compitum is the unified pollen tube transmitting tract, which allows the distribution of pollen tubes. In the Apocynaceae, where the overwhelming majority of species are apocarpous, a special type of compitum is formed through the postgenital fusion of the carpel apices, allowing pollen that has been deposited only on one side to be distributed to both carpels (or in the case of the higher pollinium-bearing Apocynaceae, a single inserted pollinium to distribute pollen tubes into both ovaries) (Endress et al., 1983; Kunze, 1991). Secamonoideae have been reported to lack a true style (Swarupanandan et al., 1996), but there is a true style above the carpels in *Pervillaea* and *Calyptranthera*, both of which belong to this subfamily (Omlor, 1996; Klackenberg, 1997). Upwardly directed, sterile placental margins are found in *Secamone*, but also in *Mandevilla* (basal Apocynaceae), whereas in most basal Apocynaceae, Periplocoideae, and Asclepiadoideae the sterile margins are directed downward (Woodson & Moore, 1938; Safwat, 1962; Nicholas & Baijnath, 1994). A critical reexamination of Brown's (1810) palynological characters has led to the proposal of a new set of diagnostic pollinium characters (Civeyrel et al., 1998), which, at present, are the only characters that reliably define the subfamily Secamonoideae. Members of the Secamonoideae can be distinguished from Periplocoideae and Asclepiadoideae by "having 20 pollinia, with their inner walls reduced, and which are connected to a translator apparatus composed of a corpusculum and one (or rarely two) caudicula, in addition to various degrees of staminal synorganization" (Civeyrel et al., 1998: 523). Pollinarium characters, especially the combination of number of pollinia and the way they are attached to the translator apparatus, remain the most valuable characters to distinguish this group. In Asclepiadoideae there are only 10 pollinia, which become attached to the caudiculae of the translator apparatus during ontogeny, whereas in Periplocoideae the 20 pollinia, when they are present, are not attached to the translator apparatus via caudiculae, but are shed onto it, which is very distinctive.

The Secamonoideae, which contain 7 generally recognized genera (*Secamone*, *Toxocarpus*, *Genianthus*, *Pervillaea*, *Secamonopsis*, *Calyptranthera*, and *Trichosandra*) and under 200 species, are restricted to the Old World tropics. There are also two genera of uncertain taxonomic position, i.e., *Goniostemma* and *Schistocodon*. The monotypic African genus *Rhynchostigma* has recently been put into synonymy under *Secamone* (Klackenberg, in press). *Secamone* is the largest genus with more than 80 species, which occur mainly in Madagascar (Klackenberg, 1992a), Africa (Goyder, 1992), and Asia (Forster & Harold, 1989; Klackenberg, 1992b). *Toxocarpus* with almost 40 species occurs mainly in Asia, as does *Genianthus* with 16 Asiatic species (Klackenberg, 1995a). The other four genera, *Pervillaea* (Klackenberg, 1995b), *Secamonopsis* (Civeyrel & Klackenberg, 1996), *Calyptranthera* (Klackenberg, 1996a; Klackenberg, 1997), and *Trichosandra* (Friedman, 1990) are restricted to Madagascar or to the Mascarene Islands, with less than 10 species each. The main center of endemism is Madagascar, where half of the known species and genera occur, followed by southeast Asia and Africa.

Malagasy genera of Secamonoideae, e.g., *Secamone*, especially the *S. cristata* group (*S. cristata* and its four subspecies, as well as *S. bosseri* and *S. polyantha*), *Pervillaea*, and *Secamonopsis*, show a remarkable range of growth habits, from erect to partially procumbent, small-bodied shrubs to larger-bodied twining lianas. Phylogenetic analysis of Secamonoideae offers the opportunity to analyze changes in growth habit during the evolutionary radiation of this group of plants in Madagascar, particularly with reference to changes from lianoid to shrubby habits. The former Asclepiadaceae are a predominantly lianoid family, but previous analyses have demonstrated reversals from lianoid growth forms to self-supporting habits (Civeyrel, 1996). Biomechanical and anatomical studies have been recently carried out to characterize different plant growth forms and to critically assess the developmental characters that underlie changes in stem mechanics (Rowe & Speck, 1998; Speck & Rowe, 1999). For the Secamonoideae we were interested in changes in growth form, in particular transitions from climbing forms to self-supporting species. We also wanted to see whether the mechanics and underlying anatomical development would be similar for lianas of different species, and how historical developmental constraints might have influenced the evolution of growth forms. Our initial investigation of the Secamonoideae presented here illustrates the differences in biomechanical behavior between two species of *Secamonopsis* also included in the phylogenetic analysis, with one represented by a self-supporting shrub and the other a twining liana. Our preliminary analysis represents a basis for examining the developmental homologies underlying the lianescent growth forms within the group and for investigating those sporadic switches to self-supporting growth forms within a predominantly lianoid group.

The Secamonoideae also show a range of interesting synorganizations in their flowers. In the former Asclepiadaceae, there is an unusual synorganization between floral parts and also between organs of different categories (Endress, 1990, 1996). Endress (1990) has described synorganization as the intimate structural connection of two or several neighboring structures to form a functional system or apparatus. In Secamonoideae there is a special kind of synorganization that occurs between pollinia and the translator apparatus, as well as within pollinia (Civeyrel, 1994, 1996). The pollinarium of Secamonoideae is composed of four pollinia connected to the translator apparatus, which in turn is made up of a corpusculum and one or two caudicula. Two caudicula have been observed in *Secamone* (Civeyrel, 1994), *Genianthus* (Civeyrel, 1996), and *Secamonopsis* (Civeyrel, 1996; Civeyrel & Klackenberg, 1996; Omlor, 1996). The four pollinia belonging to one pollinarium are each derived from a different pollen sac coming from one theca each of two adjacent anthers and are attached to the translator apparatus, which is secreted by the stigma head. This is the most common form of synorganization found in most taxa of the former Asclepiadaceae (Fig. 1A). In some taxa of the Secamonoideae there is, additionally, a special type of synorganization between pollinia from the same anther (intrapollinial synorganization) (Fig. 1B), as well as synorganization between pollinia from adjacent anthers (interpollinial organization; Fig. 1C). Synorganization within pollinia of an anther, and especially this special type of synorganization of pollinia from the pollen sacs of different anthers constitute the only record of this sort of synorganization in the angiosperms.

THE PLASTID GENE *mat*K

Systematists use cladistic analyses to study relationships among taxa but also to observe character evolution (Sibley & Ahlquist, 1987; Mickevich & Weller, 1990). Changes in morphological characters on a cladogram may also be evaluated simply by mapping characters onto molecular phylogenies, or observed directly in analyses combining molecular and morphological characters. One gene frequently used in phylogenetic reconstruction in recent years has been *mat*K (Steele & Vilgalys, 1994; Johnson & Soltis, 1994, 1995; Johnson et al., 1996; Liang & Hilu, 1996; Plunkett et al., 1996; Soltis et al., 1996; Hilu & Liang, 1997; Manos, 1997; Plunkett et al., 1997; Sang et al., 1997; Matsumoto et al., 1998; Xiang et al., 1998; Hilu & Alice, 1999; Kron et al., 1999; Les et al., 1999; Li et al., 1999; Thiv et al., 1999; Wang et al., 1999; Yokoyama et al., 2000) because of its suitable rate of mutation and resolution for infrafamilial relationships. The plastid gene *mat*K (Liere & Link, 1995; Neuhaus & Link, 1987; Sugita et al., 1985; Wolfe, 1991; Wolfe et al., 1991, 1992) is a single-copy gene of approximately 1530 base pairs in length, situated in the large single-copy region of the chloroplast. The plastid gene *mat*K has been previously used to assess the complex relationships within Apocynaceae (Endress et al., 1996; Civeyrel, 1996; Civeyrel et al., 1998), and this new set of molecular, morpho-palynological, and biomechanical characters should help to resolve the relationships and shifts in reproductive morphology and growth habit outside and inside the subfamily Secamonoideae with other groups of Apocynaceae sensu lato.

Indels have been shown to be useful in phylogenetic reconstruction. Indels in coding regions are generally useful to circumscribe lineages and define evolutionary trends (Hilu & Alice, 1999). In the plastid gene *mat*K, indels occur quite frequently and some are phylogenetically informative (Johnson & Soltis, 1994, 1995; Steele & Vilgalys, 1994; Plunkett et al., 1996, 1997; Xiang et al., 1998; Kron et al., 1999).

Materials and Methods

MATERIALS

Ten sequences belonging to the Secamonoideae are published here for the first time and added to the 46 sequences of Gentianales and Solanales previously published (Civeyrel et al., 1998; Endress et al., 1996). Table 1 provides information on these taxa, their voucher specimens and source, as well as EMBL accession numbers for new sequences. The family Apocynaceae constitutes the ingroup with taxa from Solanaceae, Rubiaceae, Loganiaceae, and Gentianaceae forming the outgroup. In the former Asclepiadaceae, representatives of all subfamilies and tribes were included.

METHODS

DNA preparation. Detailed protocols used have been published by Civeyrel et al. (1998) and will only be summarized here. Total DNA was extracted using the 2X CTAB protocol of Doyle and Doyle (1987). DNA was precipitated using ethanol or propan-2-ol, and proteins were removed with SEVAG (24:1, chloroform and isoamyl alcohol). DNA was purified by ultracentrifugation on a CsCl-ethidium bromide gradient (1.55 g/ml). Double-stranded products of *mat*K were amplified from total DNA

