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FOLIAR SCLEREIDS IN *DIONYSIA* (*PRIMULACEAE*) FROM A PHYLOGENETIC PERSPECTIVE

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Sclereid presence and distribution were studied in leaves from 50 species of *Dionysia* (*Primulaceae*). Of 16 species not previously investigated, 11 were shown to possess sclereids. The sclereids are dermal, terminal or diffused (according to the position in the leaf) and develop from different tissues, and hence are not homologous structures. The presence of different types of sclereids in different species is discussed. Sclereid characters are optimized on a cladogram based on an analysis of three DNA regions. Some clades are associated with certain types of sclereids. Terminal sclereids are most common in Iran, and diffused sclereids in Afghanistan. The evolution of sclereids in the genus is discussed, and a redefinition of what can be called a sclereid in *Dionysia* is presented.

Keywords. *Dionysia*, leaf sclereids, phylogeny, *Primula*, *Primulaceae*, stone cells.

Introduction

The genus *Dionysia* Fenzl comprises 51 species, of which 13 have been discovered since 1976. This genus of tuft- or cushion-forming xerophytic subshrubs has been shown to have evolved within the larger genus *Primula* L., its sister group being *Primula* subgen. *Sphondylia* (Mast *et al.*, 2001; Trift *et al.*, 2002, 2004). A phylogenetic study of *Dionysia* based on three DNA regions, morphology and biogeography has recently been undertaken (Trift *et al.*, 2004). The resulting cladograms indicated that the sections and subsections of *Dionysia* used in previous divisions of the genus (Wendelbo, 1961, 1964) are not monophyletic. Trift *et al.* (2004) found that many character states were size dependent. More specifically, characters like scape and petiole were reduced or absent in small species. The need for more size-independent morphological characters was noted in the conclusion. The results of Bokhari & Wendelbo (1976, 1985) and Bokhari (1988) showed that the presence of leaf sclereids was one such potential character.

Leaf sclereids in angiosperms can be described according to their position in the leaf, their ontogeny, or their shape (Foster, 1949). The many forms of sclereid found in plants have induced a rich and varied terminology. This study attempts to follow the terminology of Dickison (2000), which is representative of those adopted by most authors. Sclereids are referred to as being either dermal, terminal or diffused depending on their position in the leaf. Dermal sclereids are found in the epidermis;

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terminal sclereids are found at the ends of the veins, and diffused sclereids are found dispersed in the mesophyll. The origins of sclereids are related to their position in mature leaves. Sclereids with terminal positions normally originate from the tracheary elements, but they can in some cases originate from the parenchyma and become positioned next to the vein ends during leaf ontogeny (Rao, 1951). This only applies to some terminal sclereids; there is no indication that dermal sclereids can develop from anything but epidermal cells, and diffused sclereids from anything but parenchymatic cells (Rao, 1951). When sclereids develop from different tissues, they cannot be considered homologous. However, they form in response to a common factor, hormonal or otherwise regulatory, that directs the development of the differentiating cells in all plant tissues. Bokhari & Wendelbo (1976, 1985) and Bokhari (1988) found dermal, terminal and diffused sclereids in different species, and it is interesting to see that a relatively small genus such as *Dionysia* possesses all three kinds. In Bokhari & Wendelbo (1985) the xeromorphic features in *Dionysia* were described in detail, and they noted that sclerenchyma in leaves is thought to protect against damage from wilting. They made the following observations on sclerenchyma:

I. Dermal sclereids in *Dionysia* were strongly sclerified epidermal cells, long and narrow as opposed to the normal barrel-shaped, thin-walled cells. They were always of the macrosclereid type when described according to shape. Dermal sclereids were found only on the exposed side of the leaves.

II. Terminal sclerenchyma in *Dionysia* were of three types: polymorphic sclereids, fibrous caps, and tracheoids.

a. Terminal polymorphic sclereids were not confined to the ends of the veins, but also found along the veins. These sclereids varied considerably in size, form and degree of branching, and could accompany the entire vascular tissue.
b. Fibrous caps (consisting of groups of fibres) were located on the adaxial side of the veins in the upper half of the leaves.
c. Terminal tracheoids were described as idioblasts, resembling tracheids in their pitted walls, but differing from typical tracheary elements in their form (shorter and wider), size (larger) and position. Tracheoids are assumed to have different functions from typical tracheary elements and in other genera they are typically not found in association with veins (Bokhari & Burtt, 1970). There were significant differences between the tracheoids described from *Cyrtandra* (*Gesneriaceae*) (Bokhari & Burtt, 1970) and those later described from *Dionysia* (Bokhari & Wendelbo, 1976, 1985; Bokhari, 1988).

III. Diffused sclereids in *Dionysia* were found dispersed singly or in groups in the leaf mesophyll. Six types of diffused sclereid were described in *Dionysia*. Some of these types occurred together in the same leaf, but none of them was very common. Each type was found only in one to three species.

a. Brachysclereids: short isodiametric cells resembling parenchyma cells.
b. Fusiform sclereids: elongated and pointed at the ends.
c. Filiform sclereids: very long and narrow, showing very intrusive growth.
d. Macrosclereids: somewhat elongated cells, roughly cylindrical, with uneven secondary walls.
e. Polymorphic sclereids: bent, curved or branched, of all sizes.
f. Vesiculose sclereids: little different in shape from neighbouring parenchyma cells and not showing intrusive growth.

Of the 51 *Dionysia* species, 16 were not included by Bokhari & Wendelbo (1976, 1985) or Bokhari (1988), either due to lack of material (*D. hissarica*, *D. involucrata*, *D. kossinskyi*), or because they were not described at that time (*D. aubrietioides*, *D. bazoftica*, *D. iranica*, *D. khatamii*, *D. khuzistanica*, *D. lurorum*, *D. mozaffarianii*, *D. sarvestanica*, *D*. sp. nov. 1, *D*. sp. nov. 2, *D*. sp. nov. 3, *D*. sp. nov. 4, *D*. sp. nov. 5).

When the sclereid character states from Bokhari & Wendelbo (1976) and Bokhari (1988) were plotted on the cladogram based on three DNA regions (Trift *et al.*, 2004), terminal and dermal leaf sclereids (not terminal tracheoids) appeared to have evolved late in the history of the genus and only in western species from Iran and Iraq. Diffused leaf sclereids appeared only in species growing in Afghanistan and Pakistan. Species with peripheral distributions, growing in Oman, Tadzjikistan, Turkmenistan and Turkey, either lacked sclereids or had not been investigated. Thus, the absence or presence of leaf sclereids was a potentially informative character that could merit another investigation. A new study with new samples could also increase the knowledge on infraspecific variation.

The aim of this study was to investigate the presence of leaf sclereids in *Dionysia*, particularly in species that were not available to Bokhari and Wendelbo, and to compare the distribution of each type with recent DNA-based hypotheses of the phylogeny of the genus.

Material

Dry leaves (herbarium material or silica dried) were used. Dickison (2000) noted that sclereids originate late in the ontogeny of the leaf, and fully developed sclereids are found only in mature leaves. The best way to make sure that all sclereids were fully developed was to use the wilted, remaining leaves from previous seasons that are found on the older parts of most *Dionysia* species. Only one species was not included in the present study, due to lack of material (*D. lacei*). With four exceptions, only one specimen from each species was examined. The voucher numbers are given in Table 1, which also shows the tentative division of the genus from Trift *et al.* (2004).

Methods

In their study Bokhari & Wendelbo (1976) referred to the earlier study by Bokhari & Burtt (1970) for a description of the methods, which in turn referred to Bokhari's

TABLE 1. *Dionysia* species, in the order of the tentative new classification of the genus (Trift *et al.*, 2004), with voucher numbers for sampled specimens

Species	Voucher number, comments
CLADE A	
D. balsamea Wendelbo & Rech.f.	No 21767, 919111 (E)
D. hissarica Lipsky	JJH 918037, *Halda* 92, 960605, Gothenburg Bot. g. cult.
CLADE B	
Southern and eastern species, clade C	
D. mira Wendelbo	2002-02-01, leg. Reginald Victor (S)
D. denticulata Wendelbo	1999-1320f, Gothenburg Bot. g. cult.
D. tapetodes Bunge	MK 8809/1, Gothenburg Bot. g. cult. (no sclereids) and *Hewer* 1164, Gothenburg Bot. g. cult. (fili, fusi) and T4Z 1086-1, Gothenburg Bot. g. cult. (brac, macr, fili, fusi)
D. kossinskyi Czerniak.	*Gaudan* 29.04.1912, V. Lipsky (LE)
D. lindbergii Wendelbo	*Grey-Wilson & Hewer* 1304, 30.06.1971 (GB)
D. microphylla Wendelbo	*Trift* 57, 1999-04-19 (S)
D. involucrata Zapr.	*Trift* 201 (S)
D. hedgei Wendelbo	*Grey-Wilson & Hewer* 836, 12.05.1971 (GB)
D. viscidula Wendelbo	*Grey-Wilson & Hewer* 1305, Gothenburg Bot. g. cult.
D. afghanica Grey-Wilson	2003-893, Gothenburg Bot. g. cult. and Kammerlander Czdenek Zvolaner MK 2003-884, Gothenburg Bot. g. cult. Hybrid individual
D. freitagii Wendelbo	*Grey-Wilson & Hewer* 8479, Gothenburg Bot. g. cult.
Southern and eastern species, part clade E	
D. paradoxa Wendelbo	*Wendelbo* 744, Gothenburg Bot. g. cult. (no sclereids) and *Grey-Wilson & Hewer* 1026 (E) (macr, poly) (photo)
D. lacei Clay	Not examined
D. saponacea Wendelbo & Rech.f.	*Rechinger* 19092 (S)
Western species, clade F	
D. archibaldii Wendelbo	*Archibald* 3010, 05.08.1966 (GB)
D. esfandiarii Wendelbo	SLIZE 259:4, Gothenburg Bot. g. cult.
D. sp. nov. 1	T4Z 35 (UPS)
D. revoluta subsp. *revoluta* Boiss.	SLIZE 252, Gothenburg Bot. g. cult.
D. oreodoxa Bornm.	*Zschummel* 300-6 (UPS)
D. rhaptodes Bunge	*Wendelbo & Foroughi* 15880, 12.04.1975 (GB)
D. teucrioides Davis & Wendelbo	*Trift* 200 (S)
D. bornmülleri (Pax) Clay	*Davis* 42833, 1966-05-11, 919118 (E)
D. leucotricha Bornm.	SLIZE 289, Gothenburg Bot. g. cult.
D. aretioides (Lehm.) Boiss.	*Trift* 56, 1999-04-19 (S) and SLIZE 35, Gothenburg Bot. g. cult.

