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Sarcostemma R.Br. (Apocynaceae – Asclepiadoideae) – a controversial generic circumscription reconsidered: evidence from *trn*L-F spacers

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Abstract. The polyphyly of *Sarcostemma* sensu latu, suspected on morphological and karyological reasons is confirmed by analysis of the *trn*L-F spacer region. With the exception of *Cynanchum montevidense* (subgen. *Mellichampia,* Sundell 1981), Old World and New World taxa belong to different clades. *Sarcostemma* sensu stricto and *Platykeleba* show a strong affinity to Old World *Cynanchum;* the affinities of *Oxystelma* cannot be determined at present. *Philibertia* and *Blepharodon* are sister groups, both are sister to *Funastrum,* and *Pentacyphus* is basal to the New World clade.

Key words: Gentianales, Apocynaceae, Asclepiadoideae, *Blepharodon, Funastrum, Pentacyphus, Philibertia, Platykeleba, Sarcostemma,* chloroplast DNA, phylogeny, *trn*T-*trn*L spacer, *trn*L intron, *trn*L-*trn*F spacer.

The concept of *Sarcostemma* R.Br. s.l., a genus of ca. 40 species, has been subject of much debate. Holm (1950) recognized one genus, *Sarcostemma,* with three subgenera: "*Eusarcostemma*", restricted to the Old World; *Ceramanthus* Kunze, restricted to the New World; and *Oxystelma* (R.Br.) R.W. Holm, comprising species of both the Old and the New World. The more recent treatment by Bullock (1956), however, recognizes five genera in the complex and follows the suggestion of Schlechter (1914) to circumscribe the genera according to their geographical distribution patterns, thus restricting *Sarcostemma* R.Br. and *Oxystelma* R.Br. to the Old World, and *Philibertia* Kunth, *Pentacyphus* Schltr. and *Funastrum* E. Fourn. to the New World. Following the concept of Holm (1950), Descoings (1961) merged the monotypic Madagascan *Platykeleba* N.E.Br. with *Sarcostemma.* Liede (1996) has re-evaluated the concept of *Sarcostemma* R.Br. sensu Holm using morphological and karyological characters. She came to the conclusion that the enlarged circumscription of Holm (1950) and Descoings (1961) was untenable and therefore suggested to return to the concept of Bullock (1956) to recognize a total of six genera, *Oxystelma* (3–4 spp.), *Sarcostemma* s.str. (ca. 10 spp.) and *Platykeleba* (1 sp.) in the Old World, and *Funastrum* (14 spp. acc. to Holm 1950), *Pentacyphus* (3 spp.) and *Philibertia* (ca. 7 spp.) in the New World.

The morphological character analysis (Liede 1996), however, was hampered by a few difficulties. First, the circumscription of *Cynanchum* L., the most likely sister genus, is so vague and variable from author to author that in most characters almost every character state can be found in some odd species and a satisfactory coding was very difficult, even though the extremes were coded as different outgroup taxa ("CYN1" and "CYN2"). Second, chromosome numbers, which play an important role in the New World genera (Albers et al. 1993, Albers and Meve in press) were (and still are) unknown for several species. Third, homology problems occurred in particular for characters of the corona, for which homology of the outer corona ring between members of the ingroup was as much a problem as the interpretation of the inner or outer corona as homologous to the single corona of the outgroups. This study therefore uses an additional, molecular dataset to test monophyly or polyphyly of *Sarcostemma* sensu Holm. Of the markers available, the *trn*L-F chloroplast DNA region was chosen because its variability allows unambiguous alignments while producing good resolution for generic questions in Asclepiadoideae (Liede, unpubl. data).

