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A NEW SPECIES OF COLLETOECEMA (RUBIACEAE) FROM SOUTHERN CAMEROON WITH A DISCUSSION OF RELATIONSHIPS AMONG BASAL RUBIOIDEAE

B. SONKÉ[1], S. DESSEIN[2], H. TAEDOUMG[1],
I. GROENINCKX[3] & E. ROBBRECHT[2]

SUMMARY

Colletoecema magna, a new species from the Ngovayang Massif (southern Cameroon) is described and illustrated. A comparative morphological study illustrates the similar placentation and fruit anatomy of the novelty and *Colletoecema dewevrei*, the only other species of the genus. *Colletoecema magna* essentially differs from *C. dewevrei* by its sessile flowers and fruits, the corolla tube that is densely hairy above the insertion point of the stamens and the anthers that are included. Further characters that separate the novelty are its larger leaves, more condensed inflorescences, and larger fruits. Its position within *Colletoecema* is corroborated by *atpB-rbcL* and *rbcL* chloroplast sequences. The relationships among the basal lineages of the subfamily Rubioideae, to which *Colletoecema* belongs, are briefly addressed. Based on our present knowledge, a paleotropical or tropical African origin of the Rubioideae is hypothesized.

Key words: Rubioideae, Rubiaceae, *Colletoecema*, chloroplast DNA, Ngovayang massif.

INTRODUCTION

Up to now, *Colletoecema* was known from a single species, i.e. *C. dewevrei* (De Wild.) E.M.A.Petit, a Guineo-Congolian endemic. The genus was established by Petit (1963) based on '*Plectronia*' *dewevrei* (Rubiaceae, Vanguerieae), a species described by De Wildeman (1904). Petit (1963) demonstrated that this species does not belong to the *Canthium* complex and described a new genus, i.e. *Colletoecema*. He also showed that the original position in Vanguerieae could not be upheld. Based on the presence of raphides and the downward orientation of the embryo radicle (versus absence of raphides and upward orientation in Vanguerieae), he considered Morindeae sensu Schumann (1891) a better alliance to place the genus. This position was maintained in Robbrecht's (1988) classification.

Igersheim & Robbrecht (1993) proposed a new delimitation of Morindeae thereby restricting the tribe to five genera, i.e. *Appunia* Hook.f., *Caelospermum* Blume, *Gynochthodes* Blume, *Morinda* L., and *Pogonolobus* F.Muell. Subsequently, Robbrecht (1993) placed *Lasianthus* Jack, *Trichostachys* Hook.f., and *Colletoecema* tentatively

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1) Plant Systematic and Ecology Laboratory, Higher Teacher's Training College, University of Yaounde I, P.O. Box 047, Yaounde, Cameroon.
2) National Botanic Garden of Belgium, Domein van Bouchout, BE-1860 Meise, Belgium.
3) Laboratory of Plant Systematics, K.U.Leuven, Kasteelpark Arenberg 31, P.O. Box 2437, BE-3001 Leuven, Belgium.

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Table 1. Species included in the molecular study with reference to GenBank accession numbers. Voucher data are given for newly produced sequences.

Taxon	*rbcL*	*atpB-rbcL*	Voucher information
Alstonia scholaris (L.) R.Br.	AJ431032	DQ359161	
Appunia guatemalensis Donn.Sm.	AJ288593	AJ234009	
Argostemma hookeri King	Z68788	AJ234032	
Argostemma pumilum Benn.	FJ209065	FJ226535	Gabon, *Dessein et al. 1690* (BR)
Bouvardia ternifolia (Cav.) Schltdl.	X81093	X76478	
Caelospermum balansaeanum Baill.	AJ288598	AJ234010	
Calycophyllum candidissimum (Vahl) DC.	X83627	DQ131708	
Chiococca alba (L.) Hitchc.	L14394	DQ131711	
Cinchona pubescens Vahl	X83630	AJ233990	
Coccocypselum guianense (Aubl.) K.Schum.	FJ209066	FJ226536	Cultivated at BR, voucher: *Van Caekenberghe 170* (BR)
Coffea arabica L.	X83631	X70364	
Colletoecema dewevrei (De Wild.) E.M.A.Petit	FJ209067	DQ131713	*Lejoly 82/882* (BR)
Colletoecema magna Sonké & Dessein	FJ209068	FJ226556	Cameroon, *Dessein & Sonké 1608* (BR)
Coprosma repens A.Rich.	FJ209069	FJ226537	Cultivated at BR, voucher: *Van Caekenberghe 267* (BR)
Coussarea brevicaulis K.Krause	FJ209070	FJ226538	Brazil, *De Granville et al. 5812* (BR)
Cruckshanksia hymenodon Hook. & Arn.	AJ288599	AJ234004	
Damnacanthus indicus C.F.Gaertn.	Z68793	AJ234015	
Danais xanthorrhoea (K.Schum.) Bremek.	Z68794	AJ234019	
Didymaea alsinoides (Cham. & Schltdl.) Standl.	Z68795	AJ234036	
Faramea multiflora A.Rich.	FJ209071	FJ226539	French Guiana, *Billiet & Jadin 7414* (BR)
Geophila repens (L.) I.M.Johnst.	Z68798	AJ234017	
Hamelia papillosa Urb.	AY538487	AJ233992	
Hydnophytum formicarum Jack	X83645	X76480	
Hymenodictyon floribundum (Hochst. & Steud.) B.L.Rob.	AY538488	DQ131742	
Lasianthus pedunculatus E.A.Bruce	Z68802	AJ234003	
Lerchea bracteata Valeton	AJ288610	X83648	
Luculia grandifolia Ghose	X83648	AJ233986	
Manettia luteorubra (Vell.) Benth.	Z68803	DQ131750	
Morinda citrifolia L.	AJ318448	AJ234013	
Mostuea brunonis Didr.	L14404	DQ131697	
Mycetia malayana (G.Don) Craib	Z68806	AJ234033	
Neurocalyx zeylanicus Hook.	Z68807	AJ233995	
Oldenlandia goreensis (DC.) Summerh.	Z68808	EU542988	
Ophiorrhiza japonica Blume	FJ209072	FJ226540	Taiwan, *Jer-haur Lii 881* (BR)
Ophiorrhiza mungos L.	FJ209082	FJ226541	Cultivated at BR, voucher: *Van Caekenberghe 15* (BR)
Paederia foetida L.	AF332373	AJ234006	
Pauridiantha callicarpoides (Hiern) Bremek.	FJ209086	FJ226542	Gabon, *Dessein et al. 2502* (BR)
Pauridiantha floribunda (K.Schum. & K.Krause) Bremek.	FJ209084	FJ226543	Gabon, *Dessein et al. 1821* (BR)
Pauridiantha letestuana (N.Hallé) Ntore & Dessein	FJ209083	FJ226544	Gabon, *Dessein et al. 1835* (BR)
Pauridiantha liebrechtsiana (De Wild. & T.Durand) Ntore & Dessein	FJ209079	FJ226545	Gabon, *Dessein et al. 2470* (BR)
Pauridiantha talbotii (Wernham) Ntore	FJ209087	FJ226546	Gabon, *Dessein et al. 1758* (BR)
Pentaloncha humilis Hook.f.	FJ209074	FJ226547	Gabon, *Dessein et al. 1694* (BR)

