

What is the subtribe Glossonematinae (Apocynaceae: Asclepiadoideae)? A phylogenetic study based on cpDNA spacer

SIGRID LIEDE* FLS, ULRICH MEVE and ANGELIKA TÄUBER

Lehrstuhl f. Pflanzensystematik, Universität Bayreuth, Bayreuth, Germany

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Evidence from the chloroplast *trnT-L* spacer, *trnL* intron and *trnL-F* spacer shows the subtribe Glossonematinae of the tribe Asclepiadeae, hitherto composed of the Arabian and North African genera *Glossonema*, *Odontanthera* and *Solenostemma*, not to be monophyletic. While the affinities of *Solenostemma* cannot be determined with certainty at present, molecular, karyological and morphological evidence suggests that *Glossonema* and *Odontanthera* are closely allied to *Pentarrhinum*, an African genus of five species, belonging to the Cynanchinae. © 2002 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2002, 139, 145–158.

ADDITIONAL KEYWORDS: Asclepiadeae – classification – DNA-sequences – karyology – morphology – *trnT-L* spacer – *trnL* intron – *trnL-F* spacer.

INTRODUCTION

The Asclepiadeae, characterized by hanging pollinia, constitute a large (c. 100 genera), well-defined monophyletic tribe that has held up in recent molecular analyses (Sennblad, 1997; Civeyrel *et al.*, 1998; Sennblad & Bremer, 2000). Schumann (1895) circumscribed five subtribes in the Asclepiadeae, solely based on corona characters. In his system (Schumann, 1895), the subtribe Glossonematinae was defined by the presence of a corolline corona and accommodated 16 genera with a wide geographical range. However, a corona attached to the corolla is generally thought to be a plesiomorphic character in the Asclepiadeae (Liede & Kunze, 1993), unless a secondarily corolline position of the corona can be argued for. One such example can be found in *Metastelma* (e.g. *M. parviflorum* (Sw.) R. Br. ex Schult.), a genus of the Metastelminae in which the corona is normally gynostegial. True to his philosophy, Schumann (1895) placed *Irmischia*, a segregate genus for *Metastelma* species with a corolline corona, in the Glossonematinae, while he placed *Metastelma* in the Asclepiadinae. To remedy such apparent incongruencies without abolishing the

subtribal category altogether, Liede (1997a) attempted to define natural subtribes for the tribe Asclepiadeae based on morphological characters. Of the 16 genera of Schumann's (1895) Glossonematinae (Table 1), two are not even members of the tribe Asclepiadeae. *Conomitra* was recognized as a member of the tribe Ceropegieae (Field, 1982), and *Sacleuxia* as a Periplocoideae (Liede & Albers, 1994). Of the 14 remaining genera, Liede (1997a) transferred one (*Parapodium*) to Asclepiadinae, a position supported both by its erect habit and corona structure (Kunze, 1997). Two genera (*Araujia*, *Turrigera*) were transferred to Oxypetalinae (Liede, 1997a) because of their long corolla tube combined with a pronounced stylar head. Three genera (*Fischeria*, *Macroscopis*, *Schubertia*) were transferred to Gonolobinae (Liede, 1997a) because they possess the crested pollinarium and gynostegial structure typical for this subtribe (see Kunze, 1995). Four genera, the palaeotropical *Oxystelma* and the neotropical *Irmischia*, *Philibertia* and *Rhyssostelma*, were transferred to Metastelminae (Liede, 1997a). Both *Philibertia* and *Oxystelma* were included in *Sarcostemma* R. Br. by Holm (1950) because of their almost identical corona structure, but recent morphological, karyological and molecular analysis (Liede, 1996c; Liede & Täuber, 2000) has shown that *Oxys-*

*Corresponding author. E-mail: sigrid.liede@uni-bayreuth.de

Table 1. Glossonematinae *sensu* Schumann (1895)

Genus	Currently accepted name	Tribal and subtribal affiliation ¹
<i>Araujia</i> Brot. ²		Asclepiadeae – Oxypetalinae
<i>Conomitra</i> Fenzl		Ceropegieae
<i>Fischeria</i> DC.		Asclepiadeae – Gonolobinae
<i>Glossonema</i> Decne. ²		Asclepiadeae – Glossonematinae
<i>Irmischia</i> Schldt. ²	<i>Metastelma</i> R. Br.	Asclepiadeae – Metastelminae
<i>Macroscepis</i> Kunth		Asclepiadeae – Gonolobinae
<i>Oxystelma</i> R.Br. ²		Asclepiadeae – unclassified as to subtribe
<i>Parapodium</i> E. Mey.		Asclepiadeae – Asclepiadinae
<i>Philibertia</i> Kunth ²		Asclepiadeae – Metastelminae
<i>Prosopostelma</i> Baill.		Asclepiadeae ³
<i>Rhyssostelma</i> Decne.		Asclepiadeae – Metastelminae
<i>Sacleuxia</i> Baill.		Periplocoideae
<i>Schubertia</i> Mart. ²		Asclepiadeae – Gonolobinae
<i>Solenostemma</i> Hayne ²		Asclepiadeae – Glossonematinae
<i>Steinheilii</i> Decne. ²	<i>Odontanthera</i> Wight	Asclepiadeae – Glossonematinae
<i>Turrigera</i> Decne.	<i>Tweedia</i> Hook. & Arn.	Asclepiadeae – Oxypetalinae

¹Based on Liede & Albers, 1994; Liede, 1997a, 2001; and Liede & Täuber, 2002.

²Genera included in this study.

³Baillon (1890) neither indicated a species nor a specimen for *Prosopostelma*, thus making the name a *nomen invalidum*. In the Baillon herbarium in P a specimen annotated '*Prosopostelma angolense* Welw.' (a species never published) in Baillon's handwriting is extant, representing a species of *Tylophora* R. Br. (Asclepiadeae – Tylophorinae). The later application of the name to the succulent Madagascan *Cynanchum* – relative *Folotsia* Costantin & Bois (Asclepiadeae: Cynanchinae) by Jumelle & H. Perrier (Rev. gén. Bot. 23: 256 (1911) et Compte Rend. Hebd. Séances Acad. Sci. 152: 1017 (1911) for *Folotsia madagascariense* (Jum. & H. Perrier) Jum. & H. Perrier clearly represents a misinterpretation of Baillon's (1890) description.

telma occupies an isolated position in Old World Asclepiadeae and *Philibertia* is more closely related to other New World genera than to either *Sarcostemma* or *Oxystelma*. One genus (*Prosopostelma*) is a *nomen invalidum* (see Table 1 for authors and current placement of genera).

Only three genera, *Glossonema* (five species, one with two subspecies, according to Bullock, 1955; Raimondo & Fici, 1993), and the monotypic genera *Odontanthera* and *Solenostemma*, remained in the smallest subtribe Glossonematinae. All three genera occur in Arabia, with *Glossonema* extending as far as Pakistan and northern Kenya. *Glossonema* is the only genus of Asclepiadoideae for which andromonoecy has been reported (Ali & Ali, 1996). *Solenostemma* is of potential medicinal value (Tharib, El-Migirab & Veitch, 1986). As a possible synapomorphy for the Glossonematinae, Liede (1997a) suggested the plump, often mottled fruits, which can be arranged in a morphological series from smooth (*Solenostemma*) to tuberculate (*Odontanthera*) to possessing soft spines

(*Glossonema*). Bruyns (1999) has argued that mottled fruits cannot constitute a synapomorphy because they are frequent in the Ceropegieae; however, they are exceedingly rare in Asclepiadeae and thus might constitute a synapomorphy within this tribe.

