

Nanothamnus sericeus THOMSON, a derived species of *Blumea*

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Abstract

Nanothamnus sericeus THOMSON was described as a member of the tribe Mutisieae because of its distinctly bilabiate corolla. Later, the genus has often been treated as a member of the Inuleae but its position there has also been questioned. We investigated the systematic position of this small Indian endemic by jackknife analysis of DNA sequence data from the plastid gene *ndhF* and from the nuclear ribosomal internal transcribed spacers (ITS). It was concluded that *Nanothamnus* belongs to the Inuleae-Inulinae, and that it is a member of the genus *Blumea*, characterized by a number of autapomorphic character states. The new combination *Blumea sericea* (THOMSON) ANDERB. & A. K. PANDEY is made.

Introduction

The genus *Nanothamnus* was described in 1867 by THOMAS THOMSON (THOMSON 1867) on material from the western Indian province Concan. The strikingly two-lipped (4+1 lobes) hermaphroditic flowers made THOMSON place *Nanothamnus* in the Mutisieae, the tribe characterized by flowers with bilabiate corolla. The floral morphology has made this genus difficult to place with certainty. BENTHAM (1873) moved the genus from Mutisieae to the Inuleae-Plucheinae and noted that its corolla made it differ from other Plucheinae, but suggested affinities with *Thespidium* and *Epaltes*. HOOKER (1882) placed *Nanothamnus* between *Pluchea* and *Epaltes* and made the note that: "This curious plant bears some resemblance to a *Blumea* of the *oxyodonta* group in habit and foliage". HOFFMANN (1890) followed BENTHAM's opinion on the position of *Nanothamnus*, whereas NAIRNE (1894) erroneously stated that the florets in *Nanothamnus* were as in *Pluchea indica*, without mentioning the characteristic two-lipped corolla.

MERXMÜLLER et al. (1977) included *Nanothamnus* in the *Pluchea* group of Inuleae-Inulinae where it has remained since, albeit with some doubt. In the first cladistic study of the Inuleae, ANDERBERG (1989) described a variation in the shape and distribution of sweeping-hairs in different groups of the Inuleae sensu MERXMÜLLER et al. (1977), and also showed that members of the *Inula* group were characterized by having a large oxalate crystal in each cypsela epidermis cell. Apart from its bilabiate corolla, *Nanothamnus* was found to resemble the Inuleae s. str. in character states such as the large elongated crystal in the epidermis cells, and acute sweeping-hairs on the abaxial surface of the style-branches. Both these features were at the time considered indications of a relationship with the Inuleae s. str. and *Nanothamnus* was therefore tentatively accepted in that tribe, as a possible relative of *Blumea*. In a later paper, ANDERBERG (1991) modified the view, and expressed doubts on the actual position of *Nanothamnus*, pointing out its bilabiate disc florets, soft anther appendages, and styles with sweeping-hairs situated far below the bifurcation as anomalous character states making the taxonomic position uncertain in the tribe. THOMSON (1867) had mentioned that the anthers were very shortly caudate, but ANDERBERG (1991) found them to be calcarate with short tails. Calcarate anthers are more common among taxa of the Cichorioid tribes than among the Asteroideae to which the Inuleae belongs, and this also contributed to the doubts on its systematic position in that tribe. ANDERBERG (1994) once more tentatively included *Nanothamnus* in the Inuleae because of its elongated cypsela epidermis crystals, but again pointed out its anomalous morphological characters and ANDERBERG & ELDENÄS (2007) listed *Nanothamnus* among the Inuleae genera incertae sedis, possibly being a relative of *Blumea*, but again they pointed out its morphological differences compared to other Inuleae. Considering the many character states that make *Nanothamnus* differ from other Inuleae it is now of great interest to test its systematic position by analysis of DNA sequence data.

Material and methods

DNA was extracted from leaves taken from a herbarium specimen of *Nanothamnus sericeus* [Voucher: India, SARDESAI 2547 (S)]. To test the tribal position of *Nanothamnus* in the family, a *ndhF* sequence was analysed together with the data set (184 taxa) from ANDERBERG et al. (2005) and with an additional number of unpublished sequences, mainly from the Inuleae, a total of 254 sequences of taxa representing all Asteraceae tribes. An ITS sequence was analysed together with other sequences obtained from GenBank (Accession Numbers EF210932-EF210951, EF210953, EF210955-EF210962, EF210967, EF210972-EF210975).

