

An anatomical investigation of the stem and leaf of the South African species of *Lycium* L. (Solanaceae)

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The anatomy of the leaf and stem of the eleven South African *Lycium* L. species is described and discussed. Owing to poor differentiation between palisade and spongy parenchyma the leaves are considered to be isobilateral. The most important diagnostic characteristic of the leaves is the trichomes. Short-stalked glandular trichomes with either globular or elongated heads are found on all the species and long-stalked glandular trichomes on *L. pilifolium* C.H. Wr. and *L. hirsutum* Dun. only. A non-glandular multicellular hair type is present on *L. hirsutum* only. Another less important diagnostic characteristic is the presence or absence of collenchyma on the adaxial and/or abaxial side of the main vascular bundle.

No anatomical differences were found between the stems of the species examined. The vascular bundles are bicollateral with a well developed monofacial internal cambium. Phloem fibres are absent. Trichomes, similar to those present on the leaves, occur on the young stems.

S. Afr. J. Bot. 1984, 3: 219–230

Die anatomie van die blaar en stingel van die elf Suid-Afrikaanse *Lycium* L.-spesies word beskryf en bespreek. Die blare word as isobilateraal beskou weens die swak differensiasie tussen die palissadeparenchium en sponsparenchium. Die belangrikste kenmerke met diagnostiese waarde is die trigome, waar kortgesteelde klierhare met óf 'n bolvormige óf 'n verlengde kop by al die spesies aangetref word, asook langgesteelde klierhare by *L. pilifolium* C.H. Wr. en *L. hirsutum* Dun. 'n Nie-klieragtige meersellige haartipe word slegs by *L. hirsutum* aangetref. 'n Ander minder belangrike eienskap is die aan- of afwesigheid van kollenchium aan die adaksiale en/of abaksiale kant van die hoofaar.

Anatomies is daar geen verskil in die bou van stingels van die ondersoekte spesies nie. Die vaatbondels is bikollateraal met 'n goedontwikkelde monofasiale interne kambium. Floëemvesels kom nie voor nie. Trigome op die jong stingels stem ooreen met dié van die blare.

S.-Afr. Tydskr. Plantk. 1984, 3: 219–230

Keywords: Anatomy, leaf, *Lycium*, Solanaceae, South Africa, stem

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Accepted 20 March 1984

Introduction

Lycium L. is one of three indigenous genera of the Solanaceae found in southern Africa. In a taxonomic revision of *Lycium* in southern Africa (SWA/Namibia excluded) Joubert (1981) suggested the reduction of the 38 species described for this region (Dean 1974) to eleven clearly definable species. These species are *L. acutifolium* E. Mey. ex Dun., *L. afrum* L., *L. cinereum* Thunb., *L. echinatum* Dun., *L. ferocissimum* Miers, *L. hirsutum* Dun., *L. horridum* Thunb., *L. oxycarpum* Dun., *L. pilifolium* C.H. Wr., *L. schizocalyx* C.H. Wr. and *L. shawii* Roem. et Schult.

All South African species are densely branched shrubs or dwarf shrubs with stem thorns, measuring up to 70 mm, occurring on the branches. The leaves are simple and clustered, occurring on condensed lateral branchlets and on thorns.

The anatomy of *Lycium* has received little attention in the past. Metcalfe & Chalk (1950) mention certain anatomical aspects of the genus while Fukuda (1967) studied the histogenesis and development of the internal phloem of the Solanaceae using the European *L. chinense* Mill. as the representative of the genus.

Various taxonomic studies (Dunal 1852; Miers 1854; Hitchcock 1932; Joubert 1981) suggest that the floral morphology presents the only constant characteristics for distinguishing between species, because of the considerable variability in external morphology of the vegetative parts. Delimitation as well as identification of species when only vegetative parts are available is, therefore, difficult and often impossible. The anatomy of the South African species was studied in the hope of finding distinctive anatomical characteristics to confirm the distinction between species and to be used as a means of identifying plants when not in flower. Hitchcock (1932) considered the pubescence of the species he studied to be a possible useful characteristic. The trichomes are multicellular in all instances. Those of the South American species are often forked or branched, while short or long-stalked glandular hairs are more commonly found in the species from North America. Owing to the significance attached to the trichomes by Hitchcock (1932), this feature of the South African species was especially noted in this study as a possible distinctive characteristic of vegetative parts.

Materials and Methods

Fresh material collected from a variety of habitats (specimens of Reyneke and of Joubert) was fixed in FAA (Johansen 1940). Five of the species were cultivated under identical conditions in the botanical garden of the University of the Orange Free State. These were *L. cinereum*, *L. echinatum*, *L. ferocissimum*, *L. hirsutum* and *L. horridum*. No fresh material of *L. acutifolium* and *L. shawii* was available.

Material for light microscopy was dehydrated by means of the tertiary butyl alcohol method and embedded in wax. The sections were cut at 15 μm on a rotary microtome, differentially stained in safranin and fast green (Sass 1958) and mounted in Canada balsam.

Material prepared for the scanning electron microscope (SEM) was fixed in 3% glutaraldehyde (0,1 mol dm⁻³ phosphate buffer, pH 7,0), post-fixed in 1% phosphate buffered osmium tetroxide, dehydrated in ethyl alcohol, critical point dried and sputter coated with gold.

Jeffrey's solution (Johansen 1940) was used to prepare macerated material of the stems. The structure of the xylem elements was studied both under the light microscope and SEM. The macerated material prepared for SEM study was washed several times with water, dropped onto a specimen stub and allowed to dry. The material was then coated with gold and examined with an ISI-100 SEM at 20 kV.

The following specimens were used in the anatomical study:

L. afrum:— 3418 (Simonstown): Cape of Good Hope Nature Reserve (-AD), *H.C. Taylor 10321* (STE); *H.C. Taylor 10322* (STE).

L. cinereum:— 2926 (Bloemfontein): Botanical garden, UOFS (-AA), *Joubert 155* (BFLU); *Joubert 156* (BFLU); 3025 (Colesberg): 50 km

WNW of Aliwal North (-BD) *Reyneke 95* (BFLU); 3027 (Lady Grey): 15 km N of Zastron (-AC), *Reyneke 90* (BFLU); *Reyneke 91* (BFLU); 3125 (Steynsburg): 68 km from Venterstad to Steynsburg on gravel road (-BD), *Reyneke 59* (BFLU).

L. echinatum:— 2827 (Senekal): 15 km SE of Winburg (-AC), *Reyneke 120* (BFLU); 2926 (Bloemfontein): Botanical garden, UOFS (-AA) *Joubert 151* (BFLU); 3126 (Queenstown); Just SW of Molteno (-AD), *Reyneke 60* (BFLU); Boesmanshoek Pass, Sterkstroom (-BC), *Reyneke 61* (BFLU); 3226 (Fort Beaufort): Rasfonteinpoort near Cradock (-AB), *Reyneke 63* (BFLU).

L. ferocissimum:— 2926 (Bloemfontein): Botanical garden, UOFS (-AA), *Joubert 153* (BFLU); Rayton (-AA), *Reyneke 134* (BFLU); 3319 (Worcester): Brewelskloof NE of Worcester (-CB), *M.B. Bayer 1531* (NBG); 3418 (Simonstown): Cape of Good Hope Nature Reserve (-AD), *H.C. Taylor 10323* (STE).

L. hirsutum:— 2821 (Upington): Near Groblershoop (-DD), *Reyneke 52* (BFLU); 2925 (Jagersfontein): Near Steunmekaar (-CD), *Reyneke 98* (BFLU); 2926 (Bloemfontein): Botanical garden, UOFS (-AA), *Joubert 150* (BFLU); 40 km S of Bloemfontein (-CA), *Reyneke 107* (BFLU).

L. horridum:— 2925 (Jagersfontein): 40 km SW of Bloemfontein (-DA), *Reyneke 96* (BFLU); 2926 (Bloemfontein): Botanical garden, UOFS (-AA), *Joubert 154* (BFLU); 3025 (Colesberg): 5 km SW of Springfontein (-BC), *Reyneke 114* (BFLU); 3220 (Sutherland): 26 km NW of Sutherland (-BC), *M.B. Bayer 1561* (NBG); 3226 (Fort Beaufort): Bloemhof Farm, Mostertshoek, Tarkastad district (-AC), *Reyneke 62* (BFLU).

L. oxycarpum:— 2924 (Hopetown): Witput Station (-CB), *Joubert 158* (BFLU); 12 km NW of Luckhoff (-DB), *Reyneke 103* (BFLU); 3224 (Graaff-Reinet): 20 km NW of Graaff-Reinet (-BC), *Reyneke 67* (BFLU); 3225 (Somerset East): 15 km W of Cradock (-BA), *Reyneke 64* (BFLU).

L. pilifolium:— 2824 (Kimberley): Kimberley to Barkly West (-DA), *Joubert 159* (BFLU); 2924 (Hopetown): Rooipan-Belmont vicinity (-DA), *Joubert 157* (BFLU).

