Taxonomic relationships in the genus *Dichilus* (Fabaceae — Crotalarieae)

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Taxonomic relationships within the genus *Dichilus* have been investigated. A phenetic analysis of 25 different localities as OTU's (five of each species) and 67 characters resulted in five distinct groups, corresponding to the five species that were recognized in a recent synopsis. For cladistic analyses, 15 characters were used and the genera *Melolobium, Argyrolobium* and *Lebeckia* were used as outgroups. In a further analysis the characters were intuitively polarized. Current cladistic methodology indicated only one fully resolved cladogram of minimal length, irrespective of the choice of outgroup. This cladogram is also supported by several additional characters and biogeographical evidence and is therefore proposed as the best estimate of the phylogeny of the species of *Dichilus*.

Taksonomiese verwantskappe binne die genus *Dichilus* is ondersoek. 'n Fenetiese ontleding van 25 verskillende lokaliteite as OTE's (vyf van elke spesie) en 67 kenmerke het vyf duidelik-onderskeibare groepe tot gevolg gehad, wat ooreenstem met die vyf spesies wat in 'n onlangse sinopsis erken is. Vir kladistiese ontledings is 15 kenmerke en die genera *Melolobium, Argyrolobium* en *Lebeckia* as buitegroepe gebruik. In 'n verdere ontleding is die kenmerke intuïtief gepolariseer. Hedendaagse kladistiese metodologie het slegs een volledig-opgeloste kladogram van minimale lengte aangedui, ongeag die keuse van buitegroep. Hierdie kladogram word ook ondersteun deur verskeie bykomstige kenmerke en biogeografiese getuienis en word dus voorgestel as die beste skatting van die filogenie van die spesies van *Dichilus*.

Keywords: *Dichilus*, infrageneric relationships, phylogeny, taxonomy

Introduction

The genus *Dichilus* DC. differs from other genera of the tribe Crotalarieae in the chromosome number, spurred wing petals and distinctive combination of major alkaloids (Schutte & van Wyk 1988; van Wyk *et al.* 1988, van Wyk & Schutte 1989). Relationships between *Dichilus* and other genera of the tribe are discussed in detail elsewhere (van Wyk & Schutte 1989).

A detailed study of variation patterns within the genus, using phenetic and cladistic methods, formed part of a taxonomic revision (Schutte 1988). Our aim with this study was to investigate and summarize infrageneric relationships in an explicit and repeatable way and not to test and compare various methodologies. A phenetic analysis was used to show the degree of similarity between the five species, each represented by a selected sample from five different localities. Cladistic methods were used to examine phylogenetic relationships within the genus. These investigations have led to an improved understanding of infrageneric variation and the results are presented here.

Material and Methods

Material examined included a representative sample of five geographically isolated provenances (two or three individuals per provenance) for each of the five species. Voucher specimens are given by Schutte (1988). The 25 provenances and their geographical distribution are shown in Figure 1. Authorities for the names of the species are also given in Figure 1 and are not repeated elsewhere.

The characters and character states used in this study are listed in Table 1 (phenetic analysis) and in Tables 2 to 5 (cladistic analyses). Some of these characters are illustrated and discussed below.

For the phenetic analysis, a matrix of the 25 proven-

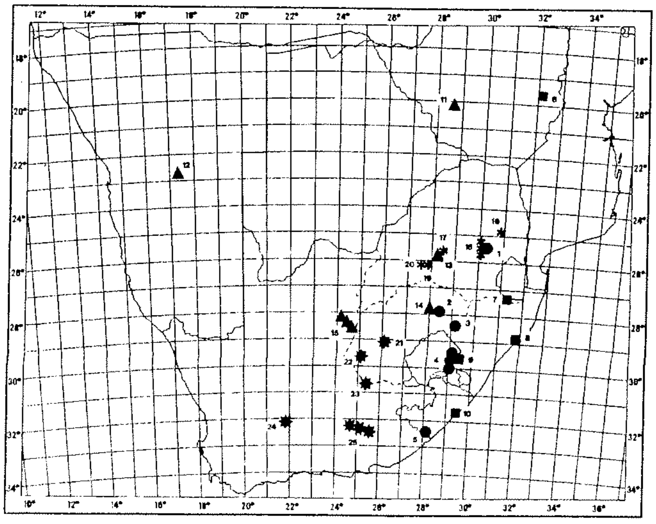

Figure 1 Geographical distribution of the 25 provenances used for variation studies. *D. strictus* E. Mey. (●): 1, Dullstroom; 2, Bethlehem; 3, Platberg; 4, Sehlabathebe; 5, Butterworth. *D. reflexus* (N.E. Br.) A.L. Schutte (■): 6, Melsetter; 7, Louwsburg; 8, Mtunzini; 9, Sani Pass; 10, Port St. Johns. *D. lebeckioides* DC. (▲): 11, Bulawayo; 12, Windhoek; 13, Pretoria; 14, Frankfort; 15, Kimberley. *D. pilosus* Conrath ex Schinz (✱): 16, Pilgrims Rest; 17, Pretoria; 18, Lydenburg; 19, Helderkruin; 20, Krugersdorp. *D. gracilis* Eckl. & Zeyh. (✸): 21, Bloemfontein; 22, Fauresmith; 23, Colesberg; 24, Layton; 25, Graaff Reinet.

Table 1 Characters and character states used for the phenetic analysis (for complete matrix see Appendix 1)

1, Branchlet angle (1 = mostly > 45°; 2 = mostly < 45°). 2, Leaf arrangement on flowering branches (1 = alternate; 2 = opposite). 3, Maximum leaf length (mm). 4, Mean leaf length (mm). 5, Maximum leaf width (mm). 6, Mean leaf width (mm). 7, Maximum leaf length: maximum leaf width. 8, Mean leaf length:mean leaf width. 9, Number of leaflets (1 = invariably 3-digitate; 2 = 3-digitate, sometimes 4- or 5-digitate; 3 = often 4- or 5-digitate). 10, Maximum leaflet length (mm). 11, Mean leaflet length (mm). 12, Maximum leaflet width (mm). 13, Mean leaflet width (mm). 14, Maximum leaflet length: maximum leaflet width. 15, Mean leaflet length:mean leaflet width. 16, Maximum petiole length (mm). 17, Mean petiole length (mm). 18, Maximum leaflet length:maximum petiole length. 19, Mean leaflet length:mean petiole length. 20, Stipules (1 = totally absent; 2 = rarely present; 3 = often present; 4 = consistently present). 21, Adaxial leaf traces (1 = absent; 2 = sometimes present; 3 = mostly present). 22, Leaf vestiture abaxial (1 = pubescent; 2 = pilose). 23, Leaf vestiture adaxial (1 = absent; 2 = present). 24, Inflorescence position (1 = invariably leaf-opposed; 2 = mostly terminal). 25, Maximum inflorescence length (mm). 26, Mean inflorescence length (mm). 27, Maximum peduncle length (mm). 28, Maximum inflorescence axis length (mm). 29, Maximum number of flowers per inflorescence. 30, Mode of flower number per inflorescence. 31, Maximum flower length (mm). 32, Standard vestiture (1 = only a few hairs; 2 = sparsely hairy; 3 = densely hairy). 33, Mean standard length (mm). 34, Mean wing length (mm). 35, Maximum number of sculpture rows on wing petals. 36, Mean keel length (mm). 37, Keel apex (1 = consistently pointed; 2 = rarely pointed). 38, Mean calyx length (mm). 39, Calyx teeth (1 = acuminate; 2 = acute). 40, Calyx deepest sinus:calyx upper sinus. 41, Calyx deepest sinus:calyx lower sinus. 42, Standard length:keel length. 43, Wing petal length:keel petal length. 44, Keel petal length:calyx length. 45, Wing petal length:calyx length. 46, Standard length:calyx length. 47, Maximum pedicel length (mm). 48, Maximum bract length (mm). 49, Maximum bracteole length (mm). 50, Maximum pod length (mm). 51, Mean pod length (mm). 52, Maximum pod width (mm). 53, Mean pod width (mm). 54, Maximum number of seeds per pod. 55, Mode of seed number per pod. 56, Maximum pod length:maximum pod width. 57, Mean pod length:mean pod width. 58, Pod vestiture (1 = mature pods glabrous; 2 = mature pods mostly hairy). 59, Orientation of pod (1 = nodding; 2 = mostly ascending). 60, Mean seed length (mm). 61, Mean seed length:mean seed width. 62, Mean seed width:mean seed thickness. 63, Seed colour (1 = orange to yellow-brown; 2 = olive-green; 3 = brown to black). 64, Stains on seed surface (1 = absent; 2 = scarcely mottled black; 3 = distinctly mottled black; 4 = mottled yellow and green). 65, Nectar guide (1 = absent; 2 = present). 66, Peak flowering period (1 = summer; 2 = winter). 67, Mean hair length, abaxial leaf surface (µm × 10).