Pentas lanceolata (Forssk.) Deflers	X83659	X76479	
Pentodon pentandrus (Schumach. & Thonn.) Vatke	X83660	AJ234024	
Phyllis nobla L.	Z68814	AJ234031	
Phylohydrax carnosa (Hochst.) Puff	AJ288615	EU543003	
Plocama calabrica (L.f.) M.Backlund & Thulin	AJ288620	X81672	
Plocama pendula Aiton	Z68816	AJ234035	
Poecilocalyx crystallinus N.Hallé	FJ209076	FJ226548	Gabon, *Dessein et al. 1961* (BR)
Poecilocalyx schumannii Bremek.	FJ209075	FJ226549	Gabon, *Dessein et al. 2419* (BR)
Pravinaria leucocarpa Bremek.	FJ209073	FJ226550	Indonesia, *Sands 3814* (BR)
Psychotria poeppigiana Müll.Arg.	Z68818	AJ234018	
Rubia tinctorum L.	X83666	X76465	
Sarcocephalus latifolius (Sm.) E.A.Bruce	X83667	DQ131785	
Schradera subandina K.Krause	Y11859	AJ234014	
Sipanea biflora (L.f.) Cham. & Schltdl.	AY538509	DQ131788	
Stelechantha cauliflora (R.D.Good) Bremek.	FJ209080	FJ226551	Gabon, *Dessein et al. 1714* (BR)
Stelechantha makakana N.Hallé	FJ209081	FJ226552	Cameroon, *Dessein & Sonké 1483* (BR)
Temnopteryx sericea Hook.f.	FJ209085	FJ226555	Gabon, *Dessein et al. 1768* (BR)
Theligonum cynocrambe L.	X83668	X81680	
Triainolepis africana subsp. *hildebrandtii* (Vatke) Verdc.	AJ288625	AJ234020	
Triainolepis xerophila (Bremek.) Kårehed & B.Bremer	AJ288633	DQ131759	
Trichostachys aurea Hiern	FJ209077	FJ226553	Gabon, *Dessein et al. 1696* (BR)
Trichostachys microcarpa K.Schum.	FJ209078	FJ226554	Gabon, *Dessein et al. 2411* (BR)
Urophyllum ellipticum (Wight) Thwaites	AJ288627	AJ234002	
Xanthophytum capitellatum Ridl.	AJ288628	AJ233996	

in Psychotrieae, calling for more detailed investigations to confirm this position. Based on molecular and morphological data, Piesschaert et al. (2000) came to the conclusion that *Colletoecema* is not a member of Psychotrieae nor a member of Morindeae or any other formal or informal group close to Psychotrieae. Their analysis of *rps16* intron sequences placed *Colletoecema* as sister to *Ophiorrhiza* in a weakly supported clade. This clade is part of a basal trichotomy with the strongly supported Urophylleae and a weakly supported clade comprising all other Rubioideae. In the supertree of Robbrecht & Manen (2006), *Colletoecema* is sister to the rest of the subfamily Rubioideae, a position that confirms the morphological isolation of the genus stressed by the authors. This position was confirmed by Rydin et al. (2008) based on the analysis of five chloroplast regions (*rbcL*, *ndhF*, *trnT-L-F*, *rps16 intron*, *atpB-rbcL* spacer).

During fieldwork in the Ngovayang massif, one of the largest intact forest remnants in the South Province of Cameroon, material was collected from a yet unknown Rubiaceae species. The presence of raphides and the valvate corolla aestivation points to a position within Rubioideae. The ribbed fruits, each containing one bilocular and very thick-walled pyrene, allows us to conclude that the material belongs to *Colletoecema*. This is further supported by the triangular stipules with a ± acute apex and ciliate margin, the axillary inflorescences paired at the nodes, and the cupuliform calyces. However, the material clearly differs from *C. dewevrei* in the larger leaves with more numerous secondary nerves, the much more condensed inflorescences, the hairy upper part of the corolla tubes, the included anthers, and the larger, sessile fruits. Therefore, the new species *C. magna* is here described and illustrated. In addition detailed morphological observations and inference of its phylogenetic position based on chloroplast DNA are presented.

