

## ***Cynanchum* and the Cynanchinae (Apocynaceae – Asclepiadoideae): a molecular, anatomical and latex triterpenoid study**

Sigrid Liede<sup>1,\*</sup>, Henning Kunze<sup>2</sup>

<sup>1</sup> Department of Plant Systematics, University of Bayreuth, Germany

<sup>2</sup> Minden, Germany

Received 22 April 2002 · Accepted 29 July 2002

### **Abstract**

The phylogeny of the genus *Cynanchum* s. str. is studied using cpDNA spacers and *ITS*. Morphological, anatomical and latex triterpenoid data are interpreted in light of the molecular results, and discrepancies are discussed. Vegetative characters are better indicators of relationship than floral characters, especially corona characters. The monophyly of all Malagasy species and, nested within the latter, of all stem-succulent taxa is ascertained and the genera *Folotsia*, *Karimbolea*, *Platykeleba* and *Sarcostemma* are subsumed under *Cynanchum*. One African species, *C. galgalense*, is excluded from *Cynanchum*.

**Key words:** Asclepiadoideae, cpDNA, *ITS*, latex triterpenoids, molecular phylogeny, stem anatomy

### **Introduction**

The speciose genus *Cynanchum* L. has long been a “dustbin genus” for everything with a gynostegial, at least basally fused corona, C(is) sensu Liede & Kunze (1993). In the New World, the concept of *Cynanchum* was particularly confused since Woodson (1941) united a whole range of genera under *Cynanchum*, based mainly on North American material. But unlike *Asclepias* L., New World *Cynanchum* was never homogeneous. Sundell (1981) revised the most distinct subgenus *Mellichampia* (A.Gray ex S. Watson) Sundell, characterized by comparatively large flowers and heart-shaped leaves with “stipules” (extremely reduced short shoots with a pair of smaller and sometimes differently shaped leaves). Analysis of New World and Old World members of *Cynanchum* L. using cpDNA spacers (Liede & Täuber in press) has shown that in the New World only subgen. *Mellichampia* is correctly associated with *Cynanchum* s. str., whereas all other former members of the genus belong to an unrelated, exclusively American group of genera. Liede & Täuber (in press) restricted subtribe Metastelminae Endl. ex Meisn. to the American genera, and resurrected subtribe Cynanchinae K.

Schum. for *Cynanchum* s. str. (incl. subgen. *Mellichampia*) and some smaller, related genera, exclusively of Old World origin.

In the Old World, *Vincetoxicum* Wolf was suggested by Liede (1996a) to be a relative of *Tylophora* R. Br., rather than of *Cynanchum*, based on chemistry. This has been supported by various molecular studies (Civeyrel et al. 1998; Sennblad & Bremer 2000, 2002; Liede 2001). The cpDNA spacer analysis (Liede & Täuber in press), however, showed that even in the Old World the generic concept of *Cynanchum* is more complex than hitherto assumed, and that some of the small Asian genera (e.g., *Metaplexis* R. Br.) probably ought to be included in *Cynanchum*. In addition, this study indicated that the Old World stem succulent genera *Folotsia* Costantin & Bois, *Karimbolea* Desc., *Platykeleba* N. E. Br., and *Sarcostemma* R. Br. are nested within the Malagasy subclade of *Cynanchum*. However, Liede & Täuber (in press) refrained from nomenclatural consequences both in the New World and in the Old World, because a single dataset was not considered sufficient for the extensive nomenclatural changes necessary to reflect these new results. The present paper focuses on *Cynanchum* s. str., in particular on the status of the small succulent genera

\*Corresponding author: Sigrid Liede, Department of Plant Systematics, University of Bayreuth, D-95440 Bayreuth, Germany; e-mail: sigrid.liede@uni-bayreuth.de

(*Folotsia*, *Karimbolea*, *Platykeleba*, *Sarcostemma*), using evidence from a second molecular marker, *ITS*, as well as from stem anatomy and triterpenoid analysis.

## Materials and methods

### DNA sequence analysis

**Taxa.** Following the results of Liede & Täuber (in press), *Schizostephanus alatus* was chosen as outgroup. More distantly related outgroups such as members of the Asclepiadinae (sensu Liede 1997b) or Tylophorinae (sensu Liede 2001) were not included in the analysis, because alignment of *ITS* sequences is problematic between members of different subtribes, and no other basal member of Cynanchinae has yet been identified among the 67 out of approx. 100 genera of Asclepiadeae for which cpDNA sequences are available (Liede 2001; Liede & Täuber 2000, in press; Liede et al. 2002, and unpubl.). The ingroup comprises 91 accessions and 85 species, because two different accessions were included for six species (*Pentarrhinum somaliense*, *C. falcatum*, *C. galgalense*, *C. gerrardii*, *C. orangeanum*, *C. thesioides*; Table 1, see there for authors of species). For *C. chouxii*, *C. ligulatum*, and one accession of *C. galgalense*, no *ITS* sequence could be obtained, for *C. schistoglossum*, the *trnT-L* spacer of cpDNA is missing. Eight species of the ingroup are members of the American *Cynanchum* subgen. *Mellichampia* and *Metalepis* Griseb., four species are Asian, including two members of *Cynanchum* sect. *Rhodostegiella* (Pobed.) Tsiang and one member of *Metaplexis*, one species (*C. floribundum*) is Australian. From the African mainland, twenty leafy species were included, constituting more than half of all known African *Cynanchum* species (Liede 1996b). From Madagascar, eighteen leafy species were included, again almost half of all known leafy Malagasy species. Of stem succulents, twenty Malagasy *Cynanchum* were included, as well as a collection of *C. gerrardii* from the African mainland. Eight species belonging to the small succulent genera *Folotsia*, *Karimbolea*, *Platykeleba* and *Sarcostemma* were also included, six of them from Madagascar.

**DNA extraction and PCR.** DNA was isolated from fresh or dried leaf tissue according to Doyle & Doyle (1987). PCR primers and protocol for the chloroplast DNA (cpDNA) *trnT-L* and *trnL-F* spacers and the *trnL* intron correspond to Taberlet et al. (1991). cpDNA spacers of 19 species were sequenced for the present study, the remaining species have been used in previous studies of the senior author.

The entire internal transcribed spacer region (*ITS*) including 5.8S of ribosomal DNA (rDNA) was amplified using the flanking primers *ITS4* and *ITS5* following a

modified protocol based on Baldwin (1992) described in Meve & Liede (2001). *ITS* sequences of 87 species were obtained for the present study, two sequences have been used in a previous study of the senior author.

**Data analysis.** cpDNA sequences were pre-aligned with Perkin Elmer Sequence Navigator Version 1.0.1, the alignment then adjusted manually. The alignment comprises 92 taxa and 2178 characters; 129 data cells and the whole *trnT-L* spacer of *C. schistoglossum* were unknown. In the *trnL-F* spacer, bp755–806 were excluded from all analyses, because alignment in this region is ambiguous.

Indels were coded as “missing characters”; parsimony informative indels were coded separately as present/absent following the “simple gap coding” method of Simmons & Ochoterena (2000). Indels present or absent only in different accessions of the same species were not coded. Indels of doubtful homology (e.g., bp186–188, 186–189 in the *trnT-L* spacer of *C. obtusifolium* and *C. madagascariense*, respectively) were not coded, nor were indels caused by variable length of a chain of at least four repeats of the same base, because the length of these chains was found to vary even within the same species (Verhoeven et al. in press). In total, 48 indels were coded, 32 in the *trnT-L* spacer, 9 in the *trnL* intron and 7 in the *trnL-F* spacer.

Sequences of rDNA were pre-aligned with Perkin Elmer Sequence Navigator Version 1.0.1, the alignment then adjusted manually. The alignment comprises 89 taxa and 827 characters, 27 data cells are unknown. Separate coding of indels was not conducted because of the heterogeneity of the dataset. All alignments are available from the senior author and can be viewed in TreeBase (Sanderson et al. 1994): study accession number = S776, matrix accession numbers = M1230, M1231.

Sequence analysis, phylogenetic analysis and tests for clade support were performed using PAUP (Swofford 1998), version 4.0b5 (PPC), on a MacIntosh G3 Powerbook.

Sequence divergence among taxa was calculated using the “Show Pairwise Distance” option for the *trnT-L* spacer, the *trnL* intron and the *trnL-F* spacer, as well as for 5.8s, *ITS1* and *ITS2* (excluding the end of 18s and the beginning of 26s). Beginning and end of *ITS1* and *ITS2* were determined by comparison with Asclepiadoideae sequences submitted to EMBL (AJ402152–AJ402162, Meve & Liede 2001).

As the dataset was too large for complete analysis, a search strategy in two steps was performed in all parsimony analyses in order to find as many tree islands as possible. First, starting trees were obtained (addition sequence random, 1000 replicates, TBR branch swapping, “MulTrees” off, “steepest descent” off). The resulting shortest starting trees were then subjected to TBR

**Table 1.** Materials used for DNA, anatomical, and triterpenoid studies. **Taxa in boldface** have been analyzed for stem anatomy, underlined taxa have been analyzed for latex triterpenoids.

Taxon	Country: Province	Voucher	EMBL Accession No. <i>trnT-L spacer</i> <i>trnL intron</i> <i>trnL-F spacer</i>	EMBL Accession No. <i>ITS</i>
<b>OUTGROUP</b>				
<i>Schizostephanus alatus</i> Hochst. ex K. Schum.	Kenya: s. loc.	<i>Noltee s.n. sub</i> <i>IPPS 8111</i> (UBT)	AJ410247 AJ410248 AJ410249	AJ329451
<b>INGROUP – Old World Cynanchum relatives</b>				
<b><i>Folotsia grandiflora</i></b> (Jum. & H. Perrier) Jum. & H. Perrier ( <i>Cynanchum grandidieri</i> Liede & Meve)	Madagascar: s. loc.	BG Munich <i>s.n.</i> (UBT)	AJ290854 AJ290855 AJ290856	AJ492801
	Madagascar: s. loc.	<i>Rauh 68584</i> (HEID)	–	–
<b><i>Folotsia madagascariensis</i></b> (Jum. & H. Perrier) Desc. ( <i>Cynanchum toliari</i> Liede & Meve)	Madagascar: Toliara	<i>McPherson 14770</i> (MO)	AJ428756 AJ428757 AJ428758	AJ492802
	Madagascar: Toliara	<i>Liede et al. 2695</i> (UBT) – anat.	–	–
	Madagascar: Toliara	<i>Liede et al. 2667</i> (MO, P, UBT) – latex	–	–
<i>Glossonema boveanum</i> (Decne.) Decne.	Kenya: Northern Frontier	<i>Liede &amp; Newton 3239</i> (ULM)	AJ428804 AJ428805 AJ428806	AJ492803
<i>Karimbolea macrantha</i> (Jum. & H. Perrier) Liede & Meve ( <i>Cynanchum macranthum</i> Jum. & H. Perrier)	Madagascar: Toliara	<i>Liede et al. 2808</i> (in cult. Bayreuth)	AJ492372 AJ492373 AJ492374	AJ492804
<i>Karimbolea mariensis</i> Meve & Liede ( <i>Cynanchum mariense</i> (Meve & Liede) Liede & Meve)	Madagascar: Toliara	<i>Liede et al. 2825</i> (K, MSUN, UBT)	AJ428768 AJ428769 AJ428770	AJ492805
<b><i>Karimbolea verrucosa</i></b> Desc. ( <i>Cynanchum verrucosum</i> (Desc.) Liede & Meve)	Madagascar: Toliara	<i>Liede et al. 2826</i> (MSUN, UBT)	AJ290878 AJ290879 AJ290880	AJ492806
<i>Metaplexis japonica</i> Makino	Japan: s. loc.	ex BG Tartu (UBT)	AJ428810 AJ428811 AJ428812	AJ492807
<i>Odontanthera radians</i> (Forssk.) D.V. Field	North Yemen: Hodeidah	<i>Müller-Hohenstein</i> & <i>Deil 1967</i> (UBT)	AJ428813 AJ428814 AJ428815	AJ492809
<i>Pentarrhinum abyssinicum</i> Decne.	Tanzania: Ufipa	<i>Bidgood et al. 2440</i> (K; MWC 8418)	AJ428816 AJ428817 AJ428818	AJ492810
<i>Pentarrhinum gonoloboides</i> (Schltr.) Liede	Kenya: Naivasha	<i>Liede &amp; Newton 3157</i> (UBT)	AJ428819 AJ428820 AJ428821	AJ492811

Table 1. (Continued).

Taxon	Country: Province	Voucher	EMBL Accession No. <i>trnT</i> -L spacer <i>trnL</i> intron <i>trnL</i> -F spacer	EMBL Accession No. <i>ITS</i>
<i>Pentarrhinum insipidum</i> E. Mey.	South Africa: Orange Free State	<i>Liede 2940</i> (UBT)	AJ410232 AJ410233 AJ410234	AJ492812
	Namibia: Karas Region	<i>Meve &amp; Liede 584a</i> (MO)	–	–
<i>Pentarrhinum somaliense</i> (Schltr.) Liede	Ethiopia: Sidamo	<i>Mesfin &amp; Vollesen 4238</i> (UPS)	AJ428822 AJ428823 AJ428824	AJ492813
	Kenya: Northern Frontier	<i>Liede &amp; Newton 3225</i> (UBT)	AJ492376 AJ492377 AJ492378	AJ492814
<i>Platykeleba insignis</i> N.E. Br. ( <i>Cynanchum insigne</i> (N.E. Br.) Liede & Meve)	Madagascar: Antananarivo	<i>Rauh 68500</i> (HEID)	AJ290906 AJ290907 AJ290908	AJ492815
	Madagascar: Antananarivo	<i>Lavranos s.n.</i> (UBT)	–	–
<i>Sarcostemma arabicum</i> Bruyns & P. I. Forst.	Yemen: Sana'a	<i>Radcliffe-Smith &amp; Henchie</i> <i>4624</i> (K, UBT)	–	–
<i>Sarcostemma australe</i> R. Br.	Australia: Queensland	<i>Kunze 205</i> (Minden)	–	–
<i>Sarcostemma pearsonii</i> N.E.Br.	South Africa: Northern Cape	<i>Liede &amp; Meve 582</i> (K, MSUN)	AJ290909 AJ290910 AJ290211	AJ492816
	South Africa: Northern Cape	<i>Liede &amp; Hammer</i> <i>2518</i> (UBT) – anat.	–	–
	South Africa: Northern Cape	<i>Liede &amp; Hammer</i> <i>2520</i> (UBT) – latex	–	–
<i>Sarcostemma resiliens</i> B. R. Adams & R. W. K. Holland	Zimbabwe: Makoni	<i>Albers et al. 515</i> (UBT)	–	–
<i>Sarcostemma stolonifera</i> B. R. Adams & R. W. K. Holland	Tanzania: Arusha	<i>Noltee 199</i> (MSUN)	–	–
<i>Sarcostemma vanlessenii</i> Lavranos	Kenya: s. loc.	<i>Lavranos s.n.</i> (UBT)	–	–
	Yemen: Dhamar	<i>Noltee 870</i> (MSUN)	–	–
<i>Sarcostemma viminale</i> (L.) R.Br. ssp. <i>viminale</i>	Madagascar: Toliara	<i>Liede et al.</i> <i>2738</i> (MO, P, UBT) – latex	–	–
		<i>Liede et al.</i> <i>2706</i> (MO, P, UBT) – anat.	–	–
ssp. <i>suberosum</i> Meve & Liede	Zimbabwe: Bulawayo	<i>Albers et al.</i> <i>540</i> (MSUN, UBT)	AJ290912 AJ290213 AJ290214	AJ492817
ssp. <i>thunbergii</i> (G. Don) Liede & Meve	South Africa: Western Cape	<i>Liede &amp; Hammer</i> <i>2507</i> (UBT) – latex	–	–
	South Africa: Northern Cape	<i>Liede &amp; Hammer</i> <i>2522</i> (UBT) – latex	–	–
	South Africa: Northern Cape	<i>Liede &amp; Hammer</i> <i>2514</i> (UBT) – anat.	–	–
	South Africa: Western Cape	<i>Liede &amp; Hammer</i>	–	–
ssp. <i>nov.</i>				

Table 1. (Continued).

