

Origin of the Taxaceae aril: evolutionary implications of seed-cone teratologies in *Pseudotaxus chienii*

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- **Background and Aims** Fleshy structures that promote biotic dispersal by ingestion have evolved many times in seed plants. Within the yew family Taxaceae *sensu lato* (six genera, including *Cephalotaxus*), it remains controversial whether the characteristic fleshy structure surrounding the seed is interpreted as a novel outgrowth of the base of the ovule (i.e. an aril) or a fleshy seed coat that is entirely derived from the integument (i.e. a sarcotesta). This paper presents a detailed study of both wild-type and teratological seed cones of *Pseudotaxus chienii*, including morphology, anatomy and ontogeny.
- **Methods** Wild-type and teratological seed cones were investigated with the classical paraffin technique and subsequent astrablue/safranin staining and scanning electron microscopy.
- **Key Results** The wild-type seed cone of *Pseudotaxus* possesses a fleshy white aril that is cup-like and not entirely fused to the seed. In the teratological seed cones investigated, the aril was bilobed and consisted of two free halves. In both wild-type and teratological cones, the aril was initiated as two lateral primordia in a transverse plane, but in wild-type cones the two primordia became extended into a ring primordium, which grew apically, leading to the cup-like shape. The teratological seed cones lacked a ring primordium and the two lateral aril lobes remained free throughout their entire ontogeny, alternating with the scale-like leaves inserted below them on the same branch; in some cases, these leaves also became fleshy.
- **Conclusions** Based on the ontogeny and arrangement of the two fleshy aril lobes in the teratological seed cones of *Pseudotaxus*, we suggest that the typical aril of Taxaceae could be readily interpreted as a fused pair of strongly swollen leaves rather than a modified integument. Our investigations of the cup-like aril of *Pseudotaxus* demonstrate a similarity not only with other Taxaceae but also with relatively distantly related conifers such as *Phyllocladus* (Podocarpaceae).

Key words: Aril, evolution, *Pseudotaxus*, seed cone, Taxaceae, teratology.

INTRODUCTION

Reproductive structures of conifers are typically arranged in compact unisexual cones, either as ‘male’ (staminate) pollen cones or as ‘female’ (ovulate) seed cones. A characteristic feature of coniferous seed cones is the presence of a bract scale/seed scale complex, in which the bract scale is widely interpreted as a leaf that subtends a modified shoot, the axillary seed scale, though in some conifers the entire complex is so strongly modified that neither scale type is readily distinguishable (Coulter and Chamberlain, 1917; Florin, 1951, 1954; Farjon and Ortiz-Garcia, 2003; Jagel and Dörken, 2014, 2015a, b). Some conifer seeds possess associated fleshy structures that make the seed attractive to birds and animals and hence are implicated in biotic dispersal; these structures include the fleshy fused bracts of *Juniperus* (Cupressaceae), the fleshy seed scale (epimatium) of *Podocarpus* (Podocarpaceae) and the fleshy aril of the yew family Taxaceae (Tomlinson and Takaso, 2002; Contreras *et al.*, 2017; Lovisetto *et al.*, 2012). The seed of *Taxus* (Taxaceae) is toxic due to the presence of cyanogenic glycosides in the seed coat; however, the fleshy structure

is edible and seeds pass through the bird undigested (Farjon, 2007; Dörken and Hetzel, 2017).

Within Taxaceae, it remains controversial whether the fleshy structure surrounding the seed is interpreted as an outgrowth of the funicle (an aril) or a fleshy seed coat derived from the integument (a sarcotesta), or indeed whether there exists a continuum between them. The fleshy structure is widely accepted as an aril in *Austrotaxus*, *Taxus*, *Pseudotaxus* and *Torreya*. Some authors also interpret the fleshy structure in *Cephalotaxus* as an aril (e.g. Stützel and Röwekamp, 1999; Mundry, 2000; Eckenwalder, 2009; Farjon, 2010), but others regard it as a sarcotesta (e.g. Page, 1990; Contreras *et al.*, 2017). The fleshy structure in *Amentotaxus* was earlier interpreted as a specialized type of ovuliferous scale termed an epimatium (e.g. Krüssmann, 1955), a structure that is typically found in Podocarpaceae (e.g. Buchholz and Gray, 1948; Tomlinson, 1992; Vázquez-Lobo *et al.*, 2007). However, based on comparative expression patterns of orthologues of the AG gene family, Englund *et al.* (2011) rejected a primary homology between the aril of *Taxus globosa* and the epimatium of *Podocarpus reichei*, which they considered homologous with the ovuliferous scale

of *Pinus*. Although arillate Taxaceae seeds are known from the Triassic, comparative data from the fossil record are also difficult to interpret and do not conclusively resolve the issue (e.g. Meyen, 1987).

In this paper, we evaluate the controversial homologies and possible evolutionary origin of the aril structure in Taxaceae *sensu lato* using a detailed comparison of wild-type and teratological seed cones in *Pseudotaxus chienii*. No previous comparative studies of seed-cone ontogeny exist for *Pseudotaxus*, though descriptions exist for some other Taxaceae; for example, Mundry (2000) described the ontogeny of the fleshy structure surrounding the ovules/seeds in *Taxus*, *Torreya* and *Cephalotaxus*. However, comparison of mature seed cones shows that *Pseudotaxus* is very similar to *Taxus*, differing only in the number of scale leaves at the stalk of the seed cone and the colour of the aril: white in *Pseudotaxus* and red in *Taxus* (Florin 1948a; Krüssmann, 1983; Farjon, 2007, 2010; Eckenwalder, 2009). In both genera, the aril is strongly swollen, fleshy, cup-like and not fused to the seed, and the distal collar is unlobed. The similarities in their seed cones indicate a close relationship between the two genera, which is confirmed in morpho-anatomical (e.g. Ghimire and Heo, 2014) and genetic (e.g. Cheng *et al.*, 2000) studies.

Taxaceae *sensu lato* consists of six extant genera, including two formerly segregated as Cephalotaxaceae (*Amentotaxus*, *Cephalotaxus*) and four 'core' Taxaceae: *Austrotaxus*, *Pseudotaxus*, *Taxus* and *Torreya* (e.g. Harris, 1976; Stevens, 2001; Eckenwalder, 2009; Lang *et al.*, 2013; Ghimire *et al.*, 2014). In many respects, cone morphology in Taxaceae is unique among living (extant) conifers. The seed cones of all Taxaceae lack a distinct bract scale/seed scale complex (Ghimire *et al.*, 2014) and the pollen cones of *Cephalotaxus* and *Pseudotaxus* are interpreted as polyaxial, in contrast with other conifers, in which the entire pollen cone is interpreted as uniaxial (e.g. Coulter and Chamberlain, 1917; Wilde, 1944, 1975; Krüssmann, 1955, 1983; Mundry and Mundry, 2001; Dörken *et al.*, 2011). Taxaceae (excluding *Amentotaxus* and *Cephalotaxus*) were formerly placed in a separate order, Taxales, but both morphological and molecular analyses demonstrate that they are true conifers, closely related to Cupressaceae (e.g. Chaw *et al.*, 2000; Quinn *et al.*, 2002; Burleigh and Matthews, 2004).

