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Nuclear rDNA ITS sequence data used to construct the first phylogeny of Vanguerieae (Rubiaceae)

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Abstract. The morphologically homogenous tribe Vanguerieae was investigated phylogenetically using sequence data from the Internal Transcribed Spacer (ITS) region in the nuclear ribosomal DNA. Sequences from 41 Vanguerieae species representing 19 genera were produced, and a parsimony analysis was performed. The phylogenetic analysis has several clades with strong support, among which three new informal groups are discussed, i.e. the *Vangueria* group, the *Fadogia-Rytigynia* group and the Spiny group. Also found monophyletic with strong support are *Multidentia*, *Keetia*, *Lagynias*, and *Pyrostria*. *Canthium* and *Rytigynia* are revealed as polyphyletic; *Vangueria*, *Tapiphyllum*, and *Fadogia* are paraphyletic. Results from this first phylogenetic analysis of the tribe clearly demonstrate a need for new circumscriptions of several genera. Morphological characters are discussed and putative synapomorphies are mentioned. Jackknife, bootstrap, and Bremer support are calculated and differences found in support are discussed.

Key words: Rubiaceae, Ixoroideae, Vanguerieae, ITS, phylogeny, support.

Although a widespread and common group in the paleotropics, Vanguerieae are a tribe that has received little attention. They are a member of the large and important family Rubiaceae, a family of mostly woody species distributed over large parts of the world, but centred in the tropics. Rubiaceae are perhaps best known for coffee (*Coffea* sp.), but also include many ornamentals and are an important constituent of many tropical habitats. The main characteristics of the family are opposite leaves, interpetiolar stipules, sympetalous corollas and inferior ovaries. While many phylogenies for the family have been produced in the last ten years, all from the subfamilial level down to genera, no phylogeny has so far been produced for Vanguerieae. This might in part be due to a strong morphological similarity between the taxa in the tribe, which has made the tribe a very difficult group to work with. The taxonomy has in recent years become refined, but no real consensus has been reached on how to delimit the genera.

Consisting of about 600 species (Robyns 1928, Cavaco 1969, Smith and Darwin 1988, Bridson 1998), Vanguerieae are among the larger tribes in the family Rubiaceae. The major part of the described taxa can be found in Africa (almost all parts south of the Sahara), but some can also be found in southern Asia, the Pacific, and Australia.

As Vanguerieae are delimited today they form a strongly supported monophyletic group (Bremer et al. 1995, Andreasen and Bremer 1996). Dumortier first mentioned the tribe in 1829 (as "Vaugnerieae"; Dumortier 1829). He included not only "Vaugneria" but also several other genera that now have been excluded from the tribe. Later authors also had opinions on Vanguerieae that deviate from the present classification (e.g. Hooker 1873, Hiern 1877), and it was not until 1958 that Verdcourt finally delimited the tribe as it is known today (Verdcourt 1958). Several subfamilial placements have been suggested, e.g. Cinchonoideae (including Ixoroideae; Verdcourt 1958) and Antirheoideae (Robbrecht 1988), but now most studies place it in Ixoroideae (e.g. Bremer et al. 1995, Andersson and Rova 1999).

The tribe is easily identified by a combination of characters: axillary inflorescences, valvate aestivation, single pendulous ovules and above all, the apex of the style swollen into a pollen presenter serving in secondary pollen presentation. This way of presenting pollen to the pollinator is a rather common phenomenon in the family (Puff et al. 1996), especially in the subfamily Ixoroideae of which Vanguerieae are a member, and was also used as a character to circumscribe the subfamily (Bremekamp 1954). However, in the case of Vanguerieae the pollen presenter is represented by a 'stylar head'-complex (Igersheim 1993), a character unique for the tribe (see Fig. 1). The complex consists of both the pollen presenting tissue and of the stigma lobes and is in most cases globose to cylindrical, often with the style recessed into the structure. Furthermore, several anatomical characters support the complex as a unique structure (Igersheim 1993). However, following the example set in Flora of Tropical East Africa (F.T.E.A.; Verdcourt and Bridson 1991) and Flora Zambesiaca (Bridson 1998), the more general term pollen presenter will be used here.

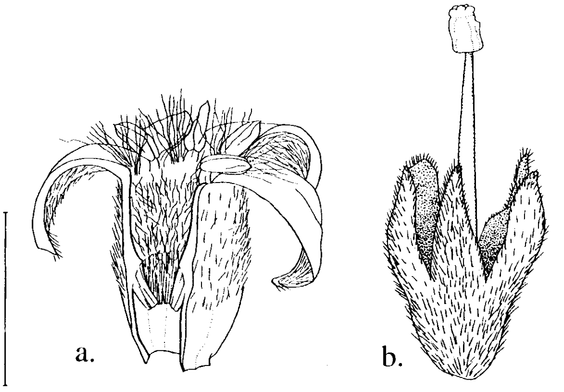

Fig. 1. *Tapiphyllum velutinum*. **a** Corolla with one lobe and corresponding part of wall removed. **b** Calyx and gynoecium with pollen presenter. From J. D. & E. G. Chapman 8939 (UPS). Bar = 0.5 cm. Drawn by H. Lantz

Of genera later to be included in Vanguerieae, the two first were *Canthium* (Lamarck 1783) and *Vangueria* (Jussieu 1789). *Canthium* was partly defined by having two locules and *Vangueria* by five. The number of locules was the main character used when later species were described; species with two locules were placed in *Canthium* (or *Plectronia*, an invalid synonym; Hiern 1898) and species with more than two locules were placed in *Vangueria*. New genera were later described, sometimes including species earlier described as *Canthium* or *Vangueria*, and today 28 generic names are in use. For a more complete discussion on the history of Vanguerieae, see Verdcourt (1987).