Materials and methods

Taxa. A total of 16 ingroup species representing the entire range of *Sarcostemma* sensu Holm (1950) was included in the present analysis. (Table 1). As in Liede (1996), the generic concept of ingroup follows the subgenera and sections of Holm (1950). Some species of *Funastrum* as well as "*Philibertia wurdackii*" present in Liede (1996) have been omitted in the present analysis due to lack of material. As sequences between members of each (sub-)genus proved remarkably similar, a larger number of ingroup taxa was not included. For a complete phylogeny of each (sub-)genus, a more highly resolving marker will be necessary.

As most distant outgroup, a member of the tribe Marsdenieae, *Gymnema* R.Br., was chosen. Members of the tribe Asclepiadeae, but from different subtribes are more closely related (*Gomphocarpus* R.Br. and *Pergularia* L. as representatives of the Asclepiadinae and *Tylophora* R.Br. as representative of the Tylophorinae). As presumably more closely related outgroups, *Blepharodon* Decne. (considered as probably closest relative to *Sarcostemma* by Holm 1950) and several species of *Cynanchum* L. (considered as only slightly more distantly related to *Sarcostemma* than *Blepharodon* by Holm 1950) were chosen, representing as broad a range of the latter genus as possible. *Cynanchum montevidense* (for authors of species, see Table 1) and *C. nummulariaefolium* represent morphologically very distinct New World species and *C. ellipticum* and *C. compactum* represent leafy and leafless Old World species, respectively. One representative each of *Folotsia* Cost. & Bois and *Karimbolea* Desc., small leafless Madagascan genera with presumed relations to *Sarcostemma* (Liede et al. 1993) were also included.

DNA extraction and PCR. DNA was isolated from fresh or silica dried leaf tissue according to Doyle and Doyle (1987). PCR primers and protocol for the *trn*L-F spacers correspond to Taberlet et al. (1991).

Sequences were obtained on an ABI Prism Model 310 Version 3.0 sequencer. All sequences have been deposited at EMBL (accession numbers AJ402118, AJ402137, AJ402142 for *Gymnema sylvestre*, AJ290836 – AJ290917 for the remaining taxa).

Data analysis. Sequences were pre-aligned with Perkin Elmer Sequence Navigator Version 1.0.1; the alignment was cleaned manually. The alignment is available from the author or to be viewed on the World Wide Web (http://www.uni-bayreuth.de/departments/planta2/). For parsimony analysis, gaps were coded as "missing" and excluded by deletion of "missing/ambiguous" and "uninformative". In a second analysis, indels 3 bp and longer were coded separately as present/absent to test the influence of longer insertions/deletions, which are presumably the result of a single event in the phylogeny.

Phylogenetic analysis and tests for clade support were performed using PAUP version 4.0d65 (PPC). Bootstrap search (1000 replicates) was conducted under the "full heuristic" search option, starting trees were obtained by "stepwise addition" and "random" addition sequence with 10 replicates; swapping algorithm was set to "TBR" and "MulTrees". Jackknife resampling (1000 replicates) was set to 50% deletion, and "Jac" resampling; the other settings were identical to the bootstrap settings. For both statistical analyses, maxtrees was set to 1000. A decay analysis (Bremer 1988) has been conducted including trees four steps longer than the minimal trees.

Distances were computed using the Neighbor-joining algorithm. All sequence characters were

