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Research article

Calycosiphonia or *Kupeantha* (Coffeae, Rubiaceae)? A morphological and molecular study of a new species from the Albertine Rift

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Abstract. Field work in the Kibira National Park (Burundi), located in the Kivu-Ruwenzori system of the Afromontane Region, revealed the existence of a new species clearly belonging to the *Argocoffeopsis-Calycosiphonia* clade (Coffeae, Rubiaceae). The species shows striking heterophylly: the plagiotropous branches have several nodes bearing reduced or even scaly leaves. For the rest, it shares characters with *Calycosiphonia* and *Kupeantha*. Therefore, a morphological comparison with the clade is done, as well as molecular phylogenetic analyses. The morphology of the novelty is closer to *Kupeantha* than to *Calycosiphonia*, inter alia because the anthers have no transverse septa, in contrast to the multilocellate anthers of *Calycosiphonia*. However, the molecular data advocate for a position in *Calycosiphonia* – a result weakening the morphological distinction between *Calycosiphonia* and *Kupeantha*. The former genus is no longer restricted to species with transverse septa in the anthers and with placental outgrowths around the seed. The new species is formally described as *Calycosiphonia albertina* Ntore & Robbr. sp. nov. Nomenclaturally, this placement is also the most conservative option. A taxonomic treatment, illustrations, a geographical distribution map, and a preliminary conservation assessment are provided. The previous inclusion of *Calycosiphonia pentamera* in *Kupeantha* based on morphology is here corroborated by molecular analyses.

Keywords. Central Africa, Coffeae, heterophylly, pollen, taxonomy.

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Introduction

The tribe Coffeae DC. has a long and intricate taxonomic history (Robbrecht & Manen 2006; Davis *et al.* 2007), inter alia because its representatives possess uniovulate as well as multiovulate ovary chambers – for decades thought to be a diagnostic character for distinguishing the major groups of Rubiaceae Juss. As a consequence, different views on the tribe can be found in literature (Robbrecht & Puff 1986; Robbrecht 1988; Andreasen & Bremer 1996, 2000; Persson 2000), and it was not until the beginning of the 21st century and using molecular techniques that an agreement on the delimitation of Coffeae was found. The first molecular data on relationships between some genera came from Andreasen & Bremer (2000), demonstrating that Coffeae in the strict sense of Robbrecht & Puff (1986) needed to be expanded with the addition of some members of the Gardenieae-Diplosporinae (sensu Robbrecht & Puff 1986). Six years later, the first molecular study specifically focusing on the tribe Coffeae was published (Davis *et al.* 2007). This work, based on sequence data from four plastid regions and morphological data, proposed an enlarged circumscription of the tribe, comprising 11 genera: the African *Argocoffeopsis* Lebrun, *Belonophora* Hook.f., *Calycosiphonia* Pierre ex Robbr., *Coffea* L., *Psilanthus* Hook.f., *Sericanthe* Robbr., and *Tricalysia* A.Rich., and the Asian *Diplospora* DC., *Discospermum* Dalzell, *Nostolachma* T.Durand, and *Xantonnea* Pierre ex Pit.; i.e., it concluded that the Gardenieae-Diplosporinae had to be merged with Coffeae (except for *Cremalespora* Benth.). Later, it was shown that the African *Tricalysia* was biphyletic, and its subgenus *Empogona* Hook.f., sister to the Asian genus *Diplospora*, was resurrected to the genus level, bringing the total number of genera to 12 (Tosh *et al.* 2009). More recently, it was proposed to reduce *Psilanthus* to synonymy of *Coffea* (Davis 2010), and a genus *Kupeantha* Cheek was recognized (see further in this Introduction). A second major step was taken by Arriola *et al.* (2018) who in their molecular analysis focused on the Asian representatives of Coffeae. Their work confirmed the results of Davis *et al.* (2007); the details are beyond the context of this study dealing with African representatives.

In the analysis of Coffeae by Davis *et al.* (2007), several suprageneric clades were supported, among others a clade with *Argocoffeopsis*, *Calycosiphonia*, and ‘*Calycosiphonia* cf.’ (these three lineages in a tritomy). In this context, a new species *Calycosiphonia pentamera* Sonké & Robbr. was described shortly after (Sonké *et al.* 2007). It showed important differences with the then bispecific genus *Calycosiphonia*, inter alia by the absence of multilocellate anthers, but it was found to be even more different from *Argocoffeopsis*. Therefore, the authors decided that the novelty should be placed in an expanded genus *Calycosiphonia*. Simultaneously, Davis & Sonké (2008) published the former ‘*Calycosiphonia* cf.’, after an extensive morphological investigation, as *Argocoffeopsis spathulata* A.P.Davis & Sonké, seeing “no logic in describing *Calycosiphonia* cf. in a new genus, as apart from the size and shape of its fruit, it shows no major morphological differences when compared with either *Argocoffeopsis* or *Calycosiphonia*.” In a scenario where *Argocoffeopsis* and *Calycosiphonia* would be considered as congeneric, i.e., clade (2) or the *Argocoffeopsis*-*Calycosiphonia* clade (hereafter called the AC clade) would represent a single genus, the name *Argocoffeopsis* would have priority. Therefore, Davis & Sonké (2008) prudently placed ‘*Calycosiphonia* cf.’ under *Argocoffeopsis*. A few years later, Cheek & Thiengué (2010) discovered another species related to *A. spathulata* and considering that *Calycosiphonia* “probably deserves to be synonymised”, they recognized the novelty as *Argocoffeopsis fosimondi* Tchiengué & Cheek.

Cheek *et al.* (2018) used morphological and plastid sequence data to clarify the generic relationships in the AC clade and assess the generic placement of the species *Calycosiphonia pentamera*, *Argocoffeopsis spathulata*, and *A. fosimondi*. Their phylogenetic results confirmed the monophyly of the AC clade, which comprises three lineages: (a) a *Calycosiphonia* clade matching the bispecific concept of Robbrecht

(1981b), (b) a clade with *Argocoffeopsis spathulata* and *A. fosimondi*, and (c) an *Argocoffeopsis* clade matching the concept of the genus by Robbrecht (1981b). However, the relationships between these three lineages within the AC clade were not resolved (Cheek *et al.* 2018: fig. 1). Morphological characters corroborated the three evolutionary lineages, and the authors proposed to recognize lineage (b) at the generic level, naming it *Kupeantha*. According to Cheek *et al.* (2018), *Kupeantha* has supra-axillary buds, another type of tertiary venation, less conspicuous calyx lobes, and non-locellate anthers. Since sequence data for *Calycosiphonia pentamera* was not available, that species was transferred to *Kupeantha* on morphological grounds alone.

To date, the AC clade comprises three genera and 15 species: *Argocoffeopsis* with eight species (Robbrecht 1981b), *Calycosiphonia* with two species (Robbrecht 1981b), and *Kupeantha* with six species (Cheek *et al.* 2018; Alvarez-Aguirre *et al.* 2021). In July 2014, during field work in Kibira National Park (Burundi), a montane forest reserve in the Kivu-Ruwenzori system of the Afromontane Region, the first author of this study noticed a peculiar population of coffee-like shrubs. We believed to have found a novel species related to *Calycosiphonia spathicalyx* (K.Schum.) Robbr., but this assumption could not be verified because only young flower buds were collected. In July 2022, the first two authors were able to return to Kibira National Park and collect material with mature flowers and fruits. The new species appeared to have anthers without transverse septa and this, in combination with other characters, casted doubt on its affiliation to *Calycosiphonia* and rather seemed to suggest a position in *Kupeantha*.

In this paper, we produce a morphological and biogeographical analysis of the new species from the Kivu-Ruwenzori system (hereafter called the KR species), comparing it in detail with the other members of the AC clade and performing a molecular study to clarify its generic position. We also provide a full taxonomic treatment with a formal name, a description, and a preliminary conservation assessment.

Material and methods

Morphological analysis

This study is based on the investigation of living plants in situ and preserved herbarium material kept in BJA and BR (acronyms according to Thiers 2023), with duplicates distributed to BRLU, C, E, EA, G, GENT, GIS, K, L, LG, M, MO, P, S, WAG, and YA. Macro-characters, such as plant height, measurements, colours, and other details given in the descriptions are based on living material, spirit, and herbarium specimens, and data derived from field notes. Morphological micro-characters are observed using a stereoscopic binocular. Pollen of the KR species and its relatives were examined with light and scanning electronic microscopy after acetolysis (Supp. file 1).

Geographical coordinates of the herbarium collections were extracted from the labels and used to present a distribution map of the KR species. The map was produced with ggplot2 ver. 3.4.2 (Wickham 2016) using map tiles by Stamen Design (<http://maps.stamen.com>) and data by OpenStreetMap (<https://www.openstreetmap.org>).

The preliminary IUCN conservation assessment of the KR species was carried out according to the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Subcommittee 2022). The extent of occurrence (EOO) and the area of occupancy (AOO) were calculated with GeoCAT (Bachman *et al.* 2011).

Molecular analyses

The phylogenetic placement of the KR species was established by phylogenetic analyses of chloroplast and nuclear data. High-quality genomic DNA was extracted from silica-dried leaf samples and herbarium samples using an adapted CTAB (cetyltrimethyl-ammonium bromide) protocol (Doyle & Doyle

1987). Quantification and purity assessment of the DNA was done with a Fragment Analyzer (Agilent Technologies, Inc.).

The analysis of the chloroplast data was done by combining newly sequenced material with the published dataset of Cheek *et al.* (2018). Voucher information and accession numbers are provided in Supp. file 2. The markers *accD*, *rpl16*, and *trnLF* were sequenced using the primers of Davis *et al.* (2007). The newly obtained and existing sequences were automatically aligned with MAFFT ver. 7.490 (Kato & Standley 2013), as a plugin in Geneious Prime ver. 2023.1.2 (<http://www.geneious.com>). The best-fit nucleotide substitution model for each marker was selected using the Akaike information criterion in jModelTest ver. 2.1.10 (Darriba 2012). The model selection test showed that the SYM model is the most optimal model for all three markers. The partitioned chloroplast dataset was analysed with MrBayes ver. 3.2.7 (Ronquist & Huelsenbeck 2003), running for 3 million generations and sampling every 1000th generation. Chain convergence and ESS parameters were checked with Tracer ver. 1.7.2 (Rambaut *et al.* 2018) and the 50% majority rule consensus tree was calculated with a burn-in of 10%. The tree with the posterior probabilities was displayed using FigTree ver. 1.4.4 (Rambaut 2018).