TABLE 1. (*Cont'd*).

Species	Voucher number, comments
D. khatamii Mozaff.	T4Z 13 (UPS)
D. janthina Bornm. & Wink.	19932488 (E)
D. curviflora Bunge	SLIZE 42:268 (UPS)
Western species, clade G	
D. lurorum Wendelbo	*Zschummel* 00-40-3, Gothenburg Bot. g. cult.
D. aubrietioides Jamzad & Mozaff.	T4Z 133 (UPS)
D. iransharii Wendelbo	*K.H. Rechinger* 47433 (S)
D. iranica Jamzad	T4Z 119 (UPS)
D. khuzistanica Jamzad	SLIZE 176:4, Gothenburg Bot. g. cult.
D. zagrica Grey-Wilson	*Hewer* 2023 (GB)
D. caespitosa (Duby) Boiss.	*Zschummel* 300-14 (UPS)
D. mozaffarianii Lidén	SLIZE 232:8, Gothenburg Bot. g. cult.
D. odora Fenzl	99-0713-1, Gothenburg Bot. g. cult.
D. gaube Bornm.	*Archibald* 1633 (GB)
D. haussknechtii Bornm. & Strauss	SLIZE 322:2, Gothenburg Bot. g. cult.
D. lamingtonii Stapf	*Hewer* H1909, 13.04.1973 (GB)
D. sp. nov. 2	*Zschummel* 300-18 (UPS)
D. sawyeri (Watt) Wendelbo	*Alexeenko* 2722 (LE) (*D. bachtiarica* cotype Bornm. et al. ex.)
D. termeana Wendelbo	*Hewer* H1987, 08.05.1973 (UPS)
D. bryoides Boiss.	SLIZE 236:2, Gothenburg Bot. g. cult.
D. diapensiifolia Boiss.	SLIZE 253:2, Gothenburg Bot. g. cult. (photo) and *Wendelbo & Fouroghi* 17548, 30.05.1975 (TARI)
D. sarvestanica Jamzad & Grey-Wilson	SLIZE 241:3, Gothenburg Bot. g. cult.
D. michauxii (Duby) Boiss.	SLIZE 254:3, Gothenburg Bot. g. cult.
D. sp. nov. 3	T4Z 125, isotype (UPS)
D. sp. nov. 4	*Wendelbo & Assadi* 16761, holotype (TARI)
D. sp. nov. 5	*Dehgani* 5466, holotype (TARI)
Primula* subgen. *Sphondylia	
P. verticillata Forssk.	*Trift* 202, 04.09.2002, cult. (S)
P. simensis Hochst.	*St Andrews* 1997-0923, Gothenburg Bot. g. cult.
P. floribunda Wall.	*Trift* 1999-04-19 (S)

study on *Limonium* leaves (Bokhari, 1970). In our study we made no leaf macerations or transections. The method for leaf clearing and staining was slightly modified from that of Bokhari (1970). Leaves were placed in glass dishes with 10% KOH solution overnight. The KOH solution was then replaced with water that was exchanged for fresh water three times in 1 hour. The water was then replaced with 4.4% sodium hypochlorite (a common household bleach) and the leaves soaked until they were transparent. The bleaching time varied with the size and texture of the leaves from 20 minutes to 5 hours. Once the leaves were bleached, they were boiled in water for 3 minutes. This process loosened the epidermis. The boiled leaves were

placed in a drop of water on a microscope slide and the epidermis was peeled off. The peeled leaves were stained with safranin in water. After 5 minutes, excess safranin was removed with tissue paper, and the leaves were washed with water and then with 95% ethanol. This was done by adding the washing liquid with a pipette to the slide surface, and then carefully removing it with tissue paper. The leaves were then dehydrated with 99.5% ethanol in the same manner. To make a permanent slide, a few drops of euparal were placed on the peeled, stained leaves and topped with a cover slide, the edges of which were sealed with varnish. The prepared slides were photographed with a digital camera mounted on a light microscope.

When removing the epidermis care was taken not to tear off the edges of the leaf mesophyll, which adhere more strongly to the epidermis than do the flat sides. This was especially important for leaves with strongly revolute leaf margins. Large, soft leaves were the most difficult to prepare and could fall apart completely if not treated gently. The presence of large numbers of sclereids generally made the leaves easy to prepare as they stabilized the tissue. The powdery farina present on the leaf surface in some species can be confused with sclereids as it loosens during preparation, but this problem is easily avoided if the farina is examined separately before the leaves are prepared. When measuring sclereid bundles the maximum width was taken at the mid vein, and the smaller measurement on the largest side vein towards the tip of the leaf. There were always much smaller bundles on veinlets that were not measured.

Sclereid characters were plotted onto a cladogram from Trift *et al.* (2004) using MacClade software (Maddison & Maddison, 1992) (Fig. 1). This cladogram was the result of a Bayesian inference analysis of the combined sequence data from three DNA regions: *rps*16, *trn*L–*trn*F and ITS. The cladogram was based on an analysis of sequences from 39 *Dionysia* species; hence 12 species were not included. These were assigned hypothetical positions based on biogeography and their affinities according to previous classifications based on morphology (Grey-Wilson, 1989). These have been drawn with dotted lines to indicate that their positions are uncertain. The support values for the tree are not included here. Some branches had a support lower than 67%; these are drawn with zigzag lines. All other branches had a support of 74–100%. The sclereid results were also plotted on a distribution map from Trift *et al.* (2004) (Fig. 2).

RESULTS

Dermal (I), terminal (II) and diffused sclereids (III) were found in *Dionysia*, and no sclereids were found in *Primula* subgen. *Sphondylia*. The category 'terminal tracheoids' was not used; instead 'tracheoid' was used as an adjective to describe vein ends resembling those in leaves of *D. lindbergii*. The vein ends were described as plain, thick or tracheoid. We will return to this matter in the discussion. We classified the diffused sclereids into five categories:

a. brachysclereids
b. fusiform sclereids
c. filiform sclereids
d. macrosclereids
e. polymorphic sclereids.

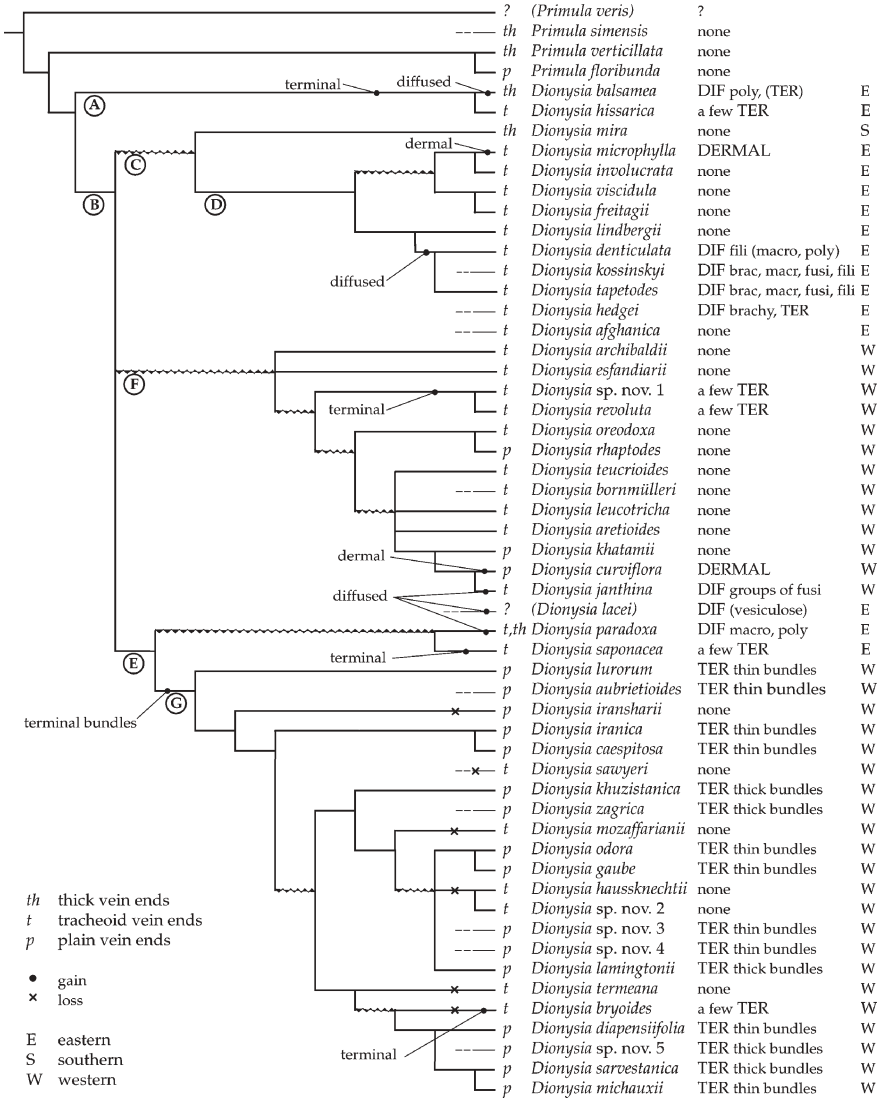

FIG. 1. Sclereid characters plotted on a cladogram taken from Trift *et al.* (2004). Species in parentheses were not included in this study. Sclereid characters in parentheses were not encountered in this study. Zigzag lines indicate clades with a weak support (67% or less in the Bayesian inference analysis). Twelve species were not included in Trift *et al.* (2004) and their positions in the tree are not known. They have been positioned with dotted lines based on their affinities as suggested by their respective authors when first described.

