UNIVERSITY OF SOUTHAMPTON

THE INTER AND INTRA-GENERIC RELATIONSHIPS BETWEEN *PSOPHOCARPUS* SPP. AND THEIR ALLIES

A Thesis submitted for the Degree of
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in the

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by

Nigel Maxted

Department of Biology

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I would like to dedicate this thesis to the memory of a special friend,
Alan Graves

FLEUR Si je livrois mon imaginatiion aux douces sensations que ce mot semble appeller, je pourrois faire un article agréable peutêtre aux Bergers, mais fort mauvais pour les Botanistes. Ecartons donc un moment les vives couleurs, les odeurs suaves, les formes élégantes, pour chercher premiérement à bien connoître l'être organisé qui les rassemble.

Jean-Jacques Rousseau
(Dictionnaire de Botanique)

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ABSTRACT

FACULTY OF SCIENCE
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The aim of this study was to produce a classification, firstly of the relationships between the legume genus *Psophocarpus* and its allies and secondly, between *Psophocarpus* species.

To provide a background and to clarify the problems associated with *Psophocarpus* taxonomy the taxonomic history of *Psophocarpus* species and their allies was reviewed. Due to the large number of taxa involved in the investigation (approx. 489 species) and that these taxa are endemic to the tropics, practically the investigation was limited to morphological studies of herbarium material gathered from major herbaria. Both phenetic and phylogenetic studies of the material were undertaken.

The phenetic investigation was divided into four component studies: the survey of 31 genera of the Phaseolinae (sensu Lackey, 1977a); the detailed study of a subset of seven genera shown in the previous survey to be most closely allied to *Psophocarpus*; the study of the nine *Psophocarpus* species and the study of the two closely allied species *P.palustris* and *P.scandens*. A gross character set was formulated of 315 morphological characters, containing vegetative, floral, legume and seed characters. From this appropriate character sets were selected for the component studies. The choice of characters was largely intuitive, but an objective character analysis program was also utilised. Over 800 specimens were scored and the data were analysed using both cluster analysis and ordination methods.

The phylogenetic investigation used the cladistic method of analysis and may be separated into two component studies: the six *Psophocarpus* allied genera study and the *Psophocarpus* species study. Apomorphic characters were selected and character state polarity ascribed to each, nested synapomorphies were found and cladograms produced for both studies.

After extensively reviewing the non-morphological evidence available in the literature classifications were proposed at the generic and specific levels. Separate phenetic and phylogenetic classifications were proposed for the relationship between *Psophocarpus* * species classification. In the discussion these classifications are reviewed in the light of other workers concepts, as well as discussing the methods used and suggesting areas for future research.

* and its close allies as they could not be married to form one general classification. However, this was possible for the proposed Psophocarpus

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Problem elucidation

Much current tropical agricultural research is being concentrated on locating and developing underexploited crops as one step towards alleviating the Third World protein deficit. One legume crop that has proved particularly interesting in this context is the winged bean, *Psophocarpus tetragonolobus* (L.) DC. The United States National Academy of Science refers to the winged bean (NAS 1975) as "a legume with a multitude of exceptionally large nitrogen-fixing nodules . . . producing seeds, pods and leaves (all edible to humans and livestock) with exceptionally high amounts of protein and edible seed oil". Haq (1982) underscores the possibilities of turning the winged bean from a 'backyard' plant of South and South East Asia to a field scale crop of the entire tropics.

Before any crop can be efficiently exploited it is essential that the crop's systematic relationships with other species of the same genus and with species of allied genera are clearly understood. The importance of this statement is predicated on the number of attempts being made by plant breeders to introduce genes for various characters into crop plants from allied wild species. Marechal, Mascherpa and Stainier (1978a), while stressing this point, go further to accentuate the practical importance of taxonomy in realising the limits of utilisation of the immense natural genetic resources available to plant breeders:

> *"Il n'en reste pas moins vrai que le success d'un programme d'hybridations inter-specifiques dependra essentiellement de la proximite phyletique des especes par entales"*

The general aim of this project therefore is to ellucidate the natural relationships between the winged bean and its allies.

1.2 Aims

The aims of this study may be summarised as follows:

1. At the generic level and to a lesser extent at the specific level *Psophocarpus* has proved problematic for the taxonomist. The first aim of this study is to clarify what are the taxonomic problems associated with *Psophocarpus*.

2. The natural position of *Psophocarpus* within the classification of the Leguminosae is here accepted as being within the tribe Phaseoleae DC., which contains more crop plants than any other legume tribe. As a large proportion of taxonomic effort is focused on crop plants and their allies, the taxonomic literature relating to the Phaseoleae is copious. Thus the second aim of this study is to collate and review the literature concerning *Psophocarpus* and its possible allies.

3. The third aim is to undertake novel morphological studies, both at the generic and specific levels. So that with this information and non-morpholoigical evidence available from the literature, the relationships between the taxa under investigation may be better understood.

4. The fourth and primary aim of the project is to produce a usable revision of both the relationships, between *Psophocarpus* species and between the genus *Psophocarpus* and its Phaseoleae allies. This primary aim may be subdivided into two components.

(a) The production of an inter and intra-generic classification based on phenetic principles. Phenetic here being defined in terms of overall similarity as it is perceived through the senses (Davis and Heywood, 1973).

(b) The production of an inter and intra-generic classification based on phylogenetic principals. Phylogenetic meaning to imply propinquity of decent (Davis and Heywood, 1973).

The final classifications of *Psophocarpus* spp. and *Psophocarpus* in relation to its allies will attempt to marry both the phenetic and phylogenetic results to produce the most natural overall classification. Though if the phenetic and phylogenetic classification are irreconsilable then they will be presented separately.

1.3 Delimitation of taxon study

Psophocarpus as a genus is distinctive, its combination of diagnostic characters effectively isolate it from its nearest allies, so that for the intra-generic study there is a clear taxon delimitation which includes the nine species described by Verdcourt and Halliday (1978) as the species of the genus *Psophocarpus*.

The scope of the inter-generic study was not so clearly delimitated; time, facilities and material availability were necessarily balanced against the number of taxa to be studied. Marchal et al (1978a) when faced with a similar problem of taxon delimitation within the *Phaseolus-Vigna* complex comment, "Un choix a priori des genres a considerer comme annexes risque donc d'etre tres subjectif. Il nous a semble preferable de faire coincider le materiel etudie avec un groupe botanique aussi naturel que possible."

A similar attitude has been adopted in the present study. After reviewing the literature relating to *Psophocarpus* taxonomy, it is apparent that the natural position of *Psophocarpus* in the classification of the Phaseoleae is within the subtribe, Phaseolinae Benth., but the genera with which it is allied within this grouping is unclear. The relative classification of the Phaseolinae will be discussed in more detail in Chapter 2, but it is important to understand modern concepts of the Phaseolinae to enable discussion of taxon delimitation for this study.

Two workers have recently proposed revised classifications of the Phaseoleae (Baudet 1978 and, Lackey 1977a and 1981). However both retain relatively similar concepts to Bentham's subtribe Phaseolinae. Baudet (1978) divides the Phaseolinae into two supergenera *Phaseolastrae* and *Dolichostrae* and excludes two genera traditionally considered

members of the Phaseolinae, *Neorautanenia* and *Macrotyloma*. Lackey (1977a) includes these two genera and four other genera (*Clitoria, Clitoriopsis, Periandra* and *Centrosema*). The latter four genera he later (1981) reinstates in the separate subtribe Clitoriinae Benth. Lackey (1977b) argues that the Clitoriinae are a pheripheral group of the Phaseolinae, but in Lackey (1981) alters his opinion believing the Clitoriinae to have an independent origin with unknown allies, possibly *Wisteria*.

Before the final choice on taxon delimitation was made the problem was discussed with Drs. B. Verdcourt and R.M. Polhill (of the Royal Botanic Gardens, Kew). It was decided that the most appropriate solution would be to include the 27 genera of the Phaseolinae and Clitoriinae (Sensu Lackey 1981) for investigation in the inter-generic study.

The species of *Psophocarpus* and genera of the Clitoriinae and Phaseolinae to be studied are listed in Tables 1.1 and 1.2 respectively.

Due to both the large number of taxa contained within the limits of the study (485 species, Lackey 1981) and the difficulties of obtaining viable seed for these taxa (only seed of two *Psophocarpus* spp. was available), the project concentrated on scoring morphological characters from dried specimens. Davis and Heywood (1973) stress the importance of obtaining herbarium material from as many herbaria as possible, so that a true estimate of the range of within taxa variation can be made. To this end herbarium specimens were loaned from the following herbaria: Royal Botanic Gardens, Kew (K); British Museum (Natural History) (BM); Museum National D'Histoire Naturelle, Paris (P); Jardin Botanique National De Belgique (BR); Conservatoire et Jardin Botaniques De Geneve (G); Tohuku University Herbarium, Biological Institute, Sendai, Japan (TUS) and National Herbarium and Botanic Garden, Harare, Zimbabwe (SRGH).

TABLE 1.1

Taxa included in Intra-Generic Study
(after Verdcourt and Halliday, 1978)

Psophocarpus Neck. ex DC., Prodr. 2 : 403 (1825).

1. *P.grandiflorus* Wilczek in Bull. Jard Bot. Brux. 24 : 414 (1954).

2. *P.tetragonolobus* (L.) DC., Prodr : 403 (1825).

3. *P.palustris* Desv. in Ann. Sc. Nat. Ser. 1, 9 : 420 (1826).

4. *P.scandens* (Endl.) Verdc. in Taxon 17 : 539 (1968).

5. *P.obovalis* Tisserant in Bull. Mus. Hist. Nat. Paris II, 2 : 575 (1930).

6. *P.monophyllus* Harms in Engl., Bot. Jahrb. 40 : 43 (May 1907).

7. *P.lecomtei* Tisserant in Bull. Mus. Hist. Nat. Paris II, 2 : 574 (1930).

8. *P.lancifolius* Harms in Mildbr., Deutsch. Zentr-Afr.-Exp. 1907 - 1908, 2 : 270 (1911).

9. *P.lukafuensis* (De Wild.) Wilczek in Fl.Congo Belge 6 : 286 (1954).

TABLE 1.2

Taxa included in Inter-Generic Study

(after Lackey 1981, including numbers of species and distributions)

Clitoriinae Benth. (1837)

1.1 *Centrosema* (DC) Benth.(1837) 45 spp., neotropics and neosubtropics

1.2 *Periandra* Benth. (1837) 6 spp., S.Domingo and Brazil.

1.3 *Clitoria* L. (1753) 70 spp., pantropical, mostly neotropical.

1.4 *Clitoriopsis* Wilczek (1954) 1 sp., Zaire and Sudan.

Phaseolinae Benth. (1837)

2.1 *Dysolobium* (Benth.) Prain (1897) 4 spp., Asia.

2.2 *Psophocarpus* DC. (1825) 9 spp., paleotropical.

2.3 *Physostigma* Balf. (1861) 4 spp., tropical Africa.

2.4 *Vatovaea* Chiov. (1951). 1 sp., Africa.

2.5 *Decorsea* R.Viguier. (1951) 4 spp., Africa and Madagascar.

2.6 *Spathionema* Taub. (1895) 1 sp., Africa.

2.7 *Otoptera* DC. (1825) 2 spp., Africa.

2.8 *Sphenostylis* E. Mey. (1836) 7 spp., Africa and India.

2.9 *Nesphostylis* Verdc. (1970) 2 spp., Africa and Burma.

2.10 *Austrodolichos* Verdc. (1970) 1 sp., Australia.

2.11 *Neorautanenia** Schinz (1899) 3 spp., Africa.

2.12 *Lablab* Adans. (1763) 1 sp., Africa.

2.13 *Alistilus* N.E. Br. (1921) 2 spp., S.tropical Africa and Madagascar

2.14 *Dipogon* Lieb. (1854) 1 sp., S.Africa.

2.15 *Dolichos* L. (1753) 60 spp., Africa to East Asia.

2.16 *Macrotyloma* (Wight & Am.) Verdc. (1970) 24 spp., Africa and Asia.

2.17 *Vigna* Savi (1824) 150 spp., pantropical, mostly paleotropical.

2.18 *Ramirezella* Rose (1903) 8 spp., Mexico and El Salvador.

2.19 *Oxyrhynchus* Brandegee (1912) 4 spp., Central American and New Guinea.

2.20 *Dolichopsis* Hassler (1907) 2 spp., South America.

2.21 *Strophostyles* Elliott (1823) 3 spp., Canada and Mexico.

2.22 *Macroptilium* (Benth.) Urban (1928) 20 spp., neotropics and neo subtropics.

2.23 *Phaseolus* L. (1753) 50 spp., neotropics, neosubtropics and eastern U.S.A.

(* corrected spelling)

1.4 Plan

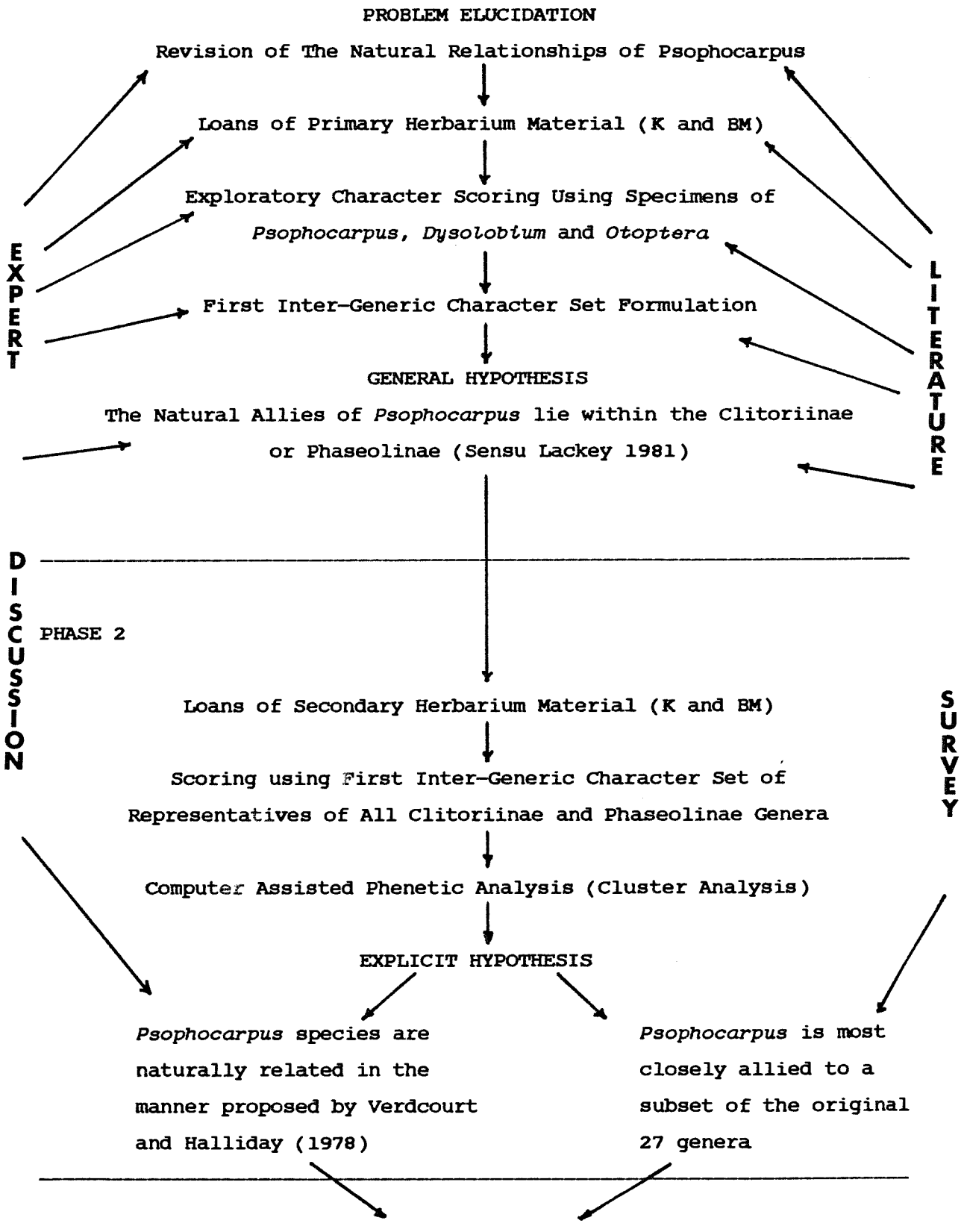
It is important when undertaking a revision of this kind to progress in a logical sequence and to this end an attempt has been made to incorporate the methodological approach suggested by Davis and Heywood (1973) and Lawrence (1951). Dr Mascherpa's chapter in Marechal, Mascherpa and Stainier (1978a) on, "Les methodes informatiques" also proved useful in developing the idea of a logical study plan. The study plan thus formulated is provided in Figure 1.1.

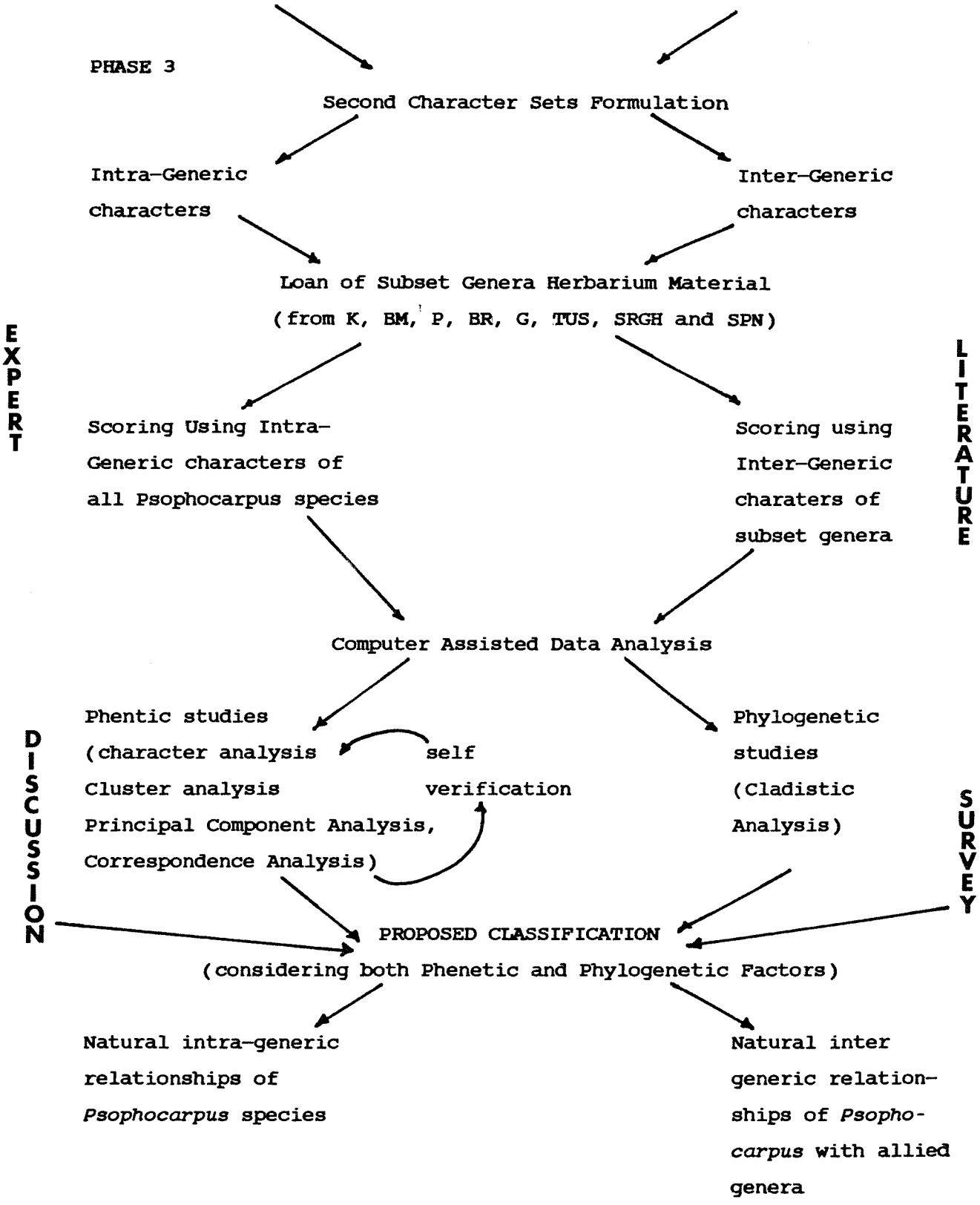
The research divides naturally into three phases. The first in which the problems concerning *Psophocarpus* taxonomy are clarified, the first attempts at constructing a generic character set from exploratory character scoring of the primary herbarium material are made and a general hypothesis is formulated.

The second phase, in which the general hypothesis is tested, uses phenetic characters chosen to form clusters of genera within the study taxon delimitations. The data produced from scoring the broad range of genera represented in the secondary herbaria loans were then analysed using cluster analysis. This analysis enabled the number of genera in the inter-generic study to be narrowed to a subset of genera most closely allied to *Psophocarpus*. Which in turn enabled a new, more explicit hypothesis to be put forward which proposed that the closest allies of *Psophocarpus* lie within the subset of genera selected from the original 27 genera of the Clitoriinae and Phaseolinae.

The final section tests the explicit inter-generic hypothesis that *Psophocarpus* is most closely allied to one of the subset genera and that the intra-generic relationships between *Psophocarpus* species proposed by Verdcourt and Halliday (1978) are natural. Two character sets were used, one for each relationship tested. The character sets were refined and extended from the first generic character set, and now included both phenetic and phylogenetic characters. Specimens were loaned from several large herbaria and the material scored for the appropriate character sets. The data produced were then analysed using several different methods of analysis to verify the classifications that were

Figure 1.1 Study Plan




PHASE 3
Second Character Sets Formulation
Intra-Generic characters
Inter-Generic characters
Loan of Subset Genera Herbarium Material
(from K, BM, P, BR, G, TUS, SRGH and SPN)
EXPERT
LITERATURE
Scoring Using Intra-Generic characters of all Psophocarpus species
Scoring using Inter-Generic charaters of subset genera
Computer Assisted Data Analysis
Phentic studies (character analysis Cluster analysis Principal Component Analysis, Correspondence Analysis)
self verification
Phylogenetic studies (Cladistic Analysis)
DISCUSSION
SURVEY
PROPOSED CLASSIFICATION
(considering both Phenetic and Phylogenetic Factors)
Natural intra-generic relationships of *Psophocarpus* species
Natural inter generic relation-ships of *Psopho-carpus* with allied genera

proposed. In the proposed classifications of *Psophocarpus* species and the relationship between *Psophocarpus* and its allies and in the discussion of these classifications an attempt was made to consider both phenetic and phylogenetic factors.

CHAPTER TWO

TAXONOMIC HISTORY OF PSOPHOCARPUS WITHIN THE PHASEOLEAE

2.1 Introduction

Historical understanding of the taxonimic treatments referring to any taxa under investigation is an essential pre-requisite of any effective taxonomic study. With a group like the Phaseoleae DC. (1825) this becomes a mammoth task due to the large number of genera (approx. 84; Lackey. 1981), mutable genus delimitations, complex nomenclatural problems and inclusion of so many important crop plants. Thus this chapter is not an attempt at a comprehensive history of Phaseoleae taxonomy, but is an attempt to abstract a posteriori major taxonimic advances as they have affected *Psophocarpus* Neck. ex DC. and its close allies, as well as the genus *Psophocarpus* itself.

In this thesis it has been accepted that the natural taxonomic position of *Psophocarpus* lies within the legume tribe, Phaseoleae, but before discussing the position of *Psophocarpus* within the Phaseoleae the Leguminosae must first be introduced.

The tribe Phaseoleae DC. (1825) belongs to the family Leguminosae, subfamily Papilionoideae (Fabaceae, Faboideae, Papilionaceae). The Leguminosae are morphologically diverse, ranging from trees to aquatics to xerophytes, and only rank second to the Gramineae in economic importance to man (Heywood, 1978). The family contains approximately 650 genera and 18,000 species (Polhill, Raven and Stirton, 1981). It is normally divided into three subfamilies, of which the largest is the Papilionoideae. The Papilionoideae are distributed through the temperate, sub-tropical and tropical regions. They are mostly herbs with some trees and shrubs among the 400-500 genera and 10,000 species (Polhill, Raven and Stirton, 1981). The leaves are usually pinnate; the flowers irregular with lateral petals enclosed by the standard in the bud; there are usually 10 stamen, commonly diadelphous but sometimes monodelphous or free. The Papilionoideae have been traditionally divided into 10 or 11 tribes on habit, vegetative and floral characters.

More recently legume taxonomists have tended to increase the number of tribes; Gillett, Polhill and Verdcourt (1971) detail 17 tribes and Polhill (in Lackey 1977b) has suggested a complete splitting of traditional tribal delimitations to form 31 Papilionoideae tribes. The tribe Phaseoleae (Sensu Lackey, 1981) is the largest Papilionoideae tribe with about 84 genera and is economically the most important, containing *Phaseolus* spp., *Vigna* spp., *Glycine max*, *Cajanus cajun*, *Clitoria ternatea*, *Macrotyloma* spp., *Lablab purpureus*, *Pachyrhizus* spp., *Pueraria lobata* and *Psophocarpous tetragonolobus*. Lackey (1981) provides the following description of the Phaseoleae:

PHASEOLEAE DC (1825)

Dextrorotatory twining, prostrate, or erect herbs, occasionally subshrubs or rarely trees; leaves usually pinnately 3-foliate, less commonly 1-9 foliolate or palmate; leaflets sometimes lobed; stipels and stipules present; foliar and foliolar pulvini present; inflorescence consisting of fascicles of flowers scattered along an axis, sometimes reduced to solitary flowers or expanded into panicles; calyx with (4-5) teeth; corolla papilionaceous; stamens in 9+1 arrangement, the vexillary stamen free or partially or completely united to the others; pods 2-valved. Seedlings with opposite first leaves. 2n usually = 20,22. 84 genera worldwide, primarily in tropical and subtropical regions.

Lackey in sub-dividing the Phaseoleae recognises 8 sub-tribes, he discriminates sub-tribel limits using leaf, seed but largely floral characters. The key to Lackey (1981) Phaseoleae subtribes is provided in Figure 2.1. Lackey then places *Psophocarpus* in the seventh subtribe, the Phaseolinae Benth. (1837), the generic key to which is included in Figure 2.2 . The key is necessarily extensive but important as it provides an important introduction to Lackey's concept of the Phaseolinae, which is followed in this thesis. *Psophocarpus* the genus has had a 'peacemeal' taxonomic history, with taxonomists only covering the most essential work (as will be shown in Section 2.3, of this chapter). However, Verdcourt and Halliday (1978) have recently thoroughly revised the genus and their description of *Psophocarpus* with synonomy is provided below.

Figure 2.1 Key to subtribes of Phaseoleae (taken from Lackey, 1981)

1 Leaflets and calyx generally with yellowish gland-dots; bracteoles 0 (except *Adenodelichos*); style slender below, hardened and a little thickened distally, glabrous except *Adenodolichos*; inflorescences not nodose 10h. *Cajaninae*.

1 Leaflets and calyx eglandular:

2 Style complicated by expansion, flattening, coiling or specialised hairs, or if rarely both unbearded and terete then petals elaborate with appendages on standard and keel-petals adaxially joined; hila usually covered with spongy tissue 10g *Phaseolinae*.

2 Style generally terete and unbearded (sometimes a few hairs below the stigma), occasionally coiled in Erythrininae, sometimes bearded or flattened in Clitoriinae but then petals less complex; hila rarely (some *Erythrina*) covered with tissue:

3 Flowers generally resupinate; calyx naked inside; style narrowed or expanded to naked, penicillate or bearded distal part; petals often hairy; leaflets 3, 1 or 5-9, with minute hooked hairs.
10f. *Clitoriinae*.

3 Flowers not resupinate or if so differing in other respects:

4 Standard silky hairy outside, rather small, without appendages inside; seeds smooth, with a prominent aril; inflorescences not or only slightly nodose.
10d *Ophrestiinae*.

4 Standard glabrous or if hairy then inflorescence generally nodose or flowers much modified:

5 Bracteoles 0; seeds with a prominent horsecollar-like aril and thick endosperm - Australia, New Guinea 10e *Kennedyinae*.

5 Bracteoles generally present:

6 Flowers mostly adapted to birds or bats, the petals generally unequal in length, the fertile parts loosely housed or exserted, sometimes with small bee-type flowers but then either style coiled (*Apios*, *Cochlianthus*) or flowers in extensive panicles and pod samaroid (*Spatholobus*) 10a *Erythrininae*.

6 Flowers mostly adapted to bees or if bird-flowers then petals subequal in length:

7 Inflorescence generally nodose, ocassionally paniculate or axillary and few-flowered; seeds diverse, with short to long hilum
10b *Diocleinae*.

7 Inflorescence not or scarcely nodose (sometimes branches in *Pueraria*); seeds smooth, granular or shagreened, with short hilum 10c *Glycininae*.

(Note: 10b-10c not sharply differentiated - if in doubt check both subtribes).

Figure 2.2 Key to Genera of Phaseolinae (taken from Lackey, 1981)

1 Plants native to America:
  2 Uncinate hairs always present (x 25 magnification); style coiled 2–3 revolutions, with a lateral flattened stigma ; bracts usually persistent to fruit set, or at least to anthesis; bracteoles, if present, equally or slightly less persistent 10.71 *Phaseolus*
  2 Uncinate hairs absent; style erect, curved, or sigmoid, rarely coiled through 3–5 or more revolutions (*Vigna* subgen. *Cochlianthus* with bracteoles never persistenting past open flower, and usually falling earlier); bracts and bracteoles variously persistent:
    3 Stipules peltate, or at least with a portion above the point of attachment and a generally smaller often bifid, portion below 10.65 *Vigna*
    3 Stipules with only 1 lobe above point of attachment, although often reflexed on mature stems.
      4 Style ± erect (perhaps slightly sigmoid in *Strophostyles*):
        5 Inflorescence subumbellate; bracts and bracteoles persistent past seed set; upper calyx-lobes completely united; seeds frequently pubescent 10.69 *Strophostyles*
        5 Inflorescences elongate; bracts rarely remaining on open flowers, bracteoles rarely remaining at pod set; upper calyx-lobes separated by a distinct notch; seeds not pubescent:
          6 Style constricted in the middle and with a distinct bulge above; flowers 22–25 mm; minute hairs on inside of the standard and wings 10.65 *Vigna*
          6 Style rarely with even the suggestion of a construction, not bulged; flowers 6–15mm; petals glabrous.
            7 Hilum 50% or more of seed circumference – Central America and vicinity 10.67 *Oxyrhynchus*
            7 Hilum less than 50% of seed circumference – southern South America 10.68 *Dolichopsis*
      4 Style coiled, or inrolled and becoming clearly sigmoid at maturity;
        8 Style coiled 3–5 or more revolution 10.65 *Vigna*
        8 Style inrolled, becoming sigmoid:
          9 Style bent sharply to form a squarish hook ; calyx-teeth all distinct to the same depth on the tube; petals glabrous; leaflets sometimes lobed; keel with a transverse fold; wings longer than other petals; inflorescence-nodes slightly swollen at most; flowers (excluding wings) 3.5–16mm 10.70 *Macroptilium*
          9 Style bent gradually; upper calyx-teeth partially or completely united; standard and wings often with minute hairs;

leaflets never lobed; keel with a longitudinal fold (only rarely approaching transverse); wings not conspicuously larger than other petals; inflorescence-nodes usually conspicuously swollen; flowers (8-) 15-38mm long:

10 Flowers often clustered into strobilus-like inflorescences; inflorescence-nodes not swollen; pedicels usually longer than the calyx at late flower or fruit stage 10.66 *Ramirezella*

10 Flowers usually not in strobilus-like inflorescences; inflorescence-nodes swollen; pedicels short, even in late flower and fruit stages rarely exceeding the calyx length 10.65 *Vigna*

1 Plants native to the Old World:

11 Style flattened:

12 Standard face with a single large appendage

13 Style without flattened margins, a line of hairs along the inner margin 10.60 *Lablab*

13 Style with a flattened blade along each margin, glabrous 10.61 *Alistilus*

12 Standard face with 2 small appendages, or no appendages:

14 Stamen-filaments filiform; vexillary stamen lacking hooks; calyx naked inside 10.56 *Sphenostylis*

14 Stamen-filaments dilated above; vexillary stamen with a hook at the base; calyx pubescent inside:

15 Style expanded at tip 10.57 *Nesphostylis*

15 Style not expanded at tip 10.58 *Austrodolichos*

11 Style terete:

16 Style with a flap behind the stigma :

17 Basal corner of keel with erect spur ± 1cm long ; standard appendages 0; style coiled 1-1.25 turns 10.51 *Physostigma*

17 Basal corner of keel not spurred; standard appendages present; style curved .5 turn. 10.52 *Vatovaea*

16 Style with at most a short projection beyond the stigma:

18 Tip of style expanded into a horizontal spoon-like cover, from which is suspended a spherical stigma ; upper wing spurs huge - S.Africa 10.55 *Otoptera*

18 Style not so expanded into a cover; upper wing spurs not particularly large - worldwide:

19 Standard appendages present:

20 Stigma lateral, oblique or rarely ± terminal 10.65 *Vigna*

20 Stigma terminal:

21 Standard appendage 1 large bilobed structuere ; style bent in (thickest at the bend), bent out and then in again, tapering, hardened with 2-line beard on supper

half 10.62 *Dipogon*

21 Standard appendages 2-4 separate:

22 Style bearded; alternate filaments expanded below the anthers 10.54 *Spathionema*

22 Style glabrous or hairs only around the stigma; filaments uniform:

23 Pollen not spinulose; standard appendages short ; flowers mostly red to blue 10.63 *Dolichos*

23 Pollen grains tuberculate or spinulose; standard appendages long and narrow ; flowers usually yellow or orange 10.64 *Macrotyloma*

19 Standard appendages absent or (*Psophocarpus*. *Decorsea*) small:

24 Style with an excentric bulging callous at the base, whole style less than half as long as the ovary ; bracteoles absent 10.59 *Neorautanenia*

24 Style without such a callous:

25 Style not bearded, but with a ring of branched hairs around the stigma 10.53 *Decorsea*

25 Style bearded, without branched hairs:

26 Style curved ± 180°, distinctly hardened, tapered but slightly thickened just below lateral stigma, so apex rather like a snake's head, bearded on upper third, pods oblong to elliptic, with thick valves; seeds 2-3 with a hilum 1/2 - 2/3 circumference. 10.67 *Oxyrhynchus*

26 Style bearded only a little below the stigma ; pods winged or more seeded; seeds with a shorter hilum:

27 Stipules not produced; lower calyx-lobe prominently upturned 10.49 *Dysolobium*

27 Stipules produced; lower calyx-lobe not prominently upturned 10.50 *Psophoarpus*

PSOPHOCARPUS Neck. ex DC., Prodr. 2: 403 (1825); Benth., Gen. Pl. 1(2): 540 (1865); Taub. in Engl. & Prantl., Pflanzenf. 3(3): 384 (1894); Hutch, Gen. Fl.Pl. 1: 442 (1964) *nom. cons.*

Botor Adans., Fam. Pl. 2: 326 (1763) *nom. rejic.*
Diesingia Endl. in Flora 15: 117 (1832) & Atakta bot. 1, t. 1 & 2 (1833).
Psilocarpus Pritzel, Ic. Ind. 2: 233 (1865).
Vignopsis De Wild. in Ann. Mus. Congo IV, 1: 69 (1902).

Herbs or subshrubs, mostly climbing or prostrate, less often erect. Leaves 1-foliate or pinnately 3-foliate; stipules prolonged below the point of insertion; stipels present. Inflorescences axillary, falsely racemose or flowers solitary or fasciculate; rhachis swollen at the point of insertion of the pedicels.

Calyx 5-lobed; upper pair of lobes forming an entire or bifid lip. Corolla blue or purplish; standard broad, auriculate and appendaged, glabrous; keel bent at a right angle. Vexillary stamen free or somewhat connate in the middle, anthers 5 dorsifixed alternating with 5 basifixed. Ovary 3-21-ovuled, winged; style thickened above the ovary, bent, flattened towards the apex, glabrous, bearded longitudinally or with a ring of hairs below the stigma, that part of the style situated above this row of hairs slightly bent; stigma terminal or internal, penicillate. Pods oblong, ± distinctly 4-winged along the angles, dehiscent, ± septate between the seeds. Seeds ovoid or oblong-ellipsoid, with or without an aril.

2.2 The taxonomic history of Psophocarpus as a member of the Phaseoleae

Current concepts of Phaseoleae circumscription are far removed from those early attempts of associating *Phaseolus* with its relatives made by Linneus in the 'Species Plantarum' (1753). Linneus lists five genera which would be placed in the Phaseoleae by contempory taxonomists: *Erythrina Phaseolus; Dolichos; Clitoria* and *Glycine*.

The first taxonomist to suggest the erection of a sub-division of the Leguminosae centred around *Phaseolus* was Adanson, whose 'Familles Des Plantes' (1763) divides the legumes into 6 sections, section 4 of which he named 'Les Haricots' (the beans). Phaseoli Les Haricots contained 24 genera, detailed in Table 2.1, four of these genera would be included in the Phaseoleae subtribe Phaseolinae (sensu Lackey, 1981): *Lablab* (6); *Phaseolus* (7); *Dolichos* (9); and *Botor* (12), the latter being a synonym of *Psophocarpus* (numbers in brackets indicate the authors placement in their classification). The generic name *Psophocarpus* was not proposed until 1790 by Necker, although not having temporal priority *Psophocarpus* have been consistently used and has now been conserved over *Botor*.

The first comprehensive attempt at a classification of the Leguminosae was proposed by Bronn in 1822. He divided the legumes into 2 suborders, the second of which, Curvembryae is further dicotimised into the Decandra and Diadelphae. The second tribe, Diadelphae he splits into six subtribes, the third of which, being the Phaseoleae containing 24 genera. His Phaseoleae contains the modern Phaseolinae genera *Voandzeia* (106); *Phaseolus* (110); and *Dolichos* (113). Bronn neglects to include either *Botor* or *Psophocarpus* in his circumscription, however his Phaseoleae are listed in Table 2.1 to add understanding of the original conception of the Phaseoleae.

In the same year as Bronn published his classification of the leguminosae, Savi (1822) erected the *Psophocarpus* allied genus *Vigna*, splitting it from the true *Phaseolus* by the following characters (detailed in Savi, 1826):

1. Upper calyx tooth entire
2. Appendages on the standard that converge towards the apex

Table 2.1 Comparative Classifications of Psophocarpus and Their Relatives

Author citations follow the respective classifications author

Adanson (1763)	Bronn (1822)	DeCandolle (1825)		
Leguminsae Sect.4.	Curvembryae Diadelphae	Papilionaceae		
Phaseoli	Subtribe C Phaseoleae	Tribe 2 Loteae	Tribe 5 Phaseoleae	
Medica Tour.	95 *Lupinus* Lin.	Subtribe 3 Clitoria	162 *Arbus* Linn.	176 *Vigna* Savi.
Ternatea Tour.	96 *Cajanus* DeC.	72 *Psoralea* Linn.	163 *Sweetia* DC.	177 *Lablab* Adans.
Mucuna Marg.	97 *Phyllolobium* Fisch.	73 *Indigofera* Linn.	164 *Macranthus* Poir.	178 *Pachyrhizus* Rich.
Canavali Bran.	98 *Rudolphia* Willd.	74 *Clitoria* Linn.	165 *Rothia* Pers.	179 *Parochetus* Hamilt.
Plaso H.M.	99 *Erythrina* Lin.	Sect 1 *Ternatea* Kunth.	166 *Teramnus* P.Br.	180 *Dioclea* H.B.et Kunth.
Lablab Alp.	100 *Butea* Roxb.	2 *Euclitoria* DC.	167 *Amphicarpaea* DC.	181 *Psophocarpus* Neck.
Phaseolus Tour.	101 *Clitoria* Lin.	3 *Centrosema* DC.	168 *Kennedya* Vent.	182 *Canavalia* DC.
Lotus Tour.	102 *Galactia* P.Br.	4 *Glycinopsis* DC.	169 *Rhynchosia* Lour.	183 *Mucuna* Adans.
Dolichos Theoph.	103 *Rhynchosia* Lour.	75 *Neurocarpum* Desv.	170 *Fagelia* Neck.	184 *Cajanus* DC.
Cajan Indor.	104 *Glycine* Lin.	76 *Martiusia* Schult.	171 *Wisteria* Nutt.	185 *Lupinus* Tourn.
Mouricou H.M.	105 *Amphicarpa* Elliot	77 *Cologania* Kunth.	172 *Apios* Boerh.	186 *Cylista* Ait.hort.Kew
Botor Rumph.	106 *Voandzeia* Pet. Th.	78 *Galactia* P.Br.	173 *Phaseolus* Linn.	187 *Erythrina* Linn.
Scandalida Fta.l.	107 *Martia* Leandr.	79 *Odonia* Bertol.	Sect 1 *Euphaseolus*	188 *Rudolphia* Willd.
Piscidia Lin.	108 *Grona* Lour.	80 *Vilmorinia*	2	189 *Butea* Roxb.
Agati HM.	109 *Macranthus* Lour.	81 *Barbieria* DC.	3 *Strophostyles* Ell.	
Toulichiba Plum.	110 *Phaseolus* Lin.	82 *Grona* Lour.	174 *Soja* Moench.	
Emerus Tour.	111 *Cylista* Roxb.	83 *Collaea* DC.	175 *Dolichos* Linn.	
Indigo Amm.	112 *Teramnus* P.Br.	84 *Otoptera* DC.	Sect.1 *Eudolichos*	
Sesban P.Alp.	113 *Dolichos*	85 *Pueraria* DC.	2 *Catiang*	
Arbus P. Alp.	114 *Mucuna* Marcg Adans.	86 *Dumasia* DC.	3 *Unguicularia*	
Solori Bram.	115 *Kennedia*	87 *Glycine* DC.		
Colinil H.M.	116 *Apios* Pursh.	88 *Chaetocalyx* DC.		
Securidaca Tour.	117 *Arbus* Lin.			
Onobrychis Tour.	118 *Poiretia* Vent.			

Table 2.1 cont.

Bentham (1837)

Phaseoleae

Subtribve 1 Clitoreae	Subtribe 6 Euphaseoleae
Dumasia DC.	Phaseolus
Amphicarpaea Ell.	*Vigna* Savi.
Cologania Kunth.	*Dolichos* Linn.
Amphodus Lindl.	*Lablab* Adans.
Clitoria Linn.	*Sphenostylis* E. Mey.
Neurocaroum Kunth.* ?	*Pachyrhizus* Rich.
Vexillaria	*Psophoscarpus* Neck.
Centrosema	*Diesingia* Endl.
Periandra	*Dunbaria* W et Arm.
Platysema	*Taentocarpum* Desv.

Bentham (1865)

Phaseoleae

Subtribe 1 Glycineae	Subtribe 5 Euphaseoleae
189 *Centrosema* DC.	220 *Physostigma* Balf.
including *Crumitium* Desv.	221 *Phaseolus* Linn.
Steganotropis Lehn.	Sect 1 *Drepanospron*
Vexillaria Benth.	2 *Euphaseotus*
190 *Periandra* Mart.	3 *Leptospron*
191 *Clitoria* Linn.	4 *Strophostyles*
Sect 1 *Ternatea*	5 *Macrotyloma*
2 *Neurocarpum*	6 *Dysolobium*
3 *Clitorianthes*	222 *Minkelesia*
192 *Cologania* Kunth.	223 *Vigna* Savi
193 *Amphicarpaea* Ell.	including *Otoptera* DC.
194 *Dumasia* DC.	*Sphenostylis* E. Mey.
195 *Shuteria* W.et Arn.	*Strophostylis* E. Mey.
196 *Glycine* Linn.	224 *Voandzeia* Thouars
including *Soja* Savi.	225 *Pachyrhizus* Rich.
Leptolobium Benth.	226 *Psophocarpus* Neck.
Bujacia E. Mey.	including *Diesingia* Endl.
197 *Teramnus* Swatz.	227 *Dolichos* Linn.
198 *Hardenbergia* Benth.	including *Lablab* Savi.
199 *Kennedya* Vent Jard Malm.	*Chloryllis* E. Mey.
including *Amphodus* Lindl.	*Dipogon* Liebm.
200 *Platycyamnus* Benth.	

* Note: Question marks refer to Benthams doubts about the true position of the genus in his classification.

Table 2.1 cont.

Taubert (1894)

Phaseoleae

Subtribe 1 Glycininae

383 *Clitoria* L.
Sect 1 *Ter atea* HBK.
2 *Neurocarpum* Desv.
3 *Clitorianthes* Benth.
384 *Bradburya* Rafin.
(Centrosema DC, syn.)
385 *Periandra* Mart

386 *Amphicarpa* Ell.
387 *Dumasia* DC.
388 *Eminia* Taub.
389 *Shuteria* W.et.Am.
390 *Glycine* L.
Sect 1 *Soja* Benth.
2 *Johnia* W.et.Am.
3 *Leptocyamus* Benth.
391 *Teramnus* Sw.
392 *Baukea* Vatke.
393 *Kennedya* Vent.
Sect 1 *Eukennedya* Taub.
2 *Zichya* Hueg.
3 *Physolobium* Hueg.
394 *Platycyamus* Benth.

Subtribe 6 Phaseolinae

422 *Physostigma* Balf.
423 *Phaseolus* L.
Sect 1 *Euphaseolus* Benth.
2 *Drepanospron* Benth.
3 *Leptospron* Benth.
4 *Macroptilium* Benth.
5 *Dysolobium* Benth.

6 *Strophostyles* Ell.
424 *Minkelersia* Mart.et Gal.
425 *Voandzeia* Thouars
426 *Vigna* Savi
Sect 1 *Euvigna* Bak.
2 *Plectotropis* Schum
including *Otoptera* DC.
Strophostyles E. Mey.
Sphenostyles E. Mey.
427 *Pachyrrhizus* Rich.
428 *Dolichos* L.
Sect 1 *Lablab* Savi
2 *Eudolichos* Taub.
3 *Choloryllis* E. Mey.
including *Dipogon* Liebm.
Macrotyloma W.et.Am.
429 *Psophocarpus* Neck.
including *Diesingia* Endl.

Harms (1914)

Phaseoleae

Subtribe 1 Glycininae

383 *Clitoria* L.
384 *Centrosema* DC.
385 *Periandra* Mart.
386 *Amphicarpa* Ell.
387 *Durnasia* DC.
388 *Eminia* Taub.
389 *Shuteria* W.et.Am.
(syn. *Psophocarpus*)
390 *Glycine* L.
390a*Neorautanenia* Schinz
391 *Teramnus* Sw.
391a*Herpyza* Ch.Wright.
392 *Baukea* Vatke.
393 *Kennedya* Vent.
394 *Platycyamus* Benth.

Subtribe 6 Phaseolinae

422 *Dolichos* L.
422a*Kerstingiella* Harms
423 *Chloryllis* E. Mey.
424 *Lablab* Adans.
424a*Dolichopsis* Hassler
425 *Adenodolichos* Harms
426 *Vignopsis* de Wild.
427 *Vigna* Savi
428 *Otoptera* DC.
429 *Voandzeia* Thou.
430 *Spathionema* Taub.
431 *Psophocarpus* Neck.
432 *Sphenostylis* E. Mey.
433 *Pachyrhizus* Rich
434 *Dysolobium* Prain
435 *Phaseolus* L.
436 *Minkelesia* Mart.et Gal.
437 *Physostigma* Balf.

Table 2.1 cont.

Hutchinson (1964)

Papilionaceae

Tribe 37 Phaseoleae

333 *Physostigma* Balf.
334 *Phaseolus* Linn.
335 *Alepidocalyx* Piper
336 *Minkelersia* Mart/Gal.
337 *Macroptilium* Urb.
338 *Peekelia* Harms.
339 *Oxyrhynchus* T5 Brand.
340 *Condylostylis* Piper
341 *Vigna* Savi
342 *Haydonia* Wilczek
343 *Pachyrrhizus* Rich ex DC.
344 *Dolichopsis* Hassler
345 *Otoptera* DC.
346 *Sphenostylis* E. Mey.
347 *Alistylus* N.E. Br.
348 *Lablab* Savi
349 *Adenodolichos* Harms.
350 *Neorautanerlia* Schinz
351 *Dolichos* Lam.emend DC.
352 *Monoplegma* Piper
353 *Spathionema* Taub.
354 *Psophocarpus* Neck.
355 *Ramirezella* Rose
356 *Endomallus* Gagnep.
357 *Baukea* Vatke.
358 *Dysolobium* Prain
359 *Voandzeia* Thou.

Tribe 38 Glycineae

360 *Clitoria* Linn.
361 *Centrosema* Benth.
362 *Platycyamus* Benth.
363 *Dumasia* DC.
364 *Periandra* Mart ex Benth.
365 *Diphyllarium* Gagnep.
366 *Pseudoeriosema* Hauman
367 *Cologania* Kunth.
368 *Herpyza* Ch.Wright
369 *Pseudoglycine* F.J. Hermann
370 *Paraglycine* F.J. Hermann
371 *Shuteria* Wight & Am.
372 *Amphicarpa* Elliot
373 *Teyleria* Backer
374 *Glycine* Linn.
375 *Kennedya* Vent.
376 *Hardenbergia* Benth.
377 *Vandasia* Domin.
378 *Clitoriopsis* Wilczek
379 *Teramnus* (P.Br) Swartz

Lackey (1977)

Phaseoleae

Subtribe 4 Phaseolinae

30?*Dysolobium* (Benth.) Prain
31?*Psophocarpus* DC.
32 *Physostigma* Balf.
33 *Vatovaea* Choiv.
34 *Decorsea* Viguier
35 *Spathionema* Taub.
36?*Otoptera* DC.
37 *Oxyrhynchus* Brandegee
38 *Peekelia* Harms.
39 *Dolichopsis* Hassler
40 *Macroptilium* (Benth.)Urban
41 *Alepidocalyx* Piper
42 *Minkelersia* Mart.& Gal.
43 *Condylostylis* Piper
44 *Ramirezella* Rose
45 *Phaseolus* L.
46 *Strophostyles* Ell.
47 *Vigna* Savi
48 *Voandzeia* Thouars
49 *Kerstingiella* Harms
50 *Lablab* Adanson
51 *Alilstilus* N.E.Bronn
52 *Dipogon* Liebn.
53 *Dolichos* L.
54 *Macrotyloma* (W&fl) Verdc.
55 *Sphenostylis* E. Meyer
56 *Nesphrostylis* Verdc.
57 *Austrodolichos* Verdc.
58?*Centrosema* Bentham.
59?*Periandra* Bentham.
60?*Clitoria* L.
61?*Clitoriopsis* Wilczek
62?*Neurautanenia* Schinz

Table 2.1 cont.

Baudet (1978)
Phaseoleae

Subtribe Glycininae

Supergenera *Glycinastrae*

Hesperothamnus
Platycyamus
Spatholobus
Apios
Cochlianthus
Butea
Erythrina
Rhodopis
Strongylodon
Macropsychanthus
Dioclea
Cleobulia
Camptosema
Cymbosema
Cratylia
Canavalia
Galactia
Collaea
Centrosema
Pachyrhizus
Periandra
Herpyza
Amphicarpa
Cologania
Glycine
Neonotonia
Teyleria
Shuteria
Diphyllarium
Mastersia
Neorautanenia
Teramnus
Calopogonium
Mucuna
Macrotyloma
Dumasia
Cruddasia
Ophrestia
Pseudoeriosema

Supergenera Clitoriastrae

Clitoria
Clitoriopsis

Supergenera *Phaseolastrae*

Vigna
Voandzeia
Physostigma
Vatovaea
Dipogon
Lablab
Spathionema
Dysolobium
Peekelia
Oxyrhynchus
Condylostylis
Dolichopsis
Macroptilium
Ramirezella
Alepidocalyx
Minkelersia
Phaseolus
Strophostyles

Subtribe Phaselinae

Supergenera *Dolichastrae*

Dolichos
Decorsea
Psophocarpus
Otoptera
Alistilus
Sphenostylis
Nesphostylis
Austrodolichos

Table 2.1 *cont*.

Lackey (1981)
Phaseoleae

Subtribe 6 Clitorinae
10.45 *Centrosema* (DC) Benth.
10.46 *Periandra* Benth.
10.47 *Clitoria* L.
10.48 *Clitoriopsis* Wilczek

Subtribe 7 Phaseolinae
10.49 *Dysolobium* (Benth.) Prain
10.50 *Psophocarpus* DC.
10.51 *Physostigma* Balf.
10.52 *Vatovaea* Chiov.
10.53 *Decorsea* R. Viguier
10.54 *Spathionema* Taub.
10.55 *Otoptera* DC.
10.56 *Sphenostylis* E. Mey.
10.57 *Nesphostylis* Verdc.
10.58 *Austrodolichos* Verdc.
10.59 *Neorautanenia* Schinz
10.60 *Lablab* Adans.
10.61 *Alistilus* N.E.Br.
10.62 *Dipogon* Lieb.
10.63 *Dolichos* L
10.64 *Macrotyloma* (Wright & Am.) Verdc.
10.65 *Vigna* Savi
10.66 *Ramirezella* Rose
10.67 *Oxyrhynchus* Brandegee
10.68 *Dolichopsis* Hassler
10.69 *Strophostyles* Elliot
10.70 *Macroptilium* (Benth.) Urban
10.71 *Phaseolus* L.

3. Presence of a disc nectary at the base of the ovary
4. Presence of a curved cylindrical pod
5. Seeds with a caruncle and hilum ventrally positioned.

He mentions two species which he refers to *Vigna*, *V.glabra* (syn. V.luteola) and *V.villosa*. Savi's conception of *Vigna* seems rather artificial in comparison to the modern views of *Vigna* set out in Verdcourt (1970c) and Marechal et al (1978a).

A more comprehensive classification of the legumes was published in 1825(a) by DeCandolle in his 'Prodromus Systematis Naturalis Regni Vegetabilis'. The proposed classification is extensively annotated in his 'Memoires sur la Famille des legumineuses' published in the same year (1825b). DeCandolle divides the leguminosae into four suborders, the first being the Papilionaceae. He then splits the Papilionaceae into six tribes, the fifth being the Phaseoleae. He does however include some genera included by modern taxonomists in the Phaseoleae (sensu Lackey 1981) in his second Papilionaceae tribe the Loteae, subtribe Clitoriae (e.g. *Clitora* (74) and *Otoptera* (84)). In his tribe Phaseoleae he includes the modern Phaseolinae genera *Phaseolus* (173); *Dolichos* (175); *Vigna* (176), *Lablab* (177), *Pachyrhizus* (178), *Psophocarpus* (181). Both the Phaseoleae and Loteae Sect. *Clitoriae* are detailed in Table 2.1 so that the relative position of *Psophocarpus* and its allies can be clearly seen in DeCandolles classification. It can be seen that DeCandolle places *Psophocarpus* at a distance from the other modern Phaseolinae genera, the closest being *Pachyrhizus*. DeCandolle does note in the 'Memoires' (p.182) that several of the twinning genera included in his Lotees-Clitoriees might require transference to the Phaseoleae when their mode of germination was known. Thus presumable he would have transferred many of the allies of *Psophocarpus* to their more natural position in the Phaseoleae. The *Psophocarpus* ally *Otoptera* DC. was in fact first described by DeCandolle in the 'Memoires' part 6. He suggests that *Otoptera* has close affinities with *Clitoria* based on leaf characters, but the pointed produced stipule opposes this relationship. Based on stamen arrangement he allies *Otoptera* to *Psoralea*, where like *Otoptera* some species are monodelphous. However the presence of stipels, lack of glandular hairs on the leaves and calyx, presence of a linear ovary

with numerous ovules questions any close relationship with *Psoralea*. DeCandolle finally suggests that the staminal arrangement and presence of stipels may even link *Otoptera* to *Pueraria*, but he concludes:

> "l'*Otoptera* forme un genre distinct, il l'est beaucoup moins de dire à quelle division de la famille il faut le repporter".

A statement which many contempory taxonomists would agree with 150 years after DeCandolle.

DeCandolle discusses in the 'Memoires' next the tribal placement of *Otoptera*, suggesting that the climbing stem, presence of stipules and linear polyovule ovary might suggest placement in the Phaseoleae, but he rejects this on the grounds of the monodelphous stamen. He finally allies *Otoptera* to *Clitoria* because of the taxonomic importance of the presence of stipels, it's resemblence to *Clitoria* in mode of germination, legume and seed morphology. When describing *Otoptera* DeCandolle erected one species *O.burchellii*, a second species was added by Vigier (1952), *O.madagascariensis*, as the name suggests, it is endemic to Madagascar.

In 1835 another genus closely allied to *Psophocarpus* was published, *Sphenostylis*, described by E. Meyer as a monotypic genus with, one species *S. marginata*. Sphenostylis is a very characteristic genus with it's distinctive flattened style. Since publication *Sphenostylis* has had numerous binomials added to it, but contempory concepts of *Sphenostylis* limit the genus to 7 or 8 true species from Africa and one species located in the Bombay area of India (Verdcourt, 1970a and 1971).

Bentham was the next taxonomist to extensively revise legume intra-family relationships in his "De Leguminsarum Generibus Commentationes" of 1837. Here Bentham takes the Phaseoleae and Lotees-Clitoriees (sensu DeCandolle 1825a) and forms what with minor variances is regarded as the modern Papilionoideae tribe, Phaseoleae. He subdivides the tribe into eight subtribes:

1. Clitorieae
2. Kennedyeae
3. Glycineae
4. Diocleae
5. Erythrineae
6. Euphaseoleae - containing *Psophocarpus*
7. Cajaneae
8. Rhynchosieae

Details of Benthams circumscription of the sub-tribes containing *Psophocarpus* allies are provided in Table 2.1. From this it can be seen that he allies *Psophocarpus* to *Pachyrhizus* as did DeCandolle, *Psophocarpus* is placed on the edge of the genera currently included in the Phaseolinae (sensu Lackey, 1981). Bentham obviously noted the similarities between *Psophocarpus* and *Diesingia* End. as he juxtaposes the genera and then unites them in the Genera Plantarum (1865).

Bentham when compiling the legumes for Bentham and Hooker's 'Genera Plantarum' (1865), retains his overall concept of the Phaseoleae, but re distributes the genera into six sub-tribes:

1. Glycineae
2. Erythrineae
3. Galactieae
4. Dioclieae
5. Euphaseoleae - containing *Psophocarpus*
6. Cajaneae

The Euphaseoleae remains virtually in-tact, with only *Dunbaria* being removed to the Cajaneae, the full details of the Euphaseoleae are given in Table 2.1. It can be seen that Bentham has extended his views by dividing *Phaseolus* into 6 sections and including several genera as synonyms under both *Vigna* and *Dolichos*. The noteworthy change to his conception of the Euphaseoleae is the movement of *Dolichos* away from *Vigna* towards *Psophocarpus*. In fact he introduces the idea of *Psophocarpus* having an intermediate position closely linked to *Vigna* and *Dolichos* which was to prove important in later classifications.

Of the second sub-tribe containing *Psophocarpus* allies, the Cliltorieae (sensu. Bentham 1837), Bentham (1865), merges it with the Glycineae, a change concured with by many later taxonomists. It is interesting to note how few changes have been made to Bentham's sub-tribes since his work. Within the Euphaseoleae these alterations can largely be refined to raising sub-genera to generic rank, which might be taken to infer Bentham's skill and foresight.

Taubert's (1894) circumscription of the Phaseoleae for Engler and Prantl's 'Die Naturlichen Pflanzenfamilien' follows Bentham's 1865 treatment (see Table 2.1). He retains Benthams concepts of the Glycininae and Phaseolinae. Within the Phaseolinae Taubert does however reverse the positions of *Psophocarpus* and *Dolichos*, again re-emphasising the peripheral nature of *Psophocarpus* to the Phaseolinae.

Within the context of *Psophocarpus* one genus commonly linked with it, is *Dysolobium* Prain. *Dysolobium* was raised to generic rank by Prain in 1897 from Bentham's (1851) *Phaseolus* sect. *Dysolobium*. Bentham (1865) does question his inclusio n of *Dysolobium* as a section of *Phaseolus* and suggests it might be more 'natural' if transferred to *Vigna*. Probably as a result of this, J.G. Baker (1876) does transfer the three section *Dysolobium* species to *Vigna*.

Prain's original delimeation of *Dysolobium* included the three species from Bentham's (1851) *Phaseolus* sect. *ysolobium*: *Phaseolus grande*, *P.lucens* and *P.dolichoicles*, plus the new species *Dysolobium tetragonum* Prain. Prain comments:

> "that the group as originally recognised by Bentham forms, in consequence of its form, septate pods and its hirsute seeds one of the most natural and definite genera in the whole of the Phaseolidae".

Though not all authors have followed this view, Gagnepain (1915) comments that, "*Dysolobium* is indistinguishable from *Vigna*". He then rather perversely in 1916 for the 'Flore Generale De L'Indo-Chine'

transfers *Dysolobium* (including subgen. *Dysolobium* and *Dolichovigna*, sensu Marechal et al, 1978b) to *Dolichos*, as well as describing two new species, *Dolichos apioides* and *D.schomburgkii*. These two species are reduced to synonyms of *Vigna pilosa* and *Dysolobium dolichoides* respectively by Van Welcen and den Hengst (in press).

In 1920 Hayata erected the genus *Dolichovigna* which has subsequently been linked to *Dysolobium*. Hayata suggests possible relationships with *Dolichos*, *Phaseolus*, *Vigna*, *Pueraria*, *Canavalia* and *Galactia*. He suggests it is possibly closest to *Dolichos* because of its terminal stigma, though the generic name he gave the genus suggests intermediacy between *Dolichos* and *Vigna*. The genus *Dolichovigna* contained two species *D.formosana* and *D.rhombifolia*, the former Hosokawa (1932) transfers to *Dolichos* as *Dolichos pilosus*. Then in 1970, Verdcourt (1970c) investigated the natural position of the problematic *Dolichos pilosus* and concluded that it should be removed to *Vigna* as the separate subgenus *Dolichovigna*. He comments specifically on *Vigna pilosa*:

> "The style is clearly that of a *Vigna* but the characteristic lateral tuft of hairs is distinctive enough to merit a subgenus, in fact, bearing in mind the extraordinary shape of the wings, many botanists may feel constrained to follow Hayata. Dr Bell kindly examined the seeds biochemically and reported that the amino-acid pattern is that of typical *Vigna*; the sculpture of the pollen-grains is also in accordance with this placing."

Verdcourt thus moves *Dilochos pilosus* to *Vigna* sect. *Dolichovigna* and places with it *Vigna clarkei*.

However, Marechal et al (1978b) transfer Hayata's (1920) *Dolichovigna* into *Dysolobium* as *Dysolobium* subgen. *Dolichovigna*, comprising *D.pilosum* and *D.apioides*. They justify this opinion as *Vigna pilosa* (syn. D.pilosum) had a 76.9% coefficient of similarity with *Dysolobium*, a figure they take to be high enough to warrant *V.pilosa* inclusion in *Dysolobium*, a view supported by Thuan (1979). In their argument Marechal et al believe that Verdcourt (1970c) over stresses the affinity between *Vigna* and *Dolichovigna*. They point out

that *Dolichovigna* and *Dysolobium* share two distinct features, the presence of a wing tooth and the septate pod, as well as shared inflorescence and seed characters. Though Marechal et al do feel *Dolichovigna* is distinct enough to warrant a separate subgenus to *Dysolobium* (sensu stricta).

In a recent revision of *Dysolobium*, Van Welzen and den Hengst (in press) on the grounds of extensive morphological investigations, reject Marechal et al's decision and propose adopting Prain's original circumscription and transfer *Dysolobium* sect *Dolichovigna* back to *Vigna*, a view supported by Verdcourt (pers. comm).

To return to the classifications of the Phaseoleae, Taubert died soon after completion of his 1894 classification. So that the revisions and supplements were completed by Harms (1897, 1900, 1906 and 1914). Harms summarises his final position in 1914, like Taubert (1894) he retains *Clitoria* and its allies in the Glycininae along with the inclusion of the new genus, *Neorautanenia* Schinz. Harms (1914) concepts of the Phaseolinae is much altered to Taubert's, see Table 2.1. Harms reverses the orientation of the Phaseolinae, moves *Psophocarpus* away from *Dolichos* and nearer *Phaseolus*, placing *Psophocarpus* in the 'spectrum' of genera that lie between *Phaseolus* and *Vigna*. Harms believed the closest genera to *Psophocarpus* were *Sphenostylis* and *Spathionema*, the latter due to its distinctive style-stigma arrangement, dilated stamen at apex, conspicuous standard appendages and oblong not septate pod, seems erronious. Harms (1914) includes *Vignopsis* (syn. *Psophocarpus*), distantly to *Psophocarpus* and juxtaposed it between *Adenodolichos* and *Vigna*, perhaps a more suitable positioning for *Psophocarpus* away from *Phaseolus* and closer to *Dolichos*.

Harms (1914) as previously stated includes *Neorautanenia* in his classification, although Harms includes it in the Glycininae it is one of the genera found closely allied to *Psophocarpus*. It was described by Schinz in 1899 with the one species *N.amboensis*. Schinz himself suggests its placement in the Glycininae possibly near *Glycine* due to *Neorautanenia* possession free vexillary stamen. Since 1899 numerous new species have been described by C.A. Smith and Harms. Verdcourt

(1970a), however comments:

> "As a generic unit it (*Neorautanenia*) is compact and is easily recognised but the division into species is exceedingly difficult unless a wide view is taken . . . . . I am convinced that only three species are clearly recognisable."

The three species referred to by Verdcourt are *N.amboensis* Schinz, *N.ficifolius* (Benth.) C.A. Smith and *N.mitis* (A. Rich) Verdc.

In the early part of this century many legume taxonomists switched from preparing full scale classifications to producing legume classifications for particular regional floras, especially of Africa. Thus their treatment of legume genera allied to *Psophocarpus* is not comprehensive, however most the the genera allied to *Psophocarpus* are represented in Africa and so these partial classifications are presenting in Table 2.2. Within these six classifications it is interesting to note the degree of variation in juxtoposing *Psophocarpus* with its allies. J.G. Baker (1871) placing *Psophocarpus* near *Dolichos*, *Pachyrhizus* and *Vigna*, but more remote to *Phaseolus* and *Physostigma*. Harms (1915) is little changed to Harms (1914) placing *Psophocarpus* in the centre of the small genera which are positioned betwen *Vigna* and *Phaseolus*, only distantly relating *Psophocarpus* to *Dolichos*. Harms includes *Vignopsis* separately to *Psophocarpus*, next to *Vigna* and much nearer to *Dolichos*, but curiously relatively remote to *Psophocarpus* itself (*Psophocarpus* = syn. *Vignopsis*). E.G. Baker (1926) places *Psophocarpus* between *Vigna* and its 'satellite' genera, and *Dolichos*, but curiously at least in the context of other classifications, juxtoposes it next to *Clitoria*. Baker includes *Vignopsis* near *Dolichos*, but places it on the periphery of the Euphaseoleae. Wilczek (1954) includes *Psophocarpus* sandwiched between the Clitoriinae (sensu Lackey 1981) and the larger Phaseolinae genera *Dolichos*, *Phaseolus* and *Vigna*. More specifically *Psophocarpus* is juxtoposed between *Lablab* and *Neorautanenia*. Wilczek classification is important because he is the first author to unite *Psophocarpus* with *Vignopsis* retaining each in a separate sub-genus. Both Hepper (1958) and Verdcourt (1971a) take a similar view of *Psophocarpus* being only peripherally associated with

Table 2.2 Comparative Classifications of African Regional Floras

Baker J.G. (1871)	Harms (1915)	
Papilionaceae	Papilionatae	
Tribe 7 Phaseoleae	Tribe 10 Phaseoleae	
51 *Centrosema* Benth.	Subtribe 1 - Glycininae	Subtribe 5 - Phaseolinae
52 *Clitoria* Linn.	*Clitoria* L.	*Dolichos* L.
53 *Shuteria* Wright & Am.	*Dumasia* DC.	Sect 1 *Eudolichos* Harms.
54 *Glycine* Willd.	*Shuteria* W.& Am.	2 *Rhynchodolichos* Harms.
55 *Teramnus* P.Br.	*Glycine* Willd.	3 *Pogonodolichos* Harms.
56 *Erythrina* Linn.	*Teramnus* P.Br.	4 *Pseudopachyrrhizus* Harms.
57 *Mucuna* Adans.	*Errina* Taub.	5 *Pseudovigna* Harms.
58 *Spatholobys* Hassk.		*Chlorylis* E. Mey.
59 *Galactia* P.Br		*Kerstingiella* Harris
60 *Dioclea* Kunth.		*Lablab* Adans.
61 *Canavalia* Adans.		*Adenodolichos* Harms.
62 *Physostigma* Balfour		*Vignopsis* De Wild.
63 *Phaseolus* Linn.		*Vigna* Siva
64 *Vigna* Savi		*Otoptera* DC.
65 *Voandzeia* Thouars		*Voandzeia* Thou.
66 *Pachyrhizus* Rich		*Spathionema* Taub.
67 *Psophocarpus* Neck.		*Psophocarpus* Neck.
68 *Dolichos* Linn.		*Sphenostylis* E. Mey.
69 *Cajanus* DC.		*Pachyrrhizus* Rich
70 *Rhynchosia* Lour.		*Phaseolus* L.
71 *Eriosema* (DC) G.Don.		*Physostigma* Balf.
72 *Flemingia* Ait.f.		

Table 2.2 cont.

Baker E.G. (1926)
Papilionaceae
Tribe 7 - Phaseoleae
Subtribe 4 - Euphasedeae
75 *Physostigma* Balf.
76 *Phaseolus* Linn.
77 *Vigna* Savi
Sect.1 *Microdontae* Harms
2 *Appendiculatae* Harms
3 *Macrodontae* Harms
4 *Vexillatae* Harms
5 *Liebrechtsia* De Wild.
6 *Procerae* Baker
78 *Otoptera* DC.
79 *Spathionema* Taub.
80 *Sphenostylis* E. Mey.
81 *Alistilus* N.E. Bronn.
82 *Voandzeia* Thouars
83 *Kerstingiella* Harms
84 *Pachyrhizus* Rich
85 *Psophocarpus* Neck.
86 *Clitoria* Linn.
Sect 1 *Ternatea* DC.
2 *Neurocarpum* Benth.
87 *Dolichos* Linn.
Sect 1 *Eudolichos* Harms
2 *Pogonodolichos* Harms
3 *Pseudopachyrhizus* Harms
4 *Pseudovigna* Harms
5 *Lablab* Harv.
88 *Adenodolichos* Harms
89 *Vignopsis* De Wild.

Wilczek (1954)
Papilionaceae
Tribe 9 - Phaseoleae
Subtribe 5 - Phaselinae
67 *Haydonia* Wilczek
68 *Clitoria* L.
69 *Clitoriopsis* Wilczek
Centrosema Benth.(introduced)
70 *Sphenostylis* E. Mey.
71 *Lablab* Adans.
72 *Psophocarpus* Neck.
Subgen 1. *Psophocarpus* Wilczek
2. *Vignopsis* (De Wild.)Wilczek
73 *Neorautanenia* Schinz
74 *Dolichos* L.(divided into 3 groups)
75 *Phaseolus* L.
76 *Physostigma* Balf.
Voandzeia Thouars (introduced)
77 *Vigna* Savi (divided into 5 groups)
78 *Adenodolichos* Harms

Hepper (1958)
Papilionaceae
Tribe 7 - Phaseoleae
Genera 34 to 63
Below *Psophocarpus* allies abstracted
39 *Clitoria* Linn.
40 *Amphicarpa* Elliot
41 *Centrosema* DC.
42 *Mucuna* Adans.
43 *Erythrina* Linn.
44 *Calopogonium* Desv.
45 *Neorautanenia* Schinz
46 *Galactia* Adans.
47 *Glycine* Linn.
48 *Pseudoeriosema* Hauman
49 *Physostigma* Balfour
50 *Sphenostylis* E. Mey.
51 *Haydonia* Wilczek
52 *Phaseolus* Linn.
53 *Vigna* Savi
54 *Dolichos* Linn.
55 *Lablab* Adans.
56 *Adenodolichos* Harms
57 *Voandzeia* Thouars
58 *Kerstingiella* Harms
59 *Psophocarpus* Neck.

Table 2.2 cont.

Verdcourt (1971)
Papilionaceae
Tribe 10 - Phaseoleae
42 *Amphicarpa* Nuttal
43 *Durnasia* DC.
44 *Clitoria* L.
45 *Centrosema* (DC.) Benth.
46 *Pseudoertosema* Hauman
47 *Ophrestia* Forbes
48 *Glycine* Willd.
49 *Teramnus* P.Br.
50 *Erythrina* L.
51 *Mucuna* Adans.
52 *Canavalia* Adans.
53 *Calopogonum* Desv.
54 *Galactia* P.Br.
55 *Macrotyloma* (Wight & Am.) Verdc.
56 *Pueraria* DC.
57 *Pseudovigna* (Harms) Verdc.
58 *Emina* Taub.
59 *Pseudeminia* Verdc.
60 *Psophocarpus* DC.
61 *Physostigma* Balf.
62 *Valovaea* Choiv.
63 *Decorsea* Viguier
64 *Phaseolus* L.
65 *Vigna* Savi
Subgen 1. *Vigna* Verdc.
2. *Sigmoidotropis* (Piper) Verdc.
3. *Cochliasanthus* (Trew) Verdc.
4. *Plectrotropis* (Schum) Bak.
5. *Ceratotropis* (Piper) Verdc.
6. *Dolichovigna* (Hayata) Verdc.
7. *Macrorhynchus* Verdc.
8. *Haydonia* (Wilczek) Verdc.
66 *Spathionema* Taub.
67 *Voandzeia* Thouars
68 *Nesphostylis* Verdc.
69 *Sphenostylis* E. Mey.
70 *Dolichos* L.
71 *Lablab* Adans.
72 *Neorautanenia* Schinz
73 *Adenodolichos* Harms
74 *Atylosia* Wight & Am.
75 *Cajanus* DC.
76 *Rhynchosia* Lour.
77 *Eriosema* (DC) Desv.
78 *Flemingia* Ait.f.

the Phaseolinae (senus stricta) genera, though Hepper places it closest to *Dolichos* while Verdcourt believes it closer to *Phaseolus* and *Physostigma*. Certainly at least one point can be observed from the study of these regional Floras, and this is how confused the natural position of *Psophocarpus* is in their classification.

In 1964 J. Hutchinson published Volume I of his 'The Genera of Flowering Plants', in which he began to circumscribe the flowering plant genera. In Volume I the legume genera are detailed. Hutchinson made few alterations to Bentham (1865), Taubert (1894) and Harms (1914) conceptions of the Leguminosae; he includes new genera and raises many sub-tribes to tribal status. Hutchinson 's family Fabaceae (Papilionaceae) is divided into 50 tribes, he raises both Bentham's (1865) sub-tribes Euphaseoleae and Glycineae to his tribes 37 Phaseoleae and 38 Glycineae respectively. Details of these tribes are provided in Table 2.1. He takes the view that *Psophocarpus* is near the periphery of the Phaseoleae, most closely associated with *Dolichos*. Though specifically placing Psophocarpus between *Spathionema* and *Ramirezella* a curious placement as both these genera possess such distinctive stigma-style arrangement and petal sculpturing.

Verdcourt (1970a) separates from the *Psophocarpus* allied genus *Sphenostylis* E.Meyer, the new genus *Nesphostylis* Verdc. Verdcourt describes one species *N.holosericea* (Bak.) Verdc. in the new genus. Lackey (1977b) observes on this separation, "Verdcourt removed this species from Sphenostylis in which it has always been an uncomfortable fit". Lackey then goes on to list the characters which separate *Nesphostylis* from *Sphenostylis* "large bracteoles, ciliated calyx interior, keel joined along the upper as well as lower sutures, toothed vexillary stamen and the dilated stamen filaments". A second species of *Nesphostylis* was described from Burma by Tateishi and Ohashi (1977), *N.lanceolata*. The original species described by Verdcourt *N. holosericea* is endemic to tropical Africa and so this genus provides an interesting example of phytogeographical links between Africa and Asia. A similar situation is found in *Psophocarpus* where one of the nine species is located in Asia, *P.tetragonolobus* and the other eight species are endemic to tropical Africa. Verdcourt (1970a) states that *Nesphostylis* is closely allied to *Sphenostylis*, but Tateishi and Ohashi

(1977) emphasise that it is also closely related to *Dolichos* due to the following characters shared by the two genera:

(i) vexillum with two appendages.
(ii) vexillary filament with a tooth or an appendage at the base.
(iii) persistant, large bracteoles.

Lackey (1977b) states that Taubert (1894) and Hutchinson (1964) when classifying the Phaseoleae, "followed Bentham's system and casually allotted new genera to pre-existing subtribes". So encouraging Lackey to undertake, "a revised subtribal classification of the Phaseoleae on the basis of a study of herbarium material and a consideration of research published since Bentham's time". Lackey (1977a) divides the Phaseoleae into seven subtribes, the fourth of which being the Phaseolinae (detailed in Table 2.1). Lackeys major alteration to Bentham (1865), Taubert (1894), Harms (1914) and Hutchinson (1964) classifications of the Phaseolinae is to extract the *Clitoria* allied genera (*Clitoria*, *Centrosema*, *Periandra* and *Clitoriopsis*) from the Glcininae and include them in the Phaseolinae. His justifications for this transference are as follows:

1. *Centrosema* has floral and legume similarities to *Sphenostylis* and *Nesphostylis*.

2. Possession of bearded or flattened styles; typical of Phaseoliniae genera.

3. *Centrosema* and *Periandra* have keel petals fused along their upper margin, also typical of Phaseolinae genera.

Lackey refers to some anomalous genera which have been traditionally included in the Phaseolinae, but remain discordant when placed alongside other Phaseolinae genera. However he retains their membership of the Phaseolinae due to their even less allied nature with other subtribes. He refers specifically to *Otoptera* and *Neorautanenia's* lack of beard and terete style; *Dysolobium's* habit being similar to some *Pueraria*, *Dioclea*, or in young stages to *Physostigma*, possessing the seed of some Glycininae but the bearded

style typical of the Phaseolinae. He states that *Psophocarpus* and *Dysolobium* are 'surely allied' due to their:

1. Curiously curved stigma
2. Winged pods
3. Long straight style hairs
4. Flucose seed (inconstant in *Psophocarpus*)

Lackey retains these anomolous genera in the Phaseolinae, simply for lack of a more obvious placement, but puzzles over their relationship with other Phaseolinae genera. These genera are thus peripherals positioned in his classification of the Phaseolinae, (see Table 2.1).

Lackey (1977a) also abstracted *Clitoria* and its allies from their established placement in the Glycininae and merges them with the Phaseolinae as a peripheral grouping. He comments that the four genera, *Clitoria*, *Centrosema*, *Periandra* and *Clitoriopsis*, "have no certain affinity with any Phaseoleae subtribes, although formerly placed in the Glycininae are surely nothing like *Glycine*". Then notes similarities with Phaseolinae genera as justification; *Centrosema* has the flattend style of *Austrodolichos*, *Sphenostylis* and *Nesphostylis*; carina fused above as in many Phaseolinae; naked calyx inside of *Sphenostylis*, which it shares with *Periandra*; *Clitoria* shares the bearded style with most Phaseolinae, while *Clitoriopsis* is closely related to *Clitoria* and shares a naked calyx interior with *Sphenostylis*.

Lackey's classification of the Phaseoleae (1977a) is extracted from his doctoral work published also in 1977(b). In his thesis Lackey extends his hypothesis that *Dysolobium* and *Psophocarpus* are closely allied. He lists the following points to corroborate his hypothesis:

1. The fruits are heavy, septate within and often four winged,
2. The beard consists of straight, multiseriate hairs,
3. The stigma is concave and oblique,
4. The carina petals are joined weakly and intermittently,
5. The lower calyx lobe is prominent,
6. The fascide nodes are cushion-like and black,

7. The seeds are velvety in *Dysolobium* and sometimes hairy in *Psophocarpus*.

He comments further that initially he considered that *Dysolobium* and *Psophocarpus* might be peripheral members of the Glycininae "because of the hairy seeds, *Eminia-Puerana* floral and bract arrangement, dark swollen fascicle nodes, and *Pueraria*-like produced stipules in *Psophocarpus*". Opposing this he suggests, "the heavy, woody nature of the plants, and sometimes thick flowers suggest the Diocleinae". However in both cases these characters are not complemented by others important for the subtribe and thus he retains *Dysolobium* and *Psophocarpus* in the Phaselinae.

Lackey believes that Prain (1897) hints at links between the two genera *Psophocarpus* and *Dysolobium*. Prains remarks referring to the similarity between the legume of *Dysolobium tetragonum* which is distinctly winged and the typical *Psophocarpus* legume. Baker (1876) in the "Flora of British India", actually tentatively refers *D.tetragonum* to *Psophocarpus* because he only knew the plant in fruit. Prain (1897) subsequently observed numerous flowering specimens and returns the species to *Dysolobium*. However if as Lackey believes, Prain considered *Dysolobium* and *Psophocarpus* to be so closely allied, why does Prain in 'Bengal Plants' (1903) separate them by placing *Phaseolus*, *Vigna*, *Pachyrhizus* and *Dolichos* between them. This appears to clearly refute Lackey's agrument, unless Prain changed his view in the intervening six years.

Marechal et al (1978a), while investigating the *Phaseolus-Vigna* complex, commented in detail on Lackey's (1977b) hypothesis of a close *Psophocarpus* - *Dysolobium* relationship. Marechal et al doubted the validity of Lackey's hypothesis and raised the following questions with reference to Lackey's seven corroborative points:

"The fruit are heavy, septate within and often four winged"

"Chez *Psophocarpus*, *les grains sont séparées par une matière papyracée spongieuse. Facilement détachable comme chez la plupart des Vigna* alors que chez *Dysolobium*, les cloisons sont franchement

ligneuses, et les gousses, si elles ont une section souvent plus ou moins quadrangulaire, ne sont nullement ailées.

"The beard consists of straight multiseriate hairs"

Il n'existe pas chez *Psophocarpus*, une véritable barbe unilatérale sous le stigmate, comme chez les *Dysolobium*, mais une couronne de poils sous le stigmate. C'est d'ailleurs la raison pour laquelle. *Psophocarpus* ne figure pas parmi les Phaseolastrae.

"The stigma is concave and oblique"

La position latérale oblique du stigmate se retrouve chez certains *Vigna*, par suite d'une legere torsion due style.

"The carina petals are joined weakly and intermittently"

Contrairement à *Psophocarpus*, la carène de *Dysolobium* est plus ou moins longuement rostrée, et au niveau du rostre, la soudure est relativement solide.

"The lower calyx lobe is prominent"

Ce caractére se retrouve chez de nombreux genres, notamment chez *Vigna (V.speciosa)*.

"The fascicle nodes are cushion-like and black"

Les noeuds enflés du rachis, glandeux et devenant noirâtes sont observés chez beaucoup de *Vigna* et espèces appartenent à des genres satellites.

"The seeds are velvety in *Dysolobium* and sometimes hairy in *Psophocarpus*"

L'aspect veloute des graines de *Dysolobium* est dû à l'adhérence de l'endocarpe sur le téguments. Un simple brossage de celui-ci rend la graine complètement lisse. Le même phénomene se retrouve chez *Vigna*

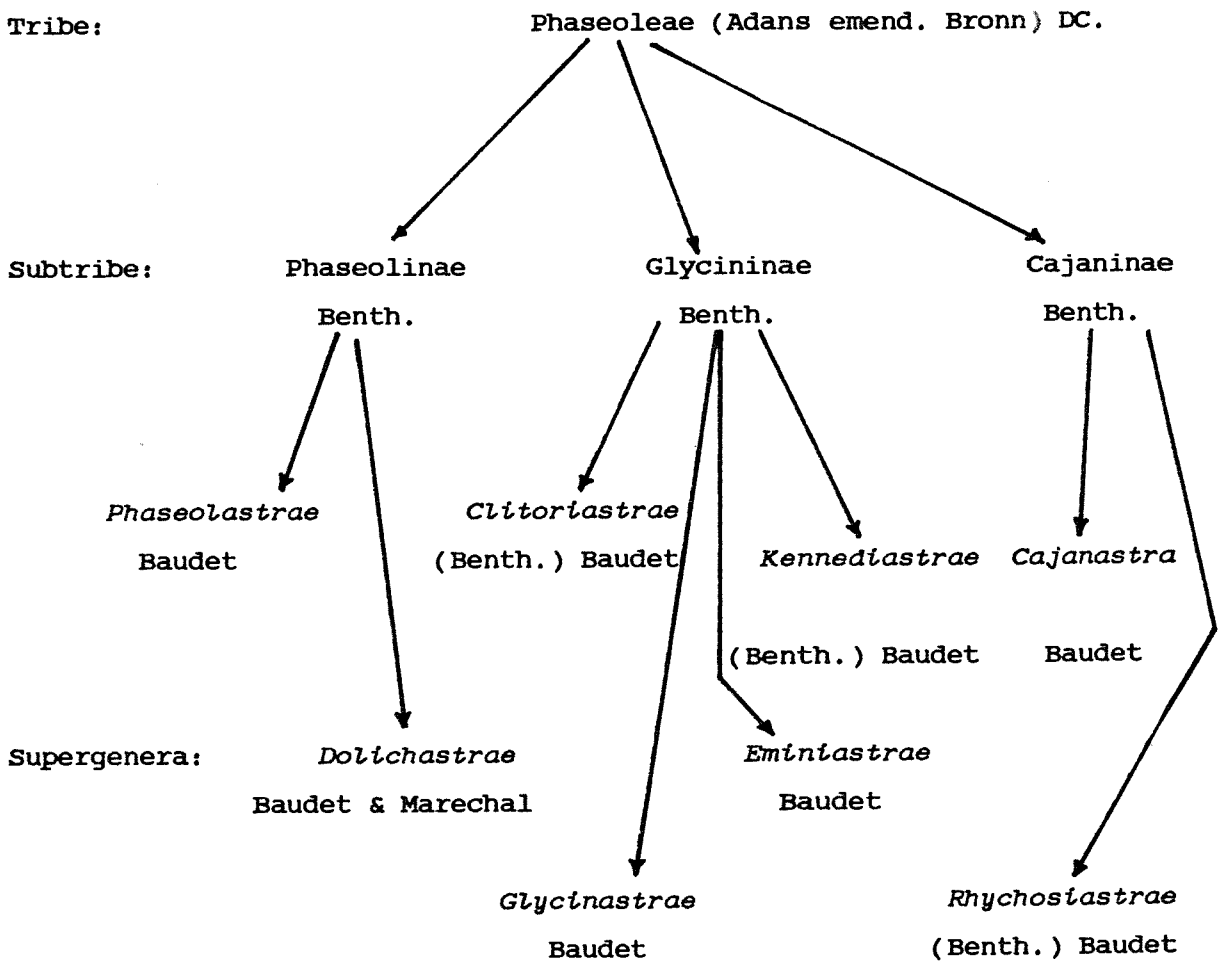
radiata, *V. mungo* et *V. trilobata*, etc., et chez *Strophostyles*. Nous n'avons jamais observe de graines velues chez *Psophocarpus* mais bien l'aspect velouté chez *P.scandens* (Endl.) Verd.

Nous admettons l'existence d'une certaine similitude entre les deux genres, en ce qui concerne les caractères séminaux, forme sphérique, aspect de l'arille s'il est présent. En revanche, certains autres caractères de *Psophocarpus* interviennent pour accentuer les dissemblances: bractées florales plus longuement persistantes, style enflé à la base et persistant sur la gousse, etc. Il semble donc finalement que les similitudes de *Dysolobium* sont tout aussi probantes avec *Vigna* qu'avec *Psophocarpus*.

Literally Marechal et al's criticism are correct, but they have taken Lackey's points too narrowly. Lackey was surely intending with these points to indicate some shared characters which might indicate a close relationship rather say these characters were unique to these genera. Lackey put forward the points as general phylogenetic indicators of relatedness. For example, Lackey's fourth point, that both genera show carina petals which are joined weakly and intermittently. Marechal et al's comment on this that contrary to *Psophocarpus*, *Dysolobium* more or less spirals lengthwise and at the beginning of the spiral the union is relatively solid. Marechal et al's observation is correct, but is does not abrogate Lackey's original point that *Psophocarpus* and *Dysolobium* both share a weakly joined or intermittent upper keel fusion. These points of argument will be discussed in the light of the present studies results later in this thesis.

At the same time as Lackey in America was engaged in revising the generic classification of the Phaseoleae, so another worker Baudet in belgium was also attempting to resolve the same problem, though from a phylogenetic stance as opposed to Lackey's phenetic study. Baudet (1977a) proposes a radical, re-classification of the Phaseoleae, first suggested by Baudet and Marechal (1976), using sub-tribes and super-genera as shown in Figure 2.1. Baudet emphasises the phylectic nature of his classification, that groups have been formed on what Baudet regards as major evolutionary trends, such as pollen aperture type, presence of hairs with hooked apices and less specific eco-geographical characters. He attempts to distance the classification from traditional taxonomic concepts of relationships based on morphology and moving towards the non-morphological, e.g. chorological, palynological, dermological, embryological and phytochemical. This produces what Baudet refers to as a choro-ecological hypothesis, which is summarised in his classification. Baudet splits the *Psophocarpus* allied genera into two sub-tribes the Phaseolinae and Glycininae as shown in Table 2.1. The sub-tribes are then split into supergenera, four for the Glycininae and two for the Phaselinae. The two Phaselinae supergenera are the *Phaseolastrae* centred around the *Phaseolus-Vigna* complex and the *Dolichastrae* centred on *Dolichos* and its close allies. The division between the Phaselinae supergenera is not clearly set out

Figure 2.1 Baudet's (1977) Classification of the Phaseoleae



in a key, but is based upon the presence of a beard below the stigma in the *Phaseolastrae* and this beards absence in the *Dolichastrae*. Phenetisists would certainly argue Baudet's classifications artificiality in the way *Psophocarpus* is separated from *Dysolobium* and *Vigna*, traditionally considered close allies, thus warrants detailed discussion. It will be discussed later in this thesis in the light of the present studies findings. Baudet published his classification in 1978, which summarises much of the work contained in his thesis. His published classification is almost identical to that in his thesis apart from some minor alteration of taxonomic position in the Glycininae-*Glycinastrae*.

For the International Legume Conference at Kew in 1978 Lackey prepared the treatment of the Phaseoleae, published in 1981. Lackey (1981) revises his classification of 1977, though the alterations are generally minor intra sub-tribal genera movements. He does however reinstate Bentham's (1837) sub-tribe Clitoriinae including: *Clitoria*, *Centrosema*, *Periandra* and *Clitoriopsis*. Lackey comments on his change of attitute that they, (the Clitoriinae genera),

> "form a well circumscribed subtribe as shown by the generally resupinate flowers, naked calyx interior and constant presence of hooked hairs"

Lackey's revised classification of the sub-tribes allied to *Psophocarpus* is provided in Table 2.1. *Psophocarpus's* position is still peripheral with *Dysolobium*, remote to *Phaseolus*, *Vigna* and *Dolichos*. Though within the Phaseolinae the *Psophocarpus* allies *Sphenostylis* and *Neph ostylis* are moved away from *Psophocarpus*, while *Neorautanenia* is positioned closer.

2.3 Taxonomic history of Psophocarpus Species

Of the nine legitimate species of *Psophocarpus* Neck. ex DC., (Verdcourt and Halliday 1978) only one was known to Linneus. In his 'Systema Natura' (1759) Linneus described *Dolichos tetragonolobus* L. (Syn. *Psophocarpus tetragonolobus* (L.) DC.) as a "Twining legume with 4 membranaceous angles, India" and then provides a reference to an earlier work by Rumphius. This being the 'Herbarii Amboinensis' of 1747 in which Rumphius describes the plants of Indonesia, as well as nomenclatural information, synonymy, location and uses of the plants. Rumphius refers to *Lobus quadrangularis* Rumphius (syn. *P.tetragonolobus*), suggests it is allied to *Phaseoli* species, noted its geographical distribution as Java and Bali and that it is locally known as *Botor*. *Botor* being derived from the Arabic word Batr meaning significantly lobed. Rumphius provides an even earlier reference to *P.tetragonolobus* as *Lobum quadrangularem* of Clusius in 1605. Verdcourt and Halliday (1978) comment that these were the earliest written descriptions, but *P.tetragonolobus* had been known for many centuries as a widely grown vegetable of the tropics, especially in South East Asia where it is known as the winged or Goa bean. Adanson's (1763) section 4, Les Haricots, Phaseoli of his 'Familles des Plantes' contains the generic name *Botor* followed by a brief description of *P.tetragonolobus*, thus initially separating *Botor* (syn. *Psophocarpus*) from *Dolichos*. The generic name, *Psophocarpus*, meaning from the greek, "psophos = "noise", and Karpos = "fruit" (the ripe fruit emits a cracking sound on dehiscence) was proposed by Necker in 1790. However it was DeCandolle who first legitimately published the generic name in 1825 (Prodr. 2:403). DeCandolle describes one species *P.tetragonolobus* (L.) DC., cultivated in North West Africa as 'Pois carre' and suggests a second, "alter minor forsan diversus reperitur in Madagascar secus rivos ex Pet. Th. l.c." This second species, Verdcourt and Halliday (1978) state is, "clearly *P.scandens* (Endl.) Verdc.", the only *Psophocarpus* spp. as yet found in Madagascar. DeCandolle in his 'Memoire' (1825) states his preference for the conservation of *Psophocarpus* Neck. over *Botor* Adans, even though *Botor* has temporal priority because he, "m'a paru i barbare". He also expands on the second smaller species, commenting that it is separated by M. Du Petit-Thouars and can be found along the stream banks of Madagascar.

However the second *Psophocarpus* species to be validly published was not *P.scandens* but *P.palustris*, published in 1826 by Desvaux. He does not detail the collector or precise location of the species other than "Hab. in locis humidis Senegaliae". Soon after in 1832 a third species, *P.palmettorum* was published by Guillemin, Perrottet and Richard. This has been a much used binomial, but was reduced to a synonym of *P.palustris* in 1978 by Verdcourt and Halliday.

The *Psophocarpus* synonomous genus *Diesingia* was erected in 1832 by Endlicher in his Flora 15 : 113 with the single species *D.scandens* (Flora 15 : 117). The following year, 1833, in Atakta Botanika he provides fully comprehensive descriptions of the genus and species with observations on relationships with allied genera. Endlicher links *Diesingia* distantly with *Dolichos*, but believes it to be mostly closely allied to *Psophocarpus* and *Lablab*. He details *D.scandens* natural habitat as being Brazil, but *Psophocarpus scandens* (syn. *D.scandens*) is geographically much more widespread, see Table 7.2. *Diesingia* Endl. did not survive long as a genus, it was sunk into *Psophocarpus* by Bentham in 1859. However, Verdcourt (1968) when splitting the *P.palustris* complex, uses the specific epithet of Endlicher's *D.scandens* in his *Psophocarpus scandens* (Endl.) Verdc. comb. nov. thus separating the East African populations from the true West African *P.palustris*.

Hasskarl in his 'Hortus Bogoriens' (1844) includes both *P.longepedunculatus* and *P.tetragonolobus* with brief descriptions of both, this being the first published description of the former. Hasskarl also attempts a subspecific splitting of *P.tetragonolobus* into two subspecies or varieties (unspecified which), A.macropterus Hasskl. and B.micropterus Hasskl. Separating the subspecies or varieties out as follows:

A. *macropterus* - legume more or less compressed (with wing 1-2 x pod width), wings more unrolled (0.5 x pod width), wing edge complete or with inclined serrations.

B. micropterus - legume more or less compressed (with wing 0.7 - 1.0 x pod width), wings more rolled (0.25 x pod width), wing edge complete.

This separation into subspecies or varieties is rather artificial, being entirely based on legume characters and has not been followed by other authors.

Bentham (1855) makes some astute observations in a footnote to his *Psophocarpus* Neck, in 'Plantae Junghuhnianae'. He distinguishes two *Psophocarpus* species *P.longipedunculatus* (syn. *P.scandens*) and *P.palmetorum* (syn. *P.palustris*), but goes on to comment that *P.longipedunculatus* is very similar to *Diesingia scandens* and that *P.palmetorum* is close to *P.palustris*. Thus preempting Verdcourt's (1968) division of *P.scandens* from *P.palustris* and Verdcourt's attitude to their respective synonymy. In 1859 Bentham actually includes *Diesingia scandens* as a synonym of *P.longepedunculatus* and *Diesingia* as a synonym of *Psophocarpus*. In the Genera Plantarum (1865) Bentham refers to four *Psophocarpus* species of Asia and Africa, presumably being: *P.tetragonolobus*, *P.longepedunculatus*, *P.palmettorum* and *P.paulustris*.

Kuntze (1891) argues that the temporal priority of *Botor* Adan. (1763) over *Psophocarpus* Neck. (1790) means that the generic name *Botor* should be conserved. Thus he publishes the binomials *B.tetragonoloba* (L.) O.K. and *B.palustris* (Desv.) O.K.; for the latter he provides a synonymy of "*Psophocarpus palustris* Desv 1826 = Ps. palmettorum Guill. & Perr. = *Ps. longepedunculata* Hassk." Apparently taking the attitude that what are referred to as *P.scandens* and *P.palustris* (sensu Verdcourt 1968) are the same species. Although Kuntze was nomenclaturally correct in wanting conservation of *Botor* over *Psophocarpus*, this has not occurred probably due to the small number of binomials published using *Botor* in comparison to *Psophocarpus*.

Taubert (1891) in his outline of *Psophocarpus* includes *Diesingia* Endl. as a synonym and refers to 5 species of tropical Asia and Africa, but does not provide details of which species he regards as valid.

De Wild. in 1902 published another genus, *Vignopsis* and one species, *V.lukafuensis*, which Verdcourt and Halliday (1978) regard as synonyms of *Psophocarpus* and *P.lukafuensis* respectively. DeWilde with the

description, geographically locates it in the Belgium Congo (Zaire) and suggests *Vigna* as a closest allied genera, but notes the difference between *Vignopsis* and *Vigna* as follows:

> "Il se differencie de ce dernier par un stigmate peu ou pas oblique et par un style glabre, muni vers le sommet, en dessous de l'insertion due stigmate, d'une collerette de poils"

These characteristics of *Vignopsis*, the shape and position of the stigma and the collar of hairs on the style behind the stigma means that *Vignopsis* fits awkardly within *Psophocarpus*: this will be discussed more extensively in latter chapters. In his observation of *V.lukafuensis*, DeWilde links it with *Vigna coerulea*, *Vigna triloba*, *Vigna huilensis*, *Vigna nilotica* and Vigna *sinensis*, because of the shared glabrous stem and distinctly spurred stipules, but again refers to the marked differences in style-stigma shape and position of style hairs. Today these five *Vigna* species have been reduced to two as *V.coerulea*, *V.triloba*, *V.huilensis* and *V.sinensis* are synonyms of *V.ungiculata*, and *V.nilotica* is a synonym of *V.luteola*. In the later analysis chapters it will be seen that DeWild was fairly accurate in his observations as *V.luteola* and *V.ungiculata* are among the closest *Vigna* species allied to *Psophocarpus lukafuensis* (syn. *Vignopsis lukafuensis*).

Harms in 1907 describes the forth, currently valid species of *Psophocarpus* from Western Sudan, *P.monophyllus*. This was a particularly shrewd taxonomic placement as *P.monophyllus* was so superficially differenet to the other *Psophocarpus* species known at that time, *P.tetragonolobus*, *P.palustris* and *P.longepedunculatus*; *P.monophyllus* being unifoliate, having quite a different leaf and stigma-style shape. Harms obviously realised this and hence the pun in the specific epithet. In a footnote to this publication Harms suggests that *P.palustris* = *P.longepedunculatus*, thus uniting the admittedly closely allied East and West African populations as the one species *P.palustris*. This unification Verdcourt (1968) was to suggest was erronious, as is corroborated by the present studies findings. Harms (1911) was to publish another *Psophocarpus* species, *P.lancifolius*, from material gathered by Mildbraed's 1907-8 German Central African Expedition. He

details the habitat as being Rutschuru-Steppe (Zaire): 1200–1300m among grass and herbs. Harms believed *P.lancifolius* to be closely allied to *P.palustris*, differing most importantly in its leaflet shape. He goes as far as suggesting it may only be a narrow leafleted form of *P.palustris*. This proposed relationship in retrospect seems rather carelessly suggested as *P.lancifolius* possesses long brown epidermal hairs and a quite different stigma-style arrangement to *P.palustris*. Both of which make the two species easily distinguishable as separate species.

In his "The Leguminosae of Tropical Africa" (1926), E.G. Baker lists three *Psophocarpus* species; *P.palustris*, *P.lancifolius* and *P.monophyllus*. Baker does not distinguish between the West and East African populations of the *P.palustris* complex. Interestingly though in the light of Verdcourt's (1968) splitting of the complex into two species, Baker refers to the true *P.palustris* coming from Senegal as does Verdcourt. He then lists four specimens: Scott Elliot 7788; Wollaston (1906); Godman 360 and Flamigni 505 and comments that they "differ from the rest in having larger ovate-lanceolate leaflets, ± truncate at the base and larger flowers". All four of these specimens were collected in East Africa; Verdcourt and Halliday (1978) refer the first three specimens to *P.grandiflorus* and the last, Flamigni 505 to *P.scandens*. This can be taken to indicate that Bakers *P.palustris* actually contained three species; *P.palustris* Desv., *P.scandens* (Endl.) Verdc. and *P.grandiflorus* Wilczek.

Baker (1926) comments about *P.palmettorum* Guill. & Perr. that it does not differ specifically from *P.palustris* Desv., a view later to be taken up by Verdcourt (1968) who reduces the former to a synonym of the latter. Under his description of *Vignopsis*, Baker suggests that *Vignopsis* "is very closely allied to the genus *Dolichos*, from which it differs in having an oblique collarette of hairs a little way below the stigma". Baker does not note the other obvious difference between the two genera, e.g. stigma-style shape is markedly different, but in broad terms Baker was correct *Vignopsis* (as is *Psophocarpus*) is closely allied to *Dolichos*, as will be shown in later chapters.

In 1930 Tisserant published descriptions of the two rarest species

of *Psophocarpus*: *P.lecomtei* and *P.obovalis*. The type for the former being Tisserant 375 collected 35Km North-East of Bambari in the Central African Republic and the latters' type being Tisserant 749 collected 25Km East of Moroubas, Central African Empire. In his notes on the species, Tisserant suggests for *P.lecomtei* that because of its unifoliate leaf it is most closely allied to *P.monophyllus*, but then adds that the two species are easily distinguished by their different habits, leaf shape and narrow inflorescence with few small flowers. For *P.obovalis* he suggests because of the tri-foliate leaves, it is closest to *P.palustris* which has equally large flowers but has quite different shaped leaflets. Both observations on inter-specific relationships are concured with by Verdcourt and Halliday (1978) and will be discussed in greater depth in later chapters.

The next important step in the taxonomy of *Psophocarpus* was taken by Wilczek in 1954(b) when preparing the "Flore Du Congo Belge" but first published as the "Groupes Nouveaux Des Phaseoleae - Phaseolinae", also of 1954(a). Wilczek publishes the ninth and last currently legitimate *Psophocarpus* species, *P.grandiflors* Wilczek, accepted by Verdcourt and Halliday (1978). He also united DeWild's genus *Vignopsis* with *Psophocarpus* though keeping *Vignopsis* in a separate subgenus. He provides a thorough description of *P.grandiflorus*, lists its distribution, gives vernacular names and suggests an affinity with *P.palustris*. By merging *Psophocarpus* with *Vignopsis* the creation of subgenera became necessary due to the degree of intra-generic variability. Wilczek splits *Psophocarpus* into subgenera as follows:-

"Subgenus *Eupsophocarpus* Wilczek subg. nov.; stylus apice incurvatus, stigma introrsum vel terminale densissiume penicillatovillosum.

Subgenus Vignopsis (DeWild.) Wilczek comb. nov.; stylus infra stigma corona pilorum longonum ornatus stigma terminale, glabrum."

In the Flora (1954b) Wilczek comments that there are about ten species of *Psophocarpus* in Africa and Asia, of which five are found in the Belgium Congo, then he keys out the subgenera and species as shown in Table 2.3, (translated from the French).