The analysis of the nuclear data was done by sending extracted DNA to Diversity Arrays Technology (Canberra, Australia) for DArTseq analysis, a sequencing technology based on genome complexity reduction (Kilian *et al.* 2012). A reduced sampling of the tribe Coffeae was used for this analysis; voucher information and accession numbers are provided in Supp. file 3. The quality of the obtained reads was checked with FastQC ver. 0.11 (Andrews 2010), and barcodes and restriction site remnants were removed with Cutadapt ver. 3.5 (Martin 2011) as part of GBprocess ver. 4.0.0.post1 (Schaumont 2020). The reads were mapped against the *Coffea canephora* Pierre ex A.Froehner reference genome (Denoeud *et al.* 2014) using BWA-MEM ver. 0.7.17-r1188 with default settings (Li & Durbin 2009). Variant calling was performed with Unified Genotyper as part of GATK ver. 3.7 (McKenna *et al.* 2010). Variants with SNP quality or genotype quality below 20, non-polymorphic sites, and sites with more than one alternative allele were removed with a custom Python script (<https://gitlab.com/sander.debacker/vcffilter>). Another custom Python script (<https://gitlab.com/ybawin/smapapps>) converted polymorphic loci into nucleotide alignments to be analysed with IQ-TREE ver. 2.2.0 (Minh *et al.* 2020) under the GTR+I+G model and ultrafast bootstrapping with 1000 replicates (Hoang *et al.* 2018). All consensus locus trees were inferred into one final tree with ASTRAL-III (Zhang *et al.* 2018).

Results and discussion

Morphology

A full organographical description of the KR species is given in the taxonomic treatment. We here describe and discuss morphological characters of interest to compare the KR species with other members of the AC clade. Table 1 summarizes the morphological and biogeographical comparison of the taxa of the AC clade.

Life form and architecture

The KR species is an evergreen shrub with the habit of *Coffea* (Fig. 1B). The architecture follows Roux's model (Hallé *et al.* 1978: 200). Through torsion of the internodes, the plagiotropous flowering branches have all leaves in the same horizontal plane (distichous leaf arrangement), ensuring a more efficient photosynthesis in the forest understorey.

This architecture agrees with most taxa in the AC clade and in Coffeae. The evergreen condition is standard in the tribe, too. However, a number of Zambezian Coffeae are deciduous, e.g., in *Tricalysia* (Robbrecht 1979); in the AC clade, deciduousness is restricted to *Argocoffeopsis eketensis* (Wernham) Robbr. and *A. rupestris* (Hiern) Robbr. (Robbrecht 1981b). The latter genus is also the sole member of the AC clade that is lianescent (Robbrecht 1981b). This is the result of a modification of Roux's model:

Table 1 (continued on next page). Morphological comparison of the taxa in the AC clade. Core *Argocoffeopsis* = *A. subcordata* (Hiern) Lebrun, *A. scandens* (K. Schum.) Lebrun, *A. kivuensis* Robbr., and related species. *Argocoffeopsis eketensis* (Wernham) Robbr. and *A. rupestris* (Hiern) Robbr. (formerly considered to belong to the genus *Argocoffea* (Pierre ex De Wild.) Lebrun) are kept separate. Core *Kupeantha* = *Kupeantha* Cheek with the exclusion of *K. pentamera* (Sonké & Robbr.) Cheek because its inclusion in *Kupeantha* was based on morphology only (Cheek *et al.* 2018). Character states in bold are characteristic for *Calycosiphonia albertina* Ntore & Robbr. sp. nov., when compared to other species of *Calycosiphonia* and *Kupeantha*.

	Core <i>Argocoffeopsis</i>	<i>Argocoffeopsis rupestris</i>	<i>Kupeantha pentamera</i>	Core <i>Kupeantha</i>	<i>Calycosiphonia albertina</i> sp. nov.	<i>Calycosiphonia spathecalyx</i>	and <i>C. macrochlamys</i>
1	Habit	lianas	shrubs	shrubs	shrubs	shrubs	shrubs
2	Foliage	evergreen	deciduous	evergreen	evergreen	evergreen	evergreen
3	Lateral buds	axillary	axillary	supra-axillary	axillary	axillary	axillary
4	Stems and leaves	mostly hairy	hairy	glabrous	glabrous	glabrous	glabrous
5	Suberisation bark	late	late	late	late	late	early, sometimes late
6	Colour old rhytidome	brownish	brownish or greyish	greyish to brownish	whitish	reddish brown	brownish (<i>C. s.</i>); greyish (<i>C. m.</i>)
7	Herbarium material, colour in dried state	greenish or brownish	brownish	blackish	blackish	blackish	blackish
8	Heterophylly – blades on orthotropous branches reduced	yes	? (field study of these deciduous taxa needed)	no	no	yes	yes (<i>C. s.</i>); ? (<i>C. m.</i>)
9	Anisophylly	no	no	no	no	yes	occasionally
10	Differentiation in macro- and microphyllous nodes	very occasional	no	no	no	yes, pronounced	occasionally, not pronounced
11	Leaf blade tip	acuminate	acuminate	acuminate or spatulate	acuminate	acuminate	acuminate or spatulate
12	Domatia	tuft, pocket, pit, or crypt type	pocket	absent	absent	tuft type	crypt type (<i>C. s.</i>); absent (<i>C. m.</i>)
13	Bracts & bracteoles	pre-calyculi	pre-calyculi or scale-like calyculi	calyculi, very rarely upper calyculus with foliaceous appendages	calyculi without foliaceous appendages	calyculi, upper one with foliaceous appendages	calyculi, at least upper one with foliaceous appendages
14	Flowers	(4–)5–8-merous	6–8-merous	5-merous	(4–)5-merous	5–6-merous	7–8-merous
15	Calyx	short tube, often with irregular margin	short tube with short teeth or vague lobes	short tube with short teeth or vague lobes	short rim surpassed by nectary disc	short tube, truncate	long tube splitting at anthesis (<i>C. s.</i>); truncate short tube (<i>C. m.</i>)

Table 1 (continued). Morphological comparison of the taxa in the AC clade. Core *Argocoffeopsis* = *A. subcordata* (Hiern) Lebrun, *A. scandens* (K.Schum.) Lebrun, *A. kiviensis* Robbr., and related species. *Argocoffeopsis eketensis* (Wernham) Robbr. and *A. rupestris* (Hiern) Robbr. (formerly considered to belong to the genus *Argocoffea* (Pierre ex De Wild.) Lebrun) are kept separate. Core *Kupeantha* = *Kupeantha* Cheek with the exclusion of *K. pentamera* (Sonné & Robbr.) Cheek because its inclusion in *Kupeantha* was based on morphology only (Cheek et al. 2018). Character states in bold are characteristic for *Calycosiphonia albertina* Nitore & Robbr. sp. nov., when compared to other species of *Calycosiphonia* and *Kupeantha*.

	Core <i>Argocoffeopsis</i>	<i>Argocoffeopsis rupestris</i> and <i>A. eketensis</i>	<i>Kupeantha pentamera</i>	Core <i>Kupeantha</i>	<i>Calycosiphonia albertina</i> sp. nov.	<i>Calycosiphonia spathecalyx</i> and <i>C. macrochlamys</i>
16	Corolla tube/lobes ratio	1–1.5	1.2–1.5; lobe much shorter than tube	0.5–0.7	0.7–0.8; ± rotate with tube much shorter than lobes	1–1.5
17	Style/stigmatic arms ratio	3–5	3.2	1.2–6	1; massive stigmatic arms on thin and short style	4–5
18	Ovules per placenta	1	1	1	1	1–2
19	Anther thecae	without transverse septa	without transverse septa	without transverse septa	without transverse septa	multilocellate
20	Anther tip	apiculate or mucronate	apiculate	apiculate	blunt	apiculate
21	Anther connective	not widened	not widened	not widened	not widened	widened
22	Fruit shape	spherical, ± didymous; foliaceous lobes of pre-calyculi accrescent	spherical, somewhat flattened; foliaceous lobes of pre-calyculi accrescent	ellipsoid	ellipsoid	spherical
23	Fruit colour	red	red	orange	red	orange (<i>C. s.</i>); unknown (<i>C. m.</i>)
24	Placental outgrowth around seeds	not present	not present	not present	not present	seeds entirely surrounded
25	Pollen	(3–)4–5-colporate; exine rugulate	(3–)4–5-colporate; exine rugulate	3-colporate; exine rugulate	3-colporate; exine rugulate	3-colporate; exine rugulate
26	Biogeography	Guineo-Congolian	Guineo-Congolian & Zambezian	Lower Guinean	Afromontane (Kivu-Ruwenzori)	Guineo-Congolian and Indian Ocean coastal belt

the branches are strictly horizontal or even recurved and, as such, function as climbing hooks. Lianas are otherwise very rare in Coffeae; this habit characterizes for example the two species of *Sericanthe* subgen. *Macrocarpus* Robbr.: *S. adamii* (N.Hallé) Robbr. and *S. auriculata* (Keay) Robbr. (Robbrecht 1981a).

Suberisation of the bark

The bark of the branches of the KR species turns very distinctly corky. While the youngest parts of the branches are glossy and vivid green (blackish when dried) (Fig. 1D), the older parts undergo suberisation and become reddish brown (brown when dried) (Fig. 1C). The bark of the older stems is dull, fissured, and greyish brown (Fig. 2B).

Suberisation of the bark is universal in the AC clade, though in *Argocoffeopsis* the bark usually becomes flaky or pellicular. Suberisation starts mostly late, i.e., herbarium specimens have one to several nodes below the stem apex that are unsuberised. Cheek *et al.* (2018) stated that suberisation in *Calycosiphonia* develops within millimetres of the stem apex, while in *Kupeantha* it develops (1–)2(–4) internodes below the stem apex. However, this is not fully correct, as we have observed that in the two species of *Calycosiphonia*, suberisation is indeed often early, but specimens showing late suberisation occur as well (e.g., *Letouzey 2108*, BR0000006986506).

In the KR species, the colour of the corky parts of the stem is reddish brown (Fig. 1C). This seems unique in the AC clade, where the older rhytidome is generally greyish to brownish, and white-pale in *Kupeantha* (Cheek *et al.* 2018).

Leaf acumen

The KR species has leaves with a drip tip (Fig. 3A), as is standard in the AC clade and common in rain forest species in general. In some members of the clade, the drip tip is spatulate at the end, e.g., in *Kupeantha spathulata* (Cheek *et al.* 2018) and occasionally in *Calycosiphonia spathicalyx* (Robbrecht 1981b). Spatulate leaves are also known from *Psilanthus lebrunianus* (Germ. & Kesler) J.-F.Leroy ex Bridson (Moens 1962, as *Coffea lebruniana* Germ. & Kesler). This character is otherwise rare in Rubiaceae and only has taxonomic importance at the specific level.

Heterophylly

The KR species exhibits a remarkable case of ‘triple’ heterophylly. First, the leaves of a pair are often slightly different in size (Figs 2C, 3A; character 9 in Table 1). However, compared to other members of Rubiaceae (e.g., *Sabicea* Aubl., Robbrecht *et al.* 2020: fig. 6D), this anisophylly is weak.