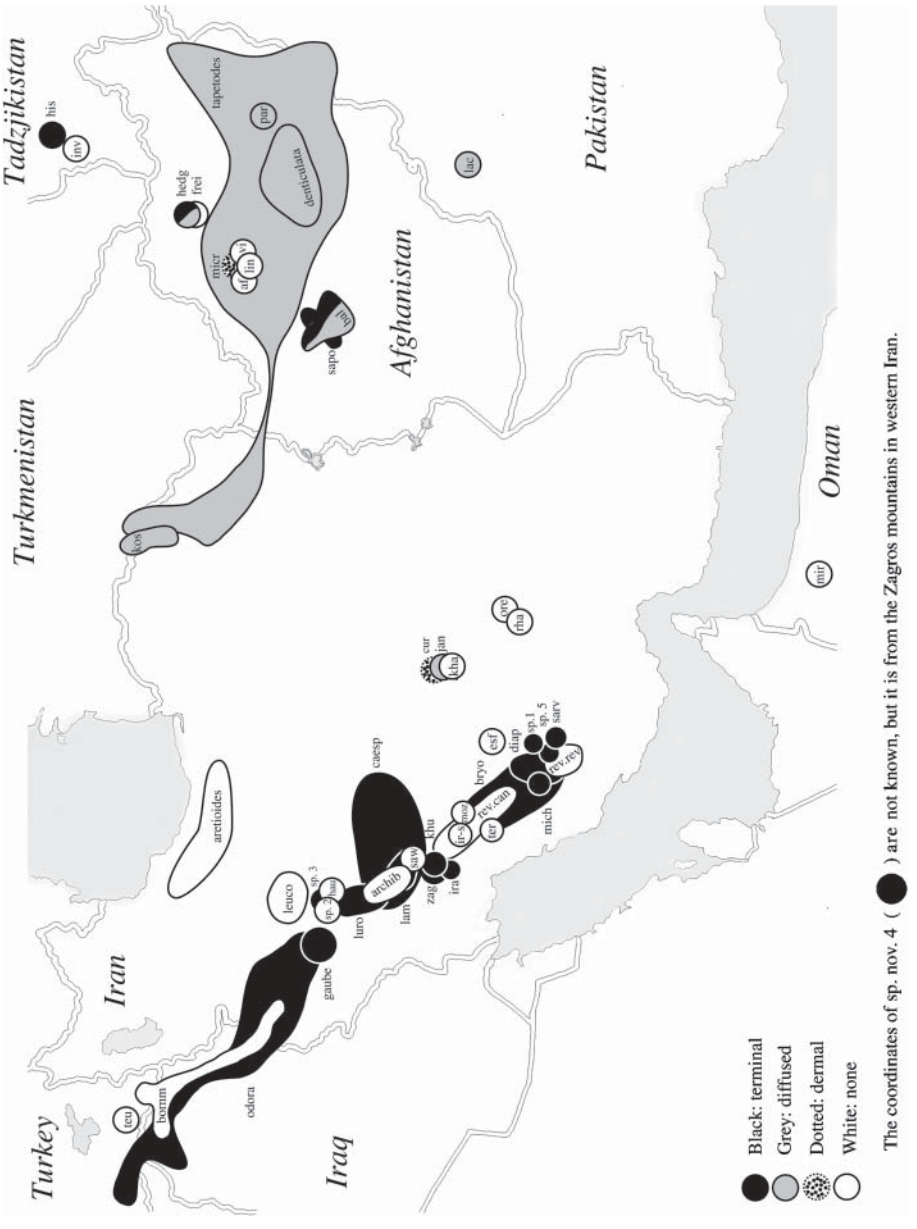

FIG. 2. Sclereid characters plotted on a distribution map taken from Trift *et al.* (2004).

Unlike Bokhari & Wendelbo (1976) and Bokhari (1988), we did not include the category vesiculose sclereids as these were reported only from *D. lacei*, of which we had no material.

I. Dermal sclereids (Figs 3, 4) were found in *D. curviflora* and *D. microphylla*. In both species, sclereids were confined to the distal part of the lower side of the leaves (the exposed part). *Dionysia curviflora* had elongated, thick-walled dermal sclereids, whereas the dermal sclereids in *D. microphylla* were rhomboid, of the same shape as surrounding epidermal cells.

II. Terminal sclereids, found at the ends of veins and along the veins, were found in many species. These sclereids resemble the vessel elements in their initial annular thickening, but are more irregular in shape and have thicker walls. Table 2 lists measurements of bundle width in relation to the width of the leaves. By definition, a thick-walled sclerenchyma cell that is long and has tapered ends and is associated with the vessels is a fibre (Dickison, 2000). Some of the terminal sclereids found are therefore more correctly called fibres. Bokhari & Wendelbo (1985) called this feature fibrous caps.

Species with leaves containing a few terminal sclereids along the veins were *D. bryoides*, *D. hissarica*, *D. revoluta* subsp. *revoluta*, *D. saponacea* and *D.* sp. nov. 1.

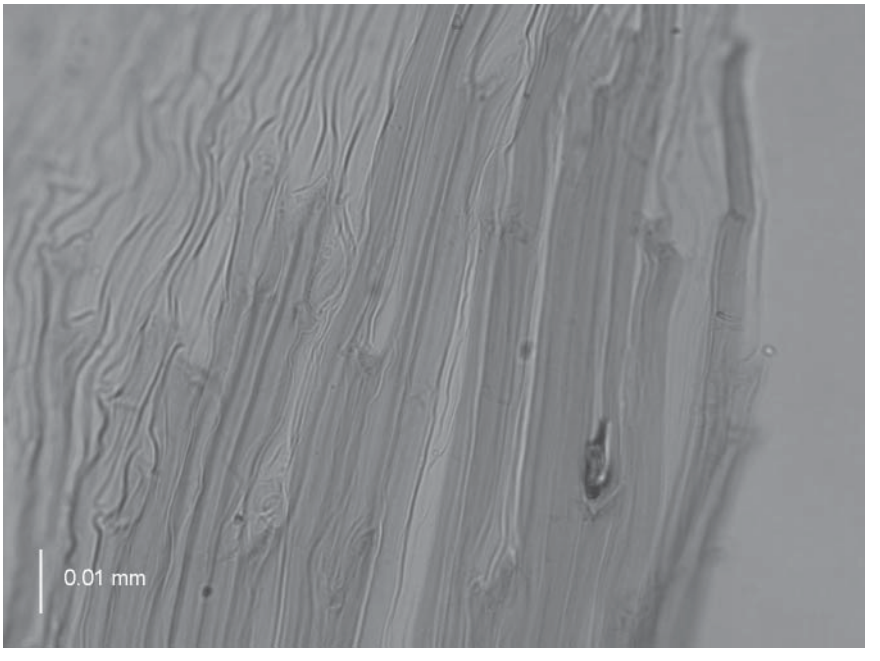

FIG. 3. Elongate dermal sclereids in *Dionysia curviflora*. Pale dermal cells are not sclerified. See Table 1 for accession details for Figs 3–24.

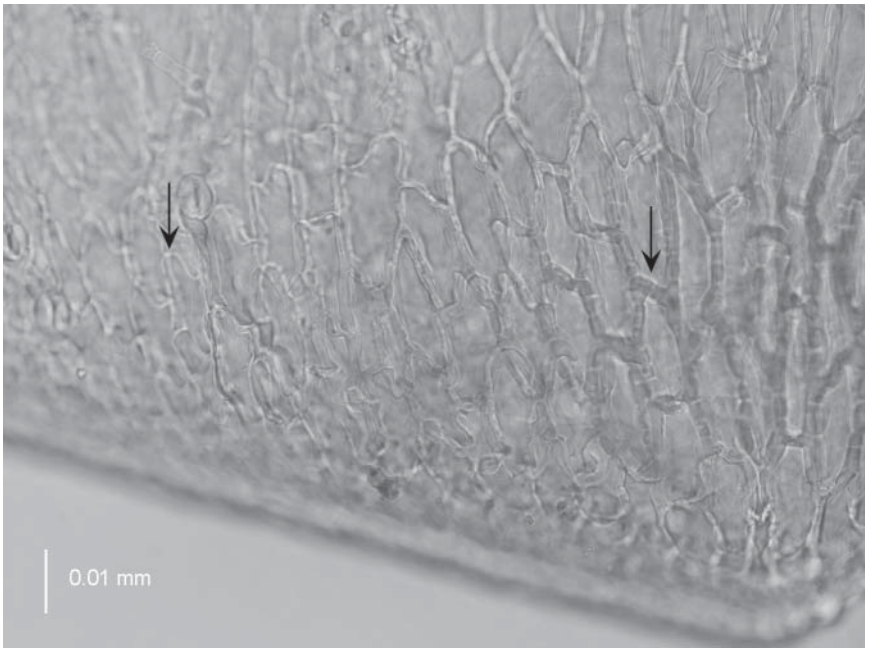

FIG. 4. Dermal sclereids in *Dionysia microphylla*. Note that the epidermal cells above the leaf tip have thicker walls than the cells to the left (arrows).