Table 2 Characters and polarization of character states in the genus *Dichilus* with *Melolobium* as outgroup

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D. gracilis	0	1	2	2	0	2	2	1	0	1	0	0	1	2	2
D. lebeckioides	0	0	1	2	0	2	1	1	1	0	1	0	1	2	2
D. pilosus	1	0	1	1	0	2	1	1	0	0	0	1	1	2	2
D. reflexus	1	0	0	2	0	1	1	1	0	0	1	0	1	2	2
D. strictus	1	0	0	0	1	0	1	0	0	0	1	0	1	2	2
Melolobium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Characters and character states— **1. Number of leaflets**: 0 = invariably 3-digitate; 1 = sometimes 4- or 5-digitate. **2. Leaf arrangement of flowering branches**: 0 = alternate; 1 = opposite. **3. Maximum number of flowers per inflorescence**: 0 = mostly more than 4-flowered; 1 = mostly less than 4-flowered; 2 = invariably 1-flowered. **4. Standard vestiture**: 0 = densely hairy; 1 = sparsely hairy; 2 = only a few hairs. **5. Stipules**: 0 = at least sometimes present; 1 = totally absent. **6. Inflorescence position**: 0 = terminal; 1 = terminal, sometimes leaf-opposed; 2 = leaf-opposed only. **7. Keel shape**: 0 = oblong, widest part in the middle; 1 = obovate, widest part terminal; 2 = obovate, widest part terminal, slightly pointed apex. **8. Calyx**: 0 = scarcely bilabiate; 1 = deeply bilabiate. **9. Nectar guide**: 0 = absent; 1 = present. **10. Seed surface**: 0 = mottled; 1 = not mottled. **11. Petiole anatomy**: 0 = adaxial leaf traces at least sometimes present; 1 = adaxial leaf traces absent. **12. Hairs**: 0 = short, appressed; 1 = long, spreading. **13. Wing petals**: 0 = auriculate; 1 = markedly spurred. **14. Chromosome base number** (see van Wyk & Schutte 1989): 0 = base number 9; 1 = base number 8; 2 = base number 7. **15. Alkaloids**: 0 = mainly sparteine and lupanine type (quinolizidine); 1 = mainly α-pyridone type (quinolizidine); 2 = mainly piperidyl type

ances as OTU's and 67 characters were used (Appendix 1). The suggestions of Sneath & Sokal (1973) were followed in the choice of characters. Most of the characters chosen were quantitative. The BMDP P2M program for single-linkage clustering [no weighting; data standardized; Euclidian distance (Engelman 1981)] was used to generate a phenogram (illustrated in Figure 12).

A review of the application of cladistic methodology in botany is given by Linder (1988) and in the Fabaceae in particular, by Crisp & Weston (1987). Despite critisism by Cronquist (1987) and various others, there appears to be no alternative method for reconstructing phylogenies in an empirical way.

Very few cladistic studies have so far been done in the Fabaceae. Crisp & Weston (1987) refer to a total of nine such studies where taxa in the family have been cladistically analyzed, with variable degrees of success. Lavin

Table 3 Characters and polarization of character states in the genus *Dichilus* with *Argyrolobium* (and *Polhillia*) as outgroup

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D. gracilis	1	1	2	2	0	2	2	0	0	1	0	0	1	2	2
D. lebeckioides	1	0	1	2	0	2	1	0	1	0	1	0	1	2	2
D. pilosus	0	0	1	1	0	2	1	0	0	0	0	1	1	2	2
D. reflexus	0	0	0	2	0	1	1	0	0	0	1	0	1	2	2
D. strictus	0	0	0	0	1	0	1	1	0	0	1	0	1	2	2
Argyrolobium	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

Characters and character states (2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14 & 15 as in Table 2) — **1. Number of leaflets**: 0 = sometimes 4- or 5-digitate; 1 = invariably 3-digitate. **8. Calyx**: 0 = deeply bilabiate; 1 = scarcely bilabiate

Table 4 Characters and polarization of character states in the genus *Dichilus* with *Lebeckia* (and *Wiborgia*) as outgroup. Character 10 is omitted due to uncertainty about this character in the outgroup

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D. gacilis	0	1	2	2	1	2	2	2	0	–	0	0	1	2	2
D. lebeckioides	0	0	1	2	1	2	1	2	1	–	1	0	1	2	2
D. pilosus	1	0	1	1	1	2	1	2	0	–	0	1	1	2	2
D. reflexus	1	0	0	2	1	1	1	2	0	–	1	0	1	2	2
D. strictus	1	0	0	0	0	0	1	1	0	–	1	0	1	2	2
Lebeckia	0	0	0	0	0	0	0	0	0	–	0	0	0	0	0

Characters and character states (1, 2, 3, 4, 6, 7, 9, 11, 12, 13, 14 & 15 as in Table 2) — **5. Stipules**: 0 = totally absent; 1 = at least sometimes present. **8. Calyx**: 0 = subequal; 1 = scarcely bilabiate; 2 = deeply bilabiate. **10. Seed surface** (omitted)

Table 5 Characters and polarization of character states in the genus *Dichilus* by allocating values intuitively (no specific outgroup is used, see text). Characters which caused homoplasy in other cladograms are omitted

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D. gracilis	–	1	2	–	0	2	2	1	0	1	–	0	1	2	2
D. lebeckioides	–	0	1	–	0	2	1	1	1	0	–	0	1	2	2
D. pilosus	–	0	1	–	0	2	1	1	0	0	–	1	1	2	2
D. reflexus	–	0	0	–	0	1	1	1	0	0	–	0	1	2	2
D. strictus	–	0	0	–	1	0	1	0	0	0	–	0	1	2	2
Hypanc	–	0	0	–	0	0	0	0	0	0	–	0	0	0	0

Characters and character states (2, 3, 5, 6, 7, 8, 9, 10, 12, 13, 14 & 15 as in Table 2) — **1. Number of leaflets** (omitted). **4. Standard vestiture** (omitted). **11. Petiole anatomy** (omitted)

(1987) (tribe Robinieae), Zandee & Geesink (1987) (tribe Milletieae) and Crisp & Weston (1987) (tribes Bossiaeeae, Brongniartieae and Mirbelieae) are recent examples. The latter is the most comprehensive study so far.

To analyse phylogenetic relationships within *Dichilus*, three different matrices were compiled, using the genera *Melolobium* Eckl. & Zeyh., *Argyrolobium* Eckl. & Zeyh. (including *Polhillia* Stirton) and *Lebeckia* Thunb. (including *Wiborgia* Thunb.) as outgroups. There is some doubt about the correct outgroup, but according to Polhill (1976, 1981a) and van Wyk & Schutte (1989) these genera are closely related to *Dichilus* and are therefore the most logical choice. Polarity of character states were determined by comparison with the corresponding character states in the outgroup. A fourth matrix was also compiled in which we based our hypotheses of character state transformations on a consideration of apparent evolutionary trends in the subfamily (Polhill 1981b) and particularly in the tribe (Dahlgren 1970; Polhill 1976). In this matrix those characters that caused homoplasy in the first three analyses were omitted.

Cladograms were constructed manually according to the algorithmic method of Kluge & Farris (1969) and also by using the computer program PAUP (version 2.4) (Swofford 1985). Various internal options of the program were used to make sure that the shortest possible cladograms were found.

Results and Discussion

The morphology of the leaves contributed 21 characters to the phenetic analysis (Table 1). Figure 2 shows the variation in the shape, size, venation and vestiture, as well as the number of leaflets. Of these, only the vestiture and leaflet number seemed useful as cladistic characters. *D. pilosus* has conspicuously pilose hairs in contrast to the appressedly pubescent vestiture of the other four species. In *D. lebeckioides* and *D. gracilis* the leaves are consistently trifoliolate, while 4- or 5-foliolate leaves are commonly found in *D. reflexus, D. pilosus* and *D. strictus*. Although stipules are very small and often caducous, they are totally absent only in *D. strictus*. Stipules are invariably present in *D. pilosus* and *D. gracilis*, often in *D. lebeckioides* and rarely in *D. reflexus*.

Transverse sections through the middle of the petiole of five different individuals of each species are shown diagrammatically in Figure 3. This figure illustrates the range of variation found in at least three petioles from between 8 and 10 samples (i.e. herbarium collections) per species. Two small adaxial leaf traces are mostly present in *D. gracilis*. In *D. pilosus*, one or two adaxial traces occur occasionally, but these are totally absent in *D. lebeckioides, D. reflexus* and *D. strictus*.

Inflorescences are invariably racemose, but the position (insertion) may be terminal or leaf-opposed. The terminal position is confined to *D. strictus* and *D. reflexus* during the flowering season and changes to a leaf-opposed position only when active vegetative growth resumes from the terminal leaf axil (directly below the inflorescence). Since vegetative growth continues during the flowering period in *D. pilosus* and *D. lebeckioides*, the inflorescences in these two species are always leaf-opposed. *D. gracilis* has a specialized type of inflorescence. During the vegetative phase, stems and leaves develop in an alternate position. Just before flowers are formed however, every second internode shortens and the alternative ones lengthen, resulting in an opposite phyllotaxis. Figure 4 illustrates the variation in the number of flowers per inflorescence. *D. strictus* normally has four to six flowers (maximum nine), *D. reflexus* three to four (maximum 10), *D. lebeckioides* and *D. pilosus* mostly one, and *D. gracilis* invariably one flower per inflorescence. A distinct line of evolutionary development can be distinguished (Figure 5). It is suggested that the ancestral condition was a long terminal raceme (as in *Melolobium, Lebeckia* and *Wiborgia*). The inflorescence structure of *D. strictus* and *D. reflexus* is easily derived from this type simply by a reduction in the length of the inflorescence axis and the number of flowers. A further reduction in flower number and the continuation of growth during the