The second type of heterophylly is much more apparent: on the plagiotropous branches, a number of (mainly flowering) nodes have a much-reduced pair of leaves (i.e., microphyllly; Figs 1D, 3A; character 10 in Table 1). The number of ‘microphyllous nodes’ on a branch is lower than the number of proper nodes. The variation of this feature is shown in Supp. file 4. The microphyllous nodes may have leaf blades that are only 50–75% smaller than those at normal nodes, but more often the leaves are more extremely reduced, even to vestigial scales. Microphyllous nodes are often anisophyllous at the same time (Fig. 2C).

The internode below a microphyllous node is often shorter than the other internodes. The combination of several short internodes having scale-like leaves may then result in a ‘pseudo-node’ – in reality, two or three nodes tightly placed together – with an increased number of flowers (Supp. file 5). The most extreme case was observed in *Ntore et al. 13* (BR0000027468784V), with a pseudo-node composed of 3–4 microphyllous nodes.

We also observed occasional anisophyllous as well as microphyllous nodes in *Calycosiphonia macrochlamys* (K.Schum.) Robbr. and *C. spathicalyx*. In these two species, the microphyllly is much

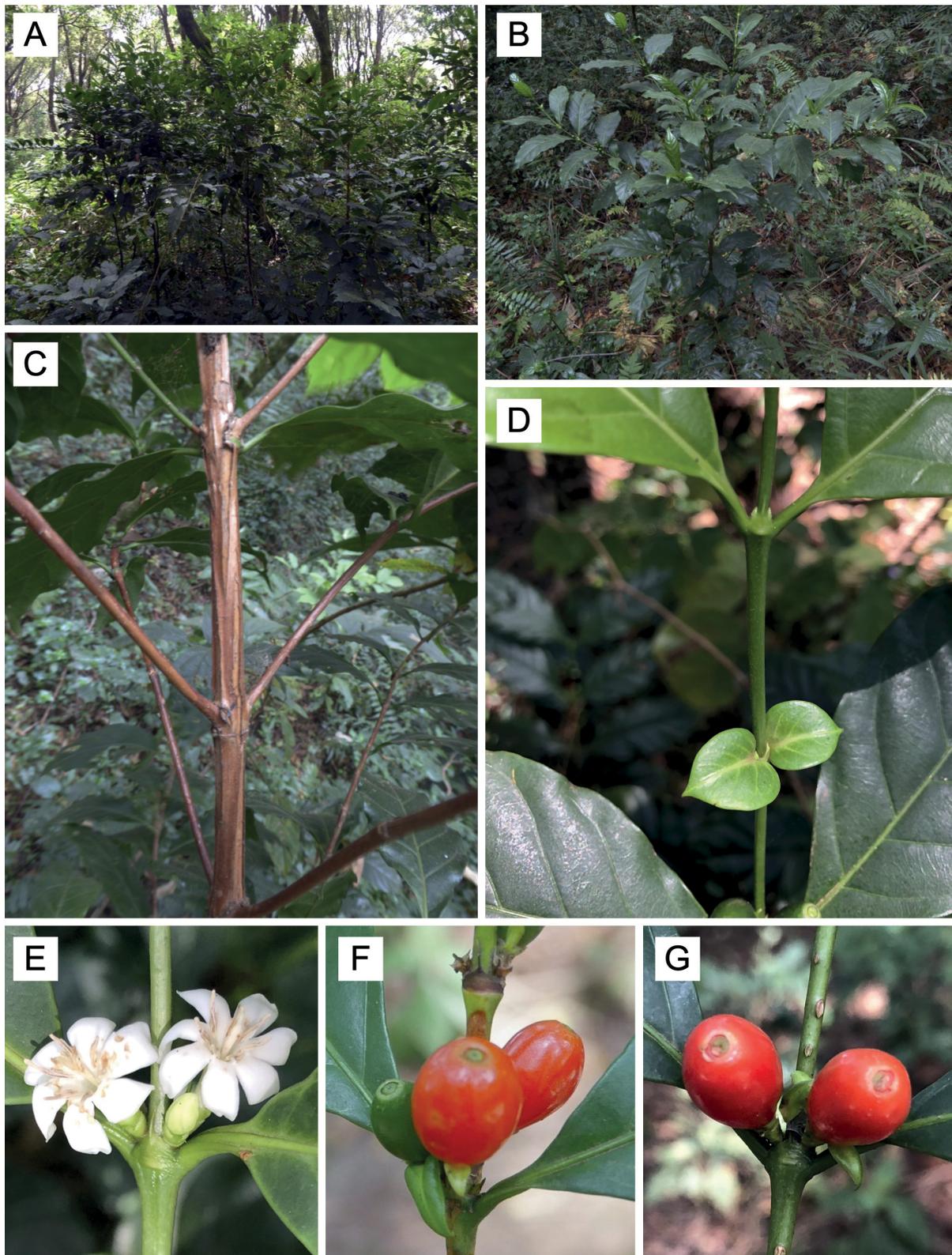


Fig. 1. *Calycosiphonia albertina* Ntore & Robbr. sp. nov. **A.** Habitat. **B.** Habit. **C.** Reddish brown bark of older branches. **D.** Microphyllous node. **E.** Flowering node. **F.** Fruiting node, lateral view of red fruits. **G.** Fruiting node, apical view of red fruits. Photographs by Salvator Ntore from the subpopulation in Kibira forest, Burundi.

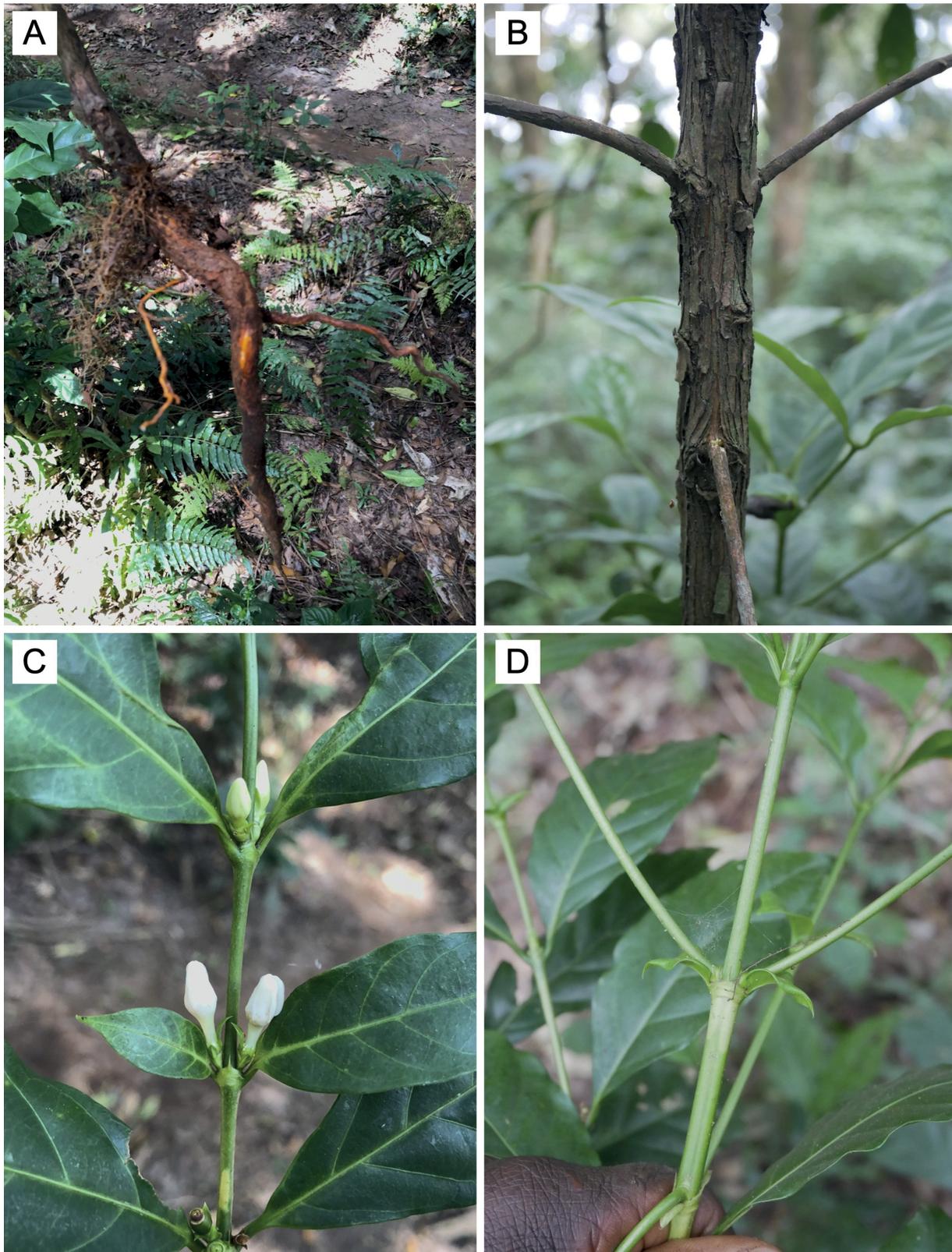


Fig. 2. *Calycosiphonia albertina* Ntore & Robbr. sp. nov. **A.** Root system of a young individual. **B.** Greyish brown bark of an older stem. **C.** Anisophyllous node. **D.** Reduced leaves on the main axis. Photographs by Salvator Ntore from the subpopulation in Kibira forest, Burundi.

less pronounced than in the KR species, and we only observed it in a few specimens (Supp. file 6). Only because we were struck by the strong expression of this feature in the KR species did we recognise its rare presence in the two species of *Calycosiphonia*. We also observed a single specimen with a microphyllous node in *Argocoffeopsis scandens* (K.Schum.) Lebrun (Supp. file 6). In *Kupeantha*, anisophylly and microphyllous seem absent.