TABLE 2. Measurements on the width of bundles of terminal sclereids, in order by increasing maximum relative width

Species	Bundle width (mm)	Leaf width (mm)	Relative width of bundle/leaf (%)
D. caespitosa	0.02–0.05	2.0–2.8	0.7–2.3
D. lurorum	0.04–0.06	2.0–3.0	1.3–3.0
D. sp. nov. 5	0.04–0.07	1.6–2.0	2.2–3.9
D. aubrietioides	0.05–0.08	2.0–2.6	1.9–4.0
D. diapensiifolia	0.04–0.09	2.0–2.2	2.0–4.1
D. odora	0.06–0.09	2.0–2.2	2.7–4.1
D. iranica	0.03–0.06	1.2–1.5	2.0–5.0
D. gaube	0.03–0.05	1.0–1.4	2.1–5.0
D. michauxii	0.03–0.06	1.1–1.6	2.1–5.5
D. sp. nov. 3	0.02–0.06	0.7–1.8	2.2–5.7
D. sarvestanica	0.04–0.10	0.9–1.2	3.6–9.1
D. zagrica	0.04–0.10	0.9–1.4	3.6–10.0
D. sp. nov. 4	0.04–0.10	0.7–1.0	4.0–10.0
D. khuzistanica	0.07–0.19	0.8–1.5	6.6–13.8
D. lamingtonii	0.06–0.11	0.5–1.3	4.6–22.0

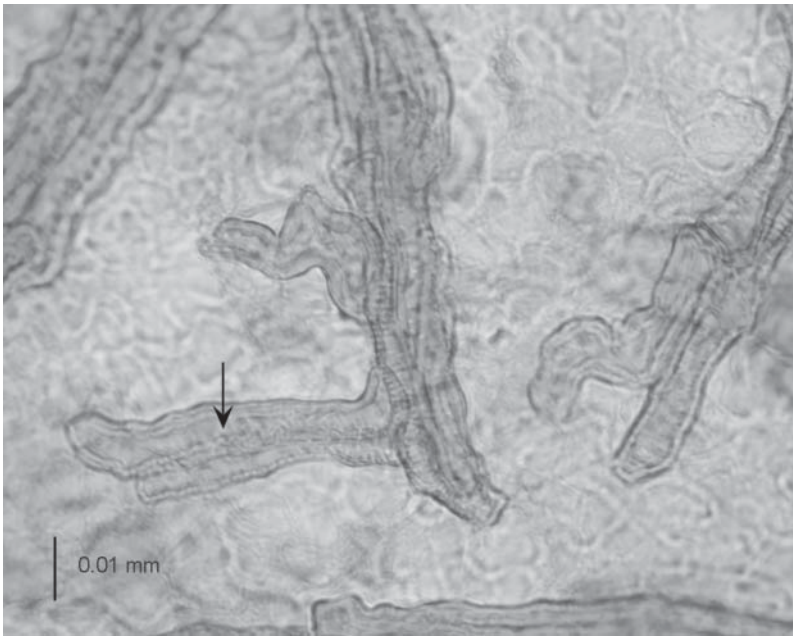

FIG. 5. Terminal sclereids in *Dionysia diapensiifolia*. Note that one sclereid shows annular thickening (arrow) while others have uniform walls.

Species with leaves containing terminal sclereids forming thin bundles (with a maximum width less than 6% of the width of the leaf) along the veins (Figs 5, 6) were *D. aubrietioides*, *D. caespitosa*, *D. diapensiifolia*, *D. gaube*, *D. iranica*, *D. lurorum*, *D. michauxii*, *D. odora*, *D.* sp. nov. 3 and *D.* sp. nov. 4. The sclereids in *D. diapensiifolia* were the most varied in shape, often bent or branched (Fig. 5). Compared with the sclereids in species with thick bundles, these sclereids were shorter.

Species with leaves containing terminal sclereids forming thick bundles (with a maximum width more than 9% of the width of the leaf) along the veins (Figs 7, 8) were *D. khuzistanica*, *D. lamingtonii*, *D. sarvestanica*, *D.* sp. nov. 5 and *D. zagrica*. In *D.* sp. nov. 5, the mid-vein bundle was unusually thick towards the tip of the leaf.

In *D. hedgei*, filiform sclereids were found along the main vein towards the base of the leaves (Figs 9, 10). They had blunt ends but were still close to fibres in shape.

III. Diffused sclereids, dispersed singly or in groups in the mesophyll, were not as common as terminal sclereids. No type was found in more than three of our sampled species. Sclereids dispersed singly were more common than sclereids in groups.

a. Brachysclereids were found dispersed singly only. There were many small (c.0.005 mm) spherical brachysclereids in the mesophyll in the leaves of *D. hedgei* (Fig. 11) and some brachysclereids at the base of the leaves in one sample of *D. tapetodes*.

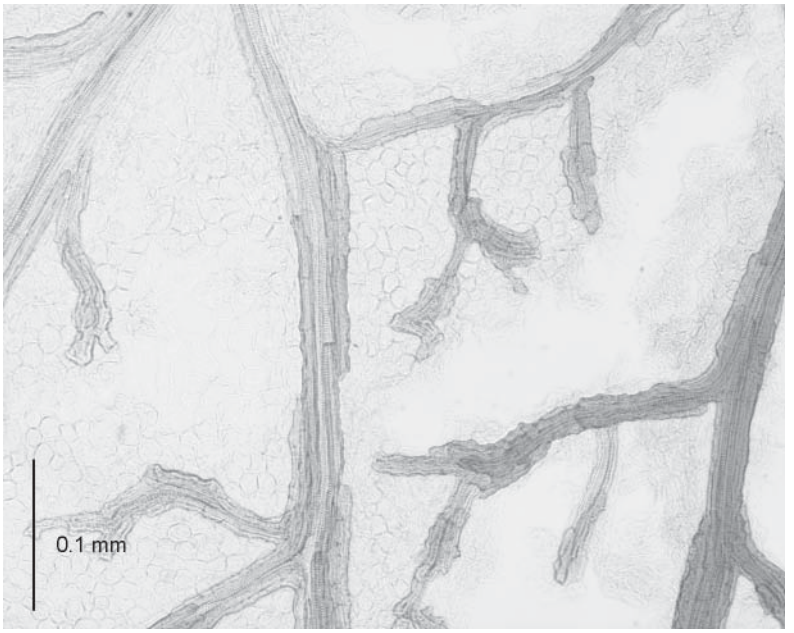

FIG. 6. Terminal sclereids forming thin bundles along the veins in *Dionysia odora*.

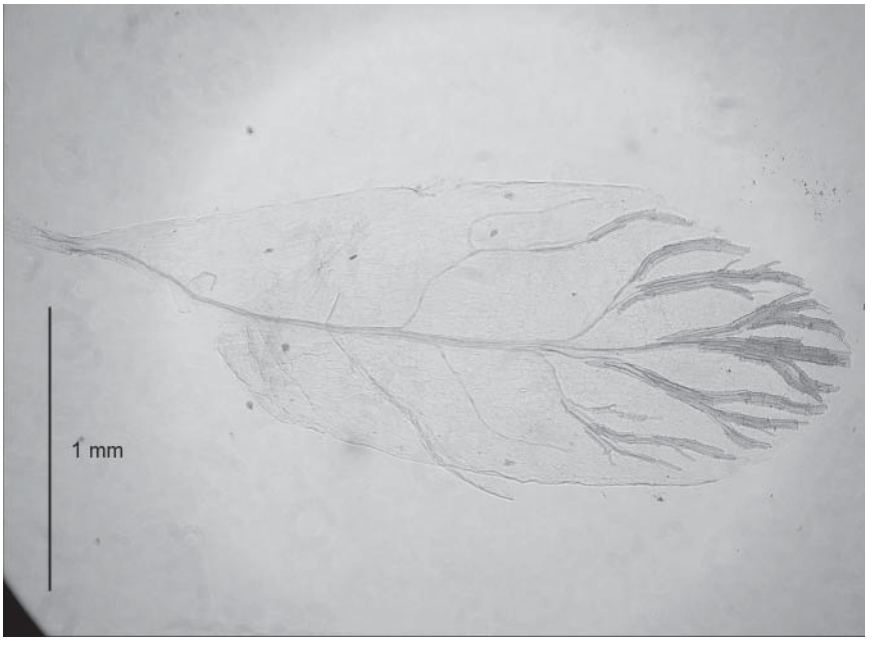

FIG. 7. A whole cleared leaf of *Dionysia sarvestanica*.

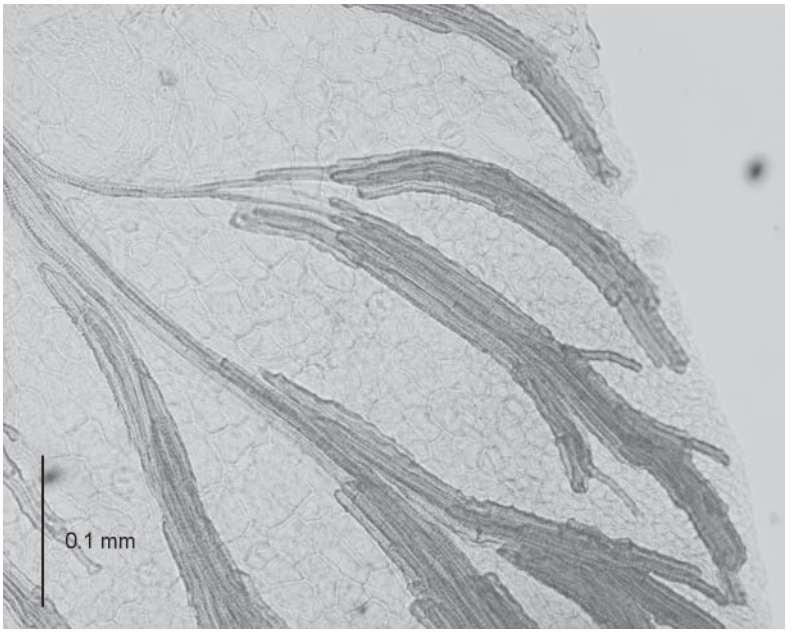

FIG. 8. Terminal sclereids forming thick bundles/fibrous caps along the veins in *Dionysia sarvestanica*.

b. Fusiform sclereids were found dispersed singly and in groups. In *D. kossinskyi* (Figs 12–14) and *D. tapetodes* the fusiform sclereids were common between the veins in the middle portion of the leaves. The leaves of *D. janthina* contained large groups of fusiform sclereids in the mesophyll, towards the base of the leaf (Figs 15–17). The groups were round and consisted of 10–20 small sclereids packed closely together. Some leaves had only one group, others up to seven groups.
c. Filiform sclereids were found dispersed singly and in groups. In *D. denticulata* they were found parallel to the long axis and not to the veins (Fig. 18). Some of them had forked ends and could be as long as 0.7 mm, 20% of the total length of the leaves. *Dionysia kossinskyi* and *D. tapetodes* had filiform sclereids in the mesophyll towards the edge of the leaf, singly and in groups between (not in contact with) the large veins, running more or less in parallel with these (Figs 13, 14).
d. Macrosclereids were found dispersed singly and in groups. In *D. kossinskyi* and one sample of *D. tapetodes* wide macrosclereids were found at the leaf base (Figs 12, 14). *Dionysia paradoxa* contained slender macrosclereids in groups of two to five in the mesophyll (Figs 19, 20). These macrosclereids were sometimes bent and branched, and c.0.1 mm long.

