

# New circumscription of the tribe Limoselleae (Scrophulariaceae) that includes the taxa of the tribe Manuleeae

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Cladistic analyses by maximum parsimony and Bayesian inference methods of chloroplast and nuclear sequence data indicate a new position for *Limosella* (Scrophulariaceae). Following this result, a new circumscription of the tribe Limoselleae is presented where the tribe Manuleeae is included in Limoselleae. Further, the study discloses that the genus *Sutera* is paraphyletic in its present circumscription, but that the two sections of *Sutera*, *Sutera* and *Chaenostoma*, are monophyletic. To accommodate these findings the genus *Chaenostoma* is re-established. Furthermore, the genus *Jamesbrittenia* recently expanded by Hilliard is shown to be a highly supported monophyletic group in its current circumscription. © 2004 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2004, 146, 453–467.

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## INTRODUCTION

Members of the tribe Manuleeae Benth (Scrophulariaceae) are common elements of the Cape Flora. Recently, the tribe Selagineae Horan. (former family Selaginaceae Choisy) was included in the Manuleeae by Kornhall, Heidari & Bremer (2001). The tribe now comprises about 625 species in 28 genera and is nearly entirely southern African in its distribution. The only exceptions to this are one species of *Jamesbrittenia* Kuntze that extends as far as to India, one of *Hebestretia* L. that extends northwards to Eritrea, and the monotypic genus *Barthlottia* Fischer that, together with a species of *Selago* L., occurs on Madagascar. The aforementioned study indicated that the current circumscriptions of the genera *Sutera* Roth and *Manulea* L. are incorrect. Fieldwork in the year 2001 (by the first author) provided material that made it possible to look more closely into these relationships and also to

test more thoroughly the taxonomic status of *Jamesbrittenia*, a genus that encompasses many taxa formerly considered to belong to *Sutera* (Hilliard, 1994). New material of the cosmopolitan genus *Limosella* L. was also included in the study after an unpublished molecular analysis of Scrophulariaceae (B. Oxelman, P. Kornhall, R. G. Olmstead & B. Bremer, unpubl. data) pointed towards a connection between the tribe Manuleeae and the genus *Limosella*.

Historically, the plants now belonging to *Manulea*, *Sutera* and *Jamesbrittenia* have an intertwined taxonomy. *Manulea*, today with 74 species, was erected by Linnaeus 1767, *Sutera*, erected by Roth 1807, now has 49 species, and *Jamesbrittenia*, now encompassing 83 species, was erected by Kuntze 1891. Many plants of *Sutera* have synonyms in *Manulea*, and *Jamesbrittenia* in *Sutera*.

The genus *Manulea* fide Hilliard (1994) is characterized by indumentum with balloon-tipped eglandular hairs; inflorescence a thyrse, raceme or panicle; corolla tube cylindrical, often abruptly expanded below limb and bent; stamens inserted halfway up in the corolla tube or higher with anthers always

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included or the anterior pair just visible in the mouth of the corolla. The genus was divided by Hilliard (1994) into four sections: *Dolichoglossa*, *Thyrsoflorae*, *Manulea* and *Medifixae*.

*Sutera* fide Hilliard (1994) is characterized by flowers usually alone in leaf-axils; corolla tube most often funnel-shaped, broad in the mouth; antecous pair of anthers exerted (in all but two species). Hilliard (1994) divided the genus into two sections; a small section *Sutera* encompassing three species and among them the type of the genus, *S. foetida* Roth, and a larger section *Chaenostoma* with 46 species. The latter section corresponds closely to Bentham's (1846) description of the genus *Chaenostoma*. The name means gaping mouth, pointing to the funnel-formed corollas. One of the main differences between *Sutera* and *Manulea*, according to Hilliard (1994), is the inclusion or exertion of the anthers, but it should be noted that in the three species of section *Sutera*, two have included anthers and thus resemble the genus *Manulea*. According to Hilliard (1994), *Sutera* has semi-annular nectariferous glands at the base of the ovaries and *Manulea* has lateral glands. Other characters are shared by the two genera, indicating that they are closely related.

The use of the name *Jamesbrittenia* was, until Hilliard's (1994) monograph of the Manuleeae, more or less restricted to the species *J. dissecta* Kuntze. The genus, fide Hilliard, has 83 species, most of which were earlier considered parts of *Sutera*. They are characterized by a calyx divided almost to the base, an abruptly expanded corolla tube with a band of clavate hairs in the throat, the anthers usually included, the posterior filaments usually pubescent and decurrent down the corolla tube, and a short included and minutely bifid stigma. Most of our understanding of the morphology of Manuleeae emanates from the detailed monographs written by Hilliard (1994, 1999). As her monographs are the most complete taxonomic treatments of taxa belonging to the tribe, we also follow her terminology in our descriptions and keys.

The genus *Limosella*, the mudworts, has a world-wide distribution with 18 described species. They are small, aquatic to semi-aquatic herbs, typically growing in mud and/or shallow waters. The leaves are more or less rosulate, subulate or cylindrical in shape, but can be differentiated into a petiole, and with a spatulate to ovate blade. Many species are heteroblastic and form submerged forms as well as land forms, and forms with swimming leaves. The small flowers are usually pedicellate, seldom sessile, and are open when present in air, but half to wholly closed when submerged in mud or water. The calyx is campanulate, five- (rarely four-) lobed. The white, pink, blue or lilac corolla consists of a cylindrical tube and a limb with five (rarely four) lobes with an indumentum of eglandular

and glandular hairs. The number of stamens is typically four and the anthers are unithecal. The two-locular ovary carries a lingulate style with a rounded, sometimes weakly bifid, stigma. The fruit is small and opens with two valves, with the number of yellowish to brown seeds varying from three to over a hundred. The genus was placed in the tribe Sibthorpieae by Bentham (1846) and in Gratioleae by Hallier (1903), and was last revised by Glück (1934). Dumortier (1827) established the tribe Limoselleae for *Limosella*.

The aims of this study were to establish a phylogeny that could be used to answer the following questions: are *Manulea*, *Jamesbrittenia* and *Sutera* monophyletic in their current circumscriptions, do the diagnostic features used to circumscribe and distinguish these genera reflect evolutionary history, and what is the taxonomic position of *Limosella*?

## MATERIAL AND METHODS

### CHOICE OF TAXA AND GENES

We generated molecular data sets of sequences from the chloroplast regions *ndhF* and *trnT-F*, and from the nuclear ITS region, using already published sequences as well as new sequencing. To achieve a sampling as representative as possible, we sought taxa from all subgeneric groups of *Jamesbrittenia*, *Manulea* and *Sutera* recognized by Hilliard (1994). We sampled major groups of the Lamiales *sensu* APGII (The Angiosperm Phylogeny Group, 2003) to ascertain the position of *Limosella*. *Olea europea* was chosen as outgroup for the analysis of *ndhF* as Oleaceae has been shown to occupy a basal position in the Lamiales (Oxelman, Backlund & Bremer, 1999). In the combined and in the ITS analyses, we used *Buddleja* as outgroup, since it has been shown to be closely related to the *Scrophularia/Verbascum/Manuleeae* clade (Kornhall *et al.*, 2001; Olmstead *et al.*, 2001; Bremer *et al.*, 2002). We sequenced *ndhF* as the region has been shown to carry information at this level of phylogeny in related taxa (Oxelman *et al.*, 1999). *TrnT-F* was chosen to enhance resolution in more closely related taxa as the introns seem to have a faster substitution rate and hence yield higher phylogenetic resolution. By *trnT-F* we mean the whole region between the *trnT* (UGU) and the *trnF* (GAA) genes, including exons and intron of the *trnL* (UAA) gene and the two intergenic spacers. After indication of a hybrid event we also sequenced the nuclear internal transcribed spacer, ITS, in order to reveal discrepancy between the evolutionary histories of the nuclear and the chloroplast genome. Sequences from the ITS region have been widely used for phylogenetic purposes especially in closely related taxa (Baldwin, 1992; Baldwin *et al.*,

1995; Andreasen & Bremer, 2000; Zimmer *et al.*, 2002). All investigated species together with EMBL/GenBank accession numbers are shown in Appendix 4. From the sequences achieved we produced three data sets for analysis, one of *ndhF* to obtain a broader overview of the position of *Limosella* and *Jamesbrittonia*, and two data sets, one with the nuclear ITS sequences, and a combined of all three genes in order to investigate the *Manulea/Sutera* complex.