Molecular methods. DNA extraction was carried with QIAGENs DNeasy Plant Mini Kit using the manufacturer's protocol. PCR reactions were performed with PuReTaq Ready-To-Go PCR Beads, 95°C 5 min, followed by 40 cycles of 95°C 30 sec, 50°C 30 sec, 72°C 1 min 30 sec, and finally 72° 8 min. Purification of PCR products was done with Omega Bio-Tek, Inc. E. Z. N. A. Cycle-pure Kit following the manufacturer's protocol. Sequencing reactions were made using the BigDye Terminator v3.1 Cycle Sequencing Kit and unincorporated dye terminators were removed using QIAGEN's DyeEx 96 Kit. Fragments were separated and analysed on an ABI PRISM 3100 Genetic Analyzer. Primers used for PCR and sequencing of *ndhF* are presented in Table 1, and for ITS in Table 2. The two new sequences have been submitted to GenBank (EU562213 and EU562214).

Alignment. Alignment of *ndhF* was performed with the BioEdit software (HALL 1999) ver. 6.0.5. The aligned *ndhF* data set included 254 taxa with several representatives of each tribe of the Asteraceae. Alignment of ITS was performed with ClustalX included in the BioEdit package using default settings. The aligned ITS data included 35 sequences from 34 taxa.

Phylogenetic analyses. The aligned sequences of *ndhF* and of ITS were analysed with parsimony jackknifing using the software XAC (FARRIS 1997) with the following settings: 1000 replications, each with branch-swapping and 10 random-additions of sequences. For the analysis of *ndhF*, *Boopis* (Calyceraceae) was used as outgroup (FARRIS 1972). The ITS data was analysed in the same way using *Duhaldea cappa* as outgroup.

Results

The results of the XAC analysis of *ndhF* placed *Nanothamnus* in the Inuleae, together with *Blumeopsis* and species of the genus *Blumea* (Fig. 1). A jackknife support of 100 % was retrieved for a group comprised of *Blumea riparia*, *B. saxatilis*, *B. integrifolia*, *B. psammophila*, *Blumeopsis flava*, and *Nanothamnus sericeus* (Fig. 2). The sister to this group was *Blumea balsamifera* (76 %), at the next lower node *Caesulia axillaris* was sister to the rest (100 %), and then *Duhaldea cappa* appeared as the sister of all taxa at the base of this clade. The close relationship between *Duhaldea* and *Blumea* was discovered by ANDERBERG (1991), and ANDERBERG et al. (2005) found that also the morphologically specialized *Caesulia* was a member of this clade, another morphologically anomalous genus for which the systematic placement has been discussed. In a recent study, PORNPONGRUNGRUENG et al. (2007) analysed phylogenetical relationships in *Blumea* and showed that *Blumeopsis* and *Merrittia* were ingroups in *Blumea*, and this is congruent with our present results. In the small sample

ndhF tree, *Blumeopsis* is sister to *Nanothamnus*, albeit with weak support (65 %), but their position as ingroups in *Blumea* is strongly supported. The analysis of ITS sequences included a larger sample of *Blumea* species and gave strong support (100 %) for a position of *Nanothamnus* in a *Blumea* clade together with the species *B. hieraciifolia*, *B. mollis* and *B. oxyodonta* (Fig. 3).

Discussion

We conclude that *Nanothamnus* is a member of the Inuleae-Inulinae as circumscribed by ANDERBERG et al. (2005), and that it is in fact a derived member of the genus *Blumea*. Within that genus, it shares a common ancestor with *Blumea hieraciifolia*, *B. mollis*, and *B. oxyodonta* (Fig. 3). It is noteworthy that HOOKER (1882) noted that *Nanothamnus* resembled a species of the *Blumea oxyodonta* group. The bilabiate flowers, the epappose fruit, the soft anther appendages, the calcarate anthers, and the sweeping-hairs extending below the bifurcation are apparently autapomorphies that have evolved in *Nanothamnus*, making it unique within the genus *Blumea*. Whereas the chromosome number in *Blumea* is complex with many basic numbers, the meiotic chromosome number in *Nanothamnus* is $n=10$ (DARUWALLA 1995), which would indicate a somatic number of $2n=20$, and this is fully congruent with that of several *Blumea* species. Soft wrinkled anther appendages are rare in the family but constitute a synapomorphy of the Arctoteae-Arctotidinae (KARIS 2006). The appendages in *Nanothamnus* are however less soft and wrinkled than those of the Arctoteae and most likely represent an independent character state transformation.

