Chapter 2. Phenetic analysis and species delimitation in the *Drosera peltata* complex (Droseraceae)

2.1 Introduction

The morphological species concept has a long history of use in resolving taxonomic questions (Mayr and Ashlock 1991; Mayr 1993). This concept is based on the premise that taxa are defined by suites of co-varying characters that can be qualified and quantified by statistical and graphical analysis: that is, phenetic analysis (Sneath and Sokal 1973). The basic premise of defining taxonomic entities is that their samples, or Operational Taxonomic Units (OTUs), cluster together in branching tree diagrams, or phenograms, and also that they group together in ordination space, and are distinctly separated from other OTUs of other taxa (Quicke 1993). The delimitation of species is a fundamental aspect of biology, and is an essential first step before evolutionary relationships can be investigated (Crisp and Weston 1993). The integrity of the putative taxa of the *D. peltata* complex therefore can be tested by use of phenetic analysis. This would also clarify the use of names applied to this complex for over 200 years (see Chapter 1).

Cluster analysis and ordination are commonly used quantitative methods in biology for testing the membership and robustness of groups (Sneath and Sokal 1973; Stuessy 1990; Quicke 1993). There are several steps to accomplish phenetic analyses (Table 4). Once the OTUs have been selected and the characters determined and measured, an OTU x character matrix is produced. Depending upon the types of characters scored, e.g. overlapping (quantitative) or non-overlapping (qualitative), the data may need to be modified to downweight multistate characters or to implement gap-coding (Thiele 1993; Ariyanti and Conn 2005). The data matrix then needs to be transformed by applying an association measure to determine the degree of similarity or dissimilarity, or distance, between the individual OTUs. The Gower's metric association is commonly used in phenetic analysis because it is range standardized, and is thus applicable to continuous biological data. It also works for datasets containing both overlapping and non-overlapping data (Gower 1971). Finally the OTUs are grouped based on either similarity (agglomerative methods) or dissimilarity (divisive methods) and the results are presented as a phenogram (Quicke 1993).

Table 4. Summary of the steps required in phenetic analysis (after Quicke 1993).

Step	Action
1	Selection of OTUs and characters to be measured.
2	Measurement of characters to produce an OTU x character matrix.
3	Weighting, where needed, of multistate characters.
4	Transforming the OTU x character matrix using the Gower's metric association measure.
5	Applying a hierarchical, agglomerative clustering algorithm to the transformed data matrix to find relationships between OTUs. The data from this step is commonly displayed as a **phenogram**.
6	Hybrid multidimensional scaling (HMDS) of the transformed data matrix to produce an **ordination plot.**
7	Interrogation of the results of ordination using the stress value, r-squared value and Kruskal-Wallis coefficient.

Ordination analyses show relationships between OTUs in a single step. The most robust ordination method for biological data has been found to be semi-strong hybrid multidimensional scaling (SSH MDS), which is both robust and works with combined overlapping and non-overlapping character datasets (Faith *et al.* 1987; Belbin 1991). The ordination results may be shown in two, or three dimensions. The results can be assessed by a number of tests. A measure of fit, or stress value, may be calculated by comparing the distances between OTUs in both transformed and untransformed datasets; the smaller the number the better the fit (Quicke 1993). The relative strength of each character in the analysis can be determined to identify the factors behind the ordination pattern. And, the characters can also be ranked according to the hypothesis that they come from the same population using the Kruskal-Wallis measure: the higher the number the more likely the character(s) and hence the OTU come from a different population (Kruskal and Wallis 1952). Finally, an advantage of ordination and cluster analyses is that discrete subsets of the data matrix may be removed, typically where they form clear and distinct groups. The remaining data can then be reanalysed to resolve internal patterns within groups (Kruskal and Wallis 1952).

Phenetic analysis has been used for a wide range of plant groups, such as *Telopea* species (Proteaceae) (Crisp and Weston 1993), *Acacia* species (Fabaceae: Mimosoideae) (Conn and Tame 1996), *Bromus catharticus* (Poaceae) (Aulicino and Arturi 2002), *Homopholis* and *Whaylleya* (Poaceae) (Wills *et al.* 2000), *Lepidosperma* species (Cyperaceae) (Hodgon *et al.* 2006), *Leucopogon* species (Epacridaceae)

(Brown and Wiecek 1996), *Dendrobium speciosum* (Orchidaceae) (Burke and Adams 2002), *Banksia integrifolia* (Proteaceae) (Thiele and Ladiges 1994), *Zieria prostrata* (Rutaceae) (Hogbin and Crisp 2003), *Drosera indica* (Droseraceae) (Susandarini *et al.* 2002), and *Sarracenia* species (Sarraceniaceae) (Schnell and Krider 1976). The authors cited above chose phenetic analyses in order to test competing classifications (Burke and Adams 2002), test and define species limits (Schnell and Krider 1976; Crisp and Weston 1993), or to investigate species complexes (Thiele and Ladiges 1994; Conn and Tame 1996; Susandarini *et al.* 2002; Hogbin and Crisp 2003; Downing *et al.* 2004). The degree of similarity between specimens studied is determined using clustering and ordination, both statistical processes now conveniently and quickly done using readily available computing power (Belbin 1995).

Susandarini *et al.* (2002) used phenetic analysis to explore the morphological variation of the polymorphic *Drosera indica* complex (Droseraceae, subgenus *Drosera,* section *Arachnopus*) using 62 samples from across its range. Twenty-six morphological characters were measured. The phenetic data matrix was analysed by cluster analysis and multi-dimensional scaling (MDS) ordination. They found that the plant samples occurred in three groups, on the phenogram and in ordination space. The groupings correlated with seed surface characters and leaf base characters and two of the groups were considered likely to represent new taxon. The third group, which was heterogeneous with relation to the characters of the OTUs it contained, was not investigated further. The authors could have investigated the data further by running further iterations of the dataset after clearly grouped OTUs were excluded (Belbin 1995). In addition, the authors missed the character of non-glandular emergences at the base of the leaves, which have been since used to differentiate a new species in the complex, *D. hartmeyerorum* (Schlauer 2001). The *Drosera peltata* complex has much in common with the *D. indica* complex, and so the methodology and results of Susandarini *et al.* (2002) are of much interest in this project.

The *D. peltata* complex occurs within subgenus *Ergaleium,* section *Ergaleium* (Schlauer 1996). To date only one study, Conn (1984), has been published testing taxonomic limits of another species within this section of the genus. Conn (1984) tested the hypothesis that *D. macrantha* consisted of two subspecies (Marchant and

George 1982). He did this by measuring sepal shape, and sepal marginal hairs from 160 specimens from across their range. The results were presented as scatter diagrams. The two subspecies did not form discrete groups on these plots and Conn suggested that the two subspecies did not deserve recognition. Since then, Lowrie (1987; 1998) has suggested that the Western Australian populations of *D. macrantha* may contain cryptic species based on other characters. In addition, Conn conducted a preliminary phenetic analysis of members of the *Drosera peltata* complex of herbarium collections from across the range, however, this study was not published (B. Conn pers. comm. 2002).

The aims of this chapter are to test the taxon limits of putative taxa within the *Drosera peltata* complex and to determine intra- and inter-specific limits.

2.2 Materials and methods

2.2.1 Specimens studied

Two hundred and thirteen herbarium specimens, from 98 collections, of the *Drosera peltata* complex, and 22 herbarium specimens of comparator species, from 8 collections, from NSW, NE, K, BM and BRU were studied. These specimens represented both morphological variation and geographic spread of the complex (Figure 7). Type specimens from K, BM, UPS and BRU were included in this study in order to test the application of names within the complex. A list of specimens studied is presented in Appendix 1.

All but six of the specimens examined were ascribed to a putative taxon based on the literature (Marchant and George 1982; Lowrie 1987; Gibson 1993; Lowrie 1998). The six unattributed herbarium specimens were included in the phenetic analyses as "*D. peltata*" (p1, p2, p3) and *D. peltata* "New Guinea" (pNG1, pNG2 and pNG3) (Table 5). The phenetic similarity of these taxa were assessed *a posteriori* based on the ordination and cluster analysis.

Table 5 Six OTUs in the phenetic analysis that were not assigned *a priori* to any entity within the *D. peltata* complex.

OTU code	Collection details	Herbarium
p1	"*D. petiolaris*", no. loc., no date, *anon. 176*	BRU
p2	No loc., 1770, *J. Banks and D. Solander s.n.*	BM514
p3	Penquite, 09x1841, *R. C. Gunn 448*	NSW146505
pNG1	West Papua: Mt. Sensenemes, Anggi Gigi Lake, 133.8833° E, -1.3667° S, alt. 2550m. 20i1962, *Sleumer and Vink, No BW 14225.*	NSW
pNG2	Slopes of Mt. Giluwa, c. 3200 m. alt., 6deg05'S, 143deg52'E, 16vi1967, *M. Coode 32526*	K
pNG3	Pilike Kula Swamp near Kondo Valley, 8 miles (c. 13 km) south of Tambul, c. 2500 m alt., 6° 05'S, 143° 55'E, 13iv1969, *J. S. Womersley 43534*	K

Each plant examined was considered as a single operational taxonomic unit (OTU). Where possible three plants were measured per herbarium collection to document variation within a population. It also provided a way to measure as full a range of characters for a population where not every individual exhibited all characters. In some cases herbarium specimens consisted of a single specimen, and they were still examined where they expanded geographic coverage of the complex or where they included the type specimen (such as *D. insolita*) (Figure 3).

Two hundred and thirty five OTUs were included in the phenetic analyses. These included all but two of the taxa within the *Drosera peltata* complex: *Drosera peltata* Thunb. var. *glabrata* Y. Z. Ruan and *D. peltata* Thunb. var. *multisepala* Y. Z. Ruan (Figure 4). No specimens of either variety were studied since they are only lodged in Chinese herbaria. A putative taxon code and a unique identifier was assigned to each OTU (Table 6; Appendix 1).

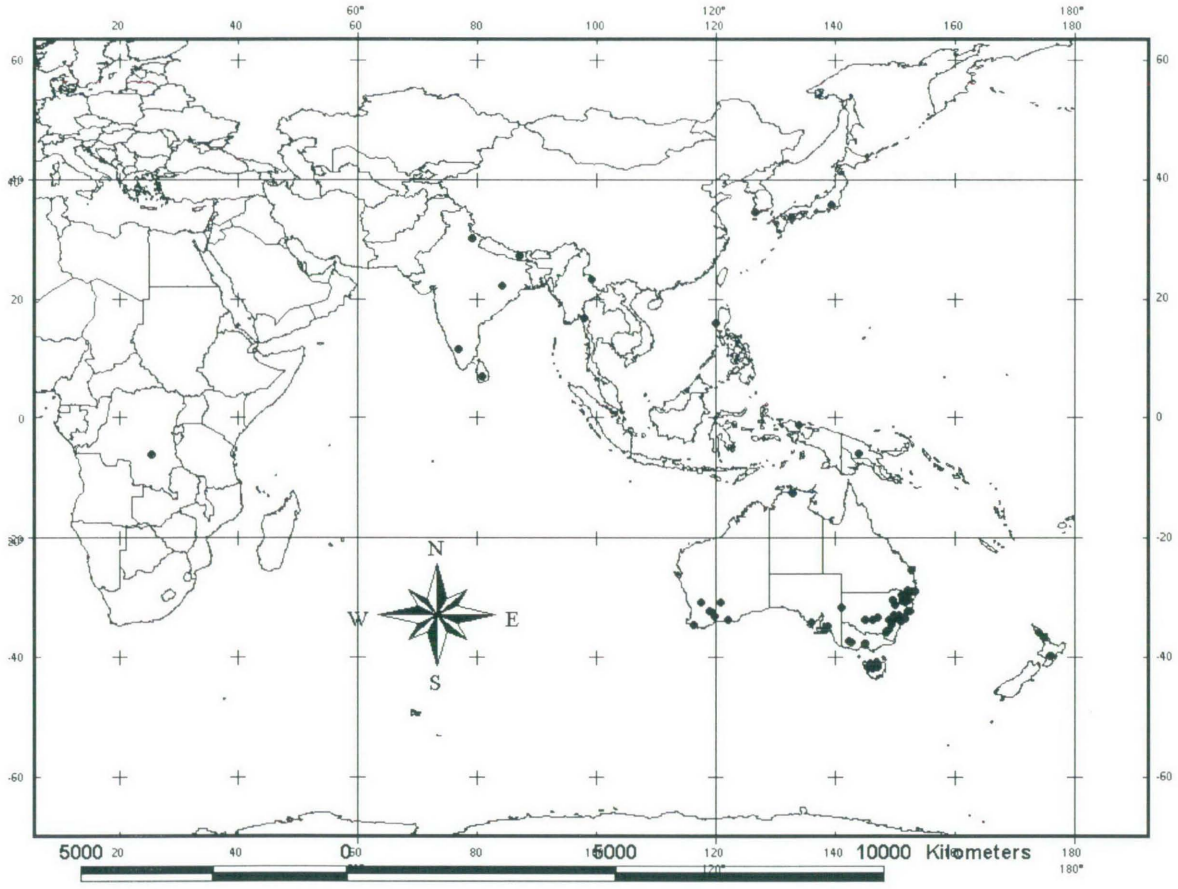


Figure 7. Geographic origin (black dots) of OTUs used in the phenetic study. Herbarium vouchers are listed in Appendix 1

2.2.2 Characters and scoring

In this study the literature was reviewed to determine an initial set of characters to analyze. The preliminary list of morphological characters was drawn from previous treatments of the genus *Drosera* and subgenus *Ergaleium* (Chapter 1) as well as circumscriptions, and descriptions, of members of the *Drosera peltata* complex (Planchon 1848; Diels 1906; Taton 1951; Conn 1981; Marchant and George 1982; Schlauer 1996; Lowrie 1998). In addition, several novel characters were added to the list as the process of data collection commenced (see Appendix 2).

Characters were scored from dried herbarium specimens for all cases. Some destructive sampling was conducted on my own samples in order to maximize the number of characters available per OTU. This typically involved removing some sepals and petals to observe the flower parts. The list of characters measured is presented in Appendix 2. The choice of characters measured is presented in the following section (with the corresponding character number in parentheses).

Table 6. Summary of OTUs used in the phenetic analysis of the *Drosera peltata* complex. Names in **bold** indicate that the **type** of the OTU was included in this study. Specimens= number of individual plants (often more than one per herbarium sheet).

Putative Taxon	Code	Vouchers	Specimens
Drosera andersoniana	AND	1	7
D. auriculata	au	19	35
D. bicolor	BIC	1	5
D. binata	bin	2	6
D. insolita	INS	1	1
D. microphylla	MIC	1	3
D. lunata	lun	1	3
D. lobbiana	lob	3	4
D. peltata	p1	1	1
D. peltata	p2	1	1
D. peltata	P3	1	1
D. peltata 'Black Mountain, A.C.T.'	pBM	28	51
D. 'foliosa'	fol	4	13
D. peltata 'gracilis'	gra	4	14
D. peltata 'Isla Gorge, Qld.'	pIG	3	11
D. peltata 'nipponica'	pN	9	20
D. peltata 'New Guinea1'	pNG1	1	1
D. peltata 'New Guinea2'	pNG2	1	1
D. peltata 'New Guinea3'	pNG3	1	1
D. peltata 'Red Rosette'	pRR	11	11
D. peltata	TYPE	1	1
D. peltata 'Western Australian Form'	pWA	3	9
D. peltata 'Western Sydney, N.S.W.'	pWS	3	8
D. salina	SAL	1	3

2.2.2.01 Root system characters

All members of *Drosera* subgenus *Ergaleium* are characterized by a root system that comprises a globous to ovoid tuber connected to the soil surface by a vertical stolon. Plants aestivate as a dormant tuber during seasonally unfavourable conditions. The size, shape and colour of tubers has been found to vary between many taxa (characters 1 and 2) (Planchon 1848; Diels 1906; Marchant and George 1982; Pietropaolo and Pietropaolo 1986). In some taxa, and the entity *D. peltata* '*gracilis*', adventitious stolons may be produced that lead to the formation of daughter tubers a distance from the parent tuber (character 3) (Vickery 1933; Rowe and Gibson 1999). Species of *Drosera* in other subgenera typically have a sparse root system of a few cylindrical roots (character 1) (Diels 1906). Two species, *D. arcturi* Hook. and *D regia* Stephens, produce a horizontal rhizome (Diels 1906; Stephens 1926; Salmon 2001). Therefore measurements of root system characteristics were considered suitable for identifying members of *Drosera* subgenus *Ergaleium* from other

subgenera and sections of the genus, and differentiating between several species of Rainbow Sundew.

2.2.2.02 Plant habit and stem characters

Diels (1906) elegantly depicted the different habits of the infraspecific groups of *Drosera* known at the time. Members of *Drosera* subgenus *Ergaleium* section *Ergaleium* develop an erect stem that may be self-supporting, particularly in short species such as *D. bulbigena*, *D. peltata s. str.*, *D. andersoniana* and the entity *D.* 'foliosa', as well as in the robust species *D. gigantea*. Entities, such as *D. peltata* 'Isla Gorge, Qld.', and species such as *D. huegelii* and *D. marchantii* subsp. *marchantii*, commonly lean on adjacent plants or rocks for support. The species with the longest stems, such as *D. erythrogyne*, *D. macrantha* subsp *macrantha* and *D. pallida*, actively climb over and through surrounding vegetation (Lowrie 1987; Lowrie 1989) (characters 4, 5 and 7). The habit of other subgenera within *Drosera* were typified by a rosulate habit, such as *D. binata* Labill. (*Drosera* subgenus *Phycopsis*, sensu Schlauer 1996).

Stem branching patterns were found to differ between different members of the *D. peltata* complex. The majority of entities are reported as having either an unbranched or apically branched stem (Lowrie 1987; Lowrie 1998; Salmon 2001). In contrast, the entity *D. 'foliosa'* (Planchon 1848) is characterized by a basal branching stem (characters 9 and 10).