#### SEQUENCING AND ALIGNMENT

Extraction and PCR amplification was carried out following the protocols described in Kornhall *et al.* (2001). The ITS primers used are shown in Appendix 1. We performed sequencing on a MegaBACE 1000 DNA analysis system (Amersham Biosciences) following the protocol of the manufacturer. Sequenced fragments were assembled and edited using the software SEQUENCHER 3.1.1 (Gene Codes Corporation, 1991), and were thereafter imported into SE-AL alignment software (Rambaut, 1995) for alignment by eye. We excluded from the analyses very variable parts of the *trnT-F* and ITS matrices when we could not ascertain homology. The aligned matrices are available from the correspondence author.

#### PHYLOGENETIC METHODS

Phylogenetic methods used were maximum parsimony (MP) and Bayesian posterior probabilities (PP). We performed parallel analyses with MP and PP on all data sets. All MP analyses were performed with the PAUP\* ver. 4.0b2a software (Swofford, 1999). Since several authors (Källersjö, Albert & Farris, 1999; Senblad & Bremer, 2000) have pointed out that there is no justification for a priori weighting of codon positions when using parsimony, we weighted all positions equally.

For the PP analyses we used the program MRBAYES ver. 2.01 (Huelsenbeck & Ronquist, 2001). To evaluate the runs we ran three independent analyses from random prior trees on all data sets, each with four heated chains. We plotted the support values for important nodes to obtain a measure of how well the chains had reached stationary following Huelsenbeck *et al.* (2002) and Leache & Reeder (2002), and estimated the burn-in by plotting the logarithm of the likelihood. We made preliminary runs with different models and as choice of model did not apparently change the result of the runs, we chose a model that made it possible to evaluate different parameters and followed our previous study (Kornhall *et al.*, 2001). The model chosen for PP analyses was the general time reversible (GTR) model.

For the *ndhF* data set, the MP analyses consisted of a heuristic and a jackknife analysis. The heuristic analysis was run with 15 addition sequence replicates and TBR branch swapping. The jackknife was run with 10 000 jackknife replicates, 37% of characters deleted, 'jac' resampling method used (Farris *et al.*, 1996), and we used NNI branch-swapping. The PP analysis on the *ndhF* was made with site-specific rates and site partition by codon. The chains were run for 220 000 generations.

For the ITS, a heuristic MP search was carried out with a restraint of no more than 1000 trees saved in every addition sequence replicate. This was done to shorten computing time. The analysis was run with ten addition sequence replicates. Otherwise, the MP analysis was carried out as on the *ndhF* data set with the exception that the jackknife was done with 20 000 replicates. In the Bayesian analysis we used a model with gamma distributed rates instead of site-specific rates, otherwise the settings were identical to the *ndhF* analysis.

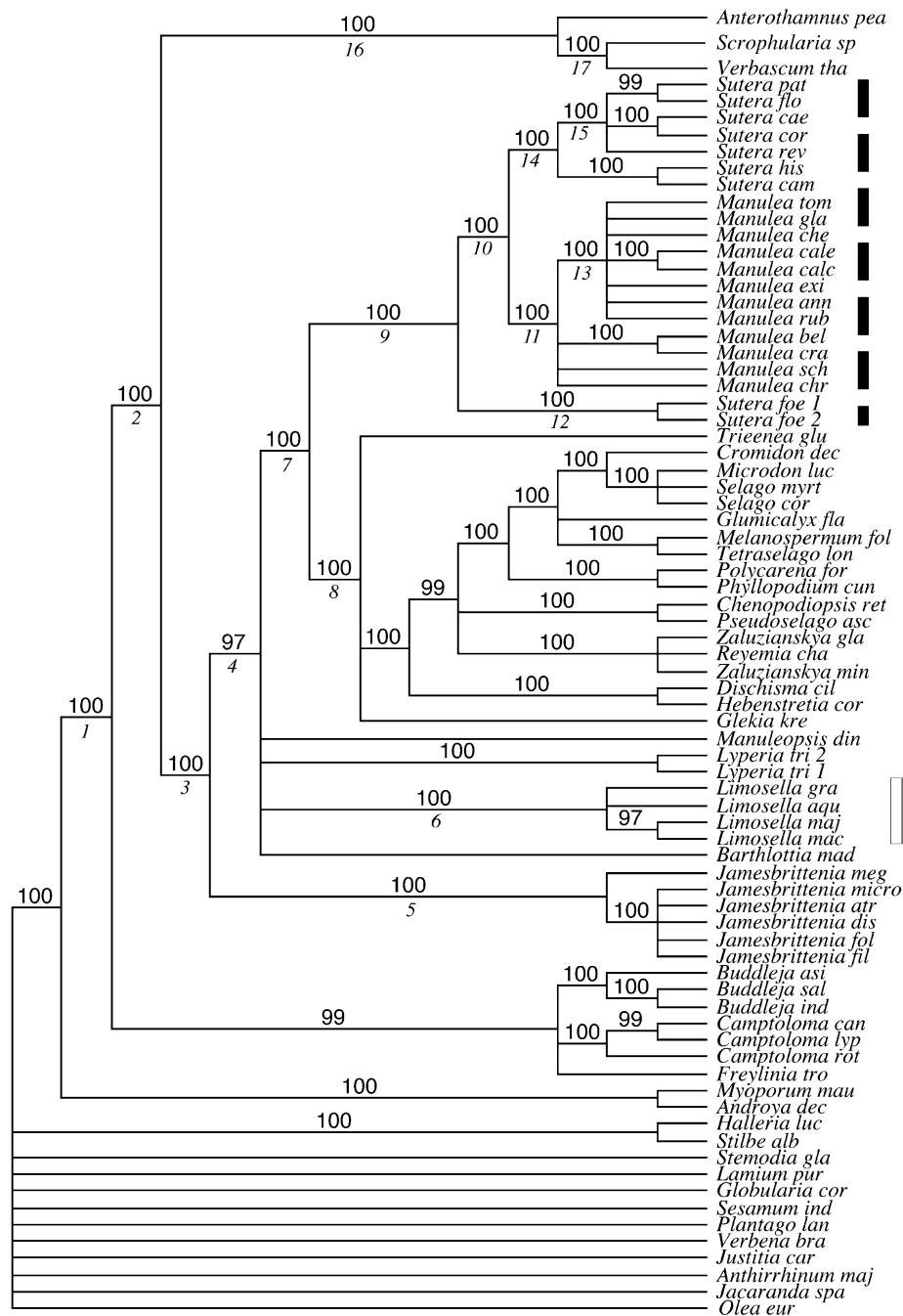
The setting of the analyses of the combined data set was identical to the ITS, with the exception that the MP heuristic search was done as on the *ndhF* data.