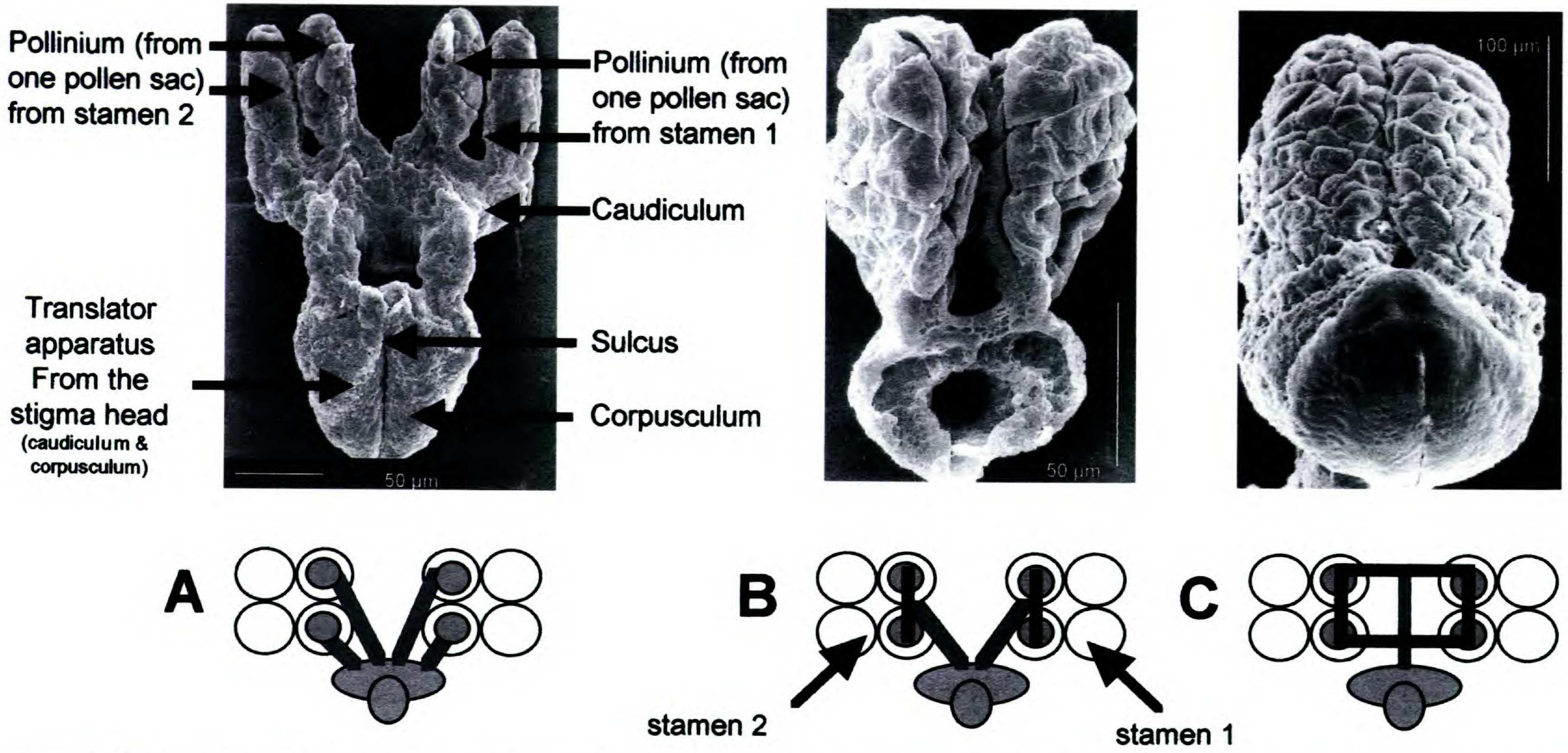

Figure 1. Synorganization within Secamonoideae. —A. *Secamone ligustrifolia*, synorganization only between pollinia and translator apparatus (in gray), no pollinial synorganization. —B. *Secamone dewevrei* subsp. *dewevrei*, intrapollinial synorganization (in black). —C. *Secamone alba*, intra- and interpollinial synorganization (in black).

Table 1. Taxa used in this study. For each taxon, the major subdivisions to which it belongs, geographic area of origin, the voucher for the material used, and EMBL accession numbers used for the 10 newly reported DNA sequences of the plastid gene *mat*K are given; * indicates a sequence published in Endress et al. (1996); ** indicates a sequence published in Civeyrel et al. (1998). Pol indicates when used for pollen. Samples used for biomechanical studies are at the end of the table.

Taxonomic divisions	Genus, species author(s)	Voucher	*Collector, #*	Herbarium	DNA	Pol	EMBL
OUTGROUP							
GELSEMIACEAE							
Gelsemieae	*Gelsemium sempervirens* (L.) J. St.-Hil.	N. Amer.	*Civeyrel 1069*	TL	DNA		*
GENTIANACEAE							
Gentianoideae	*Gentiana verna* L.	Yugoslavia	*Civeyrel 1108*	TL	DNA		*
LOGANIACEAE							
Loganieae	*Geniostoma rupestre* J. R. Forst. & G. Forst.	New Zealand	*Garnock-Jones 2200*	WELTU	DNA		*
Strychneae	*Strychnos nux-vomica* L.	India	*Civeyrel 1096*	TL	DNA		*
PLOCOSPERMATACEAE							
Plocospermeae	*Plocosperma buxifolium* Benth.	Mexico	*Salinas 8050*	Z	DNA		*
RUBIACEAE							
Cinchonoideae							
Cinchoneae	*Cinchona pubescens* Vahl	Peru	*Civeyrel 1063*	TL	DNA		*
Coptosapelteae	*Luculia gratissima* (Wall.) Sweet	India	*Civeyrel 1073*	TL	DNA		*
Ixoroideae							
Gardenieae	*Gardenia thunbergia* L. f.	South Africa	*Civeyrel 1068*	TL	DNA		*
SOLANACEAE							
Solaneae	*Solanum tuberosum* L.	Cultivated	*Du Jardin s.n.*	/	DNA		unpublished
INGROUP							
APOCYNACEAE							
Rauvolfioideae							
Alstonieae	*Alstonia scholaris* (L.) R. Br.	India	FK 212	FTG	DNA		*
Vinceae	*Kopsia fruticosa* (Ker Gawl.) A. DC.	Malaya	*Bremer 3033*	UPS	DNA		*
	Rauvolfia mannii Stapf	Tanzania	*Sennblad 218*	UPS	DNA		*
Tabernaemontaneae	*Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult.	India	*Civeyrel 1097*	TL	DNA		*
	Molongum laxum (Benth.) Pichon	Venezuela	*Romero et al. 3016*	Z	DNA		*
Alyxieae	*Chilocarpus suaveolens* Blume	Indonesia	*Chase 1208*	K	DNA		*
Pleiocarpeae	*Picralima nitida* (Stapf) T. Durand & H. Durand	Ivory Coast	*Leeuwenberg 12025*	UPS	DNA		*
Plumerieae	*Plumeria rubra* L.	Nigeria	*Civeyrel 1087*	TL	DNA		*

Table 1. Continued.

Taxonomic divisions	Genus, species author(s)	Voucher	*Collector, #*	Herbarium	DNA	Pol	EMBL
	Thevetia peruviana (Pers.) K. Schum.	Peru	*Civeyrel 1100*	TL	DNA		*
	Allamanda cathartica L.	Brazil	*Civeyrel 1054*	TL	DNA		*
Carisseae	*Acokanthera oblongifolia* (Hochst.) Codd	South Africa	*Civeyrel 1053*	TL	DNA		*
Apocynoideae							
Wrightieae	*Beaumontia grandiflora* Wall.	India	*Civeyrel 1071*	TL	DNA		**
	Nerium oleander L.	France	*Civeyrel 1079*	TL	DNA		**
	Strophanthus divaricatus (Lour.) Hook. & Arn.	China	*Civeyrel 1094*	TL	DNA		*
Apocyneae	*Apocynum androsaemifolium* L.	U.S.A.	*Civeyrel 1058*	TL	DNA		*
Periplocoideae	*Camptocarpus mauritianus* Decne.	Reunion	*Civeyrel 1062*	TL	DNA		**
	Cryptostegia grandiflora R. Br.	Madagascar	*Civeyrel 1221*	TL	DNA		**
	Hemidesmus indicus (L.) R. Br. ex Schult.	India	*Civeyrel 1008*	TL	DNA		**
	Periploca graeca L.	Greece	*Civeyrel 1083*	TL	DNA		**
	Raphionacme welwitschii Schltr. & Rendle	Zaire	*Civeyrel 1088*	TL	DNA		**
	Schlechterella abyssinica (Chiov.) Venter & Verhoeven (ex. *Triodoglossum*)	Ethiopia	*Civeyrel 1102*	TL	DNA		**
Secamonoideae	*Genianthus laurifolius* (Roxb.) Hook. f.	India	*Saldanha & Ramamoorthy 1164*	HIFP		Pol	
	Pervillaea venenata (Baill.) Klack.	Madagascar	*Civeyrel 1248*	TL	DNA	Pol	**
	Pervillaea phillipsonii Klack.	Madagascar	*Civeyrel 1241*	TL	DNA	Pol	AJ312408
	Secamone alba Jum. & H. Perrier	Madagascar	*Humbert & Capuron 2395*	P		Pol	
	Secamone bosseri Klack.	Madagascar	*Civeyrel 1267*	TL	DNA	Pol	**
	Secamone cristata subsp. *densiflora* Klack.	Madagascar	*Civeyrel 1320*	TL	DNA		AJ312400
	Secamone cristata Jum. & H. Perrier	Madagascar	*Decary 15759*	P		Pol	
	Secamone dewevrei De Wild. subsp. *dewevrei*	Zaire	*Becquaert 7052*	K		Pol	
	Secamone buxifolia Decne.	Madagascar	*Civeyrel 1322*	TL	DNA	Pol	AJ312405
	Secamone ecoronata Klack.	Madagascar	*Civeyrel 1261*	TL	DNA		AJ312407
		Madagascar	*Humbert 13408*	P		Pol	
	Secamone elliottii K. Schum.	Madagascar	*Civeyrel 1304*	TL	DNA	Pol	AJ312402
	Secamone falcata Klack.	Madagascar	*Civeyrel 1228*	TL	DNA	Pol	AJ312404
	Secamone geayii Costantin & Gallaud	Madagascar	*Civeyrel 1200*	TL	DNA		**
		Madagascar	*Civeyrel 1241*	TL		Pol	
	Secamone ligustrifolia ssp. *angustifolia* (Decne.) Klack.	Madagascar	*Civeyrel 1324*	TL		Pol	
	Secamone minutifolia Choux	Madagascar	*Civeyrel 1257*	TL	DNA		AJ312406
		Madagascar	*Perrier 16701*	P		Pol	
	Secamone parvifolia (Oliv.) Bullock	Tanzania	*Goyder et al. 3960*	K	DNA		**
	Secamone parvifolia (Oliv.) Bullock	Kenya	*Gillet 13225*	K		Pol	

Table 1. Continued.

Taxonomic divisions	Genus, species author(s)	Voucher	*Collector, #*	Herbarium	DNA	Pol	EMBL
	Secamone sparsiflora Klack.	Madagascar	*Civeyrel 1244*	TL	DNA	Pol	AJ312401
	Secamone uncinata Choux	Madagascar	*Civeyrel 1309*	TL	DNA	Pol	AJ312403
	Secamone volubilis (Lam.) Marais	Reunion	*Civeyrel 1092*	TL	DNA		**
		Reunion	*Bosser 21424*	P		Pol	
	Secamonopsis microphylla Civeyrel & Klack.	Madagascar	*Civeyrel 1206*	TL	DNA	Pol	AJ312409
	Secamonopsis madagascariensis Jum.	Madagascar	*Civeyrel 1262*	TL	DNA	Pol	**
Asclepiadoideae							
Fockeeae	*Fockea capensis* Endl.	South Africa	*Civeyrel 1067*	TL	DNA		**
Marsdenieae	*Dregea sinensis* Hemsl.	China	*Civeyrel 1066*	TL	DNA		**
	Tylophora indica (Burm. f.) Merr.	India	*Civeyrel 1009*	TL	DNA		**
Ceropegieae	*Riocreuxia burchellii* K. Schum.	Africa	*Civeyrel 1109*	TL	DNA		**
Gonolobeae	*Gonolobus xanthotrichus* Brandegee	Mexico	*Civeyrel 1060*	TL	DNA		**
	Matelea quirosii (Standl.) Woodson	Mexico	*Civeyrel 1076*	TL	DNA		**
Asclepiadeae	*Araujia sericifera* Brot.	South Amer.	*Civeyrel 1059*	TL	DNA		**
	Pentarrhinum insipidum E. Mey.	South Africa	*Civeyrel 1081*	TL	DNA		**
	Pergularia daemia (Forssk.) Chiov.	India	*Civeyrel 1000*	TL	DNA		**
	Vincetoxicum nigrum (L.) Moench	France	*Civeyrel 1106*	TL	DNA		**
Samples for biomechanical studies							
	Secamonopsis microphylla Civeyrel & Klack.	Madagascar	*Civeyrel 1386*				
	Secamonopsis madagascariensis Jum.	Madagascar	*Civeyrel 1398*				

Table 2. External and internal primers for *mat*K. The boldfaced letters represent multiple bases present in the primer sequences: K represents the base G T, M represents the base A C and Y represents the base C T.