Taxon	Country: Province	Voucher	EMBL Accession No. <i>trnT-L</i> spacer <i>trnL</i> intron <i>trnL-F</i> spacer	EMBL Accession No. <i>ITS</i>
<b><i>Cynanchum</i> sect. <i>Cynanchum</i> (Old World)</b>				
<i>Cynanchum abyssinicum</i> Decne.	Tanzania: Arusha	Liede & Meve 3373 (UBT)	AJ428579 AJ428580 AJ428581	AJ492734
<b><i>Cynanchum aculeatum</i></b> (Desc.) Liede & Meve	Madagascar: Toliara	Liede et al. 2828	–	–
<i>Cynanchum acutum</i> L.	Portugal: s. loc.	BG Lisboa s.n. (UBT)	AJ428582 AJ428583 AJ428584	AJ492735
<i>Cynanchum adalinae</i> K. Schum. ssp. <i>adalinae</i>	Cameroon: Sud (Mt. Cameroon)	Meve 902 (K, LBG)	AJ428585 AJ428586 AJ428587	AJ492736
<i>Cynanchum africanum</i> Hoffsgg.	South Africa: Western Cape	Liede 2550 (MO)	AJ428588 AJ428589 AJ428590	AJ492737
	South Africa: Western Cape	Meve & Liede 624 (UBT)	–	–
<i>Cynanchum altiscandens</i> K. Schum.	Kenya: Kiambu	Liede & Newton 2873 (UBT)	AJ428591 AJ428592 AJ428593	AJ492738
<b><i>Cynanchum ampanihense</i></b> Jum. & H. Perrier	Madagascar: Toliara	Liede et al. 2817a (MSUN)	AJ428594 AJ428595 AJ428596	AJ492739
	Madagascar: Toliara	Liede et al. 2824 (UBT)	–	–
<i>Cynanchum angavokeliense</i> Choux	Madagascar: s. loc.	Specks s.n.(UBT)	AJ428597 AJ428598 AJ428599	AJ492733
<i>Cynanchum appendiculatopsis</i> Liede	Madagascar: s. loc.	Février s.n. (UBT)	AJ492322 AJ492323 AJ492324	AJ492732
<b><i>Cynanchum arenarium</i></b> Jum. & H. Perrier	Madagascar: Toliara	Liede et al. 2686 (in cult. Bayreuth)	AJ428600 AJ428601 AJ428602	AJ492740
	Madagascar: Toliara	Liede et al. 2713 (UBT) – anat. Liede et al. 2739 (MO, P, UBT) – latex	– –	– –
<b><i>Cynanchum bisinuatum</i></b> Jum. & H. Perrier	Madagascar: Toliara	Hardy 2901 (PRE 14664)	–	–
<i>Cynanchum chouxii</i> Liede & Meve	Madagascar: Fianarantsoa	Kotozafy 442 (MO, UBT)	AJ492325 AJ492326 AJ492327	–

Table 1. (Continued).

Taxon	Country: Province	Voucher	EMBL Accession No. <i>trnT-L spacer</i> <i>trnL intron</i> <i>trnL-F spacer</i>	EMBL Accession No. <i>ITS</i>
<i>Cynanchum clavidens</i> N.E.Br. <i>ssp. hastifolium</i> (N.E.Br.) Liede	Kenya: Northern Frontier	Liede & Newton 3226 (UBT)	AJ428609 AJ428610 AJ428611	AJ492743
<i>Cynanchum comorense</i> Choux	Comores: Mayotte	Pignal 1104 (P)	AJ428612 AJ428613 AJ428614	AJ492744
<b><i>Cynanchum compactum</i></b> Choux <i>ssp. compactum</i>	Madagascar: s. loc.	Barad s.n. (UBT)	AJ290842 AJ290843 AJ290844	AJ492745
	Madagascar: s. loc.	Lavranos & Newton 12161 (UBT)	–	–
<i>Cynanchum crassiantherae</i> Liede	Somalia: Balad	Hedberg & Warfa 90 (UPS)	AJ428615 AJ428616 AJ428617	AJ492746
<i>Cynanchum crassipedicellatum</i> Meve & Liede	Madagascar: Toliara	Hardy 2852 (K, MSUN)	AJ492328 AJ492329 AJ492330	AJ492747
<i>Cynanchum cucullatum</i> N.E.Br.	Madagascar: Antananarivo	Liede et al. 2868 (MO, MSUN, P)	AJ428618 AJ428619 AJ428620	AJ492748
<i>Cynanchum danguyanum</i> Choux	Madagascar: Antsiranana	Allorge 2026 (P)	AJ428621 AJ428622 AJ428623	AJ492749
<b><i>Cynanchum decaryi</i></b> Choux	Madagascar: Toliara	Liede et al. 2691 (UBT)	–	–
<b><i>Cynanchum descoingsii</i></b> Rauh	Madagascar: Toliara	Descoings 28244 (UBT)	AJ428624 AJ428625 AJ428626	AJ492750
<b><i>Cynanchum ellipticum</i></b> R. Br.	South Africa: Eastern Cape	Liede 2933 (UBT)	AJ290845 AJ290846 AJ290847	AJ320444
	South Africa: Eastern Cape	Liede 2916 (UBT)	–	–
<i>Cynanchum erythranthum</i> Jum. & H. Perrier	Madagascar: Antsiranana	Rauh 74816 (HEID)	AJ428627 AJ428628 AJ428629	AJ492751
<i>Cynanchum falcatum</i> Hutchinson & E.A. Bruce	Ethiopia: Ogaden	Kuchar & Abdirizak 21226 (UPS)	AJ492331 AJ492332 AJ492333	AJ492753
	Ethiopia: Sidamo	Friis 3169 (K; MWC 8410)	AJ428630 AJ428631 AJ428632	AJ492752

Table 1. (Continued).

Taxon	Country: Province	Voucher	EMBL Accession No. <i>trnT-L spacer</i> <i>trnL intron</i> <i>trnL-F spacer</i>	EMBL Accession No. <i>ITS</i>
<i>Cynanchum floribundum</i> R.Br.	Australia: Northern Territory	<i>Latz 12579</i> (MO)	AJ428633 AJ428634 AJ428635	AJ492754
<i>Cynanchum folotsioides</i> Liede & Meve	Madagascar: Toliara	<i>Rauh 21847</i> (MSUN)	AJ492334 AJ492335 AJ492336	AJ492755
<i>Cynanchum galgalense</i> Liede	Somalia: Bari	<i>Thulin &amp; Warfa 6205</i> (K, UPS)	AJ492337 AJ492338 AJ492339	AJ492756
	Somalia: Bari	<i>Thulin et al. 9433</i> (UPS)	AJ492341 AJ492342 AJ492343	–
<b><i>Cynanchum gerrardii</i></b> (Harvey) Liede	Yemen: Al Hudaydah Kenya: Northern Frontier	<i>Noltee 995</i> (MSUN) – latex <i>Meve 962</i> (ULM)	– AJ428645 AJ428646 AJ428647	– AJ492757
	Madagascar: Toliara	<i>Liede et al. 2797</i> (MSUN)	AJ428642 AJ428643 AJ428644	AJ492758
	Madagascar: s. loc.	<i>Rauh s.n.</i> (HEID, UBT)	–	–
<b><i>Cynanchum hardyi</i></b> Liede & Meve	Madagascar: Mahajanga	<i>Mangelsdorff RMM 43</i> (UBT)	AJ492343 AJ492344 AJ492345	AJ492759
	Madagascar: Toliara	<i>Hardy &amp; Jacobsen 3571</i> (PRE)	–	–
<i>Cynanchum implicatum</i> Jum. & H. Perrier	Madagascar: Antsiranana	<i>Mangelsdorff 24</i> (UBT)	AJ428648 AJ428649 AJ428650	AJ492760
<i>Cynanchum itremense</i> Liede	Madagascar: Fianarantsoa	<i>Phillipson et al. 3857</i> (MO)	AJ492346 AJ492347 AJ492348	AJ492761
<b><i>Cynanchum juliani-marnieri</i></b> Desc.	Madagascar: Toliara	<i>Teissier s.n.</i> (UBT)	AJ492349 AJ492350 AJ492351	AJ492762
<i>Cynanchum junciforme</i> (Decne.) Liede	Madagascar: Fianarantsoa	<i>Liede et al. 2864</i> (MO, P, UBT)	–	–
<i>Cynanchum leucanthum</i> (K. Schum.) K. Schum. ssp. <i>leucanthum</i>	Madagascar: Antsiranana	<i>Mangelsdorff 420</i> (UBT)	AJ428654 AJ428655 AJ428656	AJ492764
<i>Cynanchum lineare</i> N.E.Br. ssp. <i>lineare</i>	Madagascar: Fianarantsoa	<i>Röösli &amp; Hoffmann 198</i> (UBT)	AJ428660 AJ428661 AJ428662	AJ492765

Table 1. (Continued).

Taxon	Country: Province	Voucher	EMBL Accession No. <i>trnT</i> -L spacer <i>trnL</i> intron <i>trnL</i> -F spacer	EMBL Accession No. <i>ITS</i>
<b><i>Cynanchum luteifluens</i></b> (Jum. & H. Perrier) Desc. var. <b><i>luteifluens</i></b>	Madagascar: Toliara	<i>Liede et al. 2699</i> (MO, P, UBT) – anat.	–	–
var. <b><i>longicoronae</i></b> Liede	Madagascar: Fianarantsoa	<i>Liede et al. 2731</i> (MO, P, UBT) – latex <i>Liede et al. 2624</i> (MO, P, UBT)	–	–
<i>Cynanchum longipes</i> N.E.Br.	Ghana: Brong-Ahafo Region	<i>Jongkind &amp; Schmidt</i> 1739 (MO)	AJ428663 AJ428664 AJ428665	AJ492766
<i>Cynanchum madagascariense</i> K. Schum.	Madagascar: Toliara	<i>Liede et al. 2756</i> (UBT)	AJ428666 AJ428667 AJ428668	AJ492767
<b><i>Cynanchum mahafalense</i></b> Jum. & H. Perrier	Madagascar: Toliara	<i>Liede et al. 2831</i> (UBT)	AJ428669 AJ428670 AJ428671	AJ492768
	Madagascar: Toliara	<i>Liede et al. 2649</i> (UBT)	–	–
<b><i>Cynanchum marnieranum</i></b> Rauh	Madagascar: Toliara	<i>Rauh s.n.</i> (MSUN)	AJ492352 AJ492353 AJ492354	AJ492769
<b><i>Cynanchum menarandrense</i></b> Jum. & H. Perrier	Madagascar: Toliara	<i>Rauh 7593</i> (HEID, UBT)	–	–
<b><i>Cynanchum messeri</i></b> (Buchenau) Jum. & H. Perrier	Madagascar: Toliara	<i>Liede et al. 2721</i> (MO, P, UBT)	AJ428672 AJ428273 AJ428274	AJ492770
<b><i>Cynanchum mevei</i></b> Liede	Madagascar: Toliara	<i>Teissier 215</i> (UBT)  <i>Liede et al. 2780</i> (MO, P, MSUN)	AJ428675 AJ428676 AJ428677 –	AJ492771  –
<i>Cynanchum meyeri</i> Schltr.	Namibia	<i>Van Wyk 9030</i> (PRE)	AJ428678 AJ428679 AJ428680	AJ492772
<i>Cynanchum moramangense</i> Choux	Madagascar: Toamasina	<i>Rakotomalaza et al. 1202</i> (MO)	AJ492355 AJ492356 AJ492357	AJ492774
<b><i>Cynanchum natalitium</i></b> Schltr.	South Africa: s. loc.	<i>Nicholas 2583</i> (NH)	AJ428687 AJ428688 AJ428689	AJ492775
	South Africa: s. loc.	<i>Kunze 316</i> (Minden)	–	–
<b><i>Cynanchum obovatum</i></b> Choux	Madagascar: Antsiranana	<i>Mangelsdorff M14</i> (UBT)	AJ428801 AJ428802 AJ428803	AJ492776
	Madagascar: Fianarantsoa	<i>Liede et al. 2859</i> (MO, P, UBT)	–	–



Table 1. (Continued).

Taxon	Country: Province	Voucher	EMBL Accession No. <i>trnT-L spacer</i> <i>trnL intron</i> <i>trnL-F spacer</i>	EMBL Accession No. <i>ITS</i>
<i>Cynanchum obtusifolium</i> L.f.	South Africa: Eastern Cape	<i>Liede 2925</i> (UBT)	AJ428690 AJ428691 AJ428692	AJ492777
	South Africa: s. loc.	<i>Kunze 303</i> (Minden)	–	–
<i>Cynanchum orangeanum</i> N.E.Br.	Botswana: Kgalagadi	<i>Cole 347</i> (PRE)	AJ492358 AJ492359 AJ492360	AJ492778
	South Africa: Northern Cape	<i>Van Rooyen 4537</i> (PRE)	AJ428693 AJ428694 AJ428695	AJ492779
<i>Cynanchum ovalifolium</i> Wight	Philippines: Zamboanga	<i>Liede 3297</i> (ULM)	AJ428696 AJ428697 AJ428698	AJ492780
<i>Cynanchum pachycladon</i> Choux	Madagascar: Toliara	<i>Liede et al. 2741</i> (MO, P, UBT)	AJ428699 AJ428700 AJ428701	AJ492781
	Madagascar: Toliara	<i>Liede et al. 2663</i> (MO, P, UBT)	–	–
<i>Cynanchum papillatum</i> Choux	Madagascar: Fianarantsoa	<i>Liede et al. 2862</i> (MSUN, UBT)	AJ428702 AJ428703 AJ428704	AJ492782
	Madagascar: Fianarantsoa	<i>Liede et al. 2622</i> (MO, TAN)	–	–
<i>Cynanchum perrieri</i> Choux	Madagascar: s. loc.	BG Berlin <i>027-03-74-80</i> (B)	AJ428705 AJ428706 AJ428707	AJ492783
	Madagascar: Fianarantsoa	<i>Liede et al. 2851</i> (UBT)	–	–
<i>Cynanchum phillipsonianum</i> Liede & Meve	Madagascar: Antsiranana	<i>Mangelsdorff M 25</i> (UBT)	AJ428708 AJ428709 AJ428710	AJ492784
<i>Cynanchum polyanthum</i> K. Schum.	Uganda: Buganda	<i>Synnott 688</i> (K; MWC 8413)	AJ428711 AJ428712 AJ428713	AJ492785
<i>Cynanchum praecox</i> Schltr. ex S. Moore	Tanzania: Ufipa	<i>Goyder et al. 3828</i> (PRE)	AJ428714 AJ428715 AJ428716	AJ492786
<i>Cynanchum pycnoneuroides</i> Choux	Madagascar: Fianarantsoa	Service Forestier <i>26466</i> (P)	AJ428717 AJ428718 AJ428719	AJ492787
	Madagascar: Fianarantsoa	<i>Rauh 10605</i> (HEID)	–	–
<i>Cynanchum radiatum</i> Jum. & H. Perrier	Madagascar: Toliara	<i>Liede et al. 2744</i> (UBT)	AJ492361 AJ492362 AJ492363	AJ492788

Table 1. (Continued).