Table 2.3 Wilczek's Key to Psophocarpus Subgenera and Species
(taken from Wilczek 1954)

SUBGENERA

A. Stigma terminal or internal, dense penecilate on the internal face; leaves 3 or 1 foliate; leaflets oval, sub-orbicular ord rhomboid, entire or sub-3 lobed. 1. *Psophocarpus*

B. Stigma terminal; collar of hairs just below the stigma around style; 3-foliate; leaflets lanceolate or rhomboid-lanceolate. 2. *Vignopsis*

1

Subgenus *Psophocarpus* Wilczek

A. Leaves unifoliate; petiole 0.15-0.25cm long; leaves with cordate base; stipule with spur ± 3.5mm long; flowers 1.3-1.6cm long; 3-4 ovules; legume lightly winged, long tapering, ± 1.5cm long and 0.6-0.5cm wide; 1-2 seeds, oval, aril, present *P.lecomtei*

B. Leaves trifoliate; petiole 2.5-18cm long; leaves cuneate or rounded at the base; stipule and spur 5-9mm long; flowers 1.6-4.5cm long; 7-10 ovules; legume strongly winged, 3.5-8cm long and 1-2cm wide; 4-8 seeds, round, aril and absent:

1. Leaflets with 5-7 lateral veins, the 2 lowest pairs being more spaced out than those above; lamina ovate-rhomboid, ± 3 lobed; flowers 1.6-2.4cm long; legume wings emarginate at apex, style rapidly deciduous post-anthesis 2. *P.palustris*

2. Leaflets with 7-10 lateral veins, equidistant; lamina ovate, entire; flowers 3-4.5cm long; pedicel 10-20mm long; standard falciform-curved, twisted; style 25-35mm long; legume wings tapering to apex, style ± persistant 3. *P.grandiflorus*

2

Subgenus *Vignopsis* (De Wild.) Wilczek

A. Plant glabrous or glabrescent; leaflets lanceolate, 0.4-0.8cm wide; raceme, peduncle, and pedicel glabrous; flowers 0.8-1.4cm long; bracteoles oblong-lanceolate, 5-7mm long and 0.7mm wide, glabrous 4. *P.lukafuensis*

B. Plant pubescent; leaflets lanceolate or lanceolate-rhomboid, 0.7-6cm wide; raceme, peduncle and pedicel pubescent; flowers 1.5-2.5cm long; bracteoles oblong-oval, 8-12mm long and 5-9mm wide, pubescent; 5. *P.lancifolius*

Note: the *P.palustris* referred to by Wilczek is *P.scandens* sensu Verdcourt (1968).

For each species Wilczek provides a thorough description, distribution details, habitat details, vernacular names and brief observations. He observes after *P.lukafuensis* that, "Les jeunes gousses sont munies de 4 ailes bein distinctes; la connaiscance de ce caractere permet de considerer *Vignopsis* De Wild. comme synonyme de *Psophocarpus*", which justifies his merging of *Psophocarpus* and *Vignopsis*, which has been followed by all other authors since Wilczek.

Hutchinson (1964) refers to five species of *Psophocarpus* of tropical Asia, Africa and Madagascar, one species of which has been naturalised to tropical America. He does not detail which five species he is refering to and why he restricts the genus to five species. This becomes more puzzling when it is considered that his work followed Wilczek (1954) chronologically and Wilczek includes five obviously "good" species without including *P.tetragonolobus* which must have been known to Hutchinson.

While examining specimens for the 'Flora of Tropical East Africa' Verdcourt (1968) noted that material refered to *Psophocarpus palustris* Desv. with a range throughout Tropical Africa was clearly divisable into subordinate taxa. The West African populations being consistantly different in a number of minor characters to those populations found elsewhere, these characters are detailed in Table 2.4. Verdcourt points out that this division into two species is not new, "Hutchinson and Dalziel in the first edition to 'Flora of West Tropical Africa' maintain two species which they call *P.palustris* Desv., and *P.palmettorum* Guill & Perr. The key they give will more or less separate out the two entities I have already mentioned". Though Verdcourts' implication that Hutchinson and Dalziel had similar ideas to his own must be questioned as they in the second edition of the F.W.T.A. (1973) regard *P.palmettonum* as a synonym of *P.palustris*. Verdcourts hypothesis is also questioned by the fact that all the specimens cited for both taxa in the first edition are referable to *P.palustris* and both *P.palustris* and *P.palmettorum* have type localities in West Africa. However there are good examples of taxonomists wanting to splits what is here referred to as the *P.palustris* complex. J.G. Baker (1871) in his description of *P.longepedunculatus* Hassk. (syn. *P.scandens* (Endl.) Verdc.) describes a

Table 2.4 Character Differentiation of Psophocarpus palustris and P.Scandens

Adapted from Verdcourt (1968) and Verdcourt and Halliday (1978).

Character	*P.palustris*	*P.scandens*
Leaflet shape	less rhomboid	± rhomboid
Terminal leaflet widest point	near middle	near base
Lamina abaxial pubescence	Densely pubescent	± glabrous
Bracteole/calyx length ratio	< 1 (± 0.5)	≥ 1
Bracteole pubescence	densely pubescent	± glabrous
Bracteole length	5 – 6.5 mm	10 – 14 mm
Bracteole width	3.5 – 5 mm	5 – 7 mm
Wing shape	Broad and curved	Narrower and longer
Legume length	2.3 – 5.5 cm	3.5 – 8.0 cm
Seeds/legume	(3–)4(–5)	4–8
Distribution	Senegal to Nigeria eastwards to Sudan	Cameroun to Angola, Zaire, E.Africa, Malawi, Madagascar, etc.

variety *Barteri* as follows:

> "Barteri, flowers white, leaflets large, subrotund, thinly grey pubescent all over beneath, bracteoles silky, blunt, herbaceous, shorter than the calyx."

This is a clear reference to *P.palustris* Desv. (sensu Verdcourt 1968). Verdcourt continues by observing:

> "the large bracteoled species has been known in the past (before the blanket-name *P.palustris* came to be so widely employed) as *P.longepedunculatus* Hassk., described from material cultivated at Bogor, but there is an earlier name on plant material cultivated in Brazil."

This earlier name applied to the Brazilian material was *Diesingia scandens* Endl. (1832), Verdcourt used the specific epithet of this to construct his new combination *Psophocarpus scandens* (Endl.) Verdc. Verdcourt (1968) having established his thesis that the *P.palustris* complex can be subdivided, then details the distribution and synonymy of *P.palustris* and *P.scandens* (sensu Verdcoudrt 1968).

Verdcourts hopes of clearly separating *P.scandens* from *P.palustris* were short lived. For in his revision of *Psophocarpus* (1978) he provides details of a few specimens which are anomalous in some way, e.g. Letouzey 13155 which has glabrescent bracteoles much shorter than the calyx, but with foliage clearly of the *P.scandens* form, and Letouzey 3566 with typical bracteoles of *P.palustris* but the slightly lobed leaves of *P.scandens*. These specimens were taken from the geographical border between the two species and so possible hybridisation or introgression would not invalidate Verdcourt's distinction of the two species, even though *P.palustris* and *P.scandens* are obviously very closely related.

Westphal in 'Pulses in Ethiopia' (1974) provides details on what he refers to as *Psophocarpus palustris* Desv., however he uses this binomial in the sense of Baker (1926), which incompasses *P.grandiflorus*, *P.palustris* and *P.scandens*; distribution of all three species; a

description and drawing of *P.grandiflorus* (he notes the description is based on one sample, unfortunately it was the wrong sample!); ecology of *P.palustris* and uses of *P.scandens*. In his taxonomic notes he refers to an Ethiopian specimen of *P.palustris* Westphal and Westphal-Stevels 2666 which is clearly *P.grandiflorus* (the basis of his description). He continues by criticising Wilczek (1954b) for not detailing the specimens of *P.palustris* collected from Ethiopia, given in a footnote to Wilczek's distribution of *P.palustris* in the Belgium Congo. It would seem unlikely that *P.palustris* (or *P.scandens*) would occur in Ethiopia as pointed out by Cufodontis (1955). However as Wilczek originally described *P.grandiflorus* it seems even less likely he would confuse it with *P.palustris*, this might possibly be explained as with the Sudanese specimen of *P.scandens* in Verdcourt and Halliday (1978), by the introduction of a specimen of *P.palustris* to Ethiopia.

Westphal considers the separation by Verdcourt (1968) of West and East African populations of *P.palustris* as premature, as no revision of the whole genus was then available. He believes the "number of admittedly small characters" (quoted from Verdcourt 1968) is insufficient to delimit species and will lead only to further confusion, especially as both leaflet shape and bract/bracteole pubescence are variable characters. Verdcourt would not claim the difference between *P.scandens* and *P.palustris* are major, they are closely allied species, however the characters Verdcourt provides are relatively constant for these taxa and as will be shown later in the thesis provide a good specific separation.

Westphal (1974) concludes his taxonomic notes on the *P.palustris* complex after emphasising the specific confusion by the following, "A revision of the genus *Psophocarpus* Neck. ex DC. is therefore most desirable". An extremely comprehensive revision of *Psophocarpus* was to follow 4 years later; Verdcourt and Halliday's (1978) - 'A Revision Of *Psophocarpus* (Leguminosae-Papilionoideae-Phaseoleae)'. This revision and the nine species is taken as the 'taxonomic foundations' of this thesis. The revision comprises a taxonomic history; major references; synonymy; descriptions; distributions, with maps; details from palynological and biochemical studies; habitats; a key; suggested classification; extensive specimen citation and theories of geographical

distribution. The revision is so extensive in its comprehension that it is tempting to quote large sections of it wholesale, but I will here restrict discussion to the sections relating to specific arrangement.

Verdcourt and Halliday (1978) restric *Psophocarpus* to nine species (detailed in Chapter 1) reducing many binomial to synonyms of these nine and excluding *P.cienkowskii* ex Bak. as a synonym of *Canavalia ensiformis* (L.) DC. They divide the genus into two,

> "subgen. *Psophocarpus* with the stigma terminal or internal but with hairs to the tip of the style and subgen. *Vignopsis* with the stigma terminal and hairs limited to a ring some short distance below the style tip. Subgen. *Psophocarpus* contains two sections, sect. *Psophocarpus* with 3-foliate leaves and sect. *Unifoliatae* A. Chev. ex. Verdc. with unifoliolate leaves".

Chevalier (1907) proposes at the end of his account of *P.monophyllus* Harms that it be taken as the type of sect. *Unifoliate* Harms. However Harms does not mention this division into sections in his original description of *P.monophyllus* so that Verdcourt validates it. The reasons for division of *Psophocarpus* into subgenera follows those of Wilczek (1954b) and the separation of section *Unifoliate* from section *Psophocarpus* due to the different number of leaflets was an obvious second step. The full details of the divisions of *Psophocarpus* may be understood from the key to *Psophocarpus* species provided in Table 2.5.

Table 2.5 Key to *Psophocarpus* species
(After Verdcourt and Halliday, 1978)

1 Leaflets 1-foliate 2
Leaflets 3-foliate 3

2 Plant totally prostrate with leaves flat on the ground; leaflet rounded elliptic or rounded ovate, 1.8–8 x 1.5–6 cm, cordate at the base with a distinct narrow sinus; inflorescence bracts 3–4 mm long. **7.P.lecomtei**
Plant trailing; leaflet ovate or rounded-elliptic, 6.5–15 x 3.5–9.5 cm, cuneate or truncate to shallowly emarginate at the base; inflorescence bracts longer, 0.6–1 cm long **6. P.monophyllus**

3 Style with a ring of hairs below the apex; seeds with raised median line (where known); leaflets narrow 4
Style laterally hairy to tip so as to form a tuft rather than a subapical ring; seeds without a raised median line; leaflets broad 5

4 Stems glabrous; calyx glabrous save for the ciliolate lobes; leaflets 1.7–8 x 0.4–1.4 cm; standard 0.8–1.5 cm long **9. P.lukafuensis**
Stems adpressed-pubescent to densely spreading pilose; calyx glabrous to densely covered with long hairs; leaflets 2.2–11 x 0.7–3.5(–6) cm; standard 1.5–2.5 cm long . . **8. P.lancifolius**

5 Leaflets narrowly oblong-elliptic or oblong-obovate, narrowed to the base; prostrate creeper . . . . . **5. P.obovalis**
Leaflets rhomboid or ovate, broader at the base 6

6 Standard 2.5–4 cm long 7
Standard 1.5–2.1 cm long 8

7 Bracteoles short, 2.5–4.5 x 2.5–3.5 mm; plant mostly glabrous; fruit 6–40 cm long, containing 5–21 seeds; known only in cultivation **2. P.tetragonolobus**
Bracteoles longer, 7–10 x 5–7 mm; plant pubescent; fruit 4–9cm long containing about 8 seeds; wild in Ethiopia, E.Zaire and Uganda **1. P.grandiflorus**

8 Leaflets much less rhomboid, the terminal one widest nearer to the middle, usually much more densely hairy beneath; bracteoles shorter than the mature calyx, often only half its length, densely pubescent, 5–6.5 x 3.5–5 mm; fruits 2.3–5.5 cm long and mostly (3–)4(–5) seeded; of restricted distribution; Senegal to Nigeria, eastwards to sudan **3. P.palustris**
Leaflets ± rhomboid, the terminal one widest near to the base, usually ± glabrous beneath; bracteoles nearly always as long as or longer than the calyx; glabrous to sparsely pubescent, 1–1.4 cm x 5–7 mm; fruits often longer, 3.5–8 cm long, 4–8 seeds; Cameroun to Angola, Zaire, E Africa, Malawi, Madagascar, etc. **4. P.scandens**

CHAPTER THREE

AN INTRODUCTION TO DATA GATHERING AND ANALYSIS

3.1 Introduction

Once the extent of the taxon to be studied has been delimitated, the subsequent steps in a taxonomic study involve skillful decision making. The choice must be made as to: the appropriate level for operational taxonomic units (OTU), the choice of suitable characters, and the choice of relevant methods of analysing the data produced. The purpopse of this chapter is to take these three points and provide each with a theoretical background and then explain the reasons for the decision taken in the present study.

For practical purposes the third decision to be made, choice of relevant method of analysis, will be divided into two sections, based on phenetic and phylogenetic or cladistic principles respectively. This dicotomy is necessitated by the fundamental differences in these two modes of analysis and their approach to the problem of association. Phenetic techniques use the occurence of character combinations as they are now perceived to construct taxonomic groups, while phylogenetic techniques add the dimension of time and ancestry to describing organisms. (Dunn and Everitt, 1982 and Davis and Heywood, 1973). This distinction between modes of analysis has historical importance to the progress of taxonomy; Sneath and Sokal (1973) state, "the separation of overall similarity (phenetics) from evolutionary branching sequence (cladistics) is an important advance in taxonomic thinking."

This chapter is not meant as a justification of the underlying philosophies of the technique to be employed, though this may be inherent in the a posteriori comparative discussion of the particular techniques used in this study. Surely twenty one years after the publication of Sokal and Sneath's 'Principles of Numerical Taxonomy' the case for the use of numerical (= phenetic in this thesis) methods has been validated. Perhaps the case for cladistic analysis is more controversial: however, this will be discussed in the light of

experience gained from the present study in later chapters.

3.2 Choice of operational taxonomic units

3.2.1 Theoretical foundation

The fundamental taxonomic units employed in computer aided taxonomy are referred to as operational taxonomic units (OTU's). An OTU may be defined as the lowest taxonomically ranked unit to be studied in a particular investigation, an element in a set that is to be classified. Operational is used instead of fundamental to imply that the taxonomic rank can be of any level; for example, they may be individuals, examplars of genera, or averages representing species (Sneath and Sokal, 1973).

The choice of the level of taxonomic rank at which the OTU's will be established is important. Jardine and Sibson (1971) advise that the choice should be preceeded by a statement of purpose of the investigation, this may effectively constrain the choice to a particular taxonomic level. A simple example might be a study of intraspecific variants in *Psophocarpus tetragonolobus*, where the ideal taxonomic unit would be the individual and not, say, avera ged varieties which would provide no idea of intra-varietal variation. In effect, the choice of taxonomic rank to be OTU's is a compromise between providing enough information on intra taxa variation adequatly to describe the taxa, but not providing so much information that the similarity matrices are of excessive size and lead to computer processing problems.

Sneath and Sokal (1973) pose a poignant question, "Should numerical taxonomy rely on the validity of prior classifications for its choice of OTU's?" Should every study be based on individuals, to be taxonomically consistant and rigorous? the answer must be yes to the first question and no to the second. It would be impossible to sample every individual, time and facilities are always limiting factors. In answering any taxonomic question, the question must be formulated within the framework of the pre-existing classification; however this does imply sampling of OTU's. Williams and Lance (1965) discuss the problems involved in choosing a sample of OTU's and conclude that the sample must

be representative of the taxa for the study to be probabilistic. Though where this is not possible, the study can remain valid within the limitations of non probabilistics. Sneath and Sokal (1973) conclude their argument by stating that if, "we wish to reexamine the relationship of numerous genera in a family we cannot reassert the validity of every genus from a study of its species (and the validity of the species from a study of their individuals)". Thus OTU's must be sampled.

Within taxon variation may present a problem, this may be especially true above the species level where within a taxon the majority of characters will vary. This may be compensated for by including one OTU to represent each varying character combination per original OTU. An alternative is to use a median representative for the polymorphic OTU in the expectation that variance in the polymorphic OTU does not exceed taxon variation. Perhaps, though, a more commonly applied solution is to limit the number of characters to those that show little intra OTU variation.

3.2.2 Reasoned choice of OTU's

As suggested by Jardine and Sibson (1971) the first step is to state the purpose of the investigation. The investigation in the present study has two main objectives, firstly to locate the 'natural' position of the genus *Psophocarpus* within the legume tribe Phaseoleae and secondly to elucidate the inter-specific relationships between *Psophocarpus* species. Thus the investigation is concentrated at two levels, the generic and the specific, which must be discussed separately. The generic study was limited, as discussed in Chapter 1, to the Phaseoleae sub-tribes Phaseolinae and Clitoriinae (sensu Lackey, 1981), which include 27 geneera and approximately 489 spoecies. With an investigation of this dimension, the taxonomic rank attributed OTU status could not be individual specimens and so it seemed best to locate OTU status at the specific level. Even at this level, however, it still left too large a number of species to be all scored individually. So, as described in section 4 of Chapter 1, the OTU scoring was divided into a preliminary survey, using a few OTU's from each genus systematically chosen to represent that genus, and a more detailed survey of the genera

shown from the preliminary survey to be closely allied to *Psophocarpus*. Even in the second more detailed survey of the seven genera shown to be most closely allied to *Psophocarpus* the survey would have proven still too comprehensive for the time available as it included approximately 237 species (150 from *Vigna* and 60 from *Dolichos* alone). Accordingly, although the species was the OTU level, not all 237 species were included in the study. Within the smaller genera: *Psophocarpus*, *Dysolobium*, *Otoptera*, *Sphenostylis*, *Neph ostylis* and *Neorautanenia* all species were represented by OTU's; in most cases the averaged scores of numerous scored specimens were used in the analysis. But for *Vigna* and *Dolichos* an attempt was made to include OTU's (=specimens=species) representing the intra-generic diversity of these larger genera.

The second objective of the study was to investigate the intra-generic relationships of *Psophocarpus* species. As circumscribed by Verdcourt and Halliday (1978) *Psophocarpus* contains nine species of which only two *P.scandens* and *P.palustris* are not clearly defined species. Thus the scope of the investigation being limited to nine taxa, the rank of OTU could be set at the individual specimen, which would provide enough information to account for intra-specific variability while not presenting so many individuals that scoring or computer time became a problem.

It should be added that for the *Psophocarpus* specific study not an equal number of OTU's (specimens) were scored from each species. By far the largest number of specimens was scored from *P.scandens* and *P.palustris* simply because of the controversy surrounding their specific distinction by Verdcourt (1968). At the other extreme only two specimens of *P.obovalis* were scored and used as OTU's because as Verdcourt (1971b) points out, the species is only known via these two specimens. He comments that it is presumably a rare species but is easily distinguishable as a separate *Psophocarpus* species from the other *Psophocarpus* species growing in the Central African Empire and Sudan.

3.3 Choice of Characters

3.3.1 Theoretical foundation

Life is necessarily mediated by the concept of characters, as a result of the accummulation of sense perception data man is able to define his existence. The concept of characters is no less important to taxonomy and provides the information on which classifications are founded. It may at first seem curious with something so fundamental as a character, that each taxonomic text book provides a virtually unique definition, perhaps because characters seem to fall into the category of concepts that are so basic as to defy accurate definition and yet are universally understood.

That is not to say that attempts at definition should not be made, Davis and Heywood (1973) provide the general definition as follows: "any attribute (or descriptive phase) referring to form, structure or behaviour which the taxonomist separates from the whole organism for a particular purpose such as comparison or interpretation". Within the context of numerical taxonomy the concept of a character is more restricted. Sneath and Sokal (1973) realise this and thus refer more specifically to 'unit characters' and their definition is as follows, "a taxonomic character of two or more states, which within the study at hand cannot be further subdivided logically, except for subdivision brought about by changes in the method of coding the state". The later definition reflects the general philosophy of numerical taxonomy by defining and utilising a larger number of 'simple' characters in an attempt at presenting a more accurate reflection of the genome in the resultant classification, 'simple' being here used in terms of lack of logical subdivision.

Davis and Heywood (1973) stress the abstract nature of characters, it is character states that taxonomists actually utilise. An example of this might be the degree of keel apex spiralling distinctive of some Phaseolinae genera; this would be the character. The degree of spiralling might vary between no spiral at all as in *Psophocarpus* species to four full 360^{o} spirals as in some *Phaseolus* species: this would represent the two extreme character states which would be used by the taxonomist in his analysis.

An important distinction is also pointed out by Davis and Heywood

(1973) as to the characters used in phenetic and phylogenetic studies. Phylogenetic characters may be used in phenetic studies, but are generally chosen for a specific purpose (to infer phylogeny) and so produce not a general but a special classification based usually on much fewer characters than phenetic studies. For this reason and because phylogenetic character definition is so intimately tied in with the practice of cladistics, phylogenetic characters will be discussed separately in the last section of this chapter.

Characters may be divided into numerous categories both depending on the source of the character and its intrinsic nature. Sneath and Sokal (1973) group the sources of taxonomic characters into the following 'rough' categories:

"a) morphological characters (external, internal, microscopic, including cytological and developmental characters).

b) physiological and chemical characters.

c) behavioural characters.

d) ecological and distributional characters (habitats, food, hosts, parasites, population dynamics, geographical distribution)"

While this list is not suggested to be completely comprehensive, it does show the wide variety of character sources available to taxonomists, and they would agree that the broader the range of information used in forming a general classification, the better the resultant classification.

Davis and Heywood (1973) list three divisions of characters which relate to their intrinsic nature. Two they distinguish being analytic and synthetic characters, analytic characters being those used in identification, characterisation and delimitation. In the classifications that result from the use of analytic characters, synthetic characters are used which show a wide occurrence and are constant for a particular taxon. They then differentiate qualitative

from quantitative characters, features assessed by size, length, number, etc., being quantitative, e.g. staminal tube length, and those assessed by form being qualitative, e.g. staminal tube apex shape.

The third division of Davis and Heywoods intrinsic characters categories is the practically important division into 'good' and 'bad' characters. They recognise good characters as being those that:

1. are not subject to wide variation within the samples being considered.

2. do not have a high intrinsic genetic variability.

3. are not easily susceptible to environmental modification.

4. show consistency, i.e. agree with the correlations of characters existing in a natural system of classification which was constructed without their use.

Stace (1980) refers to good and bad characters as those with greater or lesser taxonomic value, then continues by stressing the relativity of these terms. What may be a good character in a generic study may be poor at the species level, e.g. in the present study, extra keel tooth is a good inter-generic discriminating character but at the intra-generic level is invariable and so a bad character.

When trying to locate 'good' characters there are certain types of character that should be avoided if the resultant analysis is to have validity. Sneath and Sokal (1973) list 5 character types which should be excluded from taxonomic studies:

a. meaningless characters, attributes of an organism which do not reflect the organism's inherenet nature, e.g. specimen names or numbers, etc.

b. logically correlated characters, any property which is the logical consequence of another property, e.g. leaf length and half

leaf length.

c. partial logical correlation, where characters are only partially correlated with one another, e.g. a series of 'n' leaf measurements along a leaf blade.

d. invariant characters, characters which throughout the sampled OTU's have the same character state and so add nothing to the information content of the analysis.

e. empirical correlations, where characters are not logically correlated but empirically. Sneath and Sokal give the example of albino's having white skin and pink eyes.

To the above list might be added another category of characters to be avoided, those scored from non-homologous structures, Sneath and Sokal (1973) define homology 'loosely' (thier word) as compositional and structural correspondence, compositional correspondence being qualitative resemblance in terms of biological or chemical constituents and structural correspondence referring to similarity in terms of spatial or temporal arrangement of parts, or in structure of biochemical pathways or in sequential arrangement of substances or organised structures. With a group like the Phaseoleae avoidance of comparing, via characters, non-homologous structures is difficult because of the problems in establishing which are non-homologous structures or compositions. Sneath and Sokal (1973) realised this general problem of discovering non-homology and proposed the use of operational homology as a practical solution. Where characters shared by OTU's are said to be homologous if they are very much alike in general and in particular.

The Adansonian principles that provided the foundations for numerical taxonomy pose two important questions relating to characters; (a) whether each attribute selected should be equally weighted, and (b) how many characters should be used in a particular taxonomic study.

Adanson believed that all attributes should be weighted equally, meaning that all characters should be ascribed equal importance. The

most implicit form of weighting is that of inclusion, whether a particular character is included or rejected from the analysis. Dunn and Everitt (1982) point out that since there is an unlimited number of characters available, a subset must be chosen to base the classification upon; thus in fact every taxonomic study uses weighted characters. Weighting of characters may take two forms, a priori and a posteriori, the former being characters previously considered useful in the group, those characters known to be good diagnostically or those thought to be phylogentically important. Sneath and Sokal (1973) argue that any a priori weighting is inappropriate as it, "presupposes a knowledge that is not yet available, either about the classification of the organism, or about the presumed significance of their characters". For this reason no weighting of characters was used in this study, other than via inclusion/exclusion from the appropriate character sets. A posteriori characters are those chosen after study of the group of organisms and are used for keys or descriptions.

As stated in the previous paragraph each organism possesses a limitless number of characters; however, the taxonomist will always be limited temporally and economically. Thus the number of characters employed in a study requires skillful consideration if a stable classification is to be produced. Sokal and Sneath (1963) recommended a figure of 60 characters minimum, but Sneath and Sokal (1973) retract this fixed figure and suggest generally that the more characters used the better. Since the non-specificity hypothesis was at least partially invalidated by congruence studies, the larger the number and their origin from different sources has become increasingly important. The non-specificity hypothesis states that no distinct large class of genes affects exclusively one class of characters, such as morphology, physiology, ethology, etc., so justifying those studies obtaining data from one source, e.g. morphology, biochemistry, etc. However, congruence studies (studies where classifications are produced form different data sets then compared, e.g. comparing a morphological with a biochemically produced classification) may show a degree of mistmatching. Sneath and Sokal (1973) point out that if numerous characters are measured the estimate of similarity will be less easily changed, and would require a much larger number of characters with quite different phenetic information to change it markedly. However they

comment that it would be very difficult statistically to prove this. Dunn and Everitt (1982) rather mysteriously comment that, "it might be possible in some circumstances to achieve a stable classification using fewer characters". They do not elaborate, but presumably if, as discussed earlier, a posteriori weighted characters are used then the number could be reduced, this is surely what happens in practice.

Stace (1980) points out that practically characters are often selected because they are most easily scored or if they show promise as being reliable and discriminating in taxon delimitation. He continues, "it is most convenient if taxa are delimited by obvious features rather than by cryptic ones". Traditionally these characters have been morphological, mostly based on flowers because of their conservatism, defined as their ability to remain relatively unchanged over a long period of evolutionary development. So that between closely related taxa reproductive structures will vary little. However, as pointed out earlier congruence studies have challenged these classically orientated beliefs and Stace himself stresses the importance of using a wide range of information from diverse sources to produce a more predictive classification.

The formalisation of character selection has been the least successful aspect in the application of numerical taxonomy. Davis and Heywood (1973) comment, "character selection is the weak link in this whole aproach". This comment is unfortunately still pertinent today, perhaps due to the problems involved in defining the process of character selection, which a classical taxonomist undertakes in seconds purely intuitively. Bisby (1970) in a study of character evaluation and selection using taximetric procedures concludes that character selection methods will prove of great use to taxonomists. He comments that for:

> "taxonomists working with large amounts of data, the evaluation and selection of characters may be one of the most difficult and time-consuming stages of taxonomic work. Using 'character analysis' to discover the most useful characters can save a large amount of time."

This is obviously true, and he might have continued by pointing out that as the data set increases in size so the accuracy in selecting 'good' characters must diminish. This is a particular area of taximetric research which needs greater development to formulate the algorithms which will help surmount the problems detailed in this section.

3.3.2 Reasoned choice of characters

Having detailed the problems involved in 'good' character selection above, it must be reluctantly admitted that character selection in this study was almost entirely intuitive and thus subjective. The characters and character definitions chosen would not necessarily have been chosen by another taxonomist faced with the same problems. He or she might have subsequently produced quite different classifications (Bisby and Nicholls, 1977). However a strong effort was made in selecting characters to act logically and follow the precepts outlined above.

The initial set of characters was limited, as are all taxonomic studies, by temporal, economic and material availability factors. The group of plants to be studied was vast, nearly 500 species and endemic to the tropics. This quickly limited the study to scoring morphological characters from herbarium material, being the only way to enable observation of the taxa in the time available of plants that could not practically be grown in this country.

The gross character set was formulated from the literature and from original plant observation. The choice of characters was made a degree of magnitude easier by following temporally four major taxonomic works on taxa related to *Psophocarpus*: Verdcourt's 'Studies in the legumeinosae-Papilionoideae for the Flora of Tropical East Africa, II-IV' (1970a, b and c); and Marechal et al's, 'Etude taxonomique d'un groupe complexe d'espèces des genres *Phaseolus* et *Vigna* (Papilionaceae)' (1978a) and two PhD thesis revisions of the Phaseoleae (Papilionoideae) by Lackey (1977b) and Baudet (1977a). The characters chosen for the present study were largely adapted from these works, but other characters were added from numerous other literature sources. As detailed in the study plan (see Chapter 1.4), Phase 1 of the project included a period of exploratory character scoring using specimens of

Psophocarpus, *Dysolobium* and *Otoptera*. In this period, the characters from the literature were tested and as the plants became familiar so further novel characters were added to the gross character set. The gross character set (phenetic) is detailed in Chapter 4.

As will be seen in Chapter 5 the analysis falls into four studies or sections: (a) broad survey of Phaseolinae and Clitoriinae genera; (b) subset generic study; (c) *Psophocarpus* species study and (d) *P.scandens-P.palustris* complex study. Each of the four studies' analysis uses a different character set and the latter three each use several different character sets to accommodate the different method of analysis used. The use of multiple character sets, all abstracted from the gross character set, is necessitated by the different taxonomic questions posed by each of the four studies to be analysed. Phenetic character set selection was complicated by the different taxonomic levels of the various studies and the limitations of the computer programs in accepting certain types of data. Thus the actual character selection will be explained prior to the discussion of that study's analysis in Chapter 5. In each study however it is generally true that characters were chosen which show variation between OTU's but were constant for a particular OTU.

To aid the selection of characters for the subset of genera most closely allied to *Psophocarpus* the character analysis program CHARANAL was utilised, it being available on the ICL2970 mainframe computer of the University of Southampton. As pointed out above, as the data set increases in size so the selection of 'good' characters becomes more difficult. In the analysis of the subset generic study the data set was both large and character scores were divergent, making character selection especially problematic.

The program CHARANAL is discussed in detail in the handbook written for the program (Fleming and Appan, 1971) and its application is discussed in Bisby (1970). CHARANAL performs comparisons between pairs of characters, to assess the amount of information (biologically meaningful in the sense of being discriminating in a classification) held in common by two characters. Most commonly prior to CHARANAL this was undertaken by statistical methods, which require ordered characters (ordered characters being those in which the character states are

related as integers are in a number series). CHARANAL has the advantage of being able to utilise both ordered and non-ordered characters. It studies the actual distribution of the data using information theory (see Estabrook, 1967). The user can obtain for each pair of characters: conditional probabilities, information content of each, information quantity held in common and information quantity held in common expressed as a fraction of the information content of each. If purely character selection is required then by choosing the SUMRAT and SAMRAT option, figures are provided on which character selection may be based. The CHARANAL handbook does however stress that in this case character selection should be supplemented by biological judgement.

CHARANAL lists individuals with missing data and eliminates them from that character pair comparison for those invididuals. The user specifies which characters he or she wishes to compare. This initially will be all of the possible combinations as the program was used in the present study. Details of this study's usage of CHARANAL are discussed in Chapter 5, with the discussion of the more subjective choice of characters for each character set.

3.4 Choice of phenetic analysis

3.4.1 Theoretical foundations

This of all the four sections of this chapter is the most problematic to define under the heading 'theoretical foundations', the reason being the speed with which this field has progressed since the publication of Sokal and Sneath's 'Principles of Numerical Taxonomy' (1963). However this section will attempt to introduce phenetic

analysis and will be followed by a discussed of the chosen methods of analysis and the actual computer programs utilised. Anyone wishing a more comprehensive introduction should turn to Dunn and Everitt (1982) and from there to Sneath and Sokal (1973).

Phenetic analysis is based on overall affinity, the presence of consistent character combination defining a particular taxon, using as many chracters and as much evident as is available (Davis and Heywood, 1973). Sneath and Sokal (1973) define numerical taxonomy as, "the grouping by numerical methods of taxonomic units into taxa on the basis of their character states". Many contempory taxonomists regard phenetics and numerical taxonomy as being synomymous, but this is misleading as the latter by definition requires the application of numerical methods. Davis and Heywood (1973) point out that the traditional technqiues of taxonomists in neurally estimating resemblance on observable features is a form of phenetic analysis. However, Sneath (1961) criticises these traditional methods as being subjective and employing imprecise statistical Q-techniques. The criticisms being irrefutable, this discussion will be confined largely to the development and practice of numerical analysis.

Historically classifications were based on characters that were thought to have inherent importance to the plant, in each case a few morhpological characters were chosen. Caesalpinus, who produced the first methodical classification of plants, 'De Plantis' (1583) considered nutritional and reproductive characters to be the most important chracteristics and so his classification was based on these characters. Tournefort (1700) used flowers and fruits primarily in his classification, Linneus (1753) used floral characters, de Jussieu (1789) used number of cotyledons, nature of perianth and position of ovary, while De Candolle (1824-1873) used a more complex system, retaining the use of number of cotyledons but basing his sub-classes on the degree of reduction and fusion of floral parts.

Two botanists, however, stand out as opposing this system of artificial classifications (classifications based on a few characters), Ray and Adanson. Ray formulated the principle that all part of plants should be used for classifying plants. His classification system is

presented in his 'Methodus Plantarum' (1682) in which he retains the traditional division of plants on habit, but then uses coteledon number, fruit type, leaf and floral characters. Adanson, after having tried to apply Tournefort and Linnaeus' classifications, rejected artificial systems as being hopelessly inadequate and therefore set about creating a more natural system (Davis and Heywood, 1973). For the 'Familles des Plants' (1763) Adanson produced 65 single character systems, in which generic position was very variable. He then united these systems so that agreement between the greatest number of systems reflected the natural (phenetic) relationships. Cain (1959) comments that Adanson's approach was basically in correspondence with numerical principles. However De Candolle rejected Adanson's work on two counts; (a) it presupposes that we know not only all organs of plants but also all points of view from which they can be considered, and (b) it did not allow for a priori character weighting. The first point is probably still as relevant today as then. In the present study lack of legumes and seed on specimens led to missing data problems. However, the second of De Candolles' points is rejected (see Section 3.2 of this chapter), a priori weighting of characters is considered deleterious. With the publication of the 'Origin of Species' by Darwin (1859) there was in principle little change in classifactory procedure; taxonomists simply looked for phylogenetically important characters but retained the artificiality of using a small number of characters. Then in the 1950's, as the potential sources of taxonomic character began to mushroom, so some taxonomists began to advocate a more objective method of classification. Michener and Sokal (1957) argued that taxa should be classified on the total degree of difference or similarity, when as many characters as possible from different sources were taken into account. This was an obvious revival of Adansonian principals, and finally with the development of computer technology, multivariant analysis became a practical option.

Sneath and Sokal (1973) accept their debt to Adanson and so refer to their fundamental principles of numerical taxonomy as being Neo-Adansonian. The principles formulated by Sneath and Sokal are as follows:

1. The greater the content of information in the taxa of a classification and the more characters on which it is based, the better a given classification will be.

2. A priori, every character is of equal weight in creating natural taxa.

3. Overall similarity between any two entities is a function of their individual similarties in each of the many characters in which they are being compared.

4. Distinct taxa can be recognised because correlations of characters differe in the groups of organisms under study.

5. Phylogenetic inferences can be made from the taxonomic structures of a group and from character correlations, given certain assumptions about evolutionary pathways and mechanisms.

6. Taxonomy is viewed and practised as an empirical science.

7. Classifications are based on phenetic similarity.

Stace (1980) comments on these principles thus:

"These seven principles underlie not only Sneath and Sokal's concepts of numerical taxonomy, but the subject as a whole and nowadays essentially the same aims and methods are being applied to all groups of microorganisms, animals and plants".

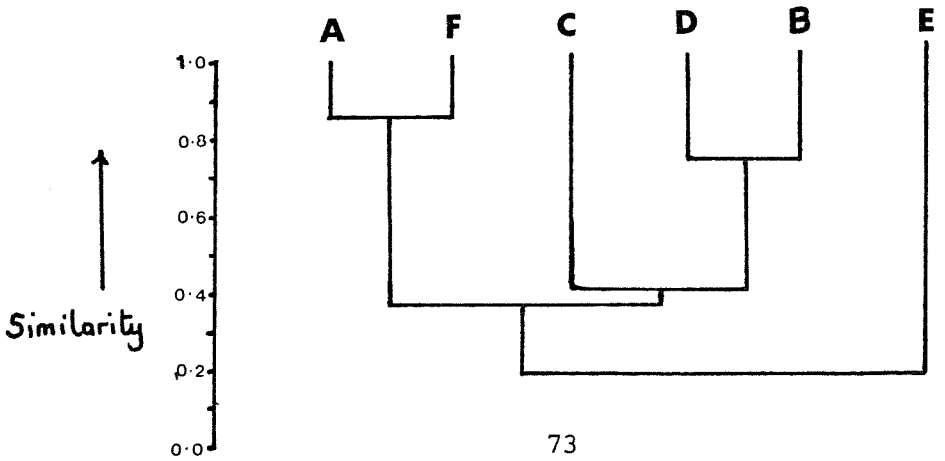
Perhaps Stace should have pointed out that the reason these principles are so important to taxonomy as a whole today is largely due to the impact of numerical taxonomy on taxonomy in the past twenty years.

Sneath and Sokal continue by outlining the practical sequence of numerical taxonomy:

"organisms and characters are chosen and recorded, the resemblances between organisms are calculated, taxa are based upon these resemblances and last, generalisations are made about the taxa (such as inferences about their phylogeny, choice of discriminating characters, etc.)".

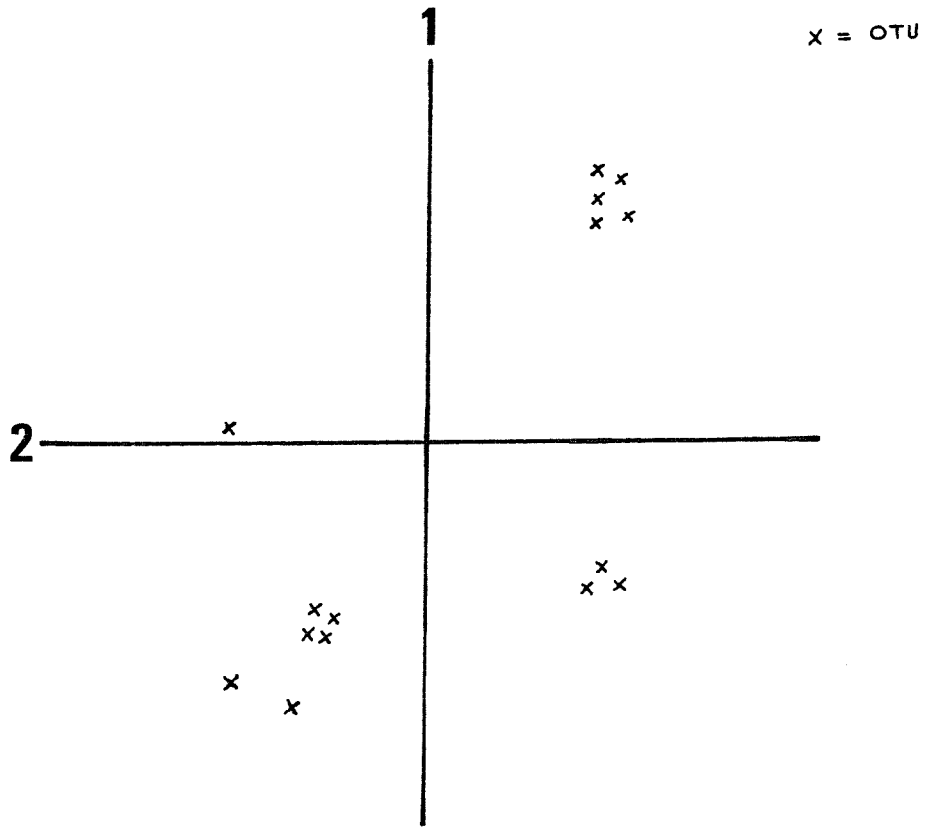
The choice of organisms and characters has been discussed in separate sections of this chapter above. Following these choices the resemblance between operational taxonomic units (OTU's) is calculated. This can be divided into two sub-stages, scoring the material and organising it in a data matrix table (OTU's x characers or t x n); this following codification is then fed into the computer and the second stage of producing a similarity or dissimilarity matrix between OTU's (t x t) is calculated. Numerous different measures of similarity and dissimilarity are available (see Dunn and Everitt (1982), Chapter 3). Sneath and Sokal recommend that the simplest coefficient that is applicable to the study's data should be chosen as it will ease interpretation of the final results.

The triangular similarity matrix is then analysed using a possible range of techniques which genereally fall into two categories, cluster analysis and ordination methods. For both these categories there are numerous variations on the basic techniques (for cluster analysis see Everitt, 1974 and for ordination see Dunn and Everitt, 1982). Both these categories of analysis produce distinctive visual result displays. Cluster analysis usually yields a hierarchical classification of OTU's which is depicted in the form of a dendrogram (see below).



OTU's are linked at particular levels of similarity, the higher the level of similarity the more closely allied are the OTU's. The abscissa has no special significance, it spaces the OTU's. However, the ordinate represents the linking of OTU's at a particular level of similarity. Linkage could extend upwards until OTU's exhibit maximum similarity (1.0) and extend downwards as far as is needed to unite all OTU's in the particular study, (in the above example 0.2).

The second category of analysis techniques, ordination, usually present their results in the form of a scatter diagram, in which the most closely allied OTU's are spatial juxtaposed (see below).



Sneath and Sokal stress the importance that numerical taxonomy enables the delimitation of taxonomic groups in an objective manner, but as the resemblances are based on phenetic observation, drawing conclusions about phylogenetic relationships must remain tentative.

The advantages of using numerical taxonomy are discussed and detailed by Sneath and Sokal (1973). They emphasise the two major advantages of repeatability and objectivity. Repeatability implies that two taxonomists will produce comparable classification if presented with the same problem. Objectivity is a relative concept, but use of numerical taxonomy will reduce subjectivity and bias, so enhancing the predictivity of the classification produced. They provide an extensive list of advantages of numerical taxonomy over traditional methods which may be summarised as follows:

1. Use of numerical method permits the integration of data from a variety of sources (morphology, anatomy, cytology, biochemistry, etc.)

2. Numerical taxonomy promotes efficiency because a large part of its procedure is automated and can be carried out by less skilled workers.

3. Once the data is coded into the computer, it can be used for various end products (keys, descriptions, distribution maps, etc.)

4. As the method is quantitative it allows greater discrimination of closely allied taxa.

5. The creation of explicit data matrices has forced the taxonomist to put more thought into character definition and selection.

6. The adoption of the canon of numerical taxonomy has forced taxonomy as a whole to re-examine its fundamental principles.

7. Numerical taxonomy has stimulated reinterpretation of a number of biological concepts and posed new questions, both biological and evolutionary.

In their concluding remarks on the potential value of numerical taxonomy Davis and Heywood in 1963 comment that, "It would be rash to predict the reaction of Angiosperm taxonomists in general to numerical procedures, but clearly a cautious welcome should be extended". Twenty one years later the note of caution can be withdrawn; numerical procedures have proved a major advantage to the taxonomist, allowing him thoroughly to tackle problems and use sources of information that would have been beyond his scope previously.

3.4.2 Reasoned choice of phenetic methods

For the phenetic analysis three computer programs were utilised; LINKAGE, CLUSTAN 1C and DECORANA, all available on the ICL 2970 mainframe computer at the University of Southampton. These programs were chosen to carryout particular forms of analysis on the different data sets. Different programs with different forms of analysis were required so the group formations produced by each could act as a verification of the other program's group formations. Each program will be discussed in turn, an introduction to concepts underlying the program will be detailed, as well as the program's advantages and disadvantages.

(a) LINKAGE

LINKAGE is a FORTRAN program written by Wirth, Estabrook and Rogers (1966) and introduced in a handbook to the program written by Fleming and Appan (1972).

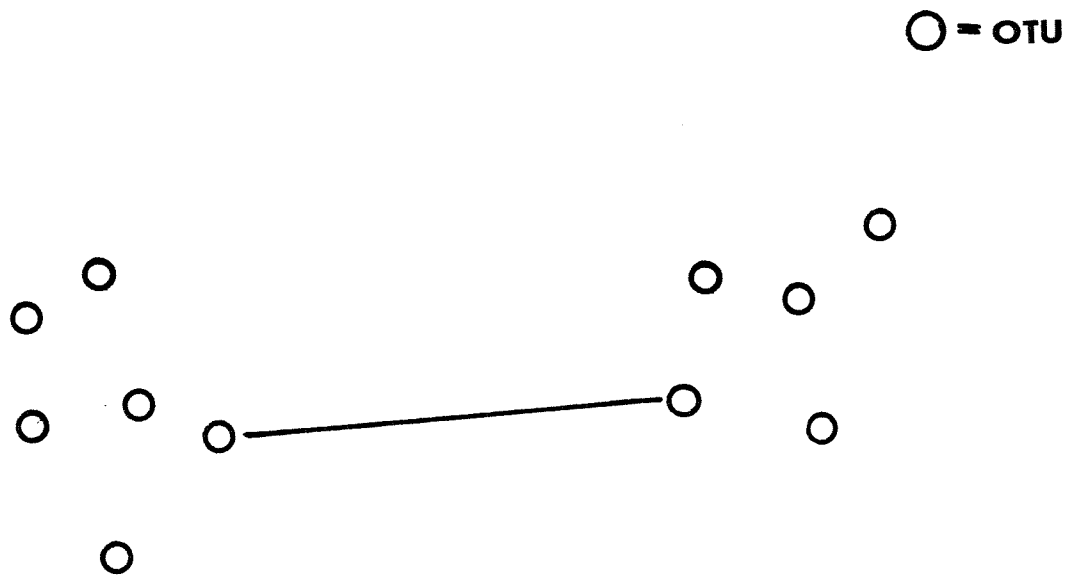
The mode of analysis used by LINKAGE can be divided into two main steps. Firstly the similarity matrix is produced from calculating the similarity coefficients between all the pairs of OTU's involved in the study. Secondly in decreasing similarity order, the objects at a specific similarity level are connected to form clusters. The clusters gradually become more inclusive as the similarity level drops; the procedure stops when all OTU's are contained in a single cluster. Measures of connectedness and isolation are calculated at each level for each cluster to aid the taxonomist interpret results. The similarity coefficient used by LINKAGE is the simple matching coefficient. The

similarity S(a,b) attributed to the pair of OTU's a and b will be the number of characters for which the same state has been attributed to OTU's a and b, divided by the total number of characters used in the comparison.

$$S(a,b) = \frac{\text{Number of characters in which states are shared by a and b}}{\text{Number of characters on which a and b have been compared}}$$

An extremely useful modification has been added to the measure of similarity, which enables the taxonomist to specify that two or more character states are partially similar instead of completely different. This allows the character states of logically ordered character to be given partial allegiance, e.g. if there are ten states to the character, keel shape, the character states are ordered but one can use this facility to infer that, say, character state 2 is partially allied to character state 5, but not with character state 7 which is quite different. The program will also compensate for missing data. As often occurs the taxonomist may not be able to score data from each OTU for every character. The 'overall similarity' of a pair of OTU's is calculated from those characters for which data is present, ignoring those characters for each OTU that are absent. The type, ordering (unordered or well ordered) and number of character states per character are specified individually for each character and so have the advantage of permitting mixtures of character types to be used.

When the similarity matrix has been calculated for all pairs of OTU's the matrix is analysed using single linkage (nearest neighbour) clustering with graph theory. Clustering follows the general rules given above, but with single linkage an OTU is admitted to a cluster once it has reached a level of similarity similar to that of any one of the OTU's already present in the cluster. Similarly any pair of clusters will coalesce if the similarity between any pair of OTU's (one representing each cluster) exceeds a threshold level of similarity. Thus fusions are based on single links between particular OTU's, see diagram below. (Note: other members of a cluster might be quite dissimilar to the OTU or cluster of OTU's with which they link).



With LINKAGE prior to actual clustering, the computer orders the similarity measures between pairs of OTU's into decreasing similarity order, so that OTU's pairs with a similarity level of 1.0 will be presented at the beginning of the print out. Following this the program instigates a cycle of cluster forming interations, with decreasing similarity so that successive OTU's are incorporated into clusters. Every time a cluster is modified, by the addition of a new OTU or cluster of OTU's, the measure of cluster isolation is printed called 'moat'. It states how long on the axis of decreasing similarity it will be before the cluster is further modified, eg:

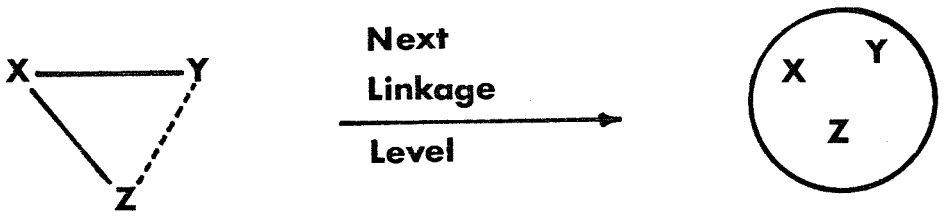
"MOAT = .07500 NEXT PAIR TO JOIN (506, 504) (507, 201)"

which means that the present cluster will be modified after a further decrease in similarity of .07500 and that the next pair to join the cluster will be OTU's 506 and 201, these will join 504 and 507

respectively.

Practically, the drawing of linkage diagrams or sub-graphs from LINKAGE output requires a brief introduction. The LINKAGE output details a sub-graph for each level of similarity at which a novel clustering occurs. At a given threshold level of similarity each pair of OTU's will cluster and this is demonstrated in the sub-graph by a line connecting the OTU's. In effect this connecting line could be of three types: a double line indicating a new clustering of either OTU's or clusters, a single line indicating a linkage established at a higher level of similarity, which demonstrates internal cluster structure, and a broken line indicating a new internal connection at that particular level of similarity.

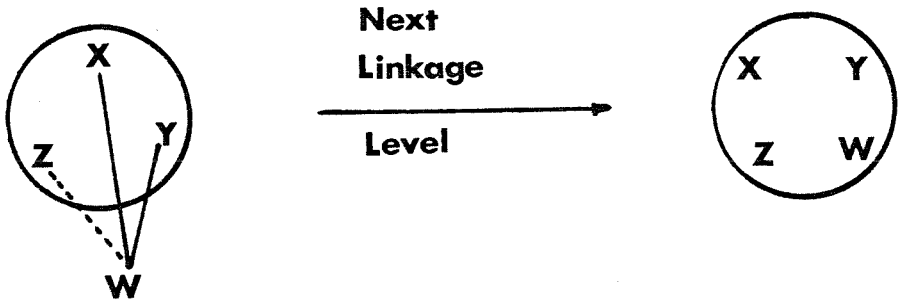
With large numbers of OTU's, especially at lower levels of similarity, the OTU's become complicatedly inter-connected. To simplify interpretation of the sub-graphs highly connected OTU's are encircled. The criterion for inclusion into a circle is that each OTU should have at least three connections with other members of the same cluster. As an example, suppose that three OTUs X, Y and Z are connected as shown below:



It can be seen that XY and XZ are established intra-cluster links and at that particular linkage level a new intra-cluster link is formed between YZ indicated by the broken line joining Y to Z. Then within the cluster

all three OTU's are joined so at the next linkage level the three linked OTU's will be drawn inside a circle as shown.

If then a fourth OTU, W, forms links with the encircled OTU's X, Y and Z, as shown below, then it will also be included in the circle as shown.



LINKAGE uses graph theory, which enables it to overcome the main criticism of the single linkage algorithm; that clusters consisting of loosely linked chains of outliers and intermediates cannot always be distinguished from clusters of highly interconnected OTU's. LINKAGE records all links between OTU's and these can be seen by drawing the individual subgraphs detailed in the output.

Several workers have concluded that single linkage cluster analysis in conjunction with graph theory provides the most satisfactory taxonomic arrangement of studied taxa (Prance, Rogers and White, 1969; Stearn, 1971). Bisby (1973) after undertaking a comparative study of multivariant data analysis using the legume genus *Crotalaria L* comments on this method:

> "It gives an excellent indication of the pattern of similarities within the groups formed, and an indication of the affinities of marginal and isolated species excluded from these groups. Some of these results have been sufficiently useful for improvements to be made to the classification of *Crotalaria*".

(b) CLUSTAN 1C

CLUSTAN is a suite of FORTRAN IV programs for the collective study and use of cluster analysis and other multivariate method written by David Wishart. Generation 1C was published in July, 1975, the original being published between 1966-70 as CLUSTAN 1A, CLUSTAN 1C is introduced in a manual written by Wishart (1975).

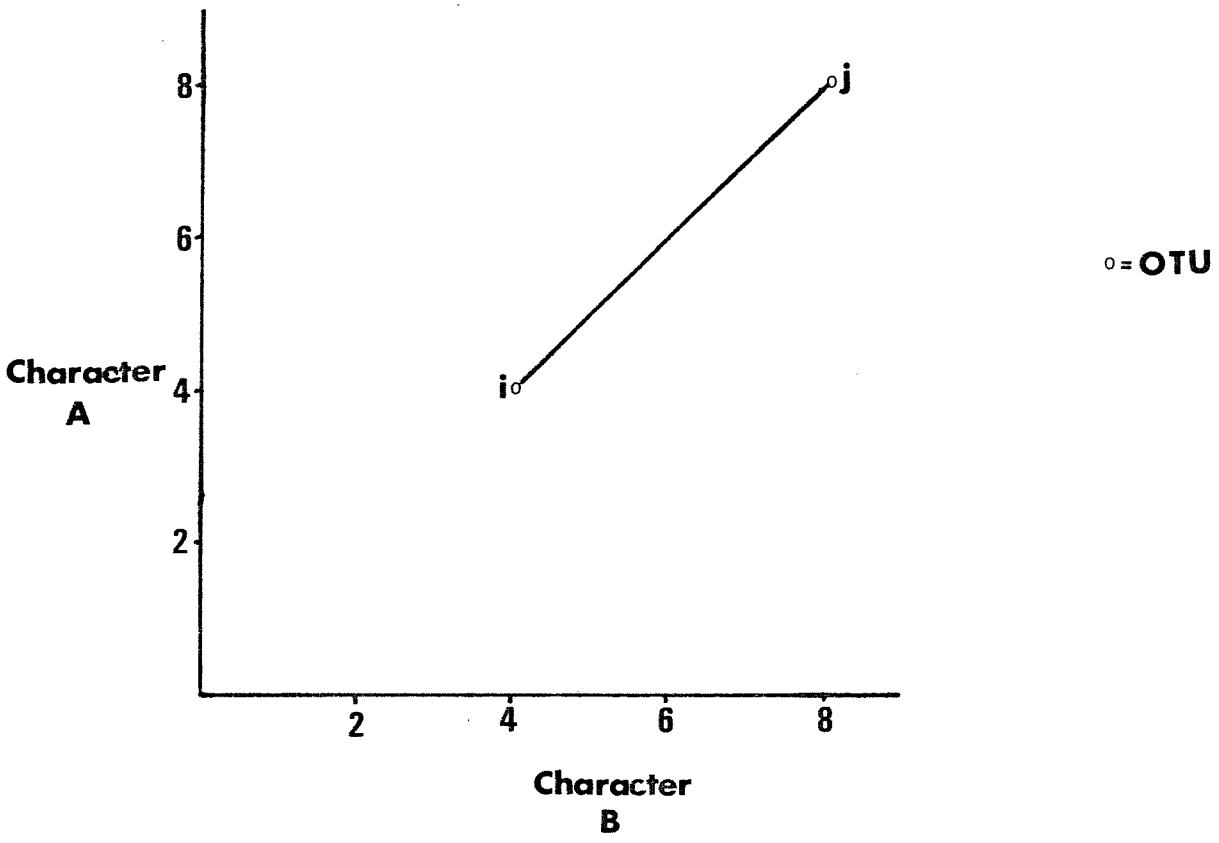
CLUSTAN has been designed as an integrated suite of programs of separate procedures within a single program, which are activated by the CLUSTAN driver. Wishart has attempted in developing CLUSTAN to simplify the program specifications so that routines are easy to use, while incorporating special features and non-standard options.

The CLUSTAN driver recognises keywood cards, which contain the name of subroutine procedures. Each keyword initiates one CLUSTAN step: thus a single CLUSTAN job can comprise any number of different steps, which provides CLUSTAN with a versatility very useful to taxonomists. The program will accept data in the form of continuous measurements, multi-state attributes or as binary data. The program is also able to accept a matrix of similarity or distance in a triangular table (t x t). Both these procedures were utilised in the present study. Details of the actual combinations of subroutines will be detailed in Chapter 5 with the results of the analysis.

CLUSTAN was used to carry out cluster analysis, principal component analysis and find the minimum spanning tree. the theoretical background and advantages of each of these techniques will now be discussed.

Following the keyword HIERARCHY a range of eight different cluster analysis programs can be selected. Following the use of single linkage with graph theory in the previous program, LINKAGE, it was decided to use two programs which undertake cluster analysis in a different manner. To this end, average linkage (unweighted pair-group) and Ward's method (error sum of squares) were selected. HIERARCHY assumes that procedure CORREL has been used to produce a similarity coefficient which is compatible with the cluster analysis method chosen. HIERARCHY may also be used after acceptance of a similarity matrix produced exterior to the program, though this limits the resultant choice of cluster analysis techniques available. The similarity coefficients selected were: simple matching coefficient (produced by LINKAGE) for the group average method, and distance coefficient for Wards' method.

The simple matching coefficient has been discussed under the program LINKAGE above. The distance coefficient is based on Euclidean distances, a simple two character example is shown below:



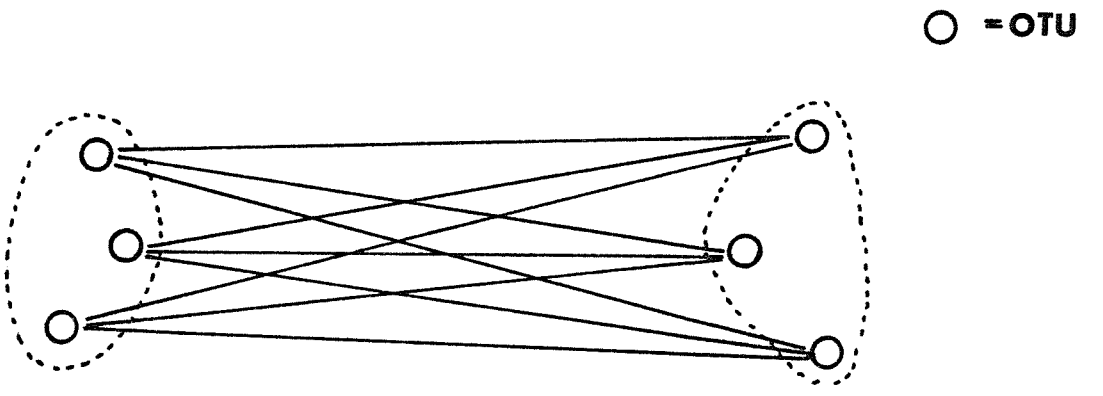
The line drawn between OTU i and j demonstrates the Euclidean distance. To calculate the Euclidean distance between OTUi and OTUj the following formula is applied:

$$dij = [(x_{ja} - X_{ia})^2 + (X_{jb} - X_{ib})^2]^{1/2}$$

This can be generalised for p characters per OTU thus:

$$dij = \left[\sum_{k=1}^{p} (X_{ik} - X_{jk})^2 \right]^{1/2}$$

Average linkage cluster analysis was chosen as a comparison with the single linkage with graph theory produced by LINKAGE. As the same similarity matrix was used for both, a direct comparison could be made of the two techniques and the validity of the criticisms of single linkage assessed. Average linkage cluster analysis was one of the earliest attempts at taking group structure into account when clusters are formed. Dunn and Everitt (1982) describe average linkage as the method that, "defines the proximity between two clusters as the average of the proximities between all pairs of OTU's that are made of one OTU from each group". This is demonstrated in the diagram below.



However, Wishart (1975) does comment that this method does, "tend to find spherical clusters", which is quite a criticism if spherical clusters are not naturally inherent in the data. It would appear to have the opposite problems of single linkage, on which Wishart comments, "will find 'straggling' clusters but often fails with large populations due to chaining".

The second cluster analysis method employed from the CLUSTAN suite was Ward's method, recommended by Wishart (1975) as "possibly the best of the HIERARCHY options". Ward (1963) proposes that at any stage of the analysis the loss of information which results from the grouping of individuals into clusters can be measured by the total sum of squared deviations of every point from the mean of the cluster to which it belongs. At each step in the analysis, union of every possible pair of clusters is considered and the two clusters whose fusion results in the minimum increase in the error sum of squares are combined (Everitt, 1974). Wishart (1975) states that, "this method finds minimum-variance spherical clusters" and Dunn and Everitt (1982) conclude that group average clustering and Ward's method are generally the best methods of clustering available, though Sneath and Sokal (1973) point out that Ward's methods of clustering on the minimum increase in the error sum of squares is questionable and "may correspond to unacceptable partitions". So it would appear that all three methods of cluster analysis used in this study have their proponents and their detractors, which should make the comparative interpretation of the results interesting in the context of comparing techniques of clustering.

The program CLUSTAN was also used to carry out ordination procedures; using the sub-routine SCATTER principal components were calculated. Before production of principal components the data matrix had to be standardized (unit variance zero mean) producing a matrix of n x n product-moment correlation coefficients between pairs of characters, and was computed using the formulae:

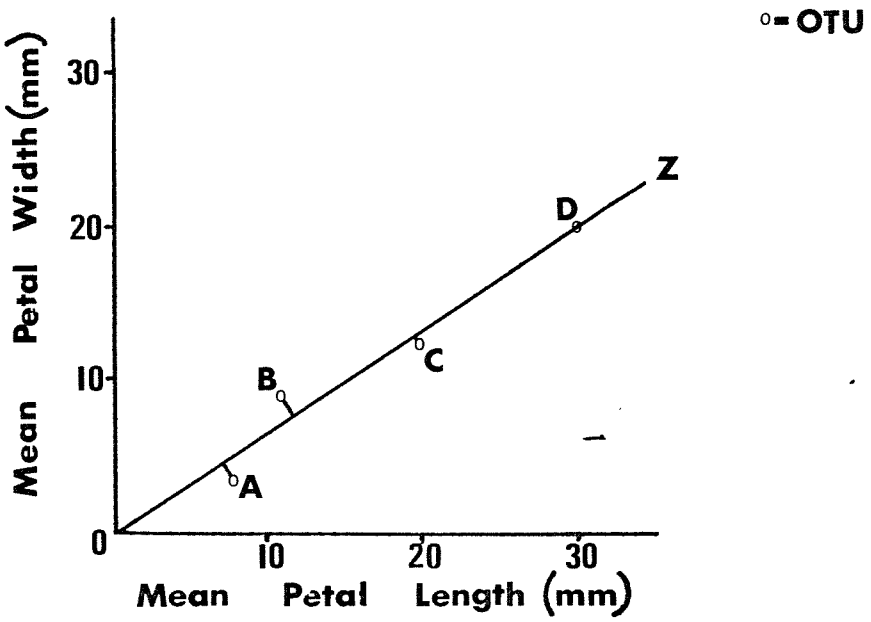
$$R = (1/t - 1)\, XX'$$

From this matrix R, the principal components, were computed involving the calculation of eigenvalues and eigenvectors. The importance of

these eigenvectors is that they describe the relationship between OTU's with economy, meaning that a large proportion of the dispersion engendered by the characters over the OTU's can be accounted for in few dimensions or principal components. The normalised vectors give the directions of a set of orthogonal axes which are the principal axes. The coordinates of these axes are linear combinations of the original variables and summarise the major dimensions of variation. An eigenvalue is equal to the variance along its corresponding axis, so that the principal axis corresponding to the largest eigenvalue is the dimension that accounts for the greatest amount of variance from the sample of OTU's. The second principal axis drawn at right angles to the first accounts for the second largest amount of variance and so on. Quite often as few as three principal axes will account for the majority of the variation (Sneath and Sokal, 1973). Dunn and Everitt (1982) provide a simple example to demonstrate the transformation involved in calculating principal components. Consider the hypothetical set of data consisting of pairs of measurements relating to the petal dimensions of four buttercups:

OTU	A	B	C	D
mean petal length (mm)	8	10	20	30
mean petal width (mm)	4	9	11	18

This data is plotted below.



To express the variation of these two characters on a single axis the line Z would be drawn which maximises the variance of the projections of the four points onto it. This will show the maximum discrimination of the four OTU's. This line is equivalent to the first principal axis. Projection of the four points onto this line gives the following first principal component scores for each OTU.

OTU	A	B	C	D
Score	9	13	23	35

The second principal axis drawn at right angles to the first would give second principal component scores of:

OTU	A	B	C	D
Score	1	-1.5	0.5	0

Dunn and Everitt (1982) note, "that the values of the first principal component are all positive and are clearly related to the size of the buttercup flowers. Those of the second component, however, can have either positive or negative value and may give information about the variation in the shape of the flowers".

Rohlf (1968) has pointed out that principal components analysis faithfully represents the distances between major groups or clusters of OTU's but it is unreliable for demonstrating the distances between near neighbours.

Dunn and Everitt (1982) point to two other problems associated with the use of principal components analysis. The first is the tendency to prdoduce principal components plots with a Gaussian distribution if non continuous data is used. Points tend to lie on the horseshoe-shaped curve rather than reflect the inherent structure of the data. This is especially noticable with binary data where the points tend to occupy the apex of a hypercube. Although Williamson (1978) has described a method to overcome these problems, the solution is not incorporated in CLUSTAN 1C. The present study involved very little binary

(presence-absence) data; proportionally continuous data predominates and so it is hoped that this malformation of data structure will not affect the results seriously. Dunn and Everitt go on to point out that in principal components plots the Euclidean distance between OTU points is an approximation of the Euclidean distance between OTU points in p-dimensional character space. This implies that if Euclidean distance is not considered the appropriate metric for analysis of the data, then using PCA would be inappropriate also. This second point is not considered to be a problem because the data gathered in the present study is compatible with the Euclidean distance metric.

The fourth problem associated with PCA is that it is scale sensitive, meaning that the most abundant character states will dominate the analysis, to the detriment of rarer character states. However this problem may be overcome by transforming the original data either by taking their square or cubed roots.

The sub-routine SCATTER which carries out the PCA can be used secondarily to produce a minimum spanning tree. Dunn and Everitt (1982) state that the usefulness of any ordination technique can be judged by how well it preserves the inherent structure of the data. Using a minimum spanning tree allows assessment of how well the original proximities of the data are preserved by two-dimensional mapping. Dunn and Everitt define it as follows:

"Suppose n points are given (possibly in many dimensions), the tree spanning these points, i.e. a spanning tree, is any set of straight-line segments joining pairs of points such that;
(a) no closed loops occur,
(b) Each point is visited by at least one line
(c) The tree is connected, that is, it has paths between any pair of points.

If a weight is assigned to each segment in the tree then its length is defined to be the sum of these weights. The minimum spanning tree of the n points is then defined as the spanning tree of minimum length."
They then continue by illustrating the use of a minimum spanning

tree in assessing the data distortion of ordination techniques, using the example described by Gower and Ross (1969). In this example canonical variate analysis is used to analyse skull measurements of white-toothed shrews from the Scilly and Channel Islands. Their two-dimensional representation of the results incorporates 89% of the variance. However distortion of the data by the ordination method can be clearly seen, e.g. in the two-dimensional representation shrews from Jersey and Sark are closely allied, but when the minimum spanning tree of the original distance matrix is superimposed onto the two-dimensional representation, it indicates that the Jersey and Sark races are closer to the Tresco race than to each other.

Wishart (1975) points out that a minimum spanning tree is analogoous to single linkage clustering, where the pair of nearest neighbours at each fusion step defines an edge of the graph. It has been used to partition a population with respect to its error sum of squares. For the above reasons the option of obtaining the first and second principal components with minimum spanning tree overlayed was chosen.

The sub-routine SCATTER also has the facility of plotting cluster cricles onto the scatter diagram of the principal component ansalysis, the cluster circle being an indicator of cluster variance. Wishart (1975) explains the use of cluster circles as follows:

> "Suppose that XM, YM are the means and VX, VY the variances of the X and Y distributions for the subset of individuals which belong to cluster L, then the circle for cluster L will be drawn with its centre located at (XM, YM) and radius proportional to the square root (VX +VY) . . . Note that if the radius is less than 0.1 inches then a small square is drawn surrounding (XM, YM) instead of a circle".

Cluster circles are drawn for the results of the following PCA plots to provide an extra aid in interpreting cluster conformity.

(c) DECORANA

DECORANA is a FORTRAN program for detrended correspondence analysis and reciprocal averaging, written by Hill (1979) and described by him in the associated manual. DECORANA was designed primarily by Hill for use by ecologists who have collected data on the occurrence of a set of species in a set of samples. It may, however, be used in taxonomic analysis if the species and samples are taken as characters and OTU's respectively. The fact that DECORANA was designed for ecological data may have some effect on the results obtained, because ecological and taxonomic data will not necessarily have similar distribution patterns, and this must be considered when interpreting the results.

Detrended correspondence analysis (DCA) is an improved refinement of correspondence analysis or reciprocal averaging (the latter two being synonyms), which are themselves refinements of principal component analysis. DCA differs from reciprocal averaging (RA) in two respects, explained in more detail below. One difference lies in the scaling of the axes, the other in the way in which the second and subsequent axes are calculated.

Hill (1979) points out that the most conspicuous fault of RA is the tendency of the second (and possibly higher axes) to be strongly related to the first axis, referred to as the arch (Gauch, Whittaker and Wentworh, 1977) or horseshoe effect (Kendall, 1971), also noted in the previous section to occur in PCA. With RA the second and subsequent axes are constrained to be uncorrelated with the first, but this does not guarantee their independence. DCA circumvents this problem and prevent systematic relationships betweeen the first and later axes.

The algebraic explanation of how this is achieved is detailed in Hill (1979), so will not be reiterated here as it required a, "firm grasp of linear algebra" (Hill, 1979). He does however explain in simple steps how the arch effect is circumvented.

1. Assign arbitary character scores, between 0 and 100, to each character in the OTU x character matrix.

2. Construct OTU scores so that the score of each OTU is the average of the scores of the characters which have been scored for it.

3. Detrend the OTU scores so that they have no systematic relationship to the first axis. This is done by dividing the first axis into segments. Within each segment of the first axis the OTU scores are readjusted to give a zero mean, resulting in a detrended set of scores. To avoid edge effects, three separate detrendings are made, with different starting positions for the segments. The final OTU scores are then derived by averaging those obtained by each separate detrending.

4. Using the detrended OTU scores, calculate new character scores using the normal RA method and then return to stage 2. After several iterations the OTU and character scores fail to change with successive iterations. Then finally the OTU scores are calculated by taking the mean character scores but not detrending.

Following this procedure the character and OTU scores are given positions on the first axis of the ordination. The second axis is produced in a similar fashion after subtraction of a multiple of the first axis. The third axis follows the same route of formulation but with respect to the second axis, and so on.

Hill (1979) points out a second inherent fault in RA, that the scaling of the axes does not have any clearly defined meaning. Typically the ends of the axes are contracted so that real point separation is contracted, compared to those points in the centre. DECORANA attempts to surmount this problem by producing a unit standard deviation for the characters of each OTU. The methodology is complex, but basically involves rescaling the characters (not the OTU's) by expanding the local scaling in proportion to the reciprocal of the local mean-square deviation.

DCA has the advantage over PCA in not being unduly influenced by the magnitude of the character states and by producing character and species ordinations of the results (Hill, 1979). Further, Gauch, Whittaker and

Singer (1981) in a compartative study of non metric ordinations with RA and DCA conclude that DCA has the following advantages over non metric ordination:

1. The quality of the results is higher.

2. Computation time is much less and so cheaper, because of the exceptionally fast algorithm.

3. Non metric ordination does not allow species and character analysis to run concurrently whereas DCA does this.

4. The subjectivity of choice involved in selecting distance measures and number of dimensions for non metric ordination is not required for DCA.

Their final conclusion is that DCA produces less distorted results than any other metric or non metric method of ordination available at present. However both RA and DCA have opposite problems to PCA in that undue weighting is given to rarer character states in the analysis, which distorts the data just as much as undue weighting of common character states in PCA.

So to conclude this section it should be noted that each method of phenetic analysis has its advantages and disadvantages, it proponents and detractors. This is one reason why the combination of the methods of analysis was chosen internally to verify the results of the phenetic analysis. The different methods each providing an impression of the actual, 'natural', relationships of the plants, which could then be used in constructing more natural composite formulation of the plants' relationships.

3.5 Choice of phylogenetic analysis

3.5.1 Theoretical foundation

Publication of the Darwinian theory of evolution in 1859 and the theory's general acceptance necessitated a fundamental change in taxonomic thinking. Darwin accentuated the dimension of time in relation to evolutionary development, phylogenetic history linking all organisms as they had diverged from common ancestors. Sneath and Sokal (1973) comment that post-Darwin, "a taxon was interpreted as a monophyletic array of related forms".

Darwin by implication posed an important problem for taxonomists by introducing the concept of organism possessing a phylogenetic history, the question whether or not phylogenetic implications should be reflected in classifications. Stace (1980) suggests that, "There is, in fact, a general belief that a perfect phenetic system would also be a perfect phylogenetic one". This is a logically satisfying argument, but taxonomists do not work with perfect systems. The argument assumes that the rate of evolution (in this context phenetic morphological divergence) for all organisms is comparably constant. This is not necessarily true, as examplified in the debate over "the salmon, the lungfish and the cow" (see Gardiner et al, 1979 and Halstead et al, 1979). Gardiner uses cladistic formulations to relate the cow more closely to the lungfish than the lungfish to the salmon. Halstead, on the other hand, argues that the cow, like all tetrapod, arose from the salmon (crossoptegrygian) group of fishes, based on the grounds of a large number of shared characteristics. More practically the problem arises, if the lungfish is accepted as the primitive ancestor of the cow, should the lungfish be placed in the same class as the cow or reflect gross resemblances by allying it with the salmon? The answer must depend on the purpose of the classification, but if a classification is desired which is of general use then the second option should obviously be taken (general is used in the sense of not being required to reflect ancestory).

Abbott, Bisby and Rogers (1985) list three essential areas that require definition if the phylogenetic pattern is to be fully

understood: (i) the branching sequence, known as the cladistic tree or cladogram, (ii) the rates of evolution in phyletic (evolutionary) lines and (iii) the actual age or time of divergence. The latter two points remain difficult to clarify, if in fact clarification is possible. However, techniques have developed to deduce possible branching sequences, though problems of convergence make deducation problematic. Abbott et al divides convergence into three components: parallelism, (the same change or mutation occurring at least twice, independently); reversal (a change or mutation subsequently being reversed); and mistaken homology (apparent resemblance occurring in non-homologous characters). The first two of these, collectively known as homoplasy, produce the major problems and lead to reticulate patterns of resemblance which are difficult to define using either phenetic and or phylogenetic techniques.

Abbott et al then divides phylogenetic analysis techniques into four "schools", traditional, parsimonic, cladistic and incompatability studies. The third method using techniques derived from Hennig (1966) is applied in this study and so will be discussed specifically in the following section of this thesis, but the other three method will be discussed briefly as follows.

Traditional methods of phylogenetic analysis are ill-defined and subjective, they involve a taxonomist 'sensing' the course of evolution in a particular group of plants he or she is interested in. The results of the taxonomists meditations are summarised usually in balloon diagrams in the form of a directed tree. This method fails to appreciate two inherent logical problems, firstly that using Traditional methods of phylogenetic analysis major groups within taxa are usually arranged on a phenetic basis and secondly that modern day taxa are shown as having originated from other modern day taxa (Abbott et al, 1985). Both these problems are crucial, but the accuracy of intuitive taxonomists must not be under estimated as was demonstrated by the introduction of objective numerical methods to phenetic analysis, where traditionally produced classifications were largely found to agree with those numerically produced.

The second phylogenetic method outlined in Abbott et al (1985) is

the parsimonic method, developed by phenticists to elucidate the evolutionary trees (cladograms) using the shortest possible evolutionary route. This method is based on the application of Ockham's razor (the simplest solution to the problem being the least unlikely) in attempting to define the tree of minimum length. To apply numerical algorithms to the parsimony method is difficult because of the sheer quantity of possible minimum trees. Cavalli-Sforza and Edwards (1967) have calculated the number of possible directed trees using 10 items at 34,459,425. The number of possible undirected trees is smaller, but still very large, 2,027,025. Several workers have developed methods to overcome the size of the problem, but each involves subjective assumptions which partially invalidate the results. (see Jensen, 1981 for worked examples and discussion).

The problem has not been simplified by the demonstration by Estabrook (1968) theoretically, and the practical results of other workers, showing there may be a large number of equally short, shortest directed trees for a given data set. Abbott et al (1985) criticises parsimony because of the often required subjective stipulation of character polarity and statement of ancestral form. They also comments that parsimonic methods are, "doomed by the assumption of parsimony". Evolution proceeds in an unparsimonioius manner: would not parsimony require functional cacti to have evolved from the Euphorbiaceae? (Abbott et al, 1985).

The fourth school of phylogenetic reconstructionists listed by Bisby (Abbott et al, 1985) are those who expound incompatability analysis. This method depends on the logical detection of homoplasies (parallelisms and reversals). Incompatability analysis can be performed manually or by computer; a good introduction to manual methods is presented by Meacham (1981). He comments that for a reasonably small data set the necessary calculations can easily be performed 'by hand', and demonstrates this by showing the working of two examples. Abbott et al (1985) provide a concise proof of the method of incompatability studies as follows: If two binary characters, a with states a_1 and a_2 and b with states b_1 and b_2, it follows that four character state combinations are possible; a_1b_1, a_1b_2, a_2b_1 and a_2b_2. There are only two evolutionary changes that can occur without homoplasy occuring, the

mutation of a_1 to a_2 and b_1 to b_2. If in nature all four character state combinations are found then three evolutionary steps are required to join them in an evolutionary tree. As only two evolutionary steps can occur, the third step must involve a repeat or a reversal of one of the other two. Thus in this case the logical conclusion is that the result is incompatible with there being no homoplasy. In contrast pairs of characters which do not exhibit homoplasy are referred to as being compatable. Some progress has been made in numerical phylogenetics, Estabrook and Meacham (1979) have developed an algorithm which will not only detect compatible character pairs but do so on large sets of characters.

Abbott et al (1985) in their conclusion on phylogenetic methods states, "whilst great progress is being made on trying to solve the problems of phylogenetic reconstruction, none as yet can provide an authoritative solution. Each is flawed by the biological assumptions needed". They continue, however, by commenting, "Hennigian analysis is of particular interest because it alone derives all present day forms from ancestors, and becuase even if one disregards its utility in tracing evolution, it can be used to produce a classification with interesting properties".

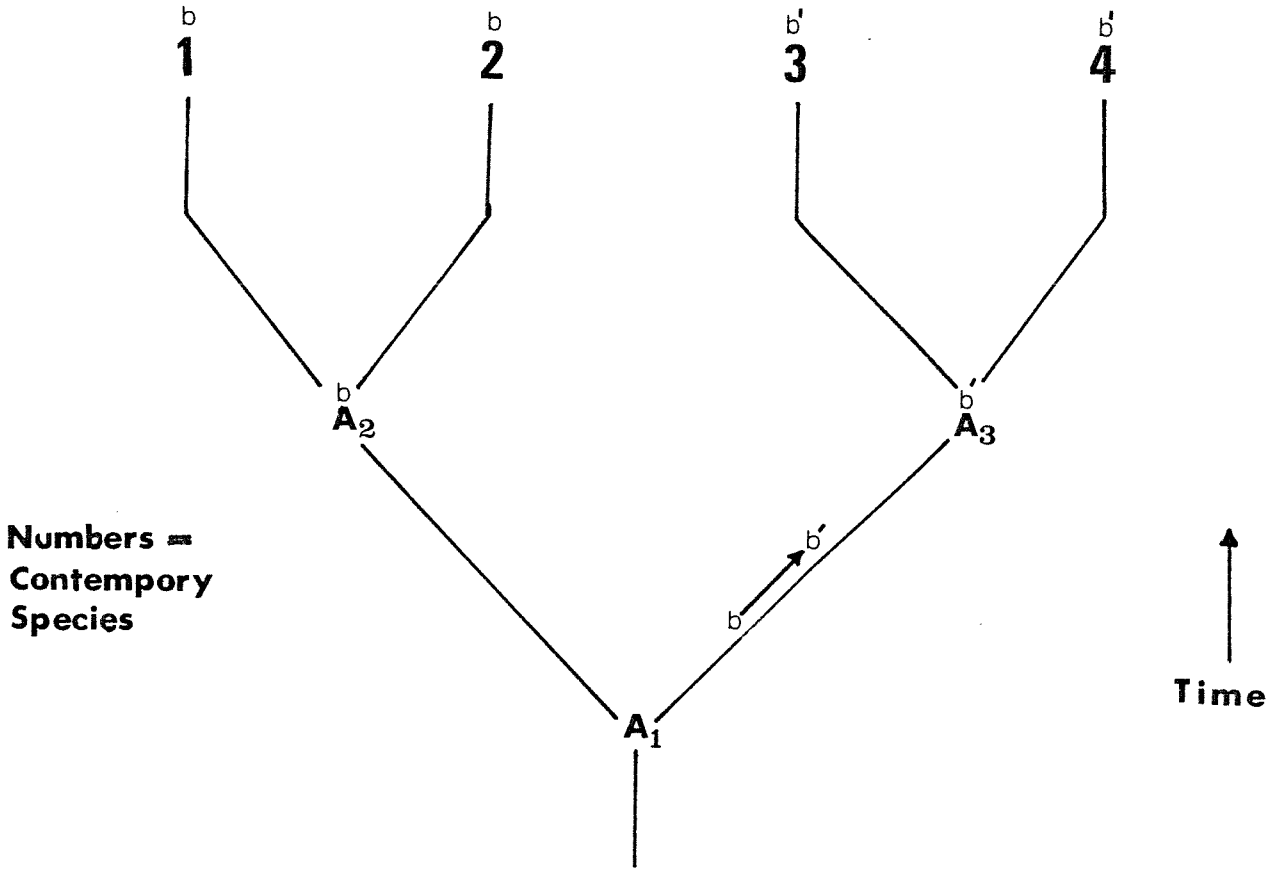
3.5.2 Reasoned choice of phylogenetic analysis techniques

For the present study Hennigian or Cladistic techniques of phylogenetic analysis were chosen partly for the above reasons detailed by Abbott et al, they allow derivation of present day forms from ancestors, and partly because they can be used to create a classification, although artificial (being based on a few characters = monothetic), that may produce interesting taxa juxtapositions which could lead to further, deeper considerations of relationships. The method also has the advantage of being relatively simple to apply and does not require computer assistance, as is shown below.

Hennig published his, 'Grundzuge einer Theorie der phylogenetischen Systematik' in 1950, but it was not until its translation into English (Hennig, 1966) that his theories raised interest and excitement for Anglo-American taxonomists. His approach to the problem of phylogenetic

association was not entirely novel, but rather involved an explicit formulation of traditional methods detailed in the previous section.

Cladistic analysis can be effectively divided into two stages: selection of apomorphic characters and ordering of organisms in a nested, evolutionary sequence or tree (cladogram) to demonstrate the logical expression of their synapomorphies. Before discussing these two stages, as cladistics uses a specialised language, some terms must be defined and this may be easily demonstrated in the diagram below.



The ancestral population A_1 splits forming two daughter species A_2 and A_3, a process known as *cladogenesis*. At least one of the daughter species must undergo a character mutation, so this daughter now possesses a unique derived character. In the diagram this characters is b and it mutates to b' with the polarity B→B'. Character state B' is referred to as *apomorphous* (derived) relative to B, and state B is referred to as *plesiomorphous* (primitive) relative to B'. Species 3 and

Figure 3.1: Demonstration of the concepts of monophyletic, polyphyletic and paraphyletic groups. (Taken from Hennig, 1966)

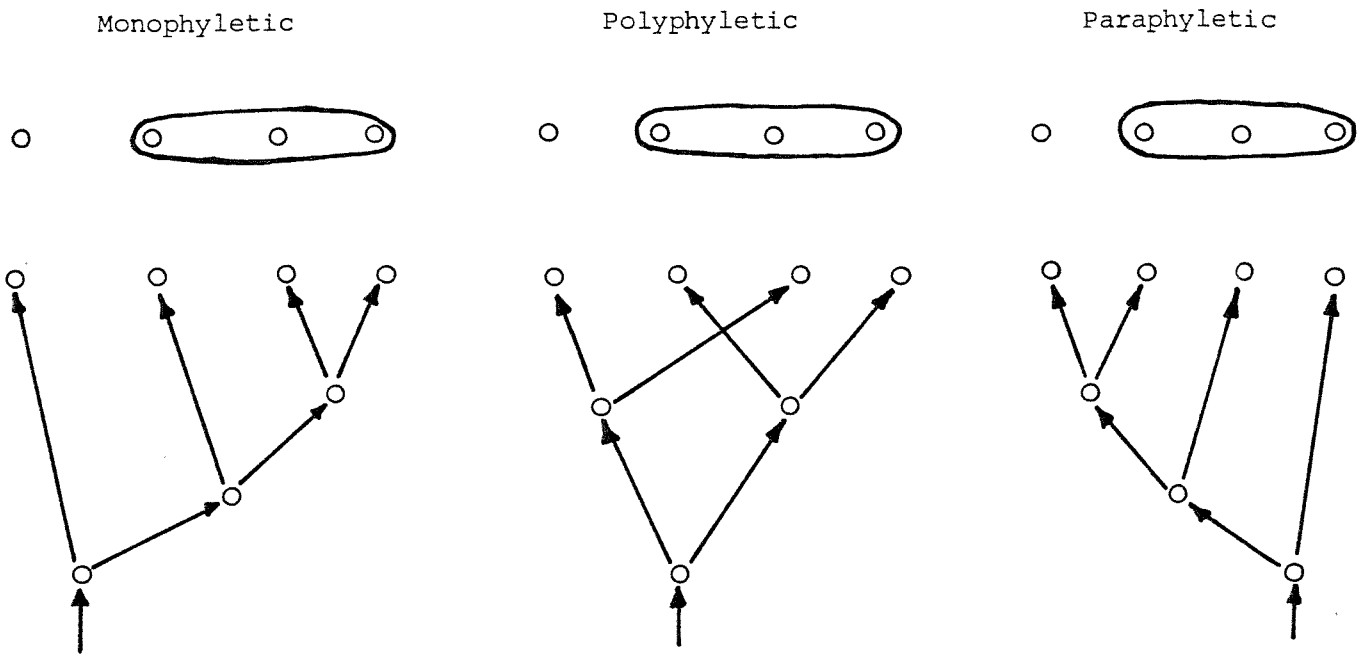
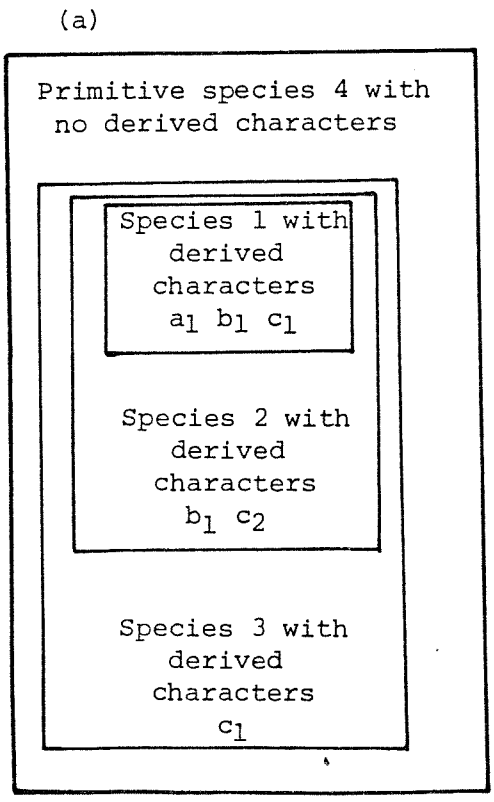
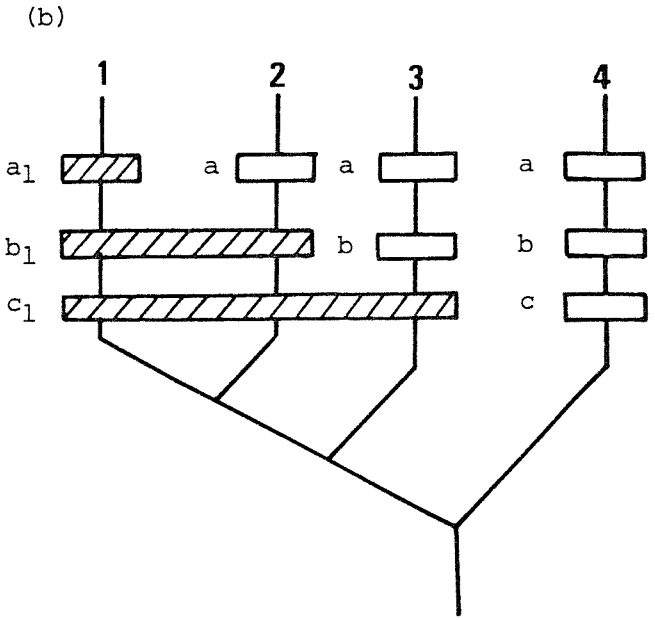


Figure 3.2: A hyothetical nested sequence of synapomorphies displayed in the form of a (a) venn diagram and a (b) cladogram





4 both possess the same derived state B' as a result of their common ancestry: shared derived character states such as B' are referred to as *synapomorphous*. Species 1 and 2 share the original character state (plesiomorphous): shared primitive character states such as B are referred to as *symplesiomorphous*. If in the diagram B' had been inherited by only one species, as it is in the ancestral species A_3 then the character state would be referred to as being *autoapomorpohic*. Species1-4 are said to be *monophyletic*, meaning that relative to other taxa they share a unique common ancestor, in this case A_1. If only species 2-4 were included then they would be referred to as *paraphyletic*. These species again share an unique common ancestor but not all the ancestor's (A_1) descendants are included in the group, species 1 is omitted. Furthermore, if one group of species due to convergence has more than one ancestor, such as the oroboid species of *Lathyrus* and *Vicia* (see Cresswell, 1983) then the group of species is referred to as *polyphyletic*, (see Figure 3.1). Finally a classification based on the results of a phylogenetic reconstruction is referred to as *holophylectic*.

To return to stage 1 of the description of cladistic analysis, selection of apomorphic characters. Characters are chosen which the taxonomist intuitively believes have mutated from one state to another and that the latter state is rare (meaning absent outside the group of study plants). Apomorphic characters must be homologous and demonstrate as clearly as possible a sense of evolutionary - directed change, so that the characters' polarity can be determined.

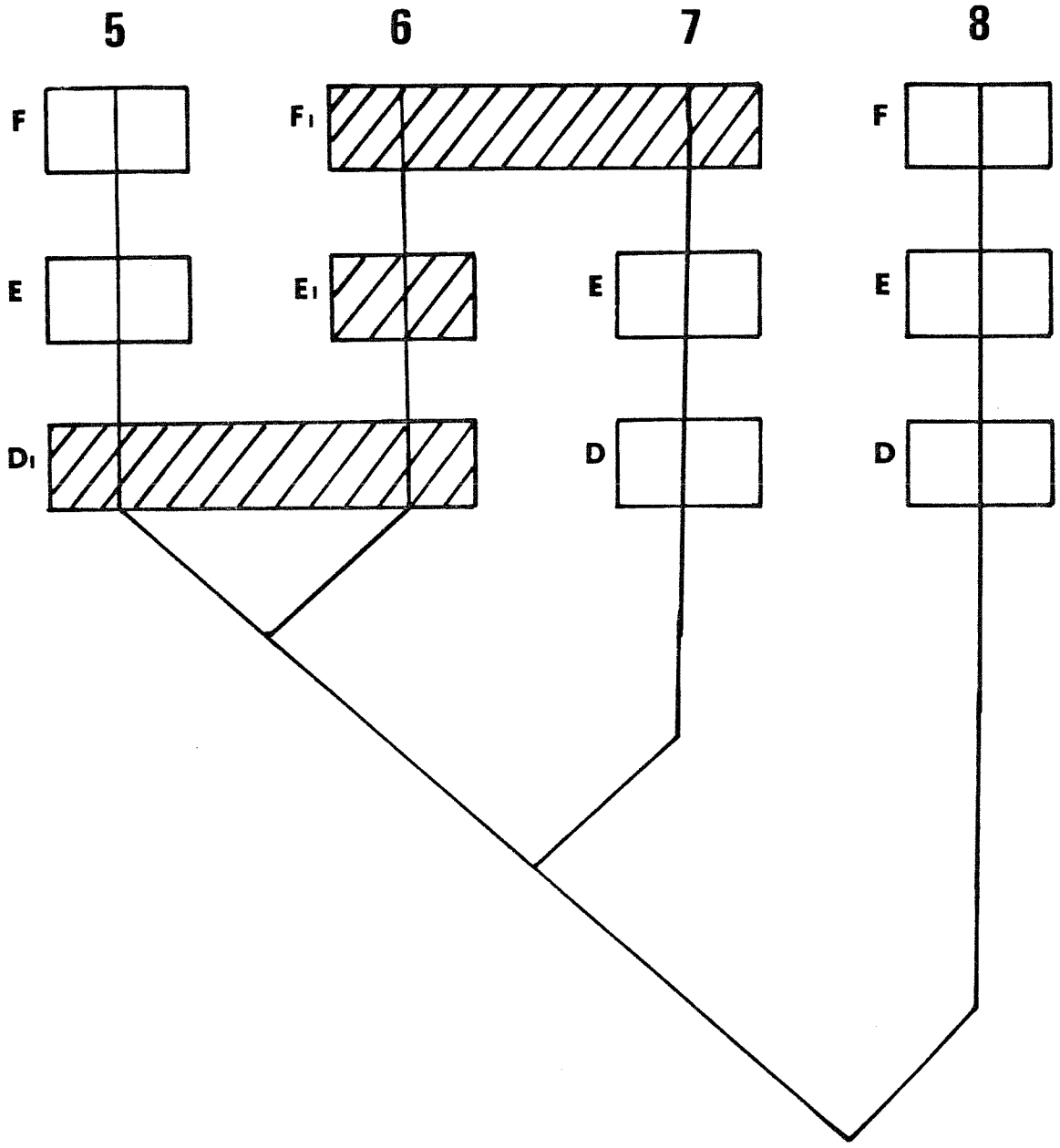
The second stage of cladistic analysis is to try to form a logically nested sequence (fitting one within another without clashing) of synamorphies, so that the taxa which has undergone the largest number of cladogenic bifurcations contains the largest number of synampomorphic characters. The nested sequence may be demonstrated visually in the form of a Venne diagram or cladogram, as shown in Figure 3.2. Problems may arise with implementing the second stage; it may be impossible to work out the nested sequence of synopomorphies. These synapomorphies may appear to conflict as shown in Figure 3.3, show parallelism and/or reversal. In Figure 3.3 character state F_1 appears to have arisen twice both in hypothetical species 6 and 7, demonstrating parellilism, while species 5 appears to have undergone reversal in the d_1 reverted to d.

One means of overcoming these problems of homoplasy is to apply Ockham's razor, as used in the parsimony method and choose the hypothesis which includes the least number of contradictions (parallilisms or reversals). Another solution is to re-examine the grounds on which the conflicting characters were defined and thus chosen as apomorphic. Perhaps character state a_1 is not homologous with a or the polarity is incorrect. Either solution may, to a pheneticist, appear unacceptably subjective, but as Patterson (1980) points out in answer to such criticism,

> "cladistics is not necessarily about evolution-speciation, ancestry and such things. It is about a simpler and more basic matter, the pattern in natural-groups, hierarchies or nested sets of groups and characters of groups."

It is in this spirit that the method of cladistic analysis was chosen and applied in this study.

Figure 3.3: A demonstration of conflicting synapomorphies in a cladogram of hypothetical species



CHAPTER FOUR

PHENETIC CHARACTER SET SELECTION

4.1 Introduction

The study's inclusion of such a large number of taxa, and the fact that these taxa are endemic to the tropics and sub-tropics, necessitated the restriction of the character set to morphological characters scored from almost wholly herbarium material. The reason for this restriction being the lack of seed availability for the taxa under investigation and the limited facilities av ailable for cul tivating plants in a tropical environment where seed was available. However both as regards time and availability, the morphological characters chosen were readily scored from herbarium material, though this restriction to dried plants had certain disadvantages.

The restriction of the study to the use of mostly herbarium material limited the choice of characters to those that could be easily scored from dried specimens. Thus some characters used by Marechal et al (1978a) in discriminating the *Phaseolus-Vigna* complex, which would probably have proved useful in the present study, could not be tested practically. These characters relate to attributes which the process of preservation makes unscoreable, e.g. presence of stipal gland secretions, presence of extra-floral nectaries, number and shape of these nectaries, flower petal colour, etc. (Note: Marechal et al used both preserved and live material in their study).

Within the limitations stipulated above, characters were chosen which followed the broad principles detailed in Section 3.3 of this thesis. The broadest range of morphological characters that could be scored from each specimen were selected. This large phenetic character set was experimented with, using *Psophocarpus* and its two closely allied genera *Otoptera* and *Dysolobium* in Phase I of the project (see Project Plan section 1.4 of this thesis). As a result of this experimentation, the gross character set was formulated. This was then used to score each OTU in the study and from the subsequent data, character subsets

were selected for particular analysis of individual taxonomic groups.

The gross character set is detailed in the following section, 4.2. The third section of this chapter explains the reasons behind the character subset selection for each set of taxa and the particular method of phenetic analysis. Discussion of the choice of phylogenetic (cladistic) characters will be detailed in Chapter 6 as their selection is so intimately bound to the process of cladistic analysis.

4.2 Gross character set

The gross character set which contains the characters scored for all OTU's in the present study is provided in Table 4.1; details of character number, character description, character state coding and character state descriptions are provided. The code of ϕ was taken to represent missing data for the cluster analysis using LINKAGE, ϕ was not used in the other methods of analysis as they could not compensate for missing data.

The gross character set includes 315 characters of which 241 were used in the final analysis. The 74 characters excluded from the analysis were not used either because they did not a posteriori conform to the definition of 'good' characters given in Section 3.3, or were not scored consistently throughout the study. These characters have however been included in the gross character set as they may prove useful to other workers in subsequent investigations.