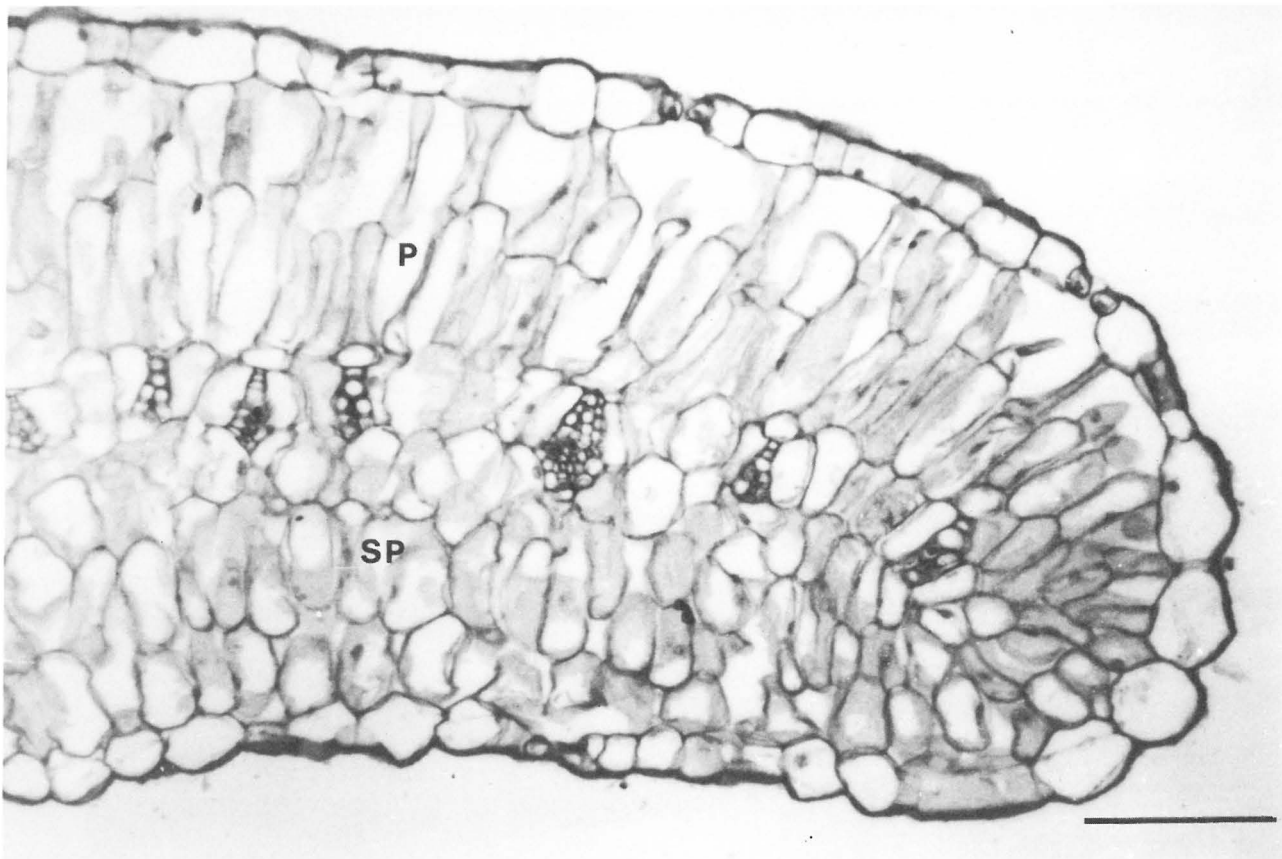


Figure 1 Transection of part of a leaf of *L. horridum* showing the palisade (P) and spongy parenchyma (SP). Bar = 100 μm . (Reyneke 62).

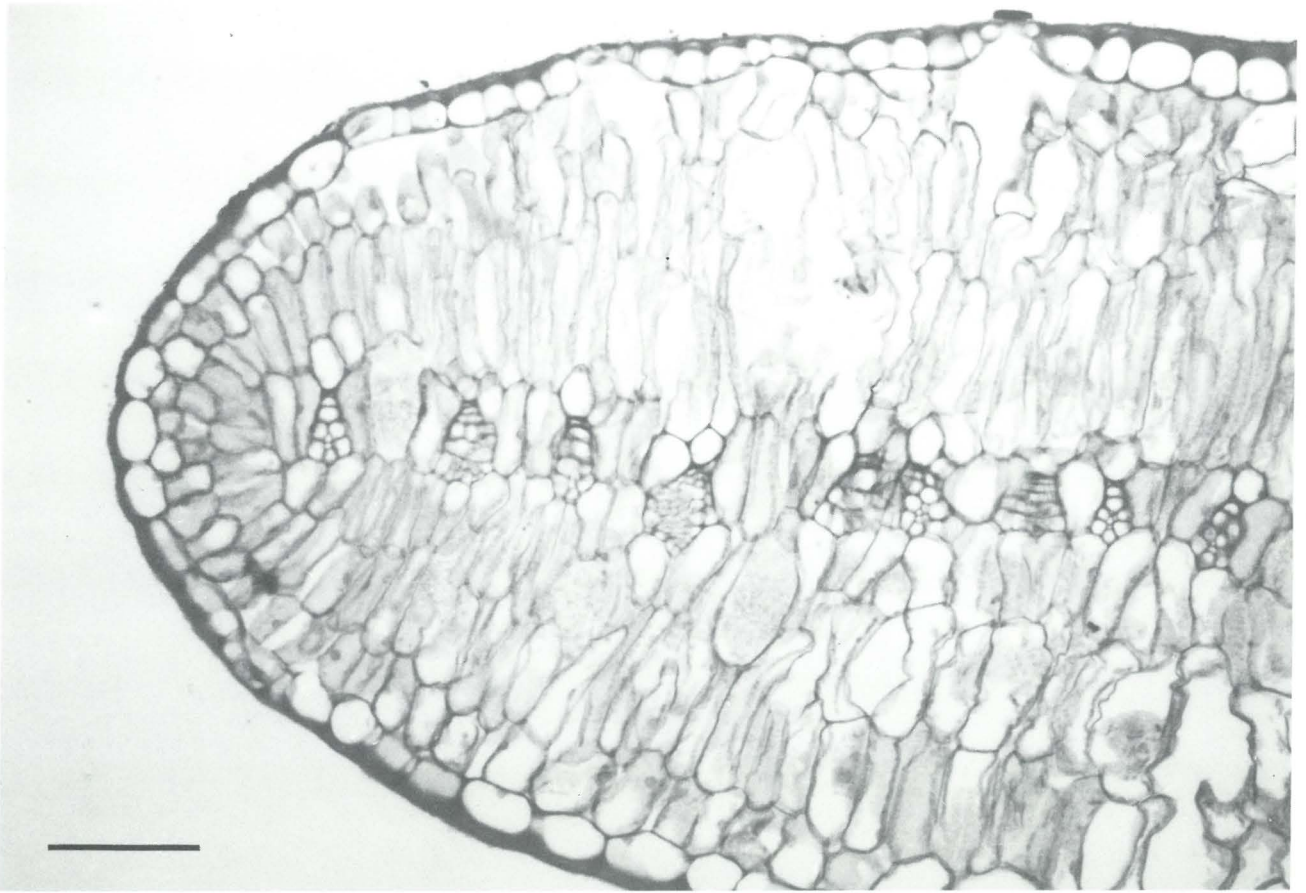


Figure 2 Transection of part of a leaf of *L. cinereum* showing the centric nature of the mesophyll. Bar = 100 μ m. (Reyneke 95).