Apart from the differences compared to other genera of the tribe, *Nanothamnus* shares some diagnostic synapomorphies with other genera of Inuleae-Inulinae (except *Caesulia*), such as the presence of a large oxalate crystal in the cypsela epidermis cells. It also shares the characteristic 3 bp (CCT) insertion in *ndhF* gene that is found in all Inuleae-Inulinae without exception, and which was described first by ELDENÄS et al. (1999). An autapomorphy in *Nanothamnus* is also a 9 bp insertion in *ndhF* (AAATTAGAT) that seems to be unique in the Asteraceae. This 9 bp insertion is located 66 bases closer to the 3' end than the unique CCT insertion.

The bilabiate flowers in *Nanothamnus* are unique in the Inuleae. They have evidently evolved from ordinary tubular hermaphroditic flowers, possibly as an adaptation to a specific pollinator but nothing is yet known about the pollination of this species. Similar odd and sporadic occurrences of bilabiate corollas are found in representatives of many tribes outside the Mutisieae, but they may be somewhat different, and the kind found in *Nanothamnus* was considered unique

by SMALL (1918). The genetic background to the shift in corolla shape is not known but would be interesting to study. Also the lack of pappus makes *Nanothamnus* unique in *Blumea*. The lack of pappus bristles makes it more difficult for the fruits to disperse any longer distances, and that may be one reason why it is confined to a rather restricted area in western India.

Nanothamnus cannot be retained as a separate genus as its closest relatives are found within *Blumea*. Therefore we propose that *Nanothamnus* is transferred to *Blumea* to maintain the generic monophyly of the latter. The genus *Blumea* now includes species from the monotypic genera *Nanothamnus*, *Merrittia* and *Blumeopsis*. Of these *Nanothamnus* may be the most surprising addition of the three.

***Blumea sericea* (THOMSON) ANDERB. & A. K. PANDEY, comb. nov.**

Basionym: *Nanothamnus sericeus* THOMSON, J. Linn. Soc., Bot. 9: 342 (1867).

Illustr.: Fig. 4; THOMSON 1867, tab. 3.

Low, strongly aromatic, generally much branched herb, branches often prostrate to ascending. Leaves alternate, oblong to ovate-lanceolate, with conspicuously reticulate venation, acutely serrate, sericeous; leaf-teeth with distinct mucro. Capitula aggregated terminally, or axillary, generally heterogamous, disciform, few-flowered, small, ovoid, 3–5 mm long and 2–4 mm wide. Receptacle flat, epaleate. Involucral bracts narrow, imbricate, in 2–3 rows. Outer florets when present, 1–4, functionally female; corolla yellow, c. 2 mm long, 2–3-dentate. Pappus lacking. Central florets 5–6, perfect, corolla yellow, deeply pseudobilabiate with 4+1 lobes. Anthers distinctly but shortly calcarate; thecae shortly caudate; anther appendage soft, somewhat wrinkled; endothecial tissue radial. Style bifid; style-branches with short, triangular, acute sweeping-hairs abaxially, below the bifurcation with longer more obtuse sweeping-hairs; style-base with prominent star-shaped crystal druses. Cypsela ovoid, dark brown with 10 distinct white ribs forming a short five-dentate ring distally; cypsela epidermis cells each with one large elongated oxalate crystal. Pappus missing.

Geographic range: *Blumea sericea* occurs only in western India. It is an endemic of the Western Ghats, growing in the Nasik, Pune, and Shimoga districts of Maharashtra and Karnataka where it grows in dry hills among grasses in forests and forest clearings. Flowers and fruits have been seen from February to April (KUMAR 1995).

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Table 1

Primer sequences (5'-3') for *ndhF*. F = forward, R = reverse.

