Phylogeny inferred from morphology and DNA data: characterizing well-supported groups in Vanguerieae (Rubiaceae)

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The Vanguerieae is a species-rich tribe of the Rubiaceae with problematic generic circumscriptions. Here we address this issue by presenting a phylogeny in which strongly supported groups are identified. We use DNA sequence data from the nuclear ribosomal internal transcribed spacer (ITS) region, *trnT-F* sequences from the chloroplast genome, and 30 morphological characters. Over 70 taxa are sampled, representing 23 out of the 27 currently recognized genera. We also present a detailed discussion of the morphological variation present in the tribe, in which we focus on features that are taxonomically informative. We show that there are several strongly supported groups in the tribe, but that these rarely coincide with traditional genera. *Canthium*, *Rytigynia*, *Tapiphyllum* and possibly *Pygmaeothamnus* are polyphyletic, and *Fadogia* is paraphyletic. *Keetia*, *Lagynias*, and *Multidentia* are monophyletic with strong support, while the monophyly of *Psydrax* is weakly supported. Several morphological characters are mapped onto the phylogeny to visualize how these can be used to delimit monophyletic groups. *Canthium* subgenus *Afrocanthium* is given generic rank as *Afrocanthium* and 17 combinations for species in this genus are made. New combinations are also made for *Canthium sensu stricto*.   © 2004 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2004, **146**, 257–283.

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INTRODUCTION

Rubiaceae is a species-rich family containing many taxonomically difficult groups at various taxonomic levels. Examples include *Psychotria*, one of the largest angiosperm genera (e.g. Nepokroeff, Bremer & Sytsma, 1999), the *Hedyotis–Houstonia–Oldenlandia* complex (e.g. Terrell, 2001), and the tribe Vanguerieae is clearly also one such group. Not identified or incorrectly determined Vanguerieae specimens are common in many herbaria. The tribe is recognized by a combination of characters, i.e. axillary inflorescences, valvate corolla aestivation, ovaries with solitary pendulous ovules, and most importantly a special kind of pollen presenter, a 'stigmatic head complex' (Igersheim, 1993), used in secondary pollen presentation.

This structure is a unique synapomorphy for the tribe and consists of a combination of the stigmatic lobes and surrounding tissue and usually looks like a globose to cylindrical knob on the apex of the style. While many other Rubiaceae species have secondary pollen presentation (Puff *et al.*, 1996), none have the very specialized structure of Vanguerieae. This synapomorphy and the above-mentioned combination of characters makes the tribe easy to recognize as a group, but unclear generic delimitations and a profusion of species make it difficult to identify a specimen to genus and species levels.

The tribe Vanguerieae contains 27 genera and about 600 currently recognized species, but this number is likely to change in the future. Many species remain to be described, especially from Madagascar and southeast Asia, and several already described are poorly known. So far, species from eastern and southern

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Africa have been investigated most carefully, but the distribution of the tribe extends over all areas in Africa south of the Sahara into Madagascar, south and south-east Asia, the Pacific and Australia.

Like most tropical Rubiaceae, Vanguerieae species are usually trees or shrubs. The most common exception from this type is the geofrutescent habit that *Fadogia*, *Fadogiella* and some other taxa share, but there are also lianescent shrubs, e.g. *Keetia* and *Psydrax* subgenus *Phallaria*. Flowers are usually small and inconspicuous with green to white corollas most common. Notable exceptions are found in the genera *Ancylanthos* and *Temnocalyx,* and in a few members of *Fadogia* that have ≤4-cm-long corollas in bright yellow and red colours.

Few genera are delimited in such a way that they are easily recognized and some genera grade into each other, e.g. *Pachystigma* and *Tapiphyllum* (Bridson, 1996). Several of the characters used to delimit genera are unfortunately often continuous in nature, e.g. amount of indumentum and length of calyx lobes, or characters are shared by many genera, e.g. plurilocular ovaries and inflorescences fasciculate. This complicates the identification of Vanguerieae specimens. Without a better knowledge of the morphological variation, the circumscription of many species and genera will continue to be uncertain.

Vanguerieae, with the exception of the large and widely spread genus *Canthium* and the West African genus *Cuviera*, was revised by Robyns (1928). In several cases the generic definitions published there still remain, although they are not unquestioned. While *Ancylanthos* (Bridson, 1996) and *Temnocalyx* (Verdcourt, 1981) have been reduced to the type species, only small changes have been implemented in other genera such as *Pachystigma* and *Tapiphyllum*, although they have been suggested to be weakly delimited (Verdcourt, 1981). *Cuviera* was later revised (Hallé, 1959), as were the African members of *Canthium* (Bridson, 1985, 1986, 1987a, b, 1992), of which the majority were transferred to *Keetia*, *Multidentia*, *Psydrax* and *Pyrostria*. Four subgenera of *Canthium* are now recognized (e.g. Bridson, 1987b, 1992), but the majority of the Asiatic *Canthium* species have not been formally referred to any of these subgenera or any other genus (but see comments in Bridson, 1987b, 1992; Wong, 1989).

In the present study, we perform a combined phylogenetic analysis of one morphological and two molecular datasets in order to generate a well-resolved phylogeny in which strongly supported groups can be identified. Such groups can be used as the basis for an improved classification of the tribe. Of the two DNA regions that are sequenced, one is nuclear and one is from the chloroplast. The nuclear genome is represented by Internal Transcribed Spacer (ITS) sequences (cf. White *et al*., 1990) from the ribosomal DNA region, the chloroplast by the *trnT-F* region (cf. Taberlet *et al*., 1991). We also provide an overview of the morphological variation present in Vanguerieae in a phylogenetic context, to clarify which morphological characters can be used to delimit monophyletic groups. Of the 27 genera accepted today (Robyns, 1928; Bridson, 1998; Schatz, 2001), we include representatives from 23 in our analysis and also sample a majority of the often distinct infrageneric groups of the larger genera. Four monotypic genera are not represented (owing to a lack of material), but a discussion of their phylogenetic affinities based on morphological features is included in the discussion.

MATERIAL AND METHODS

TAXON SAMPLING

A total of 69 ingroup taxa is included representing 23 genera (Table 1). The focus is on East African species, but taxa from other parts of Africa, south and south-east Asia, and the Pacific are also included. In phylogenetic studies of the subfamily Ixoroideae (Andreasen & Bremer, 2000), *Alberta*, *Ixora* and *Mussaenda* have been shown to be close to Vanguerieae and were therefore chosen as outgroup. We include two specimens of *Ancylanthos rubiginosus* due to the presence of an ITS paralogue for this species (see Results). In Lantz, Andreasen & Bremer (2002) *Peponidium* sp. was annotated as *Pyrostria* sp. and *Pygmaeothamnus* cf. *chamaedendrum* was incorrectly determined to *Pygmaeothamnus zeyheri* (Sond.) Robyns var. *zeyheri* Verdc.

A few specimens determined only to cf. or sp. levels need further explanation. The specimen annotated as *Leroya* cf. *richardiae* has the typical fruits of *Leroya* and is very similar in vegetative characters to *L. richardiae*. It is undoubtedly close taxonomically, but its thicker indumentum compared to the type specimens of *L. richardiae* precludes its certain identification to species. *Peponidium* sp. is a specimen of an undescribed species from central Madagascar. It is dioecious, glabrous with small leaves (up to 30 × 14 mm), has male fasciculate inflorescences with 'false involucres' (see Discussion on inflorescences below), and corolla throats congested with crisped hairs; thus, it is well in agreement with the diagnostic characters of *Peponidium*. *Pygmaeothamnus* cf. *chamaedendrum* is a geofrutex collected in South Africa with paired setulose leaves and large bilocular fruits. These characters support a clear affinity with *Pygmaeothamnus chamaedendrum* (Kuntze) Robyns var. *setulosus* Robyns (see Retief, 2003), but the lack of flowers inhibits us from determining the specimen to species. *Vangueriopsis* cf. *longiflora* is a fruiting specimen that exhibits some of the typical features of

Table 1. Voucher information and EMBL accession numbers. Herbarium abbreviations according to Holmgren, Holmgren & Barnett (1990). We were not able to sequence *Fadogia cienkowskii* for ITS and *Ancylanthos rubiginosus* (specimen B) for *trnT-F*. The *trnT-F* sequence for *Pseudopeponidium ampijoroense* was submitted in two parts owing to missing sequence and therefore has two accession numbers. New sequences are underlined

Species	Voucher	EMBL accession number ITS/*trnT-F*
Alberta magna E. Mey.	Middleton s. n. 94101, Tonkin 200 (UPS)	AJ224842/AJ620118
Ixora coccinea L.	Bremer 3104 (UPS)	AJ224826/AJ620117
Mussaenda erytrophylla Schumach. & Thonn.	Gillis 10838 (FTG)	AJ224823/AJ620116
Ancylanthos rubiginosus Desf. (specimen A)	Zimba *et al*. 776 (UPS)	AJ617747/AJ620119
Ancylanthos rubiginosus Desf. (specimen B)	Bingham 8652 (K)	AJ617748/–
Canthium burttii Bullock	Mwiga 241 (UPS)	AJ617749/AJ620120
Canthium ciliatum (Klotzsch) Kuntze	Bremer *et al*. 4345 (UPS)	AJ617750/AJ620121
Canthium coromandelicum (Burm.f.) Alston	Andreasen 36 (UPS)	AJ315081/AJ620122
Canthium gilfillanii (N.E. Br.) O.B. Miller	Bremer *et al*. 4259 (UPS)	AJ617751/AJ620123
Canthium glaucum Hiern ssp. *glaucum*	Kuchar 17410 (UPS)	AJ617752/AJ620124
Canthium inerme (L.f.) Kuntze	Bremer & Bremer 3686 (UPS)	AJ315120/AJ620125
Canthium keniense Bullock	Luke & Luke 8373 (UPS)	AJ617753/AJ620126
Canthium lactescens Hiern	Luke & Luke 9045 (UPS)	AJ617754/AJ620127
Canthium mundianum Cham. & Schltdl.	Bremer & Bremer 3704 (UPS)	AJ315107/AJ620128
Canthium oligocarpum Hiern ssp. *captum* (Bullock) Bridson	Borhidi *et al*. 85449 (UPS)	AJ617755/AJ620129
Canthium parasiebenlistii Bridson	Lantz 129 (UPS)	AJ617756/AJ620130
Canthium pseudosetiflorum Bridson	Friis *et al*. 3671 (UPS)	AJ617757/AJ620131
Canthium pseudoverticillatum S. Moore	Luke & Luke 9032 (UPS)	AJ617758/AJ620132
Canthium siebenlistii (K. Krause) Bullock	Luke *et al*. 9121 (UPS)	AJ617759/AJ620133
Cuviera angolensis Welv. ex K.Schum.	McPherson 16297 (MO)	AJ315088/AJ620134
Cyclophyllum barbatum (Forst.f.) A.C.Smith & S.P.Darwin	Lorence 8672 (UPS)	AJ617760/AJ620135
Fadogia ancylantha Hiern	Chapman & Chapman 9109 (UPS)	AJ315103/AJ620136
Fadogia cienkowskii Schweinf.	Lantz 101 (UPS)	–/AJ620137
Fadogia elskensii De Wild.	Taylor *et al*. 8318 (UPS)	AJ315118/AJ719191
Fadogia tetraquetra K.Krause	Bremer & Bremer 3799 (UPS)	AJ315099/AJ620139
Fadogia verdcourtii Tennant var. *verdcourtii*	Gereau *et al*. 6010 (UPS)	AJ315116/AJ620140
Fadogiella stigmatoloba (K.Schum.) Robyns	Lawton 1318 (S)	AJ315100/AJ620141
Hutchinsonia barbata Robyns	Adam 20599 (UPS)	AJ315102/AJ620142
Keetia gueinzii (Sond.) Bridson	Bremer 3083 (UPS)	AJ315117/AJ620143
Keetia lukei Bridson	Luke 8341 (UPS)	AJ617761/AJ620144
Keetia venosa (Oliv.) Bridson	Luke 8347 (UPS)	AJ617762/AJ620145
Keetia zanzibarica (Klotzsch) Bridson ssp. *zanzibarica*	Bremer 3069 (UPS)	AJ315105/AJ620138
Lagynias dryadum (S.Moore) Robyns	Bremer & Bremer 3811 (UPS)	AJ315090/AJ620146
Lagynias lasiantha (Sond.) Bullock	Bremer & Bremer 3792 (UPS)	AJ315089/AJ620147
Leroya cf. *richardiae* Cavaco	Davis and Rakotonasolo APD 2501 (K)	AJ617763/AJ620148
Meyna tetraphylla (Hiern) Robyns ssp. *comorensis* (Robyns) Verdc.	Bremer 3074 (UPS)	AJ315083/AJ620149
Multidentia concrescens (Bullock) Bridson & Verdc.	Bidgood *et al*. 845 (K)	AJ315086/AJ620150
Multidentia fanshawei (Tennant) Bridson	Lovett *et al*. 3311 (K)	AJ315087/AJ620151
Neoleroya verdcourtii Cavaco	23339 SF (TEF)	AJ617764/AJ719192
Pachystigma pygmaeum Robyns	Pawek 12335 (BR)	AJ315091/AJ620152
Peponidium horridum (Baill.) Arènes	Labat *et al*. 2236 (K)	AJ617765/AJ620153
Peponidium sp.	Pettersson & Nilsson 752 (UPS)	AJ315112/AJ620154
Plectroniella armata (K.Schum.) Robyns	Bremer & Bremer 3790 (UPS)	AJ315082/AJ620155
Pseudopeponidium ampijoroense Arènes	SF 33726 (TEF)	AJ617766/AJ719193 AJ719194
Pseudopeponidium asosa Arènes	Anthony 1390 (TEF)	AJ617767/AJ620156
Psydrax kraussioides (Hiern) Bridson	Lantz 114 (UPS)	AJ617768/AJ620157

Table 1. *Continued*

Species	Voucher	EMBL accession number ITS/*trnT-F*
Psydrax livida (Hiern) Bridson	Lantz 109 (UPS)	AJ617769/AJ620158
Psydrax locuples (K. Schum.) Bridson	Bremer *et al*. 4290 (UPS)	AJ617770/AJ620159
Psydrax nitidum (Craib) Wong	Ryding 599 (UPS)	AJ315108/AJ620160
Psydrax obovata (Klotzsch ex Eckl. & Zeyh.) Bridson ssp. *obovata*	Bremer & Bremer 3762 (UPS)	AJ315109/AJ620161
Psydrax parviflora (Afz.) Bridson ssp. *parviflora*	Gilbert & Phillips 9069 (UPS)	AJ315110/AJ620162
Psydrax schimperiana (A.Rich.) Bridson ssp. *schimperiana*	Simon *et al*. 462 (UPS)	AJ617771/AJ620163
Psydrax sp. A of F.T.E.A.	Luke & Luke 9031 (UPS)	AJ617772/AJ620164
Pygmaeothamnus cf. *chamaedendrum* (Kuntze) Robyns	Bremer & Bremer 3800 (UPS)	AJ315119/AJ620165
Pygmaeothamnus zeyheri (Sond.) Robyns var. *zeyheri*	Bremer *et al*. 4356 (UPS)	AJ617773/AJ620166
Pyrostria bibracteata (Baker) Cavaco	Bremer 3036 (UPS)	AJ315113/AJ620167
Pyrostria hystrix (Bremek.) Bridson	Bremer & Bremer 3791 (UPS)	AJ315114/AJ620168
Pyrostria phyllantoidea (Baillon) Bridson	Taylor *et al*. 8486 (UPS)	AJ315115/AJ620169
Robynsia glabrata Hutchinson	Hall & Amponsah 46545 (K)	AJ617774/AJ620170
Rytigynia bagshawei (S.Moore) Robyns var. *bagshawei* Verdc.	Borhidi *et al*. 84439 (UPS)	AJ315101/AJ620171
Rytigynia bugoyensis (K.Krause) Verdc.	Rwburindore 3536 (UPS)	AJ315084/AJ620172
Rytigynia fuscosetulosa Verdc.	Frimodt Moller *et al*. NG117 (K)	AJ315097/AJ620173
Rytigynia mrimaensis Verdc.	Luke & Luke 9020 (UPS)	AJ617775/AJ620174
Rytigynia senegalensis Blume	van den Berghen 8746 (BR)	AJ315104/AJ620175
Scyphochlamys revoluta Balf.f.	Chase 12564 (K)	AJ617776/AJ620176
Tapiphyllum cinerascens (Welv. ex Hiern) Robyns var. *cinerascens*	Milne-Redhead 3292 (BR)	AJ315096/AJ620177
Tapiphyllum velutinum (Hiern) Robyns	Emanuelsson 672 (S)	AJ315098/AJ620178
Vangueria apiculata K.Schum.	Kårehed & Odhult 161 (UPS)	AJ315095/AJ620179
Vangueria infausta Burch.	Bremer *et al*. 4254 (UPS)	AJ617777/AJ620180
Vangueria madagascariensis Gmelin	Bremer 3077 (UPS)	AJ224839/AJ620184
Vangueria parvifolia Sond.	Bremer & Bremer 3771 (UPS)	AJ315092/AJ620181
Vangueriella spinosa (Schumach & Thonn.) Verdc.	Merello *et al*. 1494 (K)	AJ315085/AJ620182
Vangueriopsis cf. *longiflora* Verdc.	Luke 8316 (UPS)	AJ617778/AJ620183

V. longiflora, such as extremely long stipule apices and calyx lobes, and a ribbed fruit. However, it was collected in Kenya (K7, Shimba hills), far from the other known collections in Tanzania (T6, T7), and the specimen had no flowers. Although certainly close to *V. longiflora*, we cannot be certain that it is not an undescribed species.

