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A standard method for predicting conservation status from herbarium specimens in the context of a new phylogeny and taxonomy of Loudetia

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2008-04-08T09:12:41Z
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CHAPTER 2

Species clarification in the *Loudetia simplex* (Nees) C.E. Hubb. complex

2.0 Abstract

Loudetia simplex was once split into distinct groups both at specific and infraspecific levels, but all of these taxa have been perceived to intergrade and have since been subsumed under this species. The resultant species is morphologically highly variable. A morphometric analysis was conducted with the aim of determining if distinct groups can be formed within the *L. simplex* complex and at what taxonomic level those groups should be recognized. Cluster analysis and principal coordinates analysis suggest that the *Loudetia simplex* complex can be split into 2 distinct groups, based primarily on the occurrence of tubercle-based hairs on glumes and lower lemma. Comparison with a standard species, *L. demeusei,* suggests that the 2 groups can be recognized at the specific level. Therefore a proposal is made to resurrect the name *L. camerunensis* for the form without tubercle-based hairs on glumes and the lower lemma and apply the name *L. simplex* only to the form with tubercle-based hairs on glumes and the lower lemma.

2.1 Introduction

The tribe Arundinelleae Raddi was the subject of active taxonomic research between 1934 and 1974 (Hubbard, 1934, 1937, 1949, 1957; Conert, 1957; Jacques-Félix, 1960; Phipps, 1964, 1966, 1970, 1972a, 1972b, 1972c, 1972d, 1972e; Li *et al.,* 1966; Clayton, 1967, 1972, 1974; Phipps & Mahon, 1970; Li & Phipps, 1973; Lubke & Phipps, 1973). During this period, emphasis was placed on resolving generic limits, whereas comprehensive delimitations of species within constituent genera received less attention. To date, some species, including *Loudetia simplex* (Nees) C.E. Hubb., are still not satisfactorily delimited. *Loudetia simplex* occurs in two forms based primarily on the presence of tubercle-based hairs on glumes and lower lemma. Workers have noted that the species exhibits variation in morphological characteristics, including lengths of culm, spikelet, leaf blade and inflorescence; presence and distribution of tubercle-based hairs on glumes and lower lemma, spikelet coloration and shape of lower glume apex (Stapf, 1898; Hubbard, 1934; Chippindall, 1955; Clayton, 1974). *Loudetia simplex* without tubercle-based hairs on glumes and lower lemma is associated with callus teeth of equal lengths, while the tuberculate form has a slightly oblique callus apex due to slightly unequal teeth lengths (Figure 2.1). Whereas the basic chromosome number is x = 10 (Metcalfe, 1960), tetraploids (2n = 40) and hexaploids (2n = 60) have been documented (Li *et al.,* 1966). However, it is not known whether either form is associated with a particular ploidy level.

Attempts were made to split the species into subspecific and varietal ranks (Stapf, 1898: 450; Conert, 1957). Three varieties were proposed under *Trichopteryx simplex* Hack. ex Engl. (= *Loudetia simplex*) namely *Trichopteryx simplex* var. *minor* Stapf, *T. simplex* var. *crinita* Stapf and *T. simplex* var. *sericea* Stapf (Stapf, 1898). Tubercle-based hairs on glumes and lower lemma were absent in *T. simplex* var. *minor,* but both *T. simplex* var. *crinita* and *T. simplex* var. *sericea* were variably bristly. However, these closely related

forms were included in *T. simplex* because they intergraded into it in morphological characters when the whole range of the species was considered (Stapf, 1898). In addition, the Madagascan *Loudetia stipoides* (Hack.) Conert and *Stipa madagascariensis* Baker were treated as synonyms of *Loudetia simplex* (Clayton, 1974).



Figure 2.1. Morphological variation within the *Loudetia simplex* complex. (a) = form without tubercle-based hairs on glumes and lower lemma, *Kamundi 2485* (J); (b) lower glume of the non-tuberculate form, (c) = lower glume of the tuberculate form, (d) = form with tubercle-based hairs on glumes and lower lemma, *Kamundi* 2484 (J); (e) = upper floret, (f) = callus of the non-tuberculate form, (g) = callus of the tuberculate form, (h) = upper floret; ar = apex rounded, at = apex truncate. Tubercle-based hairs are absent on glumes and lower lemma in (a) and present in (b) and callus teeth are equal in length in (a), but slightly different in (b).

Tubercle-based hairs are present on the glumes in *Loudetia stipoides* from Madagascar, but absent in *L. camerunensis* (Stapf) C.E. Hubb. from Cameroun. These species form two segregates, but they were treated as extreme forms of *L. simplex* into which characters intergrade in the East African population (Clayton, 1974: 420). In addition, *L. simplex* as a whole comprises distinct, but closely related forms, which were impossible to satisfactorily distinguish at specific or infraspecific levels, using herbarium specimens alone (Hubbard, 1934: 27; Stapf, 1898: 451). The lumping of the distinct forms under *L. simplex* resulted in a broadly circumscribed species, which probably engulfs distinct entities, including *L. stipoides* and *L. camerunensis* once treated as separate species and the then recognized infraspecific taxa, *L. simplex* var. *crinita, L. simplex* var. *minor*, *L. simplex* var. *sericea* and *L. simplex* var. *gracilis* Rendle now synonymized under *Loudetia simplex* (Table 2.1). The extent of variation therefore, shows that *L. simplex* may be poorly delimited and clarifying this problem forms the central theme of this study.

2.1.1 **Aim and questions**

The aim of this chapter is to offer a contribution towards the delimitation of the *L. simplex* complex by determining if distinct groups can be recognized within the species and at what taxonomic level can the groups so formed, if any, be recognized.

2.2 **Materials and methods**

Materials used were 59 herbarium specimens of the *L. simplex* complex and a distinct but related species, *L. demeusei* C.E. Hubb. housed at BM, ET, J, MO, PRE, SRGH, UPS and UWO - acronyms are in accordance with Holmgren *et al.* (1991). Examination of herbarium specimens suggests that the *L. simplex* complex may represent two putative groups based primarily on the presence of tubercle-based hairs on glumes and lower lemma. The groups were designated as *L. simplex* nt and *L. simplex* tb for non-tuberculate and tuberculate forms, respectively. In this study, individual plant specimens constituted operational taxonomic units (OTUs) (Stace, 1980: 55; Woodland, 1997). Authors of species names are abbreviated according to Brummitt and Powell (1992).

2.2.1 *Selection of OTUs.* Specimens were selected to obtain a representative sample with respect to the geographical distribution of the putative groups, *Loudetia simplex* nt and *Loudetia simplex* tb (Appendix 2.1). Only mature specimens and those containing a complete suite of characters were selected to minimize age-related variation (Krauss, 1996; Mikkelsen & Selberg, 2001). Premature specimens showed incomplete development of the base of the awn (at the sinus of the upper lemma), absence or incomplete development of callus of the upper floret and tender paleas & free margins of the lemma of the upper floret. These were excluded from the character coding exercise.

2.2.2 *Preparation of materials and character state scoring.* Character definitions were obtained from the literature and personal observation (Mikkelsen & Selberg, 2001). The initial and final lists of potential taxonomic characters are presented in Appendices 2.2 and 2.3, respectively. Spikelets were sampled from the middle part of the inflorescence. Five or more sets of each character per OTU were measured and average values recorded. Widths and/or lengths of the culm, leaf blade, inflorescence, pedicel, awn (softened in

"Glass Master" solution) and column of the awn of the upper lemma were measured using a ruler while culm diameter and spikelet width were measured with vernier calipers. Glume, lemma, palea and callus dimensions were viewed under a Wild Heerbrugg M7A dissecting microscope, photographed using a Nikon Digital Camera, DXM 1200, with ACT-1 computer software and measured using Simplepc computer database. Qualitative character states were coded as binary and multistate (Stace, 1980; Thiele, 1993).

Table 2.1. Synonyms of *Loudetia simplex.* After Clayton (1974: 420)

Synonym	Reference (Abbreviations after Lawrence *et al.*, (1968) &
Tristachya simplex Nees	Fl. Afr. Austr. : 269 (1841)
Trichopteryx simplex (Nees) Engl.	Hochgebirgsfl. Trop. Afr.: **129** (1892)
Arundinella simplex (Nees) Roberty	In Bull. Inst. Fr. Afr. Noire **17**: 56 (1955)
Loudetia elegans A. Br.	Flora **24**: 713 (December 1841)
Tristachya elegans (A. Br.) A. Rich.	Tent. Fl. Abyss. **2**: 418 (1851)
Trichopteryx elegans (A. Br.) Engl.	Hochgebirgsfl. Trop. Afr.: **129** (1892
Arundinella stipoides Hack.	In Sitz. Akad. Wien, Math.-Nat. **89**: 123 (1884)
Trichopteryx stipoides (Hack.) Hack.	In Journ. Linn. Soc. **29**: 65 (1891)
Loudetia stipoides (Hack.) Conert	In Engl. Bot. Jahrb. **77**: 256 (1957)
Trichopteryx incompta Franch.	In Bull. Soc. Hist. Nat. Autun **8**: 373 (1895)
Trichopteryx elegans (A. Br.) Engl. var. *subulifolia* Franch.	In Bull. Soc. Hist. Nat. Autun **8**: 373 (1895)
Trichopteryx camerunensis Stapf	In Kew Bull. **1897**: 296 (1897)
Loudetia camerunensis (Stapf) C.E. Hubb.	In Kew Bull. **1934**: 431 (1934)
Trichopteryx nigritiana Stapf	In Kew Bull. **1897**: 297 (1897)
Trichopteryx simplex var. *gracilis* Rendle	In Cat. Afr. Pl. Welw. **2**: 214 (1899)
Trichopteryx gracilis Peter	Feddes Repert. Beih. **40,** 1, Anhang: 95, fig. 54/1 (1930)
Trichopteryx simplex (Nees) Engl. Var. *crinita* Stapf	In Flora Capensis **7**: 450 (1898)
Trichopteryx simplex (Nees) Engl. Var. *minor* Stapf	In Flora Capensis **7**: 450 (1898)
Trichopteryx simplex (Nees) Engl. Var. *sericea* Stapf	In Flora Capensis **7**: 450 (1898)
Trichopteryx stipoides (Hack.) Hack. ex Elliot var. *natalensis* Hack.	In Mém. Herb. Boiss. **20**: 9 (1900)
Trichopteryx kapirensis De Wild.	Notes Fl. Katanga. **5**: 34 (Ann. Soc. Sc. Brux. **39**. Mém.: 155 (1920))
Trichopteryx ellisabethvilleana De Wild.	Notes Fl. Katanga. **5**: 31 (Ann. Soc. Sc. Brux. **39**. Mém: 156 (1920))
Arundinella hilderbrandtii Mez.	In Fedde Rep. **17**: 84 (1921)
Trichopteryx simplex (Nees) var. *longiflora* Peter	Feddes Repert. Beih. **xl**.1, Anhang: 96, fig. 54/1 (1930)
Stipa madagascariensis Baker	In Journ. Linn. Soc. **20**: 300 (1883)

2.2.3 *Preparation of data*

Data matrices were prepared separately for qualitative and quantitative morphology (Appendix 2.4). Invariant and noisy characters were removed from the qualitative data matrix. Only character states, which are considered conservative within and variable between putative groups, were retained.