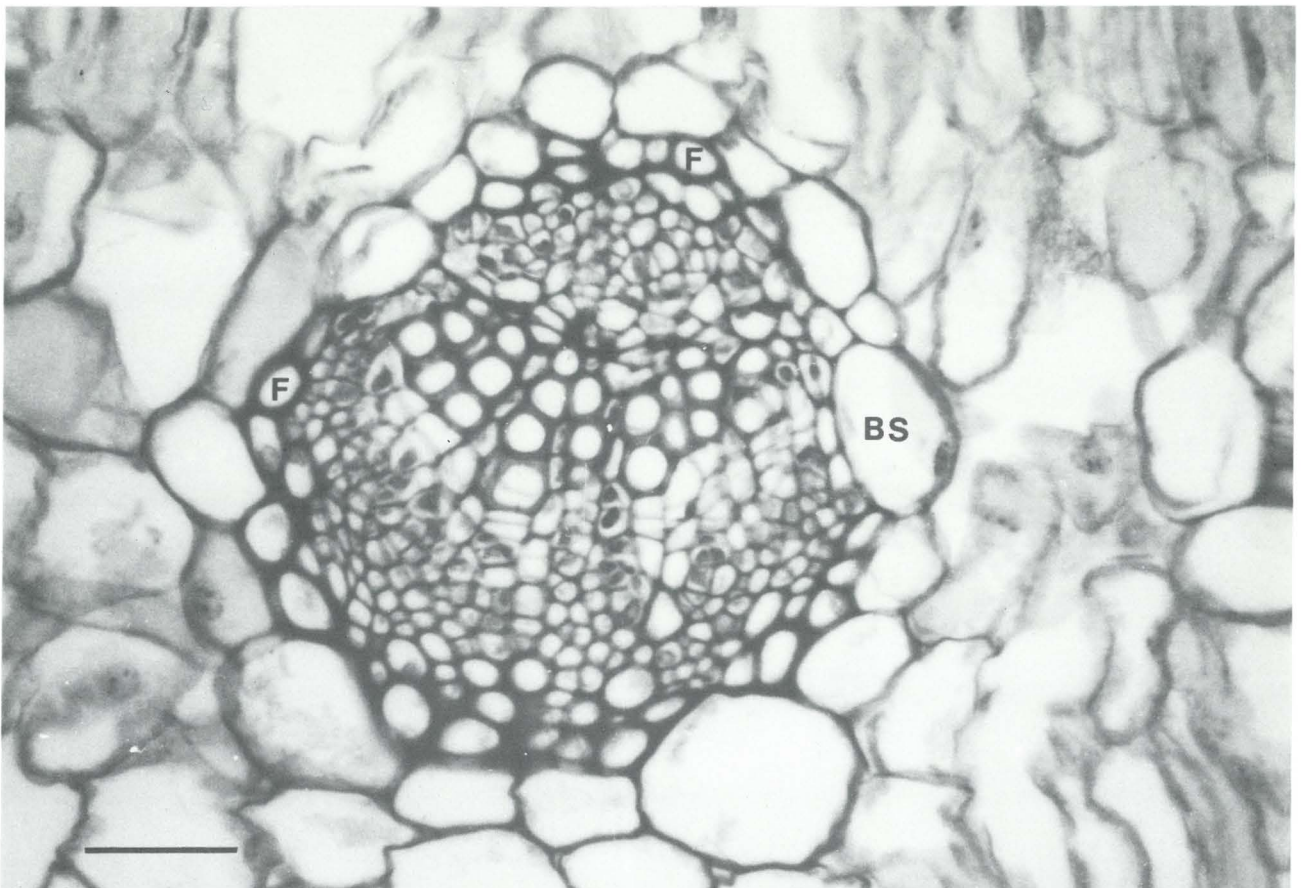


Figure 3 Transection of the midvein of a leaf of *L. echinatum* showing the bicollateral vascular bundle, fibres (F) and bundle sheath (BS). Bar = 40 μ m. (Reyneke 63).

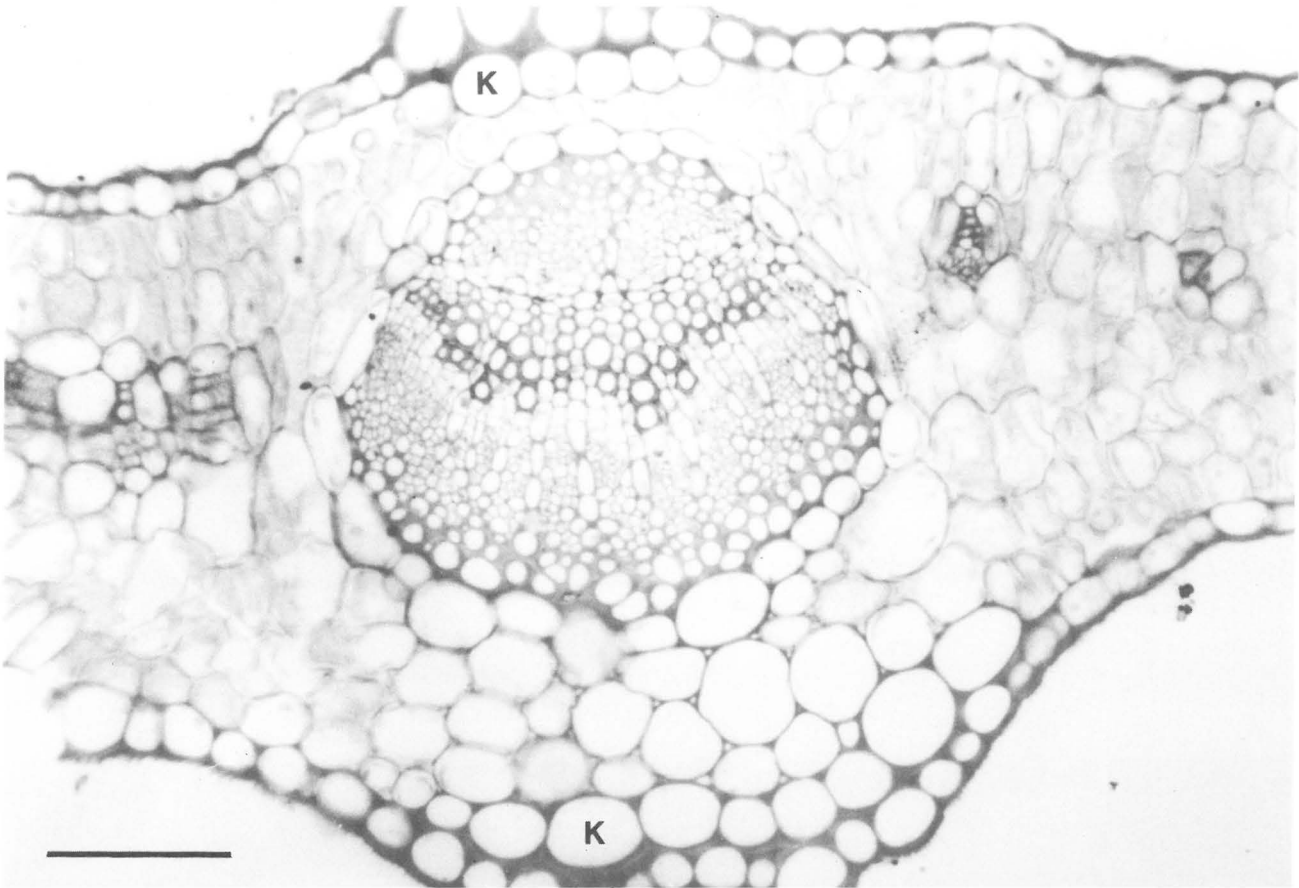


Figure 4 Transection of the midvein of a leaf of *L. hirsutum* showing collenchyma (K) on both sides of the vascular bundle. Bar = 100 μ m. (Reyneke 98).

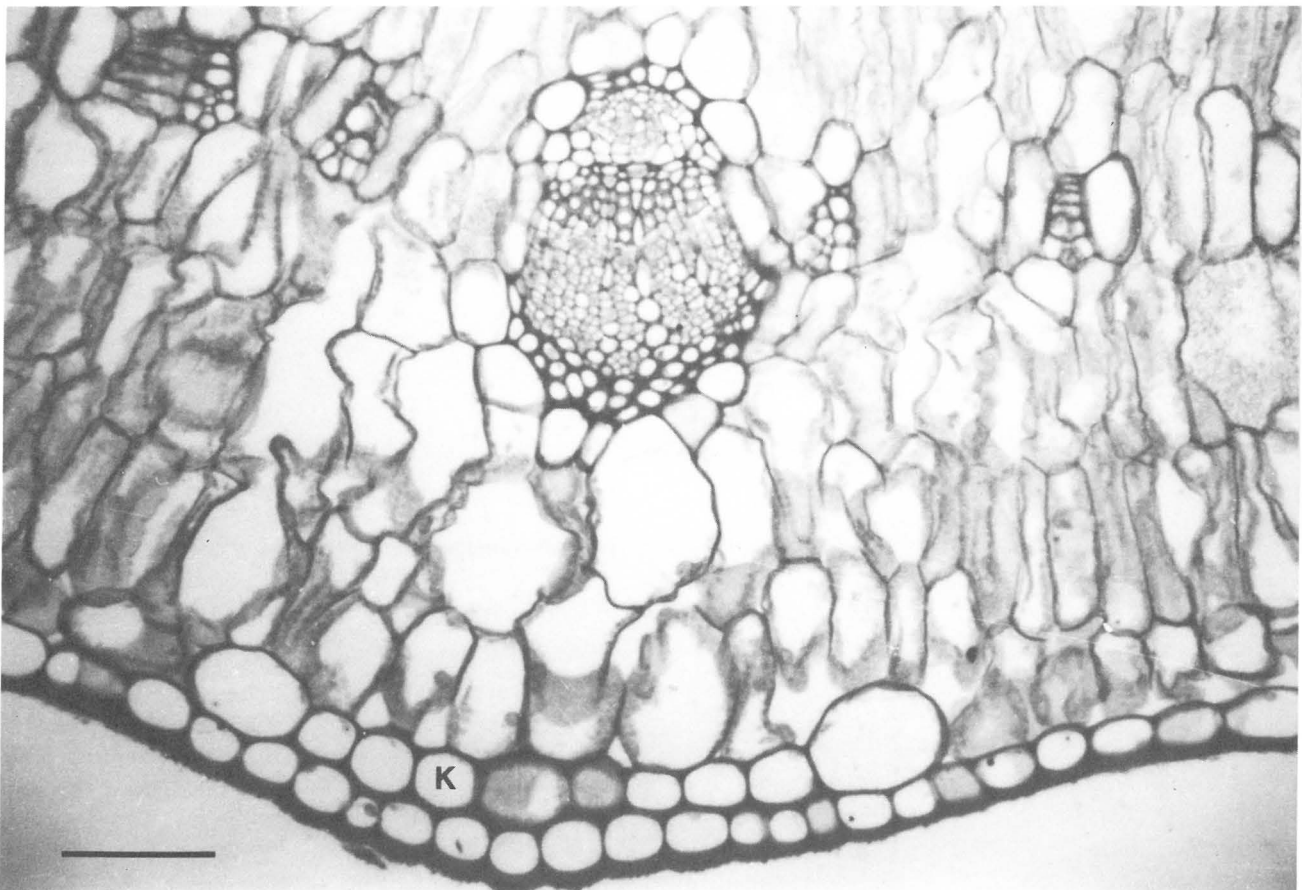


Figure 5 Transection of the midvein of a leaf of *L. cinereum* showing the single hypodermal collenchyma layer (K). Bar = 100 μ m. (Reyneke 91).

