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

#### Comments for authors

The discovery of this new genus of the Rubiaceae in West Africa is surprising. The authors have explored the importance of its finding truly in depth and provide many data that other students of this large angiosperm family want to have at disposal. From the evidence set out, I am convinced that a new genus and species is at hand, and the molecular phylogenetic study strongly confirms this. The morphological comparison with the other representatives of this group of the Pavetteae is nevertheless to some degree superficial. Bringing the knowledge of Kindia at the same level of the knowledge of other genera of the Pavetteae would genuinely justify the recognition at genus level.

I suggest to elaborate Table 1. Morphologically, Kindia is closest to the ex genus Dictyandra (Leptactina arborescens on the tree), also with short (though hypocrateriform) corolla tubes (illustrations in Robbrecht 1984). The fact then that arborescens is nested within Leptactina s.l. and Kindia sister to the rest of the Leptactina clade is in my opinion a strong argument for recognition at generic level.

Some strange reasoning from the introduction onwards should be adapted. The introduction states that the discovery of additional flowering specimens (proving contorted aestivation) allowed the identification at tribal level Pavetteae. In fact this only shows that Kindia belongs to the supertribe Ixoridinae (sensu Robbr & Manen). The combination contorted aestivation and axillary inflorescences would rather indicate the tribes Coffeeae or Octotropideae of that supertribe. However, it is the combination contorted aestivation / black glossy seeds with deep adaxial excavation that immediately points to Pavetteae.

The latter carpological characteristic should therefore be much better documented (now only a vague photo, fig. 1 and some poor drawings, fig. 4 L, N). Illustrating the dried seeds makes no sense, and the rehydrated seeds should be represented in a view that shows the excavation (remove the placental remnant attached in fig 4L). Adding a longitudinal hand section would be worthwile: this would enable to verify if the annular thickening around the opening of the seed excavation (characteristic of the Pavetteae) is present in Kindia too. The mention of a fingerprintlike seed epidermis in the description is not informative at all. It is not clear what this might be (because camera lucida observation in surface view only made?). This is perhaps to be interpreted as exotesta type b3 of Robbrecht & Puff 1986, fig. 20?

See Robbrecht 1984 & 1986 and Robbrecht & Puff 1986 for examples of detailed carpological examination (and illustrations) of this group of the Pavetteae; the new genus needs to be documented in a similar way.

The position and structure of the inflorescences is another feature that needs a more clear documentation. Arguments for the truly axillary position (paired at the nodes and opposite) of the inflorescences should be added, because figures given are not conclusive. If the axillary position holds, it should be compared to the inflorescences of Dictyandra congolana, stated by Robbrecht (1986) to be terminal but mostly borne on short-shoots. The truly axillary position in Kindia may thus find its origin in a strong reduction of such brachyblasts. The

inflorescences of Coleactina then (also terminal on axillary short-shoots; here the reduction of the lateral shoots is even stronger) seem to be in a stage intermediate between D. congolana and Kindia. Further, the description of the bracts and bracteoles also needs clarification (see notes with text).

The description of the new genus and species needs careful rewording. The descriptive language style is not smoothly applied and difficult to read, inter alia because of incorrect punctuation. Shortening is possible by a better structure (e.g. describe outer indumentum in one single section instead of repeating for each organ), and by omitting the repetitions of generic characters in the species description

The red colour of the colleter secretion was no doubt the motivation to study the chemical composition of the exudate in detail. This investigation is hardly providing any comparative evidence to place the new genus in the Rubiaceae, because similar data are sparsely available. This part should eventually be removed from the article. If it is kept, the motivation should be added to the aims of the article at the end of the Introduction, and results should definitely be discussed in the context of global knowledge of colleter secretion. Not a single reference dealing with colleters is mentioned in the paper, however. Authors should consult and cite classical literature (Hallé 1967, Robbrecht 1988, review by Thomas 1991), and explore recent literature. I attach below a list of some examples of missed recent literature.

A number of further remarks is with the text (notes & track changes).

Elmar Robbrecht, December 2017 – January 2018

#### References

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- E. Robbrecht 1988 Tropical woody Rubiaceae

Robbrecht & Puff 1986 A survey of the Gardenieae and related tribes

Thomas V. (1991) Structural, Functional and Phylogenetic Aspects of the Colleter. *Annals of Botany* Vol. 68, No. 4), pp. 287-305

#### Literature on colleters

Some examples of recent publications with information on chemical nature of colleter exudates; no doubt more literature exists, this is just a rapid check.

Emilio de Castro Miguel et al. 2017 Outer cell wall structure and the secretion mechanism of colleters of *Bathysa nicholsonii* K. Schum. (Rubiaceae) Acta Bot. Bras.vol.31no.3 http://dx.doi.org/10.1590/0102-33062016abb0420

<u>Juliana Lischka Sampaio Mayer</u> et al. A functional role for the colleters of coffee flowers <u>AoB Plants</u>. 2013; 5: plt029.

Published online 2013 Jun 28. doi: 10.1093/aobpla/plt029

Silvia Rodrigues Machado et al. Dendroid colleters on vegetative and reproductive apices in *Alibertia sessilis* (Rubiaceae) differ in ultrastructure and secretion. Flora 207, Issue 12, December 2012, Pages 868-877 https://doi.org/10.1016/j.flora.2012.09.013

<u>Victor Peçanha de Miranda Coelho</u> (2013) Colleters in Bathysa cuspidata (Rubiaceae): Development, ultrastructure and chemical composition of the secretion. Flora - Morphology Distribution Functional Ecology of Plants 208: 579–590 DOI 10.1016/j.flora.2012.08.005

1*Kindia* (Pavetteae, Rubiaceae), a new cliff-dwelling genus with chemically profiled colleter 2exudate from Mt Gangan, Republic of Guinea

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### 20ABSTRACT

21A new genus Kindia (Pavetteae, Rubiaceae) is described with a single species, K. gangan, based 22on collections made in 2016 during botanical exploration of Mt Gangan, Kindia, Republic of 23Guinea in West Africa. The Mt Gangan area is known for its many endemic species including the 24only native non-neotropical Bromeliaceae *Pitcairnia feliciana*. *Kindia* is the fourth endemic 25vascular plant genus to be described from Guinea. Based on chloroplast sequence data, the genus 26is part of Clade II of tribe Pavetteae. In this clade, it is sister to Leptactina sensu lato (including 27Coleactina and Dictyandra). Kindia gangan is distinguished from all species in Clade II by its 28epilithic habit; many-flowered axillary inflorescences; distinct calvx tube as long as the lobes; a 29dimorphic corolla tube an arrow proximal section widening abruptly to the distal section; 30 presence of a dense hair band near base of the corolla tube; anthers and style deeply included, 31midlength of the corolla tube; anthers lacking connective appendages and with sub-basal 32insertion; pollen type 1; pollen presenter (style head) winged and glabrous; red colleters that 33encircle the calyx-hypanthium, occur at base and inside calyx and stipules and produce vivid red 34exudate. *Kindia* is a subshrub that appears restricted to bare, vertical rock faces of sandstone. 35Fruit dispersal and pollination by bats is postulated. It is here assessed as Endangered EN D1 36using the 2012 IUCN standard. High resolution LC-MS/MS analysis revealed over 40 37triterpenoid compounds in the colleter exudate, including those assigned to the cycloartane class. 38Triterpenoids are of interest for their diverse chemical structures, varied biological activities, and 39potential therapeutic value.

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41**Subjects** Biodiversity, Conservation Biology, Plant Science, Taxonomy
42**Keywords** Cliff-dwelling, Conservation, Epilithic, Guinea-Conakry, Rubiaceae, Tropical
43Important Plant Areas

### **44INTRODUCTION**

45Plant conservation priorities are often poorly represented in national and global frameworks due 46to a lack of publicly available biodiversity data to inform conservation decision making (*Corlett*, 472016; *Darbyshire et al.*, 2017), despite the fact that one in five plant species are estimated to be 48threatened with extinction mainly due to human activities (*Brummitt et al.*, 2015; *Bachman et al.*, 492016). West Africa represents a priority target area for future efforts in botanical exploration to 50inform conservation action and biological resource use (*Sosef et al.*, 2017).