Within the "Rainbow Sundews" (the colloquial name applied to all members of *Drosera* subsp. *Ergaleium* section *Ergaleium* (Erickson 1968; p. 16)) the lowest nodes on the stem may not develop leaves (Marchant and George 1982; Lowrie 1987; Salmon 2001). In some species, such as *D. gigantea*, the lowest nodes of the stem have triangular bracts instead of leaves (see Marchant and George 1982, Fig. 8) (character 8). Within the *D. peltata* complex, the development of leaves in the lower portion of the stem, including the basal rosette (see next section) is variable (Salmon 2001). The petiole may develop normally or in shortened form, but is terminated by an aborted lamina, to form a prophyll (Salmon 2001). Personal observations of plants in the wild and in cultivation suggests whether the leaf develops fully or not may be a function of the amount of light exposure during growth (characters 12 and 48).

Members of *Drosera* subgenus *Ergaleium* are characterized by the absence of stipules which, in other members of the genus, are present at the base of the leaf (character 6) (Planchon 1848; Diels 1906; Marchant and George 1982; Schlauer 1996).

Personal observation of herbarium specimens suggested that members of the *D. peltata* complex from Asia, except some collections from New Guinea, had shorter stem internodes than plants from Australia, New Zealand and parts of New Guinea (characters 49, 50, 51 and 52).

2.2.2.03 Basal rosette characters

Only a few species in *Drosera* subgenus *Ergaleium* section *Ergaleium* produce basal rosettes in mature plants. These species are: *D. andersoniana*, *D. auriculata*, *D. bicolor*, *D. peltata* and *D. salina*, as well as the other entities within the *D. peltata* complex. The size, shape, colour and number of the basal leaves differ between the different taxa and entities (characters 12 to 23) (Marchant 1978; Lowrie 1987; Salmon 2001). In addition, personal observations suggest that basal leaf shape within some entities in the *D. peltata* complex are variable (characters 24 to 31). The presence or absence of a basal rosette, and its persistence, or not, to flowering has been given taxonomic significance by some authors (Turczaninow 1854; Clarke 1879; Salmon 2001).

One of the comparator taxa used in this analysis is *D. binata*. This species, whilst considered basal to *Drosera* section *Ergaleium* from molecular data (Albert *et al.* 1992; Williams *et al.* 1994; Rivadavia *et al.* 2003), has linear leaves with long, often semi-erect petioles with a stipule at their base (characters 6 and 32). The lamina is dichotomously divided at least once, but in some populations plants with multiply divided leaves occur (characters 33 to 36) (Clemesha 1972; Slack 1980; Lowrie 1998).

2.2.2.04 Cauline leaf characters

The different species of Rainbow Sundews produce cauline leaves of a range of shapes. There are two broad types of shape: orbicular and crescentic (characters 71, 98 and 125), but there are many variations on these broad classes (characters 59 to 66, 88 to 93, 115 to 119) (Planchon 1848; Diels 1906; Marchant and George 1982; Lowrie 1987; Lowrie 1989; Lowrie 1998). In all species the leaves are produced alternately up the stem. Axillary leaves may be produced in plants of some species and entities of Rainbow Sundews (character 139), and these may differ in size and shape to the primary leaves (characters 143 to 147) (Lowrie 1987).

Commonly the petiole of the cauline leaf is attached on the abaxial surface of the lamina away from the leaf margin, that is, in a peltate attachment. However, this is not true in all Rainbow Sundews, such as *D. bulbigena* (Lowrie 1987). Occasionally, plants with dimorphic leaves (spathulate in the basal rosette, and peltate cauline leaves), may produce leaves of intermediate shape at the base of the stem (character 47) (Salmon 2001).

Members of the *D. peltata* complex have cauline leaves that are generally crescentic in shape. However, there are some differences in leaf size (characters 59 to 66, 88 to 93, 115 to 120, 143 to 147), leaf length to width ratio (characters 79, 80, 106, 107, 133, 134, 159, 160), the angle on the upper leaf margin (characters 67 to 69, 94 to 96, 121 to 123 and 148 to 150), and development of narrow, linear to acicular leaf tissue with stalked retentive glands, or auricles, on the edges of the upper leaf margins (characters 81 to 84, 108 to 111, 136 to 138 and 161 to 164) (Marchant and George 1982; Lowrie 1987; Salmon 2001).

During an assessment of the literature it became apparent that the same character, particularly leaf shape, had been described differently by different authors. For example, the crescent shaped leaves of *Drosera peltata* from southern Asia has been variably described as "orbicular" (Thunberg 1797), "sub-orbicular" (Labillardiére 1805), "orbiculari vel oblata" (Ruan 1981), "triangular" (Smith 1804), "lunate" (Planchon 1848; Turczaninow 1854), "broadly crescent shaped"(Bentham 1864), "zygomorpha semiorbicularis" (Taton 1951), and "crescentic" (Marchant and George 1982; Lowrie 1987). Clearly there is a need for the consistent use of terminology when applied to describing the same plant characters, such as using the

Committee for Descriptive Biological Terminology (1962) for consistently describing shapes and (Hewson 1993) for describing hair characters.

The adaxial surface and abaxial surface of cauline leaves of most Rainbow Sundews are glabrous. However, in *D. andersoniana*, *D. macrantha* subsp. *macrantha*, *D. macrantha* subsp. *planchonii*, *D. macrantha* subsp. *eremaea*, *D. stricticaulis*, *D. modesta* and *D. subhirtella*, the stems, at least in the apical half, and the petioles of the cauline leaves, are covered in shortly stalked glands. In most of these species, the abaxial surface of the cauline leaves is also glandular hairy (character 11) (Marchant and George 1982; Lowrie 1987; Lowrie 1999).

Personal observations of *D. bicolor*, in the wild and in cultivation, revealed that cauline leaves vary in size, shape and petiole attachment up the stem (Figure 8) Based on this observation it was then decided that examination of cauline leaves from the lower, middle and upper third of the stem would be needed to examine cauline leaf variation on a single plant. Therefore it was decided to measure mature cauline leaves from the lower, middle and upper third of the stem, and also any axillary leaves when they were present (characters 54, 57 to 164).

Leaf characters were scored on well-developed leaves, in which the glandular hairs on the adaxial surface of the leaf were fully grown. Up to three leaves were measured from the basal rosette, lower, middle and upper third of the stem, and from the nodes in the cauline leaf axils. Leaves were also selected that showed the least amount of folding and distortion from the pressing process. Mean values were calculated for each measured character and these were used in the phenetic analyses.

2.2.2.05 Inflorescence characters

The flowers of members of the Rainbow Sundews are produced in either a terminal raceme or a panicle, and in some cases may be reduced to a solitary flower (Diels 1906; Marchant and George 1982; Lowrie 1987). Flowers may also be produced at the end of axillary branches. The flowers open acropetally and in most species are only open for a few hours. The common stalk that connects the panicle or raceme to the uppermost node, or peduncle, and the pedicels that support individual

flowers, vary in length between many species (characters 165 to 168 and 195 to 200) (Lowrie 1987; Lowrie 1989).

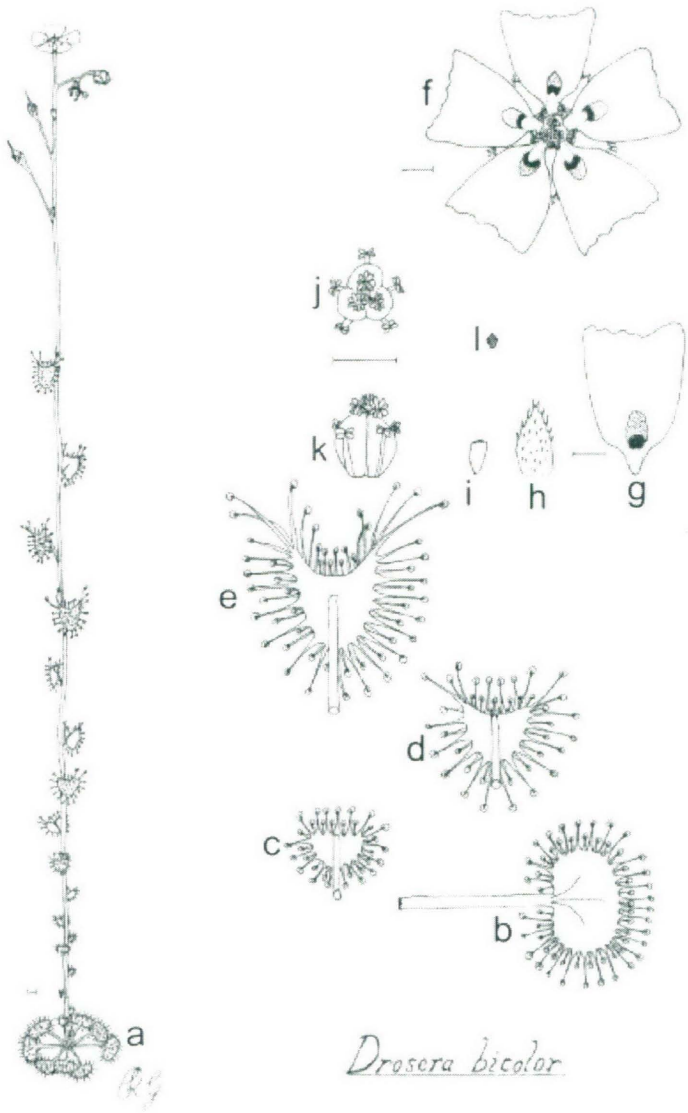


Figure 8 *D. bicolor* illustration by R. Gibson: (a) entire plant, (b) rosette leaf, (c) cauline leaf from the lower part of the stem, (d) cauline leaf from the middle part of the stem, (e) cauline leaf from the upper part of the stem, (f) open flower, (g) petal, (h) sepal, (i) bracteole, (j) gynoecium and stamens from above, (k) gynoecium and stamens from the side, and (l) seed. Scale bars = 1 mm.

At, or around, the base of many of the flowers in an inflorescence are small bracteoles of various shapes and sizes (see Figures 3, 4 and 8). Bracteoles in some Rainbow Sundews have been described as having black dots (characters 179 and 192), and in some species the margins and adaxial surface, or both, may have stalked glandular hairs (characters 173, 174, 177, 178, 180, 181, 185, 187, 190, 193, 194) (Lowrie 1987; Lowrie 1999; Salmon 2001). Bracteole characters have been used to

distinguish between some species of Rainbow Sundews (Lowrie 1999), including within the *D. peltata* complex (Ruan 1981; Schlauer 1996). Observations of bracteole shape, size, margin and surface characters were found to vary between some members of the *D. peltata* complex (characters 169 to 194). Furthermore, the surface and margin characters of the bracteoles often correlated with sepal margin and surface characters on the same plant (characters 216 to 248) (section 2.2.2.6).

2.2.2.06 Sepal characters

The sepals of the Rainbow Sundews vary in shape, size, length, width and margin (entire, dentate, serrate, with or without stalked glandular hairs or sessile glands) and adaxial surface (with or without black dots, with or without stalked glandular hairs) (characters 203 to 249). Sepal characters have long been used in *Drosera* taxonomy (Planchon 1848; Diels 1906; Marchant and George 1982; Schlauer 1996).

Traditionally *D. peltata* and *D. auriculata* have been differentiated from each other by the sepal surface and sepal margin characters. *Drosera peltata* has been characterized by a fimbriate sepal margin, with a hirsute abaxial sepal surface for Australian, New Zealand and alpine New Guinea plants (e.g. Planchon 1848; Diels 1906; van Royen 1973; Erickson 1978; Salmon 2001) whereas *D. peltata* plants from Asia have sepals with a glabrous surface and usually have an irregularly dentate margin (Malesia (van Steenis 1953), Bhutan (Grierson and Long 1984), India (Clarke 1879; Haines 1921; Gamble 1935; Balakrishnan 1981; Srinivasan 1983; Collett 1984; Ramamoorthy 1984), Sri Lanka (Trimen 1974; Amaratunga 1988), China (Jingwei 1982), and Nepal (Hara 1966; Polunin and Stainton 1985). *Drosera peltata* plants with glabrous sepals and fimbriate sepal margin have been reported from Sri Lanka (Trimen 1974), Myanmar and Singapore, 'var.1 *typica*' (Clarke 1879), Thailand (Larsen 1987), Malesia (van Steenis 1953), Taiwan (Liu 1976), Japan (Masamune 1933; Ohwi 1965), Okinawa and the Ryuku Islands (Walker 1976) and South Korea (Lee 1979).

Drosera auriculata has been characterized by sepals with an entire to weakly toothed margin and a glabrous abaxial margin (Planchon 1848; Diels 1906; Erickson 1978; Marchant and George 1982; Salmon 2001). Conn (1981) commented on the

variation in sepal margin and indumentum within the complex, and used this as an argument for reducing *D. auriculata* to a subspecies of *D. peltata*.

2.2.2.07 Floral characters

Flowers of species of *Drosera* generally exhibit pentamerous radial symmetry with five petals alternating with five sepals and five stamens (e.g. Figure 5). The superior ovary usually has three united locules and is surmounted by three, four or five styles, each style segment may be further divided into many style segments (Planchon 1848; Diels 1906; Marchant and George 1982; Schlauer 1996). The petals of most sundew species are usually white or pink (characters 261 to 263). However, within the Rainbow Sundews some species also have yellow, orange or red petals (Erickson 1978; Lowrie 1987; Lowrie 1999). The size and shape of the ovary (characters 265 to 267), stamen details (characters 268 to 270), pollen colour (character 271), degree and nature of style division (characters 272 to 284), and seed size and shape (characters 285 to 293) vary between species (Planchon 1848; Diels 1906; van Steenis 1953; Curtis 1956; van Royen 1973; Balakrishnan 1981; Marchant and George 1982; Lowrie 1987; Gibson 1993a; Lowrie 1998; Salmon 2001).

The degree of style division and position of this division has been used as a means of differentiating between members of the *D. peltata* complex (Planchon 1848; Marchant and George 1982; Lowrie and Carlquist 1992). Therefore, style characters (including the ratio of the undivided basal part of the style to the variably divided distal portion) were measured in this project.

2.2.2.08 Seed characters

The seeds of Rainbow Sundews exhibit the widest range of size and shape of any section in the genus (Planchon 1848). However, details of seed size and shape have yet to be published for all members of this section (e.g. Marchant and George 1982). In general, the seeds of members of this section are either ovoid or tabular, and range in length from 0.2 mm to over 10 mm (characters 285 to 293) (Planchon 1848; Colenso 1894; Taton 1951; Erickson 1978; Marchant and George 1982; Lowrie and Carlquist 1992; Lowrie 1999).

Within the *Drosera peltata* complex, the seeds of *D. auriculata* and *D. peltata* '*gracilis*' are tabloid and the rest are ovoid (Planchon 1848; van Royen

1973; Marchant and George 1982; Gibson 1993a; Salmon 2001). The seeds are usually black in colour and have a reticulate surface sculpture. Initial observations of the seeds suggested that the surface sculpturing of seeds of *D. peltata* 'Black Mountain A.C.T.' and *D. 'foliosa'* had deeper pits than seeds of other entities. In the revisions of the genus by Diels (1906) and Schlauer (1996), and of the *D. peltata* complex by Conn (1981), the characters of seed shape and seed length retained taxonomic significance in differentiating between members of even the most conservative classification of the complex. Therefore seed characters were collected wherever possible during this study.

2.2.2.09 Plant colour

Plant colour in *Drosera* is a function of genetic and environmental factors. Whilst many *Drosera* plants develop strong red pigmentation, particularly in the stalked retentive glands, the underlying green background to the plant may be an olive green or a yellow green (Erickson 1978; Lowrie 1987; Salmon 2001). Personal observations of live plants of members of the *Drosera peltata* complex indicated that the entities *D. peltata* 'Black Mountain, A.C.T.' and *D. 'foliosa'* were yellow green, even when growing in full sunlight. Plants of the other entities, however, were either an olive green colour or were suffused with red. There was no guarantee that live plant colour would be possible to determine from herbarium specimens, so the use of this novel character in phenetic analysis was tested in this study (character 294).

2.2.2.10 Ratios

Characters 45, 46, 79, 80, 106, 107, 133, 134, 159, 160 and 275 (Appendix 2) are ratios. Some authors have expressed doubt in the use of ratios in phenetic analysis (Phillips 1983), where they may change the weightings of some characters, and closely link size to shape. However, they do provide a means of quantifying otherwise intangible characters (Atchley *et al.* 1976; Thiele 1993), such as leaf shape, and also in providing a means of describing the relative length of the basal part of the styles to the often-divided style apex. Therefore, ratios were used in this study.

Not all characters were available to be scored from each specimen examined, particularly with regards to flower structure and seed. This was due to either the total absence of such characters, for example the specimens were sterile or had flowers but

no ripe fruit, or that some characters could only be obtained through destructive sampling, but that was not appropriate for all specimens examined. The phenetic dataset is presented in Appendix 3.

- Based on observations and analysis of a pilot dataset, several characters appeared useful in differentiating between entities within the complex. These characters were therefore used in the phenetic analysis, including characters of leaf shape in the lower, middle and upper third of the stem, sepal margin and abaxial surface of sepals, and seed size and shape.

2.2.2.11 Data preparation

Phenetic data measurements were recorded in a DELTA (Dallwitz *et al.* 1993 onwards) database and an MS-Excel spreadsheet. A character list was exported from DELTA (Appendix 2). Prior to analysis the veracity of the data was checked: redundant characters, invariant characters, and notes were removed. Multistate characters were expanded to one column per character state and each column was divided by the number of each column to ensure that characters were not over-weighted. Qualitative and quantitive characters were separated into different data matrices and analysed separately. In similar studies, these character types are also referred to as "over-lapping" and "non-overlapping" characters, respectively (Ariyanti and Conn 2005).

2.3 Phenetic analyses

Ordination and clustering were conducted on the different data matrices using the PATN for Windows program (Belbin and Collins 2006) to examine overall similarities and differences between the OTUs of the *Drosera peltata* complex based on morphological features. A distance matrix of these data was produced using the Gower metric association measure (Gower 1971). This association measure was chosen because it is able to deal effectively with qualitative and quantitive characters (Gower 1971; Belbin 1995). Cluster analysis was performed using flexible Ungrouped Pair-Group Method Using Averages (flexible UPGMA), with a ß-value of –0.1 to produce a phenogram (Belbin 1995). The dataset was also analysed using multidimensional scaling (MDS) ordination, with at least 200 random starts and the maximum number of iterations set to at least 200, both representing a change from the

default settings. This change from the default settings was done in order to explore the relationships between the dataset in more depth than the default settings allow. The relative positions of OTUs were examined in three-dimensional space (e.g. Susandarini *et al.* 2002). The relative influence of each individual character in ordination space could be determined using principal co-ordinate correlation (PCC) in which the relative strength and its direction in ordination space could be determined (Sneath and Sokal 1973).