In the last 20 years a vast amount of work on Vanguerieae has been produced for the F.T.E.A. and Flora Zambesiaca projects (Verdcourt and Bridson 1991, Bridson 1998). These publications have greatly helped the understanding of the complicated groupings within the tribe. However, very few studies have attempted to study Vanguerieae as a whole. Only two general works have been produced. The first one is 'Tentamen Monographiae Vangueriae' by Robyns (1928). In this study Robyns describes many new species and genera but avoids many problems by not dealing with *Cuviera* and the large genus *Canthium*, which is perhaps the most problematic one. The other study is the thesis of

Igersheim (1989). He investigates morphological and anatomical characters to help with the generic delimitation of the tribe and also suggests some groupings of genera.

Although the work produced for the Floras has helped alleviate the shortcomings of earlier classifications, Vanguerieae remain a complicated group of species. Very little is known about phylogenetic relationships within the tribe and the monophyly of several genera is questionable. This study uses sequence data from the Internal Transcribed Spacer (ITS) region in the nuclear ribosomal DNA to gain insight into these questions and to identify groups where further studies are most needed. There are two reasons why ITS was chosen as the DNA region to be sequenced. (1) Vanguerieae are a group of closely related species, in many cases comparable to large genera, e.g. the Rubiaceae genus *Psychotria* (Nepokroeff et al. 1999) where ITS has been shown to be very informative. (2) A study where ITS was used on the Ixoroideae (Andreasen et al. 1999) also suggested that ITS would be useful at lower taxonomic levels in the subfamily.

The aim of this study is to produce a phylogeny of the tribe that can be used as a basis for later taxonomic work. By having a broad taxonomic and geographic sampling it is hoped that the results will be generally applicable and that this will facilitate the finding of morphological characters useful for delimiting genera.

Material and methods

The strategy when sampling, was to identify all type species of all published genera, subgenera, and sections, and to include these in the study when possible. This was done to simplify taxonomic conclusions resulting from the analyses, and since the species are described on a morphological basis and thus should represent a lot of the morphological variation in the tribe, it is believed that they also represent a broad sampling in a phylogenetic sense. Fifty-three such types were identified and 17 are included here, 13 of which are types of genera in current use (Robyns 1928, Bridson 1987b, Verdcourt and Bridson 1991, Bridson 1998, Reynolds and Henderson 1999). Effort was also put into getting species from a wide geographic range. In total 41 in-group taxa were sequenced for this study (Table 1) and 19 genera were represented. Authors of names are included in Table 1, or for species not sequenced, when first mentioned in the text. In many cases the availability of good material determined what species were sequenced, especially for the larger genera where more species were needed. As outgroups *Mussaenda*, *Ixora*, and *Alberta* were chosen. *Ixora* and *Alberta* have been shown to be closely related to Vanguerieae (e.g. Andreasen et al. 1999) but not belonging to the in-group. *Mussaenda*, which is basal to all these taxa (Andreasen and Bremer 2000), was also included in order to orient the trees. *Polysphaeria* (Octotropideae, Robbrecht 1994) was included since a molecular study by J. Rova (unpubl. thesis) placed an unspecified member of the genus at an internal position in Vanguerieae.

DNA was extracted using the CTAB method (Doyle and Doyle 1987) from silica-dried, fresh or herbarium material and cleaned either with QiaQuick PCR purification kit (QIAGEN) or caesium chloride/ethidium bromide gradient centrifugation. The cocktail for PCR amplification was mixed as follows (25 µl): 2.5 µl 10xbuffer, 2.5 µl 25 mM $MgCl_2$, 2.5 µl 10 mM dNTP, 1.25 µl 10 µM forward primer, 1.25 µl 10 µM reverse primer, 1.25 µl glycerol, 0.1 µl Taq DNA polymerase, 12.65 µl dH_2O, and 1 µl DNA. Several additives were also tried, alone or in combination (replacing some of the water): 2.5 µl 6.5 M betaine, 2.5 µl 0.1 M TMACl (Chevet et al. 1995) or 0.5 µl BSA. The amplification primers chosen were ITS4 (White et al. 1990) and Leu1 (Andreasen et al. 1999). Reactions were run on a Geneamp® PCR system 9600 or 9700 system (Applied Biosystems) with 1 min. at 97 °C followed by 40–50 cycles of 97 °C 10 s; 48 °C 30 s; 72 °C 20 s + 4 °C/cycle, finishing with 72 °C for 7 min.

Sequencing reactions were done using the ABI PRISM® BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems) and Thermo Sequenase Dye Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech) using primers ITS2, ITS3, ITS4, and ITS5 (White et al. 1990). All sequencing was performed on an ABI Prism® 377 DNA Sequencer (Applied Biosystems). For the Amersham Pharmacia kit each reaction contained 4 µl mix, 2 µl 1 µM sequencing primer, 1 µl DMSO,

Table 1. Voucher information and EMBL acession numbers. Voucher information is given for new sequences only. Herbarium abbreviations according to Holmgren et al. (1990)

