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Species delimitation in the *Chlorophytum andongense* complex

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Abstract

Molecular, morphological and chromosome research have identified several subgroups in *Chlorophytum*. 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 taxonomic boundaries between species, molecular phylogenetic analyses were 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 within "The paniculate spongy-rooted group". A closer examination of the roots of *C. hirsutum* showed they were different from roots of the other members of this group. Two species, *C. andongense* and *C. macrosporum,* have been suggested to be conspecific, however, this study found that the taxa were genetically and morphologically distinct. The analyses further revealed considerable genetic variation in the geographically widespread species *C. andongense,* something that should be studied further.

Key words: Anthericaceae, Asparagaceae, *Chlorophytum hirsutum*, paniculate, phylogeny

Introduction

Chlorophytum Ker Gawl (1807: 1071) (Asparagaceae) has during the last years 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". "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. All species in this subclade are robust plants with heavily branched inflorescence and thick, spongy roots (Fig. 1A, D). These characters are separately 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 from tropical and subtropical Africa 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).

Chlorophytum zambiense differs from the other species in the group 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 by Poulsen & Nordal. *Chlorophytum pseudocaule* differs from the other species 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 having anthers that are twisting after anthesis (Kativu *et al*. 2008).

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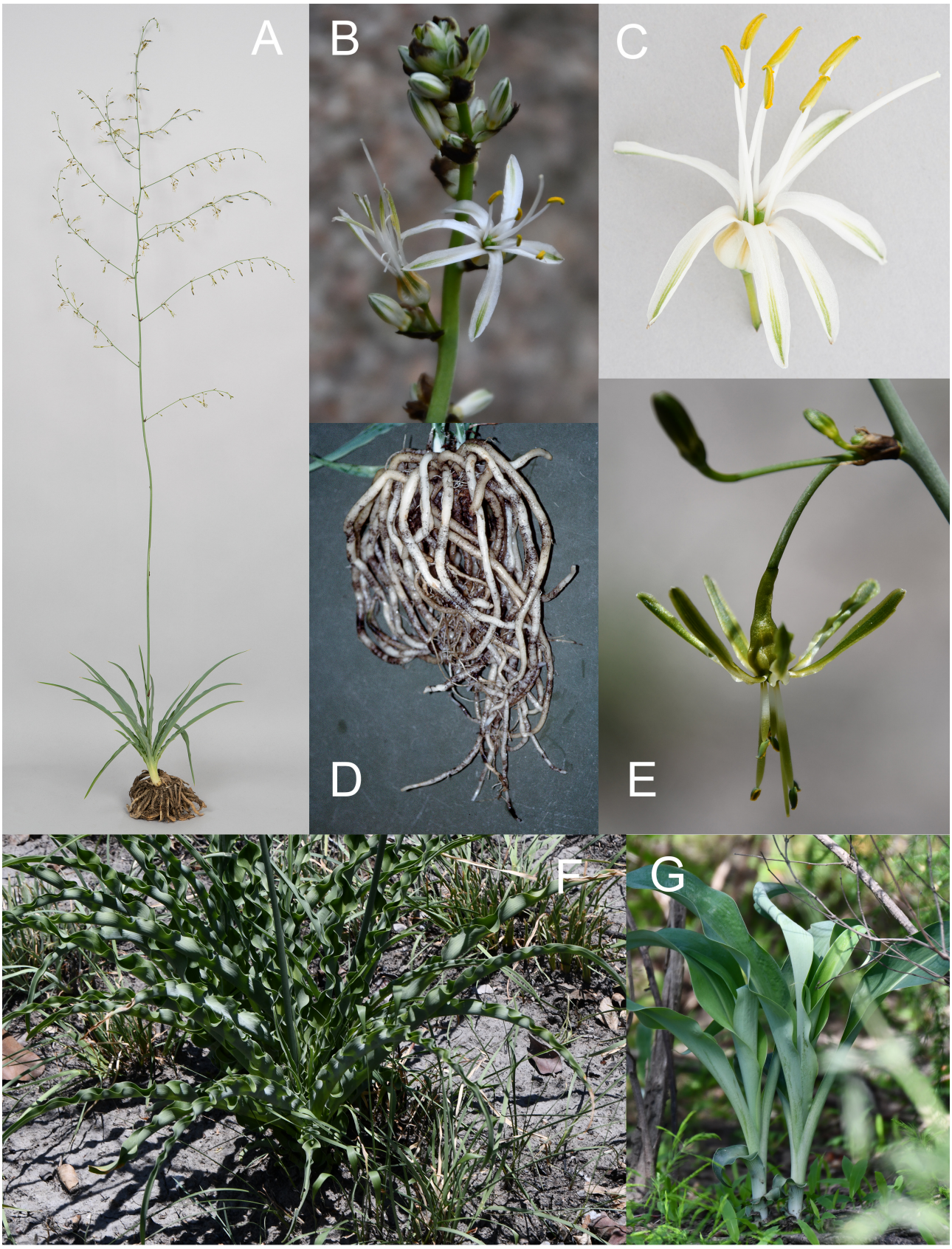

