EDINBURGH JOURNAL OF BOTANY 59 (3): 377–403 (2002)
DOI: 10.10M/S0960428602000239

MOLECULAR CONSIDERATIONS IN THE *TYLOPHORINAE* K. SCHUM. (*APOCYNACEAE-ASCLEPIADOIDEAE*)

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More than 30 collections of *Tylophora* from all over its geographic range were analysed together with representatives of all other genera in subtribe *Tylophorinae* except *Rhyncharrhena*. Regions of the chloroplast genome (*trn*T-L spacer, *trn*L intron and *trn*L-F spacer) as well as the nuclear genome (internal transcribed spacer including 5.8S of nuclear ribosomal DNA) were sequenced. Both the cpDNA dataset and the rDNA dataset yield a number of stable clades, but a completely resolved and stable phylogeny is not obtained from either alone or by combining them. The small East African and Arabian genera *Blyttia*, *Diplostigma*, *Goydera* and *Pleurostelma* form a well-supported clade in both datasets. *Pentatropis* is always recognized as monophyletic. *Vincetoxicum* is not clearly separable from *Tylophora*, and probably covers the temperate species, while *Tylophora* covers those in the tropics and subtropics.

Keywords. Apocynaceae, *Asclepiadaceae*, *Asclepiadoideae*, ITS, molecular systematics, *trn*T-L spacer, *trn*L intron, *trn*L-F spacer, *Tylophorinae*.

Introduction

The position of *Tylophora* R. Br., a genus of the Old World tropics comprising 100–150 species, has long been misunderstood because the tribe-defining character of pollinia orientation (Bruyns & Forster, 1991; Liede, 1996a) is expressed somewhat ambiguously with usually horizontal, rarely slightly erect or hanging pollinia. Swarupanandan *et al.* (1996) showed clearly that the morphology of the stamens, however, conforms to that of the *Asclepiadeae*. The flowers of *Tylophora* and its relatives are usually rather small and simple for members of the *Asclepiadoideae*; the corona is in most cases purely or at least predominantly (e.g. *T. anomala*) staminal and attached to the back of the anthers. This simplicity has probably misled researchers into constructing a link to the *Marsdenieae* or at least to consider *Tylophora* as a primitive genus in the *Asclepiadeae*. (For a thorough discussion of morphological characters see Omlor, 1998.) However, the position of *Tylophora* and *Vincetoxicum* in the *mat*K (Civeyrel *et al.*, 1998) and *rbc*L (Sennblad, 1997) trees points to a derived position for the *Tylophorinae* in the *Asclepiadoideae-Asclepiadeae* and thus supports the interpretation that floral structure is of secondary simplicity.

The position of *Tylophora* as sister group to the speciose temperate genus *Vincetoxicum* Wolf has been deduced from chemical characters (Liede, 1996b) and

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confirmed by molecular analysis of *rbc*L (Sennblad, 1997) and *mat*K (Civeyrel *et al*., 1998). Liede (2001) has shown that the *Tylophorinae* (*Biondia* Schltr., *Blyttia* Arn., *Diplostigma* K. Schum., *Goydera* Liede, *Pentatropis* R. Br., *Pleurostelma* Baill., *Tylophora*, *Vincetoxicum* and probably *Rhyncharrhena* F. Muell.) constitute a monophyletic, very closely interrelated subtribe, only very distantly related to the *Astephaninae* s. str., with which it was previously combined for morphological reasons, namely long, slender, non-verrucose hairs on the adaxial corolla surface and clear sparse latex (Liede, 1994, 1997). However, Forster (1992) included some species in *Tylophora* that contain milky latex, and reports on latex colour for some other taxa in the *Tylophorinae* (e.g. *Vincetoxicum carnosum*; Forster, 1988) are contradictory.

This paper aims to answer the following questions:

1 Is the present circumscription of *Tylophora* correct or should *Tylophora* or some of the small *Tylophorinae* genera (*Blyttia*, *Diplostigma*, *Goydera*, *Pentatropis* and *Pleurostelma*) be included in *Vincetoxicum*?

2 Is there a recognizable subgeneric structure in *Tylophora*?

3 Have the species with milky latex rightfully been included in *Tylophora* (and the *Tylophorinae*) and if so, are they related or does this trait represent parallel evolutionary events?

Materials and Methods

Taxa

Thirty-two accessions in the *Tylophorinae sensu* Liede (2001) were analysed, among them 21 species of *Tylophora* from Africa, Asia and New Caledonia. Outgroup choice was based on the results of Liede (2001); a member of the *Asclepiadinae* (*Gomphocarpus* R. Br.) and two of the Old World *Metastelminae* (*Cynanchum* L. and *Schizostephanus* Hochst. ex Benth. & Hook.) were selected (see Table 1 for species names and authors).

DNA extraction and PCR

DNA was isolated from fresh or dried leaf tissue according to the method of Doyle & Doyle (1987). PCR primers and protocols for the chloroplast DNA (cpDNA) *trn*T-L and *trn*L-F spacers and the *trn*L intron correspond to Taberlet *et al*. (1991).

The entire internal transcribed spacer (ITS) region 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).

Data analysis

cpDNA sequences were pre-aligned with Perkin Elmer Sequence Navigator Version 1.0.1; the alignment was then adjusted manually. The alignment comprised 35 taxa

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

Species	Origin	Voucher	EMBL Accession No. *trn*T-L spacer *trn*L intron *trn*L-F spacer	EMBL Accession No. ITS (incl. 5.8S)
Outgroups				
Gomphocarpus physocarpus E. Mey. (*Asclepiadeae-Asclepiadinae*)	South Africa	*Nicholas* 2829 (UDW)	AJ290875 AJ290876 AJ290877	AJ320446
Cynanchum ellipticum (Harv.) R.A. Dyer (*Asclepiadeae-Metastelmatinae*)	South Africa	*Liede* 2933 (UBT)	AJ290845 AJ290846 AJ290847	AJ320444
Schizostephanus alatus Hochst. ex K. Schum. (*Asclepiadeae-Metastelmatinae*)	Kenya	*Noltee* s.n. sub IPPS 8111 (UBT)	AJ410247 AJ410248 AJ410249	AJ320451
Ingroup (*Asclepiadeae-Tylophorinae sensu* Liede, 2001)				
**Biondia henryi* (Warb. ex Schltr. & Diels) Tsiang & P.T. Li	China	*Deng* 90203 (MO)	AJ410190 AJ410191 AJ410192	–
Blyttia fruticulosum (Decne.) D.V. Field	Kenya	*Liede & Newton* 2946 (UBT)	AJ410193 AJ410194 AJ410195	AJ320443
Diplostigma canescens K. Schum.	Kenya	*Liede & Newton* 3214 (UBT)	AJ410199 AJ410200 AJ410201	AJ320445
Goydera somaliense Liede	Somalia	*Thulin & Bashir* 6882 (UPS)	AJ410208 AJ410209 AJ410210	AJ320447
Pentatropis madagascariensis Decne.	Madagascar	*Liede* 2749 (UBT)	AJ410235 AJ410236 AJ410237	AJ320448
Pentatropis nivalis (J.F. Gmel.) D.V. Field & J.R.I. Wood	Kenya	*Meve* 949 (B, MSUN, UBT)	AJ410238 AJ410239 AJ410240	AJ320449
Pleurostelma cernuum (Decne.) Bullock	Tanzania	*Liede & Meve* 3377 (B, UBT)	AJ410241 AJ410242 AJ410243	AJ320450
Tylophora anomala N.E. Br.	Cameroon	*Meve* 916 (K, MSUN)	AJ410250 AJ410251 AJ410252	AJ320452
Tylophora apiculata K. Schum. s.l.	Kenya	*Robertson* 7016 (UBT)	AJ410253 AJ410254 AJ410255	AJ320453

TABLE 1. (*Cont'd*)