Several analyses were run, the first with a complete dataset of all OTUs and all characters. The stress value of the ordination was recorded, as were the characters that most strongly influenced the grouping pattern. Where clear and distinct groups were identified, from both the phenogram and the corresponding ordination, then these were removed from the data set. Unexpectedly placed OTUs in the phenogram and ordination, where they occurred away from other members of the same putative taxon, were investigated. If, upon examination of the data behind these anomalously placed OTUs were found to be unscored (due to lack of suitable material) or inapplicable for many attributes, particularly for suites of characters pertaining to flowers and seeds, the OTUs were removed from the dataset.

The resulting smaller dataset was re-examined and any invariant or data-poor characters were removed. The analyses were then re-run on the smaller dataset and the process of investigating groups and anomalies was continued. The influence of each character was measured by two values: the Kruskal-Wallis Test (Kruskal and Wallis 1952), and the principal co-ordinate correlation (PCC) value (measured by r-squared). These measures were chosen because they are able to be used with mixed datasets containing both over-lapping and non-overlapping characters, where the data does not necessarily have a normal distribution and where variance may be heterogeneous (Zar 1974).

2.4 Results and discussion

2.4.1 Pilot study of 28 sympatric OTUs of the *D. peltata* complex

To test the veracity of putative taxa within the *D. peltata* complex, an initial phenetic study was conducted using OTUs from locations where the same putative taxa grew sympatrically. Twenty-eight OTUs from six locations (Figure 9) representing four putative taxa (Table 7) were analysed by cluster analysis (Figure 10)

and ordination (Figure 11). In this analysis all but two taxa were placed according to putative taxon rather than geographic origin. The samples of *D. auriculata* and *D. peltata* 'Black Mountain, A.C.T.' from Picnic Point plotted together. This placement appeared to be anomalous, and when checked these two samples were found to lack all flowering and fruiting characters.

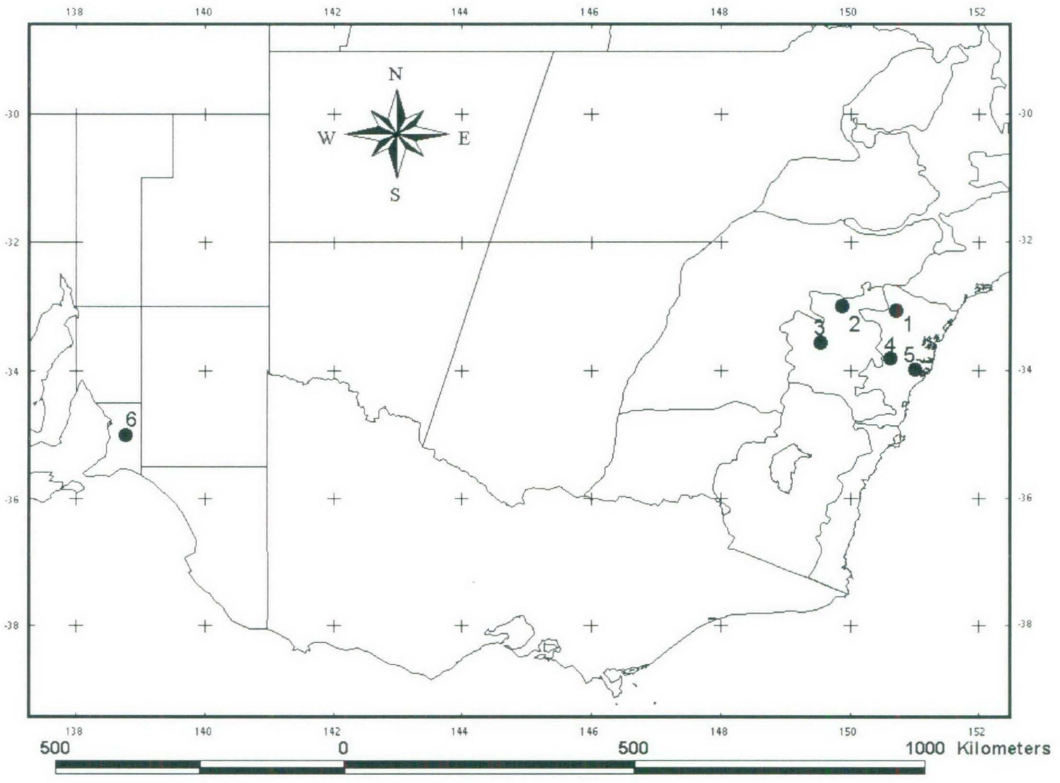


Figure 9. Map of southeastern Australia showing the location of pilot study OTUs from six sympatric populations. Site codes: (1) Wallaby Swamp, (2) Ilford, (3) Rockley Mountain, (4) Mulgoa, (5) Picnic Point, and (6) Mt. George.

Table 7. Six locations where two or more putative taxa grew sympatrically. The 28 OTUs and putative taxa here listed were included in the initial study.

Location	Putative Sympatric Taxon	OTU Codes
(1) Wallaby Swamp, N.S.W.	*D. peltata* 'Black Mountain, A.C.T.'	pBM_WlSwmp1, pBM_WlSwmp2, pBM_WlSwmp3
	D. peltata 'Red Rosette'	pRR_WlSwmp1, pRR_WlSwmp2
(2) Ilford, N.S.W.	*D. peltata* 'Black Mountain, A.C.T.'	pBM_ilf1, pBM_Ilf2, pBM_Ilf3
	D. peltata 'Red Rosette'	pRR_Ilf1, pRR_Ilf2, pRR_Ilf3
(3) Rockley Mountain, N.S.W.	*D. auriculata*	au_Rockley1
	D. peltata 'Black Mountain, A.C.T.'	pBM29
(4) Mulgoa, N.S.W.	*D. auriculata*	auMulgoa1
	D. peltata 'Black Mountain, A.C.T.'	pBM1,
	D. peltata 'Red Rosette'	pRR_Mul1, pRR_Mul2, pRR_Mul3
(5) Picnic Point, N.S.W.	*D. auriculata*	au_PP
	D. peltata 'Black Mountain, A.C.T.'	pBM_PP
	D. peltata 'Red Rosette'	pRR_PP1, pRR_PP2, pRR_PP3
(6) Mt. George, S.A.	*D. auriculata*	auMG1, auMG2, auMG3
	D. 'foliosa'	folMG1, folMG2

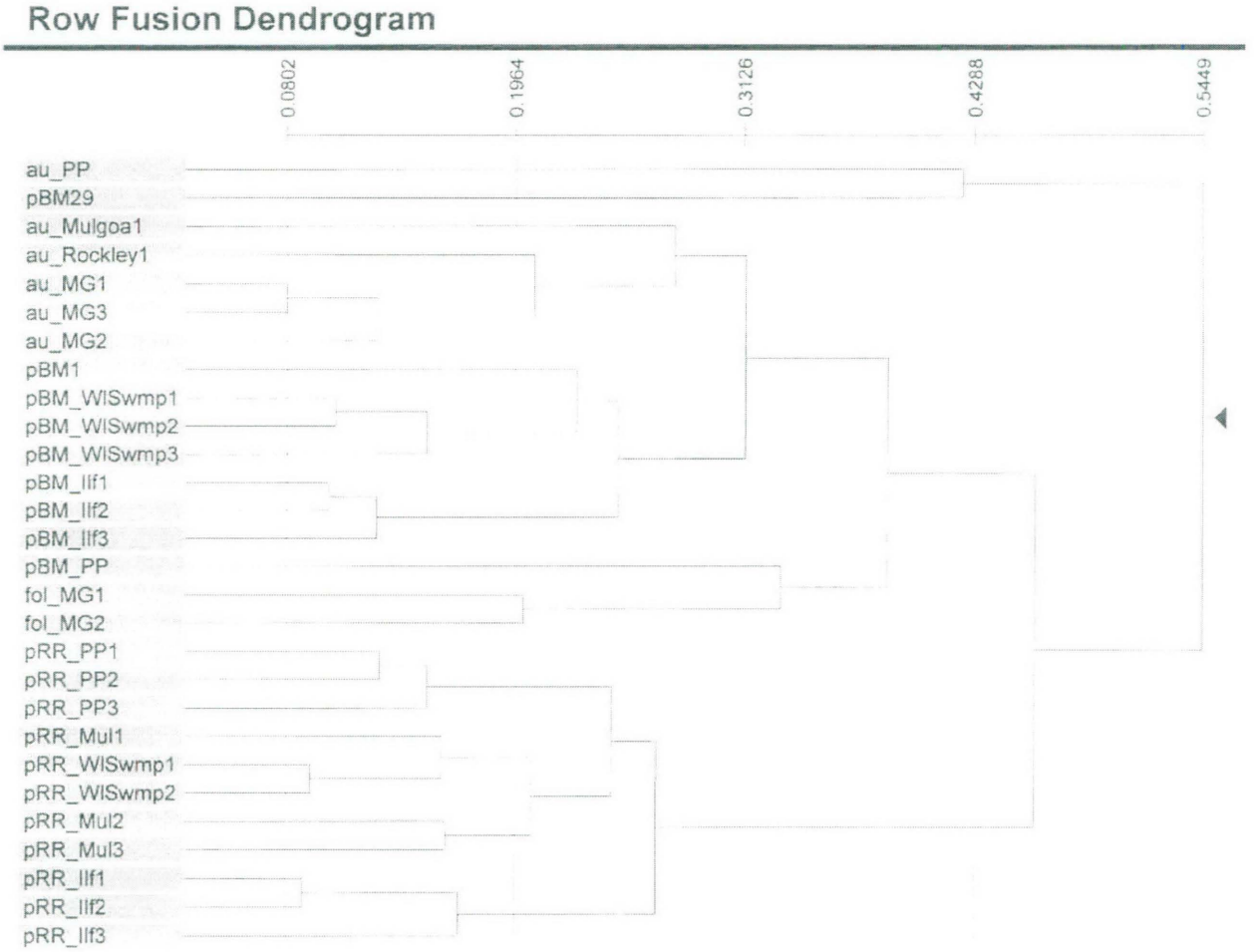
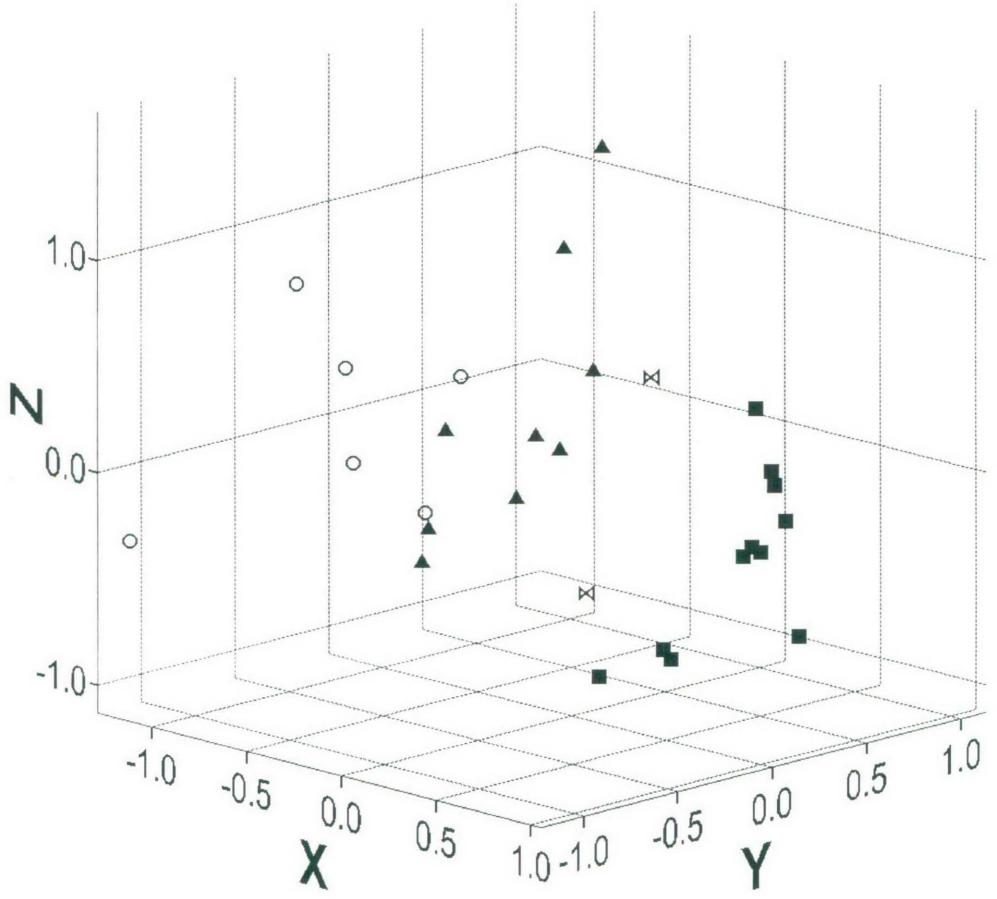


Figure 10. Phenogram of 28 OTUs that represent entities of the *D. peltata* complex that grow sympatrically in six locations. Most OTUs identified as the same putative taxa, but from different locations, plot together; the exceptions are the sterile specimens au_PP and pBM29.



○ *D. auriculata*
⋈ *D. 'foliosa'*
▲ *D. peltata* 'Black Mountain, A.C.T.'
■ *D. peltata* 'Red Rosette'

Figure 11. 3D ordination plot of the 28 sympatric putative taxa. Stress value = 0.1395.

Table 8. The top five characters based on Kruskal-Wallis value in the ordination of 28 sympatric OTUs.

Character	KW value
52. INTav	19.395768
51. INTx	19.36944
94. MCLang_n	18.318966
96. MCLang_av	18.26601
5. Height	18.039185

Table 9. Correlation between attributes and ordination vectors, and maximum correlation for 28 sympatric OTUs. Only the top five characters are listed.

Character	Vector 1	Vector 2	Vector 3	Correlation
97. (a) MCLum_1	-0.544	-0.587	-0.600	0.724
97. (c) MCLum_3	-0.544	0.587	0.600	0.724
104. MCL_Wx	0.951	0.307	-0/047	0.674
105. MCL_Wav	0.967	0.253	-0.009	0.662
103. MCL_Wn	0.981	0.192	0.033	0.636

The 28 OTUs grouped according to their *a priori* assigned putative taxa, rather than by geographic location, in this analysis indicating support for their taxonomic recognition. It is worth noting that glabrous sepalled OTUs (*D. auriculata*) group together away from the other three entities that all share hirsute sepals. An analysis of the characters behind the ordination analysis found that the shape and width of cauline leaves of the middle third of the stem (Table 9), internode maximum and average length, the angle-class of the upper margin of cauline leaves on the middle third of the stem, and plant total height (Table 8) were the strongest character vectors in the ordination analysis. However, none of these vectors could be considered to be strong and no single character used could explain the location of the groups in ordination space. None of the OTUs formed tight groups. The scatter in the ordination space may be due to the gaps in the phenetic database, particularly due to the absence of some characters. This is based on the observations of samples from the same herbarium collection, and assigned to the same taxon, with different sets of characters scored plotted in different locations in the phenogram and in ordination space. From these results a larger set of OTUs from the *D. peltata* complex was used to test taxon limits.

2.4.2 Analysis of the full dataset

Analysis of the full dataset of 235 OTUs with only the 144 quantitive characters provided poor resolution of the data. Using only the 198 qualitative data provided a clearer grouping of putative taxa and comparators, with strong groupings of OTUs of *D. andersoniana, D. salina* and *D. microphylla* respectively, that is, most of the comparator taxa were recovered as discrete groups. Within the members of the *D. peltata* complex, the putative taxa were grouped together but fell largely into two groups that correlated to OTUs with glabrous or hairy sepals (Figures 12 and 13). No single character strongly influenced the groupings of the OTUs. In fact the correlation values of even the most influential characters were poor (Tables 10 and 11).

Figure 12. Phenogram of full dataset of OTUs of *Drosera*. Data from Appendix 3. See text for discussion. The comparator taxa *D. binata, D. salina, D. microphylla* and *D. andersoniana*, as well as *D. bicolor,* form discrete groups.

