Species delimitation in the *Chlorophytum andongense* complex

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Forord

Denne masteroppgaven har åpnet øynene mine for gleden med planter og for viktigheten med å klassifisere dem. Det skylder jeg min veileder Charlotte en stor takk for. Du er en fantastisk veileder som alltid har vært imøtekommende og gitt meg god veiledning. Takk for alle morsomme og lærerike stunder med deg. Vil også rette en takk til Tor for din gode veiledning, kunnskap og øye for detaljer som har hjulpet meg i denne oppgaven. Veldig takknemlig for at dere begge har tatt dere tid og for de mange zoom-møtene. Takk til Inger for ditt engasjement og imponerende kunnskap om botanikk. Takk til Solveig for hjelp når det oppstod frustrerende problemer med de fylogenetiske analysene.

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Vil til slutt takke venner og familie som har lyttet og hjulpet meg når det trengtes. Spesielt mamma og pappa som laget hjemmekontor for meg under koronakrisen og for deres jevnlige telefonsamtaler og oppmuntringer. Setter også stor pris på mine fantastiske medstudenter og gode venner Martine og Karianne som har gjort studieløpet mitt så bra, og holdt ut med meg når konsentrasjonen min ikke alltid var på topp.

Masteren er skrevet på artikkelfrom i forhold til Phytotaxa sine retningslinjer med unntak av plasseringene til figurer og tabeller.

Table of Content

Abstract
Several subgroups in Genus *Chlorophytum* has been identified by molecular, morphological and chromosome research. One of the subgroups has been referred to as "The paniculate spongy-rooted group", where the delimitation of several species have been questioned. To determine if this is a natural group and to clarify the taxonomic boundaries between the species, molecular phylogenetic analyses was performed based on ITS, *rps*16 intron, *trn*L intron, *trn*L-F spacer, *psb*A-*trn*H spacer, and *rps*12-*rpl*20 spacer. *Chlorophytum hirsutum,* hypothesized to be sister to *C. andongense,* did not resolve in "The paniculate spongy-rooted group". A closer examination of the roots of *C. hirsutum* showed they were not similar to the other members of this group. The two species *C. andongense* and *C. macrosporum,* were genetically and morphologically distinct, and are therefore not synonymized. The analyses revealed considerable genetic variation in the geographically widespread species *C. andongense*. This should be studied further.

Introduction

The Genus *Chlorophytum* Ker Gawl (1807: 1071) (Asparagaceae) has recently been subjected to molecular phylogenetic investigations where several subgroups have been identified (Bjorå 2008, Bjorå *et al*. 2017). The different subgroups are not only supported by molecular data, but also by morphology and chromosome number. One of the groups has been referred to as "The paniculate spongy-rooted group" here after referred to as "the paniculate group". All species in this subclade are more or less robust plants with heavily branched inflorescence and thick, spongy roots (fig. 1A, D). These characters are not unique for this group as such, but the two characters in combination are. The species are further characterized by having greenish or whitish urceolate flowers with a green median midrib, large, flat seeds and basic chromosome number x = 8. The species are most often found in woodland to shrubland or in wet grassland. Members of this group includes *Chlorophytum andongense* Baker (1878: 260), *C. hirsutum* A.D.Poulsen & Nordal (1999: 941), *C. macrosporum* Baker (1876: 330), *C. pseudocaule* Tesfaye & Nordal (2007: 129), *C. viridescens* Engler (1895: 140), and *C. zambiense* Bjorå & Nordal (2008: 228).

"The paniculate group" was first recognized as a natural group and a subdivision of *Chlorophytum* in Hoell (2005). This was the first molecular phylogenetic analysis of the genus, using two DNA regions: nuclear ribosomal ITS and plastid *trn*L-F spacer. Two species have been included in the group since Hoell's thesis; *C. pseudocaule* and *C. hirsutum*. There have been no later studies focusing on "the paniculate group" or including a wide taxon sampling of this group.

The mentioned characters thick or spongy roots and panicule are uniting this group, while other characters are discriminating the species in this group. *Chlorophytum zambiense* differs from rest by having smaller flowers and ebracteate peduncles (Bjorå *et al*. 2008). *Chlorophytum hirsutum* on the other hand, is somewhat deviating by being densely pubescent and having slightly thinner roots with tubers (Poulsen & Nordal 1999). It was referred to *C. andongense* as sister species when it was described in 1999 (Poulsen & Nordal). *Chlorophytum pseudocaule* differs from the others by forming a distinct pseudostem by the leaf bases (Awas & Nordal 2007). *Chlorophytum viridescens* is less obvious to separate from the rest of the group, but is described as often being hysteranthous and anthers twisting after anthesis (Kativu *et al*. 2008).