Species	Origin	Voucher	EMBL Accession No. *trn*T-L spacer *trn*L intron *trn*L-F spacer	EMBL Accession No. ITS (incl. 5.8S)
Tylophora biglandulosa Endl.	New Caledonia	*Veillon* 7899 (P)	AJ320401 AJ320402 AJ320403	AJ320454
Tylophora conspicua N.E. Br.	Tanzania	*Liede & Meve* 3366 (B, UBT)	AJ320404 AJ320405 AJ320406	AJ320455
Tylophora coriacea (Decne.) Marais	Mauritius	*Bernardi* s.n. (BR, UBT)	AJ320407 AJ320408 AJ320409	AJ320456
Tylophora flanaganii Schltr.	South Africa	*Nicholas* 2839 (UDW)	AJ410256 AJ410257 AJ410258	AJ320457
**Tylophora flexuosa* R. Br.	Borneo	*Schneidt* JS95-84 (ABD)	AJ320410 AJ320411 AJ320412	–
Tylophora flexuosa R. Br.	Philippines	*Schneidt* JS96-40 (UBT)	AJ320413 AJ320414 AJ320415	AJ320458
Tylophora perrottetiana Decne.	Philippines	*Schneidt* JS96-31 (UBT)	AJ320416 AJ320417 AJ320418	AJ320459
Tylophora perrottetiana Decne.	Philippines	*Liede* 3252 (UBT)	AJ290915 AJ290916 AJ190917	AJ320460
Tylophora heterophylla A. Rich.	Kenya	*Liede & Newton* 3155 (UBT)	AJ410259 AJ410260 AJ410261	AJ320461
Tylophora hirsuta Wight	India	*Chaturvedi* s.n. (UBT)	AJ320419 AJ320420 AJ320421	AJ320462
Tylophora indica (Burm.f.) Merrill	India	*Bruyns* s.n. (MSUN)	AJ410262 AJ410263 AJ410264	AJ320463
Tylophora oblonga N.E. Br.	Cameroon	*Meve* 915 (B, MSUN, UBT)	AJ320422 AJ320423 AJ320424	AJ320464
Tylophora parviflora (Wight) Meve, Omlor & Liede	Philippines	*Liede* 3290 (B, UBT)	AJ320425 AJ320426 AJ320427	AJ320465
Tylophora sylvatica Decne.	Africa (ex hort.)	*Valck* s.n. (UBT)	AJ410265 AJ410266 AJ410267	AJ320466

TABLE 1. (*Cont'd*)

Species	Origin	Voucher	EMBL Accession No. *trn*T-L spacer *trn*L intron *trn*L-F spacer	EMBL Accession No. ITS (incl. 5.8S)
Tylophora tenuipedunculata K. Schum.	Kenya	*Liede & Newton* 3200 (MSUN)	AJ320428 AJ320429 AJ320430	AJ320467
Tylophora tenuis Blume	Borneo	*Schneidt* JS95-110 (ABD)	AJ320431 AJ320432 AJ320433	AJ320468
Tylophora villosa Blume	Borneo	*Schneidt* JS95-39 (ABD)	AJ320434 AJ320435 AJ320436	AJ320469
Tylophora villosa Blume	Philippines	*Schneidt* JS96-109 (ABD)	AJ320437 AJ320438 AJ320439	AJ320470
Tylophora yunnanensis Schltr.	China	*Schneidt* JS96-134 (ABD)	AJ320440 AJ320441 AJ320442	AJ320471
Vincetoxicum atratum Morr. & Decne.	China	*Schneidt* JS96-137 (ABD)	AJ410268 AJ410269 AJ410270	AJ320472
Vincetoxicum carnosum Benth.	Borneo	*Schneidt* JS95-97 (ABD, L)	AJ410271 AJ410272 AJ410273	AJ320473
Vincetoxicum hirundinaria Medic.	Germany	*Meve* 970 (UBT)	AJ410274 AJ410275 AJ410276	AJ320474
Vincetoxicum stocksii S.I. Ali & S. Khatoon	Pakistan	*Ali & Khatoon* s.n. (GA)	AJ410277 AJ410278 AJ410279	AJ320475

*ITS sequence not available.

and 1805 characters; 80 data cells were unknown, affecting the beginning of the *trn*T-L spacer in *Biondia henryi* and *T. apiculata*, the end of the *trn*T-L spacer in *T. yunnanensis*, the beginning of the *trn*L intron in *B. henryi*, the end of the *trn*L intron in *P. nivalis*, and the end of the *trn*L-F spacer in *T. coriacea* and *T. hirsuta.*

Indels were coded as 'missing characters'; parsimony-informative indels were coded separately as present or absent following the 'simple gap coding' method of Simmons & Ochoterena (2000). Indels resulting from a different number of the same base in a chain of four or more repeats of the same base were not coded separately, as the length of these chains has been found to vary even within the same species (Liede, unpubl. data). This affected bp 565–570 and 590–601 in the *trn*T-L spacer, bp 295–320 in the *trn*L intron, as well as two of the three potentially informative indels

in the *trn*L-F spacer. The 11 separately coded indels were added to the matrix in a second analysis. The alignment is available from the senior author and can be viewed in TreeBase (SN940; Sanderson *et al.*, 1994).

Not all rDNA sequences could be aligned unambiguously (see Appendix; TreeBase SN941). Manual alignment of sequences produced a lower number of parsimony-informative characters than computer programs tested for different algorithms and settings (Jotun-Hein algorithm in 'Megalign' of DNAStar (Lasergene) and Clustal Algorithm in Perkin Elmer Sequence Navigator Version 1.0.1) and was thus considered the most conservative approach. Even so, both ITS1 and ITS2 contain large areas of more or less ambiguous alignment. Excluding those areas introduces a subjective view as to which areas are sufficiently well aligned to include and which ones need to be excluded. Nevertheless, in a second analysis, the 135 most ambiguously aligned characters were excluded, 68 in ITS1 and 67 in ITS2 (see Appendix). This selective approach was preferred over a general exclusion of missing or ambiguous characters, which would have reduced the number of parsimony-informative characters to 73. Separate coding of indels was not carried out because of the heterogeneity of the dataset.

As a partition homogeneity test did not show significant discordance between the cpDNA and the rDNA dataset, a combined analysis was performed. Again, all sequence characters were analysed, and, finally, the 11 separately coded indels for the cpDNA dataset were added. The two taxa for which no rDNA data were available (*Biondia henryi* and *Tylophora flexuosa* JS95-84) were coded unknown for all rDNA data and included in the combined dataset.

Sequence analysis, phylogenetic analysis and tests for clade support were performed using PAUP version 4.0d65a (PPC) (Swofford, 1998) on a Macintosh G3 Powerbook.

Sequence divergence among taxa was calculated using the 'show pairwise distance' option for the *trn*T-L spacer, the *trn*L intron and the *trn*L-F spacer, as well as for 5.8S, ITS1 and ITS2 (excluding the end of 18S and the beginning of 26S). The beginning and end of ITS1 and ITS2 were determined in comparison with *Asclepiadoideae* sequences submitted to EMBL (AJ402152–AJ402162, Meve & Liede, 2001).

For parsimony analysis, heuristic searches were conducted on all datasets (addition sequence random, 100 replicates, TBR branch swapping, 'MulTrees' on, 'steepest descent' off). Bootstrap search (1000 replicates) was conducted under the 'full heuristic' search option, swapping algorithm was set to 'TBR', 'MulTrees' on, 'steepest descent' off, and 'maxtrees' set to 1000. Jackknife resampling (1000 replicates) was set to 50% deletion, and 'Jac' resampling; the other settings were identical to the bootstrap settings.

Finally, a maximum likelihood analysis was performed on both single and combined datasets under the HKY85 model (Hasegawa *et al.*, 1985), using the standard settings as implemented in PAUP 4.0b5 (PPC) (Swofford, 1998). As computational limits were a serious concern, all taxa representing duplicate accessions of the same taxon and closely related species were excluded, resulting in 31 taxa for the cpDNA

dataset and 30 for the rDNA and the combined dataset. Ten replicate searches were conducted for each dataset with the heuristic search algorithm as implemented in PAUP 4.0b5 with random addition sequence and TBR options invoked.

Results

Sequence characteristics for both datasets are summarized in Table 2.

Sequence divergence is higher in ITS1, with 1.5–15.7% between ingroup taxa and 6.2–17.3 between ingroup and outgroup taxa, than in ITS2, with 0.8–13.0% and 3.0–15.1%, respectively. For the cpDNA, the *trn*L intron has the lowest sequence divergence, with 0.0–1.3% between ingroup taxa and 0.4–1.7% between outgroup and ingroup taxa, while the *trn*L-F spacer has the highest sequence divergence, with 0.0–3.0% and 0.5–3.3%, respectively. Thus, sequence divergences in rDNA are between five and ten times higher than in cpDNA.

As an analysis of the sequence characters of the cpDNA dataset (independent of separately coded indels) resulted in more than 60,000 most parsimonious trees and thus exceeded computer memory capacity, the topology of the strict consensus trees was obtained excluding uninformative characters. Both the strict consensus tree (Fig. 1A) and the 'semistrict' consensus tree show a number of well-supported clades, but no resolution between them. The addition of the indels hardly changes these clades, only adding *T. parviflora* to the *Pentatropis* clade, but without any support (Fig. 1A).

The maximum likelihood analysis under the HKY85 model (Hasegawa *et al.*, 1985) yielded one most likely tree (–Ln likelihood = 3687.90, Fig. 1B). The *Tylophorinae* are clearly monophyletic, but comprise several unresolved groups: the small African genera, *Pentatropis–T. parviflora*, *T. sylvatica–T. apiculata–T. tenuipedunculata*, and the remainder of *Tylophora–Vincetoxicum* with subgroups corresponding largely to the clades of the parsimony analysis.

