

The systematics of Knoxieae (Rubiaceae)—molecular data and their taxonomic consequences

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The tribe Knoxieae consists of genera formerly included in (at least) four tribes of the Rubiaceae (the coffee family). Apart from *Knoxia* itself, which is mainly Indomalesian, the group is from Africa and Madagascar. Most genera are herbaceous or shrubby, but small trees are also present. They generally have fimbriate, colleter-tipped stipules and five-merous flowers with unequal calyx lobes. We present a molecular phylogeny of the group (based on two chloroplast markers, *rps16* and *trnT-F*, and the nuclear ITS) and discuss the morphological support for the resulting groups and the taxonomic consequences of the phylogeny. Two hundred and sixty-one new sequences from 90 species have been obtained. Of the 19 genera of the Knoxieae, all but three (two monotypic and one with two species) are represented. The genera *Calanda*, *Chlorochorion*, *Neopentania*, and *Paraknoxia* are merged with *Pentania*. *Pentas* is shown to be not monophyletic and is split into the new genera *Phyllopentas*, *Dolichopentas*, and *Rhodopentas*. We also suggest the *Pentas* subgenera *Megapentas* and *Chamaepentadoides* be included in *Chamaepentas*. The genus *Carphalea* is also shown to be not monophyletic and for the African members of the genus (section *Dirichletia*) the name *Dirichletia* should be used. *Placopoda* is suggested to be merged with *Dirichletia*. *Triainolepis* is enlarged to include *Paratriaina* and *Thyridocalyx*. The tribal position of *Lathraeocarpa* is discussed. Twenty-nine new combinations for species and seven for infraspecific taxa are made. A key to the genera of Knoxieae is provided.

KEYWORDS: ITS, key, Knoxieae, phylogeny, *rps16*, Rubiaceae, taxonomy, *trnT-F*

INTRODUCTION

The coffee family, Rubiaceae, with its more than 10,000 species is generally easily recognised due to characters such as the opposite leaves with interpetiolar stipules and tetracyclic, sympetalous flowers with inferior ovaries. Although the family as such is well delimited, dividing it into subfamilies and tribes has been more controversial. The genera here considered have previously been placed in two subfamilies and four to five tribes (Table 1). Molecular analyses (see Table 1) have made clear that the tribes Knoxieae and Triainolepideae as well as several genera from Hedyotideae, the *Pentas* group, form a monophyletic group within the subfamily Rubioideae. The *Pentas* group was first recognised on morphological grounds (Bremer, 1987), but several of the included genera had already been regarded as being closely related (e.g., Verdcourt, 1950). We here treat all these taxa as members of an enlarged Knoxieae as argued for by Andersson & Rova (1999; Triainolepideae were not included in their study) and Dessein (2003; Knoxieae emended). Their views were followed in the classification of Rubiaceae by Robbrecht & Manen (2006) and are in contrast to the classification of Rubioideae by Bremer & Manen (2000) who included the Knoxieae in a widely interpreted Spermaceae.

The members of the Knoxieae are herbs, shrubs, or small trees generally characterised by fimbriate, colleter-tipped stipules, terminal inflorescences, and five-merous, heterostylous flowers with unequal calyx lobes, sometimes enlarged and foliaceous (Fig. 1). The most well known species is the widely cultivated star cluster, *Pentas lanceolata* (Fig. 1I). Apart from *Knoxia* itself the Knoxieae are from Africa and Madagascar. *Knoxia* (Fig. 1F) is centred in Indomalesia, but is also represented in continental Africa by two species. In Table 1 all the genera under study are listed and the reasons for their inclusion are given. Their placements according to earlier classifications, as well as their number of species, are also given in Table 1.

Originally, Knoxieae were erected for the two genera *Knoxia* and *Pentania* (Fig. 1K), both characterised by a solitary pendulous ovule in each locule (Hooker, 1873). Hedyotideae (Chamisso & Schlechtendal, 1829), on the other hand, have always been considered as a tribe with numerous ovules in each locule. That the former Knoxieae and several genera of Hedyotideae are related has been suggested on several occasions. In fact, Hutchinson & Dalziel (1931) included Knoxieae in Hedyotideae and Verdcourt (1953d) actually regarded his new genus *Neopentania* as a member of Hedyotideae, albeit related to *Pentania*. Later, he (Verdcourt, 1958) acknowledged

Table 1. Genera included in the present study, the number of species in each genus, and the number of sequenced species for each genus. For some species more than one individual are sequenced (see text). The total number of sequenced taxa for a genus is given after a slash. The previous classification of the genera into subfamily and tribe according to Robbrecht (1988; also according to Verdcourt, 1958, and Bremekamp, 1966, when there were deviating views on the placement of a genus) is given, as are the reasons for their inclusion in the study (for molecular studies the first study using a DNA marker is cited).

Genus	No. of spec. (sequenced spp./taxa)	Subfamily/tribe	Reason for inclusion in the study
<i>Batopedina</i>	3 (1)	Rubioideae/Hedyotideae	Segregated from <i>Otomeria</i> and maintained as close to or even included in that genus (Verdcourt, 1953a, 1989); <i>rbcL</i> data place <i>Batopedina</i> in the <i>Pentas</i> group (Bremer & Manen, 2000).
<i>Calanda</i> *	1 (1)	Antirheoideae/Knoxieae ^a	A member of Knoxieae (Verdcourt, 1958).
<i>Carphalea</i>	10 (8)	Rubioideae/Hedyotideae ^b	Mentioned as a member of the <i>Pentas</i> group on morphological grounds (Bremer, 1987), also supported by <i>rbcL</i> and <i>ndhF</i> data (Bremer, 1996; Bremer & al., 1999).
<i>Chamaepentas</i> *	1 (0)	Rubioideae/Hedyotideae	Described as close to <i>Pentas</i> (Bremekamp, 1952), suggested to be merged with it (Verdcourt, 1976a).
<i>Chlorochorion</i> *	2 (2)	Antirheoideae/Knoxieae ^a	Described in Knoxieae as a segregate from <i>Pentanisia</i> (Puff & Robbrecht, 1989).
(<i>Gomphocalyx</i>)	1	Rubioideae/Spermacoaceae	Suggested as a member of Lathraeocarpeae (Capuron, 1973) or even to include <i>Lathraeocarpa</i> (Piesschaert, 2001). Not included here because <i>rps16</i> data place the genus elsewhere in Rubioideae (Dessein & al., 2005).
<i>Knoxia</i>	ca. 11 (3/4)	Antirheoideae/Knoxieae ^a	Type genus of Knoxieae. Morphological support (Bremer, 1996) and <i>rbcL</i> (Bremer & Manen, 2000) and <i>rps16</i> data (Andersson & Rova, 1999).
<i>Lathraeocarpa</i> *	2 (0)	Rubioideae/Lathraeocarpeae	The type species was first designated as <i>Triainolepis</i> and there are morphological similarities to <i>Triainolepis</i> and <i>Paratriaina</i> (Bremekamp, 1957).
(<i>Neohymenopogon</i> *)	3	Cinchonoideae/Cinchoneae or Rubioideae/Hedyotideae	Mentioned as a member of the <i>Pentas</i> group on morphological grounds (Bremer, 1987), probably because of the shared presence of semaphylls. But the semaphylls in <i>Neohymenopogon</i> are enlarged bracts and not calyx lobes. In other characteristics this genus of 3 species from Himalaya, e.g., the epiphytic <i>N. parasiticus</i> , shows little resemblance to the rest of the genera and is not further considered.
<i>Neopentanisia</i> *	2 (1)	Antirheoideae/Knoxieae ^a	Segregated from <i>Pentanisia</i> (Verdcourt, 1953d).
<i>Otiophora</i>	17 (12/13)	Rubioideae/Hedyotideae ^c	Morphological similarities to <i>Otomeria</i> , <i>Pentas</i> , and <i>Pentanisia</i> (Verdcourt, 1950); <i>rbcL</i> (Bremer & Manen, 2000) and <i>rps16</i> data (Andersson & Rova, 1999).
<i>Otomeria</i>	8 (4)	Rubioideae/Hedyotideae	Morphological similarities to <i>Pentas</i> (Verdcourt, 1953a; Bremer, 1987), even considered to be merged with that genus (Verdcourt, 1989); <i>rbcL</i> (Bremer & Manen, 2000) and <i>rps16</i> data (Andersson & Rova, 1999).
<i>Paraknoxia</i> *	1 (1)	Antirheoideae/Knoxieae ^a	A member of Knoxieae (Bremekamp in Germain, 1952), included in <i>Pentanisia</i> (Verdcourt, 1952) or kept as a separate genus (Verdcourt, 1976a; Puff & Robbrecht, 1989).
<i>Parapentas</i>	3 (3)	Rubioideae/Hedyotideae	Mentioned as a member of the <i>Pentas</i> group on morphological grounds (Bremer, 1987); <i>rbcL</i> (Bremer & al., 1995) and <i>atpB-rbcL</i> data (Bremer & Manen, 2000).
<i>Paratriaina</i>	1 (1)	Rubioideae/Triainolepideae ^d	A member of Triainolepideae (Bremekamp, 1956), <i>rbcL</i> data (Bremer & Manen, 2000).
<i>Pentanisia</i>	14 (11/12)	Antirheoideae/Knoxieae ^a	A member of Knoxieae, <i>rbcL</i> (Bremer & al., 1995), <i>atpB-rbcL</i> (Bremer & Manen, 2000), and <i>rps16</i> data (Andersson & Rova, 1999).

Table 1. Continued.

Genus	No. of spec. (sequenced spp./taxa)	Subfamily/tribe	Reason for inclusion in the study
<i>Pentas</i>	ca. 39 (30/31)	Rubioideae/Hedyotideae	Mentioned as a member of the <i>Pentas</i> group on morphological grounds (Bremer, 1987), supported by <i>rbcL</i> (Bremer & al., 1995), <i>ndhF</i> (Bremer & al., 1999), <i>atpB-rbcL</i> (Bremer & Manen, 2000), and <i>rps16</i> data (Andersson & Rova, 1999).
<i>Placopoda</i>	1 (1)	Rubioideae/Hedyotideae	Morphological similarities to <i>Carphalea</i> (Puff, 1988) and <i>rbcL</i> data (Bremer, 1996).
<i>Thecorchus</i>	1 (1)	Rubioideae/Hedyotideae	Suggested as close to <i>Otomeria</i> (Bremekamp, 1952) and as a member of Knoxieae on morphological grounds, albeit with hesitation (Dessein, 2003).
<i>Thyridocalyx</i> *	1 (0)	Rubioideae/Triainolepideae ^d	A member of Triainolepideae (Bremekamp, 1956).
<i>Triainolepis</i>	ca. 11 (4/7)	Rubioideae/Triainolepideae ^d	Type genus of Triainolepideae (Bremekamp, 1956), <i>rbcL</i> and <i>atpB-rbcL</i> data (Bremer & Manen, 2000).
Total:	ca. 129 (84/91)		

*For genera marked with an asterisk, no DNA data have previously been reported.

^aPlaced in Rubioideae by Verdcourt (1958) and Bremekamp (1966).

^bPlaced in Cruckshanksieae by Bremekamp (1966).

^cMoved to Spermaceae (Robbrecht, 1994), placed in Anthospermeae by Verdcourt (1958); suggested as a tribe of its own by Verdcourt & Bridson (1991).

^dPlaced in Psychotrieae by Verdcourt (1958).

similarities especially in flower structure, but refrained from merging the tribes. Instead, he suggested affinities between Knoxieae and Psychotrieae. In retrospect this is not so far fetched, since he placed *Triainolepis* (Fig. 1D) in the latter tribe. In fact, when comparing the two tribes he emphasised the similarities in fruit between *Triainolepis* and *Pentanisia* (subgenus *Holocarpa*), both with fleshy fruits, as well as the unequal calyx present in *Triainolepis* and Knoxieae. A separate tribe was, however, already erected for *Triainolepis* by Bremekamp (1956), who considered his Triainolepideae close to Psychotrieae (see also Bremekamp, 1966). The reason for acknowledging Triainolepideae, was the unique combination of shrubs or small trees with two- to ten-locular ovaries with two (to three) collateral, basal ovules in each locule and drupes with a single pyrene and single seeded locules in Rubioideae. Included in Triainolepideae were also the two new monospecific genera *Paratriaina* and *Thyridocalyx*.

Despite the many similarities between the former Knoxieae and some Hedyotideae, Robbrecht (1988) not only kept the taxa in separate tribes, but also placed Knoxieae in another subfamily, the previously recognised Antirheoideae (this is also argued for in Puff & Robbrecht's 1989 revision of the Knoxieae). Robbrecht thus emphasised the single pendulous ovule in each locule of the ovary, but the presence of heterostylous flowers without stylar pollen presentation, fimbriate stip-

ules, and raphides, the latter being the main diagnostic character for Rubioideae (Verdcourt, 1958; Bremekamp, 1966), made the tribe rather aberrant in Antirheoideae. That Knoxieae were misplaced in Antirheoideae was also indicated by their trinucleate pollen, a condition otherwise restricted to tribes of Rubioideae (Robbrecht, 1994). Furthermore, Verdcourt & Bridson (1991: 749) stated that “[s]ome *Pentanisia* are so similar to *Pentas* that only an examination of the ovary will separate them and we cannot believe this is due to convergence over a wide range of characters”.

Another tribe, *Lathraeocarpeae*, might also be related to the enlarged Knoxieae. The reason for this is that two species initially referred to as *Triainolepis* were described as a new genus, *Lathraeocarpa* (Bremekamp, 1957). *Lathraeocarpa* agrees with *Triainolepis* in having pluri-locular ovaries, but have solitary ovules in each locule and pluricolporate pollen grains instead of tri-colporate. These differences kept Bremekamp from including his new genus in Triainolepideae. *Lathraeocarpa* did not fit into any of the tribes of Rubioideae with a single ovule per locule either, for example due to stipules united with the leaves and a calyx with doubled number of lobes. Bremekamp (1957) consequently created a new tribe for the genus.

With the availability of molecular phylogenies (e.g., Andersson & Rova, 1999; Bremer & Manen, 2000), it now seems certain that the genera in this study once thought



Fig. 1. Characteristics and morphological variation of Knoxiaceae (for details see text). A, *Chamaepentas longituba* (*Pentas longituba*); B, *Chamaepentas hindsioides* (*Pentas hindsioides*); C, *Carphalea kirondrion*; D, *Triainolepis africana*; E, *Dolichopentas decora* (*Pentas decora*); F, *Knoxia sumatrensis*; G, *Phyllopentas madagascariensis* (*Pentas ionolaena* subsp. *madagascariensis*); H, *Phyllopentas schimperiana* (*Pentas schimperiana*); I, *Pentas lanceolata*; J, *Rhodopentas bussei* (*Pentas bussei*); K, *Pentanisia gossweileri* (*Neopentanisia gossweileri*); L, *Otomeria elatior*; M, *Batopedina pulvinellata*; N, *Otiophora scabra*. (A, I, J by BB; B by Frank van Caekenberghe; C, N by Torsten Eriksson; D, G by JK; E by Mats Thulin; F, H, K, L, M by Steven Dessen).

to belong to several more or less related tribes do form a monophyletic group, the Knoxieae. One of the aims of this study is to investigate whether this group is still monophyletic with the inclusion of additional hitherto unsequenced genera and species. We also aim to infer relationships within this group—are the genera as traditionally circumscribed monophyletic or is a new classification necessary? If new groupings are found, is there morphological support for these? The molecular markers used here are the mainly non-coding chloroplast regions *rps16* and *trnT-F* and the nuclear internal transcribed spacer (ITS).