MATERIALS AND METHODS

Materials

Pseudotaxus chienii (Taxaceae) is a rare Chinese species of Taxaceae, which is rarely grown in cultivation. *Taxus baccata* is native to Europe and adjacent regions and frequently in cultivation. Both taxa are evergreen, dioecious (exceptionally monoecious) shrubs or small trees. Cones of both genders develop in axillary positions on lateral branchlets. Within *Pseudotaxus* two cone types were investigated here: (1) wild-type seed cones; and (2) teratological seed cones with anomalous strongly divided bilobed arils, mostly consisting of two entirely separate halves. Within *Taxus* only wild-type seed cones were investigated. All cones were collected by one of the authors (V.M.D.) in the extensive living collection of another author (H.N.) in St Ulrich, Bollschiweil, Germany. Wild-type and teratological seed

cones of *Pseudotaxus* were collected from two different trees that are cultivated as pot plants and placed outdoors in the summer and in a temperate glasshouse in the winter. Seed cones of *Taxus* were collected from a tree cultivated outdoors.

Methods

Freshly collected material was photographed, then fixed in formalin acetic alcohol (FAA: 70 % alcohol, formaldehyde solution and glacial acetic acid, in proportions 90:5:5) before being stored in 70 % ethanol. Cone anatomy was studied using the classical paraffin technique and subsequent astra blue/safranin staining (Gerlach, 1984). 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 an X–Y stage and bright-field illumination (Keyence VH-S5). For scanning electron microscope (SEM) analysis, FAA-fixed material was dehydrated in formaldehyde dimethyl acetal (FDA) for at least 24 h (Gerstberger and Leins, 1978) and critical-point dried, then mounted onto SEM stubs and sputter-coated using a sputter coater (Bal-Tec SCD 50, Balzers). Specimens were examined using an Auriga Zeiss TM SEM.

RESULTS

Pseudotaxus chienii: wild-type seed cones (Figs 1 and 2)

Seed cones develop on lateral shoots in the axil of a typical needle leaf, with a single seed cone per leaf (Figs 1A and 2B). At pollination time, the ovules and pollination drops are oriented in a strictly downward-facing position. Whereas typical foliage leaves on other branches are needle-like with a pointed tip and spiral arrangement (Fig. 1A), the leaves on the branchlet that bears the seed cones are scale-like and decussate. There are seven or eight pairs of such scale-like leaves per branchlet (Fig. 2A). Each leaf is keeled with a distinct hyaline margin (Fig. 1B) and supplied by a single collateral vascular strand. A single ovule is usually developed per seed cone (Fig. 2A), or exceptionally two (Fig. 1C). Two collateral vascular bundles supply the ovule. The aril develops relatively late and lacks vasculature entirely. At the time of pollination, the ovule is almost entirely surrounded by the decussate scale-like leaves; only the micropyle remains free (Fig. 1A). Aril formation commences after pollination with the initiation of two lateral primordia directly below the ovule, located in the transverse plane (Fig. 1D–F) and alternating with the lowermost pair of leaves. Subsequently, the broad bases of the two primordia become extended to form a ring primordium, which grows apically. Initially the ring primordium grows weakly; it first becomes visible externally ~2–3 months after pollination when the aril starts to exceed the surrounding leaves in length, though at this stage the aril is still green (Fig. 2A). After the aril has exceeded the leaves in length, it starts to grow more rapidly so that at maturity it is as long as (or sometimes longer than) the mature seed (Fig. 2B). During maturation, the tissues of the aril become strongly enlarged to form a succulent, fleshy, cup-like structure. The distal collar is roundish and unlobed. Its

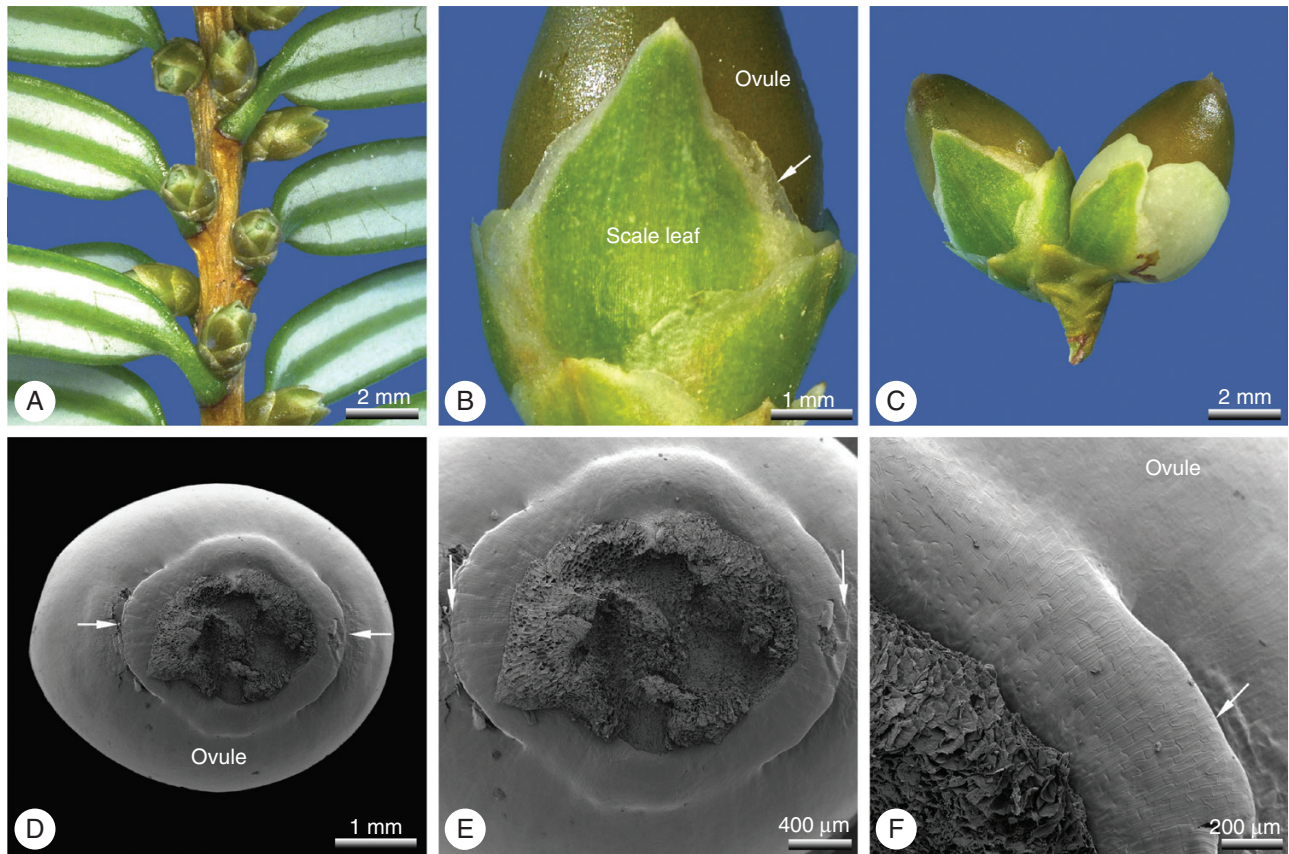


FIG. 1. *Pseudotaxus chienii*, seed cones (wild-type). (A) Seed cones shortly before pollination. (B) Detail of decussate scale-like leaves that develop at the stalk of the seed cone and surround the juvenile ovule. (C) Seed cones with two ovules. (D) Young ovule from below; the aril is initiated by two lateral primordia (arrows) located in the transverse plane. (E) Detail of (D). (F) Detail of transitional zone between the transverse free aril initials (arrows) and the adjacent median part.