L. schizocalyx:— 2924 (Hopetown): 12 km NW of Luckhoff (–DB), *Reyneke 105* (BFLU); 2925 (Jagersfontein): 5 km E of Petrusburg (–AB), *Reyneke 73* (BFLU); *Reyneke 124* (BFLU); 3125 (Steynsburg): 8 km W of Middelburg (–AC), *Reyneke 144* (BFLU).

The trichomes of all eleven species were studied from herbarium specimens received from the following herbaria: BOL; NBG; NH; PRE; STE; UN.

Results and Discussion

Anatomy of the leaf

The leaves of all species studied are simple and semi-sessile with acutely cuneate bases. In those species where a short petiole may occur, it represents a lengthening of the leaf base rather than a clearly distinguishable petiole. The leaves are often covered by a mealy excrescence, consisting primarily of calcium oxalate (Hitchcock 1932). Metcalfe & Chalk (1950) consider the leaves of the Solanaceae as usually being dorsiventral but some species are isobilateral. The leaves of the *Lycium* species investigated are all regarded as being isobilateral since the mesophyll is not clearly differentiated into palisade and spongy parenchyma. The spongy parenchyma cells are slightly elongated and palisade-like with the result that the distinction between the two layers of mesophyll becomes rather arbitrary. In certain instances this distinction is possible (Figure 1) but is, however, not constant for a particular species. In the species with narrow leaves like *L. afrum*, *L. cinereum*, *L. horridum* and *L. schizocalyx* the leaf tends to become centric (Figure 2). Crystals of calcium oxalate (Metcalfe & Chalk 1950) occur in the form of crystal sand in the mesophyll.

The midvein of all the investigated species consists of a single bicollateral vascular bundle enclosed by a parenchymatous bundle sheath. Fibres (Figure 3) are present between the two phloem groups and the bundle sheath. Collenchyma occurs on both sides of the vascular bundle (Figure 4) in *L. hirsutum*. In *L. afrum*, *L. cinereum*, *L. echinatum*, *L. horridum*, *L. pilifolium* and *L. schizocalyx* a single hypodermal collenchyma layer (Figure 5) is present on the abaxial side of the midvein. In *L. ferocissimum* and *L. oxycarpum* there is no collenchyma.

Adaxial phloem is absent from the smaller lateral veins in all species studied, as found by Fukuda (1967) in *L. chinense*. These collateral veins are enclosed by a parenchymatous bundle sheath.

The epidermal cells of the adaxial leaf surfaces appear to be similar in both surface view and cross-section, although a slight and inconsistent variation in size may be observed. A thick grooved cuticle is present (Figure 10). Leaves are amphistomatic and stomata are of the anomocytic type.

Five types of trichomes occur on the leaves of which three are glandular and two non-glandular. Two of the glandular types consist of three to four-celled uniseriate stalks (short-stalked) differing only in having spherical (Figures 6 a & b) or elongated heads (Figures 7 a & b). The third type of glandular trichome consists of a five to six-celled uniseriate stalk (long-stalked) with a multicellular head (Figures 8 & 10). One type of non-glandular trichome is uniseriate and consists of six to eight cells (Figures 9 & 10) while the other is variously branched (Figure 10).

Short-stalked glandular trichomes with spherical heads occur in *L. cinereum*, *L. ferocissimum*, *L. horridum* and *L.*

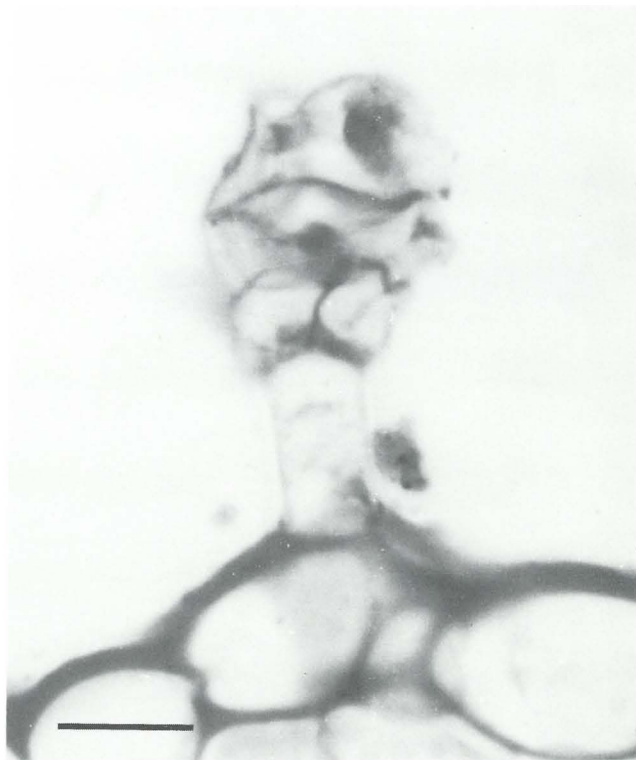


Figure 6a Longitudinal section of a short-stalked glandular trichome with spherical head of *L. schizocalyx*. Bar = 20 μ m. (Reyneke 105).



Figure 6b Scanning electron micrograph of a short-stalked glandular trichome with spherical head of *L. cinereum*. Bar = 10 μ m. (Reyneke 91).

schizocalyx, while glandular trichomes with elongated heads are found in *L. acutifolium*, *L. afrum*, *L. echinatum*, *L. oxycarpum* and *L. shawii*. Glandular trichomes, both short-stalked (elongated head) and long-stalked, occur in *L. pilifolium*. Except for short-stalked (spherical head) and long-stalked glandular trichomes, non-glandular trichomes



Figure 7a Longitudinal section of a short-stalked glandular trichome with elongated head of *L. echinatum*. Bar = 50 μm (Reyneke 61).

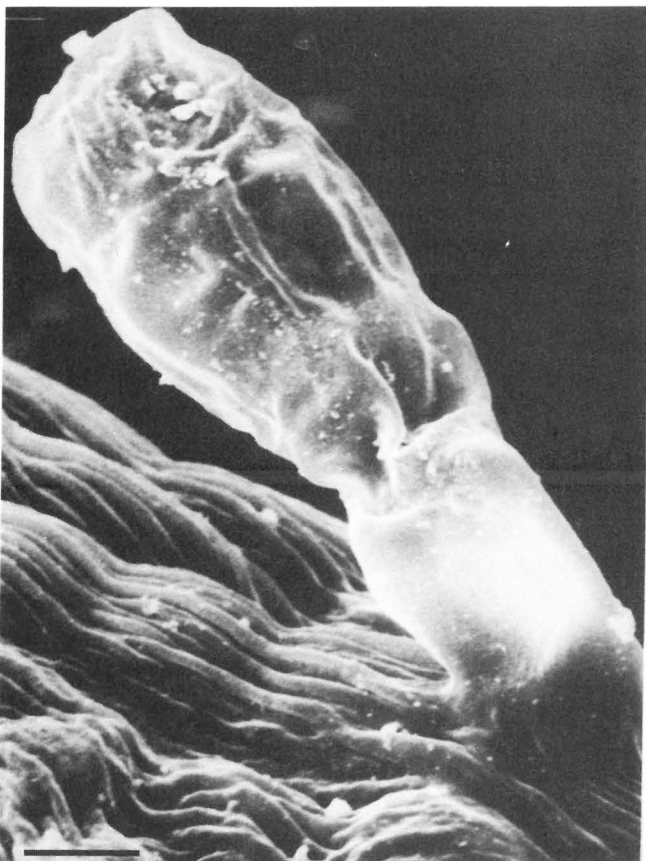


Figure 7b Scanning electron micrograph of a short-stalked glandular trichome with elongated head of *L. echinatum*. Bar = 10 μm . (Joubert 151).