The present paper continues the effort started by Liede (1999, 2001) to examine the monophyly of the six subtribes suggested by Liede (1997a) based on morphological considerations. In this study, the smallest subtribe, Glossonematinae, is studied in detail, integrating all available morphological and karyological information. An independent molecular dataset not only provides a means to test the monophyly of the subtribe, but also to assess the value of specific morphological (corona, fruits and seeds) and karyological characters as synapomorphies. CpDNA spacers (*trnT-L* spacer, the *trnL* intron and *trnL-F* spacer, Taberlet *et al.*, 1991) have been used widely to clarify phylogenies at the subfamilial and subgeneric level, while resolution is often too low for species level problems (for literature and discussion, see Gielly & Taberlet, 1996).

In the Asclepiadoideae this region has been used successfully to address phylogenetic problems at the generic and (sub-) tribal level as well (e.g. Liede & Täuber, 2000; Meve & Liede, 2001).

MATERIAL AND METHODS

DNA SEQUENCE ANALYSIS

Taxa

Material was available for analysis of all three genera of Glossonematinae *sensu* Liede (1997a) (for authors of species and vouchers, see Table 2). Of the five *Glossonema* species, material suitable for sequencing was available only for the widespread *G. boveanum* (ssp. *boveanum*). However, these five species are very similar in the morphological structure of all organs, none is considered of doubtful generic affiliation and none is geographically isolated. Species delimitation is based on quantitative characters (such as floral size or number of flowers per inflorescence, Raimondo & Fici, 1993) or the shape of corona lobes and leaves (Bullock, 1955). Therefore, in a study focusing on generic relationships, one representative species can be considered sufficient.

Secamone R. Br. of the Secamoneae was chosen as a most distant outgroup, and *Gymnema* R. Br. as representative of the Marsdenieae and *Ceropegia* L. as a member of the Ceropegieae. In the Asclepiadeae, representative members of all subtribes were selected. As far as material availability would permit, representatives of genera placed in the Glossonematinae by Schumann (1895), but attributed to other subtribes by Liede (1997a), were selected, resulting in a total of eight of the original 16 genera. *Calotropis* R. Br. and *Pergularia* L. were selected as representatives for the Asclepiadinae, *Astephanus* R. Br. and *Microloma* R. Br. for the Astephaninae *s.str.*, *Schubertia* for the Gonolobinae, *Araujia* for the Oxypetalinae, *Tylophora* R. Br. and *Pentatropis* R. Br. for the Tylophorinae. For the subtribe Metastelminae, four representatives were selected, one species each of *Blepharodon* Decne., *Funastrum* E. Fourn., *Metastelma* and *Philibertia*. For the Cynanchinae, which have been split from the Metastelminae recently following molecular study (Liede & Täuber, 2002), several representatives of the large genus *Cynanchum* L. were selected to cover the whole geographical range of the genus. Of section *Cynanchum*, *C. ellipticum*, an African species, *C. obovatum*, a leafy Malagasy species, and *C. compactum*, a leafless Malagasy species, were chosen. The Asian *C. auriculatum* and the widespread South American *C. montevidense* were selected to represent sect. *Rhodostegiella* (Pobed.) Tsiang and subgen. *Mellichampia* Sundell, respectively. Of the African *Pentarrhinum* E.Mey. and the Asian *Metaplexis* R. Br. one species

of each was chosen as further representatives of Cynanchinae. After an initial analysis showed close and well-supported relationships between *Pentarrhinum*, *Glossonema* and *Odontanthera*, sampling was extended so that four of the five *Pentarrhinum* species were included in the final analysis; the fifth species, *P. balense* (Liede) Liede is only known from the type specimen and could not be included. In addition, *Oxystelma*, an Asclepiadeae genus not affiliated with any of the presently recognized subtribes according to recent molecular results (formerly Metastelminae, but see Liede & Täuber, 2000), was included. Of the 29 taxa studied, 14 were sequenced for the present study, the remaining 15 were part of previous studies.

DNA extraction and PCR

DNA was isolated from fresh or dried leaf tissue according to Doyle & Doyle (1987). PCR primers and protocol for the plastid *trnT-L* and *trnL-F* spacers and the *trnL* intron followed Taberlet *et al.* (1991).

Sequences were obtained on an ABI Prism Model 310 Version 3.0 sequencer. All sequences have been deposited at EMBL (Table 2).

Data analysis

Sequences were prealigned with Perkin Elmer Sequence Navigator Version 1.0.1; the alignment was cleaned manually. The alignment [TreeBASE study accession number S704, matrix accession numbers M1125, M11226, Sanderson *et al.*, 1994) and available from the authors] comprises 29 taxa and 2153 characters [1109 sequence characters and 16 indels in the *trnT-L* spacer (primers a-b), 572 sequence characters and 7 indels in the *trnL*-intron (primers c-d), and 440 sequence characters and 9 indels in the *trnL-F* spacer (primers e-f)]; three data cells are unknown, affecting the beginning of the *trnL* intron in *Cynanchum obovatum*.

Phylogenetic analysis and tests for clade support were performed using PAUP* version 4.0 (Swofford, 1998) on a MacIntosh Powerbook G3.

In a first analysis, indels were coded as 'missing', but all sequence characters were analysed, regardless of gaps and potential ambiguities (dataset 1). In a second analysis, indels were excluded by deletion of 'missing/ambiguous' characters. In addition, the ambiguously aligned region between 692–780 bp (poly A in the *trnT-L* intron) was removed (dataset 2). Finally, potentially parsimony-informative indels were coded separately following the 'simple gap coding' method of Simmons & Ochoterena (2000) and added to dataset 2. In the ambiguous region of the *trnT-L* spacer between 692 and 780 bp no separate gap

Table 2. *Species included in molecular analysis* (tribal and subtribal affiliation of genera is based on Liede & Albers, 1994; Liede, 1997a and updated following Liede, 2001 and Liede & Täuber, 2002)

Species	Origin	Voucher	EMBL acc. number		
			<i>trnT-L</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer
OUTGROUPS					
¹ <i>Secamone alpinii</i> Schult. (Secamoneae)	South Africa	Liede 2931	AJ428828	AJ428829	AJ428830
¹ <i>Gymnema sylvestri</i> (Retz.) Schult. (Marsdeniaceae)	Cameroon	Meve 919	AJ402118	AJ402137	AJ402142
¹ <i>Ceropegia juncea</i> Roxb. (Ceropegieae)	India	Hanaček <i>et al.</i> 92	AJ428798	AJ428799	AJ428800
Asclepiadeae					
<i>Araujia hortorum</i> E. Fourn. (Oxypetalinae)	Argentina	Liede & Conrad 3007	AJ428792	AJ428793	AJ428794
<i>Astephanus triflorus</i> R.Br. (Astephaninae)	South Africa	Williams 659	AJ410187	AJ410188	AJ410189
<i>Blepharodon grandiflorum</i> Benth. (Metastelminae)	Venezuela	Liede & Meve 3318	AJ290836	AJ290837	AJ290838
¹ <i>Calotropis procera</i> (Ait.) Ait.f. (Asclepiadinae)	Gambia	Huber s.n.	AJ428795	AJ428796	AJ428797
¹ <i>Cynanchum auriculatum</i> Royle (Cynanchinae)	China	ex hort. Nanjing	AJ410196	AJ410197	AJ410198
¹ <i>Cynanchum compactum</i> Choux (Cynanchinae)	Madagascar	Barad s.n.	AJ290842	AJ290843	AJ290844
¹ <i>Cynanchum ellipticum</i> (Harv.) R.A. Dyer (Cynanchinae)	South Africa	Liede 2933	AJ290847	AJ290846	AJ290845
¹ <i>Cynanchum montevidense</i> Spreng. (Metastelminae)	Argentina	Liede & Conrad 3100	AJ290848	AJ290849	AJ290850
¹ <i>Cynanchum obovatum</i> (Decne.) Choux (Cynanchinae)	Madagascar	Mangelsdorff 14	AJ428801	AJ428802	AJ428803
¹ <i>Funarium clausum</i> (Jacq.) Schult. (Metastelminae)	Mexico	Liede & Conrad 2559	AJ290860	AJ290861	AJ290862
¹ <i>Metaplexis japonica</i> Makino (Cynanchinae)	Russia	ex BG Tartu s.n.	AJ428810	AJ428811	AJ428812