outer surface is strongly papillate, with cells arranged in vertical longitudinal rows (Fig. 2C). Some stomata are sunken in the outer epidermis of the aril (Fig. 2C). The inner tissue of the aril consists of fairly homogeneous isodiametric cells with thin walls and large vacuoles (Fig. 2D). The aril lacks vasculature entirely and remains unfused to the seed throughout ontogeny. At maturity, seeds are 7–8 mm long and 5–8 mm in diameter. The mature cup-like aril is ~8–10 mm long and 10–13 mm in diameter (Fig. 2B).

Pseudotaxus chienii: teratological seed cones (Figs 3–6)

On one of the trees investigated, all the seed cones displayed an unusual strongly bilobed or entirely divided aril consisting of two free halves (Figs 3–6). In these teratological seed cones, the number and morpho-anatomical features of the scale-like leaves on the cone stalk resembled the wild-type. As in the wild-type, aril development commenced with two opposite primordia in the same plane, located directly below the ovule and precisely alternating with the distalmost pair of leaves. However, in these teratological seed cones the two lateral primordia did not fuse to each other (Figs 4 and 5) and never formed a ring primordium as in the wild-type; the two lobes remained independent from each other throughout their entire ontogeny, maintaining their initial location and orientation (Figs 3 and 4). As in the

wild-type, the young lobes were green (Fig. 3B), maturing to white (Fig. 3C–F). At maturity, they represented an opposing pair of unfused distal lobes, which were as long as the seeds or slightly exceeded them in length (Fig. 3C–F); they still alternated with the distalmost pair of leaves on the stalk of the seed cone (Fig. 4A, B). The mature lobes were strongly swollen, fleshy (Fig. 3) and lacked vasculature entirely (Fig. 5).

One of the teratological seed cones contained two ovules (Fig. 6) rather than the more typical single ovule, but one of the two ovules was aborted. The aril of each ovule consisted of two separate free lobes (Fig. 6A–C), which were identical to those described for the teratological seed cones above and illustrated in Figs 3–5. However, the free lobes of the aril were not the only fleshy structure within these seed cones; several of the lower (formerly scale-like) leaves inserted on the seed-cone stalk also became strongly swollen and fleshy, except at their hyaline margins, which remained membranaceous. In these teratological seed cones, the fleshy lobes of the aril alternated perfectly with the lowermost anomalously swollen pair of leaves (Fig. 6D–F).

Taxus baccata: wild-type seed cones (Fig. 7)

The position of seed cones within the branching pattern is similar to *Pseudotaxus* (Figs 1A and 2B). At pollination time,

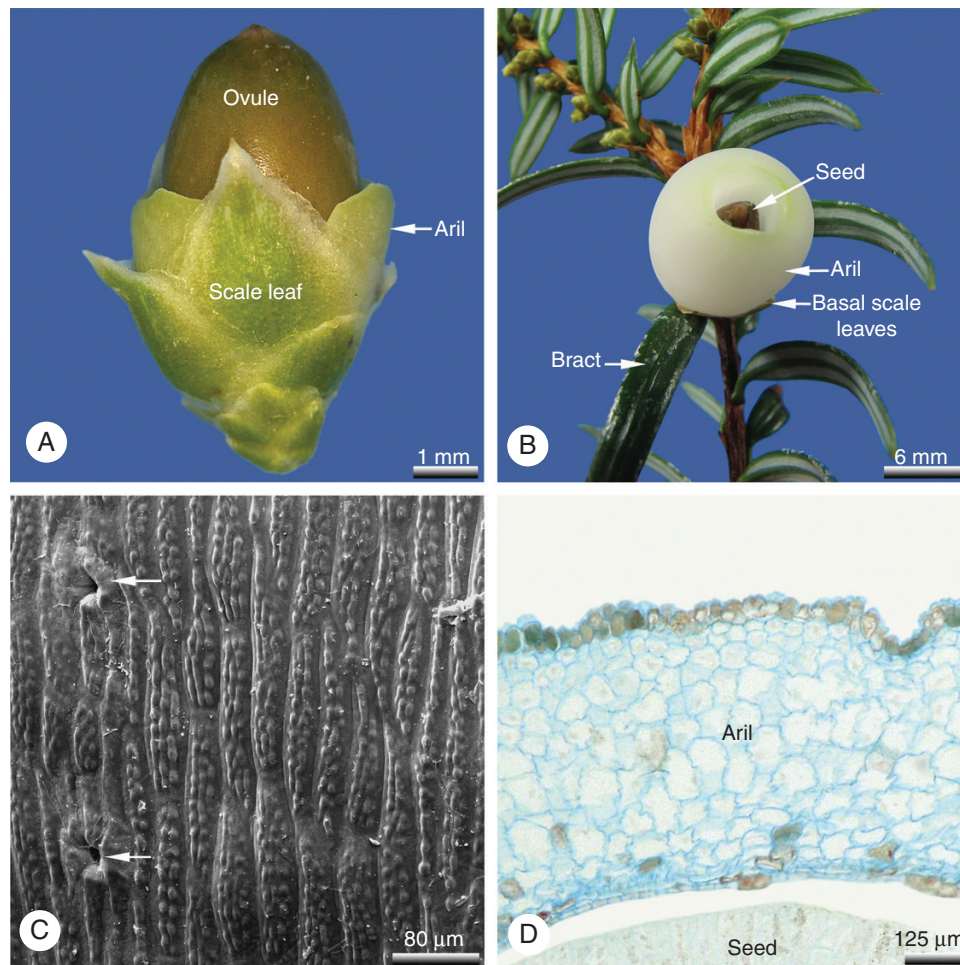


FIG. 2. *Pseudotaxus chienii*, seed cone (wild-type). (A) Immature seed cone ~3 months after pollination; the aril is still green at this stage and starts to exceed the lower scale leaves. (B) Mature seed cone; seed surrounded by the fleshy, cup-like, white aril. (C) Outer epidermis of aril; cells axially oriented and with micropapillae on their surfaces; sparse sunken stomata (arrowed) are also present. (D) Transverse section of a mature aril showing tannin-filled epidermal cells surrounding parenchymatous internal tissue lacking vasculature.