2.2.4 *Data analyses*

The degrees of overlap of culm height, culm diameter, leaf blade length, inflorescence length, ratio of the longer pedicel length to shorter pedicel length, spikelet width, lengths of glumes, lower and upper lemmas, lobes of upper lemma and lower and upper paleas, and callus of the upper floret in *Loudetia demeusei*, *L. simplex* nt and *L. simplex* tb were compared by plotting the means, ranges and standard deviations on box and whisker graphs using Statsoft (2001) computer algorithm.

Cluster analysis (CA), principle components analysis (PCA) and principal coordinates analysis (PCOORDA) were performed using NTSYS-pc version 2.02j phenetics program (Rohlf, 1998) based on the combined and separate (qualitative and quantitative) data sets. *Loudetia demeusei* was included in the data matrices to calibrate the phenogram and to help make an objective taxonomic decision (Sebola & Balkwill, unpublished). Data sets were standardized and used to compute dissimilarity indices (including correlation and Euclidean distance coefficients) for interval classes.

Association coefficients for qualitative and quantitative data sets were computed using SIMINT and SIMQUAL algorithms, respectively. The products of SIMINT and SIMQUAL were used in the cluster analysis technique of Unweighted Pair Group Method of Arithmentic Averages (UPGMA) via the SAHN option. These were graphically displayed as dendrograms using the tree plot option. A cophenetic value matrix of ultrametrics was calculated from a tree matrix produced by the SAHN algorithm using COPH and the MXCOMP options for computing the cophenetic correlation coefficient.

Ordination techniques were used to determine whether the *L. simplex* complex can be delimited in multivariate space. Principal components analysis was performed by standardizing qualitative and quantitative data matrices separately and computing matrices of correlation among the variables by rows, extracting 3 eigenvectors from the correlation matrix, projecting these eigenvectors and making 2-dimensional and 3-dimensional plots of the OTUs.

Principal coordinates analysis was performed on the combined data matrix by standardizing a combined data matrix by variables, computing and double-centering a matrix of distance between OTUs, factoring the double-centred matrix and plotting the results in 2-dimensional and 3-dimensional space (Rohlf, 1993, 1998).

2.3 Results

2.3.1 *Comparisons of means and ranges of quantitative characters*

There are overlaps in culm height, culm diameter, leaf blade length and width, ratio of longer pedicel length to shorter pedicel length and length of inflorescence, upper glume and lobes of the upper lemma and callus of the upper floret between *L. simplex* nt and *L. simplex* tb (Figure 2.2*a* to 2.2*j*). Important characters in distinguishing the two putative groups include lengths of lower glume; lower lemma, lower palea, upper lemma and awn of the upper lemma (Figure 2.2*k* to 2.2*o*). The ratio of length of longer to shorter pedicel, length of the lower and upper lemma and palea overlap between *L. demeusei* and the *L. simplex* putative groups (Figure 2.2*f*, *h, l, m n*, respectively).

2.3.2 *Cluster analysis*

The average taxonomic distances for qualitative, quantitative and the combined data matrices yielded dendrograms with correlation coefficients r = 0.77, r = 0.76 and r = 0.75, respectively, which indicate a poor fit (Rohlf, 1993). *Loudetia demeusei, L. simplex* nt and *L. simplex* tb did not form distinct clusters in the analyses based on separate quantitative and qualitative data matrices (Figure 2.3). The combined data set yielded 3 distinct clusters representing distinct taxa (Figure 2.4). The phenon line has been placed at a coefficient of dissimilarity of 1.422 within the range between 1.26 and 1.45 at which the reference species, *L. demeusei*, clusters (Figure 2.6). The distinct clusters of OTUs were not recovered when characters relating to the presence of tubercle-based hairs on the glumes, lower lemma and adaxial leaf blade surface and overlapping quantitative characters (lengths of the culm, leaf blade, inflorescence, lower glume, lobes of the upper lemma, upper palea and callus of the upper lemma; leaf blade width and ratio of length of the longer to shorter pedicels) were excluded (Figure 2.5).

2.3.3 *Principal components analysis*

PCA based on only the quantitative data matrix did not recover distinct clusters of OTUs representing the *Loudetia demeusei, L. simplex* nt and *L. simplex* tb groups in both 2-dimensional and 3-dimensional scatter diagrams (Figure 2.6*a* and 2.5*6,* respectively). However, *Loudetia simplex* nt forms a distinct cloud while OTUs of *L. demeusei* and *L. simplex* tb mix in both 2-dimensional and 3-dimensional scatter diagrams in the analysis based on only qualitative data matrix (Figure 2.7*a* and 2.7*b,* respectively). In this analysis, NT09, TB45 and TB49 are not clearly separated in a 2-dimensional plot, but TB45 is separated from *L. simplex* nt group in the *Y*-axis, in which it clusters with *L. demeusei* and *L. simplex* tb in a 3-dimensional representation.

2.3.4 *Principal coordinates analysis*

Eigenvalues obtained from a run of principal coordinates analysis based on the combined data matrix with the exclusion of leaf blade length and width indicate that the first 15 axes account for 90.88% of the variation (Table 2.3). The first axis contributes 21.20% followed by 15.42% and 8.91% for the second and third, respectively. Subsequent axes have successively less eigenvalues, indicating decreasing correlation of characters on those axes.

The OTUs agglomerate into 3 distinct clusters in both 2-dimensional and 3-dimensional representations in the analysis based on the combined data matrix with the exclusion of leaf blade length and leaf blade width, although the status of TB35 and NT09 is not clear because these OTUs mix with either the *Loudetia simplex* nt cluster or the *Loudetia simplex* tb cluster (Figure 2.8*a* and 2.8*b*). The results indicate a broad agreement with the cluster analysis by yielding distinct clusters for *L. demeusei, L. simplex* tb and *L. simplex* nt (Figure 2.4). A 2-dimensional scatter diagram shows that clusters of the OTUs are dispersed (Figure 2.8*b*).

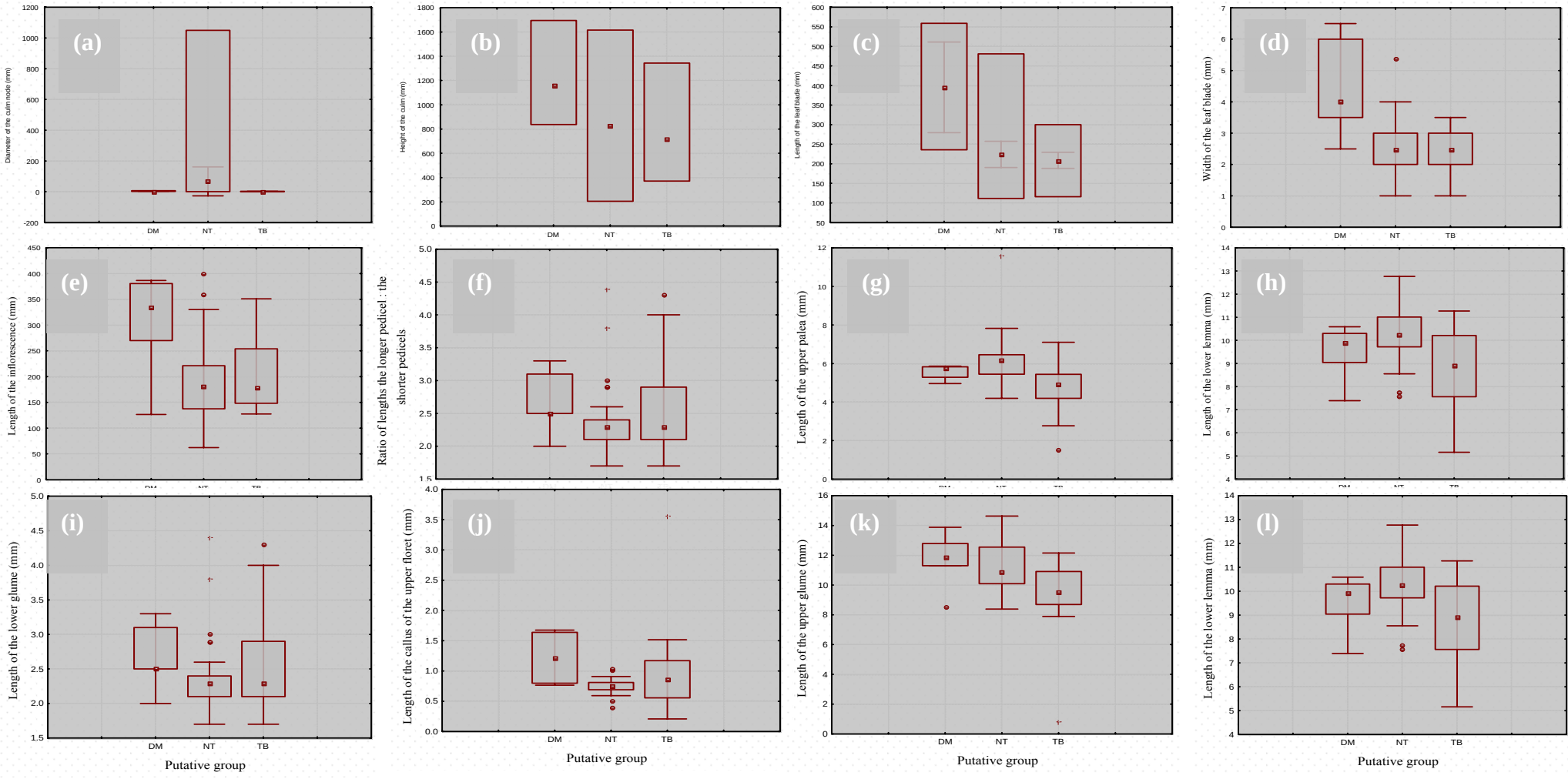


Figure 2.2. Variation in quantitative characters in *Loudetia demeusei*, *L. simplex* nt and *L. simplex* tb. Box = range of the variable within which the mean is highlighted, whisker = standard deviation, dots above or below whiskers = extreme values. Abbreviations: DM = *Loudetia demeusei*, NT = *L. simplex* without tubercle-based hairs on glumes and the lower lemma and TB = *L. simplex* with tubercle-based hairs on glumes and the lower lemma.

