

The enigmatic genus *Dipterocome*

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Abstract

The systematic position of the enigmatic genus *Dipterocome*, which has either been placed in the Calenduleae, or treated as Asteraceae incertae sedis is investigated by jackknife analysis of DNA sequence data from the plastid gene *ndhF*, and from the internal transcribed spacers (ITS) of the nuclear genome. It is concluded that the genus is a member of the tribe Cardueae, but that its exact relationships within that tribe remain to be found.

Introduction

The genus *Dipterocome* was described 1835 by F. E. L. VON FISCHER and C. A. VON MEYER (FISCHER & MEYER 1835) on material from Azerbaidjan, but they could not place their new genus. They concluded:

“Genus sane distinctissimum in nulla ex tribubus a cl. CASSINI conditis, apte collocandum; quoad fructus structuram ad *Koelpinia* accedit, sed ab omnibus Lactuceis tota flosculorum et stylorum conformatione abhorret, neque melius inter Calenduleas vel inter Adenostyleas collocari potest.”

[A genus that clearly does not fit into any of the tribes which the celebrated CASSINI surrected; with respect to the structure of the fruit it approaches *Koelpinia*, but it differs entirely from all the Lactuceae in the shape of florets and styles, neither is it possible to place it better among the Calenduleae or the Adenostyleae.]

Also CANDOLLE (1838) looked with disbelief on the strange morphological features in *Dipterocome* hence leaving it unplaced among "Genera compositarum incertae sedis". BENTHAM (1873) included *Dipterocome* in his tribe Calenduleae but referred to it as a "genus anomalum". HOFFMANN (1892) followed BENTHAM and included *Dipterocome* in the Calenduleae where its male central florets and curved spiny cypselas seemed to be in agreement with the situation in the majority of taxa of the Calenduleae. However, members of the Calenduleae do not have a pappus, and *Dipterocome* with its florets provided with up to 5 or 10 flattened bristles differs. The fact that the bristles readily fall off could have been interpreted as a stage in a reduction trend, from ancestors of the Calenduleae with a persistent pappus, via *Dipterocome* with caducous bristles, to a total lack of pappus elements in the other members of the tribe. In most flora treatments, *Dipterocome* is mentioned together with the genera of the Calenduleae (e. g. RECHINGER 1989, TAKHTAJAN 1995).

In his treatment of the Calenduleae, NORLINDH (1977) included *Dipterocome* in his taxonomic review, although also he described the genus as deviating in morphology and as a "satellite" genus only remotely related to the Calenduleae proper. NORLINDH described the differences in *Dipterocome* as: 1) ray-florets sub-bilabiate; 2) anther filaments connate; 3) different pollen morphology (based on then unpublished data provided by PRAGLOWSKI), and in summary, he considered the tribal placement of *Dipterocome* was uncertain and in need of further study. Later, PRAGLOWSKI & GRAFSTRÖM (1980) published their palynological investigations in the Calenduleae, where they had found that pollen of *Dipterocome* differed from that of other Calenduleae by having supratectal spines being reduced to minute solid spinules, by presence of well-developed infratectal baculae, lack of caveae, and a comparatively thick nexine (Fig. 2). The authors proposed a position of *Dipterocome* in the Cardueae or the Anthemideae, but NORDENSTAM (1994) presented several arguments against the Anthemideae alternative. He included *Dipterocome* in his cladistic analysis of the Calenduleae but stated that it with certainty did not belong to that tribe. The 4- or 5-veined cypselas of the central male florets were found to be plesiomorphic in contrast to the 2-veined cypselas of other Calenduleae. Other plesiomorphic features were presence of pappus bristles and elongated prismatic cypsela crystals (pappus absent and crystals druse-shaped in Calenduleae s. str.). NORDENSTAM concluded that the genus should be excluded entirely from the Calenduleae. As noted already in the protologue (FISCHER & MEYER 1835), the minute rays in *Dipterocome* are somewhat bilabiate, a feature that is otherwise characteristic of taxa in the Mutisieae. This had earlier led BREMER (1987) to suggest that it belonged there. Later, BREMER (1994) moved the genus back to the Asteroideae including it among genera of the subfamily with uncertain systematic position. In a preliminary molecular study of the phylogeny of the Calenduleae, NORDENSTAM et al. (2006), omitted *Dipterocome*, in consequence with his earlier work. It seems clear that morphological characters argue against a position