Name	Direction	Primer sequence
RJ1	F	AGG TAA GAT CCG GTG AAT CGG AAA C
1b	F	TGG GACTTC TTC TTT TTC C
431	F	GAT ACAAAT TTA TAT TTT TTG GG
520	R	CAA ATG CTT TTT GACAAG CAT TTG CCG C
5	F	GTC TCA ATT GGGTTA TAT GAT G
5B	F	GGA GCT ACT TTA GCT CTT G
16	R	GTT AAACCT CCC ATA AGC ACC ATA TTC TGA C
1260	F	TCT TAA TGA TAG TTG GTT GTA TTC ACC
1700	R	CAT AGT ATT ATC TGA TTC ATA AGG ATA
1750	R	ACT GAAAAA ATT GCATCT TTT
10	R	CCC CCT AYA TAT TTG ATA CCT TCT CC
10B	R	CCT ACT CCA TTT GGAATT CCA TC
RJ14	R	ACC AAGTTC AAT GTT AGC GAGATT AGT C

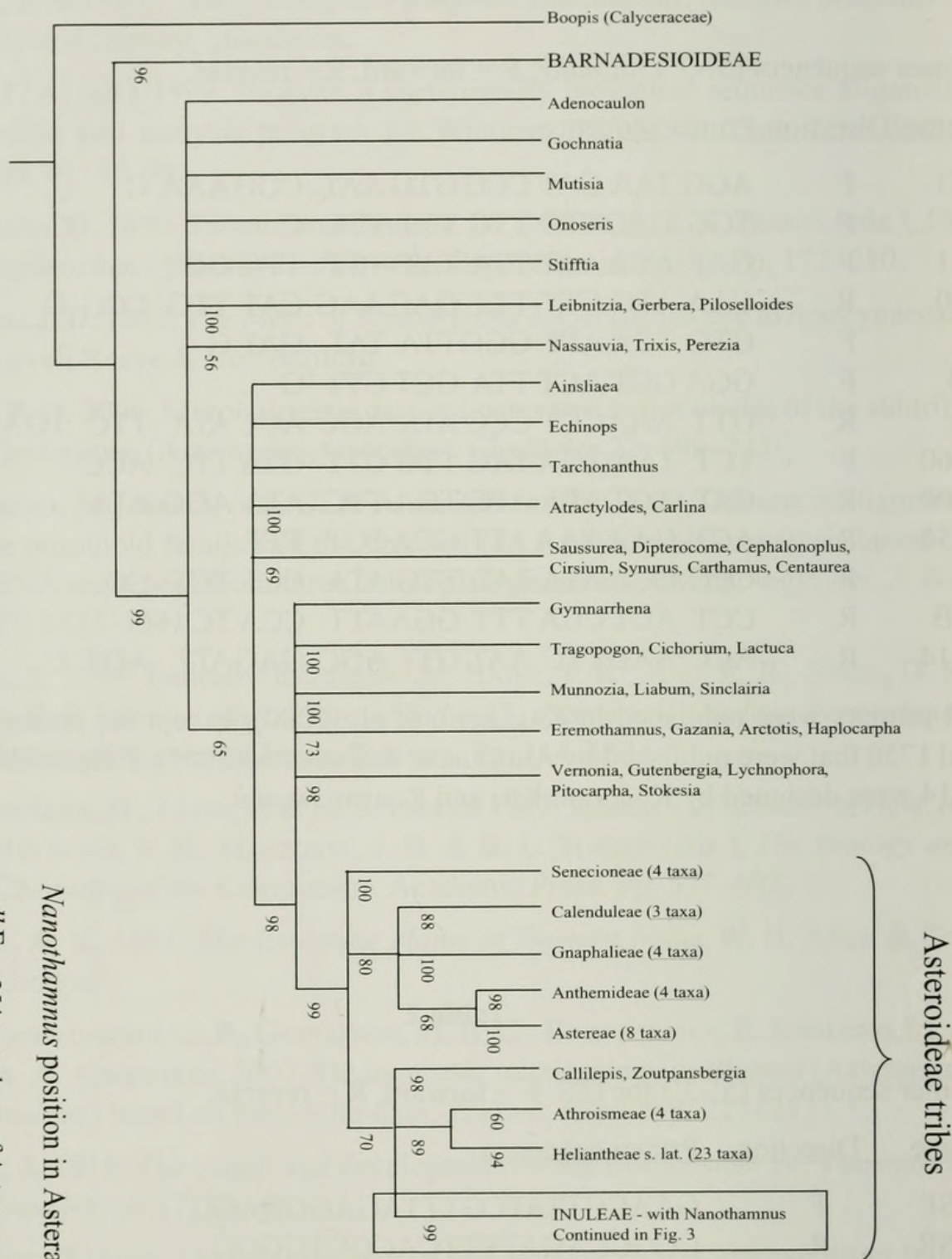
All primers were published in KÄLLERSJÖ et al. (2000), except for primers 520 and 1750 that were published by ANDERBERG & SWENSON (2003). Primers RJ1 and RJ14 were designed by KI-JOONG KIM and ROBERT JANSEN.