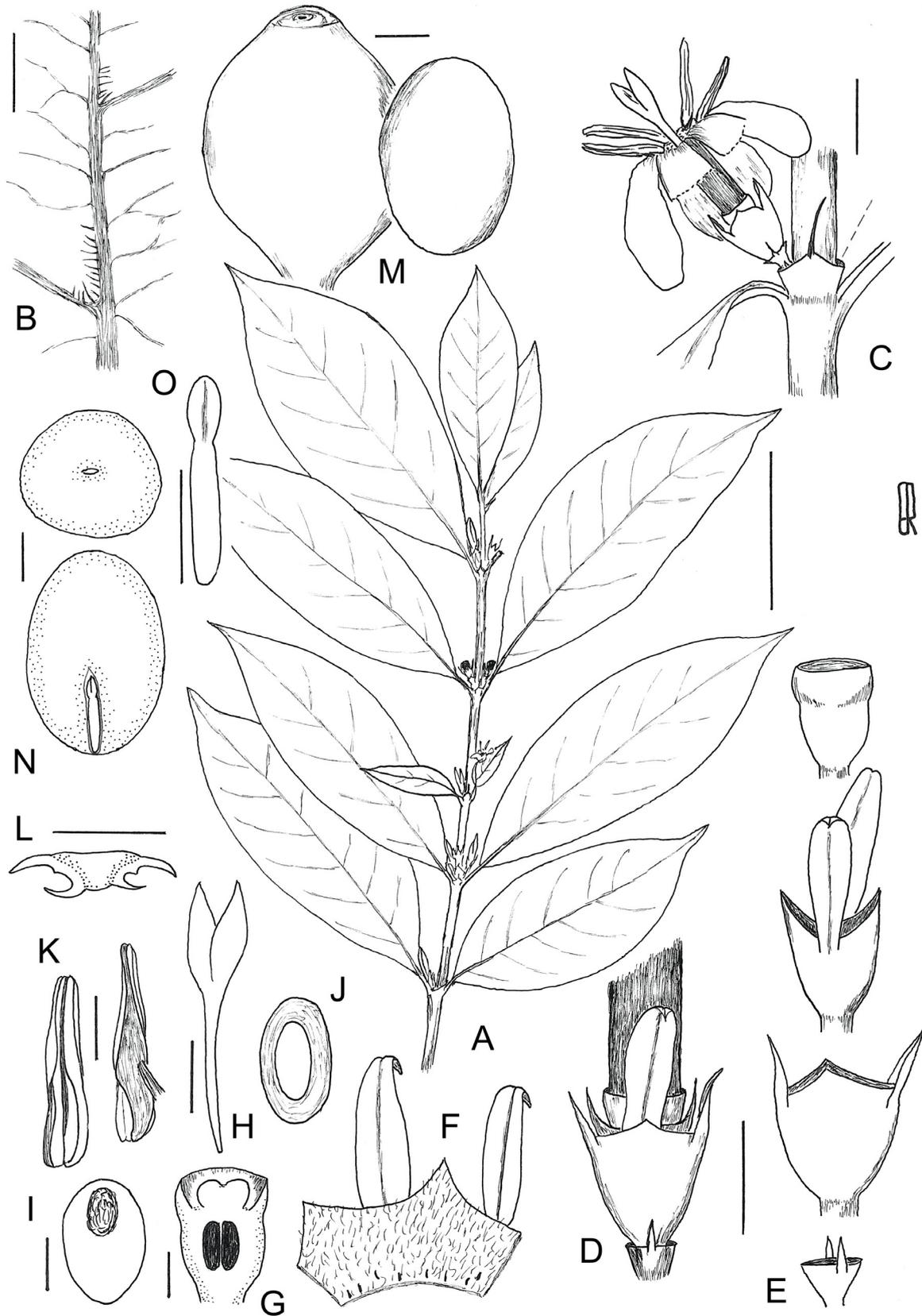
Finally, the KR species possesses a third kind of heterophylly: the leaves on orthotropous branches are much reduced (Fig. 2D; character 8 in Table 1). This type of heterophylly is not uncommon in Coffeae and has been reported for the AC clade before, e.g., in *Argocoffeopsis kivuensis* Robbr. (Robbrecht 1981b: fig. 1A). In that species, the leaves on orthotropous branches may be smaller and more rounded than the leaves on plagiotropous branches. The same is also known from the related tribe Octotropideae Bedd. (illustrated for *CremaSpora* in Robbrecht *et al.* 2020: fig. 6C).

To our knowledge, microphyllous flowering nodes for Rubiaceae are extremely rare. Verdcourt (1977: 816) reported a similar feature in *Chazaliella coffeosperma* (K.Schum.) Verdc. and *C. ramisulca* Verdc. (now in *Eumachia* DC., Palicoureeae Robbr. & Manen, Rubioideae Verdc.). In these species, he stated, “there are curious leafless nodes in some specimens with stipules and minute leaf-vestiges situated about one third to one half of the distance between two proper nodes, from the node above.” According to Olivier Lachenaud (pers. comm. Feb. 2023), this is characteristic for the whole genus *Eumachia*. However, these microphyllous nodes are not associated with flowering because the genus is characterised by terminal inflorescences (Taylor *et al.* 2017). A similar type of heterophylly was also reported for SE Asian *Damnacanthus* C.F.Gaertn. In this genus, sympodial branches display an alternation of normal and microphyllous nodes (Robbrecht *et al.* 1991: figs 1C, 2A, 3). This alternation is closely linked to the occurrence of thorns (i.e., modified inflorescences). Also in this case, the situation is not fully comparable to what is here described for *Calycosiphonia albertina* sp. nov. Note that *Damnacanthus* is not closely related to Coffeae; it belongs to the tribe Mitchellleae Razafim. & B.Bremer (Rubioideae).

Inflorescences and calyculi

The inflorescences of the KR species are axillary and paired at the nodes (Fig. 1E) and strongly congested if more than one flower occurs per inflorescence. The bracts and bracteoles are fused into cupular calyx-like structures called ‘calyculi’. Inflorescences may superficially appear many-flowered as a result of the above-described feature of pseudo-nodes. Supp. file 5 shows the dissection of such a pseudo-node consisting of one proper node and one microphyllous node, separated by an internode only 2.5 mm long. At the microphyllous (upper) node, the inflorescence on the right is uniflorous, with three calyculi. On the left two flowers occur, a mature 3-calyculate flower and a 2-calyculate flower bud. These two sessile flowers sit next to each other without any sign of a common pair of bracts. The proper node has three flowers on each side, with in total one 2-calyculate and five 3-calyculate flowers. All flowers are sessile

Fig. 3. *Calycosiphonia albertina* Ntore & Robbr. sp. nov. **A.** Plagiotropous branch with flowers, flower buds, and young fruits. **B.** Underside of a leaf with two sparse tuft domatia. **C.** Node with stipule and a 1-florous inflorescence (the dashed line shows the position of the removed second inflorescence). **D.** Base of flower showing calyculi enfolding each other and the ovary; the calyx is visible. **E.** Ovary and calyx (uppermost detail) and three calyculi separated from each other. **F.** Longitudinally opened upper calyculus from E, showing inside covered with fine curled hairs and some colleter at the base. **G.** Longitudinal section of ovary and calyx. **H.** Style and stigma. **I.** Adaxial view of placenta. **J.** Abaxial view of placenta. **K.** Side and frontal views of stamen. **L.** Transverse section of an anther. **M.** Fruit and seed. **N.** Transverse and longitudinal section of seed, showing the position of the embryo. **O.** Embryo. A from *Nkengurutse 136*; B–L from *Ntore et al. 16*; M–O from *Ntore et al. 15*. Scale bars: A = 5 cm; B–C, K = 2 mm; D–F, H, M–O = 3 mm; G, I–J, L = 1 mm. Drawn by Elmar Robbrecht.



and sit next to each other, also without any sign of a common peduncle with a pair of bracts. The available space is so limited that the six flowers sit all around the stem, which makes it difficult to distinguish the left and right groups of flowers. It is clear that ontogenetic studies are needed for a complete understanding of the morphology. Due to the absence of a common bracteal calyculus, one may deduce that the apparently 2- or 3-flowered inflorescences are, in fact, 2 or 3 coaxillar uniflorous inflorescences. Lebrun (1941: 20–24, pl. II 1–2, 6) showed this for *Argocoffeopsis* and *Calycosiphonia*.

The calyculi of the KR species envelop both each other and the ovary. However, the calyx is not ‘hidden’ as is the case in *Calycosiphonia macrochlamys* (Robbrecht 1981b: fig. 6G). The calyculi are composed of a sheathing stipular part that bears a foliar appendix on either side. Only in the upper calyculus this foliar portion is truly leafy, with a distinct midnerve. In the lower calyculi, the foliar parts have the aspect of scales, the smallest ones observed on the lowermost calyculus. Bracts and bracteoles fused into calyculi (sometimes called ‘epicalyces’) are common to all Coffeae (Davis *et al.* 2005). Their presence gave rise to the generic name of one of the largest genera of the tribe, *Tricalysia* (Robbrecht 1987). In the calyculi of species of Coffeae (and thus also in the KR species), it is usually possible to distinguish the two parts of foliar origin from the two stipular parts because the latter are somewhat wider and shorter. This is obviously the case in the KR species, as well as in *Calycosiphonia* and *Kupeantha*; these three taxa have very similar calyculi.

The situation in *Argocoffeopsis* deserves some further consideration. The flowers are solitary and terminal on reduced axillary short-shoots (brachyblasts) paired at the nodes (Robbrecht 1981b). In *A. kivuensis*, the brachyblasts consist of 2–4 very distinct internodes bearing pairs of almost ‘normal’ leaves with ‘normal’ stipules (Robbrecht 1981b: fig. 1A). In the other species, there is a trend to reduction: a shortening of the internodes and a decrease in the size of the leaves. Another condition occurs in the deciduous *A. eketensis* where the leaves on the brachyblasts are strongly reduced to become membranous and scaly (Bridson & Verdcourt 2003: 520). The reduced brachyblasts of *Argocoffeopsis* might be at the base of the axillary inflorescence of the tribe Coffeae, but the bracts and bracteoles represent ‘pre-calyculi’ that never reach the modified calyx-like structure of the other genera. This hypothesis for the origin of the axillary inflorescence can be linked to the many-flowered compound thyrsoid inflorescences that exist in other members of the Coffeae, e.g., in species of *Tricalysia* (Robbrecht 1987). Lebrun (1941: 20–24, pl. II 5–12) demonstrated that in *Coffea* this is caused by the development of lateral primordia in the axils of the calyculi that can grow into lateral inflorescence axes, bearing flowers in triplets.

Stamens

The KR species has inframedifixed stamens with a short filament inserted at the throat and hence they are exerted from the corolla tube at anthesis. The anther connective is \pm triangular in cross-section, and the thecae are without transverse septa (Fig. 3K–L).

The stamens of the KR species are similar to those of *Argocoffeopsis* and *Kupeantha* and belong to a type that is almost universal in Rubiaceae. *Calycosiphonia* deviates with regard to its stamens because the connective is broadened (Robbrecht 1981b: fig. 7H), resembling the connective in *Sericanthe*, another member of Coffeae. However, *Sericanthe* anthers are basifixed, and the enlarged connective is even more pronounced (Robbrecht 1978). The thecae of *Calycosiphonia* anthers have transverse septa (Robbrecht 1981b: fig. 7G) and such multilocellate anthers are otherwise absent in the AC clade and very rare in Rubiaceae.