Species	Voucher	EMBL number
Mussaenda erytrophylla Schumach. & Thonn.		AJ224823
Alberta magna E. Mey.		AJ224842
Polysphaeria parvifolia Hiern	Iversen et al. 87279 (UPS)	AJ315079
Polysphaeria aethiopica Verdc.	Puff & Kelbessa 821227-1/5 (UPS)	AJ315080
Ixora coccinea L.		AJ224826
Canthium coromandelicum (Burm. f.) Alston	Andreasen 36 (UPS)	AJ315081
Canthium inerme (L.f.) Kuntze	Bremer & Bremer 3682 (UPS)	AJ315120
Canthium mundianum Cham. & Schltdl.	Bremer & Bremer 3704 (UPS)	AJ315107
Canthium cf. *mundianum* Cham. & Schltdl.	Bremer & Bremer 3772 (UPS)	AJ315106
Cuviera angolensis Welv. ex K. Schum.	McPherson 16297 (MO)	AJ315088
Fadogia ancylantha Hiern	Chapman & Chapman 9109 (UPS)	AJ315103
Fadogia elskensii De Wild.	Taylor et al. 8318 (UPS)	AJ315118
Fadogia tetraquetra K. Krause	Bremer & Bremer 3799 (UPS)	AJ315099
Fadogia verdcourtii Tennant var. *verdcourtii*	Gereau et al. 6010 (UPS)	AJ315116
Fadogiella stigmatoloba (K. Schum) Robyns	Lawton 1318 (S)	AJ315100
Hutchinsonia barbata Robyns	Adam 20599 (UPS)	AJ315102
Keetia zanzibarica (Klotzsch) Bridson ssp. *zanzibarica*	Bremer 3069 (UPS)	AJ315105
Keetia gueinzii (Sond.) Bridson	Bremer 3083 (UPS)	AJ315117
Lagynias dryadum (S. Moore) Robyns	Bremer & Bremer 3811 (UPS)	AJ315090
Lagynias lasiantha (Sond.) Bullock	Bremer & Bremer 3792 (UPS)	AJ315089
Meyna tetraphylla (Hiern) Robyns ssp. *comorensis* (Robyns) Verdc.	Bremer 3074 (UPS)	AJ315083
Multidentia concrescens (Bullock) Bridson & Verdc.	Bidgood et al. 845 (K)	AJ315086
Multidentia fanshawei (Tennant) Bridson	Lovett et al. 3311 (K)	AJ315087
Pachystigma pygmaeum Robyns	Pawek 12335 (BR)	AJ315091
Plectroniella armata (K. Schum.) Robyns	Bremer & Bremer 3790 (UPS)	AJ315082
Psydrax nitidum (Craib.) Wong	Ryding 599 (UPS)	AJ315108
Psydrax obovata (Klotzsch ex Eckl. & Zeyh.) Bridson var. *obovata*	Bremer & Bremer 3762 (UPS)	AJ315109
Psydrax parviflora (Afz.) Bridson ssp. *parviflora*	Gilbert & Phillips 9069 (UPS)	AJ315110
Psydrax sp.	Cult. (Indonesia, Bogor)	AJ315111
Pygmaeothamnus zeyheri (Sond.) Robyns var. *Zeyheri* Verdc.	Bremer & Bremer 3800 (UPS)	AJ315119
Pyrostria bibracteata (Baker) Cavaco	Bremer 3036 (UPS)	AJ315113
Pyrostria hystrix (Bremek.) Bridson	Bremer & Bremer 3791 (UPS)	AJ315114
Pyrostria phyllantoidea (Baillon) Bridson	Taylor et al. 8486 (UPS)	AJ315115
Pyrostria sp.	Pettersson & Nilsson 752 (UPS)	AJ315112
Rytigynia bagshawei (S. Moore) Robyns var. *bagshawei*	Borhidi et al. 84439 (UPS)	AJ315101
Rytigynia bugoyensis (K. Krause) Verdc.	Rwburindore 3536 (UPS)	AJ315084
Rytigynia fuscosetulosa Verdc.	Frimodt Moller et al. NG117 (K)	AJ315097
Rytigynia senegalensis Blume	van den Berghen 8746 (BR)	AJ315104

Table 1 (continued)

Species	Voucher	EMBL number
Tapiphyllum cinerascens (Welv. ex Hiern) Robyns var. *cinerascens*	Milne-Redhead 3292 (BR)	AJ315096
Tapiphyllum velutinum (Hiern) Robyns	Emanuelsson 672 (S)	AJ315098
Vangueria apiculata K. Schum.	Kårehed & Odhult 161 (UPS)	AJ315095
Vangueria infausta Burch.	Blomberg et al. BMP 466 (UPS)	AJ315093
Vangueria madagascariensis Gmelin.	Bremer 3077 (UPS)	AJ224839
Vangueria parvifolia Sond.	Bremer & Bremer 3771 (UPS)	AJ315092
Vangueriella spinosa (Schumach & Thonn.) Verdc.	Merello et al. 1494 (K)	AJ315085
Vangueriopsis lanciflora (Hiern) Robyns	Cult. (Belgium, Meise)	AJ315094

and 3–4 μl DNA adding up to a final volume of 10–11 μl. The reactions for BigDye contained 2 μl mix, 0.4 μl sequencing primer, 0.6–2.6 μl DNA and if necessary up to 2 μl dH_2O to a final volume of 5 μl. Sequences were analysed with the Sequencher® (Gene Codes Corporation) software package and after a preliminary alignment in ClustalW (Thompson et al. 1994) aligned by eye in a text editor. Sequences are available from EMBL (see Table 1). The alignment is available on request to the first author.

The phylogenetic analysis was performed with PAUP* 4.0b5 (Swofford 1998). All phylogenetically informative insertion/deletion events (indels) were given the same weight independent of size. A heuristic parsimony search of 100 random addition replicates with TBR and MULTREES on was conducted. Branch support was calculated with the jackknife and bootstrap procedures implemented in PAUP*. The jackknife analysis used a deletion frequency of 37%, 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. Bremer support was calculated with Autodecay 4.0 (Eriksson 1998) using a heuristic search with 100 random addition sequence replicates.

Results

The sequences varied in length from 611 bases (*Psydrax obovata*) to 671 bases (*Fadogiella stigmatoloba*). The aligned matrix had 786 positions including 14 indel-characters (9 in ITS1, 5 in ITS2). Due to uncertain alignment 122 characters were excluded, 664 characters were included in the phylogenetic analysis. Of these, 428 were constant, 62 variable were non-informative and 174 were informative. *Fadogia verdcourtii*, *F. elskensii*, and *Pygmaeothamnus zeyheri* could not be sequenced for a region (up to 68 bases) in the 5.8S region, missing bases were replaced with N (N = any base).