of *Dipterocome* in Calenduleae, but there is no prevailing modern view of where it belongs, although JEFFREY (2007) had it as an isolated carduoid genus of the Cichorioideae.

In connection with ongoing research in the Asteraceae based on sequence data it became interesting to test the systematic position of the enigmatic *Dipterocome* also in a molecular framework.

Material and methods

DNA was extracted from leaves taken from a herbarium specimen of *Dipterocome pusilla* [Voucher: Iran, JOHARCCHI & ZANGOORI 19925 (S)]. To test the systematic position of *Dipterocome* in the Calenduleae, 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, and included 253 taxa representing all Asteraceae tribes. Based on the results of that analysis, an ITS sequence was subsequently included in a smaller data set together with 128 ITS sequences retrieved from GenBank (AY826222-AY826349).

Molecular methods. DNA extraction was carried out with QIAGENs DNeasy Plant Mini Kit using the manufacturer's protocol. For *ndhF*, 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. For ITS the same protocol was followed with the exception that the annealing temperature was raised to 55°C. 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 Analyser. Primers used for PCR and sequencing of *ndhF* are presented in Table 1. Primers used for PCR and sequencing of ITS are presented in Table 2. The ITS sequence, as well as a sequence of *trnL-F* which we did use in any analysis were compared to other deposited sequences in GenBank using BLAST.

Alignment. Alignment of *ndhF* was unproblematic and performed with the BioEdit software (HALL 1999) ver. 6.0.5. The aligned *ndhF* data set included 253 taxa with several representatives of each tribe of the Asteraceae. Alignment of ITS was partly difficult and therefore performed with the ClustalW function included in the BioEdit package. Two different alignments were analysed. The first alignment was performed with default gap opening or gap extension penalties. The second alignment used gap opening penalty 2, and gap extension penalty 5 and was also somewhat adjusted manually. The first alignment of the ITS data set included 683 characters. The second

alignment included 717 characters.

Phylogenetic analyses. The aligned sequences 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* of the Calyceraceae was used as outgroup (FARRIS 1972). The analysis of ITS included sequences from 129 taxa and used *Tarchonanthus* as outgroup.

Results

The aligned *ndhF* data set contained 2286 characters of which 693 were informative. The results of the XAC analysis placed *Dipterocome* with taxa of one of the three Cardueae clades (70 % jackknife support), together with genera such as *Saussurea*, *Synurus*, *Carthamus*, *Centaurea*, two species of *Cirsium*. In that analysis, the taxa from the Cardueae form three clades with unresolved relationships. The two other clades comprise *Echinops* in one clade and *Atractylodes* together with *Carlina* (98 % jackknife support) respectively (Fig. 3).

In the subsequent analysis of ITS, the first alignment had 365 informative characters, and gave a tree with *Dipterocome* in an unresolved position in a well supported clade (94 %) comprising most of the genera of Cardueae, a clade which has *Atractylodes*, *Atractylis*, and *Carlina* as its sister group. Sister to both these is a clade comprising the two genera *Cardopatium* and *Cousiniopsis* (Fig. 4). The second alignment with 357 informative characters of ITS (using the gap penalties mentioned above) also gave a tree with included *Dipterocome* in the large Cardueae clade that excluded the *Atractylodes*, *Atractylis* and *Carlina* clade as well as the *Cardopatium*, *Cousiniopsis* clade. Although *Dipterocome* was again found to be within the Carduinae/Centaureinae its position this time was not unresolved but instead with very low support at the base of the *Xeranthemum*, *Siebera*, *Amphoricarpos* clade that also included *Echinops* and *Acantholepis* (Fig. 5).