MOLECULAR METHODS

Thirty-five specimens were amplified from herbarium material, the rest from silica-dried or fresh material. Extraction protocols and PCR cocktails are as outlined in Lantz *et al*. (2002), unless otherwise noted. In order to increase the likelihood that all possible ITS paralogues of different stabilities were amplified, 8–10% DMSO (Buckler, Ippolito & Holtsford, 1997) was included in all steps of the ITS amplification and sequencing. For the ITS region, primers ITS4 (White *et al*., 1990), ITS-I (Urbatsch, Baldwin & Donoghue, 2000), P17, and 26S-82R (Popp & Oxelman, 2001)

were used in different combinations. The PCR reactions were run for 1 min at 97 °C followed by 35–50 cycles of 97 °C for 10 s; 48 °C–55 °C for 30 s; 72 °C for 20 s + 4 s/cycle; finishing with 72 °C for 7 min.

The *trnT-F* region was amplified by various combinations of primers A, B, C, D, E, F (Taberlet *et al*., 1991), I (Bremer *et al*., 2002), but also primers A1, G, H, and rps4, published here. A1 (5′-ACAAATGCGAT GCTCTAACC-3′) is based on primer A, but moved four bases in the 3′-direction. This modification reduces the number of possible base-pairings both within and between primer-molecules and simplified the amplification of the region between the *trnT*(UGU) and *trnL*(UAA)5′-exon genes, which is usually hard to amplify. To construct the primer, sequences were produced by amplifying with primer rps4 (5′-AAACGAG GTCCTCGRTAACG-3′), located about 200 bases in the 5′ direction from primer A, in combination with primer B or I. Primers G (forward; 5′-GGAAAGCTGA CAGGGAGATA-3′) and H (reverse; 5′-TATCTCCCT GTCAGCTTTCC-3′) are located in the intergenic

spacer between *trnT*(UGU) and *trnL*(UAA)5′-exon, approximately 500 bases downstream of primer A. The PCR cycle was constructed as follows: 95 °C for 2 min followed by 35–50 cycles of 95 °C for 35 s; 55 °C for 1 min; 72 °C for 2 min, finishing with 72 °C for 10 min. The sequencing reactions were either performed as in Lantz *et al.* (2002) or with the DYEnamic ET terminator Cycle Sequencing Kit (Amersham Biosciences) following the protocol of the manufacturer and run on a MegaBACE 1000 DNA Analysis System. Primers ITS2, ITS3, ITS4, ITS5 (White *et al.*, 1990), P16 (Popp & Oxelman, 2001) and P25 (Lidén, Fukuhara & Axberg, 1995) were used for the ITS sequencing reactions. The sequencing reactions for the *trnT-F* region used the same primers as in the PCR amplification. For four specimens (*Ancylanthos rubiginosus* A and B, *Canthium glaucum* and *Robynsia glabrata*), it was impossible to produce readable ITS sequences using direct sequencing, which necessitated gel-cleaning of the PCR fragment and cloning. The full amount of PCR product was run on an agarose gel with TAE buffer. The gel was allowed to soak for 10 min in an ethidium-bromide solution and bands were visualized and cut out under a low power UV-light. DNA was extracted from the gel slices using the Ultrafree-DA kit (Millipore Corporation). We used the Topo Ta Cloning kit (Invitrogen life technologies) according to the manufacturer's instructions for the cloning, and screened between five and ten colonies for each taxon by PCR followed by sequencing.

MORPHOLOGICAL METHODS

The morphological dataset is based on an investigation of herbarium material. With few exceptions the characters have been examined by the first author on actual material; only when material has been missing from the specimens (e.g. fruits or flowers) has the information been taken from literature. A list of specimens examined is available from the first author. We could not find flowering material for *Canthium burttii*, *C. parasiebenlistii*, *Cuviera angolensis*, *Fadogia verdcourtii*, *Keetia lukei*, *Leroya* cf. *richardiae*, *Peponidium horridum*, *Pygmaeothamnus* cf. *chamaedendrum*, *Pyrostria hystrix*, *P. phyllantoidea*, *Rytigynia bugoyensis*, *R. fuscosetulosa*, *R. mrimaensis*, *Scyphochlamys revoluta*, and *Vangueriopsis* cf. *longiflora*. Fruits were missing from our material of *Peponidium* sp. and *Pseudopeponidium ampijoroense*. Flowers, leaves and/or fruits were boiled and examined under a mounted lens and measurements were taken. Hairs from inside the corolla and from the leaf surface were mounted in Hoyer's solution (Anderson, 1954) and examined under a phase contrast microscope. The boiled material was preserved in Copenhagen Mixture (70 parts 95% ethanol, 29 parts distilled water and 1 part glyc-

erol). Thirty characters were coded and included in the morphological matrix (Appendix 2). When a character can be absent or present, and if present occurs in different states, we chose to divide the character into two (with separate coding; Lee & Bryant, 1999). All characters were traced on one of the most parsimonious trees (tree no. 1) using MacClade v.4.04 with ACCTRAN optimization. MacClade v.4.04 (Maddison & Maddison, 2001) was also used to calculate consistency index values for the morphological characters on the same tree used in the tracing. We used the *Flora of Tropical East Africa* (Verdcourt & Bridson, 1991) and *Flora Zambesiaca* (Bridson, 1998) as main taxonomic references because the results of most of the recently performed morphological/taxonomical studies of the tribe are included in these publications and they constitute the best general works on Vanguerieae in Africa.

PHYLOGENETIC ANALYSES

Sequences were aligned by eye. The phylogenetic analysis was performed using PAUP* v.4.0b10 (Swofford, 2002). All three datasets were included in a combined analysis but were also analysed separately. Phylogenetically informative insertion/deletion events (indels) were coded for the molecular datasets and given the same weight independent of size. We preferred not to introduce any hypothesis of evolution prior to the analyses, and thus all characters are treated as unordered. A heuristic parsimony search was performed with 100 random addition replicates, TBR and MULTREES on. Branch support was calculated using the jack-knife and bootstrap procedures implemented in PAUP*. The jack-knife analysis used a deletion frequency of 37.5%, 10 000 replicates, TBR branch swapping, 5 random addition sequence replicates, and MULTREES off. Bootstrap used 10 000 replicates, TBR branch swapping, 5 random addition sequence replicates, and MULTREES off. Bootstrap (bp) and jack-knife (jk) values are listed in the text as (bp; jk).

RESULTS

SEQUENCE AND ALIGNMENT DATA

Thirty-two new ITS sequences and 73 new *trnT-F* sequences were produced for the present study. ITS sequences in the ingroup varied in length from 611 bases for *Psydrax obovata* to 671 bases for *Fadogiella stigmatoloba*. When the PCR product of *Fadogia cienkowskii* was visualized on a gel, two bands were seen; one strongly amplified of about 400 bases and one faint band of a size comparable to other ITS sequences. We were unable to sequence any of the fragments. A paralogue was found for *Ancylanthos rubiginosus* characterized by a deletion of 13 bases in

ITS1. Two out of seven clones shared the deletion, but the deletion is not shared by any other species in the tribe and should not affect the phylogenetic position of the species. After alignment and removal of unalignable regions, the ITS matrix included 668 characters; 188 of these were informative, including 12 indel-characters.

TrnT-F sequences in the ingroup varied in length from 1559 bases for *Keetia zanzibarica* to 1785 bases for *Canthium gilfillanii*. Alignment resulted in a matrix of 2118 positions for the *trnT-F* sequences of which 155 were informative, including 23 indel-characters. All investigated species of *Psydrax* (apart from *P. kraussioides* which we were unable to sequence for this part) share a sequence of 40 bases not present in the other genera. The sequences of *Fadogia elskensii*, *Neoleroya verdcourtii* and *Pseudopeponidium ampi-joroense* are partial as a consequence of difficulties in sequencing.

MORPHOLOGICAL DATA

All 30 morphological characters included in the analysis are informative. Characters 11 and 27 are nonreversed synapomorphies for the ingroup. The homoplasy of the characters as measured by the consistency index (CI) (Kluge & Farris, 1969; Goloboff, 1991) exhibit a wide range of values (from tree no. 1). The highest values, suggesting low levels of homoplasy, for the characters informative in the ingroup are found for supra-axillary spines (character no. 2; CI = 1.00), sex distribution (no. 4; CI = 0.67), bracts enclosing the inflorescence when young (no. 13; CI = 1.00), and filament reflexed (no. 23; CI = 1.00). Consistency index values are lowest for indumentum (no. 6; CI = 0.06), calyx lobe length (no. 16; CI = 0.08), corolla lobe apex tailed (no. 19; CI = 0.07), dark connective on the anthers (no. 24; CI = 0.08), and fruit size (no. 30; CI = 0.08), indicating high levels of homoplasy for these characters.

PHYLOGENETIC ANALYSES

The combined matrix of ITS, *trnT-F* and morphological data included 2816 characters. An heuristic search of the combined matrix (uninformative characters removed) resulted in 6207 most parsimonious trees with a length of 1332 (CI = 0.4062, RI = 0.7776). The strict consensus tree (Fig. 1) is well-resolved and contains several strongly supported clades. *Canthium*, *Rytigynia*, *Tapiphyllum* and possibly *Pygmaeothamnus* are polyphyletic, *Fadogia* is paraphyletic and the monophyly of *Vangueria* is uncertain. For several strongly supported groups we use informal names (see Fig. 1), some of which have already been suggested in Lantz *et al*. (2002). Some nodes in the tree (Fig. 1)

have been marked by A, B, C, etc., which are referred to in the discussion. A tree in which the new classification here suggested has been implemented is presented in Figure 5.

The two specimens of *Ancylanthos rubiginosus* group together with strong support (95; 94). We also ran a separate analysis of an ITS dataset where all clones of A. *rubiginosus* were included, and the clones form a monophyletic group (results not shown). It thus seems that the variation found for the different paralogues of *A. rubiginosus* does not pass the species boundary.

The two molecular datasets were also analysed separately (results not shown). One strongly supported incongruence is seen in the *Fadogia–Rytigynia* group. *Rytigynia bagshawei* is sister to the rest of the group in the *trnT-F* tree but groups with *Hutchinsonia barbata* in the ITS tree. The result of the *trnT-F* analysis is favoured by the combined analysis. There is also some uncertainty concerning the position of *Ancylanthos rubiginosus*, and the taxa in the large-flowered group not included in the *Vangueria* group or in the *Fadogia–Rytigynia* group. Taxa with incongruent positions are restricted to the large-flowered group, and the incongruencies will be addressed in more detail in a later study on the large-flowered group (work in progress). The incongruencies do not affect the statements of homology made in the morphological discussion or the phylogeny for those species outside the large-flowered group.

The unexpected position of *Pygmaeothamnus* cf. *chamaedendrum* in the spiny group caused us to re-extract and re-sequence the specimen to check for possible contamination. The new sequences were identical to the first ones and we can thus dismiss contamination as a cause behind the phylogenetic position of *Pygmaeothamnus* cf. *chamaedendrum*. A position in the spiny group is also supported by both the ITS and *trnT-F* datasets; there is no strongly supported topological incongruence indicative of hybridization.

DISCUSSION

As one of the main aims of this study is to give an overview of the morphological variation in the tribe, we will open this section with a rather comprehensive account on the various useful characters for delimitation of groups and genera. Characters much dependent on quality and age of the material, or characters that vary continuously and lack discrete states have been excluded from the phylogenetic analysis. However, because some of these characters are certainly useful at other levels and as we also wish to present a more inclusive discussion on the variation present in the tribe, the account encompasses more characters
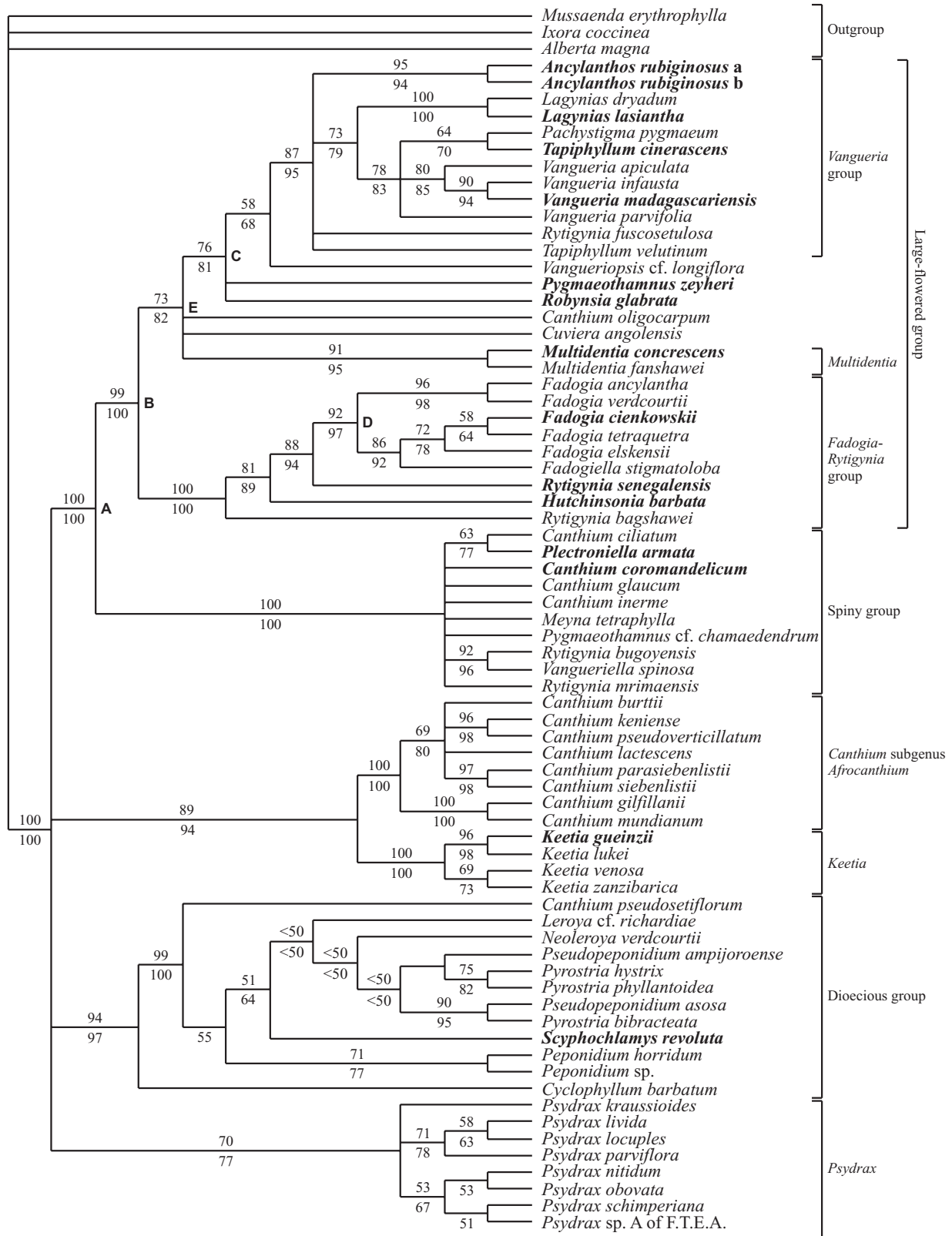
Figure 1. The strict consensus tree from the combined analysis (ITS + *trnT-F* + morphology). Bootstrap support values are above the branches, jackknife below. A, B, C, etc. refer to clades mentioned in the text. Type species of currently recognized genera in bold.

than used in the analysis. We include comments on how the morphological characters were coded for cases where we feel it is necessary to do so.