In the phylogenetic analysis, 18 equally parsimonious trees with a length of 630 were found ($CI = 0.47$ (uninformative characters removed) $RI = 0.72$). The jackknife and bootstrap values differed more than expected, sometimes with as much as twelve percent units (Fig. 2). Few nodes are collapsed in the strict consensus tree (Fig. 2) and several receive strong support. *Canthium* and *Rytigynia* are polyphyletic with members appearing in distantly related clades. *Vangueria*, *Tapiphyllum*, and *Fadogia* are paraphyletic and the monophyly of *Psydrax* is very weakly supported. With the sampling here presented, *Multidentia*, *Lagynias*, *Keetia*, and *Pyrostria* turn out monophyletic with good support. *Polysphaeria* has a basal position to the Vanguerieae.

Discussion

From the resulting trees it is evident that ITS indeed is a useful DNA region for phylogenetic studies in Vanguerieae. Several groups with

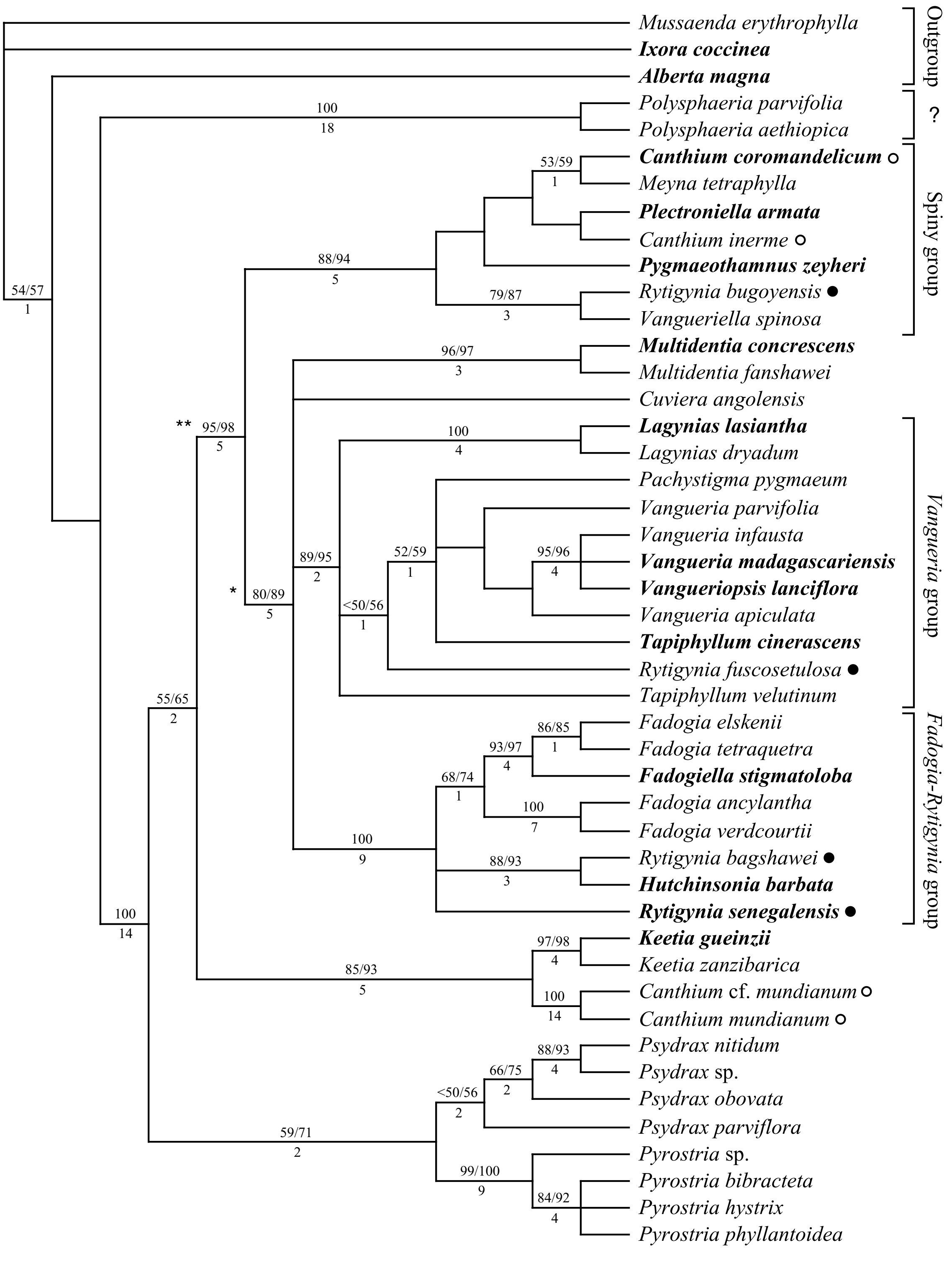

Fig. 2. Strict consensus tree of the 18 most parsimonious trees. Bootstrap/jackknife values are above the branches, Bremer support below. When bootstrap and jackknife values are equal only one value is noted. Where the bootstrap and jackknife values both are < 50 and the Bremer support is one, no support is shown. The large suprageneric groups discussed in the text are marked with brackets, the question-mark (?) denotes the

good support exist, although few of them agree with genera as presently conceived. Some main groups can be seen and the discussion following will be centred around these groups. Since very few supra-generic groups have been discussed in the tribe (the exceptions being Robyns 1928, Igersheim 1989) and the ones that have been mentioned rarely comply with the groups found here, informal names will be used (see Fig. 2). The basic taxonomic framework and terminology generally followed is the one used in the Vanguerieae volumes of F.T.E.A. (Verdcourt and Bridson 1991) and Flora Zambesiaca (Bridson 1998). The exception is the use of geofrutex instead of suffrutex, following the recommendation of Robbrecht (1988). Support values are listed in the text as (bootstrap/jackknife/Bremer support).

