Molecular phylogenetic and morphological study of *Kohautia* (Spermacoceae, Rubiaceae), with the recognition of the new genus *Cordylostigma*

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Abstract *Kohautia* Cham. & Schltdl. belongs to the predominantly herbaceous Rubiaceae tribe Spermacoceae. Species of *Kohautia* can easily be distinguished from other Spermacoceae by their monomorphic short-styled flowers in which anthers and stigma are included in the corolla tube, with the stigma always positioned below the anthers. Mainly because of this unique floral morphology, *Kohautia* was considered to be a distinct genus. Molecular data (*atpB-rbcL*, *petD*, *rps16*, *trnL-trnF*, ETS, ITS) confirm that the genus is biphyletic. Two distantly related clades correspond to the subgenera *Kohautia* and *Pachystigma* Bremek. A similar type of floral organisation thus seems to have evolved twice independently, resulting in similar, but distantly related lineages. In order to translate the biphyletic nature of *Kohautia* into a formal classification, the two subgenera are recognized at generic level. A substitute name, *Cordylostigma* Groeninckx & Dessein is proposed for *K.* subg. *Pachystigma* because of the existence of *Pachystigma* Hochst. in the Rubiaceae tribe Vanguerieae. Floral, pollen and seed characters were studied to morphologically characterize *Kohautia* s.str. and *Cordylostigma*. By optimizing pollination syndromes and pollen characters onto the molecular phylogeny, we investigated pollination shifts and pollen evolution within the two genera. Detailed floral morphological studies show that the nectar guides in the psychophilous species of *Kohautia* s.str. and *Cordylostigma* evolved in different ways but result in the same visual effect.

Keywords *Cordylostigma*; *Kohautia*; molecular phylogeny; morphology; *Pachystigma*; pollination syndromes; Rubiaceae; Spermacoceae

INTRODUCTION

Kohautia Cham. & Schltdl. was first described in 1829 by Chamisso and von Schlechtendal who named the genus after its collector Franz Kohaut. The genus belongs to the (predominantly) herbaceous tribe Spermacoceae of the asterid family Rubiaceae. In the current circumscription, *Kohautia* comprises 36 species (Govaerts & al., 2006; Table 1) distributed from India, Pakistan, Iran to the Arabian Peninsula, the Sinai, Eastern Egypt, most of Africa south of the Sahara (including Socotra and Cape Verde Islands), Madagascar and Australia. Approximately two thirds of the species are perennial herbs; the remaining third are annuals.

The African species of *Kohautia* were first revised by Bremekamp in 1952. Later, Mantell (1985) provided a comprehensive revision of the genus, taking into account infraspecific variation, pollination biology, anatomy, and distribution. Both Bremekamp and Mantell divided *Kohautia* into two subgenera based on the number of stigma lobes; *K.* subg. *Kohautia* including all species with two thin filiform stigma lobes and *K.* subg. *Pachystigma* Bremek. including all species characterized by having a single, ovoid to cylindrical stigma lobe. In addition, Mantell (1985) divided *K.* subg. *Kohautia* into two series. *Kohautia* ser. *Kohautia* is the phalaenophilous (pollinated by moths) and *K.* ser. *Diurnae* Bremek. is the psychophilous/micro-melittophilous (pollinated by butterflies or small bees). All representatives of *K.* subg. *Pachystigma* (with the exception of *Kohautia virgata* (Willd.) Bremek.) are described as being butterfly-pollinated.

Species of genus *Kohautia* are easily distinguished from other Spermacoceae by their monomorphic short-styled flowers in which anthers and stigma are always included, with the stigma held well below the anthers or occasionally just touching them (Bremekamp, 1952; Mantell, 1985). Although recognized as a distinct group of species, there have been different opinions at the generic level. Some authors included *Kohautia* in *Hedyotis* L. (Wight & Arnott, 1834: 405–418), others in *Oldenlandia* L. (Hooker, 1873, 1882: 64–71; Schumann, 1891), whereas Bremekamp (1952) and all later authors (e.g., Verdcourt, 1976; Mantell, 1985) considered *Kohautia* to be distinct enough to deserve the generic rank.

Recent molecular studies within Spermacoceae based on chloroplast (*atpB-rbcL*, *petD*, *rps16*, *trnL-trnF*) and nuclear (ITS, ETS) DNA have shed new light on the phylogeny of *Kohautia* and several other representatives of the tribe (Kårehed & al., 2008; Groeninckx & al., 2009). These molecular studies provide answers to many taxonomic debates within the tribe, but also evoke numerous new questions because detected relationships contradict previous taxonomic treatments. The new clades await morphological support before a new classification can be proposed. For example, Kårehed & al. (2008) and Groeninckx & al. (2009) demonstrated that *Kohautia* is not monophyletic. Species of the genus fall in two unrelated clades, which correspond to the subgenera *Kohautia* and *Pachystigma*. Because of their limited sampling within *Kohautia* (respectively ten and nine *Kohautia* species), Kårehed & al. (2008) and Groeninckx & al. (2009) postponed proposing a new generic circumscription. Nevertheless, both studies suggest that similar growth and floral traits evolved independently in the common ancestor of both *K.* subg. *Kohautia* and *K.* subg. *Pachystigma*, resulting in two very similar but distantly related lineages (see Fig. 1).

By increasing sampling density, the present study investigates the evolution of the species traditionally referred to *Kohautia* into greater detail. The phylogeny of the genus is reconstructed using four plastid markers (*atpB-rbcL*, *petD*, *rps16*, *trnL-trnF*) and two nuclear markers (ETS, ITS). Floral, pollen, and seed characters were studied to morphologically characterize the two lineages. By optimizing pollination syndromes and pollen characters onto the resulting molecular phylogenies, we traced the evolutionary path of pollen characters and pollinators within the two clades.

■ MATERIALS AND METHODS

Taxon sampling. — Sequences from previous studies (Kårehed & al., 2008; Groeninckx & al., 2009) were used as a basis for the phylogenetic analysis presented in this paper. The Appendix lists all taxa included in this study with author names, voucher information and GenBank accession numbers.

Our sampling includes 41 ingroup taxa, which represent 34 species of Spermacoceae. This set of taxa covers the major evolutionary lineages within the tribe (following Groeninckx & al., 2009). Our dataset contains 26 *Kohautia* taxa, representing 19 species currently described within the genus. *Kohautia* subg. *Kohautia* is thereby represented by 14 species, and *K.* subg. *Pachystigma* by 5 species. Despite many efforts, we were not able to amplify and/or sequence DNA from herbarium specimens of the other *Kohautia* species listed in Table 1 (indicated with an asterisk). DNA isolations were either of poor quality or contaminated with fungi. Three species from the sister tribe Knoxieae (*Batopedina pulvinellata* Robbr., *Carphalea madagascariensis* Lam., *Pentanisia parviflora* Stapf ex Verdc.) were chosen as outgroup taxa.

Table 1. Most recent classification of *Kohautia* according to Mantell (1985) and adapted to Govaerts & al. (2006). Taxa not included in our sampling are indicated with an asterisk.

K. subg. *Kohautia*	*K.* ser. *Kohautia*	*K. amatymbica, K. australiensis,*[a] *K. caespitosa, K. cynanchica, K. dolichostyla, K. euryantha,* K. gracilis,* K. gracillima,* K. kimuenzae,* K. microflora,* K. nagporensis,* K. pappii,* K. quartiniana,* K. ramosissima, K. retrorsa, K. socotrana,* K. subverticillata, K. tenuis*[b]
	K. ser. *Diurnae*	*K. angolensis,* K. aspera, K. azurea, K. coccinea, K. confusa,* K. grandiflora, K. huilensis,* K. platyphylla, K. pleiocaulis**
K. subg. *Pachystigma*		*K. amboensis,* K. cicendioides,* K. cuspidata, K. microcala, K. longifolia, K. obtusiloba, K. prolixipes,* K. stellarioides,* K. virgata*

[a] *Kohautia australiensis* was not yet described when Mantell wrote her revision in 1985. Based on the presence of a bifid stigma, we conclude that the species belongs to *K.* subg. *Kohautia*. According to Halford (1992) who described the species, *K. australiensis* resembles *K. coccinea* in inflorescence and capsule shape. However, based on floral morphology and general habit we believe *K. australiensis* to be closer related to *K. caespitosa*. Therefore, *K. australiensis* is placed within *K.* ser. *Kohautia*.

[b] *Kohautia senegalensis*, type of *Kohautia*, is a synonym of *K. tenuis* (Brunel & al., 1984).

Molecular phylogenetic reconstruction. — Methods for DNA extraction, PCR amplification, sequencing, sequence assembly, alignment and gap coding are as described by Kårehed & al. (2008) and Groeninckx & al. (2009). Equally weighted parsimony analyses were performed using Nona v.2.0 (Goloboff, 1993) launched through WinClada v.1.0 (Nixon, 2002). The different DNA regions were first analyzed separately to check for potential incongruence, but since the results were compatible the matrices were combined using a simultaneous approach (Nixon & Carpenter, 1996a). Heuristic searches for the shortest trees were performed using the parsimony ratchet (Nixon, 1999). Traditional searches were repeatedly conducted using TBR on 1000 Wagner trees constructed with random addition of taxon sequences, holding 10 trees per search and expending the memory to conduct more thorough analyses holding up to 10,000 trees (10 times h 100,000 mu*1000 h/10 max*). Ratchet runs of 200 iterations each, holding one tree per iteration and randomly weighting 10% of the potentially informative characters were carried out until the most parsimonious trees (MPT) were repeatedly found. All trees were collected, unambiguously supported branches collapsed, duplicate trees were identified and removed and a (strict) consensus tree was calculated using WinClada. In order to evaluate the relative support of the clades, jackknife (JS) and bootstrap (BS) analyses were executed using the 'new technology' option of TNT (Goloboff & al., 2008). In both cases, 1000 replications were conducted combining Sectorial Searches and Tree Fusion (Goloboff, 1999) using 100 random initial sequences on each of the 1000 replications, saving the consensus for further calculation of frequencies using WinClada (Nixon, 1999). Frequency values above 64% were plotted onto the consensus of the MPT.