51

## 52Botanical exploration and new species discovery in Guinea

53Guinea has numerous endemic species and a high diversity of species in the context of West 54Tropical African countries (c. 3000 species; *Lisowski*, 2009), including several endemic genera, 55i.e. Fleurydora A.Chev., Feliciadamia Bullock, Cailliella Jacq.-Fél. However, botanical 56exploration, discovery and publication of new species appeared to have nearly stopped after 57Independence in 1960. Those few species that were published in the period 1960–2010 were 58based on specimens collected in the French Colonial period, e.g. *Phyllanthus felicis* Jean F.Brunel 59(1987) and Clerodendrum sylvae J.-G.Adam (1974). In recent years, this has begun to change as 60botanical exploration, often associated with environmental impact assessments for more 61environmentally responsible mining companies such as Rio Tinto (*Harvey et al., 2010*; 62Magassouba et al., 2014), has restarted. Xysmalobium samoritourei Goyder (2009), 63Gymnosiphon samoritoureanus Cheek (Cheek & van der Burgt, 2010), Eriosema triformum 64Burgt (van der Burgt et al., 2012), Brachystephanus oreacanthus Champl. (Champluvier & 65Darbyshire, 2009), Striga magnibracteata Eb.Fisch. & I.Darbysh. (Fischer et al., 2011), 66Isoglossa dispersa I.Darbysh. & L.J.Pearce (Darbyshire et al., 2012), Eriocaulon 67cryptocephalum S.M.Phillips & Mesterházy (Phillips & Mesterházy, 2015), Napoleonea alata

68Jongkind (*Prance & Jongkind, 2015*) and *Psychotria samouritourei* Cheek (*Cheek & Williams,* 692016) are examples of recent new discoveries from Guinea resulting from this impetus. Just 70across the border in Mali, *Calophyllum africanum* Cheek & Q.Luke (*Cheek & Luke, 2016*) was 71recently found, and in Ivory Coast *Macropodiella cussetiana* Cheek (*Cheek & Ameka, 2016*). 72Even a new rheophytic genus, *Karima* Cheek & Riina has come to light in Guinea (*Cheek et al.,* 732016). Many of the new species being described are range-restricted endemics and are threatened 74by habitat clearance for subsistence agriculture, open-cast mining, urban expansion, quarrying 75(*Couch et al., 2014*) and invasive species (*Cheek et al., 2013*).

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## 77Mt Gangan: a Tropical Important Plant Area

78The criteria of the Important Plant Areas (IPAs) programme, developed by Plantlife International 79(2004), offer a pragmatic yet scientifically rigorous means of delivering biodiversity datasets, 80enabling informed site-based conservation priorities (*Darbyshire et al.*, 2017). IPAs are aligned to 81Target 5 of the Convention on Biological Diversity (CBD)'s 'Global Strategy for Plant 82Conservation' and so offer an important step towards fulfilling national CBD targets (*Darbyshire* 83et al., 2017). IPAs are identified on the basis of three criteria: the presence of threatened species, 84exceptional botanical richness and threatened habitats (*Anderson*, 2002; *Plantlife International*, 852004). These criteria were recently revised for a global approach (*Darbyshire et al.*, 2017), and 86are used in the Tropical Important Plant Areas programme of the Royal Botanic Gardens, Kew. In 87Guinea, botanical exploration is used to aid in aligning the existing forest reserve network, which 88focuses on maintaining timber resources for exploitation, and the existing few National Parks 89protecting large mammals or wetlands, to cover global priority areas for plant conservation.

90 The Mt Gangan area was identified as a prospective Tropical Important Plant Area

91(Larridon & Couch, 2016; Herbier National de Guinée, 2017; Darbyshire, continuously

92*updated*). This outlier of the Fouta Djallon Highlands of Guinea consists of two parallel ranges of 93small sandstone table mountains separated by a narrow N–S valley that appears to be a geological 94fault. Bedding of the sandstone is horizontal. Uneven erosion on some slopes has resulted in the 95formation of frequent rock ledges, overhangs and caves. In contrast, other flanks of the mountains 96are sheer cliffs extending 100 metres or more high and wide. Yet other parts of the Mt Gangan 97area 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 99trees, shrubs, subshrubs and perennial herbs, many of which are rock specialists, such as 100Fegimanra afzelii Engl., Fleurydora felicis A. Chev., Clerodendrum sylviae J.G. Adam, 101Phyllanthus felicis J. Brunel, Cyanotis ganganensis R. Schnell, Dissotis pygmaea A. Chev. & 102Jacq.-Fél., Dissotis: humilis A. Chev. & Jacq.-Fél. and Dissotis controversa (A. Chev. & Jacq.-103Fél.) Jacq.-Fél. Except Fegimanra afzelii, the abovementioned species are all either endemic or 104near-endemic to the Mt Gangan complex of precipitous sandstone table mountains. Mt Gangan 105also famously contains the entire global ulation of Pitcairnia feliciana Chev) Harms & 106Mildbr., the only non-neotropical Bromeliaceae which is in the course of being assessed 107Critically Endangered.

108

## 109A new Rubiaceae from Mt Gangan

110In February 2016, a survey was initiated of the vegetation types, plant species, and threats at Mt 111Gangan, During the survey an unusual Rubiaceae was observed with more or less sessile leaf 112rosettes (*Cheek 18345*), growing only on vertical faces of bare sandstone cliffs that form the 113flanks of parts of some of the sandstone table mountains that comprise Mt Gangan. *Cheek 18345* 114has fruits and only old, dried flowers. Because the old flowers were mistakenly interpreted as 115likely to have had valvate corolla aestivation, and because the inflorescences were axillary, with

116two-celled, fleshy fruits, containing numerous seeds, the species was initially placed in tribe 117Mussaendeae sensu Hepper & Keay (1963: 104), using the key to the tribes of Rubiaceae in the 118Flora of West Tropical Africa. Within this tribe, it keyed out as Sabicea Aubl. However, it 119matched no known species of that genus, being bizarre in several features. Checks with all other 120genera of Rubiaceae in West Tropical Africa, and indeed tropical Africa, also produced no 121matches, leading to the hypothesis that this taxon represented a new genus to science. In June and 122September 2016, additional specimens (18541A Cheek 18602) of the taxon were obtained 123during the flowering season, at which time the corolla aestivation was found to be contorted to 124the left, consistent with tribe Pavetteae Block et al., 2015), although the axillary 126chloroplast sequence data are employed to test the hypothesis that the new Rubiaceae from Mt 127Gangan is: (1) part of tribe Pavetteae, and (2) represents a new genus to science. To achieve this, 128we aim to investigate the overall morphology and the pollen morphology and compare them 129those found in other tribe Pavetteae genera, and place the taxon in a molecular phylogenetic 130framework of the tribe. Ecology, conservation status and colleter exudate biochemistry enew 131Rubiaceae are also investigated.

132

### 133MATERIALS AND METHODS

### 134Ethics statement

135The specimens studied were collected as a part of field surveys for the 'Important Plant Areas in 136the Republic of Guinea' project funded by a Darwin Initiative grant of the Department of the 137Environment food and Rural Affairs (DEFRA) of the government of the United Kingdom.

138Permits to export these specimens were issued by the Ministère de l'Environnement et des Eaux 139et Forêts of the Republic of Guinea, Certificat d'Origine n°0000344 (date 21 June 2016) and

140n°0000399 (dated 28 October 2016). Specimens were collected under the terms of Memorandum 141of Understanding between the Board of Trustees, RBG, Kew and the Herbier National de Guineé, 142Université Gamal Abdel Nasser de Conakry, renewed and extended for 5 years in December 1432015. The study area at Mt Gangan reported in this paper is controlled by the government of the 144Republic of Guinea and is not privately owned, nor protected. The taxon studied here is not yet a 145protected species.

146

# 147Taxonomy

148The electronic version of this article in Portable Document Format (PDF) will represent a 149published work according to the International Code of Nomenclature for algae, fungi, and plants 150(ICN), and hence the new names contained in the electronic version are effectively published 151under that Code from the electronic edition alone. In addition, new names contained in this work 152which have been issued with identifiers by IPNI will eventually be made available to the Global 153Names Index. The IPNI LSIDs can be resolved and the associated information viewed through 154any standard web browser by appending the LSID contained in this publication to the prefix 155"http://ipni.org/". The online version of this work is archived and available from the following 156digital repositories: PeerJ, PubMed Central, and CLOCKSS.

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## 158Morphological study

159Herbarium material was examined with a Leica Wild M8 dissecting binocular microscope fitted 160with an eyepiece graticule measuring in units of 0.025 mm at maximum magnification. The 161drawing was made with the same equipment with a Leica 308700 *camera lucida* attachment. For 162dissection, structures were first rehydrated by soaking in water with surfactant. The overall 163morphology was documented, described and illustrated following botanical standard procedures

164(*Davis & Heywood, 1963*). Information about habit, habitat, and distribution was taken from 165specimen labels and field observations.