FIGURE 1. Photographs of plants in the "the paniculate spongy-rooted group" in the genus *Chlorophytum*. A) *Chlorophytum macrosporum,* B) inflorescence part of C*hlorophytum andongense*, C) flower of *Chlorophytum andongense*, D) spongy roots of *Chlorophytum viridescens* E) Flower of *Chlorophytum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

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. zambiense* 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).

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*.

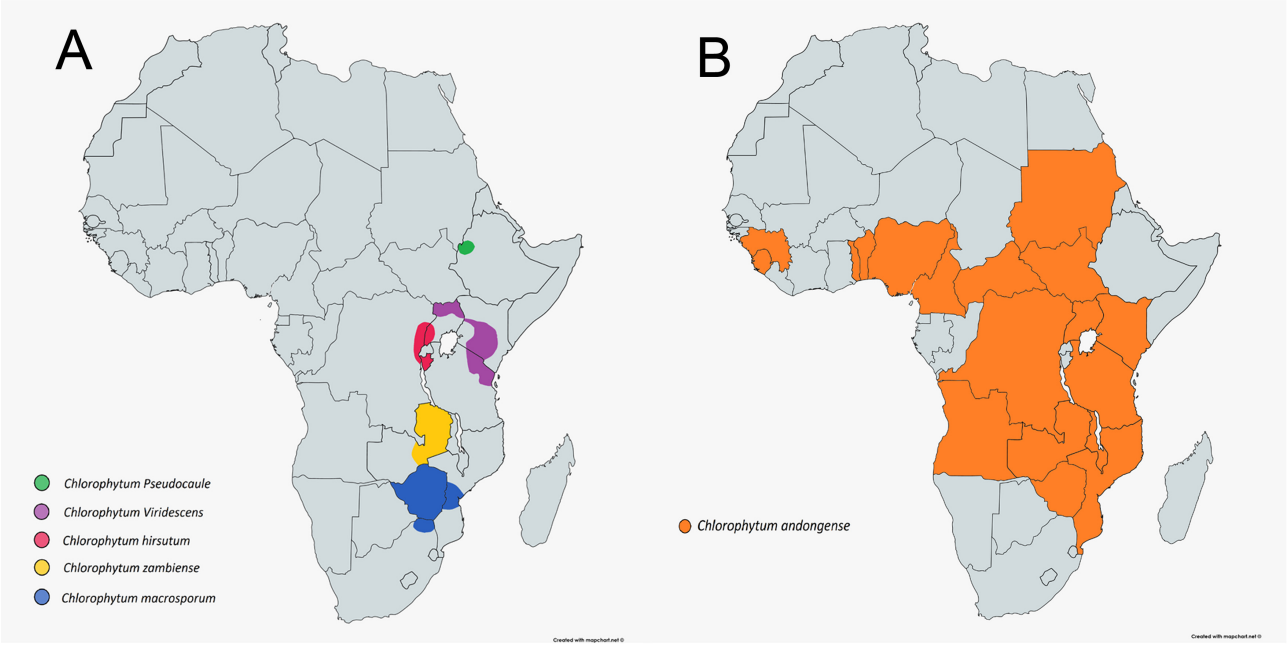

FIGURE 2. Distribution of *Chlorophytum* species in "the paniculate spongy-rooted group". A) The distribution of: *C. pseudocaule*—green, *C. viridescens*—purple, *C. hirsutum*—pink, *C. zambiense*—yellow and *C. macrosporum*—blue B) The distribution of *C. andongense* colored orange. (Awas & Nordal 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 the greenhouse in the botanical garden at the Natural History Museum in Oslo, Norway, and from herbarium specimens from ETH, MHU, MO, O, and SRGH either as silica-dried leaf samples or herbarium specimens.

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: we 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

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	ON496618	n/a
A. ramosum L.	O	Bjorå 855	Switzerland, Cult	KU880778	KU880877	KU880823	ON417757	ON417790
Chlorophytum affine (Poelln.) Hanid	O	Nordal & Bjorå 4552	Zambia, N	EF999985	EU000019	KU880830	ON417758	ON417791
C. andongense Baker (1)	O	Nordal & Bjorå 5013	United Republic of Tanzania, T3	ON462420	EU128940	ON496606	ON496569	ON496550
C. andongense (2)	MHU	MP59a	Uganda, U1	ON462421	ON496594	ON496607	n/a	ON496623
C. andongense (3)	MHU	MP57b	Uganda, U1	ON462422	ON496595	ON496608	n/a	ON496624
C. andongense (4)	O	Harder 3995	Zambia, S	ON462423	ON496596	ON496609	n/a	n/a
C. andongense (5)	SRGH	Chapano *et al.* 1841	Zimbabwe, N	ON462424	ON496597	ON496610	n/a	ON496625
C. andongense (6)	SRGH	Chapano *et al.* 1879	Zimbabwe, N	ON462425	ON496598	ON496611	n/a	ON496626
C. andongense (7)	MO, O	Gereau 3824	United Republic of Tanzania, T7	ON462426	ON496599	n/a	n/a	n/a
C. andongense (8)	SRGH	Chapano *et al.* 1852	Zimbabwe, N	ON462427	ON496600	ON496612	n/a	ON496627
C. andongense (9)	O	Hoell & Nordal 30	Zambia, B	EF999987	EU000021	ON496613	n/a	n/a