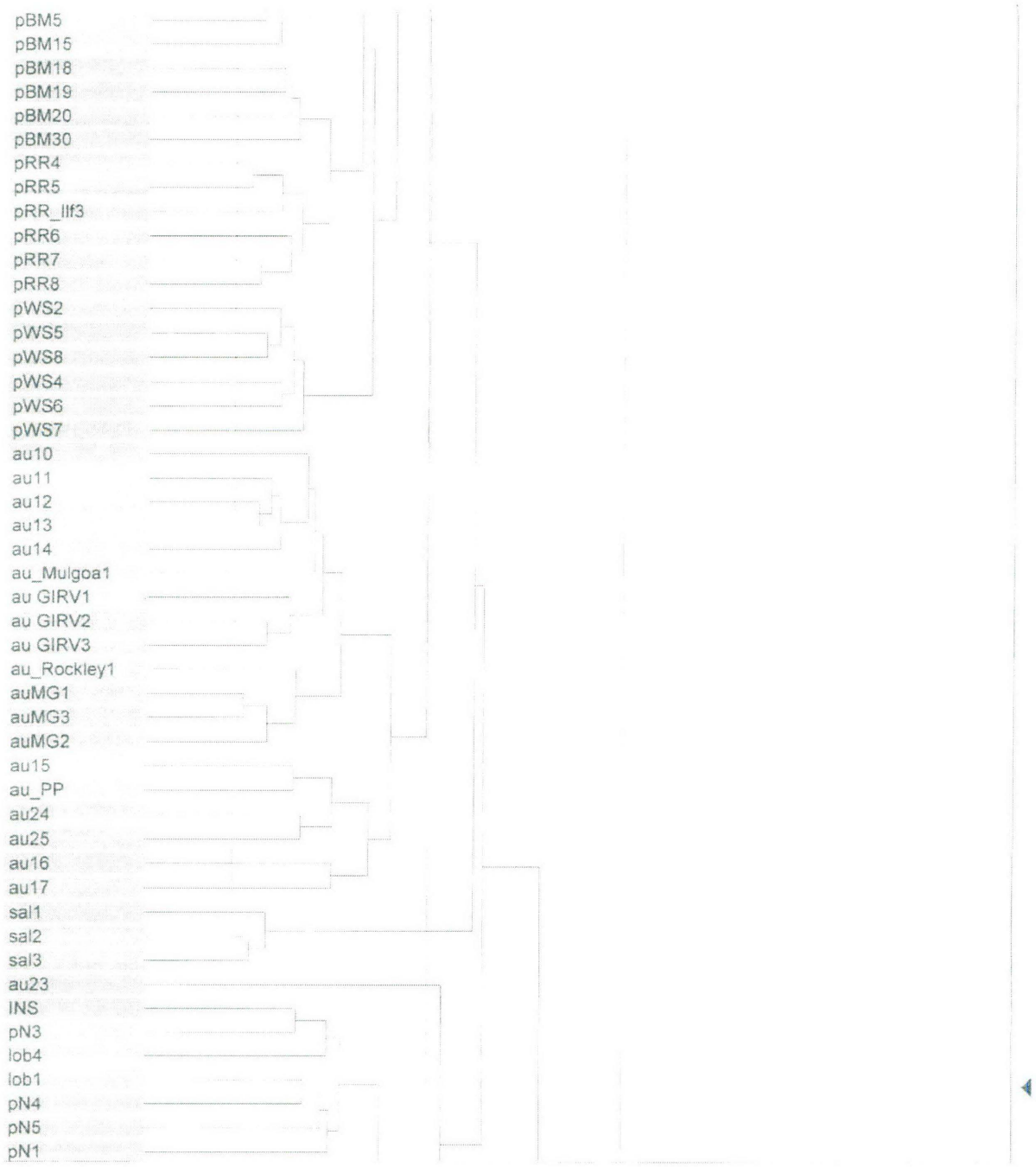


Figure 12. (continued)

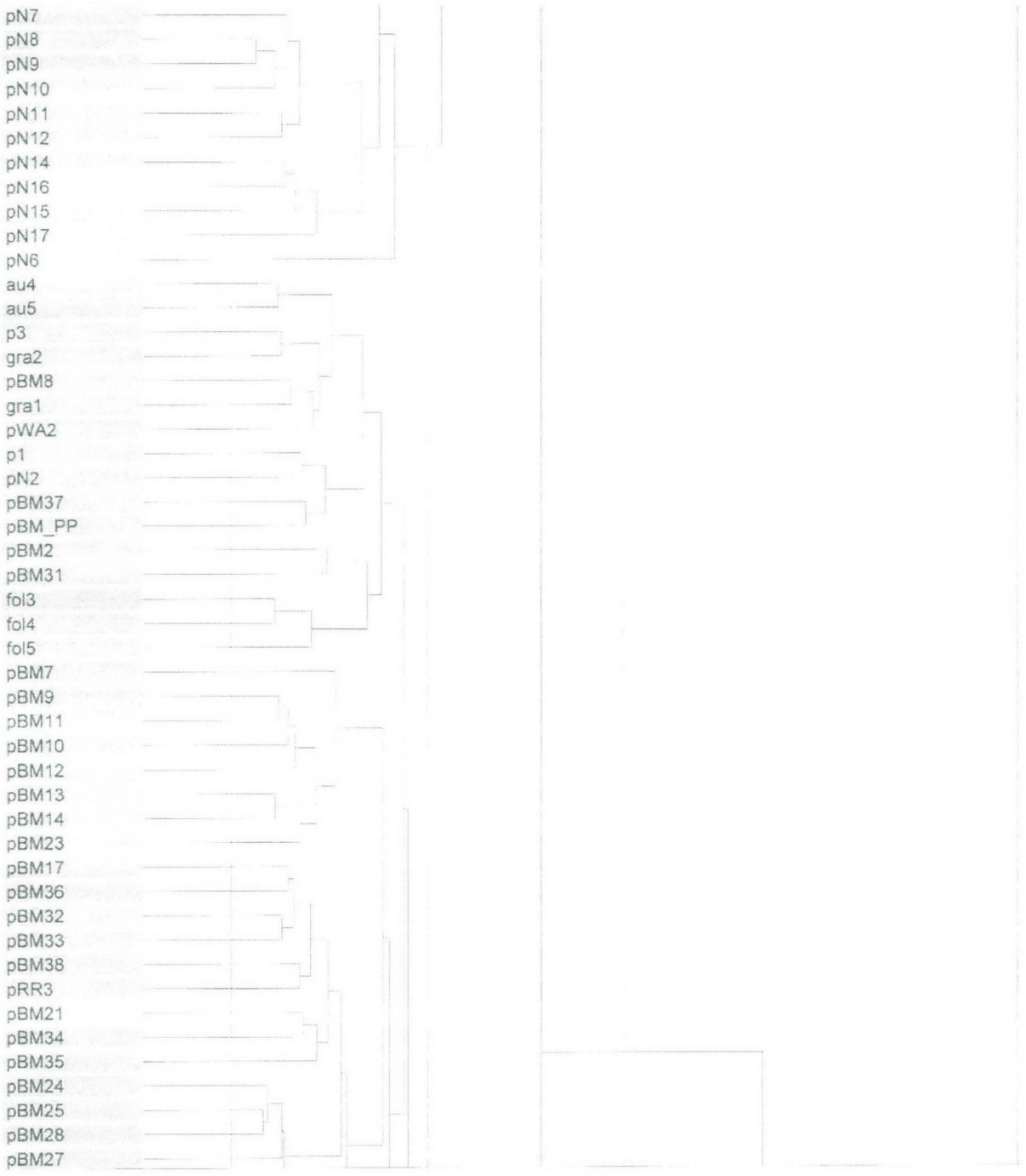


Figure 12. (continued)

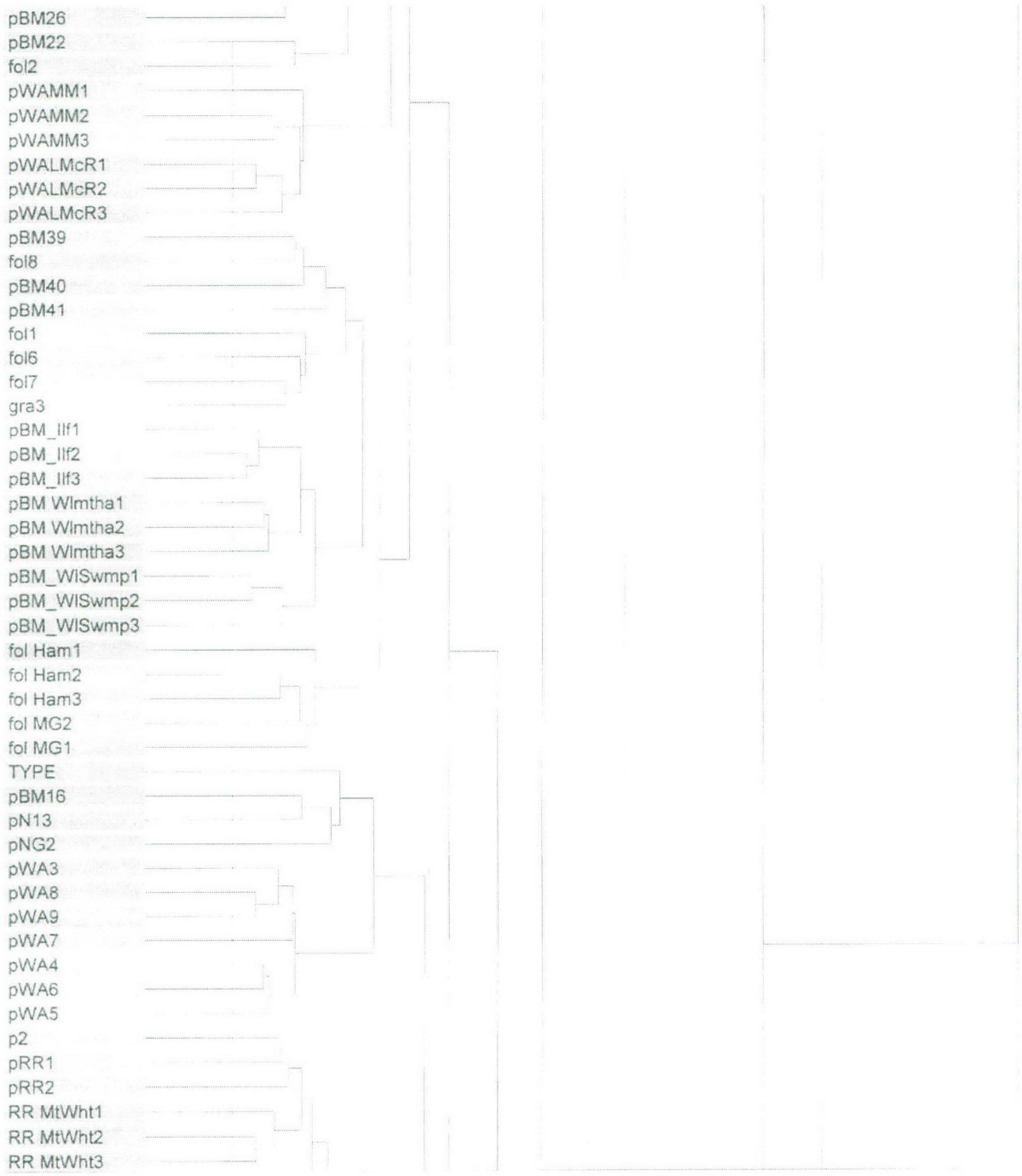


Figure 12. (continued)

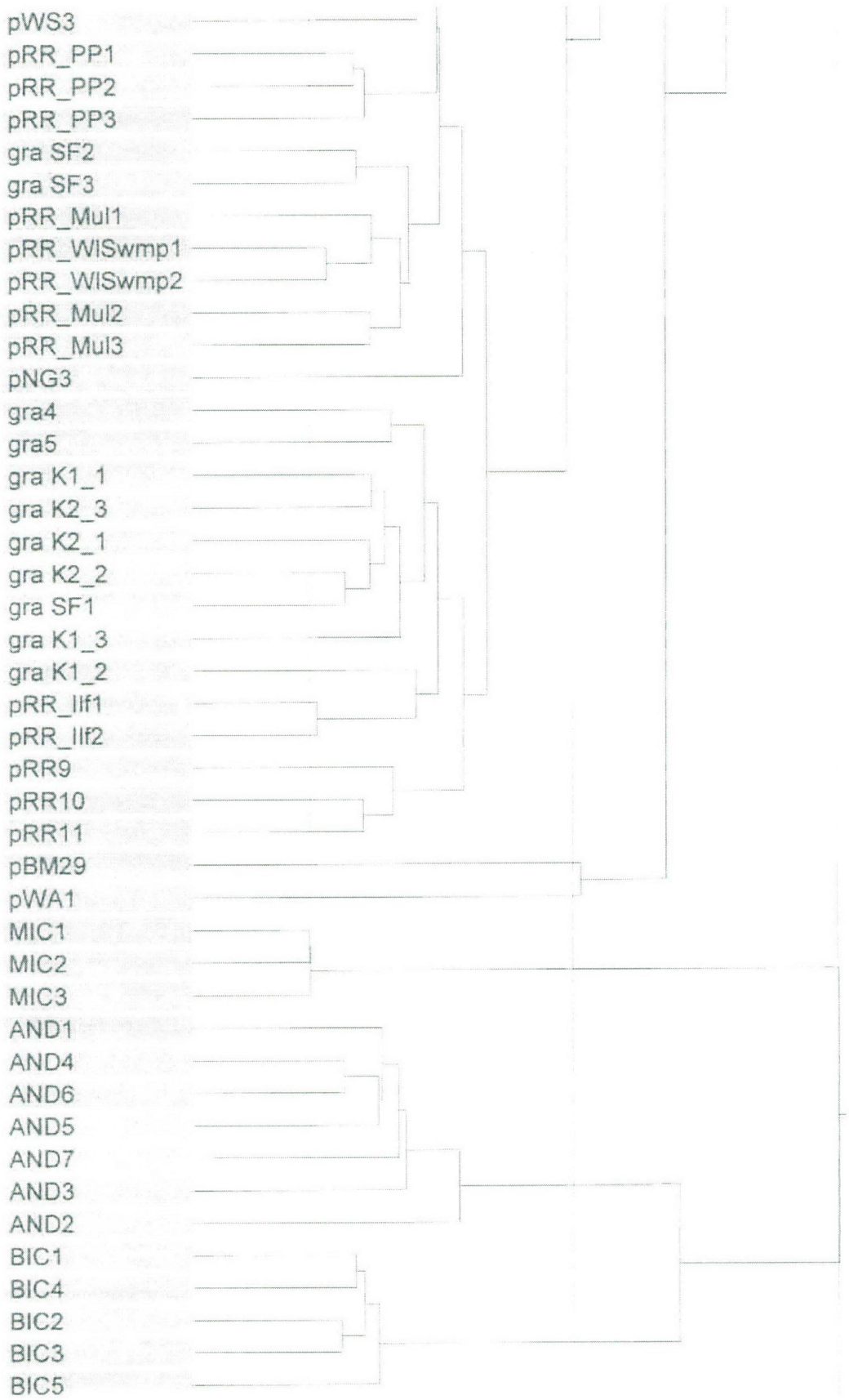
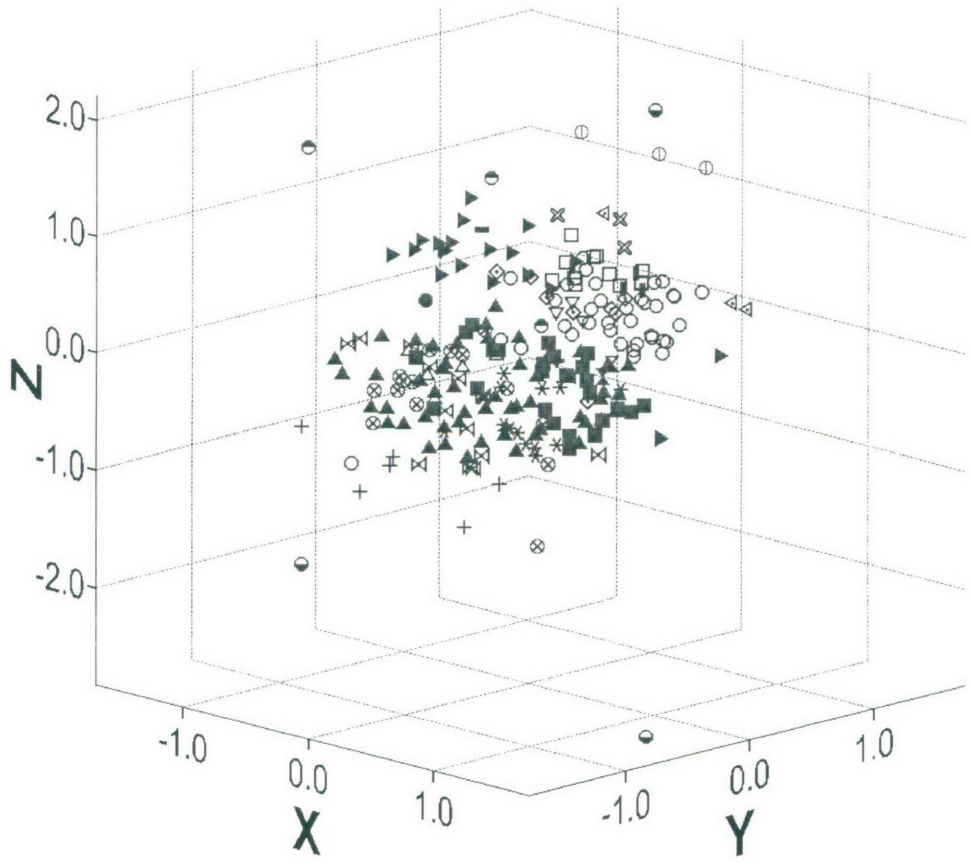


Figure 12. (continued)



+ *D. andersoniana*
○ *D. auriculata*
△ *D. bicolor*
◓ *D. binata 'multifida'*
◒ *D. binata* 'T-form'
▲ *D. peltata* 'Black Mountain, A.C.T.'
⋈ *D. 'foliosa'*
⋇ *D. gigantea* subsp. *gigantea*
∗ *D. peltata 'gracilis'*
□ *D. peltata* 'Isla Gorge, Qld.'
▬ *D. insolita*
▮ *D. lobbiana*
▽ *D. lunata*
⊕ *D. microphylla*
▶ *D. nipponica*
⬠ *D. peltata* New Guinea 1
⧖ *D. peltata* New Guinea 2
✙ *D. peltata* New Guinea 3
⋈ *D. peltata* 1
⧗ *D. peltata* 2
✖ *D. peltata* 3
■ *D. peltata* 'Red Rosette'
◁ *D. salina*
● *D. peltata* TYPE
⊗ *D. peltata* 'Western Australian Form'
◈ *D. peltata* 'Western Sydney'

Figure 13. Three-dimensional ordination plot of all 235 OTUs by all 348 characters. The stress value is 0.2039.

Table 10. The top five characters based on Kruskal-Wallis value in the ordination of 235 OTUs.

Character	KW value
239. SEP_HD	141.791565
104. MCL_Wx	119.994873
105. MCL_Wav	119.252686
75. LCL_Lav	117.591248
238. SEPapHirs	117.009521

Table 11. Correlation between attributes and ordination vectors, and maximum correlation for 235 OTUs. Only the top five characters are listed and all these have very low correlation values.

Character	Vector 1	Vector 2	Vector 3	Correlation
240. SEP_Hloc	-0.14	0.773	-0.619	0.507
241. SEPsurfHLn	-0.289	0.79	-0.54	0.436
242. SEPsurfHLx	0.289	-0.79	-0.54	0.436
243. SEPsurfHLav	-0.289	0.79	-0.54	0.436
245. SEPusHno_av	-0.307	0.784	-0.54	0.431

Most of the OTUs of comparator taxa form discrete groups along side a larger cloud of OTUs of the *D. peltata* complex. This is most likely due to the comparator taxa having suites of characters not shared by members of the *D. peltata* complex. The comparator taxon *D. gigantea*, shares many characters with members of the *D. peltata* complex, such as crescentic cauline leaves. Thus it is perhaps not surprising that the three *Drosera gigantea* OTUs did not form a discrete group, and instead grouped with the glabrous sepalled members of the *D. peltata* complex. For the next analysis only members of the *Drosera peltata* complex were included. In removing the 22 comparator OTUs, 27 characters were made invariant (Table 12). The smaller dataset was then analysed by cluster analysis and ordination.