Taxon	Country: Province	Voucher	EMBL Accession No. <i>trnT</i> -L spacer <i>trnL</i> intron <i>trnL</i> -F spacer	EMBL Accession No. <i>ITS</i>
<i>Cynanchum rauhianum</i> Desc.	Madagascar: Toliara	<i>Röösli s.n. sub Noltee 2662</i> (in cult. Bayreuth)	AJ428723 AJ428724 AJ428725	AJ492789
	Madagascar: Toliara	<i>Liede et al. 2630</i> (P, UBT)	–	–
<i>Cynanchum repandum</i> Choux	Madagascar: Antananarivo	<i>Liede et al. 2867</i> (MO, P, UBT)	AJ428726 AJ428727 AJ428728	AJ492791
<i>Cynanchum rossii</i> Rauh	Madagascar: Toliara	<i>Singer 072478</i> (ZSS)	AJ428729 AJ428730 AJ428731	AJ492792
<i>Cynanchum rubricorona</i> Liede	Somalia: Hiiraan	<i>Kuchar 16793</i> (K, MWC 8414)	AJ428735 AJ428736 AJ428737	AJ492794
<i>Cynanchum rungweense</i> Bullock	Tanzania: Mbeya	<i>Mwasumbi 16518</i> (MO, K, MWC 8415)	AJ428738 AJ428739 AJ428740	AJ492795
<i>Cynanchum schistoglossum</i> Schltr.	Burundi: Bujumbura	<i>Lewalle 5435</i> (MO)	– AJ492364 AJ492365	AJ492796
<i>Cynanchum sessiliflorum</i> (Decne.) Liede	Madagascar: Antsiranana	<i>Mangelsdorff M13</i> (UBT)	AJ428741 AJ428742 AJ428743	AJ492797
<i>Cynanchum sigridiae</i> Meve & Teissier	Madagascar: Toliara	<i>Teissier 135</i> (K, MSUN)	AJ492366 AJ492367 AJ492368	AJ492798
<b><i>Cynanchum</i> sect. <i>Rhodostegiella</i> (Old World)</b>				
<i>Cynanchum auriculatum</i> Buch.-Ham. ex Wight	China	ex hort. Nanking s.n. (UBT)	AJ410196 AJ410197 AJ419198	AJ492741
<i>Cynanchum thesioides</i> K. Schum.	China	<i>Qingru 97-81</i> (MO)	AJ492369 AJ492370 AJ492371	AJ492799
	China: Gansu	<i>Wang et al. 93-414</i> (MO)	AJ428747 AJ428748 AJ428749	AJ492800
<b><i>Cynanchum</i> subgen. <i>Mellichampia</i> (New World)</b>				
<i>Cynanchum blandum</i> (Decne.) Sundell	Ecuador: Napo	<i>Burnham 1668</i> (MO)	AJ428403 AJ428604 AJ428605	AJ492742

Table 1. (Continued).

Taxon	Country: Province	Voucher	EMBL Accession No. <i>trnT-L</i> spacer <i>trnL</i> intron <i>trnL-F</i> spacer	EMBL Accession No. <i>ITS</i>
<i>Cynanchum foetidum</i> Kunth	Mexico: Oaxaca	<i>Campos 3956</i> (MO)	AJ428636 AJ428637 AJ428638	AJ492818
		<i>Kunze 624</i> (Minden)	–	–
<i>Cynanchum laeve</i> (Michx.) Pers.	USA: Missouri	<i>Liede s.n.</i> (UBT)	AJ428651 AJ428652 AJ428653	AJ492763
<i>Cynanchum ligulatum</i> (Benth.) Woodson	Mexico: Sonora	<i>Martin &amp; McWorther s.n.</i> (MO)	AJ428657 AJ428658 AJ428659	–
<i>Cynanchum montevidense</i> Spreng.	Argentina: Salta	<i>Liede &amp; Conrad 3100</i> (ULM)	AJ290848 AJ290849 AJ290850	AJ492773
<i>Cynanchum cf. racemosum</i> Jacq.	Mexico: Tamaulipas	<i>Liede &amp; Conrad 2609</i> (ULM)	AJ428720 AJ428721 AJ428722	AJ492790
<i>Cynanchum roulinooides</i> (E. Fourn.) Rapini ( <i>C. contrapetalum</i> Sundell)	Bolivia: Chuquisaca	<i>Wood et al. 13300</i> (K, UBT)	AJ428732 AJ428733 AJ428734	AJ492793
<i>Metalepis albiflora</i> Urban	Ecuador: Napo	<i>Burnham 1611</i> (MO)	AJ428774 AJ428775 AJ428776	AJ492808
	Ecuador: Napo	<i>Gentry et al. 64103</i> (MO)	–	–

branch swapping (“MulTrees” on) to the limit of computer capacity (between 30,000 and 40,000 trees). Internal support was assessed using 1000 bootstrap replicates with random addition of taxa (10 addition sequence replicates), with a limit of 10 trees kept at each step. Decay analyses were performed with AutoDecay 4.0 (Eriksson 1998) in combination with the reverse constraint option of PAUP\*.

A partition homogeneity test (as implemented in PAUP, 1000 replicates) showed significant discordance between the cpDNA dataset and the rDNA dataset ( $p = 0.01$ ), but if the partial cpDNA datasets were tested singly, no discordance was found (*trnT-L* spacer:  $p = 0.1$ , *trnL* intron:  $p = 0.99$ , *trnL-F* spacer:  $p = 0.59$ ). With this result, the argument of Soltis et al. (2000) – that tests of incongruence are often too coarse and that the best way to detect true incongruence is to examine the internal support of nodes in separate and combined

analysis – was followed, and a combination of the two datasets was analyzed. In addition, a Neighbor-Joining analysis of all sequence data (Saitou & Nei 1987) was conducted as well, employing Jukes-Cantor (Jukes & Cantor 1969) and Kimura 2-parameter (Kimura 1980) distance models.

Finally, data from the morphological analysis of African *Cynanchum* (Liede 1997a) and the two molecular datasets were combined and analyzed, even though a partition homogeneity test showed highly significant discordance ( $p = 0.01$ ) between the morphological data and both molecular datasets. Taxa for which either no molecular data or no detailed morphological data were available were omitted, as were double accessions of the same species, leaving 63 ingroup taxa. Liede (1997b) regarded *Tylophora* as a primitive Asclepiadeae genus and thus a suitable outgroup. Since then it has been established that *Tylophora* belongs to a different subtribe (Ty-

lophorinae) in the Asclepiadeae (Liede 2001), and that *ITS* sequences of *Tylophora* and *Cynanchum* cannot be aligned unambiguously (Liede et al. in press). Therefore, *Schizostephanus alatus* was coded for morphological characters following Liede (1993b, as *Cynanchum validum*). To the morphological dataset of 87 characters (multistate characters treated as polymorphisms), the parsimony informative sequence characters of two molecular datasets (145 for *ITS* and 114 for cpDNA) were added. In addition to a parsimony analysis of the three combined datasets, an analysis of the molecular data alone was performed with the reduced number of taxa, and morphological characters were plotted on the resulting tree using MacClade 4.0 (Maddison & Maddison 2000).

### Stem anatomy

Twenty-seven leafless, stem-succulent members of *Cynanchum* and related genera, three leafy Malagasy *Cynanchum* species, and a total of six non-succulent African, Asian and American species of *Cynanchum* s. l. were studied anatomically (Table 1). In addition, two American *Funastrum* E. Fourn. species, *F. clausum* Schltr. and *F. pannosum* Schltr., were studied. For the stem-succulent taxa, mature but not basal internodes were selected. Material was fixed in FAA, dehydrated via tertiary butanol, and embedded in paraffine. Sections were stained for 5 minutes each in 0.1% aqueous safranine and 0.5% Astra blue (Gerlach 1969). Sections were washed, deparaffinated with Roticlear® (Roth Chemicals), and coverslipped with Entellan®. The data were tabulated and plotted onto the tree resulting from analysis of the combined molecular data using MacClade 4.0 (Maddison & Maddison 2000) for the taxa for which both datasets were available.

### Latex triterpene analysis

Latex samples collected in the field were dried overnight in low heat on top of the plant dryer. Latex samples from cultivated plants were dried overnight at 60 °C. All samples were analyzed for their acetone-extracted profile by Paul G. Mahlberg at Indiana University. The latex samples were redissolved in acetone and the supernatant analyzed for triterpenoid components by gas-liquid chromatography (Mahlberg et al. 1988). Analyses were performed on a Hewlett-Packard 5710 chromatograph equipped with a flame ionization detector and the oven programmed from 240–290 °C at 4 °C/min. Nitrogen was the carrier gas, 20 ml/min. Injection port and detector temperatures were 250 and 350 °C, respectively. Columns contained 3% OV-1 on 100/120 mesh Supelcoport. Individual compounds were quantified on a Hewlett Packard 3380.

## Results

### DNA sequence analysis

Sequence characteristics for both datasets are summarized in Table 2.

Sequence divergences are higher in *ITS1* with 0.0–19.4% than in *ITS2* with 0.0–11.9%. In both cases, divergence between *Glossonema boveanum* and an American *Cynanchum* species (*Metalepis albiflorum* for *ITS1*, *C. blandum* for *ITS2*) is larger than between any ingroup taxon and the outgroup. For *ITS1*, the largest divergences are found between *C. galgalense* and *Metalepis albiflora*. For the cpDNA, the *trnL* intron has the lowest sequence divergence with 0.0–4.2% between ingroup taxa, and the *trnL*-T and *trnL*-F spacers are almost equally variable with 0.0–7.8 and 0.0–8.4%, respectively. Again, highest sequence divergence is not between any ingroup taxon and the outgroup, but between a New World and an Old World species: *C. foetidum* and *C. acutum* (*trnT*-L spacer), *Metalepis albiflorum* and *Metaplexis japonica* (*trnL*-F spacer), *M. albiflorum* and *Glossonema boveanum* (*trnL* intron). Pairs of the same species are identical in all regions studied in *C. gerrardii* and *C. thesioides*. Between the remaining species pairs, divergences of up to 1.2% occur in one or several regions.

Analysis of the cpDNA dataset without *C. schistoglossum* (268 parsimony informative characters) resulted in more than 40,000 trees ( $l = 484$  steps,  $CI = 0.677$ ,  $RI = 0.84$ ,  $RC = 0.562$ ; strict consensus see Fig. 1). Adding the separately coded indels (312 parsimony informative characters) resulted in more than 44,000 most parsimonious trees ( $l = 575$  steps,  $CI = 0.649$ ,  $RI = 0.828$ ,  $RC = 0.537$ ; strict consensus see Fig. 1).

Analysis of the rDNA dataset without *C. ligulatum*, *C. chouxii* and the second accession of *C. galgalense* (200 parsimony informative characters) resulted in more than 44,000 most parsimonious trees ( $l = 605$  steps,  $CI = 0.514$ ,  $RI = 0.774$ ,  $RC = 0.40$ ; strict consensus see Fig. 2) and retrieved largely the same main clades as the cpDNA dataset. However, the Malagasy clade is much better resolved, both for the leafy and the leafless taxa. The leafless taxa together with the stem-succulent but leafy *C. pycnoneurooides* form a reasonably well supported subclade of the Malagasy clade also including the leafless succulent taxa shared with mainland Africa (*Sarcostemma* and *C. gerrardii*).

Analysis of the combined cpDNA–rDNA dataset did not change the well-supported clades of the single analyses. The combined analysis (505 parsimony informative characters) resulted in 2268 most parsimonious trees ( $l = 1175$  steps,  $CI = 0.568$ ,  $RI = 0.785$ ,  $RC = 0.446$ ; strict consensus tree see Fig. 3). Bootstrap values increased for all clades retrieved in the single analyses,

**Table 2.** Sequence characteristics.

	<i>trnT-L</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>ITS1</i>	<i>ITS2</i>	<b>5.8s</b>
Aligned total length (bp)	1179 <sup>a</sup>	578	421	337	299	162
Length range (bp)	685 ( <i>C. acutum</i> ) — 867 ( <i>Metalepis albiflora</i> )	393 ( <i>C. longipes</i> ) — 533 ( <i>C. montevidense</i> )	265 ( <i>C. longipes</i> ) —371 ( <i>Schizostephanus alatus</i> )	207 ( <i>Pentarrhinum gonoloboides</i> ) —262 ( <i>Odontanthera radians</i> )	221 ( <i>C. rubricorona</i> ) —259 ( <i>C. blandum</i> )	159–162
Length mean (bp)	787.79	502.84	361.35	232.63	244.04	159.76
Number of parsimony informative indels <sup>a</sup>	28 (indels coded 32)	9	7	—	—	—
Sequence divergence (ingroup/outgroup) (%)	1.0–5.6	0.4–3.0	0.0–5.0	7.6–14.7	3.7–10.5	0.0–0.2
Sequence divergence (ingroup) (%) <sup>b</sup>	0.0–7.8	0.0–4.3	0.0–8.4	0.0–19.4	0.0–11.9	0.0–0.2
Sequence divergence (pairs of same species) (%)	0.0–1.2	0.0–0.4	0.0–0.5	0.0–1.0	0.0–1.2	0.0

<sup>a</sup> 52 characters permanently excluded because of ambiguous alignment

<sup>b</sup> not taking into account different accessions of the same species

indicating higher congruency of the two datasets than the partition homogeneity test. The Madagascar clade is supported by 100% bootstrap value, and the succulent clade, including the succulent but linear-leaved *C. pycno-neuroides*, by 79% bootstrap support. Interestingly, the likewise stem-succulent but large-leaved *C. pachycladon* is now basal to the succulent clade s. str., though only with 68% bootstrap support. Clades retrieved in the rDNA dataset but not in the cpDNA dataset were largely preserved in the combined analysis, but equally unsupported; nevertheless, some basal resolution is apparent.

The results of the two separate Neighbor-Joining analyses based on Jukes-Cantor and Kimura 2-parameter distance models produced trees almost identical in topology (Fig. 4), the only difference concerning the *C. floribundum-C. falcatum* branch, which is unresolved in the Kimura-2-parameter analysis but neighbor to the *Pentarrhinum/Mellichampia/C. acutum* branch following the Jukes-Cantor model. The Neighbor-Joining tree is highly congruent with the parsimony-based strict consensus phylogeny (Fig. 3). It illustrates clearly the structure of *Cynanchum* with short distances between the groups and long distances between the taxa in a group in African, American and Asian taxa. The Malagasy taxa are separated by a comparatively long distance, but distances are very short within the Malagasy group, and even shorter within the group of succulent species.