Outgroup. The support in the outgroup is poor, with the exception of the two *Polysphaeria* species, which group together with very strong support (100/100/18). They were included to test if *Polysphaeria* is nested within Vanguerieae or not (for phylogenetic relationships of other Octotropideae genera, see Andreasen and Bremer 2000). Although the position of this group is weakly supported ($<50/<50/1$), there is nothing that points to a nested position within Vanguerieae. The internal position of *Polysphaeria* found in an earlier study was probably based on a Vanguerieae specimen misidentified as a *Polysphaeria*.

***Canthium* and *Rytigynia*.** Before discussing the strongly supported groups found, the polyphyletic nature of *Canthium* and *Rytigynia* needs to be addressed. As mentioned earlier, *Canthium* and *Vangueria* were the first genera described in the tribe, in part based on the number of locules. The character was often given such importance that other information was ignored in favour of it, and this resulted in two large heterogeneous groups. In the case of *Canthium*, several closely knit groups of species have now been removed from the genus to form genera of their own (Bridson 1985; 1986; 1987a, b), leaving a very heterogeneous genus. Bridson was fully aware of this, but avoided raising the probably more homogeneous subgenera of *Canthium* to generic rank "…for pragmatic reasons, the need to avoid unnecessary name changes in a tribe where the generic concepts are still unstable being paramount." (Bridson 1992). The consensus tree clearly demonstrates that *Canthium* is a polyphyletic assemblage of species, in part due to the historical emphasis on a single character. This use of a 'cardinal character' has also been criticised in the family-level classification of Rubiaceae where too much importance was ascribed to the number of ovules per locule (e.g. Bremer et al. 1995).

For a long time *Rytigynia* included only one species, *R. senegalensis*, until Robyns (1928) added 67 species. Twenty-two of these were new, the rest were new combinations. Today *Rytigynia* is thought to have a central core that is easily recognised, but also includes some satellite groups (Verdcourt 1987) intermediate between *Rytigynia* s. str. and other genera. In this study *R. fuscosetulosa* and *R. bugoyensis* would be considered belonging to such groups.

Spiny group. In the strongly supported (88/94/5) spiny group, representatives from a wide geographic range are found. *Canthium coromandelicum*, the type species of *Canthium*, is restricted to southern India and Sri Lanka while the other species in the group together cover most of Vanguerieae's African range. Even though they are distributed over a wide area many morphological similarities exist. All species except *Pygmaeothamnus zeyheri* have spines (modified branches), a character rare in other parts of Vanguerieae. In the case of

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uncertain tribal placement of *Polysphaeria*. Type species of genera are marked in **bold.** The polyphyletic genera *Canthium* and *Rytigynia* are marked with the symbols ○ (*Canthium*) and ● (*Rytigynia*). The symbols * and ** indicate strongly supported, unnamed groups discussed in the text

C. coromandelicum, *Meyna tetraphylla*, *Plectroniella armata*, *R. bugoyensis*, and *Vangueriella spinosa*, the spines are associated with brachyblasts (lateral shoots with extremely short nodes). *Canthium inerme* has no such shoots and *Pygmaeothamnus zeyheri* differs in several aspects from the other species. It lacks spines and brachyblasts, has a woody rhizome and whorled leaves, in contrast to the other species that are spiny shrubs or trees with opposite leaves.

Canthium coromandelicum shows a strong resemblance to *Meyna tetraphylla*. The only major difference lies in the number of locules (2 for *Canthium*, 4–5 for *Meyna*) and it has been suggested that *Meyna* perhaps ought to be included in *Canthium* s. str. (Bridson 1992). The two species group together in the strict consensus tree but the support is much too low (53/59/1) for a transfer to be based on the result. *Plectroniella armata* also shares a very similar habit with these two species, i.e., it has large spines and brachyblasts, but the genus has been considered closer to other spiny South African *Canthium* species than to *Canthium* s. str. (Bridson 1992). No such species are included here, but it is quite likely that they too belong to the spiny group, considering that the presence of spines is very rare outside the group. Before a more resolved tree can be constructed and *Canthium* s.l. investigated further, *Plectroniella* is best left as it is.

Rytigynia bugoyensis and *V. spinosa* form the only strongly supported (79/87/3) group within the clade. *Rytigynia bugoyensis* is a mainly East to Central African species while *V. spinosa* is restricted to West Africa. *Vangueriella spinosa* is the type species of *Vangueriella* subgenus *Stenosepalae* and *R. bugoyensis* is doubtfully included in *Rytigynia* (Bridson 1998). They share a number of morphological features, and *R. bugoyensis* has been considered for transfer to *Vangueriella* (Bridson 1992). However, when the similarities are investigated it becomes clear that finding any good synapomorphies that could support the transfer of *R. bugoyensis* to *Vangueriella* is very difficult. *Vangueriella* is partly defined by having large fruits and a mitriform pollen presenter; *R. bugoyensis* lacks these features. There are a number of similarities, including lack of a deflexed ring of hairs in the corolla, the size of the corolla-tube, and a glabrous disk, but these character states can be found in several other species in the spiny group, although not in that combination. To conclude, the transfer of *R. bugoyensis* to *Vangueriella* subgenus *Stenosepalae* is supported by the molecular data, but the morphological support is inconclusive.

***Vangueria* group.** As in the case of the spiny group, the *Vangueria* group is well supported (95/96/2), but good internal support is largely lacking. With the exceptions of *Rytigynia* and *Vangueriopsis*, the group confirms with Igersheim's 'Gattungskomplex *Vangueria*' (Igersheim 1989). This group was based on mostly anatomical and embryological characters, but later some doubt was put to the reliability of the characters (Bridson 1996). Bridson considered *Vangueria*, *Pachystigma*, *Lagynias*, and *Tapiphyllum* to form a close alliance together with *Ancylanthos*, *Fadogia*, and *Fadogiella*, but with problematic generic delimitations.

