Seed morphology and anatomy of *Austrotaxus spicata* (Taxaceae) and its systematic position

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The anatomy and ultrastructure of seed envelopes of a New Caledonian endemic *Austrotaxus spicata* were examined for the first time. The systematic position and phylogenetic relations of *Austrotaxus* were analysed in light of these data. The structure of aril and spermoderm were investigated to demonstrate the similarities with *Phyllocladus* as well as with *Taxus* and *Pseudotaxus*. On the basis of all female reproductive organ characters, *Austrotaxus* appeared to be fairly isolated and its placing in the independent family Austrotaxaceae was confirmed from the standpoint of comparative anatomy of the seed coat. Taking into consideration that the heterobathmy of features can be the most distinctively traced in the structure of reproductive organs, evaluating the extent of evolutionary advancement of *Austrotaxus* seems to be rather difficult. However, it is evident that the relationship of *Austrotaxus* either with Taxaceae or with Podocarpaceae s.l. is considerably remote. © 2004 The Linnean Society of London, Botanical Journal of the Linnean Society, 2004, 145, 437–443.


INTRODUCTION

The flora of New Caledonia contains abundant endemics. Many monotypic genera, both gymnosperms and flowering plants, pose problems for botanists. *Austrotaxus spicata* Compton can be added to these taxa. The problem of *Austrotaxus* and its phylogenetic relations has been constantly disputed since its first description (Compton, 1922) to the present day. There are three interpretations of its systematic position and phylogeny. Compton (1922), Saxton (1934) and many other scientists (Koidzumi, 1932; Florin, 1944, 1948b, 1951, 1954; Janchen, 1949; Sporne, 1967; Gaussen, 1979; Hart, 1987; Page, 1980) included *Austrotaxus* in the family Taxaceae s.l. Compton (1922) pointed out the close similarities in seed morphology of representatives of *Austrotaxus*, *Taxus* L. and *Amentotaxus* Pilg. *Amentotaxus* also resembles *Austrotaxus* in habit, leaf morphology, terminal position of seed on the axillary pedicle and presence of scales at the base of a seed (Bobrov, 1997d). Based on studies of embryology of *Austrotaxus* and its reproductive organs, Saxton (1934) considered the genus to be an archetype of the genus *Taxus*. Some features are present in both genera, e.g. late aril development (it never fuses with the spermoderm and covers most of the seed), morphological specialities of pollen grains, gametogenesis and embryogeny. As Saxton assumed, the aril in *Taxus*, morphologically slightly reduced, its specialized peltate microsporophylls as well as other significant traits of reproductive and vegetative organs were derived from the morphological peculiarities of *Austrotaxus*. Li (1953), Greguss (1955) and Takhtajan (1956) placed *Austrotaxus spicata* in the Podocarpaceae s.l.,...
based on close similarities in wood structure of *Austrotaxus* and *Podocarpus* L’Herit. ex Pers. s.l., the existence of microstrobilate aggregations in *Austrotaxus* and two species of *Podocarpus* s.l. (*P. andina* Poep. ex Endl. and *P. taxifolia* Humb., Boupl. & Kunt), and their common geographical distribution (*Austrotaxus* and podocarps occur chiefly in the Southern Hemisphere whereas taxads grow mostly in the Northern). Takhtajan (1956) inclined to support Nakai’s (1938) opinion in placing the genus in its own family Austrotaxaceae. In one of his last papers, Florin (1958) came to the same conclusion. Takhtajan (1986) acknowledged the family Austrotaxaceae Nakai, including it, however, in the order Taxales. Vegetative characters and peculiar leaf anatomy shows clearly that *Austrotaxus* is intermediate between taxads and podocarps (Ferre, Rouane & Woltz, 1977; Hu, Wang & Wang, 1992).

It is impossible therefore to avoid the conclusion that the data on structural specialities of *Austrotaxus* alone cannot resolve the problem of its systematic position in relation to other coniferous plants.