Table 2

Primer sequences (5'-3') for ITS. F = forward, R = reverse.

Name	Direction	Primer sequence
18SF	F	GAACCTTATCGTTTAGAGGAAGG
26SR	R	CCGCCAGATTTTCACGCTGGGC
5.8SC	F	TGCGTTCAAAGACTCGAT
5.8SN	R	ATCGAGTCTTTGAACGCA

Primers 18SF and 26SR were designed by CATARINA RYDIN, and primers 5.8SC and 5.8SN by YOUNGBAE SUH.



Nanothamnus position in Asteraceae
ndhF - 254 taxa, base of Asteraceae phylogeny
Tribe Inuleae with *Nanothamnus* continued in Fig. 3

Fig. 1. Parsimony jackknife tree based analysis on *ndhF* sequences from 254 taxa showing the position of Inuleae with *Nanothamnus* (Box) in the Asteraceae phylogeny. Support values > 50 % are shown for each clade. Inuleae clade expanded and continued in Fig. 2.

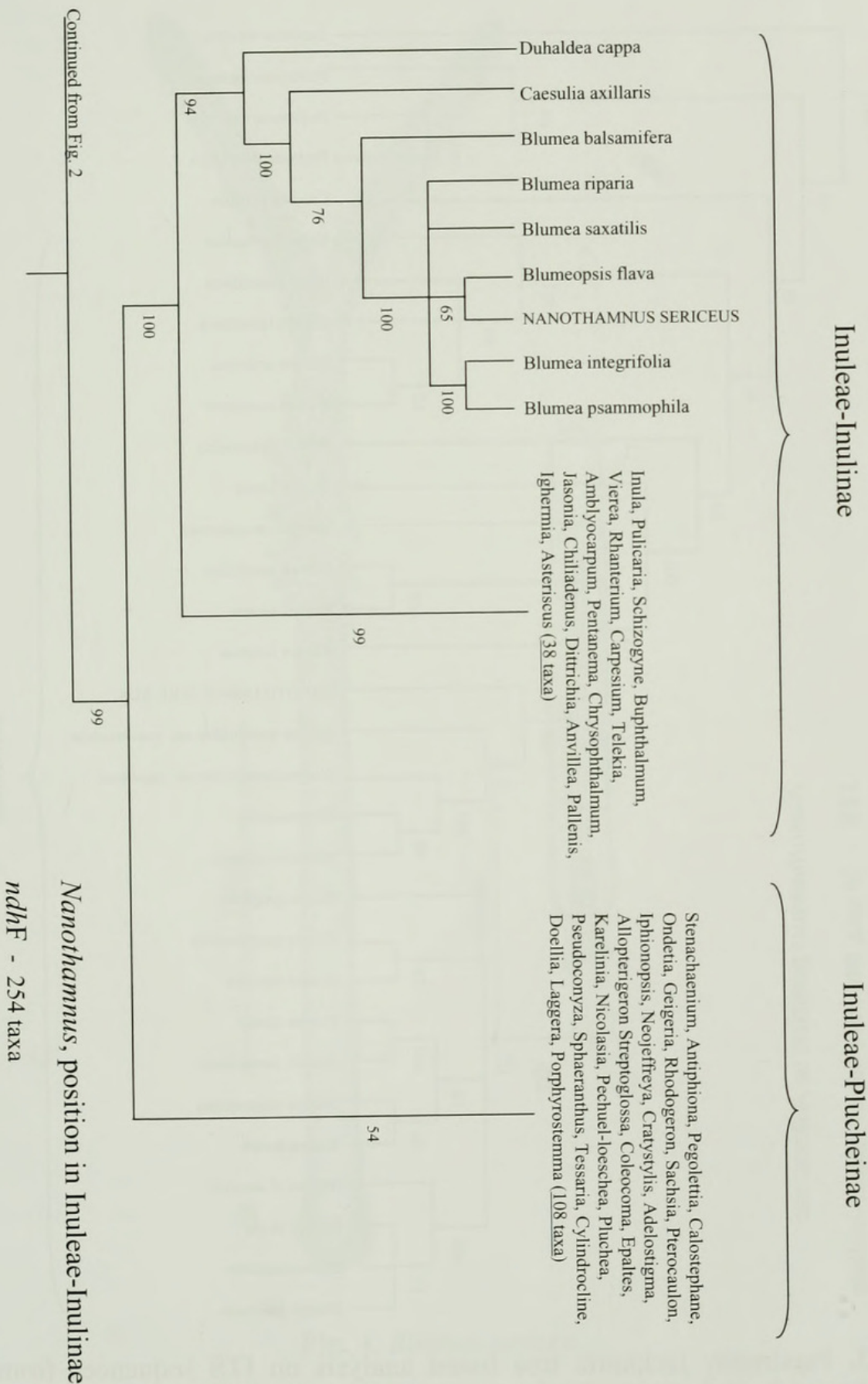


Fig. 2. Parsimony jackknife tree (continued from Fig. 1) showing the position of *Nanothamnus sericeus* in tribe Inuleae, a derived representative of *Blumea*. Support values > 50 % are shown for each clade.