#### MORPHOLOGY

Morphological traits were investigated using herbarium material. Flowers were studied after rehydration in heated water with a little detergent added. Ovaries from selected taxa were studied under a dissecting microscope. Along with the voucher material for the sequencing, type material of all species of *Sutera* was studied. We checked especially characters used by Hilliard (1994) to discriminate between *Manulea* and *Sutera*.

#### RESULTS

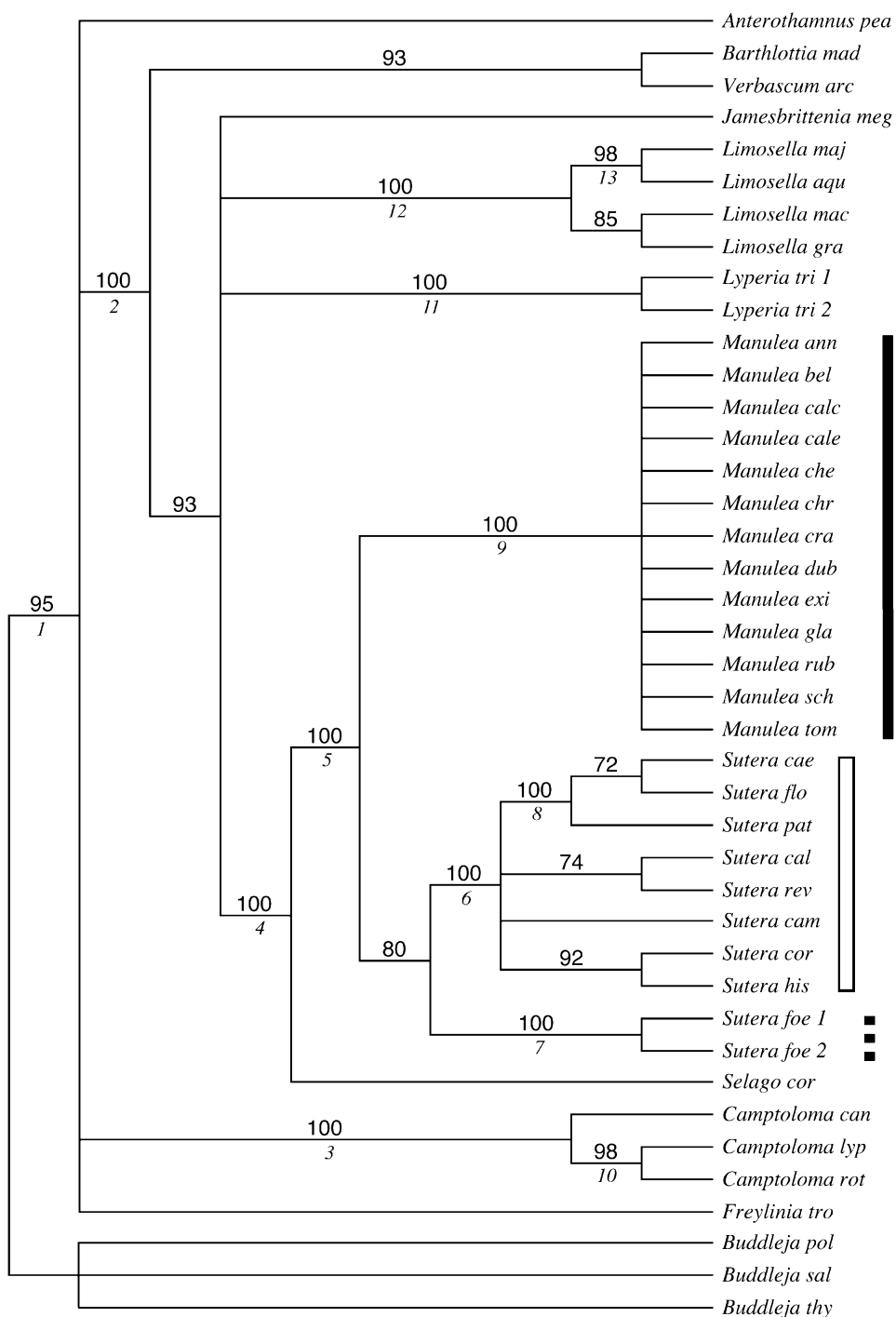
This study presents 22 new *trnT-F*, 26 new ITS and 41 new *ndhF* sequences. The *ndhF* data set consists of 77 taxa and 2260 unordered equally weighted characters, 1337 of which are constant and 548 (24%) parsimony-informative. The heuristic MP search yielded 18 157 equally parsimonious trees of length 2374, with consistency index, CI = 0.57, and retention index, RI = 0.78. The topologies of the trees obtained in the MP jackknife analyses were the same as in the PP analysis and are not shown here. The tree obtained in the PP analysis is shown in Figure 1. All PP runs gave the same topology and none of the nodes plotted varied more than 3% (see Appendix 1). Parameter values obtained in the runs are shown in Appendix 1. The rate of substitution for the third position is higher, as expected, followed



**Figure 1.** The majority rule consensus tree from the three Bayesian analyses of the *ndhF* data set. Only nodes with posterior probabilities above or equalling 95% are shown, with values above branches. The black vertical line shows the position of *Jamesbrittenia*, the white line shows the position of *Limosella*, and the dashed line shows the *Manulea/Sutera* complex. Numbers in italics below branches are used for evaluation of the runs (see Appendix 3).

by the first position and then the second. The burn-in was estimated to 50 000 generations. The likelihood was then stable for approximately 30 000 generations in all three runs. The genus *Sutera* is, in this analysis, paraphyletic. *Sutera* sect. *Chaenostoma* constitutes a monophyletic clade (100% PP) as

does *Manulea* (also 100% PP). These two form a well supported clade (100% PP) that is a sister-clade to the representatives of *Sutera* sect. *Sutera*. The whole *Manulea/Sutera* complex is monophyletic with 100% posterior probability. *Jamesbrittenia* is, according to our results, a well-defined and highly supported



**Figure 2.** The majority rule consensus tree from the three Bayesian analyses of the ITS data set. Only nodes with 70% posterior probability or more are shown, with values above branches. The black vertical line shows the position of *Manulea*, the white line shows the position of *Sutera* section *Chaenostoma*, and the dashed line, *Sutera* sect. *Sutera*. Numbers below branches were used for evaluation of the runs (see Appendix 3).

(100% PP) monophyletic clade, and *Limosella* is clearly positioned inside the Manuleeae.

The ITS data set consists of 41 taxa and 904 unordered equally weighted characters, 533 of which are

constant and 205 (23%) parsimony-informative. The shortest trees produced in the heuristic MP search were 784 steps long. Only the result from the PP analysis is shown (Fig. 2). Parameter and node values are

shown in Appendices 2 and 3. No nodes varied more than 3% from the average PP. Burn-in was estimated to 20 000 generations. All three runs were then stable for approximately 10 000 generations. In the analysis of the ITS data set, *Sutera* is monophyletic, though not significantly supported (80% PP). *Manulea* is monophyletic (100% PP) as is the *Manulea/Sutera* clade (100% PP). An anomaly exists in the analysis, namely the position of *Verbascum*. This position inside *Manuleae* of a rather distant basal taxon is probably caused by scarce sampling and/or alignment problems. The latter are possibly due to the fast evolving nature of the ITS.