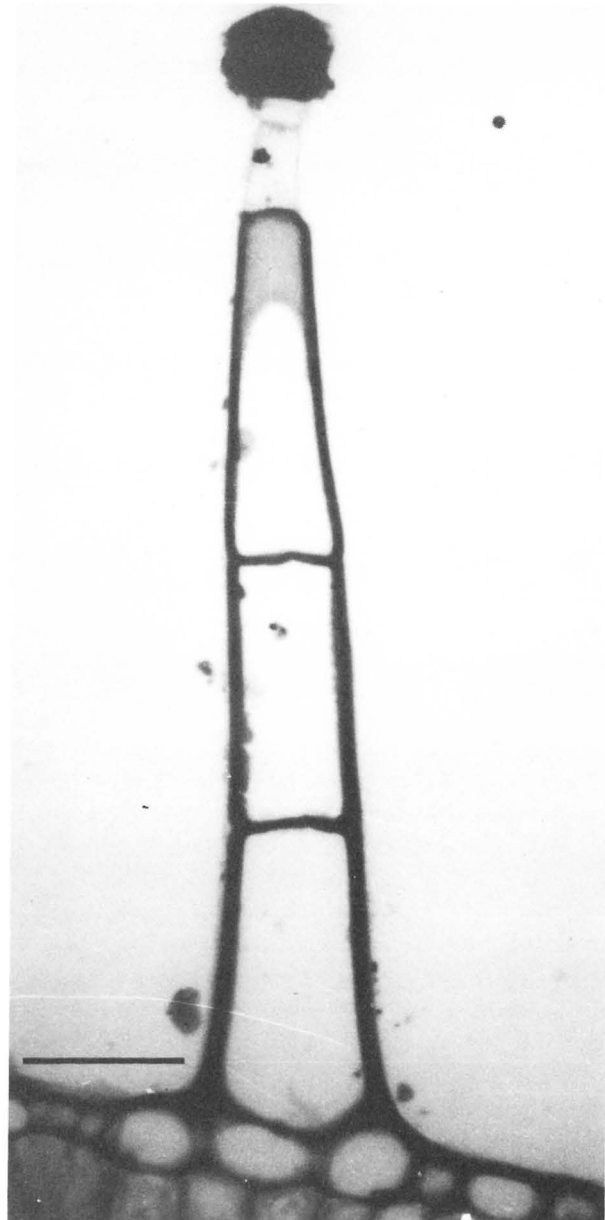


Figure 8 Longitudinal section of a long-stalked glandular trichome of *L. hirsutum*. Bar = 50 μm . (Reyneke 107).

both uniseriate and branched are found in *L. hirsutum* (Figure 10).

Anatomy of the stem

The anatomy of the stem showed very little variation in the investigated species. Longitudinal ridges are present in the young stems of all species (Figure 11). In cross-section three to four ridges consisting of sclerenchymatous cells are observable.

As periderm formation occurs at an early stage in the development of the stem, an epidermis, with trichomes similar to those of the leaves, is present only in the young stems.

The cortex (Figure 11) of the primary stem consists of several layers. The two to three layers of hypodermal collenchyma are followed internally by three to four layers of larger parenchyma cells which become sclerenchymatous (Figure 12) in the older stems. The inner three to five cortical layers consist of smaller parenchyma cells. In all the species

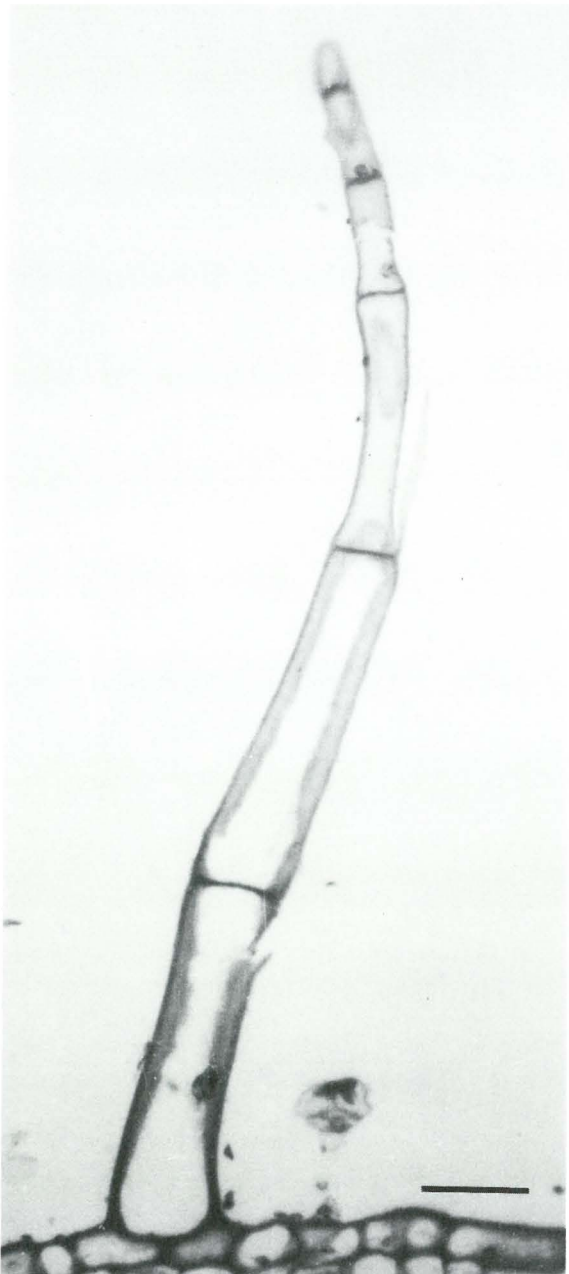


Figure 9 Longitudinal section of a non-glandular trichome of *L. hirsutum*. Bar = 50 μm . (Reyneke 98).

studied the bifacial phellogen originates from the outer layer of parenchyma just below the sclerenchymatous layer (Figure 12).

The interfascicular regions are narrow so that the vascular bundles are compactly arranged in the vascular cylinder. The external and internal phloem consists of sieve tubes, companion cells and associated parenchymatous cells. No fibres are associated with the phloem.

Secondary growth occurs at an early stage. Relatively few layers of secondary phloem are formed, while a wide cylinder of secondary xylem is formed.

The wood is diffuse porous. The vessels are grouped together to form a radial pore multiple (Figure 13). Metcalfe & Chalk (1950) also observed the grouping of the larger vessel elements. The average length of the vessel elements of all the species studied is 260,4 μm with lengths ranging from 186,0 μm to 372,0 μm . The average diameter is 37,2 μm with diameters ranging from 27,9 μm to 46,5 μm . Pit distribution is diffuse (Figure 14) and the perforation plates are simple (Figure 15). The rays are uniseriate and homogeneous. Axial parenchyma is apotracheal and diffuse.

A monofacial (unidirectional) cambium is present between the xylem and internal phloem (Figure 16) which forms secondary phloem centripetally. The occurrence of an internal cambium is rare in the family Solanaceae (Fukuda 1967).

The pith is parenchymatous and crystal sand is usually present. Brachysclereids occur in the pith of *L. oxycarpum* and *L. schizocalyx*.

Conclusion

The anatomical characteristics proved to be constant features. The five species grown under identical conditions in the garden of the Botany Department, U.O.F.S. to ascertain the influence of environment and habitat on structure, showed no significant differences, morphological as well as anatomical, in comparison with plants from their natural habitats. Neither could any significant anatomical variation be detected amongst plants collected from different habitats. It can, therefore, be concluded that difference in habitat has little influence on anatomical characteristics.

The anatomical characteristics of the leaves, especially the trichomes, proved to be of some taxonomic value. As the distinction between species is often difficult when no

Table 1 Comparison of the anatomical characteristics of the leaves of the South African species of *Lycium*

Characteristics	<i>L. acutifolium</i>	<i>L. afrum</i>	<i>L. cinereum</i>	<i>L. echinatum</i>	<i>L. ferocissimum</i>	<i>L. hirsutum</i>	<i>L. horridum</i>	<i>L. oxycarpum</i>	<i>L. pilifolium</i>	<i>L. schizocalyx</i>	<i>L. shawii</i>
Glandular, short-stalked, with spherical head			*	*	*					*	
Glandular, short-stalked, with elongated head	*	*	*					*			*
Glandular, short-stalked (elongated head) and long-stalked									*		
Glandular, short-stalked (spherical head), long-stalked. Non-glandular uniseriate and variously branched						*					
Collenchyma on both sides of the midvein						*					
Collenchyma below the midvein		*	*	*			*		*	*	
Collenchyma absent					*			*			