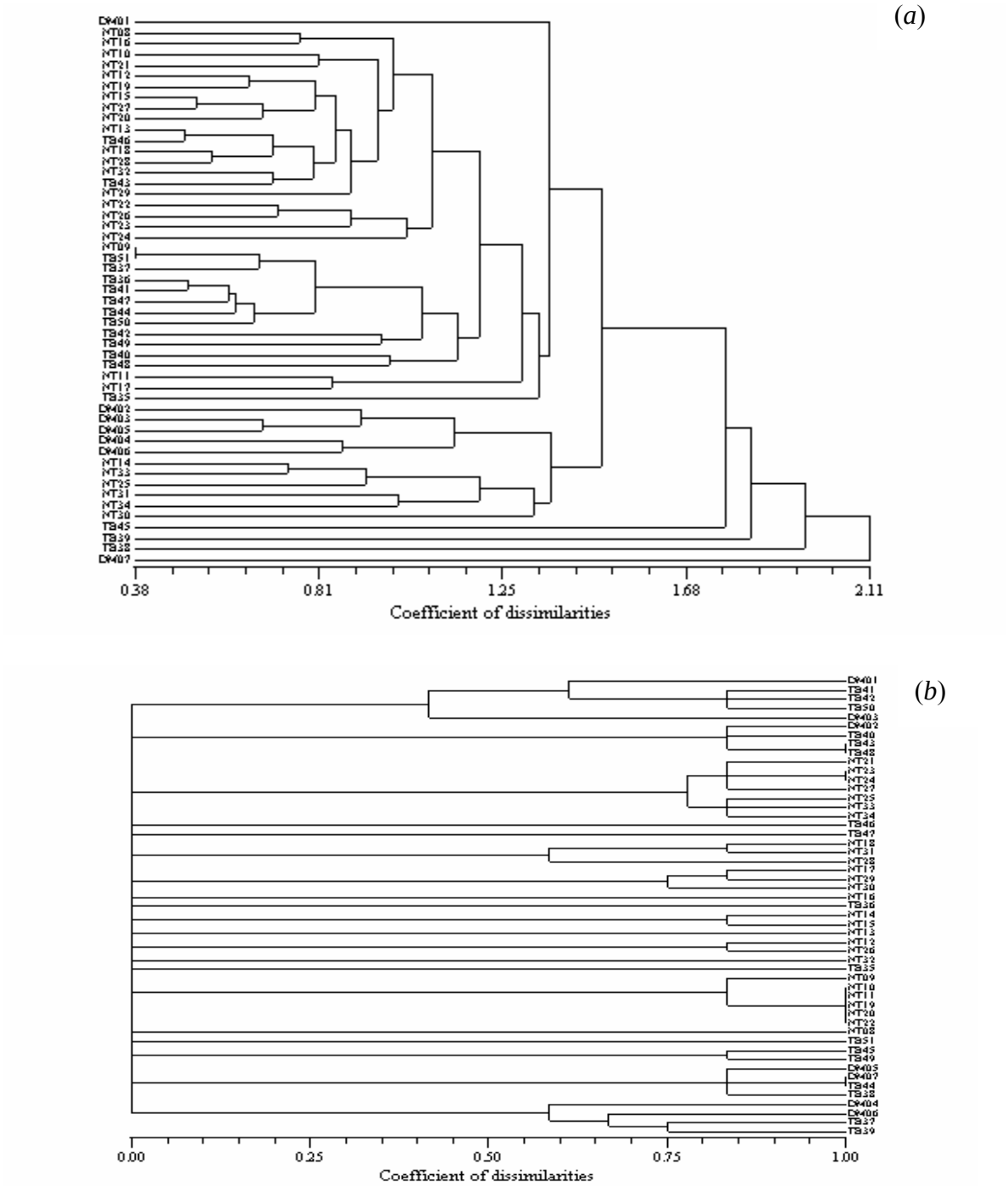


Figure 2.3. UPGMA dendrogram using average taxonomic distance based on (*a*) quantitative and (*b*) qualitative data matrices. Abbreviations used: DM = *Loudetia demeusei* (n = 7), NT = *L. simplex* without tubercle-based hairs on glumes and the lower lemma (n = 27) and TB = *L. simplex* with tubercle-based hairs on glumes and the lower lemma (n = 25).

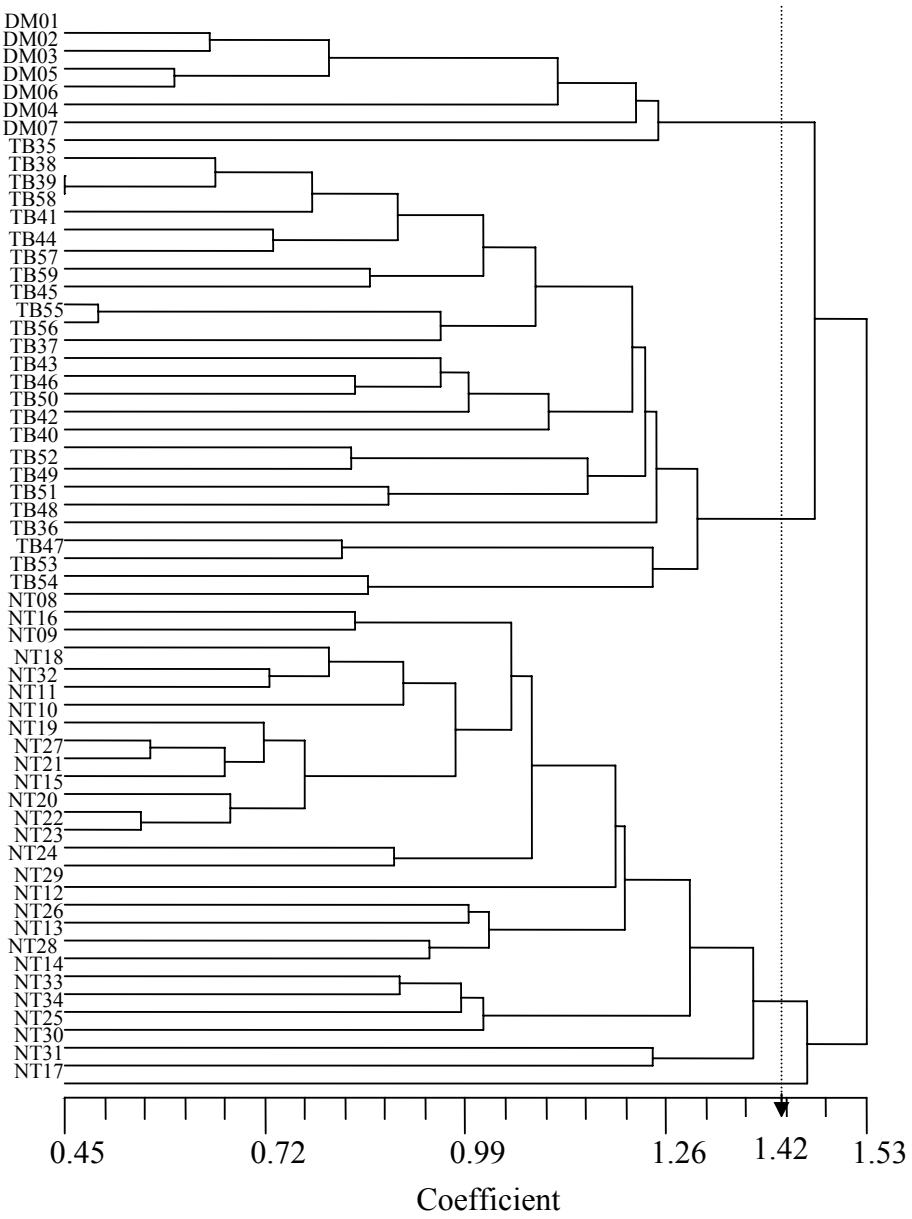


Figure 2.4. UPGMA dendrogram using average taxonomic distance on the combined (qualitative and quantitative) data matrix. A phenon line (dotted line) has been drawn at a coefficient of dissimilarity of 1.42 based on the range between 1.26 and 1.45 within which OTUs of the standard taxon, *L. demeusei,* cluster. Abbreviations and sample sizes are the same as in Figure 2.3.

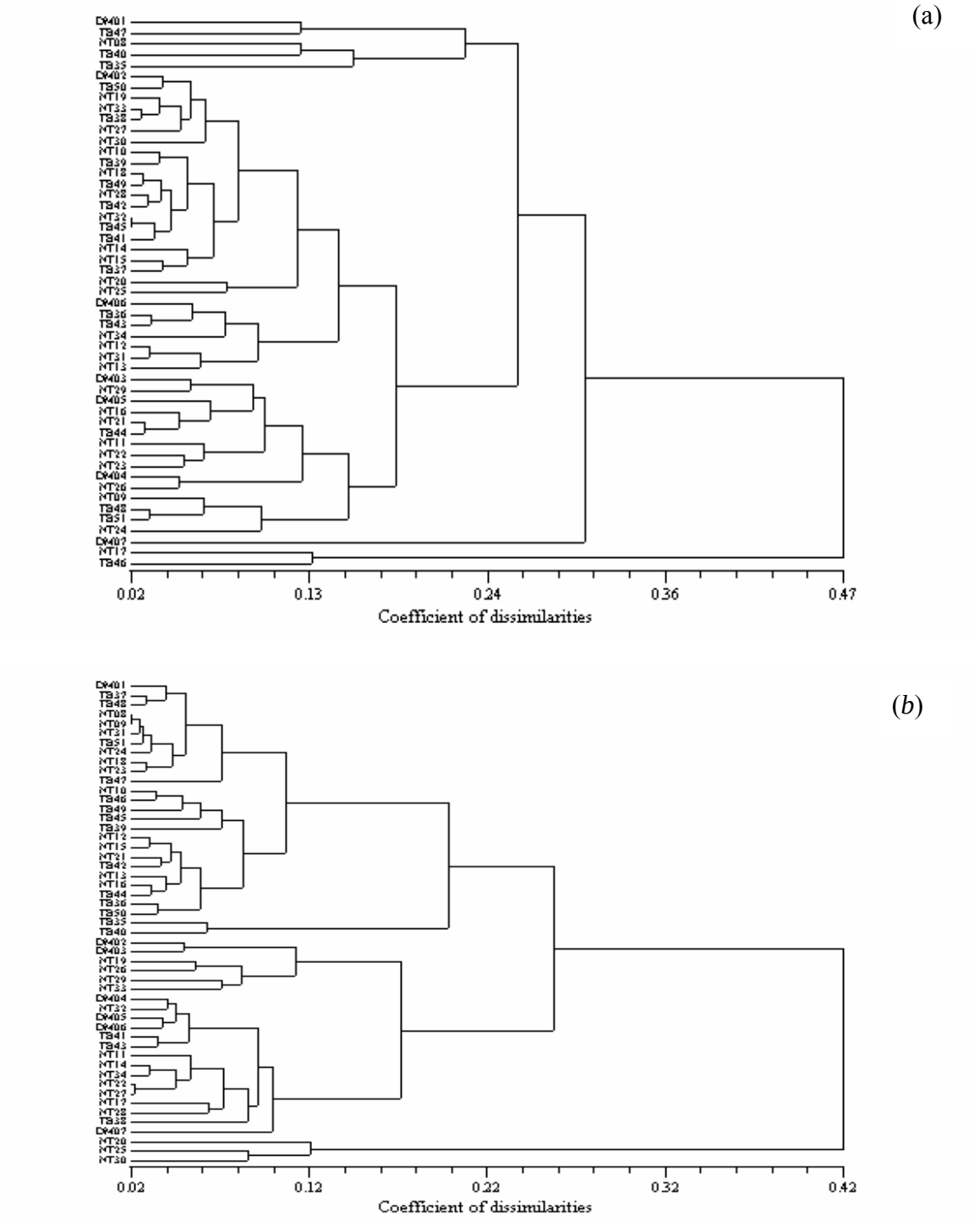


Figure 2.5. UPGMA dendrogram using average taxonomic distance based on the combined data matrix. (*a*) = exclusion of tubercle-based hairs on glumes and lower lemma and (*b*) exclusion of the overlapping quantitative characters: lengths of the upper lemma, upper palea and callus of the upper floret; width of the leaf blade, ratio of the lengths of the longer to shorter pedicels. Distinct groups were not recovered in both (*a*) and (*b*). Abbreviations and sample sizes are the same as in Figure 2.3.