The combined data set consists of 34 taxa and 4862 unordered equally weighted characters, 3669 of which are constant and 778 (16%) parsimony-informative. The heuristic search gave 240 equally parsimonious trees of length 1924, with CI = 0.76 and RI = 0.88. The strict consensus tree of these (not shown) has the same topology as the consensus tree obtained in the jackknife (not shown) and PP analyses. One randomly chosen tree from the heuristic search is shown as a phylogram in Figure 3. The tree from the PP analysis is shown in Figure 4. Parameter and node values are shown in Appendices 2 and 3. Two nodes varied more than 3%, namely nodes 4 and 23 (see Appendix 2). The former node had a very low average PP (61.7%) and the latter had a nonsignificant PP support value, 84% in average and below 70% MP jackknife support. Burn-in was estimated to 50 000 generations. All three runs then had stable likelihoods for approximately 10 000 generations. The phylogeny resembles the *ndhF* tree. *Sutera* is paraphyletic. *Manulea* and *Sutera* sect. *Chaenostoma* constitute a clade with 100% PP, *Sutera* sect. *Sutera* is sister to that clade, and the whole *Manulea/Sutera* complex is monophyletic (100% PP). The position of *Limosella* is also in accordance with the results from the *ndhF* analysis.

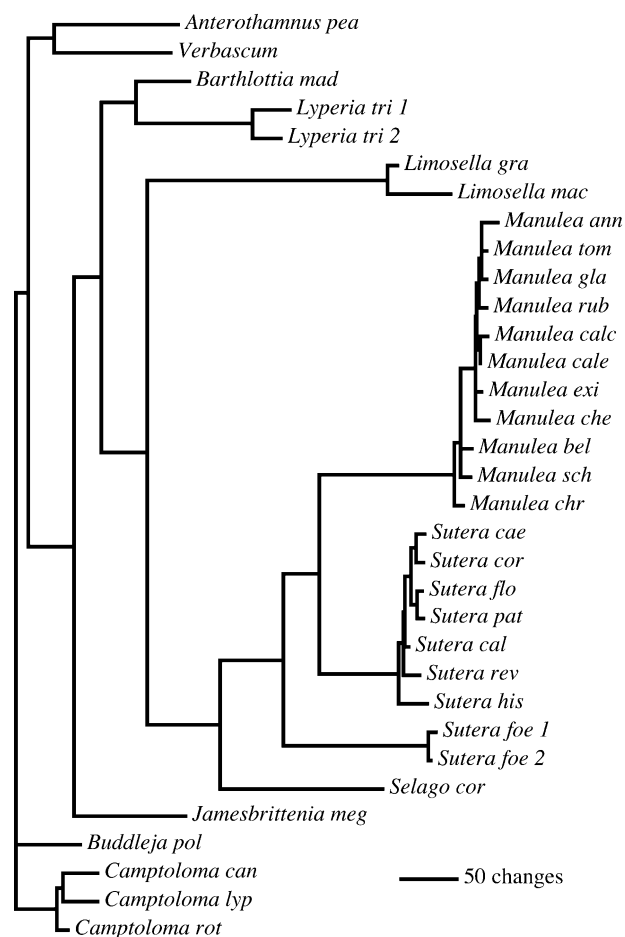
In the morphological study we found that *Sutera foetida* (All Batten 1164, S) had an evident semi-annular gland, which contradicts the findings of Hilliard (1994). Other examined specimens of *Sutera* had lateral nectariferous glands, e.g. *Sutera campanulata* Kuntze (All Batten 998, S), *Sutera patriotica* Hiern (Bremer & Bremer 3818, UPS), *Sutera floribunda* Kuntze (Bremer & Bremer 4315, UPS) and *Sutera calciphila* Hilliard (Kornhall 52, UPS).

## DISCUSSION

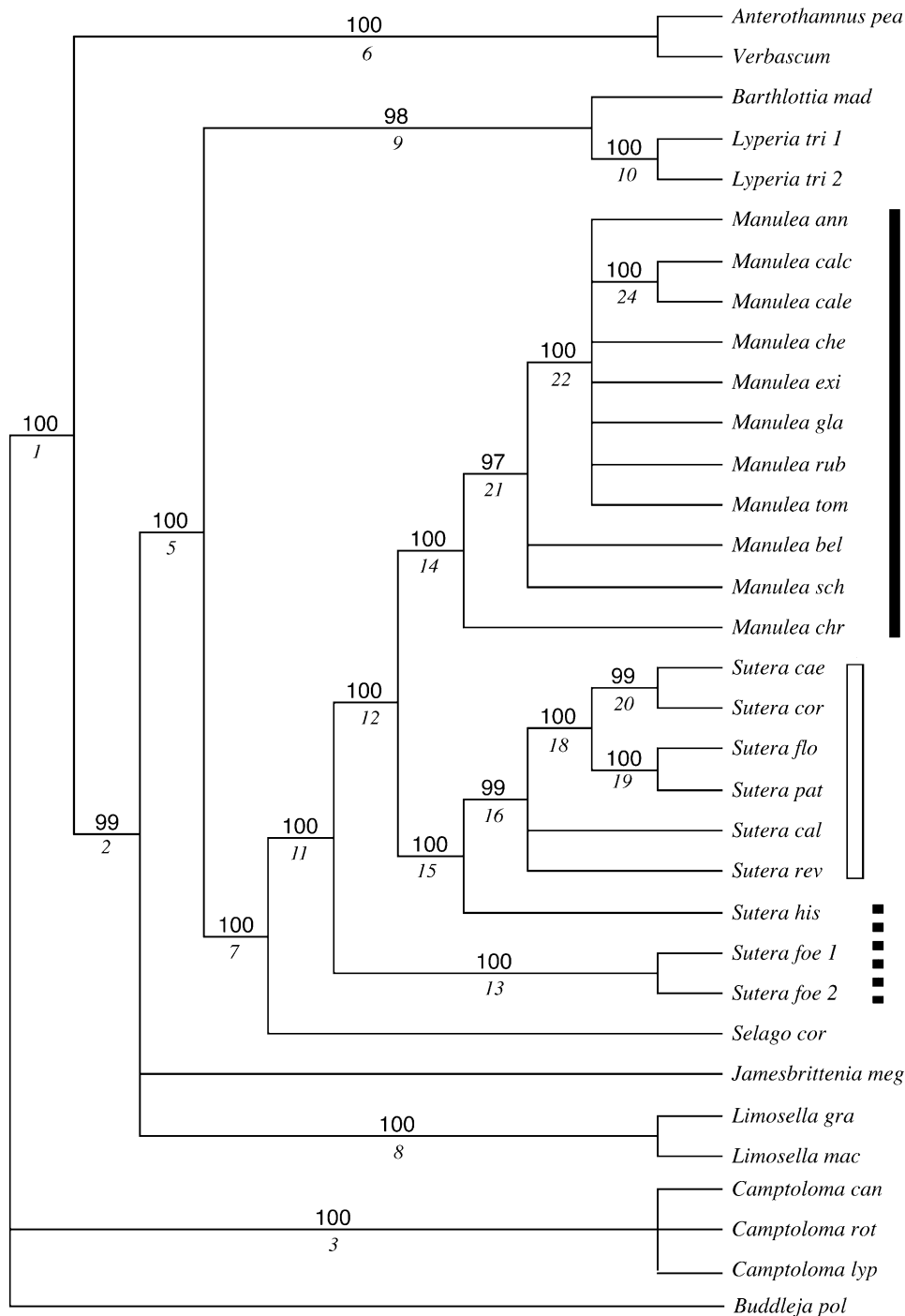
### PARSIMONY AND BAYESIAN INFERENCE

In the last ten years there has been a growing interest in the use of model-based methods in phylogenetic reconstruction, especially in view of the introduction of methods for Bayesian inference (e.g. Larget &

Simon, 1999; Huelsenbeck *et al.*, 2001; Wilcox *et al.*, 2002; Zanis *et al.*, 2002; Archibald, Mort & Crawford, 2003). New software for these statistics has made it possible to analyse phylogenetic data with model-based methods and obtain support values within a reasonable time scale. In this study Bayesian inference was applied in parallel with parsimony as we think that comparisons from real data sets between measures of support from two methods are desirable. Our experience from this and from an earlier study (Kornhall *et al.*, 2001) shows that results obtained with Bayesian statistics do not differ from parsimony in well supported nodes. This also seems to be the general picture emerging from other studies, e.g. Kårehed (2002), Schneider *et al.* (2002) and Wilcox *et al.* (2002). Bayesian probabilities generally tend to have a numerically higher value than bootstrap support. Bootstrap support of 70–80% roughly corresponds to 95% posterior probability in studies where both methods have been applied to the same data (Huelsenbeck



**Figure 3.** Phylogram of one of the 240 most parsimonious trees obtained in the heuristic MP analysis of the combined data set.