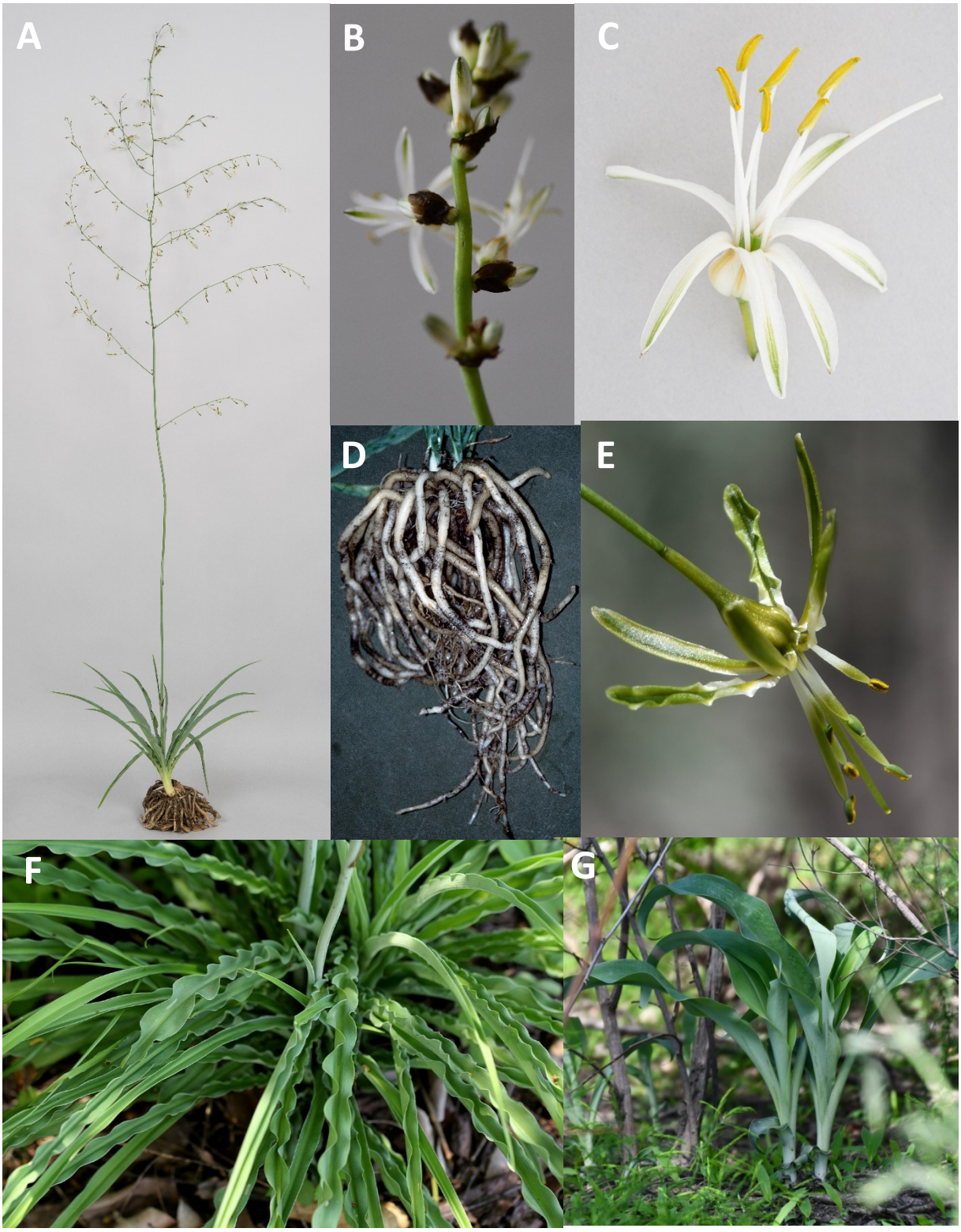


FIGURE 1. Photographs of plants in the "the paniculate spongy-rooted group" in the genus *Chlorophytum*. A) *Chlorophytum macrosporum,* B) inflorescence part of C*hlorophytum andongense*, C) flower of *Chlorophytum andongense*, D) spongy roots of *Chlorophytum viridescens* E) Flower of *Chlorophytum macrosporum* F) Leaves of *Chloropytum macrosporum* G) Leaves of *Chlorophytum andongense*. Photographed by: Kasten Sund (A, C), Charlotte S. Bjorå (B, E, G), Inger Nordal (D), Bart Wursten (F).

Meerts and Bjorå (2012) questioned if both *C. andongense* and *C. macrosporum* deserve recognition at species rank. This doubt was based on collections from Katanga in D.R. Congo that displayed a combination of defining characters with *C. macrosporum*-like fruits and *C. andongense*-like leaves. They strongly recommended a closer examination of this group.

Most species in the paniculate group are narrow endemics like *C. pseudocaule* from Ethiopia and *C. zambience* from Zambia (fig. 2A), but *C. andongense* is a notable exception with a distribution that spans the African continent from Mozambique to Guinea (fig. 2B).

Aims

The aims of this study are to test if "The paniculate group" constitute a monophyletic clade and to clarify the taxonomic boundaries between *C. andongense* and *C. macrosporum*.