Table 4.1: Gross Character Set

VEGETATIVE

No	Character description	Char.State No	Character State Description
1	Growth habit	1	Erect
		2	Ascending
		3	Climbing
		4	Scrambling
		5	Procumbent
2	Stipule length (A)		mm
3	Stipule spur length (B)		mm
4	Stipule width		mm
5	Stipule length÷spur length (A÷B)		
6	Stipule apex shape	1	Obtuse, see Figure 4.1
		2	Rounded
		3	Acute
		4	Mucronate
		5	Accuminate
7	Stipule base shape	1-7	See Figure 4.2
8	Number of stipule veins		
9	Stipule vein prominence	1	Prominant
		2	Not prominant
10	Stipule spur presence	1	Present
		2	Absent
11	Stipule reflexed	1	Reflexed
		2	Not reflexed
12	Stipule abaxial hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous

Figure 4.1: Apex shapes

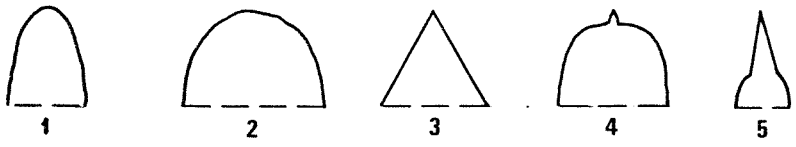


Figure 4.2: Stipule base shapes (Adapted from Maréchal et al, 1978)

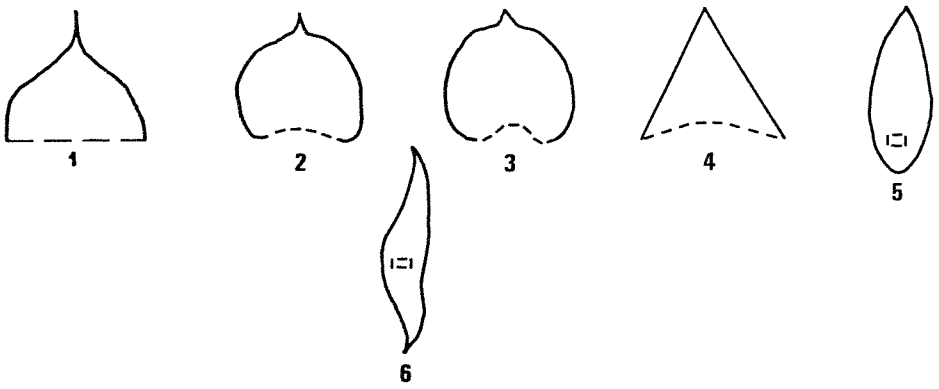
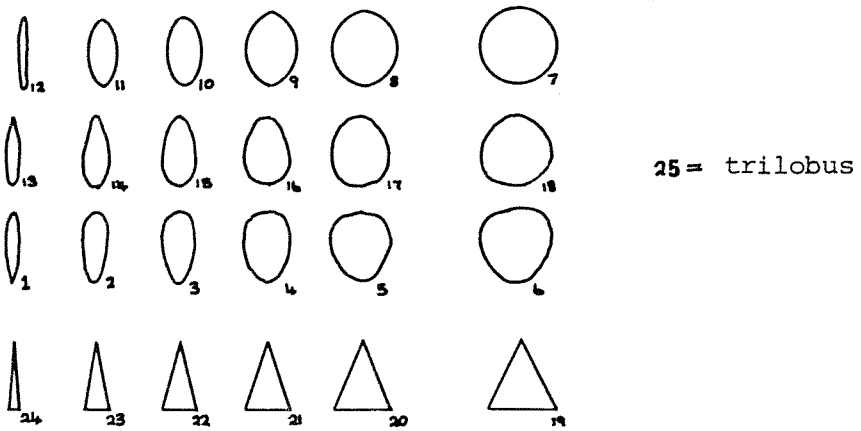


Figure 4.3: Shapes (Adapted from Systematics Association Committee for descriptive Biological Terminology in Taxon, 11; 1962)



13	Stipule abaxial hair length	1	Short
		2	Medium
		3	Long
		4	Very long
14	Stipule abaxial hair elevation	1	Adpressed
		2	±adpressed
		3	Not adpressed
15	Stipule abaxial hair colour	1	Clear
		2	Brown
16	Stipule hair apex shape	1	Hooked (see Baudet & Marechal, 1976)
		2	Straight
17	Stipule adaxial hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous
18	Stipule adaxial hair length	1	Short
		2	Medium
		3	Long
		4	Very long
19	Stipel length (lateral)		mm
20	Stipel width		mm
21	Stipel curvature	1	Straight
		2	Curved
22	Stipel shape	1-25	See Figure 4.3.
23	Stipel apex	1	Obtuse (see Figure 4.1)
		2	Rounded
		3	Acute
		4	Mucronate
		5	Accuminate
24	Stipel vein number		
25	Stipel lateral to terminal length	1	Lateral longer
		2	Equal length
		3	Terminal longer

26	Stipel adaxial hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous
27	Stipel adaxial hair length	1	Short
		2	Medium
		3	Long
		4	Very long
28	Stipel adaxial hair elevation	1	Adpressed
		2	±adpressed
		3	Not adpressed
29	Stipel adaxial hair colour	1	Clear
		2	Brown
30	Stipel hair apex shape	1	Hooked
		2	Straight
31	Stipel abaxial hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous
32	Stipel abaxial hair length	1	Short
		2	Medium
		3	Long
		4	Very long
33	Stipel abaxial hair elevation	1	Adpressed
		2	±adpressed
		3	Not adpressed
34	Ratio of stipule to stipel length		
35	Petiole length		mm
36	Petiole upper groove prominence	1	Absent
		2	Slight
		3	Prominent
37	Petiole ridging	1	Absent
		2	Slight
		3	Moderate
		4	Strong

38	Petiole hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous
39	Petiole hair length	1	Short
		2	Mediuim
		3	Long
		4	Very long
40	Petiole hair elevation	1	Adpressed
		2	±adpressed
		3	Not adpressed
41	Petiole hair colour	1	Clear
		2	Brown
42	Petiolule length		mm
43	Petiolule upper groove prominence	1	Absent
		2	Slight
		3	Prominent
44	Petiolule ridging	1	Absent
		2	Slight
		3	Moderate
		4	Strong
45	Petiolule hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous
46	Petiolule hair length	1	Short
		2	Mediuim
		3	Long
		4	Very long
47	Petiolule hair elevation	1	Adpressed
		2	±adpressed
		3	Not adpressed
48	Petiolule hair colour	1	Clear
		2	Brown
49	Ratio of petiole to Petiolule length		

50	Basal pulvinus length		mm
51	Lateral pulvini length		mm
52	Terminal pulvinus length		mm
53	Basal pulvinus hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous
54	Basal pulvinus hair length	1	Short
		2	Medium
		3	Long
		4	Very long
55	Basal pulvinus hair apex	1	Hooked
		2	Straight
56	Basal pulvinus hair position	1	Absent
		2	Adaxial surface only
		3	Adaxial and adaxial surface
57	Lateral pulvini hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous
58	Lateral pulvini hair length	1	Short
		2	Medium
		3	Long
		4	Very long
59	Lateral pulvini hair position	1	Absent
		2	Adaxial surface only
		3	Abaxial and adaxial surface
60	Terminal pulvinus hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous
61	Terminal pulvinus hair length	1	Short
		2	Medium
		3	Long
		4	Very long

62	Terminal pulvinus hair position	1	Absent
		2	Adaxial surface only
		3	Abaxial and adaxial surface
63	Ratio of basal pulvinus length to lateral length		
64	Number of leaflets	1	One
		2	Three
65	Terminal leaflet length		mm
66	Terminal leaflet width		mm
67	Lateral leaflet length		mm
68	Lateral leaflet width		mm
69	Ratio of terminal leaflet length to petiolule length		
70	Ratio of terminal leaflet length to width		
71	Colour of leaflets on drying	1	Green
		2	Intermediate
		3	Blackened
72	Leaflet lobing	1	Absent
		2	Seen on some leaflets
		3	Seen on all leaflets
73	Number of secondary leaflets veins		
74	Pattern of leaflet veination	1	Reticulate
		2	Parellel
75	Extension of secondary veins in leaflet	1	Reach leaflet margin
		2	Do not reach leaflet margin
76	Prominence of veins on abaxial surface of terminal leaflet	1	Not prominent
		2	Intermediate
		3	Prominent
77	Shape of terminal leaflet		See Figure 4.3.
78	Lateral leaflet symmetry	1	Symmetric
		2	Asymmetric
79	Terminal leaflet apex shape	1	Obtuse (see Figure 4.1)
		2	Rounded
		3	Acute
		4	Mucronate
		5	Accuminate

80	Terminal leaflet base shape	1	Angustatus (see Figure 4.4)
		2	Angustatus to truncate
		3	Truncate
		4	Cordate
81	Terminal leaflet apex form	1	Grooved
		2	Ungrooved
82	Terminal leaflet broadest point	1	Broadest at base
		2	Broadest in middle (see Figure 4.5)
		3	Broadest at apex
		4	Parallel
83	Leaflet position relative to ground	1	Adpressed to ground
		2	Not adpressed to ground
84	Leaflet adaxial surface hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous
85	Leaflet adaxial surface hair length	1	Short
		2	Medium
		3	Long
		4	Very long
86	Leaflet adaxial surface hair elevation	1	Adpressed
		2	±adpressed
		3	Not adpressed
87	Leaflet abaxial surface hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous
88	Leaflet abaxial surface hair length	1	Short
		2	Medium
		3	Long
		4	Very long
89	Leaflet abaxial surface hair elevation	1	Adpressed
		2	±adpressed
		3	Not adpressed

Figure 4.4: Leaflet base shape

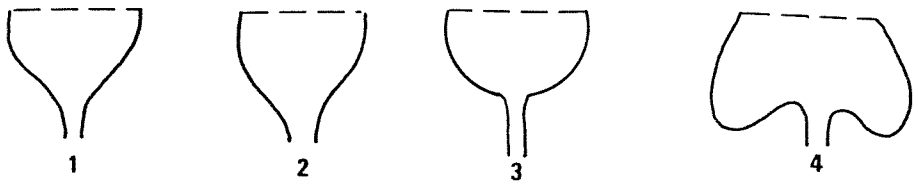


Figure 4.5: Leaflet broadest section (After Maréchal et al, 1978)

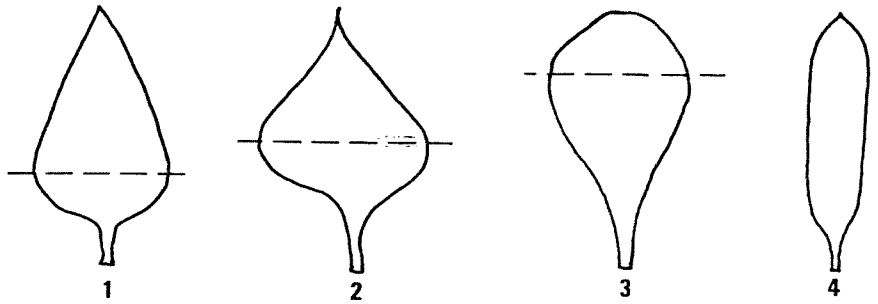


Figure 4.6: Inflorescence lowest node measurement

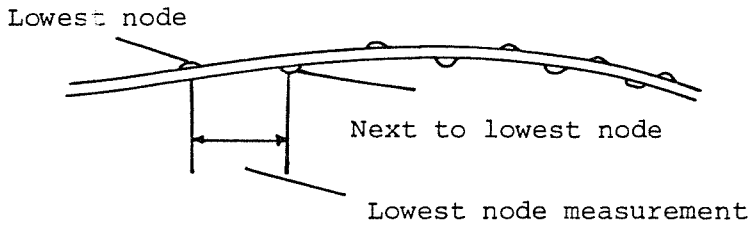
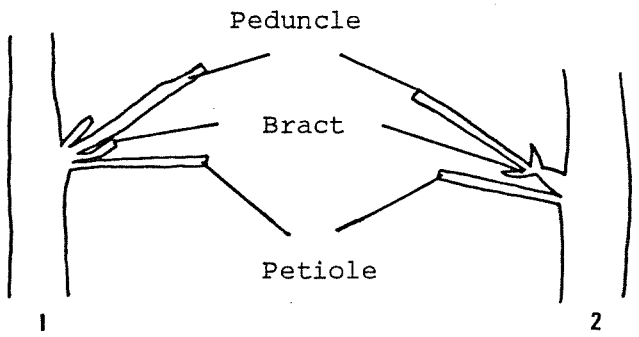


Figure 4.7: Bract position (After Maréchal et al, 1978)



90	Leaflet abaxial surface hair colour	1	Clear
		2	Brown
91	Leaflet abaxial surface hair apex	1	Hooked
		2	Straight
92	Ratio of terminal leaflet length to width		
93	Ratio of terminal leaflet length to petiolule length		
94	Stem ridging	1	Smooth
		2	Ridged
		3	Strongly ridged
95	Stem shape in transverse section	1	Rounded
		2	Angular
96	Stem hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous
97	Stem hair length	1	Short
		2	Mediuim
		3	Long
		4	Very long
98	Stem hair elevation	1	Adpressed
		2	±adpressed
		3	Not adpressed
99	Stem hair colour	1	Clear
		2	Brown
100	Stem hair apex	1	Hooked
		2	Straight

INFLORESCENCE

101 Type of inflorescence	1	Panicle (see Lackey 1981)
	2	Nodose pseudoraceme
	3	Pseudoraceme
102 Position of inflorescence	1	Terminal
	2	Auxilliary
	3	Terminal and auxilliary
103 Type of inflorescence nodes	1	Not nodose
	2	Intermediate
	3	Nodose
104 Number of nodes/ inflorescence		
105 Number of flowers/node		
106 Distance from basal node to next node		mm (see Figure 46)
107 Flower orientation	1	Not resupinate
	2	Resupinate
108 Peduncle length		mm
109 Peduncle ridging	1	Smooth
	2	Ridged
	3	Strongly ridged
110 Peduncle hair number	1	Absent
	2	Sparse
	3	Moderate
	4	Numerous
	5	Very numerous
111 Peduncle hair length	1	Short
	2	Medium
	3	Long
	4	Very long
112 Peduncle hair elevation	1	Adpressed
	2	±adpressed
	3	Not adpressed
113 Peduncle hair colour	1	Clear
	2	Brown

114 Peduncle hair apex	1	Hooked
	2	Straight
115 Rachis length		mm
116 Rachis hair number	1	Absent
	2	Sparse
	3	Moderate
	4	Numerous
	5	Very numerous
117 Rachis hair length	1	Short
	2	Medium
	3	Long
	4	Very long
118 Rachis hair colour	1	Clear
	2	Brown
119 Rachis hair apex	1	Hooked
	2	Straight
120 Pedicel length		mm
121 Pedicel hair number	1	Absent
	2	Sparse
	3	Moderate
	4	Numerous
	5	Very numerous
122 Pedicel hair length	1	Short
	2	Medium
	3	Long
	4	Very long
123 Pedicel hair colour	1	Clear
	2	Brown
124 Pedicel hair apex	1	Hooked
	2	Straight
125 Pedicel shape	1	Swollen at base
	2	Unswollen
	3	Swollen at apex
126 Ratio of peduncle to rachis length		
127 Ratio of rachis to pedicel length		

128 Bract persistance	1	Deciduous prior to anthesis
	2	Deciduous at anthesis or just after
	3	Seen on immature pod
129 Bract positioin	1	Join below base of peduncle (see Figure 4.7)
	2	Join above base of peduncle
130 Bract length		mm
131 Bract width		mm
132 Bract shape		See Figure 4.3.
133 Bract apex shape	1	Obtuse
	2	Rounded
	3	Acute
	4	Mucronate
	5	Accuminate
134 Bract base production	1	Not produced
	2	Produced
135 Bract vein number		
136 Bract vein prominence	1	Not prominent
	2	Prominent
137 Bract abaxial hair number	1	Absent
	2	Sparse
	3	Moderate
	4	Numerous
	5	Very numerous
138 Bract abaxial hair length	1	Short
	2	Medium
	3	Long
	4	Very long
139 Bract abaxial hair elevation	1	Adpressed
	2	±adpressed
	3	Not adpressed
140 Bract abaxial hair colour	1	Clear
	2	Brown
141 Bract abaxial hair apex	1	Hooked
	2	Straight

142 Bract adaxial hair number	1	Absent
	2	Sparse
	3	Moderate
	4	Numerous
	5	Very numerous
143 Bract adaxial hair length	1	Short
	2	Medium
	3	Long
	4	Very long
144 Bracteole persistance	1	Deciduous prior to anthesis
	2	Deciduous at or near anthesis
	3	Seen on young pod
145 Bracteole position	1	Join calyx (see Figure 4.8)
	2	Join pedicel
146 Bracteole length		mm
147 Bracteole width		mm
148 Bracteole shape		See Figure 4.3.
149 Bracteole apex shape	1	Obtuse
	2	Rounded
	3	Acute
	4	Mucronate
	5	Accuminate
150 Bracteole base production	1	Not produced
	2	Produced
151 Bracteole vein number		
152 Bracteole vein prominence	1	Not prominent
	2	Prominent
153 Bracteole abaxial hair number	1	Absent
	2	Sparse
	3	Moderate
	4	Numerous
	5	Very numerous
154 Bracteole abaxial hair length	1	Short
	2	Medium
	3	Long
	4	Very long

Figure 4.8: Bracteole position

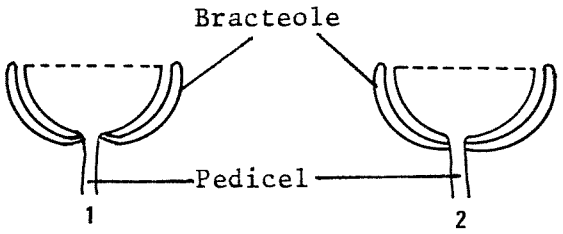


Figure 4.9: Calyx measurements

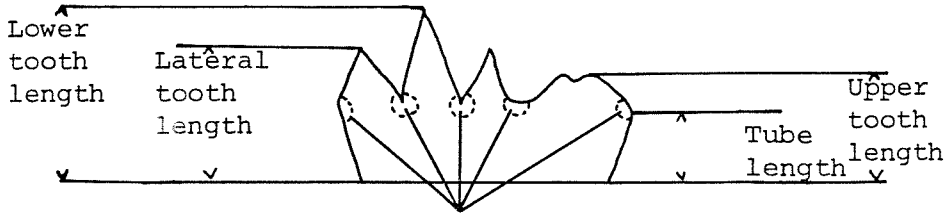


Figure 4.10: Corolla measurements

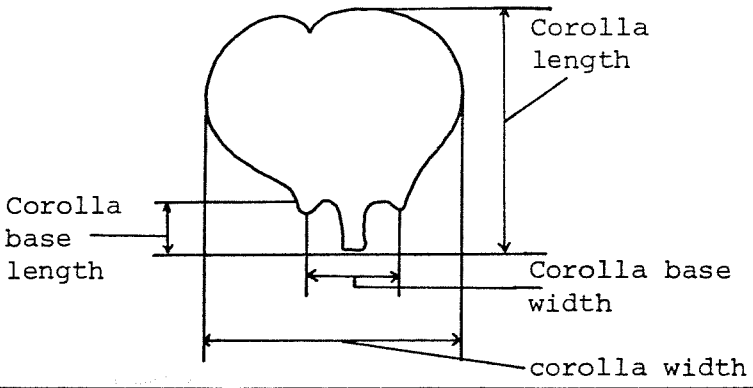
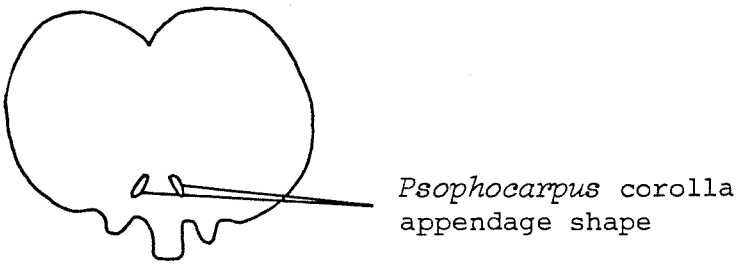


Figure 4.13: Corolla appendage shape



155	Bracteole abaxial hair elevation	1	Adpressed
		2	±adpressed
		3	Not adpressed
156	Bracteole abaxial hair colour	1	Clear
		2	Brown
157	Bracteole abaxial hair apex	1	Hooked
		2	Straight
158	Bracteole adaxial hair number	1	Absent
		2	Sparse
		3	Moderate
		4	Numerous
		5	Very numerous
159	Bracteole adaxial hair length	1	Short
		2	Medium
		3	Long
		4	Very long
160	Shape of upper calyx tooth	1	Obtuse
		2	Rounded
		3	Acute
		4	Mucronate
		5	Emarginate
		6	Bifid
161	Shape of lateral calyx teeth	1	Obtuse
		2	Rounded
		3	Acute
		4	Accuminate
162	Shape of lower calyx tooth	1	Obtuse
		2	Rounded
		3	Acute
		4	Accuminate
163	Lower tooth and calyx tube length (A)		mm (see Figure 4.9)
164	Lateral tooth and calyx tube length (B)		mm
165	Upper tooth and calyx tube length (C)		mm
166	Calyx tube length (D)		mm

167 Ratio of calyx length to bracteole length		
168 Ratio of calyx length to lateral tooth length (A÷B)		
169 Ratio of calyx length to upper tooth length (A÷C)		
170 Ratio of calyx length to tube length (A÷D)		
171 Calyx symmetry	1	Bilateral
	2	Asymmetric
172 Calyx exterior hair number	1	Absent
	2	Sparse
	3	Moderate
	4	Numerous
	5	Very numerous
173 Calyx exterior hair length	1	Short
	2	Medium
	3	Long
	4	Very long
174 Calyx exterior hair elevation	1	Adpressed
	2	±adpressed
	3	Not adpressed
175 Calayx exterior hair colour	1	Clear
	2	Brown
176 Calyx exterior hair apex	1	Hooked
	2	Straight
177 Calyx exterior hair position	1	Absent
	2	Only on lower tooth
	3	All over exterior
178 Calyx interior hair position	1	Absent
	2	Only on teeth edges
	3	Only on teeth
	4	On teeth and top of tube
	5	On teeth and upper half of tube
	6	On teeth and tube

179 Calyx interior hair tufts	1	Absent (see Figure 4.9)
	2	Present
180 Corolla exterior papillate	1	Absent
	2	Present
181 Corolla length		mm (see Figure 4.10)
182 Corolla width		mm
183 Corolla claw length		mm
184 Corolla claw width		mm
185 Ratio of corolla length to width		
186 Ratio of corolla length to claw length		
187 Ratio of corolla width to claw width		
188 Corolla apex shape	1	Emarginate
	2	Rounded
	3	Obtuse
189 Corolla base shape		See Figure 4.11.
190 Corolla symmetry	1	Bilateral
	2	Asymmetric
191 Corolla auricle shape		See Figure 4.12
192 Corolla appendage shape	1	Absent
	2	Like *Psophocarpus* (see Figure 4.13)
	3	Other shapes
193 Corolla pubescence	1	Glabrous
	2	Pubescent
194 Wing length (A)		mm (see Figure 4.14)
195 Wing width (B)		mm
196 Wing claw length (C)		mm
197 Wing spur length (D)		mm
198 Wing width above claw (E)		mm
199 Wing constriction length (F)		mm
200 Wing shape		See Figure 4.15.
201 Wing base shape		See Figure 4.16.
202 Wing pouch	1	Absent
	2	Present

Figure 4.11: Corolla base shape

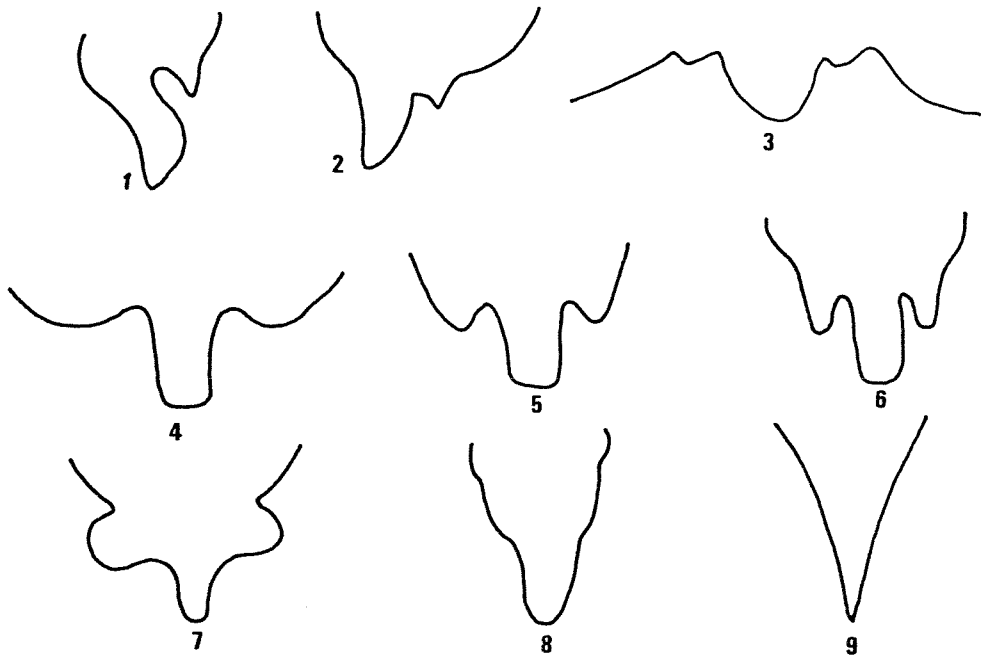


Figure 4.12: Corolla auncle shape

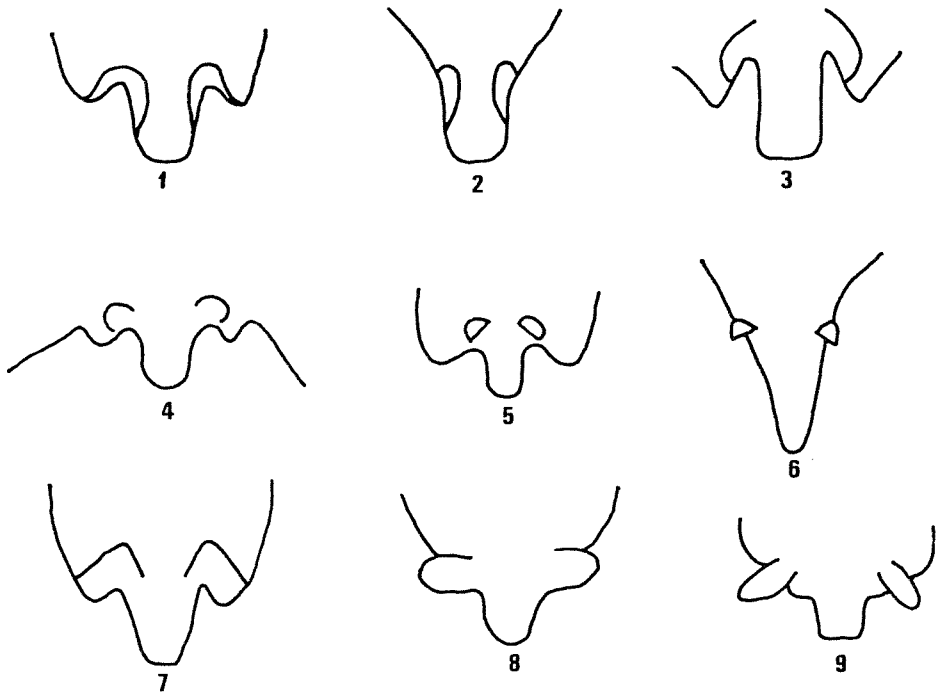


Figure 4.14: Wing measurements

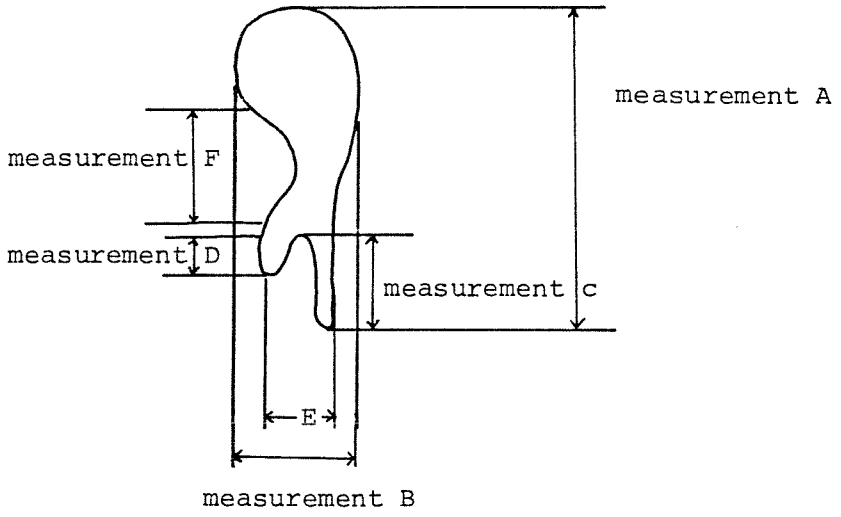


Figure 4.19: Staminal tube apex shape

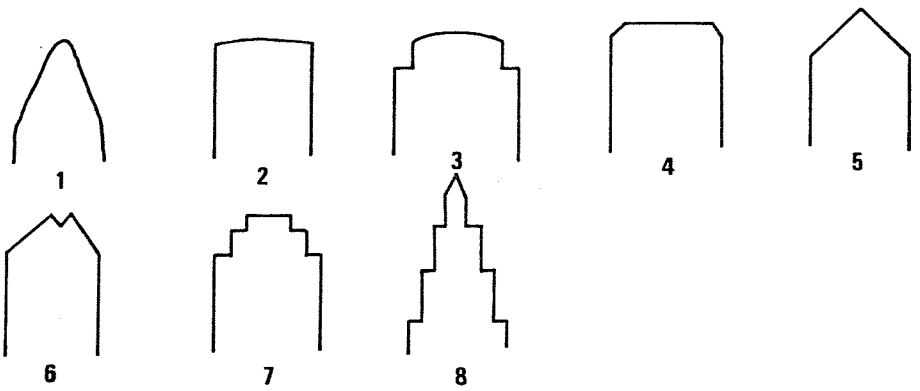


Figure 4.15: Wing shapes

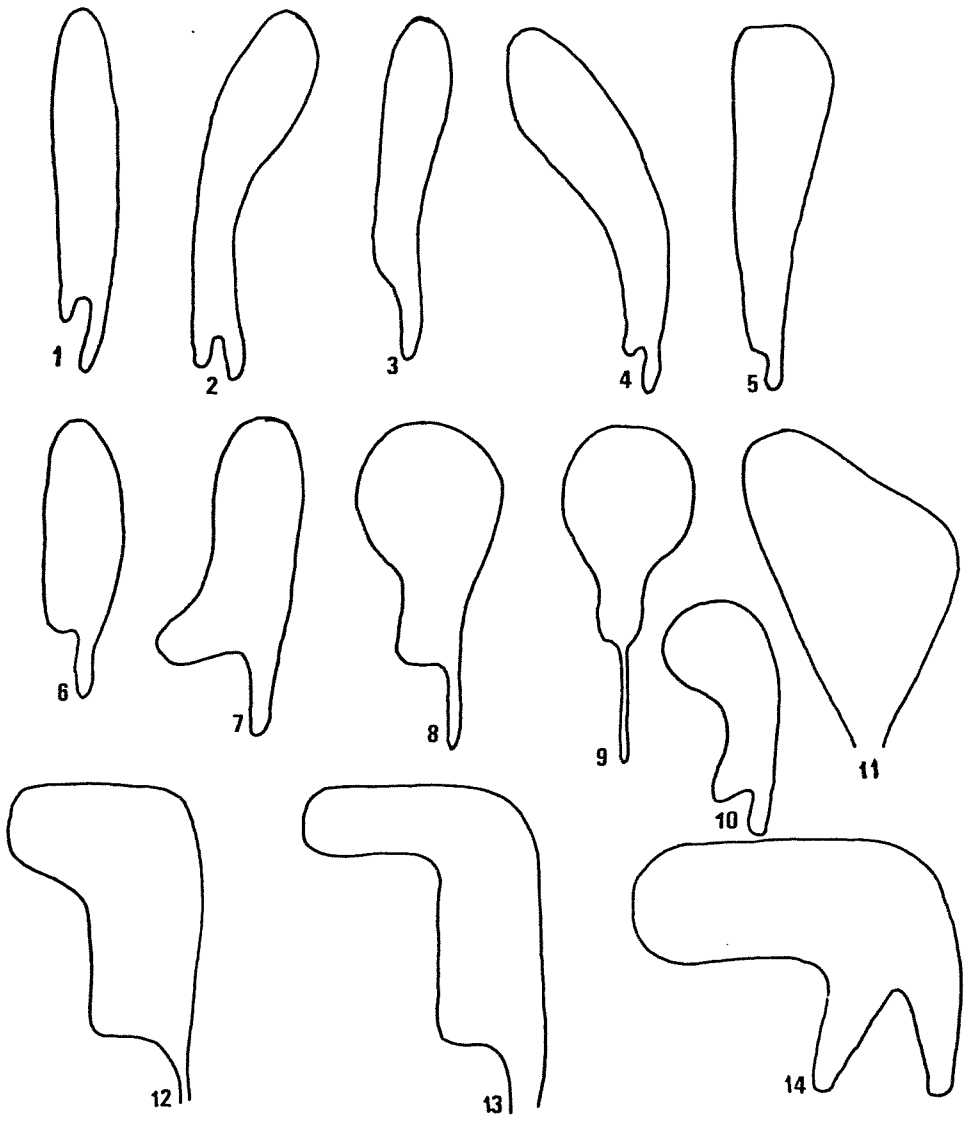


Figure 4.16: Wing base shape

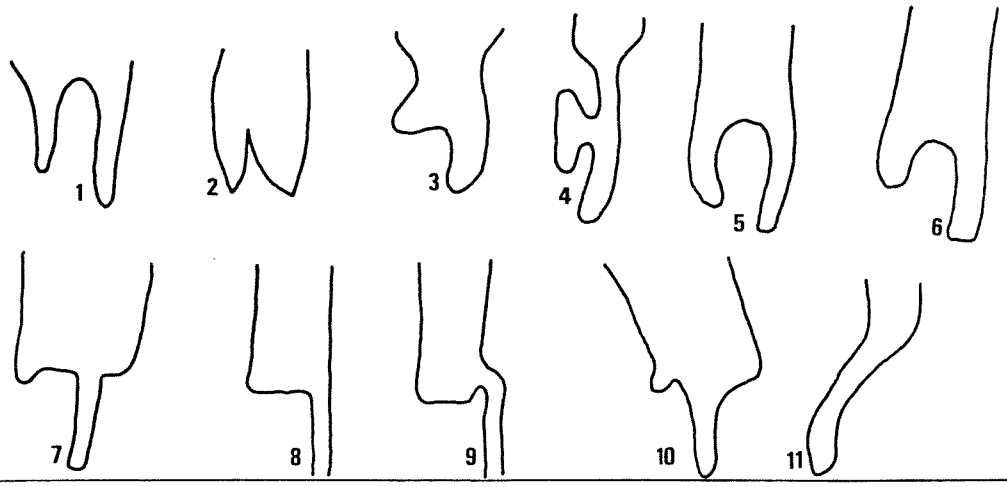
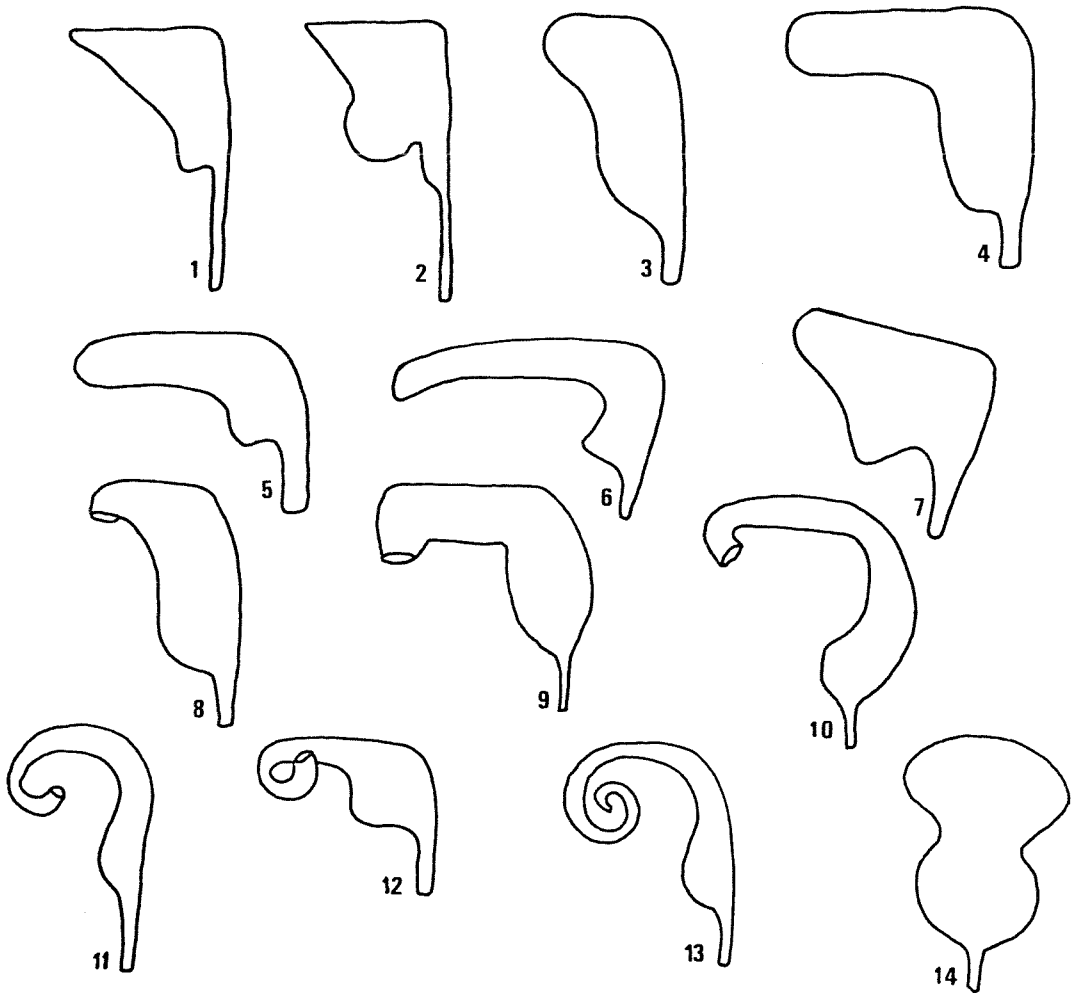


Figure 4.17: Keel shape



203 Wing auricle	1	Absent
	2	Present
204 Wing corrugation (type= lamellate, after Stirton, 1981)	1	Absent
	2	Present
205 Wing spiralling	1	Absent
	2	Present
206 Wing pubescence	1	Glabrous
	2	Pubescent
207 Wing-Keel adhesion	1	Wing free from Keel
	2	Wing adheres to Keel
208 Wing extra tooth	1	Not present
	2	Extra tooth present (see Marechal et al, 1978a)
209 Ratio of wing length to width (A÷B)		
210 Ratio of wing length to claw length (A÷C)		
211 Ratio of wing length to constriction length (A÷F)		
212 Ratio of corolla length to wing length		
213 Keel length		mm
214 Keel width		mm
215 Keel claw length		mm
216 Ratio of Keel length to width		
217 Ratio of Keel length to Keel claw length		
218 Ratio of Corolla length to Keel length		
219 Keel shape		See Figure 4.17.
220 Keel base shape		See Figure 4.18.
221 Keel pouches	1	Absent
	2	1 Side
	3	Both sides
222 Keel spiralling	1	Absent

Figure 4.18: Keel base

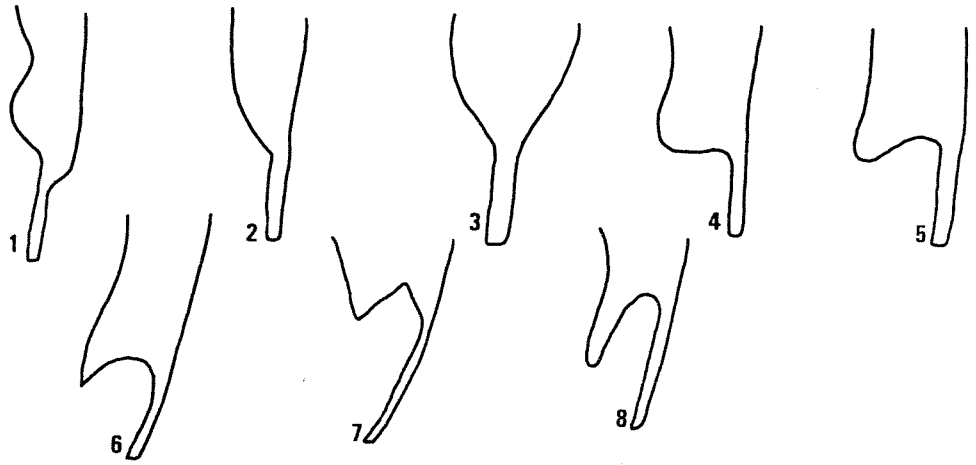


Figure 4.20: Vexillary stamen base shapes

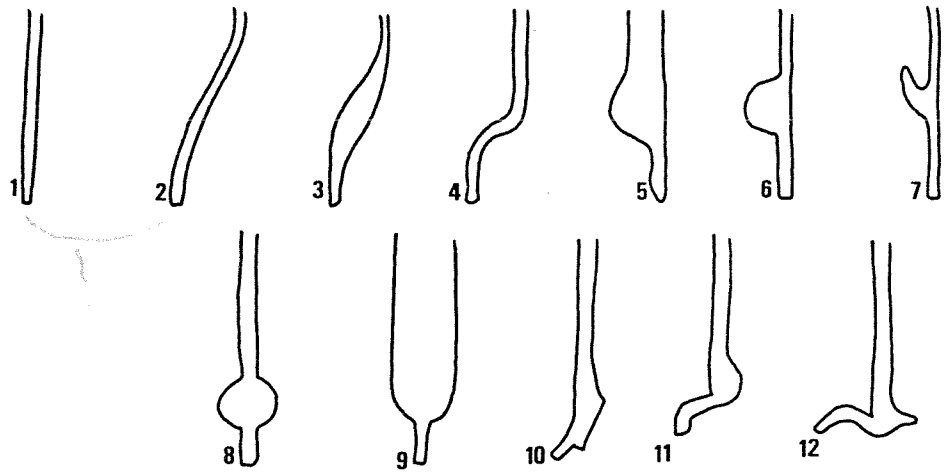
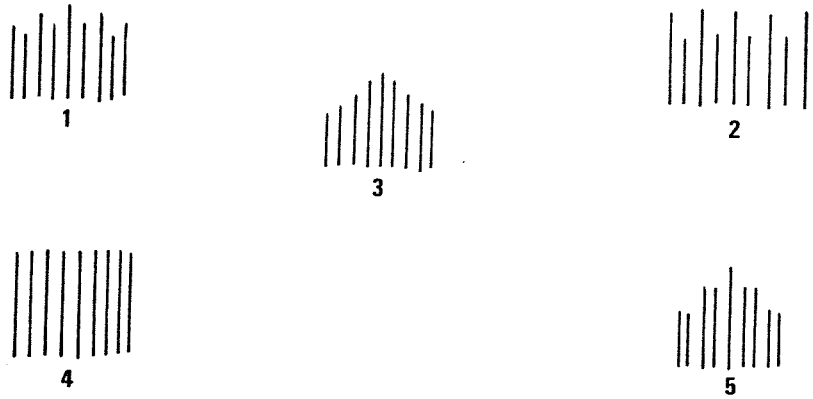
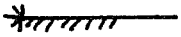
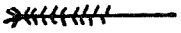


Figure 4.21: Staminal filament length pattern



	2	Present
223 Keel corrugation	1	Absent
	2	Present
224 Keel pubescence	1	Absent
	2	Present
225 Keel fusion	1	Lower surface only
	2	Upper and lower surface
226 Keel fusion type	1	Complete fusion
	2	Slight toothing
	3	Strong toothing
227 Staminal tube length		mm
228 Filament length		mm
229 Ratio of staminal tube to filament		
230 Filament dilation	1	Absent
	2	Present
231 Filament size	1	Fuliform
	2	Intermediate
	3	Large
232 Staminal tube apex shape		See Figure 4.19.
233 Staminal tube curvature	1	Straight
	2	Curved
234 Vexillary stamen attachment	1	Free
	2	Slightly joined to tube
	3	Joined to tube
235 Vexillary stamen base		See Figure 4.20
236 Anther shape	1	
	2	
	3	
	4	
237 Anther fixation	1	All basi-fixed
	2	Basi and dorsi fixed
	3	All dorsi-fixed
238 Filament pattern		See Figure 4.21.
239 Staminal pubescence	1	Glabrous
	2	Pubescent
240 Ovary length		mm
241 Stigma length		mm

242 Ratio of ovary length to stigma length		
243 Ovary shape	1	Linear
	2	Intermediate
	3	Oblong
244 Ovary ϕ shape	1	
	2	
	3	
245 Ovary pubescence	1	Glabrous 
	2	Abaxial surface only 
	3	Abaxial and adaxial surfaces 
	4	Adaxial surface only 
	5	All over 
246 Style base ϕ	1	Laterally flattened
	2	Round
	3	Ventrally flattened
	4	Triangular
247 Style apex ϕ	1	Laterally flattened
	2	Round
	3	Ventrally flattened
	4	Triangular
248 Style thickness	1	Fuliform
	2	Intermediate
	3	Large
249 Style spiralling	1	Absent
	2	Present
250 Style pubescence	1	Absent
	2	Present
251 Style post stigma	1	Absent
	2	Present
252 Style apex hairs	1	
	2	
	3	
	4	

253 Style thickening	1	No thickening (see Figure 4.22)
	2	Thickened at base
	3	Thickening in middle
	4	Thickened at apex
254 Style curvature		See Figure 4.23.
255 Style degree of curvature		See Figure 4.24.
256 Style apex bification	1	Absent
	2	Present
257 Style apex spatulate	1	Absent
	2	Present
258 Stigma shape	1	Round
	2	Elongated
259 Stigma position	1	Lateral
	2	Apical
	3	Terminal
260 Style chanelling	1	Absent
	2	Present
261 Style apex shape A (for subset generic study)		See Figure 4.25
262 Style apex shape B (for *Psophocarpus* study)		See Figure 4.26

LEGUME

263 Persistance of style	1	Absent
	2	Seen on immature pods only
	3	Seen on mature pods
264 Persistance of calyx	1	Absent
	2	Seen on immature pods only
	3	Seen on mature pods
265 Legume length		mm
266 Legume width		mm
267 Legume depth		mm

Figure 4.22: Style thickening

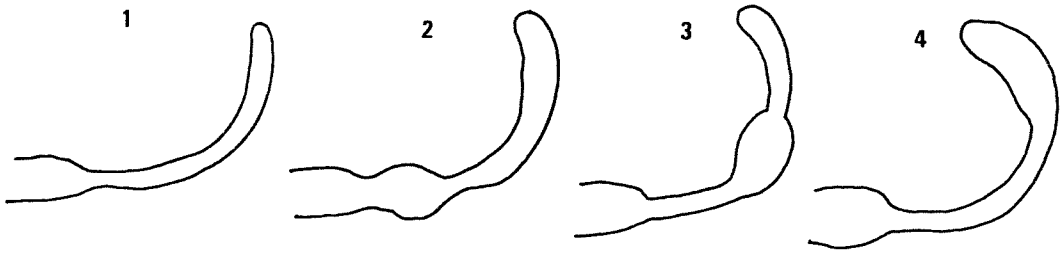


Figure 4.24: Degree of style spiraling

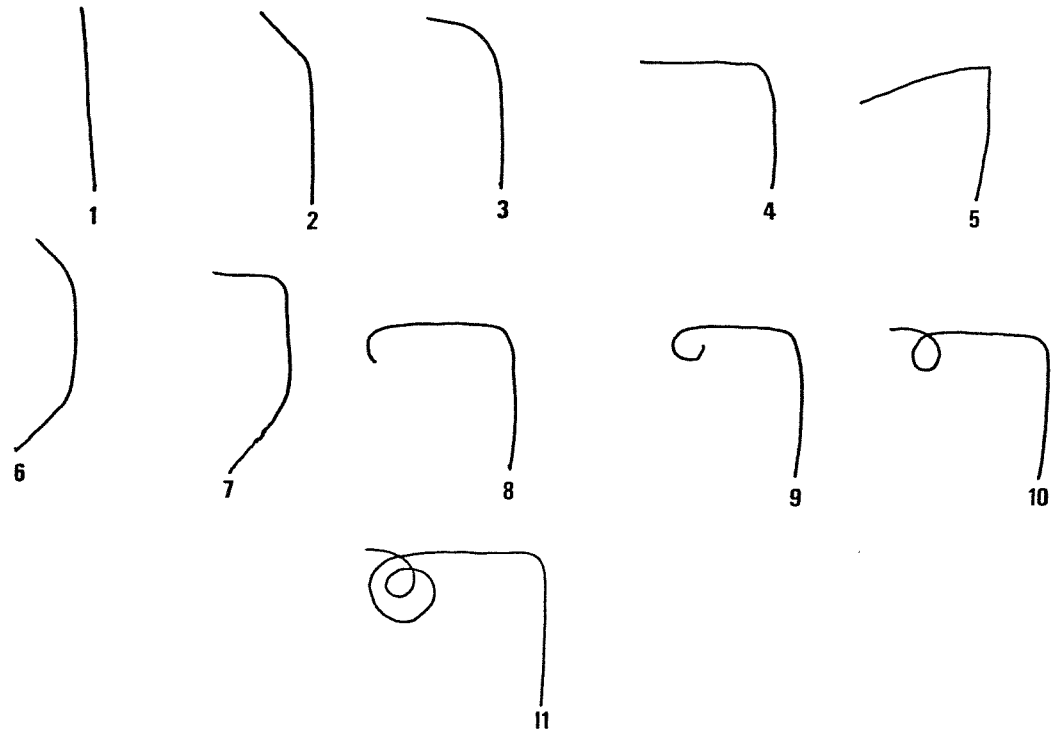


Figure 4.23: Style curvature

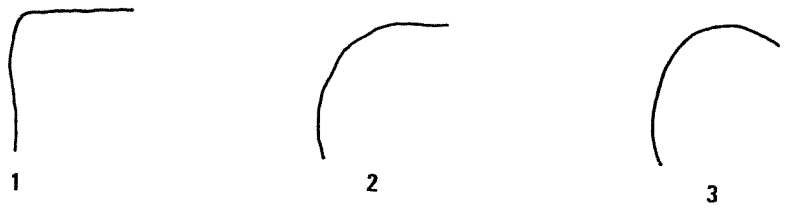


Figure 4.25: Style apex shape A (for subset inter-generic study)

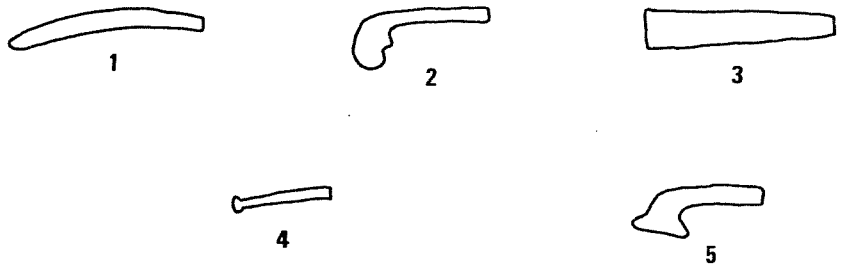
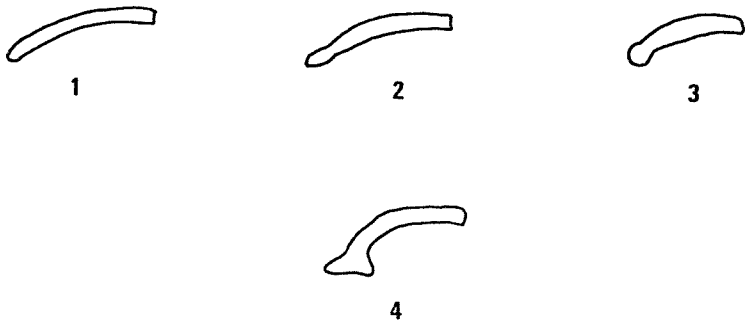


Figure 4.26: Style apex shape B (for *Psophocarpus* study)



268 Legume wing width		mm
269 Legume suture width		mm
270 Ratio of legume length to width		
271 Ratio of legume width to wing width		
272 Legume shape	1	Linear
	2	Oblong
	3	Rectangular
273 Legume wing	1	Absent
	2	Present
274 Legume wing edge	1	Ragged
	2	Entire
275 Legume wing end shape	1	See Figure 4.27
	2	
276 Legume shape ϕ	1	Round
	2	Laterally flattened
	3	Square
277 Legume enlargement	1	Distal section enlarges first
	2	Enlarges along whole length
	3	Proximal section enlarges first
278 Legume aspect	1	Upward
	2	Outward
	3	Downward
279 Legume curvature	1	Absent
	2	Present
280 Legume hair number	1	Absent
	2	Sparse
	3	Moderate
	4	Numerous
	5	Very numerous
281 Legume hair length	1	Short
	2	Medium
	3	Long
	4	Very long

Figure 4.27: Wing end shape

Figure 4.29: Seed lateral view (After Maréchal et al, 1978)

Figure 4.30: Seed attachment (After Maréchal et al, 1978)

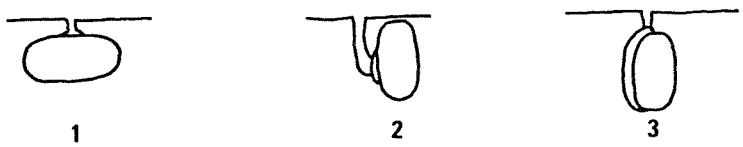


Figure 4.31: Hilum shape (After Maréchal et al, 1978)

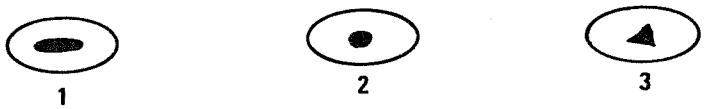


Figure 4.32: Hilum shape in lateral view (After Maréchal et al, 1978)

282 Legume hair elevation	1	Adpressed
	2	tadpressed
	3	Not adpressed
283 Legume hair colour	1	Clear
	2	Brown
284 Suture pubescence	1	Glabrous
	2	Pubescent
285 Suture width	1	Narrow
	2	Slightly projecting
	3	Projecting
286 Legume surface	1	Smooth
	2	Ridged
	3	Rough
	4	Ciliate
287 Legume dehiscence	1	Along one suture
	2	Along both sutures
288 Legume twisting once dehissed	1	Very loose
	2	Loose
	3	Medium
	4	Tight
	5	Very tight
289 Legume partition type	1	Absent
	2	Woolly
	3	Spongy
	4	Papery
290 Legume Colour	1	Cream
	2	Pale Brown
	3	Brown
	4	Reddish brown
	5	Dark brown
	6	Black
291 Legume wing colour	1	Cream
	2	Pale brown
	3	Brown
	4	Reddish brown
	5	Dark brown
	6	Black

292	Legume colour and wing colour	1 2	Different colours Same colour
293	Number seed/legume		
294	Shape of distal end of legume		See Figure 4.28

SEED

295	Seed length		mm
296	Seed width		mm
297	Seed depth		mm
298	Seed circumference		mm
299	Hilum length		
300	Ratio of seed length to width		
301	Ratio of seed circumference to hilum length		
302	Shape of seed in lateral view		See Figure 4.29
303	Position of seed attachment		See Figure 4.30.
304	Hilum shape	1 2 3	Elongated (see Figure 4.31) Oval Triangular
305	Hilum shape in lateral view	1 2 3	Raised (see Figure 4.32) Slightly raised Sunken
306	Hilum position	1 2	Central Off centre
307	Surface of hilum in profile	1 2 3	Convex Levewl Concave

Figure 4.33: Shape of aril in lateral view (After Maréchal et al, 1978)

Figure 4.34: Shape of aril from end view (After Maréchal et al, 1978)

Figure 4.28: Shape of distal end of legume

308 Micropyle prominence	1	Flush with seed surface
	2	Prominent
309 Lens presence	1	Absent
	2	Present
310 Aril presence	1	Absent
	2	Present
311 Aril shape in lateral view	1	Centrally placed (see Figure 4.33)
	2	Off Centre
312 Aril shape in end view	1	Raised upward (see Figure 4.34)
	2	Flattened to testa
313 Seed finish	1	Brilliant
	2	Shiny
	3	Matt
	4	Variable
	5	Velvet
314 Seed surface	1	Smooth
	2	Bumpy
315 Seed pubescence	1	Glabrous
	2	Pubescent

4.3 Character Subset Selections

Effectively the taxonomic investigation of the present study may be sub-divided into four component studies: the extensive inter-generic survey of *Psophocarpus* allied genera, the intensive inter-generic survey of *Psophocarpus* closely allied genera, the intra-generic *Psophocarpus* study and the *P.scandens* - *P.palustris* complex study. For each of the component studies characters were selected from the gross character set, which showed variance between taxa, but were constant for a particular taxon. The details of the characters selected are provided in Table 4.2.

For the initial extensive inter-generic study of the *Psophocarpus* allied genera in the Clitoriinae and Phaseolinae, the 73 characters were chosen intuitively and then analysed using the cluster analysis program LINKAGE. For the latter three component studies it was planned to utilise the character analysis program CHARANAL to help select phenetic characters, that would then be analysed using the relationship analysis programs, LINKAGE, CLUSTAN and DECORANA. However in practice CHARANAL was utilised only for the intensive subset generic study.

The reasons why CHARANAL was not used for all three of the latter studies as planeed required further explanation. There were two basic problems: first, the characters were all initially envisaged for use with the program LINKAGE which will accept characters with up to 35 states per character. Thus in formulating the gross character set many quantitative characters had a large number of character states, a few the full quota of 35. In LINKAGE this is an advantage because it allows maximum use of the partial correlation option. However with CHARANAL the algorithms pair-wise comparisons are greatly complicated by large number of character states per character, so much so that the computer time allocated for a particular 'runjob' expires before the job is complete. The second basic problem with using CHARANAL was that the program requires knowledge of unfilled chracter states. Since the characters have larger numbers of character states there were numerous unfilled states, but working out exactly which they were would have proved very complex in a data set of 315 characters by approximately 800 OTU's.

Table 4.2 Character Set Selections

Char. Nos.	Ex 73	Ch 132	Ch 49	LER 102	LER 51	CER 102	CER 51	FER 66	FER 43	DER 66	DER 43	LRA 152	LRA 97	CRA 152	CRA 97	FRA 75	DRA 75	LPS 75	CPS 75	FPS 72	DPS 72
1	●	●										●	●	●	●	●	●				
2												●	●	●	●	●	●				
3																					
4																					
5												●		●				●	●	●	●
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7	●	●		●	●	●	●	●	●	●	●										
8												●	●	●	●	●	●				
9																					
10		●		●		●		●		●											
11		●																			
12												●	●	●	●	●	●	●	●	●	●
13												●	●	●	●	●	●	●	●	●	●
14																					
15		●										●	●	●	●	●	●				
16		●	●	●		●		●		●											
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29												●		●							
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31																		●	●	●	●
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39												●		●				●	●	●	●
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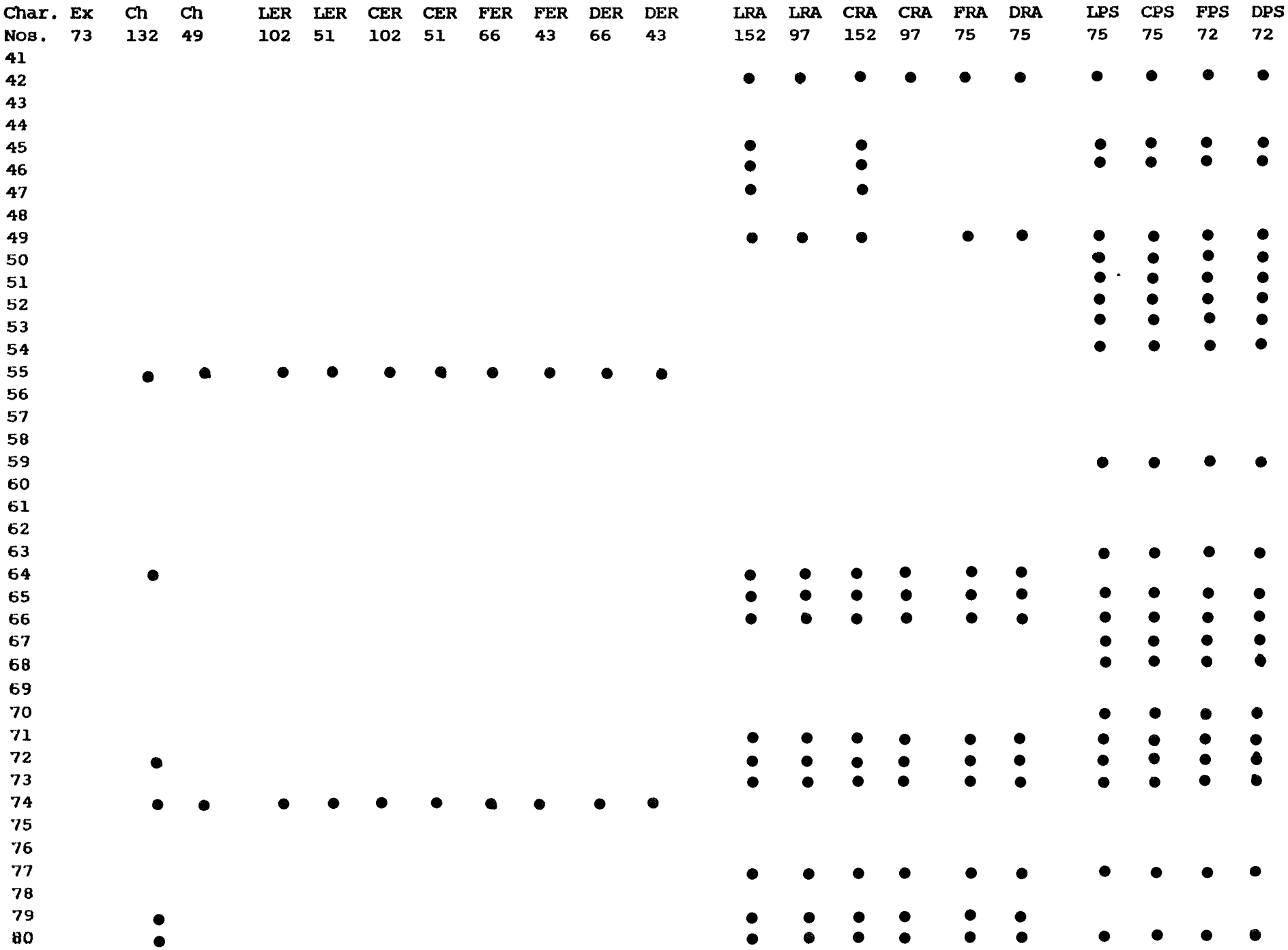
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Nos. 73 132 49 102 51 102 51 66 43 66 43 152 97 152 97 75 75 75 75 72 72
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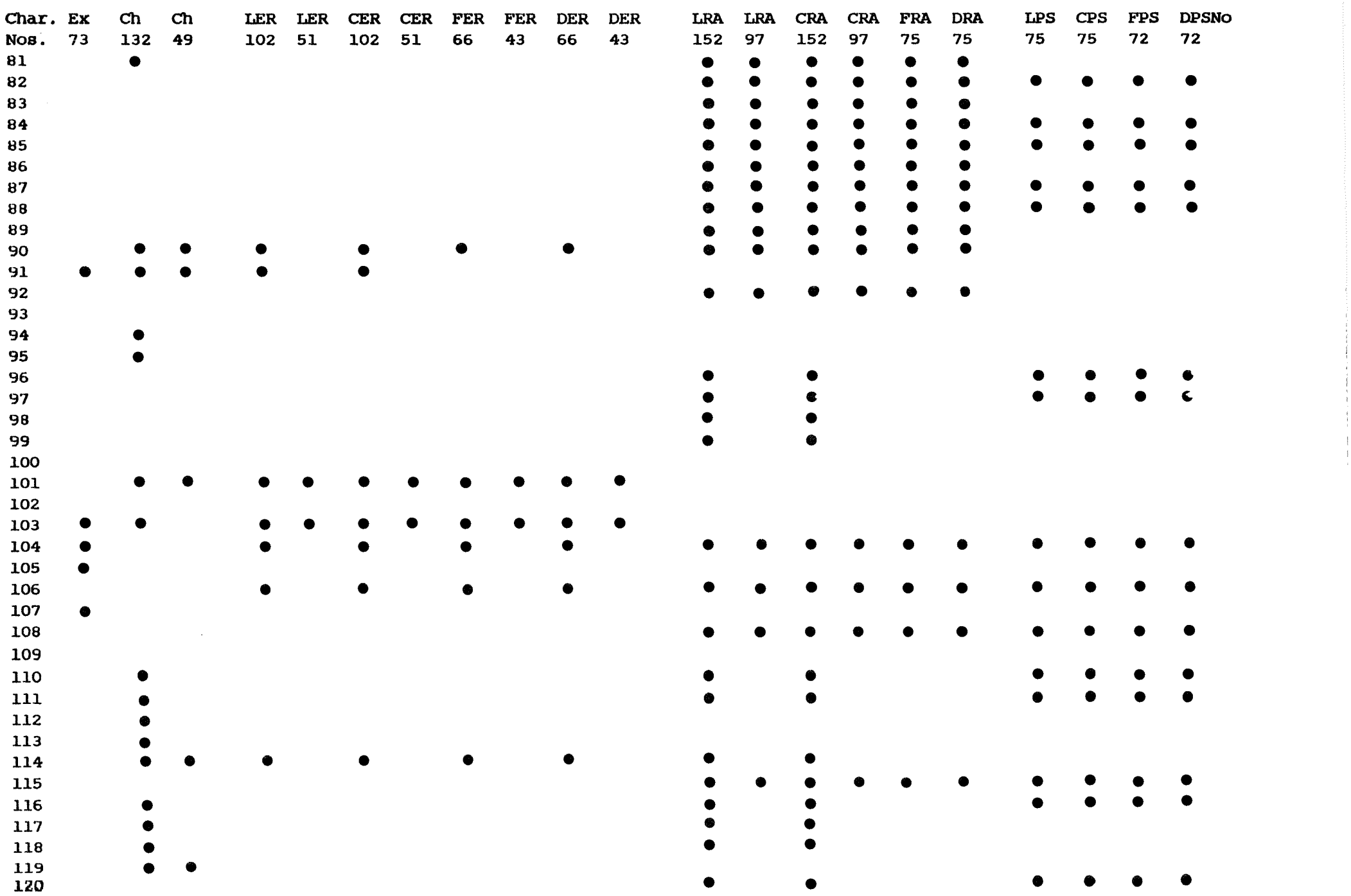
Char. Ex Ch Ch LER LER CER CER FER FER DER DER LRA LRA CRA CRA FRA DRA LPS CPS FPS DPSNo
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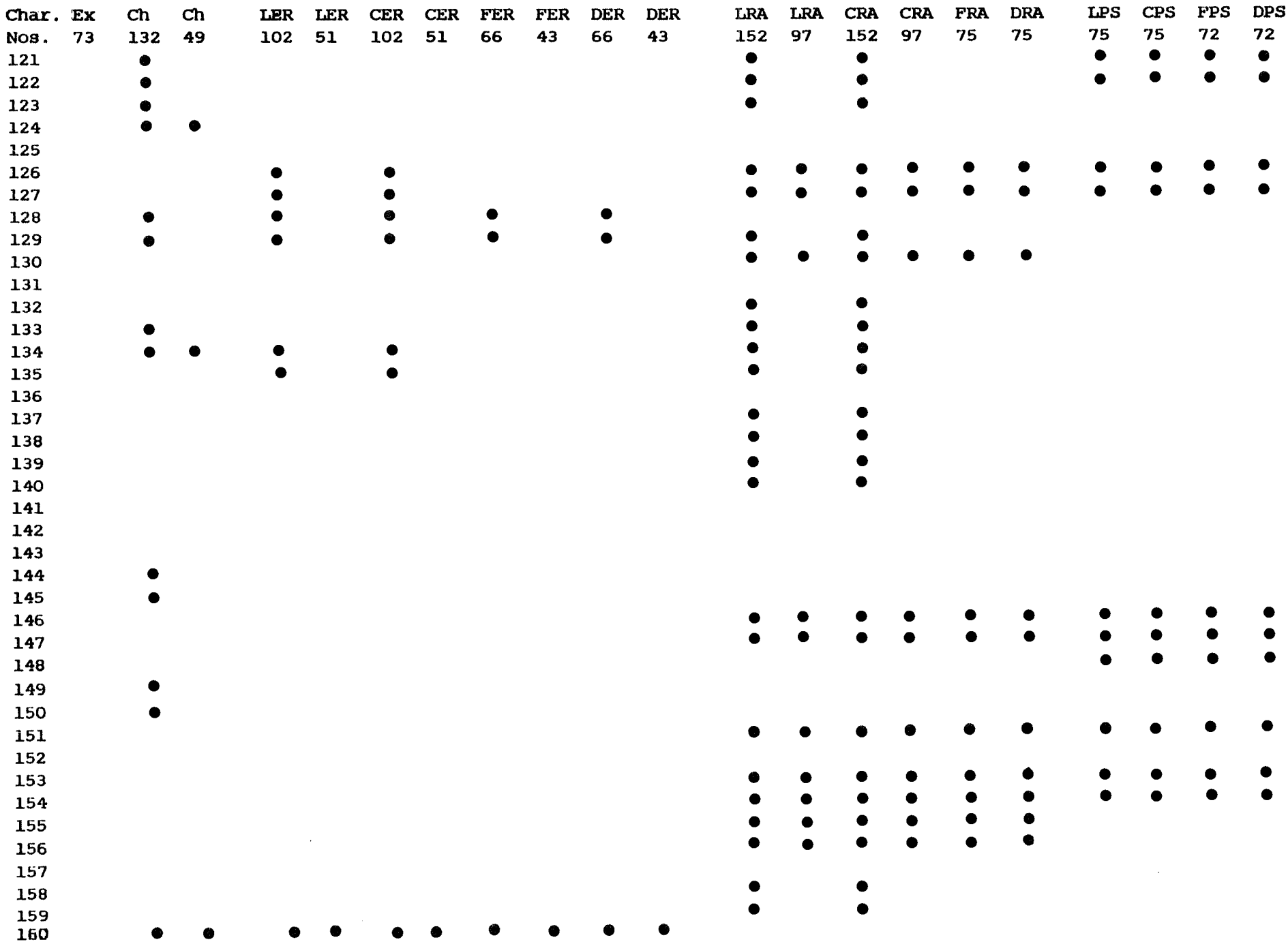
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Ch 132
Ch 49
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LER 51
CER 102
CER 51
FER 66
FER 43
DER 66
DER 43
LRA 152
LRA 97
CRA 152
CRA 97
FRA 75
DRA 75
LPS 75
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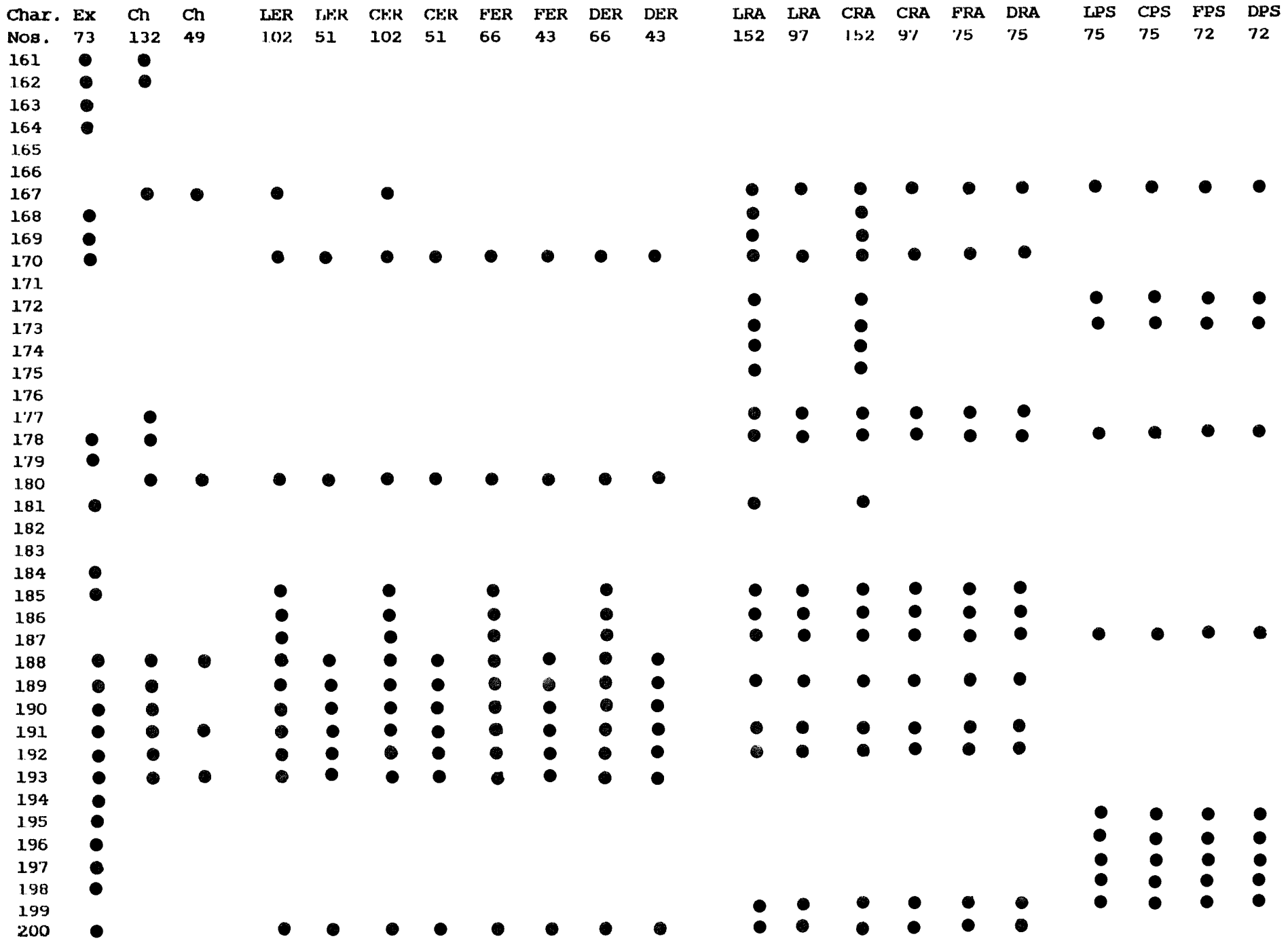
Char. Ex Ch Ch LER LER CER CER FER FER DER DER LRA LRA CRA CRA FRA DRA LPS CPS FPS DPS
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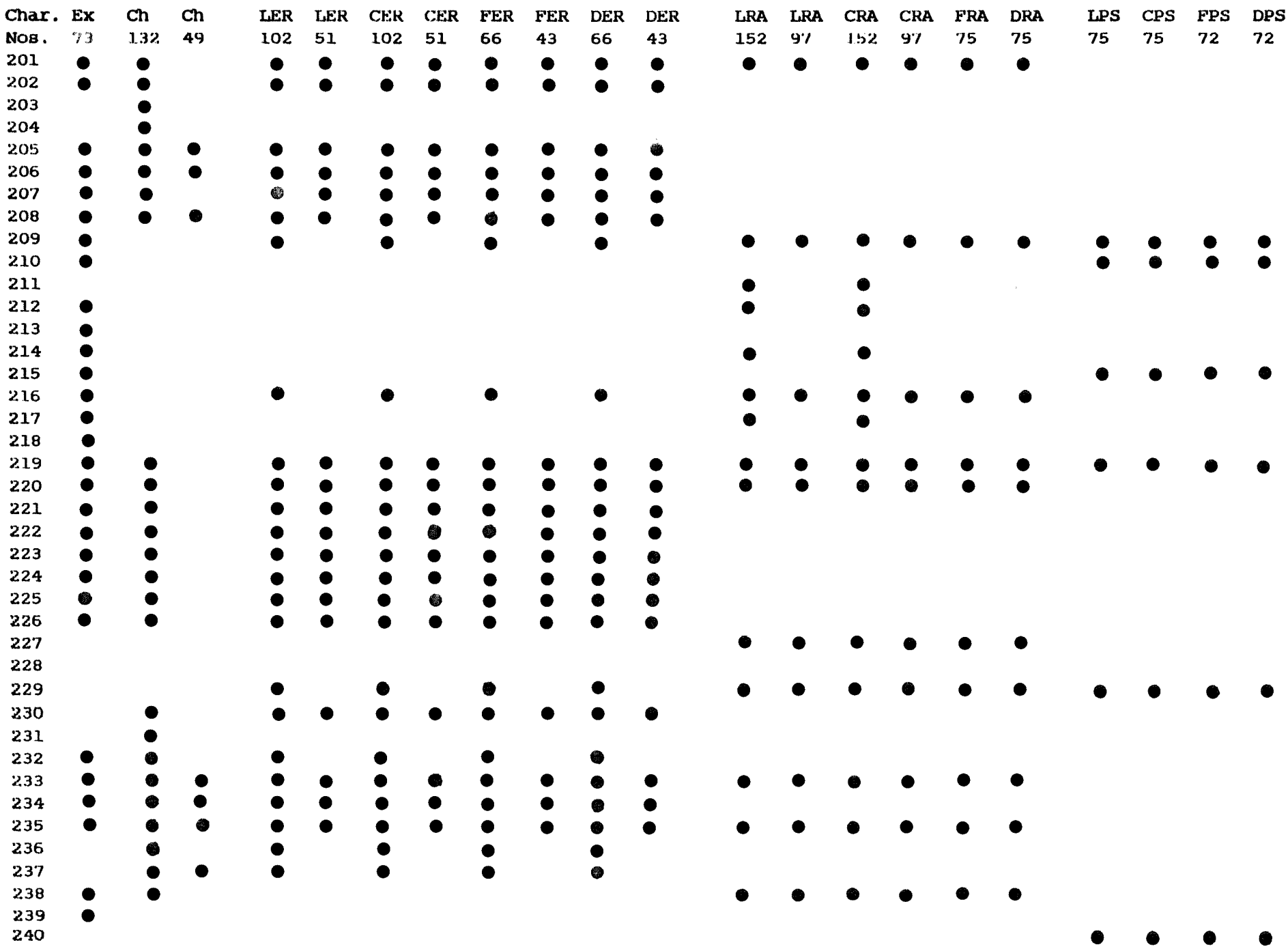
Char. Ex Ch Ch LER LER CER CER FER FER DER DER LRA LRA CRA CRA FRA DRA LPS CPS FPS DPS
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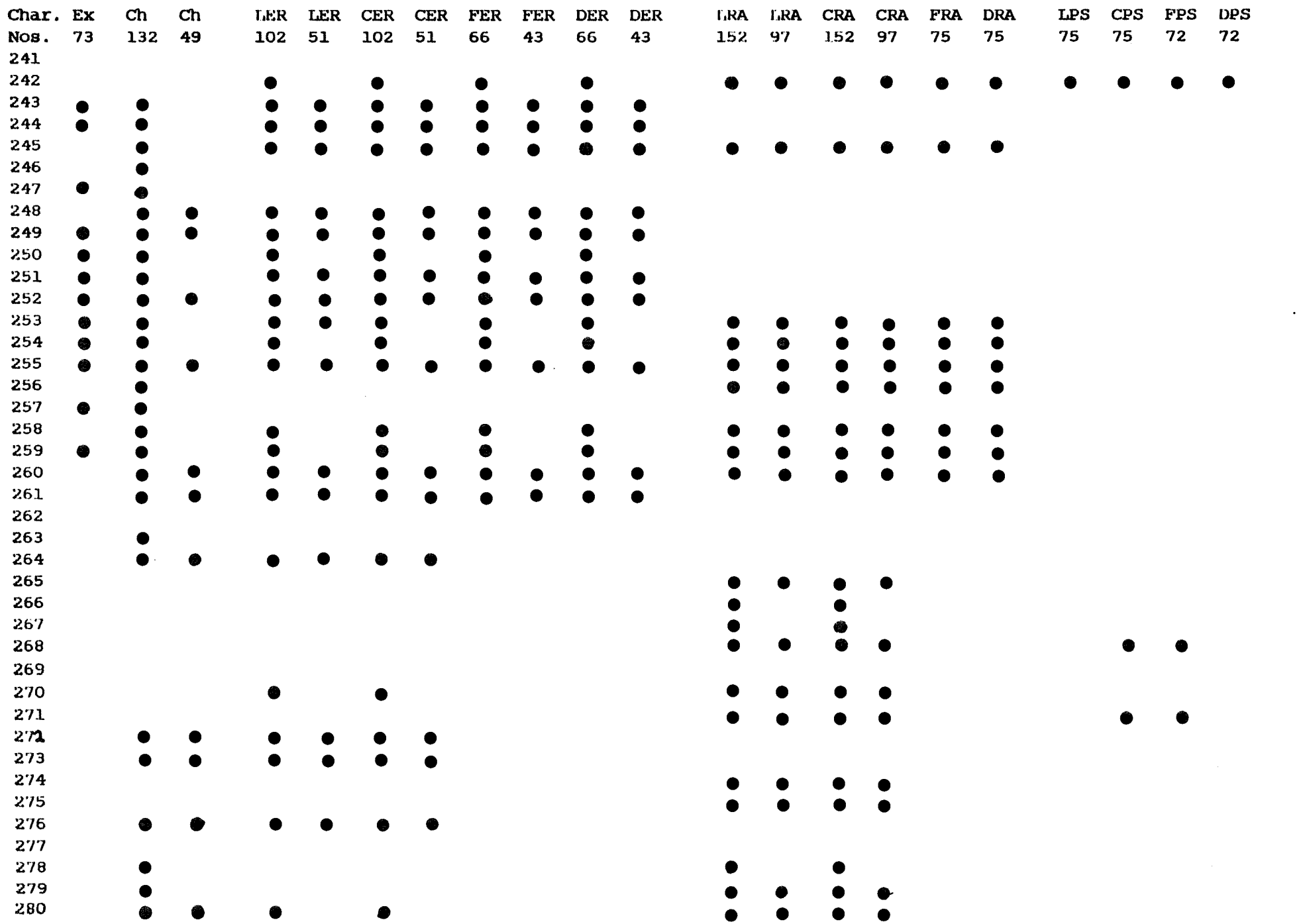
Char. Ex Ch Ch LER LER CER CER FER FER DER DER LRA LRA CRA CRA FRA DRA LPS CPS FPS DPS
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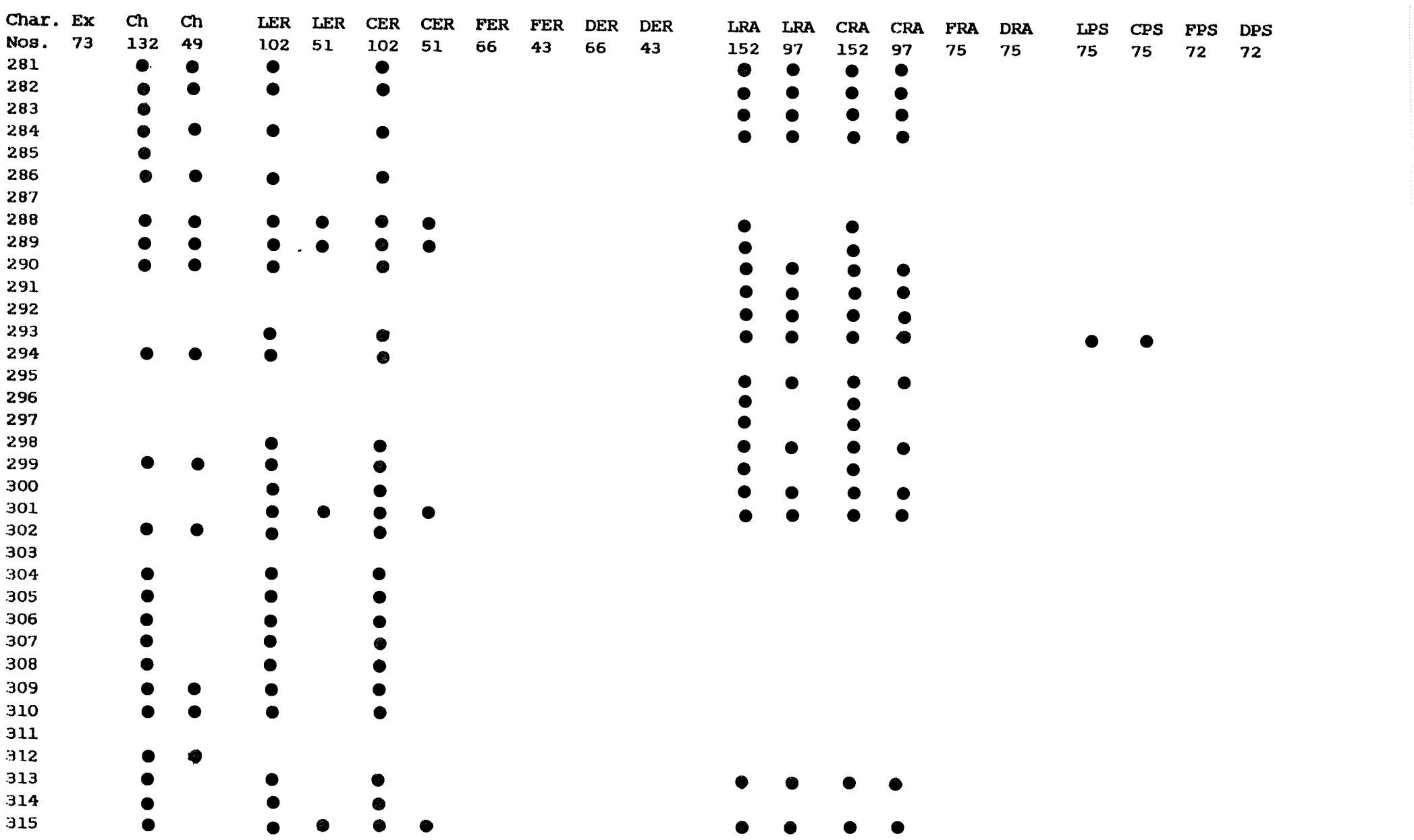
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Note: ● = Character use

Ex = Extensive inter-generic survey characters;

Ch = CHARANAL analysed and chosen characters;

LER = Subset inter-generic characters used with LINKAGE;

CER = Subset inter-generic characters used with CLUSTAN (DISTIN);

FER = Subset inter-generic characters used with CLUSTAN (FILE);

DER = Subset inter-generic characters used with DECORANA;

LRA = *Psophocarpus* species study characters used with LINKAGE;

CRA = *Psophocarpus* species study characters used with CLUSTAN (DISTIN);

FRA = *Psophocarpus* species study characters used with CLUSTAN (FILE);

DRA = *Psophocarpus* species study characters used with DECORANA;

LPS = *P. palustris* - *P. scandens* analysis characters using LINKAGE;

CPS = *P. palustris* - *P. scandens* analysis characters using CLUSTAN (DISTIN);

FPS = *P. palustris* - *P. scandens* analysis characters using CLUSTAN (FILE);

DPS = *P. palustris* - *P. scandens* analysis characters using DECORANA;

The number in each case (73, 102, 51 etc.) indicate the total number of selected characters.

To attempt to surmount these problems the data was repeatedly split until the character number was low enough so that the program CHARANAL would run in the computer time available. This was eventually achieved when the character number was 7% of the original size. This percentage of the character set being so small compared to the original character set indicated that the character selections in the sixteen subsets were possible being chosen on different criteria. To try to produce character selection on uniform criteria and obtain character selection within one CHARANAL run, all characters with over ten states were excluded from the analysis. This reduced the number of characters to 132, which had to be split in half before they would run in the time available (1000 seconds).

Unfortunately it still could not be guaranteed that the characters being chosen in the two halves were being selected on the same criteria. To overcome this it was decided to split the 132 characters into four approximately equal subsets, A, B, C and D, then run these subsets in all possible combinations (AB, AC, AD, BC, BD and CD). Following this the SUMRAT and SAMRAT values for each analysis were added together (the information content for each character being constant) and the characters with the highest overall figures used in the further analysis.

However, as the CHARANAL manual (Fleming and Appan, 1971) emphasises, when selecting characters using SUMRAT and SAMRAT the information content of each character is high, as in a character with many states, it will tend to make SUMRAT larger and SAMRAT smaller. So this factor with biological judgement was considered in choosing the 49 CHARANAL selected characters for the subset inter-generic study. Appendix 1 contains an example of the file submission for a CHARANAL run.

Within the time limits for the study it was decided that character selection for the intra-generic and *P.scandens/P.palustris* complex studies should be undertaken in the traditional, intuitive method. The characters were more easily chosen using this method than for the inter-generic study because of the small number of taxa involved, nine

for the *Psophocarpus* intra-generic study and two for the *P.scandens/P.palustris* complex study. The characters were chosen a posteriori from the data available after scoring the gross character set for each OTU.

Once the total number of characters had been selected for each of the three main studies (subset inter-generic, intra generic and *P.scandens/P.palustris* complex) the file containing the gross character set for the appropriate OTU's was edited to reduce the number of characters to those to be used in the analysis. Each study was analysed following the same route: LINKAGE, CLUSTAN (DISTIN), CLUSTAN (FILE) and DECORANA, firstly using the program LINKAGE, the formate for which is displayed in the example provided in Appendix 2. The option of LINKAGE was chosen which produces the lower triangle of the similarity matrix as a separate file. This similarity matrix was then analysed using the program CLUSTAN 1C, via the sub-routine DISTIN. However the lower triangle of similarity produced by LINKAGE is not in a format acceptable to CLUSTAN: it first requires transformation using the program JAQUIE. JAQUIE was written specifically for this purpose by Mr A Cotton of the Computing Centre, University of Southampoton, and is listed in Appendix 3. The formate for the file of a sub-routine DISTIN run is exampled in Appendix 4.

Utilising the sub-routine DISTIN restricts the choice of other sub-routines within the CLUSTAN suite and so sub-routine FILE was also used which accepts raw data and allows the full choice of CLUSTAN options. Unfortunately neither subroutine FILE nor the detrended correspondence analysis program DECORANA will accept missing data and require a quite different deck layout to LINKAGE and so the data sets required complicated rearrangement. Editing out of the few OTU's with a lot of missing data and the characters which commonly had missing data (mostly legume and seed) was undertaken. The subsequent steps to rearrange the data for FILE and DECORANA are, as previously emphasised, complex, including the use of a second program, JUDITH specifically written for the purpose by Mr A Cotton. These steps and listing of the program JUDITH are provided in Appendix 5.

The same data set was used for both the CLUSTAN (FILE) and DECORANA analysis. The file formats for these two programs are given in Appendix 6 and 7 respectively.

CHAPTER FIVE

PHENETIC STUDIES ANALYSIS RESULTS

5.1 Introduction

The purpose of this chapter is to display and discuss the results of the phenetic analysis of the four component studies. The four component studies were: the extensive survey of Clitoriinae and Phaseolinae genera for *Psophocarpus* allies; the intensive survey of Phaseolinae subset genera for close *Psophocarpus* allies; the *Psophocarpus* intra-generic study and the *P.scandens-P.palustris* complex study. This conveniently divides the results into four sections which will be described and discussed subsequently. For each of these sections the results will be discussed under the appropriate sub-heading of the relationship analysis program utilised.

5.2 Extensive inter-generic survey for Psophocarpus allies

In the second phase of the project (as outlined in Section 1.4), representatives of Phaseolinae and Clitoriinae genera were investigated to identify a smaller sub-set of genera most closely allied to *Psophocarpus*. In all 151 specimens (OTU's) were scored for the vegetative and inflorescence related characters detailed in Table 4.1. Scoring was restricted to vegetative and inflorescence characters to allow relatively rapid scoring and because the vast majority of specimens used lacked legumes and/or seeds. The specimens used in all the component studies are listed in Appendix 8, with their present study identification number, collector identification and herbarium from which they were loaned.

5.2.1 LINKAGE

A posteriori the number of characters considered to be of use in discriminating *Psophocarpus* allies was 73, as detailed in Table 4.2. These characters were used with the single linkage cluster program LINKAGE. There were 136 linkage levels with the level of similarity

decreasing from 0.88 to 0.59. The linkage diagrams (sub-graphs) showing the most closely allied genera linking with *Psophocarpus* are drawn in Figures 5.1 to 5.5. The linkage diagrams are drawn following the rules detailed in Section 3.4.2. The symbols used in the linkage diagrams in this chapter are explained in the key provided in Table 5.1.

The first linkage diagram to display a linking between the *Psophocarpus* cluster and another genus is drawn in Figure 5.1. At a threshold similarity of 0.6961 three specimens (OTU's) of *Neorautanenia* link with a *Psophocarpus scandens* specimen. The second external linkage of *Psophocarpus* with another genus is drawn in Figure 5.2 and shows the same *P.scandens* specimen linking up with a small cluster of *Sphenostylis* OTU's, at a similarity level of 0.6725. This linkage diagram also shows that *Neorautananta* has formed links with *Dolichos* OTU's. It is worth noting how OTU's from the same genera are tending to cluster together (see symbol and approximate specimen number correlations). This indicated that the choice of characters which remained constant for a particular genus, but varied between genera had been successful.

Otoptera is the next genus to form links with *Psophocarpus* directly, as is shown in Figure 5.3, at a similarity level of 0.6540. The linkage between *Otoptera* and *Psophocarpus* is marked by a broken line as *Otoptera* is already a member of the cluster containing *Psophocarpus* via its link with a *Neorautanenia* OTU. *Otoptera* forms a link with a specimen of *P.lancifolius*, it should also be noted that in Figure 5.3 the *Neorautanenia* specimen 284 has joined the encircled *Psophocarpus* OTU's, indicating its close links with *Psophocarpus*.

In Figure 5.4 the OTU's can be seen to be gravitating to form encircled sub-clusters, though all except the Clitoriinae genera are distantly linked within one Phaseolinae cluster. At this level of similarity, 0.6394 *Psophocarpus* can be seen to strengthen its linkage with *Otoptera* and *Sphenostylis* by the formation of multiple direct links.

When the similarity level is decreased slightly to 0.6389, as shown in Figure 5.5, numerous new internal links are formed between the

Table 5.1 Key to Generic and Species Symbols used in Chapter Five

(arranged after Lackey, 1981)

Clitoriinae

Centrosema (DC.) Benth.

Periandra Benth.

Clitoria L.

Clitoriopsis Wilczek

Phaseolinae

Dysolobium (Benth.) Prain

Subgen.*Dysolobium*, Marechal et al

Subgen.*Dolichovigna* (Hayata) Marechal

Psophocarpus grandiflorus Wilczek

P.tetragonolobus (L.) DC.

P.palustris Desv.

P.scandens (Endl.) Verdc.

P.obovalis Tisserant

P.monophyllus Harms

P.lecomtei Tisserant

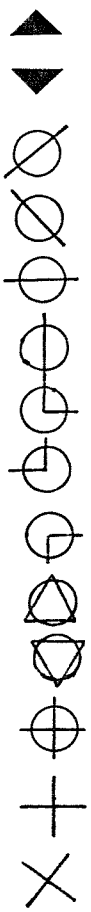
P.lancifolius Harms

P.lukafuensis (DeWild.) Wilczek

Possible *P.Palustris x P.scandens*

Physostigma Balf.

Vatavaea Choiv.



Decorsea R.Viguier

Spathionema Taub.

Otoptera DC.

Sphenostylis E.Mey.

Nesphostylis Verdc.

Austrodolichos Verdc.

Neorautanenia Schinz

Lablab Adans.

Alistilus N.E.Br.

Dipogon Lieb.

Dolichos L.

Macrotyloma (Wight & Am.) Verdc.

Vigna Savi

Subgen.*Vigna*, Marechal el al

Haydonia (Wilczek) Verdc.

Plectotropis (Schumach) Baker

Ceratotropis (Piper) Verdc.

Lasiospron (Benth.emend Piper)
Marechal et al

Sigmoidotropis (Piper) Verdc.

Macrorhyncha Verdc.

Ramirezella Rose

Oxyrhynchus Brandegee

Dolichopsis Hassler

Strophostyles Elliott

Macroptilium (Benth.) Urban

Phaseolus L.

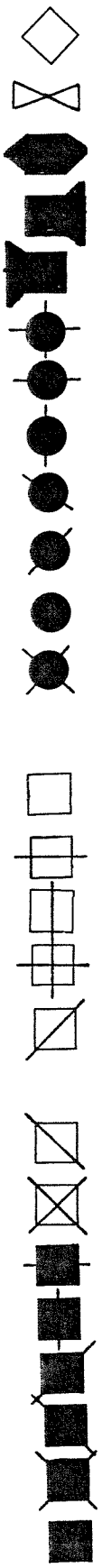


Figure 5.1: Extensive inter-generic survey - linkage level 84

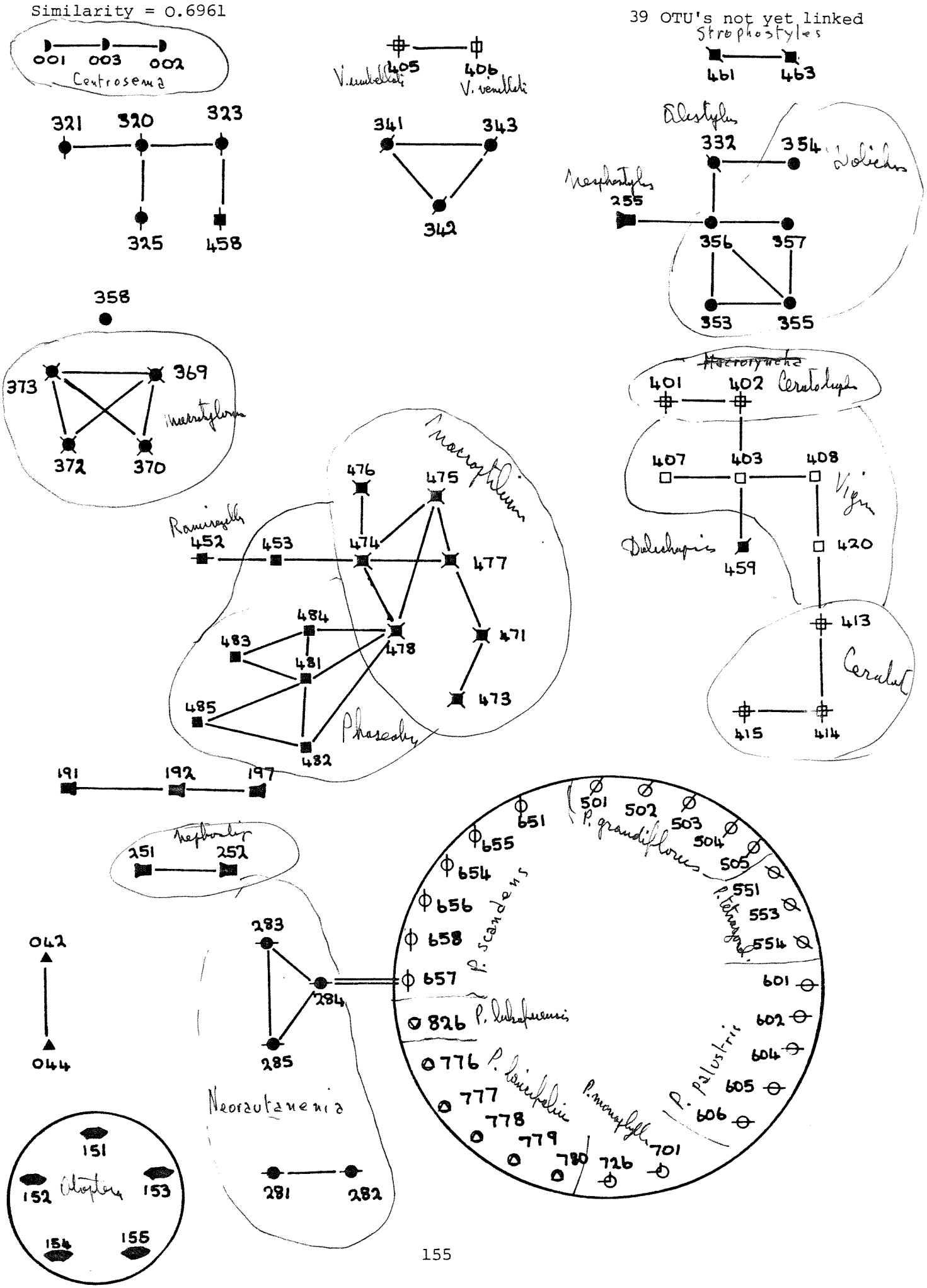


Figure 5.2: Extensive inter-generic survey - linkage level 102

Similarity = 0.6725

24 OTU's not yet linked

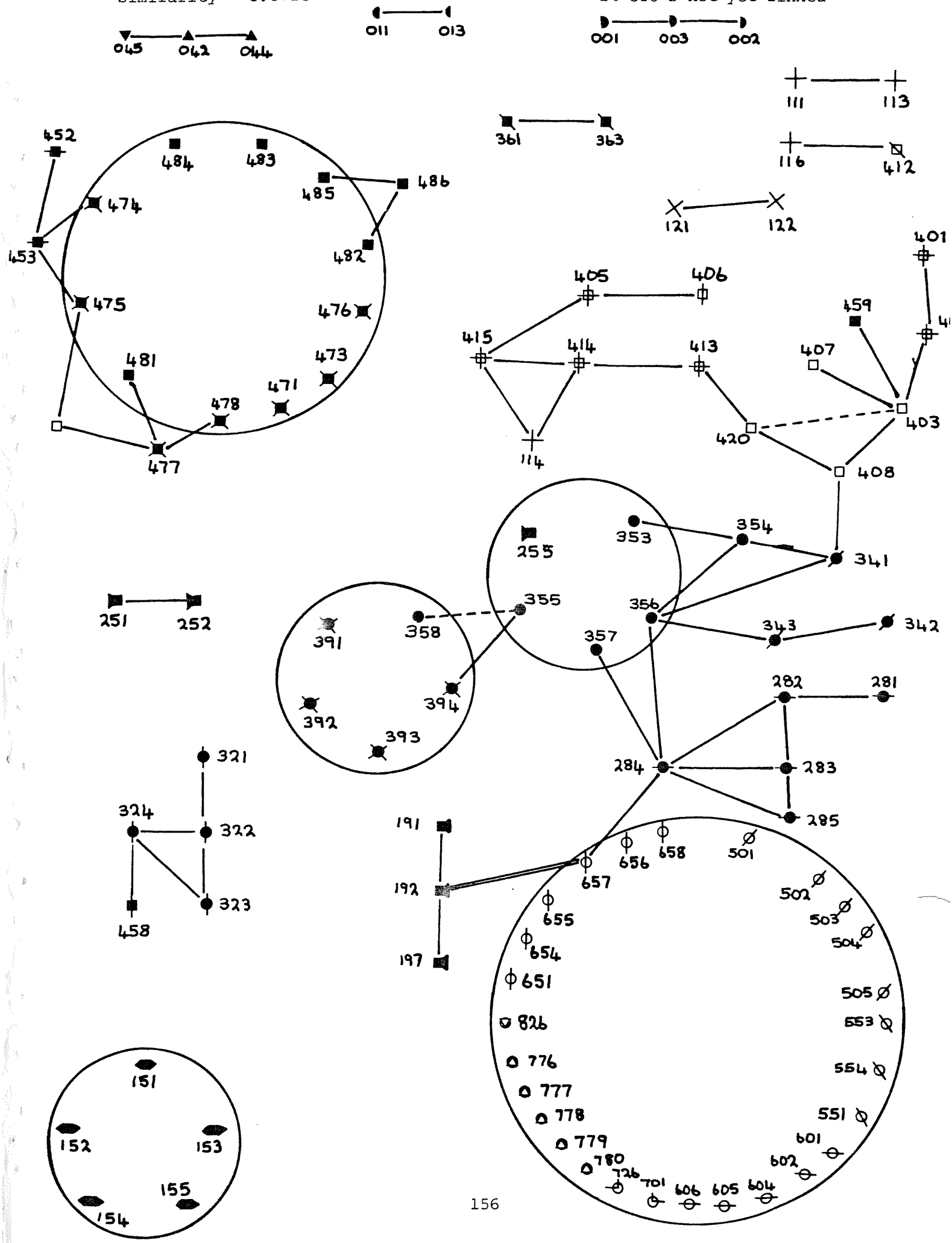


Figure 5.3: Extensive inter-generic survey - LINKAGE level 122

Similarity = 0.6540

11 OTU's not yet linked

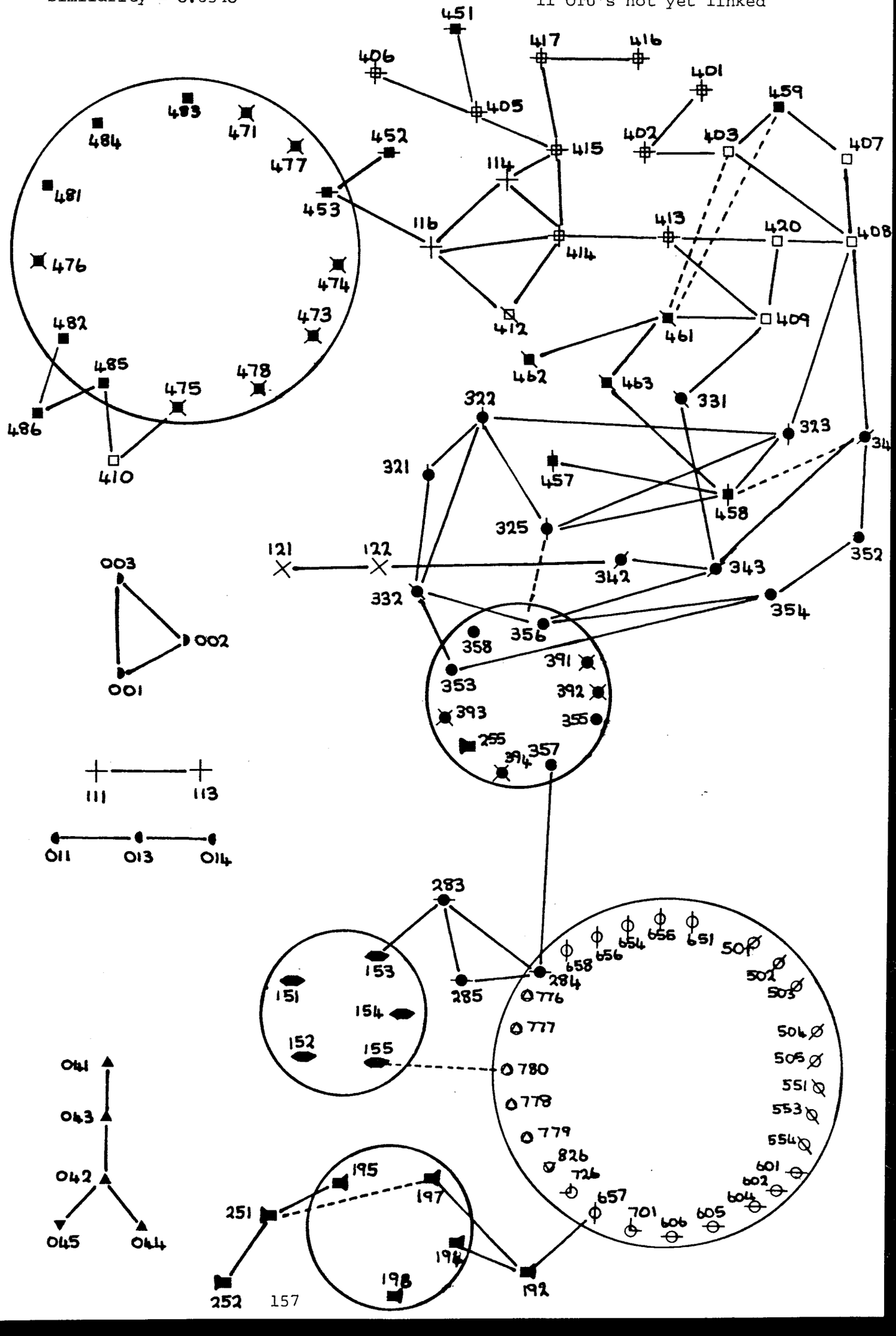


Figure 5.4: Extensive inter-generic survey - LINKAGE level 128

Similarity = 0.6394 7 OTU's not yet linked

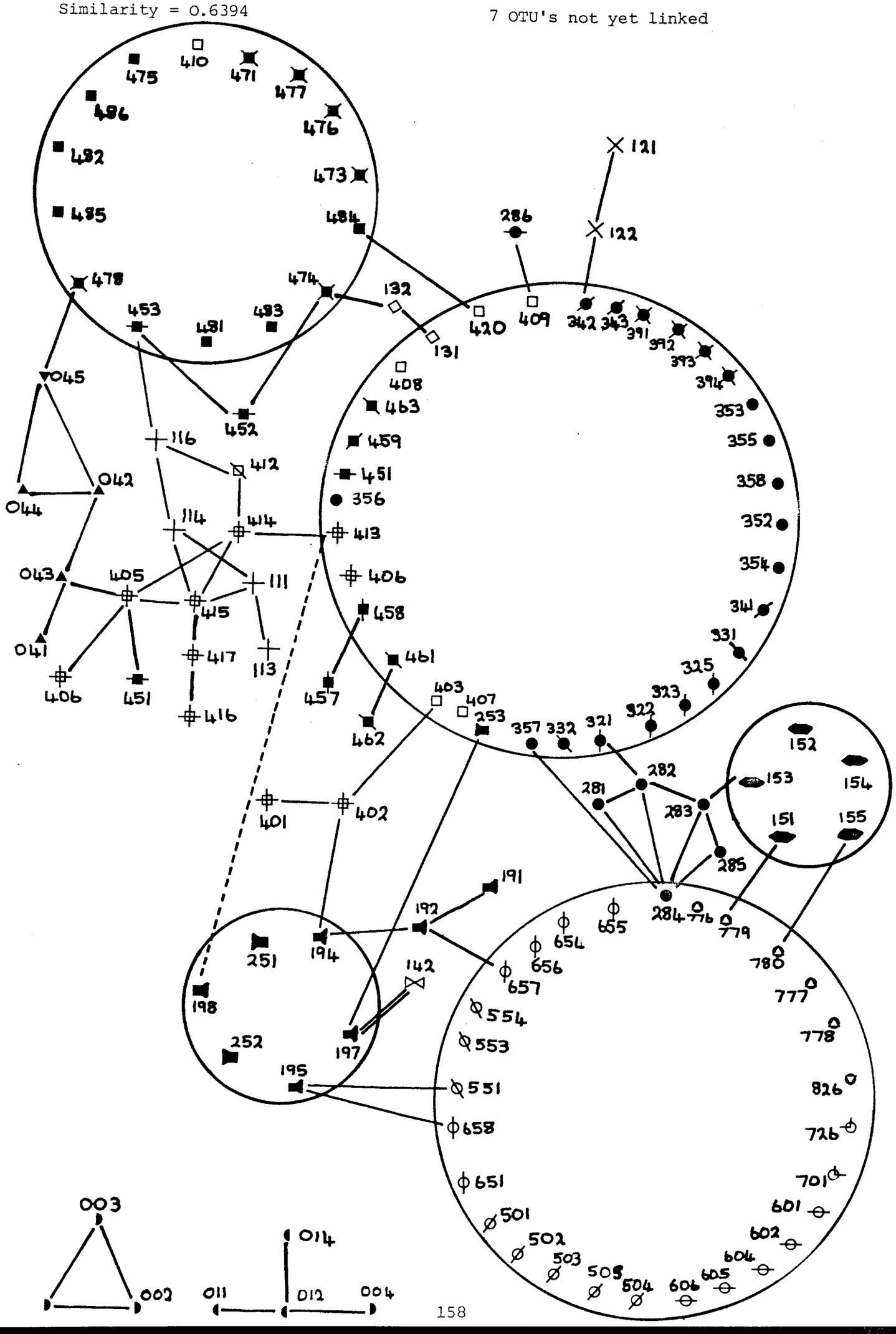
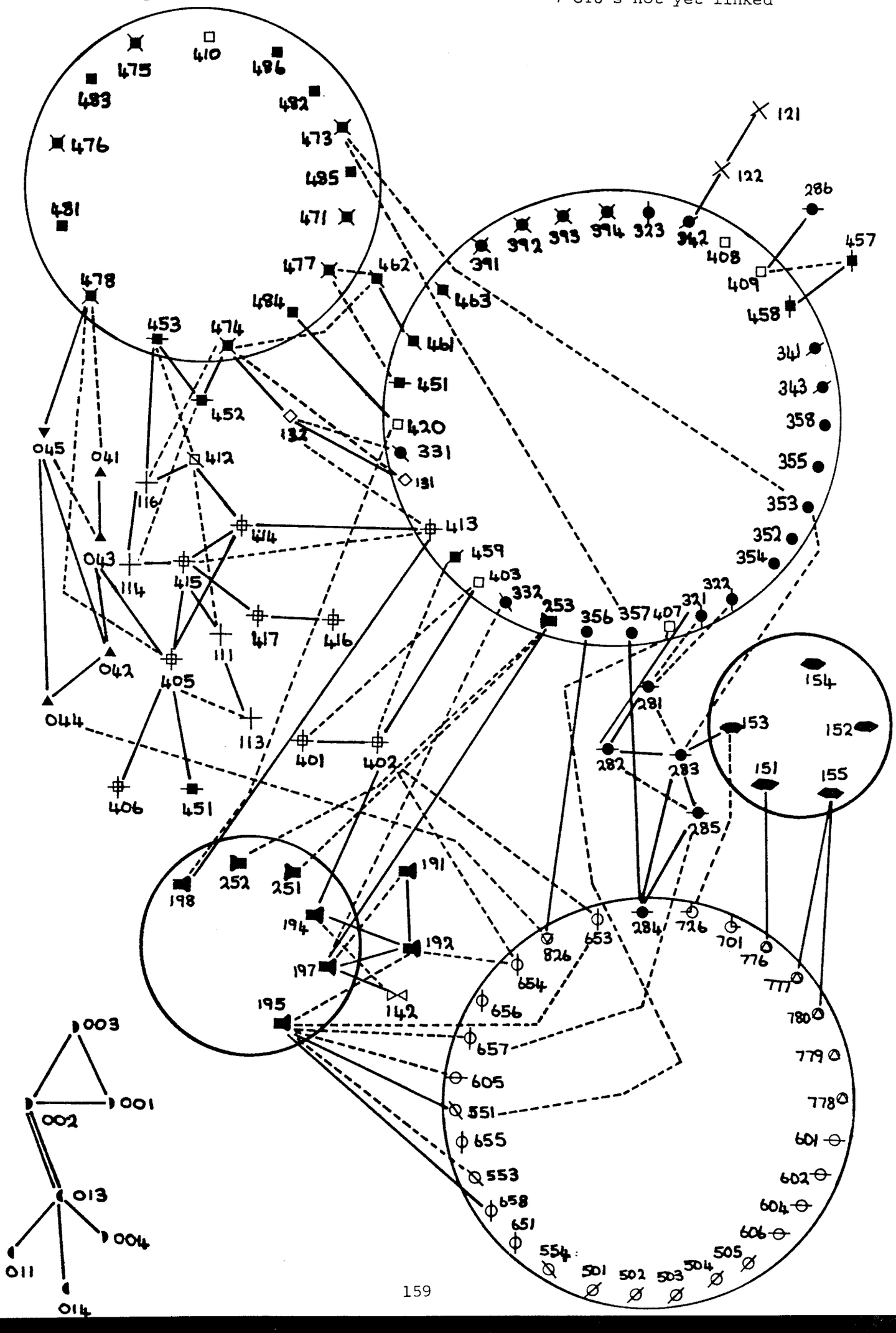


Figure 5.5: Extensive inter-generic survey - LINKAGE level 129

Similarity = 0.6389 7 OTU's not yet linked



sub-clusters. This is indicated in the complexity of new relationship indicated for linkage level 129. Note that the Clitoriinae cluster is now united but remains isolated from the Phaseolinae cluster. In Figure 5.5 *Psophocarpus* strengthens its links with *Otoptera*, *Sphenostylis* and *Neurautanenia* by forming new internal links with these genera. *Psophocarpus* also forms new direct links with three other genera, *Vigna*, *Dolichos* and *Dysolobium*. After this level of similarity, 0.6389, all the genera start to form multiple inter-generic links.