Table 1. Voucher and locality information for plant material used in this study

Outgroups		
Gymnema sylvestre (Retz.) Schult. (Marsdenieae)	Meve 919 (B, MSUN, UBT)	CAMEROON: Mokolo
Gomphocarpus physocarpus E. Mey. (Asclepiadeae–Asclepiadinae)	Nicholas 2829 (NH)	SOUTH AFRICA
Pergularia daemia (Forssk.) Chiov. (Asclepiadease–Asclepiadinae)	Masinde 888 (cult. Bayreuth)	TANZANIA: N Arusha
Tylophora perrottetiana Decne. (Asclepiadeae–Tylophorinae)	Liede 3252 (UBT)	PHILIPPINES: Los Baños
Blepharodon grandiflora Benth. (Asclepiadeae–Metastelmatinae)	Liede & Meve 3318 (UBT)	VENEZUELA: San Francisco
Blepharodon mucronatum Decne. (Asclepiadeae–Metastelmatinae)	Liede 3243 (UBT)	BELIZE: San Ignacio
Cynanchum compactum Choux (Asclepiadeae–Metastelmatinae)	BG Johannesburg (UBT)	MADAGASCAR
Cynanchum ellipticum R.Br. (Asclepiadeae–Metastelmatinae)	Liede 2933 (UBT)	SOUTH AFRICA: Grahamstown
Cynanchum montevidense Spreng. (Asclepiadeae–Metastelmatinae)	Liede & Conrad 3100 (ULM)	ARGENTINA: La Candelaria
Cynanchum nummulariaefolium Hook. & Arn. (Asclepiadeae–Metastelmatinae)	Liede & Conrad 3050 (MO, MSUN, ULM)	ARGENTINA: Las Leñas
Folotsia grandiflora Jum. & H. Perrier (Asclepiadeae–Metastelmatinae)	BG Munich s.n. (UBT)	MADAGASCAR
Karimbolea verrucosa Descoings (Asclepiadeae–Metastelmatinae)	Liede et al. 2826 (MSUN, UBT)	MADAGASCAR: Cap Ste Marie
Ingroups		
Funastrum arenarium (Decne. ex Benth.) Liede	Liede & Conrad 2952 (GA, ULM)	MEXICO: San Carlos
Funastrum clausum (Jacq.) Schult.	Liede & Conrad 2599 (MO, MSUN)	MEXICO: Tomellin
	Liede & Conrad 2958 (cult. Bayreuth)	MEXICO: Todos Santos
Funastrum elegans Decne.	Liede & Conrad 2605 (MO, MSUN)	MEXICO: Tehuacán
Funastrum odoratum (Hemsl.) Schltr.	Liede & Conrad 2560 (UBT)	MEXICO: Comitán de Domingues
	Liede & Conrad 2575 (cult. Bayreuth)	MEXICO: Las Margaretas
Oxystelma bornouense R.Br.	Liede & Newton 3210 (UBT)	KENYA: Sala Gate, Tsavo East
Oxystelma esculentum Wall. ex Decne.	ex Shirley s.n (cult. Bayreuth)	EGYPT: Elephantine Island
Pentacyphus lehmannii (Schltr.) Liede	Liede 3333 (cult. Bayreuth)	ECUADOR: Nudo de Sabanilla
Philibertia gilliesii Hook. & Arn.	Liede & Conrad 3054 (MO, ULM)	ARGENTINA: La Crucecita
	Liede & Conrad 3098 (MO)	ARGENTINA: San Pedro de Colalao
Philibertia lysimachioides (Wedd.) T. Meyer	Liede & Conrad 3139 (MSUN, ULM)	BOLIVIA: Copacabana Peninsula
Philibertia vailiae (Rusby) Liede	Liede & Conrad 3104 (K, MO, ULM)	ARGENTINA: Salta
Platykeleba insignis N. E. Br.	Rauh 68500 (HEID)	MADAGASCAR: Antananarivo
Sarcostemma pearsonii N. E. BR.	Albers, Liede & Meve 582 (K, MSUN)	SOUTH AFRICA: Upington
Sarcostemma viminale (L.) R.Br.	Albers, Liede & Meve 540 (MSUN, UBT)	ZIMBABWE: Bulawayo

analyzed using the three options under "DNA-RNA distances": "uncorrected (p)", "Jukes-Cantor" and "Kimura-2-parameter".

Results

The total matrix comprises 28 × 2053 cells, 9 of which are unknown; 28 × 2027 cells are sequence characters (1045 in the *trn*T-*trn*L spacer, 560 in the *trn*L intron and 422 in the *trn*L-*trn*F spacer), 28 × 26 cells presence/absence of gaps. Of these, 113 sequence data and the 26 gaps are parsimony-informative.

