Kindia (Pavetteae, Rubiaceae), a new cliff-dwelling genus with chemically profiled colleter exudate from Mt Gangan, Republic of Guinea

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ABSTRACT

A new genus *Kindia* (Pavetteae, Rubiaceae) is described with a single species, *K. gangan*, based on collections made in 2016 during botanical exploration of Mt Gangan, Kindia, Republic of Guinea in West Africa. The Mt Gangan area is known for its many endemic species including the only native non-neotropical Bromeliaceae *Pitcairnia feliciana*. *Kindia* is the fourth endemic vascular plant genus to be described from Guinea. Based on chloroplast sequence data, the genus is part of Clade II of tribe Pavetteae. In this clade, it is sister to *Leptactina sensu lato* (including *Coleactina* and *Dictyandra*). *Kindia gangan* is distinguished from all species in Clade II by the combination of the following characters: its epilithic habit; ~~many~~few-flowered axillary inflorescences; distinct calyx tube as long as the lobes; a dimorphic corolla tube with narrow proximal section widening abruptly to the distal section; presence of a dense hair band near the base of the corolla tube; deeply included anthers and style ~~deeply included~~, midlength of the corolla tube; anthers lacking connective appendages and with sub-~~basal~~ apical insertion; pollen type 1; pollen presenter (style head) winged and glabrous; red colleters that encircle the calyx-hypanthium, occurring at base and inside calyx and stipules and producing~~e~~ vivid red exudate. *Kindia* is a subshrub that appears restricted to bare, vertical rock faces of sandstone. Fruit dispersal and pollination by bats is postulated. It is here assessed as Endangered EN D1 using the 2012 IUCN standard. High resolution LC-MS/MS analysis revealed over 40 triterpenoid compounds in the colleter exudate, including those assigned to the cycloartane class. Triterpenoids are of interest for their diverse chemical structures, varied biological activities, and potential therapeutic value.

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Subjects Biodiversity, Conservation Biology, Plant Science, Taxonomy

Keywords Cliff-dwelling, Colleter Exudate Chemical Profile, Conservation, Epilithic, Guinea-

Conakry, Rubiaceae, Tropical Important Plant Areas

INTRODUCTION

Plant conservation priorities are often poorly represented in national and global frameworks due to a lack of publicly available biodiversity data to inform conservation decision making (*Corlett, 2016*; *Darbyshire et al., 2017*), despite the fact that one in five plant species are estimated to be threatened with extinction mainly due to human activities (*Brummitt et al., 2015*; *Bachman et al., 2016*). West Africa represents a priority target area for future efforts in botanical exploration to inform conservation action and biological resource use (*Sosef et al., 2017*).

Botanical exploration and new species discovery in Guinea

Guinea has numerous endemic species and a high diversity of species in the context of West Tropical African countries (c. 3000 species; *Lisowski, 2009*), including several endemic genera, i.e. *Fleurydora* A.Chev., *Feliciadamia* Bullock, *Cailliella* Jacq.-Fél. However, botanical exploration, discovery and publication of new species appeared to have nearly stopped after Independence in 1960. Those few species that were published in the period 1960–2010 were based on specimens collected in the French Colonial period, e.g. *Phyllanthus felicis* Jean F.Brunel (1987) and *Clerodendrum sylvae* J.-G.Adam (1974). In recent years, this has begun to change as botanical exploration, often associated with environmental impact assessments for more environmentally responsible mining companies such as Rio Tinto (*Harvey et al., 2010*; *Magassouba et al., 2014*), has restarted. *Xysmalobium samoritourei* Goyder (2009), *Gymnosiphon samoritoureanus* Cheek (*Cheek & van der Burgt, 2010*), *Eriosema triformum* Burgt (*van der Burgt et al., 2012*), *Brachystephanus oreacanthus* Champl. (*Champluvier & Darbyshire, 2009*), *Striga magnibracteata* Eb.Fisch. & I.Darbysh. (*Fischer et al., 2011*), *Isoglossa dispersa* I.Darbysh. & L.J.Pearce (*Darbyshire et al., 2012*), *Eriocaulon cryptocephalum* S.M.Phillips & Mesterházy (*Phillips & Mesterházy, 2015*), *Napoleonea alata*

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Jongkind (*Prance & Jongkind, 2015*) and *Psychotria samouritourei* Cheek (*Cheek & Williams, 2016*) are examples of recent new discoveries from Guinea resulting from this impetus. Just across the border in Mali, *Calophyllum africanum* Cheek & Q.Luke (*Cheek & Luke, 2016*) was recently found, and in Ivory Coast *Macropodiella cussetiana* Cheek (*Cheek & Ameka, 2016*). Even a new rheophytic genus, *Karima* Cheek & Riina has come to light in Guinea (*Cheek et al., 2016*). Many of the new species being described are range-restricted endemics and are threatened by habitat clearance for subsistence agriculture, open-cast mining, urban expansion, quarrying (*Couch et al., 2014*) and invasive species (*Cheek et al., 2013*).

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Mt Gangan: a Tropical Important Plant Area

The criteria of the Important Plant Areas (IPAs) programme, developed by Plantlife International (2004), offer a pragmatic yet scientifically rigorous means of delivering biodiversity datasets, enabling informed site-based conservation priorities (*Darbyshire et al., 2017*). IPAs are aligned to Target 5 of the Convention on Biological Diversity (CBD)'s 'Global Strategy for Plant Conservation' and so offer an important step towards fulfilling national CBD targets (*Darbyshire et al., 2017*). IPAs are identified on the basis of three criteria: the presence of threatened species, exceptional botanical richness and threatened habitats (*Anderson, 2002*; *Plantlife International, 2004*). These criteria were recently revised for a global approach (*Darbyshire et al., 2017*), and are used in the Tropical Important Plant Areas programme of the Royal Botanic Gardens, Kew. In Guinea, botanical exploration is used to aid in aligning the existing forest reserve network, which focuses on maintaining timber resources for exploitation, and the existing few National Parks protecting large mammals or wetlands, to cover global priority areas for plant conservation.

The Mt Gangan area was identified as a prospective Tropical Important Plant Area (*Larridon & Couch, 2016*; *Herbier National de Guinée, 2017*; *Darbyshire, continuously* *updated*). This outlier of the Fouta Djallon Highlands of Guinea consists of two parallel ranges of small sandstone table mountains separated by a narrow N–S valley that appears to be a geological fault. Bedding of the sandstone is horizontal. Uneven erosion on some slopes has resulted in the formation of frequent rock ledges, overhangs and caves. In contrast, other flanks of the mountains are sheer cliffs extending 100 metres or more high and wide. Yet other parts of the Mt Gangan area have a staircase formation, the step intervals reaching up to 2 m high.

The rock formations create a variety of microhabitats and are inhabited by sparse small trees, shrubs, subshrubs and perennial herbs, many of which are rock specialists, such as *Fegimanra afzelii* Engl. *Fleurydora felicis* A. Chev., *Clerodendrum sylviae* J.G. Adam, *Phyllanthus felicis* J. Brunel, *Cyanotis ganganensis* R. Schnell, *Dissotis pygmaea* A. Chev. & Jacq.-Fél., *D. humilis* A. Chev. & Jacq.-Fél. and *Dissotis controversa* (A. Chev. & Jacq.-Fél.) Jacq.-Fél. Except *Fegimanra afzelii*, the abovementioned species are all either endemic or near-endemic to the Mt Gangan complex of precipitous sandstone table mountains. Mt Gangan also famously contains the entire global population of *Pitcairnia feliciana* (A. Chev) Harms & Mildbr., the only non-neotropical Bromeliaceae which is in the course of being assessed as Critically Endangered.