the seed cones and their pollination drops are oriented in a downward position (Fig. 7A). There are seven or eight pairs of decussate scale leaves inserted on the stalk of the seed cone (Fig. 7A, B). At pollination time, the scale leaves almost entirely surround the ovule, except the micropylar region (Fig. 7A). The aril develops relatively late, and is not visible externally at pollination time (Fig. 7A). It is initialized as two small lateral primordia directly below the ovule, which are located in the transverse plane, in an alternating arrangement with the lowermost pair of scale leaves (Fig. 7B, C). Subsequently the broad bases of the two primordia become extended, fuse to each other and form a ring primordium (Fig. 7D, E), which is characterized by apical growth. The ovule is supplied by two collateral vascular bundle strands, whereas the aril lacks vasculature entirely. The initial ring primordium is slow-growing and becomes visible externally ~2–3 months after pollination, when the aril starts to exceed the surrounding scale leaves in length (Fig. 7D). At this ontogenetic stage, the aril is still green and not fleshy (Fig. 7F). After the aril has exceeded the scale leaves in length, further development is rapid (Fig. 7E). Mature seeds are surrounded entirely or even exceeded by the aril, which

becomes red and fleshy (Fig. 7F). The mature aril is a cup-like structure that remains unfused to the seed (Fig. 7F). All other features are similar to *Pseudotaxus* (Figs 1 and 2).

DISCUSSION

Comparison with other Taxaceae

Our data show that the wild-type aril of *Pseudotaxus* closely resembles that of *Taxus* in both structure and development. In both genera, the aril is green before turning white (in *Pseudotaxus*) or red (in *Taxus*). In both genera, aril initiation commences with formation of two lateral primordia in a transverse plane. The initials are located directly below the ovules and their tips are free at early stages, but later they form a ring primordium and ultimately a cup-shaped fleshy structure that is not fused to the ovule/seed (Mundry, 2000). Thus, in both *Taxus* and *Pseudotaxus* the aril could be interpreted as two fused, strongly swollen and fleshy scale leaves rather than as a modified integument, as other authors have also suggested for *Taxus* (e.g. Van Tieghem, 1869; early literature summarized by

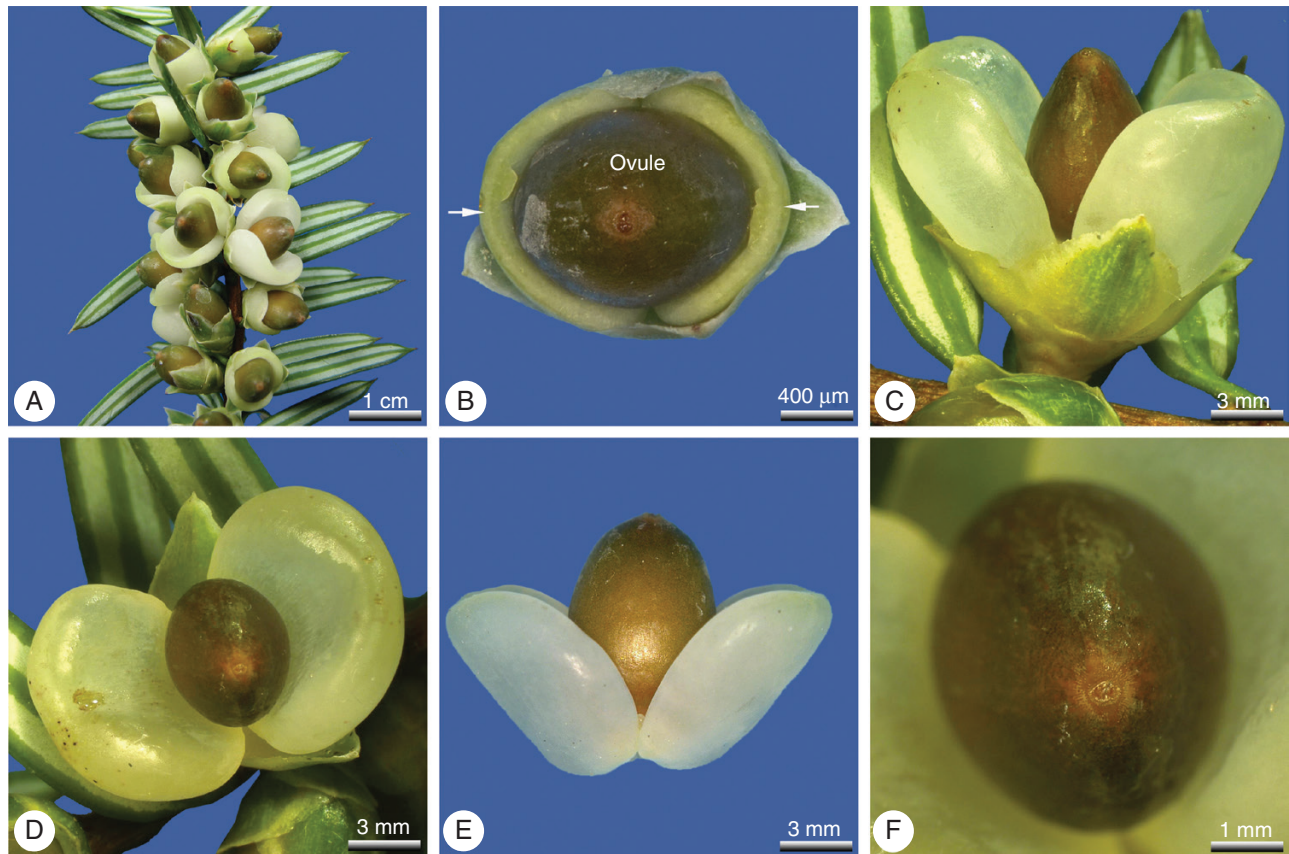


FIG. 3. *Pseudotaxus chienii*, mature teratological seed cone with a bilobed aril. (A) Branch with teratological seed cones. (B) Immature seed cone in top view; the two fleshy aril lobes (arrows) alternate with the lower pair of scale leaves. (C, D) Mature seed cone showing a bilobed aril in lateral view (C) and top view (D). (E) Mature seed surrounded by fleshy aril consisting of two separate, free lobes (lower stalk with scale leaves removed for a better overview). (F) Mature seed in top view; seed and aril not fused to each other.

Dupler, 1920). Mature aril structure in the closely related genus *Austrotaxus* also resembles that of *Taxus* and *Pseudotaxus* (e.g. Florin, 1948a, b; Krüssmann, 1983; Bobrov *et al.*, 2004), though detailed comparative ontogenetic studies are lacking. Based on comparative morphology, Florin (1948b) described the free upper collar in arils of *Austrotaxus*, *Taxus* and *Pseudotaxus* (treated as *Nothotaxus*) as their main similarity with the arils of *Torreya* and *Amentotaxus*, suggesting that the first three genera represent the more ‘primitive’ condition in Taxaceae.