Vangueria, the type genus of the tribe, is paraphyletic. Grouped with the type species *V. madagascariensis* are *V. infausta* and *Vangueriopsis lanciflora*. *Vangueria infausta* is a variable species with a large distribution in eastern and southern Africa thought to be very close to *V. madagascariensis* (Bridson 1998). *Vangueriopsis lanciflora* is the type species of *Vangueriopsis*, a genus that in earlier circumscriptions was much larger but two subgenera were removed to *Vangueriella* when that genus was erected (Verdcourt 1987). *Vangueriopsis* is today a small genus of only four species. There are several similarities between *V. infausta* and *V. lanciflora* (Coates-Palgrave 1991) but the latter exhibits some rare features including hairy anthers and very long corolla lobes. It is also worth mentioning that *Vangueriopsis* has two ovary locules instead of five, otherwise the much more common state in the *Vangueria* group. *Vangueria apiculata* has been suspected to hybridise with *V. madagascariensis* (Verdcourt 1981), and in the strict consensus tree, this species is sister to the earlier mentioned species but with very low support ($<50/<50/1$). Also with low support ($<50/<50/1$), *Vangueria parvifolia* is the sister taxon to all four already mentioned species.

Basal to the *Vangueria*-*Vangueriopsis* clade five other genera are found in the *Vangueria* group. Apart from the two *Lagynias* species that group together with very strong support, the support is negligible. *Tapiphyllum* is represented by two species, *T. cinerascens* (type species) and *T. velutinum*. *Tapiphyllum* has been thought to be close to *Vangueria* (Bridson 1996) and *Vangueria parvifolia* was recently transferred from *Tapiphyllum* (Bridson 1998). *Tapiphyllum* is mainly recognised by having large calyx lobes and velvety indumentum and is considered to be rather vaguely defined (Verdcourt 1981, Havard and Verdcourt 1987). The genus also has ties to other genera in the group. Verdcourt for example considered *Tapiphyllum* to be "...no more than a velvety *Pachystigma*..." (Verdcourt 1981). *Pachystigma* is in this analysis represented by *P. pygmaeum*, a geofrutex with a wide distribution ranging from South Africa to Tanzania. This genus is also thought to be heterogeneous (Bridson 1998) with species showing affinity to *Tapiphyllum*, *Fadogia*, *Lagynias*, and *Vangueria* and it certainly is difficult to find any good morphological support for the genus. *Pachystigma* seems to be a genus based more on a lack of characters (e.g. the velvety indumentum of *Tapiphyllum*, the larger fruits and different inflorescences of *Vangueria*) than on actual defining characters.

Rytigynia fuscosetulosa, together with *R. saliensis* Verdc., are the only member of *Rytigynia* subgenus *Sali*. This subgenus forms an aberrant group within the genus, differing from the core group in having long calyx lobes among other characters. In the description of the species (Verdcourt 1987, Verdcourt and Bridson 1991) it is mentioned that they show affinity to not only *Rytigynia* but also *Vangueria* and *Vangueriella*. The position of *R. fuscosetulosa* in this analysis supports a close relationship with *Vangueria* but not with *Vangueriella* subgenus *Stenosepalae*. *Vangueriella* is a diverse genus and it is possible that *R. fuscosetulosa* might be closer related to other *Vangueriella* species not included in this study.

Lagynias is a genus of five well defined species divided into two subgenera, subgenus *Lagynias* and subgenus *Bembea* (Verdcourt 1987). The genus is by its large calyx lobes, lax inflorescences and discolorous leaves easily recognisable. Here two species are included, *L. lasiantha* (type species) and *L. dryadum*, both from subgenus *Lagynias*. The two species group together with very strong support (100/100/4) in an unresolved basal position in the *Vangueria*-group. Morphologically the genus is rather isolated apart from some shrubby *Pachystigma* species, e.g. *P. macrocalyx* and *P. triflorum*, that are very close to *Lagynias* and have been considered for transfer to *Lagynias* (Bridson 1996). Otherwise the above-mentioned combination of characters is rare in the tribe.

Cuviera **and** ***Multidentia*** belong in a strongly supported, mostly pluri-locular clade together with the *Vangueria*-group and the *Fadogia*-*Rytigynia* group. Their position within that clade is however unresolved. *Cuviera* has a mainly Guineo-Congolian distribution (Robbrecht 1996), with only a few poorly known species occurring in East Africa. The most obvious feature of the genus is the large, often many-flowered cymes with enlarged linear-foliose bracts and bracteoles. The calyx lobes are often similarly enlarged. It is also common that the base of the pollen presenter is markedly widened, and several species have hairy styles (Hallé 1959). All these characters combined make for one of the most derived genera of the tribe. *Cuviera* was together with *Canthium* the only genera that Robyns (1928) did not look into and many authors have been involved in describing the approximately 20 species now accepted. However, due to the rare characters mentioned above, the genus seems better delimited than most other genera in

Vanguerieae. Only one species is included here, *Cuviera angolensis*, a central African member of subgenus *Cuviera* and although it seems clear that it belongs neither in the *Vangueria*-group nor in the *Fadogia-Rytigynia* group its precise relationships are unresolved. It is clear, however, that it belongs to the pluri-locular clade. This is in agreement with the fact that with few exceptions *Cuviera* has more than two locules. It has been suggested that the genus has affinity with *Vangueriella* (Verdcourt 1987). This is not supported, but since both genera are represented by only one species each and, as this study shows, no monophyly can be taken for granted, it is much too early to dismiss the possibility.