Floral, pollen, and seed morphology. — Flowers of the psychophilous *Kohautia coccinea* Royle (Zambia: *Dessein & al. 751*, BR), the psychophilous *K. microcala* Bremek. (Zambia: *Dessein & al. 1321*, BR), the phalaenophilous *K. subverticillata* (K. Schum.) D. Mantell (Zambia: *Dessein & al. 462*, BR) and the micro-melittophilous *K. virgata* (Willd.) Bremek. (Madagascar: *Groeninckx & al. 121*, BR) preserved in 70% ethanol were studied with scanning electron microscopy (SEM). Flowers were dissected under a Wild M3 stereomicroscope (Wild Heerbrugg Ltd, Heerbrugg, Switzerland). The floral material

Fig. 1. Flowers of *Kohautia* species from *K.* subg. *Pachystigma* (= *Cordylostigma*) **(A–E)** and *K.* subg. *Kohautia* (= *Kohautia* s.str.) **(F–G)**. **A,** *Kohautia microcala* (Zambia: *Dessein & al. 1149*, BR); **B,** *Kohautia longifolia* (Zambia: *Dessein & al. 1119*, BR); **C,** *Kohautia microcala* (Zambia: *Dessein & al. 1149*, BR); **D,** *Kohautia microcala* (Zambia: *Dessein & al. 1321*, BR); **E,** *Kohautia microcala* (Zambia: *Dessein & al. 1321*, BR); **F,** *Kohautia caespitosa* subsp. *brachyloba* (Zambia: *Dessein & al. 506*, BR); **G,** *Kohautia coccinea* (Zambia: *Dessein & al. 751*, BR). Photographs by Steven Dessein.

was washed repeatedly in 70% ethanol and dehydrated in a 1 : 1 ethanol-dimethoxymethan mixture (DMM or formaldehyde-dimethylacetal) for 5 minutes and in pure DMM for 20 minutes. After critical-point drying (CPD 030, BAL-TEC AG, Balzers, Liechtenstein), the dried material was mounted on aluminum stubs using Leit-C and coated with gold (SPI Module Sputter Coater, Spi Supplies, West Chester, Pennsylvania, U.S.A.) prior to observation with a JEOL JSM-6360 SEM (Jeol Ltd, Tokyo, Japan).

A palynological investigation was carried out for 13 species of *K.* subg. *Kohautia* and 4 species of *K.* subg. *Pachystigma*. Pollen samples were collected from herbarium specimens of BR (Table 2). Pollen grains were acetolysed according to the 'wetting agent' method (Reitsma, 1969). Under the SEM, external features were observed on grains that had been suspended in 70% alcohol and left to dry. Glycerin jelly slides were observed under a light microscope. Polar axis length (P) and equatorial diameter (E) were measured on ten grains of each specimen using Carnoy (Schols & al., 2002), and P/E ratios were calculated. Pollen terminology follows Punt & al. (2007).

Seeds from herbarium specimens were directly mounted on aluminium stubs, coated with gold and observed under the SEM as described above. We investigated the seed morphology of nine species of *K.* subg. *Kohautia* and five species of *K.* subg. *Pachystigma* (Table 2).

Pollination shifts and pollen evolution. — We investigated pollination shifts within *K.* subg. *Kohautia* and *K.* subg. *Pachystigma* by unambiguously optimizing pollination syndromes onto the consensus tree from the combined parsimony analysis in WinClada v.1.0 (Nixon, 2002). We made sure that the polytomies resulting on the consensus did not create artefacts on the character evolution interpretation (Nixon & Carpenter, 1996b). Information on the pollination biology of genus *Kohautia* was gathered from own field observations and from Mantell (1985), who based her descriptions on field observations in Ethiopia and, to a more limited extent, on observations of greenhouse-cultivated plants and studies of herbarium and fixed material. The number of pollen apertures and the presence/absence of a secondary reticulum were also optimised onto the consensus tree.

■ RESULTS

Molecular evidence. — Table 3 lists the characteristics of each data matrix used in the phylogenetic analysis. All tree searches of the combined analysis found the same tree lengths (L = 1603). Only two equally parsimonious trees were found after collapsing the unambiguously supported branches, with a consistency index (CI) of 0.60 and a retention index (RI) of 0.79.

Figure 2 shows the (strict) consensus tree from the parsimony analysis of the combined matrix (L = 1615, CI = 0.60, RI = 0.79).

Similar to the studies of Kårehed & al. (2008) and Groeninckx & al. (2009), species of genus *Kohautia* fall in two well supported, biphyletic clades, which correspond to the two described subgenera; *K.* subg. *Kohautia* (BS = 100, JS = 100) and *K.* subg. *Pachystigma* (BS = 100, JS = 100). *Kohautia* subg. *Kohautia* is sister to a clade that includes *Pentanopsis fragrans* Rendle and *Oldenlandia herbacea* (L.) Roxb. (BS = 90, JS = 96). *Kohautia* subg. *Pachystigma* is sister to a clade containing species of *Oldenlandia* including *O. corymbosa* L., the type of the generic name, and *O. capensis* L. f. (BS = 100, JS = 100).

Kohautia ramosissima Bremek. and *K. cynanchica* DC. form a grade sharing a most recent common ancestor with the rest of *K.* subg. *Kohautia* (respectively BS = 91, JS = 97 and BS = 71, JS = 74). *Kohautia aspera* (B. Heyne ex Roth) Bremek. and *K. azurea* (Dinter & K. Krause) Bremek., originally described within *K.* ser. *Diurnae*, are resolved as sister taxa, but without significant support (JS and BS both <65). The two species are more closely related to *K. subverticillata* (K. Schum.) D. Mantell of *K.* ser. *Kohautia* (BS = 99, JS = 100) than to other species of *K.* ser. *Diurnae*. Further internal tree resolution reveals *Kohautia caespitosa* Schnizl. subsp. *caespitosa* sister to *K. amatymbica* Eckl. & Zeyh., and *Kohautia dolichostyla* Bremek. sister to *K. australiensis* Halford. Both clades lack significant support. *Kohautia caespitosa* Schnizl. subsp. *brachyloba* (Sond.) D. Mantell is sister to a highly supported clade including *K. caespitosa* Schnizl. subsp. *amaniensis* (K. Krause) Govaerts and *K. retrorsa* (Boiss.) Bremek. (BS = 99, JS = 99). Except for *Kohautia aspera* and *K. azurea*, all other species of *K.* ser. *Diurnae* included in our sampling fall within one clade together with *K. tenuis* Cham. & Schltdl. (BS = 95, JS = 96), the type of the generic name. Within this clade, *K. grandiflora* DC. is highly supported as sister to *K. tenuis* (BS = 97, JS = 98). Relationships between *K. coccinea* Royle, *K. platyphylla* (K. Schum.) Bremek. and the clade of *K. grandiflora* and *K. tenuis* remain unresolved.

Within *K.* subg. *Pachystigma*, *Kohautia obtusiloba* (Hiern) Bremek. is sister to a clade including the remaining *Pachystigma* species in our sampling. In this clade, *Kohautia virgata* (Willd.) Bremek. is resolved as sister to *K. cuspidata* (K. Schum.) Bremek., *K. longifolia* Klotzsch and *K. microcala* Bremek. (BS = 99, JS = 99). Relationships between the latter three species remain unresolved.

Pollen morphology. — In general, the pollen grains of genus *Kohautia* are very small. The smallest pollen grains are observed in *K.* subg. *Kohautia*; P 17.60–25.99 µm, E 13.77–18.84 µm. The hexaploid *K. amatymbica* is the only species within the subgenus with much larger pollen (P 26.81–30.49 µm, E 19.80–23.91 µm). Species of *K.* subg. *Pachystigma*

Table 3. Characteristics of each data matrix used in in phylogenetic analyses I, II and III, and the corresponding tree statistics. Chars = total number of characters, PI = potentially informative characters, MPT = number of most parsimonious tree(s), CI = consistency index (Kluge & Farris, 1969), RI = retention index (Farris, 1989).

	No. of taxa	Chars	PI	PI indels	MPTs	CI	RI
atpB-rbcL	37	1030	81	15	9	0.64	0.81
petD	41	1339	171	27	27	0.69	0.87
rps16	39	614	110	12	12	0.71	0.86
trnL-trnF	42	628	106	23	4	0.72	0.90
ETS	32	266	88	3	9	0.49	0.68
ITS	33	815	150	11	3	0.54	0.70
Combined	44	4692	706	91	2	0.60	0.79

Table 2. Herbarium specimens of which pollen (p) and/or seeds (s) were studied, and their voucher information.

Taxon	Voucher information	
K. amatymbica	South Africa, *Schlieben 7910* (BR)	p
K. angolensis	Angola, *Bamps 527* (BR)	p
K. aspera	Tanzania, *Kayombo & Kitaba 4230* (BR)	p, s
K. azurea	Namibia, *Seydel 3491* (BR)	p, s
K. caespitosa		
subsp. *amaniensis*	Ethiopia, *de Wilde 5976* (BR)	p, s
subsp. *brachyloba*	Tanzania, *Kuchar 25133* (BR)	p, s
	Zambia, *Dessein & al. 432* (BR)	p, s
	Zambia, *Dessein & al. 790* (BR)	p
K. coccinea	Congo, *de Witte 430* (BR)	p, s
	Zambia, *Dessein & al. 751* (BR)	p, s
K. cuspidata	Angola, *Hess-Wyss 52/108* (BR)	p, s
K. cynanchica	South Africa, *Dessein & al. 469* (BR)	p, s
K. grandiflora	Ethiopia, *Friis & al. 6864* (BR)	p, s
K. longifolia	Tanzania, *Bidgood & al. 2595* (BR)	p, s
K. microcala	Zambia, *Dessein & al. 1149* (BR)	p, s
	Zambia, *Dessein & al. 1321* (BR)	p, s
	Zambia, *Dubois 1309* (BR)	s
K. obtusiloba	Tanzania, *Gobbo 307* (BR)	s
K. platyphylla	Ethiopia, *Friis & al. 439* (BR)	p
	Cameroon, *De Wilde & De Wilde-Duyfjes 8950* (BR)	s
K. ramosissima	Namibia, *Merxmüller & Giess 32496* (BR)	p
K. retrorsa	Oman, *Ghazanfar 1823* (BR)	p
K. subverticillata	Zambia, *Dessein & al. 462* (BR)	p, s
	Botswana, *Dessein & al. 470* (BR)	p
	Zambia, *Dessein & al. 489* (BR)	p, s
K. tenuis	Cameroon, *De Wilde & De Wilde-Duyfjes 4870* (BR)	p
	Senegal, *Vanden Berghen 6262* (BR)	s
K. virgata	Madagascar, *De Block 539* (BR)	p, s