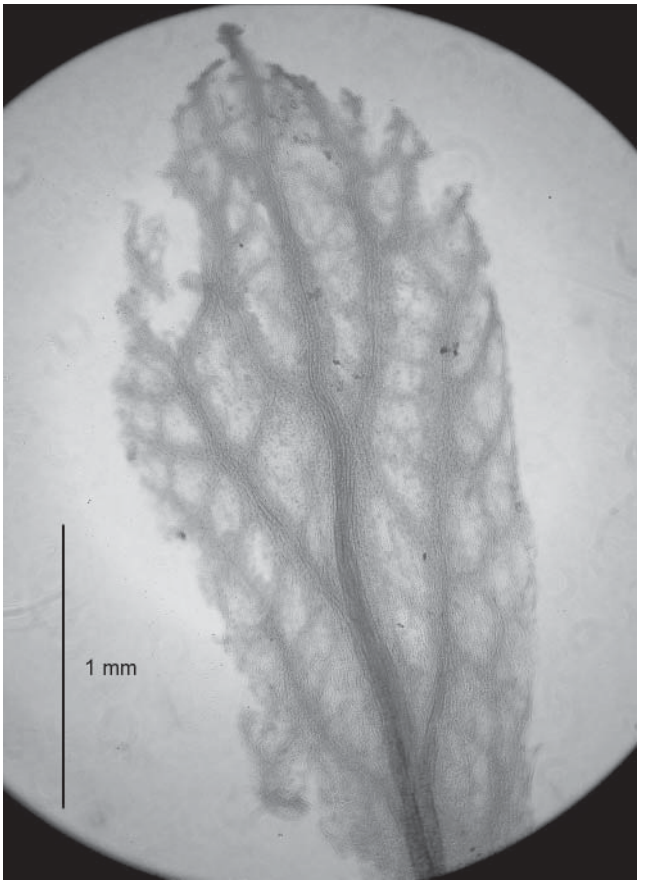

FIG. 9. A whole cleared leaf of *Dionysia hedgei*.

e. Polymorphic sclereids in the mesophyll were found dispersed singly, sometimes touching each other but these were not considered to be groups. Two leaves from two individuals of *D. paradoxa* were examined. One contained no sclereids, probably because it was too young; the other contained plenty of branched polymorphic sclereids in the mesophyll (Fig. 20). *Dionysia balsamea* contained similar large polymorphic unbranched sclereids in the mesophyll. Unlike in the terminal sclereids, the thickening of the walls in diffused polymorphic sclereids was never annular.

Tracheoid vein ends (Figs 21, 22) were found in *D. lindbergii*, and also in *D. archibaldii*, *D. aretioides*, *D. bornmülleri*, *D. bryoides*, *D. denticulata*, *D. esfandiarii*, *D. freitagii*, *D. haussknechtii*, *D. hedgei*, *D. hissarica*, *D. involucrata*, *D. janthina*, *D. kossinskyi*, *D. leucotricha* (where the tracheoid ends were smaller than in other species), *D. microphylla*, *D. mozaffarianii*, *D. oreodoxa*, *D. paradoxa*, *D. revoluta*

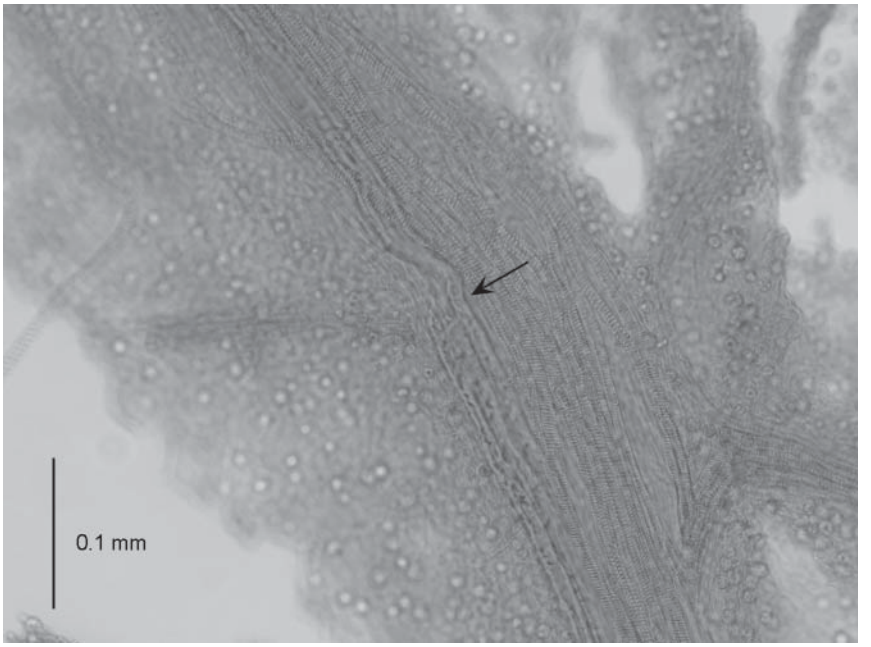

FIG. 10. Terminal sclereid or fibre (arrow) in association with a large vein in *Dionysia hedgei*.

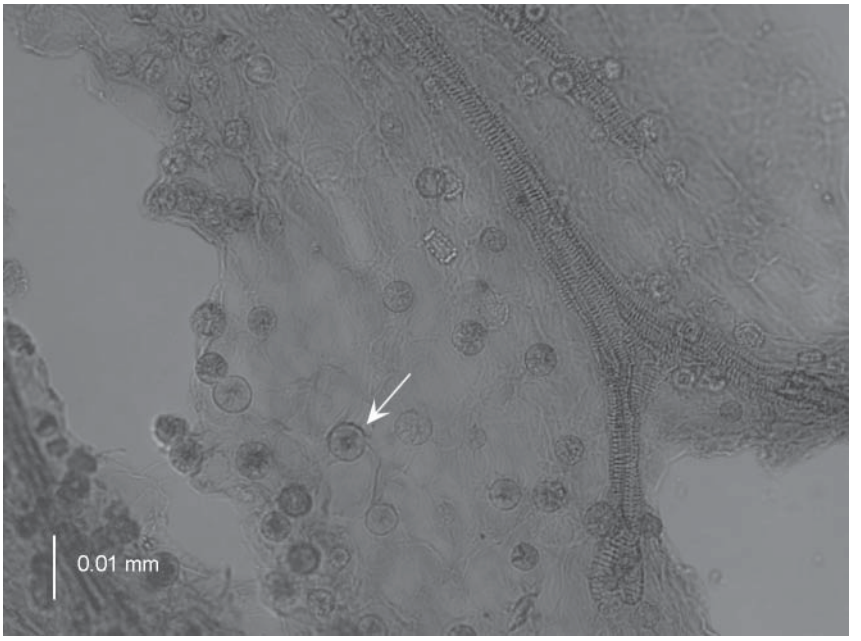

FIG. 11. Diffused spherical brachysclereids (arrow) in *Dionysia hedgei*. Tracheoid vein ends at the right.

FIG. 12. Two cleared leaves of *Dionysia kossinskyi*, one small and one large.

subsp. *revoluta*, *D. saponacea*, *D. sawyeri*, *D.* sp. nov 1, *D.* sp. nov. 2, *D. tapetodes*, *D. termeana*, *D. teucrioides* and *D. viscidula*. Tracheoid vein ends had cells that were wider, shorter and of a more irregular shape than the other vessel elements.

Plain vein ends (Fig. 23) were found in very different situations. One species with plain vein ends (*Primula floribunda*) had very large leaves (5 cm long), but more often they were found in tiny leaves (2–10 mm long) with thin veins (*D. curviflora*, *D. iransharii*, *D. khatamii* and *D. rhaptodes*). They were also found in all species where the veins were sheathed by sclereids/fibrous caps (see above): *D. aubrietioides*, *D. caespitosa*, *D. diapensiifolia*, *D. gaube*, *D. iranica*, *D. khuzistanica*, *D. lamingtonii*, *D. lurorum*, *D. michauxii*, *D. odora*, *D. sarvestanica*, *D.* sp. nov. 3, *D.* sp. nov. 4, *D.* sp. nov. 5 and *D. zagrica*.

Thick vein ends (Fig. 24) were found only in large leaves: *Primula simensis*, *P. verticillata*, *Dionysia balsamea*, *D. mira* and *D. paradoxa*.

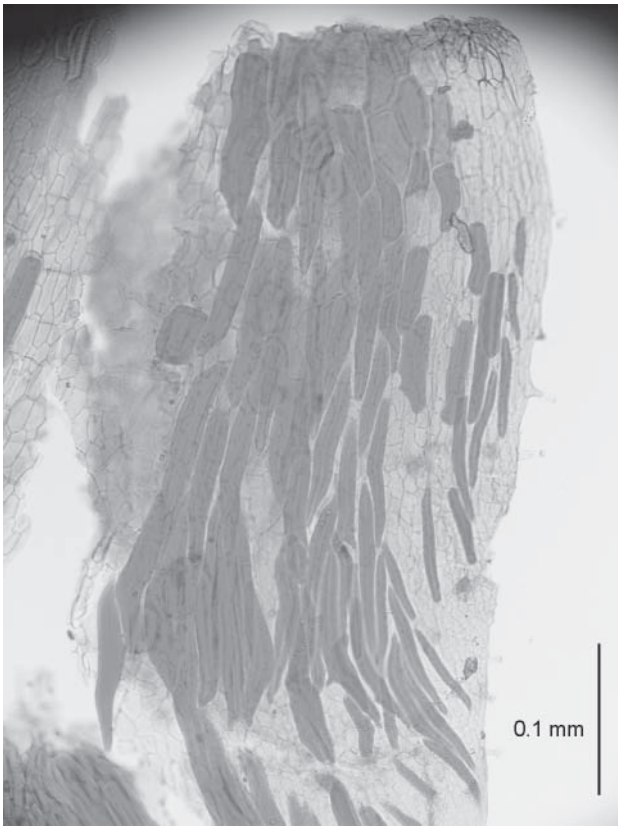

FIG. 13. Diffused macrosclereids and fusiform sclereids in *Dionysia kossinskyi*.