C. andongense (10)	O	Lund 826	South Sudan, Kiliu	ON462428	n/a	n/a	n/a	n/a
C. blepharophyllum Schweinf. ex Baker	SRGH	Chapano *et al.* 1846	Zimbabwe, N	OM127870	OM212349	OM212378	n/a	ON417794
C. comosum (Thunb.) Jacques	O	Nordal 3162	Zimbabwe, S	EF999993	EU000027	KU880840	ON417760	ON417795
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	ON417762	ON417798
C. galpinii (Baker) Kativu	SRGH	Chapano *et al.* 1879	Zimbabwe, N	OM127871	OM212350	OM212379	ON417763	ON417799
C. geophilum Peter ex Poelln.	O	Hoell & Nordal 26	Zambia, B	EF999998	EU000032	EU128972	ON417769	ON417804
C. hirsutum Poulsen & Nordal	O	Lye 22892	Uganda, U2	ON462429	ON496601	n/a	ON496619	n/a
C. longifolium Schweinf.	O	Nordal 1507	Zimbabwe, S	EU000001	EU000034	KU880851	ON496577	ON496560
C. macrosporum Baker (1)	O, SRGH	Kativu 255	Zimbabwe, C	ON462430	ON496602	ON496614	ON496620	ON496628
C. macrosporum (2)	SRGH	Chapano *et al.* 1815	Zimbabwe, N	OM127872	OM212351	OM212381	ON417773	ON417807
C. macrosporum (3)	SRGH	Chapano *et al.* 1877	Zimbabwe, N	ON462431	ON496603	ON496615	ON496621	ON496629
C. pauper Poelln (1)	SRGH	Chapano *et al.*1817	Zimbabwe, N	OM127873	OM212353	OM212383	ON417775	ON417810
C. pauper (2)	O	Hoell & Nordal 13	Zambia, B	OM179837	OM212352	OM212382	n/a	n/a
C. polystachys Baker	SRGH	Chapano *et al.*1884	Zimbabwe, N	OM127874	OM212354	OM212384	ON417776	ON417811
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	ON417780	ON417815
C. silvaticum Dammer	O	Nordal & Bjorå 4621	Kenya, K3	EU000008	EU000041	OM212385	ON417781	ON417816
C. subpetiolatum (Baker) Kativu (1)	SRGH	Chapano *et al.* 1832	Zimbabwe, N	OM127875	OM212356	OM212387	ON417785	ON417820
C. subpetiolatum (2)	O	Hoell & Nordal 15	Zambia, B	OM179836	OM212355	OM212386	n/a	n/a
C. suffruticosum Baker	O	Nordal 5014	United Republic of Tanzania, T3	KU880921	KU880938	KU880930	ON417786	ON417821
C. viridescens Engler (1)	O	Nordal & Bjorå 5012	United Republic of Tanzania, T2	ON462432	ON496604	ON496616	ON496585	ON496567
C. viridescens (2)	O	I. Bjørnstad 265	Kenya, K4	OM127876	OM212357	OM212388	ON417788	ON417823
C. zambiense Bjorå & Nordal	O	Nordal & Bjorå 4538	Zambia, N	ON462433	ON496605	ON496617	ON496622	ON496630
Paradisea liliastrum Bertol.	O	Bjorå 852	Switzerland, Cult	OM179835	OM212358	OM212389	ON417789	ON417824

