

Chlorophytum delicatulum (Asparagaceae), a newly described species from 7ambia

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Summary. Chlorophytum delicatulum Osborne, Vollesen & Bjorå from Zambia is described, illustrated and placed within the context of recent taxonomic and phylogenetic work on Chlorophytum in Africa. This delicate herb has leaf bases furnished with conspicuous dark red setae resembling eyelashes. Its phylogenetic position, ecology, phytogeography and conservation status are discussed. Chlorophytum delicatulum is assessed as being of Least Concern using the categories and criteria of the IUCN Red List.

Key Words. Africa, conservation status, ecology, new species, phylogeny, taxonomy.

Introduction

Chlorophytum Ker Gawl. (Asparagaceae: Agavoideae Anthericeae) is a genus of approximately 150 species found across the Old World Tropics (Stevens 2001). The genus is particularly diverse in eastern and southern central Africa, with 52 species occurring in the Flora of Tropical East Africa (FTEA) area (Nordal et al. 1997), 57 species in the Flora Zambesiaca (FZ) area (Kativu et al. 2008) and 52 species in the Flore d'Afrique Centrale (FAC) area (Meerts 2015). Molecular analyses of the species of Chlorophytum have shown that they can be divided into subgroups that correlate with morphological characters and number of chromosomes (Bjorå 2008; Bjorå et al. 2017).

Material of the species described here was first collected at Mutinondo Wilderness Area in Eastern Zambia in 2010, and subsequent collections were made in 2015, 2016 and 2019. An earlier collection from 1955 from Serenje, also in the same general part of Zambia, has been referred to the same taxon. When detailed studies were carried out at Kew, this material could not be matched with any known species. It is described here as *Chlorophytum delicatulum* Osborne, Vollesen & Bjorå.

Materials and Methods

Morphological studies

Specimens were collected and studied by S. Bidgood, L. Merrett, J. Osborne and K. Vollesen at Mutinondo and extensively photographed by L. Merrett. Live specimens were studied *in situ* at Mutinondo. Herbarium specimens were studied at Royal Botanic Gardens, Kew (K). SEM photographs of seeds from *Bidgood et al.* 8600 (K) were prepared by Aurelie Grall at Kew.

Conservation status

In order to assess the conservation status of this new species, georeferences were taken or estimated from each herbarium collection, and extent of occurrence was calculated from a minimum convex polygon using the online GeoCAT tool (Bachman *et al.* 2011). A provisional assessment of conservation status was obtained by applying the categories and criteria of the IUCN Red List (IUCN 2012).

Molecular studies

Sequences from 33 accessions were aligned using MUSCLE v3.8.425 (Edgar 2004) within Geneious Prime v2020.2.4 (Kearse et al. 2012), under default parameter settings. Two datasets were established for the final analyses: (1) ITS1 with 33 accessions; and (2) plastid DNA (rps16 and trnL-F intergenic spacer) with the same 33 accessions. The best-fit model of nucleotide substitution for the alignments was selected using jModelTest v2.1.10 (Guindon et al. 2003; Diego et al. 2012) under the Akaike Information Criterion (AIC). The data were analysed using Maximum Likelihood and Bayesian inference phylogenetic methods. Maximum Likelihood analyses were conducted using the Randomized Axelerated Maximum Likelihood (RAxML) v8.2.11 (Stamatakis 2014) as implemented in Geneious Prime. Rapid Bootstrapping and search

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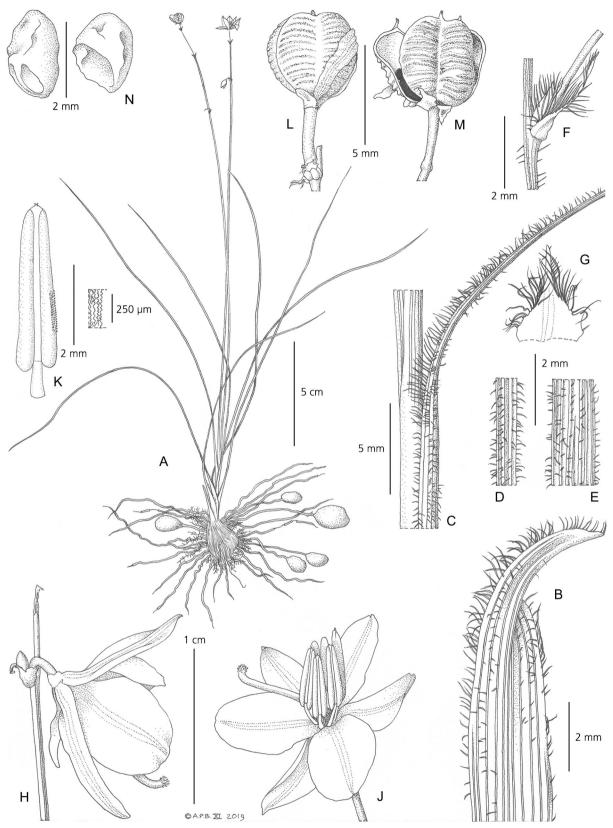