CHARACTERS

Habit

All Vanguerieae species are woody. The geofrutices (Robbrecht, 1988) are sometimes mentioned as semi-herbacous, because the aboveground parts of some species are only slightly woody or completely herbaceous, but the underground parts are always woody. *Fadogia* is a large genus characterized by this habit, but there are geofrutices in several other genera as well. Apart from *Fadogia*, geofrutices included in this study are *Ancylanthos rubiginosus*, *Fadogiella stigmatoloba*, *Multidentia concrescens*, *Pachystigma pygmaeum*, *Pygmaeothamnus* cf. *chamaedendrum*, *P. zeyheri* and *Tapiphyllum cinerascens*. All of these species belong in a strongly supported (100; 100) clade (A; Fig. 1), but the growth form has evolved several times in this clade (Fig. 2). An anatomical investigation of the wood of Vanguerieae (Lens *et al.*, 2000a) revealed that the underground parts of *Fadogiella stigmatoloba* exhibit features characteristic of roots, while these structures in other geofrutices (including the closely related genus *Fadogia*) more resemble the aboveground parts. There is also some variation in the woodiness of the aboveground parts, and it is possible that the geofrutex character could be further divided, but further anatomical data are needed for such a division to be performed.

The species of *Keetia* and *Psydrax* subgenus *Phallaria*, here represented by *P. kraussioides*, are without exceptions climbers or scandent shrubs. However, there are climbers in other genera as well (e.g. *Canthium*, *Lagynias*, *Meyna*, *Multidentia* and *Vangueriella*, and also a few species in *Psydrax* subgenus *Psydrax*), although only as rare exceptions to the otherwise common habit in those groups. The majority of the species in the tribe are not geofrutices or climbers but are usually described as either shrubs, treelets or trees, and the distinction between these growth forms is difficult to make. Treelet seems to be especially difficult to define; the term is usually used for large shrubs or small trees and is thus based on size and not on homology. Not only are the definitions of these habits vague and used differently by different authors and collectors (Coates Palgrave, 2002), but many species also have a very variable habit, further increasing the difficulty of defining states for the character. We code for three states: geofrutices, climbers, and shrubs and trees. Efforts to further divide the character into more states did not change the support of any groupings (results not shown), but it should be noted that one state (shrubs or trees) is perhaps more inclusive than the other ones.

Spines and shoots

There are at least two types of spines present in Vanguerieae. Synapomorphic for the spiny group (except *Pygmaeothamnus* cf. *chamaedendrum*; Fig. 2) are paired supra-axillary spines, i.e. they are positioned at a short distance from the nodes. The spines are usually present on all parts of the plant, but for some species (e.g. *Canthium inerme*) they are more common on sucker shoots or on seedlings. Another type can be seen for *Canthium oligocarpum* where the spines are present at the nodes, usually in whorls of three, and only occur on coppice shoots and young branches. Of the other spiny species included in this study (Fig. 2), *Cuviera angolensis*, *Hutchinsonia barbata*, *Lagynias lasiantha* and *Robynsia glabrata* only carry spines occasionally or only on older parts rarely collected and we have not examined any spiny material from these species. Given this lack of information, we code only for supra-axillary spines and not for any other type. Spines are also known from *Rytigynia* subgenus *Rytigynia*, a few South African *Canthium* species not placed to subgenus (Tilney, 1986; Bridson, 1992), and several south-east Asian *Canthium* species (Wong, 1989).

Some species, especially in the spiny group, have brachyblasts (lateral shoots with contracted internodes). Similar but not identical shoots occur in several other genera, e.g. the lateral shoots of *Pyrostria hystrix*. For *Canthium pseudoverticillatum* the lateral shoots are extremely reduced which makes the leaves appear as in whorls of four. This is different from the other type of whorled leaves common in the tribe (e.g. *Fadogia*), in which the leaves are present on the main shoot at the same node with stipules present between all leaves and usually are in whorls of three. There is a correlation between a geofrutescent habit and whorled leaves, but the correlation is not absolute, and the characters are coded as two separate characters.

Trichomes

Hairs (trichomes) vary in abundance and on where they are positioned. Hairs on leaves, stems, fruits and outer parts of the corollas are all of the same type and exhibit only a slight variation in one individual. This external indumentum is not homologous to the types found inside the corolla (see Discussion on the corolla) or inside the stipules. This seems to be the case for most Rubiaceae species (Verdcourt, 1958; Robbrecht, 1988). There is also a strong correlation between the presence of hairs for the different structures, e.g. species with hairs on the fruits always have hairs on the leaves, species with glabrous leaves never have hairs on the outside of the corollas, etc. Creating separate
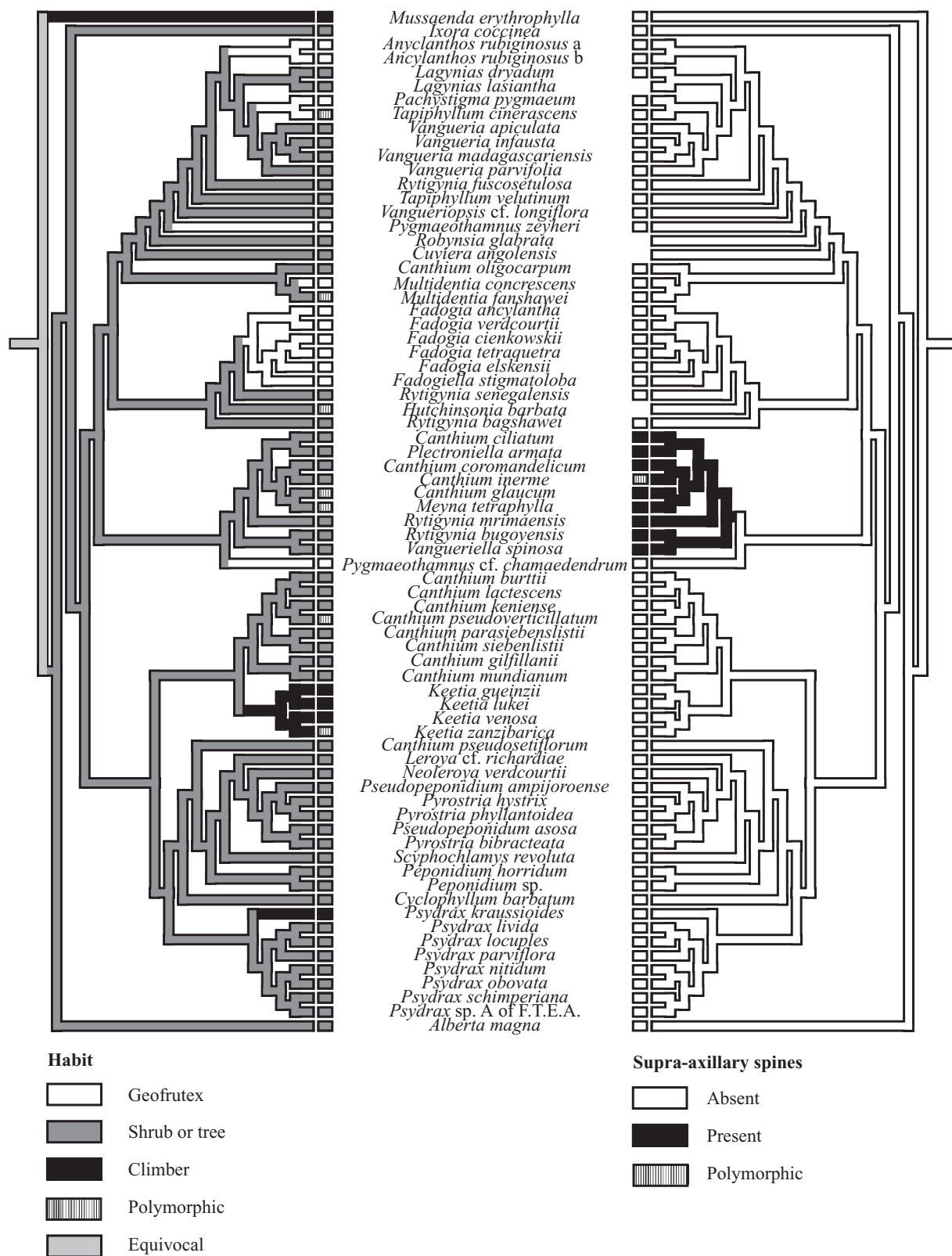
Mussaenda erythrophylla
Ixora coccinea
Ancylanthos rubiginosus a
Ancylanthos rubiginosus b
Lagynias dryadum
Lagynias lasiantha
Pachystigma pygmaeum
Tapiphyllum cinerascens
Vangueria apiculata
Vangueria infausta
Vangueria madagascariensis
Vangueria parvifolia
Rytigynia fuscosetulosa
Tapiphyllum velutinum
Vangueriopsis cf. *longiflora*
Pygmaeothamnus zeyheri
Robynsia glabrata
Cuviera angolensis
Canthium oligocarpum
Multidentia concrescens
Multidentia fanshawei
Fadogia ancylantha
Fadogia verdcourtii
Fadogia cienkowskii
Fadogia tetraquetra
Fadogia elskensii
Fadogiella stigmatoloba
Rytigynia senegalensis
Hutchinsonia barbata
Rytigynia bagshawei
Canthium ciliatum
Plectroniella armata
Canthium coromandelicum
Canthium inerme
Canthium glaucum
Meyna tetraphylla
Rytigynia mrimaensis
Rytigynia bugoyensis
Vangueriella spinosa
Pygmaeothamnus cf. *chamaedendrum*
Canthium burttii
Canthium lactescens
Canthium keniense
Canthium pseudoverticillatum
Canthium parasiebenslistii
Canthium siebenlistii
Canthium gilfillanii
Canthium mundianum
Keetia gueinzii
Keetia lukei
Keetia venosa
Keetia zanzibarica
Canthium pseudosetiflorum
Leroya cf. *richardiae*
Neoleroya verdcourtii
Pseudopeponidium ampijoroense
Pyrostria hystrix
Pyrostria phyllantoidea
Pseudopeponidium asosa
Pyrostria bibracteata
Scyphochlamys revoluta
Peponidium horridum
Peponidium sp.
Cyclophyllum barbatum
Psydrax kraussioides
Psydrax livida
Psydrax locuples
Psydrax parviflora
Psydrax nitidum
Psydrax obovata
Psydrax schimperiana
Psydrax sp. A of F.T.E.A.
Alberta magna

Habit

☐ Geofrutex

▨ Shrub or tree

■ Climber

▥ Polymorphic

▨ Equivocal

Supra-axillary spines

☐ Absent

■ Present

▥ Polymorphic

Figure 2. Habit and spines (characters 1 and 2; Appendix 1) mapped onto one of the most parsimonious trees using MacClade v.4.01 with ACCTRAN optimization. The absence of a square at the end of the branches for some species indicates that the character state is unknown for that species.

characters for the presence of hairs on all mentioned structures would result in an overweighting of the presence of hairs and we have coded only for the presence of hairs on the leaf blades. When comparing species, hairs can have a varying number of cells, different colour and variable length. Unicellular hairs are only found in *Psydrax* (Tilney, van Wyk & Kok, 1988; Tilney, Kok & van Wyk, 1990). This distinction between unicellular and multicellular hairs is the only clear distinction we can make when looking at the variation present in the whole tribe. In more restricted groups, characters such as colour and exact number of cells are likely to be more useful.

Leaves

Size, shape and texture of leaves vary within the tribe, but are of use primarily at the species level. Leaf texture is of some use to delimit *Psydrax*, which, with very few exceptions, has coriaceous leaves. Apparent or obscure tertiary nerves have been used to diagnose some genera (*Multidentia* and *Pyrostria*, respectively), but the character is not otherwise useful at the generic level. Domatia are commonly present and vary in structure and amount of hairs present. This information is potentially useful taxonomically but has not been investigated in detail here (or elsewhere). *Fadogia* seems to lack domatia altogether, although domatia can be difficult to see in some species owing to a thick indumentum, and domatia are also very rare in the *Vangueria* group. Of the *Vangueria* group species studied here, only *V. apiculata* has domatia, and this only rarely. Anatomical leaf characters potentially useful at the genus level are differentiation and arrangement of parenchymatous cells and arrangement of collenchyma in the midrib area (Tilney *et al.*, 1990).

Amount of indumentum varies from glabrous to densely pubescent. We have coded one absent/present character and one character for amount of indumentum, if present. The criteria used for the states are indumentum present but not obscuring the surface or thick indumentum present, obscuring the surface at least below. This distinction has been used in the *Flora of Tropical East Africa* (Verdcourt & Bridson, 1991) and *Flora Zambesiaca* (Bridson, 1998) to key out some genera (e.g. *Tapiphyllum*).

Stipules in Vanguerieae are always connate, but sometimes only slightly so. They usually consist of a basal broadly ovate to triangular part and a narrow apical part, but this differentiation is not always present. The narrow apices, when present, tend to become less pronounced with increasing age of the stipule, sometimes disappearing completely. Some species have stipule apices widest above the midpoint, and this has been coded as an absence/presence character. Presence of hairs within the stipules is phylogenetically informative. These hairs are completely lacking in *Psydrax*, rare in *Keetia* and also lacking in *Canthium mundianum*. It is one of the few morphological characters that indicate a relationship between *Canthium* subgenus *Afrocanthium* and *Keetia*.

Sex distribution

Hermaphrodite flowers are the most common type in the tribe, but there is also a large group of dioecious species (the dioecious group; Fig. 1). *Pyrostria* (incl. *Pseudopeponidium*, *Peponidium*, *Leroya* and *Neoleroya*; see Schatz, 2001), *Scyphochlamys*, and *Canthium* subgenus *Bullockia*, here represented by *C. pseudosetiflorum*, all belong to this group. Male flowers have a pollen presenter and sometimes rudimentary ovules and female flowers have sterile anthers. However, it is possible that on rare occasions a male plant could produce a few fruits or a female plant form some pollen (Bridson, 1987b), although no detailed study on the sex expression of these species has been made. *Canthium mundianum* is one of the rare recorded examples of species not belonging to this group that is not an obligate hermaphrodite. It is gynodioecious (Balkwill, Sebola & Robinson, 1996); single individuals either have hermaphrodite or female flowers. A study of *Psydrax odorata* (Skottsberg, 1945) noted an occurrence of functionally female flowers for some specimens of this species also. It is possible that further studies on the sexual expression of Vanguerieae would show that dioecious or gynodioecious flowers are more common than presently known.

The number of flowers in the inflorescences is correlated with the sex of the flowers. Individuals with female flowers in the dioecious group tend to have one or two flowers in each inflorescence compared to individuals with male flowers that have up to 20 flowers. For *Pyrostria* p.p. (*Pseudopeponidium*) the number of corolla lobes also varies – female flowers usually having eight lobes and male have four to five. This sex-correlated variation complicates the coding for these species, and there are several possible solutions. Polymorphism has been defined as 'variation within species that is (at least partly) independent of ontogeny and sex' (Wiens, 1999). Based on this definition, the sex-correlated variation we see is different from the polymorphic variation present for other characters. This distinction would not be seen if we scored all states that are present for the sex-correlated characters. For species that exhibit a sex-correlated variation for a character, that character is coded as unknown (?).

A difference in number of locules between male and female flowers is also known for many members of the dioecious group (Bridson, 1987b). The number of locules reported for male individuals (two to three) is based on the rudimentary ovaries that sometimes are visible. We suggest that the lesser

amount of ovaries in male flowers is due to their being nonfunctional, and we therefore do not consider this variation to be sex-correlated variation in the same sense as number of flowers per inflorescence discussed earlier. The number of locules coded and included in the morphological matrix is based on functional structures, i.e. ovaries and fruits of female or hermaphrodite flowers.