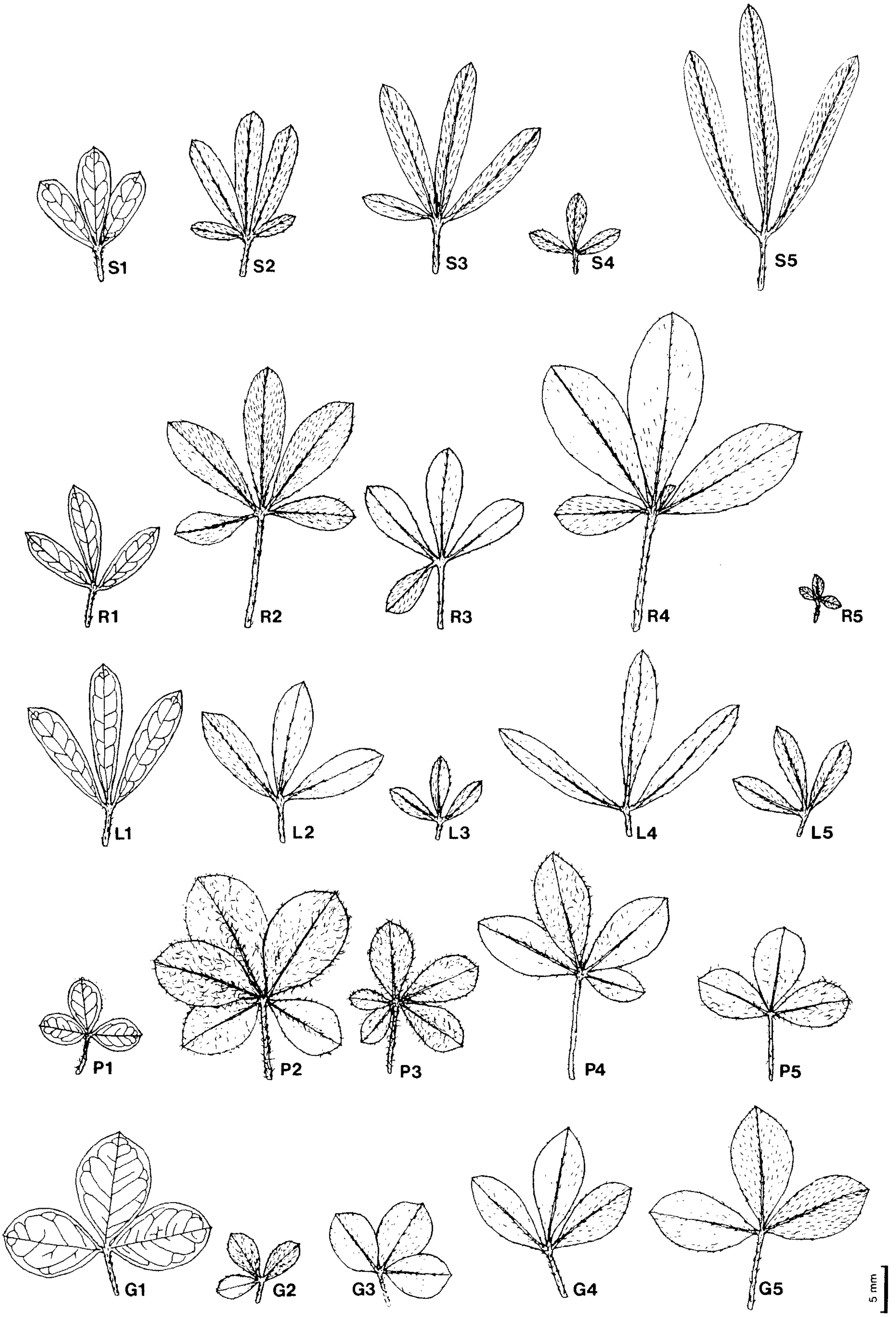

Figure 2 Variation in the size, shape, vestiture, venation and leaflet number of leaves of the genus *Dichilus*. [S, *D. strictus* (S1, *Schutte 157*; S2, *Schutte 156*; S3, *Schutte 155*; S4, *Schutte 157*; S5, *Acocks 10055*). R, *D. reflexus* (R1, *Killick & Vahrmeyr 3766*; R2, *Acocks 13032*; R3, *Schutte 183*; R4, *Acocks 13032*; R5, *Schutte 161*). L, *D. lebeckioides* (L1, *Brueckner 247*; L2, *Botha & Ubbink 1836*; L3, *Sutton 636*; L4, *Acocks 20806*; L5, *Schutte 152*). P, *D. pilosus* (P1, *Schutte 124*; P2, *Schutte 94*; P3, *Schutte 124*; P4, *Schutte 130*; P5, *Schutte 124*). G, *D. gracilis* (G1, *Acocks 14665*; G2, *Schutte 228*; G3, *Acocks 14665*; G4, *Schutte 193*; G5, *Gilfillan s.n. sub Herb. Galpin 2989*)].

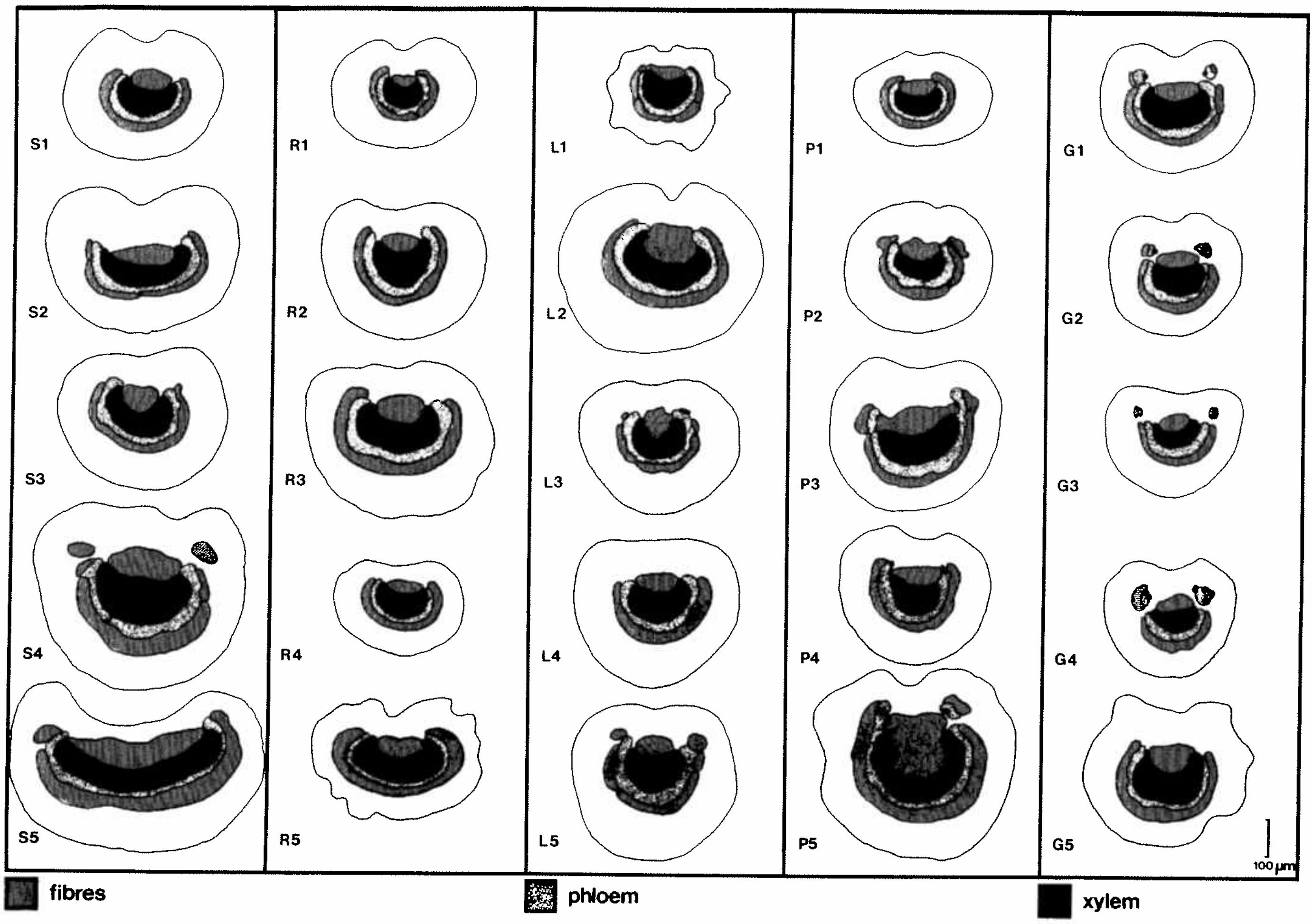

Figure 3 Transverse sections through the middle of the petiole of five different individuals of each species of the genus *Dichilus* to show the range of variation found in a much larger sample. [S, *D. strictus* (S1, *Schutte 131*; S2, *Schutte 156*; S3, *Schutte 146*; S4, *Acocks 10055*; S5, *Pegler 1806*). R, *D. reflexus* (R1, *Corby 1114*; R2, *Schutte 182*; R3, *Smuts & Evans 877*; R4, *Schutte 161*; R5, *Flanagan 2495*). L, *D. lebeckioides* (L1, *Eyles 1004*; L2, *Seydel 3787*; L3, *van Wyk 1382*; L4, *van Wyk 1538b*; L5, *Acocks 2143*). P, *D. pilosus* (P1, *Mogg 15133*; P2, *Krynauw 158*; P3, *Schutte 130*; P4, *Schutte 122*; P5, *Schutte 94*). G, *D. gracilis* (G1, *Schutte 193*; G2, *Dix s.n. sub Herb. BLFU 3550*; G3, *Wilman 3318*; G4, *van Wyk 1811*; G5, *Schutte 227*)].

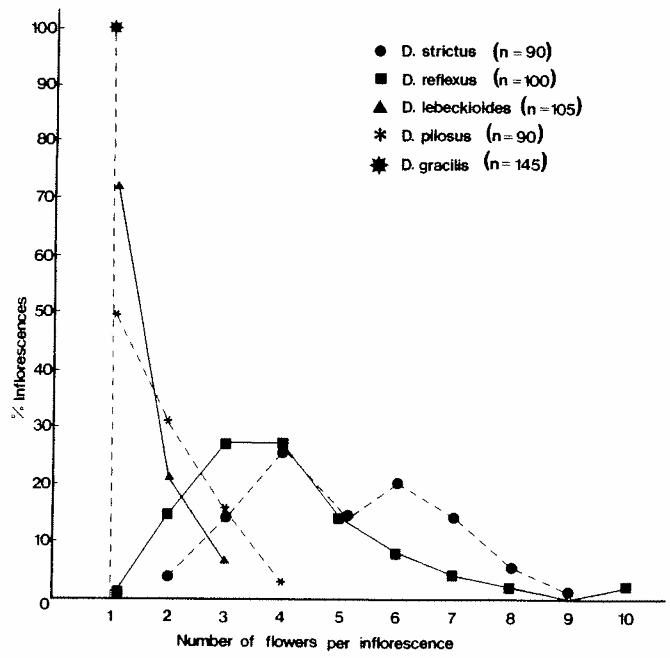

Figure 4 Variation in the number of flowers per inflorescence in the genus *Dichilus*.

flowering season (from the terminal leaf axil directly below the inflorescence) resulted in the leaf-opposed racemes of *D. lebeckioides* and *D. pilosus*. A total loss of the inflorescence axis and the peculiar growth mentioned above may have led to the single-flowered raceme at two opposite leaves as in *D. gracilis*.