Fig. 1. Chlorophytum delicatulum. A habit; B distal portion of cataphyll; C proximal part of leaf; D – E leaf surfaces F floral bract; G floral bract, flattened; H flower, outside; J flower, inside; K anther and (right) enlarged surface of anther; L capsule; M open capsule; N seeds. A, F, G, M & N from *Bidgood et al* 8600; B – E from *Merrett* 2449; H, J, K & L from photos taken by L. Merrett. DRAWN BY ANDREW BROWN.



Fig. 2. Chlorophytum delicatulum at Mutinondo Wilderness Area. A inflorescence; **B** flower showing underside of tepals; **C** seed capsule; **D** leaf bases showing dark red marginal setae. PHOTOS: L. MERRETT.

for best-scoring ML tree algorithms were used, and bootstrap analyses were performed with 1000 replicates. For the Bayesian phylogenetic analyses, Bayesian inference was performed using MrBayes v3.2.7a (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Analyses were started using a random starting tree and run for four million generations, sampling every 1000 generations. Two Markov runs were conducted with four chains per run. To check whether the Markov Chain had converged well before finishing the analysis, the standard deviation of split frequencies (SDSF) was monitored to be below 0.01. The 1000 first generations (25%) were discarded as burn-in. The remaining trees were used for calculation of posterior probabilities and building a 50% majority-rule consensus tree. The trees were displayed with iTOL (Letunic & Bork 2016) and Keynote v10.0, Apple Inc.

Taxonomic Treatment

Chlorophytum delicatulum Osborne, Vollesen & Bjorå **sp. nov.** Type: Zambia, Muchinga Province, Mpika Distr., Mutinondo Wilderness Area, Mayense Camp, 3

May 2016, Bidgood, Merrett & Vollesen 8600 (holotype K! K000569940; isotypes O!, UZL!).

http://www.ipni.org/urn:lsid:ipni.org:names:77297514-1

Erect, perennial herb, 15 - 55 cm tall, forming small tufts. Rhizome short, vertical, mostly covered by leaf bases and fibrous remains of old leaf bases. Roots wirv with fusiform distal tubers $0.8 - 1.5 \times 0.7 - 1$ cm. Cataphylls papery, 1 – 3 cm long, glabrous or apical part hispid to setose with dark red or white hairs. Leaves basal, distichous, linear, erect to spreading, 10 - 40 cm long, with distinct setose, sheathing bases. Leaf bases $2 - 7 \times 0.1 - 0.25$ cm, with papery margins and long dark red (occasionally white) setae 1 -2 mm long along veins and margins. Leaf blades flat (with in-rolled margins or plicate when dry), 0.5 -1.5 mm wide, nerves prominent, seven abaxially and four adaxially, hispid along the nerves with white hairs or a few red at the leaf tip. Inflorescences appearing with the leaves, 15 - 50 cm long, solitary or 2 (- 3) per plant, equal to or taller than the leaves. Peduncle terete or slightly flattened, longitudinally ridged, 10 - 45 cm tall, glabrous or sparsely hispid, without sterile bracts. Inflorescence racemose,

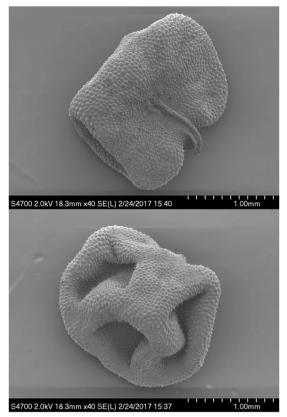


Fig. 3. Chlorophytum delicatulum seeds. Scanning Electron Microscope Images: A. Grall.