(*b*)

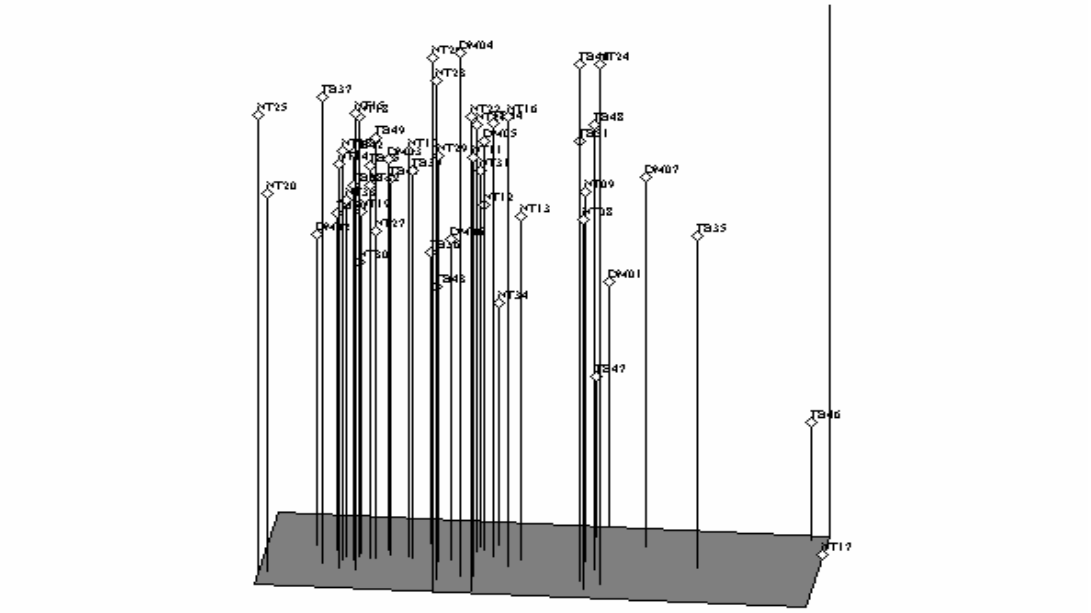


Figure 2.6. Scatter diagrams generated by the quantitative data matrix alone. OTUs did not form distinct groups in both 2-dimensional 3-dimensional representations. Abbreviations and sample sizes are the same as in Figure 2.3.

Table 2.2: Eigenvalues obtained from the principal coordinates analysis of the combined data matrix. Variation is concentrated in the first 15 axes (90.88%, with the first 3 axes accounting for 45.53%.

Axis	Eigenvalue	Percentage of total variance contribution	Cumulative percent
1	11.236	21.20	21.20
2	8.170	15.42	36.62
3	4.723	8.91	45.53
4	3.806	7.18	52.71
5	3.090	5.83	59.54
6	2.612	4.92	63.47
7	2.435	4.59	68.06
8	2.006	3.78	71.85
9	1.878	3.54	75.39
10	1.676	3.16	78.55
11	1.626	3.07	81.62
12	1.490	2.81	84.43
13	1.262	2.38	86.81
14	1.142	2.15	88.97
15	1.014	1.91	90.88
16	0.919	1.73	92.61
17	0.838	1.58	94.19
18	0.636	1.21	95.40
19 to 54	2.385	4.60	100

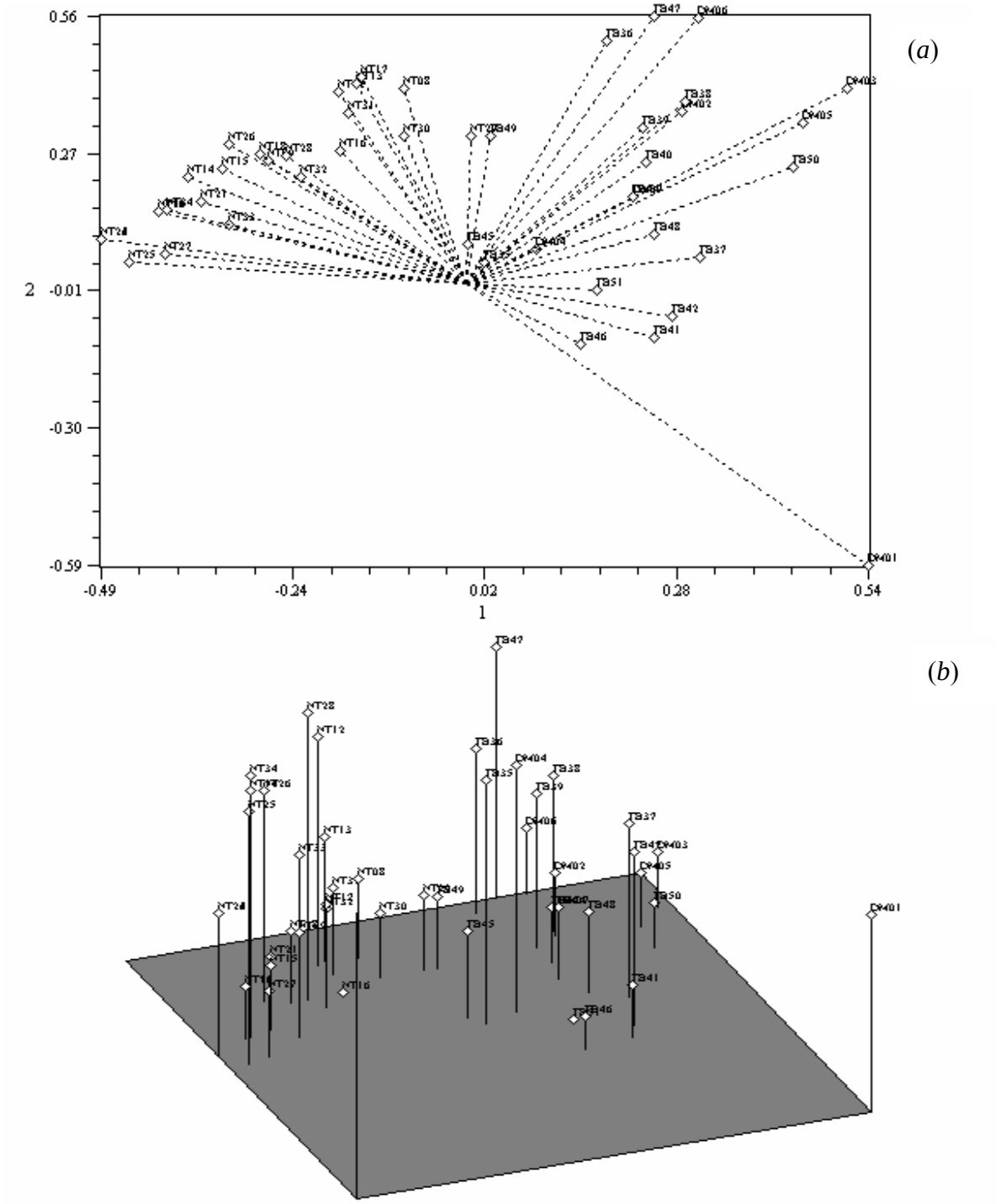


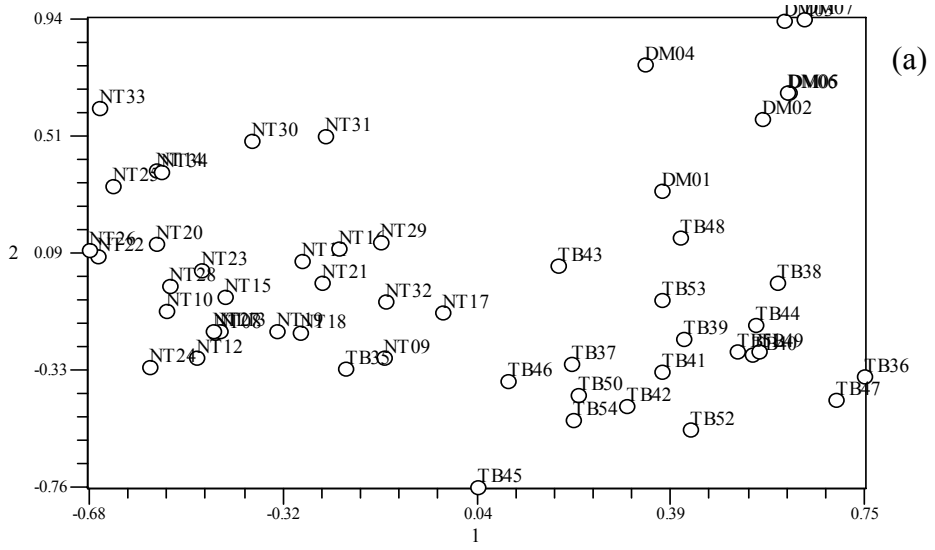
Figure 2.7. Scatter diagrams generated by principal components analysis based on the qualitative data matrix. *Loudetia demeusei* and *L. simplex* tb are not distinguishable in both 2-dimensional and 3-dimensional representations (a & b), but *L. simplex* nt forms a distinct scatter of OTUs in both 2-dimensional and 3-dimensional representations (a & b). Abbreviations and sample sizes are the same as in Figure 2.3.

2.3.5 *Reproductive versus vegetative quantitative characters*

All of the vegetative characters were overlapping while 64% of the reproductive characters were overlapping and 36% non-overlapping (Table 2.3).

Table 2.3. Comparison of potential taxonomic values of vegetative and reproductive quantitative characters within *Loudetia simplex* nt and *L. simplex* tb. OL = overlapping.