MATERIAL AND METHODS

Material

Colletoecema magna was collected and studied in the in field March 2007 (specimen *Dessein & Sonké 1608*). Herbarium material of *Colletoecema* was consulted at BR, K, and WAG (abbreviations after Holmgren et al. 1990). Measurements, colours and other details given in the descriptions are based on living material, spirit and herbarium specimens, and data derived from field notes.

To confirm the generic placement of the novelty with molecular data and to determine the position of *Colletoecema* within Rubioideae, a broad sampling of taxa from both subfamilies of Rubiaceae (Table 1) was selected from GenBank and new sequences were produced, especially for taxa belonging to the 'basal Rubioideae'. In total the sampling includes 55 taxa of subfamily Rubioideae, representing 44 genera, and 9 of subfamily Cinchonoideae sensu Robbrecht & Manen (2006). As outgroup we selected two members of Gentianales outside Rubiaceae. Table 1 provides a list of taxa included in the phylogenetic analyses of *rbcL* and *atpB-rbcL*, with GenBank accession numbers and voucher information for the here newly produced sequences. New *rbcL* sequences have been produced for 23 taxa and there are 22 new *atpB-rbcL* spacer sequences.

Terminology

Phytogeographical considerations follow White (1979). Descriptive terminology for simple symmetrical plane shapes follows Anonymous (1962).

Micromorphological and anatomical observations

Pollen data are based on observations of acetolyzed pollen grains obtained by following the protocol outlined by Dessein et al. (2002). Scanning electron microscopic observations were made with a Jeol JSM 5800 LV (25 kV) and LM observation with an Olympus BX51 with a x100 oil immersion lens. Equatorial diameter (E) and polar axis (P) were measured under LM in at least ten mature pollen grains.

Anatomical ovary sections were made from material preserved in 70% ethanol dehydrated through a graded ethanol series. Gynoecia were embedded in KULZER's Technovit 7100 (based on hydroxyethyl-methacrylate, HEMA) as detailed by Igersheim (1993). The material was gradually infiltrated with LR white resin (hard grade) (London Resin) using 1:3, 1:1, 3:1, and 1:0 solutions of resin and 100% ethanol for at least 5 h each. The ovary samples were placed in closed capsules filled with fresh resin, and polymerized at 60 °C for 48 h. Ovary sections of 2–3 μm in thickness were made with a rotary microtome (Microm HM 360, Walldorf, Germany) and stained with 0.1% toluidin blue. Photographs were taken under a Leitz Dialux 20 microscope (Wetzlar, Germany) equipped with an Olympus DP50 camera (Hamburg, Germany).

Molecular study

Leaf material was sampled from herbarium specimens or taken from silica gel dried material. Genomic DNA was extracted using the Puregene Genomic DNA Purification Kit (GENTRA Systems, Minnesota) following the manufacturer's Plant Tissue extraction protocol. Amplification reactions were prepared for a 50 μl final volume containing 5 μl 10x Taq Buffer (Roche), 2.5 μl of each of the 20 μM primers, 1 μl of 10 mg ml^{-1} bovine serum albumin (Ambion # 2616), 1 μl of 25 mM $MgCl_2$, 0.25 μl Taq DNA polymerase (Roche), 5 μl dNTP and 1 μl of template genomic DNA. PCR reactions were performed on Peltier Thermal Cyclers PTC-100 or PTC-150 (MJ Research). Amplification of the *atpB-rbcL* spacer was done with oligonucleotide primers 2 and 5 (Manen et al. 1994) as described by Groeninckx et al. (In press). *RbcL* sequences were amplified using the following primer pairs: z1/1020R and 427BS/3'. For sequencing reactions the following primers were used: z1, 1020R, 1204R, and 895 (Andreasen & Bremer 2000). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen). The yield of the PCRs was verified by running the products on a 1% agarose gel using ethidium bromide. PCR products were sequenced by the Macrogen (Seoul, South Korea) sequencing facilities. These sequences were assembled with Sequencher version 4.6 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences of *atpB-rbcL* were first aligned using Clustal X (Thompson et al. 1997) applying the default parameters. This alignment was further manually corrected in MacClade 4.04 (Maddison & Maddison 2005). Regions of ambiguous alignment were removed. The edited sequences were analyzed with gaps treated as missing data and phylogenetic informative insertions and/or deletions coded according to the 'simple indel coding' method of Simmons & Ochoterena (2000). The aligned matrix contained 1134 characters of which 320 were parsimony informative. Of these, 36 were indel characters. Alignment of *rbcL* sequences was straightforward and without indels. The matrix contained 1401 characters of which 309 were parsimony informative.

Phylogenetic analyses

Preliminary analyses showed that the *atpB-rbcL* spacer trees and the *rbcL* trees are very similar, as already demonstrated by Bremer & Manen (2000) and Rydin et al. (2008). Therefore, the two datasets were combined, which resulted in better resolution within the trees and better support values for many branches.

Maximum parsimony

Heuristic tree searches were carried out in PAUP* version 4.0b10 (Swofford 2002) using 10 000 replicates of random taxon sequence addition, holding 10 trees at each step, with tree-bisection-reconnection (TBR) branch swapping, MulTrees in effect and saving no more than 10 trees per replicate. Support values were calculated using bootstrap analysis (Felsenstein 1985). One thousand replicates of simple sequence addition, TBR swapping, MulTrees in effect, and saving 10 trees per replicate were performed in PAUP*. Clades with bootstrap values greater than 85% were considered well supported, while clades with 75–84% and 50–74% bootstrap support were treated as moderately and weakly supported, respectively.