**Figure 4.** The majority rule consensus tree from the three Bayesian analyses of the combined data set. Only nodes with 95% posterior probability or more are shown, with values above branches. The black vertical line shows the position of *Manulea*, the white line, the position of *Sutura* sect. *Chaenostoma* and the dashed line *Sutura* sect. *Sutura*. Numbers in italics below branches were used for evaluation of the runs (see Appendix 3).

*et al.*, 2002; Kauff & Lutzoni, 2002). There is criticism that Bayesian statistics overestimate the support from data (Suzuki, Glazko & Nei, 2002) and studies that point to a risk of overparameterization (Rannala,

2002; Rydin & Kallersjo, 2002). However, there is also a study by Wilcox *et al.* (2002) which claims that Bayesian inference gives more accurate support values. We think Bayesian statistics has an advantage in that the

results are easily interpreted according to biological praxis, i.e. posterior probabilities can be treated as 'normal' statistical probabilities, and 5% significance level used to test hypotheses (Huelsenbeck *et al.*, 2002; Leache & Reeder, 2002). In bootstrap or jack-knife analyses, where to place confidence levels is more or less arbitrary as the statistical meaning of the support is difficult to interpret.

#### TAXONOMY

Our results have implications for the taxonomy of the group. We will give below a new broadened circumscription of the Limoselleae to encompass the taxa of the Manuleeae. We will also give a new circumscription of the genus *Sutera* and revive the name *Chaenostoma* for a genus consisting of *Sutera* sect. *Chaenostoma*.

#### About *Jamesbrittenia*

We conclude that Hilliard's (1994) wide circumscription of *Jamesbrittenia* is strongly supported by molecular evidence. We note that ITS was comparably difficult to amplify from the genus. This, together with the rather high chromosome number of  $n = 24$  compared with a normally lower number of 14–16 in the Manuleeae, could indicate polymorphism in the nuclear genome. This could be tested by cloning and subsequent sequencing.

#### *Limosella* – *Limoselleae*

The position of the sampled *Limosella* species within a clade with members from the tribe Manuleeae is indisputable from our molecular analyses (Figs 1, 2, 4) and is also supported by B. Oxelman, P. Kornhall, R. G. Olmstead & B. Bremer (unpubl. data). However, only a few morphological traits support this position, one of which is the occurrence of synthealous anthers. On the other hand, there are no characters that contradict such a placement. The name Limoselleae, erected in 1827 by Dumortier, has priority over Manuleeae that was erected by Bentham & Hooker f. in 1876, and Selagineae and Hebenstretieae that were erected by Horaninov in 1847. We therefore propose below a new circumscription of the tribe Limoselleae that also includes the taxa of the Manuleeae.

As implied by the geographical distribution of extant Limoselleae (in the above sense) taxa, the ancestors of the present day Limoselleae probably were confined to southern Africa. According to an earlier study of the tribe Manuleeae (Kornhall *et al.*, 2001) the more basal taxa of the group have ovaries that contain many and hence smaller seeds. The taxa with larger seeds and fewer ovules appear further up in the cladograms. We could envisage that at some point, the lineage leading to *Limosella* evolved even

smaller seeds and a mud-loving ecology. The traits for propagation in and through mud facilitated a wide distribution by migrating birds and thus made *Limosella* the first and only part of the Limoselleae to achieve a global distribution. That water plants with small seeds are widely dispersed is noted by, among others, Charles Darwin (1872). In *The origin of species* he writes: '... it has long been known what enormous ranges many fresh-water, and even marsh species, have, both over continents and to the most remote oceanic islands... for the latter seem immediately to acquire, as if in consequence, a wide range'. A wide dispersion of aquatic plants is also noted by more recent authors such as Ridley (1930) and Gleason & Cronquist (1964). It is a fascinating thought that the cosmopolitan genus *Limosella* may have arisen from predecessors in southern Africa, especially when it is remembered that many of the Limoselleae are adapted to southern Africa's rather dry conditions.

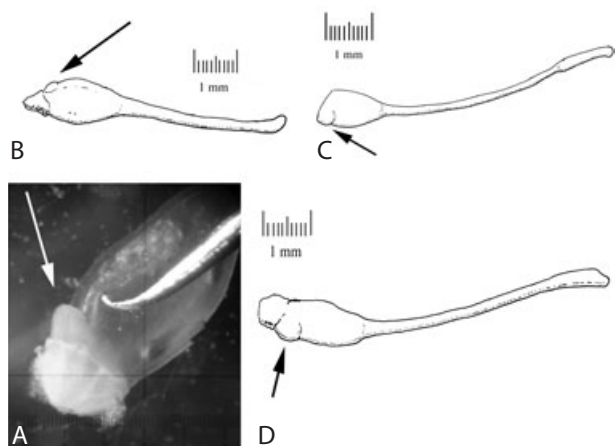
#### *Manulea/Sutera* and *Chaenostoma*?

The chloroplast and the combined analyses clearly show a paraphyletic *Sutera*, but the ITS analysis indicates monophyly of the genus. The latter result is weak (not significant 80% PP), but taken together with the morphological similarity of the sections *Sutera* and *Chaenostoma*, the result is noteworthy.

The phylogram (Fig. 4) shows short branches in both *Manulea* and *Sutera* sect. *Chaenostoma*. The small sequence variation in *Manulea* and *Sutera* sect. *Chaenostoma* in all analyses indicates that they have either diverged very rapidly or have an extensive rate of hybridization and introgression. There is no support in our data for the subgeneric classification of *Manulea* by Hilliard (1994). None of the subgenera constitute monophyletic groups. The difference in nectaries, which is one of the characters Hilliard (1994: 2) used to discriminate between *Manulea* and *Sutera*, seems to be a misconception. We found a conspicuous semi-annular gland in *S. foetida* from sect. *Sutera* (see Fig. 5), i.e. the character does not discriminate between *Manulea* and *Sutera*.