A new Rubiaceae from Mt Gangan

In February 2016, a survey was initiated of the vegetation types, plant species, and threats at Mt Gangan. During the survey an unusual Rubiaceae was observed with more or less sessile leaf rosettes (*Cheek 18345*), growing only on vertical faces of bare sandstone cliffs that form the flanks of parts of some of the sandstone table mountains that comprise Mt Gangan. *Cheek 18345* has fruits and only old, dried flowers. Because the old flowers were mistakenly interpreted as likely to have had valvate corolla aestivation, and because the inflorescences were axillary, with two-celled, fleshy fruits, containing numerous seeds, the species was initially placed in tribe Mussaendeae sensu *Hepper & Keay (1963: 104)*, using the key to the tribes of Rubiaceae in the *Flora of West Tropical Africa*. Within this tribe, it keyed out as *Sabicea* Aubl. However, it matched no known species of that genus, being bizarre in several features. Checks with all other genera of Rubiaceae in West Tropical Africa, and indeed tropical Africa, also produced no matches, leading to the hypothesis that this taxon represented a new genus to science. In June and September 2016, additional specimens (*Cheek 18541A* and *Cheek 18602*) of the taxon were obtained during the flowering season, at which time the corolla aestivation was found to be contorted to the left, consistent with tribe Pavetteae (*De Block et al., 2015*), although the axillary inflorescences are unusual in that tribe (*De Block et al., 2015*). In this study, morphological and chloroplast sequence data are employed to test the hypothesis that the new Rubiaceae from Mt Gangan is: (1) part of tribe Pavetteae, and (2) represents a new genus to science. To achieve this, we aim to investigate the overall morphology and the pollen morphology and compare them tothose found in other tribe Pavetteae genera, and place the taxon in a molecular phylogenetic framework of the tribe. Ecology, conservation status and colleter exudate biochemistry of the new Rubiaceae are also investigated.

MATERIALS AND METHODS

Ethics statement

The specimens studied were collected as a part of field surveys for the 'Important Plant Areas in the Republic of Guinea' project funded by a Darwin Initiative grant of the Department of the Environment, Food and Rural Affairs (DEFRA) of the government of the United Kingdom. Permits to export these specimens were issued by the Ministère de l'Environnement et des Eaux et Forêts of the Republic of Guinea, Certificat d'Origine nº0000344 (date 21 June 2016) and nº0000399 (dated 28 October 2016). Specimens were collected under the terms of a Memorandum of Understanding between the Board of Trustees, RBG, Kew and the Herbier National de Guineé, Université Gamal Abdel Nasser de Conakry, renewed and extended for 5 years in December 2015. The study area at Mt Gangan reported in this paper is controlled by the government of the Republic of Guinea and is not privately owned, nor protected. The taxon studied here is not yet a protected species.

Taxonomy

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants (ICN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. In addition, new names contained in this work which have been issued with identifiers by IPNI (continuously updated) will eventually be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix "http://ipni.org/". The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central, and CLOCKSS.

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Morphological study

Herbarium material was examined with a Leica Wild M8 dissecting binocular microscope fitted with an eyepiece graticule measuring in units of 0.025 mm at maximum magnification. The drawing was made with the same equipment with a Leica 308700 *camera lucida* attachment. For dissection, structures were first rehydrated by soaking in water with surfactant. The overall morphology was documented, described and illustrated following botanical standard procedures

(*Davis & Heywood, 1963*). Information about habit, habitat, and distribution was taken from specimen labels and field observations.

Material of *Cheek 18345*, *Cheek 18529*, *Cheek 18541A* and *Cheek 18602*, the new Rubiaceae of Mt Gangan, was first compared morphologically against reference material of all Pavetteae genera held at K. The study was then extended to include the BM, HNG, P and WAG herbaria. Codes for cited herbaria follow Index Herbariorum (Thiers, continuously updated). The main online search address used for retrieving specimen data from P (which globally has the largest holdings of herbarium specimens from the Republic of Guinea) was https://science.mnhn.fr/institution/mnhn/collection/p/item/p00179355?listIndex=128&listCount=610; that for WAG was http://bioportal.naturalis.nl/geographic-search?language=en. Special focus was given to taxa shown to be closely related by the molecular phylogenetic results. All specimens marked '!' have been seen.

Pollen morphology has been shown to be useful in characterising clades, and sometimes genera within tribe Pavetteae (*De Block & Robbrecht, 1998*). Pollen samples were collected from *Cheek 18541A* (K). Whole, unacetolysed anthers were placed on a stub using double-sided tape and sputter-coated with platinum in a Quorom Q150T coater for 30 s and examined in a Hitatchi 54700 scanning electron microscope at an acceleration voltage of 4kV.

Molecular methods

In this study, previously published chloroplast sequence data was used (*De Block et al., 2015*), supplemented with new sequences from selected regions (*rps16* and *trn*T-F) (Appendix 1). The DNA extraction protocol and material and methods for amplification and sequencing used in this study follow De Block et al. (*De Block et al., 2015*).

Sequences were assembled and edited in Geneious R8 (http://www.geneious.com; *Kearse* *et al., 2012*), aligned using MAFFT 7 (*Katoh et al., Asimenos & Toh, 2009*; *Katoh & Standley, 2013*),); afterwards, alignments were checked manually in PhyDE 0.9971 (*Müller et al., 2010*). The alignments used to produce the phylogenies are available as a Supplementary File Data S1.

Based on *De Block et al. (2015)*, the alignments of the two chloroplast regions were concatenated for the downstream analyses, each marker was treated as a separate partition, and both partitions were analysed using the GTR+G model. Maximum likelihood (ML) analyses were performed using RAxML 8.2.10 (*Stamatakis, 2014*). The search for an optimal ML tree was combined with a rapid bootstrap analysis of 1000 replicates. Bayesian Inference (BI) analyses were conducted in MrBayes 3.2.6 (*Ronquist et al., 2012*). Rate heterogeneity, base frequencies, and substitution rates across partitions were unlinked. The analysis was allowed to run for 100 million generations across four independent runs with four chains each, sampling every 10000 generations. Convergence, associated likelihood values, effective sample size values and burn-in values of the different runs were verified with Tracer 1.5 (*Rambaut et al., 2014*). The first 25% of the trees from all runs were excluded as burn-in before making a majority-rule consensus of the 7500 posterior distribution trees using the "sumt" function. All phylogenetic analyses were run using the CIPRES portal (http://www.phylo.org/; *Miller, Pfeiffer & Schwartz, 2010*). Trees were drawn using TreeGraph2 (*Stöver & Müller, 2010*) and FigTree 1.4.3 (*Rambaut, 2016*), and adapted in Adobe Photoshop CS5.

Ecology and conservation status

Field studies were conducted in the Mt Gangan complex north of Kindia in February (fruit), June and September (flower) 2016, and in November 2017 (fruit). Plants of the new taxon were mostly inaccessible on vertical sandstone cliffs, so were studied and counted with binoculars. Voucher specimens were made in the usual way (*Bridson & Forman, 1998*) from the few accessible plants that could be reached from the base of the cliffs. The conservation assessment was prepared following *IUCN (2012)* with the help of *Bachmann et al. (2011)*. The distribution of the species was mapped using SimpleMappr (*Shorthouse & David, 2010*).