Table 2. Sequence characteristics

	*trn*T-L spacer	*trn*L intron	*trn*L-F spacer	ITS1	ITS2	5.8S
Aligned total length (bp)	877	533	384	322	291	158
Length range (bp)	790–832	504–533	354–374	194–242	217–247	158
Length mean (bp)	800.80	517.40	369.14	225.27	234.36	158
Number of parsimony-informative indels*	7	3	1	–	–	–
Sequence divergence (ingroup) (%)	0.0–1.9	0.0–1.3	0.0–3.0	(0)† 1.5–15.7	(0)† 0.8–13.0	0.0–3.8
Sequence divergence (ingroup/outgroup) (%)	1.0–2.9	0.4–1.7	0.5–3.3	6.2–17.3	3.0–15.1	0.0–2.5

*Not considering variable lengths of chains >4 bp.
†Between *T. perrottetiana* SL3252 and JS96-40 (see text).

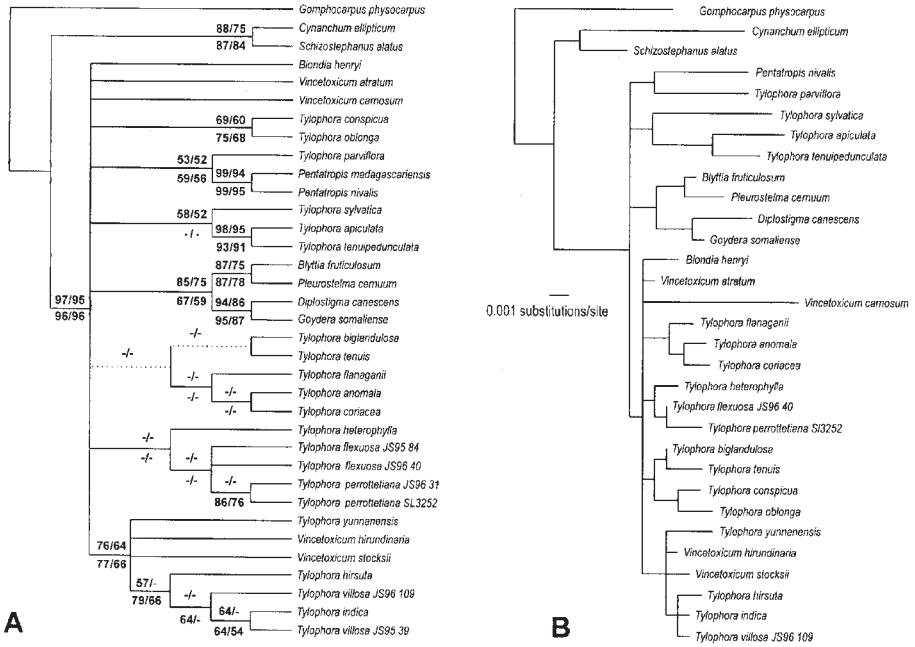

FIG. 1. **cpDNA dataset.** A, Strict consensus of more than 60,000 most parsimonious trees in one island (17 trees without uninformative characters) resulting from parsimony analysis of all sequence characters and strict consensus of more than 60,000 most parsimonious trees in one island (79 without uninformative characters) resulting from parsimony analysis of all sequence characters and indels. Numbers above branches indicate bootstrap/jackknife percentages for the analysis without indels, those below the branches for the analysis with indels. Dotted lines indicate clades not retrieved without indels. -/- denotes values below 50%. B, Maximum likelihood tree of cpDNA sequence characters (Ln = 3687.9).

For the rDNA dataset, difficulties in alignment are reflected in low indices for measuring homoplasy (Table 3). However, the clades retrieved are identical to those retrieved from the cpDNA dataset, only the most basal taxa of single clades (*T. yunnanensis*, *T. heterophylla*) are unresolved for the rDNA (Fig. 2A). Not even the *Cynanchum–Schizostephanus* clade is present in all most parsimonious trees, and therefore it does not occur in the strict consensus, despite bootstrap and jackknife values of more than 80% (Fig. 2A).

Excluding the ambiguously aligned characters of the rDNA dataset retrieves all clades found with all rDNA characters, plus the *Cynanchum–Schizostephanus* clade, the *T. conspicua–T. oblonga* clade and the *V. hirundinaria–V. stocksii* clade (Fig. 2B). The most obvious difference is that the *Cynanchum–Schizostephanus* clade is now a member of the ingroup, with *T. tenuis* in a basal position; this unexpected position for *T. tenuis* is without bootstrap or jackknife support. If the monophyly of the *Tylophorinae* is enforced by constraint, the most parsimonious trees are three steps longer ($l = 411$) than without constraint, a result considered not significant in a

TABLE 3. Results of DNA analyses using different primers and different settings (see text)

Dataset	Setting	Total taxa	Total characters	Unknown data cells	Parsimony-informative character	Most parsimonious trees	Islands	L	CI	RI	RC	Figure
cpDNA	− indels	35	1794	80	48	>60,000 (17)*	1	178 (70)*	0.8933	0.8208	0.7331	1A
	+ indels		1805	80	59	>60,000 (79)*	1	198 (90)*	0.8586	0.7926	0.6805	
rDNA		33	814	53	141	12	3	505	0.6455	0.6134	0.3960	2A
	− ambiguously aligned areas		679	53	113	110	1	408	0.6471	0.6150	0.3979	2B
Combined datasets	all rDNA	35	2609	133+2×815 (rDNA)	189	396	1	699	0.6938	0.6239	0.4329	3A
	− cpDNA indels all rDNA + cpDNA indels		2619		200	33	1	720	0.6889	0.6254	0.4308	3B
	− ambiguously aligned rDNA − cpDNA indels	35	2473	133+2×680 (rDNA)	161	5232	2	605	0.6992	0.6208	0.4341	4A
	− ambiguously aligned rDNA + cpDNA indels		2484		172	444	2	626	0.6933	0.6228	0.4318	

*Excluding uninformative characters.

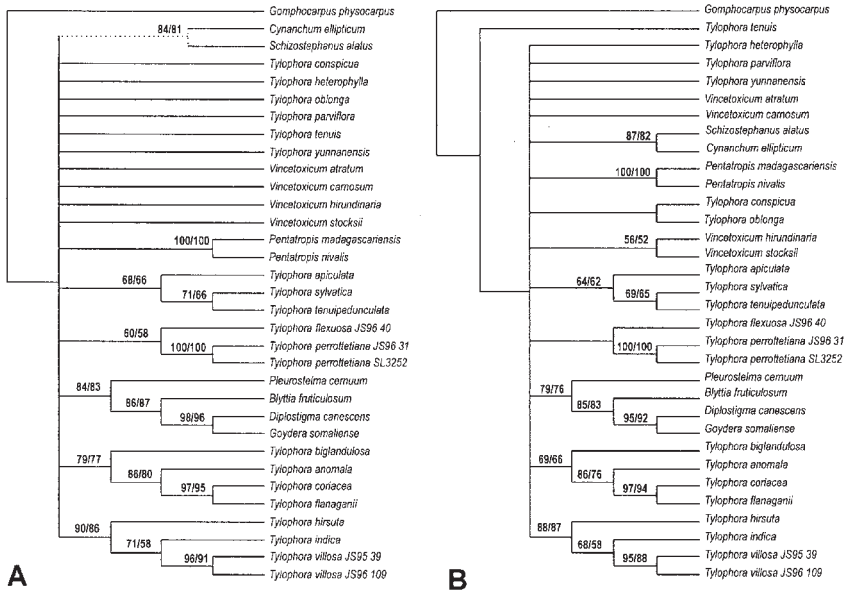

FIG. 2. **rDNA dataset.** A, Strict consensus of the 12 most parsimonious trees in three islands resulting from parsimony analysis of all sequence data. The dotted line indicates a clade not present in the strict consensus, but with a high bootstrap/jackknife support. B, Strict consensus of the 110 most parsimonious trees in one island resulting from parsimony analysis of sequence data without 135 ambiguously aligned characters. Numbers indicate bootstrap/jackknife percentages.

PTP (Permutation Test for the Existence of Phylogenetic Structure), but significant in a T-PTP (Topology Dependent PTP) as implemented in PAUP. If ITS1 is analysed alone, with ambiguous characters removed (228 characters, 48 parsimony-informative ones), all ingroup clades and *Cynanchum–Schizostephanus* are unresolved; if ITS2 is analysed alone with the ambiguous characters removed (250 characters, 54 parsimony-informative ones), all ingroup clades and *Cynanchum–Schizostephanus* remain unresolved, but *T. tenuis* retains a basal position.

The maximum likelihood trees of the rDNA data, with and without ambiguous characters (−Ln likelihood = 3292.67 and 3289.86, respectively), show no additional information; in the tree without ambiguous characters, *T. tenuis* again takes a basal position.