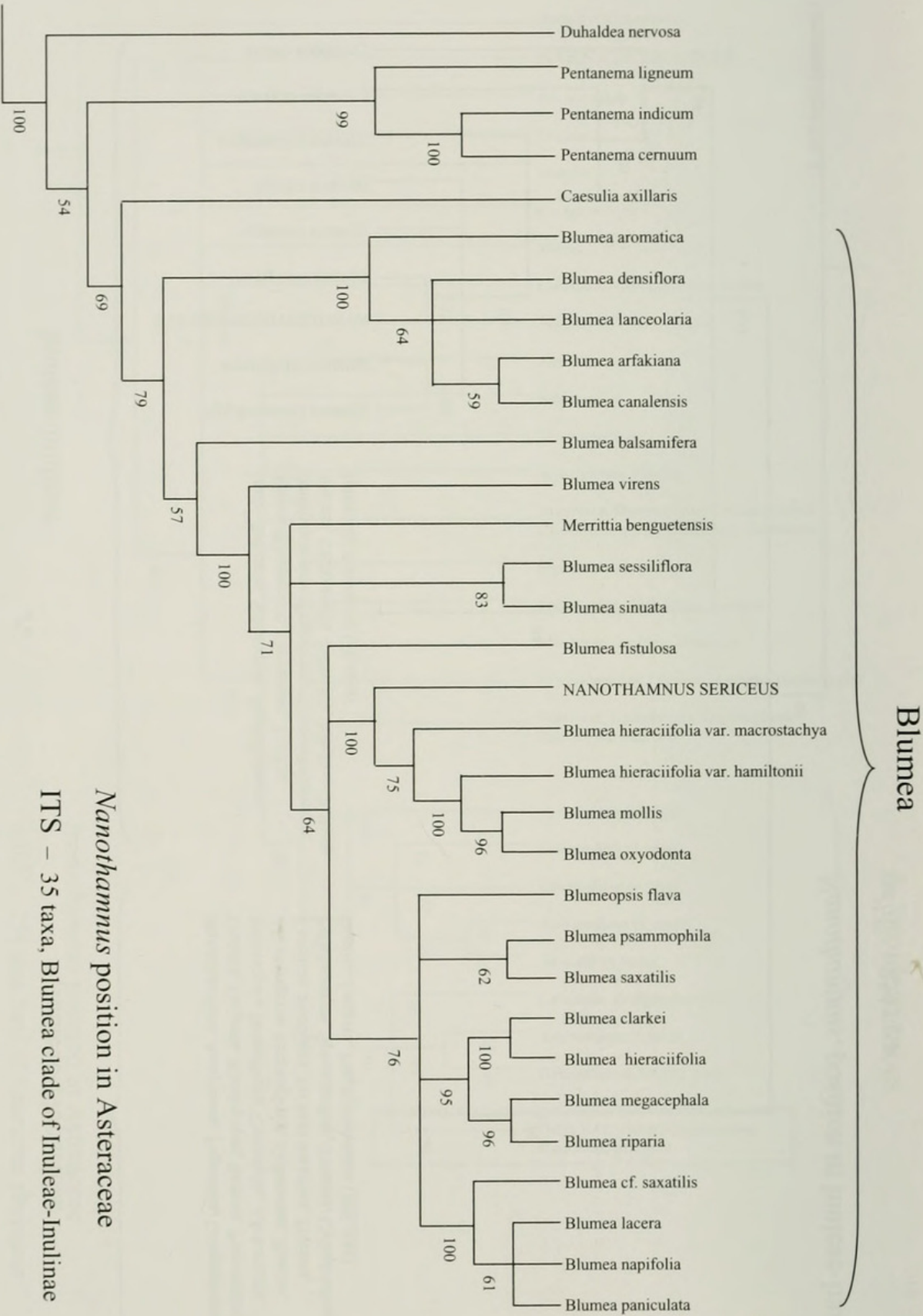


Fig. 3. Parsimony jackknife tree based analysis on ITS sequences from 35 sequences from 34 taxa showing the position of *Nanothamnus* as an ingroup in *Blumea* in a clade with *Blumea hieraciifolia*, *B. mollis* and *B. oxyodonta*. Support values > 50 % are shown for each clade.