Bayesian analysis

Evolutionary models for each plastid region were selected using Modeltest v3.06 (Posada & Crandall 1998) under the Akaike information criterion. The nucleotide substitution model that best fits the *atpB-rbcL* data was TVM+G and for the *rbcL* dataset the GTR+G+I model. As the TVM+G model is not implemented in MrBayes, the next more complex model, in our case GTR+G model, was used as recommended by Ronquist et al. (2005). The combined dataset was partitioned into three discrete units. Apart from the two plastid regions, there was a third partition for the phylogenetic informative indels. The restriction site (binary) model of evolution was implemented for the indel data, following Ronquist et al. (2005). Four independent Bayesian searches, each consisting of two simultaneous parallel analyses, were carried out using MrBayes 3.1 (Huelsenbeck & Ronquist 2001). In each Bayesian analysis four Markov Chains (three heated, one cold) were run simultaneously for 1 000 000 generations, sampling trees every 500 generations. The initial 25% of trees were discarded as a conservative burn in. After confirming by eye that trees generated from separate analyses had consistent topologies, the 'post-burn in' trees from each analysis were pooled together, imported into PAUP* and summarized by majority rule consensus, with values on tree equating to posterior probabilities (PP). Only PP values equal to or higher than 0.95 were considered significant (Suzuki et al. 2002).

RESULTS

DESCRIPTION

Colletoecema magna Sonké & Dessein, *spec. nov.* — Fig. 1–3

> Affinis *Colletoecemae dewevrei* sed ab illa differt foliorum majoribus laminis, nervorum secundariorum numero utrinque 7–10 (versus 4–6 in *C. dewevrei*), floribus sessilibus, corolla intus pubescenti super antheras (versus corolla intus pubescenti infra antheras in *C. dewevrei*) atque fructibus multo majoribus. — Typus: *Dessein & Sonké 1608* (holo BR; iso BR, K, MO, P, WAG, YA), Cameroon, Ngovayang massif, near Bidjouka, 03°09'N 10°28'E, c. 526 m alt.

Tree 8 m high; dbh 17 cm; bark greyish; young twigs glabrous. *Stipules* triangular, free at the base, 7–10 by 5–7 mm, glabrous outside, beset with colleters inside, apex acute, rarely ± rounded. *Petioles* 12–23 mm long, glabrous; leaf blades obovate, 15.2–28 by 5–9.8 cm, coriaceous, glabrous, dark green and glossy above, lighter green below; leaf apex shortly acuminate, 8–13 mm long; leaf base cuneate; midrib prominent below; secondary nerves 7–10 pairs, somewhat prominent below, very slightly prominent above; intersecondary veins forming a fine reticulate network; domatia absent. *Inflorescences* axillary, paired at the nodes, congested, sessile, few-flowered. *Flowers* possibly isostylous. *Calyx* green, cupuliform; tube c. 1.5 mm high, glabrous; lobes 0.3–0.5 by c. 1.5 mm, ciliate. *Corolla* tube white, cylindrical or slightly funnel-shaped, 2.3–5 mm long, glabrous outside, densely pubescent above the insertion point of the anthers; corolla lobes with valvate aestivation, white, triangular, thickened towards the margins and the apex, 2–3 by 1.2 mm, glabrous outside, pubescent at the base inside. *Stamens* white, inserted between the middle and the top of the corolla tube, tips shortly exceeding the corolla tube but included in the hairy throat; anthers elliptic, shortly