The combined dataset was run with all rDNA characters and with the ambiguous rDNA characters excluded, both with and without addition of the separately coded cpDNA indels (Table 3). In the analysis with all rDNA characters, but without indels, *Pentatropis* is basal in the *Tylophorinae*, while all other *Tylophorinae* are unresolved, forming largely the same subclades as in the previous analyses, though with higher bootstrap/jackknife support (Fig. 3A). Adding the indels makes *Pentatropis* and *T. parviflora* sister to *T. sylvatica–T. apiculata–T. tenuipedunculata*,

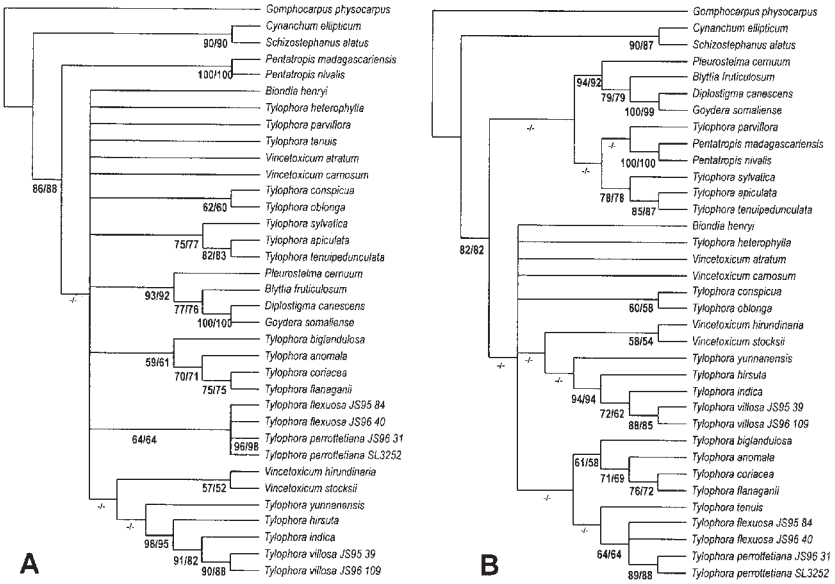

FIG. 3. **Combined cpDNA and rDNA dataset, all rDNA data included.** A, Strict consensus of the 396 most parsimonious trees in one island resulting from parsimony analysis of all sequence data. -/- denotes values below 50%. B, Strict consensus of the 33 most parsimonious trees in one island resulting from parsimony analysis of all sequence data and the indels. -/- denotes values below 50%.

and the two together to the small African genera (Fig. 3B). This whole group is sister to the remaining *Tylophorinae*, in which the *T. flexuosa* clade is sister to the *T. biglandulosa–T. anomala–T. coriacea–T. flanaganii* clade. All this additional structure, however, is without any support.

Excluding the ambiguously aligned rDNA characters, *Pentatropis* and the *T. sylvatica–T. apiculata–T. tenuipedunculata* group are basal to the remaining *Tylophorinae* if the indels are not added, otherwise the same clades as in the single analyses are retrieved (Fig. 4A).

Maximum likelihood analysis of the combined dataset without ambiguously aligned rDNA characters places the *T. sylvatica–T. apiculata–T. tenuipedunculata* group basal to all *Tylophorinae*, and leaves a *Pentatropis–T. conspicua–T. oblonga* group unresolved with a *T. heterophylla–T. parviflora*–small African genera group and a group comprising the remainder of *Tylophorinae* in three subgroups (Fig. 4B).

DISCUSSION

Clade structures

In the small genera, the outcome of *Pentatropis* as a distinct clade is unambiguous, and it occupies a rather isolated position in the ingroup. A basal position for

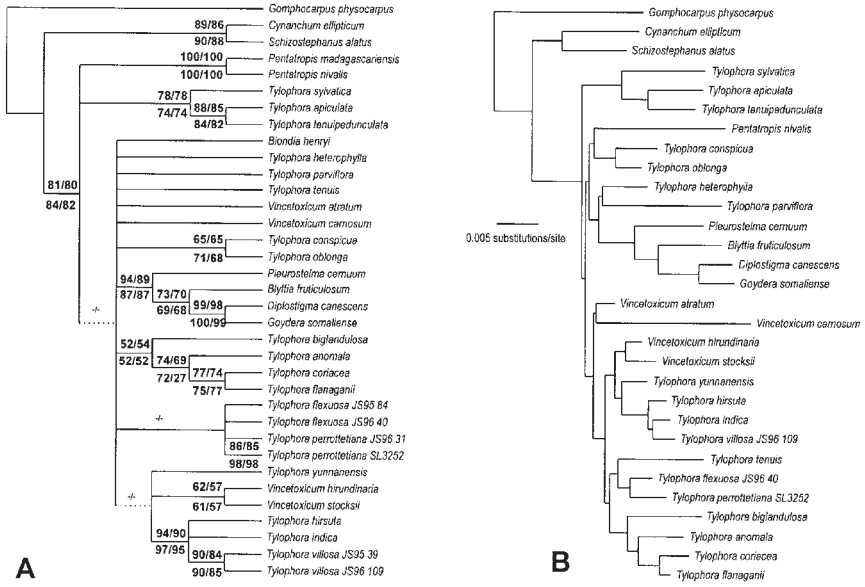

FIG. 4. **Combined cpDNA and rDNA dataset, ambiguously aligned rDNA characters excluded.** A, Strict consensus of the 5232 most parsimonious trees in two islands resulting from parsimony analysis of all sequence data and of 444 trees in two islands resulting from parsimony analysis of all sequence data and the indels. Numbers above branches indicate bootstrap/jackknife percentages for the analysis without indels, those below the branches for the analysis with indels. Dotted lines indicate clades not found with indels. -/- denotes values below 50%. B, Maximum likelihood tree resulting from analysis of cpDNA and rDNA, ambiguously aligned rDNA characters excluded (Ln = 7465.55).

Pentatropis in the *Tylophorinae*, however, is suggested only by all rDNA data (Fig. 3A). *Vincetoxicum carnosum*, despite considerable morphological similarity with *Pentatropis* (Liede, 1994), is always very isolated in the *Tylophorinae* and does not join the *Pentatropis* clade. The two species of *Pentatropis* are very closely related and show only three differences in the cpDNA dataset and six in the rDNA dataset. Floral structure is also very similar, so that possibly the much more tender, small-leaved Madagascan *P. madagascariensis* might be considered a subspecies under *P. nivalis* (see Liede & Meve, 2001).

Very constant throughout is the *Diplostigma–Goydera* clade, indicating a very close relationship between these two monotypic East African genera. The morphological analyses by Liede (1994) and Bruyns (1999) did not suggest this affinity for the morphologically very unusual *Goydera* (Liede, 1993). In all analyses, the four small African genera form a well-supported clade. While the cpDNA data suggest a *Blyttia–Pleurostelma* clade as sister to *Diplostigma–Goydera* (Fig. 1), the rDNA data and the combined datasets place *Blyttia* as sister group to the *Diplostigma–Goydera* clade and *Pleurostelma* basal to the whole clade. In both morphological analyses

(Liede, 1994; Bruyns, 1999), *Pleurostelma*, mostly because of its long stylar head, was placed basal to the (*Oncinema*)–*Astephanus*–*Microloma* clade that has been shown to be of different affinity (Liede, 2001).

Of the *Tylophora* species analysed, three African clades can be discerned: *T. sylvatica* always comes out with *T. tenuipedunculata* and *T. apiculata*, though the basal position in the clade is sometimes occupied by *T. apiculata*, and sometimes by *T. sylvatica*. *Tylophora sylvatica* and *T. tenuipedunculata* share small flower size and show similar corona structure, but *T. tenuipedunculata* has rather reduced inflorescences. *Tylophora apiculata* shares with *T. sylvatica* the more floriferous inflorescences, but in addition has larger flowers. The second clade of African *Tylophora* species comprises *T. anomala*, *T. coriacea* and *T. flanaganii*. Morphologically, the well-developed, erect, firm corona lobes and the large pollinaria with hanging pollinia characterize this group of species. In the rDNA and the combined datasets, this clade is associated with the New Caledonian *T. biglandulosa*, a species with white milky latex (Forster, 1991). Forster (1991) is thus supported in his view that there is no reason to separate *T. biglandulosa* from *Tylophora* as *Hybanthera* Endl. The third clade of African *Tylophora* species, *T. conspicua* and *T. oblonga*, is retrieved in all analyses except for that which includes all rDNA data, but the clade is more strongly supported by cpDNA data than by rDNA data. Morphologically, corona structure is very different in the two species, but both possess unusually large flowers and broad, apically twisted corolla lobes. *Tylophora oblonga*, originally in the monotypic genus *Oncostemma* K. Schum. because of its unique corona structure (Meve & Omlor, 2000), has thus correctly been included in *Tylophora*. *Tylophora conspicua* possesses whitish, milky latex.