PRIMER	SEQUENCE
*trn*K 3914F	GGG GTT GCT AAC TCA ACG G
*mat*K -8F	AAT TTC AAA TGG AAG AAA TC
*mat*K 174F	TGT GAA ACG TTT AAT TAA TC
*mat*K 174R	CGA **K**TA ATT AA**M** CGT TTC AC
*mat*K 503F	TCG CTA TTG GGT AAA AGA TGC
*mat*K 503R	GCA TCT TTT ACC CAA TAG CG
*mat*K 681F	GTG AAT ACG AAT C**Y**A TTT TC
*mat*K 900F	TGG AAA TTT TAC CTT GTC AA
*mat*K 1309F	GAC TTT CTT GTG CTA GAA CT
*mat*K 1628R	CAT GCT ACA TCA ACA TTT CAG
*trn*K -2R	AAC TAG TCG GAT GGA GTA G

using one of the *trn*K primers in combination with an internal primer (list given in Table 2). Direct sequencing of the double-stranded PCR products was performed using the Taq Dye Deoxy™ Terminator Cycle Sequencing Kit. Excess dye terminators were removed using Centri-sep spin columns. Direct sequencing was performed on an ABI 373A DNA sequencer, and sequences were edited using the programs Sequence Navigator™ and Auto-Assembler™ (Applied Biosystems, Warrington, Cheshire).

ANALYSIS

For this study all but an average of the first 50 bases at the 5′ end of *mat*K were sequenced (our primers were not located so that we could accurately determine the sequence near the forward PCR primer). In all cases, sequences were aligned visually against the published *Solanum tuberosum* L. sequence (EMBL-Z11741, Du Jardin, unpublished) (aligned matrix available from author upon request). Alignment was straightforward; the length of the individual *mat*K sequences varied between 1509 and 1551 bp, and there were 24 insertions and deletions (indels), which are in triplets (involving 3 to 21 bp). Indels often consisted of the repetition of a sequence of base pairs present just before the indel itself. Seven of these indels are phylogenetically informative, and two are homoplasious (occurring in two distant genera). None of the indels were coded in the analysis, but inserted regions were retained and coded as missing. In total, the matrix was 1653 bp long with 443 potentially parsimony-informative characters (27%).

Cladistic analysis was performed using PAUP 3.1.1. (Swofford, 1993) with the following options: heuristic search, 1000 replicates of random taxon-additions, and TBR swapping. Two separate phases were performed: the first one with 1000 replicates, with complete swapping on all trees accumulated in the replicates (which should have found all trees at that length); the second, successive approximations weighting (hereafter SW; Farris, 1969), with characters reweighted according to their rescaled consistency index (RC) based on the best fit of characters on any of the trees. Reasons for using SW have been explained in a previous paper (Civeyrel et al., 1998). Rounds of re-weighting were repeated until the tree length did not change in two consecutive iterations. Confidence in specific clades of the resulting topology was estimated by bootstrap analysis. The following settings were used: 1000 replicates, keeping bootstrap frequencies from 1 to 100%, which were compatible with the 50% majority rule consensus tree, simple addition of taxa, sampling characters with equal probability but applying weights (from SW), and NNI swapping (nearest-neighbor interchange) but holding only 25 trees per step. All illustrated trees use the ACCTRAN optimization. The base weight of 1000 applied in SW was removed for tree presentation.

POLLINARIUM PREPARATION

Samples examined are from the herbarium collection at the Royal Botanical Gardens, Kew, from the author's alcohol collection, and from fieldwork in Madagascar. Pollinaria were removed from flowers under a dissecting microscope, transferred to 100% ethanol, air dried on stubs, and coated with platinum using a Balzers Sputter Coater SPD 050. Entire flowers were prepared for observation by critical-point drying in a Balzers CPD 030; tissues were dehydrated in a graded ethanol series, transferred to acetone and to the CPD, and then observed with a Hitachi S2400 scanning electron microscope (SEM).

BIOMECHANICS AND ANATOMY

Measurements of flexural stiffness, *EI* (Newtons times square meters: Nm^2), structural Young's modulus, *E* (Mega Newtons per square meter: MNm^{-2}), and axial second moment of area, *I* (mm^4), of *Secamonopsis microphylla* and *S. madagascariensis* were taken during fieldwork in Madagascar in April 1999 near Tulear (23°24′S, 43°47′E). Flexural stiffness represents the tangible resistance to bending of a structure and is the product of the structural Young's modulus and the axial second moment of area. Structural Young's modulus is a value that

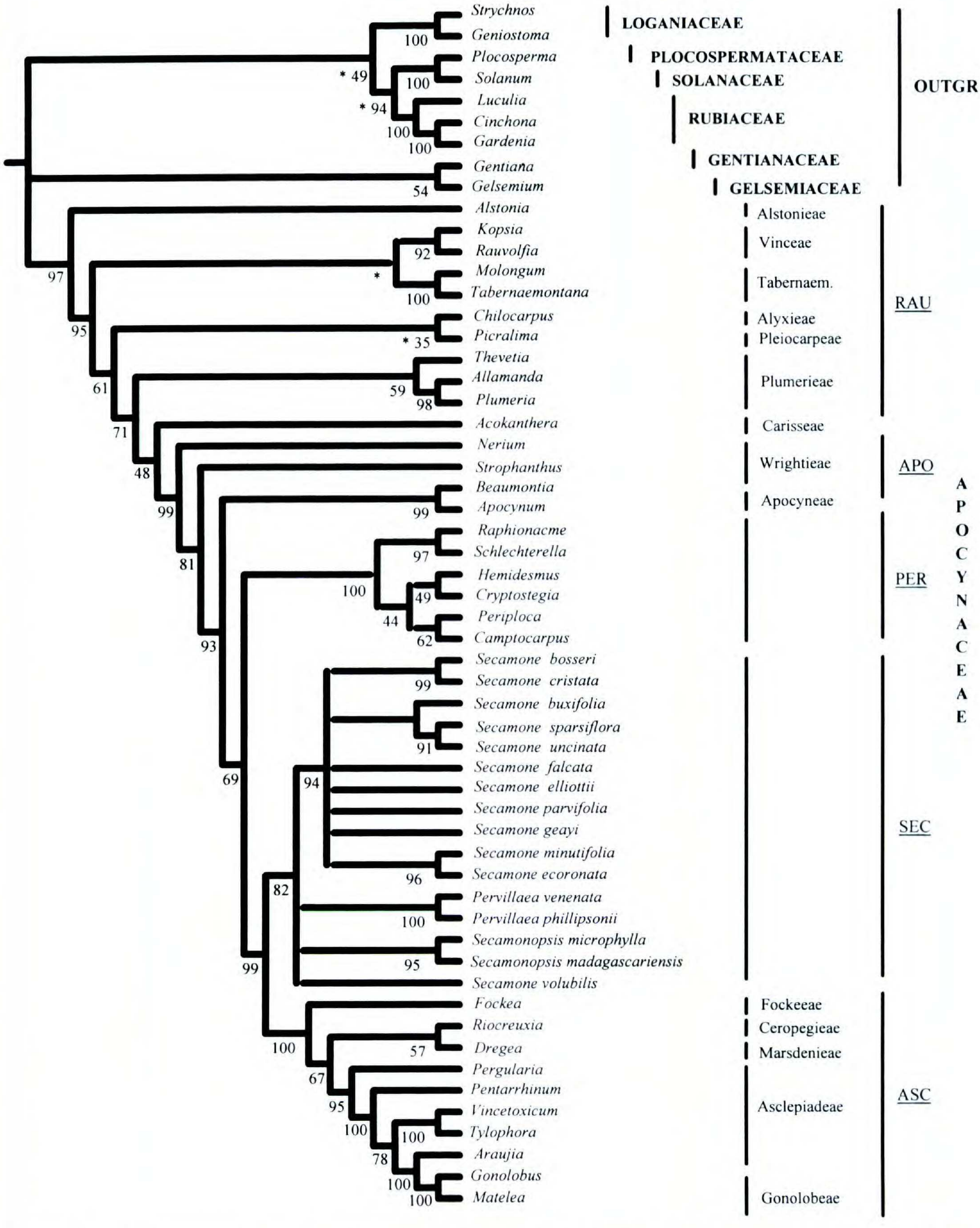

Figure 2. Strict consensus tree of the 25 most parsimonious trees obtained with the successive weighting analysis. Bootstrap values are shown below the branch. Abbreviations: APO, Apocynoideae; RAU, Rauvolfioideae; PER, Periplocoideae; SEC, Secamonoideae; and ASC, Asclepiadoideae; * denotes branches not present in the unit weighted strict consensus tree. This follows the classification published by Endress and Bruyns (2000).

describes the elastic mechanical properties of materials and is currently used for describing and comparing quantitatively changes in mechanical properties of plant stems during ontogeny (Rowe & Speck, 1998; Speck & Rowe, 1999): compliant or flexible materials have low Young's moduli, whereas stiffer materials have higher moduli. Stem segments from basal to distal parts of plants were pruned from living plants and submitted to mechanical bending tests within several hours of being cut.

	Indel A	**Indel C**
		111111111111
	111111111111111111111111111	111111111222
	000000111111111222222222 3	999999999000
Taxon	456789012345678901234567890	234567890123
Riocreuxia burchellii	CAT------AGTTTAAA**TTTAAAC**CGA	ATTAG**G**AATAAG
Pentarrhinum insipidum	CAT------AGTTTAAAC------CGA	ATTAGCAATAAG
Dregea sinensis	CAT------AGTTTAAAC------CGA	ATTAG**G**AATAAG
Pergularia daemia	CAT------AGTTTAAAC------CGA	ATT------AAG
Vincetoxicum nigrum	CAT------AGTTTAAAC------CGA	ATTAGCAATAAG
Tylophora indica	CAT------AGTTTAAAC------CGA	ATTAGCAATAAG
Araujia sericifera	C-T------AGTTTAAAC------CGA	ATTAGCAATAAG
Gonolobus xanthotrichus	CAT------AGTTTAAAC------CGA	ATTAGCAATAAG
Matelea quirosii	CAT------AGTTTAAAC------CGA	ATTAGCAATAAG
Fockea capensis	CAT------AGTTTAAAC------CGA	ATTAG**G**AATAAG
Secamone bosseri	CAT--------------------CGA	ATT------AAG
S. cristata ssp. densiflora	CAT--------------------CGA	ATT------AAG
S. sparsiflora	CAT--------------------CGA	ATT------AAG
S. uncinata	CAT--------------------CGA	ATT------AAG
S. elliottii	CAT--------------------CGA	ATT------AAG
S. geayi	CAT--------------------CGA	ATT------AAG
S. falcata	CAT--------------------CGA	ATT------AAG
S. buxifolia	CAT--------------------CGA	ATT------AAG
S. minutifolia	CAT--------------------CGA	ATT------AAG
S. ecoronata	CAT--------------------CGA	ATT------AAG
S. parvifolia	CAT--------------------CGA	ATT------AAG
S. volubilis	CATGACCATGGTTTAAAC------CGA	ATT------AAG
Pervillaea venenata	CAT------AGTTTAAAC------CGA	ATT------AAG
P. phillipsonii	CAT------AGTTTAAAC------CGA	ATT------AAG
Secamonopsis microphylla	CAT------GGTTTAAAC------CGA	ATT------AAG
Se. madagascariensis	CAT------GGTTTAAAC------CGA	ATT------AAG
Hemidesmus indicus	CAT------AGTTTAAAC------CGA	ATT------AAG
Raphionacme welwitschii	CAT------AGTTTAAAC------CGA	ATT------AAG
Schlechterella abyssinica	CAT------AGTTTAAAC------CGA	ATT------AAG
Cryptostegia grandiflora	CAT------AGTTTAAAC------CGA	ATT------AAG
Periploca graeca	CAT------AGTTTAAAC------CGA	ATT------AAG
Camptocarpus mauritianus	CAT------AGTTTAAAC------CGA	ATT------AAG
Allamanda cathartica	CAT------AGTTTAAAC------CGA	ATT------AAG
Nerium oleander	CAT------AGTTTAAAC------CGA	ATT------AAG
Apocynum androsaemifolium	CAT------AATTTAAAC------CGA	ATT------AAT
Beaumontia grandiflora	AAT------AGTTTAAAC------CGA	ATT------AAG
Strophanthus divaricatus	CAT------AGTTTAAAC------CGA	ATT------AAG
Acokanthera oblongifolia	CAT------AGTTTAAAC------CGA	ATT------AAG
Alstonia scholaris	CAT------AGTTTAAAC------CGA	ATT------AAG
Chilocarpus suaveolens	CAT------AGTTTAAAC------CGA	ATT------AAG
Kopsia fruticosa	CAG------AGTTTAAAC------CGA	ATT------AAG
Molongum laxum	CAT------AGTTTAAAC------CGA	ATT------AAG
Picralima nitida	CAT------AGTTTAAAC------CGA	ATT------AAG
Plumeria rubra	CAT------AGTTTAAAC------CGA	ATT------AAG
Rauvolfia mannii	CTA----------AAC------CGG	ATT------AAG
Tabernaemontana divaricata	CAT------AGTTTAAAC------CGA	ATT------AAG
Thevetia peruviana	CAT------AGTTTAAAC------CGA	ATT------AAG
Gentianales outgroups	***------**********------***	***------***
Solanum tuberosum	CAT------GGTTTAAATAG**AAATAG**G	***------***

