

Selagineae and Manuleeae, two tribes or one? Phylogenetic studies in the Scrophulariaceae

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Abstract. Based on results from a cladistic analysis of molecular characters using parsimony and Bayesian inference, the tribe Selagineae (Scrophulariaceae, sometimes Selaginaceae or Globulariaceae), is found to have arisen within Manuleeae (Scrophulariaceae). The inclusion of Selagineae into Manuleeae is therefore proposed. The result is corroborated by morphological characters, and indicates that the typical Selagineae condition with one ovule per ovary locule has arisen several times within the Manuleeae. Two former Manuleeae taxa are excluded from Manuleeae; *Antherothamnus* and *Camptoloma*. The former shows affinity with *Scrophularia* and *Verbascum*, and the latter groups with *Buddleja* and related genera. The controversy between parsimony and likelihood based methods are briefly discussed as is the possibility of historical bias in prior studies.

Key words: Manuleeae, *ndhF*, Scrophulariaceae, Selagineae, *trnL*, parsimony, Bayesian inference.

Among the angiosperms, a few families have been difficult to characterize on morphological grounds. One of these is Scrophulariaceae. Recent molecular investigations have shown that the family is paraphyletic and needs a totally new circumscription (Olmstead and Reeves 1995). Manuleeae and Selagineae are two currently recognized tribes that are a part of, or at least have been associated with, Scrophulariaceae s. str. Manuleeae have

always been included in Scrophulariaceae, while Selagineae have had a more complicated taxonomic history, having also been recognized as a distinct family, Selaginaceae. Both tribes consist almost exclusively of heath-like herbs to sub-shrubs, growing in sub-Saharan Africa. The taxa of the two groups share many similarities, and during fieldwork in South Africa, the senior author (BB) found it difficult to distinguish between them.

Manuleeae are characterized by the imbricate corolla-aestivation with posterior lobes external in the bud, by unithecal anthers, and by a four- to many-seeded septicidal capsule further opening by loculicidal slits. Most species have marginal stigmatic papillae on a lingulate stigma and a lateral nectariferous gland at the base of the ovary. Manuleeae consist of 17 genera and 344 species according to the latest revision (Hilliard 1994). There is considerable morphological variation within the tribe. The sub-spinescent shrub *Antherothamnus*, the glandulose herb *Camptoloma*, and the heath-type sub-shrub *Tetrasselago* could exemplify this. In the field the latter is hardly distinguishable from certain genera of Selagineae. Nearly all genera of Manuleeae have a mainly southern African distribution. The genus *Barthlottia* is found only on Madagascar. Species outside the African continent

are only found in the genera *Camptoloma* and *Jamesbrittenia*. Bentham and Hooker (1873) described Manuleeae, and the position of Manuleeae as tribe of Scrophulariaceae has never been questioned.

Selagineae and Manuleeae share several characteristic features; the same aestivation, anther, stigma, and nectary-characters that characterize Manuleeae also characterize Selagineae. The one important distinguishing character is the number of ovules in each locule of the ovary. There is one ovule per locule in Selagineae, compared to two to many in Manuleeae. Selagineae are morphologically more homogenous than Manuleeae, and have, consequently, from time to time been treated as a distinct family. Selagineae consisted before Hilliard's revision (1999) of 11 genera and about 280 species (Rolfe 1912, Hilliard 1994). Hilliard (1999) argues for an inclusion of *Walafrida* into *Selago* and of *Agathelpis* into *Microdon* making the number of genera nine in her revision. The distribution is nearly exclusively in Africa south of the equator. The exceptions to this are *Walafrida* (one species on Madagascar) and *Hebenstretia* (one species distributed from Lesotho to Eritrea). Linnaeus (1753) described the genus *Selago*, named because of its superficial resemblance to *Lycopodium* (*Huperzia*) *selago*. Choisy (1822) described the family Selaginaceae, comprised of *Polycenia*, *Hebenstretia*, *Dischisma*, *Agathelpis*, *Microdon*, and *Selago*. That Selagineae deserved family rank was accepted by Bentham and Hooker (1873), Rolfe (1912), and Stapf (1929). However, several subsequent authors did not agree and placed the group in Scrophulariaceae (Wettstein 1895, Hartl 1913, Phillips 1926, Junell 1961, Thieret 1967, Argue 1993). Cronquist (1981) disagreed with most authors and placed Selagineae as a tribe of the Globulariaceae because of the pseudo-monomerous gynoecium in some genera. Finally Hilliard, in her excellent monographs, firmly argues for a placement in Scrophulariaceae close to Manuleeae (1994) and that Selagineae have arisen within Manuleeae (1999). Hilliard also recognizes a new segregate from *Selago*, namely the

genus *Pseudoselago*. Earlier molecular studies placed the few included genera of Manuleeae and Selagineae in Scrophulariaceae s. str., (Scrophulariaceae I of Olmstead and Reeves (1995), and Oxelman et al. (1999)).

Due to the observed connections between these two tribes, we decided to investigate their relationship more closely. We have sequenced two DNA markers, *ndhF* and *trnL*, and using both parsimony-methods and likelihood-based Bayesian inference methods to reconstruct their phylogeny. The aim was to answer the following questions. 1) Do taxa formerly ascribed to Manuleeae and Selagineae form monophyletic groups corresponding to the two tribes? 2) If so, how will they be circumscribed? 3) Do all taxa belong to Scrophulariaceae s. str.? 4) How do morphological characters correspond to a molecular phylogeny? 5) Will there be any discrepancies between parsimony and likelihood-based methods on the resulting datasets? 6) Is there any molecular support for the taxonomical changes imposed by O.M. Hilliard in her monographs over Manuleeae and Selagineae?

Material and methods

Choice of taxa and genes. We generated two molecular data sets. One of *ndhF* sequences with a broad sampling to resolve the position of Manuleeae and Selagineae in Lamiales. A second combined of *ndhF* and *trnL* was analyzed to resolve relationships between taxa of the two tribes. To achieve the latter, we sought representatives for all genera of Manuleeae and Selagineae recognized by Hilliard (1990, 1994), Rolf (1912) and Fischer (1996). The choice of taxa for the broader analysis was made following results of earlier investigations (Bentham and Hooker 1873, Junell 1961, Cronquist 1981, Olmstead and Reeves 1995, Oxelman et al. 1999). *NdhF* has been shown to carry information at this level of phylogeny in related taxa (Oxelman et al. 1999). *TrnL* was chosen to reveal relations in the phylogenetic vicinity of Manuleeae and Selagineae. Introns in the *trnL* sequence may have a faster substitution rate and hence yield higher phylogenetic resolution on closely related taxa. By *trnL*, 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. Investigated taxa along with voucher information, references, and EMBL/GenBank accession numbers are given in Table 1.

Sequencing. We obtained DNA from small amounts of dried plant material. The protocol for extraction was the CTAB protocol of Doyle and Doyle (1987) with minor modifications. We cleaned DNA with the QIAquick PCR Purification Kit (QIAGEN). For PCR amplification we added 1 μ l template to a cocktail consisting of: 5 μ l 10X PCR buffer; 5 μ l MgCl₂ (25 mM); 5 μ l TMACl (0.1 M); 4 μ l dNTP; 0.25 μ l Taq (5U/ μ l); 0.5 μ l 5' primer (100 ρ mol/ μ l); 0.5 μ l 3' primer (100 ρ mol/ μ l); 0.5 μ l BSA (1%), and 28.25 μ l water. In some *trnL* amplification reactions we used 0.65 M betaine Hengen (1997), and ThermoPrimePlus Taq (Advanced Biotechnologies). In Fig. 1, we show primers used for *ndhF*. Primer sequences used for *ndhF* amplification and sequencing were constructed for this study by the authors and Bengt Oxelman or kindly shared by Robert K. Jansen and Richard G. Olmstead. For *trnL*, we used the universal primers published by Taberlet et al. (1991). We always tried to amplify *ndhF* and *trnL* in two fragments each. If herbarium material would not amplify, we used internal primers, thus shortening the fragments. We performed sequencing on an ABI 377 automatic sequencer following standard protocols. Sequenced fragments were assembled and edited using Sequencher 3.1.1 (Gene Codes Corporation).

Alignment and indel coding. The *ndhF* sequences were imported into the Se-Al alignment software (Rambaut 1995), and aligned manually. The *trnL* matrix was first imported into Clustal-X (Thompson et al. 1997) for a rough alignment but was subsequently modified by eye. We excluded parts of the very variable non-coding regions of the first spacer in the *trnL* matrix from the analyses. The parsimony analyses were performed with indel data added to the molecular matrices. We used the alignment and mutational interpretation criteria described in Golenberg et al. (1993) and Oxelman et al. (1997). The aligned matrices are available from the corresponding author.

Optimization of non-molecular characters. Ten features important for infrageneric classification in Manuleeae (Hilliard 1994) were optimized on the resulting cladogram from the combined DNA matrix with McClade (Maddison and Maddison

1992). Most morphological characters were checked on herbarium material, floral parts after rehydration. Further information on habit and chromosome base numbers were gathered from literature (Rolfe 1912; Hilliard 1990; Jong 1993; Hilliard 1994, 1995). We show the chosen characters and states in Table 2. Since our goal with this part of the study was to show the distribution of characters between Manuleeae and Selaginiae only taxa from the two tribes were included here.

