

Some ultrastructural aspects of the stem phloem in *Phacellaria* (Santalaceae) and *Korthalsella* (Viscaceae)

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SUMMARY

We present documentation of the occurrence of normal sieve-tube members in *Korthalsella dacrydii* (Ridley) Danser and *K. japonica* (Thunb.) Engler (Viscaceae) and *Phacellaria malayana* Ridley (Santalaceae), three squamate parasites. From this we may now state that morphological reduction (particularly the squamate condition and small size) in Santalales is not necessarily correlated with the evolutionary disappearance of sieve elements; the latter condition is probably unique to some species of *Arceuthobium* (Viscaceae). Sieve-tube plastids in *Phacellaria* are comparable to the S-type plastids known from all other known parasitic angiosperms. In *Korthalsella* sieve-tube members, regularly stacked and corrugated endoplasmic reticulum complexes are present as known from studies of Ranunculaceae and Papaveraceae.

Key-words: phloem, sieve-tube plastids, *Phacellaria*, *Korthalsella*.

INTRODUCTION

Those parasitic angiosperms which are recognized as morphologically reduced are also known to have highly specialized vascular tissues, a tendency which may be expressed in both xylem and phloem (Kuijt 1969). The extreme example of this tendency in the latter tissue seems to be in *Arceuthobium*, the mostly highly reduced genus of Viscaceae. Until very recently, it was accepted that sieve elements were completely absent in this genus (Kuijt 1969; Hawksworth & Wiens 1972). Calvin *et al.* (1984) have recently demonstrated, however, that this does not apply to all species. Using fluorescence microscopy only, these authors showed the existence of (relatively infrequent) sieve plates in stem tissue of what is stated to be a primitive species, the Mexican *A. globosum* Hawksworth & Wiens. The same technique, however, confirmed the absence of sieve elements in *A. douglasii* Engelm. and *A. tsugense* Rosend. of northwestern North America; these species are thought to be more advanced. The size of the stems from which samples were taken was not stated.

There are other genera in parasitic Santalales which are equally reduced morphologically as *Arceuthobium*, and the question 'whether in those plants sieve elements are present' is an appropriate one. The aphyllous condition is scattered throughout several predominantly leafy Santalalean genera such as *Thesium* and *Exocarpos* (Santalaceae), *Tristerix* and *Ixocactus* (Loranthaceae), *Dendrophthora*, *Phoradendron* and *Viscum*

(Viscaceae), but in all these genera it represents the minority of species. In the latter genus, the most highly reduced species, *V. minimum* Harvey, has recently been tested and was found to have normal sieve-tube members (Olson & Kuijt 1985). However, extrapolation from *Arceuthobium* leads one to predict that a phloem-less condition may similarly have evolved in the few small genera which are completely squamate, i.e. *Phacellaria* (Santalaceae; Danser 1939), *Daenikera* (Santalaceae; Huerlimann & Stauffer 1957), and *Korthalsella* (Viscaceae; Danser 1937). The latter genus, in fact, has been claimed to lack phloem (Stevenson 1934). Very recently, however, Touw (1984) has spoken of conductive phloem cells which retain their nuclei in at least two species of *Korthalsella*, which suggests at the same time that species such as *K. dacrydii* may lack phloem completely. However, no illustrations were published and the status of such cells has to be regarded as uncertain. The present paper attempts to answer the question with respect to some species of the two above aphyllous genera, *Korthalsella* and *Phacellaria* (Fig. 1). In the former genus the smallest known species, *K. dacrydii*, is included, which is found only in Timor, Java, Sumatra and the Malay Peninsula (Wasscher 1941).

The genus *Korthalsella* has frequently been considered to be closely related to *Arceuthobium* and, indeed, is often strikingly similar (Danser 1937). Its androecium and inflorescence (where present) are very different, however, and a close affinity may be more apparent than real. It is basically a genus of the western Pacific, reaching from Japan and Hawaii to Australia and New Zealand, and across Southeast Asia to Madagascar and Ethiopia (van Balgooy 1966). There can be little question of the highly advanced status of *Korthalsella* in Viscaceae. A recent publication has established the basic vascular patterns of the stems of a number of species, including the ones discussed here (Touw 1984). In that contribution, xylem in *Korthalsella* is stated to include tracheids as well as short vessel members with simple perforation plates.

Phacellaria is much more restricted geographically, being found from Kuala Lumpur, Malaysia, and north to southern China. It also bears a striking resemblance to *Arceuthobium*. Interestingly, all *Phacellaria* species are apparently obligate parasites on other mistletoes, perhaps only of Loranthaceae (Danser 1939). From the point of view of morphology and host specificity, it is no exaggeration to call *Phacellaria* the most advanced genus of Santalaceae. The internal structure of the plant has not previously been referred to in the literature.