The BLAST search in GenBank gave one single ITS sequence with the highest similarity score, viz. a species of *Atractylodes* (Cardueae). The BLAST of the *trnL-F* sequence gave six sequences with the highest score, 2 spp. of *Centaurea*, and four species of *Saussurea*, all Cardueae.

Discussion

We conclude that *Dipterocome* has been misplaced in Calenduleae, as previously noted by several authors. Its true systematic position is apparently in the Cardueae. This may seem surprising, as *Dipterocome* does not share any obvious

synapomorphies with taxa of that tribe. On the other hand, *Dipterocome* is strongly derived in morphology and does not share any obvious diagnostic characters with taxa of any tribe. Only the pollen wall morphology described by PRAGLOWSKI & GRAFSTRÖM (1980) indicated a position in the Cardueae.

Our molecular data obtained from *ndhF* and ITS DNA sequences unequivocally place *Dipterocome* within the Cardueae, probably somewhere at the base of the Carduinae/Centaureinae complex as defined by SUSANNA et al. (2006). In the tree from a combined analysis of ITS, *trnL-F* and *matK*, as well as in the tree from a Bayesian analysis of ITS alone, SUSANNA et al. (2006) found that there are a few major groups of the Cardueae, but with poorly supported basal relationships. They identified a Cardopatiinae, Carlininae, and Echinopinae in an unresolved basal complex together with the Carduinae/Centaureinae that comprise the majority of Cardueae genera. At the lowermost node of the Carduinae/Centaureinae in their ITS tree, there is a group called the *Xeranthemum* group with *Xeranthemum*, *Siebera*, *Chardinia*, and *Amphoricarpos*. The first two have a somewhat bilabiate corolla, like *Dipterocome*, whereas the florets in latter two are actinomorphic. Members of the Carlininae and Echinopinae have a basal attachment point for the cypselas in contrast to many genera of Carduinae/Centaureinae, which have an oblique scar.

The anthers in *Dipterocome* are very small but the base seems to be somewhat calcarate, i.e. the attachment point of the filament is above the lowest fertile point of the thecae. This is not a feature characteristic of the Cardueae but rather a plesiomorphic character state that is not present in the vast majority of species of the Asteroideae tribes.

As noted already by FISCHER & MEYER (1835), *Dipterocome* has mature cypselas that much resemble the ones in *Koelpinia linearis*, a plant that belongs in the Lactuceae and therefore differs in many other respects. However, the two inhabit the same kind of environment and are partly sympatric. Apart from their obvious differences they display some interesting similarities. Also in *Koelpinia linearis* the stems are sometimes prostrate, the leaves linear-lanceolate and the flowering capitulum small, becoming much enlarged when the fruits mature. Both species have dorsally spiny fruits that enlarge very much during maturity. The fruits seem to be adapted to zoochory and the two plants are good examples of convergent evolution in plants inhabiting similar climatological and geographical conditions.

Dipterocome pusilla FISCH. & C. A. MEY.

Index Sem. Hort. Petrop. i. 26 (1835).

Type: In collibus salsis ad Ulabanlı rarissimus. Distr. Khoi, Provinz Aderbeidschan 3.V.1828. Leg. SZOVITS 174 (LE, lecto-, selected by G. MENITSKY 1999). Digital images of

lectotype and isoelectotypes seen.

Syn.: *Jaubertia koelpinioides* SPACH, Ill. Pl. Orient. 3: 131, (1850) (n. v.); *Koelpinia sessilis* BOISS., Diagn. Pl. Orient. Ser. 1, 11: 34 (1853) (n. v.).

Illustr.: Fig. 1.