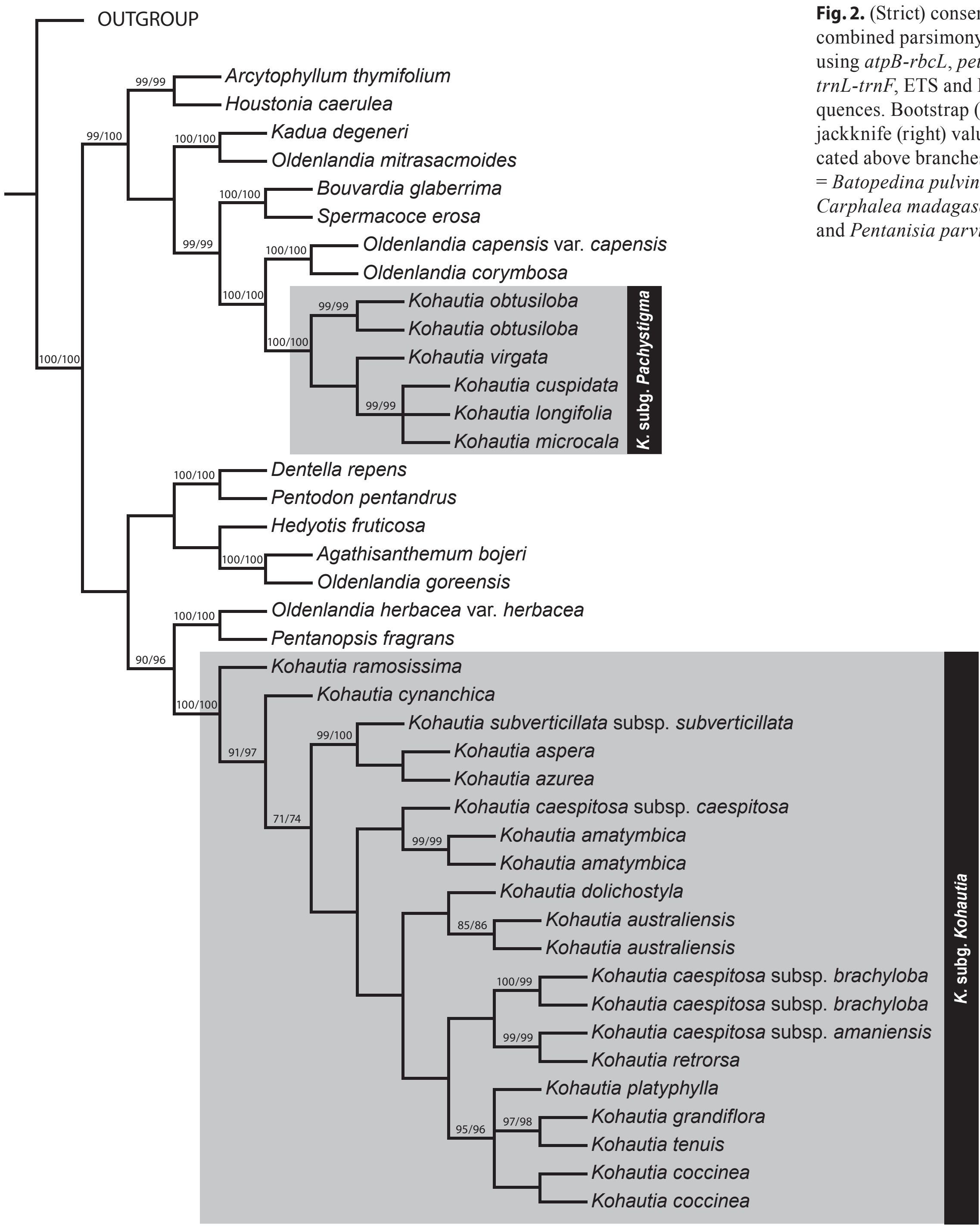


Fig. 2. (Strict) consensus of the combined parsimony analysis using *atpB-rbcL*, *petD*, *rps16*, *trnL-trnF*, ETS and ITS sequences. Bootstrap (left) and jackknife (right) values are indicated above branches. Outgroup = *Batopedina pulvinellata, Carphalea madagascariensis* and *Pentanisia parviflora*.

have distinctly larger pollen grains than species of *K.* subg. *Kohautia* (P 20.76–29.33 µm, E 18.98–26.96 µm). The pollen shape within both subgenera varies from (oblate/prolate) spheroidal to prolate. Most species are characterized by having prolate spheroidal to subprolate pollen.

Both subgenera have compound apertures consisting of an ectocolpus, a mesoporus and an endocolpus. In *K.* subg. *Kohautia*, the endoaperture is a relatively short colpus often with fish tail endings (Fig. 3A). The mesoporus is surrounded by an annulus (Fig. 3A, G, H). In *K.* subg. *Pachystigma*, on the other hand, the endocolpus is an endocingulum (Fig. 3I) and an annulus is absent. Pollen has three to six apertures. Eight-colporate pollen grains, as reported by Bremekamp (1952), were not observed. Within *K.* subg. *Kohautia*, *K. cynanchica*, *K. ramosissima* (Fig. 3B), *K. angolensis* Bremek. (not included in our molecular sampling), and *K. azurea* are characterized by having almost exclusively 3-colporate pollen grains. The remaining species are characterized by having a mixture of

4- to 5- (rarely 6-)colporate pollen grains. Within *K.* subg. *Pachystigma*, all species have 4- to 6-aperturate pollen, except *K. virgata* with 3-colporate pollen.

In both subgenera, the inner nexine surface is granular (Fig. 3A, I). The sexine of most species is heterobrochate varying from reticulate to micro-reticulate (Fig. 3B–F, K). In general, species of *K.* subg. *Pachystigma* have larger lumina (Fig. 5K) than species of *K.* subg. *Kohautia* (Fig. 3D–F). Moreover, the tectum of *K.* subg. *Pachystigma* is subtended by longer columellae than in *K.* subg. *Kohautia* creating larger intercolumellar spaces (Fig. 3I). Within *K.* subg. *Kohautia*, large lumina were observed in the earlier derived taxa *K. angolensis*, *K. cynanchica*, and *K. ramosissima* (Fig. 3B), and in the sister species *K. coccinea*, *K. platyphylla*, *K. tenuis* and *K. grandiflora* (Fig. 3C, F). The smallest lumina were found in *K. amatymbica*. This species has a micro-reticulate to perforate sexine (Fig. 3D).

Most species of *K.* subg. *Kohautia* have a double reticulum (Fig. 3E), except for the earlier derived *K. cynanchica* and *K. ramosissima* (Fig. 3B), and *K. coccinea* (Fig. 3F), *K. platyphylla* (Fig. 5C), *K. tenuis* and *K. grandiflora*. All studied species within *K.* subg. *Pachystigma*, lack a secondary reticulum (Fig. 3K).

Seed morphology. — According to Mantell (1985), the seed shape is quite different in the two subgenera. She describes the seeds of *K.* subg. *Kohautia* as being angular-conic to angular-subconic, whereas seeds of *K.* subg. *Pachystigma* are more rounded. In our study, we did not observe these differences (Fig. 4A, C, E, G). There is, however, a difference in the seed surface of the two subgenera. In *K.* subg. *Pachystigma*, the seed coat is always distinctly reticulate with prominent radial walls, which are strait, curved or undulating (Fig. 4F, H). The tangential walls are sparsely or densely punctate (Fig. 4F, H). Similar punctate micro-sculpturing is observed in a number of *Oldenlandia* species (sister group of *K.* subg. *Pachystigma*) but is lacking in *K.* subg. *Kohautia*. In most species of *K.* subg. *Kohautia*, the seed coat is alveolate to reticulate-alveolate with the radial walls only slightly raised (Fig. 4B). Some species (e.g., *Kohautia coccinea* and *K. platyphylla*; Fig. 4C–D) are exceptional in having prominent radial walls similar to species of *K.* subg. *Pachystigma*. The tangential walls in *K.* subg. *Kohautia* are, however, never punctate as in *K.* subg. *Pachystigma* but favulariate (Fig. 4B) or with a central protuberance which is either ridged (e.g., *K. platyphylla*) or tuberculate (e.g., *K. coccinea*; Fig. 4D).