<i>Metastelma linearifolium</i> A. Rich. (Metastelminae)	Cuba	Urquiola, Zizka & Dressler 561	FR	AJ428807	AJ428808	AJ428809
¹ <i>Microloma tenuifolium</i> K.Schum. (Astphaninae)	South Africa	Albers s.n.	MSUN	AJ410220	AJ410221	AJ410222
¹ <i>Oxytelma bornouense</i> R.Br. (presently unclassified as to subtribe)	Kenya	Liede & Newton 3210	UBT	AJ290883	AJ290882	AJ290881
<i>Pentarrhinum abyssinicum</i> Decne. (Cynanchinae)	Tanzania	Biggood, Mbago & Vollesen 2240	K (MWC 8418)	AJ428816	AJ428817	AJ428818
<i>Pentarrhinum gonoloboides</i> (Schltr.) Liede (Cynanchinae)	Kenya	Liede & Newton 3157	UBT	AJ428819	AJ428820	AJ428821
¹ <i>Pentarrhinum insipidum</i> E.Mey. (Cynanchinae)	South Africa	Liede 2940	UBT	AJ410232	AJ410233	AJ410234
<i>Pentarrhinum somaliense</i> (Schltr.) Liede (Cynanchinae)	Ethiopia	Mesfin & Vollesen 4238	UPS	AJ428822	AJ428823	AJ428824
¹ <i>Pentatropis nivalis</i> (J.F. Gmel.) D.V. Field & J.R.I. Wood (Tylophorinae)	Kenya	Meve 949	B, UBT	AJ410238	AJ410239	AJ410240
¹ <i>Pergularia daemia</i> (Forssk.) Chiov. (Asclepiadinae)	Kenya	Masinde 888	in cult. Bayreuth	AJ290891	AJ290892	AJ290893
¹ <i>Philibertia gilliesii</i> Hook. & Arn. (Metastelminae)	Argentina	Liede & Conrad 3098	ULM, MSUN	AJ290897	AJ290898	AJ290899
<i>Schubertia grandiflora</i> Mart. & Zucc. (Gonolobinae)	Argentina	Liede & Conrad 3033	ULM, MSUN	AJ428825	AJ428826	AJ428827
¹ <i>Tylophora indica</i> (Burm.f) Merrill (Tylophorinae)	India	Bruyns s.n.	MSUN	AJ410262	AJ410263	AJ410264
INGROUP (Glossonematinae s. Liede, 1997a)						
¹ <i>Glossonema boveanum</i> (Decne.) Decne. ssp. <i>boveanum</i>	Kenya	Liede & Newton 3239	ULM	AJ428804	AJ428805	AJ428806
<i>Odontanthera radicans</i> (Forssk.) D.V. Field	Yemen	Müller-Hohenstein & Deil 1967	UBT	AJ428813	AJ428814	AJ428815
<i>Solenostemma oleifolium</i> (Nect.) Bullock & E.A. Bruce ex Bullock	Egypt	Aziz s.n.	MO	AJ428831	AJ428832	AJ428833

¹Species for which karyological study was carried out, though not necessarily on the same voucher specimen.

coding was performed (dataset 3). Contrary to the argument of Simmons & Ochoterena (2000), indels of only 1 bp length were not coded, if they occurred in multiple repeats of the same base as the length of these repeats has been found to vary even within the same species (S. Liede, unpubl. data).

For each dataset, 1000 replicates were carried out using a random addition sequence, TBR branch swapping, Mulpars off, steepest descent off. The resulting minimal trees were used as starting trees for a second round of heuristic search, with Mulpars set on. Bootstrap analysis (1000 replicates) was conducted under the 'full heuristic' search option; swapping algorithm was set to 'TBR' and 'MulTrees'; 'maxtrees' was set at 1000. Jackknife analysis (1000 replicates) was also performed; settings were as follows: 50% deletion, and 'Jac' resampling; the other settings were identical to the bootstrap settings. Bremer support (Bremer, 1988) was calculated using AutoDecay version 4.0 (Eriksson, 1999).

Maximum likelihood analysis was conducted under the HKY85 model (Hasegawa, Kishino & Yano, 1985) as implemented in PAUP* 4.0 (Swofford, 1998). Because the search is very time consuming, only ten replicate searches were conducted using the heuristic search algorithm as implemented in PAUP* with the random addition sequence and TBR branch swapping options invoked.

MORPHOLOGY

A total of 279 specimens of *Glossonema* (89), *Odontanthera* (12), *Solenostemma* (4), and *Pentarrhinum* (174) mainly from BM, BOL, BR, EA, FT, MO, NIBG, P, PRE, UBT, UPS, WAG, and Z (acronyms according to Holmgren, Holmgren & Barnett 1990) was examined. Corona morphology was studied from alcohol-preserved flowers from our own collections (as indicated in Table 2) or from dried flowers rehydrated over night at 60°C in a mixture of five parts water, four parts ethanol, one part glycerine and three drops of dishwashing detergent in 200 mL solution. A stereomicroscope with attached drawing tube was used to draw pollinaria and seeds.

KARYOLOGY

Glossonema boveanum, *Pentarrhinum insipidum* and 14 of the outgroup species could be studied karyologically (Table 2). Of *Glossonema boveanum* several seedlings raised from the seeds of a single fruit were investigated (Voucher: *Liede & Newton* 3239). Of *Pentarrhinum insipidum*, two individuals originating from South Africa (Vouchers: *Liede* 2940 et 2491), two from Namibia (Vouchers: *Meve* 171 et 584a) and one

from Kenya (Voucher: *Liede & Newton* 3225) were investigated. The investigations were carried out using squash preparations of adventitious root tips. The root tips were pretreated in 0.002 M hydroxyquinoline for 4 h at 20°C (Tjio & Levan, 1950), fixed in Carnoy's solution for 24 h at 20 °C and stained with carmine for 24 h at 60°C (Snow, 1963).

RESULTS

DNA SEQUENCE ANALYSIS

Parsimony analysis of all sequence characters regardless of gaps and ambiguities (dataset 1, 183 parsimony informative characters) resulted in 78 most parsimonious trees if uninformative characters are excluded and 90 trees for all characters (length = 322 steps, CI = 0.696, RI = 0.795, RC = 0.553); the strict consensus tree is shown in Figure 1A.

Parsimony analysis of dataset 2 (156 parsimony informative characters) resulted in 240 most parsimonious trees (length = 272 steps, CI = 0.688, RI = 0.797, RC = 0.55). The strict consensus tree is almost identical to the one resulting from analysis of dataset 1 (Fig. 1A), except that the weakly supported subclades of the New World clade and the basal Asclepiadeae clade collapse.

