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A Numerical Taxonomic Study Of The Genus Loudetia, Hochst, Ex Steud (gramineae)

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A NUMERICAL TAXONOMIC STUDY OF THE GENUS LOUDETIA HOCHST. EX STEUD. (GRAMINEAE)

by

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ABSTRACT

Loudetia is one of the larger genera of the tribe Arundinelleae (Gramineae). Modern taxonomists have regarded the genus as consisting of three sections, one of which is further divided into six subsections, or, alternatively one of these sections is transferred to a different genus. In this work the broader of the two delimitations of Loudetia is used. The criteria for this subdivision are mainly morphological and in this thesis new anatomical evidence is presented as a basis for classification by numerical taxonomic means.

One hundred and sixty-two characters were assembled from a study of (a) the anatomy of the leaf, as seen in transverse section (79 characters); (b) the leaf epidermis, as seen in surface view (52 characters); and (c) the anatomy and morphology of the awn (31 characters), when approximately 50 representative taxa were examined. Numerical taxonomic analyses were performed using each of these character sets separately and then in a combined study. Principal components analysis was used to represent the data in a simpler structure than the original, and classifications were obtained using various methods of cluster analysis.

The general conclusion is reached that the taxonomy of

Loudetia constructed by conventional means is very similar to that using anatomical characters in multivariate analyses. The latter are shown to be very powerful techniques of showing relationships between taxa which would otherwise not be obvious when anatomical studies are made. The importance of character analysis as well as OTU analysis is stressed, and some anomalies in the results are recognized as being due to local environmental or genetic variation.

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CHAPTER I

INTRODUCTION

"Schematisation is always necessary when we are trying to bring observed facts together and arrive at a comprehensive view. But the conceptions which we form and the terms we employ or invent in the process should never be mistaken for the facts of nature. They are simply creations of the human mind which assist in the understanding and correlation of the facts of nature. As such, they are inevitably subject to differences of opinion, for different minds will always tend to prefer different criteria in constructing classifications. Ultimately the classifications that do least violence to the facts will survive . . .." Tansley, (1953).

In a taxonomic study of a group of organisms, every effort must be made to produce a classification which is an unbiased representation of the facts as they occur in nature. This is, however, not an easy task, since it is often very difficult to interpret the relationships between organisms satisfactorily. Furthermore, there are many different ways in which the same facts may be interpreted, thus resulting in a variety of classifications. An examination of the history of the

taxonomy of the tribe Arundinelleae (Phipps, 1967a) shows that this is a perfect example of the way different authors have chosen different criteria for classification, consequently arriving at different conclusions.

The major problem in the classification of this tribe is the application of a concept of generic limits. Whereas in many other tribes of grasses there are well defined species groups which can be easily recognized, such is not the case in the Arundinelleae. The genus Loudetia Hochst. ex Steud. is one of the main "species groups" which has been subject to much taxonomic confusion. Although the central core of the genus is now fairly stable and well established, sections of the genus have been reclassified by all the recent authors (Conert, 1957; Jacques-Félix, 1962; Phipps, 1967a and Clayton, 1967). The major problem of delimitation between Loudetia and its neighbours Tristachya, Loudetiopsis and Danthoniopsis is being investigated by Phipps (1969). The present study is confined to Loudetia sensu stricto (Phipps, 1966) since it is a good example for investigating the relationships between sections and subsections of a genus. Each of these subgeneric groups shows a different amount of homogeneity and by making a more thorough study of the anatomy of their members, it is hoped that an understanding of their relationships may be furthered.

1. Loudetia Hochst. ex Steud. and its relationship to the other members of the tribe Arundinelleae

The Arundinelleae were first described as a separate

tribe by Stapf (1898). Prior to this, the genera described in the nineteenth century, viz. Arundinella (1823), Tristachya (1829), Trichopteryx (1836) and Loudetia (1854) were variously placed in other tribes such as the Aveneae, Melinideae and Tristegineae (Hubbard, 1936). In fact even after the description of the tribe no consolidatory work was done on all the genera prior to that of Hubbard (1934, 1936), and the genera were still placed in different tribes by some authors. Hubbard emphasized the work of Stapf and this tribal concept of the Arundinelleae is now generally accepted. Further details may be found in Hubbard (1934) and Conert (1957).

The position regarding generic limits during the early period was even more confusing. A review of the taxonomic history of Loudetia (Phipps, 1967a) illustrates this point. The name Loudetia was proposed by Hochstetter in 1841 for an Ethiopian grass, Loudetia elegans, collected by Schimper (Hubbard, 1936). No description was given for the genus, though Braun (1841) did give a description for the type species Loudetia elegans Hochst. Braun points out that the genus was named in honour of a member of the travel association, the dentist Loudet of Karlsruhe, Germany. Braun's description of Loudetia as a genus is illegitimate, according to the later established International Rules of Botanical Nomenclature, since he also proposed that there was no distinction between this genus and Tristachya, and although he described Tristachya as a section of Loudetia, by priority

the former genus remained a valid name.

The first valid description of Loudetia was consequently given by Steudel (1854):

"Loudetia Hochst. Panicula; spiculae biflorae; glumae 2; inferior minor oblonga obtusa, superior lanceolata apice truncatula flosculos aequans; flosculus inferior neuter vel masculus superior major; valvula exterior chartacea interior hyalina minor, utraque glabra; flosculus superior undique pilosus apice truncatus bisetulosus inter setulas longissime valide aristatus; stamen 1 (an semper?); stylus brevis, stigmata 2 glabra; lodiculae 2 carnosulae rugosae."

This description was not really adequate in describing the characteristics of the genus and as a result, in the years following, most species of Loudetia were described as Trichopteryx or Tristachya.

During the 1930's Hubbard made some major contributions to Arundinellean taxonomy when working on the grasses of tropical Africa. His 1936 paper on the genera of the tribe has remained the basis for all subsequent taxonomies. Only two species of Loudetia viz. L. simplex (L. elegans) and L. arundinacea had been correctly described as members of the genus at this time. Hubbard recognised Loudetia as a large genus and the two species were increased to 27, most of them drawn from Trichopteryx where they had been initially placed. The latter was recognised as a closely knit group of seven species. Hubbard (1936) also clarified the position regarding the other genera, but recognised that there was a

difficult boundary between Loudetia and Tristachya. He distinguished Tristachya from Loudetia by the fact that the spikelets of the former occur in threes at the ends of the branches of panicles or racemes. The section Pseudotristachya also has spikelets in threes, but Hubbard felt that these species had more characters in common with Loudetia than Tristachya; consequently Pseudotristachya was included under Loudetia.

The work of Hubbard has formed the basis for subsequent argumentations of generic limits and some of the more important characteristics of the sections and subsections of Loudetia which Hubbard recognised are listed (Table 1). A more complete table of taxonomic characters for all the genera and sections is given by Phipps (1964).

In the modern period (Phipps, 1967a) of Arundinelleae taxonomy interest has been mainly centred around solving the demarcations within the Loudetia-Tristachya complex. Jacques-Félix (1950) postulated a division of the tribe based on characters of the awn and lemma of the upper floret. He suggested that a subtribe Arundinellineae consisting of Arundinella, Loudetia and Tristachya be formed, these genera being characterised by a fertile lemma that is entire or only feebly lobed, not ornamented with hairs, and a filiform awn which is twisted when dry. A second subtribe Trichopterineae, containing Trichopteryx, Danthoniopsis and Gilgiochloa was distinguished from that above in that its members have a fertile lemma that is deeply bifid, ornamented with hairs,

its lobes often bristled or setaceous, and with a ribbon-like awn which is spiral when dry. This subdivision of the tribe was not very satisfactory as Conert (1957) pointed out.

Conert (1957) was mainly concerned with the position of the Arundinelleae with respect to the other tribes, though he did introduce generic changes into the tribe as well. His contribution was accompanied by leaf and awn anatomical studies of many of the species, though about 36% were omitted (Phipps, 1966). Although Conert gave extensive descriptions of leaf and awn anatomy he did not make much use of these characters in delimiting the genera and species of the tribe. His main contribution with respect to Loudetia was the removal of the section Pseudotristachya because of the grouping of spikelets in lax triads and the construction of a new genus Loudetiopsis primarily on this characteristic. Also placed in this genus were two sections of Tristachya; Diandrostachya and Dilophotriche, since they also had the loudetiod type lemma of the lower floret with three nerves as well as the lax triads. Tristachya was considered by Conert to include those species with a 5-7 nerved lower lemma, a spikelet length of 10-45 mm and a thick, many-layered awn column. He transferred the section Paratristachya of Loudetia to Tristachya. Thus the genus Loudetia was reduced to three sections, viz. Loudetia, Lophanthera and Pleioneura.

Jacques-Félix (1962), in his treatment of the Gramineae of tropical Africa, still mentions his two subtribes Arundinellineae and Trichopterineae that were based on

characters of the awn column and lemma of the upper floret. Jacques-Félix (1960, 1962) restricted Loudetiopsis to the former section Pseudotristachya of Loudetia (sensu Hubbard) and created two new genera, Dilophotriche and Diandrostachya. In this way he could maintain his subtribes and yet not introduce heterogeneity into the genus Danthoniopsis as he had done in his earlier work (1950) which was criticised by Conert (1957). Jacques-Félix (1962) chose to maintain the section Paratristachya of Hubbard in Loudetia.

Hubbard (1936), Conert (1957) and Jacques-Felix (1960) had all made suggestions that the various sections of Tristachya be elevated to generic rank. This was done by Phipps (1964, 1966) by the formation of nine new genera. He followed the position of Conert (1957) and combined the section Paratristachya of Loudetia and the section Tristachya of the genus Tristachya to form a smaller genus-Tristachya sensu Phipps. Loudetia was once again recognised as consisting of the three sections Lophanthera, Loudetia and Pleioneura.

Phipps (1964) also described a new species of Arundinelleae which grows in and near the Zambezi river gorge below Victoria Falls, Rattraya petiolata. This grass is extraordinary in that the leaf, which is very large and broad, has a pseudopetiole. In other respects it is very similar to species of the section Pleioneura of Loudetia.

Phipps (1966) formulated major groups of genera of the tribe, the loudetioid genera (Group B) being Loudetia,

Rattraya and Loudetiopsis. Loudetiopsis sensu Jacques-Félix has more loudetioid characters than tristachyoid and was, therefore not combined with Tristachya, Diandrostachya and the other smaller tristachyoid genera. Phipps (1966), although using lemma and awn criteria in the division of the tribe, does not follow the position of Jacques-Félix (1950, 1960, 1962). This author grouped Diandrostachya, Trichopteryx, Dilophotriche and Gilgiochloa on the basis of awn and upper lemma characteristics (op. cit.), whereas Phipps felt that Diandrostachya is more tristachyoid in nature. Jacques-Felix (1962) does not make strong emphasis of this subdivision of the tribe, although it is the criterion for the first division in the key to the genera.

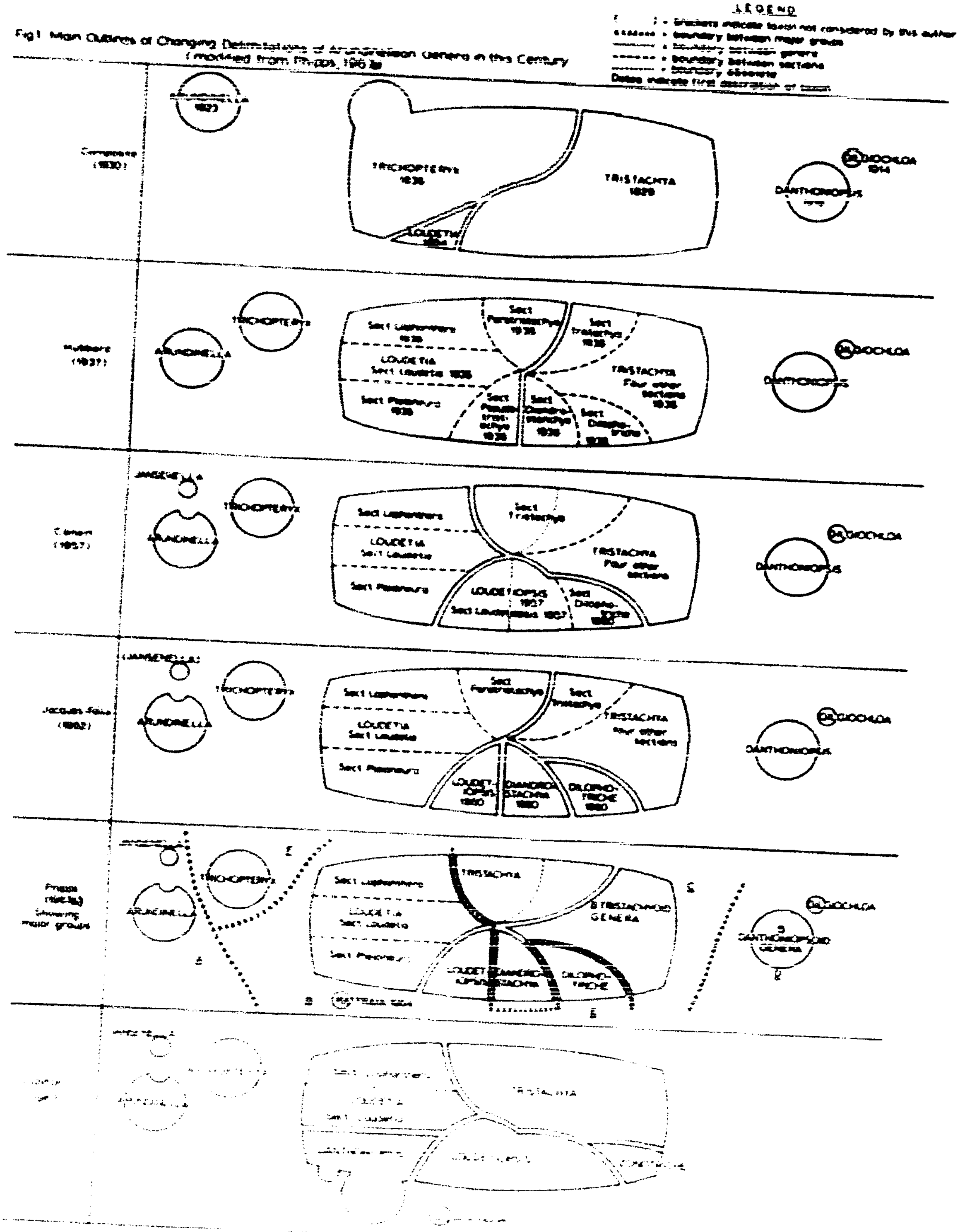
Clayton (1967) again reviewed the generic concepts and suggested that nine genera be recognised, mainly on the basis of nervation of the lower lemma and callus shape of the upper floret. Other criteria for generic delimitation in this tribe, formation of the spikelets in triads and the presence of lateral awns or hair tufts on the upper lemma received less attention. His classification is similar to that of Conert but is new inasmuch as it affects the subdivision of Loudetia. Since the section Pleioneura and Rattraya have a lower lemma which is 5-7 nerved and a short, more or less obtuse callus, Clayton transfers these species to Danthoniopsis which is similar with respect to these characters. Thus Loudetia sensu Clayton would be reduced to the two sections Loudetia and Lophanthera. Phipps (1967b) also

indicates the close relationship between the section Pleioneura, Rattraya and the Danthoniopsoid genera in the phylogeny constructed for the tribe. The change in generic boundaries is illustrated in a figure by Phipps (1967a) which is reproduced here (Fig. 1), updated to include the more recent concepts of Clayton (1967).

The concept of the genus Loudetia and the tribe in general has thus been outlined up to the present time. It is hardly likely that the present generic concepts will remain unchanged in view of the intensive work that is still being carried out on the tribe (e.g. Phipps, 1969, Li, 1969). It will have been noticed that, although many characters are available (Phipps, 1964, lists 39), none of the authors in the previous years has found sets of many characters suitable in forming specific groups. The classification of Hubbard (1936), though the most conservative, has been the most stable and has formed the basis for all the later classifications. In an effort to explain the generic limits Jacques-Félix (1950) first suggested using anatomical characters of the awn and later Conert (1957) pursued this with anatomical studies of the awn and leaf. It would appear that neither of these authors examined their data in sufficient detail to arrive at solutions of general acceptance. It is therefore obvious that the problem of classifying Loudetia should be approached from other than classical taxonomic means.

2. The criteria conventionally used in the subdivision of Loudetia

Fig 1 Main Outlines of Changing Delimitations of Arundinelloid Genera in this Century (modified from Phipps, 1967a)



Many of the criteria used by previous authors have already been discussed. The main criteria used by Hubbard (1936, 1937) were tabulated in detail by Phipps (1964) and an abbreviated form of this table is given as Table 1.

(a) Spikelet morphology

The most important characters used here are those of the spikelet. These are the most widely used criteria for all grass classifications. Although the only part of the spikelet that will be studied in detail is the awn, it is appropriate to review the morphology of the spikelet as has been described by Phipps (1964, p.89):

Spikelet 2-flowered: lower floret usually ♂; upper usually ☿; ± lanceolate and gaping; disarticulating most frequently only between the florets.

Glumes most commonly ± lanceolate, acute, 3-nerved, the lower well-developed but the smaller of the two, the upper frequently as long as the spikelet.

Lower floret: lemma similar to upper glume in all ways; palea rather shorter, narrow, 2-keeled along the 2 nerves.

Upper floret: lemma smaller than that of lower floret, variously nerved, practically always geniculately awned from the sinus between the 2 lobes; palea rather smaller than lemma, ± similar in general to that of the lower floret except in being more delicate.

Lodicules 2, ± fleshy, truncate above and cuneate below; 3 (sometimes 2) linear anthers usually present in both florets.

Caryopsis tightly enclosed in lemma and palea of upper

Table 1: Principal Morphological Characters of Loudetia and Rattraya

	Vegetative		Inflorescence		S Low
	Longevity	Pseudo-petiole	Type	Spikelet Arrangement	
Loudetia					
Section 1: Loudetia					
Sub-section a. Typicae	**Perennial**	**-**	**Panicle**	**1 - 2**	
b. Pungentes	**Perennial**	**-**	**Panicle**	**1 - 2**	
c. Acuminatae	**Perennial**	**-**	**Panicle**	**1 - 2**	
d. Densispicae	**Annual, perennial**	**-**	**Densely spiciform**	**1 - 2**	
e. Annuae	**Annual**	**-**	**Panicle**	**1 - 2**	
f. Flammidae	**Perennial**	**-**	**Densely contracted panicle**	**1 - 2**	
Section 2: Plecioncura	Perennial	-	Panicle	1 - 2	
Section 3: Lophanthera	Annual (?)	-	Panicle	3	
Section 4: Pseudotristachya	Perennial	-	Panicle	3	
Section 5: Paratristachya	Perennial	-	Panicle (often v. narrow)	1 - 3	
Rattraya	Perennial	+	Panicle	1	
Incertae sedes Loudetia jaegeriana	Perennial	-	Panicle	1 - 2	

			Spikelet				
			Lower Glume	Upper Glume		Lemma 1	
Sex of Lower Floret	Stamen Number	Anther Tips.	Nervation	Tip	Nervation	Tip	Nervatic
♂	2	glabrous	3	truncate, obtuse or subacute	3	truncate, obtuse or subacute	3
♂	2	glabrous	3	truncate or obtuse	3	obtuse, subacute, truncate or acuminate	3
♂	3	glabrous	3	acute or setaceously acute	3 - 5	acute or acuminate	3
♂	2	glabrous	3	truncate, acute, obtuse	3	subobtuse or acute	3
♂	2	glabrous	3	obtuse or truncate	3	subacute, obtuse, acuminate	3
♂, or neuter	2	glabrous	3	obtuse	3	obtuse	3
♂	3	glabrous	3 - 5	acute	3 - 9	acute	5 - 7
neuter	2	penicill-ate	3	obtuse or acute	3	obtuse or acute	3 - 5
♂	2, rarely 3	glabrous	3 (-5)	entire	3 - 5	entire	3 - 7
♂	3	glabrous	3	entire	3	entire	5 - 7
♂	3	glabrous	5	acute to acuminate	7	trilobed	7
♂	2	glabrous	3	acuminate	3	acute	3

ma 1	Palea 1	Lemma 2				
Nervation		Callus Type	Callus Length (mm)	Nervation	Indumentum	Awn Length (cm)
3	+	truncate to bipointed	0.4-1.0	(5-)7	pubescent	1.4-6.5
3	+	pungent	1.0-2.0	7(-9)	pubescent	3.5-6.0
or)						
3	+	truncate or pungent	0.5-1.0	5-9	pubescent	1.5-4.5
3	+	various	0.5-1.5	5-7	pubescent	1.3-8.0
3	+	bipointed	0.6-1.5	5-7	pubescent	5.0-12.0
3	+	obtuse	Ca. 0.3	5	pilose	0.7-1.9
5 - 7	+	obtuse	< 1.0	7-11	glabrous to pilose	0.8-1.4
3 - 5	-	pungent	2.0-3.0	7	pubescent	12.0-16.0
3 - 7 (-8)	+	obtuse to bipointed	0.2-0.8	5-9	glabrous to pilose	0.4-3.2
5 - 7	+	pungent	1.5-4.0	5-9	pubescent	2.0-20.0
7	+	obtuse	1.0	9	glabrous to pilose	1.1-1.5
3	+	?	minute	?	pubescent	1.7

floret.

Conert, (1957 p.229) has compared the Arundinellean spikelet morphology against the norms for the subfamilies Festucoideae and Panicoideae and concludes that it corresponds to neither type. In fact, the Arundinellean spikelet resembles the Festucoid type on most points: disarticulation occurs between the upper and lower florets, the spikelets are more or less narrow, pointed and laterally flattened, the glumes are large and more or less membranous and the lemma and palea enclose the fruit as in the Festucoideae. However, in the Arundinelleae, the lower floret is male or sterile and the upper is hermaphrodite, as in the Panicoideae, and the Arundinelleae differ from most Festucoideae in this character. Conert also points out that there are vegetative features of the Arundinelleae (which will be considered later) which place them intermediately between the two subfamilies and he suggested that a separate sub-family could be considered for this tribe.

There are a number of spikelet variations which have been discussed in more detail by Phipps (1964). The most significant of these is the tendency for spikelets to be disposed in triads. As seen in Table 1, many species of Loudetia have spikelets arranged in ones or twos, the arrangement in triads being more tristachyoid in nature. A second trend that may be considered is the size of the spikelet. Large spikelets tend toward the tristachyoid type. This is accompanied by an increase in awn size. The nervation of the lemma

of the lower floret and the lower glume show some variation in the loudetioid genera and have been used as diagnostic criteria in formation of sections and genera. The type of callus of the upper floret is quite variable in some subgroups of the genus (e.g. Typicae) and in others it is of diagnostic value (e.g. Pungentes, Flammidae). Finally one trend which is noteworthy with respect to the whole tribe is the variation in the number of stamens which is found to be consistently three in some taxa (e.g. Acuminatae, Pleioneura) but more usually two in Loudetia as a whole.

(b) Inflorescence

The inflorescence of Loudetia varies from an open panicle in its typical form to a panicle in subsection Flammidae and a dense spiciform panicle in subsection Densispicae.

(c) Vegetative Characters

Most species of Loudetia are perennial, though annuals do occur. The annual species are generally very robust and often have very large spikelets. Other vegetative characters have been mentioned by Phipps (1964), and the anatomical work of Conert (1957) will be considered later.

(d) The application of conventional criteria in the classification of Loudetia and Rattraya

The following keys to the genera, sections and subsections have been adapted from Hubbard (1936) and Phipps (1964, 1966):

Key to the genera

Lower glume 5-nerved, leaf blade with a pseudopetiole

..... Rattraya Phipps

Lower glume 3-5 nerved, leaf blade without a pseudo-petiole ..... Loudetia Hochst. ex Steud.

Key to sections of Loudetia

Perennials, or occasionally annuals; palea of the lower floret present; stamens 2 or 3, lower floret male, anthers with glabrous tips; awn up to about 13.0 cm long:

Lemma of the lower floret 3-nerved; lower glume 3-nerved; anthers 2 or 3 ... Section: Loudetia

Lemma of the lower floret 5-7 nerved; lower glume 3-5 nerved; anthers 3 ..... Section: Pleioneura

Annual; lower floret neuter; palea of lower floret absent; stamens 2; anthers with penicillate tips; awn up to about 17.5 cm long ..... Section: Lophanthera (Loudetia togoensis)

Key to the subsections of section Loudetia

Lemma of the upper floret glabrous to pubescent; callus pungent, truncate, emarginate or bipointed, 0.4-2 mm long; awn mostly 2.5 - 12.5 cm long; culms slender or stout:

Perennials mostly; lemma of the lower floret at least 3/4 length of the spikelet; awn up to 7 cm long:

Panicle sometimes long and narrow but never dense spiciform, branches usually more than 1 cm long:

Stamens 2; upper glume and lemma of the

upper floret truncate, obtuse or sub-acute; upper lemma 5-7 nerved:

Callus of the upper floret truncate, emarginate or bidentate 0.4 - 1 mm long .... Subsection: Typicae

Callus of the upper floret sharp, pungent, 1-2 mm long ..... Subsection: Pungentes

Stamens 3; upper glume and lemma of the lower floret mostly acute or setaceously acute; upper lemma 5-9 nerved, but mostly 9-nerved ..... Subsection: Acuminatae

Panicle dense and spiciform, its branches at most 6 mm long .... Subsection: Densispicae

Annuals; lemma of the lower floret 1/2-1/3 length of the spikelet, awn 5 - 13 cm long ..... Subsection: Annuae

Lemma of the upper floret long pilose; callus very short (ca.0.3 mm); truncate; awn up to about 2.5 cm long; reed-like perennials; culms stout; panicle up to about 60 cm long and densely contracted ..... Subsection: Flammidae

The Loudetioid Spikelet

The spikelets of Loudetia and Rattraya show more specific features than are mentioned in the general account of the whole tribe by Phipps (1964) and these are included in the description which follows:

Spikelet: lanceolate and gaping, 2-flowered, lower floret usually male (neuter in Loudetia phragmitoides and Lophanthera (L. togoensis); rachilla short, glabrous, readily disarticulating between the florets.

Glumes: ± lanceolate, acute or acuminate, the lower well-developed but the smaller of the two, usually three-nerved (3-5 nerved in Pleioneura and 5 nerved in Rattraya petiolata). The upper frequently as long as the spikelet, usually 3-nerved (3-5 nerved in the Acuminatae, 3-9 nerved in the Pleioneura and 7-nerved in R. petiolata).

Lower floret: Lemma similar to upper glume, usually 3-nerved (3-5 nerved in the section Lophanthera and 7-nerved in R. petiolata); Palea somewhat shorter, narrow, 2-keeled along the 2 nerves (absent in the section Lophanthera).

Upper floret: narrowly lanceolate with a short bearded or basal callus; lemma smaller than that of the lower floret, 5-11 nerved (the number often being characteristic of the section or subsection), geniculately awned from the sinus between the two apical lobes; palae smaller than the lemma, similar to that of the lower floret but the keels are narrowly winged in the section Pleioneura.

Lodicules 2, ± fleshy, truncate above and cuneate below;

Stamens 2 (or 3 in section Pleioneura, subsection Acuminatae and R. petiolata).

Ovary glabrous, with 2 distinct styles and plumose stigmata.

Caryopsis oblong or ellipsoid, tightly enclosed in the

lemma and palea.

The sections and subsections of Loudetia

The most comprehensive work on the morphological facies of the species is that of Hubbard (1937). Information on species described since this work is mostly available only as the type descriptions. Some of the general features of the subgeneric taxa will be considered here.

Section I. Loudetia C.E. Hubbard

Type: L. elegans Hochst. ex A.Br.

= L. simplex (Nees) C.E. Hubbard

Morphologically this section appears quite heterogeneous, though the consistent features mentioned in the key have warranted its separation from the other sections. It contains the largest number of species and is divided into six subsections.

Subsection a. Typicae C.E. Hubbard

Type: L. elegans Hochst. ex Steud.

= L. simplex (Nees) C.E. Hubbard

The 'typical' subsection is also found to be the most variable. There are nine species in this subsection which may be roughly divided into two groups on the basis of the callus of the upper floret and the size of the plants. The L. arundinacea complex consists of four species which are somewhat robust, up to 3 feet or more tall and with a truncate or slightly emarginate callus. The L. simplex complex comprises 5 species, L. simplex itself being separated into two sub-

species. These grasses are often smaller and the callus is always emarginate and 2-toothed.

There is a great deal of variability within species such as L. simplex and L. arundinacae and there is some question as to the rank of some of the so-called species in this subsection, but more studies are necessary before any conclusions can be reached.

These species come from west, tropical and southern Africa and have an important secondary geographical range on Malagasy.

Subsection b. Pungentes C.E. Hubbard

Type: L. demeusei (De Wild.) C.E. Hubbard

The four species of the Pungentes are characterised by a fairly long sharply pointed callus. They appear to be a fairly homogeneous group, occurring mainly in West-Central and Southern Africa.

Subsection c. Acuminatae C.E. Hubbard

Type: L. acuminata (Stapf) C.E. Hubbard

The taxonomy of this subsection has recently been reviewed by Lubke and Phipps in Correia, Lubke and Phipps (1967). The six species are distinguished from all the others by the presence of three anthers and usually, a 9-nerved upper lemma. Also, the upper glumes and lemma of the lower floret are mostly acute or setaceously acute. The subsectional characters are well defined but one finds a fair range of variability in overall spikelet morphology of the taxon and much in vegetative morphology. The species range from west tropical

Africa to southern Africa, Malagasy and Arabia.

Subsection d. Densispicae C.E. Hubbard

Type: L. densispicae (Rendle) C.E. Hubbard

The six species of the Densispicae appear rather questionably grouped since they apparently have little in common apart from the fact that the spikelets are arranged in a dense, spike-like panicle. There is a fairly large variation in panicle and spikelet size and in morphological characters. Two of the species are annual and the others perennial. They occur sparingly throughout tropical and southern Africa.

Subsection e. Annuae C.E. Hubbard

Type: L. hordeiformis (Stapf) C.E. Hubbard

Four species have been described in this subsection, though as there is a great deal of similarity between L. annua and L. bidentata, the latter may be a synonym of L. annua. The Annuae are all annuals with long awns which have a well-developed column and bristle. They occur from West Africa, stretching eastwards to the Sudan.

Subsection f. Flammidae C.E. Hubbard

Type: L. flammida (Trin.) C.E. Hubbard

The two species of this subsection have been held to be very similar although they occur on separate continents, i.e. tropical Africa and tropical South America. The spikelets are small and the lemma of the upper floret is loosely pilose with long hairs, and bears a short awn with a much reduced column. The plants are reed-like perennials and have a long, dense, somewhat contracted panicle.

Section 2. Pleioneura C.E. Hubbard

Type: L. ramosa (Stapf) C.E. Hubbard

The nervation of the lemma of the lower floret is the character which distinguishes the three species of this section from the others and allies them with Rattraya petiolata. The section is also well segregated from the others on the short truncate type of callus and the purple variegation of the spikelets. Most loudetioid species have a buff to orange colour to the spikelets whereas Pleioneura has spikelets which are predominently green but purple variegated as in the Danthoniopsoid genera or Arundinella. They occur in South, South West and North Central Africa.

Section 3. Lophanthera C.E. Hubbard

Type: L. togoensis (Pilger) C.E. Hubbard

The solitary species in this section has many peculiar features, such as penicillate anther tips and the lack of a palea on the lower floret which is also neuter. The spikelets and awn are large and resemble those of Tristachya in many respects. This widespread annual occurs in west and north central Africa.

Incertae sedae

L. jaegeriana A. Camus

The single specimen of this plant from Sierra Leone is reported to be close to subsection Typicae (Phipps, 1966) or section Pleioneura (Camus, 1954). It is however only represented by immature material and consequently is imperfectly understood.

Rattraya Phipps

Type: R. petiolata Phipps

As has been mentioned previously, this species is considered to be very similar to members of section Pleioneura.

3. The numerical taxonomic method

A brief survey thus indicates that in general only a few characters are used in characterising any one section or subsection of the genus. The question that one is faced with is whether one should operate on the basis of a monothetic classification, whereby, for example, all species with a dense spike-like panicle would be classified in the Densispicae? Does this system sufficiently reflect the relationships between the different taxa of the genus? The obvious solution to a study of a different tribe such as the Arundinellae is to adopt an objective numerical approach such as was first recognised by Adanson in the 1760's. Adanson was one of the first botanists to recognise that certain parts of the plant were not more important than others in classification. Though other botanists used this method before him, the results of Adanson's efforts recorded in "Familles des Plantes," published in 1763, are the most publicised. Working with almost 1700 genera he used 65 different characters taken from all parts of the plant to establish his 65 systems of classification. The task which he accomplished has led Stafleu (1966) to observe that "the extreme diligence with which he composed his systems has created the impression of a statistical method."

The Adansonian approach was in fact 200 years ahead of

its time. It is only in the last few decades that the use of many characters simultaneously in a taxonomic study has become feasible with the introduction of the digital computer to biological research. Adanson was thus the first to oppose a priori weighting of characters, an aristotelian philosophy which has existed to the present day (Stafleu, 1966; Sokal and Sneath, 1963), and introduce an inductive and empirical approach to classification. Stafleu (1966) has also suggested that the methods of Adanson have been misunderstood when numerical taxonomy is referred to as Adansonian. Adanson did not rest content with equal weighting, but used his method to discover which characters are important and which immaterial. This is, however, one of the approaches that may be taken in a numerical taxonomic study and is in fact one of the most important aspects of a study where numerous characters are considered simultaneously. The Adansonian approach may be considered very similar to that used in numerical taxonomy, in both cases the final judgement resting with the investigator.

Many arguments have been given for and against numerical taxonomy (e.g. Sokal and Sneath, 1963; Mayr, 1965). Some of the points in favour have been very aptly summarised by Gilmartin (1967). The taxonomist must carefully define, defend and explain his techniques and approach. By using computer methods he is able to handle many more data in the form of characters and individuals. He is forced to define his characters clearly, thereby facilitating reproducibility

of the work and is able to relate the chosen characters to each other. As with the classical approach, the problem is basically the choice and use of characters, and the more attention given to this point the better an understanding of the relationships between individuals will result.

With the aim of moving towards a better understanding of generic limits of the tribe, Phipps (1969) has used a very large number of morphological characters in a numerical taxonomic study. Moreover, a numerical approach is not limited to the use of morphological characters, and since Conert (op. cit.) and Jacques-Félix (op. cit.) have indicated the importance of anatomical characters in the Arundinelleae, a more detailed taxonomic investigation has been carried out. Li (1969) has made important contributions to a study of the leaf anatomy of a large number of species and in the present work, the anatomical relationships among members of Loudetia are studied in detail.

4. The use of anatomy in grass taxonomy

The introduction of anatomy into grass taxonomy is not new. The classical work of Duval-Jouve on awn anatomy appeared in 1871. Later, in 1875, he apparently was the first to attempt to use leaf anatomy in the systematics of a number of grasses. The work of Grob (1896) on the grass leaf epidermis, though quite comprehensive, did not make any major contribution to taxonomy at that time. Other workers of this era such as Pée-Laby (1898), Lewton-Brain (1904) and Schwendener (1890) presented a good deal of anatomical information which

was to serve as a basis for more study later in the twentieth century. Grass taxonomists of the early twentieth century were often more concerned with homology and phylogeny (e.g. Arber, 1923; Bugnon, 1921) and it was not until the work of Avdulov (1931) that the importance of anatomy as well as cytology and embryology became fully realised. Prat (1932, 1936) continued the use of microscopic characters and subsequently more and more importance has been given to this type of study. Prat (1936, 1960) was the first to formulate a classification of the grass family taking into account all characters (holotaxonomy). Other systems such as those of Pilger (1954), Auquier, (1963), Tateoka (1957) and Jacques-Félix (1962) have all been based partly on anatomical information. Similarly, phylogenetic theories such as those of Stebbins (1956) on the evolution of grass genera and Tran (1966) using awn anatomy have emerged using anatomical data. At the tribal or lower level anatomical studies have also proved useful (e.g. Conert (1961), Soderstrom (1967). The most comprehensive work that has been done on the family is that of Metcalfe (1960) in which a large number of species have been covered.

Metcalfe (loc. cit.) has indicated that the most useful organs for anatomical study of grasses are the leaves. Many different characters are available from leaf epidermis and leaf anatomy studies as he has indicated. Arber (1934) in her work on the Gramineae has shown how spikelet anatomy may be useful in taxonomy. The use of awn anatomy in taxonomy is however relatively recent. The work of Jacques-Félix (1950)

and Conert (1957) on the Arundinellean awn anatomy has already been discussed. The most comprehensive study of the awn anatomy of the whole family is that of Tran (1965).

Advantages and limitations in the anatomical method

There are advantages and limitations to an anatomical study of a group of plants, some of which will be considered below.

a. Availability of material

Although it is desirable for fresh material to be studied, for the most part suitable material can only be provided from herbarium specimens, which also presumably will provide an accurate identification. Except in the case of very poor material or incomplete specimens, each taxon can be adequately studied. In instances where there is immature floral material of a specimen, the use of leaf anatomy can be to distinct advantage.

b. Interpretation of the results

Limitations in the anatomical method have become evident in the last decade in that there is an abundance of information obtainable, the results of which cannot easily be subjectively comprehended. However, with the advent of digital computers, this limitation is now lifted and greater understanding is possible from the data at hand. The use of anatomical characters in numerical taxonomy is as yet a rarity, though Stant (1963), for instance has used anatomical characters in making a numerical taxonomic study of the Alismataceae.

c. The level of application of anatomical characters

Metcalfe (1954) has considered the use of microscopic characters at different hierarchial levels in angiosperm classification and points out that:

i. Above the rank of family, taxa are highly heterogeneous when these characters are used.

ii. Families show a good deal of homogeneity and appear more or less natural. These characters have indicated where revisions of families are desirable.

iii. The boundaries between genera are usually not very clearly defined on anatomical characters, except in the Gramineae where epidermal characters of the leaves appear quite diagnostic.

iv. Species are not readily distinguished by microscopical differences unless the genus is first established. This is partly due to intraspecific variation, (see below) and partly due to similar variations in more than one genus, e.g. Stipa hookeri Stapf is very similar to Ammophila arenaria (L.) Link with respect to leaf anatomy. When making an anatomical study at any one of these levels it is thus important to bear these points in mind. Failure to observe some anomalies that occur, e.g. the similarity between Stipa hookeri and Ammophila arenaria, could lead to erroneous conclusions.

d. Intraspecific variation

Metcalf (loc. cit.) recognises two factors to be considered with respect to variation within a species.

i. Structure in relation to the environment

When there are minor structural variations in a plant,

there is the possibility that these are controlled by heredity, environment or a mixture of both. Variation of features such as layers of palisade cells in the leaf or numbers of stomata per unit area are known to occur due to environmental factors (see Daubenmire, 1959; Davis & Heywood, 1963).

ii. Sporadic intraspecific variation

It is also well known that variations occur in different parts of the same plant, since the structure of any species varies within certain limits. The problem is to estimate the limit of this variation if this is possible.

Bailey (1951) points out that the intraspecific variation cannot be ignored, although it is less significant when discussing the relationships of larger groupings. Although this study is primarily concerned with higher groupings, an attempt has been made to estimate this variability. Naturally an autecological study with the aim of showing the effect of the environment is impossible in a taxonomic treatment of this magnitude. Likewise, a study of leaves from different parts of the plant to estimate variability would be a project in itself. Instead, representatives of some taxa from more than one locality have been included in this study, the exemplar method of Sokal and Sneath (1963) (discussed in chapter 3 - Numerical methods). In addition, in order to minimise variability due to age, position on the plant etc., samples were taken from the same area of the plant in each case.

The effects of the environment on grass anatomy has been studied to some extent (e.g. Nikolaevsky and Nikolaevskya,

1967). Some effects of environmental conditions on the anatomy may be inferred from results reported in the literature.

5. The objectives and approach to an anatomical taxonomic study of Loudetia

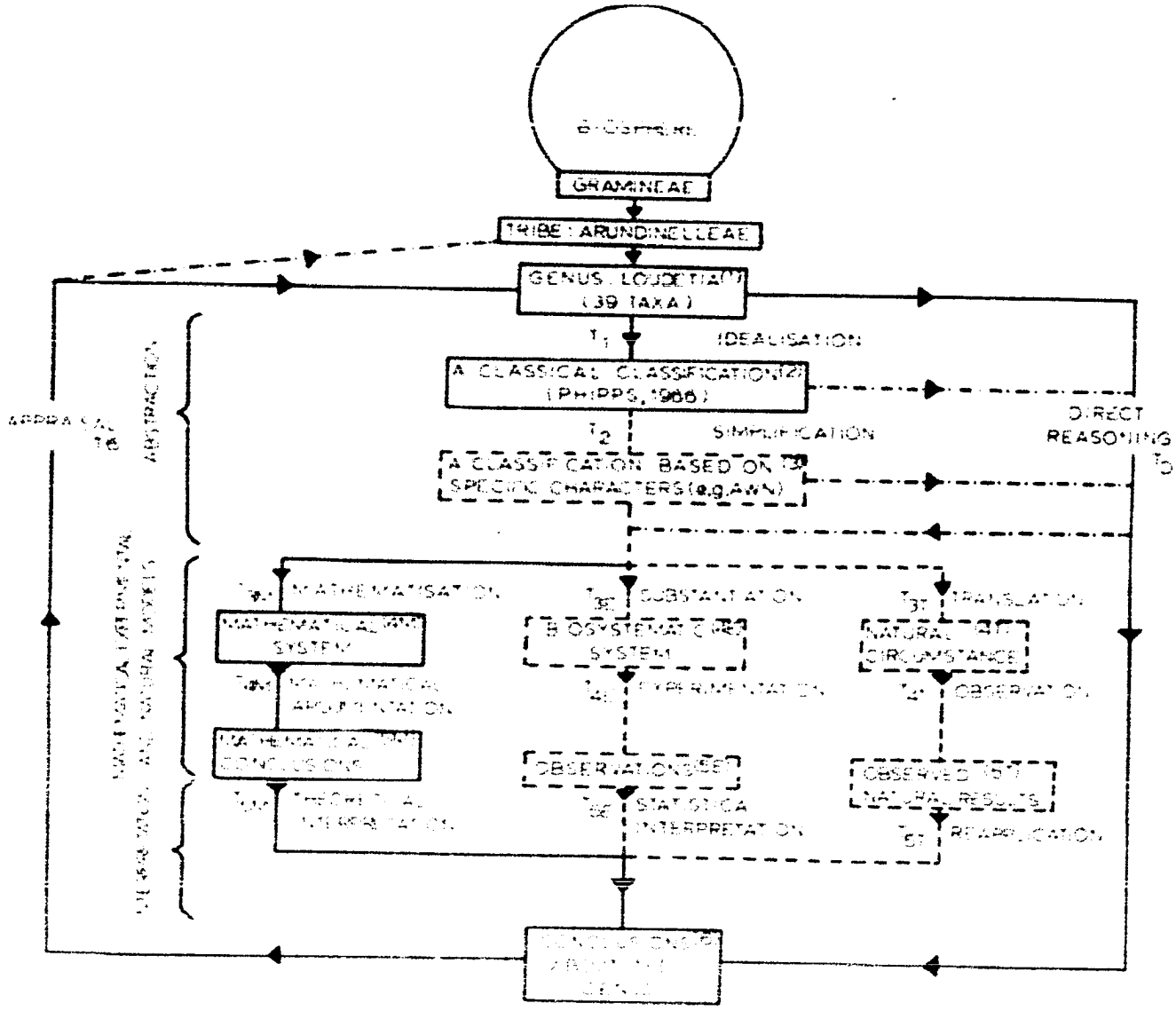
(a) A model for a classification study

It has already been indicated that the object of study is the genus Loudetia sensu Phipps, and anatomical characters will be used in a numerical taxonomic manner. A clear definition of the objectives and the process of this approach is more easily conceived if a model such as Figure 2 is constructed. This concept has been adapted from Chorley (1964) who applied a similar model in geography.

The diagram is composed of a series of steps (1-6) in each of which, some aspect of the genus Loudetia is considered, e.g. classification, observation etc. These are connected by transformations (T1 - T6) illustrating the method of reasoning. The transformations at each step introduce the possibility of "noise" (cf. information theory) in that the process of translation results in a certain loss of information, some of which may have been useful and on the other hand, the introduction of new, irrelevant information. The most efficient model would therefore be that which introduces the least noise. The model can be examined in three sections:

a. Abstraction

In this sequence of steps a conceptual model is developed and huge amounts of available information are discarded (T1 and T2) thus introducing much noise. The genus Loudetia rep-


GRAMINEAE
TRIBE : ARUNDINELLEAE
GENUS : LOUDETIA
(39 TAXA)
IDEALISATION
A CLASSICAL CLASSIFICATION (2)
(PHIPPS, 1966)
SIMPLIFICATION
DIRECT
REASONING
A CLASSIFICATION BASED ON
SPECIFIC CHARACTERS (e.g. AWN)
MATHEMATISATION
SUBSTANTIATION
TRANSLATION
OBSERVATION
STATISTICAL
INTERPRETATION
REAPPLICATION

resents a tiny segment of the biosphere on which the study is conducted. In arriving at this section of the biosphere a certain amount of noise is already introduced in that there is confusion as to the composition of the genus Loudetia. However, this abstract process is one of the most important and difficult portions of the system since it forms the basis for subsequent steps and its success depends largely on the taxonomic ability of the author. The source of noise is largely due to the subjective judgement of the author, viz. his concepts of what is important in arriving at his classification. The result of this process in the Arundinelleae has already been considered in the review of the literature. The classifications of Phipps (1966), Conert (1957) Clayton (1967), among others all bypass the major portion of the model via direct reasoning (T_D) to arrive at conclusions about the classification (6).

The model is often subjected to simplication (T2) by discarding some of the information. This transformation is often less noisy than T1 because it is often more fully understood. In this case, the classification of Phipps was not subjected to simplification, i.e. to a further classification by abstraction since this may have been even less satisfactory than the original classification.

b. Mathematical, Experimental and Natural models

Once the classification is formulated it is possible to subject it to various methods of exploitation.

1. Mathematical models

This is the most important aspect of the investigation. The first stage is the transformation of the information from words into mathematical symbols ($T_{3\,M}$) in order to produce the system on which mathematical operations may be performed ($T_{4\,M}$) resulting in the mathematical conclusions (5M). These conclusions are then susceptible to theoretical interpretation (T_{5M}) so that conclusions about the genus may be reached. Chorley (1964) points out that these mathematical conclusions do not provide explanations about the genus but merely allow conclusions to be drawn from the original mathematical assumptions.

ii. Experimental models

In this case experimental models may be considered as biosystematic studies of the genus which would lead to observations (5E) about the taxa which could then be subjected to statistical interpretation. Biosystematic studies have proved of great value in establishing and confirming relationships in grasses, but have not been pursued here.

iii. Natural models

This system involves the observation (T_{4T}) of the organisms in their natural environment or a simulated natural environment (4T) to examine what results occur in nature (5T). Although this method was not carried out in this thesis, the environmental effects are taken into account when conclusions are drawn about the classification of the genus.

c. Interpretation and appraisal

There is a certain amount of noise introduced in the

interpretation transformation (T5) since there are different ways in which the results may be interpreted.

Finally, appraisal (T6) involves the checking of conclusions that have been arrived at with all the information known about the members of the genus, so that a hypothesis may be developed. Some conclusions about the tribe as a whole may be drawn, or indeed the Gramineae or the whole biosphere which is also subject to appraisal.

This final step is the most important one since the success or failure of the process depends upon the appraisal of the investigator.

(b) The Application of the model

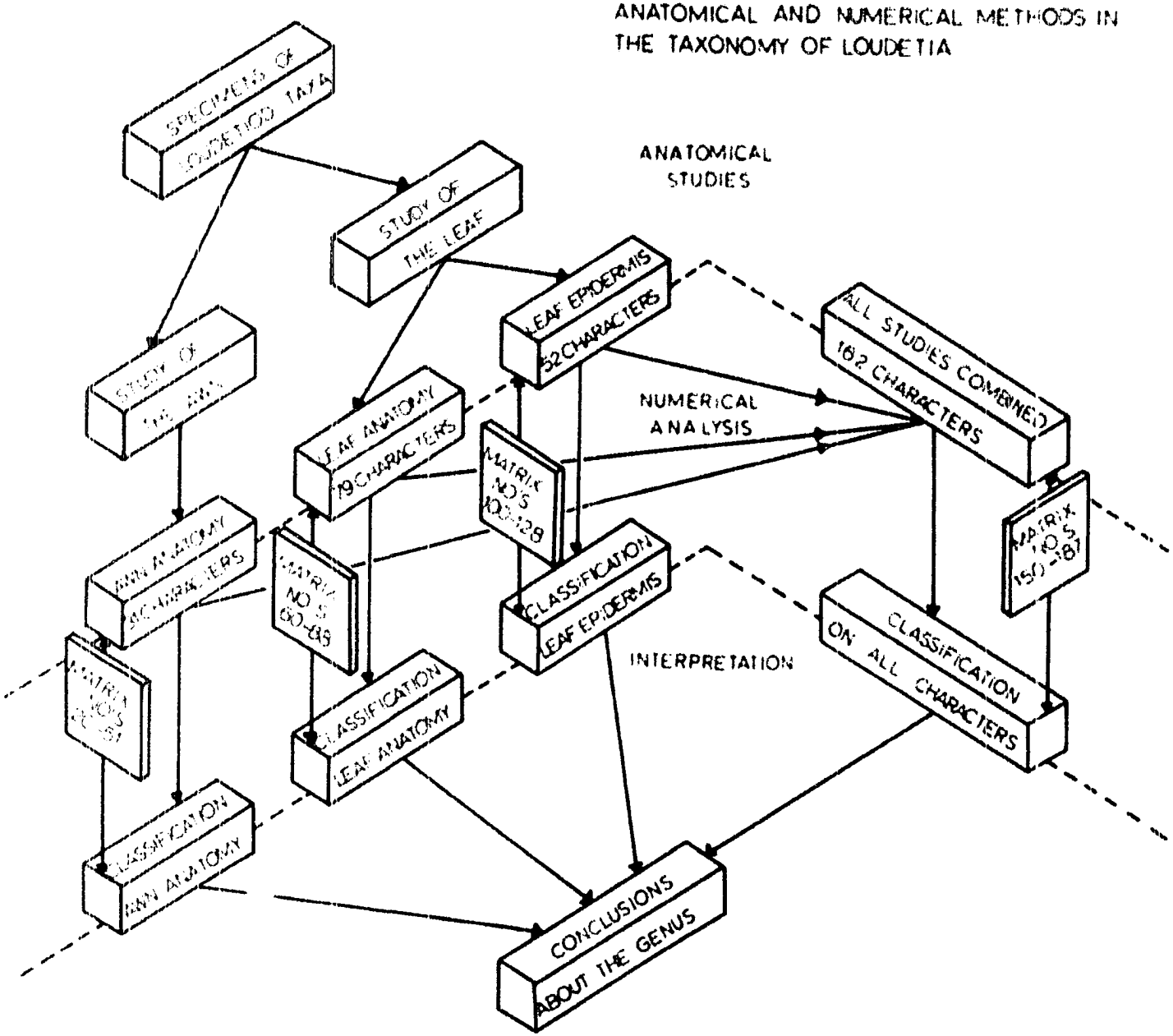
The approach that was used in applying this model to a study of the genus Loudetia may be summarised as follows. This summary may be followed in the model of the whole system (Fig. 2) or the sectional flow chart (Fig. 3) which shows the principal steps in more detail.

1. The theoretical model of the genus Loudetia is abstracted from the biosphere, after the principles of Phipps, (1966) (Fig. 2, no. 2). This forms a nucleus of 39 taxa which were studied in detail.

2. The main task of the thesis is concerned with the make-up of the different groups (Sections, Subsections, etc.) of Loudetia and whether these are valid or whether an alternative hypothesis may be generated.

3. Anatomical methods, which are now commonly being used in grass systematics are applied in an investigation of: a)

FIG. 3. FLOW CHART OF THE OVERALL APPLICATION OF ANATOMICAL AND NUMERICAL METHODS IN THE TAXONOMY OF LOUDETIA



the leaf epidermis and cross sectional anatomy (Chapter 4) and, b) the awn of the lemma of the fertile floret (Chapter 5).

4. The leaf anatomy, leaf epidermis and awn anatomy were considered in some detail so that as many useful characters as possible could be compiled. The characters were coded in quantitative terms before this information was subjected to analysis (Fig. 2, T_{3M}).

5. The numerical methods that are used in analysing the results of the anatomical investigations are reviewed in Chapter 3.

6. The numerical taxonomic study is concerned with the classification and ordination of the representatives of the genus in four different ways, based on different sets of characters (Figure 3):

a. Characters of the leaf anatomy (79) are used separately (Chapter 4).

b. Characters of the leaf epidermis (52) are considered separately (Chapter 4).

c. Awn characters (31) are used in a separate study (Chapter 5).

d. All the characters (162) are combined to give a final comprehensive classification (Chapter 6).

7. Additional information about representatives of the genus was obtained from an investigation of the evolution of the awn. This is particularly important when phylogenetic relationships are considered.

8. The results of the different classifications are

compared and using all the available information, conclusions about the genus are reached (Figure 2, no. 6), (Figure 3) and some hypotheses are formed (Fig. 2, no. T_6).

CHAPTER 2

MATERIALS

Plants and seeds have been collected in the field by a number of botanists and sent to UWO where the plants are grown in a greenhouse under uniform conditions. A large number of the plants originated from seed collected by Professor J.B. Phipps during a collecting trip to Africa and Malagasy in 1963.

Viable seed of most Loudetia species was quite easy to germinate and grow, except for Loudetia togoensis, so that a large number of accessions produced healthy plants. However, in many cases the plants of Loudetia grow in remote areas from which no new collections have been possible since these studies were initiated, so that in these cases, the examination of herbarium specimens was inevitable. Better anatomical sections can be obtained from living material and plants grown in a uniform environment should not reflect environmental abnormalities. The manner in which these difficulties were accounted for when herbarium specimens were studied are discussed later. Herbarium specimens were obtained from a number of institutions; which are listed in Table 2.

A list of the specimens which were examined and used in the anatomical or morphological studies are listed in Appendix

Table 2

List of Herbaria which supplied specimens

Herbarium and Locality	Abbreviation
Botanisches Museum Berlin-Dahlem, Germany.	B
British Museum (Natural History) London, Great Britain.	BM
Herbarium Universitatis Florentinae Instituto Botanico, Firenze, Italy.	FI
Kew, Great Britain	K
Muséum National d'Histoire Naturelle Paris, France.	P
Botanical Research Institute, National Herbarium, Pretoria, South Africa.	PRE
Rhodesia Government Herbarium, Salisbury, Rhodesia.	SRGH
U.S. National Museum (Department of Botany), Washington, D.C., U.S.A.	US
University of Western Ontario (Department of Botany), London, Ontario, Canada.	UWO

1. The collector and collector's number, origin and source of the material are also given. Each specimen was designated a certain OTU number which is referred to in the subsequent studies. More information about the specimens or OTUs in the anatomical and numerical studies is given in Chapter 3, the numerical methods, and subsequent chapters.

CHAPTER 3

NUMERICAL TAXONOMIC METHODS

1. Introduction

The numerical methods which are now commonly being used in biological taxonomy are not confined to this discipline. Other fields such as psychology, geography and ecology which are also concerned with the relationships between individuals, all use similar methods and in some cases have been using them for some time prior to the advent of rapid computational techniques. Unlike psychology, for example, where the data is normally in the form of numerical tests, biological taxonomy is hampered by the fact that much of the data may be qualitative, requiring certain adjustment or quantification, before it can be analysed by numerical techniques. For the most part it has been, and still is, largely descriptive and has consequently lagged behind its sister subjects.

Most of the numerical techniques that were first used in taxonomy have been covered by Sokal and Sneath (1963). An abundance of literature on numerical taxonomy has followed, and many of the more recent numerical taxonomic works in botany are reviewed by Williams and Dale (1965), and Crovello (1966). It would be out of place to consider much of the previous work

here so only those references that are pertinent to the methods of this study will be reviewed.

In a multivariate taxonomic study the principle entities that one is concerned with are the taxa, which may be variously defined as the individual plants, species, genera etc. Sokal and Sneath (1963: 121) refer to these entities as operational taxonomic units (OTU's). Of great importance are the characters that are capable of variation or may occur in various states from one OTU to the next. Since there are several to many such variables characterising an OTU the analysis is referred to as multivariate. If only one character or variable is considered per OTU at a time, the analysis would be a univariate one. The outcome of a numerical study is largely dependent on the choice of OTU's and characters, and these important aspects will be considered in detail below.

When the data has been suitably quantified to produce an n x t data matrix whose t columns represent the OTU's, and n rows represent the characters, various mathematical techniques may be applied to extract the maximum information from this data. The data matrix may be analysed from two points of view:

1) OTU analysis, where attention is focused on relationships between all pairs of OTU's, and

2) Character analysis, where the principal interest lies in relationships between all pairs of characters. Various mathematical models are used in defining these relationships. The definition of relationships between OTU's or characters is not readily subject to interpretation unless further analyses are

involved. These usually take the form of a classification where the individuals are sorted into groups, the members of which have certain properties in common, or ordination where the individuals are arranged on axes with their properties determining their positions.

Finally the results must be suitably presented so as to show the relationships between individuals. The presentation must lend itself to comparison between different types of analyses and perhaps further analysis in comparing the results.

2. The Operational Taxonomic Unit (OTU)

Since taxonomy is primarily concerned with the entities that are to be classified, these must be clearly defined.

The hierarchic level of taxonomic units may vary from one numerical study to the next and consequently the term operational taxonomic unit (OTU) has been applied by Sokal and Sneath (1963: 121) to refer to whichever taxon is being used in the study.

The major aim of this thesis is to investigate the genus Loudetia with respect to subgeneric groups. The most suitable taxa for study are therefore the specimens which have been identified as members of the genus. As far as was possible specimens or OTU's were selected from every species of the genus. Seeing that the relationships among and between species per se was not of particular concern, the number of specimens was limited and the exemplar method of Sokal and Sneath (1963: 161) was followed. Each species was normally represented by a single representative, these OTU's being referred to as the

exemplars of the taxa that they represent.

The problems in the choice of OTU's and especially exemplars of taxa have been outlined by Crovello (1966) and Sokal and Sneath (1963: 158). In this study the choice of the exemplars has been mainly restricted by available material for study. When there are a number of specimens available there are often indications of variability of some characters. A possible solution is thus to introduce into the study one representative of each varying constituent of each species, which is the approach made by Moss (1967), and in this study. If the preconceived ideas about a species is correct, these representatives (exemplars) should be the first to cluster together. A more recent paper by Moss (1968) indicates that the choice of a number of different exemplars for species of mites had little effect on the similarity values that were obtained.

In each individual study of leaf anatomy, leaf epidermis, and awn anatomy exemplars are taken which represent the 39 taxa of Loudetia and Rattraya. Two of the species of Loudetia have subspecies each of which has been represented by an OTU. Some of the species, particularly the widespread and variable ones, such as Loudetia arundinacea and L. simplex, are represented by additional exemplars. In this way it was hoped to obtain some indication of the intraspecific variability since this had been expected to be fairly large in an anatomical study of this nature. The list of the OTU's for each of the studies is given in Appendix 1.

The present work is primarily concerned with the constitution of the infra-generic groups, and whether there is continuity or discontinuity between them. If one was to treat these groups (sections and subsections) as separate entities additional information might be forthcoming about their inter-relationships. The same characters which were obtained for the treatment of species as OTU's were used to describe the sections and subsections as OTU's in order to accomplish this. There are six subsections of the section Loudetia and together with the sections Pleioneura and Lophanthera, the genus Rattraya and L. jaegeriana (uncertainly placed), ten different OTU's are under consideration. These OTU's are listed in Appendix 1. Some of the subsections of section Loudetia exhibit a great deal of varibility so it is unreliable to assume a great deal from this analysis but an additional indication of affinity between the groups can be obtained. The methods whereby the characters for the group OTU's were determined will be described below.

3. The Characters

As Sokal and Sneath (1963) have indicated there are many points to be considered such as the numbers of characters, weighting, coding, etc. prior to compilation of data. Since the choice of characters may greatly influence the classification produced, numerical taxonomy has been open to much criticism on this basis. Kendrick and Weresub (1966) for instance, have shown how different sets of characters may produce vastly conflicting results in the classification of the Basidiomycetes.

In view of the bias, either intentional or unintentional, that may be introduced at this point, there are a number of factors which should be considered in making this first important step in numerical taxonomy as stable and as reliable as possible.

Firstly, it is important that a character is suitably defined. Sokal and Sneath (1963: 65) define a 'unit character' as "a taxonomic character of two or more states which within the subject at hand cannot be subdivided logically except for subdivision brought about by changes in method of coding." The characters used in this study will be considered according to this definition. All OTU's possess a number of characters and each is defined by the state in which the characters exist.

(a) Numbers of Characters

As a general rule a minimum of 60 characters are suggested for use in a numerical taxonomic study (Michener and Sokal, 1957). Sokal and Sneath (1963: 115) point out that there is no definite solution to this problem, but as the number of characters increases so the confidence limits with respect to a hypothetically true similarity value between two OTU's will narrow (page 115, fig. 5-7).

In this study the anatomical sections were examined with the object of obtaining every possible character which appeared useful in a classification of the OTU's under question. During the course of character coding, additional characters were found while others which proved to be invariant were excluded.

The characters that were used will be considered in more detail later.

(b) Weighting of Characters

Although it is traditionally held that in a numerical taxonomic study all characters are given equal weight there have been some proponents of character weighting. Lambert and Dale (1964) describe two types of weighting: a) a priori weighting or selection of characters and b) a posteriori weighting which occurs internal to the analysis. The first method has been largely proposed by Kendrick et al (1964, 1966) and was introduced since equally weighted characters resulted in "haphazard classifications" of the Basidiomycetes. The arguments of Kendrick and Weresub (1966) are essentially similar to those critics of numerical taxonomy who feel that such techniques cannot replace the experienced taxonomist who has many years of experience to draw on (Mayr, 1965).

The proponents of unequal weighting feel that the selection of characters or character weighting is necessary, because secondary or dependent characters very often occur which cannot be considered, unless some other primary feature is present. An example of secondary characters are those describing hairs when hairs may or may not be present. The secondary characters are given unequal weight for they are dependent on the more important primary characters. These methods have been adequately described in Procter and Kendrick (1963), Kendrick and Procter (1964), Kendrick (1965) and Procter (1966).

The second method of weighting has been used by Goodall

(1964, 1966a, 1966b) in the form of a probabilistic similarity index. Use of this method has been made by Clifford and Goodall (1967) in a numerical taxonomic study of the Gramineae. Statistically this method of weighting is sound, since a significance test is used and objective weighting is introduced by rarity of the characters.

Neither of these two forms of weighting have been applied in this study since the first is not theoretically applicable, and the programmes were not available to make use of Goodall's techniques. Long (1966) points out that the definition of Sokal and Sneath (1963: 65) of a unit character (op. cit) was not used by Kendrick and Proctor (1964) since a primary character can be further subdivided. The "secondary" characters should therefore be treated as unit characters. This is the approach that is followed in this study.

Unequal weighting of characters may be inadvertently introduced in the coding of the characters, or by the use of raw measurements without transformation. Two methods have been employed to transform or standardise the characters so that each one has equal weight (Sokal and Sneath, 1963: 144; Crovello, 1966). Firstly the rows of the data matrix, i.e. the characters, may be standardised by computing the mean and the standard deviation for the states of each character, and expressing each state as a deviation from the mean in standard deviation units (Sokal 1961). The second method, described by Cain and Harrison (1958), has been called condensation (Crovello, 1966). The range of the row measurments or character

states is condensed to a new range of zero to one. Condensation is simply the transformation of values from one scale to another. A variation of this method was used in coding of the characters in this study (see below).

(c) Types of Characters and Character Coding

In classical taxonomic studies the characters are generally considered in a descriptive sense. In numerical taxonomy, however, the approach is more precise since the characters must be coded or transformed into numerical values without loss of information. This is not always achieved as it largely depends on the approach used. Since coding is one of the important aspects of numerical taxonomy, there has been much discussion about the methods of coding (e.g. Sokal and Sneath, 1963: 74; Kendrick, 1964). According to the definition of a unit character (op. cit.) given by Sokal and Sneath each character has one or more states. When a character has only two states, coding is relatively simple as a + or -, 1 and 0, or +1 and -1. When characters with more than two states, multi-state characters, occur, different methods of coding must be sought.

Before entering with any further discussion on coding, however, it is appropriate to consider the types of characters that one is concerned with. These are essentially two broad categories, viz. (a) qualitative and (b) quantitative characters.

(a) Qualitative Characters

These characters represent features such as colour, shape

type, etc. They may be two-state or multi-state characters. An example of each in this study is presence or absence of midrib of the leaf, and shape of the vascular bundle in the leaf, respectively.

(b) Quantitative Characters

Measurements of size or abundance are examples of quantitative characters. Specific examples are the width of the leaf or amount of thickening or numbers of fibre groups, etc. Quantitative characters may be divided into:

(i) Discrete Characters, in which the states are represented by integer numbers; they may be two or multiple state characters. For example, the number of groups of phloem cells in a vascular bundle may be one, two, three or more than three.

(ii) Continuous Characters, where the character, such as length of the awn, is represented by a real variable. Continuous characters may be divided into a number of states so as to form discrete characters. Taking the example of length of the awn $\leq$ 5 mm may represent state 1, 6 - 10 mm - state 2, 11 - 20 mm - state 3, etc. The major drawback to this type of coding is that an OTU with an awn length of 6 mm (state 2) would be considered to be the same length as a second OTU with an awn length of 10 mm (state 2), whereas an OTU with an awn length of 11 mm (state 3) is more distant from the second OTU. The advantage of this method is that it can be used when a true mean value of a character has not been obtained, due to insufficient sampling, and a more suitable decision is to consider a range for that character. It does, however, lend itself to

bias since the range is determined by the investigator. It is not obligatory to adjust real variables to discrete states and wherever possible they were handled in a continuous form.

Coding of the Characters

The method of condensation, which gives equal weight to all characters, has already been described above. Many of the characters used in this study were multi-state or two state discrete characters with only a few continuous or real characters. It was therefore convenient to use a method similar to condensation. This term is not suitable for the method used because the values for the character states are often expanded, so it is referred to simply as adjustment.

Each character was given a range of variation represented by integers in the interval from 100 to 900. This range proved to be the most suitable one for coding and punching data on IBM cards. Two state characters, such as presence or absence of the midrib of the leaf were represented by the extremes of the range, 100 and 900 respectively. Multi-state characters which could be arranged in a linear order, i.e. discrete quantitative characters, were arranged at equal intervals along this range, e.g. amount of thickening along cell walls - none (100); little (366); moderate amount (633); abundant (900). Real variables, e.g. awn length, were adjusted to this same integer system as follows. The minimum value, i.e. the lowest for any OTU, was taken as 100 and the maximum value, the largest from any OTU, represented 900 and all other values were ranged between them. The computations were made using equation:

$$L = 800 \frac{A - MIN}{MAX - MIN} + 100 \qquad (1)$$

where L is the new integer value

A is the real value of X in question

MIN is the minimum value of X, and

MAX is the maximum value of X.

In the case of multi-state qualitative characters where it was not possible to arrange the states in a unique linear order, e.g. shape of vascular bundle, these were coded as follows. Taking the above example, states were recorded as round (100), otherwise (500); elliptical (100); otherwise (500); etc. so that each OTU would be represented by the presence of one or other of these states (in some cases more than one state may have been recorded as present for any one character) and a number of otherwise values. When the characters of every OTU had been coded the information for each multi-state character complex was condensed into a single variable. The method by which this was done is similar to that of Quadling (1967) who extracted the information from a number of attributes into a single attribute complex.

The techniques will be discussed in more detail below, but briefly this method consists of performing an analysis on each of the character-complexes, using the correlation coefficient to estimate affinity. The correlation matrix is subject to a principal components analysis, and the component scores for the OTU's are determined for each of the components. The component scores of the first principal component are taken

to represent the new character values for each of the OTU's. The component scores were adjusted by the method given above for real variables so that all character states were represented by integers in the range of 100 to 900. Only the first principal compoment was used in each case, although in some cases this represented as little as 40% of the variation. A more correct method could be to use all components which, in total, account for say 70% or more of the variance. Quadling (1967) used all components accounting for 80% or more of the variance, but one is limited in the amount of characters one can accommodate so only the first component was considered.

Problems arise in taxonomy when certain parts of an organism are absent so that missing characters occur. Various solutions have been offered to overcome this (Sneath 1957; Sokal and Sneath 1963: 162). There were only a few instances in which information was not available in this study and in the event of missing data the character was scored as zero.

4. The Hypothesis of Non-Specificity

According to the hypothesis of non-specificity (Sokal and Sneath 1963: 85), no distinct large classes of genes affect exclusively one class of characters such as morphological or physiological characters, or affect specific regions such as the leaves or flowers. If such a hypothesis is true, it might be thought that characters could be sampled randomly from different areas of the organism and yet achieve equivalent classifications. Rohlf (1965) applied a randomization test to the non-specificity hypothesis, and his results indicate that the

varibility that occurs between classifications based on different character sets occurs too often to be due to chance. He had suggested previously (Rohlf, 1963) that large portions of genes are limited to a single stage in the life cycle, sex or region of the organism, so that samples of characters from any one stage, sex or region would not give an overall estimate of phenetic resemblance. Furthermore it has been shown in a number of separate cases (Erlich and Erlich, 1967; Michener and Sokal, 1966; Johnston and Holm, 1968) that this hypothesis does not always hold. Due to the non-validity of the non-specificity hypothesis Michener and Sokal (1966) suggested that characters should be selected from many parts of the organism and represent different stages in the life history. Moss (1967) has taken this approach in his study of mites, though he has also used different sets of characters to test the hypothesis of non-specificity (Moss 1968).

The evidence from botanical investigations on the importance of the non-specificity hypothesis is somewhat limited, and consequently one of the major facets of this investigation is to test this hypothesis. Different organs were examined for anatomical characters so that natural character sets from the leaf anatomy, leaf epidermis, and awn anatomy were obtained. By classifying the OTU's characterised by each of these character sets independently, and then obtaining an overall classification based on all characters, a valuable test of the hypothesis of non-specificity is presented.

5. The Choice of Strategy

Following an examination of the OTU's to decide the states of the various characters the data is represented in n x t matrix whose t column prepresent the OTU's and n rows represent the characters. Representation of data in this form may be considered geometrically.

The data is considered to have a certain structure. In geometrical terms the structure may be visualised as the placement of points (OTU's here) relative to each other in space (Orloci, 1968a). The object of any analysis of this data is to represent this structure in more comprehensible terms - in other words to find a simple structure. Orloci (1968a) has pointed out that when many mathematical models are applied they destroy the original structure such that the analysis will be on a new structure.

The simplest form in which the structure of the raw data may be conceived is by presenting the results in the form of scatter diagrams as was done by Anderson (1949, 1956). The individuals or OTU's are represented in two dimensions by two characters, further characters of the individuals being represented by variously ornamenting the OTU points. This method is sometimes useful when a few characters are being considered. (Pettet, 1960; Davis and Heywood, 1963). At the most a further dimension can be added at right angles to the other two in the original diagram. Although the same geometry is used in additional dimensions, the hyperspace created cannot be visualised.

The conception of the data in a simple form as illustrated by Anderson results in the loss of a large amount of inform-

ation. If the n x t matrix is examined with more rigorous mathematical techniques a great deal more information may be obtained. There are two possible approaches or strategies which may be used. The n x t matrix may be represented either as n points (characters) in t-dimensional (OTU) space, or as t points (OTU's) in n-dimensional (character) space. The first approach is called R-analysis and the second is called Q-analysis. Cattell (1952, 1965) mentions other approaches when time is a factor but these do not apply here.

The decision on which strategy to use is up to the investigator once the data matrix is prepared. According to what he wishes to show and the decision as to what is an individual (in taxonomy the OTU) and what is an attribute (in taxonomy - the character), he defines the strategy. In taxonomy where one is dealing with individuals as OTU's and one is interested in relationships between them, the approach is generally a Q-type analysis of OTU's. This is the approach followed by Sokal and Sneath (1963) through most of their book. However it has been pointed out by Proctor (1967) that much is to be gained from a character analysis in taxonomy, and this important aspect has not been sufficiently dealt with by Sokal and Sneath. The analysis of characters may be performed by either treating the characters as individuals in a Q-type strategy, or alternatively an R-type strategy may be used. By using character analysis it is possible to illustrate relationships among characters, and clusters of characters (Crovello, 1968; Estabrook, 1967). It has also shown to be a very power-

ful method in other disciplines such as phytosociology, the inverse analysis of the William's school (Lambert and Dale, 1964). The use of an R-type approach provides information about the contribution of the characters to the respective roots of the principle components as has been shown by Orloci (1968b).

The main emphasis in numerical taxonomy, however, as we have said before, is on OTU studies (Sokal and Sneath, 1963: 125). The relationships between all possible pairs of OTU's are of interest, based on preferably a large number of characters. Any information about the relationships between all the pairs of characters, i.e. character analysis, will only supplement the knowledge which is being accumulated about the OTU's.

Whichever strategy one employs the object is to seek a simpler structure than that of the original raw data. There are two methods that may be used, viz. classification or ordination. In classification, the individuals (OTU's or characters) are arranged in groups, the members of which have certain properties in common; in ordination the individuals (OTU's or characters) are arranged on axes with their properties determining their position (Lambert and Dale, 1964). These two approaches will be considered below but first the methods by which resemblance is estimated between entities will be discussed.

6. Coefficients and Pair Functions

There are many different techniques for computing resem-

blances between taxa. Sokal and Sneath (1963: 125), Dagnelie (1960), Orloci (1968a) and others reviewed a number of these so-called "similarity coefficients" or pair functions. Orloci (1968a) outlines two types of pair functions or models: (i) distance or scalar product functions with metric properties and (ii) non-metric pair functions.

The application of a particular pair function or model is a matter of judgement which should take into account the actual problem to be solved, the previous decisions concerning the space in which the operation will take place, and the techniques of analysis by which a solution will be attempted (Orloci, 1968a). A discussion of the types of models will indicate how these decisions have been made in this particular study.

A. Metric Models

The features that determine whether a model is metric have been discussed by Williams and Dale (1965). Two types of metric models will be considered.

(a) Euclidean Distance

The distance d(j,h) between two OTU's or indivuduals j,h is defined in terms of their characters or attributes thus:

$$d(j,h) = \sqrt{\sum_{i=1}^{n} (a_{ij} - a_{ih})^2} \qquad (2)$$

where n is the number of characters and the a's are the raw observations ($a_{ij} = x_{ij}$) or values derived by adjustment of standardisation from the raw data. Three types of distance

are described by Orloci (1967b, 1968a).

(i) Absolute Distance

Absolute distance, which is the shortest distance between two points, is calculated from the unstandardised data such that $a_{ij} = x_{ij}$. If j and h are identical i.e. j = h, d(j,h) = 0, but this distance has no maximum value. The disadvantage of this measure have been pointed out by Orloci (1967b) though some desirable features are given by Eades (1965). This distance function is the one commonly used in cluster analysis (Sokal, 1961; Rohlf and Sokal, 1965; Moss, 1967, among others) and ordinations (Orloci, 1966, among others).

The absolute distance function is the one used in cluster analysis in this study. It has widespread appeal in taxonomy and for cases when not many of the characters are measurements, and the OTU's do not show great differences in size, this function has been recommended by Rohlf and Sokal (1965).

(ii) Relative Distance

Relative distance as defined by Orloci (1967b) has the following properties: (i) it is equal to zero if OTU's j,h are identical ($x_{ij} = x_{ih}$), or if they have the character values in the same proportion ($x_{ij}/x_{kj} = x_{ih}/x_{kh}$) and (ii) it is a maximum if OTU's j,h have no characters in common. Standardisation is such that $a_{ij} = x_{ij}/v_j$ (where v_j is the length of the jth column vector) giving relative distance as expressed in Eq. 2 with values lying between zero and $\sqrt{2}$.

Though this model is of advantage in ecological studies it is inapplicable to the raw data of this study since it is

quite possible that different OTU's may have character values in the same proportion and yet their absolute difference would have to be regarded as important. They would be considered identical with this measure of affinity. For example, two qualitative characters 1 and 2 may have the scores of 100 and 200 for one OTU and 400 and 800 for another. Proportionally their character scores would be the same, viz. 100/200 = 400/800 = ½, and d(j,h) would be zero, yet the 2 OTU's are not identical.

(iii) The D^2 Statistic

This is the probabilistic measure which has been described by Mahalonobis (see Rao, 1952). It was not used in this study since such a probability based model cannot be applied to the data unless it is of a normal distribution.

(b) Scaler Product Functions

These functions have been applied in R- and Q- type analyses in principle component analysis as described by Orloci (1967a). The general expression as applied as an estimate of similarity between characters i and k in an R- type analysis is:

$$r'(i,k) = \sum_{j=1}^{t} a_{ij} a_{kj} \qquad (3)$$

This function may take three different forms, viz. sum of squares and products, variance-covariance, and correlation. Sum of squares and products (dispersion coefficient) and correlation coefficients were calculated as expressions of similarity in this study. Eq. 3 represents the former if the a's

are defined as deviates from the respective row mean $\left[a_{ij} = (x_{ij} - \bar{x}_{i.}) \text{ and } a_{kj} = (x_{kj} - \bar{x}_{k.})\right]$. When standardisation is such that $a_{ij} = (x_{ij} - \bar{x}_{i.})/(\sum_{e=1}^{t}(x_{ie} - \bar{x}_{i.})^2)^{\frac{1}{2}}$ and $a_{kj} = (x_{kj} - \bar{x}_{k.})/(\sum_{e=1}^{t}(x_{ke} - \bar{x}_{k.})^2)^{\frac{1}{2}}$ then Eq. 3 is defined as the correlation coefficient.

A similar expression may be used as an estimate of similarity between OTU's j and h in a Q-type analysis:

$$q(j,h) = \sum_{i=1}^{n} a_{ij}a_{ih} \qquad (4)$$

The Q-expressions of sum of the squares and products (dispersion coefficient) and correlation coefficient were considered in the analyses in this study, also. When $a_{ij} = (x_{ij} - \bar{x}_{i.})$ and $a_{ih} = (x_{ih} - \bar{x}_{i.})$ Eq. 4 represents the dispersion coefficient. Standardisation such that $a_{ij} = (x_{ij} - \bar{x}_{i.})/(\sum_{e=1}^{t}(x_{ie} - \bar{x}_{i.})^2)^{\frac{1}{2}}$ and $a_{ih} = (x_{ih} - \bar{x}_{h.})/(\sum_{e=1}^{t}(x_{ie} - \bar{x}_{i.})^2)^{\frac{1}{2}}$ gives the Q- expression of correlation.

If the a's are identically defined in Eqs. 3 and 4 then component analysis of the corresponding R- and Q- matrix can yield identical component scores for the OTU's (see Orloci, 1967a).

When the R-type analysis was applied using the correlation coefficient as defined above it was sometimes desirable to use this coefficient for the classification of characters as well as for principal components analysis. The sum of

squares agglomeration technique, described below, functions on a distance measure and therefore the correlation coefficient values had to be transformed to distance values before classification could be effected. This was easily achieved since the correlation coefficient is directly related to the relative distance measure of Orloci (1967b). The correlation coefficient between two OTU's j and h may be defined as the cosine of an angle between their position vectors. Orloci (1967b) illustrates that the relative (standardised) distance (D') may be determined from this angle (α), as follows:

$$D'j,h = \sqrt{2(1 - \cos \alpha_{j,h})} \qquad (5)$$

Normally the angle has a range of between zero and 90 and thus Cos α varies between +1 and zero. In the case of the correlation coefficient the value of cos α varies from +1 to -1 and thus the distance between j and h would be zero when they are identical, and 2 when they are the maximum distance apart, i.e. when the correlation is -1.

B. Non-metric Models

Many different non-metric functions with ill-defined properties have been used in taxonomy and ecology (Orloci, 1968a). Some of these are referred to by Sokal and Sneath (1963: 125) as coefficients of association. Some have been reviewed also by Dagnelie (1960) and Williams and Dale (1965). Very often these methods have found favour due to their simplicity, and they have been used extensively in bacteriology (e.g. Sneath, 1957). However, increasingly more use is being

made of models which although non-metric, have properties which are meaningful in probability or information space. Goodall's probability based model (1966a) is one such model. It applies objective weighting of characters and has been used recently by Clifford and Goodall (1967) in a taxonomic study of the Gramineae. Another probabilistic model, that of Williams and Lambert (1959, 1961) makes use of χ^2 or χ^2/N) in 'association analysis'. More recent studies have been shown how information theory based models may be applied to taxonomy (Orloci, 1968a, 1968c; Williams et al 1966; Pielou, 1966; Estabrook, 1967). Orloci (1968c) considers two types of information theory models, viz. disorder functions as considered by Shannon (1948) and Brillouin (1962), and the mutual information function as given by Kullback (1959).

Though these types of pair functions are becoming of greater importance in taxonomy none were applied on this study of Loudetia.

Returning to the choice of model as applied to this study the factors that had to be considered were: (a) the problem to be solved; (b) the space in which the operations take place, and (c) the technique of analysis. In this case the main problem is the relationship among OTU's or groups of OTU's, although additional information was obtained by examining relationships between characters. The data is such that analysis could be in metric or non-metric space and the analyses involved both classification and ordination techniques. Operations were carried out in metric space using the absolute distance func-

tion in cluster analysis and as principal components analysis was used as a means to simplify the structure, the sum of squares and products (dispersion coefficient) and correlation coefficient in their Q- and R- forms were applied.

7. Classification

The aim of classification is to sort the individuals or OTU's into groups according to some system or criterion. There are different ways in which classification may be accomplished, these having been reviewed by Sokal and Sneath (1963: 175), Lambert and Dale (1964), and Williams and Dale (1965). There are two fundamental distinctions in classification:

(a) Reticulate or Non-hierarchial Classification

Groups of individuals are found directly from the set to be classified. The number of groups to be formed or already existing groups must be known. As soon as any such formed groups are combined into super-groups or split into "sub-groups" the classification becomes hierarchial. In order to form classes directly, the parameters of the classes must be known as well as a means of allocation to the classes. Discriminant analysis falls into this category. Techniques have been discussed by Rao (1952), Cooley and Lohnes (1962), and Jancey (1966). Although we have existing groups in the genus Loudetia about which more information could be obtained by using this method, unfortunately there are not sufficient members of representative OTU's of some of the groups to apply discrimination analysis. The versatility of this method in indicating character relationships in existing groups of

Salmonoid fish has been shown by Ouelette and Qadri (1968).

(b) Hierarchial Classification

In this approach groups of individuals are established from previously unclassified groups. The groups are extracted at successive levels of relationship. The individuals are compared at one level, individuals are extracted or combined to form new groups, and then the cycle is continued at a new level with the comparison of new individuals or groups so formed. There are two basic types of hierarchial classification:

(i) Subdivisive. One begins with the complete set of individuals (or OTU's) and divides it successively into small groups. Each of these groups is then further subdivided into smaller groups. In this type of classification one concentrates on differences in making the divisions, i.e. all the individuals are treated as being the same and one looks for differences.

(ii) Agglomerative. This method is the reverse of subdivisive in that one begins at the bottom and combines individual OTU's which are most alike until all are in one population. The whole set of individuals is tested as being different and one looks for similarities. Williams and Dale (1965) have pointed out some of the advantages and disadvantages of each of these methods.

Classification methods may be further divided into "polythethic" and "monothetic" types.

(a) Monothetic Classification

This method has principally been used in subdivisive classifications. Division occurs into two groups on the presence or absence of a single character or at an inflexion point. The association analysis methods of Williams and Lambert (1959, 1961) use the presence/absence criterion in phytosociology.

(b) Polythetic Classification

This method is used in both subdivisive and agglomerative classifications. A combination of characters is used to form groups.

This type of classification is more stable and informative, but requires more computation than the monothetic type. Edwards and Cavalli-Sforza (1965) have developed a subdivisive polythetic method but is limited to the number of individuals since it requires a large number of computations.

A number of polythetic agglomerative methods have been described. These have been reviewed by Sokal and Sneath (1963), Williams and Dale (1965), Macnaughton-Smith (1965) and Gower (1967) among others. The method that will be used in this study is that of sum of squares agglomeration as described by Orloci (1967b). This technique operates on a distance matrix utilizing the within-group sum of squares. Agglomeration is carried out in successive cycles such that the within-group sum of squares is minimized and the difference between the groups are maximized at each clustering cycle. In this manner a hierarchy of dichotomous branching is constructed. Methods of representing the classification will be

discussed later. Further details of agglomeration may be found in Orloci (1968c) where it is similarly applied using information analysis.

Another type of classification used in this study is that of graph theory. First used in taxonomy by Estabrook (1966) and Wirth et al (1966), graph theory has its basis in mathematics (Busacker and Saaty, 1965) and is commonly used in disciplines such as geography that deal with maps and networks. Essentially the clustering process is a modification of that described by Sneath (1957) and defined as the single linkage method by Sokal and Sneath (1963: 180). It is an agglomerative method of classification in that aggregates of OTU's are built up through association of each OTU with its nearest neighbour. Those pairs of OTU's with the highest possible similarity value (distance or other pair function) are first clustered, and all other pairs of OTU's are clustered up to a given fixed similarity value. This constitutes the clusters of OTU's within a single partition. The next partition in the hierarchical series is formed by lowering the linking similarity value until a different partition is found. This continues until all the OTU's are agglomerated into one cluster.

Graph theory has certain advantages over the clustering by single linkage method of Sneath, in that several aids in the decision making are presented which help in the delimiting of taxa. Firstly, the series of positions may be represented in the form of subgraphs which show the clusters formed and

the similarity between linked OTU's. The fraction of possible connections made between the OTU's within a cluster at any level of clustering is measured. This connectedness indicates the internal "tightness" of the cluster. The amount of separation, the moat, of any one cluster from the rest of the OTU's is helpful in making decisions about the hierarchy. Intermediate forms between clusters may be found, which are referred to as articulation points. These are also of interest taxonomically. The methods of graph theory have been more fully explained by Estabrook (1966) and Wirth et al (1966).

8. Ordination

Techniques of ordination in botany first found favour in phytosociology (e.g. Goodall, 1954; Bray and Curtis, 1957) since it is possible to show that communities are not distinct entities as was the view of early phytosociologists, but represent a continuum. Whereas in classification one searches for discrete groups of OTU's, with ordination one shows the relationships between OTU's in a simpler structure than the original. The representation of OTU's in this new structure has sometimes been regarded as a method of classification (Goodall, 1954; Sokal, 1958; and Crovello, 1966), but some method of cluster analysis is necessary in making a decision about the formation of groups.

Ordination may be defined as a parsimonious summarization of variability within a sample in terms of a new set of variables that are more efficient than the original characters in accounting for the variability (Orloci, 1966). The n x t

data matrix is best visualized in geometrical terms where there are t OTU's whose positions are defined in hyperspace by the n characters as axes. Ordination amounts to finding new perpendicular axes. Each new axis represents a new variable and positions along the axes are the OTU scores on the variables. The new variables will be most efficient where the origin of the new axes coincides with the centre of gravity of the system and the axes are so orientated that the first axis lies in the direction of maximal dispersion within the point cluster; the subsequent axes also conform to this requirement while being orthogonal to the previous axes. The new variables so defined will be independent; in contrast to this the original characters are frequently correlated.

There are three types of ordination: (a) simple ordinations such as that of Bray and Curtis (1957) or Austin and Orloci (1966), Orloci (1966), (b) principal components analysis and (c) factor analysis. Of the simple ordinations, the method of Orloci (1966) is the best approximation to principle component analysis. More details of factor analysis may be found in Cattell (1965), Lawley and Maxwell (1963), Seal (1964), among others. The so called closed model of factor analysis (Cattell, 1965) is equivalent to principal component analysis.

Principal Components Analysis

Principal components analysis is the most efficient means of ordination. All the above mentioned conditions for efficiency, are satisfied in this type of analysis. Component analysis has been used in psychology for a number of years (Hotel-

ling, 1933; Girshick, 1936; Lorge and Morrison, 1938). It is only recently that its importance in biological work has been emphasized (Goodall, 1954; Seal, 1964; Orloci, 1966, 1967a, among others).

The methods by which principal components have been applied in this study are best covered by Orloci (1967a). Both R- and Q- type analyses were used, the measures of similarity for both types of analysis having been discussed above. The method of data centering is by row (character) as compared with the other techniques of column centering or double centering (Orloci, 1967a). If the n x t matrix of data is designated by $\underset{\sim}{x}$ with elements x_{ij}, after centering or standardisation the matrix becomes A with elements a_{ij}. The n rows represent characters and the t columns correspond to OTU's.

$$X = \begin{bmatrix} x_{11} & x_{12} & x_{1j} & \cdots & x_{1t} \\ x_{21} & x_{22} & x_{2j} & \cdots & x_{2t} \\ x_{i1} & x_{i2} & x_{ij} & \cdots & x_{it} \\ \vdots & \vdots & \vdots & & \\ x_{n1} & x_{n2} & x_{nj} & \cdots & x_{nt} \end{bmatrix}$$

After centering the row totals for the characters vanish,

i.e. $$\sum_{j=1}^{t} a_{ij} = 0$$

$$A = \begin{bmatrix} a_{11} & a_{12} & a_{ij} & \cdots & a_{1t} \\ a_{21} & a_{22} & a_{2j} & \cdots & a_{2t} \\ a_{i1} & a_{i2} & a_{ij} & \cdots & a_{it} \\ \vdots & & & & \\ a_{n1} & a_{n2} & a_{nj} & \cdots & a_{nt} \end{bmatrix}$$

The similarity between characters is defined by one of the scalar product functions, resulting in the matrix:

$$\underset{\sim}{R} = \underset{\sim}{A}\,\underset{\sim}{A}^{T} \qquad (6)$$

with elements $r_{ik} = \sum_{j=1}^{t} a_{ij}a_{kj}$

The matrix R is symetric, with n rows and n columns.

$$R = \begin{bmatrix} r_{11} & r_{12} & \cdot\cdot & r_{1k} & \cdots & r_{1n} \\ r_{21} & r_{22} & \cdot\cdot & r_{2k} & \cdots & r_{2n} \\ \vdots & & & & & \\ r_{i1} & r_{i2} & \cdot\cdot & r_{ik} & \cdots & r_{in} \\ \vdots & & & & & \\ r_{n1} & r_{n2} & \cdot\cdot & r_{nk} & \cdots & r_{nn} \end{bmatrix}$$

Similarly the similarity between OTU's in Q-analysis is defined by a symetric matrix:

$$\underset{\sim}{Q} = \underset{\sim}{A}^{T}\,\underset{\sim}{A} \qquad (7)$$

with elements $q_{jh} = \sum_{i=1}^{n} a_{ij}a_{ih}$

$$Q = \begin{bmatrix} q_{11} & q_{12} & \cdots & q_{1h} & \cdots & q_{1t} \\ q_{21} & q_{22} & \cdots & q_{2h} & \cdots & q_{2t} \\ q_{j1} & q_{j2} & \cdots & q_{jh} & \cdots & q_{jt} \\ q_{t1} & q_{t2} & \cdots & q_{th} & \cdots & q_{tt} \end{bmatrix}$$

Using the Jacobi-iterative-method (Greenstadt, 1960) the characteristic roots or eigenvalues ($\lambda 1 \ldots \lambda t$) and corresponding eigenvectors ($\alpha 1 \ldots \alpha t$) can be extracted from the matrix R. More details of the computational method are given by Seal (1964). The eigenvectors of the matrix R, are a set of direction cosines such that $\alpha_i^T \alpha_i = 1$. The component scores for the individuals or OTU's are found by a linear transformation

$$y_i = A^T \alpha_i \qquad (8)$$

where α_i is the ith characteristic column vector of R.

If the component scores are to be determined directly, the Q matrix is utilized and the eigenvalues ($\lambda 1 \ldots \lambda n$) and eigenvectors ($\alpha 1 \ldots \alpha n$) are extracted from the matrix Q by the same method. In this case $y_i = \beta_i$ where β_i is the ith characteristic column vector of Q so adjusted that $\beta_i^T \beta_i = \lambda_i$, the characteristic root. The non-zero roots of matrices R and Q are identical and the corresponding vectors are directly related:

$$\beta_i = A^T \alpha_i \qquad (9)$$

Which strategy that is to be used, therefore, depends on the number of OTU's and characters. The Q-strategy is less tedious if the number of OTU's is only slightly more, and particularly, when less than the number of characters.

Principal components analysis results in the summarization of the variation due to the n characters of the original matrix X which is expressed in the new variables ($y_1 \ldots y_n$). It is found that a few of these variables may account for a great deal of the variation. This is indicated by the roots. The proportion of the variance that each of the roots accounts for is calculated from the expression, $\frac{\lambda_1}{\sum_{i=1}^{n} \lambda_i}$. These values are usually calculated as a percentage. If the matrix A is assumed to be a sample from a multi-variate normal universe (which is not so in this study), then Bartlett's test of the roots (Lawley and Maxwell, 1963: 52) may be applied. This probability test may show how many of the roots are significant, so that only these need to be considered further.

By use of the R-type analysis it is further possible to evaluate the relative contribution of the individual characters to variation in the direction of the principal components (Orloci, 1968b). It is thus possible to provide more information about the characters and their effect on the component scores of the OTU's. The proportion of each root accounted for by different characters may be calculated from the equation:

$$p_{ji} = (\alpha_{ij}^{2} \, r_{ii}) / \sum_{k=1}^{n} (\alpha_{kj}^{2} \, r_{kk}) \qquad (10)$$

Where α_{ij} is the ith element of the jth column of α, r_{ii} is the i,i (diagonal) element of R, and r is the number of characters. The proportions of the roots calculated by the characters may be expressed as a percentages (Orloci, 1968b).

The results of a principle components analysis may be expressed in various ways, the details of which will be considered below.

9. Representation of Results

Along with the development of more sophisticated methods of analysis more refined techniques have been developed for representing the results of a numerical study in a more meaningful and clearly illustrated way. An examination of the various facets of the study will illustrate this point.

(a) Data

Since the number of characters and OTU's from a numerical study is often very numerous, it is not easy to represent the data in the form of a table. Therefore, a list of the characters and their corresponding states, the OTU's, and the n x t data matrix with n rows of coded characters and t columns of OTU's are given in the appendix.

(b) Symmetrical similarity Matrices

The symmetrical matrix produced by the application of one or other coefficient or pair-function is not readily subject to interpretation. Sneath (1957) and Sneath and Cowan (1958) have illustrated how the different class intervals of the similarity values in a half symmetric matrix may be differentially

shaded, and then the sequence of the OTU's may be rearranged. This results in a clustering of the OTU's that are similar to one another as indicated by the intensity of the shading. This method is however, very difficult if the numbers of the OTU's is large, and very often and OTU is not very conveniently arranged next to two neighbours.

Another method has been devised by Lysenko and Sneath (1959) for representing taxonomic relationships between bacteria. A model is constructed using rubber balls to represent the OTU's which are held together by lengths of brass pinwire. The lengths of the wire are determined by the similarity values of the different OTU's. The model building is carried out by trial and error, some distortion being necessary.

Another method of presenting results from the similarity matrix is given by Moss (1967). So called graph diagrams are constructed from the similarity values. The OTU's are plotted in two dimensions and by the addition of contour lines around each OTU their relationship to every other OTU is visualised. The OTU's are represented by "mountain" peaks and they are separated by "valleys" from other OTU's. The distances between peaks of OTU's can be obtained by direct measurements and these values compared with those of the original similarity matrix by the computation of a cophenetic correlation coefficient (see below).

The similarity matrix is more usually subjected to further analysis, the results of which may be more readily displayed.

(c) Representation of Classificational Hierarchies

The most common form of representation of the results of cluster analysis is in the form of a dendrogram. Since these resemble phylogenetic trees, Mayr (1965) suggests that as they are based on phenetic evidence alone, they should be called phenograms. The abscissa of the dendrogram has no special meaning, serving only to separate the OTU's, while the ordinate is based on a scale which is a measure of similarity of OTU's. More details of dendrogram forms are given in Sokal and Sneath (1963: 198). The dendrograms in this study are arranged on their sides so that the OTU names can be entered. The fusions of the OTU's at different levels are plotted against a scale indicating the within group sum of squares expressed as a percentage of the total sum of squares.

In order to define taxa from the dendrogram constructed as above, Sneath and Sokal (1962) have introduced the concept of a phenon. A horizontal line is drawn across the dendrogram at a level usually determined arbitrarily by the investigator, and all taxonomic units carried by single stems crossed by this phenon line are called phenons. The number of phenons may be varied by taking the line at different levels along the similarity value scale. Although this system of phenon lines has been used in many investigations it seems too dogmatic a decision to make in a numerical analysis, especially when more than one dendrogram may be produced and each yields quite a different set of phena.

In graph theory the inter-relationship of the different OTU's may be expressed in the form of subgraphs at different

clustering levels (Wirth et al 1966). The only drawback to this method is that the arrangement of the OTU's in two dimensions requires much trial and error for the most effective result to be achieved. An alternative suggested by Wirth et al (loc. cit.) is that of a "skyline plot" which summarizes the results of the clustering process. In representing the graph theory results in this study, dendrograms were constructed in the normal way with a similarity scale drawn along one side of the dendrogram. This is not the most satisfactory method of representing the results but it renders the method comparable with the other classification hierarchies and is easier to accomplish when there are a largish number of OTU's.

(d) Representing the results of Ordination

The results of an ordination technique such as principal components analysis are usually represented in the form of a scatter diagram. Since most of the information is contained in the few components, (the exact amount may be tabulated for the respective components), the position of the OTU's in relation to the first and second component, first and third component and so on, may be indicated by plotting these points in two dimensions. In recent years newer techniques have been developed for plotting the OTU's on the first three components often by use of an automatic plotter connected to the digital computer (e.g. Fraser and Kovats, 1966; Moss, 1967; Rohlf, 1968). These types of plots generally may be of three forms. Firstly they may be simple two dimensional views, using two axes at a time (Rohlf, 1968), and secondly they may be in the

form of a three-dimensional projection taking into account three axes at a time (e.g. Jancey, 1966; Greig-Smith et al, 1967; Moss, 1967, fig. 20). Finally they may be in the form of stereograms which require the use of a 3-D viewer to obtain the 3-D effect. Two types of stereograms have been developed, viz. those that give the appearance of the points or OTU's suspended in space, and those that represent a pin and ball type model. More details of these illustrations are found in Rohlf (1968).

Various types of hardware models may be constructed to emphasize the relationships between OTU's or groups of OTU's. The pin and ball type model has been described by Rohlf, (1968). Another form of model is as that of Phipps (1969) where beads representing the OTU's are suspended by fine threads so they appear to be floating in space. The advantage of models of this type is that they may be viewed from all angles to obtain more of an understanding of the affinity between taxa. However they are not easily presented for publication. They may be photographed (Rohlf, 1968) but as the OTU's are normally represented by coloured beads balls or pinheads, these are not useful in publication. In this study models have been constructed using pins with coloured heads and these have been photographed from different angles in colour.

10. Comparison of Results from different Classifications

The use of different mathematical models or pair-functions and different clustering techniques will lead to somewhat different similarity matrices and different classification hier-

archies. Dendrograms and similarity matrices may be compared by the computation of cophenetic correlation coefficients (Sokal and Rohlf, 1962; Sokal and Sneath, 1963: 200).

The different dendrograms of the same set of OTU's are compared as follows. The range of similarity values as indicated by the scale is divided into a number of equal intervals by drawing phenon lines at class limits across the dendrogram. The number of class limits depends on the number of OTU's, roughly 4 classes for ten OTU's or about 10 for 100 OTU's. The classes are coded from unity at the end with the lowest similarity value, going up in unit steps. The cophenetic value between two OTU's obtained from the class mark of the class in which stems between the two OTU's are connected. Cophenetic values are obtained between all pairs of OTU's and represented in matrix form. (Sokal and Rohlf, 1962). Cophenetic matrices from different dendrograms may be compared by simply calculating the product-moment correlation coefficient between corresponding elements of the two matrices. These are called cophenetic correlations.

In a similar way cophenetic correlation may be calculated between similarity matrices and as mentioned above (see above, p. 74) between the graphic representation of results and the similarity matrix (Moss, 1967). The various cophenetic matrices composed in this way are represented in tabular form by the half matrix of cophenetic correlation coefficients.

Orloci (1968c) has discussed an information theory model for predicting the degree to which two classifications are

related. Either the mutual information function as given by Kullback (1959) or the coherence coefficient of Rajski (1961) may be used to measure the relatedness. This test is only valid, however, if the two classifications are produced by independent criteria and Orloci (1968c) has illustrated the technique with an example comparing vegetational and environmental hierarchies.

In the present study the cophenetic correlation coefficients were calculated between dendrograms derived from different data sources, and each dendrogram was compared with its absolute distance matrix. In this way an estimate of the similarity between a dendrogram and its distance matrix, and also the similarity between different classifications was obtained.

11. A summary of the application of numerical methods

As has been described in Chapter 1 the taxa were studied according to four character subsets. In each the numerical methods which are used follow the same general form and this is illustrated in a flow chart (Fig. 4). A breakdown of the methods will be considered here.

Firstly, for each of the studies approximately 50 specimens or OTU's were selected as exemplars of the species in the study. Some of the more variable species are represented by more than one exemplar. The data preparation consists of the collection of characters, each of which was given equal weight, and the coding of characters on a linear scale. Some of the multi-state character sets had to be condensed to a single character and these, together with the real variables, had to

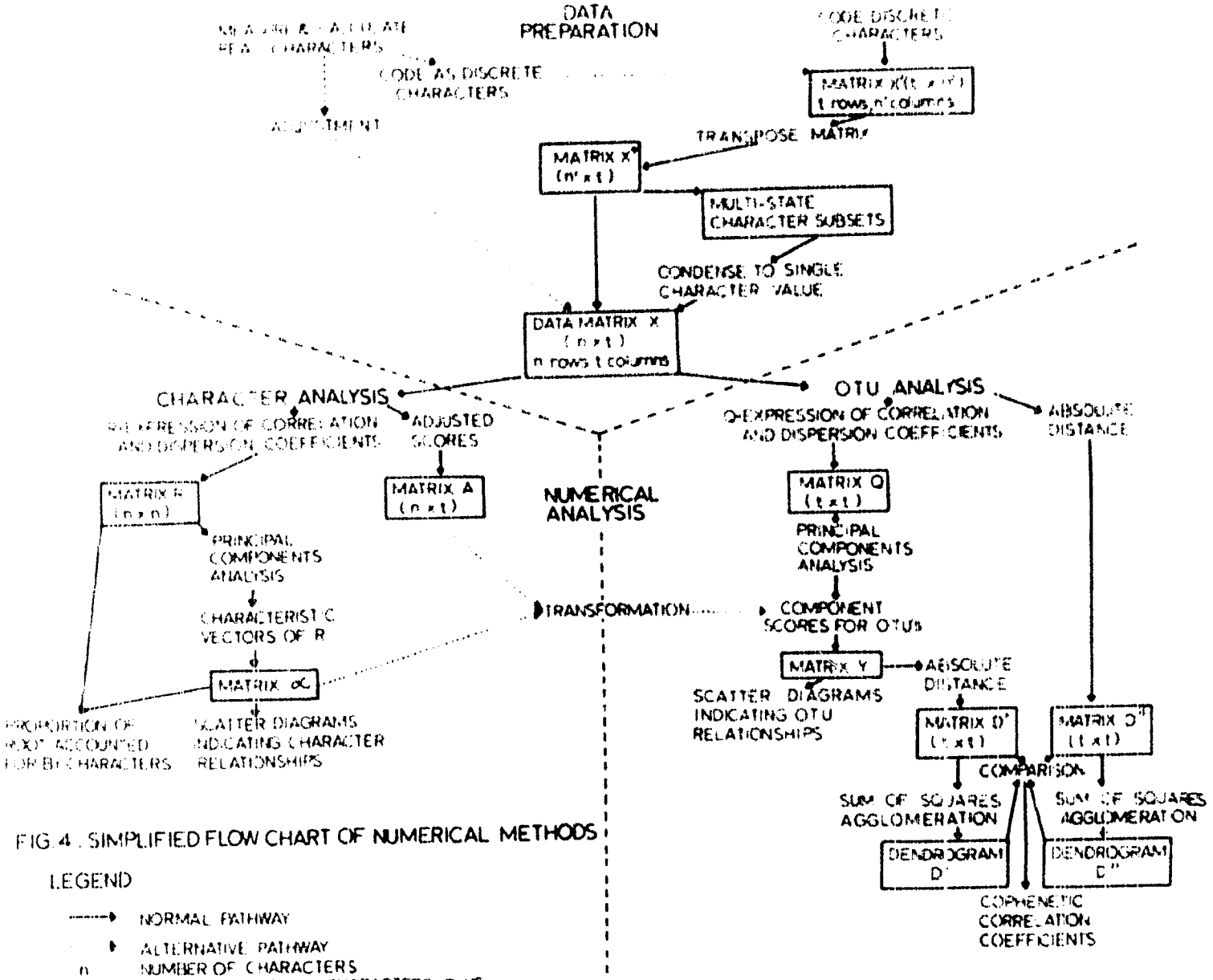


FIG. 4. SIMPLIFIED FLOW CHART OF NUMERICAL METHODS

LEGEND

——→ NORMAL PATHWAY

⇢ ALTERNATIVE PATHWAY

n NUMBER OF CHARACTERS

n' NUMBER OF DISCRETE CHARACTERS, PLUS MULTI-STATE CHARACTER SUBSETS

t NUMBER OF OTU's

be adjusted to the linear scale of values between 100 and 900.

After the n x t data matrix, with n rows of characters and t columns of OTU's, had been prepared it was subjected to analysis using both R- and Q- techniques. In the analysis of characters the R-type expressions of correlation and dispersion were calculated between characters. These were used in principal components analysis. In some instances the R-type correlation coefficient matrix was subjected to a transformation to a distance function so that a classification of characters could be achieved. When the number of OTU's exceeded the number of characters, the R-type analysis of principal components was used to produce the component scores of OTU's by transformation.

The correlation and dispersion functions appropriate for Q-analysis were chosen to measure affinity between OTU's. These coefficient matrices were subjected to principal components analysis. The results of these analyses were represented in the form of scatter diagrams.

Classification of OTU's was achieved using the absolute distance pair function and sum of squares agglomeration and graph theory methods of cluster analysis. Classification was achieved on three different forms of structure of the data. Firstly, the absolute distance matrix was calculated from the data matrix, thus hoping to measure affinity on the original structure. The structure of the sample after being subjected to principal components analysis using the dispersion coefficient and correlation coefficient was also analysed by classifi-

cation. The absolute distance matrices in this case were generated using the component scores from the principal components analysis. The distance matrices were then analysed using sum of squares agglomeration and in one case graph theory as well. The results of the classification hierarchies were represented in the form of dendrograms.

A comparison between similarity matrices and dendrograms was made by calculating the cophenetic correlation coefficients. Comparison was made between the results achieved within one particular study using one set of characters and also between the studies using different sets of characters and all the characters combined.

In addition to using the species as OTU's, the ten main groups, comprising sections and subsections, etc. were considered as OTU's and similar analyses were conducted using the same characters as in the main study.

All the computations were made on the University of Western Ontario's IBM 7040 digital computer (32K). Plotting was achieved for many of the figures with the aid of programmes written for the CalComp 563 Plotter. A list of the programmes used in each phase of the study are listed in table 3.

Table 3: List of Computer Programmes (for details see text).

Programme Name	Programme Description	Computer Language	Author
1. Data preparation			
Ratio between two variables a and b	Calculates b/a, mean, standard deviation and standard error of b/a, b and a.	Fortran IV	R.A. Lubke
Adjustment	Calculates new (integer) values on a range of 100 to 900 for real variables.	Fortran IV	Y.H.C. Li
2. Coefficients and pair functions			
Absolute distance	Measures the distance between two OTU's in terms of their characters.	Algol	L. Orloci (1967b)
R- and Q-expressions of correlation and dispersion	Measures the similarity between pairs of characters or pairs of OTU's.	Algol	L. Orloci (1967a)
3. Classification:			
Sum of squares agglomeration	Forms a classification of individuals based on distances.	Algol	L. Orloci (1967b)
Graph theory	Forms a classification of individuals based on distances.	Fortran IV	R. Chapman

4. Principal component analysis	Extracts the eigenvectors and eigenvalues (roots) of the correlation or dispersion matrices.	Fortran IV	Cooley and Lohnes (1962), modified by R.A. Lubke
5. Representation of results			
Dendrograms	Utilises the output of the optimal agglomeration programme to plot a dendrogram.	Fortran IV	R.A. Lubke
Scatter diagrams	Plots the position of individuals on two components at a time utilising the component scores of principal component analysis.	Fortran IV	R.A. Lubke
6. Comparison of dendrograms and similarity matrices			
Product moment correlation coefficient	Computes the cophenetic correlation coefficient between pairs of entities.	Fortran IV	Golden (1965), modified by R.A. Lubke

CHAPTER 4

THE ANATOMY OF THE LEAF AND ITS USE IN THE TAXONOMY OF LOUDETIA

1. Introduction

As with most other plants, grasses remain in a vegatative state for a large period of their life-cycle. In contrast to other plant groups, however, grasses appear monotonously uniform vegetatively at the morphological level. Such is not the case when the anatomy, and especially that of the leaf, is examined for identifying or classifying members of the family. Taxonomically useful criteria have been found from morphological but more frequently from anatomical investigations.

Studies on grass leaf anatomy over the last century have resulted in a wealth of information about a large number of species. An examination of the general morphology and anatomy of the grass leaf will indicate the extent of the differentia which may be used taxonomically.

The history of the use of anatomy in grass systematics may be considered in three periods. Prior to the twentieth century and in the early 1900's the use of such characters was revealed to be beneficial, though mainly for the practical purpose of identification of grasses. During the late teens

and early twenties of this century various theories on the origin and homology of the grass leaf were examined. Finally, in the 1930's the full value of anatomy and of many other characters became apparent in the classification of the grasses, such that many standard monographs on grasses will nowadays frequently include anatomical studies. The history of the development of the leaf anatomical studies will be considered, especially with respect to previous works on the Arundinelleae, but first the general form of the grass leaf will be reviewed.

A. The morphology and anatomy of the grass leaf

The grass leaf is commonly examined in different ways. Certain morphological features may be seen with the naked eye, hand lens or dissecting microscope, and the leaves may be examined anatomically with the light microscope. The leaf anatomy is generally examined from transverse sections of the lamina, and the leaf epidermis is studied in surface view. The general features of the grass leaf as described by Arber (1934), Metcalfe (1960), Jacques-Félix (1962) and others are reviewed below.

(a) External Morphology of the Leaf

Typically, the leaf consists of a sheathing base and a lamina which protrudes from the summit of the sheath. The leaf blade may be separated from the sheath by a short petiole in some grasses. The blade is the most important part of the leaf taxonomically. Variations in shape, width, length, pubescence, degree of inrolling, etc. are characters very

often used for diagnostic purposes. The ligule which is a membranous or hairy structure occurring at the summit of the sheath is also important in taxonomy. Phipps (1966) used characters of the ligule in classifying major groups in the Arundinelleae.

(b) Anatomical Structure

Seeing that the structure of the lamina has been found to be more important in taxonomy than other parts of the leaf (Metcalfe, 1960) it alone will be considered here.

I Leaf anatomy as observed from transverse sections of the lamina.

The midrib, if present, is prominent in the centre of transverse sections with an abaxial keel. The lamina is often symmetrical about the midrib, and consequently only one half and the midrib are usually illustrated. The adaxial surface and to a lesser extent, the abaxial surface of the lamina are often longitudinally ribbed and furrowed. The vascular bundles are generally arranged in a single row in the middle of the lamina. They are of different orders depending on the constituent amounts of xylem, phloem and phloem fibres they contain. The vascular bundles are surrounded by one or two bundle sheaths, the outer sheath being referred to as the parenchymatous sheath and the inner as the mestome sheath. Externally to the bundle sheath is the mesophyll tissue which consists of chlorenchyma and varying amounts of colourless cells. The latter are usually associated with the bulliform cells, which occur at the bases of furrows on the adaxial surface. The bulliform cells

have been called "motor-cells" or hinge-cells" because of their supposed function of inrolling of the leaves, but the term bulliform (Duval-Jouve, 1875) is the best one in that it presupposes no function. Finally, the leaves are adequately endowed with supporting tissue in the form of sclerenchyma. This occurs as strands or girders above and/or below the vascular bundles, and at the margins. Strands are defined as bands of sclerenchyma which do not make contact with the bundle sheath, whereas girders are attached to both the epidermis and the bundle sheath. (Metcalfe, 1960).

II Leaf epidermis in surface view

The leaf epidermis is divided into longitudinal zones, the intercostal zone (between the veins) and the costal zone (over the veins). Metcalfe (1960) has established a convention of orientating the leaf epidermis, because this is particularly important in discussing shapes of silica bodies. The epidermis strip is examined so that the long axis of the leaf and hence the zones of epidermal cells are horizontal in the field of view. This convention has been adopted by some authors, e.g. de Winter (1965), Stewart (1965), but others orientate the epidermis at right angles to the way Metcalfe pictures it, e.g. Prat (1948), Sørensen (1953).

The abaxial epidermis is often the more usual surface that is examined as the other surface often possesses large ridges and bulliform cells so that its epidermal preparations are difficult to obtain. The bulliform cell zone is seen as a region within the intercostal zone, cells being large and smooth

walled whereas the other constituent cells, the long cells and short cells often have sinuous walls and are not as wide. The long and short cells are variously arranged in both the costal and intercostal zone. The short cells, and very occasionally some of the other cells may contain silica bodies of various shapes (Parry and Smithson, 1964). In addition one may find stomata and the associated cells and interstomatal cells in the intercostal regions.

The epidermis may or may not be provided with various dermal appendages, viz. macro-hairs, micro-hairs, prickle-hairs and papillae. The macro-hairs are large though unicellular and often referred to as cushion hairs ('polsterhaare') because of the inflated epidermal cells at their base. Micro-hairs are much smaller and are commonly two-celled but the distal cell is often missing or not readily seen, as it is very thin-walled. Prickle-hairs are short robust and sharply pointed. They may be categorized as prickles or hooks. The former are usually larger whereas the latter often have a rounded base and curved point. Papillae may be observed as slight protrusions of the outer walls of epidermal cells.

B. The homology and evolution of the leaf

It is generally agreed that the monocotyledons have descended from the dicotyledons (Arber, 1923; Hutchinson, 1934; and others) and most theories on the origin of the grass leaf accept this as factual. The dicotyledonous leaf is assumed to have been derived from a modified stem (Esau, 1953: 338), and in its simple form consists of a leaf base, which is atta-

ched to the stem, petiole and lamina.

There are two conflicting views on the origin and homology (in the phylogenetic sense) of the grass leaf. Arber (1918, 1923) believes that the leaves of most monocotyledons are equivalent to the leaf base and petiole (or leaf base alone) of the dicotyledon, the lamina being unrepresented. The Gramineae are included in this general theme. The leaf sheath of the grasses is thus considered to be equivalent to the basal region of the leaf of the dicotyledons, the lamina corresponds to the petiole of the dicotyledon, the blade of lamina of the dicotyledon being absent. Bugnon (1921) suggested a different homology for the grass leaf. He treats the lamina of the grass as being equivalent to the basal sheath of the dicotyledon, and the sheath of the grass leaf as a new organ. Arber (1923) is opposed to this view, although no morphological evidence can be presented, because the production of an entirely new organ is unlikely. Known evolutionary changes among flowering plants generally involve modification or loss of existing organs.

Also of interest in the morphology of the leaf is the ligule. This structure was considered by some botanists to consist of fused stipules. However, Bugnon (1921), Philipson (1934a, 1934b) and others have shown that the ligule is more likely to consist of the free upper border of the sheath and a median upgrowth of the adaxial epidermis of the leaf. More details of the development and morphology of the ligule are given in Philipson (1934b).

C. The use of the leaf in grass taxonomy

One of the earliest investigators of grass anatomy, Duval-Jouve, was quick to realise the value of the leaf in the systematics of the family. His first work (1870) described the general anatomy of all the vegetative organs - root, rhizome, culm and leaf, and later (1875), in his paper on leaf anatomy, he used the position of bands of bulliform cells in relation to the veins or vascular bundles as a taxonomic criterion. Some species have bulliform cells in both ad- and abaxial epidermis, in others the bulliform cells occur over the tertiary vascular bundles, and so on. Schwendener (1890) first observed that the vascular bundles of some grasses possessed two bundle sheaths whereas others did not, and he based his classification of the tribes of grasses on the presence or absence of the mestome or inner sheath. This proved to be one of the characters which was used later in dividing the family into the 'festucoid' and 'panicoid' grasses, respectively.

Pée-Laby (1898) divided the grasses into five main groups which he claimed to give an exact indication of the habit, vegetative period, and soil that the plants required. The main characters which he used were (1) whether or not the upper and lower leaf surfaces are parallel; (2) the relative number of stomata on the two sides; (3) the arrangement of the chlorophyll tissue; (4) the degree of development of the motor cells. Though he was optimistic about the use of leaf characters in identifying grasses for practical purposes, Lewton-Brain (1904) felt that this task was more difficult since even some genera

were very alike anatomically. He also approached the problem of grass classification with respect to the environment. He recognized four types of grass leaf on the presence and absence of ribs and furrows and the extent to which the leaf is ribbed and furrowed. Leaves from grasses of a number of different environments were studied on this basis since he suggested that the presence of ribs is correlated with a xeric habitat, though many exceptions were found to occur.

The most notable early contribution in the realm of leaf epidermis structure was that of Grob (1896). He studied the leaf epidermis of 193 species clearly describing the many features that occur in the different species. His main interest centred around the silica bodies and micro-hairs and using the different types of these structures formed groups of the different tribes.

During this early period of anatomical work on the Gramineae it appears that there was either insufficient information from a large number of species, or what was known was inconsistently applied so that the systematic treatments produced then proved unsatisfactory. Some authors (e.g. Pee-Laby, Lewton-Brain) were more interested in the variation of vegetative features in different habitats, or the use of these characters in identifying grasses.

A period of quiescence in anatomical studies that followed was succeeded by the advancement of various theories on the homology and the origin of the leaf as discussed above. Consequently it was only with the work of Avdulov (1931) who used

leaf anatomy and chromosome number as criteria for classifying the grasses that the anatomical approach was revived. Although Avdulov first introduced holotaxonomy to the Gramineae it was Prat (1932, 1936) who was able to assimilate all the knowledge and formulate a system reflecting "true" relationships more exactly. Avdulov (1931) recognized two types of leaf anatomy which were later named 'festucoid' and 'panicoid' by Prat (1936). The leaf characteristics of these two groups are tabulated as follows:

Character	Festucoid	Panicoid
Mestome bundle sheath	Present	Poorly developed or absent
Parenchymatous bundle sheath	Poorly represented or lacking	Present
Chlorenchyma	Irregularly arranged	Radiating and in one of two layers
Colourless parenchyma	Absent	Present
Silica bodies	Long or round	Cross-shaped, saddle-shaped or dumb-bell shaped
Micro-hairs	Absent	Present
Macro-hairs	Absent	Present

Prat (1936) further subdivided the panicoid grasses into the eupanicoid and chloridoid subtypes. The former has rod-like micro-hairs and dumb-bell-shaped silica cells, while the latter has globose or club-shaped micro-hairs and saddle-shaped silica cells.

There appears evidence for further subdivision of the grasses as later authors have indicated. Stebbins (1956), using all the information available to him, recognised four groups of grasses, viz. panicoid, chloridoid, festucoid, and banbusoid. Brown (1958) examined 101 species of 72 genera of grasses with respect to leaf anatomy and recognized 6 major groups: festucoid, banbusoid, arundinoid, panicoid, aristidoid and chloridoid. Brown introduced some physiological and ecological factors to explain the anatomical structure of the leaf. Alexandrov (1926) reported the active formation of starch in the parenchymatous sheath of Setaria viridis by large specialised chloroplasts. Rhodes and Carvalho (1944) found this function common in some other panicoid species (e.g. Zea mays and Sorghum) whereas in the festucoid species they examined (oats, wheat and barley) the chloroplasts are smaller in the parenchyma sheath and starch formation occurs in much smaller amounts in both the parenchymatous sheath and the chlorenchyma. Brown (1958) expanded upon this physiological difference between the festucoid and panicoid grasses, and using characters which were associated with it, viz. type of inner or mestome bundle sheath, and the arrangement of the chlorenchyma cells between the bundles, formulated a phylogenetic based system for the Gramineae.

Although classification of tribes into sub-families on a number of criteria, including leaf anatomy, continues (e.g. Tateoka, 1957; Auquier, 1963; among others), the application of leaf anatomy at the sub-tribal level is more of interest

to this study. Many studies are now being conducted using anatomical methods in distinguishing tribal, generic or specific limits. (e.g. Decker, 1964; Tateoka, 1958). Revisions and monographs often include detailed anatomical studies (e.g. Sørensen, 1953; de Winter, 1965; Soderstrom, 1967). In a revision of the Greenland species of Pucinellia Sørensen (1953) made extensive use of epidermal characters and was able to draw up a key to the species on this basis. Similarly Stewart (1965) has shown the use of epidermal characters in identifying East African plain species of grasses. Borrill (1961) studied the subspecies of Dactylis glomerata and found quantitative and qualitative characters of the epidermal cells to be of diagnostic value in distinguishing the taxa. Another practical application of leaf epidermal studies is in the analysis of the diet of grazing animals. Since the cuticle is resistant to digestion, examination of the animals' gut contents or faeces reveals quantitative or qualitative data on the animals' diets, provided the remains can be accurately identified (Martin, 1955).

Of great assistance to some of the above and to subsequent students of grass anatomy is the work of Metcalfe (1960). In his treatise on grass anatomy he compiled diagnostic features of the leaf epidermis and internal anatomy of 206 genera, representing about 413 species. In addition, he summarized information of other species from the literature.

D. The use of the leaf in Arundinellean taxonomy

The anatomy of the leaves of a number of species of Arun-

dinelleae has already been undertaken by Conert (1957). However, Conert studied only 64% of the species known at that time (Phipps, 1967) and the intention of his work was to indicate the value of using anatomy to determine the position of the Arundinelleae in relation to the other grasses, rather than to solve the generic problems of the tribe. Although the Arundinelleae have more spikelet and other morphological characters in common with the festucoid grasses Conert (1957) found that there were some leaf anatomical characters which placed them more in line with the panicoid tribes. The chlorenchyma is very often in radiating layers around the bundles; the mestome sheath is lacking in many species and in the remainder it is poorly developed; there are always large round parenchymatous sheath cells; dumb-bell shaped and sometimes cruciform silica bodies are found on the epidermis, and micro-hairs and macro-hairs may be present. One of the conclusions that Conert reaches is that the Arundinelleae could be considered as part of a separate subfamily but he delays making this change until further evidence is forthcoming about other tribes which may have similar characteristics.

With respect to leaf anatomy of the species of the genus Loudetia, Conert (p.248) maintains that it manifests little diversity though descriptions are given of the anatomy under the different sections and subsections. A closer examination of the leaf anatomy of the different species as has been carried out here will indicate that this is not true.

Tateoka (1956a & b, 1958) has studied the leaf structure

of Arundinella spp. and has found that there is some close resemblances in the anatomy of some of these species to some Garnotia species. On the basis of these similarities Tateoka (1958) suggests that the genus Garnotia be placed in a separate tribe, Garnotieae, placed near to the Arundinelleae.

Jacques-Félix (1962) has studied the leaf anatomy of a number of different species of Arundinelleae but does not use characters from this source in his classification of the tribe.

Stewart (1965) describes the characters of the abaxial epidermis of the four species of Loudetia which occur on the East African plains, viz. L. arundinacea, L. flavida, L. kagerensis and L. simplex. Seeing that his work includes a study of 163 species from 57 genera, obviously a large number of representatives of each species could not be covered and consequently it will be found that some of these species show more intraspecific variation than Stewart has recorded in his descriptions. For example, L. arundinacea and L. simplex commonly have some macro-hairs and always have some micro-hairs whereas Stewart observed no macro-hairs in L. arundinacea and L. simplex and no micro-hairs in L. arundinacea.

Metcalfe (1960) gives full descriptions of the leaf anatomy and leaf epidermis of 2 species of each of the genera Arundinella, Loudetia and Tristachya (sensu Conert) and also summarizes the descriptions of the other genera of Conert (1957). Of the two Loudetia species he describes anatomically Loudetia superba de Not. is now recognised as Tristachya superba (de Not.) Scheinf. and Aschers, and the unidentified Loudetia sp.

from Japan from the presence of the petiole is undoubtedly Rattraya petiolata which was only described later by Phipps (1964). There is an error as to its origin since the species comes from the Zambesi River Gorge area, near Victoria Falls, Rhodesia, but the identity has been clarified by Metcalfe (1965, personal communication) in consultation with Dr. C.E. Hubbard.

At present, apart from the anatomical studies on Loudetia which will be reported here, Li (1969) has studied the leaf anatomy from transverse sections of the lamina of other genera of the Arundinelleae the results of which are examined numerically.

E. The approach to this study of the leaf

As described previously the leaf of the grasses is usually considered to provide taxonomically useful characters in two different ways. The leaf anatomy as seen from transverse sections of the lamina yields a number of characters and another set of characters may be obtained from examining the leaf epidermis in surface view. The adaxial and abaxial epidermis both show similar features but in most species the ridges and bulliform cells on the adaxial surface make it very difficult to obtain epidermal strips for study. It more than doubles the amount of work for a few extra characters and, therefore, in many cases the adaxial epidermis is examined only superficially (e.g. Stewart, 1965). In this work only the characters of the abaxial epidermis will be considered.

The studies of leaf anatomy in transverse section, and the

leaf epidermis may be considered separately (e.g. Stewart, 1965; Brown, 1958) or both together (e.g. Conert, 1957; de Winter, 1965). In this study the characters of the leaf anatomy and those of the leaf epidermis will be considered separately in formulating separate classifications of the genus. Later all the leaf characters will be considered together with those of the awn anatomy to obtain an overall classification (see fig. 3, chap. 1.). In approaching the problem in this way a comparison may be made between the different classifications and the contribution of the respective character sets may be more readily appreciated.

2. The taxonomy of Loudetia based on leaf anatomy

A. Anatomical and morphological methods

Material was used from living specimens wherever possible for, although the harder tissues such as the sclerenchyma or vascular bundles were usually well preserved, in leaves from herbarium specimens, the chlorenchyma and bulliform cells had often collapsed.

Metcalfe (1960) notes that leaves from different parts of the plant, for instance those from the higher regions of the culm, especially just below the inflorescence, and the basal leaves, differ somewhat structurally. Though the differences are comparatively insignificant he suggests that when leaves are to be compared taxonomically, it is as well to select them from corresponding positions on the different plants. Therefore, the third leaf up from the base of the plant was selected in each case. The anatomy of this leaf was examined

in a region approximately mid-way between the tip of the blade and its base.

About 1" long strips of leaves from herbarium specimens were either boiled in water for about 10 minutes (Metcalfe, 1960), or alternatively placed in a mixture of equal parts of 50% ethyl alcohol and pure glycerine for a few days (de Winter, 1965), and then washed in water. Either method proved satisfactory in restoring the material to its original form if the specimens had been preserved reasonably well on the herbarium sheets. The material was stored in 70% ethyl alcohol. Fresh material was collected into formalin acetic alcohol, and transferred to 70% alcohol after about 18 hours (Johansen, 1940). The leaves were placed in ± 25% solution of hydrofluoric acid for one to six hours to remove the silica, after which they were thoroughly washed in running water for about an hour and then transferred to 70% alcohol.