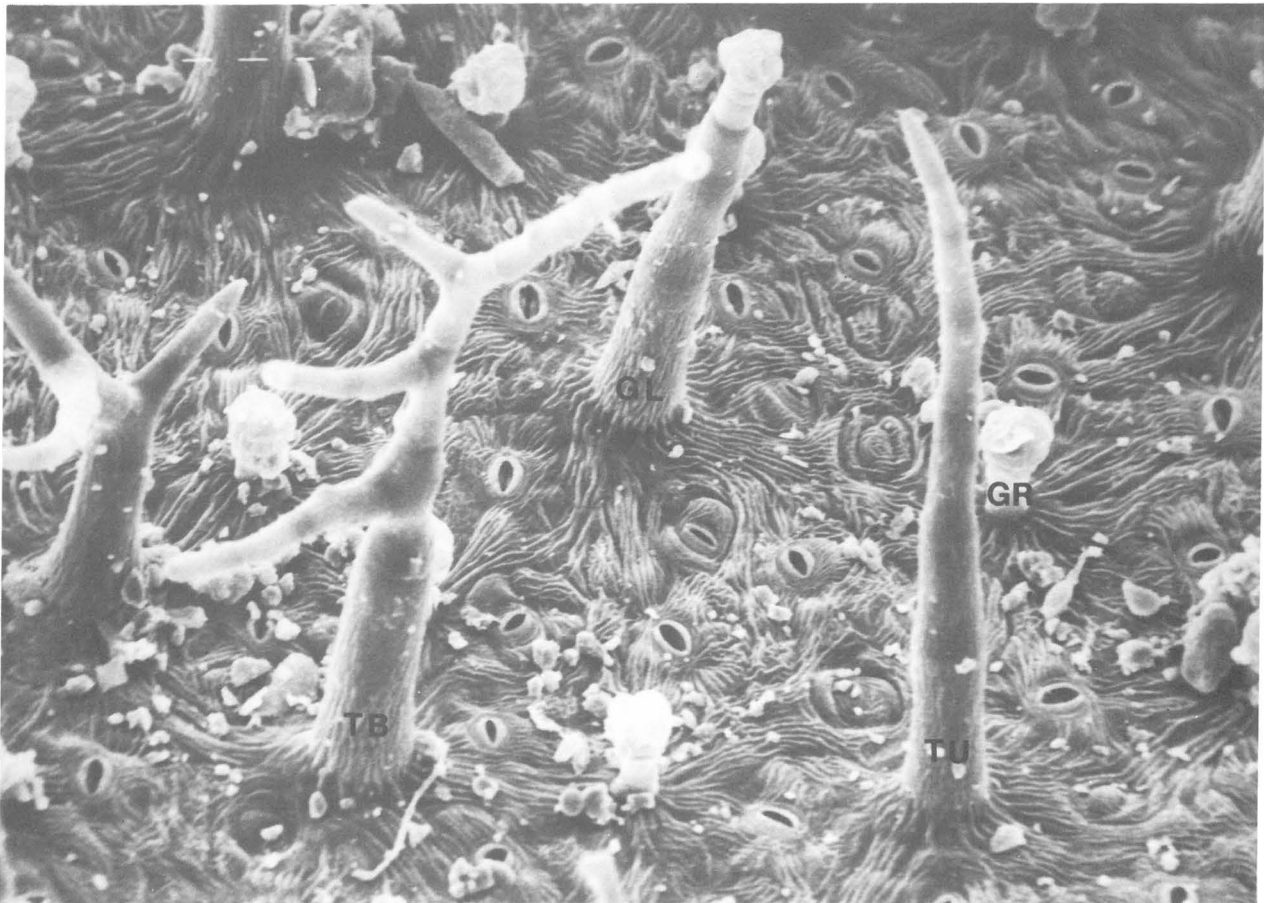


Figure 10 Scanning electron micrograph of non-glandular trichomes, branched (TB) and unbranched (TU), a long-stalked glandular trichome (GL) and a short-stalked glandular trichome (GR) of *L. hirsutum*. Bar = 10 μm . (Joubert 150).

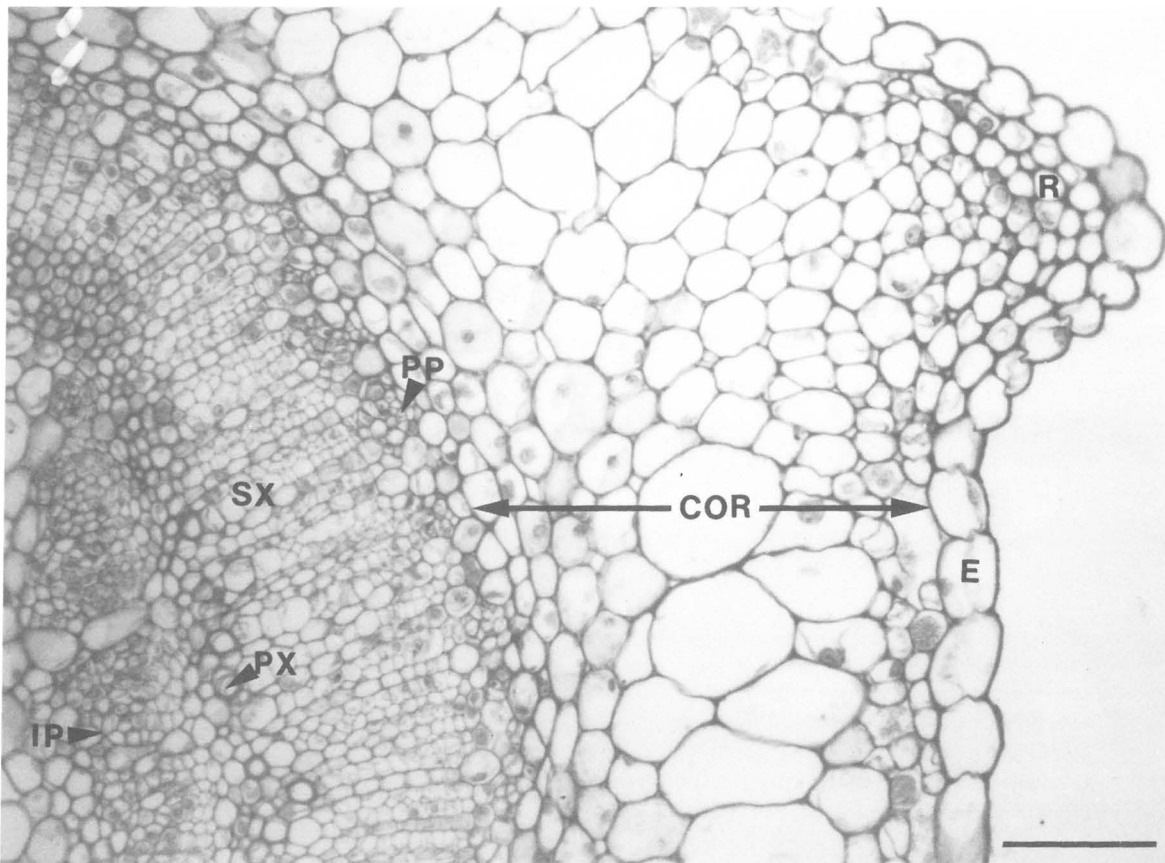


Figure 11 Transection of part of a young stem of *L. horridum* showing a longitudinal ridge (R), epidermis (E), cortex (COR), primary phloem (PP), secondary xylem (SX), primary xylem (PX) and internal phloem (IP). Bar = 100 μm . (Joubert 154).