MATERIALS AND METHODS

Molecular data and taxon sampling. — Three molecular markers were used. First, two chloroplast datasets were compiled. One consisted of DNA sequences of the *rps16* intron (Oxelman & al., 1997) and the other of sequences from the region between the *trnT* (UGU) and *trnF* (GAA) genes (*trnT-F*), including the spacer between *trnT* (UGU) and the *trnL* (UAA) 5' exon, the *trnL* (UAA) 5' exon, an intron in *trnL* (UAA), and the spacer between the *trnL* (UAA) 3' exon and *trnF* (GAA) (Taberlet & al., 1991). Secondly, a dataset with the nuclear ITS region (ITS1, 5.8 S gene, and ITS2; White, & al., 1990) were compiled.

The aim was to include all species suggested to belong to the Knoxieae in the molecular study. With the available material, either freshly collected, silica-gel dried material or herbarium specimens (from AAU, BR, C, L, P, PRE, S, UPS) we were able to obtain sequences from 84 out of ca. 129 species representing 16 of 19 genera (Table 1, Appendix 1). For four of the species we included sequences from different individuals representing different parts of the distributions, to test if these individuals do represent the same taxon (*Otomeria pauciflora* and *Pentas ionolaena* both represented with one subspecies in Africa and one in Madagascar; *Pentania ouranogyne* represented by both a Kenyan and a Somali specimen; and *Triainolepis africana* represented by one individual from Kenya, one from the Comoros and two from Madagascar). Voucher specimen information and EMBL/Genbank accession numbers are given in Appendix 1.

Most taxa are included in all three datasets. The *rps16* dataset includes 90 sequences from Knoxieae, 89 of which are obtained for this study and one from Genbank. The *trnT-F* and ITS datasets include 86 and 82 newly obtained sequences from the Knoxieae, respectively. For the previously published sequences of the *trnT-F* dataset only the region from the *trnL* (UAA) 5' exon to *trnF* (GAA) was available (i.e., for all outgroup sequences except *Conostomium quadrangulare*). Likewise, for four of the taxa

sequenced from herbarium specimens, only the *trnL-F* region could be sequenced (*Otiophora pauciflora* subsp. *pauciflora*, *Otomeria elatior*, *Knoxia sumatrensis*, *Pentas ionolaena* subsp. *ionolaena*).

To be able to test the monophyly of Knoxieae and to orient the phylogenies, 17 taxa from outside the group were included. Since Knoxieae are a member of Rubioideae and their probable sister group is Spermacoceae (Bremer & Manen, 2000; Robbrecht & Manen, 2006), we sampled all available genera with sequences for both *rps16* and *trnL-F* for Spermacoceae as well as one genus from each of the remaining tribes of Rubioideae (also restricted to the ones with both *rps16* and *trnL-F*) from Genbank. For ITS only 6 of the 17 outgroup taxa were available. Sequences from *Conostomium quadrangulare* were newly obtained.

Preliminary analyses with more outgroup sequences from Spermacoceae and Rubioideae (up to 46/286 sequences for *rps16* and 48/64 for *trnL-F*) all yielded a monophyletic Knoxieae in agreement with previous results. These analyses are not described here, since they were intended to assure that the chosen outgroup was sufficiently large to assure that none of the included sequences ended up in the studied group for dubious reasons, for example due to long-branch attraction.

Sequencing. — DNA was extracted from fresh, silica-gel dried material or herbarium specimens using the CTAB method (Doyle & Doyle, 1987) and purified with QIAquick® PCR kit (QIAGEN, Solna, Sweden/Hilden, Germany) following the instructions provided by the manufacturer. Polymerase chain reactions (PCR) were run on an Eppendorf® Mastercycler® gradient (Bergman & Beving Instrument, Stockholm, Sweden). The 50- μ l reactions included 5 μ l reaction buffer, 5 μ l MgCl₂, 5 μ l TMACL (Chevet & al., 1995), 4 μ l, 0.25 μ l Taq (5U/ μ l), 0.5 μ l 5' primer (20 μ M), 0.5 μ l 3' primer (20 μ M), 0.5 μ l BSA 1%, and 1–2 μ l of DNA templates and sterilised H₂O adding up to 50 μ l. The amplifications consisted of an initial denaturation for 1 min at 95°C, followed by 35 to 37 cycles of 1 min at 95°C, 1 min 30 s at 50 or 55°C, 1 min 30 s at 72°C (usually +1 s/cycle), and a final extension phase of 7 min at 72°C. The PCR products were purified with the MultiScreen® Separations System (Millipore, U.S.A.). The purified products were subsequently sequenced with the DYEnamic™ ET Terminator Kit (Amersham Pharmacia Biotech, Uppsala, Sweden) on a GeneAmp PCR System 9700 (Applied Biosystems, Stockholm, Sweden) and analysed on a MegaBACE™ 1000 DNA Analysis System (Amersham Pharmacia Biotech) or on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems) with the BigDye™ terminator cycle sequencing kit (Applied Biosystems). Primers used for both PCR and sequence reactions were for *rps16* F and R2 (Oxelman & al., 1997), for *trnT-F* rps4, a1, b, c, d, e, f, h, and i (Taberlet & al., 1991;

Bremer & al., 2002; Lantz & Bremer, 2004), and for ITS P17 and 26S-82R (Popp & Oxelman, 2001).

Phylogenetic analyses. — In order to be able to perform phylogenetic analyses the three datasets (*rps16*, *trnT-F*, ITS) were aligned by eye and insertion/deletion events were coded using simple indel coding (Simmons & Ochoterena, 2000) as implemented in the computer program SeqState (Müller, 2005).

The three datasets (*rps16*, *trnT-F*, ITS) were analysed both in combination and separately. We performed a Bayesian inference of phylogeny (Huelsenbeck & al., 2001) as well as Maximum Parsimony (MP) analyses. Since this is the first Bayesian analysis of Rubioideae, we performed the MP analyses to investigate if a probabilistic model based approach would give results deviating from parsimony based conclusions.

The Bayesian analyses were performed using the computer program MRBAYES (v3.1; Huelsenbeck & Ronquist, 2001a, b) under the general time reversible model (GTR) with a gamma distribution of substitution rates for the molecular data and under the standard discrete (morphology) model for the indel data. The same model was used for all molecular datasets as suggested by the computer program MrAIC (Nylander, 2004), which estimates likelihood scores under different models using the program PHYML (Guindon & Gascuel, 2003). PHYML is used to estimate the maximum of the likelihood function under all models considered, i.e., all models are not evaluated on the same, approximate topology as with the program Modeltest (Posada & Crandall, 1998). When running the combined analysis the dataset was partitioned and the partitions unlinked so each had its own set of parameters. The Markov chain was run for 2,000,000 generations for each dataset and every 100th tree was sampled. Three additional “heated” chains were used for each run (Metropolis-coupled Markov chain Monte Carlo; Huelsenbeck & Ronquist, 2001b) to reduce the probability of the first chain getting stuck on a local optimum. Furthermore (at least) two separate runs for each dataset were performed to evaluate if the chain had reached stationarity interpreted as when the standard deviation of split frequencies between the separate runs were less than 0.05. As a consequence, the first 500,000 generations were generously discarded as a burn-in period for all the separate datasets and 750,000 generations for the combined dataset.

For the MP analyses PAUP* (ver. 4.0b10; Swofford, 2001) was used. No attempts to find most parsimonious trees are reported, since we are only interested in finding well supported clades. Support for individual clades were estimated using bootstrapping (10,000 replicates with 5 random addition replicates per replicate, TBR branch swapping, and the MULTREES option turned off). Uninformative characters were excluded from the analyses and indels were treated as missing data.

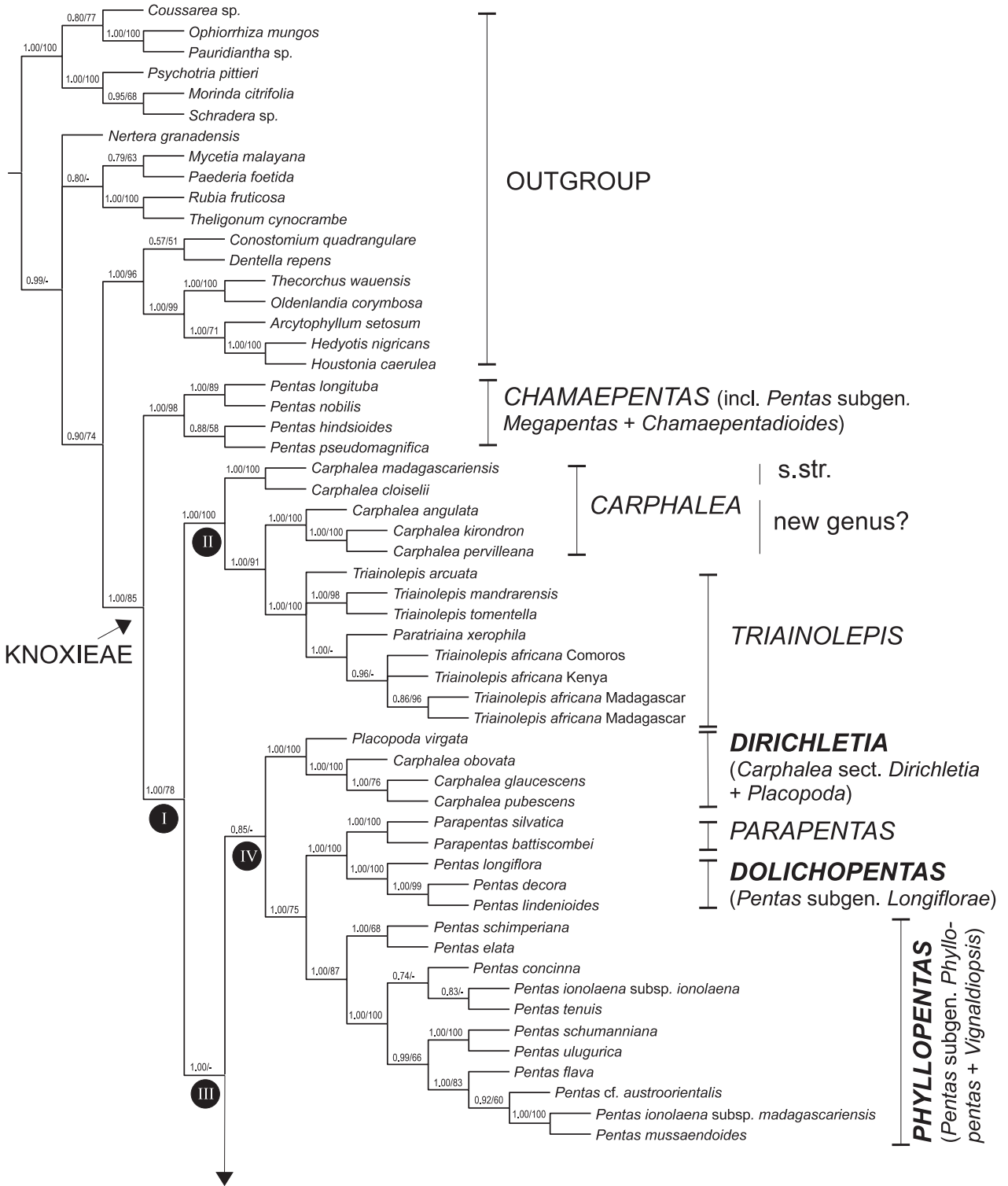
RESULTS

The lengths of the alignments were 2,071 bp for *rps16*, 3,229 bp for *trnT-F*, and 1,318 bp for ITS, resulting in 6,628 bp in the combined matrix. The numbers of phylogenetically informative characters under parsimony criteria in these matrices were 253, 433, 323, and 1,009, respectively. The numbers of phylogenetically informative insertion/deletion characters added to the four matrices were 128, 185, 122, and 435, respectively. No major differences were found if the datasets were analysed without the insertion/deletion characters—only a few clades with low support were not retrieved in those analyses. In the following we therefore only report results from the analyses with the insertion/deletion characters included.

The analyses of the three datasets combined (Figs. 2, 3). — Knoxieae (i.e., all taxa of the newly expanded tribe; Table 1) form a well supported monophyletic group (posterior probability, PP 1.00; bootstrap value, BS 85%) excluding *Thecorchus*, which in our sample is sister to *Oldenlandia* (PP 1.00, BS 100%), one of the outgroup taxa. Neither the taxa from the former Knoxieae (Knoxieae s.str.; fide Robbrecht 1988, 1994; Table 1) nor Hedyotideae (fide Robbrecht 1988, 1994; Table 1) form monophyletic groups, i.e., of the former tribes only the taxa from Triainolepideae are monophyletic (PP 1.00, BS 100%), but nested within Knoxieae. Apart from the monotypic genera (*Calanda*, *Paraknoxia*, *Paratriaina*, *Placopoda*) or those represented by a single species (*Batopedina*, *Neopentansia*), only *Chlorochorion*, *Knoxia*, and *Otiophora* are monophyletic (all three have PP 1.00 and BS 100% except *Knoxia* with BS 78%). The other supported clades more or less correspond to previously recognised subgeneric taxa. Details of their characteristics and circumscriptions are found in the Discussion together with details on relationships within, and morphological support for the resulting clades, as well as the taxonomic consequences of the phylogeny.

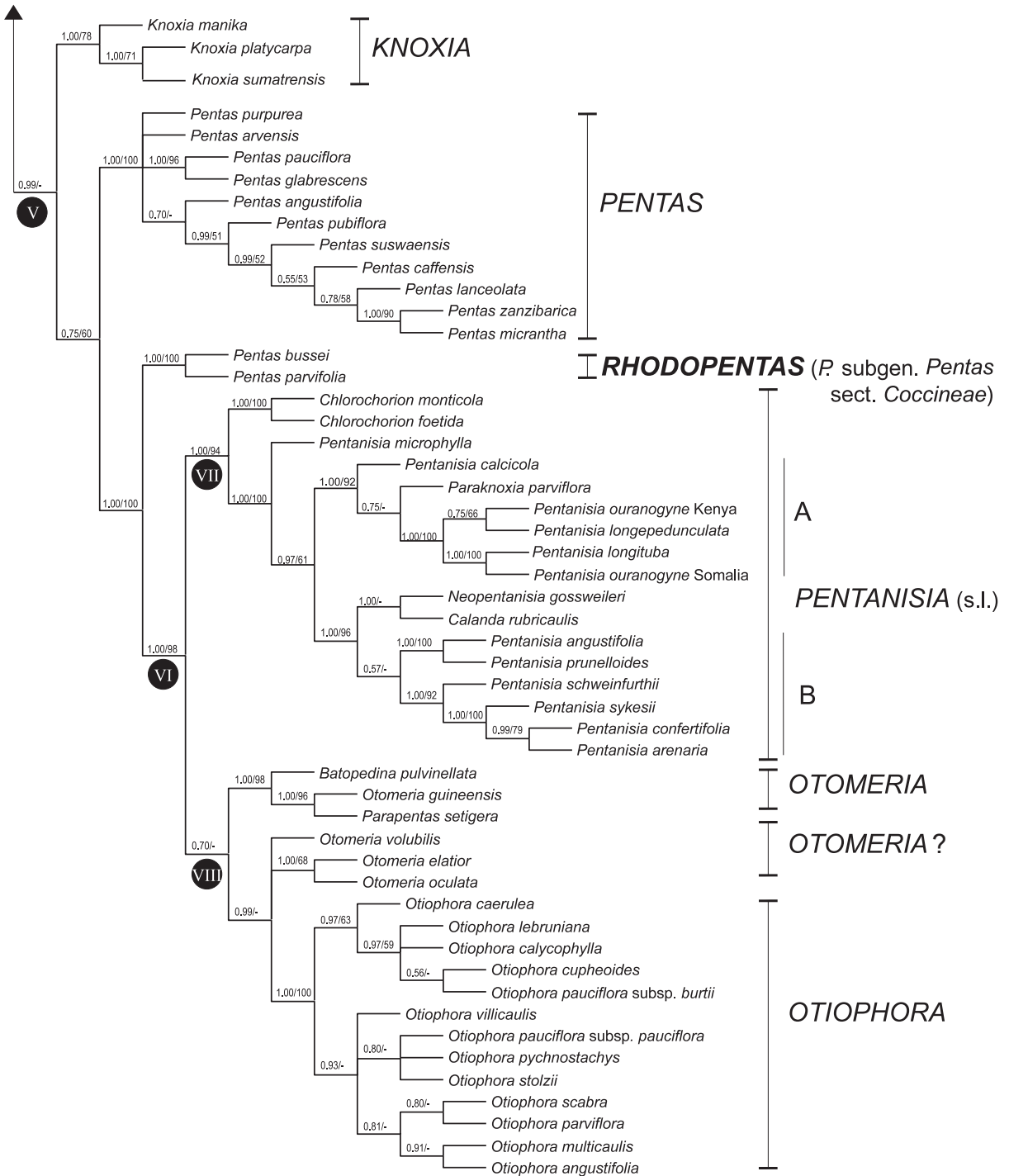
As sister to the rest of the Knoxieae (PP 1.00, BS 78%; the phylogeny is described from the top of Figs. 2 and 3) are species from *Pentas* subgen. *Megapentas* together with the single species from subgen. *Chamaepentadoides* (PP 1.00, BS 98%), *Pentas hindsioides*. The next node in the phylogeny (node I; PP 1.00, BS 78%) splits off a clade (node II; PP 1.00, BS 100%) consisting of parts of *Carpophalea* (Malagasy species), *Paratriaina*, and *Triainolepis*. Within this clade, *C. cloisellii* and *C. madagascariensis* (PP 1.00, BS 100%) are sisters to the rest (PP 1.00, BS 91%) and *C. angulata*, *C. kirondron*, and *C. pervilleana* (the latter two sisters, PP 1.00, BS 100%) together (PP 1.00, BS 100%) are sister to *Triainolepis*. *Paratriaina* nested within this clade (PP 1.00, BS 100%).