Various methods of sectioning and staining grass leaves are given in the literature. Brown (1958) reported that satisfactory transverse sections of leaves were obtained by embedding the material in paraffin wax and sectioning on a microtome. It was found that most of the Loudetia leaves were not satisfactorily sectioned in this way. The tissue softening techniques that were found to be reasonably good for softening awns before sectioning (see chap. 5) were tried with leaves also but the results were no better than with material that was sectioned without softening treatment. Other authors have found that good leaf sections can be obtained on a freezing

microtome (Brown, 1958) or by placing the material between pith and using a sledge microtome (Metcalfe, 1960; de Winter, 1965) but these methods were not attempted. By using a razor blade for cutting transverse sections by hand the best results were obtained for Loudetia leaves.

The sections were transferred to a watch glass of distilled water and thence to full strength "Javex" (a hypochlorite bleach) for 1 - 2 minutes to bleach the contents of the cells. (Metcalfe, 1960; de Winter, 1965). Some of the sections were not bleached since it was also desirable to observe the arrangement of the chlorenchyma and colourless cells. After washing in distilled water the sections were stained in safranin and counterstained in fast green after the techniques of Johansen (1940, p.80). It was found most suitable to transfer sections from one solution to the next in a small receptacle made of a piece of glass tubing with a nylon gauze patch firmly tied to one end.

The staining technique used differed from that described by Johansen (1940) in that sections were transferred to absolute alcohol after rinsing with clove oil and emptied from the staining container into a clean watch glass. This solution was replaced by euparal essence and then the sections were mounted on a slide in euparal.

The slides were examined with the aid of an Olympus EH binocular microscope, and measurements of dimensions of the leaf were made with an eyepiece micrometer. The transverse sections were drawn with the aid of a Carl Zeiss GFL microscope

equiped with a drawing apparatus.

The leaves of both herbarium and living material were examined morphologically with the aid of a Bausch and Lomb dissecting microscope. Photographs were taken of the specimens using an Asahai Pentax Spotmatic camera and Kodak Plus X film. Some of the photographs were made with close-up rings and others with the f1.8 lens alone.

B. The external morphology of the leaf

Phipps (1969) has used twelve morphological characters of the leaf, which include characters of the sheath, ligule and the blade, in his study on all the representatives of the Arundinelleae. Since the anatomical features of the blade are dependent on its morphology some of these characters also are considered below.

The leaves of two representatives of the genus are illustrated in plate 1. The typically shaped blade of the genus (e.g. L. coarctata, plate 1.1) may be described as narrowly linear and tapering to a fine point. The length and breadth of the blade varies somewhat and the tip may or may not be pungent. R. petiolata (plate 1.2) which has a distinct petiole at the summit of the leaf sheath, is the most atypical of all the taxa with respect to leaf morphology. The leaf blade is very broad and almost lanceolate in shape. Two other variations of the general morphological form that are not illustrated are the leaves of species such as L. filifolia or L. perrieri, and secondly the leaves of L. jaegeriana and L. togoensis. Species of the former type often come from xeric condit-

Plate 1

General morphological form of the leaf.

1. Loudetia coarctata (37)

2. Rattraya petiolata (50)

Magnification as indicated;
OTU numbers in parentheses.

Region of T/S
2
1

ions and it is found that the leaf blade is often tightly rolled and appears quite filiform or setaceous. The basal leaves of these species may be of this type whereas those situated along the culm are more like the general leaf type. Leaves of L. jaegeriana are found to be somewhat similar to those of L. togoensis and in both species the blades are shorter and broader than those of the majority of species. These quite broad leaves are also characterised by the absence of a midrib.

Some of the features of the leaf blade, such as the presence or absence of a midrib, inrolling of the margins, etc. will be considered in more detail from the anatomical studies. The position of the blade from which transverse sections were taken is indicated in plate 1.2.

C. The leaf anatomy of Loudetia

Contrary to what was expected from a superficial morphological examination of the leaves, there are a number of anatomical characters which proved to be descriptive of both species and higher taxa, viz. the sections and subsections of the genus. An examination of the general anatomy of the leaf will illustrate this point.

(i) The presence or absence of a midrib

The presence and size of the midrib varied both between sections and subsections and within these groups. The section Pleioneura (fig. 5. 30-32) is conspicuous due to a large midrib which often contains more than one vascular bundle. The midrib of these species contained many colourless cells above

the bundles with one to three adaxial sclerenchyma strands beneath the epidermis. Rattraya petiolata (fig. 5. 35) may also be included in this category.

The subsections Pungentes (fig. 5. 10-13), Densispicae (fig. 5. 22-25) and Annuae (fig. 5. 26-27) are characterised by an almost complete absence of a midrib. L. imegerigna (fig. 5. 34) also has no midrib and L. togoensis has only a slight midrib. However, Conert (1957: 277) reported a prominent midrib for the latter species and the reason may be that his section was taken lower down the blade or from a leaf on a different part of the plant.

The leaves of the species of subsections Typicae (fig. 5. 1-9) and Acuminatae (fig. 5. 14-20) were found to be most variable and with respect to a midrib this is certainly the case. The leaves of the species of the L. arundinacea-complex (fig. 5. 1-3) are generally larger and have a distinct midrib, whereas the L. simplex species (fig. 5. 4-9) are somewhat smaller and have either an indistinct midrib or none at all. The leaves of species of the subsection Acuminatae generally do not have a midrib or it is poorly developed. Finally the two species of the subsection Flammidae (fig. 5. 28-29) differ on the type of midrib. Loudetia flammida has a large midrib similar to that of the Pleioneura species whereas L. phragmitoides has a very insignificant midrib. This again is contrary to the findings of Conert (1957: 251) who decided the latter species was very much like L. flammida and should be considered a synonym of it.

In general the nature of the midrib reflects the form of the rest of the lamina. Those species with large midribs usually have a broad, fairly thick blade, whereas those without a midrib often have a somewhat smaller leaf. Exceptions to this rule are L. togoensis for example, which has an ill-defined midrib and a broad yet rather thin lamina. The lamina of L. phragmitoides is somewhat similar to that of L. flammida apart from the nature of the midrib. Species which have xeromorphic leaves are generally found to have a thick lamina and no midrib (e.g. L. madagascariensis, L. longipes, L. filifolia subsp. humbertiana, (figs. 5. 8, 5. 11, 5. 20).

(ii) Surface ribs and furrows

The adaxial leaf surface is usually more ribbed than the abaxial one. This feature occurs in varying forms throughout the genus and it seems to be connected with other xeromorphic features such as an abundance of mechanical tissue, rolling of the leaf, etc. Those species which have particularly prominent ribs are L. madagascariensis, L. perrieri, L. longipes, L. migiurtina, and L. simulans (figs. 5.8, 5.9, 5.11, 5.16 and 5.32).

(iii) Mesophyll

The chlorenchyma cells are arranged in radial rows around the vascular bundles. This was more apparent in some species than others, but unfortunately it was not possible to make good comparisons of all species because the material from the herbarium specimens was very often not satisfactorily preserved and the chlorenchyma cells had collapsed.

(iv) Vascular Bundles

Three major features of the vascular bundles may be considered here, viz. the size, arrangement and shape. The vascular bundle composition is generally referred to as the type or order of bundle. Various authors have described the different bundles in different ways and as many as five orders may be distinguished. Conert (1957: 230) describes three orders of bundles for the Arundinelleae, viz. primary, secondary and tertiary, the latter type with two variations. In this study three orders of bundles are distinguished, following the convention of de Winter (1965: 202):

Primary vascular bundle: possessing proto- and metaxylem vessels; protoxylem canal is usually present. The phloem is very often divided into a number of groups.

Secondary vascular bundle: possessing only metaxylem, with or without large vessels, but no protoxylem canal.

Tertiary vascular bundle: the xylem and phloem not clearly demarcated, there being no large xylem vessels at all. These bundles were generally much smaller and usually occurred below the bulliform cells.

In the illustrations, the primary vascular bundles have the metaxylem, and protoxylem vessels and protoxylem canal outlined, the secondary bundles have xylem and phloem diagrammatically indicated and the cells of the xylem and phloem are not differentiated in the drawings of the tertiary bundles.

The arrangement of the three orders of vascular bundles was found to vary in the leaves of different species. The

species of the section Pleioneura (fig. 5. 30-32) and Rattraya petiolata (fig. 5.35) have no tertiary bundles. Though some of the secondary bundles are rather small it was still possible to distinguish the xylem from the phloem in these bundles. Apart from these taxa all orders of vascular bundles will always be seen to alternate through the sections of the leaf. There are usually large primary bundles at regular intervals across the lamina, and secondary bundles occur beneath the ridges at intervals between them. Tertiary vascular bundles alternate between the secondary and primary vascular bundles. In L. togoensis and L. jaegeriana (fig. 5. 33-34) there appear to be more secondary bundles than in most other species, the number of primary and tertiary bundles being greatly reduced. When a large midrib is present the central bundle is always a large primary one which may be solitary or have adjacent secondary, tertiary and in some cases primary bundles, on either side.

The shape of the vascular bundles varies from round, through oval to triangular, and some are even quite angular in outline. This character is not very adequately illustrated in the figures because there is a certain amount of rounding of the bundles when the sheath is drawn diagramatically, and the sheath is not included when the shape of the bundle is considered.

(v) Bundle sheath

The vascular bundles of Loudetia are always surrounded completely or almost so by a parenchymatous or outer sheath.

In the primary and secondary bundles it may be incomplete adaxially or abaxially where sclerenchyma, chlorenchyma or colourless parenchyma with slightly thickened walls may occur. The cells of the outer sheath are often quite swollen and fairly thick-walled. It has been reported that in the panicoid grasses these cells contained large green plastids which are envolved with starch production (see above). In most of the species of Loudetia the chloroplasts of the parenchymatous sheath were much larger than those of the chlorenchyma and were assumed to be of the panicoid type. In order to test if these sheath cells contained starch, leaves of a number of species representing a cross-section of the genus were collected from the greenhouse on a sunny afternoon, fixed in FAA, sectioned and the sections stained with potassium iodiode. In all cases it was found that the cell contents or plastids stained dark blue or black indicating the presence of starch (plate 2. 1-4). This phenomenon was not confined to any one order of the vascular bundle, but occurred throughout the leaf. It was not possible to test the reaction in all members of the genus because living material was not available of all species. It would be interesting to see if the presence of starch in the parenchymatous sheath is uniform throughout the tribe in view of the uncertainty of its position with relation to the panicoid and festucoid grasses.

Within the parenchymatous sheath there is occasionally a mestome sheath of thick walled cells which are a lot smaller than those of the outer sheath. The mestome sheath is in most

cases incomplete and represented by only a few cells, and it may be completely absent.

(vi) Sclerenchyma

The variation in the amount of sclerenchyma is also evident in the diagrams. Almost every vascular bundle has some sclerenchyma associated with it in the form of strands or girders. The girders on the abaxial surface are often continuous with the phloem fibres especially in the higher order bundles, and in some cases sclerenchyma tissue extends in a girder from ad- to abaxial epidermis (e.g. L. crassipes, fig. 5. 13).

On the adaxial surface strands of sclerenchyma are more common, since there are very often one or more rows of colourless cells between the sclerenchyma and the bundle sheath. Especially in the region of the midrib one or more broad strands is very common (e.g. Rattraya petiolata, fig. 5. 35; L. flammida, fig. 5. 29).

The amount of sclerenchyma in the adaxial region increases as one moves from the midrib towards the margin in some leaves (L. perrieri, fig. 5. 9) whereas in others the reverse is true (L. filifolia subsp. humbertiana, fig. 5. 20). The margin of the leaf contains a large strand of sclerenchyma in many species and the margin may be quite swollen (L. simulans, L. jaegeriana, figs. 5.32, 5.34). This strand is of various shapes and forms.

(vii) Bulliform and colourless cells

In most cases the bulliform cells occur in distinct groups

Plate 2

Cross-sections of the lamina, stained with potassium iodide to show the presence of starch in the parenchymatous or outer sheath.

1. *L. arundinacea* (3)
2. *L. coarctata* (37)
3. *L. ramosa* (48)
4. *Rattraya petiolata* (50)

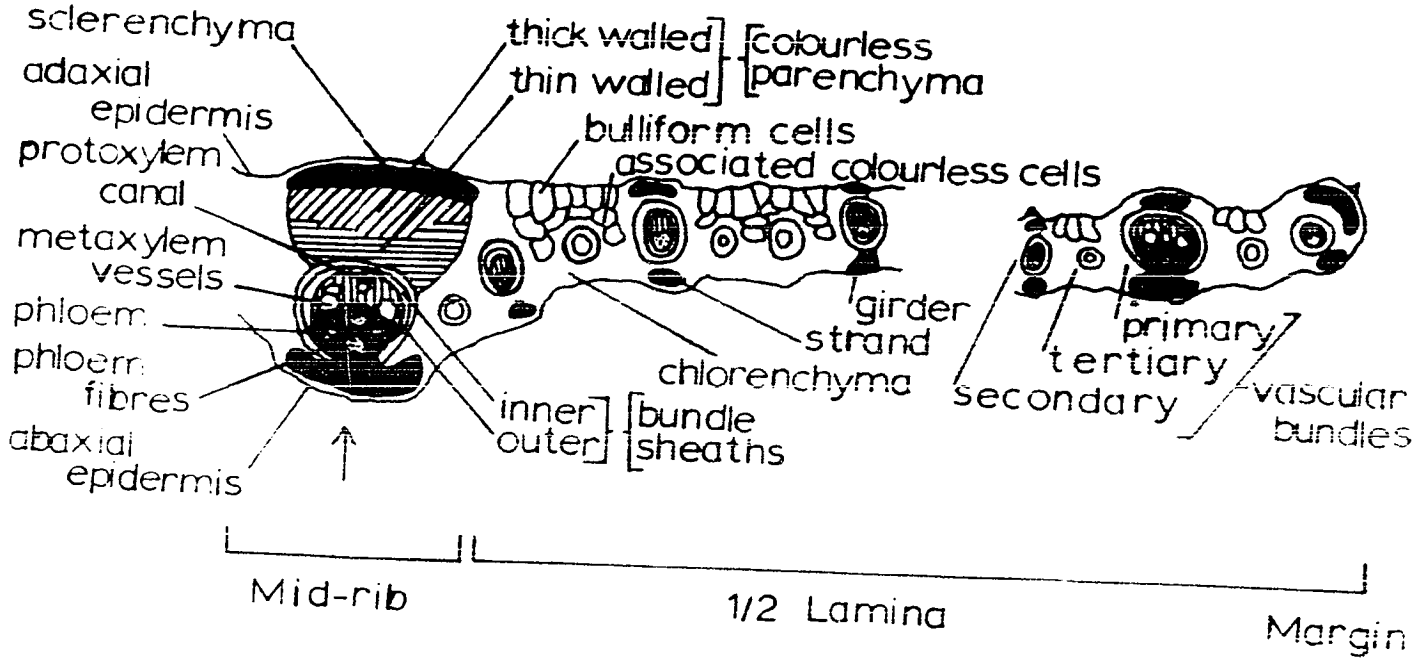
OTU numbers in parentheses.

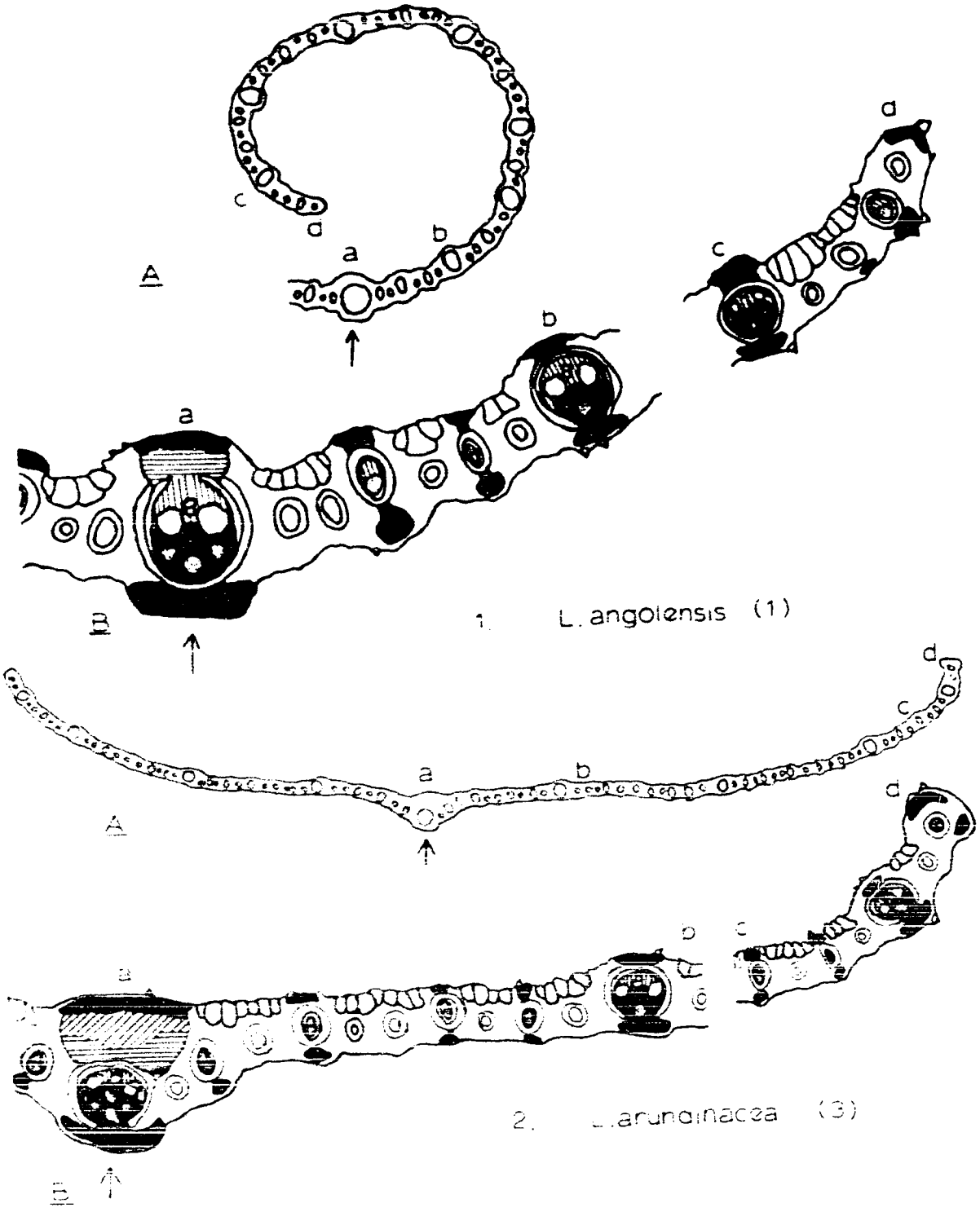
2
4
1
100μ
400μ
3

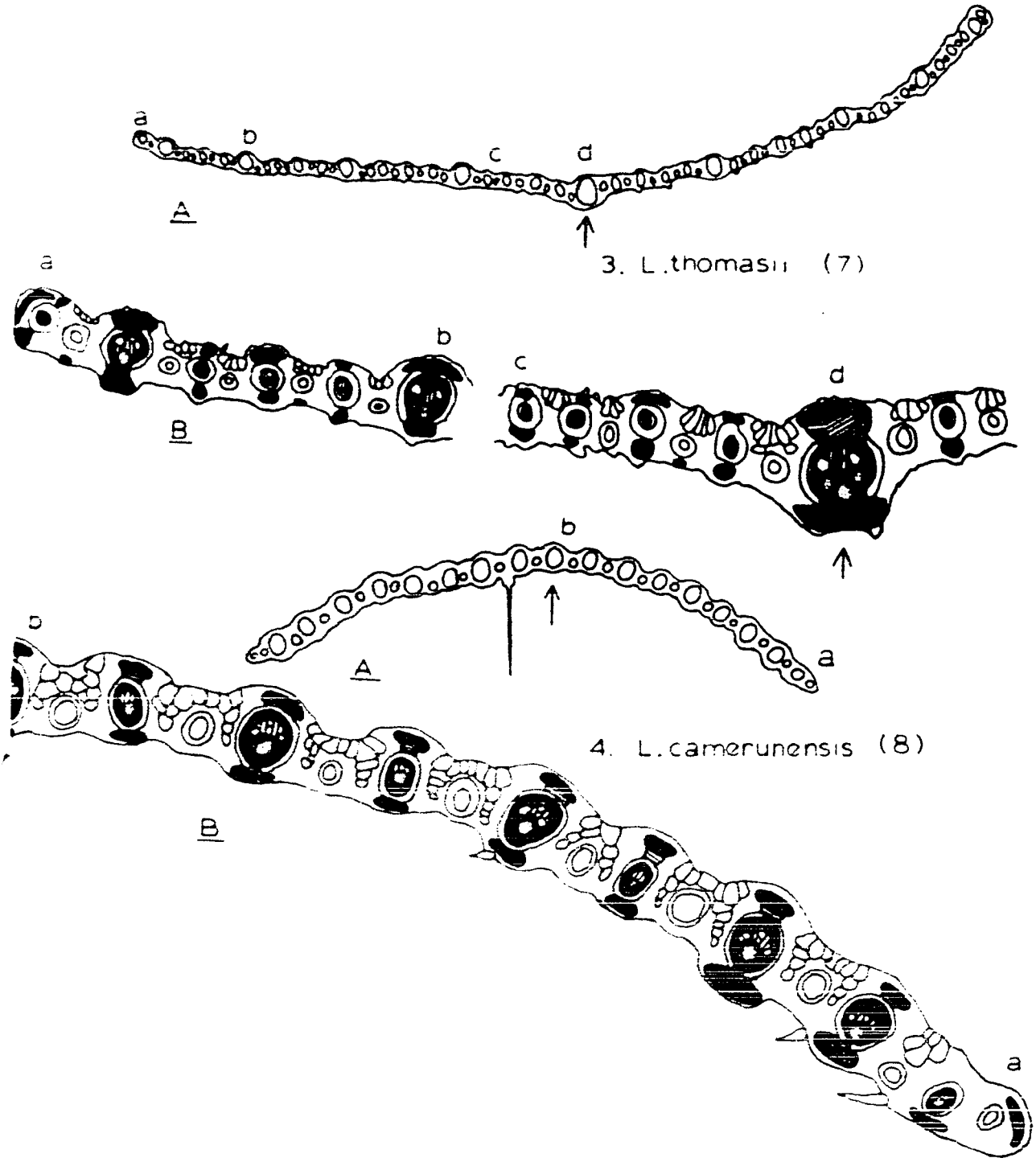
Fig. 5.1-5.35 Schematic diagrams of cross-sections of the leaves of Loudetia spp. and Rattraya. OTU numbers in parentheses.

Magnification [A 0 1mm. ; B 0 200μ]

Annotated example of B

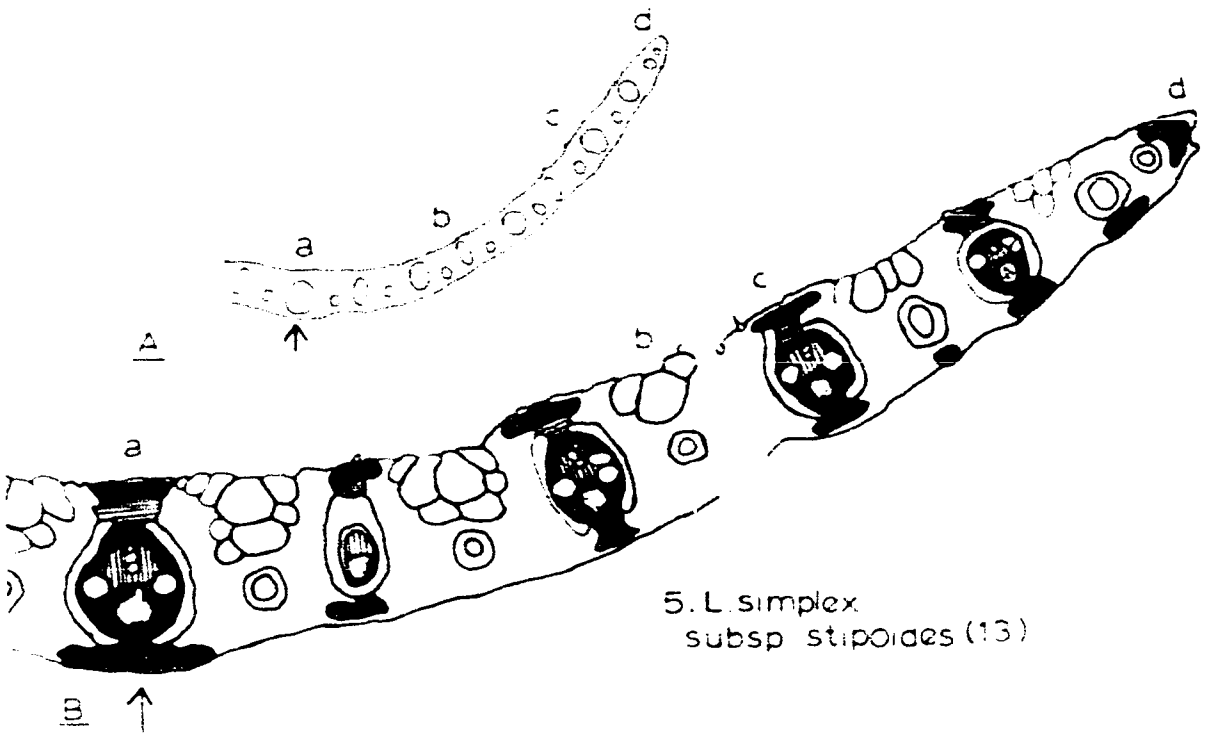



A
a
b
c
d
B
a
b
c
d
1. L. angolensis (1)
A
a
b
c
d
B
a
b
c
d
2. L. arundinacea (3)

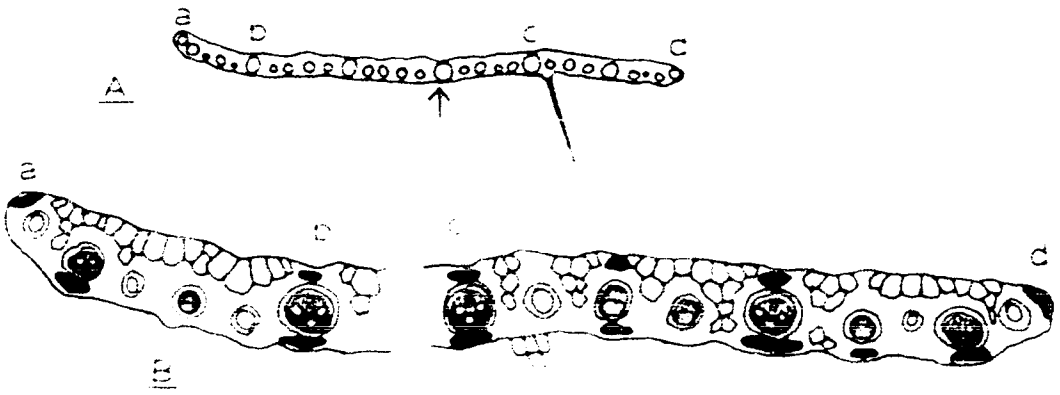


3. L.thomasii (7)

4. L.camerunensis (8)

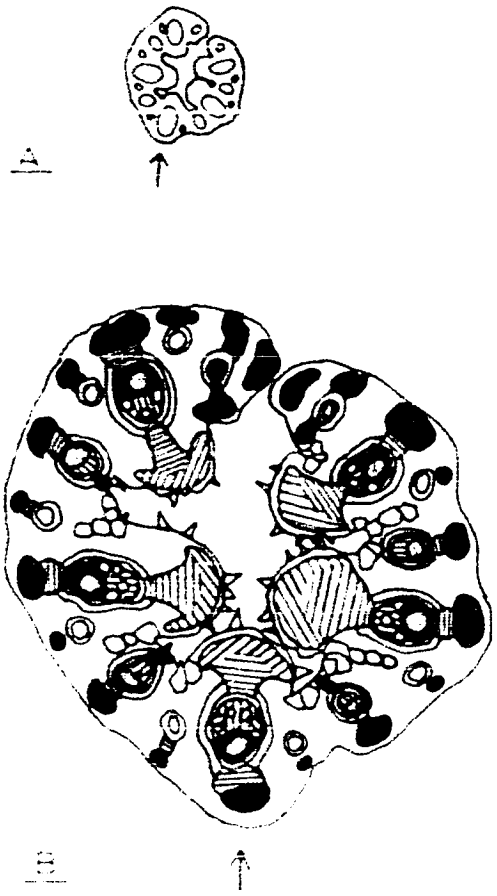


5. L. simplex subsp stipoides (13)

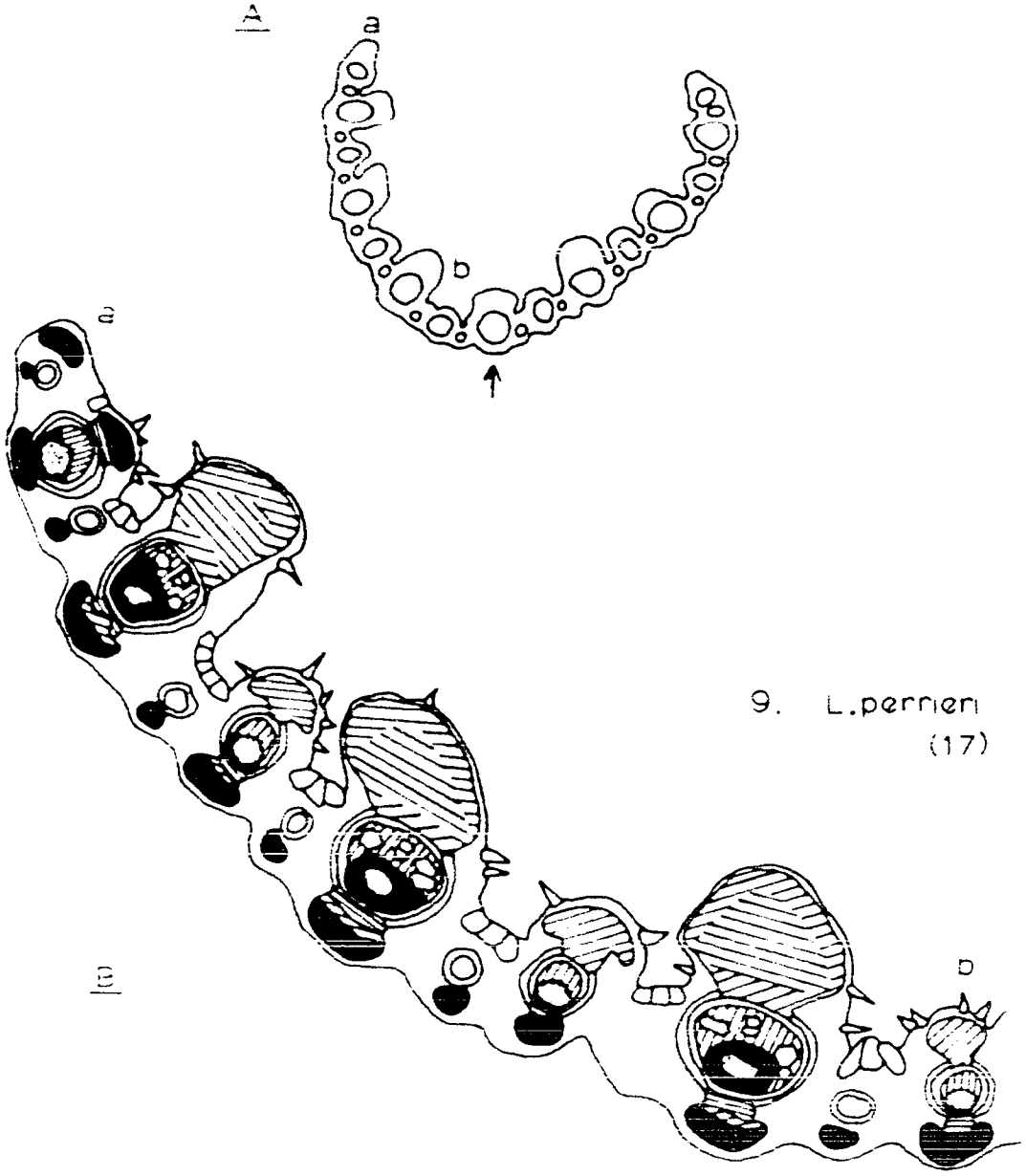


6 L. kagerensis (14)

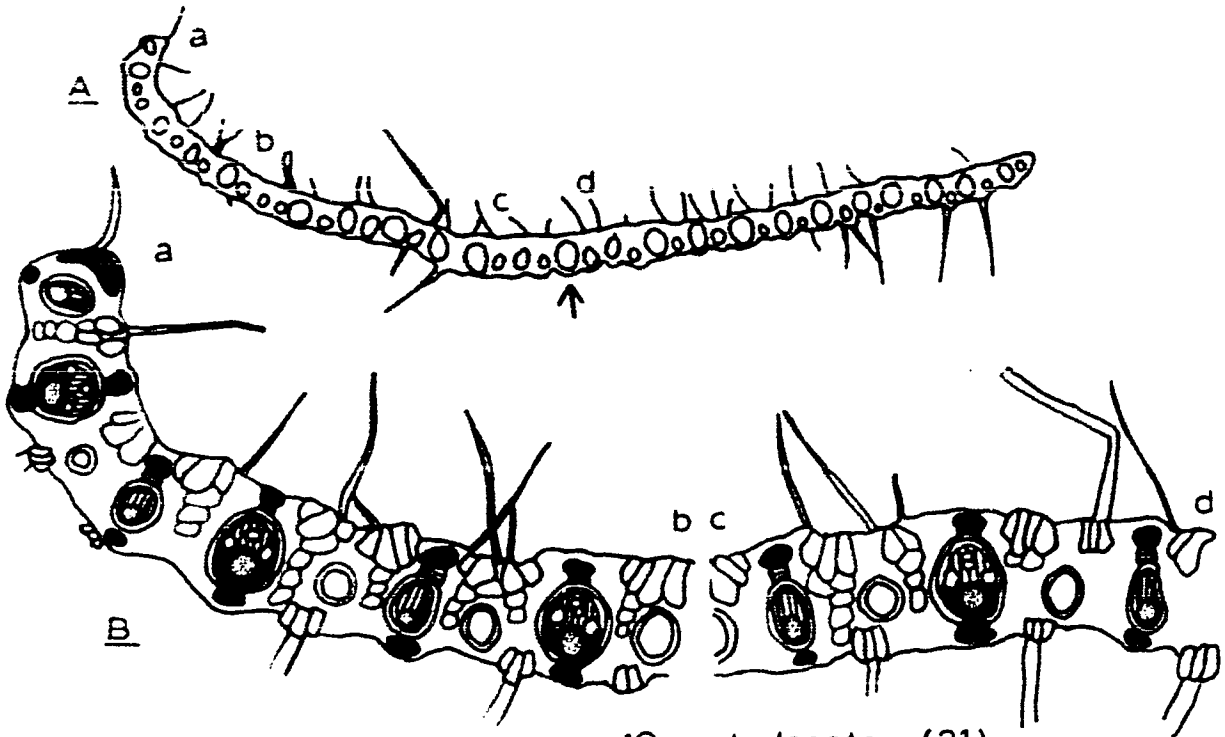

b
a
A
b
B
simplex
subsp. simplex (11)



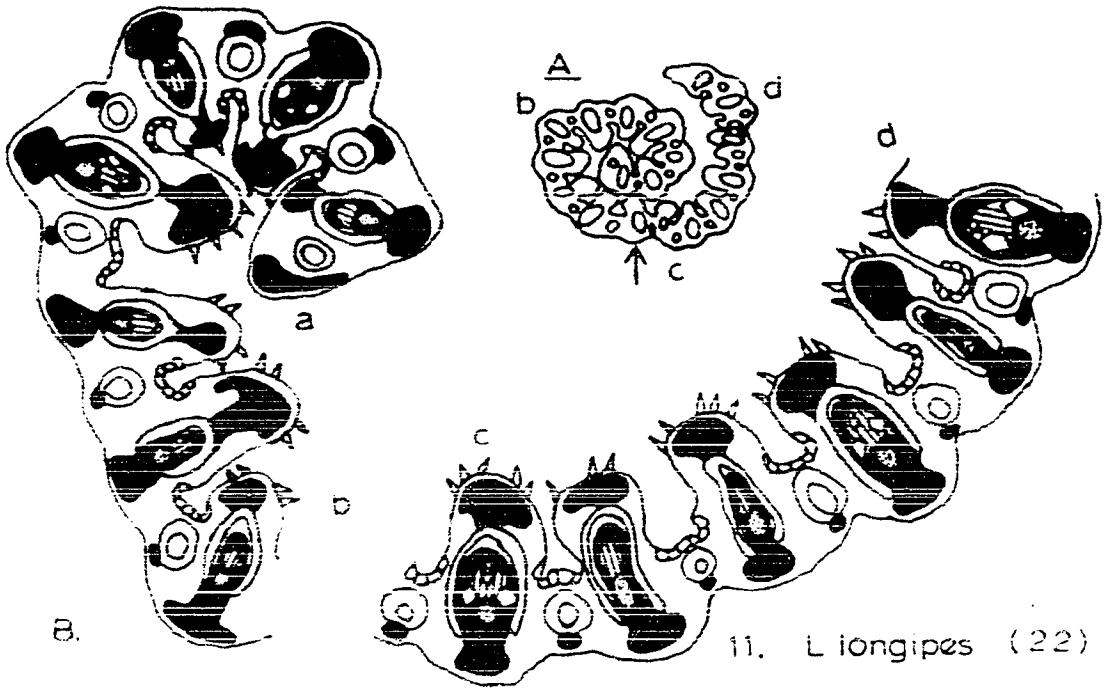
8. L. madagascariensis
(1E)



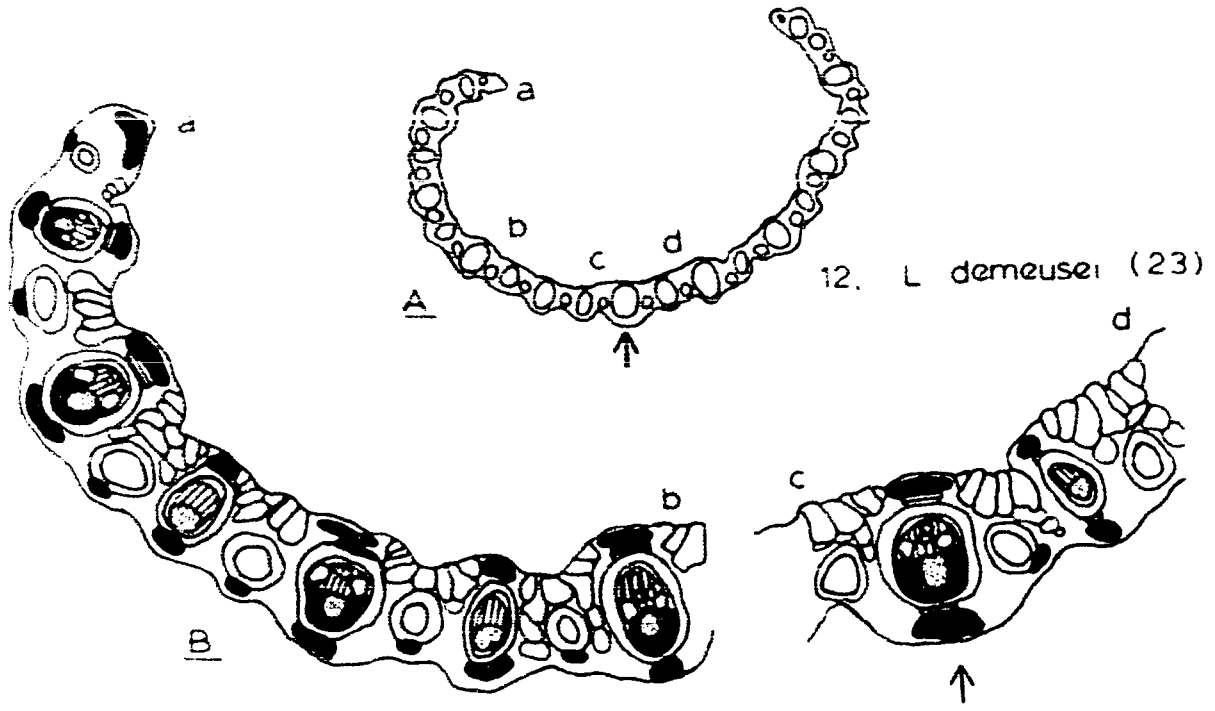
9. L.perrieri
(17)



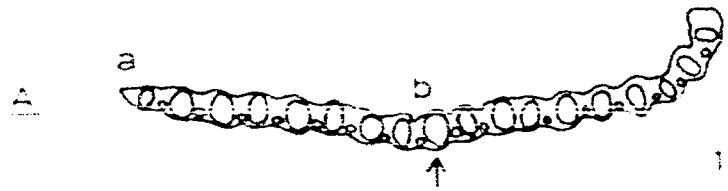
10. L. lanata (21)



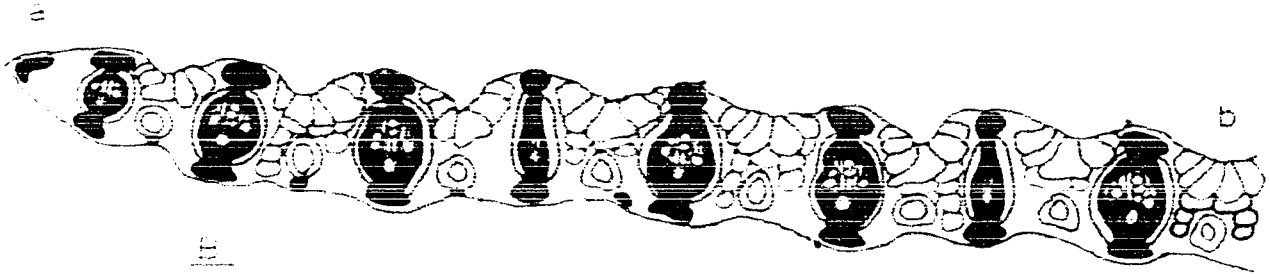
11. L. longipes (22)

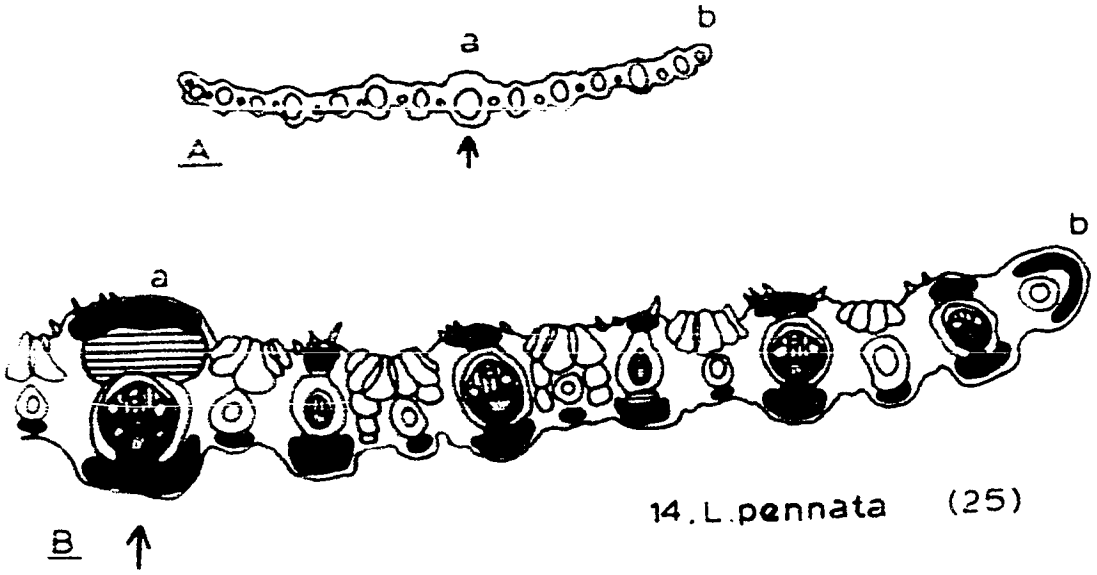


12. L demeusei (23)

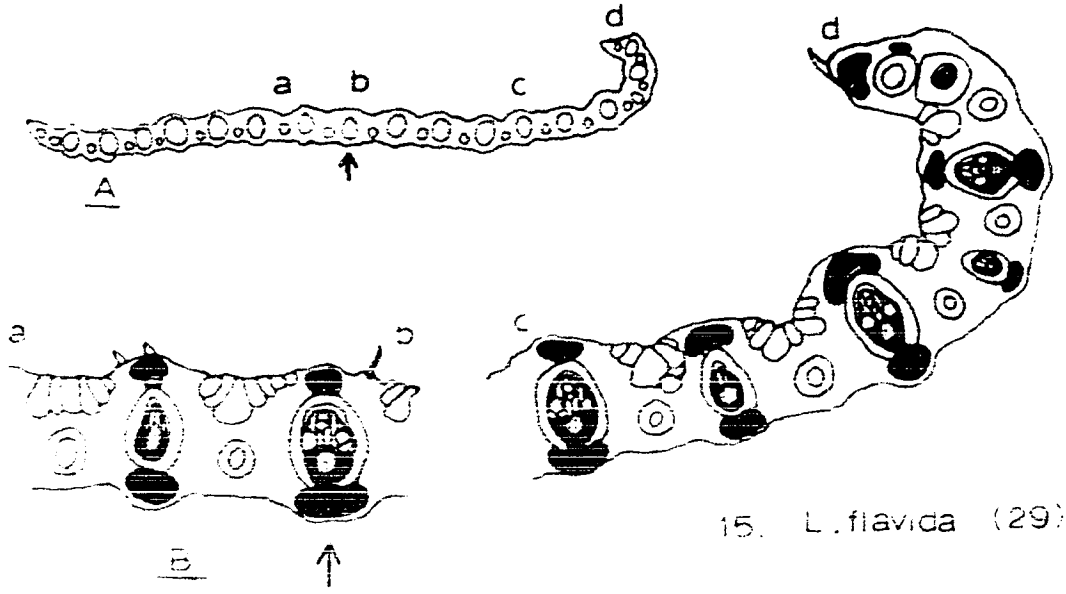


13. L crassipes (24)

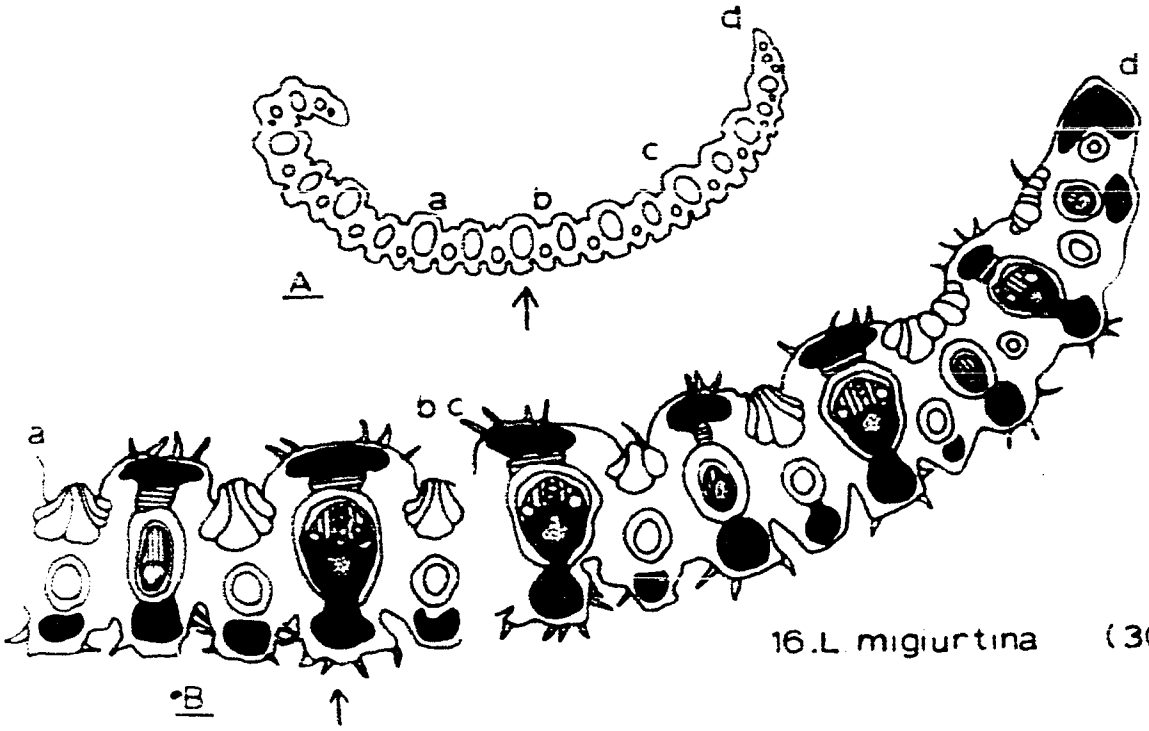




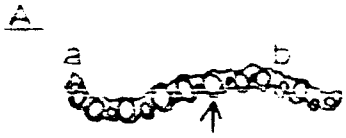
14. L. pennata (25)



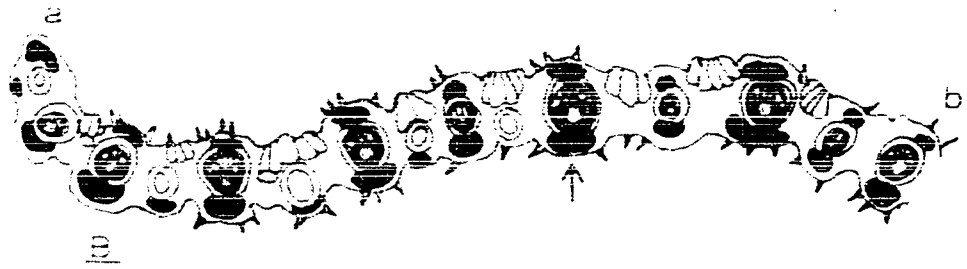
15. L. flavida (29)

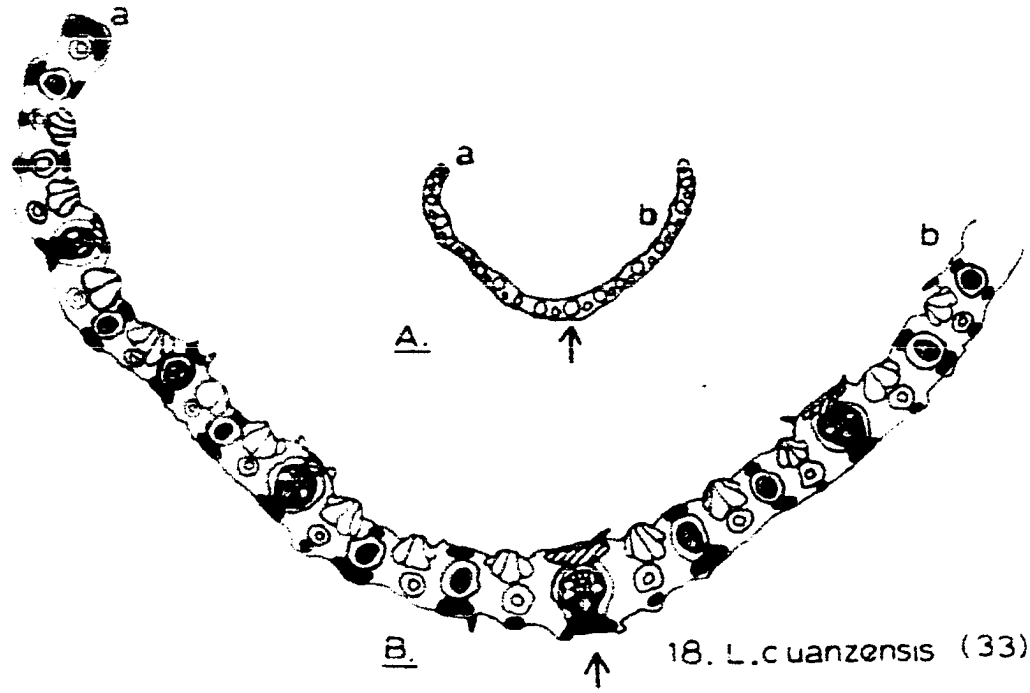


16.L migiurtina (30)



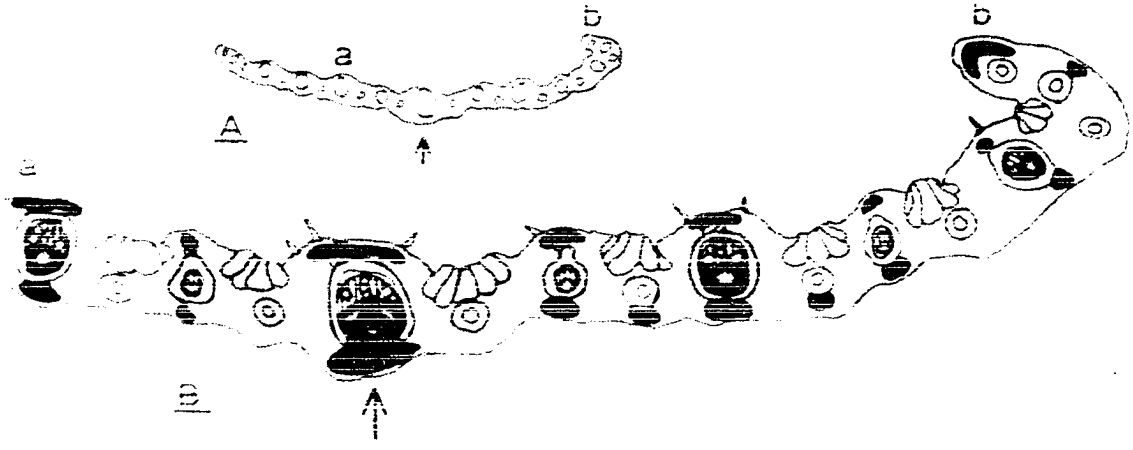
17. L acuminata (31)

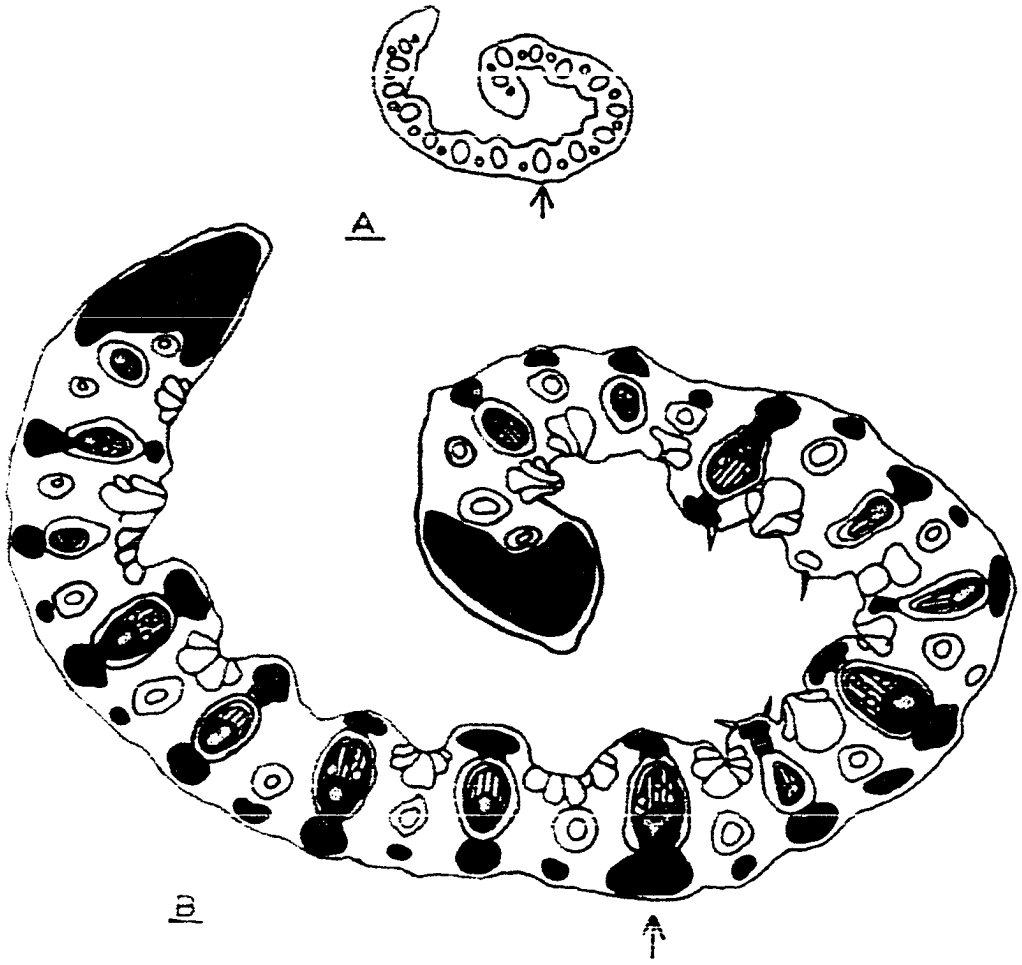




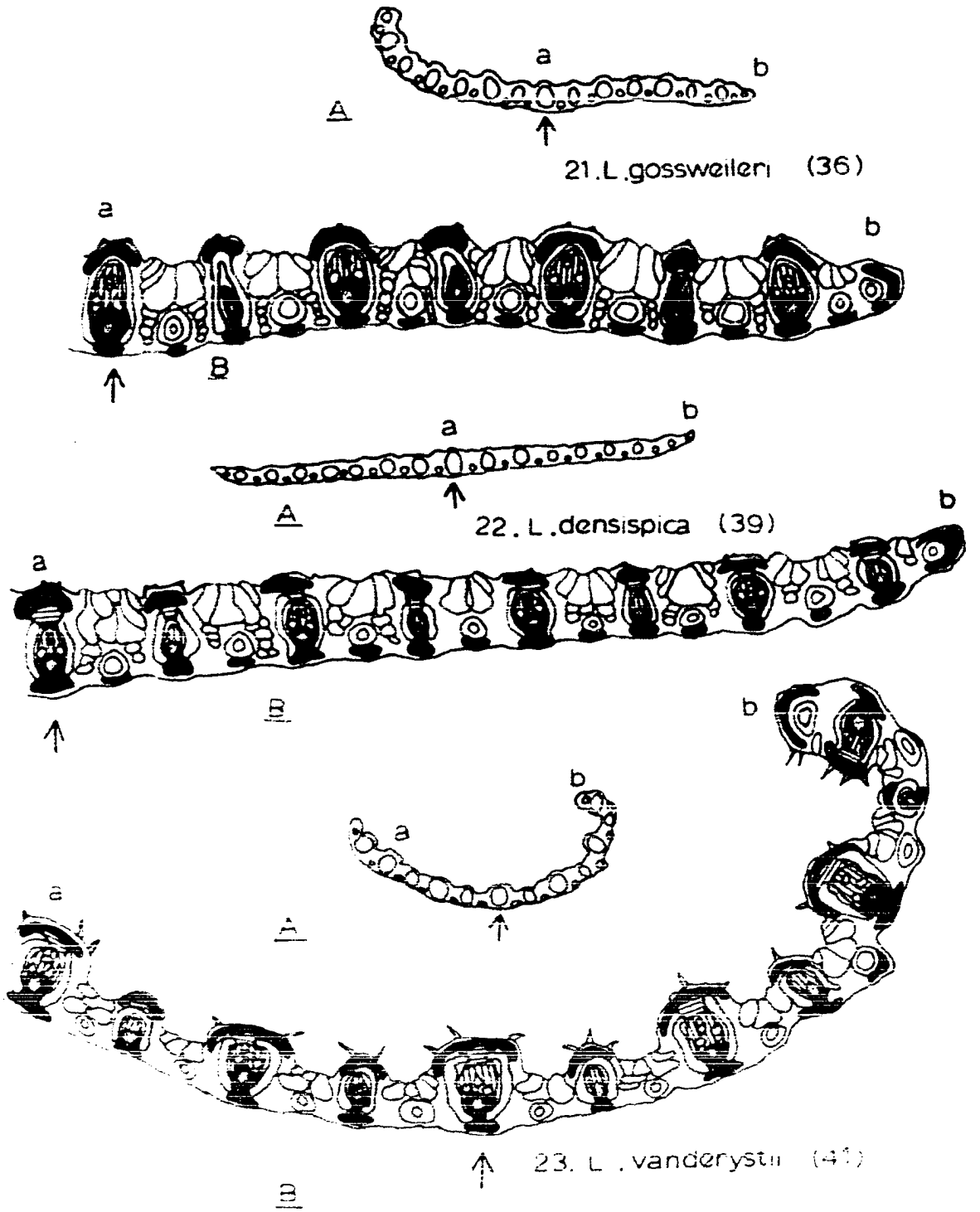
18. L.cuanzensis (33)

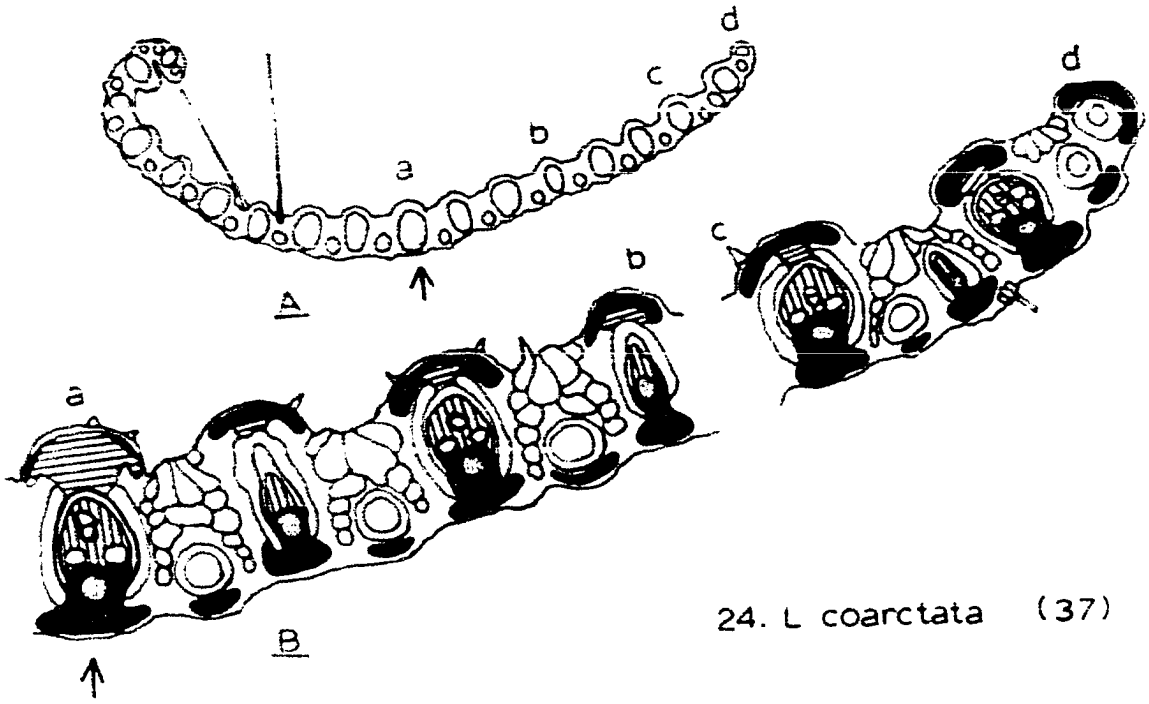
19. L.filifolia subsp.filifolia (34)



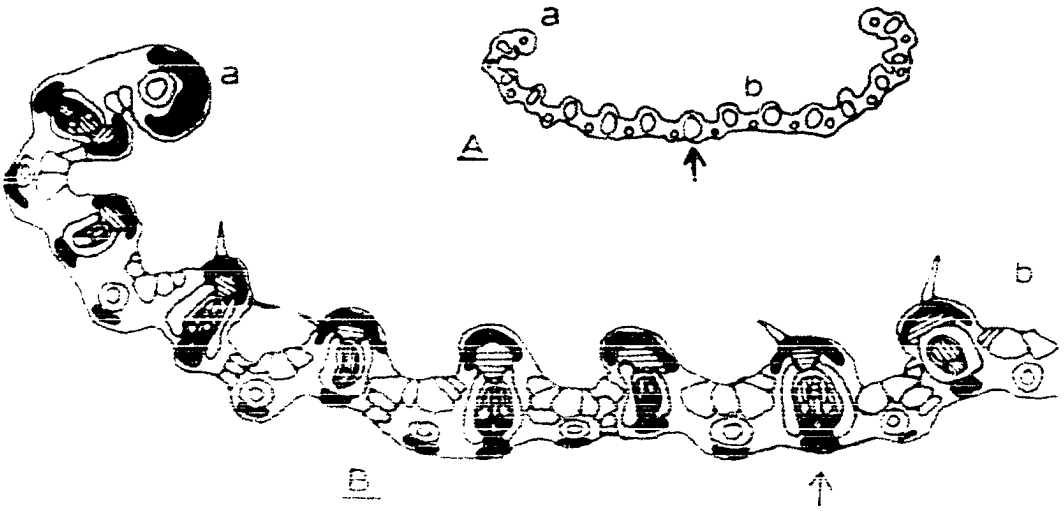


20. L. filifolia subsp. humbertiana (35)

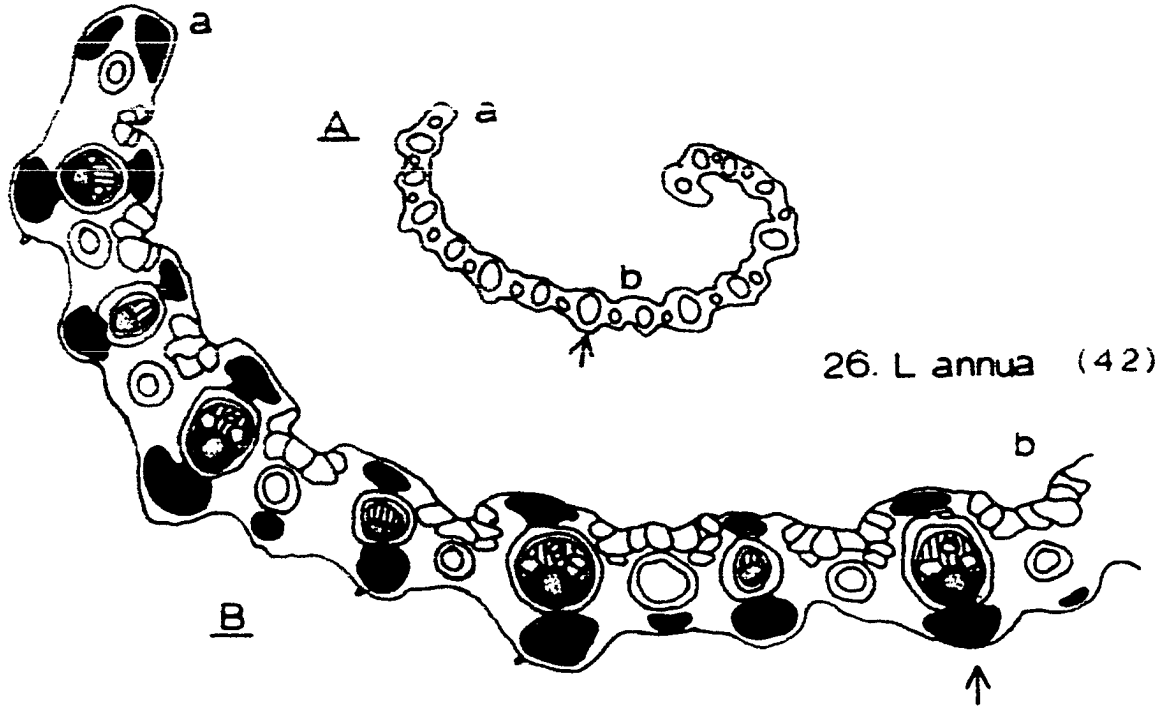

a
b
A
21. L. gossweileri (36)
a
b
B
a
b
A
22. L. densispica (39)
b
a
B
b
b
a
a
A
23. L. vanderystii (41)
B



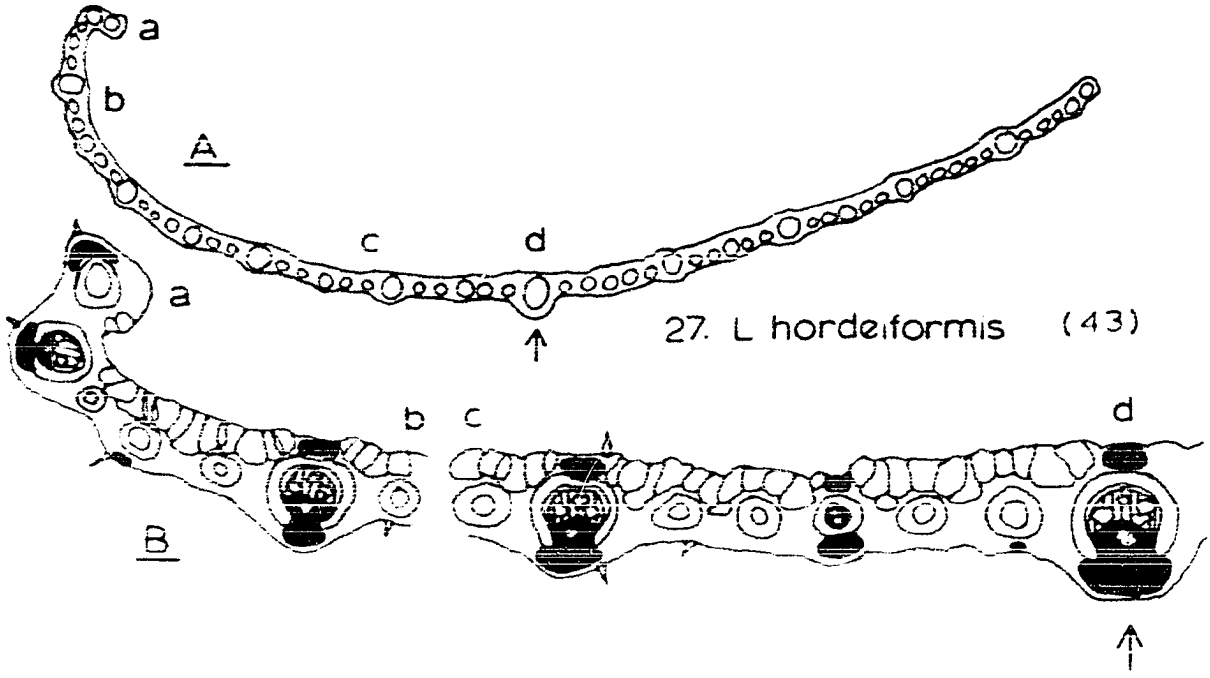
24. L coarctata (37)



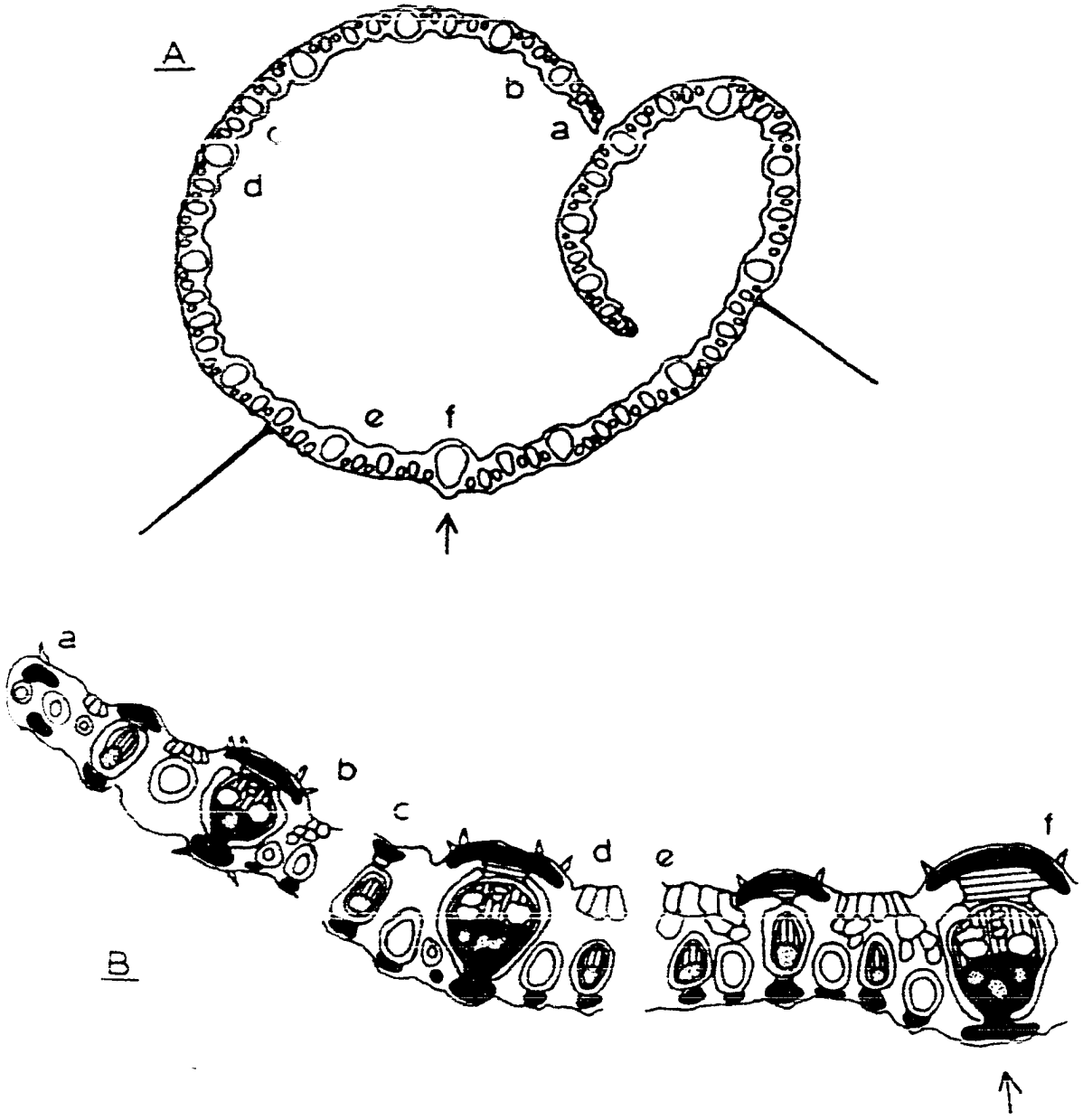
25. L. tisserantii (40)



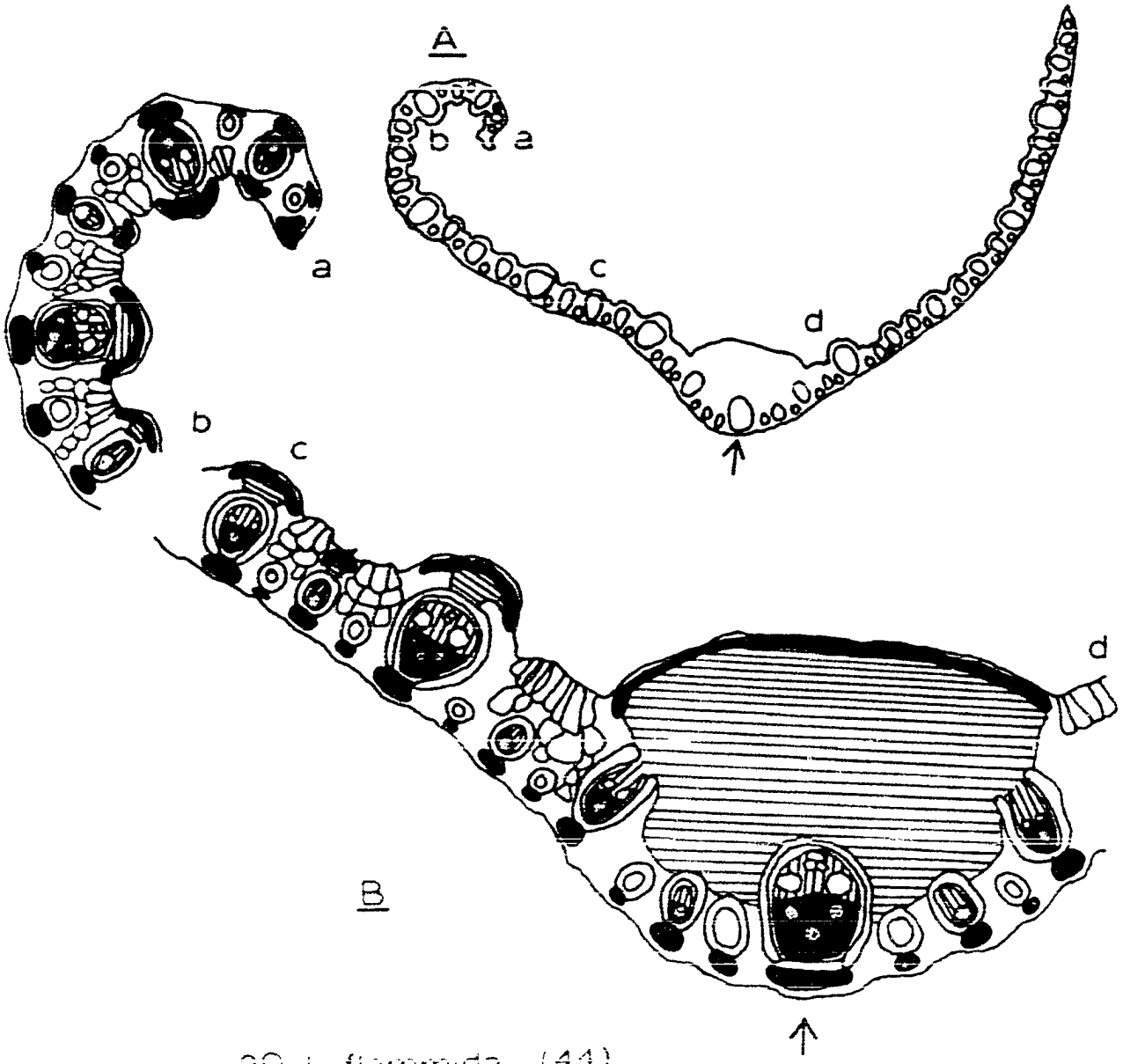
26. L annua (42)



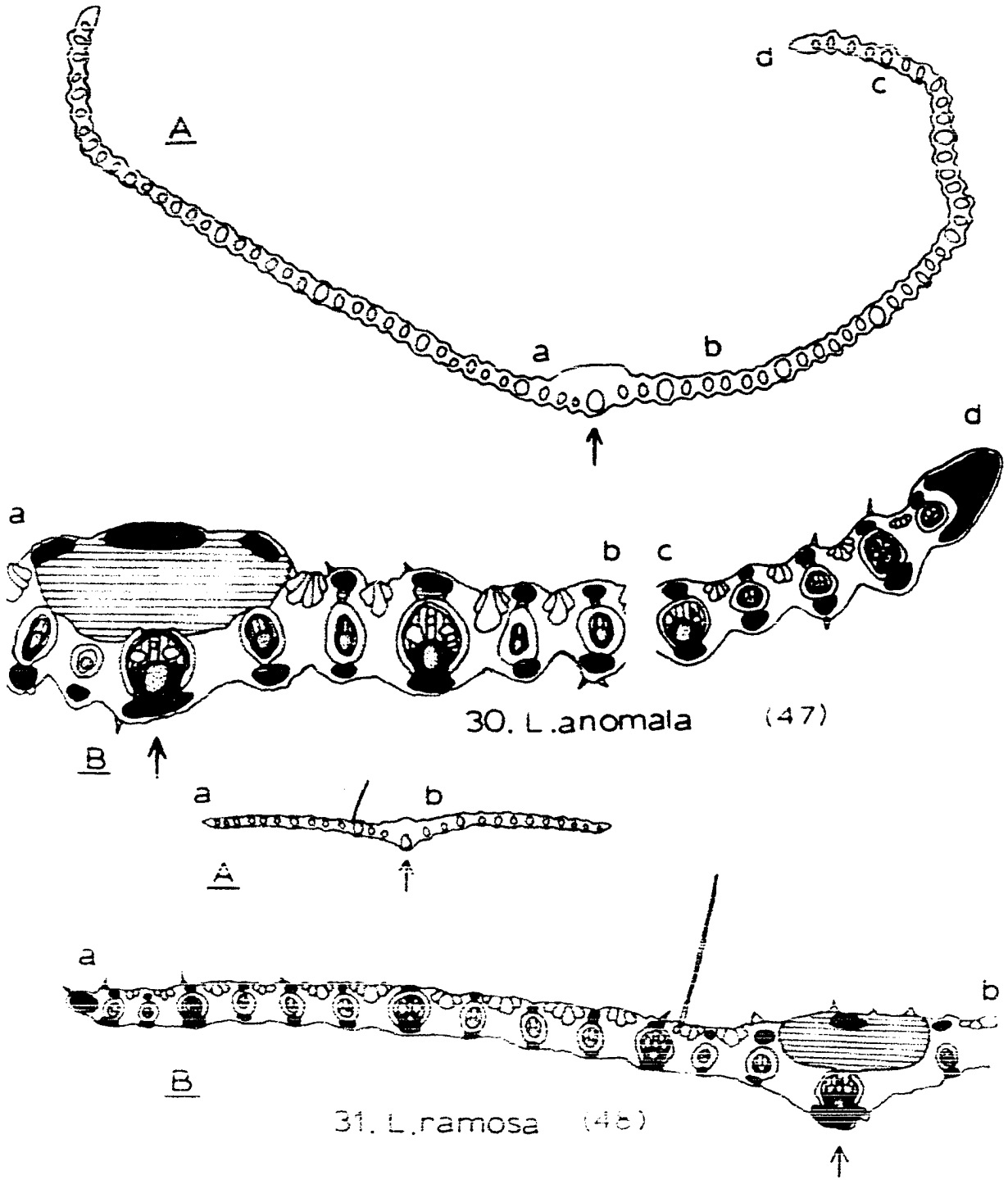
27. L hordeiformis (43)

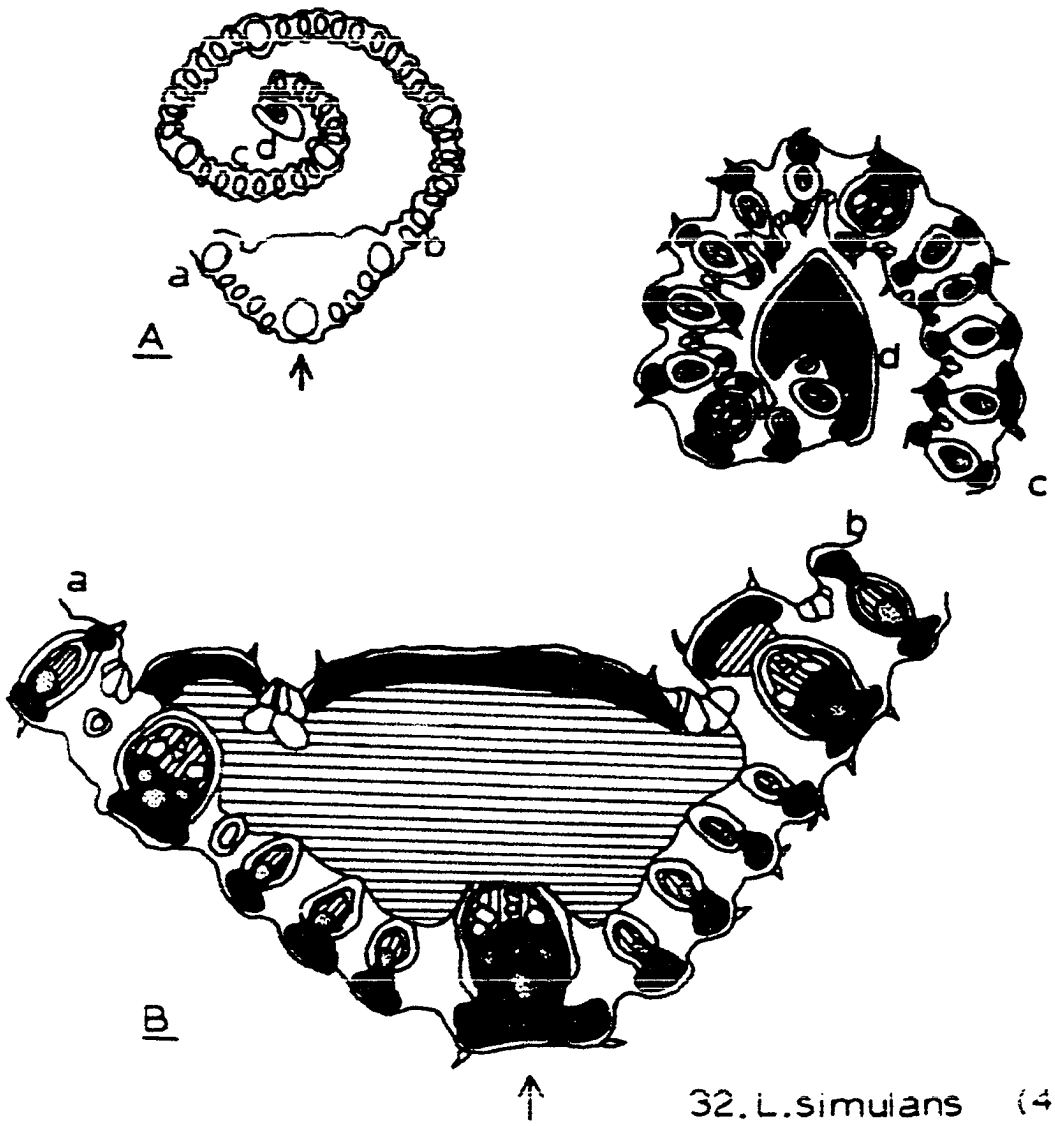


28. L phragmitoides (45)

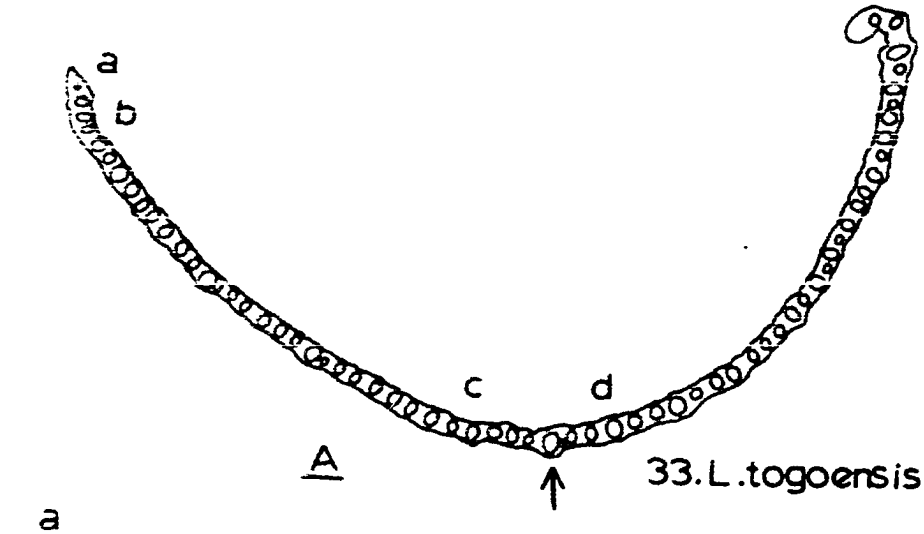


29. L. flammida (44)

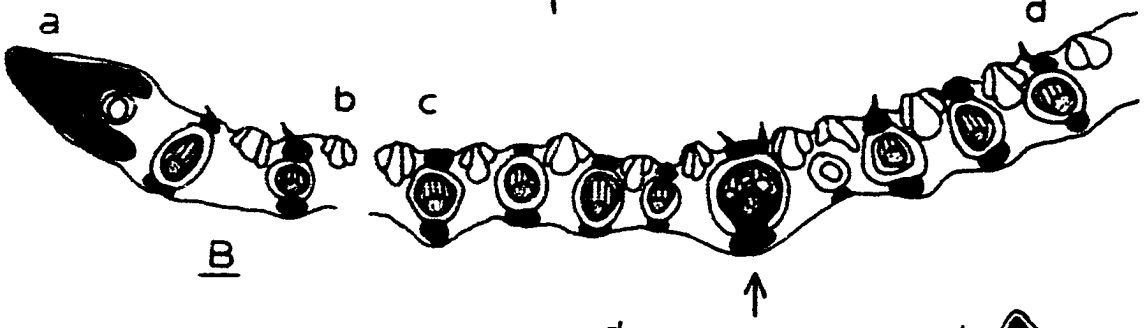

A
d
c
a
b
d
a
b
c
30. L.anomala (47)
B
a
b
A
a
b
B
31. L.ramosa (46)

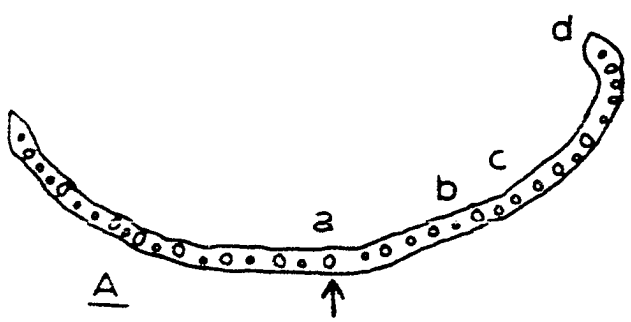


32. L. simulans (46)

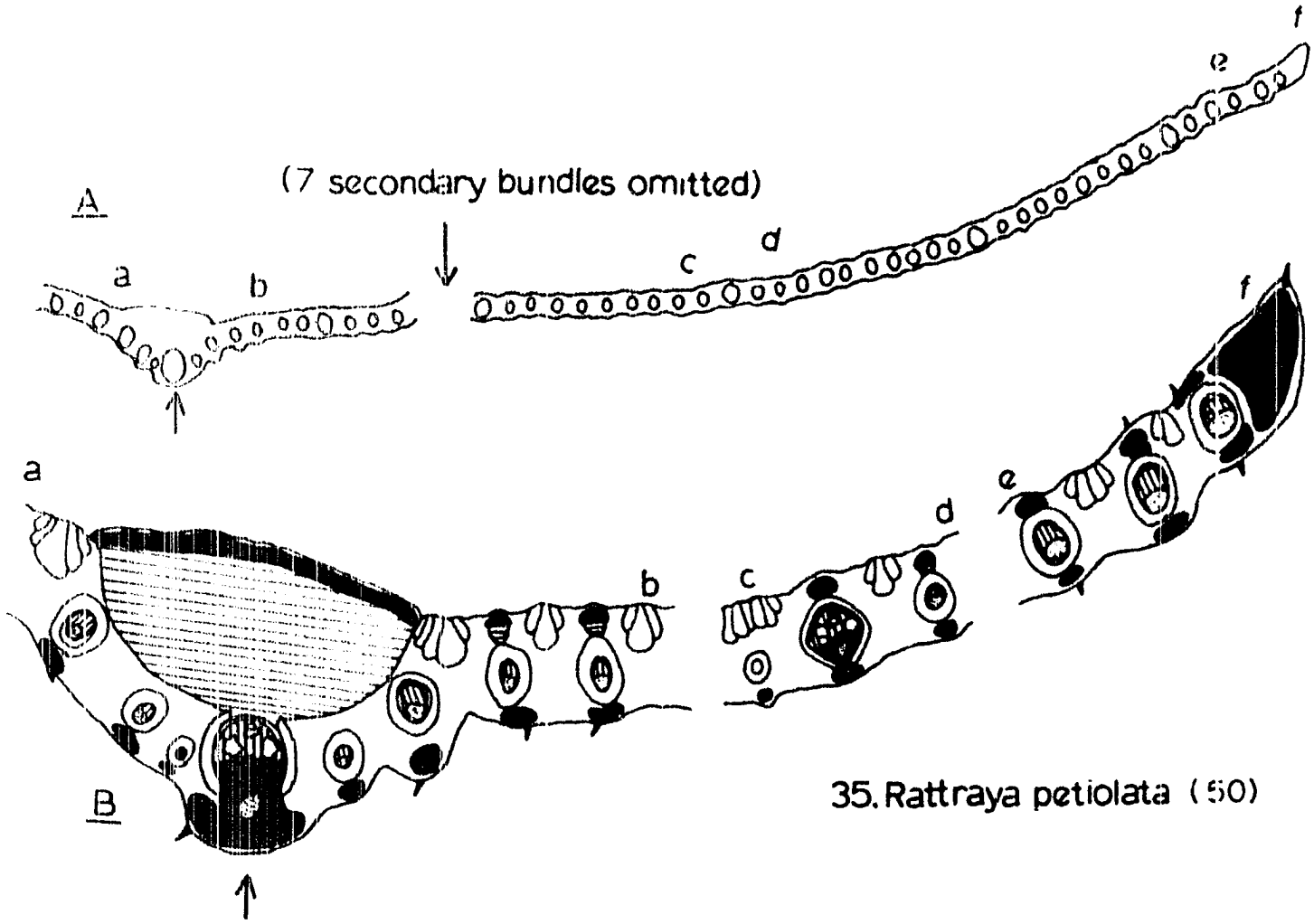


33. L. togoensis (49)





34. L jaegeriana (20)



35. Rattraya petiolata (50)

at the base of a furrow or in the corresponding region. The groups are not very distinct in the subsection Annuae (L. hordeiformis, fig. 5.27) the cells occurring over a large portion of the adaxial epidermis. The bulliform cells are in some cases no different from the other epidermal cells (L. longipes, fig. 5.11).

Two types of groups of bulliform cells are described: (a) inflated cell groups, e.g. the subsection Typicae (L. angolensis, fig. 5.1) and (b) fan-shaped groups, e.g. most species of subsection Acuminatae (L. cuanzensis, fig. 5.18). The fan-shaped group may be distinguished from the former type by the way the cells narrow on the epidermal side and fan out internally.

Associated with the bulliform cells are large inflated colourless cells which are very often quite numerous and form a large group below the bulliform cells (e.g. L. flammida, fig. 5.29). In other cases they form an archway over the tertiary vascular bundles (e.g. L. gossweileri, fig. 5.21) or they may be infrequent, or as in the case of section Pleioneura and R. petiolata, absent.

The colourless cells that are associated with the vascular bundles are of a quite different form. They are not large bubble-shaped cells and they may have thickened walls. Very often there is thin walled parenchyma adjacent to the bundle and external to this the cells become thick-walled. These cells are more numerous adaxially than abaxially.

D. The numerical taxonomy using characters of leaf anatomy

A short digression from the anatomical study of the leaf to consider the objectives of this research once again, will help to clarify the rationale behind a numerical taxonomic approach (see Figs. 2 and 3). The leaf anatomy of the various Loudetioid taxa has been examined and the differentia, which are quite adequate in formulating a classification of the taxa based on leaf anatomy, have been pointed out. If this classification was made (Fig. 2, no. 3), by direct reasoning (T_D), conclusions could be drawn about the genus (6) and an appraisal (T_6) of these conclusions made. A subjective approach such as this, however, is open to question because it is a matter for interpretation and the abundance of information cannot be satisfactorily assimilated. The alternate pathway whereby the information is quantified (T_{3M}) and analysed mathematically (T_{4M}) to provide conclusions about the genus is therefore used. Using 79 characters of the leaf anatomy a classification of the taxa is produced, and the way in which this fits into the whole thesis is indicated in Figure 3.

The general numerical methods have already been considered (chapter 3) and a flow chart (Fig. 6) shows their application using the leaf anatomy characters and OTU's represented by the species of the genus. Another flow chart (Fig. 7) illustrates the application of numerical methods with the supra-specific taxa as the OTU's. As will be seen from these diagrams there are three main processes involved, viz. data preparation and character and OTU analyses.

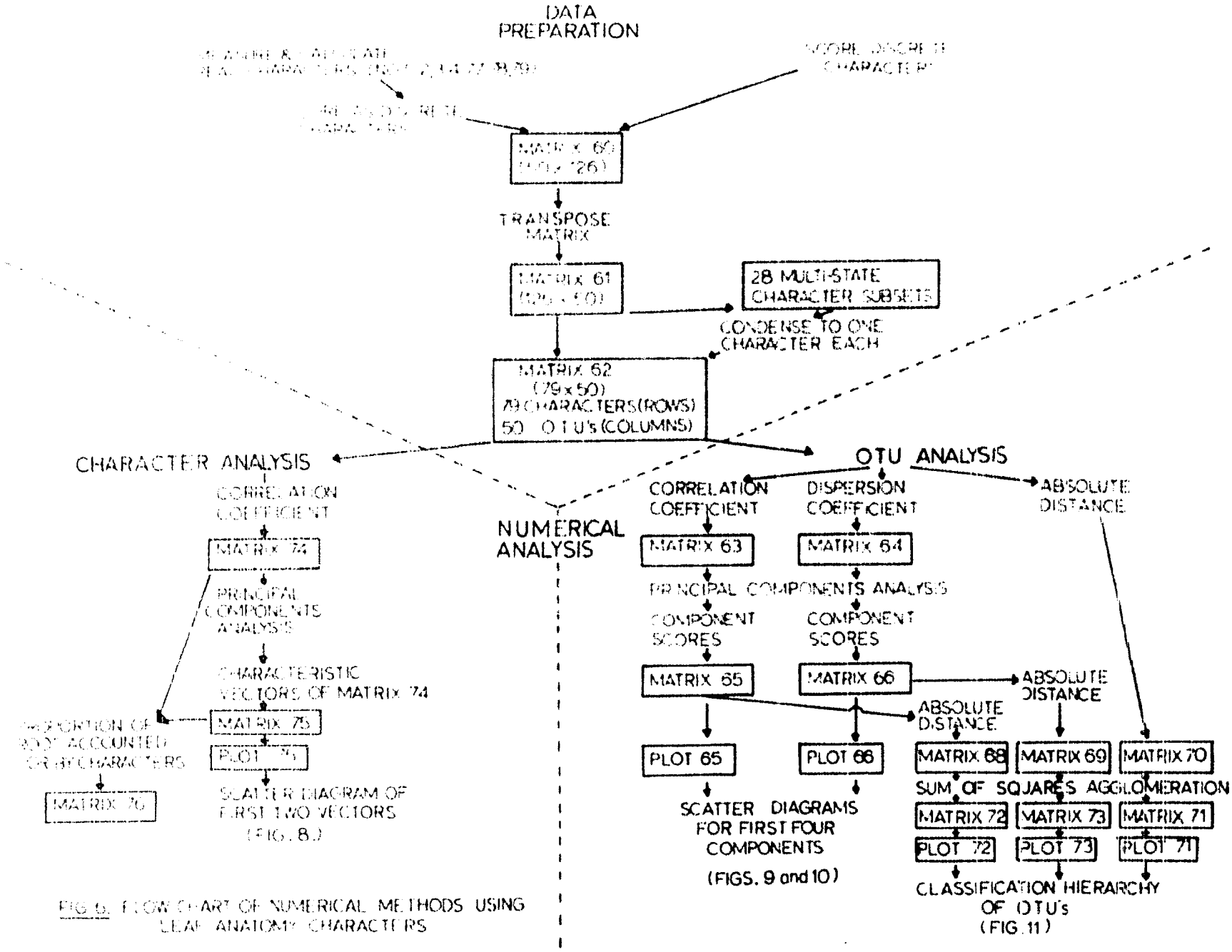


FIG. 6. FLOW CHART OF NUMERICAL METHODS USING LEAF ANATOMY CHARACTERS

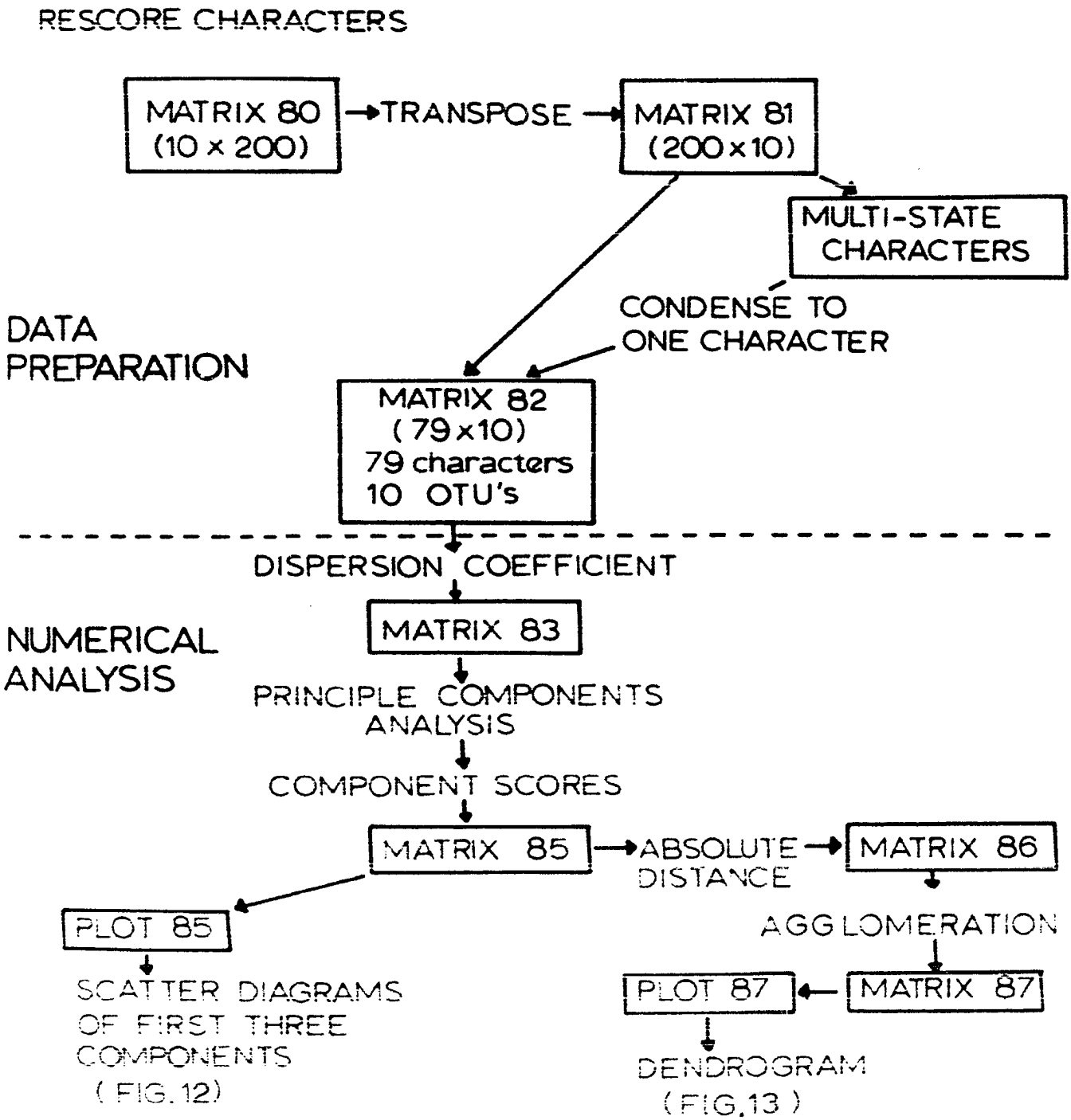


FIG. 7. FLOW CHART OF NUMERICAL METHODS USING SUPRA-SPECIFIC TAXA AS OTU's AND LEAF ANATOMY CHARACTERS

1. Data Preparation

An anatomical study of the leaf, as described above, provides the information needed for the numerical analysis, viz. the n x t data matrix. The data has to be suitably quantified to produce this matrix and this involves a detailed examination of the t OTU's so that their n characters may be scored in numerical terms.

(a) The OTU's

There are fifty OTU's included in this particular study (table 4). Each of the supra-specific groups is designated by a particular symbol which is used in the diagramatic results. These taxa are numbered from 1 to 10, as indicated, and the 50 OTU's are also numbered so that this table may be referred to from the figures. It will be noted that some of the species are represented by more than one exemplar. Some of the exemplars of a species are all cultivated under uniform conditions, e.g. L. arundinacea (3, 4, 5, 6) and L. simplex subsp. simplex (9, 10, 11), others are all from the wild, e.g. L. madagascariensis (18, 19) and L. densispica (38, 39), and yet others are from both wild and cultivated conditions, e.g. L. angolensis [1(cultivated), 2 (wild) , and L. flavida 27, 28 (cultivated) and 29 (wild)].

(b) The characters of the leaf anatomy

Seeing that the leaf anatomy of other genera of the Arundinelleae (Li, 1969) as well as that of Loudetia was being studied an attempt was made to list as many useful characters and their corresponding states as possible for the whole tribe.

Table 4 : List of OTU's used in the taxonomy based on leaf anatomy characters (OTU numbers and symbols used in Figures 9 - 13).

Section: Loudetia

1. Subsection: Typicae

- 1. L. angolensis
- 2. L. angolensis
- 3. L. arundinacea
- 4. L. arundinacea
- 5. L. arundinacea
- 6. L. arundinacea
- 7. L. thomasii
- 8. L. camerunensis
- 9. L. simplex subsp. simplex
- 10. L. simplex subsp. simplex
- 11. L. simplex subsp. simplex
- 32. L. simplex subsp. simplex
- 12. L. simplex subsp. stipoides
- 13. L. simplex subsp. stipoides
- 14. L. kagerensis
- 15. L. kagerensis
- 16. L. kagerensis
- 17. L. perrieri
- 18. L. madagascariensis
- 19. L. madagascariensis

10. Incertae sedes

- 20. L. jaegeriana

2. Subsection: Pungentes

- 21. L. lanata
- 22. L. longipes
- 23. L. demeusei
- 24. L. crassipes

3. Subsection: Acuminatae

- 25. L. pennata
- 26. L. pennata
- 27. L. flavida
- 28. L. flavida
- 29. L. flavida
- 30. L. migiurtina
- 31. L. acuminata
- 33. L. cuanzensis
- 34. L. filifolia subsp. filifolia
- 35. L. filifolia subsp. humbertiana

4. Subsection: Densispicae

▲ 36. L. gossweileri
▲ 37. L. coarctata
▲ 38. L. densispica
▲ 39. L. densispica
▲ 40. L. tisserantii
▲ 41. L. vanderystii

5. Subsection: Annuae

■ 42. L. annua
■ 43. L. hordeiformis

6. Subsection: Flammidae

△ 44. L. flammida
△ 45. L. phragmitoides

7. Section: Pleioneura

○ 46. L. simulans
○ 47. L. anomala
○ 48. L. ramosa

8. Section: Lophanthera

□ 49. L. togoensis

9. Rattraya

⊗ 50. R. petiolata

A number of these turned out to be invariant for Loudetia and were rejected. A list of the 79 characters used is given in table 33 (Appendix 2). The states of many of these characters are easily decided upon, even from the diagrams (Fig. 5. 1-35) but in some cases an explanation is necessary.

General Characters

The width and thickness of the leaf were coded as discrete characters because only a single leaf was sectioned in most cases and a satisfactory sample for any one specimen was not obtained. The three states for the width of the leaf (character 2) are as follows:

narrow	< 2 mm
average	2 - 5 mm
wide	> 5 mm

The actual widths of the lamina for each OTU are recorded in table 34 (Appendix 2).

The character states for the thickness of the lamina (characters 3 and 4) are:

relatively thin	< 165 μ
moderate	165 - 330 μ
relatively thick	> 330 μ

In some cases there was some indecision as to the state of either of these two characters, e.g. leaf width 2 mm, but the states were usually readily distinguished.

Margin of the leaf

The leaf margin (character 17) was very rarely strongly swollen, this condition being attained when the margin was

almost or greater than twice the thickness of the adjacent sections of the blade. This character was correlated with the amount of fibres and the shape of the fibre group.

Midrib

The degree of prominence of the keel (character 22) was decided as follows:

absent - no keel obvious

slightly prominent - keel obvious and yet not as large as follows:

strongly prominent - the keel portion of the midrib equal to or greater than one third of the thickness of the lamina in the midrib region.

Largest midrib or central vascular bundle

When there was no obvious midrib the largest primary bundle at the centre of the leaf was taken as being the equivalent.

Vascular Bundles

Obviously there is some variation in the characters of the vascular bundles of any one order. The vascular bundles of the primary type were surveyed and the most typical characters were scored, this being repeated for the secondary and tertiary bundles. Some subjective judgement was necessary in deciding what the typical characters were but this approach seemed a more reasonable one than selecting a bundle randomly and scoring its characters.

Shape of the Vascular Bundle

In all cases the shape of the vascular bundle was consid-

ered as if the bundle had no sheaths, i.e. the shape of the xylem and phloem (and fibrous tissue if present) alone.

Strands and Girders

If the group of fibres above or below a bundle were isolated and did not touch the sheath, they are often separated by colourless cells, they were regarded as strands. Groups of fibres attached to one or other of the epidermis surfaces and the bundle or bundle sheath were called girders.

Proportion of different orders of vascular bundles

It was found that there was some variation in numbers, arrangement and proportion of the different orders of bundles and although only a single leaf was examined, it seemed that the proportion of the different orders of bundles might be important taxonomically. The number of primary bundles, for example, often appeared to be about one half, one third, etc. of the number of secondary bundles, so a coding system was devised which indicated the more usual ratio of one order of bundle to another (characters 77-79). The exact numbers of the different orders of vascular bundles in the leaf are tabulated for all OTU's (table 34, Appendix 2).

Production of the data matrix

All characters used here are of the discrete type and are simply coded as integers, according to the states as shown in table 33 (Appendix 2). For each of the OTU's the characters were scored and the data was punched on IBM computer cards (Matrix 60, Appendix 2). For the convenience of our computer programmes the matrix was transposed so that the 50 OTU's were

in the columns and the characters in the rows (Matrix 61). Twenty-eight of the multi-state characters were represented by states in a non-linear order (e.g. characters 6, 7, 9, 12 etc.) and these were first subjected to a principal components analysis to obtain the maximum amount of information in a single character. These characters were returned to their position in Matrix 61 so that a data matrix (No. 62) was produced with 79 characters represented by rows and 50 OTU's as columns.

The data preparation was carried out in the same way for the 10 OTU's each representing a combination of one or more 50 OTU's. Matrix 82, with 79 characters (rows) and 10 OTU's (columns) was the end product in this case.

2. Analysis of Characters

This approach will be discussed first because it is useful to see what characters are important in the classification of the OTU's. The large numbers of characters of the leaf anatomy (79) makes the analysis very cumbersome and apart from the fact that the computing time is greatly increased, it is difficult to interpret the results. This is especially so because one has no preconceived ideas on definite groups of characters as is the case with OTU's.

Only the correlation coefficient was used in this case. The correlation matrix (No. 74) was subjected to a principal component analysis to extract the characteristic vectors (matrix 75) and roots (table 5). The proportion of each root accounted for by the characters was also calculated and these are listed as percentages in table 37 (Appendix 2). Some

Table 5 : Roots of the correlation matrix (No. 74)

Root	Value of Root	% of total variance accounted for	Accumulated percentage
1	10.325	13.07	13.07
2	8.981	11.37	24.44
3	6.255	7.92	32.36
4	5.858	7.42	39.77
5	4.730	5.99	47.76
6	4.297	5.44	51.20
7	3.766	4.77	55.96
8	3.061	3.88	59.84
9	2.831	3.58	63.42
10	2.247	2.84	66.27
11	2.205	2.79	69.06
12	2.049	2.59	71.65
13 - 50	22.395	28.35	100.00
Total Variance	79.000	100.00	-

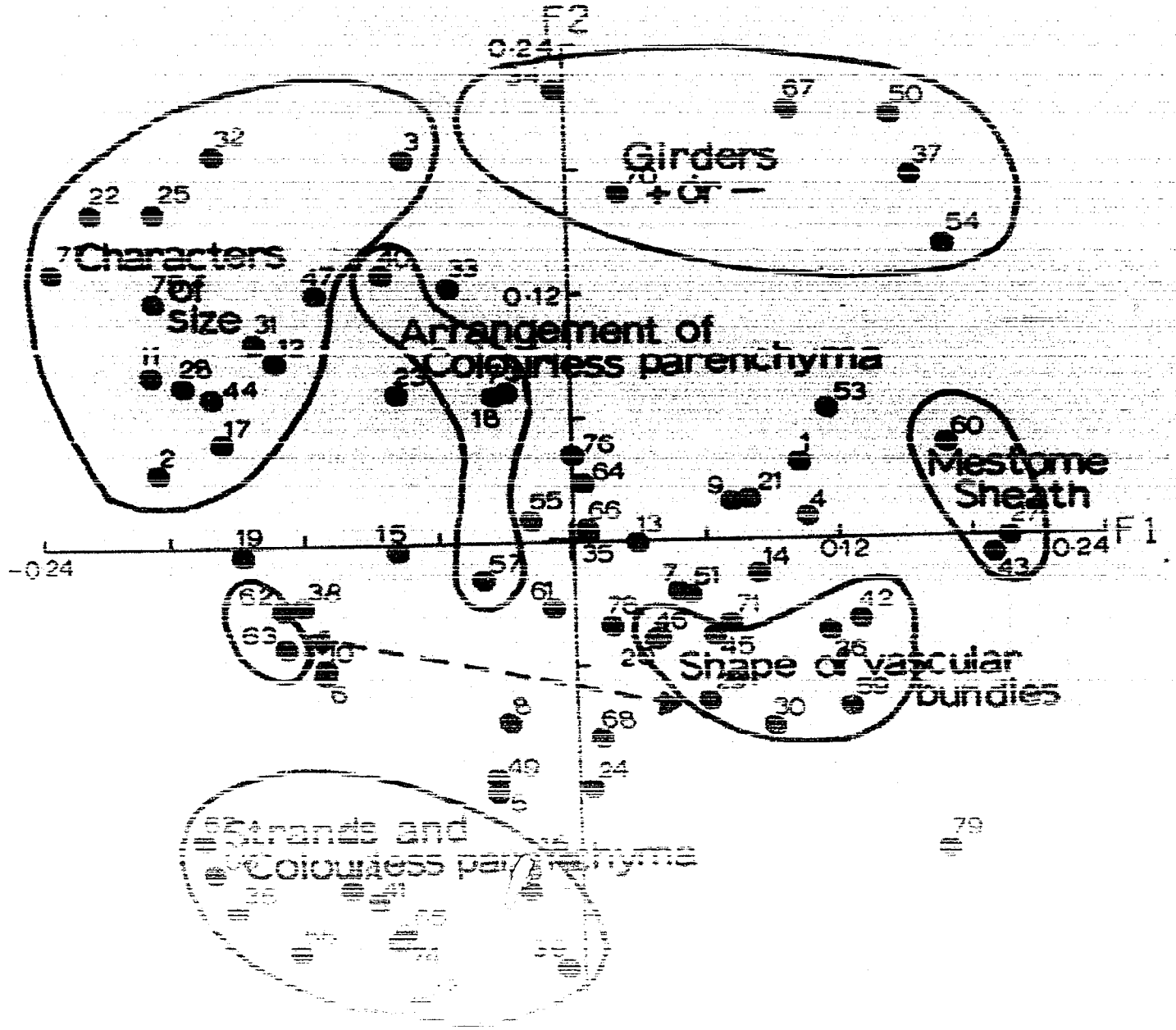
information on the importance of the characters may be obtained from an examination of this table. However, the character-character relationships are more readily seen when a scatter diagram of the character scores on the first two vectors from matrix 75 is produced (fig. 8).

Character groupings

The distribution of characters on the first two vectors appears to be quite uniform but closer analysis reveals that similar characters, or characters measuring similar properties are associated with one another although these do not fall into distinct groups, which could be clustered as such. The broad range of six "groups" of similar characters have been indicated on the overlay to figure 8.

I Characters of size or related to size

This is a somewhat obscure group of characters usually with high positive scores on the first axis. The characters are usually related to size of the lamina. Those which are connected directly with leaf size are width, thickness, type of leaf margin, presence or absence of a keel, number of vascular bundles in the midrib (2, 3, 17, 22, 25). Large leaves generally have more fibrous tissue in the bundles (28 and 44), more than one adaxial strand in the midrib (32), and a greater number of primary vascular bundles (77). The leaves of the section Pleioneura are often large and thus characters which differentiate this section are in this group. Presence or absence of colourless cells and their arrangement (11 and 12) and presence and absence of tertiary bundles (75) fall into


F2
0·24
0·12
Girders
+ or —
Characters
of
Size
Arrangement of
Colourless parenchyma
Mestome
Sheath
Shape of vascular
bundles
Strands and
Colourless parenchyme
F1
−0·24
0·12
0·24

this category.

The contribution of the above characters to the variance of the first root amounts to 39.8% and 20.88% of the variance of the second root. This amounts to about 7.5% of the total variance (table 5).

II Presence or absence of girders of sclerenchyma

All of the characters that record presence or absence of both adaxial (38, 50, 87) and abaxial girders (37, 54, 70) are grouped together with high positive scores on the second axis. The adaxial girders have higher scores than the abaxial girders and they contribute 13.31% to the variance of the second root. The abaxial girders contribute 7.96% so that combined the girders contribute 21.27% to the variance of the second root. In contrast the girders contribute only 8.44% to the first root and 3.47% to the third root.

III Presence or absence of sclerenchyma strands, and associated colourless parenchyma

The presence of strands (36, 48, 52, 65, 69) is positively correlated with the presence of associated colourless cells (39, 41, 56, 58, 72, 74). These characters occur when girders are absent and they have negative scores on the second axis. The colourless parenchyma characters generally have the higher negative scores, but combined, all these characters contribute 37.5% to the variance of the second root, and 13.44% to that of the first root. Seeing that the second root accounts for 11.37% (table 5), these characters contribute over 4% to the total variance on this root alone.

IV The amount of adaxial colourless parenchyma

The amount or way the adaxial colourless parenchyma is arranged above the different vascular bundles (40, 57, 73) would be thought to be associated with the former group of characters. However, it is common that the large leaves have a mass of colourless cells and so there is a tendency for these characters to be associated with characters of size. This is particularly true in the midrib region (No. 40). The characters assume a midway position in the scatter diagram of the first two axes.

V Prevalence of the mestome sheath

The characters measuring the prominence of the mestome sheath in all the bundle types (27, 43, 60) have high positive scores on the first axis and contribute 10.3% to the variance in this direction.

VI Shape of the vascular bundles and the nature of the parenchymatous sheath

The parenchymatous sheath may be interrupted either adaxially or abaxially or in both positions (29, 30, 45, 46, 62, 63) and these characters are generally found to be associated with the shape of the vascular bundles (26, 42, 59). In the case of the secondary bundles, however, these characters (62, 63) are remote from the others in the scatter diagram.

All the characters mentioned above contribute 82.47% to the variance of the first root, and 85.37% to the variance of the second. Thus 20.48% of the total variance is due to these 46 characters, as determined from the first two roots alone.

They also contribute a large amount to the higher order roots (table 37, Appendix 2).

VII Other characters

The remaining 33 characters are not organized into any apparent "grouping" when projected onto the first two vectors. Some of them contribute significant amounts to the roots as will be seen in table 7 or matrix 76. The first six roots account for over 50% of the total variance (table 5) and some of the characters used in this study contribute very little to this amount. The more noteworthy ones are: the shape of the adaxial and abaxial ribs (Nos. 7, 15), the height of the abaxial ribs (14), the shape of the margin of the leaf (18), the shape of the fibres at the margin of the leaf (20), the presence of distinctive cells (21), the shape of the adaxial girder on the central vascular bundle (34) and the shape of the vascular bundle and the type of sclerenchyma fibres of the tertiary bundles (76).

The results of this analysis, therefore, indicate definite character relationships which prove to be useful in interpreting the results of the analysis of OTU's.

3. Analysis of OTU's

This analysis has been performed in two ways. The principal components analysis results in a simplification, so that the trends and relationships among OTU's can be observed and then by classification techniques clusters of OTU's are found.

(a) Principal Components Analysis

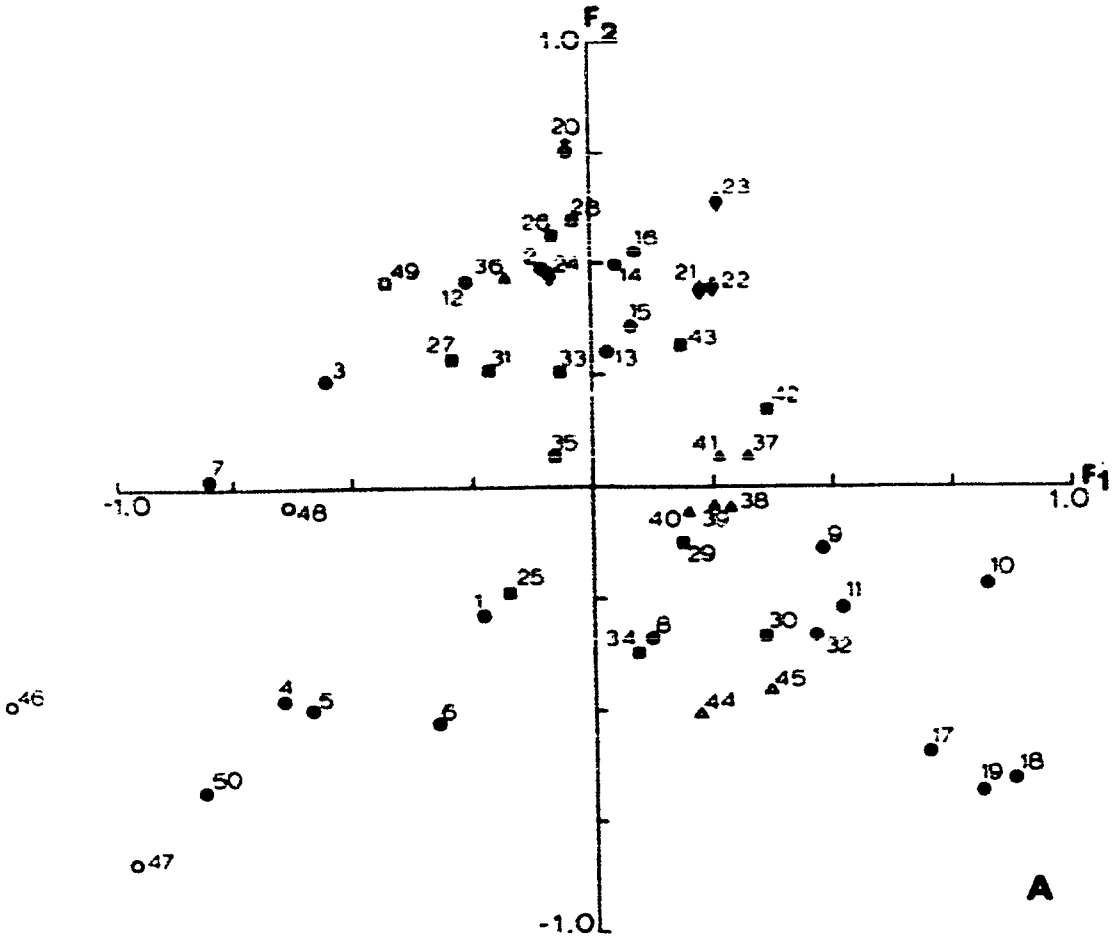
(i) Correlation coefficient

The matrix of correlation coefficients were computed (matrix No. 63) and by component analysis the characteristic roots, which are identical with those obtained above (table 5), and the component scores of the OTU's (matrix 65) were produced. The first 12 roots account for over 70% of the variance but only the results for the first four roots will be considered here, these accounting for almost 40% of the variance.

The projection of the OTU's in the two dimensional spaces determined by the first four components of the analysis, are presented in the form of scatter diagrams (Fig 9 A-D). An examination of these figures reveals that the three main sections of Loudetia are fairly well distinguished, Rattraya is usually closest to the section Pleioneura and L. jaegeriana tends to be isolated from the other species.

The OTU's of section Pleioneura have high negative component scores on the first and second components (F1 and F2). L. ramosa (48) is an exception, often having dissimilar component scores from the other members of the tribe (see fig. 9C). The trend toward separation of this section is clearly seen in fig. 9D.