In *Torreya*, aril development does not commence until the integument has already formed the micropyle. As in *Taxus* and *Pseudotaxus*, the aril is initiated by two lateral opposite primordia in the transverse plane, but the primordia become strongly extended into a ring primordium very early in ontogeny. Mundry (2000) demonstrated a clear correlation between the arrangement of the aril initials and the bract that bears the seed cone; the initials always develop in a transverse plane and the transition zone between the transverse primordia is located in the median plane. Thus, the juvenile *Torreya* aril is strongly bilobed; at its earliest ontogenetic stages it resembles *Taxus* and *Pseudotaxus* but differs considerably from them at later stages. Soon after pollination, the two free aril lobes exceed the distal micropyle and are pushed upwards to envelop the entire ovule. Subsequently, the aril becomes basally fused to the ovule and develops further by intercalary basal growth, so that at maturity

the seed and aril are almost entirely fused to each other, only the micropylar parts remaining free. The outer surface of the mature aril is also characterized by elongated cells arranged in axial rows (Mundry, 2000).

In *Cephalotaxus*, aril development begins significantly later than in *Taxus*, *Pseudotaxus*, *Austrotaxus* and *Torreya*, so that the aril is invisible before fertilization. It is initiated as a ring primordium and is almost entirely congenitally fused to the ovuliferous integument; hence some authors have interpreted this structure as a fleshy seed coat (sarcotesta), comparable with the outer fleshy structure in seeds of cycads and *Ginkgo* (e.g. Vierhapper, 1910; Page, 1990; Contreras *et al.*, 2017). However, in cycads and *Ginkgo* the maturing integuments differentiate into three layers (endotesta, sclerotesta and sarcotesta), which are initiated regularly over the entire integument (e.g. Coulter and Chamberlain, 1917; Obermann, 2003). Thus, the term ‘sarcotesta’ should be avoided in describing the fleshy outer layer of the *Cephalotaxus* seed, which is best interpreted as an aril. The ring primordium of *Cephalotaxus* shows distinct intercalary growth, so that the aril envelops the entire seed except for the distal micropylar region (Mundry, 2000). *Cephalotaxus* lacks the free distal tips as developed in *Torreya*, but the outer surface of the mature aril resembles that of *Torreya* (Mundry, 2000).

Keng (1969) described aril development in *Amentotaxus* as closely similar to that of *Cephalotaxus*; the ovule is embedded in

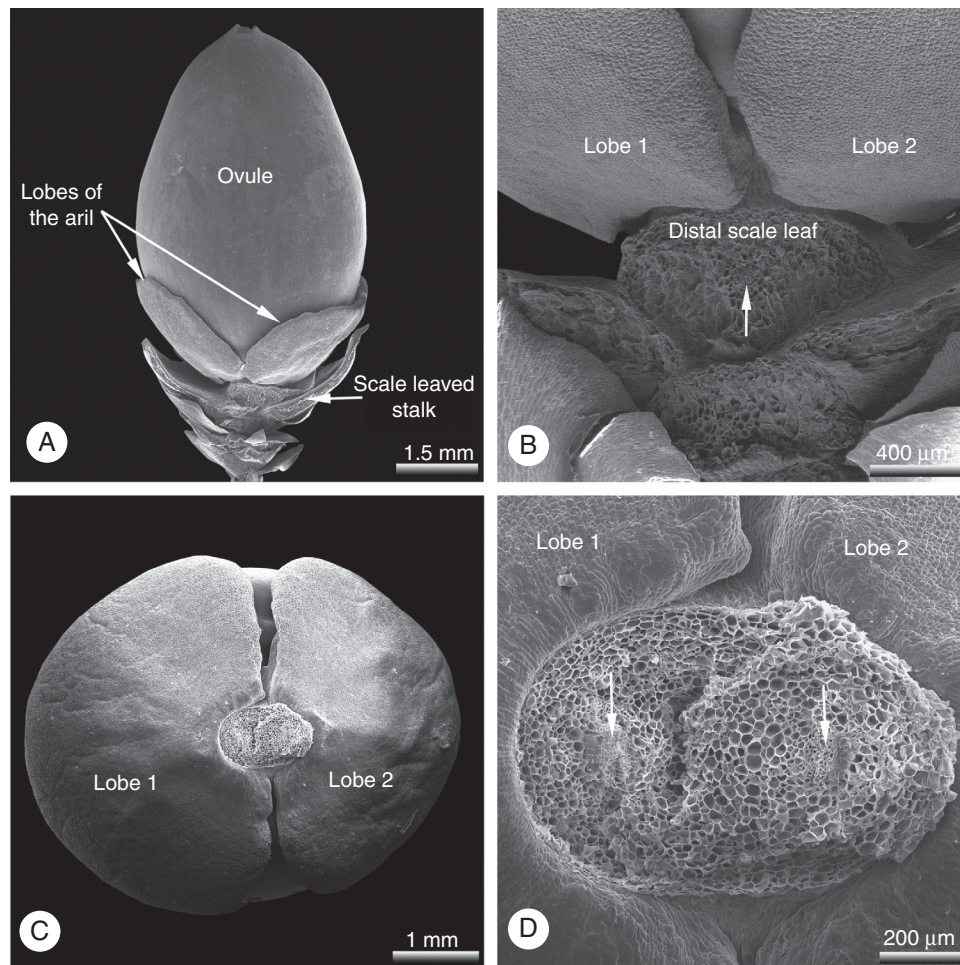


FIG. 4. *Pseudotaxus chienii*, SEM micrographs of a juvenile teratological seed cone shortly after pollination; the aril consists of two lateral free lobes located in the transverse plane (distal pair of decussate scale leaves removed for a better overview). (A) Lateral view of entire cone including stalk with scale-like leaves; the two lobes of the aril alternate with the lower scale-like leaves of the stalk. (B) Detail of (A); the lower scale leaves are supplied with a single collateral vascular bundle strand (arrow). (C) Young seed from below; the two transverse aril lobes are not fused to each other or to the ovule. (D) Detail of (C) showing funiculus with two vascular bundles supplying the ovule (arrows).

the aril at an early stage. However, the earliest cones examined were already 3 mm long, so it remains undetermined whether the *Amentotaxus* aril is initiated from a ring primordium (as in *Cephalotaxus*) or two lateral primordia in the transverse plane that later form a ring primordium, as in *Taxus*, *Pseudotaxus* and *Torreya*. Subsequent aril development in *Amentotaxus* is characterized by fusion of the aril to the integument and intercalary growth (Ghimire and Heo, 2014). Ultimately, the aril is entirely fused to the seed except at the micropylar region (Krüssmann 1955, 1983; Keng 1969; Ghimire and Heo, 2014; Eckenwalder, 2009; Farjon, 2010), as in *Cephalotaxus*. However, vasculature is reduced within the seed cone and the ovule is supplied by a single bundle strand (Keng, 1969); in all other Taxaceae the ovule is supplied by two vascular bundles.