Pollen

The KR species has spheroidal (P/E 0.84–0.97), radiosymmetric, isopolar, 3-zonocolporate pollen (Fig. 4). The grains are medium-sized (polar axis P 15.5–16.9 μm , equatorial axis E 17.7–20.0 μm). The exine is vermiculate to rugulate, with narrow grooves and convex wide muri in the mesocolpium, while in the

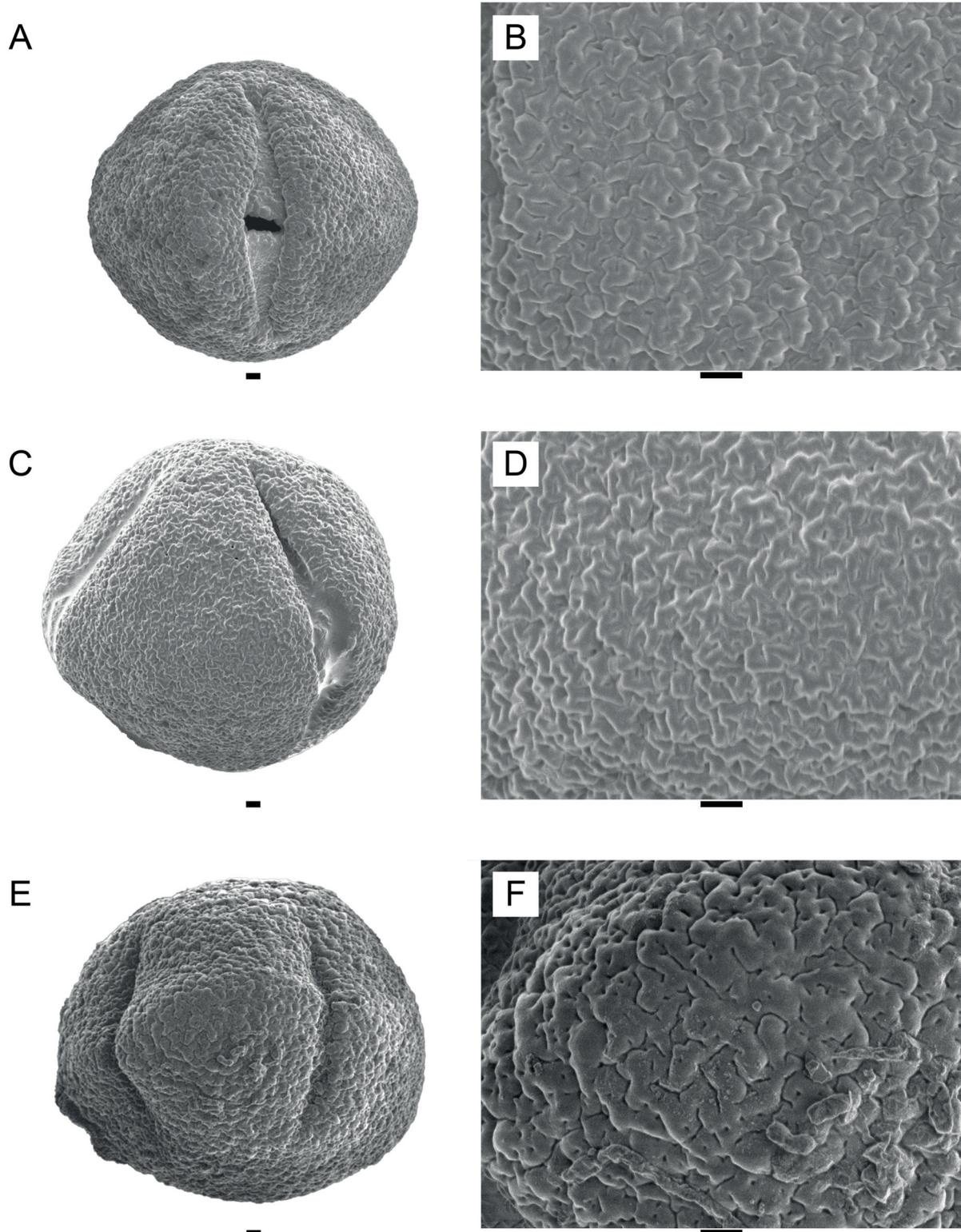


Fig. 4. Scanning electron micrographs of pollen grains. **A–B.** *Calycosiphonia albertina* Ntore & Robbr. sp. nov. (from *Nkengurutse 130*). **C–D.** *Kupeantha spathulata* (A.P.Davis & Sonké) Cheek (from *Sonké 4117*). **E–F.** *Argocoffeopsis rupestris* subsp. *rupestris* (Hiern) Robbr. (from *Essou 1472*). A, C, E = equatorial views; B, D, F = mesocolpia. Scale bars = 1 μ m.

apocolpium the grooves tend to be replaced by pores. The colpus membrane is verrucate. The exine is ca 1 μm thick, with the tectum representing ca 60% of the thickness, the bacula ca 25%, and the foot layer ca 15%.

The same pollen type is reported for *Calycosiphonia spathicalyx* (Robbrecht 1981b) and *Kupeantha kupensis* (Cheek *et al.* 2018; exine there described as vermiculate-reticulate). *Argocoffeopsis* also possesses pollen grains with a similar exine but differs in having (3–)4–5 apertures (Robbrecht 1981b). Our observations of a few species confirm and complete this earlier pollen morphological work (Fig. 4; Supp. file 1). The AC clade is stenopalynous, the number of apertures being the only variable character: (3–)4–5-colporate in *Argocoffeopsis* vs 3-colporate in *Calycosiphonia* and *Kupeantha*. The exine type universally observed in the AC clade is unique in Coffeae and clearly should be considered as a synapomorphy for the AC clade. Therefore, pollen characters are not informative with regard to the genus position of the KR species.

Most other Coffeae (*Belonophora*: Robbrecht 1980: pl. 2.48; *Empogona* and *Tricalysia*: Robbrecht 1979, 1987; *Diplospora* and *Discospermum*: Ali & Robbrecht 1991) have the standard pollen type of Rubiaceae (i.e., 3-zonocolporate with reticulate exine) except for *Sericanthe* that has pollen grains 6-sinuate or hexagonal in polar view, with verrucate exine (Robbrecht 1978). In contrast to other Coffeae, the clade *Coffea/Psilanthus* is remarkably eurypalynous (Stoffelen *et al.* 1997). *Coffea* has 3-colporate pollen with a perforate exine, grading to rugulate in some species, while *Psilanthus* has 4–5-colporate grains with a remarkable intricately curved spaghetti-like tectum. In the two genera, many species possess supratectal granules.

Fruits and seeds

The fruits of the KR species are red (Fig. 1F–G) and fleshy with a thin endocarp (Fig. 3M–O). They have two hemi-ellipsoidal seeds, one in each locule. There is no placental outgrowth around the seeds. The seed-coat is rudimentary.

The fleshy fruits and plano-convex seeds are generally similar to those of the other members of the AC clade. Placental outgrowths are characteristic of most genera of the related tribe Gardenieae (Robbrecht & Puff 1986). The same is true for *Calycosiphonia*: the two species have seeds completely surrounded by placental pulp (Robbrecht 1981b: fig. 8). In the other members of the AC clade, *Argocoffeopsis* and *Kupeantha*, outgrowths are absent (Robbrecht 1986; Cheek *et al.* 2018). In other Coffeae, placental outgrowths characterize the Asian genus *Discospermum* (Ali & Robbrecht 1991) but are otherwise restricted to scattered species in several genera (*Empogona*, *Sericanthe*, and *Tricalysia*; Robbrecht 1978, 1979, 1987).

Plano-convex seeds and rudimentary seed coats are synapomorphies of the AC clade. In rehydrated herbarium material, such seed coats are hardly detectable as a thin film. Spirit-preserved material of *Argocoffeopsis rupestris* allowed Robbrecht (1986) to describe the seed coat in more detail: “seed-coat rudimentary, detaching from the endosperm as a thin membrane of crushed cells lying \pm loose between endocarp and endosperm.” In other Coffeae, rudimentary seed coats are only known in *Belonophora* (Cheek & Dawson 2000). The genera *Coffea* and *Psilanthus* have a unique type of reduced seed coat – known as the ‘silver skin’ in coffee culture – composed of thin elongated reduced parenchyma-like exotestal cells that hold together dispersed sclereids (Robbrecht & Puff 1986: 102, 123, and references therein). In other Coffeae, seed-coats are comprised of an exotesta with mostly parenchyma-like cells (e.g., *Empogona*, Robbrecht 1979: fig. 2; *Tricalysia*, Robbrecht 1987: fig. 9) and an endotesta crushed by the development of the endosperm.

Dispersal

In July 2020, we gathered mature seeds in Kibira National Park to establish plants for ex situ conservation; however, germination at Meise Botanic Garden four weeks after harvesting failed. In June 2023, seeds

were gathered again in Burundi and were sown locally immediately after harvesting. This germination attempt was successful. The seeds of the KR species are therefore highly recalcitrant. Fruits fallen on the ground under the shrub will thus ensure local propagation. This explains the density of the subpopulation that we observed in the Kibira forest. We also observed that the fruits of the KR species are eaten by birds (Ntore *et al.* 8, 9, 16; Ntore & Bukuru 1212, 1213, 1214). According to information received from a guide at the Kibira National Park (i.e., Deus D. Niyiburana), two species are observed: *Colius striatus* (Gmelin, 1789) (Coliidae (Swainson, 1837)) and *Crithagra striolata* (Rüppell, 1840) (Fringillidae Leach, 1819). Avichory therefore likely ensures dispersal of the KR species over larger distances. Endozoochory is common in the AC clade and in other Coffeae (Kainulainen *et al.* 2017).

Very early germination was also reported for *Calycosiphonia spathicalyx* (Robbrecht 1981: fig. 8D) and *Kupeantha pentamera* (Sonké *et al.* 2008: fig. 2D, as *Calycosiphonia pentamera*). Spirit collections of these species contained fruits with seeds that started germination, embryo radicles having left the endosperm and perforating the fruit wall. Recalcitrant seeds are typical for equatorial forest species and known from other Coffeae, e.g., *Coffea* (von Teichman & van Wyk 1994).

Biogeography

We discovered the KR species in Kibira National Park in northwestern Burundi, but it had already been collected in the Bururi Forest Nature Reserve in southwestern Burundi by Jan Rammeloo in September 1974 (Rammeloo 4672, BR0000008651358). The material was provisionally identified as *Coffea* sp. by the last author in 1982 but because it bears only young fruits, no further conclusions were possible at that time. However, the occurrence of microphyllous nodes is in full morphological agreement with



Fig. 5. Distribution of *Calycosiphonia albertina* Ntore & Robbr. sp. nov. in western Burundi, southern Rwanda, and eastern DR Congo.

the material from Kibira National Park and confirms that it belongs to the KR species. During our investigation, we also found two specimens from the montane Nyungwe forest in southwestern Rwanda, filed under *Calycosiphonia spathicalyx*, but clearly belonging to the KR species. Photographic material of a plant with young fruits from Kahuzi-Biega National Park in eastern DR Congo was provided to us by Landry Dumbo (Supp. file 7). The microphyllous nodes and the reddish brown branch confirm that it matches the KR species. These findings establish the known distribution of the KR species to western Burundi, southwestern Rwanda, and eastern DR Congo (Fig. 5).