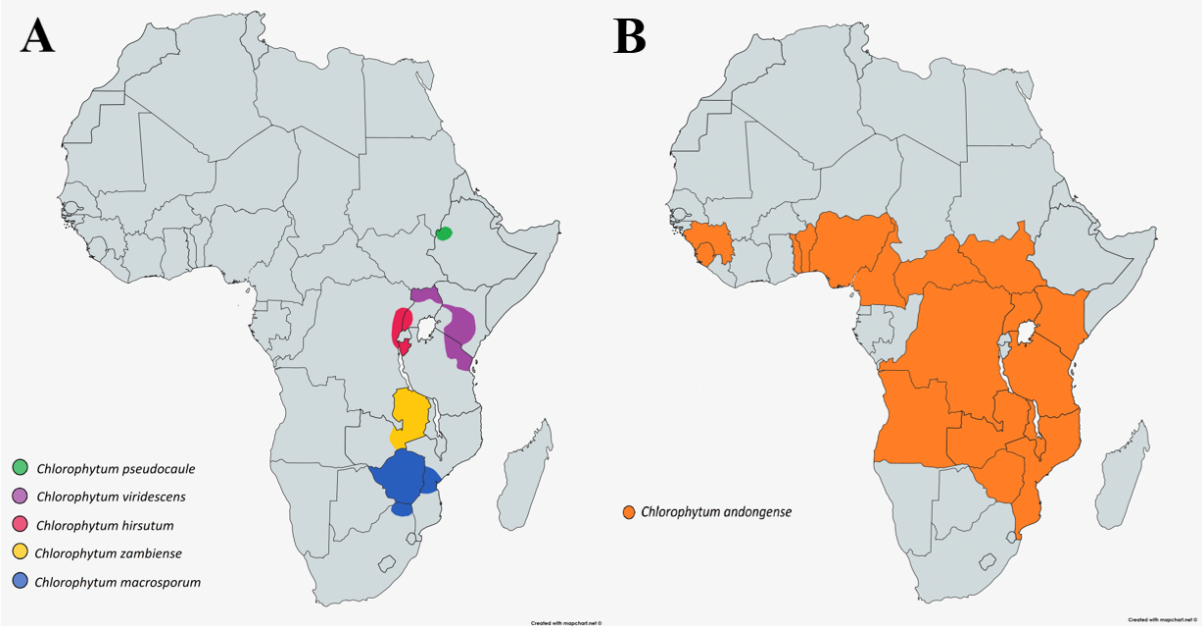


FIGURE 2. Distribution of *Chlorophytum* species in the "paniculate spongy-rooted group". A) The distribution of: *C. pseudocaule* -green, *C. viridescens* - purple, *C. hirsutum* - pink, *C. zambiense* - yellow and *C. macrosporum* -blue B) The distribution of *C. andongense* colored orange. (Awas & Nordal 2007, GBIF 2020, Nordal *et al.* 1997, Kativu *et al*. 2008). Map created with mapchart.net

Materials and methods

Plant materials

Plant material used in this this study are from silica- dried leaf samples collected during field work in Zimbabwe, from the greenhouse in the botanical garden at the Natural History Museum in Norway, and from herbarium specimen from ETH, MHU, MO, O and SRGH,

DNA extraction, amplification, and sequencing.

Total genomic DNA was extracted using E.Z.N.A SP Plant mini kit (Omega Bio-Tek, Atlanta, USA) following the manufacturer's protocol with the following modifications: incubated SP1 buffer, RNase A and powdered tissue sample for 1 hour instead of 10 minutes and incubated samples at 65°C for 5 minutes during elution to increase yield. Six different DNA regions were PCR amplified and sequenced. One nuclear region, the ribosomal internal transcribed spacer region (ITS) and five plastid regions, *rps*16 intron, *trn*L intron, *trn*L-F spacer, *psb*A-*trn*H spacer, and *rps*12-*rpl*20 spacer. ITS regions were amplified using ITS4 and ITS5 primers for all samples. The internal primers ITS3 and ITS2 were used when samples yielded insufficient PCR product. All primers from White *et al.* (1990). For *trn*L intron and *trn*L-F spacer primer c and f were used for all samples. The internal primers d and e were used for samples that yielded insufficient PCR product. All primers from Taberlet *et al*. (1991). For *rps*16 intron the primers rps16F and rps16R2 where used (Oxelman *et al.* 1997). For *psb*A-*trn*H spacer and *rps*12-*rpl*20 spacer, primers from Hamilton (1999) were used.

Depending on the concentration on the DNA extracts, 1-3 µl diluted or undiluted DNA extract where added to a 12.5 µl PCR-mix reaction consistent of buffer, $MgCl_2$, dNTP (10mM), BSA (1g/L), water, primers (10µM) and AmpliTaq (Applied Biosystems, Foster City, CA, USA). PCR cycling conditions were: 94°C for 2.5 min; 32 cycles of 94°C for 30 s, 53°C for 30 s, 72°C for 50 s; followed by 72°C for 4 min. PCR products were purified using 2 µl 10 times diluted ExoStar™ (GE healthcare UK limited) added to 8 µl PCR product, followed by incubation at 37°C for 30 min then 15 min at 80° C. If the PCR mix had high DNA concentration, 30 µl milliQ water was added to dilute the purified PCR product, otherwise 10 µl milliQ water was added. Made amplicons for sequencing containing 7.5 µl purified PCR product and 2.5 µl primer. For weak PCR products 1.3 µl primer were diluted with 1.2 µl milliQ water. The primers are the same as used for the PCR. The aliquots were sent to Macrogen Europe in the Netherlands for sequencing. In this study were 118 new sequences generated and 38 were collected from GenBank. All sequences are listed in table 1.