Material of *Cheek 18345*, *Cheek 18529*, *Cheek 18541A* and *Cheek 18602*, the new 167Rubiaceae of Mt Gangan, was first compared morphologically against reference material of all 168Pavetteae era held at K. The study was then extended to include the BM, HNG, P and WAG 169herbaria. Codes for cited herbaria follow Index Herbariorum (Thiers, continuously updated). The 170main online search address used for retrieving specimen data from P (which globally has the 171largest holdings of herbarium specimens from the Republic of Guinea) was 172https://science.mnhn.fr/institution/mnhn/collection/p/item/p00179355?

173<u>listIndex=128&listCount=610</u>; that for WAG was <a href="http://bioportal.naturalis.nl/geographic-search?">http://bioportal.naturalis.nl/geographic-search?</a>
174<u>language=en</u>. Special focus was given to taxa shown to be closely related by the molecular
175phylogenetic results. All specimens marked '!' have been seen.

Pollen morphology has been shown to be useful in characterising clades, and sometimes 177genera within tribe Pavetteae (*De Block & Robbrecht, 1998*). Pollen samples were collected from 178*Cheek 18541A* (K). Whole, unacetolysed ers were placed on a stub using double-sided tape 179and sputter-coated with platinum in a Quorom Q150T coater for 30 s and examined in a Hitatchi 18054700 scanning electron microscope at an acceleration voltage of 4kV.

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### 182Molecular methods

183In this study, previously published chloroplast sequence data was used (*De Block et al., 2015*), 184supplemented with new sequences from selected regions (*rps16* and *trn*T-F) (Appendix 1). The 185DNA extraction protocol and material and methods for amplification and sequencing used in this 186study follow De Block et al. (*De Block et al., 2015*).

Sequences were assembled and edited in Geneious R8 (http://www.geneious.com; *Kearse* 

188et al., 2012), aligned using MAFFT 7 (Katoh, Asimenos & Toh, 2009; Katoh & Standley, 2013), 189afterwards, alignments were checked manually in PhyDE 0.9971 (Müller et al., 2010). The 190alignments used to produce the phylogenies are available as a Supplementary File Data S1. 191 Based on De Block et al. (2015), the alignments of the two chloroplast regions were 192concatenated for the downstream analyses, each marker was treated as a separate partition, and 193both partitions were analysed using the GTR+G model. Maximum likelihood (ML) analyses were 194performed using RAxML 8.2.10 (Stamatakis, 2014). The search for an optimal ML tree was 195combined with a rapid bootstrap analysis of 1000 replicates. Bayesian Inference (BI) analyses 196were conducted in MrBayes 3.2.6 (Ronquist et al., 2012). Rate heterogeneity, base frequencies, 197 and substitution rates across partitions were unlinked. The analysis was allowed to run for 100 198million generations across four independent runs with four chains each, sampling every 10000 199generations. Convergence, associated likelihood values, effective sample size values and burn-in 200 values of the different runs were verified with Tracer 1.5 (Rambaut et al., 2014). The first 25% of 201the trees from all runs were excluded as burn-in before making a majority-rule consensus of the 2027500 posterior distribution trees using the "sumt" function. All phylogenetic analyses were run 203using the CIPRES portal (http://www.phylo.org/; Miller, Pfeiffer & Schwartz, 2010). Trees were 204drawn using TreeGraph2 (Stöver & Müller, 2010) and FigTree 1.4.3 (Rambaut, 2016), and 205adapted in Adobe Photoshop CS5.

206

### 207Ecology and conservation status

208Field studies were conducted in the Mt Gangan complex north of Kindia in February (fruit), June 209and September (flower) 2016, and in November 2017 (fruit). Plants of the new taxon were mostly 210inaccessible on vertical sandstone cliffs, so were studied and counted with binoculars. Voucher 211specimens were made in the usual way (*Bridson & Forman*, 1998) from the few accessible plants

212that could be reached from the base of the cliffs. The conservation assessment was prepared 213following *IUCN* (2012) with the help of *Bachmann et al.* (2011). The distribution of the species 214was mapped using SimpleMappr (*Shorthouse & David, 2010*).

215

### 216LC-MS/MS analysis of colleter exudate

217A sample of *Cheek 18345* was prepared by extracting the colleter exudate fragments in 218EtOH:MeOH: H<sub>2</sub>O (5:4:1) (1mg/ml) for 24 h, prior to centrifugation. The supernatant was then 219subjected to LC–MS/MS analysis. Analyses were performed on a Thermo Scientific system 220consisting of an 'Accela' U-HPLC unit with a photodiode array detector and an 'LTQ Orbitrap 221XL' mass spectrometer fitted with an electrospray source (Thermo Scientific, Waltham, MA, 222USA). Chromatography was performed with a 5 μl sample injection onto a 150 mm x 3 mm, 3 223μm Luna C-18 column (Phenomenex, Torrance, CA, USA) using the following 400μl/min mobile 224phase gradient of H<sub>2</sub>O/CH<sub>3</sub>CN/CH<sub>3</sub>CN +1% HCOOH: 90:0:10 (0 min), 0:90:10 (20 min), 0:90:10 225(25 min), 90:0:10 (27 min), 90:0:10 (30 min). The ESI source was set to record high resolution 226(30 k resolution) MS1 spectra (*m/z* 125–2000) in negative mode and data dependent MS2 and 227MS3 spectra using the linear ion trap. Detected compounds were assigned by comparison of 228accurate mass data (based on ppm), and by available MS/MS data, with reference to the 229published compound assignment system (*Schymanski et al.*, 2014).

230

### 231RESULTS

### 232Morphology

233Characters separating the new Rubiaceae from Mt Gangan from related genera in tribe Pavetteae 234are provided in Table 1. A detailed description is given in the taxonomic treatment below.

The pollen grains (Fig. 2) ricolporate, overall spheroidal, but usually triangular in

236polar view (Fig. 2), 20—25 μm in diameter, with an apocolpium of 3.5—4.5 μm diameter, giving 237an apocolpial index of 0.125 (Fig. 2). The mesocolpium sculpturing is microperforate- reticulate 238(Fig. 2), the reticulum units are obscurely pentagonal, about 900-1000 nm in diameter, the muri 239broad and rounded, the central perforations c. 0.1 μm. The apołcolpium exine sculpturing grades 240to microporate (Fig. 2). The colpi are about 4-6 μm wide at the equator, 2 μm wide at the poles. 241The colpal membrane ensely granular, the granular units 0.2—0.5 μm diameter, the margin 242with the mesocolpium well-defined but irregular; (Fig. 2) and the pores 3—5 μm in diameter.

## 244Molecular phylogeny

245The concatenated ML and BI analyses did not significantly differ in topology, therefore the 246results discuss the relationships shown in the majority consensus multiple-locus BI tree with the 247associated posterior probability (PP) values and the bootstrap (BS) values of the multiple-locus 248ML tree (Supplementary Fig. S1), and summarised in Fig. 3. As the data used here is largely 249based on the dataset used by *De Block et al. (2015)*, the relationships recovered here largely 250match those published in that study. Within a well supported tribe Pavetteae (BS=100, PP=1), 251 four major clades (I–IV) were retrieved. However, although in De Block et al. (2015) Clade I was 252retrieved as sister to a polytomy of Clades II–IV, in this study Clade I+III (BS=90, PP=0.99) and 253Clade II+IV (BS=79, PP=0.87) are supported as separate clades. Clade I (BS = 100, PP = 1) 254included the African genera *Nichallea* Bridson and *Rutidea* DC. Clade II (BS = 100, PP = 1) 255comprised the African genus *Leptactina* Hook.f. sensu *De Block et al.* (2015) and the new 256Rubiaceae from Mt Gangan, with the latter sister to *Leptactina* of which the monophyly is well 257supported (BS=99, PP=1). Clade III (BS = 87, PP = 0.87) consisted of the paleotropical genus 258Pavetta L., the monotypic East African genus Cladoceras Bremek, and the African species of 259 Tarenna Gaertn. In our BI analysis, the species Tarenna jolinonii N.Hallé was recovered as sister

260to the rest of a weakly supported Clade III, as was found in the results of *De Block et al. (2015)*.