The Asian *Tylophora* species fall largely into two clades. The first consists of *T. indica* and the two specimens of *T. villosa*, indicating a relationship supported by various morphological traits (Schneidt, 1999). This clade is always basally joined by *T. hirsuta*, which has also been considered a member of this complex for morphological reasons. For the cpDNA dataset and both combined datasets, this clade is basally joined by the Chinese *T. yunnanensis* and also by *Vincetoxicum hirundinaria* and *V. stocksii*. Only in the rDNA dataset analyses (Fig. 2) do *T. yunnanensis* and the *V. hirundinaria–V. stocksii* clade remain unresolved; in the analysis of all rDNA data, the latter two species also remain unresolved. Flower structure also supports an association of these species with the *T. indica* group.

The second clade comprises most members of the *T. flexuosa* group. Schneidt (1999) proposes four varieties in this complex, of which three were included in the present analysis. The two Philippine accessions of *T. perrottetiana* (SL3252 and JS96-31) are almost identical in the regions studied, with no difference in their rDNA sequences and only three in the *trn*T-L spacer. Characteristic of this species are two deletions in the *trn*L intron, one being unique in the dataset and the other unique in the ingroup. The other specimen from the Philippines (JS96-40) is clearly more distinct, with 20 differences from JS96-31 in the rDNA dataset. Owing to insufficient material no rDNA sequence could be obtained from the Bornean specimen

(JS95-84). This differs from JS96-40 in nine sites in the cpDNA dataset. This number of differences is the same as between the two specimens of *T. villosa*, one from Borneo (JS95-39) and one from the Philippines (JS96-109), with five sites differing in both the cpDNA dataset and the rDNA dataset. The last specimen of the *T. flexuosa* complex, JS95-110, represents *T. tenuis*, also proposed as a variety of *T. flexuosa* by Schneidt (1999). However, this specimen joins the *T. flexuosa* clade only in the analysis combining all data (Fig. 3B) and the maximum likelihood analysis of all sequence data without the ambiguously aligned characters (Fig. 4B); it is usually unresolved or weakly attached to other species. In the analysis of rDNA data without ambiguously aligned characters, *T. tenuis* is actually basal to all *Tylophorinae* and *Cynanchum–Schizostephanus*. Schneidt (1999) distinguished *T. perrottetiana* from *T. flexuosa* by corona differences, and from *T. tenuis* by differences in inflorescence structure. These differences in corona structure may be best recognized at varietal rank. However, differences in inflorescence structure indicate differences in species level, as none of the molecular datasets gives any indication that *T. tenuis* is not an independent species, despite its floral similarities to *T. flexuosa*.

The remaining species of *Tylophora* (the aberrant Philippine *T. parviflora* (Meve *et al.*, 2002) and the African *T. heterophylla*) and *Vincetoxicum* (*V. atratum*) are unambiguous members of the *Tylophorinae*, but show no constant affinities to any clade. No rDNA sequence was available for *Biondia*, and the cpDNA sequences confirm its membership of the *Tylophorinae*, but give no indication as to its position within the subtribe.

Evolutionary implications

Both molecular regions recognize the *Tylophorinae sensu* Liede (2001) as a well-defined monophyletic group with high bootstrap and jackknife support. While the poor resolution of the cpDNA results is easily understood because of lack of variation between the sequences, the problems with the rDNA dataset are unexpected. Sequence divergence is much lower in rDNA datasets from other *Asclepiadoideae* genera and genera complexes (e.g. $<10\%$ in the succulent *Stapelieae*, Meve & Liede, unpubl. data; $<15\%$ in *Cynanchum* s. str., Liede, unpubl. data), but not unusual for this region in other angiosperms (e.g. *Streptocarpus*, Möller & Cronk, 1997; *Heliantheae*, Urbatsch *et al.*, 2000). However, the problem of this dataset lies in the high number of very variable indels interspersed with short, hardly variable regions. This probably reflects conservation of secondary structure, but not of primary sequence (Coleman *et al.*, 1998).

Therefore, both datasets result in groups of species clustering together, though basal resolution is undetectable. Under almost all settings, the 'semistrict' consensus does not show any additional structure compared with the strict consensus. The clades regularly retrieved largely coincide in both rDNA and cpDNA analyses, and most of them are supported in one of the single datasets or one of the combined ones by satisfactorily high bootstrap/jackknife values ($>80\%$), so that they can be

regarded with some confidence as monophyletic units. These monophyletic units are composed largely of species of close geographic distribution, though the New Caledonian *T. biglandulosa* shows more affinities to the African *T. anomala–T. coriacea–T. flanaganii* clade than to any of the Asian clades.

In terms of an evolutionary scenario, poor differentiation of the chloroplast spacer regions and the *trn*L intron and the patchwork indels of ITS1 and ITS2 point to very rapid evolution of geographical groups from a common, possibly widely distributed, ancestor. As previous studies of the *Apocynaceae* as a whole have shown (Sennblad, 1997; Civeyrel *et al*., 1998), *Tylophora* is one of the most derived genera of the *Asclepiadoideae*, so that longevity of the group as reason for high ITS variation is unlikely. However, *Tylophora* and *Vincetoxicum* are the only genera in *Asclepiadoideae* for which autogamy and apomixis have been demonstrated (Chaturvedi, 1988; Naumova, 1992; Lumer & Yost, 1995). Furthermore, their smooth seeds are very effectively wind dispersed, and the normally small, rather simple, but often very nectariferous flowers are commonly visited by small insects not covering long distances. Thus, effective isolation mechanisms together with good dispersal capacity might have led to the pattern observed. On Krakatau, three out of 15 *Asclepiadoideae* reported 111 years after the catastrophic eruption are members of *Tylophora* (Bush *et al*., 1995). The rapid spread of *Vincetoxicum* in the Americas (e.g. Pringle, 1973; Sheeley & Raynal, 1996) and of *Tylophora indica* in the West Indies (Schlechter, 1899) further demonstrates the vigour of this group. Possibly several groups evolved at different times in some regions, which would explain the African clades having similar distribution but not close relationships, indicating independent colonization events in Africa. The Asian clades also appear distantly interrelated. The association of the type species of *Vincetoxicum* (*V. hirundinaria*) with the *T. indica* clade suggests that *Tylophora* comprises the tropical and *Vincetoxicum* the temperate representatives of the same complex. This view is supported by numerous intermediate species in the Far East, which have been shuffled back and forth between *Vincetoxicum* and *Tylophora*.

The two possible approaches to translate this phylogeny into a classification both create more problems than they solve. First, all the taxa studied here could be integrated into a large genus *Vincetoxicum*. To lump large and complex genera such as *Vincetoxicum*, *Tylophora* and *Biondia* would create a genus relatively easy to delimit, but very difficult to differentiate at the species level. Small, but easily recognizable, entities such as *Blyttia*, *Diplostigma*, *Goydera* and *Pleurostelma* would have to be lumped into this conglomerate as well, marking the extremes of morphological variation, and thus blurring the genus concept.

The second possibility, giving each clade generic recognition, would result in numerous small genera of uncertain delimitation that cannot be identified morphologically. However, the four morphologically very distinct East African genera could then remain distinct.

The comparatively low morphological differentiation in the whole complex and the slightly smaller number of name changes involved support the first possibility.

However, before such far-reaching changes are made, a thorough study at species level needs to be carried out for the whole complex. Especially in mainland Asia and Japan, the relationships of *Biondia*, *Tylophora* and *Vincetoxicum* need to be re-examined morphologically, karyologically and with a different set of molecular regions, based on the results presented here. The insufficiently known monotypic genera *Merrillanthus* Chun & Tsiang and *Pentastelma* Tsiang & P.T. Li possibly represent the extremes of morphological variation in the complex in China and should be included in such a study.

Finally, the present study answers the question of whether latex colour is a reliable synapomorphy for the *Tylophorinae*. While it is certainly indicative, it is neither unique (also being found in the *Ceropegieae* and the *Astephaninae* s. str.), nor present throughout. *Tylophora parviflora* with milky, yellowish latex is always placed in the main clade, but shows no clear affinities to any of the constant clades. The other two species reported to have white milky latex (*T. conspicua* and *T. biglandulosa*) show no close affinity to each other nor to *T. parviflora*. For *V. carnosum*, contradictory statements have been found on herbarium labels, so that seasonal changes or changes between populations cannot be excluded. From these results it is likely that a reversal to milky latex has occurred more than once in the *Tylophorinae*.

Acknowledgements

Data for this paper were gathered in the Department of Plant Systematics, University of Bayreuth, during the PhD project of J. Schneidt at the University of Aberdeen, supervised by C.C. Wilcock. Two samples of plant material were donated by A. Nicholas, Pietermaritzburg. The directors of P and UPS are acknowledged for permission to use material under their care for sequencing (one specimen each). We also wish to thank one anonymous reviewer for valuable and detailed comments.

References

Baldwin, B. G. (1992). Phylogenetic utility of the Internal Transcribed Spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Mol. Phyl. Evol.* 1: 3–16.

Bruyns, P. V. (1999). The systematic position of *Eustegia* R.Br. (Apocynaceae-Asclepiadoideae). *Bot. Jahrb. Syst*. 121: 19–44.