Small, glabrous, annual herb, often more or less prostrate. Leaves alternate, linear-lanceolate, entire, glabrous. Capitula axillary, heterogamous, disciform, few-flowered, very small, 3–4 mm long and 1–2 mm wide, later larger due to the growth of the cypselas. Receptacle epaleate. Involucral bracts arranged in two–three rows, herbaceous with whitish margins. Outer florets 4–7, c. 2 mm long, female, radiate, two-lipped; upper lip 2–3-dentate, longer than the very small lower lip. Fruits much larger than corolla, curved outwards, dorsally spiny, with two protruding horn-shaped spiny appendages apically; pappus of a few, soon caducous, flattened bristles. Central florets, 2–3, functionally male, c. 2 mm long, 5-lobed; corolla-lobes shortly triangular. Anther filaments connate. Anthers ecaudate but probably somewhat calcarate; anther appendage well developed, acute; endothecial tissue polarized. Style undivided or shortly divided but with branches not separating, with elongated, apically rounded sweeping hairs distally. Pappus of a few caducous, flattened bristles. Fruit not developing.

Geographic range: *Dipterocome pusilla* grows in desert and semi-deserts, in the Middle East from Jordan, Syria, to Armenia, Azerbaidjan, Iran and Afghanistan.

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Table 1.

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

<u>Name</u>	<u>Direction</u>	<u>Primer sequence</u>
RJ1	F	AGG TAA GAT CCG GTG AAT CGG AAA C
1b	F	TGG GAC TTC TTC TTT TTC C
431	F	GAT ACA AAT TTA TAT TTT TTG GG
520	R	CAA ATG CTT TTT GAC AAG CAT TTG CCG C
5	F	GTC TCA ATT GGG TTA TAT GAT G
5B	F	GGAGCTACTTTAGCTCTTG
16	R	GTT AAA CCT CCC ATA AGC ACC ATA TTCTGA 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 GAA AAA ATT GCA TCT TTT
10	R	CCC CCT AYA TAT TTG ATA CCT TCT CC
10B	R	CCT ACT CCA TTT GGA ATT CCA TC
RJ14	R	ACC AAG TTC AAT GTT AGC GAG ATT 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.

<u>Name</u>	<u>Direction</u>	<u>Primer sequence</u>
18SF	F	GAA CCTTATCGTTTA GAG GAA GG
26SR	R	CCGCCA GATTTT CAC GCT GGGC
N18L	F	AAG TCG TAA CAA GGT TTC CGT AGG TG
C26A	R	TTT CTT TTC CTC CGC T
5.8SN	F	ATC GAG TCT TTG AAC GCA
5.8SC	R	TGC GTT CAA AGA CTC GAT
ITS5	F	GGA AGT AAA AGT CGT AAC AAG G
ITS4	R	TCC TCC GCT TAT TGA TAT GC
ITS3	R	GCATCGATGAAGAACGCAGC
ITS2	F	GCTGCGTTCTTCATCGATGC

18SF and 26SR were designed by CATARINA RYDIN. N18L, C26A, 5.8SN, and 5.8SC were designed by YOUNGBAE SUH. ITS2-ITS5 are designed by WHITE et al.



Fig. 1.

Dipterocome pusilla. Scanned herbarium specimen. -
JOHARCCHI & ZANGOORI 19925 (S).