261However, in the ML analysis, this species was weakly supported as sister to Clade I. Clade IV

262(BS = 92, PP = 1) included the East African monotypic genus *Tennantia* Verdc., Asian/Pacific and

263Madagascan species of *Tarenna*, the Madagascan endemics *Homollea* Arènes, *Robbrechtia* De

264Block and *Schizenterospermum* Homolle ex Arènes and the African/Madagascan genera

265*Paracephaelis* Baill. and *Coptosperma* Hook.f. As in the results of *De Block et al. (2015)*, the

266nodes in this clade were poorly supported and the relationships between subclades remained

268

## 269LC-MS/MS analysis of colleter exudate

270High resolution LC-MS/MS analysis revealed the detection of a range of triterpenoids in the 271exudate, including those assigned as the cycloartane class (Table 2). This included a compound 272eluting at the retention time (Rt) 14.3 min with *m/z* 499.3068 that was assigned the molecular 273formula C<sub>30</sub>H<sub>44</sub>O<sub>6</sub> from the observed [M - H]<sup>-</sup> ion, which is that of dikamaliartane A, or isomer. 274Four compounds eluting at Rt 23.8, 25.3, 25.9 and 26.9 min were assigned the molecular formula 275C<sub>30</sub>H<sub>46</sub>O<sub>4</sub>, from their observed [M - H]<sup>-</sup> ions, which is that of dikamaliartane D, F, or isomer. The 276cycloartane triterpenoids, dikamaliartanes A, D and F, have previously been reported to occur in 277dikamali gum, which is the colleter exudate of *Gardenia gummifera* L.f. and *G. resinifera* Roth. 278(Kunert et al., 2009), in the Rubiaceae.

Also detected in the colleter exudate of *Cheek 18345* by LC-MS were two compounds 280eluting at Rt 20.8 and 21.8 min that were both assigned the molecular formula  $C_{30}H_{50}O_5$  from 281their observed [M - H]<sup>-</sup> ions, which is that of gummiferartane 3, a cycloartane triterpenoid 282previously reported to occur in *G. gummifera* (*CCD*, 2017). Chemically related triterpenoids are 283gummiferartanes 4 and 9 that have the molecular formula  $C_{30}H_{48}O_4$  and also occur in *G.* 

284*gummifera* (*CCD*, 2017); four compounds were assigned with this molecular formula in the 285colleter exudate, from their observed [M - H] ions, eluting at Rt 24.3, 24.9, 25.7 and 27.8 min. 286Other cycloartane triterpenoids have previously been reported to occur in species of *Gardenia* 287(*Kunert et al.*, 2009; *CCD*, 2017), with some of these in agreement with the molecular formulae 288of 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 290eluted at Rt 20.9 min with m/z 463.3281, and at Rt 21.6 min with m/z 391.3069, that were 291assigned the molecular formulae  $C_{24}H_{48}O_8$  and  $C_{20}H_{42}O_4$ , respectively. These molecular formulae 292are those of 1,2,3,4-octadecanetetrol; 1-*O*- rhamnoside and 1,2,3,4-eicosanetetrol, respectively, 293which have been reported as components of the resin from *Commiphora* species in other studies, 294as indicated in Table 2.

295

#### 296DISCUSSION

297Employing chloroplast sequence data of tribe Pavetteae, largely based on *De Block et al. (2015)*, 298placed the new Rubiaceae from Mt Gangan as sister to the rest of Clade II of that study, in which 299three genera, *Leptactina*, *Dictyandra* Hook.f. and *Coleactina* N.Hallé were traditionally 300maintained, although the two latter genera were recently subsumed into *Leptactina s.l.* (*De Block* 301*et al., 2015*). Morphologically, the new Rubiaceae from Mt Gangan was consistent with these 302genera, especially *Leptactina s.s.* and *Coleactina*, yet showed significant character disjunctions, 303sufficient to support generic status. new genus shares with the other members of Clade II 304large broad stipules and large calyx lobes, large flowers with pubescent corollas, intrusive 305placentas with numerous ovules and numerous small, angular seeds. However, morphological 306differences are marked (Table 1), notable the highly winged, glabrous pollen presenter (versus 307smooth and hairy in *Leptactina s.l.*), the absence of staminal connective appendages, the

308difference in ratio of calvx tube: lobe (calvx tube well-developed and conspicuous in the new 309taxon, versus absent or minute in *Leptactina s.l.*), and the difference in ratio of corolla tube 310length:width. The new Rubiaceae from Mt Gangan differs from all other genera avetteae by 311having many-flowered axillary inflorescences (Fig. 4). Tribe is generally characterised by 312terminal inflorescences (De Block et al., 2015). However, in Clade II, the remarkable monotypic 313genus Coleactina from Gabon, now included in Leptactina s.l., and the species Leptactina 314deblockiae Neuteba & Sonké (Neuba et al., 2016) also have axillary inflorescences, albeit 1-315flowered and not many-flowered. Finally, the copious and conspicuous bright red exudate from 316the apical bud of the new Rubiaceae from Mt Gangan appears to be unique in Pavetteae and 317probably Rubiaceae. While colleter-derived exudates are known in some genera ibe Coffeeae. 318e.g. Coffea L. and Kupeantha Cheek (Cheek et al., submitted) and in genera of other tribes such 319as Gardenia J.Ellis, they appear not to have been reported in Pavetteae before (Hallé, 1970; 320Bridson & Verdcourt, 1988; De Block et al., 2015). However, we have observed such exudates in 321some specimens of Leptactina (e.g. Fofana 188, Jacques-Felix 7422, both from Guinea, 322Leptactina senegambica Hook.f.; Goyder 6258, from Angola, Leptactina benguellensis (Benth. & 323Hook.f.) Good, all K!). As with all previously known Rubiaceae exudates, these are colourless 324and translucent, pright red and opaque as in the new Rubiaceae from Mt Gangan. 325 Plant exudates, including resins and gums, can occur as complex mixtures of different 326compound classes including carbohydrates, mono-, di- and tri- terpenoids (*Rhourrhi-Frih et al.*, 3272012). In this study, the colleter exudate of the new Rubiaceae from Mt Gangan was subjected to 328high resolution LC-MS/MS analysis for the first time to investigate the chemical composition and 329over 40 triterpenoids were detected including those assigned as the cycloartane class. These 330included those with the molecular formulae of dikamaliartanes A, D and F, or their isomers. The 331cycloartane triterpenoids, dikamaliartanes A – F have previously been subjected to antimicrobial

332assays using Staphylococcus aureus, Candida albicans and Mycobacteria but they did not reveal 333significant activity against these human pathogens (*Kunert et al.*, 2009). Any potential role they 334may have against plant pathogens or as defence compounds requires further evaluation. 335Cycloartane triterpenoids are widely distributed in the plant kingdom and it has been suggested 336that cyclization of of (3S)-squalene 2,3-epoxide in higher plants occurs with formation of 337cycloartenol, which has been considered to have a role in sterol biosynthesis, analogous to that of 338lanosterol in animals and fungi (Boar & Romer, 1975). Furthermore, some plant triterpenoids, 339including those derived from cycloartane, have been suggested to have a function in cell 340membrane composition (Nes & Heftmann, 1981), thus any evolutionary role they may have in 341members of the new Rubiaceae from Mt Gangan would be of interest to explore in further 342studies. Many triterpenoids of plant origin have been of interest for their chemical diversity, 343biological activities and potential therapeutic applications (Hill & Connolly, 2017; Howes, 2018). 344The triterpenoids detected in the exudate in this study would be of interest to explore further, not 345 only for their biological activities that might aid understanding of their rationale for synthesis by 346this species, but also for their potential uses by humanity, if this can be done in a way consistent 347with the conservation of this rare and threatened species.

In order to better characterise the new genus, a scanning electron microscope study was 349made of the pollen which provided additional characters to support its generic status. The 350palynological differences of *Kindia* from *Leptactina s.l.* are extensive. All *Leptactina s.l.* have 351pollen type 2 (*De Block & Robbrecht, 1998*), i.e. the grains are circular to quadrangular in polar 352view, (3–)4-zonocolporate, with an apocolpial index of 0.39–0.68. In comparison, those of the 353new Rubiaceae from Mt Gangan are pollen type 1, since they are triangular in polar view (Fig. 2), 3543-zonocolporate, with an apocolpial index of 0.125.

Possession of pollen type 1 by *Cheek 18541A* rather than pollen type 2, is consistent with

355

356its position as sister to Clade II since pollen type 1 'predominates in the whole of Rubiaceae and 357can be considered primitive' (*Robbrecht, 1988*), that is, plesiomorphic. Pollen type 1 also occurs 358in Pavetteae Clades III and IV (*De Block & Robbrecht, 1998*; De *Block et al., 2015*). The four 359apertures of pollen type 2 are considered as derived (*De Block & Robbrecht, 1998*) and likely 360represent a synapomorphy for *Leptactina s.l.* in Clade II.