The section Lophanthera, comprising only L. togoensis (49), is somewhat separated from the OTU's of the section Loudetia, having a high negative component score on the F4 in particular. L. jaegeriana, which has a similar leaf anatomy, is in close proximity to L. togoensis especially when the



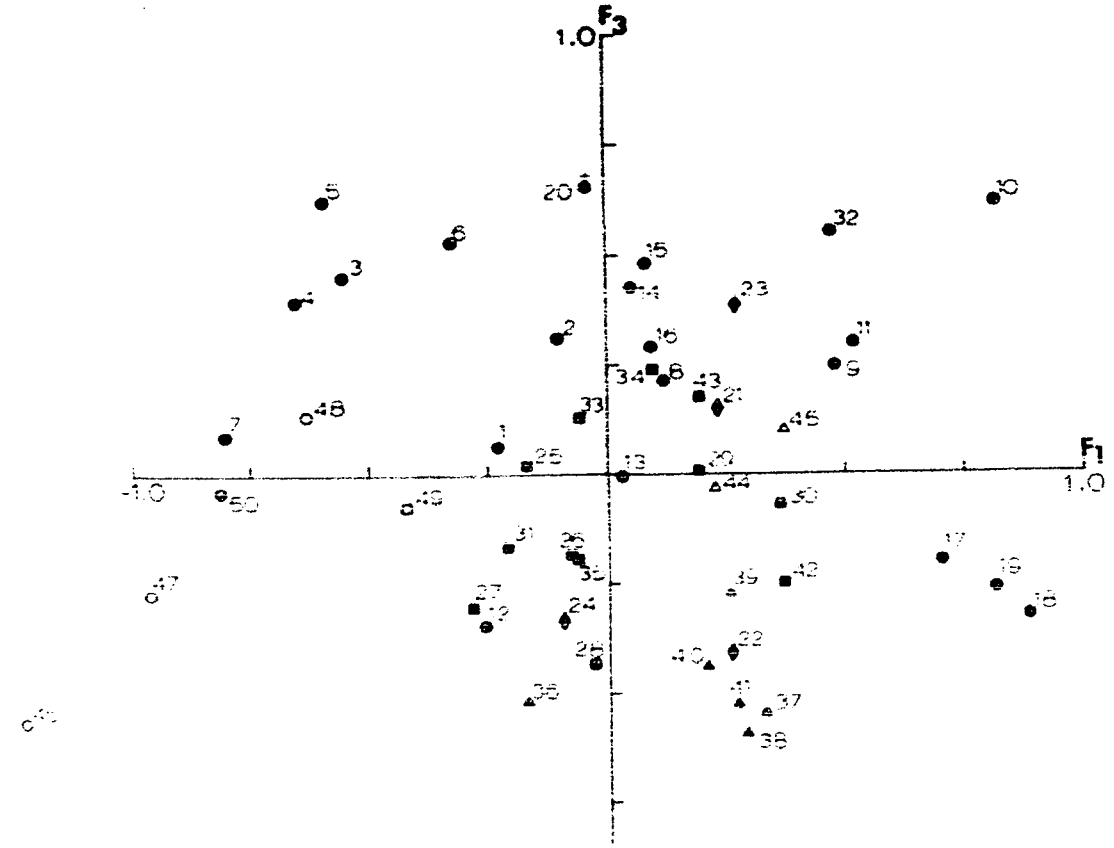
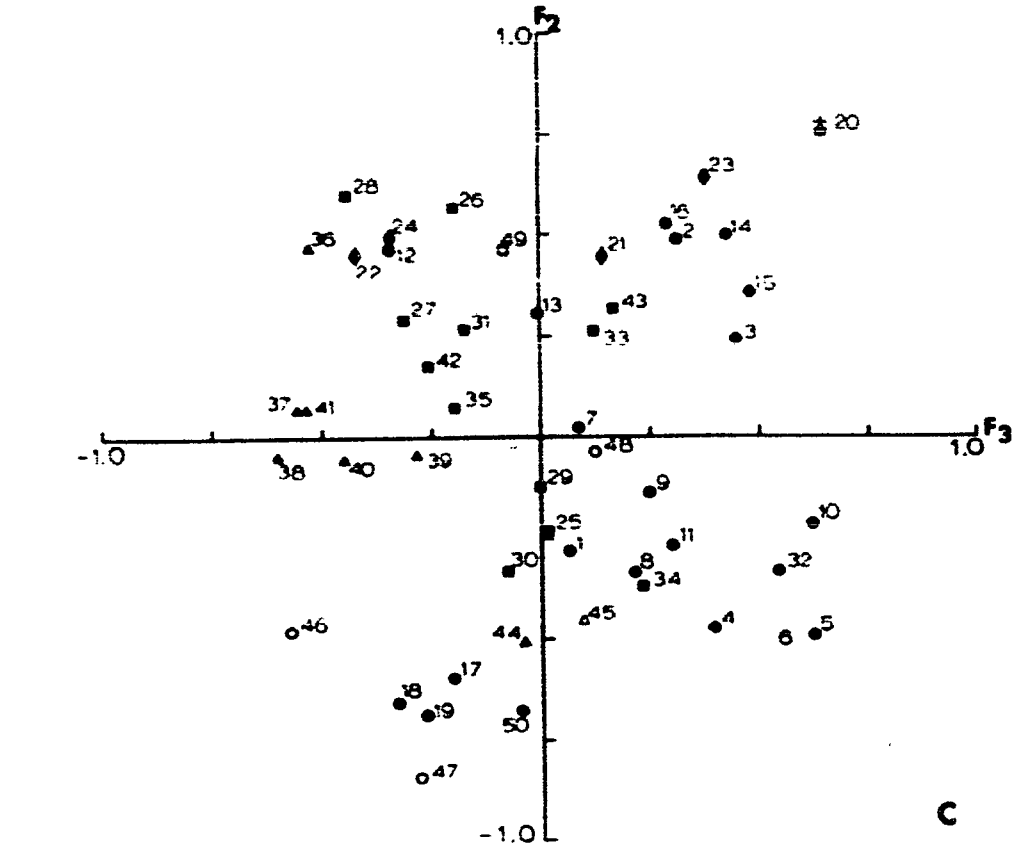
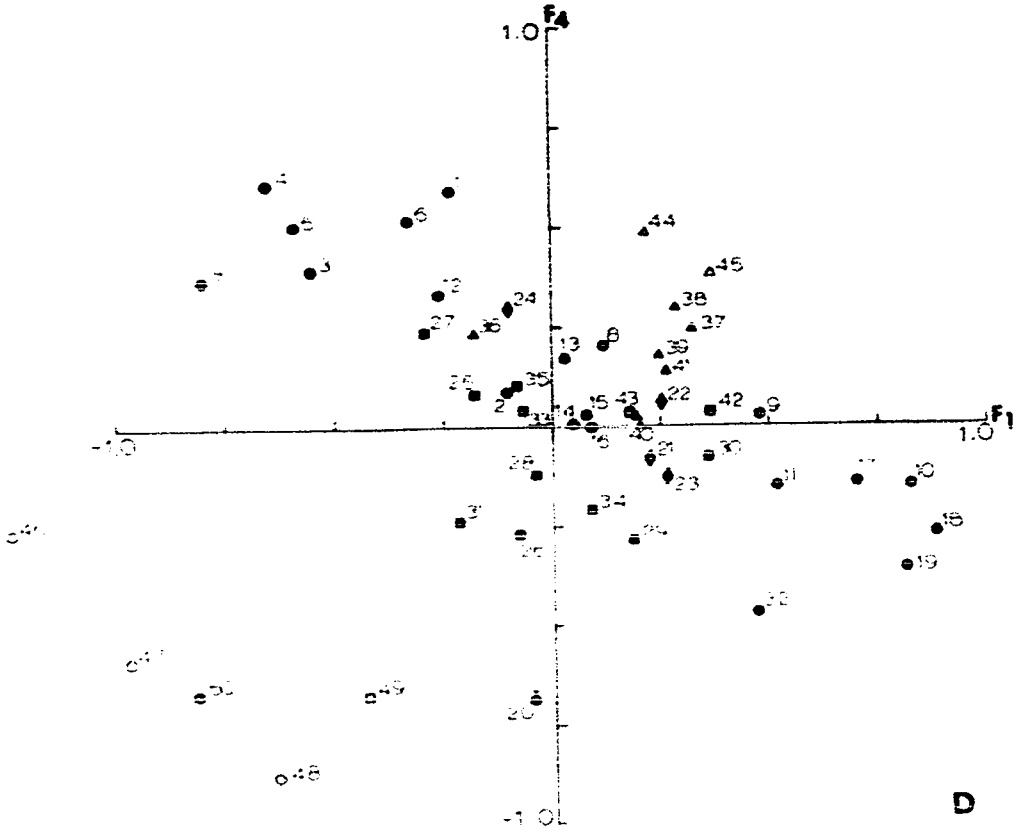


Fig. 7 A-L. Scatter diagrams showing the positions of the OTU's on the first four components of a component analysis of the correlation matrix 63, derived from leaf anatomy characters. For legend see Table 4.


F2
1.0
-1.0
F3
1.0
-1.0
C


F4
1.0
-1.0
F1
1.0
-1.0
D

distribution of OTU's on the first and second (F1 and F2), or first and fourth components (F1 and F4) is examined. In fig. 9A they appear far removed from the section Pleioneura OTU's but in fig. 9D they seem to be close to these OTU's, all of them having high negative component scores on the F4.

The section Loudetia, with a large number of OTU's is seen to be very diverse although in some instances these OTU's may be separated from those of the other two sections. In fig. 9D this is seen to be so. Some of the subsections of this section show tendencies to have OTU's grouped together but others appear very variable.

The subsection Typicae, the largest, is very variable though there are apparent subgroups. The so-called L. arundinacea-complex (1 - 7) have large leaves, with corresponding similarities in the leaf anatomy, such as a mid-rib and associated characters. In this respect these OTU's are close to some members of the section Pleioneura (fig. 9A). L. angolensis (2) usually is quite distant from the other OTU's, commonly closest to L. arundinacea (3). Most of the exemplars of the latter species (3 - 6) appear close to one another OTU 3 being the most dissimilar one. Of the three exemplars of L. simplex subsp. simplex (9, 10, 11), OTU 10 is separated from the other two. The two exemplars of the subsp. stipoides (12, 13) are often distantly placed, OTU 13 usually being close to the three exemplars of L. kagerensis (14, 15, 16). Finally, another cluster of the subsection Typicae are L. perrieri (17) and L. madagascarensis (18, 19).

The OTU's of the subsection Pungentes are also not separated from the main body of the section Loudetia. L. lanata (21) and L. longpipes (22) are very close in fig. 9A. In this case L. demeusei (23) and L. crassipes (24) are also fairly closely grouped.

The subsection Acuminatae also has a large number of OTU's and shows great diversity. The exemplars of the different species are also found to be quite different, e.g. L. pennata (25, 26) examplars are usually far removed from one another. In one case (fig. 9D) the OTU's of the Acuminatae are fairly close to one another, grouped around the centroid.

The species of the Densispicae (36 - 41) appear to be very similar on the basis of leaf anatomy (fig. 9A). The one exception is L. gossweileri (36) which always is separate from the other OTU's.

The two species of subsection Annuae (42 and 43) are often close to one another, though not separate from the main cluster of OTU's. The same is true for the two species of Flammidae, though in fig. 9D they are separate from the other OTU's with somewhat high positive component scores on both axes.

(ii) Dispersion Coefficient

A Q-type analysis of dispersion resulted in the matrix of coefficients (No. 64) and by component analysis the roots (table 6) and the component scores (matrix 66), were produced. The efficiency of the new components in accounting for the variation is better than with the correlation coefficient matrix. The first twelve roots account for over 75% of the vari-

TABLE 6: ROOTS OF THE DISPERSION MATRIX (NO. 64)

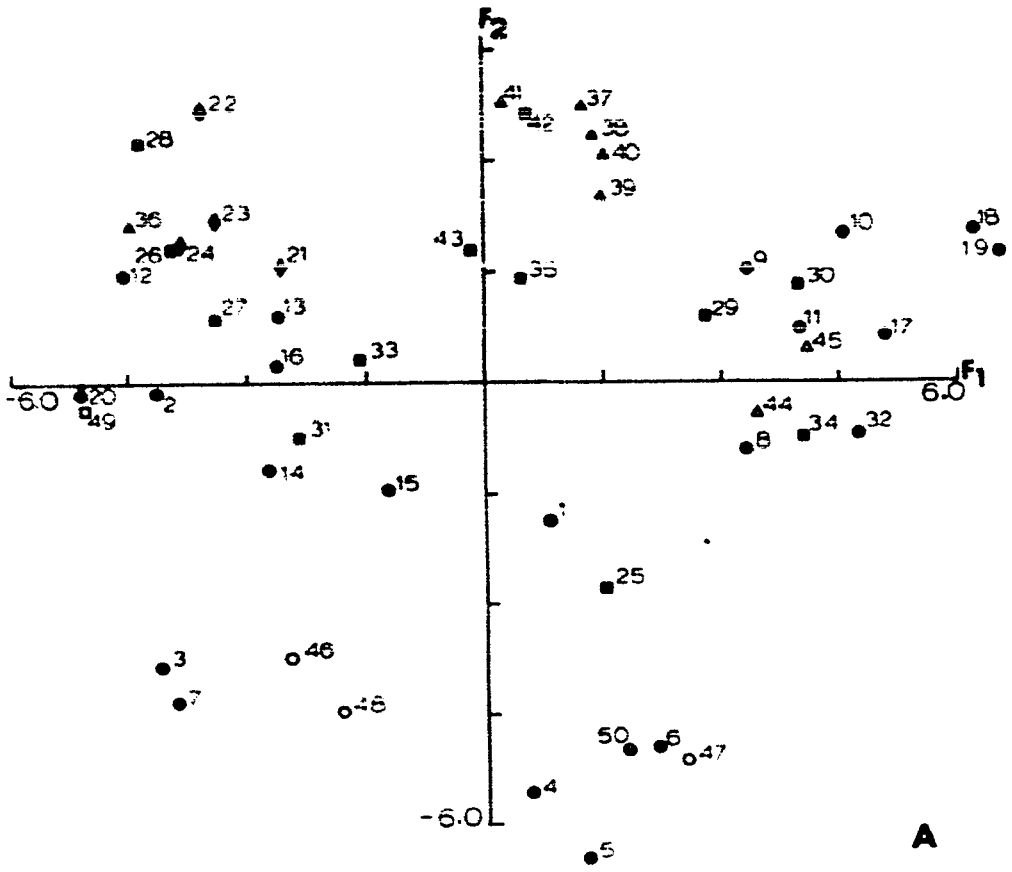
Root	Value of Root	% of total variation accounted for	Accumulated percentage
1	556.465	16.55	16.55
2	369.931	11.00	27.56
3	307.302	9.14	36.70
4	221.469	6.59	43.29
5	199.531	5.93	49.20
6	163.366	4.86	54.08
7	158.459	4.71	58.79
8	145.731	4.33	63.13
9	124.890	3.72	66.84
10	106.103	3.16	70.00
11	94.372	2.81	72.81
12	79.870	2.38	75.18
13-50	834.289	24.82	100.00
Total Sum of Squares	3,361.778	100.00	-

ance and the first four, which will be considered in more detail, account for 43.29%.

The results are presented in the form of scatter diagrams (fig. 10 A-D). There is an overall similarity between the results using the different coefficients as may be seen from the figures. The three sections of Loudetia are not as strongly emphasized as previously. The OTU's of the subsection Typicae with extreme leaf forms are more markedly separate from the main cluster of OTU's.

With respect to the section Pleioneura, L. ramosa (48) is not so far removed from the others, often being close to L. simulans (46). L. togoensis (section Lophanthera, (OTU 49) and L. jaegeriana (No. 20) are once again close to one another (fig. 10A).

The section Loudetia is most diverse, mainly due to the most variable subsections Typicae and Acuminatae. With respect to the former, the L. arundinacea-complex is more spread out, though they are mostly seen to have high negative scores on the F2 (fig. 10A). They are similar to the Pleioneura species in this respect. The OTU's 2, 3, and 7 are separated from OTU's 1, 4, 5, and 6 on the F1. L. camerunensis (8) and L. simplex subsp. simplex (9, 10, 11, 32), are often closely grouped forming the typical L. simplex-complex (fig. 10B). Once again the exemplars of subsp. stipoides (12, 13) are apart, and those of L. kagerensis (14, 15, 16) are close together. L. perrieri (17) and L. madagascariensis (18, 19) are grouped with high positive component scores on the F1 component



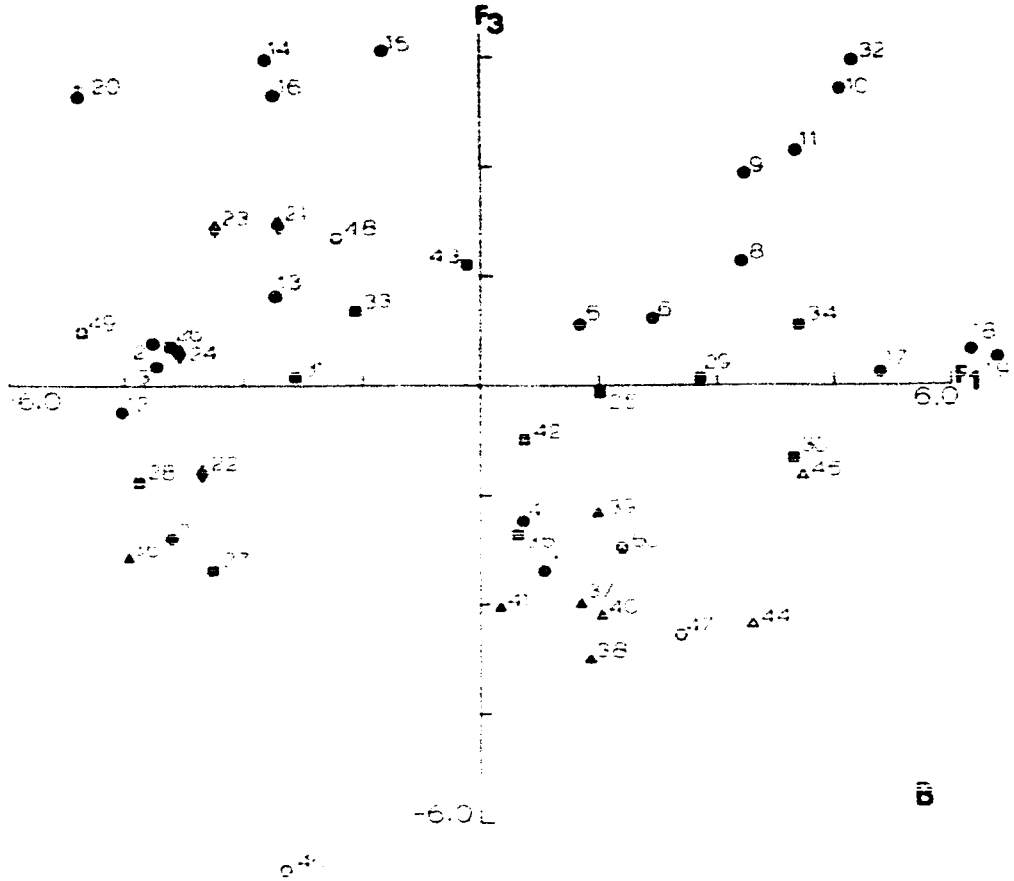
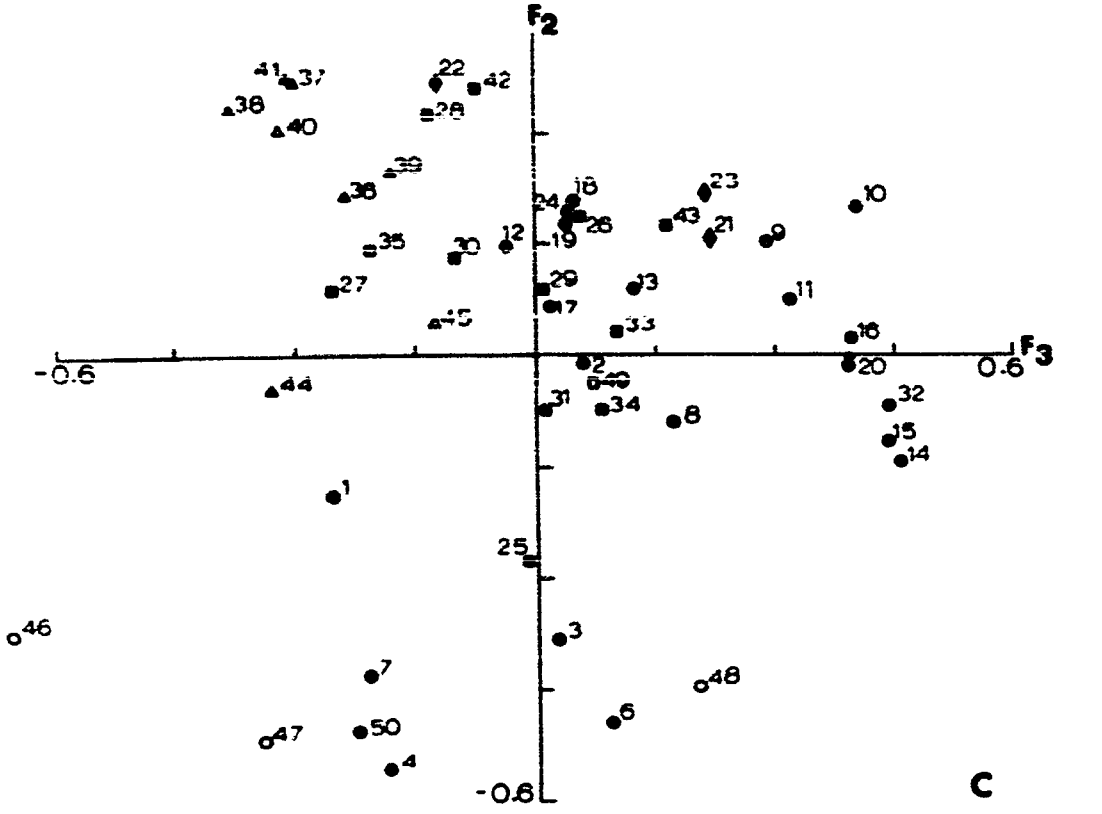
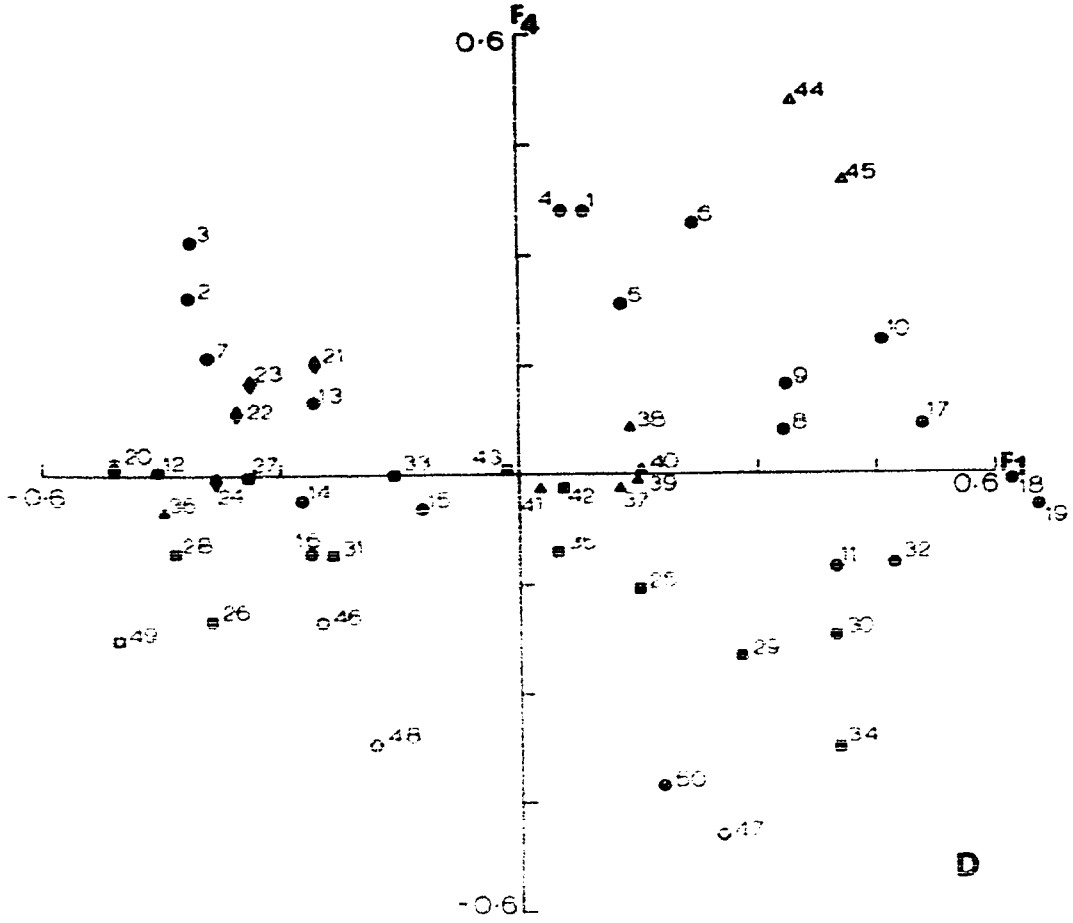


Fig. 10 A-B. Scatter diagrams showing the positions of the OTU's on the first four components of a component analysis of the dispersion matrix E4, derived from leaf anatomy characters. For legend see Table 4.


F2
F3
-0.6
0.6
-0.6
C


F4
F1
0.6
-0.6
0.6
-0.6
D

(fig. 10A, 10D).

The OTU's of the subsection Pungentes (21, 22, 23, 24) are often closely grouped together in this analysis (fig. 10D). In contrast, those of the subsection Acuminatae (25-35) are scattered throughout the diagrams. Further evidence for the close relationship between members of the Densispicae (36-41) is indicated (fig. 10A), though L. gossweileri (36) is separate from the others.

The Annuae (42, 43) and the Flammidae (44, 45) are subsections with representatives closely related. The latter is separated from other OTU's with high positive scores on the F4 and F1 (fig. 10D) as was the case previously (fig. 9D).

(b) Classification

The results of the ordination reveal certain trends and relationships among the OTU's, whereas a classification indicates the actual grouping of the OTU's. The structure which results following principal components analysis differs from the original depending on the type of coefficient used. As the principal components analysis has revealed a simpler structure than the original, a classification of the OTU's on the basis of the principal components would prove useful. Therefore, classification was performed using the component scores of the principal components analyses as data. In order to compare the results with a classification based on the original structure the raw data matrix was also used as data source. Absolute distance was computed in all cases to calculate the similarity. The three distance matrices (Nos. 68, 69 and 70)

were subjected to the sum of squares agglomeration method of cluster analysis and the results presented in the form of dendrograms (fig. 11 A-C).

(i) Classification produced using the component scores of the principal components analysis derived from the correlation coefficient matrix, as data source (Fig. 11A)

A comparison of this dendrogram with fig. 9A reveals some of the details of the structure of the data that is classified. Three major groups are formed which roughly correspond to those OTU's with (a) negative component scores on the F1 and F2, (b) positive component scores on the F2 and positive and negative scores on the F1, and (c) positive scores on the F1 and negative scores on the F2. These three main groups are characterized by different OTU's.

(a) The first group contains most of the L. arundinacea-complex of OTU's, and the section Pleioneura and Rattrava which form two subgroups.

(b) This group contains OTU's from all the subsections of section Loudetia and section Lophanthera. L. angolensis (No. 2), the only member of the L. arundinacea-complex missing from the former group, occurs here with apparent affinities with L. demeusei (23) and L. jaegeriana (20). Of the OTU's of subsection Typicae that occur in this group the exemplars of L. kagerensis (Nos. 14, 15, 16) cluster together, otherwise these OTU's are somewhat diverse, as are those of the subsections Acuminatae, Pungentes, and Annuae.

(c) Finally one finds the bulk of the L. simplex-complex of

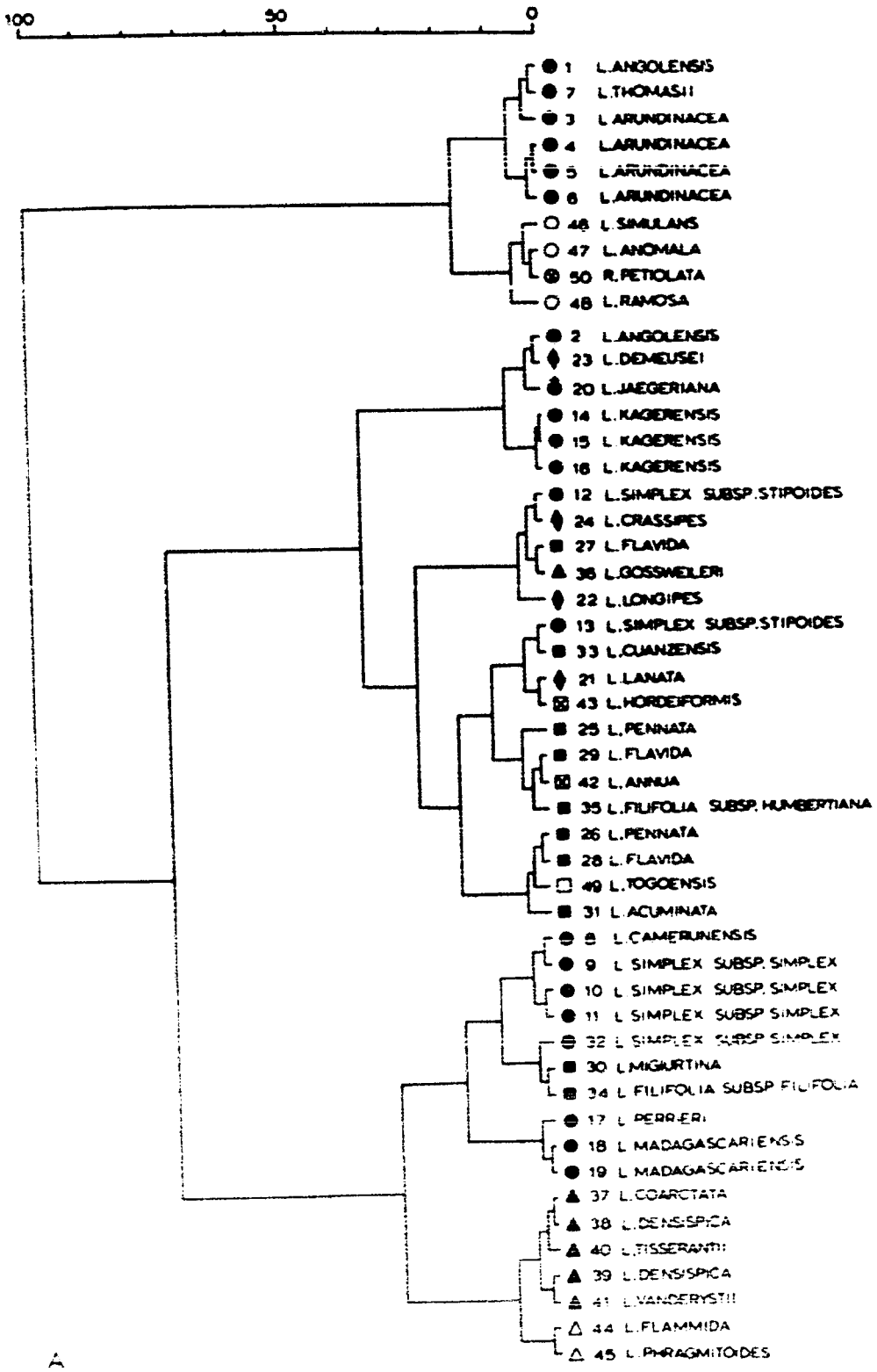
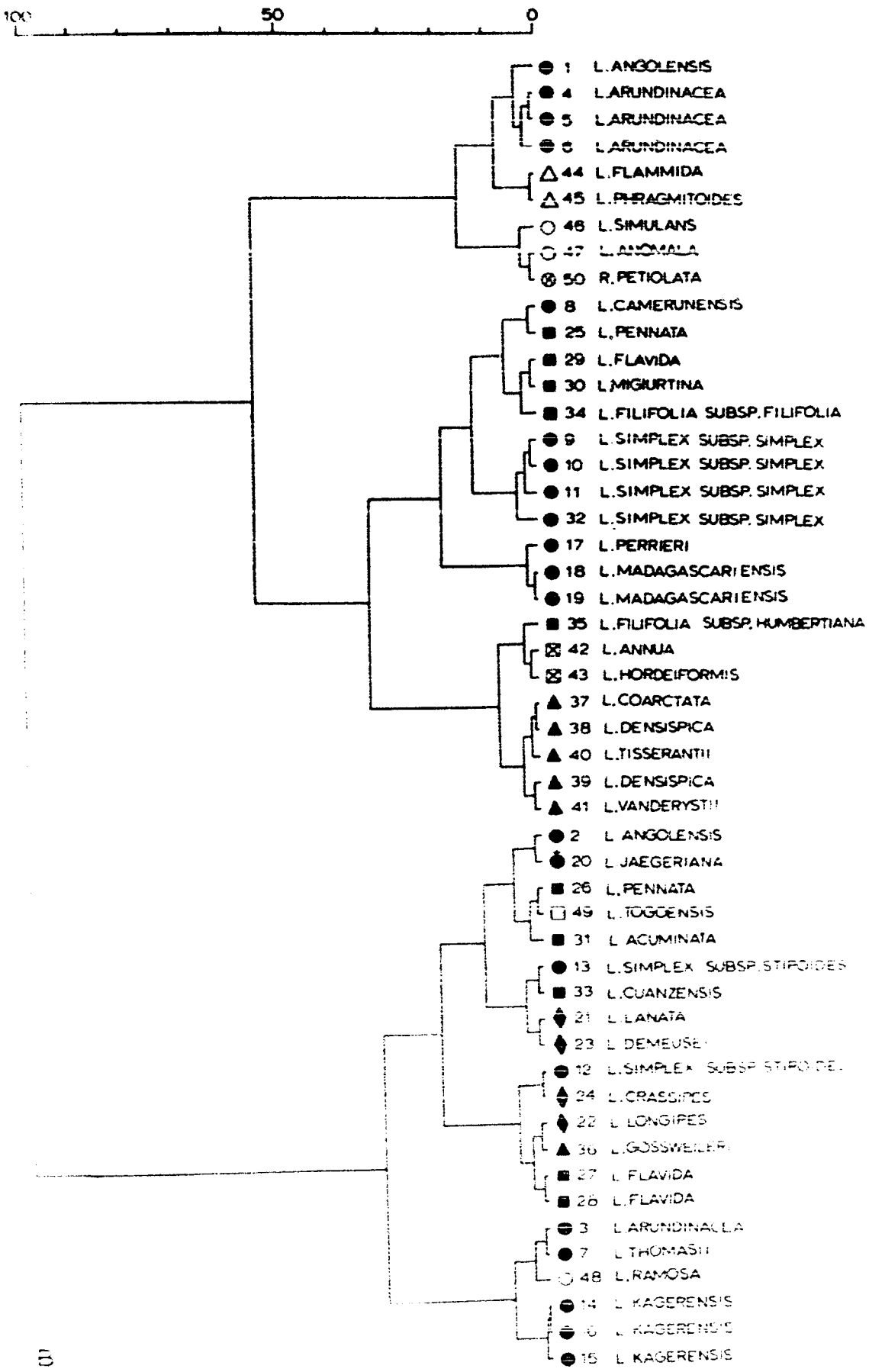
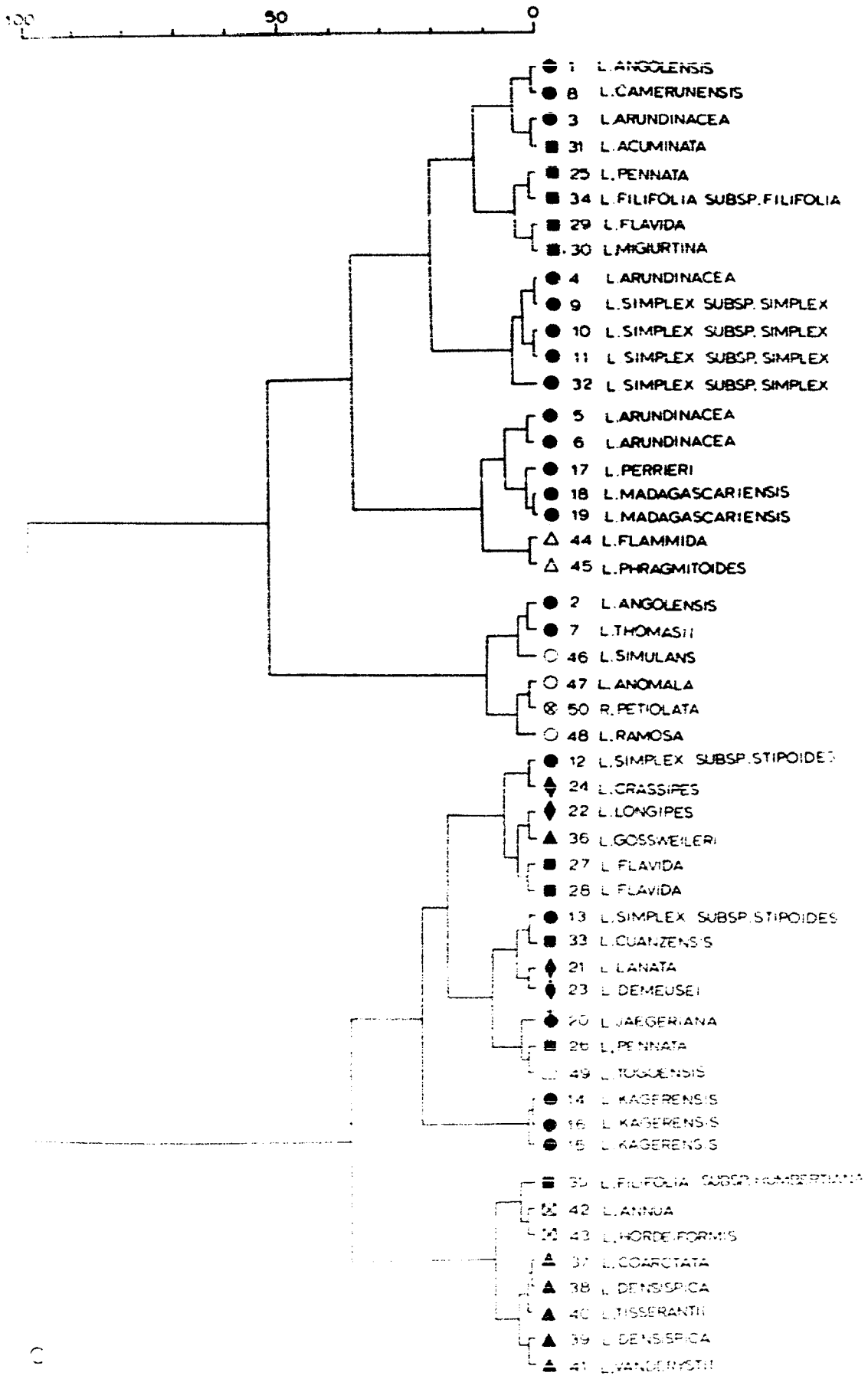


Fig. 11 Dendrograms of 50 OTU's of Loudetia and Rottboellia derived from leaf anatomy characters using three different data matrices; A-Component scores matrix derived by component analysis of the correlation matrix; B-Component scores matrix derived by component analysis of the dispersion matrix; C-Data matrix 62.
Scale indicates the within-group sum of squares expressed as a percentage of the total sum of squares. For legend see Table 4.


100
50
0
1 L.ANGOLENSIS
4 L.ARUNDINACEA
5 L.ARUNDINACEA
6 L.ARUNDINACEA
44 L.FLAMMIDA
45 L.PHRAGMITOIDES
46 L.SIMULANS
47 L.ANOMALA
50 R.PETIOLATA
8 L.CAMERUNENSIS
25 L.PENNATA
29 L.FLAVIDA
30 L.MIGIURTINA
34 L.FILIFOLIA SUBSP.FILIFOLIA
9 L.SIMPLEX SUBSP. SIMPLEX
10 L.SIMPLEX SUBSP. SIMPLEX
11 L.SIMPLEX SUBSP. SIMPLEX
32 L.SIMPLEX SUBSP. SIMPLEX
17 L.PERRIERI
18 L.MADAGASCARIENSIS
19 L.MADAGASCARIENSIS
35 L.FILIFOLIA SUBSP. HUMBERTIANA
42 L.ANNUA
43 L.HORDEIFORMIS
37 L.COARCTATA
38 L.DENSISPICA
40 L.TISSERANTII
39 L.DENSISPICA
41 L.VANDERYSTII
2 L.ANGOLENSIS
20 L.JAEGERIANA
26 L.PENNATA
49 L.TOGOENSIS
31 L.ACUMINATA
13 L.SIMPLEX SUBSP.STIPOIDES
33 L.CUANZENSIS
21 L.LANATA
23 L.DEMEUSEI
12 L.SIMPLEX SUBSP.STIPOIDES
24 L.CRASSIPES
22 L.LONGIPES
36 L.GOSSWEILERI
27 L.FLAVIDA
28 L.FLAVIDA
3 L.ARUNDINACEA
7 L.THOMASII
48 L.RAMOSA
14 L.KAGERENSIS
15 L.KAGERENSIS


100
50
0
1 L.ANGOLENSIS
8 L.CAMERUNENSIS
3 L.ARUNDINACEA
31 L.ACUMINATA
25 L.PENNATA
34 L.FILIFOLIA SUBSP.FILIFOLIA
29 L.FLAVIDA
30 L.MIGIURTINA
4 L.ARUNDINACEA
9 L.SIMPLEX SUBSP.SIMPLEX
10 L.SIMPLEX SUBSP.SIMPLEX
11 L.SIMPLEX SUBSP.SIMPLEX
32 L.SIMPLEX SUBSP.SIMPLEX
5 L.ARUNDINACEA
6 L.ARUNDINACEA
17 L.PERRIERI
18 L.MADAGASCARIENSIS
19 L.MADAGASCARIENSIS
44 L.FLAMMIDA
45 L.PHRAGMITOIDES
2 L.ANGOLENSIS
7 L.THOMASII
46 L.SIMULANS
47 L.ANOMALA
50 R.PETIOLATA
48 L.RAMOSA
12 L.SIMPLEX SUBSP.STIPOIDES
24 L.CRASSIPES
22 L.LONGIPES
36 L.GOSSWEILERI
27 L.FLAVIDA
28 L.FLAVIDA
13 L.SIMPLEX SUBSP.STIPOIDES
33 L.CUANZENSIS
21 L.LANATA
23 L.DEMEUSEI
20 L.JAEGERIANA
26 L.PENNATA
49 L.TOGOLENSIS
14 L.KAGERENSIS
16 L.KAGERENSIS
15 L.KAGERENSIS
35 L.FILIFOLIA SUBSP.HOMBERTIANA
42 L.ANNUA
43 L.HORDEIFORMIS
37 L.COARCTATA
38 L.DENSISPICA
40 L.TISSERANTII
39 L.DENSISPICA
41 L.VANDERYSTII

the Typicae, some Acuminatae, the subsections Densispicae and Flammidae agglomerated together. The exemplars of L. simplex subsp. simplex are clustered together, including OTU No. 32 which was formerly incorrectly identified as L. acuminata. The Malagasian species of the Typicae group together as suggested by fig. 9A. All but L. gossweileri of the subsection Densispicae form a compact group.

The cophenetic correlation coefficient between the distance matrix 68 and the resulting dendrogram was 0.482. This value is rather low indicating that a lot of information is lost in the production of the dendrogram from the distance matrix.

(ii) Classification produced using the component scores of the principal components analysis derived from the dispersion coefficient matrix, as the data matrix (Fig. 11B)

It is more difficult to relate this dendrogram to the distribution of the OTU's on the first four components (fig. 10). However, many of the trends observed above are present here. If the dendrogram is examined at the 20% level five major groups of OTU's may be discerned.

(a) Once again part of the L. arundinacea-complex and section Pleioneura occur together, being accompanied also by the subsection Flammidae.

(b) The OTU's that occur in this group all have similar leaves in many respects. The bulk of the L. simplex-complex occur here and some OTU's of the subsection Acuminatae. All the examplars of L. simplex subsp. simplex occur together, and

the Malagasian species form a group. The OTU's of this group generally have high positive component scores on the F1 (fig. 10A).

(c) The subsections Annuae and Densispicae form two subgroups within this group of OTU's. One exceptional OTU that occurs here is L. filifolia subsp. humbertiana (Acuminatae) which is seen to be in close proximity to the other OTU's on the F1 - F2 scatter diagram (fig. 10A).

(d) A large group of OTU's which appears quite variable. Every section and subsection is represented in this group, except the section Pleioneura. These OTU's have high negative component scores on the F1 (fig. 10A).

(e) Combined with the exemplars of L. kagerensis are three OTU's of the L. arundinacea and Pleioneura type which in the previous dendrogram were not separate from the main body of these OTU's.

The cophenetic correlation coefficient between this dendrogram and the distance matrix 69 was 0.515 which also indicates a large loss of information in the production of the dendrogram.

(iii) Classification produced from the raw data matrix (fig. 11C)

Some of the features that are different in this dendrogram are worthy of note. The L. arundinacea-complex appears very divided, the OTU's being variously clustered with OTU's of the subsections Typicae, Acuminatae or even of section Pleioneura. This dendrogram is most similar to that produced using

the component scores (dispersion coefficient) as data, in that the L. simplex-complex is grouped with some OTU's of the subsection Acuminatae, the subsections Annuae and Densispicae once again occur in one group, and one large group of OTU's includes a representative from almost every section or subsection.

The cophenetic correlation between this dendrogram and its distance matrix 70 is also quite low, viz. 0.494.

(iv) A comparison of the three different classifications

The cophenetic values obtained from the dendrograms were used to compute the cophenetic correlation coefficients between them. The correlations between the distance matrices derived from the different data sources where also computed (Table 7).

The correlation between distance matrices was high whereas the correlation between dendrograms was very low. The highest correlation in each case was between the component scores (correlation) and component scores (dispersion) as the data source.

4. Analysis of the supra-specific groups

The data matrix (No. 82) was prepared in the same way as above only this time the ten supra-specific taxa were treated as OTU's. A principal components analysis was performed and a classification of the OTU's was also produced (Fig. 7).

(a) Principal Components Analysis

The Q-type dispersion coefficient matrix (No. 83) was calculated from the data matrix and this was subjected to a

Table 7: Cophenetic correlation coefficients produced by comparison of the distance matrices and dendrograms derived from the three different data sources (leaf anatomy characters).

		Data Source: Component Scores - Correlation (Matrix 65)	Data Source: Component Scores - Dispersion (Matrix 66)	Data Source: Raw Scores - (Matrix 62)
Dendrograms	Matrix 65	1.000	0.922	0.771
	Matrix 66	0.395	1.000	0.836
	Matrix 62	0.345	0.371	1.000

Distance Matrices

component analysis. The roots of the dispersion matrix are given in table 8. The first three roots account for over 60% of the variance and the projection of the 10 OTU's on these first three components is indicated in the scatter diagrams (fig. 12 A-C).

The subsections Typicae (No. 1) and Acuminatae (No. 3) which had exhibited a great deal of variability in the other analyses, one finds closely associated. However, on the whole, all the subsections of the section Loudetia (Nos. 1-6) appear closer to one another than to the other taxa. The close association of the section Pleioneura with Rattraya is also evident, they both having high positive component scores on the F2 axis (fig. 12A). Another observation to be expected now is the affinity between L. jaegeriana (No. 10) and Lophanthera (No. 8).

(b) Classification

The absolute distance matrix (No. 86) was calculated from the component scores matrix, and sum of squares agglomeration was used to classify the OTU's and produce the resulting dendrogram (fig. 13).

The relationships indicated in the scatter diagrams are equally significant here. The Typicae and Acuminatae cluster together, and the remaining subsections of section Loudetia are united in a group. Pleioneura and Rattraya, and Lophanthera and L. jaegeriana form two other groups.

E. Discussion

A study of the leaf anatomy of Loudetia reveals a far larger amount of variability than might at first be realized.

TABLE 8: ROOTS OF THE DISPERSION MATRIX (NO. 85)

Root	Value of Root	% of total variation accounted for	Accumulated percentage
1	1,695.463	26.51	26.51
2	1,488.761	23.28	49.80
3	810.319	12.67	62.47
4	600.929	9.40	71.86
5	525.248	8.21	80.08
6	420.660	6.58	86.66
7	398.411	6.23	92.89
8	282.267	4.41	97.30
9	139.689	2.18	99.49
10	32.849	0.51	100.00
Total Sum of Squares	6,394.596	100.00	-

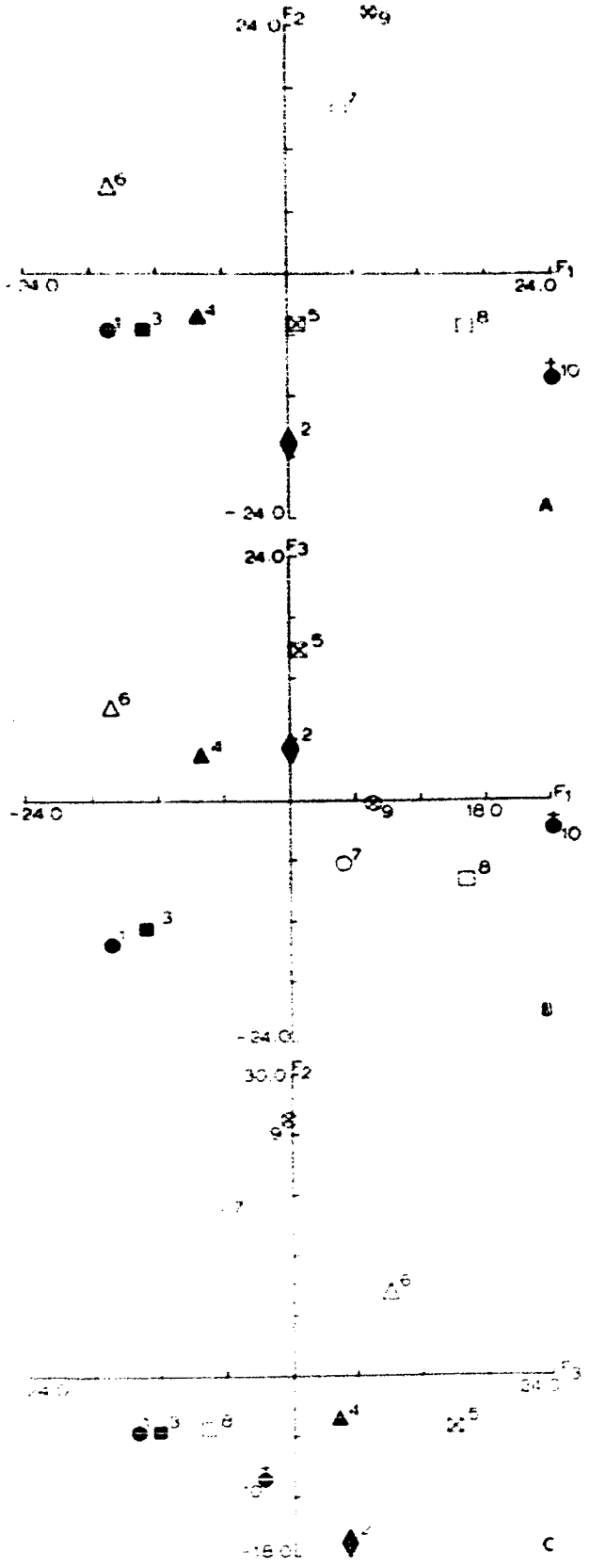


Fig. 12 A-C. Scatter diagrams showing the positions of the supra-specific taxa on the first three components of a component analysis of the dispersion matrix 83, derived from leaf anatomy characters. For legend see Table 4.

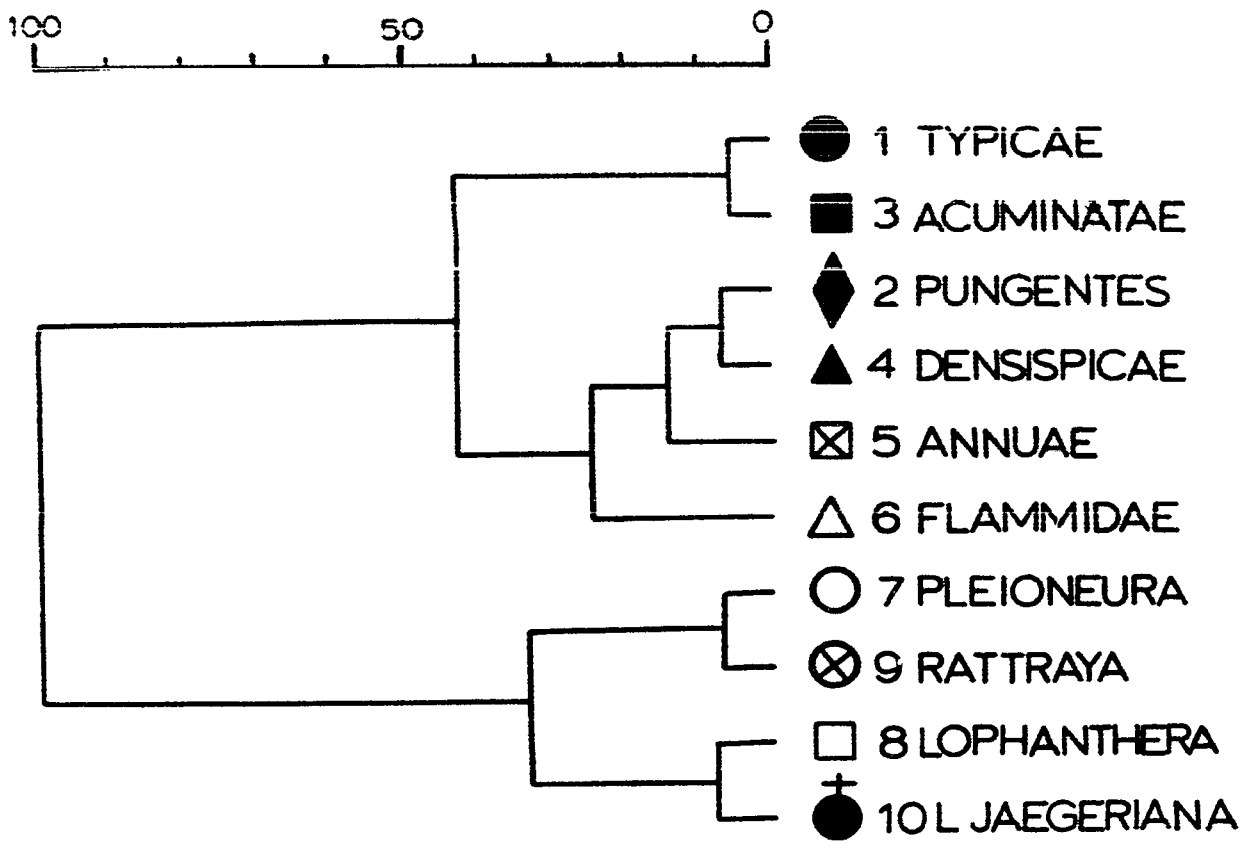


Fig. 13 Dendrogram of supra-specific taxa of Loudetia and Rattraya derived from leaf anatomy characters. Scale indicates the within-group sum of squares expressed as a percentage of the total sum of squares.

Morphologically, the leaves do not appear significantly different. The bulk of the species have narrow linear leaves which taper to a fine point. Broader leaves are found in some of the larger species, the maximum width being reached in Rattrava petiolata which also has a pseudopetiole at the summit of the leaf sheath. Deviating from the general morphological pattern one also finds leaves reduced in length yet fairly broad, as in L. jaegeriana or L. togoensis. Another trend is found towards narrow leaves which have a tendency to be involute (e.g. L. perrieri). The morphological pattern of the leaf was also found to reflect similar anatomical patterns.

In general those species with a large broad leaf had a well developed mid-rib with one or more vascular bundles and adaxial strands in this region. The species with narrower leaves usually have an ill-defined mid-rib or none at all and there is a greater tendency for the vascular bundles to be surrounded by adaxial and abaxial girders rather than strands. Leaves exhibiting xeromorphic features are found in L. perrieri and L. madagascariensis. In these species there is generally no mid-rib, but there are involute margins and ribbed surfaces to the leaves which are also often endowed with an abundance of fibres. The short, broad leaves of L. togoensis and L. jaegeriana are characterized by the lack of a mid-rib, rather pointed margins and angular vascular bundles surrounded by a large celled parenchymatous sheath.

The loudeticid leaf, therefore, is quite variable and the value of a numerical approach in extracting a maximum amount

of information from a study of this nature is revealed. The principal components analysis of characters indicated which ones proved to be critical in deciding relationships between OTU's. The characters of size and those related to size were found to be most important. These characters (thickness and width of leaf type of margin, presence or absence of a keel, the number of vascular bundles in the mid-rib, the presence or absence of adaxial strands, the number of groups of phloem, etc.) all have common the fact that they are prevalent in one extreme state in the larger leaves. They were found to account for 7.5% of the total variance. The effects of these characters on the ordination of the OTU's is seen in the scatter diagrams (fig. 9A and 10A). The L. arundinacea-complex of the subsection Typicae, the section Pleioneura and Rattraya, and the subsection Flammidae all have large leaves and may be distinguished from most other OTU's in these figures. The dendrograms (fig. 11) also illustrate the effect of these characters in classification where some or all of these OTU's are always grouped separately from the others. Coupled with characters related to size are those which measure the presence or absence of sclerenchyma strands and the accompanying colourless cells. These 11 characters account for 4% of the total variance. Whereas strands usually occur above and below the vascular bundles in the large leafed species, those with smaller leaves are more likely to have girders. Consequently, one finds the OTU's with smaller leaves fairly widely separated from those with large leaves in the scatter diagrams.

There are a number of other characters which were important in indicating OTU relationships in the ordination and classification. In all 46 characters were found to be "grouped" when projected on the first two vectors. Of the remaining 33 characters, some were found to contribute very little to the variance when the first six roots of the correlation matrix were considered. It is, therefore, important to re-examine these characters to see whether one is justified in spending the extra time in coding them. In particular it was the characters of shape, e.g. that of the ribs, margin of the leaf, fibre groups, etc. which did not contribute much to the analysis. Since these characters are difficult to code and it requires a matter of judgement to decide on a particular character state, the fault in this study may have been the method of coding, or scoring of the characters.

The results of the numerical analysis reveal distinct groups of OTU's, some of which seemed obvious from the outset while others were more obscure. In no instance is any rigid classification or grouping to be concluded because this is beyond the aims of the thesis and in fact would not provide a satisfactory solution to the taxonomy. The most obvious group of OTU's is produced due to characters of size of the leaf and the factors which accompany it. The L. arundinacea-complex of the Typicae may be included in this category and all but L. angolensis (2), which appears to be different, are grouped together (fig. 11A). Also of this type are the Pleioneura species and Rattraya. They differ from all other species

of Loudetia on a number of characters, and it is surprising that their separation is not more distinct. For example, their leaves lack tertiary vascular bundles and also have no associated colourless cells below the bulliform cells. L. ramosa has a somewhat smaller narrow leaf than the other species and may be remote from the other species (fig. 11B). Two species which have large leaves and are sometimes grouped with the above (fig. 11B) are the Flammidae. In the cross-section figures (fig. 5. 28-29) they are very similar to one another.

The smaller-leaved species do not always show consistent groups in either the ordination or classifications. There are a number of groups which occur fairly regularly. The OTU's of the Densispicae (figs. 5. 21-25) are alike though their characteristics are difficult to define. All except L. gossweileri (36) form a compact cluster. The differences between this OTU and other Densispicae appear to be small and accumulative and they separate it from the others. It has adaxial girders and no colourless parenchyma adaxially, complete bundle-sheaths, and many phloem fibres, whereas the others have strands and colourless parenchyma adaxially, incomplete bundle-sheaths and a distinct mestome sheath rather than phloem fibres in the secondary bundles.

The Annuae (fig. 5. 26-27) have a quite consistent leaf anatomy which is somewhat like that of the Densispicae and they are clustered with the latter (fig. 11B and 11C). In L. hordeiformis the bulliform cells extend across a large part of the adaxial surface, a condition that is not found in

L. annua.

The Pungentes are a variable subsection when leaf anatomy is considered. L. crassipes and L. longipes are similar in that they both have an abundance of sclerenchyma and may occur close together (fig. 9A, 11C). Similarly L. demeusei and L. lanata show certain features in common (fig. 11C).

Probably the most variable subsection from the point of view of leaf anatomy is the Acuminatae. One OTU of L. pennata (25) has a fairly well developed midrib, whereas the other (26) does not. The subspecies of L. filifolia are usually quite far apart, their differences being shown in fig. 5.19, 5.20. The clustering together of OTU's from this subsection does occur in some cases (fig. 11B, 11C) as L. pennata (25), L. flavida (29), L. migiurtina (30) and L. filifolia subsp. filifolia (34) are similar to one another (fig. 5.14 - 16, 5.19).

The smaller-leaved species of the Typicae are somewhat different in anatomy. L. madagascariensis and L. perrieri exhibit xeromorphic features and are often quite isolated from the other respresentatives, (fig. 9A, 10A, 11A). They are characterised by markedly involute leaves which have high ridges on the adaxial surface. These ridges contain thick-walled parenchymatous cells. The exemplars of L. simplex subsp. simplex (9, 10, 11, 32) are all very similar, as are those of L. kagerensis (14, 15, 16), though the two species are different from one another. On the other hand L. simplex subsp. stipoides exemplars (12, 13) do not show a strong resemblance to one another and their position in the classification varies.

Finally two species with similar leaf anatomy are L. jaeg-

eriana and L. togoensis (fig. 5.33, 5.34), but in the classification do not always occur particularly close to one another (fig. 11A). The similarities between the leaves of these two species have already been mentioned above.

From the results of the analysis conducted using the supraspecific groups of taxa as OTU's, a few additional points may be noted. The major distinction between the OTU's, viz. characters of size or related to size, are no longer conspicuous in this analysis. The Typicae (1) and Acuminatae (3) are two variable subsections and they show affinity for one another. The section Pleioneura and Rattraya are more notably separated from the other taxa as they did not have many characters in common with the Typicae. The three sections of the genus are more or less clearly divided and L. jaegeriana, at least on leaf anatomy, appears closer to Lophanthera than any other section.

As has been seen above, the results of the principal components analysis and the classifications performed in different ways, led to some variation in the results. Particularly in the case of the classifications based on different sources of data; the fact that in no case did the cophenetic correlation coefficient between dendrograms exceed 0.4, warrants some explanation. The fact that the distance matrices were all somewhat similar (see table 10) indicates that the differences are due to the clustering process. Since there are large differences between the different methods of analysis, it would seem advisable not to be dogmatic in one's approach, but to accept

the facts as they occur in each of the dendrograms, as a source of additional information about the OTU's under study.

The question arises whether the previously constructed sections and subsections of Loudetia are justified on the basis of these results. Although there is a great deal of variability in some of the subsections of Loudetia, the overall results of the analyses indicate that in fact there are very strong resemblances between the taxa of a section or subsection. Particularly as leaves are subject to a great deal of environmental and morphological variation, the taxonomic relationships appear very sound. It is desirable to base one's evidence on more information and therefore, additional studies using other characters have been performed.

F. Summary

The use of leaf anatomy as a taxonomic criterion in grass taxonomy has proved very useful in many theoretical and practical situations. Although Conert (1957) examined the anatomy of leaves of a number of species of Loudetia his subjective approach precluded much advantage arising from such a study. In this thesis 79 leaf anatomy characters were recorded for 50 OTU's of Loudetia and Rattraya. By analysing the data using numerical taxonomic methods, information about the characters and OTU's is presented. The importance of the characters was assessed in describing the variation in a principal components analysis. It was found that those characters describing the size of the leaf were most important, followed by characters which measured the amount and arrangement of sclerenchyma in

the leaf. Principal components analysis also indicated the OTU relationships as determined by the above characters. The major division of OTU's was based on size of the leaf. No distinct groups were obvious in the principal components analysis. The classification of the OTU's into groups was performed on three different sources of the data using the sum of squares agglomerative method, and discloses somewhat different results in each case. When the results are examined in detail, however, the same general trends of the classification become evident. Some of the subsections of the section Loudetia are clearly quite distinct anatomically while others are very variable.

Analysis of the 10 supra-specific taxa shows that the three main sections of the genus are fairly well distinguished, Rattraya is most closely allied to the section Pleionura, and L. jaegeriana is most similar to the section Lophanthera when characters of leaf anatomy are considered.

3. The taxonomy of Loudetia based on the leaf epidermis

A. Anatomical Methods

The material used from herbarium and living specimens has been described above. One piece of the leaf was examined in surface view for leaf epidermal characters, and the adjacent piece was sectioned transversely for leaf anatomy characters. The methods of collecting leaf material and the initial pretreatment for anatomical sections applies here also. However, as silica bodies were of interest the leaves were not treated with hydrofluoric acid.

There are various methods for preparing sections of the

leaf epidermis, some of which are described by Prat (1948) and Stewart (1965). There are three different categories:

(i) Peels

The quickest way to obtain preparations of the epidermis is simply to peel off a portion with forceps. Unfortunately this is only possible in a very few grasses.

(ii) Scraping

This is one of the commonest methods which is quite satisfactory after some practice. The method has been described by Prat (1948), Metcalfe (1960) and de Winter (1965). The epidermis which is to be examined is placed face down on a glass slide and the underlying tissues are carefully scraped off with a scapel or razor blade. It is usually better to flood the tissue with a hypochlorite bleaching colution such as 'Javex' which acts as a lubricant and also softens the tissues. When most of the overlying cells have been removed a section of epidermis which is free of other cells is cut from the leaf and washed in water in a watch glass. Other tissues still attached may be brushed off with a camel hair brush. The preparation is then ready for staining.

(iii) Chemical treatment of the leaf to dissolve the mesophyll

Other methods have been described which cause the breakdown of the mesophyll cells and fibres which hold the two epidermis surfaces together. A summary of these methods is given in Table 9.

The leaves are first trimmed along at least one margin before they are subjected to boiling. The reagents cause the

Table 9: Methods of obtaining leaf epidermis fragments by dissolving the mesophyll.

Plant Material	Reagent(s)	Methods	Author
Various plants grazed by animals.	50% nitric acid	Leaves macerated in the solution over a water bath and then washed in water.	Martin (1955) Croker (1959) Hercus (1960)
Various plants grazed by animals, ferns, dicotyledonous herbs and trees, and grasses.	5 ml 10% nitric acid 5 ml 10% chromic acid	Leaves boiled in a 150 ml flask under a reflux condenser for 3-10 minutes, until mesophyll disintegrates.	Vasquero (1958) Storr (1961) Stewart (1965)
Sugarcane.	Concentrated nitric acid containing crystals of $KClO_3$	Material boiled in a test tube over a flame.	Artschwager and Brandes (1958)
Various taxa of grasses.	40-80% nitric acid with the addition of saturated aqueous $KClO_3$	Material boiled in a test tube with the reagents. Various concentrations are suggested for different species. Bambusoideae particularly require $KClO_3$.	Pohl (1967)

Puccinellia spp. (Gramineae)	Alcohol Water	Leaves boiled first in alcohol and then in water.	Sørensen (1953)
Dactylis glomerata L. (Gramineae)	70% ethyl alcohol 88% lactic acid	Leaves boiled in alcohol to decolorize, softened in hot lactic acid for 7-25 minutes and then in cold lactic acid for 5-10 minutes. Time period depends on type of material. Epidermis fragment easily obtained by scraping method.	Clarke (1960) Borrill (1961)

two epidermis surfaces to separate and has the advantage that preparations of both surfaces may be obtained at once. After obtaining fragments in this way they are washed in water and are then ready for staining. The method of Clarke (1960), using lactic acid, merely softens the tissue so that the scraping off of the overlying tissues, as described in (ii), is more easily accomplished.

It was found that good epidermis preparations were obtained of Loudetia leaves by the scraping method of Metcalfe (1960) and Dore (1964, personal communication). In some cases, however, where the abaxial epidermis was rigid it proved difficult to obtain whole sections. The method of boiling leaves in a mixture of nitric and chromic acids as described by Storr (1961) was used but it was found that the epidermal fragments tended to curl very tightly. These methods are very popular and it is possible that successful preparations of the Arundinellean leaf epidermis may be obtained by this means if suitable concentrations of the reagent are used. For example, Pohl (1967) obtained adequate epidermal fragments of Rattraya petiolata by boiling in concentrated nitric acid, $KClO_3$ (sat. aq.) and distilled water in the ratio of 2:1:2.

A number of authors have used different staining techniques for the leaf epidermis, and some of these methods have been summarized (Table 10). Most of the features of the epidermis can be observed without staining, except that it is often very difficult to observe the thin-walled distal cells of the micro-hairs. The characters that are to be observed

Table 10: Some methods of staining and mounting the leaf epidermis.

Stain	Method	Author
Acid fuchsin in equal parts of 95% ethyl and butyl alcohols.	Material transferred through ethyl alcohol series up to 95%, stained for ½ to 1 hour, washed in 95% butyl alcohol and mounted in Euparal.	Martin (1955)
Amann's lactophenol.	Epidermis simply mounted in the lactophenol on a slide.	Sørensen (1953)
Fast green or Chlorozol black E.	Epidermis stained in either dye, dehydrated in absolute alcohol and mounted in diaphane, or in Canada balsam, after cleaning with xylol.	Pohl (1967)
1. Methylene blue and alum 2. Ruthenium red Alternatively 1a. Iodine green 2a. Alum carmine	Epidermis stained in 1. for ½ minute, washed in distilled water and to this a small quantity (less than a pin head) of 2. was added. Stained for ½ hour. Alternatively double stained in 1a. and 2a. Dehydrated in alcohol series (70, 90, 100%), cleared in xylol and mounted in Canada balsam.	Prat (1948) Stewart (1965)
Alcoholic solution of gentian violet.	Stained, dehydrated in ethyl alcohol series, mounted in Euparal.	Storr (1961)

1% solution of cotton blue.	Material placed in the stain on a slide and heated. After staining for 20 minutes mounted in 88% lactic acid.	Clarke (1960)
94 parts 1% safranin in 70% ethyl alcohol. 6 parts Delafield's haematoxylin.	Epidermis material transferred through ethyl alcohol series up to the stain. Stained for 16-24 hours, washed in 50% alcohol, dehydrated through alcohol series (70, 90, 100%), cleared in xylol and mounted in Canada balsam.	Metcalfe (1960)
Safranin	Stained in safranin, dehydrated in alcohol, cleared in xylol and mounted in Canada balsam.	de Winter (1965)

are all at the tissue or cellular level so it is desirable to stain only the cell walls and not the contents of the cells. In some cases where some mesophyll cells are attached to the epidermis, these may stain if an unsuitable stain is used.

With loudetioid leaves the most favourable results were obtained by staining with safranin (1% aqueous solution) for 30 seconds to 2 minutes. This was followed by 95% picro-alcohol (Johansen, 1940). The picro-alcohol removed the excess safranin and the time that the epidermis was left in this solution depended on the intensity of the stain that was required. Dehydration followed using an ethyl alcohol series. The epidermis was then mounted in euparal or diaphane on a slide with the outer abaxial surface facing upwards. This surface was easily recognized because the epidermis tends to curl inwards slightly.

Stewart (1965) describes a mounting technique which is perhaps simpler, in that the end of the slide is placed into the alcohol and the epidermis fragment slid on to it, having been previously orientated with the proper surface uppermost. The epidermis pieces were mounted with the long axis of the leaf parallel to that of the slide so that they were correctly orientated for viewing.

The slides were examined with the aid of an Olympus EH binocular microscope. Photographs were taken on an Ashai Pentax Spotmatic camera fitted with a microscope adapter to the above microscope. Kodak high contrast copy film was used since no fine detail of the cell contents was required in the photo-

graphs.

The epidermis was also examined with a Zeiss GFL microscope, fitted with a projection attachment. Measurements were made of the lengths of cells, hairs, etc. from the image projected on the screen of the projection attachment using a pair of dividers and a calibrated scale which was drawn with the aid of a micrometer slide.

B. The leaf epidermis of Loudetia

The most striking feature about the epidermis, when examined in surface view, is the number, type and arrangement of appendages, and in fact these will be seen to have a marked influence in a taxonomy based on leaf epidermis characters. Also quite obvious is the division of the leaf into longitudinal zones or strips (Plate 3.1 - 3.6). The width of the zones varies, the costal zones being quite large when over primary bundles, the largest costal zone occurring at the midrib. Variation in the width of the zones from one leaf to another may be observed but no taxonomic significance appears to be attached to this. The characters that are more important in the taxonomy of Loudetia are those which describe the variation in the constituent cells of the epidermis and the dermal appendages.

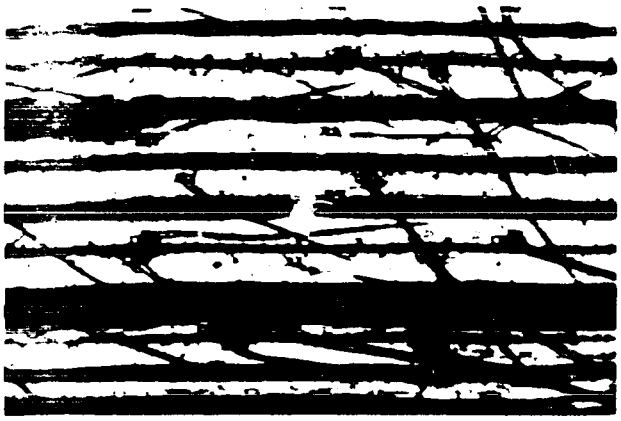
As has been mentioned above, only the abaxial epidermis surface is considered here, and most illustrations are of this surface. The general form of the adaxial epidermis of some specimens was examined (Plate 4.1 - 4.2). In adaxial view, the large almost isodiametric bulliform cells are seen to occur

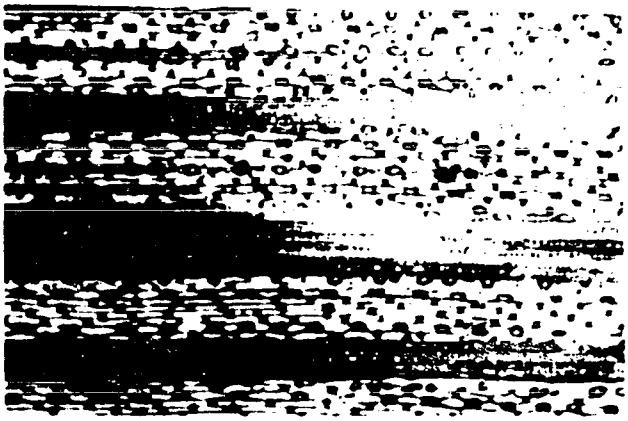
Plate 3.

Low power magnification photomicrographs of the abaxial leaf epidermis of *Loudetia* spp. and *Rattraya*.

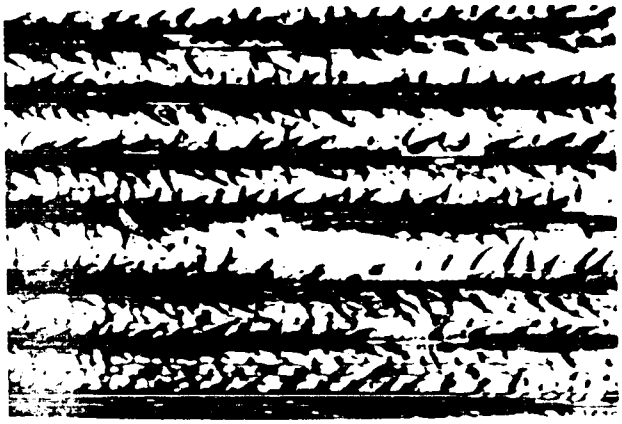
1. *Loudetia simplex* subsp. *simplex* (UWO S5)
2. *L. pennata* (28)
3. *L. migiurtina* (32)
4. *L. annua* (43)
5. *L. jaegeriana* (23)
6. *Rattraya petiolata* (52)

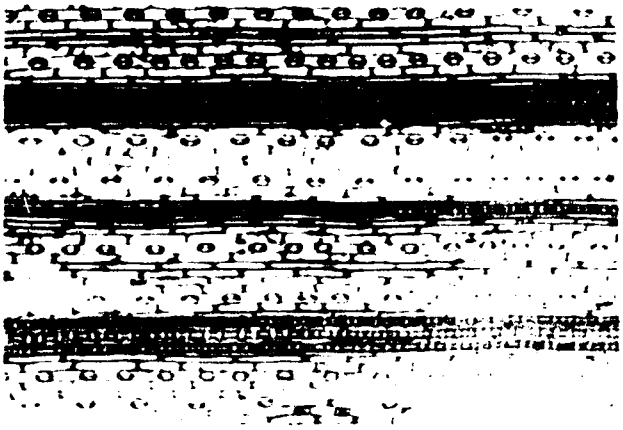
OTU or specimen number in parentheses.



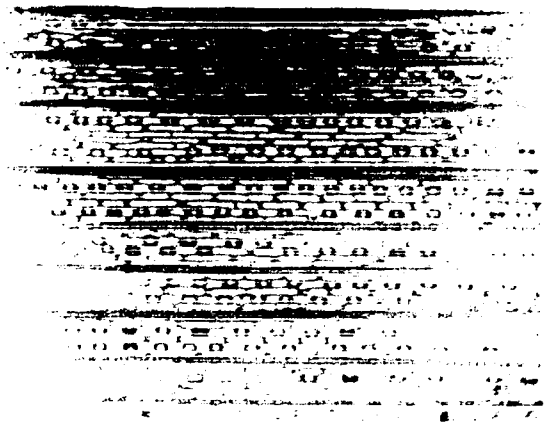


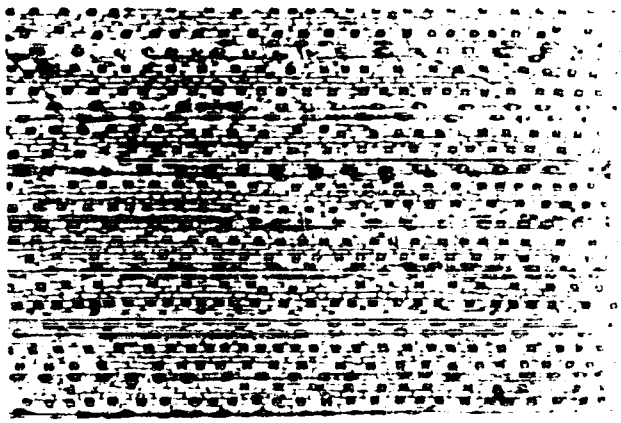
MPLEX SUBSP. SIMPLEX 2. L.PENNATA





L.MIGIURTINA 4. L.ANNUA





AEGERIANA 5. R.PETIOLATA

Plate 4.

Photomicrographs of the leaf epidermis of *Loudetia* spp.

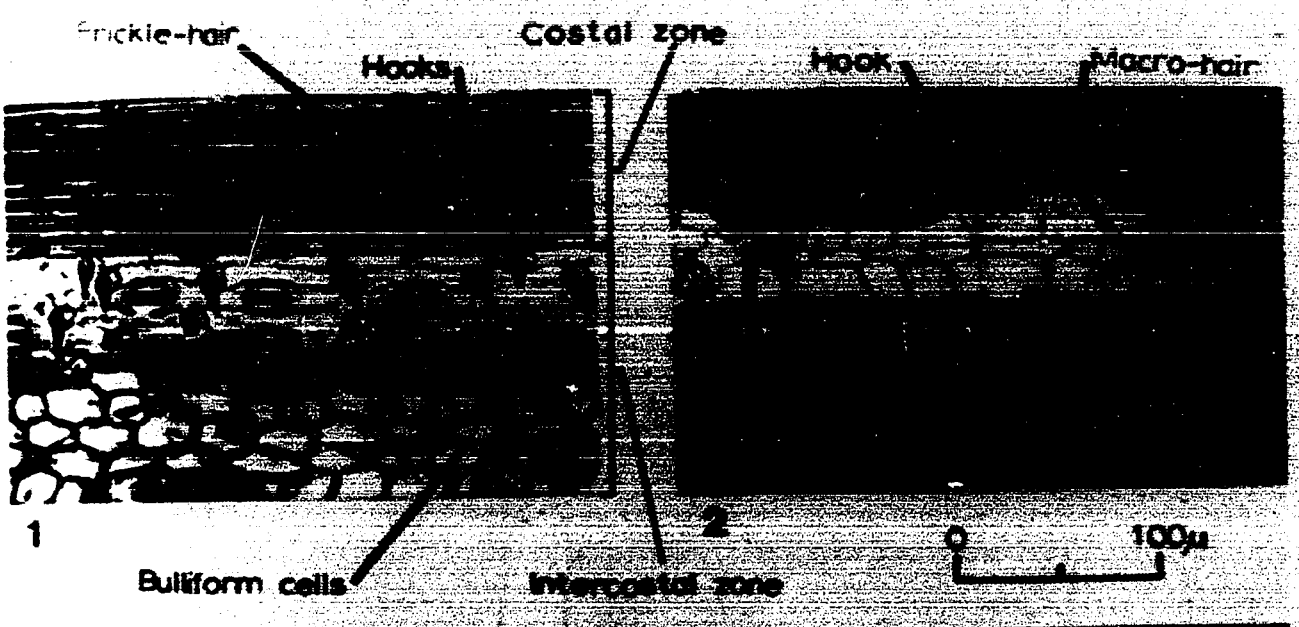
1 and 2. Adaxial epidermis of *L.flavida* (UWO S 17)

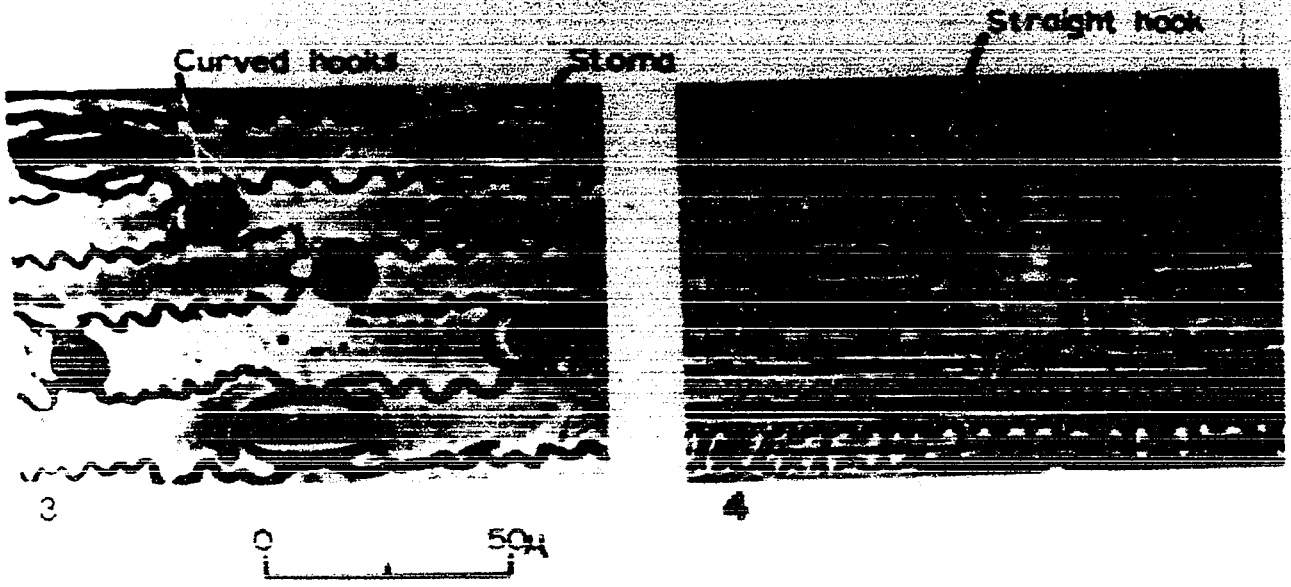
3 and 4. High power magnification photomicrographs of the abaxial surface.

3. *L. arundinacea* (UWO S 40)

4. *L. phragmitoides* (OTU No.46)

OTU or specimen number in parentheses.


Prickle-hair
Hooks
Costal zone
Hook
Macro-hair
1
2
0
100µ
Bulliform cells
Intercostal zone


Curved hooks
Stoma
Straight hook
3
4
0
50µ

in the inter-costal zone, and very often macro-hairs arise from this region. Apart from this the other festures appear very similar to those of the abaxial surface.

(a) Constituent cells of the epidermis

(i) The long cells (Plate 5.4)

The long cells in the intercostal region are always shorter and wider than those of the costal region. Also their walls are often thicker and more sinuous. Not a great deal can be inferred from the data on lengths and ratios (Table 38, Appendix 3) of the long cells, though it will be noticed that species from more xeric habitats have somewhat shorter and broader long cells, which have very thick walls (e.g. L. perrieri, L. madagascariensis, Plates 6.21 - 6.22). The species of section Pleioneura, particularly L. simulans and L. anomala (Plates 6.47 - 6.48), also have short long cells in the intercostal zone.

(ii) The short cells (Plate 5.4)

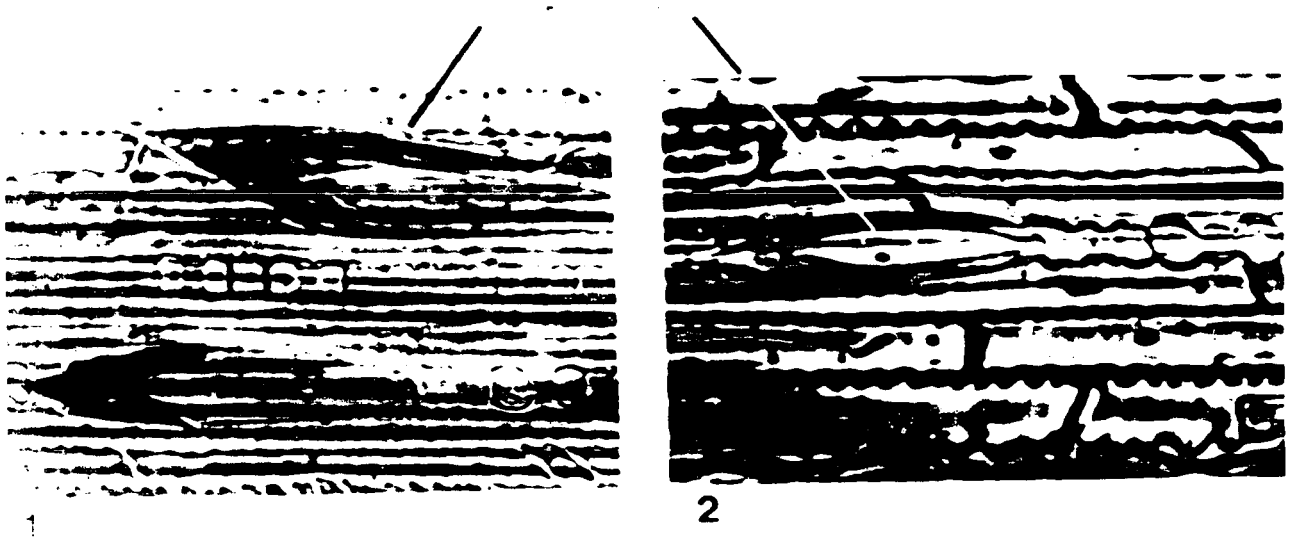
The arrangement of short cells appears fairly consistent in all of the specimens examined. In the intercostal zone they are usually solitary although they may occur in pairs, and in the costal zone, they occur in rows of many cells. The short cells of the intercostal zone are usually very short, being less than half as long as wide, but exceptionally long short cells are found in the subsection Densispicae (e.g. L. coarctata, plate 6.37, 7.2 and L. densispica Plate 6.39). The short cells in the intercostal region show little significant variation throughout the genus (Table 38, Appendix 3).

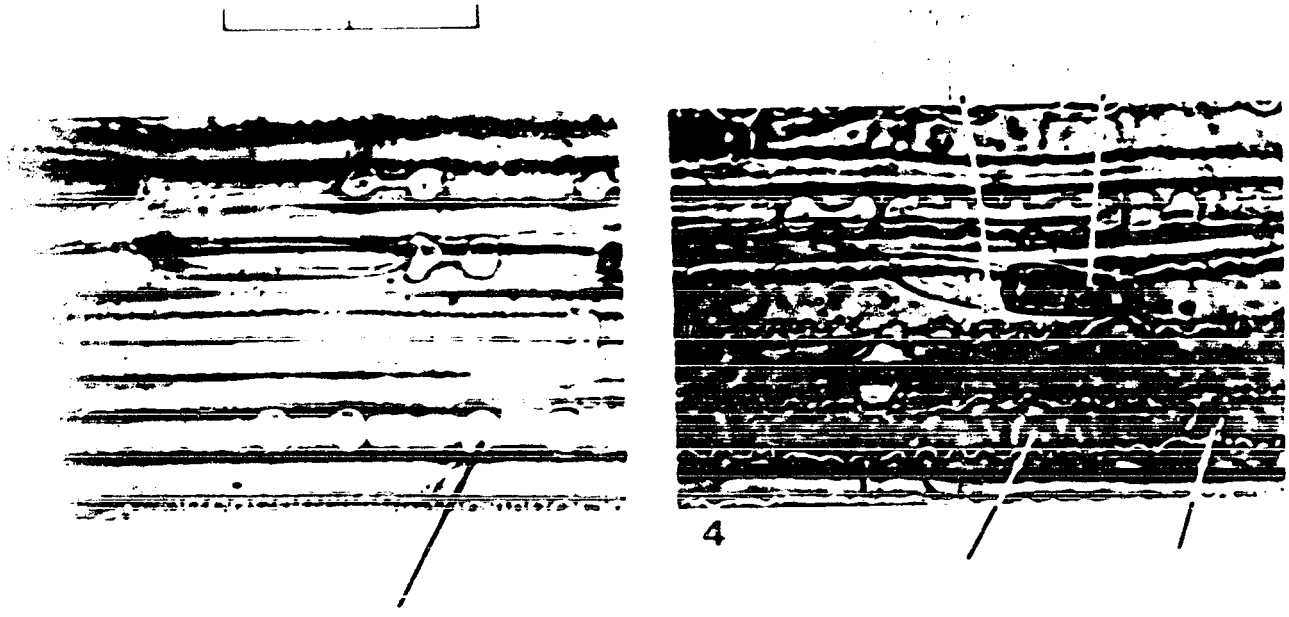
Plate 5

High power magnification photomicrographs of the abaxial leaf epidermis of *Loudetia* spp.

1. *L. arundinacea* (3)
2. *L. angolensis* (1)
3. *L. simplex subsp. simplex* (11)
4. *L. phragmitoides* (47)

OTU numbers in parentheses.


1
2


4

Silica bodies (Plate 5.3) are fairly abundant in the short cells over the veins. The density data (Table 39, Appendix 3) and the measurements (Table 38, Appendix 3) for short cells in the costal region refer only to short cells containing silica bodies. The silica bodies occur in a variety of different shapes and forms (Fig. 14). In the intercostal short cells silica bodies are not as common as in the costal region. They are not found at all in the intercostal zones of leaves of the Densispicae or Flammidae. In the Pungentes they are exclusively of the tall and narrow type, whereas in the Typicae and Acuminatae, the irregularly shaped or cross-shaped silica bodies are common.

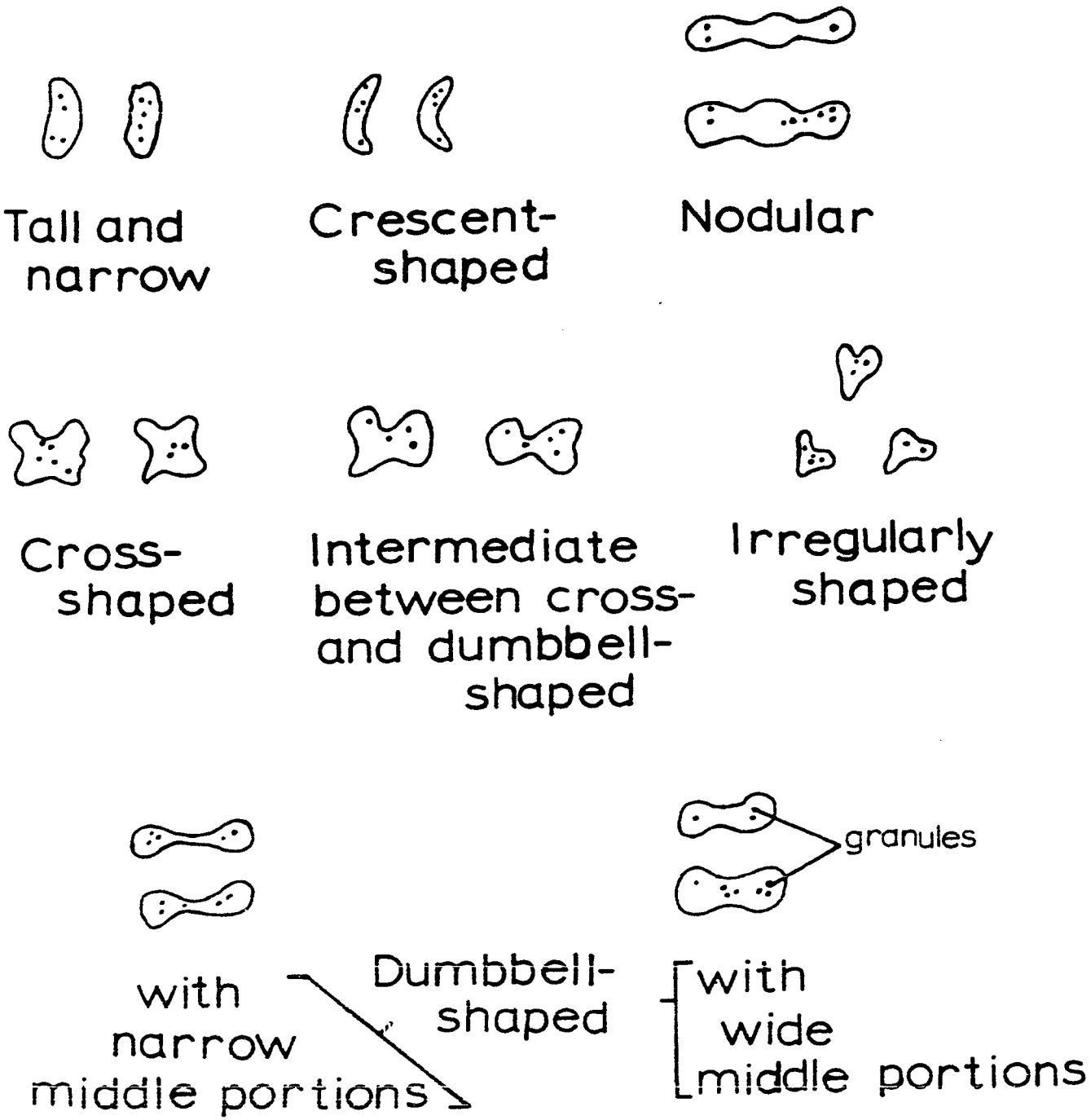
(iii) Intercostal cells differing from long and short cells

In the section Pleioneura (Plates 6.47 - 6.49) bands of cells occur in the intercostal region which have a similar appearance to the bulliform cells of the adaxial epidermis. In transverse sectional view, these cells appear to be similar to bulliform cells though they are somewhat smaller, and have thicker walls. Metcalfe (1960) refers to these cells as bulliform cells.

(iv) Stomata (Plate 4.3)

The Stomata occur in bands in the intercostal zone their density varying somewhat within and between species (Table 39, Appendix 3). The size of the stomata is quite constant within some subsections and sections of the genus, most notably the Annuae which have large stomata (Table 38, Appendix 3).

Fig.14. Types of silica-bodies



(b) Dermal Appendages

(i) Macrohairs (Plate 4.2)

The presence of macrohairs varies greatly within species as does the relative abundance of the hairs. L. arundinacea, L. simplex subsp. simplex and L. flavida are all represented by specimens which either do or do not have macrohairs.

(ii) Microhairs (Plate 5.4)

The micro-hairs on the other hand are only rarely absent. Only in L. perrieri and L. madagascariensis (Plates 6.21 - 6.22) is the absence of appendages extended to micro-hairs. The micro-hairs vary in length and width. As the distal cell was often absent or not visible, measurements had to be confined to the proximal cell (Table 38, Appendix 3). There appears to be a general consistency in the lengths and ratios of the proximal cell of the micro-hairs in the subsection Typicae. Although those of the Acuminatae are more variable it will be noticed that the cells of some are much longer and narrower (L. cuanzensis, Plate 6.33). The Annuae and Flammidae have somewhat short wide cells (L. annua, Plate 6.42 L. flammida, Plate 6.44).

(iii) Prickle-hairs (Plates 5.1 - 5.3)

Prickle-hairs are fairly common, alternating with rows of short cells in the costal zone. They are very abundant in members of the Acuminatae (L. migiurtina, Plate 3.3, 6.31, L. acuminata, Plate 6.32), and completely absent in the Densisubicae. The density, length and ratio of length to width have been recorded in Table 39, Appendix 3.

Plate 6.1 - 6.52.

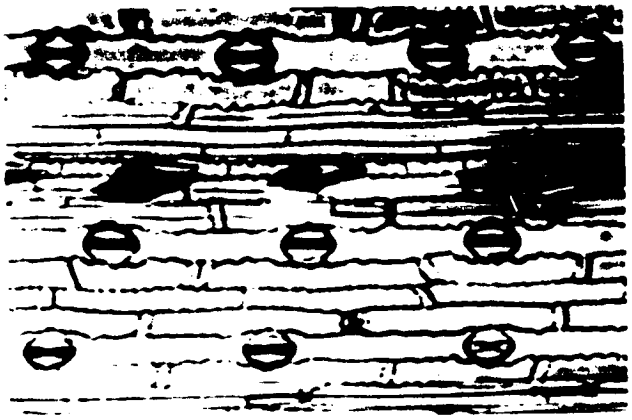
Photomicrographs of the abaxial leaf epidermis of all the OTU's studied taxonomically.

OTU numbers the same as the plate number unless otherwise indicated in parentheses.

1 L.ANGOLENSIS

2. L.ARUNDINACEA

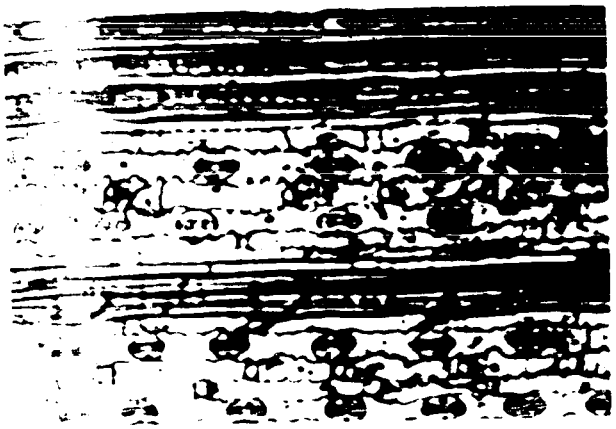
3. L.ARUNDINACEA



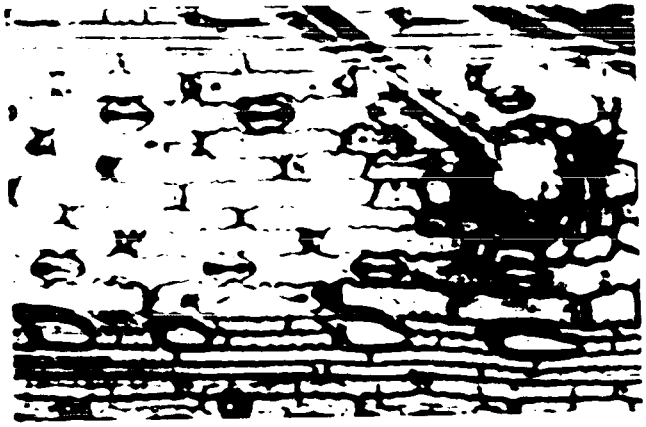
4. L.ARUNDINACEA

5. L.ARUNDINACEA

6. L.ARUNDINACEA



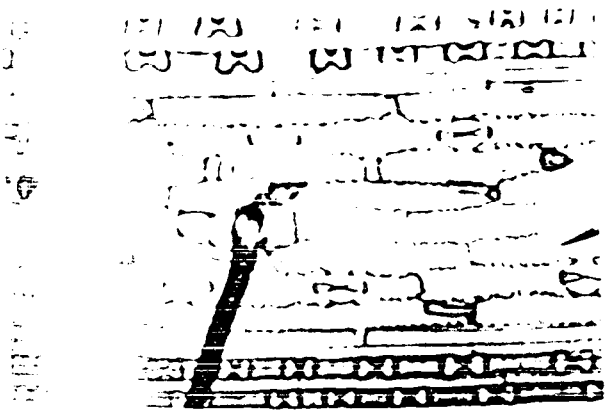
ARUNDINACEA

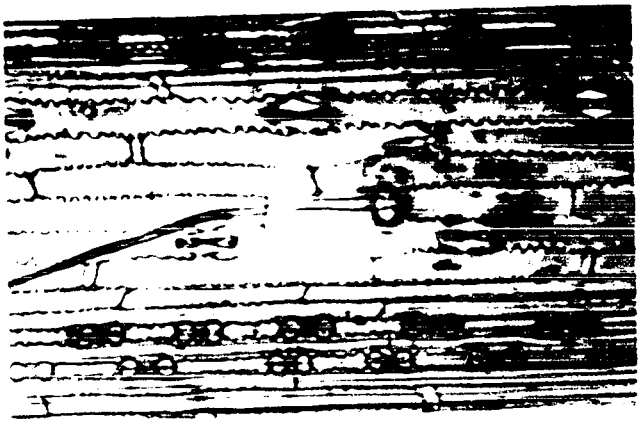


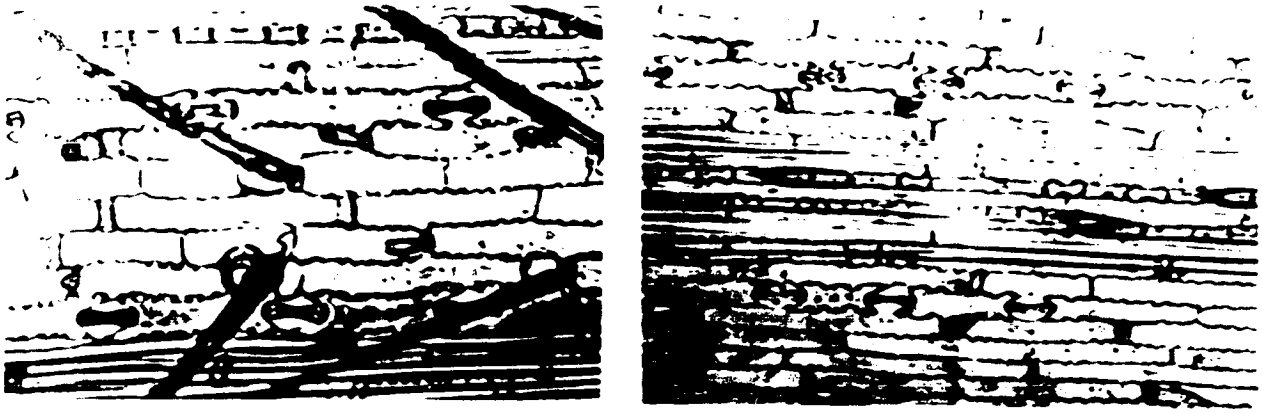
8. L.ARUNDINACEA

[illegible]OMAS

10. L.CAMERUNENSIS

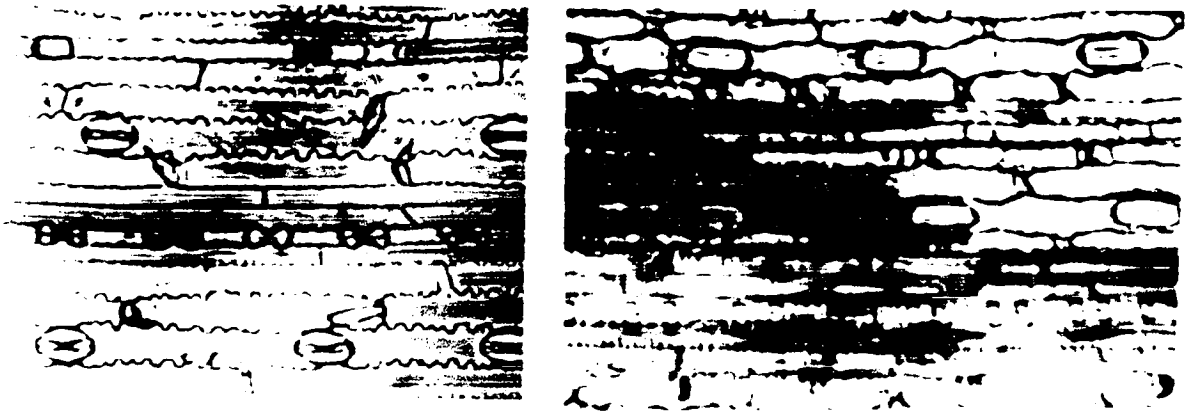


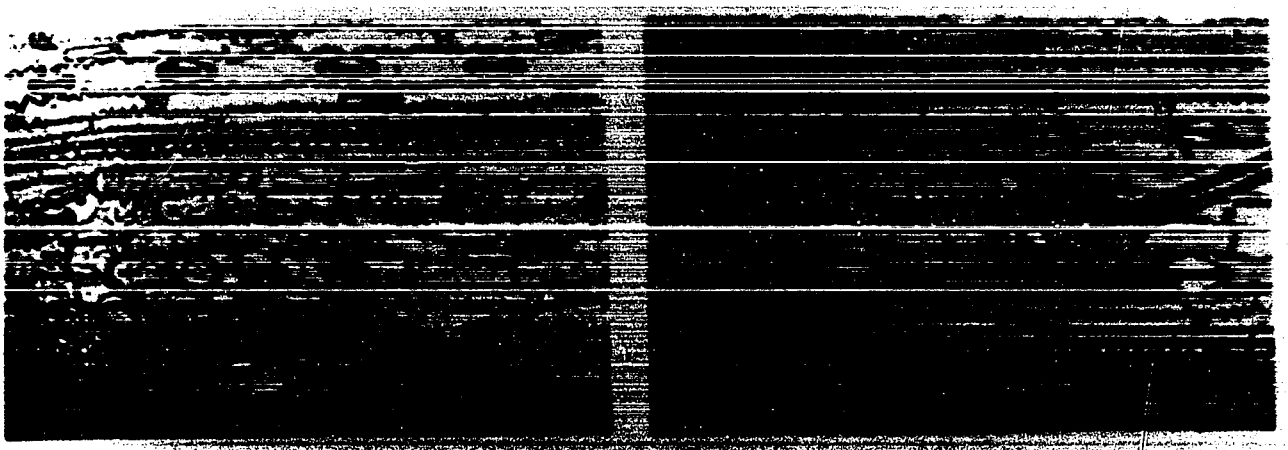




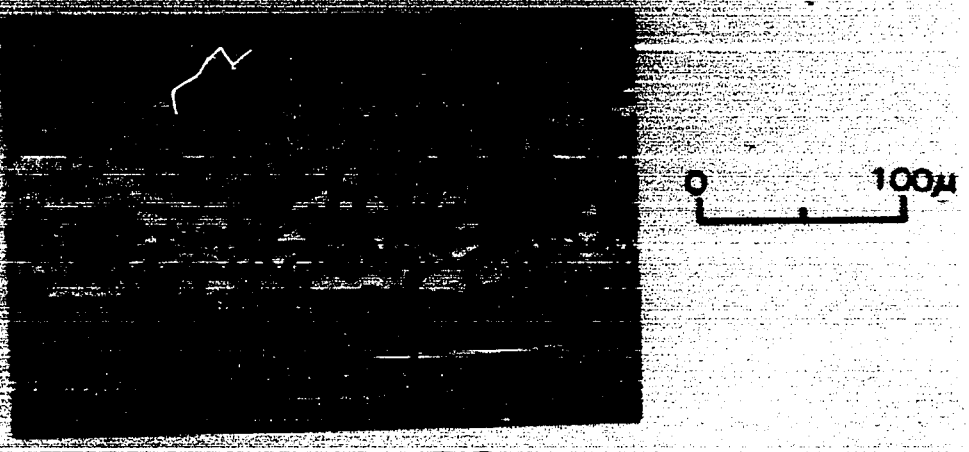
L. SIMPLEX SUBSP. SIMPLEX 14

15. L. SIMPLEX SUBSP. SIMPLEX

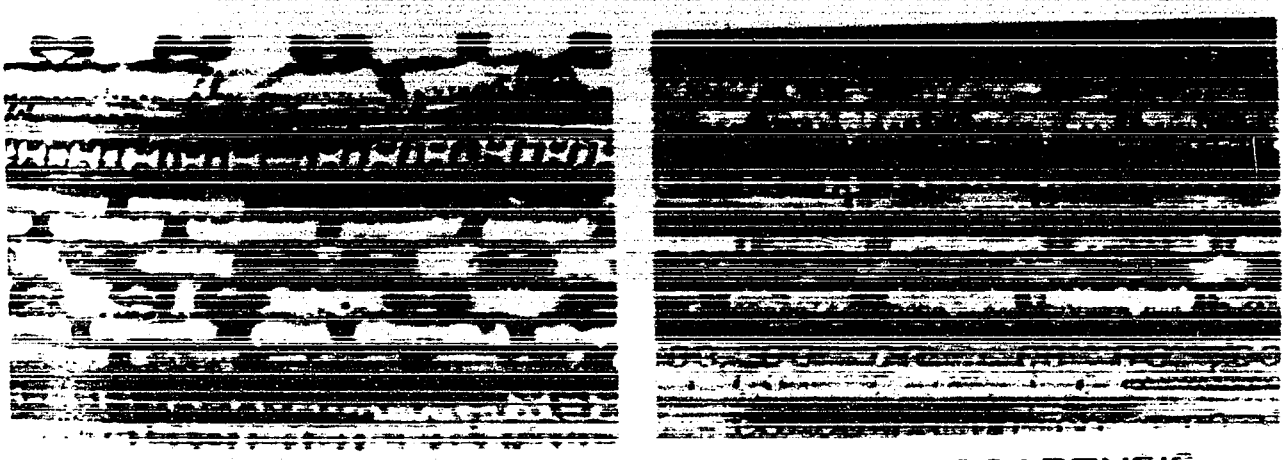




18. L.KAGERENSIS 19. L.KAGERENSIS



20. L.KAGERENSIS

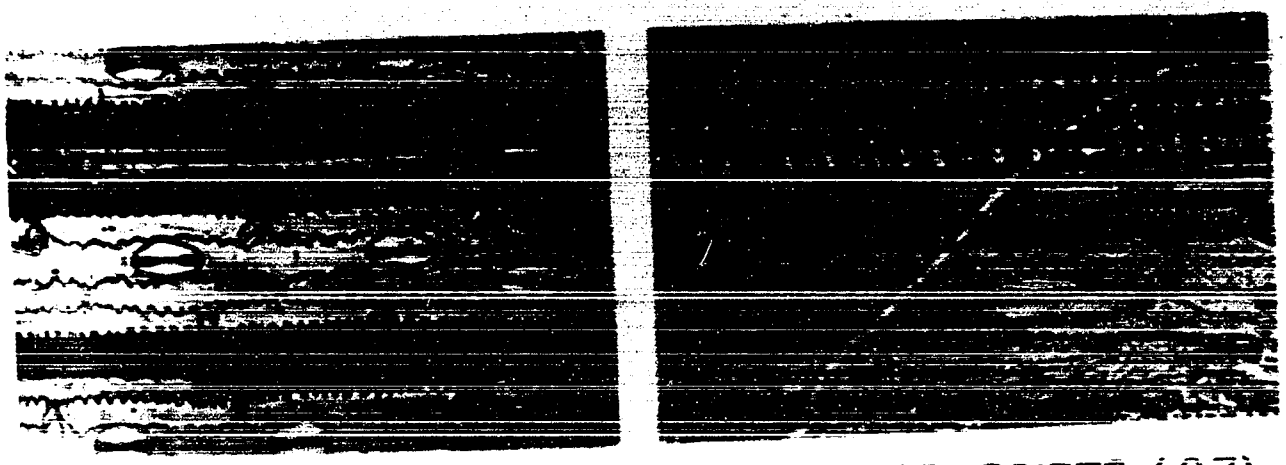


21. L.PERRIERI 22. L.MADAGASCARENSIS

23. L.LANATA (24)

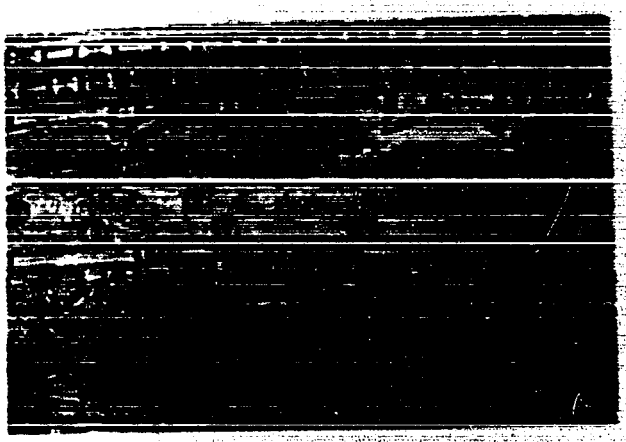
24. L.LONGIPES (25)

0 100μ

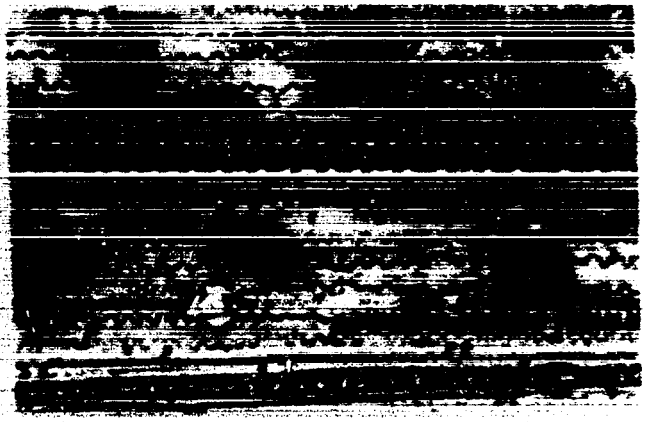


25. L.DEMEUSEI (26)

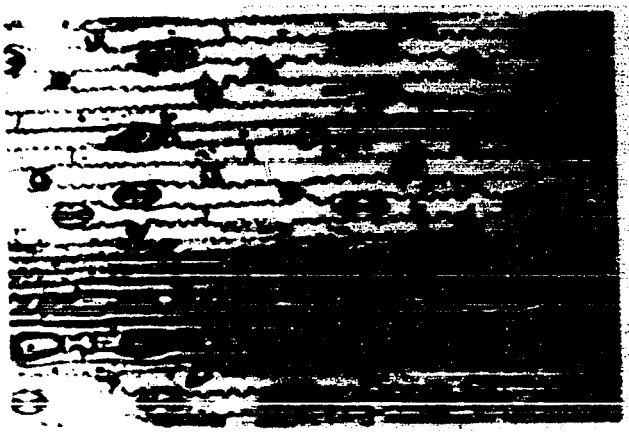
26. L.CRASSIPES (27)



27. L.PENNATA (28)

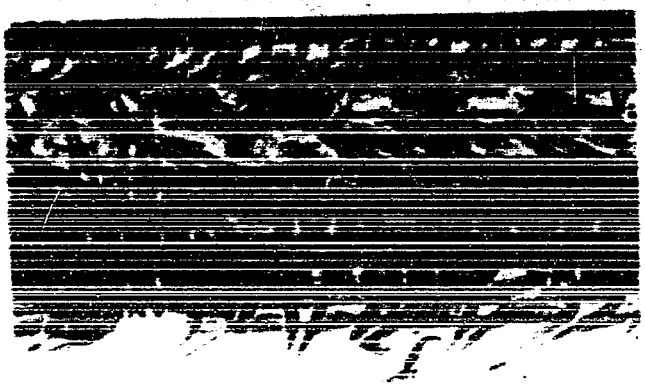


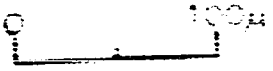
28. L.FLAVIDA (29)



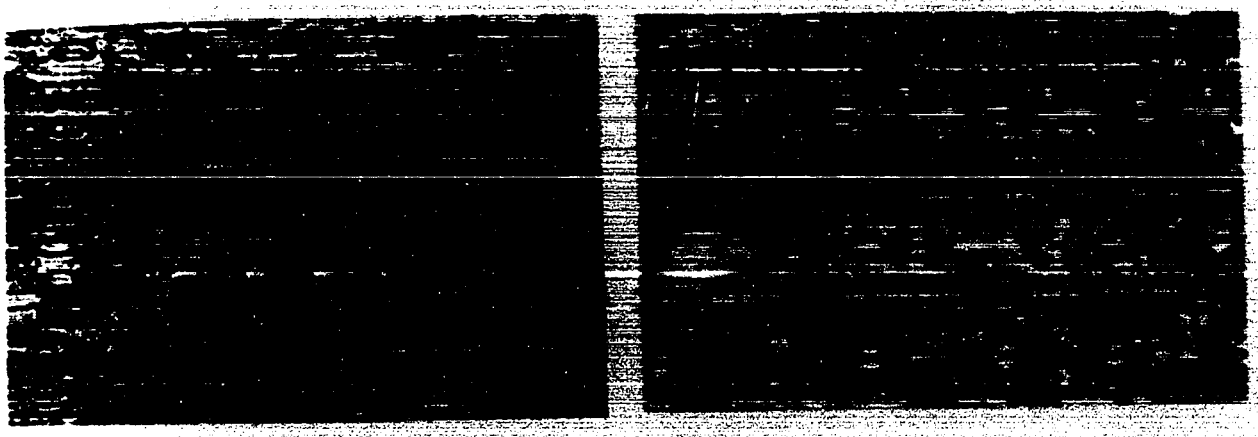
29. L.FLAVIDA (30)

30. L.FLAVIDA (31)



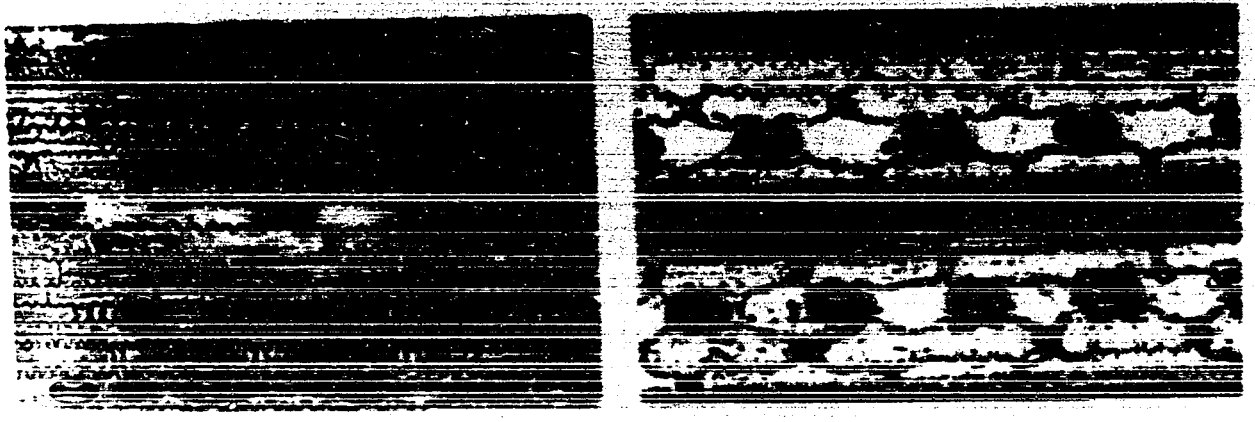


31. L.MIGIURTINA (32)

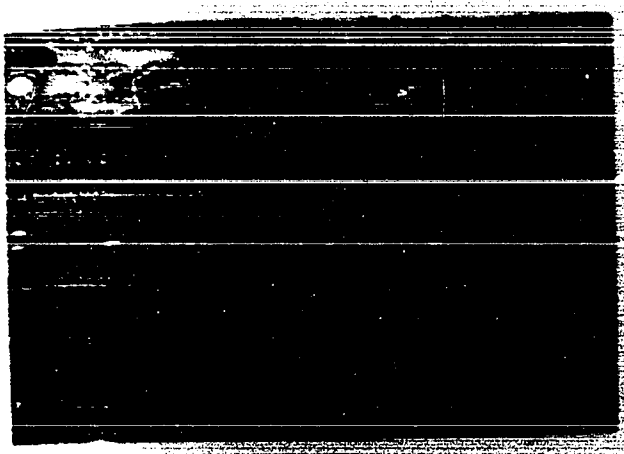


32. L.ACUMINATA (33) 33. L.CUANZENSIS (34)

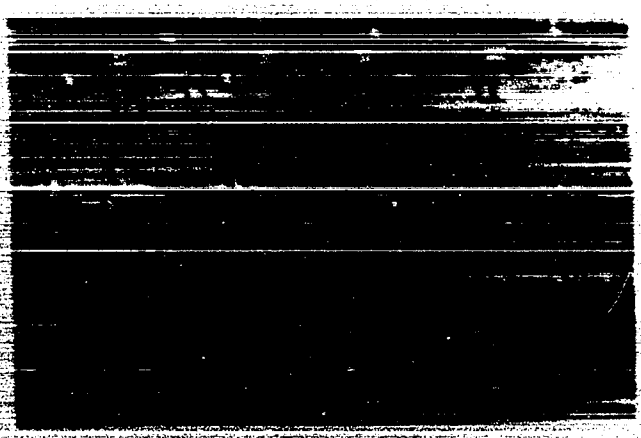
0 100µ



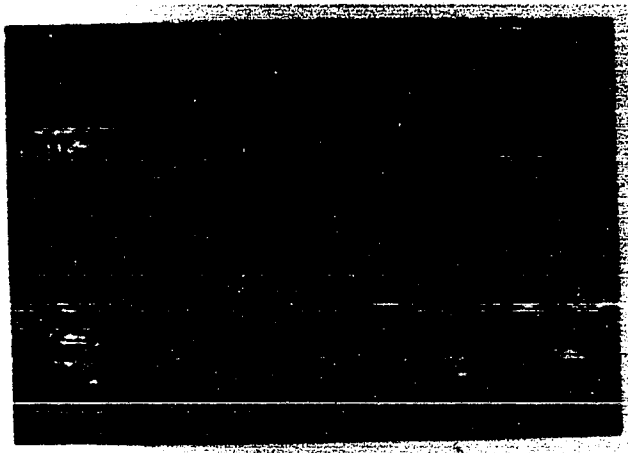
34. L.FILIFOLIA SUBSP.FILIFOLIA (35) 35. SUBSP. HUMBERTIANA (36)



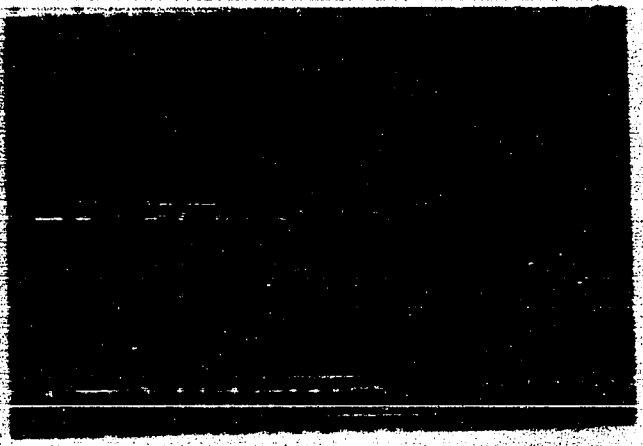
36. L.GOSSWEILERI (37)



37. L.COARCTATA (38)

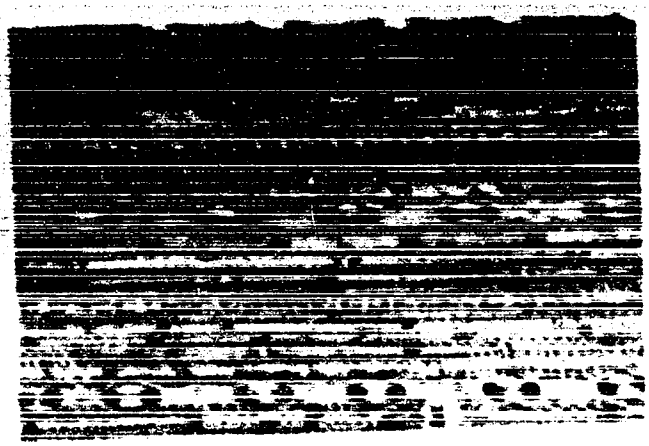


38. L.DENSISPICA (39)



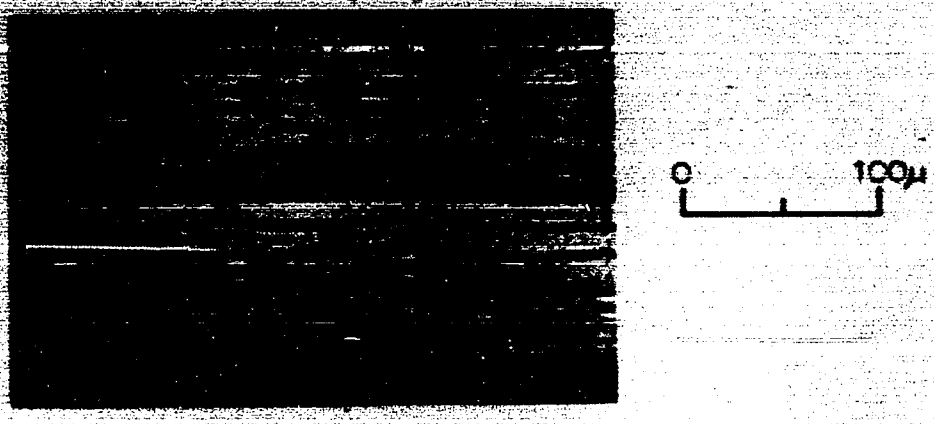
39. L.DENSISPICA (40)

L.TISSERANTII (41)

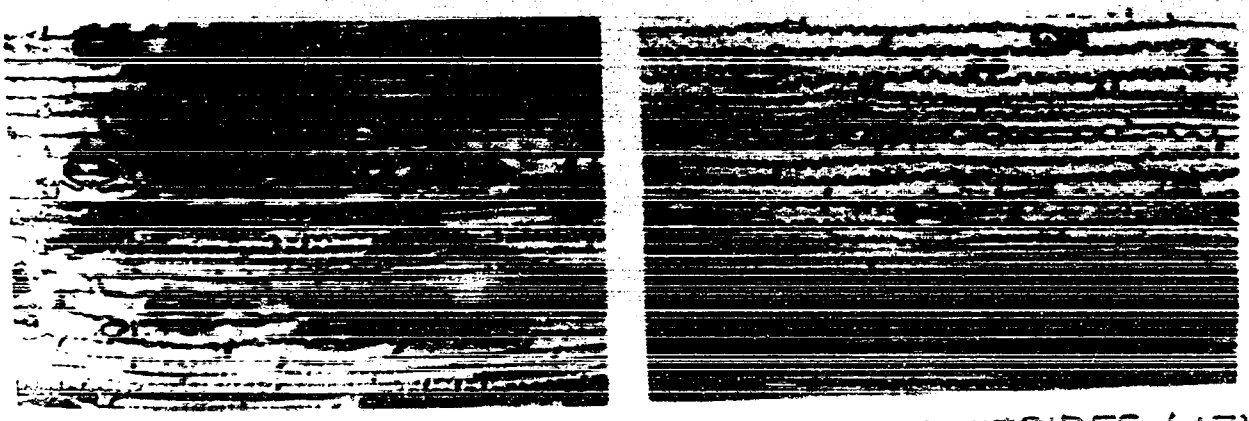


41. L.VANDERYSTII (42)

42. L.ANNUA (43) 43. L.HORDEIFORMIS (44)



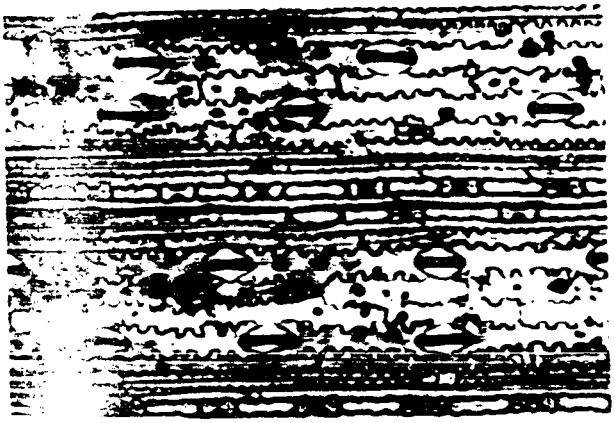
44. L.FLAMMIDA (45)



45. L.PHRAGMITOIDES (46) 46. L.PHRAGMITOIDES (47)

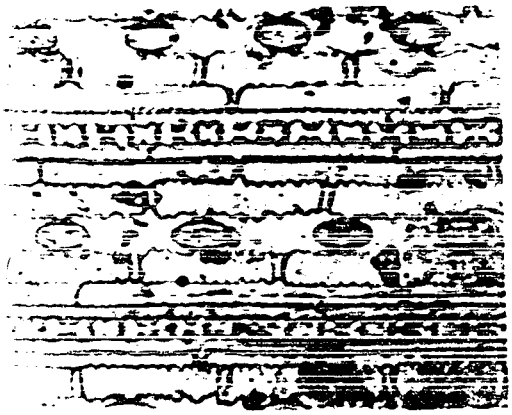
_. SIMULANS (48)

48. L. ANOMALA (49)



_. RAMOSA (50)

50 R. PETIOLATA (52)



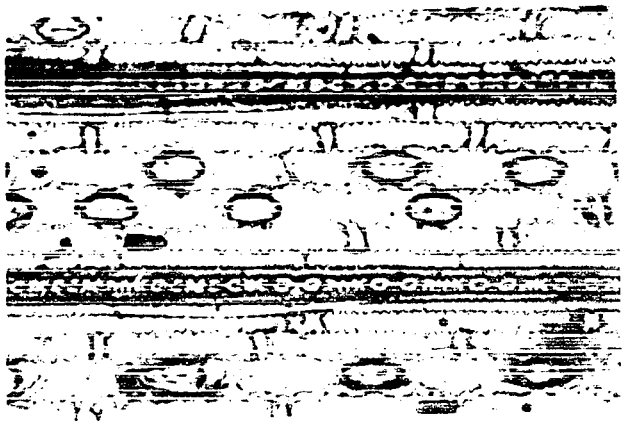


Plate 7

Photomicrographs of the abaxial leaf epidermis of *Loudetia* spp. and *Rattraya*.

1. *L. arundinacea* (4)
2. *L. coarctata* (38)
3. *L. kagerensis* (18)
4. *R. petiolata* (52)

OTU numbers in parentheses.

100μ
0
1
2
3
4

(iv) Hooks (Plate 5.1 - 5.4)

In conjunction with prickle-hairs in the costal zone one finds hooks in the intercostal zone, which may have straight points and appear very much like prickle-hairs, or have curved points. Hooks are also absent in the subsection Densispicae, and all except L. simulans of section Pleioneura. The density of both hooks and prickles is greatest in the Typicae and Acuminatae (Table 39, Appendix 3). These subsections also have the largest prickles and hooks (Table 38, Appendix 3).