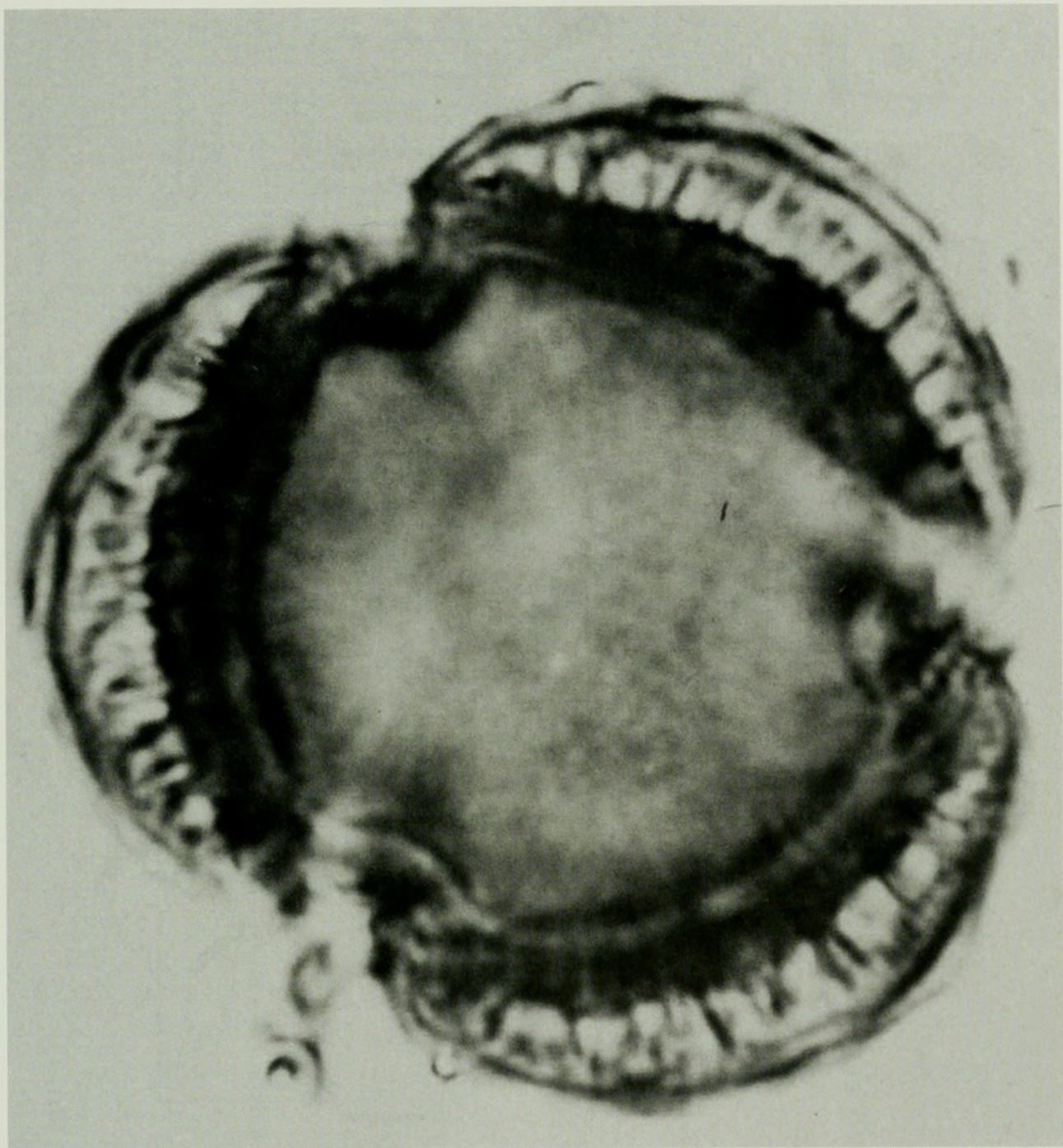


Fig. 2.

Pollen of *Dipterocome pusilla* - GROSSHEIM & GURVITSH s. n. (S).