The object of this component study, the extensive inter-generic survey, was to select a sub-set of genera that could be studied more intensely to try to elucidate the closest allies of *Psophocarpus* and the results indicated six genera to be most closely allied: *Neorautanenia*, *Sphenostylis*, *Otoptera*, *Vigna*, *Dolichos* and *Dysolobium*. To these six genera for the intensive study the small genus *Nesphostylis* was added because it is so closely allied to *Sphenostylis* and *Phaseolus* was also included for two reasons: firstly because in this extensive survey the specimens used as OTU's were largely cultivated and so it could be argued were not truly representative of the genus. Secondly, because as *Phaseolus* is obviously not closely related to *Psophocarpus* the more representative *Phaseolus* OTU's could act as useful outliers in the analysis, giving the proposed relationships extra scale and aiding result interpretation. These eight genera with *Psophocarpus* were included in the intensive subset inter-generic study.

Detailed interpretation of the linkage diagrams drawn in this section, for instance pointing out that *Otoptera* tends to link particularly with *Psophocarpus lancifolius*, would be inappropriate here. This component study was designed only as a 'crude' means of restricting the number of genera for the intensive subset generic study. Any inferences such as the *Otoptera* - *P.lancifolius* linkage will be discussed after the taxa have been subjected to the more thorough intensive subset generic study.

5.3 Intensive inter-generic survey for close Psophocarpus allies

In the third phase of the project (as outlined in Section 1.4)

systematic representatives of the nine genera were chosen (*Neorautanenia, Sphenostylis, Nesphostylis, Vigna, Otoptera, Dolichos, Dysolobium, Phaseolus* and *Psophocarpus*). Over 600 specimens representing the nine genera were scored for the 315 characters detailed in Table 4.1. The final number of OTU's analysed in this study was 88, thus for all except the three larger genera, *Vigna, Dolichos* and *Phaseolus*, many specimens socres were averaged to give a representative OTU. For the larger genera specimens (OTU's) were selected to represent the sub-generic variation. Specimens were scored, where possible. for vegetative, inflorescence, legume and seed characters. To ensure that the results obtained from the analysis reflected the natural relationships between the nine genera being studied, the data was analysed: first using a combination of characters selected by CHARANAL and intuitively. Secondly by intuitively selecting a smaller subset of the first character set containing the characters which CHARANAL and Phaseolinae taxonomists considered most useful.

5.3.1 LINKAGE

The intensive subset inter-generic data were analysed using 102 and a subset of 51 characters. For the first analysis using the 102 characters there were 88 linkage levels, which decreased in similarity from 0.8905 to 0.6208. The linkage diagrams or sub-graphs which show the coalescence of inter-generic cluster are shown in Figures 5.6 to 5.13. All the linkage diagrams are drawn that show inter-generic coalescence, rather than just those showing clustering with the *Psophocarpus* cluster, because, as will be seen, *Psophocarpus* remains distinct from the other genera, except *Otoptera*, until the final iteration of the analysis. To draw the two linkage diagrams where *Psophocarpus* unites with other generic clusters would not provide very much helpful information about the inter-generic relationships, other than the obvious, that *Psophocarpus* is most closely related to *Otoptera* and that both these genera are remote compared to the other seven genera being investigated. It is hoped by drawing the eight diagrams that an overall picture will be obtained of the nine genera's inter-relationships.

The first inter-generic coalescence of clusters is shown in Figure

Figure 5.6: Intensive subset inter-generic study (102) - Linkage level 64

Similarity = 0.7137 15 OTU's not yet linked

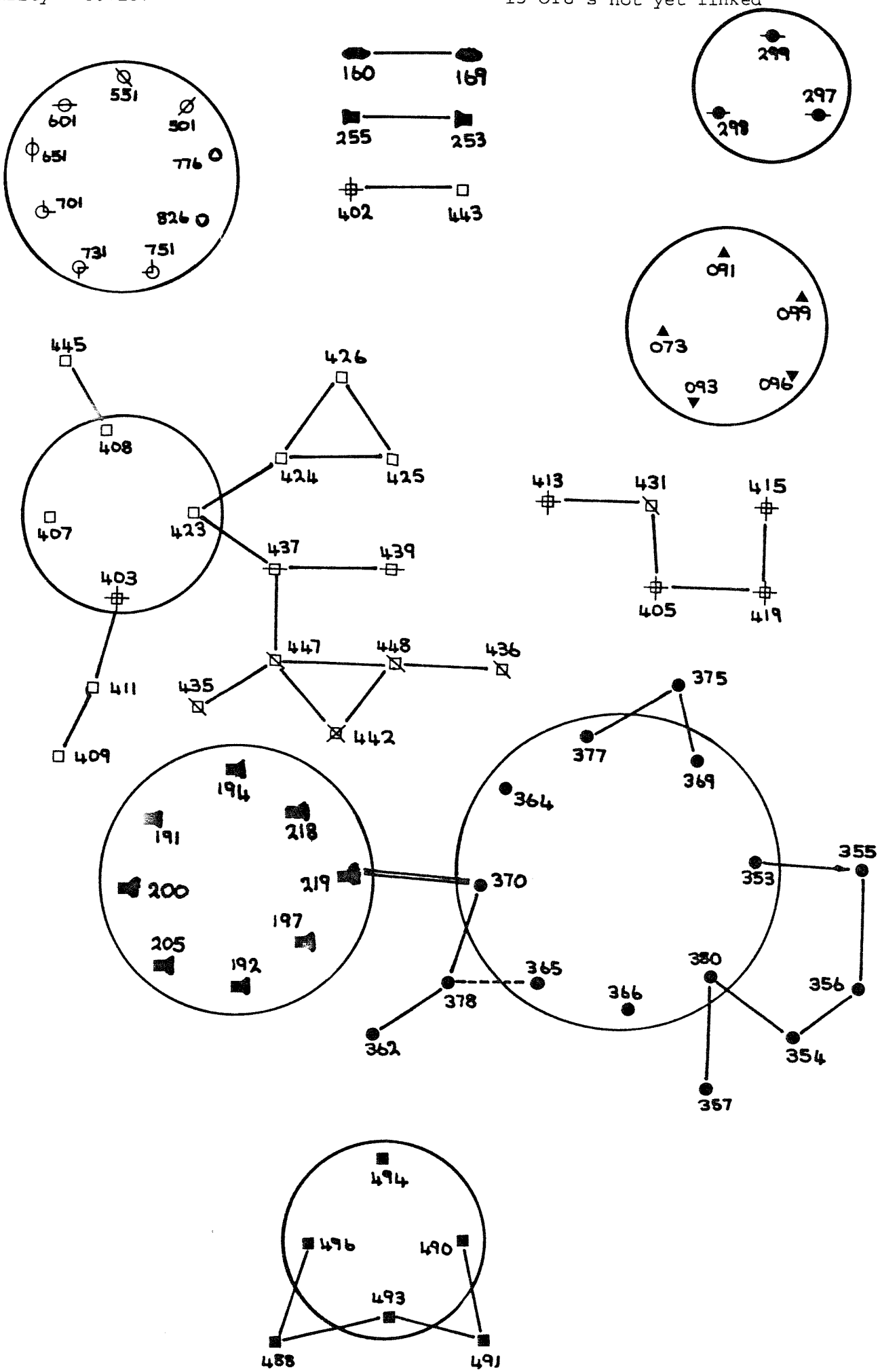


Figure 5.7: Intensive subset inter-generic study (102) - Linkage level 70

Similarity = 0.7042 10 OTU's not yet linked

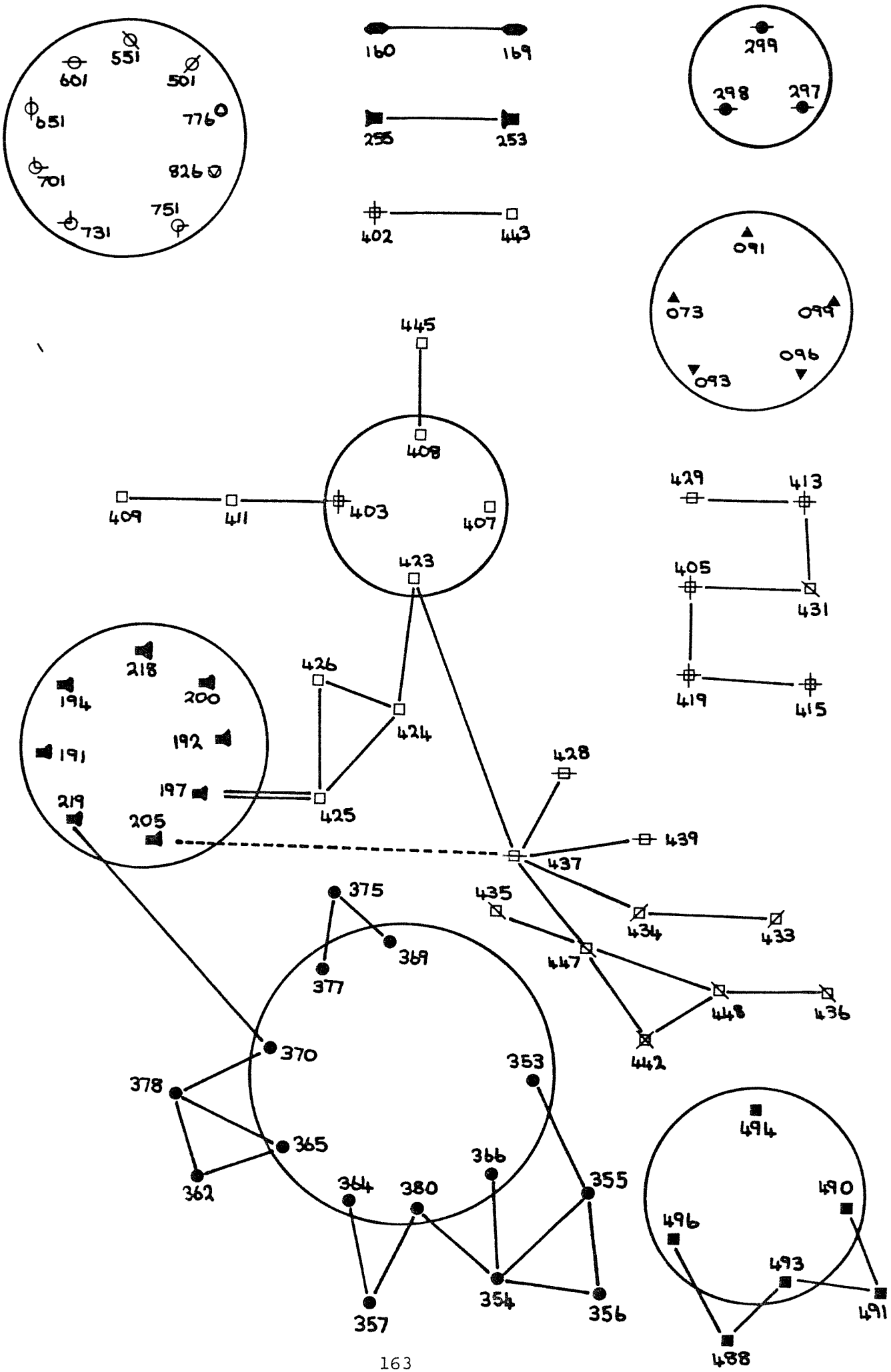


Figure 5.8: Intensive subset inter-generic study (102) - Linkage level 71

Similarity = 0.7039

10 OTU's not yet linked

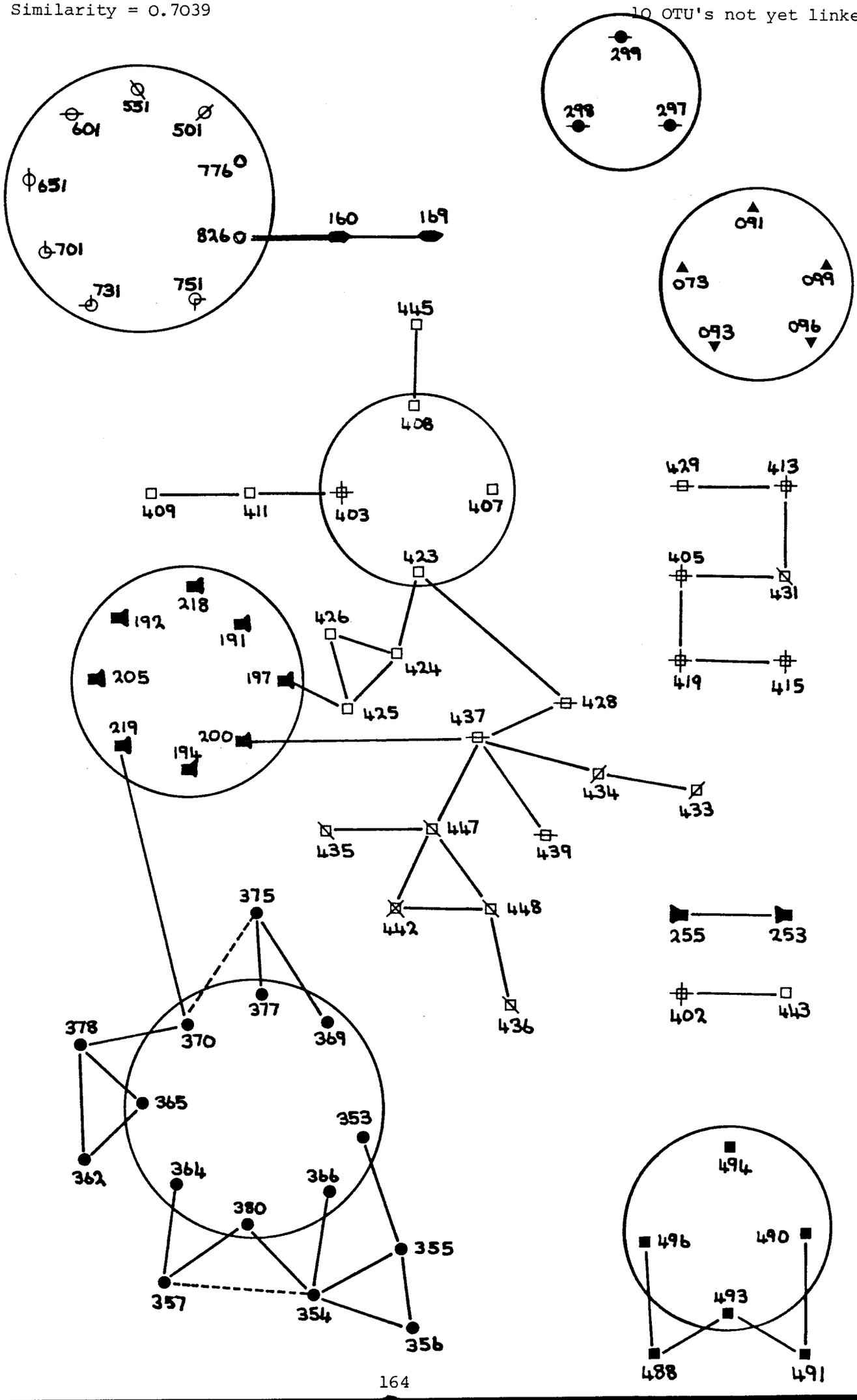


Figure 5.9: Intensive subset inter-generic study (102) - Linkage level 73
Similarity = 0.7009 10 OTU's not yet linked

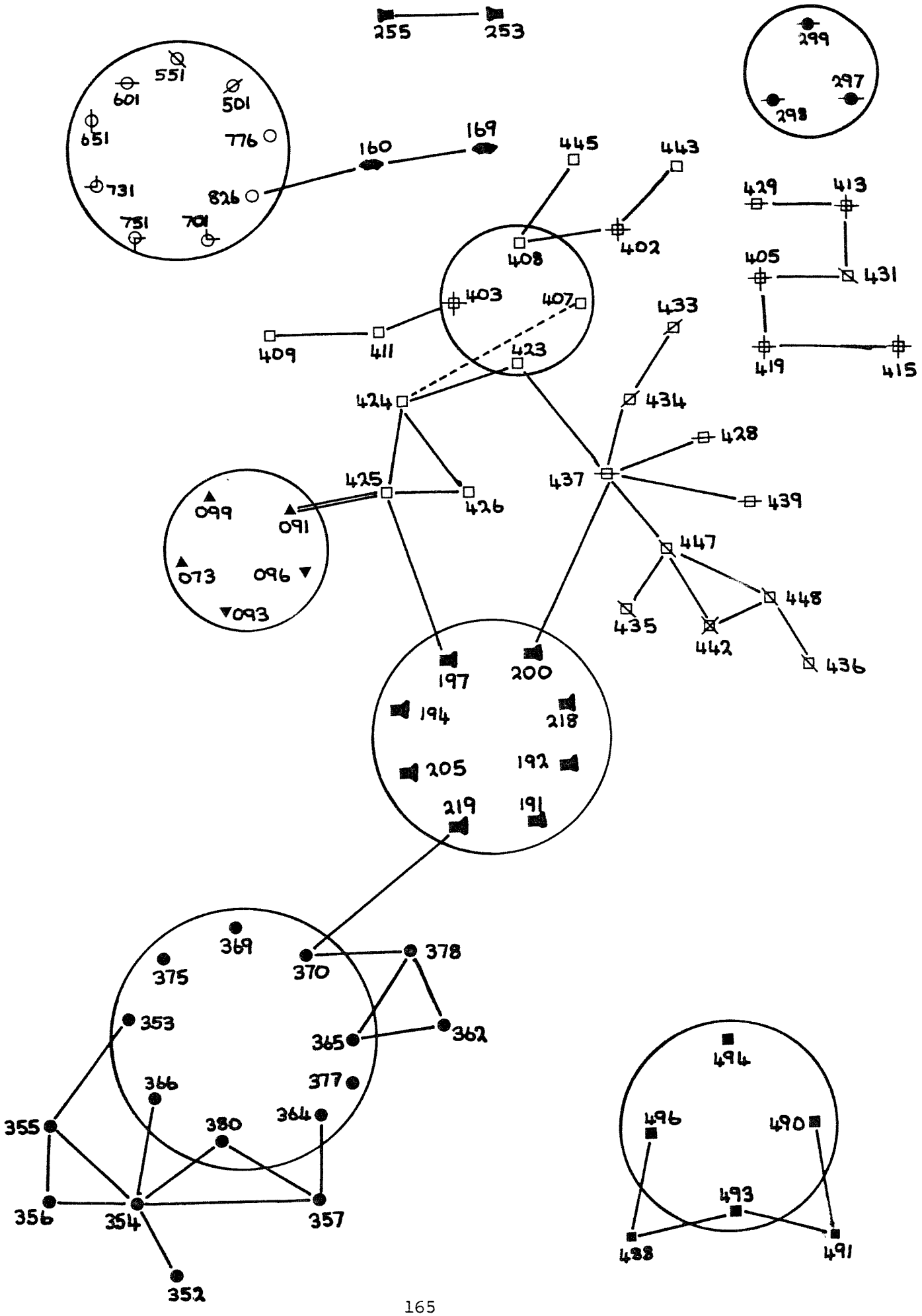


Figure 5.10: Intensive subset inter-generic study (102) - Linkage level 76

Similarity = 0.6946 9 OTU's not yet linked

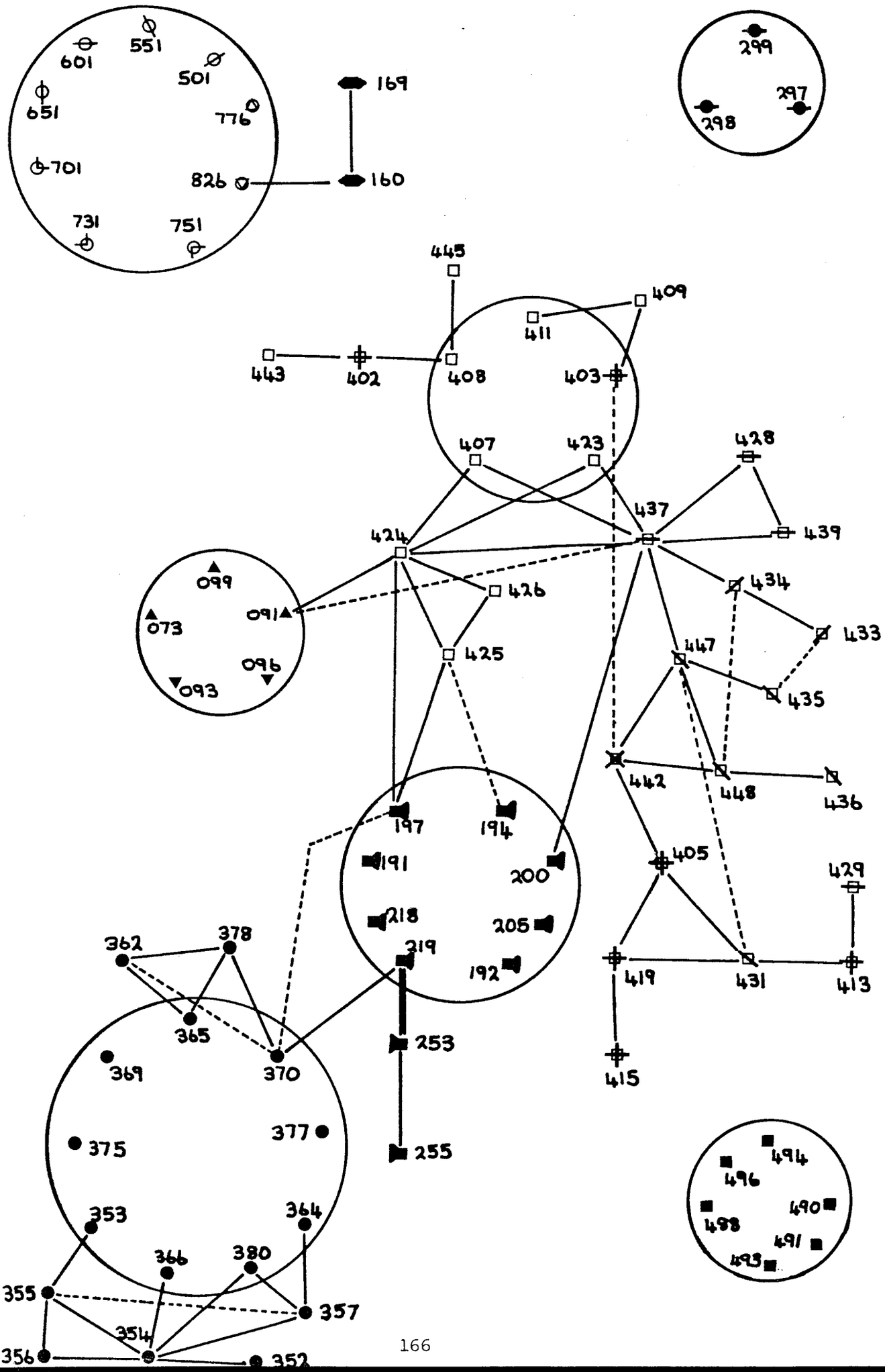


Figure 5.11: Intensive subset inter-generic study (102) - Linkage level 77

Similarity = 0.6900 9 OTU's not yet linked

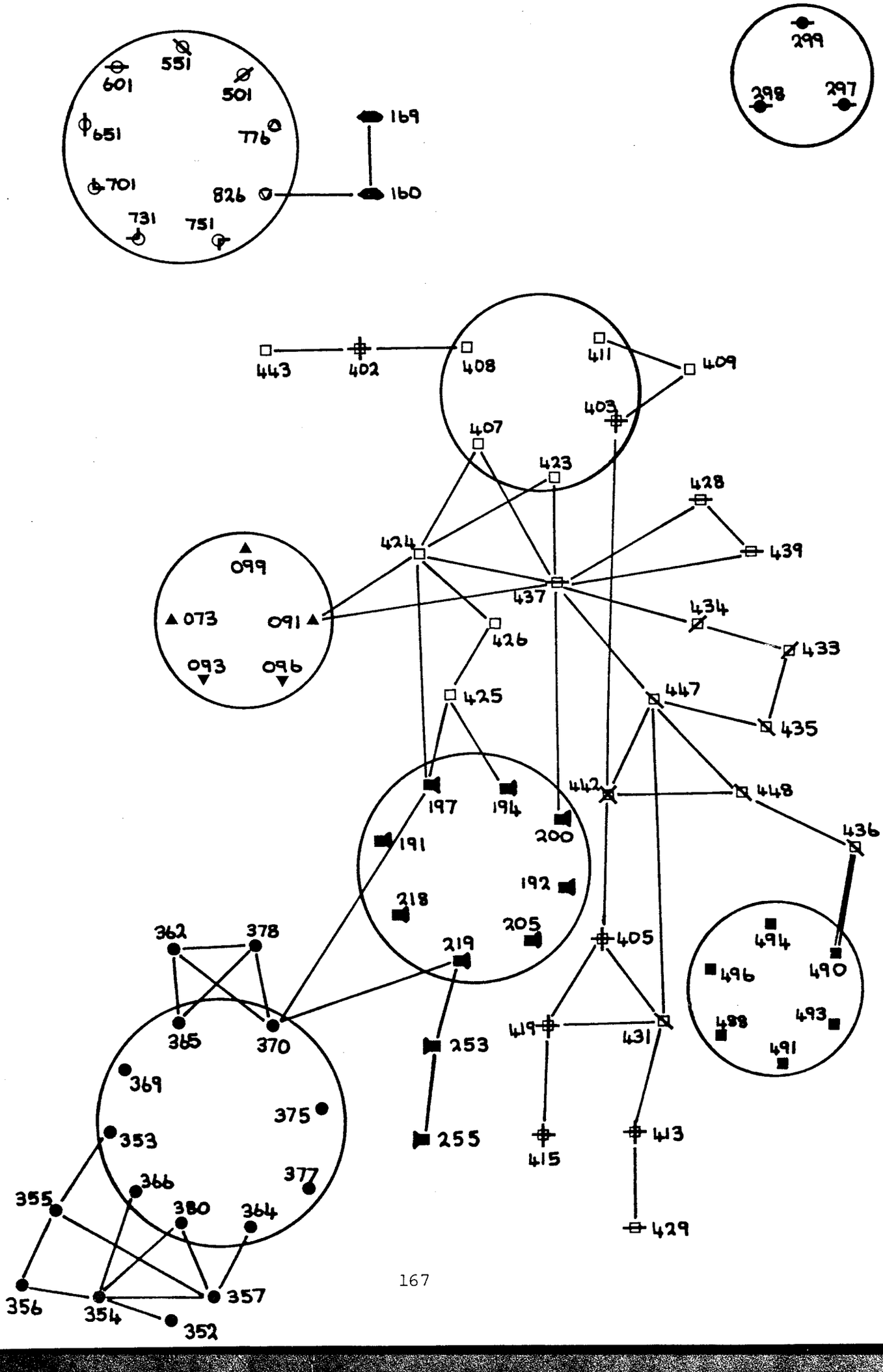


Figure 5.12: Intensive subset inter-generic study (102) - Linkage level 86

Similarity = 0.6510 1 OTU not yet linked

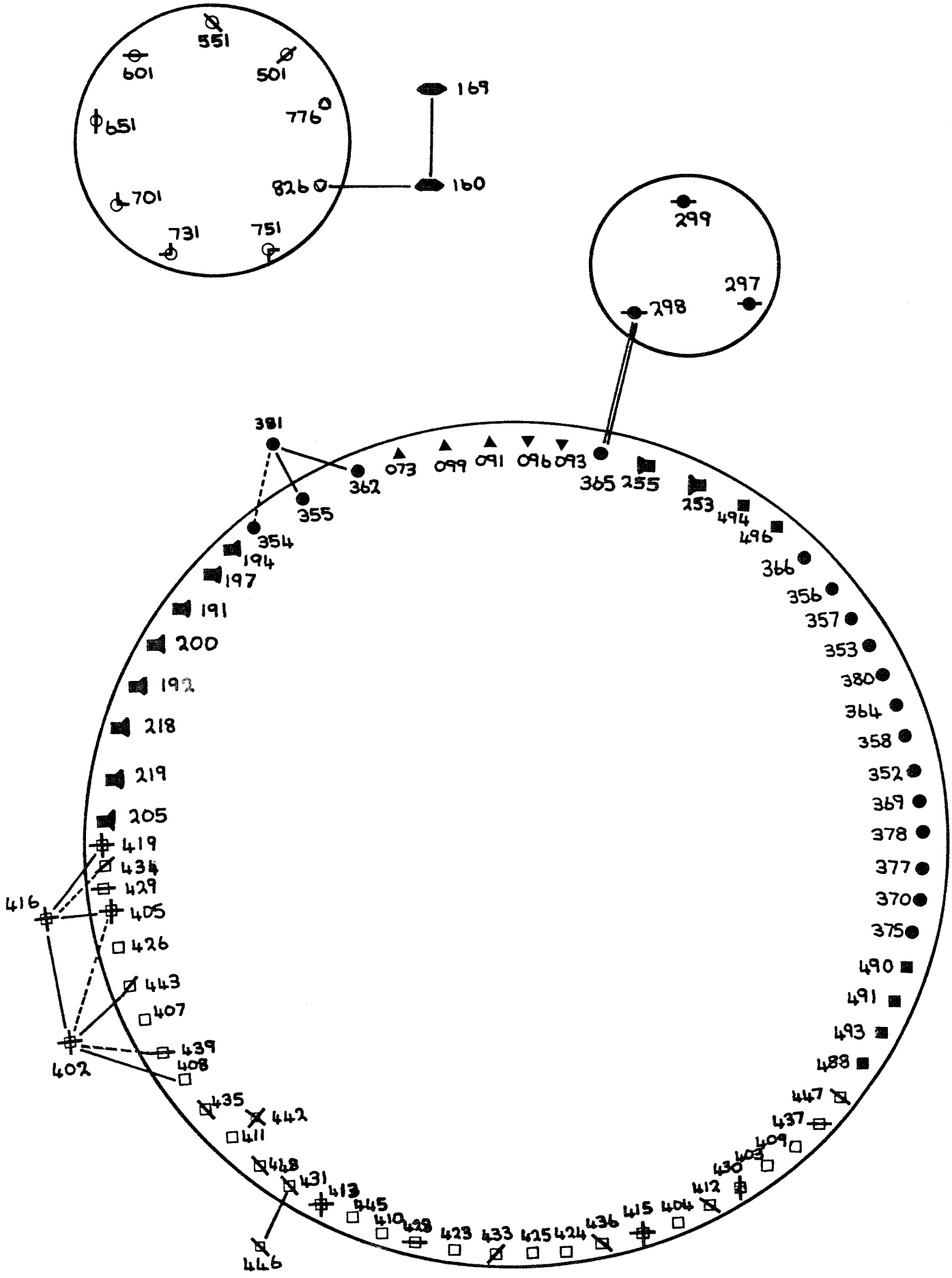
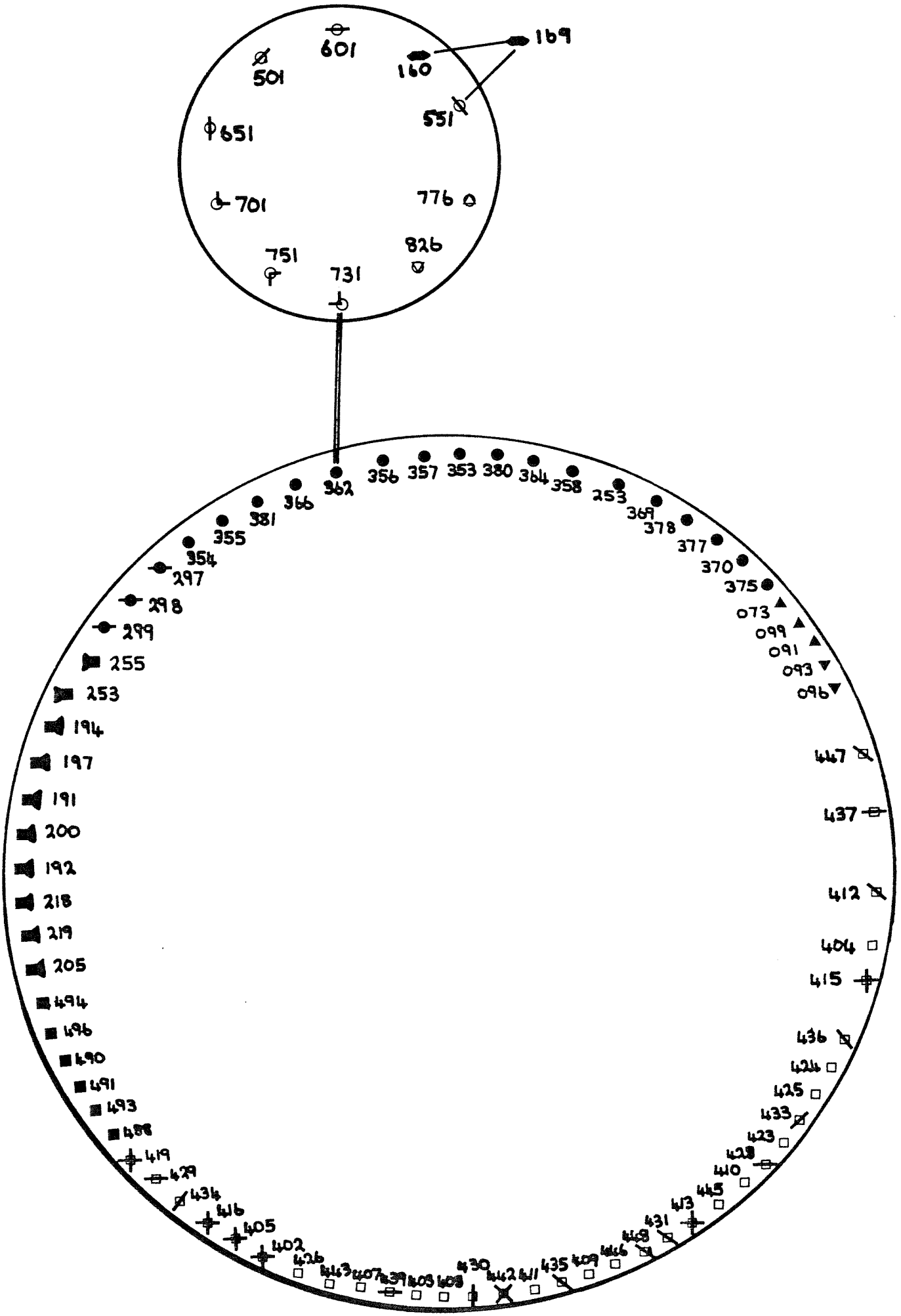


Figure 5.13: Intensive subset inter-generic study (102) - Linkage level 88

Similarity = 0.6208 All OTU's linked



5.6, where at a similarity level of 0.7137 the *Sphenostylis* cluster (containing all *Sphenostylis* OTU's) links with the *Dolichos* cluster, indicating that of the nine genera being investigated these two genera are most closely allied. Figure 5.7 shows the next genus to unite with *Sphenostylis* and *Dolichos*. At a similarity level of 0.7042 a large cluster of *Vigna* OTU's joins *Sphenostylis* via an OTU from *Vigna* subgen. *Vigna*, though at the same level a second link is made between another *Sphenostylis* OTU and an OTU from *Vigna* subgen. *Haydonia*.

The first link between the *Psophocarpus* cluster and another genus is demonstrated in Figure 5.8. At a level of similarity of 0.7039 *Psophocarpus* links with *Otoptera*, in fact the *P.lukafuensis* OTU joins the *O.burchellii* OTU, it is interesting to note from this sub-graph that apart from the initial links between the *Sphenostylis - Dolichos - Vigna* cluster, the genera are remaining isolated; secondary links are not as yet forming. It should be noted also that the clusters of OTU's forming are entirely based on generic limits apart from the single inter-generic links mentioned above. This can be taken to indicate the usefulness of the characters chosen in uniting particular genera but distinguishing between them.

The next genus to cluster with *Sphenostylis*, *Dolichos* and *Vigna* is *Dysolobium* as shown in Figure 5.9. At a level of similairty of 0.7009 the OTU of *Dysolobium lucens* links to an OTU of *Vigna* subgen. *Vigna*. This might at first seem rather surprising, perhaps it would have been expected for one of the *Dysolobium* subgen. *Dolichovigna* OTU's to form the initial link between *Dysolobium* and *Vigna*, because subgen. *Dolichovigna* is considered by some authors (Verdcourt 1970c, Van Welzen and den Hengst, in press) to be a subgenus of *Vigna*. It should be stressed here that the characters chosen for this component study were chosen to specifically elucidate the relationship between *Psophocarpus* and its allies. So any broader conclusion about the precise relationship between other genera must be interpreted with caution.

The next genus to join the main cluster is *Nesphostylis* which as can be seen in Figure 5.10, links with *S*phenostylis, from which it was originally split by Verdcourt (1970a). At this level of similarity, 0.6946, it can be seen that secondary links are beginning to form

between the genera within the main cluster.

Phaseolus is next to join the main cluster (shown in Figure 5.11). At a similarity level of 0.6900 a *Phaseolus* OTU links with a *Vigna* subgen. *Sigmoidotropis* OTU. This linkage is corroborated by Marechal et al (1978a) results, who also found Phaseolus to be most closely allied to subgen *sigmoidotropis* of the *Vigna* subgenera.

By the level of similarity of 0.6510, as shown in Figure 5.12, generic boundaries within the main cluster have broken down sufficiently for all the genera, except the new member *Neorautanenia*, to be included in a circle. The genus which joins the main cluster at this level of similarity, *Neorautanenia*, links with a *Dolichos* OTU.

The final linkage diagram of the 102 character analysis shows the linkage of the *Psophocarpus* - *Otoptera* cluster to the main cluster containing the other genera. At the similarity level of 0.6208, as shown in Figure 5.13, the *Psophocarpus monophyllus* OTU links with a *Dolichos* OTU. The general conclusions that can be drawn from the 102 character LINKAGE analysis are that the closest ally of *Psophocarpus* is *Otoptera* and that *Psophocarpus* and *Otoptera* are relatively isolated from other related genera, but the closest of these other genera is *Dolichos*.

The second LINKAGE analysis results of the intensive subset, inter-generic data, using the reduced character set of 51 characters, are summarised in Figures 5.14 to 5.21. There were 88 linkage levels, decreasing in similarity from 0.9920 to 0.7099. As might have been expected the reduction in character number has increased the similarity between OTU's in comparison to the 102 character analysis. The initial inter-generic linkage is shown in Figure 5.14, where at a similarity level of 0.8249 the cluster of *Sphenostylis* OTU's links with two *Dolichos* OTU's. The next union of genera is shown in Figure 5.15 where at 0.8067 similarity level *Psophocarpus* joins *Otoptera*. As with the 102 character analysis the fusion is between the *P.lukafuensis* OTU and the *Otopera burchellii* OTU. However, it should be noted that with the 51 character analysis *Psophocarpus* has clustered with *Otoptera* earlier in the order of inter-generic coalescence than with the 102 character

Figure 5.14: Intensive subset inter-generic study (51) - Linkage level 45

Similarity = 0.8249 30 OTU's not yet linked

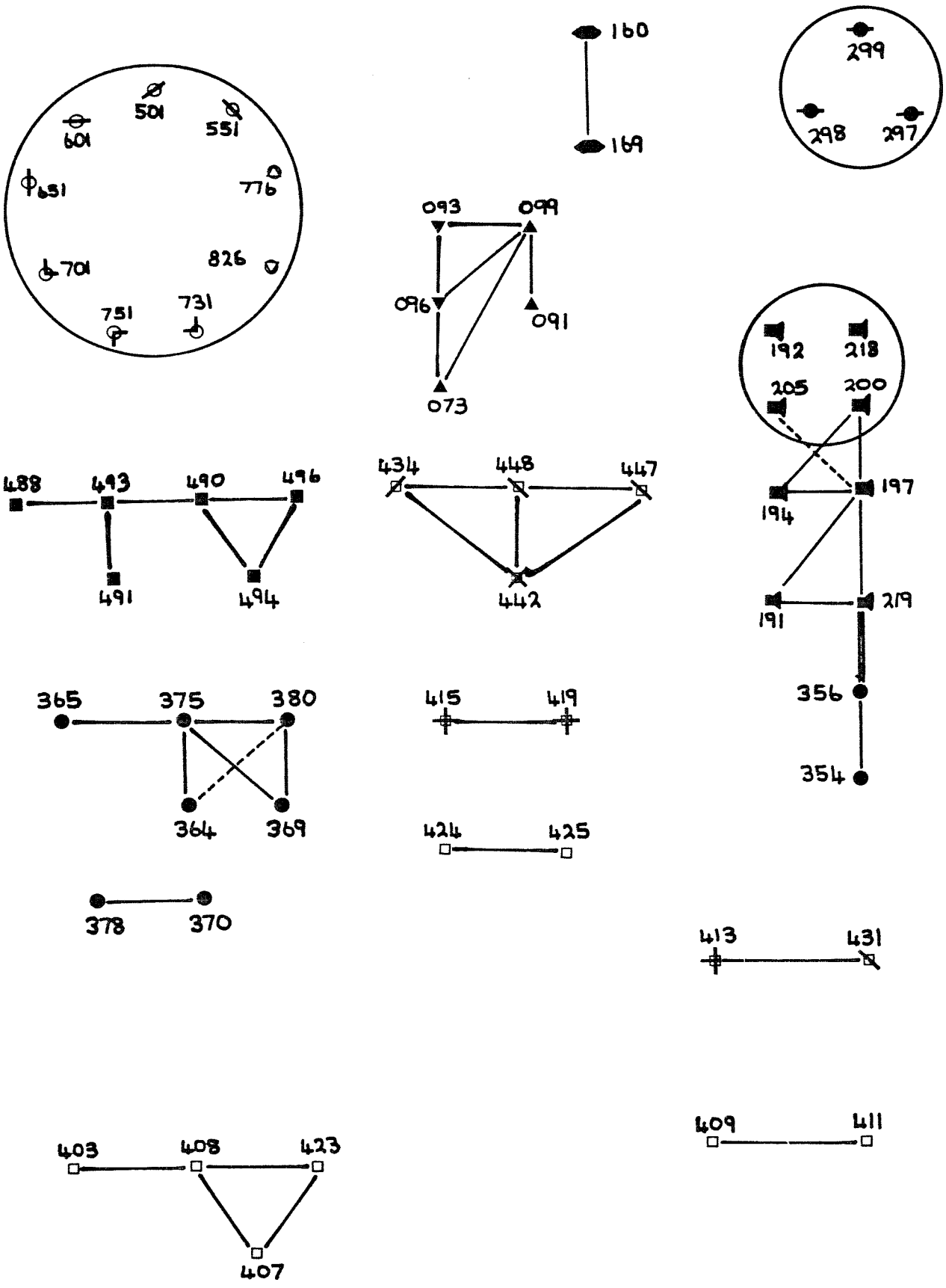


Figure 5.15: Intensive subset inter-generic study (51) - Linkage level 58

Similarity = 0.8067 21 OTU's not yet linked

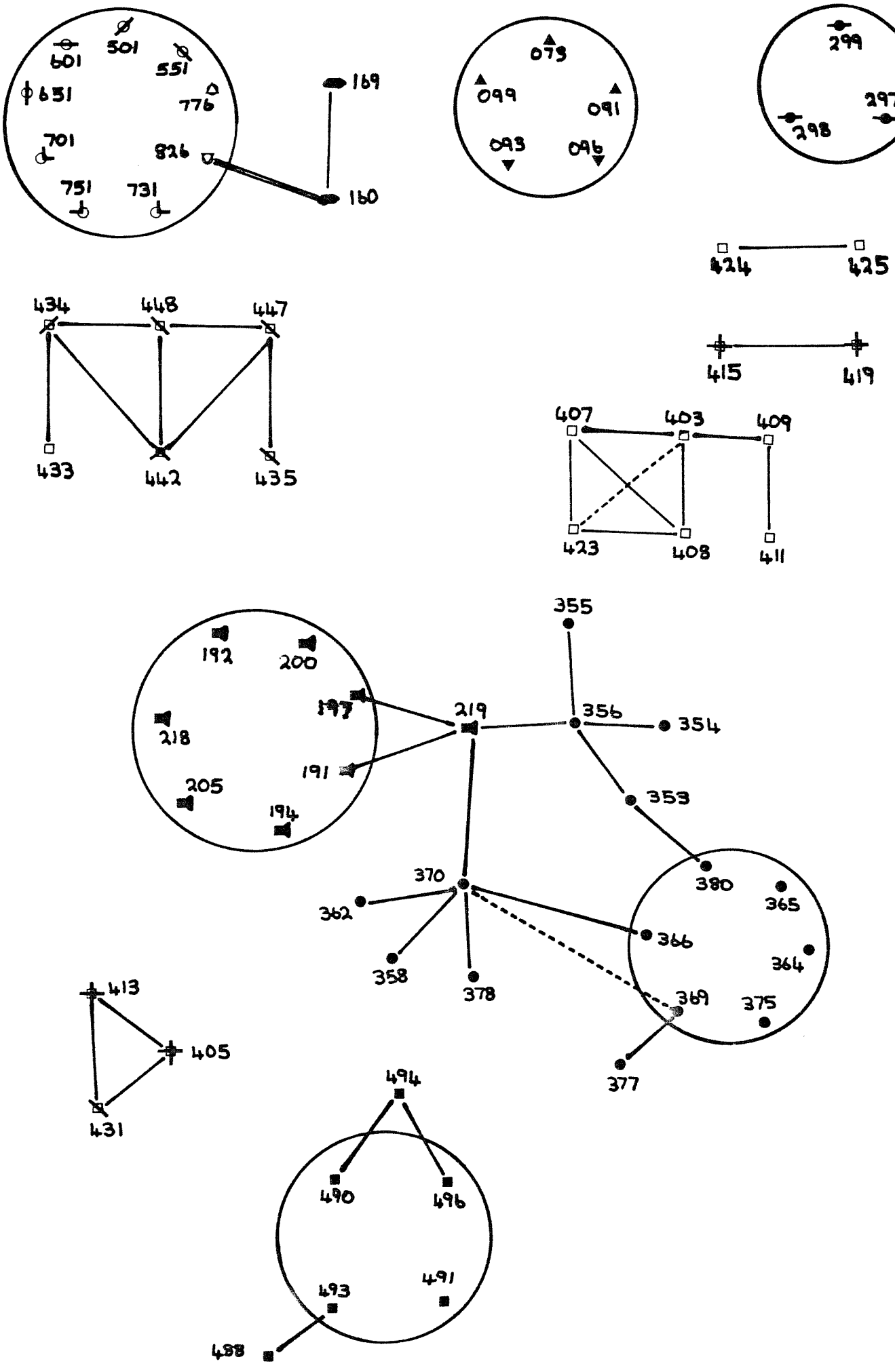


Figure 5.16: Intensive subset inter-generic study (51) - Linkage level 63

Similarity = 0.7952

16 OTU's not yet linked

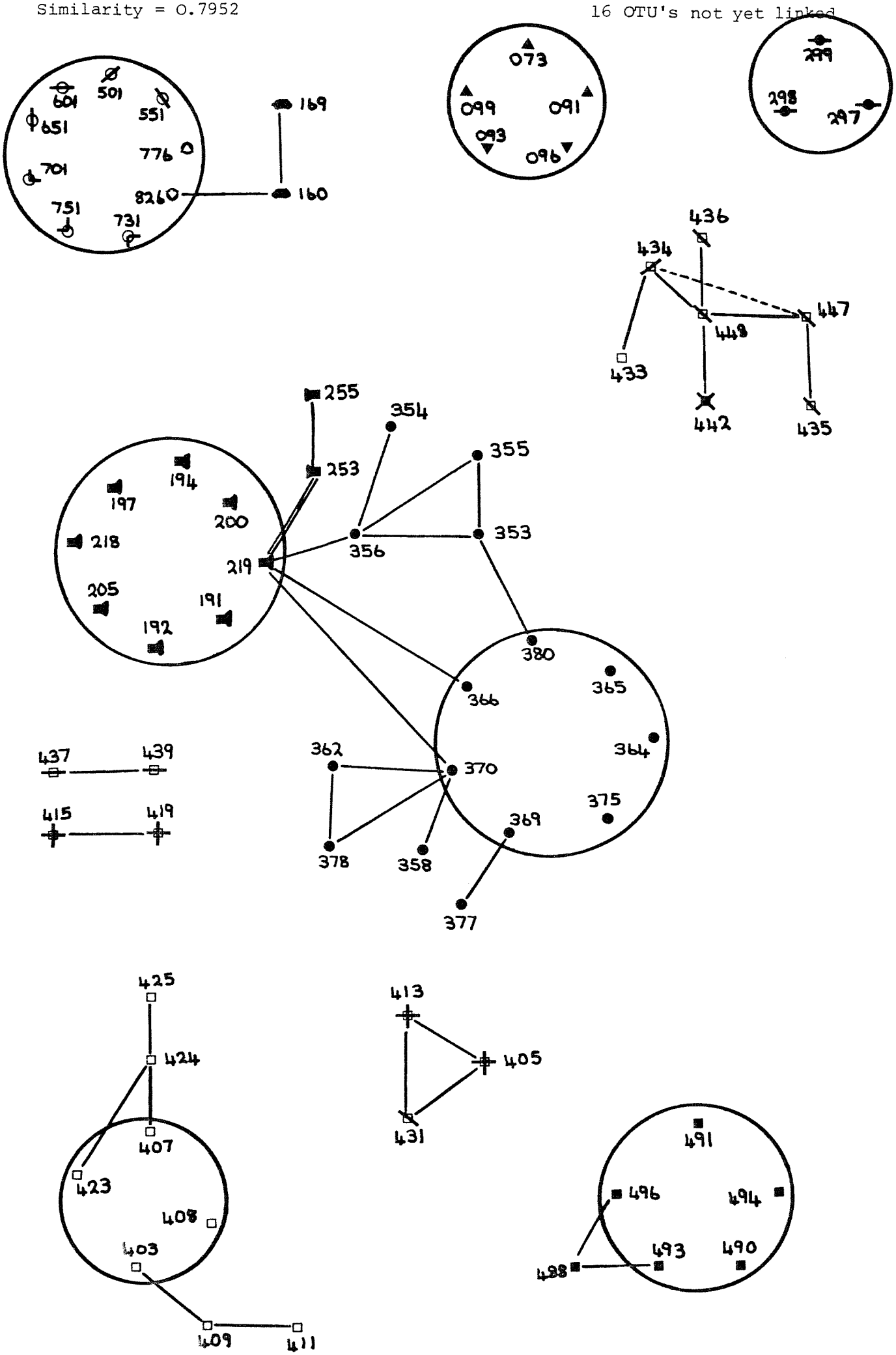


Figure 5.17: Intensive subset inter-generic study (51) - Linkage level 67

Similarity = 0.7866 15 OTU's not yet linked

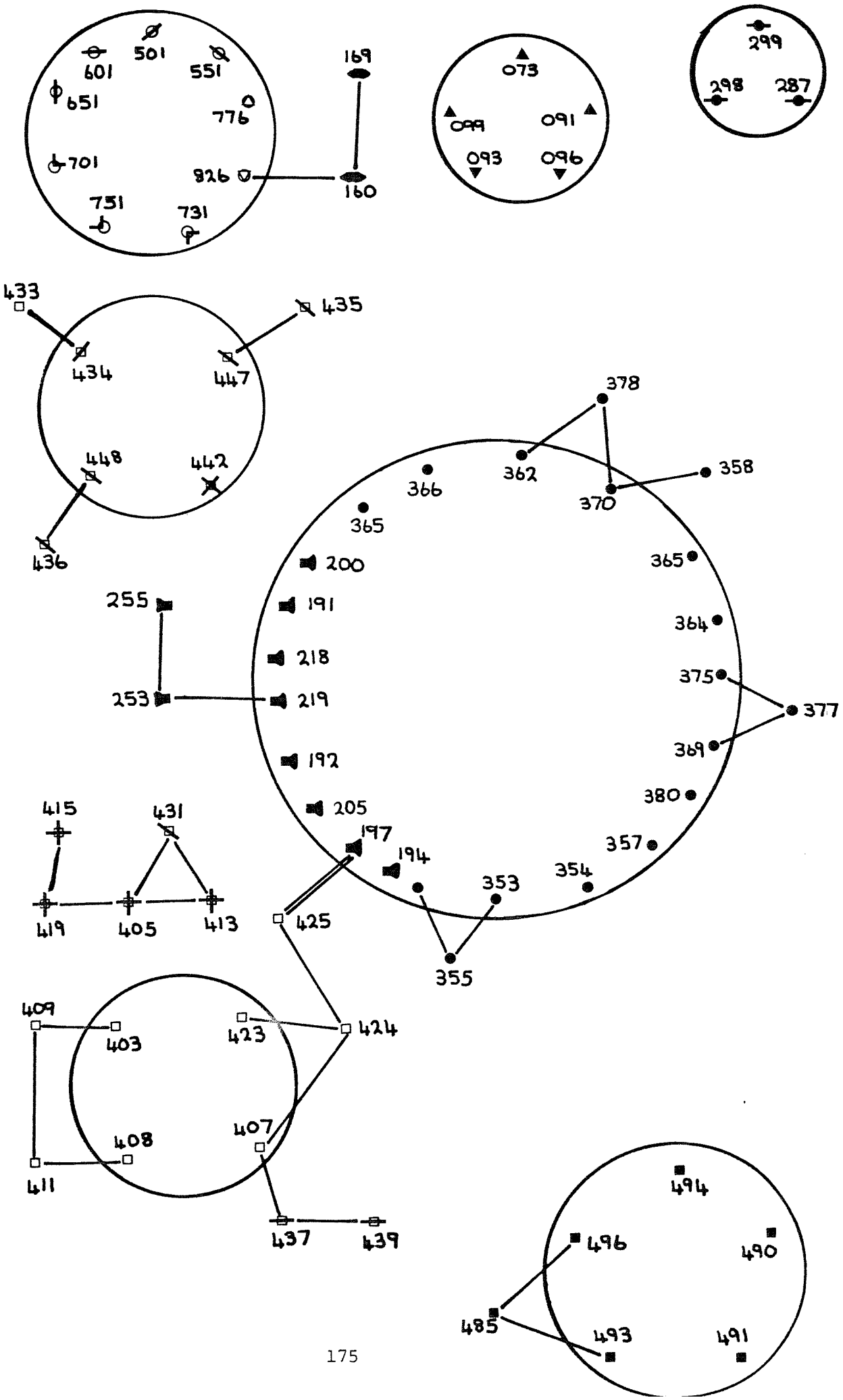


Figure 5.18: Intensive subset inter-generic study (51) - Linkage level 77

Similarity = 0.7572 8 OTU's not yet linked

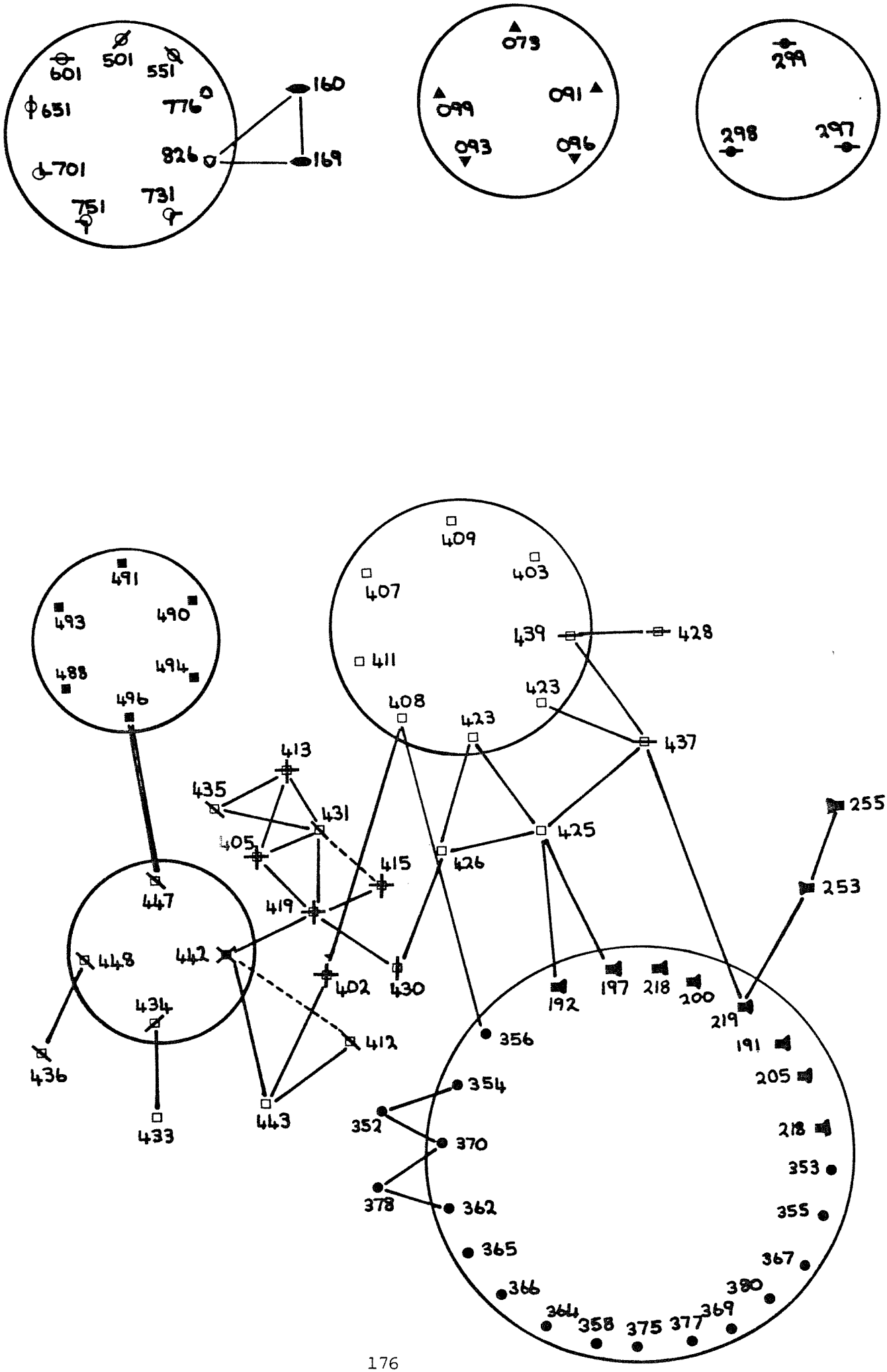


Figure 5.19: Intensive subset inter-generic study (51) - Linkage level 84

Similarity = 0.7385 2 OTU's not yet linked

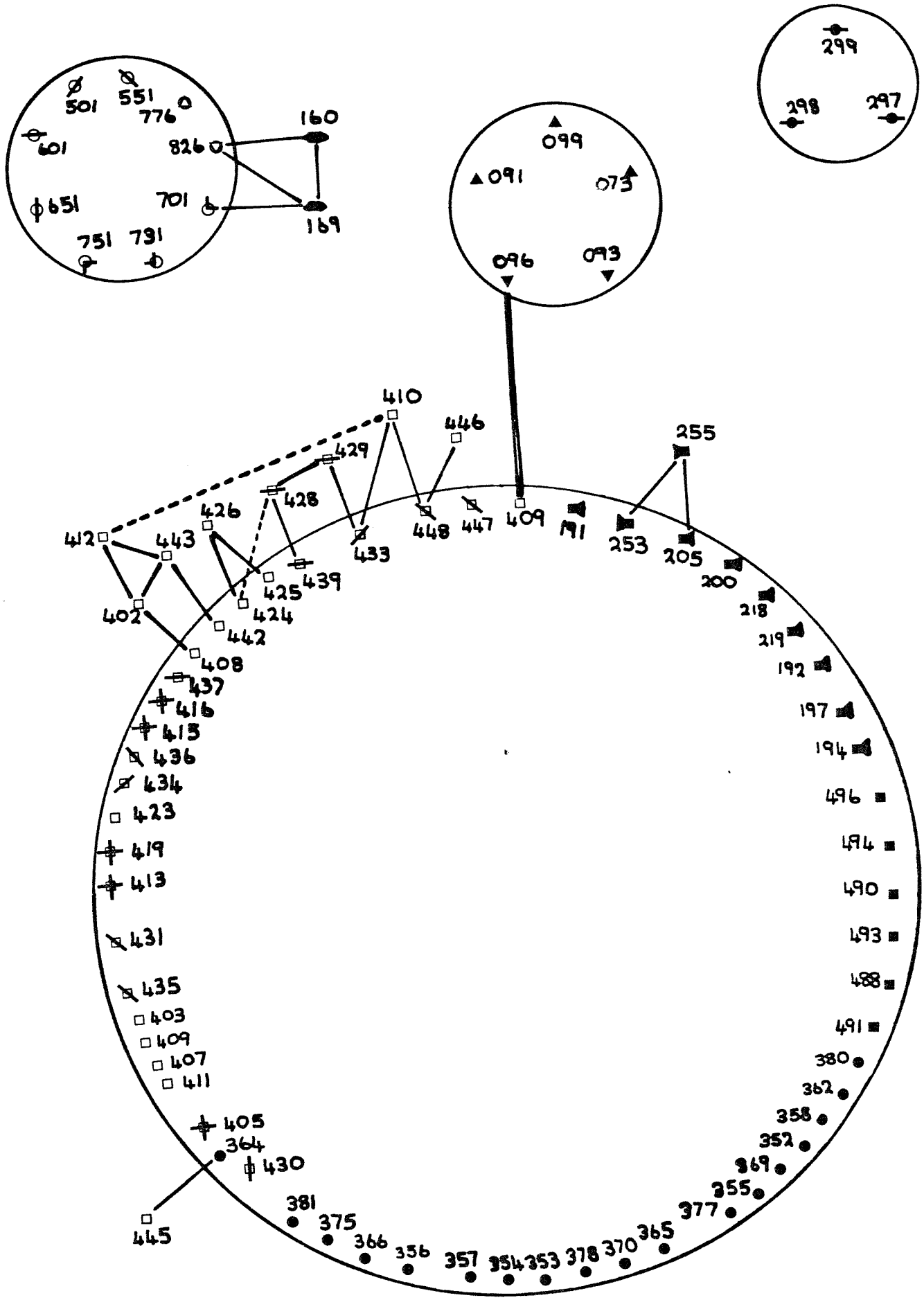


Figure 5.20: Intensive subset inter-generic study (51) - Linkage level 85

Similarity = 0.7343 2 OTU's not yet linked

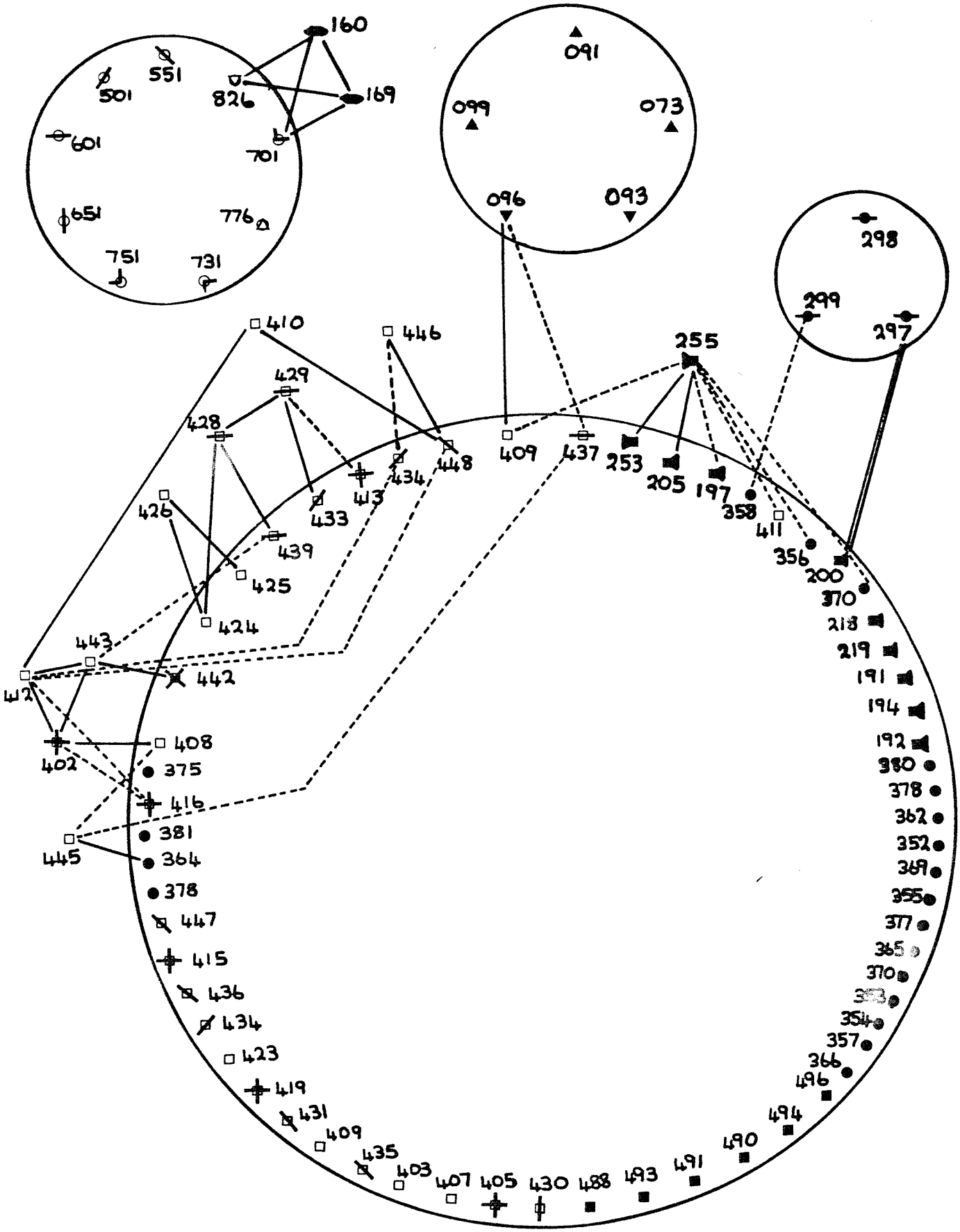
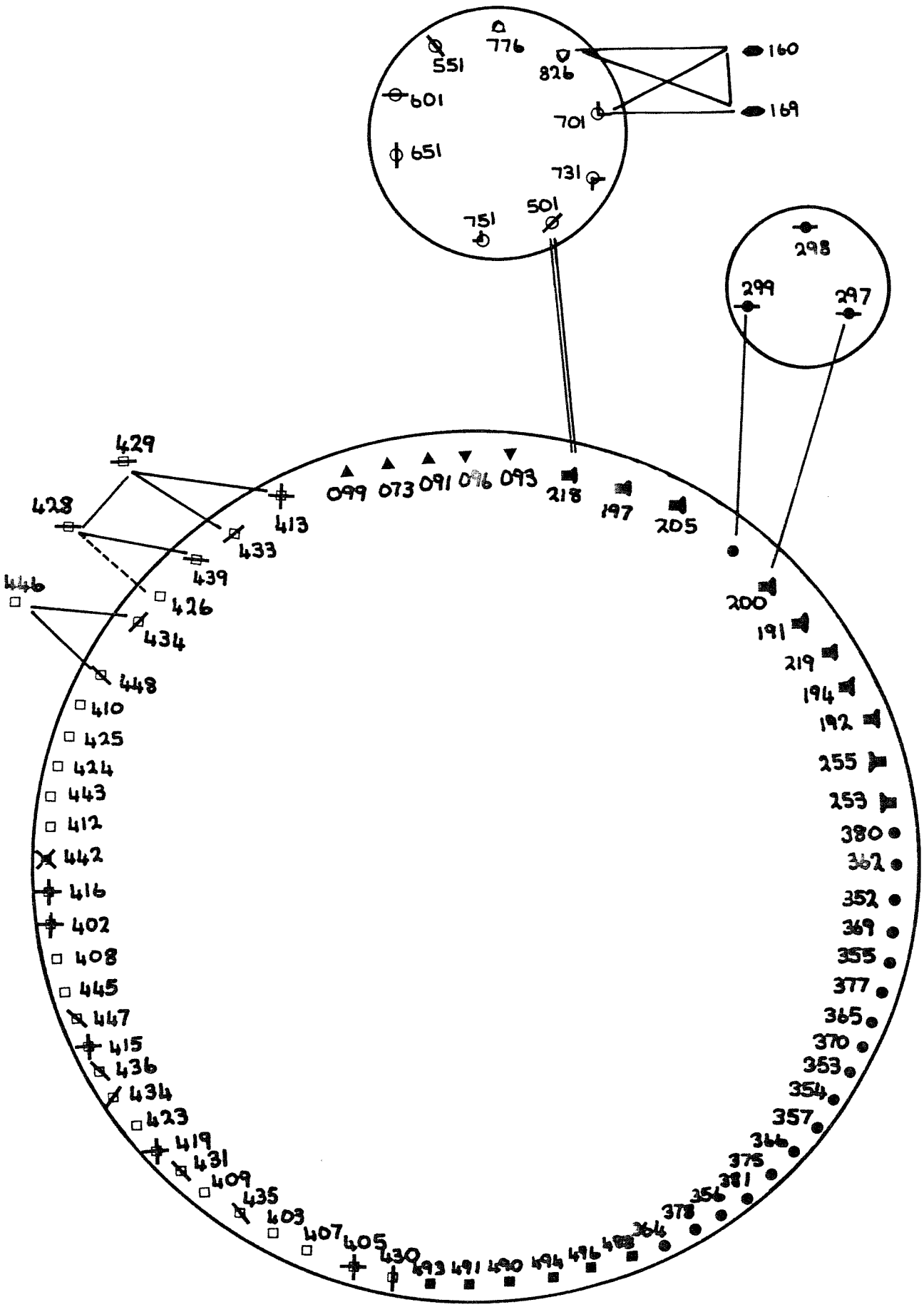


Figure 5.21: Intensive subset inter-generic study (51) - Linkage level 86

Similarity = 0.7164 2 OTU's not yet linked



analysis. In effect the subset of characters chosen for the second analysis indicates a closer relationship between *Psophocarpus* and *Otopera*.

Figure 5.16 shows the third inter-generic fusion. At a similarity level of 0.7952 *Nesphostylis* links with *Sphenostylis*. As with the linking of *Otoptera* and *Psophocarpus*, this fusion is earlier in the order of inter-generic coalescence than with the 102 character analysis. This earlier fusion of *Nesphostylis* and *Sphenostylis* perhaps reflects their closely allied nature better than the larger character set analysis.

The next genus to form links with the main cluster is shown in Figure 5.17, where at the similarity level of 0.7866 a cluster of largely *Vigna* subgen. *Vigna* OTU's links with a *Sphenostylis* OTU. It can also be seen that at this similarity level, *Sphenostylis* and many *Dolichos* OTU have formed enough internal links to warrant inclusion in a circled cluster. Though the other clusters are retaining their generic identity, as with the larger character set analysis, this may be taken to indicate the usefulness of the characters chosen in uniting each particular genus, but distinguishing between them.

The generic cluster of *Phaseolus* joins the main cluster next, at a similarity level of 0.7572, as shown in Figure 5.18. *Phaseolus* joins the main cluster via an OTU of *Vigna* subgen. *Sigmoidotropis* as in the 102 character analysis. This may be taken to indicate a true affinity between *Phaseolus* and *Vigna* subgen. *Sigmoidotropis*.

In the next linkage diagram drawn in Figure 5.19, the main cluster of genera can be seen to have formed sufficient internal links to warrant inclusion in an encircled cluster. At this level of similarity, 0.7385, a *Dysolobium* OTU links with a *Vigna* subgen. *Vigna* OTU. Interestingly, however, the link is with a *Dysolobium* OTU from subgen. *Dolichovigna* not subgen. *Dysolobium* as in the 102 character analysis. As mentioned in discussing this link in the 102 character analysis above, the link would be expected to form between *Vigna* and *Dysolobium* subgen. *Dolichovigna* because of *Dysolobium* subgen. *Dolichovigna* possibly being more naturally placed in *Vigna*. This may be interpreted as one

indication that the second, 51 character subset results, reflect the natural relationships of the taxa better than the 102 character analysis.

The next genus to join the main cluster of genera is *Neorautanenia*. This forms two links at a similarity level of 0.7343, one with an OTU of *Sphenostylis* the other with a *Dolichos* OTU, as indicated in Figure 5.20. It should also be noted that *Otoptera* is forming multiple links with *Psophocarpus*, though these are insufficient as yet to warrant the inclusion of the two genera in the same encircled cluster.

The final linkage level drawn shows in Figure 5.21 the linkage of the *Psophocarpus* - *Otoptera* cluster to the main clluster. At a similarity level of 0.7164 the *P.grandiflorus* OTU joins a *Sphenostylis* OTU. This is quite different from the 102 character analysis result where the *P.monophyllus* OTU links with a *Dolichos* OTU. However, as the analysis shows *Dolichos* and *Sphenostylis* are closely related, so it is not so surprising that *Psophocarpus* links with different genera after *Otoptera* in the two LINKAGE analyses undertaken.

To summarise the results of the two LINKAGE analyses using 102 and 51 characters; *Psophocarpus* is clearly indicated to be most closely allied to *Otoptera*. These two genera are relatively isolated from the other genera being studied. However, of the other seven genera *Dolichos* and its allies seem more closely allied than the *Phaseolus* - *Vigna* complex genera. Of *Dolichos* and its allies, *Dolichos* and *Sphenostylis* seem to be closest to *Psophocarpus*.

5.3.2 CLUSTAN (DISTIN)

For this analysis the same two character sets were used as for the analysis in the previous section. In both cases the lower triangle of the similarity matrix produced by LINKAGE, was used by the CLUSTAN sub-routine DISTIN and HIERARCHY to carry out average linkage cluster analysis. The method used is discussed in full in Section 3.4.2(b).

The two dendrograms produced by procedure PLINK after the average linkage cluster analysis are drawn in Figures 5.22 and 5.23, being for

Figure 5.22: Intensive subset inter-generic study (102) - CLUSTAN (DISTIN)

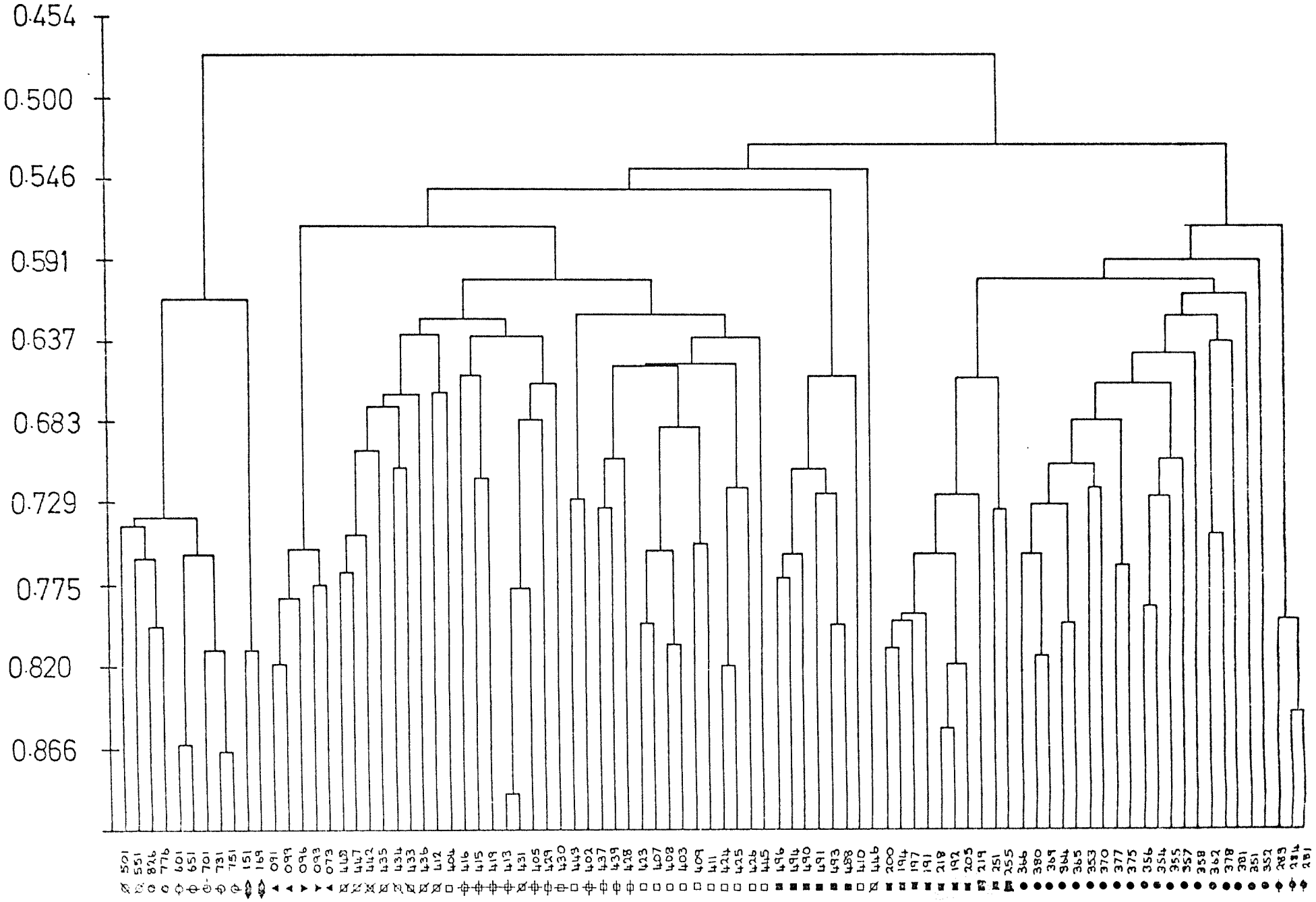
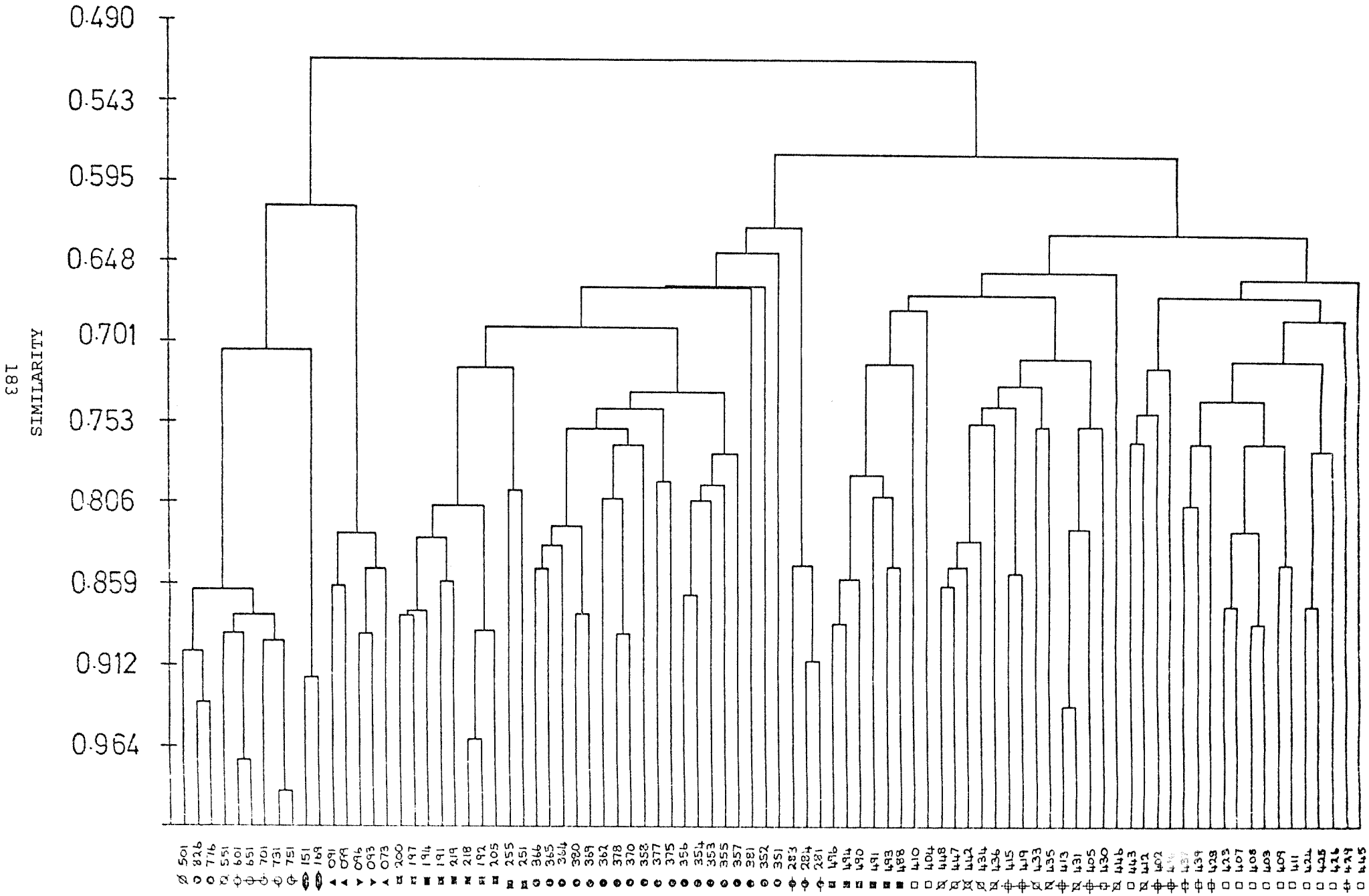


Figure 5.23: Intensive subset inter-generic study (51) - CLUSTAN (DISTIN)



the 102 and 51 character analysis respectively.

The first thing to note about Figure 5.22 is that as with the LINKAGE analysis *Psophocarpus* can be seen to be most closely allied to *Otoptera* and that these two genera are distinct from the other genera in the analysis. Any further conclusions about the relationship of *Psophocarpus* with the other genera in the analysis must be speculative as *Psophocarpus* and *Otoptera* are so clearly isolated from the main cluster. It is worth noting, however, that the main cluster divides neatly into two, comprising the *Phaseolus* - *Vigna* complex (including *Dysolobium*) and *Dolichos* with its allies (*Sphenostylis*, *Nesphostylis* and *Neorautanenia*).

The OTU's representing genera can be seen to cluster neatly toghether, with perhaps only one possible misplacement where OTU 410, a specimen of *Vigna gracilis* clusters with the *Phaseolus* OTU's rather than with the *Vigna*. This finding contrasts strongly with the results of Marechal et al (1978a) and was not reflected in the previous analyses using LINKAGE. The reason for this OTU's misplacement is not clear, because had erronious character scoring been the cause then it would also have been reflected in the LINKAGE analysis.

It is very tempting to comment on the specific arrangement of the *Psophocarpus* species OTU's, which seem to appear oddly ordered. However as pointed out previously the characters chosen for this study are in large different from those chosen for the inter-specific study, and so discussion of inter-specific relationships will be confined to the appropriate sections of this chapter.

The second dendrogram drawn in Figure 5.23, produced from the average linkage analysis results of the 51 character subset of the larger 102 character set, shows some interesting differences to the former dendrogram. The most important is the movement of the *Dysolobium* cluster from beside *Vigna* and *Phaseolus* in the main cluster to join *Psophocarpus* and *Otoptera* in smaller cluster. As with the previous dendrogram *Psophocarpus* is shown to be most closely allied to *Otoptera*. The placement of *Dysolobium* with these two obviously allied genera, is interesting in that it is the first evidence to support Lackey's (1977b)

hypothesis that *Dysolobium* is closely allied to *Psophocarpus*. With the larger character set it would appear that the characters which link *Psophocarpus* and *Dysolobium* are diluted while in the smaller character set the phylogenetically useful characters have proportionally greater expression, thus explaining the differences in results between the two dendrograms. As will be shown in the next chapter phylogenetic characters are important in assessing the relationship between *Psophocarpus* and *Dysolobium*.

Apart from the change in association of *Dysolobium* the relationships of the genera remain as in the previous dendrogram. With the larger cluster splitting into two, one side containing *Dolichos* and its allies, the other containing *Phaseolus* and *Vigna*. As previously, the *Vigna* subgen. *Vigna* OTU 410 clusters with the *Phaseolus* OTU's rather than with the other *Vigna* OTU's. In both dendrograms the clustering of *Vigna* subgenera OTU's does not follow faithfully the scheme of subgeneric classification according to Marechal et al (1978a). However, as pointed out previously, the characters were selected to test the relationship of *Psophocarpus* with its close allies and so these characters will not necessarily reflect natural intra-generic relationships.

To summarise the results of the CLUSTAN (DISTIN) average linkage cluster analysis, it is clear that *Otoptera* is the most closely allied genus to *Psophocarpus*. *Dysolobium* was shown to be close to these two when the smaller character set was used. The other genera fall into two groups, that based on *Phaseolus* and *Vigna*, and that based on *Dolichos* and its allies (*Sphenostylis*, *Nesphostylis* and *Neorautanania*). Within the grouping of *Dolichos* and its allies, *Sphenostylis* was shown to be most closely allied to *Nesphostylis* and *Neorautanenia* most remote from these genera and *Dolichos* itself.

5.3.3 CLUSTAN (FILE)

For the CLUSTAN(FILE) and DECORANA analysis the number of characters in both character sets was reduced from 102 and 51 to 66 and 43 respectively. This was necessary as both CLUSTAN (FILE) and DECORANA could not make allowance for missing data. The actual characters used

are detailed in Table 4.2.

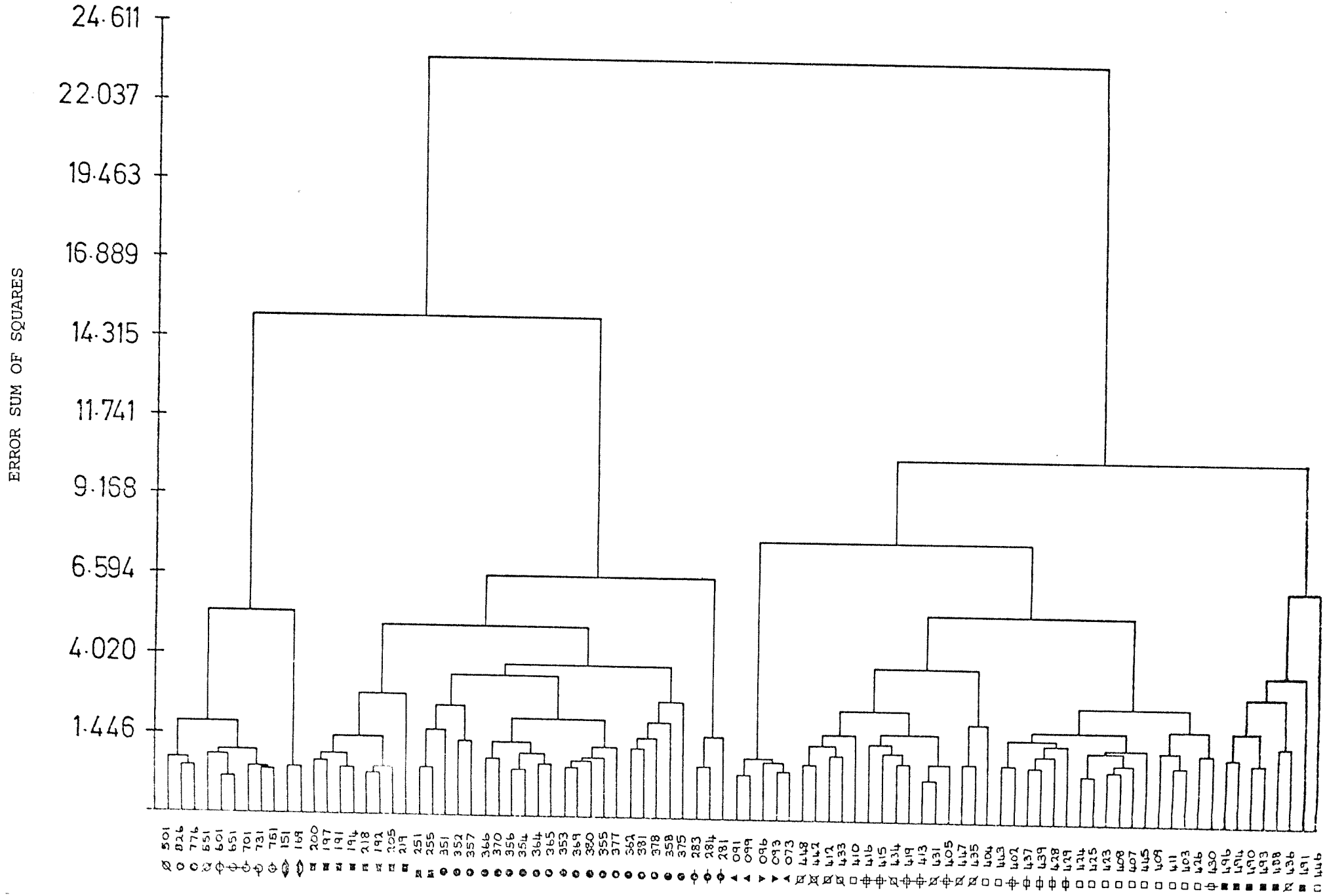
A) Ward's Method: The results of cluster analysis using Ward's method are displayed in the dendrograms drawn in Figures 5.24 and 5.25 for the 66 and 43 character sets respectively.

Broadly the dendrogram shown in Figure 5.24 produced by analysing the 66 character set, shows a dicotomy into two groups, *Dolichos* and its allies (including *Psophocarpus* and *Otoptera*) and *Phaseolus*, *Vigna* and *Dysolobium*. This it should be noted, agrees with Baudet's (1978) division of the Phaseolinae into two supergenera, the *Dolichastrae* and the *Phaseolastrae*. The implications of Baudet's use of supergenera will be discussed in the final chapter.

The dendrogram indicates, as has been shown with the other forms of analysis, that *Otoptera* is the most closely allied genus to *Psophocarpus*. Next in relatedness come the *Dolichos* allied genera, within whose cluster the true *Dolichos* OTU's seem most closely allied to *Nesphostylis*, and then *Sphenostylis* and *Neorautanania* are positioned more remotely. Within the *Vigna* related cluster, both *Dysolobium* and *Phaseolus* form distinct sub-cluster. However, the *Phaseolus* sub-cluster does include two *Vigna* OTU's, *V.caracalla* and *V.longifolia* both of which appear by this method to have been misplaced at least in terms of Verdcourt (1970c) and Marechal et al (1978a). The OTU's of the six *Vigna* sub-genera can be seen to cluster into sub-generic groupings, but the clustering is not without misplacement, if Marechal et al's (1978a) concepts are followed. It should be reiterated though that the object of the study was not to discriminate between *Vigna* sub-genera but to elucidate the relationships of *Psophocarpus* with its allies and thus characters were chosen with different uses in mind.

The dendrogram showing the results of the Ward's method cluster analysis of the 43 character set is shown in Figure 5.25. In general the OTU's in this dendrogram do not form such discrete generic clusters as with the 66 character set. However the close relationship between *Psophocarpus* and *Otoptera* can still be clearly seen: in fact in the dendrogram these two genera are split off from the other genera of the

Figure 5.24: Intensive subset inter-generic study (66) - CLUSTAN (FILE) using Ward's Method



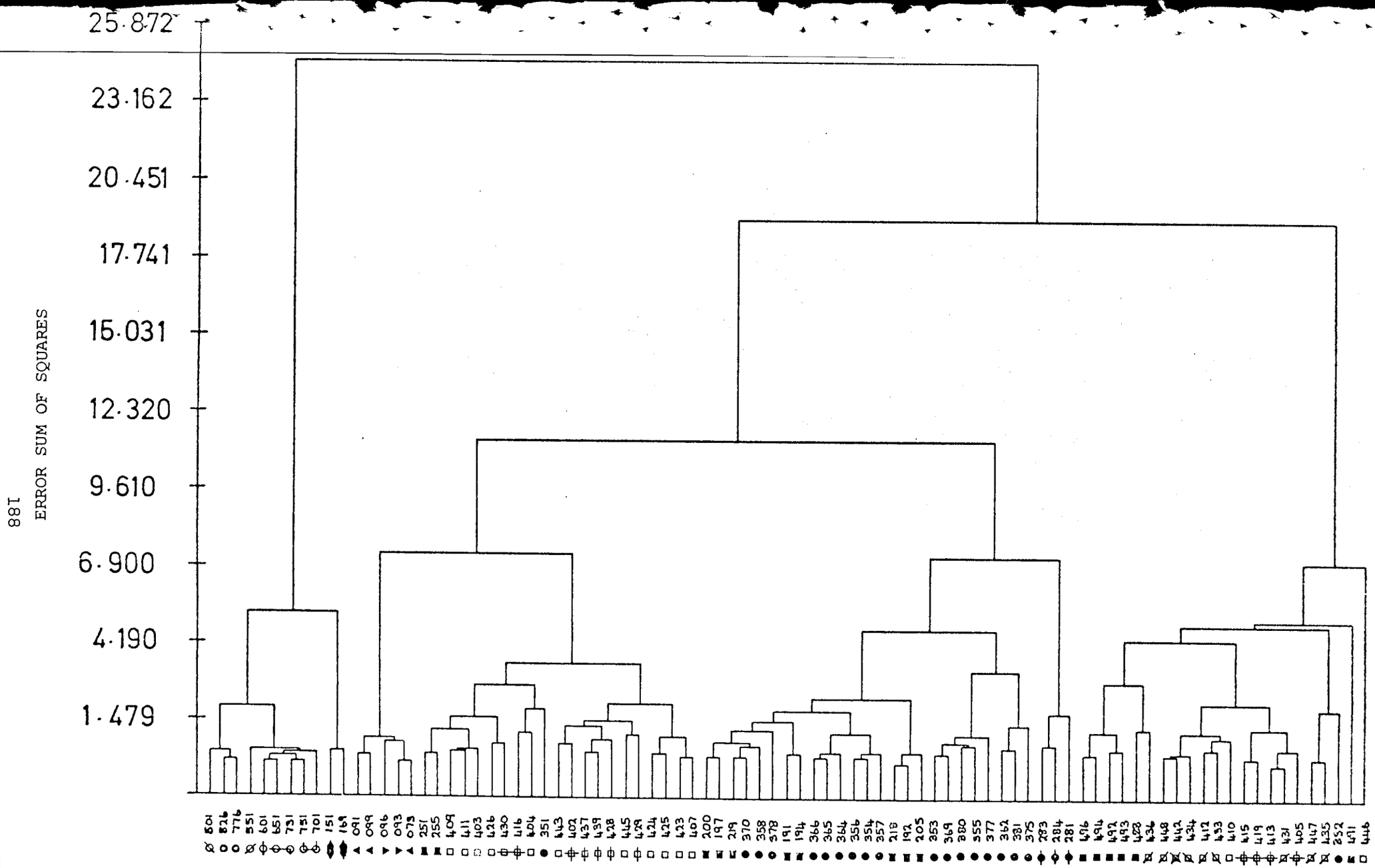


Figure 5.25: Intensive subset inter-generic study (43) - CLUSTAN (file) using Ward's method

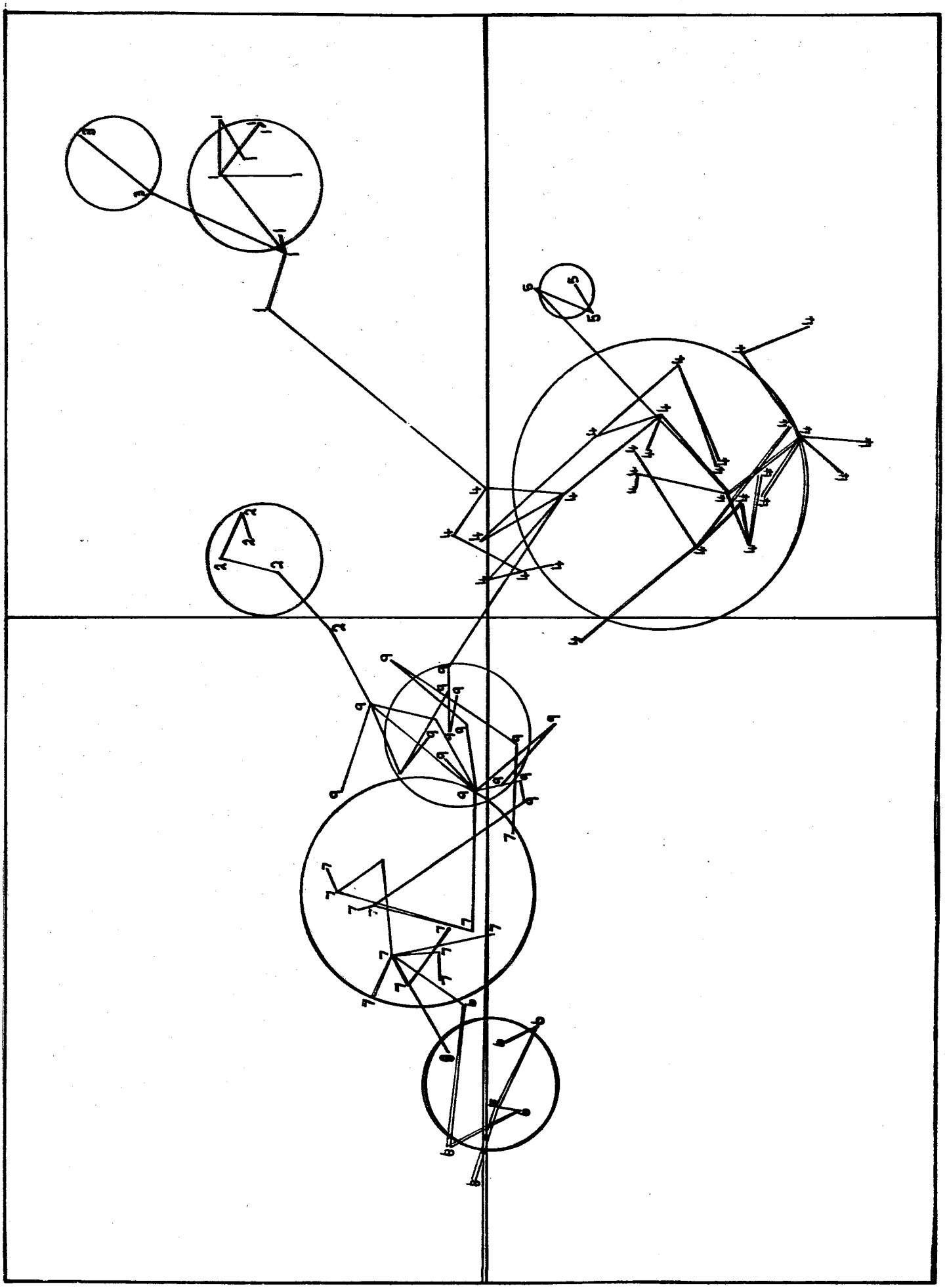
main cluster at a relatively high error sum of squares level indicating their isolation. As the genera in the main cluster do not form discrete clusters, except for *Dysolobium* and *Neorautanenia* their inter-relationships will not be discussed in detail. It is interesting to note how *Sphenostylis* has been inter-mingled with *Dolichos* OTU, even though *Sphenostylis* has many distinctive characters associated with its stima-style arrangement that might have been expected to keep it as a uniform cluster. Also the placement of *Nesphostylis* juxtaposed with *Vigna* and in a different major cluster to *Sphenostylis* must be a misplacement. *Nesphostylis* shares numerous characters with *Sphenostylis* from which it was split by Verdcourt (1970a). *Nesphostylis* is clearly much closer to *Sphenostylis*, both sharing the spatulate style apex, than to *Vigna*. Perhaps the results of this analysis using Ward's method should act as a warning against using a relatively small character set and a cluster analysis method which is biased towards locating minimum-variance spherical clusters.