Vegetative characters	Status		Reproductive characters	Status	
	Non- OL	OL		Non- OL	OL
Culm height		x	Inflorescence length		x
Culm node diameter		x	Ratio of the longer to shorter pedicel length		x
Culm internode diameter		x	Lowe glume length		x
Leaf blade length		x	Upper glume length	x	
Leaf blade width		x	Lower lemma length	x	
		x	Lower palea length		x
			Upper lemma length		x
			Length of lobes of the upper lemma	x	
			Length of the awn of the upper lemma		x
			Upper palea length	x	
			Length of the callus		x
Total	0	6		4	7
Total (%)	0	100		36	64



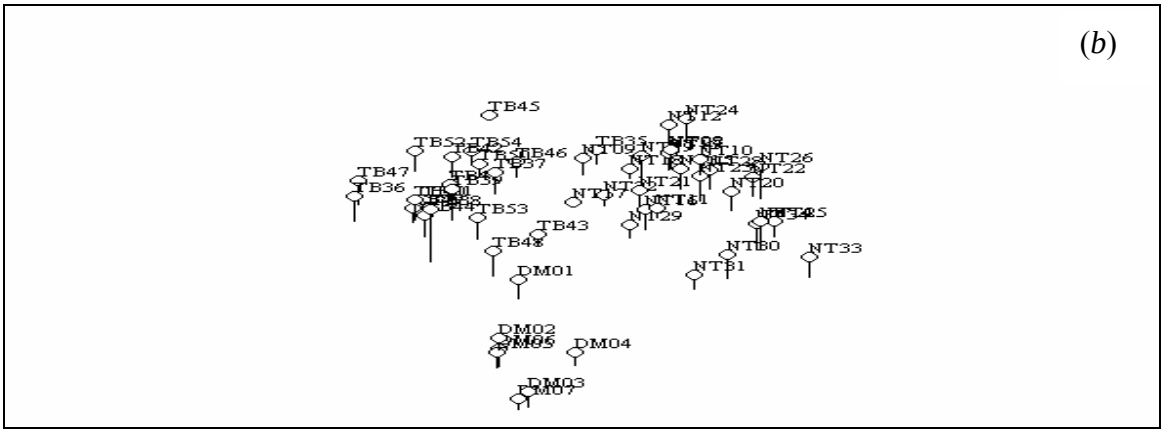


Figure 2.8: Scatter diagrams generated by PCOORDA based on the combined data matrix with the exclusion of the overlapping characters, culm height and leaf blade length. The OTUs are clearly separated in both 2-dimensional (*a*) and 3-dimensional (*b*), but NT09 and TB35 are not clearly separated in a 3-dimensional representation. Abbreviations and specimens examined are the same as in Figure 2.3.

2.4 **Discussion**

2.4.1 *Characters used*

A taxon defined by few characters may become obscured when large numbers of characters are used as exemplified by the taximetrix of danthoniopsoid, loudetioid and tristachyoid groups based on 65 and 109 characters (Phipps, 1972c, 1972d). Thus, there is a need to use as many characters as possible to achieve a stable taxonomy in the Arundinelleae, which is in accordance with Sokal & Sneath (1963) and Woodland (1997). However, sometimes it becomes impossible to find large numbers of taxonomically valuable characters because some characters with taxonomic potential become invariant between groups (Figure 2.1) and few have been used instead (Shaw,

1998). For example, *Senecio germanicus* and *S. memorensis* (Asteraceae) and *Merxmuellera cincta* subsp. *sericea* (Gramineae) have been compared and delimited by 11 and 8 characters, respectively (Hodálová & Marhold, 1993; Barker, 1999). In the present study, a data set with 35 characters (Appendix 2.4) did not yield distinct clusters of *L. demeusei, L. simplex* nt and *L. simplex* tb. The exclusion of some of the characters that show a high degree of overlap between *L. simplex* nt and *L. simplex* tb putative groups (including lengths of the culm and leaf blade) resulted in OTUs clustering in distinct groups (Figures 2.4 & 2.8).

Comparisons of lengths of the lower lemma, lower palea, upper lemma and awn of the upper lemma suggest that these characters are important in segregating *L. simplex* nt from *L. simplex* tb. However, variation in lengths of culm, leaf blade, inflorescence, lower glume, lobes of the upper lemma, upper palea and callus of the upper floret; leaf blade width and ratio of lengths of the longer to shorter pedicels overlap considerably between *L. simplex* nt and *L. simplex* tb. Overlapping characters are not taxonomically valuable (Wilkin, 1999). Therefore, these overlapping characters may not be useful in the delimitation of the *Loudetia simplex* complex. Consequently, *L. simplex* nt and *L. simplex* tb show independent clustering when the analysis excludes all of the overlapping characters. On the other hand, the ratio of the longer to shorter pedicel lengths, lengths of lower lemma and lower palea show overlapping variation between *L. demeusei* and either of *L. simplex* nt and *L. simplex* tb. The clustering of the OTUs of *L. demeusei* within the *L. simplex* tb putative group may be attributed to the influence these overlapping characters have on the agglomeration of OTUs.

The noisy morphometric characters occur in both vegetative and reproductive morphology. Vegetative characters are known to be plastic under the influence of environmental factors (Davis & Heywood, 1963; Heywood, 1967), particularly rainfall and soil nutrients. Factors, including climate, water regimes and soil nutrient contents might have caused the overlapping variation on lengths of the culm, leaf blade, inflorescence or pedicel of mature plants. Field observation might be useful in determining the association of environmental factors with sizes of quantitative characters in *L. simplex* nt and *L. simplex* tb. On the other hand, reproductive morphology is known to be conservative within and it may be variable between taxa (Senblad & Bremer, 2000). In this study, ranges of all of the vegetative quantitative characters did not vary between *L. simplex* nt and *L. simplex* tb, whereas 36% of reproductive characters were potentially taxonomically valuable in delimiting these two groups (Table 2.3). However, there are no significant differences between ranges that are useful to distinguish taxa in the length of the inflorescence, ratio of lengths of the longer to the shorter pedicel; lengths of lower glume, lower palea, upper lemma lobes and callus of the upper floret (Table 2.3), suggesting that some of the reproductive characters are not useful in distinguishing between *L. demeusei, L. simplex* nt and *L. simplex* tb (Figure 2.2*f* to 2.2*i*). The mixing of clusters of OTUs of *L. simplex* nt and *L. simplex* tb when culm height and leaf blade length are included in analyses based on only quantitative or the combined data sets may indicate that overlapping characters are exerting greater influence on the agglomeration of OTUs at the level of 35 characters. In other words, overlapping quantitative characters swamp non-overlapping characters, which may be attributable to the former comprising a

greater proportion of characters than the latter (Hillis, 1987; Bull *et al.*, 1993). The results are thus not consistent with previous reports in which the length and width of leaf blade were found to be important in the agglomeration of large-leaved *L. arundinacea* and *L. phragmitoides* at the level of 65 characters (Phipps, 1972d).

2.4.2 *Clustering of OTUs of the* Loudetia demeusei, L. simplex *nt and* L. simplex *tb*

The UPGMA clusters of *L. simplex* nt and *L. simplex* tb OTUs and principal coordinates analysis (Figures 2.4 and 2.8) support the existence of distinct groups within the *Loudetia simplex* complex. Congruence of the results from cluster analysis and principal coordinates analysis may indicate that the groups so formed are taxonomically stable and can therefore be recognized. The results suggest that *L. simplex* nt and *L. simplex* tb can be recognized at species level at a coefficient of dissimilarities between 1.26 and 1.45, based on the clustering of the standard taxon, *L. demeusei* (Figure 2.4). This is contrary to the previous assertion that *L. simplex* exhibits continuous morphological character variation so that distinct infraspecific taxa cannot be recognised (Hubbard, 1934, 1937; Clayton, 1974).

In the present study, the 2-dimensional diagram (Figure 2.7) yields a dispersed cloud of OTUs of *L. demeusei, L. simplex* nt and *L. simplex* tb in the ordination space, which may indicate that there are low correlations among character axes during the principal components analysis (Table 2.2). *Trichopteryx simplex* (= *Loudetia simplex*) was previously split into distinct entities primarily based on the presence of tubercle-based hairs on glumes and / or lower lemma (Stapf, 1898). The tuberculate forms were treated as two distinct varieties, *Trichopteryx simplex* var. *crinita* and *T. simplex* var. *sericea* (Stapf, 1898). However, the clustering of OTUs for *L. Simplex* tb and *L. simplex* nt do not suggest the existence of boundaries of taxonomic value that can be recognized at infraspecific level.

The overlapping distribution of character states between *L. simplex* and *L. camerunensis* is a general pattern in grasses (Clayton & Renvoize, 1986). In a cladistic analysis, *L. simplex* and *L. camerunensis* appear in different clades, indicating that they are not closely related (Chapter 3, Figure 3.14). The separation between *L. simplex* and *L. camerunensis* in the tree topology of Figure 3.14 is consistent with field observations, in which *L. simplex* grows mainly in hill slopes with shallow and rocky soils with a good drainage systems whereas *L. camerunensis* grows mainly in poorly drained sandy soils, sometimes close to stream banks. Therefore the considerable overlap in character states between *Loudetia simplex* and *L. camerunensis* may suggest reticulate character distribution between two distinct species. For example, a monotypic genus *Gilgiochloa* clustered with species of *Danthoniopsis* in a phonetic analysis, but it was maintained as a distinct genus based on one character, almost woody palea of the lower floret (Clayton, 1972).

2.4.3 *Nomenclature of* L. simplex nt and L. simplex tb

Tristachya simplex Nees in Fl. Afr. Austr.: 269 (1841) is the basionym for *Loudetia simplex* Nees. The plants have tubercle-based hairs on glumes and lower lemma. In 1884, the name *Arundinella stipoides* Hack. was proposed for Madagascan plants with tubercle-

based hairs on glumes and lower lemma (Hackel in Sitz. Acad. Wien, Math.-Nat. **89**: 123 (1884)). In 1893, it was transferred to *Trichopteryx* (*T. stipoides* (Hack.) Hack.) (Hackel in Elliot, 1893) and then to *Loudetia* (*L. stipoides* (Hack.) Conert (Conert, 1957)). *Trichopteryx camerunensis* Stapf in Kew Bull. **1897**: 296 (1897), *T. simplex* var. *gracilis* Rendle in Cat. Afr. Pl. Welw. 2: 214 (1899) and *T. simplex* var. *minor* Stapf in Flora Capensis **7**: 450 (1899) were described for plants without tubercle-based hairs on the glumes and lower lemma and were later transferred to *Loudetia simplex* (Hubbard, 1934). Later, *L. stipoides* and *L. camerunensis* were then treated as synonyms under *L. simplex* (Clayton, 1974). *Loudetia simplex* has priority over *L. stipoides* and *L. camerunensis* is an older name than *T. simplex* var. *minor* or *T. simplex* var. *gracilis* (Greuter *et al.,* 2000). Therefore, in the light of present results, the *Loudetia simplex* complex can be split into *L. simplex* (Nees) C.E. Hubb. for plants with tubercle-based hairs on glumes and lower lemma and *L. camerunensis* (Stapf) C.E. Hubb. for plants without tubercle-based hairs on glumes and lower lemma.