Analysis of all sequence characters yields 113 parsimony-informative characters. Branch-and-Bound search results in 32 trees (l = 345, CI = 0.8957, HI = 0.1043, RI = 0.9095, RC = 0.8146; Fig. 1).

Excluding gaps, "missing/ambiguous" characters and uninformative characters results in 96 parsimony-informative characters. Branch-and-Bound search results in 14 trees (l = 136, CI = 0.7721, HI = 0.2279, RI = 0.9096, RC = 0.7023).

Inclusion of gaps, exclusion of "missing/ambiguous" and uninformative characters results in 122 parsimony-informative characters. Branch-and-Bound search also results in 14 trees (l = 185, CI = 0.7081, HI = 0.2919, RI = 0.8811, RC = 0.6239).

The strict consensus tree of all three parsimony analyses shows an identical topology (Fig. 1). All New World taxa except *Cynanchum montevidense* form a reasonably well supported clade ("NW clade"). The taxa constituting the NW clade have all been classified as members of the subtribe Metastelmatinae by Liede (1997). In the NW clade, the sister group relationship between *Blepharodon* and *Philibertia* is supported by a bootstrap percentage of 89%, and the relationship between these two and *Funastrum* by 79%. Bootstrap percentages supporting the genera *Blepharodon* and *Philibertia* are 100%, and 95% for *Funastrum*.

The Old World taxa fall into several unresolved clades, reflecting in part the subtribal concept, proposed by Liede (1997). *Tylophora perrottetiana* is representing the Tylophorinae, *Gomphocarpus physocarpus* and *Pergularia daemia* are representing the Asclepiadinae. *Cynanchum montevidense*, *C. ellipticum* and all the leafless taxa form a well supported clade representing a part of the subtribe Metastelmatinae (Liede 1997). *Oxystelma bornouense* and *O. esculentum,* considered as members of *Sarcostemma* by Holm (1950) and considered to belong to the Metastelmatinae by Liede (1997), form a clade not joining any of the remaining ones and thus occupy at present an isolated position in the tribe Asclepiadeae.

The Neighbor-joining tree (Fig. 2) largely confirms the topology of the parsimony consensus tree independent of the algorithm chosen. Here, all Old World taxa (and *C. montevidense*) form a branch that is as well supported as the New World branch. *Oxystelma* is placed on a branch with the Asclepiadinae, though with low bootstrap support. Within the highly supported (bootstrap value of 94%) Old World Metastelmatinae branch, two features stand out. The first is the extremely long branch of *C. montevidense*, suggesting that this group of *Cynanchum* has diverged considerably since its arrival in the New World and the second is the close relationship of all leafless succulent taxa.

Discussion

These results strongly support the conclusions drawn by Liede (1996) that *Sarcostemma* sensu