With the discovery, characterisation and placement of the new Rubiaceae of Mt Gangan as 362sister to Clade II, re-interpretation of the polarity of some characters in the rest of the clade is in 363order. Features of *Coleactina papalis* N.Hallé (now *Leptactina papalis* (N.Hallé) De Block), 364previously interpreted as apomorphies for the genus *Coleactina* now appear to be plesiomorphic 365with regard to the newly discovered taxon. These are: the well-developed calyx tube, and the pair 366of involucral cups (epicalycular bracts) surrounding the ovary (Fig. 4H). Additional potentially 367plesiomorphic characters for Clade II are the axillary inflorescences found in several *Leptactina* 368species including *L. papalis* and *L. deblockieae* (*Neuba et al., 2014*), and the new Rubiaceae of 369Mt Gangan. The newly discovered lineage, sister to the rest of Clade II, may represent an 370evolutionary relict, as it is only known from a single morphologically and molecularly isolated 371species, which is rare, with less than 100 individuals found in the wild. The unexpected discovery 372of this lineage from West Africa, sister to *Leptactina s.l.*, which is most diverse in terms of 373species and morphology in Central Africa, e.g. in Gabon (*Hallé, 1970*) may also provide insights 374into the geographical origins of Clade II.

The unique habit of the new taxon within tribe Pavetteae may derive from adaptation to 376its unusual epilithic habitat: narrow fissures in vertical sandstone cliff faces (Fig. 1). In this 377habitat, the well-developed aerial stems present in the rest of the tribe risk pulling the plants, by 378their mass, from the tiny fissures and pockets in which they are rooted. This circumstance appears 379to parallel the situation of *Mussaenda epiphytica* Cheek (tribe Mussaendeae, Rubiaceae; *Cheek*,

3802009), a rare epiphytic species, similarly threatened with extinction (*Onana & Cheek, 2011*), in a 381genus of shrubs and twining terrestrial climbers. *Mussaenda epiphytica* also appears to have lost 382its ability to produce long stems, which was similarly be conjectured to be disadvantageous in an 383epiphytic life form (*Cheek, 2009*).

384

## 385TAXONOMIC TREATMENT

386 Kindia Cheek, gen nov.

387Type: *Kindia gangan* Cheek

388

389Epilithic, homostylous shrub, lacking underground rootstock, stems short, unbranched, erect 390or appressed to substrate, reiterating from base, completely sheathed in marcescent stipules, stem 391 indumentum simple, short. Leaves opposite, equal in shape and size at each node, each stem with 3922-3 pairs of leaves held  $\pm$  appressed to the vertical substrate, blades simple, entire; domatia 393absent, nervation pinnate; petiolate; stipules broadly ovate, midline with a raised ridge; base of 394adaxial surface with a mixture of hairs and standard type colleters producing a vivid red exudate 395 from the apical bud, conspicuous in dried specimens. Inflorescences axillary, opposite, in 396successive nodes, pedunculate-fasciculate, 1–4(–6)-flowered; bracts cupular, 2, sheathing, with 397two large and two small lobes (Fig. 1H). Ovary-hypanthium sessile, cylindric, with a ring of 398 orange colleters inserted above the base, continuous with the calvx tube and about twice as long 399as broad, inner part of calyx tube with dense band of colleters at base, calyx lobes 5, oblong-400elliptic, about as long as tube. Corolla twice as long as calyx; tube cylindric-funneliform, 401exceeding calyx, outer surface densely sericeous, inner surface glabrous apart from a dense band 402of hairs just above the base; corolla lobes 5, elliptic-triangular, about one third as long as tube, 403aestivation contorted to the left in bud. Stamens epipetalous, five, inserted midway up corolla

404tube, alternating with corolla lobes, anthers narrowly oblong, sessile, attached near base, 405connective and apical appendage not developed. Ovary 2-celled, placentation axile, placentae 406intrusive, swollen, ovules numerous; style included, distal half hairy, basal part glabrous; pPollen 407presenter (stylar head) dilated, outer surface glabrous, fluted-ridged, with two appressed stigmatic 408lobes at apex, apices tapering, acute, at same level as anthers. Fruit globose, ripening greenish-409yellow or white, glossy, semi-translucent, outer surface hairy; pericarp succulent, thick, calyx 410persistent. Seeds numerous, truncated, 4–5-sided pyramidal (frustrums), glossy black, hilar area 411white, deeply excavated; embryo occupying c. 5-10% of the seed volume, horizontal, cotyledons 412barely detectable.

413

414Kindia gangan Cheek sp. nov. —Fig. 4

415*Type*. Republic of Guinea, Kindia Prefecture, Mt Gangan area, Kindia-Télimelé Rd, km 7, N of 416Mayon Khouré village, fr. 5 Feb. 2016, *Cheek* 18345 (holotype HNG!, isotypes BR!, K!, P!, 417US!).

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429or sometimes pendulous, not rooting at the nodes, woody, reiterating from base, completed 421sheathed in persistent dark brown stipules, 6(-35) cm long, each stem with 2-3 pairs of leaves 422held ± appressed to the substrate, roodes (0.25–)0.5 cm long, 5–7 mm diam., indumentum 423simple hoosed of, short white hairs, 0.1–0.2 mm long. *Leaves* opposite, equal in shape and 424size at each node, blade elliptic (-obovate), (7.5–)9.4–11.7 by (3.2–)4.2–6.6(-7) cm, apex obtuse 425to shortly acuminate, acumen 1–2 mm, long base acute, abruptly decurrent to the upper 2–5 mm 426of the petiole, upper blade surface bullate, indumentum white, simple subappressed, 0.1–0.3 mm 427long, 30 % cover, midrib hairs 0.3–0.4 mm long, 80 % cover, midrib c. 1 mm broad, yellow

428drying white, secondary nerves (7-)8-10(-11) on each side of the midrib; lower surface of blade 429with indumentum as upper, denser, c. 40% cover, midrib 1.2–1.3 mm wide, divided showing 4303 dsitinct longitudinal portions areas, the central portion arearaised, convex, 40 % covered in 431hairs; the lateral areaportions flat, 90% covered in hairs; domatia absent; secondary nerves 432 and at c. 60° from the midrib, curving near the margin and looping towards the leaf apex and 433uniting with the nerve above (brochidodromous); tertiary nerves conspicuous, raised, white 434puberulent, scalariform, (5–)6–8 between each pair of secondary nerves; quaternary nerves 435apparent only in the tertiary cells (areolae) towards the margin, each tertiary cell with 8–12 bullae 436(not always visible in the pressed specimens). *Petiole* semi-circular in transverse section, 3–4 mm 437long at the distal-most node, elongating to 6-10(-14) mm long at the second and third node from 438the apex. *Interpetiolar stipule* broadly ovate 3–5.5 by 3–5 mm, apex acute or rounded – shortly 439acuminate, outer surface midline with a raised ridge, indumentum as leaf blade; adaxial surface 440 with colleters in line at the base, producing a vivid red exudate over the apical bud, conspicuous 441in dried specimens. Colleters standard type, orange, cylindric, 0.5–1.5 by 0.2 mm long, gradually 442tapering to a rounded apex, interspersed with bristle hairs 1–2 mm long at stipule base, otherwise 443hairs sparse, 0.2–0.4 mm long, 10–20 % cover. *Inflorescences* axillary, opposite, and in 444successive nodes, pedunculate-fasciculate, 1–4(–6)-flowered. *Peduncle* 4–15 by 1.5–2.5 mm, 445indumentum as leaf-blade; epicalycular bracts cupular, (proximal) sheathing the smaller 446inner (distal), 3.5–4 by 5–7 mm, xx-lobed, with the large lobes oblong-elliptic 4.5–6.5 by 2.5 447mm, and the short lobes gular 1–2 by 2 mm. Ovary-hypanthium sessile (pedicel absent). 448partly concealed, and sunken inside the epicalycular cup (ovary locules extending below the 449junction of ovary with epicalycular cup), free part subcylindrical, 2 mm long, 4 mm in diameter 450at junction with calyx, hairs white, more or less patent, 0.5 mm long, ring of orange colleters 4510.75 mm long, appressed, inserted about 1/3 up from base, ovary-hypanthium as wide as calyx