In northwestern Burundi, the KR species is found in the Kibira forest – Kibira means forest in the Kirundi language – a patch of montane rainforest on the Congo-Nile Divide, a narrow and steep north-south oriented mountain chain, generally less than 16 km wide, which extends across the entire length of Burundi, with an elevation between 1700 and 2670 m. The forest extends from the northwest of the Muramvya province to the northern border with neighbouring Rwanda where it is contiguous with the Nyungwe forest in southwestern Rwanda. The Kibira and Nyungwe forests are the two main locations where the new species can be found. The outlier in southwestern Burundi is the Bururi Forest Nature Reserve, a small patch of forest immediately west of the town of Bururi, on the extreme southern edge of the Congo-Nile Divide. The Kibira and Bururi forests are the last vestiges of a rainforest that once covered a large part of the high mountains of western Burundi. The part that connected them has now disappeared completely as the result of human occupation, which is accompanied by forest clearing for conversion to agriculture and other basic services. Some small remnant patches of rainforest between Kibira and Bururi, such as Monge, Mpotsa, and Vyanda forest, testify to this former continuous extent. The Bururi, Kibira, and Nyungwe forests are part of the Albertine Rift montane rainforest, a biodiversity hotspot that covers the western parts of Rwanda and Burundi, the eastern edge of the Democratic Republic of the Congo, and parts of western Uganda and Tanzania. The area is part of the Afromontane Region (White 1978) and harbours numerous endemic species, e.g., *Dewildemanian burundiensis* Lisowski, *Impatiens bururiensis* Grey-Wilson, *Pavetta troupinii* Bridson (Ntore *et al.* 2018), and *Renalmia susanneae-katziae* Eb.Fisch., Killmann & Dhetchuvi (Fischer *et al.* 2023).

For the DR Congo, the occurrence of the KR species in Kivu makes sense biogeographically. Several other species show the same distribution pattern and occur in western Burundi and western Rwanda, as well as on the western ridge of the Albertine Rift in the eastern DR Congo: e.g., *Ixora burundiensis* Bridson, *Oxyanthus troupinii* Bridson, *Pavetta pierlotii* Bridson, and *Pavetta rwandensis* Bridson (Ntore *et al.* 2018). Therefore, the KR species is another endemic element of the Kivu-Ruwenzori system of the Afromontane Region (*sensu* White 1978).

Coffeae are a paleotropical tribe also reaching NE Australia (Davis *et al.* 2007). In Africa, the Guineo-Congolian Region is the centre of diversity of Coffeae. The tribe also reached the Afromontane, Sudanian, and Zambezian Regions and even occurs in South Africa and Madagascar. This is exemplified by the chorological patterns found in the larger genera *Empogona* (Robbrecht 1979: 256, figs 4–5) and *Tricalysia* (Robbrecht 1987: 58, figs 10–12; Ranarivelo-Randriambovonjy *et al.* 2007). However, the distribution of the AC clade is more restricted. *Argocoffeopsis* is mainly Guineo-Congolian; *A. eketensis* also occurs in the Zambezian Region, and *A. kivuensis* is an Afromontane species endemic of the Kivu-Ruwenzori system (Robbrecht 1981b). The genus mainly occurs between 0 and 1200 m a.s.l. (GBIF.org 2023a), except for *A. kivuensis* that occurs between 1650 and 2100 m a.s.l. (Robbrecht 1981b). *Calycosiphonia* is Guineo-Congolian, with *C. spathicalyx* also occurring in the Indian Ocean coastal belt (Robbrecht 1982). It is essentially a lowland genus, occurring between 100 and 1240 m a.s.l. (GBIF.org 2023b). *Kupeantha* is restricted to the West African mountain system of the Afromontane Region and surrounding lowland, occurring between 400 and 1600 m a.s.l. (with *K. kupensis* perhaps up to 2000 m) (Cheek *et al.* 2018; Alvarez-Aguirre *et al.* 2021; GBIF.org 2023c).

Molecular analyses

The topology of the phylogenetic tree resulting from the analysis of the chloroplast data, comprising representatives of all African and two Asian genera of Coffeae (the Asian genera *Nostolachma* and *Xantonnea* are not represented because of lack of material) (Supp. file 8) did not deviate much from that of Cheek *et al.* (2018). In both analyses, the relationships between *Argocoffeopsis*, *Calycosiphonia*, and *Kupeantha* are not resolved: this resulted in a polytomy in our tree and an unsupported clade (*Kupeantha*, *Argocoffeopsis*) in Cheek *et al.* (2018). The tribe Coffeae is recovered as monophyletic (PP = 1), with the genus *Coffea* as sister to the rest of the tribe. The rest of Coffeae is divided into two clades: the AC clade containing the genera *Argocoffeopsis*, *Calycosiphonia*, and *Kupeantha* (PP = 1), and a clade containing the other genera of the tribe (*Belonophora*, *Diplospora*, *Discospermum*, *Empogona*, *Sericanthe*, *Tricalysia*) (PP = 0.96). Within the AC clade, the genus *Argocoffeopsis* is recovered as monophyletic (PP = 1). Most species of *Kupeantha* are found in a single, supported clade (PP = 1), except for *K. pentamera*. This species is part of a molecular analysis for the first time, with two out of the three studied markers (Supp. file 2), and falls unresolved in the AC clade. The two current species of *Calycosiphonia* are sister lineages (PP = 0.55). The KR species is undoubtedly part of the AC clade but its relationship with the three genera is unclear.

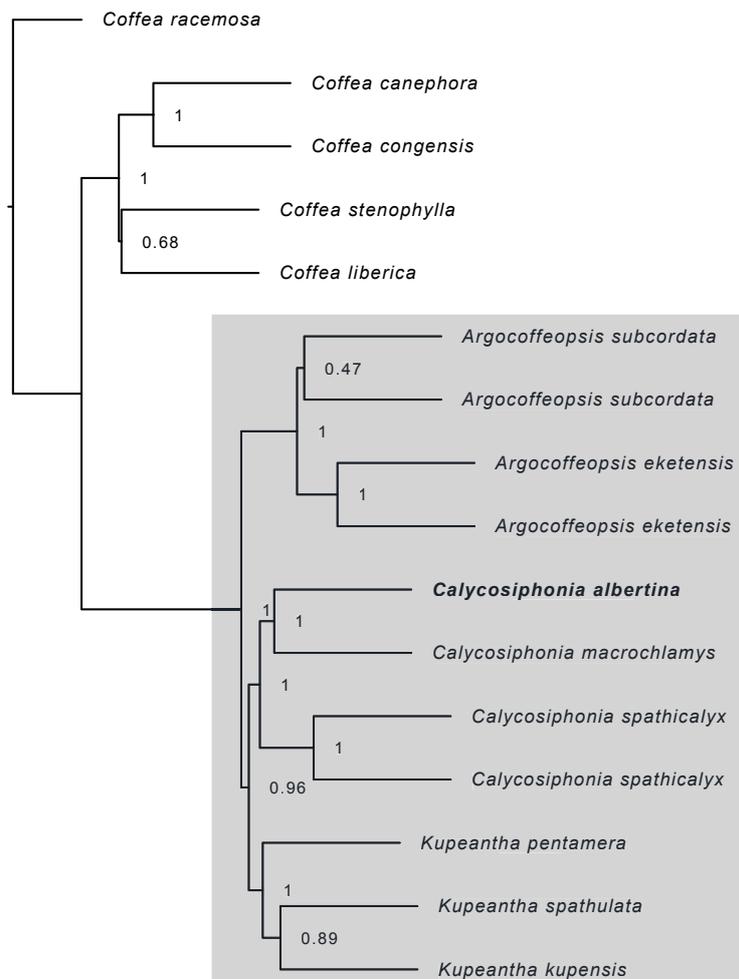


Fig. 6. Phylogenetic tree of the AC clade (in grey) based on nuclear data (3720 loci). The new species *Calycosiphonia albertina* Ntore & Robbr. sp. nov. is indicated in bold. Bootstrap values are indicated at the nodes.

The result of the analysis of the nuclear data is based on 6387 SNPs in 3720 loci from a reduced sampling of the tribe Coffeeae. The species of *Coffea* were used as outgroup for the AC clade (Fig. 6). The AC clade as a whole (BS = 1) and the genera *Argocoffeopsis*, *Calycosiphonia*, and *Kupeantha* are found to be monophyletic (BS = 1). *Calycosiphonia* and *Kupeantha* are sister to each other (BS = 0.96), and *Argocoffeopsis* is sister to both. *Kupeantha pentamera* is strongly supported as part of the genus *Kupeantha* and it falls as sister to the other species. The KR species is strongly supported as part of the genus *Calycosiphonia*.

Taxonomic scenarios

It is beyond any doubt that the KR species belongs to the AC clade of Coffeeae, as demonstrated by the molecular analyses (Fig. 6; Supp. file 8). The presence of uniflorous inflorescences with distinct calyculi, rugulate pollen exine, and planoconvex seeds without hilar scar and with extremely reduced seed coat also confirms this (Table 1).

To solve the issue of the generic position of the KR species, different taxonomic scenarios are possible. (1) Uniting all taxa of the AC clade in an enlarged genus *Argocoffeopsis* should be rejected, as was also mentioned by Cheek *et al.* (2018) when describing the genus *Kupeantha*. Based on habit alone, *Argocoffeopsis* is very different from *Calycosiphonia* and *Kupeantha* (i.e., lianas vs shrubs). (2) Recognizing the KR species as a separate genus would imply that it should be distinguished from the other genera of the AC clade by a few unique characters, mainly the occurrence of microphyllous nodes and the red colour of the fruits. However, this is morphologically weak and we also showed that microphyllous nodes can occasionally occur in the two species of *Calycosiphonia*. (3) Merging *Kupeantha* into *Calycosiphonia*. (4) Including the KR species in either *Calycosiphonia* or *Kupeantha*. Scenarios (3) and (4) seem to be the only viable options.