Separate coding of indels (dataset 3, 186 parsimony informative characters; indel 6 in the *trnL-F* spacer and indel 22 in the *trnL* intron are not parsimony informative) yielded 132 most parsimonious trees (length = 322 steps, CI = 0.678 RI = 0.8, RC = 0.544). The strict consensus tree again shows the same clades as in the analyses without separately coded gaps. The subclades of the New World clade weakly supported by dataset 1 are collapsed and so is the *Solenostemma* – *Oxystelma* clade, that was also only weakly supported by datasets 1 and 2. However, additional resolution is found in the *Cynanchum* clade, with the *Glossonema* – *Odontanthera* – *Pentarrhinum* clade nested in *Cynanchum*, although with weak support. *Gymnema* and *Ceropegia* also form an unsupported clade.

The maximum likelihood analysis under the HKY85 model (Hasegawa *et al.*, 1985) yields one most likely tree (– Ln likelihood = 6739.57). This tree (Fig. 2) corresponds in most respects to the strict consensus trees derived from parsimony analysis, except that *Cynanchum ellipticum* is sister to *Metaplexis* – *C. auriculatum*, and *C. montevidense* is sister to the two Malagasy *Cynanchum* species (Fig. 2).

CORONA MORPHOLOGY

Superficial examination of the shape and organization of the corona in the three Glossonematae genera

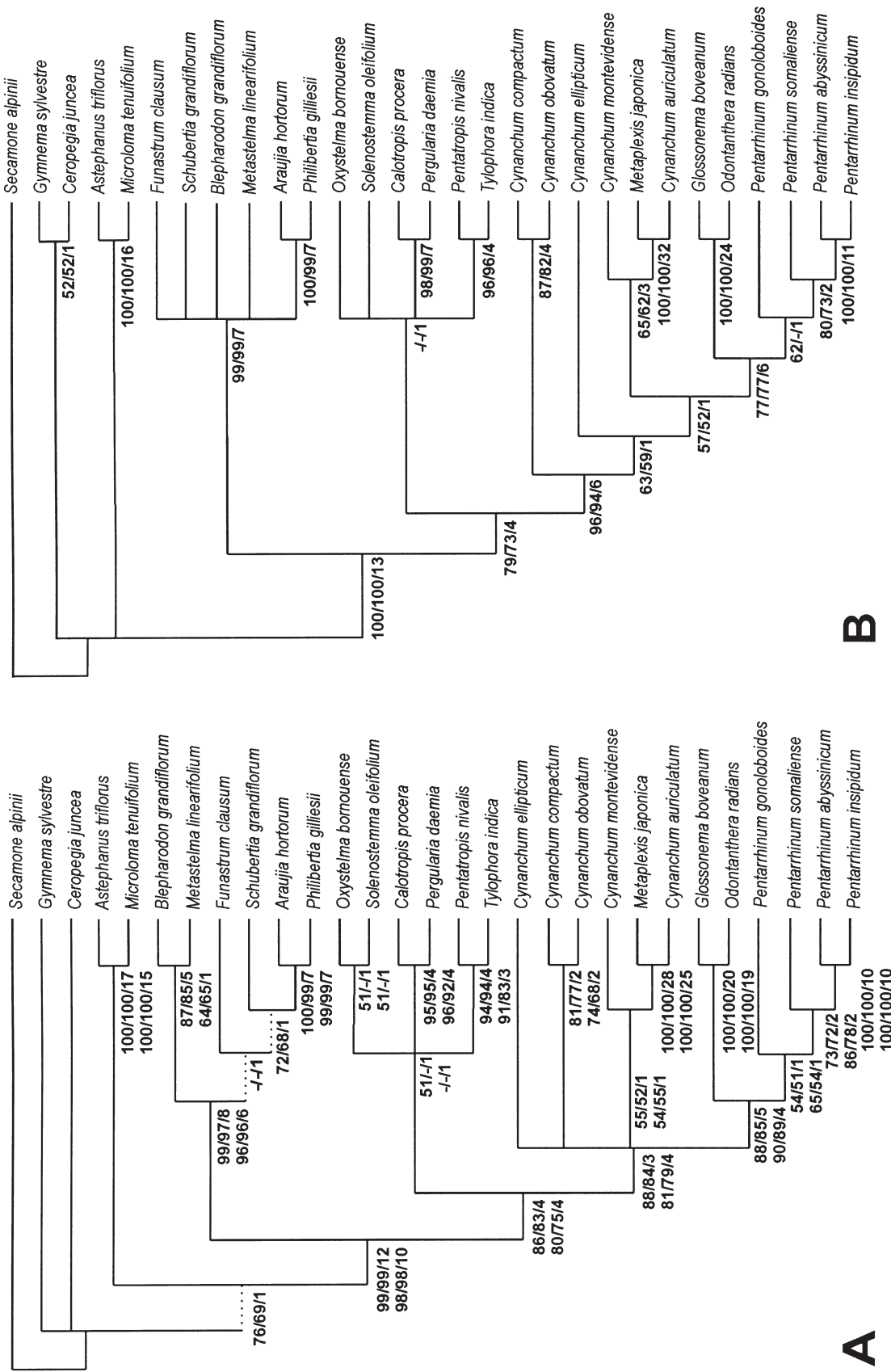


Figure 1. A. Strict consensus tree resulting from parsimony analysis of dataset 1. All sequence characters, gaps not coded separately, 183 parsimony informative characters; 78 (90) most parsimonious trees, length = 322 steps, CI = 0.696, RI = 0.795, RC = 0.553 and dataset 2 (gaps not coded separately, slightly ambiguously alignable characters 692–780 and ‘missing/ambiguous’ characters excluded; 156 parsimony informative characters; 240 most parsimonious trees, length = 272 steps, CI = 0.688, RI = 0.797, RC = 0.55). B. Strict consensus tree resulting from parsimony analysis of dataset 3 (sequence characters, characters 692–780 excluded, separately coded gaps added, 186 parsimony informative characters; 132 most parsimonious trees, length = 322 steps, CI = 0.678, RI = 0.8, RC = 0.544). Numbers below branches indicate bootstrap/jackknife values of 1000 replicates, the third figure indicates the Bremer support value. In part (A) the upper set of numbers refers to dataset 1, the lower set of numbers to dataset 2. Dotted lines indicate clades not retrieved for dataset 2.