Table 12. Twenty-seven characters became invariant in the dataset after the removal of twenty-two comparator OTUs from the main dataset. These characters were then removed from the dataset prior to reanalysis.

Character No.	Character Code	Character Description
1	ROOT	Root system type
6	Stip_P	Stipules <whether present>
7	Vert_Stem	Vertical stem <whether present>
8	Stem_Brcts	Stem bracts <whether present>
32	BRLshp_lin	Basal rosette with linear leaves <whether present>
33	L_dich	Leaves <whether dichotomously divided>
34	L_dich_2	Once-forked leaves <whether present>
35	L_dich_3_4	Multiply-divided leaves with 3 or 4 terminal lobes <whether present>
36	L_dich_5	Multiply-divided leaves with 5 or more terminal lobes <whether present>
53	STEMglab	Stem <whether glabrous>
55	CL_cup	Cauline leaves <whether flat or cupped>
89	MCLshp_ov	Ovate middle cauline leaves <whether present>
93	MCLshp_ren	Reniform middle cauline leaves <whether present>
98	MCLgshp	Middle cauline leaf general shape <orbicular or crescent shaped>
116	UCLshp_ov	Ovate leaves <whether present> in the apical third of the stem
120	UCLshp_ren	Reniform leaves <whether present> in the apical third of the stem
125	UCL_gshp	Upper cauline leaf general shape <orbicular or crescent-shaped>
144	AXLshp_ov	Ovate axillary leaves <whether present>
151	AXLgshp	Axillary cauline leaf general shape <orbicular or crescent-shape>
268	FILcol	Filaments <colour>
273	STYdiv	Styles <whether divided into numerous style segments>
276	STYcol4	Styles <colour> (white with a distinct orange base)
278	STYapx_ovo	Ovoid stigma apices <whether present>
280	STYapx_cra	Crassiform stigmas <whether present>
282	STIGundiv	Undivided stigmas <whether undivided>
283	STIGbifid	Bifid stigmas <whether present>
284	STIGmul	Multiply divided stigmas <whether present>

2.4.3 Second iteration - 213 OTUs of the *D. peltata* complex

The second iteration of the full data set comprised 213 OTUs and 323 characters and included only OTUs of the *D. peltata* complex. The three dimensional ordination stress value was 0.2087. The most influential characters in ordination space are all relatively weak (Tables 13 and 14) and all are based on the characters of sepal surface

hair distribution, density and length. In the phenogram for the cluster analysis (Figure 14) and the three-dimensional ordination (Figure 15). *Drosera bicolor* OTUs plot away from the remaining OTUs. The latter form a cloud within clearly defined groups.

Table 13. The top five characters based on Kruskal-Wallis value in the ordination of 213 OTUs of the *D. peltata* complex.

Character	KW value
105. MCL_Wav	131.165521
239. SEP_HD	130.425442
104. MCL_Wx	129.78742
75. LCL_Lav	128.268052
103. MCL_Wn	127.930887

Table 14. Correlation between attributes and ordination vectors, and maximum correlation for 213 OTUs of the *D. peltata* complex. Only the top five characters are listed and all these have low correlation values and pertain to sepal hair characters.

Character	Vector 1	Vector 2	Vector 3	Correlation
240. SEP_Hloc	-0.290	0.377	0.879	0.693
241. SEPsurfHLn	-0.337	-0.191	0.922	0.566
242. SEPsurfHLx	-0.337	-0.191	0.922	0.566
243. SEPsurfHLav	-0.337	-0.191	0.922	0.566
245. SEPusHno_av	-0.353	-0.188	0.917	0.551

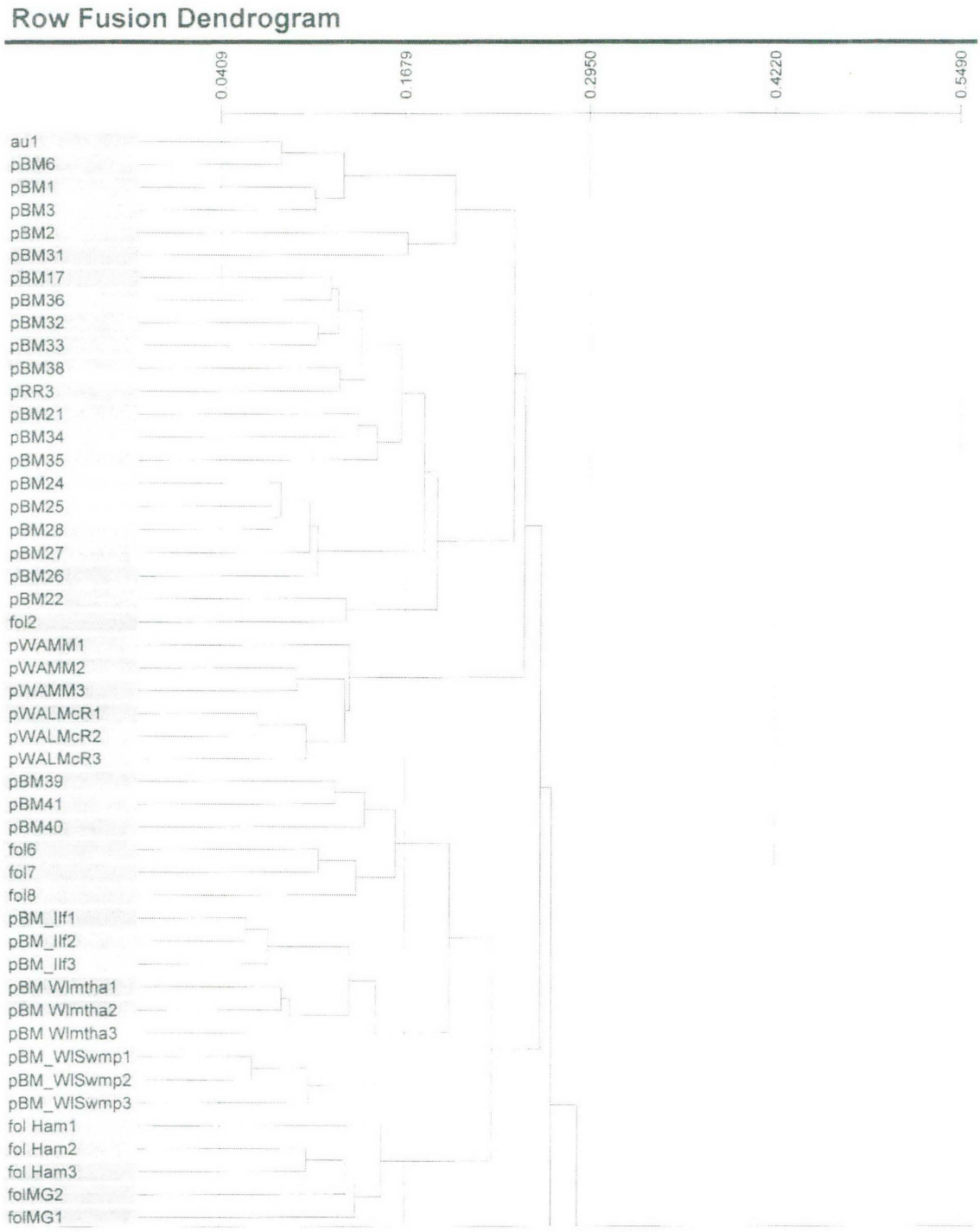


Figure 14. Phenogram of the second iteration of the *D. peltata* complex dataset. Five OTUs of *D. bicolor* occur well away from other members of the complex.

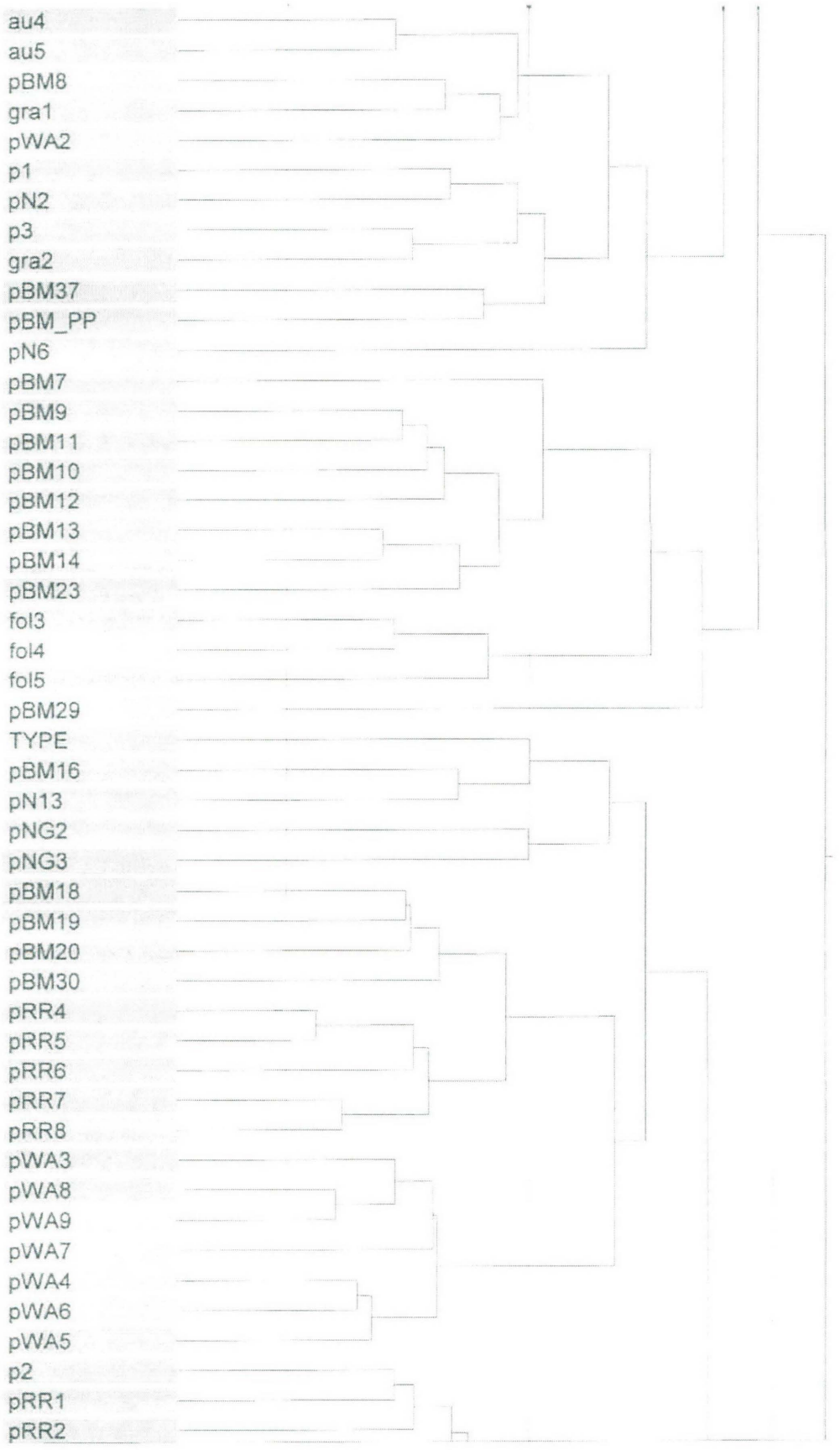


Figure 14. (continued)

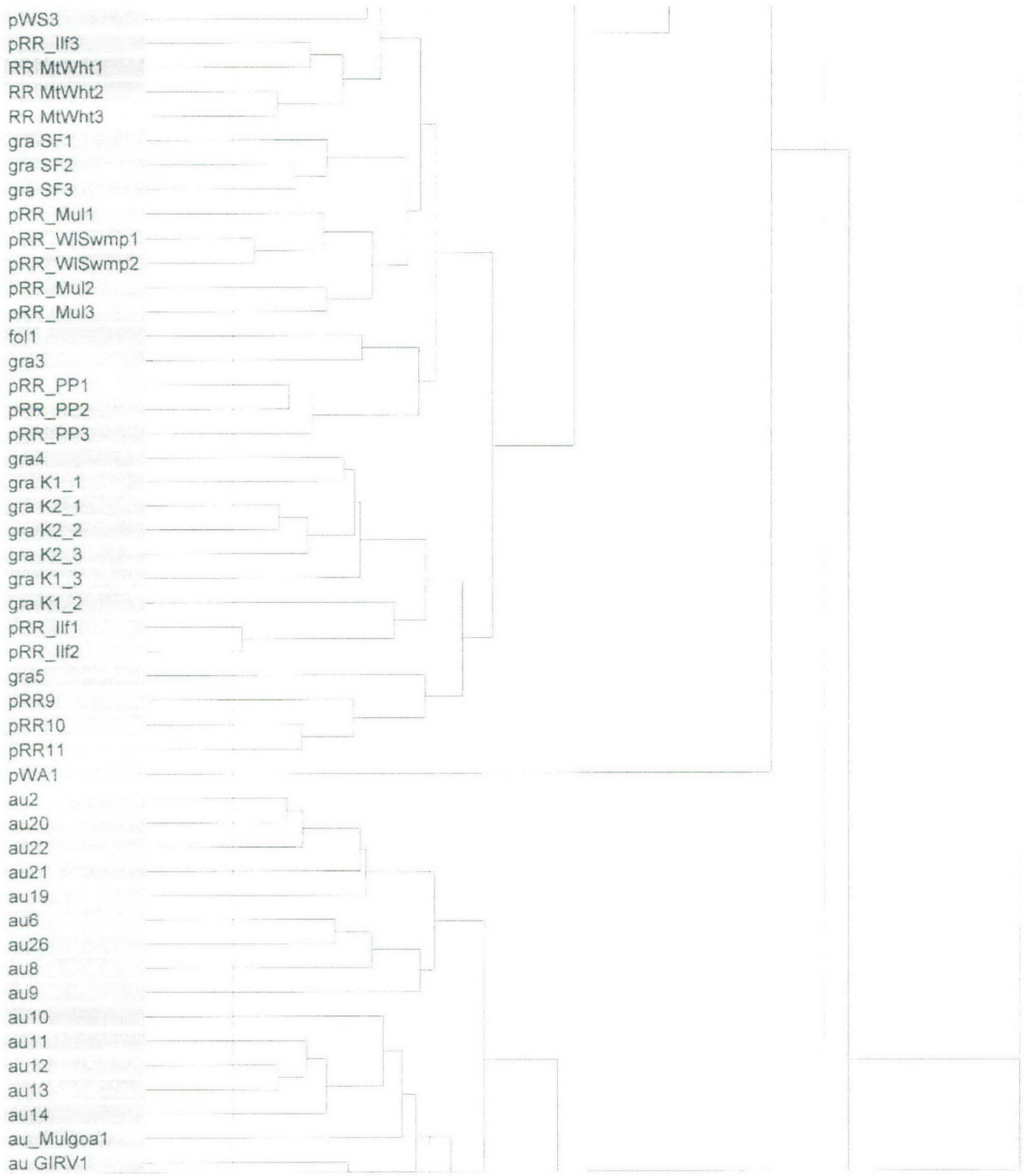


Figure 14. (continued)

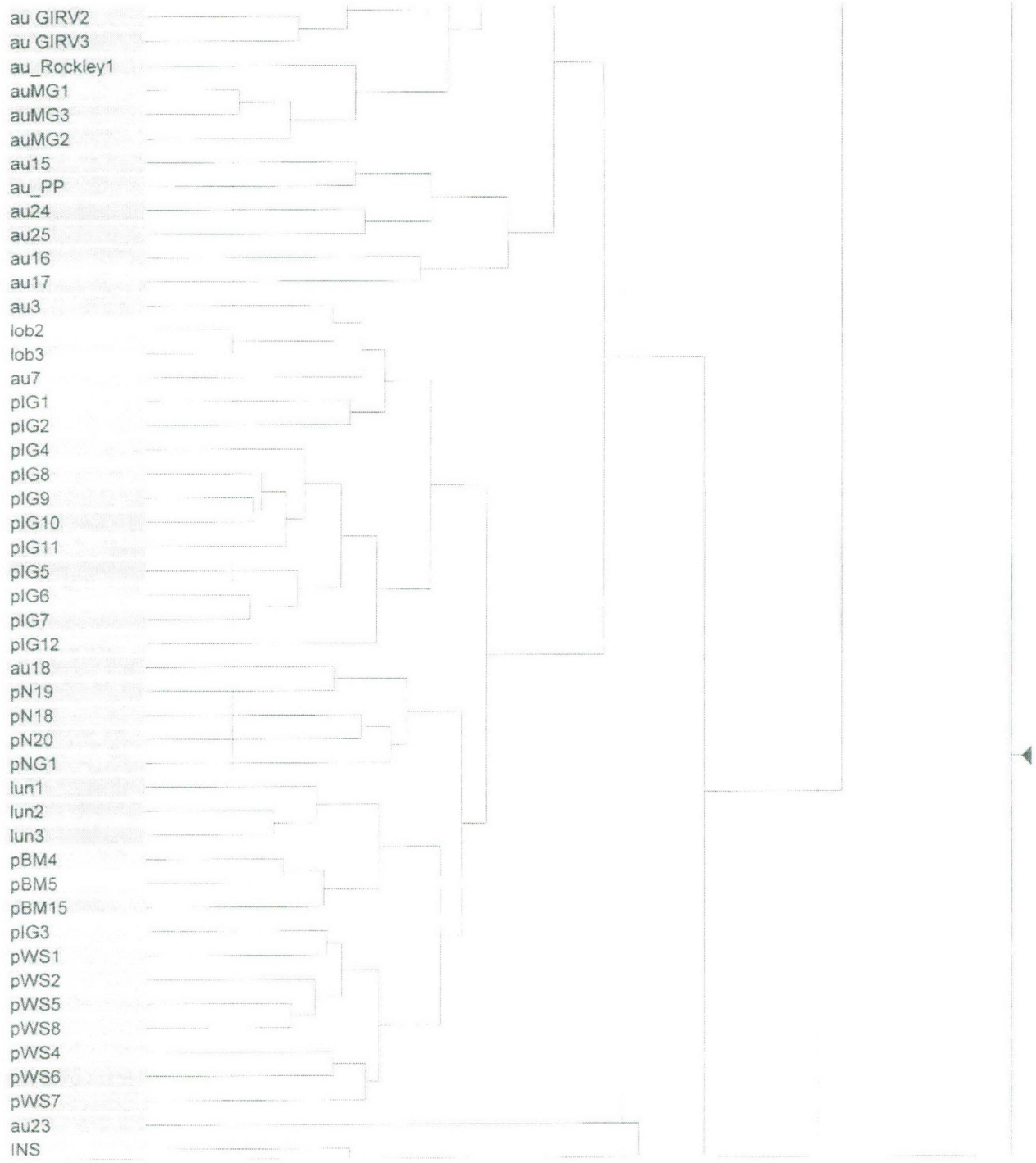


Figure 14. (continued)