Morphologically, the KR species seems to better match with *Kupeantha* than *Calycosiphonia* (Table 1). It agrees with *Kupeantha* inter alia because the corolla tube is much shorter than the lobes, the anthers do not have a broadened connective, and the pollen sacs lack transverse septa. With *Calycosiphonia*, the KR species mainly shares the foliaceous appendages of the calyculi. Compared to these two genera, the KR species shows several particular features, not in the least the universal occurrence, on every plagiotropous branch, of microphyllous nodes (Fig. 1D; Table 1) – as mentioned above, this feature also comes to expression, although weakly and only rarely, in *Calycosiphonia*. The strongest morphological argument against the inclusion of the KR species in *Calycosiphonia* might seem the presence of anther thecae without transverse septa. Sonké *et al.* (2007) encountered the same situation when describing *Calycosiphonia pentamera*: “Some will consider the absence of multilocellate anthers in *C. pentamera* as a serious argument against our generic placement of the novelty. However, horizontal division of the pollen sacs has a very scattered distribution in Rubiaceae (Robbrecht 1988) and in general occurs in a limited number of species or even individuals of a genus (inter alia in *Pavetta*, *Leptactina*, *Tricalysia*).” The same is true for the KR species, and therefore the presence of its ‘standard’ anthers cannot be used to argue that a position in *Calycosiphonia* is untenable. It should be noted, though, that *C. pentamera* is now placed firmly in *Kupeantha* (confirmed by our phylogenetic analysis; Fig. 6). The absence of a placental outgrowth around the seeds in the KR species is another argument against placing the species in *Calycosiphonia* because the two species of *Calycosiphonia* have seeds surrounded by placental pulp. However, here as well, the importance of this character should not be overstated. Within Coffeeae, the genera *Empogona* and *Sericanthe* are characterised by uncovered seeds, but isolated species have seeds covered with placental outgrowths (e.g., *Empogona breteleri* (Robbr.) Tosh & Robbr., *Sericanthe jacfelicis* (N.Hallé) Robbr., and *S. pellegrinii* (N.Hallé) Robbr.) (Robbrecht 1978, 1979). In summary, based on morphology, the KR species has similarities with both *Calycosiphonia* and *Kupeantha*.

Biogeographically, the montane KR species is the only species in *Calycosiphonia* or *Kupeantha* that occurs in the Kivu-Ruwenzori system of the Afromontane Region. The genus *Calycosiphonia* has a wide distribution in West Central and Tropical Africa (Robbrecht 1982; POWO 2023), and specimens of *C. spathicalyx* have been recorded in eastern DR Congo. The genus *Kupeantha* is found in Lower Guinea and the West African mountain system (Cheek *et al.* 2018; Alvarez-Aguirre *et al.* 2021; GBIF.org 2023c). However, macrodisjunctions between Lower Guinea and the Albertine Rift have been reported before, even at species level, e.g., *Hymenocoleus rotundifolius* (A.Chev. ex Hepper) Robbr. (Robbrecht 1996), *Impatiens mannii* Hook.f. (GBIF.org 2023d), and *Tricalysia anomala* E.A.Bruce (Robbrecht 1983). In summary, the biogeography does not offer a decisive argument for the inclusion in either *Calycosiphonia* or *Kupeantha*.

Morphology and biogeography do not allow a clear-cut decision with regard to the position of the KR species because *Calycosiphonia* and *Kupeantha* are not easily distinguished. However, the nuclear data leave little doubt and establish three clades: *Argocoffeopsis*, *Calycosiphonia*, and *Kupeantha* (Fig. 6). The here demonstrated sister relationship of the latter two genera would even not hamper their merger (scenario 3 above). However, we refrain to do so because we believe this decision should be taken in the framework of a thorough analysis of all five *Kupeantha* species. In any case, the KR species belongs to *Calycosiphonia* in its possible strictest sense; placing it in *Calycosiphonia* (scenario 4 above) is therefore the most conservative option.

Taxonomic treatment

Class Magnoliopsida Brongn.
Order Gentianales Juss. ex Bercht. & J.Presl
Family Rubiaceae Juss.
Tribe Coffeae DC.
Genus *Calycosiphonia* Pierre ex Robbr.

Calycosiphonia albertina Ntore & Robbr. sp. nov.

urn:lsid:ipni.org:names:77340650-1

Figs 1–3, 4A–B

Diagnosis

A member of the tribe Coffeae because of its axillary inflorescences, paired at the nodes, its contorted corolla aestivation, and its ovules impressed in fleshy placentas attached to the middle of the septum. In that tribe belonging to the *Argocoffeopsis-Calycosiphonia* clade for its plano-convex seeds (mostly two per fruit) with rudimentary seed coat. Peculiar in the reddish brown suberisation of the bark and the plagiotropous branches with numerous nodes having reduced to scaly leaves besides nodes with normal leaves. Belonging to *Calycosiphonia* as indicated by nuclear DNA data, and readily distinguished from *C. macrochlamys* and *C. spathicalyx* by the non-septate anthers with narrow connective that are reminiscent of *Kupeantha*.

Etymology

The epithet ‘*albertina*’ refers to the Albertine Rift in which the species is found.

Type material

BURUNDI • Rugazi, Ruce, P.N. de la Kibira; 03°12'06.2" S, 29°31'43.8" E; 2004 m a.s.l.; 19 Jul. 2022; Ntore *et al.* 14; holotype: BR[BR0000026554747V]; isotypes: BJA, BR[BR0000027462416V], MO, P, K, YA.

Other material examined

BURUNDI • Bubanza, Rugazi, Ruce, Musezero, Parc national de la Kibira; 03°11'39.5" S, 29°32'12.8" E; 2205 m a.s.l.; 2 Oct. 2019; *Nkengurutse 6*; BJA, BR[BR0000025464368V] • Bubanza, Rugazi, Ruce, Musezero, Parc national de la Kibira; 03°11'45" S, 29°31'53" E; 2070 m a.s.l.; 2 Oct. 2019; *Nkengurutse 8*; BJA, BR[BR0000025464382V] • Rugazi, Ruce, P.N. de la Kibira; 03°12'04.8" S, 29°31'42.3" E; 2001 m a.s.l.; 8 Jun. 2022; *Nkengurutse 130*; BJA, BR[BR0000027462409V] • Rugazi, Ruce, P.N. de la Kibira; 03°12'04.8" S, 29°31'42.3" E; 2001 m a.s.l.; 8 Jun. 2022; *Nkengurutse 131*; BJA, BR[BR0000027462430V] • Rugazi, Ruce, P.N. de la Kibira; 03°12'04.6" S, 29°31'41.36" E; 2000 m a.s.l.; 8 Jun. 2022; *Nkengurutse 132*; BJA, BR[BR0000027462508V] • Rugazi, Ruce, P.N. de la Kibira; 03°12'05" S, 29°31'42.3" E; 2005 m a.s.l.; 8 Jun. 2022; *Nkengurutse 133*; BJA, BR[BR0000027462423V] • Rugazi, Ruce, P.N. de la Kibira; 03°12'05.2" S, 29°31'42.5" E; 2004 m a.s.l.; 8 Jun. 2022; *Nkengurutse 134*; BJA, BR[BR0000027462324V] • Rugazi, Ruce, P.N. de la Kibira; 03°12'06.1" S, 29°31'43.6" E; 2009 m a.s.l.; 8 Jun. 2022; *Nkengurutse 135*; BJA, BR[BR0000027462331V] • Rugazi, Ruce, P.N. de la Kibira; 03°12'06.7" S, 29°31'43.4" E; 2011 m a.s.l.; 8 Jun. 2022; *Nkengurutse 136*; BJA, BR[BR0000027450451V] • Rugazi, Ruce, P.N. de la Kibira; 03°12'06.5" S, 29°31'43.7" E; 1997 m a.s.l.; 8 Jun. 2022; *Nkengurutse 137*; BJA, BR[BR0000027462317V] • Rugazi, Ruce, P.N. de la Kibira; 03°12'06.6" S, 29°31'44" E; 1997 m a.s.l.; 8 Jun. 2022; *Nkengurutse 138*; BJA, BR[BR0000027462300V] • Rugazi, Ruce, P.N. de la Kibira; 03°12'06.3" S, 29°31'44.4" E; 1990 m a.s.l.; 8 Jun. 2022; *Nkengurutse 139*; BJA, BR[BR0000027450413V] • Parc national de la Kibira, à env. 8 km de Teza, versant ouest, le long d'un sentier menant vers Bubanza (non loin de la lisière); 03°12'06" S, 29°31'45" E; 2008 m a.s.l.; 19 Jul. 2014; *Ntore 700*; BR[BR0000013563493, BR0000013563615], LG • Parc national de la Kibira, à env. 8 km de Teza, versant ouest, le long d'un sentier menant vers Bubanza (non loin de la lisière); 03°12'06" S, 29°31'45" E; 2008 m a.s.l.; 19 Jul. 2014; *Ntore 701*; BR[BR0000013563653], LG, MO, P • Parc national de la Kibira, 8 km ouest de Teza, versant ouest, le long du sentier menant vers Bubanza (non loin de la lisière); 03°12'06" S, 29°31'45" E; 2008 m a.s.l.; 19 Jul. 2014; *Ntore 702*; BJA, BR[BR0000013563479] • Parc national de la Kibira, à env. 8 km de Teza, versant ouest, le long du sentier menant vers Bubanza (non loin de la lisière); 03°12'06" S, 29°31'45" E; 2008 m a.s.l.; 19 Jul. 2014; *Ntore 703*; BJA, BR[BR0000013563486] • Parc national de la Kibira, à env. 8 km de Teza, versant ouest, le long du sentier menant vers Bubanza (non loin de la lisière); 03°12'06" S, 29°31'45" E; 2008 m a.s.l.; 19 Jul. 2014; *Ntore 704*; BJA, BR[BR0000013563462, BR0000013563622] • Ruce, Parc national de la Kibira; 03°12'06" S, 29°31'45" E; 2004 m a.s.l.; 4 Jul. 2023; *Ntore & Bukuru 1212*; BJA, BR[BR0000026650685V, BR0000026650692V] • Ruce, Parc national de la Kibira; 03°12'06" S, 29°31'45" E; 2004 m a.s.l.; 4 Jul. 2023; *Ntore & Bukuru 1213*; BJA, BR[BR0000026650722V, BR0000026650739V] • Ruce, Parc national de la Kibira; 03°12'06" S, 29°31'45" E; 2004 m a.s.l.; 4 Jul. 2023; *Ntore & Bukuru 1214*; BJA, BR[BR0000026650708V, BR0000026650715V] • Rugazi, Ruce, P.N. de la Kibira; 03°12'06" S, 29°31'45" E; 2008 m a.s.l.; 19 Jul. 2022; *Ntore et al. 8*; BJA, BR[BR0000027450475V], K, P • Rugazi, Ruce, P.N. de la Kibira; 03°12'06" S, 29°31'45" E; 2008 m a.s.l.; 19 Jul. 2022; *Ntore et al. 9*; BJA, BR[BR0000027467633V], S • Rugazi, Ruce, P.N. de la Kibira; 03°12'06.4" S, 29°31'45" E; 2008 m a.s.l.; 19 Jul. 2022; *Ntore et al. 11*; BJA, BR[BR0000027467688V], MO, P • Rugazi, Ruce, P.N. de la Kibira; 03°12'06.4" S, 29°31'45" E; 2008 m a.s.l.; 19 Jul. 2022; *Ntore et al. 12*; BJA, BR[BR0000027467756V], L • Rugazi, Ruce, P.N. de la Kibira; 03°12'06.2" S, 29°31'43.8" E; 2004 m a.s.l.; 19 Jul. 2022; *Ntore et al. 13*; BJA, BR[BR0000027468609V, BR0000027468784V], BRLU, G • Rugazi, Ruce, P.N. de la Kibira; 03°12'06.2" S, 29°31'43.8" E; 2004 m a.s.l.; 19 Jul. 2022; *Ntore et al. 15*; BJA, BR[BR0000027468791V, BR0000026554761V], C, E, EA, YA • Rugazi, Ruce, P.N. de la Kibira; 03°12'06.2" S, 29°31'43.8" E; 2004 m a.s.l.; 19 Jul. 2022; *Ntore et al. 16*; BJA, BR[BR0000026554365V] • [Bururi Forest Nature Reserve], forest gallery of the Sikuvyaye W of the road Mutambara–Bururi; [03°58'07.2" S, 29°36'00.8" E]; [1828 m]; 19 Sep. 1974; *Rammeloo 4672*; BR[BR0000008651358], GENT.