To summarise, the results of cluster analysis using Ward's method, *Psophocarpus* was shown to be most closely allied to the genus *Otoptera*, then more remotely to the *Dolichos* allied genera, but most distantly to *Phaseolus*, *Vigna* and *Dysolobium*.

B) Principal Components Analysis (PCA): The results of the PCA produced by sub-routine SCATTER of CLUSTAN are shown in Figure 5.26 and 5.27. The data sets used for the analysis are the same as those used for Ward's method above.

The scatter diagram shown in Figure 5.26 shows a clear separation of the OTU's into groups based on genera. As with the methods of analysis detailed above *Psophocarpus* and *Otoptera* can be seen to be closely allied to each other, but these genera are relatively remote to the other seven genera examined. The closest other genus to *Psophocarpus* and *Otoptera* are *Sphenostylis*, *Neorautanania* and *Dysolobium*, though as the overlay shows, *Psophocarpus* is linked to *Sphenostylis* by the minimum spanning tree.

The relationship between the genera other than *Psophocarpus* and *Otoptera* is similar to that indicated in the above forms of analysis.



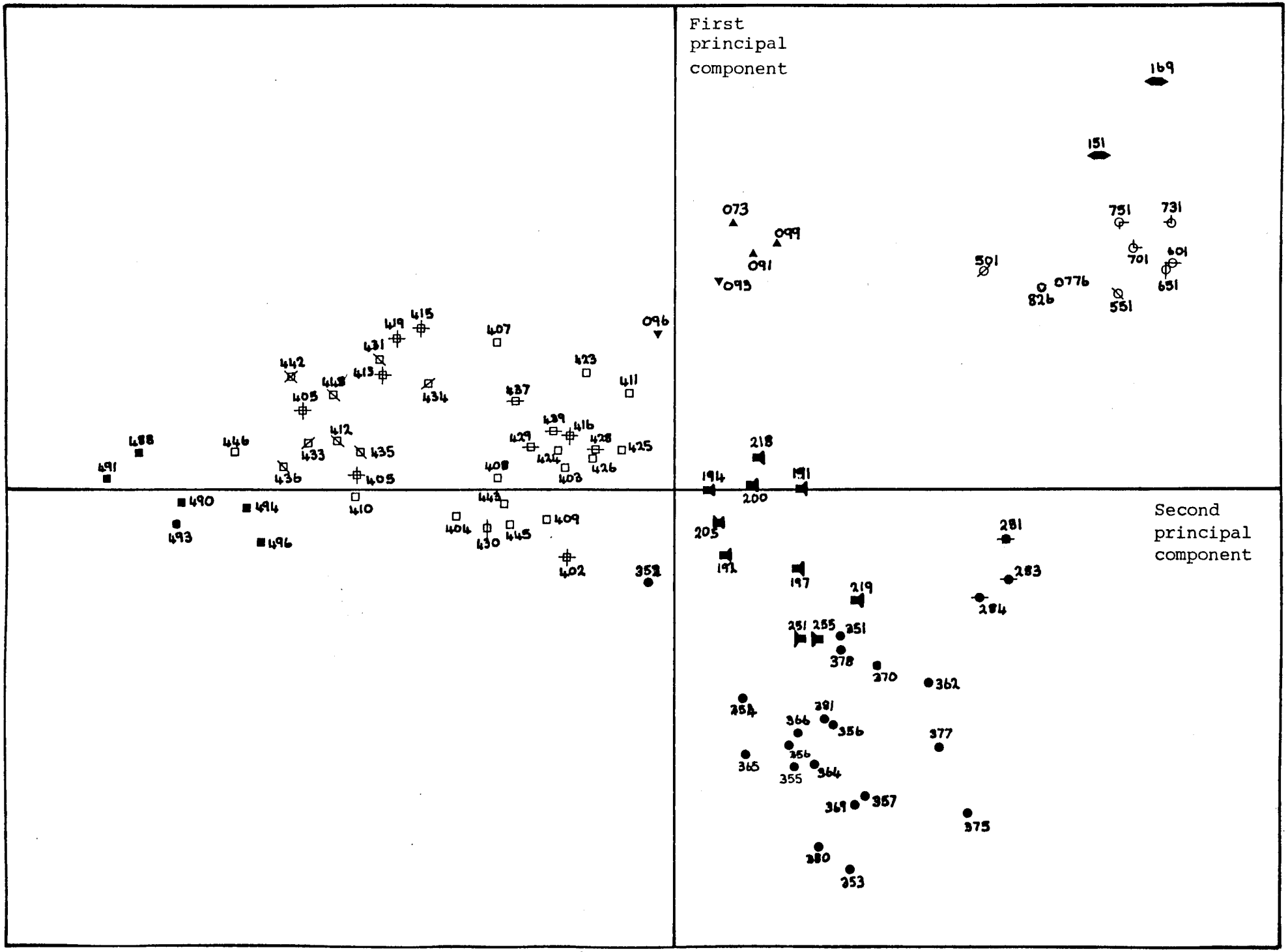


Figure 5.26: Intensive subset inter-generic study (66) - CLUSTAN (FILE) Principal components analysis

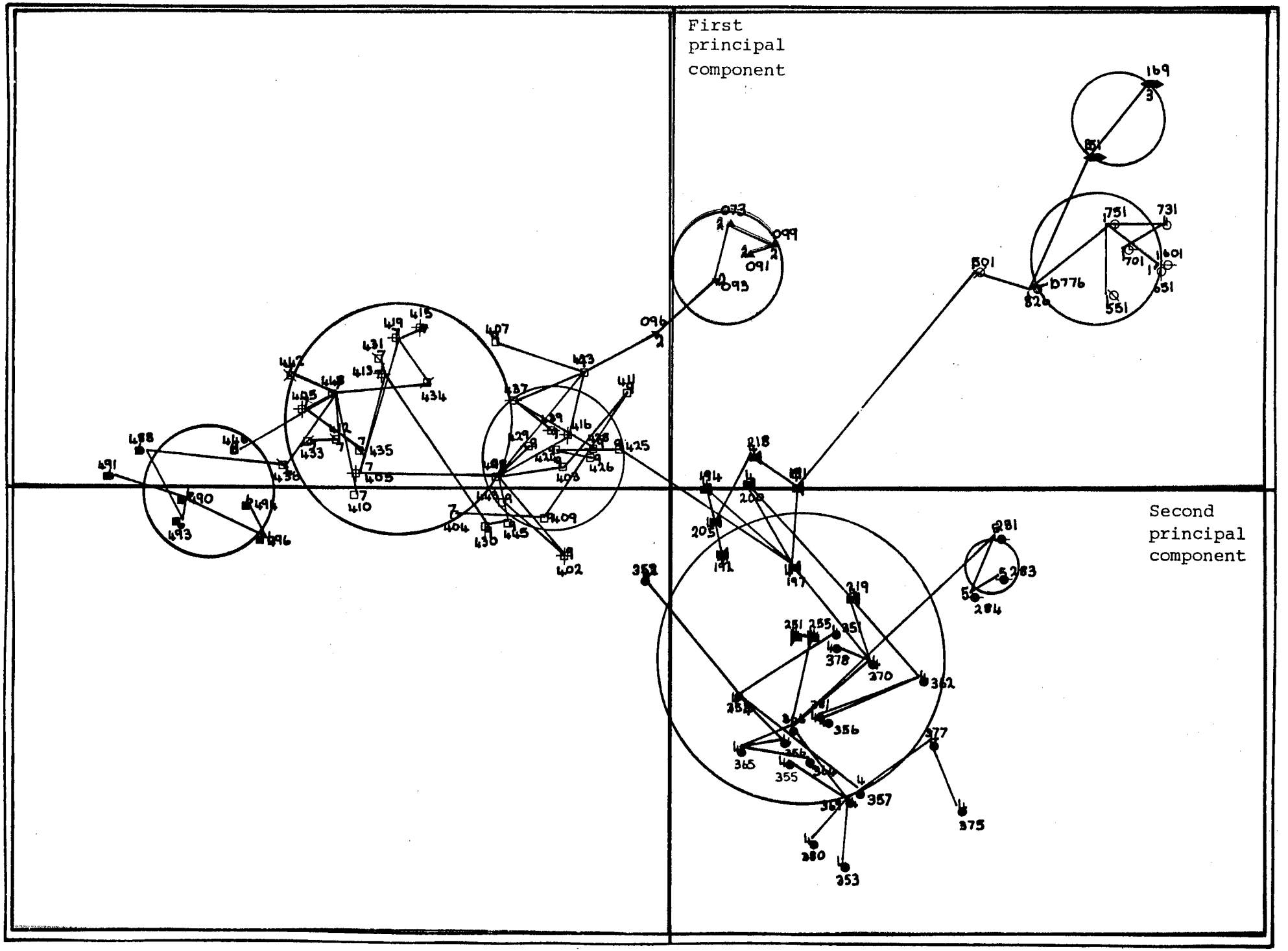
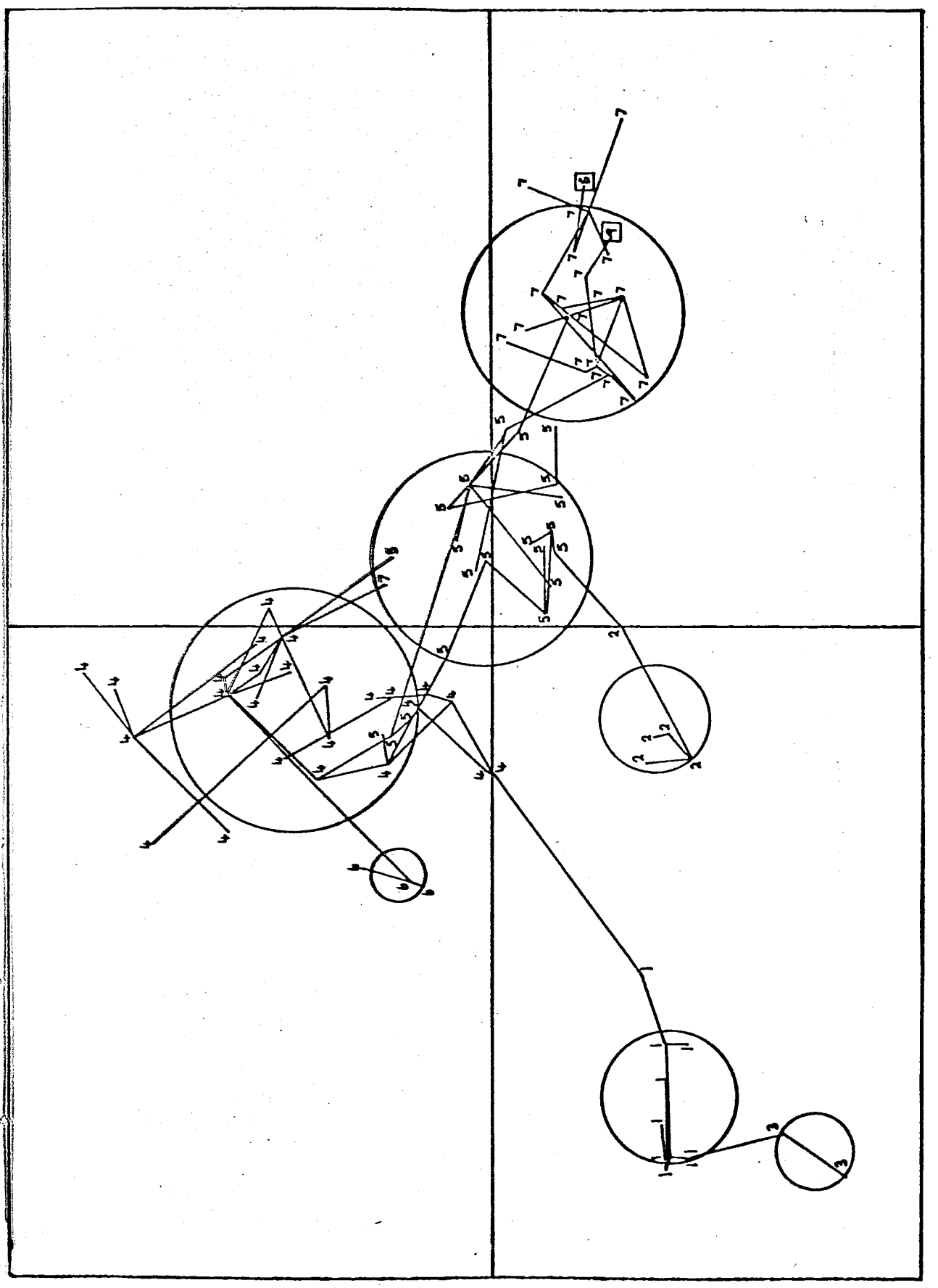


Figure 5.26: Intensive subset inter-generic study (66) - CLUSTAN (FILE) Principal components analysis



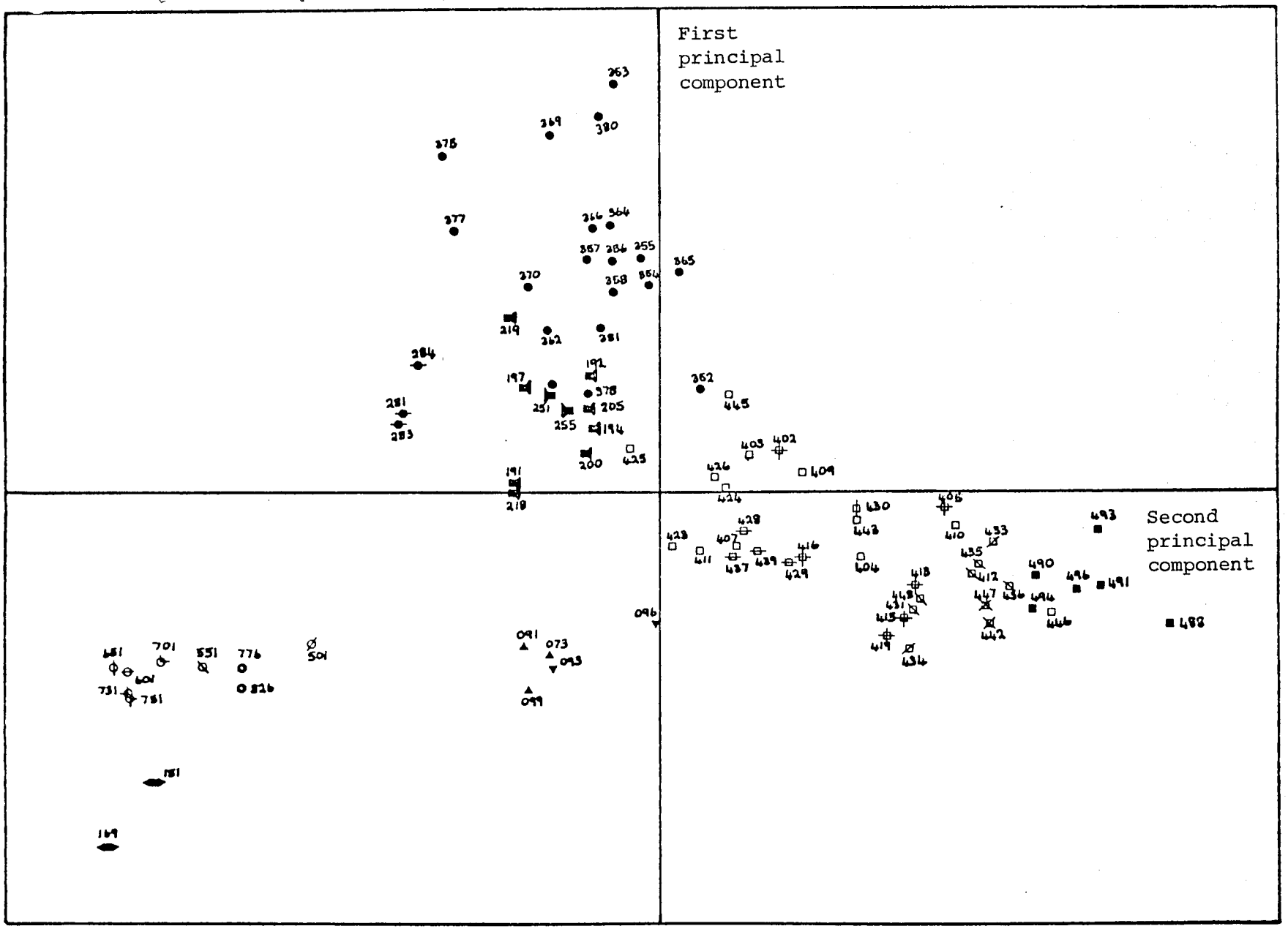


Figure 5.27: Intensive subset inter-generic study - CLUSTAN (file) Principal components analysis

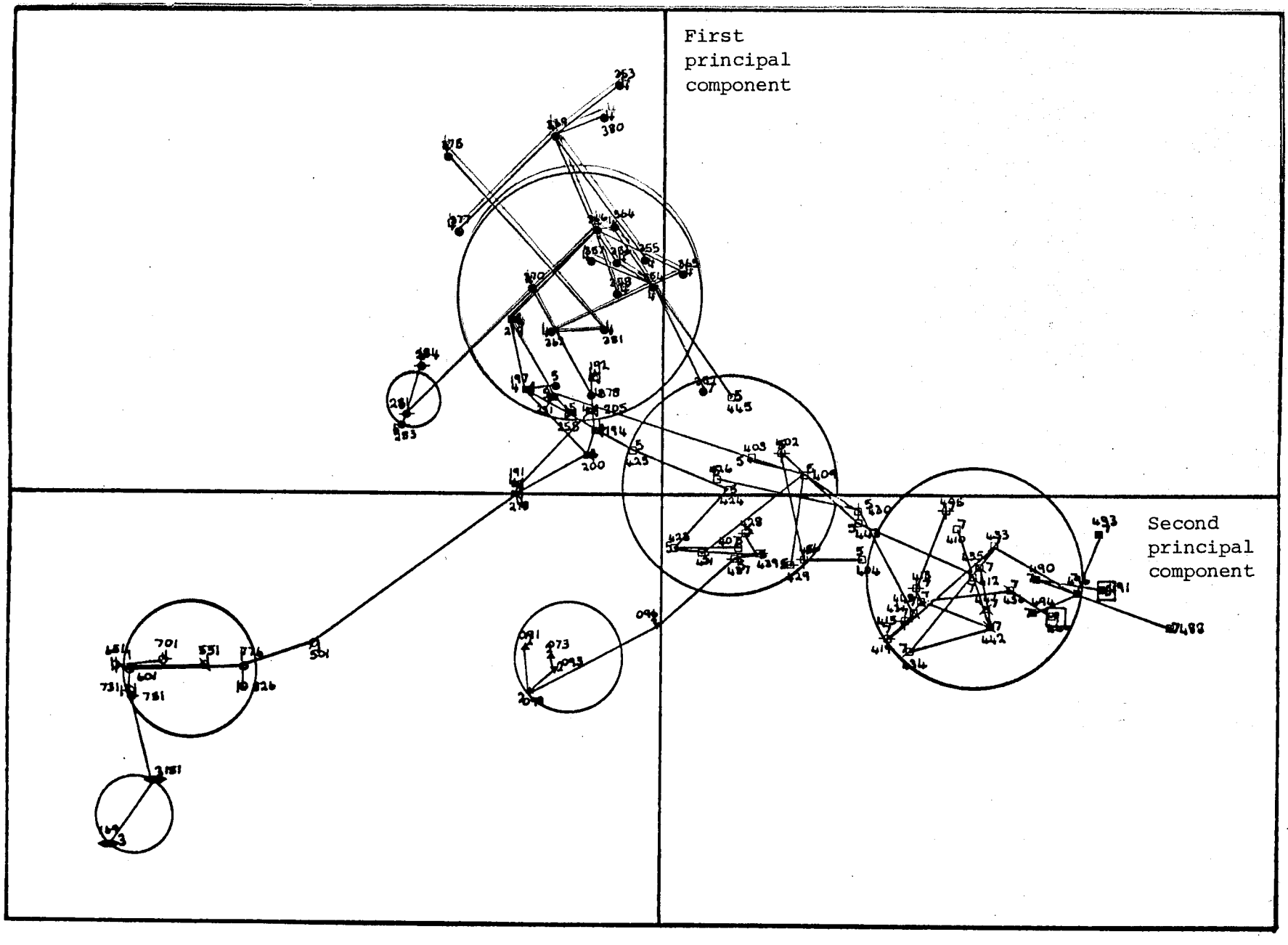


Figure 5.27: Intensive subset inter-generic study - CLUSTAN (file) Principal components analysis

The OTU's cluster broadly into two groups, the first containing *Dolichos* and its allies and the second containing *Phaseolus, Vigna* and *Dysolobium*. Within the *Dolichos* centred cluster, *Sphenostylis* and *Neorautanenia* both form outlying sub-clusters positioned nearest to *Psophocarpus*, while *Nesphostylis* takes up an intermediate placement between *Sphenostylis* and *Dolichos*.

The second cluster based on *Phaseolus, Vigna* and *Dysolobium* is more straggling than the *Dolichos* centred cluster. *Phaseolus* as might have been expected, is located furthest away from *Psophocarpus*, then *Vigna* and closest to *Psophocarpus, Dysolobium*. Within the *Dysolobium* cluster subgenus *Dysolobium* is closest to *Psophocarpus* and subgenus *Dolichovigna* takes up a position intermediate between *Dysolobium* subgen. *Dysolobium* and *Vigna*. This, as stated above, would be expected as some authors believe subgen. *Dolichovigna* to be better placed in *Vigna*. The *vigna* OTU's can be seen to fall into two sub-clusters, these do not follow sub-generic groupings, though the cluster closest to *Sphenostylis* contains the majority of the subgenus *Vigna* OTU's.

If the overlay containing the minimum spanning tree (MST) is placed over Figure 5.26 it can be seen that there are clearly distortions of the data by the PCA. Nevertheless, the MST does not indicate distortion in the placement of the genera in relation to each other and as this study was designed to elucidate generic relationships the MST can be said to have validated the PCA findings. The MST can be seen to help resolve problems of misplacement, e.g. one OTU that could be considered misplaced is 352, a *Dolichos* OTU which lies closer to the *Vigna* cluster than to the other *Dolichos* OTU's, but the MST shows this OTU to be linked to the *Dolichos* cluster and not the *Vigna*.

The overlay also shows the cluster circles and cluster circle membership numbers. Cluster 1 is *Psophocarpus* which shows only *P.grandiflorus* lying away from the circle. The second cluster is based on *Dysolobium* with four OTU's within its circle and *D.apolodes* positioned outside nearer the *Vigna* cluster 9. The third cluster contains the two *Otoptera* OTU's: there only being two OTU's, the OTU's necessarily lie within the circle. The fourth large cluster circle is composed of *Dolichos* and its closely allied genera *Sphenostylis* and

Nesphostylis. Although there are many OTU's which lie outside the fourth cluster, these are not so distantly placed that they may be considered misplaced by the PCA. The fifth cluster circle is relatively small and is restricted to the small genus *Neorautanenia*. The small size of the cluster circle indicates the small intra-generic variation associated with *Neorautanenia*. The sixth cluster circle is composed of the *Phaseolus* OTU's, plus OTU 436 (*Vigna caracalla*). This placement of *V.caracalla* with *Phaseolus* was also found by Marechal et al (1978a) and may justify the species movement to Phaseolus. The seventh and ninth cluster circles formed are, as discussed above, composed of two *Vigna* sub-clusters, though here it should be noted that the cluster circles overlap indicating the two clusters' allied nature. The eigth cluster circle is composed of one OTU 446 (*Vigna longifolia*) and lies within the *Phaseolus* cluster circle. It is difficult to justify this OTU being given a cluster circle of its own, though its close association with the *Phaseolus* OTU's suggests that it is not a typical *Vigna* species. Marechal et al (1978a) found this species to be linked to *Vigna jurana* and *Phaseolus antillanus*, but this association would still not explain why it warrants a separate cluster circle.

The results of PCA using 43 character set are shown in Figure 5.27. An almost identical scatter diagram is produced as was seen in Figure 5.26 for the 66 character analysis. *Psophocarpus* and *Otoptera* can be seen to be closely allied to each other but at a distance from the other genera. Of the other genera *Neorautanenia*, *Sphenostylis* and *Dysolobium* are spatially closest to *Psophocarpus*. The main cluster can be split easily into two sub-clusters, those associated with *Dolichos* and those with *Vigna*. As with the cluster analysis using Ward's method and this character set, some OTU's appear to be slightly misplaced between the two sub-clusters of the main cluster. The *Vigna* OTU 425, of subgen *Vigna*, is located close to the *Dolichos* cluster, while the *Dolichos* OTU 352 is placed with the *Vigna* OTU's. The general conclusion for the CLUSTAN (FILE) analysis must be that the larger character set better describes the natural variation in the OTU's studied because of the more discrete specific clusters produced by its analysis results.

The indications of the MST and cluster circles on the overlay of Figure 5.27 are largely consistent with those found for Figure 5.26, the

few differences being brought about by the OTU misplacements as discussed above. However, this time unlike in Figure 5.26, the MST does not draw the misplaced OTU's back into their natural clusters. There are several OTU's whose cluster circle membership number indicates further apparent misplacement, three *Sphenostylis* OTU's are linked into cluster 5, one of the *Vigna* clusters. The *Dolichos* OTU which as mentioned above is placed in the *Vigna* cluster circle 5, is drawn back into the *Dolichos* cluster by the MST, but is curiously given cluster circle membership of cluster 7, which is composed of some *Vigna* and *Phaseolus*. This misplaced *Dolichos* OTU is a specimen of *D.dinklagei* and there is no logical explanation to justify its placement.

The main point, however, remains valid that in Figure 5.27 the generic clusters are relatively distinct and similar in membership to those in Figure 5.26. The differences between the two scatter diagrams, MST's and cluster circles appears to indicate that the larger character set provides, for the CLUSTAN (FILE) analysis, a better reflection of the natural relationship between the investigated genera.

Figure 5.28: Intensive subset inter-generic study (66) - DECORANA - OTU plot

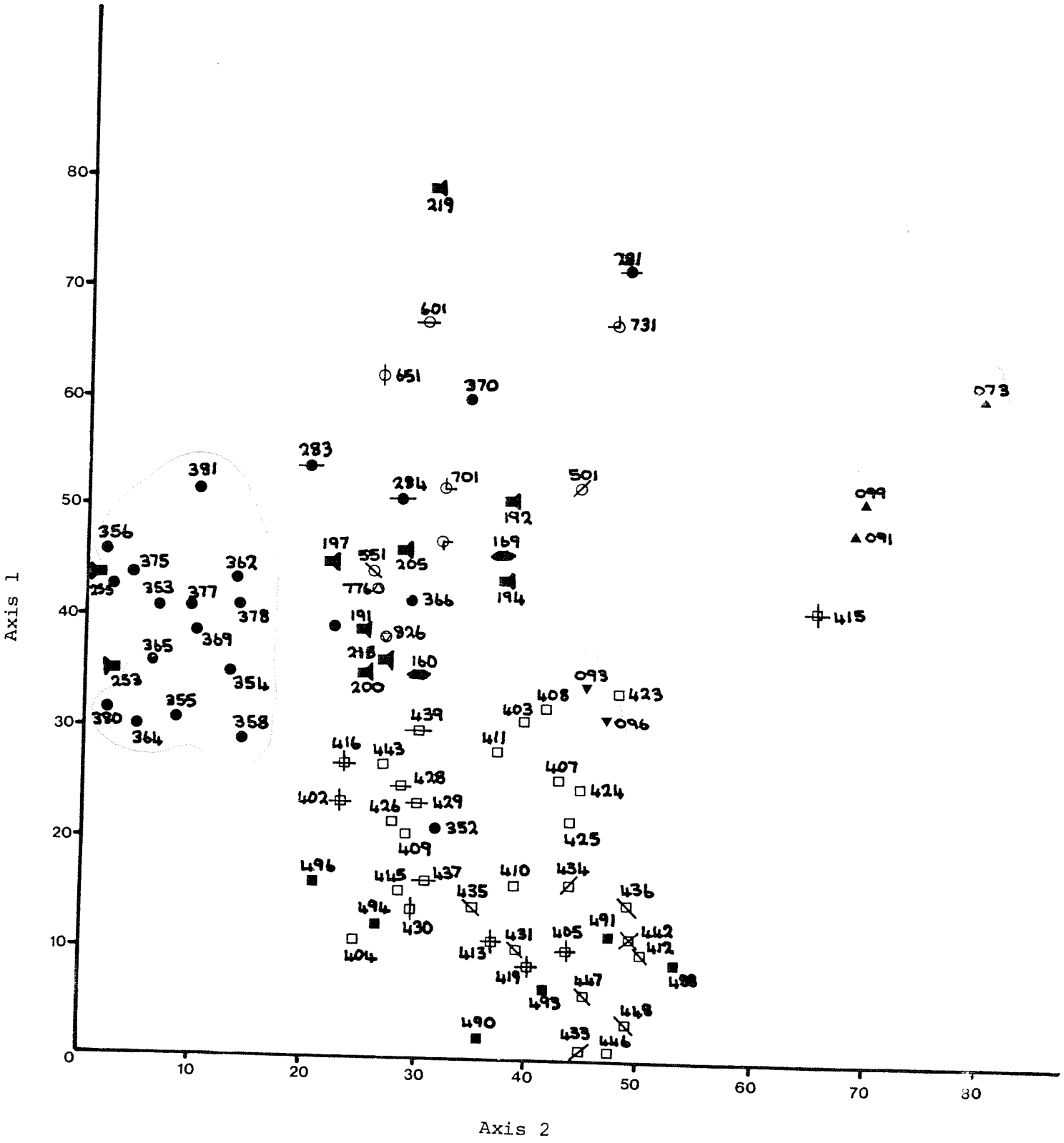
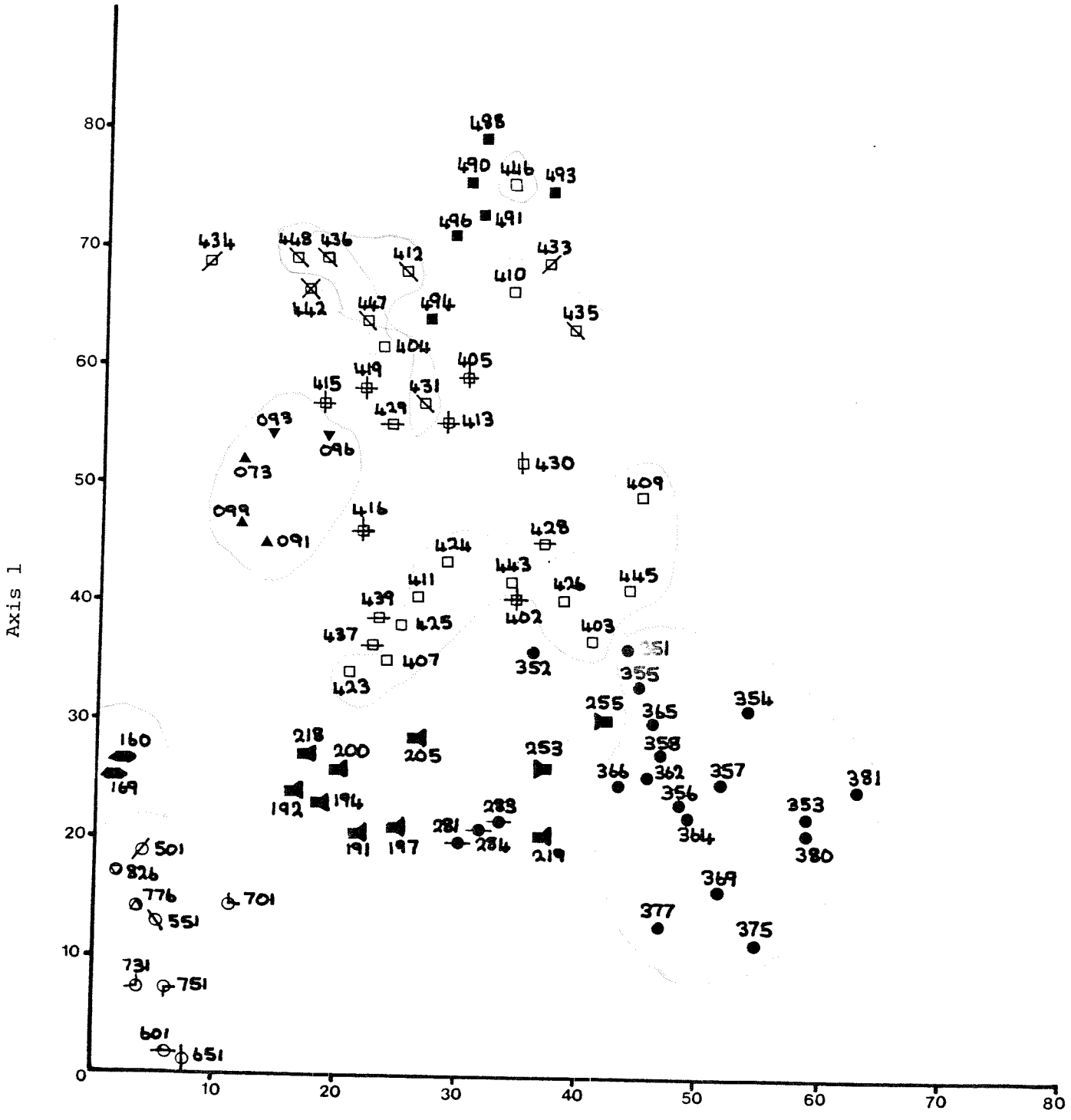


Figure 5.29: Intensive subset inter-generic study (43) - DECORANA - OTU plot



5.3.4 DECORANA

The same two character sets used for the CLUSTAN (FILE) analysis were used for the DECORANA; the results of the 66 and 43 character analysis are displayed in Figures 5.28 and 5.29 respectively. In general the clusters of OTU's produced by this method are not as generically uniform or as distinct as with the other methods used. Compared with the results of the other forms of analysis used, a few OTU's seem rather misplaced, note the position of OTU's 219 and 415 in Figure 5.28 though these OTU's associate with their generic allies in Figure 5.29. Having made these points, however, four main clusters can be distinguished in Figure 5.28; a cluster of *Psophocarpus*, *Otoptera*, *Neorautanenia*, *Sphenostylis* with a few *Dolichos* OTU's a second cluster containing most of the *Dolichos* OTU's with the *Nesphostylis* OTU's, the third composed of *Phaseolus*, *Vigna* and *Dysolobium* subgen. *Dolichovigna* OTU's, while the fourth cluster of OTU's contains *Dysolobium* subgen. *Dysolobium* and a *Vigna* OTU of *Vigna umbellata* of cultivated origin.

As with the previous methods of analysis, *Psophocarpus* can be seen to be most closely allied to *Otoptera* and *Sphenostylis*, though some *Dolichos* OTU's and *Neorautanania* also appear close. Interestingly the *Nesphostylis* OTU's cluster with the majority of the *Dolichos* OTU's rather than with their close ally *Sphenostylis*. Perhaps the most important difference in the results of this DECORANA analysis from the other methods of analysis used to interpret *Psophocarpus's* inter-generic relationships is the clear splitting up of the two *Dysolobium* subgenera. Subgen. *Dolichovigna* as some authors belive (Verdcourt 1970c, van Welzen and den Hengst, in press) is linked with the *Vigna* OTU's, rather than with *Dysolobium* subgen. *Dysolobium* OTU's. However, the importance of this should not be over-emphasised because as is shown in Figure 5.29, where the results of the smaller character set are analysed using DECORANA, all *Dysolobium* OTU's are closely linked.

The results of the analysis using the 43 characters are displayed in Figure 5.29. The general division of OTU's into clusters is indistinct. However *Psophocarpus* and *Otoptera* do form a separate distinct cluster from the other main cluster of OTU's. The main cluster of OTU's forms a 'generic rainbow' with the *Dolichos* allied genera at its base, merging

into the *Vigna* OTU's and *Phaseolus* furthest away from *Psophocarpus*. The *Dysolobium* OTU's forming a tightly knit group, associated near the *Vigna* subgenus *Ceratatropis* OTU's with the *Dolichos* allies closest to *Psophocarpus* and *Otoptera*. *Sphenostylis*, *Neorautanenia* and *Nesphostylis* are more closely allied to *Psophocarpus* than *Dolichos* itself.

To summarise the results of the inter-gteneric analysis using DECORANA, *Otoptera* as with the other methods of analysis has been shown to be the closest ally of *Psophocarpus*. After *Otoptera* the other genera closely related are *Dolichos* and its allies, most notably *Sphenostylis* and *Neorautanenia*, with *Nesphostylis* and *Dolichos* itself more remote. The *Phaseolus* - *Vigna* complex genera are most distant from *Psophocarpus*. Interestingly, *Dysolobium* is shown in both scatter diagrams to be of equal distance from *Psophocarpus* as *Vigna* subgen. *Vigna* and subgen. *Haydonia*. This refutes Lackey's (1977b) hypothesis that *Psophocarpus* and *Dysolobium* are especially closely allied, at least on the ground of phenetic evidence.

As well as the scatter diagram of OTU's, DECORANA also produces a scatter diagram of the characters used in the analysis. The four character scatter diagrams for the three studies analysed using DECORANA are discussed in Section 8.4 of this thesis. Due to the degree of difference in scale of the axes of the OTU and character scatter diagrams, they cannot be plotted in the same figure and thus it was considered more appropriate to present them in the section discussing phenetic character choice.

5.4 Intra-generic Psophocarpus Study

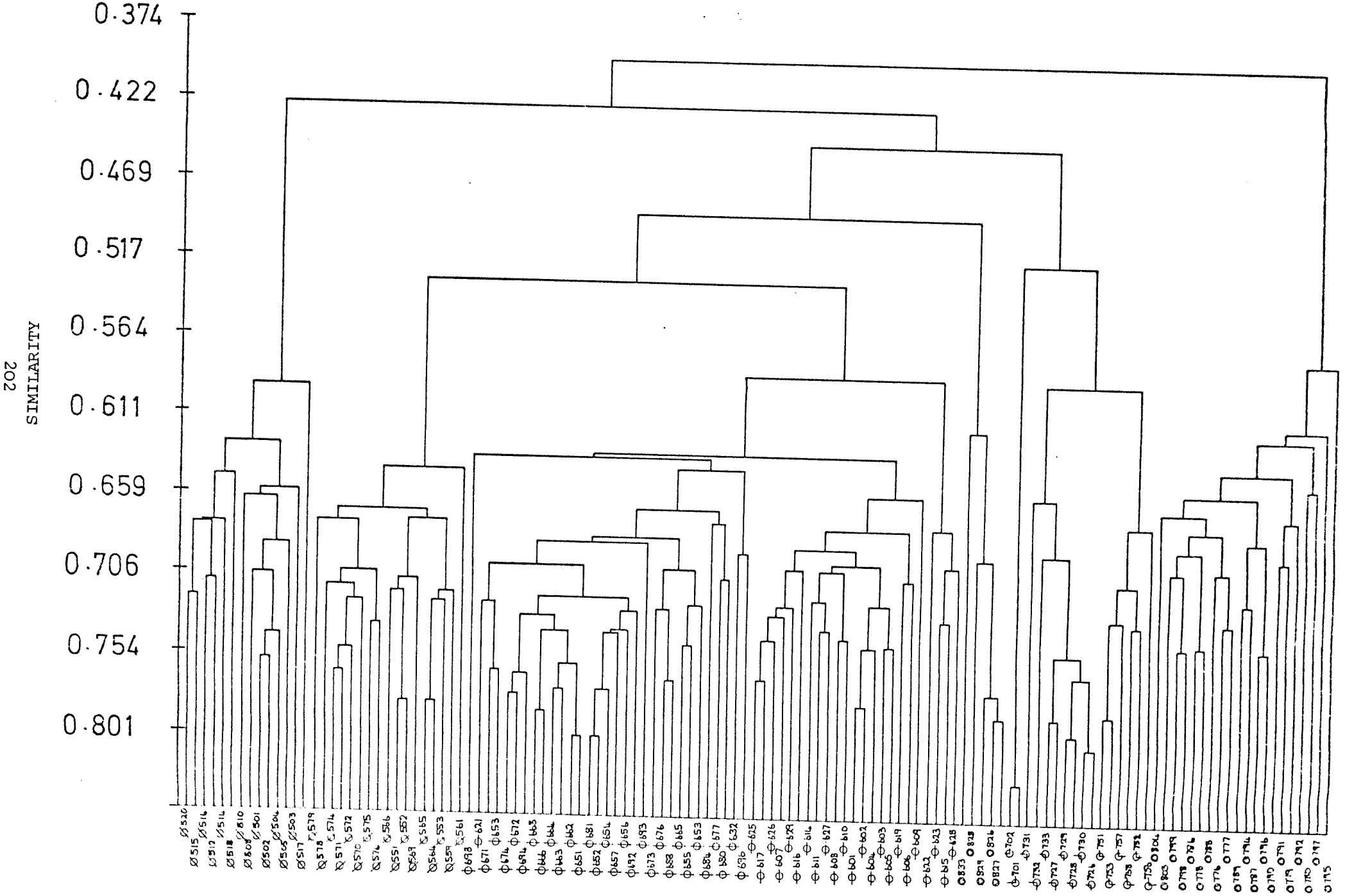
The third phase of the project (as outlined in Section 1.4), as well as elucidating *Psophocarpus's* inter-generic relationships, was designed to clarify the relationships between the nine *Psophocarpus* species circumscribed by Verdcourt and Halliday (1978). Section 5.4 details the results of the analysis of the nine species inter-relationships. The relationship of two of these species, *P.palustris* and *P.scandens*, is critical, in that Verdcourt (1968) separates these species, but Westphal (1974) considers this splitting "rather premature". For this reason the particular relationship between these two species will be examined separately in the following section (5.5).

For the analysis 126 specimens of the nine species were scored where possible for the 315 characters (vegetative, inflorescence, legume and seed) detailed in Table 4.1. Each specimen was regarded as an OTU for the purposes of the analysis. An inequal number of specimens was scored for each species, a larger number being scored of the more common species, while for the rare species *P.obovalis*, where only two specimen are known, only these two could be scored.

Characters for this study were chosen intuitively and for the first analysis run 152 characters were chosen in an attempt to reflect the inter-specific variation, from consideration of the LINKAGE and CLUSTAN (DISTIN) results, it was considered that the 152 characters chosen were too heavily weighted by number in favour of hair character and organ size and so the character set was reduced to 97 (as detailed in Table 4.2). Upon these 97 characters the analysis and results detailed below are based.

The dendrogram drawn in Figure 5.30 is that produced following the CLUSTAN (DISTIN) procedure for the 152 character, character set, the implications of which will not be discussed in detail here because of the bias produced by the excessive inclusion of hair and size characters. This is demonstrated by the clear separation of *P.lancifolius* from the other species based largely on hair characters and the separation of *P.grandiflorus* from the remaining species on size characters. These two species separation from the other species is not thought to reflect the natural relationships between the nine species as well as the 97 character, character set. The results of the analysis of

Figure 5.30: *Psophocarpus* Inter-generic study (152) - CLUSTAN (DISTIN)



the latter are discussed in detail below.

5.4.1 LINKAGE

For the inter-specific *Psophocarpus* study using 97 characters there were 125 linkage levels, which decreased in similarity from 0.8138 to 0.5147. The linkage diagrams or sub-graphs which show the linking of the species of *Psophocarpus* are drawn in Figure 5.31 to 5.38.

The first two species to form links are as might have been expected were *P.palustris* and *P.scandens* as shown in Figure 5.31 at a similarity of 0.7312. It can be seen that one specimen (OTU) of *P.palustris*, 621, was already a member of the main *P.scandens* cluster prior to this linkage level. This OTU was not taken as the first true *P.palustris* to link with *P.scandens* as the specimen was considered of intermediate form by Verdcourt and Halliday (1978). It should be noted how quickly these two species have formed links compared to the other species. The next species does not link until the similarity has decreased by 0.1 and by then the specific clusters containing encircled OTU's are clearly formed.

This difference can clearly be seen in Figure 5.32 where at a similarity level of 0.6342 a *P.tetragonolobus* OTU links with a *P.scandens* OTU. The OTU's linked within the other clusters are entirely linked with other members of the same species, with only a few OTU's not included within encircled clusters. It can be seen that both *P.scandens* and *P.palustris* are by this level of similarity linked in the same encircled cluster.

In the following linkage level drawn in Figure 5.33 *P.grandiflorus* forms a new link with the *P.scandens*, *P.palustris* and *P.tetragonolobus* cluster. At this level of similarity, 0.6323, it is shown that a second *P.tetragonolobus* OTU links with the same *P.scandens* OTU as the original link, thus strengthening the ties between *P.scandens* and *P.tetragonolobus*.

By the time the next inter-specific link is made at a similarity level of 0.6110, *P.tetragonolobus* has joined the *P.palustris* -

Figure 5.31: *Psophocarpus* intra-generic study (97) - Linkage level 38

Similarity = 0.7312

75 OTU's not yet linked

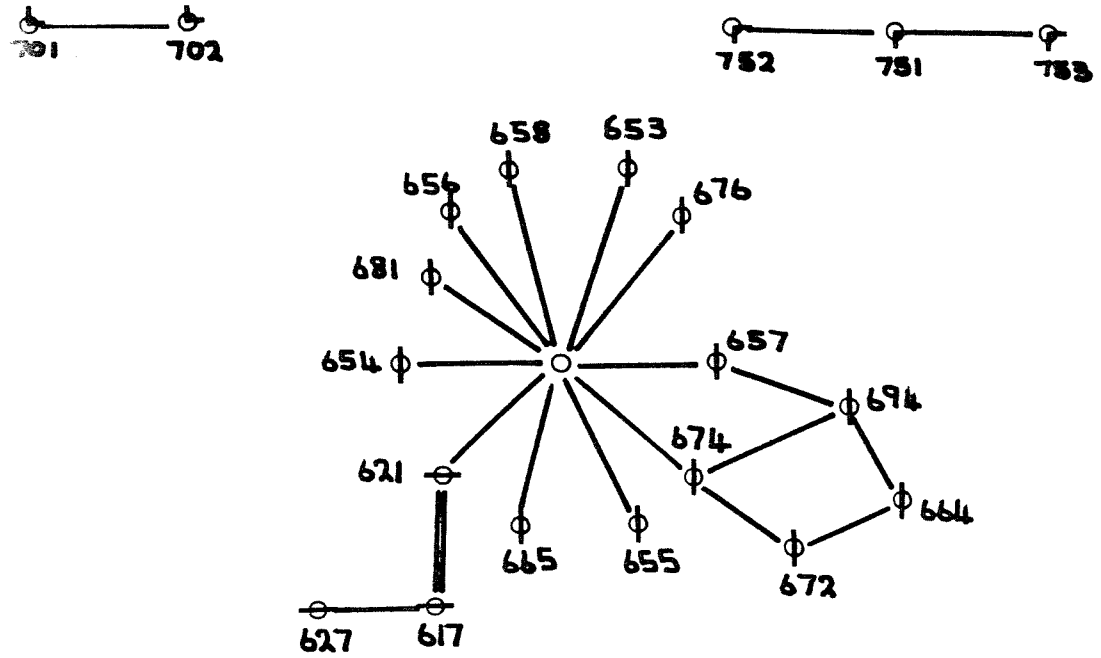


Figure 5.32: *Psophocarpus* intra-generic study (97) - Linkage level 114

Similarity = 0.6342 5 OTU's not yet linked

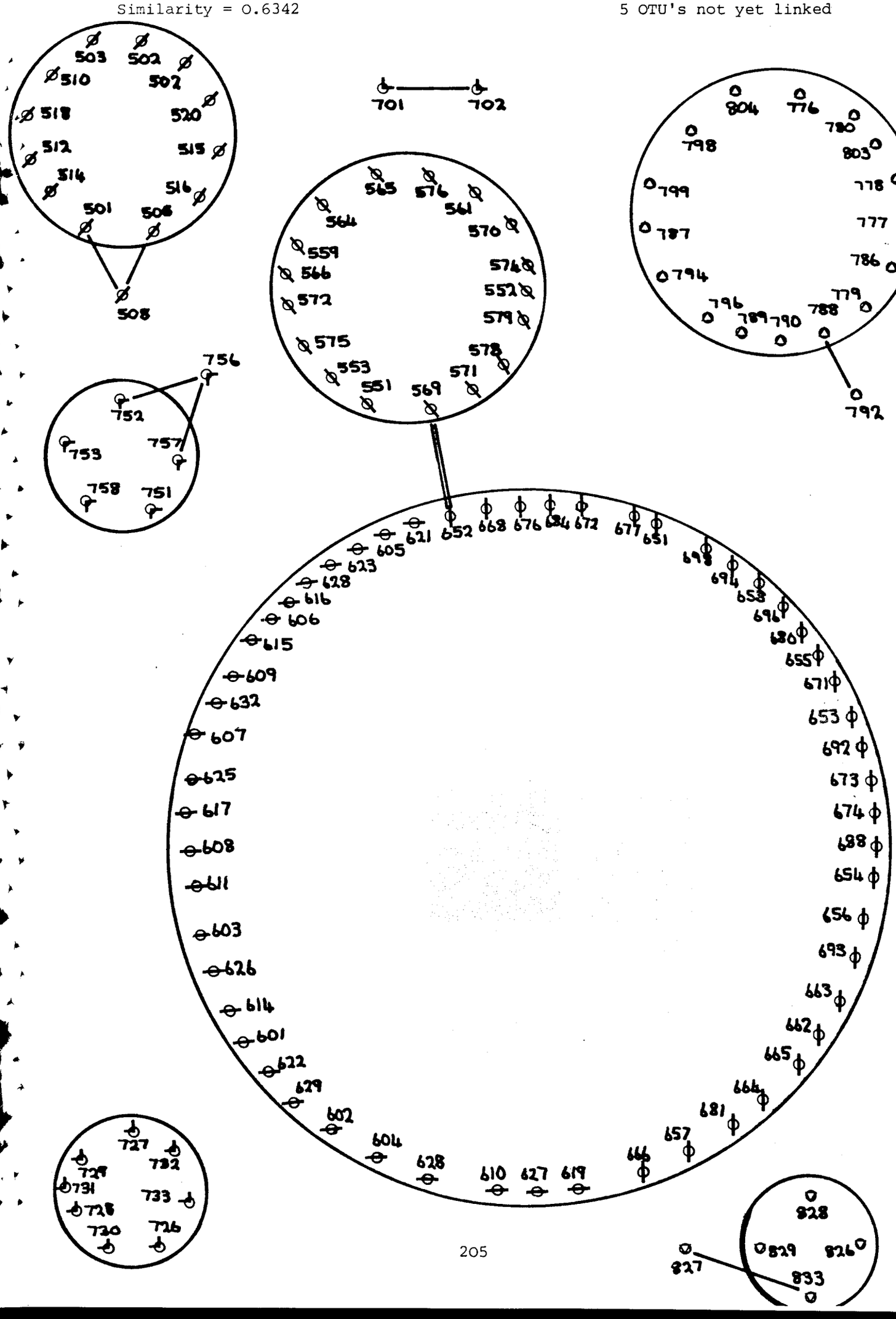


Figure 5.33: *Psophocarpus* intra-generic study (97) - Linkage level 115

Similarity = 0.6323 5 OTU's not yet linked

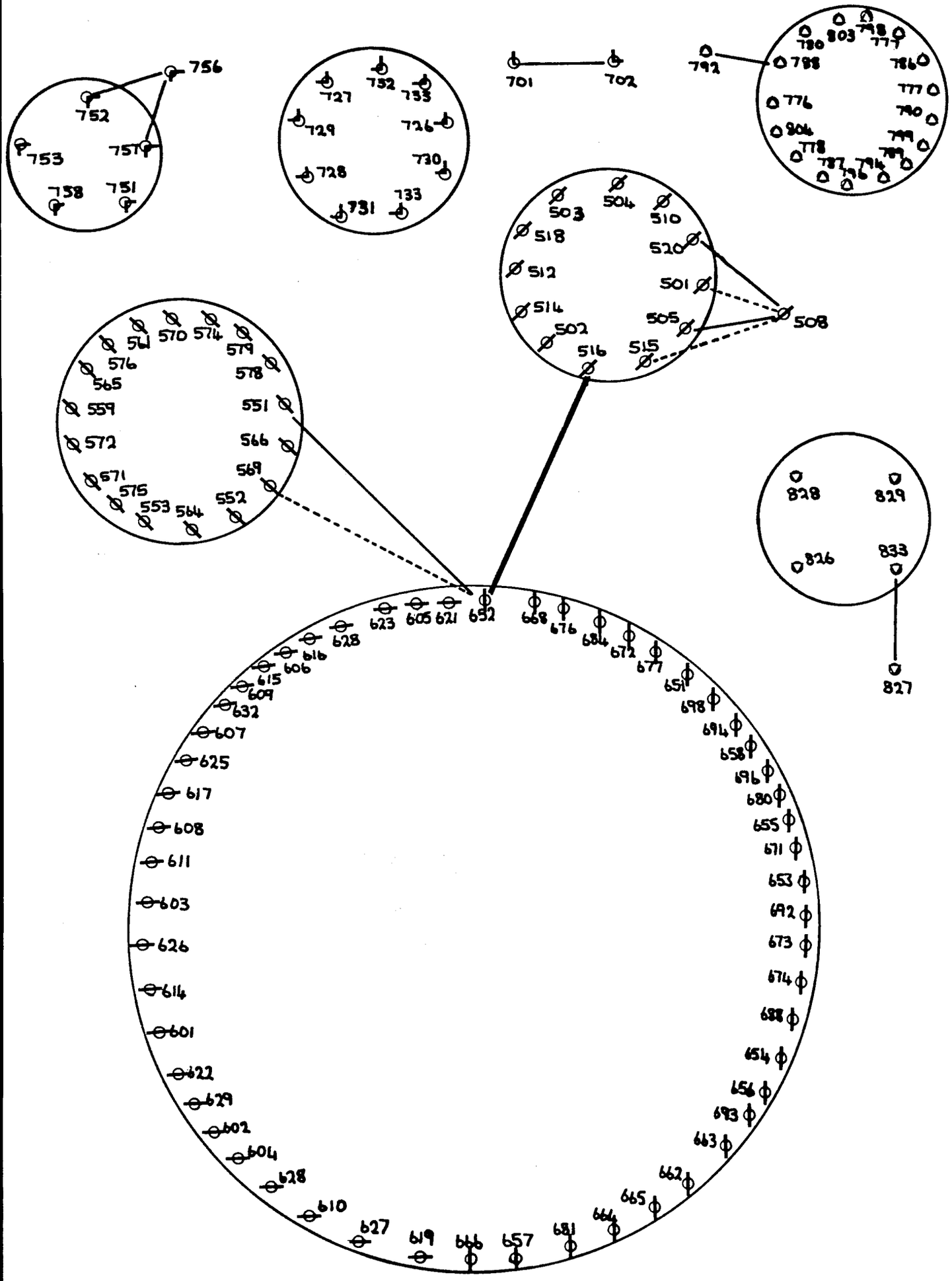


Figure 5.34: *Psophocarpus* intra-generic study (97) - Linkage level 119

Similarity = 0.6110 2 OTU's not yet linked

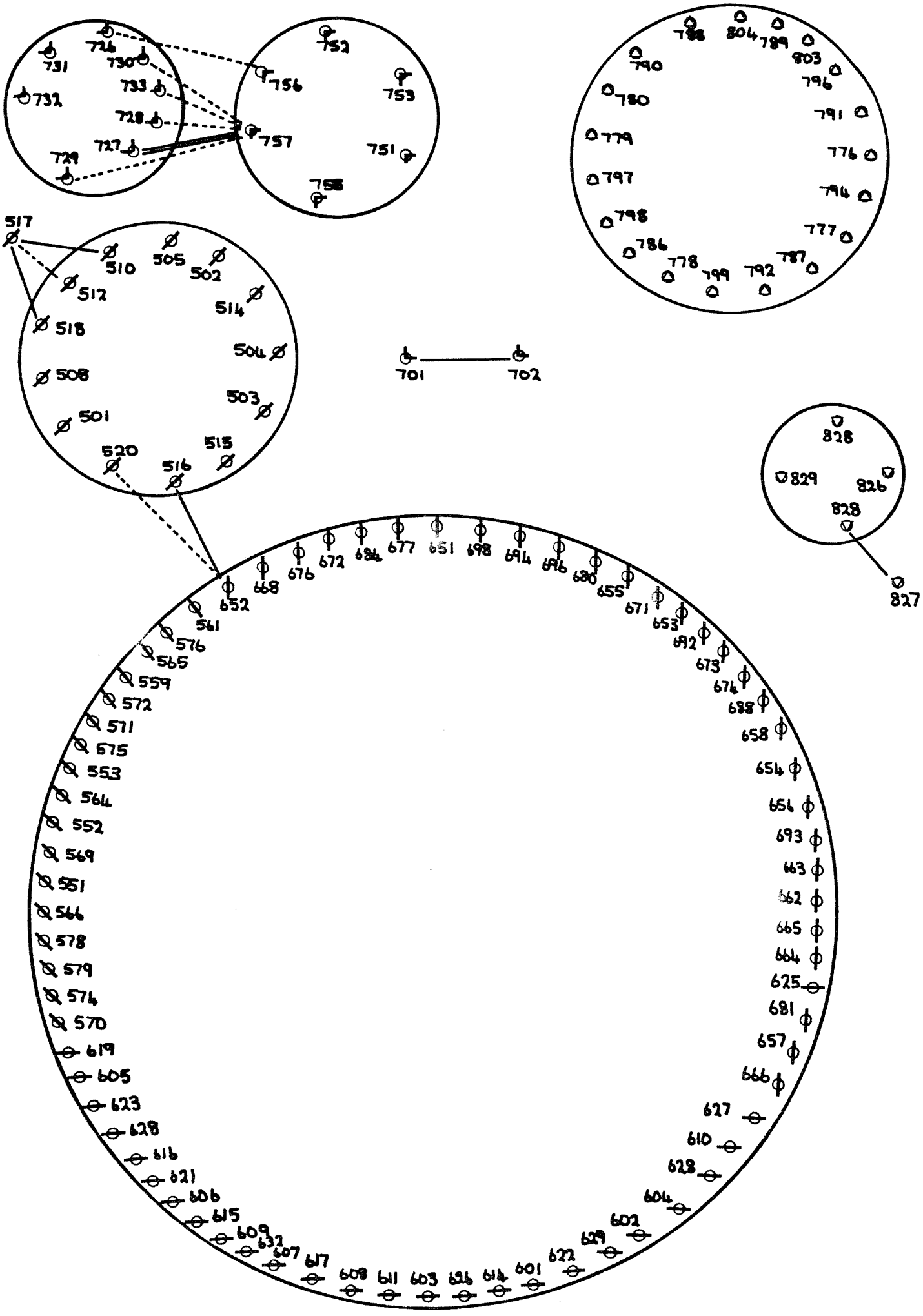


Figure 5.35: *Psophocarpus* intra-generic study (97) - linkage level 121

Similarity = 0.5807 1 OTU not yet linked

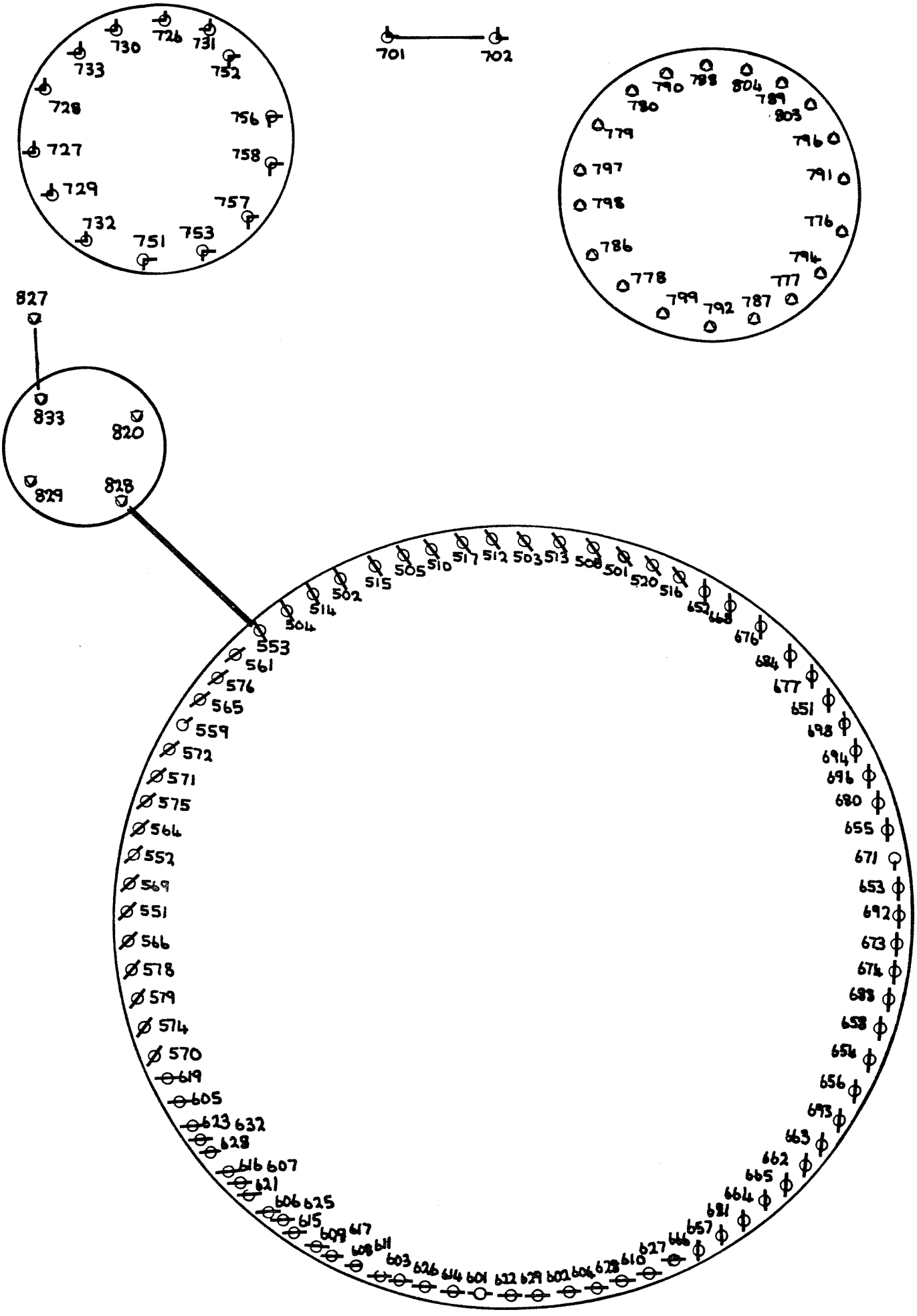


Figure 5.36: *Psophocarpus* intra-generic study (97) - Linkage level 122

Similarity = 0.5755 1 OTU not yet linked

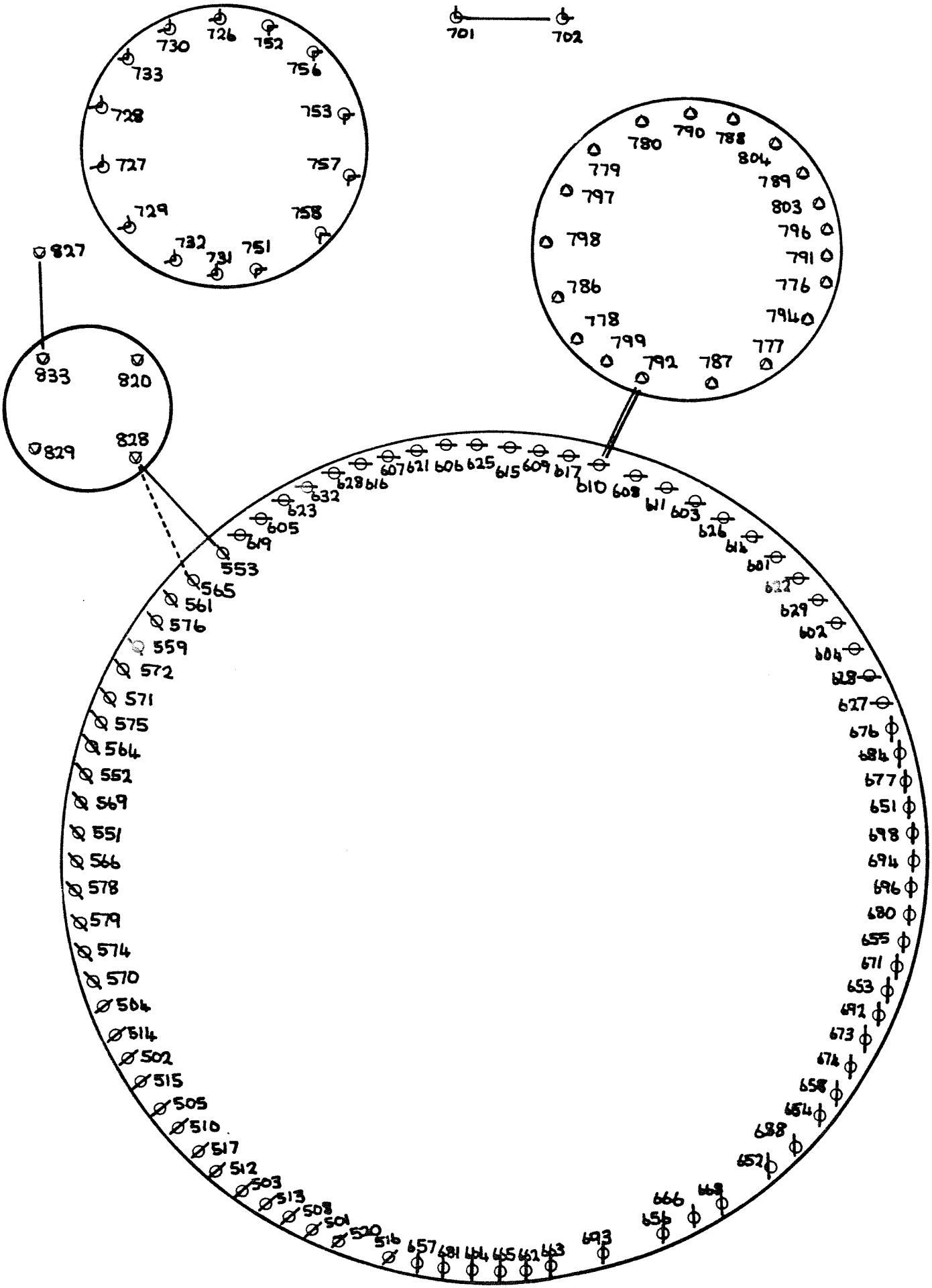


Figure 5.37: *Psophocarpus* intra-generic study (97) - Linkage level 124

Similarity = 0.5493 All OTU's linked

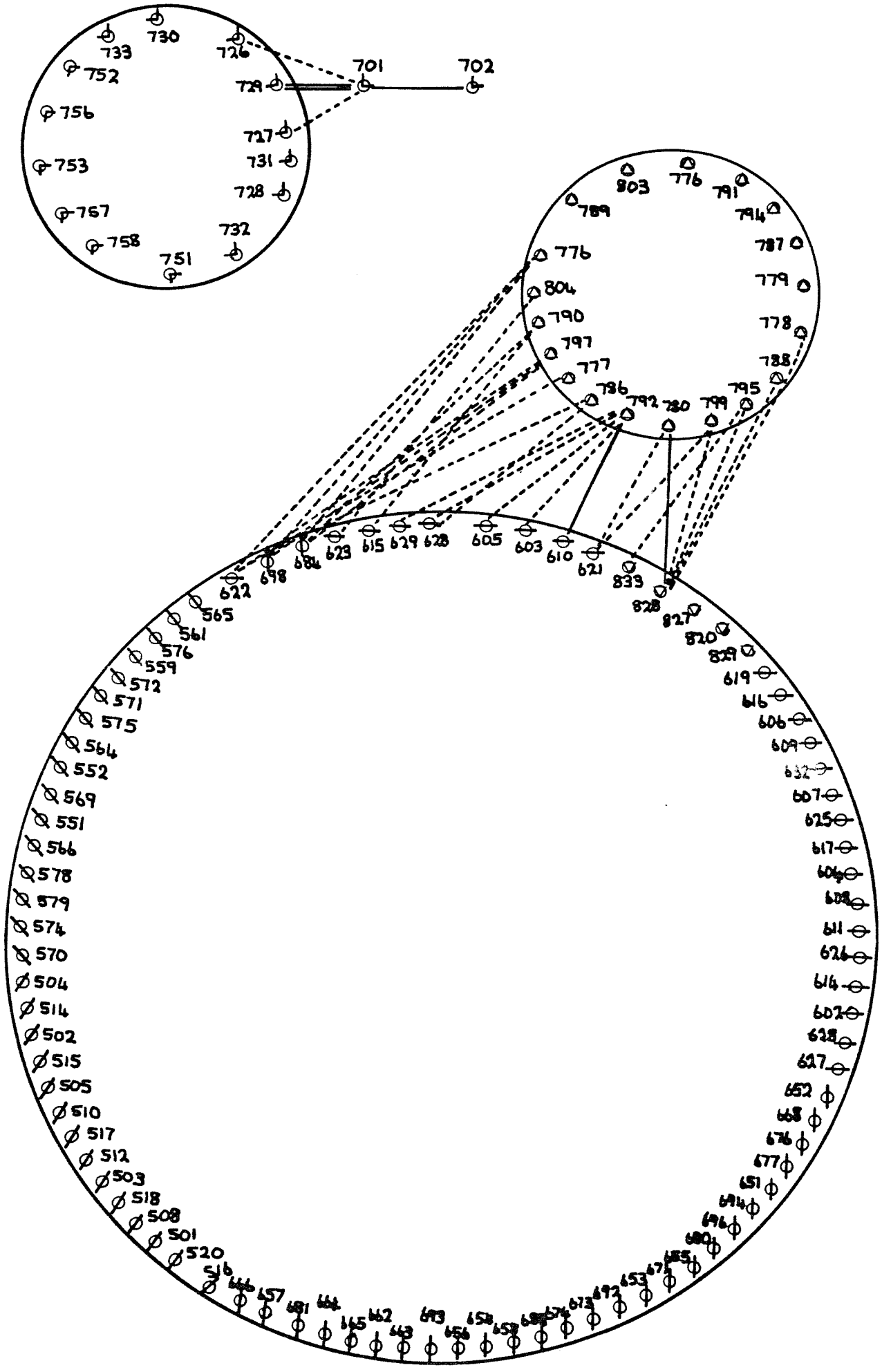
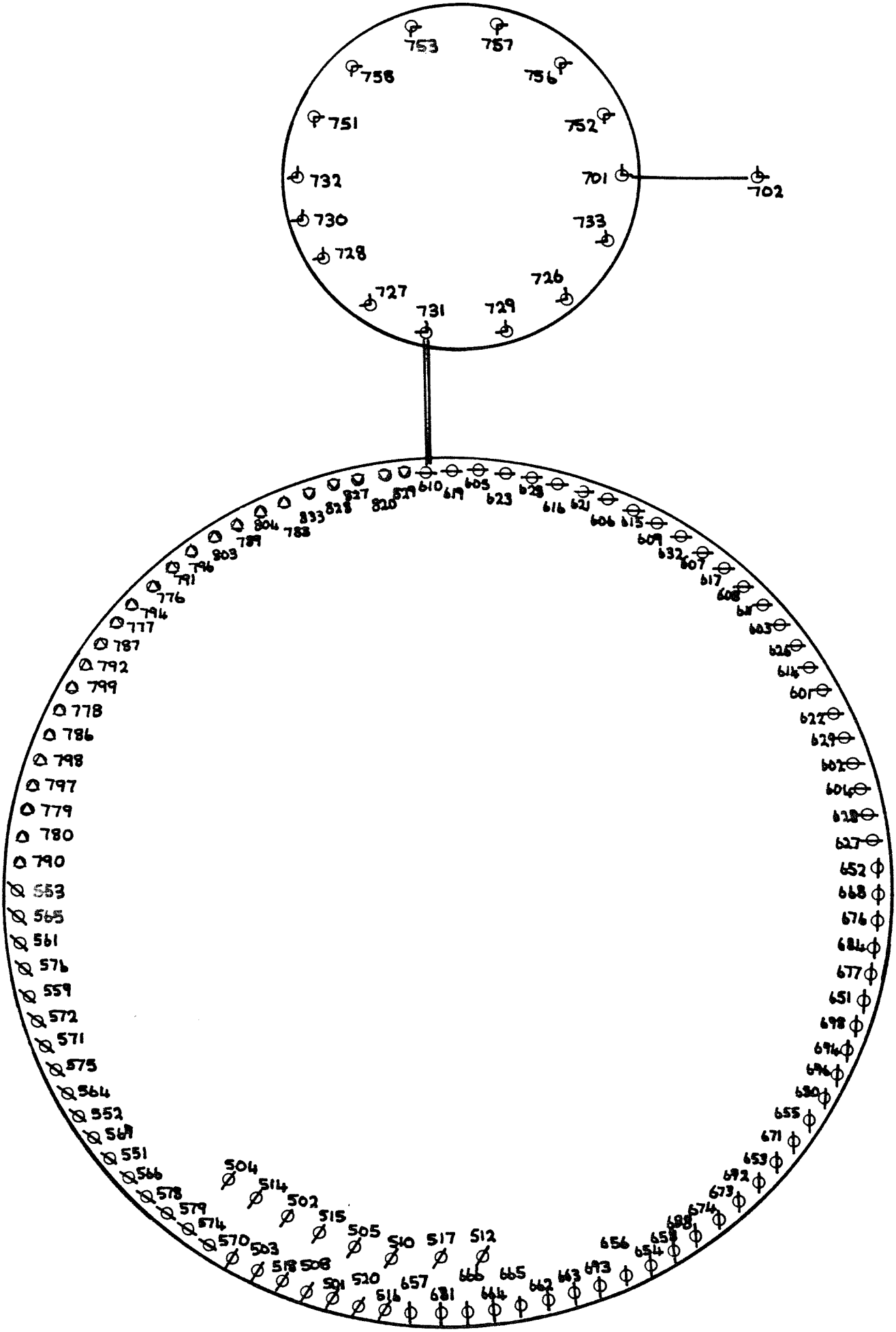


Figure 5.38: *Psophocarpus* intra-generic study (97) - Linkage level 125
Similarity = 0.5147 All OTU's linked



P.scandens encircled cluster, *P.tetragonolobus* being drawn into the latter cluster by links between *P.scandens* and itself, indicating the closer relationship of *P.tetragonolobus* to *P.scandens* than *P.palustris*. As well as showing this, Figure 5.34, also shows the first links between *P.monophyllus* and *P.lecomtei*. The links between these two species are multiple and so by the next level of similarity they will both be included in one encircled cluster.

Figure 5.35 shows *P.lukafuensis* joining the main cluster via a *P.tetragonolobus* OTU at a similarity level of 0.5807. There is quite a large decrease in similarity level between this and the previous level where *P.grandiflorus* joined this cluster. Indicating that *P.lukafuensis* is considerably more remote than the other four species already present in the cluster. At this linkage level *P.grandiflorus* can be seen to have been incorporated in the main encircled cluster, this it achieved by links with both *P.scandens* and *P.palustris*, indicating its equidistant relationship with these two species.

The next species cluster to join the main cluster of species is *P.lancifolius*, which at a similarity level of 0.5755 links with a *P.palustris* OTU. It should be noted that in Figure 5.36 the *P.lukafuensis* cluster forms a second link with a *P.tetragonolobus* OTU, underscoring their allied nature.

Figure 5.37 indicates the next species to link, which at a similarity level of 0.5493, are *P.obovalis* with the *P.monophyllus* - *P.lecomtei* cluster. A multiple link is formed between a *P.obovalis* OTU and *P.*monophyllus OTU's, so that the *P.obovalis* OTU 701 will be included in the encircled cluster of *P,monophyllus* and *P.lecomtei* in the next linkage diagram. It can be seen that *P.lukafuensis* has been drawn into the main encircled cluster, containing *P.palustris*, *P.scandens*, *P.tetragonolobus* and *P.grandiflorus*, via multiple links with *P.tetragonolobus* and one link with *P.scandens*. This infers closer links with *P.tetragonoloubs* and *P.scandens* than with *P.lancifolius* as proposed by Verdcourt and Halliday (1978). Figure 5.37 also shows *P. lancifolius* forming numerous links with the main cluster OTU's, particularly specimens of *P.palustris*, *P.scandens* and *P.lukafuensis*: drawing *P.lancifolius* into the main encircled cluster.

The linkage of the two remaining multiple species clusters occurs after a marked decrease in similarity to 0.5147. This marked drop in similarity may be taken to indicate the relative isolation of these two clusters. The actual link shown in Figure 5.38 is between a *P.monophyllus* OTU and a *P.palustris* OTU. It should also be noted that the two multiple species clusters at this stage are entirely encircled, except for OTU 702, which indicates the internal cohesion of both clusters and displayes possibly their natural relationship. *P.obovalis* (of which 702 is a specimen) is always going to be the anomolous *Psophocarpus* species as will be explained below, due to its tri-foliate leaf and the *P.monophyllus* - *P.lecomtei* stigma style arrangement.

To summarise the results of the intra-generic LINKAGE analysis it was shown that the nine species fall into two groups, the first containing *P.palustris*, *P.scandens*. *P.tetragonolobus*, *P.grandiflorus*, *P.lukafuensis*, and *P.lancifolius*, while the second contains *P.*monophyllus, P.lecomtei and *P.obovalis*. Of the six species in Group 1, *P.palustris* and *P.scandens* are clearly most closely related, then more remote are *P.tetragonolobus* and *P.grandiflorus* related to *P.scandens* and *P.palustris* respectively. The other two species *P.lukafuensis* and *P.lancifolius* of group 1 are most distant from the other species, particularly *P.lancifolius* which seems distantly related to *P.palustris*, *P.scandens* and *P.lukafuensis*. *P.lukafuensis* seems most closely related to *P.tetragonolobus* based on the 97 character set although by the taxonomically important characters of the stigma and style, as Verdcourt and Halliday (1978) pointed out, it shows a closer relationship to *P.lancifolius*.

Of the second group of species, *P.monophyllus* and *P.lecomtei* are clearly most closely related, sharing the distinctive style apex shape, stigma position and unifoliate leaf. *P.obovalis* as pointed out above, is an anomolous *Psophocarpus* species, having the stigma style arrangement of *P.monophyllus* and *P.lecomtei*, but the tri-foliate leaf of the group 1 species. Verdcourt and Halliday (1978) associate this species as a distant relative of the group 1 species, but the LINKAGE results indicate it to be closer to the group 2 species. Although obviously relatively distant to the other group 2 species, its inclusion

with them seems more natural, especially as stigma style characters are considered so important taxonomically.

5.4.2 CLUSTAN (DISTIN)

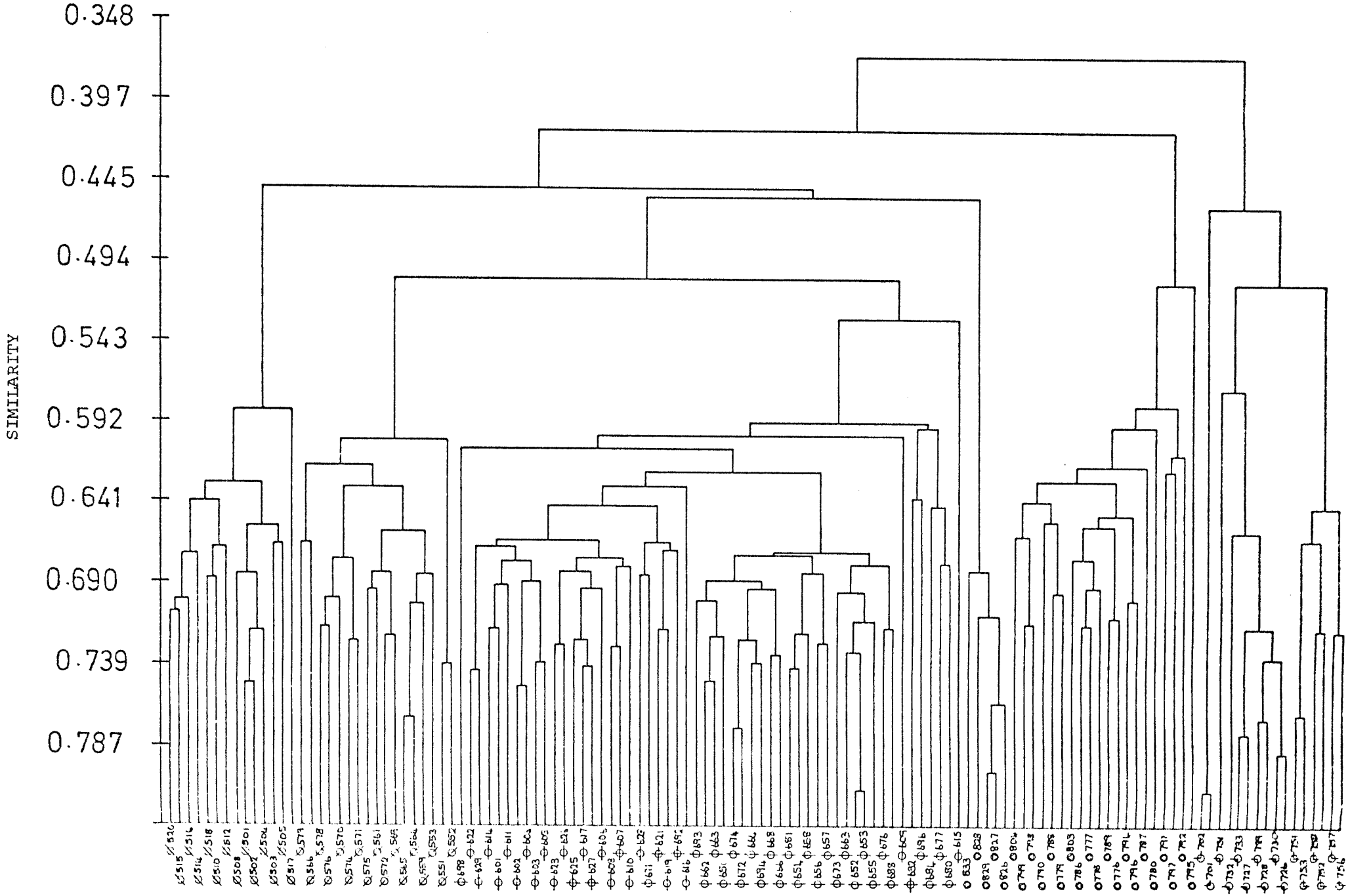
The dendrogram displaying the results of the average linkage cluster analysis, produced using CLUSTAN sub-routine DISTIN and the lower half of the similarity matrix taken from LINKAGE, is shown in Figure 5.39. Using the similarity coefficients produced by LINKAGE means that the dendrogram is based on the same 97 characters that were used for LINKAGE and are detailed in Table 4.2

The first comment to make about the dendrogram is how OTU's representing particular species tend to form discrete clusters, except for the two closely allied species *P.palustris* and *P.scandens*, of which there is doubt that they are in fact true species. To attempt to resolve this doubt the *P.palustris* - *P.scandens* complex OTU's were studied separately to the other *Psophocarpus* species. The results of this study are detailed in Section 5.5 of this chapter.

The main branching of the dendrogram divides the species into two groups; one group containing *P.grandiflorus*, *P.tetragonolobus*, *P.palustirs*, *P.scandens*, *P.lukafuensis* and *P.lancifolius*, while the other contains *P.obovalis*, *P.monophyllus* and *P.lecomtei*. This dicotomy reflects the major dicotomy produced by the LINKAGE results.

The most remote of the first six species is *P.lancifolius*, due to its coloured, long vegetative hairs and its particular stigma style arrangement. The next species to be split from the main cluster is *P.grandiflorus* whose difference from the other group 1 members relates to the size of its flower and the resultant petal and style shape changes. With a small increase in similarity *P.lukafuensis* is split from the remaining group 1 members. *P.lukafuensis* having quite separate distinctions from *P.grandiflorus* to the remaining three species of group 1. *P.lukafuensis* has smaller flowers and a quite different stigma style arrangement, similar to *P.lancifolius*. *P.tetragonolobus*, *P.palustris* and *P.scandens* can be seen to be relatively closely allied, but *P.tetragonolobus* shows some important differences (associated with

Figure 5.39: *Psophocarpus* Intra-generic study (97) - CLUSTAN (DISTIN)



larger flowers, pods and seeds) to the other closely allied species. The two most closely allied *Psophocarpus* species are *P.palustris* and *P.scandens* and they with their intermediate specimens will be disucssed separately as mentioned previously.

The group 2 species (*P.obovalis*, *P.monophyllus*, *P.lecomtei*) form three discrete specific clusters, *P.monophyllus* and *P.lecomtei* being closely allied as both are unifoliate, but *P.obovalis* though tri-foliate and so more distantly related shares the same stima style arrangement as the other two species. The shared stigma style arrangement is the base of a large number of characters, which appear to link these three species naturally together.

To summarise the results of the CLUSTAN (DISTIN) analysis, they mirror closely the findings of the LINKAGE. However, the different modes of displaying the results has introduced minor deviation in particular OTU allegiances. The clusters formed are specifically uniform and the two major groupings can be detected, composed of the same species as indicated from the LINKAGE results.

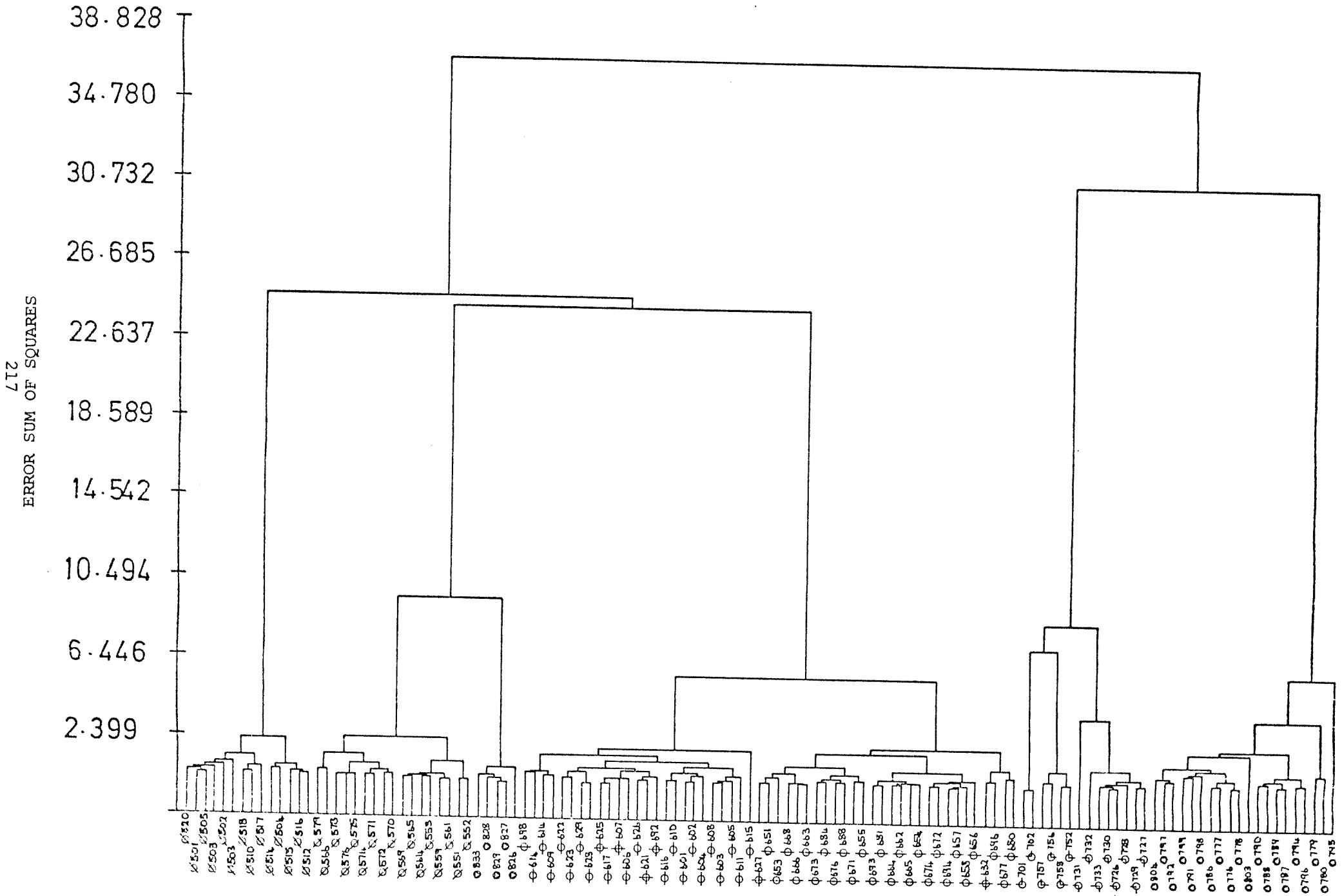
5.4.3. CLUSTIN (FILE)

For this and the DECORANA analysis the character set used was reduced from 97 to 76 characters to eliminate those characters for which there was missing data. Both CLUSTAN and DECORANA were unable to compensate for missing data. This restriction also meant that three OTU's (652, 751 and 753) with missing data for the reduced character subset of 76 characters were necessarily excluded from the analysis. This meant that 123 OTU's were analysed using CLUSTAN(FILE) and DECORANA.

A) Ward's Method: The results of the intra-generic *Psophocarpus* analysis using Ward's method of cluster analysis are shown in Figure 5.40.

As with the previous methods of analysis OTU's representing particular species can be clearly seen to link closely together, forming discrete clusters. Even the closely allied species *P.palustris* and

Figure 5.40: *Psophocarpus* Intra-generic study (76) - CLUSTAN (file) - Ward's method



P.scandens form uniform specific cluster except for OTU 698, a *P.scandens* specimen that joins the *P.palustris* cluster. This particular OTU was shown both by LINKAGE and CLUSTAN (DISTIN) to be a particularly isolated member of the *P.palustris* - *P.scandens* complex. However, in the following section (5.5) where the *P.palustris* - *P.scandens* complex is studied in detail it is clearly shown that 698, although relatively distinct from other *P.palustris* and *P.scandens*, is more closely allied to *P.scandens* than *P.palustris*.

The species of *Psophocarpus* are split up in a slightly different manner than was indicated by the LINKAGE and CLUSTAN(DISTIN) analysis; largely due to the change in position of *P.lancifolius*, which in this analysis is grouped, although distantly, with the *P.obovalis*, *P.monophyllus* and *P.lecomtei* OTU's. *P.lancifolius* is like *P.obovalis*, a peripheral *Psophocarpus* species in that it forms no obvious close relationship with the other *Psophocarpus* species, except perhaps with *P.lukafuensis* as suggested by Verdcourt and Halliday (1978). To ally *P.lancifolius* with *P.obovalis*, *P.monophyllus* and *P.lecomtei* to which it is so clearly not naturally allied perhaps underlines its anomalous position and that Ward's method results must be interpreted with biological insight, rather than literally. The strongest evidence against this relationship is the quite different stigma style arrangement of *P.lancifolius* to the other three species.

As with the other methods of analysis the three comparatively rare species from Central and West Africa, *P.obovalis*, *P.monophyllus* and *P.lecomtei* are shown to link closely together. This recurrent demonstration of a close relationship surely indicates a naturally allied grouping, even though *P.lecomtei* and *P.monophyllus* are uni-foliate while *P.obovalis* is tri-foliate. The similarity in floral characteristics underlines this close relationship and is considered more important than the vegetative differences (Verdcourt pers. comm).

The other main cluster of the dendrogram in Figure 5.40 splits off at a high level of error sum of squares the *P.palustris* - *P.scandens* complex OTU's from *P.tetragonolobus* and *P.lukafuensis*. The latter two form a second cluster and the more remote *P.grandiflorus* forms an unispecific third cluster. Of these species it is expected that

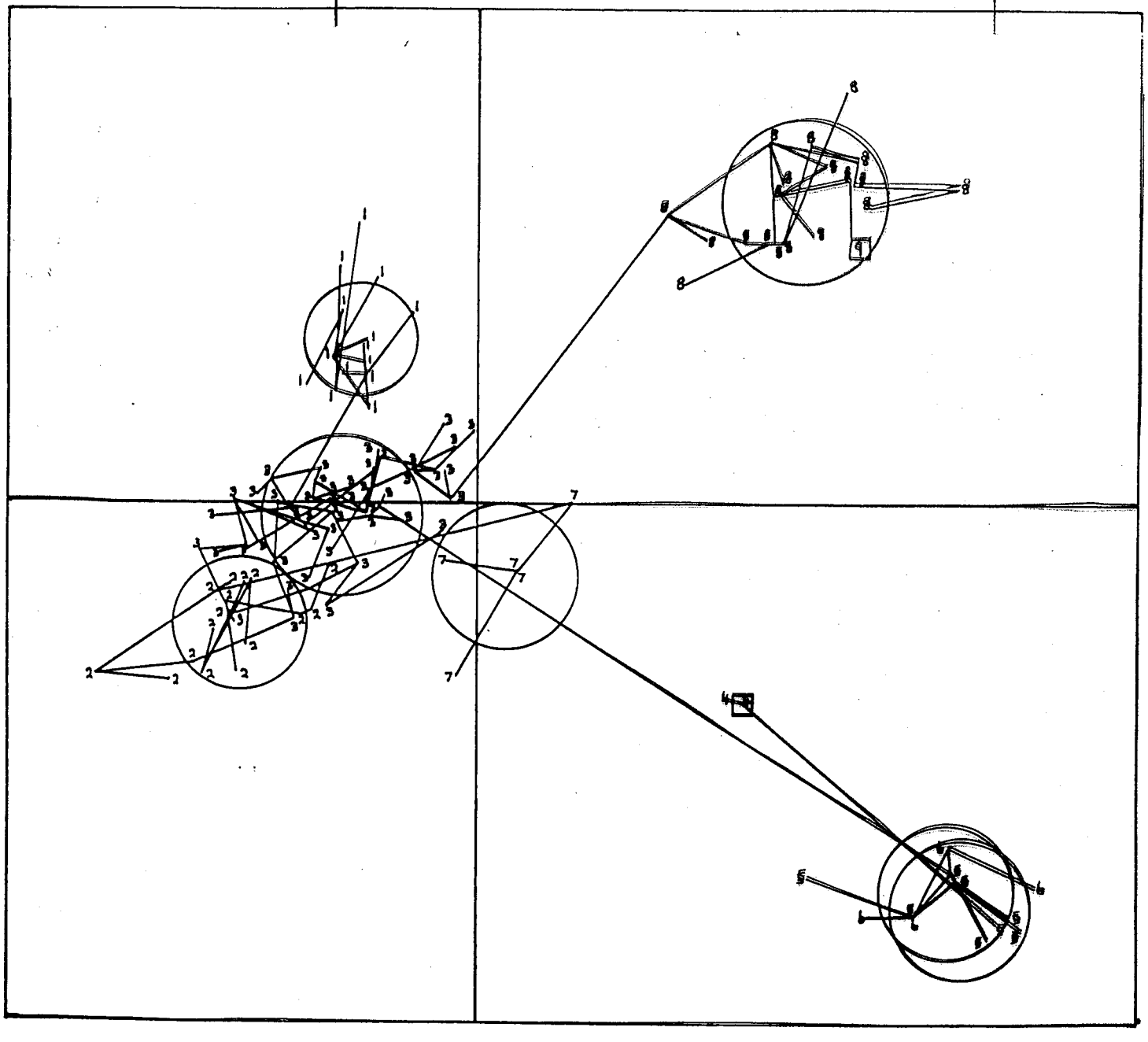
P.grandiflorus would be found most remote, its larger overall foral dimensions bring with it necessary changes in petal shape. Its stigma style arrangement is also different from the other four species of this major cluster. However the linking of *P.tetragonolobus* to *P.lukafuensis* rather than to the *P.palustris* - *P.scandens* complex is puzzling in the context of the above results from the other analysis methods. It would have been expected that *P.tetragonolobus* would join *P.scandens* then form links with *P.lukafuensis*. This might possibly be another consequence of using Ward's method which forces discrete spherical clusters on the data. However it does seem to be apparent that *P.lukafuensis* is closer to *P.tetragonolobus* than was thought by Verdcourt and Halliday (1978).

B) Principal Components Analysis: The results of ther PCA on the 76 characters and 123 OTU's representing the nine *Psophocarpus* species are shown in the scatter diagram drawn in Figure 5.41.

The results displayed in Figure 5.41 show close agreement with those produced by single linkage cluster analysis (LINKAGE) and average linkage cluster analysis (CLUSTER-DISTIN). Three basic clusters can be discriminated. The central cluster containing *P.tetragonolobus*, *P.grandiflorus*, *P.lukafuensis*, *P.palustris*, and *P.scandens*; the second cluster with the anomolous *P.lancifolius* and the third containing *P.obovalis*, *P.monophyllus* and *P.lecomtei*. Within each cluster OTU's referable to the same species group most closely together, indicating the usefulness of the chosen characters in retaining specific identity, but discriminating between species.

The central cluster shows three very closely allied species *P.palustris*, *P.scandens* and *P.tetragonolobus*, though the latter can be seen to form a slightly more distinct grouping than *P.palustris* and *P.scandens*. *P.lukafuensis* clusters closely to these three species and is spatially closest to *P.palustris* and *P.scandens* OTU's. The most remote member of this cluster, *P.grandiflorus* forms a distinct sub-cluster which is spatially most closely allied to *P.palustris* and *P.scandens*.

P.lancifolius again is shown to be a peripheral *Psophocarpus* species



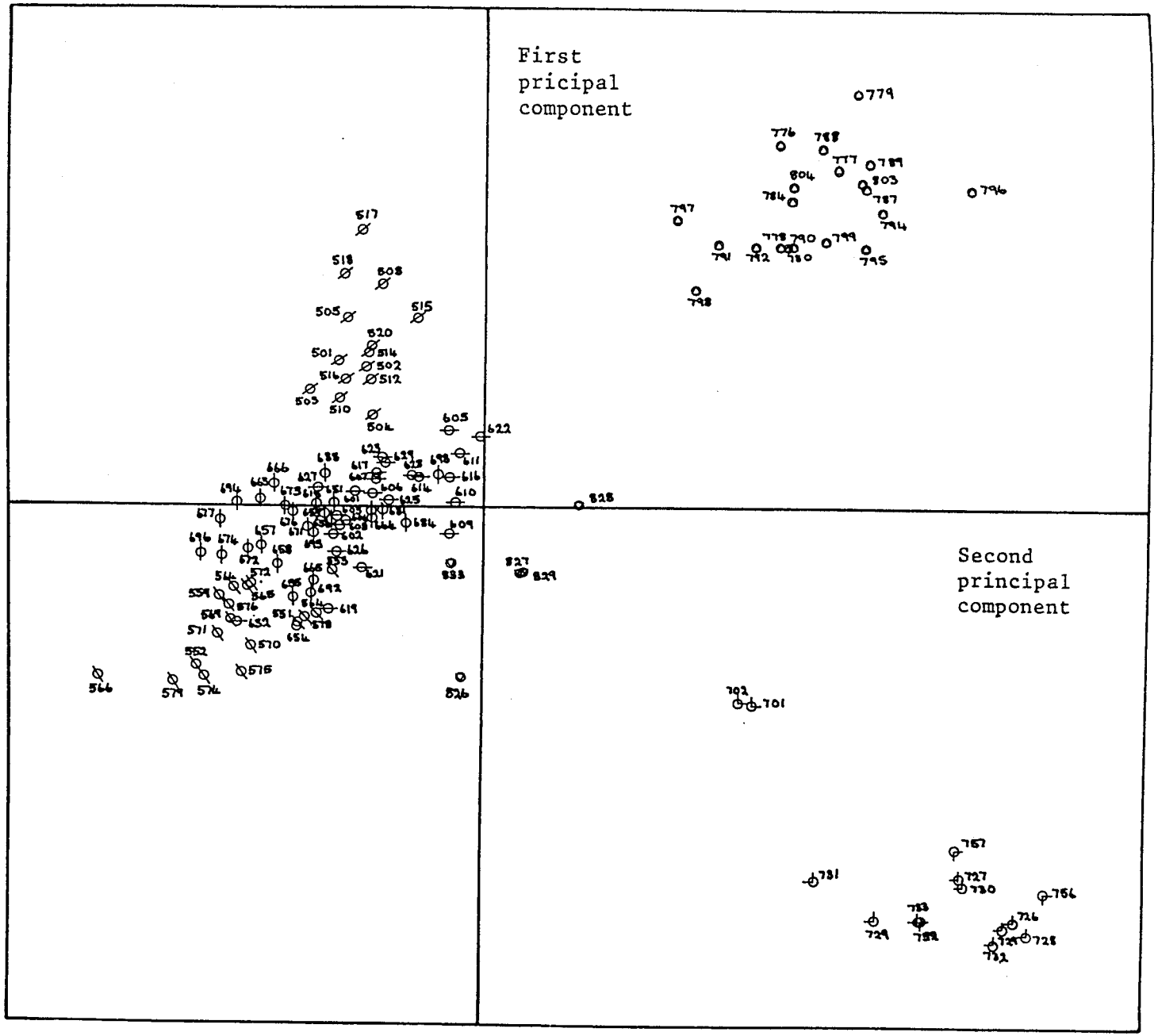


Figure 5.41: Intra-generic Psophacarpus study (76) - CLUSTAN (file) - Principal components analysis

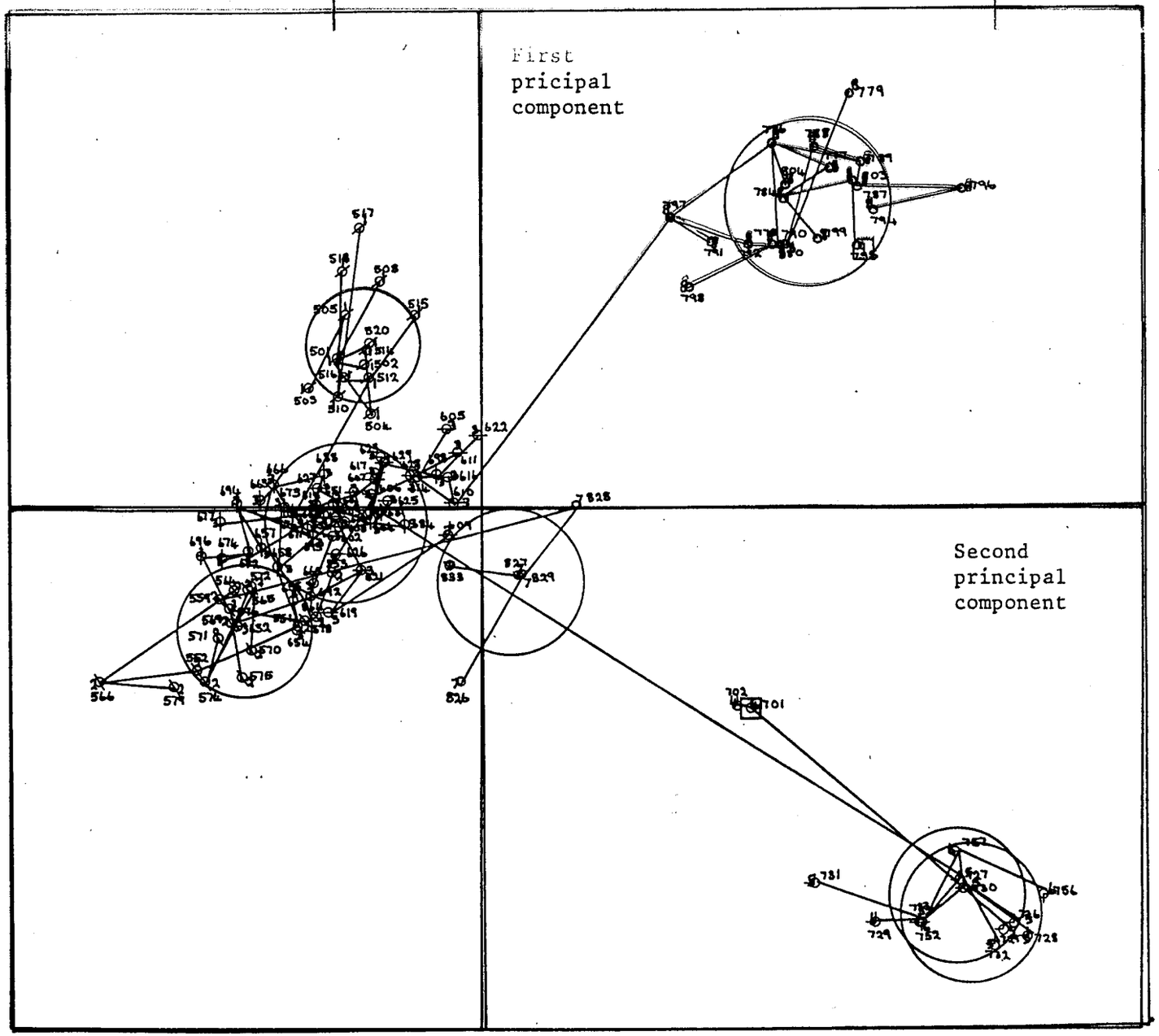


Figure 5.41: Intra-generic Psophacarpus study (76) - CLUSTAN (file) - Principal components analysis

with no clear allies within the genus. The third cluster is composed of the two closely allied speices *P.monophyllus* and *P.lecomtei*, of which there appears no clear separation into specific clusters, with *P.obovalis*. As previously emphasised these three species share a unique stigma style arrangement, but interestingly in the scatter diagram the two *P.obovalis* OTU's are placed closer to the central cluster. As *P.obovalis* is tri-foliate it would be expected that it would be placed in an intermediate position between the uni-foliate *P.monophyllus* and *P.lecomtei* and the tri-foliate central cluster species.