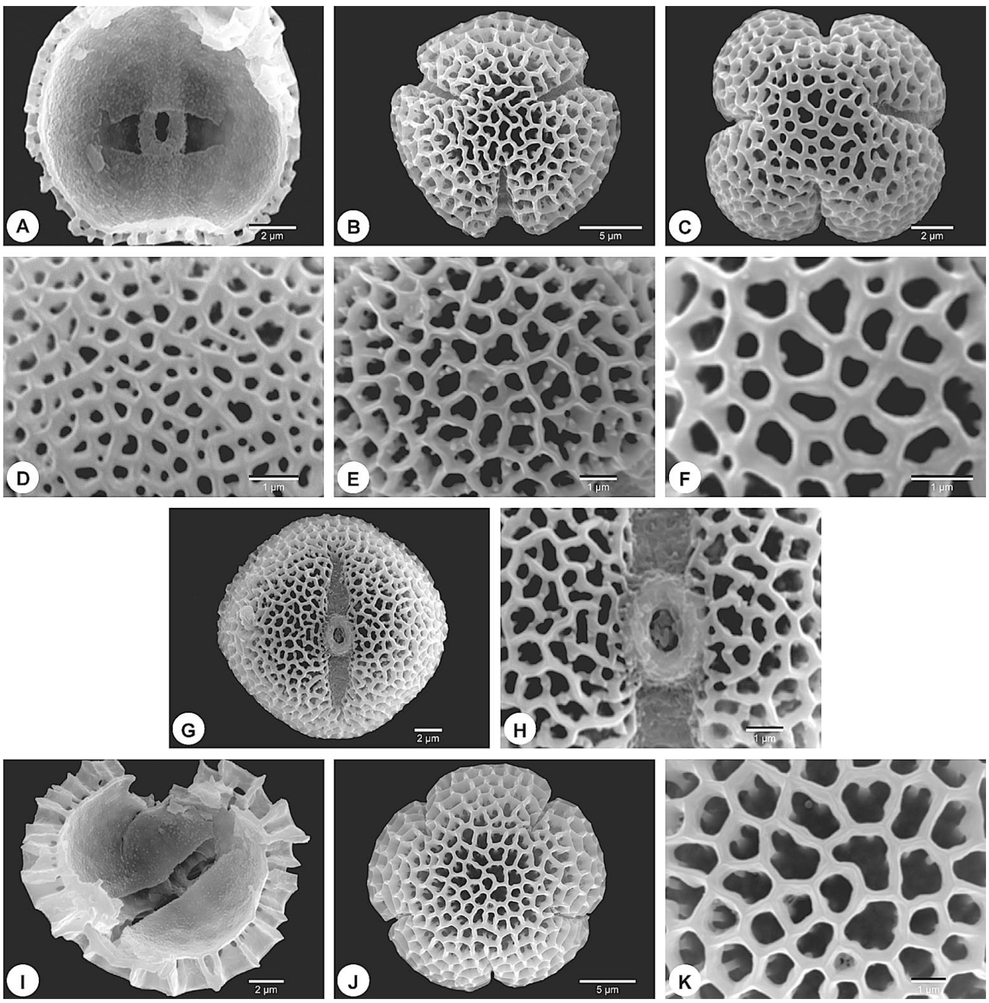


Fig. 3. Pollen morphology of *K.* subg. *Kohautia* **(A–H)** and *K.* subg. *Pachystigma* **(I–K)**. **A,** Broken pollen grain of *K. platyphylla*; endocolpus with fish tail endings and mesocolpus surrounded by an annulus; **B,** polar view on 3-colporate pollen of *K. ramosissima*; **C,** polar view on 4-colporate pollen of *K. platyphylla*; **D,** detail of micro-reticulate/perforate apocolpium of *K. amatymbica*; **E,** detail of (micro-)reticulate apocolpium of *K. subverticillata* with secondary reticulum; **F,** detail of (micro-)reticulate apocolpium of *K. coccinea* without secondary reticulum; **G,** equatorial view on pollen of *K. caespitosa* subsp. *brachyloba*; **H,** detail of aperture of *K. caespitosa* subsp. *brachyloba*; **I,** broken pollen grain of *K. microcala*; view on endocingulum; **J,** polar view on 5-colporate pollen of *K. longifolia*; **K,** detail of reticulate apocolpium of *K. longifolia* without secondary reticulum.

Floral morphology. — Psychophilous species of the two subgenera *Kohautia* and *Pachystigma* have flowers with long, narrowly cylindrical corolla tubes, mostly inconspicuously coloured, and with barrel-shaped, inflated apical parts where the anthers are included (Fig. 1A, D, G). The corolla lobes are broadly lanceolate-elliptic to roundly elliptic, usually brightly coloured above, and paler below (Fig. 1A–E, G). The colour of the corolla lobes usually falls into the red end of the colour spectrum, i.e., red, pink, orange or lilac. The nectar is hidden at the base of the corolla tube, around the style. In the psychophilous species of *K.* subg. *Kohautia*, the fused basal parts of the corolla lobes are turgid and swollen at anthesis (Figs. 1G and 5A–B). Seen from above, these swollen parts slightly project into and over the corolla throat, forming a distinct cross or 'bullseye' in the centre of the flower (Fig. 5A). These intrusions are characterized by epidermal cells with a different shape and orientation (Fig. 5A–B) and may function as nectar guides. In *K.* subg. *Pachystigma*, the fused basal parts of the corolla lobes are beset with hairs (Fig. 5C–D). These hairs are paler or darker than the rest of the corolla, or contrastingly coloured (Fig. 1A–C, E), and may also function as nectar guides.

Most species of *K.* subg. *Kohautia* are phalaenophilous. These moth-pollinated flowers are similar to butterfly-pollinated flowers in having long and narrow corolla tubes with an upper inflated, barrel-shaped portion containing the anthers

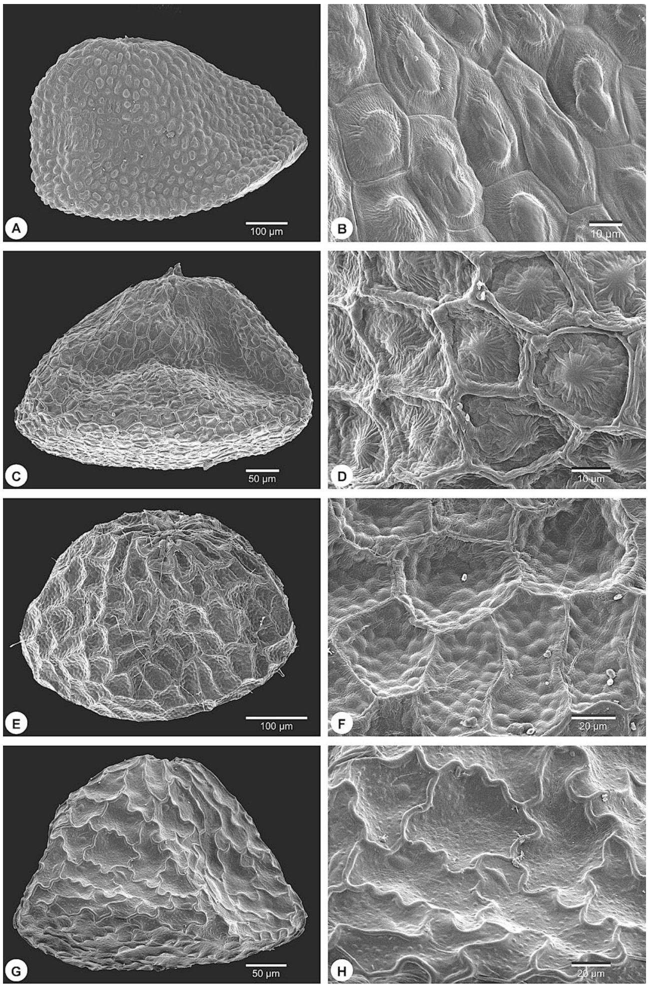


Fig. 4. Seed morphology of *K.* subg. *Kohautia* **(A–D)** and *K.* subg. *Pachystigma* **(E–H)**. **A–B,** Seed and detail of the seed coat of *K. subverticillata*; **C–D,** seed and detail of the seed coat of *K. coccinea*; **E–F,** seed and detail of the seed coat of *K. microcala*; **G–H,** seed and detail of the seed coat of *K. virgata*.

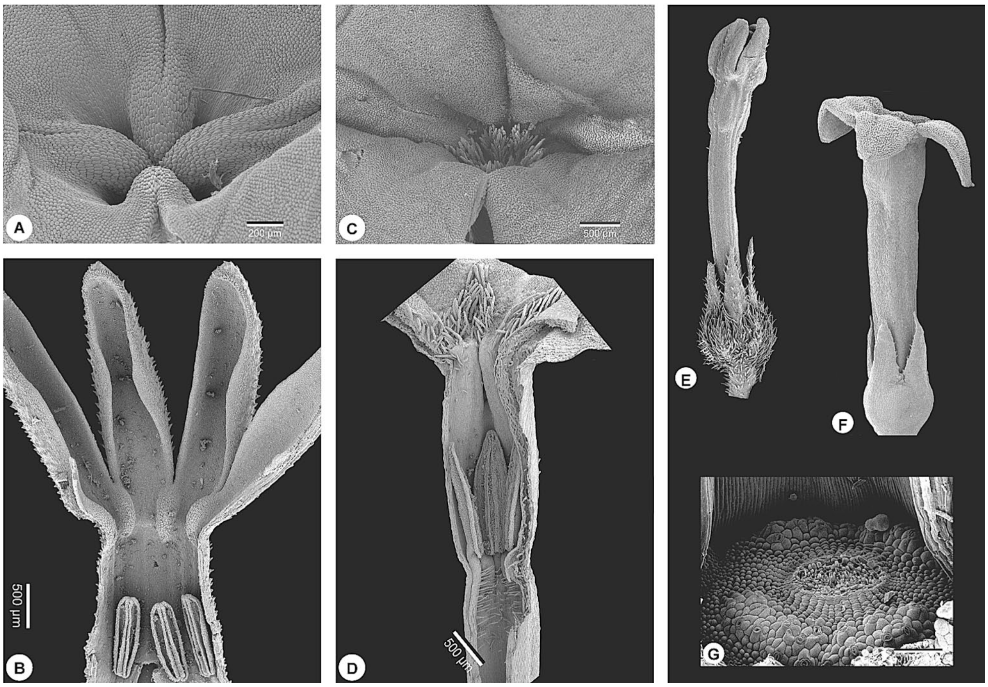


Fig. 5. Flower morphology of psychophilous **(A–D)**, phalaenophilous **(E)** and micro-melittophilous (**F–G**) species. **A,** top view on psychophilous flower of *K. coccinea* with swollen, fused basal parts of the corolla lobes ('bullseye'); **B,** dissected psychophilous flower of *Kohautia coccinea*; **C,** top view on psychophilous flower of *K. microcala* with hairs on the fused basal parts of the corolla lobes; **D,** dissected psychophilous flower of *K. microcala*; **E,** phalaenophilous flower of *K. subverticillata*; **F,** micro-melittophilous flower of *K. virgata*; **G,** nectary disc above the ovary surrounding the base of the style in *K. virgata*. Note the nectarostomata.