Thus, among extant Taxaceae, two generic groupings exist with respect to the structure and ontogeny of the fleshy structure (aril): (1) *Austrotaxus*–*Pseudotaxus*–*Taxus* (the APT group) and (2) *Amentotaxus*–*Cephalotaxus*–*Torreya* (the ACT group). The most striking differences between the two groups relate to fusion and growth (Table 1). The aril is strongly fused

to the ovule in the ACT group, compared with a free cup-like structure in the APT group. Aril growth is intercalary in the ACT group compared with apical (distal) growth in the APT group. In the ACT group, the aril has several vertical intercellular spaces and a non-papillate epidermis, compared with a strongly papillate epidermis and internal tissues lacking intercellular spaces in the APT group. The strong initial distal growth of the free lobes in *Torreya* represents a marked difference not only with *Amentotaxus* and *Cephalotaxus* but also with all other Taxaceae.

Conversely, the genera share some morpho-anatomical similarities. In five genera (*Amentotaxus*, *Austrotaxus*, *Cephalotaxus*, *Pseudotaxus*, *Taxus*) the micropyle remains visible externally even at maturity, but in *Torreya* it is soon enveloped by the two free distal lobes of the aril. In three genera (*Pseudotaxus*, *Taxus*, *Torreya*), aril development is initiated by two lateral primordia in the transverse plane before later forming a ring primordium, but in *Cephalotaxus* and *Amentotaxus* such distinct transverse initial lobes are not visible and aril development commences with a ring primordium (Table 1). No

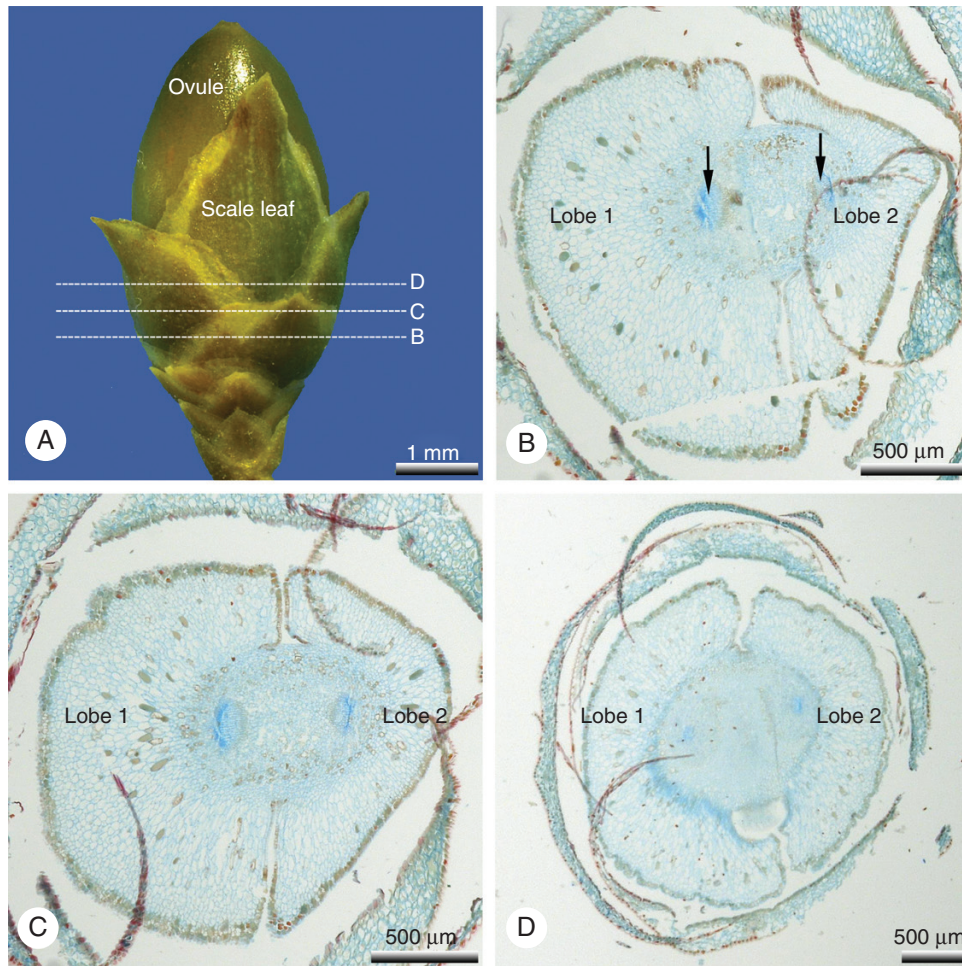


FIG. 5. *Pseudotaxus chienii*, teratological seed cone with a bilobed aril ~3 months after pollination. (A) Ovule still enclosed by the basal pairs of scale leaves. (B–D) Series of transverse sections from basal to distal as marked in (A); the two lateral lobes of the aril are free and inserted at the same level below the ovule; the right lobe is slightly larger than the left one; the fleshy lobes are parenchymatous and lack vasculature; within the funiculus two vascular bundles (arrowed in B) supply the ovule.

comparative data exist for *Austrotaxus* for this character, and further studies are desirable for aril initiation and ontogeny in *Austrotaxus* and early ontogeny in *Amentotaxus*.

Interpretation of the *Pseudotaxus* aril

The wild-type aril of *Pseudotaxus* originates at a relatively late stage from an abaxial ring primordium adjacent to the ovule, making it difficult to resolve its homologies based on typical seed cones. Thus, the teratological seed cones investigated here provide significant relevant data. In the numerous teratological seed cones examined, the aril was bilobed or entirely divided into two free lobes. In both wild-type and teratological seed cones, the aril initiates with two opposite primordia in the transverse plane, but in wild-type seed cones the two primordia rapidly fuse at their margins to form a ring primordium, even at early developmental stages. In contrast, in the teratological seed cones the two lateral primordia remain free throughout the entire maturation of the seed cone. In both wild-type and teratological seed cones, the two transverse aril primordia alternate

with the distalmost pair of scale-like leaves inserted on the seed-cone stalk, and this alternation is maintained to maturity in mature teratological seed cones.

Thus, the two lobes of the aril could readily be interpreted as two opposite leaves that become strongly swollen and fleshy during development. The following data support this hypothesis: (1) the seeds and fleshy aril lobes are not fused to each other; (2) the two aril lobes are inserted below the ovule in the same plane; (3) the two fleshy aril lobes precisely alternate with the distalmost pair of scale-like leaves on the seed-cone stalk and therefore form part of the same organ series; (4) juvenile lobes are green and chlorophyll-rich before ultimately turning white. Finally, further convincing evidence (5) was provided by a teratological seed cone that formed additional fleshy structures below it, apart from the distal fleshy aril lobes (Fig. 6); our study shows that these additional fleshy structures are transformed leaves of the seed-cone stalk that have become unusually swollen, fleshy and white.