LC-MS/MS analysis of colleter exudate

A sample of *Cheek 18345* was prepared by extracting the colleter exudate fragments in EtOH:MeOH: H_2O (5:4:1) (1mg/ml) for 24 h, prior to centrifugation. The supernatant was then subjected to LC–MS/MS analysis. Analyses were performed on a Thermo Scientific system consisting of an 'Accela' U-HPLC unit with a photodiode array detector and an 'LTQ Orbitrap XL' mass spectrometer fitted with an electrospray source (Thermo Scientific, Waltham, MA, USA). Chromatography was performed with a 5 µl sample injection onto a 150 mm x 3 mm, 3 µm Luna C-18 column (Phenomenex, Torrance, CA, USA) using the following 400µl/min mobile phase gradient of $H_2O/CH_3CN/CH_3CN$ +1% HCOOH: 90:0:10 (0 min), 0:90:10 (20 min), 0:90:10 (25 min), 90:0:10 (27 min), 90:0:10 (30 min). The ESI source was set to record high resolution (30 k resolution) MS1 spectra (*m/z* 125–2000) in negative mode and data dependent MS2 and MS3 spectra using the linear ion trap. Detected compounds were assigned by comparison of accurate mass data (based on ppm), and by available MS/MS data, with reference to the published compound assignment system (*Schymanski et al., 2014*).

RESULTS

Morphology

Characters separating the new Rubiaceae from Mt Gangan from ~~related genera in tribe Pavetteae~~its sister genus *Leptactina* are provided in Table 1. A detailed description is given in the taxonomic treatment below.

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The pollen grains are tricolporate, overall spheroidal, but usually triangular in polar view (Fig. 2) 20-25 µm in diameter, with an apocolpium of 3.5-4.5 µm diameter, giving an apocolpial index of 0.125 (Fig. 2). The mesocolpium sculpturing is microperforate-reticulate (Fig. 2), the reticulum units are obscurely pentagonal, about 900-1000 nm in diameter, the muri broad and rounded, the central perforations c. 0.1 µm. The apolcolpium exine sculpturing grades to microporate (Fig. 2). The colpi are about 4-6 µm wide at the equator, 2 µm wide at the poles. The colpal membrane is densely granular, the granular units 0.2-0.5 µm diameter, the margin with the mesocolpium well-defined but irregular (Fig. 2), and the pores 3-5 µm in diameter.

Molecular phylogeny

The concatenated ML and BI analyses did not significantly differ in topology, therefore the results discuss the relationships shown in the majority consensus multiple-locus BI tree with the associated posterior probability (PP) values and the bootstrap (BS) values of the multiple-locus ML tree (Supplementary Fig. S1), and summarised in Fig. 3. As the data used here is largely based on the dataset used by *De Block et al. (2015)*, the relationships recovered here largely match those published in that study. Within a well supported tribe Pavetteae (BS=100, PP=1), four major clades (I–IV) were retrieved. However, although in De Block et al. (2015) Clade I was retrieved as sister to a polytomy of Clades II–IV, in this study Clade I+III (BS=90, PP=0.99) and Clade II+IV (BS=79, PP=0.87) are supported as separate clades. Clade I (BS = 100, PP = 1) included the African genera *Nichallea* Bridson and *Rutidea* DC. Clade II (BS = 100, PP = 1) comprised the African genus *Leptactina* Hook.f. sensu *De Block et al. (2015)* and the new Rubiaceae from Mt Gangan, with the latter sister to *Leptactina* of which the monophyly is well supported (BS=99, PP=1). Clade III (BS = 87, PP = 0.87) consisted of the paleotropical genus *Pavetta* L., the monotypic East African genus *Cladoceras* Bremek. and the African species of

Tarenna Gaertn. In our BI analysis, the species *Tarenna jolinonii* N.Hallé was recovered as sister to the rest of a weakly supported Clade III, as was found in the results of *De Block et al. (2015)*. However, in the ML analysis, this species was weakly supported as sister to Clade I. Clade IV (BS = 92, PP = 1) included the East African monotypic genus *Tennantia* Verdc., Asian/Pacific and Madagascan species of *Tarenna*, the Madagascan endemics *Homollea* Arènes, *Robbrechtia* De Block and *Schizenterospermum* Homolle ex Arènes and the African/Madagascan genera *Paracephaelis* Baill. and *Coptosperma* Hook.f. As in the results of *De Block et al. (2015)*, the nodes in this clade were poorly supported and the relationships between subclades remained unclear.

LC-MS/MS analysis of colleter exudate

High resolution LC-MS/MS analysis revealed the detection of a range of triterpenoids in the exudate, including those assigned as the cycloartane class (Table 2). This included a compound eluting at the retention time (Rt) 14.3 min with *m/z* 499.3068 that was assigned the molecular formula $C_{30}H_{44}O_6$ from the observed $[M - H]^-$ ion, which is that of dikamaliartane A, or isomer. Four compounds eluting at Rt 23.8, 25.3, 25.9 and 26.9 min were assigned the molecular formula $C_{30}H_{46}O_4$, from their observed $[M - H]^-$ ions, which is that of dikamaliartane D, F, or isomer. The cycloartane triterpenoids, dikamaliartanes A, D and F, have previously been reported to occur in dikamali gum, which is the colleter exudate of *Gardenia gummifera* L.f. and *G. resinifera* Roth. (Kunert et al., 2009), in the Rubiaceae.

Also detected in the colleter exudate of *Cheek 18345* by LC-MS were two compounds eluting at Rt 20.8 and 21.8 min that were both assigned the molecular formula $C_{30}H_{50}O_5$ from their observed $[M - H]^-$ ions, which is that of gummiferartane 3, a cycloartane triterpenoid previously reported to occur in *G. gummifera* (*CCD, 2017*). Chemically related triterpenoids are gummiferartanes 4 and 9 that have the molecular formula $C_{30}H_{48}O_4$ and also occur in *G. gummifera* (*CCD, 2017*); four compounds were assigned with this molecular formula in the colleter exudate, from their observed [M - H]$^-$ ions, eluting at Rt 24.3, 24.9, 25.7 and 27.8 min. Other cycloartane triterpenoids have previously been reported to occur in species of *Gardenia* (*Kunert et al., 2009*; *CCD, 2017*), with some of these in agreement with the molecular formulae of the triterpenoids detected in the colleter exudate of *Cheek 18345*, as indicated in Table 2.

Other compounds detected in the colleter exudate of *Cheek 18345* included those that eluted at Rt 20.9 min with *m/z* 463.3281, and at Rt 21.6 min with *m/z* 391.3069, that were assigned the molecular formulae $C_{24}H_{48}O_8$ and $C_{20}H_{42}O_4$, respectively. These molecular formulae are those of 1,2,3,4-octadecanetetrol; 1-*O*- rhamnoside and 1,2,3,4-eicosanetetrol, respectively, which have been reported as components of the resin from *Commiphora* species in other studies, as indicated in Table 2.