There are three possible solutions to the taxonomic enigma of the *Manulea/Sutera* complex: i) keep the current taxonomy; ii) lump all taxa of the *Manulea/Sutera* clade into an expanded *Manulea* (*Manulea* published 1767 has priority over *Sutera* and *Chaenostoma*); or iii) split *Sutera* into two genera. Of these solutions the first, though appealing for reasons of taxonomic stability, does not take into account the strong evolutionary evidence from the chloroplast genome. The second, to lump all taxa of the *Manulea/Sutera* clade into an expanded *Manulea*, does not give recognition to the morphological diversity that in the first hand motivated the creation of the genera. There are characters, such as the non-decurrent pos-





**Figure 5.** Nectaries in *Sutera* taxa. Arrows indicate nectaries. A, ovary with semi-annular gland of *S. foetida*. B–D, pistils of *S. patriotica*, *S. calciphila* and *S. floribunda*, respectively, with lateral glands. (Drawings and photo by Per Kornhall.)

ticous filaments, that could be used as diagnostic features for an expanded *Manulea*, but the morphological heterogeneity would be quite high in the genus. *Manulea* and *Chaenostoma* are, in almost all cases, easy to distinguish in the field. *Manulea* differs from *Chaenostoma* by having reddish or brownish flowers, abruptly inflated and often bent at the apex, in racemes, thyrses or panicles and with leaves more or less rosulate. This is in contrast to *Chaenostoma* that most often have white, solitary flowers on stems that are leafy throughout. This solution would

also require very many new combinations to be made in *Manulea*. We believe that the third alternative is the best, as it is supported by both molecular and morphological data. It also requires relatively few new combinations since many of the *Sutera* sect. *Chaenostoma* species already have synonyms in *Chaenostoma*. One or two species and hybrids of *Sutera*, e.g. *S. hispida* Druce and *S. cordata* (sometimes sold under the erroneous name 'Bacopa'), have lately become quite popular as garden plants. These will now be transferred to *Chaenostoma*. To avoid this, the section *Chaenostoma* could possibly be conserved with a new type, but that would leave section *Sutera* without any legitimate name. We therefore propose revival of the nomen conservandum *Chaenostoma* for a genus consisting of the morphologically distinct species in *Sutera* sect. *Chaenostoma* sensu Hilliard, leaving *Sutera* consisting of *Sutera* sect. *Sutera* (sensu Hilliard, 1994). The type of section *Chaenostoma*, *Sutera aethiopicum* Kuntze, is unfortunately not represented in our molecular sampling, but *S. calciphila*, *S. patriotica* and *S. caerulea* Hiern, represent its closest allies (Hilliard, 1994). We conclude that the characters used to subdivide the *Manulea/Sutera* complex into two genera do not demonstrate the evolutionary history of the species involved. We think that the division of the complex into three genera better reflects both the phylogeny and the morphological diversity of the species involved. A key to the genera *Manulea*, *Chaenostoma* and *Sutera*, and generic descriptions are given below. A list of the names and combinations in *Chaenostoma* can be found in Appendix 2.

#### KEY TO THE GENERA *MANULEA*, *CHAENOSTOMA* AND *SUTERA*

- Aa. Posticous stamens included, inserted halfway up the tube or higher. Anticous pair sometimes visible in mouth but not exerted..... *Manulea*  
 Ab. At least one pair of anthers exerted (in two species of *Sutera* all stamens included but then inserted near base of tube). ..... B  
 Ba. At least some cymules with 3–11 flowers in every inflorescence. Hairs present on upper surface of corolla lobes around mouth..... *Sutera*  
 Bb. Flowers solitary in leaf axils. Corolla lobes glabrous on upper surface ..... *Chaenostoma*

#### DESCRIPTIONS OF *SUTERA* AND *CHAENOSTOMA*

*Sutera* Roth, Bot. Bemerk. 172 (1807).  
 Syn. *Sutera* Roth section *Sutera* Hilliard 1994: 221.  
 Bushy perennial or annual (*S. foetida*) herbs, glandular and sometimes foetid. Stems leafy throughout. Leaves opposite (*S. cooperi*), or alternate (*S. griquensis*), or opposite becoming alternate upwards (*S. foetida*), bases cordate, cuneate, abruptly contracted or tapering into a petiolar part, margins toothed or serrate. Inflorescence  $\pm$  racemose, flowers in

cymules or cymose racemes, rarely solitary, sometimes paniced. Bracts present or wanting, not adnate to base of pedicel. Calyx bilabiate, sometimes obscurely so, anticous lip 2-lobed, posticous lip 3-lobed, lobes  $\pm$  linear-lanceolate, usually pubescent. Corolla tube cylindrical, or narrowly funnel-shaped (*S. foetida*), mouth round, limb nearly regular, lobes spreading, suborbicular to oblong, entire, glandular-pubescent or glabrous (*S. foetida*) outside, often with glistening glands as well, inside with clavate hairs extending from throat out onto lower part of lobes. Sta-

mens 4, didynamous, filaments not decurrent, anticous pair shortly exerted in one species (*S. foetida*), posticous pair included (in *S. cooperi*, *S. griquensis* deeply included and inserted in lower part of tube); all anthers synthecous. Stigma usually lingulate with 2 marginal bands of stigmatic papillae, included in two species and shortly exerted in *S. foetida*. Ovary  $\pm$  elliptic in outline, ovules many in each loculus. Fruit a septicidal capsule with a short loculicidal split at tip of each valve, glabrous or with glistening glands. Seeds roughly elliptic in outline, sometimes angled by pressure, amber-coloured, testa thin, tightly investing the endosperm, which is alveolate, with several longitudinal rows of transversely elongated pits arranged in chequer-board fashion, under the SEM seen to be ornamented with  $\pm$  oblong reticulations.

*Distribution:* Southern Africa from the Orange Free State and Transvaal to the Cape.

*Three species:* *S. cooperi*, *S. griquensis* and *S. foetida*. *Chaenostoma* Benth. in Hook., *Comp. Bot. Mag.* 1: 374 (1836), nom. cons.

Syn. *Sutera* Roth section *Chaenostoma* (Benth.) Hilliard 1994: 221.

SHRUBLETS, suffrutices or perennial herbs, rarely annual, mostly glandular, and sometimes aromatic or foetid. STEMS leafy throughout. LEAVES usually opposite, sometimes alternate upwards, bases either  $\pm$  connate or decurrent in narrow wings or ridges, simple, entire to toothed, rarely more deeply lobed. INFLORESCENCE  $\pm$  racemose, flowers mostly solitary in axils of leaves or bracts, sometimes in cymules or cymose racemes, sometimes paniced. BRACTS at most adnate to extreme base of pedicel. CALYX bilabiate, sometimes obscurely so, anticous lip 2-lobed, posticous lip 3-lobed, or rarely regularly divided into 6–9 lobes, lobes  $\pm$  linear-lanceolate, usually pubescent. COROLLA tube funnel-shaped, mouth round, limb nearly regular, lobes spreading, suborbicular to oblong, entire, usually glandular-pubescent outside, often with glistening glands as well, inside usually with either 1–5 longitudinal bands of clavate hairs in throat, or glabrous. STAMENS 4 (a 5th occasionally developed), didynamous, filaments usually inserted in upper part of corolla tube, not decurrent, anticous pair exerted, posticous pair either included or exerted; all anthers synthecous. STIGMA usually lingulate with 2 marginal bands of stigmatic papillae, exerted. OVARY  $\pm$  elliptic in outline, often with glistening glands at least on the sutures, rarely glandular-pubescent as well, nectariferous gland semi-annular, ovules many in each loculus. FRUIT a septicidal capsule with a short loculicidal split at tip of each valve, glabrous or with glistening glands. SEEDS roughly elliptic in outline, sometimes angled by pressure, amber-coloured, pallid or grey- to violet-blue,

testa thin, tightly investing the endosperm, which is alveolate, with several longitudinal rows of transversely elongated pits arranged in chequer-board fashion, under the SEM seen to be ornamented with irregular pustules.

*Distribution:* Africa south of the Cunene and Zambezi rivers, mainly Cape, Natal, Transvaal. 46 species.

#### *New description of the tribe Limoselleae*

Scrophulariaceae tribe Limoselleae Dumort., *Florula Belgica* p.52 (1827).