Phylogenetic methods. Phylogenetic methods used were parsimony and Bayesian inference. All parsimony analyses were performed with the PAUP* ver. 4.0b2a software (Swofford 1999), using heuristic search and jackknife analysis. The combined matrix was analyzed with parsimony only. Since several authors (Källersjö et al. 1999, Sennblad and Bremer 2000, Broughton et al. 2000) have pointed out that there is no justification for a priori weighting of codon positions, when using parsimony, we weighted all positions equally. We used the following settings in PAUP:

Heuristic search (parsimony): gaps = "missing"; random addition sequence, number of replicates = 1000, (TBR) branch-swapping algorithm; MulTrees' option in effect.

Parsimony jackknife analyzes: jackknife method with heuristic search; replicates = 10,000; percentage of characters deleted in each replicate = 37; "Jac" re-sampling method; NNI branch-swapping algorithm used.

Bayesian inference is a quite novel technique in phylogenetics. Applications of the method can be found in Li et al. (1996), Mau et al. (1999), Rannala and Yang (1996), Yang and Rannala (1997), Newton et al. (1998), and Simon and Larget (1999). We used Bayesian inference on the *ndhF* matrix only and without indel data. We chose to perform this analysis on the *ndhF* only, since we judged that any inconsistencies between the methods would be more likely to show up in the bigger matrix than in the smaller. Before conducting the Bayesian analysis, we used a likelihood ratio test (Huelsenbeck and Rannala 1997) to propose a model of evolution to implement in the Bayesian analysis. For this, we used the software MODELTEST (Posada and Grandall 1998) and PAUP* (Swofford 1999). The software MODELTEST evaluates different nested sets of substitution models and score their likelihoods. For the Bayesian

Table 1. Voucher or reference information and EMBL/GenBank accession numbers for the taxa studied. Acc. numbers marked with asterix are here published for the first time

Family	Tribus	Species	Voucher or reference	EMBL, GenBank acc. for ndhF	EMBL, GenBank acc. for trnL
Acanthaceae		<i>Justicia</i>	Kim et al. (1999)	AF130155	
Bignoniaceae		<i>Jacaranda</i>	Spangler et al. (1999)	AF102631	
Buddlejaceae		<i>Buddleja</i>	Bayliss 8518 (S)	AJ401396* AJ401397*	
Buddlejaceae		<i>Buddleja</i>	Oxelman et al. (1999)	AF027277	
Buddlejaceae		<i>Nicodemia</i>	Olmstead et al. (1995)	L36405	
Globulariaceae		<i>Globularia</i>	Oxelman et al. (1999)	AF027282	
Lamiaceae		<i>Lamium</i>	Wagstaff et al. (1995)	U78694	
Myoporaceae		<i>Androya</i>	Oxelman et al. (1999)	AF027276	AJ296513*
Myoporaceae		<i>Myoporum</i>	Bremer 3927 (UPS)		
Myoporaceae		<i>Myoporum</i>	Olmstead et al. (1995)	L36403	
Oleaceae		<i>Olea</i>	Oxelman et al. (1999)	AF027288	
Pedaliaceae		<i>Sesamum</i>	Olmstead et al. (1995)	L36413	
Plantaginaceae		<i>Plantago</i>	Olmstead et al. (1995)	L36408	
Scrophulariaceae		<i>Antirrhinum</i>	Olmstead et al. (1995)	L36392	
Scrophulariaceae		<i>Freylinia</i>	Bremer 3765 (UPS)	AJ401402*	
Scrophulariaceae		<i>Scrophularia</i>	Freeman C.E. (1999)		AF118803
Scrophulariaceae		<i>Scrophularia</i>	Olmstead et al. (1995)	L36411	
Scrophulariaceae		<i>Verbascum</i>	Olmstead et al. (1995)	L36417	
Scrophulariaceae		<i>Verbascum</i>	Gustafsson M.H.G. 134 (UPS)		AJ401460* AJ296522*
Scrophulariaceae	Manuleeae	<i>Anterotheramus</i>	Hansen 3472 (UPS)	AJ401392*	AJ401442* AJ296509*
Scrophulariaceae	Manuleeae	<i>Barthlottia</i>	Guillaumet J.-L. 3861 (P)	AJ401438*	AJ401443* AJ401444*
Scrophulariaceae	Manuleeae	<i>Campptoloma</i>	Jonsell 5558 (UPS)	AJ401398* AJ401399*	AJ401445* AJ401449*
Scrophulariaceae	Manuleeae	<i>Campptoloma</i>	Thulin 9655 (UPS)	AJ401401*	AJ296514*
Scrophulariaceae	Manuleeae	<i>Campptoloma</i>	Nordenstam & Lundgren 869 (S)	AJ401431* AJ401432*	AJ401450* AJ296515*
Scrophulariaceae	Manuleeae	<i>Glekia</i>	MacOwan 1363 (S)	AJ401422*	AJ296519*
Scrophulariaceae	Manuleeae	<i>Glumicalyx</i>	Hedberg 82054 (UPS)	AJ401413*	AJ296497*
Scrophulariaceae	Manuleeae	<i>Jamesbrittenia</i>	Örtendahl 691 (UPS)	AJ401404*	AJ296511*
Scrophulariaceae	Manuleeae	<i>Jamesbrittenia</i>	Täckholm 17/1 1927 (S)	AJ401435* AJ401436*	AJ401451*
Scrophulariaceae	Manuleeae	<i>Jamesbrittenia</i>	Hilliard & Burt 14560 (S)	AJ401439*	
Scrophulariaceae	Manuleeae	<i>Lyperia</i>	Bremer 3717 (UPS)	AJ401406*	

Scrophulariaceae	Manuleeae	<i>Lyperia</i>	<i>antirrhinoides</i>	Hagström & Acock 1162 (S)	AJ401405*	AJ401446*	AJ401452*
Scrophulariaceae	Manuleeae	<i>Manulea</i>	<i>cheiranthus</i>	Acock J.P.H. 4777 (S)	AJ401418*	AJ401419*	
Scrophulariaceae	Manuleeae	<i>Manulea</i>	<i>tomentosa</i>	Bremer 3781 (UPS)	AJ401394*		
Scrophulariaceae	Manuleeae	<i>Manulea</i>	<i>glandulosa</i>	Bremer 3519 (UPS)	AJ401395*		AJ296520*
Scrophulariaceae	Manuleeae	<i>Manulea</i>	<i>crassifolia</i>	Hilliard & Brutt 12073 (S)	AJ401428*	AJ401429*	AJ401453* AJ2965118*
Scrophulariaceae	Manuleeae	<i>Manuleopsis</i>	<i>dinterii</i>	Strey 2340 (UPS)	AJ401410*		AJ401454* AJ296500*
Scrophulariaceae	Manuleeae	<i>Melanospermum</i>	<i>foliosum</i>	Hansen 3151 (UPS)	AJ401415*		AJ401456* AJ296507*
Scrophulariaceae	Manuleeae	<i>Melanospermum</i>	<i>transvaalense</i>	Hilliard & Brutt 14432 (S)	AJ401426*	AJ401427*	AJ401455* AJ296508*
Scrophulariaceae	Manuleeae	<i>Phyllopodium</i>	<i>cuneifolium</i>	All Batten AB 1125 (S)	AJ401430*		AJ401457* AJ296496*
Scrophulariaceae	Manuleeae	<i>Polycarena</i>	<i>formosa</i>	Fellingham 3219 (UPS)	AJ401423*		AJ296515*
Scrophulariaceae	Manuleeae	<i>Reyemia</i>	<i>chasmantiiflora</i>	All Batten AB 1016 (S)	AJ401425*		AJ296505*
Scrophulariaceae	Manuleeae	<i>Strobilopsis</i>	<i>wrightii</i>	Hilliard & Brutt 10510 (S)	AJ401441*		
Scrophulariaceae	Manuleeae	<i>Sutera</i>	<i>patriotica</i>	Bremer 3818 (UPS)	AJ401393*		
Scrophulariaceae	Manuleeae	<i>Sutera</i>	<i>foetida</i>	All Batten 1107 (S)	AJ401407*	AJ401408*	AJ296510*
Scrophulariaceae	Manuleeae	<i>Tetraselago</i>	<i>longituba</i>	Bremer 3814 (UPS)	AJ401417*		AJ296506*
Scrophulariaceae	Manuleeae	<i>Trieneea</i>	<i>glutinosa</i>	Vlok 2634 (S)	AJ401400*		AJ401459* AJ296516*
Scrophulariaceae	Manuleeae	<i>Zahizianskya</i>	<i>benthamiana</i>	Bremer 3510 (UPS)	AJ401409*		
Scrophulariaceae	Manuleeae	<i>Zahizianskya</i>	<i>katherinae</i>	Olmstead R.G. et al. (1999)	AF123693		AJ401448* AJ296504*
Scrophulariaceae	Manuleeae	<i>Zahizianskya</i>	<i>glareosa</i>	Bremer 3817 (UPS)	AJ401424*		AJ401447*
Scrophulariaceae	Manuleeae	<i>Zahizianskya</i>	<i>minima</i>	Bremer 3542 (UPS)	AJ401437*		AJ296491*
Scrophulariaceae	Manuleeae	<i>Zahizianskya</i>	<i>angustifolia</i>	Bremer 3506 (UPS)	AJ401411*		AJ296502*
Selagineae	Selagineae	<i>Agathelpis</i>	<i>retrorsa</i>	Vlok et al. 68 (S)	AJ401421*		AJ296503*
Selagineae	Selagineae	<i>Chenopodiopsis</i>	<i>decumbens</i>	Goldblatt 7092 (S)	AJ401403*		AJ296501*
Selagineae	Selagineae	<i>Cromidon</i>	sp.	Bremer 3654 (UPS)	AJ401412*		
Selagineae	Selagineae	<i>Dischisma</i>	<i>dentata</i>	Oxelman et al. (1999)	AF027285		
Selagineae	Selagineae	<i>Hebenstretia</i>	<i>cordata</i>	Bremer 3667 (UPS)	AJ401414*		AJ296499*
Selagineae	Selagineae	<i>Hebenstretia</i>	<i>lucidus</i>	Schlechter s.n. (UPS)	AJ401416*		AJ296492*
Selagineae	Selagineae	<i>Microdon</i>	<i>ascendens</i>	Esterhuisen 22750 (S)	AJ401433*		AJ296495*
Selagineae	Selagineae	<i>Pseudoselago</i>	<i>serrata</i>	Bremer 3544 (UPS)	AJ401440*		
Selagineae	Selagineae	<i>Pseudoselago</i>	<i>subglabra</i>	Bremer 3522 (UPS)			
Selagineae	Selagineae	<i>Selago</i>	<i>thumberghii</i>	Olmstead et al. (1995)	L36412		AJ296498*
Selagineae	Selagineae	<i>Selago</i>	sp.	Bremer 3732 (UPS)			AJ296493*
Selagineae	Selagineae	<i>Selago</i>	<i>corymbosa</i>	David Hummel 1960-04-14 (S)	AJ401434*		AJ401458* AJ296494*
Scrophulariaceae	Selagineae	<i>Walafriida</i>	<i>nitida</i>	Bremer 3766 (UPS)	AJ401420*		AJ296500*
Stilbaceae		<i>Stilbe</i>	<i>albiflora</i>	Oxelman et al. (1999)	AF027287		
Verbenaceae		<i>Verbena</i>	<i>bracteata</i>	Olmstead et al. (1995)	L36418		