0.5-5 cm long, with one to four widely spaced nodes; basal internode (1-) 1.5-4 cm long. Floral bracts uniformly dark reddish-purple or straw coloured with dark reddish-purple margins and apex, 3-6 mm long, ovate or broadly ovate, narrowing abruptly or gradually into a long caudate apex, with 3-5 veins, setose along veins and margins, setae 0.5-1 mm long, dark reddish-purple, turning whitish. Flowers open and star-shaped at anthesis, single or two per node; pedicels 2-5 mm long at anthesis, extending to 4-8 mm in fruit, articulated below the middle, glabrous. Tepals white with pale green 3-5-

veined central stripes, glabrous; three outer ones linear-lanceolate, $6 - 9 \times 1.5 - 2$ mm, minutely papillose at apices, three inner ones broadly elliptic, $5 - 8 \times 3 - 6$ mm. Filaments white, glabrous, smooth, c. 1 mm long; anthers yellow, 2 - 3 mm long, papillose. Ovary sessile, ellipsoid, $1 - 2 \times 0.75 - 1$ mm; style 5 - 6.5 mm long, glabrous, bent outwards at anthesis; stigma truncate. Capsule partially covered by marcescent tepals when young, sub-globose, 3locular, c. 5 × 4 mm, truncate to slightly retuse, with dense transverse ridges, style base persistent. Seeds black, irregularly folded, $1.7 - 2.5 \times 1.5 - 1.7$ mm; hilum forming a ridge 0.5 - 0.8 mm long, terminating in a minute acute projection (sometimes the hilum is not clearly visible, depending on how the seed is folded); testa glossy, echinulate-papillose. Figs 1 - 3.

RECOGNITION. Chlorophytum delicatulum is easily recognised by the combination of wiry roots with distal tubers, grass-like linear hairy leaves, racemose inflorescence, conspicuous dark red setae (resembling eyelashes) on cataphylls and basal part of the leaf margins, and bracts with long dark reddish-purple setae on veins and margins. It resembles narrow-leaved forms of C. galpinii (Baker) Kativu var. galpinii, which differ by almost always having a paniculate inflorescence, longer glabrous or very finely ciliate bracts (6 – 15 mm long, 3 - 6 mm long in C. delicatulum), and generally larger flowers (tepals 10 – 14 mm long, 5 – 9 mm long in C. delicatulum). Chlorophytum delicatulum is also similar to narrow-leaved forms of C. rubribracteatum (De Wild.) Kativu which typically have a reddish-purple coloration on cataphylls, leaf bases, bracts and tepal apices, and paniculate inflorescences. Some forms of C. sphagnicolum Meerts from NW Zambia with racemose inflorescences resemble the new species but differ in having generally broader leaves (1 - 2.5 (-3)) mm wide, 0.5 - 1.5 mm wide in C. delicatulum), and longer glabrous or very finely ciliate bracts ((5-) 7 – 11 mm long, 3 – 6 mm long in C. delicatulum). A character matrix illustrates the

Table 1. Character matrix comparing Chlorophytum delicatulum with closely related taxa.

	C. rubribracteatum	C. galpinii var. galpinii	C. galpinii var. matabelense	C. galpinii var. norlindhii	C. sphagnicolum	C. delicatulum
Dark red setae	absent	absent	absent	absent	absent	present on cataphylls, leaf bases and bracts
Cataphyll colour	purple	green	green	green	green	green
Inflorescence	racemose (unbranched)	usually branched	usually branched	usually branched	racemose (unbranched)	racemose (unbranched)
Leaf width (mm)	0.5 - 5 (-8)	1 - 5 (-9)	1 - 10	2 - 7 (-9)	1 - 2.5 (-3)	0.5 - 1.5
Length of floral bracts (mm)	5 – 20 (– 30)	6 – 15	5 – 20 (– 25)	5 – 18 (– 40)	(5 –) 7 – 11	3 – 6
Tepal length (mm)	$10 - 17 \ (-20)$	10 - 14	8 – 15	10 - 15	5 – 11	5 – 9