RWANDA • Préfecture de Cyangungu, Commune de Busozo, Forêt de Busoro, sous-bois de forêt à *Cleistanthus polystachyus*, *Diospyros gabunensis* au sommet de la montagne; 02°34'5.0448" S,

29°14'27.402" E; 1740 m a.s.l.; 2 Jul. 1999; *Ewango 2087*; BR[BR0000009717664], GIS, M, MO • Préfecture de Cyangungu, Commune de Busozo, Bweyeye, Forêt de Uruvirevire, sous-bois de la forêt à *Chrysophyllum rwandense*; 02°34'26.7384" S, 29°12' 43.7256" E; 1740 m a.s.l.; 9 Jul. 1999; *Ewango 2108*; BR[BR0000009698956], GIS, M, MO, WAG.

Description

Shrub 1.5–4 m tall, with the habit of *Coffea*, often drying blackish in herbarium; branches first glossy and vivid green, soon becoming reddish brown, corky (colours as seen in vivo; brown when dried); bark of older stems greyish brown, fissured; vegetative parts glabrous, except for the domatia; plagiotropous stems with microphyllous nodes in between nodes with normal leaves; internodes below microphyllous nodes shortened to almost reduced. *Leaves* with petioles 5–9 mm long; blades elliptic to obovate, 9–16 × 4–6 cm, with cuneate base and an apical acumen 10–15 mm long; (4–)6–7 pairs of lateral nerves; sparsely hairy domatia, with the hairs perpendicular to the main or lateral nerve; leaves of microphyllous nodes often with rounded base, (20–)40–90% smaller than normal leaves or even reduced to minute scales. *Stipules* sheathing, triangular, ca 1 mm long, bearing a central awn 1–2 mm long, inside hairy, rapidly deteriorating and becoming corky. *Inflorescences* 1-florous, often appearing 2–3-florous (2 or 3 coaxillar 1-florous inflorescences); peduncle 0–2 mm long; bracts and bracteoles fused into 2–4 calyculi per flower, with 2 triangular stipular parts and 2 foliar appendages, the foliar appendices leafy on the upper calyculus, scale-like on the lower ones; inside of the calyculi covered with curled hairs and bearing colleters at the base. *Flowers* 5–6-merous, sessile; calyx shortly tubular, ca 1 mm long, truncate at margin; corolla white, glabrous inside and outside, with a tube 5 mm long, shorter than the lobes of 6–7 × 3 mm; style ca 6 mm long, topped by 2 massive stigmatic arms of almost the same length; ovary 2-locular, with in each cell a peltate placenta bearing 1 embedded ovule; stamens inframedifixed, with filaments 1 mm long and inserted at the throat; anthers ca 5 mm long, exerted, blunt at apex; thecae without transverse septa; connective as wide as the thecae. *Fruits* fleshy, ellipsoidal, ca 15 × 12 × 12 mm, vivid red, glabrous, 2-locular, crowned by the nectary disc surrounded by the slightly accrescent calyx. *Seeds* hemi-ellipsoidal, ca 12 × 10 × 10 mm, one in each locule, without placental outgrowth around the seeds, flat at the adaxial side (less so in case of abortion of the ovule in the second locule) and convex at the abaxial side; seed coat rudimentary; embryo straight, with short cotyledons and long inferior radicle.

Distribution

The species has been collected in western Burundi and southwestern Rwanda (Fig. 5). Photographs of the species were taken in September 2021 in Kahuzi-Biega National Park, Mulolo 2 (or Idunga) village, South Kivu province in eastern Democratic Republic of the Congo (approximate coordinates: 02°21'37" S, 28°21'52" E) (Supp. file 7).

Habitat and ecology

The species grows in the understorey of wet ombrophilous montane or gallery forest, at an elevation of 1740–2205 m (the occurrence in DR Congo is at ± 1438 m). The label of the specimen *Ewango 2108* mentions that the plant occurs in the understorey of *Chrysophyllum rwandense* Troupin forest, while the specimen *Ewango 2087* occurs in the understorey of a forest with *Cleistanthus polystachyus* Hook.f. ex Planch. and *Diospyros gabunensis* Gürke. The specimens *Nkengurutse 6, 8* occur in the understorey of ombrophilous forest of *Xymalos monospora* (Harv.) Baill., while the specimens *Ntore & Bukuru 1212, 1213, 1214* are found in a forest dominated by *Syzygium guineense* subsp. *parvifolium* (Engl.) F.White.

Phenology

Most specimens were collected in June and July and have both flowers and fruits. This is also the case for a single alcohol collection (*Bukuru 01*, BR) made in April 2023. Two specimens were collected in September: *Rammeloo 4672* has fruits and mentions white flowers on the label; *Dumbo s.n.* (photograph)

has fruits. The two specimens collected in October (*Nkengurutse* 6, 8) bear fruits. The labels of *Ntore & Bukuru* 1212, 1213, 1214 mention that the species flowers year-round.

Vernacular names

In Kirundi, the species is known as ikawa, ikawakawa, ikawa y'ishamba, or umukawakawa, which means coffee, wild coffee, or similar to coffee.

Preliminary IUCN conservation assessment

The extent of occurrence (EOO) is estimated at 8313 km², which falls within the range of the Vulnerable category under subcriterion B1. The area of occupancy (AOO) is estimated at 28 km², which falls within the range of the Endangered category under subcriterion B2. The species occurs at four locations that all lie inside nominally protected areas: the Kibira National Park and the Bururi Forest Nature Reserve in Burundi, the Nyungwe National Park in Rwanda, and the Kahuzi-Biega National Park in Democratic Republic of the Congo. In the single location in Kibira National Park, we observed a large subpopulation and no obvious threats were noticed. The single location in Bururi Forest Nature Reserve is within a well-protected area and no threats to the subpopulation could be perceived. The single location in Nyungwe National Park occurs in a well-managed park and therefore no threats to the subpopulation were observed. The single location in the Kahuzi-Biega National Park (Mulolo 2) in DR Congo is nominally within a protected area but the forest is heavily used by the local people and this could pose a threat to the species (Aveling 2010; UNESCO 2021). However, apart from the decline in quality habitat in this area, the species seems generally safe. It is conserved *ex situ* at the University of Burundi. In summary, *Calycosiphonia albertina* sp. nov. is preliminary assessed as Endangered B2ab(iii).

Conclusion

We include the KR species in the genus *Calycosiphonia* and provide its formal recognition in the taxonomic treatment. With its inclusion in *Calycosiphonia* based on molecular data, we widen the delimitation of the genus: it is no longer restricted to species with transverse septa in the anthers and with placental outgrowths around the seed. This decision also strongly reduces the morphological distinction between *Calycosiphonia* and *Kupeantha*.

The inclusion of *Calycosiphonia pentamera* in *Kupeantha* based on morphology by Cheek *et al.* (2018) is corroborated by our molecular analysis. The species is sister to the rest of *Kupeantha*.

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Supplementary files

Supp. file 1. References for the pollen data, from literature and/or from herbarium material.

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Supp. file 2. Voucher table for the chloroplast tree.

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Supp. file 3. Voucher table for the nuclear tree.

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Supp. file 4. Silhouettes of plagiotropous branches of *Calycosiphonia albertina* Ntore & Robbr. sp. nov. to illustrate microphyllly. Short bars represent vestigial scales. Parts of branches drawn more thickly indicate suberisation. Microphyllous nodes with leaf blades that are only 50–75% smaller than those at normal nodes: A2, D5, E2, E6. Nodes with extremely reduced leaves: B4, C3, F3. Nodes with leaves reduced to vestigial scales: A3, A4, A7, B5, F6. Microphyllous anisophyllous nodes: B4, E6. Nodes numbered from stem tips downwards. A = *Nkengurutse 11*; B = *Nkengurutse 136*; C = *Ntore et al. 11*; D = *Ntore et al. 13*; E = *Ntore et al. 14*; F = *Rammeloo 4672*. Drawn by Elmar Robbrecht.

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Supp. file 5. **A.** Dissection of a pseudo-node (rehydrated from *Ntore et al. 9*). **B.** Diagram of the pseudo-node in A. Black circles are mature flowers (corollas fallen off) and open circles are flower buds. Drawn by Elmar Robbrecht. **C.** Photograph of a pseudo-node with almost identical structure. Photograph by Salvator Ntore from the subpopulation in Kibira forest, Burundi.

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Supp. file 6. Microphyllous nodes as in *Calycosiphonia albertina* Ntore & Robbr. sp. nov. were detected in five specimens of the two other species of *Calycosiphonia* Pierre ex Robbr. and in one specimen of *Argocoffeopsis scandens* (K.Schum.) Lebrun, although less pronounced.

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Supp. file 7. Occurrence of *Calycosiphonia albertina* Ntore & Robbr. sp. nov. in South Kivu, DR Congo. The record on Fig. 5 is based on photographic material sent to the first author by Landry Dumbo. The photograph was taken in September 2021 at Kahuzi-Biega National Park, Mulolo 2 (or Idunga) village, South Kivu province in eastern Democratic Republic of the Congo (approximate coordinates: 02°21'37" S, 28°21'52" E; approximate elevation: 1438 m). The morphology shown on the photograph (e.g., the microphyllous nodes) is a perfect match for *C. albertina*.

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Supp. file 8. Phylogenetic tree of the Coffeae tribe based on three chloroplast markers (*accD*, *rpl16*, and *trnLF*). The AC clade is indicated in grey and the new species *Calycosiphonia albertina* Ntore & Robbr. sp. nov. is indicated in bold. Bayesian posterior probability values are indicated at the nodes.

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