452pre-anthesis and with identical indumentum; calvx tube (3–)4–5(–10) mm long, 4–5 mm wide at 453base, 5–6(–10) mm wide at apex; calvx lobes 5, oblong elliptic, 7–11 by 2–3(–4.5) mm, apex 454acute, indumentum on both surfaces 0.4–0.6(–1.1) mm long, c. 50 % cover on tube, 20–30 % 455cover on lobes; inner surface also with a dense band of colleters at base, extending in lines a short 456distance up from the base of the calyx tube. *Corolla* white, cylindrical 4.5 cm long, 2–2.3 cm 457 wide at mouth; with two distinct sections, proximal and distal; proximal section slender, 6 by 2 458mm, glabrous in proximal part; middle portion of the proximal tube with a densely puberulent 459band 1—2 mm long, hairs white 2 mm long forming a seal with the style; distal section of corolla 460tube abruptly wider, 2.6 by 1.4 cm, outer surface densely pale brown sericeous, hairs simple, 0.5 461mm long, covering the surface; lobes 5, oblong-elliptic, 9–12 by 6.5–9 mm, apex obtuse, then 462extending into a filiform appendage 3–4 mm long, apex acute, margins involute; inner surface of 463corolla glabrous in proximal 2.2–2.4 cm, distal part of tube with thinly scattered hairs 0.1–0.2 464mm long, 30–40 % cover. Stamens five, alternating with corolla lobes, elliptic c. 1.5 by 0.1 mm 465anthers sessile, attached near the base and inserted 1.5 cm from corolla base. *Disc* bowl-shaped, 466adnate to base of corolla tube, 1mm wide, 2 mm deep, glabrous, lacking surface sculpture. Ovary 4672-celled, placentation axile, placentae intrusive, shield-shaped, 2 x 1.25 mm, 0.5mm thick 468(including ovules); ovules 40-50 per locule, elliptic, 0.25 mm long; style included, 2.2 cm long, 1 469mm diam. at base, proximally 9–9.5 mm, glabrous, above which the median 5–6 mm length is 470patent-hairy, the hairs 0.3–0.5 mm long, distal 10.5–11 mm of length glabrous; pollen presenter 471(stylar head) dilated, with two appressed lobes 3 by 1–1.2 mm, outer surface fluted-ridged, apices 472tapering, acute. Fruit globose, 9–10 mm diam. ripening greenish-white, glossy, semi-translucent, 473outer surface with appressed white hairs 0.6–0.9 mm long; pericarp succulent, 2–3 mm thick, 474calyx persistent. Seeds numerous 30–50 per fruit, truncated, 4–5-sided, having the shape of a 475knotted pyramideal (frustrums), 1.5–2 by 1.5–2 by 1.5 mm, the proximal (hilar end) white

476distal two-thirds glossy black; epidermis finger-print like, bryo minute, c. 0.3 mm long, 477cotyledons about 1/4 of length, not well demarcated.

478

#### 479Distribution

480Republique de Guinée, Kindia Prefecture, northeastern boundary of Mt Gangan area, west of 481Kindia-Telimélé Rd (Fig. 5).

482

# 483Ecology

484The area of the Mt Gangan complex in which we found plants of *Kindia* consists of two parallel 485ranges of small sandstone table mountains separated by a narrow N-S valley that appears to be a 486geological fault. Bedding of the sandstone is horizontal. Uneven erosion on some slopes has 487resulted in the formation of frequent rock ledges, overhangs and caves. In contrast other flanks of 488the mountains are sheer cliffs extending 100 metres or more high and wide. It is on the cliff areas 489at 230–540 m a.s.l that *Kindia gangan* occurs as the only plant species present, usually as 490 scattered individuals in colonies of (1-3-)7-15 plants, with no other species of vascular plant 491present on the bare expanses of rock that are shaded for part of the day due to the orientation of 492the cliffs or to overhangs or due to a partial screen of trees in front of the rockfaces. *Pitcairnia* 493*feliciana* (Bromeliaceae), in contrast is found in fully exposed sites where there is, due to the rock 494bedding, a horizontal sill in which to root. These two species can grow within metres of each 495other if their cliff microhabitats occur in proximity. The rock formations create a variety of other 496microhabitats, including vertical fissures, caves, shaded, seasonally wet ledges, and are inhabited 497by sparse small trees, shrubs, subshrubs, perennial and annual herbs, many of which are narrow 498endemic rock specialists discovered in the French colonial period (see Introduction). We 499speculate that the seed of this species might be bat-dispersed because of the greenish yellow500white colour of the berries (not attractive to birds) and the position of the plants high on cliff 501faces, where nothing but winged creatures could reach them, apart from those few plants at the 502base of the cliffs. However, fruit dispersal is not always effected since we found numerous old 503dried intact fruits holding live seeds on the plants at the type locality in February 2016. It is 504possible that the robust, large white flowers are pollinated by a small species of bat since in June 505and September we saw signs of damage to the inner surface of the corolla inconsistent with visits 506by small insects. The very broad, short corolla is not consistent with pollination by sphingid 507moths (which prefer long, slender-tubed flowers), but this cannot be ruled out.

508

#### 509Local names and uses

510None are known. The local communities in the area when interviewed in November 2017, stated 511that they had no uses nor names for the plant (Molmou & Doré, pers. obs.).

512

## 513Etymology

514The genus is named for the town and prefecture of Kindia, Guinea's fourth city, and the species is 515named for Mt Gangan to its north, which holds the only known location for the species. Both 516names are derived as nouns in apposition.

517

### 518Conservation status

519Knowledge of *Kindia gangan* is based on 15 days of searching in sandstone rock outcrops around 520the Mt Gangan complex in 2016-2017 by teams each comprising 3–5 botanists, together with 521local community representatives. This area was previously visited by several excellent botanists 522in the colonial period, notably by Jacques-Felix in 1934-37. Only 86 mature plants of *Kindia* 523*gangan* were seen at seven sites at two locations (as defined by *IUCN*, *2012*). The two locations

524are separated by 19 km. Within locations, the sites are separated by 150 m – 1.5 km. The Extent 525of Occurrence and Area of Occupancy were calculated as 27.96 km² and 20 km² respectively 526(*Bachmann et al., 2011*). At each site (1–7–)10–20 plants occur gregariously. Accordingly, since 527less than 250 mature individuals are known of this species, it is here assessed as Endangered 528under Criterion D1 of *IUCN (2012)*. It is to be hoped that more plants will be found, enabling a 529lower assessment of the threat to this species. Currently, threats to the plants at the two known 530locations of this species are low. Quarrying of sandstone for building construction in nearby 531Kindia, Guinea's fourth city occurs nearby, but fortunately one of the locations of *Kindia gangan* 532has no road access, so the known plants are not immediately threatened, while at the second 533location, plants are within reach of roads and so more threatened by future quarrying. It is to be 534hoped that further sites for the species will be found, lowering the extinction risk of the species. 535As a precautionary measure it is intended to feature the species in a poster campaign to raise 536public awareness, and to seedbank it in the newly created seed bank at the University of Gamal 537Abdel Nasser, Conakry and also at the Royal Botanic Gardens, Kew.

538

## 539Additional specimens examined

540Republic of Guinea, Kindia Prefecture, Mt Gangan area, Mt Gnonkaoneh, NE of Mayon Khoure 541village which is W of Kindia-Télimelé rd., fl. 19 June 2016, *Cheek 18529* (HNG!, K!); Mt 542Khonondeh, NW of Mayon Khoure village which is W of Kindia to Telimele rd., fl.20 June 2017, 543*Cheek 18541A* sight observation; ibid, *Cheek 18545* (HNG!, K!); ibid. Mt Gnonkaoneh, NE of 544Mayon Khoure village, fl. 30 Sept. 2016, *Cheek 18602* (HNG!, K!); near Kalakouré village, 545Kindia-Télimelé rd, fr. 1 Nov. 2017, *Doré 136* (HNG!, K!); Sougorunyah near Fritaqui village, fr. 5466 Nov. 2017, *Molmou 1669* (HNG!, K!); Kebe Figuia near Fritaqui village, fr. 6 Nov. 2017, sight 547observation by Doré and Molmou.

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556

### 557APPENDIX 1

558Sampled plants and DNA sequences. For each plant the provenance, followed by collector and 559collector number, herbarium for deposition of voucher specimen (in parentheses), and GenBank 560accession numbers for *rps16* and *trn*T-F. FTEA: *Flora of tropical East Africa*. Abbreviation 's.n.' 561indicates no collection number.