Dionysia lacei was not examined in our study. Bokhari & Wendelbo (1976) reported vesiculose sclereids scattered singly in the mesophyll of *D. lacei*. Bokhari & Wendelbo reported filiform sclereids, straight macrosclereids and polymorphic sclereids in the mesophyll from *D. denticulata*, whereas we found only filiform sclereids. We included these previously reported types when plotting the sclereid characters on the cladogram (Fig. 1).

DISCUSSION

The optimization of sclereid characters on the cladogram (Fig. 1) corroborated the hypothesis that dermal, diffused and terminal sclereids are not homologous structures, as one type never evolved into another. A secondary loss of sclereids was common. When one type of sclereid was lost, it was never replaced by sclereids of a different type. Two types, terminal and diffused sclereids, occurred together only in *D. hedgei* and *D. balsamea.* Note that tracheoid vein ends were not considered to

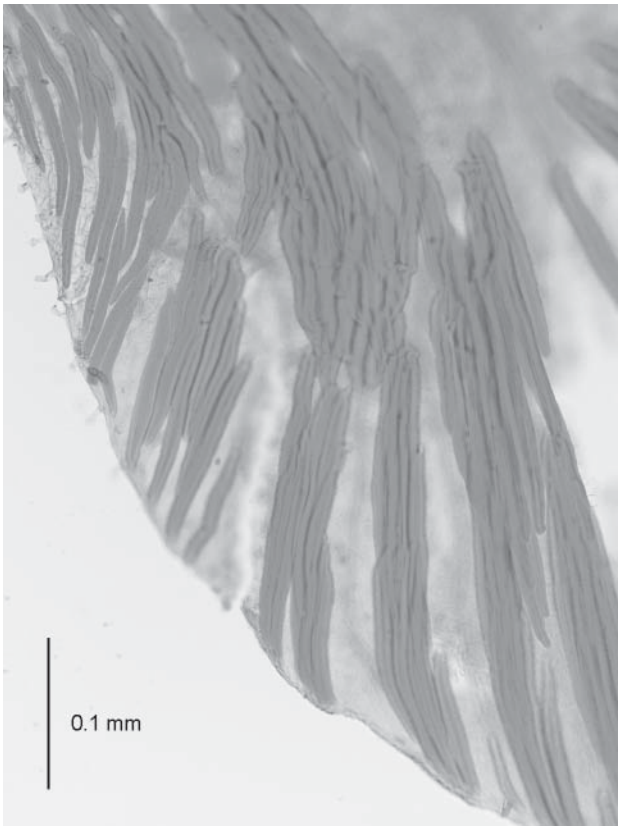

FIG. 14. Diffused fusiform and filiform sclereids in groups in *Dionysia kossinskyi*.

be sclereids (see below). Even closely related species, such as *D. janthina*/*D. curviflora* or *D. balsamea*/*D. hissarica*, had different types of sclereids. We could not see any correlation between xeromorphic habit and presence of sclereids. Some of the species without sclereids were very xeromorphic in growth habit, hairs, stomatal grooves or cuticle (e.g. *D. rhaptodes*, *D. afghanica*) and some species with sclereids were not particularly xeromorphic (e.g. *D. paradoxa*, *D. balsamea*) (Bokhari & Wendelbo, 1985).

I. Dermal sclereids. Dermal sclereids were previously reported from *D. curviflora*. We also saw a tendency towards sclerified epidermal cells in *D. microphylla*. Bokhari (1988) classified the latter as cuticularized and cutinized epidermal cells. Even while preparing the leaves of *D. microphylla* the hard epidermis was evident, as the peeled epidermis would not lie flat unless pressed under a cover slide. The walls of the dermal sclereids in *D. microphylla* were not as thick as those in *D. curviflora*, and the sclerified area was smaller. The cells in *D. microphylla* still had the same shape as

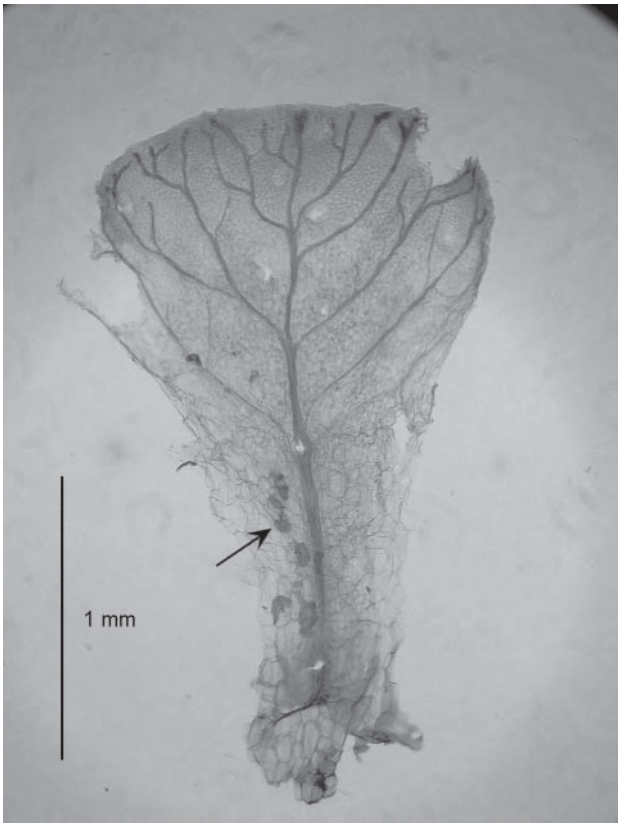

FIG. 15. A whole cleared leaf of *Dionysia janthina* showing position of sclereids (arrow).

surrounding dermal cells and had not reached the same high degree of specialization. Considering the differences between the two, it was not surprising to see that dermal sclereids appeared to have evolved in parallel in these two taxa.

II. Terminal sclereids. Terminal sclereids are initially annular but become uniformly thickened as they mature (Fig. 5). In species with large terminal sclereids, the walls of the sclereids can be very thick and the lumen reduced. Species with a few terminal sclereids along the veins appeared in three clades in the genus, which indicates that this character state is a parallelism.

There was no qualitative difference between a few sclereids along the veins (as in *D. bryoides*, not illustrated), thin bundles (as in *D. odora*) and thick bundles of sclereids/fibrous caps (as in *D. sarvestanica*). These types formed a continuum and the extreme was sclerenchymatic cells that, by definition, are fibres and not sclereids. The thicker the bundles were, the less varied were the shapes of the terminal sclereids. The really thick bundles consisted of straight, tapered cells. Bundles were found only in clade G and their presence was a good anatomical character for this group.

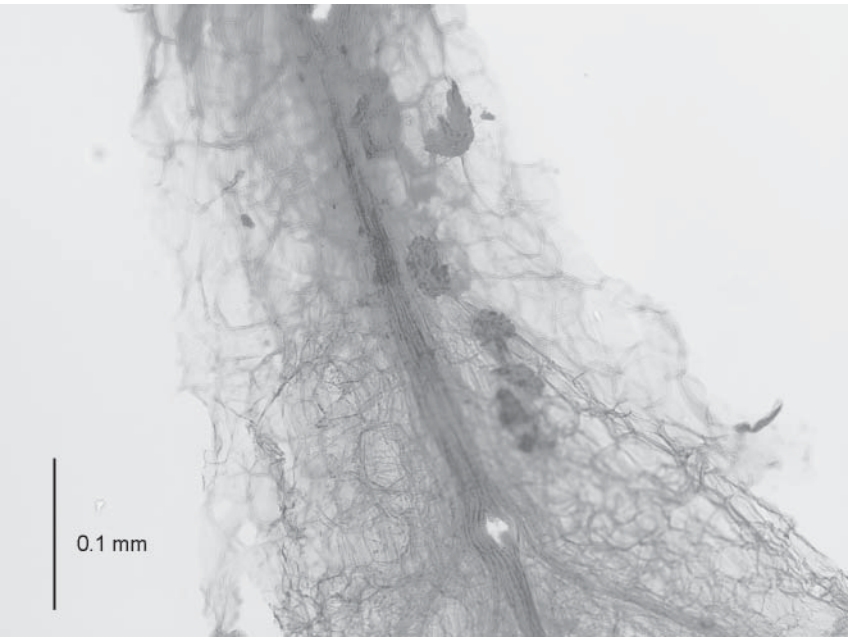

FIG. 16. Diffused groups of fusiform sclereids in *Dionysia janthina*.

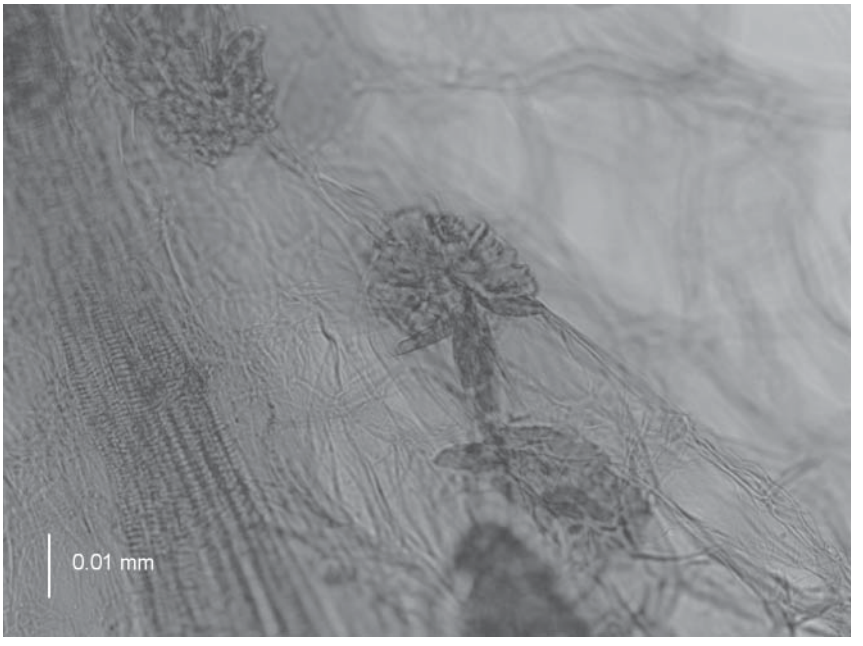

FIG. 17. Diffused groups of fusiform sclereids in *Dionysia janthina* at larger magnification.