Multidentia is a morphologically distinct genus occurring in Tropical Africa. It was described by Gilli (1973), but received its current circumscription when Bridson (1987a) added more species to a final of eleven taxa. Common to most of the included species is their cupular calyx limb (the connate part of the calyx), a deeply cleft and strongly ribbed pollen presenter with a usually tapering base, and thickly woody pyrenes. Included in this study are the type species *M. concrescens* and *M. fanshawei*, the type species of the now rejected *Canthium* section *Granditubum*. These species group together with very strong support (96/97/3) and the genus seems monophyletic. All *Multidentia* species have two locules and earlier investigations (Bridson 1987a, Igersheim 1989) have suggested an affinity with other two-locular genera. Bridson (1987a) suggested an affinity with *Vangueriella* sect. *Vangueriella* (not included in this study) and Igersheim (1989) included *Multidentia* in his 'Gattungskomplex *Canthium*' together with *Canthium*, *Eriosemopsis*, *Meyna* (this genus is however pluri-locular) and *Pygmaeothamnus*. Here the genus groups together with the largely pluri-locular species but in an unresolved position.

The *Fadogia-Rytigynia* group contains two of the larger Vanguerieae-genera, *Fadogia* and *Rytigynia*. Both genera include around 50 species (Bridson 1998). The other two genera in the group, *Hutchinsonia* and *Fadogiella*, are on the other hand very small with three species each (Robyns 1928, Bremekamp 1952, Bridson 1998). The same grouping of genera as found in the *Fadogia–Rytigynia* group was suggested by Igersheim (Gattungskomplex *Rytigynia*; 1989) based on anatomical and embryological characters. *Fadogia* is represented by four species and is paraphyletic (68/74/1) with the type species of *Fadogiella* nested within. With few exceptions, *Fadogia* species have a geofrutescent habit, whorled leaves, reduced calyx lobes and three to five locules. *Fadogiella stigmatoloba* shares all these characters but is further characterised by its velvety indumentum, a defining character for the genus. The *Fadogia* species form two clades. *Fadogia elskensii* is considered very close to the type species of *Fadogia*, *F. cienkowskii* Schweinf., and groups with *F. tetraquetra* (86/85/1). Both were included in the same subsection (section *Subulatae* subsection *Imparisepalae*) by Robyns (1928) and are considered very close (Verdcourt and Bridson 1991). The other group consists of *F. ancylantha* and *F. verdcourtii* (100/100/7), both of which have been suggested to have an affinity with *Temnocalyx* Robyns (Tennant 1968, Verdcourt and Bridson 1991). *Temnocalyx* is very close to *Fadogia* but is characterised by having large flowers with curved corolla-tubes. The character was however considered too variable and the genus is now monotypic after the transfer of several species to *Fadogia* (Verdcourt 1981). Awaiting an analysis where the type species of *Temnocalyx* and more *Fadogia* species are included no recommendation is presented about how to deal with the paraphyletic *Fadogia*. Accommodating the *Fadogiella* species in *Fadogia* would, however, necessitate only slight changes in the description of *Fadogia*.

As noted above, *Rytigynia* is polyphyletic. Included in the *Fadogia-Rytigynia* group are two *Rytigynia* species, *R. senegalensis* and *R. bagshawei*. The type species of *Rytigynia*, *R. senegalensis*, has an unresolved position in the group and *R. bagshawei* is sister to *Hutchinsonia barbata* (88/93/3), the type species of a West African genus with very long and narrow corolla-tubes. Both *Rytigynia* species are members of subgenus *Rytigynia*. Interestingly, *R. bagshawei* is a two-locular species while both *R. senegalensis* and *H. barbata* are plurilocular, which further demonstrates the risks of ascribing too much weight to number of locules.

Keetia is one of the distinct groups removed from the earlier very large *Canthium* in connection with the F.T.E.A. (Verdcourt and Bridson 1991) and Flora Zambesiaca (Bridson 1998) projects. It shares several characters with *Psydrax*, e.g. style much longer than corolla-tube, pollen presenter cylindrical and usually longer than wide, and cotyledons oriented parallel to ventral face of seed, but there are also important differences (Bridson 1986). Of extra importance is considered the presence of a lid-like area surrounding a central crest on the pyrenes, which is lacking in *Psydrax*. *Keetia gueinzii* is the type species of the genus, an extremely widespread species that can be found in almost all of Vanguerieae's continental African range. *Keetia zanzibarica* occurs on large parts of the African east coast. The strong support from the molecular data (97/98/4) in combination with the morphological characters gives added weight to the conclusion that *Keetia* is one of the best-delimited Vanguerieae genera.

***Canthium* subgenus *Afrocanthium*.** Considering the many morphological similarities between *Keetia* and *Psydrax*, one would expect to find *Psydrax* as the sister group of *Keetia* and not two *Canthium* specimens as seen in this study. The two specimens might be representatives of the same species, *C. mundianum*, a member of *Canthium* subgenus *Afrocanthium* (Bridson 1992). The vegetative specimen annotated as *Canthium* cf. *mundianum* was collected in South Africa. Based on domatia and other macromorphological characters it seems closely related to *C. mundianum*, but differs in having an abnormal leaf shape (broader, size up to 8.5 × 6.0 cm) and an almost complete lack of tanniniferous cells (see Tilney et al. 1988, 1990 for a discussion on tanniniferous cells in southern African Vanguerieae). Whether the specimen is a new species, subspecies, or if the variation is just dependent on growth conditions is not known, but it was decided that it remains in the study awaiting a better determination. The subgenus *Afrocanthium* is based on a combination of characters of which the deciduous habit, reduced calyx lobes, and absence of a ring of reflexed hairs in the corolla-tube is perhaps of most importance. This analysis clearly separates *C. mundianum* from subgenus *Canthium* and subgenus *Lycioserissa*, but without including the type species of the subgenus (*C. lactescens* Hiern) it is too early to decide what to do with the subgenus name *Afrocanthium*. *Canthium mundianum* needs to be transferred to another genus, but which cannot be decided from the present data. Including the species in *Keetia* would be very problematic since it lacks many of the morphological synapomorphies of that genus.