All these features combined support the hypothesis that the typical, cup-like aril of wild-type *Pseudotaxus* could have originated as two fused and strongly swollen leaves. Thus, an

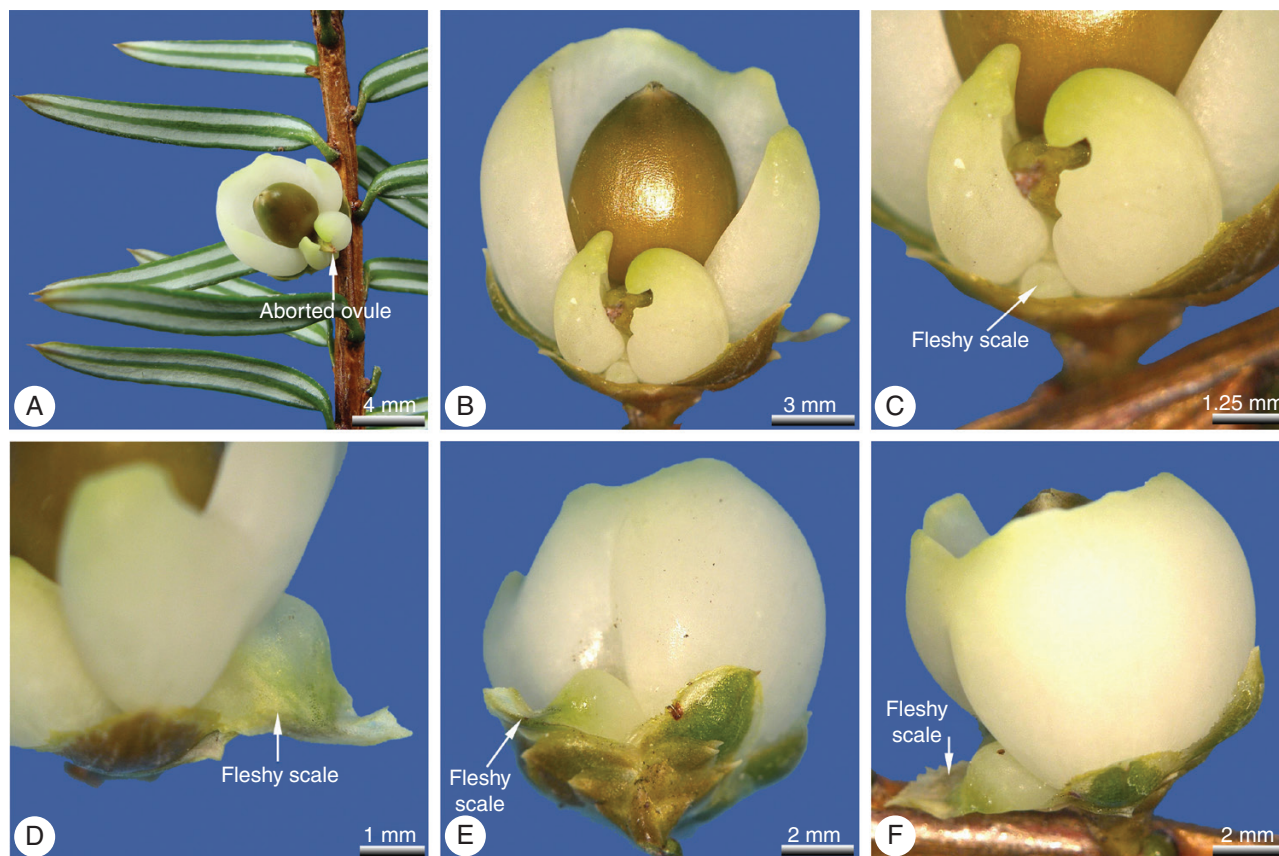


FIG. 6. *Pseudotaxus chienii*, mature teratological seed cone showing two ovules (rather than the more typical single ovule), one of them aborted. (A, B) Both ovules are surrounded by a fleshy, bilobed aril, consisting of two free lobes. (C–F) Below the ovules, several pairs of scale leaves are present; these are typically keeled and green, but some are strongly swollen and white, comparable with the lobes of the aril.

origin of the aril as an outgrowth of the seed scale, shoot axis, funiculus or integument is unlikely. These teratological data are congruent with the hypothesis of some previous authors (e.g. Taylor *et al.*, 2009) that the taxaceous aril is derived from modified sterile scales at the base of the ovule-carrying short shoot. A remarkably similar condition occurs among other gymnosperms, in some species of the gnetalean genus *Ephedra*, in which the seeds are surrounded by several decussate fleshy and intensely coloured bracts (e.g. Stützel and Mundry, 2001; Huang *et al.*, 2005; Rydin *et al.*, 2010; Yang and Wang, 2013). These fleshy bracts are attractive to animals that disperse the seeds, such as frugivorous birds or seed-caching rodents (Ickert-Bond and Wojciechowski, 2004; Hollander and Vander Wall, 2009, 2010). Each pair of fleshy bracts is connate to a degree that is species-specific, with fusion of one- to two-thirds of their length (Huang *et al.*, 2005), comparable with the bilobed arils in the teratological seed cones of *Pseudotaxus* investigated here (Figs 3–6).

Conversely, other factors could contradict this interpretation, including spatial constraints, the relatively late development of the aril, and the absence of vasculature. Spatial constraints during early aril development could result in the formation of two apparently distinct primordia. Initiation and subsequent development of the aril represent the final steps in seed-cone formation in this species, after all other structures are formed. If the aril represents a fusion product of the modified distal pair

of scale leaves, as suggested here, then much earlier initiation would be expected, directly subsequent to the formation of the distal pair of scale leaves and not after a break of several weeks. One possibility is that aril initiation does take place earlier, but long before the first initials become visible externally. Furthermore, while each scale leaf of the stalk is supplied with a single collateral vascular strand, the *Pseudotaxus* aril lacks vasculature entirely, as also in *Taxus* (Dupler, 1920; Mundry, 2000). However, the absence of aril vasculature in *Pseudotaxus* does not exclude derivation of the aril from fused scale leaves. In contrast, within *Torreya*, the aril is supplied with two vascular bundle strands, one at each lateral side (Vierhapper, 1910; Herzfeld, 1914), perhaps due to the relatively large size of the mature *Torreya* seeds compared with the small mature seeds of *Taxus* and *Pseudotaxus*.

Conclusions

Fleshy structures that promote biotic dispersal by ingestion (either as fleshy seeds or fleshy fruits) have evolved many times in seed plants. Examples include *Ginkgo*, cycads, *Ephedra* (Gnetales), some conifers (e.g. *Juniperus*, *Podocarpus*, Taxaceae) and angiosperms (e.g. fleshy-seeded *Magnolia*: Lovisetto *et al.*, 2015) and some extinct seed plants such as some Cordaitales (e.g. Arber, 1914). The ‘fleshiness’