Inflorescences

Inflorescences at anthesis can either be borne at nodes where the leaves have fallen or be subtended by leaves. The character closely approaches deciduous (leaves not present) or not deciduous (leaves present). Defining homologous structures in inflorescences is generally a difficult matter (Weberling, 1965), and this is definitely the case for the Vanguerieae. There is a lot of variation in the inflorescences, but apart from number of flowers per inflorescence and the presence of a discrete type of paired bracts on the peduncle (see below), we refrain from coding any more characters. Length of peduncle is a potentially useful character; some species have inflorescences that look fasciculate owing to a much shortened peduncle. Some have an elongate peduncle, but there are many intermediates. Length and presence of secondary inflorescence branches vary; several species have very reduced secondary branches resulting in an umbel-like inflorescence. Bracts and bracteoles occur in varying size and shape. Comparisons of these characters from the literature are further complicated by the imprecise usage of the terms. Here we use bracts for structures present on the peduncle and bracteoles for structures occurring further up in the inflorescence. Several species have paired bracts on the peduncle (e.g. *Keetia gueinzii*). Others have a structure more reminiscent of a connate sheath, usually splitting up with increasing age (e.g. *Vangueria madagascariensis*). Bracteoles are commonly unpaired, but can be paired or lacking. The paired, large bracts of *Pyrostria* and related genera are distinct. They completely surround the young inflorescences and later split into two lobes, and are in structure similar to the stipules. A further development has occurred in the genus *Scyphochlamys*, where the flowers are collected in an involucre (Verdcourt, 1983). In *Pyrostria* p.p. (*Peponidium*) a 'false involucre' (Arènes, 1960) is sometimes present at the base of the peduncle. We view these structures as homologous to the bracts of *Pyrostria*.

Number of flowers has, with some hesitation, been included in the analysis. Defining discrete states for the character has been exceedingly difficult and some species fall in between the categories suggested and are polymorphic in the current coding. The character is informative for some genera (e.g. *Fadogia* and *Keetia*), and is often used in the description of genera.

Calyx

Two features of the calyx have been coded; length of the lobes and length of the calyx limb tube. Calyx lobes are useful for identifying species, but seem to be of limited use for delimiting genera. Most species have small, shortly triangular or indistinct lobes, but in some genera the lobes are elongate (defined here as length > 1 mm and ratio of length : width > 1). Elongate lobes can either be linear, lanceolate, obovate, ovate, rounded or triangular. The distinction between the different shapes is hard to make and we have only coded for elongate or not elongate lobes. The calyx limb tube (the part of the calyx free from the ovary, but not lobed) can either be short, with the disk of the gynoecium clearly visible when a flower with the corolla removed is viewed from the side, or well developed and hiding the disk.

Corolla

Corolla aestivation is valvate. Most species are five-lobed, but four lobes are common in *Keetia*, *Psydrax* and *Pyrostria*, and also occur in *Plectroniella*, and all subgenera of *Canthium* apart from subgenus *Lycioserissa*. Some species are obligately four-lobed but many have a varying amount of either four or five lobes. Six lobes (or even more) are sometimes present in *Fadogia*, *Multidentia* and a few other genera. We include a character for the presence of appendages on the corolla lobe apices but do not make any distinction between appendages of different lengths. An apiculum is here interpreted as a short appendage and species with apiculate corolla lobe apices are scored as 'appendages present'. The distinction between the two states is sometimes very hard to make and the character is certainly open to some interpretation. Corolla tube length varies from around 1 mm to 30 mm. There are two discrete groups; tubes usually <8 mm or tubes usually >9 mm. A further division can be made in the group with long tubes between robust, wider tubes and weaker, more slender ones. Species with long tubes are rare in the tribe and were formerly thought to be closely related (e.g. Robyns, 1928), especially those that also are more robust. These species were usually placed in *Fadogia*, *Ancylanthos* or *Temnocalyx*. Today, the importance of the character has been re-evaluated and the two latter genera have been reduced to the type species and the remaining species moved to several other genera (Verdcourt, 1981; Bridson, 1996). In the current circumscriptions, *Fadogia* houses most of the species with long and robust corolla tubes, but they can also be found in *Ancylanthos*, *Fadogiella*, *Lagynias*, *Multidentia*, *Tapiphyllum* and *Temnocalyx*. *Hutchinsonia* and some members of *Cyclophyllum* (Smith & Darwin, 1988; Reynolds & Henderson, 2001) have long but slender corollas, clearly different from the robust

type. There is a tendency for the whole clade (B; Fig. 1) consisting of the *Fadogia–Rytigynia* group and its sister group to have larger corollas compared with the rest of the tribe, both in length and width of the tube and length of the lobes. We therefore annotate this clade as the large-flowered group (Fig. 1), with the caveat that this is not based on a discrete state but instead on a trend, and is not without exceptions. *Cyclophyllum* is the only genus with long corolla tubes not in the large-flowered group.

Most Vanguerieae species have hairs in the corolla tube. Diffusely spreading or crisped hairs present at the throat are common, and a ring of retrorse hairs at varying positions in the corolla tube is also often present. There are, however, intermediates and these can be difficult to assign to one of the mentioned groups. An investigation of the hairs with the help of a phase contrast microscope reveals that apart from the variation noticeable by eye, there are differences in shape and surface only visible under the microscope. We suggest, albeit with some hesitation, that there are indeed two kinds of hairs: verrucose and smooth. The verrucose type has a surface covered with elongate warts and is usually moniliform to a varying degree, at least towards the apex, but can also be straight in outline, tapering to the usually obtuse apex. The hairs are usually diffusely spreading but can be rather retrorse (and thus easily mistaken for the other kind of hairs, see below) and often form a tangled mass close to the opening of the corolla tube. The smooth kind has a smooth or slightly striate surface without warts, is straight or only slightly undulating in outline and has an acute apex. This type can be further divided into (1) retrorse hairs, straight, placed in a well-defined ring usually separate from other kinds of hairs, hairs parallel to each other, and (2) hairs usually diffusely spreading (often bent and with a wrinkled surface on herbarium specimens), only slightly retrorse or not at all, sometimes attached at the same point as verrucose hairs or alone and easily mistaken for the verrucose hairs. Verdcourt (1958) mentions the presence of flat, ribbon-like hairs inside the corolla of most Rubiaceae. The question of whether the two hair types discussed here, i.e. verrucose and smooth, also are flat is difficult to answer owing to the very transparent nature of the hairs, but the smooth kind does appear to be flat. As for the verrucose hairs, we can clearly see that the hairs have a volume, at least when they are moniliform. The two hair types co-occur in several species, but the two states of the smooth hairs never do, supporting our coding of the characters (Patterson, 1988). We have constructed three characters for these hairs: absence or presence of verrucose and smooth hairs, respectively, and type of smooth hairs if present. This investigation can be thought of as a first effort to more clearly understand the very complex and variable nature of the internal corolla hairs in Rubiaceae. Presence of smooth retrorse hairs is clearly informative (Fig. 3), but the verrucose hair character is more homoplastic (Fig. 3). Whether this homoplasy is the result of a mistaken homology assessment on our part or whether the character for some reason is subject to common reversals and parallel evolution is not known. We welcome further investigation into this character.

Several species also have hairs on the outside of the corolla. In some cases the hairs are restricted to the tube or to the lobes, but they can also cover the whole outer part of the corolla. The type of hair present is the same type as other external indumenta.

Androecium

In Vanguerieae, the pollen is released from the anthers in bud directly onto the pollen presenter, which when the flower opens presents the pollen to the pollinators. The stamens are attached to the corolla close to the opening of the tube, alternate to the corolla lobes, and remain in the mature flowers. The anthers may be included in the corolla tube (or just exserted), exserted on long straight filaments, or exserted on reflexed filaments. This is a function of the length and orientation of the filament. Anthers exserted on reflexed filaments is the common situation in *Psydrax*, and a good synapomorphy for the genus. The orientation of the anthers also varies; some species have flexed anthers with the dorsiventral plane perpendicular to the axis of the flower. This is more a function of where the filament attaches on the anther rather than the length of the filament. All anthers are dorsifixed, but the point of attachment ranges from close to the base of the anther to the middle of the anther, and it is especially when the filament is attached close to the middle that the anther tends to be perpendicular to the axis of the flower. Filament attachment on the anther has not been included in the analysis because the variation is gradual. The point of attachment cannot easily be extrapolated from the orientation of the anthers, which varies depending on the developmental stage of the flowers and the quality of the material.

Sterile apical appendages on the anthers are known from several genera in the tribe. The structure is correlated with type of pollen presenter, of which there are two types, type 1 and 2 (Igersheim, 1993; see Gynoecium section in the Discussion). However, we are unable to distinguish between the appendages present in some type 1 species (e.g. *Canthium inerme*) and those of the type 2 species (e.g. *Vangueria parvifolia*) and can find no discrete states for the character. There is too much variation in the apical appendages for the type of pollen presenter to be deduced from them.

Darkened connectives on the dorsal face of the anthers are a feature of many species, but have been
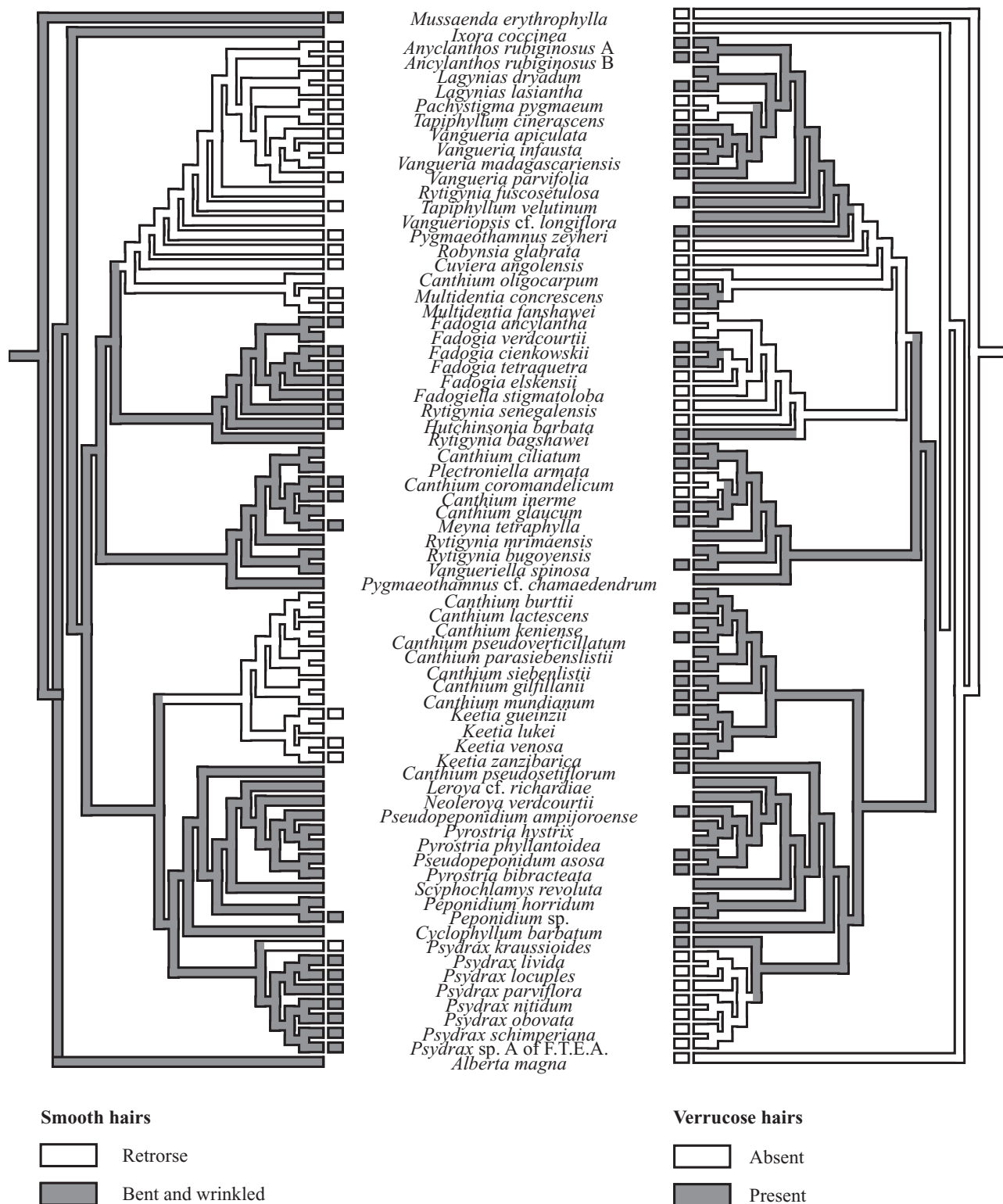
Figure 3. Smooth and verrucose hairs from the corolla inside (characters 21 and 22; Appendix 1) mapped onto one of the most parsimonious trees using MacClade v.4.01 with ACCTRAN optimization. An absence of a square at the end of the branches indicates that the character is either not present or not known for the species.

used to define the subgenera of *Canthium* (Bridson, 1992). Some species have no darkened connective or sometimes just a slight coloration present at the point of filament attachment. Others have the whole dorsal side dark or only the margin pale. The character is only just visible in recently collected or living material, which tends to be very pale; the colour darkens as the specimen dries.

Gynoecium

Style at least twice as long as corolla tube is a feature of all *Psydrax* and *Keetia* species, and is also known from a few other species that are not closely related (see Appendix 2). Structure of the pollen presenter, especially the amount by which the style is recessed into the presenter, has been used successfully for delimiting some of the genera earlier included in *Canthium*, e.g. *Pyrostria* (Bridson, 1987b), and *Keetia* (Bridson, 1986). In *Pyrostria*, the style is not recessed at all; in *Canthium* (as circumscribed by Bridson, 1992) it is slightly recessed; and in *Keetia* it is very deeply recessed. In some cases the difference between recessed and not recessed is very slight, and the character can in difficult cases be subject to opinion. The character is also somewhat connected to the shape of the pollen presenter, i.e. for the style to be deeply recessed the presenter has to be elongate. We code for recessed and not recessed, but do not include the shape of the presenter because it is correlated with the amount by which the style is recessed into the presenter. The variation in shape is gradual throughout the tribe and is also dependent on how deeply lobed the presenter is. The presenters are usually globose to cylindrical, but tend to look coroniform or mitriform when they are deeply divided by the stigmatic lobes. For all examined species, the number of stigmatic lobes is the same as the number of locules in the ovary (before any ovules are aborted). Based on developmental studies of flower buds in different stages of development, Igersheim (1993) suggested that there are two types of pollen presenters in the tribe and that these types are correlated with the presence of sterile apical appendages on the anthers. In type 1, the stigmatic surfaces are tightly pressed together in bud and not exposed, and the anthers lack or have very short appendages. This type is the most common in Vanguerieae. In type 2, the stigmatic surfaces are exposed from early development but protected by the sterile appendages of the anthers. According to Igersheim (1989, 1993), type 2 is present in *Vangueria*, *Fadogia*, *Pachystigma*, *Tapiphyllum*, *Lagynias*, *Ancylanthos* and part of *Rytigynia*, a group of genera that with a few additions agree with the large-flowered group found in this study (also discussed by Bridson, 1996). The type of presenter thus seems to be of taxonomic

importance, but a lack of developmental data stops us from coding the character.

The number of locules in the ovaries and fruits varies from two to 20, with more than five locules only regularly occurring in female individuals of *Pyrostria* and *Scyphochlamys* (Bridson, 1987b; Schatz, 2001). It is a useful character for defining larger groups of genera, e.g. the strongly supported suprageneric *Vangueria* group that with few exceptions has three to five locules. We divide the character into three states: 2 locules, 3–5 locules, and usually > 5 locules. As seen from Figure 4, plurilocular ovaries are likely to have evolved several times and bilocular ovaries is the plesiomorphic condition.

Fruits and seeds

All species have fleshy drupes with one to ten pyrenes. Fruits with one pyrene are, however, the result of faulty development because the number of locules is always two or more. Pyrene characters have been used successfully in several genera earlier included in *Canthium* (Bridson, 1985, 1987a, b). The number of pyrenes is a major factor in determining the shape of the fruit; fruits with two pyrenes tend to be bilobed, fruits with more pyrenes are more globose. The presence of a lid-like area surrounding the apical crest of the pyrenes is a useful character to differentiate *Keetia* from *Psydrax* (Bridson, 1986). This character has not been reported for the sister group of *Keetia*, *Canthium* subgenus *Afrocanthium* (Fig. 1). Other variation of the pyrenes includes degree of woodiness of the pyrene walls; *Multidentia* is partly defined by having thickly woody pyrenes (Bridson, 1987a).