2.5 Conclusions

This study reports the existence of 2 distinct taxonomic entities within the *L. simplex* complex. These entities can primarily be distinguished by the presence of tubercle-based hairs on glumes and lower lemma. Comparison with a standard species, *Loudetia demeusei* (Figure 2.4), suggests that the 2 entities can be recognized at the specific level. This study thus proposes that the *Loudetia simplex* complex be split into *L. simplex* (Nees) C.E. Hubb. for tuberculate plants and *L. camerunensis* (Stapf) C.E. Hubb. for non-tuberculate plants. Field observation at Buffelskloof near Lydenburg and Pullen Farm near Nelspruit, Mpumalanga Province, South Africa showed that the two species exhibit habitat specificity, in which *L. camerunensis* grows in poorly-drained sandy soils and *L. simplex* occurs in well-drained shallow, stony soils. Although the flowering period overlaps, *L. simplex* appeared to flower first. These differences suggest that the two species are distinct.

2.7 References

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Appendix 2.1. Specimens studied

OUT	Species	Coll. & No.	Locality
DM01	*L. demeusei*	Trauseld 786	Natal, South Africa
DM02	*L. demeusei*	Devred 1598	Leopoldville (= Kinshasa), the DRC
DM03	*L. demeusei*	Devred 2641	Kenge, Kwango, DRC
DM04	*L. demeusei*	Devred 2907	Tono Territ-Fischi, DRC
DM05	*L. demeusei*	Devred 3568	Kianga (Territi - Cungu), DRC
DM06	*L. demeusei*	Drummond 10065	Kawambwa, Zambia
DM07	*L. demeusei*	Germain 2165	Popokabaka, DRC
NT08	*L. simplex* nt	Adam 20351	Mont Nimba, Liberia

OUT	Species	Coll. & No.	Locality
NT09	*L. simplex* nt	Bogdan 1415	Kitale, Kenya
NT10	*L. simplex* nt	Bouxin 1643	Kindama, Rwanda
NT11	*L. simplex* nt	Burtt 1441	Singida District, Tanzania
NT12	*L. simplex* nt	Chapman 7355	Mulanje Mt., Malawi
NT13	*L. simplex* nt	Chase 3636	Nyanga, Zimbabwe
NT14	*L. simplex* nt	Crook 651	Harare, Zimbabwe
NT15	*L. simplex* nt	Davidse 6614	Mutare, Zimbabwe
NT16	*L. simplex* nt	du Toit 2359	KwaZulu-Natal, South Africa
NT17	*L. simplex* nt	Killick 1232	KwaZulu-Natal, South Africa
NT18	*L. simplex* nt	Loveridge 1358	Golomonzi, Zimbabwe
NT19	*L. simplex* nt	Malaisse 2401	Kolwezi, DRC
NT20	*L. simplex* nt	Milne-Redhead 4493	Mwinilunga, Zambia
NT21	*L. simplex* nt	Pauwels 7435	Malamville, Kalimama, Benin
NT22	*L. simplex* nt	Pawek 9091	Viphya Plateau, Mzimba, Malawi
NT23	*L. simplex* nt	Phillips 1353	Nyika Plateau, Malawi
NT24	*L. simplex* nt	Procter 2055	Njombe Dist., Lupembe, Tanzania
NT25	*L. simplex* nt	Rattray 1627	Matobo, Zimbabwe
NT26	*L. simplex* nt	Reekmans 3168	Kumuyange, Burundi
NT27	*L. simplex* nt	Reekmans 8763	Bujumbira, Burundi
NT28	*L. simplex* nt	Reekmans 11004	Bujumbira, Burundi
NT29	*L. simplex* nt	Rose Innes 30184	Ghana
NT30	*L. simplex* nt	Rose Innes 30313	Ghana
NT31	*L. simplex* nt	Rose Innes 31138	Ghana
NT32	*L. simplex* nt	Salesiens 740	Ellisabethville, DRC
NT33	*L. simplex* nt	Simon 172	Lomagundi, Mpinga, Zimbabwe
NT34	*L. simplex* nt	Zimba 839	Kalabo, Zambia
TB35	*L. simplex* tb	Adam 11	Belya, Guinea
TB36	*L. simplex* tb	Biegel 1145	Nyanga, Zimbabwe
TB37	*L. simplex* tb	Burrows 2179	Mutare, Zimbabwe
TB38	*L. simplex* tb	Burrows 7329	Mpumalanga
TB39	*L. simplex* tb	Cleghorn 1854	Mutare, Zimbabwe
TB40	*L. simplex* tb	du Toit 1099	KwaZulu-Natal, South Africa
TB41	*L. simplex* tb	Fisher 1523	Mutare, Zimbabwe
TB42	*L. simplex* tb	Harris 2218	Vakaga, Central Africa Republic
TB43	*L. simplex* tb	Jackson 2	Tegina, Ibadan, Nigeria

Appendix 2.2. A preliminary list of characters and a proposed scoring strategy.
A. Quantitative characters

Char. No.	Character	Within OTU Character variation					Total	Average
		1	2	3	4	5		
1	Length of the culm (mm)							
2	Length of the leaf blade (mm)							

Char. No.	Character	Within OTU Character variation					Total	Average
		1	2	3	4	5		
3	Width of the leaf blade (mm)							
5	Length of the inflorescence (mm)							
7	Length of the longer (longest) pedicel (mm)							
8	Length of the shorter (shortest) pedicel (mm)							
9	Width of the spikelet at the widest point (mm)							
10	Length of ventral lodicule (mm)							
11	Length of dorsal lodicule (mm)							
12	Length of the larger lobe of the lodicule (mm)							
13	Length of lower glume (mm)							
14	Width of lower glume (mm)							
15	Length of the upper glume (mm)							
16	Width of the upper glume (mm)							
17	Length of the lower lemma (mm)							
18	Width of the lower lemma (mm)							
19	Length of the upper lemma (mm)							
20	Width of the upper lemma (mm)							
21	Length of the upper palea (mm)							
22	Width of the upper palea (mm)							
23	Length of awn of the upper lemma (mm)							
24	Length of wing of the upper palea (mm)							
25	Width of wing of the upper palea (mm)							
26	Length of lateral lobes of the upper palea (mm)							
27	Length of callus of the upper floret (mm)							
28	Width of callus of the upper floret at the widest point (mm)							
29	Width of callus of the upper floret at the narrowest point (mm)							
30	Length of the long cell (µm)							
31	Width of the long cell (µm)							
32	Length of the short cell (µm)							
33	Width of the short cell (µm)							
34	Length silica body (µm)							

Char. No.	Character	Within OTU Character variation					Total	Average
		1	2	3	4	5		
35	Width of silica body (μm)							
36	Diameter of the cell of the outer bundle sheath (μm)							
37	Diameter of the cell of the inner bundle sheath (μm)							
38	Length of the basal cell of micro-hair (μm)							
39	Width of the basal cell of micro-hair (μm)							
40	Length of the apical cell of micro-hair (μm)							
41	Width of apical cell of micro-hair (μm)							
42	Length of bulliform cell (μm)							
43	Width of bulliform cell (μm)							
44	Diameter of the largest colourless cell (μm)							
45	Diameter of chlorenchyma cell (μm)							
46	Diameter of mesophyll cell (μm)							
47	Diameter of the 1st order vascular bundle (μm)							
48	Diameter of the 2nd order vascular bundle (μm)							
49	Diameter of the 3rd order vascular bundle (μm)							
50	Distance between the 1st order vascular bundles (μm)							

No.	Definition of the character	Definition of character states and codes assigned
	Qualitative characters	
51	Growth duration	Annual (1) perennial (2)
52	Culm characteristics	Erect (1) decumbent (2)
53	Presence of rhizomes	Absent (1) present (2)
54	Culm branching habit	Simple (1) branched (2)
55	Nature of culm	Slender or wiry (1) stout (2)
56	Presence of coarse hairs on basal leaf sheaths	Absent (1) present (2)
57	Splitting of basal leaf sheaths	Not splitting (1) splitting (2)
58	Presence of coarse hairs on basal	Absent (1) present (2)

No.	Definition of the character	Definition of character states and codes assigned
	culm nodes	
59	Tufting of plants	Not tufted (1) tufted (2)
60	Shape of leaf blade	Filiform (1) linear or expanded (2)
61	Nature of leaf blade	Involute (1) flat (2)
62	Leaves basal or cauline	Mainly basal (1) Mainly cauline (2)
63	Texture of adaxial leaf surface	(Smooth (1) rough or scabrid (2)
64	Presence of coarse hairs on adaxial surface of the basal leaf	Absent (1) present (2)
65	Type of coarse hairs on adaxial surface of the basal leaf	Non-tuberculate (1) tuberculate (2)
66	Presence of fine hairs on adaxial surface of the basal leaf	Absent (1) present (2)
67	Presence of leaf pseudopetiole	Absent (1) present (2)
68	Presence of ligule	Absent (1) present (2)
69	Inflorescence type	Spikelike panicle (1) contracted panicle (2) open panicle (3)
70	Presence of tufts of hairs at the lowest infloresence branch	Absent (1) present (2)
71	Presence of a collar-like structure at the base of inflorescence primary branch	Absent (1) present (2)
72	First inflorescence node whorled or not	Absent (1) present (2)
73	Type of lower floret	Neuter (1) male (2)
74	Presence of rachilla extension	Absent (1) present (2)
75	Spikelet aggregation	Diads (1) triads (2)
76	Relative lengths of pedicels per spikelet aggregation	More or less equal (1) unequal (2)
77	Spikelet aggregation consistently with short and long pedicels	No (1) Yes (2)
78	Shape of apex of lower glume	Acute (1) rounded (2), truncate (3)
79	Presence of fine hairs on lower glume back	Absent (1) present (2)
80	Type of fine hairs on lower glume back	Linear (1) club-shaped (2)
81	Distribution of fine hairs on lower glume back	Restricted to 2 lateral veins (1) distributed along 3 veins (2)
82	Presence of coarse hairs on lower glume back	Absent (1) present (2)