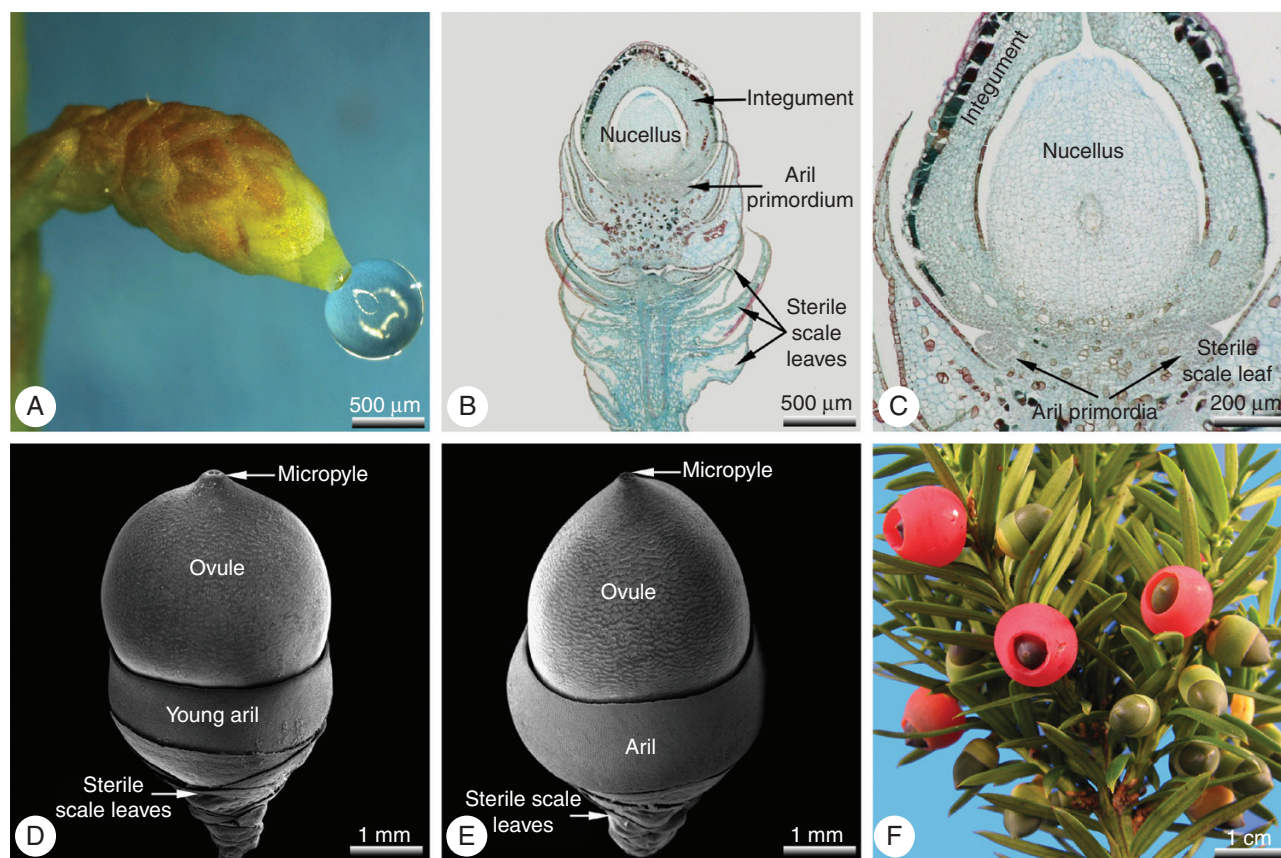


FIG. 7. *Taxus baccata*, aril development. (A–C) Seed cones at pollination time. (A) Seed cone with pollination drop; no aril structures visible externally. (B) Longitudinal section of the seed cone illustrated in (A); the aril primordia located below the ovule develop at a late ontogenetic stage. (C) Detail of (B); ovule base showing aril primordia in the transverse plane. (D, E) After pollination the aril starts enclosing the seed; in wild-type seed cones it forms a cup-like structure with apical growth. (F) Lateral branch with seed cones in different ontogenetic stages: immature ones with a green aril, mature ones with a fleshy, red aril.

TABLE 1. Comparison of fleshy ovule/seed structures in genera of Taxaceae

Fleshy structure (aril)	<i>Austrotaxus</i> , <i>Pseudotaxus</i> , <i>Taxus</i> (APT group)	<i>Amentotaxus</i> , <i>Cephalotaxus</i> , <i>Torreya</i> (ACT group)
Attachment	Aril free, cup-like (not strongly fused to the ovule)	Aril strongly fused to the ovule
Early development and growth	Development initiated by two lateral primordia with mostly apical growth; subsequently a ring primordium is formed (<i>Austrotaxus</i> unknown)	In <i>Amentotaxus</i> and <i>Cephalotaxus</i> , growth commences with a ring primordium, and is intercalary (though there are few data on <i>Amentotaxus</i>). In <i>Torreya</i> , an initial distal growth phase forms two free distal lobes; subsequently a ring primordium is formed, with an intercalary growth phase.
Epidermis	Papillate	Non-papillate
Anatomy	Internal tissues lacking large intercellular spaces	Internal tissues with several large, vertical intercellular spaces
Vasculature	Absent	In <i>Torreya</i> , two vascular strands are present; in <i>Amentotaxus</i> absent; in <i>Cephalotaxus</i> they are probably absent (based on images in Mundry, 2000)

syndrome, in which the relevant structure becomes not only enlarged, soft and sweet-tasting but also colourful and attractive, encompasses a complex suite of changes in tissue texture and physiology and pigment synthesis. A suite of MADS-box genes is implicated in the development of fleshy fruits and seeds in both angiosperms and gymnosperms (e.g. Prasad *et al.*, 2010; Lovisetto *et al.*, 2012, 2013, 2015). Lovisetto *et al.* (2012) found that although *AGAMOUS* (*AG*) is widely

expressed in both male and female structures in *Taxus*, *AG* expression is particularly localized to the young developing aril during later stages of seed development, and that other (*AGL6* and *TM8-like*) MADS-box genes were also apparently involved. They hypothesized that this suite of genes represents a basic genetic toolkit that is common to all fleshy structures, regardless of their morpho-anatomical origin (Lovisetto *et al.*, 2015).

The hypothesis that iterative gene co-option results in iterative evolution of anatomically similar but morphologically non-homologous structures accords with the structural diversity of the fleshy seed structures within conifer families such as Taxaceae and Podocarpaceae, in which the fleshy region is variously interpreted as an aril, epimatium or sarcotesta. Our investigations of the cup-like aril of *Pseudotaxus* demonstrate a similarity not only with other Taxaceae but also with some other extant conifers, such as *Phyllocladus* (Podocarpaceae s.l.), in which development of the fleshy structure associated with the seed shares several similarities with *Taxus* and *Pseudotaxus*, despite their relatively distant relationship. As in some Taxaceae, the aril of *Phyllocladus* is free and not fused to the seed. As in *Pseudotaxus*, aril development is relatively late in *Phyllocladus glaucus*, initially as an outgrowth at the ovule base on its lateral margins, but rapidly forming a ring primordium, leading to a closed collar and finally a cup-like aril with the two lateral initials visible as distinct lobes on the distal collar (Tomlinson and Takaso, 1989). Vascularization of the ovule in *Phyllocladus* also resembles most Taxaceae (except *Amentotaxus*), with two vascular strands. Thus, fleshy structures in gymnosperms could well be highly homoplastic. Recent studies on gene expression in conifers and other living gymnosperms are contributing to the debate on their homologies, but more comparative data are needed to untangle the diverse range of structures and development in these taxa. Our comparative investigation of teratological seed cones in *Pseudotaxus* strongly indicates that the typical aril of Taxaceae could be interpreted not as a modified integument but as a fused pair of strongly swollen leaves.

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