DISCUSSION

Employing chloroplast sequence data of tribe Pavetteae, largely based on *De Block et al. (2015)*, placed the new Rubiaceae from Mt Gangan as sister to the rest of Clade II of that study, in which three genera, *Leptactina*, *Dictyandra* Hook.f. and *Coleactina* N.Hallé were traditionally maintained, although the two latter genera were recently subsumed into *Leptactina s.l.* (*De Block et al., 2015*). Morphologically, the new Rubiaceae from Mt Gangan was consistent with these genera, especially *Leptactina s.s.* and *Coleactina*, yet showed significant character disjunctions, sufficient to support generic status. The new genus shares with the other members of Clade II large broad stipules and large calyx lobes, large flowers with pubescent corollas, intrusive placentas with numerous ovules and numerous small, angular seeds. However, morphological differences are marked (Table 1), notable the highly winged, glabrous pollen presenter (versus smooth and hairy in *Leptactina s.l.*), the absence of staminal connective appendages, the difference in ratio of calyx tube:lobe (calyx tube well-developed and conspicuous in the new taxon, versus absent or minute in *Leptactina s.l.*), and the difference in ratio of corolla tube length:width. The new Rubiaceae from Mt Gangan differs from all other genera of Pavetteae by having ~~many~~few--flowered axillary inflorescences (Fig. 4). The tribe is generally characterised by terminal inflorescences (*De Block et al., 2015*). However, in Clade II, the remarkable monotypic genus *Coleactina* from Gabon, now included in *Leptactina s.l.*, and the species *Leptactina deblockiae* Neuba~~Nueba~~ & Sonké (*Neuba et al., ~~2016~~2014*) also have axillary inflorescences, albeit 1-flowered and not ~~many~~few--flowered. Finally, the copious and conspicuous bright red exudate from the apical bud of the new Rubiaceae from Mt Gangan appears to be unique in Pavetteae and probably Rubiaceae. While colleter-derived exudates are known in some genera in tribe Coffeeae, e.g. *Coffea* L. and *Kupeantha* Cheek (*Cheek et al., submitted*) and in genera of other tribes such as *Gardenia* J.Ellis, they appear not to have been reported in Pavetteae before (*Hallé, 1970*; *Bridson & Verdcourt, 1988*; *De Block et al., 2015*). However, we have observed such exudates in some specimens of *Leptactina* (e.g. *Fofana 188, Jacques-Felix 7422*, both from Guinea, *Leptactina senegambica* Hook.f.; *Goyder 6258*, from Angola, *Leptactina benguellensis* (Benth. & Hook.f.) Good, all K!). As with all previously known Rubiaceae exudates, these are colourless and translucent, not bright red and opaque as in the new Rubiaceae from Mt Gangan.

Plant exudates, including resins and gums, can occur as complex mixtures of different compound classes including carbohydrates, mono-, di- and tri- terpenoids (*Rhourrhi-Frih et al., 2012*). In this study, the colleter exudate of the new Rubiaceae from Mt Gangan was subjected to high resolution LC-MS/MS analysis for the first time to investigate the chemical composition and over 40 triterpenoids were detected including those assigned as the cycloartane class. These

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Commented [p9]: See De Block 2003; Copious colleter exudate in Robbrechtia included those with the molecular formulae of dikamaliartanes A, D and F, or their isomers. The cycloartane triterpenoids, dikamaliartanes A – F have previously been subjected to antimicrobial assays using *Staphylococcus aureus*, *Candida albicans* and *Mycobacteria* but they did not reveal significant activity against these human pathogens (*Kunert et al., 2009*). Any potential role they may have against plant pathogens or as defence compounds requires further evaluation. Cycloartane triterpenoids are widely distributed in the plant kingdom and it has been suggested that cyclization of of (*3S*)-squalene 2,3-epoxide in higher plants occurs with formation of cycloartenol, which has been considered to have a role in sterol biosynthesis, analogous to that of lanosterol in animals and fungi (*Boar & Romer, 1975*). Furthermore, some plant triterpenoids, including those derived from cycloartane, have been suggested to have a function in cell membrane composition (*Nes & Heftmann, 1981*), thus any evolutionary role they may have in members of the new Rubiaceae from Mt Gangan would be of interest to explore in further studies. Many triterpenoids of plant origin have been of interest for their chemical diversity, biological activities and potential therapeutic applications (Hill & Connolly, 2017; Howes, 2018). The triterpenoids detected in the exudate in this study would be of interest to explore further, not only for their biological activities that might aid understanding of their rationale for synthesis by this species, but also for their potential uses by humanity, if this can be done in a way consistent with the conservation of this rare and threatened species.

In order to better characterise the new genus, a scanning electron microscope study was made of the pollen which provided additional characters to support its generic status. The palynological differences of *Kindia* from *Leptactina s.l.* are extensive. All *Leptactina s.l.* have pollen type 2 (*De Block & Robbrecht, 1998*), i.e. the grains are circular to quadrangular in polar view, (3–)4-zonocolporate, with an apocolpial index of 0.39–0.68. In comparison, those of the new Rubiaceae from Mt Gangan are pollen type 1 (De Block & Robbrecht, 1998), since they are

Commented [p10]: Move part on pollen to the morphological section in the beginning of the discussion triangular in polar view (Fig. 2), 3-zonocolporate, with an apocolpial index of 0.125.

Possession of pollen type 1 by *Cheek 18541A* rather than pollen type 2, is consistent with its position as sister to Clade II since pollen type 1 'predominates in the whole of Rubiaceae and can be considered primitive' (*Robbrecht, 1988*), that is, plesiomorphic. Pollen type 1 also occurs in Pavetteae Clades III and IV (*De Block & Robbrecht, 1998*; De *Block et al., 2015*). The four apertures of pollen type 2 are considered as derived (*De Block & Robbrecht, 1998*) and likely represent a synapomorphy for *Leptactina s.l.* in Clade II.

With the discovery, characterisation and placement of the new Rubiaceae of Mt Gangan as sister to Clade II, re-interpretation of the polarity of some characters in the rest of the clade is in order. Features of *Coleactina papalis* N.Hallé (now *Leptactina papalis* (N.Hallé) De Block), previously interpreted as apomorphies for the genus *Coleactina* now appear to be plesiomorphic with regard to the newly discovered taxon. These are: the well-developed calyx tube, and the pair of involucral cups (epicalycular bracts) surrounding the ovary (Fig. 4H). Additional potentially plesiomorphic characters for Clade II are the axillary inflorescences found in several *Leptactina* species including *L. papalis* and *L. deblockieae* (*Neuba et al., 2014*), and the new Rubiaceae of Mt Gangan. The newly discovered lineage, sister to the rest of Clade II, may represent an evolutionary relict, as it is only known from a single morphologically and molecularly isolated species, which is rare, with less than 100 individuals found in the wild. The unexpected discovery of this lineage from West Africa, sister to *Leptactina s.l.*, which is most diverse in terms of species and morphology in Central Africa, e.g. in Gabon (*Hallé, 1970*) may also provide insights into the geographical origins of Clade II.

The unique habit of the new taxon within tribe Pavetteae may derive from adaptation to its unusual epilithic habitat: narrow fissures in vertical sandstone cliff faces (Fig. 1). In this habitat, the well-developed aerial stems present in the rest of the tribe risk pulling the plants, by their mass, from the tiny fissures and pockets in which they are rooted. This circumstance appears to parallel the situation of *Mussaenda epiphytica* Cheek (tribe Mussaendeae, Rubiaceae; *Cheek, 2009*), a rare epiphytic species, similarly threatened with extinction (*Onana & Cheek, 2011*), in a genus of shrubs and twining terrestrial climbers. *Mussaenda epiphytica* also appears to have lost its ability to produce long stems, which was similarly ~~be~~ conjectured to be disadvantageous in an epiphytic life form (*Cheek, 2009*).

TAXONOMIC TREATMENT

Kindia Cheek, gen nov.