The length of bracts and bracteoles (Figures 6 and 7) follows the same phylogenetic trend, i.e. the species with terminal racemes (*D. strictus* and *D. reflexus*) tend to have longer bracts and bracteoles than those with leaf-opposed racemes (*D. pilosus* and *D. lebeckioides*). Bracts and bracteoles are significantly shorter in *D. gracilis* and therefore seem to support the view that the inflorescence of this species is the most specialized.

Limited variation was observed in the flower structure (Figure 8) and the five species are indeed remarkably uniform. The most conspicuous difference lies in the short, scarcely bilabiate calyx of *D. strictus* in contrast to the long, deeply bilabiate calyces of the other species. It is perhaps significant that the increase in the length of lobes and the depth of sinuses (Figure 9) are correlated with the trends shown in Figures 4 to 7 and that *D.*

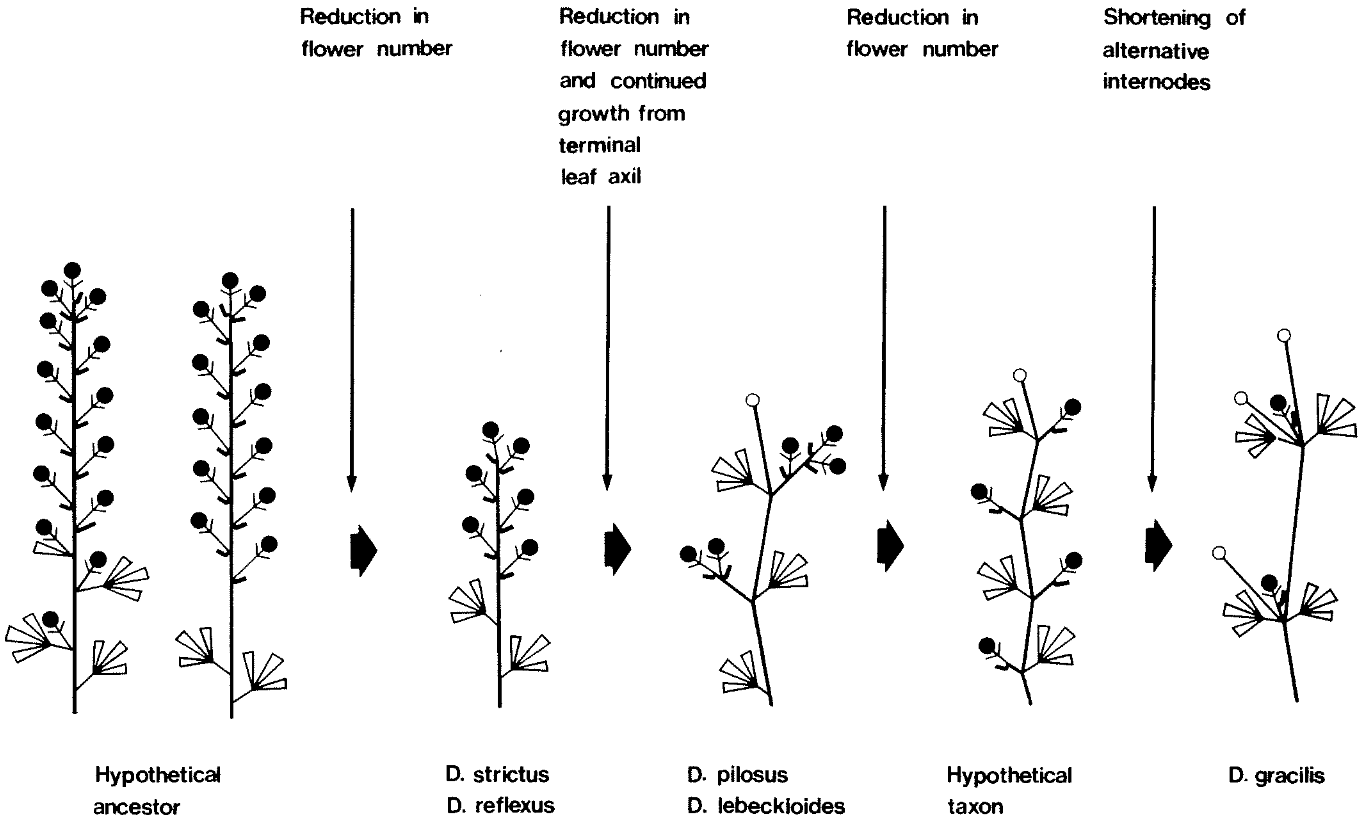

Figure 5 Schematic representation of the proposed phylogenetic development of inflorescence structure in the genus *Dichilus*.

strictus and *D. gracilis* again represent the extremes of the spectrum of variation. The same is true for the vestiture of the standard petal. *D. strictus* has a densely hairy vestiture abaxially on the upper part of the standard; in *D. pilosus* the standard is sparsely hairy and in the other three species only a few hairs are present along the middle of the standard. The conspicuously spreading hairs (also on the calyx and elsewhere) is a

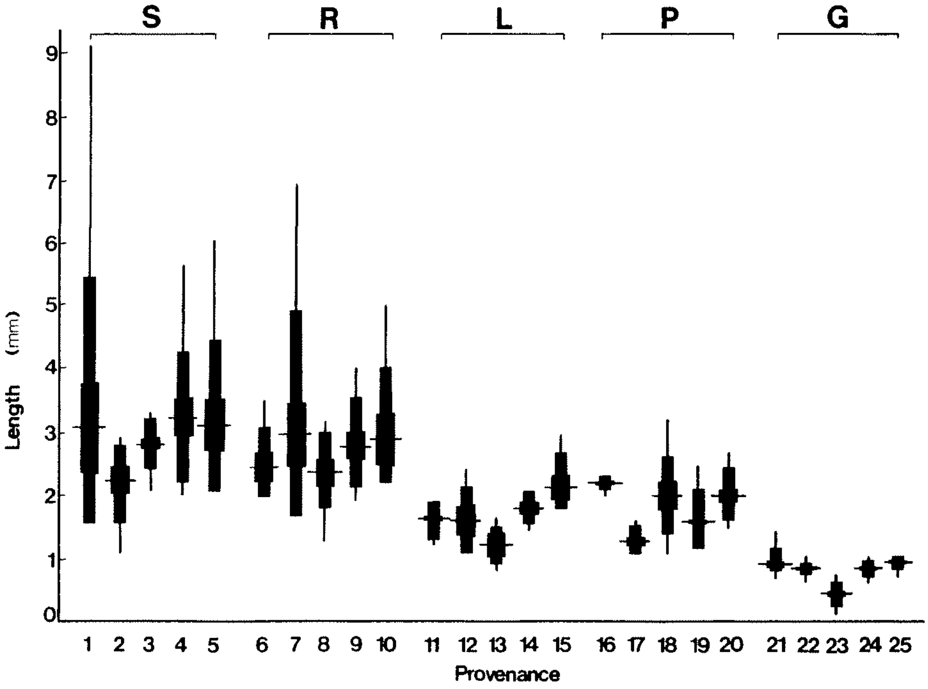

Figure 6 Variation in the length of bracts in the genus *Dichilus*. The range, mean, standard deviation and standard error of 10 measurements for each of the 25 provenances are given. [S, *D. strictus*; R, *D. reflexus*; L, *D. lebeckioides*; P, *D. pilosus*; G, *D. gracilis*. Provenance numbers as in Figure 1].

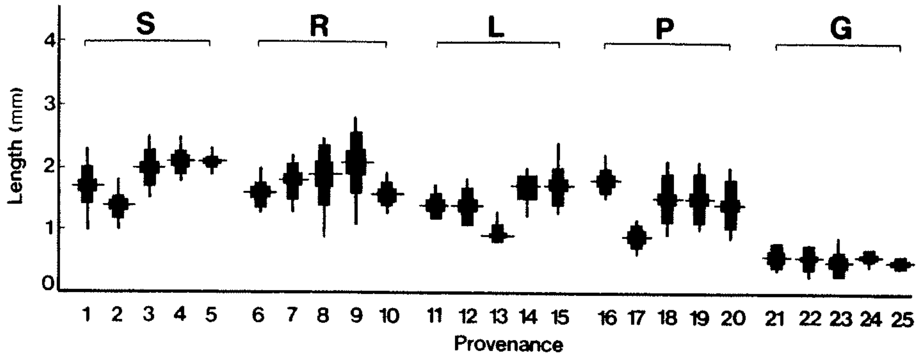

Figure 7 Variation in the length of bracteoles in the genus *Dichilus*. The range, mean, standard deviation and standard error of 10 measurements for each of the 25 provenances are given. [S, *D. strictus*; R, *D. reflexus*; L, *D. lebeckioides*; P, *D. pilosus*; G, *D. gracilis*. Provenance numbers as in Figure 1].

useful diagnostic character for *D. pilosus*. A distinct nectar guide (orange mark at the base of the standard) occurs only in *D. lebeckioides*. *D. gracilis* may be distinguished by the slightly more pointed keel petals.

There are some conspicuous differences in the number of seeds per pod. *D. strictus, D. reflexus* and *D. pilosus* have 1–5–7 seeds per pod. The seed number in *D. lebeckioides* is 2–7–9 and that of *D. gracilis* 1–4–5, (These figures are based on a random sample of 150 pods from various individuals of each species.) The colouration pattern of the seed surface is a particularly useful character to distinguish between some of the species. Figure 10 illustrates the differences between the five species.

In Figure 11 the relative proportions of piperidyl and quinolizidine alkaloids extracted from three samples of each of the species (van Wyk *et al.* 1988) are shown. The presence of alkaloids follows the same general trend (from *D. strictus* to *D. gracilis*) as observed in other characters. The pattern is one of a decreasing percentage of quinolizidine alkaloids and an increasing percentage of piperidyl alkaloids.

The result of the phenetic analysis is given in Figure 12. The five species of *Dichilus* are shown to be phenetically quite distinct and it seems unlikely that a significantly different result will be obtained with other clustering routines. Single-linkage clustering often leads to chaining, so that more 'sophisticated' methods (such as UPGMA) are usually recommended. However, we clearly did not encounter this problem. The provenances of each species are very similar, particularly those of *D. gracilis*. This indicates that geographical isolation has a limited effect on infraspecific variation. Although *D. reflexus* and *D. lebeckioides* appear to be phenetically similar, there is no evidence of any distinct supraspecific groups.