The next split (node III; PP 1.00, BS < 50%) is between (node IV; PP 0.85; BS < 50%) the remaining (Afri-



Figs. 2, 3 (overleaf). Phylogenetic tree of the Knoxieae resulting from the Bayesian inference and maximum parsimony analysis (bootstrapping) of the combined *rps16*, *trnT-F*, and ITS data. Numbers above branches are posterior probabilities followed by bootstrap percentages. Bootstrap values below 50% are indicated with a dash. A few nodes from the bootstrap analysis with bootstrap values above 50% are not consistent with the tree presented here: The clade of the three *Triainolepis africana* individuals from Madagascar and the Comoros has a bootstrap value of 81%. *Pentas concinna* is sister to the ►

Figure 2



► rest of *Phyllopentas* excluding *Pentas elata* and *P. schimperiana* (BS 59%). *Otiophora stolzii* is sister to the rest of the genus (BS 68%). In *Pentania* A, *Paraknoxia* is the first branching taxon and *Pentania calcicola* is sister to the rest (BS 62%). *Calanda* and *Neopentania* are not each other's sisters, but sisters to *Pentania angustifolia* and *P. prunelloides* (BS 56%) and the rest of *Pentania* B (BS 58%), respectively. Proposed new genera are indicated in bold. See Tables 1 to 3 for previous classifications of the taxa.

can) species of *Carphalea* (PP 1.00, BS 100%), *Placopoda* (as sister to those *Carphalea* species; PP 1.00, BS 100%), members from three subgenera of *Pentas*, and two species of *Parapentas* and the remaining taxa of Knoxiaceae (Fig. 3; node V; PP 0.99, BS < 50%). In the first clade of this split (node IV), *Carphalea* p.p. + *Placopoda* are sisters to the other taxa (PP 1.00, BS 75%). The two species of *Parapentas* (PP 1.00, BS 100%) are sister to *Pentas* subgen. *Longiflorae* (PP 1.00, BS 100%) and together with *Longiflorae* sister to the species from *Pentas* subgen. *Phyllopentas* and *Vignaldiopsis* (PP 1.00, BS 87%).

In the second clade of the split at node III (node V), *Knoxia* (PP 1.00, BS 78%), *Pentas* subgen. *Pentas* (PP 1.00, BS 100%; except section *Coccineae*), and *Pentas* subgen. *Pentas* section *Coccineae* (PP 1.00, BS 100%) are consecutive sisters (PP 0.99 BS < 50%, PP 0.75 BS 60%, and PP 1.00 BS 100%, respectively) to the remaining taxa (node VI, PP 1.00, BS 98%). These are a clade hereafter referred to as *Pentania* s.l. (node VII; PP 1.00, BS 94%) and its sister clade (node VIII; PP 0.70, BS < 50%), which consists of the clade *Batopedina* + *Otomeria guineensis* + *Parapentas setigera* (PP 1.00, BS 98%; the latter two sisters with PP 1.00, BS 96%) and a clade (PP 0.99, BS < 50%) including *Otomeria volubilis*, *Otomeria elatior* + *O. oculata* (PP 1.00, BS 68%), and *Otiophora* (PP 1.00, BS 100%).

Within *Pentania* s.l. (node VII), *Chlorochorion* (PP 1.00, BS 100%) and *Pentania microphylla* are consecu-

tive sisters (PP 1.00, BS 100%) to the rest (PP 0.97, BS 61%). Those remaining taxa split into two clades: *Pentania* A and B (both PP 1.00, BS 92% and 96%, respectively). The former consists of *Paraknoxia* and *Pentania* subgen. *Ouranogyne* (sensu Verdcourt, 1952; see Table 2 for subgeneric classification of *Pentania*) and the latter of the remaining species of *Pentania* (PP 0.57) and *Calanda* + *Neopentania* (PP 1.00) as sister to these (PP 1.00). *Pentania* subgen. *Pentania* (sensu Verdcourt, 1952) is paraphyletic with respect to *P.* subgen. *Holocarpa* (sensu Verdcourt, 1952; PP 1.00, BS 100%). Compared to the Bayesian inference, the parsimony analysis retrieves different relationships within *Pentania* B, although with low bootstrap values (results not shown). *Calanda* and *Neopentania* are not supported as sisters; *Calanda* is weakly supported (BS 56%) as sister to *P. angustifolia* + *P. prunelloides* (BS 100%) and *Neopentania* is weakly supported (BS 58%) as sister to *P. schweinfurthii* + *P.* subgen. *Holocarpa* (BS 92%).

Differences between the combined and the separate analyses. — The well supported clades mentioned above, that will be discussed in further detail are generally also supported by the separate analyses. The relationships between and within the clades receive, however, less support when the datasets are analysed separately. Here the most significant differences are presented, i.e., differences that are not consistent with the total evidence approach of the combined analyses.

Table 2. Taxa of *Pentania* s.l. listed according to previous revisions compared to our results and the characters initially used for distinguishing the different taxa.

Latest revision; Puff & Robbrecht (1989)		Verdcourt's (1952) revision	This study; sequenced taxa in bold	Characters/notes
<i>Calanda</i>	<i>rubricaulis</i>	—	<i>Pentania rubricaulis</i>; <i>Pentania B</i>	Perennial; head-like inflorescences; fused flower aires; dehiscent fruits
<i>Chlorochorion</i>	<i>foetida</i>	subg. <i>Pentania</i> sect. <i>Axillares</i>	<i>Pentania foetida</i>; included in <i>Pentania</i> s.l.	Perennial; pseudo-axillary inflorescences; 2-locular, dehiscent fruits. Split from <i>Pentania</i> by Puff & Robbrecht (1989)
	<i>monticola</i>	subg. <i>Pentania</i> sect. <i>Axillares</i>	<i>Pentania monticola</i>; included in <i>Pentania</i> s.l.	
<i>Neopentania</i>	<i>annua</i>	—	<i>Pentania annua</i> ; <i>Pentania B</i>	Annual; terminal inflorescences; fruits of 2 cohering, warty spheres. Split from <i>Pentania</i> by Verdcourt (1953d)
	<i>gossweileri</i>	—	<i>Pentania gossweileri</i>; <i>Pentania B</i>	
<i>Paraknoxia</i>	<i>parviflora</i>	<i>Pentania</i> subg. <i>Micropentania</i>	<i>Pentania parviflora</i>; <i>Pentania A</i>	Annual; pseudo-axillary inflorescences; 2-locular, dehiscent fruits

Table 2. Continued.

Latest revision; Puff & Robbrecht (1989)	Verdcourt's (1952) revision	This study; sequenced taxa in bold	Characters/notes
<i>Pentania</i>			
subg. <i>Pentania</i>			
			Perennial; terminal inflorescences; 2-locular (tardily) dehiscent fruits
<i>angustifolia</i>	subg. <i>Pentania</i> sect. <i>Pentania</i>	<i>Pentania</i> B	
<i>prunelloides</i>	subg. <i>Pentania</i> sect. <i>Pentania</i>	<i>Pentania</i> B	
subg. <i>Holocarpa</i>			
			Perennial; terminal inflorescences; 2–5-locular, indehiscent fruits
<i>schweinfurthii</i>	subg. <i>Pentania</i> sect. <i>Pentania</i>	<i>Pentania</i> B	2-locular ovary
<i>veronicoides</i>	subg. <i>Holocarpa</i>	<i>Pentania</i> B	<i>Holocarpa</i> sensu Verdcourt (1952): 3–5-locular ovaries, ± succulent fruits
<i>sykesii</i>	subg. <i>Holocarpa</i>	<i>Pentania</i> B	
<i>arenaria</i>	subg. <i>Holocarpa</i>	<i>Pentania</i> B	
<i>renifolia</i>	subg. <i>Holocarpa</i>	<i>Pentania</i> B	
<i>confertifolia</i>	subg. <i>Holocarpa</i>	<i>Pentania</i> B	
<i>ouranogyne</i>	subg. <i>Ouranogyne</i>	<i>Pentania</i> A	Verdcourt (1952) separated <i>Holocarpa</i> and <i>Ouranogyne</i> due to drupaceous fruits in the former
<i>longituba</i>	subg. <i>Ouranogyne</i>	<i>Pentania</i> A	
<i>longepedunculata</i>	subg. <i>Ouranogyne</i>	<i>Pentania</i> A	
<i>calcicola</i>	subg. <i>Ouranogyne</i>	<i>Pentania</i> A	
<i>microphylla</i>	incertae sedis	included in <i>Pentania</i> s.l.	2-locular, schizocarpous fruits (included in <i>Holocarpa</i> by Puff & Robbrecht [1989] because erroneously interpreted as indehiscent)
incertae sedis			
<i>procumbens</i>	subg. <i>Pentania</i> sect. <i>Repentes</i>	?	Perennial, procumbent; minute leaves; terminal inflorescences; 2-locular fruits

The *rps16* dataset is the least informative and provides no support for the inclusion of *Pentas elata* and *P. schimperiana* in the *Phyllopentas* + *Vignaldiopsis* clade. There is also no support for *Chlorochorion* as a member of *Pentania* s.l. In the Bayesian analysis, *Carphalea angulata*, *C. kirondron*, and *C. pervilleana* (PP 0.66) form a clade, which is the sister group (PP 1.00) to a *Triainolepis* + *Paratriaina* and *C. cloiselii* + *C. madagascariensis* clade (PP 0.98), while in the MP analysis they do not

group with that clade at all. In the MP analysis *Knoxia* is unresolved, as is the clade of those *Carphalea* species that grouped with *Placopoda* in the combined analyses.

The *trnT-F* dataset also does not place *Pentas elata* and *P. schimperiana* in the *Phyllopentas* + *Vignaldiopsis* clade. *Carphalea angulata* and *C. kirondron* are not supported as sister taxa (*C. pervilleana* not sequenced), but are with PP 0.98 closer to the *Triainolepis* + *Paratriaina* clade than to *C. cloiselii* + *C. madagascariensis* as in

the combined analyses. *Knoxia manika* is resolved in the outgroup as sister to *Thecorchus* + *Oldenlandia* (PP 0.99, BS 97%).

The ITS data give some indication that the Malagasy *Carphalea* species form a monophyletic sister group (PP 0.92, BS 73%) to *Triainolepis* + *Paratriaina*.

DISCUSSION

The newly expanded Knoxieae are well-supported and can generally be recognised by the suite of characters mentioned in the Introduction. However, the genera previously referred to Knoxieae and Hedyotideae (Robbrecht, 1988, 1994; Table 1) do not form monophyletic groups. The only former tribe that remains monophyletic is Triainolepideae, but treating it as a separate tribe would make Knoxieae paraphyletic. Synapomorphies of Knoxieae are difficult to assess considering which taxa of the Spermaceae alliance are closest to them and to their presumed sister taxon Spermaceae (cf. Bremer & Manen, 2000). In the following, we refer to the combined analyses of the three datasets (Figs. 2, 3), if not otherwise indicated. Since the genera now included in Knoxieae are treated in separate tribes in all major Floras and revisions, we will discuss them under headings referring to their previous tribal placements (Table 1). The main treatment of a taxon is indicated with the taxon name in bold face.

The former Knoxieae. — The former Knoxieae species (Knoxieae s.str.; Robbrecht 1988, 1994; Table 1) are not supported as monophyletic. Thus, solitary, pendulous ovules seem to have evolved twice, once in *Knoxia* and once in the lineage leading to *Pentansia* s.l., i.e., the other genera of Knoxieae s.str. The character most emphasised and used in the recognition of Knoxieae s.str. is apparently homoplastic and the suite of other characters used to define the group (predominately herbaceous habit, fimbriate stipules, presence of raphides, heterostylous flowers with frequently irregular calyx lobes, valvate aestivation, and salver-shaped corollas, uniform 3-colporate pollen, chromosome base number of $x = 10$; cf. Puff & Robbrecht, 1989) are particularly common in the expanded Knoxieae as a whole.

Knoxia (Fig. 1F) differs from *Pentansia* s.l. in its four-merous flowers in inflorescences where the flowers are arranged spirally, but solitary and not in pairs as in *Pentansia* s.l. (Puff & Robbrecht, 1989; Puff, 1983; also in, e.g., *Otiophora*, *Otomeria*, *Pentas*). *Knoxia* also has true carpophores, i.e., a structure holding the mericarps formed by paired vascular strands in the centre of the septum (Puff & Robbrecht, 1989). Furthermore, it is the sole genus under study not restricted to Africa and Madagascar. Only two of the ca. eleven species of *Knoxia* are African, otherwise the genus is Indomalaysian

with one of the species extending to China and Australia (Bhattacharjee & Deb, 1985; Ridsdale, 1996, 1998). The African *Knoxia* species were recently included in the genus (Puff & Robbrecht, 1989); earlier they were placed in two monotypic genera, *Afroknoxia* (*Knoxia manika*) from D.R. Congo (Verdcourt, 1981) and *Neobaumannia* (*Knoxia hedyotideae*; Hutchinson & Dalziel, 1931), which occurs from Cameroun west to Ghana. Puff & Robbrecht (1989) merged these African species with *Knoxia* based on the shared presence of true carpophores and close similarities in habit, stipules, inflorescence, flowers, and fruits. Upon their description (Schumann, 1896; Verdcourt, 1981), the African species were in fact suggested as being closely related to the Asian ones, although they were recognised as genera. The shape of the carpophore readily distinguishes the African from the Asian species; in the former it is arch-shaped, while in the latter it is column-like (Verdcourt, 1981; Puff & Robbrecht, 1989).

In our study *Knoxia* is monophyletic. We were not able to sequence *Knoxia hedyotideae*, but we see no reason not to accept the morphology based inclusion of it (Puff & Robbrecht, 1989).