Recently, anatomical features of seeds have been used as characters in plant taxonomy and phylogeny, but in the case of *Austrotaxus* they have been apparently ignored by investigators. Data on ovules and seed morphology of *Austrotaxus* in Schnarf’s (1937) monograph, appeared to be taken from Saxton’s (1934) article. Studies conducted by Woltz & Bailly (1982) brought some light on the morphology and anatomy of *Austrotaxus* embryos and seedlings, but the anatomical structure of the seed envelope remains uninvestigated.

In order to bridge this gap and to reveal the significance of anatomical traits of seeds in solving systematic and phylogenetic problems, the present study was carried out and the seed morphology, anatomy and ultrastructure of *Austrotaxus spicata* were investigated.

**MATERIAL AND METHODS**

The material was very kindly provided by Prof. E. S. Chavchavadze, the Head of the Botanical Museum, the Komarov Botanical Institute, Russian Academy of Science, Saint-Petersburg. Prior to anatomical investigations, dry seeds were soaked in Strasburger’s mixture and than embedded in paraffin. The seeds were sectioned in transverse and longitudinal directions in relation to their longitudinal axis. Sections with a thickness of 10–20 μm were obtained by using a slide microtome. All sections were stained with phloroglucinol and hydrochloric acid to reveal details of lignification of cell walls in different topographical zones of the seed coat. Sections were preserved in glycerine. All experimental investigations were executed in accordance with standard morphological and anatomical procedures (Bondartzev, 1954; Prozina, 1960; O’Brien & McCully, 1981). Anatomical figures were prepared from transverse sections, which are the most informative in the investigation of seed coat anatomy (Schnarf, 1937).

**RESULTS**

**MORPHOLOGY**

The brown seed is orthotropous (Fig. 1A), up to 25 mm long and 10–12 mm thick, with undulating surface covered (except in the micropyle area) with the smooth brownish-orange aril, which is not fused with the seed coat (Fig. 1C, D), ellipsoidal, but in cross section, round or oval-quadrangular (Fig. 1C). The micropyle is narrow and bilobed (Fig. 1B). Five to ten decussate scales are situated at the base of the seed. The seed usually has a long pedicle of axillary origin.

**ANATOMY**

Aril structure (Fig. 2) varies depending on the region being examined. In the central part, the aril adheres tightly to the seed coat while in the upper part it forms a free collar rising above the micropyle. In the central part, the aril is composed of 23–27 cell layers, differentiated in the following manner. Almost square in outline, epidermal cells are characterized by heavily

![Figure 1. Morphology of the seed of Austrotaxus spicata.](https://academic.oup.com/botlinnean/article-abstract/145/4/437/2420232/figure-1)  
A, general view. Scale bar = 6 mm. B, view from micropyle. Scale bar = 3.6 mm. C, schematic diagram of longitudinal section of seed. D, schematic cross-section of seed. Abbreviations: a, aril; sc, seed coat.
thickened outer tangential walls (their thickness accounts for two-thirds of the total cell height) and transparent cytoplasm. The cuticle is rather thin. The hypodermis consists of small cells also with clear cytoplasm but with the cell walls only slightly thickened. Three zones can be readily distinguished in the main aril-forming tissue. The three or four outermost cell-layers are represented by large parenchymatous cells with small intercellular ducts. Their walls are weakly thickened; cell content is colourless. Minute thin-walled cells filled with green granulated contents form 15–17 middle layers. Larger cells with weakly coloured cytoplasm and thickened dark orange walls constitute the innermost two or three layers. The inner epidermis is made up of very small cells with slightly and uniformly thickened walls; both walls and cytoplasm are colourless. A thin cuticle is present. No vascular elements were found. At the sheath-like part surrounding the micropyle, the aril consists of four or five cell layers. Outer and inner epidermis are fairly close in structure, being composed of large cells with heavily thickened outer tangential walls covered with relatively thin cuticle. The transitional zone between ground tissues is represented by two or three layers of markedly compressed thin-walled tannin-containing cells.