Analyses

Sequences were trimmed, edited and aligned using Geneious Prime 2020.0.5 (https://www.geneious.com, Kearse *et al.* 2012). Made multiple sequence alignment using Muscle algorithm (Edgar 2004) for making. Followed by manual editing of alignments in BioEdit 7.2.6.1 (Hall 1999). Gap were coded manually using same method for simple indel coding as in Simmons & Ochoterena (2000). Maximum parsimony analyses were performed using TNT (Goloboff *et al.* 2008). Made separate analyses for the nuclear region and the five plastid regions. Heuristic searches were performed with TBR branch swapping and 2000 replicates, otherwise default setting was used. Performed Jackknife analyses (Farris *et al*. 1996) with 36% removal probability and 1000 replicates with cut off value of 50%, otherwise default settings were used. Bayesian analyses were performed in MrBayes v3.2.7 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Used JModeltest (Darriba *et al.* 2012, Guidon & Gascuel 2003) to do AIC calculation for each DNA region separately. Different prior models of nucleotide substitution were used for the different DNA regions in the MrBayes analyses. Separate analyses were made for the nuclear region and the five plastid regions. The analyses were run two times independently for 4 million generations for the ITS dataset and 5 million generation for the plastid dataset. Used four chains in the runs, one cold and three heated, sampling trees every 1000th generation. Burn-in set to 25%. The average standard deviation of split frequencies (ASDSF) was below 0.01 confirming that the Markov Chains of the two independent runs were convergent.

TABLE 1. List with voucher information (taxon name, herbarium, voucher identification and country of origin) and GenBank accession numbers for DNA used in the present study. Abbreviation: A.= *anthericum*, C. = *Chlorophytum*, Herb.= voucher-holding herbarium, n/a = not available.

Taxon/Specimen No.	**Herb.**	**Voucher ID**	**Locality**	**ITS**	***trn*L-F**	***rps*16**	***trn*H,*psb*A**	***rps*12,rpl20**
Anthericum corymbosum Baker	O	Nordal 2276	Ethiopia, Bale	KU880775	KU880874	KU880820	x	n/a
A. ramosum L.	O	Bjorå 855	Switzerland, Cult	KU880778	KU880877	KU880823	x	x
Chlorophytum affine (Poelln.) Hanid	O	Nordal & Bjorå 4552	Zambia, N	EF999985	EU000019	KU880830	x	x
C. andongense Baker (1)	O	Nordal & Bjorå 5013	Tanzania, T3	x	EU128940	x	x	x
C. andongense (2)	MHU, O	MP59a	Uganda, U1	x	x	x	n/a	x
C. andongense (3)	MHU, O	MP57b	Uganda, U1	x	x	x	n/a	x
C. andongense (4)	O	Harder 3995	Zambia, S	x	x	x	n/a	n/a
C. andongense (5)	SRGH	Chapano *et al.* 1841	Zimbabwe, N	x	x	x	n/a	x
C. andongense (6)	SRGH	Chapano *et al.* 1879	Zimbabwe, N	x	x	x	n/a	x
C. andongense (7)	MO, O	Gereau 3824	Tanzania, T7	x	x	n/a	n/a	n/a
C. andongense (8)	SRGH	Chapano *et al.* 1852	Zimbabwe, N	x	x	x	n/a	x
C. andongense (9)	O	Hoell & Nordal 30	Zambia, B	EF999987	EU000021	x	n/a	n/a
C. andongense (10)	O	Lund 826	Sør-Sudan, Kiliu	x	n/a	n/a	n/a	n/a
C. blepharophyllum Schweinf. ex Baker	SRGH	Chapano *et al.* 1846	Zimbabwe, N	x	x	x	x	x
C. galpinii (Baker) Kativu	SRGH	Chapano *et al.* 1879	Zimbabwe, N	x	x	x	x	x
C. comosum (Thunb.) Jacques	O	Nordal 3162	Zimbabwe, S	EF999993	EU000027	KU880840	x	x
C. filipendulum Baker	O	Poulsen 956	Uganda, U2	EF999994	EU000028	EU128968	n/a	n/a
C. gallabatense Schweinf. ex Baker	O	Hoell & Nordal 25	Zambia, B	EF999996	EU000030	EU128971	x	x
C. geophilum Peter ex Poelln	O	Hoell & Nordal 26	Zambia, B	EF999998	EU000032	EU128972	x	x
C. hirsutum Poulsen & Nordal	O	Lye 22892	Uganda, U2	x	x	n/a	x	n/a
C. longifolium Schweinf.	O	Nordal 1507	Zimbabwe, S	EU000001	EU000034	KU880851	x	x
C. macrosporum Baker (1)	O, SRGH	Kativu 255	Zimbabwe, C	x	x	x	x	x