Syn. Manuleae, Benth. & Hook. f., *Genera plantarum* 2: 915–919 (1876); Selagineae, Choisy in *Memoires de la Société de Physique et d'Histoire Naturelle de Genève* 2. 2 (1822); Hebenstretieae, Horaninov (1847); Selagineae, Horaninov (1847).

Herbs or shrubs, often glandular. Leaves simple, without stipules, often opposite at the base of the plant and alternate upwards. Bracts (if present) often adnate to calyx. Flowers often solitary in leaf axils, often in racemes of cymes, or in panicles, occasionally cymbose, bisexual, zygomorphic to subactinomorphic. Calyx (3-)5(-9) lobed, obscurely to distinctly bilabiate. Corolla gamopetalous, tube cylindrical or funnel-shaped, more or less bilabiate, posterior lip 2-lobed, anterior lip 3-lobed, sometimes lower lip seems wanting and posterior lip 4-lobed, with unicellular clavate hairs inside. Stamens, dorsifixed, synthecous (2-)4,(-5), inserted in corolla tube. Stigma often lingulate with marginal bands of stigmatic papillae, rarely bifid, or entire with terminal papillae. Style solitary, terminal, and filiform. Ovary superior, 2-celled or rarely 1-celled by abortion. Ovules one to many in each locule. Nectary often a small dorsal gland, sometimes annular. Fruit, when many-seeded a septicidal capsule, otherwise indehiscent. Seeds small, often with copious endosperm.

*Genera included:* *Barthlottia*, *Chaenostoma*, *Chenopodiopsis*, *Cromidon*, *Dischisma*, *Glekia*, *Globulariopsis*, *Glumicalyx*, *Gosela*, *Hebenstretia*, *Jamesbrittenia*, *Limosella*, *Lyperia*, *Manulea*, *Manuleopsis*, *Melanospermum*, *Microdon*, *Phyllopodium*, *Polycarena*, *Pseudoselago*, *Reyemia*, *Selago*, *Strobilopsis*, *Sutera*, *Tetraselago*, *Trieneea* and *Zaluzianskya*.

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## APPENDIX 1

ITS primers used for amplification and sequencing. \*= used for amplification.

## Forward:

P16	tca ctg aac ctt atc att tag agg a	Popp & Oxelman (2001)
P17*	cta ceg att gaa tgg tcc ggt gaa	Popp & Oxelman (2001)
P16,5	gac gtc gcg aga agt yca ytg a	B. Oxelman, unpubl. data
P16B	cca ytg aac ctt atc att kag agg a	B. Oxelman, unpubl. data
ITS.LEU1	gtc cac tga acc tta tca ttt ag	Andreasen, Baldwin & Bremer (2000)
Reverse:		
P25	ggg tag tcc cgc ctg acc tg	Oxelman & Lidén (1995)
P26sR	gat atg ctt aaa ytc ggc ggg t	B. Oxelman unpubl. data
ITS4	tcc tcc gct tat tga tat gc	White <i>et al.</i> (1990)
26S-82R*	tcc cgg ttc gct cgc cgt tac ta	Popp & Oxelman (2001)

## APPENDIX 2

Evaluation of the different PP runs for the different data sets (see text). Node = chosen nodes, see Figs 2, 3, 5. Mean = mean values from the three runs in the PP-analysis. % = maximum deviation from the mean values in percent. Pars. = value from MP Jackknife analyses on comparable nodes.

<i>NdhF</i>				ITS			
Node	Mean	%	Pars.	Node	Mean	%	Pars.
1	100	0	100	1	99.7	0.7	75
2	100	0	94	2	100	0	71
3	100	0	91	3	100	0	97
4	97.3	1.7	80	4	99.7	0.7	82
5	100	0	100	5	100	0	99
6	100	0	100	6	100	0	100
7	100	0	100	7	100	0	100
8	100	0	100	8	100	0	71
9	100	0	98	9	100	0	100
10	100	0	74	10	98.7	1.7	96

APPENDIX 2 *Continued*

<i>NdhF</i>				ITS			
Node	Mean	%	Pars.	Node	Mean	%	Pars.
11	100	0	100	11	100	0	100
12	100	0	100	12	100	0	100
13	100	0	93	13	98.7	1.7	94
14	100	0	100				
15	100	0	79				
16	100	0	100				
17	100	0	96				

Comb.							
Node	Mean	%	Pars.	Node	Mean	%	Pars.
1	100	0	97	14	100	0	100
2	98.7	0.7	84	15	100	0	100
3	100	0	100	16	99	0	94
4	61.7	10.3	Missing	17	94	0	74
5	100	0	Missing	18	100	0	97
6	100	0	99	19	100	0	95
7	100	0	100	20	99.7	0.7	Missing
8	100	0	100	21	96.3	3.5	77
9	98	1	76	22	100	0	100
10	100	0	100	23	83.7	4.4	Missing
11	100	0	100	24	100	0	97
12	100	0	99	25	92	2.2	Missing
13	100	0	100	26	93	2.2	Missing

## APPENDIX 3

Posterior distributions for the parameters from the PP runs. Mean = the mean values from the three runs/PP analysis. % dev = the greatest deviation from the mean values in per cent.  $r(x-y)$  = the substitution rate for the transition/transversion from  $x$  to  $y$ ,  $\pi(x)$  = stationary frequency of nucleotide  $x$ ,  $\alpha$  = the shape parameter for the gamma distribution,  $ss(z)$  = the substitution rate for the codon position  $z$ .

Parameter	<i>ndhF</i>		ITS		Total	
	Mean	% dev.	Mean	% dev.	Mean	% dev.
$r(g-t)$	1.00	0.00	1.00	0.00	1.00	0.00
$r(c-t)$	3.37	1.76	1.65	0.05	1.81	0.35
$r(c-g)$	2.53	1.67	0.66	0.47	1.35	0.11
$r(a-t)$	0.25	4.12	1.98	1.50	0.52	1.13
$r(a-g)$	3.12	1.50	3.30	0.25	2.07	0.21
$r(a-c)$	2.26	0.68	0.88	1.21	1.08	0.43
$\pi(a)$	0.30	0.18	0.21	0.11	0.29	0.01
$\pi(c)$	0.13	0.66	0.29	0.32	0.18	0.19
$\pi(g)$	0.16	0.09	0.28	0.10	0.18	0.15
$\pi(t)$	0.42	0.36	0.22	0.44	0.34	0.19
$\alpha$	0.39	0.03	0.36	0.23		
ss1	0.68	0.21				
ss2	0.48	1.15				
ss3	1.84	0.22				

## APPENDIX 4

Species list with voucher information and/or accession numbers in GenBank/EMBL. \* = sequences new in this publication. Numbers in parentheses after species names are for identification of sequences from the same species.