C. The numerical taxonomy using leaf epidermis characters

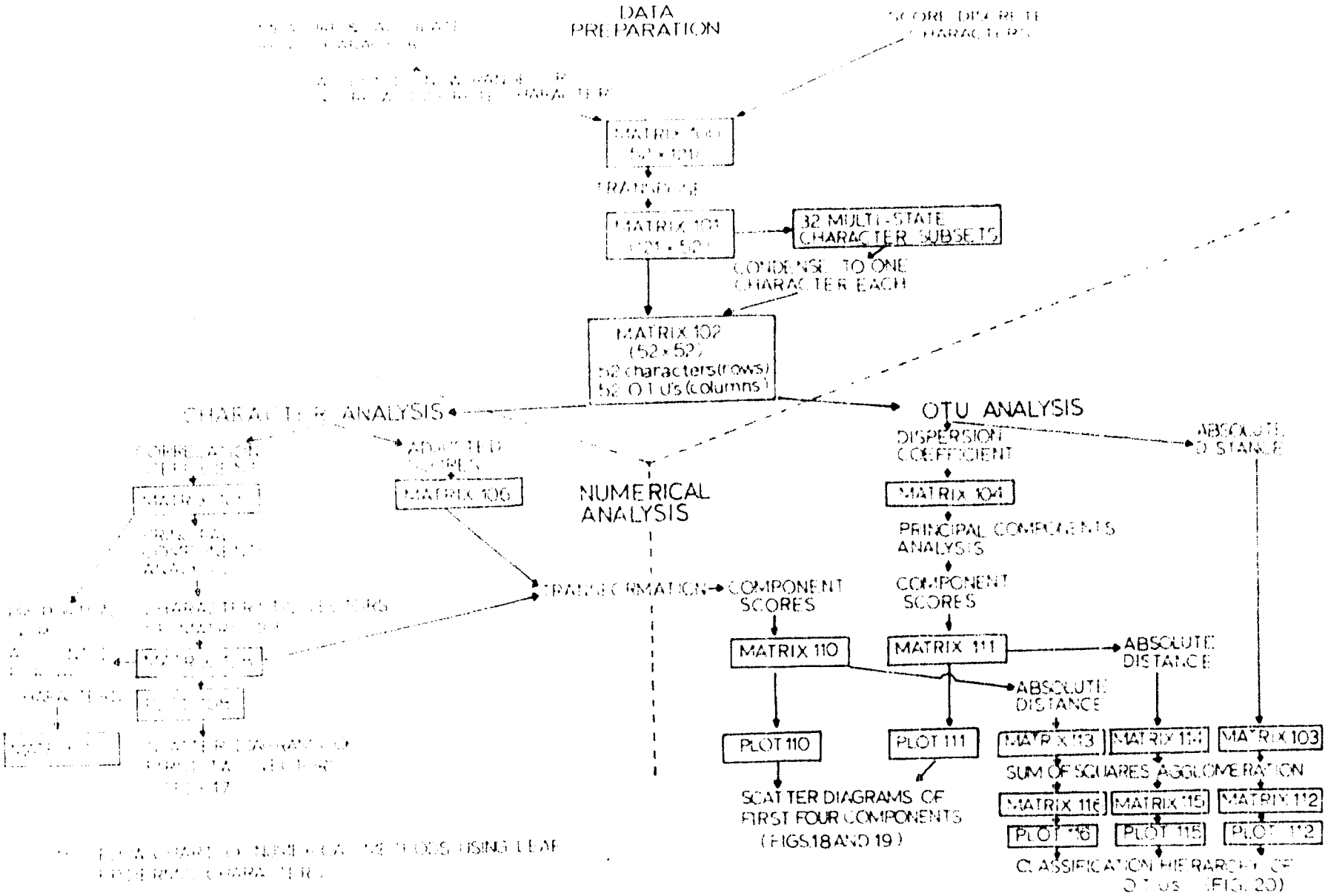
From a subjective analysis of the information available on the leaf epidermis of the specimens of Loudetia it would be difficult to formulate a classification. By subjecting the information to a multivariate analysis, however, as was done with the leaf anatomy data, some interesting conclusions may be drawn. Some 52 characters of the leaf anatomy are used in this analysis, and the relation of this segment of the study to the whole thesis is outlined in Figures 2 and 3.

The application of the numerical methods using the leaf epidermis characters and 52 specimens as OTU's is presented in a flow chart (Fig. 15). Numerical methods were also applied to the 10 supra-specific taxa (Fig. 16).

1. Data Preparation

(a) The OTU's

Fifty-two specimens of Loudetia were taken as the representative OTU's in this study (Table 11). The same symbols are used as previously (Table 4), but the OTU's have been


DATA PREPARATION
TRANSPOSE
32 MULTI-STATE CHARACTER SUBSETS
CONDENSE TO ONE CHARACTER EACH
MATRIX 102 (52 × 52) 52 characters (rows) 52 O.T.U's (columns)
CHARACTER ANALYSIS
OTU ANALYSIS
NUMERICAL ANALYSIS
DISPERSION COEFFICIENT
MATRIX 104
PRINCIPAL COMPONENTS ANALYSIS
COMPONENT SCORES
MATRIX 110
MATRIX 111
ABSOLUTE DISTANCE
PLOT 110
PLOT 111
SCATTER DIAGRAMS OF FIRST FOUR COMPONENTS (FIGS. 18 AND 19)
MATRIX 113
MATRIX 114
MATRIX 103
SUM OF SQUARES AGGLOMERATION
MATRIX 116
MATRIX 115
MATRIX 112
PLOT 116
PLOT 115
PLOT 112
CLASSIFICATION HIERARCHY OF O.T.U's (FIG. 20)

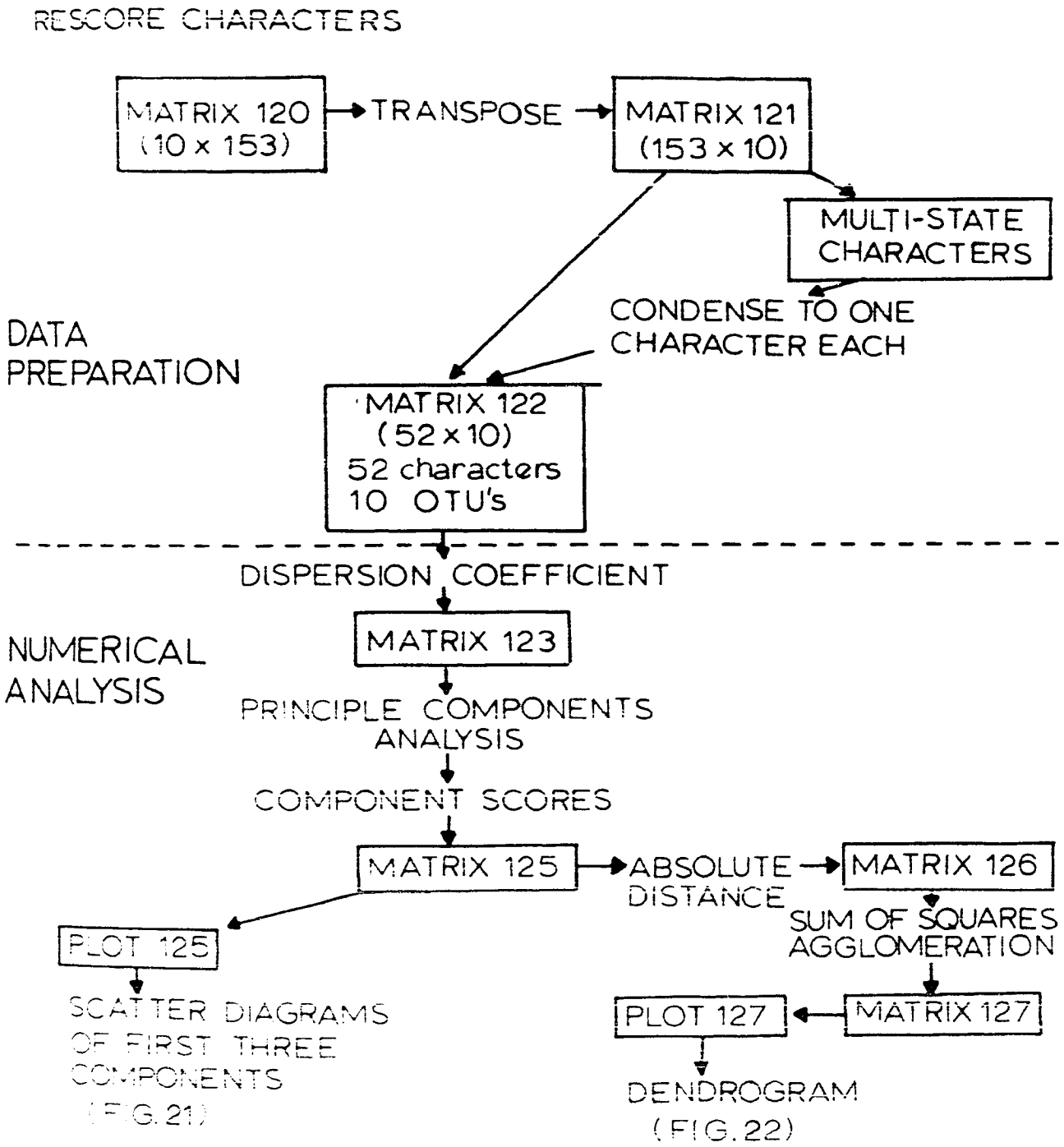


FIG. 16 FLOW CHART OF NUMERICAL METHODS USING SUPRA-SPECIFIC TAXA AS OTU's AND LEAF EPIDERMIS CHARACTERS

Table 11: List of OTU's used in the taxonomy, based on leaf epidermis characters. (Numbers and symbols used in Figures 18-22)

Section: Loudetia

1. Subsection: Typicae

1. L. angolensis
2. L. arundinacea
3. "
4. "
5. "
6. "
7. "
8. "
9. L. thomasii
10. L. camerunensis
11. L.simplex subsp. simplex
12. "
13. "
14. "
15. "
16. L.simplex subsp. stipoides
17. "
18. L. kagerensis
19. "
20. "
21. L. perrieri
22. L. madagascariensis

10. Incertae sedes

23. L. jaegeriana

2. Subsection: Pungentes

24. L. lanata
25. L. longipes
26. L. demeusei
27. L. crassipes

3. Subsection: Acuminatae

28. L. pennata
29. L. flavida
30. "
31. "
32. L. migiurtina
33. L. acuminata
34. L. cuanzensis
35. L. filifolia subsp. filifolia
36. L. filifolia subsp. humbertiana

4. Subsection: Densispicae

37. L. gossweileri
38. L. coarctata
39. L. densispica
40. "
41. L. tisserantii
42. L. vanderystii

5. Subsection: Annuae

⊠ 43. L. annua
44. L. hordeiformis

6. Subsection: Flammidae

△ 45. L. flammida
46. L. phragmitoides
47. "

7. Section: Pleioneura

○ 48. L. simulans
49. L. anomala
50. L. ramosa

8. Section: Lophanthera

□ 51. L. togoensis

9. Genus: Rattraya

⊗ 52. R. petiolata

assigned different numbers so that the specimens of the higher taxa are still in numerical order. L. arundinacea (2-8) and L. simplex subsp. simplex (11-15) are each represented by seven and five cultivated examples respectively. L. densispica (39, 40) is represented by two wild examplars, and L. kagerensis (18, 19) and L. flavida (29-31) are represented by both wild and cultivated examplars.

(b) The leaf epidermis characters (Table 40, Appendix 3)

A total of 52 characters were found to be useful in describing differences and similarities between the OTU's. Twenty-five of these characters describing the appendages are secondary in the sense that they are dependant on the presence of a primary character, viz. the appendage. Each character was, however, given equal weight.

Measurements

The lengths and widths of the cells and appendages were measured from representative cells taken at random from the section of epidermis being examined. Only a few measurements were made in each case so that no mean value was calculated. The values are given in Table 38 (Appendix 3). These measurements were used to formulate discrete character states.

Densities

Counts were made of the number of cells or appendages in a 200 x 200 micron square grid attached to the screen of the projection attachment. Twenty counts were made in each case and the mean values were converted to numbers per square mm of leaf epidermis (Table 39, Appendix 3). Short cells and

stomata are always present so the density values were used directly following adjustment to the 100 to 900 scale. In the case of appendages, discrete character states were formed.

Silica bodies (Fig. 14)

The shape or type of silica bodies are given in Metcalfe (1960), whose convention was followed here. Correct orientation of the epidermis is important in deciding upon the shape of a silica body. The irregularly shaped silica bodies which frequently occurred in the short cells of the intercostal zone are not mentioned by Metcalfe (1960). Frequently more than one type of silica body occurs in the same leaf. Measurements and counts of the short cells of the costal zone were always made on cells which contained silica bodies.

Stomata

The measurements were made on the length and breadth of the whole complex, i.e. the length of the subsidiary cells at the widest point and breadth across both subsidiary cells.

Macrohairs

The relative frequency or abundancy of these hairs was decided as follows:

Rare - one or fewer hairs per sq mm

Frequent - 2-5 hairs per sq mm

Abundant - more than 5 hairs per sq mm

The point of attachment, and type of base of the macrohairs, were observed from transverse sections of the leaves.

Microhairs

Usually only the proximal cell was present and in some

cases the characters for the distal cell (35 and 36) were not recorded.

The prickle-hairs and hooks differ at the margin of the leaf and characters for these appendages were not scored from this region.

Production of the data matrix

For each specimen the characters were scored according to their states as indicated in Table 40 (Appendix 3). The densities (characters 10, 15, 23) were adjusted to a new integer range between 100 and 900. The data was punched on IBM computer cards (matrix 100, Appendix 3) and rearranged so that the 52 OTU's were in the columns and the characters in rows (Matrix 101). Thirty-two of the characters were represented by states in a non-linear form and these were subjected to a principal component analysis so as to condense the information into 32 single characters. The characters were returned to their positions in matrix 101 so that the data matrix (102) was formed with 52 characters as rows and 52 OTU's as columns.

The data preparation was carried out in the same way for the 10 OTU's representing supra-specific taxa, data matrix 122 with 52 rows (characters) and 10 columns (OTU's) being the result.

2. Analysis of Characters

The results of the component analysis are not as informative as the previous analysis using leaf anatomy characters. The same methods were followed in this instance, the correlation matrix (105) being analysed into its characteristic

vectors (matrix 108) and roots (Table 12), and the proportion of the root accounted for by the characters also determined (matrix 109). These values for the first 7 roots, which account for over 50% of the variation, are given in Table 43 (Appendix 3). A scatter diagram showing the position of the characters when projected on the first two vectors is also given (Fig. 17).

Character "groups"

Some "groups" of characters measuring similar properties are evident from the scatter diagram.

I Characters measuring lengths and ratios

The characters measuring the length and/or ratios of the long cells (1, 2, 5, 6), short cells - intercostal zone (16, 17), stomata (21, 22) and microhairs (32) have high positive scores on the first axis. These characters account for 46.42% of the variance on the first and 30.60% of the variance of the third root, a value of 8.1% of the total variance, on these two roots alone. There are some characters which measure these same properties which do not fall in this category, and the reasons for this will be discussed below.

II Occurrence, density and distribution of cells and appendages

A number of characters have high positive scores on the second axis and these fall into the category of the heading. The occurrence and distribution of microhairs (29, 30) prickle-hairs (37, 38) and hooks (45, 46), are the most important ones. Others are the density of short cells in the inter-costal zone (10) and the type of cells adjacent to the prickles (44) and hooks (52). In all these characters account for

TABLE 12: ROOTS OF CORRELATION COEFFICIENT MATRIX (NO. 105)

Root	Value of Root	% of total variance accounted for	Accumulated percentage
1	6.171	11.87	11.87
2	5.708	10.98	22.85
3	4.403	8.47	31.32
4	3.579	6.88	38.20
5	3.073	5.91	44.11
6	2.459	4.73	48.84
7	2.304	4.43	53.27
8	1.984	3.82	57.09
9	1.827	3.51	60.60
10	1.680	3.23	63.83
11	1.564	3.01	66.84
12	1.507	2.90	69.74
13	1.405	2.70	72.44
14 - 52	14.334	27.57	100.00
Total Variance	52.000	100.00	-

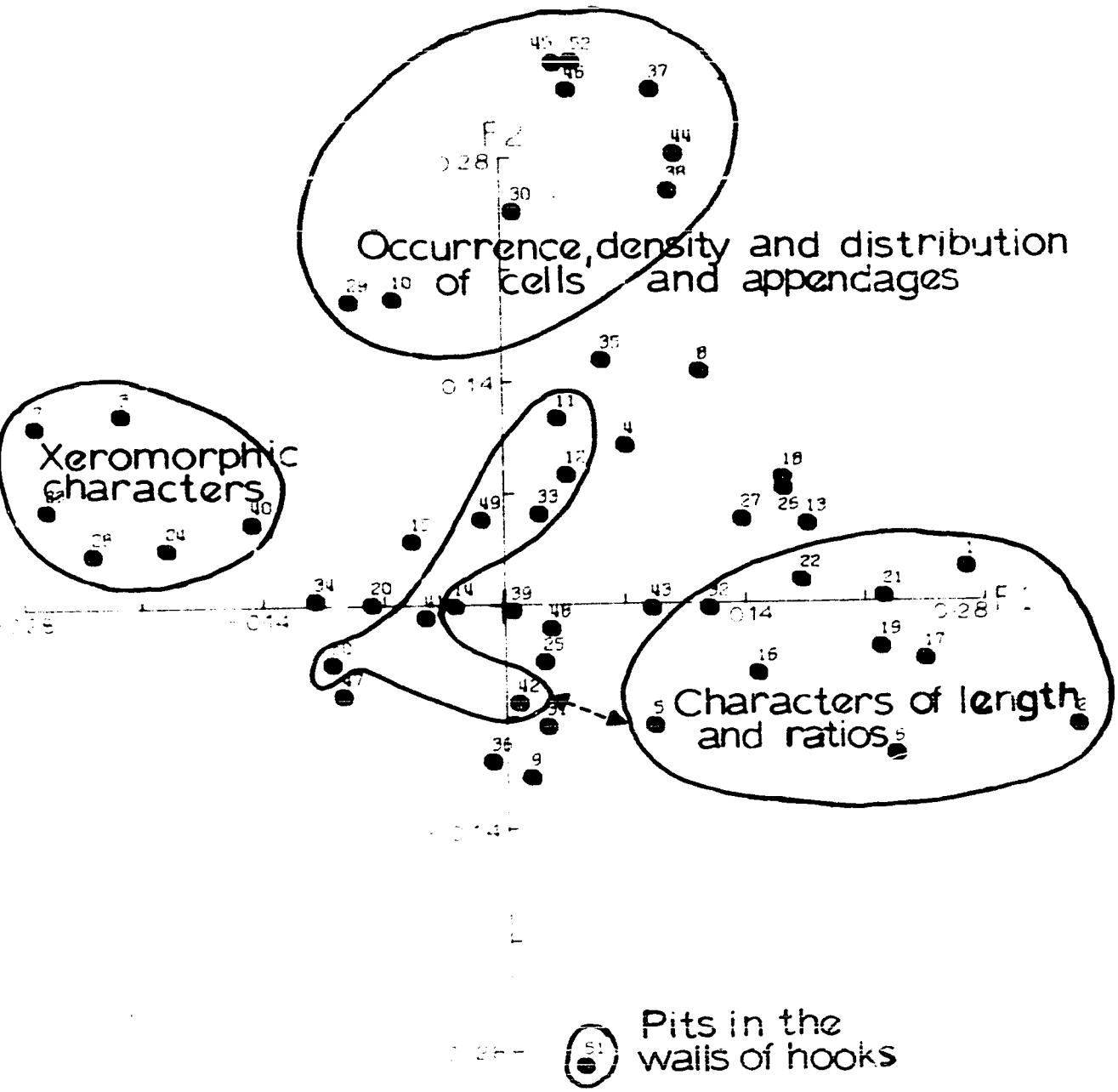


Figure 17: Scatter diagram showing the projection of leaf epidermis characters on the first two vectors produced by component analysis of correlation matrix 105. To identify characters, see Table 40, Appendix 3.

61.68% of the variance of the second root which is 6.77% of the total variance.

III Xeromorphic characters

Some characters which are typically associated with zeromorphic modifications, viz. thickness of the long cell walls (3, 7), density of stomata (23), occurrence of macrohairs (24), and density of prickle-hairs (40), fall into a "group." The type of epidermal cells at the base of the macrohairs (28) also has high negative scores on the first axis. Over 31% of the variance of the first root is accounted for by these characters which amounts to 3.68% of the total variance.

IV Intermediate characters measuring lengths and ratios

In a position on the first axis midway between the group of xeromorphic characters and characters measuring lengths and ratios, one finds the rest of the characters of lengths and ratios. These are the length and ratio for the short cells of the intercostal zone (11, 12), prickle-hairs (41, 42), and hooks (49, 50), and proximal cell length (33). These characters may be partly associated with xeromorphic features but are also similar to other characters of lengths and ratios.

V Other characters

One character worthy of attention is that which registers the presence or absence of pits in the walls of hooks. It has a high negative score on the second axis and accounts for 8.37% of the variance of this root. The taxonomic importance of this character is not obvious.

Of the remaining characters, one may note close associa-

tion between some, such as the type of silica bodies (13, 18), or characters concerning the attachment and type of base of the macrohairs (26, 27). Some of the characters appear to be of little importance. The frequency of macrohairs (25), density of microhairs (31), and length of the prickle-hairs (42), contribute very little to the variance on the first seven roots (Table 19).

Further consideration will be given to these results when the OTU relationships are examined by principal components analysis.

3. Analysis of OTU's

(a) Principal Component Analysis

(i) Correlation Coefficient

The correlation matrix 105 had already been analysed into its characteristic vectors (matrix 108) and, therefore, by use of the adjusted scores (matrix 106) the component scores for the 52 OTU's were produced indirectly by transformation (matrix 110). The first 13 roots in this case account for over 70% of the variance and the first four roots which account for 38.2% of the variance, will be considered further. Scatter diagrams illustrate the projection of the OTU's in the two dimensional space determined by these first four components (Fig. 18 A-D).

Of the three sections of the genus only Pleioneura is more or less separate from the others, and it is close to Rattraya with high negative component scores on the F_1 (Fig. 18A). L. ramosa (50) is the most dissimilar representative of the

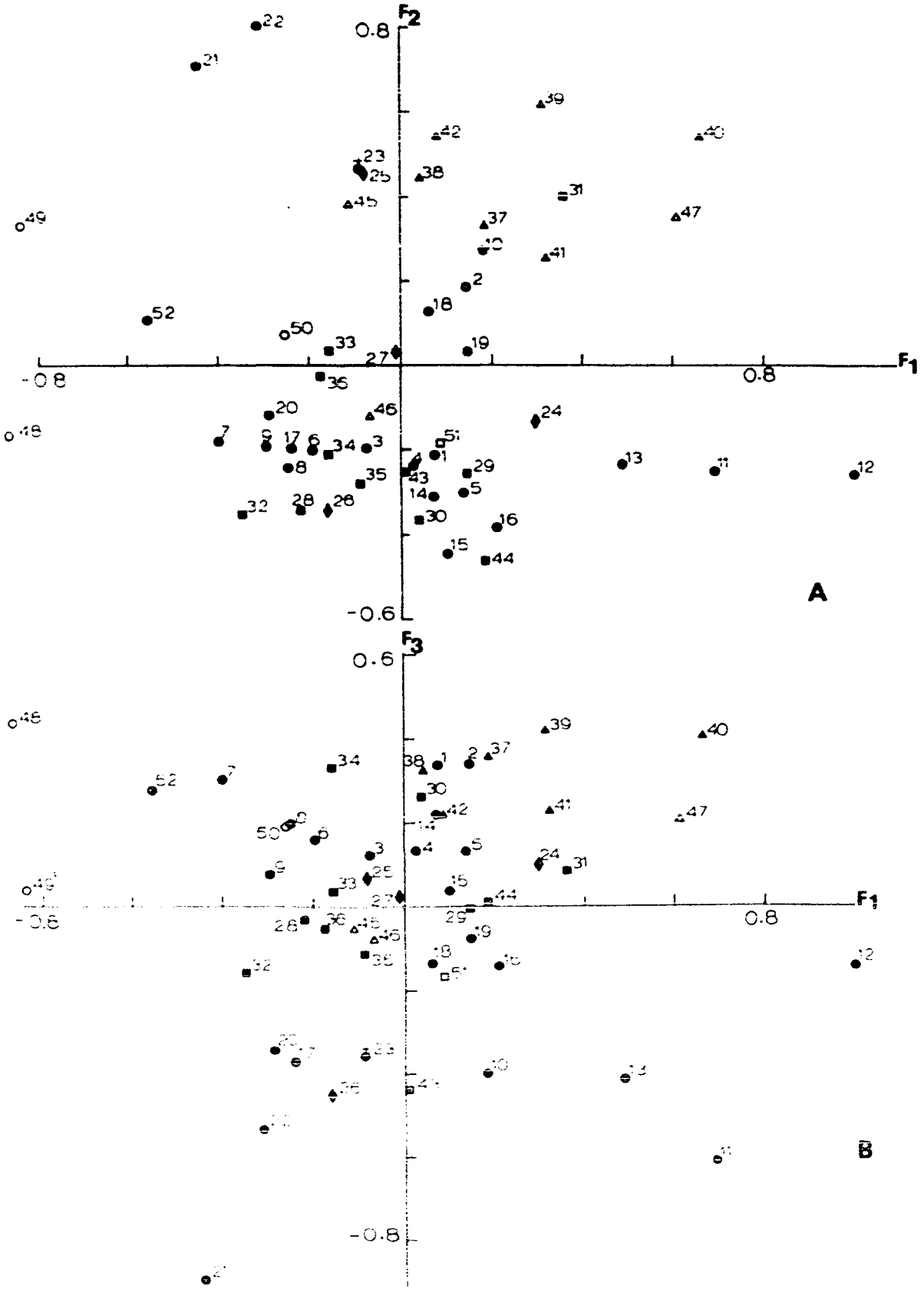
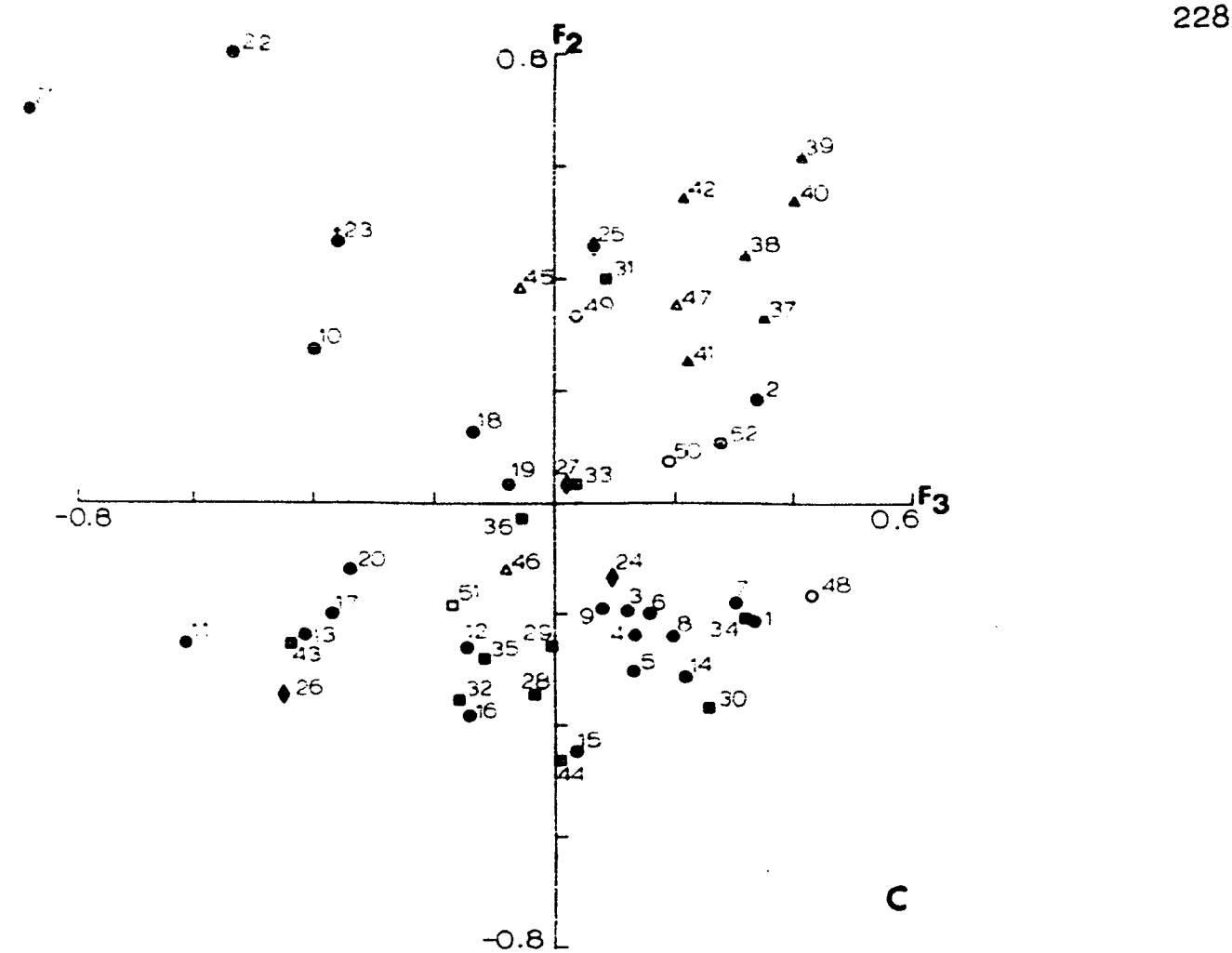
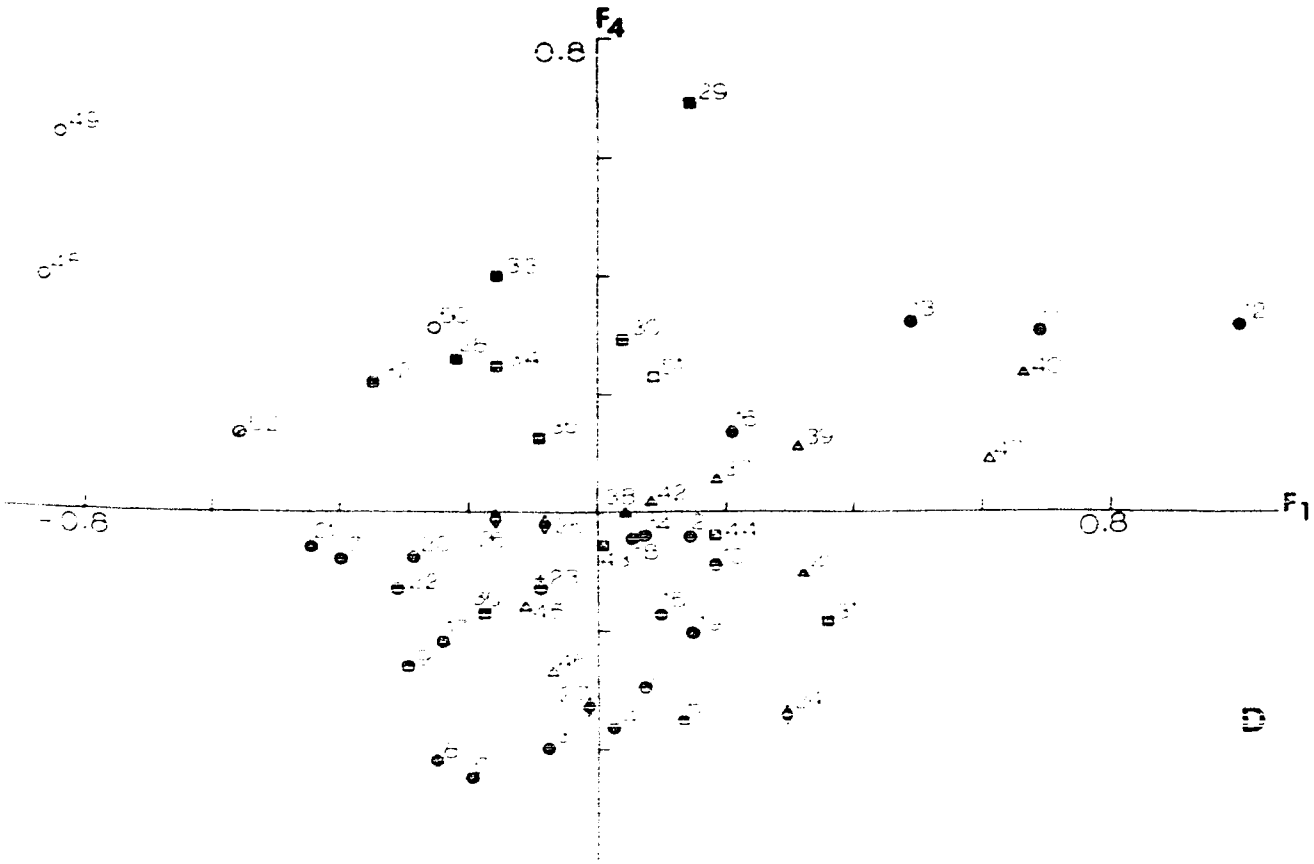


Fig. 10 A-B. Scatter diagram showing the positions of OTU's on the first four components of a principal component analysis of the correlation matrix 105, derived from leaf epidermis characters. For legend see Table II.


F2
0.8
-0.8
F3
0.6
-0.8
C


F4
0.8
-0.8
F1
0.8
D

group. Lophanthera (51) is not separated at all from section Loudetia whereas L. jaegeriana (23) tends to be slightly separate (Fig. 18C).

The subsections of Loudetia do not have many characteristic epidermal features. The Typicae is quite the most variable. L. perrieri and L. madagascariensis (21, 22) are well isolated from other OTU's (Fig. 18A - 18C). At the other extreme are three exemplars of L. simplex subsp. simplex (11, 12, 13). The OTU's of subsection Densispicae again have many features in common (Fig. 18A, 18C). Other subsections such as the Annuae and Flammidae which had similar representatives with respect to leaf anatomy are more diverse when leaf epidermis characters are considered.

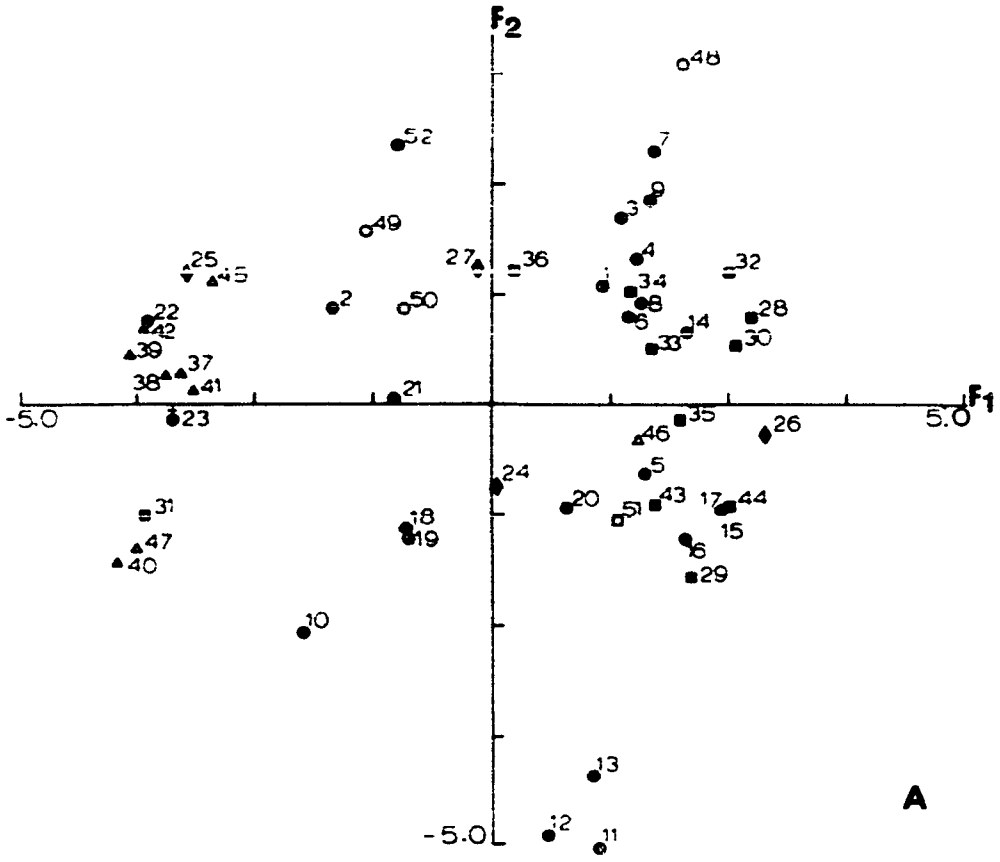
(ii) Dispersion coefficient

The dispersion matrix (104) was analysed into principal components and the roots expressed in Table 13. The first 12 roots account for over 70% of the variance, this being greater than the value arrived at with the correlation matrix. The first four roots account for 39.84% of the total variance. Similar scatter diagrams were produced for this analysis (Figs. 19 A-D).

Although illustrating similar relationships to the previous analysis it will be noted that the section Pleioneura is not now so strongly demarcated from the other OTU's (Fig. 19A). More remote are some representatives of the section Loudetia, three exemplars of L. simplex subsp. simplex (11, 12, 13) having particularly high negative scores on the F_2

TABLE 13: ROOTS OF DISPERSION COEFFICIENT MATRIX (NO. 104)

Root	Value of Root	% of total variation accounted for	Accumulated percentage
1	260.550	14.87	14.87
2	174.543	9.96	24.83
3	132.065	7.54	32.36
4	131.097	7.48	39.84
5	115.454	6.59	46.43
6	87.536	4.99	51.42
7	80.340	4.58	56.01
8	77.217	4.41	60.41
9	67.071	3.83	64.24
10	57.146	3.26	67.50
11	53.187	3.03	70.54
12	47.670	2.72	73.26
13-52	468.708	26.74	100.00
Total sum of squares	1,752.584	100.00	-



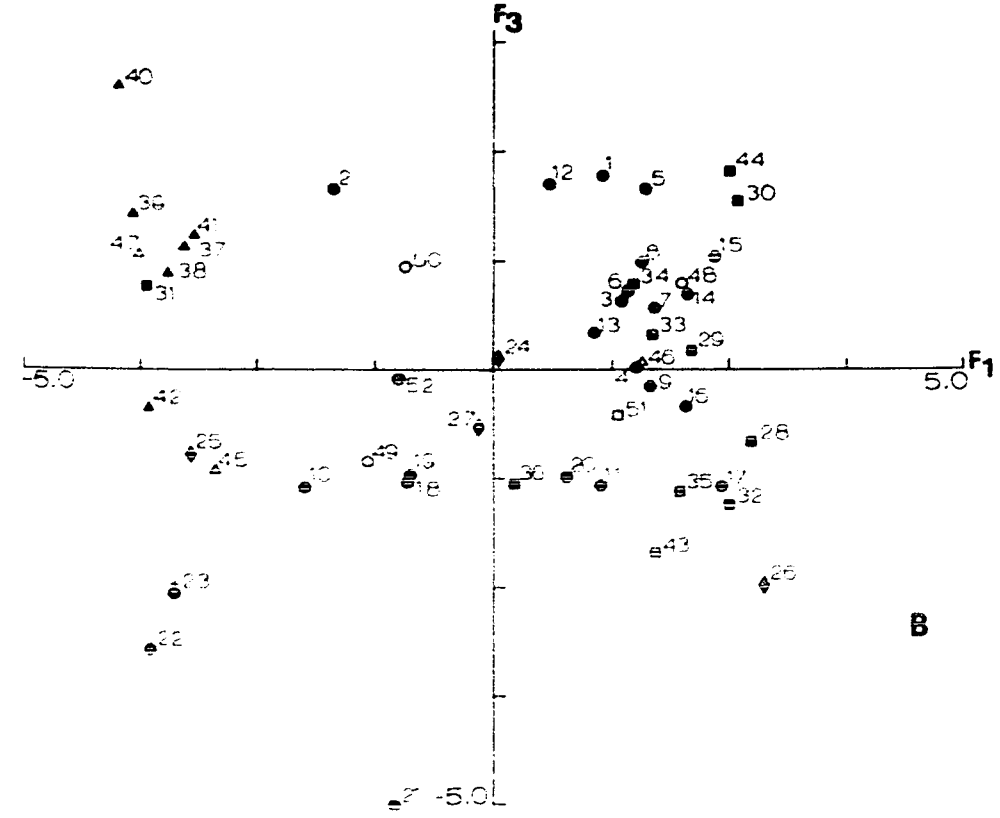
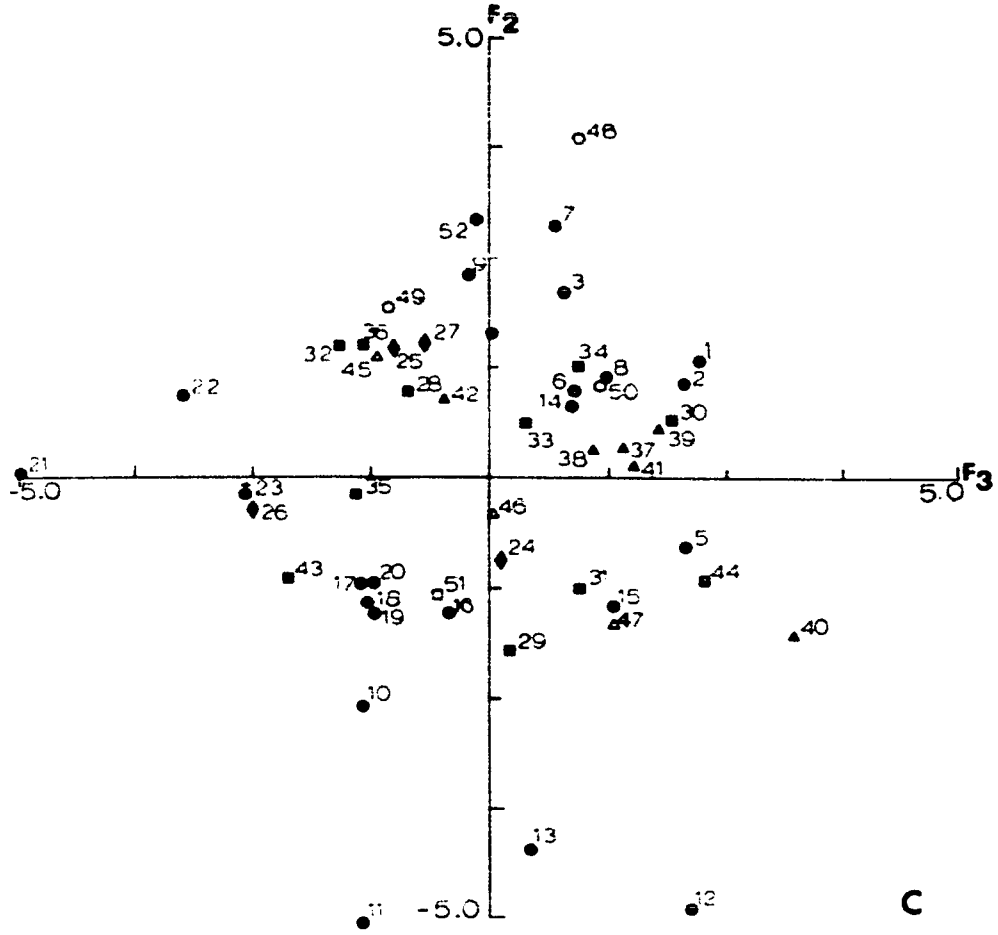
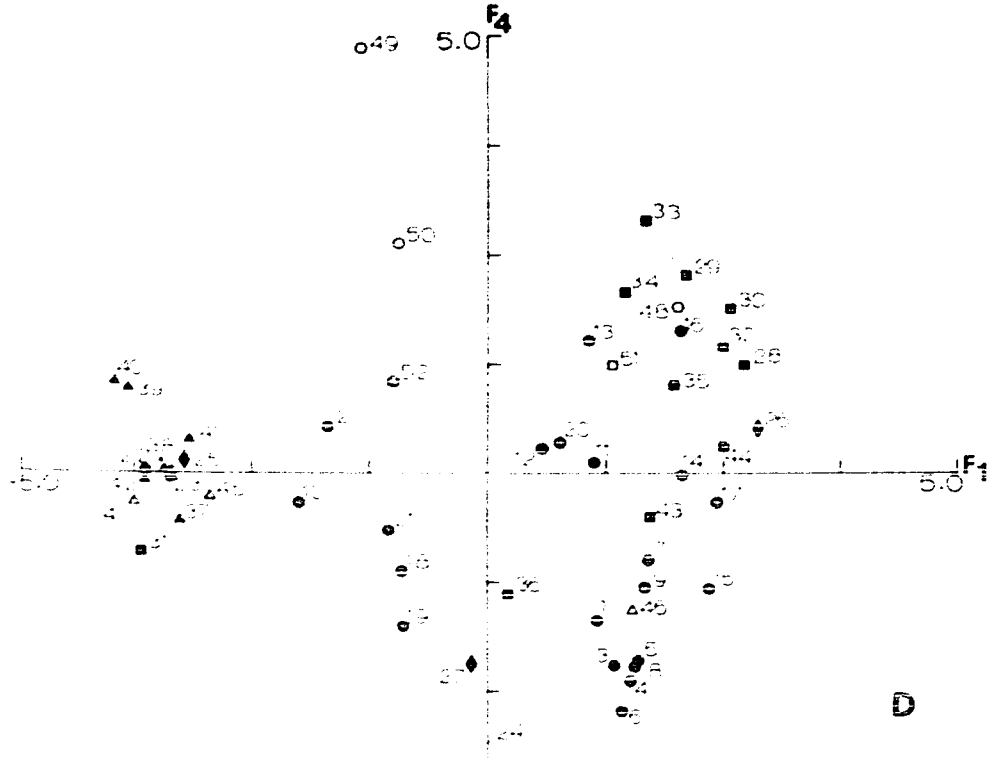


Fig. 19 A-B. Scatter diagrams showing the positions of OTU's on the first four components of a component analysis of the dispersion matrix 104, derived from leaf epidermis characters. For legend see Table 11.


F2
5.0
-5.0
F3
5.0
-5.0
C


F4
5.0
-5.0
F1
5.0
D

(Fig. 19A and 19C). The Densispicae (37-42) all have high negative scores on the F_1 and are "clustered" with L. flammida (45) and L. phragmitoides (47), L. jaegeriana (23), L. madagascariensis (22) and L. flavida (31) which have similar values on this component (Fig. 19A and 19D). The subsection Typicae is again found to be very variable. The OTU's of the L. arundinacea-complex (1-9) form a loose cluster on the F_1 and F_4 except for OTU 2 (L. arundinacea) which is far removed. L. kagerensis (18-20) exemplars are often closely clustered with L. simplex subsp. stipoides (16, 17) (Fig. 19C). Finally one may notice that some of the Acuminatae (28-36) form a cluster on the F_1 and F_4 (Fig. 19D), though some are quite remote from it, e.g. L. flavida (31) and L. filifolia subsp. L. humbertiana (36).

(b) Classification

The classification of OTU's was carried out in the same way as previously, using three different sources of data. Firstly, the component scores (matrix 110) produced from component analysis of the correlation matrix 105 were used to arrive at the absolute distance matrix 113. Secondly, the component scores (Matrix 111) arrived at using the dispersion coefficient were used to develop an absolute distance matrix (114), and finally the raw data matrix 102 was the source for producing absolute distance matrix 103. The sum of squares agglomeration method of cluster analysis gave the dendrograms showing the classification hierarchy of OTU's (Fig. 20 A-B).

(i) Classification resulting from the use of component scores derived from the correlation coefficient matrix, as the data matrix (Fig. 20A)

Although the sections and subsections do not appear to fall into well demarcated groups in the principal components analysis (Fig. 18), the dendrogram at the 15% level shows a number of groups which closely follow the conventional classification:

(a) This group comprises all of the L. arundinacea-complex of the Typicae except for one L. arundinacea OTU (7). L. crassipes (27) and L. filifolia subsp. humbertiana also fall into this category.

(b) The second group contains one subgroup of the subsection Annuae (43, 44) and the section Lophanthera (57) and another subgroup of the L. kagerensis (18-20) exemplars, L. camerunensis (10), L. lanata (24) and L. phragmitoides (46).

(c) On epidermal characters the L. simplex subspecies appear more similar, three of this species occurring together here.

(d) Almost all the Acuminatae are clustered together, although they have with them two odd OTU's, (L. simplex subsp. simplex - 14 and L. demeusei - 26).

(e) Three closely similar exemplars of L. simplex subsp. simplex (11-13) form a tight cluster.

(f) The section Pleioneura (48-50) and Rattraya (52) have the odd L. arundinacea (7) with them.

(g) As has been noted L. perrieri (21) and L. madagascariensis (22) form an isolated group.

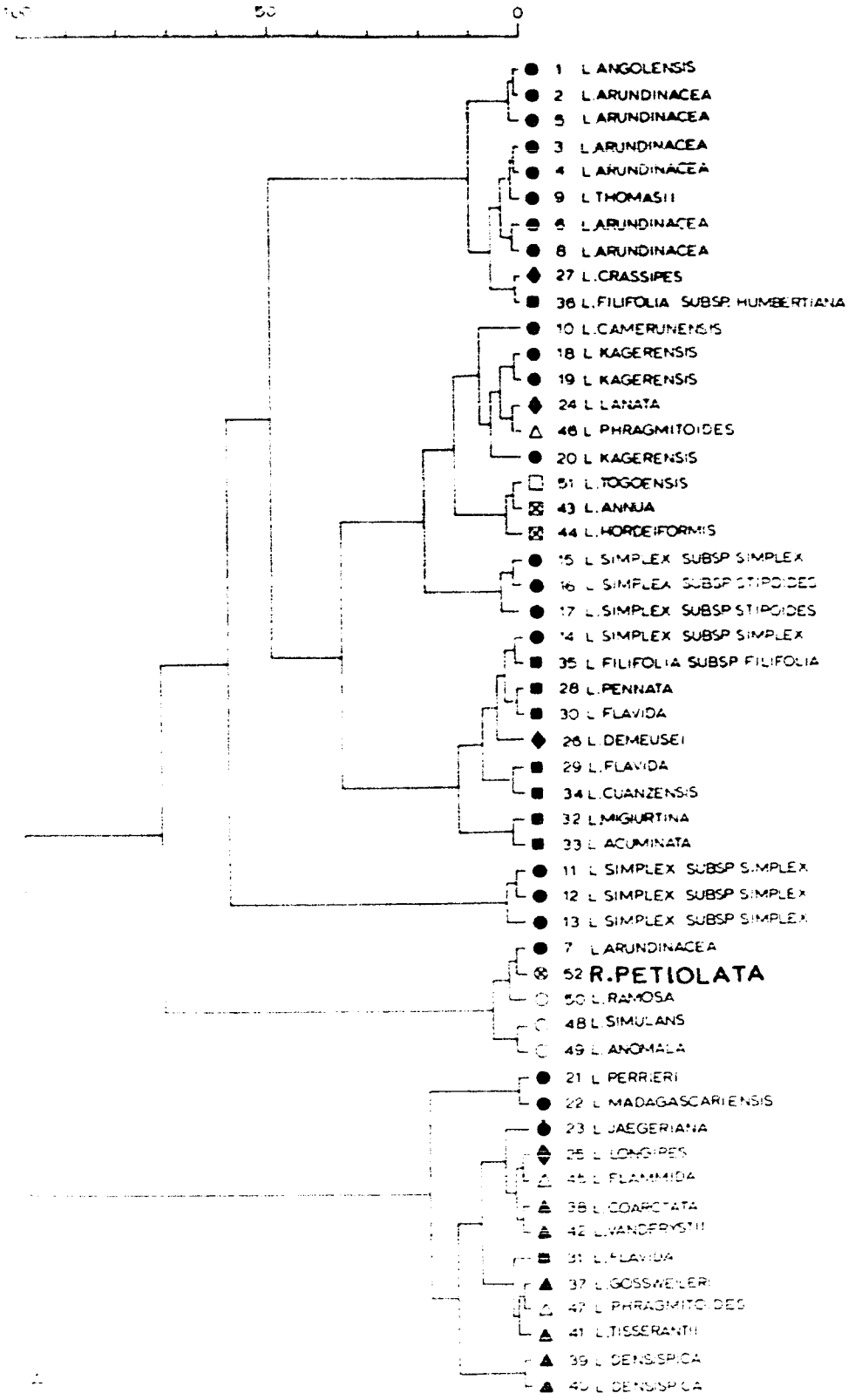
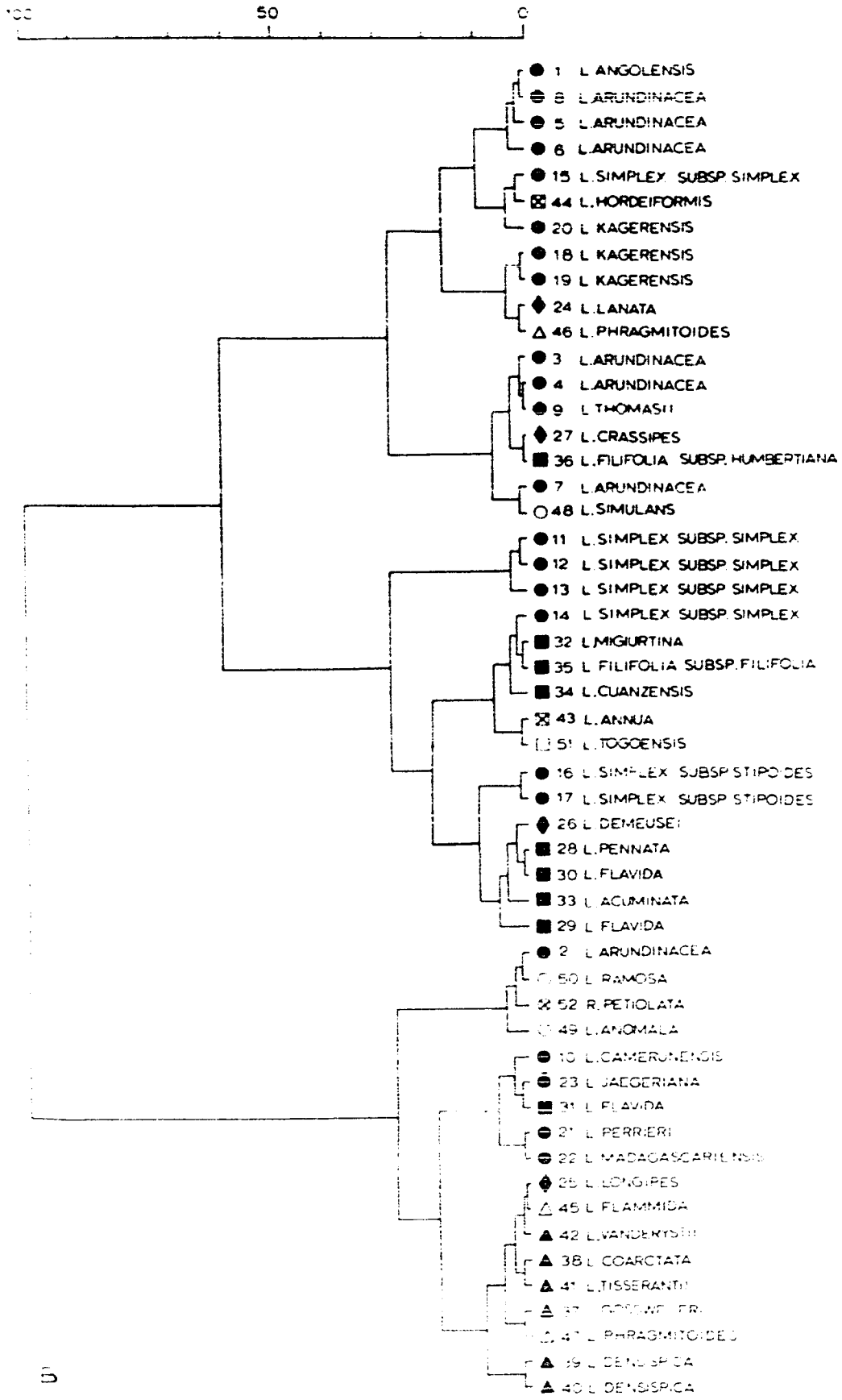


Fig. 2. Dendrograms of 52 OTU's of Loudetia and Hattrava derived from leaf epidermis characters using two different data matrices: A-Component scores matrix derived by component analysis of the correlation matrix; B-Data matrix 102.
Scale indicates the within-group sum of squares expressed as a percentage of the total sum of squares. For legend see Table II.


100
50
0
1 L.ANGOLENSIS
8 L.ARUNDINACEA
5 L.ARUNDINACEA
6 L.ARUNDINACEA
15 L.SIMPLEX SUBSP.SIMPLEX
44 L.HORDEIFORMIS
20 L.KAGERENSIS
18 L.KAGERENSIS
19 L.KAGERENSIS
24 L.LANATA
46 L.PHRAGMITOIDES
3 L.ARUNDINACEA
4 L.ARUNDINACEA
9 L.THOMASII
27 L.CRASSIPES
36 L.FILIFOLIA SUBSP.HUMBERTIANA
7 L.ARUNDINACEA
48 L.SIMULANS
11 L.SIMPLEX SUBSP.SIMPLEX
12 L.SIMPLEX SUBSP.SIMPLEX
13 L.SIMPLEX SUBSP.SIMPLEX
14 L.SIMPLEX SUBSP.SIMPLEX
32 L.MIGIURTINA
35 L.FILIFOLIA SUBSP.FILIFOLIA
34 L.CUANZENSIS
43 L.ANNUA
51 L.TOGOENSIS
16 L.SIMPLEX SUBSP.STIPOIDES
17 L.SIMPLEX SUBSP.STIPOIDES
26 L.DEMEUSEI
28 L.PENNATA
30 L.FLAVIDA
33 L.ACUMINATA
29 L.FLAVIDA
2 L.ARUNDINACEA
50 L.RAMOSA
52 R.PETIOLATA
49 L.ANOMALA
10 L.CAMERUNENSIS
23 L.JAEGERIANA
31 L.FLAVIDA
21 L.PERRIERI
22 L.MADAGASCARIENSIS
25 L.LONGIPES
45 L.FLAMMIDA
42 L.VANDERYSTII
38 L.COARCTATA
41 L.TISSERANTII
37 L.GOSSWEILERI
47 L.PHRAGMITOIDES
39 L.DENSISPICA
40 L.DENSISPICA

(h) The OTU's of subsection Densispicae (37-42) form the bulk of the final group also having two OTU's of the subsection Flammidae (45, 47), L. flavida (31), L. longipes (25) and L. jaegeriana (23) along with them.

The cophenetic correlation coefficient between the distance matrix 113 and the cophenetic values from the dendrogram was 0.374, indicating a very low transfer of information in the production of the classification.

(ii) Classification produced from the raw data matrix (Fig. 20B)

It was found the absolute distance matrix (103) produced in this way had a correlation of 1.00 with the absolute distance matrix (114) derived from the use of component scores (Matrix 111) produced by components analysis of the dispersion matrix (104). The classifications produced were, therefore, identical and this dendrogram can be considered as a classification of the structure as represented in the scatter diagrams (Figs. 19 A-D).

This dendrogram is quite similar to the former, similar groups being apparent at the 15% level. The L. arundinacea-complex is, however, split into two at this level. L. hordeiformis (44) is also separated from L. annua (83), and L. togoensis (51) with which it was clustered. The OTU's of subsection Acuminatae are not all together in one group as they almost all were before. L. simulans (48) clusters with L. arundinacea (7) and not with the other Pleioneura OTU's. The similarities between OTU's of the Densispicae (37-42) are

further illustrated though the Flammidae exemplars (45, 47) still cluster in this position.

The cophenetic correlation between distance matrix and dendrogram was 0.476.

(iii) A comparison of the classifications

The cophenetic values from the dendrograms and the distance matrix values were used in computation of cophenetic correlation coefficients (Table 14). The values obtained for distance and for dendrograms were fairly high.

4. Analysis of the supra-specific groups

(a) Principal Components Analysis

The component analysis of the dispersion matrix (123) resulted in the characteristic roots (Table 15) and component scores for the individuals (Matrix 125). The first three roots account for over 70% of the variation and the plot of OTU's on the first three components illustrates their relative position in R-space (Fig. 21 A-C).

Unlike the results from the use of leaf anatomy characters, the sections are not sharply defined here. Of the section Loudetia, the subsections Acuminatae (3) and Typicae (1) both have high negative component scores on the F_1 (Fig. 21A). The subsections Pungentes (2), Annuae (5) and Flammidae (6) appear close to one another (Fig. 21B), while the Densispicae (4) are more remote. Rattraya (9) and the section Pleioneura (7) show the usual affinity as do L. jaegeriana (10) and the section Lophanthera (8).

Table 14: Cophenetic correlation coefficients produced by comparison of the distance matrices and dendrograms derived from the three different data sources, using leaf epidermis characters.

		Data Source: Component Scores - Correlation Coef. (Matrix 110)	Component Scores - Dispersion Coef. (Matrix 111)	Raw Scores (Matrix 102)	
Dendrograms	Matrix 110	1.000	0.883	0.883	Distance Matrices
	Matrix 111	0.646	1.000	1.000	
	Matrix 102	0.646	1.000	1.000	

TABLE 15: ROOTS OF DISPERSION COEFFICIENT MATRIX (NO. 123)

Root	Value of Root	% of total variation accounted for	Accumulated percentage
1	168.763	40.09	40.09
2	78.999	18.77	58.86
3	51.049	12.13	70.99
4	31.053	7.38	78.37
5	28.983	6.89	85.25
6	22.562	5.36	90.61
7	16.915	4.02	94.63
8	12.686	3.01	97.65
9	9.907	2.35	100.00
10	0.000	0.00	100.00
Total Sum of Squares	420.917	100.00	-

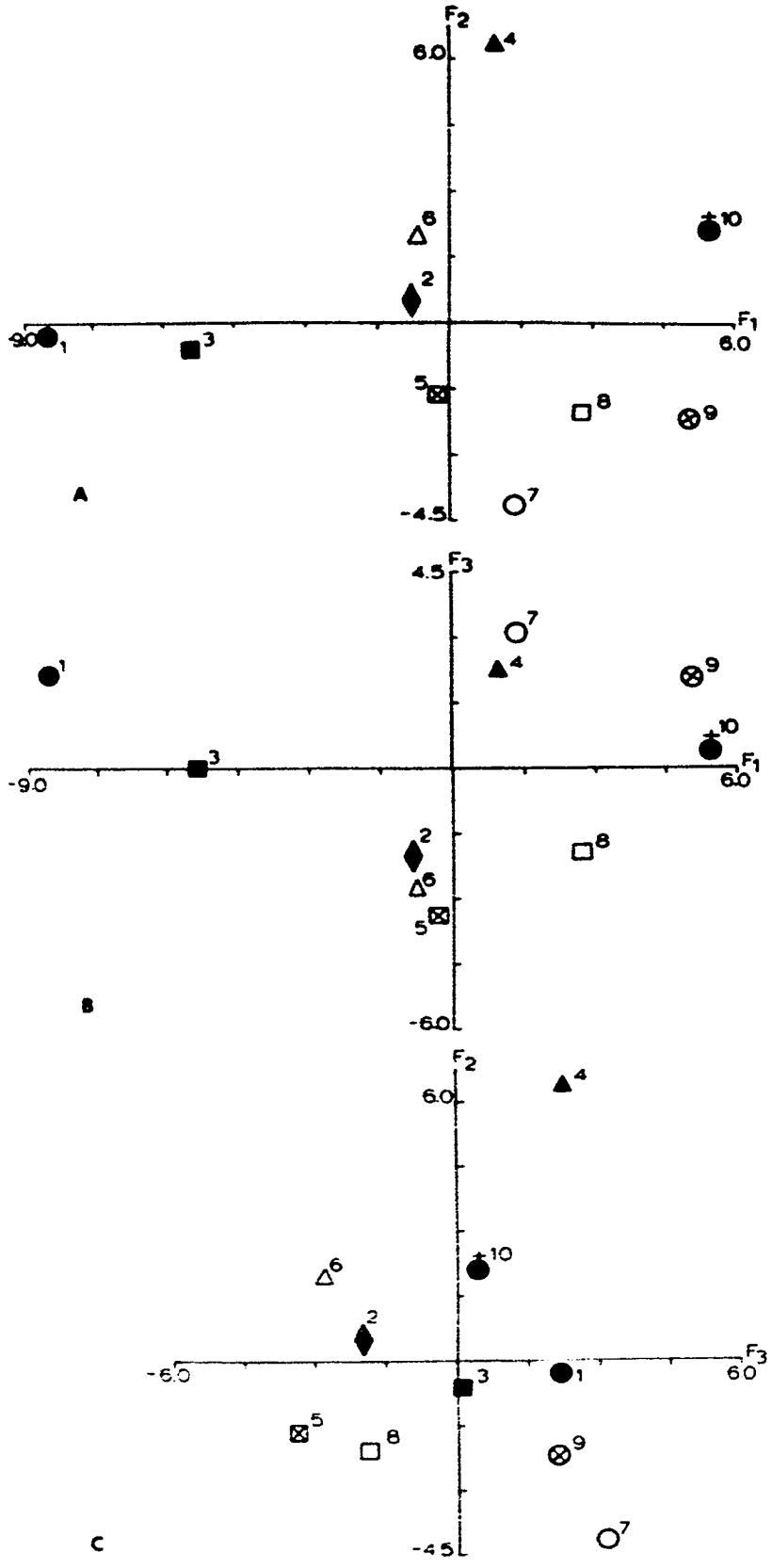


Fig. 21 A-C. Scatter diagrams showing the positions of the supra-specific taxa on the first three components of a component analysis of the dispersion matrix 123, derived from leaf epidermis characters. For legend see Table 11.

(b) Classification

The absolute distance matrix (126) computed from the component scores matrix, was used to produce a dendrogram (Fig. 22) by sum of squares agglomeration.

The clusters formed were readily apparent from the structure shown in the scatter diagrams. The Typicae and Acuminatae form one group, the remainder of the section Loudetia another, and the sections Pleioneura and Lophanthera, Rattraya and L. jaegeriana a third.

D. Discussion

The relative ease in obtaining good leaf epidermal strips from both living and herbanium material, has provided the grass taxonomist with an additional source of characters which may be used for identifying species when flowering material is not available, or for providing additional information in classification. In Loudetia where the leaves of most species appear very similar, 52 characters were obtained from examining the abaxial epidermis alone, so the potential for taxonomic criteria in other taxa, where more variation occurs in the vegetative parts, is very good.

Unlike transverse sections of the leaf, however, the immediate impression of the leaf epidermis as a source of differentia is less apparent. The most striking feature is the presence or absence of the appendages and in fact these may be very important as in the study. Very often the presence or absence of appendages, especially macrohairs, is a feature which frequently varies within a species. Closer examination

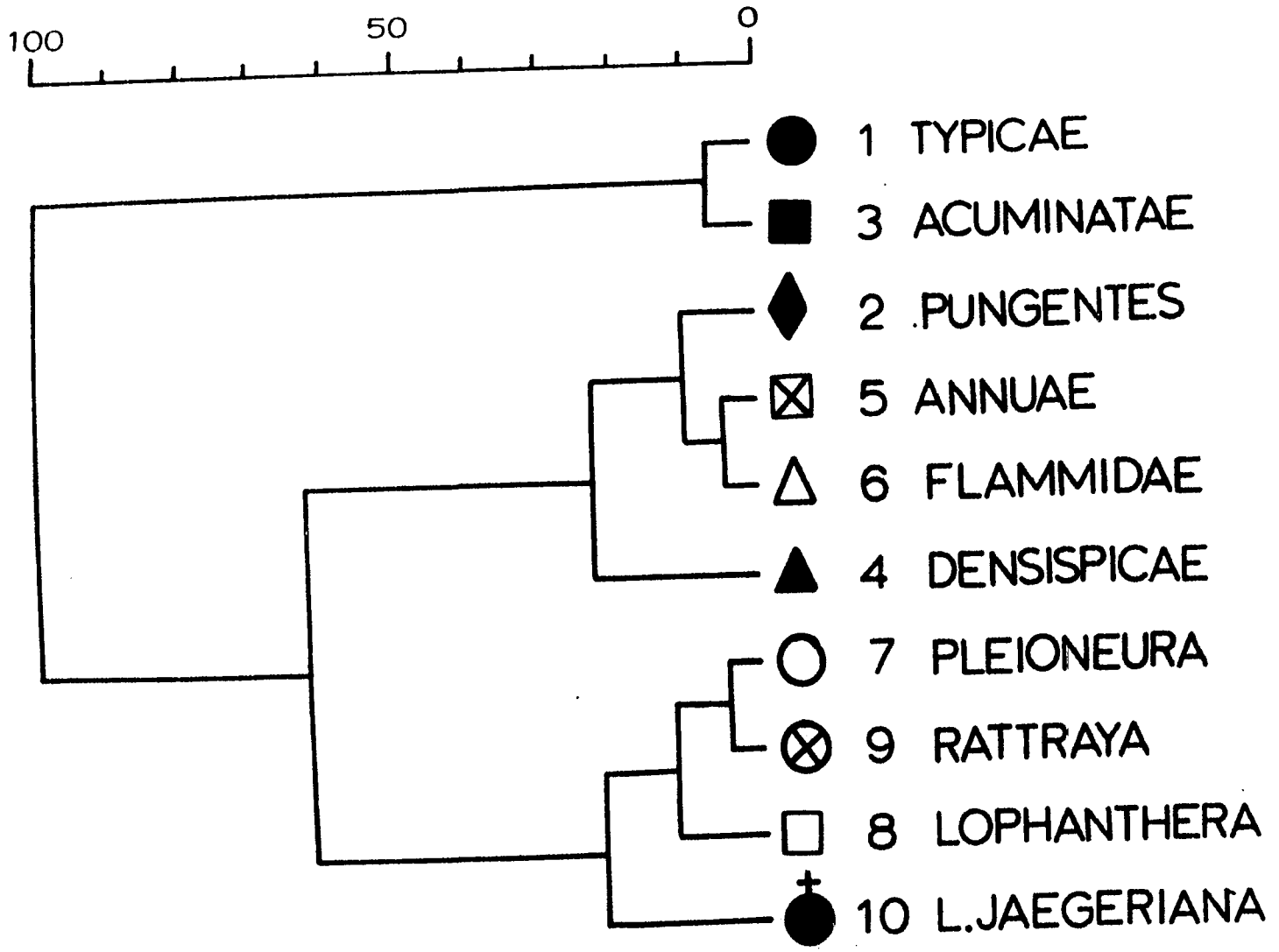


Fig. 22 Dendrogram of supra-specific taxa of Loudetia and Rattraya derived from leaf epidermis characters. Scale indicates the within-group sum of squares expressed as a percentage of the total sum of squares.

of the leaf epidermis reveals the distinct costal and intercostal zones and the difference in cells and appendages in the two regions. The length and width of the long cells was found to be a characteristic useful in classification. The smaller size of these cells in the leaf epidermis of OTU's of Pleioneura was one of the features which enables one to distinguish the section. The complete absence of appendages of any kind was found to occur in some species, e.g. L. perrieri, though it was uncommon for microhairs to be lacking. The lack of prickles and hooks proved to be a characteristic of subsection Densispicae.

Whereas on a subjective basis it is difficult to visualize any groups of OTU's when leaf epidermis characters alone are considered, the results of a multivariate analysis were very promising. The principal components analysis using the correlation coefficient gave strong indications of the importance of different characters. A number of the characters which gave a measure of cell or appendage length, or ratio of length-to-width, proved to be the most important on the first and third components, accounting for some 8% of the total variation. Not all these characters had the same effect, some of them occurring in an intermediate position between the above characters and the xeromorphic characters on the first axis (Fig. 17). These characters were the length and ratio of the cells of the intercostal zone, and prickle hairs, hooks and microhairs, characters which are probably associated to some extent with the xeromorphic characters. The latter include density

of stomata, thickness of cell walls, occurrence of macrohairs, the density of prickle-hairs, and are important with respect to the first root. The main characters in describing variation on the second axis, were those measuring the occurrence and distribution of microhairs, prickle-hairs and hooks. Those characters found to be of very little importance were the frequency of macrohairs, density of microhairs, and length of prickle-hairs.

The effect of the characters in describing OTU relationships was indicated in the component analysis of OTU's (Fig. 18). The OTU's of section Pleioneura and Rattraya are most markedly separated on the F_1 as these OTU's have short broad long cells, small stomata and the differentially shaped cells of the intercostal zone. On the F_2 axis, which was mainly influenced by the occurrence and distribution of microhairs, prickle-hairs and hooks the OTU's of L. perrieri and L. madagascariensis, which have none of these appendages, have maximum positive scores. In close proximity are the OTU's of subsection Densispicae which have no prickles or hooks. In the opposite direction, one finds the three exemplars of L. simplex subsp. simplex (11, 12, 13) which have all appendages including macrohairs, which positions them apart from the other exemplars of the taxon (14, 15).

The results of the component analysis using the dispersion coefficient as similarity measure, reveal similar trends. Some of these are magnified, such as the position of OTU's 11, 12, 13 (Fig. 19A), and the cluster of Densispicae OTU's.

It will be noticed that all the OTU's in this cluster with the Densispicae have no prickles or hooks. L. madagascariensis (22) occurs in this group apart from L. perrieri (21) since the latter species has hooks. Also L. flavida (31) is far apart from the other Acuminatae which always are abundantly supplied with hooks and prickles. The OTU's of subsection Acuminatae are for the most part closely clustered on the basis of leaf epidermis characters because of this fact. L. filifolia subsp. humbertiana (36) is, however, another exception. Also in the same series of figures (No. 18 A-D), it will be observed that the L. arundinacea-complex of the Typicae are close to one another except for L. arundinacea (2) which has no hooks and is closer to the Densispicae group on the F_1.

The classifications that were produced by cluster analysis show results of marked conformity with the classical division of the genus. The exceptions that occur may usually be traced to the complete lack of one or other of the appendages which the "nearest neighbours" all possess. The Densispicae and a few other OTU's are separated from all others in the one dendrogram (Fig. 20A) on this basis. The section Pleioneura has been shown to have a number of characters different from other OTU's but it is interesting to note that one exemplar of L. arundinacea (7) has similarities with that section. A similar situation was found when leaf anatomy characters were considered, and this may indicate a similarity due to similar size of the leaves of all these OTU's. For the most part, the

exemplars of the different species such as L. arundinacea, L. simplex, L. kagerensis, showed greater affinity for one another than for other species. The subsection Pungentes again appears very variable on the basis of leaf epidermis characters. On the other hand the Annuae and Flammidae do show some features in common. Finally, two OTU's which were similar on leaf anatomy characters, viz. L. jaegeriana and L. togoensis, are seen to differ markedly on leaf epidermis characters, because the former lacks both prickle-hairs and hooks.

The results of the analysis based on supra-specific OTU's are vaguely similar to those achieved with the leaf anatomy characters. The two variable subsections of the section Loudetia are, however, sharply defined as a group in the classification. L. jaegeriana comes out closest to Lophanthera in this classification, in spite of the differences already mentioned. The subsection Densispicae is shown to be quite different in the scatter diagrams (Fig. 21A, C).

The cophenetic correlation coefficients do not reveal such a marked difference in the classifications based on different sources of the data. The fact that the distance matrices produced from the raw scores and the component scores resulting from the use of the dispersion coefficient were identical indicates that the structure following principal components is the same as the original. Although the two dendrograms are quite similar, the transfer of information from distance matrix to dendrogram was found to be low in both cases.

As with the results using leaf anatomy characters, it has been shown that strong relationships between OTU's of the subsections and sections of Loudetia previously constructed, do exist when the leaf epidermis is considered as a source of characters. Some features which account for unexpected differences, e.g. the lack of one or another of the appendages, may be strongly influenced by the environment, or may be evidence of infraspecific variation.

E. Summary

The epidermis of the leaf of Loudetia spp. proves to be a valuable source of taxonomic characters. Fifty-two OTU's were examined and 52 characters were scored. Of these, 25 were dependent on the presence of the appendages because they described the appendages in one way or another. The numerical taxonomic results which were obtained from this data illustrated the importance of the different characters and relationships between the OTU's. The component analysis of characters stressed the importance of characters describing the length and ratio of length-to-width of cells and appendages, the occurrence, density and distribution of short cells and appendages, and characters of a xeromorphic nature. Groups of OTU's by principal components analysis and the sum of squares method of agglomeration, were formed mainly on the basis of characteristics mentioned above. The presence or absence of any of the appendages had a strong effect. In general the trends in the classification and ordination emphasized the division of the genus along the classical lines, although odd groups of OTU's

occurred which showed distinctive features. These may be accounted for by sporadic morphological or ecological variation rather than strictly taxonomic relationships. A comparison of the classifications arrived at from the different data sources, showed close agreement. The variability of the subsections Acuminatae and Typicae is apparent from the ordination and classification based on the 10 supra-specific taxa, since they cluster together, separate from the other species. The results of these analyses conform to the results previously achieved with leaf anatomy characters.

CHAPTER 5

THE AWN AND ITS USE IN THE TAXONOMY OF LOUDETIA

1. Introduction

Although the awn of the Gramineae has long been the subject of study in terms of morphology, function and homology, its use in a detailed anatomical investigation for taxonomic purposes is relatively unknown. Previous work on the awn of the Arundinelleae by Jacques-Félix (1950) and Conert (1957) has indicated that in this tribe such an investigation might be useful. With the intention of elucidating some of the relationships between the various taxa of the genus Loudetia, a study of the anatomy and morphology of the awn was therefore initiated. The awn is an integral part of the spikelet, which in many cases is highly specialized, and a study of the spikelet as a whole was necessary as a preliminary step, to help explain many of the features of the awn useful in taxonomy and phylogeny.

Seeing that homology has featured prominently in studies of the spikelet and the awn the current concepts of homology are reviewed. Meeuse (1966) points out that the concept of homology is much older than the theory of evolution. He named the early concept of homology, typological or phenetic homology.

This concept has its basis in mathematics or more precisely, set theory (Mason, 1957; Meeuse, 1966). Organs are considered homologous when they are composed of more or less identical elements or characters. This approach is used by Sokal and Sneath (1963) in numerical taxonomy. Following the advent of the theory of evolution, phylogenetic homology was introduced. The range of similarity between organs which had previously been regarded as a typological series, was now replaced by a phylogenetic series. Although endeavouring to explain historical events, this form of homology is not usually supported by fossil or other evidence. A third type of homology called serial homology, is given by Meeuse (1966). The principle of this concept is based on the assumption that if organs develop in the same way in morphologically corresponding places they are homologous. Although the morphogenetic processes observed in this way may indicate homology between organs, these processes are subject to change and the previously homologous organs may no longer be homologous. Meeuse (1966) states that there is no worthwhile correlation between phylogenetic homology and serial homology. The concepts of homology that will be examined with respect to the spikelet, lemma and awn include all three types.

A. Morphology and homology of the spikelet

A full description of the Loudetioid spikelet has already been given in Chapter 1, and this description may be briefly summarized as follows. The spikelet consists of two flowers, the lower being male or neuter and the upper hermaphrodite,

borne on a short rachilla, each subtended by a palea and lemma, and the whole subtended by two sterile bracts or glumes at the base of the rachilla (Plate 8). When considered in this simple form, the spikelet can easily be conceived as a reduced shoot, the homology being aptly defined by Hitchcock (1950, p.7). The spikelet is described as "a reduced modified shoot in which the rachilla is a stem bearing at each node a reduced leaf (bract). The flowers are secondary reduced shoots borne in the axils of the bracts, the first bract (palea) on the secondary shoot being a modified prophyllum and the stamens and pistil being modified leaves or bracts". This phylogenetic homology has been accepted for some time, though it was originally thought that the lemma and palea together constitute a perianth. The lodicules, which are absent in some grasses, are now commonly regarded as the reduced perianth and the lemma and palea are recognized as a modified leaf and prophyll respectively (Bews, 1929).

Although the phylogenetic homology of the spikelet is generally accepted, more recent work by Sharman (1947, 1960) and Barnard (1955, 1957) on floral histogenesis in the Gramineae, has revealed that this homology is an over-simplification. Barnard (1957) concludes that both the spikelet and the flower are reduced branched systems. The lemma is then a foliar bract which subtends the branch systems of the flower; the palea and lodicules are also foliar structures upon its main axis; the stamens represent lateral branches bearing microsporangia while the gynaecium is composed of fused foliar structures. The

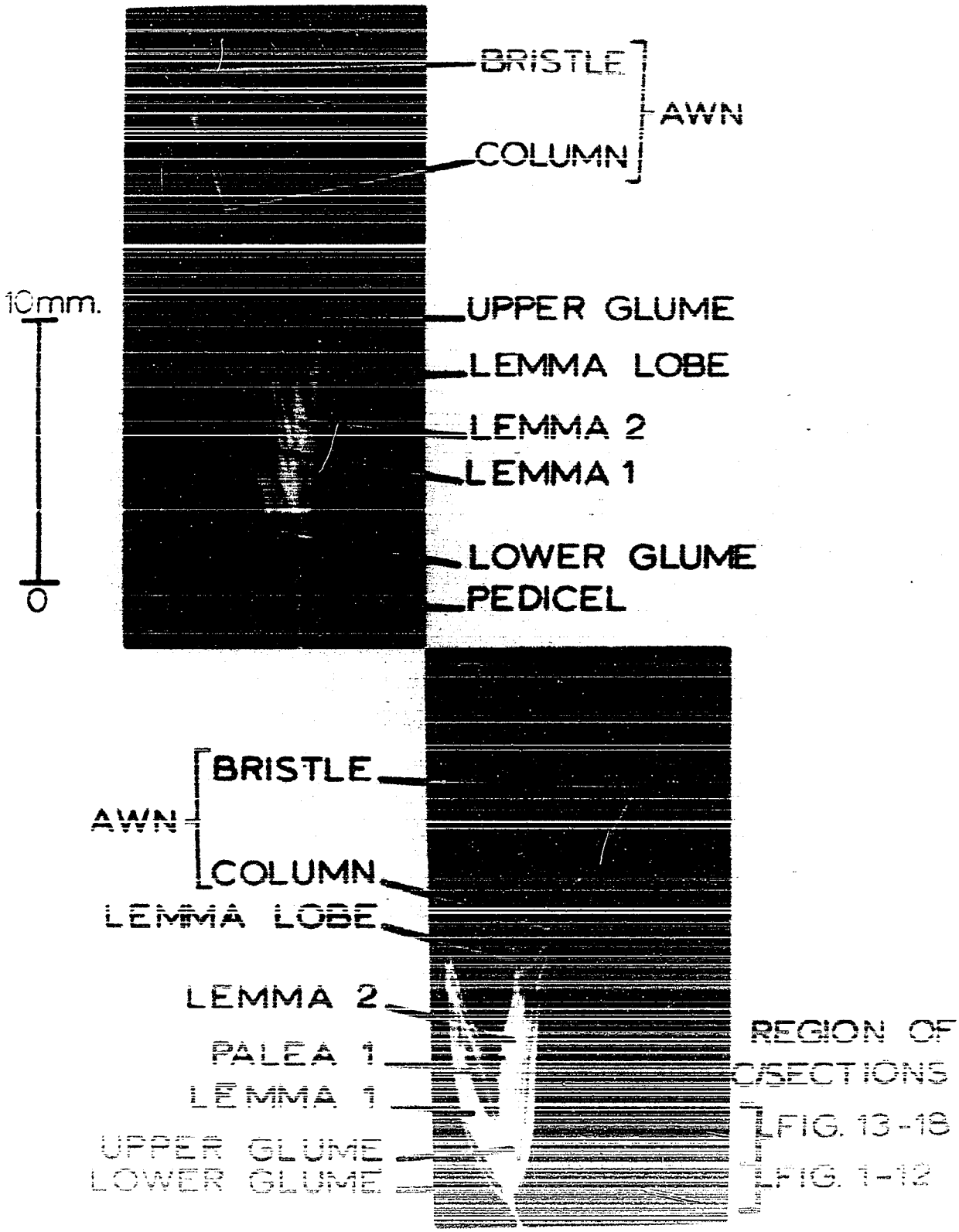
Plate 8

The external morphology of the spikelet.

1. *Loudetia simplex* subsp. *simplex* (8)

2. *Rattraya petiolata* (50)

OTU numbers in parentheses.


BRISTLE
AWN
COLUMN
10mm.
0
UPPER GLUME
LEMMA LOBE
LEMMA 2
LEMMA 1
LOWER GLUME
PEDICEL
BRISTLE
AWN
COLUMN
LEMMA LOBE
LEMMA 2
PALEA 1
LEMMA 1
UPPER GLUME
LOWER GLUME
REGION OF
SECTIONS
FIG. 13-18
FIG. 1-12

evidence for the stamens being homologous with lateral branches and the other floral parts being of foliar nature is based on the similarity in primordia and early development of the organs. These findings do not, however, effect the classical phylogenetic homology in an overall manner, as the spikelet is still regarded as a reduced branch system.

B. The lemma and awn of the upper floret

The fertile lemma with its apical awn is one of the most important parts of the spikelet, being both functionally beneficial to the plant, and of great value taxonomically. Elias (1942) considers the lemma to consist of three parts, viz. from the base up - the callus, the body or lemma proper, and the awn. The callus is, however, usually regarded as a separate entity since it is actually part of the rachilla which disarticulates with the fertile floret. The lemma is consequently thought of as a fertile bract borne on the callus and which may or may not bear an awn (Plate 16).

As with the other floral bracts, the lemma is regarded as being a specialized vegetative leaf. There is a general resemblance between the leaf and the awned lemma; the awn is homologised with the blade, the body of the lemma with the sheath, and the lobes of the lemma with the ligule. This classical homology has developed largely from the work of Duval-Jouve (1871), who was the first to make an anatomical study of a number of grass awns. Two types of awn were distinguished by Duval-Jouve, viz. (a) the complete awn, which is composed of two regions - a basal spirally twisted part,

the column ("colonne"), which is the larger and has approximately the same diameter throughout its length, and a terminal untwisted part or bristle ("subule"), which tapers to the tip from its basal region where it is geniculate with the lower portion; (b) the incomplete awn which does not have the twisted column and is therefore reduced to the bristle. This type may occur on the lemma or glumes, whereas the former occurs only on the lemma.

Duval-Jouve's homology is that the lemma and complete awn represents a vegetative leaf; the lemma corresponding to the sheath, and the superior part to the ligule, the column to the petiole and the bristle to the blade.

This view has been supported by many authors following him, but was attacked by Bugnon (1921) and again by Philipson (1934a & b, 1935). Bugnon (1921) made comparative anatomical studies of the vegetative leaf and lemma and concluded that the whole of the lemma is homologous with the blade of the leaf, the sheath being absent and that the ligule is not homologous with the fused lobes of the lemma.

Philipson (1934a), however, is not in agreement with Bugnon though he feels that the classical theory is not always correct. He made a detailed study of the morphology of the lemma of species which possess a dorsal awn in various states of proliferation.* On this basis he concludes that the apical

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* Proliferation occurs, quite commonly in some grasses, when the spikelets after apparently normal development, once more become active meristematically so that leafy shoots are produced; the rachilla elongates and one or more of the floral bracts develops into leaves (Evans, 1964).

part of the lemma and the awn represent the blade, the basal part of the lemma the sheath, and part of the margin of the ligule may be represented by the lateral lobes of the lemma. Philipson (1934a, 1935) concedes that in species with a large terminal awn such as Stipa pennata L. the awn may represent all of the blade of the leaf, since he did not investigate proliferation of spikelets of species with this type of awn.

The tribe Stipeae contains species with some of the most elaborate awns and Elias (1942) has devoted much of his monograph on the North American Tertiary Prairie grasses to this tribe. He is in general agreement with the classical interpretation of the lemma and awn, though he is doubtful about the column of the awn resembling the petiole as was the view of Duval-Jouve. He considers the lobes of the lemma to be homologous with the auricles of the vegetative leaves and not the ligule. In search of a structure homologous with the ligule, he found a labrum or lip within the crown of the lemma below the base of the awn to be present in some species.

Elias also considered the homology of the callus, the lemma and the palea in some detail. He compared the disarticulated floret with an intravaginal lateral shoot which has been forcefully pulled off. The base of the shoot removed in this way has a sharp point and oblique scar and resembles the callus; the first leaf sheath and the prophyll resemble the lemma and the palea respectively. Elias' account of this

homology is very clear and he attempts to explain every detail, so much so that one feels he has not taken heed of his own advice that "we must certainly also take into account that they (homologous organs) subsequently drifted widely apart in their evolutionary adaptation for the special function which they perform and correspondingly modified their original organization and developed new details".

A most comprehensive work on the awn was produced recently by Tran (1965) in which detailed anatomical studies of the awn and lemma of representatives of a number of the tribes have provided a good basis for the understanding of the evolution of the awn. Tran (1965) also draws attention to the work of Roeper (1826) who originally proposed the theory of homology between the awn and lemma and the leaf blade and sheath, which has erroneously been credited to Duval-Jouve (1871).

Tran (1965) does not distinguish between bristles and complete awns, basing her classification of awns into three morphological types on the longitudinal elements of the aristate lemmas:

(a) Median awns are inserted in the sinus of a bifid tip of the lemma. The Arundinelleae and Andropogoneae fit this category.

(b) Terminal awns appear to continue the apex of the lemma, e.g. Aristideae and Stipeae.

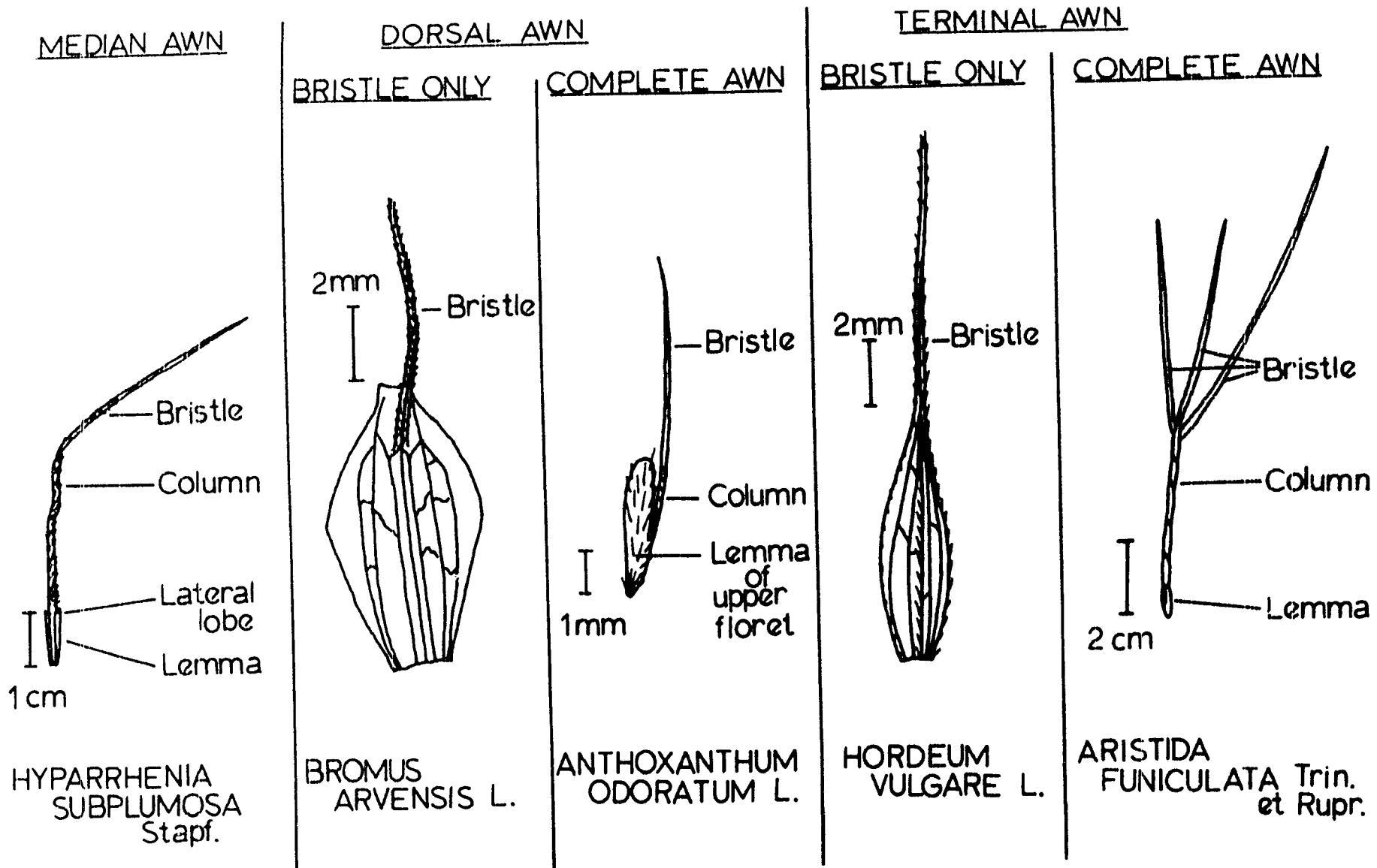
(c) Dorsal awns may be inserted basally or higher up the lemma but they arise from the dorsal side of the lemma e.g. Aveneae.

Some of the awned lemmas studied by Tran are illustrated in Fig. 23.

The median awned lemmas exhibit the three separate longitudinal elements; dorsal awned lemmas indicate that fusion of the two lateral elements has come about, the central element now being dorsally placed; and finally, terminally awned lemmas contain all three elements fused in the awn. Tran's evidence for the presence of three longitudinal elements in the terminal awn is based on the fact that there are three vascular elements in these awns. The lemmas of dorsal awned type have no central vascular bundle above the insertion of the awn and the lateral elements are therefore taken to be fused. The median awned lemma is apparently the most primitive type, the evidence for three elements in the primitive form being that Bugnon (1921) observed three vegetative points at the origin of each lemma. These are consisdered to be the primordia of the three elements, the two secondary lateral elements possibly only becoming active apically, after being raised to this position by the inter-calary growth of the primary median element.

Although Tran (1965) does not account for the homology of the lemma in any way other than this, one assumes that she is in agreement with the theory of Bugnon (1921). The theories on the homology of the lemma and the awn are all of a phylogenetic nature. It is very difficult, if it is at all possible, to present adequate evidence for phylogenetic homology especially as so little is known about floral development. Meeuse

FIG. 23 : THE THREE DIFFERENT MORPHOLOGICAL CATEGORIES OF AWNS
(Redrawn from TRAN, 1965)



(1966) and Sharman (1967) have pointed out that it is not reliable to use abnormalities such as spikelet proliferations to establish phylogenetic homologies. They do, however, indicate the plasticity in morphogenesis (Sharman, 1967), and more work on the genetic control of these abnormalities (e.g. Stebbins, 1965), may provide more useful evidence on homology. The fact that the lemma is of foliar nature has been accepted, though homologies of its various parts with the vegetative leaf are merely speculative.

In summary:

1. The spikelet in the grasses has generally been accepted to represent a reduced modified shoot, bearing modified leaves (floral bracts) at its nodes with florets in the axils of some.
2. The lemma and palea are recognized as a modified leaf and prophyll respectively.
3. There are three principal opinions on the phylogenetic homology of the lemma and the awn:

(a) that the awn represents the blade of a vegetative leaf and the lemma the sheath (Roeper, 1826, modified by Duval-Jouve, 1871).

(b) That the whole of the lemma is homologous with the blade of the leaf (the awn representing the apical part of the blade), the sheath being absent (Bugnon, 1921).

(c) That the sheath of the leaf is represented by the lower part of the lemma, and the upper portion of the lemma and the awn represents the blade (Philipson, 1934).

C. The function of the awn

The theories on the homology and evolution of the awn and the lemma are in most cases unrelated to the function of these structures. In view of the important modifications of the awn and lemma with respect to function, it seems imperative that this aspect be considered before conclusions can be drawn about homology and evolution.

There are three basic functions of the awn of the lemma of the fertile floret, the success of any one of these functions depending on which is most adaptive to the plant in a particular environment:

(a) Physiological functions - respiration and photosynthesis.

(b) Dispersal mechanism.

(c) Seed-burying mechanism.

(a) Physiological functions of the awn have been reviewed by Grundbacher (1963). This review is confined to cereal awns in which about one-third of the tissue is chlorenchyma which is overlain by stomata, and awns are found to have a significant role in photosynthesis and transpiration.

As assimilatory organs awns may contribute more than ten per cent of the total kernel dry weight, the amount that they contribute depending on their size and the environmental conditions. In a warm and semi-arid climate, awns have a favourable influence on grain yield which may be explained in a number of ways. The awns are in a favourable photosynthetic position since they are located far above the foliage leaves, and are relatively free from shading. Awns may also be better

adapted to their xeromorphic structure, than leaves under dry conditions. It has been found that under humid conditions, awns may be heavily attacked by fungi, such as rust and mildew, so that awns would be of a disadvantage under such conditions. Also under humid conditions awned spikelets accumulate water and are subject to lodging. Finally they may be subject to damage by wind in these regions since wind usually accompanies rain.

Compensation for yield would have to be provided by the leaves or other photosynthetic areas when awns are reduced due to unfavourable conditions.

Awns are also an important site for transpiration. The effects of transpiration are unclear. It has been claimed that transpiration in the awn effects the transportation of assimilates towards the developing grain; however, other views are that there is no vital consequence in transpiration (Grundbacher, 1963).

(b) Dispersal of the fruits may be aided by the awn in many species. Disarticulation of the rachilla often occurs at the base of the callus, when the caryopsis is at an advanced state of development, resulting in a fruit with suitable adaptations for dispersal. The grain is very often tightly enclosed within the lemma and palea, and the callus and awn have adaptive features for dispersal by animals or wind.

When fruits are dispersed by animals, the awn has rigid bristles and the callus is sharp pointed so that the fruit becomes attached to the animal's fur. Bessey (1884) has

explained the consequences of dispersal by this mechanism in Stipa spartea, in that the wool of sheep becomes entangled with the fruits.

Fruits that are dispersed by wind are frequently well supplied with hairs on both the callus and body of the lemma. Elias (1942) has illustrated that the awn in the Stipeae may be elaborately branched and feathery.

(c) The hygroscopic seed-burying mechanism is one of the most interesting features of the awn and many authors have devoted their attention to it, those most noteworthy being Hildebrand (1873), Francis Darwin (1876), Murback (1900) and Haberlandt (1914). The ingenious method by which the awn is able to accomplish this feat involves both the gross morphology and anatomy and fine cell structure of the awn.

In species exhibiting this peculiarity, the awn consists of a basal column, which is spirally twisted when dry, and straight when wet, and the narrower distal bristle. When the grain lands on the soil after dispersal, the column is invariably spirally twisted as the awn is dry. Following wetting by rain or dew, the hygroscopic action of the awn becomes apparent. If the awn is suitably placed on the soil, the bristle, which is bent almost at right angles to the twisted column, will be pushed down against the soil surface, consequently, as the column takes up water and untwists the whole body of the lemma and callus revolves and the point of the callus will be forced into the soil. The backward pointing hairs of the callus serve to hold the lemma containing the

caryopsis in the soil and each successive turn of the column will cause the body to move further into the soil. The success of the awn in burying the grain has been discussed by Darwin (1876). The hygroscopic mechanism which causes torsion in the dry column was found to be due to the individual thickened cells. By isolating cells and submitting them to solutions of different concentrations, Darwin (1876) and Murback (1900) have shown that torsion occurs in the cells in the same way as in the awn, and they become spirally twisted in an anticlockwise direction when there is a low content of water.

The essential features in the torsion of the cells is the thickening of the secondary cell wall. Much work has been done on cell wall structure in recent years (e.g. Roelofsen, 1959; Setterfield and Bayley, 1961; Mühlethaler, 1967; among others) and it is reported that the thickening is composed of rows of microfibrils which are orientated in different layers of the cell wall. The microfibrils are subdivided at the submicroscopic level into micelles, which are chains of cellulose molecules, these being orientated in the same direction as the microfibrilles. In fibres of monocotyledons it is reported by Roelofsen (1959) that the bulk of the microfibrils are orientated in a *Z-spiral. The angle of the spiral with respect to the long axis of the fibre varies in different species and different fibres.

* The direction of the spiral is now commonly designated as Z or S rather than clockwise and anti-clockwise.

When water is incorporated into the cell wall inter- and intrafibrillar swelling occurs (Roelofson, 1959). As the fibrils are mostly arranged in a Z-spiral the resulting forces increase the diameter of the cell and also, by being resolved in different directions on either side of the cell produce torsion. The torsion which occurs in the individual cells coupled with the fact that the thickening is produced eccentrically to the outside of the cell, accounts for the torsion and anticlockwise twisting of the column (Murback, 1900). Although many studies have been made on the mechanics of cells of woody plants (e.g. Côté, 1965; Mark, 1967) apparently little work has been done on the hygroscopic cells of the awn with the view of explaining this mechanism. Seifriz (1952) states that, since it is impossible to twist old cellulose without rupturing it, spirality must be established when the tissue is young, probably when it is formed. As a result of many observations on Myxomycetes he concluded that spirality is a property of the protoplasm, and a state of torsion is established when the protoplasm is in motion. It is conceivable, therefore, that the property of torsion is an inherent characteristic of the cells of the column. It is most probable that the nature of these cells is determined at the time that the thickening is formed.

All of these functions of the awn are not exhibited in perfection in any one species. In many cereals (e.g. wheat, barley, rye) the awns are sites of physiological activity and

are of the bristle type and not adapted for dispersal or seed-burying. In other grasses, awns are more likely to be more efficient in the latter two functions although the physiological role may be present.

D. The use of the awn in taxonomy

The awn has rarely been considered as an important structure in itself with anatomical and morphological characters useful in classification. Some authors have found that because of its great diversity and specialization it is of great advantage as a taxonomic feature (e.g. Duran and Rossengurtt, 1953; Launert, 1965; Sedulsky, 1966; Baum, 1968). Elias (1942) found the awn most useful in studying fossil grasses since it is one of the few structures which is preserved.

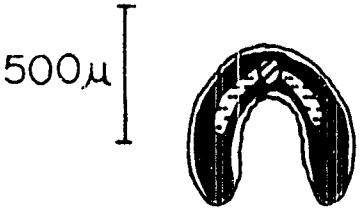
Tran's (1965) work on the systematics of all the grasses using awn characters is worthy of special mention. In addition to the morphological types, Tran distinguished four different anatomical categories of awns (Fig. 24):

(a) Awns where there is a lack of chlorenchyma, and both bristle and column are very highly sclerified. e.g. Arundinelleae and Andropogoneae.

(b) In the Festuceae and Hordeae the awn (or bristle) is composed entirely of parenchyma (usually chlorenchyma) surrounded by a thickened epidermis and sclerified zone. Two species illustrated by Tran perhaps also fit this category, viz. Anthoxanthum odoratum L. and Alopecurus myosuroides Huds., since they both completely lack any sclerification and the

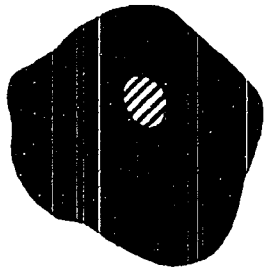
FIG. 24: THE DIFFERENT ANATOMICAL TYPES OF AWNS; TRANSVERSE SECTIONS THROUGH THE MIDDLE OF THE BRISTLE OR COLUMN (redrawn from TRAN, 1965)

ALL TISSUES SCLERIFIED



COLUMN

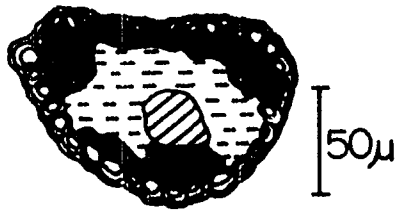
HYPARRHENIA SUBPLUMOSA Stapf.



BRISTLE

TETRAPOGON SPATHACEUS (Hochst. ex Steud.) Hack. ex Th. Dur. et Schinz.

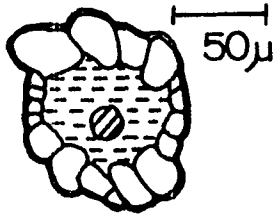
PARENCHYMA AND EXTERNAL SCLERENCHYMA



BRISTLE

BROMUS ARVENSIS L.

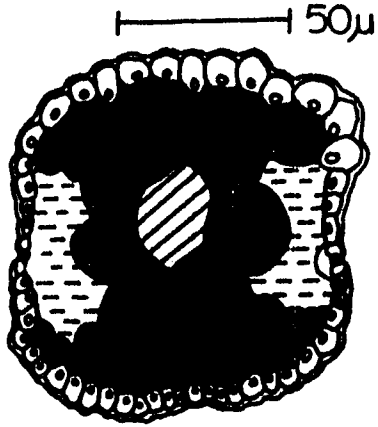
ENTIRELY PARENCHYMA



COLUMN

ALOPECURUS MYOSUROIDES Huds.

TWO LATERAL GROOVES OF PARENCHYMA



COLUMN

LAGURUS OVATUS L.

RADIAL CHLORENCHYMA

BRISTLE

ARISTIDA FUNICULATA Trin. et Rupr.

KEY

SCLERENCHYMA

CHLORENCHYMA

VASCULAR TISSUE

epidermis is not thickened. Tran does not mention this type.
(c) A third type of anatomical structure is that where most of the awn is sclerified except for two lateral grooves of parenchyma which may be chlorenchymatous, e.g. Agrosteae, Aveneae, Stipeae.
(d) Finally awns which have radically arranged chlorenchyma around the vascular bundles are considered as a separate type. e.g. Aristideae and Paniceae.

Using both the anatomical and morphological types, Tran suggests several large groupings of the tribes. The first criterion is the type of chlorenchyma found in the awn. One sees a range from the generalised structure to the development of the radial chlorenchyma. The second factor in the division of these groups is "le mode de coalescence des eléménts . . . dans les lemmes" in which the medianly inserted awn is regarded as the type from which the lemmas with dorsal or terminal awns were derived. A comparison of the affiliation of the tribes based on awn anatomy is compared with that of leaf anatomy, and close agreement is seen. Tran (1965) also compares this system of the Gramineae with those of Prat (1960) and Auquier (1963) based on other characters and also finds great similarities in the classifications.

While agreeing with Tran on many points of this systematic treatment, research on the awn of Loudetia and the Arundinelleae in general, has indicated that it involves an oversimplification both in the classification and evolution of awn. The morphological classification of awns is in many cases

inconsistent within tribes for there appears to be an overlap particularly between terminal and median awned lemmas. The anatomical classification is not developed further when the classification of tribes is considered because it is quite artificial and does not relate to tribal affinities. The anatomical classification, therefore, appears to be irrelevant. The main point, which has been entirely neglected by Tran, is that the awn is a highly specialized organ which has many adoptive features acquired through selection for a specific function. Finally the homology of the awns and lemmas that is implied in Tran's work is not clear and no hypothetical basis for an understanding of the evolution of the awn is given.

Work on the anatomy of the awns of the Arundinelleae began with a study made by Jacques-Félix (1950). He proposed the subdivision of the tribe into two subfamilies, the Arundinellées and Trichopteryxinées mainly on the basis of awn and lemma characters. The consequence of these changes have already been discussed in the review of the literature of the tribe (Chapter 1). Although Jacques-Félix's argument was not sound in this case, he did show that there are two different types of awns in the Arundinelleae, those which appear as an inverted horseshoe in cross-section and are tightly twisted when dry, and those more flattened in cross-sectional shape and spiral when dry. Other anatomical characters of the awn appeared to be of use and Conert (1957) was encouraged to examine the awn anatomy of the Arundinelleae. His work res-

sulted in a number of changes, the most important with respect to awn anatomy being that Tristachya sensu stricto was distinguished by a large awn of many layers of cells. The work of Conert (1957) did not disclose much with respect to the awn anatomy of Loudetia, so it was felt that a more thorough analysis of the awn anatomy was warranted.

Tran (1965) studied one species of Loudetia, viz. L. togoensis which has an anatomy somewhat like members of the Andropogoneae studied by Tran (1965) and Duval-Jouve (1871). Tran gives an illustrated description of the anatomy of the awn of L. totoensis as seen from transverse sections from the junction with the lemma to the summit of the bristle.

E. Objectives of this study of the awn

At the outset of this study the aim was to distinguish suitable characters which could be used in a numerical taxonomic study of the group. Previous authors had indicated that the column was the most important part taxonomically so its anatomy was examined in detail with the object of classification. However, differences are not confined to the column and, therefore subsequent studies were made on the anatomy of the complete awn of some species and the morphology of the awns of all the representatives were examined in greater detail. An examination of serial sections of the spikelets proves particularly useful in investigating the differences in the morphology of the spikelets of different species (Arber, 1934), so serial sections of spikelets of some representatives of Loudetia were made.

A study of the function of the awn of Loudetia has revealed much of the perfection of this organ especially as a mechanism for burying the grain. Some of the theoretical aspects of this function when applied to awns of different shapes and anatomical structure will be considered. These factors are very important in the evolution of the awn and the lemma as a whole, and should be considered when phylogenetic relationships are examined. The objectives of this study may be summarized as follows:

(1) To detect what relationships exist between the representative OTU's of the genus Loudetia using characters of the awn in a numerical taxonomic study. The importance of the characters will also be considered.

(2) To examine the morphology and anatomy of the awns of the species of Loudetia with respect to their function, so as to help explain the evolution of the awn and consequently phylogenetic relationships between the species.

2. Anatomical and Morphological methods

The specimens examined are listed in appendix 1 (see Chapter 2). Awns were selected from spikelets just prior to anthesis. Those from herbarium specimens were softened in the same way as the leaves and fresh material was killed and fixed in F.A.A. (see Chapter 4).

The awns are often small and difficult to handle, thus a method used by Decker (1965, personal communication) for handling grass caryopses was adapted for use here. The awns were embedded in agar in a petri dish, either singly or in

pairs. Increasing concentrations of ethyl alcohol were added to the petri dish at 2 hour intervals and when in a 70% concentration, cylindrical sections of agar containing the awns were cut out. Dehydration was completed using the tertiary butyl alcohol schedule of Johansen (1940) and the agar blocks were embedded in paraffin wax. By adding a few grains of erythrosin to the 75% or absolute tertiary butyl alcohol the agar was stained and could be more easily observed in the paraffin wax.

The awns are very hard, so that the sections tend to fragment unless the embedded tissue is softened. Various methods of softening hard material are reported in the literature and those used on Loudetia awns are given in Table 16. Some other methods not attempted are those of Larkin et al (1952), which is especially for grain kernels, Gamapathy and Palser (1964), Burkart (1966) and Ayensu (1967).

Some of the techniques proved successful, the mixture containing glycerine and hydrofluoric acid being the best. Maze (1966, personal communication) who has studied Stipa awns anatomically, recommended soaking the paraffin blocks in three parts 70% ethyl alcohol and one part glycerine for about a week. This method was modified to include one part 25% hydrofluoric acid. The mixture penetrates the material and removes the pink erythrosin stain from the agar so it is obvious to what depth the material has been treated.

Sections were made on an American Optical rotary microtome at a thickness of 10-15 microns. The paraffin ribbons

Table 16: Methods for softening hard material embedded in paraffin wax.

Schedule	Solution	Time Period	Temperature	Comments in source	Source
1.	10-15% hydrochloric acid	3-7 days	Room temp. ±22 C or 32 C oven	If left too long, material may show signs of maceration	Foster and Gifford, 1947
2.	20 ml glycerine 80 ml 70% ethyl alcohol followed by 10% hydrofluoric acid	1 week	''	Usually yields smooth ribbons because of lubricating action of glycerine	''
3.	10 ml glycerine 10 ml hydrofluoric acid 80 ml 95% ethyl alcohol	3 days - 2 weeks	''	'' recommended for general use	''
4.	10 ml glacial acetic acid 90 ml 60% ethyl alcohol	2-5 days	Room temp. or 35-37 C oven	recommended for general use	Gifford, 1950
5.	20 ml glacial acetic acid 80 ml 60% ethyl alcohol	''	''	recommended for relatively hard material	''

6.	10 ml glycerol 90 ml aqueous solution 'Dreft'	2-3 days	37 C oven	-	Alcorn and Ark, 1953
7.	<u>Stock solution</u> Ethyl alcohol 30 parts Glycerine 10 parts Acetic acid 3 parts Hydrochloric acid 1 part - Stock solution 5 parts Water 2 parts - Stock solution 4 parts Water 1 part	 4-5 days ''	 - -	 For young material For older material	Reeve, 1954

were mounted on microscope slides with Haupt's adhesive and stained using the safranin fast green procedure of Johansen (1940). Decker (1965, personal communication) recommends that the agar be removed from the slides but as this was difficult and was not attempted unless it was loose and liable to cover the sections.

The slides were examined with the aid of an Olympus EH binocular microscope and photographs taken using an Asahi Pentax Spotmatic camera on Kodak high contrast copy film.

The spikelets and awns were examined with a Bausch and Lomb dissecting microscope. Photographs were taken of the awns and spikelets using an Asahi Pentax Spotmatic camera on Kodak Plus X film. Some of the photographs were taken with the aid of a Wild dissecting microscope and others using the camera fitted with the f 1.8 lens and close up rings alone.

3. Detailed Morphology of the Spikelet

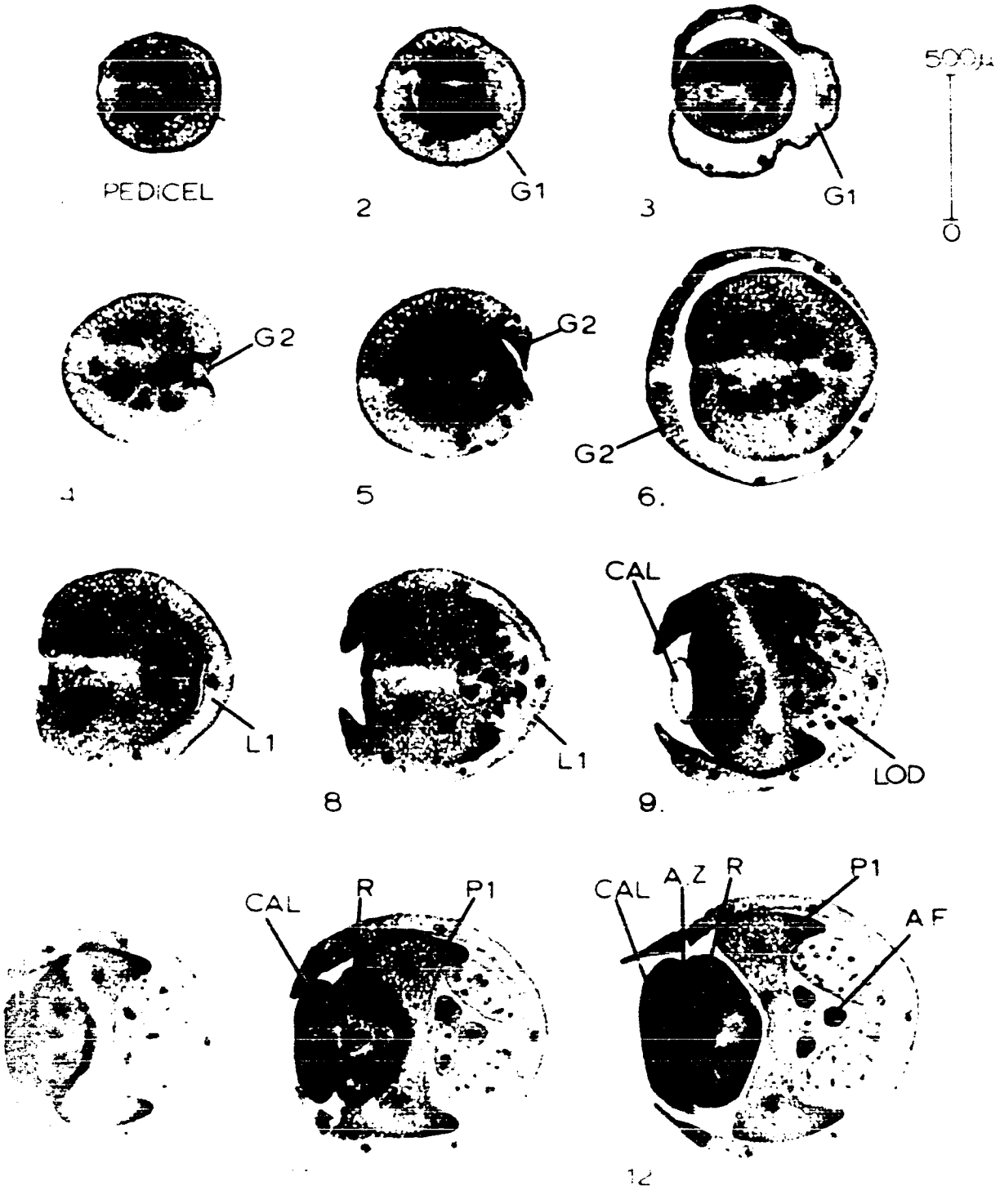
The morphology of the spikelets of a number of different species was examined. Rattraya petiolata (Plate 8) is used here as an example, the photographs in plates 9.1 to 9.18 showing selected sections from the pedicel, just below the first glume to part-way up the second floret. The lower glume (G1) is the lowermost bract (Plate 9.1-3). It has 5 vascular traces, evident first in the periphery of the pedicel (9.1) and then in the glume itself (9.2-3). These are the 5 vascular bundles or nerves of the glume. Higher up the upper glume (G2) has vascular traces connected with the central axis or rachilla (9.4-6). Although there are apparently 9 vascular

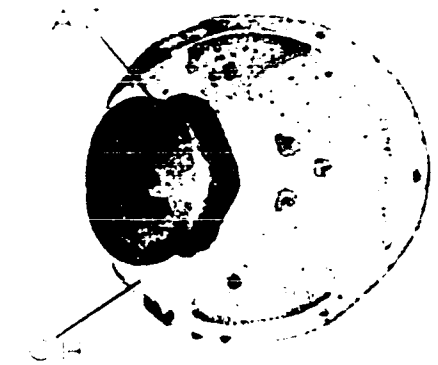
Plate 9.1 - 9.18

Cross-sections at intervals through a spikelet of **Rattraya petiolata** (OTU No.

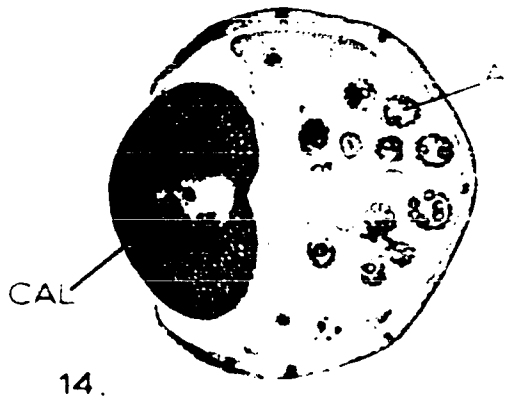
Legend

A - anther	G2 - upper glume
A.F. - anther filament	L1 - lemma of the lowe
A.Z. - abscission zone	L2 - lemma of the uppe
CAL - callus	LOD - lodicule
C.H. - callus hairs	P1 - palea of the lowe
G - gynoecium	P2 - palea of the uppe
G1 - lower glume	R - rachilla

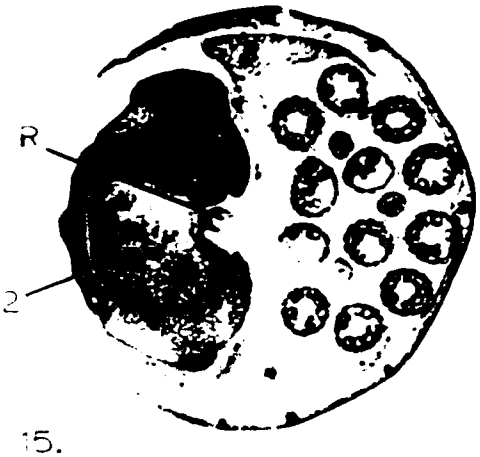

PEDICEL
2
G1
3
G1
500µ
0
G2
4
G2
5
G2
6.
L1
8
L1
CAL
LOD
9.
CAL
R
P1
CAL
A Z
R
P1
A F
12



13.



14.



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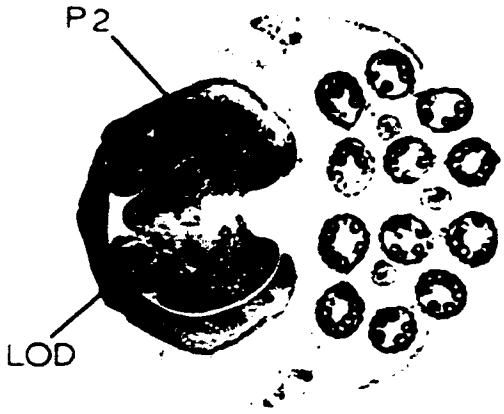
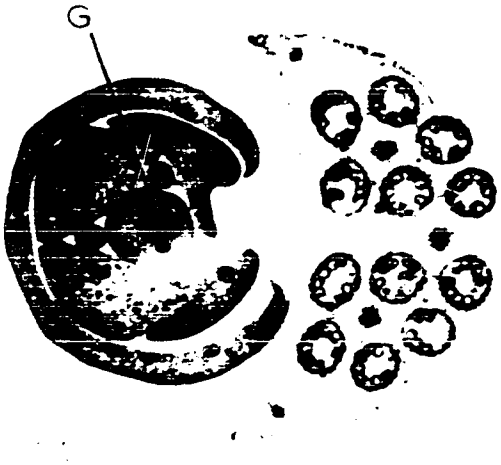
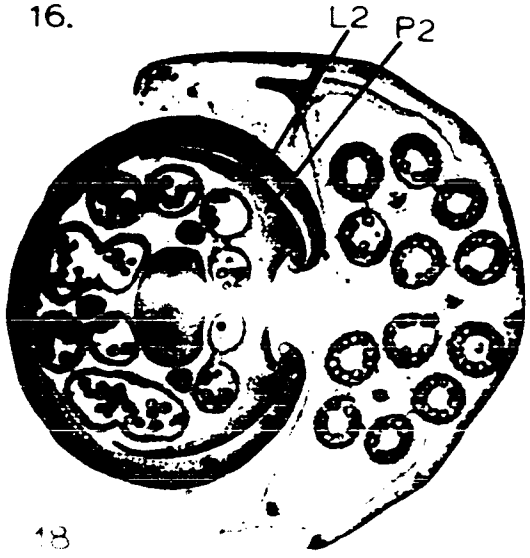


16.





18

bundles evident at this level, the upper glume only has 7 nerves because the smaller bundles at the margins do not extend very far into it.

Above the attachment of the sterile bracts or glumes, the rachilla is unbranched for a short distance below the region of the first floret. The lemma of the lower floret (L1) encircles the rachilla (9.7-9) in this region and has a slit-shaped lacuna to its interior. Seven vascular traces are visible (= 7 nerves of the lemma). The inner part of the lower floret is attached at the same level as the lemma, the base of the two fleshy lodicules (LOD), rudimentary gynoecium and stamens are seen in Plate 9.8 and 9.9. On the adaxial side is the base of the palea (Pl) which has two vascular bundles and is two-keeled (9.9-12).

The oval rachilla (R) with three large vascular bundles is apparent, and one can trace the lateral branch of the upper floret quite clearly in the succeeding plates (9-12). The base of the callus of the upper floret (CAL) is attached at the same level as the lodicules of the lower floret (9.9), but, as it was lost during sectioning and staining, its position has been outlined in this figure. The callus is larger higher up the spikelet, and the vascular traces are evident in Plate 9.12-13.

There is a clear abscission zone (A.Z.) between the callus and the rachilla (9.12) thus resulting in a wedge shaped, pointed callus (e.g. L. flavida, plate 16) when viewed from the side. The callus is a fairly large long body which

supports the bracts and essential parts of the upper floret, so that there are a number of sections not illustrated between Plates 9.13-14 and 9.14-15. The callus is copiously endowed with long epidermal hairs (C.H.) (9.12-14) which are mostly attached laterally in tufts.

The base of the upper lemma (L2) is illustrated (9.15-16) with 9 vascular bundles (nerves) in this particular species. At this level (9.15) the summit of the rachilla is seen (R).

The form of the upper floret is similar to that of the lower floret except that it is much more compact, this fact being evident in that the upper lemma closely encloses the palea (P2) around the two keels (9.18).

What may be noticed from these sections of the spikelet is that the upper and lower florets appear quite different in their basal attachment to the rachilla and in their anatomy at this level. The upper part of the rachilla at the base of the upper floret forms the callus which disarticulates in an oblique longitudinal direction from the lower part of the rachilla. It is also noted that the summit of the rachilla is apparent at the base of the upper lemma.

4. The morphology of the lemma of the upper floret

The lemmas of all the different taxa were not studied in detail, although they were examined with respect to their basic structure and morphological relationship with the awn and the callus.

The lemma varies from very small and narrow in relation to the awn, to quite large and broad. In pubescence it ranges

from quite glabrous to long pilose, although it is most frequently shortly pilose or hispid. The lemma usually tightly surrounds the palea, and the caryopsis is firmly enclosed within.

The callus varies in different species and has been of great advantage taxonomically in subdividing the genus. It is frequently sharply pointed and generally has stiff white hairs projecting upwards, mostly from its lateral margins.

5. The morphology of the awn of Loudetia

In gross morphology, the awns are found to differ greatly (Plates 11-14). The basal column is of uniform thickness throughout its length, generally thicker than the bristle and is spirally twisted in an anticlockwise direction. The bristle tapers from its base to the tip. The awn is generally geniculate at the junction of the bristle and the column, but the column may also be bent half or two thirds of the way up from the base when tightly spiralled (e.g. L. arundinacea (3), Plate 11.3). The column may be very short (e.g. Flammidae, plate 13.7, 13.8) or more usually is almost as long as the bristle (e.g. L. flavida, Plate 12.5).

Both the column and bristle are variously hairy, though the former is usually more so. It may be quite hispid in some species, whereas in others it is merely scabrous. The column and bristle are differently coloured, ranging from dark brown to pale yellow. The column is usually darker than the bristle, but it was found that the colouring varied somewhat with the age of the awns; the older the awns the darker the colouring.

Disarticulation of the awn from the lemma may occur in some species. In the very large awned species such as L. togoensis or subsection Annuae, disarticulation is quite common and abscission occurs at the base of the column (Plate 10). In most other species disarticulation is also in this position although it does not always occur. In the species of subsection Flammidae, which have small awns, disarticulation is at the summit of the column rather than the base.

6. Anatomy of the awn of Loudetia

The anatomy was studied from serial cross-sections of the column, the transition zone between the column and the bristle, and the bristle, examples of these are Loudetia cerata (Plate 15), L. flavida (Plate 16) and L. anomala (Plate 17).

Three basic types of anatomy were distinguishable. In the Loudetia form the column is an inverted U-shape in cross-section and consists of 5 to 6 layers of cells. The bristle is terete in cross-section, but may be flattened or angled. In the Lophanthera type the column is also an inverted U-shape in cross-section but consists of ten or more layers of cells. In the Pleioneura form, the awn is slightly curved in the same direction as the previous types or almost flat in cross-section and invariably consists of 6 layers of cells, having a double layer of thickened cells in the upper region of the mesophyll. The bristle is more or less oval in cross-section, but may be angled. Only the Loudetia and Pleioneura type awns were examined in the transition zone and bristle region. The differences in the anatomy of these regions are discussed below.

Plate 10

The apex of the lemma and the base of the awn of the fertile floret.

1. *R. petiolata* (50)
2. *L. togoensis* (49)

OTU numbers in parentheses.