All genera of *Pentansia* s.l. except *Calanda* were originally regarded as congeneric with *Pentansia*. Our data show that the recognition of several smaller genera was premature in the sense that it renders *Pentansia* paraphyletic (Fig. 3). In Table 2 we give an overview of previous classifications of the genera and the subgeneric taxa of *Pentansia* s.l. and the characters used to define them. Sister to all other species of *Pentansia* s.l. is the genus ***Chlorochorion***, described by Puff & Robbrecht (1989) who argued that the two species of Verdcourt's (1952) subgenus *Pentansia* section *Axillares* certainly deserved generic status because of their habit (more straggling), pseudo-axillary inflorescences, considerably smaller flowers, and smaller fruits dehiscent into mericarps. Although the habit is more straggling than in *Pentansia*, we question the recognition of *Chlorochorion* since axillary inflorescences occur in addition to terminal ones in some *Pentansia* species (*P. arenaria*, *P. prunelloides*, *P. schweinfurthii*). Furthermore, there is some overlap in flower size between the two genera (*P. schweinfurthii*, *P. veronicoides*), and schizocarps are present also in *P. microphylla* (Thulin, 2006).

In Verdcourt's 1952 revision, *Pentansia* was divided into four subgenera and the little known *P. microphylla* was regarded as incertae sedis (Table 2). Our results support the Somalian *P. microphylla* as the next branching taxon after *Chlorochorion*. Both *Chlorochorion* and *Pentansia microphylla* have schizocarpous fruits. Schizocarpous fruits, thus, seem to be plesiomorphic for *Pentansia* s.l.. Another plesiomorphic character is likely fruits with a wedge-shaped false carpophore (false carpophores are formed by the uppermost pedicel portion; Puff &

Robbrecht, 1989), since such are present in both *Chlorochorion* and *Pentania microphylla* (also present in *P.* subgenus *Pentania*).

One of the subgenera was the monospecific *Micropentania*. Based on its annual habit, four-merous flowers, short corolla tube, and the often pseudo-axillary inflorescences, *Pentania parviflora* had already been treated as the sole species of the new genus *Paraknoxia* (Bremekamp in Germain, 1952). It is also the only species with a ring-shaped false carpophore. The treatment of the species as a separate genus is followed in the Flora of Tropical East Africa (Verdcourt, 1976a) and by Puff & Robbrecht (1989), who stated that generic rank is certainly justified and further argued for an isolated position within Knoxieae s.str., i.e., with no obvious affinities to any of the other genera. However, as previously mentioned, *Pentania* is not monophyletic in our study. *Paraknoxia* groups with the species of Verdcourt's subgenus *Ouranogyne* (*Pentania* A). *Ouranogyne* was characterised by a three- to five-locular ovary and a woody, indehiscent fruit. *Paraknoxia* occurs in central and eastern Africa (an eastern Sudano-Zambesian distribution), *Ouranogyne* consists of species from the Horn of Africa and tropical East Africa with two of the four species restricted to Somalia (*P. calcicola* and *P. longepedunculata*) as is *P. microphylla*. *Pentania* A, thus, has a more northern distribution compared to the members of *Pentania* B, which are centred from south central Africa westwards to Angola and south to South Africa; *P. schweinfurthii* is widespread in tropical Africa from Nigeria and Sudan south to Angola and Zimbabwe.

Verdcourt's other two subgenera were *Holocarpa* with three- to five-locular ovaries and indehiscent fruits with a well developed, more or less succulent fruit wall, and *Pentania* with two-locular ovaries and dry fruits. Puff & Robbrecht's (1989) view that *Ouranogyne* sensu Verdcourt should be included in *Holocarpa* because of their shared presence of a several-seeded stone is, as mentioned, not supported by our results. They also included *Pentania microphylla* and *P. schweinfurthii* from subgenus *Pentania* although both species only have two-locular ovaries because their fruits were regarded as indehiscent. *P. microphylla*, has, however, schizocarps (Thulin, 2006). We have not seen any mature fruits of *P. schweinfurthii*, but immature ones are very similar to the ones of *P. microphylla*. That the fruits of the latter really split open is impossible to tell from immature fruits. Our results show that the species of Verdcourt's *Holocarpa* group together with subgenus *Pentania* section *Pentania*. *Pentania schweinfurthii* is sister to *Holocarpa*, thus rendering section *Pentania* paraphyletic.

A third section of subgenus *Pentania*, section *Repentes*, was recognised by Verdcourt (1952). The sole species of *Repentes* was the little known *P. procumbens* from

Angola. It has two-locular fruits, but Puff & Robbrecht (1989) argued that more fruiting material was needed before its affinities could be settled and consequently regarded it as incertae sedis. We have not seen any material of this species, but its two-locular fruits and its distribution indicate a relationship with *Pentania* B. As sister to the species of the subgenera *Holocarpa* and *Pentania* (*Pentania* B) is the monotypic *Calanda* together with *Neopentania*. *Calanda rubricaulis* is an Angolan endemic and is morphologically very distinct from all other species of *Pentania* s.l. as well as from the rest of Knoxieae. A number of characters (such as triangular stipules without fimbriae, flowers in heads arranged in umbel-like inflorescences, and fused flower pairs, i.e., the ovaries and enlarged calyx lobes are fused but the corollas are free from each other; Puff & Robbrecht (1989) indicate that the species has been isolated and evolved into a very characteristic plant. A closer look at the above characters reveals that they are probably modifications of characters present in *Pentania*. We have for example in contrast to earlier reports found stipules with colleters. Taking this into consideration, it seems that *Calanda* might just be a derived species of *Pentania*, and according to our results most related to *Pentania* B, which also fits with the more southern distribution of that group. To keep *Calanda* as a genus would render *Pentania* paraphyletic and we consequently argue for transferring *Calanda* to *Pentania*.

Neopentania was segregated from *Pentania* because of its annual habit and fruits consisting of two cohering spheres covered with wart-like papillae (Fig. 1K; Verdcourt, 1953d). Another character distinguishing it from other members of *Pentania* s.l. is its U-shaped false carpophores (Puff & Robbrecht, 1989). The genus was first regarded as an Angolan endemic, but one of the two species, *Neopentania gossweileri*, was later found in D.R. Congo (Puff & Robbrecht, 1989) and Zambia (*Dessein & al.* 1037, BR).

In summary we suggest, based on the above arguments, that *Chlorochorion*, *Paraknoxia*, *Calanda*, and *Neopentania* should be merged into *Pentania* (see Tables 2, 3, Appendix 2). Even though subgroups (*Chlorochorion*, *P. microphylla*, *Pentania* A, and *Pentania* B either including or excluding *Calanda* + *Neopentania*) could be recognised at subgeneric level we refrain from doing so. With the observation that fruit type (e.g., locule number and fruit dehiscence) seems to have a more complex pattern than previously thought, unambiguous characters diagnostic for the subgroups seem to be lacking. Distribution may actually best reflect the phylogeny, although there are taxa from both *Pentania* A and B with rather wide distributions.

The former Hedyotideae. — Like the members of the former Knoxieae, the former Hedyotideae taxa do not form a monophyletic group.

Table 3. Synopsis of genera and species we recognise in Knoxiaceae and previous placements for taxa affected by nomenclatural changes; sequenced species in bold. Unsequenced species are placed according to morphological similarities or previous infrageneric classifications (see text); for *Pentanisia* see also Table 2.

Species	Previous placement	Species	Previous placement
<i>Knoxia</i>		<i>Dolichopentas</i>	
<i>hedytidea</i>		<i>decora</i>	<i>Pentas</i> subg. <i>Longiflorae</i>
<i>hirsuta</i>		<i>liebrechtsiana</i>	<i>Pentas</i> subg. <i>Longiflorae</i>
<i>manika</i>		<i>lindenoides</i>	<i>Pentas</i> subg. <i>Longiflorae</i>
<i>plantaginea</i>		<i>longiflora</i>	<i>Pentas</i> subg. <i>Longiflorae</i>
<i>platycarpa</i>			
<i>rosettifolia</i>		<i>Parapentas</i>	
<i>roxburghii</i>		<i>battiscombei</i>	
<i>spicata</i>		<i>silvatica</i>	
<i>sumatrensis</i>			
<i>wightiana</i>		<i>Phyllopentas</i>	
<i>zeylanica</i>		<i>austrorientalis</i>	<i>Pentas</i> subg. <i>Phyllopentas</i>
		<i>concinna</i>	<i>Pentas</i> subg. <i>Vignaldiopsis</i>
<i>Pentanisia</i>		<i>decaryana</i>	<i>Pentas</i> subg. <i>Phyllopentas</i>
<i>angustifolia</i>		<i>elata</i>	<i>Pentas</i> subg. <i>Vignaldiopsis</i>
<i>annua</i>	<i>Neopentanisia</i>	“flava”^b	<i>Pentas</i> subg. <i>Phyllopentas</i>
<i>arenaria</i>		<i>hirtiflora</i>	<i>Pentas</i> subg. <i>Phyllopentas</i>
<i>calicicola</i>		<i>ionolaena</i>	<i>Pentas</i> subg. <i>Phyllopentas</i>
<i>confertifolia</i>		<i>ledermanii</i>	<i>Pentas</i> subg. <i>Vignaldiopsis</i>
<i>foetida</i>	<i>Chlorochorion</i>	<i>madagascariensis</i>	<i>Pentas</i> subg. <i>Phyllopentas</i>
<i>gossweileri</i>	<i>Neopentanisia</i>	<i>mussaendoides</i>	<i>Pentas</i> subg. <i>Phyllopentas</i>
<i>longepedunculata</i>		<i>schimperiana</i>	<i>Pentas</i> subg. <i>Vignaldiopsis</i>
<i>longituba</i>		<i>schumanniana</i>	<i>Pentas</i> subg. <i>Phyllopentas</i>
<i>microphylla</i>		sp. ^c	<i>Pentas</i> subg. <i>Phyllopentas</i>
<i>monticola</i>	<i>Chlorochorion</i>	<i>tenuis</i>	<i>Pentas</i> subg. <i>Vignaldiopsis</i>
<i>ouranogyne</i>		<i>ulugurica</i>	<i>Pentas</i> subg. <i>Vignaldiopsis</i>
<i>parviflora</i>	<i>Paraknoxia</i>		
<i>procumbens</i>		<i>Rhodopentas</i>	
<i>prunelloides</i>		<i>bussei</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Coccineae</i>
<i>renifolia</i>		<i>parvifolia</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Coccineae</i>
<i>rubricaulis</i>	<i>Calanda</i>		
<i>schweinfurthii</i>		<i>Otomeria</i>	
<i>sykesii</i>		<i>cameronica</i>	<i>Otomeria</i> subg. <i>Otomeria</i>
<i>veronicoides</i>		<i>guineensis</i>	<i>Otomeria</i> subg. <i>Otomeria</i>
		<i>lanceolata</i>	<i>Otomeria</i> subg. <i>Otomeria</i>
<i>Pentas</i>		<i>micrantha</i>	<i>Otomeria</i> subg. <i>Otomeria</i>
<i>angustifolia</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Monomorphi</i>	(new genus?) ^d	
<i>arvensis</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Pentas</i>	<i>elatior</i>	<i>Otomeria</i> subg. <i>Neotomeria</i>
<i>glabrescens</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Papillopilosae</i>	<i>madiensis</i>	<i>Otomeria</i> subg. <i>Neotomeria</i>
<i>caffensis</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Hirtistylus</i>	<i>oculata</i>	<i>Otomeria</i> subg. <i>Neotomeria</i>
<i>cleistostoma</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Pentas</i>	(new genus?) ^d	
<i>herbacea</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Monomorphi</i>	<i>volubilis</i>	<i>Otomeria</i> subg. <i>Volubilis</i>
<i>lanceolata</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Pentas</i>	(<i>Parapentas</i>)	
<i>micrantha</i>^a	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Monomorphi</i>	<i>setigera</i>^c	
<i>pauciflora</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Papillopilosae</i>	(<i>Batopedina</i>) ^c	
<i>pubiflora</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Pentas</i>	<i>linearifolia</i>	
<i>purpurea</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Pentas</i>	<i>pulvinellata</i>	
<i>purseglovei</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Pentas</i>	<i>tenuis</i>	
<i>suswaensis</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Pentas</i>	<i>Otiophora</i>	
<i>zanzibarica</i>	<i>Pentas</i> subg. <i>Pentas</i> sect. <i>Pentas</i>	<i>angustifolia</i>	
		<i>caerulea</i>	
<i>Chamaepentas</i>		<i>calycophylla</i>	
<i>graniticola</i>	<i>Pentas</i> subg. <i>Megapentas</i>	<i>cupheoides</i>	
<i>greenwayii</i>		<i>inyangana</i>	
<i>hindsiioides</i>	<i>Pentas</i> subg. <i>Chamaepentadoides</i>		
<i>longituba</i>	<i>Pentas</i> subg. <i>Megapentas</i>		
<i>nobilis</i>	<i>Pentas</i> subg. <i>Megapentas</i>		
<i>pseudomagnifica</i>	<i>Pentas</i> subg. <i>Megapentas</i>		

Table 3. Continued.

Species	Previous placement	Species	Previous placement
▶ <i>lanceolata</i>		<i>obovata</i>	<i>Carphalea</i> sect. <i>Dirichletia</i>
<i>lebruniana</i>		<i>pubescens</i>	<i>Carphalea</i> sect. <i>Dirichletia</i>
<i>multicaulis</i>		<i>somaliensis</i>	<i>Carphalea</i> sect. <i>Dirichletia</i>
<i>parviflora</i>		<i>virgata</i>	<i>Placopoda</i>
<i>pauciflora</i> ^f		<i>Triainolepis</i>	
<i>pyncoclada</i>		<i>africana</i>	
<i>pyncostachys</i>		<i>ampandrandavae</i>	<i>Thyridocalyx</i>
<i>rupicola</i>		<i>arcuata</i>	
<i>scabra</i>		<i>brevituba</i>	
<i>spirospicata</i>		<i>emirnsensis</i>	
<i>stolzii</i>		<i>hirtiflora</i>	
<i>villicaulis</i>		<i>mandrarensis</i>	
<i>Carphalea</i> ^g		<i>opaca</i>	
<i>angulata</i>		<i>polyneura</i>	
<i>cloisellii</i>		<i>rhyncopetala</i>	
<i>kirondron</i>		<i>sancta</i>	
<i>linearifolia</i>		<i>tomentella</i>	
<i>madagascariensis</i>		<i>xerophila</i>	<i>Paratriaina</i>
<i>pervilleana</i>		<i>Lathraeocarpa</i>	
<i>Dirichletia</i>		<i>acicularis</i>	
<i>glaucescens</i>	<i>Carphalea</i> sect. <i>Dirichletia</i>	<i>decaryi</i>	

^aWe suspect the Malagasy subspecies subsp. *micrantha* is a separate species, in such case the name *Pentas wylei* should be taken up for the African mainland taxa.

^bFor this undescribed species we use the name Homolle used on the herbarium label of *Decary 13125* (P). We have seen a newly collected specimen (*ATH 211*, TAN) matching both Verdcourt's (1953c) description and the cited specimen *Decary 13125* (P) from the same locality as that specimen. Note: *Decary 5427* (P) is also listed by Verdcourt (1953c) as this undescribed species, but the herbarium sheet is labelled *Pentas micrantha* and is checked as such by Verdcourt; there is evidently a slip in the published work.

^cVerdcourt (1953c) listed this little known species, which he considered close to *P. mussaendoides*, as *Pentas* sp. 34.

^dProbably constitutes a genus separate from *Otomeria*; no formal taxonomic change made pending better sampling of *Otomeria* and *Batopedina*.

^eProbably to be included in *Otomeria*; no formal taxonomic change made pending better sampling of *Otomeria* and *Batopedina*.

^fThe African subspecies subsp. *burtii* probably constitutes a separate species distinct from the Malagasy subsp. *pauciflora*.

^gChloroplast data indicate that *Carphalea* should better be split into a new genus including *C. angulata*, *C. kirondron*, *C. pervilleana*, and possibly also *C. linearifolia*.