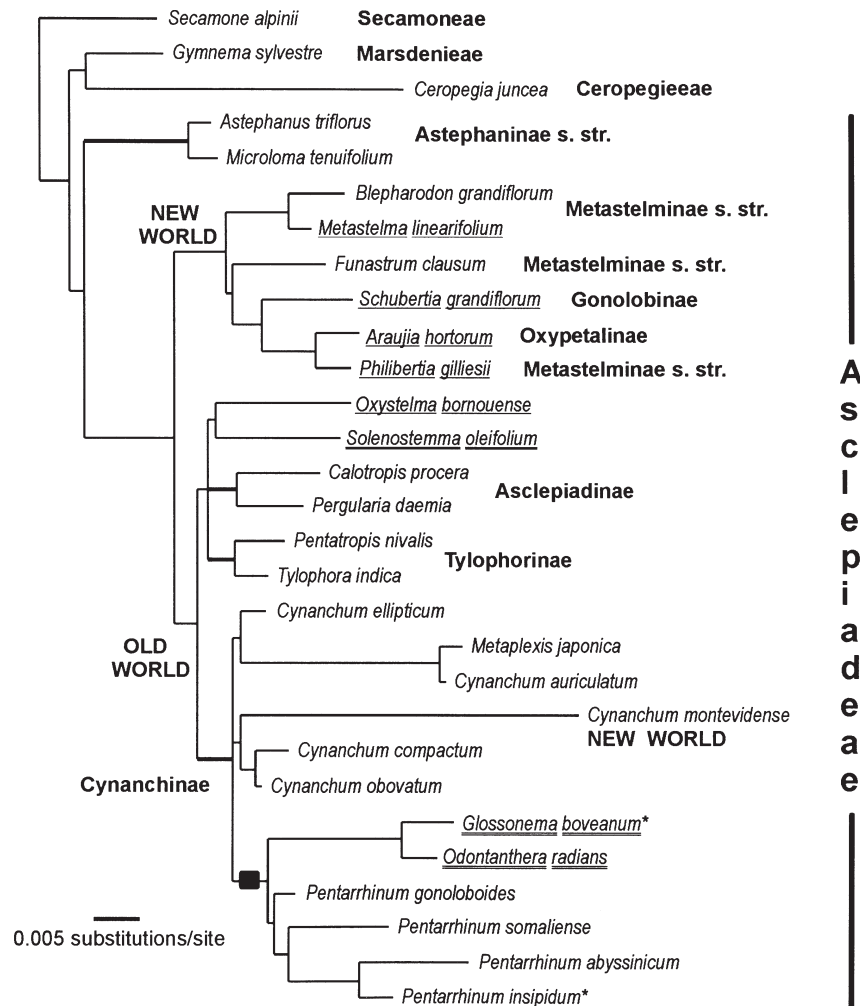


Figure 2. Maximum likelihood tree ($-\ln$ likelihood = 6739.57). Single underlines mark Glossonematinae genera *sensu* Schumann (1895), double underlines, Glossonematinae genera *sensu* Liede (1997a). Thick lines indicate monophyletic subtribes. The black square indicates the group of taxa with thick-walled, ornamented fruits and a gynostegial corona displaced toward the corolla. Asterisks denote the taxa for which a large chromosome pair has been found. Classification according to Liede & Albers (1994), Liede (1997a, 2001) and Liede & Täuber (2002) is indicated.

might lead to the conclusion that they are rather dissimilar. In *Odontanthera* the corona appears to be of true corolline origin, because the corona lobes, that are fused in a ring-like fashion, are placed on and fused with the upper parts of the corolla tube (see plate 'Odontandra reniformis' in Wight, 1838). In *Glossonema* (e.g. *G. varians*, Miller & Morris, 1988: 44, 45E), and in *Pentarrhinum gonoloboides* (Fig. 3A) the corona is basally fused with the corolla, while in *P. insipidum* the corona is completely separate from the corolla (Kunze, 1990: Fig. 110; Nicholas & Liede, 1992). However, careful dissections revealed (Kunze, unpubl.) that all coronal structures have been initiated by the gynostegium either in staminal position alone (*Glossonema*), or in both staminal and intersta-

mental position (*Odontanthera*, *Pentarrhinum*: Fig. 3A; Kunze, 1997: fig. 7) to form a gynostegial corona of staminal and interstaminal parts [*C(is)* *sensu* Liede & Kunze, 1993], the typical coronal structure of Cynanchinae. However, in all three genera the *C(is)* has undergone a centrifugal shift to fuse either with the corolla base (*G. varians*, *P. gonoloboides*), or with (almost) the basal half of the corolla tube (*Odontanthera*, see plate in Wight, 1838), so that the gynostegial origin of the corona is obscured. The lack of corolline vascular bundles in the corona lobes (Fig. 3A; Kunze, 1990: fig. 110 for *Pentarrhinum insipidum*) and the considerable distance of the corona lobes to the sinus of the corolla lobes, in which a true corolline corona originates, support a gynostegial rather than a

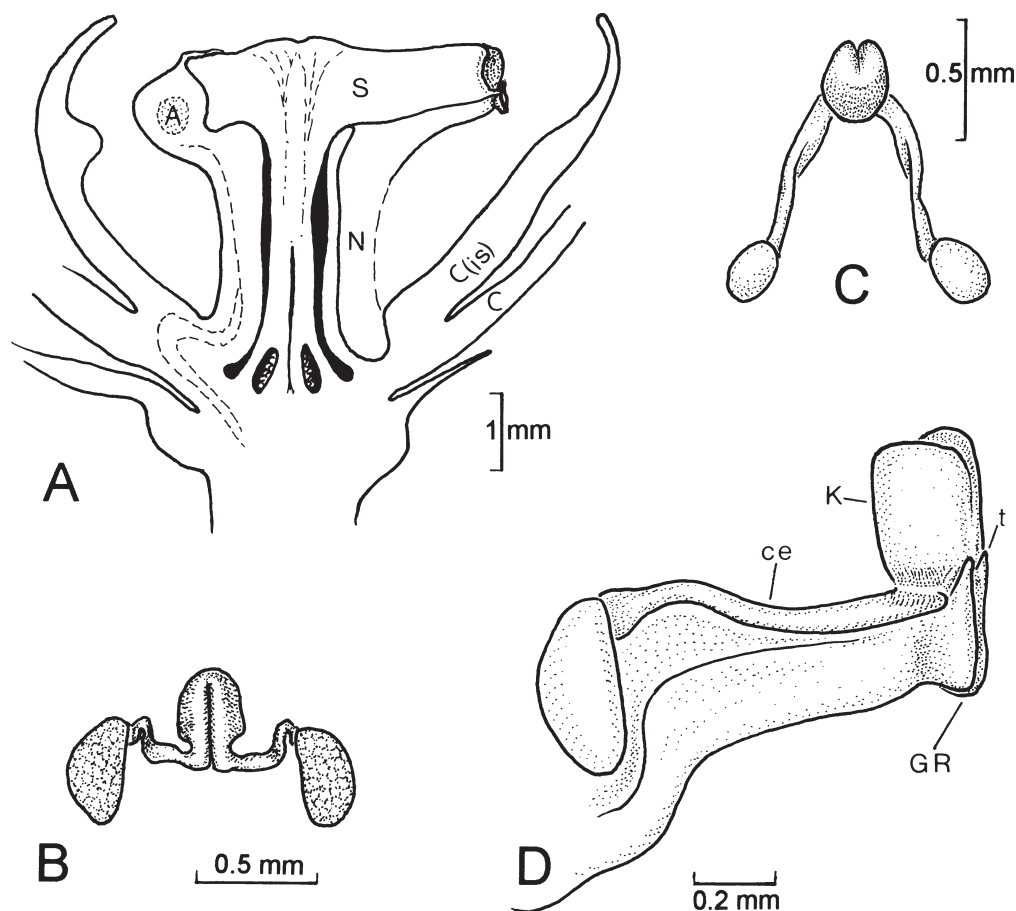


Figure 3. *Pentarrhinum gonoloboides*. A. Longitudinal section of flower. B. Pollinarium in frontal view, when attached to the stylar head. C. Pollinarium after removal from the stylar head. D. Section of gynostegium showing a guide rail with apical tooth and one pollinarium naturally attached to the stylar head. (A–D from *Liede & Newton* 3157 (UBT); A–D drawn by U. Meve, A from a sketch provided by H. Kunze. (A, anther; C, corolla; C(is), corona of fused interstaminal and staminal parts; ce, caudicle; GR, guide rail; K, corpusculum; N, nectary; S, stylar head; t, anther wing extended in a tiny tooth).

corolline origin of the corona in *Pentarrhinum*, *Glossonema* and *Odontanthera*.

The name *Odontanthera* refers to the little tooth atop each anther wing (see plate in Wight, 1838), a structure also found in *Pentarrhinum gonoloboides* (Fig. 3D), but not in any other Cynanchinae known to us. Interestingly, both *O. radians* (l.c) and *P. gonoloboides* (Fig. 3B–D) also possess very similar, quite unusual pollinaria with long, frog-leg-shaped caudicles and small pollinia that are ovate in lateral view (Fig. 3B, D) and almost spherical in apical view (Fig. 3C).

