus chienii* W.C. Cheng is a dioecious, evergreen shrub. It is native to a very discontinuous range in SO-China from Hunan to Zhejiang, south to Guangxi and Guangdong (Eckenwalder, 2009). In its natural habitat it reaches heights up to 4 m (Dallimore and Jackson, 1966).

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The position of *Pseudotaxus*-cones within the branching system is the same as in *Taxus* (Fig. 1A), *Torreya* (Fig. 1D) and *Cephalotaxus*. They appear in the axils of pherophylls (Fig. 2) on the last seasonal growth unit. Each cone bears 8–12 sporangiophores (Fig. 3A–C). They are arranged spirally but may have a nearly whorled appearance. The cones are sessile (Fig. 2) and start with 2–3 small sterile cataphylls (Fig. 4A and B). The following 2–4 cataphylls are much larger and thus overtop the cone (Fig. 3F and 4A). They are fertile and bear in their axils in median position a single perisporangiate sporangiophore (Fig. 4C and D). These large cataphylls are followed by additional 1–2 smaller cataphylls (Fig. 3C and D), which bear also sporangiophores in their axils. In the distal part of the cone cataphylls are lacking, so that the sporangiophores are not subtended by a pherophyll (Fig. 3A–C and 4A). Each sporangiophore is composed of a flat, roundish, terminal scutellum, a stalk, which is attached in the center of the scutellum and 3–8 sporangia arranged parallel round the stalk (Fig. 5). The size of the sporangiophores appears relatively uniform despite the high variable number of sporangia. If there are only few sporangia (Fig. 5A and C) the sporangiophores are markedly larger than if there are many (Fig. 5B and D). Median sections through sporangiophore and pherophyll show a vascular bundle entering the sporangiophore. The pherophyll lacks any vascular supply even in the insertion zone (Fig. 4C). There is always a terminal sporangiophore (Fig. 4A). Due to the spiral arrangement and the dense packing in the bud the terminal sporangiophore is frequently dislocated laterally.

The male cones studied were collected in a relative adult stage of development. Further ontogenetic studies on juvenile cones are necessary to get the conclusive evidence that the sporangiophores actually arise in the axils of pherophylls. Furthermore it would be helpful, to see if there are rudiments of pherophylls in the distal part of a cone and if the proximal cataphylls are really sterile or if rudiments of sporangiophores can be seen there.

Discussion

The results of our study about male cones of *Pseudotaxus chienii* give strong support for the evolutionary concept for male reproductive structures of Taxales suggested by Mundry and Mundry.
(2001). This concept combines the partly conflicting morphological hypotheses suggested by Dluhosch (1937) and Wilde (1975) into a single consistent concept. Dluhosch regarded perisporangiate sporangiophores, as they can be found in Taxus, as primitive in gymnosperms, and hyposporangiate sporangiophores, as they can be found in Torreya, as derived by reduction of the adaxial sporangia. Wilde (1975) regarded the perisporangiate sporangiophore of Taxus as derived from a reduced lateral cone (flower) in a compound cone (inflorescence) similar to those, which can be found in Cephalotaxus. She regarded cones of Torreya as derived from the ter-
minal flower of such a compound cone. Mundry and Mundry (2001) stated that in a first step the lateral cones of a compound cone should have got reduced to a perisporangiate sporangiophore as it occurs in Taxus. Thereafter, in a second step the adaxial sporangia of the perisporangiate sporangiophore got reduced. So a hypso­porangiate (dorsiventral) sporangiophore, as it occurs in Torreya would have been formed. At least for Taxaceae we reject therefore the idea of primitive perisporangiate sporangiophores as proposed by Dluhosch earlier.

The reduction of lateral cones of Cephalotaxus has been described by Wilde (1975) and Mundry and Mundry (2001). The compound cone of Cephalotaxus harringtonia is mostly composed of 2–12 simple lateral cones and a single terminal cone. Well developed lateral cones are composed of 8–10 sporangiophores. Sometimes the cones are reduced to 2–3 sporangiophores. Each sporangiophore is hypso­porangiate and has a prominent phyllom­like structure and 2–3 sporangia.