Pentas is not monophyletic according to our results. Below we will discuss monophyletic subgroups and suggest how to taxonomically treat these. Previously, *Pentas* has been divided into six subgenera (Verdcourt, 1953c; Table 3): *Megapentas* comprising large-flowered, semi-succulent species with subequal calyx lobes, the monotypic *Chamaepentadoides* characterised by a small inflorescence and spatulate calyx lobes, *Phyllopentas* with one or two of the calyx lobes enlarged and petal-like (Fig. 1), *Vignaldiopsis* with equal linear calyx lobes, a red-brown indumentum, and leaves with a characteristic venation, *Longiflorae* (as *Longiflora*; cf. *International Code of Botanical Nomenclature* [McNeill & al., 2006], Article 21.2) with equal deltoid calyx lobes, often verticillate leaves, and styles tomentose with scaly emergences, and *Pentas*

with unequal calyx lobes. Verdcourt himself stated that “some of the subgeneric groupings which have been employed would doubtless be considered of generic standing by many workers but such disagreement about the status of groups is unavoidable and of little importance” (Verdcourt, 1953c: 246). Had *Pentas* been monophyletic his argument is naturally sound, but our results indicate that *Pentas* might have to be split into smaller monophyletic genera. Such genera seem to correspond well with some of Verdcourt's subgenera.

Megapentas is a very characteristic subgenus, in particular with its large flowers (corolla tubes 6.5–16 cm long). Of the four species of this subgenus, three (*Pentas graniticola*, *P. longituba* [Fig. 1A], and *P. pseudomagnifica*) are endemic to Tanzania and there confined to

small areas. *Pentas nobilis* is more widespread (Congo and southern Tanzania down to Mozambique), but is, as the other species, more or less restricted to rocky places (Verdcourt, 1953c, 1976a, 1989). The unsequenced species, *Pentas graniticola*, is the only glabrous species, but is considered very close to *P. nobilis* (Verdcourt, 1953c).

Together with subgenus *Chamaepentadoides*, *Megapentas* is the sister taxon to the rest of the *Knoxia* group. *Chamaepentadoides* consists of the sole species *Pentas hindsioides* (Fig. 1B) from Kenya and Tanzania. As the species of the previous subgenus it is confined to small areas. According to Verdcourt (1953c) it is very close to *Pentas pseudomagnifica*, but also to *P. longiflora* (subgenus *Longiflorae*). The monotypic genus *Chamaepentas* is hardly generically separable from *Chamaepentadoides*. They share the spatulate calyx lobes, very similar corollas (Bremekamp 1952, Verdcourt 1953c), and nearly identical pollen (also almost identical to the pollen of *Megapentas*; Dessein & al., 2000). *Chamaepentas* differs mainly in being a creeping herb with reduced inflorescences and placentas attached towards the septum (Bremekamp, 1952; the drawing of *Chamaepentas* in the *Flora of Tropical East Africa*; Verdcourt, 1976a), not around the middle of the septum as in *Pentas*.

Although *Chamaepentas* was not sequenced as a part of this study, it seems very likely that it would group with *Chamaepentadoides*. Following Verdcourt (1976a) we suggest the two taxa to be merged and in accordance with our results we also suggest that *Megapentas* is merged with *Chamaepentas*.

Phyllopentas from Tanzania, Malawi, and Madagascar consists of seven species, two of which are undescribed (Verdcourt, 1953c, 1976a). The five species sequenced here form a strongly supported group (PP 1.00, BS 87%) together with *P. tenuis*, *P. concinna*, and *P. ulugurica* of subgenus *Vignaldiopsis*. The two subspecies of *P. ionolaena* included here do not form a sister relationship. The Malagasy subspecies is strongly supported (PP 1.00, BS 100%) as the sister to *P. mussaendoides*, also from Madagascar. Consequently this subspecies is raised to specific level (*Phyllopentas madagascariensis*; Appendix 2; Fig. 1G), as had been implied by Verdcourt (1953c, 1976a). The new species differs, for example, in the shape of the calyx lobe, which is more similar to the Malagasy species and *P. schumanniana*. The unsequenced species (the Malagasy *P. decaryana*, *P. hirtiflora* and *P. sp. 34*, the latter two closely allied to *P. mussaendoides*; Verdcourt, 1953c) are also expected to belong to this group, especially considering that they have the very characteristic petal-like calyx lobe restricted to the subgenus. In the absence of other evidence, these calyx lobes are unlikely to have evolved twice within *Pentas* s.l. (or at least more than twice if the occurrence in *P. ionolaena* subsp. *ionolaena* is considered to have a separate origin).

Of the species from *Pentas* subgenus *Vignaldiopsis*, *Pentas tenuis* from Ethiopia was considered related to *P. ionolaena* and, moreover, to link *Phyllopentas* and *Vignaldiopsis* (Verdcourt, 1966). The two species are sisters according to our data, but the PP is only 0.83. Also from Ethiopia is *P. concinna*, similar to *P. tenuis* but ecologically and geographically distinct (Puff, 2003). *Pentas concinna* is weakly supported as the sister to all the above species. *Pentas ulugurica*, originally described in *Tapinopentas* (Verdcourt, 1953b) but later transferred to *Pentas* (Hepper, 1960), was also considered close to the above species and a link between the two subgenera, in particular because of a capsule structure as in *Phyllopentas* but without the foliaceous calyx lobe (Verdcourt, 1976a). Both *P. ulugurica* and *P. ionolaena* are endemic to the same part of Tanzania (the Uluguru mountains), but our data suggest a sister group relationship between *P. ulugurica* and *P. schumanniana* from southern Tanzania and northern Malawi.

The remaining species of *Vignaldiopsis*, *P. elata*, *P. ledermannii*, and *P. schimperiana* (Fig. 1H), were the only ones recognised in Verdcourt's revision (*P. ledermannii* then included in a subspecies of *P. schimperiana*, subsp. *occidentalis*; some specimens were also erroneously included in *Pentas pubiflora* subsp. *bamandensis*; Verdcourt, 1976b). In our analysis *P. schimperiana* and *P. elata* form a sister relationship and, moreover, are sister to *Phyllopentas* + the above species of *Vignaldiopsis*. *P. elata* is a rare Tanzanian species and is kept as a separate species only because of geographical isolation and the relative constancy of characters within *P. schimperiana*; otherwise it could be regarded as a subspecies of the latter (Verdcourt, 1976a). The unsequenced species *Pentas ledermannii* is also considered close to *P. schimperiana*, but has shorter calyx lobes and corolla tubes and a western distribution (Cameroon; Verdcourt, 1976b). That *Phyllopentas* and *Vignaldiopsis* are closely related is further corroborated by palynological data (Dessein & al., 2000). Since neither of the two seem to be monophyletic, but together form a well supported group, we suggest a new genus *Phyllopentas* including all species of the former two subgenera (Appendix 2).

In the subgenus *Longiflorae* four species are recognised, all considered very closely related, if not conspecific (Verdcourt, 1953c, 1976a). Intermediate forms blur the species delimitations. The pyrophytic herb *Pentas lindenoides* from southern Tanzania, Malawi and Zambia could perhaps better be regarded as a montane pyrophytic ecotype, which together with large-flowered variants of *P. decora* from southern Tanzania would constitute a large-flowered variety of the latter (Verdcourt, 1976a). *Pentas decora* (Fig. 1E) with a wide distribution in tropical Africa is, possibly better considered a synonym of *P. liebrechtsiana* from D.R. Congo and Angola, the type of

which, moreover, is intermediate between *P. decora* and *P. longiflora* (Verdcourt 1953c, 1976a).

Considering the supposed close relationships of the species in the subgenus, not surprisingly our analyses show *Longiflorae* to be monophyletic (PP 1.00, BS 100%). That *Parapentas* is the sister to *Longiflorae* (PP 1.00, BS 100%) has, however, never been suggested before. *Parapentas* is a genus of three species, two of which occur in tropical East Africa and the third with a wider Guineo-Congolian distribution (Bremekamp, 1952; Verdcourt 1953b, 1976a; Bridson & Verdcourt, 2003). In contrast to the erect, often woody herbs of *Longiflorae*, *Parapentas* constitutes herbs creeping on the forest floor. That *Parapentas* has not been associated with *Longiflorae* is not surprising, in addition to its deviating habit it differs in the absence of the scaly emergences of the style, and there is no tendency towards verticillate leaves.

Interestingly, it is only the East African *Parapentas* that is placed sister to subgenus *Longiflorae*. The Guineo-Congolian *Parapentas setigera* (possibly also present in Malawi; Verdcourt, 1953b; Bridson & Verdcourt, 2003) is unexpectedly resolved as sister to *Otomeria guineensis* and should be excluded from the genus (see below). *Parapentas setigera* is, however, a tetraploid ($2n = 40$; Kiehn, 1985) and has a capsule splitting into four valves (Verdcourt, 1976a), whereas the East African species are diploid (*P. battiscombei* $2n = 20$, *P. silvatica* $n = ca. 10$; Lewis, 1965) and have loculicidal capsules. Considering the morphological and palynological (Dessein & al., 2000) differences between *Longiflorae* and *Parapentas* we argue for keeping the taxa apart and a new genus, *Dolichopentas*, to be recognised for the species of *Longiflorae* (Appendix 2; the name *Longiflorae* can not be used according to the *International Code of Botanical Nomenclature* [McNeill & al., 2006], Article 20.2).

In his revision Verdcourt (1953c: 295) himself discussed that subgenus *Pentas* was possibly polyphyletic. The subgenus was divided into five sections: *Coccineae*, *Monomorphi*, *Papillopilosae* (as *Papillopilosa*), *Hirtistylus*, and *Pentas*.

The two species of section *Coccineae* (Fig. 1J) have been suggested as being close to *Otomeria* subgenus *Neotomeria* (see below; Verdcourt, 1953a, 1953c, 1976a) based on similarities of the fruits. Our results do not confirm such close affinities. Nevertheless, *Coccineae* seems to be a distinct group intermediate between the remaining sections of *Pentas* and a larger clade including *Otomeria* (see Fig. 3). *Coccineae* should best be treated as a new genus and we suggest the name *Rhodopentas* (Appendix 2).

Species from the other four sections of subgenus *Pentas* form a well supported clade (PP 1.00, BS 100%), but only *Papillopilosae* and the monospecific *Hirtistylus* are monophyletic.

Monomorphi, the other section of subgenus *Pentas* that was regarded as close to *Otomeria* (see below), is characterised by having only long-styled flowers. Of the three species, *Pentas herbacea*, the only one not sequenced, is considered most closely related to *Otomeria*. *Pentas micrantha* and *P. angustifolia* are not each other's closest relatives, and consequently the validity of the section and the character defining it is dismissed. The closest species to *P. micrantha* is interestingly, *P. zanzibarica* (PP 1.00, BS 90%), since Tanzanian specimens of *P. micrantha* (subsp. *wylei*) often have been referred to *P. zanzibarica* (Verdcourt, 1953c) and two varieties of *P. zanzibarica* (var. *membranacea* and var. *pembensis*) have been transferred to *P. micrantha* (Verdcourt, 1976a).

Papillopilosae with its two species, *P. glabrescens* and *P. pauciflora*, are restricted to Somalia and form a well supported clade (PP 1.00, BS 96%). The Ethiopian *Pentas caffensis* is the only species in section *Hirtistylus*. This section is mainly characterised by the style, which is covered with white scaly emergences and mostly a few longer hairs (Verdcourt, 1960). Of the eight species of section *Pentas* (Fig. 1I), six are sequenced here. That the remaining two species (*Pentas cleistostoma*, *P. purseglovei*) should not be placed within this group is unlikely considering the supposed close relations between the species (Verdcourt, 1953c). This section is the most widespread in the genus. It occurs from south west Arabia to southern Africa.

To conclude our discussion on *Pentas* subgenus *Pentas*, the section *Coccineae* should be raised to generic level and the remaining sections are what still should be treated as the genus *Pentas* after the removal of the other subgenera.

Otomeria has always been regarded as closely related to *Pentas* and at times the two genera have been suggested to be merged (Scott Elliot, 1896; Schinz, 1923). In his revision of *Otomeria*, Verdcourt (1953a) acknowledged a close relationship and saw more than one link between the two. Of the three subgenera of *Otomeria* he recognised, subgenus *Otomeria*, and especially *O. micrantha*, was thought to be very close to *Pentas* section *Monomorphi*. The latter has, however, a more branched inflorescence and obtriangular fruits as commonly in *Pentas*. Subgenus *Neotomeria* (Fig. 1L) with larger, often bright scarlet flowers compared to the small white flowers of *Otomeria*, was considered related to *Pentas* section *Coccineae*. The third subgenus, *Volubilis*, is distinct from the other two in having a condensed infructescence and a scandent habit. There is also a tendency towards being scandent in *Pentas* section *Coccineae*.

The genus *Tapinopentas* (Bremekamp, 1952) was suggested as a possible new subgenus of *Otomeria* by Verdcourt (1953b), and was subsequently also included in *Otomeria* (*O. cameronica*; Hepper, 1960). It had earlier been distinguished because of its creeping habit and

small inflorescences, but Hepper (1960) argued that there were no valid characters to separate the genus and that *Otomeria guineensis* was extremely close to it.

Despite the similarities between *Otomeria* and *Pentas*, Verdcourt kept the two genera separate because “[i]t is nevertheless convenient and probably more correct” (Verdcourt, 1953a), “the result would be no more satisfactory ... [and] it seems practical to retain the classification” (Verdcourt, 1976a). Verdcourt (1953a) also mentioned a certain resemblance of *Otomeria* to certain species of *Pentanisia*; the latter view actually seems to fit best with our results.

Had Verdcourt been right, we would not have hesitated to merge *Otomeria* with *Pentas* in order to avoid a polyphyletic genus. However, there seems to be no evidence for the idea that parts of *Otomeria* are more related to parts of *Pentas*. Furthermore, our data do not support a monophyletic *Otomeria*. Our representatives from subgenus *Neotomeria* (*Otomeria elatior* [Fig. 1L] and *O. oculata*) are closely related (PP 1.0, BS 68%), but do not group with *Pentas* section *Coccineae* as Verdcourt (1953a) expected. They belong to a well supported clade (PP 0.99, BS < 50%) together with *Otomeria volubilis* and *Otiophora* (PP 1.0, BS 100%). *Otomeria volubilis* is the sole species of subgenus *Volubilis*. Consequently, the subgenera *Neotomeria* and *Volubilis* form either a monophyletic group or a grade to *Otiophora*, but our data provide too little information to resolve this. *Otomeria guineensis* (subgenus *Otomeria*) is strongly supported as sister to *Parapentas setigera* (PP 1.0, BS 96%) and the two are sister to *Batopedina* (PP 1.0, BS 98%). Together the three taxa form a weakly supported (PP 0.70, BS < 50%) sister clade of the above clade of the other *Otomeria* species and *Otiophora*.

That *Parapentas setigera* should not group with the other *Parapentas* species is totally unexpected. As a precaution we have sequenced another specimen (*Sonké & Nguembo 3422*, BR). The obtained sequences are identical. Obviously the morphological similarities between the species of *Parapentas* reflect ecological adaptations rather than phylogeny. The Guineo-Congolian *Parapentas setigera* clearly has an independent origin from the East African taxa.

Batopedina (Fig. 1M) was erected when two species were transferred from *Otomeria* because of their paired axillary flowers at lower nodes (Verdcourt, 1953a). Later a third species was added (Robbrecht, 1981), which mostly has solitary flowers that may be positioned terminally. Thus, the validity of the genus is doubtful and indeed Verdcourt (1989) actually questioned the status of *Batopedina* and suggested that it may have to be combined with *Otomeria*; at least a glabrous variety with spike-like inflorescences, *B. linearifolia* var. *glabra*, was considered a distinct species, which should be transferred. The species sequenced, *Batopedina pulvinellata* (Fig. 1M), is the

third species mentioned above. It was initially thought to be a new species of *Parapentas* but placed in *Batopedina* mainly because of its sub-shrubby habit and similar testa cells (Robbrecht, 1981).