The two American sections – the North and Central American *C. foetidum*, *C. laeve* and *C. racemosum* (sect. *Mellichampia*) and the South American *C. blandum*, *C. montevidense* and *C. roulinioides* (sect. *Roulinia*) – together with *Metalepis albiflorum* form reasonably to well-supported clades in all analyses (Figs 1–3). The Central American *C. ligulatum*, for which no *ITS* sequence was available, falls into its expected place with *C. foetidum* and *C. racemosum* in the cpDNA analysis (Fig. 1). These two subclades form a well supported clade in the cpDNA and combined analyses (Figs 1, 3), whereas they are unresolved with subclades of the *Pentarrhinum*-clade (Liede et al. 2002) in the analysis resulting from *ITS* data. In the combined analysis, the Afro-Arabian *Pentarrhinum*-clade is sister to the American clade (Fig. 3).

Most Asian taxa form a very well supported clade, including *Metaplexis japonica* (Figs 1–3). *Cynanchum ovalifolium*, a widespread coastal Australasian species, joins this clade with low support in the *ITS* and the combined datasets (Figs 2, 3); the Neighbor-Joining analysis (Fig. 4) shows very long branches both for the core Asian taxa and for *C. ovalifolium*. The only available Australian species, *C. floribundum*, is even more weakly joint to the Asian clade in the *ITS* and combined analyses (Figs 2, 3), but in the Neighbor-Joining analysis *C. floribundum* is next to the African *C. falcatum*.

African *Cynanchum* fall into three main groups. Stable and well supported by both datasets is the *C. clavidentis*-clade, comprising the widespread *C. clavidentis*, the two Somalian twiners *C. crassiantherae* and *C. rubricorona*, as well as the geophytic *C. orangeanum* and *C. praecox*. In the combined analysis (Fig. 3), this clade is basal in *Cynanchum*.

The South African *C. africanum*, *C. ellipticum*, *C. meyeri* and *C. natalitium*, and the East African *C. altiscandens* form a weakly supported clade for both datasets (Figs 1–3), but only the *ITS* dataset joins the South African *C. obtusifolium* without support (Fig. 2), and the East African endemic *C. rungweense* is added only in the combined analysis (Fig. 3). A tie to *C. polyanthum* is suggested by the Neighbor-Joining analysis (Fig. 4), but not by parsimony analysis. The association of *C. polyanthum* with the Australian *C. floribundum* in the cpDNA dataset without indels is most likely an artefact caused by long-branch attraction.

The positions of the remaining species have less support. The Somalian *C. galgalense* shows no affinity to any clade in any dataset, even though a second specimen has been analyzed for cpDNA to ascertain the results obtained from the first specimen. The combined analysis (Fig. 3) suggests a clade of the remaining African *Cynanchum* species, but only the subclade of the West African *C. adalinae* and *C. longipes* is reasonably well supported. Very well supported by *ITS* data is the morphologically unsuspected sister species relationship between *C. falcatum* and *C. schistoglossum* (for which cpDNA data are incomplete). The members of this African clade – to which the type species of *Cynanchum*, the circum-Mediterranean *C. acutum*, belongs – all show very long branches (Fig. 4).

Analysis of the combined morphological/molecular dataset (343 parsimony informative characters) results in 1232 most parsimonious trees ( $l = 1488$  steps,  $CI = 0.337$ ,  $RI = 0.587$ ,  $RC = 0.198$ ; strict consensus see Fig. 5). Homoplasy measures for the combined trees are considerably worse than for any of the molecular trees alone, but better than those found by Liede (1997a) for pure morphological trees. Support is improved by morphological characters only for the *Folotsia*-clade, sup-

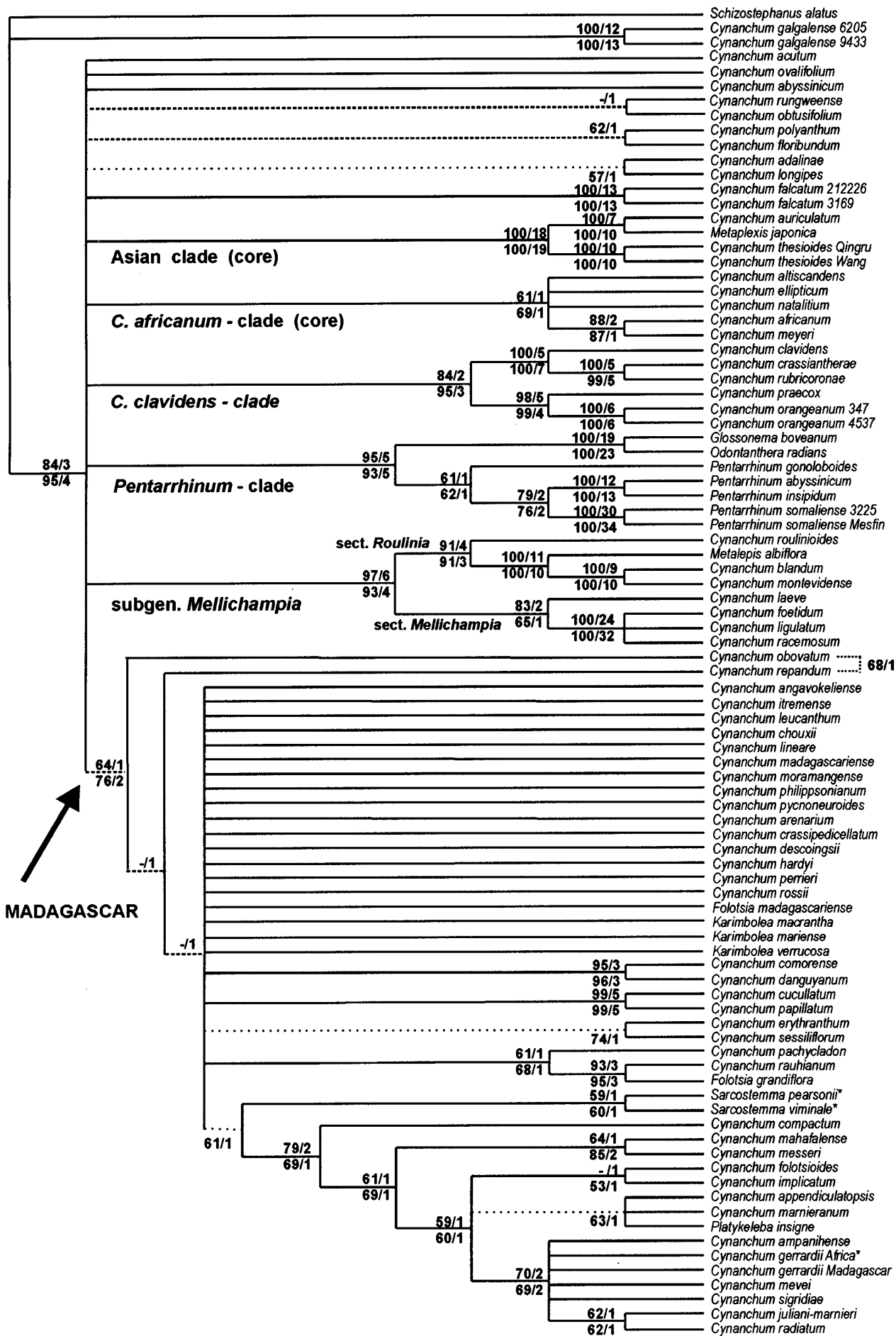
port for the other clades is either the same or worse than for the molecular data alone. In the molecularly unresolved stem-succulent *C. arenarium*-clade, morphological data strongly support respective sister group relationships between *C. arenarium* and *C. hardyi*, and *C. crassipedicellatum* and *C. descoingsii*.

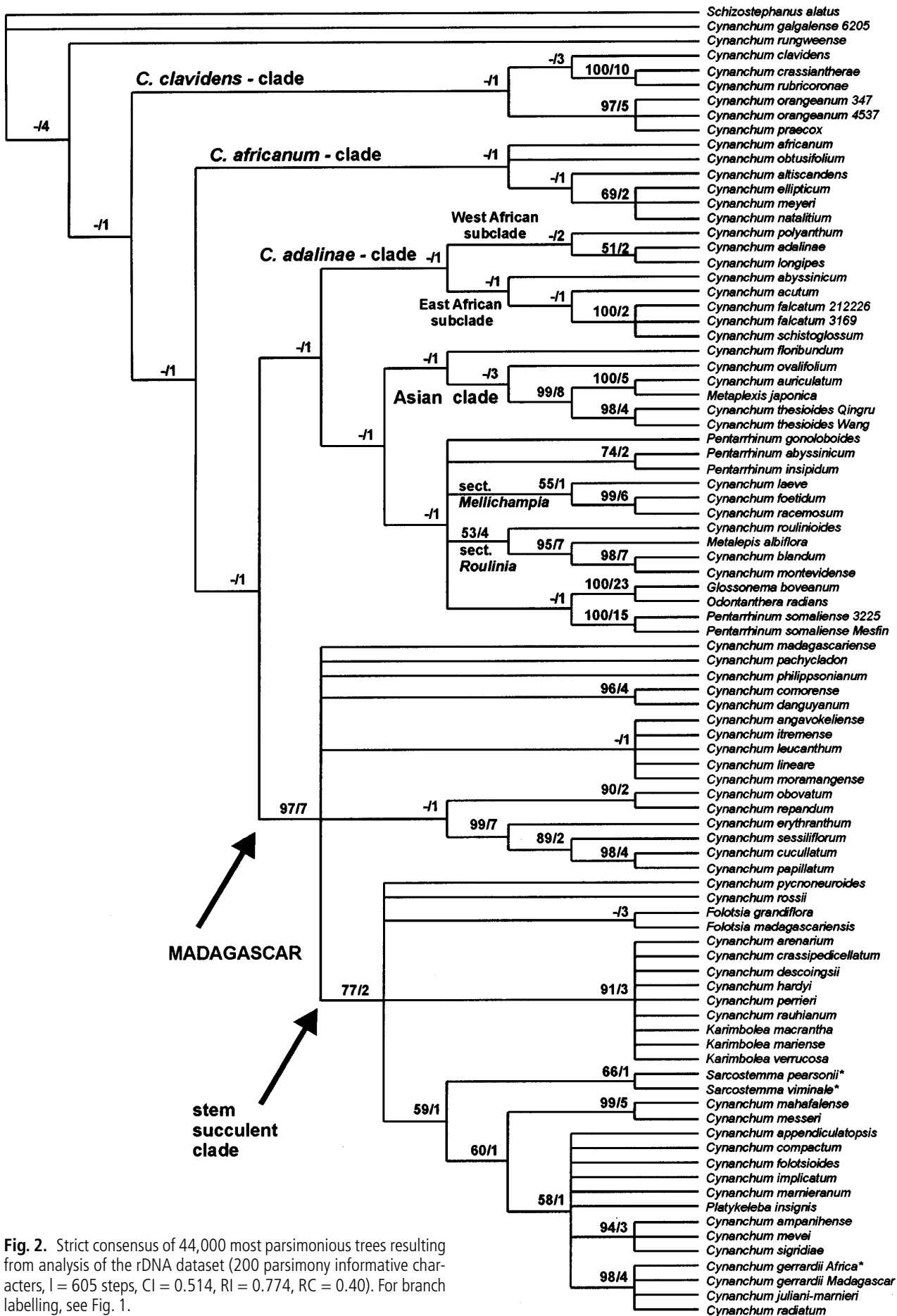
Plotting of the morphological characters defined in Liede (1993b) on the strict consensus tree resulting from analysis of the combined molecular datasets shows that almost all characters used are homoplasious. Only thick-walled, ornamented fruits are restricted to *Pentarrhinum* (and *Glossonema/Odontanthera*, see Liede et al. 2002), which forms a subclade of *Cynanchum*. Stem succulence (Fig. 6A) is almost restricted to the “stem-succulent” clade, which includes the linear-leafed *C. pycno-neuroides*. The large-leafed but succulent *C. pachycladon* is basal to this clade, though with low support. Outside this clade, weak stem succulence occurs only in *C. phillipsonianum*. Other characters, even conspicuous ones such as trichomes on the corolla, ligulate or constricted coronas, papillose connective appendages, etc., are found in several clades. Corona characters in particular are highly homoplasious, e.g. the degree of fusion of the gynostegial corona (Fig. 6B). Except for the Malagasy *C. erythranthum*-clade, all clades have members with coronas fused to various degrees. In the stem-succulent clade, possession of warts and striation of the stems can be used as a rough indicator for relationship in the leafless taxa (Fig. 7). All members of the *C. arenarium* polytomy (except for *C. rauhianum*) have warty stems, but the latter also occur in *C. marnieranum* (Fig. 7A). The wartiness of *C. pachycladon* is a feature of its corky bark and probably not homologous to the wartiness of the green stems of the remaining taxa. Striation is characteristic of the most derived subclade of the stem-succulent clade, but *C. compactum* and *C. marnieranum* are exceptions (Fig. 7B).

### Stem anatomy

A total of 16 characters was studied (Table 3). All species follow the general pattern of Apocynaceae s.l., in which the pith is surrounded by an amphi-

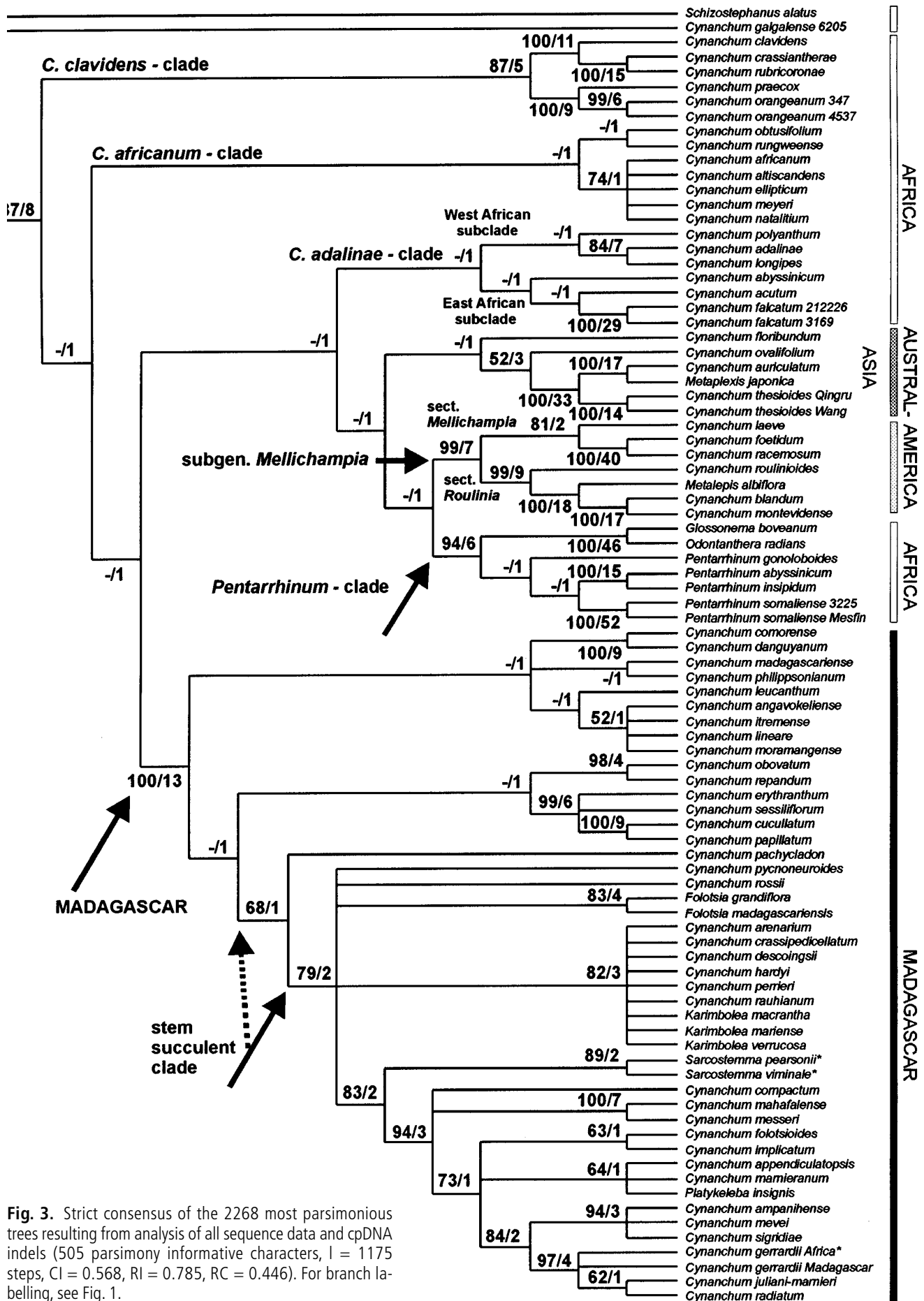
**Fig. 1.** Analysis of the cpDNA dataset without *C. schistoglossum*. Strict consensus of 44,000 most parsimonious trees resulting from analysis without indels (268 parsimony informative characters,  $l = 484$  steps,  $CI = 0.677$ ,  $RI = 0.84$ ,  $RC = 0.562$ ), and of more than 40,000 trees resulting from analysis with indels (312 parsimony informative characters,  $l = 575$  steps,  $CI = 0.649$ ,  $RI = 0.828$ ,  $RC = 0.537$ ). Clades only retrieved in the analysis without indels are represented by dashed lines, those retrieved only in the analysis with indels by dotted lines. Bootstrap percentages and decay indices are given for analysis without indels above branches, with indels below branches. The only conflicting arrangement of taxa, *C. obovatum* and *C. repandum*, is indicated at the right margin. Asterisks indicate African taxa in the Malagasy clade.