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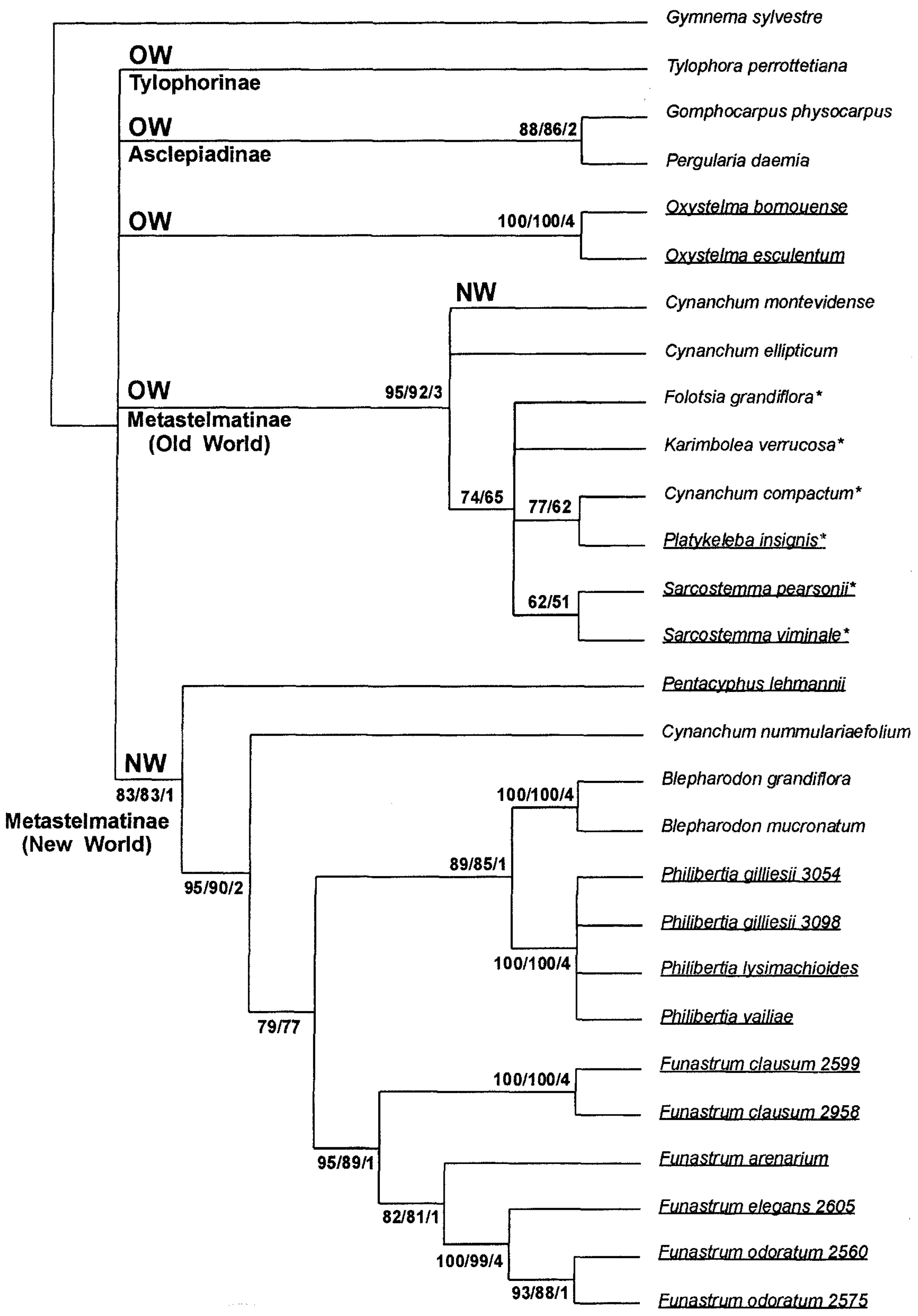
Fig. 1. Strict consensus tree resulting from Branch-and-Bound analysis of all 113 parsimony-informative sequence characters (32 trees, l = 345, CI = 0.8957, HI = 0.1043, RI = 0.9095, RC = 0.8146). Numbers indicate bootstrap percentages (1000 replicates)/jackknife percentages (1000 replicates)/Bremer support values (Bremer support values of zero are not shown). *NW* restricted to the New World, *OW* restricted to the Old World; taxa underlined have been included in *Sarcostemma* sensu Holm (1950) and Descoings (1961), taxa with an asterisk (*) are stem succulents with leaves reduced to scales