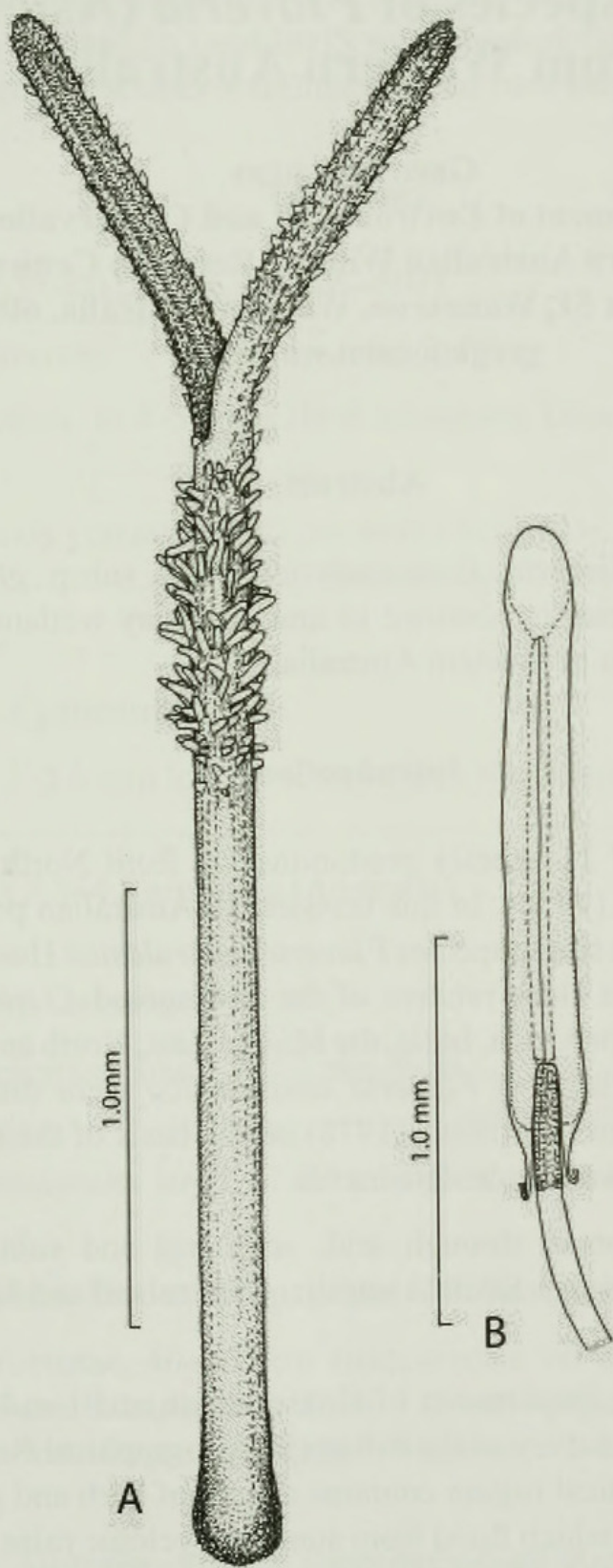


Fig. 4. *Blumea sericea*.

A: Style showing shortly triangular sweeping hairs on the branches and long obtuse sweeping hairs below the bifurcation. B: Stamen showing tailed and calcarate anther base. – A-B: SARDESAI 2547 (S).



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