C. macrosporum (2)	SRGH	Chapano *et al.* 1815	Zimbabwe, N	x	x	x	x	x
C. macrosporum (3)	SRGH	Chapano *et al.* 1877	Zimbabwe, N	x	x	x	x	x
C. pauper Poelln (1)	SRGH	Chapano *et al.*1817	Zimbabwe, N	x	x	x	x	x
C. pauper (2)	O	Hoell & Nordal 13	Zambia, B	x	x	x	n/a	n/a
C. polystachys Baker	SRGH	Chapano *et al.*1884	Zimbabwe, N	x	x	x	x	x
C. pseudocaule Tesfaye & Nordal	ETH	Awas 1731	Ethiopia, Wellega	KU880805	KU880901	KU880857	n/a	n/a
C. rubribracteatum (De Wild) Kativu	O	Bjorå 657	Zambia, C	KU880808	KU880904	KU880860	x	x
C. silvaticum Dammer	O	Nordal & Bjorå 4621	Kenya, K3	EU000008	EU000041	x	x	x
C. subpettiolatum (Baker) Kativu (1)	SRGH	Chapano *et al.* 1832	Zimbabwe, N	x	x	x	x	x
C. subpettiolatum (2)	O	Hoell & Nordal 15	Zambia, B	x	x	x	n/a	n/a
C. suffruticosum Baker	O	Nordal 5014	Tanzania, T3	KU880921	KU880938	KU880930	x	x
C. viridescens Engler (1)	O	Nordal & Bjorå 5012	Tanzania, T2	x	x	x	x	x
C. viridescens (2)	O	I. Bjørnstad 265	Kenya, K4	x	x	x	x	x
C. zambiense Bjorå & Nordal	O	Nordal & Bjorå 4538	Zambia, N	x	x	x	x	x
Paradisea liliastrum Bertol.	O	Bjorå 852	Sveits, Cult	x	x	x	x	x

Results

The sequence length in characters for the six regions were: ITS 688; *trn*L-F 800; *rps*16 876; *trn*H & *psb*A 544; *rpl*20 & *rps*12; 773. The parsimony analyses of the ITS dataset resulted in 5 most parsimonious trees (MPTs) of tree length 485 steps with a retention index (RI) = 0.764 and a consistency index (CI) = 0.650. The parsimony analyses of the plastid dataset resulted in 46 most parsimonious trees (MPTs) of tree length 649 steps with RI = 0.802 and CI =0.753. Best fit models of nucleotides substitution selected by AIC in JModeltest were: ITS: GTR+G, *trn*L-F: HKY+G, *rps*16: GTR+G, *trn*H: F81+G+I, *rpl*20: GTR+G+I. In the Bayesian analyses the Standard deviation of split frequencies (ASDSF) had fallen to 0.004154 in the ITS dataset and to 0.009928 in the plastid dataset.

The separate plastid regions rendered congruent topologies (not shown). The topology in the parsimony analysis and the Bayesian analyses where congruent in both the nuclear ITS tree and plastid tree but is further resolved in the Bayesian analysis. The ITS (fig. 3A) vs. pDNA (fig. 3B) show slightly different topology. The clade consisting of species that earlier were referred to the genus *Anthericum* Linnaeus (1753: 310) resolves as a monophyletic clade in the nuclear tree (PP 0.95, JK 52), and in the polytomy in the pDNA tree. The clade consisting of species that has distichous leaves are well-supported in both trees (ITS: PP 1, JK 100, pDNA PP 0.97). The clade denoted Euchlorophytum renders monophyletic in both trees (ITS: PP 0.97, JK 88, pDNA: PP 0.98). The species that earlier was referred to the genus *Dasystachys* Baker (1898: 490) are well-supported in the ITS tree (PP 1, JK 97), but not in the pDNA tree.