Species	Voucher	<i>ndhF</i>	<i>trnL</i>	ITS
<i>Androya decaryi</i> Perrier		AF027276		
<i>Anterotheramnus pearsonii</i> N.E. Br.	Hansen 3472 (UPS)	AJ401392	AJ401442 AJ296509	AJ550575
<i>Antirrhinum majus</i> L.		L36392		
<i>Barthlottia madagascariensis</i> Fischer	Guillaumet 3861 (P)	AJ401438	AJ401443 AJ401444	AJ550576
<i>Buddleja asiatica</i> Lour.		AF027277		
<i>Buddleja indica</i> =		L36405		
<i>Nicodemia diversifolia</i> Tenore				
<i>Buddleja polystachya</i> Fresen.	Thulin 9405 (UPS)		AJ551271	AJ550577
<i>Buddleja saligna</i> Willd.	Bayliss 8158 (S)	AJ401396, AJ401397		AJ550578
<i>Buddleja thyrsoides</i> Lam.	Bengt Oxelman pers. comm.			AJ550579
<i>Camptoloma canariense</i> Hilliard	Jonsell 5558 (UPS)	AJ401398, AJ401399	AJ401445, AJ401449	AJ550580
<i>Camptoloma lyperiiflorum</i> Hilliard	Thulin, Beier & Hussein 9655 (UPS)	AJ401401	AJ296514	AJ550581
<i>Camptoloma rotundifolium</i> Benth.	Nordenstam & Lundgren 869 (S)	AJ401431, AJ401432	AJ401450, AJ296515	AJ550582
<i>Chenopodiopsis retrorsa</i> Hilliard		AJ401421		
<i>Cromidon decumbens</i> Hilliard		AJ401403		
<i>Dischisma ciliatum</i> Choisy		AJ401412		
<i>Freylinia tropica</i> Moore	Bremer 3765 (UPS)	AJ401402	AJ550583	
<i>Glekia krebsiana</i> Hilliard		AJ401422		
<i>Globularia cordifolia</i> L.		AF027282		
<i>Glumicalyx flanaganii</i> Hilliard & Burt		AJ401413		
<i>Halleria lucida</i> L.	Bremer 3692 (UPS)	AJ550569		
<i>Hebenstretia cordata</i> L.		AJ401414		
<i>Jacaranda sparrei</i> Gentry		AF102631		
<i>Jamesbrittenia atropurpurea</i> Hilliard	Skarpe 372 (UPS)	AJ550570		
<i>Jamesbrittenia dissecta</i> Kuntze		AJ401435, AJ401436		
<i>Jamesbrittenia filicaulis</i> Hilliard		AJ401439		
<i>Jamesbrittenia foliolosa</i> Hilliard	Kornhall 96 (UPS)	AJ550571		
<i>Jamesbrittenia megadenia</i> Hilliard	Örtendahl 691 (UPS)	AJ401404	AJ296511	AJ550584
<i>Jamesbrittenia microphylla</i> Hilliard	Kornhall 58 (UPS)	AJ550572, AJ550573		
<i>Justicia carnea</i> Lindl.		AF130155		
<i>Lamium purpureum</i> L.		U78694		
<i>Limosella aquatica</i> L.	Lohammar 29.10. 1971 (UPS)	AJ550547		AJ550588
<i>Limosella grandiflora</i> Benth.	Kornhall 112 (UPS)	AJ550552	AJ550525	AJ550587
<i>Limosella macrantha</i> Fries	Hedberg 5640 (UPS)	AJ550553	AJ550526	AJ550586
<i>Limosella major</i> Diels	Hedberg & Aweke 5475 (UPS)	AJ550548		AJ550585
<i>Lyperia tristis</i> Benth. (1)	Vlok 2488 (S)	AJ401406	AJ550527	AJ550589
<i>Lyperia tristis</i> Benth. (2)	Bremer 3717 (UPS)	AJ550554	AJ550528	AJ550614
<i>Manulea annua</i> Hilliard	Kornhall 6 (UPS)	AJ550555	AJ550529	AJ550590
<i>Manulea bellidifolia</i> Benth.	Hedberg 82011 (UPS)	AJ550556	AJ550530	AJ550591
<i>Manulea calciphila</i> Hilliard	Kornhall 63 (UPS)	AJ550557	AJ550533	AJ550592
<i>Manulea caledonica</i> Hilliard	Bremer 3714 (UPS)	AJ550558	AJ550531	AJ550593

APPENDIX 4 *Continued*

Species	Voucher	<i>ndhF</i>	<i>trnL</i>	ITS
<i>Manulea cheiranthus</i> L.	Acock 4777 (S)	AJ401418, AJ401419	AJ401446, AJ401452	AJ550594
<i>Manulea chrysantha</i> Hilliard	Vlok 2514 (S)	AJ550559	AJ550532	AJ550595
<i>Manulea crassifolia</i> Benth.	Hilliard & Burt 12073 (S)	AJ401428, AJ401429		AJ550596
<i>Manulea dubia</i> Roessler	Nordenstam 330 (S)			AJ550597
<i>Manulea exigua</i> Hilliard	Kornhall 82 (UPS)	AJ550560	AJ550534	AJ550598
<i>Manulea glandulosa</i> Phillips	Bremer 3519	AJ550549	AJ296520	AJ550599
<i>Manulea rubra</i> L. f.	Kornhall 5 (UPS)	AJ550561	AJ550535	AJ550600
<i>Manulea schaeferi</i> Pilger	Örtendahl 64 (S)	AJ550562	AJ550536	AJ550601
<i>Manulea tomentosa</i> L.	Bremer 3781 (UPS)	AJ401394	AJ550537	AJ550602
<i>Manuleopsis dinterii</i> Thell.		AJ401410		
<i>Melanospermum foliosum</i> Hilliard		AJ401415		
<i>Microdon lucidus</i> Choisy		AJ401416		
<i>Myoporum mauritianum</i> A. DC.		L36403		
<i>Olea europaea</i> L.		AF027288		
<i>Phyllopodium cuneifolium</i> Benth.		AJ401430		
<i>Plantago lanceolata</i> L.		L36408		
<i>Polycarena formosa</i> Benth.		AJ401423		
<i>Pseudoselago ascendens</i> Hilliard		AJ401433		
<i>Reyemia chasmantiiflora</i> Hilliard		AJ401425		
<i>Scrophularia</i> sp.		L36411		
<i>Selago corymbosa</i> L.	Vlok 2514 (S)	AJ401434	AJ401458, AJ296494	AJ550603
<i>Selago myrtifolia</i> E. Mey.		AJ401420		
<i>Sesamum indicum</i> L.		L36413		
<i>Stemodia glabra</i> Oerst.	Nordenstam <i>et al.</i> 967 (S)	AJ550574		
<i>Stilbe albiflora</i> E. Mey.		AF027287		
<i>Sutera caerulea</i> Hiern	Vlok 00421a (S)	AJ550563	AJ550538	AJ550604
<i>Sutera calciphila</i> Hilliard	Kornhall 52 (UPS)		AJ550539	AJ550605
<i>Sutera campanulata</i> Kuntze	Batten 998 (S)	AJ550550		AJ550606
<i>Sutera cordata</i> Kuntze	Kornhall 106 (UPS)	AJ550564	AJ550540	AJ550607
<i>Sutera floribunda</i> Kuntze	Batten 1065 (S)	AJ550565	AJ550541	AJ550608
<i>Sutera foetida</i> Roth (1)	Batten 1107 (S)	AJ401407, AJ401408	AJ296510	AJ550609
<i>Sutera foetida</i> Roth	Batten 1164 (S)	AJ550551	AJ550542	AJ550611
<i>Sutera hispida</i> Druce	Kornhall 91 (UPS)	AJ550566, AJ550567	AJ550543	AJ550610
<i>Sutera patriotica</i> Hilliard	Bremer 3818 (UPS)	AJ401393	AJ551261	AJ550612
<i>Sutera revoluta</i> Kuntze	Kornhall 98 (UPS)	AJ550568	AJ551262	AJ550613
<i>Tetraselago longituba</i> Hilliard & Burt		AJ401417		
<i>Trieneea glutinosa</i> Hilliard		AJ401400		
<i>Verbascum arcturus</i> L.	Gustafsson 134 (UPS)	AJ401460, AJ296522	AJ550615	
<i>Verbascum thapsus</i> L.		L36417		
<i>Verbena bracteata</i> Cav.		L36418		
<i>Zaluzianskya glareosa</i> Hilliard & Burt		AJ401424		
<i>Zaluzianskya minima</i> Hilliard	Bremer 3542 (UPS)	AJ401437		