The total number of cell layers forming the seed coat (Fig. 3) is 21–25. Exotesta consists of an outer epidermis followed by a hypodermal zone. Epidermal cells are large and radially elongated with pronounced thickening of radial and especially outer tangential walls. Cell lumina are triangular in outline. A thin cuticle is present. There are two or three hypodermal layers of colourless parenchymatous cells with evenly thickened walls. The mesotesta may be divided into two zones – the outer parenchymatous and the inner sclerenchymatous. The former is represented by four to five layers of thin-walled tanniniferous cells. Small tightly placed cells are polygonal in outline, have uniformly thickened walls and form 14–16 layers of the sclerified zone. In the four ‘angles’ of the seed the thickness of sclerenchyma approaches 22–24 cell layers. The total number of cell layers here is about 29–33. Endotesta is obliterated.

**DISCUSSION**

**RELATIONSHIPS WITH PODOCARPACEAE S.L.**

The results of the present investigation indicate that in seed envelope structure, *Austrotaxus* has some affinity with the Taxaceae s.l. as well as with Podocarpaceae s.l., perhaps a little closer to the latter. Analysing previously published data on aril and seed coat structure of taxads and based on our own examination of *Taxus*, *Torreya* Arn., *Amentotaxus*, *Cephalotaxus* Siebold & Zucc. ex Endl. seed coat structure as well as more than 100 Podocarpaceae species from all 18 genera (*sensu* Page, 1990), we deduce that *Austrotaxus* is close to those members of the Podocarpaceae s.l. that have orthotropous ovules and seeds with reduced aril (*Phyllocladus* Rich. & Mirb.) or lack an aril (*Microstrobos* J. Garden & L. A. S. Johnson = *Pherosphaera* W. Archer).
The genus *Microstrobos* is undoubtedly isolated due to extreme specialization; its connection with *Phyllocladus* and *Austrotaxus* is hypothetical and hardly probable. In contrast, a number of *Phyllocladus* species and *Austrotaxus* share certain features of aril anatomy, namely: large-celled external epidermis with heavily thickened tangential walls, the presence of an outer and inner hypodermis with tannin-containing cell-walls and/or cavities, and the complete absence of a vascular system (Bobrov, Melikan & Yembaturova, 1999). To a certain extent, *Austrotaxus* and the most primitive representatives of the genus *Phyllocladus* resemble each other. The exotesta of both is well differentiated, with the epidermis made up of large cells and ‘stony’ hypoderm; the mesotesta has two distinct layers, free not only of distinctive topographical zonality, but also of any traces of vascular, resin or secretory system. In addition the aril and seed coat in *Austrotaxus* do not fuse. Thus, we consider that it is more correct to treat the outer coat of seeds of *Amentotaxus*, *Torreya* and *Cephalotaxus* as an outer integument (Bobrov, 1996, 1997a, b, c; Melikian & Bobrov, 1997c, 2000; Bobrov & Karpun, 1998; Bobrov & Sorokin, 2002). Anatomically the seed envelopes of *Austrotaxus* resemble each other, whereas in most of the seven *Phyllocladus* species studied it is homogeneous; the parenchotesta is situated to the periphery of the sclerotesta in the *Austrotaxus*’s mesotesta, whereas in *Phyllocladus* the location of the tissues tends to be the opposite (Ph. alpinus Hook. f., Ph. hypophyllus Hook. f., Ph. major Pilg., Ph. protractus (Warb.) Pilg.), or the mesotesta is uniformly sclerified (Ph. totoa Molloy, Ph. trichomanoides D. Don in Lamb.) and there is only one species (Ph. asplenifolius (Labill.) Hook. f) characterized by the same type of zonation as in *Austrotaxus* (Bobrov et al., 1999). Moreover, *Austrotaxus* differs greatly from *Phyllocladus* in some other structural specialities, e.g. axial parenchyma in the secondary xylem (Greguss, 1955; Chavchavadze, 1979), the absence of phylloclades (Eichler, Engler & Prantl, 1889; Compton, 1922; Keng, 1978), and pollen morphology (*Phyllocladus* grains have pronounced (though markedly reduced) air sacs, whereas *Austrotaxus* pollen grains have no sacs (Saxton, 1934; Pocknall, 1981), and overall architecture of male and female reproductive organs [Saxton, 1934; Gaussen, 1974, 1979; Tomlinson, Takaso & Rattenbury, 1989]. However, the differences between *Austrotaxus* and other genera from Podocarpaceae s.l. are even stronger. Most podocarps have an epimatium or seeds covered by testa and tegmen (Bobrov, 1996; Melikian & Bobrov, 1997a, b, 2000), features missing in *Austrotaxus*. Thus the genus can be compared only with arillate Podocarpaceae members. However, the aril in *Austrotaxus* is radial symmetrical, whereas podocarps (Saxe-Goetha Lindl., *Microcachrys* Hook. ex Hook. f., *Falcifoliolum* de Laub., *Metadacrydium* Baum.-Bod. ex A. V. F. Ch. Bobrov & Melikan, *Lagarostrobus* Quinn, *Lepidothamnus* Phil., *Corneria* A. V. F. Ch. Bobrov & Melikan, *Gaussenia* A. V. F. Ch. Bobrov & Melikan) possess an asymmetrical aril (Melikan & Bobrov, 1997b, c). Ovules and seeds in *Austrotaxus* are straight whereas true orthotropous seeds have never been reported in Podocarpaceae s.l.