Size of the fruits varies; the bilocular fruits tend to be smaller than the plurilocular ones, with the notable exception of *Multidentia*, a bilocular genus with very large fruits (Bridson, 1987a). There are also some interesting characters to be gained from the embryos (Capuron, 1969; Bridson, 1985; Verdcourt, 1987; Bridson, 1996). Investigations of the embryos have revealed two states for the plane of the cotyledons. For *Keetia* and *Psydrax*, the plane is parallel to the ventral face of the pyrenes, while for most other Vanguerieae it is perpendicular. A difference in the ratio of the length of cotyledons to radicle has also been used, but there is some doubt about how constant both of the mentioned embryonic characters are within species (Verdcourt, 1987).

Pollen

Several papers have been published on the pollen morphology of Vanguerieae (Tilney, 1986; Havard & Verdcourt, 1987; Verdcourt, 1987; Igersheim, 1989; Tilney & van Wyk, 1997; Lens *et al.*, 2000b; we follow the terminology used in the last reference). As it is clear from these studies that the pollen of
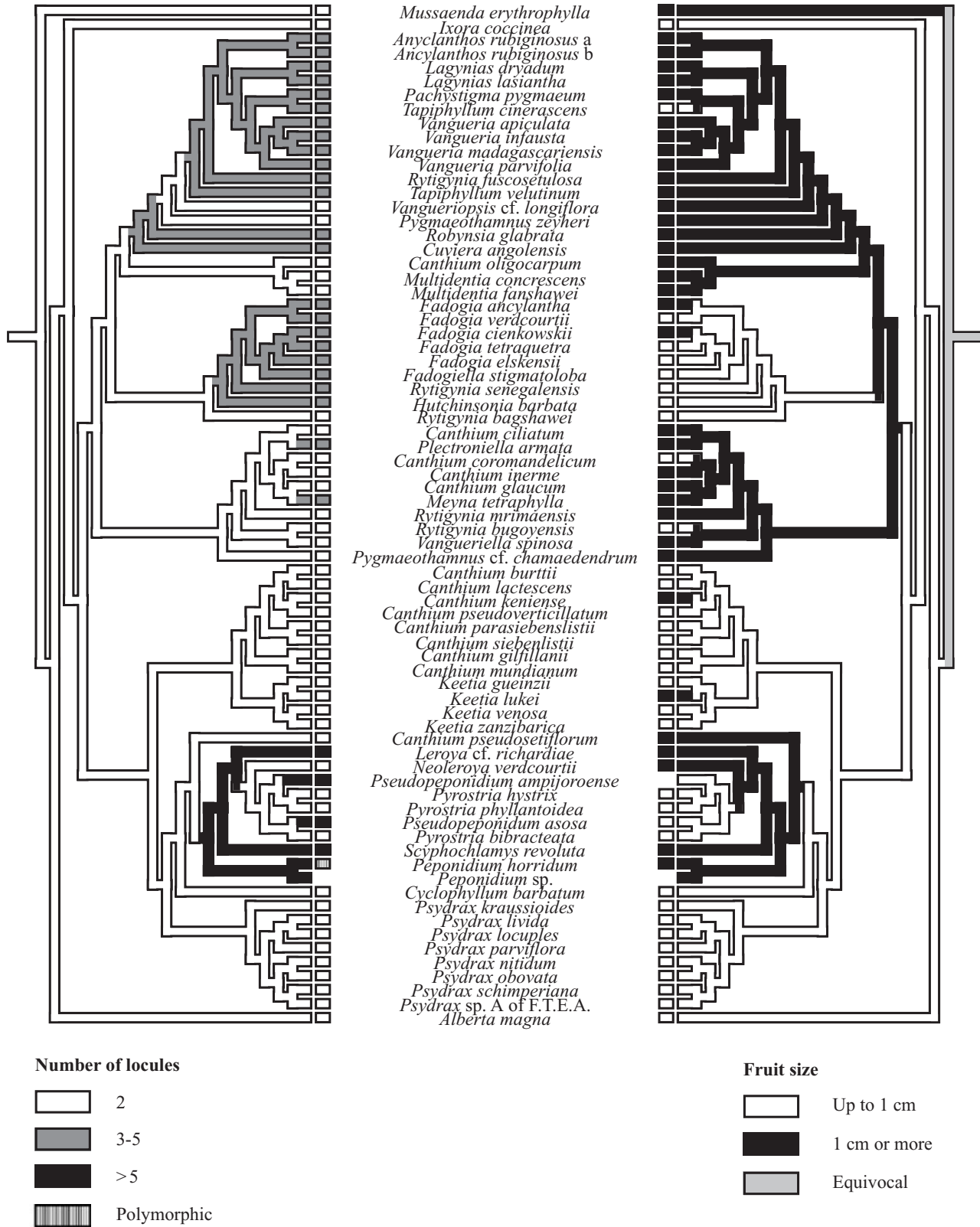
Mussaenda erythrophylla
Ixora coccinea
Ancylanthos rubiginosus a
Ancylanthos rubiginosus b
Lagynias dryadum
Lagynias lasiantha
Pachystigma pygmaeum
Tapiphyllum cinerascens
Vangueria apiculata
Vangueria infausta
Vangueria madagascariensis
Vangueria parvifolia
Rytigynia fuscosetulosa
Tapiphyllum velutinum
Vangueriopsis cf. *longiflora*
Pygmaeothamnus zeyheri
Robynsia glabrata
Cuviera angolensis
Canthium oligocarpum
Multidentia concrescens
Multidentia fanshawei
Fadogia ancylantha
Fadogia verdcourtii
Fadogia cienkowskii
Fadogia tetraquetra
Fadogia elskensii
Fadogiella stigmatoloba
Rytigynia senegalensis
Hutchinsonia barbata
Rytigynia bagshawei
Canthium ciliatum
Plectroniella armata
Canthium coromandelicum
Canthium inerme
Canthium glaucum
Meyna tetraphylla
Rytigynia mrimaensis
Rytigynia bugoyensis
Vangueriella spinosa
Pygmaeothamnus cf. *chamaedendrum*
Canthium burttii
Canthium lactescens
Canthium keniense
Canthium pseudoverticillatum
Canthium parasiebenslistii
Canthium siebenlistii
Canthium gilfillanii
Canthium mundianum
Keetia gueinzii
Keetia lukei
Keetia venosa
Keetia zanzibarica
Canthium pseudosetiflorum
Leroya cf. *richardiae*
Neoleroya verdcourtii
Pseudopeponidium ampijoroense
Pyrostria hystrix
Pyrostria phyllantoidea
Pseudopeponidium asosa
Pyrostria bibracteata
Scyphochlamys revoluta
Peponidium horridum
Peponidium sp.
Cyclophyllum barbatum
Psydrax kraussioides
Psydrax livida
Psydrax locuples
Psydrax parviflora
Psydrax nitidum
Psydrax obovata
Psydrax schimperiana
Psydrax sp. A of F.T.E.A.
Alberta magna

Number of locules

☐ 2

▦ (grey) 3-5

■ > 5

▥ Polymorphic

Fruit size

☐ Up to 1 cm

■ 1 cm or more

▨ (grey) Equivocal

Figure 4. Number of locules in the ovary and size of fruits (characters 29 and 30; Appendix 1) mapped onto one of the most parsimonious trees using MacClade v.4.01 with ACCTRAN optimization. An absence of a square at the end of the branches indicates that the character is either not present or not known for the species.

Vanguerieae is homogeneous and of little use for delimiting genera, we code no pollen characters here. The basic type of pollen in Vanguerieae can be described as 3-zonoaperturate, suboblate to oblate spheroidal, pororate or porate (rarely colporate), and with a perforate to sometimes reticulate sexine. There is a tendency for the taxa in the large-flowered group (Fig. 1), to have both larger and more spheroidal pollen (Igersheim, 1989). There are some interesting pollen structures found in the tribe worth mentioning. Tilney & van Wyk (1997) discuss the presence of intine and/or protoplasm protruding from the apertures (disappears in acetolysis), which they considered homologous to the oncus (a lens-shaped structure occurring just inside of the aperture). They suggest the term 'protruding oncus' for this structure and note that it is present in *Keetia*, *Psydrax* and most subgenera of *Canthium* (subgenus *Canthium* was not investigated). From pictures in Verdcourt (1987) and Igersheim (1989) it is also noticeable on pollen of *Pyrostria*, *Fadogia* and *Vangueria*. It is known from several other rubiaceous groups, e.g. *Myrmecodia* (Robbrecht, 1988) and *Ophiorrhiza* (Weber & Igersheim, 1994). Further studies on nonacetolysed pollen would very likely reveal the structure from more taxa. Also of interest is the presence of sporopollenin bridges that unite the pollen into large masses consisting of hundreds of pollen in the genera *Keetia* and *Psydrax* (Igersheim, 1989). It is not known if this is a regular feature of these genera or if it is only a product of faulty development; it was not reported in other studies of these genera (Tilney & van Wyk, 1997; Lens *et al.*, 2000b). The presence of baculae in several taxa (Igersheim, 1989; Tilney & van Wyk, 1997; Lens *et al.*, 2000b), especially in the large-flowered group, is potentially useful. It is known from *Canthium* subgenus *Afrocanthium*, *Cuviera*, *Fadogia*, *Fadogiella*, *Lagynias*, *Pygmaeothamnus*, *Vangueria* and *Vangueriopsis*.

PHYLOGENY

As discussed above, the morphological variation in Vanguerieae is in many cases continuous, with the consequence that there are few characters easily divided into discrete states. This follows a similar pattern to many other species-rich tropical groups, e.g. Strobilanthineae in the Acanthaceae (Carine & Scotland, 2002) and *Andira* in the Fabaceae (Pennington, 1996). This lack of useful morphological characters complicates the delimitation of genera. In some cases single, easily seen and measured characters have been used to delimit genera to the exclusion of other 'more difficult' characters. When this 'cardinal character' has been subject to parallel evolution, the resulting genera have not been monophyletic. An example of this is

the polyphyletic genus *Tapiphyllum* (Fig. 1), mostly defined by the presence of velvety indumentum obscuring the leaf surface. For *Rytigynia*, also a polyphyletic genus, several characters have been used to delimit the genus, but the characters have been too general and shared by many other genera, i.e. a shrubby habit and few-flowered inflorescences. The lack of a unique synapomorphy for *Rytigynia* has resulted in a polyphyletic genus.

In the following discussion we focus on groups that receive strong support from the phylogenetic analysis, and discuss how these groups compare to the traditionally recognized genera. We also indicate morphological characters that could be used to delimit these groups.

Spiny group

The spiny group is a strongly supported (100; 100) suprageneric group. It forms a strongly supported clade (A; Fig. 1) together with the large-flowered group. Apart from *Pygmaeothamnus* cf. *chamaedendrum*, all species in the spiny group are trees or shrubs, have paired leaves, domatia and paired supra-axillary spines that are, with the exception of *Canthium inerme*, in combination with brachyblasts. Spines are rare in the tribe outside this group, so it was therefore given an informal name based on this structure (Lantz *et al.*, 2002). *Plectroniella* and *Meyna* are plurilocular; the other taxa have two locules. *Pygmaeothamnus* cf. *chamaedendrum* is very different from the other species in the group. It is a geofrutex that lacks spines, brachyblasts and domatia.

Six genera are represented in the spiny group. The clade is largely unresolved and presents several taxonomic problems. Three *Canthium* species representing two different subgenera belong in the group; *Canthium coromandelicum* (the type species of *Canthium*) and *C. glaucum* in subgenus *Canthium* and *C. inerme*, the type of subgenus *Lycioserissa*. Species from the other subgenera of *Canthium* are found in distantly related clades, and the genus needs to be restricted. *Rytigynia bugoyensis* and *R. mrimaensis* are not close to the type species of the genus (*R. senegalensis*), and should be removed from *Rytigynia*.

Only the clade consisting of *Rytigynia bugoyensis* and *Vangueriella spinosa* has strong support (92; 96). This confirms the suggestion of Bridson (1992) that *Rytigynia bugoyensis* possibly should be transferred to *Vangueriella* sect. *Stenosepala*. The two South African species *Canthium ciliatum* and *Plectroniella armata* also form a clade, but with weaker support (63; 77), an affinity suspected also from morphology (Bridson, 1992). Based on morphology, *Meyna* has been suggested as a likely candidate for inclusion in *Canthium* subgen. *Canthium* (Bridson, 1992). *Meyna*, together

with *Canthium* subgen. *Canthium*, share a distribution with representatives in both Asia and on the African mainland. No other genus in the group is found in Asia, and this could suggest a common origin for *Meyna* and *Canthium* subgen. *Canthium*. The inclusion of *Rytigynia mrimaensis* in *Rytigynia* has earlier been questioned and it has been suggested to be close to some Indian species of *Canthium* (Group 1; Bridson, 1992). None of these species are sampled here, but they all have paired supra-axillary spines in combination with brachyblasts and thus most likely belong in the spiny group.

The unresolved nature of the clade makes it difficult to suggest how to deal with the polyphyletic genera. One solution would be to sink all taxa in the spiny group into *Canthium*. *Canthium* is one of the first genera described in Vanguerieae (Lamarck, 1785) and *C. coromandelicum* is the type species of the genus. The group is strongly supported (100; 100), but would be very heterogeneous morphologically. This is mainly due to *Pygmaeothamnus*, which differs in a number of characters from the other taxa in the tribe. A generic description of this group would include most of the morphological variation present in the whole tribe. If *Pygmaeothamnus* were excluded, the resulting group would be much better delimited in a morphological sense. This group would be characterized by the presence of paired supra-axillary spines, domatia and usually brachyblasts and thus be morphologically homogeneous.

We suggest that *Canthium* is restricted to the spiny group with the exception of *Pygmaeothamnus* cf. *chamaedendrum*. In view of the very different morphology of this species compared to the other species in the clade, we await more material, preferable flowering, before we draw any conclusions about the taxonomy. We make combinations at a generic level and also make new combinations for the species included in the analysis, but avoid making combinations for any species not sampled. No modern revision exists for *Meyna* and it is has been suggested that the species are poorly delimited (Verdcourt & Bridson, 1991).

It should be noted that we only sample one Asiatic species (*C. coromandelicum*) but the majority of the species congruent with our view of *Canthium* are Asiatic (see Bridson, 1992). In morphology these species are entirely in accordance with our definition of *Canthium*, i.e. they have paired supra-axillary spines in combination with brachyblasts and have, apart from the species in *Meyna*, already names in *Canthium*. Thus, our circumscription of *Canthium* disrupts the current taxonomy of these species to only a minor extent. It has been suggested that smaller groups within the Asiatic *Canthium* may be recognized (Bridson, 1992), but we consider the differences between these groups to be small and that the advantages of a strongly delimited

Canthium far outweigh the fact that these groups are not formally recognized. If their monophyly is eventually confirmed, they may be recognized at the subgeneric level. There remains also a number of Asiatic and Pacific species currently in *Canthium* that need to be transferred to *Cyclophyllum*, *Psydrax* or *Pyrostria* (Bridson, 1985, 1987b; Smith & Darwin, 1988). These genera are already strongly delimited in a morphological sense, and with the new circumscription of *Canthium* suggested here, this is true also for *Canthium*. What is needed now is that all non-African species of *Canthium* should be examined and formally assigned to one of these genera.

Large-flowered group

Multidentia, a genus with a wide distribution in tropical Africa, receives strong support (91; 95) in the analysis. A combination of conspicuous tertiary nerves, a well-developed calyx limb tube and thick, woody pyrenes makes the genus morphologically distinct. *Multidentia*, *Canthium oligocarpum* and *Cuviera angolensis* are at unresolved basalmost positions in a moderately supported (73; 82) clade (E; Fig. 1) whose members, with few exceptions, have large fruits and smooth, retrorse corolla tube hairs. *Canthium oligocarpum*, a member of *Canthium* subgen. *Lycioserissa*, is restricted to high-altitude evergreen forests in eastern and southern Africa. It is not close to either the type of *Canthium* or to *C. inerme*, the type of subgenus *Lycioserissa*, both in the spiny group, and while it is certain it cannot remain in *Canthium*, the unresolved position does not enable us to suggest a generic placement for the species. *Cuviera* is a genus of about 20 species centred in West Africa, exhibiting many features rare in the tribe such as myrmecophyly and swollen hairy styles (e.g. Hallé, 1959).