**Fig. 2.** Strict consensus of 44,000 most parsimonious trees resulting from analysis of the rDNA dataset (200 parsimony informative characters,  $I = 605$  steps,  $CI = 0.514$ ,  $RI = 0.774$ ,  $RC = 0.40$ ). For branch labelling, see Fig. 1.





**Fig. 3.** Strict consensus of the 2268 most parsimonious trees resulting from analysis of all sequence data and cpDNA indels (505 parsimony informative characters, I = 1175 steps, CI = 0.568, RI = 0.785, RC = 0.446). For branch labelling, see Fig. 1.

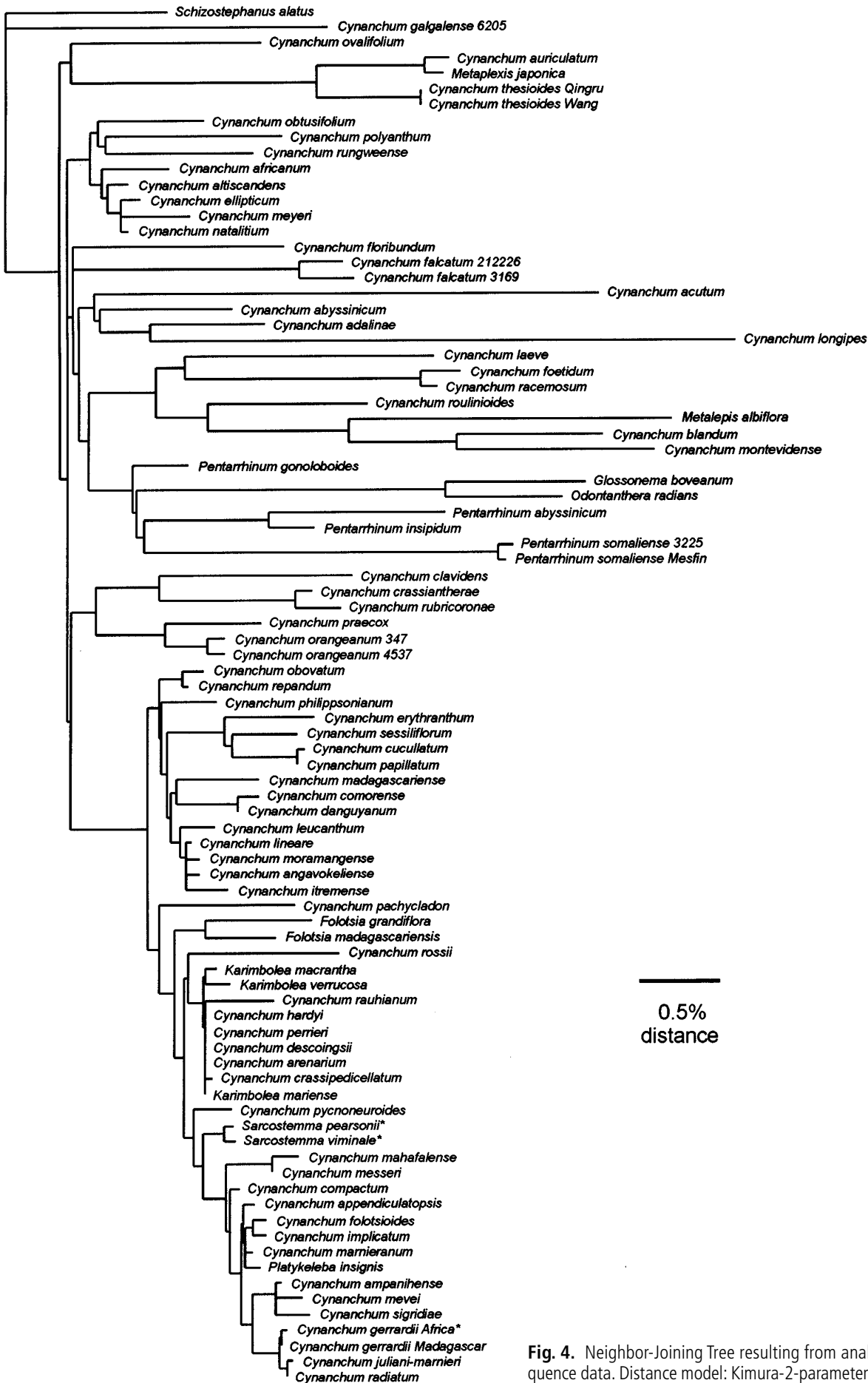


Fig. 4. Neighbor-Joining Tree resulting from analysis of all sequence data. Distance model: Kimura-2-parameter