Figure 3. Aligned sequences of the plastid gene *mat*K showing the three main phylogenetically informative indels A, B, and C. Numbers indicate position of nucleotides that are numbered consecutively from 5′ to 3′, dashes indicate gaps, question marks an unresolved sequence as for *Allamanda cathartica*, and bold sequences repeat underlined sequences. The sequences were replaced by a line of asterisks (**) for the outgroup taxa where they were present.

Indel B

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                                  6666666666666666666666666666666666666666666666666666
                                  4444455555555556666666666777777777888888888999999999
Taxon                             5678901234567890123456789012345678901234567890123456789
-------------------------------------------------------------------------------------------
Riocreuxia burchellii             AAAGAAAACCAGAAAGAAAACCAGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
Pentarrhinum insipidum            AAAGAAAACT----------------------TTTTTAACAAAAGGAACTCTAAAA
Dregea sinensis                   AAAGAAAACCAGAAAGAAAACCAGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
Pergularia daemia                 AAAGGAAATC------------GGTTTTCATTTTTTAACAAAACGAAATCTAAAA
Vincetoxicum nigrum               AAAGAAAACC------------AGTTTTCATTTTTTAACAAAAAGAAATCTAAAA
Tylophora indica                  AAAGAAAACC------------AGTTTTCATTTTTTAACAAAAAGAAATCTAAAA
Araujia sericifera                AAAGAAAACC------------AGTTTTCTTTTTTTAACAAAAAGAAATCTAAAA
Gonolobus xanthotrichus           AAAGAAATCC------------AGCTTTCTTTTTTTAACAAAAAGAAATCTAAAA
Matelea quirosii                  AAAGAAATCC------------AGTTTTCTTTTTTTAACAAAAAGAAATCTAAAA
Fockea capensis                   AAAGAAAACT------------TGTTTTCTTTTTTTAACAAAAAGAAATCTAAAA
Secamone bosseri                  AAAGAAAACC------------AGTTTTCATTTTTTGACA------------AAA
S. cristata ssp. densiflora       AAAGAAAACC------------AGTTTTCATTTTTTTAACA------------AAA
S. sparsiflora                    AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. uncinata                       AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. elliottii                      AAAGAA---C------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. geayi                          AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. falcata                        AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. buxifolia                      AAAGAAAACC------------AGTTTTCATTTTTTTA---AAAAGAAATCTAAAA
S. minutifolia                    AAAGAA---C------------TGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. ecoronata                      AAAGAAAACC------------TGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. parvifolia                     AAAGAAAACC------------AGTTTTCGTTTTTTTAACAAAAAGAAATCTAAAA
S. volubilis                      AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
Pervillaea venenata               AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
P. phillipsonii                   AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
Secamonopsis microphylla          AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
Se. madagascariensis              AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
Hemidesmus indicus                AAAGAAAACC------------AGGTTTCATTTTTTTAACAAAAAGAAATCCAAAA
Raphionacme welwitschii           AAAGAAAACC------------AGGTTTCATTTTTTTAACAAAAAGAAATCCAAAA
Schlechterella abyssinica         AAAGAAAACC------------AGGTTTCATTTTTTTAACAAAAAGAAATCCAAAA
Cryptostegia grandiflora          AAAGAAAACC------------AGGTTTCATTTTTTTAACAAAAAGAAATTCAAAA
Periploca graeca                  AAAGAAAACC------------AGGTTTCGTTTTTTTAACA------------AAA
Camptocarpus mauritianus          AAAGAAAACC------------AGGTTTCATTTTTTTAACA------------AAA
Allamanda cathartica              ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?
Nerium oleander                   AAARAAAACC------------AGKTTTCATTTTTTTAACA------------AAA
Apocynum androsaemifolium         AAAGAAACCC------------GGTTTTCTTTTTTTAACA------------AAA
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Strophanthus divaricatus          AAAGAAAACC------------AGTTTCAATTTTTTTAACA------------AAA
Acokanthera oblongifolia          AAAGAAACCC------------AGTTTTTATTTTTTTAACA------------AAA
Alstonia scholaris                AAAGAAACCC------------AGTTTTTCTTTTTTTAACA------------AAA
Chilocarpus suaveolens            AAAGAAACCC------------GGTTTTGATTTTTTTAACA------------AAA
Kopsia fruticosa                  AAAAAAACCC------------AGTTTCGATTTTTTTAACA------------AAA
Molongum laxum                    AAAGAAAGAC------------AGTTTTTATTTTTTTAACA------------AAA
Picralima nitida                  AAAGAAAGCC------------GGTTTTGCTTTTTTTCACA------------AAA
Plumeria rubra                    AAAGAAACCC------------AGTTTGGATTTTTTTAACA------------AAA
Rauvolfia mannii                  AAAGAACCCC------------AGTTTTTCTTTTTTTAACA------------AAA
Tabernaemontana divaricata        AAAGAAAGTC------------GGTTTTGATTTTTTTAACAAAAATAAATCCAAAA
Thevetia peruviana                AAAGAACCCC------------CGCTTGGATTTTTTTAACA------------AAA