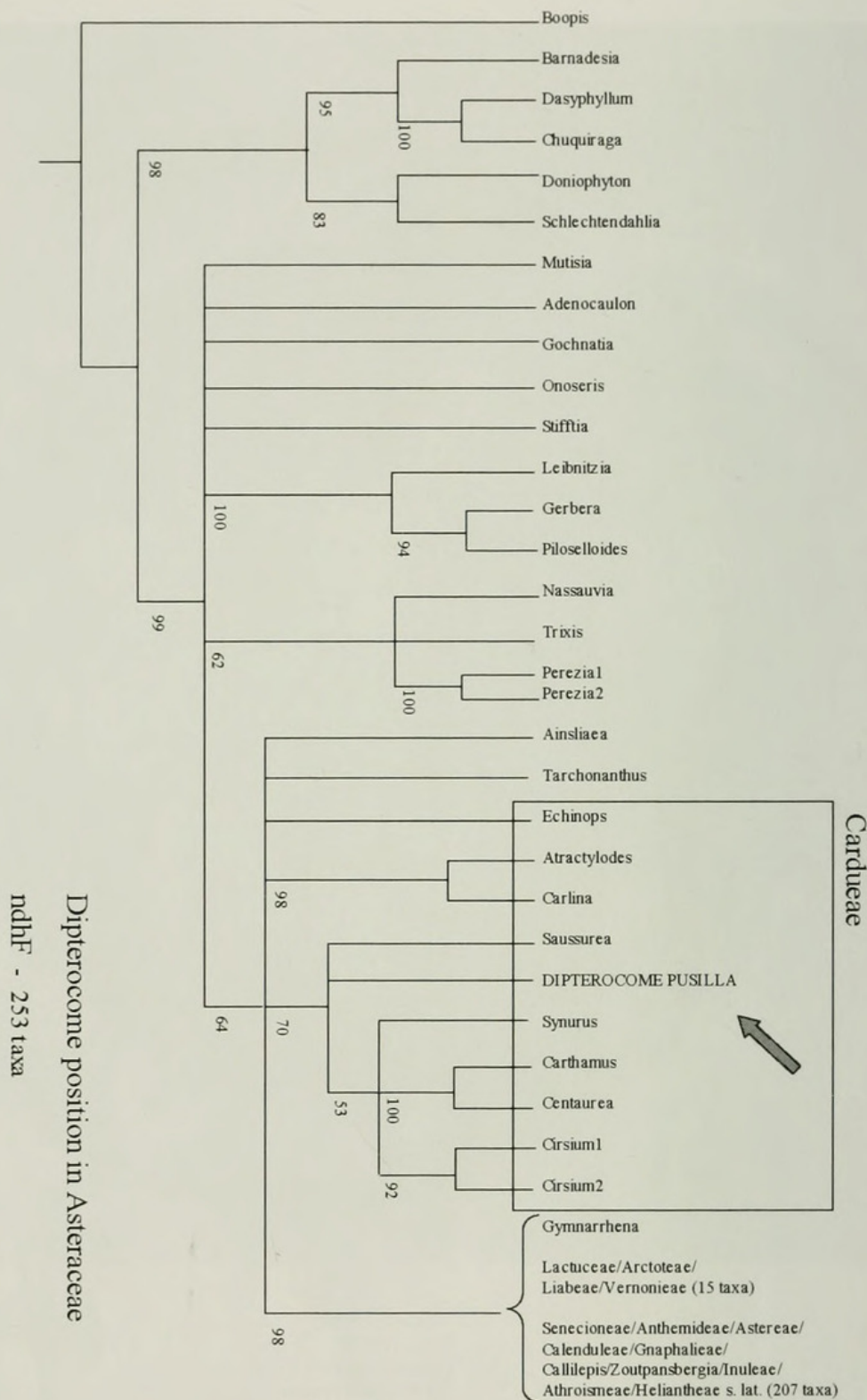


Fig. 3.

Parsimony jackknife tree based on *ndhF* sequence data showing the position of *Dipterocone* among the Asteraceae tribes.
Support values > 50 % are shown for each clade.