Psydrax has the widest geographical distribution of all Vanguerieae genera. It is also the largest by far with approximately 100 species (Bridson 1998). Thirty-four species occur in Africa (incl. Madagascar), the rest are found in southern Asia, the Pacific and Australia. However, several of these have still not been formally transferred from *Canthium* (Bridson 1998). Of the species here included, *Psydrax* sp. and *P. nitidum* were collected in southeast Asia, *P. obovata* and *P. parviflora* are from Africa. The specimen *Psydrax* sp. has been impossible to determine to species but as a representative of Asian Vanguerieae it was still included in the study, and groups together with the other Asian species, *P. nitidum* with strong support (88/93/4). *Psydrax* forms a monophyletic group in the strict consensus tree, but with very low support (< 50/56/2). The low support is somewhat surprising since the genus is very well delimited by morphological characters. Several characters are shared with *Keetia* (as noted above) but *Psydrax* differs in usually having reflexed anthers and a short calyx-limb. Two subgenera have been described (see Bridson 1985 for a discussion), subgenus *Psydrax* is the largest and occurs throughout the distribution range. Subgenus *Phallaria* consists of lianas or scandent shrubs and is restricted to continental Tropical Africa. All species represented in this study belong to subgenus *Psydrax*.

Pyrostria. As of present, *Pyrostria* contains about 45 species, but this figure is very likely to increase. *Pyrostria* is centred in Madagascar and forms the central core of a group of genera, many of which quite easily could be accommodated in *Pyrostria* (Bridson 1987b). *Pyrostria* s. str. is characterised by large bracts that enclose the young inflorescences, pluri-locular ovaries, and unisexual flowers. The generic concept has, however, been widened and the genus now includes both hermaphrodite and two-locular species. Some ebracteate groups could also be transferred to *Pyrostria* at a later stage (Bridson 1987b). Here, three species from the African mainland are included, *P. hystrix*, *P. phyllantoidea*, and *P. bibracteata*. All of these have bracts but *P. hystrix* is a hermaphrodite. *Pyrostria* sp. is a probably undescribed very distinct species collected in Madagascar. It has hairy calyx-lobes, glabrous small leaves, and domatia present as ciliate pits. The presence of bracts, unisexual flowers, and obscure secondary and tertiary nerves definitely suggest a position in *Pyrostria*. The genus receives very strong support in this analysis (99/100/9) with the non-Madagascan taxa also forming a strongly supported group (84/92/4). In its current circumscription it seems clear that *Pyrostria* is a monophyletic group with the presence of bracts as a good diagnostic character.

Other strongly supported clades. Apart from the groups discussed above, there are some additional strongly supported groups. The pluri-locular clade, which includes *Multidentia*, *Cuviera*, the *Vangueria* group, and the *Fadogia-Rytigynia* group, is one such clade (80/89/5; * in Fig. 2). Many species in this group usually have more than two locules, but *Vangueriopsis lanciflora* and *R. bagshawei* do not. There are more two-locular species not sampled which most likely belong to this group. The node uniting the spiny group and the pluri-locular group is also strongly supported (95/98/5; ** in Fig. 2), but morphological support remains to be found.

Support. There are several methods used for calculating support in phylogenetic trees. Bootstrap (Felsenstein 1985) and jackknife (Farris et al. 1996) are probably the two most widely used. These two support measures were calculated, and settings were chosen that would yield as accurate and similar results as possible (Hedges 1992, Farris et al. 1996, DeBry and Olmstead 2000). The results from the analyses are presented in Fig. 2. For several nodes, the differences are substantial, ranging up to twelve percent units. Jackknife values are usually the highest, and the largest differences are seen at nodes with the lowest support values (see Mort et al. 2000 for a similar result). Although the standard deviation is larger for lower bootstrap values, and partly could explain why the lower values differ more, the high number of replicates makes the standard deviation expected very small (Hedges 1992). A completely different kind of support, Bremer support (Bremer 1988), was also added. As a non-statistical alternative it provides a basis for interesting comparisons. No strong correlation is seen between the Bremer support and the other support measures, at least for lower support values (also seen in Norén and Jondelius 1999). It is important to note that bootstrap and jackknife are different measures of support sensitive to different parameters, and it is only under very specific circumstances rarely encountered in real datasets that the values are expected to be identical (Oxelman et al. 1999). The phenomenon does, however, need to be investigated further.

Conclusions. Vanguerieae is a tribe in great need of revision. The focus of the revisional work done so far has usually been on the East African species, and the tribe needs a revision on a more global level. This analysis reveals some strongly supported groups, but without including more species it is difficult to draw any conclusions about the classification. The situation is in many cases, e.g. the *Vangueria* group, so confused that a species by species approach is needed to get a clear picture of how to delimit the genera. Even so, the results from this analysis do improve our understanding of the tribe. Several of the traditional genera are not monophyletic, i.e. *Canthium*, *Rytigynia*, *Vangueria*, *Tapiphyllum*, and *Fadogia*. There are also some monophyletic groups that receive strong support, including some of the genera currently in use. *Multidentia*, *Lagynias*, *Keetia*, and *Pyrostria* all seem monophyletic based on the included species and well delimited also in a morphological sense. Interesting are the larger supra-general groups found that are better supported than many genera. The spiny group is especially interesting since it has the added support of a morphological synapomorphy, the presence of spines. It is much more difficult to find morphological support for the *Vangueria* and the *Fadogia-Rytigynia* groups and they require a more complete sampling and careful morphological investigation before any such conclusions can be drawn. Especially the *Vangueria* group, but also the spiny group, suffer from poor internal support from the ITS data here used. The inclusion of more characters, both molecular and morphological, will hopefully rectify the situation and such a work is in progress. The problem of generic delimitation in Vanguerieae is problematic, but not unsolvable.

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