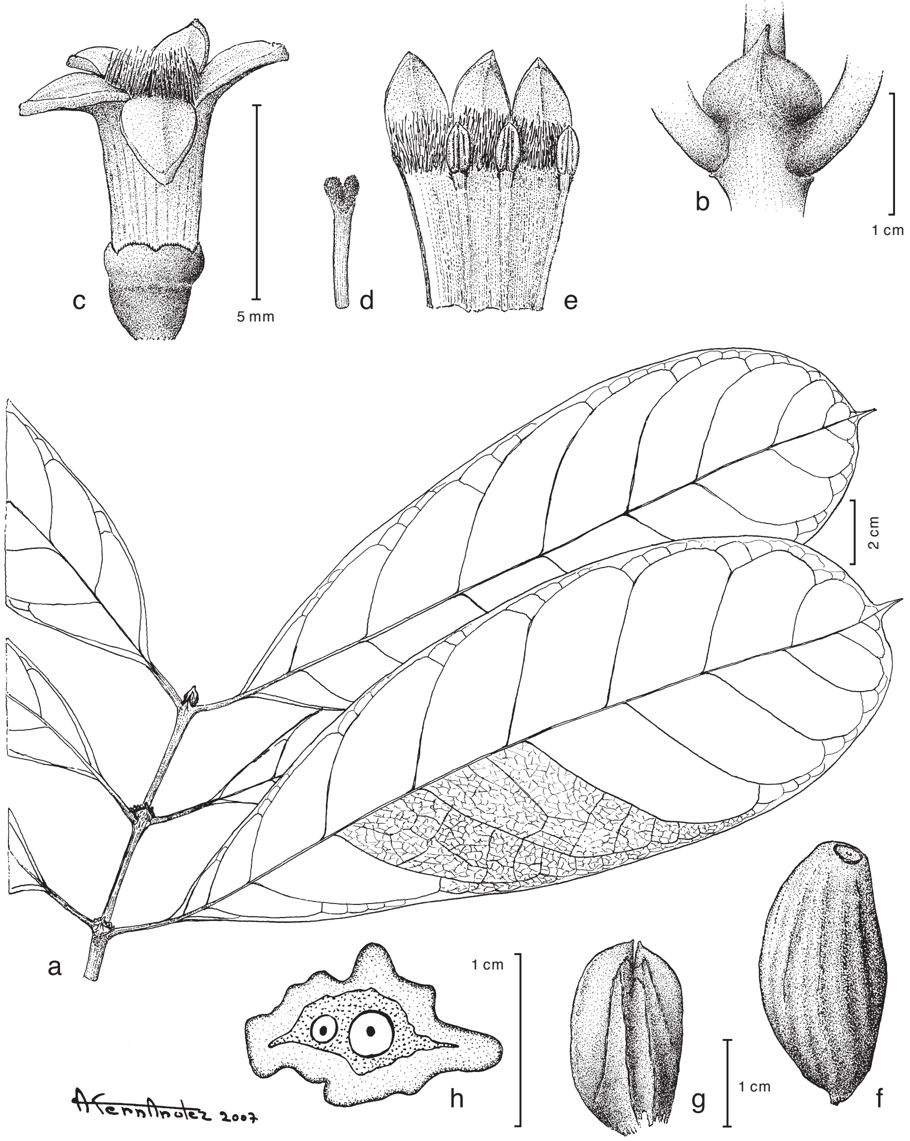

Fig. 1. *Colletoecema magna* Sonké & Dessein. a. Flowering branch with inconspicuous congested axillary inflorescences; b. stipule with ciliate margin; c. calyx and corolla; d. style; e. dissected corolla with two lobes removed; f. immature fruit; g. pyrene; h. cross section of fruit showing a single pyrene with two seeds (from outside to inside: exocarp, finely stippled mesocarp, coarsely stippled endocarp, black air-space, white seed-coat and endosperm, black embryonal cavity) (*Dessein & Sonké 1608*, BR).

appendiculate, 1.2–1.5 mm long; filaments dorsimedifixed, c. 1.3 mm, sparsely hairy. *Ovary* 2-locular, broadly obovoid, c. 2.5 by 2.3 mm, glabrous; style included, glabrous, 2–3.5 mm with two papillose stigmatic lobes to 1 mm long; nectary disk white, well developed, 1.2–1.6 mm high, 2–2.3 mm diam.; 1 erect ovule in each locule. *Drupes*

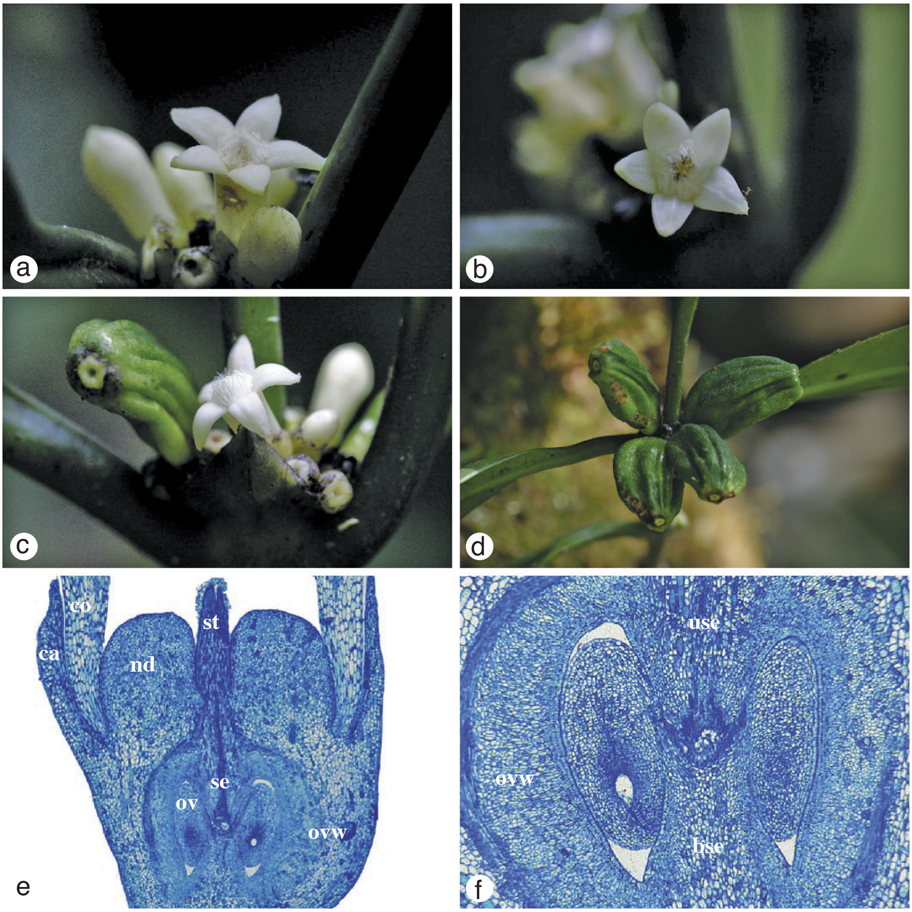

Fig. 2. Flowers, fruits and placentation of *Colletoecema magna* Sonké & Dessein. a. Side view of open flower; b. top view of open flower; c. side view of flowering and fruiting node; d. side view of fruiting node; e. longitudinal section of gynoecium; f. detail of longitudinal section of gynoecium showing attachment of ovules. Abbreviations: bse = basal part of septum; co = corolla tube; ca = calyx; nd = nectary disk; ov = ovules; ovw = ovary wall; se = septum; st = style; use = upper part of septum.

only seen immature, green with white persistent disk, elliptic, somewhat flattened, ribbed, 27–31 by 13–17 mm, glabrous; endocarp consisting of one 2-locular pyrene. *Pyrenes* elliptic, flattened, ribbed, with a split at the tip and fibrous at base, c. 24 by 15 mm, with a cylindrical seed in each locule.