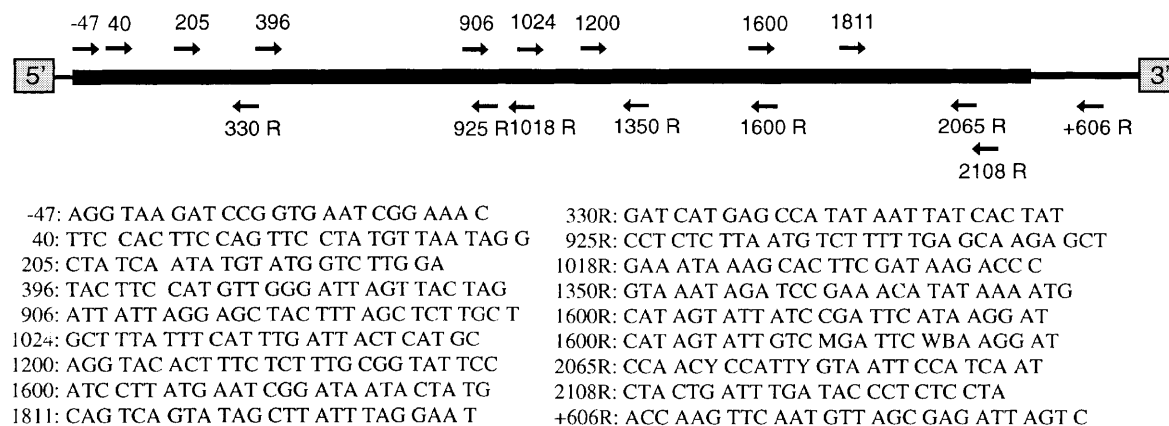


Fig. 1. Map of the chloroplast gene *ndhF* with primer sites indicated. Coding strand primers (forward) are indicated above the line, and reverse (indicated by a R) are below the line. Numbers indicate relative position of the 5' end of the primer aligned to the tobacco sequence (Shinozaki 1986, GenBank accession number Z00044), "+" denotes a position downstream from the 3' end of the gene and "-" a position upstream the 5' end

inference, we used 'Mr Bayes' (Huelsenbeck 2000) a software that uses a Metropolis-coupled Markov chain Monte Carlo algorithm (MC)³. We incorporated the transition-transversion rates proposed by the likelihood ratio test as initial values in the inferences. We conducted the inferences with a general timereversible model, GTR, and with data partitioned according to codon positions. The "temperature" of the chains and other options of the (MC)³ were all left at default, but we corrected for sequencing error, setting its probability to 1%. We did five different analyses with different starting points, each run with 4 chains and 100 000 generations. Of these, we sampled every 100:th generation. We plotted the lnL vs. time of the five runs to evaluate the chains and the 'burn in' period. From the sampled generations we discarded the first 50 000 as the burn-in, giving us 500 sampling points for each run. Then we plotted the posterior probability of ten nodes that were interesting for this study. The degree to which they agree is a relevant indicator of chain convergence (Fredrik Ronquist pers. comm.). The reason for running 5 runs is only to get an indication of chain convergence. If we from the beginning would be sure of the efficiency of the settings of the run this step would be unnecessary. The interesting properties of a Bayesian analysis lies in the MC³ chain itself. Then we summarized the five runs and constructed a majority rule consensus tree with the help of PAUP. The percentage a single node is present in a majority rule consensus tree is a good

approximation of the posterior probability of that node (Huelsenbeck 2000).

Results

Molecular data. The *ndhF* data set consists of 61 taxa and 2,130 unordered equally weighted characters, 1,257 of which are constant and 493 parsimony-informative. The matrix shows 4 indel characters and the heuristic search yielded 96 equally parsimonious trees of length 2,238, with consistency index CI=0.56, and retention index RI=0.73. The strict consensus tree of these (not shown) has the same topology as the consensus tree obtained in the jackknife analysis (Fig. 2). All Manuleeae/Selagineae taxa included in the analysis (except *Camptoloma*) are part of Scrophulariaceae s. str., with Buddlejaceae together with *Camptoloma* and *Freylinia* as a sister group (Fig. 2, I-II). The genus *Antherothamnus* groups with *Scrophularia* and *Verbascum* with 100% jackknife support. All Manuleeae and Selagineae taxa (except *Antherothamnus* and *Camptoloma*) constitute a clade with 95% jackknife support (Fig. 2, III). The result shows that Manuleeae and Selagineae do not form two separate monophyletic groups. Instead, Selagineae taxa are nested within Manuleeae. All of the former Selagineae taxa are included in a

Table 2. Characters and character states used in the optimization of non-molecular characters

1. Habit
1: shrub
2: shrublet
3: perennial
4: annual
2. Occurrence of cymes
0: absent
1: present
3. Ovules per loculus
0: one
1: two
2: more than two
4. Chromosome number (n=)
0: seven
1: six
2: eight
3: nine
5. Stem hairs
0: eglandular
1: stalked glandular
2: sessile glands
6. Bract, adherence to calyx
0: free
1: adnate
7. Calyx lobing
0: nearly to base
1: ± halfway
2: toothed
8. No. of stamens
0: two
1: four
2: two + a staminode
3: four + a staminode
9. Stigmatic area
0: lingulate
1: ± terminal
10. Nectary
0: annular
1: lateral gland

clade (Fig. 2, IV), with 100% support in the jackknife analysis. The newly described genus *Barthlottia* (Fischer 1996) groups with *Manuleopsis* in a clade with 85% jackknife support,

and *Manulea* and *Sutera* constitute a clade with 86% jackknife support. Other well supported clades are: *Chenopodiopsis* and *Pseudoselago* (100%), *Zaluzianskya* and *Reyemia* (100%), *Polycarena* and *Phyllopodium* (100%), *Glumicalyx* and *Strobilopsis* (98%), *Melanospermum* and *Tetraselago* (91%), *Selago*, *Walafrida*, *Agathelpis*, and *Microdon* (100%), the latter clade + *Cromidon* (95%), and *Hebenstretia* and *Dischisma* (100%).