To conclude, neither the present morphological data nor molecular data with our sampling is sufficient to fully understand the phylogeny of *Otomeria*, *Batopedina* and *Parapentas setigera*. Pending more data we refrain from proposing any formal taxonomic changes. Assuming that the unsequenced species of *Otomeria* subgenus *Otomeria* are related to *O. guineensis* (the type species), *Parapentas setigera* could be transferred to *Otomeria*. If *Batopedina* is monophyletic, we would also argue for combining *Batopedina* with *Otomeria* in line with previous suggestions (e.g., Verdcourt, 1989). Our data suggest that *Otomeria* subgenus *Neotomeria* and subgenus *Volubilis* are distinct from *Otomeria*, even in its suggested broader sense including *Parapentas setigera* and *Batopedina*. One or two new genera should be recognised depending on whether the two taxa form a monophyletic sister group to *Otiophora* or a grade up to that genus.

The tribal position of *Otiophora* (Fig. 1N) has been difficult as mirrored by its placement in no less than four different tribes, including one of its own (Table 1). When Verdcourt (1950) revised the genus he placed it in Anthospermeae, but acknowledged affinities with *Otomeria*, *Pentas*, and *Pentanisia*—in retrospect an idea supported by molecular data, but unfortunately not followed in classifications. Puff (1983) argued strongly for the exclusion of *Otiophora* from Anthospermeae. Differences in, for example, chromosome number, inflorescence and flower structure pointed him to Hedyotideae, but since similarities in placentation rather indicated Spermaceae he put forward the possibility of a link between the two tribes. Robbrecht (1988) accepted Puff’s view, but later moved *Otiophora* to Spermaceae, following Igersheim & Rohrhofer (1993), who disregarded Hedyotideae in favour of Spermaceae, mainly based on structural and developmental characters of the gynoecium. The reason for the difficulty to choose either Hedyotideae or Spermaceae is now evident, the genera of Hedyotideae are placed within Spermaceae. Most of the genera showed to be closely related to *Otiophora* by molecular data are, however, former members of Hedyotideae. Treating *Otiophora* as a separate tribe, as suggested by Verdcourt & Bridson (1991), would lead to loss of phylogenetic information and make Knoxiaceae paraphyletic.

The naturalness of the genus has, however, never been questioned, and a monophyletic *Otiophora* is well supported (PP 1.0, BS 100%) by our data. The genus is well characterised by, for example, isostylous flowers, extremely narrow corolla tubes, and locules with a single ovule attached to a shield-like placenta at the base of the septum. Although there is some variation in, for example

habit and inflorescence structure within the genus, no well characterised subgroups are recognised (Verdcourt, 1950). Our molecular data is not informative enough to be used for infrageneric issues. Therefore, it is not possible to tell if the two individuals of *Otiophora pauciflora* are conspecific or not. The Malagasy subspecies (subsp. *pauciflora*) and the African subspecies (subsp. *burtitii*; represented by a specimen from Kenya) are, however, not supported as sister taxa in any of the analyses, and their specific status should be investigated further. Pollen measurements have, for example, indicated a discrepancy between Malagasy and African specimens (Puff, 1981a).

Carphalea (Fig. 1C) has mostly been regarded as a member of Hedyotideae, although Bremekamp (1966) placed it in Cruckshanksieae. Later authors (e.g., Puff, 1988; Robbrecht, 1988, 1994; Bremer & Manen, 2000) have, however, questioned the validity of this tribe. Anderson & Rova (1999) did recognise it excluding *Carphalea*, but still regarded its circumscription as problematic. Puff (1988), even though he did not question the placement in Hedyotideae, regarded the position of *Carphalea* as rather isolated. Except for its presumed sister taxon *Placopoda* (Balfour, 1882; Puff, 1988) no close relatives have been proposed, apart from the fact that Hooker (1873) placed *Carphalea* (i.e., the species then placed in *Dirichletia*; Verdcourt, 1974; Puff, 1988) next to *Otomeria* (only in the sense of enumeration without actually mentioning a closer relationship).

Two sections of *Carphalea* are recognised on basis of calyx shape and distribution; one Malagasy with a usually distinctly lobed calyx (section *Carphalea*: *C. angulata*, *C. cloiselii*, *C. kirondron* [Fig. 1C], *C. linearifolia*, *C. madagascariensis*, *C. pervilleana*) and one with the African mainland and Socotran species with the calyx mostly irregularly lobed and eccentrically elliptic in outline (section *Dirichletia*: *C. glaucescens* from North Eastern and Eastern Africa, *C. somaliensis* from Somalia, *C. obovata* from Socotra, *C. pubescens* from Central Africa; Verdcourt, 1974, Puff 1988). ***Placopoda***, a monotypic genus from Socotra, is regarded as the closest relative of *Carphalea* and does agree with *Carphalea* in a number of characters, the main difference being the calyx which does not enlarge in fruit (Balfour, 1882; Puff, 1988).

Our data do not support *Carphalea* as monophyletic. *Carphalea* section *Carphalea* is paraphyletic with respect to *Triainolepis* (including *Paratriaina*; see below) and section *Dirichletia* is sister to *Placopoda* (PP 1.00, BS 100%). The latter two are in turn possibly sister to the clade consisting of *Phylloentas*, *Longiflorae*, and *Parapentas* (PP 0.85, BS < 50%). The type species of *Carphalea* is *C. madagascariensis* and together with *C. cloiselii* (PP 1.0, BS 100%) it forms the sister group to the remaining species of the section and *Triainolepis* (PP 1.00, BS 91%). *C. angulata* and the closely related *C. kirondron* and *C. pervilleana*

(PP 1.00, BS 100%) form a well supported clade (PP 1.00, BS 100%). Compared to *C. madagascariensis* with four similar, white to pinkish calyx lobes and *C. cloiselii* with an umbrella-like calyx that enlarges after flowering, these three species all have a bright red enlarged calyx mostly with one considerably larger lobe. Our results indicate that a new genus has to be recognised for this clade, but since the ITS data suggest a monophyletic *Carphalea* (section *Carphalea*; PP 0.92, BS 73%) we refrain from doing so in case additional data would further strengthen the hypothesis indicated by the ITS data.

The only Malagasy *Carphalea* species that is not sequenced is *C. linearifolia*. The species has a calyx with one or two enlarged lobes and the other lobes very small. According to Homolle (1937) the calyx is white. Data from this species might be essential to accurately resolve the phylogeny of the Malagasy *Carphalea* species and to evaluate if a new genus should be described. Unfortunately, only two collections of this species are known, the most recent is from 1910. Puff (1988) published comments about a disagreement in collection date and locality of one of the collections, *Perrier de la Bâthie 3891* (P). The sheet we have seen is annotated by Homolle and the date and locality agree with the ones given by Homolle (1937), i.e., the specimen was collected at Mt. Ambatosolo, Sakeny basin (Tsiribihina) in 1910.

All species of *Carphalea* section *Dirichletia* except *C. somaliensis* have previously been included in a separate genus, *Dirichletia*. We have no sequence data of *Carphalea somaliensis*, but the species is according to Puff (1988) doubtlessly closely related with *C. glaucescens*. Consequently, we propose the recognition of *Dirichletia* and make a new combination for *D. somaliensis* (Appendix 2). Apart from having a uniform morphology, *Dirichletia* is further distinguished by pollen data (exines with smaller lumina than in the Malagasy taxa; Puff, 1988). We also suggest *Placopoda* to be included in the re-instated *Dirichletia*, based on morphological similarities (Balfour, 1882; Puff, 1988) and the increased phylogenetic information in the classification resulting from the reduction of a monotypic genus.

The former Triainolepideae. — Bremekamp (1956) recognised twelve species of *Triainolepis*, nine endemic to Madagascar, one extending to the Comoros and Aldabra (*T. fryeri*), and two from the African east coast (*T. africana* and *T. hildebrandtii*). The latter three were merged into *Triainolepis africana* (Fig. 1D) by Verdcourt (1975) who also questioned whether all the Malagasy species actually are distinct species. He, however, described a new species from islands outside Mozambique (Verdcourt, 1989). Field work and herbarium studies have led us to the conclusion that (several of) Bremekamp's species are worthy of recognition and that there might be additional undescribed species in the genus. Our results indicate

that the Malagasy individuals of *Triainolepis africana* are more closely related to each other (PP 0.86, BS 86%) than to the individuals from Kenya and the Comoros. All included individuals represent *Triainolepis africana* subsp. *hildebrandtii*. If the Malagasy individuals of *Triainolepis africana* are to be recognised as a separate species, the name *T. leucophlebia* should be used for this taxon instead of *T. fryeri* (Verdcourt, 1974, 1976a).

The monotypic, Malagasy genera *Paratriaina* and *Thyridocalyx* were regarded as tentative synonyms to *Triainolepis* (Schatz, 2001). He presented no arguments, but Capuron (1973) had already questioned the validity of the genera. He argued that the characters defining *Paratriaina* were merely ecological adaptations common to the area where the species grows. The species was moreover originally regarded as a member of *Triainolepis* (Homolle, in sched.). Bremekamp (1956) distinguished *Paratriaina* on the presence of solitary, six-merous flowers terminating the brachyblasts. We agree with Capuron (1973) that *Paratriaina* should be included in *Triainolepis*, especially since we have seen numerous individuals of several *Triainolepis* species also having six-merous flowers.

Thyridocalyx was as *Paratriaina* initially annotated by Homolle (in sched.) as *Triainolepis* on the type collection. The genus was separated by Bremekamp (1956) mainly because of its two-locular ovaries. That the numbers of locules is of good generic value was questioned by Capuron (1973) considering that the locule number varies between four and ten in *Triainolepis*. Although we have not been able to sequence *Thyridocalyx* (only known from the type collection), we recommend in line with Capuron's reasoning that *Thyridocalyx* should better be included in *Triainolepis*. An enlarged *Triainolepis* will also not become paraphyletic if, as likely, *Thyridocalyx* would show closer affinities to the other taxa from southern Madagascar.

Lathraeocarpeae. — The two species of the monogeneric, Malagasy tribe Lathraeocarpeae are only known from four collections. They were initially included in *Triainolepis* (Homolle, in sched.) but Bremekamp (1957) argued for recognition of them at tribal level. In our view this seems unlikely, and *Lathraeocarpa* is probably related to the Knoxieae and in particular *Triainolepis*. Unfortunately the species have not been recovered although the type localities and possible other localities have been revisited (e.g., S. Razafimandimbison, Bergius Foundation, Stockholm, Sweden and A. Davis, Royal Botanic Gardens, Kew, U.K.; pers. obs.).

Capuron (1973) added *Gomphocalyx* to Lathraeocarpeae, mainly because these genera share a doubled calyx and uniovulate locules. Dessein & al. (2005) showed *Gomphocalyx* to belong to Spermaceae in an analysis of *rps16* sequences, i.e., if Capuron was right when placing *Gomphocalyx* and *Lathraeocarpa* together

Lathraeocarpa too would belong to Spermaceae and not to Knoxieae. Piesschaert (2001) proposed a fusion of the two genera, but such a treatment was dismissed by Dessein & al. (2005). There are, however, a number of morphological characters pointing to affinities with *Triainolepis* (e.g., *Lathraeocarpa* has a four-locular ovary, fleshy fruits similar to the ones in *Triainolepis*, and a shrubby habit compared to the weedy *Gomphocalyx* with two-locular ovaries and dry fruits) and pending molecular data *Lathraeocarpa* should most probably be regarded as a member of Knoxieae and close to *Triainolepis*.

Thecorchus. — *Thecorchus wauensis*, the sole species of a genus distributed from Senegal to Ethiopia, was suggested to be close to *Otomeria* by Bremekamp (1952) and suggested as a member of Knoxieae on morphological grounds by Dessein (2003), albeit with hesitation. Our results clearly place the genus outside Knoxieae and it is best treated as a member of Spermaceae close to *Oldenlandia*.

Taxonomic conclusions. — In Tables 2 and 3 we summarise how our circumscription of the Knoxieae genera compare to previous treatments. All species we regard as members of the Knoxieae are included. The formal taxonomic changes are made in Appendix 2.

In summary, we suggest that the paraphyletic *Pentania* should be expanded to include also *Calanda*, *Chlorochorion*, *Neopentania*, and *Paraknoxia*. *Pentas* subgenus *Megapentas* together with subgenus *Chamaepentadoides* should be merged with *Chamaepentas*. The two *Pentas* subgenera *Phyllopentas* and *Vignaldiopsis* are regarded as a new genus *Phyllopentas*. *Pentas* subgenus *Longiflorae* is also considered a new genus, *Dolichopentas*, as is *Pentas* subgenus *Pentas* section *Coccineae*, *Rhodopentas*. Excluding *Megapentas*, *Chamaepentadoides*, and the new genera *Phyllopentas*, *Dolichopentas*, and *Rhodopentas* from *Pentas* has resulted in a smaller *Pentas* with only 14 species compared to the ca. 40 that used to be treated under the genus name.

Otomeria is not monophyletic; on one hand we suggest to include *Batopedina* and *Parapentas setigera* and on the other hand the subgenera *Neotomeria* and *Volubilis* should perhaps be better removed and erected to one or two separate genera. *Carphalea* is also not monophyletic. The African species should be treated as *Dirichletia*, with which *Placopoda* is merged. For the Malagasy species a new genus might have to be erected for *Carphalea angulata*, *C. kirondron*, and *C. pervilleana*, but pending additional data we refrain from doing so. The genera *Paratriaina* and *Thyridocalyx* are sunk into *Triainolepis*.

Description of the tribe. — **Knoxieae** Hook. f., in Benth. & Hook. f., Gen. Pl. 2: 9, 21. 1873 – emend. Type: *Knoxia* L.

Triainolepideae Bremek., Proc. Kon. Ned. Akad. Wetensch. C 59: 3. 1956.

Lathraeocarpeae Bremek., Bull. Jard. Bot. État Bruxelles 27: 165. 1957, tentatively included.

Herbs, subshrubs, shrubs or small trees. *Leaves* decussate, sometimes whorled, pseudo-verticillate, rarely with a basal rosette (*Knoxia plantaginea*, *K. rosettifolia*, *Pentanisia annua*), or solitary (common in *Batopedina pulvinellata*). *Stipules* fimbriate or rarely triangular (*Pentanisia rubricaulis*), colleter-tipped. *Raphides* present. *Inflorescences* terminal (congested to spicate), (pseudo-) axillary, or solitary (*Lathraeocarpa*, *Triainolepis xerophila*, common in *Parapentas*); in *Pentanisia rubricaulis* calyx and ovary of a flower pair fused. *Flowers* (3–)4–5(–6)-merous, mostly heterostylous. *Calyx* mostly unequal, 1 or more lobes enlarged, often foliaceous; in *Lathraeocarpa* the calyx has twice the number of the corolla lobes (8). *Corolla* narrowly cylindrical, cylindrical to funnel-shaped with valvate aestivation. *Ovary* 2–10-locular, each locule with one pendulous ovule (*Knoxia*, *Pentanisia*), one erect ovule basally attached to the septum (*Otiophora*), (3–)4–7 ovules on a slender basal placenta (*Carphalea*, *Dirichletia*), 2(–3) collateral ovules inserted at the base (*Triainolepis*; solitary in *Lathraeocarpa*), or with numerous ovules attached around the middle of the septum (*Batopedina*, *Dolichopentas*, *Chamaepentas*, *Otomeria*, *Parapentas*, *Pentas*, *Phyllopentas*, *Rhodopentas*) or basally attached to the septum (*Chamaepentas greenwayii*). *Pollen* 3–4(–5)-colporate (*Lathraeocarpa* 7–10-colporate). *Fruits* dry, dehiscent or indehiscent, or sometimes drupaceous (*Pentanisia* p.p., *Lathraeocarpa*, *Triainolepis*). *Seeds* 1–2–many per locule. *Basic chromosome number* $x = 10$ (Lewis, 1965; Shivakumar & Chennaveeraiah, 1984; Kiehn, 1985; Philip & Mathew, 1987; Puff & Robbrecht, 1989) or 17 (*Otiophora*; Puff, 1981a, b) with $2x$ or $4x$ (*Otomeria cameronica*, *Parapentas setigera*, predominately in *Pentanisia*) ploidy level.