MATERIALS AND METHODS

Samples were taken from healthy young stems or inflorescences just below mature flowers. Materials of *Korthalsella japonica* (Thunb.) Engler and *K. dacrydii* (Ridley) Danser were both collected on 26th December 1986 in the area of the Bogor Mountain Garden at Tjibodas, Java, the former species on *Altingia excelsa* Noronha at c. 1400 m in the Garden itself, and the latter species on *Podocarpus imbricata* Blume at c. 1500 m, along the trail 1–2 km above the Garden. *Phacellaria malayana* Ridley was collected on 6th January 1987 in Cameron's Highlands near Kuala Lumpur, Malaysia, at c. 1500 m, where it was found growing on a Loranthaceous host, *Dendrophthoe* cf. *pentandra* (L.) Miquel; the host tree is unknown.

All materials were cut into cubical pieces 1–2 mm in size and fixed in 3% glutaraldehyde and 3% *p*-formaldehyde in 0.1 M sodium cacodylate buffer at pH 7.3. Fixed materials were then rinsed with the same buffer, postfixed in 1% buffered osmium tetroxide for 2 h, and embedded in Spurr's epoxy resin. Thin sections were cut with a diamond knife on a

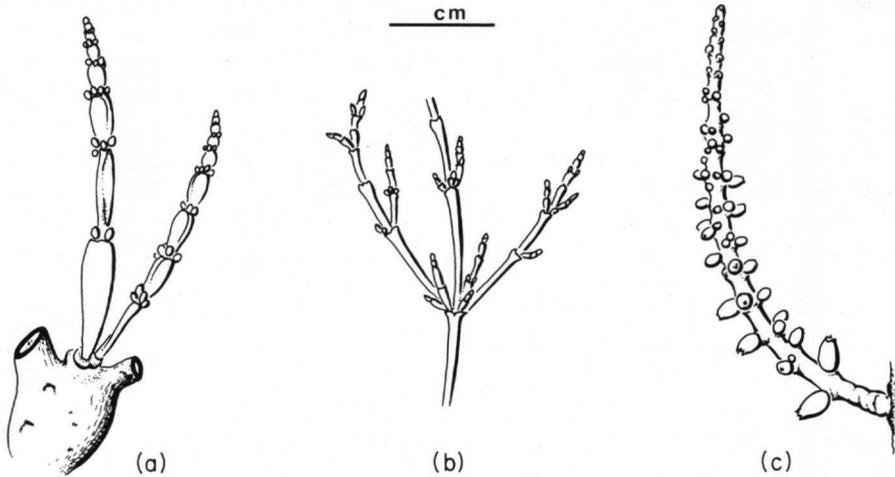


Fig. 1. Habit of young flowering plants of *Korthalsella japonica* (a), *K. dacrydii* (b), and *Phacellaria malayana* (c).

Reichert OM-02 ultramicrotome, placed on copper grids and stained with uranyl acetate and lead citrate. Sections were viewed and photographed with a Hitachi H-600 electron microscope.

For light microscopy, thick sections (1–2 μm) of the material embedded for electron microscopy were cut with glass knives on a Sorvall MT-2 ultramicrotome. Sections were mounted on glass slides, stained with 1% toluidine blue in a 1% aqueous solution of sodium borate, and viewed and photographed with a Zeiss light microscope.

RESULTS

All three species investigated are small, at most sparsely branched plants emerging directly from the host bark (Fig. 1). *K. japonica*, the largest of the three, can reach 10 cm in length, but our material was little more than half that size. *Phacellaria malayana*, in our locality, was approximately the same size. *K. dacrydii*, while rarely reaching 9 cm in length, is more commonly only a couple of centimetres long even when it first begins to flower. The flattened stem of *K. japonica* contrasts with the terete one of *K. dacrydii*, and clearly represents an adaptation to photosynthetic efficiency.