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 was added to a 12.5 µl PCR-mix reaction consisting 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. Aliquots for sequencing contained 7.5 µl purified PCR product and 2.5 µl primer. The sequencing primers were the same as used for the PCR. The aliquots were sent to Macrogen Europe in the Netherlands for sequencing. In this study 118 new sequences were 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). Multiple sequence alignments were made using the Muscle algorithm (Edgar 2004), followed by manual editing of alignments in BioEdit 7.2.6.1 (Hall 1999). Gaps were coded manually using simple indel coding as in Simmons & Ochoterena (2000). Maximum parsimony analyses were performed using TNT (Goloboff *et al.* 2008). The nuclear region and the five plastid regions were run in two separate analyses. Heuristic searches were performed with TBR branch swapping and 2000 replicates, otherwise default setting was used. Jackknife analyses (Farris *et al*. 1996) were performed with 36% removal probability, 1000 replicates, and with a 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). We 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. We used four chains in the runs, one cold and three heated, sampling trees every 1000th generation. Burn-in was set to 25%.

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 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 indicating that the Markov Chains of the two independent runs were convergent.

The separate plastid regions rendered congruent topologies (not shown). The topology in the parsimony analysis and the Bayesian analyses were 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) resolved as a monophyletic clade in the nuclear tree (PP 0.95, JK 52), and as a 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 is 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).