Included genera and number of species: *Batopedina* (3), *Carphalea* (6), *Chamaepentas* (6), *Dirichletia* (5), *Dolichopentas* (4), *Knoxia* (ca. 11), *Lathraeocarpa* (2; tentatively included), *Otiophora* (17), *Otomeria* (ca. 9), *Parapentas* (2), *Pentanisia* (including *Calanda*, *Chlorochorion*, *Neopentania*, *Paraknoxia*; 20), *Pentas* (14), *Phyllopentas* (ca. 15), *Rhodopentas* (2), *Triainolepis* (including *Paratriaina*, *Thyridocalyx*; ca. 13).

Distribution: Africa, Madagascar, south west Arabia (*Pentas lanceolata*), Indomalesia.

KEY TO GENERA

For the most part, the key is adapted from Verdcourt (1953c, 1976a). Ideas from other workers including Puff & Robbrecht (1989) and Charlotte Taylor (Missouri Botanical Garden, St. Louis, U.S.A.; pers. comm.) are also incorporated.

1. Ovule solitary in each locule 2
1. Ovules two or more in each locule 4
2. Ovules attached towards the base of the septum, erect; corolla tube generally narrowly cylindrical (filament-like) *Otiophora*
2. Ovules pendulous, apically attached; corolla tubes generally cylindrical, slender, expanded above (in long-styled flowers) 3
3. Fruits with true carpophores (formed from vascular strands in the septum of the ovary) *Knoxia*
3. Fruits lacking true carpophores or with various kinds of false carpophores (modifications of the uppermost part of the pedicel) *Pentanisia*
4. Ovules two (rarely three) in each locule, collateral, erect; ovary 2–10-locular; drupe. *Triainolepis*
4. Ovules numerous in each locule; if two or three ovules then on stalked placenta with the stalk attached towards the base of the septum, ovary 2–3(–4)-locular and fruit not drupaceous. 5
5. Ovules few (< 7) on stalked placenta with the stalk attached towards the base of the septum, only one maturing per locule; ovary 2–3(–4)-locular; fruits dry and indehiscent 6
5. Ovules many, not on stalked placenta, ovary 2-locular, fruits many-seeded capsules. 8
6. Calyx not enlarged in fruit; Socotran species *Dirichletia virgata*
6. Calyx enlarged 7
7. Calyx distinctly lobed, lobes equal or unequal, white to bright red; Malagasy species *Carphalea*
7. Calyx eccentrically elliptic, not distinctly lobed, whitish-green, white, pinkish or pale mauve; African mainland (incl. Socotra) species. *Dirichletia*
8. Ovules many, attached towards the base of the septum; creeping herb, calyx lobes spatulate at their apices; endemic to S. Pare Mts., Tanzania *Chamaepentas greenwayii*
8. Ovules many, placenta attached around the middle of the septum. 9
9. Creeping or decumbent forest floor herbs 10
9. Erect herbs, creeping perennial herbs or subshrubs growing on rock, (sub)shrubs or scandent plants 11
10. Creeping perennial herbs; inflorescences few-flowered; flowers sessile in the leaf axils or terminal *Parapentas* s.l.^a
10. Decumbent perennial herb; inflorescences terminal, laxly arranged when mature; flowers not sessile; endemic to Uluguru Mts., Tanzania. *Phyllopentas ulugurica*
11. Scandent plant; inflorescence capitate in fruit; corolla scarlet, corolla tube > 20 mm long *Otomeria volubilis* (*Otomeria* subgen. *Volubilis*)^b
11. Erect or creeping herbs or (sub)shrubs 12

- 12. Flowers solitary or paired, axillary or at apices of branches; creeping perennial herbs or small shrubs with small leaves sometimes ± pseudoverticillate *Batopedina*^c
- 12. Flowers in more complicated inflorescences 13
- 13. Flowering inflorescences capitate, later elongating into a long simple “spike”, rarely with axillary “spikes” from the upper axils, and frequently with solitary flowers at the lower nodes; fruits oblong (ovoid in *Otomeria cameronica*) 14
- 13. Flowering inflorescences capitate or lax much-branched complicated cymes, not elongating into simple “spikes” in fruit, although individual branches sometimes become spicate; fruits (sub)globose or obtriangular, less often ovoid-oblong 15
- 14. Flowers white or yellowish; corolla tube < 18 mm long *Otomeria* subgen. *Otomeria*
- 14. Flowers red or pink; corolla tube > 15 mm long *Otomeria* subgen. *Neotomeria*^b
- 15. Corolla tube 2–2.5 mm long; small herb; Malagasy species *Phyllopentas decaryana*
- 15. Corolla tube at least 4 mm long 16
- 16. Calyx with one or two lobes enlarged into a white to purple stipitate lamina in the majority of flowers (in *Phyllopentas schumanniana* rarely none or very few of the calyces have a calyx lobe developed into a lamina) *Phyllopentas*
- 16. Calyx lobes often foliaceous but then green and never enlarged into a stipitate lamina 17
- 17. Calyx lobes flat, foliaceous or deltoid, nearly always 1–3 enlarged and the rest much smaller 18
- 17. Calyx lobes subulate, narrowly spatulate or linear, subequal 20
- 18. Enlarged calyx-lobes distinctly spatulate, narrowed at the base; corolla tube 3.5–4.5 cm long; inflorescences composed of a few 3-flowered cymes *Chamaepentas hindsioides*
- 18. Enlarged calyx lobes not or scarcely spatulate; corolla tube 0.4–9 cm long, but mostly short; inflorescences many flowered 19
- 19. Flowers bright vermilion-scarlet; leaves with a very fine characteristic indumentum beneath; stem indumentum mostly ferruginous when dry; capsule ovoid-oblong, a little contracted above *Rhodopentas*
- 19. Flowers white, mauve, blue or pink, only rarely red and then of a deeper crimson shade; leaf indumentum coarser; capsule obtriangular *Pentas*
- 20. Leaves in whorls of 3–5 or if paired then plant a short pyrophyte ± 16 cm tall *Dolichopentas*
- 20. Leaves paired; plant never a short pyrophyte 21
- 21. Corolla tube 0.4–2.8 cm long; in the largest-flowered species the leaves being large and oblong *Phyllopentas*
- 21. Corolla tube 2.5–16 cm long; in the smallest-flowered

- species the leaves being linear-lanceolate 22
- 22. Stipular setae noticeably capitellate; calyx lobes mostly over 1 cm long; if shorter then corolla lobes 1.5–2 cm long and 0.5–1 cm wide or if corolla lobes up to 1.3 cm long and 2.5 mm wide then petioles up to 1 cm long, corolla tube 3.75 cm long, gradually widening from base to throat, inflorescence ± 15-flowered, lax (*Chamaepentas hindsioides*) *Chamaepentas*
- 22. Stipular setae obscurely capitellate; calyx lobes under 1 cm long; corolla lobes up to 1.3 cm long and 2.5 mm wide; corolla tube abruptly expended just below the throat; inflorescence often many-flowered *Dolichopentas*

^aIn the key *Parapentas setigera* is included in the genus *Parapentas* although it is evident from our results that the species should be transferred elsewhere. *Parapentas setigera* is distinguished from *P. silvatica* in being heterostylous and from *P. battiscombei* by its shorter corolla tube (< 9 mm; 14–23 mm in *P. battiscombei*).

^bProbably to be recognised as a separate genus; see Discussion.

^cProbably to be included in *Otomeria*.

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Appendix 1. Species names and accession numbers in the EMBL/Genbank archives for the sequences used in the molecular analyses. For the previously unpublished sequences the voucher specimens are listed.

Species, voucher specimen, accession no.: *rps16*, *trnT-F*, ITS

Knoxieae: *Batopedina pulvinellata* E. Robbrecht, *Malaisse* 7695 (UPS), AM266813, AM266902, AM266989; *Calanda rubricaulis* K. Schum, *Kers* 3414 (S), AM266814, AM266903, AM266990; *Carphalea angulata* Baill., *Gautier* 4455 (TEF), AM266815, AM266904, AM266991; *Carphalea cloiselii* Homolle, *ATH* 434 (TAN), AM266816, AM266905, AM266992; *Carphalea glaucescens* (Hiern) Verdcourt, *SMP* 215, 1988 (UPS), AM266817, AM266906, AM266993; *Carphalea kirondron* Baill., *Thulin & al.* 10390 (UPS), AM266818, AM266907, AM266994; *Carphalea madagascariensis* Lam, *Razafimandimbison* 524 (UPS), AM266819, AM266908, AM266995; *Carphalea obovata* (Balf. f.) Verdcourt, *Miller & al.* 8269 (UPS), AM266820, AM266909, AM266996; *Carphalea pervilleana* Baill., *Razafimandimbison* 544 (UPS), AM266821, AM266910, AM266997; *Carphalea pubescens* (Klotzsch) Verdcourt, *van Wyk BSA* 47 (PRE), AM266822, AM266911, AM266998; *Chlorochorion foetidum* (Verdcourt) C. Puff & E. Robbrecht, *De Block & Steperaere* 503 (BR), AM266823, AM266912, AM266999; *Chlorochorion monticola* (K. Krause) C. Puff & E. Robbrecht, *Stolz* 1286 (S), AM266824, AM266913, AM267000; *Knoxia manika* (Verdcourt) C. Puff & E. Robbrecht, *Schajjes* 3339 (BR), AM266825, AM266914, AM267001; *Knoxia platycarpa* Arn., *Lundqvist* 11302 (UPS), AM266826, AM266915, AM267002; *Knoxia sumatrensis* (Retz.) DC., *Klackenberg & Lundin* 268 (S), AM266827, AM266916, AM267003; *Neopentania gossweileri* Verdcourt, *Dessein & al.* 1037 (BR), AM266828, AM266917, AM267004; *Otiophora angustifolia* Verdcourt, *Dessein & al.* 607 (BR), AM266829, AM266918, AM267005; *Otiophora caerulea* (Hiern) Bullock, *Dessein & al.* 367 (BR), AM266830, AM266919, AM267006; *Otiophora calycophylla* (Sond.) Schlechter & K. Schum., *Bremer & al.* 4339 (UPS), AM266831, AM266920, AM267007; *Otiophora cupheoides* N.E. Br., *Bremer* 3805 (UPS), AM266832, AM266921, AM267008; *Otiophora lebruniana* (Bamps) E. Robbrecht & C. Puff, *Reekmanns* 10118 (UPS), AM266833, AM266922, AM267009; *Otiophora multicaulis* Verdcourt, *Dessein & al.* 405 (BR), AM266834, AM266923, AM267010; *Otiophora parviflora* Verdcourt, *Cholocholo, Nurmi & Steiner* 16 (UPS), AM266835, AM266924, AM267011; *Otiophora pauciflora* Baker subsp. *burtii* (Milne-Redh.) Verdcourt, *Luke* 9096 (UPS), AM266836, AM266925, AM267012; *Otiophora pauciflora* Baker subsp. *pauciflora*, *Thulin & al.* 10248 (UPS), AM266837, AM266926, AM267013; *Otiophora pycnostachys* K. Schum, *Harder & al.* 2755 (PRE), AM266838, AM266927, AM267014; *Otiophora scabra* Zucc., *Iversen & Martinson* 89078 (UPS), AM266839, AM266928, AM267015; *Otiophora stolzii* (Verdcourt) Verdcourt, *Philips* 900 (UPS), AM266840, –, –, *Otiophora villicaulis* Mildbr., *Dessein & al.* 393 (BR), AM266841, AM266929, AM267016; *Otomeria elatior* (A. Rich. ex DC.) Verdcourt, *Iwarson & Ryding* 883 (UPS), AM266842, AM266930, AM267017; *Otomeria guineensis* Benth., *Leeuwenberg* 5643 (PRE), AM266843, AM266931, AM267018; *Otomeria oculata* S. Moore, *Puff & Kelbessa* 821222-2/1 (K), AM266844, AM266932, AM267019; *Otomeria volubilis* (K. Schum.) Verdcourt, *de Wilde* 8275 (PRE), AM266845, AM266933, –, *Paraknoxia parviflora* (Verdcourt) Bremek., *Polhill & Paulo* 1986 (PRE), AM266846, –, –, *Paraknoxia parviflora* (Verdcourt) Bremek., *Verdcourt* 2454 (S), –, AM266934, AM267020; *Parapentas battiscombei* Verdcourt, *Luke* 9431 (UPS), AM266847, AM266935, AM267021; *Parapentas setigera* (Hiern) Verdcourt, *Leeuwenberg* 3722 (L), AM266848, AM266936, AM267022; *Parapentas silvatica* (K. Schum.) Bremek., *Bremer* 3091 (UPS), AM266849, AM266937, AM267023; *Paratriaina xerophila* Bremek., *Razafimandimbison & Bremer* 489 (UPS), AM266850, AM266938, AM267024; *Pentania angustifolia* (Hochst.) Hochst., *Bremer & al.* 4276 (UPS), AM266851, AM266939, AM267025; *Pentania arenaria* (Hiern) Verdcourt, *Biggood & al.* 3373 (BR), AM266852, AM266940, AM267026; *Pentania calcicola* Verdcourt, *Thulin & al.* 7280 (UPS), AM266853, AM266941, –, *Pentania confertifolia* (Baker) Verdcourt, *Dessein & al.* 730 (BR), AM266854, AM266942, AM267027; *Pentania longepedunculata* Verdcourt, *Thulin & al.* 7350 (UPS), AM266855, AM266943, AM267028; *Pentania longituba* Oliver, *Thulin & al.* 9057 (UPS), AM266856, AM266944, AM267029; *Pentania microphylla* (Franch.) Chiov., *Thulin & al.* 9206 (UPS), AM266857, AM266945, AM267030; *Pentania ouranogyne* S. Moore, *Thulin & al.* 10980, *Somalia* (UPS), AM266858, AM266946, AM267031; *Pentania ouranogyne* S. Moore, *Andreasen* 310, *Kenya* (UPS), AM266859, AM266947, AM267032; *Pentania prunelloides* (Klotzsch ex Eckl. & Zeyh.) Walp., *Bremer & al.* 4275 (UPS), AM266860, AM266948, AM267033; *Pentania schweinfurthii* Hiern, *Iwarson & Ryding* 926 (UPS), AM266861, AM266949, AM267034; *Pentania sykesii*, *Bremer & al.* 4338 (UPS), AM266862, AM266950, AM267035; *Pentania angustifolia* (A. Rich. ex DC.) Verdcourt, *Bayliss* 10414 (PRE), AM266863, AM266951, AM267036; *Pentania arvensis* Hiern, *Leeuwenberg* 7639 (BR), AM266864, AM266952, AM267037; *Pent-*

Appendix 1. Continued.