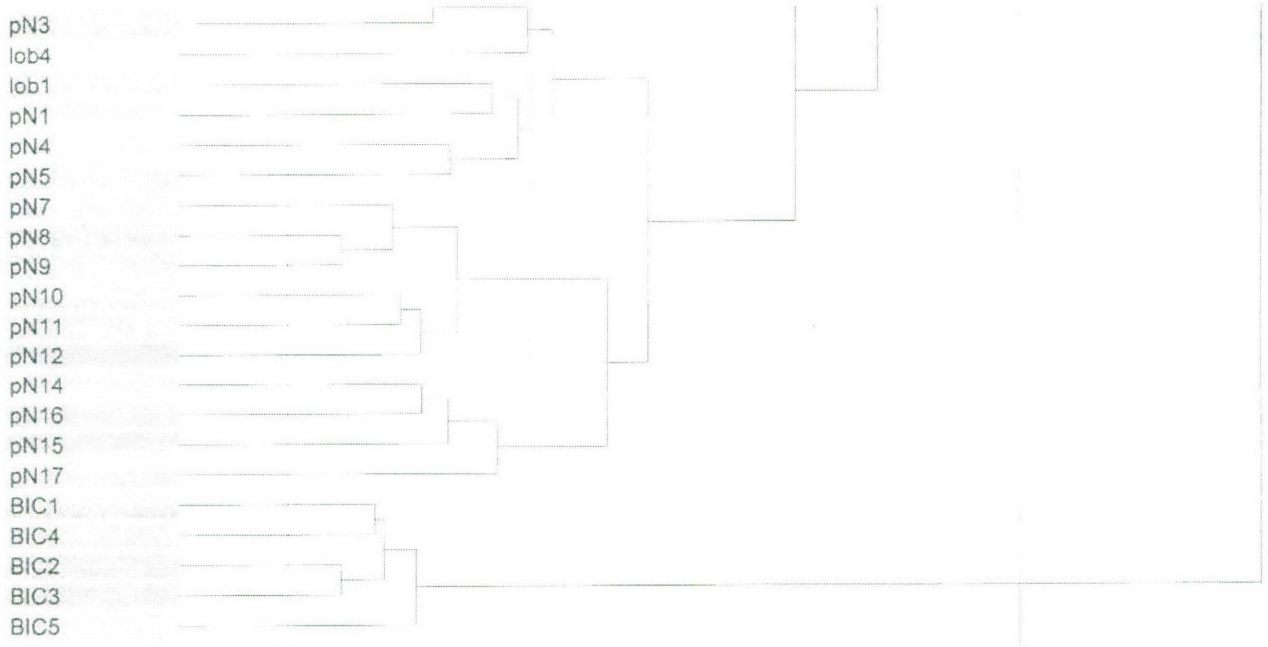
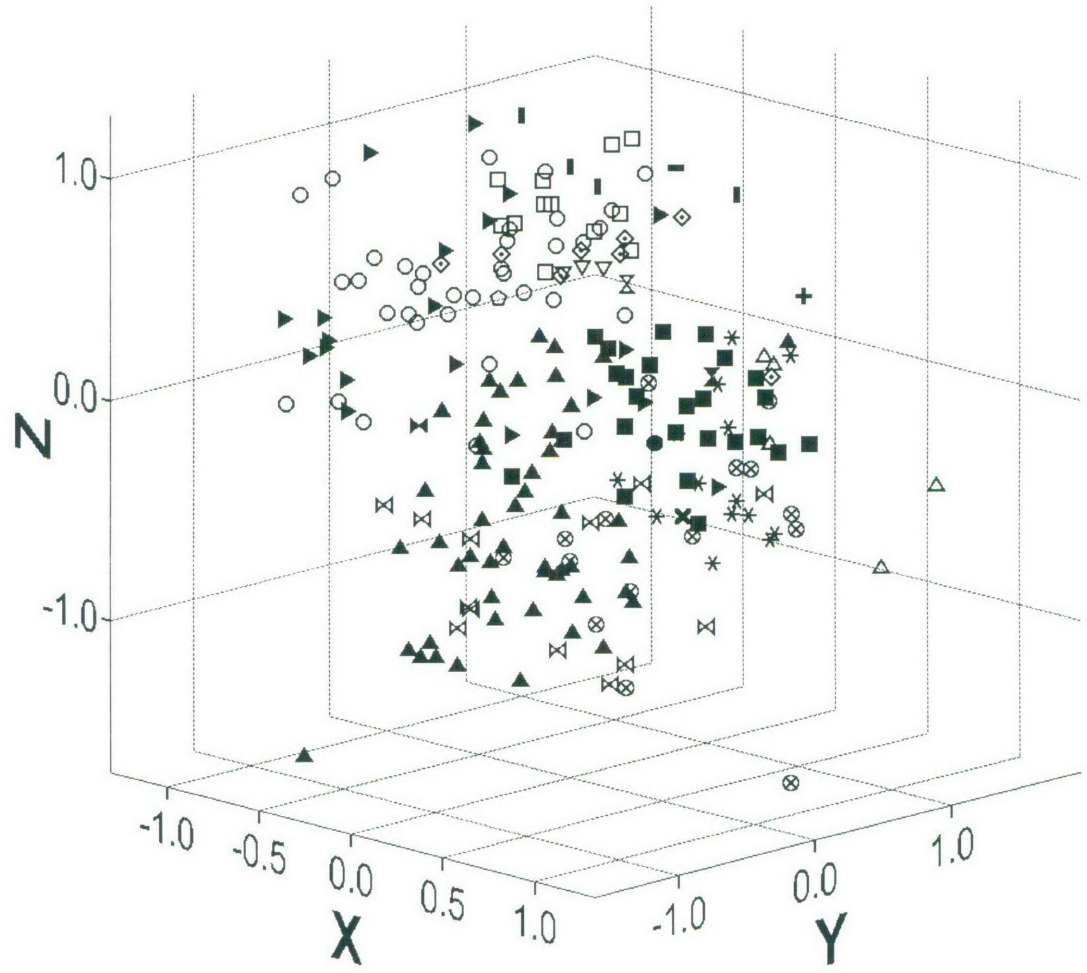


Figure 14. (continued)

From the current iteration of the dataset, *D. bicolor* was recognized as a distinct species from the *D. peltata* complex and was removed from the dataset. In order to test for internal groups within the remaining OTUs, a subset of the database was formed using only those OTUs with a more complete set of characters measured.

To produce the data rich dataset only those OTUs with a minimum of three measurements of styles and seeds were retained. This led to the removal of 82 OTUs (Table 15) which resulted in eight characters becoming invariant (Table 16). The invariant characters were removed from the dataset prior to the next analysis.



- *D. auriculata*
- *D. bicolor*
- *D. peltata* 'Black Mountain, A.C.T.'
- *D. 'foliosa'*
- *D. peltata 'gracilis'*
- *D. peltata* 'Isla Gorge, Qld.'
- *D. insolita*
- *D. lobbiana*
- *D. lunata*
- *D. nipponica*
- *D. peltata* New Guinea 1
- *D. peltata* New Guinea 2
- *D. peltata* New Guinea 3
- *D. peltata* 1
- *D. peltata* 2
- *D. peltata* 3
- *D. peltata* 'Red Rosette'
- *D. peltata* TYPE
- *D. peltata* 'Western Australian Form'
- *D. peltata* 'Western Sydney'

Figure 15. Three dimensional ordination of 213 OTUs of the *D. peltata* complex.

Table 15. 82 OTUs removed from the dataset to ensure the most data-rich OTUs were retained for subsequent analysis.

Taxon	OUT code
D. auriculata	au1, au3, au5, au7, au16, au18, au19, au20, au21, au22, au26, auMG1, auMG3
D. lunata	lun1, lun2, lun3
D. lobbiana	lob1, lob2, lob3, lob4
D. peltata	p1, p2
D. peltata 'Black Mountain, A.C.T.'	pBM1, pBM4, pBM5, pBM7, pBM8, pBM15, pBM16, pBM17, pBM20, pBM21, pBM23, pBM30, pBM31, pBM32, pBM33, pBM34, pBM35, pBM41, pBM Wlmtha1,2,3
D. foliosa	fol1, fol2, fol7
D. gracilis	gra2, gra 3, gra 4
D. peltata 'Isla Gorge, Qld.'	pIG2, pIG5, pIG7
D. peltata '*nipponica*'	pN1, pN2, pN3, pN5, pN6, pN7, pN8, pN9, pN13, pN18, pN19, pN20, pN21
D. peltata 'New Guinea'	pNG1, pNG2
D. peltata 'Red Rosette'	pRR1, pRR2, pRR3, pRR4, pRR5, pRR6, pRR7, pRR8, pRR10, pRR_WlSwmp1,2,3
D. peltata 'Western Australian Form'	PWA1, pWA2
D. peltata 'Western Sydney'	PWS3

Table 16. Eight characters made invariant by the removal of the 82 OTUs in Table 12 from the dataset following the second iteration analysis.

Character No.	Character Code	Character Description
63	LCLshp_flab	Flabellate-shaped leaves <whether present>
64	LCLshp_orb	Orbicular leaves <whether present>
66	LCLshp_tell	Transversely-elliptic leaves <whether present>
92	MCLshp_orb	Orbicular middle cauline leaves <whether present>
99 (c)	MCLW3_3	Middle cauline leaves widest<in the lower third>
169 (g)	LBshp7	Lower bract <shape: ovate>
282	STIGundiv	Undivided stigmas <whether present>
290	SEED_srf	Seed surface <texture>

2.4.4 Third iteration – 128 data-rich OTUs

The third iteration of the full dataset comprised 128 OTUs and 311 characters. In both the phenogram (Figure 16) and the ordination plot (3D stress value of 0.2101) (Figure 17), the OTUs grouped generally into samples with glabrous sepals and those with hirsute sepals, but with very little other pattern emerging. Perhaps not surprisingly, sepal characters were found to strongly influence the locations of OTUs in ordination space (Table 17 and 18).

Table 17. The top five characters based on Kruskal-Wallis value in the ordination of 128 OTUs of the *D. peltata* complex.

Character	KW value
239. SEP_HD	85.68949
238. SEPapHirs	82.69126
75. LCL_Lav	76.46313
74. LCL_Lx	75.90005
103. MCL_Wx	75.17372

Table 18. Correlation between attributes and ordination vectors, and maximum correlation for 128 OTUs of the *D. peltata* complex. Only the top five characters are listed and all these have low correlation values and pertain to sepal hair characters.

Character	Vector 1	Vector 2	Vector 3	Correlation
239. SEP_HD	0.140	-0.649	0.748	0.829
238. SEPapHirs	0.142	-0.639	0.756	0.791
240. SEP_Hloc	0.142	-0.639	0.756	0.791
241. SEPsurfHLn	0.183	-0.642	0. 744	0.735
242. SEPsurfHLx	0.183	-0. 642	0.744	0.735

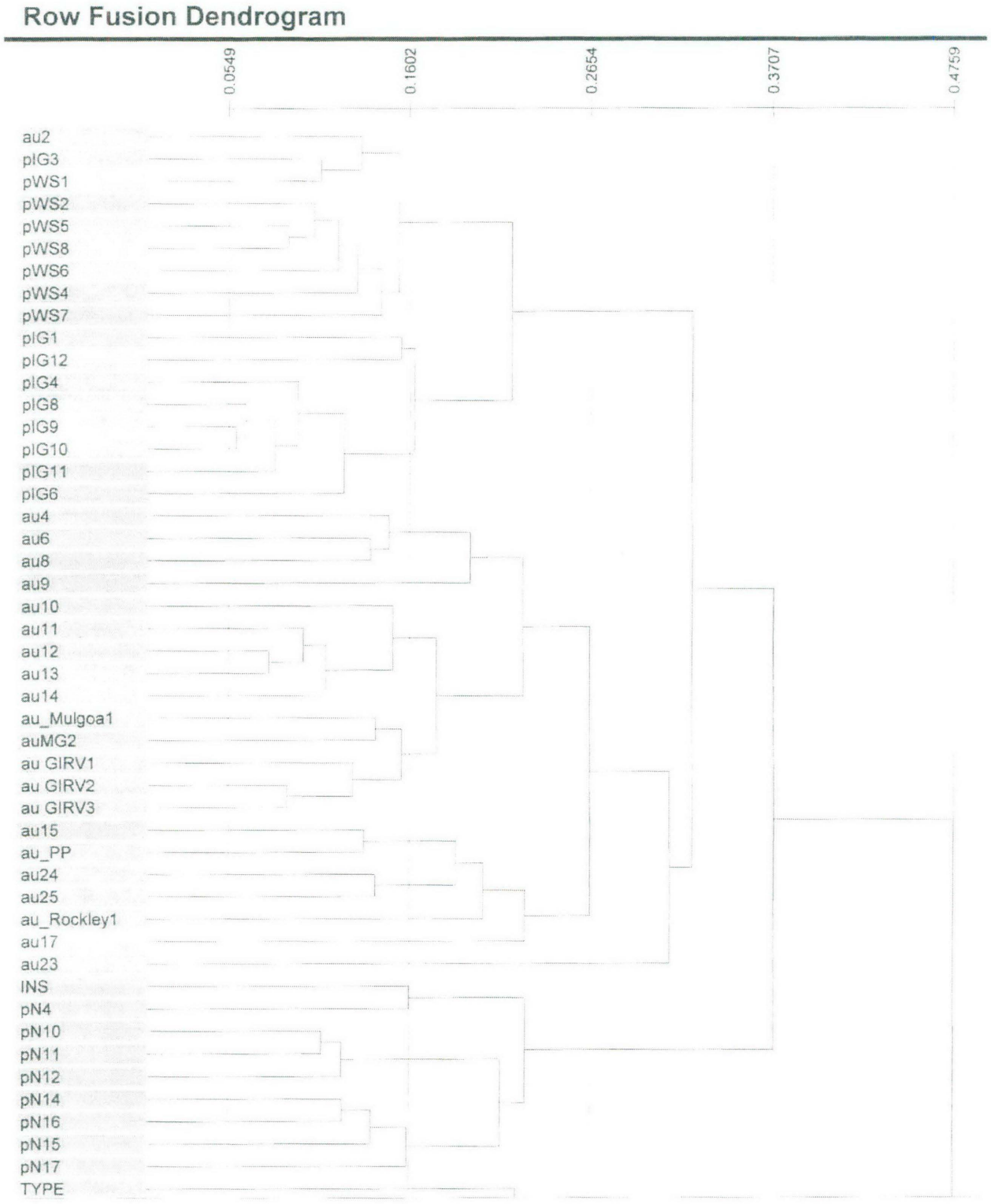


Figure 16. Phenogram of the 128 data-rich OTUs within the *D. peltata* complex. The OTUs form two broad groups, of glabrous or hirsute sepals, with mixtures of putative taxa throughout the diagram.

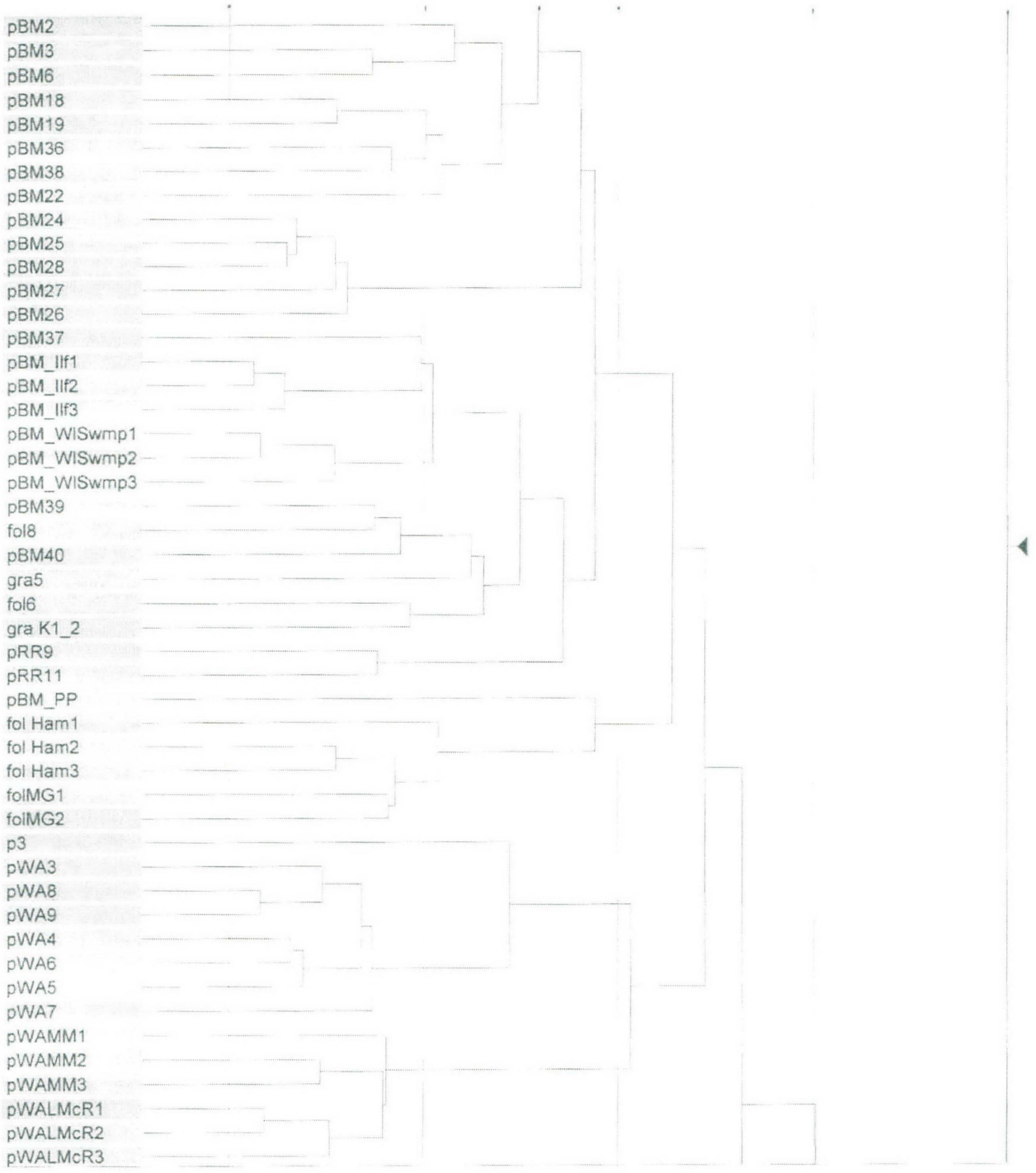


Figure 16 (continued)