No.	Definition of the character	Definition of character states and codes assigned
83	Type of coarse hairs on lower glume back	Non-tuberculate (1) tuberculate (2)
84	Distribution of coarse hairs on lower glume back	Restricted to 2 lateral veins (1) distributed along 3 veins (2)
85	Distribution of coarse hairs along the length of lower glume	Restricted to the upper ¾ of the glume (1) more than the upper ¾ of the glume (2)
86	Number of lobes on lower glume apex	Entire (1) bilobed (2)
87	Outline of the apex of lower glume	Pointed (1) emarginate (2)
88	Number of veins on lower glume	3 (1) 5 (2)
89	Presence of awn on lower glume	Absent (1) present (2)
90	Shape of apex of upper glume	Acute (1) rounded (2), truncate (3)
91	Presence of fine hairs on upper glume back	Absent (1) present (2)
92	Type of fine hairs on upper glume	Linear (1), club-shaped (2)
93	Distribution of fine hairs on upper glume back	Restricted to 2 lateral veins (1) distributed along 3 veins (2)
94	Presence of coarse hairs on upper glume back	Absent (1) present (2)
95	Type of coarse hairs on upper glume back	Non-tuberculate (1) tuberculate (2)
96	Distribution of coarse hairs on upper glume back	Restricted to 2 lateral veins (1) distributed along 3 veins (2)
97	Distribution of coarse hairs along the length of upper glume back	Restricted to the upper ¾ of the glume(1) over the upper ¾ of the glume
98	Number of lobes on upper glume apex	Entire (1) bilobed (2)
99	Outline of the apex of upper glume	Pointed (1) emarginate (2)
100	Number of veins on upper glume	3 (1) 5 (2)
101	Presence of short awn on upper glume apex	Absent (1) present (2)
102	Shape of apex of the lower lemma	Acute (1) rounded (2), truncate (3)
103	Presence of fine hairs on back of the lower lemma	Absent (1) present (2)
104	Type of fine hairs on back of the lower lemma	Linear (1) club-shaped (2)
105	Distribution of coarse hairs on back of the lower lemma	Restricted to 2 lateral veins (1) distributed along 3 veins (2)

No.	Definition of the character	Definition of character states and codes assigned
106	Distribution of fine hairs on back of the lower lemma	Only the upper ¾ of the glume (1) extended below the upper ¾ of the lemma (2)
107	Presence of coarse hairs on back of the lower lemma	Absent (1) present (2)
108	Type of coarse hairs on back of the lower lemma	Non-tuberculate (1) tuberculate (2)
109	Distribution of coarse hairs on back of the lower lemma with respect to ribs	Restricted to 2 lateral veins (1) distributed along 3 veins (2)
110	Distribution of coarse hairs along the length of the lower lemma	Only the upper ¾ of the glume (1) extended below the upper ¾ of the lemma (2)
111	Number of lobes on apex of the lower lemma	Entire (1) bilobed (2)
112	Outline of the apex of the lower lemma	Pointed (1) emarginate (2)
113	Number of veins on the lower lemma	3 (1) 5 (2)
114	Presence of short awn on the lower lemma	Absent (1) present (2)
115	Number of lobes on the lower palea	Entire (1) bilobed (2)
116	Number of veins on the lower palea	2 (1) 3 (2)
117	Size of the lower lemma relative to the upper glume	More or less equal (1), unequal (2)
118	Texture of the lower lemma	Chartaceous (1) Coriaceous (2)
119	Presence of awns on the upper lemma	Absent (1) present (2)
120	Position of awn of the upper lemma	Median only (1) median and lateral (2)
121	Presence of distinct column and bristle on the awn of the upper lemma	Absent (1) present (2)
122	Number of veins on the upper lemma	5 (1) 7 (2) 9 (3) 11 (4)
123	Presence of thickened vein on the upper lemma	Absent (1) present (2)
124	Apex of the upper lemma	Awned (1) mucronate (2)
125	Presence of coarse hairs on the upper lemma	Absent (1) present (2)
126	Presence of fine hairs on the upper lemma	Absent (1) present (2)
127	Number of lobes on the lower palea	Entire (1) bilobed (2)

No.	Definition of the character	Definition of character states and codes assigned
128	Number of veins on the lower palea	2 (1) 3 (2)
129	Number of stamens in the upper floret	2 (1) 3 (2)
130	Outline of callus of the upper floret	Parallel-sided (1) divergent- sided towards the apex (2)
131	Shape of apex of callus of the upper floret	Pungent (1) truncate (2) oblique (3)
132	Number of lobes of apex of callus of the upper floret	Entire (1) bidentate (2)
133	Extent of incision of apex of callus of the upper floret	Narrowly-incised (1), deeply-incised (2)
134	Relative size of teeth of callus of the upper floret	More or less equal (1) unequal (2)
135	Orientation of callus of the upper floret	Straight (1) curved (2)
136	Presence of rudimental tooth on callus of the upper floret	Absent (1) present (2)
137	Presence of beard on callus of the upper floret	Absent (1) present (2)

Appendix 2.3: Final list of quantitative and qualitative characters.

No.	Definition of the character	Definition of character states and codes assigned
	Quantitative characters	
1	Culm height	From rhizomes to the inflorescence base
2	Culm diameter	Measured from the plant base
3	Culm node diameter	From the plant base
4	Leaf blade length	From ligule attachment to the apex of the second leaf from the plant base
5	Leaf blade width	At the widest point on the second leaf from the base
6	Inflorescence length	From lowest node of the inflorescence to the apex
7	Spikelet width	At the widest point of spikelet from the middle of the inflorescence
8	Lower glume length	From rachis attachment to the glume apex
9	Upper glume length	From rachis attachment to the glume apex
10	Lower lemma length	From rachis attachment to the lemma apex
11	Upper lemma length	From rachis attachment to the lemma apex

No.	Definition of the character	Definition of character states and codes assigned
12	Upper lemma lobes	From base of the lobe to the lemma apex
13	Awn length	From the sinus to the apex of the awn
14	Awn column length	From lemma attachment to column / bristle attachment
15	Lower palea length	From rachis attachment to the palea apex
16	Upper palea length	From rachis attachment to the palea apex
17	Length of callus	From apex of callus to the upper lemma attachment
18	Width of callus	Across the apex of the callus
19	Ratio of leaf blade length to leaf blade width	
20	Ratio of upper glume length to lower glume length	
21	Ratio of upper glume length to lower lemma length	
22	Ratio of lower lemma length to upper lemma length	
23	Ratio of lower palea length to upper palea length	
24	Ratio of callus length to callus width	
	Qualitative characters	
25	Culms	Wiry (1); stout (2)
27	Mature basal sheaths	Not splitting (1); splitting (2)
28	Presence of hairs on culm nodes	Absent (1); present (2)
29	Shape of leaf blade	Filiform or involute (1); flat (2)
30	Presence of tubercle-based hairs on adaxial leaf blade surfaces	Absent (1); present (2)
31	Presence of hairs on the lower node of the inflorescence	Absent (1); present (2)
32	Shape of apex of lower glume	Rounded (1); truncate (2)
33	Presence of tubercle-based hairs on lower glume	Absent (1); present (2)
34	Shape of apex of upper glume	Rounded (1); truncate (2)
35	Presence of tubercle-based hairs on upper glume	Absent (1); present (2)
36	Shape of apex of lower lemma	Acute (1); rounded (2)
37	Presence of tubercle-based hairs on lower lemma	Absent (1); present (2)

Appendix 2.4. Data matrix of quantitative and qualitative characters for *Loudetia demeusei* (DM), *L. simplex* nt (without tubercle-based hairs) and *L. simplex* tb (with tubercle-based hairs on glumes and lower lemma). Abbreviations: l = length, w = width, G1 = lower glume, G2 = upper glume, L1 = lower lemma, L2 = upper lemma, P1 = lower palea, P2 = upper palea, Lf = leaf, tb = tubercle-based hair, inflo. = inflorescence, Pres = presence, bld = blade.

A Quantitative morphology

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
OTU	Culm l	Leaf l	Leaf w	Inflo. l	G1 l	G2 l	L1 l	L2 l	L2 lobe l	Awn l	P1 l	P2 l	Callus l	Callus w	Lf L : Lf w	G2 : G1	G2 l : L1 l	L1 l : L2 l	P1 : P2 l	Callus l : callus w
DM01	837	361	3.5	387	4	8.57	7.39	4.4	0.4	30	6.82	4.96	0.85	0.26	103.1	2.13	1.16	1.68	1.38	3.27
DM02	1695	241	4	381	5.4	11.5	9.92	4.8	0.6	56	6.97	5.78	1.07	0.19	60.3	2.14	1.16	2.08	1.21	5.63
DM03	1686	487	6.5	338	6	12	10.1	5.1	0.9	74	7.16	5.86	1.22	0.24	74.9	1.99	1.18	2.01	1.22	5.08
DM04	913	396	3.8	127	5.9	13.9	10.3	4.9	1.2	71	6.91	5.29	1.05	0.20	104.2	2.36	1.35	2.12	1.31	5.25
DM05	1159	487	6	280	5.2	11.9	9.42	5	0.7	59	6.73	5.83	1.02	0.25	81.2	2.30	1.26	1.90	1.15	4.08
DM06	914	236	2.5	270	7.3	12.8	10.6	4.9	0.8	65	7.26	5.78	1.11	0.18	94.4	1.76	1.21	2.17	1.26	6.17
DM07	898	559	5	335	4.8	11.3	9.04	5	0.6	65	7.12	5.61	2.22	0.23	111.8	2.33	1.25	1.81	1.27	9.65
NT08	441	130	1	158	5	12.6	11	4.6	1	46	6.36	6.17	0.57	0.31	130.0	2.50	1.14	2.39	1.03	1.84
NT09	563	224	2	197	4.4	9.9	9.56	4.2	1.1	39	5.18	4.46	0.64	0.24	111.9	2.24	1.04	2.28	1.16	2.67
NT10	1115	309	3.1	234	4.1	10.4	10.1	4.6	1.9	51	7.3	5.45	0.72	0.4	99.6	2.54	1.03	2.18	1.34	1.80
NT11	517	167	2.2	122	5	11.1	10.7	4.3	1	43	7.99	6.05	0.89	0.21	76.7	2.21	1.04	2.47	1.32	4.24
NT12	779	250	3	236	3.3	9.35	8.72	4.7	1.2	36	6.51	5.61	0.4	0.34	83.3	2.82	1.07	1.86	1.16	1.18
NT13	581	186	2	191	4.1	10.5	10.3	4.6	1.1	37	7.08	5.74	1.01	0.35	93.0	2.56	1.02	2.21	1.23	2.89
NT14	864	160	2	150	6.1	13.9	11.1	5.8	1.1	49	8.2	6.39	0.91	0.31	80.0	2.30	1.25	1.93	1.28	2.94
NT15	928	207	2	138	4.4	10.9	10.2	4.9	1.7	46	7.02	6.37	0.82	0.28	103.5	2.46	1.07	2.07	1.10	2.93
NT16	781	298	2.3	185	5.4	12.2	9.72	4.6	0.9	49	7.23	6.18	0.65	0.28	127.7	2.25	1.26	2.09	1.17	2.32
NT17	204	112	2	180	5.4	9.21	10.9	4.2	0.8	33	6.31	4.38	0.93	0.23	55.9	1.71	0.84	2.58	1.44	4.04
NT18	1070	250	2.5	173	3.9	10.5	10.2	4.8	0.5	34	6.49	5.34	0.59	0.29	100.0	2.67	1.03	2.11	1.22	2.03
NT19	787	163	3.8	179	3.8	9.75	8.55	4.6	1.8	38	6.45	5.81	0.74	0.35	42.6	2.59	1.14	1.84	1.11	2.11
NT20	953	114	2.5	139	4.1	11	10.7	5.7	1.9	61	7.65	6.72	0.84	0.31	45.6	2.71	1.03	1.89	1.14	2.71
NT21	1180	453	3.8	273	4.1	10.1	9.13	4.9	1.2	50	6.63	5.61	0.66	0.31	119.2	2.49	1.11	1.86	1.18	2.13
NT22	534	182	2	103	4.9	12.6	11.6	5.7	2.2	53	7.47	6.27	0.7	0.38	91.0	2.57	1.09	2.04	1.19	1.84
NT23	558	171	1.8	93	6.2	11.7	10.6	5.2	1.5	35	7.49	7.82	0.75	0.34	97.4	1.89	1.04	2.04	1.14	2.21
NT24	547	266	1.8	133	4.6	9.84	10.1	4.1	2.4	48	7.04	6.29	0.65	0.39	145.4	2.14	0.98	2.44	1.12	1.67
NT25	1027	168	3	85	5.2	14.6	12.5	4.6	1	57	7.62	6.21	0.75	0.25	55.8	2.84	1.17	2.75	1.23	3.00
NT26	549	218	3.3	62.5	4.9	11.5	10.2	5.7	1.3	55	7.35	6.17	0.82	0.46	65.3	2.36	1.12	1.79	1.19	1.78
NT27	882	155	2	221	3.9	10.6	9.86	4.9	1.2	47	6.62	5.92	0.71	0.34	77.5	2.69	1.07	2.00	1.12	2.09
NT28	804	173	3	138	4.1	10.7	10.7	5.2	0.7	34	7.79	6.47	0.62	0.32	57.7	2.58	1.00	2.07	1.20	1.94
NT29	845	280	4	185	4.5	10.8	11.4	5.5	0.7	54	5.9	5.42	0.96	0.35	70.0	2.41	0.94	2.09	1.09	2.74
NT30	1332	236	5.4	360	4.9	12.8	11	5.9	2.1	51	7.8	7.19	0.65	0.34	43.9	2.63	1.17	1.87	1.08	1.91
NT31	1447	481	3	401	4.8	13.8	11.3	5.6	1.1	53	7.73	6.46	0.93	0.23	160.3	2.88	1.22	2.04	1.20	4.04
NT32	986	215	3	208	4.4	10.7	9.24	3.7	0.8	50	6.23	5.23	0.68	0.29	71.7	2.47	1.16	2.50	1.19	2.34
NT33	1050	198	3	220	4.9	13.9	12.8	6.1	1	50	9.38	6.83	0.97	0.32	66.0	2.83	1.09	2.10	1.37	3.03