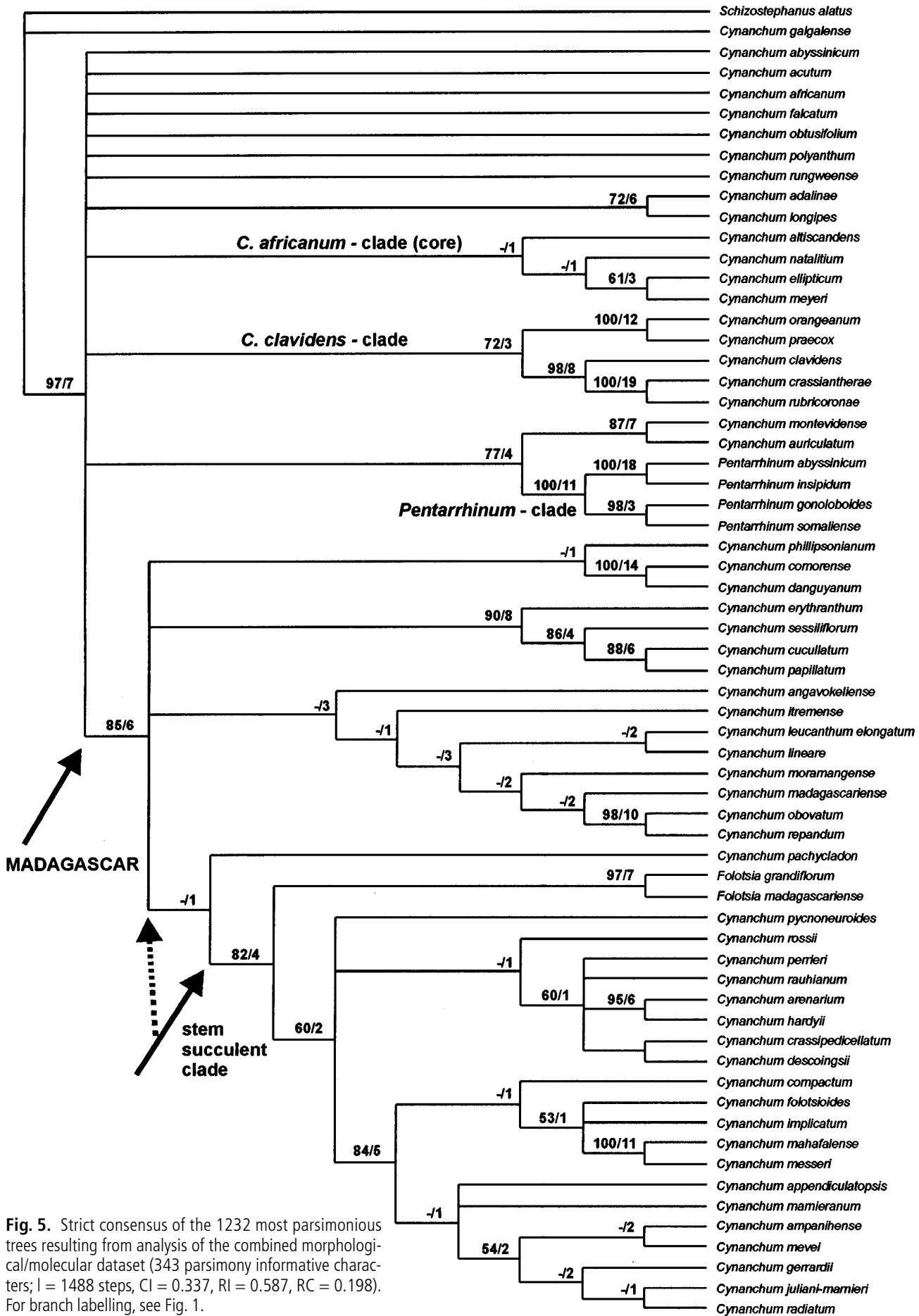
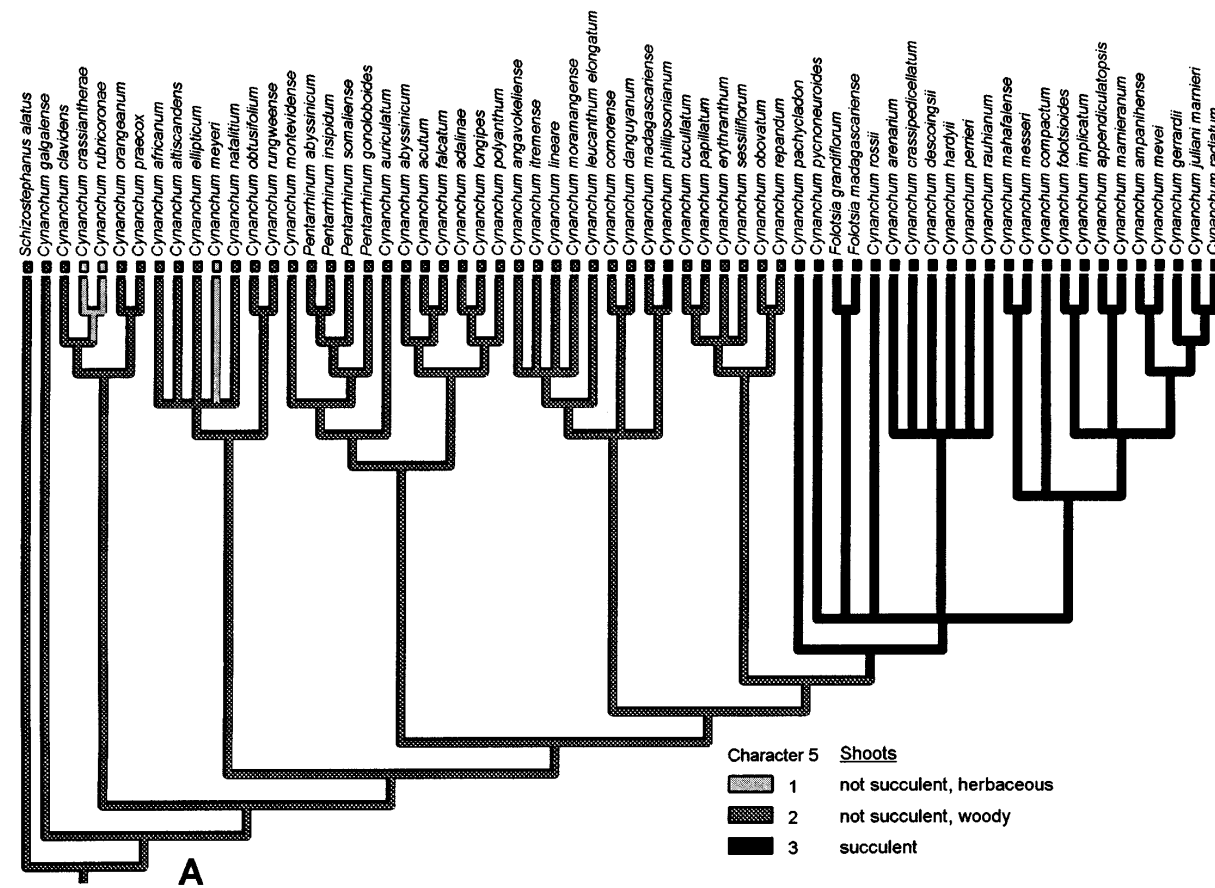
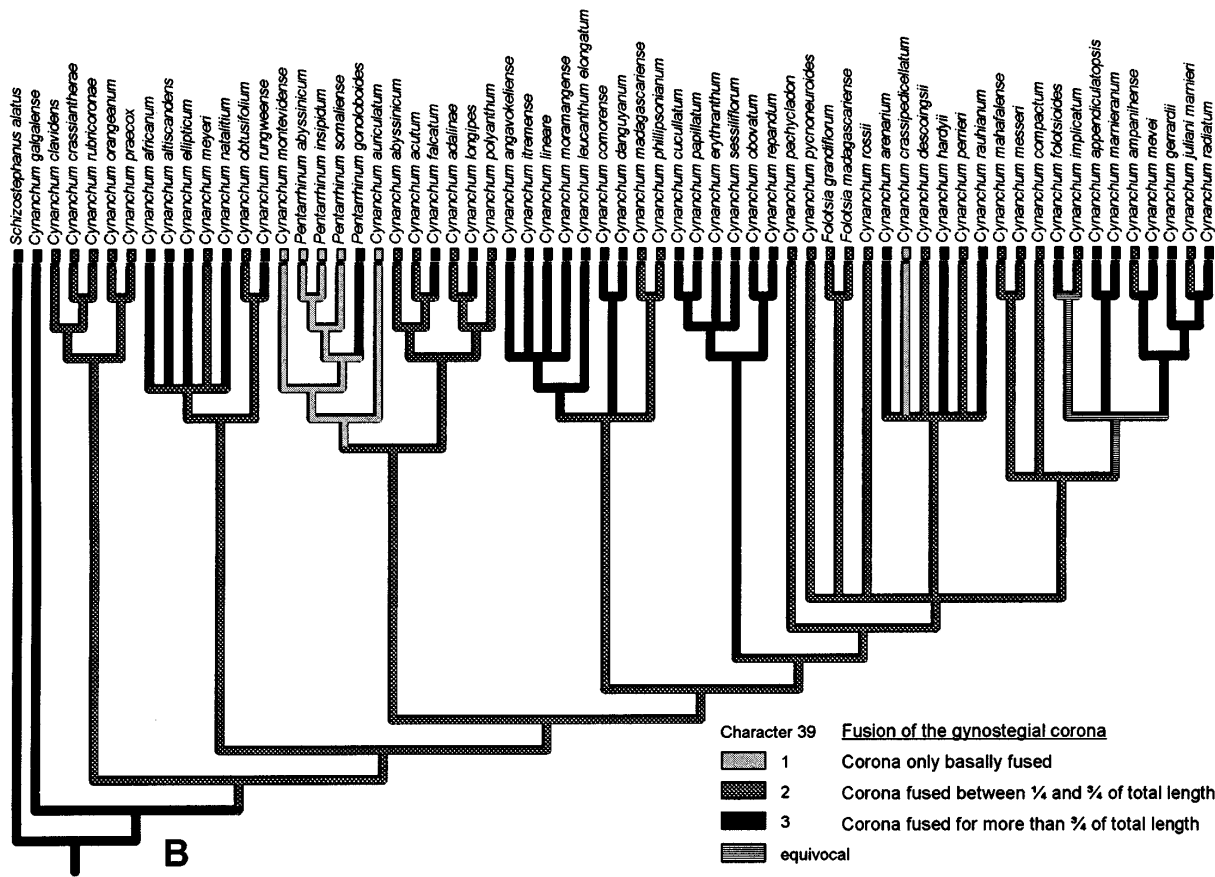
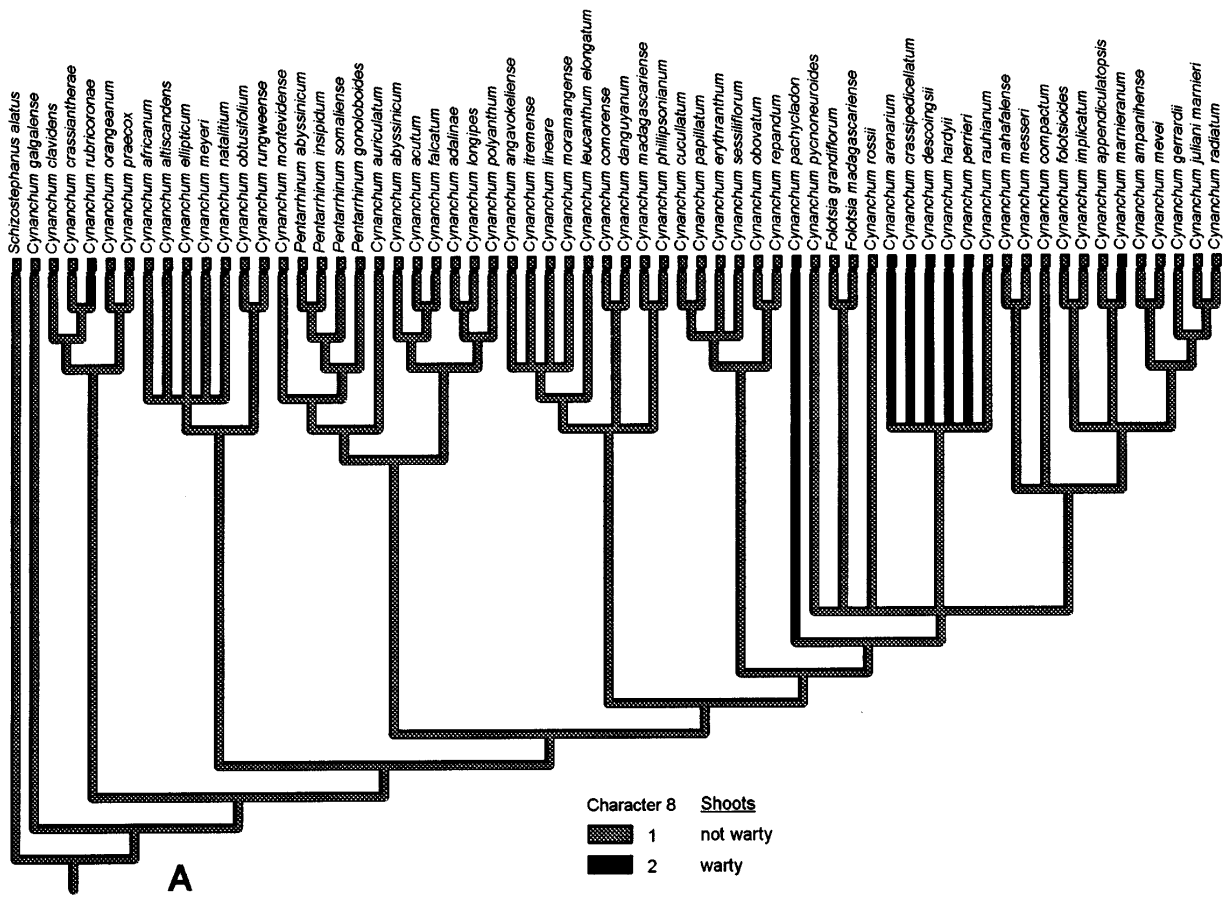
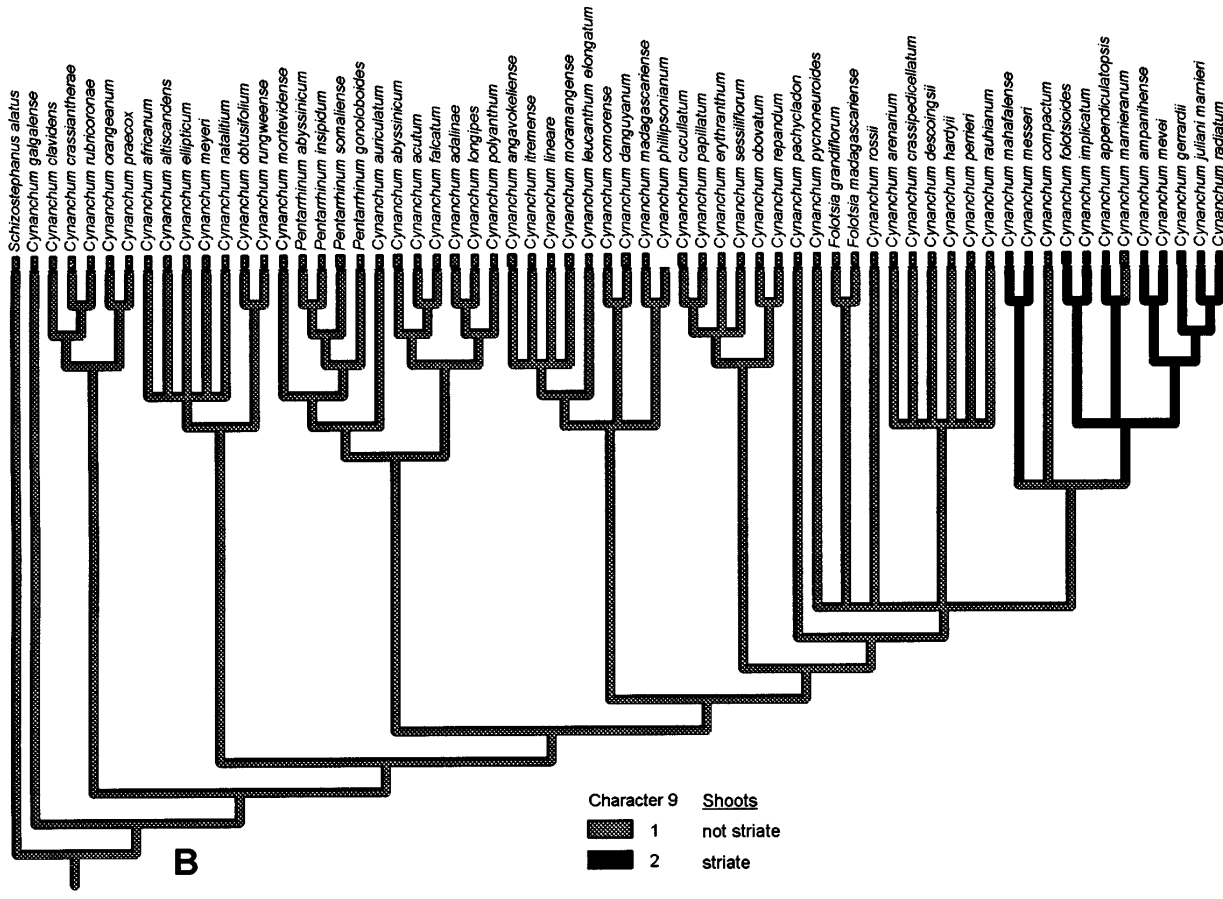


Fig. 5. Strict consensus of the 1232 most parsimonious trees resulting from analysis of the combined morphological/molecular dataset (343 parsimony informative characters;  $l = 1488$  steps,  $CI = 0.337$ ,  $RI = 0.587$ ,  $RC = 0.198$ ). For branch labelling, see Fig. 1.



**Fig. 6.** Examples of distribution of morphological characters identified by Liede (1993b) on the molecular phylogeny. A. Shoots woody, herbaceous or succulent. B. Degree of corona fusion.



**Fig. 7.** Examples of distribution of morphological characters identified by Liede (1993b) on the molecular phylogeny. A. Stems warty or smooth. B. Stems striate or uniform.

Table 3. Stem anatomy.

Species	Number	Pith				Xylem				stone cells	Sclerenchyma			Endo-dermis	Cortex		Hypo-dermis	Epidermis		Sto-mata	
		5–10 cells	more than 10 cells	single groups	reduced ring	5–10 cells	more than 10 cells	single groups	reduced ring		loose ring	no nests (0); nests in one row (1); nests in 2–3 rows (2)	reduced		different cell sizes	parenchyma undifferentiated (0); with sclerenchyma (1)		palisade parenchyma	number of layers (0: absent; 1: 1–2; 2: more than two)		papillöse
<i>Funastrum clausum</i>	2599	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Funastrum pannosum</i>	2600a	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Metalepis albiflora</i>	Gentry 64103	–	0	1	0	0	0	0	0	1	0	2	0	0	0	0	2	0	0	0	–
<i>Cynanchum foetidum</i>	HK 624	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0
<i>Cynanchum ovalifolium</i>	3279	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	–
<i>Pentarrhinum insipidum</i>	PVB s.n.	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
<i>Cynanchum natalitium</i>	HK 316	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
<i>Cynanchum obtusifolium</i>	HK 303	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
<i>Cynanchum decaryi</i>	2691	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0
<i>Cynanchum pachycladon</i>	2663	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	–	0	0	–
<i>Cynanchum pycnoneuroides</i>	10605	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
<i>Cynanchum aculeatum</i>	2828	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	2
<i>Cynanchum ampanihense</i>	2824	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	1

<i>Cynanchum arenarium</i>	2713	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2
<i>Cynanchum bisinuatum</i>	2901	0	1	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	1	0	0	2
<i>Cynanchum compactum</i>	12161	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	2
<i>Cynanchum descoingsii</i>	68638	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Cynanchum gerrardii</i>	Rauh s.n.	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1
<i>Cynanchum hardyi</i>	3571	0	1	0	0	0	0	0	0	0	1	0	0	01	0	0	0	0	0	1	0	2
<i>Cynanchum juliani-marnieri</i>	Teissier s.n.	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	2
<i>Cynanchum luteifluens</i> var. <i>luteifluens</i>	2699	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
<i>Cynanchum luteifluens</i> var. <i>longicoronae</i>	2624	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	2
<i>Cynanchum mahafalense</i>	2649	2	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	2	1	0	2
<i>Cynanchum marnieranum</i>	Rauh s.n.	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1
<i>Cynanchum menarandrense</i>	7593	0	1	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0
<i>Cynanchum messeri</i>	2721	2	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	1	2
<i>Cynanchum mevei</i>	2780	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	2
<i>Cynanchum rahianum</i>	2630	0	1	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	1	0	0	2
<i>Folotia grandiflora</i>	68584	0	0	0	1	0	0	0	0	0	1	0	0	01	0	0	0	0	1	0	0	2
<i>Folotia madagascariensis</i>	2695	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
<i>Karimbolea verrucosa</i>	2826	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1
<i>Platykeleba insignis</i>	Lavranos s.n.	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	01	1	0	2
<i>Sarcostemma arabicum</i>	R. & H. 4624	0	0	1	0	0	1	0	0	0	2	0	0	0	1	0	0	0	0	1	0	1
<i>Sarcostemma australe</i>	HK 205	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	1
<i>Sarcostemma pearsonii</i>	2518	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0
<i>Sarcostemma viminale</i> ssp. <i>viminale</i>	2706	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	1	1
<i>Sarcostemma viminale</i> ssp. <i>thunbergii</i>	2514	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	2
<i>Sarcostemma viminale</i> ssp. <i>nov.</i>	2552	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0

Table 4. Percentage composition of latex triterpenoids.