FRUIT AND SEED MORPHOLOGY

The follicles in *Glossonema* and *Odontanthera* are usually single and ovate to thick-fusiform (Figs 4, 5).

The pericarp is characteristically sculptured at least with pronounced tubercles but usually with soft spines, which can become hard and sharp during fruit ripening (e.g. *Glossonema*). The broadly ovate seeds, which are lined by a broad, more or less dentate seed wing are very similar in the two genera (Fig. 6C, D). Their surface is rather smooth, but the testa on the seed body bears papillae and short hairs. The possession of thick-walled fruits with tubercles or spines was the main reason for the transfer of three former *Cynanchum* species to *Pentarrhinum* by Liede (1997b), increasing the number of species in *Pentarrhinum* from two to five. As far as is known, all species develop single follicles, however, complete fruits consisting of two follicles can be found occasionally (see Nicholas & Liede, 1992). Except for *P. balense* (see Liede, 1996a) they all possess soft spines



Figure 4. *Glossonema boveanum*, an annual plant from northern Kenya (Liede & Newton 3239, ULM); note the widely spaced soft spines of the follicle.

Figure 5. *Pentarrhinum insipidum* (Liede 2941, UBT) during the dry season near Pretoria; note the tuberculate follicle.

or at least protuberances formed by the pericarp (Figs 4, 5, 6A). Seed morphology, too, broadly conforms to that of *Glossonema* and *Odontanthera*, but the testa can be either smooth (e.g. *Pentarrhinum insipidum*, Fig. 6D) or pubescent (e.g. *P. abyssinicum*, see Nicholas & Liede, 1992: 5b).

KARYOLOGY

The *Pentarrhinum insipidum* individuals studied were not homogeneous with regard to chromosome number; three showed $2n = 22$, while the other two, one from Kenya and one from South Africa, are (predominantly) dysploid with $2n = 24$ chromosomes. *Glossonema boveanum* showed $2n = 22$ in all prepared metaphase plates (Fig. 7). However, much more conspicuous, the genomes of the two species are not homogeneous in size. Both taxa possess one pair of exceptional large chromosomes in each chromosome set contrasting to the rest of more or less equally sized chromosomes (Fig. 7). These large chromosomes

measure *c.* $1.12\mu\text{m}$ in length, the regular chromosomes only *c.* $0.75\mu\text{m}$. Such a size anomaly of a single (or a few) chromosomes has been found in none of the other karyologically investigated species in this study (see Table 2), nor in any of the more than 600 species of Asclepiadoideae hitherto studied karyologically (see Albers & Meve, 1991, 2001).

DISCUSSION

The gene tree derived from the chloroplast *trnT-L* spacer, the *trnL* intron and the *trnL-F* spacer does not support the *Glossonematinae sensu* Liede (1997a) as a monophyletic subtribe of the Asclepiadeae. It shows that *Glossonema* and *Odontanthera* are indeed closely related, but they show strong affinities with the Cynanchinae, in particular with *Pentarrhinum* (Figs 1, 2).

Support for the close relationship of *Glossonema*, *Odontanthera* and *Pentarrhinum* is derived from morphological and karyological observations. The super-

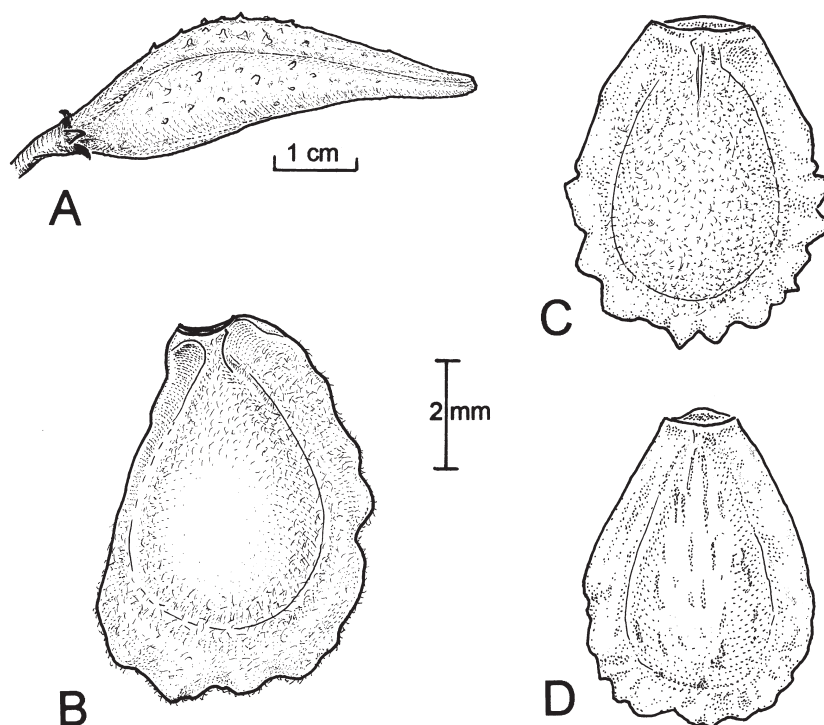


Figure 6. A. *Odontanthera radians*, follicle (drawn from a photograph in Collenette (1985: 70); B. seed, dorsal side (Turner 41, BM). C. *Glossonema boveanum*, seed, dorsal side (Liede & Newton 3239, ULM). D. *Pentarrhinum insipidum*, seed, dorsal side (Liede 3224, ULM).

ficially different corona structure of these three genera has been shown to follow the same basic pattern of organization, but with a different degree of fusion with the corolla. The 'corolline corona' defined as synapomorphy of the Glossonematinae by Schumann (1895) is in most cases a misinterpretation of a gynostegial corona displaced toward the corolla and fused with it to a variable degree. Such a displacement alone cannot be considered synapomorphic in the Asclepiadeae because it has been observed several times (e.g. *Parapodium* E. Mey., Asclepiadinae, Kunze (1997); or *Macrosepis* Kunth, Gonolobinae [U. Meve, unpubl.]).

Glossonema, *Odontanthera* and *Pentarrhinum* seem to be better characterized by thick-walled fruits with soft spines. However, in a wider Asclepiadeae context, soft-spined fruits and broadly ovate, dentate seeds are not unique, they also occur in, e.g. *Pergularia*, *Calotropis* or *Gomphocarpus* R. Br., members of the subtribe Asclepiadinae (Liede, 1996b). Fruit morphology, as well as corona morphology, thus shows a considerable degree of parallel evolution within the Asclepiadeae. Within the Cynanchinae, the combination of a gynostegial corona displaced toward the corolla and thick walled fruits characterizes the *Glossonema* – *Odontanthera* – *Pentarrhinum* clade well.