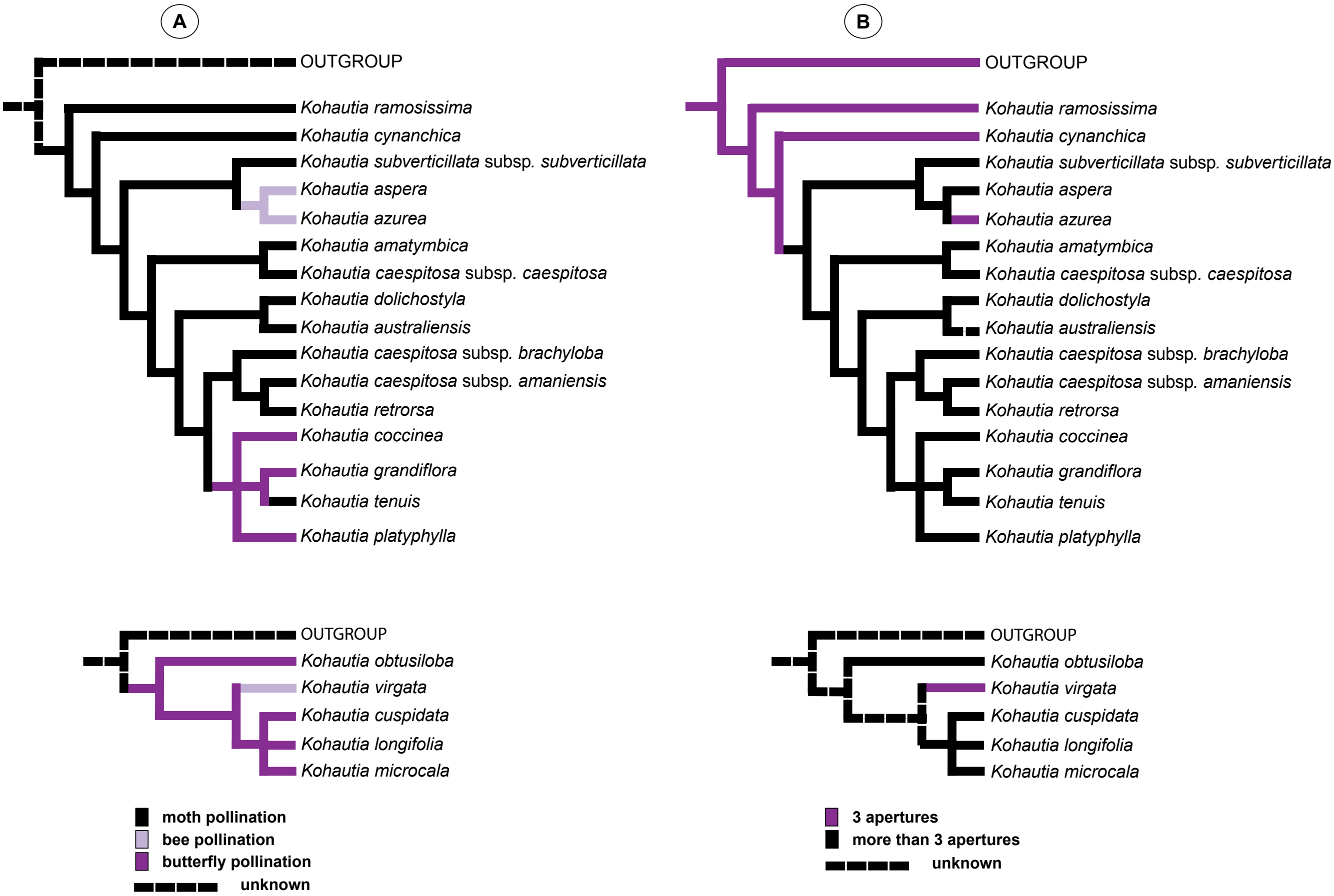


Fig. 6. Optimization of pollination syndromes **(A)**, number of pollen apertures **(B)** and the presence/absence of a secondary reticulum **(C)** onto the (strict) consensus tree of the parsimony analysis for *K.* subg. *Kohautia* and *Pachystigma*.

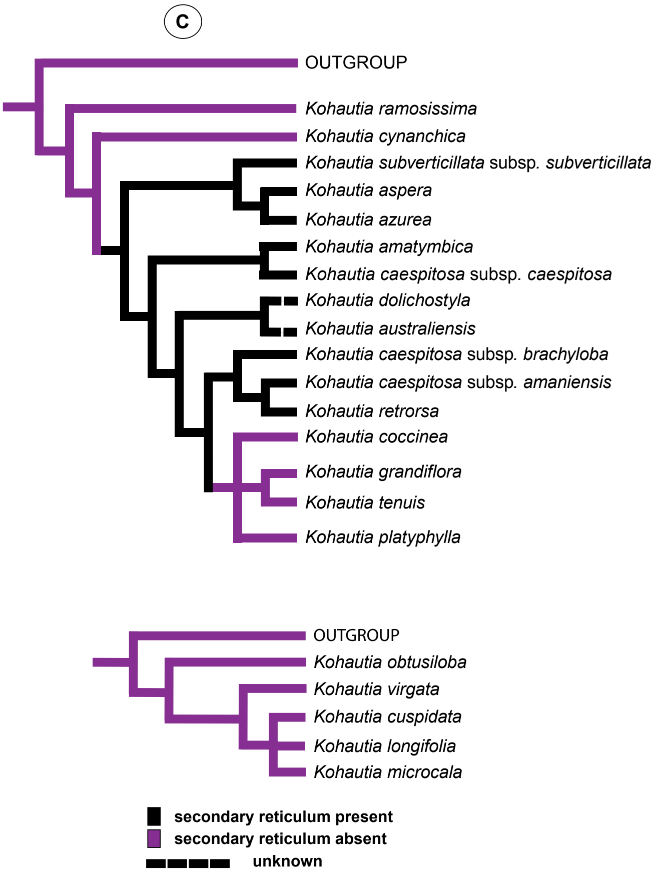
(Fig. 5E). The corolla lobes are white to greenish-white or yellowish above (Fig. 1F). Below they are darker, often olive-green or brownish-red just like the corolla tubes. As in phalaenophilous species, the nectar is hidden at the base of the corolla tubes. In contrast to psychophilous flowers nectar guides are absent.

Micro-melittophilous flowers of *Kohautia azurea*, *K. aspera* and *K. virgata* are typically blue-violet, white-blue or white with short narrowly cylindrical tubes (Fig. 5F). Micro-melittophilous flowers differ from ordinary melittophilous flowers in their small size and smaller amount of nectar production, sufficient for smaller pollinators. A nectariferous disc showing many nectarostomata is present above the ovary surrounding the base of the style (Fig. 5G). Nectar is thus hidden, not too deeply but within easy reach of a bee's short proboscis. Nectar guides are similar to those of psychophilous flowers.

Pollination shifts in *K.* subg. *Kohautia* and *K.* subg. *Pachystigma*. — Optimizing pollinators onto the molecular phylogenetic reconstruction of *K.* subg. *Kohautia*, indicates that ancestral flowers were visited by moths (Fig. 6A). During evolution three shifts occurred; one shift from moth to butterfly pollination, one reversal back to psychophily in *K. tenuis*, and one shift from moth to bee pollination for the clade of *Kohautia aspera* and *K. azurea*. Species of *K.* subg. *Pachystigma* are all psychophilous with one transition to melittophily for *K. virgata*.

Pollen evolution in *K.* subg. *Kohautia* and *K.* subg. *Pachystigma*. — Figure 6B shows the evolution of aperture number within the two subgenera. Within *K.* subg. *Kohautia*, 3-colporate pollen is considered the ancestral condition. During evolution a shift occurred from 3-colporate pollen to pluri-colporate pollen, followed by one reversal back to the 3-colporate state in *K. azurea*. In *K.* subg. *Pachystigma*, evolutionary reconstruction of the number of apertures is ambiguous. Acctran optimization supports pluri-colporate pollen as the character state in the most recent common ancestor of the subgenus with one shift to 3-colporate pollen in *K. virgata*. Deltran optimization, on the other hand, favours the 3-colporate condition as ancestral state with two parallel gains of pluri-colporate pollen.

Figure 6C shows the presence/absence of a secondary reticulum within both subgenera. In *K.* subg. *Kohautia*, absence is supported as the character state in the most recent common ancestor of the subgenus with one shift to presence of a secondary reticulum followed by another shift to absence of a secondary reticulum. Within *K.* subg. *Pachystigma*, all species studied lack a secondary reticulum. The absence of a secondary reticulum is considered the ancestral condition.



TAXONOMIC TREATMENT

Based on the morphological differences between *K.* subg. *Kohautia* and *K.* subg. *Pachystigma*, Mantell (1985) tentatively suggested that the two subgenera could also be treated as genera. Eventually, she decided to maintain a broad definition for the genus *Kohautia*.

Our molecular data and previous molecular studies (Kårehed & al., 2008; Groeninckx & al., 2009) clearly show the need to recognize the two subgenera as genera. Despite the unifying floral architecture, there are numerous morphological differences between the two subgenera. The number of stigmatic lobes is probably the most striking diagnostic field character: two in *K.* subg. *Kohautia* and one in *K.* subg. *Pachystigma*. Other differences can be found in floral, seed, and pollen morphology, and in the pollination biology of the two subgenera. Table 4 summarizes the main morphological differences between *K.* subg. *Kohautia* and *K.* subg. *Pachystigma*.

In order to translate our results into a formal classification, *Kohautia* is here restricted to comprise the species of *K.* subg. *Kohautia* only and the species of *K.* subg. *Pachystigma* are transferred to the new genus *Cordylostigma*. The name *Pachystigma* is not available at the rank of genus as *Pachystigma* Hochst. already exists in the Rubiaceae tribe Vanguerieae. The name *Cordylostigma* refers to the presence of a single stigma lobe characteristic of its representatives. We decided to no longer recognize *K.* ser. *Diurnae* and ser. *Kohautia* as they are not supported as monophyletic by our molecular data. For the same reason, we do no longer recognize *Cordylostigma* ser. *Barbatae* and ser. *Imberbae*. In the following paragraphs, a description is given for the restricted genus *Kohautia* s.str. and the new genus *Cordylostigma* and synonyms of these names are listed.