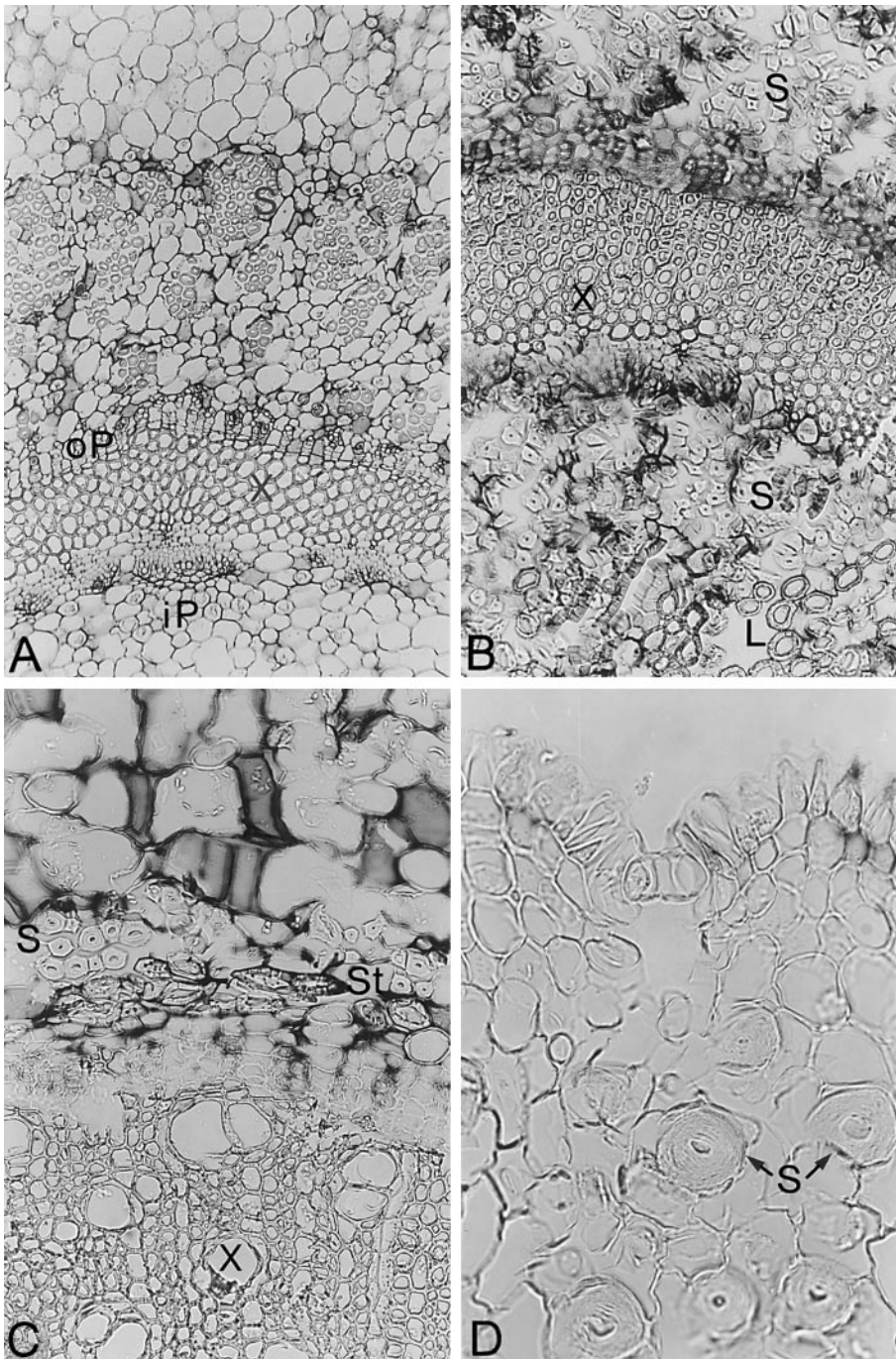
Species	Number	Retention time (min)															
		8.8	11.7	12.0	13.6	14.0	14.5	14.8	15.3	15.5	15.7	16.3	17.2	18.0	19.3	20.4	21.9
<i>Pentarrhinum insipidum</i>	584a																
<i>Cynanchum africanum</i>	624	t			t	4											
<i>Cynanchum cucullatum</i>	2868																
<i>Cynanchum junciforme</i>	2864									t							
<i>Cynanchum obovatum</i>	2859			1						1							
<i>Cynanchum pachycladon</i>	2863	t								13							
<i>Cynanchum papillatum</i>	2862									t							4
<i>Cynanchum aculeatum</i>	2828							1		1							
<i>Cynanchum arenarium</i>	2739	t								7							
<i>Cynanchum crassipedicellatum</i>	2852	1		t		2	7		6								
<i>Cynanchum gerrardii</i>	995					1	1										
<i>Cynanchum luteifluens</i>	Rauh s.n.									1							
var. <i>luteifluens</i>	2731									2							
<i>Cynanchum mairianum</i>	2835									1							
<i>Cynanchum perrieri</i>	2851	t			t	3			6								
<i>Folotia grandiflora</i>	68584							1		1							
<i>Folotia madagascariensis</i>	2667			t					1								
<i>Karimbolea verrucosa</i>	2826	t							3								
<i>Platykeleba insignis</i>	Lavranos s.n.								t								
<i>Sarcostemma pearsonii</i>	2520								4								
<i>Sarcostemma resiliens</i>	515								2								
<i>Sarcostemma stolonifera</i>	199	t							2								
<i>Sarcostemma vanlessenii</i>	Lavranos s.n.									1							
<i>Sarcostemma viminale</i>	870									t							
ssp. <i>viminale</i>	2738	t							4								
<i>Sarcostemma viminale</i>	2507								3								
ssp. <i>thunbergii</i>	2522								5								
<i>Sarcostemma viminale</i>	2552								3								
ssp. <i>thunbergii</i>	Thailand s.n.								2								
<i>Sarcostemma viminale</i> ssp. nov.	Singh s.n.								4								
<i>Sarcostemma viminale</i>									3								
<i>Sarcostemma viminale</i>									2								
<i>Sarcostemma viminale</i>									4								



phloematic xylem. Adjacent to the outer phloem is a zone of sclerenchymatic fibres arranged either in a closed ring or in separate bundles with intermittent parenchyma. The cortex is formed by parenchymatic tissue. Usually, but not always, present are a hypodermis below the epidermis consisting of smaller cells of regular size, as well as a starch sheath on the inside of the cortex. Differences between species involve mainly specialized cells occurring in the pith or the cortex.

The American *Funastrum* species differ from all other analyzed species by a division of the outer cortex into an inner part of more or less isodiametric parenchyma cells and surrounding, radially elongated palisade cells (Fig. 9B). Special cells such as brachysclereids or single fibres are absent in *Funastrum*, but present in *Sarcostemma*; stomata are level with the surface in *Funastrum*, but sunken in *Sarcostemma*.

Plotting of the characters on the cladogram derived from DNA characters shows that of the characters used



**Fig. 8.** Stem anatomy. A. Cross section of a stem succulent *Cynanchum* (*C. rauhianum*) representing the basic pattern of stem anatomy. B. *C. messeri*, specialized pith with parenchyma interspersed with sclerenchyma fibres and lignified cells. C. *Sarcostemma viminale* ssp. nov. (SL 2552), cross section showing nests of stone cells between outer phloem and sclerenchyma of inner cortex. D. *Sarcostemma pearsonii*, outer cortex with stone cells, papillose epidermis and sunken stomata. H: hypodermis, iP: inner phloem, L: lignified cells, oP: outer phloem, S: sclerenchyma, St: stone cells, X: xylem.

the anatomical ones are no better indicators of relationship than are the morphological ones in succulent *Cynanchum*. Only a papillose epidermis coincides roughly with the main clades of succulent *Cynanchum*, but *C. marnieranum* with a smooth epidermis is found in the otherwise papillose clade, and *C. hardyi* with a papillose epidermis in the otherwise smooth clade. The two *Folotisia* species also differ in this character (Table 3). Only the *C. mahafalense/C. messeri* clade, which is morphologically otherwise well supported, is uniquely characterized by both lignified cells and sclerenchyma in the pith.

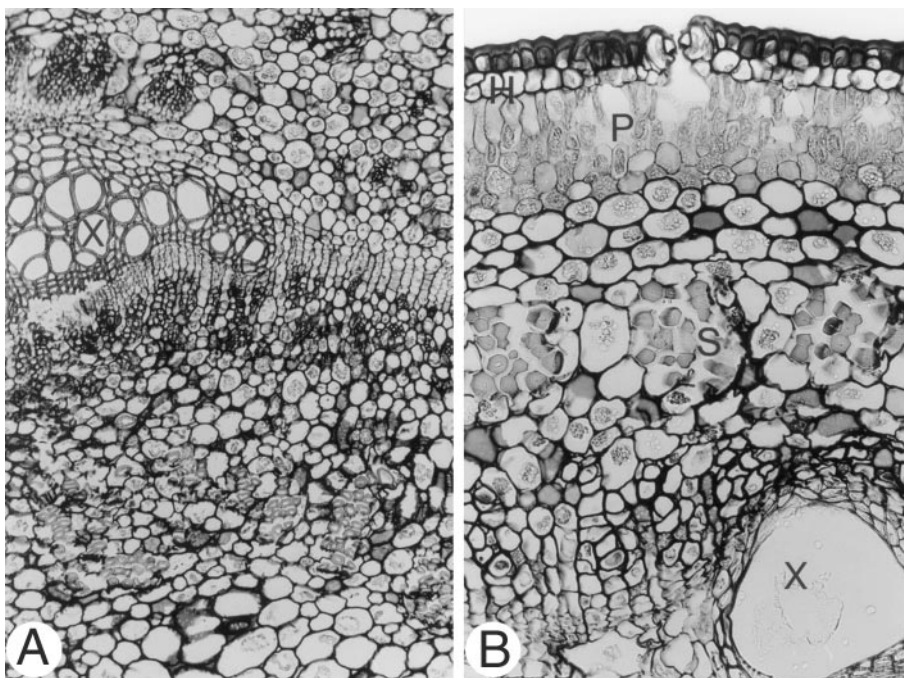
### Triterpene analysis

Liede et al. (1993) used latex triterpenoid patterns to show that *Karimbolea* constitutes a true member of Asclepiadeae despite the erect pollinia of *K. verrucosa*, the only *Karimbolea* species known at the time of that analysis. In the course of that analysis (Liede et al. 1993), the very different triterpenoid composition of *Funastrum* and Old World *Sarcostemma* (Fig. 10A, E) – then both included in *Sarcostemma* sensu Holm (1950) – together with the striking uniformity of the stem-succulent Malagasy species with *Sarcostemma* s. str. (Fig. 10C, E) – then belonging to five different genera – led to a discontinuation of the study. Almost all stem succulents show the same general triterpenoid pattern with four major peaks, the largest one at a retention time of 18 min (Fig. 10C). A small fifth peak at 15.3 min is usually but not always present (Table 4). Exceptions are *C. arena-*

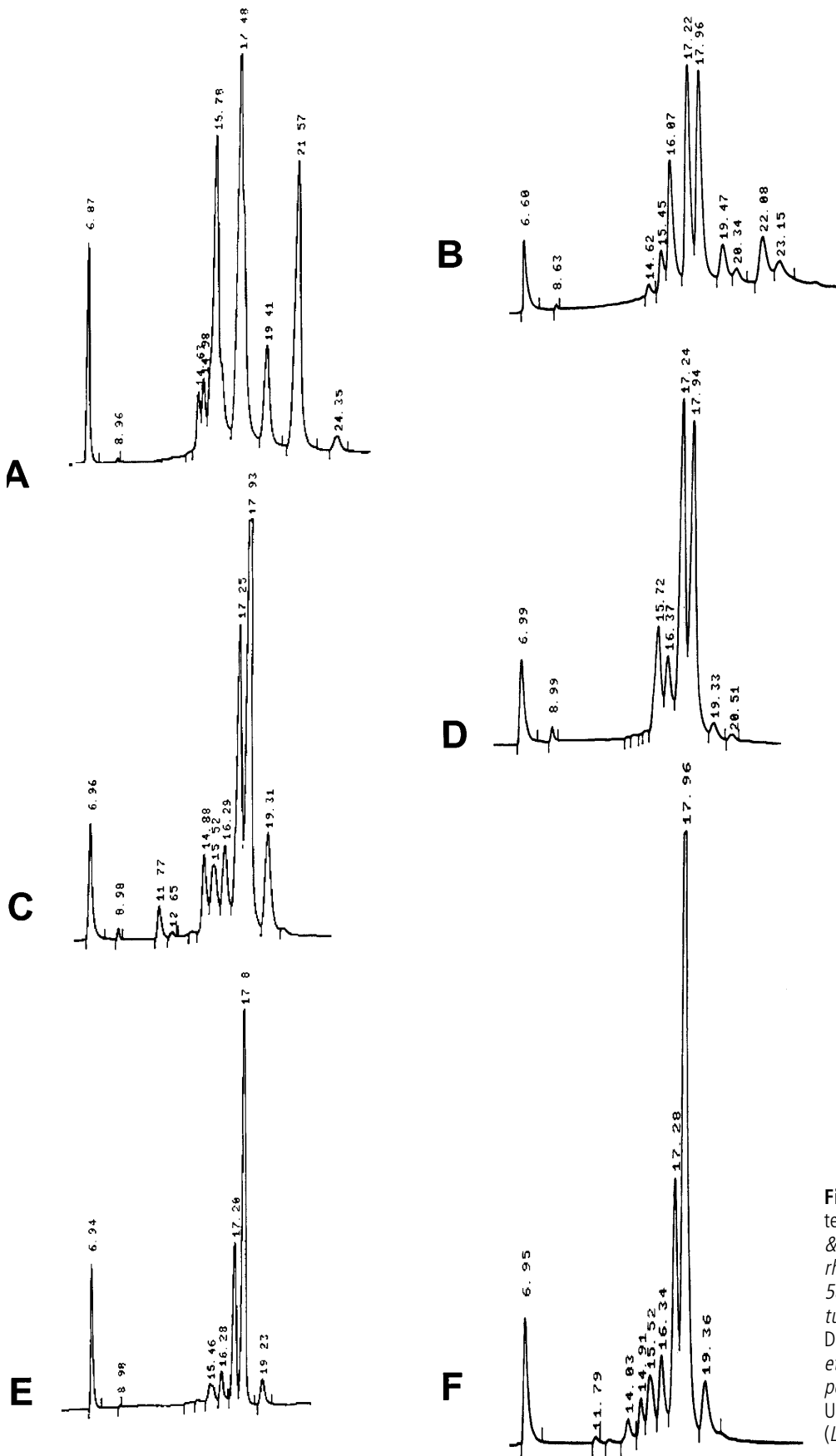
*rium* and *C. perrieri*, which both show three peaks of approx. the same size at 14.8 min, 15.5 min and 16.3 min (Fig. 10F). *Cynanchum crassipedicellatum* with an exceptionally large peak at 15.3 min and 19.3 min, *Folotisia madagascariensis* with two late peaks, and *Karimbolea verrucosa* with an exceptionally large peak at 19.3 min also differ slightly. All *Sarcostemma* species – from Madagascar, mainland Africa or even India and Thailand – are remarkably uniform, except for *S. vanlessenii* for which the peak at 15.5 min is absent and the one at 19.3 min exceptionally large. Of the five leafy Malagasy species studied, *C. pachycladon* (Fig. 10D) shows the same peaks, but at different ratios, whereas *C. papillatum* (Fig. 10C) displays a different pattern (Table 4). The two African species, *C. africanum* and *Pentarrhinum insipidum* (Fig. 10B), show triterpenoid patterns different from each other and from the Malagasy species.

### Discussion

The present results make a consistent treatment of *Cynanchum* L. difficult. The two extreme alternatives supported by the data and cladistic theory are either to recognize each clade as an independent genus, or to unite all clades to a very broadly circumscribed genus *Cynanchum*. As the first option would result in many small, morphologically poorly discernible genera, the second alternative is more practicable. On the other hand, this would mean that well established, easily recognizable



**Fig. 9.** Stem anatomy. A. *Cynanchum pycnoneuroides*, cross-section of succulent stem of the only leafy species in the succulent clade. B. *Funastrum clausum*, palisade parenchyma. Abbreviations as in Fig. 8; P: palisade parenchyma.



**Fig. 10.** Latex triterpenoid patterns. A. *Funastrum clausum* (Liede & Conrad 2599, MO). B. *Pentarrhinum insipidum* (Meve & Liede 584, MO). C. *Cynanchum papillatum* (Liede et al. 2622, MO). D. *Cynanchum pachycladon* (Liede et al. 2663, MO). E. *Sarcostemma pearsonii* (Liede & Hammer 2520, UBT). F. *Cynanchum arenarium* (Liede et al. 2739, MO).

genera would have to be abandoned. Thus, the genera *Glossonema*, *Odontanthera*, *Pentarrhinum*, *Metaplexis*, *Metalepis*, *Folotsia*, *Karimbolea*, *Platykeleba* and *Sarcostemma* are all candidates for inclusion in a large *Cynanchum*. Some genera not analyzed here due to lack of material, e.g. the Asian *Adelostemma* Hook. f., might be likely candidates as well. However, especially for the Asian taxa and the American *Metalepis*, more species would need to be analyzed before such far-reaching conclusions can be drawn.