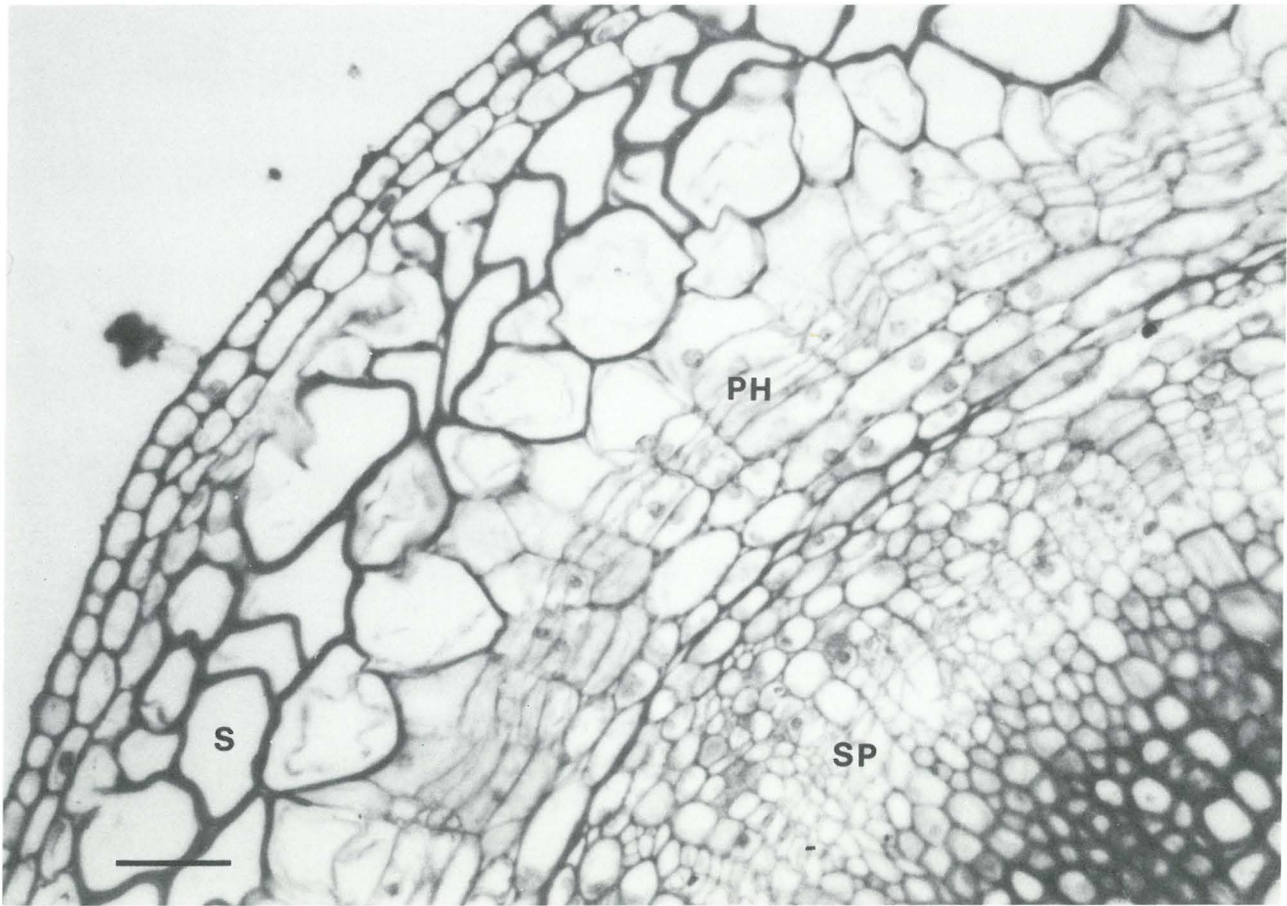


Figure 12 Transection of part of the stem of *L. ferocissimum* showing the origin of the phellogen (PH) beneath the sclerenchymatous cortex layer (S). SP = secondary phloem. Bar = 50 μ m. (Reyneke 134).

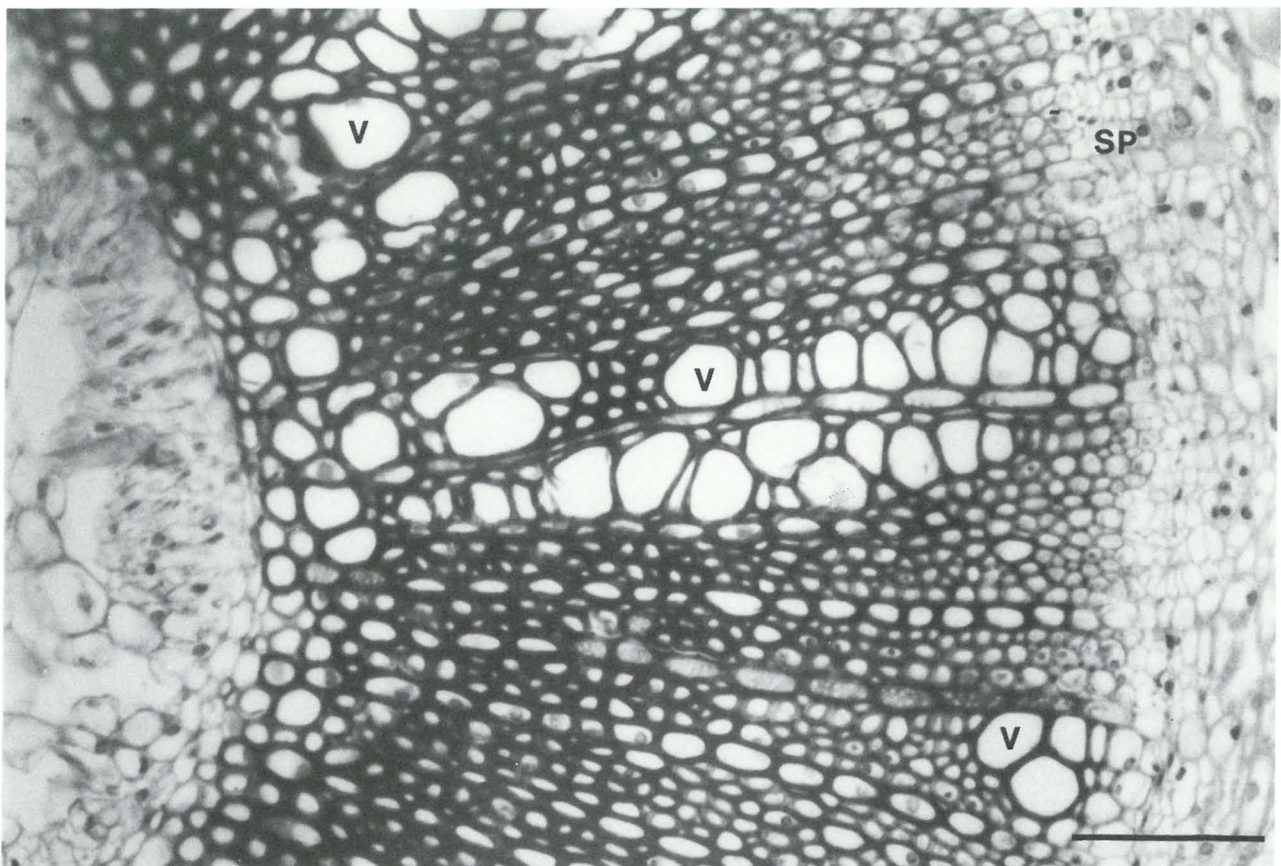


Figure 13 Transection of part of the xylem of *L. echinatum* showing the irregularly grouped vessels (V). SP = secondary phloem. Bar = 100 μ m. (Reyneke 63).

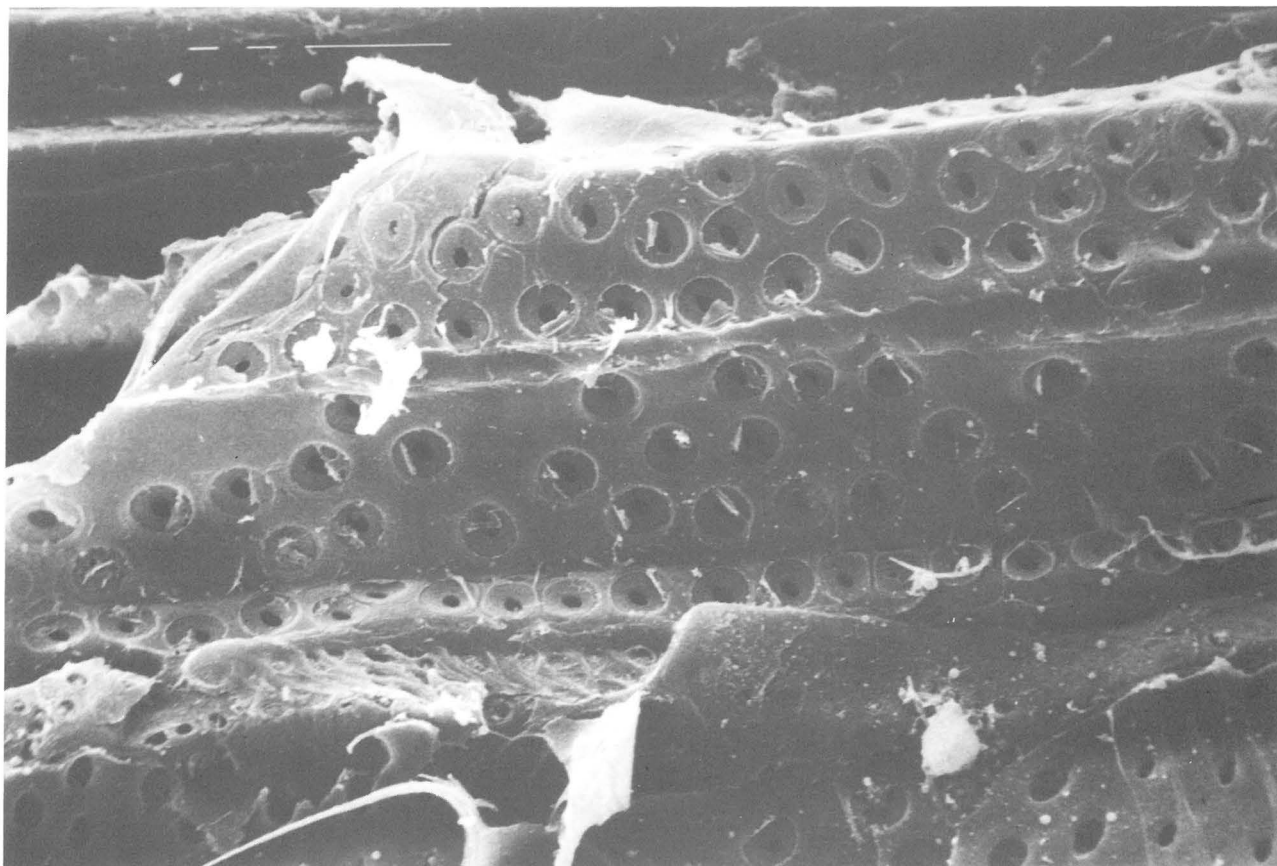


Figure 14 Scanning electron micrograph of a vessel of *L. echinatum* showing the diffuse distribution of the pits. Bar = 10 μ m. (Joubert 151).