Distribution & Habitat — *Colletoecema magna* is known from the Lower Guinean subcentre of endemism (White 1979), and is restricted to southern Cameroon. The area from which *C. magna* was collected supports a closed-canopy evergreen forest with many epiphytes and a rich herb layer, classified by Letouzey (1985) as Biafran evergreen forests, rich in Caesalpiniaceae.

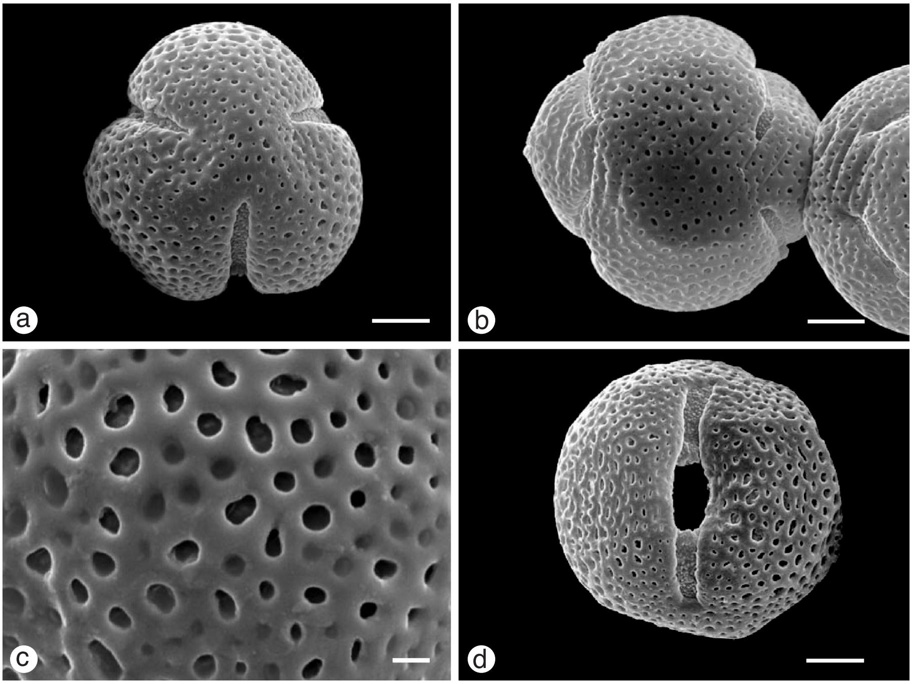

Fig. 3. Pollen morphology of *Colletoecema magna* Sonké & Dessein. a. Polar view of tricolporate pollen grain; b. polar view of tetracolporate pollen grain; c. detail of apocolpium showing perforate to (micro)reticulate sexine; d. equatorial view of pollen grain. — Scale bars: a, b, d = 5 μm, c = 1 μm.

Conservation status — Data deficient. *Colletoecema magna* on the evidence available, is highly localized, being known only from the southern edge of the Ngovayang massif. Despite the fact that the species has conspicuous fruits, and the fact that several lengthy visits have been made to the Ngovayang massif over recent years, *C. magna* is only known from one site and one single specimen. Further searching is needed to find more locations for this tree. Given its apparent rarity, *C. magna* may be a suitable subject for a propagation and reintroduction scheme. The Ngovayang massif is well known as a refuge for narrowly endemic plant species. Examples from Rubiaceae alone include *Coffea mapiana* (Sonké et al. 2006b), *Chassalia bipindensis* (Sonké et al. 2006a), and *Argocoffeopsis spathulata* (Davis & Sonké 2008).

Phenology — Flowering and young fruits March – April.

Etymology — The specific epithet '*magna*' refers to its larger leaves and fruits compared to *C. dewevrei*.

Notes — The collection *De Kruif 986* (WAG, YA) is very similar to *C. magna*, but differs in the pedicellate fruits and the somewhat different shape of the leaves, i.e. rather elliptic instead of obovate and with a longer acumen. The specimen was collected near Bipaga II, km 40 along the road Kribi-Edéa at an altitude of 30 m, c. 50 km from the site of the type collection of *C. magna*. Without flowers, its status cannot be determined.

Issembé 873 (WAG) and *Wieringa 5254* (WAG), both from Gabon, probably represent a third species of *Colletoecema*. They resemble *C. magna* in having sessile fruits, but leaf and fruit size are closer to *C. dewevrei*. Flowers are needed to confirm and describe this taxon.

Micromorphological and anatomical observations

Gynoecium — The gynoecium of *C. magna* is bicarpellate and each locule is uniovulate (Fig. 2e, f). Numerous needle-like crystals and dark coloured idioblasts are observed in the gynoecial wall. The anatropous ovules, which lack an obturator of any kind, are attached around the middle of the septum. This septum clearly consists of an apical and a basal part during its development and the ovules are attached to the basal part (Fig. 2e, f). On top of the ovary, a well-developed nectar disk, surrounding the base of the style, is present. The nectar disk of *C. magna* is, unlike the one of *C. dewevrei*, not provided with striate papillae. The style ends in a shortly bilobed, papillose stigma, but is otherwise glabrous.