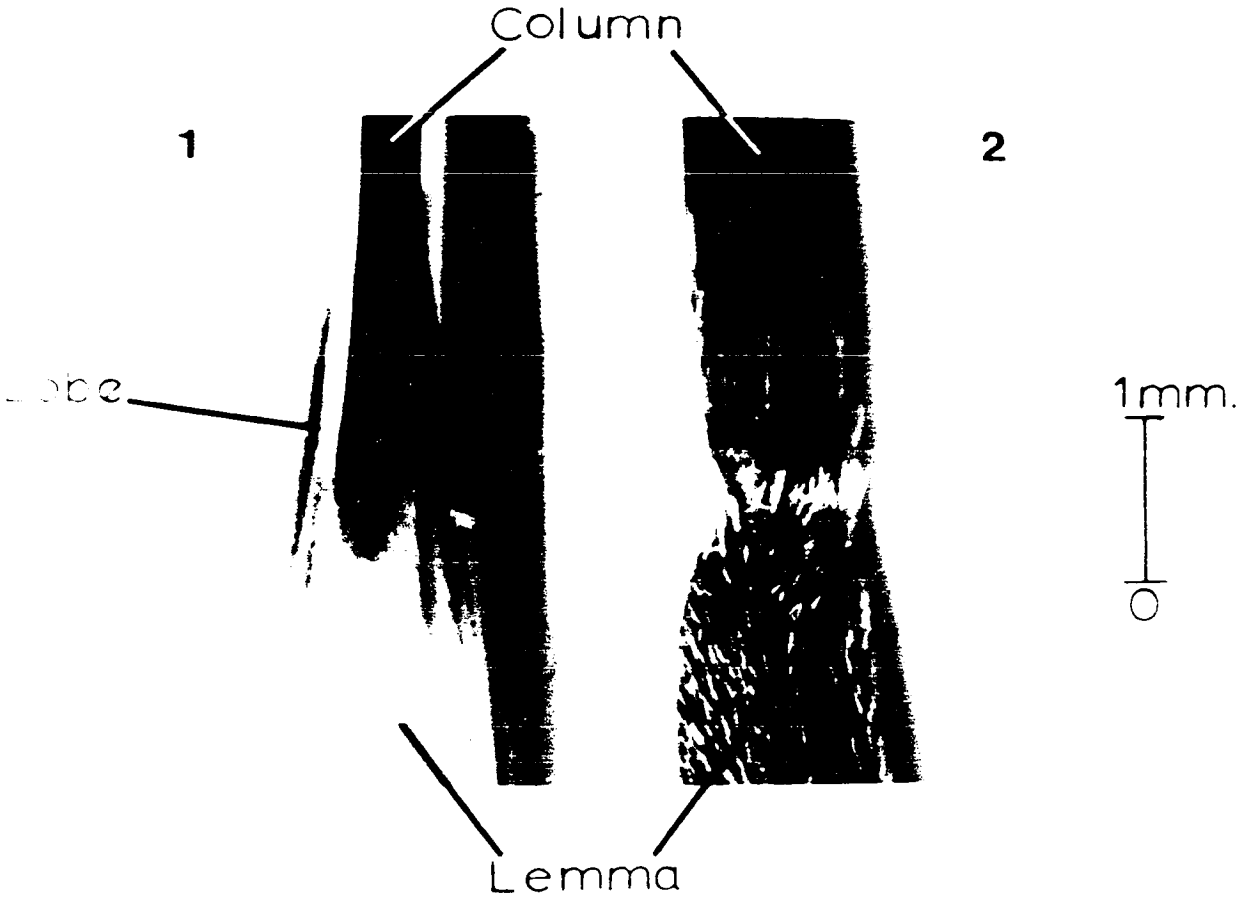
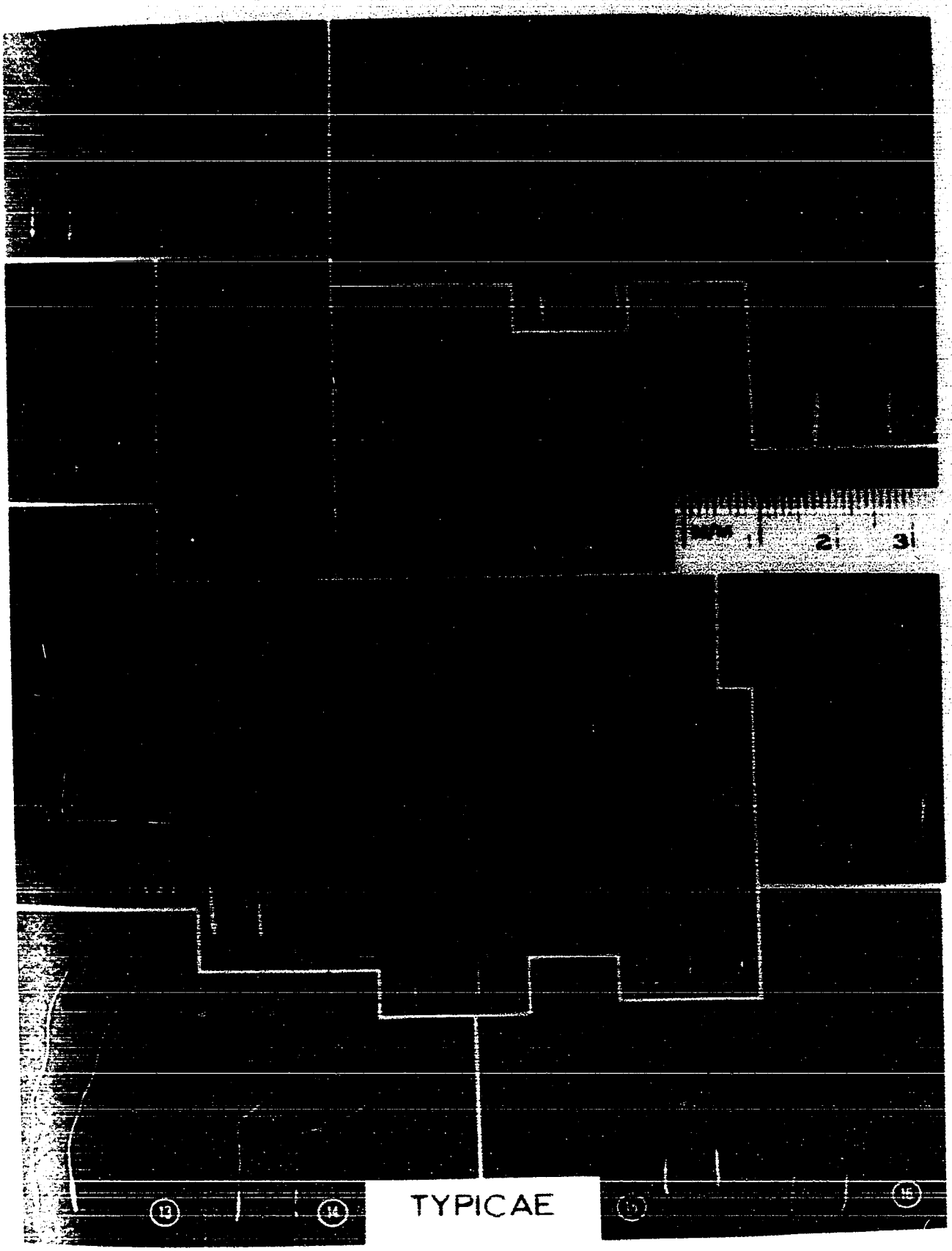

Column
1
2
Lobe
1mm.
Lemma

Plate 11

The morphology of the awn of *Loudetia* spp.

1. *L. angolensis* (1)
2. *L. angolensis* (UWO S196)
3. *L. arundinacea* (3)
4. *L. arundinacea* (5)
5. *L. arundinacea* (4)
6. *L. thomasii* (Thomas, 1027)
7. *L. eripoda* (Tessman 2728)
8. *L. simplex* subsp. *stipoides* (UWO S222B)
9. *L. simplex* subsp. *simplex* (8)
10. *L. simplex* subsp. *simplex* (7)
11. *L. simplex* subsp. *simplex* (UWO S41)
12. *L. perrieri* (13)
13. *L. kagerensis* (11)
14. *L. kagerensis* (12)
15. *L. madagascariensis* (15)
16. *L. madagascariensis* (14)

OTU or specimen numbers in parentheses.



TYPICAE

Plate 12

The morphology of the awn of *Loudetia* spp.

1. *L. demeusei* (19)
2. *L. lanata* (Crook, 655)
3. *L. crassipes* (20)
4. *L. longipes* (18)
5. *L. pennata* (Polhill & Panlo, 1324)
6. *L. flavida* (23)
7. *L. flavida* (24)
8. *L. acuminata* (28)
9. *L. filifolia* subsp. *humbertiana* (30)
10. *L. filifolia* subsp. *filifolia* (Schweickerdt, 1878a)

OTU or specimen numbers in parentheses.

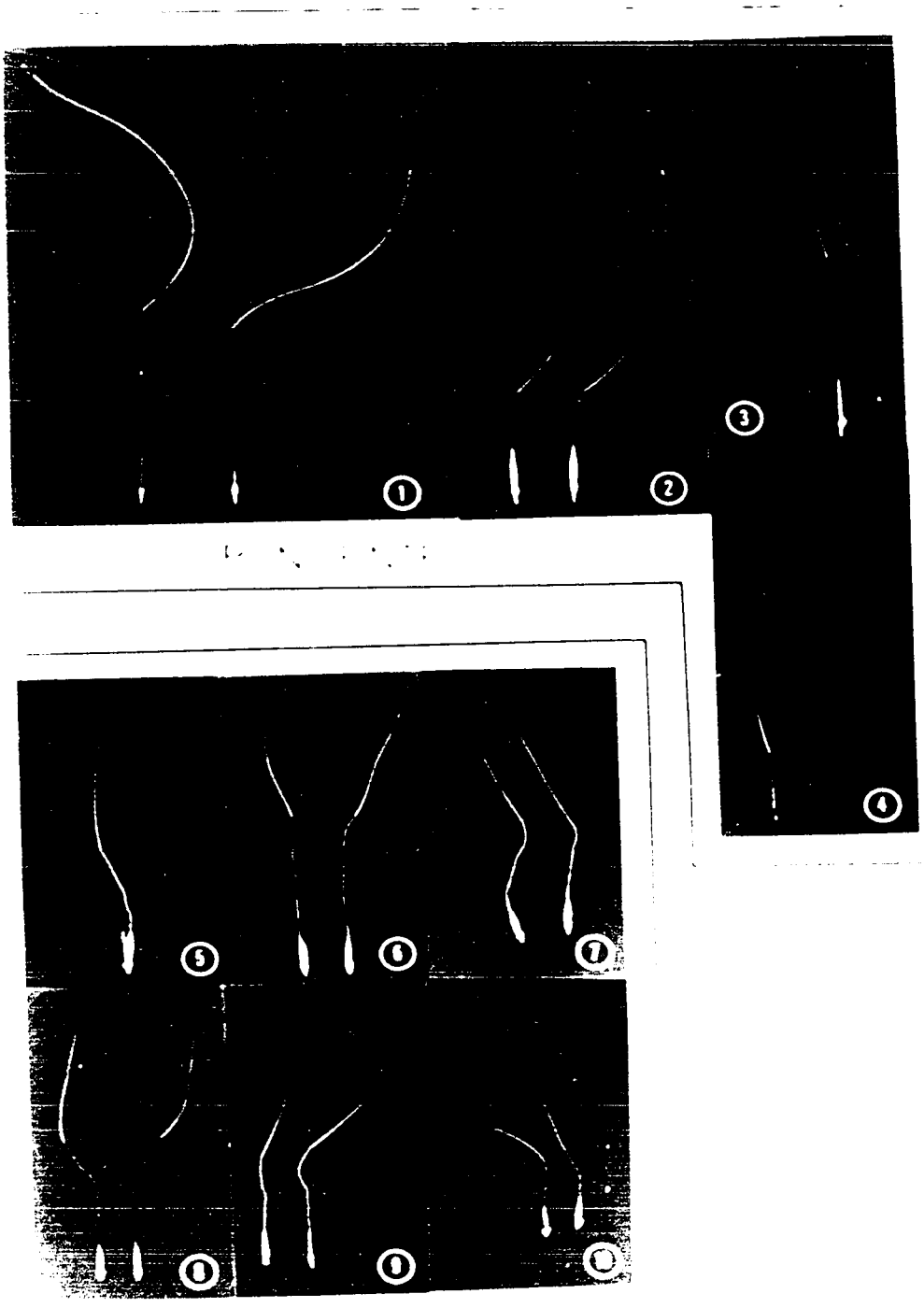

1
2
3
4
5
6
7

Plate 13

The morphology of the awn of *Loudetia* spp. and *Rattraya*.

1. *L. gossweileri* (32)
2. *L. echinulata* (34)
3. *L. coarctata* (35)
4. *L. vanderystii* (Pauwels, 4920)
5. *L. tisserantii* (Tisserant, in Kew H 31967/65)
6. *L. densispica* (Gossweiler, 11601)
7. *L. flammida* (51)
8. *L. phragmitoides* (43)
9. *L. simulans* (44)
10. *L. anomala* (45)
11. *L. ramosa* (48)
12. *R. petiolata* (50)

OTU or specimen numbers in parentheses.

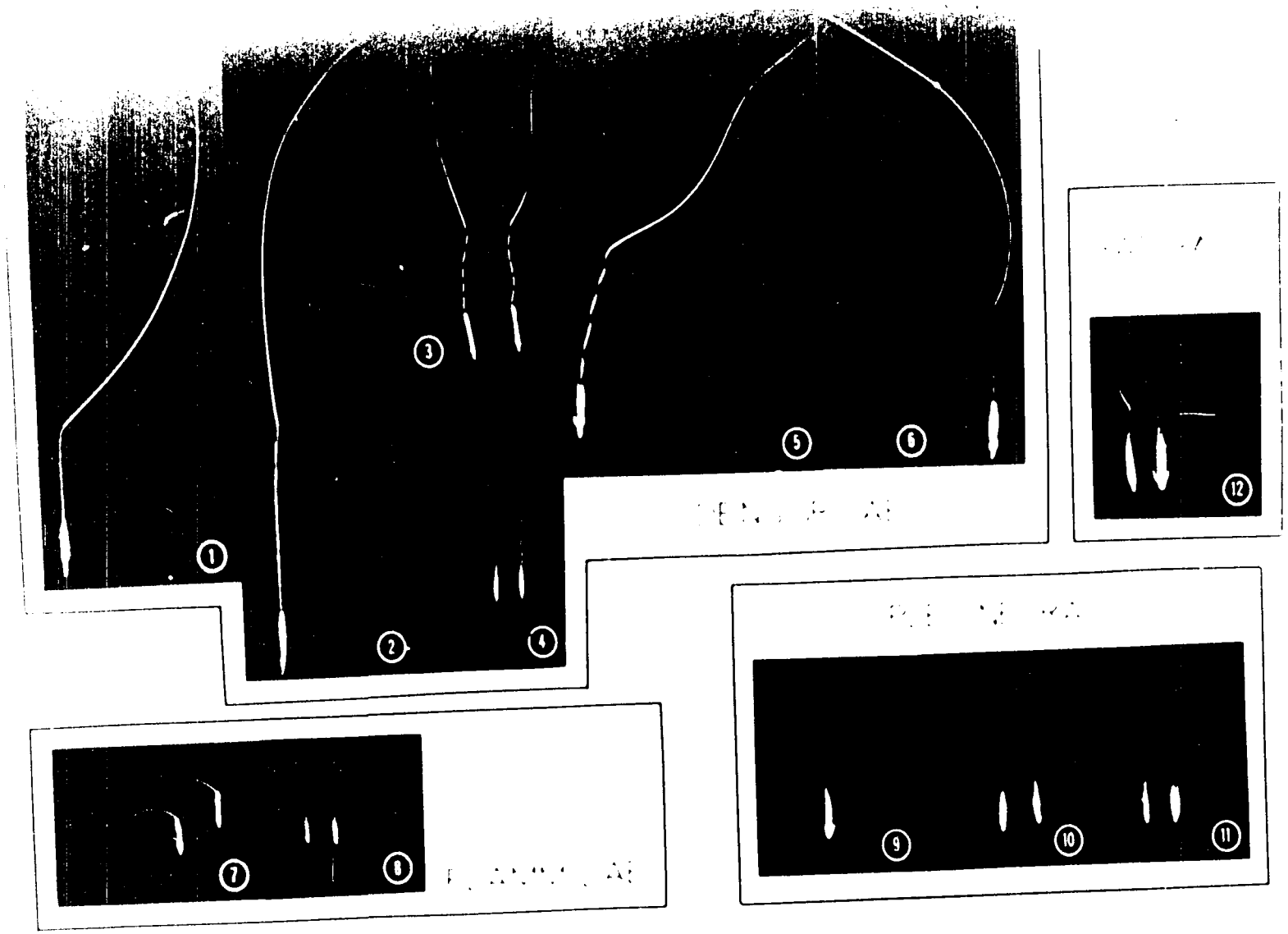
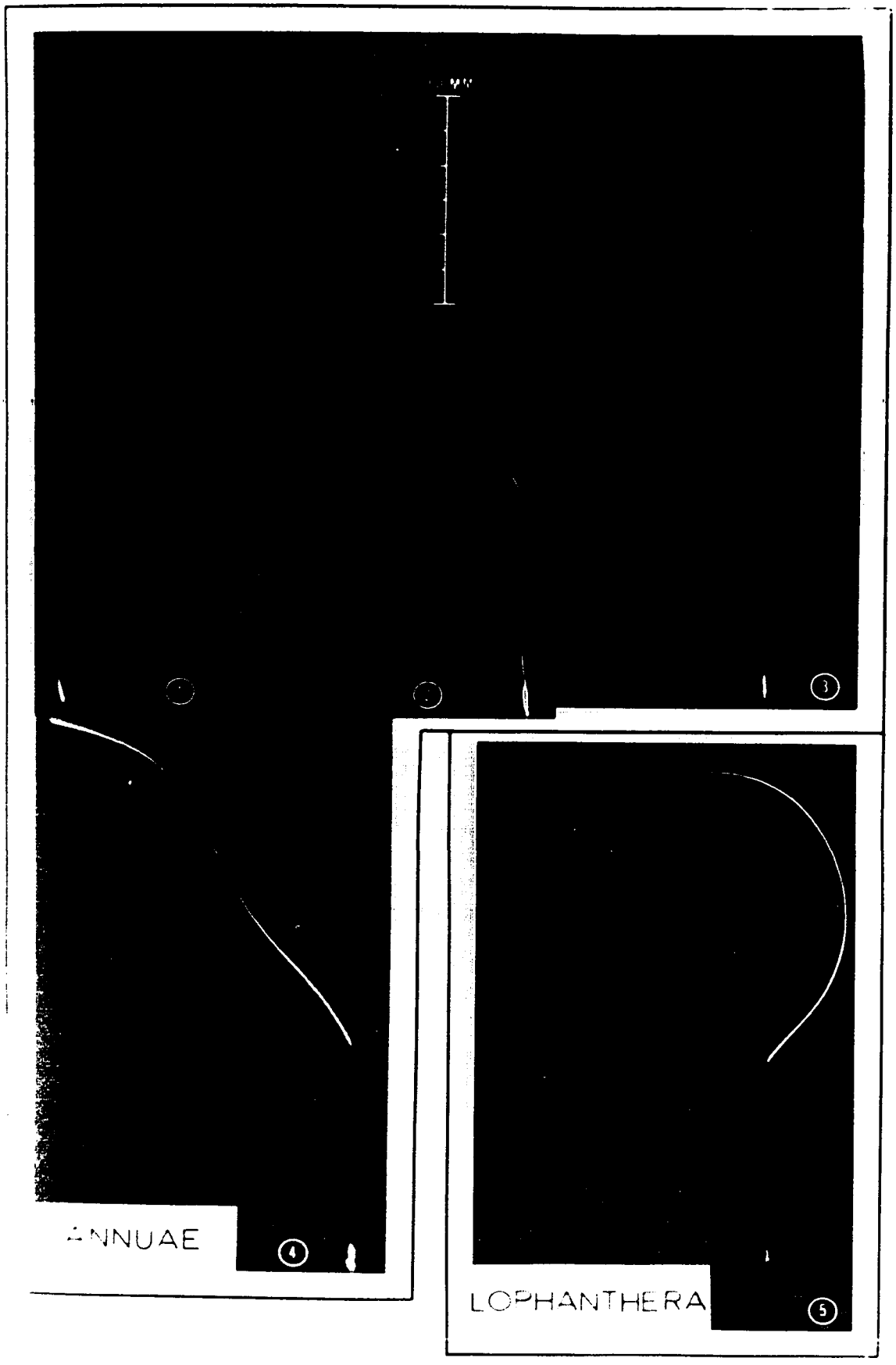

1
2
3
4
5
6
7
8
9
10
11
12

Plate 14

The morphology of the awn of *Loudetia* spp.

1. *L. cerata* (39)
2. *L. bidentata* (41)
3. *L. hordeiformis* (UWO S290)
4. *L. annua* (40)
5. *L. togoensis* (49)

OTU or specimen numbers in parentheses.


3
ANNUAE
4
LOPHANTHERA
5

The Column (Plates 18-24)

Three distinct tissue layers may be recognised in the cross-section of the column, viz. the upper or adaxial epidermis (AD), the lower or abaxial epidermis (AB) and the mesophyll (M1, M2, M3), (Plates 18-24). There is a single, centrally located vascular bundle.

The cells of the adaxial epidermis are usually quite small and have an abundance of thickening, predominantly towards the outside, so that the lumen is eccentrically situated. The cells are wider than they are long with a slightly arched outer wall, so that the adaxial epidermis has a wavy outline. Sections occur through the small prickle-hairs on the adaxial epidermis which always face towards the apex at an acute angle, and these look like small "papillae" (Plate 16). Toward the summit of the prickle-hair, the epidermal cell to which the prickle is attached has a very thin outer wall.

The abaxial epidermis has very large cells in comparison with the cells of the adaxial epidermis and thickening of the cell walls is usually not as prevalent. The cells are almost isodiametric, but the outer wall is highly arched producing the wavy outline of the abacial epidermis. In some species there are large epidermal hairs, these often occurring in great abundance (Plate 20.9).

The mesophyll is treated as three different zones, mainly for convenience, though there is a certain distinction between the three zones. The upper zone (M1) consists of one layer

of cells in the Loudetia and Pleioneura forms (Plate 23.1 - 23.4) and two or three layers in the Lophanthera type (Plate 23.5). The lower zone (M3) of the mesophyll consists of one layer in the Loudetia form, two layers in the Pleioneura type and two or three layers in the Lophanthera type. The middle zone of the mesophyll (M2) consists of a single layer of cells in most species, though often there may be two layers of cells especially towards the centre of the awn. In the Lophanthera type (Plate 23.5) there are three layers. The mesophyll shows varying degrees of thickening, but when there is an abundance, the lumina of the cells of the outer zones are eccentrically placed closer to the middle zone.

The central vascular bundle is of the first order type in the larger awns such as the Lophanthera type, possessing well developed xylem tissue. In the Pleioneura form (Plate 23.1 - 23.4) it is poorly developed and is a second order bundle. In the Loudetia form, the vascular bundle varies between these two types, depending on the size and thickness of the awn. The bundle structure is similar to that described in the section on the leaf anatomy.

The bristle

Apart from the shape, the bristle of the Loudetia type awn and the Pleioneura type are very similar. The epidermis is identical on both the upper and lower surfaces. The cells are more or less isodiametric with evenly thickened walls. The vascular bundle is centrally placed in the awn and is surrounded and supported above and below by a girder of thick-

ened mesophyll cells. Some of these supporting cells are also present laterally and they are separated from the vascular bundle by a region of thin-walled chlorenchyma, which are abundantly supplied with chloroplasts. There are intercellular spaces in this tissue and stomata in the upper and lower epidermis adjacent to these spaces (Plate 16). In some of the sections (Plate 15), the chlorenchyma cells have broken down, possibly due to the harsh softening treatment to which the tissue was subjected.

The transition zone between the column and the bristle

In the Loudetia type when the column is an inverted U-shape, the transition between column and bristle occurs as a "filling in" of the central portion between the lateral blades of the column (Plate 15). More thickened cells of the lower zone of the mesophyll are present in this region, and at the same level the middle zone of the mesophyll becomes the chlorenchyma of the bristle. In the cross-section of the column of L. flavida (Plate 16), one can see chloroplasts in the middle zone of the mesophyll and they are seen in this region in other species too.

In the Pleioneura type of awn, a flattening of the awn is observed which is accompanied by an increase in the number of chlorenchyma cells in the middle zone of the mesophyll while the lower zone remains unchanged (Plate 17). Higher up, the cells of the lower zone of the mesophyll are reduced in number in the central region and occur only at the margins and below the vascular bundle, forming supporting girders. In both

Plate 15

The morphology and anatomy of the awn of L. cerata (OTU No. 39)

Legend

Ad - adaxial epidermis

Ab - abaxial epidermis

Ch - chlorenchyma

H - hairs

IS - inner bundle sheath

OS - outer bundle sheath

M1,M2,M3 - upper, middle and lower mesophyll zones

Ph - phloem

St - stoma

X - xylem

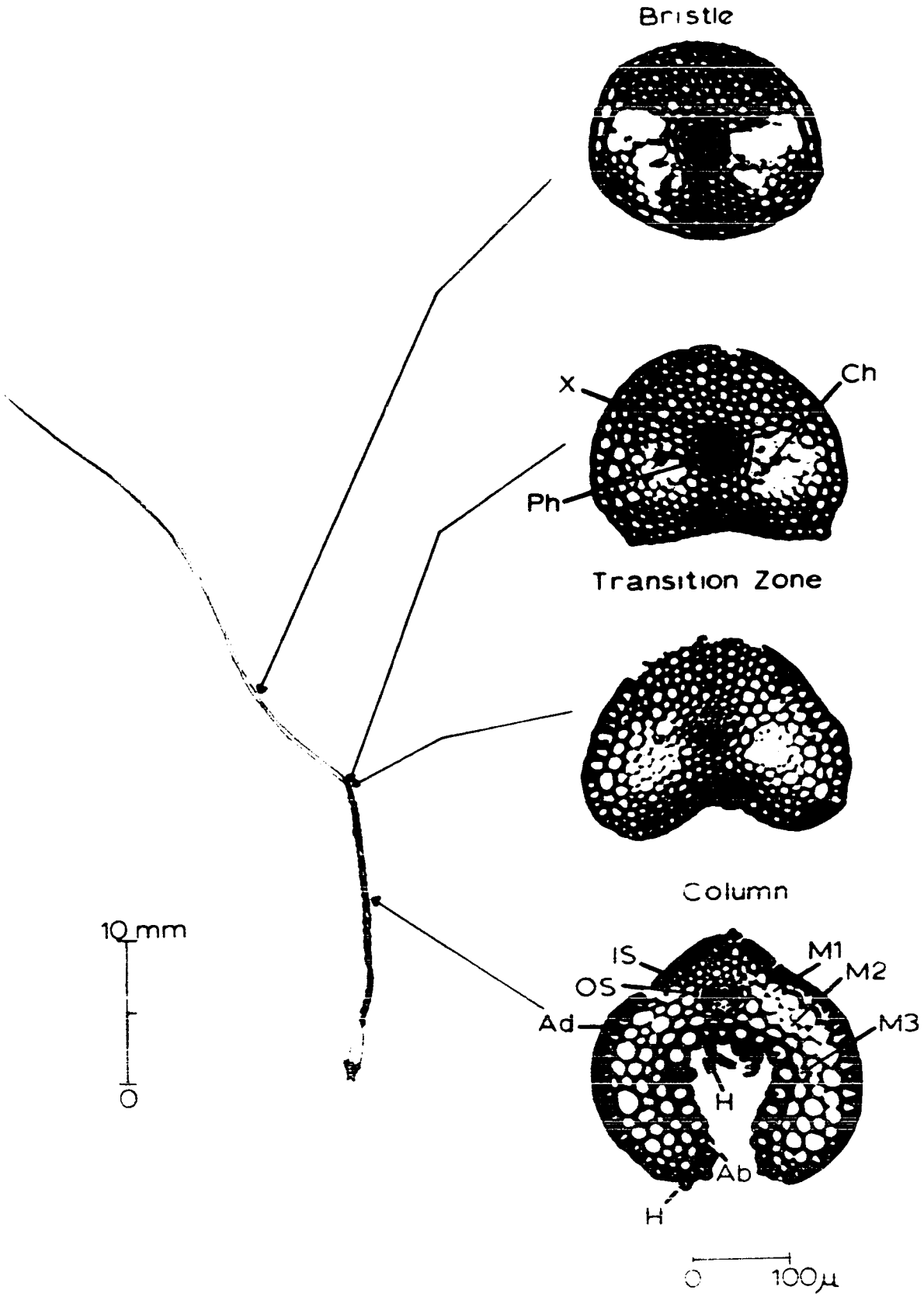

Bristle
X
Ch
Ph
Transition Zone
Column
10 mm
0
IS
OS
M1
M2
Ad
M3
H
Ab
H
0
100μ

Plate 16

The morphology and anatomy of the awn of *L. flavida* (UWO S58)

Legend

Ad - adaxial epidermis
Ab - abaxial epidermis
Ch - chlorenchyma
H - hairs
IS - inner bundle sheath
OS - outer bundle sheath
M1, M2, M3 - upper, middle and lower mesophyll zones.
Ph - phloem
St - stoma
X - xylem

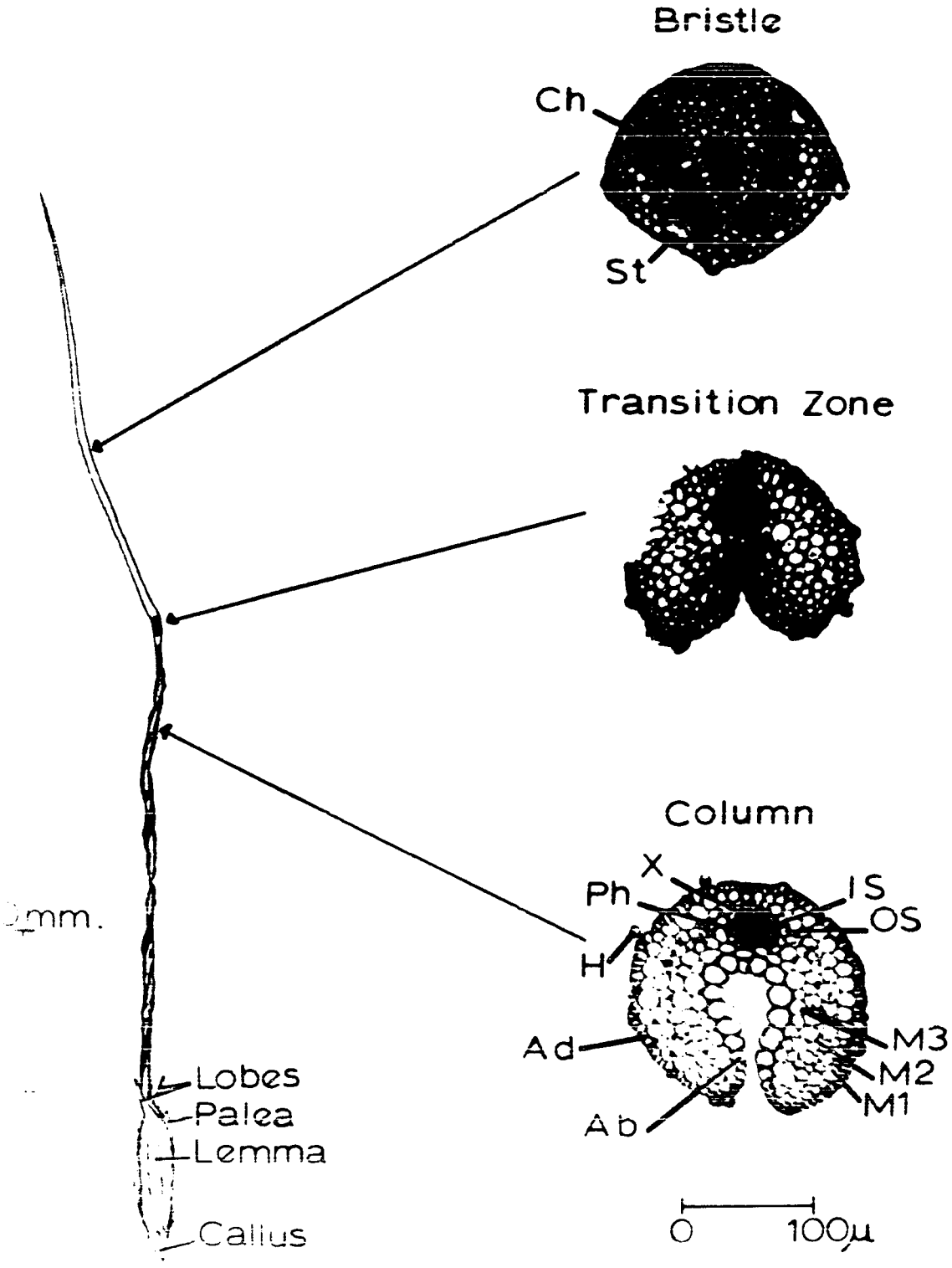

Bristle
Ch
St
Transition Zone
Column
X
Ph
IS
OS
H
Ad
M3
M2
M1
Ab
0
100μ
mm.
Lobes
Palea
Lemma
Callus
Lateral View

Plate 17

Morphology and anatomy of the awn of *L. anomala* (OTU No. 47)

Legend

Ad - adaxial epidermis

Ab - abaxial epidermis

Ch - chlorenchyma

H - hairs

IS - inner bundle sheath

OS - outer bundle sheath

M1,M2,M3 - upper, middle and lower mesophyll zones.

Ph - phloem

St - stoma

X - xylem

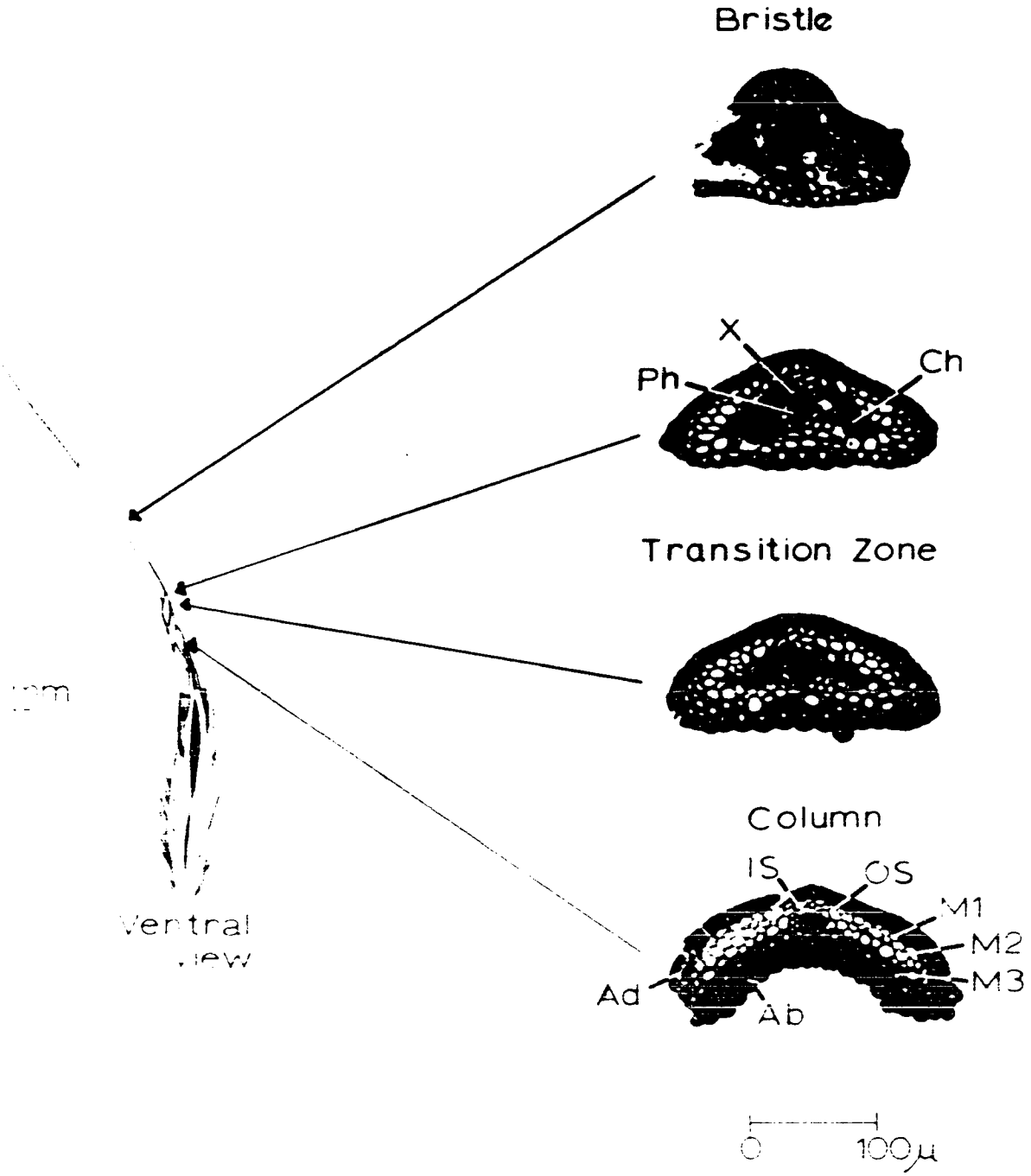

Bristle
X
Ph
Ch
Transition Zone
Column
IS
OS
M1
M2
M3
Ad
Ab
Ventral
0
100μ

Plate 18

Cross-sections through the middle of the column of the awn of the fertile floret.

1. *L. angolensis* (2)
2. *L. arundinacea* (3)
3. *L. arundinacea* (4)
4. *L. arundinacea* (5)
5. *L. camerunensis* (6)
6. *L. simplex* subsp. *simplex* (8)
7. *L. simplex* subsp. *stipoides* (9)

OTU numbers in parentheses

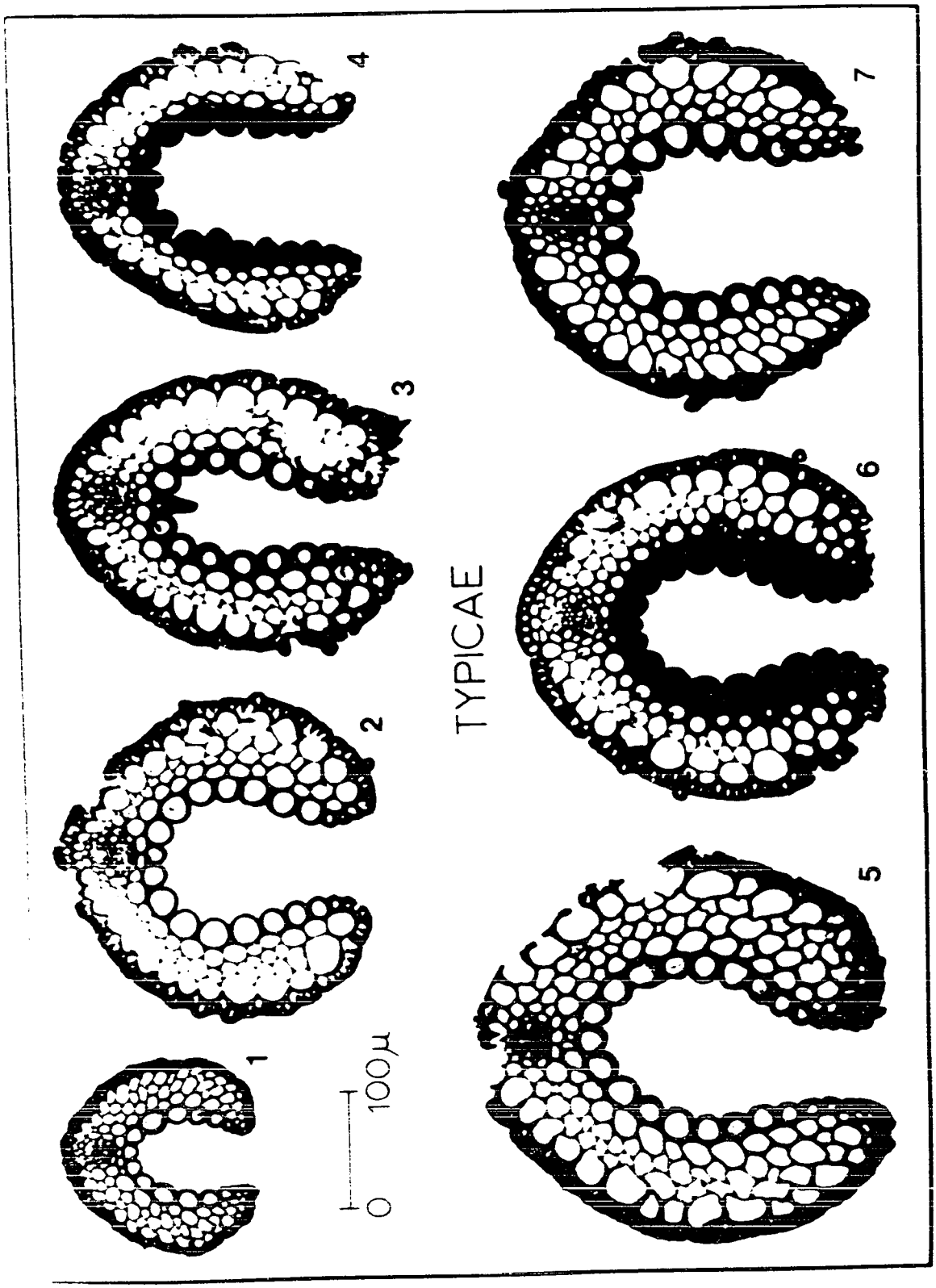

1
2
3
4
5
6
7
TYPICAE
0
100μ

Plate 19

Cross-sections through the middle of the column of the awn of the fertile floret.

1. *L. kagerensis* (11)
2. *L. kagerensis* (12)
3. *L. perrieri* (13)
4. *L. madagascariensis* (14)
5. *L. madagascariensis* (15)
6. *L. jaegeriana* (16)

OTU numbers in parentheses.

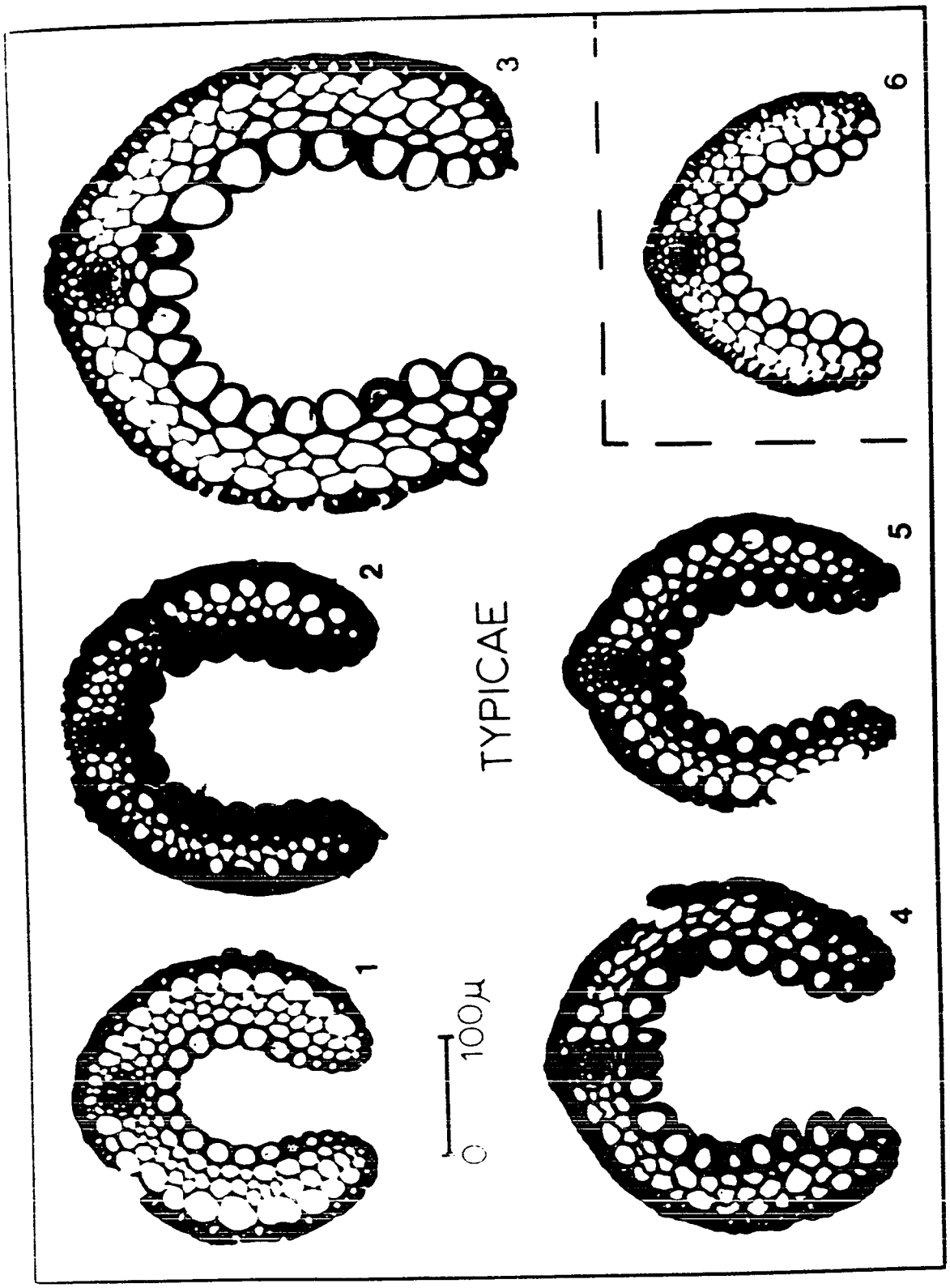

1
2
3
4
5
6
TYPICAE
0 100μ

Plate 20

Cross-sections through the middle of the column of the awn of the fertile floret.

1. *L. lanata* (17)
2. *L. longipes* (18)
3. *L. crassipes* (20)
4. *L. pennata* (21)
5. *L. pennata* (22)
6. *L. flavida* (23)
7. *L. migiurtina* (26)
8. *L. acuminata* (27)
9. *L. cuanzensis* (29)
10. *L. filifolia* subsp. *humbertiana* (31)

OTU numbers in parentheses.

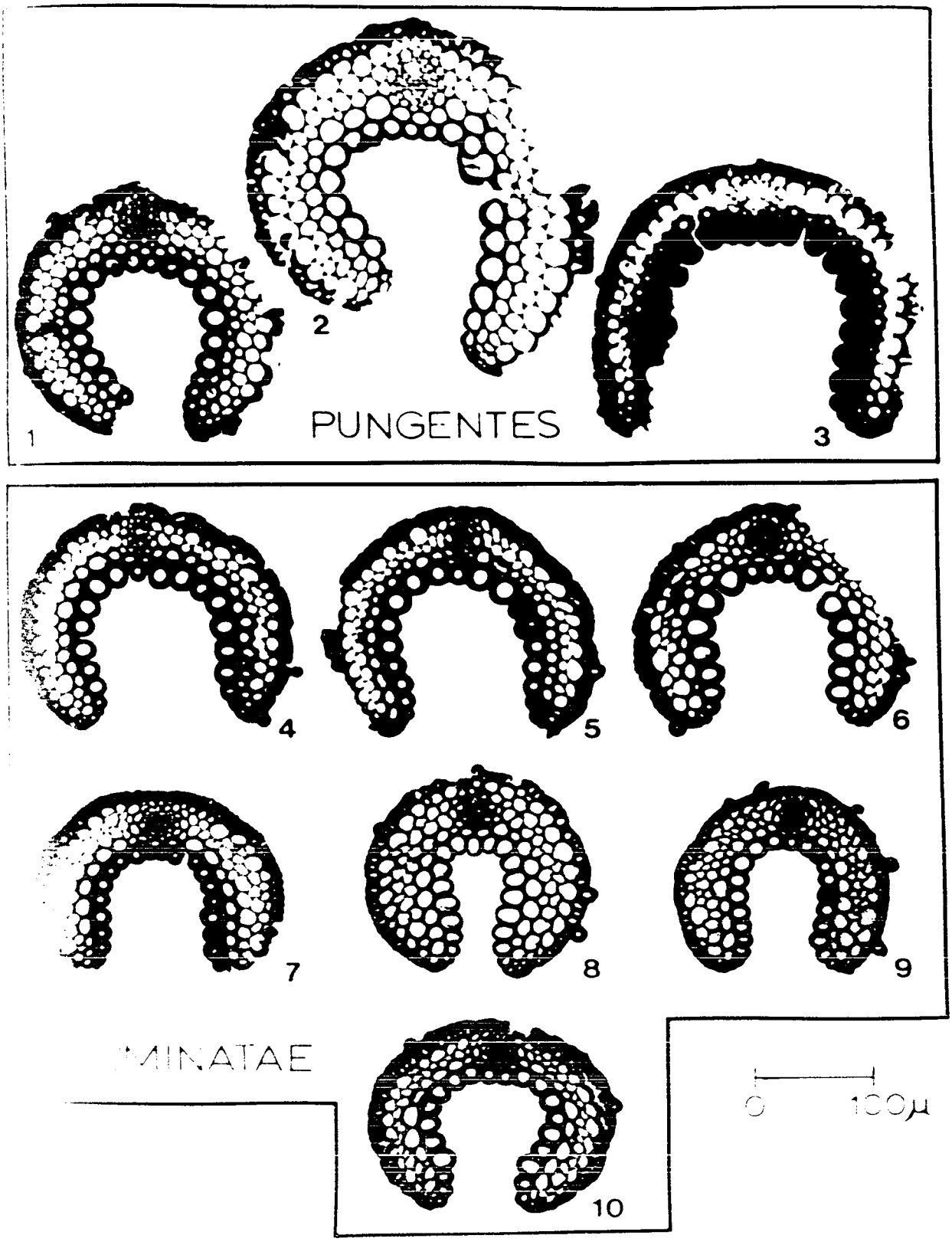

1
2
3
PUNGENTES
4
5
6
7
8
9
MINATAE
10
0
100μ

Plate 21

Cross-sections through the middle of the column of the awn of the fertile floret.

1. *L. gossweileri* (32)
2. *L. densispica* (33)
3. *L. echinulata* (34)
4. *L. coarctata* (35)
5. *L. tisserantii* (37)
6. *L. vanderystii* (38)

OTU numbers in parentheses.

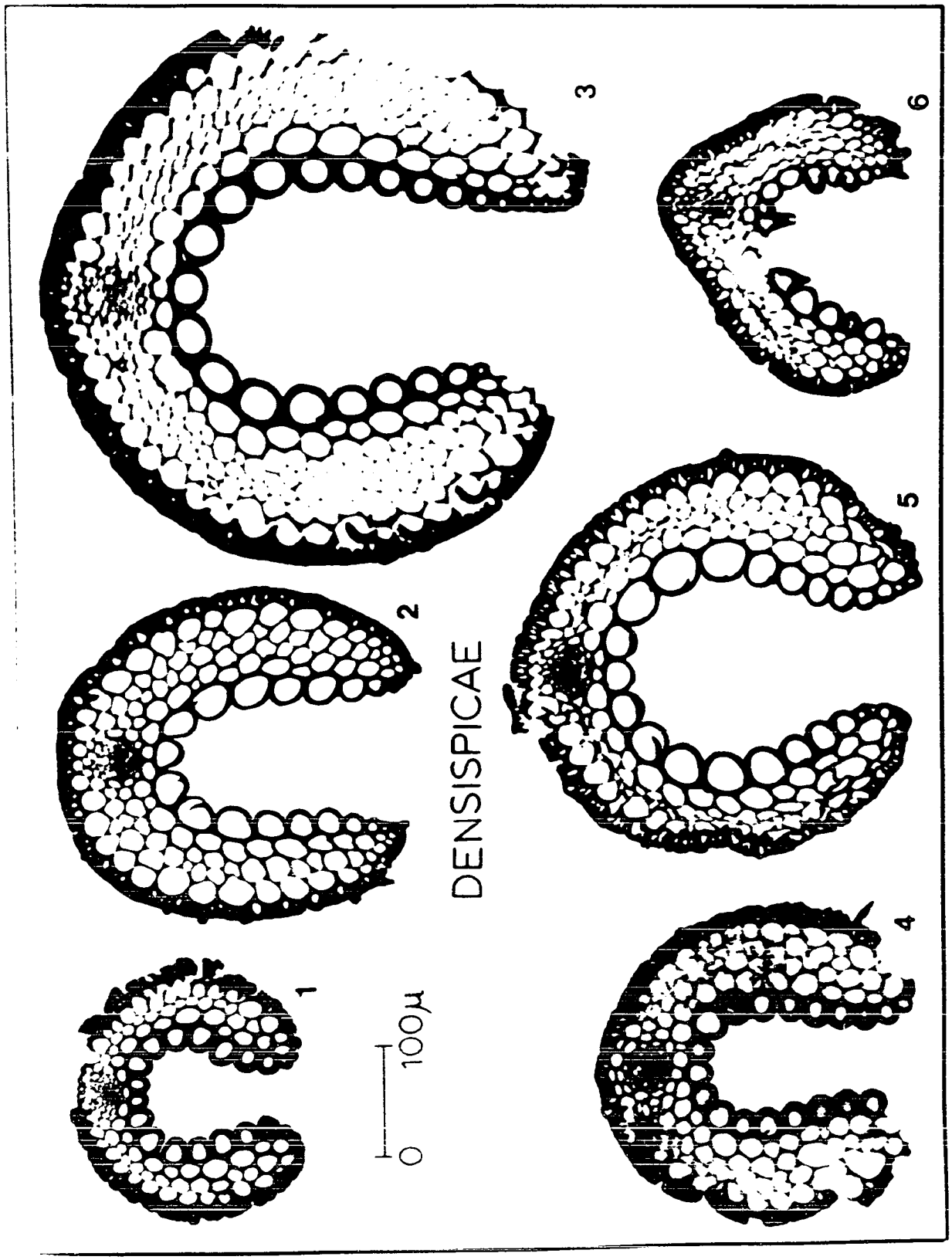

1
2
3
4
5
6
DENSISPICAE
0
100μ

Plate 22

Cross-sections through the middle of the column of the awn of the fertile floret.

1. *L. cerata* (39)
2. *L. annua* (40)
3. *L. bidentata* (41)
4. *L. hordeiformis* (42)

OTU numbers in parentheses.

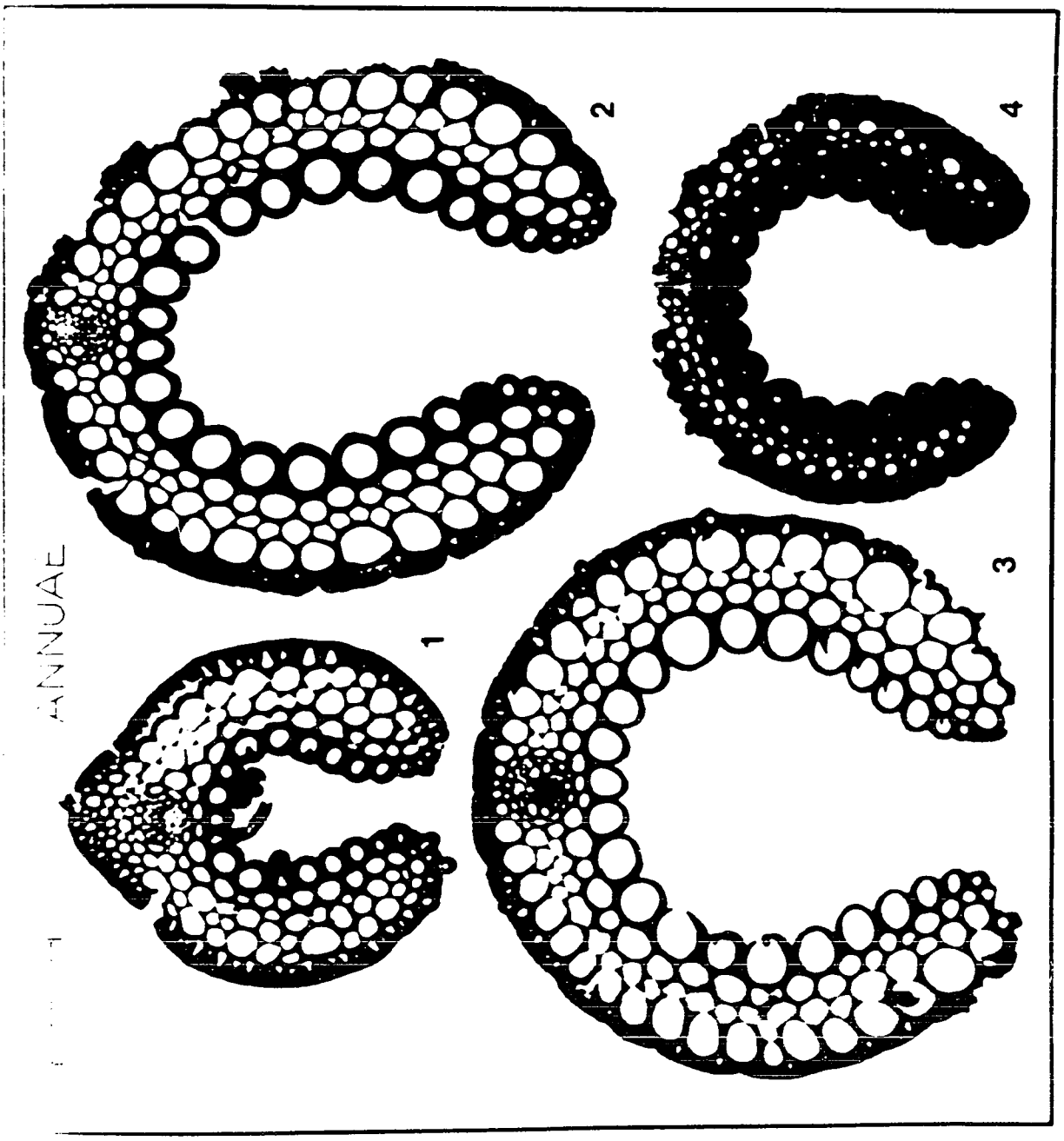
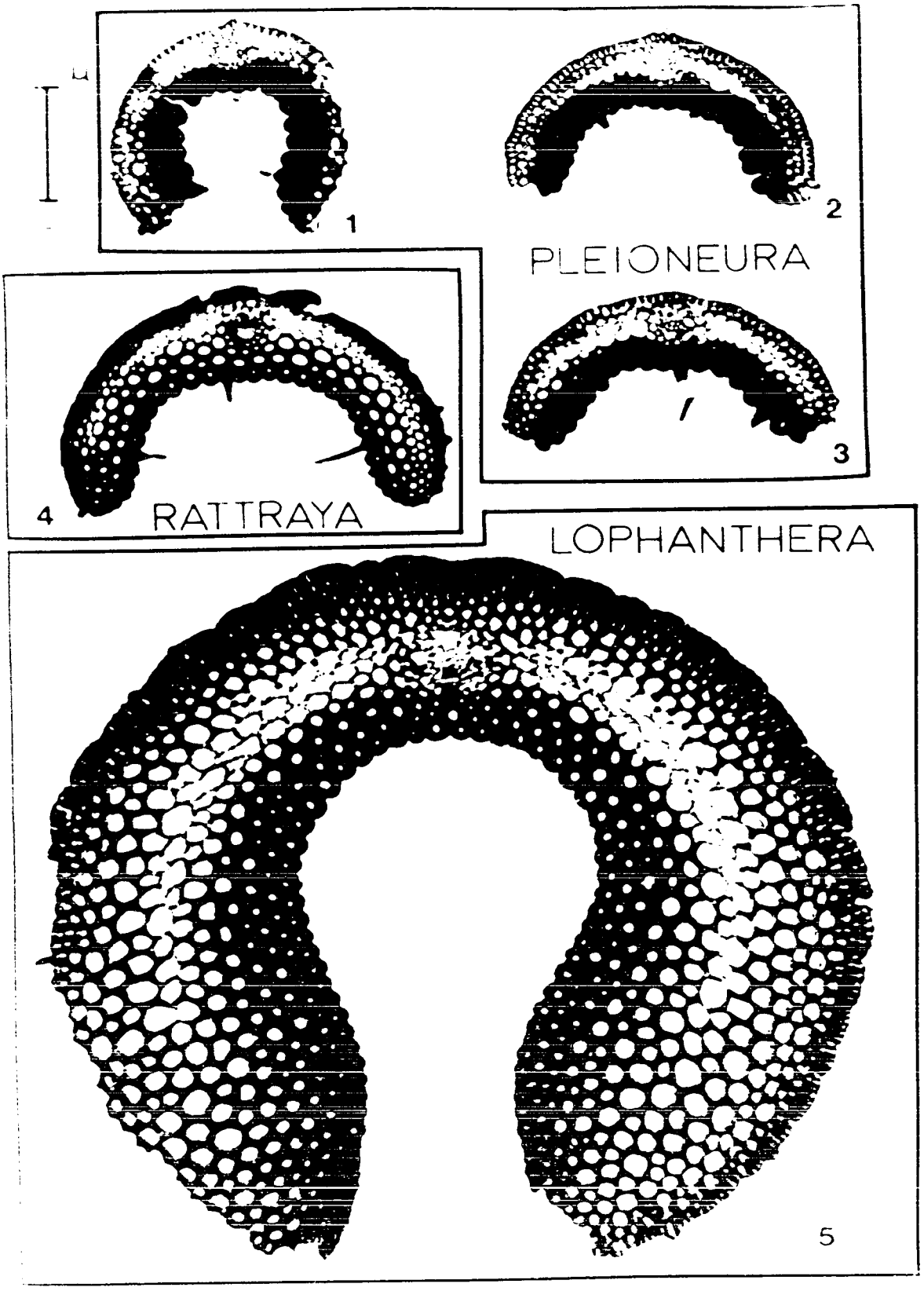

ANNUAE
1
2
3
4

Plate 23

Cross-sections through the middle of the column of the awn of the fertile floret.

1. *L. simulans* (44)
2. *L. anomala* (46)
3. *L. anomala* (47)
4. *R. petiolata* (50)
5. *L. togoensis* (49)

OTU numbers in parenthesis.


1
2
PLEIONEURA
3
4
RATTRAYA
LOPHANTHERA
5

types of awn, the upper zone of the mesophyll becomes more extensive in the transition region, and results in the upper part of the supporting girder in the bristle.

The illustrations of Tran (1965) indicate that the transition zone between bristle and column of the awn of L. togoensis (section Lophanthera) is very similar to that of L. cerata (Plate 15) or L. flavida (Plate 16), except that the awn is larger. The anatomy of the bristle is also similar to that of the above two species.

7. The numerical taxonomy of the genus based on characters of the awn

From an examination of the morphology and anatomy of the awns it is obvious that there are a number of differences between the taxa. The use of some of the anatomical characters of the awn in a classical approach has already proved to be useful (Conert, 1957). Quantification of the data so that a numerical analysis may be perfected is, however, more beneficial in drawing conclusions about the genus as will be indicated here. Thirty-one characters describing awn morphology and anatomy were used in a study of 50 OTU's. The methods of analysis are practically identical with those used on the leaf (Fig. 25). The 10 supra-specific taxa were also treated in a similar way with respect to the awn characters (Fig. 26).

1. Data Preparation

(a) The OTU's

Fifty OTU's were studied with respect to awn characters. (Table 17). L. arundinacea (3-5), L. simplex (7-10),

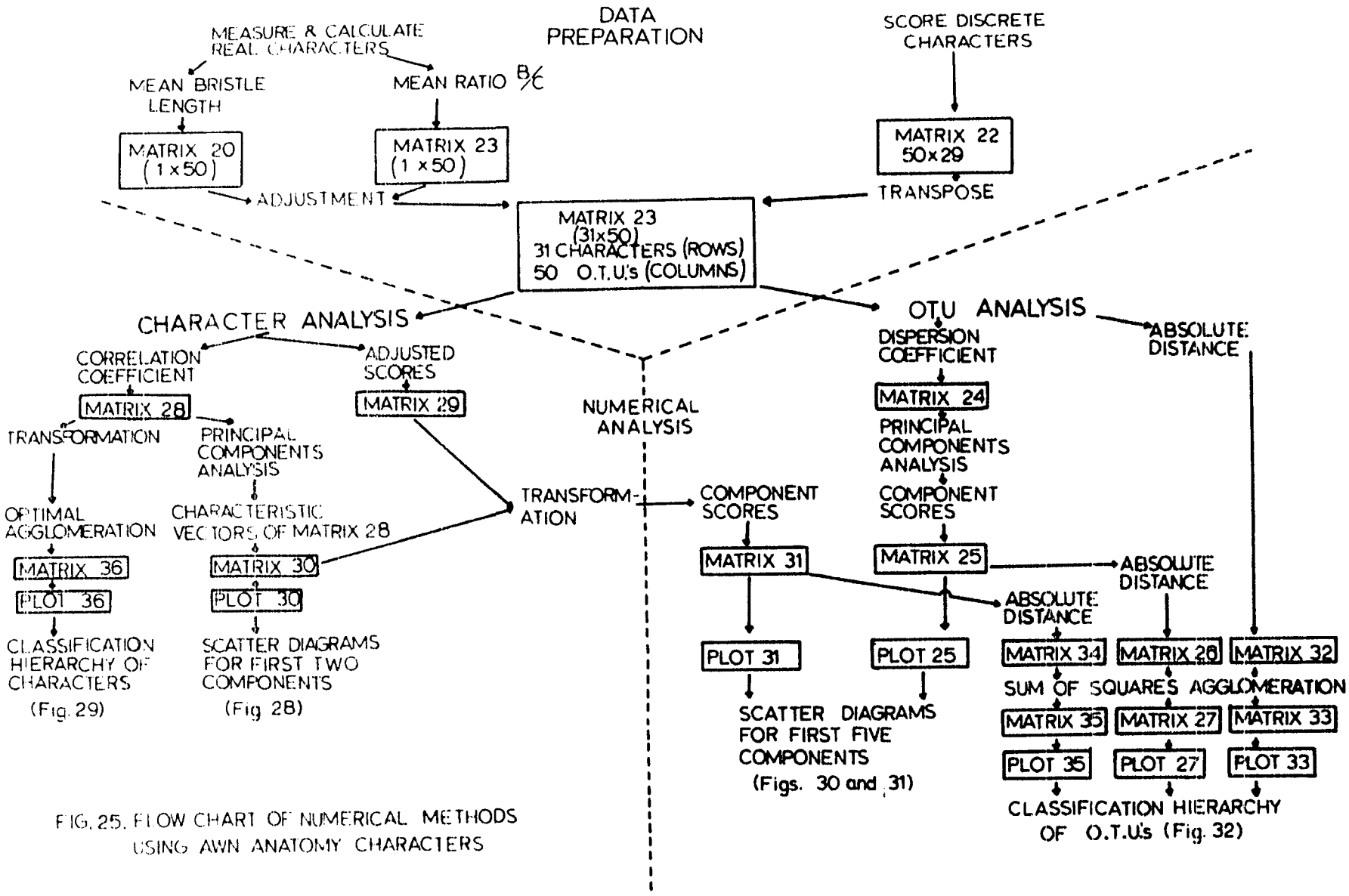


FIG. 25. FLOW CHART OF NUMERICAL METHODS USING AWN ANATOMY CHARACTERS

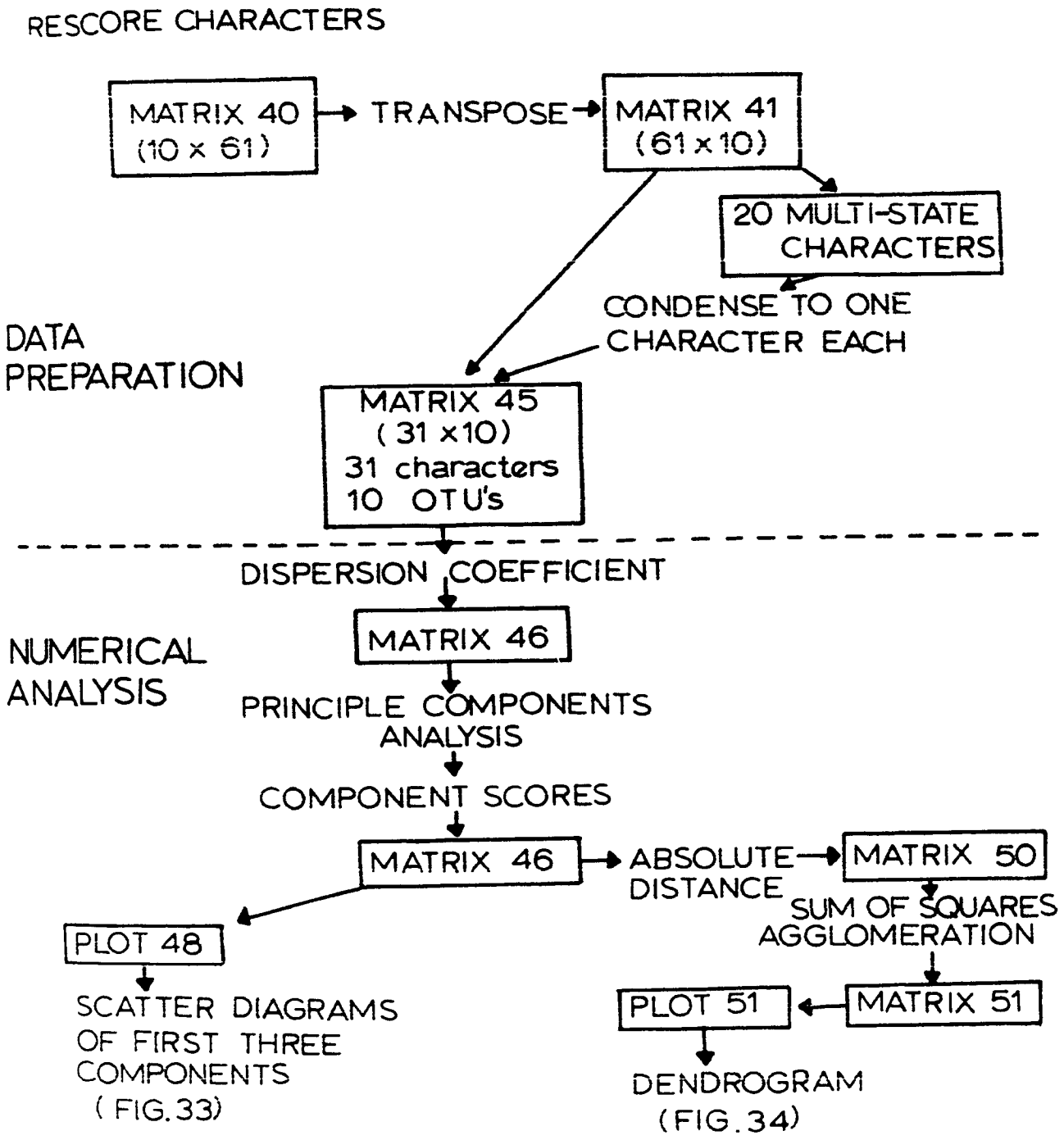


FIG.26. FLOW CHART OF NUMERICAL METHODS USING SUPRA SPECIFIC TAXA AS OTU's AND AWN ANATOMY CHARACTERS

Table 17: List of OTU's used in a taxonomic study based on awn characters. Symbols and numbers referred to in Figures 27 - 34.

Section: Loudetia

1. Subsection: Typicae

1. L. angolensis
2. "
3. L. arundinacea
4. "
5. "
6. L. camerunensis
7. L.simplex subsp. simplex
8. "
9. L.simplex subsp. stipoides
10. "
11. L. kagerensis
12. "
13. L. perrieri
14. L. madagascariensis
15. "

10. Incertae sedes

16. L. jaegeriana

2. Subsection: Pungentes

17. L. lanata
18. L. longipes
19. L. demeusei
20. L. crassipes

3. Subsection: Acuminatae

21. L. pennata
22. L. pennata
23. L. flavida
24. "
25. "
26. L. migiurtina
27. L. acuminata
28. L. acuminata
29. L. cuanzensis
30. L. filifolia subsp. humbertiana
31. "

4. Subsection: Densispicae

32. L. gossweileri
34. L. echinulata
35. L. coarctata
36. L. densispica
33. L. densispica
37. L. tisserantii
38. L. vanderystii

5. Subsection: Annuae

■ 39. L. cerata
■ 40. L. annua
■ 41. L. bidentata
■ 42. L. hordeiformis

6. Subsection: Flammidae

▲ 43. L. phragmitoides
▲ 51. L. flammida

7. Section: Pleioneura

○ 44. L. simulans
○ 45. L. anomala
○ 46. "
○ 47. "
○ 48. L. ramosa

8. Section: Lophanthera

□ 49. L. togoensis

9. Genus: Rattraya

⊗ 50. R. petiolata

L. kagerensis (11, 12) and L. flavida (23-25) all are represented by OTU's grown in the greenhouse. Species with more than one exemplar that are all from the wild are L. angolensis (1-2), L. madagascariensis (14, 15), L. pennata (21, 22), L. acuminata (27, 28), L. filifolia subsp. humbertiana (30, 31), L. densispica (33, 36) and L. anomala (45, 47). It may further be noted that some of the OTU's present in the previous studies on the leaf are lacking here, either due to lack of material or difficulty in obtaining sections. L. flammida (51) in particular had a very short column and it was not possible to obtain sections of the column so that the awn characters could be scored for inclusion in this analysis. Additional OTU's of the subsection Annuae were available for inclusion here as were additional exemplars of L. anomala of the section Pleioneura which provided more information about these groups.

(b) Characters of the Awn

When examined superficially, the awn anatomy of the different taxa appears very similar, which is quite understandable in a homogeneous genus such as Loudetia, where differences between species are very small and often very few. In a more detailed study of the awn anatomy, however, a sufficient number of diagnostic characters may be found. The comparative study of the anatomy of the awns was limited to cross-sections of the column approximately half way up from the base, due to the amount of work and time which would be involved if a complete anatomical study of the awn were to be undertaken of all species.

As mentioned above, there are differences between species in the anatomy of the bristle and the transition zone of the awn, but these would probably only further confirm the results which are to follow.

The cross sections of the columns are illustrated in Plates 18-24.

In addition to the anatomical characters a few morphological characters were obtained, and it is interesting to note the differences in size and form of the awns (Plates 11 - 14).

Table 44 (Appendix 4) gives a list of the characters used in this study. In some cases the character and states are self-explanatory, but in others a more detailed explanation is necessary.

Characters 1 and 2

The lengths of the bristles and columns of twenty different awns of a specimen were measured although in some instances only a few awns or the measurements from the literature were available.

The mean bristle length and mean ratio of bristle length-to-column length (b/c) was calculated for each OTU. The mean ratio b/c was plotted against the mean length of the bristle (Fig. 27) for each OTU to give some indication of the range of measurements in the group. It will be noted that the annual species have the longest bristles (and the largest awns), Loudetia togoensis (49) with a mean bristle length of 40.275 mm. having the highest value. The subsection Annuae, except for

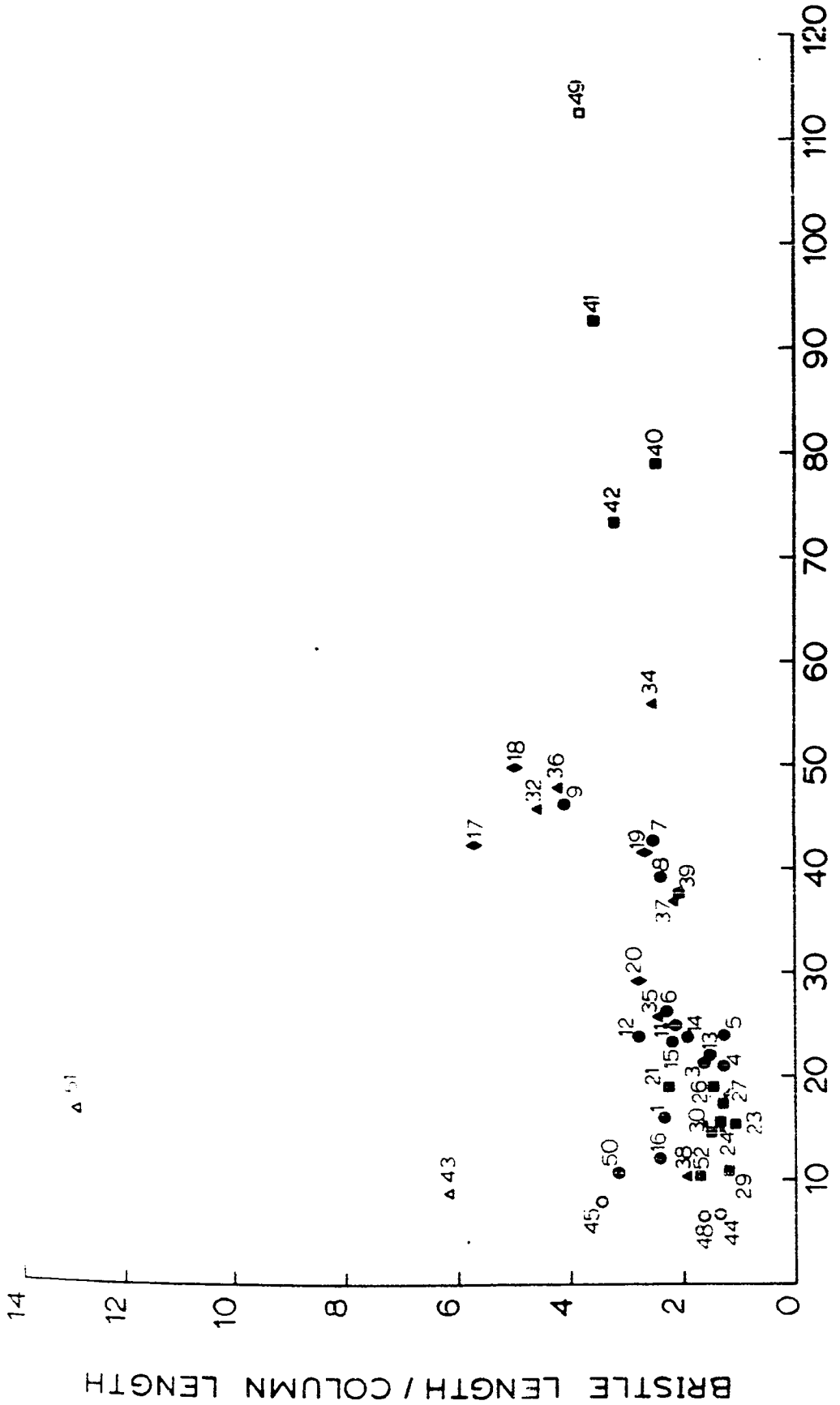


Fig. 27 Scatter diagram showing the distribution of OTU's with respect to mean bristle length and mean ratio of bristle length-to-column length. For legend see Table 17.

L. cerata (39), has species (40-42) with bristle lengths much longer than those of the majority of the rest of the genus. In contrast to this, the section Pleioneura (44, 45 and 48) has representatives with very short bristles, L. ramosa (48) has the shortest bristle (mean = 6.576 mm). The subsection Flammidae (43 and 51) is characterised by an awn with a very short column and hence the ratio is large in this group although the awns are not very long.

Characters 3 and 4

Only in the Flammidae, where the column is very short, was it found that the column is also not markedly twisted. Correlated with this is the fact that disarticulation of the awn occurs at the top of the column and not at the base. The anatomical features peculiar to this subsection which cause disarticulation at this level in the awn were not investigated.

Character 6

The thickness of the columns were measured at the centre and these are tabulated in Table 45 (Appendix 4). Only a single measurement was made in most cases, since the cross-sections were not made of a number of awns. Since this did not represent a true sample for an OTU it was decided that three discrete character states should be chosen to represent width, which are as follows:

Relatively thin	95 μ
Average	96 - 149 μ
Relatively thick	150 μ

On this basis only the L. togoensis (Plate 23.5) column

was regarded as being relatively thick, the columns of all the species in the section Pleioneura (Plate 23. 1-3), sub-section Acuminatae (Plate 20. 4-10) and Rattraya (Plate 23.4) were classed as relatively thin and in other groups both thin and average columns occur.

Character 14

If hairs or prickle-hairs are present the frequently occur in the middle of the awn below the vascular bundle (section Pleioneura Plate 23. 1-3).

Character 18

In the upper zone of the mesophyll the thickening of the cell walls is one of two types. The cell wall may be distinctly arched, with the thickening forming an arch, or the thickening may appear only slightly curved or straight across the outer wall. The latter occurs in all the Pleioneura, Rattraya and L. togoensis (Plates 23, 24). When this type of thickening occurs, the thickened walls of the upper zones of the mesophyll and the adaxial epidermis takes up less safranin and appears pink, in comparison to the brightly red stained thick walls of the lower zone of the mesophyll and abaxial epidermis, e.g. Rattraya petiolata (Plate 24.3). It is likely that there is a difference in chemical composition of the cell walls of the different tissues, though this was not investigated further.

Characters 10, 12, 15, 20 and 23

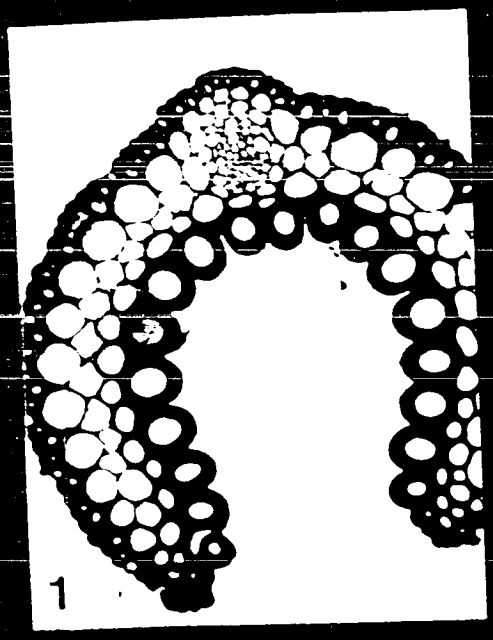
These characters refer to the width of the cells of the different tissues as seen in cross-section. It was difficult

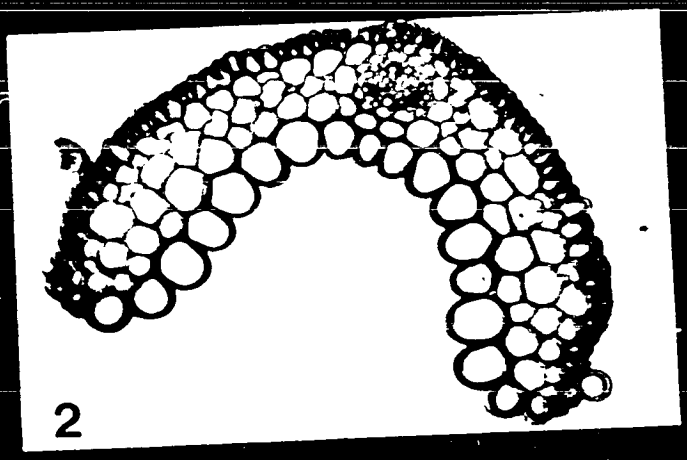
Plate 24

Cross-sections through the middle of the column of the awn of the fertile floret.

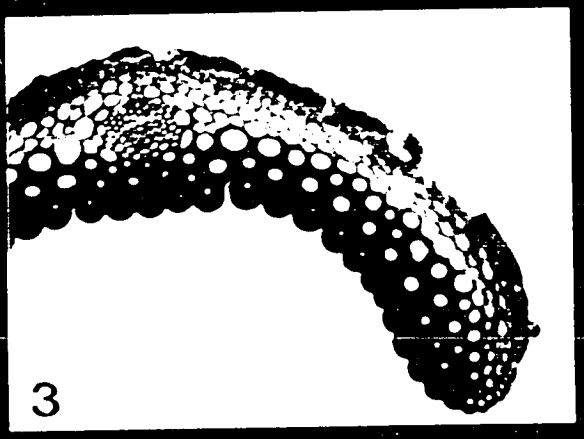
1. L. madagascariensis (15)
2. L. jaegeriana (16)
3. R. petiolata (50)
4. L. togoensis (49) - whole section
5. L. togoensis (49) - portion from the centre of the column.

OTU numbers in parentheses.

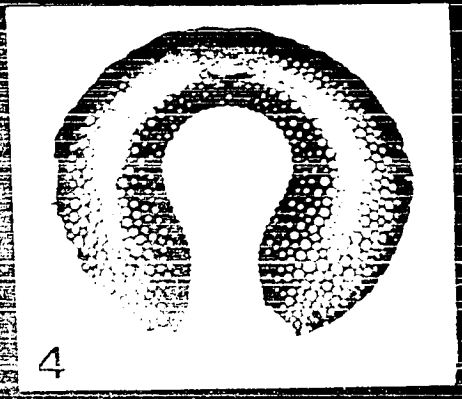

1

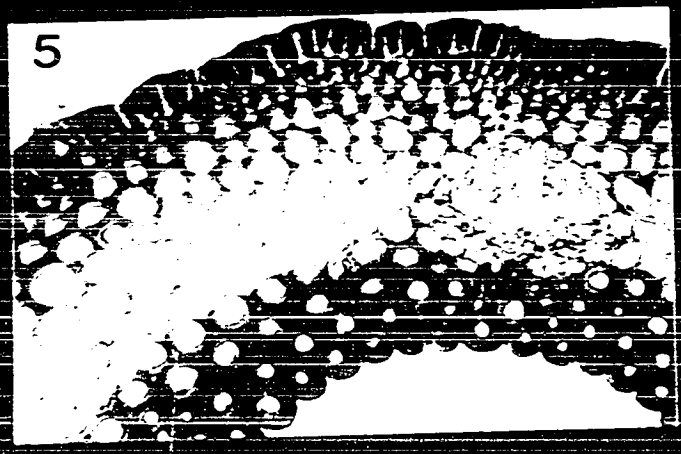

2

0
100μ


3

0
400μ


4


5

to decide which of the states would best describe the size of the cells, so the following method was devised. The thickness of the awn was measured in a region between the 'mid-rib' and the margin. This value was divided by the number of layers of cells in the region to give the mean cell width (X_c). This mean value was used as a basis in characterising the cell sizes as follows:

		Small	$\leq$	$X_c - (X_c/4)$
$X_c - (X_c/4)$	$<$	Medium	$<$	$X_c + (X_c/4)$
$X_c + (X_c/4)$	$\leq$	Large	$\leq$	$X_c + (X_c/2)$
		Very large	$>$	$X_c + (X_c/2)$

The values obtained in this way were used as a guide, because only about one or two cells of each tissue were measured and the thickness of the awn was only measured once. The cells of each tissue were measured to determine into which size category they would fall, but if there was some indecision, extra measurements were made, or a subjective decision taken.

By assigning size classes in this way, the size of the cells of a tissue layer were compared with all other tissues of the awn, so that the overall size of the awn was not accounted for in these characters.

It was found that the adaxial epidermis usually has rather small cells when compared with cells of the abaxial epidermis, which are large. The cells of the mesophyll vary from small to large.

(c) Production of the data matrix

The 50 OTU's were examined and their character states scored on coding forms. The real variables, bristle length (Matrix 20) and the ratio b/c (Matrix 21), were adjusted to the 100-900 range and amalgamated with the matrix of discrete characters (Matrix 22) to form a 31 x 50 data Matrix (Appendix 4). (Fig. 25). This matrix has been reproduced in a more readable form with the 50 OTU's rearranged in rows and the 31 characters represented as columns (Table 18).

2. Analysis of Characters

The correlation coefficient was used in the character analysis as it is a useful coefficient for observing the relationships between variables.

(a) Correlation between Characters

An examination of the correlation coefficients allows us to make some interesting observations about the characters. These will be discussed under the following headings:

a. Characters of Size or Related to Size

Thirteen of the 31 characters are, in one way or another, measurements of size of the awn. They may be treated as two sub-groups:

i. Size of the Awn (Characters 1, 6, 19, 21, 26, 30, 31)

The length of the bristle (1), width of the column (6), number of layers of cells of the upper (M1) (19), middle (M2) (21) and lower zones (M3) (26) of the mesophyll must all affect the size of the awn and most of these characters are strongly inter-correlated. In addition, these characters are usually

Table 18: Awn characteristics of *Loudetia* spp. and *Rattraya*.

		CHARACTERS OF THICKENING or related TO THICKENING												CHARACTERS of THICKENING and SIZE				
		Thickening of the Cell Walls					Morphological Characters & related ones				Characters affected by thickening			Size of cells and No. of layers			Xylem Tissue	
		Epidermis		Mesophyll										Size of Cells				
OTU No.	SPECIES	Adaxial (11)	Abaxial (13)	Hypo-dermal zone (14)	Middle zone (22)	Inner zone (24)	Ratio of bristle to column (2)	Column twisting (3)	Point of disarticulation of the awn (4)	Inner bundle sheath (29)	Adaxial epid. passing around margin (9)	Position of vascular bundle (27)	Outer bundle sheath (28)	adaxial epidermis (10)	Middle zone of mesophyll (20)	No. of layers of lower zone of mesophyll (26)	Protoxylem canal (30)	Large meta-vessel (31)
1	L.angolensis	+++	+++	+++	+++	+++	2.309	+	Base	+	+	Outer	-	Small	Medium	1	-	-
2	L.angolensis	++	++	+	+	++	2.309	+	"	+	+	"	+	"	"	1	+	-
3	L.arundinacea	++	++	+	-	++	1.986	+	"	+	-	"	-	"	Small	1	+	+
4	L.arundinacea	+++	++	++	-	++	1.326	+	"	+	-	"	-	"	"	1	+	+
5	L.arundinacea	+++	+++	++	-	++	1.278	+	"	+	+	Middle	-	"	"	1	+	+
6	L.camerunensis	+++	++	++	+	++	2.2057	+	"	+	+	Outer	-	"	Medium	1	+	+
7	L.simplex	++	+++	++	+	++	2.509	+	"	+	+	"	-	Medium	"	1	+	+
8	L.simplex	++	+++	++	+	+++	2.316	+	"	+	-	"	+	"	"	1	+	+
9	L.simplex subsp. stipoides	+++	++	++	+	++	4.083	+	"	+	+	"	+	Small	"	1	+	-
10	L.simplex subsp. stipoides	+++	+++	+++	+++	+++	4.083	+	"	+	-	"	+	"	"	1	+	-
11	L.kagerensis	++	++	+	+	+	2.177	+	"	+	+	"	+	"	"	1	+	+
12	L.kagerensis	+++	+++	++	-	+++	2.750	+	"	+	+	"	+	"	Small	1	+	+
13	L.perrieri	++	++	+	+	+	1.473	+	"	+	-	"	+	"	Medium	1	+	+
14	L.madagascariensis	+++	++	+	+	++	1.921	+	"	+	-	"	-	"	"	1	+	[illegible]
15	L.madagascariensis	+++	++	++	+	++	2.178	+	"	+	+	"	+	"	Small	1	+	
16	L.jaegeriana	++	++	+	-	+	2.10	+	O	-	-	"	+	"	Medium	1	-	-
17	L.lanata	+++	++	++	-	++	3.651	+	Base	+	+	"	+	"	"	1	+	[illegible]
18	L.longipes	++	++	++	+	++	4.980	+	"	+	+	"	+	Medium	"	1	+	
19	L.demeusei	+++	+++	+++	-	+++	2.662	+	"	+	-	"	+	Small	Small	1	+	[illegible]
20	L.crassipes	+++	+++	++	-	+++	2.762	+	"	+	+	"	-	"	"	1	+	-
21	L.pennata	+++	++	++	-	++	2.224	+	"	-	-	"	+	"	Medium	1	+	-
22	L.pennata	+++	++	+	-	++	2.224	+	"	+	+	"	+	"	Small	1	+	-
23	L.flavida	++	++	++	-	++	1.021	+	"	+	-	"	-	"	"	1	+	-
24	L.flavida	+++	+++	+++	++	+++	1.293	+	"	+	O	"	+	Medium	Medium	1	+	-
25	L.flavida	++	++	++	+	++	1.021	+	"	+	+	Middle	+	Small	"	1	+	-
26	L.migiurtina	+++	++	++	-	++	1.30	+	"	+	+	Outer	+	"	"	1	-	-
27	L.acuminata	++	++	+	+	++	1.237	+	"	+	-	"	+	Medium	"	1	+	-
28	L.acuminata	+++	+++	+++	+++	+++	1.237	+	"	+	+	"	-	Small	"	1	+	-
29	L.[illegible]	++	++	+	-	++	1.20	+	"	+	-	"	-	Medium	"	1	-	-
30	L.filifolia subsp. humbertiana	+++	++	++	-	++	1.452	+	"	+	-	"	+	"	Small	1	+	
31	L.filifolia subsp. humbertiana	++	++	++	-	++	1.452	+	"	+	-	"	+	"	"	1	-	
32	L.gossweileri	+++	++	++	+	++	4.490	+	"	+	+	"	+	Small	Medium	1	+	
33	L.densispica	++	++	++	+	++	4.490	+	"	+	+	"	+	"	"	1	+	
34	L.echinulata	+++	++	++	-	+	2.467	+	"	+	+	"	+	"	Small	1	+	
35	L.coarctata	+++	++	++	-	+	2.402	+	"	+	+	"	-	"	Medium	1	+	
36	L.densispica	+++	+++	++	+	++	4.174	+	"	+	+	"	+	"	Small	1	+	
37	L.tisserantii	++	++	+	-	+	2.1086	+	"	+	-	"	+	"	"	1	-	
38	L.vanderystii	++	++	+	+	+	1.903	+	"	+	+	Middle	+	Medium	Medium	1	-	
39	L.cereta	++	++	++	+	++	2.0833	+	"	+	+	"	+	"	"	1	-	
40	L.annua	+++	++	++	+	++	2.420	+	"	+	+	Outer	+	Small	Small	1	+	
41	L.bidentata	++	++	++	+	++	3.477	+	"	+	-	"	+	"	"	1	+	
42	L.hordeiformis	+++	+++	+++	++	+++	3.183	+	"	+	-	"	+	"	"	1	+	
51	L.flammida	+++	+++	++	++	++	12.812	-	Top	-	-	"	-	Medium	"	1	-	
43	L.phragmitoides	+++	+++	+++	+++	+++	6.176	-	Top	-	-	"	-	Small	"	1	-	
44	L.simulans	+++	+++	+++	+++	+++	1.327	+	Base	+	+	"	+	Medium	Medium	2 or more	-	
45	L.anomala	+++	+++	+++	+	+++	3.412	+	"	+	-	"	-	"	"	2 or more	-	
46	L.anomala	+++	+++	+++	++	+++	3.412	+	"	+	-	"	-	"	"	2 or more	-	
47	L.anomala	+++	+++	+++	-	+++	3.412	+	"	-	+	"	+	"	"	2 or more	-	
48	L.ramosa	+++	+++	+++	+	++	1.996	+	"	+	-	"	+	"	"	1	-	
49	L.togoensis	+++	+++	+++	++	+++	3.766	+	"	+	-	"	-	"	"	2 or more	+	
50	R.petiolata	+++	+++	+++	+	+++	3.138	+	"	+	-	"	-	"	"	2 or more	-	

	Miscellaneous		Main Characters				CHARACTERS of SIZE or related to SIZE					Miscellaneous			
				Numbers of layers of Cells		Shape of the margins (7)	Morphological charac. & related char.			Distribution of Thickening					
rge metaxylem vessels (31)	Size of cells of lower zone of mesophyll (2)	Shape above the vascular bundle (8)	Thickness of awn (6)	Hypodermal zone of mesophyll (19)	Middle zone of mesophyll (21)		Bristle length (1)	Shape in cross-section (5)	Size of cells of upper mesophyll (15)	upper zone of mesophyll (17)	lower zone of mesophyll (25)	Type of thickening of upper zone of mesophyll (19)	Size of cells of abaxial epidermis (12)	Hairs on abaxial epidermis (14)	OTU No.
-	Medium	Rounded	Thin	1	1	Pointed	16.09	Terete	Medium	External	External	Arched	Medium	-	1
-	"	"	"	1	1	"	16.09	U-shaped	"	"	Even	"	Large	-	2
+	"	"	"	1	1	"	21.25	Terete	Large	"	"	"	"	+	3
+	"	"	"	1	1	"	21.08	U-shaped	"	"	"	"	"	+	4
+	"	"	"	1	1	"	24.17	"	"	"	"	"	"	+	5
+	"	"	Moderate	1	2	Rounded	26.4	"	"	"	"	"	"	-	6
+	"	"	"	1	1	"	40.27	"	"	"	"	"	"	-	7
+	"	"	"	1	2	"	39.38	"	"	"	"	"	"	+	8
-	"	"	"	1	1	"	46.65	Terete	"	"	"	"	"	-	9
-	"	"	"	1	1	Pointed	46.65	U-shaped	"	"	"	"	"	+	10
+	"	"	Thin	1	1	Rounded	25.09	Terete	"	"	"	"	"	-	11
+	"	"	"	1	1	"	24.02	U-shaped	Medium	"	"	"	"	-	12
+	"	Arc	Moderate	1	1	"	22.2	"	"	"	"	"	Very Large	-	13
+	"	"	Thin	1	1	"	23.66	"	"	"	"	"	" "	-	14
+	Small	"	"	1	1	Pointed	23.38	"	Large	"	"	"	" "	-	15
-	Medium	Rounded	"	1	1	Rounded	12.0	Curved	Medium	Even	"	"	" "	+	16
+	Small	"	"	1	1	Pointed	42.36	Terete	"	External	"	"	" "	-	17
-	Medium	"	Moderate	1	1	Rounded	49.8	"	Large	"	"	"	Large	-	18
+	"	"	Thin	1	1	"	41.6[illegible]	U-shaped	"	"	External	"	Very Large	-	19
-	Small	"	"	1	1	"	29.0	"	Medium	"	"	"	" "	-	20
-	Medium	"	"	1	1	Pointed	19.1[illegible]7	U-shaped	"	"	Even	"	" "	-	21
-	"	"	"	1	1	Rounded	19.1[illegible]7	"	"	"	"	"	" "	-	22
-	"	"	"	1	1	"	15.1[illegible]	"	"	"	"	"	" "	-	23
-	Large	"	"	1	2	O	15.0[illegible]	"	Large	"	External	"	Large	-	24
-	Medium	"	"	1	2	Rounded	15.1[illegible]	"	Medium	Even	Even	"	"	-	25
-	"	"	"	1	2	"	19.05	"	"	External	"	"	Medium	-	26
-	Large	"	"	1	2	Pointed	17.3[illegible]	Terete	Large	"	"	"	Large	-	27
-	Medium	"	"	1	1	Rounded	17.3[illegible]	U-shaped	Medium	"	External	"	Very Large	-	28
-	"	"	"	1	2	"	10.8	"	"	"	Even	"	Large	-	29
-	"	"	"	1	1	Pointed	14.77	"	"	"	"	"	Very Large	-	30
-	"	"	"	1	1	"	14.77	Terete	"	"	"	"	" "	+	31
-	"	"	"	1	1	Rounded	45.8	"	"	"	"	"	Large	+	32
+	"	"	Moderate	1	2	Pointed	45.8	U-shaped	Large	"	"	"	"	+	33
+	"	"	"	1	3	"	56.0	"	Medium	"	"	"	Very Large	-	34
+	"	"	"	1	2	"	25.97	"	"	"	"	"	" "	-	35
+	Large	"	Thin	1	1	"	48.0	"	Large	"	External	"	" "	-	36
-	Medium	"	Moderate	1	2	"	36.9	Terete	"	"	Even	"	" "	-	37
-	"	"	"	1	2	"	10.2[illegible]	U-shaped	Medium	"	"	"	Large	+	38
-	"	Arc	"	1	1	Rounded	37.5	Terete	"	"	"	"	"	+	39
+	"	Rounded	"	1	1	"	78.8[illegible]	"	Large	"	"	"	Very Large	-	40
+	"	"	"	1	1	"	92.4[illegible]	"	"	Even	"	"	" "	-	41
+	"	"	Thin	1	1	"	73.3[illegible]	"	"	External	"	"	" "	-	42
-	"	"	"	1	1	"	16.585	Curved	Medium	"	External	"	" "	-	61
-	"	"	"	1	1	"	8.88	U-shaped	"	"	"	"	Large	-	43
-	"	"	"	1	1	Pointed	6.90	"	"	"	"	Flat	"	+	44
-	"	"	"	1	1	Rounded	7.90	Curved	"	"	Even	"	"	+	45
-	"	Arc	"	1	2	"	7.90	"	"	"	External	"	"	+	46
-	"	"	"	1	2	"	7.90	"	"	"	Even	"	"	+	47
-	"	"	"	1	1	"	6.976	U-shaped	"	"	"	"	"	+	48
+	"	Rounded	Thick	2 or more	1	Pointed	110.27	Terete	"	"	"	"	Medium	+	49
-	Large	"	Thin	1	2	Rounded	10.845	Curved	"	"	"	"	Large	+	50

correlated with the characters of the vascular bundle, viz. presence or absence of the protoxylem canal (30) and large metaxylem vessels (31), since awn size affects vascular bundle size.

ii. Size of the Cells (Characters 10, 12, 14, 15, 20, 23)

There is not always positive correlation between the size of the cells of the different tissues. The size of the cells of the adaxial epidermis (10) is positively correlated with the size of the middle zone of the mesophyll (M2) (20), and the size of the cells character for the abaxial epidermis (12) and (M2) (20) are strongly negatively correlated. The size of the cells of the abaxial epidermis (12) is positively correlated with the absence of hairs in this region (14) so that one would not expect the very large celled epidermis to have hairs. Other characters under this heading are the size of the cells of the upper (15) and lower (23) zones of the mesophyll.

b. Characters of Thickening of the Cell Walls

i. The Amount of Thickening (Characters 11, 13, 16, 22, 24)

Five characters describe the amount of thickening of the cells of the different tissues, viz. 11 - adaxial epidermis, 13 - abaxial epidermis, 16 - upper zone, 22 - middle, and 24 - lower zone of the mesophyll. These characters are all highly positively correlated confirming that when thickening is abundant in one tissue, it is abundant in the others as well. Some of these characters show an appreciable negative correlation with the type of thickening in the upper zone of the

mesophyll (18). This predicts that the OTU's which have the straight type of thickening of the lower mesophyll cells usually have highly thickened cell walls.

ii. Distribution of Thickening (Characters, 17, 25)

The character describing the distribution of thickening of the upper zone of the mesophyll (17) does not show any noteworthy relationship with other characters. The distribution of thickening in the M3 layer (25) however, is negatively correlated with most of the characters describing the amount of thickening, so it would appear that when the cells have thickened walls the M3 layer has the thickening distributed more to the outside of the awn.

iii. Type of Thickening (Character 18)

This character is only defined for the upper zone of the mesophyll (18), and shows a positive correlation with the shape of the column in cross-section (5) (because the curved awns have this type of thickening) and the presence of hairs on the abaxial epidermis (14). It is negatively correlated with a number of characters connected with size (e.g. 10) and amount of thickening of the cells (13, 16). Cells with this type of thickening have thick walls.

c. Shape and Dimensions of the Awn and Related Characters (Characters 2, 3, 4, 5, 29)

The ratio b/c (2), twisting of the column (3) disarticulation of the awn (4) and presence or absence of an inner bundle sheath (29) are strongly positively correlated. The shape of the awn in cross-section (5) is not positively assoc-

iated with these characters.

d. Miscellaneous Characters (Characters 7, 8, 9, 27, 28)

Five of the characters do not appear to be very strongly positively or negatively correlated with any of the others. These are the shape of the margins of the awn (7) the shape of the epidermis below the vascular bundle (8), whether or not the adaxial epidermis passes around the margin of the awn (9) and the characters of the vascular bundle viz. its position (27) and whether the outer bundle sheath is complete or not (28).

(b) Principal Components Analysis

The correlation coefficient matrix (No. 28) was subjected to a component analysis and the roots (eigenvalues) obtained expressed in Table 19, and the eigenvectors in Matrix 30.

The character scores were plotted on the first two vectors of Matrix 30 (Fig. 28) and the percentage efficiency of the axes in accounting for the variation is also given in Table 19. The thirty-one characters are represented in the scatter diagrams by symbols following the convention of grouping the characters described above. The proportion of each root that the characters account for was calculated. From this information, the percentages of the first five roots that the different character groups account for were calculated, and also the percentage of the total variation accounted for by these groups of characters (Table 20). Only the first five roots were considered because it is found that they account for over 55% of the variation (Table 47, Appendix 4).

Table 19: Roots of correlation coefficient matrix 28

Root	Value of root	Percentage of total variance accounted for	Accumulated percentage
1	5.925	19.11	19.11
2	3.673	11.85	30.96
3	3.332	10.75	41.71
4	2.218	7.15	48.86
5	1.882	6.07	54.93
6	1.567	5.06	59.99
7	1.479	4.77	64.76
8	1.291	4.17	68.92
9	1.251	4.03	72.96
10	1.105	3.56	76.52
11	0.958	3.09	79.61
12	0.893	2.88	82.50
13 - 31	5.426	17.50	100.00
Total Variance	31.000	100.00	-

Table 20: Percentage of the root and the total variation accounted for by the groups of awn characters.

Characters	Percentage of Root					Percentage of Total				
	Root No.					Root No.				
	1	2	3	4	5	1	2	3	4	5
1. Size										
(a) Size of awn	21.51	15.03	66.63	15.69	13.81	4.12	1.77	7.23	1.13	0.84
(b) Size of cells	16.28	24.40	8.61	7.83	20.56	3.12	2.88	0.93	0.56	1.25
Total	37.79	39.43	75.24	23.52	34.37	7.24	4.65	8.16	1.69	2.09
2. Thickening										
(a) Amount of thickening	34.90	17.63	5.62	16.66	12.13	6.69	2.08	0.61	1.20	0.74
(b) Distribution of thickening	4.55	7.53	1.30	3.15	6.42	0.87	0.89	0.14	0.23	0.39
(c) Type of thickening	10.69	3.08	0.56	1.35	3.43	2.05	0.43	0.06	0.10	0.21
3. Shape of awn	8.18	26.33	14.80	47.12	16.25	1.57	3.11	1.61	3.39	0.99
4. Miscellaneous characters	3.89	6.01	2.48	8.19	27.37	0.75	0.71	0.27	0.59	1.67

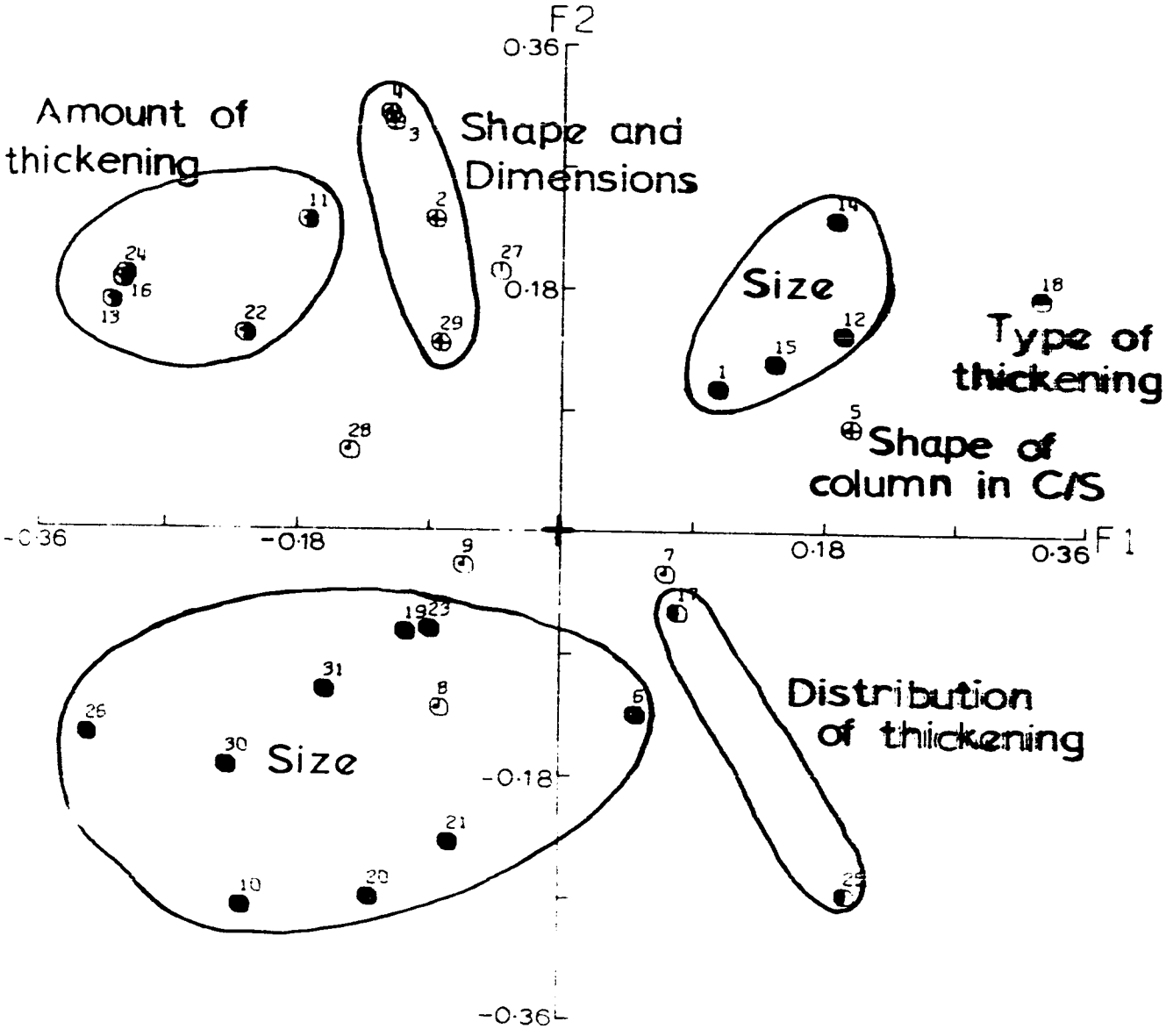


Figure 28: Scatter diagram showing the projection of awn characters on the first two vectors produced by component analysis of correlation matrix 18. To identify characters, see Table 44, Appendix 4.

From Table 31, it will be noticed that characters of size and amount of thickening of the cell walls are important on the first principle axis. They account for 37.79% and 34.9% of the variation of the first root, i.e. 7.24% and 6.69% of the total variation respectively. The characters describing thickening have high negative scores on the first axis, and high positive scores on the second axis, and thus are well clustered (Fig. 28). The characters related to size are in two groups when plotted on the first two vectors, with either positive scores on both axes or negative scores on both. The type of thickening (Character 18) is also an important one, being responsible for over 10% of the variance on the first root and it is seen to have the highest positive score on the first axis.

When the second root is examined, it will be noted that the important characters are those of the shape of the awn (26.33%), size (39.43%), and amount of thickening of the cells (17.63%).

Most of the characters describing the shape of the awn are clustered with high positive scores on the first axis. An exception is the character describing the shape of the column in cross-section (5) which is closer to some of the characters of size (Fig. 28).

The characters associated with the size of the awn are largely responsible for the variation of the third root, viz. 66.63% of the root and 7.23% of the total variation. Characters describing the shape of the awn (47.12%), size (23.52%)

and amount of thickening of the cells (16.66%) are of importance when the fourth root is considered.

The miscellaneous characters are not usually of much significance. Characters describing the shape of the margins (7), shape of the awn above the vascular bundle (8) and the outer bundle sheath (28) particularly appear to be very insignificant in this study. The character describing whether the adaxial epidermis passes around the margin or not (9) is, however, important on the fifth root (17.64%).

(c) Classification of characters

The correlation matrix (No. 28) was transformed so that the values gave a distance measure (see chapter 3) and a classification of the characters was formed using the sum of squares agglomeration technique. The results of this analysis are shown in the dendrogram (Fig. 29).

At the 10% level clusters of characters may be noted which approximately correspond to the groupings previously formed. Characters measuring shape and dimensions, amount of thickening of the cell walls, and distribution of the thickening all form groups. Some of the miscellaneous characters cluster together whereas others are solitary or cluster with other character groups. Characters describing size occur in four different clusters.

The results of the character analysis, therefore reveal that there are fairly distinct groups of characters of the awn which have varying degrees of importance in an analysis of this

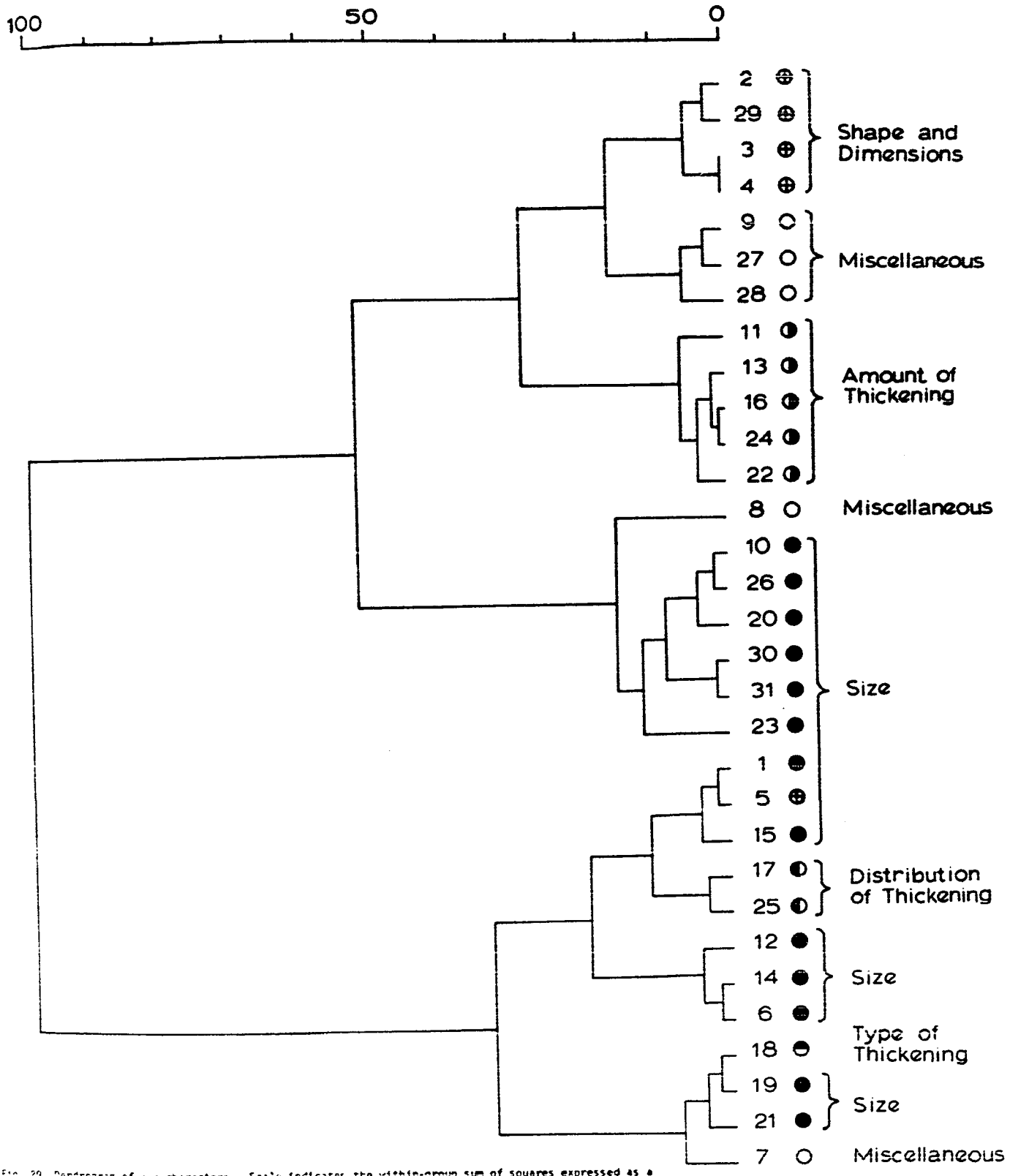


Fig. 29 Dendrogram of own characters. Scale indicates the within-group sum of squares expressed as a percentage of the total sum of squares. To identify characters see Table 44 (Appendix 4).

sort. The effect of these characters will be appreciated when an OTU analysis of the same data is performed.

3. Analysis of OTU's

(a) Principal Components analysis

(i) Correlation coefficient

The component scores for the OTU's (Matrix 31) were calculated indirectly from the adjusted scores (Matrix 29) and the transformation coefficients (Matrix 30) of the correlation matrix 28 (Fig. 25). The position of the OTU's in the space determined by the first five components is illustrated in the scatter diagrams (Fig. 30 A-E).

The section Pleioneura (44-48), Rattraya (50), the section Lophanthera (49) and L. phragmitoides (43) are quite well separated on the first component (Fig. 30A). Most of the section Loudetia is tightly clustered with negative scores on the first component, illustrating the strong homogeneity of the section with respect to awn characters. Exceptions are L. angolensis (1), L. simplex, subsp. stipoides (10), L. flavida (24) and L. acuminata (28), which have higher component scores than their nearest relatives. In fact all these species are represented by exemplars in the main group of the section also, viz. OTU Nos. 2, 9, 23 and 27 respectively.

L. phragmitoides (43, subsection Flammidae) has a number of peculiar characters of shape and it is isolated with a high negative component score on the second component (Fig. 30A, 30B). Other OTU's which are noticeable with high negative scores on the second axis are those of the Annuae (39-42)

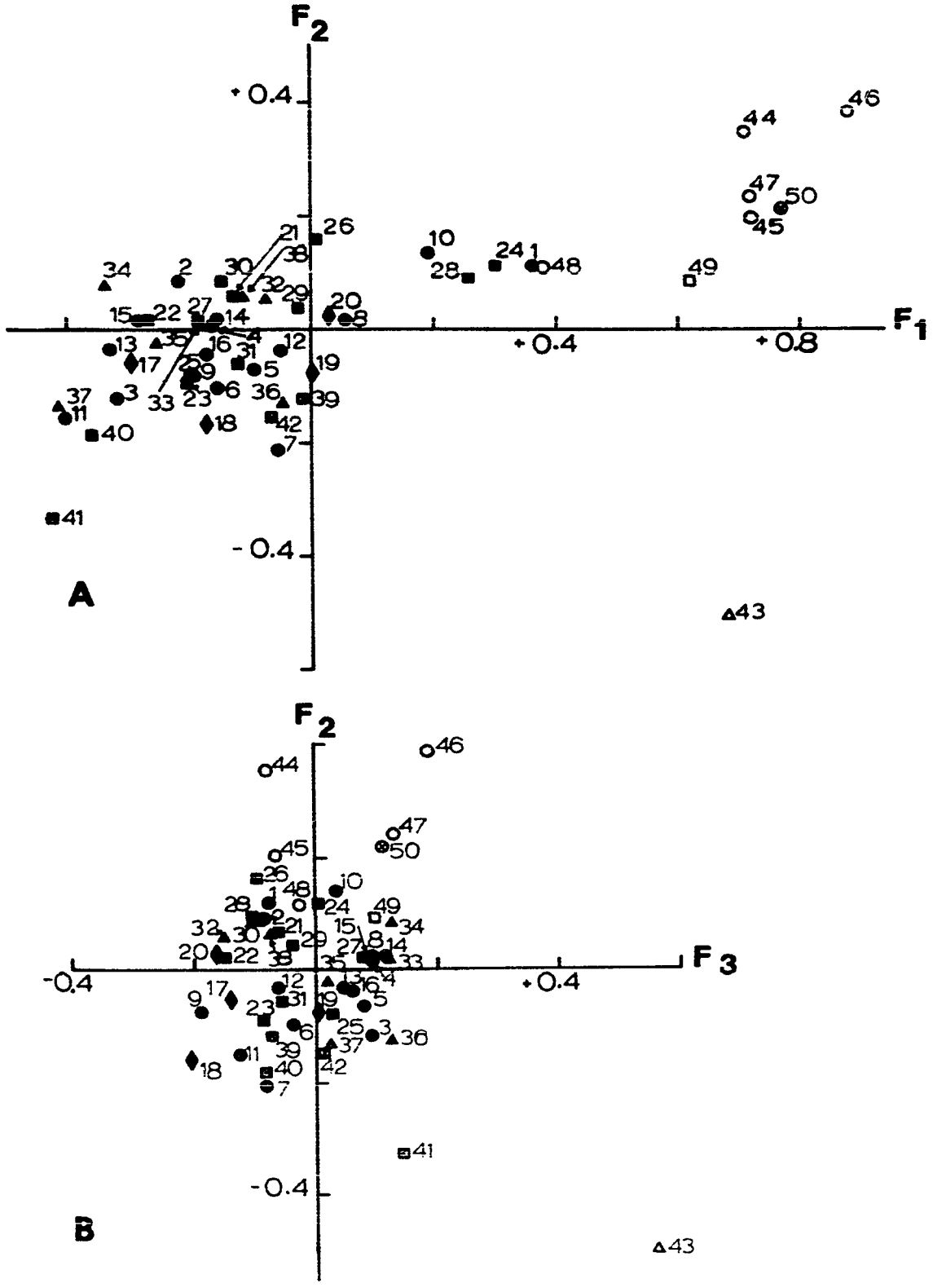
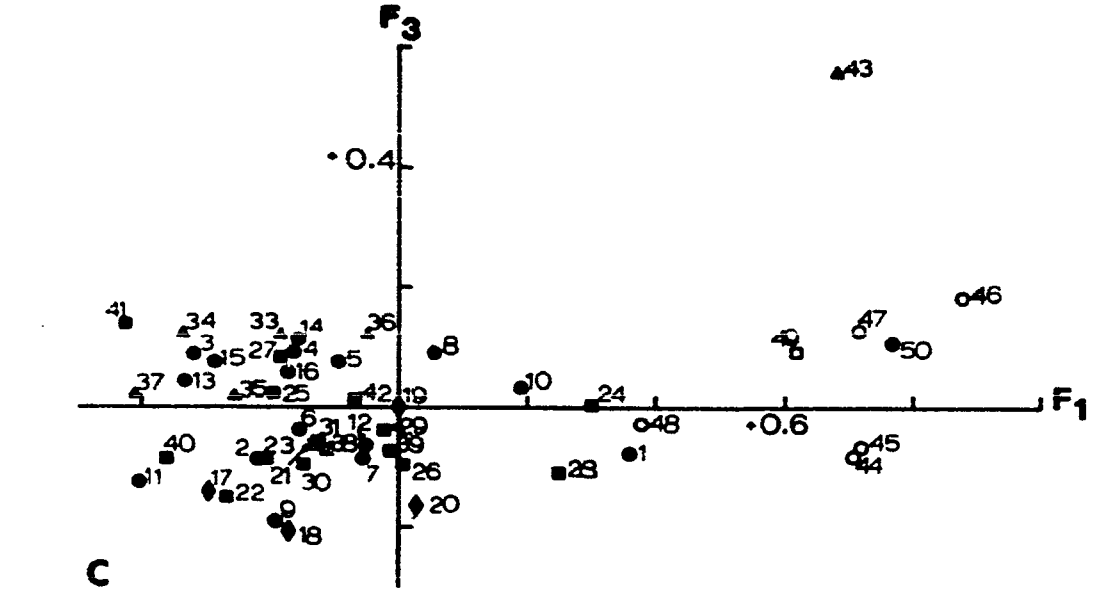
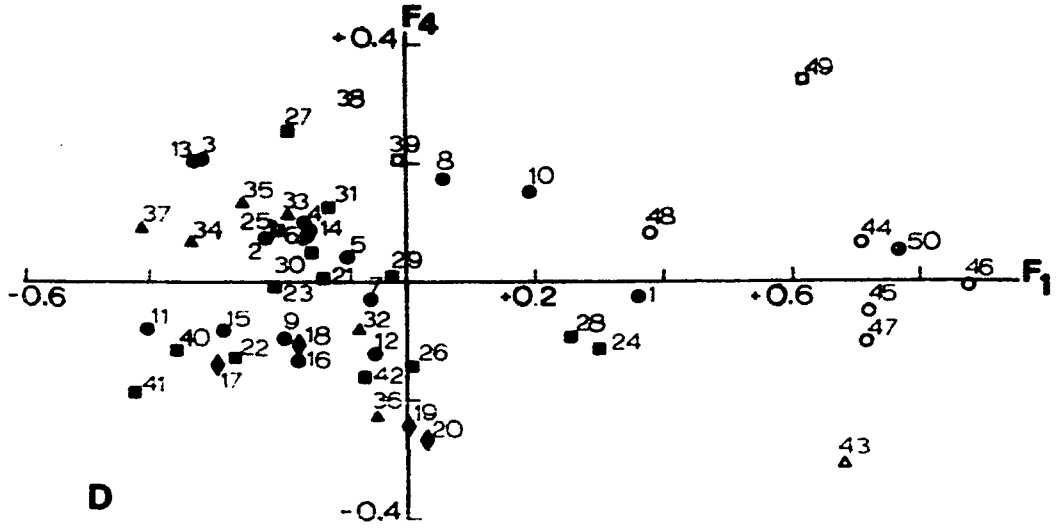
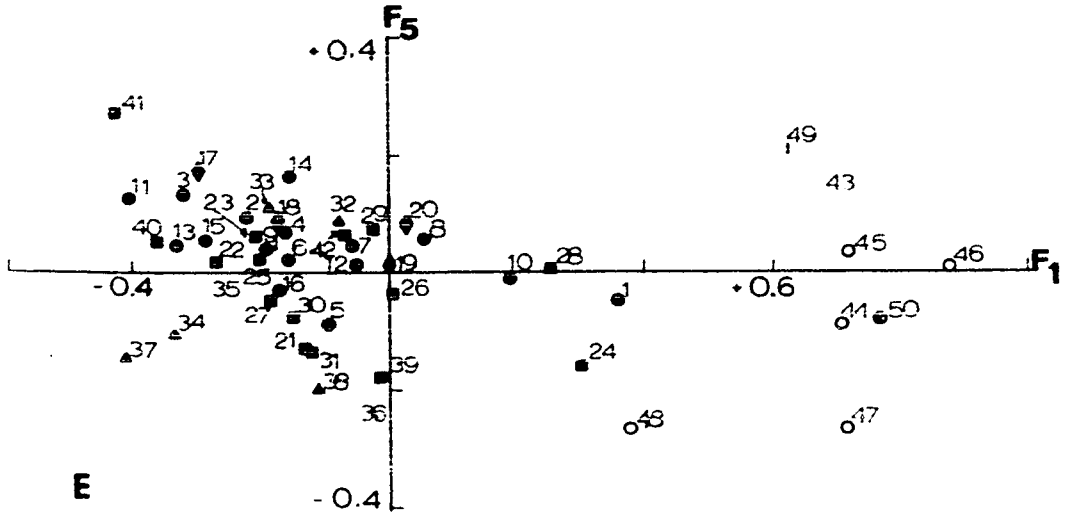


Fig. 30 A-E: Scatter diagrams showing the distribution of OTU's on the first five components of a components analysis of correlation matrix 28, derived from awn anatomy characters.
For legend see Table 17.


F3
F1
+0.4
+0.6
C


F4
F1
+0.4
-0.4
-0.6
+0.2
+0.6
D


F5
F1
+0.4
-0.4
+0.6
E

(Fig. 30A, B). The other components also emphasize the distinctions seen above. The subsections of the section Loudetia are in general always in a single cluster and little differentiation between them may be noted. One exception to this other than the Flammidae and Annuae mentioned above, is the Pungentes (17-20) which tend to have high negative scores on the F_4 (Fig. 30D).

(ii) Dispersion Coefficient

The dispersion matrix (No. 24) was subjected to a component analysis to obtain the characteristic roots (Table 21) and the component scores for OTU's (Matrix 25). Scatter diagrams were drawn showing the position of the OTU's with respect to the first four components (Figs. 31 A-D). The percentages of each of the first five roots accounted for by these characters were calculated (Table 48, Appendix 4).

The first component accounts for over 20% of the variation, the major effects being due to characters of size (58.81%) and amount of thickening of the cell walls (25.63%). The section Pleioneura (44-48) and Rattraya (50) are quite distinct with high positive scores on this component. The section Lophanthera (49) is also separate from the section Loudetia (1-43) which is somewhat variable.