The result of the likelihood ratio test proposed the use of a GTR model with equal transition rates, a proportion of invariant sites, and a gamma shaped model of substitution on the variant sites. The rates proposed were: $r(a-c) = 1.8656$, $r(a-g) = 3.0498$, $r(a-t) = 0.2135$, $r(c-g) = 2.0176$, $r(c-t) = 3.0498$, and $r(g-t) = 1.000$. These results we implemented into 'MrBayes' as a GTR model with the substitution rates from the test as initial values. The majority rule consensus from all the runs of the Bayesian analysis with posterior probabilities plotted on it is shown in Fig. 3. A plot of the lnL as a function of time for all five (MC)³ runs is shown in Fig. 4. It shows that the runs have converged towards the same likelihood (the actual value fluctuating around -15,030). It also shows that the burn-in period of 50 000 generations seems adequate since the curve then has been reasonably stable for some 10 000 generations. When comparing the posterior probabilities of the ten nodes (Fig. 3, I–X) of the five different runs only the node marked VIII differed between the runs. It received a posterior probability between 0.98–1.00. All the other nodes marked with roman figures had unambiguous posterior probabilities of 1.00, which indicates that the chains have reached the same global optimum. The topology of the tree agrees fully with that resulting from the parsimony analysis. The four nodes commented on above in the ndhF tree (Figs. 2 and 3, I–IV) all receive a posterior probability of 1.00. An aspect of Bayesian inference is that it does not only give posterior probabilities of phylogenetic trees but also the posterior distributions for

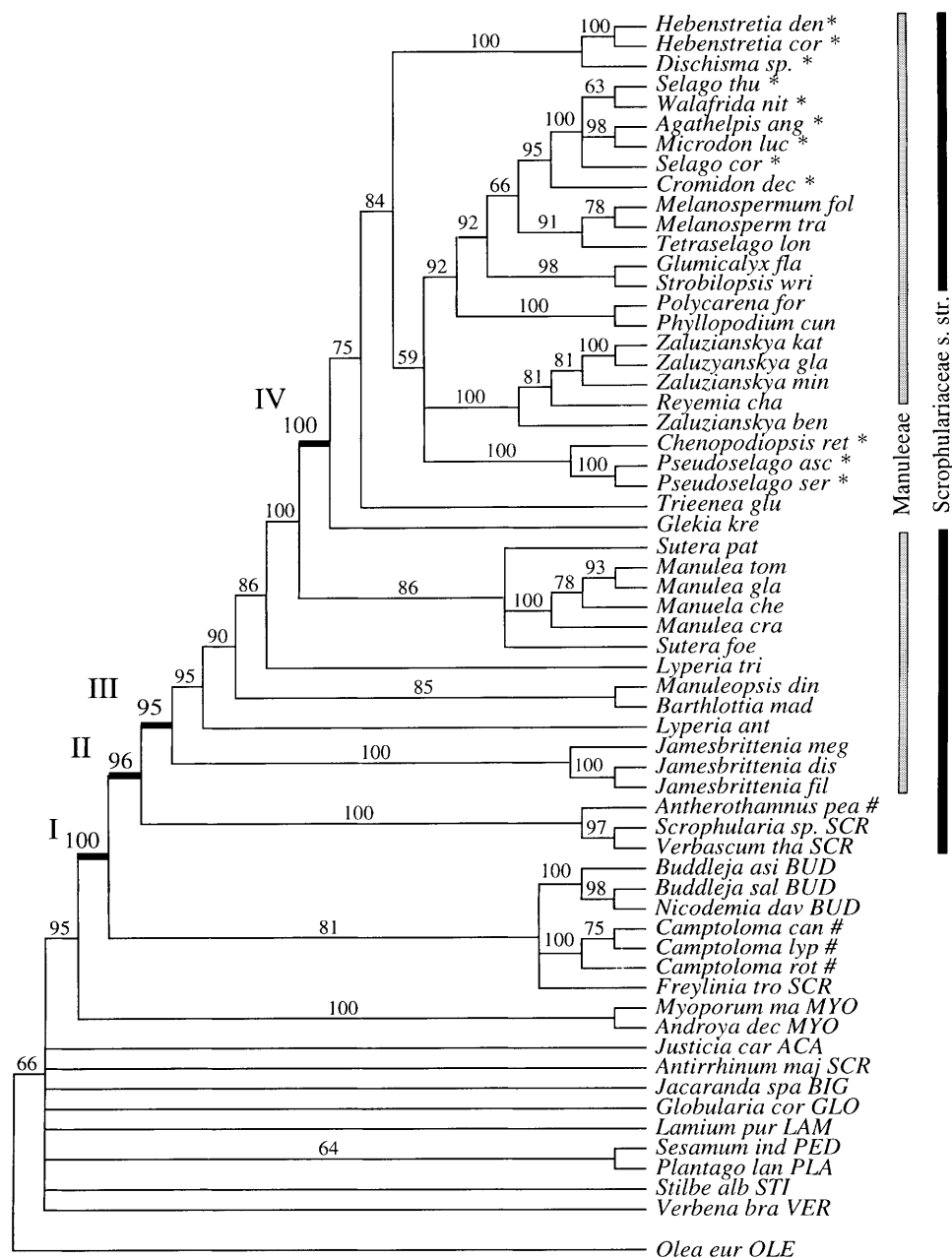


Fig. 2. The jackknife consensus tree from the analysis of *ndhF* matrix. Roman figures are commented on in text. Dark vertical line denotes Scrophulariaceae s. str. Grey vertical line Manuleeae as conceived in this paper. Figures above nodes are jackknife support in percent. Hash mark (#) denotes former Manuleeae taxa in this study found outside Manuleeae, and an asterisk (*) denotes former Selagineae taxa. Taxonomic position of taxa outside Manuleeae/Selagineae group are indicated with a three letter combination: **ACA** Acanthaceae, **BIG** Bignoniaceae, **BUD** Buddlejaceae, **GES** Gesneriaceae, **GLO** Globulariaceae, **LAM** Lamiaceae, **MYO** Myoporaceae, **OLE** Oleaceae, **PED** Pedaliaceae, **PLA** Plantaginaceae, **SCR** Scrophulariaceae, **STI** Stilbaceae, **VER** Verbenaceae

each of the parameters of the model. A summation of these distributions is given in Table 3. It shows, as expected, transition rates

to be higher than transversion rates, and shows a higher substitution rate on the third codon position.