Pollen — Pollen of *C. magna* is tri- or tetracolporate, with the tricolporate grains being by far predominant (c. 90%) (Fig. 3a, b). The polar axis (P) varies from 29.9–32.2 µm; the equatorial diameter (E) from 27.3–31.7 µm. The outline in polar view (amb) is more or less circular with the colpi somewhat sunken. In equatorial view, the pollen grains are spheroidal (P/E ranges from 0.9–1.1). The ectocolpi are relatively long with the ends acute to truncate (Fig. 3d). The mesoaperture is a porus. The colpus membrane is granular and sometimes thickened towards the mesoaperture. Endoapertures could not be observed. The sexine is perforate to (micro)reticulate and lacks supratectal elements (Fig. 3c).

Fruit/pyrene anatomy — During development of the ovary into a mature drupe, only a single, bilocular pyrene is formed. It is elliptic and strongly flattened. The pyrene wall is very hard and thick, and is surrounded by a parenchymatous mesocarp and a single-layered exocarp. At the top of the pyrene, an incision is present between the two locules. The base of the pyrene is more soft and fibrous, a feature perhaps allowing the embryo radicle to penetrate through the hard pyrene wall upon germination.

Molecular results

The majority rule consensus tree from the Bayesian analysis is shown in Fig. 4. It is slightly more resolved than the maximum parsimony consensus tree but otherwise the two trees are topologically congruent. The subfamily Rubioideae, including *Colletoecema*, *Pentaloncha*, and *Temnopteryx*, is strongly supported (bs = 100; PP = 1.00). *Colletoecema* is sister to all other Rubioideae, but without support (bs < 50; PP = 0.86). *Colletoecema magna* is highly supported as sister to *C. dewevrei* (bs = 100; PP = 1.00). The next most basal branch is *Temnopteryx*, but the basal position of this genus gets no support (bs < 50; PP = 0.76). A polytomy is formed by the well-supported clades of Ophiorrhizeae (bs = 93; PP = 1.00), Urophylleae (bs = 100; PP = 1.00) and the remainder of Rubioideae (bs = 100; PP = 1.00). Within this last clade, the well-supported tribe Lasiantheae (bs = 100; PP = 1.00) is sister to a clade comprising Coussareeae (bs = 100; PP = 1.00) and the supertribes Rubiidinae (bs = 100; PP = 1.00) and Psychotriidinae (bs = 100; PP = 1.00).

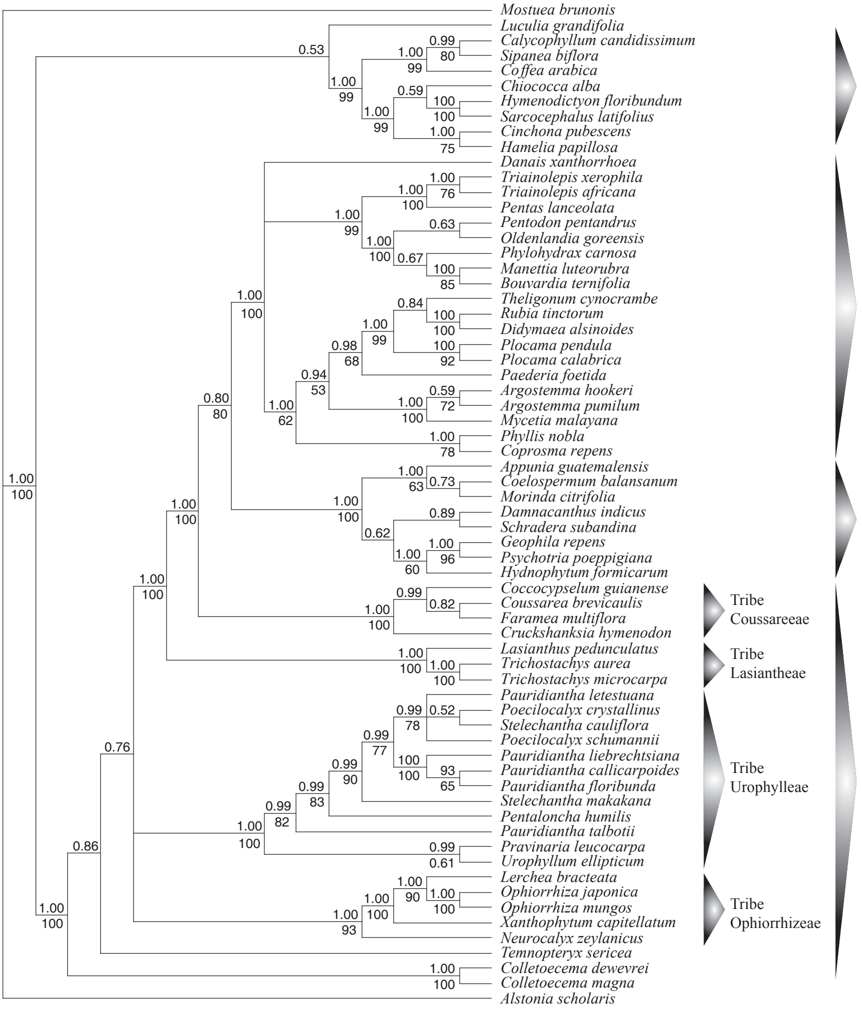

Fig. 4. Majority rule consensus tree from Bayesian analysis. Posterior probabilities of clades are given above branches and parsimony bootstrap percentages below. Classification on the right according to Robbrecht & Manen (2006).

DISCUSSION

Diagnostic characters and relationships

The single, bilocular flattened and thick-walled pyrene with apical incision of the novelty points towards a position within *Colletoecema*. This is further confirmed by the texture and venation of the leaves, triangular stipules with a ± acute apex and ciliate margin, axillary inflorescences paired at the nodes, cupuliform calyces, and very similar placentation. Its position within *Colletoecema* is also supported by sequences from the *rbcL* gene and the *atpB-rbcL* spacer. In the combined analysis, the *Colletoecema* clade gets maximum bootstrap support and Bayesian posterior probability.