Type: *Kindia gangan* Cheek

Epilithic, homostylous subshrub, lacking underground rootstock, stems short, unbranched, erect or appressed to substrate, reiterating from base, completely sheathed in marcescent stipules, stem indumentum simple, short. Leaves opposite, petiolate, equal in shape and size at each node, each stem with 2–3 pairs of leaves held ± appressed to the vertical substrate, blades simple, entire; domatia absent, nervation pinnate;~~ petiolate~~; stipules broadly ovate, midline with a raised ridge; base of adaxial surface with a mixture of hairs and standard type colleters producing a vivid red exudate from the apical bud, conspicuous in dried specimens. Inflorescences axillary, opposite, in successive nodes, pedunculate-fasciculate, 1–4(–6)-flowered; bracts cupular, 2, sheathing, with two large and two small lobes (Fig. 1H). Ovary-hypanthium sessile, cylindric, with a ring of orange colleters inserted above the base, continuous with the calyx tube and about twice as long as broad, inner part of calyx tube with dense band of colleters at base, calyx lobes 5, oblong-elliptic, about as long as tube. Corolla nearly twice as long as calyx; tube cylindric-funneliform,

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exceeding calyx, outer surface densely sericeous, inner surface glabrous apart from a dense band of hairs just above the base; corolla lobes 5, elliptic-triangular, about one third as long as tube, aestivation contorted to the left in bud. Stamens epipetalous, five, inserted midway up corolla tube, alternating with corolla lobes, anthers narrowly oblong, sessile, attached near base, ~~connective and~~ apical appendage not developed. Ovary 2-celled, placentation axile, placentae intrusive, swollen, ovules numerous; style included, distal half hairy, basal part glabrous; Pollen presenter (stylar head) dilated, outer surface glabrous, fluted-ridged, with two appressed stigmatic lobes at apex, apices tapering, acute, at same level as anthers. Fruit globose, ripening greenish-yellow or white, glossy, semi-translucent, outer surface hairy; pericarp succulent, thick, calyx persistent. Seeds numerous, truncated, 4–5-sided pyramidal (frustrums), glossy black, hilar area white, deeply excavated; embryo occupying c. 5-10% of the seed volume, horizontal, cotyledons barely detectable.

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Kindia gangan Cheek *sp. nov.* —Fig. 4

Type. Republic of Guinea, Kindia Prefecture, Mt Gangan area, Kindia-Télimelé Rd, km 7, N of Mayon Khouré village, fr. 5 Feb. 2016, *Cheek* 18345 (holotype HNG!, isotypes BR!, K!, P!, US!).

Perennial, epilithic subshrub, multi-stemmed from base, stems very short, appressed to substrate or sometimes pendulous, not rooting at the nodes, woody, reiterating from base, ~~completed~~ completely sheathed in persistent dark brown stipules, 5–6(–35) cm long, each stem with 2–3 pairs of leaves held ± appressed to the substrate, internodes (0.25–)0.5 cm long, 5–7 mm diam., indumentum simple, short white hairs, 0.1–0.2 mm long. *Leaves* opposite, equal in shape and size at each node, blade elliptic (-obovate), (7.5–)9.4–11.7 by (3.2–)4.2–6.6(–7) cm, apex obtuse to

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shortly acuminate, acumen 1–2 mm, long base acute, abruptly decurrent to the upper 2–5 mm of the petiole, upper blade surface bullate, indumentum white, simple subappressed, 0.1–0.3 mm long, 30 % cover, midrib hairs 0.3–0.4 mm long, 80 % cover, midrib c. 1 mm broad, yellow drying white, secondary nerves (7–)8–10(–11) on each side of the midrib; lower surface of blade with indumentum as upper, denser, c. 40% cover, midrib 1.2–1.3 mm wide, divided into 3 longitudinal portions, the central portion raised, convex, 40 % covered in hairs; the lateral portions flat, 90% covered in hairs; domatia absent, secondary nerves arising at c. 60º from the midrib, curving near the margin and looping towards the leaf apex and uniting with the nerve above (brochidodromous); tertiary nerves conspicuous, raised, white puberulent scalariform (5–)6–8 between each pair of secondary nerves; quaternary nerves apparent only in the tertiary cells (areolae) towards the margin, each tertiary cell with 8–12 bullae (not always visible in the pressed specimens). *Petiole* semi-circular in transverse section, 3–4 mm long at the distal-most node, elongating to 6–10(–14) mm long at the second and third node from the apex. *Interpetiolar stipule* broadly ovate 3–5.5 by 3–5 mm, apex acute or rounded – shortly acuminate, outer surface midline with a raised ridge, indumentum as leaf blade; adaxial surface with colleters in line at the base, producing a vivid red exudate over the apical bud, conspicuous in dried specimens. *Colleters* standard type, orange, cylindric, 0.5–1.5 by 0.2 mm long, gradually tapering to a rounded apex, interspersed with bristle hairs 1–2 mm long at stipule base, otherwise hairs sparse, 0.2–0.4 mm long, 10–20 % cover. *Inflorescences* axillary, opposite, and in successive nodes, pedunculate-fasciculate, 1–4(–6)-flowered. *Peduncle* 4–15 by 1.5–2.5 mm, indumentum as leaf-blade; epicalycular bracts cupular, 2, outer (proximal) sheathing the smaller inner (distal), 3.5–4 by 5–7 mm, large lobes oblong-elliptic 4.5–6.5 by 2.5 mm, short lobes triangular 1–2 by 2 mm. *Ovary-hypanthium* sessile (pedicel absent), partly concealed, and sunken inside the epicalycular cup (ovary locules extending below the junction of ovary with epicalycular cup), free part

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subcylindrical, 2 mm long, 4 mm in diameter at junction with calyx, hairs white, more or less patent, 0.5 mm long, ring of orange colleters 0.5-0.75 mm long, appressed, inserted about 1/3 up from base, ovary-hypanthium as wide as calyx pre-anthesis and with identical indumentum; calyx tube (3–)4–5(–10) mm long, 4–5 mm wide at base, 5–6(–10) mm wide at apex; calyx lobes 5, oblong elliptic, 7–11 by 2–3(–4.5) mm, apex acute, indumentum on both surfaces 0.4–0.6(–1.1) mm long, c. 50 % cover on tube, 20–30 % cover on lobes; inner surface also with a dense band of colleters at base, extending in lines a short distance up from the base of the calyx tube. *Corolla* white, cylindrical, 4–4.5 cm long, 2–2.3 cm wide at mouth; with two distinct sections, proximal and distal; proximal section slender, 6 by 2 mm, glabrous in proximal part; middle portion of the proximal tube with a densely puberulent band 1-2 mm long, hairs white 2 mm long forming a seal with the style; distal section of corolla tube abruptly wider, 2.6 by 1.4 cm, outer surface densely pale brown sericeous, hairs simple, 0.5 mm long, covering the surface; lobes 5, oblong-elliptic, 9–12 by 6.5–9 mm, apex obtuse, then extending into a filiform appendage 3–4 mm long, apex acute, margins involute; inner surface of corolla glabrous in proximal 2.2–2.4 cm, distal part of tube with thinly scattered hairs 0.1–0.2 mm long, 30–40 % cover. *Stamens* five, alternating with corolla lobes, elliptic, c. 1.5 by 0.1 mm; anthers sessile, attached near the base and inserted 1.5 cm from corolla base. *Disc* bowl-shaped, adnate to base of corolla tube, 1 mm wide, 2 mm deep, glabrous, lacking surface sculpture. *Ovary* 2-celled, placentation axile, placentae intrusive, shield-shaped, 2 x 1.25 mm, 0.5 mm thick (including ovules); ovules 40-50 per locule, elliptic, 0.25 mm long; style included, 2.2 cm long, 1 mm diam. at base, proximal 9–9.5 mm glabrous, above which the median 5–6 mm length is patent-hairy, the hairs 0.3–0.5 mm long, distal 10.5–11 mm of length glabrous; pollen presenter (stylar head) dilated, with two appressed lobes 3 by 1–1.2 mm, outer surface fluted-ridged, apices tapering, acute. *Fruit* globose, 9–10 mm diam. ripening greenish-white, glossy, semi-translucent, outer surface with appressed white hairs 0.6–

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0.9 mm long; pericarp succulent, 2–3 mm thick, calyx persistent. *Seeds* numerous 30–50 per fruit, truncated, 4–5-sided pyramidal (frustrums) 1.5–2 by 1.5–2 by 1.5 mm, the proximal (hilar end) white, the distal two-thirds glossy black, epidermis finger-print like; embryo minute, c. 0.3 mm long, cotyledons about 1/4 of length, not well demarcated.

Distribution

Republique de Guinée, Kindia Prefecture, northeastern boundary of Mt Gangan area, west of Kindia-Telimélé Rd (Fig. 5).