Our results show that all leafless stem-succulent genera (*Folotsia*, *Karimbolea*, *Platykeleba* and *Sarcostemma*) are monophyletic with stem-succulent *Cynanchum*, and that this group is derived from the likewise monophyletic Malagasy subgroup of *Cynanchum*. Evidence comes from both a chloroplast and a nuclear molecular marker, as well as from stem anatomy and triterpenoid analysis. Therefore, these genera are included in *Cynanchum* here. While the former three genera are small and endemic to Madagascar, *Sarcostemma* comprises about 20 species even after the transfer of all American members to *Funastrum*, *Philibertia* Kunth and *Tetraphysa* Schltr. (Liede 1996b; Liede & Täuber 2000). The necessary name changes on species level have already been made for the Malagasy representatives (Liede & Meve 2001), and will be made for African and Australasian *Sarcostemma* following species-level revision of the group (Liede et al., in prep.). The hypothesis of Liede (1997a) and Meve & Liede (2002), that the colonization of mainland Africa by *Cynanchum gerrardii* and of the whole Old World Tropics by *Sarcostemma* originated in Madagascar, is supported independently by the cpDNA and the rDNA data. Both *C. gerrardii* and *Sarcostemma* are members of the succulent clade, which is nested in the well-supported clade of otherwise exclusively Malagasy species. The identity of all partial sequences for the African and Malagasy accessions of *C. gerrardii* points to a fairly recent event. In *Sarcostemma*, the identity of cpDNA sequences between *S. viminale* and the morphologically most distinctive *S. pearsonii*, along with only three base changes between the two species for *ITS*, also points to recent radiation and speciation of *Sarcostemma* on the African mainland. Preliminary RAPD data (Liede et al., unpubl.) agree with this conclusion. The short distances between the Malagasy species, in particular the stem-succulent ones, compared to the African species in both datasets (Fig. 4) also allows the speculation that the radiation of the Malagasy species is a fairly recent event, because it is unlikely that both *ITS* and cpDNA should undergo a parallel slowdown in modification rate in Madagascar.

The phylogeny resulting from the molecular data explains both the triterpenoid and the stem anatomical data. Both studies were begun in the early 1990s to clarify the phylogeny of stem-succulent Malagasy *Cynanchum* rela-

tives, and were abandoned because of the then inexplicable similarity between then well-established genera on the one hand and the differences between species then believed to belong to one genus on the other hand (*Sarcostemma* sensu Holm; Liede 1996b, Liede & Täuber 2000). In the triterpenoid dataset, *C. arenarium* and *C. perrieri* share an apomorphic pattern (Fig. 10F), while all other stem-succulent species studied show slight variations of the basic pattern (Fig. 10E). In the anatomical dataset, the four basic patterns described by Puech (1912) can be retrieved: the basic pattern without any noticeable specialisations (e.g., *C. rauhianum*, Fig. 8A); the “*Sarcostemma*” pattern with single sclerenchymatic fibres dispersed in the parenchyma of the cortex (Fig. 8C, D); the *C. gerrardii* pattern with sclerenchymatic fibres in the pith; and, as a specialisation of the preceding one, the *C. messeri*–*C. mahafalense* pattern with the pith consisting almost exclusively of lignified cells and sclerenchymatic fibres (Fig. 8B). However, only the *C. messeri*–*C. mahafalense* pattern coincides with a clade retrieved by molecular analysis. The two Malagasy leafy stem-succulent species share the basic anatomical pattern (Fig. 9A). The large-leaved *C. pachycladon* is basal to the stem-succulent clade following *ITS* and combined data (Figs 2–4), whereas the linear-leaved *C. pycnoneurooides* is an undisputed member of the stem-succulent clade, so that its leaves must be understood as a secondary development.

The leafy non-succulent Malagasy species fall in two unsupported clades. Well supported is the subclade *C. erythranthum*/*C. sessiliflorum*/*C. papillatum*/*C. cucullatum*. In this clade, the former three species have reddish flowers in which the gynostegium is entirely enclosed by the corolla throughout anthesis. *C. sessiliflorum* (and *C. junciforme*, which was not available for analysis) had been described under a different genus, *Pycnoneurum*, by Decaisne (1838), but were transferred to *Cynanchum* by Liede (1993a). The high support of the sister species pairs *C. comorense*/*C. danguyanum* and *C. obovatum*/*C. repandum* is reflected in the morphological similarity of these species. It is surprising, though, that each of the two clades of leafy Malagasy species harbours a subclade with linear-leafed, tuberous species (*C. angavokeyliense*, *C. lineare*, *C. moramangense*, and *C. sessiliflorum*, *C. cucullatum*, *C. papillatum*, respectively). Therefore, this conspicuous habit must have evolved more than once in Madagascar.

The present results indicate that the small Afro-Arabian genera *Pentarrhinum*, *Glossonema* and *Odontanthera* are monophyletic, and together form a subclade within *Cynanchum*, as Liede et al. (2002) have demonstrated. This clade is characterized by thick-walled, ornamented fruits. However, as no further morphological or chemical evidence could be found, name changes are left to a species-level revision of the group.

The Asian members of subgen. *Rhodostegiella* (*C. auriculatum* and *C. thesioides*) form a clade together with *Metaplexis*, indicating that *Metaplexis* should be included in *Cynanchum* as well. Again, evidence is not considered sufficient to execute the necessary name changes. Affinities of subgen. *Rhodostegiella*, the remaining Asian species (e.g. *C. ovalifolium*) and the seven true Australian *Cynanchum* species (Forster 1991, Liede 1996a), of which only *C. floribundum* was available for the present study, need to be studied further as the *ITS* data (Fig. 2) indicate that these species might form a monophyletic subclade of *Cynanchum*.

Likewise, *Metalepis albiflora* is member of the American subgen. *Mellichampia* following both molecular datasets. Again, only one species of the genus could be analyzed and there is no additional chemical or morphological evidence, so that no name changes are made here. The distances between the American species are as long as between the members of the *C. acutum* group and the *Pentarrhinum* group (Fig. 4), and hint at an old event.

The exclusion of the Somalian *C. galgalense* from *Cynanchum* by both molecular datasets (Figs 1–4) is surprising considering its morphological characters (Liede 1993b). The only character unusual for *Cynanchum* concerns its long, bostrychoid, persistent inflorescences. Unfortunately, its latex color is still unknown. The highly fused corona of *C. galgalense* does not indicate a relationship to *C. obtusifolium* or another member of the *C. africanum* clade as suggested by Liede (1993b), but must be interpreted as parallelism. An attempt to align the cpDNA sequences of the two *C. galgalense* accessions to other published datasets (Liede 2001; Liede & Täuber in press) showed that the species occupies an isolated position in the Asclepiadoideae and is not a member of any of the circumscribed subtribes (Liede, unpubl.).

The African *Cynanchum* species fall into three clades. The problem that a morphological synapomorphy, or at least a unique character combination, could not be found even for well-supported clades is a frequent phenomenon in Asclepiadoideae (e.g., Liede 2001). In this subfamily, reticulate evolution and parallelisms are the normal condition and not the exception, both within and between genera (Liede & Täuber 2000; Meve & Liede 2001). The basal, predominantly East African/Somalian *C. clavidens*-clade is well supported. Morphologically, it consists of two subclades. The first comprises the rhizomataceous *C. orangeanum* and *C. praecox*, with linear and elliptical leaves, respectively. In the second subclade, leaves are triangular to hastate in outline. The clade is characterized by leaf bases that are never deeply cordate, and by a corona that is fused for about half of its length and possesses pronounced staminal and interstaminal

lobes. The following, mostly southern African *C. africanum*-clade (*C. altiscandens* and *C. rungeweense* are East African species) is moderately supported, at least for the core species. Morphologically it is characterized by at the most shallowly cordate leaf bases and a highly fused corona (with the exception of *C. meyeri*, a shrubby Namibian endemic). The third clade, the *C. adalinae*-clade, comprises the large African twiners with normally deeply cordate leaf bases. However, *C. falcatum* and *C. schistoglossum* have much less pronouncedly cordate leaf bases. This clade is split into a West and an East African subclade (Fig. 3). Most members of this clade are unresolved in the cpDNA analysis, and only the *C. longipes* – *C. adalinae* sister species relationship is well supported. Corona shape is highly diverse in this clade, normally the staminal lobes are longer than the interstaminal ones, and ligules are present in some species (*C. abyssinicum*). This clade combines with the Asian, the American and the *Pentarrhinum*-clade to form a subclade of *Cynanchum* in sister-group position to the Malagasy subclade (Fig. 3). All these clades are constant and some even well supported, but basal resolution is unsupported. This pattern of speciation, in which several clades can be distinguished but no or unsupported basal resolution is found between them, seems to be rather frequent in Asclepiadoideae, as a similar situation has been encountered in *Tylophora* (Liede et al. in press) and in the stapeliads (Meve & Liede in press). One might speculate that a rather old group of taxa has undergone geographic isolation and is now evolving in different parts of the world at different speeds and reacting to different selection pressures, while at the same time hardly changed members of the genus are still extant in Africa, the center of origin of Asclepiadoideae (Kunze et al. 1994).

## Taxonomic treatment

### *Cynanchum* L.

*Cynanchum* L., Sp. Pl.: 212. 1 May 1753. – Type: *C. acutum* L.

≡ *Sarmasikia* Bubani, Fl. Pyren. 1: 550. 1897, nom. illeg.

= *Bunburia* Harv. Gen. S. Afr. Pl., ed. I: 416. Jul–Dec 1838. – Type: *B. elliptica* Harv.

= *Colostephanus* Harv., Gen. S. Afr. Pl., ed. I: 417, in nota. 1838. – Type: *C. capensis* Harv.

= *Cyathella* Decne. in Ann. Sci. Nat. Bot., sér. 2, 9: 332. Jun 1838. ≡ *Cynoctonum* E. Mey., Comm. Pl. Afr. Austr.: 215. 1–8 Jan 1838 [non *Cynoctonum* J. F. Gmel., Syst. Nat. 2: 306, 443. Sep(sero)-Nov 1791. (Loganiaceae)]. – Type: non designatus.

- = *Decanema* Decne. in Ann. Sci. Nat. Bot., sér. 2,9: 338. Jun 1838. – Type: *D. bojerianum* Decne.
- = *Decanemopsis* Costantin & Gall. in Bull. Mus. Hist. Nat. (Paris) 12: 418. 1906. – Type: *D. aphylla* Costantin & Gall. **Syn. nov.**
- = *Diploglossum* Meisn., Pl. Vasc. Gen. 1: 269; 2:176. 5–11 Apr 1840. – Type: non designatus.
- = *Drepanostemma* Jum. & H. Perrier in Rev. Gén. Bot. 23: 256. 1911. – Type: *D. luteum* Jum. & H. Perrier. **Syn. nov.**
- = *Flanagania* Schltr. in Bot. Jahrb. Syst. 18, Beibl. 45: 10. 22 Jun 1894. – Type: *F. orangeana* Schltr.
- = *Folotsia* Costantin & Bois in Compt. Rend. Hebd. Séances Acad. Sci. 147: 258. 1908. – Type: *F. sarcostemmatoides* Costantin & Bois. **Syn. nov.**
- = *Karimbolea* Desc. in Cactus 15: 77. Oct-Dec 1960. – Type: *K. verrucosa* Desc. **Syn. nov.**
- = *Mahafalia* Jum. & H. Perrier in Rev. Gén. Bot. 23: 255. 1911. – Type: *M. nodosa* Jum. & H. Perrier.
- = *Mellichampia* A. Gray ex S. Watson in Proc. Amer. Acad. Arts 22: 437. 25 Jun 1887. – Type: *M. rubescens* A. Gray ex S. Watson.
- = *Monostemma* Turcz. in Bjull. Moskovsk. Obsc. Isp. Prir., Otd. Biol. 21(1): 255. 1848. – Type: non designatus. **Syn. nov.**
- = *Nematostemma* Choux in Compt. Rend. Hebd. Séances Acad. Sci. 172: 1310. 1921. – Type: *N. perrieri* Choux.
- = *Perianthostelma* Baill., Hist. Pl. 10: 247. Jul–Aug 1890. – Type: non designatus.
- = *Platykeleba* N. E. Br., Bull. Misc. Inform. 1895: 250. Oct 1895. – Type: *P. insignis* N. E. Br. **Syn. nov.**
- = *Pycnoneurum* Decne. in Ann. Sci. Nat. Bot., sér. 2,9: 340. 1838. – Lectotype: *P. junciforme* Decne.
- = *Rouliniella* Vail in Bull. Torrey Bot. Club 29: 662. 30 Dec 1902. – Lectotype: *R. corymbosa* (Decne.) Bullock (*Roulinia corymbosa* Decne.) ≡ *Roulinia* Decne. in Candolle, Prodr. 8: 516. Mar(med.) 1844 (non *Roulinia* Brongn. in Ann. Sci. Nat. Bot., sér. 2,14: 320. Nov 1840, nom. illeg., [Liliaceae]).
- = *Sarcocyphula* Harv., Thes. Cap. 2: 58. 1863. – Type: *S. gerrardii* Harv.
- = *Sarcostemma* R. Br., Prodr.: 462. 27 Mar 1810. – Lectotype: *S. viminalis* (L.) R. Br. ex R. W. Holm (*Euphorbia viminalis* L.). **Syn. nov.**
- = *Symphyglossum* Turcz. in Bull. Soc. Imp. Naturalistes Moscou 21(1): 255. 1848. – Type: *S. hastatum* (Bunge) Turcz., nom. rej. [non *Symphyglossum* Schltr. in Orchis 13: 8. 15 Feb 1919. (Orchidaceae), nom. cons.].
- = *Voharanga* Costantin & Bois in Compt. Rend. Hebd. Séances Acad. Sci. 147: 259. 1908. – Type: *V. madagascariensis* Costantin & Bois.
- = *Vohemaria* Buchenau in Abh. Naturwiss. Ver. Bremen 10: 372. 1889. – Type: *V. messeri* Buchenau.

At the species level, the necessary name changes for Malagasy species have been made in advance in order that the new names are available for the Flora of Madagascar treatment (Liede & Meve 2001). The remaining species to be renamed all belong to the former genus *Sarcostemma*, of which a RAPD study is well advanced (Liede, Gebauer & Meve, unpubl. data). To reduce name changes to a minimum, the African, Asian and Australian *Sarcostemma* species will only be renamed after completion of this study.

## Acknowledgements

Studies in the genus *Cynanchum* have been sponsored for many years by the DFG (LI 496). M. Chase, Kew, extracted an extra set of *Cynanchum* specimens. A. Nicholas, Pietermaritzburg, R. Omlor, Mainz, L. Allorge, Paris, R. Mangelsdorff, Frankfurt, P. Morat, Paris, P. Raven, St. Louis, MO, M. Teissier, Les Cèdres, and M. Thulin, Uppsala, collected fresh material for this study or made it available from collections under their care. Studies of triterpenoids were carried out in the laboratory of Prof. P. G. Mahlberg, Bloomington, IN. Dr. U. Meve, Bayreuth, offered valuable criticism on the manuscript.

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