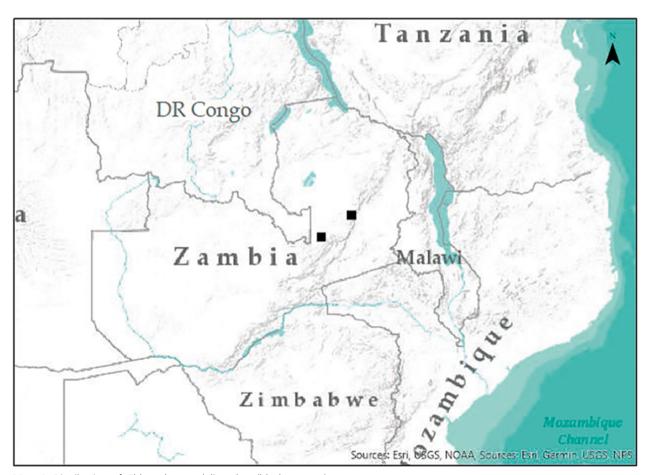
morphological differences between *C. delicatulum*, *C. rubribracteatum*, *C. sphagnicolum* and the varieties of *C. galpinii* (Table 1). These three species also differ from *C. delicatulum* in their ecology. *Chlorophytum delicatulum* grows in pockets of sandy-gravelly or sandy-peaty soils in shallow, seasonally wet depressions on large granite outcrops or in rock crevices, *C. galpinii* and *C. rubribracteatum* are typically open woodland and grassland species, while *C. sphagnicolum* grows in ephemeral wet or swampy grassland pans on ironstone or dolomite soils.

One primary separating character for species groupings within *Chlorophytum* in recent floras (Nordal *et al.* 1997; Kativu *et al.* 2008; Meerts 2015) is the number of flowers per bract (either single or two to several per bract). *Chlorophytum delicatulum* blurs the difference between the groups insofar as the populations studied. For example, *Bidgood* 8231 (K) has some individuals with all flowers in an inflorescence being solitary, while others have at least some flowers paired. This variation appears not to be associated with the plants' vigour.

DISTRIBUTION. Chlorophytum delicatulum is endemic to northern-central Zambia, in Mpika and Serenje Districts (Map 1). Due to the close proximity of this area to southern DR Congo (Katanga), it is quite possible that the species will eventually also be found there.

SPECIMENS EXAMINED. ZAMBIA. Central Province: Serenje Distr., Serenje, 23 Jan. 1955, Fanshawe 1839 (K!). Muchinga Province: Mpika Distr., Mutinondo Wilderness Area, Mayense Camp, 25 March 2010, Merrett 558 (K!) & 16 April 2015, Bidgood et al. 8231 (K!) & 3 May 2016, Bidgood et al. 8600 (K!, O!, UZL!) & 18 March 2019, Merrett 2449 (K!) & Choso Falls, 22 Feb. 2015, Osborne 1037 (UZL, K!).

HABITAT. At Mutinondo, Chlorophytum delicatulum grows in pockets of sandy-gravelly or sandy-peaty soils in shallow, seasonally wet depressions on large granite outcrops or in rock crevices. Dominant woody associates include Combretum molle R.Br. ex G.Don, Ficus ingens (Miq.) Miq., Landolphia parvifolia K.Schum., Lannea discolor (Sond.) Engl., Myrothamnus flabellifolius Welw., Ozoroa insignis Delile subsp. reticulata (Baker f.) J.B.Gillett, Tetradenia discolor Phillipson, Vitex mombassae



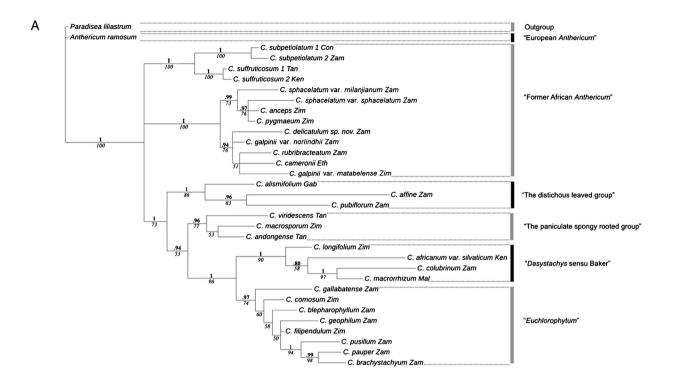
Map 1. Distribution of Chlorophytum delicatulum (black squares).