Ecology

The area of the Mt Gangan complex in which we found plants of *Kindia* consists of two parallel ranges of small sandstone table mountains separated by a narrow N-S valley that appears to be a geological fault. Bedding of the sandstone is horizontal. Uneven erosion on some slopes has resulted in the formation of frequent rock ledges, overhangs and caves. In contrast other flanks of the mountains are sheer cliffs extending 100 metres or more high and wide. It is on the cliff areas at 230–540 m a.s.l that *Kindia gangan* occurs as the only plant species present, usually as scattered individuals in colonies of (1–3–)7–15 plants, with no other species of vascular plant present on the bare expanses of rock that are shaded for part of the day due to the orientation of the cliffs or to overhangs or due to a partial screen of trees in front of the rockfaces. *Pitcairnia feliciana* (Bromeliaceae), in contrast, is found in fully exposed sites where there is, due to the rock bedding, a horizontal sill in which to root. These two species can grow within metres of each other if their cliff microhabitats occur in proximity. The rock formations create a variety of other microhabitats, including vertical fissures, caves, shaded, seasonally wet ledges, and are inhabited by sparse small trees, shrubs, subshrubs, perennial and annual herbs, many of which are

Commented [p18]: Word for word repetition of introduction narrow endemic rock specialists ~~discovered in the French colonial period (see Introduction)~~. We speculate that the seed of this species might be bat-dispersed because of the greenish yellow-white colour of the berries (not attractive to birds) and the position of the plants high on cliff faces, where nothing but winged creatures could reach them, apart from those few plants at the base of the cliffs. However, fruit dispersal is not always effected since we found numerous old dried intact fruits holding live seeds on the plants at the type locality in February 2016. It is possible that the robust, large white flowers are pollinated by a small species of bat since in June and September we saw signs of damage to the inner surface of the corolla inconsistent with visits by small insects. The very broad, short corolla is not consistent with pollination by sphingid moths (which prefer long, slender-tubed flowers), but this cannot be ruled out.

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Local names and uses

None are known. The local communities in the area when interviewed in November 2017, stated that they had no uses nor names for the plant (Molmou & Doré, pers. obs.).

Etymology

The genus is named for the town and prefecture of Kindia, Guinea’s fourth city, and the species is named for Mt Gangan to its north, which holds the only known location for the species. Both names are derived as nouns in apposition.

Conservation status

Knowledge of *Kindia gangan* is based on 15 days of searching in sandstone rock outcrops around the Mt Gangan complex in 2016-2017 by teams each comprising 3–5 botanists, together with local community representatives. This area was previously visited by several excellent botanists in the colonial period, notably by Jacques-Felix in 1934-37. Only 86 mature plants of *Kindia gangan* were seen at seven sites at two locations (as defined by *IUCN, 2012*). The two locations are separated by 19 km. Within locations, the sites are separated by 150 m – 1.5 km. The Extent of Occurrence and Area of Occupancy were calculated as 27.96 km^2 and 20 km^2 respectively (*Bachmann et al., 2011*). At each site (1–7–)10–20 plants occur gregariously. Accordingly, since less than 250 mature individuals are known of this species, it is here assessed as Endangered under Criterion D1 of *IUCN (2012)*. It is to be hoped that more plants will be found, enabling a lower assessment of the threat to this species. Currently, threats to the plants at the two known locations of this species are low. Quarrying of sandstone for building construction in nearby Kindia, Guinea's fourth city occurs nearby, but fortunately one of the locations of *Kindia gangan* has no road access, so the known plants are not immediately threatened, while at the second location, plants are within reach of roads and so more threatened by future quarrying. It is to be hoped that further sites for the species will be found, lowering the extinction risk of the species. As a precautionary measure it is intended to feature the species in a poster campaign to raise public awareness, and to seedbank it in the newly created seed bank at the University of Gamal Abdel Nasser, Conakry and also at the Royal Botanic Gardens, Kew.

Additional specimens examined

Republic of Guinea, Kindia Prefecture, Mt Gangan area, Mt Gnonkaoneh, NE of Mayon Khoure village which is W of Kindia-Télimelé rd., fl. 19 June 2016, *Cheek 18529* (HNG!, K!); Mt Khonondeh, NW of Mayon Khoure village which is W of Kindia to Telimele rd., fl. 20 June 2017, *Cheek 18541A* sight observation; ibid, *Cheek 18545* (HNG!, K!); ibid. Mt Gnonkaoneh, NE of Mayon Khoure village, fl. 30 Sept. 2016, *Cheek 18602* (HNG!, K!); near Kalakouré village, Kindia-Télimelé rd, fr. 1 Nov. 2017, *Doré 136* (HNG!, K!); Sougorunyah near Fritaqui village, fr. 6 Nov. 2017, *Molmou 1669* (HNG!, K!); Kebe Figuia near Fritaqui village, fr. 6 Nov. 2017, sight observation by Doré and Molmou.

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APPENDIX 1

Sampled plants and DNA sequences. For each plant the provenance, followed by collector and collector number, herbarium for deposition of voucher specimen (in parentheses), and GenBank accession numbers for *rps16* and *trn*T-F. FTEA: *Flora of tropical East Africa*. Abbreviation ‘s.n.’ indicates no collection number.

Tribe Alberteae: *Razafimandimbisonia humblotii* (Drake) Kainul. & B.Bremer**—**Madagascar, Tosh et al. 263 (BR), KM592238, KM592145.

Tribe Coffeeae: *Tricalysia semidecidua* Bridson**—**Zambia, Dessein et al. 1093 (BR), KM592279, KM592185.

Tribe Ixoreae: *Ixora sp.*—Thailand, Sudde 1487 (K), KM592208, KM592115.

Tribe Gardenieae: *Euclinia longiflora* Salisb.**—**Africa (country unknown), Van Caekenberghe 348 (BR), KM592203, KM592110.

Gardenia rutenbergiana (Baill. ex Vatke) J.-F.Leroy**—**Madagascar, Groeninckx et al. 24

(BR), KM592204, KM592111.

Oxyanthus troupinii Bridson—Burundi, Niyongabo 115 (BR), KM592219, KM592126.

Tribe Mussaendeae: ***Mussaenda flava*** Verdc.**—**Africa (country unknown), Van Caekenberghe 60 (BR), KM592217, KM592124.

Tribe Pavetteae: ***Cladoceras subcapitatum*** (K.Schum. & K.Krause) Bremek.**—**Tanzania, Luke et al. 8351 (UPS), AM117290, KM592094.

Coptosperma bernierianum (Baill.) De Block~~—Unknown origin~~Madagascar, Schatz et al. 3764 (MO), KJ815340, KJ815589; ***C. borbonicum*** (Hend. & Andr.Hend.) De Block**—**Comores, De Block 1389 (BR), KM592189, KM592096; ***C. borbonicum*** (Hend. & Andr.Hend.) De Block—~~Unknown~~Reunion, Kainulainen 189 (S), KJ815342, KJ815591; ***C. borbonicum*** (Hend. & Andr.Hend.) De Block—Unknown, Kroger et al. 56 (S), KJ815341, KJ815590; ***C. cymosum*** (Willd. ex Schult.) De Block—Mauritius, Razafimandimbison et al. 843 (S), KJ815343, KJ815592; ***C. graveolens*** (S.Moore) Degreef—Kenya, Mwachala 3711 (BR), KM592200, KM592107; ***C. humblotii*** (Drake) De Block—Madagascar, Bremer et al. 5167 (S), KJ815345, KJ815594; ***C. littorale*** (Hiern) Degreef—Mozambique, Luke et al. 9954 (UPS), KM592190, KM592097; ***C. madagascariense*** (Baill.) De Block—Madagascar, De Block et al. 2238 (BR), KM592191, KM592098; ***C. madagascariense*** (Baill.) De Block—Madagascar, Razafimandimbison 527 (UPS), KM592191, KM592098; ***C. mitochondrioides*** Mouly & De Block—Madagascar, Bremer et al. 5127 (S), KJ815348, KJ815597; ***C. nigrescens*** Hook.f.—Madagascar, De Block et al. 535 (BR), KM592192, KM592099; ***C. nigrescens*** Hook.f.—Kenya, Luke & Luke 9030 (UPS), KM592193, KM592100; ***C. peteri*** (Bridson) Degreef—Tanzania, Lovett & Congdon 2991 (BR), KM592201, KM592108; ***C. supra-axillare*** (Hemsl.) Degreef—Madagascar, De Block et al. 1321 (BR), KM592194, KM592101; ***C. sp. nov. A*—**Madagascar, De Block et al. 720 (BR), KM592199, KM592106; ***C. sp. nov. B***—Madagascar, De Block et al.