**RELATIONSHIPS WITH TAXACEAE s.l.**

*Austrotaxus* stands even further apart from Taxaceae s.l. (incl. *Taxus*, *Pseudotaxus* W. C. Cheng (= *Nothotaxus* Florin), *Torreya* and *Amentotaxus*) and Cephalotaxaceae. According to our original data (Melikian & Bobrov, 1997c, 2000; Bobrov & Karpun, 1998; Bobrov & Sorokin, 2002), fleshy, vascularized, extremely histologically differentiated, and supplied with resin and/or secretory ducts the outer seed envelope of *Torreya*, *Amentotaxus* and *Cephalotaxus* (usually defined as ‘aril’, but we incline to consider it an epimatium) fused with the seed coat has almost nothing in common with that of *Austrotaxus*, which is composed of fewer cell layers, free not only of distinctive topographical zonality, but also of any traces of vascular, resin or secretory system. In addition the aril and seed coat in *Austrotaxus* do not fuse. Thus, we consider that it is more correct to treat the outer coat of seeds of *Amentotaxus*, *Torreya* and *Cephalotaxus* as an outer integument (Bobrov, 1996, 1997a, b, c; Melikian & Bobrov, 1997a, b, c, 2000; Bobrov & Karpun, 1998; Bobrov & Sorokin, 2002). In our opinion, the genera *Amentotaxus*, *Torreya* and *Cephalotaxus* have bitemic ovules and seeds covered with testa and tegmen and they do not have either an epimatium or aril. The exceptionally peculiar process of ovule development in *Torreya* and *Amentotaxus* on the one hand and in *Cephalotaxus* on the other (Oliver, 1903; Kemp, 1959; Singh, 1961; Keng, 1969) considerably isolates these genera from each other and from the rest of the conifers (with the exception of *Nageza* Gaeren., *Retropilium* C. N. Page (= *Decussocarpus* de Laub. s.s.) and *Acmopyle* Pilg. – Bobrov, 1996, 1997a, b, c, d; Melikian & Bobrov, 1997a, b, c, 2000; Bobrov & Sorokin, 2002). Anatomically the seed envelopes of *Austrotaxus* seem to be somewhat close to those of *Taxus* and *Pseudotaxus*, but the difference between these genera is particularly evident in leaf morphology and anatomy (de Laubenfels, 1953; Hu et al., 1992), structure of stomata (Florin, 1931; Hu et al., 1992), xylem features (Greguss, 1955) and morphology of male reproductive organs (Saxton, 1934; Wilde, 1977; Gaussen, 1979). Even though the aril in *Taxus* and *Pseudotaxus* is not vascularized and never fuses with the spermoderm, neither external nor internal hypoderm is differentiated. The seed coat of *Taxus* and *Pseudotaxus* differs significantly from that of *Austrotaxus* in some ultrastructural traits of the exotesta (e.g. in the degree and character of wall thickening in epidermal cells). The mesotesta consists of homogenous sclerenchymatous tissue (Schnarf, 1937; Florin,
Table 1. Arillate taxa of Podocarpales and Taxales with orthotropous ovules and seeds. Abbreviations: oe, outer epidermis; oh, outer hypodermis; ih, inner hypodermis; ie, inner epidermis; e, epidermis; h, hypodermis; scl, sclerenchyma; par, parenchyma; // – ‘or’