Fig. 4. Chlorophytum delicatulum in flower at Mutinondo Wilderness Area, growing with Coleochloa setifera and Myrothamnus flabellifolius. PHOTO: L. MERRETT.

Vatke and Xerophyta trichophylla (Baker) N.L.Menezes. Dominant perennial herbaceous species include Aloe mzimbana I.Verd. & Christian, A. zebrina Baker, Aeollanthus fruticosus Gürke, Coleochloa setifera (Ridl.) Gilly and Cyperus semitrifidus Schrad. A number of grasses are common associates and these include Diectomis fastigiata (Sw.) P.Beauv., Eragrostis welwitschii Rendle, Sporobolus festivus Hochst. ex A.Rich., Stereochlaena cameronii (Stapf) Pilg. and Trachypogon chevalieri (Stapf) Jacq.-Fél. Also present are a large number of annual herbs that include Chamaecrista mimosoides (L.) Greene, Commelina subulata Roth, Grona hirta (Guill. & Perr.) H.Ohashi & K.Ohashi, Exochaenium exiguum A.W.Hill (Sebaea minuta Paiva & I.Nogueira) and rare local endemics such as Crepidorhopalon mutinondoensis Eb.Fisch. & I.Darbysh. (Fischer et al. 2014), Euphorbia jubata L.C.Leach and Exochaenium alatum (Paiva & I.Nogueira) Kissling (Sebaea alata Paiva & I.Nogueira). The species has been collected at elevations from 1200 - 1450 m. Fig. 4.

CONSERVATION STATUS. Chlorophytum delicatulum is known from only two sites in northern-central Zambia (Map 1). It has a very restricted distribution with an extent of occurrence of approximately 31 km². The exact locality of the site at Serenje in Central Province, where this species was collected in 1955, is not known but is likely to be one of the hills that surround the town. There is extensive urban development and agricultural land conversion in this area, but the hills currently support natural or semi-natural vegetation and although the vegetation here is to some degree disturbed, Chlorophytum delicatulum may well still occur here. At the Mutinondo Wilderness Area in Muchinga Province, there have been five recent collections dating from 2010 - 2019. The species has been observed to be fairly frequent here and does not appear to be under threat. The almost bare granitic domes where it occurs do not contain any valuable minerals or gemstones and are of no agricultural use. Few of the woody species reach a size where they are of use for firewood and charcoal production. For



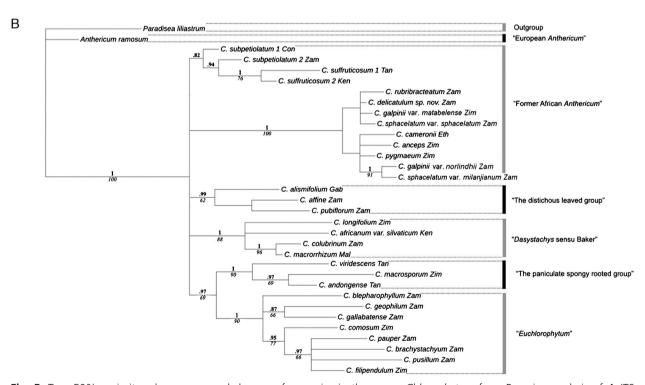


Fig. 5. Two 50% majority rule consensus phylograms for species in the genus *Chlorophytum* from Bayesian analysis of A ITS dataset, B combined plastid DNA dataset. The Bayesian posterior probability values (PP) of at least 0.9 are stated in bold above branches and maximum parsimony jackknife support (JK) of at least 50 % are stated in italics below branches. Multiple accessions of the same taxon are numbered according to Table 2. Bars with names to the right represent informal morphological groups (following Bjorå 2008). Abbreviations: C = *Chlorophytum*, Con= Dem. Rep. Congo, Eth = Ethiopia, Gab = Gabon, Ken = Kenya, Mal= Malawi, Tan = Tanzania, Zam = Zambia, Zim = Zimbabwe.