cf. *austrororientalis* Homolle ex Verdcourt, *Rakoto 283* (UPS), AM266865, AM266953, AM267038; *Pentas bussei* Krause, *Luke 8319* (UPS), AM266866, AM266954, AM267039; *Pentas caffensis* Chiov., *Puff & Kelbessa 820811–2/2* (UPS), AM266867, AM266955, AM267040; *Pentas concinna* K. Schum., *Burger 3153* (S), AM266868, AM266956, AM267041; *Pentas decora* S. Moore, *Thulin & Tidigs 183* (UPS), AM266869, AM266957, AM267042; *Pentas elata* K. Schum., *Simon & al. 486* (BR), AM266870, AM266958, AM267043; *Pentas glabrescens* Baker, *Thulin & Warfa 6194* (UPS), AM266871, AM266959, AM267044; *Pentas hindsioides* K. Schum., *Iversen, Pocs & Temu 85101* (UPS), AM266872, AM266960, –; *Pentas ionolaena* K. Schum. subsp. *ionolaena*, *Borhidi, Hall, Hedberg & Mshoo 82250* (UPS), AM266873, AM266961, AM267045; *Pentas ionolaena* K. Schum. subsp. *madagascariensis* Verdcourt, *Kårehed, Razafimandimbison & Bremer 205* (UPS), AM266874, AM266962, AM267046; *Pentas lanceolata* (Forsk.) Defl., *Bremer 2702* (S), AM266875, AM266963, AM267047; *Pentas lindenoides* (S. Moore) Verdcourt, *Gereau & al. 3498* (PRE), AM266876, –; AM267048; *Pentas longiflora* Oliver, *Luke 8834* (UPS), AM266877, AM266964, AM267049; *Pentas longituba* K. Schum. ex Engl., *Bremer 3094* (UPS), AM266878, AM266965, AM267050; *Pentas micrantha* Baker, *Luke 9038* (UPS), AM266879, AM266966, AM267051; *Pentas mussaendoides* Baker, *Razafimandimbison 564A* (UPS), AM266880, AM266967, AM267052; *Pentas nobilis* S. Moore, *Richards 19015* (S), AM266881, AM266968, AM267053; *Pentas parvifolia* Hiern, *Luke 8340* (UPS), AM266882, AM266969, AM267054; *Pentas pauciflora* Baker, *Thulin 10898* (UPS), AM266883, AM266970, AM267055; *Pentas pseudomagnifica* M.R.F. Taylor, *Borhidi, Hall & Hedberg 82270* (UPS), AM266884, –; *Pentas pubiflora* S. Moore, *Luke 8917* (UPS), AM266885, AM266971, AM267056; *Pentas purpurea* Oliver, *Huntley & al. 78* (PRE), AM266886, AM266972, AM267057; *Pentas schimperiana* Vatke, *Luke 8918* (UPS), AM266887, AM266973, AM267058; *Pentas schumanniana* Krause, *Gereau & Kayombo 4127* (BR), AM266888, AM266974, AM267059; *Pentas suswaensis* Verdcourt, *Verdcourt & Glover 3983* (PRE), AM266889, AM266975, –; *Pentas tenuis* Verdcourt, *Gilbert & Thulin 746* (UPS), AM266890, AM266976, AM267060; *Pentas ulugurica* (Verdcourt) Hepper, *Lovett & Kayombo 415* (BR), AM266891, AM266977, AM267061; *Pentas zanzibarica* Vatke, *Luke 8320* (UPS), AM266892, AM266978, AM267062; *Pentas* sp. “*flava*”, *ATH 211* (TAN), AM266893, AM266979, AM267063; *Placopoda virgata* Balf. f., *Thulin & Gifri 8528* (UPS), AM266894, AM266980, AM267064; *Triainolepis africana* Hook. f., *Kenya, AF129276*^a, –; *Triainolepis africana* Hook. f., *Kårehed, Razafimandimbison & Bremer 235, Madagascar* (UPS), AM266896, AM266982, AM267066; *Triainolepis africana* Hook. f., *Razafimandimbison 443, Madagascar* (UPS), AM266897, AM266983, AM267067; *Triainolepis africana* Hook. f., *Barthelat & Sifari 235, Comoros* (P), AM266898, AM266984, –; *Triainolepis arcuata* (Dubard & Dop) Bremek., *Razafimandimbison 552* (UPS), AM266895, AM266981, AM267065; *Triainolepis mandrarenensis* Homolle ex Bremek., *Razafimandimbison 521* (UPS), AM266899, AM266985, AM267068; *Triainolepis tomentella* Bremek., *Razafimandimbison 538* (UPS), AM266900, AM266986, AM267069; *Thecorchus wauensis* (Hiern) Bremek., *Friis & al. 2560* (C), AM266901, AM266987, AM267070.

Outgroup: *Coussarea* sp., AF004041^b, AF152612^d, –; *Ophiorrhiza mungos* L., AF004064^b, AF152610^d, –; *Pauridiantha* sp., AF004068^b, AF102467^c, –; *Psychotria pittieri* Standl., AF002746^b, AF152614^d, AF071998^e; *Morinda citrifolia* L., AF001442^b, AF152616^d, AF333844^b; *Schradera* sp., AF003617^b, AF152613^d, –; *Nertera granadensis* Druce, AF002741^b, AF152623^d, AF257928ⁱ (*Nertera holmboei*); *Myrcetia malayana* Craib, AF002771^b, AF152622^d, –; *Paederia foetida* L., AF004065^b, AF152619^d, –; *Rubia fruticosa* Ait., AF004078^b, AF102475^c, –; *Theligonum cynocrambe* L., AF004087^b, AF152621^d, –; *Conostomium quadrangulare* (Rendle) Cufod., *Tweedie 2477* (S), AM266812, AM266988, –; *Dentella repens* J.R. Forst. & G. Forst., AF333370^e, AF381540^f, –; *Oldenlandia corymbosa* L., AF333381^e, AF381543^f, AF381484^f; *Arcytophyllum setosum* (Ruiz & Pav.) Standl., AF002755^b, AF333365^e, –; *Hedyotis nigricans* (Lam.) Fosberg, AF333373^e, AF333374^e, AF381461^f + AF381494^f; *Houstonia caerulea* L., AF333379^e, AF381524^f, AF381458^f + AF381491^f.

^aPiesschaert & al., 2000; ^bAndersson & Rova, 1999; ^cAndersson & al., 2002; ^dRova & al., 2002; ^eStruwe & al., 1998; ^fChurch, 2003; ^gNepokroeff & al., 1999; ^hMalcomber, 2002; ⁱAnderson & al., 2001.

Appendix 2. Nomenclatural changes as suggested in the text.

Chamaepentas graniticola (E.A. Bruce) Kårehed & B. Bremer, **comb. nov.:** *Pentas graniticola* E.A. Bruce in Kew Bull. 1933: 146. 1933.

Chamaepentas hindsioides (K. Schum.) Kårehed & B. Bremer, **comb. nov.:** *Pentas hindsioides* K. Schum. in Bot. Jahrb. Syst. 34: 330. 1904.

Chamaepentas hindsioides var. *glabrescens* (Verdc.) Kårehed & B. Bremer, **comb. nov.:** *Pentas hindsioides* K. Schum. var. *glabrescens* Verdc. in Bull. Jard. Bot. État Bruxelles 23: 281. 1953.

Chamaepentas hindsioides var. *parensis* (Verdc.) Kårehed & B. Bremer, **comb. nov.:** *Pentas hindsioides* K. Schum. var. *parensis* Verdc. in Bull. Jard. Bot. État Bruxelles 23: 281. 1953.

Chamaepentas hindsioides var. *williamsii* (Verdc.) Kårehed & B. Bremer, **comb. nov.:** *Pentas hindsioides* K. Schum. var. *williamsii* Verdc. in Bull. Jard. Bot. État Bruxelles 23: 279. 1953.

Chamaepentas longituba (K. Schum. ex Engl.) Kårehed & B. Bremer, **comb. nov.:** *Pentas longituba* K. Schum. ex Engl. in Abh. Königl. Akad. Wiss. Berlin 1894: 57. 1894.

Chamaepentas nobilis (S. Moore) Kårehed & B. Bremer, **comb. nov.:** *Pentas nobilis* S. Moore in J. Bot. 46: 37. 1908.

Chamaepentas pseudomagnifica (M. Taylor) Kårehed & B. Bremer, **comb. nov.:** *Pentas pseudomagnifica* M. Taylor in Kew Bull. 1940: 56. 1940.

Dirichletia somaliensis (Puff) Kårehed & B. Bremer, **comb. nov.:** *Carphalea somaliensis* Puff in Bull. Jard. Bot. Belg. 58: 297. 1988.

Dirichletia virgata (Balf. f.) Kårehed & B. Bremer, **comb. nov.:** *Placopoda virgata* Balf. f. in Proc. Roy. Soc. Edinburgh 11: 836. 1882.

Dolichopentas Kårehed & B. Bremer, **nom. nov.:** *Pentas* subgen. *Longiflorae* Verdc. in Bull. Jard. Bot. État Bruxelles 23: 281. 1953 (*Longiflora*) (Type: *D. longifolia*).

Dolichopentas decora (S. Moore) Kårehed & B. Bremer,

Appendix 2. Continued.

- comb. nov.:** *Pentas decora* S. Moore in J. Bot. 48: 219. 1910 (*'decorus'*).
- Dolichopentas decora* var. *lasiocarpa* (Verdc.) Kårehed & B. Bremer, **comb. nov.:** *Pentas decora* S. Moore var. *lasiocarpa* Verdc. in Bull. Jard. Bot. État Bruxelles 23: 293. 1953.
- Dolichopentas decora* var. *pubescens* (S. Moore) Kårehed & B. Bremer, **comb. nov.:** *Pentas verticillata* var. *pubescens* S. Moore in J. Bot. 48: 220. 1910 = *Pentas triangularis* De Wild. in Feddes Repert. 13: 139. 1914 = *Pentas decora* var. *triangularis* (De Wild.) Verdc. in Bull. Jard. Bot. État Bruxelles 23: 291. 1953 = *Pentas globifera* Hutch. in Kew Bull. 1921: 374. 1921.
- Dolichopentas liebrechtsiana* (De Wild.) Kårehed & B. Bremer, **comb. nov.:** *Pentas liebrechtsiana* De Wild. in Ann. Mus. Congo Belge, Bot., sér. 4, 1: 153. 1902.
- Dolichopentas lindenoides* (S. Moore) Kårehed & B. Bremer, **comb. nov.:** *Heinsia lindenoides* S. Moore in J. Linn. Soc., Bot. 37: 301. 1906 = *Pentas lindenoides* (S. Moore) Verdc., Kew Bull. 30: 344. 1975 = *Pentas geophila* Verdc. in Bull. Jard. Bot. État Bruxelles 23: 293. 1953.
- Dolichopentas longiflora* (Oliv.) Kårehed & B. Bremer, **comb. nov.:** *Pentas longiflora* Oliv. in Trans. Linn. Soc. London, Bot. 2: 335. 1887.
- Pentanisia gossweileri* (Verdc.) Kårehed & B. Bremer, **comb. nov.:** *Neopentanisia gossweileri* Verdc. in Kew Bull. 8: 114. 1953.
- Pentanisia rubricaulis* (K. Schum.) Kårehed & B. Bremer, **comb. nov.:** *Calanda rubricaulis* K. Schum. in Warb., Kunene-Sambesi Exped.: 386. 1903.
- Phyllopentas* (Verdc.) Kårehed & B. Bremer, **stat. nov.:** *Pentas* subgen. *Phyllopentas* Verdc. in Bull. Jard. Bot. État Bruxelles 23: 254. 1953 (Type: *P. mussaendoides* – indicated by Verdcourt with the letter “G” for “genotype”, the term he used (cf. Verdcourt, 1953c: 238) for the type of the name of a genus) = *Pentas* subgen. *Vignaldiopsis* Verdc. in Bull. Jard. Bot. État Bruxelles 23: 261. 1953 (Type: *P. schimperiana*).
- Phyllopentas austroorientalis* (Homolle & Verdc.) Kårehed & B. Bremer, **comb. nov.:** *Pentas austroorientalis* Homolle & Verdc. in Bull. Jard. Bot. État Bruxelles 23: 261. 1953 (Verdcourt, l.c., ascribed the name to “Homolle & Verdc.” and under Art. 46.2, final sentence, this must be accepted).
- Phyllopentas concinna* (K. Schum.) Kårehed & B. Bremer, **comb. nov.:** *Pentas concinna* K. Schum. in Bot. Jahrb. Syst. 33: 335. 1903.
- Phyllopentas decaryana* (Homolle ex Verdc.) Kårehed & B. Bremer, **comb. nov.:** *Pentas decaryana* Homolle ex Verdc. in Bull. Jard. Bot. État Bruxelles 23: 353. 1953.
- Phyllopentas elata* (K. Schum.) Kårehed & B. Bremer, **comb. nov.:** *Pentas elata* K. Schum. in Engl., Pflanzenw. Ost-Afr. C.: 377. 1895.
- Phyllopentas hirtiflora* (Baker) Kårehed & B. Bremer, **comb. nov.:** *Pentas hirtiflora* Baker in J. Linn. Soc., Bot. 22: 482. 1887.
- Phyllopentas ionolaena* (K. Schum.) Kårehed & B. Bremer, **comb. nov.:** *Pentas ionolaena* K. Schum. in Bot. Jahrb. Syst. 28: 487. 1900.
- Phyllopentas ledermannii* (K. Krause) Kårehed & B. Bremer, **comb. nov.:** *Pentas ledermannii* K. Krause in Bot. Jahrb. Syst. 57: 25. 1920.
- Phyllopentas madagascariensis* (Verdc.) Kårehed & B. Bremer, **comb. et stat. nov.:** *Pentas ionolaena* K. Schum. subsp. *madagascariensis* Verdc. in Bull. Jard. Bot. État Bruxelles 23: 257. 1953.
- Phyllopentas mussaendoides* (Baker) Kårehed & B. Bremer, **comb. nov.:** *Pentas mussaendoides* Baker in J. Linn. Soc., Bot. 20: 165. 1883.
- Phyllopentas schimperiana* (Vatke) Kårehed & B. Bremer, **comb. nov.:** *Pentas schimperiana* Vatke in Linnæa 40: 192. 1876.
- Phyllopentas schumanniana* (K. Krause) Kårehed & B. Bremer, **comb. nov.:** *Pentas schumanniana* K. Krause in Bot. Jahrb. Syst. 39: 521. 1907.
- Phyllopentas tenuis* (Verdc.) Kårehed & B. Bremer, **comb. nov.:** *Pentas tenuis* Verdc. in Kirkia 5: 273. 1966.
- Phyllopentas ulugurica* (Verdc.) Kårehed & B. Bremer, **comb. nov.:** *Tapinopentas ulugurica* Verdc. in Bull. Jard. Bot. État Bruxelles 23: 61. 1953 = *Pentas ulugurica* (Verdc.) Hepper in Kew Bull. 14: 254. 1960.
- Rhodopentas* Kårehed & B. Bremer, **nom. nov.:** *Pentas* subgen. *Pentas* sect. *Coccineae* Verdc. in Bull. Jard. Bot. État Bruxelles 23: 296. 1953 (Type: *R. parvifolia*).
- Rhodopentas bussei* (K. Krause) Kårehed & B. Bremer, **comb. nov.:** *Pentas bussei* K. Krause in Bot. Jahrb. Syst. 43: 134. 1909.
- Rhodopentas parvifolia* (Hiern) Kårehed & B. Bremer, **comb. nov.:** *Pentas parvifolia* Hiern in J. Linn. Soc., Bot. 16: 262. 1877.
- Triainolepis ampandrandavae* (Bremek.) Kårehed & B. Bremer, **comb. nov.:** *Thyridocalyx ampandrandavae* Bremek., Proc. Kon. Ned. Akad. Wetensch. C 59: 20. 1956.
- Triainolepis xerophila* (Bremek.) Kårehed & B. Bremer, **comb. nov.:** *Paratriaina xerophila* Bremek. in Proc. Kon. Ned. Akad. Wetensch. C 59: 18. 1956.
- Triainolepis xerophila* var. *glabrescens* (Bremek.) Kårehed & B. Bremer, **comb. nov.:** *Paratriaina xerophila* Bremek. var. *glabrescens* Bremek. in Proc. Kon. Ned. Akad. Wetensch. C 59: 19. 1956.
- Triainolepis xerophila* var. *pubescens* (Bremek.) Kårehed & B. Bremer, **comb. nov.:** *Paratriaina xerophila* Bremek. var. *pubescens* Bremek. in Proc. Kon. Ned. Akad. Wetensch. C 59: 20. 1956.