Korthalsella. Notwithstanding considerable differences in stem size, no differences in phloem structure have been noted between the two *Korthalsella* species studied. In both cases, well differentiated sieve-tube elements are present in association with the xylem. Figure 2a shows two major collateral bundles of *K. japonicum*. At the ultrastructural level, sieve-tube members are recognized by their conspicuous sieve plates (Figs 2c and 3b), masses of fibrillar or tubular P-protein particularly in and near the sieve pores, less dense cytoplasm and persistent endoplasmic reticulum, mitochondria, and plastids occupying a parietal position (Figs 2b, 3c, 3d). Nuclei are absent in fully differentiated sieve-tube members. The walls of the sieve-tube elements are thick. In contrast to the usual situation in sieve-tube elements (Evert 1984), the tonoplast in *Korthalsella* sieve-tube members often seems to persist at least in part (Figs 2d and 3d). The adjacent companion cells have very large, prominently heterochromatic nuclei, extremely small vacuoles,

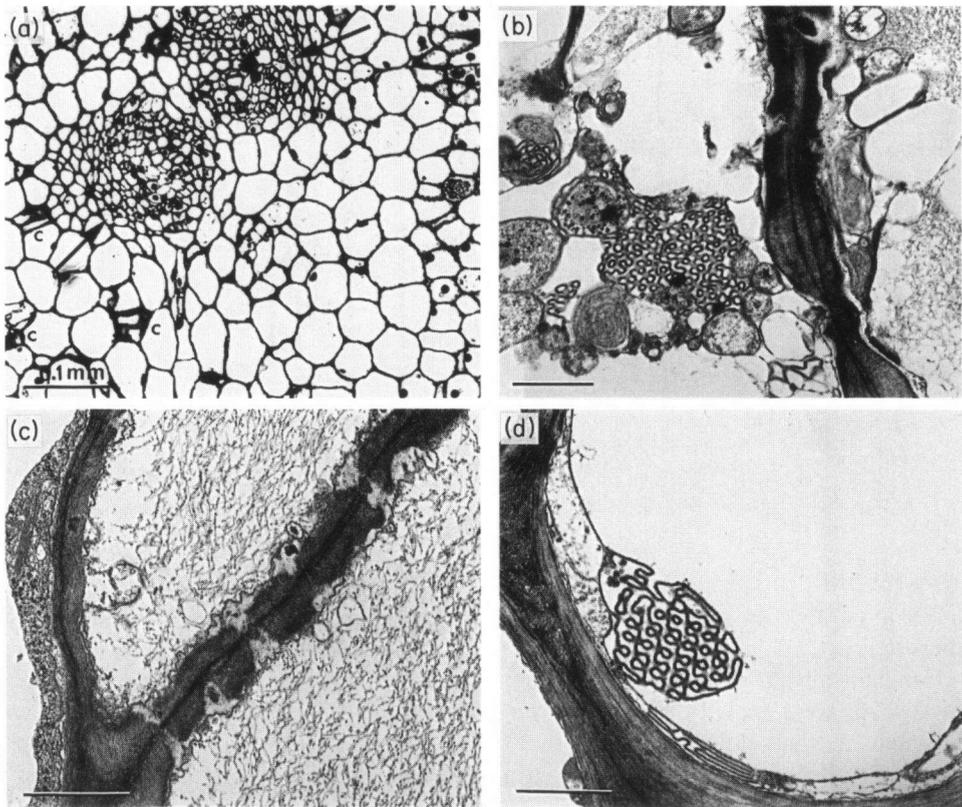


Fig. 2. *Korthalsella japonica*. The bars indicate 1 μ m. (a) Cross section of portion of mature stem showing two large vascular bundles and thick-walled crystalliferous cells = c. Arrows indicate phloem areas. (b) Longitudinal section of sieve plate between two sieve-tube members showing areas with convoluted ER complex. (c) Mature sieve plate. (d) Cross section of sieve-tube member with parallel and convoluted forms of ER.

numerous mitochondria, and dense cytoplasm (Figs 3a and d). Plastids in companion cells usually have rather few thylakoid membranes but contain numerous electron-dense plastoglobuli (Fig. 3d), thus resembling the sieve-tube plastids of *Viscum minimum* (Olson & Kuijt 1985). A noteworthy feature is the fact that the plasmodesmic connections between sieve-tube members and companion cells in *Korthalsella*, in contrast to the standard branched system of plasmodesmata (Esau & Thorsch 1985) known from other plants, seem to be simple (Fig. 3d). We cannot say that this is invariably so in *Korthalsella*, however.

***Phacellaria*.** The vascular system of the *Phacellaria* stem consists of a normal eustelic arrangement of an undetermined number of bundles. Small clusters of rather thin-walled sclereids are scattered throughout the otherwise homogeneous cortex, and the vascular bundles themselves contain many tannin-containing cells. In a mature stem, a certain amount of secondary xylem is visible (Fig. 4a). This tissue consists of rather narrow tracheary elements with scalariform-reticulate lateral walls; at least some of these elements are vessel members with simple perforation plates. The pith is made up of undifferentiated parenchyma, many cells of which contain tannin-like substances.