Gymnema sylvestre
OW
Tylophorinae
Tylophora perrottetiana
OW
Asclepiadinae
88/86/2
Gomphocarpus physocarpus
Pergularia daemia
OW
100/100/4
Oxystelma bomouense
Oxystelma esculentum
NW
Cynanchum montevidense
Cynanchum ellipticum
OW
Metastelmatinae
(Old World)
95/92/3
Folotsia grandiflora*
Karimbolea verrucosa*
74/65
77/62
Cynanchum compactum*
Platykeleba insignis*
62/51
Sarcostemma pearsonii*
Sarcostemma viminale*
Pentacyphus lehmannii
Cynanchum nummulariaefolium
NW
83/83/1
Metastelmatinae
(New World)
100/100/4
Blepharodon grandiflora
Blepharodon mucronatum
95/90/2
89/85/1
Philibertia gilliesii 3054
Philibertia gilliesii 3098
100/100/4
Philibertia lysimachioides
Philibertia vailiae
79/77
100/100/4
Funastrum clausum 2599
Funastrum clausum 2958
95/89/1
Funastrum arenarium
82/81/1
Funastrum elegans 2605
100/99/4
Funastrum odoratum 2560
93/88/1
Funastrum odoratum 2575

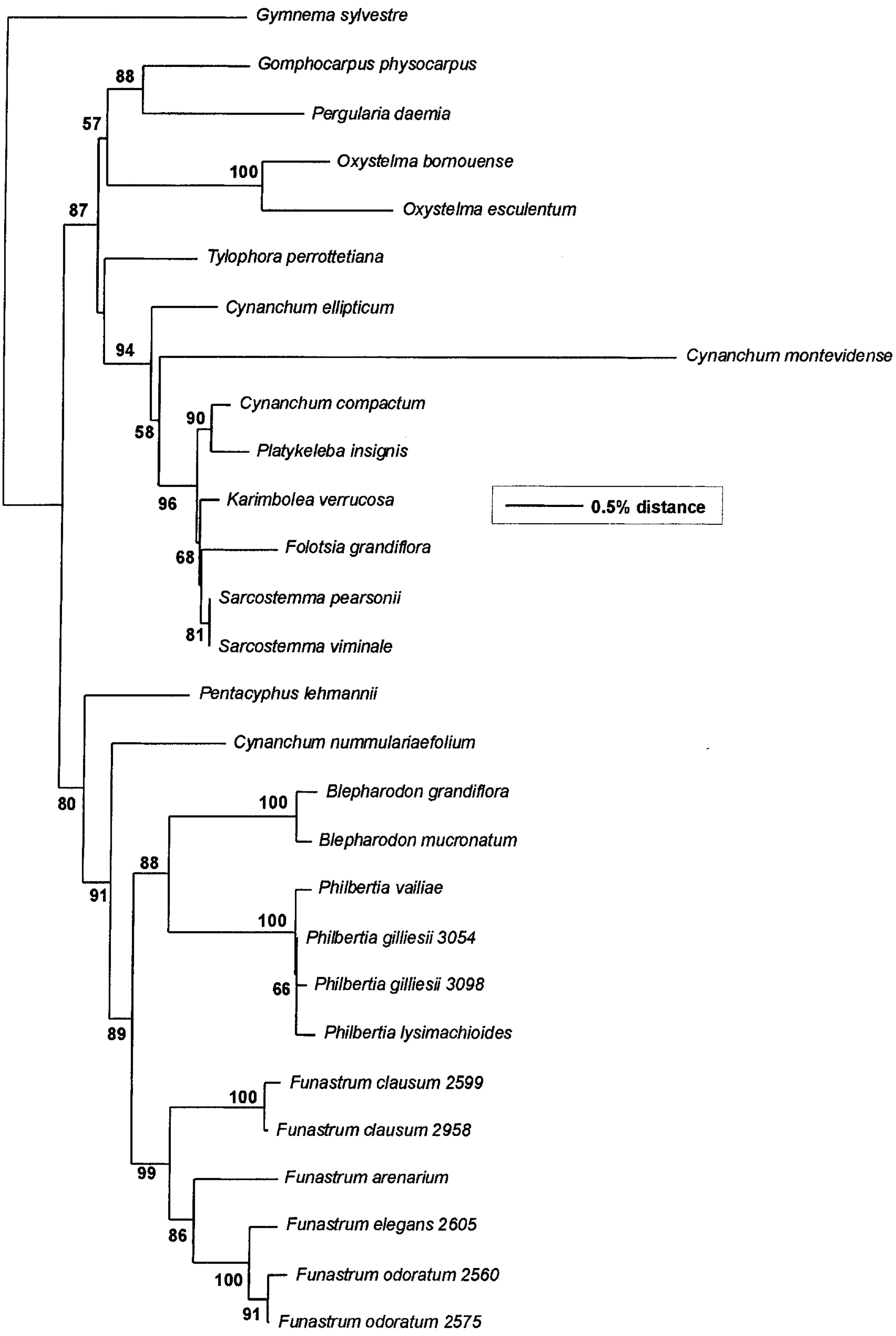


Fig. 2. Neighbor joining tree of all sequence data. Numbers indicate bootstrap percentages (1000 replicates)

Holm (1950) is not a monophyletic genus. The delimitation that fits best with the results presented here and in Liede (1996) is achieved, when the original six genera, *Funastrum*, *Pentacyphus* and *Philibertia* in the New World, *Oxystelma*, *Platykeleba* and *Sarcostemma* s.str. in the Old World are recognized. The inclusion of the "outgroups" *Blepharodon* and *C. nummulariaefolium* in he NW clade and other *Cynanchum* species in the same clade as *Sarcostemma* s.str. also sheds light on the highly outgroup-dependent topologies depicted in Liede (1996). Looking at the topologies of the analyses done with "outgroups" (here shown to be really outside all taxa belonging to *Sarcostemma* sensu Holm (Figs. 2, 3a, b, f in Liede 1996)) the general tendency of the New World taxa to form a distinct clade is reflected there as well. However, the florally very similar *Oxystelma* and *Platykeleba* were invariably joint in these analyses and placed basally to the two *Pentacyphus* species, a result clearly untenable looking at the present analysis.