Kohautia Cham. & Schltdl. in Linnaea 4: 156. 1829, nom. cons. ≡ *Hedyotis* sect. *Kohautia* (Cham. & Schltdl.) Wight & Arnott, Prodromus 1: 417. 1834 ≡ *Oldenlandia* subg. *Kohautia* (Cham. & Schltdl.) Benth. & Hook. f., Gen. Pl. 2: 59. 1877 ≡ *Kohautia* (subg. "*Eu-kohautia*") ser. *Noctiflorae* Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 91. 1952 – Type: *K. senegalensis* Cham. & Schltdl.

– "*Kohautia* subg. *Eu-kohautia*" Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 81. 1952, non rite publ. (Art. 21.3).

= *Kohautia* (subg. *Kohautia*) ser. *Diurnae* Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 81. 1952 – Type: *K. coccinea* Royle.

Table 4. Morphological differences between *Kohautia* s.str. and *Cordylostigma*.

	Kohautia s.str.	*Cordylostigma*
Stigma	Two-lobed	One-lobed
Seed coat	Alveolate to reticulate-alveolate and tangential walls never punctate	Distinctly reticulate with prominent radial walls and punctate tangential walls
Pollen	3-colporate, or 4- to 5- (rarely 6-)colporate	4- to 6-colporate (except *K. virgata* with 3-colporate pollen)
Endocolpus	Short colpus, often with fish tail endings	Endocingulum
Mesoporus	Surrounded by an annulus	Not surrounded by an annulus
Double reticulum	Present in most species	Absent in all species
Pollinators	Moths, butterflies and small bees	Butterflies (only *K. virgata* is pollinated by small bees)
Nectar guides in psychophilous flowers	Intrusions	Hairs

= *Duvaucellia* Bowdich in Bowdich & Bowdich, Exc. Madeira: 259. 1825, nom. rejic. – Type: *D. tenuis* S. Bowd.

Annual or perennial herbs or subshrubs, sometimes with short woody subterranean stems; stipular sheath in the mid-stem region mostly with one or two fimbriae; corolla lobes above and entrance to tube always glabrous, white or brightly coloured; stigma two-lobed; stigma lobes filiform; pollen 3-colporate, or 4-to 5- (rarely 6-)colporate, P 17.60–25.99(–30.49) µm, E 13.77–18.84(–23.91) µm, with short endocolpus (often with fish tail endings), with an annulus surrounding mesoporus, in most cases with secondary reticulum; seed coat alveolate to reticulate-alveolate, sometimes distinctly reticulate, with tangential walls never punctate; pollinators moths, butterflies, or small bees. — Number of species: 27. — Distribution: India, Pakistan, Iran, northern East and sub-Saharan Africa, Cape Verde Islands and Socotra.

Cordylostigma Groeninckx & Dessein, **nom. et stat. nov.** ≡ *Kohautia* subg. *Pachystigma* Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 66. 1952 ≡ *Kohautia* (subg. *Pachystigma*) ser. *Barbatae* Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 66. 1952 (non *Pachystigma* Hochst. in Flora 25: 234. 1842) – Type: *Kohautia longifolia* Klotzsch. (*C. longifolia* (Klotzsch) Groeninckx & Dessein, see below).

= *Kohautia* (subg. *Pachystigma*) ser. *Imberbae* Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 77. 1952 – Type: *K. virgata* (Willd.) Bremek.

Annual or perennial herbs, sometimes with short woody subterranean stems; stipular sheath in the mid-stem region mostly with 2–8(11) fimbriae; corolla tube hairy or papillate at the throat inside; corolla lobes broad, elliptic, not parted at the base, mostly brightly coloured, with hairs at the base inside; stigma one-lobed; stigma lobes ovoid or cylindrical; pollen 4- to 6-colporate, exceptional 3-colporate (i.e., in *C. virgata*), P 20.76–29.33 µm, E 18.98–26.96 µm, with endocingulum, without annulus around mesoporus, without secondary reticulum; seed coat distinctly reticulate, with radial walls strait, curved or undulating, and with tangential walls punctate; pollinators butterflies, in *C. virgata* small bees. — Number of species: nine. — Distribution: mainly in eastern and southern Africa, also in Madagascar; one species extending to western Africa and into Sudan.

Nomenclatural changes

The following nomenclatural changes need to be made (only homotypic synonyms are given):

Cordylostigma amboensis (Schinz) Groeninckx & Dessein, **comb. nov.** ≡ *Oldenlandia amboensis* Schinz in Vierteljahrsschr. Naturf. Ges. Zürich 68: 429. 1923 ≡ *Kohautia amboensis* (Schinz) Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 75. 1952 – Type: Namibia, Amboland, Olukonda, Ondonga, *Rautanen 824* (Z!, holo; BM, G!, K!, P!, iso; BR!, photo).

Cordylostigma cicendioides (K. Schum.) Groeninckx & Dessein, **comb. nov.** ≡ *Oldenlandia cicendioides* K. Schum. in Bot. Jahrb. Syst. 33: 333. 1903 ≡ *Kohautia cicendioides* (K. Schum.) Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 75. 1952 – Type: Angola, auf feuchtem Boden bei Pallanca, sine col., sine num. (B†); Neotype (designated by Bremekamp, 1952): Angola, between Sambos Mission Station and Cabama, *Pearson 2492* (K!, neo; BR!, photo).

Cordylostigma cuspidata (K. Schum.) Groeninckx & Dessein, **comb. nov.** ≡ *Oldenlandia cuspidata* K. Schum. in Bot. Jahrb. Syst. 23: 413. 1897 ≡ *Kohautia cuspidata* (K. Schum.) Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 74. 1952 – Type: Angola, Huila Plateau, Lopollo, December 1859, *Welwitsch 5342* (B†, holo; K, LISU, BM, G!, P!, PRE!, iso).

Cordylostigma longifolia (Klotzsch) Groeninckx & Dessein, **comb. nov.** ≡ *Kohautia longifolia* Klotzsch in Peters, Naturw. Reise Mossambique 1: 297. 1862 – Type: Mozambique, Sena, *Peters s.n.* (B†, holo). Neotype (designated by Bremekamp, 1952): Mozambique: Gonubi Hill, *Schlechter 12181* (K, neo; BM, BR!, E!, G!, W, isoneo).

Cordylostigma microcala (Bremek.) Groeninckx & Dessein, **comb. nov.** ≡ *Kohautia microcala* Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 73. 1952 – Type: Zambia, near Kalungwizi R, *Walter 5* (K!, holo; BR!, photo).

Cordylostigma obtusiloba (Hiern) Groeninckx & Dessein, **comb. nov.** ≡ *Oldenlandia obtusiloba* Hiern in Oliver, Fl. Trop. Afr. 3: 56. 1877 ≡ *Kohautia obtusiloba* (Hiern) Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 66. 1952 – Types: Tanzania, Bagamoyo District, Kingani, *Kirk s.n.* & Mozambique, *Forbes 358* (both K!, syn).

Cordylostigma prolixipes (S. Moore) Groeninckx & Dessein, **comb. nov.** ≡ *Oldenlandia prolixipes* S. Moore in J. Bot. 43: 351. 1905 ≡ *Kohautia prolixipes* (S. Moore) Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 67. 1952 – Type: Kenya, Kwale District, near Avisana, Daruma, *Kässner 442* (BM!, holo; K, iso).

Cordylostigma stellarioides (Hiern) Groeninckx & Dessein, **comb. nov.** ≡ *Oldenlandia stellarioides* Hiern, Catalogue of African Plants 1: 447. 1898 ≡ *Kohautia stellarioides* (Hiern) Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 76. 1952 – Type: Angola, Pungo Andongo, *Welwitsch 3052* (BM!, lecto, designated by Bremekamp, 1952; K, isolecto).

Cordylostigma virgata (Willd.) Groeninckx & Dessein, **comb. nov.** ≡ *Hedyotis virgata* Willd. in Sp. Pl. 1: 567. 1798 ≡ *Oldenlandia virgata* (Willd.) DC., Prodr. 4: 425. 1830 ≡

Kohautia virgata (Willd.) Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48: 77. 1952 – Type: Ghana, s.l., probably *Thonning s.n.* (B†, holo; S, isoneo, designated by Bremekamp, 1952).

DISCUSSION

Generic delimitation problems within Spermacoceae. — Generic delimitations within Spermacoceae are complicated by the strong habitual similarity between the representatives, which makes it difficult to find morphological characters to define genera. The subdivision in genera is especially problematic in the *Hedyotis*-*Oldenlandia* complex, part of Spermacoceae including most species of the former tribe Hedyotideae. For centuries the taxonomic status of *Hedyotis* L., *Oldenlandia* L. and their satellite genera (e.g., *Amphiasma* Bremek., *Arcytophyllum* Willd. ex Schult. & Schult. f., *Houstonia* L., *Kohautia*, and *Kadua* Cham. & Schltdl.) has been a subject of discussion. The main issue has been whether most species of the complex should be lumped into *Hedyotis* (advocated by inter alia Merrill & Metcalf, 1946; Wagner & al., 1989; Fosberg & Sachet, 1991; Dutta & Deb, 2004), or if many small genera should be recognized in addition to a narrow circumscription of *Hedyotis* (supported for the African taxa by Bremekamp, 1952, for the neotropical taxa by Terrell & al., 1986; Terrell, 1991, 2001a–c, and for the Asian taxa by Terrell & Robinson, 2003).

In the past, the delimitation of genera has been a somewhat subjective activity; certain morphological features were considered important for some authors to recognize a species or a group of species at the generic level, while for other authors they were not convincing enough to segregate a new genus. This has resulted in numerous taxonomic debates, which in the absence of molecular data could not be solved. However, purely molecular studies of Spermacoceae (Kårehed & al., 2008; Groeninckx & al., 2009) answer some of these taxonomic debates, but at the same time they generate numerous new taxonomic problems. The present study of the genus *Kohautia* demonstrates that an integrative approach, combining molecular data with morphological observations, can further untangle the taxonomic webs in Spermacoceae. Moreover, integrative studies also allow describing new genera and new species with more certitude, as has been demonstrated by Groeninckx & al. (2010 and in press).