Gentianales outgroups             **********------------**********------------AAA
Solanum tuberosum                 **********------------**********------------***
```

Figure 3. Continued.

Representative axis segments were selected from the entire plant in order to observe trends in mechanical properties all over the stem system for both shrub and liana. The bending tests were carried out on a steel frame bending apparatus in the close vicinity of the collected sample. Span distance of the tested stems and weight increments were varied according to the size and bending resistance of the material selected (see Rowe & Speck, 1996; Speck & Rowe, 1999). Segments of each tested stem were retained in FAA for anatomical and developmental analyses of plant axes.

Results

PHYLOGENETIC ANALYSIS

The unit weight analysis of the complete data set resulted in 600 trees 1679 steps long, with a consistency index of CI = 0.642 (0.530 excluding uninformative characters) and a retention index of RI = 0.730. The successive weighting analysis resulted in 25 trees 1407 steps long (base weight of 1000 applied in Successive Weighting, SW, removed), with a consistency index of CI = 0.867 (0.725 excluding uninformative characters) and a retention index of RI = 0.900. The strict consensus tree of the unit weighting analysis and from the successive weighting are almost identical; only four branches at the base of the tree are not present in the unit weighting analysis. Therefore, comments in the discussion will be based only on the strict consensus tree (Fig. 2) of the SW analysis. In this analysis three indels, occurring in several taxa (Fig. 3), have been examined as putative molecular markers for this phylogeny. They are usually repetitions (represented in bold) of an adjacent sequence (with this adjacent sequence underlined) (Fig. 3).

POLLINARIUM STRUCTURE AND INSERTION

The morphology of non-acetolyzed pollinaria of Secamonoideae was examined and morphological differences were observed in pollinia, corpusculum, and caudicula (Civeyrel, 1994, 1996), as well as pollinia insertion. The corpusculum is coffee bean-shaped, with a more or less narrow slit facing the pollinator. The back of the corpusculum is more or less spongy with perforations of different sizes; the front is generally more compact with a verrucate or smooth surface. There are one or two caudicula bearing the pollinia; when there is only one caudiculum, it can be hemispherical (Fig. 4A, B, G) or elongated (Fig. 4D, C, E) and with the pollinia almost sessile on the back. In some cases the corpusculum is surrounded by a caudiculum without a distinct shape bearing four fused pollinia (Figs. 4F, H, I, 5A, B, D, E). When two caudicula are present, they are either short (Figs. 1B, 5F) or long (Fig. 5C, G), and bear a pair of fused pollinia. The samples examined included pollinia arranged in all possible configurations of synorganization. They may be disposed all around the caudiculum (Fig. 4A, B, C, G) or tiered in pairs when there is no pollinial synorganization (Fig. 4D, E). They are fused in pairs when there is intrapollinial synorganization (Figs. 1B, 5C, F, G) or in a unit of four in the case of intra- and interpollinial synorganization (Figs. 4F, H, I, 5A, B, D, E). A reduction of pollen walls between fused pollinia has also been observed (Civeyrel, 1995).

Pollinium insertions have been examined in *Secamone geayi* and in *S. buxifolia*. In *Secamone buxifolia* only a part of the pollinarium was inserted into the pollination chamber, and sometimes only one pollinium, whereas in *Secamone geayi* the entire pollinarium was inserted.

BIOMECHANICAL RESULTS OF A SECAMONOID SHRUB AND LIANA

Both *Secamonopsis madagascariensis*, a twining liana, and *S. microphylla*, a shrub, show reductions in Young's modulus of the stem during ontogeny. Plant ontogeny is depicted here as increasing stem diameter as indicated by the increase in *I*, second moment of area, in Figure 6. Young distal stages of *S. microphylla* show relatively high values of *E* for the stem of just over 5100 MNm^{-2}; this value drops during ontogeny to around 2000 MNm^{-2}. The drop in Young's modulus during ontogeny is more marked in the twining liana *S. madagascariensis*; young distal stages show values of just over 2000 MNm^{-2} and this is followed by a drop to as low as just over 300 MNm^{-2} in the oldest sample measured.

Discussion

STRICT CONSENSUS TREE

The three subfamilies of the former Asclepiadaceae are monophyletic, with Secamonoideae as sister group of the Asclepiadoideae (Fig. 2). Asclepiadoideae and Periplocoideae are both strongly supported, each with a bootstrap value of 100%; the Secamonoideae are supported by a bootstrap value of 82%. The composite group formed by the three subfamilies of the former Asclepiadaceae is less well supported with a bootstrap value of only 69%. The monophyly of the former Asclepiadaceae, which is only poorly supported here, has been much questioned in recent years (Sennblad & Bremer, 1996; Sennblad et al., 1998; Sennblad & Bremer, 2000; Potgieter & Albert, 2001 this volume) and is far from being resolved. The systematic position of the Periplocoideae, however, is beyond the scope of this paper. Here we will focus on the relationships within Secamonoideae.