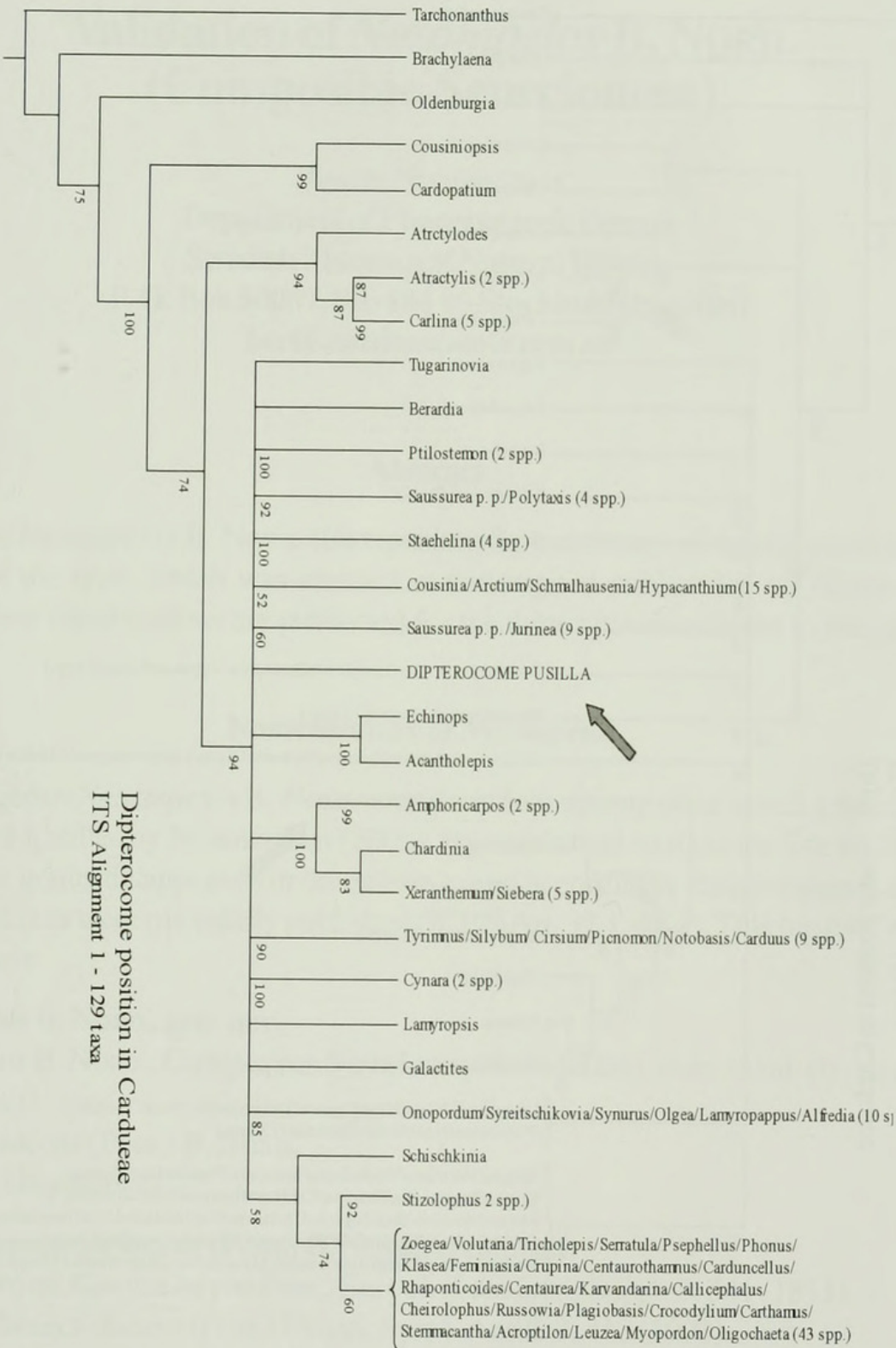


Fig. 4.

Pasimony jackknife tree based on analysis of ITS sequence data.
Alignment using default gap penalties.
Support values > 50 % are shown for each clade.