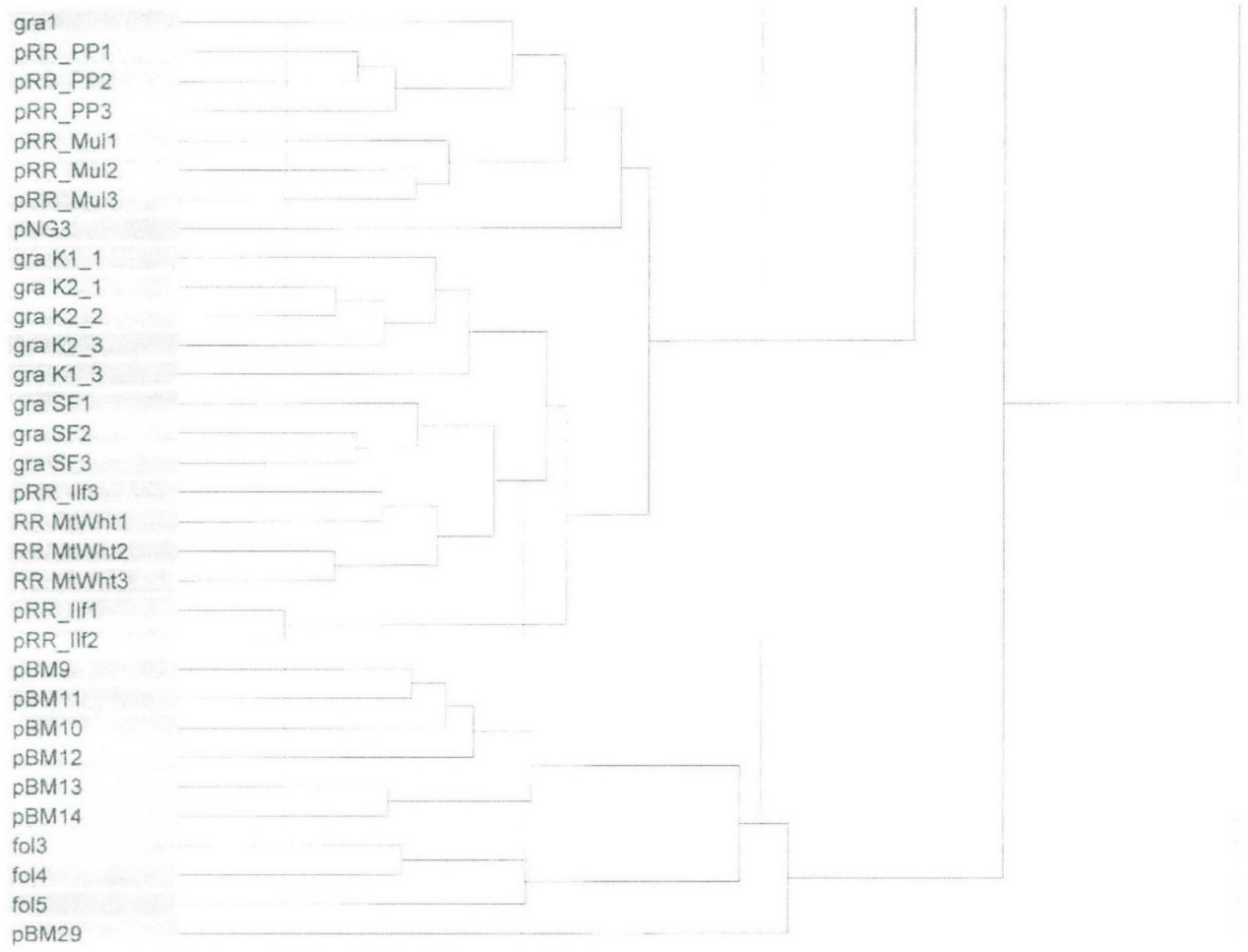
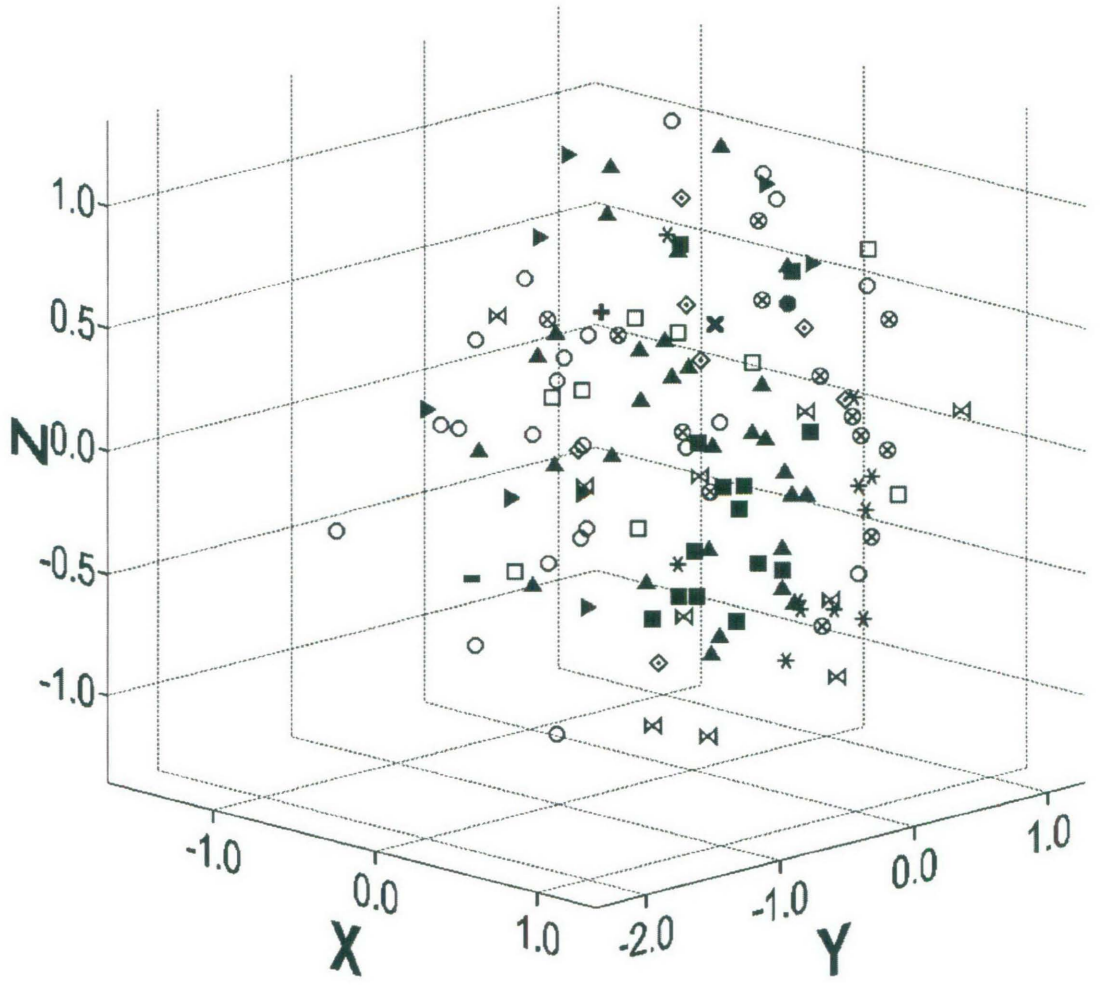


Figure 16 (continued)



○ *D. auriculata*
▲ *D. peltata* 'Black Mountain, A.C.T.'
⋈ *D. 'foliosa'*
✳ *D. peltata 'gracilis'*
□ *D. peltata* 'Isla Gorge, Qld.'
▬ *D. insolita*
▶ *D. nipponica*
✚ *D. peltata* New Guinea 3
✖ *D. peltata* 3
■ *D. peltata* 'Red Rosette'
● *D. peltata* TYPE
⊗ *D. peltata* 'Western Australian Form'
◈ *D. peltata* 'Western Sydney'

Figure 17. Three-dimensional ordination of the 128 data-rich OTUs. The OTUs form a nebulous cloud with no discernible groups.

The phenetic and ordination analysis of the 128 data-rich OTUs did not reveal any further groups within the dataset. Whilst small concentrations of OTUs of the same entity may have grouped together in the phenogram, no OTUs of the same entity grouped together and away from others. The OTUs were grouped somewhat on the presence or absence of a basal rosette, but outcomes of further phenetic and ordination analyses remained the same (results not shown) with no discrete groups of OTUs obtained.

A different approach was tried for the fourth iteration of the full dataset. OTUs from the same herbarium collections were pooled to make a new OTU per collection (Table 19). Then, in successive analyses, the data-poor OTUs were removed, and finally the comparator taxa were removed. This led to 41 characters being made invariant (Table 20) which were then removed from the dataset. The final dataset for the fourth iteration comprised 66 OTUs and 317 characters.

Table 19. Forty-one new OTUs made by combining 146 OTUs that represent individual herbarium specimens from the one collection.

New OTU code	OTU codes combined
au_NZ_av	au9, au10, au11, au12, au13,au14
au_Briggs	au20, au21, au22
au_Dunkeld	au15, au23, au24, au25
au_MG_av	auMG1, auMG2, auMG3
au_GIRV_av	au_GIRV1, au_GIRV2, au_GIRV3
BIC_av	BIC1, BIC2, BIC3, BIC4, BIC5
PBM_Grt_av	pBM3, pBM4, pBM5
PBM_Wlawa_av	pBM9, pBM10, pBM11, pBM12, pBM13, pBM14
PBM_Mundi_av	pBM15, pBM16
PBM_Guyra_av	pBM18, pBM19, pBM20
PBM_NZ_av	pBM24, pBM25, pBM26, pBM27, pBM28
PBM_Tennant_av	pBM32, pBM33, pBM34
PBM_Uralla_av	pBM36, pBM37, pBM38
PBM_Gunn_av	pBM39, pBM40, pBM41
PBM_Ilf_av	pBM_Ilf1, pBM_Ilf2, pBM_Ilf3
PBM_WlSwmp_av	pBM_WlSwmp1, pBM_WlSwmp2, pBM_WlSwmp3
PBM_WH_av	pBM_Wlmtha1, pBM_Wlmtha2, pBM_Wlmtha3
fol_Tumby_av	fol3, fol4, fol5
fol_Gunn_av	fol6, fol7, fol8
fol_HAM_av	fol_Ham1, fol_Ham2, fol_Ham3
fol_MG_av	folMG1, folMG2
gra_Gunn_av	gra2, gra3, gra3
gra_K1_av	graK1_1, graK1_2, graK1_3
gra_K2_av	graK2_1, graK2_2, graK2_3
gra_SF_av	graSF1, graSF2, graSF3
pIG_Qld_av	pIG5, pIG6, pIG7, pIG8, pIG9, pIG10, pIG11
pN_Japan_av	pN7, pN8, pN9, pN10, pN11, pN12
pN_SK_av	pN13, pN14, pN15, pN16, pN17
pN_Phil_av	pN18, pN19, pN20
pRR_Bruhl_av	pRR4, pRR5
pRR_Williams_av	pRR6, pRR7, pRR8
pRR_PL_av	pRR9, pRR10, pRR11
pRR_PP_av	pRR_PP1, pRR_PP2, pRR_PP3
pRR_Ilf_av	pRR_Ilf1, pRR_Ilf2, pRR_Ilf3
pRR_Mul_av	pRR_Mul1, pRR_Mul2, pRR_Mul3
pRR_WalSW_av	pRR_WlSwmp1, pRR_WlSwmp2
pRR_MW_av	pRR_MtWht1, pRR_MtWht2, pRR_MtWht3
PWA_HH_av	pWA3, pWA4, pWA5, pWA6, pWA7, pWA8, pWA9
PWA_MM_av	pWAMM1, pWAMM2, pWAMM3
PWA_LMR_av	pWALMcR1, pWALMcR2, pWALMcR3
PWA_av	pWS4, pWS5, pWS6, pWS7, pWS8

Table 20. Forty-one characters made invariant by the removal of the 146 OTUs listed in Table 16 from the dataset following the fourth iteration analysis.

Character No.	Character Code	Character Description
1	ROOT	Root system <type>
6	Stip_P	Stipules <whether present>
7	Vert_Stem	Vertical stem <whether present>
8	Stem_Brcts	Stem bracts <whether present>
32	BRLshp_lin	Basal rosette with linear leaves <whether present>
33	L_dich	Leaves<whether dichotomously divided>
34	L_dich_2	Once-forked leaves <whether present>
35	L_dich_3_4	Multiply-divided leaves with 3 or 4 terminal lobes <whether present>
36	L_dich_5	Multiply-divided leaves with 5 or more terminal lobes <whether present>
53	STEMglab	Stem <whether glabrous>
55	CL_cup	Cauline leaves <whether flat or cupped>
60	LCLshp_ov	Ovate-shaped leaves <whether present> on the lower third of the stem
63	LCLshp_flab	Flabellate-shaped leaves <whether present> on the lower third of the stem
71(a)	LCL_gshp1	Lowest cauline leaf general shape <crescent-shaped>
89	MCLshp_ov	Ovate middle cauline leaves <whether present>
92	MCLshp_orb	Orbicular middle cauline leaves <whether present>
93	MCLshp_ren	Reniform middle cauline leaves <whether present>
98	MCL_gshp	Middle cauline leaf general shape <orbicular or crescent-shaped>
99 (c)	MCLW3_3	Middle cauline leaves widest <with respect to the axis: in the lower third>
108	MCLaurP	Middle cauline leaf auricles <whether present>
116	UCLshp_ov	Ovate leaves <whether present> in the apical third of the stem
125	UCL_gshp	Upper cauline leaf general shape <orbicular or crescent-shaped>
135	UCLaurP	Upper cauline leaf auricles <whether present>
144	AXLshp_ov	Ovate axillary leaves <whether present>
151	AXL_gshp	Axillary cauline leaf general shape <orbicular or crescent-shaped>
268	FILcol	Filaments <colour>
272	STYLno	Styles <number>
273	STYdiv	Styles <whether divided into numerous style segments>
288 (e)	STYcol_red	Styles <colour: red>
277	STYapx_cyl	Cylindrical stigma apices <whether present>
278	STYapx_ovo	Ovoid stigma apices <whether present>
279	STYapx_obc	Narrowly obcuneate stigma apices <whether present>
280	STYapx_cra	Crassiform stigmas <whether present>
282	STIGundiv	Undivided stigmas <whether present>
284	STIGmul	Multiply-divided stigmas <whether present>

2.4.5 Fourth iteration – data-rich, pooled collection OTUs of the *D. peltata* complex

The fourth iteration of the full dataset comprised 66 OTUs and 317 characters, which were selected to enhance the chance to find any patterns in the dataset, by having as few gaps in data set as possible. The five most influential characters are shown in Tables 21 and 22. Cluster analysis produced the phenogram, shown in Figure 18 and the ordination analysis is shown in Figure 19. The three-dimensional stress value of the ordination analysis was 0.1947.

Table 21. The top five characters based on Kruskal-Wallis value in the ordination of 66 pooled OTUs of the *D. peltata* complex.

Character	KW value
104. MCL_Wx	44.58761
77. LCL_Wx	44.09955
239. SEP_HD	43.94895
105. MCL_Wav	43.18866
132. UCL_Wav	42.44642

Table 22. Correlation between attributes and ordination vectors, and maximum correlation for 66 pooled OTUs of the *D. peltata* complex. Only the top five characters are listed.

Character	Vector 1	Vector 2	Vector 3	Correlation
107. MCL_LWx	0.334	0.150	0.930	0.809
131. UCL_Wx	0.207	0.294	0.933	0.802
132. UCL_Wav	0.161	0.272	0.949	0.802
130. UCL_Wn	0.134	0.317	0. 939	0.782
239. SEP_HD	-0.821	-0. 077	0.567	0.782

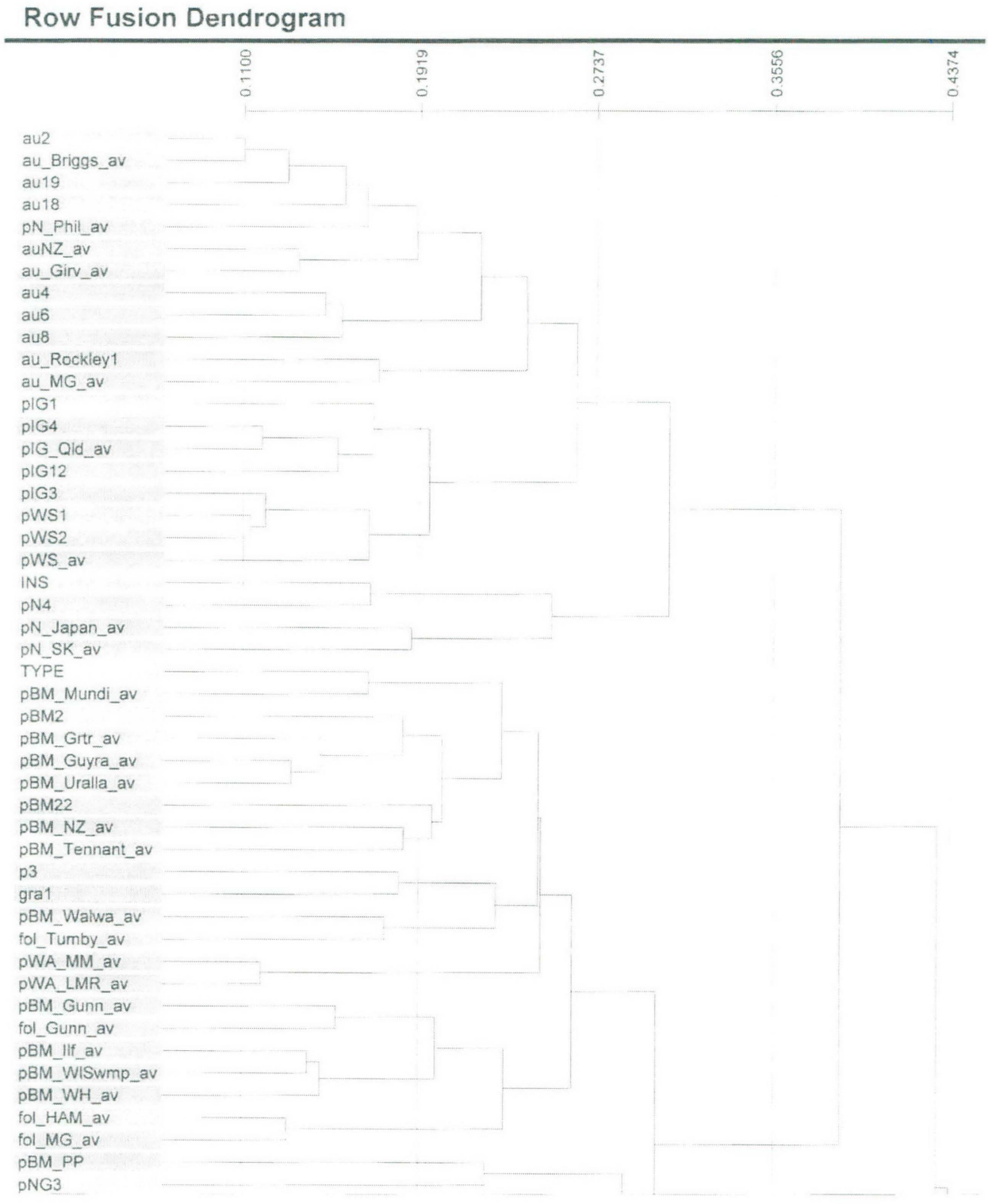


Figure 18. Phenogram of the 66 data-rich OTUs and OTUs from combined specimens per collection. Note that *D. bicolor* plots away from other putative taxa and that the putative taxa do not form discrete groups.