If the overlay containing the minimum spanning tree and cluster circles is lowered over the scatter diagram, it is seen that within each specific cluster the ordination technique has distorted the data set, e.g. OTU 731 does not link to its spatially closest *P.monophyllus* OTU 729 but to 733. However, between specific clusters, the clusters linked by the MST are those spatially related. There is one specific cluster that might be considered not to follow this rule, and that is the *P.lukafuensis* cluster, which although placed most closely to *P.palustris* and *P.scandens* OTU's is linked by the MST to a *P.tetragonolobus* OTU. Thus the ordination has slightly misplaced the *P.lukafuensis* cluster and this should be borne in mind when interpreting the results.

The cluster circles and cluster circles membership numbers are also drawn on the overlay. In general the picture corroborates the taxa relationships indicated in the scatter diagram. Most interestingly when nine cluster circles are specified the program cannot distinguish *P.palustris* and *P.scandens* into separate clusters. All their representative OTU's are included in cluster 3. The ninth non-specific cluster is formed by splitting off one *P.lancifolius* OTU (795) from the other *P.lancifolius* OTU's in cluster 8. However if eight cluster circles are specified then the cluster nine OTU is merged with the other *P.lancifolius* OTU's.

Overall the clusters show a higher degree of internal variance, leading to large cluster circles and many OTU's laying outside of the circle. Though only clusters five and six, containing *P,monophyllus* and *P.lecomtei* respectively, show overlapping cluster circles. These two species, as indicated by the other methods of analysis, are obviously

closely allied and so overlapping cluster circles would be expected. The *P.palustris - P.scandens* complex cluster circle is close to the *P.tetragonolobus*, this again reflects the relationship indicated by the other methods of analysis. It can also be seen that a few OTU's from these two clusters are found to lie within the opposing cluster cirlce, but here it should be remembered that these OTU's are drawn back within their more natural cluster by the minimum spanning tree.

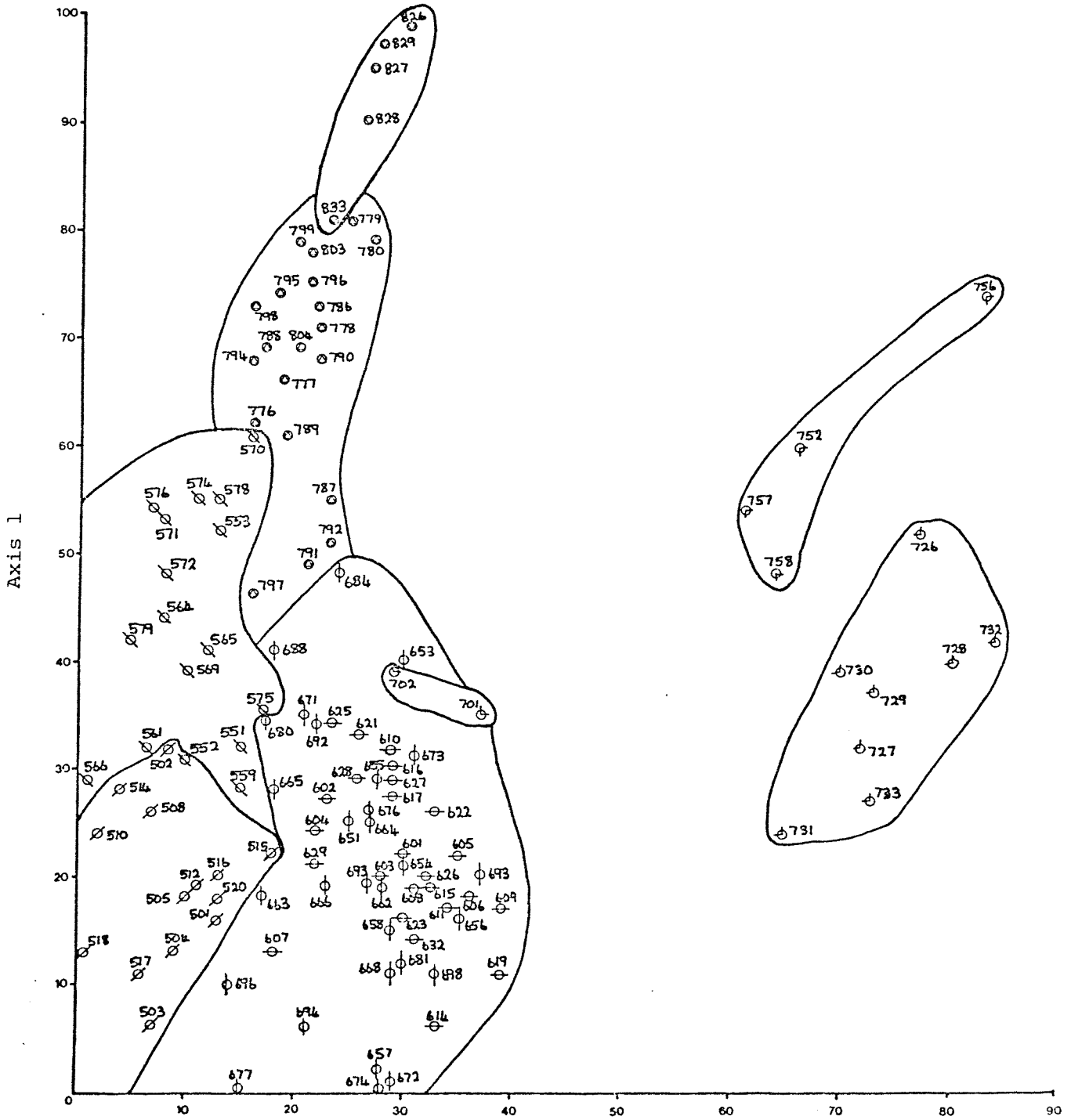
5.4.4 DECORANA

The same character set was used for the DECORANA intra-generic study analysis as for the CLUSTAN(FILE) analysis detailed above. The character set is detailed in Table 4.2. The scatter diagram displaying the results of the DECORANA analysis is drawn in Figure 5.42.

The scatter diagram shows the OTU's falling into two major groupings. The first containing *P.grandiflorus*, *P.tetragonolobus*, *P.palustris*, *P.scandens*. *P.obovalis*, *P.lancifolius* and *P.lukafuensis*, while the second grouping contains *P.monophyllus* and *P.lecomtei*. Within the larger OTU grouping the specific clusters do not form discrete sub-clusters but rather juxtapose one on another. For this reason the specific clusters in Figure 5.42 have been circumscribed to aid recognition, except for *P.palustirs* and *P.scandens*, whose OTU's are interspersed with each other and so specific clusters cannot be determined. The most interesting placement in the scatter diagram is the positioning of the *P.obovalis* OTU's in the main cluster and not with *P.monophyllus* and *P.lecomtei* with which it has been linked by all the above forms of analysis. Why DECORANA should place *P.obovalis* in the main cluster is unclear, but as it is the only method of analysis to do so it must be for intrinsic reasons of the ordination produced by DECORANA.

Within the large cluster of species *P.grandiflorus*, *P.tetragonolobus* and the *P.palustris - P.scandens* complex OTU's are found to be closely allied. The two *P.obovalis* OTU's are positioned near the *P.palustris - P.scandens* complex cluster. There is a second change in the main cluster species relationship, in that *P.lancifolius* is seen to be

Figure 5.42: *Psophocarpus* intra-generic study (76) - DECORANA - OTU plot



closely allied to *P.tetragonolobus* and *P.scandens*, while *P.lukafuensis* is more distantly placed. In the other forms of analysis the position of *P.lancifolius* and *P.lukafuensis* was reversed with *P.lancifolius* being more remote and *P.lukafuensis* showing a closer relationship with *P.tetragonolobus* and *P.scandens*.

The second cluster containing *P.monophyllus* and *P.lecomtei* is quite distinct from the main cluster and in fact the two species form discrete specific clusters. The linkage of these two species has been noticed in each method of analysis and so can be taken to reflect a close natural relationship.

5.5 Psophocarpus palustris - P.scandens Complex Study

Due to the critical relationship between *P.palustris* and *P.scandens* these two species were investigated in a separate study, as well as in the main *Psophocarpus* species study. However in this separate study characters were selected particularly which it was thought would elucidate the relationship between these two closely allied species.

The 54 specimens scored from the *P.palustrs - P.scandens* complex were scored where possible for the 315 characters detailed in Table 4.1. For the purposes of the analysis each specimen was regarded as an OTU.

Throughout this chapter in the figures detailing analysis results, each OTU has been given a symbol which indicates its generic, subgeneric or specific identity. For this particular study instead of the OTU being identified within the course of the investigation, their identity as members of either species or intermediate status is largely taken from lists of identified specimens provided in Verdcourt and Halliday (1978). A few specimens not listed by these authors were identified by the present researcher. It was felt as Verdcourt split the taxa into two species, his concept and identification should be followed. However this does not infer that the present author's identification would have varied from Verdcourt and Hallyday's, in fact only one specimen (625, *P.palustris*, Deighton 3466) ascribed to *P.palustris* by them, could possibly better have been referred to intermediate status. In all six specimens (OTU's) are given intermediate status, five described by Verdcourt and Halliday (1978) and OTU 625 shown in the analysis to warrant this position.

5.5.1 LINKAGE

For the *P.palustris - P.scandens* complex analysis 75 characters were chosen intuitively to clarify the intra-taxon relationship. The LINKAGE analysis used 53 linkage levels to complete OTU linkage, decreasing in similarity from 0.6783 to 0.5235. The linkage diagrams that show intra taxon relationship most clearly is drawn in Figure 5.43.

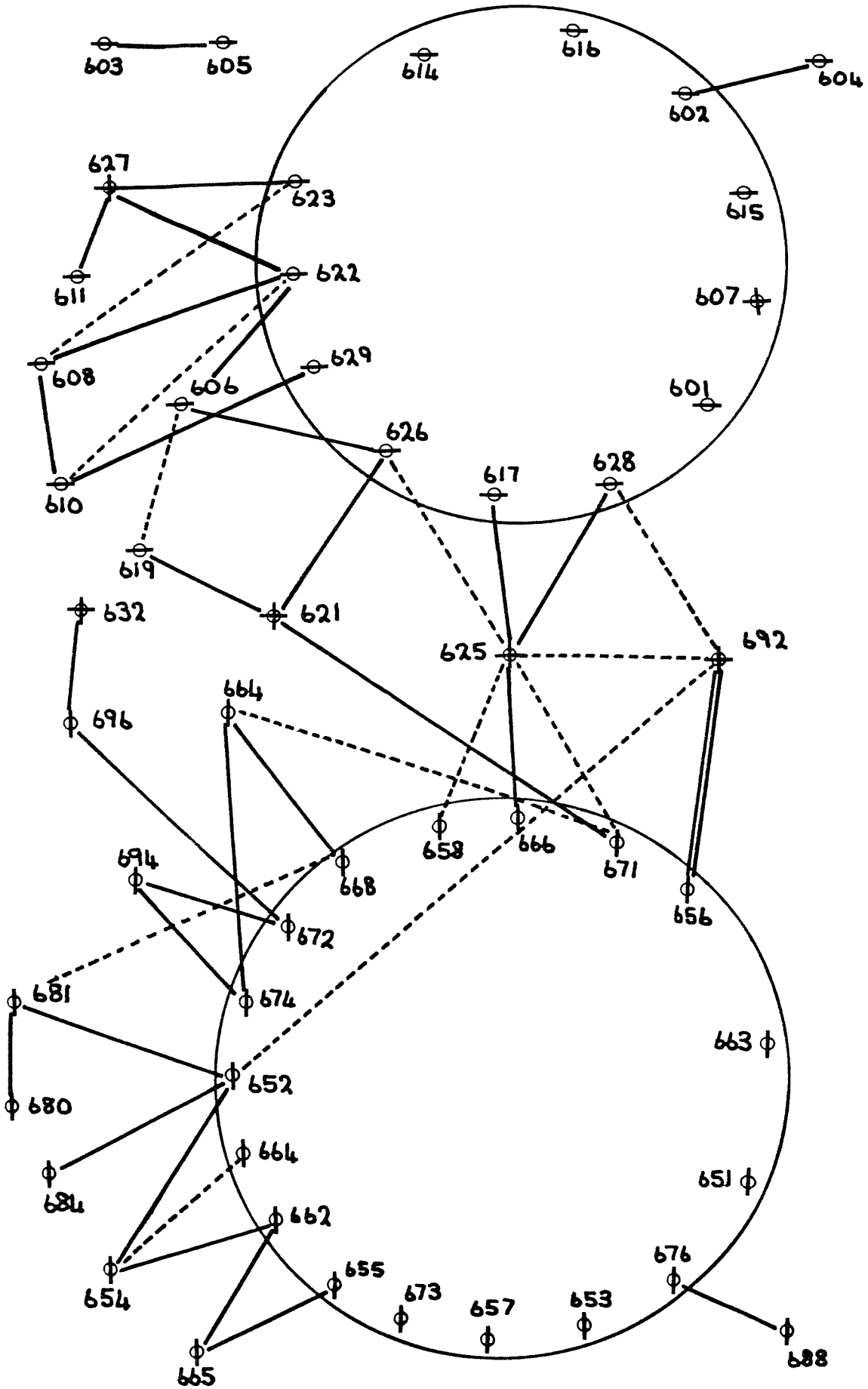
In Figure 5.43 at the similarity level of 0.5654 the OTU's can be

Figure 5.43: *Psophocarpus palustris* - *P. scandens* complex study (75) - Linkage level 49

Similarity = 0.5654

4 OTU's not yet linked

(ϕ698, ϕ677, ϕ609 and ϕ693)



seen to be arranged in a dumbell shape with two encircled clusters joined by a few bridging OTU's. The two encircled clusters are uniform specifically, in that no specimens identified as belonging to one species (cluster) appear linking directly with specimens of the second species (cluster). At this level of similarity links between the two clusters of OTU's are restricted to specimens considered to show intermediate characteristics (621, Talbot 1318; 625, Deighton 3466 and 692, Letouzey 13155). If the similarity level is further decreased then linkages begin to occur directly between the two clusters rather than via the intermediates. However the fact that two tightly knit clusters are formed prior to any direct inter-cluster linkage indicates that Verdcourt's splitting of the complex into two species was valid.

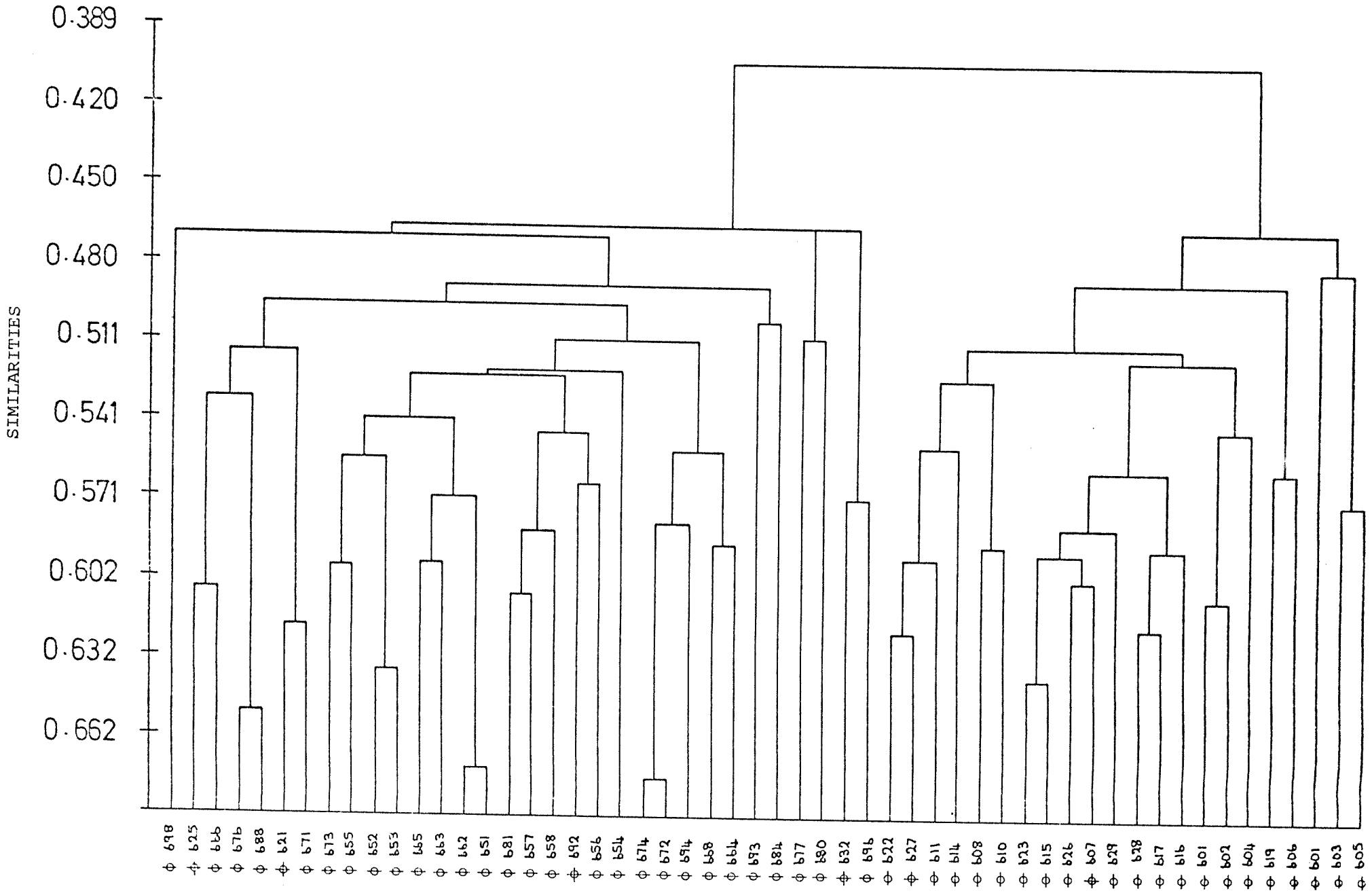
Specimens showing anomalies or intermediacy are however found as pointed out by Verdcourt and Halliday (1978) but in closely related species this does not invalidate the proposition that the species are real identifiable entities. One specimen (OTU=607) Le Testu 4187, attributed by Verdcourt and Halliday to *P.palustris*, but showing intermediacy "bracteoles long but hairy, leaflet hairy", is linked quite early (at a relatively high similarity level) into the encircled *P.palustris* cluster. This is unexpected for a specimen showing intemediacy, however size of bracteoles would only be reflected in two of the 75 characters, bracteole length and width. So it can be explained that the effect of possessing *P.scandens* type bracteoles was diluted in relation to the overall character set, when other intermediate characters were not found.

5.5.2 CLUSTAN (DISTIN)

For the average linkage analysis using the program CLUSTAN sub-routine DISTIN the same 75 character (detailed in Table 4.2) were used as for the LINKAGE analysis as in the previous section. The dendrogram displaying the results of the analysis is drawn in Figure 5.44.

The most striking feature of the dendrogram is the clear dicotomy of the OTU's into the two specific clusters of *P.palustris* and *P.scandens*.

Figure 5.44: *Psophocarpus palustris* - *P. scandens* · Complex study (75) - CLUSTAN (DISTIN)



Perhaps surprisingly, though, the specimens showing intermediate features do not take up an intermediate position in the dendrogram. In fact except for OTU 632, the other OTU referred to as intermediates are firmly placed in specific cluster, sharing relatively high similarity with 'true' *P.palustris* or *P.scandens*. OTU 632, Talbot 1318 (BM) is a specimen ascribed by Verdcourt and Halliday (1978) to *P.palustris*, but they note it possesses "short glabrescent bracteoles". From the results of this analysis it can be seen to share other *P.scandens* characters largely concerned with relative pubescence. The fact that 632 separates with 696 from the *P.scandens* cluster at the lowest level of similarity infers its true intemediacy.

Two other specimens (OTU's 625 and 621) ascribed by Verdcourt and Halliday (1978) to *P.palustris* but with intermediate features are linked into the *P.scandens* cluster. Both these specimens were found to be important in linking *P.palustris* with *P.scandens* in the LINKAGE analysis (see Figure 5.43), indicating their true intermediate nature. However, as the CLUSTAN(DISTIN) results suggest, if these specimens require identification to one of the two species then it would more appropriately be *P.scandens* than *P.palustris* as Verdcourt and Hallliday (1978) suggest.

To summarise the results of the CLUSTAN(DISTIN) analysis of the *Psophocarpus palustris - P.scandens* complex, it can be said that average linkage cluster analysis justifies Verdcourt's splitting of *P.scandens* from *P.palustris*. However, the intermediate specimens do not show up in the results as clearly as with the LINKAGE analysis.

5.5.3 CLUSTAN(FILE)

To eliminate the characters which for some OTU's there was missing data, the character set was reduced to 72 (as detailed in Table 4.2) for the CLUSTAN(FILE) and DECORANA analysis. OTU 652 was excluded from the analysis because it has other missing character scores.

A) Ward's Method: The results of the *P.palustris - P.scandens* complex analysis using Ward's method of cluster analysis are displayed in the

dendrogram in Figure 5.45.

As with the single linkage and average linkage cluster analysis programs, there is a clear dicotomy into two specific clusters. Though as noted in Section 3.4.2(b) Ward's method does have an intrinsic tendency to form isolated clusters, which may accentuate rather than reflect the nature of the data. This can be clearly seen by the position of the specimens (OTU's) showing intermediate features in the dendrogram. All of these specimens are intimately related to 'true' species specimens, none of them indicate intermediacy. Specimens (OTU's) 632 and 621 as in the average linkage (CLUSTAN-DISTIN) results show a closer affinity to *P.scandens* than to *P.palustris* to which they were ascribed by Verdcourt and Halliday (1978). Interestingly however OTU 625 the third intermediate specimen ascribed to *P.scandens* in the average linkage analysis using Ward's method is placed intimately within the *P.palustris* cluster, forming close links with 'true' *P.palustris* specimens. The reason for this change in position is not apparent as the data sets were virtually identical for both sets of analysis (Ward's method having three less legume characters than the average linkage method). In a character set of 75 the loss of three characters would be difficult to explain causing such a change in specific affinity. Thus it is thought that the change in allegiance of OTU 625 is due to the difference in the methods of cluster analysis and the fact that 625 is found in different positions in the two resultant dendrograms can be thought to justify its intermediate status.

B) Principal Components Analysis: The results of the principal components analysis (PCA) produced by sub-routine SCATTER of CLUSTAN are shown in Figure 5.46. The data set was identical to that used in the above Wards's method of cluster analysis.

As with the previous methods of analysis PCA has partitioned the OTU's into two specific clusters, with intermediate OTU joining either one or other specific cluster. The partition of the OTU's into the two clusters is further evidence to validate Verdcourt's (1968) splitting of the species.

Three of the specimens (OTU's) showing intermediate features, 625,

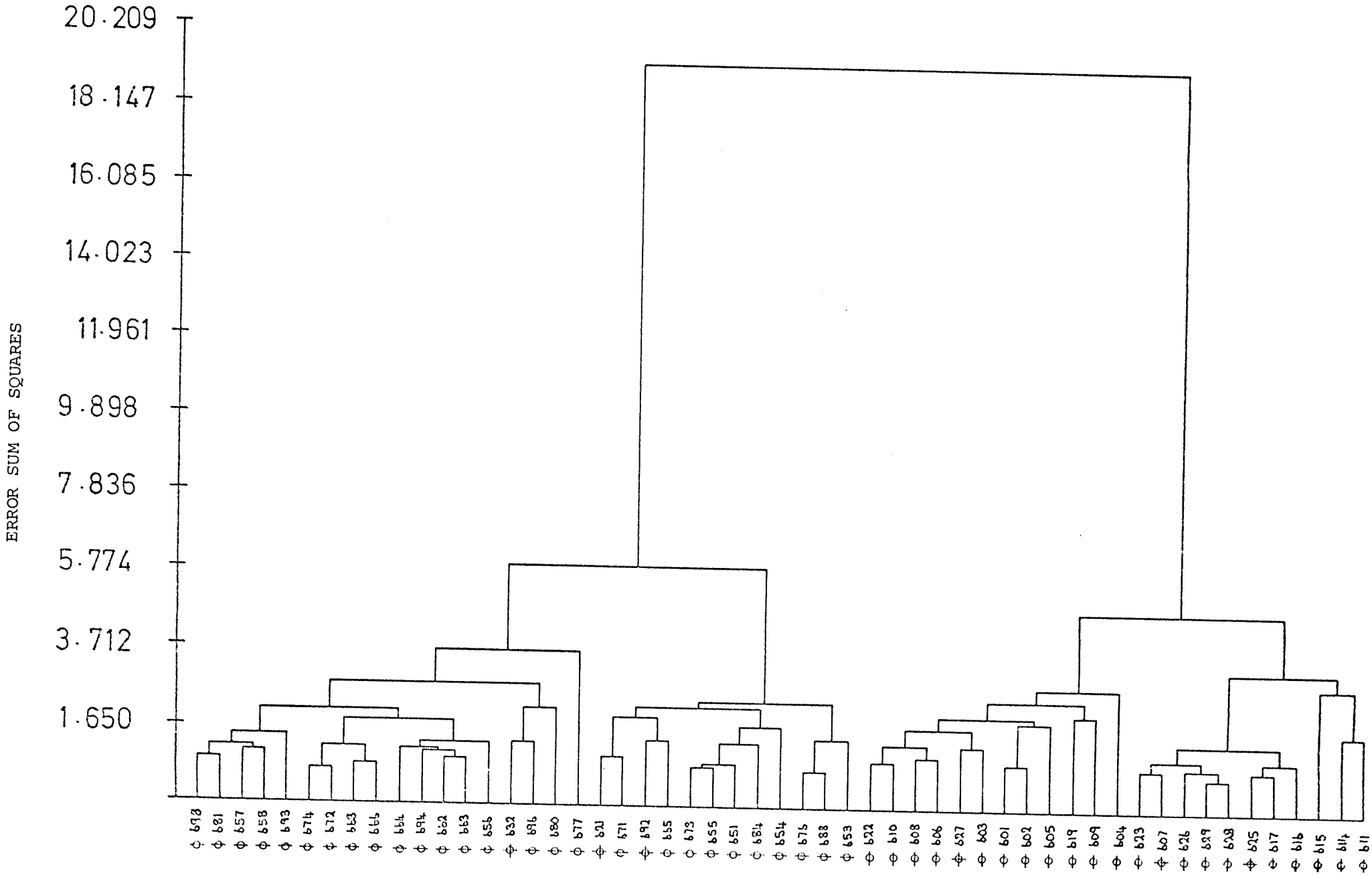
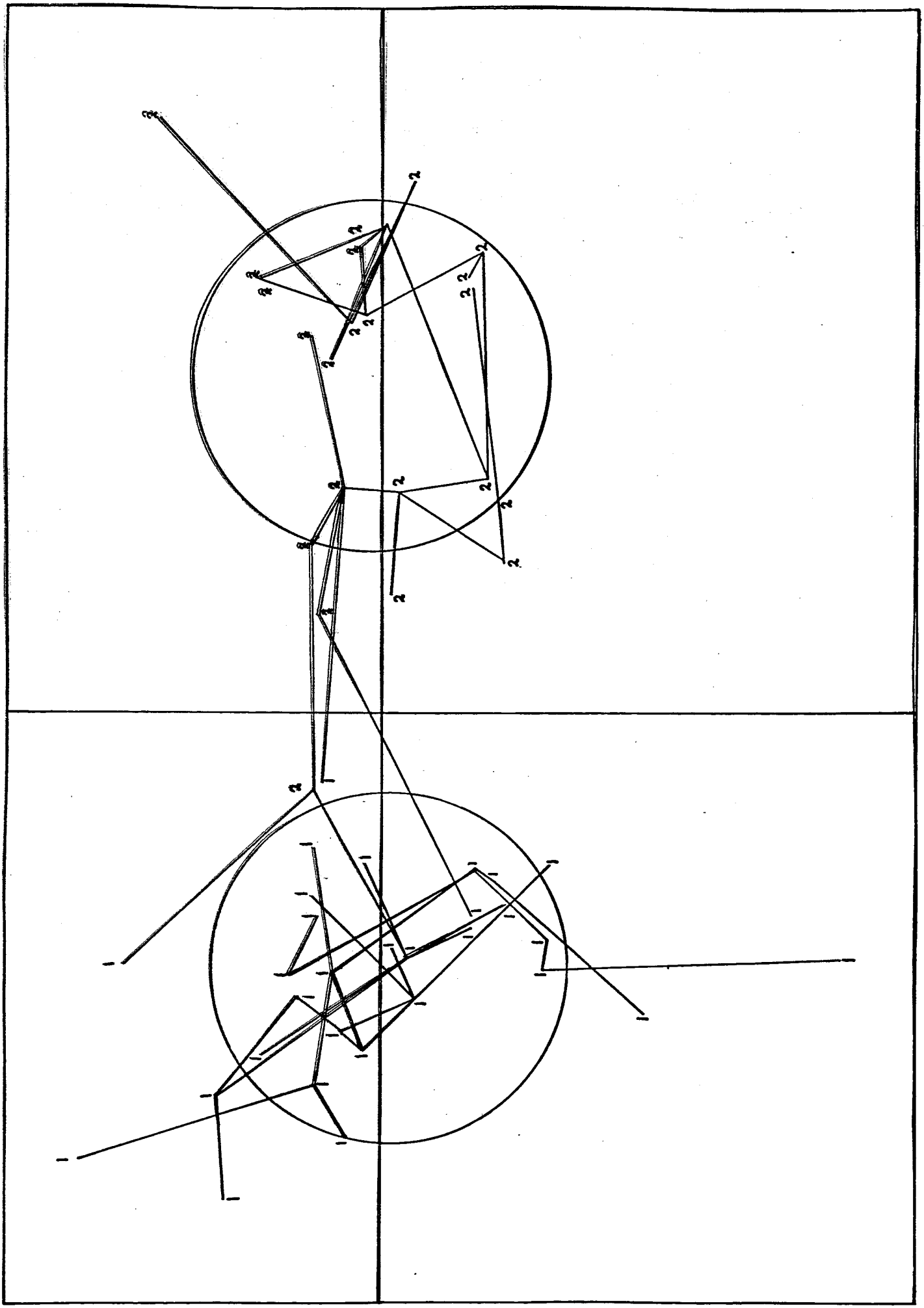


Figure 5.45: *P. palustris* – *P. scandens* Complex study (72) - CLUSTAN (FILE) - Ward's method



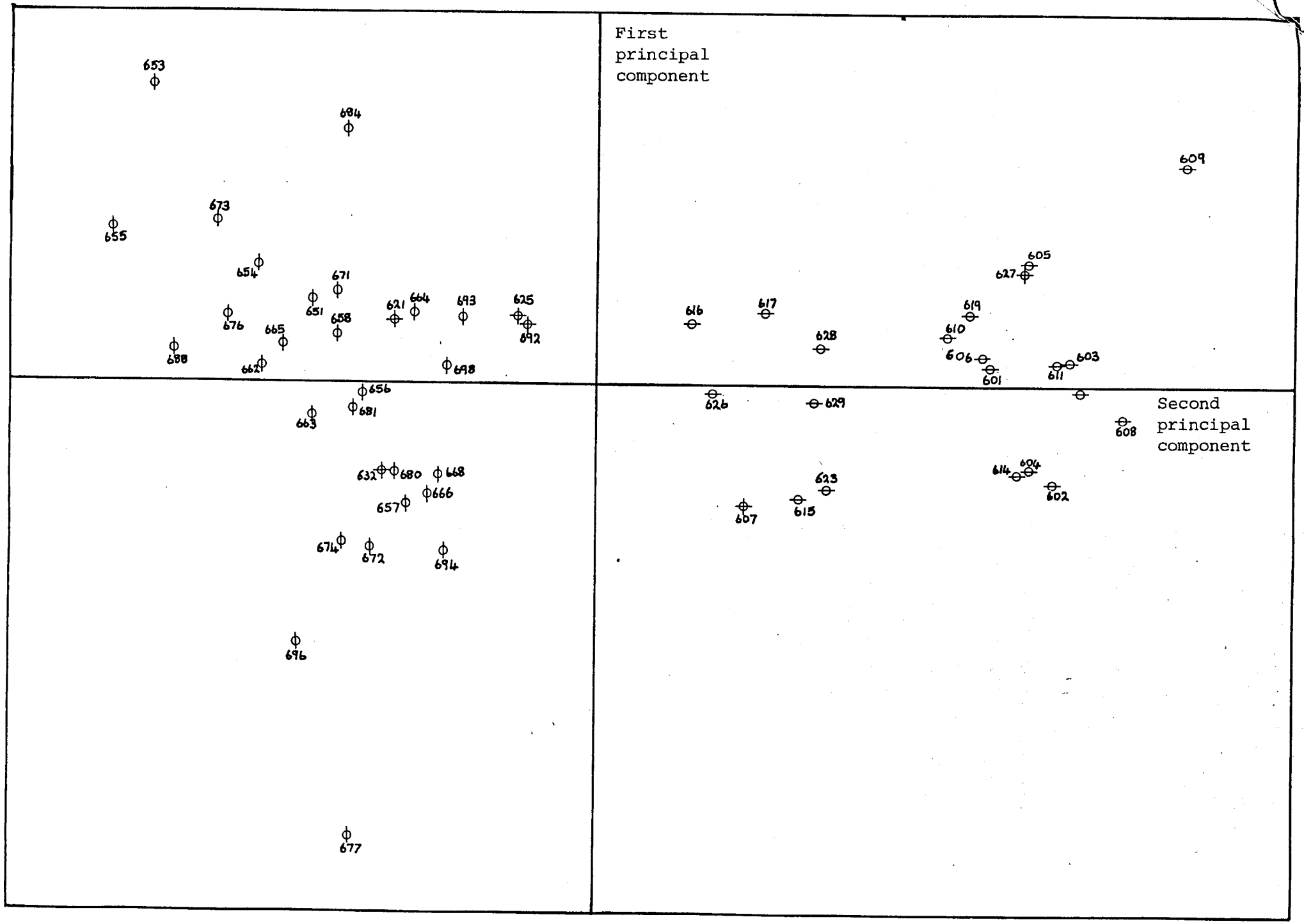


Figure 5.46: *Psophocarpus palustris - P. scandens* Complex study (72) CLUSTAN (SCATTER)

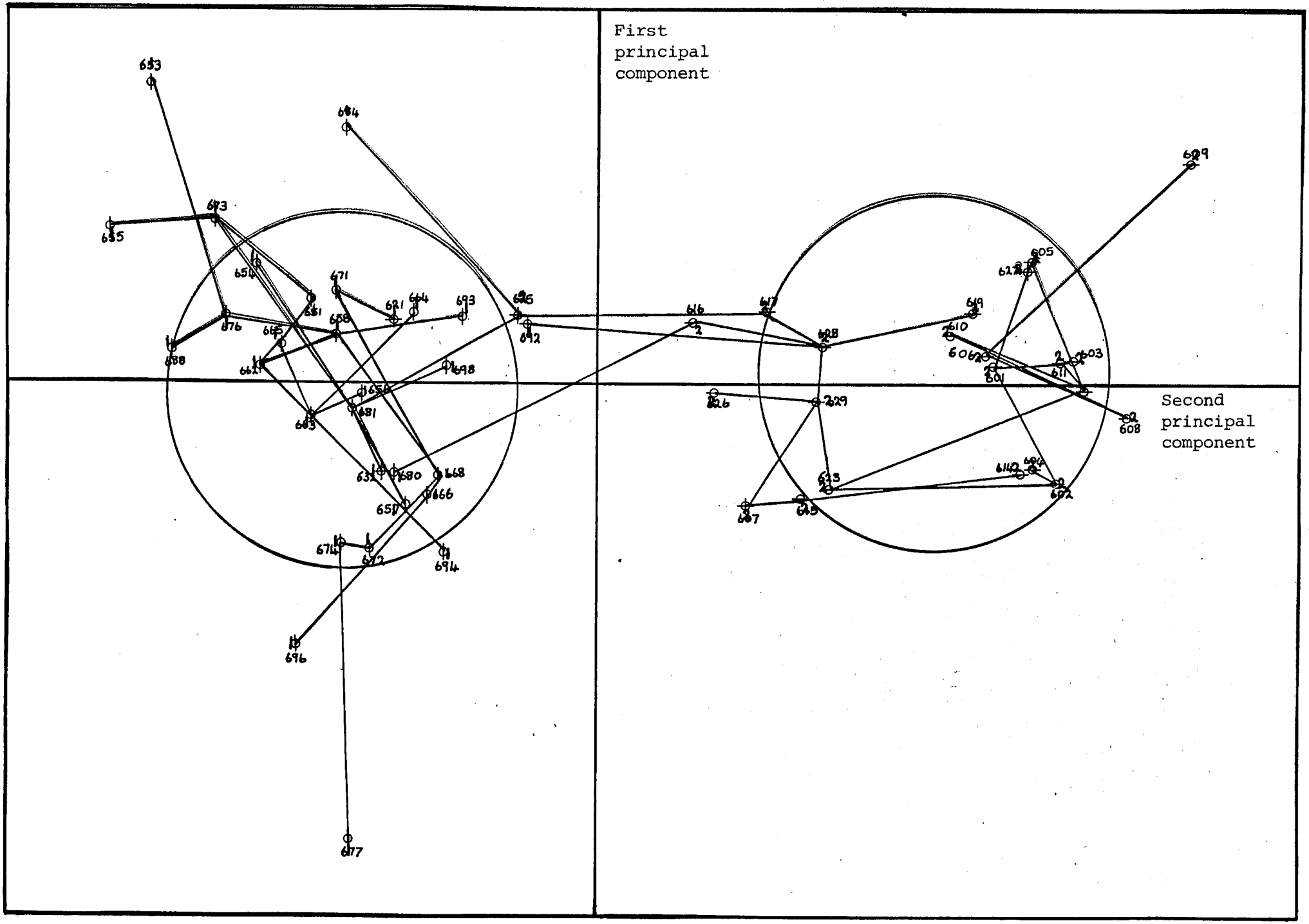


Figure 5.46: *Psophocarpus palustris - P. scandens* Complex study (72) CLUSTAN (SCATTER)

692 and 607 can be seen to be positioned in the scatter diagram at the interface between the two clusters. This positioning indicates their intermediate nature. While the other three OTU's showing intermediate features can be seen to be positioned more firmly in the centre of their respective clusters: 627 in the *P.palustris* cluster and 621 and 632 in the *P.scandens* cluster.

The overlay to Figure 5.46 shows the minimum spanning tree with the cluster circles and OTU cluster circle membership. The minimum spanning tree does indicate that the data has been distorted by the PCA ordination carried out, as demonstrated by the linking of OTU 677 to 674 rather than 696, 677's spatially closest OTU in the scatter diagram. However the important point to note is that OTU's within each specific cluster are linked more closely by the minimum spanning tree than OTU's between clusters. The point of this analysis was to establish if two specific clusters could be clearly distinguished, which can be seen to occur even after the application of the minimum spanning tree has been considered. The minimum spanning tree does not link any OTU's of either of the two true species, but shows the species linking via the mediation of the two intermediate specimens (OTU's) 625 and 632. Which further supports the separate *P.palustris* and *P.scandens* hypothesis.

The cluster circles with their cluster membership numbers are also included on the overlay to Figure 5.46. The two cluster circles (cluster 1 being *P.scandens* and 2 being *P.palustris*) are isolated enough to allow inference of substantial differences between the two sets of OTU's. Like the results of the minimum spanning tree this supports the splitting of *P.scandens* from *P.palustris*.

There is one interesting apparent misplacement in assigning cluster membership numbers, OTU 625 although placed close to the cluster 1 circle (of *P.scandens*) is referred to cluster 2 (of *P.palustris*). OTU 625 (specimen Deighton 3466, ascribed by Verdcourt and Halliday (1978) to *P.palustris*) has been shown by the other methods of analysis to possess intermediate status between *P.palustris* and *P.scandens*. The positioning of OTU 625 near the *P.scandens* circle but referring it to membership of the *P.palustris* may be taken as another indication of its intermediate nature between the two species, though as none of the other

intermediate OTU's show this degree of misplacement it indicates some distortion of the data by the ordination technique used.

5.5.4 DECORANA

The same character set was used for the *P.palustris* - *P.scandens* compex DECORANA analysis as for the CLUSTAN(FILE) analysis. The 72 characters used are detailed in Table 4.2. The result of the analysis is shown in Figure 5.47.

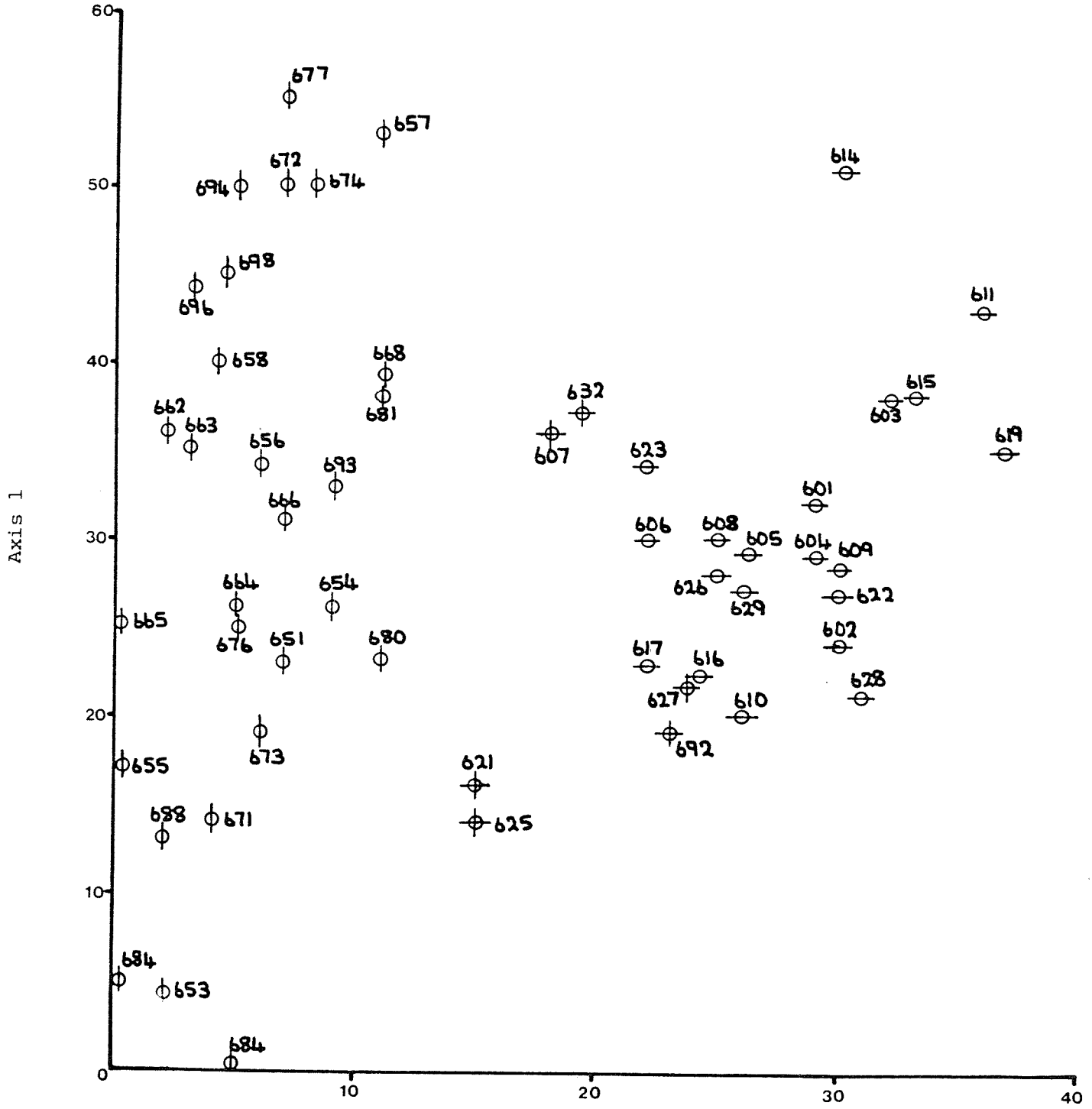
If the intermediate specimens are ignored then the two specific clusters of OTU's can be seen, though as with the other forms of analysis, the *P.scandens* cluster, on the left of the scatter diagram, can be seen to show wider internal variation than the *P.palustris* cluster. The first axis separates out the two species, as with the other forms of analysis, validating Verdcourt's splitting of the species.

The results of the DECORANA analysis do show more clearly than the other forms of analysis the intermediate position between the specific clusters of the specimens showing intermediate features. Five of the six intermediate specimens can be seen to lie between the specific clusters rather than actually within them as indicated by Ward's method of cluster analysis, and to a lesser extent by principal components analysis.

5.6 Summary of phenetic studies results

One thing that is apparent from discussing the results of the

Figure 5.47: *Psophocarpus palustris* - *P. scandens* Complex study (72) - DECORANA - OTU plot



component studies analysis is that the different methods of analysis indicate different taxa relationships. This fact itself must be a strong argument for using several methods of analysis to self-verify the results obtained. Having pointed out the different emphasis placed on certain taxa relationships by particular methods of analysis, the results do provide satisfactory answers to the questions asked about the relationship of *Psophocarpus* with its allies, *Psophocarpus* species inter relationships, and whether *P.palustris* and *P.scandens* are valid speices. The object of this summary is to distil from the results of the various methods of analysis the answers to the taxa relationship questions posed above.

5.6.1 Inter-generic survey for Psophocarpus allies

The analysis assumed that the allies of *Psophocarpus* were within the 27 genera of the Phaseoleae sub-tribes, Clitoriinae and Phaseolinae. These 27 genera were surveyed using a character set restricted to vegetative and inflorescence, morphological characters and the results of the subsequent LINKAGE analysis indicated a subset of seven genera to be most closely allied to *Psophocarpus*. The seven genera being: *Neorautanenia, Sphenostylis, Otoptera, Vigna, Dolichos, Dysolobium* and *Nesphostylis*.

The results of the generic subset intensive study all show that *Psophocarpus* is a relatively isolated genus, though of the genera studied *Otoptera* is clearly most closely related and these two genera were in turn distant to the other genera studied.

The subset genera other than *Psophocarpus* and *Otoptera* fall into two distinct groupings: *Dolichos* and its allies and the *Phaseolus-Vigna* complex genera. These two groups conform to the splitting of the Phaseolinae into supergenera by Baudet (1978), *Dolichos* and its allies being members of the *Dolichastrae* and the *Phaseolus-Vigna* complex genera being part of the *Phaseolastrae*. However members of both these supergenera show close allegiance with *Psophocarpus*, which may be interpreted as suggesting an artificiality in Baudet's splitting. This point will be discussed in more detail in the concluding chapter.

From the group of genera allied to *Dolichos*, *Sphenostylis*, *Neorautanenia* and *Dolichos* itself show a close relationship to *Psophocarpus*. Of these three *Sphenostylis* shows the most consistent close relationship to *Psophocarpus*. The second group of genera containing *Phaseolus*, *Vigna* and *Dysolobium* is more distant from *Psophocarpus* than the *Dolichos* centred group of genera. However of these three genera, *Dysolobium* is consistently shown to be most closely allied and in the CLUSTAN (DISTIN) 51 character analysis formed a discrete cluster with *Psophocarpus* and *Otoptera*. *Phaseolus* is shown, as was expected, to be the most distant genus in the subset generic study to *Psophocarpus* with *Vigna* displaying an intermediate position between *Dysolobium* and *Phaseolus*.

5.6.2 Intra-generic survey of Psophocarpus Species

The results of intra-generic survey do show some variance between the different methods of analysis, however overall a pattern of specific relationships can be detected.

The nine species clearly divided into two groups, one containing *P.palustris*, *P.scandens*, *P.tetragonolobus*, *P.grandiflorus*, *P.lukafuensis* and *P.lancifolius* and the second group containing *P.monophyllus*, *P.lecomtei* and *P.obovalis*. Within the first group are included the two most closely related *Psophocarpus* species, *P.palustris* and *P.scandens*. To these species *P.tetragonolobus* is allied with which it shares a basic stigma style arrangement, though it is closer to *P.scandens* than *P.palustris*. To a lesser extent *P.grandiflorus* is also related to these three species, though its larger flowers and different stigma style arrangement make it a more remote ally. The two species *P.lukafuensis* and *P.lancifolius* are the most remote members of the first grouping of species *P.lukafuensis* seems distantly related to *P.tetragonolobus*, while *P.lancifolius* shows no clear relationship with the other species, but its stigma style arrangement suggests its closest ally is *P.lukafuensis* (to which it was linked by Verdcourt and Halliday, 1978).

Of the second grouping of species *P.monophyllus* and *P.lecomtei* share numerous vegetative and floral characters which obviously link them

closely together. The third species of this grouping is *P.obovalis* which it might be argued should take up an intermediate position between the two major groups, because of its tri-foliate leaves but the stigma style arrangement of *P.monophyllus* and *P.lecomtei*. However the importance of shared inflorescence characters suggests that *P.obovalis* should be considered a remote member of the second grouping of *Psophocarpus* species.

5.6.3 P.palustris - P.scandens complex study

The results of the *P.palustris* - *P.scandens* complex study validate the splitting of *P.scandens* from *P.palustris* by Verdcourt (1968). In each method of analysis two specific clusters can be seen and referred to *P.scandens* and *P.palustris*, though as suggested by Verdcourt and Halliday (1978) specimens showing intermediate features are found. With the following methods of analysis; LINKAGE, DECORANA and CLUSTAN(FILE) - minimum spanning tree, the specimens with intermediate features are shown to link the two specific clusters, but the existance of intermediates does not negate the splitting of the two species.

CHAPTER SIX

CLADISTIC ANALYSIS RESULTS

6.1 Introduction

The method of cladistic or Hennigian analysis used in this chapter is based on the concepts introduced in Section 3.5.2. of Chapter 3. The analysis will be divided into two sections. The first where the apomorphic characters will be selected and the second where the taxa will be ordered on the basis of synapomorphic character states and the results displayed in the form of a cladogram.

It must be stressed here the basic difference between the results produced here and those produced in the previous chapter from the phenetic analysis. In this chapter the data will be interpreted using an evolutionary and genetic approach, with evolutionary changes being considered in relation to time. Both the analysis in this and the previous chapter was based on morphological data, but here the polarity of character state mutation adds an extra dimension to the analysis and subsequent result interpretation.

In the previous phenetic analysis chapter the investigation was divided into four component studies, but for the cladistic analysis only two of these studies will be phylogenetically analysed; the subset inter-generic study and the intra-generic *Psophocarpus* study. The extensive Phaseolinae and Clitoriinae genera data was considered inappropriate for phylogenetic analysis here as all these genera were not studied in sufficient detail to allow accurate selection of apomorphic characters. For the subset inter-generic phenetic study seven genera were studied, but two of these genera *Vigna* and *Dolichos* were large in species numbers and so representative taxa were chosen. However for the cladistic analysis the full range of variation within taxa must be understood to enable selection of apomorphies and so these two genera were necessarily excluded from the analysis. The final phenetic component study that of the *P.palustris* - *P.scandens* complex was also considered inappropriate for cladistic analysis as it involved

only two taxa.

So for the following two sections, selection of apomorphies and construction of cladograms, each section will be subdivided to differentiate the six genus inter-generic study and the nine species intra-generic study.

6.2 Selection of apomorphies

6.2.1 Six genus cladistic analysis

Apomorphic character states were chosen which would enable cladistic conclusions to be drawn from the six genera being studied: *Psophocarpus, Dysolobium, Otoptera, Neorauntanenta, Sphenostylis* and *Nesphostylis*. The characters selected with their appropriate states and polarities are detailed in Table 6.1. The fourteen characters were all used in the phenetic analysis and are illustrated in more detail in Table 4.1. Autoapomorphic characters (a derived character found in only one taxa) were included in the original character set, but as the analysis assumes that each genus has at least one autoapomorphic character to discriminate it from its allies, these autoapomorphic characters were not included in the analysis.

6.2.2 Psophocarpus species cladistic analysis

Apomorphic characters were selected for the nine *Psophocarpus* species and are detailed with their character states and polarities in Table 6.2. Polarity in this analysis, as in the previous inter-generic analysis, being decided on the relative frequency of a particular character state, if a character state is rare it is assumed that it is derived. This assumption is based on the probability that within any related group of taxa if a mutation has occurred recently, then its mutated character state will be found in fewer taxa than the original non-mutant character stat e.

Table 6.1 Six Genus Cladistic Analysis - Apomorphic Characters

NOS.	CHARACTER DESCRIPTION	PRIMITIVE STATE	DERIVED STATE
1	Stipule base production	Absent	Present
2	Type of inflorescence node	Unswollen	Swollen
3	Corolla exterior papillae	Absent	Present
4	Corolla appendage shape (see Figure 4.13)	Not like *Psophocarpus*	Like *Psopho-carpus*
5	Keel fusion	Complete	Toothed
6	Vexillalry stamen	Free	Joined
7	Style thickness	Large	Fuliform
8	Style apex spatulate	Absent	Present
9	Style chanelling	Absent	Present
10	Legume wing	Absent	Present
11	Legume shape	Linear	Non-linear
12	Degree of legume twisting post dehiscence	Tight	Loose
13	Legume partitioned	Absent	Present
14	Seed pubescence	Absent	Present

Table 6.2 Psophocarpus Species Cladistic Analysis – Apomorphic Characters

LETTER	CHARACTER DESCRIPTION	PRIMITIVE STATE	DERIVED STATE
a	Growth habit	Climbing	Prostrate
b	Petiole length	≥6mm	< 6mm
c	Petiolule length	≥5mm	< 5mm
d	Number of leaflets	3	1
e	Terminal leaflet apex shape	Acute	Mucronate
f	Terminal leaflet base shape	Angustatus/ truncate	Cordate
g	Prominence of veins on abaxial leaflet surface	Not Prominent	Prominent
h	Average number of nodes/inflorescence	> 4	≤4
i	Wing shape (see Figure 6.1)	A	B
j	Keel shape (see Figure 6.2)	A	B
k	Ovary pubescence	In specific position	All over
l	Style apex shape (see Figure 6.3)	A	B
m	Proximal legume wing end shape (see Fig 6.4)	A	B
n	Legume pubescence	Present	Absent

Figure 6.1: Wing shape

A B

Figure 6.2: Keel shape

A B

Figure 6.3: Style apex shape

A B

Figure 6.4: Legume wing end shape

A B

6.3 Construction of cladograms

For both analyses the same procedure was followed as is detailed below:

a) A table was compiled of taxa against apomorphic characters, and for each cell in the table the particular taxon was indicated as possessing the primitive or derived character state for that particular character. (See Figure 6.5).

b) The table was then cut into strips so that each strip contained all the character states for a particular taxon. Within the strips each derived cell was coloured to enable easy visual recognition.

c) A second table was constructed which contained for each character, the other characters which either shared a similar distribution of derived character states for the taxa or whose derived character states nested within its own. For the three characters in the example, characters B and C nest within A, and character B nests within C.

d) The initial cladistic taxa dicotomy is based on the character which has the largest number of other derived characters nested within it. In the simple example given in Figure 6.5, the dicotomy would be based on Character A. The procedure of constructing the table of similar derived characters or characters distributions nested within other characters is repeated until the taxa are no longer divisible on the apomorphic characters chosen.

e) A cladogram may then be built from the information provided on the divisions of taxa suggested from the previous section. For the example the cladogram is shown in Figure 6.6

6.3.1 Six genus cladistic analysis

The above procedure was followed for the six genera included in the inter-generic cladistic analysis and the resultant cladogram is shown in Figure 6.7.

Figure 6.5: Example of taxa against apomorphic character table

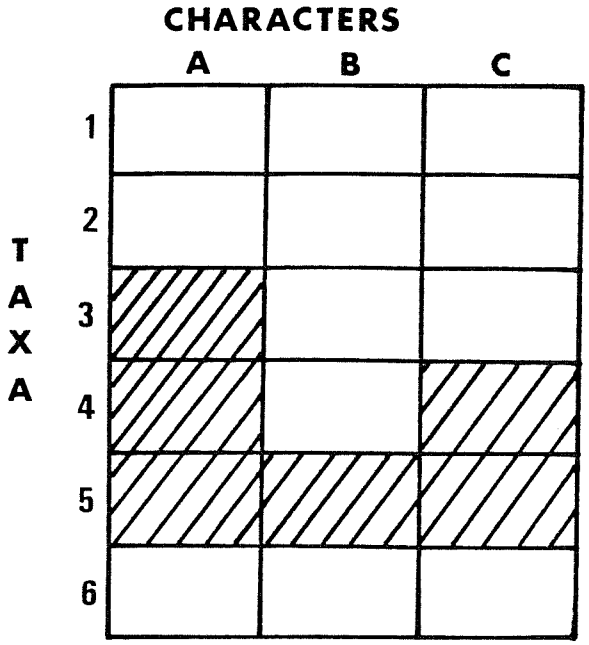


Figure 6.6: Cladogram drawn from the example information provided in Figure 6.5

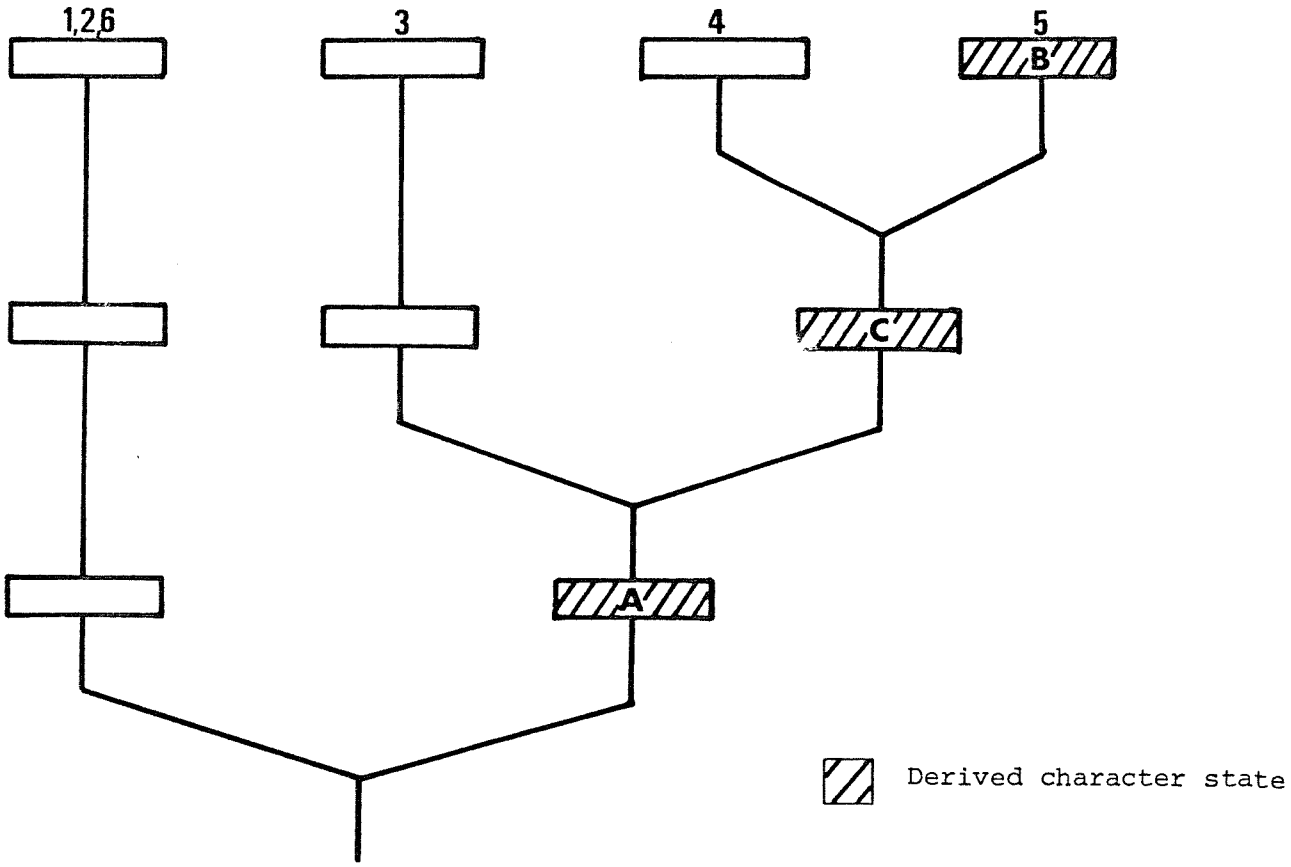
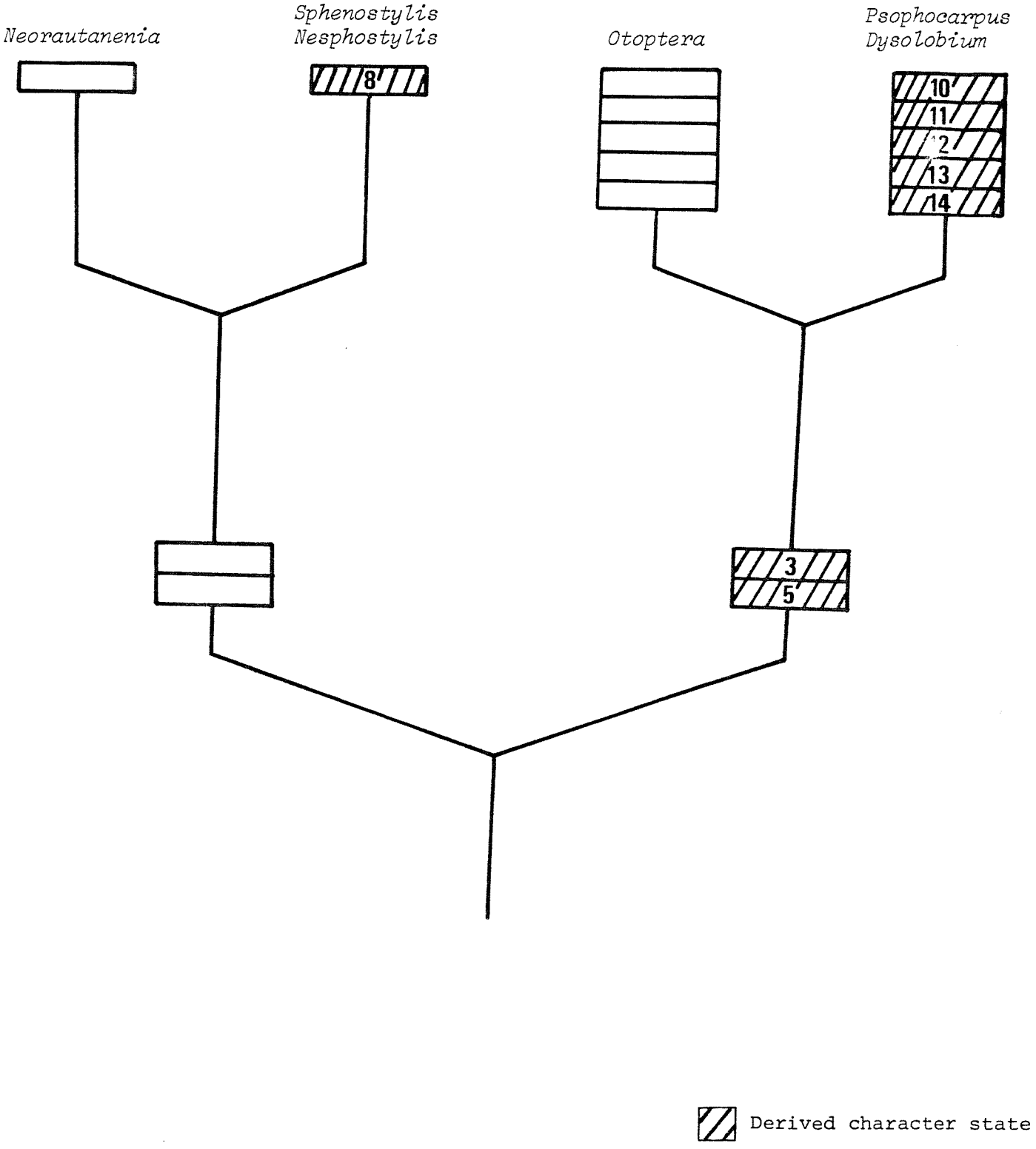


Figure 6.7: Six genus inter-generic cladogram



The first dichotomy in Figure 6.7 is made on characters 3 and 5, corolla exterior papillae and keel fusion resspectively. This separates the six genera into two groups: *Neorautanenia, Sphenostylis* and *Nesphostylis*, and secondly *Otoptera, Dysolobium* and *Psophocarpus*. The derived character state of toothed keel fusion is shown in plates 6.1, 6.2 and 6.3 for *Psophocarpus, Dysolobium* and *Otoptera* specimens. Toothed keel fusion is most obvious in *Psophocarpus*, it is also prominent in some *Dysolobium* specimens. Though in other *Dysolobium* specimens and *Otoptera* it is less obvious, but still present.

The first of the above group is then split on the basis of character 8, whether the style apex is spatulate. Both *Sphenostylis* and *Nesphostylis* possessing the derived character state spatulate style apex, this phylogenetic allegiance between these two genera concurs with the phenetic findings and is not surprising as these two genera have only recently (Verdcourt, 1970a), been distinguished as separate genera.

The second group of three genera show a 'strong' dichotomy based on five synapomorphic character states, the derived character states being shared by *Dysolobium* and *Psophocarpus*. This may be taken to indicate a 'strong' phylogenetic allegence between these two genera, which interestingly does not reflect their phenetic relationship. The five characters on which the dichotomy is based are: legume shape, legume twisting following dehiscence, legume partitioning, seed pubescence and presence/absence of a legume wing. The latter character is illustrated in Plates 6.4, 6.5 and 6.6 respectively for *Psophocarpus palustris, Dysolobium tetragonum* and *Dysolobium grande*. All *Psophocarpus* species possess a distinctive winged legume along the angles and Plate 6.5 shows that the wing is equally as prominent in *D.tetragonum*. The wing though is not found in all *Dysolobium* species, the only other *Dysolobium* to show any form of wing is *D.grande*, which as is shown in Plate 6.6, possesses a much smaller wing than *D.teragonum*.

6.3.2 Psophocarpus species cladistic analysis

PLATE 6.1

Psophocarpus scandens
Keel Petal

From live specimen

x 12

PLATE 6.2

Dysolobium dolichoides
Keel Petal

Kerr 9330

x 20

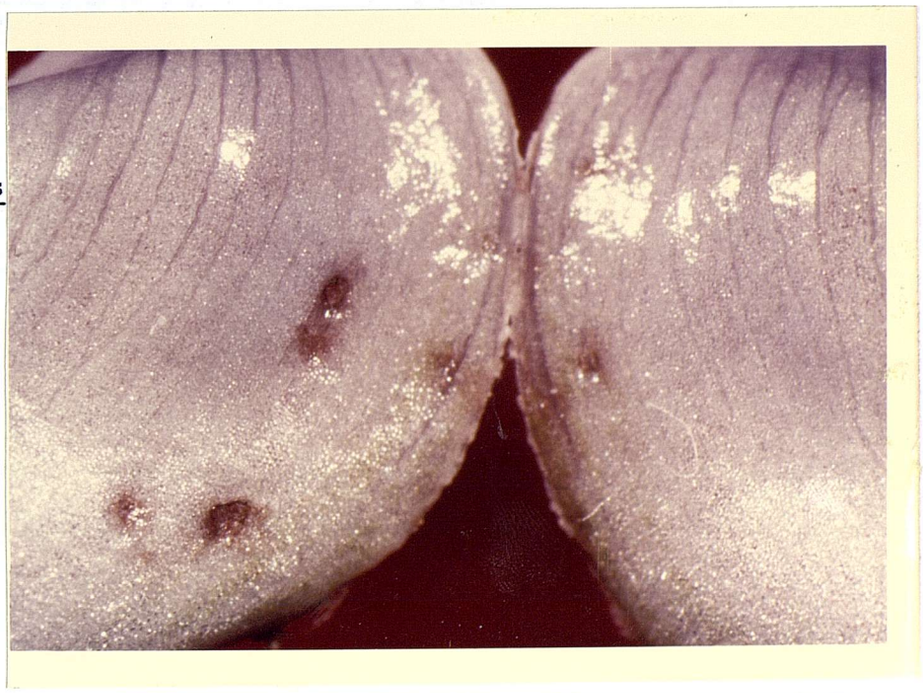


PLATE 6.3

Otoptera burchellii
Keel Petal

Pearson 3738

x 7

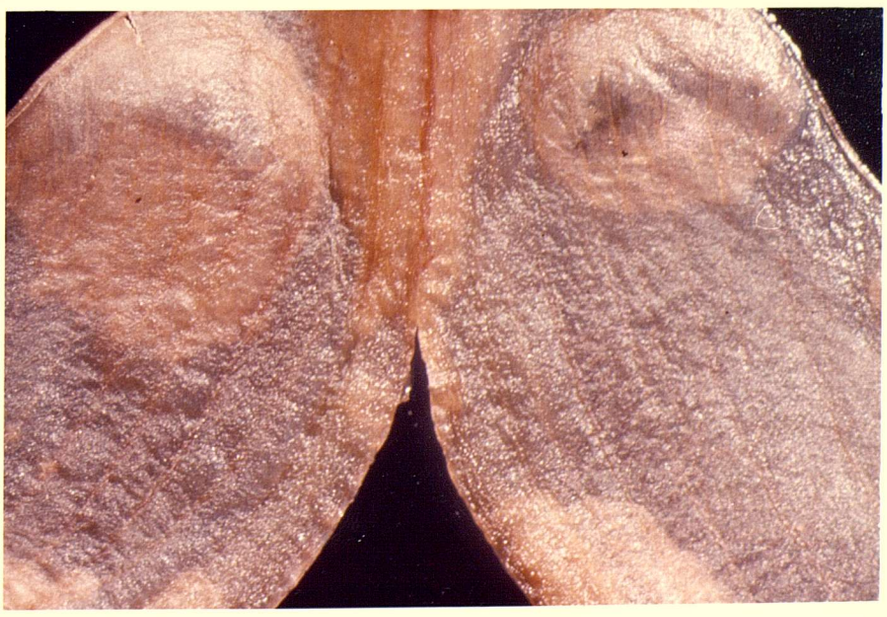


PLATE 6.4

Psophocarpus scandens
Legume

Ross 1119

x 2

PLATE 6.5

Dysolobium tetrogonum
Legume

Masters s.n.

x 3

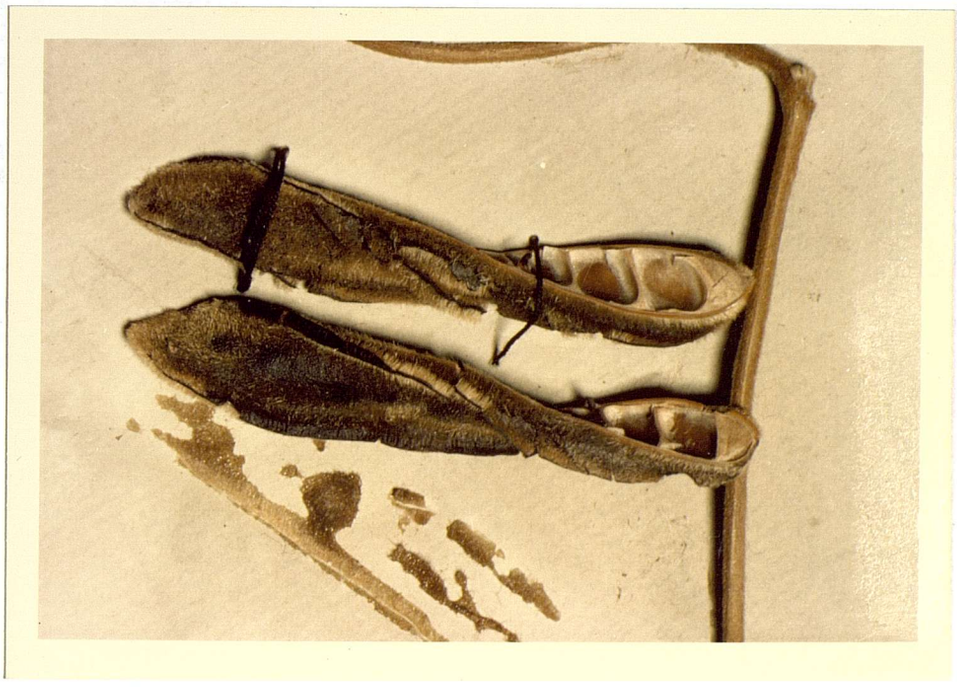


PLATE 6.6

Dysolobium grande
Legume

Kerr 1407

x 2

The analysis procedure was followed as detailed above and the resultant cladogram for the nine Psophocarpus species is presented in Figure 6.8.

The initial dichotomy of the nine species is based on character n, legume pubescence. The species divided into two groups: *P.lukafuensis*, *P.tetragonolobus*, *P.palustris* and *P.scandens*, and secondly *P.lancifolius*, *P.grandiflorus*, *P.obovalis*, *P.monophyllus* and *P.lecomtei*.

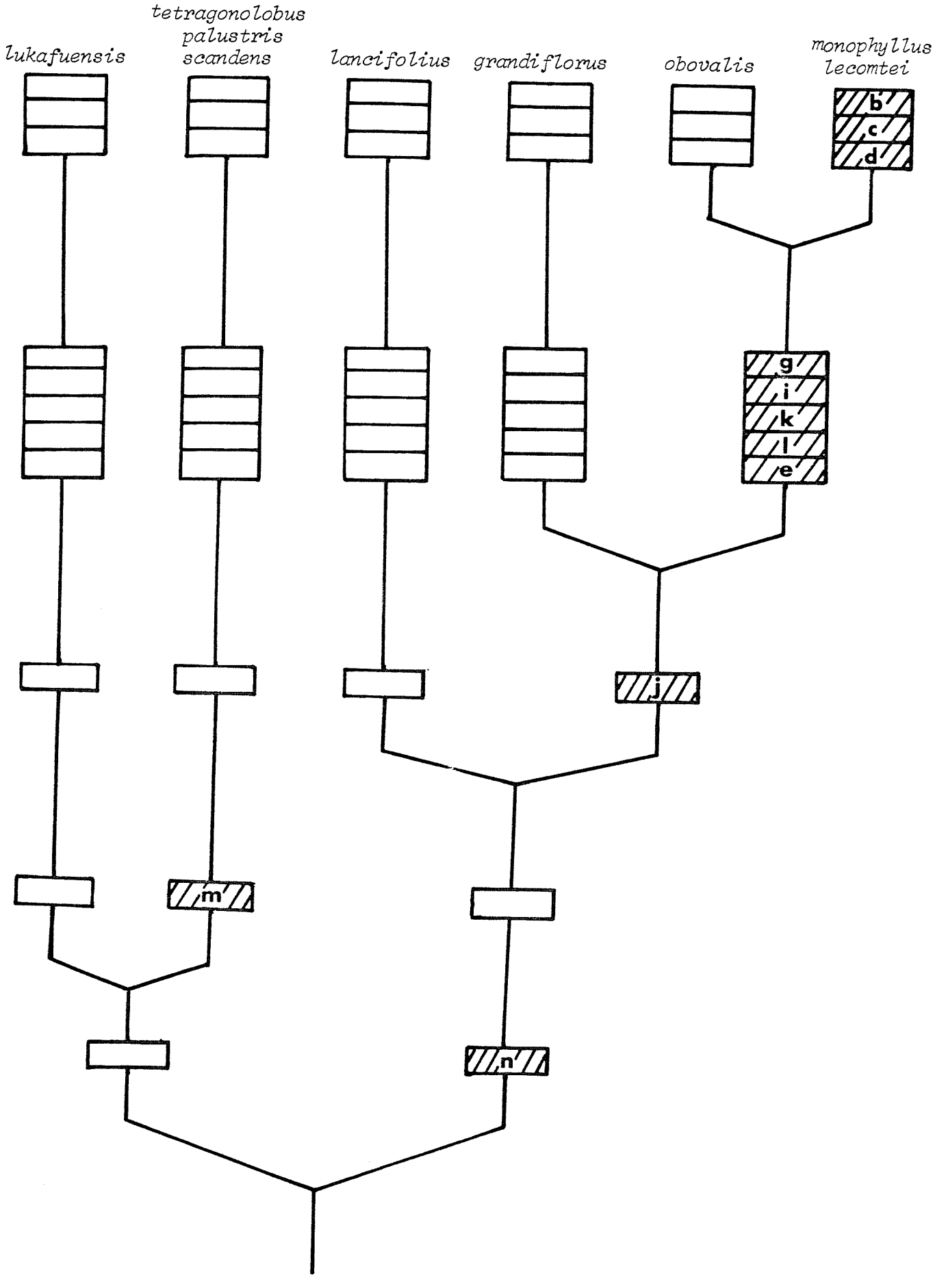
Within the first group of species *P.lukafuensis* is split off from the other three species by character m, proximal legume wing end shape. The remaining three species in this group are not separated using the synapomorphic characters chosen, which underlines the close phylogenetic, as well as phenetic, similarity between *P.tetragonolobus*, *P.palustris* and *P.scandens*.

P.lancifolius is the first species to be separated from the second group, it is discriminated on the basis of character j, keel shape. The other four species of this group all share the derived keel shape, where the upper portion of the keel petal has a pronounced beak.

The next dichotomy separated *P.grandiflorus* from the remaining species. Five characters correlate in separating off *P.grandiflorus*: vein prominence on the abaxial leaflet surface, style apex shape, ovary pubescence, wing shape and terminal leaflet apex shape. The fact that these five characters all suggest this dichotomy may be taken to indicate there is a 'strong' natural phylogenetic division between the remaining three species and the other six species already separated from this group.

The final dichotomy separates, as might be expected, the trifoliate species, *P.obovalis* from the unifoliate *P.monophyllus* and *P.lecomtei*. The three synapomorphic characters which form the basis of this separation relate to this difference in leaflet number: petiole length, petiolule length and number of leaflets.

Figure 6.8: *Psophocarpus* species cladogram



CHAPTER SEVEN

NON-MORPHOLOGICAL EVIDENCE

7.1 Introduction

So far the evidence used to postulate relationships between the taxa being investigated in this study has been entirely morphological, based on phenetic and phylogenetic use of the characters detailed in chapters four and six. Time would not permit other non-morphological studies to be undertaken. However, the taxonomic literature emphasises the importance of collecting information from as broad a range of evidence as possible if the classifications produced are to have any practical stability. Accordingly this chapter summarises the major non-morphological evidence available from the literature concerning the taxa under investigation.

The chapter is divided into eight sections, each concerning a particular information source. Each section can be sub-divided into two; that relating to the inter-generic relationships, focusing on the seven genera shown above to be most closely allied to *Psophocarpus*, and that referring to the intra-generic relationships between the nine *Psophocarpus* species. The evidence available from the literature is not comprehensive, in that for each major source of information there may not be corresponding information available for the particular group of plants being investigated. However, the following information is an attempt to summarise the evidence that is available and where possible relate it to the taxa being studied in this thesis.

7.2 Phytogeographical evidence

There has been no detailed study of the phytogeograph y of the Phaseolinae, but Brenan (1965) does attempt to relate the legume flora of Africa with that of other continents, while Baudet (1976) attempts to relate Phaseoleae genera and species numbers to world distribution.

Both these studies ask and answer questions which are different in form from the present studies. In this section, through a search of the literature, it is hoped to distinguish genera that are possible allies of *Psophocarpus* and which show similar generic distributions or at least not incompatible distributions. For example, *Psophocarpus* is largely restricted to Africa with one cultivated Far Eastern species: it would be thus unlikely to share a close relationship with a South American endemic.

The geographical distribution of Clitoriinae and Phaseolinae genera is given in Table 7.1. The bulk of the information is taken from Lackey (1981), but uses the divisions of the world after Baudet (1976).

In interpreting the distribution of *Psophocarpus* in relation to its allies, it is worth noting the remarks of Brenan (1965):

> "The genus is a particularly useful unit for studies of this sort," (Phytogeographical), "since the number of species of a given genus occurring in different parts of the world often indicate its centre of origin and directions of spread. I am of course aware that there are dangers in assuming that the area where the maximum number of species of a genus occurs is necessarily its point of origin. Nevertheless this assumption is more often likely to be true than false and where a certain pattern of distribution is found to be repeated by numerous genera it seems reasonable, unless there is difinite evidence against it, to accept the pattern at its face-value and to try to draw deductions from it."

Following Brenan's application of Occam's razor, we can assume the centre of origin for *Psophocarpus* is Tropical Africa and the one species, *P.tetragonolobus* not endemic to this areas has most likely spread to Asia from Africa. Conclusions about *P.tetragonolobus* origin must however, be treated with caution, partly due to its unique intra-generic distribution and also because of its being so widely cultivated, with no wild form or pregenitor yet being located. So in trying to validate a close relationship between *Psophocarpus* and its allies, a genus or genera with African or African and Asian distribution would be expected.

Table 7.1 Geographical Distribution of Clitoriinae and Phaseolinae Genera

GENERA CLITORIINAE	DISTRIBUTION AFRICA	AMERICA	ASIA	AUSTRALIA	AFRICA & ASIA	AMERICA & ASIA	PAN TROPICAL	REMARKS
10.45 A *Centrosema* (DC) Benth. B (45)							✓	Neo and sub-tropical.
10.46 *Periandra* Benth. (6)		✓						Mainly Brazilian
10.47 *Clitoria* L. (70)							✓	Mostly neotropical
10.48 *Clitoriopsis* Wilczek (1)	✓							Sudan and Zaire
PHASEOLINAE								
10.49 *Dysolobium* (Benth.) Prain (4)			✓					
10.50 *Psophocarpus* Neck.ex DC.(9)					✓			8spp in Africa 1spp cult in Asia
10.51 *Physostigma* Balf. (4)	✓							
10.52 *Vatovaea* Choiv.(1)	✓							
10.53 *Decorsea*	✓							Africa and

Viguier (4)			Madagascar
10.54 *Spathionema* Taub. (1)	✓		
10.55 *Otoptera* DC (2)	✓		lsp. S.Africa lsp. Madagascar
10.56 *Sphenostylis* E.Mey. (7)		✓	6sp. Africa lsp. India
10.57 *Nesphostylis* Verdc. (2)		✓	lsp. Africa lsp. Burma
10.58 *Austrodolichos* Verdc. (1)		✓	
10.59 *Neorautanenia* Schinz (3)	✓		
10.60 *Lablab* Adans. (1)	✓		
10.61 *Alistilus* N.E.Br. (2)	✓		lsp. S.Africa lsp. Madagascar
10.62 *Dipogon* Lieb. (1)	✓		S.Africa

GENERA CLITORIINAE	DISTRIBUTION							REMARKS
	AFRICA	AMERICA	ASIA	AUSTRALIA	AFRICA & ASIA	AMERICA & ASIA	PAN TROPICAL	
10.63 *Dolichos* L.(60)				✓	✓			Mostly African
10.64 *Macrotyloma* (W.& A.) Verdc. (24)					✓			
10.65 *Vigna* Savi (150)							✓	Mostly Africa and Asia
10.66 *Ramirezella* Rose (8)		✓						Mexico and El Salvador
10.67 *Oxyrhynchus* Brandegee (4)						✓		3sp C.America 1sp New Guinea
10.68 *Dolichopsis* Hassler (2)		✓						S. America
10.69 *Strophostyles* Elliott (3)		✓						N. America
10.70 *Macroptilium* (Benth.) Urban (20)							✓	Mostly American
10.71 *Phaseolus* L. (50)							✓	Mostly American

A = The numbers prior to each generic name are those used by Lackey (1981) whose generic layout is followed
B = The numbers in brackets following each generic name is the estimated number of species per genera.
The numbers quoted largely follow Lackey (1981)

Of the seven genera shown to be closely allied to *Psophocarpus* on morphological grounds in the extensive generic survey of Clitoriinae and Phaseolinae genera, only one does not follow the expected distribution pattern, and that is *Dysolobium*, which is restricted to Asia. This could be taken as an effective argument against *Dysolobium* and *Psophocarpus* being closely linked. As the one Asian *Psophocarpus* species was possible fairly recently introduced to Asia (in geological time) the two genera must have been effectively isolated for a biologically extended period, and so if related would be distant relatives.

The other six genera either share the African and Asian distribution of *Psophocarpus* or are restricted to Africa, *Vigna*, *Dolichos*, *Sphenostylis* and *Nesphostylis* falling into the first category, while *Otoptera* and *Neorautanenia* belong to the second. Of these six genera all are found congeneric with *Psophocarpus* species. So no one genera appears a more probable ally with *Psophocarpus* than the others, though it is interesting to note that *Sphenostylis* has an African centre of diversity with one species endemic to Asia. This is a similar distribution pattern to that of *Psophocarpus*.

Brenan (1965) emphasises the importance of African-Asian links and comments that migration of species appears to occur equally in both directions. He continues by stating his belief that dry savannah genera are more likely to have links with Asiatic species and genera than forest genera. *Psophocarpus* species required a moist savannah or woodland environment and so following Brenan's argument would be less likely to have close links with Asian genera. This again points towards *Dysolobium* and *Psophocarpus* not being closely linked.

The phytogeography of *Psophocarpus* species was discussed in detail by Verdcourt and Halliday (1978) in their revision of the genus and so what follows will largely comprise a summary of their views. The distribution of the nine *Psophocarpus* species is tabulated in Table 7.2 from data provided by Verdcourt and Halliday (1978). Distribution maps are also provided of the eight African species in Figures 7.1, 2 and 3.

Table 7.2 Geographical Distribution of Psophocarpus Species

Distributions adapted from Verdcourt and Halliday (1978). Species are represented by the first two letters of their specific epithet.
e.g. gr = *Psophocarpus grandiflorus*.

	gr	te	pa	sc	ob	mo	le	la	lu
Africa									
Senegal			▶						
Gambia			▶						
Guinea Bissau			▶			▶			
Guinea			▶			▶			
Sierra Leone			▶						
Liberia			▶						
Mali			▶			▶			
Ivory Coast			▶			▶			
Upper Volta						▶			
Ghana			▶						
Togo			▶						
Dahomey			▶						
Nigeria			▶	▶				▶	
Cameroon			▶	▶					
C.African Rep.			▶	▶	▶		▶		
Sudan			▶	▶	▶				
Ethiopa	▶								
Gabon				▶					
Cabinda				▶					
Congo				▶					
Uganda	▶			▶				▶	
Rwanda								▶	
Burundi				▶				▶	
Kenya								▶	
Tanzania				▶					
Zaire	▶			▶			▶	▶	▶
Zambia				▶				▶	▶
Zimbabwe								▶	
Malawi				▶				▶	
Mozambique				▶					
Comoro Is.				▶					
Madagascar				▶					
Mauritius				▶					
America									
Brazil				▶					
West Indies				▶					

Asia	gr	te	pa	sc	ob	mo	la	la	lu
India		▶		▶					
Sri Lanka		▶							
Burma		▶							
Malaysia		▶							
Thailand		▶							
Laos		▶							
Vietnam		▶		▶					

	gr	te	pa	sc	ob	mo	la	la	lu
China		▶							
Philippines		▶							
Java		▶		▶					
Christmas Is.		▶							
Indonesia		▶		▶					
Irian Jaya		▶							
Papua New Guinea		▶		▶					

Note: The distribution of *P.tetragonolobus* is undoubtedly much wider than indicated in the above because of its large scale tropical cultivation, however the above distribution is an attempt to outline its historical centre.

Figure 7.1: Distribution map of *Psophocarpus gradiflorus*, *P. palustris* and *P. scandens* (Taken from Verdcourt and Halliday, 1978)

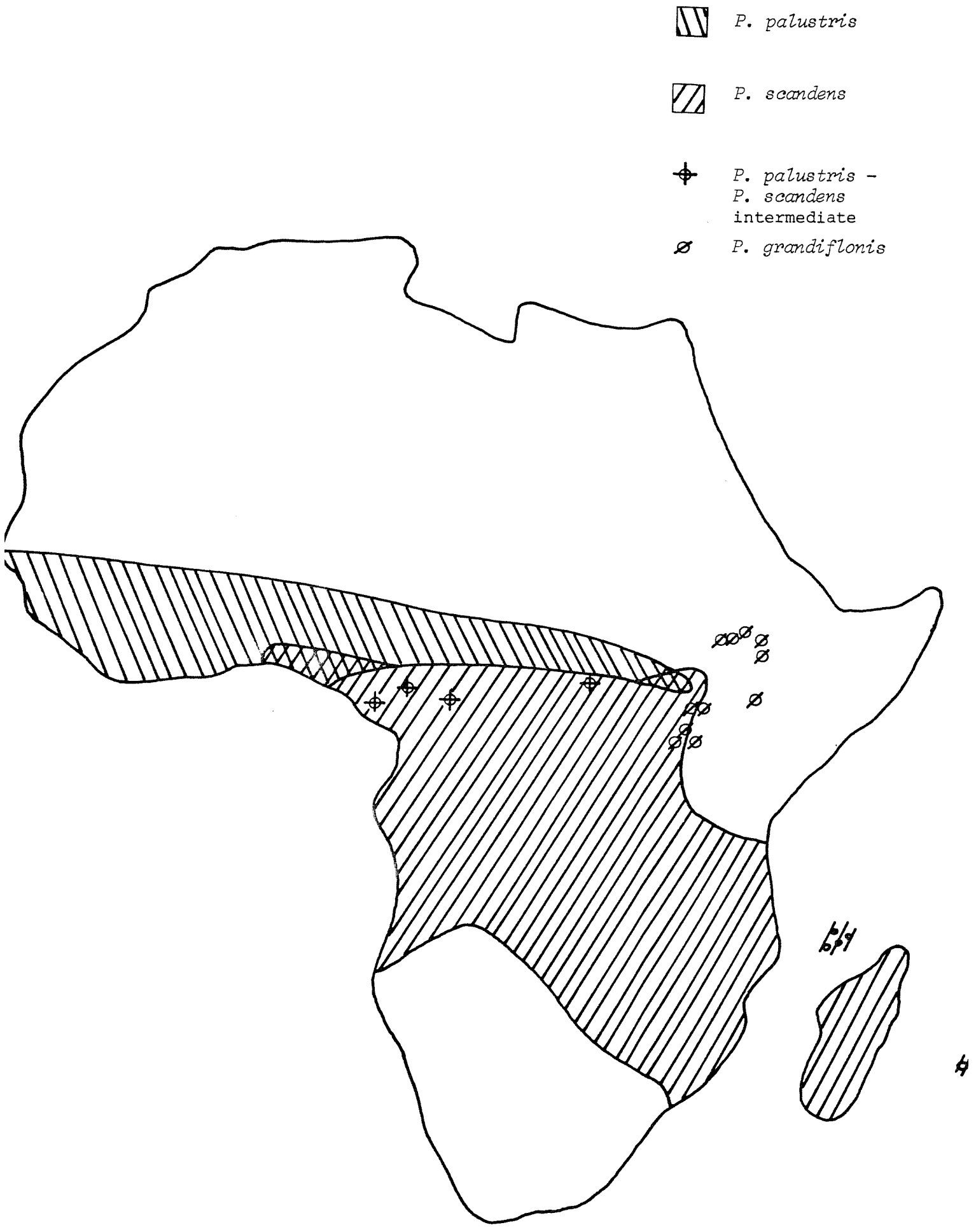


Figure 7.2: Distribution map of *Psophocarpus obovalis*, *P. monophyllus* and *P. lecomtei* (Taken from Verdcourt and Halliday, 1978)

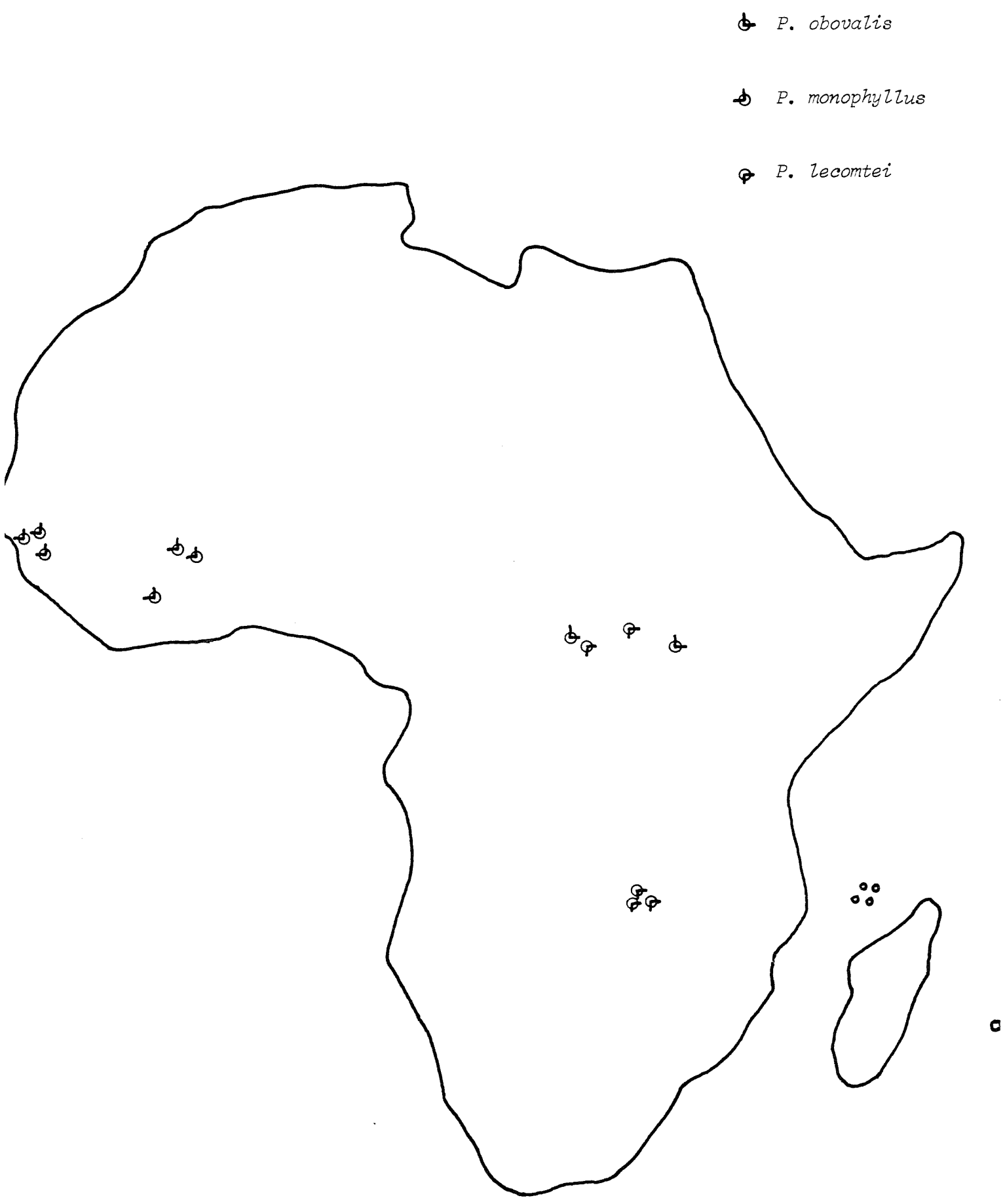
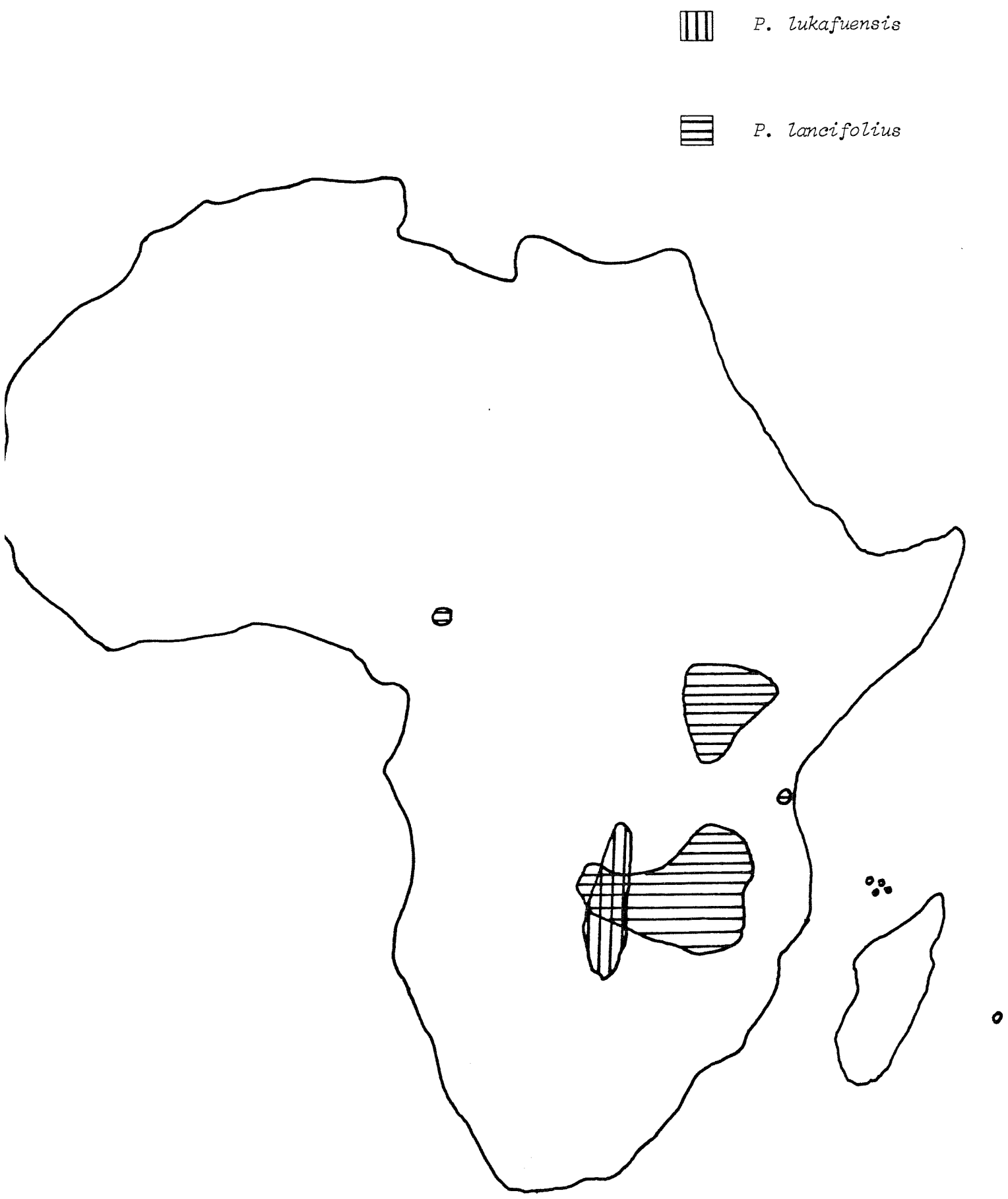


Figure 7.3: Distribution map of *Psophocarpus lancifolius* and *P. lukafuensis* (Taken from Verdcourt and Halliday, 1978)



There is a problem in discussing the phytogeography of *Psophocarpus* species, what to infer from the distribution of *P.tetragonolobus*, which is restricted to Asia, while the other *Psophocarpus* speices are all found in Africa. Discussion of the origin of winged bean needs be speculative and in Eagleton, Knan and Chai (1981) opinion is unresolvable. However, there are two basic schools of though on the origin of *P.tetragonolobus*, Burkill (1935) considers the species to have spread from Africa to South-East Asia, more specifically from Madagascar or Mauritius where *P.scandens (P.tetragonolobus's* closest morphological ally) is common. It has been reported by Rukee (1980) that *P.tetragonolobus* has existed in Mauritius for over a century, it was a popular vegetable, but for unclear reasons has now virtually disappeared from the island. De Candolle (1825a,b) also refers to *P.tetragonoloubs* or 'Pois Carre' as it is known locally being cultivated in Mauritius. Both Rukee and De Candolle's reports appear to help validate Burkill's hypothesis of the origin of *P.tetragonolobus*. Murdock (1959) points out that this migration route has been followed by numerous other species and Smart (1980a,b) uses *P.tetragonolobus* as an example of transdomestication from Africa to Asia. The opposing view is that *P.tetragonolobus* was domesticated in Asia from an unknown wild Asian ancestor, which has subsequently become extinct or at least has not yet been found. This view is supported by Vavilov (1951) and Cobely (1956) who suggest an Indian centre of origin, though Hymowitz and Boyd (1977) believe this to be unlikely. Ryan (1972) tentatively suggests that Papua New Guinea is the likely site for the origin of winged bean: Hymowitz and Boyd (1977) concur with this view and conclude:

> "Although the data presented are by no means conclusive, the meager evidence available points to Papua and New Guinea as the most likely centre of geographical origin, or at the minimum a centre of germplasm diversity, for *P.tetragonoloubs*.