Within Secamonoideae five groups are strongly supported by bootstrap values above 90% (Fig. 2). These groups are: (1) *Secamonopsis*, (2) *Pervillaea*, (3) the two species belonging to the *Secamone cristata* group sampled here (*S. cristata* subsp. *densiflora* and *S. bosseri*), and two groups

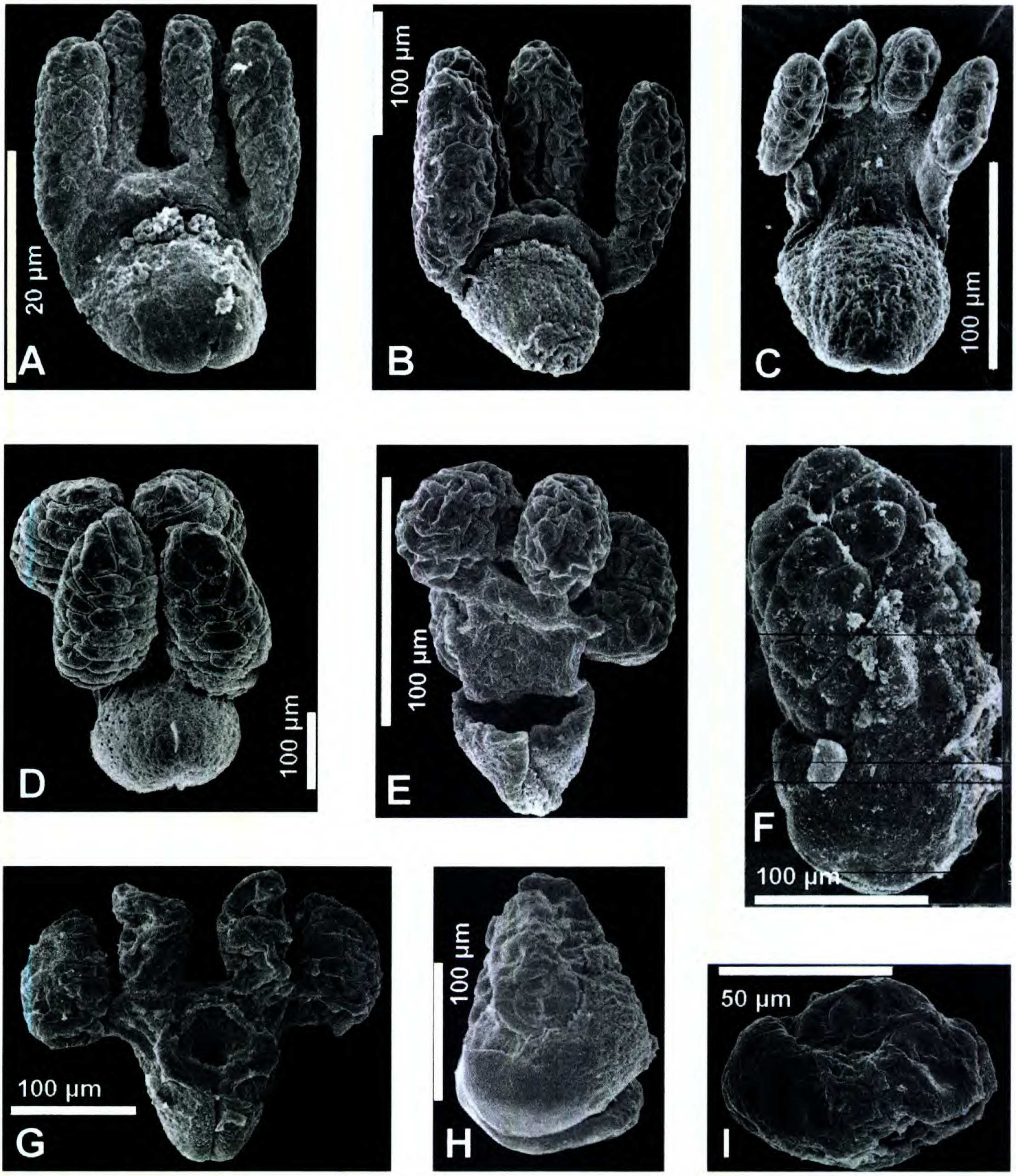
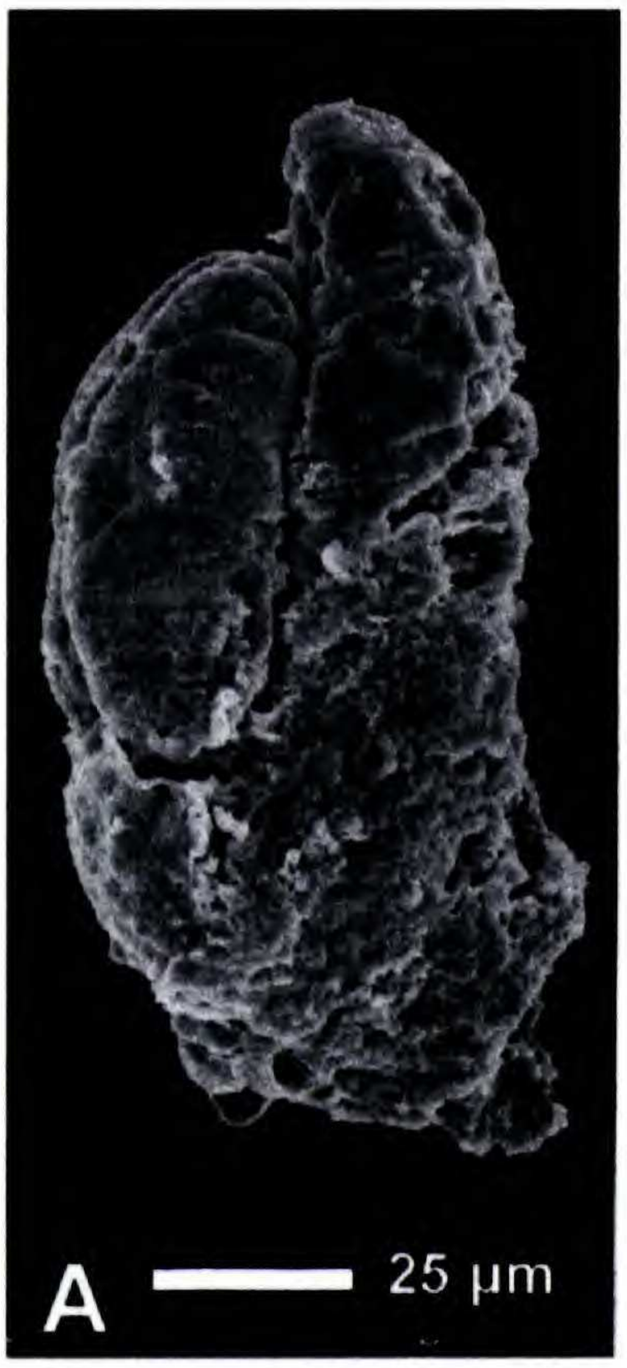


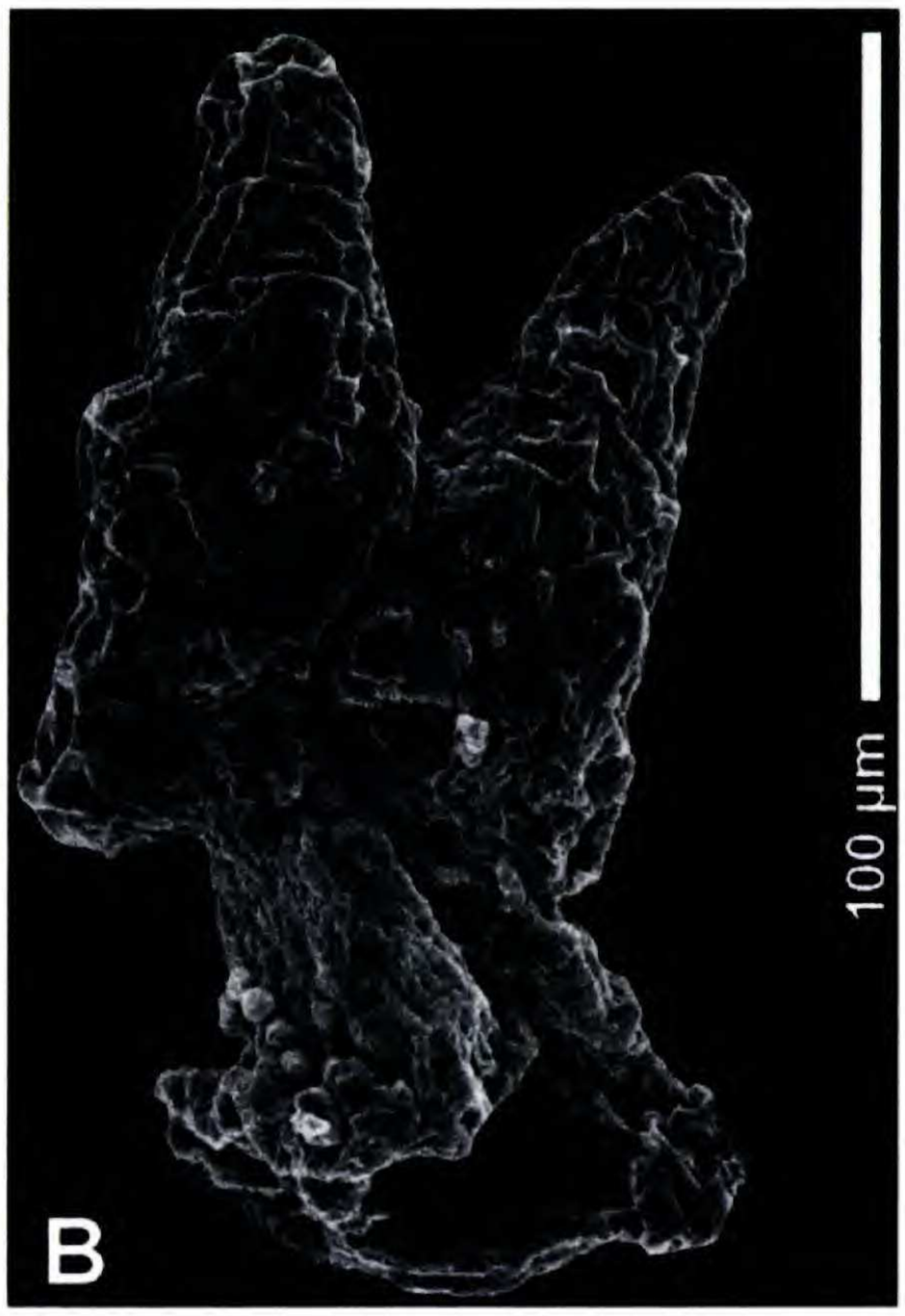
Figure 4. Pollinaria of *Secamone*, SEM photos. —A. *S. sparsiflora*. —B. *S. uniciata*. —C. *S. buxifolia*. —D. *S. parvifolia*. —E. *S. elliottii*. —F. *S. geayi*. —G. *S. falcata*. —H. *S. ecoronata*. —I. *S. minutiflora*. Voucher specimens are cited in Table 1.

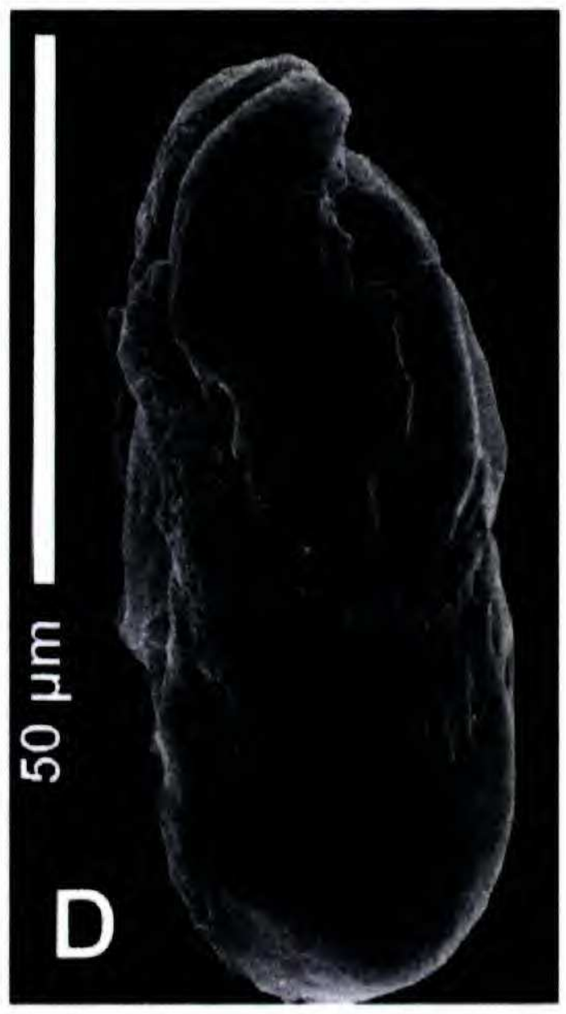
within the remainder of the *Secamone* clade, (4) *S. sparsiflora* and *S. unciata*, and (5) *S. minutifolia* and *S. ecoronata*. There is no strong support for the position of *Secamone volubilis*, endemic to Reunion Island. The only African *Secamone* included in this study, *S. parviflora*, is associated with the poorly resolved clade of Malagasy *Secamone*. Unless more sampling is done on species from the African mainland and from Asia and Australasia it will be difficult to assess the origin of the Madagascan species.

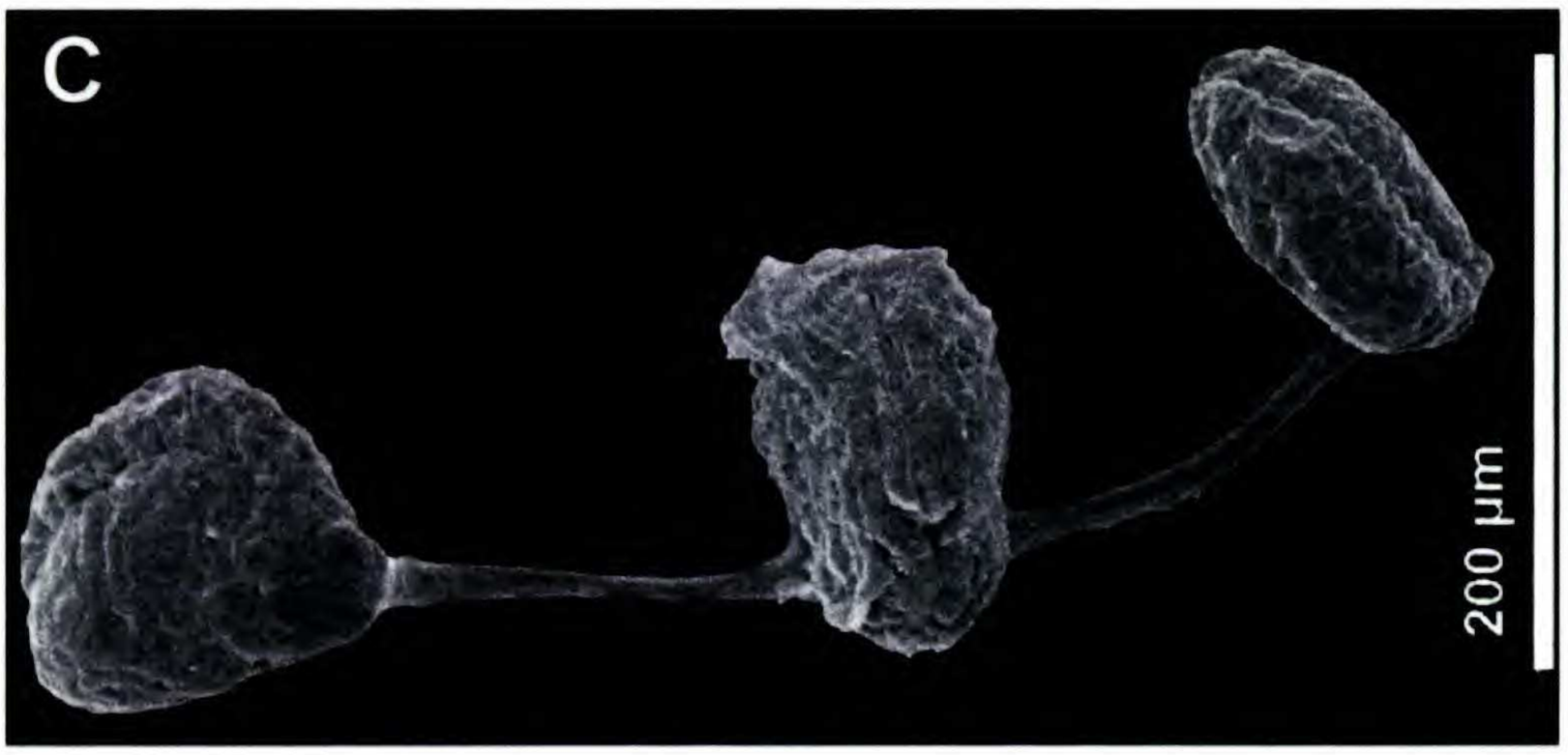
INDELS

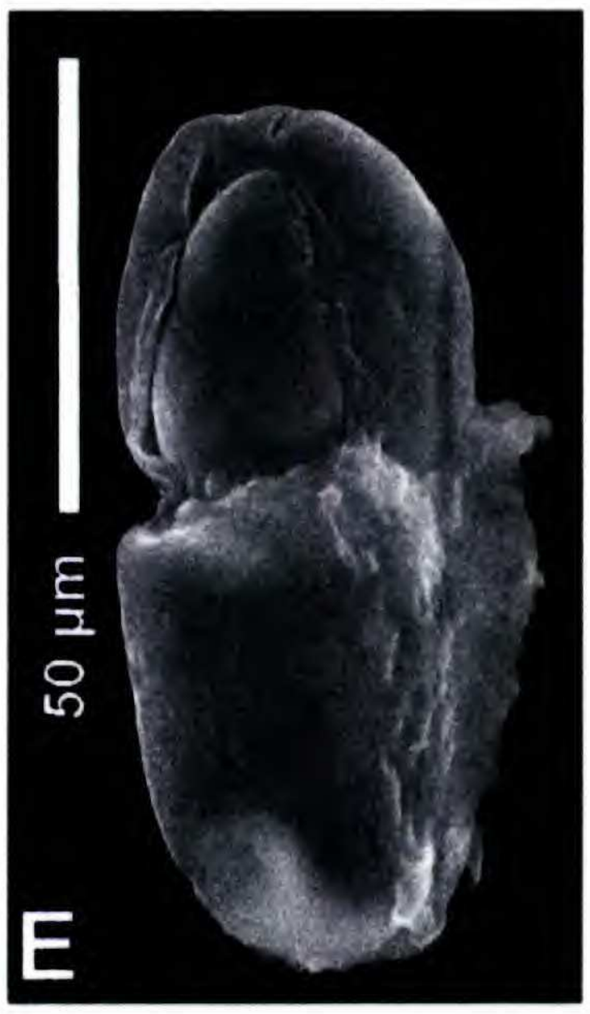
A six-base insertion from bp 1195 to 1200 characterizes the Asclepiadoideae group (with the no-

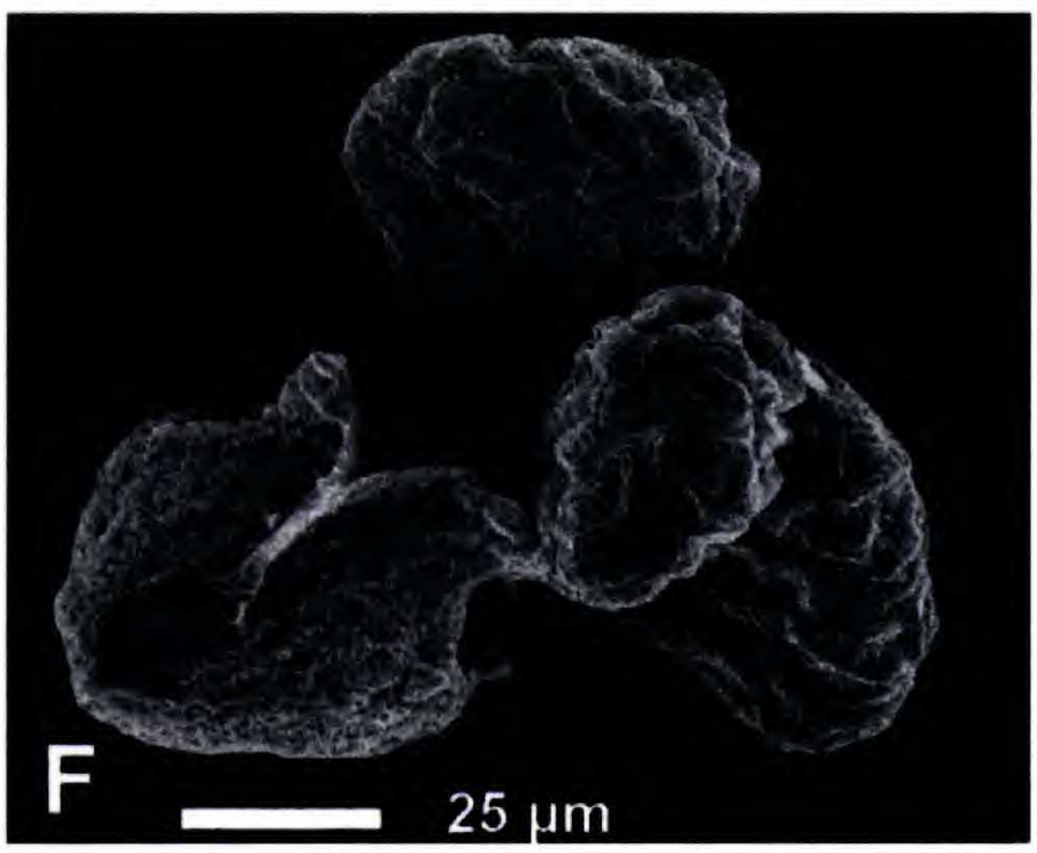












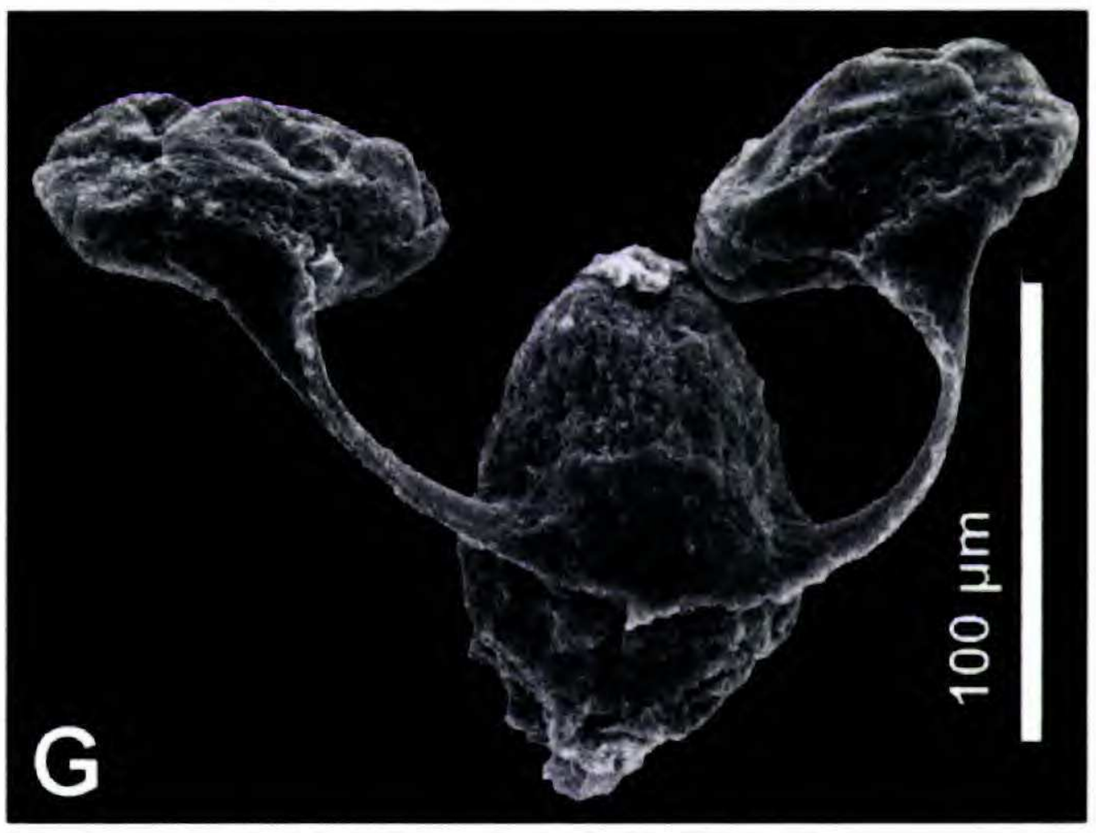


Figure 5. Pollinaria of Secamonoideae, SEM photos. —A. *Pervillea venenata.* —B. *Pervillea phillipsonii.* —C. *Secamonopsis madagascariensis.* —D. *Secamone bosseri.* —E. *Secamone cristata.* —F. *Genianthus laurifolius.* —G. *Secamonopsis microphylla.* Voucher specimens are cited in Table 1.

table exception of *Pergularia daemia*), and has not been found in any other Apocynaceae or among the outgroup taxa (Fig. 3, C region). Within this six-pair base insertion there is only one change: in the basal taxa *Fockea*, *Riocreuxia*, and *Dregea*, a G (2′-deoxyguanosine) is found on position 1197, replacing the C (2′-deoxycytidine) found in all other taxa. This insertion could be useful for identifying possible members of the Asclepiadoideae, such as sterile herbarium specimens within the family Apocynaceae, which contains between 4000 and 6000 species.

Another interesting indel that we have been investigating is actually a series of indels found in the *mat*K region from 639 to 696 bp (Fig. 3, B region). It has been found in all the former Asclepiadaceae, with the exception of two taxa belonging to the *Secamone cristata* group and two species of Periplocoideae in *Periploca* and *Camptocarpus*. This insertion has also been found in two unrelated taxa in the basal Apocynaceae: *Beaumontia* and *Tabernaemontana*. Looking closely at the base composition of this insertion, it can be seen that in all Periplocoideae and in *Beaumontia* and *Tabernaemontana*, there is a C at position 695, whereas there is a T (2′-deoxythymidine) for all Secamonoideae and Asclepiadoideae. In this same region two small indels (bp 651–653) have been found in two species of the genus *Secamone*: *S. elliottii* and *S. minutifolia*. There are also two insertions (bp 656–666) in two taxa belonging to different tribes of the Asclepiadoideae in *Riocreuxia* (Ceropegieae) and *Dregea* (Marsdenieae).

The third indel, and probably the most interesting one for our study, is a nine bp deletion (bp 118–126) that has only been found in the Madagascan *Secamone* clade including the African species, but not in *Secamone volubilis* from the Mascarenes.

SYNORGANIZATION

The type of pollinium synorganization between the contents of different pollen sacs we have described in Secamonoideae is not known to occur anywhere else in the angiosperms. A comparison of the geographic distribution of this character has shown that intrapollinial synorganization is much more widespread than interpollinial synorganization. Intrastaminal synorganization is found in taxa in both Asia and Africa and Madagascar, and occurs in *Secamone*, *Genianthus*, *Pervillaea*, *Secamonopsis* (Civeyrel, 1996), *Toxocarpus*, and *Calyptranthera*. In general this feature is linked with two caudicula such as in the pollinaria of *Secamone dewevrei* (Fig. 1B), *Secamonopsis madagascariensis* and *S. microphylla* (Fig. 5C, G), or *Genianthus laurifolius* (Fig. 5F). Interpollinial synorganization has a much narrower distribution; it has been found only in Madagascar and does not extend beyond this island, not even in the Mascarenes or the Comoro Islands. It occurs in three different genera: *Pervillaea* (Civeyrel, 1996; Klackenberg, 1996b) and *Calyptranthera* (see Klackenberg, 1996a, 1997, 1998, for illustration of pollinarium), both endemic to Madagascar, and in some species of the widely distributed genus *Secamone* (Civeyrel, 1994). Unfortunately, we do not have sequences for all species of *Pervillaea*, and those of *Calyptranthera* are yet to be sequenced. Klackenberg (1996b), however, has suggested that *Calyptranthera* is the sister group of the genus *Pervillaea*, where all the examined species have interstaminal synorganization. We have mapped the distribution of this character onto our molecular tree (Fig. 7A) to estimate character evolution among species in Madagascar. Based on the phylogeny presented here, pollinial synorganization has evolved twice within this group, since *Pervillaea* lies outside of the group of *Secamone*. It can also be seen that only one type of pollinial synorganization occurs for each of our well supported groups within Secamonoideae. This has to be confirmed by more sampling within the genus *Secamone*, however.