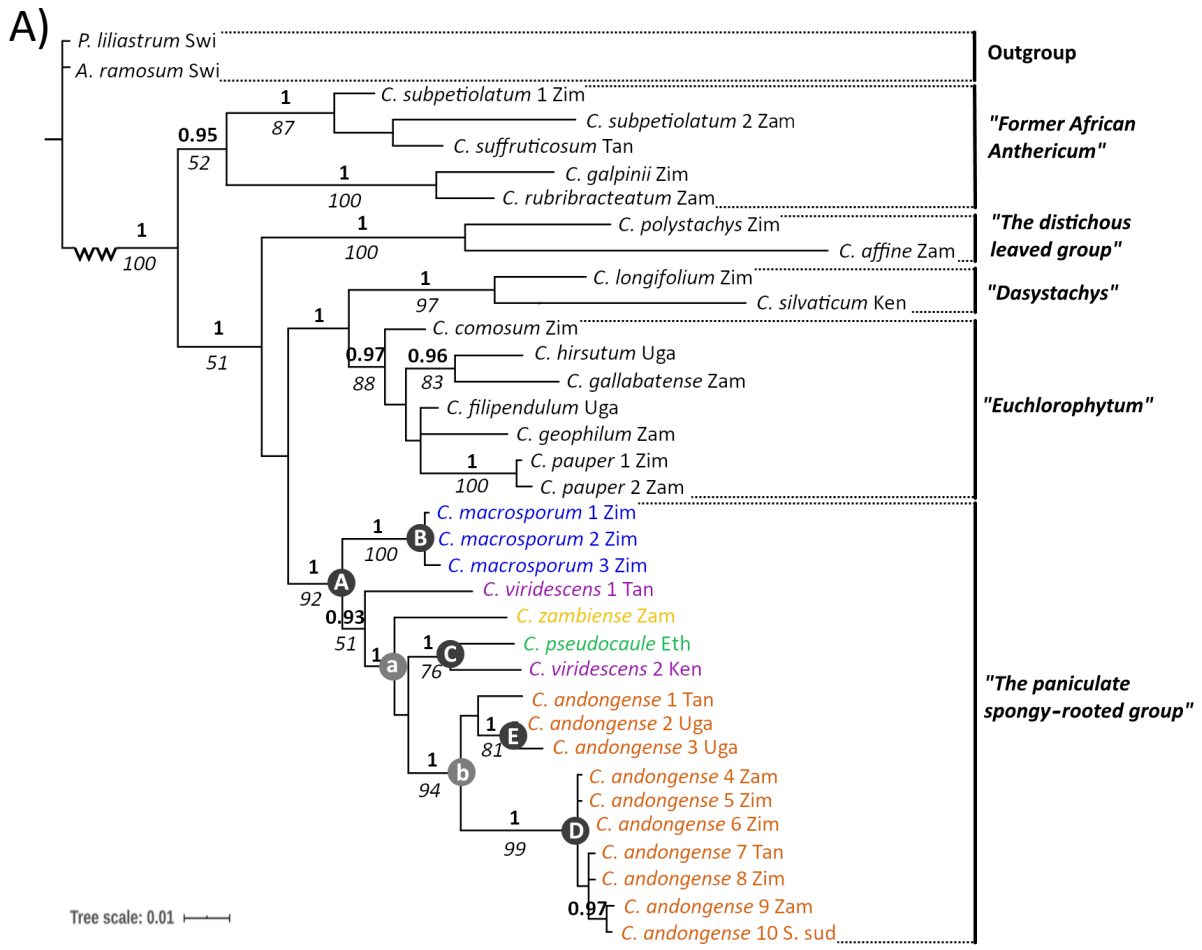
One accession, *C. gallabatense* Schweinfurth ex Baker (1876: 325) is incongruently positioned in the ITS as sister to *C. hirsutum* (PP 0.96, JK 83) vs. pDNA as sister to *C. geophilum* Peter ex Poellnitz (1943: 127. PP 1, JK 98). Apart from this incongruent pattern, is the ITS and pDNA topologies supported by JK of at least 50 % or PP of at least 0.9 were congruent, but resolved to different extents and in different parts of the trees (fig. 3A, B).

The paniculate spongy-rooted group

The species having paniculate inflorescence and spongy roots resolves in a monophyletic clade A (fig. 3A, B) in both trees, but only in the nuclear tree with high support (PP 1, JK 92). One notable exception was *C. hirsutum*, hypothesized to belong to this group, resolved in the

Euchlorophytum in a well-suppoted clade as sister to *C. gallabatense*. In the pDNA tree *C. hirsutum* came in a clade with species formerly referred to *Dasystachys*, but without support.

All accessions of *C. macrosporum* forms a well-supported clade B in both trees (ITS: PP 1, JK 100, pDNA: PP 1 JK 55). In the ITS tree, *C. macrosporum* resolves as sister to the rest of the clade A, but in the plastid tree, the *C. macrosporum* clade is part of a polytomy. Also, *C. andongense* is monophyletic in the ITS tree (PP 1, JK 94), but not in the pDNA tree. There is some internal structure in the species that has support in both trees; the two Ugandan accessions are well supported (ITS: PP 1 JK 81, pDNA: PP 1 JK 70), in the ITS tree together with a sample from northern Tanzania. The accessions from *C. pseudocaule* and *C. viridescens* from Kenya form a well-supported clade C in both trees (ITS: PP 1, JK 76, pDNA: P 1, JK 80). *Chlorophytum viridescens* from Tanzania and *C. zambiense* form the supported clade c in the pDNA tree (PP 1, JK 59). Whereas in the ITS tree has *C. zambiense* no support and *C. viridescens* from Tanzania is sister to clade a (PP 0.93, JK 51).