Pickersgill (1980) points out that whichever school is correct, it is certain that *P.tetragonolobus* has been cultivated in South-East Asia for long enough to establish considerable diversity in leaves, flowers, pods and seeds from the other species.

In their discussion of the geographical distribution of the other eight species of *Psophocarpus* Verdcourt and Halliday (1978) make use of the reciprocal expansion and contraction of savannah and forest corresponding with the oscillations between pluvial and inter-pluvial periods to explain contemporary plant distributions. They comment that the natural distribution of the genus is restricted to Africa and Madagascar including the Comoro Islands and perhaps, Mauritius.

Of the specific distributions, *P.scandens* can be seen to be most widespread as shown in Table 7.2 and Figure 7.1. Though as suggested by Verdcourt and Halliday this may be partly due to cultivation and subsequent dispersal by man. They feel, however, that the current distribution does reflect *P.scandens* natural distribution: as *P.scandens* is cultivated its natural range has been extended. As can be seen in Figure 7.1, *P.palustris* has a separate distribution with intermediates found where the two species are juxtaposed. Verdcourt and Halliday suggest the following explanation of their distribution: *P.scandens* extended over the whole area at one time and then a barrier separated off the North Eastern populations. Subsequently the barrier was removed and the two populations extended to be contiguous as they are today. The barrier may possibly be the highland forest and grassland ridge, which the boundary between the two species currently follows, and which formed a West-East arc in the pluvial maximum, though Verdcourt and Halliday doubt if this arc would have caused sufficient isolation. They further suggest it is more likely that the necessary isolation occurred when the lowland forest was fragmented during the interpluvial period. They conclude however that following isolation the West-East ridge has slowed down reintegration of the spcies, as both species are usually found at lower altitudes (930m) than the height of the ridge.

P.grandiflorus is much more of an upland species, being found from 1600-2300 m. Its actual distribution is shown in Table 7.2 and Figure 7.1. It is found in the Ruwenzori and Virunga mountains and in the Elgon and Ethiopian highlands. Verdcourt and Halliday note that this is a common distribution pattern, but that it might also thus be expected to be found in the Kenyan and Tanzanian highlands, especially as the distance between the Elgon and Kenyan highlands is less than that between the Elgon and Ethiopian localities. They hypothesise that

P.grandiflorus evolved in a restricted area from a lowland ancestor which was forced to higher altitudes during an interpluvial period and then spread during a colder, wetter period.

Of the three species morphologically linked by their shared bifid style apex, *P.obovalis*, *P.monophyllus* and *P.lecomtei*, all three are comparatively rare. Verdcou rt and Halliday (1978) suggest all three may be senescent species. *P.obovalis* has been collected from only two isolated areas (see Figure 7.2) and Verdcourt and Halliday suggest it has either been extensively overlooked or the populations were divided by the forest ridge barrier as suggested for *P.palustris* and *P.scandens*. They find it difficult to explain why this species has not spread further into habitats that would appear suitable.

P.monophyllus shows a similar lack of radiation into apparently suitable environments to *P.obovalis*. It is restricted to moist woodland and savannah in the West as shown in Figure 7.2. The third species in this group, *P.lecomtei* is found in two small areas of moist woodland and upland savannah, isolated by about 1600km and extensive areas of lowland forest. As with the other species the distribution of *P.lecomtei* may be explained by geological and subsequent vegetation changes during the pluvials, which lead to the isolation of the two populations, though Verdcourt and Halliday (1978) suggest this "species is probably more widespread than the few records suggest". The distribution of *P.lecomtei* is also shown in Figure 7.2.

The distribution of the two subgen. *Vignopsis* species is shown in Figure 7.3. *P.lukafuensis* is restricted geographically to a narrow area of moist woodland and savannah about 850km long from about 7^{o}S. This area changed markedly during the pluvials; from uplands type, evergreen forest at the height of the pluvials to being, during the interpluvials, on the border of savannah and arid steppe and scrub, then once again savannah. Verdcourt and Halliday believe the species originated near the centre of its present range and its poor radiation ability is due to an inefficient dispersal capability.

The second subgen. *Vignopsis* species is much more environmentally widespread, being found in moist woodland, savannah, forest-savannah

mosaic and particularly grassland in upland localities from 1100-2550m, though usually associated with water. Its distribution, detailed in Table 7.2 and Figure 7.3 may also be explained by changes in vegetation during the pluvials though Verdcourt and Halliday (1978) question its natural presence in Nigeria. They consider its migration to Nigeria more likely by long distance dispersal of some kind (possibly man?). Interestingly they offer no hypothesis for the evolution of subgen. *Vignopsis*, though they comment that a postulated common ancestor would, if its range were equivalent to the two species today, have been fragmented into at least three areas surrounded by arid zones during the driest periods.

So to summarise the evidence from the phytogeographical literature survey, no one genus is indicated as being most likely closely allied to *Psophocarpus*. However, the inter-generic evidence may be used negatively to exclude the genus *Dysolobium*, which is endemic to Asia and thus less likely to be a close ally of *Psophocarpus* which is naturally distributed in Tropical Africa. Of the eight *Psophocarpus* species naturally distributed in Africa, two, *P.palustris* and *P.scandens* show a situation typical of closely allied species. Their distributions juxtapose each other and along the interface intermediate forms are located. Their distant ally *P.grandiflorus* overlaps in distribution with *P.scandens* but prefers a much higher altitude and is thus practically isolated from its relatives.

Of the second group of three species, *P.obovalis*, *P,monophyllus* and *P.lecomtei*, all three prefer similar habitats, though *P.lecomtei* does favour higher altitudes. Otherwise *P.obovalis* and *P.lecomtei* are conspecific, whereas *P.monophyllus* is quite separately located in the West, and so they could not be said to form a natural group based on geographical distribution.

The two subgen. *Vignopsis* species, *P.lukafuensis* and *P.lancifolius* are conspecific for part of their range and both prefer higher altitude habitats. They thus form a natural grouping at least with respect to the phytogeographical evidence provided.

7.3 Cytotaxonomic evidence

This is the source of taxonomically important data based on chromosome number and morphology. This information may vary from simple chromosome numbers, through to highly sophisticated techniques which elucidate chromosome banding patterns. For the taxa of the Phaseolinae and Clitoriinae included in this study only the first steps have been undertaken. Some of the genera have still to have chromosome counts recorded. The chromosome counts for the taxa under study are recorded, where known in Table 7.3.

The first cytological information relating to the Phaseolinae was produced by Karpechenko (1925) who counted chromosome numbers of ten *Phaseolus* and *Vigna* species. The first review of Legume chromosome numbers followed in 1938, undertaken by Senn, who established that the overall base count for the Phaseoleae was x=11. This was extended by Turner and Fearing (1959) who added a large number of fresh chromosome counts for African species and drew phyletic conclusions after comparing these fresh counts with those already established. Following their lead, numerous workers realised the importance of establishing accurate cytological data for species as a pre-requisite of efficient breeding strategies and began extensive cytological studies: Miege (1960a, b, 1962), Turner and Fearing (1960), Frahm-Leliveld (1965, 1969), Marechal and Otoul (1965, 1966a,b), Marechal (1969, 1970), Thuan (1975), Lackey (1977b, 1979b, 1980a): all these authors contributed to the information summarised in Table 7.3.

Goldblatt (1981) in his summary of legume cytotaxonomy for the second volume of the "Advances In Legume Systematics", states that the base number for the Phaseolinae is x=11, which is true for each subtribe. However, there is common aneuploid reduction to n=10. Within the Phaseolinae this is found in *Macrotyloma*, *Dolichos*, some *Vigna* spp. and some *Sphenostylis*. He doubts the validity of this *Sphenostylis* count, as does Lackey (1980a), who believes n=11 to be correct. Within the Phaseolinae there are two exceptions to counts of n=10 or 11. One is *Oxyrhynchus* which was counted by Goldblatt as n=12 but first published in Lackey (1980a). The second exception is *Psophocarpus* which from the two species with published counts is n=9 (see below for further

TABLE 7.3 Chromosome Counts of Clitoriinae and Phaseolinae Genera
(Adapted from Lackey 1980)

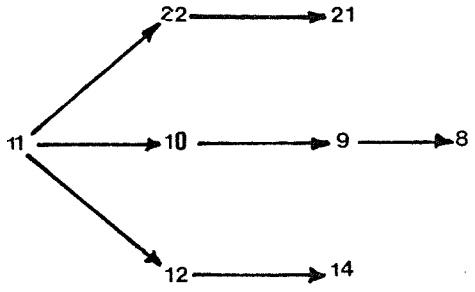
GENERA	SPECIES COUNTED PER GENUS	HAPLOID NUMBER(S)	VARIATION IN HAPLOID COUNTS
CLITORIINAE			
A			B C
10.45 *Centrosema* (DC.) Benth.	5/45		[9(1), 10(4), 11(1), 12(1)]
10.46 *Periandra* Benth.	2/6	11	11(3)
10.47 *Clitoria* L.	4/70	8,10,12	8(6), 11(1), 12(3)
10.48 *Clitoriopsis* Wilczek	0/1		
PHASEOLINAE			
10.49 *Dysolobium* (Benth.) Prain	0/4		
10.50 *Psophocarpus* Neck.ex DC.	2/9	9	9(1), 10(2), 11(1), 13(1)
10.51 *Physostigma* Balf.	1/4	11	11(2)
10.52 *Vatovaea* Choiv.	0/1		
10.53 *Decorsea* Viguier	0/4		
10.54 *Spathioriema* Taub.	0/1		
10.55 *Otoptera* DC.	0/2		
10.56 *Sphenostylis* E.Mey.	3/7	11	11(1), [9(1), 10(1)]
10.57 *Nesphostylis* Verdc.	0/2	10	10(1)
10.58 *Austrodolichos* Verdc.	0/1		
10.59 *Neorautanenia* Schinz	1/3	11	11(1)
10.60 *Lablab* Adans.	1/1	11	11(9) [10(2), 12(3)]
10.61 *Alistilus* N.E.Br.	0/2		
10.62 *Dipogon* Lieb.	1/1	11	11(4)
10.63 *Dilichos* L.	4/60	10	10(5) [12(1)]
10.64 *Macrotyloma* (W.& A.) Verdc.	7/24	10	10(15) [11(3), 12(1)]
10.65 *Vigna* Savi	36/150	10, 11	10(22), 11(15),22(6), [12(19)]
10.66 *Ramirezella* Rose	0/8		
10.67 *Oxythynchus* Brandegee	1/4	12	12(1)
10.68 *Dolichopsis* Hassler	0/2		
10.69 *Strophostyles* Elliott	2/3	11	11(3)
10.70 *Macroptilium* (Benth.) Urban	5/20	11	11(13)
10.71 *Phaseolus* L.	17/50	10, 11	10(1), 11(57), 22(1), [12(1)]

A = The numbers prior to each generic name are those by Lackey (1981) whose generic layout is followed.
B = Numbers in rounded brackets are the number of reports per particular chromosome count.
C = Numbers in square brackets indicate doubtful counts.

more detailed discussion of *Psophocarpus* cytology).

A base number of x=9 is rare in the Phaseolinae and Lackey (1980a) lists along with *Psophocarpus* only, *Butea* (subtribe Erythrininae) and *Calopogonium* (subtribe Diocleinae) with haploid numbers of n=9 and n=18 respectively. Both these other genera are morphologically quite distinct from *Psophocarpus* and so unlikely to be closely allied to it. So it seems likely that x=9 has arisen on more than one occasion in the *Phaseoleae*. This basic difference in chromosome number between *Psophocarpus* and its morphologically close allies makes it difficult to draw any conclusions about their possible relationship based on cytotaxonomic evidence. A haploid number of n=9 was recorded for *Sphenostylis stenocarpa* by Miege (1960a), but Lackey (1977a) believes this to be an incorrect count and he (1980a) suggests that n=11 is correct, though he fails to cite the author of the latter count. As *Sphenostylis* is shown by this study's morphological investigations to be closely allied to *Psophocarpus*, it will prove very interesting to obtain a conclusive count for the genus.

Datta and Saha (1975) provide some help in interpreting the cytological relationship between *Psophocarpus* and its allies. Baudet (1977b) summarises their views on the evolution of chromosome number in the Phaseoleae thus:

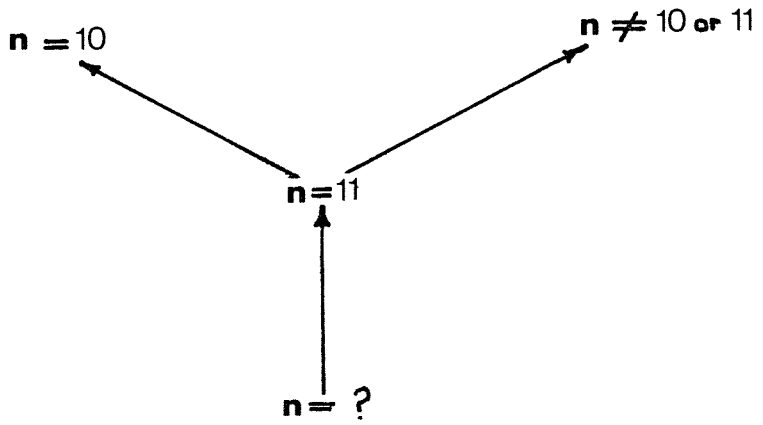


Datta and Saha believe that all haploid chromosome numbers in the Phaseoleae have radiated from the base of x=11. So for *Psophocarpus* to become n=9 there must have been a reduction from n=11 to n=9 via n=10. Logically from this it would be speculated that genera with n=10 are closer to *Psophocarpus* than those with n=11. Following this reasoning *Nesphostylis*, *Dolichos*, *Macrotyloma* and certain *Vigna* and *Phaseolus* spp. may be thought closer to *Psophocarpus* than the other genera of the Phaseolinae, known to be n=11.

Care must be taken when drawing conclusions of this kind, even though *Dolichos*, *Nesphostylis* and *Vigna* were included in the subset of seven genera most closely related to *Psophocarpus*. Neither Verdcourt (1970b,c) nor Marechal et al (1978a) draw conclusions about relating chromosome number to natural relationships. Marechal et al used haploid chromosome number as a character in their numerical analysis of the *Phaseolus-Vigna* complex comment:

> "une convergence par perte d'une paire de chromosomes est parfaitement plausible et le nombre chromosomique de bas ne presente donc ici qu'une tres fiable valeur taxonomique".

Datta and Saha's (1975) hypothesis of chromosome number radiation is not the only possible hypothesis. Another is presented by Baudet (1977b) as follows:



Which he considers might also be possible, though it would seem more likely that to get from n=11 to n=9, the stage n=10 would be passed through rather than losing two chromosomes at once.

There is little information available on karyotype morphology of the Phaseolinae, but as stressed by Pickersgill (1980) *Psophocarpus* is atypical not only in its basic chromosome number but also in its bimodal chromosome complement. Of the Clitoriinae species *Centrosema virginianum* does show a size difference within the complement, but within the Phaseolinae, reported there is a continuous gradation in size and the longest and shortest chromosomes do not differ greatly in size. This supports Lackey's (1977b) opinion and the finding of the present study that *Psophocarpus* is a relatively isolated genus within the Phaseolinae.

To summarise, at present there is only sufficient cytological data available to enable speculative interpretation of the position between *Psophocarpus* and its allies. The chromosome counts for all the Phaseolinae genera are incomplete and some of the genera shown morphologically to be most closely allied to *Psophocarpus*, *Otoptera*, *Dysoobium* and *Sphenotylis* are as yet uncounted or require verification. With the currently available information it appears that *Psophocarpus* is cytologically remote from its possible allies.

For the *Psophocarpus* species cytological study, the two species for which viable seed was available were counted. The method followed is detailed in Appendix 9 and the photographs of the mitotic squashes of *P.tetragonolobus* and *P.scandens* are presented in Plates 7.1 and 7.2 respectively.

Both species can be seen to be 2n=18, which concurs with recent reports in the literature: Tixier (1965), Lackey (1980b) and Pickersgill (1980) found *P.tetragonolobus* 2n=18 and Haq and Smartt (1977) and Pickersgill (1980) found *P.scandens* 2n=18. Other reports in the literature indicate higher chromosome numbers; n=10 (for *P.palustris*; Frahm-Leliveld, 1960), 22 (for *P.palustris*; Miege, 1960, cited in Cave, 1964) and even 26 (for *P.tetragonolobus*; Ramirez, 1960). These reports attributed to *P.palustris* as pointed out by Pickersgill (1980) should

PLATE 7.1: Somatic metaphase plate from a root-tip cell of *Psophocarpus tetragonolobus*, 2n = 18, x 4500

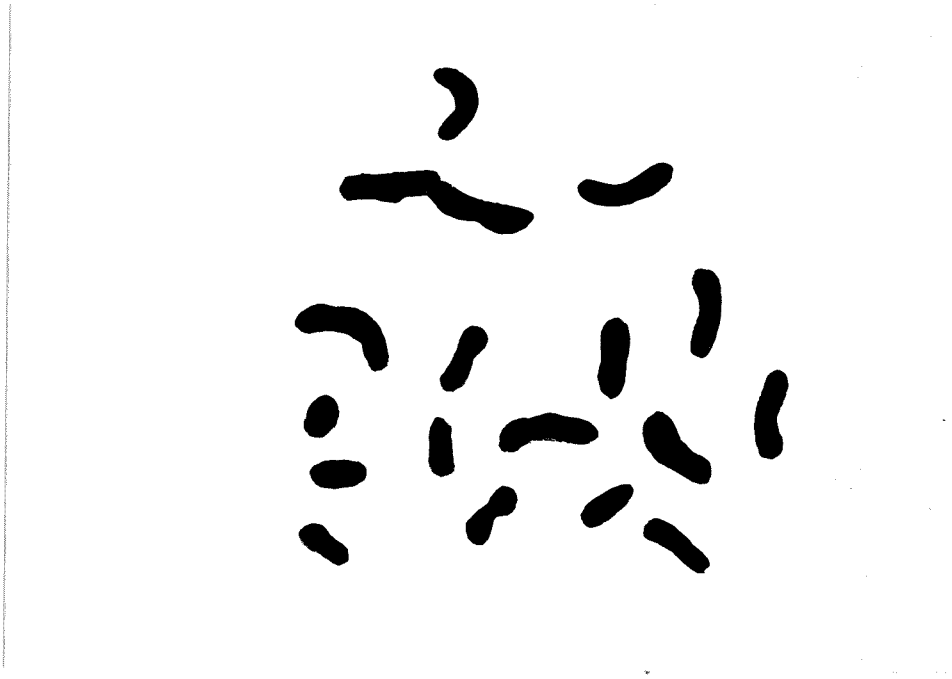
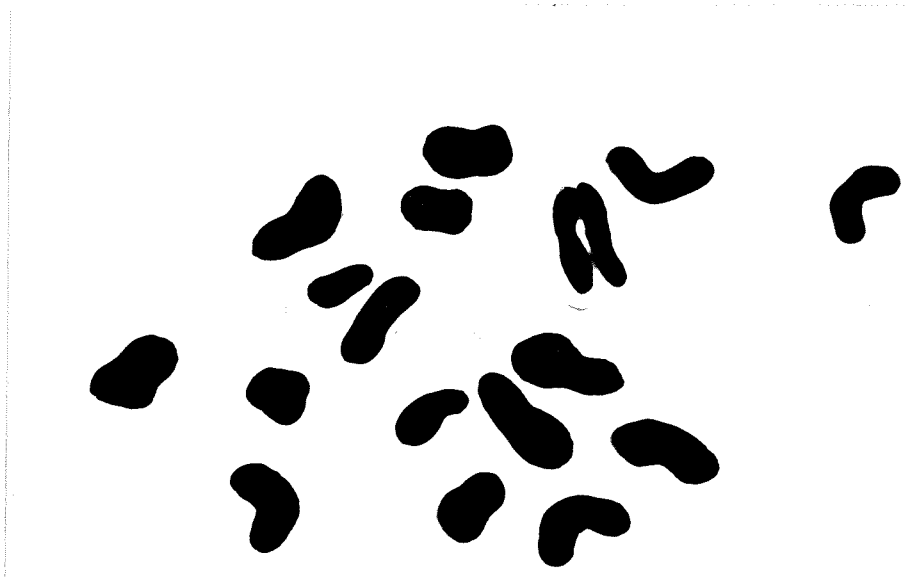


PLATE 7.2: Somatic metaphase plate from a root-tip cell of *Psophocarpus scandens*, 2n = 18, x 5000



amost certainly be attributed to *P.scandens* following Verdcourt's (1968) splitting of *P.scandens* from *P.palustris*. The exception is Miege (1960) whose count of 2n=22 may be correctly attributed to *P.palustris* as the seed was obtained in West Africa where *P.palustris* is native and where *P.scandens* is absent.

Pickersgill (1980) provides an extensive cytological study of both *P.tetragonolobus* and *P.scandens*, including discussion of counts, Karyotype morphology, and behaviour of the chromosomes during meiosis. She concludes that two classes of chromosomes are distinguishable, each species possessing 6 short and 12 long chromosomes. The short chromosomes of both species appear identical and all are metacentric or submetacentric. While the long chromosomes of *P.tetragonolobus* are mostly submetacentric or acrocentric, those of *P.scandens* mostly have arms of about equal lengths. Pickersgill (1980) does not report the presence of chromosomal satellities, but does report that in one pair of short chromosomes the centromeric region frequently appears longer than in the other chromosomes. She concludes from the mitotic studies that:

> "differentiation of *P.tetragonolobus* and *P.scandens* seems to have been accompanied by some repatterning of chromosome structure, at least in the long chromosomes, even though chromosomes number and the gross morphology of the karyotype have remained unchaged."

She goes on to describe the meiotic behaviour of the chromosomes, but does not note any distinguishing features between the two species.

Pickersgill's (1980) main conclusion from her study is that the differences in karyotype morphology and lack of success with interspecific hybridisation (see following section) make it unlikely that *P.scandens* is the immediate wild ancestor of the domesticated *P.tetragonolobus*. She puts forward the hypothesis that *P.palustris* is closer to the ancestral stock of *Psophocarpus*, because it is recorded as being 2n=22 by Miege (1960) and the *Psophocarpus* ancestor would almost certainly be 2n=22. This count does require confirmation with correctly identified material, but it is an interesting idea and would prove exciting if *P.palustris* also were to lack the bimodal chromosome. Pickersgill suggests that *P.palustris* may not only help provide a link

between *Psophocarpus* and the other Phaseolinae but may also explain how the aneuploid reduction occurred to produce the x=9.

Soon there may be evidence to support Pickergill's hypothesis about the importance of *P.palustris*. Recently Dr. N. Haq of Southampton has obtained seed believed to be of *P.palustris* and *P.grandiflorus*. While this report is being written the seed of these species is being grown and as yet no firm conclusions about its identify or cytology can be recorded. However, Dr Haq and the present author have prepared mitotic squashes from seed attributed to *P.grandiflorus* and it seems most likely that its diploid chromosome number is 2n=22, but this is a provisional finding as no photographable squashes have yet been prepared. No material of *P.palustris* has yet been observed. If Miege's (1960) count for *P.palustris* and Haq and Maxted's provisional count for *P.grandiflorus* are confirmed, then Pickersgill's hypothesis would be corroborated and it would mean that the two cultivated *Psophocarpus* species were more remote than is indicated by this investigations morphological studies. The *P.palustris* karyotype analysis will prove especially interesting, as it is so closely morphologically allied to *P.scandens* and intermediate forms between the two are found.

7.4 Biosystematic evidence

To the plant breeder and taxonomist, biosystematic or hybridisation evidence provides very useful information. For the plant breeder, inter-specific or inter-generic crosses provide potential gene pools that may be tapped for adaptive characters. For the taxonomist, hybridisation experiments provide information on the degree of isolation of taxa and would help establlish a particular taxa placement in the taxonomic hierarchy. However in relating *Psophocarpus* to its potential allies and inter-relating *Psophocarpus* species biosystematic evidence provides little useful information. Smartt (1980b) comments thus, "In general it is safe to say that interspecific hybridisation is not necessarily easy to achieve between even closely related species of legumes."

There are no reported successes in hybridising *Psophocarpus* spp. with other Phaseolinae genera, and probably no serious systematic

attempt has been made. Haq (unpublished) has attempted, unsuccessfully, crosses in both directions between *P.tetragonolobus* and *Phaseolus vulgaris*. Haq and Maxted (unpublished) have made a few crosses using cultivated *Vigna* spp. as the male parent (*V.unguiculata*, *V.umbellata* and *V.angularis*), but with no success. *Psophocarpus* is clearly isolated within the Phaseolinae (Lackey 1977b, Verdcourt and Halliday, 1878 and the present study's findings) and so it would seem unlikely that there would be success with inter-generic hybridisation, unless advanced techniques such as somatic hybridisation techniques are used. In fact there is doubt as to the possibility of any inter-generic crosses between legume genera (see McComb, 1975).

Within the genus *Psophocarpus* itself, until very recently seed was only available for two species (*P.tetragonolobus* and *P.scandens*) and so there has not been much opportunity for inter-specific hybridisation, though the two species available as shown earlier are those mostly closely allied on morphological evidence. Pickersgill (1980) records that Erskine attempted over 100 crosses between *P.tetragonolobus* and *P.scandens*, using *P.tetragonolubus* as the female parent without obtaining hybrids. Pickersgill herself attempted a more limited investigation and found that pods dropped within 48 hours of the cross, regardless of direction in which the cross was made. Haq and Maxted (unpublished) have also attempted a small number of inter-specific crosses without success. Pickersgill (1980) suggests that even were a hybrid produced between *P.tetragonolubus* and *P.scandens* the difference in karyotype are such that the hybrid would be extremely sterile.

As recently two other species of *Psophocarpus*, *P.palustris* and *P.grandiflorus*, have had viable seed collected, it will be interesting to see how these species, which are also relatively closely related to *P.tetragonolubus*, will react in hybridisation experiments with *P.scandens* and *P.tetragonolobus*. From the morphological similarity and the fact that intermediate forms are found it is possible that *P.palustris* and *P.scandens* will hybridise, though *P.grandiflorus* is probably the most remote of these four species and so would not be expected to cross. It may prove very interesting to both the taxonomist and the plant breeder to see how *P.palustris* reacts when crossed with *P.tegragonolobus*. For the plant breeder it may if successful prove to

be a means of introducing varying resistance genes into winged beans. For the taxonomist it could clarify whether *P.scandens* or *P.palustris* is most closely allied to *P.tetragonolobus*.

To summarise there is little biosystematic evidence to help relate *Psophocarpus* to its allies or *Psophocarpus* species to each other. In fact what little inter-generic evidence there is suggests that *Psophocarpus* is remote from its closest allies as there has been no success in inter-generic crossing, which concurs with morphological findings. At the intra-generic level there has been no success in hybridising *P.tetragonolobus* and *P.scandens*, though with the two other species seed available, there may be useful biosystematic evidence available soon. For the intra-generic crosses the plant breeders will obviously focus on crosses with *P.tetragonolobus* because of its commercial potential. However, based on the morphological results above, it can be speculated that they have a small chance of producing a vialbe hybrid, at least by conventional methods of hybridisation.

7.5 Palynological evidence

The pollen morphology of the Papilionoideae has not received as much attention as that of the Caesalpinioideae and Mimosoideae (Ferguson and Skvarla, 1981). However, much of the work that has been undertaken has been concentrated on the Phaseoleae, particularly by continental palynologists who have recently concentrated on a systematic study of Phaseolinae pollen. As yet their systematic survey is incomplete, concentrating on the *Phaseolastrae* genera and does not attempt to relate *Psophocarpus* pollen to potential allies.

The initial observations of Phaseolinae pollen used light microscopy alone. Bronckers and DeKeyser (1966) studied the pollen of four *Dolichos* spp. and *Lablab purpureus* (L) Sweet (syn. *Lablab niger*) and concluded that *Dolichos lignosus* had a quite different pollen type from the other *Dolichos* spp. and from the *Lablab purpureus*. This justified Wilczek in the splitting of *D.lignosus* from *Dolichos* to form a separate genus based on the one species, *Dipogon lignosus* (L.) Verdc. Verdcourt (1970b) extends this study of the pollen of *Dolichos* and its allies; he notes that Erdtman (1952) found spinuliferous pollen grains present for *Macrotyloma axillare* (E.Mey.) Verdc., Verdcourt comments that they are characteristic of *Macrotyloma* spp. Verdcourt (1970c) in investigating the pollen of *Vigna* spp. divides the genus into two: species which show a wide, open reticulation and a small number of species with very fine or practically no reticulation. Verdcourt also refers to an earlier study at Kew of *Phaseolus* labelled material, again the species may be subdivided on the degree of exine reticulation. Taylor (1966) found the more prominently reticulate species to be endemic to Asia and the finely reticulate to America, though later *in litt* he agreed that reticulate species were found in America.

In their second paper covering Phaseolinae palynology, Bronckers, De Keyser and Stainier (1972) studied *Haydonia* Wilczek (syn *Vigna* subgen. *Haydonia* Verdc.) *Phaseolus* L., *Vigna* Savi and *Voandzeia* Thouars of Zaire. Using light microscopy, they observed 43 species and provided details of exine reticulation and relative grain and pore size. They concluded that practically the species of the four genera can not be distinguished using pollen characters alone, though within genera

species may be determined. Similar conclusions were drawn from Stainier's (1974) further observations of *Phaseolus* L. *Vigna* Savi and *Ph ysostigma* Balf., however she does establish the principal useful pollen characters as being pore sculpturing, exine reticulation and thickness of the network walls.

Following this Stainier, collaborating with various authors (Stainier and Horvat, 1978a, 1978b; Horvat and Stainier, 1979, 1980; Marechal, Mascherpa and Stainier, 1978a) has concentrated on *Phaseolus-Vigna* complex genera, using both light and electron microscopy. For the latter paper Stainier observed 28 characters for 177 taxa from the *Phaseolus-Vigna* complex. She recorded size, shape and exine topographic chracters using light microscopy. However the relative character analysis showed that all the pollen characters possessed "Facteur de ponderation" figures lower than 10 which is low in comparison to the morphological characters used in the study. Two large groupings of genera are found: those possessing 3 colpi and fine reticulation and those with 3 pores and large reticulation. However these groupings do not generally reflect those produced by classsical means, but they are useful in distinguishing certain genera. Stainier concludes that more general taxonomic importance should be attached to transmission electron microscope (T.E.M.) studies of exine ultra-structure patterns for the *Phaseolus-Vigna* complex, which has the potential to yield more taxonomically useful information.

Stainier and Horvat (1978a,b) and Horvat and Stainier (1979 and 1980) have systematically undertaken T.E.M. studies. They have found marked differences and specialisation between genera and sub-genera of the *Phaseolus-Vigna* complex, especially in wall stratification. Their survey is not yet complete and none of the genera thus covered are shown by this study's results to be particularly closely allied to *Psophocarpus*. It would be interesting to note the results of observing *Dylosolium* using transmission electron microscopy. Marechal, Mascherpa and Stainier (1978a), using light microscopy, find a distinction between the two subgenera of their *Dysolobium*. While both posses tricolporate pollen, subgen. *Dysolobium* is more finely reticulate than subgen. *Dolichovigna*, which has more prominent reticulation like most *Vigna* species. Fergusion and Skvarla (1981) comment that like *Dysolobium*,

Psophocarpus is tricolporate but add that is it "generally less specialised than that of much of the subtribe, which may be taken to indicate a possible link between subgen. *Dysolobium* and *Psophocarpus*, both taxa having fine reticulate wall stratification". Ferguson and Skvarla (1981) comment on *Sphenostylis*, which in the present study has been shown on morphological evidence to be close to *Psophocarpus*, that it "has triangular, very oblate, flattened pollen with very short colpi. The wall stratification is very specialised with complex columnelae". The degree of specialisation found in *Sphenostylis* pollen does not corroborate its morphological links with *Psophocarpus*, which has a simpler wall stratification pattern.

The most interesting genus that should be observed for potential palynological links with *Psophocarpus* is *Otoptera*, which has been consistently shown throughout this study to be closely allied to *Psophocarpus* based on morphological evidence. But as *Otoptera* is a small, non-economic specied genus it seems unlikely that its pollen will be studied in detail soon.

At the instigation of B.Verdcourt, M.M.Poole undertook a generic study of *Psophocarpus* pollen, to complement Dr Verdcourt's morphological studies. The study was published in 1979 and covers light, scanning electron and transmission eletron microscopic studies of the nine species circumscribed by Verdcourt and Halliday (1978).

Poole (1979) provides a general description of *Psophocarpus* pollen morphology, systematic description of species; key to *Psophocarpus* species using pollen characters; drawings and photographs of pollen grains; as well as a discussion of taxonomic relatinships as they relate to the pollen data. In the discussion, Poole notes, "it is noteworthy that in such a small genus as *Psophocarpus*, the ornamentation of the mesocolpium should fall into four quite distinct groups." These are: Group 1, with a mesocolpium of fine-reticulate with granular luminina, containing *P.palustris*, *P.scandens*, *P.lukafuensis* and *P.obovalis*; Group 2 showing a mesocolpium of coarse-reticulate with granular lumina, containing *P.lancifolius*, *P.lecomtei and P.monophyllus*; Group 3, striate-rugulate, poles and colpal margins perforate to complete, containing the one species, *P.grandiflorus*. The final group also contains one species,

P.tetragonolobus and possesses a mesocolpium of very coarse-reticulation, often interrupted, producing irregular, isolated muri, interspersed with densely packed, pila-like columellae. Towards the poles the muri are continuous forming a complete reticulum, and at the poles the tectum is perforate. Her monospecific groups 3 and 4 appear quite distinct, but she does comment on the second group that *P.lancifolius* mesocolpium reticulation is inconsistent and might be better considered as an intermediate between groups 1 and 2. This would leave the two species *P.monophyllus* and *P.lecomtei* in group 2. She comments that she has found no reliable pollen character to separate these two, which echoes the present study's morphological findings. Within group 1 she comments that it is impossible to separate *P.palustris* and *P.scandens* on pollen morphology, but on average *P.scandens* has slightly smaller pollen than *P.palustris*. These two species' closeness is also reflected from the present investigations morphological findings. Poole comments that *P.lukafuensis* is similar in general characteristics to *P.scandens* and *P.palustris*, but is consistently smaller. She also places *P.obovalis* in this group, which is surprising in the context of the morphological finding. However, she emphasises that it fits badly into this group, it being the only sub-prolate member and with a more coarse reticulum. Reading the comparative descriptions porovided, it would appear more naturally placed in group 2 with its morphological allies, *P.monophyllus* and *P.lecomtei*.

Poole (1979) provides detailed data for the pollen characters of the nine species but other than the division into pollen types attempts no further phenetic analysis. The bulk of the data she provides is detailed in Table 7.4. This data may be easily coded for numerical analysis, so that a more objective analysis of the palynological study's results could be obtained. This was carried out using the program LINKAGE followed by CLUSTAN(DISTIN) as described in Section 3.4.2(b). The dendrogram displaying the results of the analysis is shown in Figure 7.4.

The dendrogram indicates a splitting of the species into two clusters, the first containing *P.grandiflorus*, *P.obovalis*, *P.monophyllus* and *P.lecomtei*, the second with *P.tetragonolobus*,

Table 7.4 Psophocarpus Species Pollen Description (compiled from Poole 1979)

CHARACTER DESCRIPTIONS	SPECIES								
	P.grandi-florus	*P.tetragon-olobus*	*P.palu-stris*	*P.scan-dens*	*P.obova-lis*	*P.mono-phyllus*	*P.lec-omtei*	*P.luka-fuensis*	*P.lanci-folius*
Polar length µm (P)	53.0–58.1	42.3–51.6	52.3–63.3	48.3–61.6	47.7	36.0–49.6	43.6	36.0–41.5	47.6–50.0
Equatorial axis length mm (E)	45.9–49.3	43.4–49.9	50.0–60.0	47.3–56.3	46.5	31.6–42.6	38.2	36.9–41.7	43.5–48.5
P + E	1.08–1.18	0.99–1.06	1.05–1.08	1.02–1.09	1.31	1.14–1.17	1.14	0.96–1.06	1.03–1.09
Colpus length µm	31–54	25–38	32–49	22–40	32–38	25–40	28–33	22–35	30–40
Endoaperture dia. µm	7–12	9–15	9–21	10–15	5–8	6–12	8–10	8–14	10–15
Colpa surface	S[A]toG	G	G	StoG	StoG	StoG	S	G	G
Total wall thickness µm	3.0–4.0	2.0–3.0	3.0–4.0	3.0–4.0	2.0–2.5	2.0–2.5	2.0–3.0	2.5–3.0	3.0–3.5
Mesocolpium ornamentation	SR[B]	CR	FR	FR	FR	CR	CR	FR	FR/CR
Pole & Colpa margin type	PtoC[C]	RPorC	PtoR	PtoR	FRtoP	PtoR	PtoR	PtoR	P
Lumina width µm	–	8	1–3	1–3	1–3	4–6	4–6	1–3	1–3/4–6

A = In describing colpa surface; S=smooth and G=granular
B = In describing mesocolpium ornamentation; SR=striate;rugulate, CR=coarse reticulate, FR=fine reticulate
C = In describing pole and colpa margin type; P=perforate, C=complete, R=reticulate, RP=reticulate perforate
FR=fine reticulate

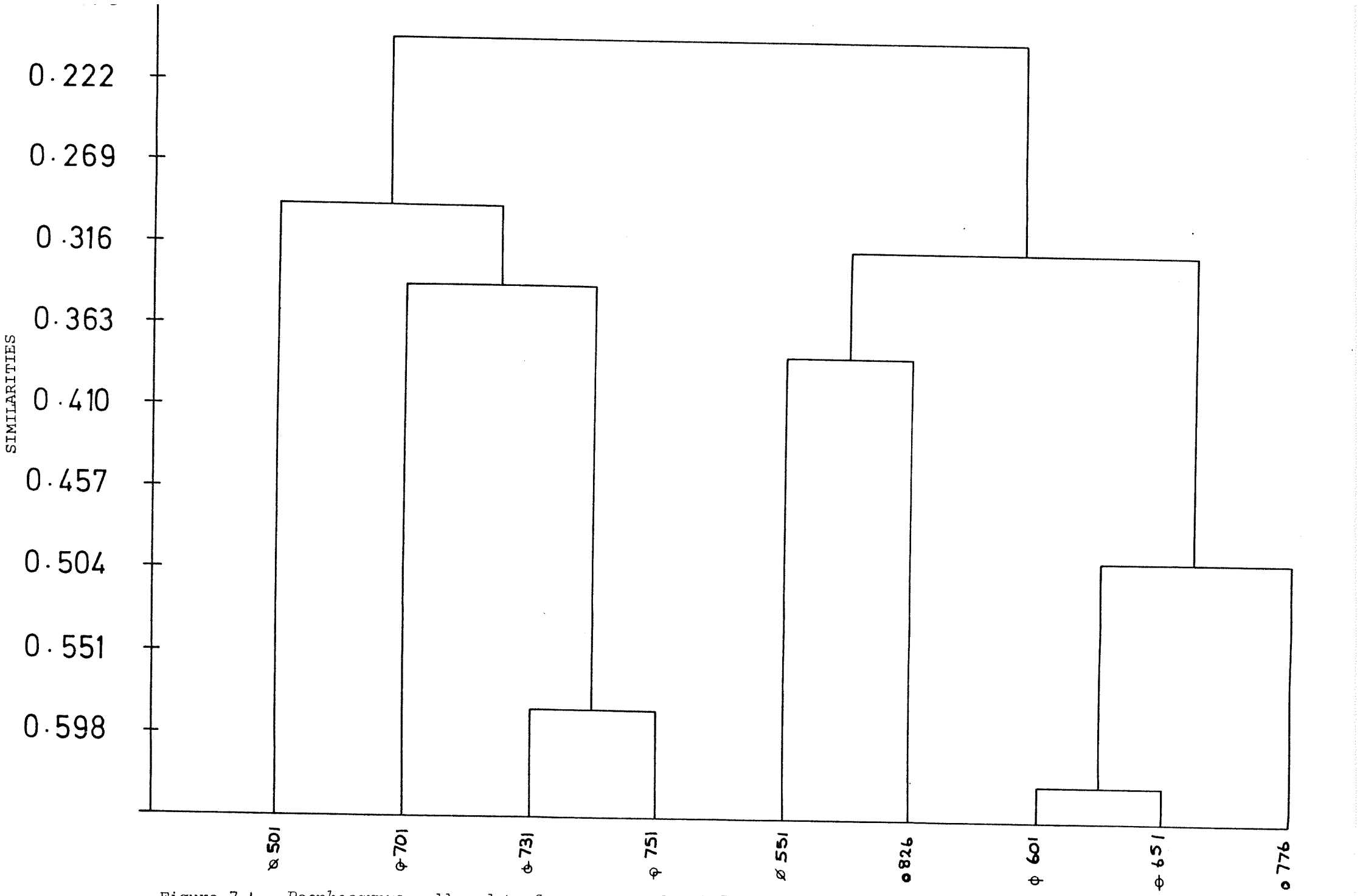


Figure 7.4: *Psophocarpus* pollen data from M.M. Poole, 1979

P.lancifolius, *P.palustris*, *P.scandens* and *P.lukafuensis*. Within the first cluster *P.grandiflorus* can be seen to form a distinct type isolated from the other species, corroborating Poole's splitting of *P.grandiflorus* into a mono-specific pollen type. The other three species form a distinct sub-cluster with *P.obovalis* joining as a distant ally the closely related *P.monophyllus* and *P.lecomtei* of Poole's group 2. Note that *P.lancifolius* has been excluded from this group as proposed by Poole, and is linked remotely to *P.tetragonolobus* in the second major cluster.

Within the second major cluster both *P.lancifolius* and *P.tetragonolobus* can be seen to be remotely related to the remaining three species: *P.palustris*, *P.scandens* and *P.lukafuensis*. Of these three species, the former two are very closely related, with *P.lukafuensis* slightly more distant; in fact the relationship follows that suggested by Poole (1979).

The results of the numerical analysis of Poole's (1979) data do vary from her own interpretations of her results. However the species placed in different positions, most notably *P.obovalis* and *P.lancifolius* are those about which she herself expressed doubts as to their natural position.

If the *Psophocarpus* palynological evidence is considered in the light of the morphological results, then they can be seen to reflect broadly the results of the morphological analysis, except that is, for the position of *P.grandiflorus*. The morphological results indicate the close allegence of *P.monophyllus* with *P.lecomtei* and more distantly *P.obovalis* but do not indicate *P.grandiflorus* to be allied to this group. Also within the second cluster shown in the palynological dendrogram, *P.tetragonolobus* might be expected as on morphological evidence to be closer to *P.palustris*, *P.scandens* and *P.lukafuensis*. However as Poole (1979) points out:

> "*P.tetragonolobus* has a very long history of cultivation, in fact no wild specimens have ever been found (Verdcourt and Halliday, 1978); it is perhaps not surprising therefore that its pollen should exhibit the greatest degree of specialisation within the genus."

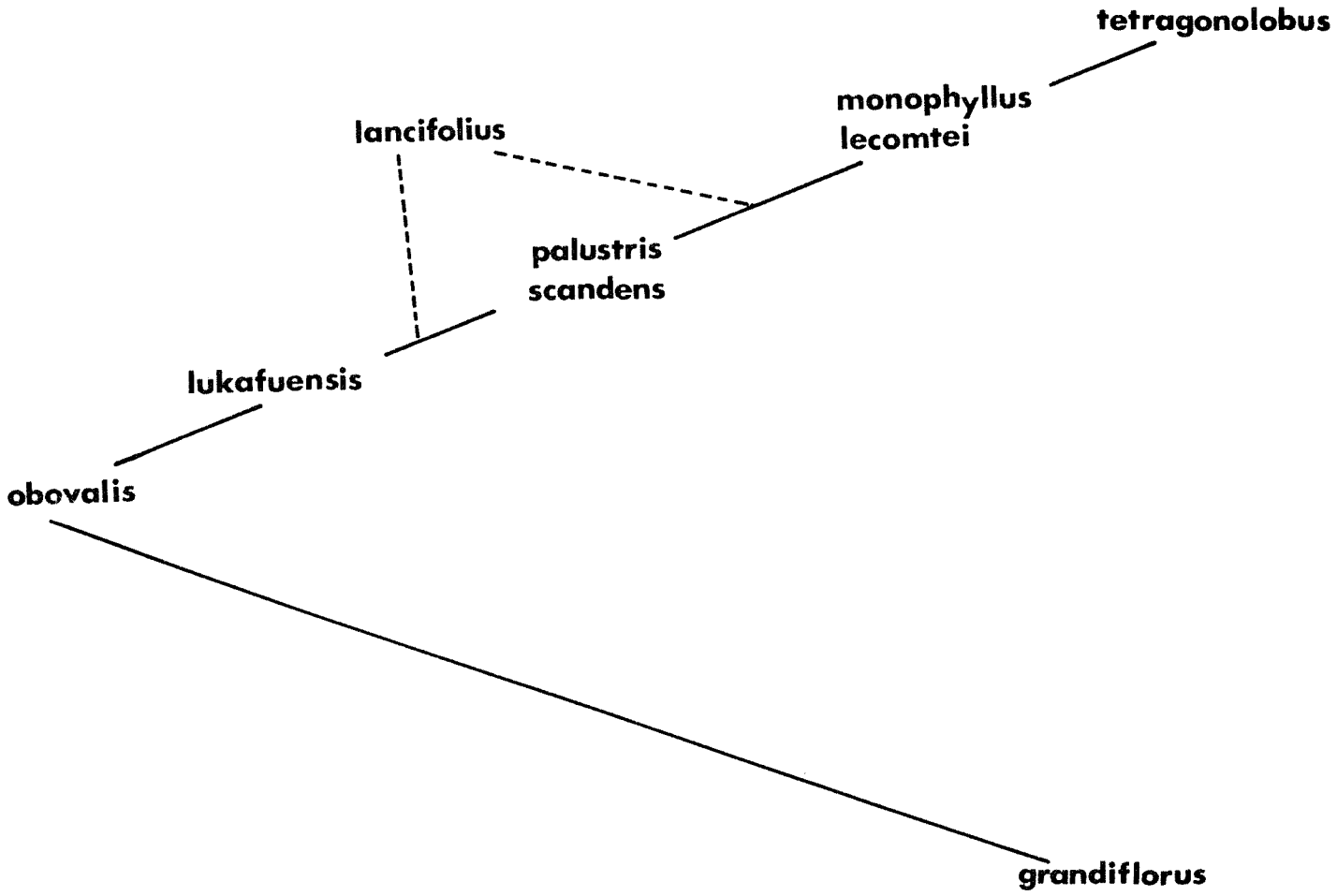
Poole (1979) includes a diagram displaying possible phylogenetic trends within the genus: this diagram is included here in Figure 7.5. She comments that *P.obovalis* and *P.lukafuensis* are possibly the least advanced because of their small pollen size and fine reticulum. The former also possessing a long colpi and small endoaperture which also indicates primitiveness. Poole then argues that the degree of specialisation shown by *P.tetragonolobus* and *P.grandiflorus* suggests a more recent evolution, though they have developed along different evolutionary lines. She finds *P.lancifolius* difficult to place in a phylogenetic context (as it is phentically) because of its inherent variability in tectum, varying from fine to coarse reticulate. She links *P.scandens* and *P.palustris*, *P.monophyllus* and *P.lecomtei*, as the two sets of closely allied species and believes the latter two to be more advanced as they possess larger lumina, which is associated with advancement.

To summarise the palynological literature relating to the present study, it was found that no true relationship could be established between the pollen of *Psophocarpus* and its allies, as the investigation of *Psophocarpus'* allies has not been systematically completed. Within the genus *Psophocarpus*, the results presented here from numerical analysis of Madalaine Poole's data show similar groupings to those produced by the morphological data, though there is one apparently misplaced species, *P.grandiflorus*. This species based on pollen characters links with the three species possessing unequally bifid style apices (*P.obovalis*, *P.monophyllus* and *P.lecomtei*). This is an interesting juxtaposition, but one which cannot be defended on morphological grounds.

7.6 Anatomical evidence

As emphasised by Lackey (1981) "little taxonomic advantage has been taken of the possible data from this field", though in recent years more systematic coverage of legume anatomy has begun to be exploited. There have been no anatomical studies comparing the nine *Psophocarpus* species, and the following account abstracted from the literature will concentrate on trying to relate *Psophocarpus* to its possible allies.

Figure 7.5: Possible phylogenetic trends in *Psophocarpus* pollen (Taken from Poole, 1979)



The first comprehensive review of legume anatomy was included in Solereder (1908) who records the presence of hooked hairs in *Clitoria, Centrosema, Periandra* (from the Clitoriinae sensu Lackey, 1981) and Phaseolus (Phaseolinae sensu Lackey, 1981). Solereder takes most of his account of Phaseoleae anatomy from Debold (1892) who worked on leaf and axis anatomy of the Phaseoleae (including *Psophocarpus* in the study). This work was later taken up and expanded by Lackey (1978), as will be shown below. Debold noted several grouping of genera based on certain characters; presence of mucilaginous epidermis in *Clitoria, Periandra, Dolichos* and *Phaseolus*, presence of tannin containing sacs within the leaflet in *Clitoria, Centrosema, Periandra, Phaseolus, Vigna* and *Dolichos*, and possession of rod-shaped crystals in the palisade tissue in *Clitoria, Centrosema, Phaseolus* and *Vigna*. But none of these generic groupings includes *Psophocarpus* and thus does not help in linking *Psophocarpus* to its allies.

A second major review of legume anatomy was included in Metcalfe and Chalk (1950), who reiterate much of the information detailed in Solereder (1908) and Debold (1892) as well as including additional material. They, however, like Solereder, suggest few characteristics that link *Psophocarpus* with its potential allies. They comment that *Psophocarpus, Vigna* and *Phaseolus* are the only Phaseolinae genera studied to possess stomata both on the adaxial and abaxial leaf lamina surfaces. It is suggested also that the browning of herbarium material is due to degradation reactions in cells or sacs containing tannins, proteins or gum-like substances. Verdcourt and Halliday (1978) use this browning on drying as a distinguishing character between the closely allied *Psophocarpus* species, *P.scandens* and *P.palustris*. *P.scandens*, leaves blacken on drying which infers it must possess more cells with degrading secondary metabolites than *P.palustris*.

Some work was published by Dormer (1945, 1946) in which he concluded that the Phaseoleae have a complicated open vascular system and that the Phaseoleae form at least a limited amount of secondary xylem, but no specific references were made to peculiarities in *Psophocarpus*. Baudet (1973) published some investigation results on the epidermis of *Phaseolus-Vigna* complex taxa. He concluded that *Phaseolus* could be distinguished from its allies *Vigna* and *Macroptilium* by the presence of

hooked hairs, and that some *Phaseolus* species have only hypostomatic stomata. He found hooked hairs to be absent from *Phaseolus adenanthus* and suggests that this species may be better transferred to *Vigna*, which Marechal et al (1978b) do. Baudet and Marechal (1976) in a more extensive survey record the presence of hooked hairs in the Phaseoleae and Hedysareae. Of the 47 genera from the Phaseleae examined, hooked hairs were recorded as present in 6: *Centrosema, Clitoria, Clitoriopsis, Phaseolus, Alepidocalyx* and *Minkelersia*. (*Periandra* which also possess hooked hairs was not studied). The latter two were reduced to subgeneric rank of *Phaseolus* by Marechal et al (1978b). They comment that the presence of hooked hairs is a stable character and can be used with others to distinguish *Phaseolus* from its allies. In the experience gained from the morphological study undertaken for the present investigation this view is supported, but it is worth noting that one specimen of *Psophocarpus grandiflorus* (Loveridge 344) possessed hooked hairs of the *Phaseolus* type, as well as normal straight hairs. Though this is an obvious exception to the rule, it is worth noting in case these rare exceptions are found in other genera.

Lackey (1977b) included in his thesis a detailed study of Phaseoleae leaflet anatomy, which follows on from the work of Debold (1892). They together investigated 79 genera and over 300 species, providing a good systematic coverage of the tribe. The characters used by Lackey were related to stomata, epidermal cells, hairs, crystals, paraveinal mesophyll and palisade layer. Not all these characters correlated with generic grouping and those Lackey considers most useful were published in Lackey (1978). Table 7.5 summarises the results of his anatomical studies for the *Psophocarpus* allied genera. In his discussion of the taxonomic grouping, Lackey (1978) concludes that, "anatomical characters parallel morphological groupings at various taxonomic levels", but that there are no characters which prove consistently taxonomically useful throughout the Phaseoleae.

For the *Psophocarpus* allied genera Lackey (1978) comments that there is a similarity with the Diocleinae, though the number of uniseriate hairs is reduced and the number of stalked glands increased. He records that *Nesphostyles* and *Austrodolichos* both possess well developed bundle sheaths with crystals less pronounced than in other genera. Lackey

Table 7.5 Anatomical Characters of Leaflects of the Clitoriinae and Phaseolinae

(Adapted from Lackey 1980a)

SPECIES	CHARACTERS								
	1A	2	3	4	5	6	7	8	9
Clitoriinae									
Centrosema angustifolium (H.B.K.) Benth.	+	+	+	+	—	—	—	—	—
Centrosema virginianum (H.B.K.) Benth.	+	+	+	+	—	—	—	—	—
Periandra heterophylla Benth.	—	+	+	+	—	—	—	—	—
Clitoria laurifolia Poir.	+	+	+	+	?	+	—	—	—
Clitoria ternatea L.	—	+	+	+	—	—	—	—	—
Clitoriopsis mollis Wilczek	—	+	+	+	+	+	—	—	—
Phaseolinae									
Dysolobium grande (Benth.) Prain	+	+	+	—	+	+	—	—	+
Psophocarpus palustris Desv.	+	+	+	—	—	—	—	—	—
Physostigma venenosum Balf.	—	+	+	—	—	+	—	—	—
Vatovae pseudolablab (Harms) Gillett	+	+	—	—	—	+	—	—	—
Decorsea schlechteri (Harms) Gillett	+	+	—	—	—	+	—	—	—
Spathionema Kilimand-schariaum Taub.	+	+	—	—	—	—	—	—	—
Otoptera burchellii DC.	+	+	+	—	—	+	—	—	—
Sphenostylis angustifolia Sond.	+	+	+	—	—	+	—	—	—
Nesphostylis holosericea (Bak.) Verdc.	+	+	+	—	+	+	—	—	—
Austrodolichos errabundus (Scott) Verdc.	+	+	+	—	—	+	—	—	+
Neorautanenia amboensis Schinz	+	+	+	—	—	—	—	—	—
Lablab purpureus (L.) Sweet	+	+	+	—	—	+	—	—	—
Alistilis bechuanicus N.E.Br.	+	+	+	—	—	+	—	—	—
Dipogon lignosus (L.) Verdc.	+	+	+	—	—	+	—	—	—
Dolichos monticola Benth.	+	+	+	—	—	+	—	—	—
Dolichos kilimand-scharicus Taub.	+	+	+	—	—	+	—	—	—
Macrotyloma biflora (Schum.& Thonn.) Lackey	+	+	+	—	—	+	—	—	—
Vigna ambacensis Bak.	+	+	+	—	—	+	—	—	—

Vigna monophylla Taub.	+	+	–	–	–	+	–	–	–
Vigna venusta (Piper) Marechal et al	–	+	+	–	–	+	–	–	–
Voandzeia subterranea (L.) Thouars	+	+	+	–	–	+	–	–	–
Ramirezella strobilophora (Robins) Rose	–	+	+	–	–	+	–	–	–
Oxyrhynchus trinervus (Donn.Sm.) Rudd	–	+	+	–	–	–	–	–	–
Dolichopsis paraguariensis Hassler.	+	+	+	–	–	+	–	–	+
Strophostyles helvula (L.) Ell.	+	+	+	–	+	+	–	–	+
Macroptilium gracile (Benth.) Urban	+	+	+	–	–	+	–	–	–
Macroptilium lathyroides (L.) Urb.	+	+	+	–	–	+	–	–	–
Phaseolus coccineus L.	+	+	–	+	–	+	–	–	–
Phaseolus parvulus (Greene) Piper	?	?	+	+	–	+	–	–	–
Phaseolus galactioides (Mart.& Gal.) Marechal et al	+	+	–	+	–	+	–	–	–

A 1 = Stomata on upper surface; 2 = stomata on lower surface; 3 = Uniseriate hairs; 4 = hooked hairs; 5 = unstalked glands; 6 = stalked glands; 7 = vesicular glands; 8 = bulbous-based hairs; 9 = paraveinal mesophyll; + = present; ? = questionable.

(1977b) comments that the most notable feature about Phaseolinae anatomy is the presence of hooked hairs and records their presence in the same genera as Baudet and Marechal (1976), plus *Periandra* which the latter workers did not investigate. He comments that there is no natural link between the Phaseolinae and Clitoriinae genera possession of hooked hairs; presumably this character has arisen more than once in this group.

In discussing the results of Lackey's (1978) study (detailed in Table 7.5) it can be seen that only *Neorautanenia amboensis* shares the same character combination as *Psophocarpus palustris*, while *Otoptera burchellii*, *Sphenostylis angustifolia*, *Dolichos* spp. and *Vigna ambacensis* of *Psophocarpus*' close allies show one character's difference. In each case these specimens possessed stalked glands, which were not shown to be present in *Psophocarpus palustris*. Before considering these results too seriously it should be stated that Lackey provides no assessment of intra-generic variation. So it would be unwise to draw anything other than preliminary conclusions from the results of one species, but based on Lackey's data it appears that *Psophocarpus* is most closely allied to *Neorautanenia*; the other close allies (on morphological grounds) are also suggested as being close, except for *Dysolobium* which has three character differences to *Psophocarpus*. Unstalked glands, stalked glands and paraveinal mosophyll are all present in *Dysolobium* but absent from *Psophocarpus*, which suggests that Lackey's (1977b) own hypothesis that *Dysolobium* and *Psophocarpus* are closely allied is invalid.

In the work of Lersten (1981) a scanning electron microscopy study of seed testa topography of the Papilionoideae, *Psophocarpus* is shown to share a testa pattern with the following genera from the Phaseolinae: *Decorsea*, *Lablab*, *Neorautanenia*, *Macrotyloma*, Some *Vigna* spp. and *Phaseolus*. All these showed a regulate surface, which he defines as being a conspicuous irregular roughened pattern. Of the other genera of the Phaseolinae he investigated he found *Strophostyles* to be levigate (smooth) and the other *Vigna* spp. and *Macroptilium* to be lophate (short ridges with irregular sides). Taxonomically these groupings seem to be rather diverse and do not help relate *Psophocarpus* to its allies. Any grouping which includes *Neorautanenia* and some *Vigna* spp. with

Psophocarpus may be of interest, but if this group also includes *Phaseolus* which is so clearly not closely related to *Psophocarpus*, then the results must be treated more cautiously.

Smith (1981) notes in his review of the cotyledons of the Leguminosae that *Psophocarpus tetragonolobus* possesses a rare pattern of cotyledon reserve mobilisation, only recorded from *P.tetragonolobus* thus far in his investigations. In this pattern of mobilisation which he refers to as C2, mobilisation begins on the adaxial colytedon surface and around the cotyledon veins. As this pattern is not found in other genera or species no conclusions may be drawn about the relationship of winged bean to other species or genera, but it would be interesting to investigate the other *Psophocarpus* species and genera shown by the morphological studies to be allied to *Psophocarpus*, to distinguish in which, if any other, this mobilisation pattern is found.

Lackey (1981) comments that little advantage has been taken of the potential anatomical data available to the taxonomist, this seems especially cogent when one is attempting to relate *Psophocarpus* to its allies, or *Psophocarpus* species to each other. Most of the anatomical studies attempted have concentrated on the Leguminosae as a whole and so provide little information of use in answering the sort of questions posed in this thesis.

7.7 Chemotaxinomic evidence

Lackey (1981) comments on the lack of use made of potential anatomical characters, quoted in the previous section, could equally well be extended the lack of exploitation of legume chemistry. He comments that, "despite the frequent use of *Phaseolus vulgaris* and other beans as subjects of physiological study and the review of legume chemistry by Harborne, Boulter and Turner (1971), there has been little comparative phytochemistry of the Phaseoleae". Smartt (1980b) suggests that non-protein amino acids, alkaloids and phenolics are all potentially useful in this context. The studies that have been undertaken are either extensive, attempting to cover the entire Leguminosae (Jay, Lebreton & Letoublon 1971; Boulter, Thurman and Derbyshire 1967' Baudet and Torck 1978) or intensive, and focus on a

small number of genera which unfortunately exclude *Psophocarpus*. (Casimir & Le Marchand 1966; Bell 1970a,b cited in Verdcourt 1970b and c; Otoul, 1971; Otoul and Dardenne, 1976). Unfortunately none of these studies is useful in trying to ally *Psophocarpus* with other Phaseoleae genera.

However, there are some studies which have included representatives of *Psophocarpus*. Boulter, Thurman and Derbyshire (1967) include *Psophocarpus palustris* (as with the cytological studies of this period, the material is likely to be referable to *P.scandens* following Verdcourt's (1968) splitting of the two species). In their disc electrophonetic analysis of globulin proteins of legume seeds they found *P.palustris* had a unique banding pattern compared to the other Phaseoleae specimens examined. Their analysis divided the Phaseoleae examined into five groups as follows:

Group 1 *Lablab purpureus* (L.) Sweet
Phaseolus acutifolius A.Grey
Phaseolus coccineus L.
Phaseolus vulgaris L.
Vigna radiata (L.) Wilczek
Vigna umbellata (Thumb.) Ohwi-Ohashi
Vigna unguiculata (L.) Walpers

Group 2 *Macroptilium atropurpureum* (DC.) Urban
Macroptilium lathyroides (L.) Urban

Group 3 *Dolichos bicontortus* Dur.

Group 4 *Psophocarpus scandens* (Endl.) Verdc.

Group 5 *Pachyrhizus angulatus* Rich.

As can be seen, *Psophocarpus palustris* is placed in their table on the periphery of the Phaseolinae genera between *Dolichos* and *Pachyrhizus*, the latter Lackey (1981) places in the Diolceinae.

In his review of canavanine distribution in the Phaseleae, Lackey (1977a) found canavanine absent from all Phaselinae genera (sensu Lackey 1981) tested. This conclusion is reiterated in an extended survey of canavanine distribution in the Papilionoideae by Bell, Lackey and Polhill (1978), with one exception *Phaseolus anisotrichos* (Bell and Hohenschutz).

Baudet (1977b) compiles from the literature a table containing the recorded presence of the non-protein amino acids, 5-methyl-cysteine and pipecolic acid. He also tabulates his own results from a survey of Phaseoleae for the presence/absence of Leuco-anthrocyanins. The results for the Phaseolineae genera (sensu Lackey 1981) are tabulate from Baudet (1977b) in Table 7.6. In considering the results included in Table 7.6, it is noted that the *Psophocarpus* species surveyed contain neither 5-methyl-cyzteine or leuco-anthocyanins. As will be discussed below Bell and Quleshi (1978) cited in Verdcourt and Halliday (1978), found pipecolic acid present in four *Psophocarpus* species, though not in *P.grandiflorus*. So in considering an ally of *Psophocarpus*, absence of 5-methly-cysteine and leuco-anthocyanins, but probable presence of pipecolic acid would be expected.

Dysolobium, though not surveyed for 5-methly-cysteine or pipecolic acid, shows the absence of leuco-anthocyanins, as does *Psophocarpus*. *Sphenostylis* appears more remote as it displays the presence of both 5-methly-cysteine and leuco-anthrocyanins, unlike *Psophocarpus*, *Neorautanenia* lacks pipecolic acid but like *Psophocarpus* it lacks the presence of leuco-anthrocyanins and so may also be considered more remote. Both *Dolichos* spp. and *Vigna* spp. show variable intra-generic presence/absence of the three chemicals, which questions the validity of using the presence of these chemicals for drawing taxonomic conclusions at the generic level. This is underlined as the table has so much missing data, so any conclusions must be speculative. It is unfortunate that *Otoptera*, the genus shown to be morphologically most closely allied to *Psophocarpus*, was not included in any of the studies; it will prove very interesting to see if it shares any chemical characters with *Psophocarpus*.

Table 7.6 Phaseolinae Chemotaxonomic Evidence
(Abstracted from Baudet 1977)

S = S-methyl-cysteine. P = pipecolic acid. L = leuco-anthocyanins
X = present, O = absent, - = not analysed.

SPECIES	S	P	L
Dysolobium grande	-	-	O
Psophocarpus lancifolius	O	-	O
Psophocarpus tetragonolobus	O	-	O
Psophocarpus palustris	-	-	O
Physostigma cylindrospermum	-	X	-
Physostigma mesoporiticum	-	-	O
Vatovaea pseudolablab	-	X	O
Decorsea schlechteri	-	O	-
Sphenostylis marginata	X	-	X
Sphenostylis stenocarpa	O	-	X
Neorautanenia mitis	-	O	O
Lablab purpureus	X	X	O
Dipogen lignosis	-	O	O
Dolichos glabrescens	O	-	-
Dolichos kilimandocharicus	-	O	-
Dolichos trilobus	O	-	O
Dolichos sericeus spp *sericeus*	O	-	X
Dolichos sericeus spp *glabrescnes*	-	-	O
Dolichos sericeus spp *pseudofalcatus*	-	-	O
Dolichos pseudocajarius	-	-	X
Dolichos tonkouiensis	-	-	X
Macrotyloma africarium	O	-	O
Macrotyloma axillare	O	X	O
Macrotyloma ellipticum	O	-	X
Macrotyloma geocarpum	O	-	-
Macrotyloma stenophyllum	O	-	X
Macrotyloma uniflorum	O	X	O
Vigna ambacensis	X	-	-
Vigna angivensis	X	-	-
Vigna fischeri	O	-	O
Vigna gracilis	O	-	O
Vigna heterophylla	X	-	-
Vigna hosei	-	-	O
Vigna kirkii	-	-	-
Vigna lasiocarpa	X	-	-
Vigna longifolia	-	X	-
Vigna luteola	O	X	-
Vigna monophylla	X	O	O
Vigna multinervis	-	-	O
Viugna nigritia	-	-	O
Vigna oblongifolia	-	O	O
Vigna pilosa	-	O	-
Vigna reticulata	-	-	O
Vigna subterranea	-	-	O
Vigna triphylla	O	-	O
Vigna unguiculata	X	O	O
Vigna vexillata	X	O	-
Vigna acountifolia	X	-	O
Vigna angularis	O	X	O

Vigna mungo	o	o	–
Vigna radiate	x	o	o
Vigna trilobata	–	–	o
Vigna umbellatta	o	o	o
Strophostyles helvola	–	–	o
Strophostyles umbellata	x	x	o
Macroptilium atropurpureum	x	–	–
Macroptilium bracteotum	–	–	o
Macroptilium enythroloma	–	–	o
Macroptilium heterophyllum	–	–	o
Macroptilium talhyroides	–	–	o
Phaseolus acutifolius	–	x	o
Phaseolus anisotrichus	x	–	o
Phaseolus coccineus	x	–	o
Phaseolus filiformis	x	x	o
Phaseolus lunatus	x	x	o
Phaseolus obvallatus	x	x	o
Phaseolus polyanthus	–	–	o
Phaseolus polystachyus	x	–	o
Phaseolus ritensis	x	–	o
Phaseolus vulgaris	x	–	o
Phaseolus (S.America) *adenanthus*	x	x	o
Phaseolus caracalla			

Sources of data: Baudet (1977)
Thompson, Morns & Zacharius (1956)
Rinderknecht, Thomas & Aslin (1958)
Casimir & Le Marchand (1966)
Bell (1970a)
Bell (1971)
Otoul & Dardenne (1977)
Otoul (1977)

There are numerous papers included in 'Advances in Legume Systematics' Vol 2 (Polhill and Raven, 1981) on the phyto-chemistry of the Leguminosae, but as with the anatomical papers in the same volume, they provide little help in the present study. In trying to indicate trends within the Leguminosae, the results are far too general to be interpreted at the generic level and supply little help in allying *Psophocarpus* with other genera.

At the specific level the only comparative phyto-chemical study of *Psophocarpus* has been undertaken by Bell and Qureshi and is presented in Verdcourt and Halliday (1978). They undertook a preliminary investigation of free amino-acid patterns on the five species detailed below with the following results:

Species	Chemical	
	R	B
P.palustris	+++	trace
P.scandens	+	trace
P.tetragonolobus	+	trace
P.grandiflorus	-	++
P.lancifolius	+	-

Bell comments that there appear to be two significant spots, due to uncommon amino-acids. These are not accumulated equally by the species studied. "One fluorescing red under ultra-violet light, is probably a substituted pipecolic acid and the other is a basic amino-acid", these he refers to as R and B respectively. *P.palustris* he comments is different from the other species in that soluble N appears concentrated in two major spots, R and a second, which he identifies as being possibly alanine and is common to all species. *P.grandiflorus* stands out as having no R and a prominent B spot. *P.scandens*, *P.tetragonolobus* and *P.lancifolius* seem very similar, though *P.lancifolius* has no B. Bell suggests this may be due to a minor concentration difference.

Verdcourt and Halliday (1978) comment on these results that they have "not thrown any light on the inter-relationships of the species and

certainly the fairly strong morphological differences between the two subgenera are not backed up by the results, neither does *P.tetragonolobus* stand apart in any way".

Even in the light of results from the present morphological study, the free amino-acids results do not concur fully with morphological findings. The strong difference in spot R between *P.palustris* and *P.scandens* is surprising as they are so morphologically intimate. The closeness between *P.scandens* and *P.tetragonolobus*, and with *P.palustris* and the distance of these three species from *P.grandiflorus* might be expected, based on the present study's morphological results, though the closeness between *P.scandens*, *P.tetragonolobus* and *P.lancifolius* is contrary to morphological expectations.

To summarise, the amount of phyto-chemical evidence available within the context of the present investigations is very limited. At the inter-generic level there have been few systematic surveys reported, and those published either have large amounts of missing data or remain constant for the taxa shown morphologically to be close to *Psophocarpus*. At the inter-specific level there is only one relevant study, by Bell and Qureshi (1978) cited in Verdcourt and Halliday (1978), and this yields results which contradict established morphological groupings. This study also does not include any of the three *Psophocarpus* species with a bificate style apex because of the rarity of their seed and so could not be considered a comprehensive inter-specific study. There is thus quite a broad scope for further research available to future Phaseoleae phyto-chemists.

7.8 Seedling evidence

The relevant seedling studies represented in the literature are restricted to the generic level: there has been no systematic study of the seedling characters of *Psophocarpus* spp. The initial studies were carried out by De Candolle (1925b), which were expanded and added to by Compton (1912) and then Baudet (1974,1977b), though other workers have also contributed evidence on a smaller scale, usually relating to a few taxa.

The evidence produced from these studies shows that in the Phaseolinae embryos are curved, except for *Vigna subterranea*, the roots are triarch and metoxylem is developed in the centre (except for *Vigna subterranea* which has central pith with usually septarch xylem). The first leaves to appear after the cotyledons are opposite and simple (again, *Vigna subterranea* is different, with trifoliolate leaves), then subsequent leaves attain the adult condition.

Baudet's (1974) seedling study, though not completely comprehensive, does provide some useful information on *Psophocarpus* and its close allies. This evidence is abstracted for the close *Psophocarpus* allies in Table 7.7. Lackey (1981) condenses this table to generic details only, except for *Vigna* for which he provides subgeneric information. However, it was considered here more appropriate to include extracts from Baudet's (1974) original table as it provides a better idea of intra-generic variation.

Of the three *Psophocarpus* species investigated there was no intra-generic variation in the seven characters scored. So based on these observations the seedling characteristics of *Psophocarpus* are: hypogeal germination, glabrous epicotyle, double cotyledon stipules, petiole of cotyledon divided into three sections, cotyledonal stipels present, cotyledon base shape is cuneate to auriculate and the first leaf is trifoliate. If these features are compared with the other taxa in the survey shown in Table 7.7, it can be seen that *Psophocarpus* is unique in possessing double cotyledon stipules and a cuneate to auriculate cotyledon base shape. *Psophocarpus* like the majority of the other species exhibits hypogeal germination, there being epigeal

TABLE 7.7 Seedling Attributes of Psophocarpus and Its Close Allies
(Taken from Baudet, 1974)

TAXA	ATTRIBUTES[A]						
	G	E	ST	P	S	B	F-1
Psophocarpus Neck ex DC.							
palustris Desv.	H			3	X	5	3
tetragonolobus (L) DC.	H	O	D	3	X	5	3
lancifolius Harms	H	O		3	X	5	3
Sphenostylis E.Mey							
Stenocarpa (Hochst.) Harms	H	O	B(E)	1	O	2	3
Neorautanenia Schinz							
mitis (A.Rich.) Verdc.	H	X	B	3	X	3	3
Dolichos L.							
Sericeus E.Mey.							
spp.*pseudofalcatus* Verdc.	E	O	B	3	O	6	3
spp.*glabrescens* Verdc.	E	(X)	B	S	O	3-4	3
spp.*sericeus*	E	O	E-B	3	O	3	3
trilobus L.	H	O	E(B)	3	O	3	3
pseudocajanus Bak.	H	X	E	3	O	3	3
gululu DeWild.	H	X	B				
Vigna Savi							
Vigna subgen. *Cochliasanthus* Verdc.							
caracalla (L.) Verdc.	H	O	E	3	X	4	3
Vigna subgen *Plectrotropsis* Bak.							
vexillata (L.) A.Rich.	H	O	E(B)	1	O	2-3	3
Vigna subgen. *Ceratotropsis* Verdc.							
radiata (L.) Wilczek	E	X	E-B	1	O	3	3
mungo (L.) Hepper	E	X	E	1	O	3	3
acontifolia (Jacq.) Marechal	E	X		3	O	3	3
angularis (Willd.) Ohwi&Ohashi	H	O	E	3	O	3	3
trilobata (L.) Verdc.	H	O	E	3	O	3	3
umbellata (Thumb.) Ohwi&Ohashi	H	O	E	3	O	3	3
Vigna subgen. *Haydonia* Verdc.							
triphylla (Wilczek) Verdc.	H	O	E	3	O	3	3
Vigna subgen. *Vigna*							
sect *Vigna*							
luteola (Jacq.) Benth.	H	O	B	1	O	3	3
marina (Burm.) Merrill	H	O	B		O		3
fischeri Harms	H	O	B	1	O	3	3
oblongifolia A.Rich.	H	O	E	1	O	2	3
heterophylla A.Rich.	H	O	E	1	O	2	3
ambacensis Bak.	H	O	E	1	O	2-3	3
filicaulis Hepper	H	O	E(B)	1	O	2	3
aff *comosa* Bak.	H	O	B	3	X	6	3
racemosa (G.Don.) Hutch.&Dalz.	H	O		3	O	3	3
laurentii DeWild	H	O	E	3	X	3	3
gracilis Hook f.	H	O	E(B)	3	X	3	3
hosei (Craib.) Backer	H	O	B	3	O	3	3
parkeri Bak.	H	O	E	3	O	3	3
kirkii (Bak.) Gillett	E	O	E-B	1	O	3	3
angivensis Bak.	H	O		1	O		1

sect.*condylostylus* Verdc.							
multinervis Hutch.& Dalz.	H	O	E		O		3
sect.*Lasiospron* Verdc.							
lasiospon (Benth.) Verdc.	H	O	E-B	3	X	3	3
longifolia (Benth.) Verdc.	H	O	B	3	X	3	3
sect.*Catiang* Verdc.							
unguiculata (L.) Verdc.							
spp.*dekindtiana* (Harms) Verdc.	E	O	E	1	O	3	3
spp.*cylindrica* (L.) Van Eseltine	E	O	E(B)	1	O	3	3
ssp.*unguiculata*	E	O	E	1	O	3-4	3
spp.*sesquipedalis* (L.) Verdc.	E	O	E	1	O	3	3
ssp.*mensensis* (Schweinf.) Verdc.	E	O				3	3
sect.*Liebrechtsia* Bak. f.							
frutescens A.Rich.							
var.*frutescens*	H	O	B	3	O	2	3
var.*buchneri* (Harms) Verdc.	H	O	B	3	O	3	3
sect.*Reticulatae* Verdc.							
reticulata Hook.f.	E	X	E	1	O	2-3	1
sect.*Glossostylus* Verdc.							
venulosa Bak	H	O	E	3	O	2	3

A, G=germination, H:hypogeal, E:epigeal; E=epicotyl, X:pubescent, O:glabrous; ST=cotyledon stipules, D:double, B:bifid, E:entire; P=petiole of cotyledon, 3:three sections, 1:one section; S=stipels of cotyledon, X:present, O:absent; B base shape of cotyledon, 1:cuneate, 2:rounded, 3:cordate, 4:truncate to auriculate, 5:cuneate to auriculata
,
6:truncate; f-1, number of leaflets for the first leaf.

germination in *Dolichos sericeus*, *Vigna radiata*, *V.mungo*, *V.acontifolia*, *V.kirkii* and *V.unguiculata*. Like *Sphenostylis*, some *Dolichos* and the majority of *Vigna* spp., *Psophocarpus* possesses a glabrous epicotyl. The colytedonous petiole of *Psophocarpus* is divided into 3 sections as in *Neorautanenia*, *Dolichos* and some *Vigna*. *Psophocarpus* possesses cotyledonal stipels as does *Neorautanenia* and some *Vigna* spp. Like all the taxa surveyed, except for *Vigna angivensis* and *V.reticulata*, *Psophocarpus* has three leaflets composing the first true leaf.

It would be foolish to draw any firm conclusions from Baudet's (1974) results, as the coverage of even those genera most closely allied to *Psophocarpus*, (indicated by the present morphological investigation), is incomplete and there were only seven characters used. However, based on these characters the genus *Neorautanenia* and certain *Vigna* species (*V.comosa*, *V.llaurentii*, *V.gracilis*, *V.lasiospron* and *V.longifolia*) appear most closely allied to *Psophocarpus*.

For thier investigation of the *Phaseolus-Vigna* complex, Marechal et al (1978a) include Baudets seven seedling characters plus the number of leaflets of the cotyledon, which in only *Vigna subterranea* they found to be three. In their discussion of the relative usefulness of the characters they used they do not regard the seedling characters as proving very useful, not even the shape of the cotyledon base which a priori they considered might be taxonomically useful. Although these workers were not studying exactly the same taxa as in the present study, Marechal et al's conclusions indicate that caution should be used in drawing inferences from Baudet's seedling data on the relationship of *Psophocarpus* to its allies.

7.9 Other evidence

The final section of this chapter contains information that is considered useful in the context of relating the taxa under study, but which would not easily fit into the previous sections.

When presenting his proposed generic classification of the Phaseoleae, Baudet (1978) includes a table laying out his classification and including characters that Baudet considers of the principal

taxonomic importance. Part of his table is presented (for *Psophocarpus* and its allies) in Table 7.8.

Psophocarpus within Baudet's (1978) classification lies in his supergenera *Dolichastrae*, though his *Glycinastrae* genera commonly considered allied with the Phaseolinae and his *Phaseolastrae* genera are also included in Table. 7.7. In trying to locate *Psophocarpus'* allies it is expected that the allies will share character states with *Psophocarpus*. In interpreting the results of Baudet's taxonomic investigation problems arise because of the amount of missing data, especially for the *Dolichastrae* of which *Psophocarpus* is a member. *Dysolobium* and *Otoptera* both have a significant proportion of missing data which invalidates any accurate comparison with *Psophocarpus* or their other allies. Of the genera for which all the data is recorded *Psophocarpus* is shown to be closest to *Dipogon* and *Lablab*, and then *Sphenostylis* and *Dolichos*. The first two genera are somewhat surprising close allies, as they were not included in the subset of genera used for the phentic analysis in Phase 3 of this study. However, the latter two genera were included and both *Sphenostylis* and *Dolichos* were found to be closely allied to *Psophocarpus*. Next in association come *Neorautanenia*, *Vigna*, *Voandzeia* and *Macroptilium*, of which only the former two were included in this study's subset genera. So broadly the results of Baudet's investigations do concur with those of the present investigations, though the close allegiance of *Dipogon* and *Lablab* to *Psophocarpus* is difficult to explain. Perhaps it is worth noting in this context that to a pheneticist any classification based on as few as fourteen characters, even a posteori chosen characters, is artificial and so should not be expected truly to reflect natural relationships. This point is not meant as a criticism of Baudet's classification, but rather a criticism of attempting to draw conclusions from only the fourteen characters he regards as most important.

As relative style shape is so important in distinguishing Phaseoleae genera, Baudet (1977b, 1978) includes sections on the possible evolution of the basic style shapes based on ontological studies of various species of Phaseoleae. His conclusions are displayed in Figure 7.6. Of the seven genera shown in the present study to be morphologically close to *Psophocarpus* some are easily ascribed style types and these are given

Table 7.8 Principal Taxonomic Characters used by Baudet (1978) to Discriminate Psophocarpus and Its Allies

(arrangement after and taken from Baudet, 1978)

TAXA	CHARACTERS[A]													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Glycininae – *Glycinastrae*														
Neorautanenia Schinz	0	0	0	0	0	X	L	3	C	0	X	E	0	0
Macrotyloma (W.& A.) Verdc.	0	0	X	0	0	X	B	3	P	0	X	E	0	(X)
Phaseolinae – *Phaseolastrae*														
Vigna Savi	X	X	X	0	0	X	B	3	P	0	0	E	0	0
Voandzeia (L.) Thouars	X	X	X	0	0	X	B	3	P	0	0	E	0	0
Physostigma Balf.	X	X	X	0	0	X	B	3	P	0	NR	NR	0	0
Vatoveae Choiv.	X	X	X	0	0	X	NR	NR	NR	0	0	E	0	0
Dipogon Lieb.	X	X	X	0	0	X	L	3	C	0	0	E	0	0
Lablab Adans.	X	X	X	0	0	X	L	3	C	0	0	E	0	0
Spathionema Taub.	X	X	X	0	0	X	NR	NR	NR	0	NR	NR	0	NR
Dysolobium (Benth.) Prain	X	X	X	0	0	X	NR	NR	NR	0	NR	NR	0	0
Oxyrhyochus Brandegee	X	X	X	0	0	X	NR	NR	NR	0	NR	NR	NR	NR
Dolichopsis Hassler	X	X	X	0	0	X	NR	NR	NR	0	NR	NR	NR	NR
Macroptilium (Benth.) Urban	X	X	X	0	0	X	E	3	C	0	X	E	0	0
Ramirezella Rose	X	X	X	0	0	X	NR	NR	NR	0	NR	NR	0	NR
Phaseolus L.	X	X	X	0	0	X	E	3	C	X	X	E	0	0
Strophostyles Elliot	X	X	X	0	0	X	NR	NR	NR	0	0	E	0	0
Phaseolinae – *Dolichastrae*														
Dolichos L.	X	0	X	0	0	X	L	3	C	0	X	E	0	(X)
Decorsea R.Viguier	X	0	X	0	0	X	NR	NR	NR	0	NR	NR	0	NRD
Psophocarpus Neck.ex DC.	X	0	X	0	0	X	L	3	C	0	0	0	0	0
Otoptera DC.	X	0	X	0	0	X	NR	NR	NR	0	NR	NR	0	NR
Alistilus N.E.Br.	X	0	X	0	0	0	NR	NR	NR	0	NR	NR	0	NR
Sphenostylis E.Mey.	X	0	X	0	0	X	B	3	C	0	0	E	0	X
Nesphostylis Verdc.	X	0	X	0	0	X	L	3	C	0	NR	NR	0	NR
Austrodolichos Verdc.	X	0	X	0	0	X	NR	NR	NR	0	NR	NR	NR	NR

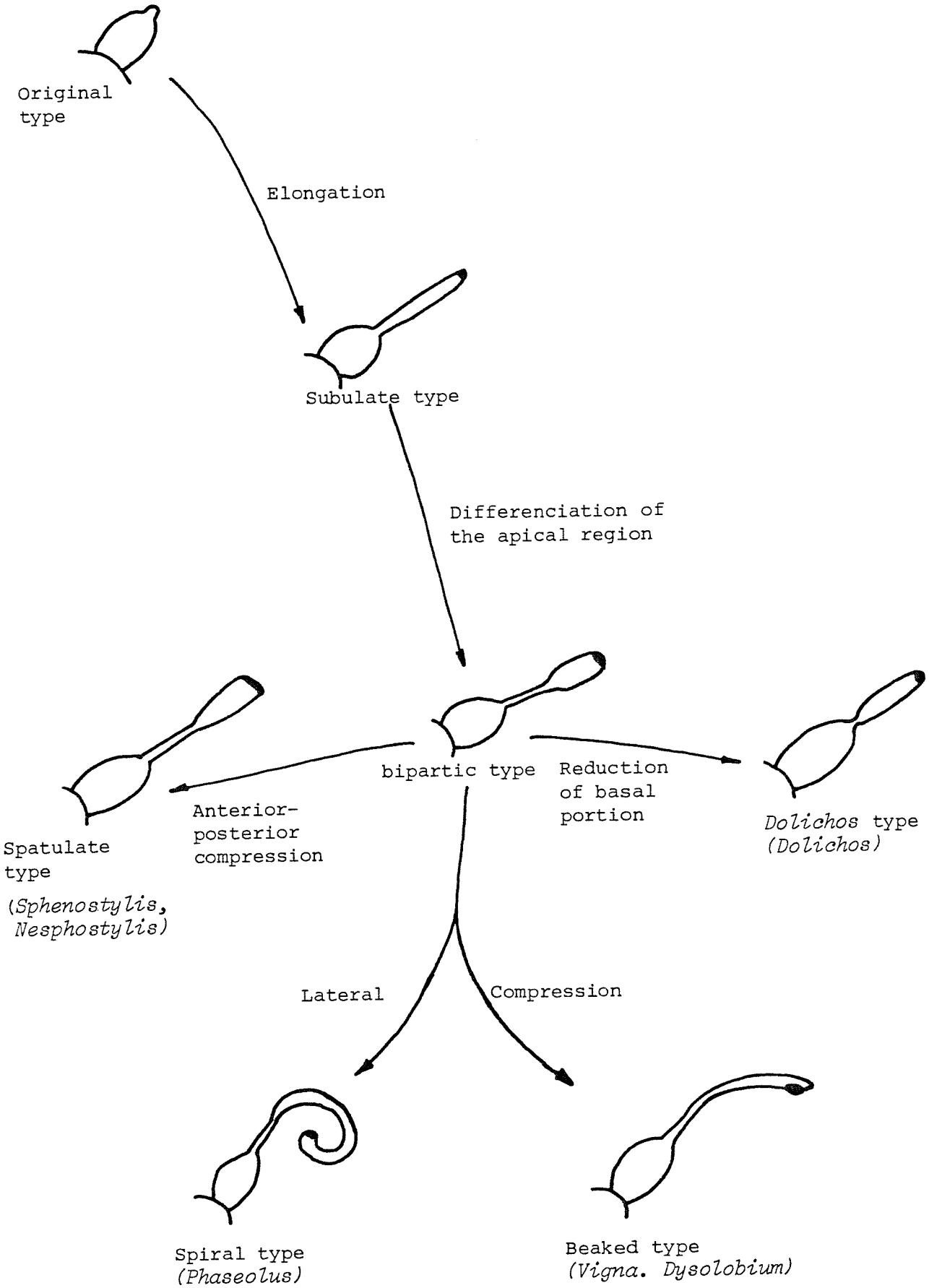
A. 1=Style: X forms two distinct parts, O forms one part; 2=Style: X hairy, O glabrous; 3=Stigma: X hairy O glabrous; 4=Glands: X present, O absent; 5=Ovary: X 1-2 Ovule, O 2 Ovules; 6=Bracteoles: X present, O absent; 7=Pollen: B polar length equatorial width, E polar length = equatorial width, L polar lenth equatorial width; 8=Pollen: 3 apertures, m 3 apertures; 9=Pollen: C colporate, P porate; 10=Hooked hairs: X present, O Absent; 11=Epicotyl: X hairy, O glabrous; 12=Cotyledonal stipules: E entire, D double, 13=Canavanine: X present, O absent; 14=Leuco-anthrocyanins: X present, O absent, (X) present in certain species, NR not recorded.

in parenthesis in Figure 7.6. However, the remaining genera *Otoptera* and *Neorautanenia* with *Psophocarpus* itself are not so easy to place. *Neorautanenia* and to a lesser extent *Psophocarpus* have both a prominant basal portion of the style, while *Otoptera* possesses a unique style form with a prominent basal portion and a prominent curved apical portion joined by a less prominent central section.

At first it might appear that *Otoptera* is closest to the spatulate type, but its style is quite different from *Sphenostylis* and *Nesphostylis*. It does in the basal and central portions resemble *Psophocarpus*, but in the apical section *Otoptera* is unique, and fits none of Baudet's types. The position of the genus *Psophocarpus* is complicated by the presence of two distinct stigma-style arrangements within the genus. The three species with a bifid style apex would presumably be placed in a different type to the other species. Baudet's style catagorisation works well for the majority of Phaseolinae genera but for *Otoptera*, *Neorautanenia* and *Psophocarpus* there is no clear position. However, these three genera can be linked together if the apical style region is not taken into consideration as each possesses a swollen basal portion and a relatively narrow central style region. These two shared features are not found in the other closely allied subset genera. Though this may appear strong evidence for linking *Neorautanenia*, *Otoptera* and *Psophocarpus*, these inferences must be treated with caution as the apical portion of the styles in each genus is so distinct.

In a review of the morphology of discoid floral nectaries in the Leguminosae, especially the Phaseoleae, Waddle and Lersten (1973) found differences between genera in size, lobing and vascular presence, but concluded that the diversity and occurrence of discoid nectaries was of little taxonomic use in the Phaseoleae. However, their studies in the Phaseolinae showed that *Psophocarpus palustris* possesses a relatively large discoid nectary, similar in size to *Phaseolus vulgaris*. What is perhaps of more taxonomic interest is that in *Sphenostylis angustifolia* no nectary was recorded as being seen. On re-examination of *Sphenostylis* specimens loaned for the morphological study, it was found that *Sphenostylis* does in fact possess a small discoid nectary. This can be taken to demonstrate the importance of using as large a number of

Figure 7.6: Evolution of Phaseolease styles types (From Baudet, 1977b)



characters as possible to obtain a better impression of the 'natural classification'. Based on the relative size of the discoid nectary *Psophocarpus* seems allied to *Phaseolus* and remote to *Sphenostylis*, which would contradict the patterns of relationships indicated by the majority of other characters.

7.10 Summary of non-morphological evidence

In summarising the non morphological evidence available in the literature which could be used to link *Psophocarpus* with its allies, there is a notable lack of helpful information, even though *P.tetragonolobus* is becoming such an important food crop of the Tropics. This most probably is because as Lackey (1977a,b and 1981) points out, *Psophocarpus* is taxonomically anomolous and is quite distinct from other Phaseoleae genera. The particular non-morphological studies that have been undertaken have generally concentrated on a group of closely related genera and as *Psophocarpus* is isolated it falls into none of these close groupings (e.g. the *Phaseolus-Vigna* complex or *Dolichos* and its allies) and has thus not been extensively studied.

The available phytogeographic data does not point to any genus being particularly closely allied to *Psophocarpus*, as most of the genera shown to be morphologically close to *Psophocarpus* share a similar global distribution. The one main exception to this is *Dysolobium* which is endemic to South-East Asia and so is arguably less likely to share a close relationship with a genus naturally restricted in the wild to Africa.

Based on cytological evidence the only conclusive counts of *Psophocarpus* species are n=9, which clearly isolated *Psophocarpus* from the other Phaseolinae which have counts of n=11 and n=10. The bimodal nature of *P.tetargonolobus* and *P.scandens* chromosomes also isolate them from possible allies in which there is a smooth gradation from long to short chromosomes. However some of the genera that are morphologically closely allied to *Psophocarpus*, such as *Otoptera*, *Dysolobium* and *Sphenostylis*, have either not yet been counted or the reported chromosome counts are questioned by some authors. When cytological information is recorded for these genera, then a clearer picture of the

overall relationships between Phaseolinae genera will enable the cytotaxonomist to establish if *Psophocarpus* is as clearly isolated from other Phaseolinae genera as it now appears to be.

There is no biosystematic evidence available which could be used to ally *Psophocarpus* to other genera, as no systematic attempts have yet been made to cross *Psophocarpus* with related genera, though the cytological evidence suggests that potential inter-generic crosses would be unlikely to succeed.

There has been no complete systematic investigation of Phaseolinae pollen and no workers have attempted to relate *Psophocarpus* pollen to its potential allies. However many Phaseolinae genera, including *Psophocarpus* have been palynologically studied. The little information there is available that may be comparatively interpreted suggests that *Psophocarpus* is most closely linked to *Dysolobium* subgen. *Dysolobium*. Both these taxa lack the usual degree of pollen specialisation which is commonly found in the Phaseolinae, but until the systematic coverage of Phaseolinae is more complete no firm conclusions can be drawn as to which genera pollen is most similar to *Psophocarpus*.

The phytochemical and anatomical inter-generic evidence is limited and could mislead interpretation of possible relationships because of the large proportion of missing data for certain genera. Often the genera which are shown earlier on morphological grounds to be most closely allied to *Psophocarpus* are those where the seed is scarce and so have not been included in investigations. What evidence there is from phytochemical and anatomical sources suggests that *Psophocarpus* is isolated from, and a peripheral member of, the Phaseolinae. Which supports the morphological findings, though the morphological study also indicates that *Otoptera* is likewise isolated and peripheral and will be very interesting when *Otoptera* seed is available and can be comparatively studied with *Psophocarpus*.

As with the phytochemical and anatomical evidence on the basis of seedling characters *Psophocarpus* is relatively isolated from the other Phaseolinae investigated, its closest allies being *Neorautanenia* and certain *Vigna* spp., though as with the other studies missing data

hampers interpretation of results.

The general picture obtained from the literature survey of non-morphological evidence is that *Psophocarpus* is a relatively isolated genus and that there is no one genus which is suggested from the different sources of evidence as being consistently most closely allied to *Psophocarpus*.

The non-morphological information available in the literature for comparing classifications of the nine *Psophocarpus* species is even more limited than for the inter-generic comparison. *Psophocarpus* is only recently receiving taxonomic interest due to the increasing importance of *P.tetragonolobus* as a high protein crop of the Tropics.

Interpretation of the phyto-geographical evidence is complicated by the presence of one species, *P.tetragonolobus* restricted to Asia, while the other eight species are all endemic to Tropical Africa. However as *P.tetragonolobus* is not naturally found in the wild and is probably an example of transdomestication from Africa, its distribution is not helpful within the context of *Psophocarpus'* phyto-geography. Of the other species, the two most closely allied species, *P.palustris* and *P.scandens* are found to have juxtoposing distributions with intermediate forms located along the interface of the two species' distributions. The distribution of *P.grandiflorus* overlaps with *P.scandens* but the former prefers a much higher altitude environment. Of the three species with bificate style apices, two, *P.obovalis* and *P.lecomtei*, are located sympatrically but the third, *P.monophyllus* is located at a distance in Western Africa, which is puzzling due to the close morphological similarity between *P.lecomtei* and *P.monophyllus*. Of the two subgenus *Vignopsis* species, both *P.lukafuensis* and *P.lancifolius* have overlapping populations in Zaire, though *P.lukafuensis* is currently much more restricted in habitat and distribution than *P.lancifolius*.

Cytologically the only two *Psophocarpus* species with confirmed chromosome counts are *P.tetragonolobus* and *P.scandens*; both have n=9 and distinctive bimodal chromosomes (6 short and 12 long). There are differences between the two species in positioning of the centromere in the long chromosomes, but the short chromosomes appear identical. Of

the other species there is a reported count of n=11 for *P.palustris* and a provisional count of n=11 for *P.grandiflorus*. It should be stressed that both these counts require verification, but if verified they will prove very interesting in an intra-generic context. It would be taken to indicate that *P.palustris* and *P.grandiflorus* are closer than *P.scandens* and *P.tetragonolobus* to the *Psophocarpus* ancestors which were almost certainly n=11. It would support the present study's morphological findings of links between *P.tetragonolobus* and *P.scandens*, but would contradict the obvious close morphological relationship between *P.palustris* and *P.scandens*.

There has been no successful hybridisations between *Psophocarpus* speices, and the current cytological evidence available does not point to success in the future. However, based on morphological findings and the fact that intermediates are already found in the wild, it seems most likely that *P.palustris* and *P.scandens* are potential crossable species, though if n=11 is confirmed for *P.palustris* this potential would be more remote.

Based on palynological evidence three species appear relatively remote from the others and from each other: *P.tetragonolobus*, *P.grandiflorus* and *P.lancifolius*. The other species fall into two groups, the first contains the closely palynologically allied *P.palustris* and *P.scandens*, with *P.lukafuensis* more distant. The second contains another pair of closely allied species, *P.lecomtei* and *P.monophyllus* with the more remote *P.obovalis*. These groupings do agree in part with morphological findings. It might perhaps have been expected that *P.tetragonolobus* and possibly *P.grandiflorus* would be found closer to the first group of three species, but the fact that *P.tetragonolobus* has been cultivated for such a long time may explain its relative pollen specialisation.

The final major source of intra-generic evidence discussed is phyto-chemical and the results of seed amino acid analysis, however, do not concur with the morphological groupings. The chemical analysis results indicate *P.grandiflorus* and *P.palustris* to be separate and distinct from each other and from the grouping of *P.tetragonolobus*, *P.scandens* and *P.lancifolius*. In comparison with the other forms of

evidence it is most surprising that *P.lancifolius* is allied with *P.tetragonolobus* and *P.scandens*. *P.lancifolius* has been consistently indicated as being distinct from the other *Psophocarpus* species and has not shown a special allegiance with these two species. *P.palustris*, to reflect morphological findings, would be expected closer to *P.scandens*, but the isolation of *P.grandiflorus* is not surprising because as with other sources of evidence it was found in this study to be distanced from the other *Psophocarpus* species.

CHAPTER EIGHT

PROPOSED CLASSIFICATIONS AND DISCUSSION

8.1 Introduction

In completing this thesis the word 'conclusion' has been deliberately avoided as this final chapter is concluding but not conclusive, it is the closing chapter of this thesis but not the closing chapter of *Psophocarpus* taxonimic research. It is surely the dynamic nature of taxonomy that sets it apart from other branches of biology and makes it for us so intellectually exciting. There are no truisms in taxonomy.

What a taxonomic study such as this does produce, however, is a set of answers to taxonomic problems which are hopefully closer to the truth, or to put it in another way, a better approximation to the intrinsic 'natural' classification. In this concluding chapter the primary aim is to propose classifications for the relationship between the genus *Psophocarpus* and its close allies, and for species within *Psophocarpus*. These classifications were derived from the results obtained by phenetic and phylogenetic studies of morphological data and considering information available from the literature.

The chapter's secondary aims are: to discuss the proposed classification in the light of the views of other authors, to discuss the characters and methods used in the investigation, and to suggest areas for future research.

8.2 Proposed Classification

Both Lackey (1977b) and Baudet (1977b) are circumspect when introducing their revisions of the Phaseoleae in their respective theses. Lackey (1977b) provides an extended quotation from Barneby (1964) who points out the ease of criticising the excesses of one's predecessors but the difficulty in providing a better replacement. Lackey's quotation from Barneby (1964) concludes:

"The professed aim of the taxonomist is often called a "natural" system, but the system can never be more than a formal reflection of the truth, standing in much the same relation to truth as a two-dimensional picture stands to the phenomenon of a three- or four-dimensional reality."

Whilst Baudet (1977b) stresses the phylogenetic foundation to his classification, he is even more self-deprecating about his classification's predictive power. He comments:

"Au sein de chaque groupe, L'ordre des genres est relativement arbitrarire' les relations ne sont evidemment pas simplement lineaires, et une sequence ordonnee serait purement illusoire, dans l'etat actual de nos connaissances."

While agreeing at least in part with the sentiments expressed by Lackey (1977b) and Baudet (1977b) it must surely be true that if taxonomy is recognised as being a truly dynamic science, then each revision although only a crude adumbration of the abstract 'natural' classification, is still preferable to no revision at all. If for instance, Baudet's listing of genera is "relativement arbitraire" then is it only by coincidence that the closest ally to *Psophocarpus* as shown in this study to be *Otoptera* is predicted correctly?

The classifications here proposed are not conclusive in the sense of mirroring the natural classification, but are attempts to communicate the relationships between the taxa being studied as accurately as possible given the current evidence available.

8.2.1 Proposed classifications of *Psophocarpus* and its close allies

In considering the results of the phenetic and cladistic analyses of the inter-generic studies (detailed in Chapters 5 and 6) it is apparent that the results of both techniques cannot be married to form one general classification of *Psophocarpus* and its close allies, for this reason the two classifications are presented in Figures 8.1 and 8.2, from consideration of the phenetic and phylogenetic analysis respectivly.