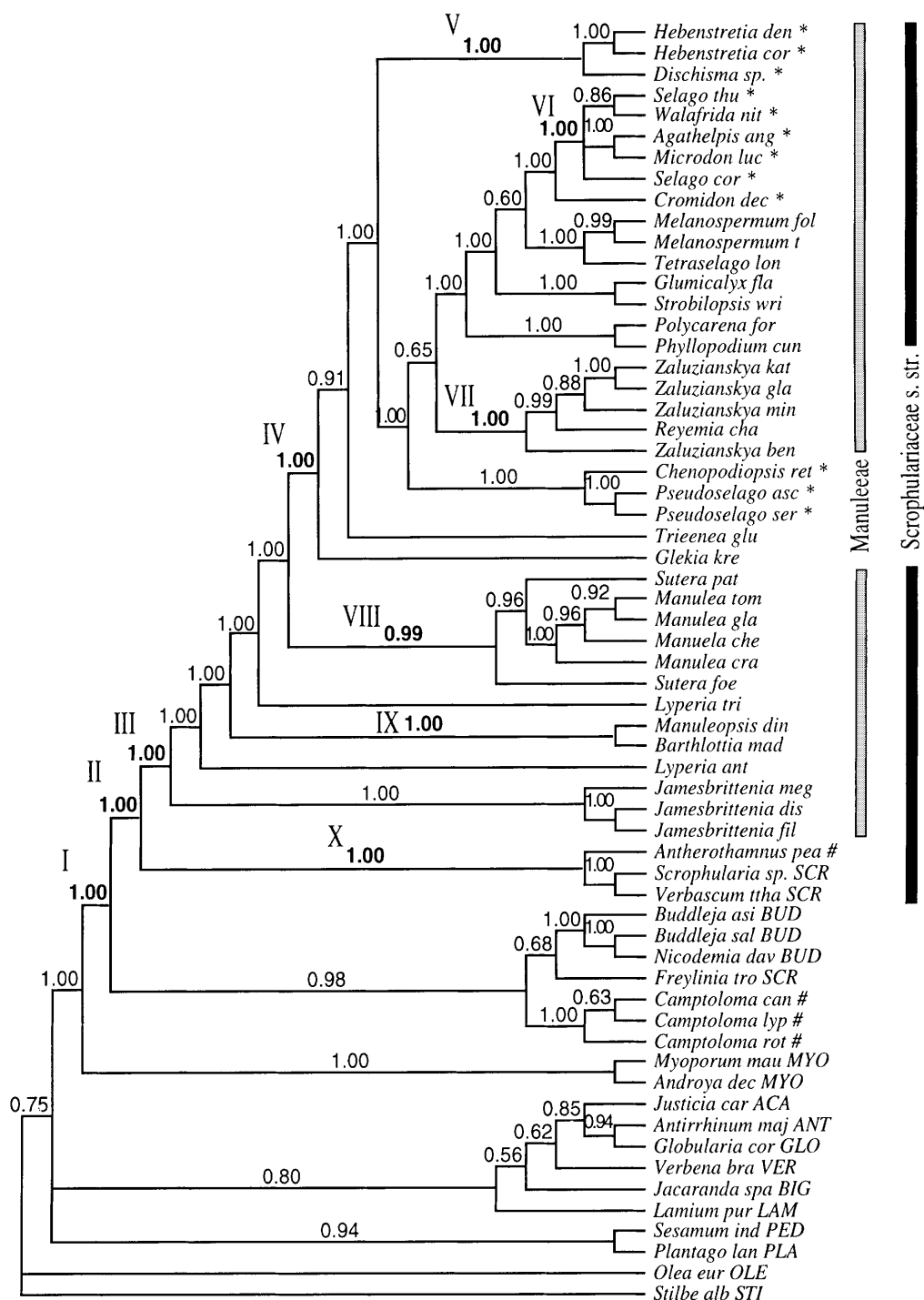


Fig. 3. The Majority rule consensus tree of the five (MC)³ runs with the ‘burn-in’ generations discarded. Values above nodes are posterior probabilities. Nodes marked with roman figures are commented on in text. Dark vertical line denotes Scrophulariaceae s. str. Grey vertical line Manuleeae as conceived in this paper. Hash mark (#) denotes former Manuleeae taxa in this studie found outside Manuleeae, and an asterisk (*) denotes former Selagineae taxa. Taxonomic position of taxa outside Manuleeae/Selagineae group are indicated with a three letter combination: **ACA** Acanthaceae, **BIG** Bignoniaceae, **BUD** Buddlejaceae, **GES** Gesneriaceae, **GLO** Globulariaceae, **LAM** Lamiaceae, **MYO** Myoporaceae, **OLE** Oleaceae, **PED** Pedaliaceae, **PLA** Plantagina-ceae, **SCR** Scrophulariaceae, **STI** Stilbaceae, **VER** Verbenaceae

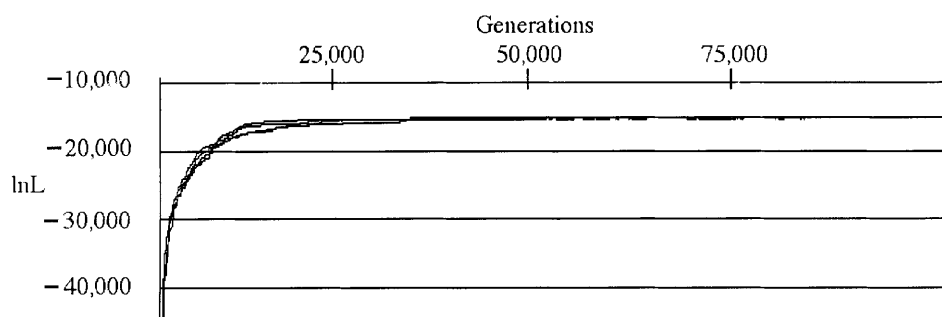


Fig. 4. Plot of $\ln L$ for the five different $(MC)^3$ runs. It shows that the five runs have converged towards the same likelihood and that a 'burn-in' period of 50,000 generations seems adequate. All chains have then been reasonable stable for some 10,000 generations

Table 3. Posterior distributions for the parameters of the model used in the Bayesian inference. $r(x-y)$ = the substitution rates for the transition/transversion x to y . $\pi(x)$ = stationary frequency of nucleotide x . $ss(x)$ = the substitution rate for the codon position x

Parameter	Mean	Variance	(95 percent CI)
$r(g-t)$	1.865600	0.000000	(1.865600 1.865600)
$r(c-t)$	9.048147	1.148381	(7.058245 11.244601)
$r(c-g)$	5.429866	0.606056	(4.047859 7.008018)
$r(a-t)$	0.330626	0.004798	(0.203193 0.469188)
$r(a-g)$	8.508037	0.767989	(6.985123 10.459041)
$r(a-c)$	5.267836	0.523832	(3.991844 6.903719)
$\pi(a)$	0.298873	0.000073	(0.281036 0.317996)
$\pi(c)$	0.125684	0.000040	(0.113377 0.138434)
$\pi(g)$	0.152747	0.000030	(0.144287 0.162896)
$\pi(t)$	0.422697	0.000094	(0.402376 0.442885)
$ss(1)$	0.501123	0.016771	(0.201152 0.661753)
$ss(2)$	0.420365	0.004296	(0.346902 0.556004)
$ss(3)$	1.662917	0.134851	(0.738605 2.037615)

The combined data matrix (*ndhF* and *trnL*) consists of 29 taxa, and 3,852 unordered equally weighted characters, 2,848 of which are constant and 450 parsimony-informative. The matrix includes 35 indel characters. The heuristic search gave two equally most parsimonious trees of which one is shown in Fig. 5. The difference between these two trees is restricted to the relations between *Selago*, *Walafrida*, *Microdon*, and *Agathelpis*. The topology of the strict consensus tree from these and the jackknife consensus tree are almost identical. The included Manuleeae and Selagineae (except *Camptoloma* and *Antherothamnus*) constitute a clade with 84% support.

Antherothamnus groups with *Scrophularia* and *Verbascum* with 100% support. Unambiguous indel characters support two nodes with a jackknife support of 100% as well. The first one from the base of the tree (Fig. 5, I) containing *Trieneea*, *Glekia*, and upwards has support of six indel characters, and the second one from *Glumicalyx* and upward (Fig. 5, II) is supported by three indel characters. Overall, the tree is consistent with the *ndhF* tree.

Optimized non-molecular characters. We show the resulting matrix in Table 4 and results of the optimizations in Figs. 6–13. We have chosen not to show character one, habit, and character eight, stamens. The genus *Lyperia*

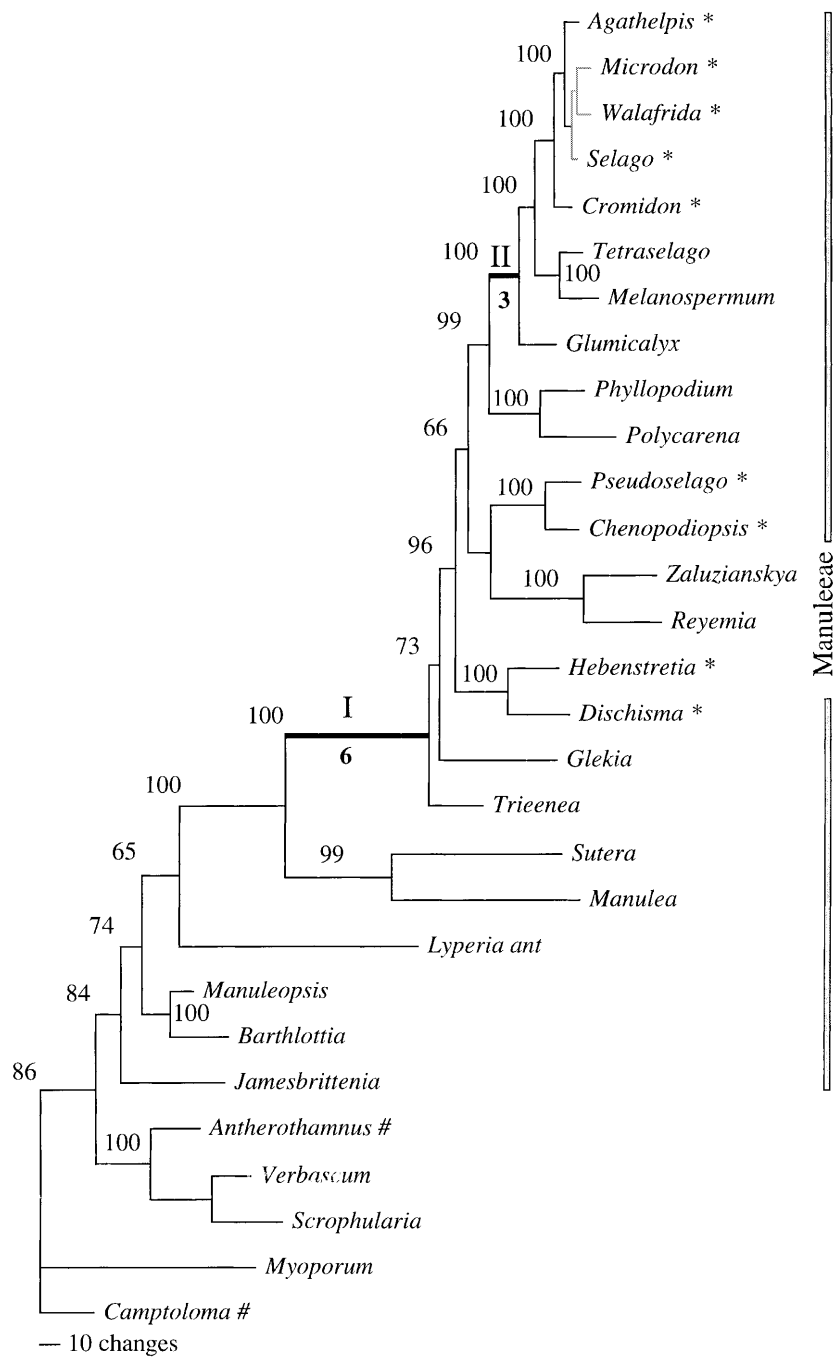


Fig. 5. One of the two most parsimonious trees from the heuristic parsimony analysis of the combined matrix of *ndhF* and *trnL*. Roman figures commented on in text. Figures above, or to the right of, nodes indicates jackknife support values, and figures in bold below number of indel characters unambiguously supporting the node. Grey vertical line indicate Manuleeae as perceived in this article. Asterisk denotes former Selagineae taxa, and hash-mark former Manuleeae taxa now found outside Manuleeae. The part of the tree disagreeing between the two most parsimonious trees are shaded in grey