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
OTU	Culm 1	Leaf 1	Leaf w	Inflo. 1	G1 1	G2 1	L1 1	L2 1	L2 lobe 1	Awn 1	P1 1	P2 1	Callus 1	Callus w	Lf L. : Lf w	G2 : G1	G2 1 : L1 1	L1 1 : L2 1	P1 1 : P2 1	Callus 1 : callus w
NT34	858	240	3	330	3.8	12.9	11.2	5.1	0.9	51	7.98	6.76	0.77	0.23	80.0	3.37	1.15	2.19	1.18	3.35
TB35	599	241	1.2	286	4.2	12	10.3	4.1	1.2	46	6.34	4.86	0.81	0.34	209.6	2.84	1.16	2.50	1.30	2.38
TB36	757	169	2.5	235	4.3	8	6.98	3.4	1.2	31	4.97	4.1	0.88	0.2	67.4	1.88	1.15	2.03	1.21	4.40
TB37	1027	204	2	128	4.5	9.46	9.22	4.3	1.4	32	6.46	5.35	0.59	0.33	102.0	2.09	1.03	2.15	1.21	1.79
TB38	738	137	2.2	151	6.1	9.79	5.16	5	1.1	36	5.8	5.03	0.59	0.34	62.1	1.60	1.90	1.03	1.15	1.74
TB39	896	225	2.4	204	4.4	10.6	8.89	7.1	0.7	44	1.74	1.52	0.91	0.42	93.8	2.43	1.19	1.25	1.14	2.17
TB40	581	203	1	150	5	9.28	7.8	4.3	1.1	32	5.77	5.22	0.98	0.23	202.5	1.87	1.19	1.83	1.11	4.26
TB41	707	177	3.4	155	4	8.75	7.56	3.7	1.2	35	5.3	4.55	0.71	0.23	52.1	2.19	1.16	2.02	1.16	3.09
TB42	1344	300	3.5	254	4.1	9.27	8.25	4.5	0.8	35	4.6	4.16	0.23	0.23	85.7	2.28	1.12	1.82	1.11	1.00
TB43	1045	230	3	351	4.8	10.3	10.5	4.7	1.1	49	6.4	5.62	0.62	0.25	76.7	2.13	0.98	2.23	1.14	2.48
TB44	619	239	3	148	5	8.69	7.41	4.3	0.7	31	5.22	4.58	0.81	0.25	79.5	1.75	1.17	1.71	1.14	3.24
TB45	897	200	2.8	180	3.1	9.57	9.58	2.8	0.4	38	3.21	2.77	0.49	0.3	72.7	3.08	1.00	3.45	1.16	1.63
TB46	374	246	2.9	281	4	10.9	9.71	4.4	0.9	42	6.42	5.27	0.83	0.35	85.4	2.72	1.12	2.20	1.22	2.37
TB47	371	116	2	195	4.6	7.89	7.54	4.1	1.2	22	4.97	4.23	0.9	0.24	58.0	1.70	1.05	1.83	1.17	3.75
TB48	485	220	2	145	6.6	11.6	8.92	4.8	1	35	7.05	6.36	0.85	0.23	110.0	1.76	1.30	1.88	1.11	3.70
TB49	1048	255	2.7	190	2.6	8.12	7.6	3.6	1	46	5.08	4.81	0.67	0.24	94.4	3.12	1.07	2.14	1.06	2.79
TB50	957	142	2	215	4.3	8.99	8.74	4.4	1.3	32	5.52	4.68	1.09	0.2	71	2.08	1.03	1.99	1.18	5.45
TB51	481	204	2	145	4.6	9.09	8.58	3.6	1	35	5.17	4.37	0.55	0.24	102	1.98	1.06	2.41	1.18	2.29

Appendix 2.4B Qualitative morphology. OTU. = operational taxonomic unit

OTU	Species	123456789	111 012
DM01	*Loudetia demeusei*	222221223	212
DM02	*Loudetia demeusei*	222221224	212
DM03	*Loudetia demeusei*	222211124	212
DM04	*Loudetia demeusei*	222221224	231
DM05	*Loudetia demeusei*	222221124	212
DM06	*Loudetia demeusei*	222212224	212
DM07	*Loudetia demeusei*	222221224	211
NT08	*Loudetia simplex* without tubercle-based hairs on glumes	112211213	111
NT09	*Loudetia simplex* without tubercle-based hairs on glumes	222211313	111
NT10	*Loudetia simplex* without tubercle-based hairs on glumes	122221313	111
NT11	*Loudetia simplex* without tubercle-based hairs on glumes	122221313	111
NT12	*Loudetia simplex* without tubercle-based hairs on glumes	211211313	111
NT13	*Loudetia simplex* without tubercle-based hairs on glumes	112211314	111
NT14	*Loudetia simplex* without tubercle-based hairs on glumes	121211313	121
NT15	*Loudetia simplex* without tubercle-based hairs on glumes	122211313	111
NT16	*Loudetia simplex* without tubercle-based hairs on glumes	122211213	111
NT17	*Loudetia simplex* without tubercle-based hairs on glumes	122212214	111
NT18	*Loudetia simplex* without tubercle-based hairs on glumes	122221314	111
NT19	*Loudetia simplex* without tubercle-based hairs on glumes	122221313	111
NT20	*Loudetia simplex* without tubercle-based hairs on glumes	122221313	111

OTU	Species	123456789	111 012
NT21	*Loudetia simplex* without tubercle-based hairs on glumes	222221313	111
NT22	*Loudetia simplex* without tubercle-based hairs on glumes	122221313	111
NT23	*Loudetia simplex* without tubercle-based hairs on glumes	122221313	121
NT24	*Loudetia simplex* without tubercle-based hairs on glumes	122211313	121
NT25	*Loudetia simplex* without tubercle-based hairs on glumes	121221313	121
NT26	*Loudetia simplex* without tubercle-based hairs on glumes	111211313	111
NT27	*Loudetia simplex* without tubercle-based hairs on glumes	122221313	111
NT28	*Loudetia simplex* without tubercle-based hairs on glumes	111221314	121
NT29	*Loudetia simplex* without tubercle-based hairs on glumes	222121214	111
NT30	*Loudetia simplex* without tubercle-based hairs on glumes	222211214	111
NT31	*Loudetia simplex* without tubercle-based hairs on glumes	222111314	111
NT32	*Loudetia simplex* without tubercle-based hairs on glumes	222221314	111
NT33	*Loudetia simplex* without tubercle-based hairs on glumes	221221313	111
NT34	*Loudetia simplex* without tubercle-based hairs on glumes	221211313	121
TB35	*Loudetia simplex* with tubercle-based hairs on glumes	112121323	221
TB36	*Loudetia simplex* with tubercle-based hairs on glumes	222222223	222
TB37	*Loudetia simplex* with tubercle-based hairs on glumes	222221223	222
TB38	*Loudetia simplex* with tubercle-based hairs on glumes	221211224	212
TB39	*Loudetia simplex* with tubercle-based hairs on glumes	222211224	222
TB40	*Loudetia simplex* with tubercle-based hairs on glumes	122221224	212
TB41	*Loudetia simplex* with tubercle-based hairs on glumes	222221223	211
TB42	*Loudetia simplex* with tubercle-based hairs on glumes	222121223	221
TB43	*Loudetia simplex* with tubercle-based hairs on glumes	222221224	211
TB44	*Loudetia simplex* with tubercle-based hairs on glumes	222211224	211
TB45	*Loudetia simplex* with tubercle-based hairs on glumes	122221324	211
TB46	*Loudetia simplex* with tubercle-based hairs on glumes	122221223	211
TB47	*Loudetia simplex* with tubercle-based hairs on glumes	212222224	222
TB48	*Loudetia simplex* with tubercle-based hairs on glumes	222221224	211
TB49	*Loudetia simplex* with tubercle-based hairs on glumes	122211324	212
TB50	*Loudetia simplex* with tubercle-based hairs on glumes	222121223	212
TB51	*Loudetia simplex* with tubercle-based hairs on glumes	122221123	111
TB52	*Loudetia simplex* with tubercle-based hairs on glumes	122111223	212
TB53	*Loudetia simplex* with tubercle-based hairs on glumes	122121223	212
TB54	*Loudetia simplex* with tubercle-based hairs on glumes	122212324	221
TB55	*Loudetia simplex* with tubercle-based hairs on glumes	122122223	212