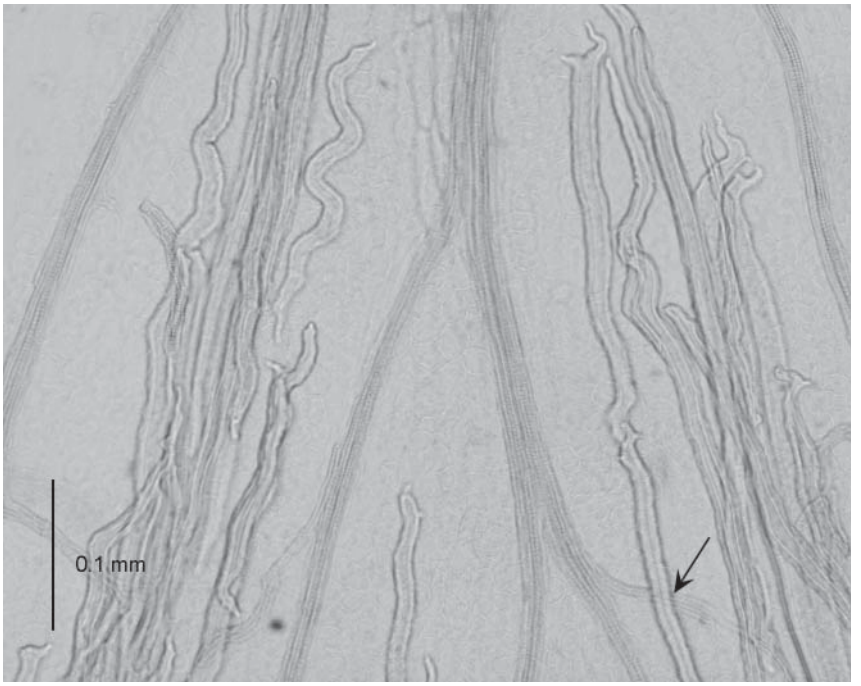

FIG. 18. Diffused filiform sclereids in *Dionysia denticulata*. Note that they cross veins (arrow).

FIG. 19. A pair of diffused macrosclereids in *Dionysia paradoxa*.

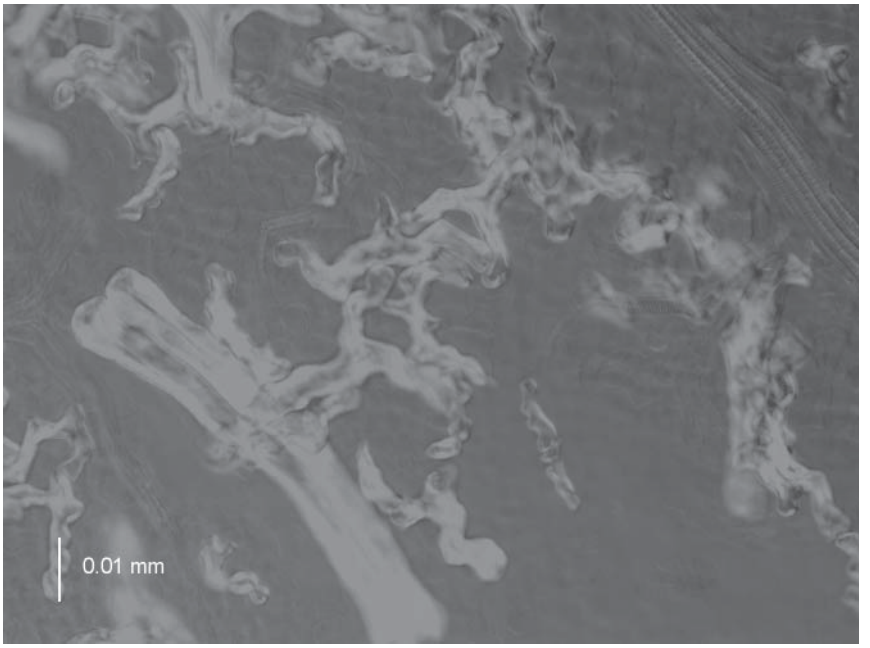

FIG. 20. Diffused polymorphic sclereids and a pair of macrosclereids in *Dionysia paradoxa*.

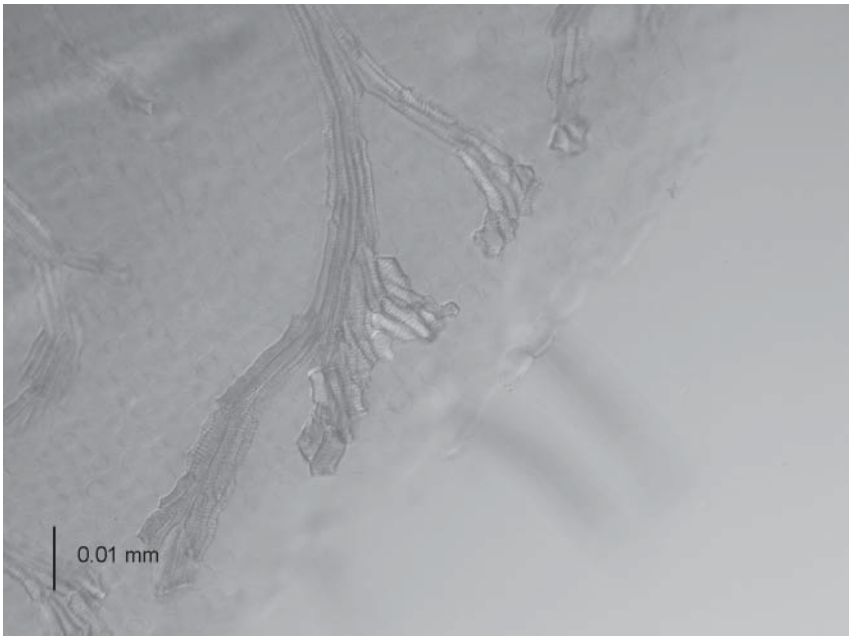

FIG. 21. Tracheoid vein ends in *Dionysia hissarica*.

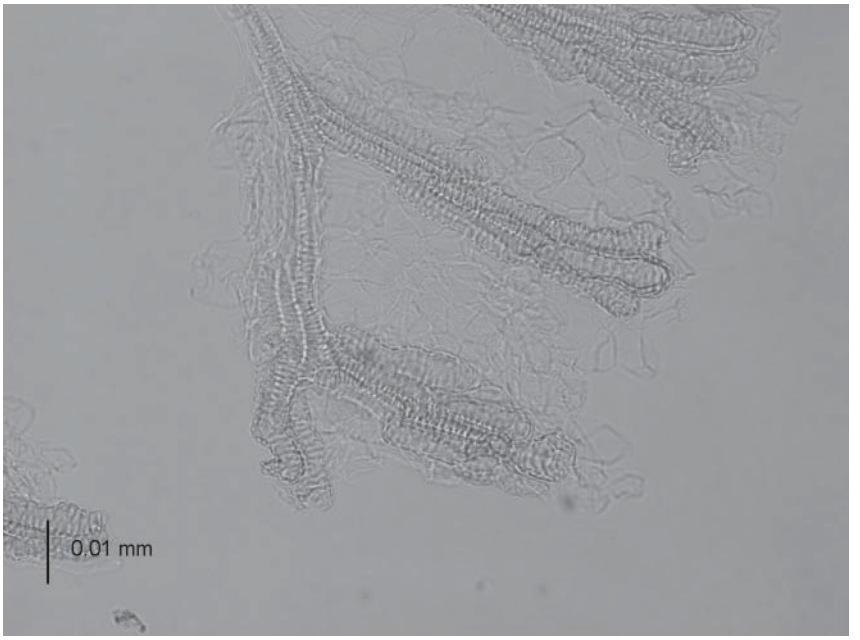

FIG. 22. Tracheoid vein ends in *Dionysia lindbergii*, described as a unique type by Bokhari & Wendelbo (1976).

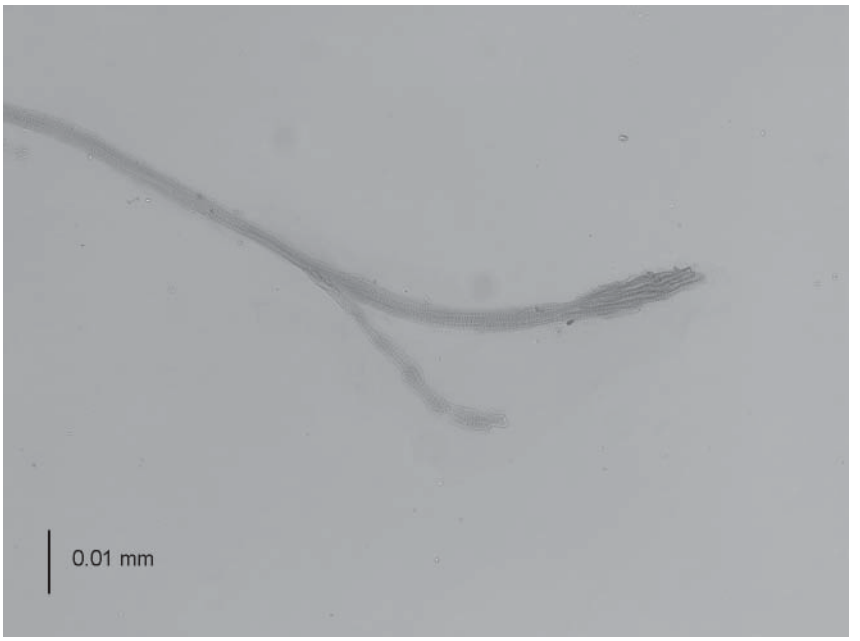

FIG. 23. Plain vein ends in *Dionysia curviflora*.