Table 4. Characters and character states chosen for the optimization of nonmolecular characters. Former Selagineae taxa denoted by an asterisk

Genera/character	1	2	3	4	5	6	7	8	9	10
<i>Agathelpis</i> *	2, 3	0	0	?	0	1	2	0	0	1
<i>Barthlottia</i>	1	1	2	?	1	0	0	1	1	?
<i>Chenopodiopsis</i> *	4	0	0	?	?	1	1	0, 1	0	0
<i>Cromidon</i> *	3, 4	0	0	?	1	1	1	1	0	1
<i>Dischisma</i> *	2, 3, 4	0	0	?	1	?	0	1	0	0
<i>Glekia</i>	2	0	2	?	0	1	1	1	0	1
<i>Glumicalyx</i>	2, 3	0	2	0	0	0	0, 1	1	0	1
<i>Hebenstreitia</i> *	2, 3, 4	0	0	?	0	1	2	1	?	1
<i>Jamesbrittenia</i>	1, 2, 3, 4	0	2	1	1, 2	0	0	1, 2	1	0, 1
<i>Lyperia</i>	3, 4	0	2	2	1	0	0	1, 2	1	1
<i>Manulea</i>	2, 3, 4	1	2	2, 3	1, 2	0	1	0, 1	0	1
<i>Manuleopsis</i>	1	1	2	?	1	0	0	1	1	0
<i>Melanospermum</i>	3, 4	0	2	?	0, 1	0, 1	1	1	0	1
<i>Microdon</i> *	2, 3	0	0	?	0	1	2	1	0	1
<i>Phyllopodium</i>	3, 4	0	2	1	0, 1	1	0, 1	1	0	1
<i>Polycarena</i>	4	0	2	0	1	0	1	0, 1	0	1
<i>Pseudoselago</i> *	2, 3, 4	0	0	?	1	1	0	1	0	1
<i>Reyemia</i>	4	0	2	?	0, 1	1	2	2	0	1
<i>Selago</i> *	2, 3, 4	0	0	0	0	0	1	1	0	1
<i>Sutera</i>	3, 4	1	2	0	1, 2	?	1	1	0, 1	0
<i>Tetraselago</i>	3	0	1	0	0	1	2	1	0	1
<i>Trieneea</i>	2, 3, 4	0	1, 2	1	0, 1	0, 1	0	1	0	1
<i>Walafriida</i> *	2, 3, 4	0	0	?	1	1	1	1	0	1
<i>Zaluzianskya</i>	2, 3, 4	0	2	1	0, 1	1	2	0, 1	0	1

is here represented by *L. anthirrhinoides*. According to the *ndhF* data the genus is polyphyletic so the genus needs further investigation. Two genera are habitually shrubs; *Manuleopsis* and *Barthlottia*. Most other genera are polymorphic and either shrublets, perennials or annuals. Character eight, numbers of stamen is polymorphic within most genera. Only *Reyemia* (two stamens plus a staminode), and *Agathelpis* (two stamens) deviates consistently from the common condition of four stamens.

The optimization of character two, occurrence of cymes, makes evident that cymes are found only in the basal parts of the cladogram (Fig. 6). Character three, the number of ovules per locule, shows a distribution supporting the former tribes Manuleeae and Selagineae (Fig. 7). In character four, haploid chromosome number, $n = \text{six or seven}$ is common

(Fig. 8). Taxa in the clade encompassing *Glumicalyx* through *Agathelpis* including *Tetraselago*, and *Melanospermum* when known all have $n = 7$ or 14 . In the basal parts of the tree $n = 8, 9$, or 12 appears. Cytological data for only 12 of 26 studied taxa were available. Character five, stem hairs, shows a distribution in which presence of eglandular stem-hairs is a more common character in the upper parts of the phylogenetic tree as opposed to glandular stem-hairs is derived on multiple occasions and is being evenly distributed among former Selagineae and Manuleeae taxa (Fig. 9). The state of character six with bracts adherent to calyx, denotes a node encompassing *Trieneea* and upward (Fig. 10). The common condition of character seven, calyx lobed no more than halfway to the base of calyx is derived and common in the upper parts of the tree, while lobing to the base is common in the basal parts

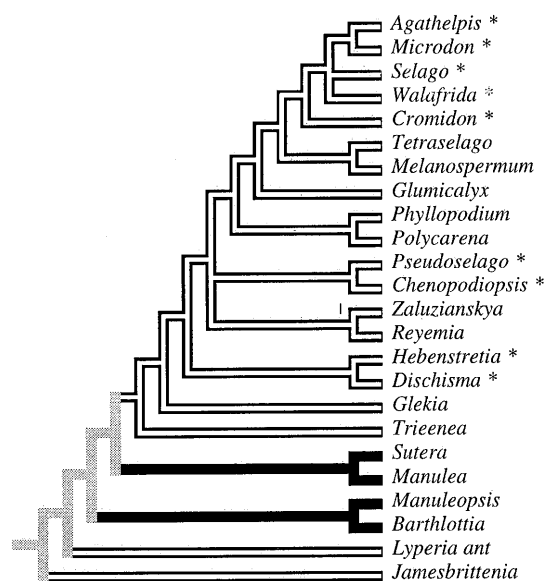


Fig. 6. Optimization of character 2, occurrence of cymes. Asterisk denotes former Selagineae taxa. Black line indicates presence of cymes, grey line equivocal parts of the tree, and unfilled line inflorescences other than cymes

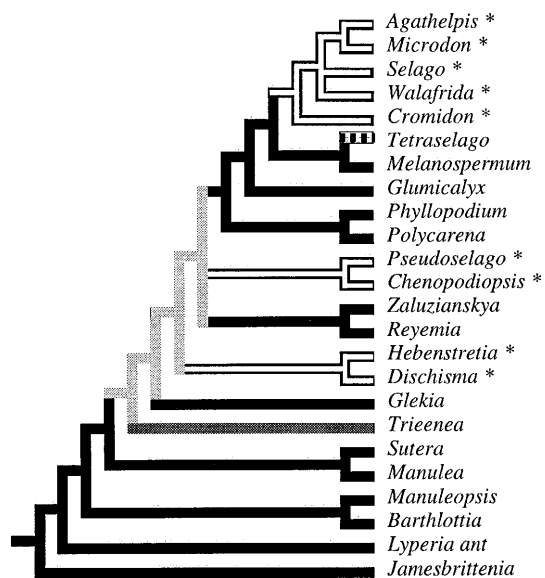


Fig. 7. Optimization of character 3, number of ovules/loculus. Asterisk denotes former Selagineae taxa. Unfilled line indicates one, dashed line two, and a black line more than two ovules per loculus. Light grey line marks equivocal parts of the tree, and a dark grey line denotes a taxon with two or more ovules/loculus

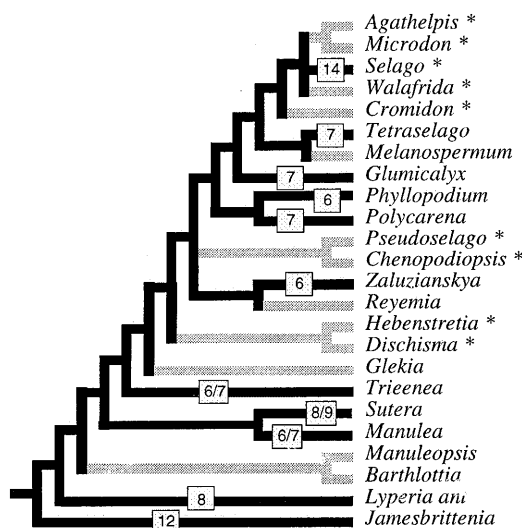


Fig. 8. Optimization of character 4, chromosome number $n =$. Asterisk denotes former Selagineae taxa. Grey line indicates no data

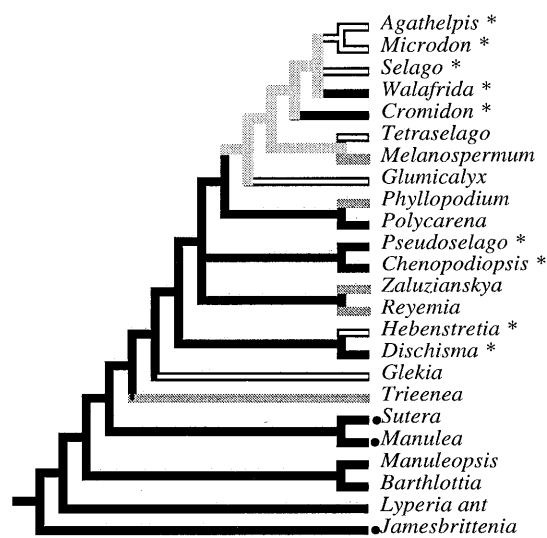


Fig. 9. Optimization of character 5, stem hairs. Asterisk denotes former Selagineae taxa. Unfilled line indicates eglandular, and black line stalked glandular stem hairs. Light grey line marks equivocal parts of the tree, dark grey denotes polymorphic taxa, eglandular and stalked glandular. Taxa with sessile glands are denoted by a black dot

of the cladogram (Fig. 11). For character nine, stigmatic area, the common situation in both Manuleeae and Selagineae is a lingulate stigmatic area (Fig. 12). This is opposed to a terminal stigmatic surface in the basal parts of the cladogram. Character ten, nectary, shows that from *Trieneea* and upward the common situation is a lateral gland as opposed to an annular gland in taxa of the basal part of the tree (Fig. 13).