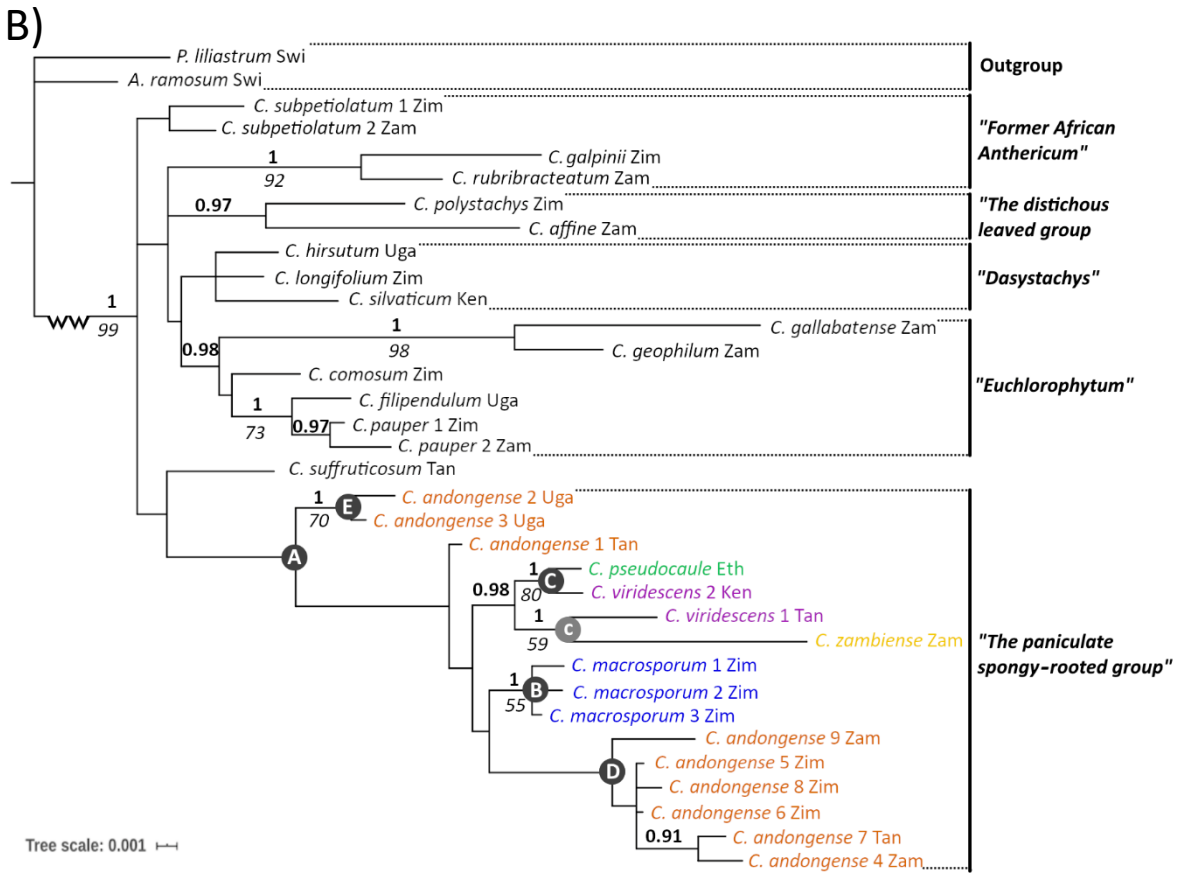


FIGURE 3. Two 50% majority rule consensus phylograms over species in the genus *Chlorophytum* from Bayesian analysis of A) ITS dataset, and B) combined plastid DNA dataset. The Bayesian posterior probability values (PP) of at least 0.9 are stated in bold above branches and maximum parsimony jackknife support (JK) of at least 50 % are stated in italics below branches. Multiple accessions of the same species are numbered according to Table 1. Black bars with names to the right represent the "Morphological" groups (following Bjorå 2008). Taxa are colored as defined in figure 2. White capital letters in dark circle represent congruent groups whereas white small letters in grey circle represent incongruence in the two trees. Manual shortening of long branches are represented by zigzag branches in each tree. Abbreviations: A = *Anthericum*, C = *Chlorophytum*, Eth = Ethiopia, Ken = Kenya, P = *Paradisea*, S. Sud = South Sudan, Swi = Switzerland, Tan = Tanzania, Uga = Uganda, Zam = Zambia, Zim = Zimbabwe.

Discussion

All accessions of *Chlorophytum* species having a paniculate inflorescence and spongy roots included in this analysis, constitute a monophyletic group, except for *C. hirsutum*. When describing their new species *Chloropytum hirsutum*, Poulsen & Nordal (1999) suggested that its closest relative was *C. andongense*. That is not supported by my analyses where *C. hirsutum* is placed in the strongly supported "Euchlorophytum" clade in the nuclear tree. Poulsen & Nordal (1999) mentions in their description that the roots of *C. hirsutum* are not very thick and spongy like those in *C. andongense,* but rather thin roots with tubers. In the nuclear tree, *C. hirsutum* resolves as sister to *C. gallabatense*. This is not very surprising, as they share the traits of haveing paniculate inflorescence and roots with tubers. They differ by *C. hirsutum* being hirsute all over the lamina and has distal tubers whereas *C. gallabatense* has mostly glabrous leaves and tubers mainly on lateral branches (Nordal 1997, Poulsen & Nordal 1999). *Chlorophytum hirsutum* appears therefore as the hairy sister to *C. gallabatense* (Inger Nordal pers.com.) In the plastid tree, *C. hirsutum* is clustering with the "*Dasystachys*" clade, but without support. Nothing in the morphology of *C. hirsutum* suggest that the species belong in the "*Dasystachys*" clade. The unexpected position in the plastid tree could be due to missing data in the analyses, as not all regions were possible to amplify for *C. hirsutum*. Based on molecular analysis and a re-evaluation of morphological characters, I conclude that *C. hirsutum* does not belong in "The paniculate group". This conclusion can be finally by counting chromosomes, as Euchlorophytum has x=7 and Dasystachys has x=8.