Bruyns, P. V. & Forster, P. I. (1991). Recircumscription of the Stapelieae (Asclepiadaceae). *Taxon* 40: 381–391.

Bush, M. B., Whittacker, R. J. & Partomihardjo, T. (1995). Colonization and succession on Krakatau: an analysis of the guild of vining plants. *Biotropica* 27: 355–372.

Chaturvedi, S. K. (1988). Abiotic pollination in *Tylophora hirsuta* Wight (Asclepiadaceae). *Asklepios* 45: 58–62.

Civeyrel, L., Le Thomas, A., Ferguson, K. & Chase, M. W. (1998). Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to molecular phylogeny obtained from plastid *mat*K sequences. *Mol. Phyl. Evol.* 9: 517–527.

COLEMAN, A. W., PREPARATA, R. C., MEHROTRA, B. & MAI, J. C. (1998). Derivation of the secondary structure of the ITS-1 transcript in Volvocales and its taxonomic correlations. *Protist* 149: 135–146.

DOYLE, J. J. & DOYLE, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11–15.

FORSTER, P. I. (1988). Studies on the Australasian Asclepiadaceae, V. *Cynanchum carnosum* (R.Br.) Schltr. and its synonymy. *Austrobaileya* 2: 525–527.

FORSTER, P. I. (1991). The distribution and synonymy of *Tylophora biglandulosa* (Asclepiadaceae). *Bull. Misc. Inform.* 46: 563–567.

FORSTER, P. I. (1992). A taxonomic revision of *Tylophora* R.Br. (Asclepiadaceae: Marsdenieae) in Australia. *Austral. Syst. Bot.* 5: 29–51.

HASEGAWA, M., KISHINO, H. & YANO, T. (1985). Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* 21: 160–174.

LIEDE, S. (1993). *Goydera*, a new genus of Asclepiadaceae from Somalia. *Novon* 3: 265–267.

LIEDE, S. (1994). Myth and reality of the subtribe Astephaninae K.Schum. (Asclepiadaceae). *Bot. J. Linn. Soc.* 114: 81–98.

LIEDE, S. (1996a). Anther differentiation in the Asclepiadaceae: form and function. In: D'ARCY, W. G. & KEATING, R. C. (eds) *The Anther: Form, Function and Phylogeny*, pp. 221–235. Cambridge: Cambridge University Press.

LIEDE, S. (1996b). *Cynanchum–Rhodostegiella–Vincetoxicum–Tylophora*: new considerations on an old problem. *Taxon* 45: 193–211.

LIEDE, S. (1997). Subtribes and genera of the tribe Asclepiadeae (Apocynaceae-Asclepiadoideae) – a synopsis. *Taxon* 46: 233–247.

LIEDE, S. (2001). New considerations on the subtribe Astephaninae Endl. (Apocynaceae-Asclepiadoideae). *Ann. Missouri Bot. Gard.* 88: 657–668.

LIEDE, S. & MEVE, U. (2001). New combinations and new names in Malagasy Asclepiadoideae (Apocynaceae). *Bull. Mus. Natl. Hist. Nat. (Paris), sect. B, Adansonia, sér. 3* 23: 347–351.

LUMER, C. & YOST, S. E. (1995). The reproductive biology of *Vincetoxicum nigrum* (L.) Moench (Asclepiadaceae), a Mediterranean weed in New York State. *Bull. Torrey Bot. Club* 12: 12–23.

MEVE, U. & LIEDE, S. (2001). Reconsideration of the status of *Lavrania*, *Larryleachia* and *Notechidnopsis* (Asclepiadoideae-Ceropegieae). *S. Afr. J. Bot.* 67: 161–168.

MEVE, U. & OMLOR, R. (2000). The identity of the African genus *Oncostemma* (Asclepiadaceae). *Bot. J. Linn. Soc.* 133: 195–201.

MEVE, U., OMLOR, R. & LIEDE, S. (2002). A new combination in *Tylophora* (Apocynaceae-Asclepiadoideae) from the Philippines. *Syst. Geogr. Pl.* 72 (in press).

MÖLLER, M. & CRONK, Q. B. (1997). Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer sequences. *Amer. J. Bot.* 84: 956–965.

NAUMOVA, T. N. (1992). Family Asclepiadaceae. In: *Apomixis in Angiosperms*, p. 63. Boca Raton, FL: CRC Press.

OMLOR, R. (1998). *Generische Revision der Marsdenieae (Asclepiadaceae).* Dissertation Universität Kaiserslautern 1997. Aachen: Shaker.

PRINGLE, J. (1973). The spread of *Vincetoxicum* species (Asclepiadaceae) in Ontario. *Canad. Field-Nat.* 87: 27–32.

SANDERSON, M. J., DONOGHUE, M. J., PIEL, W. & ERIKSSON, T. (1994). TreeBASE: a prototype database of phylogenetic analyses and an interactive tool for browsing the phylogeny of life. *Amer. J. Bot.* 81: 183.

SCHLECHTER, R. (1899). Asclepiadaceae. In: URBAN, I. (ed.) *Symbolae antillanae*, vol. 1, part 2, 11, pp. 236–384. Berlin: Borntraeger.

SCHNEIDT, J. (1999). *Taxonomy and biodiversity of the genus* Tylophora *(Apocynaceae-Asclepiadoideae) in Malesia.* PhD thesis, University of Aberdeen.

SENNBLAD, B. (1997). Phylogeny of the Apocynaceae s.l. *Acta Universitas Upsaliensis*, 295pp. Uppsala.

SHEELEY, S. E. & RAYNAL, D. J. (1996). The distribution and status of species of *Vincetoxicum* in eastern North America. *Bull. Torrey Bot. Club* 123: 148–156.

SIMMONS, M. P. & OCHOTERENA, H. (2000). Gaps as characters in sequence-based phylogenetic analyses. *Syst. Biol*. 49: 369–381.

SWARUPANANDAN, K., MANGALY, J. K., SONNY, T. K., KISHOREKUMAR, K. & CHAND BASHA, S. (1996). The subfamilial and tribal classification of the family Asclepiadaceae. *Bot. J. Linn. Soc.* 120: 327–369.

SWOFFORD, D. L. (1998). *PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods)*, Version 4. Sunderland, MA: Sinauer Associates.

TABERLET, P., GIELLY, L., PAUTOU, G. & BOUVET, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Mol. Biol.* 17: 1105–1109.

URBATSCH, L. E., BALDWIN, B. G. & DONOGHUE, M. J. (2000). Phylogeny of the coneflowers and relatives (Heliantheae: Asteraceae) based on nuclear rDNA Internal Transcribed Spacer (ITS) sequences and chloroplast DNA restriction site data. *Syst. Bot*. 25: 539–565.

Received 12 February 2001; accepted after major revision 26 June 2002

APPENDIX: Alignment of rDNA

*** denote areas excluded in one analysis because of ambiguous alignment**

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                                         0            0         00 0  00      0  0  0                                                       1
                                         0            1         22 2  33      3  4  4                                                       0
                                         1            4         45 7  01      8  1  4                                                       0
                                         ????????????????????????  ?  ????????   ?
                                         ____18s_______          ******      ****
Gomphocarpus_physocarpus                 cggaaggatcattgccaaatcctt--g---taccgaa-----cgacccgcgaacgcgttccgaagacgatg---aagcgctgctg-cgcc-c--ttg-ct
Cynanchum_ellipticum                     cggaaggatcattgtcaaatcctttttg---taccaaa---atgacctgcgaacacgttccgaaaaca---a-caa-----------gcc-c--ttg-ct
Schizostephanus_alatus                   cggaaggatcattgtcaaatcctt--g---taccgaa-----tgacccgcgaacacgttccgaaaacagt-ag---gcggtgttg-cgcc-c--ttg-ct

Blyttia_fruticulosum                     cggaaggatcattgtcgaatgctt--g---taccgaa-----tgacctgcgaacacgttccgaaaaaa-tgag-gagcgctgttg-cgcc-c--ttg-ct
Diplostigma_canescens                    cggaaggatcattgtcgaatcctc--g---taccgaa-----tgacctgcgaacgcgttccgaaaaaa-tca-caagcgctgttg-cgcc-c--ttg-ct
Goydera_somaliense                       cggaaggatcattgtcgaatcctc--g---taccgaa-----tgacctgcgaacacgttccgaaaaaa-tca-caagcgctgttg-cgcc-c-tttg-ct
Pentatropis_madagascariensis             cggaaggatcattgtcgaatcctt--g---taccgaa-----tgacctgcgaacgcgttccgaaaa---tcg-caagcgccgtcggcgcc-c--ttg-ct
Pentatropis_nivalis                      cggaaggatcattgtcgaatcctt--g---taccgaa-----tgacctgcgaacgcgttccgaaaa---tcg-caagcgccgtcggcgcc-c--ttg-ct
Pleurostelma_cernuum                     cggaaggatcattgtcaaatcctt--g---taccgaa-----tgacctgcgaacacgttccgaaaaa--tca-caagcgctgttg-cgcc-c--tcg-ct