Discussion

Selagineae are nested within Manuleeae, and thus, the two tribes can no longer be maintained as separate taxa. The Selagineae condition, with one or two ovules per ovary locule has apparently emanated several times within Manuleeae. The optimization of non-molecular characters also shows that characters used for generic delimitation within former Manuleeae are found in taxa from both former tribes.

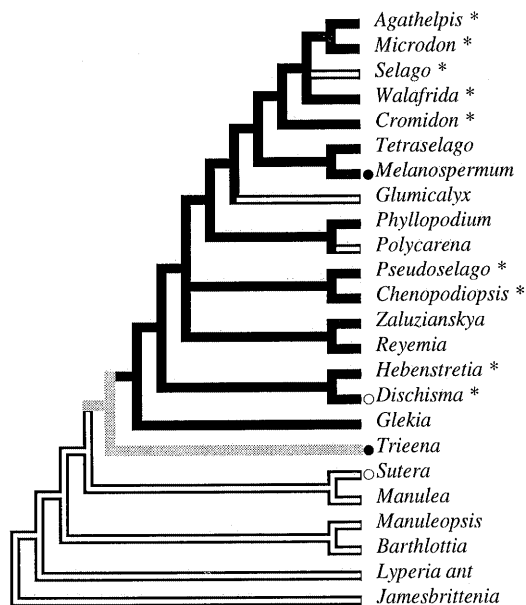


Fig. 10. Optimization of character 6, adherence of bract to calyx. Asterisk denotes former Selagineae taxa. Unfilled line indicates bracts free from calyx, black line bracts adnate to calyx. Grey line indicates equivocal parts of the tree. Polymorphic taxa are marked with a black dot, and taxa without data with an unfilled dot

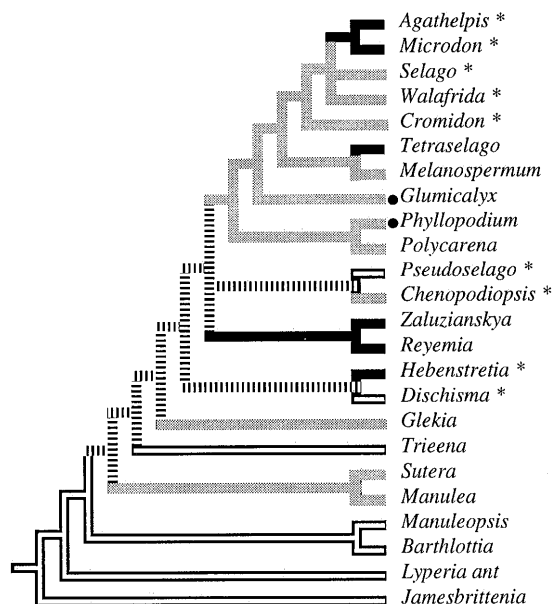


Fig. 11. Optimization of character 7, calyx lobing. Asterisk denotes former Selagineae taxa. Unfilled line indicates calyx lobed nearly to base, grey line calyx lobed halfway to base, and black line a toothed calyx. Equivocal parts of the tree marked with dashed line, and polymorphic taxa with a black dot

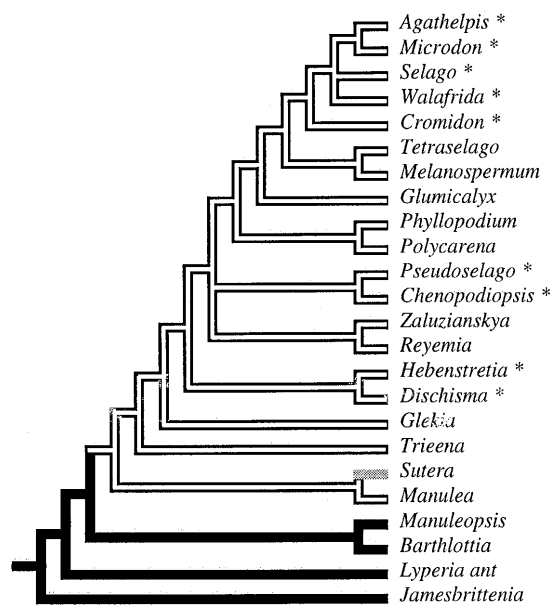


Fig. 12. Optimization of character 9, stigmatic surface. Asterisk denotes former Selagineae taxa. Unfilled line indicates lingulate, black line more or less terminal stigmatic area. Polymorphic taxa are marked with a grey line

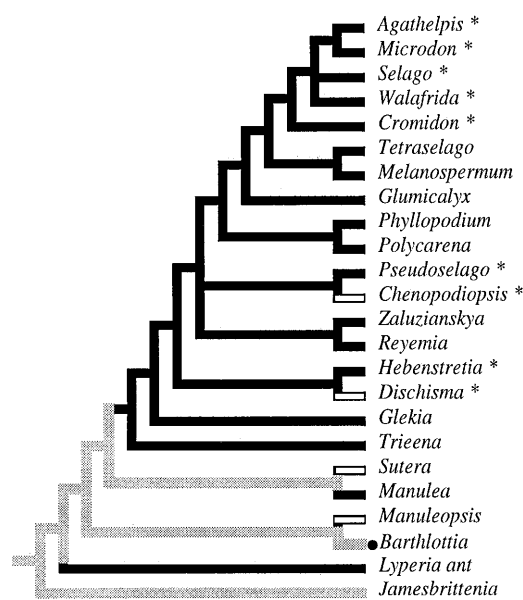


Fig. 13. Optimization of character 10, nectary. Asterisk denotes former Selagineae taxa. Unfilled line indicates that the nectary is annular, black line a lateral gland, and grey line mark poly-morphic parts of the tree. No data available are marked with a black dot

Several of the characters show patterns of distribution corroborating the molecular cladogram. Naturally, character three (Fig. 7), ovule number, supports the pre-existing taxonomy. It is the cardinal character used to separate the two tribes. Most other characters support the molecular cladograms. The state of character six, bracts adherent to calyx, and the state of character ten, nectary a lateral gland, denote a very well supported node with 100% jackknife support and six unambiguous indel characters (Fig. 2, I). All former Selagineae taxa are found above this node. The occurrence of a lateral nectary gland is mentioned by Hilliard (1994), besides lingulate stigma, and unicellular clavate hairs inside the corolla, as putative unique features of both Manuleeae and Selagineae. Another node with 100% jackknife support and supported by three unambiguous indels encompasses *Glumicalyx* to *Selago* and *Tetraselago* (Fig. 5, II). The clade defined by these characters is interesting because *Tetraselago* is said to be the missing link between Manuleeae and Selagineae. Junell (1961) described the

genus when he discovered that there existed *Selago* species with two ovules per locule. Hilliard (1977) consequently transferred the genus to Manuleeae.

Cronquist's (1981) view that Selagineae belongs in Globulariaceae, due to a single carpel with pendulous ovule, is contradicted by this study. His view has earlier been refuted on non-molecular grounds by Hilliard (1994) and Argue (1993). The presence of a single carpel with a pendulous ovule in Globulariaceae and some Selagineae is probably a matter of convergent evolution.