- Tribe Alberteae: *Razafimandimbisonia humblotii* (Drake) Kainul. & B.Bremer—563Madagascar, Tosh et al. 263 (BR), KM592238, KM592145.
- Tribe Coffeeae: *Tricalysia semidecidua* Bridson—Zambia, Dessein et al. 1093 (BR), 565KM592279, KM592185.
- Tribe Ixoreae: *Ixora sp.*—Thailand, Sudde 1487 (K), KM592208, KM592115.
- 567 **Tribe Gardenieae:** *Euclinia longiflora* Salisb.—Africa (country unknown), Van 568Caekenberghe 348 (BR), KM592203, KM592110.
- 569 *Gardenia rutenbergiana* (Baill. ex Vatke) J.-F.Leroy—Madagascar, Groeninckx et al. 24 570(BR), KM592204, KM592111.
- 571 *Oxyanthus troupinii* son—Burundi, Niyongabo 115 (BR), KM592219, KM592126.

- 572 **Tribe Mussaendeae:** *Mussaenda flava* Verdc.—Africa (country unknown), Van 573Caekenberghe 60 (BR), KM592217, KM592124.
- Tribe Pavetteae: *Cladoceras subcapitatum* (K.Schum. & K.Krause) Bremek.—Tanzania, 575Luke et al. 8351 (UPS), AM117290, KM592094.
- 576 Coptosperma bernierianum (Baill.) De Block—Unknown origin, Schatz et al. 3764 577(MO), KJ815340, KJ815589; *C. borbonicum* (Hend. & Andr.Hend.) De Block—Comores, De 578Block 1389 (BR), KM592189, KM592096; *C. borbonicum* (Hend. & Andr.Hend.) De Block— 579Unknown, Kainulainen 189 (S), KJ815342, KJ815591; *C. borbonicum* (Hend. & Andr.Hend.) De 580Block—Unknown, Kroger et al. 56 (S), KJ815341, KJ815590; *C. cymosum* (Willd. ex Schult.) 581De Block—Mauritius, Razafimandimbison et al. 843 (S), KJ815343, KJ815592; C. graveolens 582(S.Moore) Degreef—Kenya, Mwachala 3711 (BR), KM592200, KM592107; *C. humblotii* 583(Drake) De Block—Madagascar, Bremer et al. 5167 (S), KJ815345, KJ815594; C. littorale 584(Hiern) Degreef—Mozambique, Luke et al. 9954 (UPS), KM592190, KM592097; C. 585*madagascariense* (Baill.) De Block—Madagascar, De Block et al. 2238 (BR), KM592191, 586KM592098; C. madagascariense (Baill.) De Block—Madagascar, Razafimandimbison 527 587(UPS), KM592191, KM592098; *C. mitochondrioides* Mouly & De Block—Bremer et al. 5127 588(S), KJ815348, KJ815597; *C. nigrescens* Hook.f.—Madagascar, De Block et al. 535 (BR), 589KM592192, KM592099; *C. nigrescens* Hook.f.—Kenya, Luke & Luke 9030 (UPS), KM592193, 590KM592100; C. peteri (Bridson) Degreef—Tanzania, Lovett & Congdon 2991 (BR), KM592201, 591KM592108; *C. supra-axillare* (Hemsl.) Degreef—Madagascar, De Block et al. 1321 (BR), 592KM592194, KM592101; *C. sp. nov. A*—Madagascar, De Block et al. 720 (BR), KM592199, 593KM592106; *C. sp. nov. B*—Madagascar, De Block et al. 796 (BR), KM592195, KM592102; *C.* 594sp. nov. C—Madagascar, De Block et al. 1355 (BR), KM592196, KM592103; C. sp. nov. D— 595Madagascar, De Block et al. 704 (BR), KM592197, KM592104; *C. sp. nov. E*—Madagascar, De

596Block et al. 733 (BR), KM592198, KM592105.

- 597 *Homollea longiflora* Arènes—Madagascar, De Block et al. 767 (BR), KM592205, 598KM592112; *H. perrieri* Arènes—Madagascar, Morat 4700 (TAN), KM592206, KM592113.
- 599 Kindia gangan Cheek—Republic of Guinea, Cheek 18345 (K), XXX, XXX.
- Leptactina arborescens (Welw. ex Benth. & Hook.f.) De Block—Ghana, Schmidt et al. 6011683 (MO), KM592202, KM592109.; L. benguelensis (Welw. ex Benth. & Hook.f.) R.D.Good—602Zambia, Dessein et al. 1142 (BR), KM592209, KM592116; L. delagoensis K.Schum.—Tanzania, 603Luke & Kibure 9744 (UPS), KM592210, KM592117; L. epinyctios Bullock ex Verdc.—Zambia, 604Dessein et al. 1348 (BR), KM592211, KM592118; L. involucrata Hook.f.—Cameroon, Davis 6053028 (K), KM592212, KM592119; L. leopoldi-secundi Büttner—Republic of Congo, 606Champluvier 5248 (BR), KM592213, KM592120; L. mannii Hook.f.—Gabon, Dessein et al. 6072518 (BR), KM592214, KM592121; L. papalis (N.Hallé) De Block—Gabon, Dessein et al. 2355 608(BR), KM592188, KM592095; L. papyrophloea Verdc.—Tanzania, Luke & Kibure 9838 (UPS), 609KM592215, KM592122; L. pynaertii De Wild.—Republic of the Congo, Champluvier s.n. (BR), 610KM592216, KM592123.
- 611 *Nichallea soyauxii* (Hiern) Bridson—Cameroon, Dessein et al. 1402 (BR), KM592218, 612KM592125.
- Paracephaelis cinerea (A.Rich. ex DC.) De Block—Madagascar, De Block et al. 2193 614(BR), KM592220, KM592127; P. cinerea (A.Rich. ex DC.) De Block—Madagascar, Bremer et 615al. 5122 (S), KJ815372, KJ815619; P. saxatilis (Scott-Elliot) De Block—Madagascar, De Block 616et al. 2401 (BR), KM592221, KM592128; P. saxatilis (Scott-Elliot) De Block—Madagascar, 617Razafimandimbison & Kroger 937 (S), KJ815374, KJ815622; P. sericea (Arènes) De Block, 618Madagascar, De Block et al. 849 (BR), KM592207, KM592114; P. tiliacea Baill.—Madagascar, 619Groeninckx et al. 113 (BR), KM592222, KM592129; P. trichantha (Baker) De Block—

- 620Unknown, Friedmann 833385 (UPS), KJ815376, KJ815624; *P. sp.*—Unknown, De Block 1174 621(BR), AM117331, KJ815620.
- 622 Pavetta abyssinica Fresen.—Africa (unknown country), De Block 6 (BR), FM204726, 623FM207133; *P. agrostiphylla* Bremek.—Sri Lanka, Bremer B. & K. 936 (UPS), KM592223, 624KM592130; *P. batesiana* Bremek.—Gabon, Dessein et al. 2071 (BR), KM592224, KM592131; 625**P. hymenophylla** Bremek.—Tanzania, Luke et al. 9101 (UPS), KM592225, KM592132; **P. indica** 626L.—Sri Lanka, Andreasen 202 (UPS), KM592226, KM592133; *P. sansibarica* K.Schum.— 627Kenya, Luke et al. 8326 (UPS), KM592227, KM592134; *P. schumanniana* F.Hoffm. ex 628K.Schum.—Zambia, Dessein et al. 911 (BR), KM592228, KM592135; *P. stenosepala* K.Schum. 629—Kenya, Luke et al. 8318 (UPS), KM592233, KM592140; *P. suffrutico*sa K.Schum.— 630Cameroon, Lachenaud et al. 838 (BR), KM592231, KM592138; P. tarennoides S. Moore— 631Kenya, Luke et al. 8325 (UPS), KM592234, KM592141; *P. ternifolia* Hiern—Burundi, Ntore 19 632(BR), KM592235, KM592142; *P. tetramera* (Hiern) Bremek—Gabon, Van de Weghe 163 (BR), 633KM592236, KM592143; *P. vaga* S.T.Reynolds—Australia, Harwood 1290 (DNA), KM592237, 634KM592144; *P. sp. A of FTEA* Bridson—Tanzania, Luke et al. 9134 (UPS), KM592232, 635KM592139; *P. sp. B*—Vietnam, Davis et al. 4082 (K), KM592229, KM592136; *P. sp. C*—Asia 636(country unknown), Van Caekenberghe 199 (BR), KM592230, KM592137.
- 637 *Robbrechtia grandifolia* De Block—Madagascar, Kårehed 311 (UPS), KM592239, 638KM592146; *R. milleri* De Block—Madagascar, Bremer et al. 5295 (S), KM592240, KM592147.
- Rutidea decorticata Hiern—Cameroon, Maurin 14 (K), KM592241, KM592148; R. 640dupuisii De Wild.—Gabon, Dessein et al. 1802 (BR), KM592242, KM592149; R. ferruginea 641Hiern—Cameroon, Dessein et al. 1674 (BR), KM592242, KM592150; R. fuscenscens Hiern—642Tanzania, Luke et al. 9124 (UPS), KM592244, KM592151; R. membranacea Hiern—Liberia, 643Adam 21433 (UPS), KM592245, KM592152; R. olenotricha Hiern—Ghana, Schmidt et al. 1731