A chromosome number of $2n = 22$ is the typical condition for Asclepiadoideae and has been found in c. 96% of the over 600 species investigated (based on Albers & Meve, 2001; unpublished counts of Albers & Meve). The basic chromosome number of $x = 11$ is regarded as the primary condition in Apocynaceae as a whole (Van der Laan & Arends, 1985; Albers, Liede & Meve 1993). Decreasing basic chromosome numbers deviating from $x = 11$ occur rarely in Asclepiadoideae (e.g. $x = 10$ in *Microlooma*, *Funastrum*; Albers *et al.* 1993) and so do increasing ones (e.g. $x = 12$ in *Vincetoxicum* Wolf, *Pergularia*, *Heterostemma* Wight & Arn.; see Albers *et al.*, 1993; Albers & Meve, 2001). However, in all reported cases, including *Pentarrhinum insipidum*, the dysploid $2n = 24$ are restricted to individual plants. The occasional occurrence of the two additional chromosomes marks them as 'accessory chromosomes', so-called B-chromosomes (Rees, 1974), even though they do not show the smaller size characteristic for B-chromosomes. Meiotic studies could clarify their nature; however, such studies could not be conducted due to the lack of appropriate material (e.g. flower buds). The typical variation in B-chromosome frequency among different populations, as in *Pentarrhinum insipidum*, can be regarded as an adaptative response to varying envi-

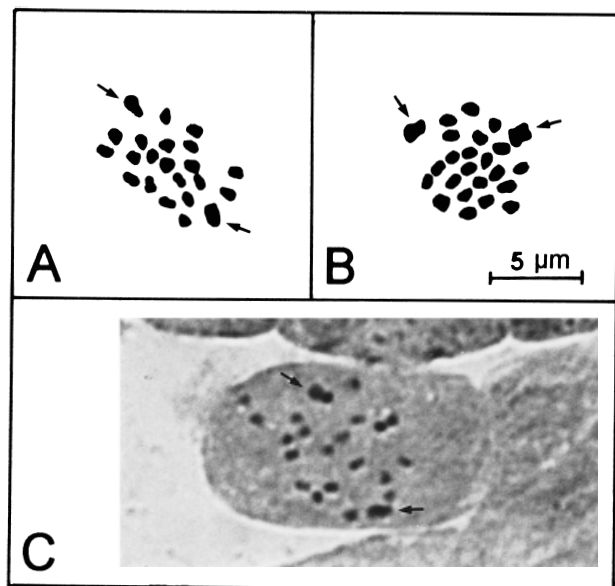


Figure 7. Mitotic metaphase plates. A. *Glossonema boveanum*, $2n = 22$ (Voucher: Liede & Newton 3239, ULM). B. *Pentarrhinum insipidum*, $2n = 24$ (Voucher for B: Liede & Newton 3225, UBT). C. *Pentarrhinum insipidum*, $2n = 22$ (Voucher: Liede 2940, UBT); photograph published in Albers *et al.* (1993; fig. 3), there erroneously identified as '*Cynanchum virens*'). Arrows mark the pair of large chromosomes.

ronmental conditions (see Rees, 1974). Therefore, the occurrence of accessory chromosomes does not represent a useful character for taxonomic considerations.

Pentarrhinum and *Glossonema* share a karyological character so far not observed in other Asclepiadoideae, namely, one pair of chromosomes surpassing all other chromosomes by almost a third of total length. Throughout the thousands of metaphase plates of six hundred different species investigated by Albers & Meve, 2001, and unpublished counts), chromosome size variation within each genome is very low (e.g. Albers & Meve, 1991). The reason for the enlargement of a pair of chromosomes in *Glossonema* and *Pentarrhinum* is unknown. Because the remaining 20 (or 22) chromosomes are fairly homogeneous in size, translocation or fusion events are unlikely, so that additional heterochromatin is most likely responsible for the chromosomal enlargement. While it would be highly desirable to test the remaining species of the *Pentarrhinum* – *Glossonema* – *Odontanthera* clade for this enlarged chromosome pair, the lack of living material or viable seeds has thus far prevented a complete study. Nevertheless, the uniqueness of this karyological peculiarity is a corroboration for a close relationship between *Pentarrhinum* and *Glossonema*.

In light of the combined results of the molecular, morphological and karyological investigations, two possibilities exist for the subtribe Glossonematinae *sensu* Liede (1997a). First, the composition of the subtribe Glossonematinae could be changed to include *Glossonema*, *Odontanthera* and *Pentarrhinum*. Second, the subtribe could be dissolved and *Glossonema* and *Odontanthera* could be included in the Cynanchinae to which they undoubtedly are closely related. For the present, the latter solution is preferred because the chromosomal abnormality needs further testing as possible synapomorphy for a subtribe.

Taking the gene tree resulting from the present analysis as the best currently available reflection of phylogenetic relationships between the genera studied, it becomes clear that *Solenostemma* belongs to none of the subtribes presently recognized in the Asclepiadoideae. The idea that it might be related to the Asclepiadoideae with which it shares the erect growth form (see Collenette, 1985) is not supported by the sequence data. The clade of *Solenostemma* and *Oxystelma*, very weakly supported by the results of parsimony analysis of the sequence data alone (Fig. 1A) is not retrieved if the indels are added (Fig. 1B). Other morphological characters do not give clues as to the affinities of *Solenostemma*; karyological data are not available at present. Again, several possibilities exist: (1) *Solenostemma* could be understood as sole member of a subtribe in its own right, leaving its affinity still unknown; (2) a subtribal concept in the Asclepiadoideae could be abolished altogether; (3) *Solenostemma* could be treated as a genus of uncertain placement in the Asclepiadoideae. While the first solution would be nomenclaturally correct, the data at hand (Liede, 1999; Liede & Täuber, 2000; Liede, 2001) show that there are several genera (e.g. *Oxystelma*) that cannot be linked at present to any larger group of genera. Thus, the number of subtribes might soon become inflationary. Conversely, abolishing subtribes altogether reduces the information on the relationships within the tribe Asclepiadoideae, because subtribes are a useful category to identify closely related groups of genera. To identify and name well-circumscribed groups of taxa and to leave the remaining ones unclassified is a practice that has been used lately in the interpretation of molecular data (e.g. Bremer, Bremer & Thulin, 1997). In the present case, a preliminarily unclassified position in the Asclepiadoideae is preferred for *Solenostemma* until all Asclepiadoideae genera have been analysed for several DNA regions so that monotypic subtribes are at least very well supported.

Therefore, the question as to the monophyly of the Glossonematinae *sensu* Liede (1997a) has to be answered in the negative. The Glossonematinae *sensu* Schumann (1895) fare even worse if his concept is

judged from the scattering of the eight genera included in this study over several clades (Fig. 2).

With regard to the other subtribes suggested by Liede (1997a, 2001; Liede & Täuber, in press), the present gene tree lends support to the suggestion of Liede (1996b), who, mainly based on chemical evidence, transferred *Pergularia* to the Asclepiadinae, in close relationship to *Calotropis* (Figs 1, 2), and also *Gomphocarpus* (not included here).

The exclusion of the predominantly Old World Cynanchinae from the Metastelminae *sensu* Liede (1997a), suggested by Liede & Täuber (2000) and executed by Liede & Täuber (2002), is well supported by the present results. However, the strongly supported New World clade comprises not only the four Metastelminae *s. str.* genera, but also *Araujia* of the Oxypetalinae and *Schubertia* of the Gonolobinae, so that Metastelminae *s. str.* might be paraphyletic without Oxypetalinae and Gonolobinae. A study exploring the relationships of these New World genera (S. Liede & D. Goyder, unpubl. data) is currently in progress.