Tylophora_anomala                        cggaaagatcattgtcaaatcctc--g---taccgaa-----tgacctgcgaacacgttccgaaag---tca-caagcgttgttg-cgcc-c--ttg-ct
Tylophora_apiculata                      cggaaggatcattgtcaaatcctt--g---taccgaa-----tgacctgcgaacgcgttccaaaaa---tca-caagcgttgttg-cgcc-c--ttg-ct
Tylophora_biglandulosa                   cggaaggatcattgtcaaatcctt--g---taccgaa-----cgacccgcgaaccggttccgaaag---tca-cgagcgt------c-cc--ttttg-ct
Tylophora_conspicua                      cggaaggatcattgtcaaatcctt--g---taccgaa---tatgacctgcgaacacgttccaaaaa---tca-caagcgctgttg-cgcc-c-tttg-ct
Tylophora_coriacea                       cggaaggatcattgtcaaatcctc--g---taccgaa-----tgacccgcgaacacgttcggaaag---tca-caagcgttgttg-cgcc-c--ttg-ct
Tylophora_flanaganii                     cggaaggatcattgtcaaatcctc--gtactaccgaa-----tgacccgcgaacacgttccgaaag---taa-caagcgttgttg-cgcc-c--ttg-ct
Tylophora_flexuosa_JS96_40               cggaaggatcattgtcaaatcctt--g---taccgaa-----tgacatgcgaacacgttccgaaaa---tca-caagcgttgttgg-gcc-c-tttg-ct
Tylophora_perrottetiana_JS96_31          cggaaggatcattgtcaaatcctc--g---taccgaa-----tgacctgcgaacgcgttccgaaaa---tca-caggcgttgttg-cgcc-c--ttg-ct
Tylophora_perrottetiana_SL3252           cggaaggatcattgtcaaatcctc--g---taccgaa-----tgacctgcgaacgcgttccgaaaa---tca-caggcgttgttg-cgcc-c--ttg-ct
Tylophora_heterophylla                   cggaaggatcattgtcaaatcctt--g---taccgaa-----tgacctgcgaacacgttaaaaaaaaatga-caagcgctgttg-cgcc-c--ttg-ct
Tylophora_hirsuta                        cggaaggatcattgtgaaatcctc-tgg--taccgaa-----tgacccgcgaacacgttccgaaaa---tca-caagcgttgttg-cgcc-c--ttg-ct
Tylophora_indica                         cggaaggatcattgtgaaatcctg--g---taccgaa-----ggacccgcgaacacgttccgaaaa---tca-caagcgttgttg-cgcc-c--ttg-ct
Tylophora_oblonga                        cggaaggatcattgtcaaatcctt--g---taccgaa-----tgacctgcgaacacgttccaaaaa---tca-caagcgctgttg-cgcc-c-tttg-ct
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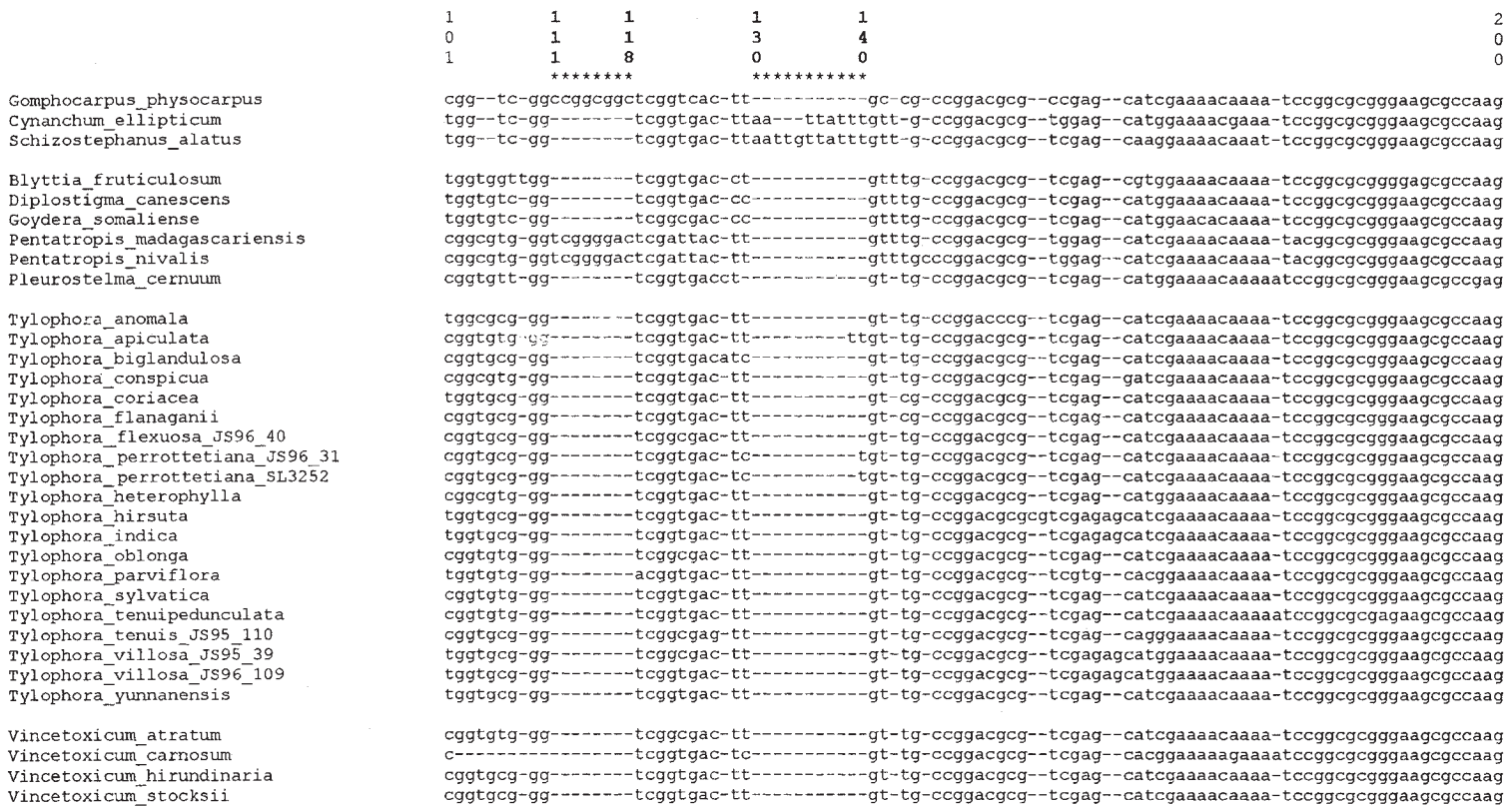
Tylophora_sylvatica                      cggaaggatcattgtcaaatcctt--g---taccgaa-----tgacctgcgaacgcgttc-gaaaaa--tca-caggcgctgtcg-cgccgccgttg-ct
Tylophora_tenuipedunculata               cggaaggatcattgtcaaatcctt--g---aaccgaaccgaacgacctgcgaacgcgttccaaaaa---tca-caagcgttgtgg-cgc-----------
Tylophora_tenuis_JS95_110                cggaaggatcattgtcaaatcctc--g---taccgaa-----cgacccgcgaacgcgttccgaaaa---tcg-caagcgctgttg-cgcc-c--ttggct
Tylophora_villosa_JS95_39                ??????????????????????????????????????????acccgcgaacacgttccgaaaa---tca-caagcgttgttg-cgcc-c--ttg-ct
Tylophora_villosa_JS96_109               cggaaggatcattgtgaaatcctg--g---taccgaa-----tgacccgcgaacacgttccgaaaa---tca-caagcgttgttg-cgcc-c--ttg-ct
Tylophora_yunnanensis                    cggaaggatcattgtcaaatcctt--g---taccgaa-----tgacctgcgaacacgttccgaaaa---tca-caagcgttgttg-cgcc-c--ttg-ct