- 644(MO), KM592246, KM592153; *R. parviflora* DC.—Liberia, Adam 20156 (UPS), KM592248,
  645KM592154; *R. seretii* De Wild.—Cameroon, Gereau 5588 (UPS), KM592249, KM592155.
  646 *Schizenterospermum grevei* Homolle ex Arènes—Madagascar, De Block et al. 2167
  647(BR), KM592250, KM592156; *S. rotundifolia* Homolle ex Arènes—Madagascar, De Block et al.
  648771 (BR), KM592251, KM592157.
- 649 Tarenna alleizettei (Dubard & Dop) De Block—Madagascar, De Block et al. 1883 (BR), 650KM592272, KM592178; *T. alleizettei* (Dubard & Dop) De Block—Madagascar, Kårehed 313A 651(UPS), KJ815382, KJ815630; *T. alpestris* (Wight) N.P.Balakr.—India, De Block 1474 (BR), 652KM592252, KM592158; *T. asiatica* (L.) Kuntze ex K.Schum.—India, Auroville 998 (SBT), 653KM592253, KM592159; *T. bipindensis* (K.Schum.) Bremek., Liberia, Jongkind 8495 (BR), 654KM592255, KM592161; *T. capuroniana* De Block—Madagascar, De Block et al. 937 (BR), 655KM592273, KM592179; *T. capuroniana* De Block—Madagascar, Bremer et al. 5041 (S), 656KJ815386, KJ815634; *T. depauperata* Hutch.—China, Chow & Wan 79063 (UPS), KM592256, 657KM592162; *T. flava* Alston—Sri Lanka, Klackenberg 440 (S), KM592257, KM592163; *T.* 658 fuscoflava (K.Schum.) S.Moore—Ghana, Schmidt et al. 2099 (MO), KM592258, KM592164; T. 659gracilipes (Hayata) Ohwi—Japan, Van Caekenberghe 149 (BR), KM592259, KM592165; T. 660grevei (Drake) Homolle—Madagascar, De Block et al. 959 (BR), KM592274, KM592180; T. 661*jolinonii* N.Hallé—Gabon, Champluvier 6098 (BR), KM592260, KM592166; *T. lasiorachis* 662(K.Schum. & K.Krause) Bremek.—Gabon, Wieringa 4432 (WAG), KM592261, KM592167; T. 663*leioloba* (Guillaumin) S.Moore—New Caledonia, Mouly 174 (P), KM592262, KM592168; T. 664*microcarpa* (Guillaumin) Jérémie—New Caledonia, Mouly 297 (P), KM592263, KM592169; *T*. 665*nitidula* (Benth.) Hiern—Liberia, Jongkind 8000 (BR), KM592264, KM592170; *T. pallidula* 666Hiern—Gabon, Dessein et al. 2215 (BR), KM592265, KM592171; *T. pembensis* J.E.Burrows— 667Mozambique, Luke et al. 10136 (UPS), KM592266, KM592172; **T. precidantenna** N.Hallé—

668Gabon, Dessein et al. 2360 (BR), KM592267, KM592173; *T. rhypalostigma* (Schltr.) Bremek.—669New Caledonia, Mouly 182 (P), KM592268, KM592174; *T. roseicosta* Bridson—Tanzania, Luke 670et al. 9170 (UPS), KM592269, KM592175; *T. sambucina* (G.Forst.) T.Durand ex Drake—New 671Caledonia, Mouly et al. 364 (P), KM592270, KM592176; *T. spiranthera* (Drake) Homolle—672Madagascar, De Block et al. 946 (BR), KM592275, KM592181; *T. thouarsiana* (Drake) Homolle 673—Madagascar, De Block et al. 655 (BR), KM592276, KM592182; *T. uniflora* (Drake) Homolle 674—Madagascar, Bremer et al. 5230 (S), KM592277, KM592183; *T. vignei* Hutch. & Dalziel—675Republic of Guinea, Jongkind 8126 (BR), KM592271, KM592177.

676 *Tennantia sennii* (Chiov.) Verdc. & Bridson—Kenya, Luke et al. 8357 (UPS), 677KM592278, KM592184.

Tribe Vanguerieae: *Vangueria madagascariensis* J.F.Gmel.—Africa (country unknown), 679Delprete 7383 (NY), EU821636, –.

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#### 698Author Contributions

- Martin Cheek conceived and designed the experiments, performed the experiments,
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- Sékou Magassouba performed the ecological study, contributed
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- Melanie-Jayne R. Howes conceived and designed the experiments, performed the
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- Charlotte Couch performed the ecological study, contributed reagents/materials/analysis
- 715 tools, reviewed drafts of the paper.
- Isabel Larridon conceived and designed the experiments, performed the experiments,
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- figures and/or tables, reviewed drafts of the paper.

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721The following information was supplied regarding data availability:

722The raw data has been supplied as a Supplementary File.

723

# 724Supplemental Information

725Supplemental information for this article can be found online at http://dx.doi.org/10.XXXX/

726peerj.XXXX#supplemental-information.

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# 909Figure captions

910Figure 1 Photographs showing the cliff-dwelling habitat, and the habit flowers and 911fruits of Kindia gangan at Mt Gangan, Kindia, Guinea. Top left, plants scattered igh 912sandstone cliff (Cheek 18345); top right, plant habit on cliff face (Cheek 18541A); bottom left, 913frontal view of flower (Cheek 18541A); bottom middle, side view of inflorescence showing 914cupular bract (Cheek 18541A); bottom right, opened fruit showing ripe seeds (Cheek 18345). 915Photos taken by Martin Cheek.

**Figure 2 Scanning electron micrographs of triangular pollen of** *Kindia gangan***.** Left, 918polar view; right, surface sculpturing (from *Cheek 18541A*).

920Figure 3 Summary phylogenetic hypothesis based on the concatenated BI analysis.

921Clades 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 924*in situ* on rock face (from photograph), (C) adaxial leaf indumentum around midrib, (D) 925abaxial leaf indumentum, (E) inner face of stipule at second node, (F) secretory colleter from 926E, (G) flower, (H) pedicel and cup of bracts below flower, corolla cut longitudinally and 927opened to display inner surface, (J) stigma, (K) transverse section of mature fruit, empty of 928seeds but showing placenta, (L) seed, hydrated, lateral view, (M) seed, dry, lateral view, (N) 929seed, dry, view from above. Scale bars: A = 5 cm; G, I, K = 1 cm; H = 5 mm; C, D, E, J = 2 930mm; F, L, M, N = 1 mm. Drawn by Andrew Brown based on *Cheek 18345*.

932Figure 5 Map of the distribution of Kindia gangan.

934Supplementary Files

935Supplementary file Data S1 Concatenated alignment of the chloroplast sequence data 936(*rps16* and *trn*T-F).

937

938Supplementary file Figure S1 Majority consensus multiple-locus BI cladogram with the 939associated PP values and the BS values of the multiple-locus ML tree. Only PP above 0.80 940and BS values above 75% are shown. Nodes with PP <0.5 support have been collapsed. Inset 941tree 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).

I recommend to divide the column Leptactina s.l. into three columns: Leptactina s.s., ex Dictyandra and ex Coleactina. The merging of these three is mainly based on molecular phylogeny, and I agree with that, but it hides the great morphological diversity of Leptactina s.l., which is necessarily to be surveyed for a correct comparison here.

Characters	Leptactina s.l.	Kindia
Pollen: apocolpial index	0.39-0.89	0.125
Pollen aperture number	4	3
Anther attachment	Sub-apical	Sub-basal
Anther apical connective appendage	Present	Absent
Multilocellate anthers present/absent	XXXX	<u>Absent</u>
Position in corolla tube of anthers and stigmas	Exserted or included at tube apex	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	Uniform throughout length	Strongly dimorphic, proximal narrow section abruptly widening to broad distal section
Presence of a dense, discrete band of hairs near	Absent	Present
base of corolla tube		
Pollen presenter	Smooth, hairy	Longitudinally winged, glabrous