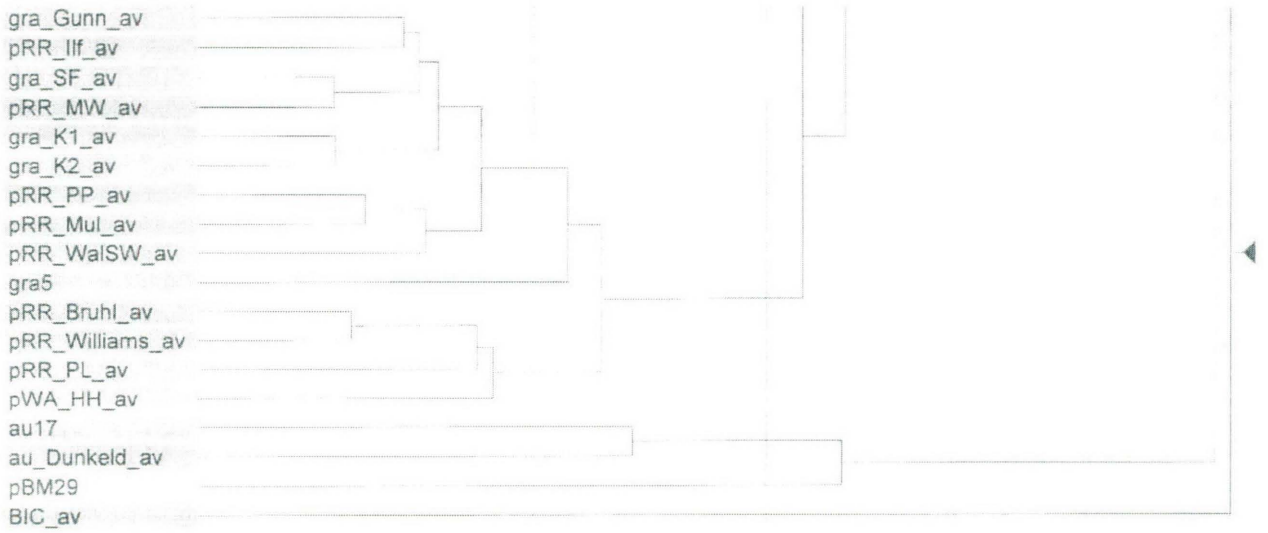
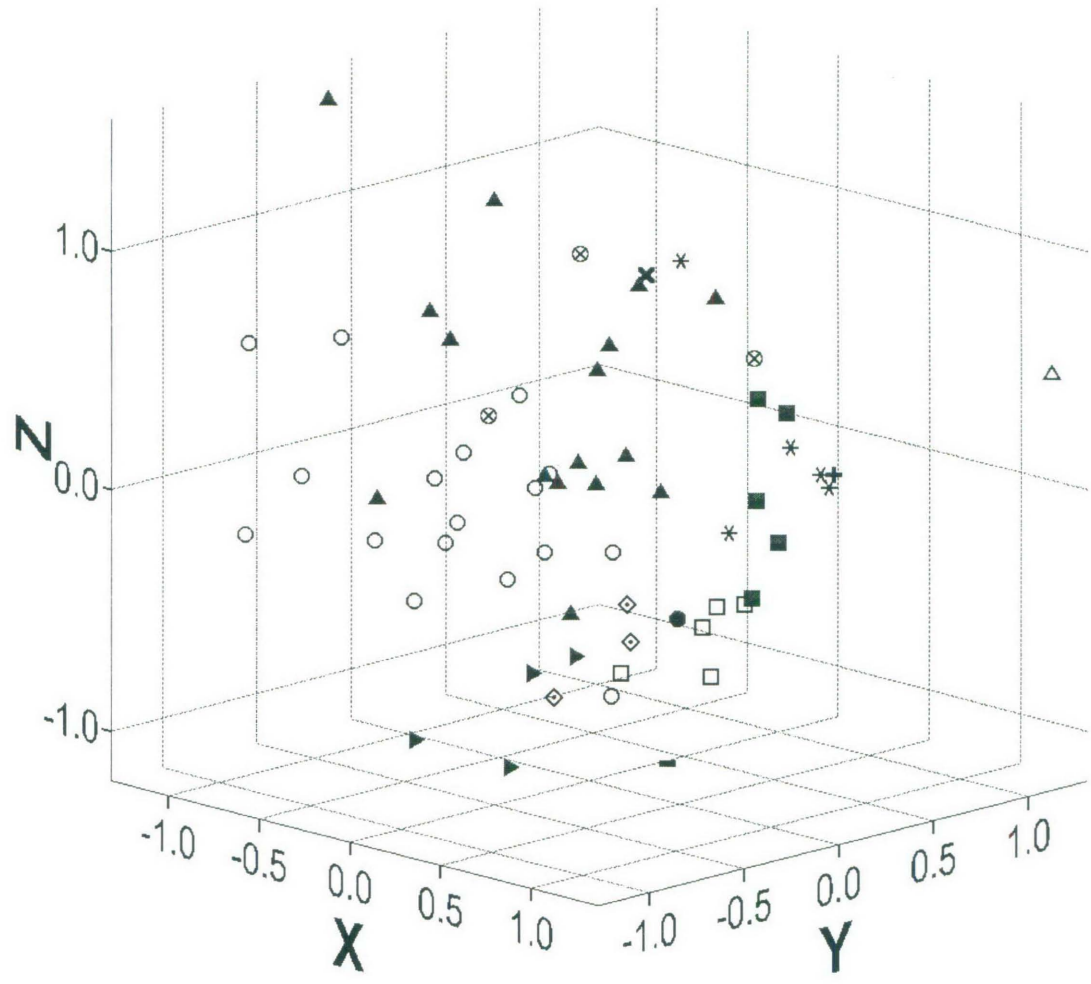


Figure 18 (continued)

From the fourth iteration of the dataset there is support for the separation of *D. bicolor* from the *D. peltata* complex but not for the recognition of other entities as distinct taxa within the complex, not even for the recognition of *D. auriculata* as separate from *D. peltata*. The remaining OTUs form a continuum in which there are no clear boundaries for any of the groups of putative taxa, this is despite some groupings of like taxa together in the phenogram. This indicates that there are OTUs which have either characters that exist in a continuum, rather than as discrete states, or that there are few c-varying suites of characters. Intriguingly the length and width characters of cauline leaves from different thirds of the stem seems to have a larger influence than sepal characters on the locations of OTUs in ordination space.

The six OTUs that were not given a priori names (Table 5) did not group with *D. bicolor* in any of the phenograms or ordination diagrams. Therefore they are all to be ascribed to the morphologically variable and widespread species *D. peltata*.



○ *D. auriculata*
△ *D. bicolor*
▲ *D. peltata* 'Black Mountain, A.C.T.'
✳ *D. peltata* '*gracilis*'
□ *D. peltata* 'Isla Gorge, Qld.'
▬ *D. insolita*
▶ *D. nipponica*
✚ *D. peltata* New Guinea 3
✖ *D. peltata* 3
■ *D. peltata* 'Red Rosette'
● *D. peltata* TYPE
⊗ *D. peltata* 'Western Australian Form'
◈ *D. peltata* 'Western Sydney'

Figure 19. Three-dimensional ordination of 66 OTUs by 317 characters.

2.5 General discussion

The results of this analysis did not support the recognition of putative species within the complex, which was surprising and counterintuitive to earlier examination of this complex, in which the different taxonomic entities appeared to be morphologically distinct (Gibson 1992; Gibson 1993a). Phenetic analysis does support the recognition of *D. bicolor* as distinct from the rest of the complex, in contrast to initial taxonomic status given to this entity by Lowrie and Carlquist (1992), and the subsequent reduction to synonymy to *D. peltata* by Walker (1993 onwards). The historical recognition of the glabrous-sepalled species '*D. auriculata*' and the hirsute sepalled species '*D. peltata*' (Planchon 1848; Diels 1906; Conn 1981; Marchant and George 1982; Schlauer 1996) was here not recognized. It appears that gaps in the phenetic dataset, due in part of phenotypic plasticity, may have blurred the results of this analysis. However, it does appear that not all characters given taxonomic credence for identifying members of this complex by other authors have the merits so claimed.

2.5.1 Data gaps in the phenetic data matrix

The phenetic dataset generated by this study had many missing values, or gaps. This occurred due to three reasons. The first was that the character to be scored was missing, most commonly because it had yet to form on the plant before it was collected, such as flowers on sterile specimens, or seeds from those plants that were picked just as they had begun to flower. The second case was when the character was present but was obscured by other plant parts and for which destructive sampling was not an option, such as examining the flower structure or seed shape of closed flowers of type specimens. The third case was where additional characters were added to the study after commencement and it was not possible to review all previously examined specimens due to their location in overseas herbaria. Having gaps in a phenetic dataset is not ideal (Belbin 1995; Wilkinson 1995). The influence of missing values was seen particularly when studying sterile and fertile specimens, and how far these samples would plot apart in ordination space; such as sterile and fertile OTUs of *D. binata* (Figure 13). Gaps in the dataset may have lead to dispersal of OTUs within ordination space. This may partly explain why many of the OTUs of putative taxa grouped in the

phenogram but that these groups did not discretely occur in ordination space. In addition it appears that presumed "good characters" had poor taxonomic value.

Members of the *Drosera peltata* complex exhibit phenotypic plasticity that is due, in large part, to local environmental conditions. Whilst all members of this complex are perennial, they are all seasonal in growth spending several months of the year aestivating as a tuber in the soil profile. They grow when conditions are seasonally or temporally suitable (Marchant and George 1982; Salmon 2001). The stored water and nutrient reserves of the tuber may be used to start a new season's growth so it coincides with, or just precedes, the return of favourable growing conditions. During the growing season the old tuber is exhausted and replaced whilst the above-ground parts of the plant go through the cycle of forming a basal rosette, forming a stem with cauline leaves, developing a terminal raceme (or rarely a cyme), flowering and setting seed. Observations of plants in the wild reveal that they become dormant rapidly with the aerial portions withering quickly when exposed to hot and dry weather. This suggests that the reserves in the tuber are not available to support all mature flowers setting seed prior to plant senescence. In contrast, under prolonged optimal growing conditions, plants respond by forming additional axillary branches, and thus often produce more inflorescences and hence generate more seed. Thus in many ways plants of the *D. peltata* complex behave in a similar way to an annual plant, such as the species of *Leavenworthia*: Brassicaceae (Solbrig and Rollins 1976) from around limestone outcrops in the United States of America.

The formation of the basal rosette stage of members of the *D. peltata* complex outside of Western Australia appears to be facultative. Observations of plants in the wild and in cultivation suggest that light levels may have something to do with whether a rosette is formed or whether the plant will abort this stage and directly form a stem with cauline leaves. It appears that the basal rosette usually develops when the soil surface receives bright light. In contrast when the stolon from the tuber reaches the soil surface where it is dark, due for example to shading by existing vegetation, then a small rosette may form, or the rosette and perhaps even the lower stem leaves may be aborted. The petioles of the aborted leaves instead remain short, sometimes retaining the aborted lamina in an embryonic state, and form what have been called

bracts. Salmon (2001) has elegantly illustrated plants of this complex exhibiting a range of forms with and without basal rosettes.

2.5.2 Characters historically used in taxonomy of the *D. peltata* complex

Several characters have been stated by different authors as having taxonomic significance in distinguishing between taxa within the complex. These include peduncle length, sepal indumentum, sepal apex and margin outline, sepal length, petal length, length of basal part of style branches and seed length (Planchon 1848; Diels and Pritzel 1905; Diels 1906; Marchant and George 1982; Salmon 2001). In the present study not all characters were available to be measured from all specimens. However, sepal surface and margin characters, and the development, or not, of a basal rosette, were available from most specimens studied.

Historically the sepal margin and outer surface characters, flower structure and seed morphology characters have been used to differentiate between different members of the *D. peltata* complex (Diels 1906; Marchant and George 1982). These characters are not always present on herbarium specimens, particularly those collected early in the growing season. From the specimens studied here, even discarding sterile specimens, the strength of such characters in differentiating between taxa in ordination space was weak at best.

The persistence of a functioning basal rosette to flowering has been used as a taxonomic character for differentiating between taxa of the *D. peltata* complex in southern Asia (e.g. Clarke 1879). However, from the course of the study, and as shown from the phenetic analysis, this character appears to be dubious. Aside from the plasticity on whether the basal rosette is produced or aborted, local seasonal conditions would play an important role in determining whether the basal rosette was withered or functioning at the time of flowering, with the latter occurring under prolonged seasonal conditions. In addition, when collecting herbarium specimens for this study it was found that plants did not always break from the rootstock at soil level. In some cases the point of detachment occurred within the rosette, so that only some of the basal rosette was collected with the specimen, or at other times the stem broke above the rosette. Post-collection deterioration of herbarium specimens also

reduces the amount of information about basal rosette development. The most extreme example found was that of the type specimen of *D. insolita*: the circumscription includes details of the basal rosette yet the type specimen, which consists of one plant and is the only collection of this taxon, does not now have a basal rosette. It appears the basal rosette leaves have become detached and lost from the specimen over time. Therefore the presence or absence of a basal rosette, and its persistence to flowering, or not, appears to be of dubious taxonomic use. It is therefore not surprising that this character did not show a strong correlation to taxa in this phenetic analysis.

Flower structure and seed characters showed promise in this study, in that they appeared to co-vary in predictable ways between different entities within the complex. However, they were either not present, or were difficult to measure, from herbarium specimens. The life cycle of most members of this complex culminates in flowering and seed set at the end of the growing season; often occurring over only a few short weeks. In addition the flowers are open for a few hours around the middle of the day. This combination of features conspires to make collecting a herbarium specimen with a pressed open flower and ripe seed an unlikely event. If it were not possible to collect such specimens during a warm day when the flowers were open, then another option would be to collect flowering and fruiting specimens and preserve them in liquid, so that the flower structure could be teased open and examined in three-dimensions.

The analysis of sympatric putative entities did indicate that there was some biological reality to the differences initially observed. However, these differences did not remain when a larger sample set was analysed. It may be that we are dealing with a highly variable species perhaps in the early stage of differentiating across its range. If so, then a phenetic study may be less useful in highlighting any differences. However, the use of molecular data of targeted genes, and conducting experimental pollinations to determine intra- and inter-taxon compatibility, may be useful tools to test the nature of variation and to test if any incipient speciation may be occurring.

2.5 Conclusions

This phenetic study found support for the recognition of *D. bicolor* as a distinct species but no support for the recognition of further taxa within the *Drosera peltata*

complex. The latter result is surprising given the prior recognition of supposedly distinct taxonomic entities (e.g. de Candolle 1824; Planchon 1848; Diels 1906; Vickery 1933; Marchant and George 1982; Stanley and Ross 1983; Harden 1990; Gibson 1993a).

From this study several conclusions were drawn:

- The test of additional novel characters, intuitively thought to have taxonomic significance, did not prove to be the case from this study;
- Lack of data across the dataset appears to have weakened the resolving of taxonomic pattern;
- Few distinct morphological entities within the *D. peltata* complex were evident in the dataset and from the nebulous cloud of OTUs in ordination space.
- Only two taxa can be recognized from within the *D. peltata* complex, viz the highly localized *D. bicolor* from south-western Australia, and the widespread and morphologically variable *D. peltata*.

There are certainly some characters that correlate with some members of the complex, such as sepal surface and margin characters and the width of cauline leaves. However, these do not play a significant role in clearly separating OTUs in ordination space. Instead the OTUs of the *a priori* assigned entities tend to occur like a series of stacked discs. Such a situation has been described by Sneath and Sokal (1973, p. 429) "...in which two taxa are sharply separated on a few characters only, so that they form in phenetic space, two flat plates separated by a small gap." This pattern of OTU distribution suggests that a weak phenetic signal may occur in the dataset.

It is possible that cryptic species exist in the complex and it is possible that the weak pattern in the phenograms represents evidence for them. Perhaps measuring characters from live samples, or liquid preserved samples, with flowers and seeds available, would help to resolve the significance of variation within the complex? Alternatively, the analysis of separate data sets, such as molecular data (Chapter 3), or experimental pollinations between putative species (Chapter 4), may provide new insight into the *D. peltata* complex.