Vincetoxicum_atratum                     cggaaggatcattgtcaaatcctc--g---taccgaa-----tgacctgcgaacacgttccgaaaa---tca-caagcgctgttg-cgcc-c--ttg-ct
Vincetoxicum_carnosum                    cggaaggatcattgtcgaatcctc--g---taccgaa-----tgacctgcgaacccgttccaaaaa---tca-caagcg-------------------t
Vincetoxicum_hirundinaria                cggaaggatcattgtcaaatcctc--g---taccgaa-----tgacctgcgaacacgttccgaaaa---tca-caagcgctgttg-cgcc-c--ttgg-t
Vincetoxicum_stocksii                    cggaaggatcattgtcaaatcctt--g---taccgaa-----tgacctgcgaacacgttccgaaaa---tca-caagc----------cc----ttg-ct
```


1 1 1 1 1 2
0 1 1 3 4 0
1 1 8 0 0 0
******** **********
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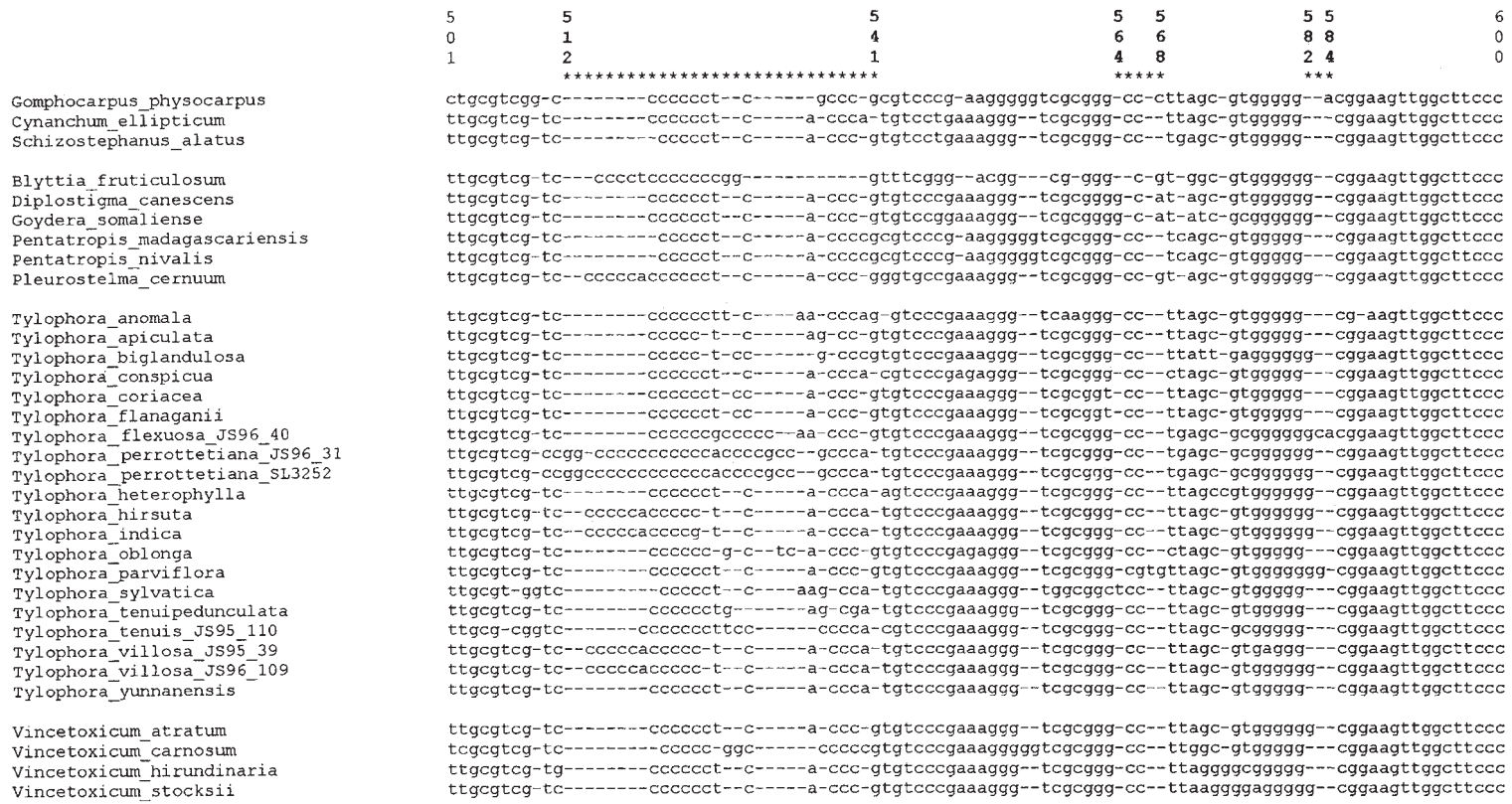
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Pleurostelma_cernuum
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Tylophora_apiculata
Tylophora_biglandulosa
Tylophora_conspicua
Tylophora_coriacea
Tylophora_flanaganii
Tylophora_flexuosa_JS96_40
Tylophora_perrottetiana_JS96_31
Tylophora_perrottetiana_SL3252
Tylophora_heterophylla
Tylophora_hirsuta
Tylophora_indica
Tylophora_oblonga
Tylophora_parviflora
Tylophora_sylvatica
Tylophora_tenuipedunculata
Tylophora_tenuis_JS95_110
Tylophora_villosa_JS95_39
Tylophora_villosa_JS96_109
Tylophora_yunnanensis
Vincetoxicum_atratum
Vincetoxicum_carnosum
Vincetoxicum_hirundinaria
Vincetoxicum_stocksii

```
                                   6                                                                       6 6                        7
                                   0                                                                       7 7                        0
                                   1                                                                       3 5                        0
                                                                                                           ***
Gomphocarpus_physocarpus           gtgcagcgtt--cgcggctagcccgaaaca-cgg-ctccc-tcgccgcggaggtcgcgacaa-gt-ggtggt---cgtcg-agatt----gtacgcgagt
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Vincetoxicum_atratum               gtgcagcgtt--tgcggctagcctgaaacaacgg-ttccc-tcggcgcggacgtcgcgacaa-gt-ggtggt---cgtcg-agatt----gtacgcgagt
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Vincetoxicum_hirundinaria          gcgcggcgtt--tgcggctagcctgaaacaacgg-ttccc-tcggcgcggacgtcgcgacaa-gt-ggtggtc--cgtcg-agatt----gtacgcgagt
Vincetoxicum_stocksii              gtgcggcgtt--tgcggctagcctgaaacgacgg-ttccc-tcggcgcggacgtcgcgacaaagt-ggtggtc--cgtcg-agatt----gtacgcgagt
```


7 7 7 8
0 8 8 9 0
1 0 6 0 0
26s
Gomphocarpus_physocarpus
Cynanchum_ellipticum
Schizostephanus_alatus
Blyttia_fruticulosum
Diplostigma_canescens
Goydera_somaliense
Pentatropis_madagascariensis
Pentatropis_nivalis
Pleurostelma_cernuum
Tylophora_anomala
Tylophora_apiculata
Tylophora_biglandulosa
Tylophora_conspicua
Tylophora_coriacea
Tylophora_flanaganii
Tylophora_flexuosa_JS96_40
Tylophora_perrottetiana_JS96_31
Tylophora_perrottetiana_SL3252
Tylophora_heterophylla
Tylophora_hirsuta
Tylophora_indica
Tylophora_oblonga
Tylophora_parviflora
Tylophora_sylvatica
Tylophora_tenuipedunculata
Tylophora_tenuis_JS95_110
Tylophora_villosa_JS95_39
Tylophora_villosa_JS96_109
Tylophora_yunnanensis
Vincetoxicum_atratum
Vincetoxicum_carnosum
Vincetoxicum_hirundinaria
Vincetoxicum_stocksii

```
                                         8        8 8
                                         0        1 1
                                         1        0 4
Gomphocarpus_physocarpus                 accccaggtcaggc
Cynanchum_ellipticum                     accccaggtcaggc
Schizostephanus_alatus                   accccaggtcaggc

Blyttia_fruticulosum                     accccaggtcaggc
Diplostigma_canescens                    accccaggtcaggc
Goydera_somaliense                       accccaggtcaggc
Pentatropis_madagascariensis             accccaggtcaggc
Pentatropis_nivalis                      accccaggtcaggc
Pleurostelma_cernuum                     accccaggtcaggc

Tylophora_anomala                        accccaggtcaggc
Tylophora_apiculata                      accccaggtcaggc
Tylophora_biglandulosa                   accccaggtcaggc
Tylophora_conspicua                      accccaggtcaggc
Tylophora_coriacea                       accccaggtcaggc
Tylophora_flanaganii                     accccaggtcaggc
Tylophora_flexuosa_JS96_40               accccaggccaggc
Tylophora_perrottetiana_JS96_31          accccaggtcaggc
Tylophora_perrottetiana_SL3252           accccaggtcaggc
Tylophora_heterophylla                   accccaggtcaggc
Tylophora_hirsuta                        accccgggtcaggc
Tylophora_indica                         accccgggtcaggc
Tylophora_oblonga                        accccaggtcaggc
Tylophora_parviflora                     accccaggtcaggc
Tylophora_sylvatica                      accccaggtcaggc
Tylophora_tenuipedunculata               accccaggtcaggc
Tylophora_tenuis_JS95_110                accccaggtcaggc
Tylophora_villosa_JS95_39                acccc?????????
Tylophora_villosa_JS96_109               accccgggtcaggc
Tylophora_yunnanensis                    accccaggtcaggc

Vincetoxicum_atratum                     accccaggtcaggc
Vincetoxicum_carnosum                    accccaggtcaggc
Vincetoxicum_hirundinaria                accccaggtcaggc
Vincetoxicum_stocksii                    accccaggtcaggc
```