Phylogenetic relationships within genus *Kohautia* s.str. — *Kohautia ramosissima* and *K. cynanchica* are the earliest diverging species within the genus *Kohautia* s.str. Both species are centred in southern West Africa, and are distinguished from the other members of *Kohautia* s.str. by their 3-colporate pollen grains without a secondary reticulum. The two species differ from each other in a number of significant characters. *Kohautia ramosissima* differs from *K. cynanchica* in the more distinctly pedicellate and smaller flowers, and the larger pollen grains.

Subsequent branching produced a highly supported clade with the two melittophilous species, *K. aspera* and *K. azurea* (previously described in *K.* ser. *Diurnae*), as sister to the phalaenophilous species *K. subverticillata*. A close relationship between *K. aspera* and *K. subverticillata* is not surprising; a great deal of confusion has always existed in separating the two species because of their similar paired small, subsessile flowers. Both species are widespread herbs, occurring in Africa, south-west Arabia and India. *Kohautia azurea* has small flowers just like *K. aspera* and *K. subverticillata*, but is restricted to Namibia and is characterized by having 3-colporate pollen and pedicellate flowers.

One of the most morphologically particular species within the genus is *Kohautia amatymbica*. This hexaploid species can be distinguished from other representatives of *Kohautia* not only by its unique chromosome number, but also by its exclusively geoxylic suffrutescent habit (weedy habit but with a short, underground woody base), capitate inflorescences with large showy flowers, and its relatively large pollen and seeds. Its distinct morphology makes it very difficult to discuss its resemblance to other *Kohautia* species. The only species to which it shows a slight morphological resemblance is *K. kimuenzae* (De Willd.) Bremek. from South West Congo, which was not included in the present study. Our molecular data suggest *Kohautia amatymbica* to be related to *K. caespitosa* subsp. *caespitosa*, but this sister relationships lacks significant support. Morphologically and biogeographically this relationship is quite surprising. *Kohautia caespitosa* subsp. *caespitosa* is distributed from Egypt to Northeast Tropical Africa and occurs also in the Arabian Peninsula, whereas *K. amatymbica* occurs in Tropical Africa and South Africa.

Unexpectedly, *Kohautia caespitosa* subsp. *caespitosa* is not closely related to the other two *caespitosa* subspecies included in our analysis. Further research will have to confirm if the three subspecies are better treated as distinct species. Molecular data strongly support a relationship between *Kohautia caespitosa* subsp. *amaniensis* and *K. retrorsa*. Mantell (1985) already discussed a close relationship between the two species. *Kohautia retrorsa* has a south-east Arabian and Iranian distribution, forming a link between the African and Indian *Kohautia* species. Surprisingly, it shows more genetic and morphologic similarities to the Somalia-Masai centred *K. caespitosa* subsp. *amaniensis* (i.e., fruit shape and fruit wall) than to the neighbouring subsp. *caespitosa* of south-western Arabia.

Molecular data support a close relationship between the psychophilous species *K. coccinea*, *K. grandiflora* and *K. platyphylla* (originally described in *K.* ser. *Diurnae*), and the phalaenophilous species *K. tenuis* (originally described in *K.* ser. *Kohautia* and the type of the generic name *Kohautia*). The phalaenophilous *K. tenuis* is closely related to the psychophilous *K. grandiflora*. The sister relationship is well supported by molecular, morphological and biogeographical data. Both species are diploid and occur more or less sympatrically (centered in the Sudanian Regional Centre of Endemism) in open areas in dry fire-prone grassland and open woodland (*K. grandiflora* is also often found in seasonally waterlogged clay soils). They resemble each other in a number of morphological characters; both species have mucronate corolla lobe apices, subglobose to spherical capsules and similar testa cell sculpturing (testa cells with indistinctly reticulate radial walls and distinctly globular or alveolate periclinal walls). The main difference between *K. grandiflora* and *K. tenuis* lies in their inflorescence structure (compact, more or less corymbiform vs. staggered) and in the shape and colour of their corolla lobes (broad and brightly coloured above with distinct intrusions at the corolla throat vs. linear and white above without intrusion in the corolla throat).

Pollination shifts within genus *Kohautia* s.str. — Optimisation of pollination syndromes onto the molecular trees suggests that pollination shifts might have triggered speciation in the genus *Kohautia* s.str. Three pollination shifts have occurred in *Kohautia* s.str.; one shift from the ancestral state of moth pollination to butterfly pollination, a second from moth to bee pollination, and the third was a reversal from butterfly to moth pollination (Fig. 6A). The occurrence of pollination shifts raises questions about the genetic mechanisms underlying such transitions. At first it might appear that a simple shift from ancestral white corolla lobes to brightly coloured ones may have caused a shift from moth to butterfly pollination. However, more complex genetic adaptations are necessary to induce a shift in the formation of nectar guides, in the timing of flower opening (night vs. day), in scent release and nectar physiology. The genetic understanding of complex traits like pollination syndromes is making progress (Bradshaw & Schemske, 2003; Galliot & al., 2006; Cronk & Ojeda, 2008). In monkeyflowers (genus *Mimulus* L.), for example, Bradshaw & Schemske (2003) demonstrated that a single allele substitution at a flower colour locus could produce a shift from bee to hummingbird pollination. In the genus *Ipomoea* L., on the other hand, changes in the control of the anthocyanin biosynthetic pathway are associated with shifts in flower colour from purple to red, and shifts from bee to hummingbird pollination (Zufall & Rausher, 2003, 2004).

Natural selection for shifts in pollinators is often generated by "competitive" interactions with sympatric congeneric species (Armbruster, 1996). It is commonly concluded that competition for pollinators lowers reproductive success, and that evolution therefore selects for plant characteristics that reduce the extent of pollinator sharing. Differences in flowering time, but also in flower morphology and in flower colour are commonly attributed among sympatric species to selection for reducing competition for pollinators. As mentioned above, the phalaenophilous *K. tenuis* and the psychophilous *K. grandiflora* occur more or less sympatrically (Sudanian Regional Centre of Endemism). The different pollination syndromes in the two species may form an effective barrier preventing hybridization. Sympatric species with the same pollination syndrome, on the other hand, may be kept apart by mechanical isolation, i.e., extreme differences in flower size as in *K. coccinea* and *K. platyphylla*, or by differences in ploidy level, e.g., *K. cynanchica* (2*n*) and *K. ramosissima* (4*n*).

Phylogenetic relationships within *Cordylostigma*. — Our molecular data support a close relationship between *Cordylostigma cuspidata*, *C. longifolia* and *C. microcala*. Based on morphological observations, Mantell (1985) already suggested a close relationship between *C. longifolia* and *C. microcala*. In her revision, she even postulated that *C. microcala* might be an extreme form of the variable *C. longifolia*. Besides resemblance in morphology, the distribution range of the two species overlaps in the Southeastern and central parts of the Zambezian Region, where they grow in seasonally waterlogged soils, in grassland and open woodland. *Cordylostigma cuspidata* differs from *C. microcala* and *C. longifolia*, by its capituliform inflorescences, with 10–15 flowers with red, purplish-red or bright pink corolla lobes. In contrast to *C. microcala* and *C. longifolia*, *C. cuspidata* occurs in the western Zambezian Regional Centre of Endemism and the Northwestern part of the Kalahari-Highveld Transition Zone.

Sister to the clade of *Cordylostigma cuspidata*, *C. longifolia* and *C. microcala* is the micro-melittophylous *C. virgata*. *Cordylostigma virgata* is a widespread species with a disjunctive distribution (Tropical Africa, South Africa and the western Indian Ocean). The absence of hairs in the corolla throat, the lack of constrictions above and below the anthers, the often emergent sterile connectives on the anthers, and the seeds with wavy periclinal testa walls set *C. virgata* apart from all the other *Cordylostigma* species. Mantell (1985) suggested *C. virgata* to be closely related to *C. longifolia* because both species occur in Madagascar. Further research is needed to falsify this biogeographic speciation hypothesis.

Cordylostigma obtusiloba is sister to the remaining *Cordylostigma* species in our analysis. Morphologically, *C. obtusiloba* is a distinct and easily recognizable species. It has very slender stems arising from a short, inconspicuous (sometimes woody) underground stem; slender, pedicellate, few-flowered inflorescences; and flowers with relatively long, funnel-shaped corolla tubes with an unusual dilated apical part.

Floral evolution in genus *Kohautia* s.str. and genus *Cordylostigma*. — Molecular data reveal that the monomorphic short-styled flowers have evolved twice independently within Spermacoceae, i.e., in *Kohautia* s.str. and in *Cordylostigma*. Additional floral ontogenetic observations are needed to determine if the floral organization of *Kohautia* s.str. and *Cordylostigma* has common grounds. By studying the floral morphology of both genera into more detail, we noticed for example that the formation of nectar guides in the psychophilous species of *Kohautia* s.str. and *Cordylostigma* is accomplished in different ways but producing the same visual effect. In genus *Kohautia* s.str., nectar guides are formed by intrusions, whereas in genus *Cordylostigma* hairs function as nectar guides. Superficially the nectar guides look the same in the two genera, and could be incorrectly interpreted as homologous. Comparative floral morphology shows that the nectar guides of the two genera have a different structure. The nectar guides can be explained as an evolutionary response to similar selective pressures (i.e., adaptations to butterfly pollination) and they are thus treated as analogous. Floral morphology studies of the psychophilous species of *Kohautia* s.str. and *Cordylostigma* have not only demonstrated convergent evolutionary characters, but identified a discriminating character supporting each clade.

Pollen and pollination. — Pollen morphology may be correlated with pollination vectors (Hesse, 2000), in particular aperture and exine ornamentation characteristics are correlated with specific pollinators (Proctor & al., 1996; Tanaka & al., 2004). Our study shows that the psychophilous species of both genera *Kohautia* s.str. and *Cordylostigma* have pollen grains without a secondary reticulum. The function of tectal columellae is undoubtedly complex, but one function of the intercolumellar spaces is the accommodation of pollen surface coatings (Heslop-Harrison, 1979). Tectal columellae increase the space for surface materials and there is evidence that 'sticky' pollen, with additional coating materials occurs frequently in flowers associated with bird-pollination (Muller, 1981) or insect-pollination (Osborn & al., 1991). Unfortunately, these surface substances are removed by acetolysis and are soluble in ethanol. We could hypothesize that the absence of a secondary reticulum may increase the space for sticky coating materials, which in turn may increase the pollination effectiveness; 'sticky' pollen could be a mechanism to increase the pollen transfer by butterflies. Unfortunately, pollination studies within the tribe Spermacoceae are scarce, making it very difficult to confirm or refute this hypothesis.