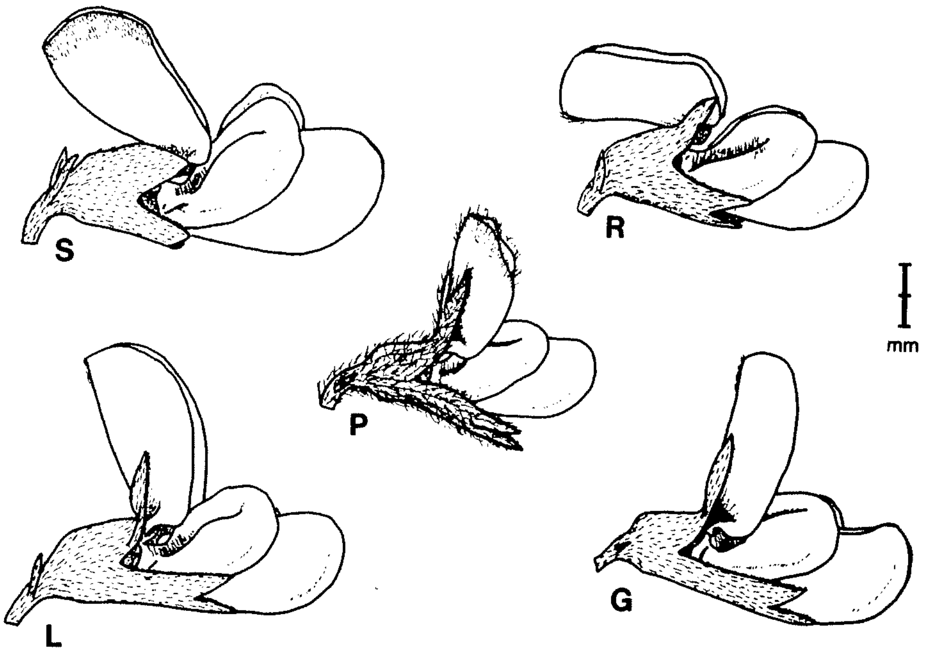

Figure 8 Flower structure of the five species of the genus *Dichilus*. Note the differences in the calyx length, vestiture and shape of the keel apex. [S, *D. strictus* (*Schutte 155*); R, *D. reflexus* (*Schutte 187*); L, *D. lebeckioides* (*van Wyk 1538b*); P, *D. pilosus* (*Schutte 94*); G, *D. gracilis* (*Schutte 227*)].

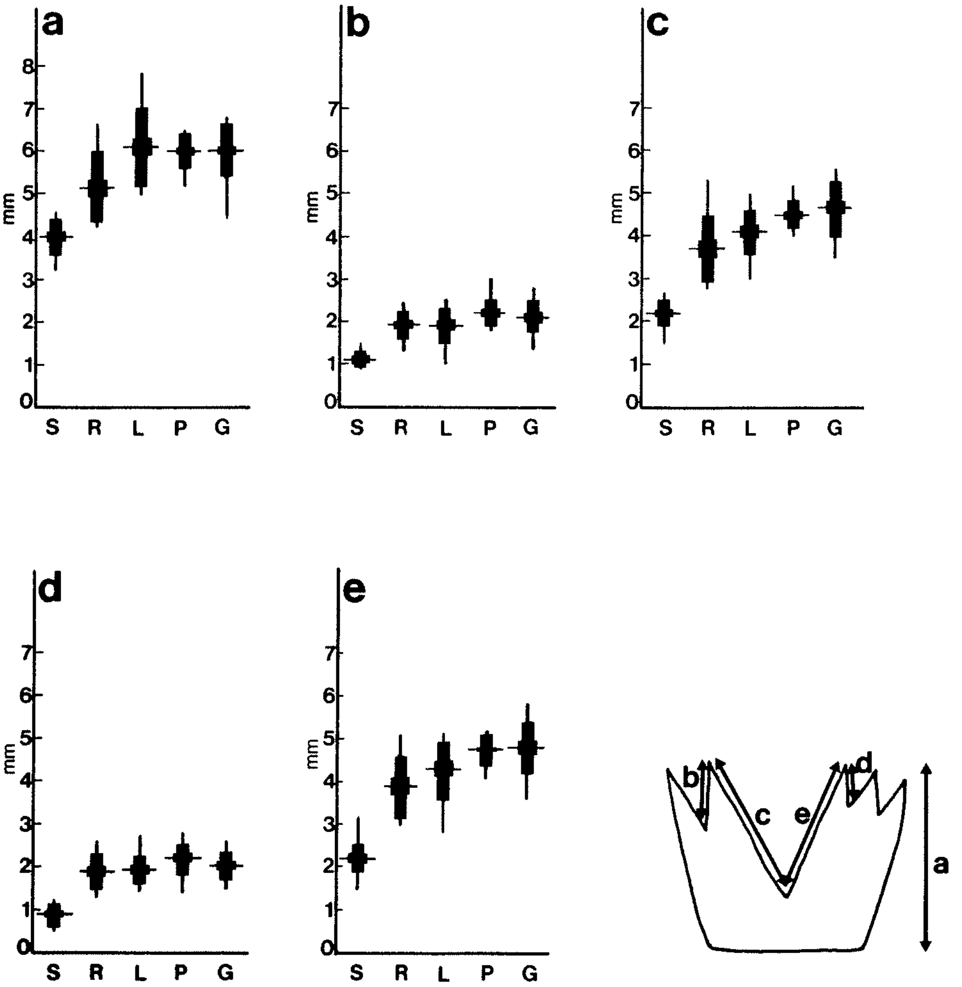

Figure 9 Variation in various measurements of the calyx in the genus *Dichilus*. The range, mean, standard deviation and standard error of 15 measurements for each species are given. (a, total length; b, upper sinus; c, length of upper lip; d, lower sinus; e, length of lower lip; S, *D. strictus*; R, *D. reflexus*; L, *D. lebeckioides*; P, *D. pilosus*; G, *D. gracilis*].

Figure 13 illustrates all cladograms of minimal length which were inferred from the data sets in Tables 2 to 5. It is important to note that all reversals (characters 1, 4 and 11) are here indicated as convergences. We find it more logical to assume that these particular character states have been lost more than once even though this increases the length of the trees. The alternative (a 'reappearance' of leaflets, hairs and adaxial leaf traces) seems less likely. Minimal lengths of the PAUP results therefore differ from the lengths shown in Figure 13. Each matrix furnished two cladograms and these had identical topologies regardless of the choice of outgroup. Only one of the two topologies (B, D and F in Figure 13) were fully resolved. The other (A, C, E, G and H) showed polychotomy with respect to *D. lebeckioides*, *D. pilosus* and *D. gracilis*. The two cladograms stayed unchanged even when the polarization of some morphoclines were reversed or when certain characters were omitted.

When *Melolobium* was used as outgroup (Figure 13A & B) the consistency index was 0.826 (minimal length 23) and each of the two cladograms showed three convergences (characters 1, 4 & 11). *D. strictus*, *D. lebeckioides*, *D. gracilis* and *D. pilosus* have at least one autapomorphy each. Cladogram B is clearly more acceptable because A is not fully resolved. In the cladograms with *Argyrolobium* as outgroup (Figure 13C & D), only characters 1 and 8 were polarized differently. In this case, the consistency index was 0.864 (minimal length 22) and the number of convergences in the second cladogram (D) reduced to two. *D. strictus* is now defined by two autapomorphies, *D. lebeckioides* and *D. pilosus* by one each and *D. gracilis* by four. *D. reflexus* has two apomorphies which show convergence in *D. strictus*, *D. lebeckioides* and *D. gracilis*. The topology which has *D. gracilis* and *D. lebeckioides* as sister groups (D) is again fully resolved. With *Lebeckia* as outgroup (Figure 13E & F), characters 5 and 8 were polarized differently. The consistency index was 0.840 but the minimal number of character state changes increased to 25. *D. lebeckioides* and *D. pilosus* are now defined by a single autapomorphy each and *D. gracilis* by three. In this case both *D. strictus* and *D. reflexus* have no autapomorphies. When no particular outgroup was used and characters 1, 4 and 11 omitted, only one partially resolved cladogram resulted (consistency index 1.000). In this case, the same polychotomous clade (Figure 13G & H) is formed as in A, C and E.

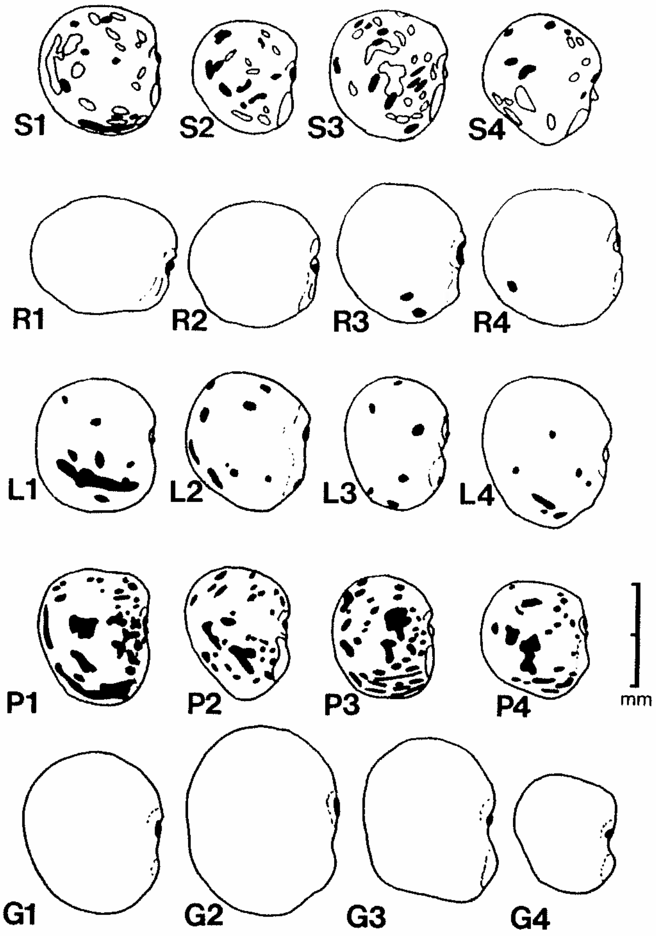

Figure 10 Variation in the size, shape and surface colouration of seeds in the genus *Dichilus*. [S, *D. strictus*: olive-green, mottled green and yellow (S1, *Schutte 154*; S2, *Schutte 138*; S3, *Schutte 157*; S4, *Schutte 140*). R, *D. reflexus*: green to almost black, occasionally mottled black (R1, *Schutte 177*; R2, *Schutte 186*; R3, *Wood 8978*; R4, *Schutte 177*). L, *D. lebeckioides*: brown to dark brown, often mottled black (L1, *Schutte 134*; L2, *Schutte 153*; L3, *Schutte 135*; L4, *Schutte 153*). P, *D. pilosus*: brown, distinctly mottled black (P1, *Schuttte 94*; P2, *Schutte 95*; P3, *Schutte 122*; P4, *Schutte 94*). G, *D. gracilis*: orange to pale yellow-brown, not mottled (G1, *Schutte 345*; G2, *Shearing C*; G3, *Schutte 338*; G4, *Schutte 335*)].