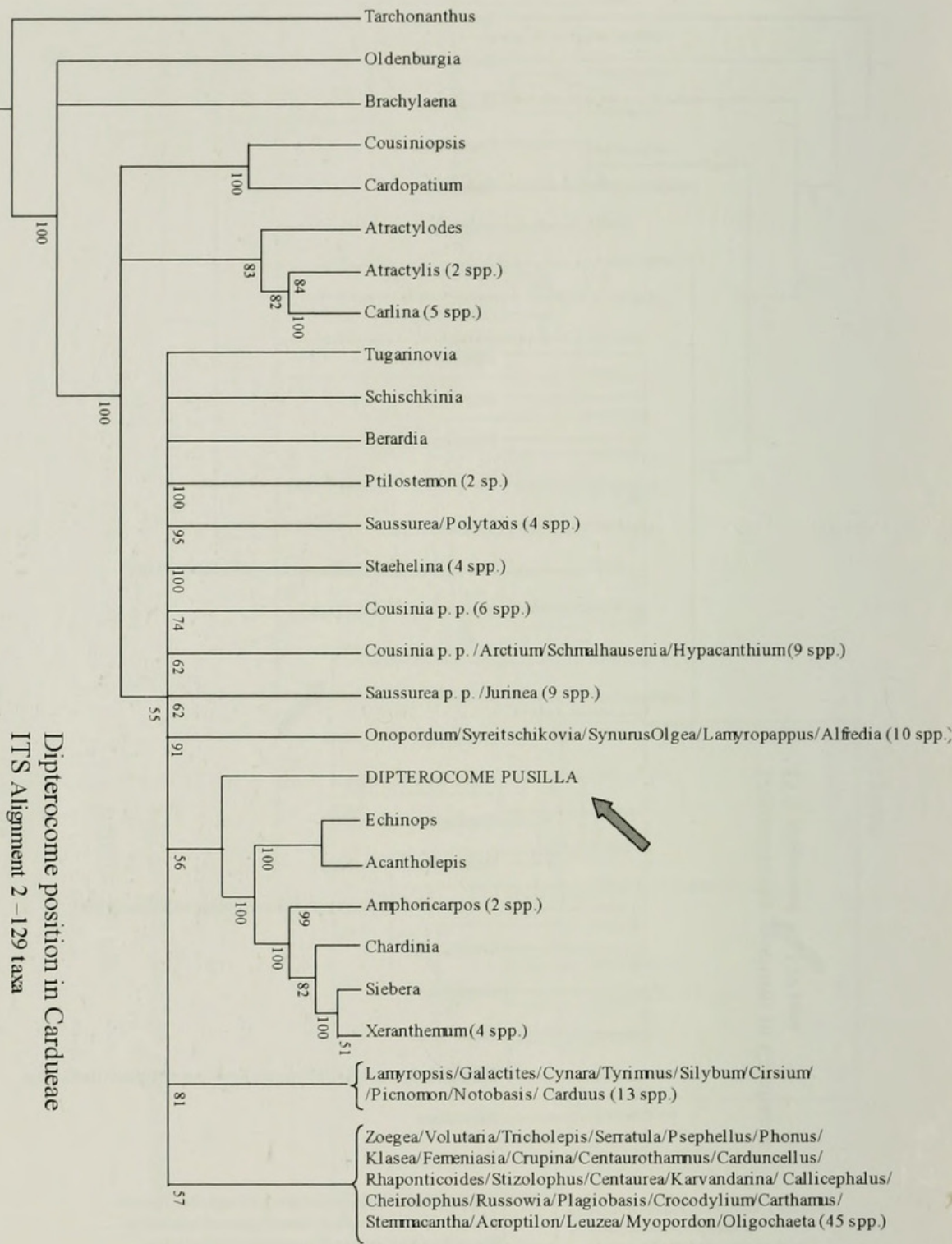


Fig. 5.

Pasimony jackknife tree based on analysis of ITS sequence data.
Alignment using non-default gap penalties.
Support values > 50 % are shown for each clade.



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