Chlorophytum zambiense and *C. pseudocaule* have a very limited distribution (fig. 2A) and are both distinctive in their morphology. Represented by only one accession in the phylogenetic analyses they both resolve within the paniculate group. All accessions of *C. macrosporum* forms a strongly supported clade in all analyses (fig. 3A, B). *Chlorophytum andongense* is strongly supported in the ITS tree as a monophyletic clade, but resolves as a polytomy in the plastid tree. There is no support from this study that *C. andongense* and *C. macrosporum* are conspecific. There are also clear morphological differences described in the Flora Zambesiaca (FZ, Kativu *et al.* 2008) between the two species (table 2); *C. andongense* is a more robust and higher plant. It has has distinctly broader leaves with margin not undulate to sometimes slightly crisped (fig. 1G), longer floral bract, and smaller fruits compared to *C. macrosporum*. Whereas *C. macrosporum* has narrow leaves with conspicuously undulate margins (fig. 1F), shorter floral bract and longer capsule (Kativu *et al.* 2008). Another difference, not mentioned in FZ is

that *C. macrosporum* often has tepals with undulate margins (fig. 1E). This morphological trait in *C. macrosporum* was observed during field work and studying greenhouse material.

When I collected the two species in the field, I found them close together on separate sides of a termite mound. *Chlorophytum macrosporum* was in flower while *C. andongense* was in its early vegetative state. If these two species differ in flowering time, it might indicate there is a phenological reproductive barrier between them. Meerts and Bjorå (2012) suggested the two species to be very closely related, perhaps even conspecific, and reported collections from Katanga having a combination of defining characters with *C. andongense*-like leaves and *C. macrosporum*-like fruits. Based on a hypothetical phenological reproductive barrier and that no "typical" *C. macrosporum* yet has been collected in D.R. Congo (fig. 2) a possible explanation could be that the Katanga collection represents variation within *C. andongense,* or possibly a new taxon that should be taxonomically recognized. This should be addressed in further studies.

TABLE 2. List over morphological differences between *C. andongense* and *C. macrosporum* recorded in Flora Zambesiaca (Kativu *et al.* 2008) and Flora of Tropical East Africa (Nordal *et al.* 1997). Apart from the morphological trait describing margin tepals which is observed during field work and studying greenhouse material. Abbreviation: *C.* = *Chlorophytum*.

Morphological traits	***C. macrosporum***	***C. andongense***
Plant high	80 – 150 cm	65 – 200 cm
Leaves length	25 – 52 x 1.4 – 2.5 cm	25 – 80.5 x 2.9 – 8.9 cm
Leaves margin	Undulate	Not undulate sometimes slightly crisped
Floral bract length	0.6 cm	5 cm
Tepals margin	Often undulate	Not undulate
Capsule length	11 – 15 mm	5 – 12 mm

Among the taxa in this group, *C. andongense* has by far the widest distribution (fig. 2), and it is perhaps not very surprising that there is genetic structure within this clade. In the nuclear tree, the *C. andongense* individuals splits into two groups representing specimens from the Flora of Tropical East Africa (FTEA, Nordal *et al.* 1997) or the FZ (Kativu *et al.* 2008) area. The FTEA clade consists of two accessions from Uganda and one from northern Tanzania. The two Ugandan accessions are also well supported in the pDNA tree. The FZ clade consists of accessions from south tropical Africa together with one specimen from South Sudan.

Morphological differences between the Flora areas are also recorded in FZ and FTEA when it comes to length of the fruits and flower color. In FZ the flower of *C. andongense* is described as having a greenish perianth and the fruit being a 5 – 9 mm long capsule, while in FTEA it is described as having greenish to whitish perianth and the fruit being a 10 – 12 mm long capsule. When studying greenhouse material of the Tanzania accession, the perianth had a clear white color with a thin green spot on the tepals (fig. 1C). It differed from the flora-description by having very dark, prominent bracts, subsessile flowers and a short pedicel in fruit (fig. 1B). Based on this analysis it is premature to give taxonomic recognition to the subclades. More material from the entire distribution area is needed to fully understand the variation in this taxon.

Chlorophytum viridescens does not resolve as monophyletic in the phylogenetic analyses. The accession from Kenya is in a well- supported clade with *C. pseudocaule,* whereas *C. viridescens* from Tanzania is sister to all other accessions in the group except *C. macrosporum*. Since there is only two accession of *C. viridescens* in this study, we cannot conclude on the phylogenetic position of this species. In addition, one accession from Benin determined as *C. andongense* had fruit characters like *C. viridescens* but found very far from its reported distribution (Sinsin 2978, O, WAG). This might represent unknown variation perhaps within the *C. andongense* complex. More collections are needed to get a better understanding of *C. viridescens*.

Conclusion

Based on both molecular and morphological evidence, *C. hirsutum* does not belong in "the paniculate group". "The paniculate group" is a well-supported monophyletic clade. *Chlorophytum andongense* and C. *macrosporum* are distinct species and should not be synonymized. The phylogenetic analyses indicate geographical structure and morphological differences within subgroups of the *C. andongense* clade that needs further studies.

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