Notwithstanding its firm position within *Colletoecema*, several qualitative floral characters of *C. magna* differ remarkably from *C. dewevrei*. The flowers of *C. magna* are sessile, the corolla tube is densely hairy above the insertion point of the stamens, and the anthers are included. These three characters are not found in *C. dewevrei*, where the flowers are at least shortly pedicellate, the corolla tube is pubescent below the insertion point of the anthers, and the anthers are exserted in the two flowering morphs observed. In addition the leaves of *C. magna* are larger and more distinctly obovate than observed in *C. dewevrei* and the fruits are also sessile and more than double in size. Also pollen is slightly different: grains of *C. dewevrei* are described as exclusively 3-colporate (Piesschaert et al. 2000), while in *C. magna* both 3- and 4-colporate pollen grains were observed. This kind of pollen variation is not uncommon in Rubiaceae. Among basal Rubioideae, only the tribe Urophylleae is characterized by exclusively 3-colporate pollen grains. The tribe Ophiorrhizeae has 3- or 4-colporate grains, while Lasiantheae and Coussareeae have (2–)3–4(–5)- and 2–4-colporate grains, respectively (Dessein et al. 2005).

Chorology

The novelty accords with the Guineo-Congolian endemism of the genus *Colletoecema*. *Colletoecema magna* appears to be a narrow endemic of a small part of the Lower Guinean subcentre of endemism. In contrast, *C. dewevrei* is a common species represented by numerous collections (cf. already Petit 1963; erroneously reported to be rare by Rydin et al. 2008); it has been collected from Cameroon, Gabon, Central African Republic, the Republic of Congo and R.D.Congo. It is a linking element of the Lower Guinean and Congolian subcentres of endemism. If our suspicion of the existence of a third species in Gabon is correct, the genus *Colletoecema* has a standard chorological pattern recorded for many Guineo-Congolian genera, i.e. having the centre of largest diversity in the Lower Guinea area (Robbrecht 1996).

Relationships among basal Rubioideae

The Bayesian analysis of the combined matrix is largely in agreement with the studies of Bremer & Manen (2000), Robbrecht & Manen (2006), and Rydin et al. (2008). Most tribes recognized in these studies have been recovered with moderate to high bootstrap support and high Bayesian posterior probability. In agreement with Robbrecht & Manen (2006), apart from a basal grade, two clades can be recognized, i.e. supertribe Psychotriidinae and supertribe Rubiidinae.

As we are mainly interested in the position of the new *Colletoecema* species, we will focus on the relationships among the basal lineages of Rubioideae. In agreement with the results of Robbrecht & Manen (2006) and Rydin et al. (2008), *Colletoecema* is sister to all other Rubioideae in our Bayesian analysis, but without support (bs < 50; PP = 0.86). This result also confirms the finding of Piesschaert et al. (2000) based on *rps16* intron sequences that *Colletoecema* does not belong to Morindeae (Petit 1963) or Psychotrieae (Robbrecht 1993). However, a relationship between *Colletoecema* and *Ophiorrhiza*, as inferred with weak support by Piesschaert et al. (2000), is not supported here. The tropical Asian Ophiorrhizeae form a strongly supported clade (bs = 93; PP = 1.00) in a trichotomy with the Urophylleae and the reminder of Rubioideae. The Urophylleae, including the African *Pentaloncha humilis*, form a strongly supported group (bs = 100; PP = 1.00), which is in agreement with the study of Smedmark et al. (2008) based on *rps16* intron sequences. Their proposal to also include the African genus *Temnopteryx* in the Urophylleae is not supported here. In their Bayesian analysis with a larger sample of outgroup taxa, *Temnopteryx* falls within Urophylleae but without support (bs < 50; PP = 0.78). By rooting the tree to lineages of Rubioideae, they get strong support for the position of *Temnopteryx* within Urophylleae. Unfortunately, they did not include *C. dewevrei* in their sampling. In our Bayesian analysis, *Temnopteryx* is sister to all other Rubioideae excluding *Colletoecema*. However, this position gets no support either (bs < 50; PP = 0.76). If *Temnopteryx* is excluded from the analysis, the position of *Colletoecema* as sister to all other Rubioideae gets somewhat stronger support. More molecular data are needed to confirm the position of *Temnopteryx* in Urophylleae. Within Urophylleae two clades can be recognized: an Asian clade (bs = 61; PP = 0.99) and an African clade (bs = 82; PP = 0.99). Based on the present analyses, it seems that most, if not all, satellite genera of *Pauridiantha* will have to be merged with *Pauridiantha*. This was already done for *Commitheca* by Ntore et al. (2003) and suggested for *Poecilocalyx*, *Rhipidantha*, and *Stelechantha* by Ntore (2004, 2008). A more fine-tuned phylogeny of the group is needed, however, before making taxonomic changes (see also Smedmark et al. 2008).

In conclusion, it is clearly demonstrated that the novelty belongs to *Colletoecema* and that the genus has its closest relatives among the basal groups of Rubioideae. The phylogenetic isolation of *Colletoecema* does not go hand in hand with a geographical isolation, as suggested by Rydin et al. (2008). Instead, *Colletoecema* occurs in one of the most important diversity centres of the Rubioideae (and the Psychotriidinae in particular), the Guineo-Congolian Region of tropical Africa. The present evidence, therefore, indicates a tropical African or paleotropical origin of the Rubioideae.

However, our knowledge about the relationships among the basal lineages of Rubioideae remains unsatisfactory and more molecular data and better sampling are needed to propose a more solid phylogenetic hypothesis.

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