The topology with *D. gracilis* and *D. lebeckioides* as sister groups is therefore proposed as the most likely representation of the phylogeny of the genus *Dichilus*. The alternative (with *D. gracilis* and *D. pilosus* as sister groups) is not supported by any apomorphies. Furthermore, the geographical distribution of *D. gracilis, D. lebeckioides* and *D. pilosus* (Figure 14), shows that *D. lebeckioides* and *D. gracilis* are more likely to be sister groups because these species are partly sympatric. An independent origin for *D. pilosus* is more likely. *Argyrolobium* proved to be the most suitable outgroup for *Dichilus* (minimum of 22 character state changes, cladograms C & D) but only marginally better than *Melolobium* and *Lebeckia* (23 and 25 character state changes respectively).

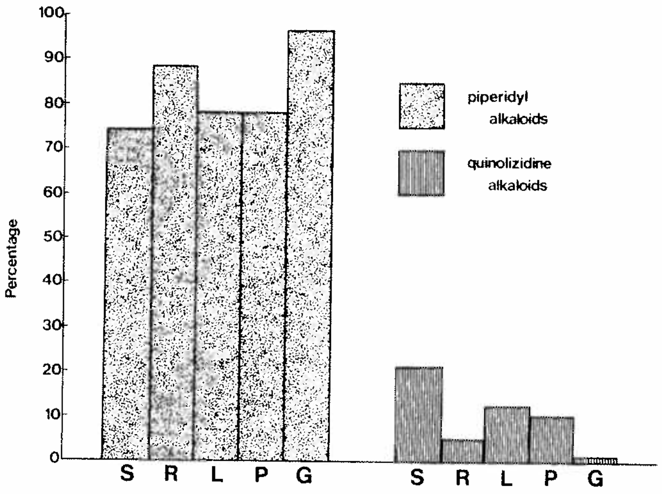

Figure 11 Concentration piperidyl and quinolizidine alkaloids in the species of *Dichilus*. The result represents mean values of three extracts from each of the five species as in van Wyk *et al.* (1988). [S, *D. strictus*; R, *D. reflexus*; L, *D. lebeckioides*; P, *D. pilosus*; G, *D. gracilis*].

In addition to the characters that were used for cladogram construction, various other characters also support the proposed cladogram. From the basal node to the terminal one, the following general trends (as illustrated earlier) are evident: a reduction in the length of the inflorescence and the length of bracts and bracteoles, an increase in the length of calyx lobes and the depth of sinuses, a reduction in the number of seeds per pod, an increase in piperidyl alkaloids and a decrease in quinolizidine alkaloids. The proposed phylogeny is so well corroborated by the available evidence that other possibilities seem unlikely. Our results provide a sound empirical basis for assuming that the present taxonomy of the genus *Dichilus* has considerable predictive value.

Acknowledgements

The assistance of Dr Herman van der Bank with data analyses and financial support from the Rand Afrikaans University is acknowledged.

References

CRISP, M.D. & WESTON, P.H. 1987. Cladistics and legume systematics, with an analysis of the Bossiaeeae, Brongniartieae and Mirbelieae. In: Advances in legume systematics 3, ed. Stirton, C.H., pp. 65–130, Royal Botanic Gardens, Kew.

CRONQUIST, A. 1987. A botanical critique of cladism. *Bot. Rev.* 53: 1–52.

DAHLGREN, R. 1970. Current topics — parallelism, convergence, and analogy in some South African genera of Leguminosae. *Bot. Not.* 123: 551–568.

ENGELMAN, L. 1981. Cluster analysis of cases. In: BMDP statistical software, eds Dixon, W.J. *et al.*, pp. 456–463, University of California Press, London.

KLUGE, A.G. & FARRIS, J.S. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* 18: 1–32.

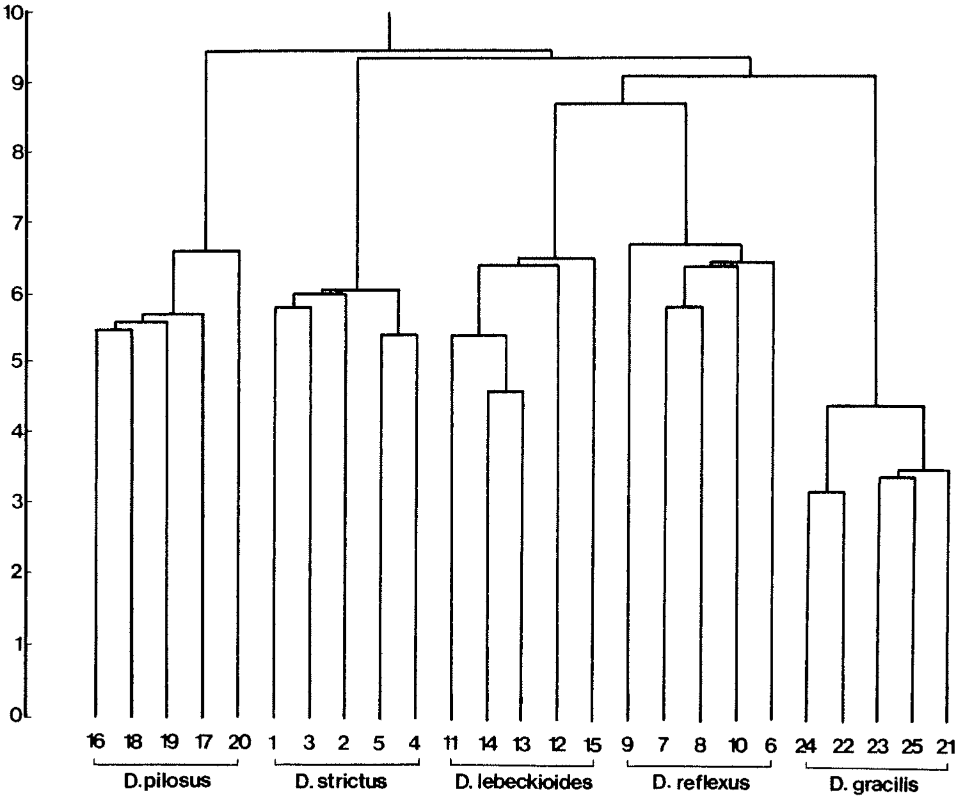

Figure 12 Phenogram of 25 provenances and 67 characters to illustrate phenetic relationships between the five species of the genus *Dichilus*. Scale shows amalgamation distance.

LAVIN, M. 1987. A cladistic analysis of the tribe Robinieae (Papilionoideae, Leguminosae). In: Advances in legume systematics 3, ed. Stirton, C.H, pp. 31–64, Royal Botanic Gardens, Kew.

LINDER, H.P. 1988. A review of cladistics for botanists. *S. Afr. J. Bot.* 54: 208–220.

POLHILL, R.M. 1976. Genisteae (Adans.) Benth. and related tribes (Leguminosae). *Bot. Syst.* 1: 143–368.

POLHILL, R.M. 1981a. Tribe 29. Crotalarieae (Benth.) Hutch. In: Advances in legume systematics 1, eds Polhill, R.M. & Raven, P.H., pp. 399–402, Royal Botanic Gardens, Kew.

POLHILL, R.M. 1981b. Papilionoideae. In: Advances in legume systematics 1, eds Polhill, R.M. & Raven, P.H., pp. 191–208, Royal Botanic Gardens, Kew.

SCHUTTE, ANNE LISE 1988. 'n Taksonomiese studie van die genus *Dichilus* DC. (Fabaceae — Crotalarieae). M.Sc. thesis, Rand Afrikaans University.

SCHUTTE, ANNE LISE & VAN WYK, B-E. 1988. A synopsis of the genus *Dichilus* (Fabaceae — Crotalarieae). *S. Afr. J. Bot.* 54: 182–186.

SNEATH, P.H.A. & SOKAL, R.R. 1973. Numerical taxonomy. Freeman, San Francisco.

SWOFFORD, D.L. 1985. PAUP, phylogenetic analysis using parsimony, version 2.4. Illinois Natural History Survey, Illinois.

VAN WYK, B-E. & SCHUTTE, ANNE LISE 1989. Taxonomic relationships amongst some genera of Leguminosae tribe Crotalarieae and *Argyrolobium* (Genisteae). *Kew Bull.* 44(3): 397–423.

VAN WYK, B-E., VERDOORN, G.H. & SCHUTTE, ANNE LISE 1988. Chemotaxonomic value of alkaloids in the genus *Dichilus*. *Biochem. Syst. & Ecol.* 16: 471–474.

ZANDEE, M. & GEESINK, R. 1987. Phylogenetics and legumes: A desire for the impossible? In: Advances in legume systematics 3, ed. Stirton, C.H., pp. 131–167, Royal Botanic Gardens, Kew.