A moderately supported (76; 81) clade (clade C; Fig. 1) positions *Pygmaeothamnus zeyheri* and *Robynsia glabrata* at unresolved basal positions. *Pygmaeothamnus zeyheri* is a geofrutex with a widespread distribution in central, eastern, and southern Africa. The other member of the genus (*P. chamaedendrum*; see spiny group) has a restricted distribution in north-eastern South Africa. *Robynsia* is a monotypic West African genus showing some morphological affinity with *Cuviera*, such as large many-flowered inflorescences with large bracts and bracteoles.

Vangueriopsis cf. *longiflora*, forms a weekly supported (58; 68) clade together with the strongly supported (87; 95) *Vangueria* group. *Vangueriopsis* (as circumscribed by Verdcourt, 1987) is a small genus of four species with a mostly Guineo-Congolian distribution (Robbrecht, 1996) but also occurring in East Africa (Verdcourt & Bridson, 1991). A member of the genus, *V. lanciflora*, was included in an earlier analy-

sis (Lantz *et al.*, 2002), where it grouped with *Vangueria madagascariensis* and *Vangueria infausta* with strong support. We are uncertain about the identity of the specimen used in the earlier analysis, and it was therefore not included in the present study. *Vangueriopsis* exhibits a number of floral features rare in the tribe. Corolla lobes are long and linear, usually > 15 mm, anthers are exserted on long filaments, and hairs are present on the anthers. These characters are not shared by the *Vangueria* group, an assemblage of several genera with especially unclear generic limits (Verdcourt, 1981; Bridson, 1996, 1998). All taxa in the Vangueria group lack domatia (except a rare occurrence in *V. apiculata*) and have three to five locules. Several nodes are collapsed in the strict consensus tree and the support for several clades is low, but *Tapiphyllum* is polyphyletic and the monophyly of *Vangueria* is uncertain. *Lagynias* is monophyletic with strong support (100; 100), but the two included species are close and the monophyly of *Lagynias* is not really tested until more aberrant species such as *L. monteiroi* (Oliv.) Bridson are included. Sister to *Lagynias* is a moderately supported (78; 83) group that includes several *Vangueria* species of which the type species, *V. madagascariensis*, is one.

The *Fadogia–Rytigynia* group is a strongly supported (100; 100) group sister to the other taxa in the large-flowered clade. *Fadogia* is paraphyletic with *Fadogiella* nested within it; the genera also share several morphological features. Synapomorphic characters for *Fadogia* including *Fadogiella* are geofrutescent habit, whorled leaves (only sometimes in *Fadogiella*) and an absence of domatia. *Rytigynia* is highly polyphyletic and is, in addition to the *Fadogia–Rytigynia* group, also represented in the spiny group and in the *Vangueria* group. The genus does not form a monophyletic group even within the *Fadogia–Rytigynia* group. *Rytigynia senegalensis*, the type species of *Rytigynia*, has an isolated position as sister to *Fadogia/Fadogiella*. *Rytigynia bagshawei* is sister to all other taxa in the *Fadogia–Rytigynia* group.

It is within the large-flowered group that we encounter some of the most difficult issues concerning the classification of Vanguerieae. At least 15 genera are represented, there are cases of incongruence between the nuclear and plastid datasets, several nodes are weekly supported or collapsed, and several genera are para- or polyphyletic. These issues will be investigated in greater detail in another study (work in progress) in which more species are sampled and more data are added to better resolve the phylogeny of the group. This will hopefully enable us to elucidate the taxonomy of the group.

Canthium subgen. Afrocanthium, Keetia and Psydrax. *Canthium* received a lot of attention during the pro-

duction of the Vanguerieae part of the *Flora of Tropical East Africa* (Verdcourt & Bridson, 1991). The transfer of many species from the large genus *Canthium* created several smaller, better-delimited genera. *Psydrax* and *Keetia* are two such genera (Bridson, 1985, 1986). The remaining species of *Canthium* were divided into subgenera (Verdcourt & Bridson, 1991; Bridson, 1992) to reflect the fact that there were still large morphological differences present in the genus and to avoid premature name changes. *Canthium* subgen. *Afrocanthium*, *Keetia*, and *Psydrax* are all supported as monophyletic in the phylogeny (Fig. 1). *Keetia* has been thought to be related to *Psydrax* (Bridson, 1985, 1986) and shares many morphological characters with that genus, including style at least twice the length of the corolla tube (except *P. kraussioides* of subgenus *Phallaria*), style deeply recessed into pollen presenter, hairy disks (not all taxa), cotyledons orientated parallel to the ventral face of seed, and the occurrence of sporopollenin bridges in the pollen. *Keetia* receives strong support (100; 100) in the phylogeny and is well delimited in a morphological sense. All species are climbers, have a lid-like area on the pyrenes and probably have smooth retrorse hairs in the corolla tube, although this last character has not been confirmed for species not included here. Two subclades are supported, but apart from the slightly longer calyx lobes in the strongly supported clade of *K. gueinzii* and *K. lukei*, morphological support is lacking for this division. The support for *Psydrax* is lower (70; 77), which is surprising considering the number of morphological synapomorphies shared by the species. Unicellular hairs, no hairs inside the stipules, and anthers exserted on reflexed filaments are all characters that are synapomorphic for the genus. The species also share a region of 40 bases in *trnT-F (*except possibly *P. kraussioides*; see Results) that is not alignable with other taxa. It does not appear to be an inversion and is probably best explained by intramolecular recombination (Kelchner, 2000). This synapomorphic molecular character further supports the monophyly of *Psydrax*. The genus is the largest in Vanguerieae comprising over 100 species and has a distribution equal to that of the tribe. The Asiatic species *P. nitidum* is nested within a clade of African species, suggesting an African origin of *Psydrax*, but the support for this relationship is weak and we only include a single species from Asia.

Keetia and *Canthium* subgen. *Afrocanthium* are strongly supported (89; 94) as sister taxa. However, apart from a lack of hairs within the stipules that is shared by *K. gueinzii* and *C. mundianum*, the morphology gives little indication for this relationship. We know of no morphological synapomorphy for this group, and the two groups should remain separate.

Canthium subgen. *Afrocanthium* receives strong support from the phylogeny (100; 100). The subgenus is restricted to eastern and southern Africa and in the most recent revision 20 species are recognized (Bridson, 1992). Eight species are included in this analysis sampled from Kenya (*C. keniense*, *C. lactescens*, *C. pseudoverticillatum*), Tanzania (*C. burttii*, *C. siebenlistii*), Malawi (*C. parasiebenlistii*), and South Africa (*C. gilfillanii*, *C. mundianum*) and thus represent a broad geographical range. The clade is divided into two clades; one strongly supported (100; 100) consisting of the two South African species *C. mundianum* and *C. gilfillanii* (perhaps conspecific and best differentiated at the infraspecific level) and one more moderately supported (69; 80) in which the East African and Zambezian species are included. *Canthium siebenlistii* and *C. parasiebenlistii*, considered close (Verdcourt & Bridson, 1991), also form a strongly supported clade (97; 98), as does *C. keniense* and *C. pseudoverticillatum* (96; 98), but the relationships within the *Afrocanthium* clade are otherwise weakly supported or unresolved. The subgenus is recognized by a combination of absence of dark connective on the stamens, inflorescences borne at nodes from which the leaves have fallen, usually evident lenticels, and very short calyx limb tubes. A style that usually only slightly exceeds the corolla tube sets it apart from *Keetia* and *Psydrax*. Within the subgenus there is variation especially in the size of leaves, type of stipules and shape of fruits, and the majority of this spectrum of variation is exhibited by the species sampled. *Canthium* subgen. *Afrocanthium* is a morphologically distinct and strongly supported subgenus clearly separate from the type species *C. coromandelicum* and should be given generic rank. The type of the subgenus, *C. lactescens*, is strongly supported as a member of the clade and the name *Afrocanthium* is therefore applicable to the clade.

Cyclophyllum and the dioecious group

The clade consisting of *Pyrostria*, *Cyclophyllum* and closely related genera is strongly supported (94; 97). *Leroya*, *Neoleroya*, *Peponidium* and *Pseudopeponidium* are restricted to Madagascar, *Canthium* subgen. *Bullockia* to the African mainland, and *Pyrostria* is represented both in Africa, on Madagascar, on the Mascarenes and the Comoro Islands, and in southeast Asia (Bridson, 1987b). *Scyphochlamys* is a monotypic genus restricted to the small island of Rodriguez located east of Madagascar. *Cyclophyllum* is a Pacific and Australian genus (Reynolds & Henderson, 2001) and is thus geographically separated from the other genera in the clade. *Pseudopeponidium* (Bridson, 1987b), *Leroya*, *Neoleroya* and *Peponidium* (Schatz, 2001) have been formally included in *Pyrostria*, but no individual combinations have been made and we

therefore use the old generic names in the following discussion.

The main morphological characters shared by the genera are fleshy corollas and a large amount of moniliform hairs in the corolla throat congesting the opening. These characters are not shared by *Canthium* subgen. *Bullockia* (here represented by *C. pseudosetiflorum*) and *Peponidium*, but are otherwise present for all species in the clade. At the base of the clade is the hermaphrodite *Cyclophyllum barbatum*, a member of a genus that has been suspected to have an affinity with *Pyrostria* (Bridson, 1987b), and this is supported here. A strongly supported clade (99; 100) that includes all dioecious Vanguerieae taxa (*Pyrostria bibracteata* is a hermaphrodite) is sister to *C. barbatum*. This clade will be referred to as the dioecious group (Fig. 1). Apart from *Canthium pseudosetiflorum*, a member of the dioecious subgenus *Bullockia*, all species in the clade also have large paired bracts, or homologous structures, on the peduncles. The clade is largely unresolved but a few clades receive moderate to strong support. Two species from the African mainland, *Pyrostria hystrix* and *P. phyllantoidea*, form a moderately supported (75; 82) clade but a third species, *P. bibracteata* with a distribution in both Africa and on Madagascar, groups with the Malagasy species *Pseudopeponidium asosa* (90; 95). The two species of *Peponidium* also group together (71; 77), but at an unresolved basal position. Although strongly supported as a group, the dioecious clade is lacking internal support and we refrain from making any new combinations pending further data (work in progress). The only solution that receives any support is the inclusion of the whole dioecious clade in *Pyrostria*, which would necessitate an inclusion also of *Scyphochlamys* and *Canthium* subgen. *Bullockia*.

PHYLOGENETIC AFFINITIES OF GENERA NOT INCLUDED IN THE PHYLOGENETIC ANALYSIS

The following conclusions are based both on comparisons of actual material and on data taken from literature.

Eriosemopsis was described by Robyns (1928) to accommodate a single species from South Africa, *E. subanisophylla*. It is a geofrutex with elongated calyx lobes, two locules in the ovary, pilose hairs at the opening of the corolla tube and a ring of retrorse hairs further down in the tube. All of these characters agree with *Pygmaeothamnus zeyheri*, that also occurs in South Africa. *Eriosemopsis subanisophylla* was probably described in a genus of its own and not included in *Pygmaeothamnus* owing to its thick indumentum and raised venation on the leaves, a unique combination in the tribe, but otherwise the diagnostic charac-

ters of both genera overlap and the Robyns (1928: fig. II) considered them closely related.

Everistia was erected for a single Australian species, *E. vaccinifolia* (Reynolds & Henderson, 1999), based on the different morphology of this species compared to other genera occurring in Australia, i.e. *Psydrax* and *Cyclophyllum*. The genus is characterized by a multi-branched habit, young branches usually resembling spines, obscure nerves in the leaves and a deeply two-lobed pollen presenter. Of the two other genera occurring in Australia, it approaches *Psydrax* the most. Both genera have long, exserted filaments and delicate corollas (compared to the fleshy corollas of *Cyclophyllum*), but in *Psydrax* the filaments are usually reflexed. We find it likely that *Everistia* is related to *Psydrax*, but it remains to be seen whether or not *Everistia* is nested within *Psydrax*.

Perakanthus is a monotypic genus endemic to peninsular Malaysia (Wong, 1989). Only a few other Vanguerieae genera, i.e. *Psydrax* (Bridson, 1985), *Pyrostria* (Bridson, 1987b), *Meyna*, and several unrevised groups currently with names in *Canthium* (Wong, 1989; Bridson, 1992) occur in south-east Asia, and none of them are similar to *Perakanthus*. The flowers of *Perakanthus* have slightly curved corolla tubes ≤12 mm long with external indumentum, some hairs present at the opening of the tube, and a ring of retrorse hairs close to the disk. Similar flowers are found in *Ancylanthos*, *Lagynias* and *Tapiphyllum* (Bridson, 1996), but these are African genera and are without exception plurilocular; *Perakanthus* has two locules. It remains to be settled whether *Perakanthus* is a rare representative of the *Vangueria* group in south-east Asia or whether it is related to any of the other genera occurring in the same area with which it has acquired several autapomorphies.

Temnocalyx is a monotypic genus with a restricted distribution in south-west Tanzania (Verdcourt, 1981). Diagnostic features of the genus are a long and robust corolla tube which is glabrous both internally and externally, and an obconic pollen presenter which is continuous with the style (Verdcourt & Bridson, 1991). Similar long and externally glabrous corolla tubes are only found in *Fadogia*, but these species usually have hairs in the corolla tube. The only exception is *F. fuchsoides*, which sometimes lacks internal hairs. Furthermore, *Fadogia* is a morphologically well-delimited genus characterized by a number of features (see above) and *Temnocalyx* does not share these features. Based on these differences it is unlikely that *Temnocalyx* is close to *Fadogia*. The plurilocular ovary together with inflorescences borne at nodes from which the leaves have fallen, suggest an affinity with the *Vangueria* group. However, *Temnocalyx* has solitary flowers and domatia, and this is not known from the *Vangueria* group. Solitary flowers are common in

Rytigynia, and this genus (i.e. excluding the species known not to belong in the *Fadogia*–*Rytigynia* group) also has short calyx lobes, a feature shared with *Temnocalyx*, but large flowers are unknown in the genus and the inflorescences are usually subtended by mature leaves. A few species in the dioecious group also have a pollen presenter continuous with the style, but these pollen presenters are usually small and globose, not obconic and deeply divided by the stigmatic lobes as in *Temnocalyx*. In a tribe diagnosed by the presence of a usually globose or cylindrical pollen presenter with the style recessed into the presenter, this is of special interest, and could possibly imply a plesiomorphic state.

The otherwise unknown combination of characters of *Temnocalyx* and the unique nature of the pollen presenter makes it hard to draw any conclusions concerning the phylogenetic affinities of the genus. At the moment, it is uncertain whether *Temnocalyx* is a genus that has acquired several autapomorphic characters or if it perhaps has retained some plesiomorphic features.

CONCLUSIONS

The well resolved and in many cases also strongly supported phylogeny presented here makes it possible for us to make several statements concerning the classification of Vanguerieae. The phylogenetic affinities of most Vanguerieae genera are now rather clear. Only four currently accepted genera are not included in our analysis. For two of these, i.e. *Eriosemopsis* and *Everistia*, there is morphological evidence that enables us to suggest possible relationships, but for *Perakanthus* and *Temnocalyx* molecular data are needed to ascertain their phylogenetic relationships. It is clear that the taxonomy now in use is in bad agreement with the phylogeny. *Canthium*, *Rytigynia*, *Tapiphyllum* and possibly *Pygmaeothamnus* are polyphyletic, *Fadogia* is paraphyletic. Some genera are monophyletic with strong support, i.e. *Keetia*, *Lagynias* (poorly sampled) and *Multidentia*, as is *Canthium* subgen. *Afrocanthium*, but most of the strongly supported clades are not identical to currently accepted genera. Four such clades (apart from the very inclusive large-flowered group) are discussed; the spiny group, the *Vangueria* group, the *Fadogia*–*Rytigynia* group, and the dioecious group.