CONCLUSIONS

Our study confirms that *Kohautia* as traditionally delimited is biphyletic. Both molecular and morphological data show the need to recognize *K.* subg. *Kohautia* as making up the entire genus *Kohautia* and *K.* subg. *Pachystigma* as the new genus *Cordylostigma*.

Morphological similarity between species of Spermacoceae has generated systematic confusion. This paper demonstrates that detailed integrated studies, combining molecular and morphological data, can help to further solve these taxonomic problems.

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Appendix. List of taxa used in the phylogenetic analysis with voucher information (geographic origin, collector, collector number, herbarium). Previous published sequences of *atpB-rbcL*, *rps16*, *trnL-trnF*, *petD*, ETS and ITS are provided with accession numbers and literature citations. New sequences are marked in bold. Key to literature citations: (1) = Andersson & al., 2002; (2) = Groeninckx & al., 2009; (3) = Kårehed & al., 2008; (4) = Kårehed & Bremer, 2007.

Agathisanthemum Klotzsch: ***A. bojeri*** Klotzsch, Zambia, *Dessein & al. 671* (BR), EU542917[2], EU543018[2], EU543077[2], EU557678[3], –, AM939424[3]. ***Arcytophyllum*** Willd. ex Schult. & Schult. f.: ***A. thymifolium*** (Ruiz & Pav.) Standl., Ecuador, *Ståhl 4481* (GB), EU542923[2], AF333366[1], EU543082[2], EU557683[3], AM932921[3], AM939431[3]. ***Bouvardia*** Salisb.: ***B. glaberrima*** Engelm., cult., *Forbes s.n.* (S), EU542925[2], EU543022[2], EU543084[2], EU557685[3], AM932922[3], AM939432[3]. ***Dentella*** J.R. Forst & G. Forst.: ***D. repens*** (L.) J.R. Forst. & G. Forst., Australia, *Andersson 2262* (GB), EU542932[2], AF333370[1], EU543091[2], EU557693[3], AM932930[3], AM939440[3]. ***Hedyotis*** L.: ***H. fruticosa*** L., Sri Lanka, *Larsson & Pyddoke 22* (S), EU542942(2), –, EU543098(4, EU557702(3), AM932941(3), AM939453(3). ***Houstonia*** L.: ***H. caerulea*** L., USA, *Vincent & Lammers s.n.* (GB), EU542953(2), AF333379[1], EU543109(2), EU557713(3), –, AM939464(3). ***Kadua*** Cham. & Schltdl.: ***K. degeneri*** (Fosberg) W.L. Wagner & Lorence, cult., *Wood 5062* (PTGB), EU542958(2), AF333371[1], EU543113[2], EU557717[3], AM932953[3], AM939470[3]. ***Kohautia*** Cham. & Schltdl.: ***K. amatymbica*** Eckl. & Zeyh., South Africa, *Bremer & al. 4307* (UPS), EU542962[2], EU543035[2], EU543117[2], EU557721[3], AM932956[3], AM939484[3]; South Africa, *Venter & Venter 10287* (MO), **GU951588, GU951636, GU951652, GU951621, GU951600,** –; ***K. aspera*** (B. Heyne ex Roth) Bremek., Tanzania, *Kayombo & Kitaba 4230* (BR), –, **GU951640, GU951656,** –, **GU951604,** –; ***K. australiensis*** Halford, Australia, *Latz 17736* (BR), **GU951589**, **GU951637**, **GU951653, GU951622**, **GU951601**, **GU951611**; Australia, *Albrecht & Latz 12201* (BR), **GU951590, GU951638, GU951654, GU951623, GU951602, GU951612; *K. azurea*** (Dinter & K. Krause) Bremek., Namibia, *Seydel 3491* (BR), **GU951591, GU951639, GU951655, GU951624, GU951603, GU951613; *K. caespitosa*** Schnizl. subsp. ***amaniensis*** (K. Krause) Govaerts, Ethiopia, *de Wilde 5976* (BR), **GU951592, GU951641, GU951657, GU951625,** –, **GU951614; *K. caespitosa*** Schnizl. subsp. ***brachyloba*** (Sond.) D. Mantell, Zambia, *Dessein & al. 432* (BR), EU542963[2], EU543036[2], EU543118[2], EU557722[3], AM932957[3], AM939474[3]; Tanzania, *Kuchar 25133* (BR), **GU951593, GU951642, GU951658, GU951626, GU951605, GU951615; *K. caespitosa*** Schnizl. subsp. ***caespitosa***, Zambia, *de Wilde 4618* (BR), –, –, **GU951659,** –, –, –; ***K. coccinea*** Royle, Zambia, *Dessein & al. 751* (BR), EU542964[2], EU543037[2], EU543119[2], EU557723[3], AM932959[3], AM939476[3]; s.l., *Wieringa 4916* (WAG), **GU951594**, **GU951643**, **GU951660, GU951627,** –, –; ***K. cuspidata*** (K. Schum.) Bremek., Malawi, *La Croix 4501* (MO), **GU951595,** –, **GU951661, GU951628, GU951606, GU951616; *K. cynanchica*** DC., South Africa, *Dessein & al. 469* (BR), EU542965[2], EU543038[2], EU543120[2], EU557724[3], AM932960[3], AM939477[3]; ***K. dolichostyla*** Bremek., Somalia, *Thulin 10819* (BR), **GU951596, GU951644, GU951662, GU951629, GU951607, GU951617; *K. grandiflora*** DC., Ethiopia, *Friis & al. 6864* (BR), **GU951597, GU951645, GU951663, GU951630, GU951608, GU951618; *K. longifolia*** Klotzsch, Tanzania, *Bidgood & al. 2595* (BR), –, **GU951646**, **GU951664, GU951631**, –, –; ***K. microcala*** Bremek., Zambia, *Dessein & al. 1149* (BR), EU542966[2], EU543039[2], EU543121[2], EU557725[3], AM932962[3], AM939479[3]; ***K. obtusiloba*** (Hiern) Bremek., Kenya, *Luke 9035* (UPS), EU542967[2], EU543040[2], EU543122[2], EU557726[3], AM939481[3], –; Kenya, *Luke & Robertson 2323* (MO), **GU951598, GU951647, GU951665,** –, –, –; ***K. platyphylla*** (K. Schum.) Bremek., Ethiopia, *Friis & al. 439* (BR), –, **GU951648, GU951666, GU951632,** –, –; ***K. ramosissima*** Bremek., Namibia, *Merxmüller & Giess 32496* (BR), –, **GU951649, GU951667,**

Appendix. Continued.

GU951633, GU951609, –; ***K. retrorsa*** (Boiss.) Bremek., Oman, *Ghazanfar 1823* (BR), –, **GU951650, GU951668, GU951634,** –, –; ***K. subverticillata*** (K. Schum.) D. Mantell, Zambia, *Dessein & al. 470* (BR), EU542968[2], EU543041[2], EU543123[2], –, EU557727[3], **GU951619; *K. tenuis*** Cham. & Schltdl., Burkina Faso, *Madsen 5940* (MO), **GU951599, GU951651, GU951669, GU951635, GU951610, GU951620; *K. virgata*** (Willd.) Bremek., Madagascar, *De Block & al. 539* (BR), EU542969[2], –, EU543124[2], EU557728[3], AM939483[3], AM932965[3]. ***Oldenlandia*** L.: ***O. capensis*** L. f. var. ***capensis***, Zambia, *Dessein & al. 843* (BR), EU542980[2], EU543048[2], EU543133[2], EU557737[3], AM932974[3], AM939496[3]; ***O. corymbosa*** L., Zambia, *Dessein & al. 487* (BR), EU542982[2], EU543050[2], EU543135[2], EU557739[3], AM932979[3], AM939502[3]; ***O. goreensis*** (DC.) Summerh., Zambia, *Dessein & al. 1286* (BR), EU542988[2], EU543055[2], EU543141[2], EU557745[3], AM932985[3], AM939510[3]; ***O. herbacea*** (L.) Roxb. var. ***herbacea***, Zambia, *Dessein & al. 463* (BR), EU542990[2], EU543057[2], EU543143[2], EU557747[3], AM932988[3], AM939552[3]; ***O. mitrasacmoides*** F. Muell., Australia, *Harwood 1516* (BR), EU542993[2], –, EU543146[2], EU557750[3], AM932992[3], AM939515[3]. ***Pentanopsis*** Rendle: ***P. fragrans*** Rendle, Ethiopia, *Gilbert & al. 7458* (UPS), –, EU543065[2], EU543153[2], EU557758[3], AM933002[3], AM939526[3]. ***Pentodon*** Hochst.: ***P. pentandrus*** (K. Schum. & Thonn.) Vatke, Zambia, *Dessein & al. 598* (BR), EU543002[2], EU543066[2], EU543154[2], EU557759[3], AM933003[3], AM939528[3]. ***Spermacoce*** L.: ***S. erosa*** Harwood, Australia, *Harwood 1148* (BR), EU543008[2], EU543070[2], EU543159[2], EU557765[3], AM933009[3], AM939537[3].

Outgroup: ***Batopedina*** Verdc.: ***B. pulvinellata*** E. Robbr., Zambia, *Dessein & al. 264* (BR), EU542924[2], EU543021[2], EU543083[2], EU557684[3], –, AM266989[4]. ***Carphalea*** Juss.: ***C. madagascariensis*** Lam., Madagascar, *De Block & al. 578* (BR), EU542926[2], EU543023[2], –, EU557686[3], –, AM267020[4]. ***Pentanisia*** Harv.: ***P. parviflora*** Stapf ex Verdc., Zambia, *Dessein & al. 678* (BR), EU543001[2], EU543064[2], EU543152[2], EU557757[3], –, AM266995[4].