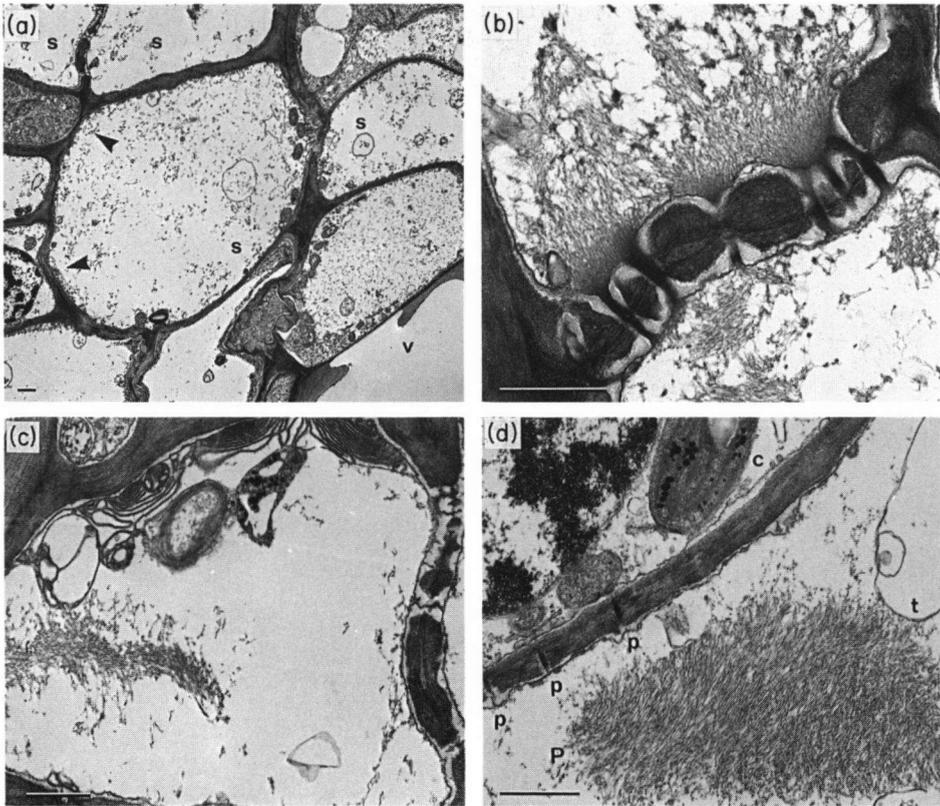


Fig. 3. *Korthalsella dacrydii*. The bars indicate 1 μ m. Cross section of the portion of the stem bundle showing sieve-tube members (s), companion cells (arrows), and vessel member (v). (b) Longitudinal section of sieve plate showing callose (white) and abundant P-protein occluding sieve pores. (c) Transverse section of sieve-tube member showing several arrays of parallel ER. (d) Longitudinal section of sieve-tube member (s) and adjacent companion cell (c). Note the P-protein (P), simple plasmodesmata (p), and persisting tonoplast (t).

External to the xylem of each bundle is a small area occupied by fully differentiated phloem, including sieve-tube members. Sieve-tube members are characterized by conspicuous sieve plates more or less perpendicular to the length direction of the stem (Fig. 4b and c). Sieve pores show a thick cylinder of callose, and P-protein fibrils can be seen to extend outward in the neighboring sieve-tube element. No nucleus, ribosomes or dictyosomes were observed in mature sieve-tube elements, but plastids persist. The endoplasmic reticulum (ER) is again conspicuous, but tends to be of a more irregular, reticular conformation (Fig. 4b and d). In *Phacellaria*, the sieve-tube plastids are clearly of the S-type as designated by Behnke (1981). The plastids have rather few, large starch bodies with electron-light centres (Fig. 4b and d).

DISCUSSION

The most important finding in our study is the fact that normal, fully differentiated sieve-tube elements are characteristic of the phloem of *Korthalsella* and *Phacellaria*. This is true even for the smallest known species of *Korthalsella*, *K. dacrydii*, which is significantly