The morphological data together with the molecular analyses show that the present taxonomical situation is unsatisfactory. However, circumscription of Manuleeae or Selagineae remains a question. The taxonomy of Scrophulariaceae either is not settled. Among the questions to be answered is whether the family should be restricted to Scrophulariaceae s. str. or include also Buddlejaceae, Myoporaceae, and additional taxa (Olmstead et al. 2001). This makes questions of ranks and delimitation of Manuleeae and Selagineae difficult. Recognition of Manuleeae s.l. as a subfamily, Manuleoideae of Scrophulariaceae, could have been a possibility. Manuleoideae would then incorporate predominantly southern African taxa characterized by corolla lobe aestivation and synthecous anthers. It would correspond to Hilliard's view of Manuleeae, with Selagineae added. The tribe Selagineae could then be retained, but expanded to contain all the taxa with normally lingulate stigma, lateral nectary, and bracts adherent to calyx. That would correspond to a very well supported clade from *Trieenea* and upward (Fig. 5, I). We would then get a classification with a high information content, since both an extended Manuleeae and a morphologically distinct Selagineae would be recognized. However, current nomenclatural practice stipulates that we then should recognize tribes for all the other taxa included in the subfamily as well. We would then either have a lot of small tribes (Manuleeae, Jamesbritteniidae, etc.) or a paraphyletic large tribe, Manuleeae, neither

of which is desirable. Still it is obvious that Selagineae and Manuleeae cannot be regarded as separate taxa of the same rank. Therefore, we propose the inclusion of Selagineae in Manuleeae:

Scrophulariaceae tribe **Manuleeae** Benth. in 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)

Herbs or shrubs often glandular. Leaves simple, without stipules, often opposite at the base of the plant and alternate upwards. Bracts often adnate to calyx. Flowers often solitary in leaf axiles, often in racemes of cymes, or in panicles, occasionally corymbose, bisexual, zygomorphic to sub-actinomorphic. Calyx (3-) 5 (-9) lobed, obscurely to distinctly bilabiate. Corolla gamopetalous, tube cylindrical or funnelshaped, more or less bilabiate, posticous lip 2-lobed, anticous lip 3-lobed, sometimes lower lip seems wanting and posticous lip 4-lobed, with unicellular clavate hairs inside. Stamens dorsifixed, synthecous, usually 4, rarely 2, inserted in corolla tube. Stigma often lingulate with marginal bands of stigmatic papillae, rarely bifid, or entire with terminal papillae. Style solitary, terminal, filiform. Ovary superior or two-celled or rarely by abortion one-celled. Ovules one to many in each locule. Nectary often a small dorsal gland, sometimes annular. Fruit when many seeded a septicidal capsule, otherwise nondehiscent. Seeds small with copious endosperm. Type genus: *Manulea* L.

Genera included: *Barthlottia*, *Chenopodiopsis*, *Cromidon*, *Dischisma*, *Glekia*, *Globulariopsis*, *Glumicalyx*, *Gosela*, *Hebenstretia*, *Jamesbrittenia*, *Lyperia*, *Manulea*, *Manuleopsis*, *Melanospermum*, *Microdon*, *Phyllopodium*, *Polycarena*, *Pseudoselago*, *Reyemia*, *Selago*, *Strobilopsis*, *Sutera*, *Tetraselago*, *Trieneea*, and *Zaluzianskya*. (We here follow Hilliard's (1999) view with *Walafrida* included in *Selago* and *Agathelpis* included in *Microdon*).

Notes: A very close relation between *Reyemia* and *Zaluzianskya* is evident in our data sets. The sequences are very similar in

both base-sequence and indel-composition. This close relation is earlier observed by Hilliard (1994).

The position of the genus *Barthlottia* (only present in the *ndhF* analysis) close to *Manuleopsis* is consistent with Fischer's (1996) view on the affinity of the monotypic genus only found on Madagascar.

A close relationship between *Manulea* and *Sutera* has been observed by Argue (1993) and is supported by this study, as is Hilliard's (1995) separation of *Pseudoselago* from *Selago*.

The affinity between *Hebenstretia* and *Dischisma* inferred from our data is in line with Roessler's (1979) and Hilliard's (1999) comprehension of the two genera. The two genera are not distinguishable except for the number of calyx-segments.

According to our analyses *Selago* is polyphyletic but due to bad resolution in the whole clade encompassing the two representatives of *Selago* we can not make any statement about this from our data. The close relationship between *Walafrida* and *Selago* proposed by Hilliard (1999) is weakly supported in the *ndhF* analyses. Hilliard has in her revision of Selagineae included *Agathelpis* in *Microdon*. This decision is also supported in our analyses of *ndhF* data (Figs. 2 and 3). In the combined tree there is no support in our data for such a decision (Fig. 5).

Scrophulariaceae s. str. The former Manuleeae and Selagineae taxa included in our study are found in a clade within the Lamiales together with Buddlejaceae and *Freylinia*. All genera (except *Camptoloma*) also form a part of Scrophulariaceae s. str. (Fig. 2, II). This is in accordance with indications from earlier molecular studies with taxa from Manuleeae or Selagineae incorporated (Olmstead and Reeves 1995, Olmstead et al. 2001, Oxelman et al. 1999). That *Camptoloma* in our study falls outside Scrophulariaceae s. str. is an unexpected result. We have not been able to find any justification for this either in literature or in a superficial morphological examination. *Camptoloma* groups with representatives of

Buddlejaceae and the genus *Freylinia*. The support for this clade is not very high, 81% in the parsimony analysis, but receives a posterior probability of 0.98 in the Bayesian inference. Hilliard (1999) raises the question on whether *Camptoloma* is a phylogenetic as well as geographic link between northern Scrophulariaceae and southern Manuleeae and Selagineae. Our data points somewhat in that direction but in a more complex manner.

The genus *Antherothamnus* is consistently in this study found in a clade together with *Scrophularia* and *Verbascum*. It is noteworthy that *Antherothamnus* is the only member of the former Manuleeae that has a fully developed staminode apart from four fertile stamens, and is thus resembling taxa in *Scrophularia* (*Verbascum* has a fully developed fifth stamen). A close relation between *Scrophularia* and *Verbascum* has earlier been proposed by Bachman (1882), Hartl (1959), Thieret (1967), Freeman and Scogin (1999), and Hilliard (1994).

Likelihood or parsimony. Likelihood-based Bayesian inference methods were used in this study as well as parsimony. To run a bootstrap analysis with maximum likelihood would take a very long computing time (Larget and Simon 1999, and Sandersson and Kim 2000) and individual bootstrap replicates would not be certain to have reached the most likely global optima (Li 1996). Bayesian inference on the other hand will do that and give a measure of support that is statistically valid in a very reasonable computing time. It seems to be a promising method.

Historical bias. It is interesting (a posteriori) that so many authors have noted the similarities between Manuleeae and Selagineae but no one has proposed to merge the two tribes. The similarities between the former Manuleeae and the former Selagineae have been noted by, among others, Cronquist (1981) who wrote: "Selagineae are in many ways similar to Manuleeae...". In addition, Hilliard, on the very first page of her excellent monograph on Manuleeae, notes that the morphological distinctiveness of Selagineae/

Selaginaceae has been superficial. Why then have not Manuleeae and Selagineae been united before? Is it because of the use of a cardinal character, here number of ovules per locule? Evidence is piling up that reduction of ovule numbers are a particularly problematic issue within the Lamiales (Wagstaff and Olmstead 1997, Olmstead et al. 2001). This is previously also known for the Rubiaceae (Verdcourt 1958, Bremekamp 1966, Bremer 1992). Maybe it is relevant to discuss historical bias in the context of Cuerrier et al. (1998). They used statistical test methods, and found that modern taxonomic systems (i.e. Dahlgren, Thorne, Cronquist, and Takhtajan) do not differ much neither from each other nor from older systems (i.e. Engler, Bessey, Gobi, and Hallier). There could be at least two different explanations for this. First, that taxonomy has evolved, from a basic relatively good framework, that has been increasingly refined, with new techniques and deeper understanding. Through this, the system has gradually become better, and more stable. Secondly, it could be the effect of historical inertia or bias. A historical bias could be at hand every time a systematist bases systematic work on preceding classifications. The old classifications will always influence the new. As a plant taxonomist, in dealing with an enormously rich and variable material, it is natural to be influenced and biased by previous authors. This has a good effect on stability of the system, and a bad effect in that change in taxonomy that ought to be done is not done. Cuerrier et al. (1998) states the following: "...For changes and modifications to become effective in future classifications of flowering plants, one will have to minimize, if not avoid, the implicit influence of the modern systems as standard systems, and to count on, among others, molecular data in redefining taxonomic concepts founded on classical morphology...". We would argue that the present study proves their statement to be true to some extent. No one has proposed to join Manuleeae and Selagineae, although there is much morphological evidence that unites them. Is this because ovule

number has been used as a taxonomic concept in the negative sense that Cuerrier et al. discusses?

Conclusion

The molecular analyses result in well-resolved trees with high support. They clearly show that neither Manuleeae nor Selagineae can be maintained as separate taxa. Former Selagineae taxa are found intertwined among former Manuleeae taxa in the cladograms. Accordingly, we have extended Manuleeae to encompass all former Manuleeae and Selagineae taxa (except *Antherothamnus* and *Camptoloma*). The extended Manuleeae constitutes a sister clade to a clade with *Scrophularia*, *Verbascum* and *Antherothamnus*. *Camptoloma* is the only taxa of the former Manuleeae and Selagineae that in our study falls outside Scrophulariaceae sensu stricto. Our analysis of morphological data corroborates the molecular data. The Selagineae condition with one or two ovules per ovary locule must according to our data have emanated several times within Manuleeae.

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