796 (BR), KM592195, KM592102; ***C. sp. nov. C***—Madagascar, De Block et al. 1355 (BR), KM592196, KM592103; ***C. sp. nov. D***—Madagascar, De Block et al. 704 (BR), KM592197, KM592104; ***C. sp. nov. E***—Madagascar, De Block et al. 733 (BR), KM592198, KM592105.

Homollea longiflora Arènes—Madagascar, De Block et al. 767 (BR), KM592205, KM592112; ***H. perrieri*** Arènes—Madagascar, Morat 4700 (TAN), KM592206, KM592113.

Kindia gangan Cheek—Republic of Guinea, Cheek 18345 (K), XXX, XXX.

Leptactina arborescens (Welw. ex Benth. & Hook.f.) De Block—Ghana, Schmidt et al. 1683 (MO), KM592202, KM592109.; ***L. benguelensis*** (Welw. ex Benth. & Hook.f.) R.D.Good—Zambia, Dessein et al. 1142 (BR), KM592209, KM592116; ***L. delagoensis*** K.Schum.—Tanzania, Luke & Kibure 9744 (UPS), KM592210, KM592117; ***L. epinyctios*** Bullock ex Verdc.—Zambia, Dessein et al. 1348 (BR), KM592211, KM592118; ***L. involucrata*** Hook.f.—Cameroon, Davis 3028 (K), KM592212, KM592119; ***L. leopoldi-secundi*** Büttner—Republic of Congo, Champluvier 5248 (BR), KM592213, KM592120; ***L. mannii*** Hook.f.—Gabon, Dessein et al. 2518 (BR), KM592214, KM592121; ***L. papalis*** (N.Hallé) De Block—Gabon, Dessein et al. 2355 (BR), KM592188, KM592095; ***L. papyrophloea*** Verdc.—Tanzania, Luke & Kibure 9838 (UPS), KM592215, KM592122; ***L. pynaertii*** De Wild.—Republic of the Congo, Champluvier s.n. (BR), KM592216, KM592123.

Nichallea soyauxii (Hiern) Bridson—Cameroon, Dessein et al. 1402 (BR), KM592218, KM592125.

Paracephaelis cinerea (A.Rich. ex DC.) De Block—Madagascar, De Block et al. 2193 (BR), KM592220, KM592127; ***P. cinerea*** (A.Rich. ex DC.) De Block—Madagascar, Bremer et al. 5122 (S), KJ815372, KJ815619; ***P. saxatilis*** (Scott-Elliot) De Block—Madagascar, De Block et al. 2401 (BR), KM592221, KM592128; ***P. saxatilis*** (Scott-Elliot) De Block—Madagascar, Razafimandimbison & Kroger 937 (S), KJ815374, KJ815622; ***P. sericea*** (Arènes) De Block, Madagascar, De Block et al. 849 (BR), KM592207, KM592114; ***P. tiliacea*** Baill.—Madagascar, Groeninckx et al. 113 (BR), KM592222, KM592129; ***P. trichantha*** (Baker) De Block—~~Unknown~~Aldabra, Friedmann 833385 (UPS), KJ815376, KJ815624; ***P. sp.—*** ~~Unknown~~Madagascar, De Block 1174 (BR), AM117331, KJ815620.

Pavetta abyssinica Fresen.—Africa (unknown country), De Block 6 (BR), FM204726, FM207133; ***P. agrostiphylla*** Bremek.—Sri Lanka, Bremer B. & K. 936 (UPS), KM592223, KM592130; ***P. batesiana*** Bremek.—Gabon, Dessein et al. 2071 (BR), KM592224, KM592131; ***P. hymenophylla*** Bremek.—Tanzania, Luke et al. 9101 (UPS), KM592225, KM592132; ***P. indica*** L.—Sri Lanka, Andreasen 202 (UPS), KM592226, KM592133; ***P. sansibarica*** K.Schum.—Kenya, Luke et al. 8326 (UPS), KM592227, KM592134; ***P. schumanniana*** F.Hoffm. ex K.Schum.—Zambia, Dessein et al. 911 (BR), KM592228, KM592135; ***P. stenosepala*** K.Schum.—Kenya, Luke et al. 8318 (UPS), KM592233, KM592140; ***P. suffruticos***a K.Schum.—Cameroon, Lachenaud et al. 838 (BR), KM592231, KM592138; ***P. tarennoides*** S.Moore—Kenya, Luke et al. 8325 (UPS), KM592234, KM592141; ***P. ternifolia*** Hiern—Burundi, Ntore 19 (BR), KM592235, KM592142; ***P. tetramera*** (Hiern) Bremek—Gabon, Van de Weghe 163 (BR), KM592236, KM592143; ***P. vaga*** S.T.Reynolds—Australia, Harwood 1290 (DNA), KM592237, KM592144; ***P. sp. A of FTEA*** Bridson—Tanzania, Luke et al. 9134 (UPS), KM592232, KM592139; ***P. sp. B***—Vietnam, Davis et al. 4082 (K), KM592229, KM592136; ***P. sp. C***—Asia (country unknown), Van Caekenberghe 199 (BR), KM592230, KM592137.

Robbrechtia grandifolia De Block—Madagascar, Kårehed 311 (UPS), KM592239, KM592146; ***R. milleri*** De Block—Madagascar, Bremer et al. 5295 (S), KM592240, KM592147.

Rutidea decorticata Hiern—Cameroon, Maurin 14 (K), KM592241, KM592148; ***R. dupuisii*** De Wild.—Gabon, Dessein et al. 1802 (BR), KM592242, KM592149; ***R. ferruginea*** Hiern—Cameroon, Dessein et al. 1674 (BR), KM592242, KM592150; ***R. fuscenscens*** Hiern—

Tanzania, Luke et al. 9124 (UPS), KM592244, KM592151; ***R. membranacea*** Hiern—Liberia, Adam 21433 (UPS), KM592245, KM592152; ***R. olenotricha*** Hiern—Ghana, Schmidt et al. 1731 (MO), KM592246, KM592153; ***R. parviflora*** DC.—Liberia, Adam 20156 (UPS), KM592248, KM592154; ***R. seretii*** De Wild.—Cameroon, Gereau 5588 (UPS), KM592249, KM592155.

Schizenterospermum grevei Homolle ex Arènes—Madagascar, De Block et al. 2167 (BR), KM592250, KM592156; ***S. rotundifolia*** Homolle ex Arènes—Madagascar, De Block et al. 771 (BR), KM592251, KM592157.