The male cones of Cephalotaxus fortunei are less branched than in Cephalotaxus harringtonia. Furthermore the cone axis and the pherophylls of the lateral cones get often fused and the pherophyll appears metatopic recalcendent near the distal end of the compound cone. The pherophylls of those simple reduced cones are usually also reduced. It is not unlikely that they sometimes are completely lacking. Occasionally the two or three remaining sporangiophores of such a cone are congenitally fused and form a single large synangium. This synangium is composed of a terminal scutellum and a centrally attached stalk. Several synangia are arranged around the stalk. To summarize, within the genus Cephalotaxus more or less all intermediates from an individual male cone (flower) to perisporangiate sporangiophore can be found.

Our results indicate that the number of sporangiophores of a Pseudotaxus-cone corresponds more or less to the number of the lateral cones of Cephalotaxus harringtonia. In contrast to this, the number of sporangiophores in a male cone of Pinaceae is distinctly higher. In Cephalotaxus fortunei pherophylls can be partly or completely reduced in the distal part of the cone. A comparable situation can be found in Pseudotaxus chienii, where pherophylls are always developed in the basal and middle part of the male cone while they are lacking in the distal part. It is fundamental for the understanding of the bauplan of plants that axillary structures are derived from shoots. In accordance to the discussion of the origin of the seed scale in Pinaceae the axillary position of the sporangiophore of Pseudotaxus is a strong argument for a shoot­like nature of the sporangiophores of Taxus. Furthermore it occurs quite often in inflorescences that pherophylls for the most distal flowers get reduced or may even be completely absent. This is well documented for several angiosperm-groups e.g. Asteraceae (Engler, 1954b) or Ericaceae (Ruhland, 1903; Stützel, 1984). In

Fig. 4. Anatomy of male cones of Pseudotaxus chienii: (A) longitudinal section; (B) basal part of the cone with several sterile cataphylls followed by several fertile pherophylls, which are lacking in most distal parts; (C) and (D) detail of basal sporangiophores, which each are inserted in the axil of a pherophyll (C = cataphyll; P = pherophyll; SP = sporangiophore).
Fig. 5. Morphology (as seen from the sporangiophore basis) and anatomy of perisporangiote sporangiophores of *Pseudotaxus chienii*; number of sporangia per sporangiophore varies strongly; if there are only few sporangia (A and C) they will be larger in size than in sporangiophores with a high number of sporangia (B and D), where they are smaller; (S = sporangium; ST = stalk).

Fig. 6. Transition series for male reproductive structures in Taxales beginning with compound cones in *Cephalotaxus* ("inflorescence") and ending with *Taxus* ("flower"); compound cones in *Pseudotaxus* with strongly reduced flowers can be regarded as an intermediate link in a transition series between *Cephalotaxus* and *Taxus*; hyposporangiote sporangiophores in *Torrey* can be regarded as derived from a *Taxus*-like perisporangiote sporangiophore by the reduction of all adaxial sporangia.
contrast to our results Page (1990) described the pherophylls of Pseudotaxus-cones as sterile bracts. Such sterile bracts, inserted in a cone could only be explained as transformed, sterile sporangio-
phores, which now take over the protection of the cone. In this case the sterile bracts would have to follow the phyllotaxis. However this is definitely not the case. It seems that the sporangio-
phores are placed directly in the axis of the pherophyll. As the distal parts of these pherophylls are frequently somewhat asymmetric and as we did not have the material to see the primary morphogenesis, we could not prove whether the sporangio-
phore is in the median plane of the pherophyll. However the position appears to be precisely enough suggesting, what has to be expected for a lateral branch in the axil of a pherophyll.

Our results are quite similar to those of Wilde (1975), but branched sporangio-
phores as they were described by Wilde, could also not be found in our material. It may be, that Wilde misinter-
preted as branched those sporangio-
phores from the distal part of the cone, where pherophylls are lacking.

Conclusions

Our results fit quite well with the phylogenetic transition series suggested by Wilde (1975) and Mundry and Mundry (2001). The studies indicate that bracts in cones of Pseudotaxus are not ster-
ile as it was supposed in earlier studies. They represent fertile pherophylls, which bear a single perisporangiate sporangio-
phore in the axils in median position. Due to this, male cones of Pseudo-
taxus represent an inflorescence with partly reduced pherophylls. Therefore Pseudotaxus could be regarded as an intermediate link in a transition series (Fig. 6) between Cephalotaxus with compound cones (inflorescences) and Taxus with uniaxial cones (flowers). For these reason sporangio-
phores of Pseudotaxus, Taxus and the ter-

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