We suggest several changes to the classification of Vanguerieae (see Fig. 5), but also refrain from making new combinations when we consider data are lacking. We restrict *Canthium* to the spiny group and make new combinations in *Canthium* for the species sampled with the exception of *Pygmaeothamnus* cf. *chamaedendrum*. The affinity between this nonspiny geofrutex and a group which without exception
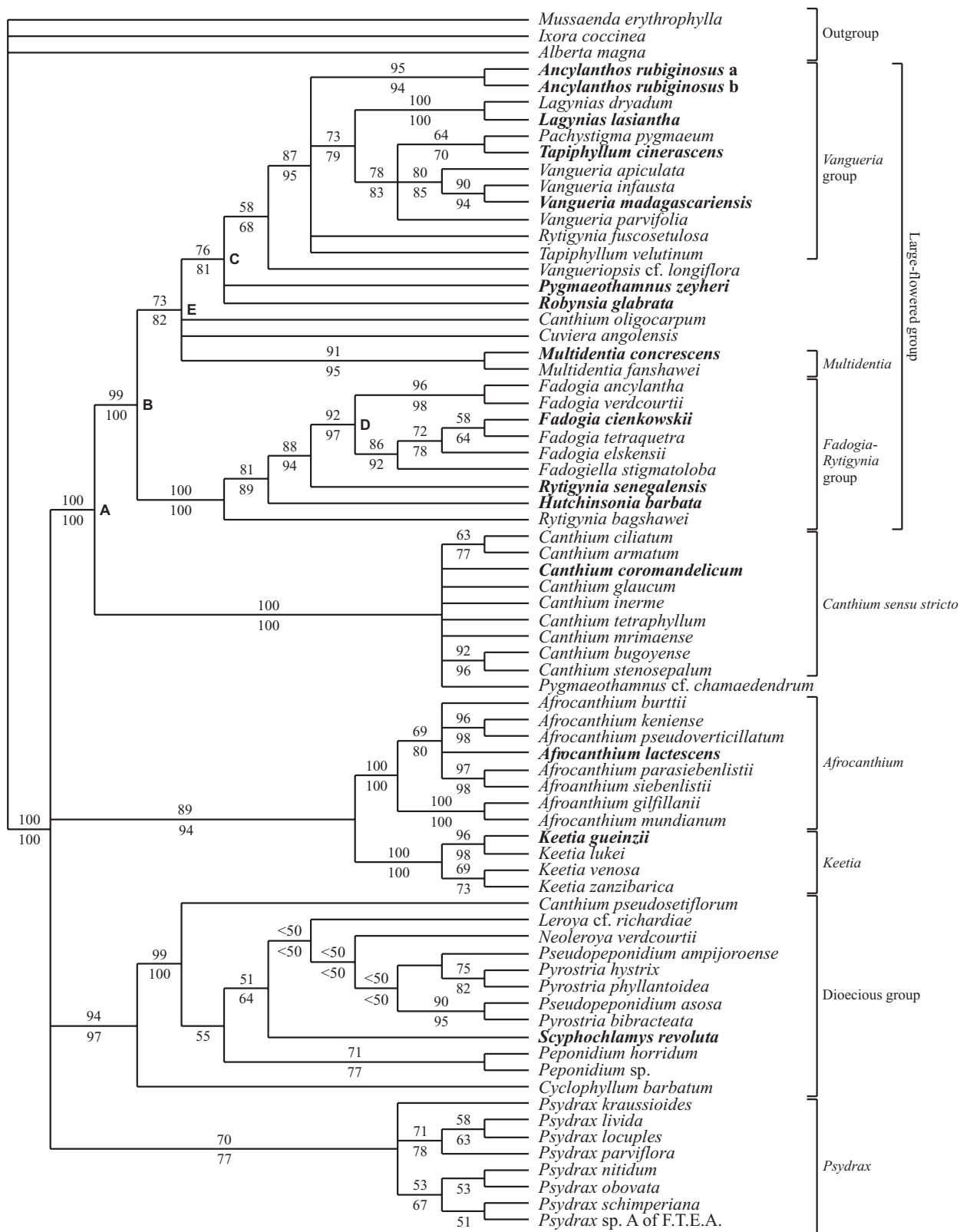
Figure 5. The strict consensus tree from the combined analysis (ITS + *trnT-F* + morphology) with the new classification implemented. Bootstrap support values are above the branches, jack-knife below. A, B, C, etc. refer to clades mentioned in the text. Type species of currently recognized genera in bold.

constitutes spiny shrubs or trees needs to be investigated further. We raise the status of *Canthium* subgen. *Afrocanthium* to genus *Afrocanthium* and make new combinations in this genus for 17 species. The *Vangueria* group, the *Fadogia–Rytigynia* group, and the dioecious group all contain more than 50 species, and these groups should be better sampled before any new classification is suggested (work in progress). With this study we now have a better understanding of how the genera of Vanguerieae are related. What is needed now is better sampled studies within the monophyletic groups identified, and revisional work in which the results of phylogenetic studies are utilized.

TAXONOMIC TREATMENT

AFROCANTHIUM

Canthium subgen. *Afrocanthium* is strongly supported as monophyletic and is also morphologically distinct. It is distanced from the type of *Canthium* and we therefore change the status of the subgenus to genus. That the subgenus was distinct from the type of *Canthium* was also known by the author of the subgenus (D. Bridson, pers. comm.). We accept the revision of *Afrocanthium* (Bridson in Verdcourt & Bridson, 1991; see also Bridson, 1992) without modifications to the circumscriptions of any species but make new combinations under *Afrocanthium*. We do not provide names for the species that in the revision were described as species *A*, *B* and *C*, but suggest that these species are formally described under *Afrocanthium* when complete material is available. See these publications also for a list of full synonyms.

Afrocanthium (Bridson) Lantz & Bremer **stat. nov.** = *Canthium* subgen. *Afrocanthium* Bridson, Fl. Trop. E. Africa, Rubiaceae 3: 864. 1991.

Description: See Bridson (1992).

Type: Afrocanthium lactescens (Hiern) Lantz

New combinations in *Afrocanthium* (Bridson) Lantz & Bremer

AFROCANTHIUM BURTTII (Bullock) Lantz **COMB. NOV.**
 Canthium burtii Bullock in Kew Bull. 1933: 146. 1933.

AFROCANTHIUM GILFILLANII (N.E. Br.) Lantz **COMB. NOV.**
 Plectronia gilfillanii N.E. Br. in Bull. Misc. Inform. Kew 1906: 105. 1906.

AFROCANTHIUM KENIENSE (Bullock) Lantz **COMB. NOV.**
 Canthium keniense Bullock in Kew Bull. 1932: 377. 1932.

AFROCANTHIUM KILIFIENSE (Bridson) Lantz **COMB. NOV.**
 Canthium kilifiense Bridson in Fl. Trop. E. Africa, Rubiaceae 3: 874. 1991.

AFROCANTHIUM LACTESCENS (Hiern) Lantz **COMB. NOV.**
 Canthium lactescens Hiern in Cat. Afr. Pl. 1: 511. 1898.

AFROCANTHIUM MUNDIANUM (Cham. & Schltdl.) Lantz **COMB. NOV.**
 Canthium mundianum Cham. & Schltdl. in Linnaea 4: 131. 1829.

AFROCANTHIUM NGONII (Bridson) Lantz **COMB. NOV.**
 Canthium ngonii Bridson in Kew Bull. 47: 371, fig. 4. 1992.

AFROCANTHIUM PARASIEBENLISTII (Bridson) Lantz **COMB. NOV.**
 Canthium parasiebenlistii Bridson in Fl. Trop. E. Africa, Rubiaceae 3: 870. 1991.

AFROCANTHIUM PETERI (Bridson) Lantz **COMB. NOV.**
 Canthium peteri Bridson in Fl. Trop. E. Africa, Rubiaceae 3: 873. 1991.

AFROCANTHIUM PSEUDORANDII (Bridson) Lantz **COMB. NOV.**
 Canthium pseudorandii Bridson in Kew Bull. 47: 385, fig. 12. 1992.

AFROCANTHIUM PSEUDOVERTICILLATUM (S. Moore) Lantz **COMB. NOV.**
 Canthium pseudoverticillatum S. Moore, J. Bot. 43: 352. 1905.

AFROCANTHIUM RACEMULOSUM (S. Moore) Lantz **COMB. NOV.**
 Canthium racemulosum S. Moore in J. Linn. Soc., Bot. 40: 87. 1911.

AFROCANTHIUM RONDOENSE (Bridson) Lantz **COMB. NOV.**
 Canthium rondoense in Fl. Trop. E. Africa, Rubiaceae 3: 868. 1991.

AFROCANTHIUM SALUBENII (Bridson) Lantz **COMB. NOV.**
 Canthium salubenii Bridson in Kew Bull. 47: 377, fig. 7. 1992.

AFROCANTHIUM SHABANII (Bridson) Lantz **COMB. NOV.**
 Canthium shabanii Bridson in Fl. Trop. E. Africa, Rubiaceae 3: 873. 1991.

AFROCANTHIUM SIEBENLISTII (K. Krause) Lantz **COMB. NOV.**
 Plectronia siebenlistii K. Krause in Bot. Jahrb. Syst. 57: 35. 1920.

AFROCANTHIUM VOLLESENII (BRIDSON) LANTZ **COMB. NOV.**

> *Canthium vollesenii* Bridson in Fl. Trop. E. Africa, Rubiaceae 3: 875. 1991.

CANTHIUM

Canthium is polyphyletic and the circumscription of the genus needs to be changed. We suggest that the genus is restricted to the spiny group with the exception of *Pygmaeothamnus* cf. *chamaedendrum*, a taxon that needs to be investigated further. New combinations at the generic level are made and we also make combinations for the species sampled in this study. It should be mentioned that the genus *Plectronia* was for a long time thought to be an earlier synonym of *Canthium* and many species now in *Canthium* were therefore described in this genus. This was made in error and the name *Plectronia* should not be used. See Ross (1975) and Verdcourt (1987) for more details. Note that even with these combinations and the change of status for subgenus *Afrocanthium* suggested here, *Canthium* is not monophyletic. The issue of *Canthium oligocarpum* and *Canthium* subgen. *Bullockia* will be addressed in later publications (work in progress). The description included below is for *Canthium s.s.*; *Afrocanthium*, *Canthium* subgen. *Bullockia*, and *C. oligocarpum* are excluded.

CANTHIUM Lam., ENCYCL. 1: 602. 1785.
Lycioserissa Roem. & Schultes, Syst. Veg. 4: 353. 1819.
Dondisia DC., Prodr. 4: 469. 1830.
Psilostoma Klotzsch ex Eckl. & Zeyher, Enum. pl. afric. austral. 361. 1837.
Meyna Link, Jahrb. Gewächsk. 1(3): 32. 1820, syn. nov.
Plectroniella Robyns, Bull. Jard. Bot. État. 11: 243. 1928, syn. nov.
Vangueriopsis sect. *Stenosepalae* Robyns, Bull. Jard. Bot. État. 11: 243. 1928; *Vangueriella* sect. *Stenosepalae* (Robyns) Verdc., Kew Bull. 42: 189. 1987, syn. nov.
Canthium subgen. *Lyciocerissa* (Roem. & Schultes) Bridson, Fl. Trop. E. Africa, Rubiaceae 3: 876. 1991, syn. nov.

Description: Shrubs, small trees, scandent shrubs, or (rarely) lianas. Paired supra-axillary spines and brachyblasts usually present. LEAVES deciduous, paired on brachyblasts or sometimes, leaves paired on main shoots, glabrous to sparsely hairy, rarely with thick indumentum; domatia present as tufts of hair, as shallow hair-covered depressions, or (rarely) lacking; stipules small, triangular or with a broad base and a subulate to filiform appendage, pubescent within. FLOWERS 4- or 5-merous, one to twenty, in fasciculate to long pedunculate inflorescences;

bracts and bracteoles inconspicuous. Calyx limb tube short, not equalling disk, or sometimes more developed, repand, shortly dentate or lobes triangular to linear-lanceolate. Corolla white to green, externally glabrous or with a few scattered hairs, internally with tangled hairs at opening of tube and/or with retrorse hairs; lobes acute to acuminate, sometimes shortly apiculate. Anthers inserted at throat or clearly exserted, with darkened connective or not. OVARY 2–5-locular. Style slightly to long exserted. Pollen presenter coroniform to globose, rarely longer than wide, hollow at base. FRUIT a fleshy drupe with 1–5 pyrenes.

New combinations in Canthium

CANTHIUM ARMATUM (K. SCHUM.) LANTZ **COMB. NOV.**
> *Vangueria armata* K. Schum. in Bot. Jahrb. Syst. 28: 69. 1899.
> *Plectroniella armata* (K. Schum.) Robyns in Bull. Jard. Bot. État. 11: 243. 1928.

CANTHIUM MRIMAENSE (VERDC.) LANTZ **COMB. NOV.**
> *Rytigynia mrimaensis* Verdc., in Kew Bull. 42: 170. 1987.

CANTHIUM BUGOYENSE (K. KRAUSE) LANTZ **COMB. NOV.**
> *Plectronia bugoyensis* K. Krause in Wiss. Erg. Deutch. Zentr.-Afr. Exped., Bot. 1907–8: 327. 1911.
> *Rytigynia bugoyensis* (K. Krause) Verdc. in Bull. Jard. Bot. État. 50: 515. 1980.

CANTHIUM STENOSEPALUM LANTZ **NOM. NOV.**
> *Phallaria spinosa* Schumach. & Thonn. in Beskr. Guin. Pl. 113. 1827.
> *Vangueriella spinosa* (Schumach. & Thonn.) Verdc. in Kew Bull. 42: 10. 1987.
> *Canthium spinosum* (Klotzsch ex Ecklon & Zeyher) Kuntze already exists. The species epithet is derived from *Vangueriella* sect. *Stenosepalae* Verdc. for which *Vangueriella spinosa* is the type.

CANTHIUM TETRAPHYLLUM (SCHWEINF. EX. HIERN) BAILL. in Adansonia 12: 192. 1878.
> *Vangueria tetraphylla* Schweinf. ex. Hiern in Fl. Trop. Afr. 3: 152. 1877.
> *Meyna tetraphylla* (Schweinf. EX. Hiern) Robyns in Bull. Jard. Bot. État. 11: 233. 1928.

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REFERENCES

Anderson LE. 1954. Hoyer's solution as a rapid mounting medium for bryophytes. *Bryologist* **57:** 242–247.

Andreasen K, Bremer B. 2000. Combined phylogenetic analysis in the Rubiaceae-Ixoroideae: morphology, nuclear and chloroplast DNA data. *American Journal of Botany* **87:** 1731–1748.

Arènes J. 1960. A propos de quelques genres Malgaches de Rubiacées (Vanguériées et Gardenieés). *Notulae Systematicae, Herbier du Muséum de Paris Phanérogamie* **16:** 6–41.

Balkwill K, Sebola JR, Robinson ER. 1996. Sex expression in *Canthium mundianum* (Rubiaceae). In: van der Maesen LJG, van der Burgt XM, van Medenbach de Rooy JM, eds. *The biodiversity of African plants*. Dordrecht: Kluwer Academic Publishers, 650–655.

Bremer B, Bremer K, Heidari N, Erixon P, Olmstead RG, Anderberg AA, Källersjö M, Barkhordarian E. 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Molecular Phylogenetics and Evolution* **24:** 274–301.

Bridson DM. 1985. The reinstatement of *Psydrax* (Rubiaceae, subfam. Cinchonoideae tribe Vanguerieae) and a revision of the African species. *Kew Bulletin* **40:** 687–725.

Bridson DM. 1986. The reinstatement of the African genus *Keetia* (Rubiaceae subfam. Cinchonoideae, tribe Vanguerieae). *Kew Bulletin* **41:** 965–994.

Bridson DM. 1987a. The recognition and recircumscription of the African genus *Multidentia* (Rubiaceae-Vanguerieae). *Kew Bulletin* **42:** 641–654.

Bridson DM. 1987b. Studies in African Rubiaceae-Vanguerieae: a new circumscription of *Pyrostria* and a new subgenus, *Canthium* subgen. *bullockia*. *Kew Bulletin* **42:** 611–639.

Bridson DM. 1992. The genus *Canthium* (Rubiaceae-Vanguerieae) in tropical Africa. *Kew Bulletin* **47:** 353–401.

Bridson DM. 1996. The tropical African genus *Ancylanthos* (Rubiaceae-Vanguerieae) reconsidered. *Kew Bulletin* **51:** 343–352.

Bridson DM. 1998. Rubiaceae (tribe Vanguerieae). In: Pope GV, ed. *Flora Zambesiaca*. London: Royal Botanic Gardens, Kew, 211–377.