One way to view the evolution of pollinial synorganization is to relate it to pollination and pollinium insertion. We have started to examine pollination in different species exhibiting different degrees of synorganization. For species without pollinial synorganization such as in the Madagascan species *Secamone buxifolia* (Fig. 4C), we have noted that when there is an insertion by an insect, only one pollinium is inserted, either alone, or sometimes still attached to the translator apparatus. Kunze (1991) has also demonstrated this with *Secamone alpinii*. The reverse occurs in species such as *Secamone geayi* (Fig. 4F), another Madagascan species, with interpollinial synorganization, where we have seen that the entire pollinarium (i.e., all four pollinia and the translator) is inserted. Fused pollinia with intrapollinial synorganization are very strongly glued together, and in *Secamone*, for example, it is almost impossible to separate the pollinia from the corpusculum without breaking them. The fusion observed may reduce the risk of losing pollinia during transport. But since all four pollinia are fused into a unit, it also means that such a unit can only be distributed once. Conversely, free pollinia (i.e., with neither intrapollinial nor interpollinial synorganization) can be distributed among up to four different flowers. When no pollinial synor-

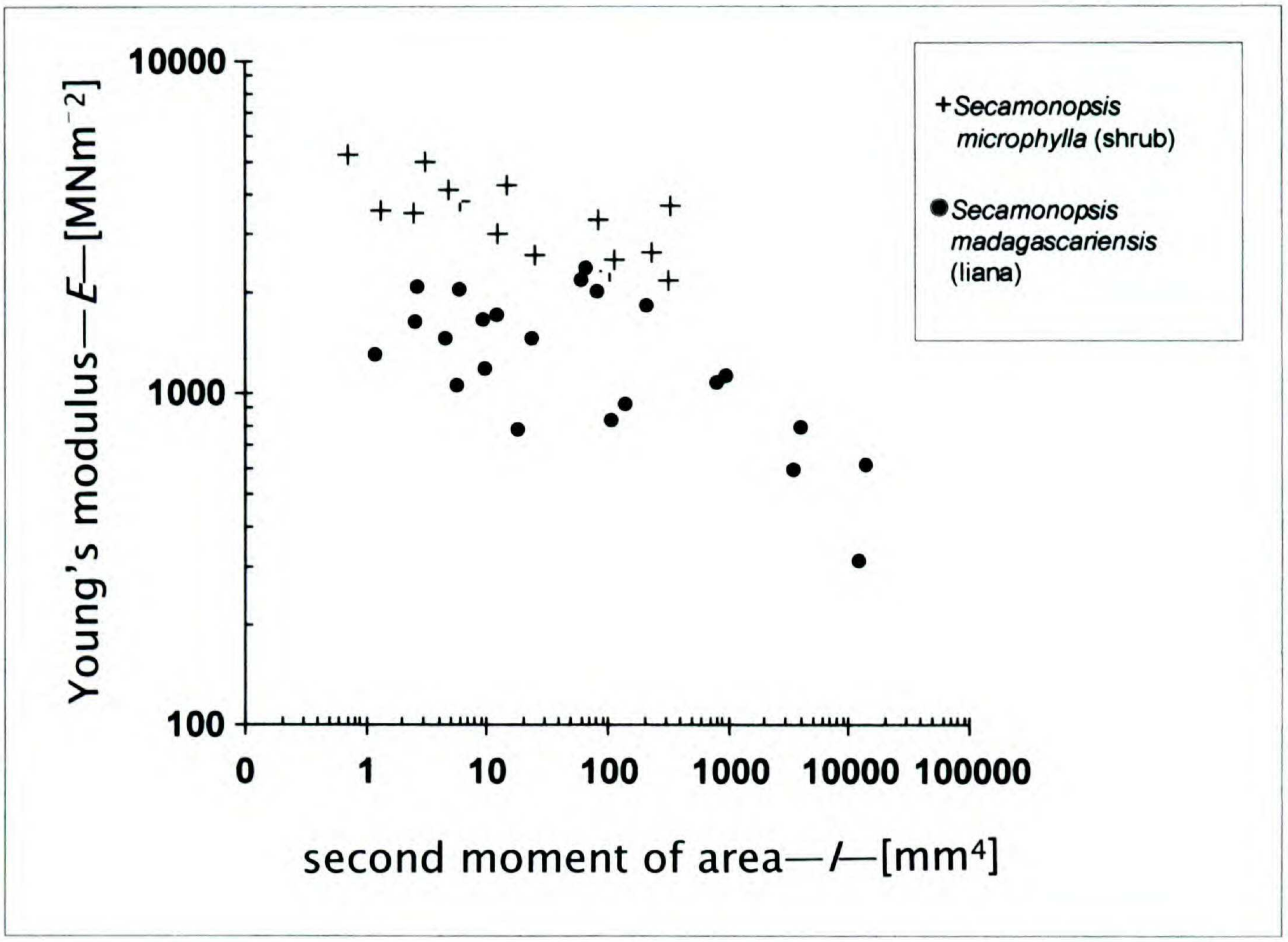


Figure 6. Double logarithmic plot of biomechanical data for two species of *Secamonopsis*. Structural Young's modulus (*E*) is plotted against axial second moment of area (*I*) of stems sampled throughout the plant body. Both the shrub (*S. microphylla*) and liana (*S. madagascariensis*) show a decrease in *E* during ontogeny which is characteristic of woody lianas (Speck & Rowe, 1999).

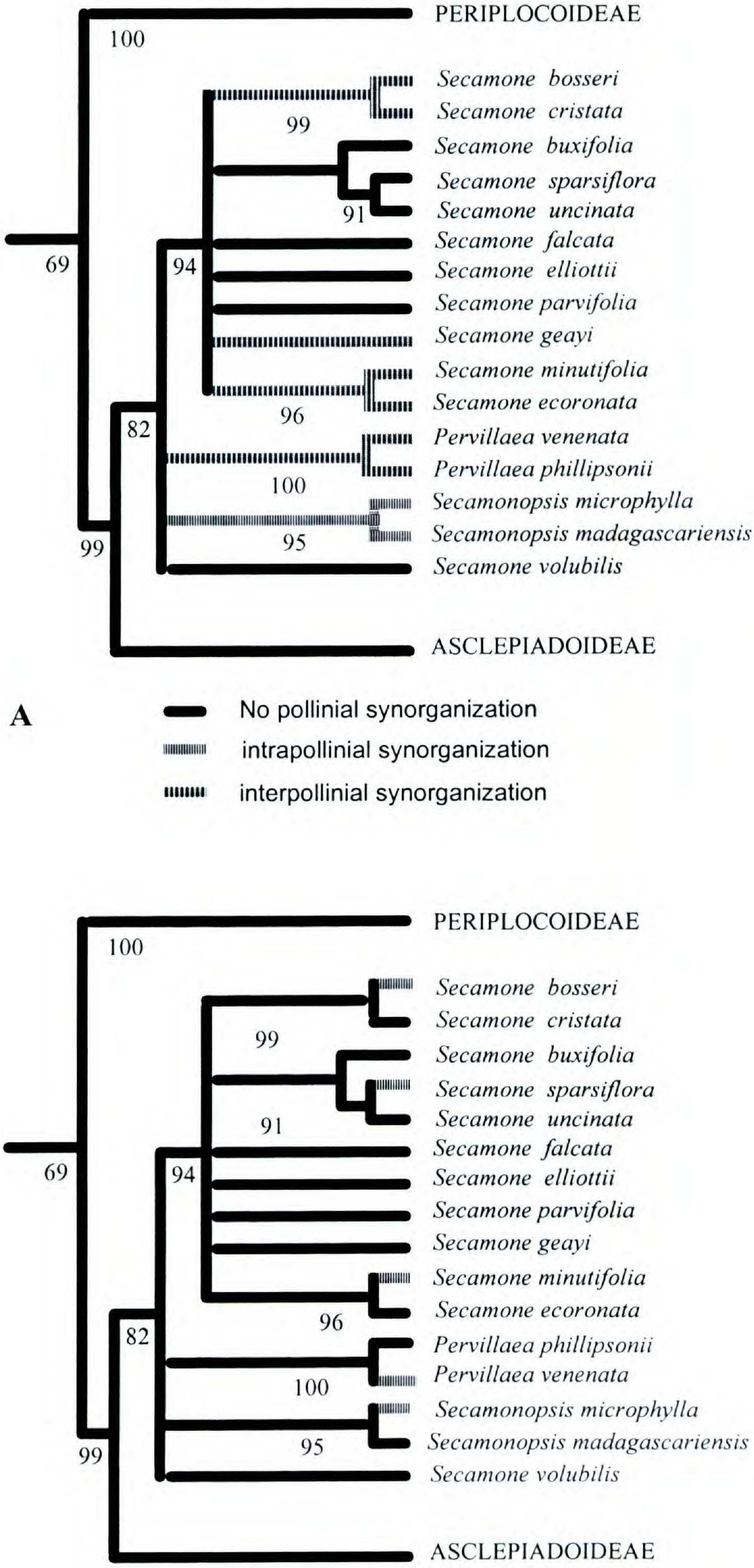
ganization is present, however, the risk of losing pollinia during transport may be high, as pollinia are sometimes only loosely attached to the translator apparatus (Civeyrel, 1996) and can easily fall from it (Friedmann, 1990). Pollination success for flowers with intrapollinial synorganization might be intermediate between these two extremes in terms of pollinia loss and numbers of flowers pollinated, and this would be interesting to investigate.

BIOMECHANICS

In addition to studying reproductive traits during the evolution of this group, we have begun to incorporate biomechanical studies for investigating evolution of growth forms. Distribution of plant growth forms mapped onto the molecular tree suggests that self-supporting shrubs have evolved from lianescent forms at least five times within the Secamonoideae for the examples investigated herein (Fig. 7B). In the genus *Secamonopsis*, *S. madagascariensis* is a twining liana with stiff searchers (young stems) and flexible basal stages; *Secamonopsis microphylla* is a small semi-erect shrub. The phylogeny suggests that the "self-supporting" growth form here is derived within the lianescent group. As expected, the liana species shows a typical drop in the value of *E* (Young's elastic modulus) for the stem during ontogeny as has been documented for a variety of woody lianas (Rowe & Speck, 1996; Speck & Rowe, 1999). What is surprising here is that the shrub actually shows a drop in stem elastic modulus during ontogeny as well. This appears to explain the remarkable habit of this shrub, in which older branches are semi-recumbent

→

Figure 7. —A. Pollinial synorganization. —B. Biomechanical aspects. Characters mapped onto the strict SW analysis consensus tree of the 25 most parsimonious trees obtained with the successive weighting analysis. Bootstrap values are shown above the branches.


PERIPLOCOIDEAE
100
Secamone bosseri
Secamone cristata
99
Secamone buxifolia
Secamone sparsiflora
91
Secamone uncinata
Secamone falcata
69
94
Secamone elliottii
Secamone parvifolia
Secamone geayi
Secamone minutifolia
Secamone ecoronata
96
82
Pervillaea venenata
Pervillaea phillipsonii
100
Secamonopsis microphylla
Secamonopsis madagascariensis
95
99
Secamone volubilis
ASCLEPIADOIDEAE
A
No pollinial synorganization
intrapollinial synorganization
interpollinial synorganization
PERIPLOCOIDEAE
100
Secamone bosseri
Secamone cristata
99
Secamone buxifolia
Secamone sparsiflora
91
Secamone uncinata
69
Secamone falcata
94
Secamone elliottii
Secamone parvifolia
Secamone geayi
Secamone minutifolia
Secamone ecoronata
96
82
Pervillaea phillipsonii
Pervillaea venenata
100
Secamonopsis microphylla
Secamonopsis madagascariensis
95
99
Secamone volubilis
ASCLEPIADOIDEAE
B
Twining liana
Self supporting shrub