On the second component, which accounts for 13.78% of the total variation, the OTU's of section Loudetia are more spread out. One group of OTU's has high positive component scores, viz. L. jaegeriana (16), L. cuanzensis (29), L. filifolia, subsp. humbertiana (31), L. vanderystii (38) and L. cerata (39),

Table 21: Roots of dispersion coefficient matrix 24

Root	Value of root	Percentage of total variation accounted for.	Accumulated percentage
1	28,841.877	22.144	22.144
2	17,932.717	13.768	35.912
3	11,901.340	9.137	45.049
4	10,566.552	8.113	53.162
5	8,676.750	6.662	59.823
6	7,711.558	5,921	65.744
7	6,616.016	5.080	70.824
8	4,684.228	3,596	74.420
9	4,382.419	3,365	77.785
10	3,767.489	2,893	80.678
11	3,560.793	2,734	83.411
12	3,402.164	2,612	86.023
13 - 31	18,204.195	13,977	100.00
Total sum of squares	130,248.098	100.000	-

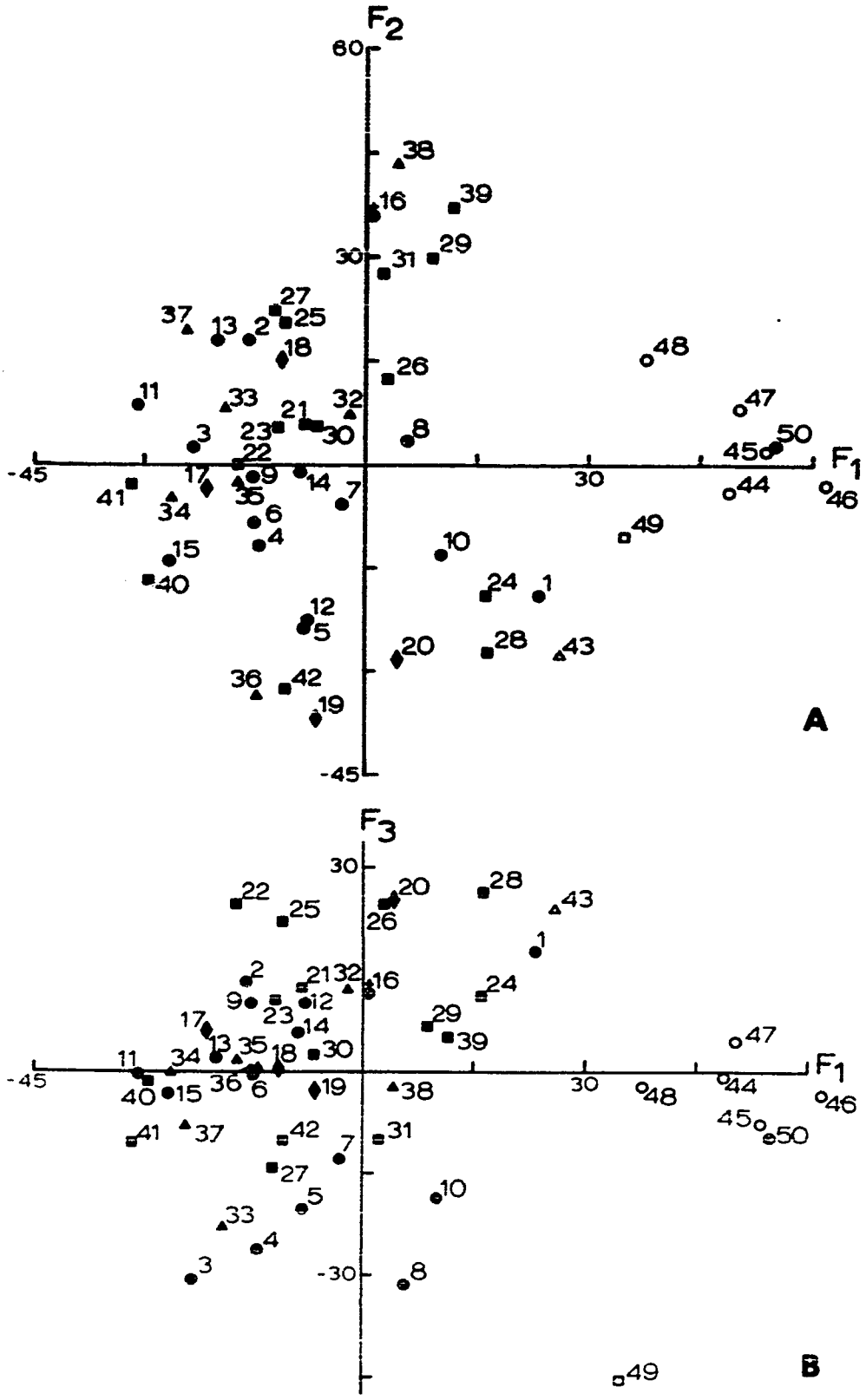
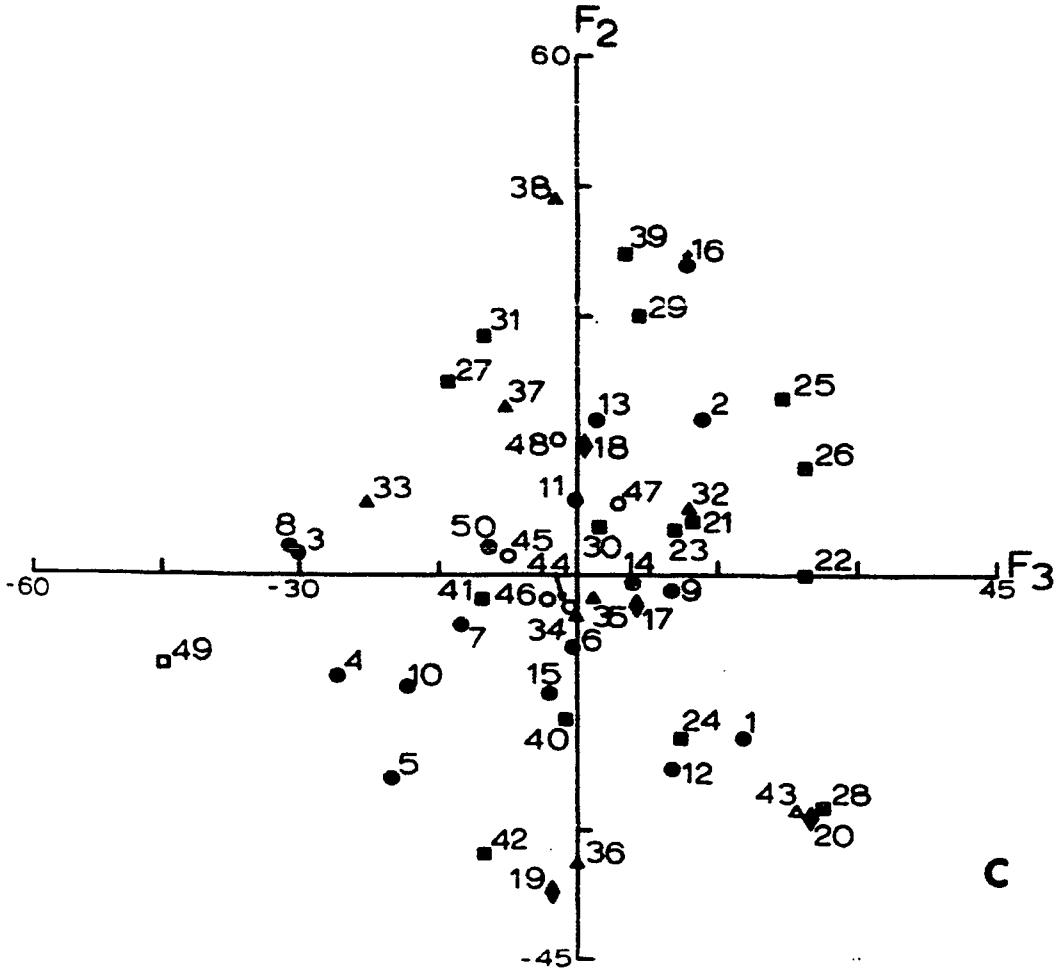
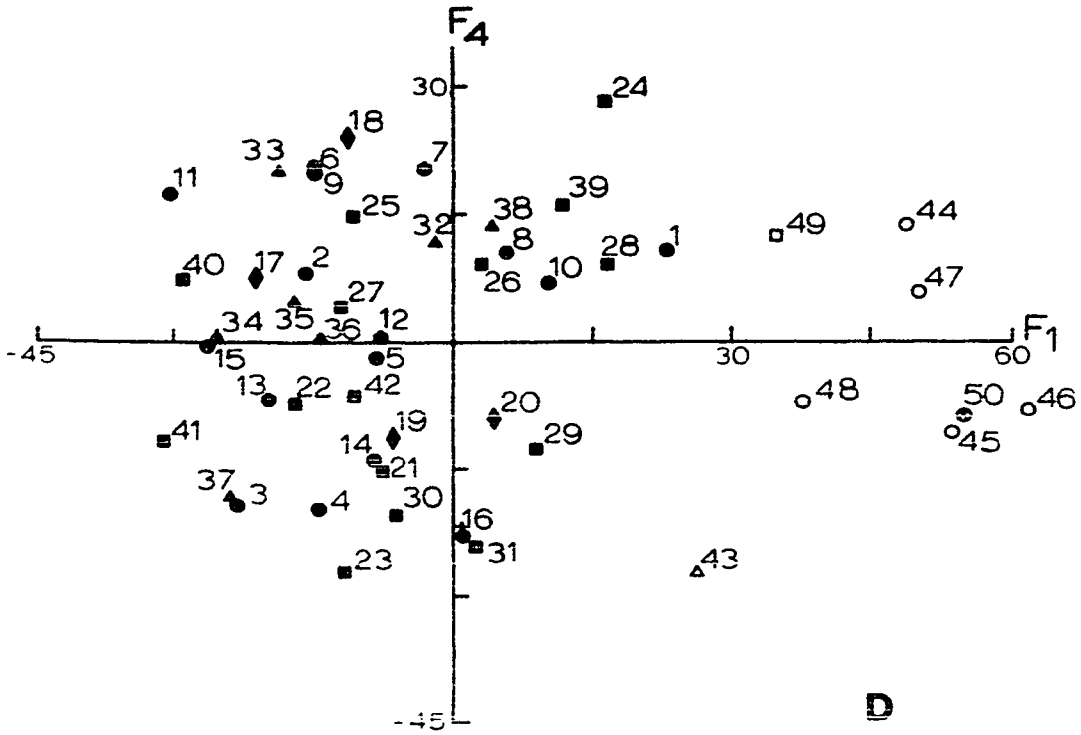


Fig. 31 A-D. Scatter diagrams showing the distribution of OTU's on the first four components of a component analysis of the dispersion matrix 25, derived from own characters. For legend see Table 17.


F2
60
38
39
16
29
31
27
37
13
2
25
48
18
26
33
11
47
32
8
3
50
45
30
21
23
44
14
22
F3
-60
-30
41
46
9
45
34
35
17
7
6
49
4
10
15
40
24
1
5
12
43
28
20
42
36
19
-45
C


F4
30
24
18
33
7
6
9
11
25
39
38
32
8
49
44
40
17
2
28
1
26
10
47
27
34
35
36
12
F1
-45
15
5
30
60
13
22
42
48
50
46
20
41
19
29
14
21
45
37
3
4
30
16
31
23
43
-45
D

and another group has high negative component scores, viz. L. demeusei (19), L. densispica (36), L. hordeiformis (42), etc., which are separated from the main body of the OTU's of section Loudetia. The characters that are important in accounting for the variation on this axis are size (40.47%) and the amount of thickening of the cell walls (47.81%). Presumably, the odd groups of OTU's have some of these characters in common, but this is not very obvious (Plates 18-24).

It will be noted that L. togoensis (49) with a high negative component score on the third component (Fig. 31B) is separated from other OTU's. The exemplars representing L. arundinacea (3-5) and L. angolensis (1, 2) are not as far apart on this component as they are on others (Fig. 31C). The exemplars of a particular species often do have a number of characters in common, although the results may not always indicate this because the differences are magnified.

Some of the miscellaneous characters are very important in describing the variation on the first five components in this analysis. The shape of the margins (7) and the nature of the adaxial epidermis (9) account for 10.38% and 13.28% of the variance of the third root, respectively. They are equally important on the fifth root. The latter character (9) also accounts for 47.86% of the variance of the fourth root.

(b) Classification

The same approach was made in classifying the OTU's as used previously. Firstly, the component scores of the principal component analysis using the correlation coefficient

(Matrix 31), secondly the component scores using the dispersion coefficient (Matrix 25) and finally the data matrix (No. 23) were used as data matrices to calculate the absolute distance matrices 34, 26 and 32 respectively (Fig. 25). The distance matrices were subjected to the sum of squares agglomeration method of cluster analysis and the results are presented as dendrograms (Fig. 32 A-C).

(i) Classification resulting from the use of the component scores derived from the correlation coefficient matrix, as the data matrix (Fig. 32A)

This dendrogram may be compared with the distribution of the OTU's on the first few components as indicated in the scatter diagrams (Fig. 30 A-E). The two major groups in this classification roughly correspond to the distribution of the OTU's on the first component. Pleioneura (44-48), Rattraya (50) and Lophanthera (49) form the major part of this group together with some of the unusual OTU's of Section Loudetia. The other large group of OTU's consists of all the remaining OTU's of section Loudetia. The awns of this section are all very similar and not a great deal can be detected from the classification.

There is a fairly good exchange of information from the distance matrix to the dendrogram. The cophenetic correlation coefficient was calculated to be 0.674.

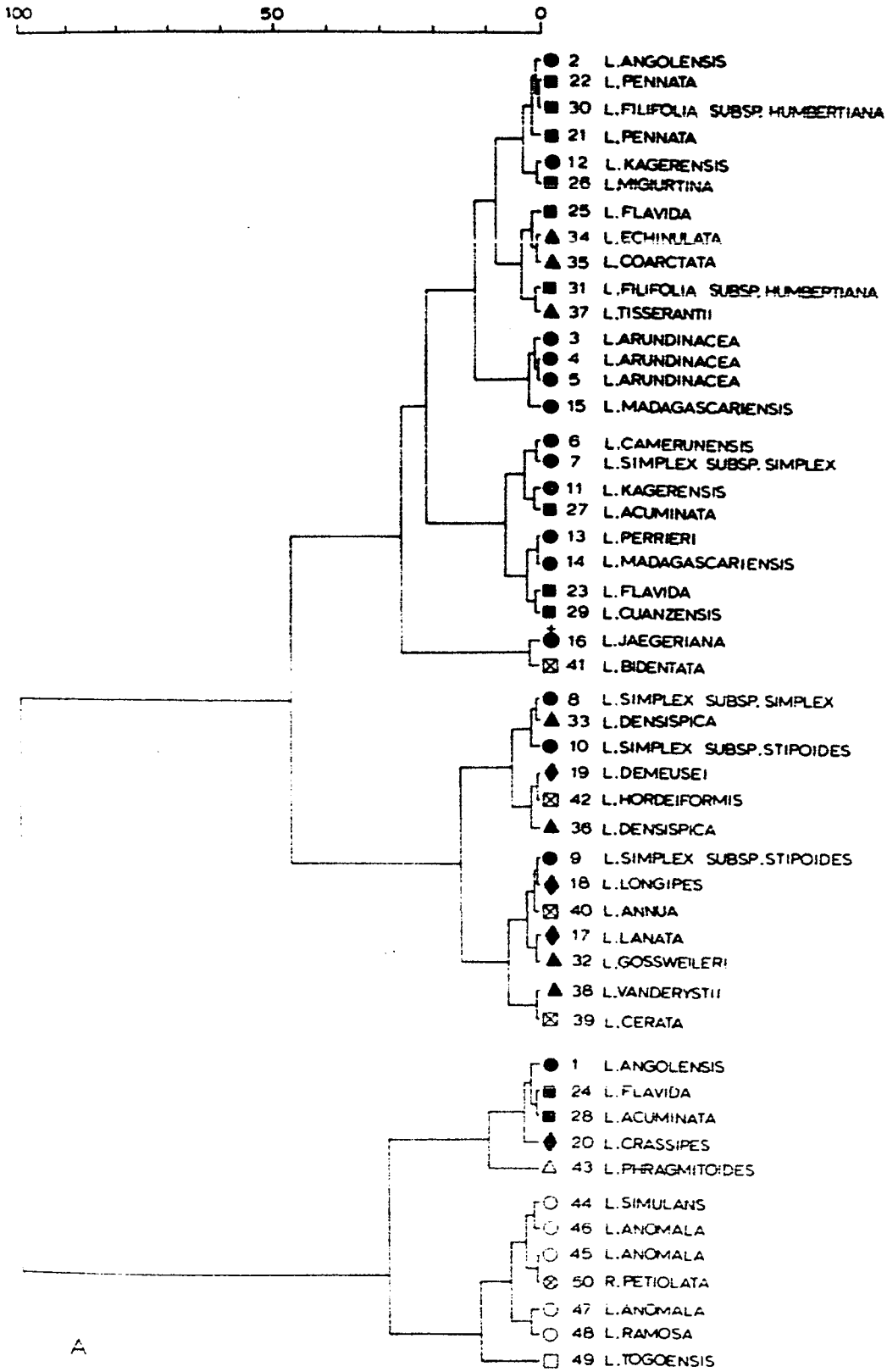
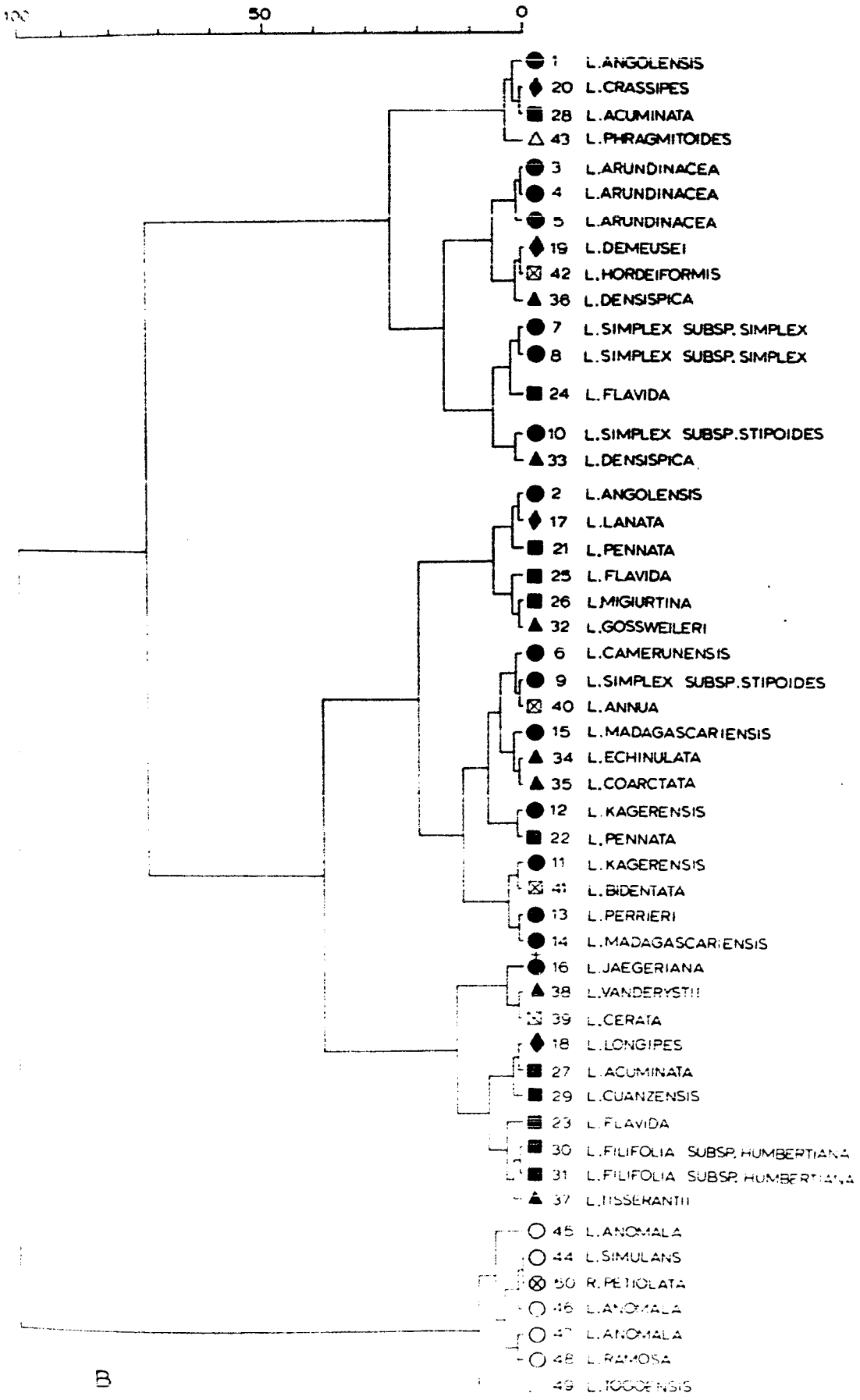
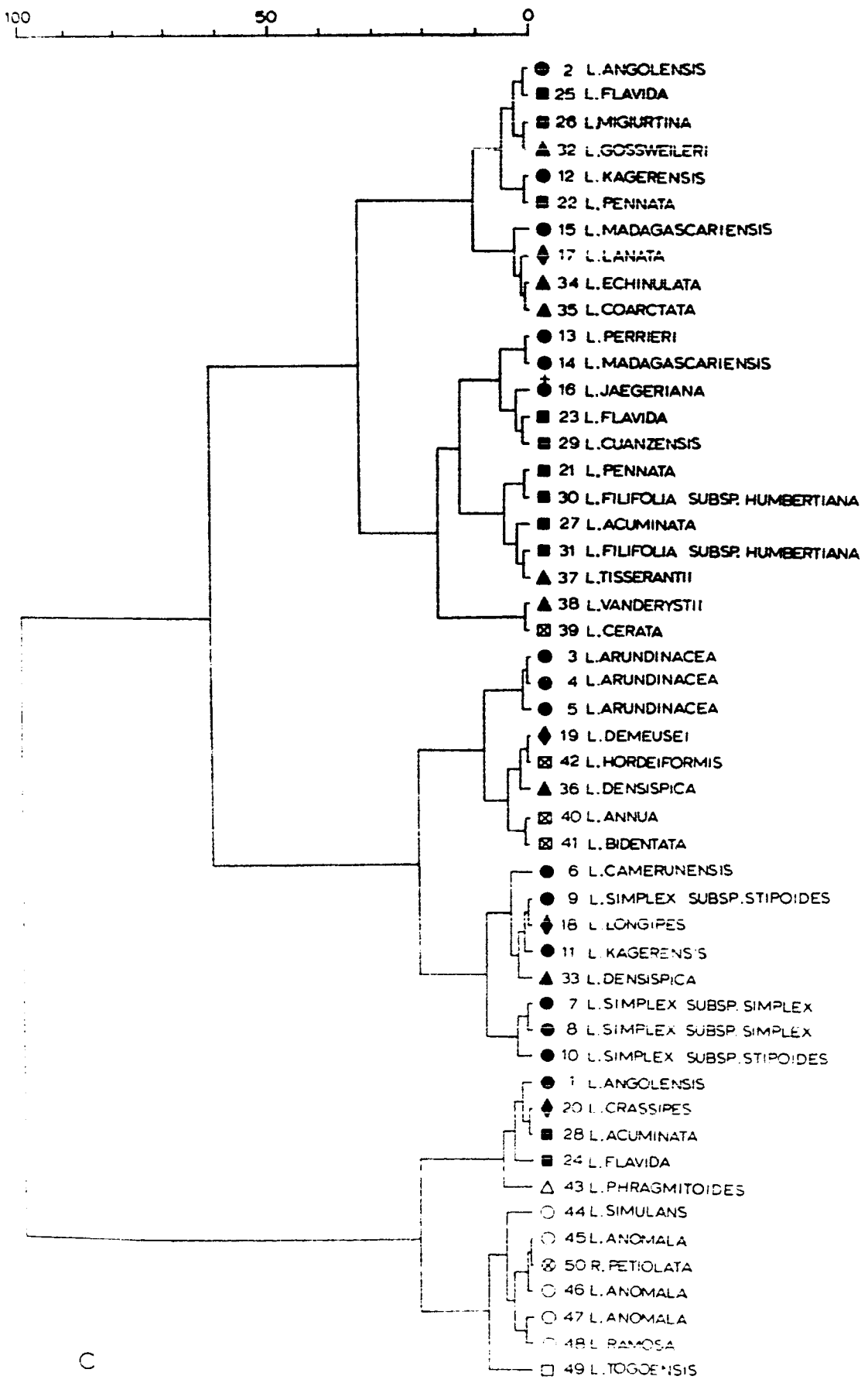


Fig. 32 Dendrograms of 50 OTU's of Loudetia and Rattraya derived from awn characters using three different data matrices: A-Component scores matrix derived by component analysis of the correlation matrix; B-Component scores matrix derived by component analysis of the dispersion matrix; C-Data matrix 23.
Scale indicates the within-group sum of squares expressed as a percentage of the total sum of squares. For legend see Table 17.


100
50
0
1 L.ANGOLENSIS
20 L.CRASSIPES
28 L.ACUMINATA
43 L.PHRAGMITOIDES
3 L.ARUNDINACEA
4 L.ARUNDINACEA
5 L.ARUNDINACEA
19 L.DEMEUSEI
42 L.HORDEIFORMIS
36 L.DENSISPICA
7 L.SIMPLEX SUBSP. SIMPLEX
8 L.SIMPLEX SUBSP. SIMPLEX
24 L.FLAVIDA
10 L.SIMPLEX SUBSP.STIPOIDES
33 L.DENSISPICA
2 L.ANGOLENSIS
17 L.LANATA
21 L.PENNATA
25 L.FLAVIDA
26 L.MIGIURTINA
32 L.GOSSWEILERI
6 L.CAMERUNENSIS
9 L.SIMPLEX SUBSP.STIPOIDES
40 L.ANNUA
15 L.MADAGASCARIENSIS
34 L.ECHINULATA
35 L.COARCTATA
12 L.KAGERENSIS
22 L.PENNATA
11 L.KAGERENSIS
41 L.BIDENTATA
13 L.PERRIERI
14 L.MADAGASCARIENSIS
16 L.JAEGERIANA
38 L.VANDERYSTII
39 L.CERATA
18 L.LONGIPES
27 L.ACUMINATA
29 L.CUANZENSIS
23 L.FLAVIDA
30 L.FILIFOLIA SUBSP. HUMBERTIANA
31 L.FILIFOLIA SUBSP. HUMBERTIANA
37 L.ISSERANTII
45 L.ANOMALA
44 L.SIMULANS
50 R.PETIOLATA
46 L.ANOMALA
47 L.ANOMALA
48 L.RAMOSA
49 L.IOCOENSIS

B


100
50
0
2 L.ANGOLENSIS
25 L.FLAVIDA
26 L.MIGIURTINA
32 L.GOSSWEILERI
12 L.KAGERENSIS
22 L.PENNATA
15 L.MADAGASCARIENSIS
17 L.LANATA
34 L.ECHINULATA
35 L.COARCTATA
13 L.PERRIERI
14 L.MADAGASCARIENSIS
16 L.JAEGERIANA
23 L.FLAVIDA
29 L.CUANZENSIS
21 L.PENNATA
30 L.FILIFOLIA SUBSP. HUMBERTIANA
27 L.ACUMINATA
31 L.FILIFOLIA SUBSP. HUMBERTIANA
37 L.TISSERANTII
38 L.VANDERYSTII
39 L.CERATA
3 L.ARUNDINACEA
4 L.ARUNDINACEA
5 L.ARUNDINACEA
19 L.DEMEUSEI
42 L.HORDEIFORMIS
36 L.DENSISPICA
40 L.ANNUA
41 L.BIDENTATA
6 L.CAMERUNENSIS
9 L.SIMPLEX SUBSP.STIPOIDES
18 L.LONGIPES
11 L.KAGERENSIS
33 L.DENSISPICA
7 L.SIMPLEX SUBSP. SIMPLEX
8 L.SIMPLEX SUBSP. SIMPLEX
10 L.SIMPLEX SUBSP.STIPOIDES
1 L.ANGOLENSIS
20 L.CRASSIPES
28 L.ACUMINATA
24 L.FLAVIDA
43 L.PHRAGMITOIDES
44 L.SIMULANS
45 L.ANOMALA
50 R.PETIOLATA
46 L.ANOMALA
47 L.ANOMALA
48 L.RAMOSA
49 L.TOGOENSIS

C

(ii) Classification resulting from the use of the component scores derived from the dispersion matrix, as the data matrix (Fig. 32B)

In this case the structure, as seen in the scatter diagrams (Fig. 31 A-D), is somewhat different when the dendrograms are compared. The sections Pleioneura (44-48) and Lophanthera (49), and Rattraya (50) form a group which is completely isolated from the section Loudetia. Some of the same features that were observed above are present in the classification of OTU's of this section. Some of the OTU's of subsection Acuminatae (23, 27, 29-31) in particular, group together but those of the Typicae (1-15) are most variable. The exemplars of the species of the Typicae do, however, cluster together, e.g. L. arundinacea (3-5), L. simplex subsp. simplex (7, 8). Also of interest is the fact that L. perrieri (13) is close to one of the examplars of L. madagascariensis (14).

The cophenetic correlation between the distance matrix (26) and the dendrogram is also fairly high in this classification (0.660).

(iii) Classification resulting from the data matrix (Fig. 32C)

The results of this analysis are very similar to those achieved with the component scores from the correlation coefficient as data matrix. Not much more need be added except that some of the large awned Annuae (40-42) are clustered, as well as many of the representatives of the Acuminatae (e.g. 21, 27, 30, 31). The cophenetic correlation between the

distance matrix (32) and the dendrogram was 0.608, slightly lower than in the previous analyses.

(iv) Comparison of the classifications

The cophenetic correlation coefficients were calculated between the distance matrices and the dendrograms (Table 22). The distance matrices are very similar in all cases and the dendrograms are also quite similar. The value of 0.827 for the correlation between the dendrograms derived from raw data and component scores using the correlation coefficient is the highest obtained in comparing dendrograms in this study.

4. Analysis of supra-specific groups

Rescoring of the data for the ten supra-specific taxa resulted in the production of the data matrix 45 which was analysed by principal component analysis and subsequently the OTU's were classified (Fig. 26).

(a) Principal components analysis

The dispersion matrix (No. 46) was analysed into principal components (Matrix 48) and the characteristic roots (Table 23). The first three components, which account for over 70% of the variation, were used to show the OTU relationships. (Fig. 33 A-C).

The most prominent feature in all the scatter diagrams is the tight cluster formed by the first five subsections of the section Loudetia.

The subsection Flammidae (6) is different and although it appears close to L. jaegeriana (10) on the F_2 (Fig. 33A) it is actually way apart as seen on the F_3 (Fig. 33B, C).

Table 22: Cophenetic correlation coefficients produced by comparison of the distance matrices and dendrograms derived from the three different data sources, using awn characters.

		Data Source: Component Scores - Correlation (Matrix 31)	Component Scores - Dispersion (Matrix 25)	Raw Scores - (Matrix 23)	
Dendrograms	Matrix 31	1.00	0.814	0.842	Distance Matrices
	Matrix 25	0.568	1.00	0.950	
	Matrix 23	0.827	0.610	1.00	

Table 23: Roots of dispersion coefficient matrix 46

Root	Value of root	Percentage of total variation accounted for	Accumulated percentage
1	8,806.176	34.195	34.195
2	5,708.055	22.165	56.360
3	3,995.094	15.513	71.874
4	3,285.033	12.756	84.630
5	1,624.325	6.307	90.937
6	977.716	3.797	94.734
7	730.373	2.836	97.570
8	412.210	1.601	99.171
9	213.603	0.829	100.0
10	0.0	0.0	100.0
Total sum of squares	25,752.585	100.0	-

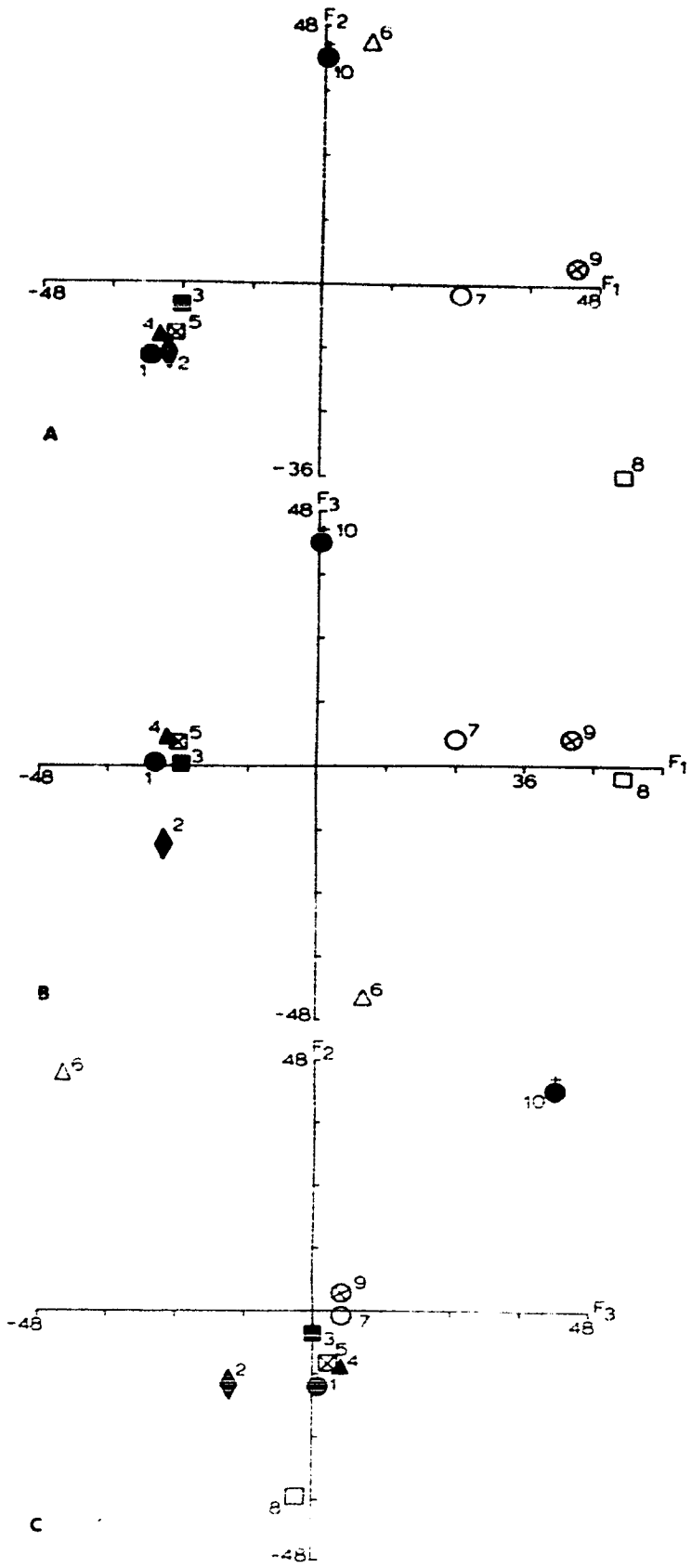


Fig. 33 A-C. Scatter diagrams showing the positions of the supra-specific taxa on the first three components of a component analysis of the dispersion matrix 46, derived from awn characters. For legend see Table 17.

The section Pleioneura (7) is quite close to Rattraya (9) and also the section Lophanthera (8).

(b) Classification

The absolute distance matrix (No. 50) was produced from the component scores matrix (No. 48) and following sum of squares agglomeration a dendrogram (Fig. 34) was drawn from the results. The tight cluster of subsections of section Loudetia seen above, form a cluster with L. jaegeriana, and Pleioneura clusters with Rattraya and Lophanthera. At the 15% level, the Flammidae, L. jaegeriana and Lophanthera would all be separate.

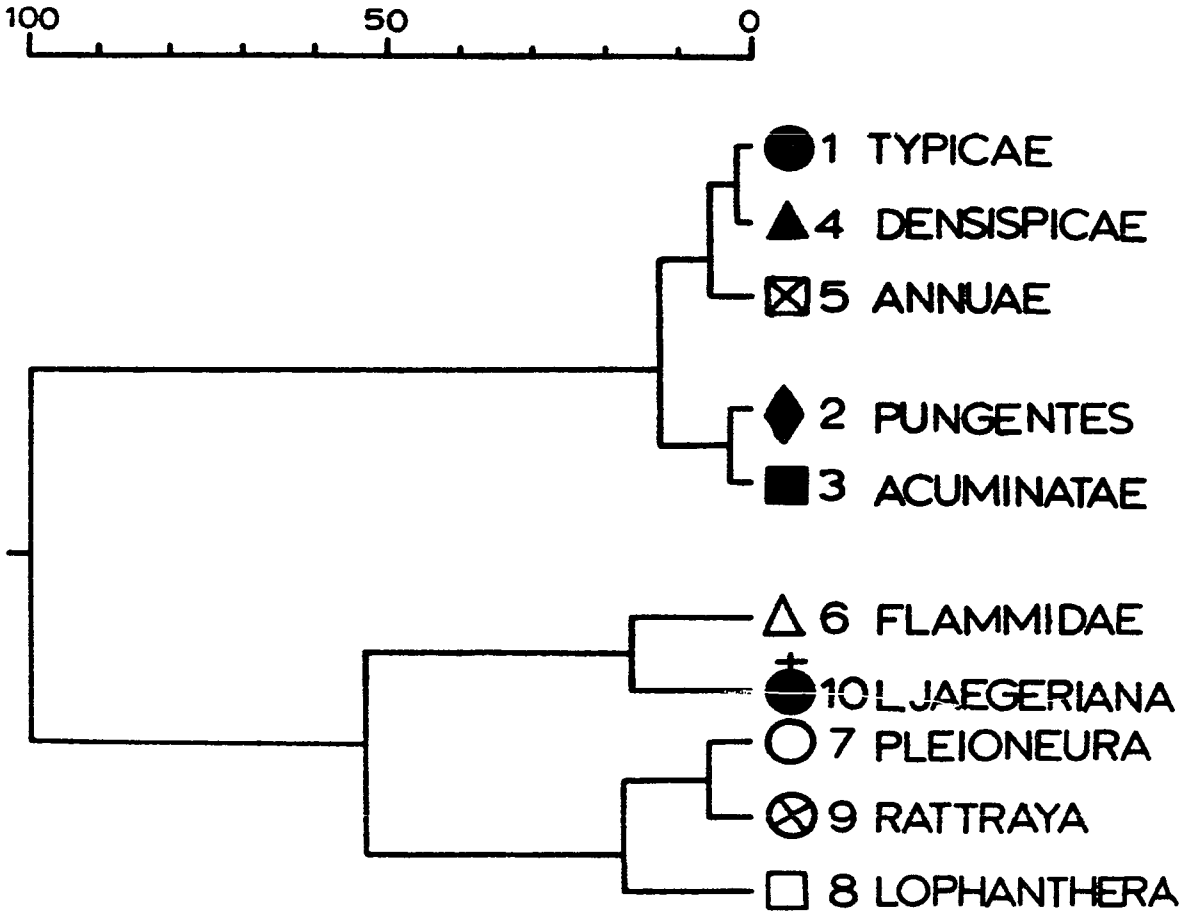


Fig. 34 Dendrogram of supra-specific taxa of Loudetia and Rattraya derived from awn characters. Scale indicates the within-group sum of squares expressed as a percentage of the total sum of squares.

5.10 Discussion

(a) The taxonomy of Loudetia based on characters of the awn

Morphologically, the awn of *Loudetia* was found to differ greatly both between species and within species. This variation was mainly in size, although the general shape of the awns differ from one species to the next. The awns of the subsection *Typicae* are variable in size, ranging from very small in *L. thomasii* to quite large in *L. simplex* subsp. *stipoides*. Similar variations in the size of awns within the subsections *Densispicae* and *Annuae* occur. The largest and most well-developed awns are in the annual species. Although all species have a complete awn, i.e. with column and bristle, in the subsection *Flammidae* a great reduction in length of the column was observed. To a lesser extent the awns of the section *Pleioneura* and *Rattraya* are also short with a short column. The differences between the supra-specific taxa on the basis of bristle length and the ratio of length of bristle-to-length of column is illustrated diagramatically (Fig. 27).

The morphological differences in the awns of the species of *Loudetia* were found to be related to anatomical differences. Three anatomical types of awn were distinguished which follow the three sections of the genus:

(a) The *Lophanthera* type, where the awns are very large and have 12-14 layers of cells across the column which is terete in cross-section.

(b) The *Pleioneura* type in which the column is only slightly curved in cross-section, the mesophyll consists of

about five layers (± 7 layers of cells in all), the uppermost zone of which has a distinctive type of thickening.

(c) The Loudetia type, in which the column is an inverted U-shape or terete in cross-section, and the mesophyll consists of three to four layers of cells (5-6 layers of cells in all).

Intermediates between the Loudetia type and Pleioneura type were found in some small awned species, viz. the subsection Flammidae and L. jaegeriana. Since the species of the section Pleioneura are small awned, this is to be expected. Rattraya petiolata awns do not differ very greatly from those of the Pleioneura species.

The comparative anatomical studies were all made from transverse sections taken from the middle of the column. These are differences at the base of the column, in the transition zone between column and bristle, and in the bristle, but the amount of work involved did not warrant making serial sections through all regions, when they would probably only reflect differences already observed from a study of the column. Judging from the results on the epidermal studies, an investigation of the epidermis of the awn would probably give a number of similar taxonomically useful characters. For instance, from the plates, it would appear that adaxial epidermis of the species of Densispicae is devoid of prickle-hairs (Plate 24), as was the situation in the abaxial leaf epidermis. Some of the awns were sectioned through the bristle region and the transition zone. It was found that the trans-

ition type of the Lophanthera and Loudetia type column differed from that of the Pleioneura type; likewise at the base of the column the attachment of the awn to the lemma, was different according to the type of the awn.

The numerical analysis of the data accumulated from a study of 31 characters of fifty OTU's revealed only little more than was already known about the OTU's, but a lot about the characters. The analysis of characters using component analysis and the correlation coefficient showed that there are a number of categories into which the characters may be divided. The most important group of characters were those of size or related to size. Some of these characters described the size of the awn and others the size of the cells of the column. On the first three components alone the thirteen characters of size account for over 20% of the total variation. Also of important are those characters describing the amount of thickening of the cell walls, because on the first two components, these five characters account for over 8% of the total variance. The shape of the awn is an influencing factor especially on the second root (26.33% of that root).

The effect of the characters on the OTU relationships was shown in the OTU analysis. On the first component the separation of the section Loudetia, except for L. phragmitoides (43), from the remainder of the OTU's is mainly due to the larger size of the former OTU's. An exception is L. togoensis (49) which, in spite of its large awn, is more closely allied to the section Pleioneura due to common characters of thickening.

The amount of thickening (34.9%) and type of thickening (10.69%) also carry a large amount of weight on this component. The cells of L. togoensis are usually abundantly thickened, have the slightly curved or arched type of thickening in the upper zone of the mesophyll and the cells of the different tissues are invariably the same size in the different OTU's. L. ramosa (48) has an inverted U-shaped awn and a number of characters in common with species of the section Loudetia and is, therefore, slightly apart from the other OTU's of Pleioneura. It is close to some anomalous OTU's of the section Loudetia, e.g. L. angolensis (1), L. simplex subsp. stipoides (10), L. flavida (24), and L. acuminata (28). Other exemplars of these species, OTU Nos. 2, 9, 23 and 27 respectively, have much less thickening to the cell walls (see Table 28). Thus it would appear that an increased amount of thickening of the cell walls leads to separations of these OTU's from their nearest relatives.

L. phragmitoides (43) which is isolated on the second component with a high score, is somewhat different in that the column is very short and other characters of shape appear associated with this character. Shape is important when this component is considered. The OTU's of subsection Annuae, which have large awns, tend to have high negative scores on this axis, thus showing that size should also be considered here.

When dispersion was used as a similarity coefficient similar results were obtained, although some differences in

the importance of the characters were indicated. The most noticeable fact is that the characters of shape are very insignificant when the first five components are considered. Some of the miscellaneous characters, e.g. whether the adaxial epidermis passes around the margin or not (9), are important when the third, fourth, and fifth roots are considered. L. phragmitoides (43) shows some peculiarities with respect to this (character 9) and other characters and is isolated on the fourth component. Generally, characters describing size and the amount of thickening are the most important ones in this analysis.

The classifications produced from the different data sources were all very similar. The final agglomeration in each case demonstrates that the two major groups are based on size and amount of thickening of the cells. In two dendrograms some of the OTU's of the section Loudetia were grouped with the sections Pleioneura and Lophanthera. These OTU's all have strongly thickened cells and are therefore different from the other OTU's of section Loudetia and are more similar to the OTU's of other sections. However, the type of this thickening is different. The clustering within the section Loudetia does not follow the subsections very closely. Some of the Acuminatae and Typicae cluster together and different exemplars of some species are very often closed to one another. The anatomy as seen in cross-sectional view of the column is very similar throughout the section, only L. phragmitoides (43) being slightly different. L. perrieri (13) is always

closest to L. madagascariensis (14); a situation which was common in the studies using leaf anatomy and leaf epidermis characters. The position of L. jaegeriana (16) is never clear in the classifications or ordinations. This species has a small awn and the column is only slightly curved in cross-section, yet the cells are not thickened like those of the OTU's of section Pleioneura, so it is grouped with the section Loudetia.

In the classification and ordination of supra-specific taxa, the position as indicated above is further exemplified. A very tight cluster of the subsections of Loudetia, except for the Flammidae (6) which appear to be closest to L. jaegeriana (10), is formed. The latter is different from the Flammidae, however, because the cell walls are not thickened and the third component illustrates the great distance between the two.

(b) The evolution of the awn and its bearing on the phylogeny of the genus

The production of an elaborate lemma and awn of the fertile floret in many grasses is seen as the result of a trend towards reduction and specialization in the spikelets of the family. These elaborate mechanisms with which the floret is endowed have been shown to have an important function in the adaptation of the plant to its environment. If we are to consider the evolution of the awn and its associated lemma, it is, therefore, important that the function of these structures be taken into account.

The earliest attempts to classify awns by Duval-Jouve (1871) produced two categories viz. the complete awn with both column and bristle and the incomplete awn with bristle alone. The lemma is an integral part of the awn-lemma adaptive mechanism and in this study a classification of lemmas has been chosen. Every indication points to the existence of three types of upper lemmas:

(1) A simple lemma with an awn.

(2) Those lemmas with a short bristle awn.

(3) Lemmas with a complete awn consisting of a basal column and distal bristle.

Intermediates exist between all three categories. Furthermore where the awn arises from the tip of the lemma there may or may not be two lateral lobes. In most cases where the awn is of the bristle type the lobes are absent. In species with a short column which is only slightly curved in cross-section (Pleioneura type) the lobes are large and elongated. Finally those species with large awns have reduced lateral lobes. In tracing the evolution of the lemma account must be taken of the ancestral type, what course the evolution has taken, and the adaptive advantage of the changes.

Although many authors have accepted the hypothesis of Roeper (1826, as modified by Duval-Jouve, 1871) that the awn represents the blade of the vegetative leaf and the lemma the sheath, there is no conclusive evidence for this phylogenetic homology. The only accepted homology is that the lemma is of foliar nature (e.g. Barnard, 1954). In view of the lack of

evidence for the support of the classical phylogenetic homology it is, therefore, more logical to look for an alternative hypothesis for the primitive lemma.

The common floral bract is typically awnless and because of the increasing specialization of the awned bract the awnless condition may be considered to be the primitive situation. This is contrary to the view of Duval-Jouve (1871) who regarded the awnless bracts as being similar to the basal leaves which lack blades and which occur in some Cyperaceae, Juncaceae and Gramineae. There is no evidence to suggest that awnless bracts are homologous with bladeless leaves, and it seems more satisfactory to consider the awnless bract as representing a complete reduction of the vegetative leaf.

The Cyperaceae and Juncaceae are generally considered to be less advanced than the grasses and they show a lesser amount of reduction and specialization (Bews, 1929). The floral bracts of sedges and rushes are typically awnless, though in some species of Cyperaecae, e.g. Scirpus validus Vahl and Carex laxiflora Lam., a bristle type awn is present at the apex of the scale. The conclusion that the awnless condition is the primitive one, is supported by this evidence.

The primary function of the lemma is, therefore, the same as that of other floral bracts, to enclose and protect the floret and later the grain. The glumes and sterile lemmas usually contain chlorophyll and they have the additional function of providing a site for photosynthesis and transpiration. The fertile lemma may also possess this function but often it

is achlorophyllous and enclosed within the other bracts. The lemma is in close proximity to the developing grain and it would be highly advantageous if it could provide a site for photosynthesis. Consequently the evolution of a bristle may be witnessed. The substantial amounts by which the yield of wheat (Triticum aestivum L.) may be increased due to the presence of awns has been indicated by Grundbacher (1963). The existence of bristle type awns on the other bracts of the spikelets and on the floral bracts in the Cyperaceae, shows the importance of these structures in increasing the site of photosynthesis and transpiration in close proximity to the flower and fruits.

In the tribe Arundinelleae, although most lemmas of the fertile floret are awned, some representatives with awnless lemmas occur in the genus Arundinella, e.g. A. hirta (Thunb.) Tanaka. Although the awnless species are not necessarily the most primitive, because there may be a secondary loss of the awn, they provide evidence for a form from which the Loudetioid forms may have evolved. The existence of species in the genus with most weakly developed bristle-type awns may also be noted, e.g. A. fluviatilis Hand.-Mazz. The smallest bristle is no more than an elongated mucronate tip of the lemma and it is conceivable that the lemma could have given rise to a bristle which would have a physiological adaptive role for the plant. The anatomy of the bristles of the complete awns of Loudetia spp. is one adapted to this function. There are lateral bands of chlorenchyma and stomata in this region of the awn, being

the required tissues for photosynthesis and respiration. In species with merely well-developed bristles on the lemmas, the anatomy is very often highly suitable for the physiological functions. (Fig. 23 and 24). The bristle may have the additional function of dispersal. Large plumose bristles have developed in species of Aristida, for example, which enable the lemma and the enclosed grain to be carried by the wind. Coupled with the development of bristles for wind dispersal are elaborate hairs or tufts of hair on the back of the lemma and also on the callus. Tufts of hair on the back of the lemma are common in the Arundinelleae e.g. all Danthoniopsoid species, some Tristachyoid species and all the Trichopteryx spp. The bristle may also serve as an accessory for dispersal by animals if it is sharp and rigid enough to catch in the animal's fur. The development of a sharp callus and straight hairs on the callus which hold the grain in the fur of the animal would follow. This is particularly noticeable in species of Loudetia of subsections Typicae, Pungentes and Acuminatae.

The evolution of a more specialized awn, i.e. with both column and bristle, could have developed from the bristle type. Francis Darwin (1876) pointed out the importance of the geniculation between the column and the bristle as applied to its success as a seed burying mechanism. Recently Harper et al. (1965), in a study of the effect of the heterogeneity of soil surfaces on seeds of selected species, showed the consequences of such a development. They found that when the grains of

Bromus rigidus Roth, which have straight awns, were sown, they "fell like darts, entering cracks in soil clods and leaving only the awn showing," whereas in B. madritensis L., the awn was bent so that the grain fell more slowly and landed horizontally on the soil. The awns of both these species are of the bristle type and consequently one may speculate on the evolution of the column from a species with an awn such as B. madritensis. Seeing that the awn of this species is curved, it floats to the ground more slowly and, therefore, has the advantage of being carried by the wind for a greater distance than B. rigidus. However, because it is bent it lands flat on the soil and another mechanism is necessary to account for the burying of the grain, which was so easily accomplished by the former species. The evolution of a specialized column below the bend or geniculations with a hygroscopically controlled twisting mechanism would be one solution. The upper part of the bristle is bent against the soil in a suitable position to give the leverage ot help in the burying process when the awn is moistened by dew or rainfall.

There are a number of factors which are important for the evolution of a column with a hygroscopically controlled torsion mechanism. Firstly the mechanism for producing torque in the thick-walled cells of the column must have developed. This property derives from the spirality of the protoplasm (Seifriz, 1952) and the method of wall formation in the cells (Murback, 1900). However, very little is known about the mechanism and the submicroscopic structure of the cells or their evolution.

Secondly, in order to be functionally efficient, the thick-walled cells or sclerenchyma of the column must be arranged in the most suitable manner and the column itself must assume the most suitable shape. Finally, it is an advantage for the column to be long since it should be twisted to a maximum when dry so as to give the grain as many turns as possible to enable the callus and the grain to enter the soil when it is wetted.

The gross morphological and anatomical details of the column may be examined in terms of structural mechanics and elasticity (see Appendix 5). The column is thought of as a solid shaft of varying cross-sectional shape to which a torque is applied. The results of an analysis of the effects of torsion on solid shafts reveals that one of the most important properties is cross-sectional shape. The shapes of the various awns in cross-section were illustrated in Fig. 24. The sclerenchyma or thick-walled cells will in all cases be accepted as the solid portion of the column. (See Appendix 5). On this basis six different shapes of columns and bristles may be recognized:

(a) Inverted U-shaped, e.g. Arundinelleae

(b) U-shaped, e.g. Aristida funiculata

(c) Solid circular, e.g. Tetrapogon spathaceus

(d) Circular-solid at circumference, e.g. Bromus arvensis

(e) Circular - no thickening, e.g. Alopecurus myosuroides

(f) I-shaped, e.g. Lagurus ovatus

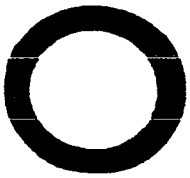
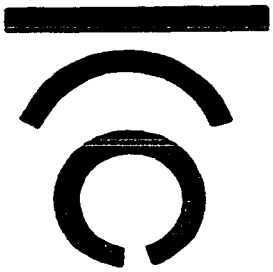
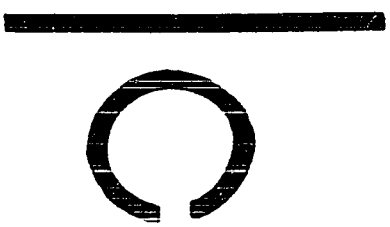
These are some of the cross-sectional shapes of awns. An

obvious omission is the slightly curved shape of the Pleioneura type column. It may also be noted that these shapes are the idealized ones, as all the tissues are sometimes not in the outline of the shape considered, e.g. I-shaped.

The torsional rigidity and strength are properties of a body which explains its efficiency when subjected to torque. A very high torsional rigidity indicates a great resistance to torque and a low value indicates that the body may be readily twisted. Torsional strength illustrates the strength of the body when it is subjected to torque. A high value indicates that the body is strong and a low value that it is weak when torque is applied. The torsional rigidity and strength were calculated for solid shafts of different cross-sectional shapes but of equal area (Table 24), and are thus directly comparable. The shapes have been arranged in decreasing values of mechanical efficiency, the most efficient shape being that which is most rigid and strongest, when subjected to torque. The solid circular shape is the standard on which all others are based. It will be noticed that the hollow tubular shaft (Table 24, 2) or hollow box (3) have very high values, whereas the I-shaped (8) and open hollow-tubular or long rectangular shaped shafts (7 and 9) have very low values. The cross-sectional shapes of the solid shafts (Table 24) closely resemble the shapes of the columns or bristles in cross-section (Fig. 24).

Whereas efficiency in the mechanical sense is considered in terms of which shape is most rigid or strong when subjected

Table 24 : Torsional Rigidity and Strength of Different Sections of equal Cross section Areas

	Shape of Cross section	Torsional Rigidity	Torsional Strength
1.		100.0	100.0
2.		637.0	332.0
3.		341.0 (approx.)	280.0 (approx.)
4.		88.0	74.0
5.		74.36	62.91
6.		70.0	62.0
7.		15.71	32.36
8.		9.9 (nearly exact)	22.2 (approx.)
9.		5.5	18.0

to torque, in the case of awns efficiency must be related to function. In the case of the column it is desirable for the body to be readily subjected to twisting and, therefore, have a low torsional rigidity, but remain quite strong. The bristle, whether it represents either the whole awn or the distal part of a complete awn, should be both rigid and strong. It will be noted that the evolution of the column and bristle has followed a pattern which is most efficient for its function (Fig. 24). The bristles which are suitably adapted for lodging in animals' fur or providing a strong rigid lever for the burying mechanism have a solid circular, or circular structure, solid at the circumference. (cf. Table 24, 1 and 2). The latter shape has a tendency to become square. Shafts of this shape are extremely rigid and strong. In contrast, the shape of the column is commonly I-shaped, U-shaped or inverted U-shaped. (cf. Table 24, 7-9). The I-shaped shaft is the most efficient in terms of maintaining its strength yet being readily subjected to twisting. The U-shaped and inverted U-shaped forms are actually identical, merely orientated differently, and comparable forms are the open hollow tubular shafts. Although very thin forms of the latter are more efficient with respect to twisting, one assumes that structural difficulties and the strength of such shapes makes them unsuitable. It will be noticed that thin rectangular shafts or slightly curved shafts are not different with respect to rigidity and strength. A final column shape which is not explained in terms of solid shafts is that which is circular with no thick-

ening. It is probable that the large fairly thin-walled epidermal cells of this column possess the torsional properties and when twisting is accomplished, warping of the awn occurs, a column of this structure not being particularly strong or rigid. The large distorted nature of the epidermal cells suggests that they might readily become distorted (Fig. 24).

The above evidence suggests that the column has evolved in a variety of different cross-sectional shapes in the different species of grasses, and yet always possessing the hygroscopically controlled torsion mechanism for burying the grain. The question arises whether the column has a polyphyletic or monophyletic origina. The fact that the columns are vastly different in cross-sectional shape and cellular structure suggests that their origin is polyphyletic. Also within taxa at the tribal or even subtribal level (e.g. Arundinella spp.), all three forms of lemma awn mechanism occur, so that it is possible that the whole evolution of lemma → lemma plus bristle → lemma plus column and bristle has occurred many times over. The view of Tran (1965) that the awn has evolved monophyletically from the three "longitudinal elements" of the lemma, or the median type of the Arundinelleae, must assume separation of the ancestors of the species with the dorsally and terminally awned lemmas, prior to the formation of a column, and preferably before or shortly after evolution of the bristle. If this is not so, many unlikely steps in the evolution of the terminal and dorsally awned species must be accepted. For example, the Arundinellean type column (median

awn), although very similar in shape is orientated, with respect to the lemma, in a direction completely opposite to that of the Aristidean type (terminal awn). The column of the latter type has two lateral vascular bundles and it is assumed that the lateral lobes of the lemma are incorporated into the awn. If evolution of this type of column occurred from an Arundinelleaen type, the inverted U-shaped column would have to have "flattened out" in intermediate forms, the lateral lobes become coalesced with the column, and then recurved to produce the U-shaped column. There is no apparent selective advantage for evolution in this direction and it seems most unlikely. The evolution of the column and possibly also the bristle on the lemma has almost undoubtedly occurred more than once.

The evolution of a column has most likely developed from a bristle which already has developed a geniculation as mentioned above. In this way the rigid structure of the bristle can be explained when the function is solely for dispersal by animals or by wind. The subsequent development of a twisted column would have to occur in a region that would be easily susceptible to change. Chance in the types of cells and shape and arrangement of cells, could most easily be accomplished at the base of the awn at the junction with the apex of the lemma.

The evolution of the column may be traced in the different species of grasses. In the Arundinelleae and Loudetia in particular, there are certain trends in the evolution of the

column which reflect the function of the body. It may be noted that species of Andropogoneae have very similar awns to those of the Arundinelleae and similar patterns in the evolution of the awn in this tribe may be found. Tran (1965) has discussed the close taxonomic proximity of these two tribes and it is possible that their origin stems from very close relatives.

The species of Loudetia with a flattened or slightly curved type of column have a different mechanism of dispersal of the grain, from those with the inverted U-shaped column. In the former species the column is much shorter and usually the bristle is sharply geniculate. The lemma is very often liberally provided with hairs, the callus is small and obtuse and the whole floret is reduced in size. This mechanism is ideally suited for wind dispersal. The species that possess this type of awn are those of the subsection Flammidae, section Pleioneura, and R. petiolata. The species of the Flammidae grow close to streams, rivers and lakes and this type of dispersal mechanism is suitable since it is not necessary to have a hygroscopically controlled mechanism for burying the grain. The species of Pleioneura and Rattraya occur in dry, rocky areas in which there is possibly not such an abundance of animals for animal dispersal of the fruits, so it is probably more advantageous for them to be dispersed by wind over a wider area. The ground is also very hard and not suitable for an adaptive burying mechanism to develop.

Most of the species of Loudetia section Loudetia generally occur in savannah or grassveld areas, where they are often the

dominant species, and one finds an unspecialized awn/lemma mechanism. The lemma may be shortly hairy, the column varies from quite short to as long as the bristle and the callus is often sharply pointed and equipped with straight hairs. Some of the fruits of these species with longer bristles may have a tendency to wind dispersal but generally, the sharp pointed callus and bristle make them adaptable to animal dispersal. The fairly well-developed column also serves as a burying mechanism. Exceptions are the species of the Flammidae (discussed previously) and the Annuae. The latter, like L. togoensis, have large well developed awns. The column being particularly long, is capable of many turns to drive the callus and the grain into the soil. The fact that this development has occurred in most of the annuals would suggest that it is important for Loudetia spp. with this lifeform to bury the grain prior to germination when the annual rains fall. Annuals cannot tolerate the low survival rates from seed that perennials can, and burying of the grain is a possible advantage in seedling establishment.

Although the function of the different awns of Loudetia is quite clear, the selective advantage of the one type of twisted column over another is obscure. The torsional rigidity and strength of a solid shaft of thin rectangular-slightly curved or U-shaped in cross-section, is identical in each case (Table 24). Presumably the round or angular rigid bristle has developed a column at the summit of the lemma. Its original shape would be curved in the direction of the lemma, unless

the summit of the lemma already consists of three lobes or longitudinal elements as Tran (1965) suggests. The fact that the apex of the lemmas of many species of grasses do occur in this form, suggests that this supposition is true. (The nature of the base of the awn and the summit of the lemma has been illustrated in Plate 10). If the column had evolved prior to the development of the two lateral lobes of the lemma, torsion at the apex of the lemma would cause the whole lemma to twist. The formation of the division between the column and lobes could then be explained as a means of overcoming this twisting. However, it seems more likely that this was not the path of evolution because in Aristida spp., where torsion occurs, there are no lobes and the awn may be regarded as an extention of the lemma (terminal). Also the column curves in the same direction as the lemma whereas in the Arundinelleae it curves in the opposite direction. The lobes had, therefore, probably evolved prior to the column and the original column was probably narrow and rectangular in cross-section.

There are two possible explanations for the evolution of a curved column. Firstly, if the cells exhibiting torsion occurred along one edge of the column, there may be a tendency for the column to curve as a consequence of unequal torsion. This situation may exist in the Pleioneura type of column where the differential thickened cells occur along the upper zone of the mesophyll. However, the cells of the column were not studied in isolation to see which ones exhibit torsion. Frances Darwin (1876) found that by gluing bundles of awns

together to represent an awn, torsion occurred in the same way as in a single awn on drying. A similar test was made by gluing fresh awns of L. arundinacea in a flat plane to simulate an ancestral Pleioneura awn. It was found that on drying this "macro-awn" assumed a curved shape in cross-section, particularly in the middle of the "column". Therefore there is a tendency for the awn to curve and the direction in which it curves could possibly be explained by the position of the cells exhibiting torsion.

It should also be considered that the column is attached at each end. Lyse and Johnson (1935) have pointed out that when a structure is submitted to a torque, it undergoes warping and pure torsion no longer exists. With this in mind, one may accept the fact that, although it appears as if the three cross-sectional shapes are not different with respect to torsional efficiency, one type may be of advantage over another, when their attachment at either end and more details about the nature of the materials are examined. Whatever the reasons it is obvious that the large well-developed columns, which are an inverted U-shape in cross-section, have a selective advantage when the function of burying the grain is the issue.

Of the other shaped columns the one most striking is the I-shaped form. This structure appears to be the most suitable for twisting and it will be noticed that it occurs in both dorsal and terminal awns. It has probably evolved more than once and one may speculate that, because of its overall square shape, it has developed from the base of a square shaped

bristle. The fact that this shape is lacking in forms with a median awn supports the hypothesis that evolution in the latter has occurred from a flattened centre of the lemma. The thin-walled circular columns with large epidermis cells, e.g. Alopecurus myosuroides Huds., also have an unusual shape with regard to their torsional efficiency. However, they probably evolved from a bristle of similar shape, the cells with the property of torsion having developed at some stage.

The evolution of the three awn types of Loudetia, as described above, may be related to the phylogeny of the genus as presented by Phipps (1967b). Although awn characters were not specifically included in the 40 morphological characters that he used to construct this phylogeny, the adaptive features and specialisation of the awn is so closely related to corresponding spikelet developments that no conflict is encountered. An early bifurcation of the section Pleioneura and Rattraya is illustrated which is in keeping with the situation when awn anatomy and morphology are considered. The awns of these species are vastly different from those of most of the other species in morphology, anatomy, and function. Although they may be considered as primitive, if the burying mechanism is considered to be advanced, the great specialisation of the spikelet places the species of this group in a more advanced position phylogenetically. The Lophanthera type awn may be considered to be the most advanced as the burying mechanism is most highly developed. Evolution of this sort of awn could have occurred from the Loudetia type since the species of

subsection Annuae have large awns approaching the Lophanthera type. This position agrees with the views of Phipps (1967b). The species of subsection Flammidae have awns similar to those of the Pleioneura type and consequently they should be placed closer to this group than illustrated by Phipps (1967b) or on a separate branch from the main Loudetia one.

Tran (1965) has applied the evolution of the different types of awns she recognizes to a system of classification of the different tribes of the Gramineae and compares her results with those of Prat (1960) and Auquier (1963) based on conventional methods. The same approach could be made with the evolutionary conclusions drawn from this study of the awn. However, no attempt has been made to study representatives of the Gramineae, other than Loudetia and in a general sense the tribe Arundinelleae. All conclusions about the awns of other species have been drawn from the studies made by others, in particular Duval-Jouve (1871) and Tran (1965). Certainly one aspect that has not been considered is the radially arranged chlorenchyma which is of importance in classification based on leaf anatomy. (Brown, 1958). Only when more of these anatomical features are studied could any overall conclusions about the evolution of species of grasses with respect to their awns be made.

11 Summary

1. The awns of the genera Loudetia and Rattraya show a great deal of morphological variation. In all cases the lemma of the fertile floret possesses an awn which consists of a basal twisted column and distal bristle. Variation occurs both in size and relative lengths of the awn components. The annual species - subection Annuae and section Lophanthera - have the longest columns. At the opposite extreme the subsection Flammidae, section Pleioneura and genus Rattraya have very short columns that are not as markedly twisted.

2. Comparative anatomical studies of the awn were made from cross-sections of the middle of the column. Three anatomical awn types were recognized for the genus mainly from these sections. The Lophanthera type is many layered, thick and terete in cross-section. The Pleioneura type is small and only slightly curved with characteristically thickened cell walls. The Loudetia type is an inverted U-shape or terete in cross-section, with not many layers of cells and not characteristically thickened walls.

3. An analysis of 50 OTU's based on 31 characters of the awn, revealed that the most important characters are size or those related to size. The characters measuring the amount of thickening of the cell walls and the shape of the awn are also found to be important.

4. The OTU's which could be classified according to the three anatomical categories mentioned above, were found to form separate clusters in the analyses. These three groups

roughly correspond to the sections of the genus, with Rattraya being very similar to the section Pleioneura. The subsections of section Loudetia are not notably distinct with the exception of the Flammidae with unusually shaped awns and the Annuae with very large awns. L. jaegeriana possesses characters akin to both the Loudetia and Pleioneura type.

5. Three types of lemma of the upper floret are recognized:
   (a) Those without awns.
   (b) Those with bristle-type awns.
   (c) Those with awns with both column and bristle.

6. All indications point to the fact that the awn is a highly specialized body which has highly adaptive functions in different species.

7. The most primitive form of floral bract, or fertile lemma, has a protective role and possibly functions as a site for photosynthesis and transpiration.

8. The first type of awn to evolve was one consisting of a simple bristle which had a physiological and dispersal function.

9. The evolution of the column followed due to the selective advantage of the grain being buried prior to germination.

10. Specialization of the lemma and callus occurred along with the awn thus providing accessory features for dispersal and the burying mechanism.

11. Six different column and bristle shapes are recognized which are important with respect to torsional rigidity and strength. These shapes are correlated with the function of

the awn or bristle.

12. The evolution of the awn of the fertile floret of Loudetia is traced with respect to its function.

13. The most efficient cross-sectional shape of a column for the burying adaptation is I-shaped.

CHAPTER 6

THE TAXONOMY OF LOUDETIA BASED ON CHARACTERS OF THE LEAF AND AWN

1. Introduction

The prime objective of this thesis, as has been outlined in the opening chapter, is to test the validity of the previously constructed classifications of the genus using anatomical and numerical methods. This aim has already been approached in three different ways. Characters of the awn, leaf anatomy and leaf epidermis were each considered as separate means of describing the variation between the taxa of the genus. A final analysis of this information in a combined study based on all the characters, would now be appropriate. The taxonomy obtained in this way will be outlined below.

Another important aspect of this study is to test the hypothesis of non-specificity (see Chapter 3). If this hypothesis is true the results of the classifications achieved using different sets of characters should be equivalent. A comparison will, therefore, be made between the classifications obtained using leaf anatomy, leaf epidermis, awn anatomy, and all the characters combined.

Finally, the application of anatomical characters to a taxonomy of the genus may be considered. The importance

of vegetative keys when flowering material is not available has been stressed by students of grass taxonomy (e.g. Turner, 1953; Hubbard, 1954; Stewart, 1965). Since the information on the anatomy of the leaves of Loudetia is available, this approach can be attempted.

2. The numerical taxonomy of the genus based on all characters of the leaf and awn anatomy

The multivariate techniques using all the characters combined were applied in much the same way as described previously. Seeing that the character analysis has been performed for each of the individual sets of characters this strategy was not repeated on the combined data matrix. Moreover, the large number of characters (162) would make the extent of computations enormous. Analysis of OTU's alone is, therefore, performed on this data. The course of the numerical methods is illustrated in the flow chart (Fig. 35).

A. Data preparation

The data matrices from the leaf anatomy (No. 62), leaf epidermis (No. 102) and awn character (No. 102) studies were the source of the data here. In each of the respective studies, the number of specimens and species were limited by the availability of material so that the same OTU's were not always used. It was, therefore, necessary to transpose the data matrices so that the common OTU's could be selected out and arranged in the same order in each case for the combined 162 x 39 data matrix considering all characters (No. 153). A list of these OTU's is given in Table 25.

B. Principal components analysis

(a) Correlation coefficient

The correlation matrix (No. 155) was computed and the characteristic roots (Table 26) and component scores for OTU's (Matrix 158) were extracted by component analysis. The first 14 roots account for over 70% of the variation. The first four roots, which will be considered further with respect to the component scores of the individuals, account for over 30% of the variance (Table 26).

The projection of the OTU's in the planes of the first four components is given in the scatter diagrams (Fig. 36 A-D). The first two components, which account for over 19% of the variation, most conveniently represent the distribution of the supra-specific taxa of OTU's. Fig. 37 shows the extent of the isolation or overlap between the sections and subsections of the genus. The third component also is responsible for a large portion of the variation (6.57%) and by constructing a model, the positions of the OTU's in three dimensional space were represented (Plate 25).

An examination of any of these illustrations reveals that the sections of the genus are easily delimited. Pleioneura (35-37) and Rattraya (38) are most distant from the other sections on the F_1. Lophanthera (38) is also fairly distinct and similarly L. jaegeriana (14). There is a large amount of overlap between OTU's of the subsections of the section Loudetia. There are, however, the same trends which have been witnessed before.

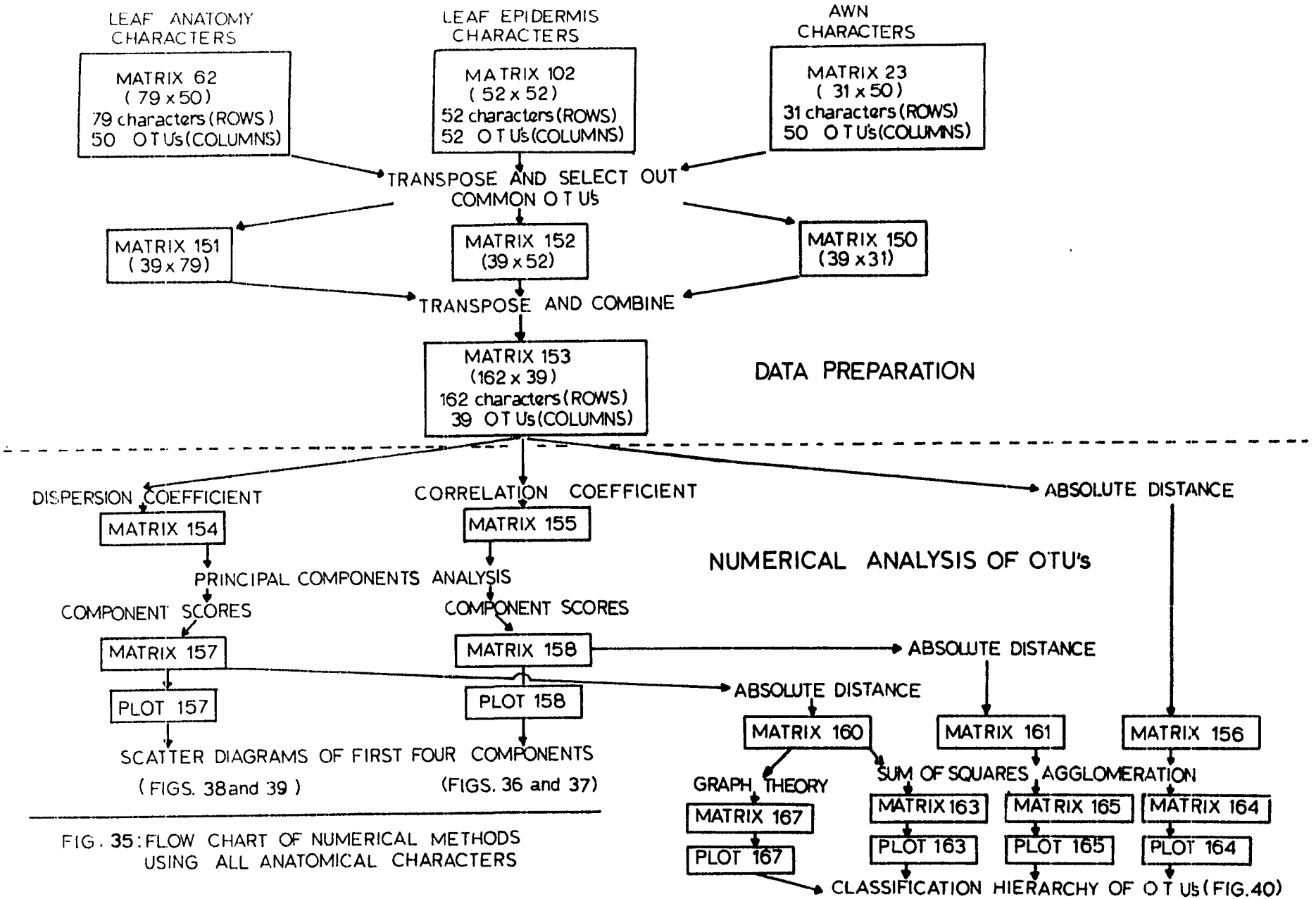


FIG. 35: FLOW CHART OF NUMERICAL METHODS USING ALL ANATOMICAL CHARACTERS

Table 25: List of OTU's used in the taxonomy based on all characters of the leaf and awn. (Numbers and symbols used in Figures 35-43)

Section: Loudetia

1. Subsection: Typicae

1. L. angolensis
2. L. arundinacea
3. "
4. "
5. L. thomasii
6. L. camerunensis
7. L.simplex subsp. simplex
8. "
9. L.simplex subsp.stipoides
10. L. kagerensis
11. L. kagerensis
12. L. perrieri
13. L. madagascariensis

10. Incertae sedes

14. L. jaegeriana

2. Subsection: Pungentes

15. L. lanata
16. L. longipes
17. L. demeusei
18. L. crassipes

3. Subsection: Acuminatae

19. L. pennata
20. L. flavida
21. L. migiurtina
22. L. acuminata
23. L. cuanzensis
24. L. filifolia subsp. filifolia
25. L. filifolia subsp. humbertiana

4. Subsection: Densispicae

26. L. gossweileri
27. L. coarctata
28. L. densispica
29. L. tisserantii
30. L. vanderystii

5. Subsection: Annuae

31. L. annua
32. L. hordeiformis

6. Subsection: Flammidae

33. L. flammida
34. L. phragmitoides

7. Section: Pleioneura

- 35. L. simulans
- 36. L. anomala
- 37. L. ramosa

8. Section: Lophanthera

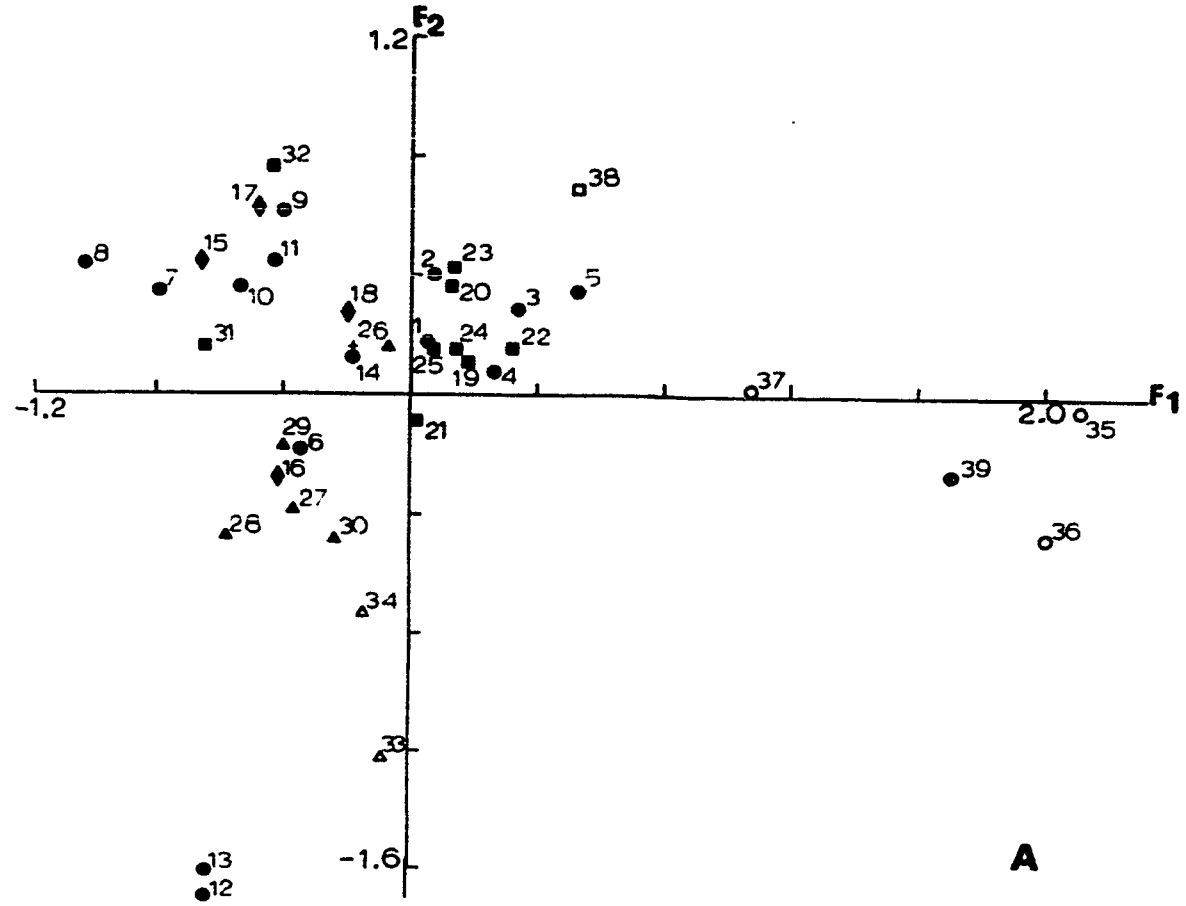
- 38. L. togoensis

9. Genus: Rattraya

- 39. R. petiolata

Table 26: Roots of correlation coefficient matrix (No. 155)

Root	Value of root	Percentage of total variance accounted for	Accumulated percentage
1	18.955	11.700	11.700
2	12.215	7.54	19.24
3	10.635	6.57	25.81
4	9.808	6.05	31.86
5	8.998	5.55	37.41
6	8.508	5.25	42.67
7	7.591	4.69	47.35
8	7.026	4.34	51.69
9	6.145	3.79	55.48
10	5.583	3.45	58.93
11	5.120	3.16	62.09
12	4.792	2.96	65.05
13	4.391	2.71	67.76
14	4.086	2.52	70.28
15 - 39	48.147	29.72	100.00
Total Variance	162.00	100.00	-



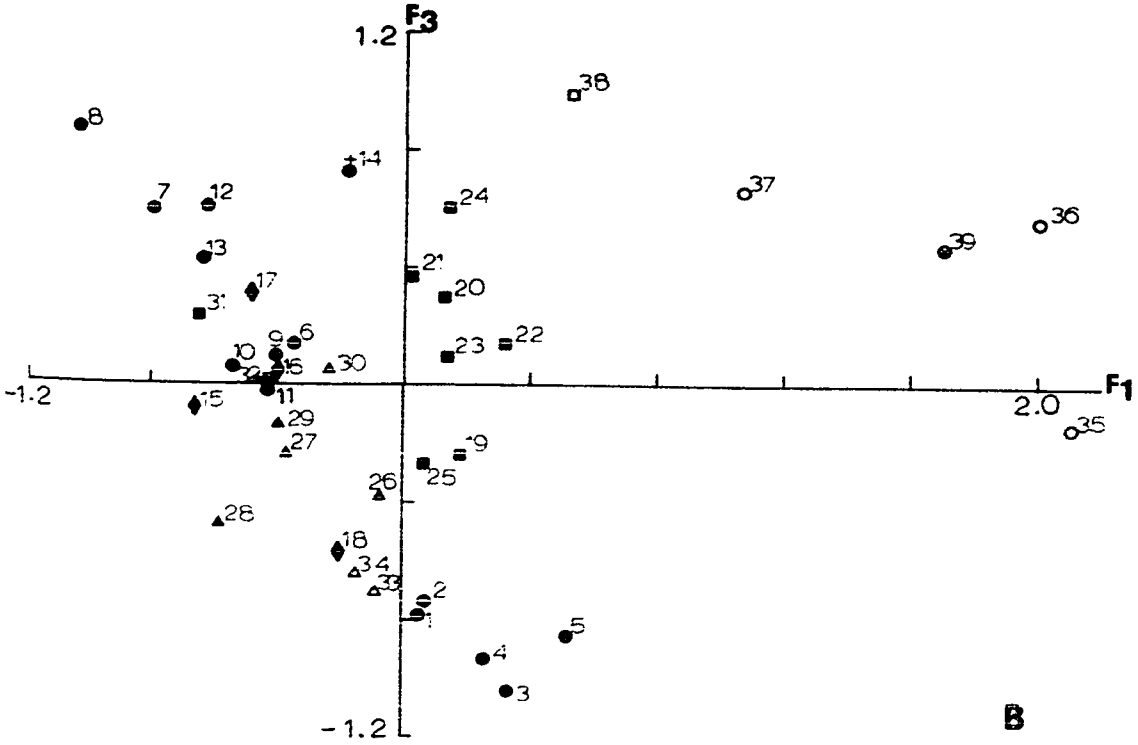
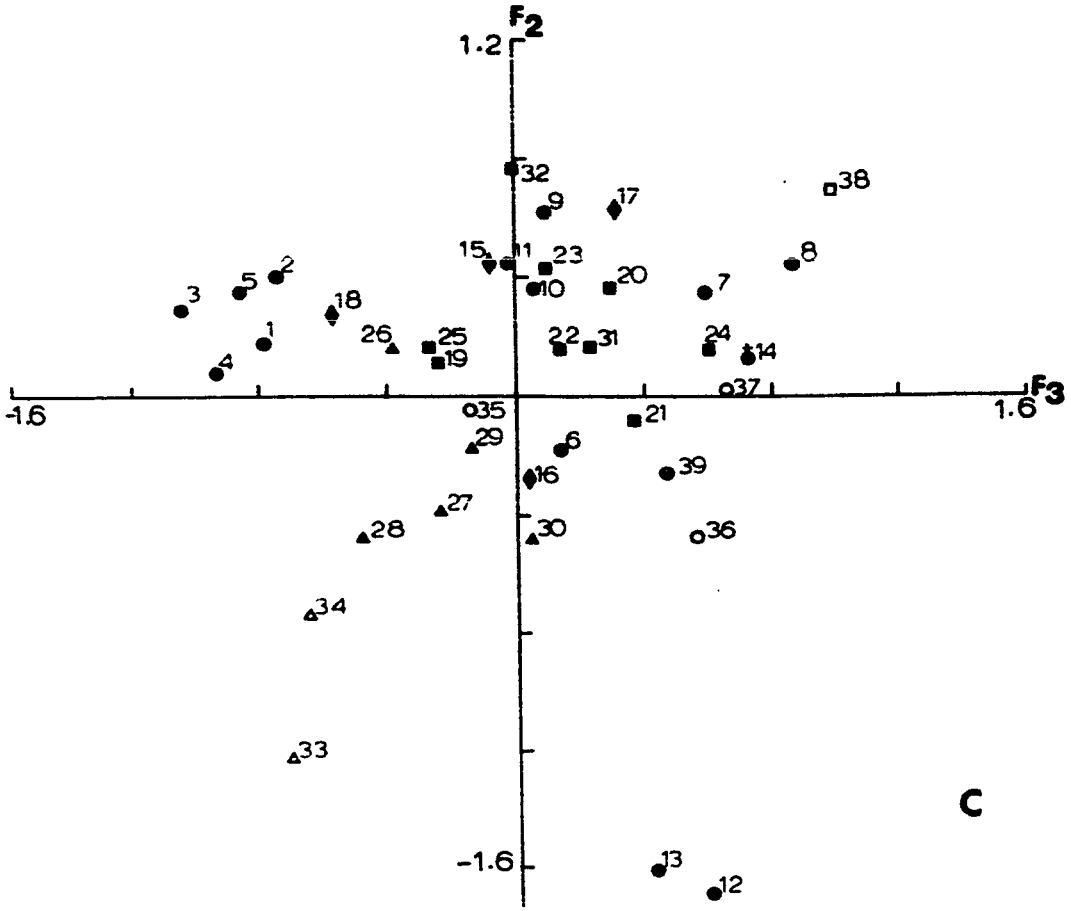
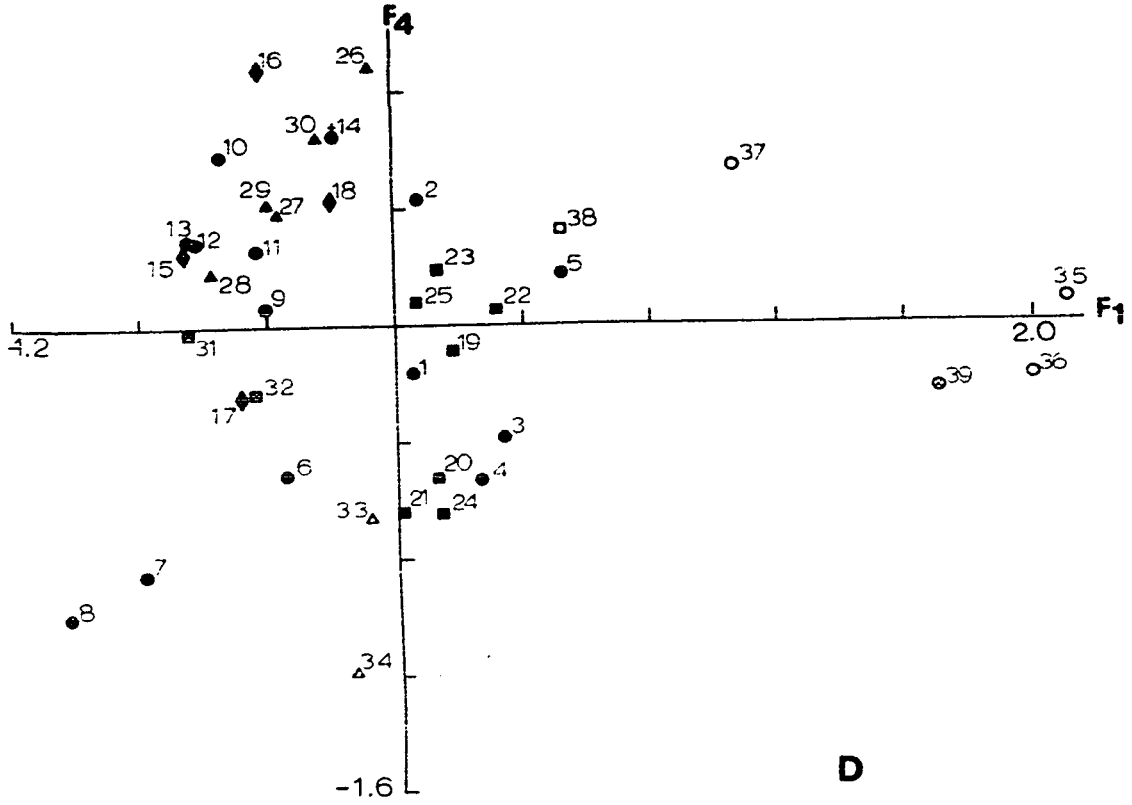


Fig. 36 A-D. Scatter diagrams showing the projection of OTU's on the first four components produced by component analysis of the correlation matrix 155, derived from all leaf and awn characters. For legend see Table 25.


F2
1.2
-1.6
F3
-1.6
1.6
C


F4
F1
-4.2
2.0
-1.6
D

The subsection Typicae is somewhat fragmented when the position of the OTU's on the first two components is plotted, (Fig. 37). We will, for the moment, call the fragments Typicae I, II, III and IV. One large group which includes all the L. arundinacea-complex (Typicae I) is centrally located. These species have high negative component scores on the F_4 (Fig. 36.D). The L. simplex-complex is split into three "subgroups". Firstly L. simplex subsp. stipoides (9) is in close proximity with L. kagerensis (10, 11) and these OTU's are called the Typicae II. L. simplex subsp. simplex (7, 8) and L. cameruneusis (6), the Typicae III, are quite close to L. perrieri (12) and L. madagascariensis (13), the Typicae IV (Fig. 36A). If a third component is considered, the Typicae III and IV are found to be more separate (Plate 25). When the F_1 and F_4 scatter diagram (Fig. 36.D) is examined, however, the L. simplex-complex OTU's form a tight cluster.

The subsection Pungentes (15-18) is shown to possess somewhat similar OTU's though they are not markedly separated from the other subsections. Conversely, the Annuae, (31, 32) Flammidae (33, 34), and Densispicae (26-30) sometimes form isolated groups (Fig. 37). L. gossweileri (26) of the latter subsection once again shows a peculiar distribution certainly due to the effect of leaf anatomy characters. The Acuminatae (19-25), although overlapping with the OTU's of the L. arundinacea-complex due to their wide range of variation, form a more compact cluster than witnessed previously (Fig. 37, 36 D).

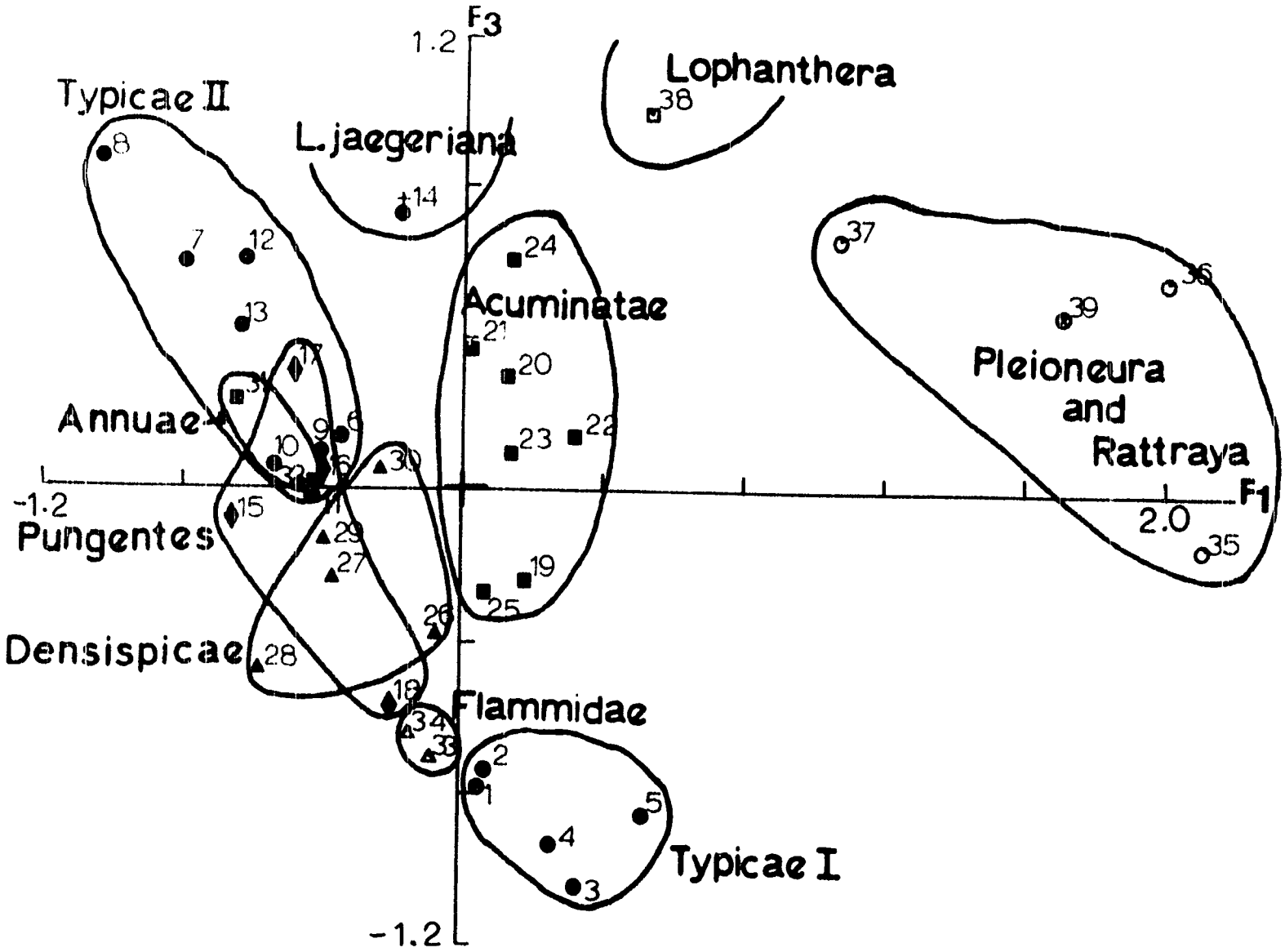


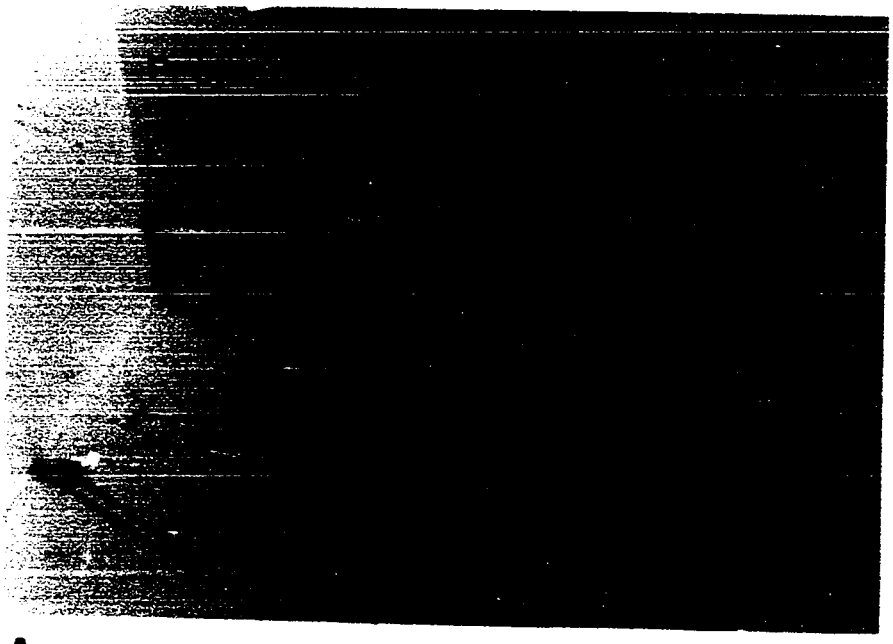
Fig. 37 Scatter diagram showing the projection of OTU's on the first and third components produced by component analysis of the correlation matrix 155. For legend see Table 25.

Plate 25

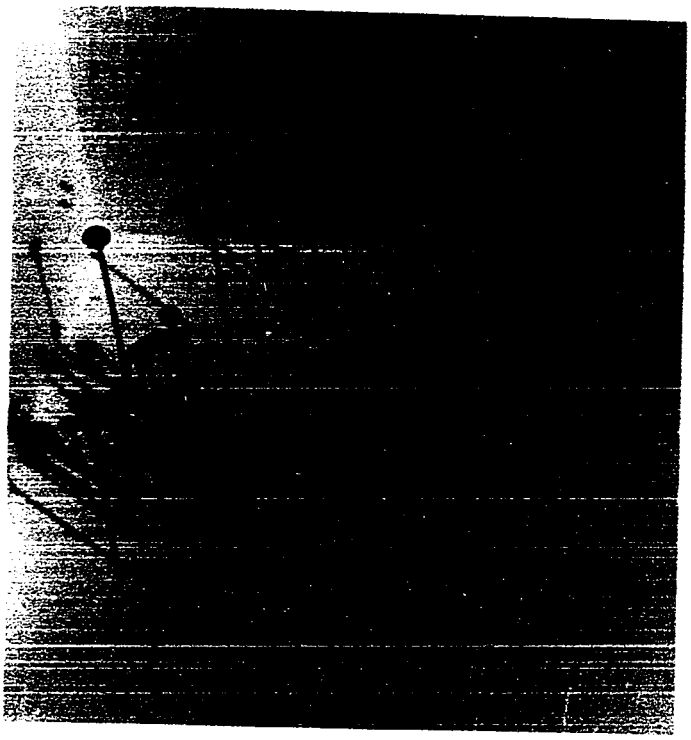
Photographs of a model showing the projection of the OTU's on the first three components of a component analysis of correlation matrix 155. The third component is represented on the vertical axis.

Legend

Typicae - black	Flammidae - mauve
Pungentes - blue	Lophanthera - black with red spot
Acuminatae - black with yellow spot	Pleioneura - yellow
Dentispicae - red	Rattrava - pink
Annuae - green	L. jaegeriana - orange



A



B

(b) Dispersion Coefficient

The dispersion matrix (No. 154) was also derived from the data matrix and subjected to a component analysis. The roots and the percentage of the variance that each root accounts for are given in Table 27. In this case the first 14 roots account for over 72% of the variance, while the first four account for 34.0% of the total variance.

The scatter diagrams showing the position of the OTU's on the first four components were drawn (Fig. 38 A-D). It was found that the scatter diagram of the first and third components show the overall relationships as discovered in the classification more aptly and this is shown in Fig. 39. The dispersion of the OTU's in a space determined by the first three components was illustrated by the construction of a model (Plate 26).

Once again, the sections of the genus are well-delimited. L. jaegeriana (14) is not as easily separated on the plot of the F_1 and F_2 (Fig. 38 A), but on the third component its positive score is higher than a number of the other OTU's (Fig. 39). The dispersion of the OTU's within section Loudetia is somewhat different. For instance, sub-section Typicae does not occur in four "groups" when the F_1 and F_2 scatter diagram is considered. However, the L. arundinacea-complex (Typicae I - Fig. 39) is distributed similarly to the previous analysis. The distribution of OTU's on the F_1 and F_3 (Fig. 38 B) is almost identical to the distribution of OTU's on the F_1 and F_4 (Fig. 36 D) of the

previous analysis. The Typicae I have high negative scores on the F_3. The OTU's of the L. simplex-complex (6 - 13) are well clustered on the F_1 and F_3 (Fig. 36 D), but their varied component scores on the F_2 positions them somewhat differently along this axis. L. perrieri (12) and L. madagascariensis (13) appear as extreme forms with high negative component scores. Both subspecies of L. simplex (7-9) and the exemplars of L. kagerensis (10, 11) are close to one another (Fig. 38A). L. camerunensis (6) is slightly apart from the other Typicae and closer to the Densispicae (26-30) form a fairly close cluster except for the anomalous L. gossweileri (26). The Flammidae (33,34) and the Acuminatae (19-25) are often quite distinct from other OTU's (Fig. 39, Plate 26).

C. Classification

Once again classifications were produced using three different sources of data. The absolute distance matrices were calculated from the component scores matrices (Nos. 157 and 158) and the raw data matrix (No. 153). It was found that the distance matrix (No. 160) derived from the component scores of the dispersion matrix was identical to that derived from the raw data (Matrix No. 156). The distance matrices were subjected to the sum of squares agglomeration method of cluster analysis and dendrograms drawn (Fig. 40. A - B). The distance matrix produced from the component scores (dispersion coefficient) was also analysed by graph theory and a dendrogram plotted (Fig. 40.C.).

Table 27: Roots of dispersion coefficient matrix (No. 154)

Root	Value of root	Percentage of total variation accounted for	Accumulated percentage
1	536.85	10.83	10.83
2	439.74	8.87	19.71
3	377.69	7.62	27.33
4	330.23	6.66	34.00
5	304.54	6.15	40.14
6	255.61	5.16	45.30
7	237.70	4.80	50.10
8	207.00	4.18	54.28
9	186.78	3.77	58.05
10	180.70	3.65	61.69
11	152.21	3.07	64.76
12	143.37	2.89	67.66
13	128.41	2.59	70.25
14	126.83	2.56	72.81
15 - 39	1,347.36	27.19	100.00
Total sum of squares	4,955.02	100.00	-

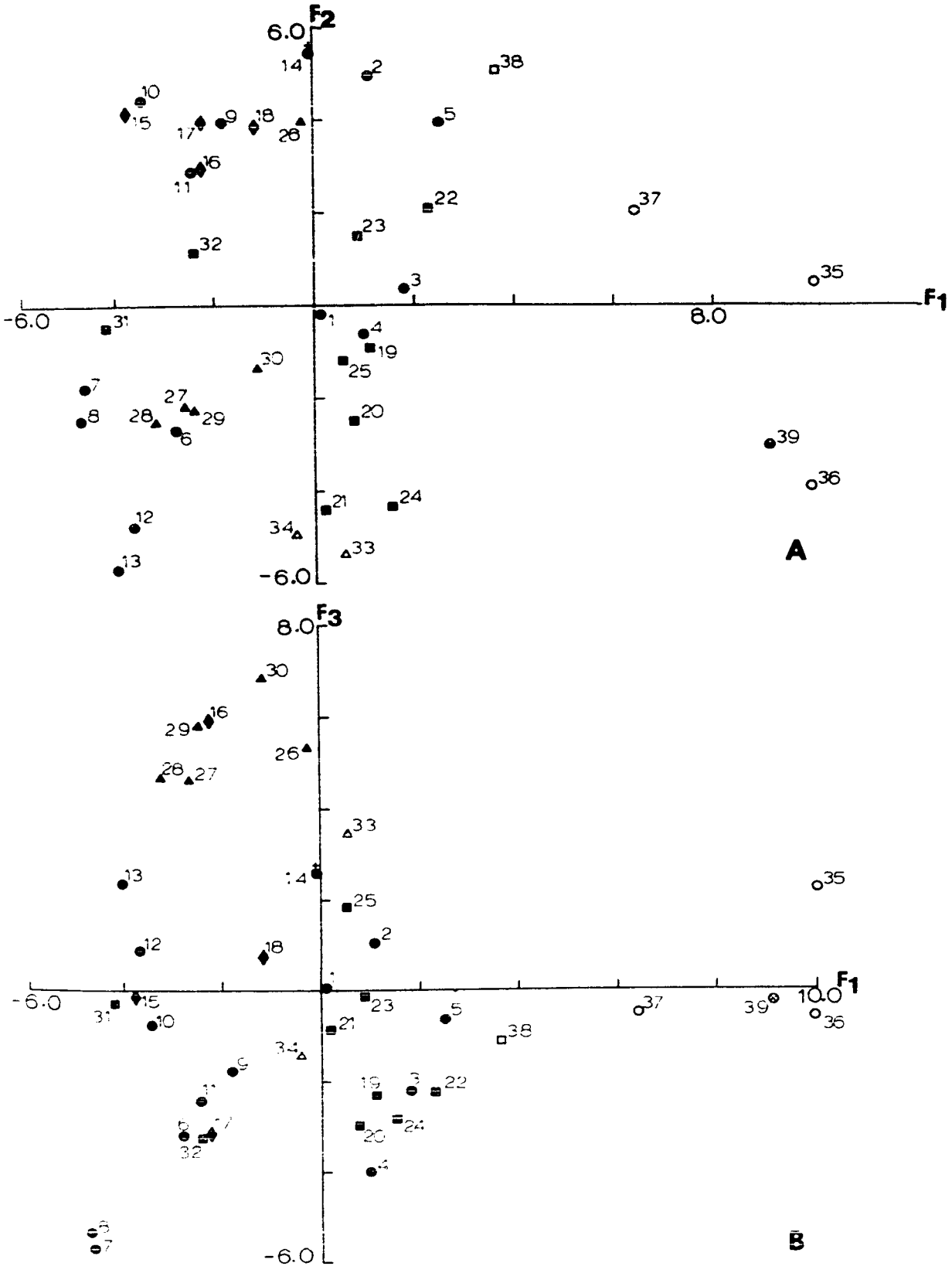
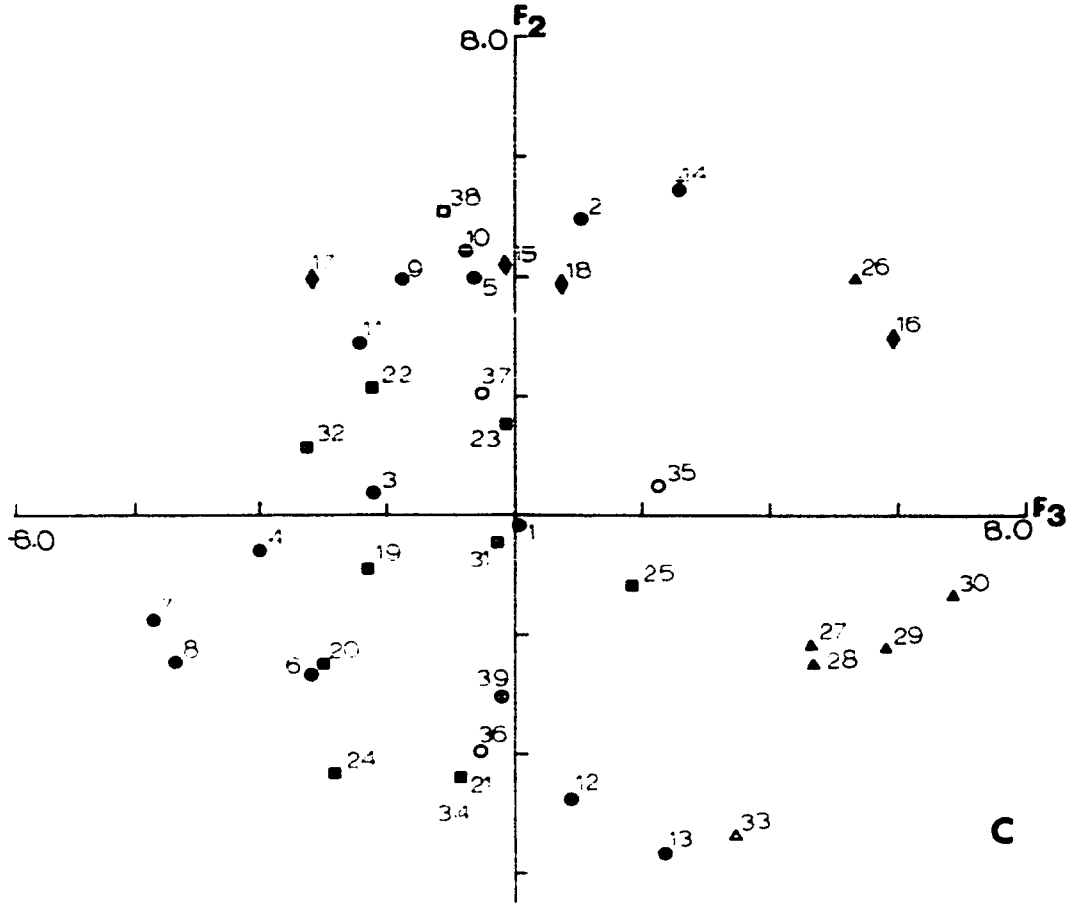
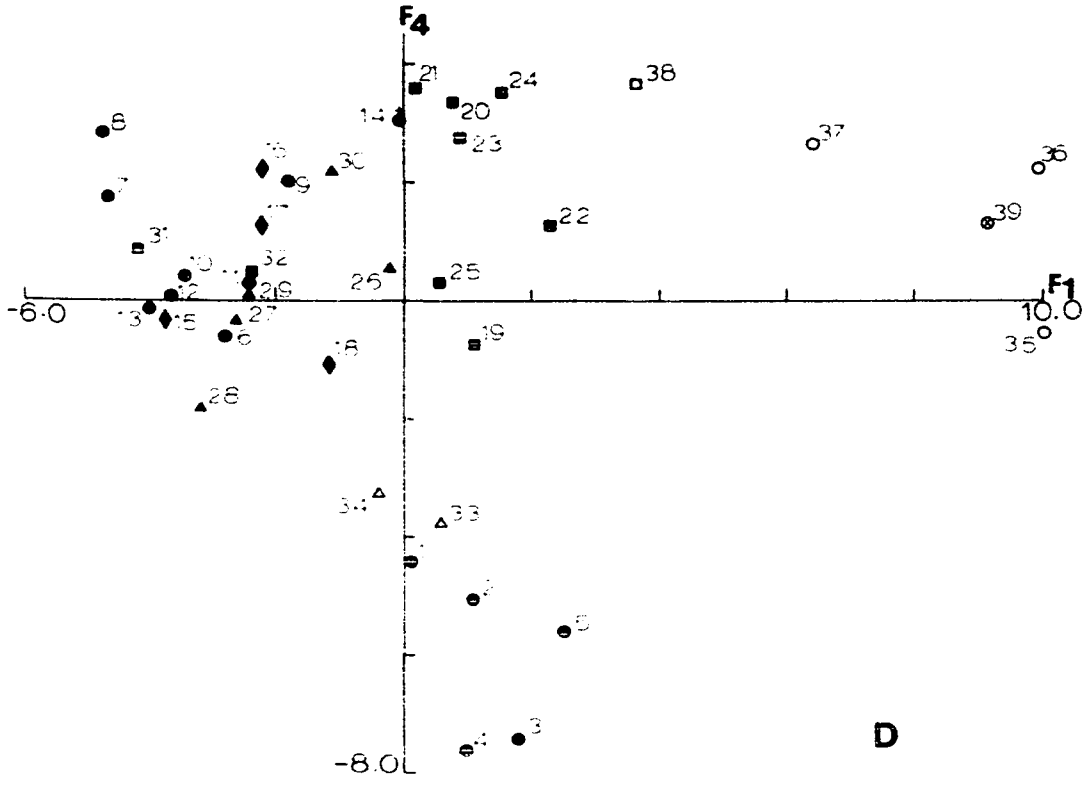


Fig. 38 A-D. Scatter diagrams showing the projection of OTU's on the first four components produced by component analysis of the dispersion matrix 154, derived from all leaf and own characters. For legend see Table 25.


F2
8.0
F3
-6.0
8.0
C


F4
F1
-6.0
10.0
-8.0
D

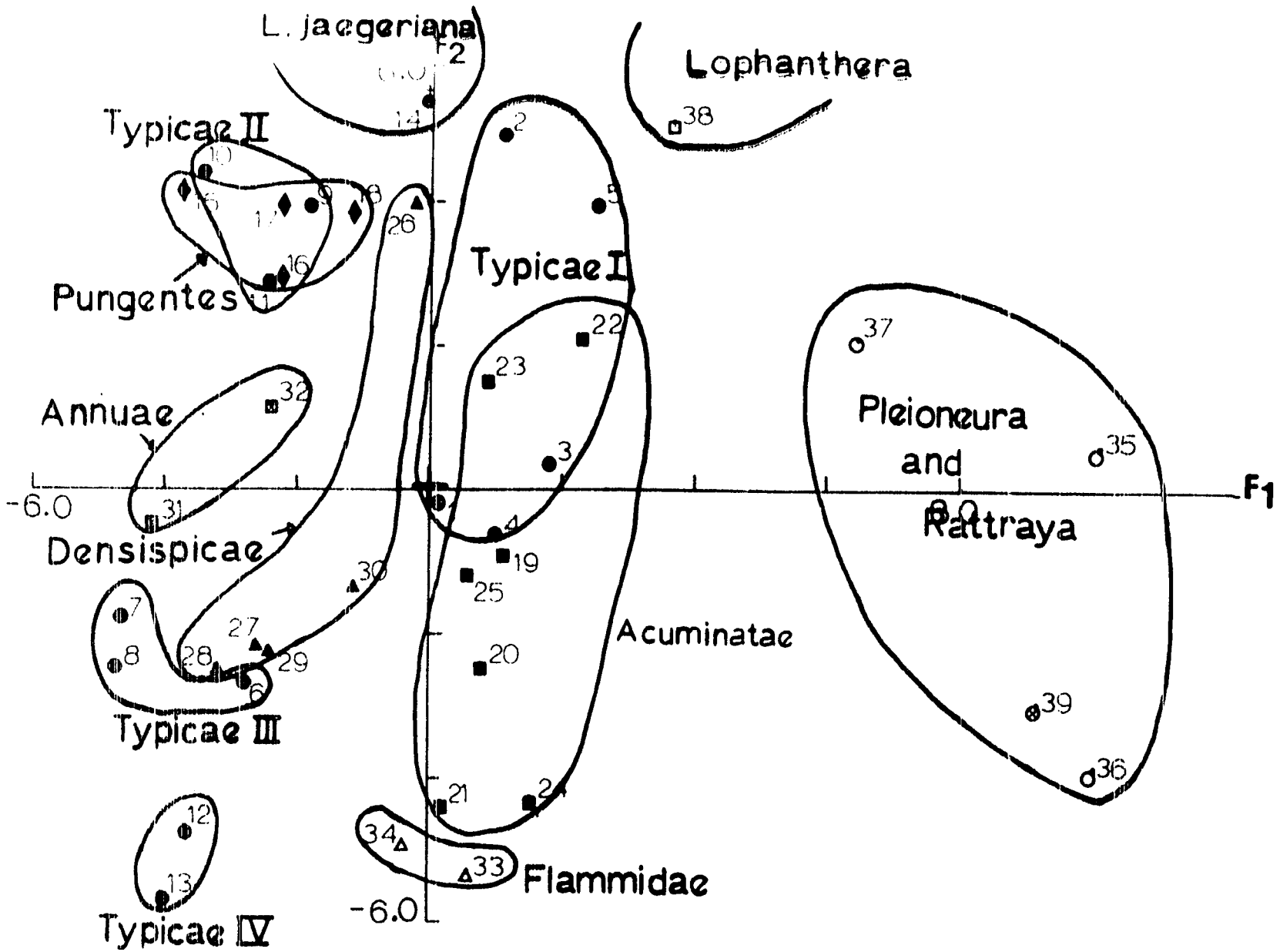


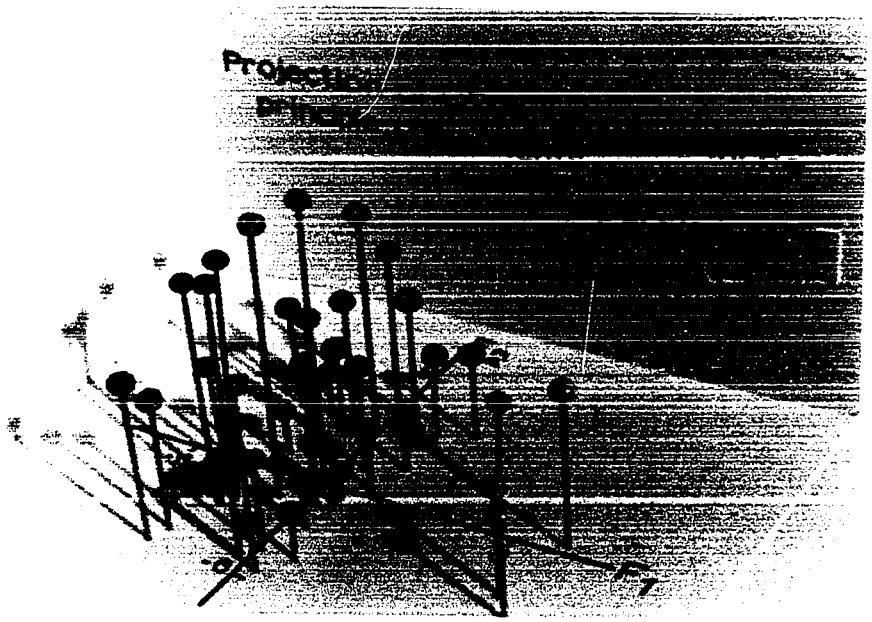
Fig. 39 Scatter diagram showing the projection of OTU's on the first two components produced by component analysis of the dispersion matrix 154. For legend see Table 25.

Plate 26

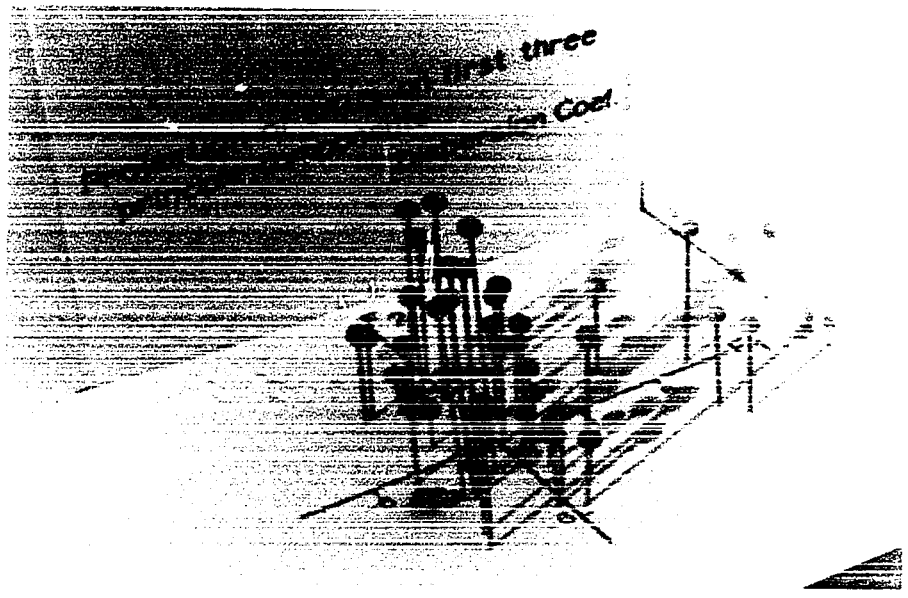
Photograph of a model showing the projection of the OTU's on the first three components of a component analysis of correlation matrix 155. The third component is represented on the vertical axis.

Legend

Typicae - black	Flammidae - mauve
Pungentes - blue	Lophanthera - black with red spot
Acuminatae - black with yellow spot	Pleioneura - yellow
Dentispicae - red	Rattraya - pink
Annuae - green	L. jaegeriana - orange



A



B

(a) Classification resulting from the use of the component scores of the principal components analysis (correlation coefficient) as the data matrix. (Fig. 40.A)

If this dendrogram is examined at the 10% level the following clusters of OTU's appear:

(i) The L. arundinacea -complex (1-5) forms a compact group (Typicae I - see above)

(ii) The OTU's of subsection Flammidae (33, 34) are closely akin to the above.

(iii) L. simplex subsp. simplex (7,8) and L. camerunensis form a group - the Typicae III of the principal components analysis.

(iv) The Typicae II, (L. simplex subsp. stipoides, 9, and L. kagerensis, 10, 11) cluster with L. cuanzensis (23).

(v) The subsection Annuae (31, 32) and two of the OTU's of subsection Pungentes (L. lanata, 15 and L. demeusei 17) form a group.

(vi) L. jaegeriana (14) is akin to L. togoensis (38).

(vii) The majority of the subsection Acuminatae cluster together, except for L. cuanzensis (23) and L. filifolia subsp. humbertiana (25).

(viii) L. longipes (16), L. gossweileri, L. crassipes (18) and L. filifolia subsp. humbertiana are an odd group.

(ix) All the OTU's of subsection Densispicae (26-30), except for L. gossweileri (16) which is nevertheless fairly close by, cluster together.

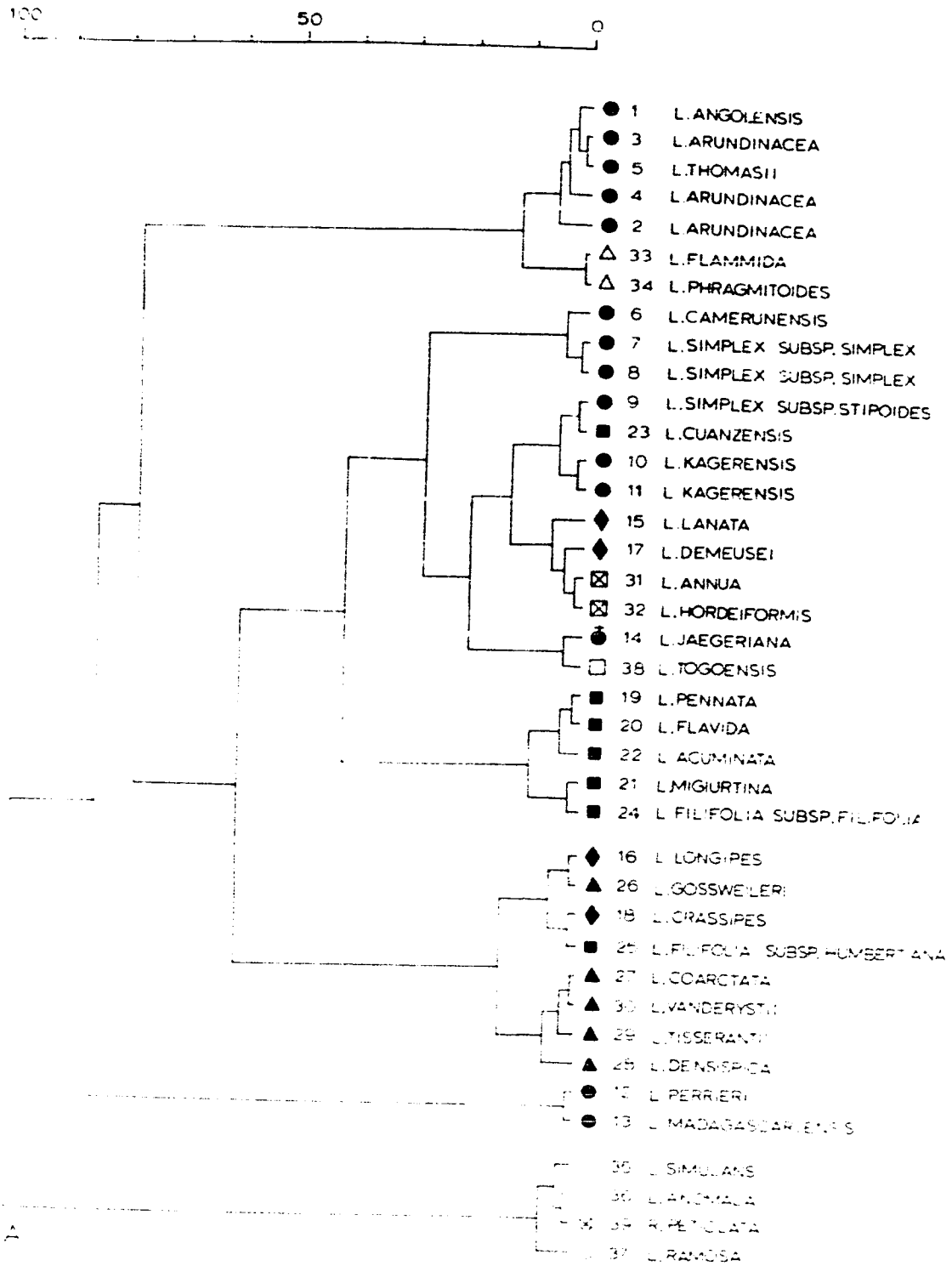
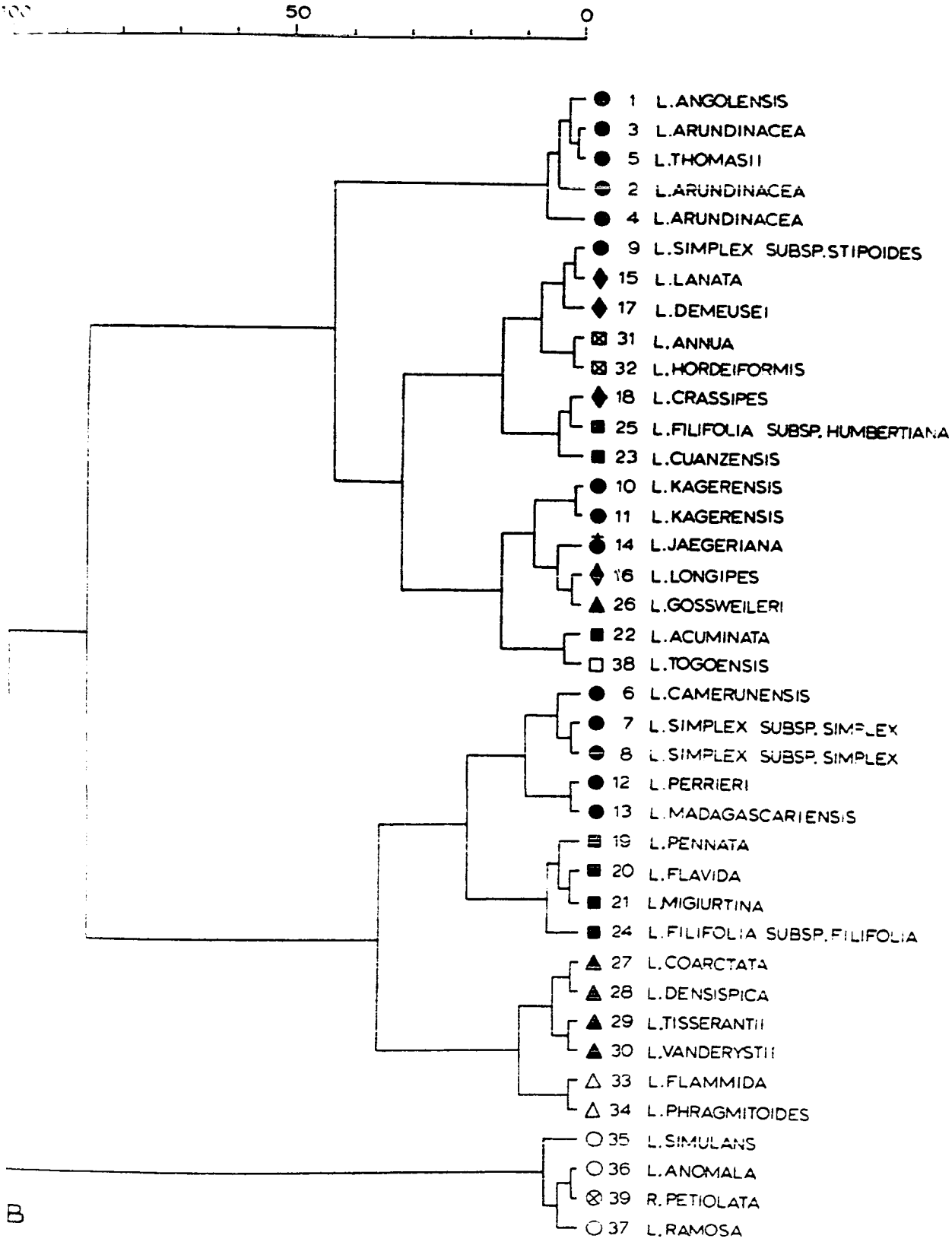
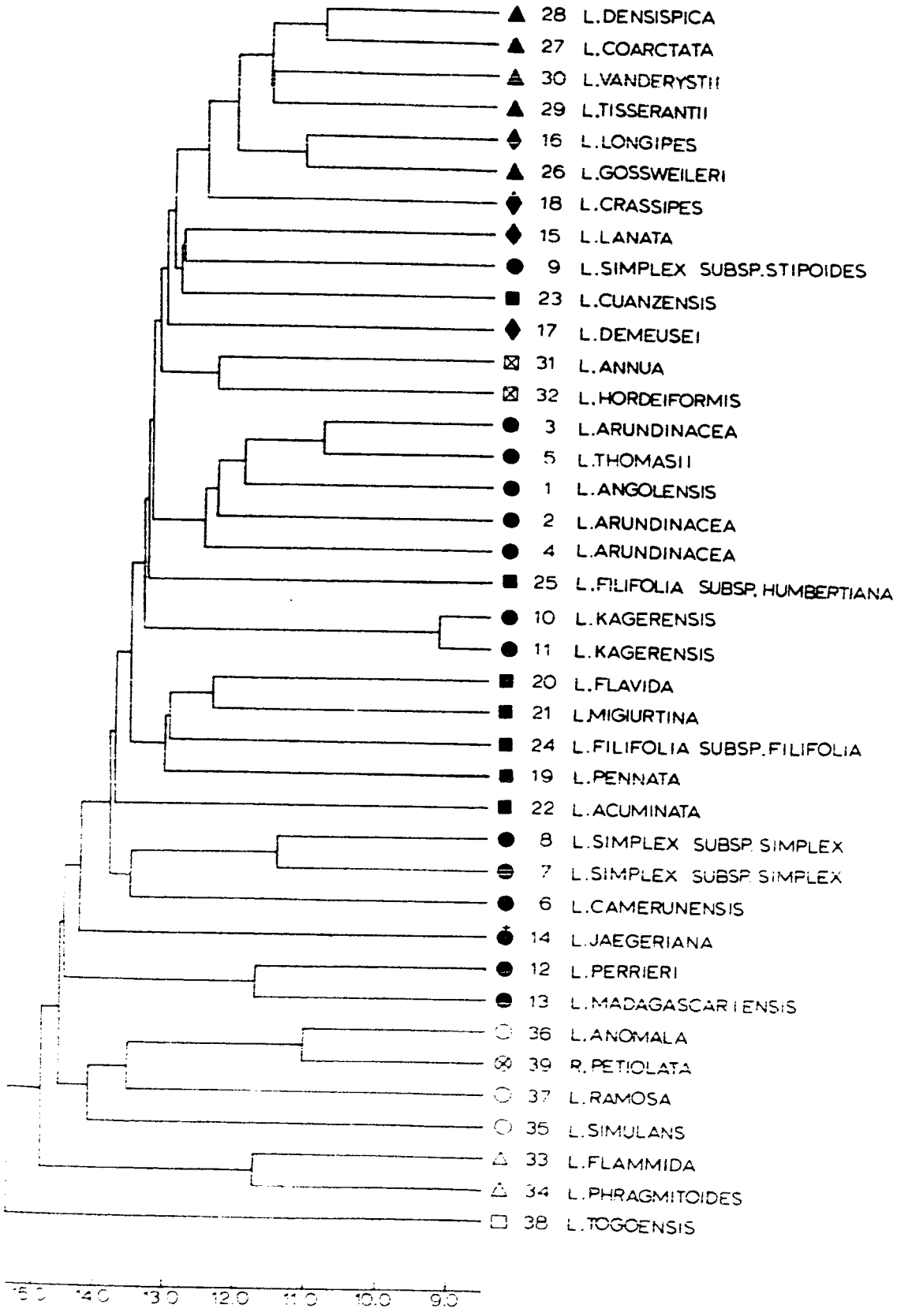


Fig. 41 Dendrograms of 39 OTU's of Loudetia and Rattraya derived from all leaf and awn characters, using different data sources and clustering methods: A-Sum of squares agglomeration and component scores (correlation coefficient) as data source; B-Sum of squares agglomeration and data matrix 153 as data source; Scale indicates the with-group sum of squares expressed as a percentage of the total sum of squares; C-Graph Theory and data matrix 153 as data source; Scale indicates absolute distance values. For legend see Table 25.