While a species-level analysis would be needed to completely clarify the phylogeny of *Funastrum*, *Philibertia* and *Blepharodon*, it is interesting to note that here, as in Liede (1996) and contrary to the suggestion of Holm (1950), *F. clausum* takes a basal position in *Funastrum*. Also *Blepharodon mucronatum*, excluded from *Blepharodon* by Morillo (1976), is unambiguously joined with *B. grandiflora* and shows only four differing sites (option "pairwise base differences" in PAUP 4.03a), indicating very close relationships. For comparison, *Oxystelma bornouense* and *O. esculentum* possess 22, *Funastrum clausum* and *F. elegans* 25, *F. elegans* and *F. odoratum* seven and *Philibertia vailiae* and *P. lysimachioides* four differing sites. The morphologically very different *Sarcostemma viminale* and *S. pearsonii* have no differing sites in the regions analyzed.

While the present results clarify the concept of *Sarcostemma*, they pose a number of new questions in asclepiad classification. The split of the subtribe Metastelmatinae into an Old World clade and a New World clade and the exclusion of *Oxystelma* from either suggests that the Metastelmatinae in their present circumscription are not monophyletic. This hypothesis needs to be tested using as many of the presently 34 recognized genera as possible (a list of the current tribal and subtribal disposition of asclepiad genera is maintained on our homepage, http://www.uni-bayreuth.de/departments/planta2/). This question is intimately associated with the concept of the largest genus of the subtribe, *Cynanchum*, which, according to the present results, is not monophyletic. A worldwide revision of *Cynanchum* s.l. is at present under study in our lab.

Species-level revisions of the New World genera *Blepharodon*, *Funastrum*, *Pentacyphus* and *Philibertia* are also needed to re-evaluate circumscription and species concepts still adhering to Holm (1950) and to derive a phylogeny for each of these genera based on the higher relationships elucidated here.

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