and lean against each other or along the ground with the more rigid younger branches oriented vertically. Interestingly, the values of *E* for the shrub are higher than the liana, and this may also reflect the difference between the mechanics of the shrub-like form and the liana. Initial observations of the anatomy of the two plants indicate that the shrub has a much denser wood, fewer and smaller vessels, and a relatively narrow band of compliant outer secondary phloem and bark compared with the liana. Ongoing investigations will quantify the contribution of each tissue to the mechanics of the stem during ontogeny; it will then be possible to determine more exactly which developmental traits cause the mechanical patterns observed and thus explain, for example, why the "shrub-like" plant has retained a basically lianescent mechanical signal. Further investigations will also sample additional genera from the Secamonoideae, particularly in terms of assessing shrub-like or self-supporting habits derived from within a largely lianescent clade. Plant growth forms have been common characters in phylogenetic analyses with character states assigned to trees or shrubs or herbs, and so on. However, growth forms themselves are clearly complex aspects of a plant's life history and are the result of a complex array of developmental traits. We hope that both biomechanical and anatomical approaches combined with phylogenetic techniques as outlined here may provide a means of determining the underlying developmental processes in the evolution of different growth forms.

The Secamonoideae have retained some ancestral characters such as four pollinia per stamen and a relatively simple translator apparatus in which the pollinia are only weakly attached to the translator, which help us to understand the evolution of the reproductive system of the entire family Apocynaceae. The Secamonoideae have also evolved some unique characters among angiosperms such as pollinial synorganization, which links together pollen from different pollen sacs and anthers. With their distribution of many endemic taxa, and their remarkable speciation in Madagascar, a detailed phylogenetic study of the Secamonoideae also enables us to study some fundamental aspects of plant evolution, such as changes in reproduction and overall growth form.

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EVOLUTIONARY INNOVATION AND DIVERSIFICATION IN THE FLOWERS OF ASCLEPIADACEAE[1]

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Abstract

Evolutionary innovation is an important mode of morphological diversification. Because explicit phylogenetic analyses are lacking for most evolutionary innovations, the patterns of origin, diversification, and homoplasy of innovations are poorly understood. Asclepiadaceae are a large angiosperm family characterized by a suite of putatively novel features that contributes to extreme floral complexity and diversity. In this paper, I use a preliminary phylogenetic hypothesis for Asclepiadaceae to explore the patterns of diversification in two novel floral characters, the pollinarium and the corona. The presence, number, and orientation of pollinia and the presence and form of corolline and gynostegial coronas are analyzed. Comparison of the histories of these structures suggests a contrast between relatively conserved evolution of pollinaria and labile evolution of coronas. I examine prior homology assessments of pollinaria and coronas and evaluate the sensitivity of evolutionary reconstructions to errors in homology assessment. These analyses point to crucial areas where additional ontogenetic studies, interpreted in a phylogenetic context, are required. This is particularly true in the phylogenetic assessment of the homology of corolline and gynostegial coronas. I also investigate the sensitivity of evolutionary reconstructions to phylogenetic uncertainty, and find this source of error to be slight.

Key words: Apocynaceae, Asclepiadaceae, character evolution, corona, diversification, innovation, novelty, phylogenetic uncertainty, pollinia.

Innovation is considered a central process in the evolutionary origin of morphological diversity (Mayr, 1960; Liem, 1974; Nitecki, 1990). Although the precise meaning of evolutionary innovation may vary from author to author, it generally refers to the appearance in a descendant of a new structure that differs "more than quantitatively" from its ancestral morphology (Mayr, 1960: 351). "Key" innovations have attracted special attention, because of their purported role in accelerating the rate of species diversification (Mitter et al., 1988; Farrell et al., 1991; Hodges, 1997). Despite keen interest in the role of evolutionary innovations in diversification, there has been remarkably little progress in understanding the ontogenetic bases of the origins of novelties and the evolutionary lability of novelties following their origin.

Species of Asclepiadaceae (including Periplocaceae) comprise a large clade of Apocynaceae sensu lato (Judd et al., 1999; Endress & Bruyns, 2000) that is notable for extreme floral complexity arising from several features that are rare or unknown outside of Apocynaceae s.l. Three floral structures merit particular attention due to their complexity and limited distribution among angiosperms: *pollinarium*, *gynostegium*, and *corona*. Each of these structures has been identified as a distinctive feature of Asclepiadaceae, although the presence of homologous structures (particularly gynostegia and coronas) in non-asclepiad Apocyna-

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