50
0
1 L.ANGOLENSIS
3 L.ARUNDINACEA
5 L.THOMASII
2 L.ARUNDINACEA
4 L.ARUNDINACEA
9 L.SIMPLEX SUBSP.STIPOIDES
15 L.LANATA
17 L.DEMEUSEI
31 L.ANNUA
32 L.HORDEIFORMIS
18 L.CRASSIPES
25 L.FILIFOLIA SUBSP. HUMBERTIANA
23 L.CUANZENSIS
10 L.KAGERENSIS
11 L.KAGERENSIS
14 L.JAEGERIANA
16 L.LONGIPES
26 L.GOSSWEILERI
22 L.ACUMINATA
38 L.TOGOENSIS
6 L.CAMERUNENSIS
7 L.SIMPLEX SUBSP. SIMPLEX
8 L.SIMPLEX SUBSP. SIMPLEX
12 L.PERRIERI
13 L.MADAGASCARIENSIS
19 L.PENNATA
20 L.FLAVIDA
21 L.MIGIURTINA
24 L.FILIFOLIA SUBSP.FILIFOLIA
27 L.COARCTATA
28 L.DENSISPICA
29 L.TISSERANTII
30 L.VANDERYSTII
33 L.FLAMMIDA
34 L.PHRAGMITOIDES
35 L.SIMULANS
36 L.ANOMALA
39 R.PETIOLATA
37 L.RAMOSA
B


28 L.DENSISPICA
27 L.COARCTATA
30 L.VANDERYSTII
29 L.TISSERANTII
16 L.LONGIPES
26 L.GOSSWEILERI
18 L.CRASSIPES
15 L.LANATA
9 L.SIMPLEX SUBSP.STIPOIDES
23 L.CUANZENSIS
17 L.DEMEUSEI
31 L.ANNUA
32 L.HORDEIFORMIS
3 L.ARUNDINACEA
5 L.THOMASII
1 L.ANGOLENSIS
2 L.ARUNDINACEA
4 L.ARUNDINACEA
25 L.FILIFOLIA SUBSP. HUMBERTIANA
10 L.KAGERENSIS
11 L.KAGERENSIS
20 L.FLAVIDA
21 L.MIGIURTINA
24 L.FILIFOLIA SUBSP.FILIFOLIA
19 L.PENNATA
22 L.ACUMINATA
8 L.SIMPLEX SUBSP SIMPLEX
7 L.SIMPLEX SUBSP SIMPLEX
6 L.CAMERUNENSIS
14 L.JAEGERIANA
12 L.PERRIERI
13 L.MADAGASCARIENSIS
36 L.ANOMALA
39 R.PETIOLATA
37 L.RAMOSA
35 L.SIMULANS
33 L.FLAMMIDA
34 L.PHRAGMITOIDES
38 L.TOGOENSIS
15.0
14.0
13.0
12.0
11.0
10.0
9.0

(x) The Typicae IV (L. perrieri, 12, and L. madagascariensis, 13) are together.

(xi) The section Pleioneura (35-37) and Rattraya (39) form the final cluster.

At the 50% level only 5 groups are formed which are:

(i) Most large leaved OTU's -L. arundinacea complex (1-5) and subsection Flammidae (33, 34).

(ii) A group which includes most of the remainder of the section Loudetia, L. jaegeriana and section Lophanthera.

(iii) The subsection Densispicae plus a few other OTU's.

(iv) The two unusual OTU's of subsection Typicae - L. perrieri, L. madagascariensis.

(v) Section Pleioneura and Rattraya.

The correlation between this dendrogram and its distance matrix was found to be 0.539.

(b) Classification produced from the data matrix 153 (Fig. 40 B)

This dendrogram is identical with that produced when the component scores (dispersion coefficient) are used as data matrix, since the two distance matrices were found to be identical. The correlation between the distance matrix and the resulting dendrogram was 0.508 in this instance.

If the OTU groups are compared at the 10% level in this case, the result is as follows:

(i) The L. arundinacea - complex (1-5)

(ii) The subsection Annuae (31, 32), L. lanata (15)

and L. demeusei (17) (Pungentes), and L. simplex subsp. simplex (9).

(iii) L. crassipes (18), L. filifolia subsp. humbertiana (25) and L. quanzensis (23).

(iv) L. kagerensis (10, 11), L. jaegeriana (14), L. longipes (16) and L. gossweileri (26).

(v) L. acuminata (22) and L. togoensis (38)

(vi) L. simplex-complex (6-13) apart from L. kagerensis (10, 11) and L. simplex subsp. stipoides (9) already mentioned above.

(vii) Subsection Acuminatae (19-25) except for those already mentioned.

(viii) Subsection Densispicae (26-30), except for the anomalous L. gossweileri (26).

(ix) Subsection Flammidae (33, 34).

(x) Section Pleioneura (35-37) and Rattraya (39).

The larger groups at the 50% level in this case are different from those in the above dendrogram:

(i) The L. arundinacea-complex, many other OTU's of section Loudetia and L. togoensis and L. jaegeriana form a group (i - v of above).

(ii) The L. simplex-complex, subsections Acuminatae, Densispicae and Flammidae are clustered (vi-ix).

(iii) Section Pleioneura and Rattraya.

(c) Classification produced from the data matrix 153 using graph theory (Fig. 40, C).

The correlation between the distance matrix and the dendrogram produced by graph theory was 0.699 so it would appear that this method is more efficient in transferring the information from the distance matrix to the classification. It is, however, difficult to interpret the results at any one level due to the formation of groups at high values on the distance scale. The arrangement of OTU's gives an indication of similarity between OTU's.

The subsections Densispicae (26-30) and Pungentes (15-18) are fairly close to one another with L. simplex subsp. stipoides (9) and L. cuanzensis (23) occurring in the same group. The Annuae (31, 32) and the L. arundinacea-complex are below this forming two groups. L. kagerensis (10, 11) are seen to be the two most similar OTU's in the whole dendrogram. The OTU's of subsection Acuminatae (19-25) with the exception of L. filifolia subsp. humbertiana (25) and L. cuanzensis (23) are clustered together. L. simplex subsp. simplex (7, 8) and L. camerunensis (6) form another group. L. jaegeriana (14) is isolated between this group and L. perrieri (12) and L. madagascariensis (13).

Finally, one comes to the usual two clusters formed by section Pleioneura (35-37) with Rattrava and subsection Flammidae (33, 34) with section Lophanthera (38), this last being most dissimilar from all other OTU's.

If one were to consider the classification at a level

Table 28: Cophenetic correlation coefficients produced by comparison of the distance matrices derived from three different data sources, using all characters.

Data Source

	Component Scores - Correlation Coef. (Matrix 158)	Component Scores - Dispersion Coef. (Matrix 157)	Raw Data (Matrix 153)
Matrix 158	1.00	-	-
Matrix 157	0.915	1.00	-
Matrix 153	0.915	1.00	1.00

Table 29: Cophenetic correlation coefficients produced by comparison of dendrograms derived from different data sources or using different clustering methods, and using all characters.

Clustering Method	Clustering Method	Sum of Squares Agglomeration		Graph Theory
Clustering Method	Data Source	Component Scores - Correlation Coef. (Matrix 158)	Component Scores - Dispersion Coef. (Matrix 157)	Component Scores - Dispersion Coef. (Matrix 157)
Sum of Squares Agglomeration	Matrix 158	1.00	-	-
	Matrix 157	0.453	1.00	-
Graph Theory	Matrix 157	0.533	0.336	1.00

just greater than a 14.0 distance, the sections Lophanthera, Pleioneura (including Rattreya) and the majority of section Loudetia would form three groups. L. jaegeriana (14), the subsection Flammidae (33, 34) and L. perrieri (12) and L. madagascariensis (13) would form another three.

(d) Comparison of the different classifications

The distance matrices which were derived from the three different data sources were compared by use of the product moment correlation coefficient (Table 28) and the results obtained showed very high correlation in all cases. The cophenetic values obtained from the dendrograms were used to calculate the cophenetic correlation coefficients. (Table 29). These results were found to be slightly higher than those obtained in other analyses.

D. Analysis of the supra-specific taxa

(a) Data preparation

The supra-specific groups were identical in each of the analyses on the separate character sets, so the data preparation was much simpler in this case. The component scores derived from the principal components analyses of the leaf anatomy, leaf epidermis and awn data matrices were combined to form a 30 x 10 data matrix (No. 180) which contained the information for all the characters (Fig. 41).

(b) Principal Components Analysis

The dispersion coefficients (matrix No. 182) were computed and subjected to component analysis. The roots are given in Table 30, and the positions of the OTU's with respect to the first three components are shown in the scatter diagrams (Fig. 42 A-C). The first three roots account for 69% of the variation.

The results of the analysis reflect the trends seen previously. All the subsections of the section Loudetia except for subsection Flammidae form a tight cluster on both the F_1/F_2, and F_1/F_3 scatter diagrams (Fig. 42 A,B). The section Pleioneura (7) and Rattraya (9) are closer to one another than to any other OTU (Fig. 42 C). The section Lophanthera (9) subsection Flammidae (6) and L. jaegeriana (10) do not show close affinities for any other OTU's.

(c) Classification

The absolute distance matrix (No. 184) was computed from the component scores matrix, and following sum of square agglomeration, the dendrogram (Fig. 43) plotted.

The clusters which are formed are very similar to what would be expected from the distribution of the OTU's in the scatter diagrams. The section Loudetia (1-5) forms one group, except for subsection Flammidae (6) which clusters with L. jaegeriana (10). The section Lophanthera (8) clusters with the section Pleioneura (7) and Rattraya (9).

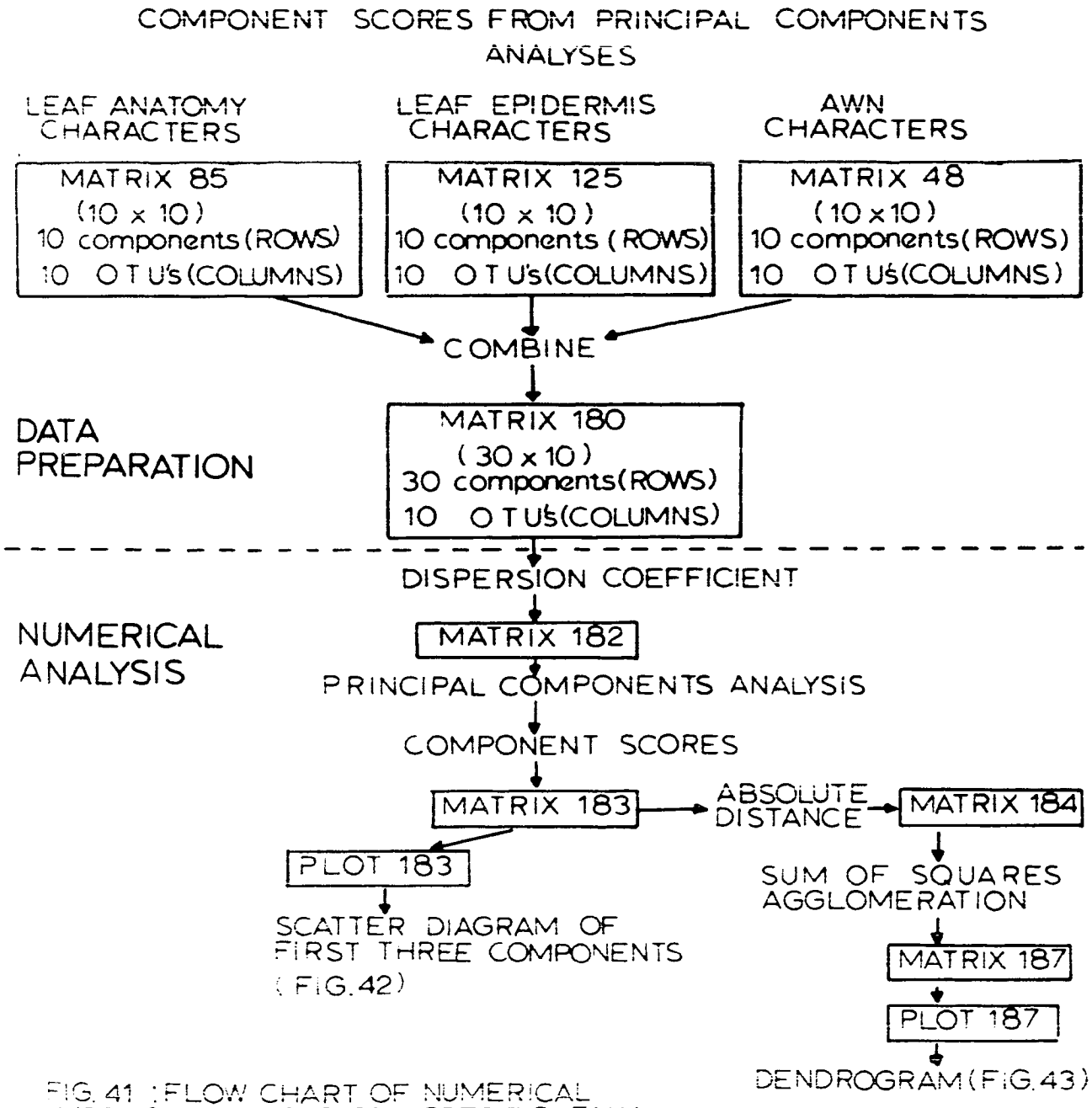


FIG. 41 : FLOW CHART OF NUMERICAL METHODS USING SUPRA SPECIFIC TAXA AS O T U's AND ALL ANATOMICAL CHARACTERS

Table 30: Roots of Dispersion coefficient matrix (No.182)

Root	Value of root	Percentage of total variation accounted for	Accumulated percentage
1	10.310	34.40	34.40
2	5.455	18.20	52.60
3	4.927	16.44	69.04
4	3.288	10.97	80.01
5	1.747	5.83	85.83
6	1.582	5.28	91.11
7	1.148	3.83	94.94
8	0.934	3.12	98.06
9	0.581	1.94	100.00
10	0.000	0.00	100.00
Total sum of squares	29.972	100.00	-

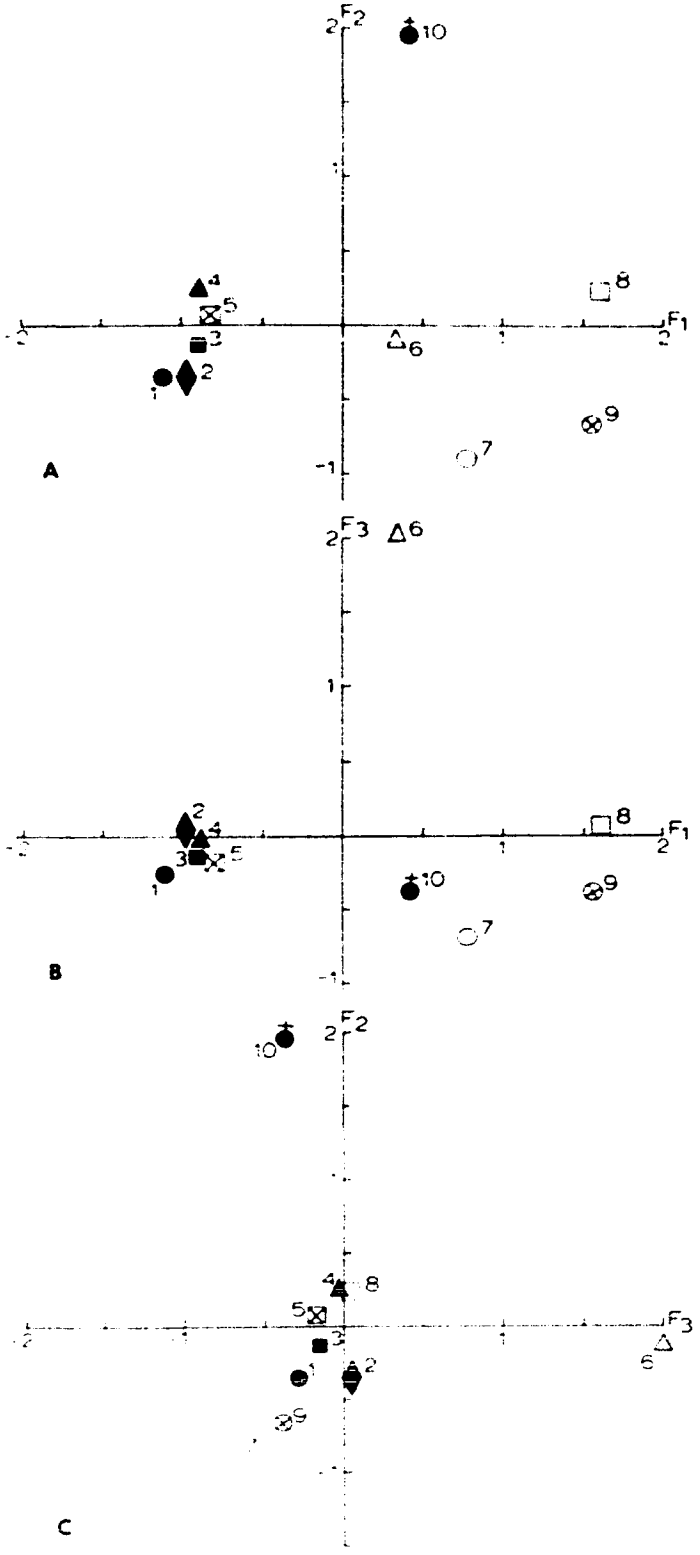


Fig. 42 A-C. Scatter diagrams showing the projection of the supra-specific taxa on the first three components of a component analysis of dispersion matrix 182, derived from all leaf and awn characters. For legend see Table 25.

3. A comparison of the classifications using different sets of characters

The use of characters from different sources resulted in classifications which, although showing definite similarities in the grouping of OTU's, were by no means identical. The cophenetic correlation coefficients were computed between the different classification hierarchies, in order to get an exact measure of comparison. The dendrograms compared were those produced by using as data source, the component scores matrix derived from the use of the dispersion coefficient. The cophenetic values of the OTU's common to each dendrogram were the only ones used in computing the cophenetic correlations.

The results of this analysis (Table 31) show that in each case the dendrogram of any particular set of characters is more similar to that in which all the characters were used, than to those derived from other character sets. The lowest correlation is between the classification based on leaf anatomy characters and that based on leaf epidermis characters. The leaf anatomy characters dendrogram is most similar to that when all the characters were used indicating that leaf anatomy characters are the most important when all the characters are used. However, there are more leaf anatomy characters (79) than those of leaf epidermis (52) and awn anatomy (31) which may influence this conclusion.

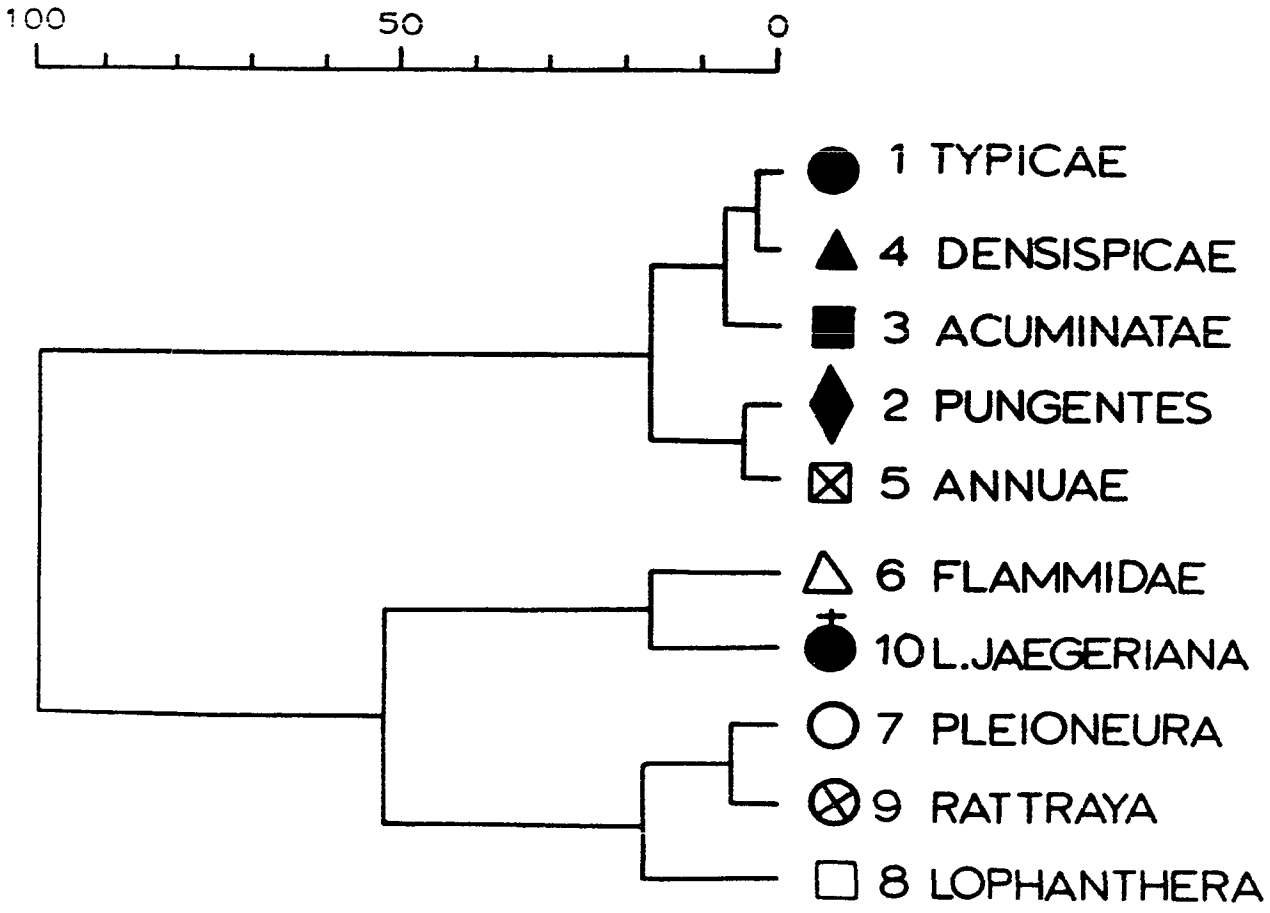


Fig. 43 Dendrogram of supra-specific taxa of Loudetia and Rattraya derived from all leaf and awn characters. Scale indicates the within-group sum of squares expressed as a percentage of the total sum of squares.

Table 31: Cophenetic correlation coefficients derived by comparison of the classifications produced using different sets of characters.

	Character Sets			
	Leaf Anatomy Characters	Leaf Epidermis Characters	Awn Characters	All Characters Combined
Leaf Anatomy Characters	1.00	-	-	-
Leaf Epidermis Characters	0.014	1.00	-	-
Awn Characters	0.019	0.041	1.00	-
All Characters Combined	0.412	0.145	0.270	1.00

4. Keys to the taxa using awn and leaf characters

Contrary to the findings of Conert (1957) it was found that the anatomy of the leaves and awns of the species of Loudetia and Rattraya was of significance taxonomically. The sections of the genus Loudetia can be easily recognized on characters of the awn and the leaf. In many cases the subsections of section Loudetia also have distinct characteristics and can be distinguished from one another. Problems arise in the identification of species within a subsection, and there are also cases where species from different subsections have very similar leaf anatomy. The extent of intra-specific variation has not been pursued in great depth in this study and problems arise when identifications are made at this level on leaf anatomy characters. Certainly the morphological variation in a group such as the Typicae would be expected to extend to anatomical variation. Discrepancies that occur in diagnostic keys based on leaf epidermal characters when only a few representatives of a species, or those from a restricted range are studied, have been pointed out in the work of Stewart (1965), (this thesis p. 97). The keys presented here at the specific level should, therefore, be considered as models for refinement as more information comes to hand.

The diagnostic keys presented below were produced from the information that had been recorded on coding forms, tables, figures, and plates. It is possible to develop keys from the data matrix in the form of punched

cards (Sokal and Sneath, 1963) but in view of the large number of characters and lack of experience in such methods, this was not attempted.

The sections of Loudetia and the genus Rattraya are mostly readily identified on the basis of awn anatomy and morphology. It is difficult to produce anatomical sections of the awn, so that alternative methods using leaf characters are also given. However, at the most, only the gross features of awn anatomy are required and these may be easily observed with a dissecting microscope. Another reason for including vegetative characters throughout the keys is that flowering material may not be available and it may, therefore, be necessary to use vegetative characters. Within sections and subsections, the awn anatomy is of less significance taxonomically and most characters used are those of leaf morphology or anatomy. As is the case with many vegetative keys, a large number of characters must be given at each dichotomy (see Hubbard, 1954) because the differences between closely allied taxa can only be defined polythetically, over a number of examplars.

(a) Key to the sections of Loudetia and the genus Rattraya

1. Awn very large (14.0 cm or longer), column in cross-section 12-14 layers thick; leaves fairly short and broad, mid-rib absent, in transverse section cells of the outer bundle sheath very large, vascular bundles angular in outline; in section margin of the leaf pointed, swollen and with many fibres.....Section: Lophanthera

1. Awn usually not very large (usually less than 12.0 cm), or if so, column in cross-section never more than 7 layered; leaves various, but not as above, or if so, (L. jaegeriana) awn very small and plants delicate:

    2. Column of awn slightly curved in cross-section; usually much shorter than the bristle; the leaf, in transverse section, lacking colourless cells associated with bulliform cells, and without tertiary vascular bundles; abaxial bulliform cells usually present:

        Leaves up to 15 mm or more wide, pseudo-petiole always present; in transverse section mid-rib with single broad adaxial strand, neither surface of blade ribbed . . .. Genus: Rattraya

        Leaves not as wide (up to 12 mm), no pseudo-petiole; if mid-rib very large, three adaxial strands present and blade surfaces ribbed or wavy ... Section: Pleioneura

    2. Column of awn inverted U-shape in cross-section, well developed and up to the length of the bristle; or if not (Flammidae and L. jaegeriana), associated colourless cells and tertiary vascular bundles present in leaf blade; abaxial bulliform cells usually absent:

        Leaf blade short and broad, mid-rib absent; in transverse section cells of outer bundle

sheath very large, vascular bundles angular in outline; margin of leaf pointed, swollen with triangular shaped group of fibres; column of awn only slightly curved in cross-section ..... Section: L. jaegeriana

Leaves various, usually long-linear and tapering, anatomically not as above; column of awn usually U-shaped in cross-section ..... Section: Loudetia

(b) Key to the subsections of the section Loudetia

1. Column of awn very short (up to 2.4 mm); leaf blade 6-12 mm wide, with or without a midrib, involute when dry; in transverse section primary vascular bundles triangular in outline, with a broad thin adaxial strand arched over them; abaxial epidermis lacking silica bodies in intercostal zone ..... Section: Flammidae

1. Column of awn much longer (5-40 mm); leaf blade usually narrower but if 6-10 mm wide then with a mid-rib and never with the above combination of anatomical characters:

2. Column of the awn up to 40 mm long, well-developed; blade in transverse section with bulliform cells appearing large, rounded, ± isodiametric, in a group of five or more or ± continuous and not in groups; stomata of abaxial epidermis ± 40 µm long, intercostal

silica bodies tall and narrow or crescent-shaped ..... Subsection: Annuae

2. Column of awn usually not as well developed (5-20 mm long); blade in transverse section with bulliform cells of various shapes, more often in groups of five or fewer cells; stomata always less than 40μ long and silica bodies of intercostal zone, if present, more often cross-, dumbell- or irregularly shaped:

3. Abaxial epidermis devoid of prickle-hairs and hooks, but microhairs always present, long cells with thick walls, intercostal silica bodies absent; leaf blade with no mid-rib, usually distinctly ribbed adaxially and with many large bulliform and accessory parenchyma cells between the ribs, bulliform cells half the thickness of leaf... Subsection: Densispicae

3. Abaxial epidermis with prickle-hairs and/or hooks, or if absent, long cells with very thick walls and microhairs absent, or intercostal silica bodies present, leaf blade not anatomically as above and bulliform cells less than half thickness of leaf:

4. Prickle-hairs usually absent on abaxial epidermis, hooks usually

present, intercostal zone silica bodies tall and narrow

..... Subsection: Pungentes

4. Prickle-hairs and hooks invariably present, intercostal zone silica bodies if present, usually differently shaped from the above:

5. Lamina in transverse section with bulliform cells in fan-shaped groups, no distinct mid-rib; prickle-hairs and hooks usually abundant on abaxial epidermis

.. Subsection: Acuminatae

5. Lamina in transverse section with bulliform cells in inflated cell groups or not in distinct groups, prickle-hairs and hooks not usually very abundant

.. Section: Typicae

(c) Keys to the species

Genus: Loudetia

Section: Lophanthera ..... 1 species - L. togoensis

Section: Loudetia

Subsection: Flammidae

Mid-rib prominent, secondary vascular bundles with

abaxial girders and without colourless parenchyma cells ..... L. flammida

Midrib not very obvious, secondary vascular bundles possessing abaxial strands and colourless parenchyma cells ..... L. phragmitoides

Subsection: Annuae

Blade in transverse section with bulliform cells appearing in large groups dispersed between secondary and primary vascular bundles ..... L. annua

Blade in transverse section with bulliform cells appearing almost continuously across adaxial surface or in very large groups, very few secondary vascular bundles ..... L. hordeiformis

Subsection: Densispicae

Blade in transverse section with adaxial girders above primary and secondary vascular bundles ..... L. gossweileri

Blade in transverse section with adaxial strands and colourless parenchyma above primary and secondary vascular bundles:

Abaxial epidermis with many intercostal zone short cells (> 250 per sq. mm):

Macrohairs absent, central vascular bundles with broad adaxial strand ... L. vanderystii

Macrohairs present, central vascular bundle without adaxial strand, colourless parenchyma only ..... L. coaretata

Abaxial epidermis with few intercostal zone short cells (< 250 per sq. mm):

Long cells of abaxial epidermis distinctly sinuous, macrohairs absent; tertiary vascular bundles with abaxial strands ..... L. tisserantii

Long cells of abaxial epidermis not distinctly sinuous, macrohairs sometimes present, tertiary vascular bundles with abaxial girders ..... L. densispica

Subsection: Pungentes

Leaf blade well supplied with fibres, vascular bundles with adaxial and abaxial girders.

Blade involute when dry, adaxial surface with larg; ribs, and very small bulliform cells ..... L. longipes

Blade flat, adaxial surface at the most wavy, and with large bulliform cells..L. crassipes

Leaf blade not as fibrous, vascular bundles with adaxial and often abaxial strands rather than girders:

Macrohairs abundant, outer bundle sheath of primary vascular bundles interrupted abaxially ..... L. lanata

Macrohairs absent, outer bundle sheath of primary vascular bundles not interrupted ..... L. demeusei

Subsection: Acuminatae

1. Hooks and prickle-hairs very abundant on abaxial epidermis surface (> 300 per sq. mm).

   Leaf blade greater than 3 mm wide and 220 μ thick ..... L. migiurtina

   Leaf blade less than 3 mm wide and 160 μ thick ..... L. acuminata

1. Hooks and prickle-hairs usually present, but not as abundant (< 300 per sq. mm):

   2. Leaf blade with many small vascular bundles, twice as many secondary as primary bundles and four times as many tertiary as primary bundles; very little sclerenchyma in leaf, no adaxial fibres over primary vascular bundles ..... L. cuanzensis

   2. Leaf blade with equal numbers of primary and secondary bundles and twice to three times as many tertiary bundles; an abundance of sclerenchyma, especially over the primary vascular bundles:

      3. Long cells of adaxial epidermis short and broad; those of intercostal zone: ± 80 μ long and length-width ratio less than 6.0; those of costal zone ± 115 μ long and ratio less than 14.0; in the leaf blade three times as many tertiary as primary or secondary bundles ..... L. filifolia

Blade relatively thin (± 160μ) and margins not swollen and with few fibres ..... subsp. filifolia

Blade thicker (± 200μ), well sclerified, margins swollen with many fibres ..... subsp. humbertiana

3. Long cells of abaxial epidermis of intercostal zone mostly longer than 80μ and ratio greater than 6.0, those of costal zone greater than 115μ and ratio greater than 15.0; in the leaf blade twice as many tertiary as primary or secondary bundles:

Abaxial leaf epidermis ribbed ..... L. pennata

Abaxial leaf epidermis at the most wavy ..... L. flavida

Subsection: Typicae

1. Mid-rib present, leaf blade usually ≥ 5 mm wide; usually 4 (rarely 3) groups of phloem present in the largest vascular bundle of the blade ..... L. arundinacea-complex

2. Mid-rib with a strongly prominent keel, usually with more than three vascular bundles in the mid-rib; bulliform cells often extending over the whole adaxial epidermis and not in distinct groups ..... L. arundinacea

2. Mid-rib lacking a prominent keel, but with a large central vascular bundle; bulliform cells arranged in distinct groups:

   Secondary and tertiary vascular bundles of equal numbers in the leaf blade and three to four times as numerous as primary bundles ..... L. thomasii

   Secondary vascular bundles twice as numerous as primary bundles, and tertiary bundles twice as numerous as secondary bundles ..... L. angolensis

1. Mid-rib absent, leaf blade usually less than 5 mm wide, usually one (sometimes 2 or 3) groups of phloem present in largest vascular bundle ..... L. simplex-complex

   3. Leaf markedly involute when dry, with large adaxial ribs containing parenchyma cells some of which may be thickened, but without sclerenchyma; abaxial leaf epidermis with short, broad, long-cells with very thick walls, micro-hairs absent:

      Leaf blade very narrow (< 2 mm wide) tightly involute and therefore almost circular in cross-section ..... L. madagascariensis

      Leaf blade wider (3-4 mm) not so tightly involute, U-shape in cross-section ..... L. perrieri

3. Leaf blade not distinctly involute, if ribs present not large and as above; abaxial leaf epidermis with long narrow long-cells with thin or moderately thick walls, micro-hairs always present:

4. Bulliform cells extending over the adaxial epidermis and not in distinct groups, macro-hairs always present ..... L. kagerensis

4. Bulliform cells always in distinct inflated cell groups, macro-hairs present or absent:

5. Phloem fibres of secondary and primary vascular bundles usually enveloping both phloem and xylem; inner bundle sheath not obvious:

Silica bodies in the intercostal zone of the abaxial epidermis present and abundant .... L. camerunensis

Silica bodies in the intercostal zone of the abaxial epidermis absent

L. simplex subsp. stipoides

5. Phloem fibres of secondary and primary vascular bundles con-

tiguous to phloem only, inner bundle sheath well defined

L. simplex subsp. *simplex*

Section: *Pleioneura*

Leaf blade narrow (up to 2.5 mm wide), flat; in transverse section without ribs, midrib with solitary vascular bundle and single central adaxial strand; bulliform cells on abaxial epidermis absent .... *L. ramosa*

Leaf blade wide (6-12 mm), involute; in transverse section blade ribbed or wavy; midrib well developed with three or more vascular bundles and three adaxial strands; bulliform cells on abaxial epidermis present:

Mid-rib with many vascular bundles, broad central adaxial strand separated from two smaller lateral ones by bulliform cells; margin of blade strongly swollen, hooks on abaxial epidermis present ..... *L. simulans*

Mid-rib with 3-5 vascular bundles, three adaxial strands, almost equal in size, separated from one another by colourless parenchyma; margin of blade only slightly swollen, hooks on abaxial epidermis absent ..... *L. anomala*

Incertae sedes ..... 1 species - *L. jaegeriana*

Genus: *Rattraya* ..... 1 species - *R. petiolata*

5. Discussion

The value of using a large number of characters in a combined analysis is illustrated in the results achieved here. Whereas the individual analyses based on sets of characters from different organs each gave indications of the relationships or groups of OTU's that are present in the genus, the combination of all the characters has produced a taxonomy very similar to that constructed from morphological characters.

The three sections of Loudetia are clearly recognized in the principal components analysis whether either the correlation coefficient or the dispersion coefficient is used as similarity measure. The subsections of the section Loudetia are not always distinct but certain trends may be seen in the scatter diagrams and models. In particular, the subsection Pungentes is rather a diverse group when anatomical characters are considered. The subsection Typicae is seen to contain a number of different forms. The L. arundinacea-complex invariably is very homogeneous but the L. simplex complex is not so. Firstly, L. perrieri, and L. madagascariensis are two species which are highly specialized with respect to leaf anatomy and their isolation from other taxa is often quite striking in the principal components analyses (Plates 28 and 29). The examplars of L. kagerensis and L. simplex subsp. stipoides form another group in this complex when leaf anatomy characters in particular are considered. Lastly, L. simplex subsp. simplex and L. camerunensis may be distinguished as another small grouping possessing distinctive properties. The OTU's of the whole complex do, nonetheless,

have many festures in common since they often group together. With respect to the subsections Densispicae, Annuae and Flammidae the same features may be seen in the ordinations considering all characters together as when analysed separately. The OTU's of the subsection Acuminatae are somewhat differently dispersed when all characters are considered. In the analyses based on leaf epidermis characters, it was found that the majority of OTU's did form a cluster. Combining all the characters gives a much greater indication of the affinity between OTU's of this section. L. jaegeriana, which is considered to have properties intermediate between other sections, was somewhat isolated in the principal components analyses, therefore supporting this view.

The clusters which are formed following classification approximate the situation that has been described for the ordinations. Firstly, the dendrogram produced by sum of squares agglomeration of the distance matrix resulting from the component scores (correlation coefficient) as data matrix, will be considered. (Fig. 40A). Five large groups of OTU's which are apparent at the 50% level may be characterized. Firstly, the L. arundinacea-complex of the subsection Typicae and the Flammidae form a group. All these OTU's have very large leaves with a mid-rib. Section Pleioneura and Rattraya also possess some of these characters but they are separated from the above group mainly because of different awn anatomy. The other three groups of OTU's all have leaves without a distinct mid-rib and the characters associated with it. The first one com-

prises mainly the L. simplex-complex of the subsection Typicae, the Acuminatae, Annuae and some OTU's of subsection Pungentes. Secondly, there is a group consisting of the Densispicae and a few other OTU's. The two OTU's which were found to differ greatly on the basis of leaf anatomy, L. perrieri and L. madagascariensis, form a third and final group. Within these large groups, it is seen that many of the affinities between particularly closely related OTU's result in sub-clusters.

The classification produced using the raw data and sum of squares agglomeration of absolute distance (Fig. 40B) is somewhat different from the above. Only three large groups are obvious at the 50% level. Most notable is the fact that subsection Flammidae is quite distant from the L. arundinacea-complex while L. perrieri and L. madagascariensis do not form an isolated group. Otherwise the results resemble the above.

Graph theory produces a different pattern of classification (Fig. 40C). Large groups of taxa similar to the first set of results (Fig. 40A) were observed, but the main sections of the genus were separated in this case. The OTU's of section Loudetia, excluding L. perrieri and L. madagascariensis and the subsection Flammidae, occur in a group which appears very heterogeneous, although some clusters do form which approximate the results obtained by the other clustering methods.

The distance matrices derived from the different data sources were very similar; in the case of the component scores (dispersion coefficient) data and raw data they were identical. The dendrograms produced by the sum of squares agglomeration

clustering methods had cophenetic correlations with much the same values as those achieved with the analyses on character-sets separately, i.e. about 0.5. The dendrogram from the graph theory technique was most similar to that derived from sum of squares agglomeration using the component scores (correlation coefficient) as data. The similarities in the two classifications have been discussed above and are apparent in the figures (Fig. 40A, 40C).

The extremely low cophenetic correlation coefficients obtained when classifications using different sets of characters are compared, indicates that the hypothesis of non-specificity does not hold well. Even when classifications derived from leaf anatomy and leaf epidermis characters, which one might expect to be especially similar, are compared, the cophenetic correlation coefficient is only 0.014, which is in fact the lowest value obtained. The different character sets have each achieved meaningful results, but the dendrograms apparently vary in different directions. In any one of the character sets studied there are main groups of characters which have a marked influence on ordination and classification of OTU's. For example, with respect to leaf characters, the OTU's with large leaves and mid-ribs were clustered together. When awn characters are considered, this group no longer appears. Consequently, it is better to consider the classifications as not being directly comparable, but each of the character sets as adding information for an ideal classification based on all characters. In all cases, the classifications

based on individual sets of characters were more similar to the classification based on all the characters, than to one another. The additive effect is, therefore, apparent, and the best possible classification is that in which all the characters are used. Similar conclusions about the non-validity of the non-specificity hypothesis are found in the literature (e.g. Johnson & Holm, 1968, Erlich & Erlich, 1967).

6 Summary

The sections of the genus Loudetia, the genus Rattraya and to a lesser extent the subsections of section Loudetia, may be distinguished when the taxa are ordinated or classified. The subsection Pungentes is most diverse and the subsection Typicae may be divided into the L. arundinacea-complex and L. simplex-complex. L. jaegeriana, which has uncertain taxonomic affinities, appears to lie in a position between the sections Lophanthera and Loudetia.

The classifications obtained with different sets of characters are very different, thus indicating that the hypothesis of non-specificity is not valid. The characters from different sources are considered to have an additive effect. The most satisfactory classification will, therefore, be obtained when all characters are used.

It was found that anatomical characters were useful in drawing up diagnostic keys for identifying both the higher taxa and the individual species. The usefulness of these keys at the specific level is not proven due to the unassessed intra-specific variation.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

Inspite of the intentions of the taxonomist to produce an unbiased classification, it is obvious from an examination of the history of the taxonomy of the Arundinelleae that this is not always done. In this investigation an attempt has been made to remove some of the bias by using an objective numerical approach. In the introduction (Chapter 1) it was noted that the subjective approach bypasses the major portion of the model constructed for this study (Fig. 2) via direct reasoning (T_D) to arrive at conclusions about the classification (6). However, the mathematical approach used in this thesis, although being more tedious, allows many more conclusions to be drawn about the classification and the model itself.

Of particular importance are the conclusions that are reached about the taxonomy of Loudetia and Rattraya. In addition, interesting conclusions may be drawn about the use of anatomy and numerical techniques and their importance in taxonomy as a whole.

1. The taxonomy of Loudetia and Rattraya

The assignment of the sections of Loudetia as first described by Hubbard (1934) are ever changing, as different

authors present their views on the structure of the genus. However, as more intensive morphological and anatomical investigations are carried out a more stable classification will be attained. The analysis of the make-up of Loudetia (sensu stricto) and Rattraya in this thesis is an attempt to reach a more satisfactory classification. The use of anatomical and numerical methods has led to a number of conclusions about the taxa being studied.

Using leaf anatomy characters results very close to the original model of the taxa were obtained, though the sections of Loudetia are not strongly separated on this basis. There is an emphasis on size of the leaf and characters associated with size, such that some very distinctive characters of section Pleioneura and Rattraya are not sufficient to separate these taxa from the other OTU's of Loudetia. Pleioneura and Rattraya are easily recognized by their lack of tertiary vascular bundles and colourless cells beneath the bulliform cells, but because of their large leaves and distinct midribs, they cluster with the L. arundinacea-complex of subsection Typicae.

The broad leaf without a midrib as found in L. togoensis and L. jaegeriana does not have sufficient characters to be differentiated from the majority of Loudetia species which lack a midrib and yet are a lot smaller. Groups of OTU's may be distinguished among those in this category, some of which follow the conventional supra-specific taxa. For example, OTU's of subsection Densispicae have many characters in common and form a cluster.

The study based on abaxial leaf epidermis characters did not reveal such obvious taxonomic relationships. This was partly due to the importance of the occurrence, distribution and density of appendages in the analyses. Although these features may indicate very sound taxonomic relationships, for example the OTU's of subsection Densispicae all lack prickle-hairs and hooks, in many cases they vary within species, and may also reflect the environmental conditions of the habitat whence the plants came. In spite of these difficulties some of the established taxa such as section Pleioneura and Rattraya and subsection Acuminatae could be identified on leaf epidermis characters.

A taxonomy based on awn characters was very suitable in describing the sections of Loudetia. Three types of awns were recognized. The Loudetia type awn is fairly well developed with an inverted U-shaped column. The Pleioneura species have a smaller awn in which the column is only slightly curved in cross-section. The well-developed awn of the section Lophanthera has many layers of cells in the cross-section of the column and is an inverted U-shape. Some subsections of section Loudetia are atypical. The Flammidae have awns more like those of the Pleioneura type as the column is short and not quite an inverted U-shape in cross-section. However, the cells are not thickened in the same way as those of the Pleioneura type. In the Annuae the awns are also quite large but they never reach the size of the Lophanthera type. The latter type is also typical of the Tristachyoids and was used by Conert (1957) as

a criterion for removing the section Paratristachya of Loudetia and placing it in the genus Tristachya. The Pleioneura type awn, on the other hand, is of the same form as that of the members of the subtribe Trichopterineae created by Jacques-Felix (1950). Although Conert (1957) indicated that there were problems in the taxonomy of the Arundinelleae if one accepted these subtribes, he did not refer to such a difficulty in the taxonomy of Loudetia. These subtribes are not generally accepted (Phipps, 1967a) so they need not be considered further, but it is interesting to note that the genus as constituted by Phipps (1967a) contains awn types of both subtribes. Clayton (1967) removed section Pleioneura to Danthoniopsis and thus Loudetia would not contain both awn types if this generic concept were accepted.

Not all the subsections of section Loudetia are easily distinguished on awn anatomy. In fact they are remarkably heterogeneous. Exceptions that occur have been mentioned above.

In a multivariate analysis using all the anatomical characters, the trends mentioned above are combined so that the overall relationships among the taxa are obvious. The suggestion of Rohlf (1963) that characters be used from all parts of the organism in a numerical taxonomic study, proved to be the most satisfactory approach in that the combined effect of the characters is presented. The three sections of Loudetia are always more-or-less distinct. Rattraya is very close to the OTU's of section Pleioneura and, in fact, L. ramosa is more different from the other taxa of Pleioneura when

anatomical characters are considered. L oudetia jaegeriana is separated from other OTU's in the ordination though it usually clusters with the section Loudetia. The OTU's of the sub-sections of section Loudetia are not always clustered in the analyses. Two groups of the Typicae may be recognized mainly on leaf characters. The L. arundinacea-complex has large leaves whereas the L. simplex-complex has smaller leaves. Of the latter group further sub-groups may be recognized though the results are not consistent. Loudetia madagascariensis and L. perrieri are two species with xeromorphic leaves and many characters in common so they are separate from the other OTU's of the L. simplex-complex.

The Densispicae, Annuae and Flammidae are each quite homogeneous when the overall results are analysed. Conert (1957) suggested that L. flammida and L. phragmitoides are the same species but evidence presented here shows that awn and leaf characters at least are sufficient to maintain them in two different species. The final subsection, the Acuminatae, although not showing much homogeneity in ordinations and classifications based on isolated character sets, forms a more compact group of OTU's when all the characters are considered together.

There are, therefore, a number of conclusions about the taxa that may be drawn from this study. There is no doubt as to the distinction between the three different sections of Loudetia. The fact that Rattraya is very similar in anatomy to the section Pleioneura suggests that it be included in this

section. Clayton (1967) proposed that the section Pleioneura, Rattraya and the Danthoniopsoid genera be amalgamated in one genus Danthoniopois. His evidence is mainly on the basis of the nervation of the upper lemma and type of short blunt callus. Phipps (1969) has also found similarities between these taxa when a large number of morphological characters are used in a multivariate analysis. The anatomy of OTU's of the section Pleioneura is quite different from Loudetia in many respects and thus the transfer seems justified on this basis.

Results of analyses presented here indicate that L. jaegeriana occupies a position remote from other OTU's of the genus, and it should possibly be given a sectional rank. Camus (1961) suggested that it was closely related to the subsection Typicae or the section Pleioneura. On the basis of awn characters it could be placed between these two groups, although it is then most similar to subsection Flammidae. However, the leaf anatomy is very much like that of the section Lophanthera.

Within the section Loudetia no real differences from the conventional taxonomy were observed, and the subsections should be maintained. On morphological characters the two complexes of species of the subsection Typicae may be distinguished and the same is found to be true when anatomical characters are considered. If further evidence warranted it these two groups could be given subsectional status.

2. The anatomical method

The use of anatomy in grass taxonomy has become increas-

ingly important as more sophisticated techniques of data analysis are used. Although Conert (1957) found little value from this type of data when the taxonomy of Loudetia was considered, in this investigation anatomical characters are shown to be very valuable in classifying the genus. The reasons for the success of this study may be attributed to the multivariate methods of analysis. A simple examination of the anatomy in an attempt to elucidate "important" characteristics or groups of species as was done by Conert is unsuitable as the many variables in the anatomy are difficult to interpret subjectively. A numerical analysis in which the characters are clearly defined and quantified reveals much more about the characters and the taxa. Furthermore, it has been shown that the use of criteria from only one organ in such an analysis reveals different results from those obtained using criteria from all the organs. The best situation is therefore one in which all characters are used simultaneously and such an approach can only be made using multivariate techniques.

A drawback in the use of anatomical characters is that they are often subject to more intraspecific variation than, for example, some floral characters. A large amount of variation is due to the environment. Lewton-Brain (1904) was one of the first workers to study the anatomy of grass leaves with respect to ecological habitats. He found that ribs and furrows in the leaf, bulliform cells, the amount of sclerenchyma tissue, macro-hairs, prickles, hooks, stomata, thickness of the cuticle and epidermal cells, were all characters which showed a large

amount of variability in different environments. Numerous other workers have reported similar findings (e.g. Starr, 1912, Sabnis, 1921, Shields, 1950, Nikalaevsky and Nikolaevskya, 1967). Daubenmire (1959) and Davis and Heywood (1963) have summarized much of the information known about the environmental factors effecting the phenotype.

The problem of intraspecific and environmental variation is acute when a study of the species or lower taxon is the unit of study. (Davis and Heywood, 1963). Although in this study the relationships between supra-specific taxa was of greater interest, by introducing additional exemplars to the analysis some estimate of the intraspecific variability was obtained. The most notable demonstration of such variability was the present or absence of appendages on the leaf epidermis. Exemplars of L. flavida and L. simplex subsp. simplex with and without some of the appendages were found to be separated on this basis. Another example was the degree of thickening of the cell walls in the awn. The exemplars of L. angolensis and L. simplex subsp. stipoides with much thickening in the cell walls were separated from other OTU's of section Loudetia which did not exhibit this condition. It is, therefore, important to allow for such situations and to attempt to recognize that variation which is due to environmental factors.

In addition to the intraspecific variation taxonomies of this kind may reveal major variations in the anatomy of higher taxa which may be attributed to particular habitat adaptations. Examples in this thesis are L. madagascariensis and L. perrieri

which are very similar to one another anatomically and have xeromorphic features of the leaf which separate them from other closely related taxa. The species of subsection Pungentes are very variable anatomically and since some have xeromorphic features, e.g. L. lanata with many macrohairs, and L. longipes with inrolled leaves, it would be inconclusive to impose an alternative classification on the basis of leaf anatomy.

The anatomical method is, therefore, sometimes limited in its applicability, and when numerical techniques of data analysis are used the results should be subjected to close scrutiny to observe unintentional bias that may occur. For this reason the character analysis is particularly important in an anatomical study.

3. The numerical method

The advantage of using numerical techniques in the analysis of anatomical data has already been pointed out above. In addition the convenience of analysing the data in different character sets has been emphasized in this study. It is important to obtain as much information about the characters as possible and indicate their effects on the ordination and classification of individuals. Conclusions may be drawn from the character analysis as to whether the clustering of OTU's is a true reflection of taxonomic relationship or whether the important characters are subject to environmental or sporadic morphological variation. For example, some "clusters" of OTU's resulting from component analysis using leaf epidermis characters, were influenced by characters, such as presence/absence

of macrohairs, which are known to be subject to environmental and morphological variation. The most useful technique in character analysis was the principal components analysis. As well as the character "groups" detected in scatter diagrams the proportion of the roots that each of the characters account for, was calculated by the method of Orloci (1968b). Although classification of characters was not as useful, in the analyses using awn characters the clusters of characters that were formed could be related to OTU relationships.

The major problem of the thesis was the taxonomy of the OTU's. In general the principal components analysis was found to be the most useful method of indicating OTU relationships because no fixed clustering of OTU's was desired. The component analysis could be directly related to the results of that obtained in the character analysis. The first two components are very often the most useful in scatter diagrams in indicating the overall OTU positions, though by constructing models in three dimensions and scatter diagrams using up to the fourth component more information was obtained. Classification of OTU's by sum of squares agglomeration was most effective when the component scores of the principal components analysis were used as data source, because the resulting dendrogram could be compared with the scatter diagrams and the relationships more easily envisaged.

A test of the nonspecificity hypothesis revealed that the classifications using different character sets could not be considered as comparable in this work. The most satisfactory

classification is regarded as that where all the possible characters are used, the different character sets each having an additive effect. Similar conclusions were reached about this hypothesis by Rohlf (1963), Erlich and Erlich (1968) amongst others but no other reports are available from anatomical taxonomic studies of plants.

4. Other conclusions

Many of the conclusions reached about the use of anatomical and numerical taxonomic methods may be inferred to other members of the Arundinelleae, or the grasses and plants in general. The success in applying a model for the study of the genus Loudetia illustrates the advantage of conducting a taxonomic study in this way. In addition, the general principles of the evolution of the awn may be applied to other species of grasses so as to obtain more information about their phylogeny.

CHAPTER 8

SUMMARY

1. The genus Loudetia is introduced in relationship to the other members of the tribe Arundinelleae as being one of the main "species groups" about which a confused concept of generic limits has reigned.

2. The central core of the genus which is studied in this thesis consists of three sections, viz. Loudetia, Lophanthera and Pleioneura. Section Loudetia is further sub-divided into six sub-sections - Typicae, Pungentes, Acuminatae, Densispicae, Annuae and Flammidae. One species, L. jaegeriana has not been assigned to an infrageneric taxon. The monotypic genus Rattraya was included in the study with the 38 taxa of Loudetia because it appeared to be very closely related to species of section Pleioneura.

3. Morphological criteria have been traditionally used in the taxonomy of the genus and some of the important characters of the inflorescence and spikelet are tabulated. In general only a few characters have been used in characterising any one section or sub-section.

4. A review of the use of anatomical characters in grass systematics indicates their importance but previous work on

the anatomy of the arundinelleae revealed no significant conclusions with respect to the internal variation of Loudetia sensu stricto. The major limitations in previous studies using anatomical data is attributed to the subjective methods of analysis.

5. In view of the dilemma in the taxonomy of the genus and the lack of conclusions from a conventional anatomical study an objective numerical approach is suggested as the obvious alternative.

6. A model for a numerical taxonomic study is outlined and in its application is shown to be divided into four separate parts: (a) The anatomy of the leaf, as seen in transverse section, is studied and characters from this source are used in a numerical classification. (b) The leaf epidermis is examined in surface view and the characters are compiled for use in another numerical taxonomic study. (c) Characters of the awn are assembled for a numerical taxonomic study. (d) All the characters are combined to give a final comprehensive classification. A model of this sort allows the different classifications to be compared and conclusions may be reached about the genus from the separate studies and the combined analysis.

7. Specimens of Loudetia species were obtained both from living and herbarium material for the anatomical studies.

8. Numerical taxonomic studies involve both analyses of relationships between pairs of OTU's (operational taxonomic units) and pairs of characters. Traditionally the former type

of study has been most important, but more recently the value of character analysis has been stressed. In this thesis the analysis of the OTU's is the most fundamental part of the work but character analysis of the different character sets also proved to be very valuable in assessing OTU relationships.

9. Principal components analysis with an appropriate coefficient was used in showing the relationships in a simpler structure than that of the original data, and absolute distance and the sum of squares agglomeration method of classification were used in formulating groups.

10. Seventy-nine leaf anatomy characters were recorded for 50 OTU's of Loudetia and Rattraya. Character analysis of these data revealed that size of the leaf, and the amount, position and arrangement of sclerenchyma were the most important characters. The OTU analyses, therefore, showed that division of the genus was mainly due to these characters. However, in general, the results of the principal components analysis and classification suggest strong resemblances between taxa within a section or sub-section.

11. The leaf epidermis also proved to be a valuable source of characters and from the 52 OTU's examined 52 characters were scored. In the character analysis the features of the epidermis that were found to be most significant were the presence or absence of appendages, such as hooks and prickles, the cell lengths and ratios and some xeromorphic characters. The same trends of classification along the classical lines were revealed from the OTU analysis. In particular, section

Pleioneura and Rattraya were readily distinguished and sub-section Densispicae was found to be characterized by the absence of prickles and hooks.

12. A study of 50 OTU's of Loudetia and Rattraya revealed 31 characters of the awn which are useful in numerical analyses. The characters of size or related to size were found to be the most important ones followed by thickening of the cell walls, and shape of the awn. Three categories of awns were recognized which correspond to the division of the genus into three sections. The numerical results supported this classification. Exceptions occur in the section Loudetia where sub-section Flammidae has small awns which are similar to those of section Pleioneura.

13. This study of the awn also revealed a number of interesting facts about its function and evolution. The awn is recognized as an integral part of the lemma of the upper floret and the whole complex has highly specialised functions. Within the genus Loudetia the functions of the awned lemma are shown to be directly related to its anatomical and morphological structure, an important factor when the phylogeny of the taxa is considered.

14. The multivariate analyses using a combination of all the characters produced a taxonomy essentially similar to the conventional one. Though, initially the same trends had been thought to exist in the classifications based on different character sets, a comparison made by calculating cophenetic correlations between these classifications showed that the

similarity was actually very slight. It is therefore concluded that the hypothesis of nonspecificity, which assumes that equivalent classifications will be produced from different character sets, does not hold very well. It is preferable to consider each of the character sets as accumulating information towards the most stable classification where all characters are used.

15. From the results using all characters the following conclusions are reached:

(a) The three sections are easily recognized and are valid taxonomic groups.

(b) Section Pleioneura is most different from the other sections and Rattraya appears to be part of this species group.

(c) L. jaegeriana is somewhat different from all other species, having leaf characteristics like those of section Lophanthera and awn characteristics similar to those of sections Loudetia and Pleioneura. It is suggested that it would be appropriate for this species to be given sectional status.

(d) The subsections of section Loudetia cannot always be satisfactorily recognized when anatomical characters alone are considered. Subsection Typicae may be divided into two groups, the L. arundinacea-complex and L. simplex-complex which should perhaps be given subsectional rank. Most of the other subsections appear quite homogeneous except for subsection Pungentes whose members vary somewhat anatomically.

16. The general conclusion is reached that the taxonomy of Loudetia as constructed by conventional means is very similar to that using anatomical characters in a multivariate analysis.

17. This work reveals the wealth of information that may be obtained from an anatomical analysis when numerical taxonomy is applied.

18. The importance of character analysis is strongly emphasized, especially as anomalies in anatomy due to environmental or infraspecific variation can be more readily recognized when this approach is taken.

REFERENCES

Adanson, M.1763. Familles des Plantes. Vol. 1. Vincent, Paris. 190 pp.

Alcorn,S.M. and P.A. Ark. 1953. Softening paraffin-embedded plant tissues. Stain Technol. 28 : 55-56

Alexandrov, W.G. 1926. Über tägliche Veränderungen des Stärkegehalts in Blättern. Deut. Bot. Gesell. Ber. 44 : 217 - 226.

Anderson,E. 1949. Introgressive hydridisation. Wiley & Sons, New York. 109 pp.

Anderson,E. 1956. Character association analysis as a tool for the plant breeder. Brookhaven Symp. Biol. 9 : 123 - 140.

Arber, A. 1918 - 19. The "Law of Loss" in evolution. Proc. Linn. Soc. Lond., Session 131 : 70 - 78.

Arber,A. 1923. Leaves of the Gramineae. Bot. Gaz.76 : 374

Arber,A. 1934. The Gramineae. Macmillan Co., Cambridge University Press. 480 pp.

Artschwager,E. and E. J. Brandes. 1958. Sugarcane. U.S.D.A. Agriculture Handbook, 122 : 45. Supt. of Documents, Washington, D.C.

Auquier,P.1963. Critères anciens et modernes dans la systematique des Graminées. Natura mosana.16: 1-63.

Austin,M.P. & L. Orloci.1966. Geometric models in ecology II. An evaluation of some ordination techniques. J. Ecol. 54 : 217 - 227

Avdulov,N.P. 1931 Karyo-systematische Untersuchung der Familie Gramineen. Bull. of Appl. Bot. and Pl. Breeding. Suppl. 44 : 4 - 425.

Ayensu,E.S. 1967. Aerosol OT solution - an effective softener of herbarium specimens for anatomical study. Stain Technol. 42 : 155 - 156.

Bailey, I.W. 1951. The use and abuse of anatomical data in the study of phylogeny and classification. Phytomorphology 1 : 67 - 69.

Barnard,C. 1955. Histogenesis of the inflorescence and flower of Triticum aestivum L. Aust.J.Bot.3: 1-20.

Barnard,C. 1957. Floral histogenesis in the Monocotyledons I. The Gramineae. Aust. J. Bot. 5 : 1-20.

Baum, B.R. 1968. Delimitation of the genus Avena (Gramineae). Can. J. Bot. 46 : 121 - 132.

Bessey,C.E. 1884. The injuriousness of porcupine grass. Am. Nat. 18 : 929 - 930.

Bews, J.W. 1929. The World's Grasses. Longmans, London.408 pp.

Borrill,M. 1961. Epidermal characteristics in the diploid subspecies of Dactylis glomerata L. J. Linn. Soc. (Bot.). 56 . 453 - 458.

Braun, A. 1841. Bemerkungen über die Floral von Abyssinien. Flora 45 : 705 - 720.

Bray, J.R. & J. T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27 : 325 - 349.

Brillouin,L. 1962. Science and information theory.(2nd.ed.) Academic Press, N.Y.

Brown, W.V. 1958. Leaf anatomy in grass systematics. Bot. Gaz. 119 : 170 - 178.

Bugnon,P. 1921. La feuille chez les Graminées. Mém. Soc. Linn. Norm. 21(2). 108 pp.

Burkart,L.F. 1966. Triethylene glycol as a woody tissue softener in preparation for microtome sectioning. Stain Technol. 41 : 255 - 259.

Busacker,R.G. & T.L. Saaty. 1965. Finite graphs and networks. McGraw-Hill, N.Y. 294 pp.

Cain,A.J. and G.A. Harrison. 1958. An analysis of the taxonomist's judgement of affinity. Proc. Zool. Soc. Lond. 131 : 85 - 95.

Camus, A. 1954. Loudetia jaegeriana. J. Agric. trop. 1: 212.

Cattell, R.B. 1952. Factor Analysis. Harper, N.Y.

Cattell,R.B. 1965. Factor Analysis: An introduction to essentials. I and II. Biometrics 21: 190-215,405-435.

Chorley,R.J. 1964. Geography and Analogue theory. Ann. Assoc. Am. Geographers. 54 : 127 - 137.

Clarke, J. 1960. Preparation of leaf epidermis for topographic study. Stain Technol. 35 : 35 - 39.

Clayton, W.D. 1967. Studies in the Gramineae XV. Arundinelleae. Kew Bull. 21 : 119 - 124.

Clifford, H.T. and D.W. Goodall. 1967. A numerical contribution to the classification of the Poaceae. Aust. J. Bot. 15 : 499 - 519.

Conert, H.J. 1957. Beiträge zur Monographie der Arundinelleae. Bot. Jb. 77 : 226 - 354

Conert, H.J. 1961. Die Systematik und Anatomic der Arundineae. J. Cramer, Weinheim. 208 pp.

Cooley, W.W. and P.R. Lohnes. 1962. Multivariate procedures for the behavioural sciences. John Wiley & Sons, N.Y.

Correia, R.I. de S., R.A. Lubke and J.B. Phipps. 1967. Estudos nas Arundinelleae (Gramineae) VII. Um Novo Género, Três Novas Espécies e Novas Combinações. Bolm. Soc. broteriana. 51 : 191 - 202.

Côte, W.A. 1965. Cellular ultrastructure of woody plants. Syracuse Univ. Press 603 pp.

Croker, B.H. 1959. A method of estimating the botanical composition of the diet of sheep. N.Z. Jl. Agric. Res. 2 : 72 - 85.

Crovello, T.J. 1966. Quantitative taxonomic studies in the genus Salix. Ph.D. Thesis. University of California, Berkeley.

Crovello, T.J. 1968. Key communality cluster analysis as a taxonomic tool. Taxon 17 : 241 - 258.

Dagnelie,P. 1960. Contribution à l'étude des communautés végétales par l'analyse fectorielle. Bull. Serv. Carte phytogéogr. Ser.B.5 : 7 - 71.

Daubenmire,R.F. 1959. Plants and Environment. A textbook of Plant Autecology. John Wiley and Sons,N.Y. 422pp.

Darwin,Francis. 1876. On the hygroscopic mechanism by which certain seeds are enabled to bury themselves in the ground. Trans. Linn. Soc. Lond.Series II. Botany. 1 : 149 - 162.

Davis,P.H. & V.H. Heywood, 1963. Principles of Angiosperm taxonomy. Oliver & Boyd, London, 556 pp.

Decker, H.F. 1964. An anatomic-systematic study of the classical tribe Festuceae (Gramineae). Am. J. Bot. 51 : 453 - 463.

de Winter,B. 1965. The South African Stipeae and Aristideae (Gramineae). Bothalia 8(3): 201 - 404.

Duran,L. & B. Rossengurtt. 1953. La flechilla de los generos stipa y Piptochaetium del Uruguay. Agros (Pelotas) 141 : 9 - 13.

Duval-Jouve,J. 1870. Etude anatomique de quelques Graminées de l'Hérault. Mém. Acad. Sci.Lett. Montpellier, Sect. Sci., 7:

Duval-Jouve,J. 1871. Etude anatomique de l'arête des Graminées. Mém. Acad. Sci. Lett. Montpellier, Sect.Sci. 8:33-78.

Duval-Jouve,J. 1875. Histotaxie des feuilles de Graminées. Annls Sci. Nat.Bot. sér.6, 1 : 227 - 346.

Eades, D.C. 1965. The inappropriateness of the correlation coefficient as a measure of taxonomic resemblance. Syst. Zool. 14 : 98 - 100

Edwards, A.W.F. and L.L. Cavalli-Sforza. 1965. A method for cluster analysis. Biometrics 21 : 362 - 375.

Elias, M.K. 1942. Tertiary prairie grasses and other herbs from the high plains. Geol.Soc.of Am. Special Papers No. 41. 176 pp.

Erlich, P.R. and A. H. Erlich. 1967. The phenetic relationships of the butterflies I. Adult taxonomy and the nonspecifity hypothesis. Syst.Zool.16 : 301-317.

Essau, K. 1953. Plant Anatomy. John Wiley & Sons, N.Y.735 pp.

Estabrook, G.F. 1966. A mathematical model in graph theory for biological classifications. J.Theor.Biol. 12: 297-310.

Estabrook, G.F. 1967. An information theory model for character analysis. Taxon 16 : 86 - 97.

Evans, L.T. 1964. Reproduction. pp 126-153. In: Barnard, C. (ed.) Grasses and Grasslands. MacMillan & Co.N.Y.

Fisher, D.R. 1968. A study of faunal resemblance using numerical taxonomy and fact r analysis. Syst. Zool. 17 : 48 - 63.

Foster, A.S. and E.M. Gifford. 1947. Improvements in the paraffin method. Stain Technol. 22 : 129-131.

Fraser,A.R. and M. Kovats. 1966. Stereoscopic models of multivariate statistical data. Biometrics. 22 : 358 - 367.

Gamapathy,P.S. & B.F. Palser. 1964. Studies of floral morphology in the Ericales VII. Embryology in the Phyllodoceae. Bot. Gaz. 125 : 280-297.

Gifford,E.M. 1950. Softening refractory plant material embedded in paraffin. Stain Technol. 25 : 161-162.

Gilmartin,A.J. 1967. Numerical taxonomy - an eclectic viewpoint. Taxon 16 : 8 - 12.

Girshick,M.A. 1936. Principal components. J. Am. Statist. Ass. 31 : 519-528.

Golden, J.T. 1965. Fortran IV. Programming and Computing. Prentice-Hall, Englewood Cliffs, N.J. 270 pp.

Goodall,D.W. 1954. Objective methods for classification of vegetation III. An essay in the use of factor analysis. Aust. J. Bot. 2 : 304 - 324

Goodall,D.W.. 1964. A probabilistic similarity index. Nature 203 : 1098

Goodall,D.W. 1966a. A new similarity index based on probability. Biometrics 22 : 882-907.

Goodall,D.W. 1966b. Numerical taxonomy of bacteria - some published data re-examined. J.gen. Microbiol. 42 : 25 - 37.

Gower, J.C. 1967. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53 : 325 - 338.

Greenstadt, J. 1960. The determination of the characteristic roots of a matrix by the Jacobi method. pp.84-91. In: Ralston, A. & H.S. Wilf (eds.): Mathematical methods for digital computers.

Greig-Smith,P.,M.P.Austin and T.C.Whitmore. 1967. The application of quantitative methods to vegetation survey I. Association-analysis and principal component ordination of rain forest. J.Ecol.55:438-503.

Grob,A. 1896. Beiträge zur Anatomie der Epidermis der Gramineen blätter. Bibliotheca botanica 7:1-122.

Grandbacher,F.J. 1963. The physiological function of the cereal awn. Bot. Rev. 29 : 366 - 381.

Haberlandt, G. 1914. Physiological Plant Anatomy. Macmillan and Co., London.

Harper,J.L., J. T.Williams and G.R. Sagar.1965. Heterogeneity of soil surfaces and its role in determining establishment of plants from seeds. J. Ecol. 53: 273 - 286.

Hercus,B.H. 1960. Plant cuticle as an aid to determining the diet of grazing animals. Eighth Int. Grassland Congress, Session 1B: Grazing Intake and Behaviour Studies.

Hildebrand,F. 1873. Die Schlenderfrüchte und ihr im anatomischen Bau begründeter Mechanism. Pringlish. Jahrb. 9 : 235 - 276.

Hitchcock,A.S. 1950. Manual of the grasses of the United States. U.S. Dept. of Agric. Miscellaneous Publications 200. 1051 pp.

Hotelling,H. 1933. Analysis of a complex of statistical variables into principal components. J. educ. Psychol. 24 : 417-441, 498-520.

Hubbard,C.E. 1934. Notes on African grasses. 17. Kew Bull. 3 : 425 - 436.

Hubbard,C.E. 1936. The genera of the tribe Arundinellene. Kew Bull. 5 : 317-332.

Hubbard,C.E. 1937. Flora of Tropical Africa. 10 : 1-84.

Hubbard,C.E. 1954. Grasses. A guide to their structure, identification, uses and distribution in the British Isles. Penguin Books, London. 428 pp.

Hutchinson,J. 1959. The families of flowering plants. Vols. I and II. Oxford University Press. 729 pp.

Jacques-Felix,H. 1950. Notes sur les Graminées d'Afrique tropicale II. Les Arundinellees. Revue int. Bot. appl. 333 - 334 : 418 - 428

Jacques-Felix,H. 1960. Notes sur les Graminées d'Afrique Tropicale, Dilophotriche et Diaindrostachya , genres nouveaux d'Arundinelleae. J. Agric. trop. Bot. appl. 7 : 407 - 408.

Jacques-Felix,H. 1962. Arundinelleae : in Flore Agrostologique. 91 - 92, 154 - 164.

Jancey,R.C.. 1966. The application of numerical methods of data analysis to the genus Phyllota Benth. in New South Wales. Aust. J. Bot. 14 : 131-149.

Johansen,D.A.1940. Plant microtechnique. McGraw-Hill, New York, 523 pp.

Johnston,M.P. & R.W. Holm. 1968. A numerical taxonomic study of the genus Sarcostemma. pp. 199-217. In: Heywood, V.H. (ed) Modern Methods in Plant taxonomy. Academic Press, London.

Kendrick,W.B. 1964. Quantitative Characters in computer taxonomy. Systematics Association Publication No. 6. Phenetic and Phylogenetic Classification: 105 - 114.

Kendrick,W.B. 1965. Complexity and dependence in computer taxonomy. Taxon 14 : 141 - 154.

Kendrick,W.B. & J.R. Proctor. 1964. Computer taxonomy in the Fungi Imperfecti. Can.J.Bot. 42 : 65 - 88.

Kendrick,W.B. & L.K. Weresab. 1966. Attempting Neo-Adansonian computer taxonomy at the ordinal level in Basidiomycetes. Syst. Zool. 15 : 307-329.

Kullback,S. 1959. Information Theory and Statistics. John Wiley & Sons, New York.

Lambert, J.M. & M.B. Dale. 1964. The use of statistics in phytosociology. Advances in Ecological Research II : 59 - 99.

Larkin, R.A.., M.M. McMasters, I.M. Cull, M.J. Wolf and C.E. Kist. 1952. Preparing serial sections of mature corn and wheat kernels. Stain Technol 27: 107 - 112.

Launert,E. 1965. A survey of the genus Leersia in Africa (Gramineae, Oryzoideae, Oryzeae). Senck. biol. 46 : 139 - 153.

Lawley,D.N. & A.E. Maxwell. 1963. Factor analysis as a statistical method. Butterworth's, London.

Lewton-Brain,L. 1904. On the anatomy of the leaves of British grasses. Trans. Linn. Soc. Lond. Ser.II, Botany, 6 : 315 - 359.

Li, Y.H.C. 1969. Leaf anatomy of the Arundinelleae (Gramineae) M.Sc. Thesis, University of Western Ontario, London. (In Preparation).

Long, C.A. 1966. Dependence in taxonomy. Taxon.15:49-51.

Lorge,I. & N. Morrison. 1938. The reliability of principal components. Science 87 : 491-492.

Lyse, I. and B.G. Johnson. 1935. Structural beams in torsion. Lehigh Univ. Publs. 9(10) : 469 - 516.

Lysenko, O. & P.H.A. Sneath. 1959. The use of models in bacterial classification. J. gen. Microbiol. 20 : 284 - 290.

Macnaughton-Smith,P. 1965. Some statistical and other numerical techniques for classifying individuals. H.M.S.O. London.

Mark,R.E. 1967. Cell wall mechanics of tracheids. Yale University Press, New Haven. 310 pp.

Martin,D.J. 1955. Features on plant cuticle. Trans.Proc. bot. Soc. Edinb. 36 : 278 - 288.

Mason, H.L. 1957. The concept of the flower and the theory of homology. Madroño 14 : 81-95.

Mayr, E. 1965. Numerical phenetics and taxonomic theory Syst. Zool. 14 : 73-97.

Meeuse, A.D.J. 1966. Fundamentals of phytomorphology. The Ronald Press Co., N.Y. 231 pp.

Metcalfe, C.R. 1954. An anatomist's views on Angiosperm classification. Kew Bull. 9 : 427-440.

Metcalfe, C.R. 1960. Anatomy of the Monocotyledons I. The Gramineae. Oxford University Press, London. 731 pp.

Michener, C.D. & R.R. Sokal. 1966. Two tests of the hypothesis of nonspecificity in Hoplitis Complex (Hymenoptera: Megachilidae). Ann. ent. Soc. Am. 57: 1211-1217.

Moss, W.W. 1967. Some new analytic and graphic approaches to numerical taxonomy, with an example from the Dermanyssidae (Acari). Syst. Zool. 16 : 177-207.

Moss. W.W. 1968. Experiments with various techniques of numerical taxonomy. Syst. Zool. 17 : 31-47.

Mühlethaler, K. 1967. Ultrastructure and formation of plant cell walls. A.Rev. Pl. Physiol. 18: 1-24.

Murback, L. 1900. Notes on the mechanics of the seed-burying awns of Stipa avenacea. Bot. Gaz. 30 : 113-117.

Nikolaevsky, W.G. and L.D. Nikolaevskya. 1967. Ecologically based differences in the structure of grass stems. Biologia Plant. (Praha) 9 : 321-329.

Orloci, L. 1966. Geometrical models in ecology I. The theory and application of some ordination methods. J. Ecol. 54 : 193 - 215.

Orloci,L. 1967a.Data centering: A review and evaluation with reference to component analysis. Syst. Zool. 16 : 208 - 212.

Orloci,L. 1967b. An agglomerative method for classification of plant communities. J. Ecol. 55 : 193-205

Orloci,L. 1968a. Definitions of structure in multivariate phytosociology samples. Vegetatio 15 : 281-291.

Orloci,L. 1968b. A model for the analysis of structure in taxonomic collections. Can.J. Bot. 46 : 1093-1097.

Orloci,L. 1968c. Information analysis in phytosociology: partition, classification and prediction. J. Theor. Biol. 20 : 271-284.

Ouelette,R.P. & S.H. Qadri. 1968. The discriminatory power of taxonomic characteristics in separating salmanoid fishes. Syst. Zool. 17 : 70-75.

Parry, D.W.. & F. Smithson. 1964. Types of opaline silica depositions in the leaves of British grasses. Ann. Bot. N.S. 28 : 169-185.

Pée-Laby,E. 1898. Etude anatomique de la feuille des Graminées de la France. Annls. Sci.Nat.Bot. sér. 8, 8 : 227-346.

Pettet,A. 1960. Variation within the British representatives of the Melanium subgenus of Viola. Ph.D. Thesis, University of Southampton.

Philipson,W.R. 1934a. The morphology of the lemma in grasses. New Phytol. 33 : 359-371.

Philipson,W.R. 1934b. The development and morphology of the ligule in grasses. New Phytol. 33 : 310-325.

Philipson,W.R. 1935. The development of the spikelet in Agrostis canina L. New Phytol. 34 : 421-436.

Phipps, J.B. 1964. Studies in the Arundinelleae (Gramineae) I. Classification of the taxa occurring in Bechuanaland, the Rhodesia's and Nyasaland and Mocambique. Kirkia. 4 : 87 - 124.

Phipps, J.B. 1966. Studies in the Arundinelleae (Gramineae) III. Check-list and key to genera. Kirkia 5:235-258.

Phipps, J.B. 1967a. Studies in the Arundinelleae (Gramineae) VI. Development of generic concepts. Bolm. Soc. Broteriana. 51 : 27-55.

Phipps,J.B. 1967b. Studies in the Arundinelleae (Gramineae) VIII. The phylogeny - a hypothesis. Blumea 15:477-517.

Phipps, J.B. 1969. The genera of Arundinelleae Stapf (Gramineae). Ph.D. Thesis, University of Western Ontario, London. (In preparation).

Pielou, E.C. 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol. 13 : 131-144.

Pilger, R. 1954. Das System der Gramineae unter Ausschluss der Bambusoideae. Bot. Jb. 76 : 281-384.

Pohl, R.W. 1967. Controlled maceration of grass leaves of epidermis for slides. Stain Technol. 42 :195-197.

Prat, H. 1932. L'epidermie des Graminées. Étude anatomique et systématique. Annls. Sci. Nat.Bot. sér. 10, 14 : 119 - 324.

Prat, H. 1936. La Systématique des Graminées. Annls. Sci Nat. Bot. sér. 10, 18: 165 - 258.

Prat, H. 1948. General features of the epidermis in Zea mays. Ann. Mo. bot. Gdn. 35 : 341 - 351.

Prat, H. 1960. Vers une classification naturelles des Graminées. Bull. Soc. bot. Fr. 107 : 32-379.

Proctor, J.R. 1966. Some processes of numerical taxonomy in terms of distance. Syst. Zool. 15 : 131 - 140.

Proctor, J.R. and W.B. Kendrick. 1963. Unequal weighting in numerical taxonomy. Nature 197 : 716-717.

Proctor, M.C.F. 1967. Review of "Principles of Numerical Taxonomy" by R. R. Sokal & P.H.A. Sneath (1963) in Watsonia 6 : 321 - 323.

Quadling,C. 1967. Evaluation of tests and grouping of cultures by a two-stage component method. Can. J. Microbiol. 13 : 1379-1400. Appendix by J. W. Hopkins.

Rajski,C. 1961. Information Theory . p.41 (C.Cherry,ed.). London: Royal Institure.

Rao, C. R. 1952. Advanced statistical methods in biometric research . John Wiley & Sons, N.Y.

Reeve, R.M. 1954. Fruit histogenesis in Rubus strigosus R.M.R. I. Outer epidermis, parenchyma and receptacle. Am. J. Bot. 41 : 152-160.

Rhodes,M.M. and A. Carvalho. 1944. The function and structure of the parenchyma sheath plastids of the maize leaf.Bull. Torrey bot. Club. 71 : 335-346.

Roelofsen, P.A. 1959. The plant cell wall. Handbach der pflanzenanatomie III (4). 335 pp.

Roeper, J. 1826. Observationes aliquot in florum inflorescentiarumque naturam. Linnaea 1: 433-466.

Rohlf, F.J. 1963. Congruence of larval and adult classifications in Aedes (Diptera: Culicidae) Syst. Zool. 12 : 97 - 117.

Rohlf, F.J. 1965. A randomization test of the nonspecificity hypothesis in numerical taxonomy. Taxon 14: 262-267.

Rohlf, F.J. 1966. Stereograms in numerical taxonomy. Syst. Zool. 17 : 246 - 255.

Rohlf, F.J. and R. R. Sokal. 1965. Coefficients of correlation and distance in numerical taxonomy. Kans. Univ. Sci. Bull. 45 : 3 - 25.

Sabnis, T.S. 1921. The physiological anatomy of the plants of the Indian Desert. J. Indian Bot. 2 : 157-167, 217-227, 271-299.

Saint-Venant, V. 1855. Mémoire sur la Torsion des Prismes. Mém. acad. sci. savants éstrangers. 14: 233-560.

Schwendener,S.1890. Die Mestomscheiden der Gramineenblätter. Sber. dt. Akad. Wiss. 405-426.

Seal, H.L. 1964. Multivariate statistical methods in biometric research. John Wiley & Sons, N.Y.

Seifriz,W. 1952. The rheological properties of protoplasm. pp. 49-52. In: Frey-Wyssling,A. (ed.) Deformation and flow in biological systems.

Sendulsky,T. 1966. Contribution to the study of fruits and associated structures of grasses from the "cerrados" II - Andropogon L. An.Acad. Brasileria Cienc. 38:207-211 (supplemento).

Setterfield, G. & S.T. Bayley. 1961. Structure and physiology of cell walls. A. Rev. Pl. Physiol. 12 : 35 - 62.

Shannon, C.E. 1948. A mathematical theory of communication. Bell. Syst. tech. J. 27: 379-423, 623-656.

Sharman, B.C. 1947. The biology and developmental morphology of the shoot apex in the Gramineae. New phytol. 46: 20-34.

Sharman, B.C. 1960. Developmental anatomy of the stamen and carpel primordia in Anthoxanthum odoratum. Bot. Gaz. 121 : 192-198.

Sharman, B.C. 1967. Interpretation of the morphology of various naturally occurring abnormalities of the inflorescence of wheat (Triticum) Can. J. Bot. 45 : 2073-2080.

Shields, L.M. 1950. Leaf xeromorphy as related to physiological and structural differences. Bot. Rev.16 : 399-447.

Sneath, P.H.A. 1957. The application of computers to taxonomy. J. gen. Microbiol. 17 : 201-226.

Sneath, P.H.A. and S.T. Cowan. 1958. An electro-taxonomic survey of bacteria. J. gen. Microbiol. 19:551-565.

Sneath, P.H.A. & R.R. Sokal. 1962. Numerical taxonomy. Nature 193 . 855 - 860.

Soderstrom, T.R. 1967. Taxonomic study of subgenus Podosemum and section Epicampes of Muhlenbergia (Gramineae). Contr. from the U.S. National Herbarium 34: 75 - 139.

Sokal, R.R.. 1958. Quantification of systematic relationships and of phylogenetic trends. Proc. 10th Int. Congr. Ent. 1 : 409 - 415.

Sokal, R.R. 1961. Distance as a measure of similarity. Syst. Zool. 10 : 70 - 79.

Sokal, R.R.. and F.J. Rohlf, 1962. The comparison of dendrograms by objective methods. Taxon 11: 33-39.

Sokal, R.R. and P.H.A. Sneath. 1963. Principles of Numerical Taxonomy. Freeman & Co. London. 359 pp.

Sørensen, T. 1953. A revision of the Greenland species of Puccinellia Parl. Meddelelser om Grønland. 136(3) : 169 pp.

Stafleu, F.A. 1966. Adanson - Labillardière - De Candole. J. Cramer, Lehre. 103 pp.

Stant, M. Y. 1963. Anatomy of the Alismataceae. J. Linn. Soc. (Bot.) 59 : 1 - 42.

Stapf, O. 1898. In: Thistleton-Dyer, W.T. (ed.) Flora Copensis 7(2) : 314, 448-454.

Starr, A. 1912. Comparative anatomy of dune plants. Bot. Gaz. 54 : 265 - 305.

Stebbins, G.L. 1956. Cytogenetics and evolution of the grass family. Am. J. Bot. 43 : 890-905.

Stebbins, G.L. 1965. Some relationships between mitotic rhythm, nucleic acid synthesis and morphogenesis in higher plants. Brookhaven Symp. Biol. 18:204-221.

Steudel, E.G.. 1854. Syn. Pl. Gram.: 114-117, 237-244.

Stewart, D.R.M. 1965. The epidermal characters of grasses, with special reference to East African plains species. Bot. Jb. 84 : 63-174.

Storr, G.M. 1961. Microscopic analysis of faeces, a technique for ascertaining the diet of herbivorous mammals. Aust. J.biol. Sci. 14(1): 157-164.

Tansley, A.G. 1953. The British Islands and their vegetation. Cambridge University Press.

Tateoka, T. 1956a. Re-examination of anatomical characteristics of the leaf in Eragrostoideae and Panicoideae (Poaceae). Jap.J. Bot. 31 : 201-218.

Tateoka, T. 1956b. Notes on some grasses 1. Bot. Mag. Tokyo 69 : 311-315.

Tateoka, T. 1957. Miscellaneous papers on the phylogeny of Poaceae (10). Proposition of a new phylogenetic system of Poaceae. J. Jap. Bot. 32 : 275 - 287.

Tateoka, T. 1958. Notes on some grasses VIII. On leaf structure of Arundinella and Garnotia. Bot. Gaz. 120: 101-109.

Thompson, D'Arcy, W. 1917. On Growth and Form. Cambridge University Press. 793 pp.

Timoshenko, S. 1953. History of Strength of Materials. McGraw-Hill, N.Y. 452 pp.

Timoshenko, S. and D.H. Young. 1962. Elements of strength of materials. (4th ed.). Van Nostrand Co., Princetown, N.J. 377 pp.

Tran, T.T.H. 1965. Les Glumelles Inférieures Aristées de Quelques Graminées: Anatomie, Morphology. Bull. Jard. bot. État. Brux. 35: 219-284.

Turner, D.M. 1953. The identification of the grasses. The Institute of Corn & Agricultural Merchants, London. 66 pp.

Uvarov, E.B. and D. R. Chapman. 1958. A dictionary of Science. Penguin Books, London.

Vaquero, J.M.R. 1958. Aplicación del método de Jeffrey para la separación de epidermis. Archivos de Bioquimica, Quimica y Farmacia-Tucuman 8: 127-131.

William , W.T. & J. M. Lambert. 1959. Multivariate methods in plant ecology I. Association analysis in plant communities. J. Ecol. 47: 83-101.

Williams, W.T. & J. M. Lambert. 1961. Multivariate methods in plant ecology III. Inverse association-analysis J. Ecol. 49: 717-729.

Williams, W.T. & M. B. Dale. 1965. Fundamental problems in numerical taxonomy. Adv. Bot. Res. 2: 35-68.

Williams, W.T., J. M. Lambert, and G.N. Lance. 1966. Multivariate methods in plant ecology V. Similarity analysis and information analysis. J. Ecol. 54 : 427 - 445.

Wirth, M., G. F. Estabrook and D.J. Rogers. 1966. A graph theory model for systematic biology, with an example for the Oncidiinae (Orchidaceae). Syst. Zool. 15 : 59-69.

Appendix 1

LIST OF SPECIMENS

Table 32: Specimens examined morphologically and anatomically and used as exemplars in the numerical taxonomy of Loudetia and Rattraya.

OTU NO.							
LEAF ANATOMY	LEAF EPIDERMIS	AXIS ANATOMY	ALL CHARACTERS	NAME	SPECIMEN	WILD OR CULTIVATED	SOURCE
				LOUDETIA Section 1: Loudetia Subsection a: Typicae			
1	1	--	1	L. angolensis C.E. Hubbard	UWO S195 (UWO)	C	J.B. Phipps, N.W. Province, Zambia
--	--	2	--	" " " "	E. Milne Redhead 4489 (K)	W	Zambia
2	--	1	--	" " " "	R.B. Drummond 8273 (UWO)	W	N.W. Province, Zambia
3	2	3	2	L. arundinacea (Hochst. ex A. Rich.) C.E. Hubbard	UWO S26 (UWO)	C	L.D.E.F. Vesey-FitzGerald 13, Abercorn, Zambia
--	3	--	--	" " " "	UWO S31 (UWO)	C	L.D.E.F. Vesey-FitzGerald 2456, Abercorn, Zambia

4	4	4	3	" " " "	UWO S44 (UWO)	C	G.D. Scott, Bukoba, Tanzania
5	5	--	--	" " " "	UWO S88 (UWO)	C	H. Jacques-Félix, Boukoko Region, Central African Rep.
6	6	5	4	" " " "	UWO S154 (UWO)	C	J.B. Phipps, Kinshasa, Congo-Kinshasa
--	7	--	--	" " " "	UWO S218 (UWO)	C	J.B. Phipps, N. Province, Zambia
--	8	--	--	" " " "	UWO S249 (UWO)	C	A. Boudet 1435, Côte d'Ivoire
7	9	--	5	L. thomasii C.E. Hubbard	Thomas 1027 (K)*	W	Toro, Uganda
--	--	--	--	L. eriopoda C.E. Hubbard	Tessman 2728 (K)*	W	Assum, Tibati Highland, Cameroons
8	10	6	6	L. camerunensis (Stapf) C.E. Hubbard	T.D. Maitland 865 (K)	W	Cameroon Mountain, Cameroons
--	--	--	--	" " " "	J. Milbraed 10841 (U.S.)	W	Cameroon Mountain, Cameroons
--	--	--	--	" " " "	J.K. Morton K857 (K)	W	Ghana
9	11	7	7	L. simplex (Nees) C.E. Hubbard subsp. simplex	UWO S4 (UWO)	C	E.M. Cooling, Kitwe, Zambia
10	12	8	8	" " " "	UWO S10 (UWO)	C	W.B. Cleghorn, Chilimanzi Distr., Rhodesia

* Type Specimen

11	13	--	--	" " " "	UWO 341 (UWO)	C	J.B. Phipps, Bulawayo, Rhodesia
--	14	--	--	" " " "	UWO S52 (UWO)	C	J.D. Chapman, Malawi
--	15	--	--	" " " "	UWO S150 (UWO)	C	R.H. Compton, Mbabane, Swaziland
13	16	9	9	L. simplex (Nees) C.E. Hubbard subsp. stipoides (Hack.) J. Bosser	UWO S225 (UWO)	C	J.B. Phipps, Tananboa, Malagasy
12	--	--	--	" " " "	UWO S222B (UWO)	C	J.B. Phipps, Tsivangambalada, Malagasy
--	--	10	--	" " " "	UWO S226 (UWO)	C	J.B. Phipps, Ambalavoa, Malagasy
--	17	--	--	" " " "	J. Bosser 7273 (UWO)	W	Malagasy
14	18	11	10	L. kagerensis (K. Schum.) C.E. Hubbard ex. Hutch.	UWO S18 (UWO)	C	A.V. Bogdan, Kitale, Kenya
15	19	12	11	" " " "	UWO S79 (UWO)	C	B.H. Bowdan, Entebbe, Uganda
16	--	--	--	" " " "	UWO S257 (UWO)	C	A.A. Mbabali, Lake Kabugab, Uganda
--	20	--	--	" " " "	G.H.S. Wood 521	W	
17	21	13	12	L. perrieri A. Camus	P. de la Bathie 2699 (UWO)	W	Antisirabé, Malagasy

--	--	14	--	L. madagascariensis (Bak.) Bosser	J. Bosser 7539 (UWO)	M	Isalo, Malagasy
18	--	15	13	" " " "	J. Bosser 15462 (UWO)	M	Malagasy
19	22	--	--	" " " "	R. Viguier & H. Humbert 1745 (B)*	M	Vakinankaratra Prov., Malagasy
				Subsection b: Pungentes			
--	--	17	--	L. lanata (Stent and Rattray) C.E. Hubbard	J.B. Phipps 2525 (SRGH)	W	Charter Distr., Rhodesia
21	--	--	15	" " " "	A.O. Crook 655 (UWO)	M	Salisbury Distr., Rhodesia
--	24	--	--	" " " "	UWO S54 (UWO)	C	Enkeldoorn, Rhodesia
22	25	18	16	L. longipes C.E. Hubbard	J. Gossweiler 4033 (K)*	W	Benguella, Angola
23	26	19	17	L. demeusei (De Wild) C.E. Hubbard	L. Pauwels 4514 (UWO)	W	Mdjili, Congo-Kinshasa
24	27	20	18	L. crassipes C.E. Hubbard	J. Gossweiler (K-H 2075/66)	W	Benguella, Angola
				Subsection c: Acuminatae			
25	28	--	--	L. pennata (Chiov.) C.E. Hubbard	R. Pohill & S. Panlo 1324 (SRGH)	W	Tanzania
--	--	22	19	" " " "	L.D.E.F. Vesey-FitzGerald (SRGH)	W	Abercorn Distr., Zambia
26	--	21	--	" " " "	N. McCallum Webster A310 (K)	W	Abercorn Distr., Zambia

* Type Specimen

28	--	23	--	L. flavida (Stapf) C.E. Hubbard	UWO 317 (UWO)	C	A.V. Bogdan, W. Pokot Distr., Kenya
29	30	24	20	" " " "	UWO 342 (UWO)	C	Bulawayo, Rhodesia
27	29	25	--	" " " "	L.D.E.F. Vesey-FitzGerald 1244 6/3 (BM)	W	J. Quara, Dhofar, Muscat and Oman
--	31	--	--	" " " "	UWO S106 (UWO)	C	A. Yousif Kordofani, Yambia Region, Sudan
30	32	26	21	L. migiurtina (Chiov.) C.E. Hubbard	Puccioni and Stefanini 738 (816 bis) (FI)*	W	Between Hongolo and Hariri, Somalia
31	33	28	22	L. acuminata (Stapf) C.E. Hubbard	J.B. Hall and Enti GC35968 (UWO)	W	Nakpanduri, Ghana
--	--	27	--	" " " "	A. Macleod 80 (SRGH)	W	Bogola, Cameroons
32	--	--	--	L. acuminata (?)	UWO S305 (UWO)	C	
33	34	29	23	L. cuanzensis Lubke and Phipps	J. Gossweiler 10695 (US)*	W	Cuanza Norte, Angola
--	--	--	--	" " " "	J. Gossweiler 9634 (US)	W	" " "
34	35	--	24	L. filifolia Schweick. subsp. filifolia	Schweickerdt 1878a (SRGH)	W	Transvaal, South Africa
35	--	30	25	subsp. humbertiana A. Camus	J. Bosser 8972 (UWO)	W	Sukaraho, Malagasy
--	36	31	--	" " " "	J. Bosser 8973 (UWO)	W	" " "

* Type Specimen

Subsection d: Densispicae

36	37	32	26	L. gossweileri C.E. Hubbard	J.H. Hinds 284 (SRGH)	W	Lakalanya Area, Zambia
--	--	34	--	L. echinulata C.E. Hubbard	C. Tisserant 2712 (K)*	W	Harama, Central African Rep.
37	38	35	27	L. coarctata (A. Camus) C.E. Hubbard	UMO 5105 (UMO)	C	Ahmed Yousif Kordofani, Wambia Region, Sudan
--	--	33	--	L. densispica (Rendle) C.E. Hubbard	G. Barbosa 9661 (SRGH)	W	Tundavala, Huila, Angola
--	--	36	28	" " " "	L.E. Codd 1583 (PRE)	W	Swaziland
38	40	--	--	" " " "	J.P.H. Acocks 11697 (PRE)	W	Ermelo, Transvaal, South Africa
39	39	--	--	" " " "	J.P.H. Acocks 13308 (PRE)	W	" " "
--	--	--	--	" " " "	Dured 1828 (PRE)	W	Kahemba-Kwango, Congo-Brazza
--	--	--	--	" " " "	J. Gossweiler 11601 (SRGH)	W	Angola
40	41	37	29	L. tisserantii C.E. Hubbard	H. Gerard 53 (B)	W	Uele, Inkproo, Congo-Kinshasa
--	--	--	--	" " " "	C. Tisserant (K H31967/65)	W	Region de Bambari, Central African Rep.
--	--	38	--	L. vanderystii (De Wild) C.E. Hubbard	J. Lebrun 6034 (B)	W	Sewane, Congo-Kinshasa

* Type Specimen

41	42	--	30	" " " "	UWO S283 (UWO)	W	L. Pauwels 4920, Congo-Kinshasa

Subsection e: Annuae

--	--	40	--	L. annua (Stapf) C.E. Hubbard	UWO S291 (UWO)	W	P. de Leeuw, Bornu Province, Northern Nigeria
42	43	--	31	" " " "	Mosnier 25 (UWO)	W	Mali
--	--	39	--	L. cerata (Stapf) C.E. Hubbard	A. Chevalier 10483 (K)*	W	Fort Archambault, Central African Rep.
--	--	42	32	L. hordeiformis (Stapf) C.E. Hubbard	A. Chevalier 22149 (B)	W	North Baoulé Distr., Côte d'Ivoire
43	44	--	--	" " " "	Harrison 109 (K)	W	Sudan
--	--	--	--	" " " "	UWO S290 (UWO)	W	P. de Leeuw, Katsina, N. Nigeria
--	--	41	--	L. bidentata Berhaut	J.G. Adam 13878 (UWO)	W	Kolda, Sénégal

Subsection f: Flammidae

44	45	--	33	L. flammida (Trin.) C.E. Hubbard	UWO S110 (UWO)	C	T. Sendulsky 41, Minas Gerais, Brazil
45	46	43	34	L. phragmitoides (Peter) C.E. Hubbard	UWO S65 (UWO)	C	Manuel Porto, Equatorial Guinea
--	47	--	--	" " " "	UWO S200 (UWO)	C	J.B. Phipps, N.W. Province, Zambia

* Type Specimen

				Section 2: Pleioneura			
46	48	44	35	L. simulans C.E. Hubbard	C. Tisserant 3601 (K)*	W	Region de Bazoum, Central African Rep.
--	--	46	--	L. anomala C.E. Hubbard and Schweick.	K. Dinter 4676 (B)	W	Demaraland, S.W.A.
47	49	45	36	" " " "	O.H. Volk 1137 (PRE)	W	Waterberg Plateau, S.W.A.
--	--	47	--	" " " "	O.H. Volk 1649 (PRE)	W	S.W.A.
48	50	48	37	L. ramosa (Stapf) C.E. Hubbard	UWO S245 (UWO)	C	Okahandja Distr., S.W.A.
				Section 3: Lophanthera			
--	--	--	--	L. togoensis (Pilg.)C.E.Hubb.	Kersting 662 (B)	W	Koukomba, Togo
--	--	49	--	" " " "	UWO S293 (UWO)	W	P. de Leeuw, Shika, N. Nigeria
49	51	--	38	" " " "	J.G. Adam 18879 (UWO)	W	Tambacounda, Sénégal
				Incertae Sedes			
20	23	16	14	L. jaegeriana A. Camus	P. Jaeger 1705 (P)*	W	S.E. of Sérelen-Kouko, Sénégal
				Rattraya Phipps			
50	52	50	39	R. petiolata Phipps	UWO P18 (UWO)	C	J.L. Stephens (P.I. 225-553), Victoria Falls, Rhodesia

* Type Specimen

Appendix 2

DATA AND TABLES PERTAINING TO THE ANALYSES USING LEAF ANATOMY CHARACTERS

Table 33 List of leaf anatomy characters and their corresponding states; coded values in parentheses

Character	States
I. General Characters	
1. Curvature of the lamina	Not involute (100); involute (900)
2. Width of leaf	Narrow (100); average (500); wide (900)
3. Thickness of the lamina at midrib	Relatively thin (100); moderate (500); relatively thick (900)
4. Thickness of lamina halfway to margin	Relatively thin (100); moderate (500); relatively thick (900)
II. Adaxial Surface	
5. Presence of ribs	Ribbed (100); wavy (500); flat (900)
6. a. Height of ribs	More or less same height (100); otherwise (500)
6. b.	Of different heights (100); otherwise (500)
7. a. Shape of ribs	Round (100); otherwise (500)
b.	Angular (100); otherwise (500)
III. Bulliform Cells	
8. Arrangement	In distinct groups (100); not in distinct groups (900)
9. a. Shape of group	Inflated cell group (100); otherwise (500)
b.	Fan-shaped group (100); otherwise (500)
10. Size of cells	Not much larger than remaining epidermal cells (100); conspicuously larger than remaining cells (900)
11. Associated colourless cells	Yes (100); no (900)

Table 33 (continued)

12. a. Arrangement	In a group penetrating the mesophyll (100); otherwise (500)
b.	Appear as archway over adaxial side of a small bundle, the ends of vertical pillars reaching the adaxial epidermis (100); otherwise (500)
IV. Abaxial Surface	
13. Presence of ribs	Ribbed (100); wavy (500); flat (900)
14. a. Height of ribs	More or less the same height (100); otherwise (500)
b.	Of different heights (100); otherwise (500)
15. a. Shape of ribs	Round (100); otherwise (500)
b.	Angular (100); otherwise (500)
16. Portion of abaxial epidermal cells between veins consisting of large cells in distinct group	Yes (100); no (900)
V. Margin of the Leaf	
17. Leaf margins	Thin (100); slightly swollen (500); strongly swollen (900)
18. Shape in transverse section	Round (100); pointed (900)
19. Associated fibre cells	Nil (100); 25 or less (366); 26-50 (633); more than 50 cells (900)
20. a. Outline of fibre group	Dot shape (100); otherwise (500)
b.	Angular (100); otherwise (500)
c.	Asymmetric arch (100); otherwise (500)
d.	Symmetric arch (100); otherwise (500)
e.	Triangular (100); otherwise (500)

Table 33 (continued)

VI. Mesophyll	
21. Presence of distinctive cells	Yes (100); no (900)
VII. Midrib	
22. Keel	Absent (100); slightly prominent (500); strongly prominent (900)
23. a. Shape of keel	Round (100); otherwise (500)
b.	Angular (100); otherwise (500)
24. Rib above keel	Yes (100); no (900)
25. Number of vascular bundles	1 solitary (100); 3 V.Bs (500); more than 3 V.Bs (900)
VIII. Largest Midrib or Central Vascular Bundle	
26. a. Shape of V.B.	Round (100); otherwise (500)
b.	Oval (100); otherwise (500)
c.	Angular (100); otherwise (500)
d.	Triangular (100); otherwise (500)
27. Mestome sheath	Absent (100); partly defined (500); well defined (900)
28. Fibrous ground tissue	Absent (100); contiguous to phloem (500); envelops phloem and xylem (900)
29. Interruption of parenchymatous sheath	Yes (100); no (900)
30. a. Position	Adaxially interrupted (100); otherwise (500)
b.	Abaxially interrupted (100); otherwise (500)
31. Groups of phloem cells	1 (100); 2 (366); 3 (633); 4 or more (900)

Table 33 (continued)

Associated sclerenchyma	
32. Adaxial strand	Nil (100); 1 (366); 2 (633); 3 (900)
33. a. Shape of adaxial strand	Narrow (100); otherwise (500)
b.	Broad (100); otherwise (500)
34. Adaxial girder	Yes (100); no (900)
35. a. Shape of adaxial girder	Narrow (100); otherwise (500)
b.	Wedge-shaped (100); otherwise (500)
c.	Broad (100); otherwise (500)
36. Abaxial strand	Yes (100); no (900)
37. Abaxial girder	Yes (100); no (900)
38. a. Shape of abaxial girder	Narrow (100); otherwise (500)
b.	Broad (100); otherwise (500)
c.	Wedge-shaped (100); otherwise (500)
Associated colourless parenchyma	
39. Between V.B. and adaxial epidermis or sclerenchyma	Yes (100); no (900)
40. a. Arrangement	A few cells in a single row (100); otherwise (500)
b.	A mass of many cells (100); otherwise (500)
41. Between V.B. and abaxial epidermis, or sclerenchyma arranged in single row.	Yes (100); no (900)
IX. First Order V.B.	
42. a. Shape	Triangular (100); otherwise (500)
b.	Round (100); otherwise (500)

Table 33 (continued)

c.	Oval (100); otherwise (500)
d.	Angular (100); otherwise (500)
43. Mestome sheath	Nil (100); partly defined (500); well defined (900)
44. Fibrous ground tissue	Absent (100); contiguous to phloem (500); envelops phloem and xylem (900)
45. Interruption of parenchymatous sheath	Yes (100); no (900)
46. a. Position	Adaxially interrupted (100); other (500)
b.	Abaxially interrupted (100); other (500)
47. Groups of phloem cells	1 (100); 2 (366); 3 (633); 4 or more (900)
Associated sclerenchyma	
48. Adaxial strand	Yes (100); no (900)
49. a. Shape of adaxial strand	Narrow (100); otherwise (500)
b.	Broad (100); otherwise (500)
50. Adaxial girder	Yes (100); no (900)
51. a. Shape of adaxial girder	Narrow (100); otherwise (500)
b.	Broad (100); otherwise (500)
c.	Wedge shape (100); otherwise (500)
52. Abaxial strand	Yes (100); no (900)
53. a. Shape of abaxial strand	Narrow (100); otherwise (500)
b.	Broad (100); otherwise (500)
54. Abaxial girder	Yes (100); no (900)
55. a. Shape of abaxial girder	Narrow (100); otherwise (500)
b.	Broad (100); otherwise (500)
c.	Wedge shape (100); otherwise (500)
d.	Trapezoid (100); otherwise (500)

Table 33 (continued)

Associated colourless parenchyma	
56. Between V.B. and adaxial epidermis or sclerenchyma	Yes (100); no (900)
57. a. Arrangement	A few cells in a single row (100); otherwise (500)
b.	A mass of many cells (100); otherwise (500)
58. Between V.B. and abaxial epidermis or sclerenchyma arranged in single row	Yes (100); no (900)
X. Second Order V.B.	
59. a. Shape	Round (100); otherwise (500)
b.	Oval (100); otherwise (500)
c.	Elliptical (100); otherwise (500)
d.	Angular (100); otherwise (500)
60. Mestome sheath	Nil (100); partly defined (500); well defined (900)
61. Fibrous ground tissue	Absent (100); contiguous to phloem (500); envelops phloem and xylem (900)
62. Interruption of parenchymatous sheath	Yes (100); no (900)
63. a. Position	Adaxially interrupted (100); otherwise (500)
b.	Abaxially interrupted (100); otherwise (500)
64. Extension of sheath cells adaxially	Yes (100); no (900)
Associated sclerenchyma	
65. Adaxial strand	Yes (100); no (900)
66. a. Shape of adaxial strand	Narrow (100); otherwise (500)
b.	Broad (100); otherwise (500)

Table 33 (continued)

67. Adaxial girder	Yes (100); no (900)
68. a. Shape of adaxial girder	Narrow (100); otherwise (500)
b.	Wedge-shaped (100); otherwise (500)
c.	Broad (100); otherwise (500)
69. Abaxial strand	Yes (100); no (900)
70. Abaxial girder	Yes (100); no (900)
71. a. Shape of abaxial girder	Narrow (100); otherwise (500)
b.	Broad (100); otherwise (500)
c.	Wedge-shaped (100); otherwise (500)
Associated colourless parenchyma	
72. Between V.B. and adaxial epidermis and sclerenchyma	Yes (100); no (900)
73. a. Arrangement	A few cells in a single row (100); otherwise (500)
b.	A mass of many cells (100); otherwise (500)
74. Between the V.B. and abaxial epidermis or sclerenchyma arranged in a single row	Yes (100); no (900)
XI. Third Order V.B.	
75. Presence	Yes (100); no (900)
76. 1. a. Shape	Round (100); otherwise (500)
b.	Oval (100); otherwise (500)
c.	Elliptic (100); otherwise (500)
d.	Angular (100); otherwise (500)

Associated sclerenchyma

76. 2. Abaxial strand	Yes (100); otherwise (500)
76. 3. Abaxial cylinder	Yes (100); otherwise (500)

XII. Proportion of Different Orders of Bundles

77. Ratio of no. of secondary V.Bs to no. of primary V.Bs	± .5 (100) ± 1 (280) ± 2 (360) ± 3 (540) ± 4 (720) more than 4 (900)
78. Ratio of no. of tertiary V.Bs to no. of primary V.Bs	0 (100) ± 1 (280) ± 2 (360) ± 3 (540) ± 4 (720) more than 4 (900)
79. Ratio of no. of secondary V.Bs to no. of tertiary V.Bs	0 (100) ± .5 (280) ± 1 (360) ± 2 (540) ± 3 (720) ± 4 (900)

Table 34 The width of the lamina, and the actual numbers of the different orders of vascular bundles observed in the transverse sections.

OTU No.	Name	Width of lamina (mm.)*	Numbers of Vascular Bundles*		
			Primary	Second-ary	Tertiary
1.	L.angolensis	(8.91)	(15)	(28)	(62)
2.	L. angolensis	(5.08)	(5-6)	(14)	(24)
3.	L.arundinacea	5.61	9	27	52
4.	L.arundinacea	9.24	13	35	50
5.	L.arundinacea	(9.64)	(15)	(52)	(64)
6.	L.arundinacea	9.41	13	35	50
7.	L.thomasii	(5.18)	(9)	(32)	(40)
8.	L.camerunensis	4.37	11	12	23
9.	L.simplex subsp. simplex	2.23	7	9	17
10.	L.simplex subsp. simplex	3.47	7	7	23
11.	L.simplex subsp. simplex	3.22	9	8	22
12.	L.simplex subsp. stipoides	(8.09)	(15)	(16)	(30)
13.	L.simplex subsp. stipoides	(4.29)	(9)	(8)	(16)
14.	L. kagerensis	2.90	7	6	24
15.	L. kagerensis	(4.13)	(9)	(10)	(36)
16.	L. kagerensis	4.36	9	9	35
17.	L. perrieri	3.71	7	8	16
18.	L.madagascariensis	1.24	5	6	8
19.	L.madagascariensis	1.07	5	6	4

OTU No.	Name	Width of lamina (mm.)*	Numbers of Vascular Bundles*		
			Primary	Second-ary	Tertiary
20.	L. jaegeriana	3.63	5	13	19
21.	L. lanata	4.70	12	12	24
22.	L. longipes	3.32	9	10	19
23.	L. demeusei	4.12	10	8	21
24.	L. crassipes	3.05	11	7	18
25.	L. pennata	2.48	7	6	14
26.	L. pennata	2.64	7	11	17
27.	L. flavida	2.92	7	8	16
28.	L. flavida	3.30	10	11	21
29.	L. flavida	2.72	6	13	20
30.	L. migiurtina	3.80	9	9	19
31.	L.acuminata	1.40	6	6	6
32.	L.acuminata	3.17	6	10	18
33.	L.cuanzensis	2.06	5	12	18
34.	L.filifolia subsp. filifolia	1.98	5	5	13
35.	L.filifolia subsp. humbertiana	2.56	7	6	18
36.	L. gossweileri	(2.41)	(7)	(6)	(16)
37.	L. coarctata	4.04	10	7	21
38.	L. densispica	(3.30)	(7-8)	(10)	(18)
39.	L. densispica	(2.71)	(7)	(8)	(16)
40.	L. vis_erantii	2.49	7	6	14
41.	L. vanderystii	2.15	6	6	14
42.	L. annua	2.81	7	7	15

OTU No.	Name	Width of lamina (mm.)*	Numbers of Vascular Bundles*		
			Primary	Second-ary	Tertiary
43.	L. hordeiformis	6.27	11	10	38
44.	L. flammida	6.77	12	27	41
45.	L. phragmitoides	(10.56)	(17)	(38)	(90)
46.	L. simulans	(10.69)	(21)	(116)	-
47.	L. anomala	8.42	(13)	(64)	-
48.	L. ramosa	2.31	6	20	-
49.	L. togoensis	(5.94)	(11)	(40)	(16)
50.	Rattraya petiolata	(14.19)	(11)	(96)	-

*Estimated value (from T/S of half of lamina) in bracts.