Buckler IVES, Ippolito A, Holtsford TP. 1997. The evolution of ribosomal DNA: divergent paralogues and phylogenetic implications. *Genetics* **145:** 821–832.

Capuron R. 1969. A propos des Rubiacées-Vanguériées de Madagascar. *Adansonia* **2** (9)**:** 47–55.

Carine MA, Scotland RW. 2002. Classification of the Strobilanthinae (Acanthaceae): trying to classify the unclassifiable? *Taxon* **51:** 259–279.

Coates Palgrave M. 2002. *Keith Coates Palgrave trees of Southern Africa*, 3rd edn. Cape Town: Struik Publishers.

Goloboff PA. 1991. Homoplasy and the choice among cladograms. *Cladistics* **7:** 215–232.

Hallé N. 1959. Sur les *Cuviera* (Rubiacées) d'Afrique intertropicale et description pour ce genre de deux espèces et de deux variétés nouvelles. *Bulletin de la Société Botanique de France* **7–8:** 342–348.

Havard A, Verdcourt B. 1987. A pollen survey of *Tapiphyllum* (Rubiaceae-Vanguerieae). *Kew Bulletin* **42:** 605–609.

Holmgren PK, Holmgren NH, Barnett LC. 1990. *Index herbariorum, Part 1: the herbaria of the world*, 8th edn. New York: New York Botanical Garden, Bronx.

Igersheim AF. 1989. Beiträge zur Klärung der Gattungsabgrenzungsprobleme innerhalb der Rubiaceae-Vanguerieae. D.Phil. thesis, University of Vienna.

Igersheim AF. 1993. Gynoecium development in Rubiaceae-Vanguerieae, with particular reference to the 'stylar-head'-complex and secondary pollen presentation. *Plant Systematics and Evolution* **187:** 175–190.

Kelchner SA. 2000. The evolution of non-coding chloroplast DNA and its application in plant systematics. *Annals of the Missouri Botanical Garden* **87:** 482–498.

Kluge A, Farris JS. 1969. Quantitative phyletics and the evolution of the anurans. *Systematic Zoology* **18:** 1–32.

Lamarck JBAPM de. 1785. *Encyclopédie méthodique 1(2)*. Paris.

Lantz H, Andreasen K, Bremer B. 2002. Nuclear rDNA ITS data used to construct the first phylogeny of Vanguerieae (Rubiaceae). *Plant Systematics and Evolution* **230:** 173–187.

Lee D-C, Bryant HN. 1999. A reconsideration of the coding of inapplicable characters: assumptions and problems. *Cladistics* **15:** 373–378.

Lens F, Jansen S, Huysmans S, Robbrecht E, Smets E. 2000b. Pollen morphological variation in Vanguerieae (Ixoroideae-Rubiaceae). *Grana* **39:** 90–102.

Lens F, Jansen S, Robbrecht E, Smets E. 2000a. Wood anatomy of the Vanguerieae (Ixoroideae-Rubiaceae), with special emphasis on some geofrutices. *IAWA Journal* **21:** 443–455.

Lidén M, Fukuhara T, Axberg T. 1995. Phylogeny of *Corydalis*, ITS and morphology. *Plant Systematics and Evolution* **9** (suppl.)**:** 183–188.

Maddison DR, Maddison WP. 2001. *MacClade 4: analysis of phylogeny and character evolution*. Sunderland, MA: Sinauer Associates.

Nepokroeff M, Bremer B, Sytsma KJ. 1999. Reorganization

of the genus *Psychotria* and tribe Psychotrieae (Rubiaceae) inferred from ITS and *rbc*L sequence data. *Systematic Botany* **24:** 5–27.

Patterson C. 1988. Homology in classical and molecular biology. *Molecular Biology and Evolution* **5:** 603–625.

Pennington RT. 1996. Molecular and morphological data provide phylogenetic resolution at different hierarchical levels in *Andira*. *Systematic Biology* **45:** 496–515.

Popp M, Oxelman B. 2001. Inferring the history of the polyploid *Silene aegaea* (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. *Molecular Phylogenetics and Evolution* **20:** 474–481.

Puff C, Robbrecht E, Buchner R, De Block P. 1996. A survey of secondary pollen presentation in the Rubiaceae. *Opera Botanica Belgica* **7:** 369–402.

Retief E. 2003. Rubiaceae. In: Germishuizen G, Meyer NL, eds. *Plants of southern Africa: an annotated checklist. Strelizia 14*, 825–841. Pretoria: National Botanical Institute.

Reynolds ST, Henderson RJF. 1999. Vanguerieae A. Rich. ex Dum. (Rubiaceae) in Australia, 1. *Everistia*. *Austrobaileya* **5:** 353–361.

Reynolds ST, Henderson RJF. 2001. Vanguerieae A. Rich. ex Dum. (Rubiaceae) in Australia, 2. *Cyclophyllum*. *Austrobaileya* **6:** 41–66.

Robbrecht E. 1988. Tropical woody Rubiaceae. *Opera Botanica Belgica* **1:** 1–271.

Robbrecht E. 1996. Generic distribution patterns in subsaharan African Rubiaceae (Angiospermae). *Journal of Biogeography* **23:** 311–328.

Robyns W. 1928. Tentamen Monographiae Vanguerieae Generumque Affinium. *Bulletin du Jardin Botanique de l'état Bruxelles* **11:** 1–359.

Ross JH. 1975. The typification of *Lycium inerme*. *Bothalia* **11:** 491–493.

Schatz GE. 2001. *Generic tree flora of Madagascar*. London: Royal Botanic Gardens, Kew/ St. Louis, MO: Missouri Botanical Garden.

Skottsberg C. 1945. The flower of *Canthium*. *Arkiv för Botanik* **32A:** 1–12.

Smith AC, Darwin SP. 1988. Rubiaceae. In: Smith AC, eds. *Flora Vitiensis Nova 4.* Lawai, Kauai, Hawaii: The Pacific Tropical Botanical Garden, 143–376.

Swofford DL. 2002. *PAUP*: phylogenetic analysis using parsimony (*and other methods), v.*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. *Plant Molecular Biology* **17:** 1105–1109.

Terrell EE. 2001. Taxonomic review of *Houstonia acerosa* and *H. palmeri*, with notes on *Hedyotis* and *Oldenlandia* (Rubiaceae). *SIDA Contributions to Botany* **19:** 913–922.

Tilney PM. 1986. The taxonomic significance of anatomical and morphological characters in the southern African species of *Canthium* Lam. (Rubiaceae). D.Phil. thesis, University of Pretoria.

Tilney PM, Kok PDF, van Wyk AE. 1990. The taxonomic significance of anatomical characters of the leaf in the southern African species of *Canthium* s.l. (Rubiaceae). *South African Journal of Botany* **56:** 363–382.

Tilney PM, van Wyk AE. 1997. Pollen morphology of *Canthium*, *Keetia* and *Psydrax* (Rubiaceae: Vanguerieae) in southern Africa. *Grana* **36:** 249–260.

Tilney PM, van Wyk AE, Kok PDF. 1988. The taxonomic significance of anatomical characters of the stem in the southern African species of *Canthium* s.l. (Rubiaceae). *South African Journal of Botany* **54:** 585–595.

Urbatsch LE, Baldwin BG, Donoghue MJ. 2000. Phylogeny of the coneflowers and relatives (Heliantheae: Asteraceae) based on nuclear rDNA internal transcribed spacer (ITS) sequences and chloroplast DNA restriction site data. *Systematic Botany* **25:** 539–565.

Verdcourt B. 1958. Remarks on the classification of the Rubiaceae. *Bulletin du Jardin Botanique de l'état Bruxelles* **28:** 209–281.

Verdcourt B. 1981. Notes on African Rubiaceae. *Kew Bulletin* **36:** 493–557.

Verdcourt B. 1983. Notes on Mascarene Rubiaceae. *Kew Bulletin* **37:** 563–570.

Verdcourt B. 1987. Notes on African Rubiaceae-Vanguerieae. *Kew Bulletin* **42:** 123–199.

Verdcourt B, Bridson DM. 1991. Rubiaceae (Part 3). In: Polhill RM, ed. *Flora of tropical East Africa*. Rotterdam/ Brookfield: A.A. Balkema, 749–956.

Weber M, Igersheim A. 1994. 'Pollen buds' in *Ophiorrhiza* (Rubiaceae) and their role in Pollenkitt release. *Botanica Acta* **107:** 257–262.

Weberling F. 1965. Typology of inflorescences. *Journal of the Linnean Society. Botany* **59:** 215–221.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T, eds. *PCR protocols: a guide to methods and applications*. San Diego: Academic Press, 315–322.

Wiens JJ. 1999. Character analysis in morphological phylogenetics: problems and solutions. *Systematic Biology* **50:** 689–699.

Wong KM. 1989. Rubiaceae. In: Ng FSP, ed. *Tree flora of Malaya 4*. Petaling Jaya: Longman Malaysia Sdn. Berhad, 324–425.

APPENDIX 1

Morphological characters and character states. All multistate characters are unordered. Characters 11 and 27 are used to define the ingroup. All characters are discussed in more detail in the text apart for numbers 11, 25 and 27, which are only mentioned here.

1. Habit (0) geofrutex; (1) shrub or tree; (2) climber
2. Supra-axillary spines (0) absent; (1) present
3. Reduced lateral branches (0) absent; (1) present
4. Sex distribution (0) hermaphrodite; (1) dioecious; (2) gynodioecious.

Leaves

5. Leaf arrangement (0) paired; (1) in whorls of three or more
6. Indumentum (0) absent; (1) present
7. Amount of indumentum (0) not obscuring the surface; (1) obscuring the surface
8. Domatia (0) absent; (1) present
9. Hairtuft inside stipules (0) absent; (1) present
10. Stipule apex widest above midpoint (0) no; (1) yes, at least sometimes.

Inflorescence

11. Inflorescence position (0) terminal; (1) axillary
12. Axillary inflorescences borne at leafless node (0) no; (1) yes
13. Bracts present on peduncle, enclosing the young inflorescence (0) absent; (1) present
14. Number of flowers per inflorescence (0) 1–2; (1) 3–10; (2) 11–20; (3) > 20

Calyx

15. Limb tube short, disk visible from side (0) no; (1) yes
16. Lobe length (0) < 1 mm, length/width ratio < 1; (1) > 1 mm, length/width ratio > 1

Corolla

17. Number of lobes (0) 4; (1) 5; (2) 6
18. Tube length (0) 8 mm or less; (1) more than 8 mm
19. Lobe apex tailed (0) no; (1) yes
20. Smooth hairs in the corolla tube (0) absent; (1) present
21. Type of smooth hairs (0) retrorse, straight, in a well defined ring; (1) diffusely spreading, rarely retrorse
22. Verrucose hairs in corolla tube (0) absent; (1) present

Androecium

23. Filament reflexed (0) no; (1) yes
24. Dark connective (0) absent; (1) present

Disk

25. Disk (0) glabrous; (1) indumentum present

Gynoecium

26. Style length (0) less than twice as long as tube; (1) at least twice as long as tube
27. Pollen presenter (0) absent; (1) present
28. Pollen presenter type (0) hollow, style recessed into presenter; (1) solid, style not recessed into presenter
29. Number of locules (0) 2; (1) 3–5; (2) > 5

Fruit

30. Size (0) ≤ 1 cm long; (1) > 1 cm long

APPENDIX 2

Morphological matrix. Polymorphic characters are abbreviated as follows: A = 0,1; B = 1,2; C = 2,3; D = 0,1,2, E = 1,2,3, ? = unknown state, – = inapplicable.

Species	Character number			
	1	10	20	30
Alberta magna	100000-0100-03011100-000000-00			
Ixora coccinea	100000-0100-03010100-0?1000-00			
Mussaenda erythrophylla	20000100100-0301110010?1000-01			
Ancylanthos rubiginosus	0000A1A?10110101111101O1001011			
Canthium burttii	10?00A?1?0110B10100???0??01?00			
Canthium ciliatum	11100101101100101010-101001001			
Canthium coromandelicum	111000-110110110000110010O1000			
Canthium gilfillanii	100?010110110B101000-100001000			
Canthium glaucum	B11000-110110100A000-101001001			
Canthium inerme	1A0000-110110110100110010O1001			
Canthium keniense	100000-110110B10100???0?001001			
Canthium lactescens	1010010A10110310A000-100001000			
Canthium mundianum	10020A0100110B1A1000-100001000			
Canthium oligocarpum	100000-110100E0A1010-001001001			
Canthium parasiebenlistii	10100A0110110B10A00??000?1000			
Canthium pseudosetiflorum	1011A10010100?011000-101001001			
Canthium pseudoverticillatum	B0100A0110110B10A000-101001000			
Canthium siebenlistii	10000A0110110B100000-100011000			
Cuviera angolensis	1?0000-011100201101100O1001011			
Cyclophyllum barbatum	100000-A101001101000-101001100			
Fadogia ancylantha	0000AA00101001001101100000101O			
Fadogia cienkowskii	0000111?10100101101111O1001011			

APPENDIX 2 *Continued*

Species	Character number			
	1	10	20	30
Fadogia elskensii	0000111?10100101		10111001001010	
Fadogia tetraquetra	00001A001010010110111101001010			
Fadogia verdcourtii	00001AA010100100111???0??01010			
Fadogiella stigmatoloba	0010A11?101101001001100100101010			
Hutchinsonia barbata	B?0001011010000111111001001010			
Keetia gueinzii	200001010010030010010101111000			
Keetia lukei	200000–111100200100???01111001			
Keetia venosa	2000010110100300D0010101111000			
Keetia zanzibarica	B000010110100300A0010101111000			
Lagynias dryadum	100001001011010110110?01011011			
Lagynias lasiantha	1?00010010110B0110110101011011			
Leroya cf. *richardiae*	100?010110111?01??????????1?21			
Meyna tetraphylla	B1100A0A101101101001110011011			
Multidentia concrescens	0000A0–010100201100101001001			
Multidentia fanshawei	B00000–11010030011010101001001			
Neoleroya verdcourtii	100100–010101?00001???01001?01			
Pachystigma pygmaeum	0000AA001011010110110000001011			
Peponidium horridum	1001010010101?01101???01?010B1			
Peponidium sp.	100?00–110101?011001110000010??			
Plectroniella armata	11100101101102000000–101011011			
Pseudopeponidium ampijoroense	100100-A00101?11?000–10100102?			
Pseudopeponidium asosa	100100–110101?11?000–101001120			
Psydrax kraussioides	200000-A0?100D1010110110001000			
Psydrax livida	1000010100110E0000011011011000			
Psydrax locuples	100000–100100E0000011011011000			
Psydrax nitidum	100000–100100100100011011111000			
Psydrax obovata	100000–001100300A0011011011000			
Psydrax parviflora	100000–100100300000011011111000			
Psydrax schimperiana	100000-A01100C0010011011011000			
Psydrax spA	100000–100100B0000011010011000			
Pygmaeothamnus cf. *chamaedendrum*	000?010010110B01?0??????0?1?01			
Pygmaeothamnus zeyheri	000011001011020110110101001001			
Pyrostria bibracteata	100000-A10101E100000–101001100			
Pyrostria hystrix	101100-A10101?10001???010?1?00			
Pyrostria phyllantoidea	101100–010101?11001???010?1?00			
Robynsia glabrata	1?000100101103011110001001011			
Rytigynia bagshawei	1000010110100010101 0–101001000			
Rytigynia bugoyensis	11100101101101111101???00001000			
Rytigynia fuscosetulosa	100001001010010110 0???00001011			
Rytigynia mrimaensis	11100101101?00?0?01???0??01?01			
Rytigynia senegalensis	101000–111100000100110010 11010			
Scyphochlamys revoluta	100100–000101?00A00???0?0?1?21			
Tapiphyllum cinerascens	A000A11?101101011011 0000001010			
Tapiphyllum velutinum	1010011?10110B0110110100001011			
Vangueria apiculata	100000-A10110311101101000001011			
Vangueria infausta	10001001011031110A10100001011			
Vangueria madagascariensis	100000–0101103111000–100001011			
Vangueria parvifolia	10A0011?10110111110A10100101011			
Vangueriella spinosa	111000–110110C111010–100011001			
Vangueriopsis cf. *longiflora*	100?010?10110?01?0?????????1?01			