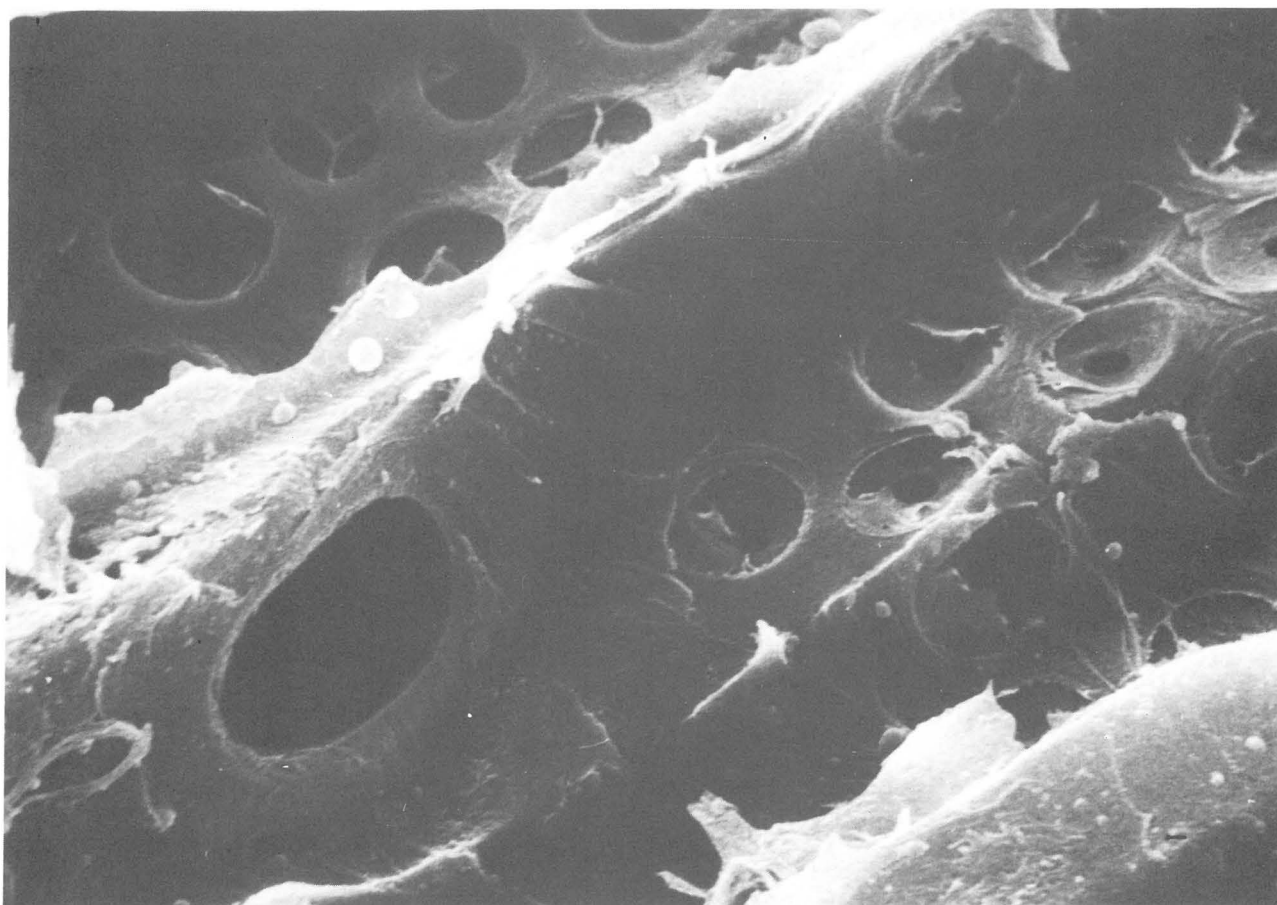


Figure 15 Scanning electron micrograph of a vessel of *L. hirsutum* showing the simple perforation plate. Bar = 10 μ m (Joubert 150).

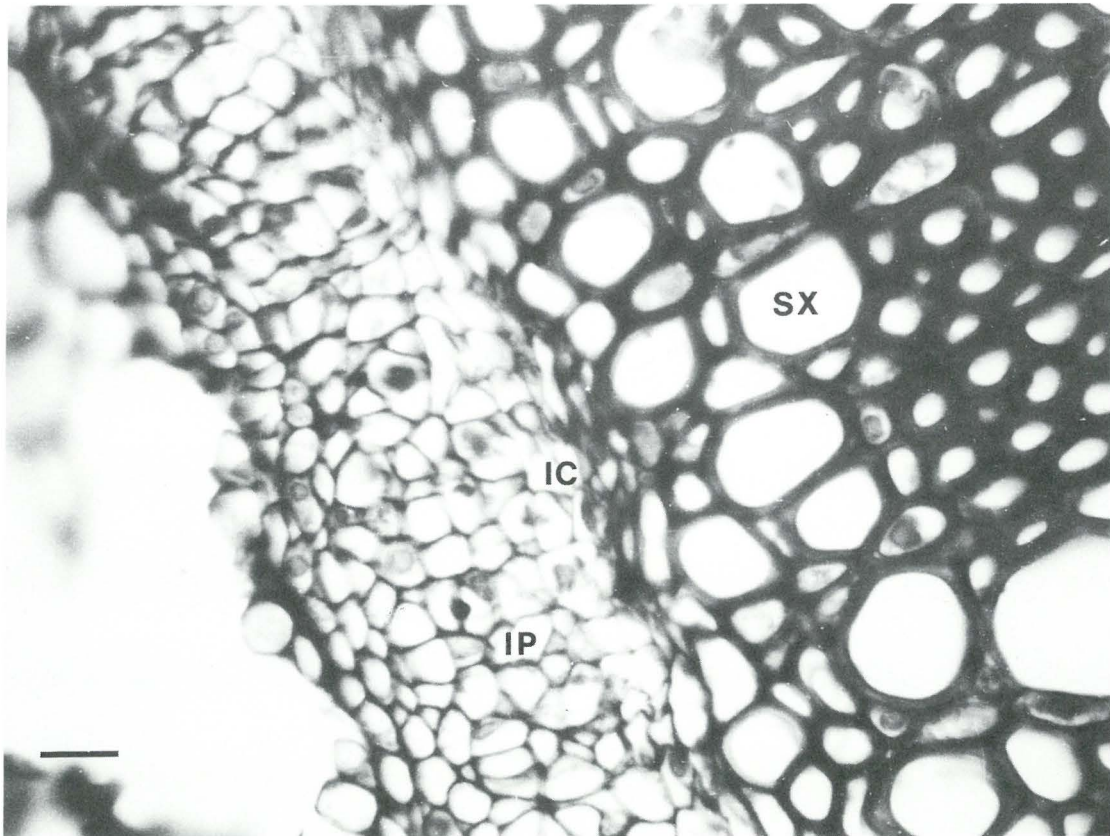


Figure 16 Transection of part of the stem of *L. echinatum* showing the monocafial internal cambium (IC). IP = internal phloem; SX = secondary xylem. Bar = 20 μ m. (Reyneke 61).

flowers are present, the anatomical differences may be valuable for identifying species as indicated in Table 1.

It is clear from Table 1 that the anatomical characteristics of the leaves of *L. horridum*, *L. cinereum* and *L. schizocalyx* do not provide any distinction. They may, however, be distinguished as a group from the other eight species with regard to the type of trichome and position of the collenchyma. The remaining species may be successfully distinguished by the type of trichomes occurring. Especially in the instance of *L. horridum* and *L. echinatum*, this distinction proved to be valuable. There had been some doubt, when reducing the number of species to eleven, as to whether *L. echinatum* and *L. horridum* should be combined as one species as florally they showed similarities. The growth form, however, differed sufficiently to separate the two species initially. The validity of the separation has been confirmed by the study of the trichomes. Short-stalked glandular trichomes with spherical heads occur in *L. echinatum* while those in *L. horridum* show elongated heads.

L. hirsutum is distinctly characterized by the occurrence of the branched and unbranched non-glandular trichomes that do not occur in any of the other South African species. This species does not have a different distribution pattern, nor is the habitat any different from the other species. In fact, *L. hirsutum* often occurs in the same locality as other *Lycium* species. Interesting is that, according to Hitchcock (1932), this type of branched non-glandular trichome occurs in the South American species of the genus but is not common in species native to Mexico and the United States of America. The short-stalked glandular trichomes occurring in the other species are, however, commonly found in the

species of North America. This also applies to the characteristic long-stalked glandular trichome that occurs with the short-stalked glandular trichome in *L. pilifolium*. The significance of this fact is not clear but it may be related to a difference in origin of *L. hirsutum* compared with the other South African species. It should be noted that according to Hitchcock (1932) none of the South African species of the genus occur in the western hemisphere.

As the aim of the study was to compare the anatomy of leaves and stems in the hope of finding distinctive anatomical characteristics to confirm the distinction between species and to be used to identify plants when not in flower, this distinction according to the trichomes is especially valuable when the variability of the vegetative parts is considered.

Acknowledgements

This research was financed by the University of the Orange Free State.

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