Table 2. Voucher information (taxon name, herbarium, voucher identification and country of origin) and GenBank accession numbers for DNA sequences used in the present study. *C. = Chlorophytum*; Herb. = voucher-holding herbarium

Taxon/Specimen No.	Herb.	Voucher ID	Locality	ITS1	$trn ext{L-F}$	rps16
Anthericum ramosum L.	О	Bjorå 855	Switzerland, Berner Oberland, Schrändli	KU880778	KU880877	KU880823
Chlorophytum affine Baker	O	Nordal & Bjorå 4552	Zambia, N: Ntumbachusi falls	EF999985	EU000019	KU880830
C. africanum var. silvaticum (Dammer) Meerts	O	Nordal & Bjorå 4621	Kenya, K3: Near Gilgil	EU000008	EU000041	EU128980
C. alismifolium Baker	BR, LBV, MO, WAG	Sosef 2515	Gabon, Ngounié	ON000252	ON012695	ON055482
C. anceps (Baker) Kativu	K, O, SRGH	Kativu 344	Zimbabwe, C: Harare, Epworth Mission area	KU880913	KU880939	KU880923
C. andongense Baker	O	Nordal & Bjorå 5013	Tanzania, T3: Pare D., SW of N Pare, near Lembeni	EU128950	EU128940	EU128960
C. blepharophyllum Schweinf. ex Baker	О	Hoell & Nordal 94	Zambia, N, Lumangwe Falls	KU880785	KU880882	KU880832
C. brachystachyum Baker	O	Bjorå 615	Zambia, E: Luangwa Valley	KU880915	KU880933	KU880925
C. cameronii (Baker) Kativu	ETH	Sebsebe et al. 6093	Ethiopia, Benshangul- Gumuz, Gojam	KU880788	KU880885	KU880834
C. colubrinum (Baker) Engl.	O	Nordal & Bjorå 4535	Zambia, C: Kasanka	EF999991	EU000025	KU880835
C. comosum (Thunb.) Jacques	O	Nordal 3162	Zimbabwe, Cult. in Harare	EF999993	EU000027	KU880840
C. delicatulum sp. nov. Osborne, Vollesen & Bjorå	K, O, UZL	Osborne 1037	Zambia, N: Mutinondo Wilderness Area	ON008452	ON012694	ON055481
C. filipendulum Baker	О	Nordal 3219	Zimbabwe, E: Chipinge D., Kiledo lodge	EU128956	EU128944	EU128969
C. gallabatense Schweinf. ex Baker	О	Hoell & Nordal 25	Zambia, B: Lukulu road	EF999996	EU000030	EU128971
C. galpinii var. norlindhii (Weim.) Kativu	О	Hoell & Nordal 17	Zambia, B: Liyoyelo to Mongu	EF999997	EU000031	KU893896
C. galpinii var. matabelense (Baker) Kativu	SRGH	Chapano 1879	Zimbabwe, Makonde	OM127871	OM212350	OM212379
C. geophilum Peter ex Poelln.	O	Hoell & Nordal 26	Zambia, B: Lukulu road	EF999998	EU000032	EU128972
C. longifolium Schweinf.	О	Nordal 1507	Zimbabwe, S: Maswingo, near Great Zimbabwe	EU000001	EU000034	KU880851
C. macrorrhizum Poelln.	О	Nordal & Bjorå 4521	Malawi: Nyika, nr. Zambian border	EU000003	EU000036	EU128976
C. macrosporum Baker	О	Kativu 255	Zimbabwe, C: Chegutu	EU000004	EU000037	KU880853
C. pauper Poelln.	O	Hoell & Nordal 11	Zambia, B: Munga	OK572497	OM174166	OM212371
C. pubiflorum Baker	О	Nordal & Bjorå 4561	Zambia, N: E of Mununga Bridge	KU880807	KU880903	KU880859
C. pusillum Schweinf. ex Baker	О	Nordal & Bjorå 4567	Zambia, N: E of Mununga Bridge	EU000007	EU000040	EU128979
C. pygmaeum (Weim.) Kativu	О	Kativu 384	Zimbabwe, Nyanga Distr. Nyanga National Park, Mare Camp	ON008453	ON012696	ON055483
C. rubribracteatum (De Wild.) Kativu	О	Bjorå 657	Zambia, C: 32 km	KU880808	KU880904	KU880860
C. sphacelatum var.	О	Hoell & Nordal 18	f Chipata to Lusaka Zambia, B: Mongu to	KU880919	KU880936	KU880929
milanjianum (Rendle) Nordal C. sphacelatum (Baker)	О	Hoell &	Mufwaya, Loyi Zambia, S: S of Zimba,	EU000009	EU000042	KU880866
Kativu var. sphacelatum C. subpetiolatum (Baker) Kativu	BRLU	Nordal 2 Meerts 10/31	Monachongwe farm D. R. Congo: Lualaba	ON000253	ON012697	ON055484
(1) C. subpetiolatum (2)	О	Hoell &	prov. Fungurume Zambia, B: Road	EU000011	EU000044	KU880867
C. suffruticosum Baker (1)	O	Nordal 15 Nordal &	to Mouyo Tanzania, T2:	KU880921	KU880938	KU880930
C. suffruticosum (2)	O	Bjorå 5014 A. Bjørnstad	North Pare Kenya K7: Teita Distr.,	EU000010	EU000043	KU880868
C. viridescens Engl.	O	2804 Nordal & Pione 5019	51 km NW of Voi Tanzania, T2:	EU000012	EU000045	EU128981
Paradisea liliastrum (L.) Bertol.	O	Bjorå 5012 Bjorå 852	Moshi-Arusha Rd. Switzerland, Cult.	OM179835	OM212358	OM212389