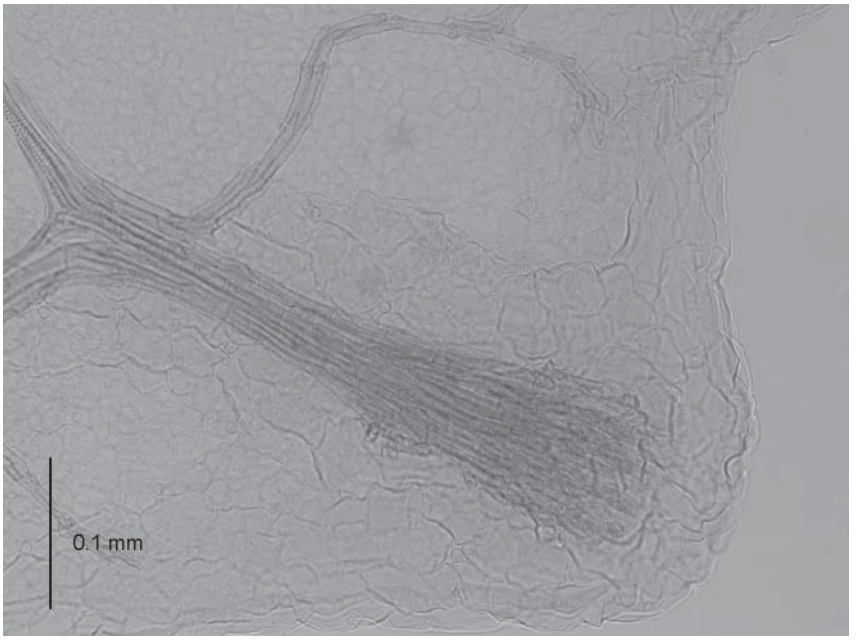

FIG. 24. Thick vein ends in *Primula verticillata*. Note that ends of small veins are not thick.

Dionysia bryoides had a few terminal sclereids along the veins but not enough to form bundles. Twelve species had bundles; seven appeared to have lost them. The fact that *D. lurorum* has bundles supports its position as part of clade G. This was useful since it has few other morphological characters in common with the rest of the clade and the relationship only appeared in one of the analyses by Trift *et al.* (2004). A secondary loss of bundles was correlated with regaining tracheoid vein ends. The distribution of thick and thin bundles, respectively, was not coupled to particular monophyletic groups within clade G. These character states have either evolved several times, or are a result of environmental factors. Considering that *D. lurorum* and *D. iransharii* have thin bundles it seems reasonable to consider thick ones a derived state, but with several reversions.

Filiform sclereids were found in *D. hedgei*, along the largest vein of the leaf (Figs 9, 10). They were concentrated towards the base of the largest vein and not towards the ends of the vein. The ends of these cells are slightly blunt, and the shape is sometimes a little bent, which makes the sclereids in *D. hedgei* more similar to the diffused filiform sclereids in *D. denticulata* than to fibres.

III. Diffused sclereids

a. Diffused brachysclereids were found in *D. hedgei*, *D. kossinskyi* and *D. tapetodes* (and previously reported from *D. trinervia*, now a synonym of *D. tapetodes*). Since brachysclereids have the shape of unspecialized parenchyma cells, they

would naturally be similar even when they result from parallel evolution. The brachysclereids in *D. hedgei* were of the same size and shape in the whole leaf and not part of a continuum of shapes as in *D. kossinskyi* and *D. tapetodes*.

b. *Dionysia janthina* was found to have round sclereid aggregates composed of small fusiform sclereids in the mesophyll at the base of the leaves. This type was not found anywhere else, and was not reported by Bokhari & Wendelbo (1976, 1985) or Bokhari (1988). When searching for this type, it was important to make sure the basal part of the leaf was not left on the stem. As an autapomorphy in this species it provided no useful phylogenetic information. Fusiform sclereids in *D. kossinskyi* and *D. tapetodes* were much larger and not in groups. Although all are classified as fusiform these sclereids have nothing in common and we believe they were of independent origins.

c. Diffused filiform sclereids were easy to detect. The sclereids of this type, found in *D. denticulata*, ran throughout the length of the leaf (Fig. 18). They lie parallel to the axis of the leaf, crossing the veins. The filiform sclereids found in *D. kossinskyi* and *D. tapetodes* were shorter and located between the veins, running more in parallel with these, and very likely originating from parenchyma cells. Both forms of filiform sclereids were found only in clade D and may be a synapomorphy, but note that four of the species in that clade had only hypothetical positions based on biogeography and relationships suggested by Grey-Wilson (1989) (Fig. 1).

d. Diffused macrosclereids were found in *D. kossinskyi*, *D. tapetodes* and *D. paradoxa*. Bokhari & Wendelbo reported macrosclereids to be present in *D. denticulata*. We could not find this in our sample and the conclusion was that absence of sclereids may depend on the material studied. The macrosclereids in *D. paradoxa* varied in shape, being branched and bent sometimes so much as to resemble polymorphic sclereids. The macrosclereids in *D. kossinskyi* and *D. tapetodes* were located at the base of the leaves and are continuously variable in shape: fusiform in the middle and filiform towards the tip of the leaf. This typical 'tapetodes-type' sclereid pattern was what Bokhari & Wendelbo (1976, 1985) and Bokhari (1988) found in *D. tapetodes* and *D. trinervia* (now a synonym). The *D. tapetodes* species complex deserves study in itself. This species has the widest distribution of all *Dionysia* species (Fig. 2) and there are several possible infraspecific taxa. It may not be a monophyletic species. Perhaps a closer investigation of the sclereid patterns in a large sample of specimens from the entire area, preferably in combination with sequences from a DNA region suitable for infraspecific studies, could yield interesting results. The macrosclereids present in clade C and in *D. paradoxa* appeared to be of independent origin according to the cladogram but this could also be considered another argument for the existence of a southern/eastern group as suggested in Trift *et al.* (2004).

e. Diffused polymorphic sclereids were found in *D. paradoxa* and *D. balsamea*, two large-leaved species, considered by some to be close relatives (Grey-Wilson, 1989). Their close relationship was not supported in our analyses of molecular

data (Trift *et al.*, 2004). Diffused polymorphic sclereids were also reported from *D. denticulata* by Bokhari & Wendelbo (1976), resulting in the character state being present in three different clades (A, D and E). Even though they are most likely to have evolved independently, they are remarkably similar.

In a study on the leaf sclereids in the genus *Cyrtandra* (*Gesneriaceae*), Bokhari & Wendelbo (1970) described the tracheoids they observed as 'idioblasts resembling tracheids in their annular thickened or pitted walls, but differing from typical tracheary elements in their form, size and general topography. [. . .] In the majority of the cases the tracheoids are more or less isolated, occurring either in the middle of the ground tissue or in connection with the vascular system or secretory organs'. There is no doubt that tracheoids are a sclereid type in *Cyrtandra*. However, we could not agree that the cells found at the ends of the veins in *Dionysia* were of that type. The leaves of *D. lindbergii* were described by Bokhari & Wendelbo (1976) as having terminal tracheoids, and this character state was said to be unique to this species of *Dionysia*. Later, in Bokhari & Wendelbo (1985), they were also reported from *D. teucrioides*, *D. saponacea* and in all species of *Dionysia* subsect. *Revolutae*. We found very similar forms to that of *D. lindbergii* at the ends of the veins in many other species (Figs 21, 22). Furthermore, Bokhari & Wendelbo (1976) presented a photograph and a drawing of vein ends in *D. lindbergii*, and the drawing of a cell with pitted walls did not rightly interpret the photograph in which the cells have annular walls. We could not find tracheoids with pitted walls in *D. lindbergii* or any other *Dionysia.* The conclusion is that terminal tracheoids are not a sclereid type in *Dionysia*. In this discussion we use tracheoid as an adjective to describe a type of vein end.

In our study tracheoid vein ends were noted in 29 species scattered throughout the genus. A tracheoid vein end has cells that are noticeably different from the ordinary vessel elements (Figs 21, 22). This type of vein end is not unusual in angiosperms (Dickison, 2000). This character state appeared in all types of leaf but was never present when the veins were sheathed by bundles of sclereids (see above). Plain vein ends (Fig. 23) were found mostly in species with tiny leaves or where the veins had bundles of sclereids around them (except for *P. floribunda* that has large leaves and no bundles). In five species (two of which were from *Primula*) the veins were sufficiently different to be characterized as having thick vein ends. Thick vein ends means that the veins, when approaching the edge of the leaf, are divided laterally and become 10–20 times as thick (Fig. 24). The cells have the shape of ordinary vessel elements and are not notably larger. This character state was noted for *P. verticillata*, *P. simensis*, *D. balsamea*, *D. mira* and *D. paradoxa* that all have large leaves. The character of thick vein ends was shown to be the ancestral state of the genus when the characters were optimized on the tree (Fig. 1).

In one case, Bokhari & Wendelbo (1976) reported sclereids that were not found in our study. In *D. denticulata* they found filiform sclereids, straight macrosclereids and polymorphic sclereids in the mesophyll, whereas we found only filiform sclereids. On

the other hand, a reported absence of sclereids depends on the material studied. We could not say, based on our material, whether this was due to infraspecific variation or to some effect of the environment during growth. Therefore, the interesting question with regard to a specimen is not whether sclereids are absent or present, but if present, then what type of sclereid is found?

Dermal sclereids were found both in a southern/eastern and in a western species (Fig. 2). Terminal bundles, thick and thin, were characteristic of the western clade G. In the other western clade F, the majority of species lacked sclereids (only four out of 13 had them), and therefore absence of sclereids may be the ancestral state of this clade (compare Figs 1 and 2). Diffused sclereids in general (all five types) appeared only in species from the southern/eastern part of the genus. The only exception was *D. janthina*, which grows in central Iran and has a unique type of diffused sclereid. Diffused sclereids were found mostly in the group of southern/eastern species, providing support for our tentative division of the genus (Table 1).

Conclusion

The results indicate that only two types of similar-looking sclereids are the result of parallel evolution, namely 'diffused polymorphic sclereids' and 'a few terminal sclereids', the latter appearing in four different clades in the tree. Bundles of terminal sclereids/fibres are characteristic of the western clade G, whereas sclereids are rare in the western clade F. Diffused sclereids of all types appear only in eastern species, with one exception.

Presence of foliar sclereids is independent of the size of the leaves and of the growth habit. Leaf sclereids provide useful phylogenetic information and should always be taken into consideration when trying to determine the position of a newly discovered species in relation to the rest of the genus *Dionysia*.

Acknowledgements

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