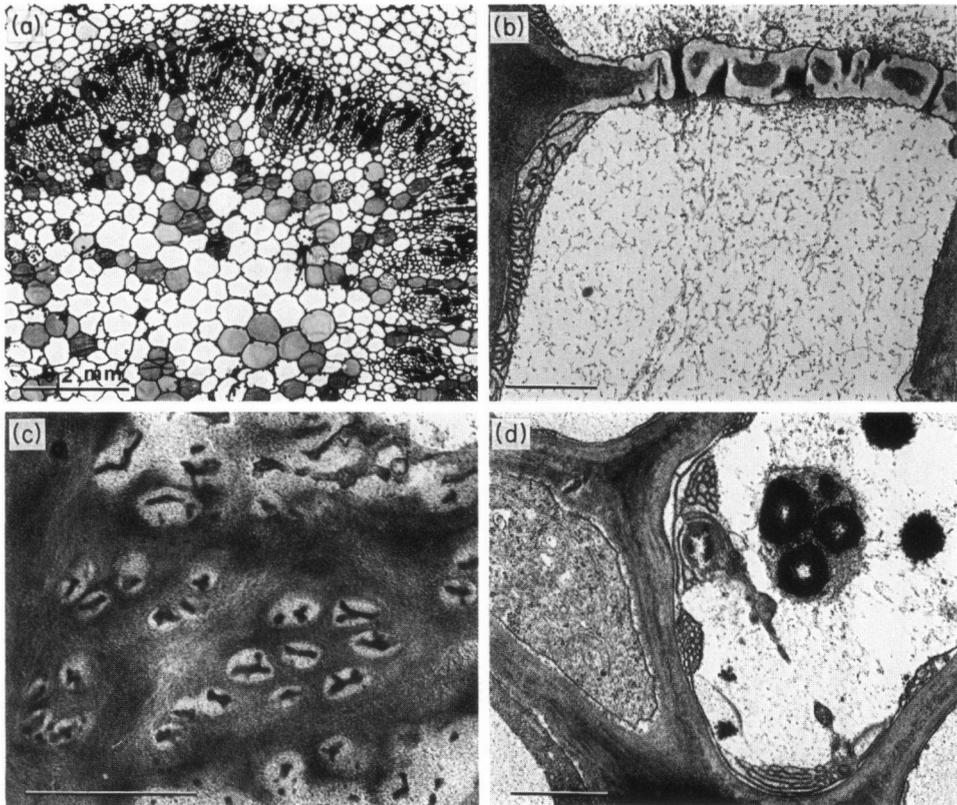


Fig. 4. *Phacellaria malayana*. The bars indicate 1 μm . (a) Cross section of a mature stem showing evidence of secondary growth, with tannin-containing cells in the pith. (b) Longitudinal section of the sieve plate between two sieve-tube members, showing abundant callose (white), P-protein occluding sieve pores, and peripheral ER arrays. (c) Sieve plate in parallel section, with abundant callose (white), the sieve pores occluded with P-protein. (d) Cross section of sieve-tube member and adjacent companion cell, the former with S-type plastid and peripheral ER arrays.

smaller than both *Arceuthobium douglasii* and *A. tsugense* in which Calvin *et al.* (1984) were unable to discover such cells. Olson & Kuijt (1985) demonstrated that normal sieve-tube elements are characteristic also for the even smaller, squamate *Viscum minimum*. We are thus justified in concluding that the evolutionary disappearance of conductive phloem cells is a condition probably unique to the genus *Arceuthobium*, and is not necessarily a consequence of a highly reduced morphological status and small size as suggested in earlier publications (Calvin 1967; Calvin *et al.* 1984; Coetzee & Fineran 1987). The nucleated condition of *Korthalsella* sieve elements, mentioned by Touw (1984), could not be confirmed. The only unusual features we have found are the fact that the plasmodesmata that connects sieve tube members with adjoining companion cells are simple instead of the plasmodesmal canal on the side of the companion cell being branched (Esau & Thorsch 1985), and that the tonoplasts of both species of *Korthalsella* seem to persist.

It is of some interest that the exceedingly regular, convoluted ER complexes which are known from especially Ranunculaceae and Papaveraceae (Behnke 1981) are also found in the sieve-tube elements of *K. japonica* (Figs 2b and d). Such complexes consist of tightly stacked and regularly corrugated layers and, while found primarily in sieve elements,

have also been described in nectaries and other tissues in the above two families. The complexes observed for *Korthalsella* are much smaller than, for example, those illustrated in Behnke's Fig. 3, but are undeniably of the same structural character. In addition, the ER may be stacked in flattened bodies against the cell walls, the cisternae being aligned more or less parallel to the latter (Figs 2d and 3c; see also Evert 1984).

The occurrence of S-type plastids in *Phacellaria* sieve-tube members (Fig. 4d) is of some interest, and conforms to the situation in at least four other species of Santalaceae (Behnke 1981). Interestingly enough, all parasitic angiosperms included in Behnke's survey have S-type phloem plastids, including Cuscutaceae, Krameriaceae, Orobanchaceae, Rafflesiaceae, Santalaceae and Visaceae.

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