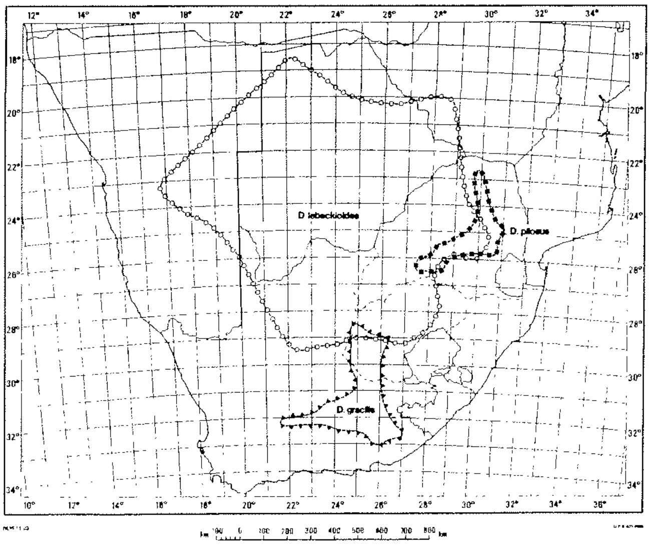

Figure 14 Approximate geographical distribution of *D. lebeckioides*, *D. pilosus* and *D. gracilis*. (Data from Schutte & van Wyk 1988).

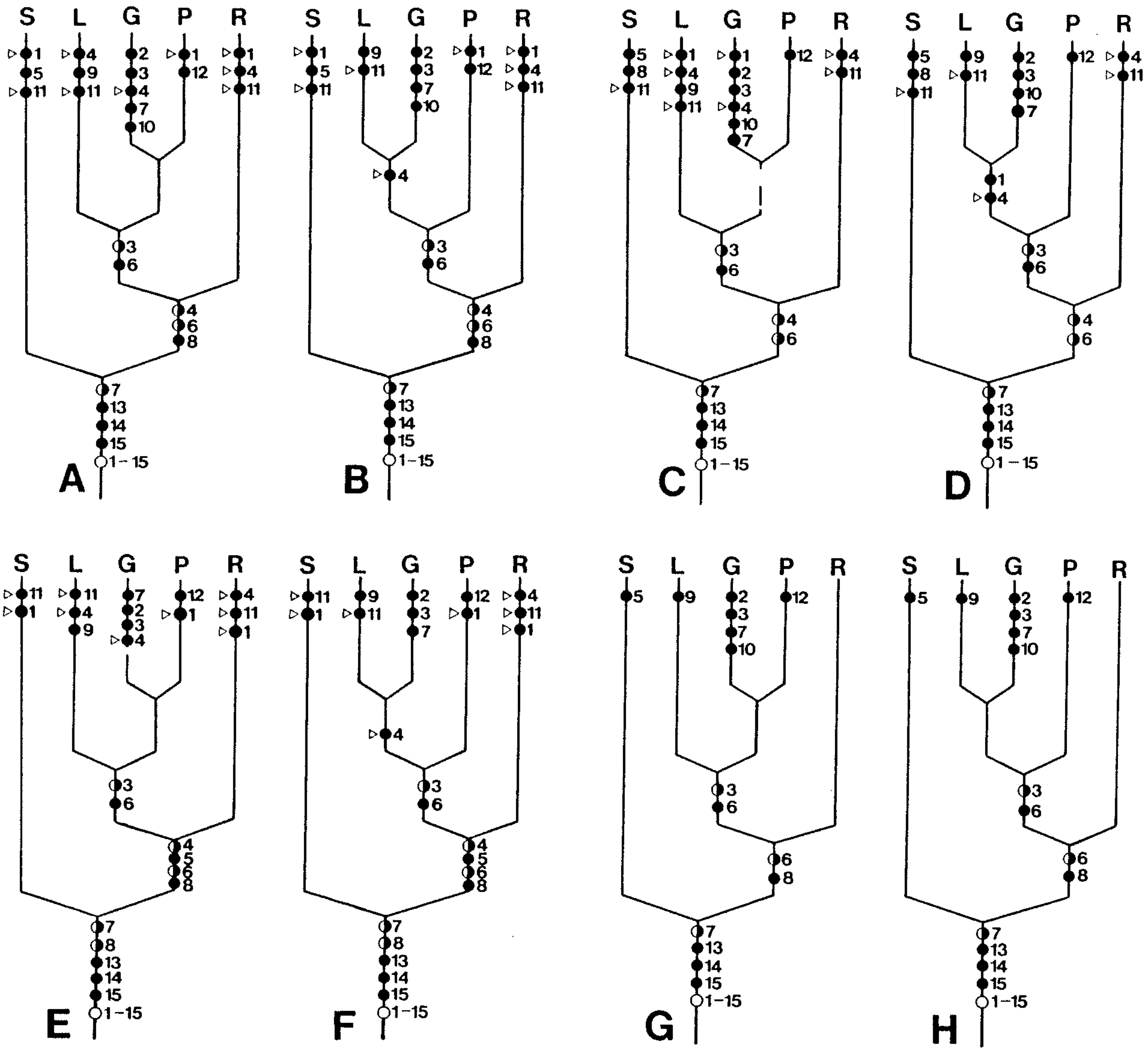

Figure 13 All cladograms of minimal length obtained in analyses of the data in Tables 2 to 5, showing hypothetical relationships between the species of *Dichilus*, with *Melolobium* (A & B), *Argyrolobium* (C & D) and *Lebeckia* (E & F) as outgroups. In G & H, no specific outgroup was used (see text and Table 5). [(○), plesiomorphic state; (◐), intermediate state; (●), apomorphic state; (▷), convergences. S, *D. strictus*; R, *D. reflexus*; L, *D. lebeckioides*; P, *D. pilosus*; G, *D. gracilis*].

Appendix 1 Matrix of 25 provenances and 67 characters used in constructing the phenogram of the genus *Dichilus* in Figure 12. (Provenances as in Figure 1; characters and character states as in Table 1)

Provenance	Character number																							
number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
01	2	2	26.0	15.9	20.0	12.0	2.1	1.4	3	21.5	11.8	3.5	2.4	7.0	5.1	10.0	3.2	9.0	4.1	1	1	1	1	2
02	2	2	28.0	16.2	25.0	12.0	2.1	1.4	3	24.0	12.6	3.0	2.0	9.6	6.4	5.0	2.8	7.8	4.4	1	1	1	1	2
03	2	2	25.0	15.7	19.0	12.0	2.6	1.3	3	20.0	12.2	4.0	2.4	7.3	5.2	5.5	2.9	8.3	4.6	1	1	1	1	2
04	2	2	18.0	15.8	13.0	8.4	2.6	2.0	3	14.5	11.7	3.0	2.5	6.0	4.8	4.0	3.2	6.0	3.9	1	1	1	1	2
05	2	2	19.0	16.5	12.0	9.5	2.3	1.8	3	15.0	13.2	3.0	2.5	7.5	5.5	4.5	3.2	6.3	4.7	1	1	1	1	2
06	2	2	13.5	11.7	15.5	10.9	1.4	1.1	1	10.5	8.3	3.5	3.0	3.2	2.8	5.5	4.0	2.6	2.1	2	1	1	1	2
07	2	2	26.0	16.0	23.5	16.2	1.3	1.0	2	14.5	10.1	6.5	3.3	4.0	3.1	8.5	5.0	3.6	2.1	2	1	1	1	2
08	2	2	21.0	14.7	24.0	14.6	1.5	1.0	1	15.0	10.7	4.5	3.5	3.4	3.0	5.5	3.8	3.6	2.8	2	1	1	1	2
09	2	2	22.5	12.6	23.5	12.6	1.5	1.0	1	16.5	9.7	5.0	3.2	4.5	3.1	7.0	4.2	3.8	2.5	2	1	1	1	2
10	2	2	21.0	15.7	18.5	14.5	1.6	1.1	1	15.5	10.3	6.5	3.5	3.3	3.0	6.5	3.9	4.2	2.7	2	1	1	1	2
11	2	2	17.0	13.6	18.0	12.6	1.4	1.1	1	13.5	10.5	3.5	2.6	5.0	4.1	4.5	3.5	4.8	3.1	3	1	1	1	1
12	2	2	20.0	12.5	14.0	9.1	1.7	1.4	1	18.5	10.6	3.0	2.3	6.2	4.7	4.5	2.7	6.2	4.0	3	1	1	1	1

Appendix 1 Continued

Provenance	Character number																							
number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
13	2	2	19.5	12.4	14.5	10.3	1.8	1.2	2	14.5	10.0	2.5	2.1	7.0	4.9	5.5	2.7	5.7	3.9	3	1	1	1	1
14	2	2	17.0	12.2	16.5	9.2	1.8	1.4	1	13.0	9.3	2.5	2.1	6.0	4.5	4.0	2.4	5.7	4.1	3	1	1	1	1
15	2	2	20.0	12.7	16.0	9.3	2.0	1.4	1	14.5	8.8	3.0	2.0	5.7	4.3	4.0	2.3	5.7	4.1	3	1	1	1	1
16	2	2	15.5	9.0	18.0	10.7	1.4	1.0	3	10.0	6.7	5.0	3.6	2.7	1.9	10.0	4.0	3.2	1.9	4	2	2	2	1
17	2	2	23.0	14.2	24.0	14.8	1.3	1.0	3	15.0	9.3	8.0	4.5	2.5	2.1	8.5	5.4	2.4	1.8	4	2	2	2	1
18	2	2	14.5	10.5	17.0	11.7	1.1	0.9	3	10.0	7.2	5.0	3.6	3.3	2.1	4.5	3.5	2.6	2.0	4	2	2	2	1
19	2	2	18.0	12.9	16.5	12.3	1.3	1.0	3	11.5	7.5	7.0	3.8	2.6	2.0	9.5	5.8	1.7	1.3	4	2	2	2	1
20	2	2	21.5	12.7	23.0	12.7	1.4	1.0	3	12.5	7.4	6.5	4.1	2.2	1.8	10.0	5.0	2.0	1.5	4	2	2	2	1
21	1	1	15.0	11.3	19.0	12.6	1.2	0.9	1	11.0	7.9	6.5	3.7	2.9	2.2	7.0	3.5	4.7	2.5	4	3	1	1	1
22	1	1	23.5	13.0	22.0	13.7	1.2	1.0	1	13.5	8.9	7.5	4.2	3.3	2.2	8.5	4.9	3.3	1.9	4	3	1	1	1
23	1	1	15.0	10.0	16.0	11.3	1.1	0.9	1	10.5	7.2	4.5	3.3	3.0	2.2	5.5	3.3	3.3	2.2	4	3	1	1	1
24	1	1	20.5	12.6	22.0	14.4	1.2	0.9	1	13.0	8.6	7.5	4.4	2.8	2.1	9.0	4.4	3.3	2.0	4	3	1	1	1
25	1	1	14.5	10.3	16.0	11.4	1.1	0.9	1	11.5	7.6	5.5	3.5	2.8	2.2	5.0	3.0	4.3	2.7	4	3	1	1	1