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REFERENCES

- Albers F, Liede S, Meve U. 1993.** Deviating chromosome numbers in Asclepiadaceae. *Nordic Journal of Botany* **13**: 37–39.
- Albers F, Meve U. 1991.** Mixoploidy and cytotypes: a study of possible vegetative species differentiation in stapeliads (Asclepiadaceae). *Bothalia* **21**: 67–72.
- Albers F, Meve U. 2001.** A karyological survey of Asclepiadoideae, Periplocoideae and Secamonoideae, and evolutionary considerations in Apocynaceae *s.l.* In: Endress M, Stevens WD, eds. Proceedings of the International Botanical Congress (IBC, St Louis 1999). *Annals of the Missouri Botanical Garden* **88**: 624–656.
- Ali T, Ali SI. 1996.** Andromonoecy in *Glossonema varians* (Stocks) Hook.f. (Asclepiadaceae). *Pakistan Journal of Botany* **28**: 25–29.
- Baillon HE. 1890.** *XCII Asclépiadacées. Histoire des plantes*, Vol. 10. Paris: Hachette, 221–304.
- Bremer K. 1988.** The limits of Amino Acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**: 795–803.
- Bremer K, Bremer B, Thulin M. 1997.** *Introduction to phylogeny and systematics of flowering plants*. Uppsala: Department of Systematic Botany, Uppsala University, 1–95.
- Bruyns PV. 1999.** Subtribes and genera of Asclepiadeae: a response to Liede. *Taxon* **48**: 23–29.
- Bullock AA. 1955.** Notes on African Asclepiadaceae VII. *Kew Bulletin* **292**: 611–626.
- Civeyrel L, Le Thomas A, Ferguson K, Chase MW. 1998.** Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to molecular phylogeny obtained from plastid *matK* sequences. *Molecular Phylogenetics and Evolution* **9**: 517–527.
- Collenette S. 1985.** *Asclepiadaceae. An illustrated guide to the flowers of Saudi Arabia*. London: Scorpion, 69–74.
- Doyle JJ, Doyle JL. 1987.** A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11–15.
- Eriksson T. 1999.** AutoDecay, Version 4.0. (Program Distributed by the Author). Stockholm, Sweden: Bergius Foundation, Royal Swedish Academy of Sciences.
- Field DV. 1982.** The identity of *Odontanthera* Wight (Asclepiadaceae) with notes on *Glossonema* Decne. & *Conomitra* Fenzl. *Kew Bulletin* **37**: 341–347.
- Gielly L, Taberlet P. 1996.** A phylogeny of the European gentians inferred from chloroplast *trnL* (UAA) intron sequences. *Botanical Journal of the Linnean Society* **120**: 57–75.
- Hasegawa M, Kishino H, Yano T. 1985.** Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **21**: 160–174.
- Holm RW. 1950.** The American species of *Sarcostemma* R. Br. (Asclepiadaceae). *Annals of the Missouri Botanical Garden* **37**: 477–560.
- Holmgren PK, Holmgren NH, Barnett LC. 1990.** *Index Herbariorum. Part 1. The Herbaria of the World*. Regnum Vegetabile. New York: Botanical Garden, 120pp.
- Kunze H. 1990.** Morphology and evolution of the corona in Asclepiadaceae and related families. *Tropische und Subtropische Pflanzenwelt* **76**: 1–51.
- Kunze H. 1995.** Floral morphology of some Gonolobaeae (Asclepiadaceae). *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **117**: 211–238.
- Kunze H. 1997.** Corona and nectar system in Asclepiadinae (Asclepiadaceae). *Flora* **192**: 175–183.
- Liede S. 1996a.** A revision of *Cynanchum* (Asclepiadaceae) in Africa. *Annals of the Missouri Botanical Garden* **83**: 283–345.
- Liede S. 1996b.** On the position of the genus *Pergularia* (Asclepiadaceae). In: Van Der Maesen LJG, Van der Burgt KM, Van Medenbach de Roy JM, eds. *The biodiversity of African plants*. Proceedings Xivth AETFAT Congress, 22–27 August 1994. Wageningen, The Netherlands: Kluwer Academic Press, 481–488.
- Liede S. 1996c.** *Sarcostemma* (Asclepiadaceae) – a controversial generic circumscription reconsidered: morphological evidence. *Systematic Botany* **21**: 31–44.

- Liede S. 1997a.** Subtribes and genera of the tribe Asclepiadeae (Apocynaceae: Asclepiadoideae): a synopsis. *Taxon* **46**: 233–247.
- Liede S. 1997b.** Phylogenetic study of the African members of *Cynanchum* (Apocynaceae: Asclepiadoideae). *Systematic Botany* **22**: 347–372.
- Liede S. 1999.** New considerations on the subtribe Astephaninae Endl. (Apocynaceae: Asclepiadoideae). XVI. St Louis: International Botanical Congress (Poster-Abstract) **356**: 352.
- Liede S. 2001.** New considerations on the subtribe Astephaninae Endl. (Apocynaceae – Asclepiadoideae). In: Endress M, Stevens WD, eds. Proceedings of the International Botanical Congress (IBC, St Louis 1999). *Annals of the Missouri Botanical Garden* **88**: 657–668.
- Liede S, Albers F. 1994.** Tribal disposition of Asclepiadaceae genera. *Taxon* **43**: 201–231.
- Liede S, Kunze H. 1993.** A descriptive system for corona analysis in Asclepiadaceae and Periplocaceae. *Plant Systematics and Evolution* **185**: 275–284.
- Liede S, Täuber A. 2000.** *Sarcostemma* R. Br. (Apocynaceae: Asclepiadoideae), controversial generic circumscription reconsidered: evidence from *trnL-F* spacers. *Plant Systematics and Evolution* **225**: 133–140.
- Liede S, Täuber A. 2002.** Circumscription of the genus *Cynanchum* (Apocynaceae: Asclepiadoideae). *Systematic Botany* in press.
- Meve U, Liede S. 2001.** Reconsideration of the status of *Laurania*, *Larryleachia* and *Notechidnopsis* (Asclepiadoideae: Ceropegieae). *South African Journal of Botany* **67**: 161–168.
- Miller AG, Morris M. 1988.** Plants of Dhofar. Sultanate of Oman: Office of the Adviser for Conservation of the Environment.
- Nicholas A, Liede S. 1992.** A revision of the genus *Pentarrhinum* E. Meyer (Asclepiadaceae). *Kew Bulletin* **47**: 475–490.
- Raimondo FM, Fici S. 1993.** A new species of *Glossonema* (Asclepiadaceae) from Somalia. *Webbia* **47**: 145–148.
- Rees H. 1974.** B chromosomes. *Science Progress (Oxford)* **61**: 535–554.
- Sanderson MJ, Donoghue MJ, Piel W, Eriksson T. 1994.** TreeBASE: a prototype database of phylogenetic analyses and an interactive tool for browsing the phylogeny of life. *American Journal of Botany* **81**: 183.
- Schumann K. 1895.** *Asclepiadaceae*. In: Engler A, Prantl K, eds. *Die natürlichen Pflanzenfamilien*, Vol. 4.1. Leipzig: Engelmann, 189–305.
- Sennblad B. 1997.** *Phylogeny of the Apocynaceae s.l.* Acta Universitatis Upsaliensis, Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 295.
- Sennblad B, Bremer B. 2000.** Is there a justification for differential a priori weighting in coding sequences? A case study from *rbcL* and Apocynaceae *s.l.* *Systematic Biology* **49**: 101–113.
- Simmons MP, Ochoterena H. 2000.** Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* **49**: 369–381.
- Snow R. 1963.** Alcoholic hydrochloric acid-carmines as stain for chromosomes in squash preparations. *Stain Technology* **38**: 9–13.
- Swofford DL. 1998.** *PAUP**. *Phylogenetic Analysis Using Parsimony (*and other methods)*, version 4. Sunderland, MA: Sinauer Associates Inc.
- Taberlet P, Gielly L, Pautou G, Bouvet J. 1991.** Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105–1109.
- Tharib SM, El-Migirab S, Veitch GBA. 1986.** A preliminary investigation of the potential antimicrobial activity of *Solenostemma argel*. *International Journal of Crude Drug Research* **24**: 101–104.
- Tjio JH, Levan A. 1950.** The use of oxyquinoline in chromosome analysis. *Anales Estación Experimental Aula Dei* **2**: 21–64.
- Van der Laan FM, Arends JC. 1985.** Cytotaxonomy of the Apocynaceae. *Genetica* **68**: 3–35.
- Wight R. 1838.** On a new genus of Asclepiadeae. *Madras Journal of Literature and Science* **7**: 142–143.