TABLE 35 - DATA MATRIX 60 - LEAF ANATOMY CHARACTERS

CTU NUMBER 1

500 900 500 100 500 500 500 500 500 100 100 500 900 100 500 100 500 500 500 500 500 900 100 100 500
500 500 100 500 500 100 500 500 100 100 100 500 500 500 500 900 900 100 100 100 900 366 500 100 900
500 500 500 900 100 500 100 500 100 500 100 100 500 100 500 900 900 900 100 100 100 900 100 500 100
900 500 900 500 900 500 900 100 500 100 500 500 100 100 500 900 500 500 100 500 500 500 900 500 [illegible]
900 900 500 500 100 500 500 100 900 100 500 500 100 100 100 500 100 100 100 500 500 500 100 500 540
720 540

CTU NUMBER 2

500 900 500 100 500 500 500 500 500 100 100 500 900 100 500 100 500 900 500 500 500 900 100 900 600
500 500 500 500 100 900 100 500 500 100 100 100 500 500 500 900 500 100 500 100 900 633 100 500 900 100
500 500 100 900 100 500 100 500 900 500 500 900 500 100 500 500 900 500 900 500 500 900 633 900 500 900
100 500 100 500 900 500 500 100 100 500 500 500 900 900 500 900 900 500 500 100 500 900 500 900 500 900
900 900 500 500 100 100 500 500 900 100 100 500 500 900 500 500 900 100 100 500 500 500 100 500 540
720 540

CTU NUMBER 3

100 900 500 100 500 500 500 500 500 900 500 500 900 100 100 100 900 500 500 500 500 900 100 100 600
500 500 100 500 500 900 900 100 500 900 900 100 500 500 500 500 900 500 100 500 900 100 900 366 500 100 900
500 500 500 900 100 500 100 500 100 500 100 900 500 100 500 500 500 900 100 500 100 100 900 500 900
100 500 100 500 900 500 500 100 100 500 500 100 900 500 500 900 500 500 100 500 500 500 900 500 900
900 900 500 500 100 100 500 500 900 100 100 500 500 900 500 500 900 100 100 500 500 500 100 500 540
900 540

CTU NUMBER 4

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500 500 100 500 500 100 900 500 100 100 900 100 500 500 500 500 500 900 100 100 900 900 500 100 900
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100 500 100 500 900 500 500 100 500 500 500 100 100 100 500 900 500 500 100 500 500 500 900 500 900
500 100 500 100 900 500 500 500 900 100 500 500 100 100 100 500 100 100 100 500 500 500 500 500 540
720 360

CTU NUMBER 5

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500 500 100 500 500 900 900 500 100 900 900 100 500 500 500 500 900 100 100 900 900 500 100 900 900
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100 500 100 500 900 500 500 100 500 100 500 500 100 100 500 900 500 500 100 500 500 100 900 500 500
900 100 500 100 900 500 500 500 100 900 500 500 500 100 100 500 100 100 100 500 500 500 500 500 540
720 360

CTU NUMBER 6

100 900 900 500 500 500 500 500 500 900 500 500 900 100 100 100 900 500 500 500 500 900 100 100 900
500 500 500 100 500 100 500 500 100 900 900 100 500 500 500 500 900 100 100 100 900 366 500 100 900
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900 500 900 500 900 500 500 100 500 100 500 500 100 100 500 900 500 500 100 500 900 500 900 500 500
900 100 900 100 900 500 500 500 100 900 500 500 500 900 500 500 900 100 100 500 500 500 900 500 540
720 360

CTU NUMBER 7

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500 500 100 500 500 100 500 500 100 100 100 500 500 500 500 100 900 100 100 100 900 366 500 100 900
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100 500 100 500 900 500 500 100 500 500 500 100 900 500 500 900 500 100 500 500 500 500 900 500 900
500 900 500 500 100 500 500 100 900 100 500 500 100 900 500 500 900 100 100 500 500 500 100 500 540
720 360

CTU NUMBER 8

500 500 500 100 500 500 500 500 500 100 100 500 900 100 100 100 500 500 500 500 500 900 100 100 600
500 500 500 100 500 900 900 500 100 100 100 500 100 500 500 500 900 100 500 100 100 366 500 100 900
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100 500 100 500 900 500 500 100 500 100 500 500 100 100 500 100 500 500 100 500 500 500 900 500 500
900 100 500 100 900 500 500 500 100 500 500 500 500 100 100 500 100 100 500 500 100 500 500 500 240
360 540

CTU NUMBER 9

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500 500 500 100 500 100 100 500 500 900 100 500 100 500 500 900 500 100 500 100 100 366 500 100 900
500 500 500 100 500 500 500 500 100 100 500 100 500 500 100 500 900 500 100 500 100 100 100 500 100
900 500 500 500 900 500 500 100 500 100 500 500 100 100 500 100 500 500 100 500 900 500 900 500 500

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CTU NUMBER   10
   100  500  500  500  500  500  500  500  100  100  500  900  100  100  100  900  500  500  500  500  900  100  100  611
   500  500  100  500  500  900  100  500  500  900  100  500  100  500  500  900  500  500  500  100  366  500  100  900
   500  500  500  100  900  500  500  500  100  100  500  100  500  500  100  500  900  500  900  500  900  100  100  500  100
   900  500  500  500  100  500  100  900  500  500  500  500  100  100  500  100  500  500  100  500  900  100  900  900  900
   900  100  500  100  900  500  500  500  900  100  500  100  500  100  100  500  100  100  100  500  500  500  100  500  200
   540  720
CTU NUMBER   11
   100  500  500  100  500  500  500  500  500  100  100  500  900  100  100  100  500  500  500  500  500  900  100  100  611
   500  500  100  500  500  900  100  500  500  900  100  500  100  500  500  900  500  100  500  100  100  366  500  100  900
   500  500  500  900  100  500  100  500  100  100  500  900  500  500  100  500  900  500  100  500  100  100  100  500  100
   900  500  500  500  100  500  100  900  500  500  500  500  100  100  500  100  500  500  100  500  900  100  900  900  900
   900  100  500  100  900  500  500  500  100  900  500  500  500  100  100  500  100  100  500  500  100  500  500  500  200
   360  720
CTU NUMBER   12
   100  900  500  500  500  500  500  500  500  100  100  500  900  100  100  100  500  500  500  500  500  900  100  100  611
   500  100  500  500  500  100  100  500  500  900  100  500  100  500  500  100  900  100  100  100  366  100  500  500  900
   500  500  100  900  100  500  100  500  100  100  500  900  500  500  100  500  100  900  100  100  100  100  900  500  900
   100  500  100  500  900  500  500  100  500  100  500  500  900  500  500  900  500  500  100  500  100  900  100  500  100
   100  900  500  500  100  500  500  100  900  100  500  100  500  900  500  500  900  100  500  100  500  900  100  100  200
   360  540
CTU NUMBER   13
   100  500  500  500  900  500  500  500  500  100  100  500  900  100  100  500  900  500  500  500  500  100  900  611
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   500  500  500  900  100  500  100  500  100  100  500  900  500  500  100  500  900  500  100  100  100  100  900  500  900
   100  500  100  500  900  500  500  100  500  100  500  500  100  100  500  900  500  500  100  500  900  500  900  500  900
   500  900  500  500  100  500  500  100  900  100  500  100  500  900  500  500  900  100  500  100  500  500  100  900  200
   360  540
CTU NUMBER   14
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   100  500  500  500  500  100  100  500  500  900  100  100  500  500  500  900  500  100  100  100  100  100  500  500  100
   500  500  100  900  100  500  100  500  900  500  500  900  500  100  500  500  900  500  100  500  100  100  900  500  500
   100  500  100  500  900  500  500  100  500  100  500  500  900  500  500  900  500  500  100  500  500  100  900  500  900
   900  100  500  100  900  500  500  500  100  900  500  500  500  900  500  500  900  100  500  500  100  500  100  500  200
   540  900
CTU NUMBER   15
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   500  500  100  900  100  500  100  500  900  500  500  900  500  100  500  500  900  500  100  100  100  100  900  500  500
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   720  900
CTU NUMBER   16
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   500  500  500  500  100  100  100  500  500  900  100  500  100  500  500  900  500  100  100  100  100  100  500  500  100
   100  500  500  900  100  500  100  500  900  500  500  900  500  500  100  500  900  500  100  100  100  100  900  500  900
   100  500  100  500  900  500  500  100  500  100  500  500  900  500  500  900  500  500  100  500  500  100  900  900  500
   900  100  500  100  900  500  500  500  100  900  500  500  500  900  500  500  900  100  500  500  100  500  100  500  200
   720  900
CTU NUMBER   17
   900  500  900  500  100  500  100  500  100  100  100  500  900  100  100  500  100  500  100  100  500  100  100  100  366
   500  500  100  500  500  100  100  500  500  100  100  500  500  500  100  900  500  100  100  100  100  100  500  500  900
   500  500  500  100  900  500  500  500  100  500  100  100  500  500  500  900  500  100  100  100  100  100  900  500  500
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   360  540
CTU NUMBER   18
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   900  500  500  500  100  500  100  900  500  500  500  500  100  500  100  100  500  500  100  500  900  500  100  100  800
   900  100  100  500  900  500  500  500  100  900  500  500  500  100  100  500  100  100  100  900  500  500  100  500  200
   360  360
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CTU NUMBER 19

500	100	500	500	100	500	100	100	500	100	100	500	100	100	100	500	900	500	500	500	500	100	500	360
500	500	500	500	100	900	100	500	500	100	100	500	100	500	500	900	500	100	100	100	100	900	500	900
500	500	500	100	900	500	500	500	100	500	500	500	100	500	100	100	500	100	500	500	900	500	100	500
900	500	500	500	100	500	100	900	500	500	500	500	100	500	100	100	500	100	100	500	100	500	500	100
900	100	100	500	900	500	500	500	100	900	500	500	500	100	100	500	100	100	100	500	500	500	100	900
360	360																					

CTU NUMBER 20

100	500	100	100	900	500	500	500	500	900	500	500	900	100	100	100	900	500	500	500	900	500	900	500
500	500	500	500	100	900	100	500	500	900	100	500	500	100	500	500	100	500	500	500	100	100	500	100
100	500	500	900	100	100	500	500	900	500	500	900	500	500	500	100	500	100	900	500	500	100	900	500
100	100	500	500	900	500	500	100	100	500	500	500	900	500	500	900	500	500	500	100	900	100	900	500
500	900	500	500	100	100	500	500	900	100	100	500	500	900	500	500	900	100	500	500	500	100	100	900
720	360																					

CTU NUMBER 21

100	500	500	500	900	500	500	500	500	100	100	100	900	100	100	100	500	900	500	500	500	900	100	100
500	500	500	500	100	100	100	500	500	900	100	500	100	900	500	900	500	100	500	100	100	900	500	500
500	500	500	900	100	100	500	500	100	100	500	100	500	500	100	500	900	500	500	100	500	900	100	500
100	100	500	500	900	500	500	100	100	500	500	500	100	100	500	900	500	500	100	500	900	900	500	500
900	900	500	500	100	100	500	500	900	100	100	500	500	900	500	500	900	100	100	500	500	500	500	500
360	540																					

CTU NUMBER 22

900	500	500	500	100	500	100	500	100	900	500	500	100	100	500	100	500	500	500	500	500	900	100	900
500	500	500	500	100	900	100	500	500	100	100	500	100	500	500	900	500	100	500	100	100	500	900	500
500	100	500	900	100	500	900	100	900	500	500	500	100	500	900	500	500	100	900	900	100	500	100	500
100	500	500	100	900	500	900	100	500	500	100	500	900	500	500	900	500	500	100	500	900	900	100	500
100	900	500	500	100	500	100	500	900	100	500	500	100	900	500	500	900	100	100	500	500	500	500	100
360	540																					

CTU NUMBER 23

900	500	500	500	900	500	500	500	500	100	100	100	900	100	100	100	500	500	500	500	500	900	100	900
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100	500	100	500	900	500	500	100	100	500	500	500	100	100	500	900	500	500	100	500	900	100	900	500
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360	720																					

CTU NUMBER 24

100	500	500	500	500	500	500	500	500	100	100	100	900	100	100	100	500	500	500	500	500	900	100	100
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100	500	500	900	100	500	100	500	100	100	500	900	500	500	100	500	100	900	100	100	100	100	900	500
100	500	100	500	900	500	500	100	500	100	500	500	100	100	500	900	500	500	100	500	100	900	100	100
900	900	500	500	100	500	100	500	900	100	500	100	500	900	500	500	900	100	100	500	500	500	100	500
360	720																					

CTU NUMBER 25

100	500	500	500	100	500	100	100	500	100	500	100	900	100	500	100	100	500	100	100	500	900	100	100
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500	500	500	900	100	500	100	500	100	500	100	900	500	100	500	500	500	900	100	500	100	100	900	500
100	500	100	500	900	500	500	100	500	100	500	500	100	100	500	100	500	500	100	500	500	100	900	500
100	100	100	500	900	500	500	500	100	900	500	500	500	100	100	500	100	100	100	500	500	500	100	900
360	540																					

CTU NUMBER 26

100	500	100	100	500	500	500	500	500	100	500	100	900	100	500	100	100	500	100	100	500	900	100	100
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100	100	500	500	900	500	500	100	500	100	500	500	900	500	500	900	500	500	100	500	500	100	900	500
900	900	500	500	100	100	500	500	900	100	500	100	500	100	100	500	900	100	100	500	500	500	100	500
360	540																					

CTU NUMBER 27

500	500	500	500	100	100	500	100	500	100	500	100	900	100	500	100	500	500	500	500	500	900	100	100
500	500	100	500	500	900	500	500	100	100	100	500	100	500	100	500	900	100	500	100	100	900	500	500
500	500	500	900	100	500	100	500	100	100	500	900	500	500	100	500	500	900	100	500	100	100	900	500
100	500	100	500	900	500	500	100	500	100	500	500	900	500	500	900	500	500	100	500	500	500	900	500
100	900	500	500	100	100	500	500	900	100	500	500	100	900	500	500	900	100	100	500	500	500	500	100
360	540																					

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OTU NUMBER   28
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  360  540
OTU NUMBER   29
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  900  500  500  500  900  500  500  100  100  500  500  500  100  100  500  100  500  500  100  500  500  100  900  500  500
  900  100  100  500  900  500  500  500  100  900  500  500  500  100  100  500  100  100  100  500  500  500  500  500  200
  540  540
OTU NUMBER   30
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  360  540
OTU NUMBER   31
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  100  100  500  500  900  500  500  100  500  100  500  500  900  500  500  100  500  100  500  500  500  500  100  500  500
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  280  360
OTU NUMBER   32
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  540  540
OTU NUMBER   33
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  500  500  500  900  100  500  100  500  100  100  100  900  500  500  100  500  500  500  100  100  100  100  900  500  500
  100  500  100  500  900  500  500  100  500  100  500  500  100  100  500  900  500  500  100  500  500  100  900  500  500
  900  900  500  500  100  100  500  500  900  100  500  100  500  100  100  500  100  100  100  500  500  500  100  500  300
  720  540
OTU NUMBER   34
  100  100  500  100  100  100  500  500  100  100  500  100  900  100  500  100  900  500  500  500  500  100  100  100  633
  500  500  500  100  500  900  500  500  100  100  100  500  100  500  500  500  900  100  500  100  900  366  500  100  900
  500  500  500  900  100  500  100  500  100  100  500  900  500  500  100  500  500  900  900  500  100  100  500  100  100
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  100  100  500  100  900  500  500  500  100  900  500  500  500  100  100  500  100  100  100  500  500  500  100  500  80
  540  720
OTU NUMBER   35
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  900  500  500  500  900  500  500  100  500  100  500  500  100  100  500  900  500  500  100  500  500  500  900  500  500
  900  100  500  100  900  500  500  500  900  100  500  100  500  100  100  500  900  100  100  500  500  500  100  500  200
  540  720
OTU NUMBER   36
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  100  900  500  500  100  500  500  100  900  100  500  500  100  900  500  500  900  100  100  500  500  500  100  900  200
  360  720
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CTU NUMBER   37
   900  500  500  500  100  100  500  100  500  100  500  100  900  100  100  100  500  500  500  500  500  900  100  100  633
   500  500  500  100  500  100  100  500  500  100  100  500  100  500  500  900  500  100  100  100  100  100  500  900  900
   500  500  500  900  100  500  100  500  100  500  100  900  500  500  100  500  900  500  100  100  100  100  100  900  100
   900  500  500  500  900  500  500  100  500  100  500  500  100  500  100  500  900  500  500  100  500  900  100  500  100
   100  100  500  100  900  500  500  500  900  100  500  100  500  100  500  100  900  100  500  500  100  500  100  900  100
   360  720
CTU NUMBER   38
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   500  500  100  500  500  100  100  500  500  100  100  500  100  500  500  900  500  100  100  100  100  366  500  100  900
   500  500  500  900  100  500  100  500  100  500  100  900  500  500  100  500  900  500  100  100  100  100  900  100  100
   900  500  500  500  900  500  500  100  500  100  500  500  100  500  100  900  500  500  100  500  900  500  100  500  100
   100  100  500  100  900  500  500  500  900  100  500  100  500  100  100  500  100  900  100  100  500  500  900  100  200
   360  540
CTU NUMBER   39
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   500  500  500  100  500  900  100  500  500  100  100  900  100  500  500  900  500  100  100  100  100  366  500  100  900
   500  500  500  900  100  500  100  500  100  500  100  900  500  500  100  500  900  500  100  100  100  100  900  100  100
   900  500  500  500  900  500  500  100  500  100  500  500  100  100  500  900  500  500  100  500  900  500  100  500  100
   100  100  500  100  900  500  500  500  900  100  500  100  500  100  100  500  900  100  100  500  500  500  100  500  200
   360  540
CTU NUMBER   40
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   500  500  100  500  500  900  100  500  500  100  100  500  100  500  500  900  500  100  100  100  100  366  500  100  900
   500  500  500  900  100  100  500  500  100  500  100  900  500  500  100  500  900  500  100  100  100  100  500  100  100
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   100  100  500  100  900  500  500  500  900  100  100  500  500  100  100  500  900  100  100  500  500  500  100  500  200
   360  540
CTU NUMBER   41
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   500  500  500  900  100  500  100  500  100  500  100  900  500  500  100  500  900  500  100  100  100  100  500  100  100
   900  500  500  500  900  500  500  100  500  100  500  500  100  500  100  900  500  500  100  500  500  500  100  500  100
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   360  540
CTU NUMBER   47
   900  500  500  500  500  500  500  500  500  100  100  500  900  100  100  100  500  500  500  500  500  900  100  100  633
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   500  500  100  900  100  500  100  500  900  500  500  500  900  500  500  100  500  900  500  500  100  500  100  100  100
   900  500  500  500  900  500  500  100  500  100  500  500  100  100  500  900  500  500  100  500  900  100  100  500  100
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   360  540
CTU NUMBER   43
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   500  500  500  100  500  900  500  500  100  900  100  500  100  500  500  900  500  100  500  100  100  366  100  500  900
   500  500  500  900  100  500  100  500  100  500  900  500  500  100  500  900  500  500  100  500  100  100  100  100  500
   900  500  500  500  900  500  500  100  500  100  500  500  100  100  500  900  500  500  100  500  500  100  900  500  100
   900  100  100  500  900  500  500  500  900  100  500  100  500  900  500  500  900  100  100  500  500  500  100  500  200
   500  900
CTU NUMBER   44
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   500  500  500  500  100  900  900  100  500  100  900  500  100  500  500  900  500  100  500  100  900  366  500  100  900
   500  500  500  100  900  500  500  500  100  500  100  100  100  500  500  500  900  500  100  500  100  900  100  500  100
   900  500  500  500  900  500  500  100  100  500  500  500  100  500  100  900  500  500  100  500  900  500  100  500  100
   900  100  500  100  900  500  500  500  900  100  100  500  500  100  500  100  900  100  500  100  500  500  500  100  366
   540  540
CTU NUMBER   45
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   100  500  500  500  500  900  100  500  500  100  100  500  500  500  100  500  100  100  900  500  100  100  366  500  100
   100  500  500  100  900  500  500  500  100  500  100  100  100  500  500  500  900  500  100  100  100  633  100  500  100
   900  500  500  500  900  500  500  100  100  500  500  500  100  100  500  100  500  500  100  500  900  100  100  500  100
   500  100  500  100  900  500  500  500  900  100  100  500  500  100  100  500  100  100  500  100  500  900  500  100  366
   500  540
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OTU NUMBER 46

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500 500 500 900 100 500 100 500 100 500 100 900 500 500 100 500 500 900 100 100 100 633 900 500 500
100 500 100 500 900 500 500 100 500 100 500 500 900 500 500 900 500 500 500 100 500 100 900 500 500
100 900 500 500 100 500 100 500 900 100 500 500 100 100 100 500 900 900 500 500 500 500 500 500 900
100 100

OTU NUMBER 47

100 900 500 100 100 100 500 100 500 100 500 100 900 900 500 500 100 100 500 100 500 100 500 900 900
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100 100

OTU NUMBER 48

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500 100 100 500 900 500 500 500 100 900 500 500 500 900 500 500 900 900 500 500 500 500 500 500 540
100 100

OTU NUMBER 49

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900 900 500 500 100 100 500 500 900 100 100 500 500 900 500 500 900 100 500 500 500 100 500 500 540
280 280

OTU NUMBER 50

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900 500 500 100 900 500 500 500 100 900 500 500 500 100 100 500 900 900 500 500 500 500 500 500 900
100 100

TABLE 36 - DATA MATRIX B2 - LEAF ANATOMY CHARACTERS AND SUPRA-SPECIFIC TAXA

CTU NUMBER	1																						
528	362	551	500	583	518	100	404	900	100	100	100	339	500	584	404	900	404	900	142	500	635	900	473	404
900	536	900	417	900	900	651	900	500	737	417	460	900	473	501	417	900	536	900	404	900	584	500	900	500
696	100	900	900	633	473	900	100	900	462	900	456	900	400	500	784	500	900	404	404	900	473	900	100	100
500	640	900	900																					
CTU NUMBER	2																						
528	324	611	900	583	518	496	404	483	100	100	100	900	100	900	100	900	404	900	608	500	726	100	473	100
712	191	900	417	900	322	395	100	500	100	100	100	100	473	900	417	491	191	900	404	900	100	900	541	900
100	900	500	100	100	473	541	900	481	816	900	456	900	400	900	217	900	100	100	100	900	100	100	900	00
633	900	364	578																					
CTU NUMBER	3																						
528	100	302	500	900	100	100	100	100	900	100	100	339	900	100	404	660	404	500	677	500	900	900	473	100
750	445	900	100	900	900	395	500	500	575	417	460	575	473	501	100	711	445	900	404	900	100	500	460	00
491	100	100	900	416	473	541	100	677	100	487	100	100	400	500	784	500	500	404	404	900	473	500	100	100
900	754	772	703																					
CTU NUMBER	4																						
528	324	302	500	900	100	100	100	483	900	100	100	577	100	900	100	660	404	900	900	500	726	100	100	100
712	445	900	100	900	100	395	900	500	737	100	100	900	473	100	100	491	445	900	100	900	100	500	900	500
696	900	500	100	839	473	900	900	481	100	900	900	456	900	500	900	500	900	100	100	900	473	900	900	00
788	693	364	578																					
CTU NUMBER	5																						
100	585	611	900	768	900	900	404	900	900	100	100	900	100	900	100	900	900	500	900	500	900	496	473	100
712	100	785	100	557	100	395	100	500	737	100	100	900	473	900	100	491	100	785	100	597	100	100	100	00
900	900	500	100	622	100	541	900	481	100	100	456	456	100	100	100	100	500	100	100	500	473	500	900	100
488	693	511	415																					
CTU NUMBER	6																						
100	900	900	900	550	518	505	100	100	900	100	100	577	100	900	100	900	100	500	100	100	460	504	100	404
863	100	785	100	900	287	645	900	500	737	900	900	858	100	100	900	556	100	785	100	900	900	100	900	100
900	900	500	100	478	100	900	100	481	455	487	900	456	100	100	900	100	500	100	100	100	900	900	100	100
482	578	536	415																					
CTU NUMBER	7																						
528	585	900	500	233	483	505	100	483	900	900	900	100	500	584	404	100	404	500	900	500	100	900	473	404
490	900	900	100	900	322	900	500	100	900	100	900	900	100	100	100	224	900	900	100	900	318	500	900	500
491	900	500	100	416	473	541	900	100	546	487	100	100	400	500	100	500	100	404	404	500	473	500	100	500
225	100	100	100																					
CTU NUMBER	8																						
900	900	100	100	768	900	900	100	100	900	900	900	900	100	900	100	403	100	100	831	900	726	100	900	100
712	100	785	100	900	100	100	500	900	737	100	100	900	900	500	100	100	100	785	100	597	100	900	541	900
696	900	500	100	900	900	100	900	100	184	100	100	100	100	900	217	900	500	100	100	100	100	100	900	100
100	322	390	130																					
CTU NUMBER	9																						
900	900	699	100	100	900	900	100	100	900	900	900	339	100	900	900	403	100	100	831	900	100	496	100	900
712	809	785	100	900	287	645	900	100	900	100	100	900	100	101	100	587	809	785	100	597	100	100	900	100
900	900	500	100	622	100	900	900	100	900	100	100	100	100	100	900	100	500	900	900	100	900	500	900	900
225	254	100	100																					
CTU NUMBER	10																						
900	324	100	100	100	900	900	900	518	900	100	100	339	100	900	100	403	100	100	390	100	726	100	900	100
100	809	100	900	100	100	100	500	900	737	100	100	533	900	501	100	100	809	100	900	100	100	900	541	900
696	900	500	100	478	900	100	900	100	184	100	100	100	100	900	217	900	500	100	100	100	100	100	900	100
218	322	390	381																					

Table 37: Percentage of roots of correlation matrix 74, accounted for by leaf anatomy characters. (Table 4)

Characters	Root Number 1	2	3	4	5	6
I General Characters						
1 Curvature	1.05	0.14	3.12	0.40	0.78	2.36
2 Width of leaf	3.42	0.13	0.55	1.79	1.80	0.98
3 Thickness-midrib	0.53	3.47	0.00	1.36	1.02	0.01
4 Thickness	1.12	0.01	1.82	0.49	0.32	0.01
II Adaxial Surface						
5 Presence of ribs	0.13	1.46	6.19	3.81	0.00	0.37
6 Height of ribs	1.27	0.39	0.64	0.04	2.50	0.93
7 Shape of ribs	0.22	0.06	0.91	0.01	0.16	0.15
III Bulliform Cells						
8 Arrangement	0.09	0.76	3.55	0.28	0.53	0.38
9 Shape of group	0.52	0.04	1.16	0.48	0.75	2.30
10 Size of cells	1.25	0.36	0.93	0.34	2.17	1.86
11 Colourless cells	3.51	0.69	0.42	6.08	0.29	0.08
12 A'ment of " "	1.74	0.79	1.59	4.40	0.26	0.19
IV Abaxial Surface						
13 Presence of ribs	0.08	0.00	2.43	0.26	0.21	0.04
14 Height of ribs	0.69	0.03	0.30	0.23	0.16	3.90
15 Shape of ribs	0.62	0.00	0.55	0.44	0.17	1.90
16 Groups of large cells	0.00	2.39	0.34	1.66	0.86	0.06
V Margin of the Leaf						
17 Type	2.43	0.25	0.52	5.08	0.13	2.07
18 Shape	0.12	0.50	0.02	0.84	1.89	1.80
19 Associated fibres	2.19	0.00	0.01	0.25	2.50	0.08
20 Outline of fibre group	0.13	0.23	0.21	0.71	0.25	1.94
VI Mesophyll						
21 Distinctive cells	0.63	0.04	0.46	0.00	0.10	0.00
VII Midrib						
22 Keel	4.56	2.62	0.73	0.38	0.22	0.03
23 Shape of keel	0.61	0.53	0.01	0.19	2.04	0.67
24 Rib above keel	0.00	1.43	6.06	0.18	0.06	0.86
25 Vascular bundles	3.41	2.62	0.42	0.43	1.14	0.48

Table 37 : (Continued)

	1	2	3	4	5	6
VIII Central Vascular Bundles						
26 Shape	1.31	0.20	4.11	0.28	0.62	1.94
27 Mestome sheath	3.87	0.00	0.04	0.01	0.91	3.82
28 Fibrous tissue	2.99	0.60	0.12	3.38	2.34	1.27
29 Parenchymatous sheath	0.36	0.61	2.46	0.95	0.51	3.24
30 Position of interruption	0.78	0.83	2.35	0.45	0.07	6.75
31 Groups of phloem	1.98	0.96	0.67	3.64	0.08	0.61
Associated Sclerenchyma						
32 Adaxial strand	2.50	3.57	0.33	0.45	0.19	1.11
33 Shape of strand	0.29	1.53	0.08	2.94	0.15	1.97
34 Adaxial girder	0.00	4.79	0.02	0.18	0.10	0.01
35 Shape of " "	0.00	0.00	0.11	0.16	0.24	0.10
36 Abaxial strand	2.40	3.09	0.64	0.02	1.56	0.72
37 Abaxial girder	2.40	3.09	0.64	0.02	1.56	0.72
38 Shape of " "	1.49	0.09	0.11	0.29	2.29	1.24
Associated Parenchyma						
39 Adaxially	0.01	4.26	0.00	0.76	0.73	0.09
40 Arrangement adaxially	0.70	1.70	1.13	0.13	6.03	0.33
41 Abaxially	0.84	2.97	1.48	0.73	1.51	0.61
IX First Order Bundles						
42 Shape	1.65	0.15	3.28	0.38	3.91	0.94
43 Mestome sheath	3.59	0.01	0.03	0.01	1.23	2.79
44 Fibrous tissue	2.57	0.52	0.21	3.91	1.41	1.00
45 Parenchymatous sheath	0.39	0.22	3.63	1.32	0.00	2.16
46 Position of interruption	0.14	0.22	3.70	2.25	0.03	3.39
47 Groups of phloem	1.28	1.47	1.05	4.98	2.91	0.42
Associated Sclerenchyma						
48 Adaxial strand	1.07	2.77	0.13	0.00	1.22	5.17
49 Shape of adaxial strand	0.13	1.28	0.51	0.13	0.22	2.00
50 Adaxial girder	2.13	4.19	0.35	0.02	0.36	2.34
51 Shape of " "	0.27	0.07	0.32	4.96	0.93	0.73
52 Abaxial strand	2.85	2.03	0.34	1.48	0.07	1.23
53 Shape of " "	1.32	0.40	0.00	0.29	2.29	1.60
54 Abaxial girder	2.85	2.02	0.34	1.48	0.07	1.23
55 Shape of " "	0.04	0.01	0.86	0.24	6.04	1.18
Associated Parenchyma						
56 Adaxially	1.61	3.87	0.01	0.49	1.51	1.09
57 Arrangement Adaxially	0.16	0.03	2.76	0.31	6.52	1.14
58 Abaxially	1.71	2.49	0.38	0.79	0.93	0.39

Table 37 (Continued)

	1	2	3	4	5	6
X Second Order Bundles						
59 Shape	1.53	0.67	0.08	6.61	0.75	0.21
60 Mestome sheath	2.84	0.21	0.04	1.40	1.45	3.79
61 Fibrous tissue	0.01	0.10	2.61	3.33	0.02	0.64
62 Parenchymatous sheath	1.71	0.10	6.22	1.02	1.88	0.45
63 Position of interruption	1.68	0.24	3.99	0.29	1.41	3.12
64 Extension of sheath	0.00	0.08	6.00	0.38	0.80	1.02
Associated Sclerenchyma						
65 Adaxial strand	0.64	3.52	0.79	0.03	1.67	0.19
66 Shape of " "	0.00	0.00	0.51	2.94	0.15	2.54
67 Adaxial girder	1.00	4.33	0.64	0.06	1.01	0.00
68 Shape of " "	0.01	0.90	1.13	1.88	0.49	1.18
69 Abaxial strand	0.06	2.85	1.48	1.92	1.01	3.23
70 Abaxial girder	0.06	2.85	1.48	1.92	1.01	3.23
71 Shape of " "	0.49	0.17	0.52	0.01	7.04	0.89
Associated Parenchyma						
72 Adaxially	0.56	4.96	0.26	0.01	1.50	0.52
73 Arrangement adaxially	0.08	0.53	0.65	0.64	4.50	0.05
74 Abaxially	0.69	3.69	1.47	0.05	1.51	0.48
XI Third Order Bundles						
75 Presence	3.44	1.40	0.42	4.53	0.03	0.04
76 Shape & sclerenchyma	0.00	0.18	0.00	0.15	0.96	0.00
XII Proportions of Bundles						
77 Secondary : Primary	5.44	1.79	0.18	1.63	1.11	0.79
78 Tertiary : Primary	0.03	0.17	5.95	4.81	0.43	0.06
79 Secondary:Tertiary	2.75	2.32	0.44	2.28	2.48	0.00

Table 38 : (Continued)

OTU No.	Name	Long Cells			
		Intercostal Zone		Costal Zone	
		Length	Ratio	Length	Rat
41.	L. tisserantii	110.0	9.16	135.0	15.8
42.	L. vanderystii	92.5	8.5	132.5	16.8
43.	L. annua	100.0	5.0	140.0	9.
44.	L. hordeiformis	115.0	6.05	160.0	13.
45.	L. flammida	75.0	5.0	115.0	10.
46.	L. phragmitoides	85.0	6.54	109.0	13.
47.	"	120.0	9.23	165.0	18.
48.	L. simulans	38.0	3.45	165.0	19.
49.	L. anomala	42.5	3.5	131.0	17.
50.	L. ramosa	75.0	5.0	135.0	18.
51.	L. togoensis	100.0	6.76	140.0	17.
52.	R. petiolata	60.0	6.67	90.0	11.

Short Cells							
Intercostal Zone		Costal Zone		Stomata		Proximal (of Micro-H	
Length	Ratio	Length	Ratio	Length	Ratio	Length	
3.0	0.25	26.0	2.48	29.0	1.81	20.0	
8.0	0.85	22.5	2.81	25.0	1.56	19.0	
7.0	0.35	30.0	1.88	40.0	1.67	30.0	
7.5	0.46	35.0	2.33	40.0	1.6	22.5	
7.5	0.5	25.0	1.99	31.0	1.55	20.0	
6.0	0.61	20.0	2.0	34.0	1.7	22.5	
6.0	0.48	29.0	2.64	34.0	2.13	26.5	
4.5	0.40	14.0	2.15	17.5	1.52	40.0	
7.5	0.75	12.0	1.50	20.5	1.17	25.0	
5.5	0.48	17.5	2.06	24.0	1.43	20.0	
5.0	0.36	25.0	2.38	30.0	1.58	21.0	
4.0	0.40	15.0	2.31	20.0	1.6	39.0	

	Proximal Cell of Micro-hairs		Prickle-hairs		Hooks	
o	Length	Ratio	Length	Ratio	Length	Ratio
1	20.0	4.0	-	-	-	-
6	19.0	2.92	-	-	-	-
7	30.0	3.0	90.0	2.83	21.0	1.27
	22.5	2.5	70.0	3.68	20.0	1.33
5	20.0	2.11	-	-	-	-
	22.5	4.23	45.0	3.10	26.0	2.89
3	26.5	2.65	-	-	-	-
2	40.0	8.0	60.0	4.0	17.5	2.92
7	25.0	3.83	26.0	2.17	-	-
3	20.0	2.5	44.0	3.38	-	-
8	21.0	3.5	40.0	2.87	11.0	1.22
	39.0	9.75	36.0	2.88	-	-

Table 39 : Density of short cells, stomata and appendages on the abaxial epidermis of Loudetia spp. and Rattraya. (Number per sq. mm.)

OTU No.	Name	Short Cells: Intercostal	Short Cells: Costal	Stomata	Micro-hairs	Prickle-hairs	Hooks
Section: Loudetia							
Subsection: Typicae							
1.	L.angolensis	123.75	78.75	126.25	47.5	56.25	113.75
2.	L.arundinacea	288.75	225.0	51.25	50.0	46.25	0.0
3.	"	208.75	110.0	62.5	87.5	60.0	67.5
4.	"	130.0	170.0	77.5	42.5	55.0	45.0
5.	"	191.25	62.5	105.0	51.25	31.25	12.5
6.	"	240.0	80.0	145.0	102.5	185.0	95.0
7.	"	177.5	230.0	425.0	12.5	72.5	197.5
8.	"	98.75	41.25	177.5	93.75	63.75	216.25
9.	L. thomasii	65.0	100.0	191.25	97.5	108.75	268.75
10.	L.camerunensis	311.25	250.0	117.5	81.25	8.75	0.0
11.	L.simplex subsp. simplex	56.25	393.75	126.25	81.0	17.5	66.25
12.	"	93.75	221.25	80.0	18.75	1.25	0.0
13.	"	62.5	105.0	96.25	36.25	10.0	16.25
14.	"	73.75	135.0	117.5	10.0	70.0	38.75
15.	"	137.5	282.5	103.75	58.75	3.75	23.75
16.	L.simplex subsp. stipoides	181.25	128.75	76.25	41.25	11.25	117.5
17.	"	206.25	152.5	121.25	18.75	17.5	308.75
18.	L. kagerensis	233.75	447.5	136.25	88.75	-	3.75
19.	"	107.5	352.5	90.0	62.5	-	2.5
20.	"	241.25	437.5	136.25	85.0	0.0	6.25
21.	L. perrieri	248.75	493.75	192.5	-	-	-
22.	L.madagascariensis	357.5	373.75	112.5	-	-	-
	Mean	174.32	221.42	130.34	-	-	-
Incertae sedes							
23.	L. jaegeriana	180.0	198.75	216.25	15.0	-	-
Subsection: Pungentes							
24.	L. lonata	183.75	297.5	97.5	15.0	-	113.75
25.	L. longipes	251.25	231.25	143.75	26.25	-	-
26.	L. demeusei	58.75	35.0	122.5	26.25	80.0	126.25
27.	L. crassipes	130.0	315.0	145.0	40.0	-	35.0
	Mean	155.94	219.69	147.19	-	-	-

OTU No.	Name	Short Cells: Intercostal	Short Cells: Costal	Stomata	Microhairs	Prickle-hairs	Hooks
	Subsection: Acuminatae						
28.	L. pennata	96.25	978.75	117.5	76.25	137.5	240.0
29.	L. flavida	422.5	893.75	116.25	7.5	23.75	2.5
30.	"	152.5	608.75	113.75	46.25	148.75	258.75
31.	"	372.5	270.0	125.0	0.0	-	-
32.	L. migiurtina	40.0	282.5	143.75	2.5	326.25	455.0
33.	L. acuminata	190.0	617.5	177.5	30.0	345.0	445.0
34.	L. cuanzensis	403.75	250.0	253.75	21.25	148.75	66.25
35.	L. filifolia subsp. filifolia	180.0	178.75	126.25	98.75	5.0	25.0
36.	L.filifolia subsp. humbertiana	240.0	418.75	118.75	55.0	-	32.5
	Mean	233.06	499.86	143.61	-	-	-
	Subsection: Densispicae						
37.	L. gossweileri	165.0	377.5	155.0	47.5	-	-
38.	L. coarctata	284.25	186.25	181.25	6.25	-	-
39.	L. densispica	142.5	272.5	165.0	15.0	-	-
40.	L. densispica	112.5	255.0	95.5	50.0	-	-
41.	L. tisserantii	52.5	300.0	102.5	22.5	-	-
42.	L. vanderystii	482.5	335.0	138.75	15.0	-	-
	Mean	207.38	287.71	140.0	-	-	-
	Subsection: Annuae						
43.	L. annua	142.5	160.0	103.75	48.75	-	15.0
44.	L. hordeiformis	15.0	122.5	75.0	57.5	20.0	182.5
	Mean	78.75	141.25	89.38	-	10.0	-
	Subsection: Flammidae						
45.	L. flammida	337.5	140.0	150.0	35.0	-	-
46.	L.phragmitoides	355.0	180.0	125.0	6.25	13.75	10.75
47.	L. "	275.0	131.25	95.0	35.0	-	-
	Mean	322.5	150.41	123.33	-	4.58	3.58
	Section: Pleioneura						
48.	L. simulans	225.0	282.5	352.5	97.5	422.5	60.0
49.	L. anomala	230.0	500.0	362.5	62.5	35.0	-
50.	L. ramosa	308.75	317.5	217.5	55.0	1.25	-
	Mean	221.25	366.67	310.83	-	-	20.0
	Section: Lophanthera						
51.	L. togoensis	177.5	267.5	130.0	27.5	1.25	-
	Rottboellia						
52.	R. petiolata	165.0	467.5	341.25	18.25	176.25	-

Appendix 3

DATA AND TABLES PERTAINING TO THE ANALYSES USING LEAF EPIDERMIS CHARACTERS

Table 40: Characters of the leaf epidermis; coded values in parentheses

Character	States
I. Long Cells	
Intercostal zone:	
1. Ratio length/width	Less than 4 (100); 4-6 (366); 6-8 (633); greater than 8 (900)
2. Length of long cells	Less than 51 μ (100); 51-75 μ (300) 76-100 μ (500); 101-125 μ (700) greater than 125 μ (900)
3. Nature of the walls	Thin (100); thick (500); very thick (900)
4.	Sinuous (100); not sinuous (900)
Costal zone:	
5. Ratio length/width	Less than 10 (100); 10-13 (300); 13-16 (500); 16-19 (700); greater than 19 (900)
6. Length of long cells	Less than 100 μ (100); 101-125 (300); 126-150 (500); 150-175 (700); greater than 175 μ (900)
7. Nature of the walls	Thin (100); thick (900);
8.	Sinuous (100); not sinuous (900)
II. Short Cells	
Intercostal zone:	
9. Distribution	Solitary (100); associated in pairs (900)
10. Frequency	
11. Ratio length/width	Less than 0.2 (100); 0.2-0.6 (366); 0.6-0.9 (633); greater than 0.9 (900)

Table 40 (continued)

12. Length of short cells	Less than 4.0 μ (100); 4.1-6.0 (366); 6.1-8.0 (633); greater than 8 μ (900)
13. a. Type of silica bodies	Tall and narrow (100); otherwise (500)
b.	Crescent-shaped (100); otherwise (500)
c.	Cross-shaped (100); otherwise (500)
d.	Intermediate between cross-and dumb-bell shaped (100); otherwise (500)
e.	Irregularly shaped (100); otherwise (500)
Costal zone:	
14. Distribution	Sometimes in pairs or rows of cells (100); solitary (900)
15. Frequency	
16. Ratio length/width	Less than 1.5 (100); 1.6-2.0 (366); 2.1-2.5 (633); greater than 2.5 (900)
17. Length of short cells	Less than 15 μ (100); 16-20 (366); 21-25 (633); greater than 25 μ (900)
18. a. Type of silica bodies	Tall and narrow (100); otherwise (500)
b.	Crescent-shaped (100); otherwise (500)
c.	Cross-shaped (100); otherwise (500)
d.	Nodular (100); otherwise (500)
e.	Intermediate between cross-and dumb-bell shaped (100); otherwise (500)
f.	Dumb-bell shaped with narrow middle portions (100); otherwise (500)
g.	Dumb-bell shaped with wide middle portions (100); otherwise (500)
h.	Irregularly shaped (100); otherwise (500)
19. Characteristic thick-walled epidermal cells in intercostal zone, which differ from long and short cells.	Yes (100); No (900)

12. Length of short cells	Less than 4.0 μ (100); 4.1-6.0 (366); 6.1-8.0 (633); greater than 8 μ (900)
13. a. Type of silica bodies	Tall and narrow (100); otherwise (500)
b.	Crescent-shaped (100); otherwise (500)
c.	Cross-shaped (100); otherwise (500)
d.	Intermediate between cross-and dumb-bell shaped (100); otherwise (500)
e.	Irregularly shaped (100); otherwise (500)
Costal zone:	
14. Distribution	Sometimes in pairs or rows of cells (100); solitary (900)
15. Frequency	
16. Ratio length/width	Less than 1.5 (100); 1.6-2.0 (366); 2.1-2.5 (633); greater than 2.5 (900)
17. Length of short cells	Less than 15 μ (100); 16-20 (366); 21-25 (633); greater than 25 μ (900)
18. a. Type of silica bodies	Tall and narrow (100); otherwise (500)
b.	Crescent-shaped (100); otherwise (500)
c.	Cross-shaped (100); otherwise (500)
d.	Nodular (100); otherwise (500)
e.	Intermediate between cross-and dumb-bell shaped (100); otherwise (500)
f.	Dumb-bell shaped with narrow middle portions (100); otherwise (500)
g.	Dumb-bell shaped with wide middle portions (100); otherwise (500)
h.	Irregularly shaped (100); otherwise (500)
19. Characteristic thick-walled epidermal cells in intercostal zone, which differ from long and short cells.	[illegible] (100); [illegible] (900)

III. Stomata

20.	a. Shape of subsidiary cells	Triangular (100); otherwise (500)
	b.	Intermediate between triangular and low dome shaped (100); otherwise (500)
	c.	Low dome shaped (100); otherwise (500)
21.	Ratio length/width	Less than 1.3 (100); 1.4-1.6 (366) 1.7-1.9 (633); greater than 1.9 (900)
22.	Length of subsidiary cells	Less than 20 μ (100); 21-25 (300); 26-30 (500); 31-35 (700); greater than 35 (900)
23.	Frequency	

IV. Macrohairs

24.	Occurrence	Present (100); Absent (900)
25.	a. Frequency	Rare (100); otherwise (500)
	b.	Frequent (100); otherwise (500)
	c.	Abundant (100); otherwise (500)
26.	a. Point of attachment	Raised (100); otherwise (500)
	b.	Superficial (100); otherwise (500)
27.	a. Type of base	Swollen (100); otherwise (500)
	b.	Uniform (100); otherwise (500)
28.	a. Epidermal cells at the base	Long cells (100); otherwise (500)
	b.	Short cells (100); otherwise (500)
	c.	Specialized small cells (100); otherwise (500)
	d.	Solitary round cells (100); otherwise (500)

Table 40 (continued)

V. Microhairs		
29.	Occurrence	Present (100); absent (900)
30. a.	Distribution	Intercostal-zone (100); otherwise (500)
b.		Costal zone (100); otherwise (500)
31. a.	Frequency	≤ 15 (100); otherwise (500)
b.		16 - 30 (100); otherwise (500)
c.		31 - 45 (100); otherwise (500)
d.		46 - 60 (100); otherwise (500)
e.		61 - 75 (100); otherwise (500)
f.		76 - 90 (100); otherwise (500)
g.		≥ 90 (100); otherwise (500)
32. a.	Proximal cell-ratio length/ width	< 3 (100); otherwise (500)
b.		3 - 4 (100); otherwise (500)
c.		4 - 5 (100); otherwise (500)
d.		5 - 6 (100); otherwise (500)
e.		> 6 (100); otherwise (500)
33. a.	Proximal cell length	< 20 μ (100); otherwise (500)
b.		21 - 25 (100); otherwise (500)
c.		26 - 30 (100); otherwise (500)
d.		31 - 35 (100); otherwise (500)
e.		> 35 (100); otherwise (500)
34. a.	Nature of wall of proximal cell	Thin (100); otherwise (500)
b.		Thick (100); otherwise (500)
35. a.	End of distal cell	Passing into a fine point (100); otherwise (500)

35.	b.	Evenly rounded (100); otherwise (500)
36.	a. Shape of distal cell	Cylindrical (100); otherwise (500)
	b.	Tapering (100); otherwise (500)
VI. Prickle-hairs		
37.	Occurrence	Present (100); absent (900)
38.	Distribution	Sometimes in intercostal zone (100): otherwise (500)
39.	a. Points	Always present (100); otherwise (500)
	b.	Sometimes absent (100); otherwise (500)
40.	a. Frequency	$\leq$25 (100); otherwise (500)
	b.	26 - 50 (100); otherwise (500)
	c.	51 - 75 (100); otherwise (500)
	d.	76 - 100 (100); otherwise (500)
	e.	101 - 125 (100); otherwise (500)
	f.	126 - 150 (100); otherwise (500)
	g.	$\geq$151 (100); otherwise (500)
41.	a. Ratio length/width	$<$3 (100); otherwise (500)
	b.	3 - 4 (100); otherwise (500)
	c.	4 - 5 (100); otherwise (500)
	d.	$>$5 (100); otherwise (500)
42.	a. Length of prickle hairs	$<$40 (100); otherwise (500)
	b.	41 - 60 (100); otherwise (500)
	c.	61 - 80 (100); otherwise (500)
	d.	$>$80 (100); otherwise (500)
43.	a. Pits in the walls	Present (100); otherwise (500)
	b.	Absent (100); otherwise (500)

Table 40 (continued)

44.	a. Adjacent cells	Long cells (100); otherwise (500)
	b.	Short cells (100); otherwise (500)
VII.	Hooks	
45.	Occurrence	Present (100); absent (900)
46.	a. Distribution	Intercostal zone (100); otherwise (500)
	b.	Costal zone (100); otherwise (500)
47.	a. Frequency	≤ 20 (100); otherwise (500)
	b.	21 - 60 (100); otherwise (500)
	c.	61 - 100 (100); otherwise (500)
	d.	101 - 140 (100); otherwise (500)
	e.	141 - 180 (100); otherwise (500)
	f.	181 - 220 (100); otherwise (500)
	g.	≥ 221 (100); otherwise (500)
48.	a. Points	Straight (100); otherwise (500)
	b.	Curved (100); otherwise (500)
49.	a. Ratio length/width	≤ 1.5 (100); otherwise (500)
	b.	1.6 - 2.0 (100); otherwise (500)
	c.	2.1 - 2.5 (100); otherwise (500)
	d.	2.6 - 3.0 (100); otherwise (500)
	e.	> 3.0 (100); otherwise (500)
50.	a. Length of hooks	≤ 15 U (100); otherwise (500)
	b.	16 - 20 (100); otherwise (500)
	c.	21 - 25 (100); otherwise (500)
	d.	26 - 30 (100); otherwise (500)
	e.	> 30 (100); otherwise (500)
51.	a. Pits in the walls	Present (100); otherwise (500)
	b.	Absent (100); otherwise (500)

Table 40 (continued)

52.	a. Adjacent cells	Long cells (100); otherwise (500)
	b.	Short cells (100); otherwise (500)

TABLE 41 - DATA MATRIX 100 - LEAF EPIDERMIS CHARACTERS

OTU NUMBER 1

633 700 500 100 700 500 500 100 100 366 366 500 500 500 500 500 900 633 366 500 500 500 100 500 100
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CTU NUMBER 13

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CTU NUMBER 16

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CTU NUMBER 17

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CTU NUMBER 18

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CTU NUMBER 19

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CTU NUMBER 20

366	500	500	100	100	100	500	100	100	366	366	500	500	500	500	100	100	366	366	100	500	100	500	100	100
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CTU NUMBER 21

100	300	900	100	100	100	900	100	100	366	633	500	500	500	500	500	900	100	366	500	500	100	500	100	100
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CTU NUMBER 22

366	500	900	100	100	100	900	100	100	366	633	500	500	500	500	500	900	366	366	500	500	100	500	100	100
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CTU NUMBER 23

366	500	500	100	300	300	500	100	100	366	633	500	500	500	500	500	900	366	366	100	500	100	500	100	100
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CTU NUMBER 24

900	700	100	100	700	700	100	100	100	366	366	100	500	500	500	500	900	633	366	500	500	500	100	100	100
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CTU NUMBER 25

366	500	900	100	500	500	500	100	100	633	900	100	500	500	500	500	900	366	366	500	500	500	100	500	100
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CTU NUMBER 26

366	500	900	100	300	500	500	100	100	366	633	100	500	500	500	500	900	366	366	500	500	100	500	100	500
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CTU NUMBER 27

633	500	500	100	300	100	500	100	100	366	100	100	500	500	500	500	900	366	900	500	500	500	100	500	100
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CTU NUMBER 28

633	500	500	100	500	300	500	100	100	366	366	500	500	100	500	100	900	633	366	500	500	500	500	100	100
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CTU NUMBER 29

633	500	500	100	900	700	500	100	100	366	633	500	500	500	500	100	900	633	366	500	500	500	500	500	100
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CTU NUMBER 30

633	500	500	100	900	500	500	100	100	633	633	500	500	500	500	100	100	900	633	500	500	500	500	100	100
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CTU NUMBER 31

500	700	500	100	500	700	500	100	100	366	366	500	500	500	500	500	900	633	366	500	500	500	500	500	100
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CTU NUMBER 32

100	300	500	100	100	100	500	100	100	633	633	500	500	500	500	100	900	633	633	500	500	500	500	100	100
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CTU NUMBER 33

633	300	100	100	500	300	500	100	100	633	633	500	500	500	500	100	100	366	633	500	500	500	500	500	100
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CTU NUMBER 34

633	500	500	100	500	300	500	100	900	366	366	500	500	500	500	100	100	900	633	500	500	500	500	500	100
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CTU NUMBER 35

366	500	500	100	500	300	500	100	100	366	633	500	500	500	500	100	900	900	366	500	500	100	500	100	100
100	500	900	100	100	500	366	500	900	500	500	500	500	500	500	500	500	500	500	500	100	100	100	500	500
500	500	500	500	100	500	500	500	500	100	500	500	500	100	500	500	100	500	100	500	500	100	100	500	100
100	100	500	500	500	500	500	500	100	500	500	500	500	100	500	500	500	500	100	100	100	100	100	500	500
500	500	500	500	500	100	500	500	500	500	100	500	500	500	100	500	500	500	100	100	100				

CTU NUMBER 36

366	500	500	100	300	300	500	100	100	366	366	500	500	100	500	100	900	366	366	500	500	100	500	100	100
100	500	900	500	100	500	633	500	900	500	500	500	500	500	500	100	500	100	500	100	500	500	100	500	500
500	100	500	500	500	500	500	500	500	100	500	500	500	500	100	500	500	500	500	500	500	500	100	500	500
500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	100	100	500	500
500	500	500	500	500	100	500	500	100	500	500	500	500	100	500	500	500	500	100	100	100				

CTU NUMBER 37
500 500 500 100 700 300 500 100 100 366 100 500 500 500 500 500 900 633 366 500 500 500 100 500 100
100 100 900 500 500 100 633 100 100 100 500 500 500 100 500 100 100 500 500 500 100 100 500 500 500
500 100 500 500 500 500 500 100 500 500 500 500 100 500 500 500 100 500 100 500 500 100 900 500 500
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CTU NUMBER 38
633 500 500 100 700 300 500 100 100 633 633 500 500 500 500 500 100 633 366 500 100 500 100 500 100
100 100 900 500 100 500 366 300 100 500 100 500 500 100 100 500 500 500 100 500 100 100 500 100 500
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500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500

CTU NUMBER 39
900 500 500 900 500 500 500 900 100 900 900 500 500 500 500 500 100 633 633 500 100 500 500 500 100
100 500 900 500 100 100 633 500 900 500 500 500 500 500 500 500 500 500 500 500 100 100 500 100 500
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CTU NUMBER 40
900 900 500 900 500 500 500 900 100 900 900 500 500 500 500 500 100 900 900 500 500 900 100 100 100
100 500 900 500 100 100 366 500 100 100 500 500 500 100 500 100 100 500 500 500 100 100 500 500 500
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CTU NUMBER 41
900 700 500 100 500 500 500 100 100 100 100 500 500 500 500 500 100 633 900 500 500 900 100 500 100
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CTU NUMBER 42
900 500 900 100 700 500 500 100 100 633 633 500 500 500 500 500 900 900 633 500 500 500 500 500 100
500 500 900 500 100 500 366 300 900 500 500 500 500 500 500 500 500 500 500 500 100 100 500 100 500
500 500 500 500 500 100 500 500 500 500 100 500 500 500 500 500 100 500 0 0 0 0 900 500 500
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CTU NUMBER 43
366 500 500 100 100 500 500 100 100 366 633 100 100 500 500 100 900 366 900 100 500 100 500 500 100
100 100 900 100 100 500 366 900 900 500 500 500 500 500 500 500 500 500 500 500 100 100 100 500 500
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CTU NUMBER 44
633 700 500 100 500 700 500 100 100 366 633 100 500 500 500 100 100 633 900 500 500 100 500 500 100
100 100 900 500 100 100 366 900 100 100 500 500 500 100 100 500 500 500 100 500 100 100 100 500 500
500 100 500 500 500 100 500 500 500 500 500 100 500 500 500 100 500 100 500 100 100 100 100 500 500
100 100 500 500 500 500 500 500 500 100 500 500 500 500 100 500 500 500 500 100 100 100 100 500 500
500 500 500 100 500 500 100 100 500 500 500 500 500 100 500 500 500 500 100 100 100

CTU NUMBER 45
366 300 500 100 100 300 500 100 100 366 633 500 500 500 500 500 900 366 633 500 500 500 500 100 100
100 500 900 500 100 100 366 700 900 500 500 500 500 500 500 500 500 500 500 500 100 100 100 500 500
100 500 500 500 500 100 500 500 500 500 100 500 500 500 500 500 100 500 100 500 500 100 900 500 500
500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 900 500 500 500 500
500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500 500

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OTU NUMBER   46
   633  500  500  100  500  300  500  100  100  633  366  500  500  500  500  500  900  366  366  500  500  500  500  100  100
   100  500  900  500  100  100  633  700  100  500  500  100  100  500  100  500  500  500  100  500  100  100  500  100  500
   900  500  500  500  500  500  500  100  500  500  500  100  500  500  500  500  100  500  100  500  500  100  100  500  100
   900  100  500  500  500  500  500  500  500  100  500  500  500  100  500  500  100  500  500  100  100  100  500  500  500
   900  100  500  500  500  100  100  500  500  500  100  500  500  500  500  100  500  500  100  100  100
OTU NUMBER   47
   900  700  500  100  700  700  500  100  100  366  366  500  500  500  500  500  900  900  900  500  500  500  100  500  100
   100  500  900  500  100  100  900  700  100  100  500  500  500  100  500  100  100  100  500  500  100  100  500  500  500
   100  500  500  500  500  100  500  500  500  500  500  500  100  500  500  500  100  500  100  500  100  500  900  500  500
   900  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  900  500  500  500
   900  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500
OTU NUMBER   48
   100  100  900  100  900  700  900  100  100  366  366  100  500  100  100  100  100  633  100  500  500  100  500  100  100
   500  500  100  500  500  100  366  100  900  500  500  500  500  500  500  500  500  500  500  500  100  100  100  500  500
   500  500  500  500  100  500  500  500  500  100  500  500  500  500  100  500  100  500  100  500  500  100  100  500  100
   500  500  500  500  500  500  500  100  500  100  500  500  500  100  500  500  100  500  500  100  100  100  500  500  100
   500  500  500  500  500  100  500  500  500  500  100  500  500  100  500  500  500  500  100  100  100
OTU NUMBER   49
   100  100  900  100  700  500  900  100  100  633  633  100  500  100  500  100  100  100  100  500  500  100  100  100  100
   100  100  100  500  500  100  100  300  900  500  500  500  500  500  500  500  500  500  500  500  100  100  500  500  500
   500  500  100  500  500  500  100  500  500  500  500  100  500  500  500  500  100  500  100  500  100  500  100  500  500
   100  500  100  500  500  500  500  500  100  500  500  500  100  500  500  500  100  500  100  100  900  500  500  500   00
   500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500
OTU NUMBER   50
   366  300  500  100  700  500  500  100  100  366  366  100  500  100  100  100  100  633  366  500  500  500  100  100  100
   100  500  900  500  500  100  366  300  900  500  500  500  500  500  500  500  500  500  500  500  100  100  500  500  500
   500  100  500  500  500  100  500  500  500  500  100  500  500  500  500  500  100  500  100  500  100  500  100  500  100
   500  100  500  500  500  500  500  500  500  100  500  500  500  100  500  500  500  100  100  100  900  500  500  500  500
   500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500
OTU NUMBER   51
   633  500  500  100  700  500  500  100  100  366  366  100  100  500  500  100  900  633  633  500  500  500  500  500  100
   100  100  900  500  100  100  366  500  900  500  500  500  500  500  500  500  500  500  500  500  100  100  500  500   00
   500  500  500  500  500  500  100  500  500  500  500  100  500  500  500  100  500  500  100  500  500  100  100  500  500
   100  100  500  500  500  500  500  500  100  500  500  500  100  500  500  500  100  500  500  100  100  100  500  100  500
   500  500  500  500  500  100  100  100  500  500  500  500  100  500  500  500  500  500  100  100  100
OTU NUMBER   52
   633  300  500  100  300  100  500  100  100  366  100  100  500  500  500  100  900  633  100  500  500  500  500  100  500
   100  500  100  500  100  100  366  100  900  500  500  500  500  500  500  500  500  500  500  500  100  100  100  500  100
   500  500  500  500  500  500  500  500  500  100  500  500  500  500  100  500  100  500  100  500  500  100  100  500  100
   500  500  500  500  500  500  500  100  100  500  500  500  100  500  500  500  500  100  100  100  900  500  500  500  500
   500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500  500
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TABLE 42 - DATA MATRIX 122 - LEAF EPIDERMIS CHARACTERS AND SUPRA-SPECIFIC TAXA

CTU NUMBER	1																						
100	808	100	100	100	100	100	900	100	414	597	116	341	100	279	460	709	556	900	790	100	167	242	900	100
100	100	100	100	900	100	153	100	100	443	600	460	558	100	100	100	100	100	100	528	100	100	100	100	100
100	100																						
CTU NUMBER	2																						
473	794	100	900	500	460	500	900	900	354	597	116	723	900	275	487	692	729	900	790	500	830	231	900	657
483	500	733	900	100	805	473	761	900	100	900	460	558	900	900	768	807	487	100	528	100	730	100	509	476
528	100																						
CTU NUMBER	3																						
100	702	487	900	100	418	900	900	100	607	597	789	518	100	900	609	692	454	900	790	230	100	288	900	344
518	100	326	900	417	319	100	640	761	100	600	460	100	900	434	392	413	100	100	528	100	540	100	100	100
100	100																						
CTU NUMBER	4																						
877	900	514	100	500	757	900	100	900	523	100	100	900	100	427	371	900	888	900	900	580	255	275	900	344
518	100	535	900	100	712	407	100	761	100	900	900	900	900	759	900	900	900	900	900	900	900	900	900	900
900	900																						
CTU NUMBER	5																						
497	794	900	900	100	625	900	900	900	101	900	773	417	900	101	487	500	100	900	790	900	680	101	900	657
518	500	733	900	417	807	900	324	761	100	900	100	558	100	641	635	288	487	100	100	100	657	505	738	596
528	100																						
CTU NUMBER	6																						
473	702	900	900	100	726	900	900	900	900	597	789	900	900	121	900	900	873	900	900	230	900	218	900	413
100	100	326	900	417	848	670	142	761	100	600	460	558	100	641	768	807	487	460	528	497	824	496	761	758
528	100																						
CTU NUMBER	7																						
474	100	514	900	900	625	500	900	900	568	597	789	100	100	603	100	292	588	100	624	771	326	865	100	900
900	900	900	900	417	440	637	499	761	100	600	100	558	100	313	635	713	100	100	528	487	807	496	761	760
528	100																						
CTU NUMBER	8																						
900	654	900	900	900	799	900	900	900	425	900	900	417	900	382	249	500	417	900	900	900	764	241	100	900
900	900	900	900	100	900	704	482	100	100	900	100	558	900	641	768	807	487	460	100	487	795	100	738	758
528	100																						
CTU NUMBER	9																						
900	347	900	900	900	778	900	900	900	384	900	571	506	900	382	249	100	900	100	900	900	590	900	100	900
900	900	900	900	417	900	848	900	761	100	900	100	558	100	617	768	807	514	100	900	900	900	900	900	900
900	900																						
CTU NUMBER	10																						
847	654	900	900	900	900	900	900	900	433	900	773	900	900	229	779	483	282	900	100	900	764	538	100	900
900	900	900	900	417	892	704	482	761	900	100	900	900	900	759	900	900	900	900	900	900	900	900	900	900
900	900																						

Table 43 : Percentage of roots of correlation matrix 105, accounted for by leaf epidermis characters. (Table 40)

Characters	Root Number 1	2	3	4	5	6	7
I Long Cells							
Intercostal zone:							
1 Ratio length/width	7.24	0.05	3.85	0.80	0.03	0.06	1.66
2 Length of long cells	11.12	0.63	0.02	2.14	0.19	0.01	0.02
3) Nature of the walls	4.89	1.46	0.09	0.00	0.78	0.79	0.01
4)	0.51	1.00	0.36	0.04	15.64	0.75	0.00
Costal zone:							
5 Ratio length/width	0.74	0.58	8.11	2.70	0.93	2.29	0.52
6 Length of long cells	5.14	0.92	1.02	2.23	0.06	2.78	0.84
7) Nature of the walls	7.43	1.29	0.20	0.01	2.21	0.65	0.24
8)	1.31	2.13	1.79	0.47	11.42	1.13	0.05
II Short cells							
Intercostal zone:							
9 Distribution	0.02	1.17	0.09	0.12	0.11	3.70	4.36
10 Density	0.40	3.68	0.08	0.05	0.81	3.90	6.25
11 Ratio length/width	0.10	1.36	1.62	1.38	12.03	0.02	1.22
12 Length of short cells	0.13	0.66	1.07	1.57	13.50	0.02	0.77
13 Type of silica bodies	3.12	0.25	0.01	4.94	0.01	3.23	3.76
Costal zone:							
14 Distribution	0.09	0.00	1.53	6.00	3.41	1.59	0.49
15 Density	0.29	0.16	0.18	8.26	1.57	0.37	8.87
16 Ratio length/width	2.15	0.20	7.85	1.04	0.16	0.96	0.01
17 Length of short cells	5.98	0.13	0.11	0.17	1.45	3.74	0.16
18 Type of silica bodies	2.64	0.64	6.47	0.02	0.78	0.28	0.01
19 Cells different from long and short cells	4.78	0.08	1.00	3.78	0.32	2.12	7.10
III Stomata							
20 Shape of subsidiary cells	0.60	0.00	8.72	0.66	0.21	1.65	0.06
21 Ratio length/width	4.88	0.00	0.10	0.15	0.53	0.87	0.49
22 Length of subsidiary cells	2.98	0.02	7.27	0.82	3.30	0.05	0.16
23 Density	7.08	0.38	1.15	2.32	0.68	0.53	1.97
IV Macrohairs							
24 Occurrence	3.82	0.13	0.15	0.46	0.10	7.04	0.31
25 Frequency	0.05	0.13	1.62	0.14	0.61	0.00	1.36
26 Point of attachment	2.61	0.53	0.23	7.21	0.92	5.20	2.65
27 Type of base	1.91	0.38	0.00	8.65	2.89	7.55	1.51
28 Epidermal cells at base	5.69	0.11	0.93	6.23	2.22	0.93	3.29

Characters	Root Number						
	1	2	3	4	5	6	7
V Microhairs							
29 Occurrence	0.78	3.62	5.46	0.14	0.10	2.40	0.33
30 Distribution	0.00	6.08	0.76	0.45	0.11	1.13	0.36
31 Density	0.06	0.58	0.06	0.02	0.21	0.06	4.75
32 Proximal cell ratio l/w	1.41	0.00	1.27	0.35	1.35	0.49	7.63
33 Proximal cell length	0.04	0.32	0.00	0.57	6.93	2.32	3.36
34 Nature of wall of p cell	1.22	0.00	6.49	0.74	0.45	0.06	3.46
36 Shape of distal cell	0.01	0.96	3.47	0.42	0.00	7.80	0.02
35 End of distal cell	0.34	2.35	6.49	0.00	2.20	0.01	1.01
VI Prickle-hairs							
37 Occurrence	0.78	10.36	0.04	1.27	0.95	0.37	2.57
38 Distribution	0.96	6.69	0.14	1.39	1.33	0.78	0.48
39 Points	0.00	0.00	0.71	7.15	0.00	0.37	7.41
40 Density	2.13	0.27	5.68	1.55	0.52	8.18	0.13
41 Ratio length/width	0.21	0.01	1.29	3.01	2.08	3.20	0.55
42 Length of prickle hairs	0.01	0.38	0.00	0.75	0.08	0.02	1.55
43 Pits in the walls	0.73	0.00	0.80	0.86	2.07	0.96	0.12
44 Adjacent cells	1.03	7.94	0.15	5.15	1.78	0.04	0.46
VII Hooks							
45 Occurrence	0.10	11.46	1.41	0.36	0.01	1.28	1.77
46 Distribution	0.16	10.34	0.90	0.21	0.99	3.00	4.73
47 Density	0.89	0.32	3.07	0.89	0.13	2.04	0.00
48 Points	0.07	0.02	0.28	5.40	0.04	0.80	4.37
49 Ratio length/width	0.02	0.29	1.87	0.01	1.38	9.41	0.82
50 Length of hooks	1.01	0.13	2.97	5.61	0.19	0.08	4.67
51 Pits in the walls	0.18	8.37	0.26	0.94	0.12	0.53	0.15
52 Adjacent cells	0.18	11.52	0.78	0.37	0.00	2.44	1.20

Appendix 4

DATA AND TABLES PERTAINING TO THE ANALYSES USING AWN CHARACTERS

Table 4: Characters of the awn of the lemma of the upper floret; coded values in parentheses

Character	States
General Characters	
1. Length of the bristle (m.m.)	
2. Ratio of length of bristle to length of column (b/c)	
3. Column markedly twisted	Yes (100); No (900)
4. Point of disarticulation of the awn	Base of the column (100); top of the column (900)
5. Shape in cross-section	Slightly curved (100); U-shaped (500); terete (900)
6. Thickness of the column	Relatively thin (100); average (500); relatively thick(900)
7. Shape of the margins	Rounded (100); pointed (900)
8. Shape above the vascular bundle	Smoothly rounded (100); raised arc (900)
9. Adaxial epidermis passing around the margin	Yes (100); No (900)
Adaxial Epidermis	
10. Size of the cells	Small (100); medium (900)
11. Amount of thickening of the walls	Moderate (100); abundant (900)
Abaxial Epidermis	
12. Size of cells	Medium (100); Large (500); Very Large (900)
13. Amount of thickening of the walls	Moderate (100); Abundant (900)
14. Hairs or prickle-hairs	Present (100); Absent (900)

Table44 (continued)

Zones of the "Mesophyll"	
Upper Zone	
15. Size of the cells	Medium (100); Large (900)
16. Amount of thickening	Little (100); Moderate (500); Abundant (900)
17. Distribution of thickening	Mostly external (100); Evenly distributed (900)
18. Type of thickening	Straight (100); arched (900)
19. Number of layers of cells	One (100); 2 or more (900)
Middle Zone	
20. Size of cells	Small (100); Medium (500); Large (900)
21. Number of layers of cells	one (100); two (500); three (900)
22. Amount of thickening	None (100); Little (366); Moderate (633); Abundant (900)
Lower Zone	
23. Size of cells	Small (100); Medium (500); Large (900)
24. Amount of thickening	Little (100); Moderate (500); Abundant (900)
25. Distribution of thickening	Mostly external (100); Evenly distributed (900)
26. Number of layers of cells	one (100); 2 or more (900)
Vascular Bundle	
27. Position of vascular bundle	Within the middle zone of mesophyll (100); within the upper and lower zones of mesophyll (900)
28. Outer bundle sheath	Complete (100); Incomplete (900)
29. Inner bundle sheath	Present (100); Absent (900)
30. Protoxylem canal	Present (100); Absent (900)
31. Large metaxylem vessels	Present (100); Absent (900)

Table 45: Thickness of the column at the centre

(Measurements in microns)

OTU No.	Name	Thickness	Character 5 Thickness of the column
1.	L. angolensis	61.25	Relatively thin
2.	"	63.0	"
3.	L. arundinacea	84.0	"
4.	"	91.0	"
5.	"	80.5	"
6.	L. camerunensis	115.5	Average
7.	L. simplex	105.0	"
8.	"	101.5	"
9.	L.simplex subsp.stipoides	101.5	"
10.	"	101.5	"
11.	L. kagerensis	80.5	Relatively thin
12.	L. kagerensis	80.5	"
13.	L. perrieri	122.5	Average
14.	L. madagascariensis	94.5	Relatively thin
15.	"	94.5	"
16.	L. jaegeriana	73.5	"
17.	L. lanata	77.0	Relatively thin
18.	L. longipes	105.0	Average
19.	L. demeusei	84.0	Relatively thin
20.	L. crassipes	77.0	"
21.	L. pennata	66.5	Relatively thin
22.	"	70.0	"
23.	L. flavida	73.5	"
24.	"	80.5	"
25.	"	94.5	"
26.	L. migiurtina	63.0	"
27.	L. acuminata	80.5	Relatively thin
28.	"	66.5	"
29.	L. cuanzensis	56.0	"
30.	L.filifolia subsp.humbertiana	66.5	"
31.	"	59.5	"

OTU No.	Name	Thickness	Character 5 Thickness of the column
32.	L. gossweileri	77.0	Relatively thin
33.	"	108.5	Average
34.	L. echinulata	140.0	"
35.	L. coarctata	98.0	"
36.	L. densispica	91.0	Relatively thin
37.	L. tisserantii	105.0	Average
38.	L. vanderystii	98.0	"
39.	L. cerata	115.5	Average
40.	L. annua	112.0	"
41.	L. bidentata	115.5	"
42.	L. hordeiformis	84.0	Relatively thin
43.	L. phragmitoides	59.5	Relatively thin
44.	L. simulans	63.0	Relatively thin
45.	L. anomala	77.0	"
46.	L. anomala	66.5	"
47.	"	77.0	"
48.	L. ramosa	63.0	"
49.	L. togoensis	161.0	Relatively thick
50.	L. Rattraya petiolata	84.0	Relatively thin

TABLE 46 - DATA MATRIX 23 - ANSI CHARACTERS

CTU NUMBER 1
174 187 100 100 900 100 900 100 100 100 900 100 900 900 100 900 100 900 100 900 100 900 900 900 100
100 900 900 100 900 900

CTU NUMBER 2
174 187 100 100 500 100 900 100 100 100 100 900 100 900 100 100 100 900 100 900 100 366 900 900 900
100 900 100 100 100 900

CTU NUMBER 3
214 139 100 100 900 100 900 100 900 100 100 900 100 100 900 100 100 900 100 100 100 100 900 900 900
100 900 900 100 100 100

CTU NUMBER 4
212 122 100 100 900 100 900 100 900 100 900 900 100 100 900 500 100 900 100 100 100 100 900 900 900
100 900 900 100 100 100

CTU NUMBER 5
236 118 100 100 900 100 900 100 100 100 900 900 900 100 900 900 100 900 100 100 100 100 900 900 900
100 900 900 100 100 100

CTU NUMBER 6
253 187 100 100 500 500 100 100 100 100 900 500 100 900 900 500 100 900 100 900 500 366 900 500 900
100 100 900 100 100 100

CTU NUMBER 7
360 201 100 100 500 500 100 100 100 900 100 500 900 900 900 500 100 900 100 900 100 366 900 500 900
100 900 900 100 100 100

CTU NUMBER 8
354 190 100 100 500 500 100 100 900 900 100 500 900 100 900 500 100 900 100 900 500 366 900 900 900
100 900 100 100 100 100

CTU NUMBER 9
410 307 100 100 900 500 100 100 100 100 900 500 100 900 900 500 100 900 100 900 100 366 900 900 900
100 900 100 100 100 900

CTU NUMBER 10
410 307 100 100 500 500 900 100 900 100 900 500 900 100 900 900 100 900 100 900 100 900 900 900 900
100 900 100 100 100 900

CTU NUMBER 11
243 179 100 100 900 100 100 100 100 100 100 500 100 900 900 100 100 900 100 900 100 366 900 100 900
100 900 100 100 100 100

CTU NUMBER 12
235 217 100 100 500 100 100 100 100 100 900 500 900 900 100 500 100 900 100 100 100 100 900 900 900
100 900 100 100 100 100

CTU NUMBER 13
221 132 100 100 500 500 100 900 900 100 100 900 100 900 100 100 100 900 100 900 100 366 900 100 900
100 900 100 100 100 100

CTU NUMBER 14
232 162 100 100 500 100 100 900 900 100 900 900 100 900 100 100 100 900 100 900 100 366 100 500 900
100 900 900 100 100 100

CTU NUMBER 15
230 179 100 100 500 100 900 900 100 100 900 900 100 900 900 500 100 900 100 100 100 366 100 500 900
100 900 100 100 100 100

CTU NUMBER 16
142 194 100 0 100 100 100 100 900 100 100 900 100 100 100 100 900 900 100 900 100 100 900 100 900
100 900 100 900 900 900

CTU NUMBER 17
377 412 100 100 900 100 900 100 100 100 900 900 100 900 100 500 100 900 100 900 100 100 100 500 900
100 900 100 100 100 100

CTU NUMBER 18
434 367 100 100 900 500 100 100 100 900 100 500 100 900 900 500 100 900 100 900 100 366 900 500 900
100 900 100 100 100 900

CTU NUMBER 19
371 211 100 100 500 100 100 100 900 100 900 900 900 900 900 900 100 900 100 100 100 100 100 900 100
100 900 100 100 100 100

CTU NUMBER 20
273 218 100 100 500 100 100 100 100 100 900 900 900 900 100 500 100 900 100 100 100 100 100 900 100
100 900 900 100 100 900

```
CTU NUMBER   21
   198  182  100  100  500  100  900  100  900  100  500  900  100  900  100  500  100  900  100  900  100  100  500  900  100
   100  900  100  500  100  900
CTU NUMBER   22
   198  182  100  100  500  100  100  100  100  100  900  900  100  900  100  100  100  900  100  100  100  100  500  500  900
   100  900  100  100  100  900
CTU NUMBER   23
   167  100  100  100  500  100  100  100  900  100  100  900  100  900  100  500  100  900  100  100  100  100  500  500  900
   100  900  900  100  100  900
CTU NUMBER   24
   172  119  100  100  500  100    0  100    0  900  900  500  900  900  900  900  100  900  100  900  500  633  900  900  100
   100  900  100  100  100  900
CTU NUMBER   25
   167  100  100  100  500  100  100  100  100  100  100  500  100  900  100  500  900  900  100  900  500  366  500  500  900
   100  100  100  100  100  900
CTU NUMBER   26
   197  133  100  100  500  100  100  100  100  100  500  100  100  900  100  500  100  900  100  900  500  100  500  500  900
   100  900  100  100  900  900
CTU NUMBER   27
   186  116  100  100  900  100  900  100  900  900  100  500  100  900  900  100  100  900  100  900  500  366  900  500  900
   100  900  100  100  100  900
CTU NUMBER   28
   186  116  100  100  500  100  100  100  100  100  900  900  900  900  100  900  100  900  100  900  100  900  500  900  100
   100  900  900  100  100  900
CTU NUMBER   29
   133  113  100  100  500  100  100  100  900  900  100  500  100  900  100  100  100  900  100  900  500  100  900  500  900
   100  900  900  100  900  900
CTU NUMBER   30
   164  130  100  100  500  100  900  100  900  900  900  900  100  900  100  500  100  900  100  100  100  100  500  500  900
   100  900  100  100  100  900
CTU NUMBER   31
   164  130  100  100  900  100  900  100  900  900  100  900  100  100  100  500  100  900  100  100  100  100  500  500  900
   100  900  100  100  900  900
CTU NUMBER   32
   403  334  100  100  900  100  100  100  100  100  900  500  100  100  100  500  100  900  100  900  100  366  500  500  900
   100  900  100  100  100  900
CTU NUMBER   33
   403  334  100  100  500  500  900  100  100  100  100  500  100  100  900  500  100  900  100  900  500  366  500  500  900
   100  900  100  100  100  100
CTU NUMBER   34
   482  198  100  100  500  500  900  100  100  100  900  900  100  900  100  500  100  900  100  900  500  100  500  100  900
   100  900  100  100  100  100
CTU NUMBER   35
   247  194  100  100  500  500  900  100  100  100  900  900  100  900  100  500  100  900  100  900  500  100  500  100  900
   100  900  900  100  100  100
CTU NUMBER   36
   420  313  100  100  500  100  900  100  100  100  900  900  900  900  900  500  100  900  100  100  100  366  900  500  100
   100  900  100  900  100  100
CTU NUMBER   37
   334  174  100  100  900  500  900  100  900  100  100  900  100  900  900  100  100  900  100  900  500  366  500  100  900
   100  900  100  100  900  900
CTU NUMBER   38
   129  160  100  100  500  500  900  100  100  900  100  500  100  100  100  100  100  900  100  900  500  366  500  100  900
   100  100  100  100  900  900
CTU NUMBER   39
   339  173  100  100  900  500  100  900  100  900  100  500  100  100  100  500  100  900  100  900  100  366  500  500  900
   100  100  100  100  900  900
CTU NUMBER   40
   658  195  100  100  900  500  100  100  100  100  900  900  100  900  900  500  100  900  100  100  100  366  500  500  900
   100  900  100  100  100  100
```

CTU NUMBER 41
763 266 100 100 900 500 100 100 900 100 100 900 100 900 900 500 900 900 100 100 100 366 500 500 900
100 900 100 100 100 100

CTU NUMBER 42
615 246 100 100 900 100 100 100 900 100 900 900 900 900 900 900 100 900 100 100 100 633 900 900 900
100 900 100 100 100 100

CTU NUMBER 43
118 448 900 900 500 100 100 100 900 100 900 500 900 900 100 900 100 900 100 100 100 900 500 900 100
100 900 900 900 900 900

CTU NUMBER 44
103 172 100 100 500 100 900 100 100 900 900 500 900 100 100 900 100 100 100 900 100 900 500 900 100
900 900 100 100 900 900

CTU NUMBER 45
111 262 100 100 100 100 100 100 900 900 900 500 900 100 100 900 100 100 100 900 100 366 500 900 900
900 900 900 100 900 900

CTU NUMBER 46
111 262 100 100 100 100 100 900 900 900 900 500 900 100 100 900 100 100 100 900 500 633 500 900 100
900 900 900 100 900 900

CTU NUMBER 47
111 262 100 100 100 100 100 900 100 900 900 500 900 100 100 900 100 100 100 900 500 100 500 900 900
900 900 100 900 900 900

CTU NUMBER 48
100 140 100 100 500 100 100 900 900 900 900 500 900 100 100 500 100 100 100 900 100 366 500 500 900
100 900 100 100 900 900

CTU NUMBER 49
900 284 100 100 900 900 900 100 900 900 900 100 900 100 100 900 100 100 900 900 900 633 500 900 900
900 900 900 100 100 100

CTU NUMBER 50
133 243 100 100 100 100 100 100 900 900 900 500 900 100 100 900 100 100 100 900 500 366 900 900 900
900 900 900 100 900 900

Table 47: Percentage of roots of correlation matrix 28, accounted for by awn characters (Table 44).

Character Number		Root Number				
		1	2	3	4	5
1. Size						
(a)Size of the awn	1	1.26	1.52	19.92	0.18	1.64
	6	0.60	1.01	15.57	5.72	0.37
	19	1.02	0.35	14.16	0.64	4.63
	21	0.52	4.80	4.60	1.92	0.24
	26	10.31	2.25	1.17	1.06	3.22
	30	5.16	3.42	4.34	3.59	0.51
	31	2.64	1.68	6.87	2.58	3.20
Total		21.51	15.03	66.63	15.69	13.81
(b) Size of the cells	10	4.62	7.65	0.30	0.20	0.16
	12	3.62	1.93	2.65	4.25	6.81
	14	3.45	5.32	0.49	0.0	3.11
	15	2.17	1.81	4.91	0.21	1.59
	20	1.64	7.15	0.22	2.56	6.63
	23	0.78	0.54	0.04	0.61	2.26
Total		16.28	24.40	8.61	7.83	20.56
Total for size		37.79	39.43	75.24	23.52	34.37
2. Thickening of cell walls						
(a) Amount of thickening	11	3.07	5.35	0.16	7.79	0.04
	13	9.44	2.91	0.74	2.55	0.66
	16	8.94	3.54	2.12	0.94	0.71
	22	4.59	2.26	1.80	3.15	8.87
	24	8.86	3.57	0.80	2.23	1.85
Total		34.90	17.63	5.62	16.66	12.13

Character Number		Root Number				
		1	2	3	4	5
2.(b) Distribution of thickening	17	0.66	0.50	0.58	2.86	0.80
	25	3.89	7.03	0.72	0.29	5.62
	Total	4.55	7.53	1.30	3.15	6.42
(c) Type of thickening	18	10.69	3.08	0.56	1.35	3.43
3. Shape of the awn	2	0.71	5.64	2.53	5.63	2.87
	3	1.35	8.89	1.70	18.32	2.01
	4	1.41	9.33	1.20	17.03	1.36
	5	4.00	0.78	5.47	2.19	2.11
	29	0.71	1.69	3.89	3.95	7.90
	Total	8.18	26.33	14.79	47.12	16.25
4. Miscellaneous characters	7	0.51	0.06	1.18	0.17	1.61
	8	0.67	1.85	1.11	0.67	1.76
	9	0.43	0.10	0.15	0.01	17.64
	27	0.19	3.64	0.03	6.98	5.94
	28	2.09	0.36	0.01	0.36	0.42
	Total	3.89	6.01	2.48	8.19	27.37

Table 48: Percentage of roots of dispersion matrix, accounted for by awn characters (Table 44)

Character Number	Root Number				
	1	2	3	4	5
1(a) Size of the awn					
1	0.16	0.16	0.49	0.18	0.03
6	0.09	0.08	1.16	1.03	0.01
19	0.01	0.00	0.09	0.01	0.01
21	0.12	0.22	0.50	0.58	0.27
26	4.06	0.00	0.88	0.01	0.28
30	11.49	6.92	1.55	2.94	0.34
31	8.97	8.92	18.77	0.42	o.02
Total	24.90	16.30	23.04	5.17	0.96
(b) Size of the cells					
10	11.79	6.64	5.71	2.13	1.56
12	0.91	0.11	0.35	2.48	0.02
14	9.08	3.82	18.74	0.13	3.24
15	7.14	4.79	18.78	5.56	8.96
20	4.96	8.81	0.29	25.95	0.26
23	0.03	0.00	0.03	0.02	0.08
Total	33.91	24.17	43.90	36.27	14.12
Total for size	58.81	40.47	66.94	41.44	15.08
2. Thickening of cell walls					
(a) Amount of thickening					
11	3.24	21.70	2.54	0.04	11.16
13	15.34	18.02	1.08	0.55	1.81
16	3.34	4.21	0.05	0.49	0.01
22	0.99	0.73	0.00	1.72	0.18
24	2.72	3.15	0.02	0.09	0.59
Total	25.63	47.81	3.69	2.89	13.75

Character Number		Root Number				
		1	2	3	4	5
2(b) Distribution of thickening	17	0.04	0.17	0.05	0.04	0.35
	25	1.91	5.83	2.98	0.02	0.01
	Total	1.95	6.00	3.03	0.06	0.36
2(c) Type of thickening	18	5.84	0.02	1.08	0.00	0.19
3. Shape of the awn	2	0.00	0.01	0.00	0.00	0.00
	3	0.01	0.02	0.02	0.04	0.01
	4	0.01	0.02	0.02	0.03	0.01
	5	1.24	0.00	0.26	0.57	0.00
	29	0.11	0.01	0.53	0.91	0.05
	Total	1.37	0.06	0.83	1.55	0.07
4. Miscellaneous characters	7	1.41	0.05	10.38	0.78	59.53
	8	0.56	0.45	0.02	0.07	0.21
	9	1.36	0.93	13.28	47.86	10.67
	27	0.00	0.63	0.10	0.85	0.00
	28	3.07	3.57	0.64	4.49	0.13
	Total	6.40	5.63	24.42	54.05	70.54

TABLE 49 - DATA MATRIX 45 - AWN CHARACTERS AND SUPRA-SPECIFIC TAXA

CTU NUMBER	1																							
263	188	100	100	900	900	404	900	100	473	404	456	404	460	900	582	900	900	100	404	743	882	900	470	404
100	100	473	900	460	400																			
CTU NUMBER	2																							
364	302	100	100	900	900	404	100	100	473	404	100	404	900	900	270	900	900	100	404	900	669	900	100	404
100	100	473	900	900	400																			
CTU NUMBER	3																							
174	131	100	100	900	798	404	100	100	473	404	456	404	460	900	582	584	900	100	404	743	900	449	100	404
100	100	473	541	460	100																			
CTU NUMBER	4																							
336	229	100	100	900	900	404	100	100	473	404	100	404	460	900	900	900	900	100	404	343	669	449	805	404
100	100	473	900	460	400																			
CTU NUMBER	5																							
594	220	100	100	731	900	100	900	100	473	404	100	404	460	900	270	584	900	100	404	900	396	700	100	100
100	100	900	900	460	400																			
CTU NUMBER	6																							
148	674	900	900	605	798	100	100	900	900	100	125	100	900	100	100	900	900	100	900	900	295	700	106	900
100	900	100	100	100	100																			
CTU NUMBER	7																							
105	175	100	100	270	798	404	900	100	100	100	125	100	100	100	100	900	100	100	100	900	900	700	100	404
900	900	473	541	100	100																			
CTU NUMBER	8																							
900	284	100	100	731	100	900	100	900	100	100	900	100	100	100	100	900	100	900	100	100	100	700	106	100
900	900	100	900	900	900																			
CTU NUMBER	9																							
133	243	100	100	100	798	100	100	900	100	100	125	100	100	100	100	900	100	100	100	343	367	100	106	100
900	900	100	900	100	100																			
CTU NUMBER	10																							
142	194	100	0	100	798	100	100	900	900	900	521	900	100	100	731	100	900	100	100	900	384	700	900	100
100	900	900	100	100	100																			

Appendix 5

THE STRUCTURAL MECHANICS AND ELASTICITY OF AWNS

Appendix 5

THE STRUCTURAL MECHANICS AND ELASTICITY OF AWNS

Seeing that awns occur in a variety of shapes and forms one is interested in understanding more about the mechanics, or behaviour of these structures under the action of force, and their elasticity, or the properties which they possess which enables them to assume their original form when the forces are removed. In the discussion of function and evolution of the awn, various terms such as torsion, rigidity and strength, have been used without any explanation. These factors which are important in the operation of the awn will be considered here, the rigidity and strength of variously shaped solid bodies will be evaluated, and compared. When these matters are understood, the function of different awns will be more apparent.

Firstly, one should consider the mechanics of the awn. The hygroscopic mechanism whereby the column of the awn twists, has been attributed to the torsion which results in the thickened cells on taking up or losing water. Torsion in a body may be defined as the "twisting" about an axis, produced by the action of two equal and opposite parallel forces acting upon the body. The force referred to in this sense is called the <u>torque</u> (Uvarov and Chapman, 1958)*. Any body subjected

* All definitions taken from this source.

to a force in such a way undergoes stress. (Stress is the force per unit area). In the case of torque in a solid shaft, however, the stress is referred to as the shear or shearing stress, which is the stress applied to the body in the plane of one of its faces. As a result of an applied stress, the body may become deformed, a condition referred to as strain. The strain is the ratio of the dimensional change to the original or unstrained dimension. The strain may be a ratio of lengths, areas or volumes.

Secondly, the elasticity, or properties which the awns possess under force, are of importance. Any material has an elastic limit, which is the limit of stress within which the strain in the material completely disappears when the stress is removed. When a stress is applied to a body (within its elastic limit) a corresponding strain is produced, and the ratio of stress to strain is a characteristic constant of the body. Thus the modulus of elasticity in shear or shear modulus is the ratio of shearing stress to shearing strain for a given material. Seeing that the torque on an awn produces a shearing stress, resulting in shearing strain, the modulus of shear is an important measure of the property of the awn.

From these basic principles on the mechanics and elasticity of the awn, it is apparent that there are two main properties which should be considered, viz. the torque that is applied and the modulus of shear of the material. If we assume the awns to represent solid shafts in which the amount of torque, the material and consequently the modules of shear are constant,

they can very readily be compared. In engineering beams of wood are regarded as solid (Lyse and Johnston, 1935) and thus all cross-sectional shapes of awn will be assumed principally from the outline of their fibres, or thickened cells. The other assumptions are also not perfectly valid, as the magnitude of torque depends on the number and size of cells producing it and the material, i.e. cells and tissues, is not identical from one awn to the next.

A comparison of the mechanics and elasticity of differently shaped awns involves the evaluation of their properties as related to function. As a burying mechanism, the awn would require a column which is readily twisted so that many revolutions can be performed when the grain is pushed into the soil. The number of revolutions may be increased by having many turns or a large angle of twist per unit length, and a longer awn. On the contrary, the bristle attached to the column should be rigid and not twist. When bristles alone occur they should be strong and fairly rigid for wind or animal dispersal. The properties that are important are rigidity and strength. From our knowledge of torsion in solid shafts, it is possible to calculate the torsional constant, which gives a measure of rigidity, and the constant of strength which determines the strength of the body for awns of various cross-sectional shapes.

Evaluation of rigidity and strength

Lyse and Johnston (1935) calculated the relative rigidity and strength of shafts of a number of different shapes but of

equal cross-sectional area. Since the other cross-sectional shapes are of interest in the study of torsion in the awn, values for these have also been calculated. Our knowledge of torsion in such structures is mainly due to the researches of Saint-Venant (1855). Timoshenko (1953) and Timoshenko and Young (1962) have applied the mathematical concepts of torsion in engineering. Apart from the work of Tompson (1917) on torsion in antelope horns, little attempt has been made to investigate torsion in biological structures.

The torsion constant, K, is the measure of torsional rigidity and twisting deflections. It is also part of any formula for torsional stresses, and may be determined for any known shape.

When torque, T, is applied to a circular shaft of radius, r, the maximum shearing stress, τ, at the surface is given by

$$\tau = \frac{Tr}{J} \qquad \text{A1}$$

in which, J = polar moment of inertia. In terms of T Equation A1 may be rearranged to read:

$$T = \frac{\tau J}{r} \qquad \text{A2}$$

The torque, T, may also be expressed in terms of Θ, the angle of twist per unit length, and G, the modules of shear, thus:

$$T = J G \Theta \qquad \text{A3}$$

The torsion constant, K, is equal to J, the polar moment of inertia for circular sections. For non-circular sections, the torsion constant is always less than the polar moment of inertia, but it may be determined similarly from equation A3 by substitution of K, in place of J, thus:

$$T = K G \Theta \qquad A4$$

The constant of strength, S, may also be evaluated for any shape shaft. As seen in Equation A2 the torque, T, is proportional to the maximum shearing stress, r. For a circular shaft, the maximum shearing stress is also dependent on the radius of the shaft and the polar moment of inertia (Equation A1). The constant of strength, S, in the case of circular shafts, is equal to the polar moment of inertia, J, divided by the radius. In non-circular shafts it may be determined from equation A2 by substitution, thus:

$$T = S\tau \qquad A5$$

If the equation for torque is known for a solid shaft of any cross-sectional shape, the torsion constant, K, and the constant of strength, S, may be calculated from Equations A4 and A5 respectively. Timoshenko and Young (1962) have given formulae for the maximum shearing stress, τ and angle of twist per unit length, Θ, for a number of shafts of different cross-sectional shape, thereby making it possible to calculate K and S.

1. Shaft with a circular cross section

The torsion constant, K, may be readily calculated when the diameter, d, of the circular cross section is known, because it is equal to the polar moment of inertia:

$$K = J = \frac{\pi d^4}{32} \qquad A6$$

The constant of strength, S, may then be determined from the torsion constant, because by comparison with equations A2 and A5.

$$S = \frac{J}{r} \qquad A7$$

K and S were calculated for a shaft with a circular cross section of area 3000 units and radius 30.9 units (fig. A1).

2. Shaft with a rectangular section

The torque for a shaft with rectangular cross section may be given by the equation:

$$T = \beta bc^3 G\theta \qquad A8$$

where b is the longer and c the shorter side of the rectangular cross section and β is a numerical factor depending upon the ration b/c (Table 50).

Alternatively torque may be expressed in terms of the maximum shearing stress:

$$T = \alpha bc^2 \tau \qquad A9$$

where α is another factor dependant on the ratio b/c (Table 50).

Table 50: Factors used in calculating torque in a shaft of rectangular cross section (Timoshenko and Young, 1962)

b/c	α	β
1.00	0.208	0.141
1.50	0.231	0.196
1.75	0.239	0.214
2.00	0.246	0.229
2.50	0.258	0.249
3.00	0.267	0.263
4.00	0.282	0.281
6.00	0.299	0.299
8.00	0.307	0.307
10.00	0.313	0.313
∞	0.333	0.333

By comparison with equations A4 and A5, it will be seen that:

$$K = \beta\, bc^3 \qquad A10$$

and

$$S = \alpha\, bc^2 \qquad A11$$

respectively.

The torsion constant, K, and constant of strength, S, were calculated for three shafts with the same cross sectional area of 3000, but of different rectangular shape (Figs. A2 - A4).

3. Shaft with a section resembling the sector of an annulus

As seen from Table A1, when the width of the rectangle becomes small, α and β approach 1/3 and the equations for

torque become

$$T = \frac{bc^3}{3} G\theta \qquad A12$$

and

$$T = \frac{bc^2}{3} \tau \qquad A13$$

These equations may be applied in cases such as the sector of the annulus where the width of the cross section is small.

Therefore:

$$K = \frac{bc^3}{3} \qquad A14$$

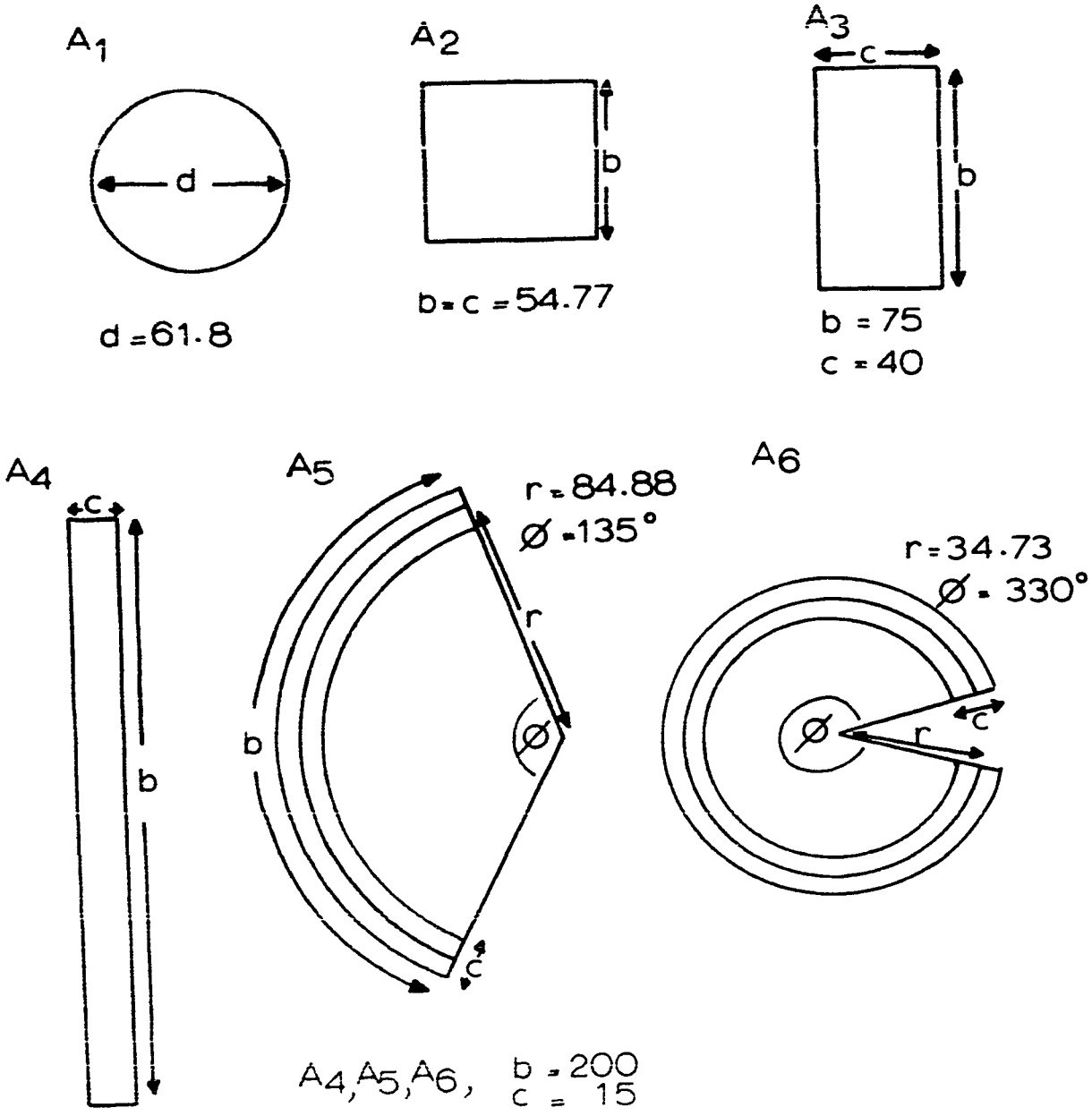
and

$$S = \frac{bc^2}{3} \qquad A15$$

for shafts with cross sections that fit this category.

The area of the Figs A5 and A6 are 3000 units and the dimensions are b = 200 and c = 15 as for Fig. A4 so that the constants are equal with those calculated for a shaft with rectangular section as in Fig. A4.

The torsion constants (k) and constants of strength (S) as determined for shafts of different cross sectional shape, but of equal area are recorded in Table 51. From these values, it is possible to make a comparison of the torsional rigidity and strength of the differently shaped sections (Lyse and Johnston, 1935). The constants obtained for the solid circular shaft is taken as a standard of 100.0 and all other values are expressed as a percentage of this value. The comparative



Figures A_1 – A_6 Shapes and Dimensions of Cross sections of equal Area (3000 units)

rigidity and strengths are listed in Table 51. Lyse and Johnson (1935) calculated the relative rigidity and strength of other cross sectional shapes and these are listed with the values from Table 51 in Table 24 (Chap. 5).

Table 51: Torsion constants (K), constants of strength (S), and the relative rigidity and strength of differently shaped sections of equal cross sectional area

Shape of section	Fig. No.	Torsion Constant (K)	Torsional Rigidity	Constant of Strength (S)	Torsional Strength
Circle	A1	1,432,221	100.0	46,350	100.0
Square	A2	1,269,000	88.60	34,176	73.74
Rectangle	A3	1,064,960	74.36	29,160	62.91
"	A4	225,000	15.71	15,000	32.36
Sector of an annulus					
$\phi = 135^\circ$	A5	"	"	"	"
$\phi = 330^\circ$	A6	"	"	"	"

Appendix 6

DATA MATRICES PERTAINING TO THE ANALYSES USING ALL CHARACTERS

TABLE 52 - DATA MATRIX 159 - ALL CHARACTERS

CTU NUMBER 1

174 187 100 100 900 100 900 100 100 100 100 500 100 900 100 100 100 900 100 900 100 300 500 900
100 900 100 100 100 900 900 900 500 100 500 514 414 100 900 900 100 477 500 484 671 900 100 100 499
100 100 500 900 100 100 100 900 900 100 100 900 366 900 900 900 900 500 900 900 900 900 540 100 100 900
100 100 900 100 100 900 445 900 660 100 900 100 900 900 900 900 500 900 900 900 900 900 540 100 100 900
100 179 100 900 100 100 100 360 720 540 633 700 500 100 700 500 500 100 100 287 366 366 900 900 139
633 366 900 900 900 633 300 261 900 461 493 464 841 100 384 900 900 900 900 100 900 100 721 100 900
465 143 900 510 100 535 747 900 100 900 900 100

CTU NUMBER 2

900 445 900 660 100 900 100 900 900 900 900 900 900 900 900 900 540 100 100 900 100 170 100 900 100
100 100 360 720 540 633 700 500 100 700 500 500 100 100 287 366 366 900 900 139 633 366 900 900 900
633 300 261 900 461 493 464 841 100 384 900 900 562 900 900 500 500 900 900 900 900 549 100 900 900
535 747 900 100 900 900 100 900 660 100 100 900 562 900 900 500 500 900 900 900 900 549 100 900 900
100 100 900 619 900 900 100 100 540 900 540 633 700 500 100 700 500 500 100 100 569 633 633 900 900 262
633 366 900 900 900 633 300 101 900 461 493 464 841 100 384 900 900 900 900 100 900 100 721 515 900
900 900 900 510 900 900 603 502 483 393 672 900

CTU NUMBER 3

900 100 900 660 100 100 900 562 900 900 500 500 900 900 900 900 549 100 900 900 100 100 900 619 900
100 100 540 900 540 633 700 500 100 700 500 500 100 100 569 633 633 900 900 262 633 366 900 900 900
633 300 101 900 461 493 464 841 100 384 900 900 900 900 100 900 100 721 515 900 900 900 900 515 900
900 603 502 483 393 672 900 900 660 100 375 100 900 900 900 500 500 900 900 900 900 549 100 900 900
100 179 100 900 100 100 247 540 720 360 633 700 500 100 500 500 500 100 100 297 366 366 900 900 225
633 633 900 900 100 366 500 157 900 461 493 464 841 100 100 500 100 205 900 100 900 100 721 100 900
900 100 101 510 100 535 900 900 900 900 900 100

CTU NUMBER 4

672 900 900 660 100 375 100 900 900 900 500 500 900 900 900 100 900 900 515 900 100 170 100 900 100
100 247 540 720 360 633 700 500 100 500 500 500 100 100 297 366 366 900 900 215 633 633 900 900 00
366 500 157 900 461 493 464 841 100 100 500 100 205 900 100 900 100 721 100 900 900 100 101 510 100
535 900 900 900 900 900 100 900 660 100 900 100 900 900 900 900 500 900 900 900 100 900 900 515 100
900 446 900 619 900 100 247 540 720 360 633 500 500 100 500 300 500 100 100 486 366 100 900 900 139
366 100 639 900 900 633 300 301 100 100 100 100 900 100 100 500 100 900 900 100 524 100 721 100 795
100 900 900 510 100 535 718 900 900 900 900 100

CTU NUMBER 5

900 100 900 660 100 900 100 900 900 900 900 500 900 900 900 100 900 900 515 100 900 446 900 619 900
100 247 540 720 360 633 500 500 100 500 300 500 100 100 486 366 100 900 900 139 366 100 639 900 900
633 300 301 100 100 100 100 900 100 100 500 100 900 900 100 524 100 721 100 795 100 900 900 510 100
535 718 900 900 900 900 100 900 660 100 375 900 562 900 140 500 500 900 900 900 900 549 100 100 900
100 179 900 619 900 100 100 540 720 360 633 500 500 100 500 300 900 100 100 186 366 366 900 900 156
366 366 343 900 807 633 300 400 900 461 493 464 841 100 100 500 363 205 900 100 900 100 721 100 704
900 143 101 510 100 535 698 499 900 900 900 100

CTU NUMBER 6

900 100 900 660 100 375 900 562 900 140 500 500 900 900 900 900 549 100 100 900 100 170 900 619 900
100 100 540 720 360 633 500 500 100 500 300 900 100 100 186 366 366 900 900 156 366 366 343 900 807
633 300 400 900 461 493 464 841 100 100 500 363 205 900 100 900 100 721 100 704 900 143 101 510 100
535 698 499 900 900 900 100 900 660 100 900 100 900 100 900 900 500 900 900 900 100 900 900 515 100
900 446 100 900 100 100 569 280 360 540 633 500 100 100 300 300 500 100 100 607 633 900 607 900 83
366 366 772 900 100 900 900 242 100 333 100 100 900 100 384 500 900 900 100 433 100 100 721 100 100
465 100 101 510 900 900 603 502 483 393 672 900

CTU NUMBER 7

900 100 900 660 100 900 100 900 100 900 500 900 900 900 900 100 900 900 515 100 900 446 100 900 100
100 569 280 360 540 633 500 100 100 300 300 500 100 100 607 633 900 407 900 283 366 366 772 900 100
900 900 242 100 333 100 100 900 100 384 500 900 100 900 100 900 900 500 900 900 900 100 900 900 515 900
900 603 502 483 393 672 900 900 660 100 900 100 900 100 900 900 500 900 900 900 100 900 900 515 900
100 900 100 900 100 100 569 280 360 540 633 700 100 100 500 700 100 100 100 171 366 366 900 900 405
366 633 476 900 100 633 500 261 100 900 900 900 100 100 100 500 900 225 100 900 100 100 721 900 100
100 900 900 510 100 535 718 900 900 100 900 100

CTU NUMBER 8

672 900 900 660 100 900 100 900 100 900 900 500 900 900 900 100 900 900 515 900 100 900 100 900 100
100 569 280 360 540 633 700 100 100 500 700 100 100 100 171 366 366 900 900 405 366 633 476 900 100
633 500 261 100 900 900 900 100 100 100 500 900 225 100 900 100 100 721 900 100 100 900 900 515 900
535 718 900 900 100 900 100 100 900 900 618 100 900 100 900 900 100 900 900 900 100 900 900 515 900
100 900 100 900 100 100 100 280 540 720 900 900 100 100 700 900 100 100 100 235 366 366 900 100 250
633 900 900 900 448 900 700 162 100 333 900 900 100 100 384 500 900 100 100 400 536 100 721 100 100
465 100 900 510 100 535 100 100 453 292 900 100

CTU NUMBER 9

900 100 100 900 900 618 100 900 100 900 900 100 900 900 900 100 900 900 515 900 100 900 100 900 100
100 100 280 540 720 900 900 100 100 700 900 100 100 100 100 366 366 900 100 294 633 900 900 900 100
900 700 182 100 333 900 900 100 100 384 900 100 100 400 536 100 721 100 100 666 900 900 100 100 900
534 100 100 453 282 900 100 900 663 100 900 100 900 100 900 900 900 900 500 900 900 100 900 100 100
100 900 900 618 900 100 588 280 360 540 633 900 900 100 700 700 900 100 100 900 100 100 100 721 900
366 633 343 900 807 366 700 154 900 461 493 464 841 100 100 500 900 100 900 100 100 100 900 100 100
465 900 900 100 100 535 747 100 439 100 900 100

CTU NUMBER 10

900 100 900 660 100 900 100 900 900 900 900 500 900 900 900 900 549 100 100 900 100 900 900 618 900
100 586 280 360 540 633 900 500 100 700 700 500 100 900 385 366 633 900 900 100 366 633 549 900 900
366 700 154 900 461 493 464 841 100 100 500 900 100 900 100 100 100 721 900 100 465 900 900 100 100
535 747 100 439 100 900 100 900 660 100 900 900 562 900 900 500 100 900 900 900 100 900 900 618 100
900 446 900 618 900 100 422 280 540 900 366 500 500 100 500 300 900 100 100 475 366 366 900 900 465
366 366 639 900 194 633 500 282 100 100 100 900 900 100 384 500 900 100 900 100 900 900 900 446
502 425 494 900 100 535 100 100 453 100 900 492

CTU NUMBER 11

900 100 900 660 100 900 900 562 900 900 500 100 900 900 900 100 900 900 515 100 900 446 900 618 900
100 422 280 540 900 366 500 500 100 500 300 500 100 100 475 366 366 900 900 494 366 366 900 900 194
633 500 282 100 100 100 900 900 100 384 500 900 100 900 100 900 900 500 486 555 502 425 494 900 100
535 100 100 453 100 900 462 900 660 100 900 900 562 900 900 500 100 900 900 900 100 900 900 618 100
900 446 100 900 100 100 422 280 720 900 900 700 500 100 700 500 500 100 100 286 366 633 900 900 370
366 366 605 900 100 366 500 183 100 100 100 100 900 100 100 500 100 205 900 100 100 900 900 486 555
502 425 494 900 100 535 100 100 453 100 900 492

CTU NUMBER 12

900 492 900 660 100 900 900 562 900 900 500 100 900 900 900 100 900 900 515 100 900 446 100 900 100
100 422 280 720 900 900 700 500 100 700 500 500 100 205 900 100 100 900 900 494 555 502 425 494 900 100
366 500 183 100 100 100 100 900 100 100 500 900 100 100 100 187 900 100 100 100 900 900 549 900 515 100
535 100 100 453 100 900 492 100 900 900 618 100 100 100 187 900 100 100 100 900 100 100 500 366 633 900 900 480
900 446 100 900 100 100 100 280 360 540 100 300 900 100 100 900 100 100 100 900 100 100 500 366 633 900 900 486 555
100 366 343 900 194 366 700 403 900 461 493 464 841 900 900 500 445 385 528 568 477 900 900 486 555
502 425 494 900 100 535 100 900 900 100 100 100

CTU NUMBER 13

900 492 100 500 900 618 100 100 100 187 900 100 100 100 900 900 549 900 515 100 900 446 100 100 100
100 100 280 360 540 100 300 900 100 100 100 900 100 100 500 366 633 900 900 489 100 366 343 900 194
366 700 403 900 461 493 464 841 900 900 500 445 385 528 568 477 900 900 486 555 502 425 494 900 100
535 100 900 900 900 100 100 100 100 900 900 618 100 100 100 187 900 100 100 900 100 100 687 366 633 900 900 480
900 446 100 900 100 100 100 280 360 360 366 500 900 100 100 100 900 100 100 687 366 633 900 900 486 555
366 366 343 900 459 366 500 232 900 461 493 464 841 900 900 500 445 385 528 568 477 900 900 486 555
502 425 494 900 900 900 603 502 483 393 672 900

CTU NUMBER 14

100 100 100 900 900 618 100 100 100 900 900 500 100 100 900 100 100 900 515 100 900 446 100 900 100
100 100 280 360 360 366 500 900 100 100 100 900 100 100 687 366 633 900 900 388 366 366 343 900 459
366 500 232 900 461 493 464 841 900 900 500 445 385 528 568 477 900 900 486 555 502 425 494 900 900
900 603 502 483 393 672 900 900 660 100 343 900 562 900 100 500 100 900 900 900 900 549 100 900 900
100 100 900 619 900 100 389 540 720 360 366 500 500 100 300 900 100 100 900 900 900 900 100 900 900 486 555
366 366 100 900 194 366 500 454 900 461 493 464 841 100 100 100 900 900 900 900 900 900 900 900 900
502 425 494 900 900 900 603 502 483 393 672 900

CTU NUMBER 15

672 900 900 660 100 343 900 562 900 100 500 100 900 900 900 900 549 100 900 900 100 100 900 619 900
100 389 540 720 360 366 500 500 100 300 300 500 100 100 383 366 633 900 900 239 366 366 100 900 194
366 500 454 900 461 493 464 841 100 100 100 900 900 900 900 100 900 900 486 555 502 425 494 900 900
900 603 502 483 393 672 900 900 660 100 343 100 900 900 900 900 500 900 900 900 900 549 100 900 900
100 100 900 619 900 100 247 280 360 540 900 700 100 100 700 700 100 100 100 389 366 366 753 900 323
633 366 900 194 366 500 199 100 900 100 900 900 100 384 100 100 900 100 384 100 100 205 900 100 900 900 900 486 555

CTU NUMBER 16

672 900 900 660 100 343 100 900 900 900 900 500 900 900 900 900 549 100 900 900 100 100 900 619 900
100 247 280 360 540 900 700 100 100 700 700 100 100 100 389 366 366 753 900 323 633 366 636 900 194
366 500 199 100 900 100 100 900 100 384 100 100 205 900 100 900 900 900 486 555 502 425 494 900 00
535 747 900 100 264 900 100 900 660 100 900 900 562 900 900 900 900 100 627 100 900 549 100 900 900
100 179 900 619 900 100 415 280 360 540 366 500 900 100 500 500 500 100 100 505 633 900 753 900 267
366 366 900 900 807 366 500 298 900 461 493 464 841 100 100 900 100 147 900 400 536 900 900 486 555
502 425 494 900 900 900 603 502 483 393 672 900

CTU NUMBER 17

500	100	900	660	100	517	900	562	900	900	900	900	100	627	100	900	549	100	330	900	100	179	900	619	900
100	415	280	360	540	366	500	900	100	500	500	500	100	100	504	633	900	753	900	267	366	366	900	900	807
366	400	258	900	461	493	464	841	100	100	500	100	147	900	400	536	900	900	486	555	502	425	494	900	900
500	603	502	483	393	672	900	900	660	100	343	100	900	900	900	900	100	900	900	900	900	549	100	900	900
100	100	900	619	900	100	100	280	360	720	366	500	900	100	300	500	500	100	100	175	366	633	753	900	101
366	366	215	900	100	366	700	253	900	461	493	464	841	100	100	500	900	147	159	100	900	100	721	900	704
900	900	101	100	100	100	747	900	900	100	900	100													

CTU NUMBER 18

672	900	900	660	100	343	100	900	900	900	900	100	900	900	900	900	549	100	900	900	100	100	900	619	900
100	100	280	360	720	366	500	900	100	300	500	500	100	100	175	366	633	753	900	101	366	366	215	900	100
366	700	253	900	461	493	464	841	100	100	500	900	147	159	100	900	100	721	900	704	900	900	101	100	100
100	747	900	900	100	900	100	900	660	100	900	100	900	900	900	100	900	100	100	900	900	549	100	900	900
100	900	900	619	900	100	100	100	360	720	633	500	500	100	300	100	500	100	100	297	366	100	753	900	900
366	900	900	900	900	633	500	301	900	461	493	464	841	100	100	500	100	100	900	400	536	900	900	486	555
502	425	494	900	100	535	900	900	900	900	100														

CTU NUMBER 19

900	100	900	660	100	900	100	900	900	900	100	900	100	100	900	900	549	100	330	900	100	900	900	619	900
100	100	100	360	720	633	500	500	100	300	100	500	100	100	297	366	100	753	900	338	366	900	900	900	900
633	500	301	900	461	493	464	841	100	100	500	100	100	900	400	536	900	900	486	555	502	425	494	900	100
535	900	900	900	900	900	100	900	660	100	900	100	900	100	900	500	100	900	900	100	100	100	100	515	100
500	446	100	900	100	100	100	280	360	540	633	500	500	100	500	300	500	100	100	240	366	366	555	900	900
633	366	503	900	900	366	300	242	900	461	493	464	841	100	100	500	363	205	900	100	900	100	721	900	739
100	900	101	100	100	100	747	900	900	100	900	100													

CTU NUMBER 20

900	100	900	660	100	900	100	900	100	900	500	100	900	900	100	100	100	900	515	100	900	446	100	900	100
100	100	280	360	540	633	500	500	100	500	300	500	100	100	240	366	366	555	900	900	633	366	503	900	900
366	300	242	900	461	493	464	841	100	100	500	363	205	900	100	900	100	721	900	739	100	900	101	100	100
100	747	900	900	100	900	100	900	660	100	343	100	900	100	900	500	100	900	900	900	100	100	900	515	100
500	446	100	900	100	100	247	360	540	540	633	500	500	100	900	500	500	100	100	336	633	633	764	100	587
900	633	503	900	900	633	500	234	900	461	493	464	841	100	100	900	286	147	900	100	900	100	721	900	739
900	900	900	100	100	100	747	499	453	100	900	100													

CTU NUMBER 21

900	100	900	660	100	343	100	900	100	900	500	100	900	900	900	100	100	900	515	100	900	446	100	900	100
100	247	360	540	540	633	500	500	100	900	500	500	100	100	336	633	633	764	100	587	900	633	503	900	900
633	500	234	900	461	493	464	841	100	100	900	286	147	900	100	900	100	721	900	739	900	900	100	100	100
100	747	499	453	100	900	100	900	660	100	343	100	900	100	900	900	500	900	900	100	100	900	900	515	100
900	446	100	900	100	100	100	280	360	540	100	300	500	100	100	100	500	100	100	143	633	633	764	900	310
633	633	503	900	807	633	500	298	900	461	493	464	841	100	617	100	286	147	900	100	900	100	100	900	795
465	100	101	100	100	100	747	100	453	292	900	100													

CTU NUMBER 22

900	100	900	660	100	343	100	900	100	900	900	500	900	900	100	100	900	900	515	100	900	446	100	900	100
100	100	280	360	540	100	300	500	100	100	100	500	100	100	143	633	633	764	900	310	633	633	503	900	807
633	500	298	900	461	493	464	841	100	617	100	286	147	900	100	900	100	100	900	795	465	100	101	100	100
100	747	100	453	292	900	100	900	660	100	900	900	562	100	140	500	500	900	900	900	900	549	100	900	900
100	900	100	900	100	100	415	280	280	360	633	300	100	100	500	300	500	100	100	400	633	633	764	100	554
366	633	767	900	900	633	300	371	900	461	493	464	841	100	617	500	100	900	900	100	100	100	721	900	795
900	900	101	100	100	100	747	100	439	100	100	100													

CTU NUMBER 23

500	100	900	660	100	900	900	562	100	140	500	500	900	900	900	900	549	100	900	900	100	900	100	900	100
100	415	280	280	360	633	300	100	100	500	300	500	100	100	400	633	633	764	100	594	366	633	767	900	900
633	300	371	900	461	493	464	841	100	617	500	100	900	900	100	100	100	721	900	795	900	900	101	100	100
100	747	100	439	100	100	100	900	660	100	900	100	900	900	900	500	100	900	900	900	900	549	100	900	900
100	900	100	900	100	100	100	360	720	540	633	500	500	100	500	300	500	100	900	766	366	366	764	100	283
900	633	767	900	900	366	100	534	900	461	493	464	841	100	100	500	286	147	900	100	900	100	721	900	795
100	100	101	510	100	535	718	100	100	264	900	100													

CTU NUMBER 24

100	100	900	660	100	900	100	900	900	900	500	100	900	900	900	900	549	100	900	900	100	900	100	900	100
100	100	360	720	540	633	500	500	100	500	300	500	100	900	766	366	366	764	100	283	900	633	767	900	900
366	100	534	900	461	493	464	841	100	100	500	286	147	900	100	900	100	721	900	739	100	100	101	510	100
535	718	100	100	264	900	100	100	100	900	618	100	900	100	140	500	100	900	900	100	100	900	900	515	100
900	446	100	900	100	100	100	100	280	540	720	366	500	500	100	500	300	500	100	100	383	366	633	764	900
900	366	343	900	100	366	500	261	900	461	493	464	841	100	100	500	286	205	900	100	900	100	721	900	100
100	100	900	100	100	535	900	100	439	100	900	100													

```
CTU NUMBER   25
 500  100  100  100  300  618  100  500  100  140  500  100  900  900  100  100  900  900  515  100  900  446  100  900  100
 100  100  280  540  720  366  500  500  100  500  300  500  100  100  383  366  633  764  900  222  900  366  383  900  100
 366  500  261  900  461  493  464  841  100  100  500  286  205  900  100  900  100  721  900  100  100  100  900  100  100
 535  900  100  433  100  900  100  900  660  100  900  100  900  900  900  500  500  900  900  900  100  900  900  515  900
 100  900  100  900  500  100  100  280  540  720  366  500  500  100  300  300  500  100  100  486  366  366  515  900  424
 366  366  383  900  459  633  500  245  900  461  493  464  841  100  100  900  286  147  900  100  900  900  900  486  515
 502  425  494  900  100  535  900  100  900  900  900  100

CTU NUMBER   26
 900  100  900  660  100  900  100  900  900  900  500  500  900  900  900  100  900  900  515  900  100  900  100  900  900
 100  100  280  540  720  366  500  500  100  300  300  500  100  100  486  366  366  555  900  426  366  366  383  900  459
 633  500  245  900  461  493  464  841  100  100  900  286  147  900  100  900  900  900  486  555  502  425  494  900  100
 535  900  100  900  900  900  100  900  660  100  375  900  562  900  900  500  900  100  627  100  900  549  100  100  900
 100  179  900  619  900  100  100  280  360  720  900  900  500  100  700  300  500  100  100  357  366  100  900  900  391
 633  366  733  900  900  633  100  323  100  333  900  900  635  100  384  900  100  100  900  100  900  900  900  486  555
 502  425  494  900  900  900  603  502  483  393  672  900

CTU NUMBER   27
 900  100  900  660  100  375  900  562  900  900  500  900  100  627  100  900  549  100  100  900  100  179  900  619  900
 100  100  280  360  720  900  500  500  100  700  300  500  100  100  357  366  100  900  900  391  633  366  733  900  900
 633  100  323  100  333  900  900  635  100  384  900  100  100  900  100  900  900  900  900  500  100  627  100  100  900
 900  603  502  483  393  672  900  900  660  100  900  100  900  900  900  900  500  100  627  100  100  900  900  515  900
 100  900  100  100  900  100  422  280  360  720  633  500  500  100  700  300  500  100  100  561  633  633  900  100  229
 633  366  843  900  459  366  300  379  100  100  900  100  900  100  384  100  100  205  900  100  900  900  900  486  555
 502  425  494  900  900  900  603  502  483  393  672  900

CTU NUMBER   28
 672  900  900  660  100  900  100  900  900  900  900  500  100  627  100  100  900  900  515  900  100  900  100  900  459
 100  422  280  360  720  633  500  500  100  700  300  500  100  100  561  633  633  900  100  229  633  366  843  900  900
 366  300  379  100  100  900  100  900  100  384  100  100  205  900  100  900  900  900  486  555  502  425  494  900  900
 900  603  502  483  393  672  900  900  660  100  900  100  100  900  900  900  500  100  627  100  100  900  900  515  900
 100  179  100  100  900  100  415  280  360  540  900  900  500  900  500  500  500  900  100  276  900  900  900  100  287
 900  900  636  900  807  366  500  199  100  333  900  900  635  100  384  900  900  900  100  900  100  900  900  900  486
 502  425  494  900  900  900  603  502  483  393  672  900

CTU NUMBER   29
 672  900  900  660  100  900  100  100  900  900  900  500  100  627  100  100  900  900  515  900  100  179  100  100  900
 100  415  280  360  540  900  900  500  900  500  500  500  900  100  276  900  900  900  100  287  900  900  636  900  807
 366  500  199  100  333  900  900  635  100  384  900  900  900  900  100  900  900  900  486  555  502  425  494  900  900
 900  603  502  483  393  672  900  900  660  100  375  100  100  900  900  900  500  100  627  100  100  900  900  515  900
 100  100  100  900  900  100  100  280  360  540  900  700  500  100  500  500  500  100  100  165  100  100  900  100  325
 633  900  900  900  807  633  500  210  900  461  493  464  841  100  384  500  900  225  900  100  900  900  900  486  555
 502  425  494  900  900  900  603  502  483  393  672  900

CTU NUMBER   30
 672  900  900  660  100  375  100  100  900  900  500  100  100  627  100  100  900  900  515  900  100  100  100  900  900
 100  100  280  360  540  900  700  500  100  500  500  500  100  100  165  100  100  900  100  325  633  900  900  900  807
 633  500  210  900  461  493  464  841  100  384  500  900  225  900  100  900  900  900  486  555  502  425  494  900  900
 900  603  502  483  393  672  900  900  660  100  900  100  100  900  900  500  500  100  627  100  900  549  100  100  900
 100  900  100  900  900  100  100  280  360  540  900  500  900  100  700  500  500  100  100  900  633  633  900  900  355
 900  633  767  900  459  366  300  288  900  461  493  464  841  100  384  100  320  225  900  400  536  900  900  486  555
 502  425  494  900  900  900  603  502  483  393  672  900

CTU NUMBER   31
 672  900  900  660  100  900  100  100  900  900  500  500  100  627  100  900  549  100  100  900  100  900  100  900  900
 100  100  280  360  540  900  500  900  100  700  500  500  100  100  900  633  633  900  900  355  900  633  767  900  459
 366  300  288  900  461  493  464  841  100  384  100  320  225  900  400  536  900  900  486  555  502  425  494  900  900
 900  603  502  483  393  672  900  900  660  100  900  100  900  900  900  900  100  100  627  900  100  100  900  515  900
 100  900  100  900  900  100  100  280  360  540  366  500  500  100  100  500  500  100  100  319  366  633  481  900  200
 366  900  236  900  100  366  900  213  900  461  493  464  841  100  100  900  320  100  900  100  900  100  721  100  100
 100  100  101  510  100  535  100  900  100  100  900  100

CTU NUMBER   32
 672  900  900  660  100  900  100  900  900  900  900  100  100  627  900  100  100  900  515  900  100  900  100  900  900
 100  100  280  360  540  366  500  500  100  100  500  500  100  100  319  366  633  481  900  206  366  900  236  900  100
 366  900  213  900  461  493  464  841  100  100  900  320  100  900  900  900  500  100  900  900  900  100  100  900  515
 535  100  900  100  100  900  100  900  660  100  900  633  700  500  100  500  700  500  100  100  101  366  633  616  100
 100  900  900  900  619  900  100  100  280  540  900  633  700  500  100  500  700  500  100  100  101  366  633  616  100
 633  900  440  900  807  366  900  151  100  333  900  100  900  100  100  900  320  900  900  100  900  100  721  900  100
 900  143  101  100  100  100  698  900  100  900  900  100
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CTU NUMBER   33
  900  100  900  660  100  900  100  900  900  900  500  100  900  900  900  100  100  900  515  900  100  900  900  619  900
  100  100  280  540  900  633  700  900  100  500  700  900  100  100  101  366  633  616  100  175  633  900  440  900  807
  366  900  151  100  333  900  100  900  100  100  900  320  900  900  100  900  100  721  900  100  900  143  101  100  100
  100  698  900  100  900  900  100  900  660  100  343  100  100  900  900  900  500  100  627  900  100  900  900  515  900
  100  100  100  100  900  100  900  360  540  540  366  300  500  100  100  300  500  100  100  682  366  633  900  900  100
  366  633  503  900  807  366  700  312  900  461  493  464  841  100  100  500  320  225  900  100  900  900  900  486  555
  900  425  494  900  900  900  603  502  483  393  672  900
CTU NUMBER   34
  900  100  900  660  100  343  100  100  900  900  900  500  100  627  900  100  900  900  515  900  100  100  100  100  900
  100  900  360  540  540  366  300  500  100  100  300  500  100  100  682  366  633  900  900  100  366  633  900  900  807
  366  700  312  900  461  493  464  841  100  100  500  320  225  900  100  900  900  100  100  627  900  100  900  900  515  900
  900  603  502  483  393  672  900  900  660  100  343  100  900  500  500  100  500  300  500  100  100  682  633  366  900  900  223
  100  100  100  900  100  100  900  360  900  540  633  500  500  100  500  300  500  100  100  682  633  366  900  900  807
  366  366  503  900  807  633  700  258  100  900  100  100  900  100  384  100  100  900  900  100  900  100  721  100  100
  900  900  101  510  100  535  747  499  439  100  900  100
CTU NUMBER   35
  672  900  900  660  100  343  100  900  100  900  900  100  100  627  900  100  900  900  515  900  100  100  100  900  100
  100  900  360  900  540  633  500  500  100  500  300  500  100  100  682  633  366  900  900  223  366  366  503  900  807
  633  700  258  100  900  100  100  900  100  384  100  100  900  900  100  900  100  721  100  100  900  100  549  100  330  900
  535  747  499  439  100  900  100  900  660  100  900  900  562  900  100  500  100  900  700  900  100  100  460  366  366  100  100  310
  100  179  100  900  900  900  455  900  100  100  100  100  900  100  900  700  900  100  100  460  366  366  100  100  310
  633  100  471  100  900  366  100  745  900  461  493  464  841  100  100  500  286  147  900  100  900  100  721  100  795
  900  900  101  510  100  535  900  100  439  900  900  100
CTU NUMBER   36
  900  100  900  660  100  900  900  562  900  100  500  100  900  900  100  900  549  100  330  900  100  179  100  900  900
  900  455  900  100  100  100  100  900  100  900  700  900  100  100  460  366  366  100  100  310  633  100  471  100  900
  366  100  745  900  461  493  464  841  100  100  500  286  147  900  100  900  100  721  100  795  900  900  101  510  100
  535  900  100  439  900  900  100  900  660  100  900  100  900  900  100  500  500  900  100  100  468  633  633  407  100  495
  900  446  100  900  100  900  455  900  100  100  100  100  900  100  700  500  900  100  100  468  633  633  407  100  495
  100  100  437  100  900  100  300  767  900  461  493  464  841  100  384  500  900  900  900  100  100  100  721  900  710
  100  100  101  100  900  900  603  502  483  393  672  900
CTU NUMBER   37
  900  100  900  660  100  900  100  900  900  100  500  500  900  900  900  100  100  900  515  100  900  446  100  900  100
  900  455  900  100  100  100  100  900  100  700  500  900  100  100  468  633  633  407  100  495  100  100  437  100  900
  100  300  767  900  461  493  464  841  100  384  500  900  900  900  100  100  100  721  900  710  100  100  101  100  900
  900  603  502  483  393  672  900  900  660  100  343  900  562  900  100  100  100  900  900  100  100  900  515  100
  900  446  900  619  900  900  455  540  100  100  366  300  500  100  700  500  500  100  100  432  366  366  100  100  340
  633  366  636  900  900  366  300  456  900  461  493  464  841  100  384  900  320  225  900  100  100  100  721  100  100
  900  900  900  100  900  900  603  502  483  393  672  900
CTU NUMBER   38
  672  900  900  660  100  343  900  562  900  100  100  100  900  900  900  100  100  900  515  100  900  446  900  619  900
  900  455  540  100  100  366  300  500  100  700  500  500  100  100  432  366  366  100  100  340  633  366  636  900  900
  366  300  456  900  461  493  464  841  100  384  900  320  225  900  100  100  100  721  100  100  900  900  900  100  900
  900  603  502  483  393  672  900  900  660  100  375  900  562  900  100  500  100  900  900  900  900  549  100  900  900
  100  100  900  619  900  100  535  540  280  280  633  500  500  100  700  500  500  100  100  379  366  366  481  900  298
  633  633  600  900  807  366  500  269  900  461  493  464  841  100  384  500  900  900  100  100  900  100  721  900  100
  100  100  101  510  100  535  100  499  100  264  900  100
CTU NUMBER   39
  672  900  900  660  100  375  900  562  900  100  500  100  900  900  900  900  549  100  900  900  100  100  900  619  900
  100  535  540  280  280  633  500  500  100  700  500  500  100  100  379  366  366  481  900  298  633  633  600  900  807
  366  500  269  900  461  493  464  841  100  384  500  900  900  100  100  900  100  721  900  100  100  100  101  510  100
  535  100  499  100  264  900  100  900  660  100  900  100  439  900  100  100  100  900  900  100  900  900  515  100
  900  446  100  900  900  900  455  900  100  100  633  300  500  100  300  100  500  100  100  357  366  100  616  900  298
  633  100  375  100  807  366  100  678  900  461  493  464  841  100  100  500  286  147  900  100  900  100  721  100  795
  100  100  900  100  900  900  603  502  483  393  672  900
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TABLE 53 - DATA MATRIX 180 - SUPRA-SPECIFIC TAXA AND COMPONENT SCORES AS DATA

OTU NUMBER	1								
28.127598	14.188360	-0.844751	-5.789468	-6.510270	-10.740070	8.896918	2.342228	10.321577	0.002033
16.202165	5.543578	14.194902	-9.444189	-3.300001	2.954859	1.278450	-1.726056	-7.157112	0.388423
8.501341	0.277799	-2.147306	-0.666325	2.185877	-1.884465	-0.338189	0.413820	0.483948	0.000203
OTU NUMBER	2								
26.691820	14.232800	14.803186	4.185857	-0.810803	7.568421	-12.259475	-13.472197	1.979440	0.002033
-0.089357	16.674655	-5.073560	7.979248	-9.650785	9.509875	3.071425	-5.842420	0.872498	2.091356
0.819455	-0.510922	2.012543	0.321208	-2.334839	-0.993227	-3.017423	0.353986	-0.546053	0.000203
OTU NUMBER	3								
23.832433	4.122375	-0.075747	0.978880	8.622185	-4.848713	-16.593386	12.434120	-1.487703	0.002033
13.194703	5.397264	12.489952	6.729907	6.045200	-9.181154	2.889699	-2.063875	4.057066	2.471947
5.372464	0.664052	-0.060028	-1.626170	-2.225247	2.771198	0.556445	-0.936405	0.625718	0.000203
OTU NUMBER	4								
25.960572	10.731216	-4.889056	9.419186	22.928237	-6.621198	11.822664	-3.641047	-6.171021	0.002033
8.101548	4.446272	-4.191069	0.729453	-5.072928	-2.920714	-15.846930	5.378795	-0.778979	1.977234
-0.967183	-6.383919	-2.257137	1.611577	-1.337310	-0.772629	1.159244	-0.505775	-0.270007	0.000203
OTU NUMBER	5								
25.002527	10.290592	-3.639192	-8.841558	-16.610257	19.540920	7.624593	5.220801	-4.061421	0.002033
-0.700643	4.999780	-14.793719	-3.899776	-2.468307	-10.921255	8.469424	1.789720	-4.136236	1.606035
0.240299	1.625295	3.384179	0.967351	1.384719	-0.845280	0.787942	-2.284147	-0.808358	0.000203
OTU NUMBER	6								
-8.064022	-44.871129	43.898377	7.699549	-3.133342	-2.032718	3.983389	1.499665	-0.657846	0.002033
16.141376	-8.033073	-8.998190	-10.077280	8.609542	8.649522	2.129073	1.717609	2.976439	2.065281
0.718138	-1.976502	2.811626	1.750605	1.979214	2.140562	0.219372	1.790404	0.043051	0.000203
OTU NUMBER	7								
-24.086042	0.855853	-4.961285	-39.042633	-9.697956	-12.571460	-2.454492	-4.047992	-4.534570	0.002033
-4.810662	-16.044970	6.311998	9.288385	-3.510761	4.455660	5.430216	9.987142	-2.170392	1.709184
-1.346196	4.174221	-3.118212	0.776714	-0.401978	0.626843	0.471939	0.767874	-1.930476	0.000203
OTU NUMBER	8								
-53.272049	35.846992	2.604670	29.977640	-9.614856	-3.315999	0.131903	1.320565	-1.027259	0.002033
-16.157221	5.101034	7.897812	-13.950961	-8.860681	-1.234747	1.805530	3.385202	5.200436	1.028554
-2.769877	2.060673	1.910675	-1.087928	-1.626571	-1.916895	1.883289	1.302150	0.963378	0.000203
OTU NUMBER	9								
-44.186160	-3.508467	-5.009031	-16.365232	21.539776	13.508536	1.020736	1.215437	4.870197	0.002033
-7.566529	-25.533920	0.320217	-1.902524	-4.104081	-2.404774	-3.138828	-9.709772	-0.998449	1.904823
-5.042999	2.184518	-2.087088	1.924511	0.664553	0.472136	-1.106646	-0.717782	1.851898	0.000203
OTU NUMBER	10								
-0.006682	-41.888587	-41.887173	17.777780	-6.712719	-0.487721	-2.172853	-2.871583	0.768602	0.002033
-24.304808	10.301193	2.716170	-0.980394	13.078367	2.723688	-3.000634	-0.571893	-3.452188	1.962539
-5.525442	-2.115214	-0.449249	-3.971543	1.711582	0.401756	-0.615973	-0.184126	-0.413099	0.000203