Provenance	Character number																				
number	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
01	32.0	17.6	6.5	5.0	9	5	10.5	3	8.9	6.7	6	9.1	2	4.3	2	1.83	2.00	0.980	0.74	2.12	1.56
02	32.0	23.5	10.0	11.5	8	6	10.5	3	8.8	7.0	6	9.0	2	3.2	2	1.50	1.88	0.977	0.78	2.81	2.19
03	30.0	23.1	12.0	12.0	7	4	10.0	3	8.6	7.0	5	9.0	2	3.3	2	2.22	3.14	0.960	0.78	2.73	2.12
04	39.0	23.6	11.0	9.0	8	6	11.5	3	9.9	8.0	6	10.0	2	4.1	2	2.05	3.58	0.990	0.80	2.44	1.95
05	33.0	20.9	12.0	5.0	7	4	11.0	3	8.5	7.9	7	9.8	2	4.0	2	2.00	3.13	0.870	0.81	2.45	1.98
06	24.0	19.4	8.5	7.0	5	4	10.0	1	8.6	6.9	5	9.0	2	4.6	1	1.85	1.81	0.960	0.77	1.96	1.50
07	30.0	20.1	8.0	10.0	7	4	10.5	1	8.7	7.3	4	8.8	2	4.6	1	1.76	2.16	0.993	0.83	1.90	1.57
08	31.5	23.3	8.0	15.0	10	5	9.5	1	8.5	6.7	4	8.5	2	4.5	1	2.33	2.47	1.000	0.79	1.89	1.49
09	46.0	26.0	24.0	14.0	6	3	10.5	1	9.0	7.0	5	8.9	2	6.1	1	2.00	1.81	1.010	0.79	1.46	1.15
10	32.0	24.9	9.0	16.0	8	5	8.5	1	7.8	6.4	4	8.2	2	4.2	1	1.50	1.60	0.950	0.78	1.95	1.52
11	16.5	14.4	3.5	0.0	1	1	11.0	1	8.5	6.7	6	9.9	2	5.1	1	1.90	2.06	0.860	0.68	1.94	1.31
12	20.0	14.6	5.0	5.0	3	1	12.5	1	11.8	7.6	6	11.8	2	7.8	1	2.38	2.38	1.000	0.64	1.51	0.97
13	17.0	13.2	5.5	3.5	3	1	10.5	1	8.8	7.2	7	9.1	2	5.4	1	2.55	2.19	0.965	0.79	1.68	1.32
14	17.0	12.9	5.0	2.0	3	1	10.5	1	9.7	7.7	6	9.8	2	6.6	1	1.89	2.10	0.987	0.78	1.51	1.18
15	21.5	15.3	9.0	10.0	3	2	11.0	1	9.3	6.9	6	9.3	2	6.0	1	2.11	2.26	0.995	0.74	1.56	1.16
16	24.0	19.4	8.5	7.0	2	1	10.0	2	8.7	7.0	5	8.7	2	6.2	1	2.17	1.86	1.000	0.80	1.40	1.13
17	30.0	20.1	8.0	10.0	1	1	10.5	2	7.8	5.7	4	7.7	2	5.9	1	2.14	2.13	1.010	0.74	1.31	0.97
18	31.5	23.3	8.0	15.0	3	1	9.5	2	7.6	6.0	5	7.9	2	6.0	1	2.54	2.78	0.955	0.76	1.33	1.01
19	46.0	26.0	24.0	14.0	3	1	9.5	2	8.3	6.6	4	8.0	2	5.7	1	1.95	2.38	1.040	0.82	1.40	1.20
20	32.0	24.9	9.0	16.0	4	2	9.5	2	8.1	6.6	4	7.9	2	5.5	1	2.17	2.27	1.025	0.84	1.44	1.20
21	17.5	15.0	9.0	0.0	1	1	9.5	1	9.1	6.9	4	9.1	1	6.5	1	2.40	2.49	0.997	0.75	1.40	1.06
22	15.0	13.4	8.0	0.0	1	1	10.5	1	9.0	7.1	4	9.5	1	6.2	1	2.05	1.93	0.945	0.75	1.54	1.14
23	16.0	14.0	6.5	0.0	1	1	10.0	1	8.5	6.5	5	8.7	1	5.4	1	2.19	2.38	0.980	0.76	1.63	1.24
24	19.0	16.2	11.0	0.0	1	1	11.0	1	9.6	6.9	4	9.7	1	6.3	1	2.30	2.54	0.983	0.71	1.56	1.11
25	20.0	16.0	10.0	0.0	1	1	11.5	1	8.8	7.3	5	9.5	1	5.9	1	2.13	2.60	0.633	0.76	1.81	1.23

Provenance	Character number																					
number	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67
01	2.07	3.5	2.2	1.8	37.0	29.2	4.5	3.7	6	5	9.4	7.8	1	1	2.6	1.3	1.1	2	4	1	1	33
02	2.75	5.5	2.9	1.8	32.5	27.6	4.0	3.5	7	6	10.0	7.9	1	1	2.4	1.2	1.2	2	4	1	1	35

Appendix 1 Continued

Provenance	Character number																				
number	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67
03	2.61	3.5	3.0	2.3	33.0	25.8	4.5	3.6	6	5	8.8	7.3	1	1	2.5	1.3	1.3	2	4	1	1	36
04	2.41	3.5	5.6	2.5	33.5	26.3	4.0	3.3	7	5	11.2	8.2	1	1	2.5	1.3	1.2	2	4	1	1	36
05	2.13	2.5	3.0	2.2	29.0	26.1	4.0	3.8	6	5	8.3	7.0	1	1	2.5	1.3	1.2	2	4	1	1	36
06	1.87	3.5	3.3	2.2	29.0	24.6	4.5	4.3	6	4	6.4	5.7	1	1	2.4	1.1	1.5	3	1	1	1	25
07	1.88	4.0	5.5	2.2	36.0	29.6	5.5	4.7	7	6	8.0	6.3	1	1	2.6	1.1	1.5	3	1	1	1	33
08	1.89	5.0	2.3	1.9	33.0	26.8	5.0	4.3	7	5	8.3	6.2	1	1	2.3	1.1	1.4	3	1	1	1	42
09	1.48	3.5	2.3	1.7	33.0	25.3	5.5	4.6	7	5	6.6	5.5	1	1	2.5	1.0	1.3	3	1	1	1	40
10	1.86	3.0	5.0	1.6	28.5	22.7	5.5	4.7	6	5	6.8	4.8	1	1	2.5	1.1	2.0	3	1	1	1	27
11	1.67	2.0	1.9	1.7	42.5	34.4	4.3	3.7	9	7	12.5	9.4	1	1	2.6	1.3	1.2	3	2	2	1	30
12	1.51	3.0	2.5	1.8	50.0	43.6	5.0	4.7	9	7	11.1	9.3	1	1	2.7	1.2	1.3	3	2	2	1	28
13	1.62	2.5	1.7	1.3	42.5	31.9	4.0	3.2	9	7	15.6	10.1	1	1	2.6	1.3	1.2	3	2	2	1	28
14	1.49	2.0	2.1	1.8	41.0	34.3	4.5	3.7	8	7	13.7	9.3	1	1	2.8	1.3	1.2	3	2	2	1	32
15	1.56	3.0	2.1	1.7	40.0	32.1	3.5	3.4	9	8	11.4	9.5	1	1	2.3	1.3	1.2	3	2	2	1	31
16	1.40	2.0	2.3	1.9	35.0	30.1	4.0	4.0	6	5	8.8	7.6	2	2	2.8	1.4	1.2	3	3	1	1	65
17	1.32	2.5	1.5	0.9	30.0	27.8	4.0	3.2	5	5	10.0	8.9	2	2	2.5	1.4	1.2	3	3	1	1	57
18	1.27	2.5	2.5	1.5	29.0	24.9	4.0	3.6	6	5	8.3	6.9	2	2	2.5	1.4	1.2	3	3	1	1	63
19	1.45	3.5	2.1	1.6	32.5	25.7	3.5	3.2	6	5	10.0	7.9	2	2	2.6	1.5	1.1	3	3	2	1	76
20	1.47	3.0	1.8	1.5	31.0	24.9	3.5	3.2	6	5	10.8	7.9	2	2	2.5	1.4	1.2	3	3	1	1	66
21	1.39	3.0	1.6	0.8	21.0	16.1	4.5	4.0	5	4	5.3	4.0	1	1	2.9	1.3	1.1	1	1	2	2	29
22	1.45	2.5	1.0	0.7	26.5	21.3	5.5	4.5	5	4	6.0	4.8	1	1	3.0	1.2	1.1	1	1	2	2	29
23	1.60	2.5	1.3	0.9	25.5	20.0	4.5	3.9	5	4	6.7	5.2	1	1	2.8	1.3	1.2	1	1	2	2	26
24	1.53	2.5	1.1	0.8	25.5	22.4	5.0	4.5	5	4	5.7	5.0	1	1	3.3	1.3	1.1	1	1	2	2	31
25	1.50	2.5	1.4	1.1	26.0	21.9	4.5	4.1	5	4	6.5	5.3	1	1	2.9	1.3	1.1	1	1	2	2	29