Tarenna alleizettei (Dubard & Dop) De Block—Madagascar, De Block et al. 1883 (BR), KM592272, KM592178; ***T. alleizettei*** (Dubard & Dop) De Block—Madagascar, Kårehed 313A (UPS), KJ815382, KJ815630; ***T. alpestris*** (Wight) N.P.Balakr.—India, De Block 1474 (BR), KM592252, KM592158; ***T. asiatica*** (L.) Kuntze ex K.Schum.—India, Auroville 998 (SBT), KM592253, KM592159; ***T. bipindensis*** (K.Schum.) Bremek., Liberia, Jongkind 8495 (BR), KM592255, KM592161; ***T. capuroniana*** De Block—Madagascar, De Block et al. 937 (BR), KM592273, KM592179; ***T. capuroniana*** De Block—Madagascar, Bremer et al. 5041 (S), KJ815386, KJ815634; ***T. depauperata*** Hutch.—China, Chow & Wan 79063 (UPS), KM592256, KM592162; ***T. flava*** Alston—Sri Lanka, Klackenberg 440 (S), KM592257, KM592163; ***T. fuscoflava*** (K.Schum.) S.Moore—Ghana, Schmidt et al. 2099 (MO), KM592258, KM592164; ***T. gracilipes*** (Hayata) Ohwi—Japan, Van Caekenberghe 149 (BR), KM592259, KM592165; ***T. grevei*** (Drake) Homolle—Madagascar, De Block et al. 959 (BR), KM592274, KM592180; ***T. jolinonii*** N.Hallé—Gabon, Champluvier 6098 (BR), KM592260, KM592166; ***T. lasiorachis*** (K.Schum. & K.Krause) Bremek.—Gabon, Wieringa 4432 (WAG), KM592261, KM592167; ***T. leioloba*** (Guillaumin) S.Moore—New Caledonia, Mouly 174 (P), KM592262, KM592168; ***T. microcarpa*** (Guillaumin) Jérémie—New Caledonia, Mouly 297 (P), KM592263, KM592169; ***T. nitidula*** (Benth.) Hiern—Liberia, Jongkind 8000 (BR), KM592264, KM592170; ***T. pallidula***

Hiern—Gabon, Dessein et al. 2215 (BR), KM592265, KM592171; ***T. pembensis*** J.E.Burrows—Mozambique, Luke et al. 10136 (UPS), KM592266, KM592172; ***T. precidantenna*** N.Hallé—Gabon, Dessein et al. 2360 (BR), KM592267, KM592173; ***T. rhypalostigma*** (Schltr.) Bremek.—New Caledonia, Mouly 182 (P), KM592268, KM592174; ***T. roseicosta*** Bridson—Tanzania, Luke et al. 9170 (UPS), KM592269, KM592175; ***T. sambucina*** (G.Forst.) T.Durand ex Drake—New Caledonia, Mouly et al. 364 (P), KM592270, KM592176; ***T. spiranthera*** (Drake) Homolle—Madagascar, De Block et al. 946 (BR), KM592275, KM592181; ***T. thouarsiana*** (Drake) Homolle—Madagascar, De Block et al. 655 (BR), KM592276, KM592182; ***T. uniflora*** (Drake) Homolle—Madagascar, Bremer et al. 5230 (S), KM592277, KM592183; ***T. vignei*** Hutch. & Dalziel—Republic of Guinea, Jongkind 8126 (BR), KM592271, KM592177.

Tennantia sennii (Chiov.) Verdc. & Bridson—Kenya, Luke et al. 8357 (UPS), KM592278, KM592184.

Tribe Vanguerieae: *Vangueria madagascariensis* J.F.Gmel.—Africa (country unknown), Delprete 7383 (NY), EU821636, – .

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Author Contributions

- Martin Cheek conceived and designed the experiments, performed the experiments, analysed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Sékou Magassouba performed the ecological study, contributed reagents/materials/analysis tools.
- Melanie-Jayne R. Howes conceived and designed the experiments, performed the experiments, analysed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Tokpa Doré performed the ecological study, contributed reagents/materials/analysis tools.
- Saïdou Doumbouya performed the ecological study, contributed reagents/materials/analysis tools.
- Denise Molmou performed the ecological study, contributed reagents/materials/analysis tools.

- Aurelie Grall performed the molecular laboratory study, contributed reagents/materials/analysis tools.
- Charlotte Couch performed the ecological study, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Isabel Larridon conceived and designed the experiments, performed the experiments, analysed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

Data Availability

The following information was supplied regarding data availability:

The raw data has been supplied as a Supplementary File.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.XXXX/peerj.XXXX#supplemental-information.

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Figure captions

Figure 1 Photographs showing the cliff-dwelling habitat and the habit of *Kindia gangan* at Mt Gangan, Kindia, Guinea. Top left, plants scattered on high sandstone cliff (*Cheek 18345*); top right, plant habit on cliff face (*Cheek 18541A*); bottom left, frontal view of flower (*Cheek 18541A*); bottom middle, side view of inflorescence showing cupular bract (*Cheek 18541A*); bottom right, opened fruit showing ripe seeds (*Cheek 18345*). Photos taken by Martin Cheek.

Figure 2 Scanning electron micrographs of triangular pollen (unacetolysed) of *Kindia gangan*. Left, polar view; right, surface sculpturing (from *Cheek 18541A*).

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Figure 3 Summary phylogenetic hypothesis based on the concatenated BI analysis. Clades I–IV were numbered according to *De Block et al. (2015)*.

Figure 4 *Kindia gangan* Cheek. (A) habit, with indication of bullate leaf surface, (B) plants *in situ* on rock face (from photograph), (C) adaxial leaf indumentum around midrib, (D) abaxial leaf indumentum around midrib, (E) inner face of stipule at second node, (F) secretory colleter from E, (G) flower, (H) pedicel and cup of bracts below flower, (I) corolla cut longitudinally and opened to display inner surface, (J) stigma, (K) transverse section of mature fruit, empty of seeds but showing placenta (in the left locule), (L) seed, hydrated, lateral view, (M) seed, dry, lateral view, (N) seed, dry, view from above. Scale bars: A = 5 cm; G, I, K = 1 cm; H = 5 mm; C, D, E, J = 2 mm; F, L, M, N = 1 mm. Drawn by Andrew Brown based on *Cheek 18345*.

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Figure 5 Map of the distribution of *Kindia gangan*.

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Supplementary Files

Supplementary file Data S1 Concatenated alignment of the chloroplast sequence data (*rps16* and *trn*T-F).

Supplementary file Figure S1 Majority consensus multiple-locus BI cladogram with the associated PP values and the BS values of the multiple-locus ML tree. Only PP above 0.80 and BS values above 75% are shown. Nodes with PP <0.5 support have been collapsed. Inset tree shows the branch lengths.

Table 1. Characters separating *Kindia* from *Leptactina s.l.*, including *Coleactina* and *Dictyandra* (i.e. the remainder of Pavetteae Clade II according to *De BBlock et al., 2015*).

Characters	***Leptactina s.l.***	***Kindia***
Pollen: apocolpial index	0.39-0.89	0.125
Pollen aperture number	(3-)4	3
Anther attachment	Sub-apical	Sub-basal
Anther apical connective appendage	Present	Absent
Position in corolla tube of anthers and stigmas	Exserted or included at tube apex or with their tips exserted; more rarely, at ½ or 1/3th of the height of the corolla tube (Puff et al. 1996)	Deeply included at about midway down tube
Style arms at anthesis	Divergent	Appressed together
Corolla tube length: breadth ratio	(15–)20–25: 1	3: 1
Corolla tube width	Only slightly dimorphic; long narrow proximal section widening subtly at the throath (where anthers are included)Uniform throughout length	Strongly dimorphic, short proximal narrow section abruptly widening to long, broad distal section
Presence of a dense, discrete band of hairs near base of corolla tube	Absent	Present
Pollen presenter	Smooth, usually hairy	Longitudinally winged, glabrous

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