Figure 8.1: Proposed phenetic classification of *Psophocarpus* and its close allies

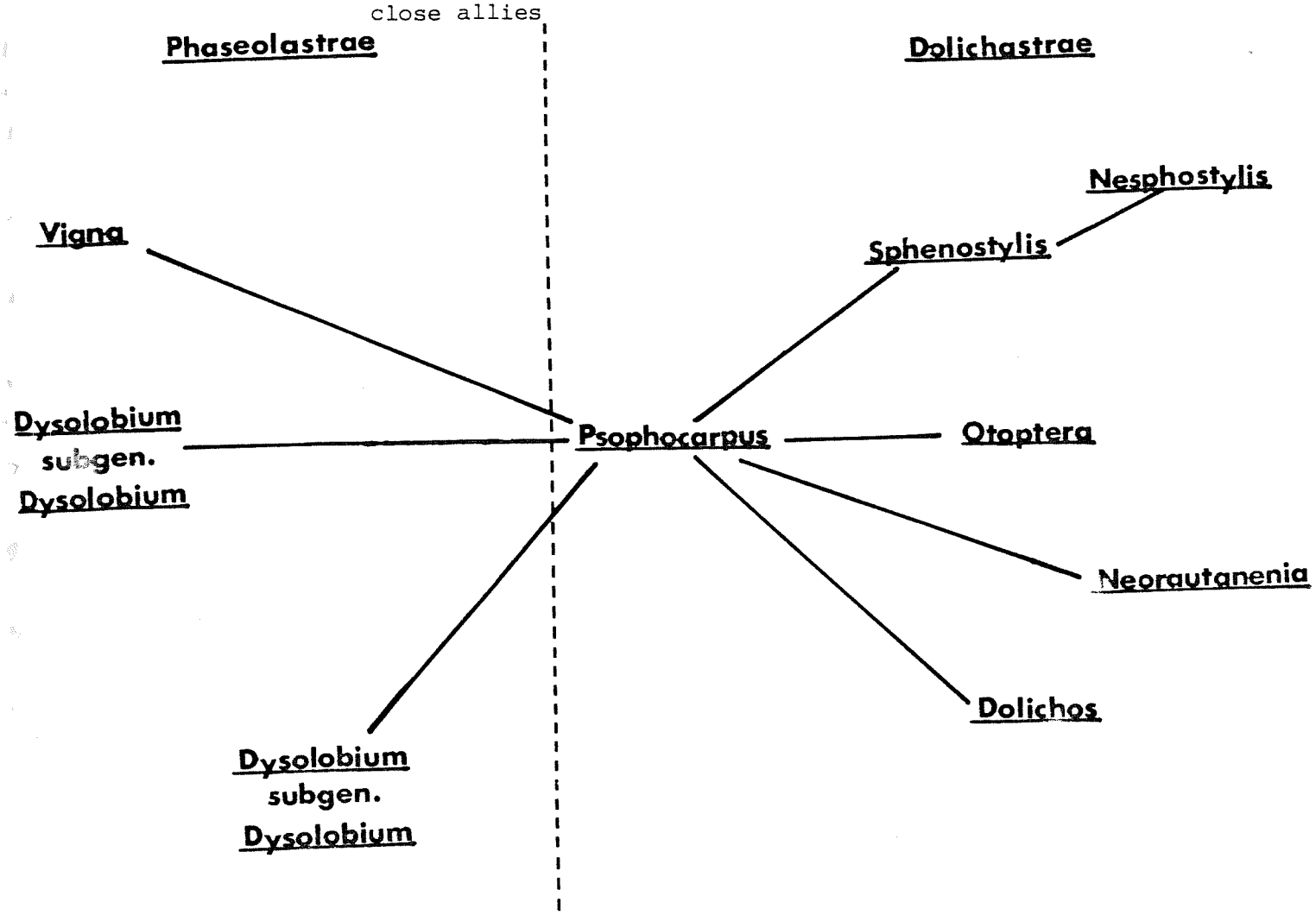
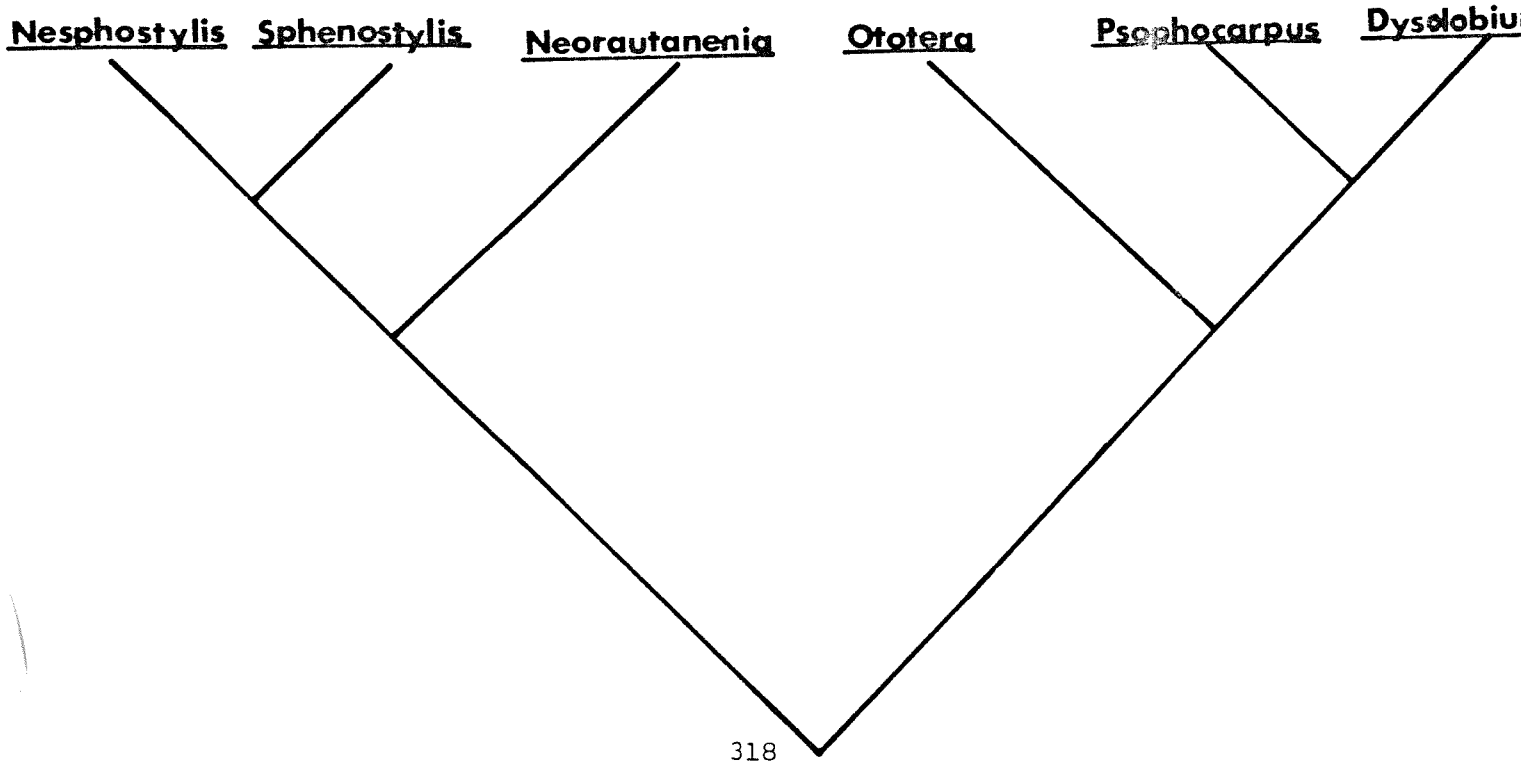


Figure 8.2: Proposed cladistic classification of *Psophocarpus* and its close allies



The reason for the incompatability of the two classifications results for the groupings of taxa is due to the difference in the phentic and cladistic methods of analysis. The phenetic classifiction shows the division of the genera into the two supergeneric groups proposed by Baudet and Marechal (1976). Of these two groups the *Dolichastrae* genera are mostly closer to *Psophocarpus* than the *Phaseolastrae* genera. The closest allied genus to *Psophocarpus* is clearly *Otoptera*. Of the other *Dolichastrae* genera studied, *Sphenostylis* and *Dolichos* follow next in similarity and then slightly more remotely *Neorautanenia*, though these latter three genera are virtually equidistant from *Psophocarpus* and so placing one of these genera closer than the other two is a subjective judgement. One of the *Dolichastrae* genera, *Nesphostylis*, has no direct link with *Psophocarpus* in the proposed classification, the reason being that *Nesphostylis* displayed no close phenetic links with *Psophocarpus*, thus in the classification it is linked to its close ally *Sphenostylis*.

Of the second grouping of *Psophocarpus* allies, the genera from the *Phaseolastrae*, *Dysolobium* subgen. *Dysolobium* is closest to *Psophocarpus*, but it is still more remote than the *Dolichastrae* genera directly linked to *Psophocarpus*. *Dysolobium* subgen. *Dolichovigna* was indicated as being intermediate between *Dysolobium* (sensu stricta) and *Vigna*. Both *Dysolobium* subgen. *Dolichovigna* and *Vigna* were the most remote of the phenetically closely allied genera.

It is difficult to display in the two-dimensional drawing of the phenetic classification the degree of isolation which *Psophocarpus* shows as a genus from all the other genera investigated. It was also shown that once *Otoptera* clusters with *Psophocarpus* these two genera were together isolated from the other genera. This degree of isolation is the reason why *Psophocarpus* and to a lesser extent *Otoptera* have been so difficult to place accurately in classifications of the Phaseoleae.

For the cladistic analysis both the larger genera in the inter-generic subset analysis were excluded, because the full range of intra-generic variation in both *Vigna* and *Dolichos* was not fully recorded, so there would have been problems in determining synopomorphic characters, in the phenetic analysis *Dysolobium* was split into

subgenera, because the genus was phentically analysed using OTU's to represent each species recorded by Marechal et al (1978a). For the cladistic analysis the genus was not represented by OTU's, it was analysed as an entire unit and so, as Marechal et al's circumscription of *Dysolobium* was followed for the cladistic analysis, the genus was not subdivided.

The proposed cladistic classification of *Psophocarpus* and its allies (see Figure 8.2) shows an initial splitting of the six genera into two groups of three genera: *Psophocarpus*, *Dysolobium* and *Otoptera*' *Sphenostylis*, *Nesphostylis* and *Neorautanenia*. Of the first grouping, of three genera. *Otoptera* is shown to be the most remote genus, while *Psophocarpus* and *Dysolobium* are shown to be close phylogenetic allies with five synopomorphies underlining the closeness of their relationship. In the second grouping of three genera, *Neorautanenia* is most remote from the phylogenetically (and phenetically) closely allied *S phenostylis* and *Nesphostylis*.

8.2.2 Proposed classification of Psophocarpus species

Unlike the inter-generic analysis results the phenetic and cladistic analysis results of *Psophocarpus* species were analogous enough to allow one general classification to be produced; the classification is drawn in Figure 8.3.

The nine species of *Psophocarpus* are divided into two subgenera, subgen. *Psophocarpus* and subgen. *Bifidstylus*. Subgenera *Psophocarpus* contains two sections, of which the first contains four species. This first section, *Psophocarpus*, contains the closely allied, ,but clearly distinct *P.palustris* and *P.scandens*. *P.tetragonolobus* is allied to these two species with which it shares a stigma-style arrangement, though *P.tetragonolobus* is more closely allied to *P.scandens* than to *P.palustris*. The remote member of this section is *P.grandiflorus*, which appears closer to *P.scandens* and *P.palustris* than to *P.tetragonolobus*. *P.grandiflorus* has a different stigma-style arrangement to the other three species of the section, as well as having distinctive characters relating to flower part size and shape.

Figure 8.3: Proposed classification of *Psophocarpus* species

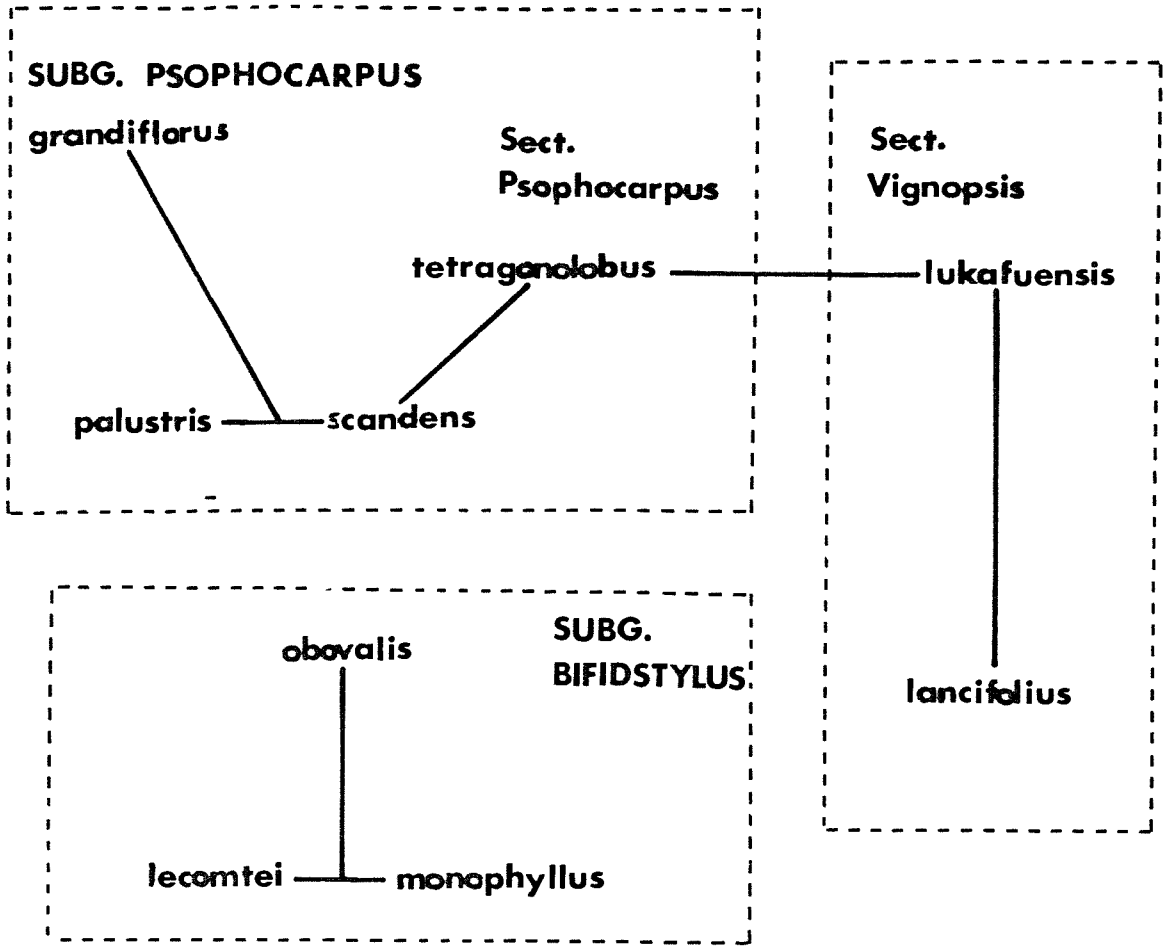
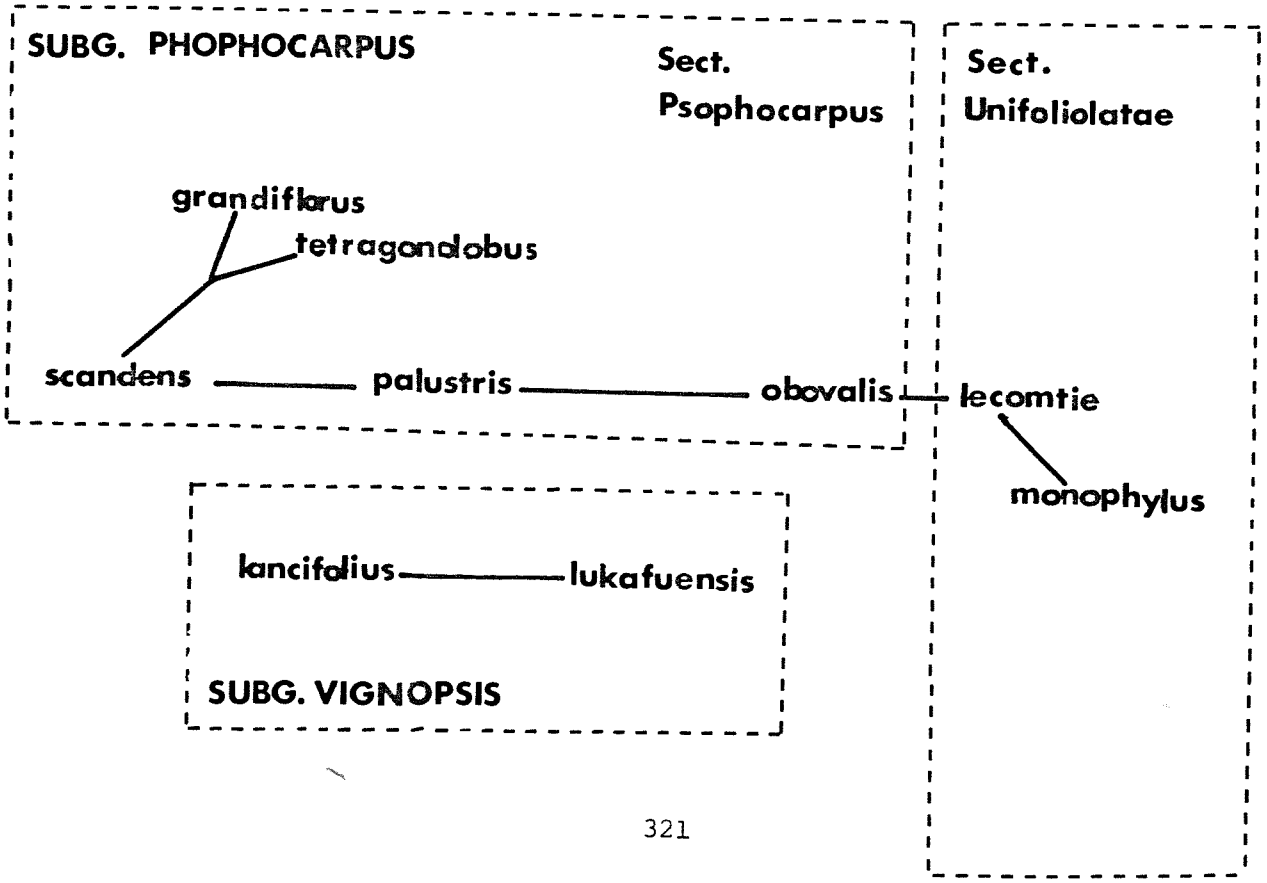


Figure 8.4: Classification of *Psophocarpus* species (From Verdcourt and Halliday, 1978)



The second section of subgen. *Psophocarpus* is section *Vignopsis*, which contains the two species *P.lukafuensis* and *P.lancifolius*. These species are linked by the shared possession of a terminal stigma and hairs positioned a short distance below the style apex, though in other than these characters the species are quite distinct. *P.lukafuensis* was indicated in the phenetic analysis particularly as having links with *P.tetragonolobus*, though this linkage seems to be based on overall similarity rather than clear single characters. The second species *P.lancifolius* is distinct from all the other *Psophocarpus* species in possessing long brown hairs, a slightly different stigma-style arrangement to *P.lukafuensis*, and a unique style hair position just below the style apex but not forming a complete ring around the style as is found in *P.lukafuensis*.

The second *Psophocarpus* subgenus contains three species, *P.obovalis*, *P.monophyllus* and *P.lecomtei*, which cluster together on the basis of their shared unequal bifid style apex. Within subgen. *Bifidstylus* the species divides into two, the closely allied unifoliate species, *P.monophyllus* and *P.lecomtei* and the trifoliate remote member of the subgenus *P.obovalis*. The difference in leaflet number is an important distinction between these three species, but it seems reasonable to suggest that the shared stigma-style arrangement as well as the other characters which correlate with it (see Chapter 6) over-weigh the leaflet number difference and suggest that the subgenus forms a natural grouping. It could be argued that the difference in leaflet number may warrant the splitting of the subgenus into two sections, but as the subgenus contains only three species this has not been suggested here to avoid over-division of what is a relatively small genus.

8.3 Discussion of Proposed Phenetic Classifications

The discussion of the classifications presented in the previous section will be divided into two as follows:

8.3.1 Discussion of proposed inter-generic classification

To enable comparison of the phentic classification here presented with those of other recent taxonomists, Table 8.1 summarises the

Table 8.1 Comparative Placements of Psophocarpus with Its Close Allies

Hutchinson (1964)	Baudet (1978)	Lackey (1981)	Proposed Placement
Phaseoleae	Glycinanae–*Glycinastrae*	Phaseolinae	
	Neorautanenia		Vigna
Vigna	33	Dysolobium	Dysolobium
3[B]		Psophocarpus	Psophocarpus
Otoptera	Phaseolinae–*Phaseolinae*	4	Otoptera
Sphenostylis	Vigna	Otoptera	Sphenostylis
3	6	Sphenostylis	Nesphostylis
Neorautanenia	Dysolobium	Nesphostylis	Dolichos
Dolichos	10	1	Neorautanenia
2		Neorautanenia	
Psophocarpus	Phaseolinae–*Dolichastrae*	3	
3	Dolichos	Dolichos	
Dysolobium	1	1	
	Psophocarpus	Vigna	
	Otoptera		
	1		
	Sphenostylis		
	Nesphostylis		

A. For each author the closely allied genera of *Psophocarpus*, as indicated by the phenetic study, are abstracted from the author's classifications for comparison. For full classifications see Chapter 2.

B. Numbers to the left of genera refer to the number of intervening genera included by the author in his full classification.

classifications of *Psophocarpus* and its allies for: Hutchinson (1964), Baudet (1978), Lackey (1981); as well as for the phenetic classification proposed here. In each case for the other author's classifications the genera shown in Chapter 5 to be most closely allied to *Psophocarpus* are abstracted from their complete classification to allow easy visual comparison.

Of the three 'abstracted' classifications, Lackey (1981) provides the most similar generic arrangement to that proposed in this thesis, with only *Vigna* being significantly altered in position. Baudet (1978) divides the close *Psophocarpus* allies into two sub-tribes and three supergenera and so it can be inferred does not consider these genera to be at all closely allied phenetically. However, if the genera within his *Dolichastrae* are considered alone then he does predict the allegiance of *Psophocarpus* and *Otoptera* by juxtaposing them in his classification, whereas Lackey (1981) has four intervening genera between *Psophocarpus* and *Otoptera* in his classification. Baudet also within the *Dolichastrae* predicts the closeness of *Psophocarpus* to *Dolichos* and *Sphenostylis*.

It is, however, the splitting of the *Psophocarpus* allies into three supergenera with a large number of intervening genera between them, which questions the validity of Baudet's classification. In comparing his classification with others, it will be compared to phenetically based classifications, which is unfair as Baudet (1977b, 1978) stresses the phylogenetic nature of his classification. This is reflected in Baudet and Marechal's (1976) splitting of the Phaseolinae into supergenera based on one character, style hair position. This is undoubtedly a character of great phylogenetic importance in the Phaseolinae, but it could be argued by a pheneticist that it produces an 'artificial' classificaiton. Baudet's classification must thus be regarded as a special purpose classification, reflecting evolutionary development rather than phenetic resemblence. It should be noted, however, that within the Phaseolinae supergenera the genera are arranged in phenetic suquence not according to further phylogenetic characters, as indicated by the resemblence of the proposed classification to Baudet's Dolichastrae.

The earliest classification to which the one here proposed is compar ed is that of Hutchinson (1964), whose arrangement of *Psophocarpus* allies is quite different from that proposed. Hutchinson does not predict the closeness of *Otoptera* and *Sphenostylis* to *Psophocarpus*, but indicates *Dysolobium* and *Dolichos* to be more probable allies.

In discussing the comparative classification of *Psophocarpus* and its allies, the discussion has necessarily concentrated on the proposed phenetic classification, as there is no other cladistic classification available for comparison with that proposed, comparison of phenetic against phylogenetic classification not being possible as previously explained. It is hoped that the cladistic classification here suggested will provide a basis for a better understanding of the evolutionary relationships between *Psophocarpus* and its allies.

8.3.2 Discussion of proposed intra-generic classification

The proposed classification of *Psophocarpus* species will be discussed in comparison with the only previous classification of *Psophocarpus* species suggested by Verdcourt and Halliday (1978), which is presented in Figure 8.4.

The genus *Psophocarpus* contains nine species, the question over the status of the *P.palustris-P.scandens* complex being clarified above. Verdcourt (1968) proposed the splitting of the distinctive West African population from the other populations referred to *P.palustris*, the West African population being designated as true *P.palustris*, and the other populations being referred to *P.scandens*. Westphal (1974) questioned this distinction and believed it premature, but Verdcourt and Halliday (1978) provide further evidence to support the separation of the two species. The findings of the present investigations support Verdcourt's view about the distinct nature of these two species.

Both the *Psophocarpus* classification proposed and the classification suggested by Verdcourt and Halliday (1978) divide *Psophocarpus* into two subgenera, one of which (subgen. *Psophocarpus*) is further divided into two sections. Section *Psophocarpus* of subgen. *Psophocarpus* in either

classification contains the four species: *P.grandiflorus*, *P.tetragonolobus*, *P.palustris* and *P.scandens*. However, beyond this level, the similarities between the classifications begins to break down.

Verdcourt and Halliday (1978) include a fifth species, *P.obovalis* in section *Psophocarpus*, which in the present study is consistently linked with their section *unifoliolatae* species, *P.monophyllus* and *P.lecomtei*. *P.obovalis* shares the distinctive stigma-style arrangement of an unequal bifid style apex and terminal sigma, with *P.monophyllus* and *P.lecomtei*, as well as the following less taxonomically important characters: mucronate leaflet apex, prominence of veins of the adaxial surface of the leaflet, wing shape (see Figure 6.1), keel shape (see Figure 6.2), complete ovary pubescence and legume pubescence. Although Verdcourt and Halliday do consider these three species to be linked (see Figure 8.4), they place *P.obovalis* in section *Psophocarpus* with the other trifolioliate subgen. *Psophocarpus* species, and separate the two unifolioliate species *P.monophyllus* and *P.lecomtei* into their section *Unifoliolatae*. The results of the analyses (both phenetic and phylogenetic) based on the distinctive stigma-style arrangement with other correlated characters warrant separation of these three species, into a separate subgenus from the other *Psophocarpus* species. The distinct stigma-style arrangement with the correlated characters is considered more important than the difference in leaflet number. Several Phaseoleae genera contain a small number of unifoliolate species; this evolutionary development appears to have arisen separately in the different genera as it does not correlate with other characters, and thus is not considered of major taxonomic importance.

The other major difference between Verdcourt and Halliday's classification and that presented here refers to the status of their subgen. *Vignopsis*, here it is treated as a second section of subgen. *Psophocarpus*. Verdcourt and Halliday divide the genus as follows:

> "The genus can be divided into two subgenera, subgen: *Psophocarpus* with the stigma terminal or internal but with hairs to the tip of the style, and subgen. *Vignopsis* with the stigma terminal and hairs limited to a ring some distance below the style tip."

The two species included in subgen. *Vignopsis* are *P.lukafuensis* and *P.lancifolius*. While both do possess hairs some short distance below the style tip, the actual ring of hairs is much more prominent in *P.lukafuensis* whereas in *P.lancifolius* it may be incomplete or is at least more prominent on the lower surface of the style. Having observed numerous live specimens of *P.tetragonolobus* and *P.scandens* for hybridisation experimnents (and from herbariuim studies of *P.palustris*) it became apparent in these species that the hairs form a ring around the apical stigma. As the hairs closely surround the apex this is difficult to see in herbarium material. Thus it would appear that the difference between *P.tetragonolobus*, *P.scandens* and *P.palustris* on the one hand and *P.lukafuensis* and *P.lancifolius* is a matter of distance of the hairs below the apex. This indicates that Verdcourt and Halliday's section *Psophocarpus* is closer to their subgen. *Vignopsis* than their section *Unifoliolatae*, the latter having the quite distinct unequal bifid style apex. Thus in the proposed classification the position of Verdcourt and Halliday's section *Unifoliolatae* and subgen. *Vignopsis* are reversed. This reversal of position is also clearly supported by other correlated characters as shown in the phenetic and phylogenetic analyses.

Within the proposed section *Vignopsis*, *P.lukafuensis* and *P.lancifolius* are quite distinct. Not only is there a difference in degree of ringing of the style by hairs, but *P.lancifolius* is a more distinctly pubescent species with long brown hairs scattered over its indumentum. The style and petal shapes also differ between the two species. In both the phenetic and phylogenetic analysis *P.lukafuensis* is shown to be allied to *P.tetragonolobus*, while *P.lancifolius* is considered remote from the other eight species, but as these two species both possess hairs a short distance below the style apex, this does justifies their inclusion in the same section.

8.4 Discussion of Phenetic Characters

It would be very helpful in discussing the phenetic characters used in the analysis if each character's taxonomic discriminating power could have been objectively assessed, so that the characters which proved most useful in the investigations could be emphasised for the benefit of

future workers. However no objective method of character selection was used, though obviously if a character was used in the analysis then it may be taken that the character was considered to be of taxonomic use.

The character analysis program CHARANAL was used in an attempt to make character selection more objective. Unfortunately, CHARANAL was used only with the subset inter-generic study and even then the results needed interpretation with biological insight. Experience suggests that if CHARANAL is to be used, then the characters originally chosen must not have a large number of character states, and a detailed record of those character states not used must be recorded. The scope of this project (large numbers of: characters, character states, OTU's and subsequent data) made the use of CHARANAL more difficult.

Another source of information that could have aided the objective choice of characters was the character plots produced by DECORANA, which might have indicated the taxoniomic usefulness of the characters used in the studies. The character plots for the subset inter-generic study using 66 and 43 characters, for the intra-generic study and for the *P.palustris - P.scandens* complex study are drawn in figures 8.5, 8.6, 8.7 and 8.8 respectively.

If the DECORANA OTU plots are compared to the character plots (see Section 5.3.4, 5.4.4 and 5.5.4) there are two differences. Firstly, the OTU plots contain only positively positioned OTU's, while in the character plots the characters may be positioned positively or negatively. Secondly, there is a large difference in the scaling of the axis, for each plot the OTU's are located within a more restricted area than the characters. Both these factors make it difficult to relate the two types of plot. It was expected that when th OTU plots were superimposed on the characters plots that characters located in the same position as OTU's would be useful in discriminating those OTU's, but this was not found to be true. For example, it would be expected that the *Phaseolus* OTU's would have a close proximity to the character style spiraling, but this was not found to occur, the *Phaseolus* OTU's were closely placed to character 2, presence-absence of stipule spur, which has no obvious, particular relationship with *Phaseolus* OTU's.

Figure 8.5: Intensive subset inter-generic study (66) - DECORANA - character plot

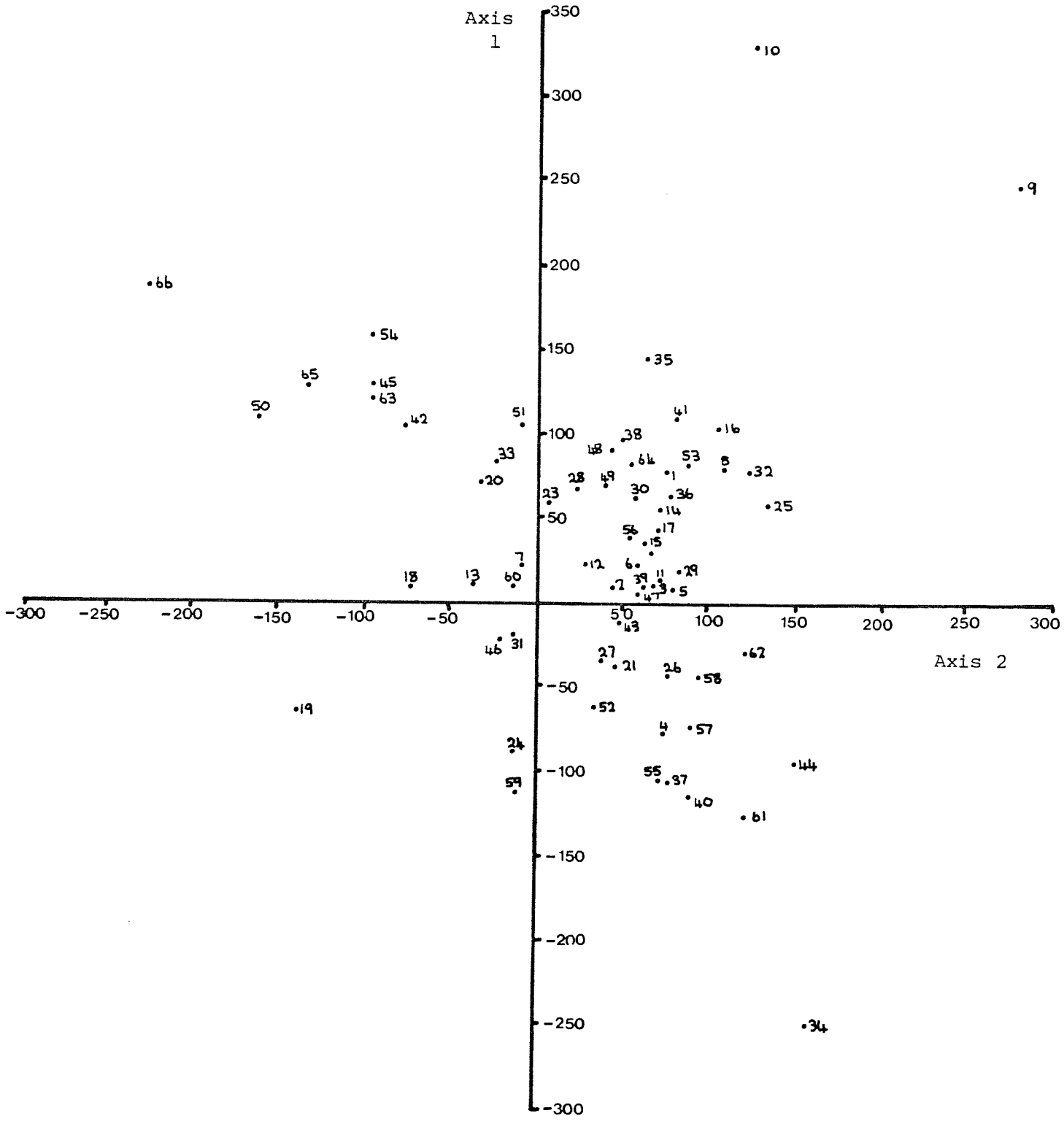


Figure 8.6: Intensive subset inter-generic study (43) - DECORANA - character plot

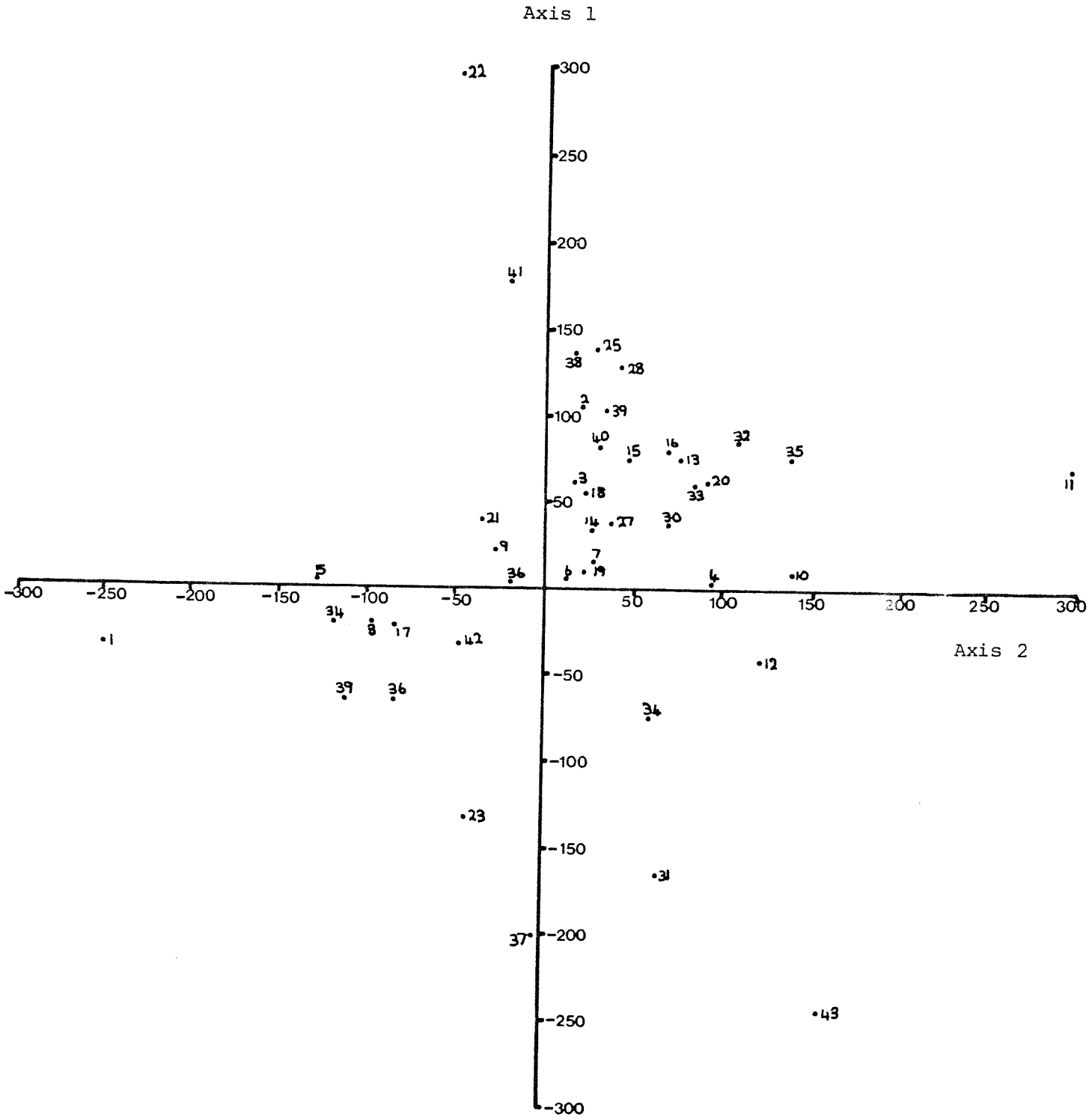


Figure 8.7: Intra-generic study (76) - DECORANA - character plot

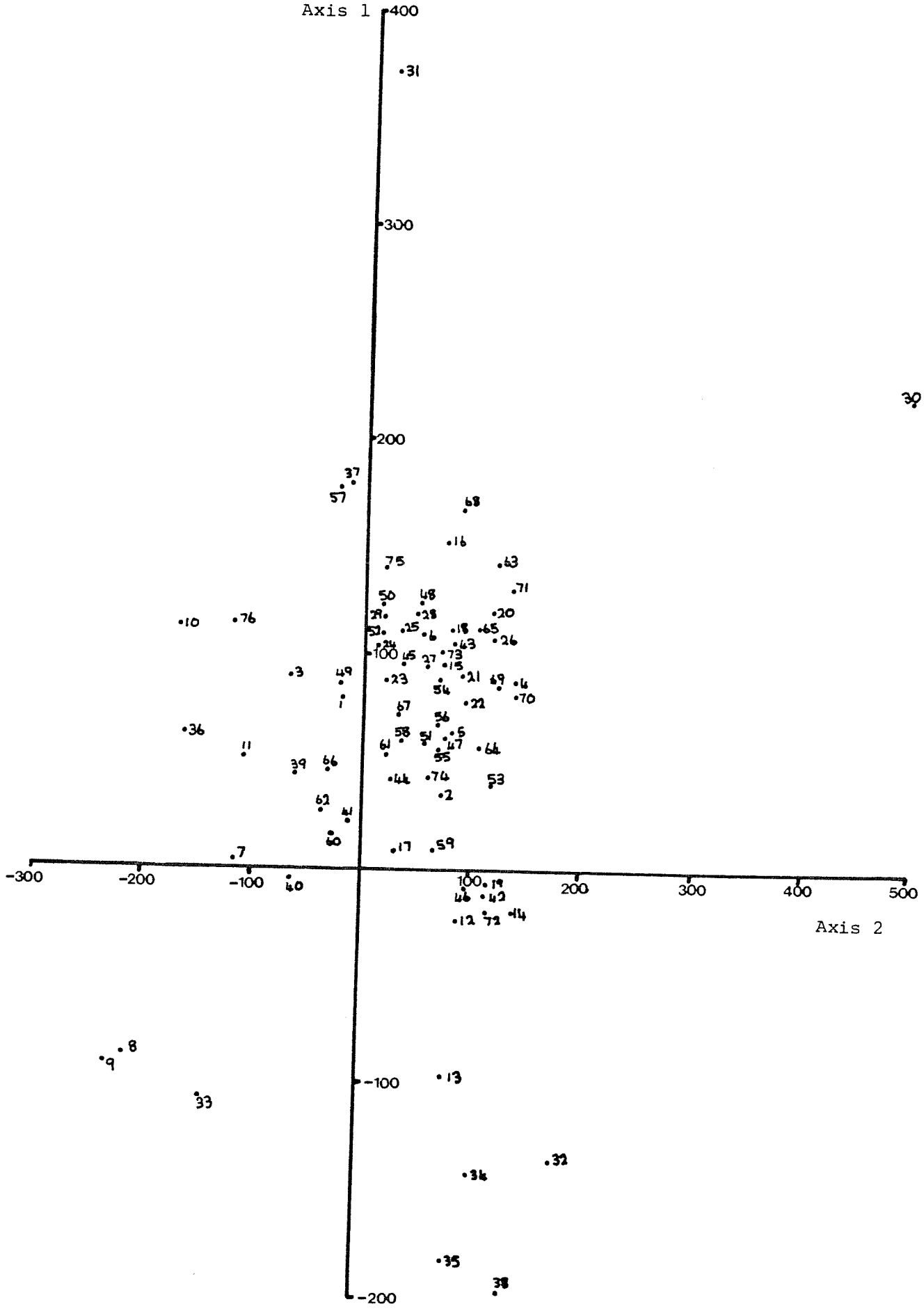
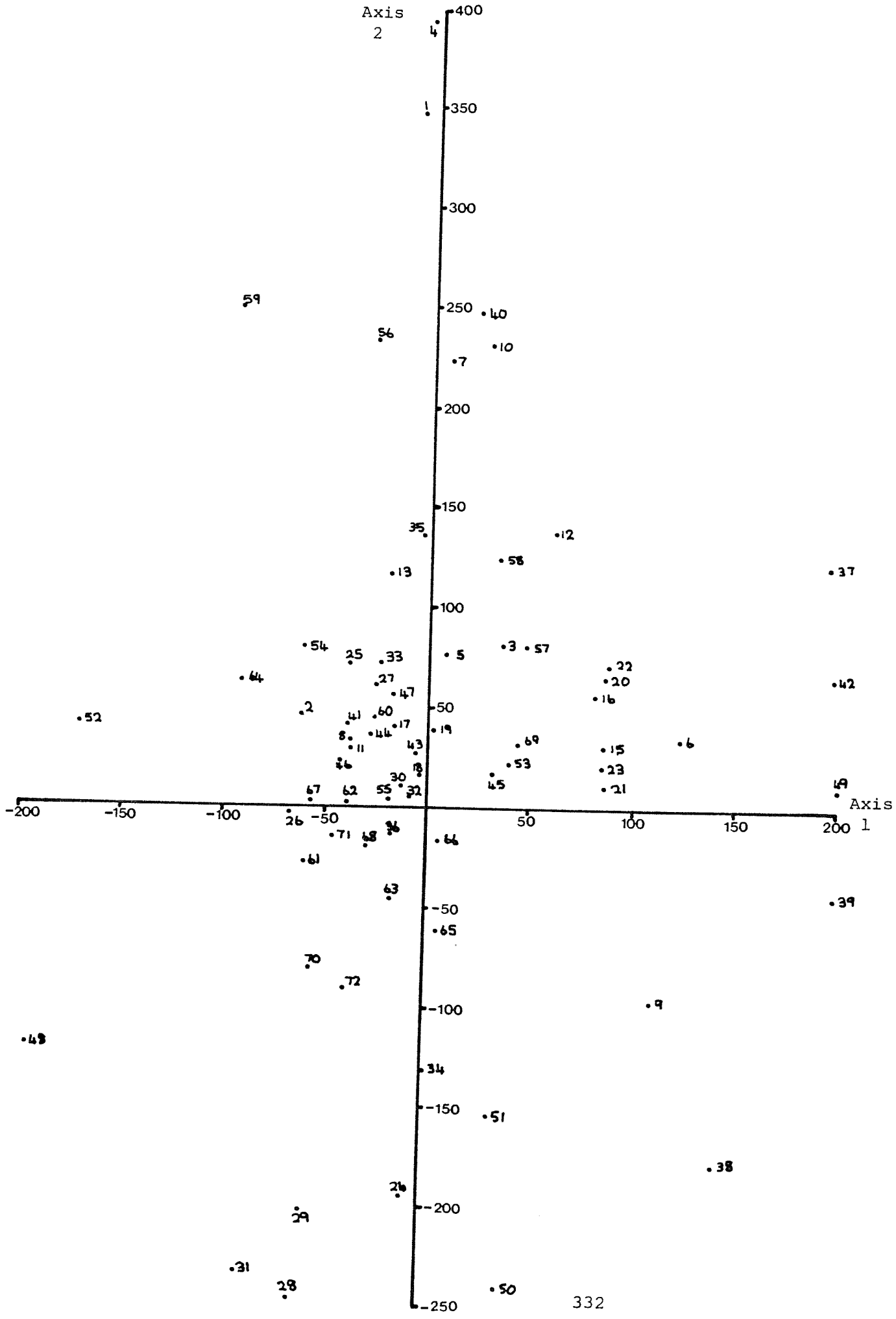


Figure 8.8: *Psophocarpus palustris* - *P. scandens* Complex study (72) - DECORANA - character plot



If the relationship between the characters themselves is interpretated then it might be expected that those characters placed most remote from the bulk of the characters would be of least importance in discriminating taxa. For example, characters 9, 10, 34 and 66 in Figure 8.5 would be expected to be of little importance, these characters were: 9, number of nodes/inflorescnece: 10, distance from basal to next node: 34, keel shape and 66, style apex shape. While with 'taxonomic judgement' it may be accepted that the characters 9 and 10 may be of little taxonomic use in discriminating taxa, characters 34 and 66 are clearly 'good' characters.

The difficulty in interpreting the character plots is difficult to explain. The DECORANA manual (Hill, 1979) provides no details on interpretation of the plots, however in this project it was assumed that a program written for specifically ecological data may be used in a systematic context. Hill (1979) does warn that assumptions about the distribution of the data are made and if the data does not follow these assumptions then the results may be unreliable. Though no problem was encountered with the OTU plots, this may explain the difficulty in interpreting the character plots. The fact that the OTU plots broadly reflect groupings of taxa found by the other methods of analyses used justifies the use of DECORANA in this project.

The provenance of characters used in the analysis is of interest, Table 8.1 details the proportions of characters taken from different 'organs' in the gross character set and those used in the inter and intra-generic analysis. To aid comparison the figures are also presented in Table 8.2 for the character sets used by Marechal et al (1978a). The basic difference between those characters selected for inter and intra generic use is the higher proportion of floral and ovary characters in the former, and adult vegetative characters in the latter. It is well established that floral characters are of more use in generic demarcation, Tutin (1956) comments on the construction of genera that the taxonomist,

"knew from experience that reproductive parts vary much less than vegetative ones so that, having mentally formed their genus largely

Table 8.2 Analysis of Morphological Character Provenance

	Present Investigation				Marechal et al	
	Gross Char. Set %	Inter-Generic 102 %	51 %	Intra Generic 97 %	Gross Char. Set %	Facteur de Ponderation
Adult vegetative	31.7	6.9	5.9	30.9	18.9	8.3
Inflore-scence	18.4	11.8	5.9	14.4	12.3	27.1
Floral	25.1	36.2	52.9	22.7	30.3	29.2
Ovary	6.9	15.7	19.6	9.3	11.5	22.9
Legume	9.8	14.7	11.8	16.5	9.8	10.4
Seed	6.3	14.7	3.9	6.2	13.1	2.1

A = Character provenance analysis from Marechal, Mascherpa and Stainier (1978a) data.

on general 'look', they searched for some reproductive character to define it."

Leonard (1955) divides generic distinguishing characters into primary and secondary, he refers to primary characters as follows, "les plus importants, resposant sur la structure de la fleur."

For the intra-generic character set it can be seen that the proportions of characters from the different sources remain relatively constant compared to the original character set. From this it may be inferred that characters from all sources are of equal use in delimitating species.

In comparing the provenance of the characters used by Marechal et al (1978a) with those used in the present study, Marechal et al's characters were selected from a wider provenance, while those used in the present studies were largely floral characters. However both studies show that a predominance of floral and ovary characters is of most use in inter-generic comparison. In concluding this discussion of phenetic characters it is relevant to reiterate Marechal et al's concluding comments:

"En conclusion avec les donnees actuellement disporibles pour l'ensemble des taxons envisages, les resultats de l'analyse discriminante ont conduit a une situation tres classique en taxonomie vegetale, ou l'importance des caracteres floraux est nettement dominante."

8.5 Discussion of Phenetic Analysis

The first point to stress, prior to discussing the relative advantages and disadvantages of the methods of analysis used, is the importance of using more than one method of analysis on which to base the classification. This propostion may be validated by comparing a classification produced by any one of the methods of analysis used with the classification presented in this chapter produced after interpreting the results obtained from each method of analysis. in the latter case using multiple analysis techniques enables mutual verification of the

results and means the subsequent classification presented is less biased by the use of the particular method of analysis chosen.

Accepting the importance of using more than one method of analysis, in the present studies, the method of analysis which produced results most similar to those presented in the proposed classifications was LINKAGE. The results of this project concur with those of Bisby (1973), who found that single linkage cluster analysis combined with graph theory (as used in LINKAGE) produces an excellent indication of similarity patterns within and between groups. The results show no indication of chaining (see Section 3.4.2.a), being avoided, by the use of subgraphs, a criticism often made against the use of single linkage cluster analysis.

The average linkage cluster analysis program operated via the CLUSTAN(DISTIN) subroutine produced very similar results in each case to LINKAGE. However, in its tendency to form spherical clusters with the data there may be occasional minor taxa misplacements. The major drawback of this method is that it tends to force intermediate OTU's or plants into major clusters, as seen in the *P.palustris - P.scandens* complex results. The results of this analysis are presented in the form of a dendrogram, which enables easy visual assimulation of the results, but this does not compare with the more detailed result analysis given by drawing the subgraphs provided by LINKAGE.

The least successful method of analysis used was Ward's method of cluster analysis. This method finds minimum variance spherical clusters, but as Sneath and Sokal (1973) point out it may lead to unacceptable partitions, and this was the case in the present study. This method is successful with data that has quite distinct clusters without intermediates, but if the clusters are indistinct or there are intermediates it will still produce discrete clusters and thus lead to misplacement of taxa. At the generic level this led to the separation of the closely allied genera *Sphenostylis* and *Nesphostyolis*, while at the specific level it caused the clustering of *P.lancifolius* with *P.obovalis*. Both these results disagree with the results produced by the other methods of analysis.

In the introduction to the phentic analysis in Chapter 3 the comments from the literature on the usefulness of principal components analysis (PCA) are summarised, the conclusion being that PCA is good at separating clusters but less accurate at assessing intra-cluster relationships; these comments are mirrored in the findings of the present results. The PCA located the major taxa under study and in relating these taxa based clusters to each other, but if there were intermediates present then these were generally forced into clusters rather than taking up separate intermediate positions between the major clusters. The minimum spanning tree (MST) was used in conjunction with PCA to assess the degree of data distortion by the PCA. In each of the component studies it was found that the PCA had distorted intra-cluster relationships to a varying degree, but that between clusters relationships were truly reflected. Thus MST proved very useful in checking the accuracy of the PCA, but for the data analysed showed that the data distortion was not serious enough to affect result interpretation. Cluster circles were also used in conjunction with the PCA and found to be useful in displaying the amount of intra-cluster variation.

The final method of analysis applied to each data set was detrended correspondence analysis (DCA), which is a more sophisticated ordination technique than PCA. However, in the studies analysed the DCA results gave clusters that were not so taxonomical uniform and discrete as the PCA. The DCA had a larger percentage of apparently misplaced OTU's than the PCA, especially at the inter-generic study level. However, having realised this difficulty it was found that DECORANA (the DCA program used) provided the best discrimination of the *P.palustris - P.scandens* complex, forming two specific clusters with the intermediate specimens being located between these clusters in an appropriate intermediate position.

To summarise the discussion of the methods of phenetic analysis used, it was found that all the methods of analysis experimented with were useful, except for Ward's method of cluster analysis, which produced an unacceptable amount of misplaced taxa. It is stressed that no single method of analysis should be used alone, as it provides no

means of result verification, and any bias introduced by any particular technique may be undetected.

8.6 Discussion of Phylogenetic Analysis

In discussing the cladistic relationships between the taxa investigated it is difficult to avoid contrasting the results with those produced by the phenetic analysis. Though both reflect inter-taxa relationships, they function on different premisses as stressed throughout this thesis. However, having underlined the different nature of phenetic and phylogenetic analysis the results broadly concur both at the inter and intra-generic level. There is one major exception, the relationship between *Psophocarpus*, *Dysolobium* and *Otoptera*. The phenetic analysis indicates that *Otoptera* is most closely related to *Psophocarpus*, while the phylogenetic analysis places *Dysolobium* closest to *Psophocarpus*. Of these two hypotheses, there is no right solution as the two forms of analysis are investigating different relationships. So that both Baudet (1978) and Lackey (1977a) are correct in their predictions of the nearest ally of *Psophocarpus*, Baudet predicted the phenetically close *Otoptera* and Lackey the phylogenetically close *Dysolobium*.

Of the actual method of phylogenetic analysis used there are two main criticisms, firstly the method used in selecting apomorphic characters and secondly, the way in which these characters were ascribed character state polarity.

Apomorphic characters were selected from the Gross Character Set detailed in Chapter 4 and were included if they were believed to have mutated from one state to another and were considered taxonomically important in discriminating taxa. In effect this meant that those characters intuitively considered of most phylogenetic use were selected for the analysis. Even though the characters were in this analysis chosen a posterior (following the phenetic analysis and thus familiarisation with the plants) the resultant classification is monothetic, being based on a few characters which were subjectively chosen. One of the advantages of phenetic analysis is that, even though the characters may be selected intuitively, the large number of

characters chosen will reduce the effect of particular character bias in the resultant classification. So the subjective choice of a small number of characters for the cladistic analysis is a serious criticism of the technique used, but this criticism must be balanced against the advantages gained from the possible novel juxtopositons of taxa as a result of cladistic anhalysis.

Secondly, using the cladistic method of analysis employed it is assumed that in ascribing character state polarities that rare character states are derived. This assumption is not necessarily correct, there are many situations in which rare character states may not be derived, see Stevens (1980) for discussion of this point. As the ascription of primitive and derived character states is basic to cladistic analysis, the classifications of taxa being based on synamorphic characters (the taxa sharing certain derived character states), this is a fundamental criticism of the method. However, as with the other main criticism of this method, provided these problems are appreciated then it does not invalidate the method, nested groups of taxa are still distinguishable and are important in attempting an overview of the inter-taxa relationships.

As well as discussing the actual cladistic analysis that was carried out on the taxa under investigation, there were other characters that might prove interesting phylogenetically. However these could not be included in the study because of their ambiguous presence. One such character was wing petal extra ventral tooth. All *Dysolobium* specimens possess the extra wing tooth, both of subgen. *Dysolobium* and subgen. *Dolichovigna* (sensu Marechal et al, 1978a) Plate 8.1 show the wing petal of a *Dysolobium dolichoides* specimen, the extra tooth is arrowed. Plates 8.2 and 8.3 show the wing petals of two specimens from the phenetic and phylogenetically allied genera of *Dysolobium*, *Psophocarpus* and *Otoptera*. Note that both these specimens also possess extra wing teeth equal in prominence to that found in *Dysolobium*. The presence of the extra tooth is rare in both *Psophocarpus* and *Otoptera*, but the fact that the mutation does occur and was not noted for other genera may be taken to indicate the natural allied relationship between the three genera.

PLATE 8.1

Dysolobium dolichoides
Wing petal

Kerr 11/11/23

x10

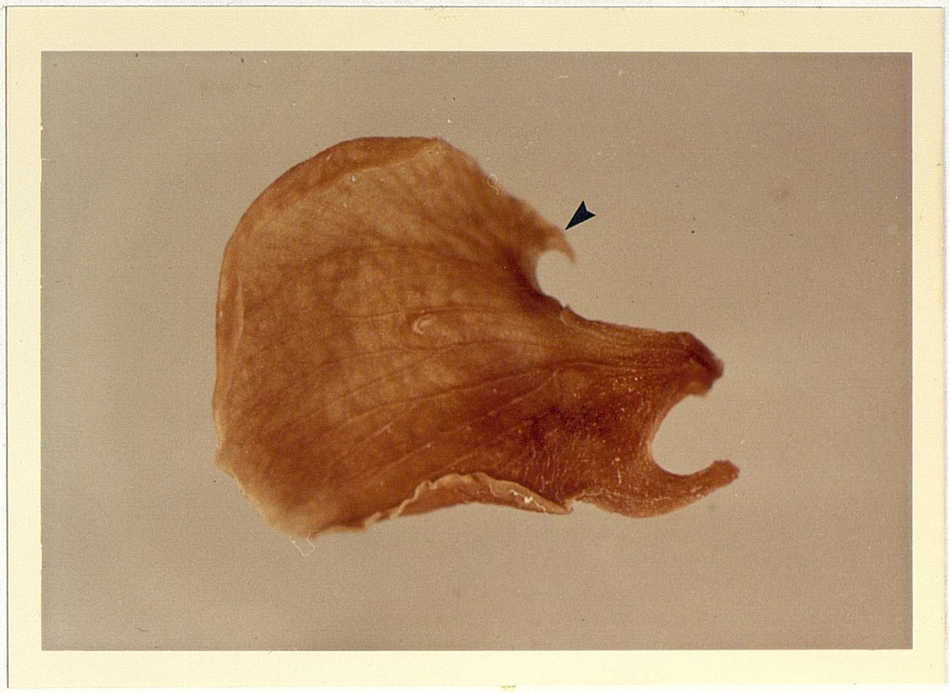


PLATE 8.2

Psophocarpus scandens
Wing petal

Linder 1019

x4.5

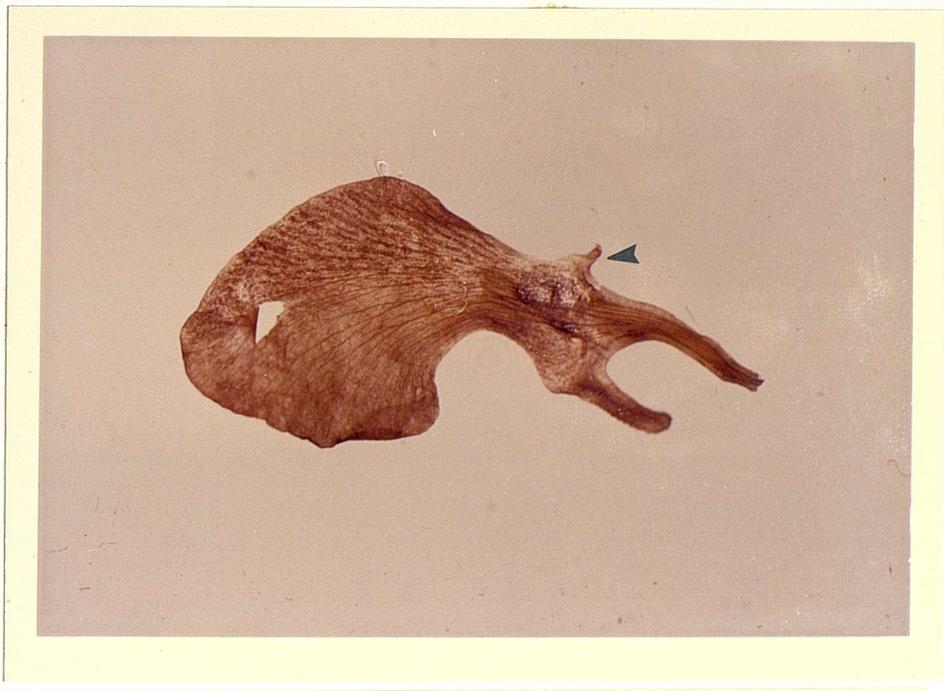
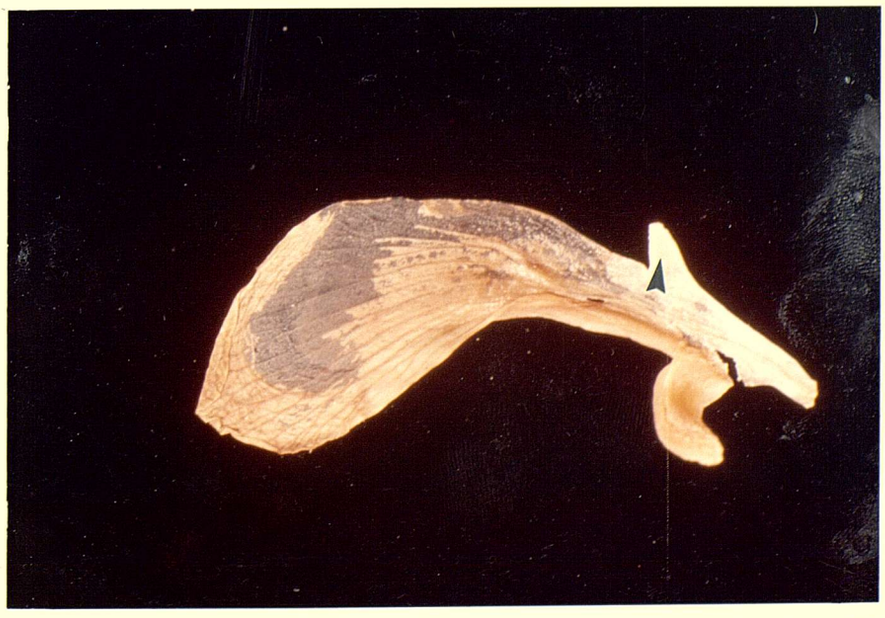


PLATE 8.3

Otoptera burchellii
Wing petal

Seydel 3903

x4



A second character which is of interest in this way is style pubescence, Baudet and Marechal (1976) split the Phaselinae into supergenera on this character alone. Their *Phaseolastrae* possessing a pubescent internal style surface, while their *Dolichastrae* have a glabrous style. Thus *Dysolobium* is placed in the Phaseolastrae and *Psophocarpus* in the *Dolichastrae*, *Dysolobium*, however, possesses a much reduced style beard concentrated near the style apex and so may be considered a somewhat ambiguously placed genus. Style pubescence is a very important character for the Phaseolinae, Plate 8.4 shows the typical *Dolichastrae* and *Psophocarpus* style hair position. Plate 8.6 shows the typical *Phaseolastrae*, but atypical *Dysolobium* hair position, note that in this *Dysolobium pilosum* specimen the beard extends along the style and form a concentrated mass around the apex. The specimen shown in Plate 8.6 is atypical because the beard does not normally extend so far along the style in *Dysolobium*. Note the similarity in style beard between the *Psophocarpus palustris* specimen shown in Plate 8.5 and that of the *Dysolobium* specimen in Plate 8.6. Both show hairs spread along the style but with a concentration at the apex. Obviously there are more hairs in the *Dysolobium* beard, but there are clearly numerous hairs in a similar position on the *P.palustris* specimen, which may be taken as another phylogenetic indicator of the close relationship of the two genera. It may also be noted that there is a more general (i.e. found in many specimens) similarity in style hair arrangements between *Dysolobium pilosum* and *Psophocarpous lancifolius*.

Within *Psophocarpus* itself, *P.tetragonolobus* possesses an intrigueing auto-apomorphic charactger, the T shaped wing spur, shown in Plate 8.7. This T shaped appendage is found in no other Phaseolinae species and it is intrigueing to conjecture its possible evolutionary advantage, possibly to help intermesh petals? The unusual appendage has been noted by Bentham (1959), Backer and Bakhurzen van den Brink (1963) and Westphal (1974), but none of these authors is able to offer a hypothesis to explain its development.

Plate 8.8 shows the standard of a *Psophocarpus palustris* specimen note the unusual base shape with the "clefts" into the standard each side over the auricles. This particular basal shape was found in several specimens of both *P.scandens* and *P.palustris*. It is unknown if

PLATE 8.4
Psophocarpus tetragonolobus
Style
Live specimen
x5

PLATE 8.5
Psophocarpus palustris
Style
Berhaut 1045
x15

PLATE 8.6
Dysolobium pilosum
Style
Haselfoot-Haines 3918
x6

PLATE 8.7

Psophocarpus tetragonolobus

Wing petal

Live specimen

x4

PLATE 8.8

Psophocarpus palustris

Standard petal

Testu 4187

x3

these "clefts" occur as a mutation or are areas of weakness in the standard, which on drying split to form the "clefts", but the fact that the clefts occur in both *P.scandens* and *P.palustris* is yet another indicator that these species are very closely allied.

The final point in this section is an observation rather than a comment on the phylogenetic analysis itself, following on from the discussion of synapomorphic charactes. Verdcourt and Halliday (1978) in their key to *Psophocarpus* species, key out their subgen. *Vignopsis* species as having, among other characters, narrow leaflets, though in the description of *P.lancifolius* they do concede that, "rarely the terminal leaflet almost round". From the present studies observation broad leafleted *P.lancifolius* do not appear to be so rare or restricted to the terminal leaflet. Three specimens (Froment 206, Pringle 6471 and Buchanan 158) were found with all three leaflets broadly rhomboid-ovate; similar to *P.palustris*. This excluded narrow leaflet being used as a synapomorphic character in the cladistic analysis.

8.7 Suggested Future Research

The most obvious suggestion for future research is to fill in the numerous gaps in the non-morphological evidence that is currently available. This will most probably remain a long term objective because the viable seed which is required for these studies has not yet been collected, but once the seed is available it would seem logical to extend the cytological, biosystematic, palynological, anatomical and phytochemical investigations.

Perhaps a more potentially productive current research topic would be a revision of *Dolichos* and its allies, taking as a model the revision by Marechal et al (1978a) of the *Phaseolastrae*. It would be logical to limit the study to Baudet's second Phaselinae supergenera, *Dolichastrae*. This grouping includes *Psophocarpus* and so the revision would follow on from the study here presented, it would also enable the true validity of splitting the Phaseolinae into supergenera to be assessed.

Having gathered an equivalent amount of information on the *Dolichastrae* to that already held by Marechal and his co-workers on the

Phaseolastrae the next logical step would be to establish a Phaseolinae data base. As mentioned throughout this thesis the Phaseolinae contain numerous important crop plants and once the information had been gathered for revising the *Phaseolastrae* and *Dolichastrae* it should then be made available for anyone who might wish to use it. The information would be entered into a computerised monographic data-base so that then classifications, lists, catalogues and keys could be produced on demand using all or chosen subsets of taxa and all or chosen characters. Much of the initial problems involved in establishing a monographic database have been overcome by the pioneering work of the Vicieae Database group at Southampton University (see Adey, Allkin, Bisby and White, 1984). Bisby et al (1983) list the following advantages to the taxonomist of using a database of the kind invisaged for the Phaselinae:

"1) that it will continue to fill as data on less well known species becomes avaialble,

2) that it will be revised as alterations, deletions or insertions become appropriate,

3) that it can accommodate different kinds of data side by side and,

4) that it can accommodate more than one classification of the species."

Surely this is the kind of versatility that a contemporary taxonomist requirres to aid the taxonomic product user, whether a fellow taxonomist or a more general scientist. Bisby et al (1983) conclude that, "in the near future the taxonomic community set up international monographic information centres for the dozen or so groups of plants such as the Vicieae which are of prime importance to man", the Phaseolinae should be one of these groups of plants.

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APPENDIX 1

Example of File Format for CHARANAL

```
INTER-CHARANAL
0089132   1
0606080303040406080404040403060503040405030304080605040408060504040806050404030 4
0603030406030808080406030309100403030804030303030303060403030303030404804120304
0506040408050504030303070504120304030604040403040403040806050403040506030406031 0
0503040304030303030603 03
99
99
99
3171222173232233122321354322643226432311111211335322142211411111112312 11      501
2132322222215123111213412111132321164312112233281121211212 11                   501
3171211163231233122311323322433224332213121312315122142211311111115311 11      551
2132321221214143111222813241132331143421112233281111211212 11                   551
3171222163231231122321353322633226332211311211335322142211411111114312 11      601
```


— BULK OF DATA —

```
1112222510131131111412100000000001111112511251335311177311511121213111 11      276
1136332235321223111223313141221211311112113105164131211212 11                   276
FINISH
```

APPENDIX 2

Example of File Format for LINKAGE

```
 10100
INTER-GENERIC STUDY USING 51 CHARACTERS
101001
 0 8 2 0 0 0 0 0 0 0 0 0 0 0 0 8 2 036 7 0 0 0 0 0 0 0 9 3 010 3 0 0 0 0 0 0
 0 0 0 015 5 0 8 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 014 4 0 6 2 0 0 0 0 0 0 0 0
 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 012 3 0 0 0 0 0 0 0 0 0 0 6 2 0 4 2 0 0 0 0 0 0
 0 7 2 012 5 0 0 0 0 6 2 0 0 0 0 0 0 0 0 0 0 0 0 0 6 2 0 0 0 021 5 0 0 0999
9999
722135G2142211841111122312112132222153112411132322 61                    501
722135E2142211831111125311112132121431128241323226 1                    551


                          - BULK OF DATA -


111215K1177311551112113111111113323213112314221231 51                   276
                                                                         99999
```

APPENDIX 3

Program Jaquie

```
1                DIMENSION A(W),B(Y,Y)
2                DATA  A/W*0.0/
3                READ(5,*,END=40)A
4          40    K=1
5                DO  10  I=1,Y
6                DO  10  J=1,I
7                IF(A(K).EQ.0.0)GOTO  30
8                B(I,J)=A(K)
9                K=K+1
10         10    CONTINUE
11         30    WRITE(6,*)I,J,K
12               DO  100  I=1,Y
13         100   WRITE(7,12)(B(I,J),J=1,I)
14         12    FORMAT(7F11.7)
15               STOP
16               END
```

NOTE: W = number of OTU's multiplied by itself divided by two.
Y = number of OTU's minus 1

APPENDIX 4

Example of File Format for CLUSTAN 1C (DISTIN)

```
DISTIN
MADELAINE M POOLE DATA
   9 4     (7F11.7)
  0.0863101
  0.2616882  0.2746369
  0.3090909  0.2722062  0.6238487
  0.3189074  0.1859503  0.2727273  0.3168831
  0.3290908  0.1925133  0.1470587  0.2272727  0.4669420
  0.2290908  0.3589832  0.1859503  0.1577539  0.2097018  0.5820542
  0.0403361  0.3783942  0.4384223  0.5064934  0.2213902  0.2226195  0.2851239
  0.1189075  0.3783942  0.3825958  0.3098685  0.1404958  0.0561497  0.1187928
  0.2862558
HIERARCHY
3  2  4
PLINK

M M POOLE DATA
STOP
```

APPENDIX 5

STEPWISE DATA TRANSFORMATION FROM LINKAGE TO DECORANA AND CLUSTAN (FILE) FORMAT

1. Call up the editor for the file you wish to transfer from LINKAGE to DECORANA format. Remove the LINKAGE prefix and suffix to the data. In the example provided in Appendix 2 this would be done as follows:- P9, T88, PE.

2. For DECORANA the identifier must be moved from the end to the beginning of the line. For the Appendix 2 example this would be done as follows:- -E(P77, T+3, P.1, T.52, P.EG)E

3. For CLUSTAN(FILE) the identifier is not required so may be erased in the Appendix 2 example by:- -E(T.52, P.EG)E

4. Next unwanted character can be removed. As DECORANA and CLUSTAN cannot compensate for missing data, in practice those characters with missing data were removed. Using the Appendix 2 data as an example the last eight characters commonly lacked scoreable attributes and so these were removed using:- -E(T.47, P.EG)E

5. As the maximum line length is 80 letters or numbers, the lines of data must be shortened so that when the spaces separating individual character scores are inserted the maximum line length is not exceeded. An example of this would be to use the command:- -E(T.25, KG)E

6. Then spaces must be inserted between each character score, using the following command:- -E(-.E(T+1, I/ /)G)E

7. Next the alpha-numeric data used by LINKAGE must be converted to numeric which is used by CLUSTAN and DECORANA. This is a process of changing A to 10, B to 11, etc., and is carried out using the following commands:- -E(-.E(A/10)G)E then -E(-.E(R/B/11)G)E and so on.

8. The data file at this stage is ready for the addition of the DECORANA data prefix and suffix and subsequent analysis as shown in Appendix 7. However CLUSTAN(FILE) will not accept free format data and so it must be converted to the format it will accept. This is done by the program JUDITH written specifically for the purpose by A.Cotton of the Computing Centre, Southampton University. The program is listed below.

```
      PROGRAM JUDITH

1           D I M E N S I O N   K ( 1 0 0 )
2           D O   2 0   I = 1 , X
3           R E A D ( 5 , * ) ( K ( J ) , J = 1 , Z )
4           W R I T E ( 7 , 1 0 ) ( K ( J ) , J = 1 , Z )
5     1 0   F O R M A T ( 2 0 I 3 )
6     2 0   C O N T I N U E
7           S T O P
8           E N D
```

NOTE: X = Number of OTU's in the study.
Z = Number of characters in the study.

9. This converts the data to the set format shown in Appendix 6 and with the addition of the CLUSTAN(FILE) data prefix and suffix the file is ready to enter the CLUSTAN analysis.

APPENDIX 6

EXAMPLE OF FILE FORMAT FOR CLUSTAN 1C (FILE)

```
SIZE
3
FILE
INTER43
  87  43   OSC10E
   0  (2013/2013/313)
  7  2  2  1  3  5  16 2  1  4  2  2  1  1  8  4  1  1  1  1
  1  2  2  3  1  2  1  1  2  1  3  2  2  2  1  5  3  1  1  2
  4  1  1
  7  2  2  1  3  5  14 2  1  4  2  2  1  1  8  3  1  1  1  1
  1  2  5  3  1  1  1  1  2  1  3  2  1  2  1  4  3  1  1  2
  8  2  4
```

- BULK OF DATA -

```
1  1  1  2  1  5  20 1  1  7  7  3  1  1  5  5  1  1  1  2
1  1  3  1  1  1  1  1  1  1  3  3  2  3  2  1  3  1  1  2
3  1  4
CORREL

HIERARCY
6  2 22
SCATTER
 -1 -2LXXX                    XX 1

PLINK

PSOPHOCARPUS INTER-GENERIC STUDY USING WARDS METHOD AND 43 CHARACTERS
STOP
```

APPENDIX 7

EXAMPLE OF FILE FORMAT FOR DECORANA

```
USE(CONVERTDATAMATRIX,WRITE=TRANSFER)
-----
DECORANA INTRA-SPECIFIC; 123 76 1 1
520 3 26 13 4 5 2 7 23 18 8 3 27 23 1 2 8 21 3 3 1 1 3 3 2 2 3 3 2 2 1
6 6 22 30 34 15 7 4 23 16 13 6 3 4 2 3 14 16 4 2
2 19 14 5 4 23 2 2 15 19 2 2 2 7 1 1 3 4 1
1 2 1 3 10 26 9
518 3 15 10 3 5 2 5 35 30 11 3 33 23 1 1 6 21 3 2 1 1 4 4 3 2 4 4 3 2 1
7 6 26 35 35 21 9 4 30 14 13 6 3 4 2 3 11 19 4 2
2 24 10 5 4 20 2 2 15 23 2 2 2 9 1 1 3 4 1
1 2 1 3 12 21 12


- BULK OF DATA -

790 3 9 11 3 3 3 5 9 6 15 3 8 4 1 2 5 9 4 1 2 1 5 4 3 3 5 4 3 3 1
10 2 1 5 3 4 13 2 17 13 17 4 4 4 3 2 11 23 4 2
2 23 8 5 4 26 2 4 21 12 2 1 2 18 5 2 3 3 1
1 4 1 6 11 24 4
791 3 16 11 4 4 3 5 10 9 11 3 15 5 1 2 7 17 4 1 2 1 5 4 2 3 6 4 2 3 1
15 6 4 18 20 12 8 3 22 18 17 5 4 2 3 2 8 15 4 2
2 23 12 5 4 28 2 5 18 18 2 1 2 15 4 2 2 3 1
1 4 1 6 10 26 12
1 0 5 5 ;0
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USE(CONDENSE,READ=TRANSFER,WRITE=TRANSFER)
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   76  123DECORANA INTRA-SPECIFIC STUDY.                              T*IN76**
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USE(DECORANA,READ=TRANSFER)
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-1 0
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APPENDIX 8

LIST OF SPECIMENS USED IN STUDIES

The list contains the specimen identification number, species identification, collector's name and number and herbarium from which the specimen was loaned. The specimen identification number was assigned arbitrarily to each specimen, the number itself having no intrinsic value other than similar numbers being ascribed to related specimens, e.g. all *P.grandiflorus* specimens have identification numbers between 501 and 520. Each specimen was identified and its specific identity is provided below. The herbarium from which the specimens were loaned are listed, using the standard abbreviation take from Index Herbariorum (Holingren, Keuken and Schofield, 1981) as follows:

Royal Botanical Gardens, Kew, Surrey, England	K
British Museum (Natural History), London, England	BM
Biology Dept., Southampton University, England	SPN
Museum National d'Histoire Naturelle, Laboratoire de Phanerogamie, Paris, France	P
Jardin Botanique National de Belgique - Nationale Plantentuin van Belgie, Meise, Belgium	BR
Conservatoire et Jardin Botaniques de la Ville de Geneve, Chambesy, Switzerland	G
National Herbarium and Botanical Garden, Causeway Harara, Zimbabwe	SRGH
Biological Institute, Faculty of Science, Tohoku University, Aoba, Sendai, Japan	TUS

SPECIMEN IDENT. NO.	SPECIMEN IDENTIFICATION	COLLECTORS NAME AND NUMBER	HERBARIUM
001	Centrosema rotundiflorum	Pires 7314	K
002	C.plumieri	Reed 1909	K
003	C.grandiflorum	Hatschbach 14229	K
004	C.brassilianum	Bisby 1230	SPN
005	C.plumieri	Hilliers 3988	BM
006	C.grandiflorum	Hunt & Ramos 6714	BM
011	Periandra coccinea	Shepherd 760	K
012	P.coccinea	Gardiner 5996	K
013	P.acutifolia	Irwin 171388	BM
014	P.mediteranea	Mori 12117	BM
015	P.mediteranea	Mori 10612	K
021	Clitoria javiterisis	Fondere K436	K
022	C.tematea	Perriott CF51	K
023	C.amazonum	Bisby 1273	SPN
024	C.javiterisis	Fendler 2201	K
025	C.ternatea	Lazier CF78	K
031	Clitoriopsis mollis	Saeger 3603	K
037	Dysolobium grande	Kanai s.n.	TUS
038	D.pilosum	Tateishi 8576	TUS
039	D.pilosum	Tateishi 8542	TUS

040	D.pilosum	Tateishi 7408	TUS
041	D.grande	Larsen 34243	K
042	D.dolichoides	Roxburgh 2397	BM
043	D.dolichoides	Hooker 1841	K
044	D.dolichoides	Henry s.n.	BM
045	D.pilosum	Henry s.n.	BM
046	D.grande	Kerr 1407	K
047	D.pilosum	Poilane 8513	K
048	D.apoiodes	Poilane 28075	BM
049	D.grande	Clarke 37158	K
050	D.grande	Kerr 39	K
051	D.pilosum	Beddome 2257	BM
052	D.apioides	Poilane 14010a	P
053	D.apioides	Poilane 14010b	P
054	D.pilosum	Clarke 40741a	BM
055	D.pilosum	Heyne s.n.	BM
056	D.pilosum	Kerr 1603	BM
057	D.pilosum	Kerr s.n.	BM
058	D.pilosum	Merrill 3674a	BM
059	D.pilosum	Wallich 5599	BM
060	D.dolichoides	Wallich 5625	BM
061	D.dolichoides	Kerr 3931	BM
062	D.grande	Hooker 15615	K
063	D.grande	Chiwiwat & Nimanang 25	K
064	D.grande	Constantino 15380	K
065	D.grande	Nujomdham et al 161	K
066	D.grande	Kerr 1407	K
067	D.grande	Kerr 2162	K
068	D.grande	Williamson 57-221	K
069	D.grande	Cavalene 3674	K
070	D.grande	Das 395	K
071	D.grande	Parry 756	K
072	D.grande	Ham s.n.	K
073	D.grande	Hooker 1867	K
074	D.grande	De Candolle 5615	G
075	D.grande	De Candolle 5602	G
076	D.grande	Clark 37258	G
077	D.grande	Kostermans 259	G
078	D.grande	Wallich s.n.	G
079	D.pilosum	Playfiar 313	K
080	D.pilosum	Mooney 1622	K
081	D.pilosum	Hooker 23	K
082	D.pilosum	Wight 1036-243	K
083	D.pilosum	Haselfoot-Haines 3918	K
084	D.pilosum	Ramos 22464	K
085	D.pilosum	Cavillier 5398	G
086	D.pilosum	Roxburgh s.n.	G
087	D.pilosum	Wallich 5598	G
088	D.pilosum	Mokim s.n.	G
089	D.pilosum	Merrill 64	G
090	D.dolichoides	De Candolle 5600	G
091	D.lucens	Cavillier 5601	G
092	D.dolichoides	Wallich s.n.	G
093	D.pilosum	Kerr 22/10/22	BM
094	D.pilosum	Clarke 40741ab	BM
095	D.pilosum	Merrill 3674b	BM
096	D.apioides	Poilane 14010c	BM

097	D.dolichoides	Wallich 5600	BM
098	D.dolichoides	Put 2606	BM
099	D.dolichodes	Marcan 435	BM
100	D.dolichoides	Horsfield s.n.	BM
101	D.dolichoides	Kerr 9330	BM
102	D.dolichoides	Kerr 11/11/23	BM
103	D.pilosum	Roxburgh 291	BM
104	D.pilosum	Put 1417	BM
105	D.pilosum	Prain 360	BM
106	D.pilosum	Kerr 13596	BM
111	Physostigma mesoponticum	Verdcourt 2824	K
112	P.mesoponticum	Gillett 17527	BM
113	P.cylindrospernum	Breteler 1791	K
114	P.cylindrospernum	Ghesquiese 2727	BM
115	P.cylindrospernum	Leonard 754	BM
116	P.venenosum	Le Testu 2010	BM
117	P.venenosum	Bamps 447	BM
121	Vatovaea pseudolablab	Richards 24854	K
122	V.pseudolablab	Richards 20325	K
131	Decorsea schlechteri	Faulkner 275	K
132	D.schlechterei	Rand 311	BM
141	Spathionema kilamandschainicum	Verdcourt 2371a	K
142	S.kilamandschainicum	Burtt 2094	BM
143	S.kilamandschainicum	Gillett 16884	BM
144	S.kilamandschainicum	Napier Box TN1/L/34	BM
151	Otoptera burchellii	Pearson 3738	BM
152	O.burchellii	Eyles 163	K
153	O.burchellii	Norman R57A	K
154	O.burchellii	Eyles 5073	BM
155	O.burchellii	Seydel 479	K
156	O.burchellii	Plowes 39803	K
157	O.burchellii	Leach & Noel 20	K
158	O.burchellii	Richards 14615	K
159	O.burchellii	Blair Rains 9	K
160	O.burchellii	Legard 240	K
161	O.burchellii	Werdermann & Oberdieck 2278	K
162	O.burchellii	Bingham 346	P
163	O.burchellii	Seydel 3909a	P
164	O.burchellii	Munchen 4118	BR
165	O.burchellii	Seydel 3747	BR
166	O.burchellii	Dinter 6904	G
167	O.burchellii	Vahrmeyer 6245	G
168	O.burchellii	Seydel 3909b	G
169	O.madagascariensis	Humbert 11568	G
170	O.burchellii	Plowes 1489	BM
171	O.madagascariensis	Perrier 16649	P
172	O.madagascariensis	Perrier 19257	P
173	O.madagascariensis	Keraudren-Aymonin 24722	P
174	O.madagascariensis	Bosser 14319	P
175	O.madagascariensis	Humbert 19795	P
176	O.madagascariensis	Humbert 11568	P

177	O.madagascariensis	Perrier 4117	P
178	O.madagascariensis	Bosser 17428	P
179	O.madagascariensis	Bosser 13941	P
180	O.madagascariensis	Dequaire 27576	P
181	O.madagascariensis	Decary 3428	P
182	O.madagascariensis	Peltier 5825	P
183	O.madagascariensis	Peltier 1275	P
184	O.madagascariensis	Peltier 1335	P
185	O.burchellii	Hansen 3073	SRGH
186	O.burchellii	Denny 347	SRGH
187	O.burchellii	Morwe 697	SRGH
191	Sphenostylis marginata	Banda 348	BM
192	S.marginata spp. erectus	Rand 227	BM
193	S.marginata	Siame 709	BM
194	S.stenocarpa	Irvine 4793	K
195	S.stenocarpa	Irvine 1821	K
196	S.stenocarpa	Dalziel 626	K
197	S.schweinfurthii	Dalziel 402	K
198	S.schweinfurthii	Okafor & Daramola 5408	K
199	S.schweinfurthii	Morton K365	K
200	S.angustifolia	Stohr N26	BM
201	S.angustifolia	Ommanney 38	BM
202	S.angustifolia	Schlieben 7944	BR
203	S.stenocarpa	Exell & Mendonca 142	BM
204	S.stenocarpa	Le Testu s.n.	BM
205	S.briertii	Banda 378	BM
206	S.briertii	Horsbrugh-Porter s.n.	BM
207	S.briertii	Polhill & Paulo 1567	BR
208	S.marginata	Head 172	BM
209	S.marginata	Kafuli 41	BM
210	S.marginata	Pawek 12323	BR
211	S.marginata spp. erecta	Coget 166	BR
212	S.angustifolia	Bayliss 1779	G
213	S.angustifolia	Schlieben 7769	G
214	S.angustifolia	Burke 1843	G
215	S.angustifolia	Zeher 524	G
216	S.briartii	Bayliss 1874	G
217	S.briartii	Robyns 1549	G
218	S.congensis	Vaughan 1909	G
219	S.calantha	Schlieben 2314	G
220	S.calantha	Schlieben 6361	G
221	S.marginata spp. erecta	Schlieben 5340	G
222	S.marginata spp. erecta	Schlieben 6043	G
223	S.marginata spp. erecta	Schlieben 6363	G
224	S.marginata spp. erecta	Adamson B5874	G
225	S.marginata	Drege 426	G
226	S.marginata	Drege 1836	G
227	S.marginata	Roberty 17099	G
228	S.marginata	Roberty 16093	G
229	S.marginata	Rudatis 1616	G
230	S.schweinfurthii	Ake Assi 7541	G
231	S.schweinfurthii	Ake Assi 8637	G
232	S.schweinfurthii	Chevalier 368	G
233	S.schweinfurthii	Chevalier 7314	G
234	S.stenocarpa	De Candolle 2259	G
235	S.stenocarpa	Goctze 1388	G

236	S.stenocarpa	Lewalle 5638	G
237	S.stenocarpa	Nicholson 21748	G
238	S.stenocarpa	Robery 14053	G
239	S.stenocarpa	Schlieben 3799	G
240	S.stenocarpa	Hundt 916	G
241	S.stenocarpa	Schlieben 6129	G
242	S.stenocarpa	Schlieben 4242	G
243	S.stenocarpa	Stolz 651	G
244	S.stenocarpa	Schlieben 2024	G
245	S.marginata	Baum 796	G
251	Nesphostylis holosericea	Lely 655	K
252	N.holosericea	de Wilde 976b	K
253	N.holosericea	Bond 137	K
254	N.holosericea	Barter s.n.	K
255	N.lanceolatus	Wallich 5546	BM
256	N.holosericea	de Wilde 9766	BR
257	N.holosericea	Ezpinto Santo 3524	BR
258	N.holosericea	Jones 5304	BM
259	N.holosericea	De Candolle 2258	BM
260	N.holosericea	HbG. 59004	G
261	N.holosericea	Roberty 6493	G
262	N.holosericea	Grundy L138	K
263	N.holosericea	Vollesen MRC4513	K
264	N.holosericea	Adams 4866	K
265	N.holosericea	Ern 2627	K
266	N.holosericea	Hooker 1867	K
267	N.lanceolatus	Le Testu 3352	BM
271	Austrodolichos errabundus	Adams 906	K
272	A.errabundus	Chippendale 7809	K
281	Neorautanenia mitis	Proctor 2143	K
282	N.mitis	Kelly 406	K
283	N.amboensis	DSV 81	BM
284	N.ficifolius	Henriques s.n.	BM
285	N.ficifolius	Rogers 129101	BM
286	N.amboensis	Le Testu 4743	BM
287	N.amboensis	Bates 15659	K
288	N.amboensis	Wild 4765	K
289	N.mitis	Milne-Redhead and Taylor 79866	K
290	N.mitis	Stolz 2368	BM
291	N.amboensis	Cap 13987	P
292	N.mitis	Angus 914	BM
293	N.mitis	Gossweiler 9569	BM
294	N.mitis	Welwitsch 2197	BM
295	N.mitis	Haerdi 118/17	BR
296	N.mitis	Powell 10711	BR
297	N.mitis	de Wilde 4946	BR
298	N.ficifolius	Merxmuller 1352	BM
299	N.amboensis	Munchen 4227	BR
300	N.amboensis	Norrgrann 280a	BR
301	N.ficifolius	Dale SKF 153	BR
302	N.amboensis	Walters 4459	BR
303	N.amboensis	Story 4982	G
304	N.mitis	Pappi 5795	G

305	N.mitis	Ern et al 2042	K
306	N.mitis	MacGregor 201	K
307	N.mitis	Dalziel 555	K
308	N.mitis	Latilo 54861	K
309	N.amboensis	Bingham 158	K
310	N.amboensis	Smith 1239	K
311	N.amboensis	Goddier 518	K
312	N.amboensis	Norrgrann 280b	K
313	N.amboensis	Ngoni 283	K
314	N.mitis	Haerdi 54890	G
315	N.mitis	Bally B9718	G
316	N.ficifolius	Eyles 165	BM
317	N.ficifolius	Burke 127	BM
318	N.mitis	Chiovenda 5795	G
321	Lablab purpureus	Jeffrey K879	K
322	L.purpureus	Bausekom 3910	K
323	L.purpureus	Stainton 517	BM
324	L.purpureus	Bally 1889	BM
325	L.purpureus	Edwards 12	K
331	Alistilus jumellii	Mabberley 912	K
332	A.bechuanicus	Codd 8454	K
333	A.bechuanicus	Richard 0117	BM
334	A.bechuanicus	Vahrmeyer 1324	K
335	A.bechuanicus	Obermeyer et al 317	K
341	Dipogon lignosus	Pollock 227	K
342	D.lignosus	Rodd 1493	K
343	D.lignosus	Schelpe 4166	BM
344	D.lignosus	Symon 9541	BM
345	D.lignosus	Breutel s.n.	P
346	D.lignosus	Rodin 1030	K
347	D.lignosus	Eckhon 1836	K
348	D.lignosus	Langley-Kitching s.n.	K
349	D.lignosus	Hubbard 3549	K
351	Dolichos fragrans	Put 4480	K
352	D.dinklagei	Thomas 2901	K
353	D.trinervatus	Richards 10538	K
354	D.trilobus	Radcliff-Smith 4934	K
355	D.homblei	Young 1311	BM
356	D.staintonii	Polunin 3220	BM
357	D.terminicaulis	Henry 12, 507a	BM
358	D.chysanthus	Faulkner 271	BM
359	D.trinervatus	Brummitt 9449	K
360	D.dinklagei	Thomas 4659	K
361	D.eriocaulis	Fanshawe F985	BR
362	D.chloryllis	Burke s.n.	BM
363	D.capensis	Bunbury s.n.	BM
364	D.angustifolius	Rogers 16943	BM
365	D.linearis	Drege 142	BM
366	D.sericeus	Eggeling 2390	BM
367	D.gululu	Gossweiler 1951	BM
368	D.tenuicaulis	Hara 6301769	BM
369	D.dongulata	Humbert 16814	BM
370	D.malosanus	Kassner 2046	BM

371	D.schweinfurthii	Keay 22900	BM
372	D.kilimondschonius	Rae A53	BM
373	D.linearifolius	Brito Teixeira 14	BM
374	D.dasycarpus	Thorel s.n.	BM
375	D.schweinfurthii	Tisserant 954	BM
376	D.linearifolius	Young 1105	BM
377	D.kilimondschonius	Zimmer 224	BM
378	D.junghuhnianus	Lugduno-Batavo 9164	K
379	D.junghuhnianus	Koorders 29267b	K
380	D.antunesii	Gossweiler 12573	K
381	D.pratensis	Haro & Lond II 246	K
391	Macrotyloma uniflorum	Bourne 3257	K
392	M.axilleri	Harley 4320	K
393	M.stipulosum	Scott-Elliot 8249	BM
394	M.strophyllum	Le Testu 3252	BM
401	Vigna angularis	Live	SPN
402	V.angularis	Live	SPN
403	V.luteola	Box 955	BM
404	V.unguiculata	Arsene 5130	BM
405	V.umbellata	Live 6/12/82	SPN
406	V.vexillata	Strey 9504	K
407	V.luteola	Schweinfwith 3/1888	K
408	V.marina	Yuncker 15009	BM
409	V.parkeri	Taylor 2698	BM
410	V.gracilis var. multiflora	Pringle 6471	BM
411	V.racemosa	Bates 632	BM
412	V.adenantha	Basurto 185	BM
413	V.mungo	Portman 10/4/70	SPN
414	V.mungo	Smart 3/4/70	SPN
415	V.umbellata	Smart 7/4/70	SPN
416	V.acontifolia	Live 14/12/82	SPN
417	V.trilobata	Live 29/1/83	SPN
418	V.radiata	Radcliff-Smith 5314	K
419	V.minima	Sinclair & Salleh 40467	K
420	V.luteola	Zohary & Amdursky 437	K
421	V.luteola	Hayne s.n.	K
422	V.membranacea	Collenette 262	K
423	V.luteola	Hiepko & Schultze-Motel 24	K
424	V.comosa	Reekmans 6106	K
425	V.haumaniana	Hooper & Townsend 577	K
426	V.reticulata	Letouzey 6033	K
427	V.frutescens	Runyinya 853	K
428	V.monophylla	Richards 3909	K
429	V.richardsian	Callum-Webster 885	K
430	V.vexillata	Wickens s.n.	K
431	V.nuda	Strid 2024	K
432	V.adanantha	Hooker 5610	K
433	V.lasiocarpa	Jenman 5506	K
434	V.juruana	Bentham 1641	K
435	V.peduncularis	Hassler 8591	K
436	V.caracalla	Montes 2146	K
437	V.venulosa	Tisserant 2038	BM
438	V.venulosa	Afzelius s.n.	BM
439	V.nigritia	Yates 1906-7	BM

440	V.nigritia	Lebroer 1982	BM
441	V.macrorhyncha	Schlieben 3389	BM
442	V.macrorhyncha	Bagshawe 235	BM
443	V.procera	Gossweiler 6313	BM
444	V.speciosa	Basurto & Duran 176	BM
445	V.multinervis	Le Testu 3331	BM
446	V.longifolia	Alston 7627	BM
447	V.speciosus	Plant Brazil 1125	K
448	V.candida	Hassler 12107	K
451	Ramirezella strobilophora	Hinton 16138	K
452	R.ornata	Calderon 2304	BM
453	R.pubescens	Hinton 3441	BM
454	R.pubescens	Hinton 7935	BM
455	R.strobilophora	Townsend & Barber 412	BM
457	Oxyrhynchus trinervus	Skutch 2738	K
458	O.papuanus	Mauriasi 11516	K
459	Dolichopsis paraquariensis	Renvoize 3552	K
460	D.parquariensis	Fiebrig 1456	K
461	Strophostylis umbellata	Ray 10302	K
462	S.helvola	Soper 2483	BM
463	S.helvola	Senn 441	K
464	S.helvola	Bisby 1095	SPN
465	S.leiosperma	Mead 211	BM
466	S.peduncularis	Short s.n.	BM
467	S.helvola	Maclagan s.n.	BM
468	S.umbellata	Rickett 134	BM
471	Macroptilium bracteatum	May 12168	K
472	M.lathyroides	Balkinshaan NBK 510	K
473	M.lathyroides	Townsend 73/279	K
474	M.lathyroides	Portman 22/4/70	SPN
475	M.atropurpureum	Z 3071	BM
476	M.atropurpureum	Portman 28/5/70	SPN
477	M.martii	Hassler 6380	BM
478	M.longepedunculatum	Hoehne 3956	BM
479	M.lathyroides	Live 4/6/82	SPN

481	Phaseolus vulgaris	Live 12/12/82	SPN
482	P.vulgaris	Smart 3/4/70	SPN
483	P.coccineus var. rubronanum	Portman 5/4/70	SPN
484	P.coccinues var. albonanus	Smart 3/4/70	SPN
485	P.acutofolius	Live 6/4/82	SPN
486	P.lunatus	Portman 15/4/70	SPN
487	P.lunatus	Daughty P1/2	BM
488	P.lunatus	Cambridge Congo Expedition 110	BM
489	P.vulgaris	Hinton 11684	K
490	P.vulgaris	Hinton 11551	K
491	P.retinensis	Lindheimer 367a	K
492	P.retinensis	Lindheimer 367b	K
493	P.grayanus	Blumer 1347	K
494	P.galactoides	McVaugh 13051	K
495	P.galactoides	Townsend & Barber 319	K
496	P.parvulus	Blumer 1351	K
501	Psophocarpus grandiflorus	Loveridge 344	K
502	P.grandiflorus	Hancock 197	K
503	P.grandiflorus	Mooney 6227	K
504	P.grandiflorus	Siegenthaler 1483	K
505	P.grandiflorus	Taylor 3207	BM
506	P.grandiflorus	Frils 2196	K
507	P.grandiflorus	Strauffer 40	K
508	P.grandiflorus	Alluaud 364	P
509	P.grandiflorus	Froment 206	BR
510	P.grandiflorus	Gutzwiller 749	BR
511	P.grandiflorus	Ghequizse 4935	BR
512	P.grandiflorus	Thomas 2587	BR
513	P.grandiflorus	Mooney 8661	BR
514	P.grandiflorus	Godman 360	BM
515	P.grandiflorus	Wollaston s.n.	BM
516	P.grandiflorus	Westphal 2666	K
517	P.grandiflorus	Reekman 10691	K
518	P.grandiflorus	Synges 51047	BM
519	P.grandiflorus	Synges 51047	BM
520	P.grandiflorus	Taylor 3175	BM
551	Psophocarpus tetragonolobus	Live PT-1119	SPN
552	P.tetrogonolobus	Jahni 1	K
553	P.tetragonolobus	Live PT-1115	SPN
554	P.tetragonolobus	Live PT-1102	SPN
555	P.tetragonolobus	Brass 21939	K
556	P.tetragonolobus	Lezon 656	K
557	P.tetragonolobus	Ford 170	K
558	P.tetragonolobus	Henry 1901	P
559	P.tetragonolobus	Westphal 9595	P
560	P.tetragonolobus	Westphal 9647	P
561	P.tetragonolobus	Cumming 656	P
562	P.tetragonolobus	Mendoza 3103	BR
563	P.tetragonolobus	Koenig	BM
564	P.tetragonolobus	Kerr	BM
565	P.tetragonolobus	Jermy 4405	BM
566	P.tetragonolobus	Cuming 656	G
567	P.tetragonolobus	Maunting	G
568	P.tetragonolobus	Boivin 1853	G

569	P.tetragonolobus	Perrottet 1819	G
570	P.tetragonolobus	Brass 21939	G
571	P.tetragonolobus	Degenere 11395	G
572	P.tetragonolobus	Knukoff 4272	G
573	P.tetragonolobus	Bon 4232	G
574	P.tetragonolobus	Bartlett & La Rue 366	BM
575	P.tetragonolobus	Kerr 3375	BM
576	P.tetragonolobus	Kerr 15/10/20	BM
477	P.tetragonolobus	Irvine 3619	K
478	P.tetragonolobus	Irvine 4792	K
601	Psophocarpus palustris	Dalziel 8016	K
602	P.palustris	Wilde 952B	K
603	P.palustris	Morton GC9674	K
604	P.palustris	Morton SL2499	K
605	P.palustris	Dalziel 1906	K
606	P.palustris	Linder 1019	K
607	P.palustris	Testu 4187	BM
608	P.palustris	Morton A135	P
609	P.palustris	Chevalier 22719	P
610	P.palustris	Berhaut 1045	P
611	P.palustris	Felix 1398	P
612	P.palustris	Haerdi 104/3914	K
613	P.palustris	Berhaut 1045	BR
614	P.palustris	Geerling et Bokdam 1619	BR
615	P.palustris	Roberty 15549	G
616	P.palustris	Roberty 13469	G
617	P.palustris	Roberty 6873	G
618	P.palustris	Wallich 1164	G
619	P.palustris	Morris 773	BM
620	P.palustris	Humboldt Stifling 2852	BM
621	P.palustris	Talbot 1318	K
622	P.palustris	Oloruferni & Macauley FHI 62047	K
623	Psophocarpus Palustris	Morton A135	K
624	P.palustris	Deighton 3611	K
625	P.palustris	Deighton 3466	K
626	P.palustris	Thomas 6537	K
627	P.palustris	Letouzey 3566	K
628	P.palustris	Morton GC9674	K
629	P.palustris	Espirito Santo 1664	K
630	P.palustris	Henri Tehe 810	G
631	P.palustris	Dalziel 17	SRGH
632	P.palustris	Talbot 1318	BM
633	P.palustris	Twillet 1827	G
634	P.palustris	De Candolle 1864	G
635	P.palustris	Schweinfurth 2852	SRGH
636	P.palustris	Stolz 1521	G
637	P.palustris	Espirito Santo 3574	SRGH
651	Psophocarpus scandens	Fanshawe 8943	K
652	P.scandens	Live 23/5/82	SPN
653	P.scandens	Pickersgill Q1586	K
654	P.scandens	Joffre Ville 1	SPN
655	P.scandens	Pickersgill Q1539	K
656	P.scandens	Baron 4560	BM
657	P.scandens	Ross 1119	BM

658	P.scandens	Yuncker 17955	BM
659	P.scandens	Jalani 6	K
660	P.scandens	Jalani 3	K
661	P.scandens	Jalani 2	K
662	P.scandens	Hepper 4086	P
663	P.scandens	Dacrumont 10	P
664	P.scandens	Schlieben 1126	P
665	P.scandens	Vasse 427	P
666	P.scandens	Wagemans 1703	BR
667	P.scandens	Breteler 2938	BR
668	P.scandens	Leeuwenberg 7023	BR
669	P.scandens	Westphal et Westphal Stevels	BR
670	P.scandens	Faulkner 2690	BR
671	P.scandens	Compere 733	BR
672	P.scandens	Gossens 2413	BR
673	P.scandens	Goudot 1833	G
674	P.scandens	De Candolle 2280	G
675	P.scandens	Germain 1921	G
676	P.scandens	Yuncker 17,955	G
677	P.scandens	Moncand 127	G
678	P.scandens	Schlieben 5283	G
679	P.scandens	De Candolle 2279	SRGH
680	P.scandens	Salzmann 185	G
681	P.scandens	Schlieben 1126	G
682	P.scandens	Zenker 415	G
683	P.scandens	Zenker 2723	G
684	P.scandens	Baum 1005	G
685	P.scandens	Stefano 10	SRGH
686	P.scandens	Blancket 239	G
687	P.scandens	Zimmermann 918	G
688	P.scandens	Salzmann s.n.	G
692	P.scandens	Letouzey 13155	K
694	P.scandens	Westphal 9647	K
696	P.scandens	Hilderbrandt 2958	BM
697	P.scandens	Le Testu 4340	BM
698	P.scandens	Eccles 43	SRGH
701	Psophocarpus obovalis	Myers 9292	K
702	P.obovalis	Tisserant 749	BM
726	P.monophyllus	Adams 347	K
727	P.monophyllus	Chevalier 21972b	P
728	P.monophyllus	Mali 3577	P
729	P.monophyllus	Chevalier 21972a	P
730	P.monophyllus	Mali 252	P
731	P.monophyllus	Laferrere 74	BR
732	P.monophyllus	Espirito Santo 3095	BR
733	P.monophyllus	Chevalier 21972c	Br
751	Psophocarpus lecomtei	Witte 3118	K
752	P.lecomtei	Verheyen 3118a	BM
753	P.lecomtei	Le Testu 4102a	BM
754	P.lecomtei	Tisserant 379	P
755	P.lecomtei	Le Testu 4102b	P
756	P.lecomtei	Verheyen 3118b	P
757	P.lecomtei	De Witte 5979	BR

758	P.lecomtei	Le Testu 4102c	BR
776	Psophocarpus lancifolius	Richards 15053a	K
777	P.lancifolius	Pawek 4641	K
778	P.lancifolius	Tweedie 3101	K
779	P.lancifolius	Mailmes 305	BM
780	P.lancifolius	Witte 163	BM
781	P.lancifolius	Baudet 350	K
782	P.lancifolius	Chandler 1590	K
783	P.lancifolius	Robinson 5051	K
784	P.lancifolius	Lebum 3874	P
785	P.lancifolius	Polhill & Paulo 1744a	P
786	P.lancifolius	Pawek 12531	BR
787	P.lancifolius	Polhill & Paulo 1744	BR
788	P.lancifolius	de Wilde 5607	BR
789	P.lancifolius	Milne-Redhead & Taylor 8961	BR
790	P.lancifolius	Richards 8751	BR
791	P.lancifolius	Homble 1920	BR
792	P.lancifolius	de Witte 163	BR
793	P.lancifolius	Lebrun 8903	BR
794	P.lancifolius	Lebrun 3874	BR
795	P.lancifolius	Stolz 803	G
796	P.lancifolius	Lebrun 8903	G
797	P.lancifolius	Froment 206	SRGH
798	P.lancifolius	Davies 77	SRGH
799	P.lancifolius	Brummitt 9149	SRGH
800	P.lancifolius	Staples 555	SRGH
801	P.lancifolius	Brummitt 10122	SRGH
802	P.lancifolius	Mutimushi 303	SRGH
803	P.lancifolius	Robinson 5051	SRGH
804	P.lancifolius	Richards 15033b	SRGH
805	P.lancifolius	Pringle 6471	K
806	P.lancifolius	Buchanan 158	K
826	Psophocarpus lukafuensis	Fanshawe 1024a	K
827	P.lukafuensis	Fanshawe 1024b	BR
828	P.lukafuensis	Kassner 2996a	BM
829	P.lukafuensis	Verdick 401	K
830	P.lukafuensis	White 7153a	K
831	P.lukafuensis	Macauley	K
832	P.lukafuensis	Fanshawe 1024c	SRGH
833	P.lukafuensis	White 7153b	SRGH

APPENDIX 9

PREPARATION OF MITOTIC ROOT SQUASHES

The following method was utilised:

1. Cut about 20mm off the root tip of a young seedling or rooted cutting.

2. Place in a saturated solution of para-dichlorobenzene (PDB) for 45 mins.

3. Remove from PDB and wash in distilled water.

4. Transfer the root tips to a watch glass containing 9:1 aceto-orecin and HCL. Then gently heat the base of the watch glass until the stain begins to fume, and leave to cool with a second watch glass inverted over the first to prevent the stain evaporating.

5. After a further 45 minutes of steeping the root tips in the stain, the apical 2mm of the root tip was mounted in 9:1 aceto-orcein and HCL. A cover slip was placed over the root tip and tapped to tease the root tip apart and obtain an even spread of cells.

6. The slides were observed using a Zeiss photo-microscope 1, using a green filter to heighten chromosomal contrast.