<table>
<thead>
<tr>
<th>Features</th>
<th>Phyllocladus</th>
<th>Austrotaxus</th>
<th>Pseudotaxus</th>
<th>Taxus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habit</td>
<td>trees/shrubs</td>
<td>tall tree</td>
<td>shrub</td>
<td>polystems trees/shrubs</td>
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<td>Wood parenchyma</td>
<td>absent phylloclades (amphistomous/hypostomous)</td>
<td>scanty to abundant hypostomotic leaves to 15 cm long</td>
<td>scanty hypostomotic needles to 25 mm long</td>
<td>scanty amphistomous needles to 35 mm long</td>
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<td>Photosynthetic structures</td>
<td></td>
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<tr>
<td>Stomata</td>
<td>monocyclic solitary</td>
<td></td>
<td></td>
<td>amphicyclic solitary</td>
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<tr>
<td>Microstrobilus position</td>
<td>helical, 30–70</td>
<td>helical, 1–2 (to 5)</td>
<td>helical, ?, perisporangiate</td>
<td>helical, 6–14, peltate</td>
</tr>
<tr>
<td>Disposition, number and morphology of microsporophylls</td>
<td>2 (abaxial)</td>
<td>2–3 (few 4–5)</td>
<td>4–5</td>
<td>(4) 5–9</td>
</tr>
<tr>
<td>No. of microsporangia per microsporophyll</td>
<td>elliptoidal with 2 reduced sacci</td>
<td>spheroidal without sacci</td>
<td>polychydrical without sacci</td>
<td>polychydrical without sacci</td>
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<tr>
<td>Pollen grain morphology</td>
<td></td>
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<tr>
<td>Structure of female reproductive organs</td>
<td>helical bracts on short shoot</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Morphology of aril</td>
<td>covers seed on ( \frac{1}{2} ) height</td>
<td>covers seed excepting micropyle</td>
<td>covers seed on ( \frac{5}{6} ) height</td>
<td>covers seed on ( \frac{3}{4} ) height</td>
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<tr>
<td>Shape of micropyle</td>
<td>bilobed</td>
<td></td>
<td></td>
<td>rounded</td>
</tr>
<tr>
<td>Histology of aril</td>
<td>oe &amp; oh, par, ie</td>
<td>oe &amp; oh, par, ih &amp; ie</td>
<td>oe &amp; oh, par; ie</td>
<td>oe &amp; oh, par, ie</td>
</tr>
<tr>
<td>Structure of exotesta</td>
<td>scl/scl /par//par + scl</td>
<td>par + scl obliterated</td>
<td>par</td>
<td>scl</td>
</tr>
<tr>
<td>Structure of endotesta</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>No. of derivatives of vascular bands in seed coats</td>
<td></td>
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1948a; Bobrov & Karpun, 1998; Bobrov & Sorokin, 2002. Vegetative and reproductive traits of Austrotaxus, Taxus, Pseudotaxus and Phyllocladus are compared in Table 1.

**SYSTEMATIC POSITION**

We conclude that based on vegetative and, more importantly, reproductive organs Austrotaxus combines the features of various genera belonging to Taxales and Podocarpales. Taking into account all the data obtained on seed envelope morphology and anatomy, we suggest it is advisable to admit the family status of Austrotaxaceae Nakai 1938 and to keep Austrotaxus in an intermediate position between taxads and podocarps.

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