these uses, there are better alternatives (e.g. *Brachystegia*) in nearby woodland habitats. Occasional trees which have died of natural causes may of course be collected for firewood, and some species (e.g. *Aloe*) are used medicinally. Grazing by wild hares, or possibly bushbuck, has been observed, but in no way represents a threat. Occasional burning, while killing all aerial parts, will not affect the tuberous root system. The Mutinondo Wilderness Area is currently managed for conservation and ecotourism, and *Chlorophytum delicatulum* is considered to be secure at this site. Because of its small stature and linear grass-like leaves the species is inconspicuous and likely to be overlooked. It may well be more widespread. *Chlorophytum delicatulum* is provisionally assessed as being of Least Concern (LC).

PHENOLOGY. At Mutinondo and Serenje, flowering specimens have been observed or collected from the middle of the rainy season to the early dry season from late January to early May. Flowers are noted to close mid to late afternoon. Fruiting specimens have been collected from March to May.

When observed in the field, it is striking that plants of *Chlorophytum delicatulum* are never seen in groups or aggregations. Plants are always solitary, often occurring some considerable distance from the next plant. The significance of this population structure is not obvious. It could be an adaptation to herbivory (see below) or a strategy to counteract pests. This is a phenomenon also observed in several other species of *Chlorophytum* at Mutinondo, and here also in other plant families, e.g. in species of several genera in Apocynaceae subfam. Asclepiadoideae.

ETYMOLOGY. The specific epithet refers to the slender and delicate form of the plant.

Phylogenetic position

Phylogenetic analyses were performed to place the undescribed species in relation to other species of Chlorophytum. The analyses revealed the same general pattern as reported previously (Bjorå 2008; Bjorå et al. 2017). Thus, the general structure of the phylogenetic tree will not be discussed in detail here. Both in the nuclear (ITS, Fig. 5A) and concatenated chloroplast (Fig. 5B) analyses, C. delicatulum clusters together with species that were formerly included in genus Anthericum (Baker 1898; Kativu & Nordal 1993) and now informally referred to as the "Former African Anthericum group". This group is highly supported (Fig. 5A & B, PP1/JK 100) in the molecular phylogeny and by morphology. The species are all characterised by wiry roots with distal tubers, most of them having distichous leaves, and all having shallow deltoid capsules that are transversely ridged and seeds that are irregularly folded. Species in this group are distributed from Ethiopia, southwards to the Cape, with more species occurring in southern Africa. Within this group, C. delicatulum resolves as sister to C. galpinii var. norlindhii (Weim.) Kativu, C. rubribracteatum, C. cameronii (Baker) Kativu and C. galpinii var. matabelense (Baker) Kativu (Fig. 5A, PP 0.94/JK 74). This clade is also seen in the chloroplast tree, but without robust support (Fig. 5B). The molecular analyses do not indicate a single sister species to C. delicatulum. Rather, it resolves as distinct from the "Former African Anthericum group" sister taxa included in the analyses (Fig. 5A & B). Since only a limited number of taxa from the "Former African Anthericum group" were included in the molecular study, further research is required to unravel the relationships among the closely related C. delicatulum, C. sphagnicolum and varieties of C. galpinii.

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Declarations

Conflicts of interest. The authors declare that they have no conflict of interest.

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