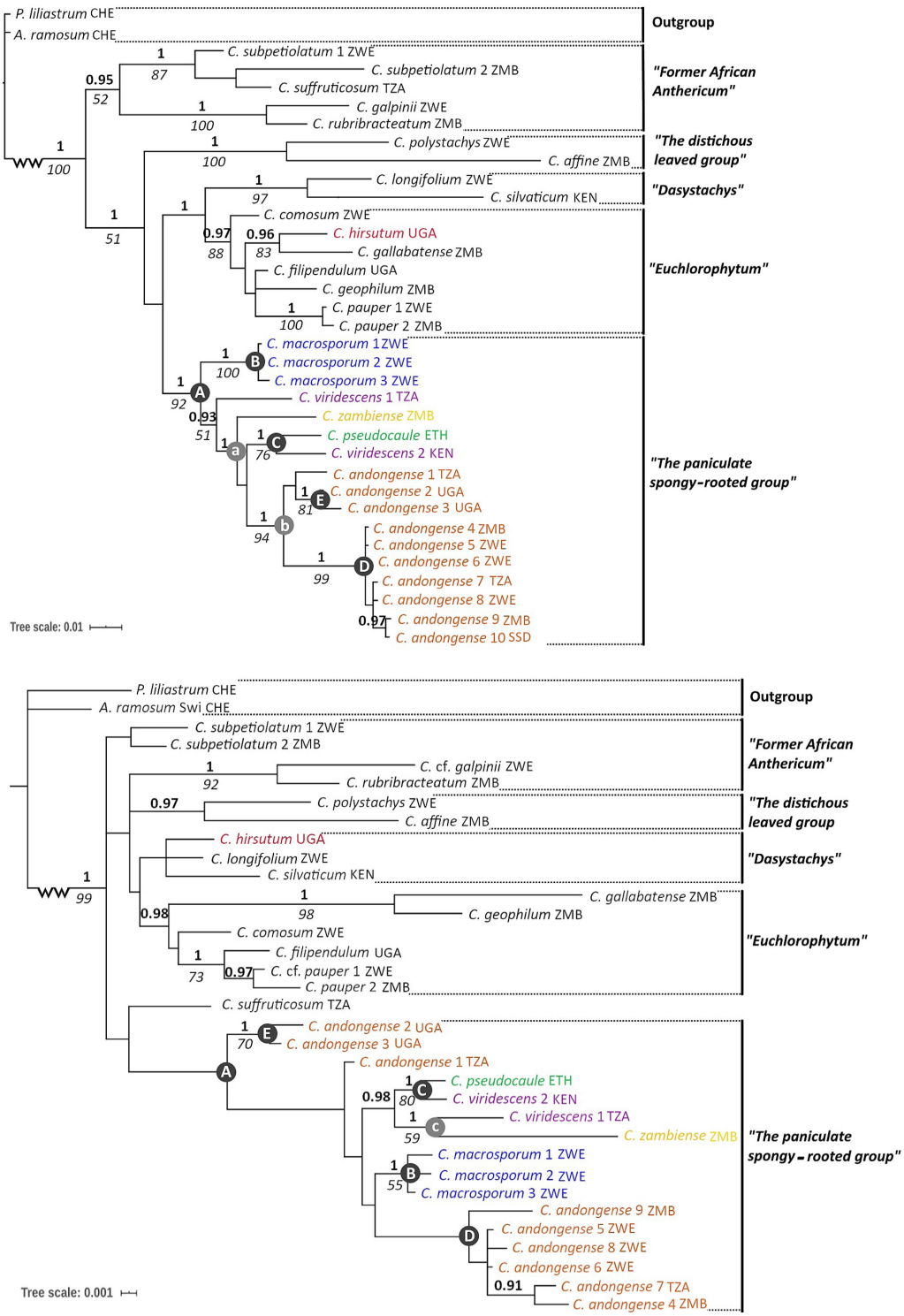

FIGURE 3. Two 50% majority rule consensus phylograms over species in the genus *Chlorophytum* from Bayesian analysis of A) ITS dataset, and B) combined plastid DNA dataset. The Bayesian posterior probability values (PP) of at least 0.9 are stated in bold above branches and maximum parsimony jackknife 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 Fig. 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*, SSD = South Sudan, CHE = Switzerland, TZA = United Republic of Tanzania, UGA = Uganda, ZMB = Zambia, ZWE = Zimbabwe.

The paniculate spongy-rooted group

The species having paniculate inflorescence and spongy roots resolved 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* resolved 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 United Republic of 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 United Republic of 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 United Republic of Tanzania is sister to clade a (PP 0.93, JK 51).

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 our 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 having 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). *C. hirsutum* appears therefore as the hairy sister to *C. gallabatense*. 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, we conclude that *C. hirsutum* does not belong in "The paniculate group". This conclusion can be tested by counting chromosomes, as "Euchlorophytum" has x = 7, while "the paniculate group" and *"Dasystachys"* has x = 8.

Chlorophytum zambiense and *C. pseudocaule* have a very limited distribution (Fig. 2A) and are both distinctive in their morphology. They resolve within the paniculate group in the phylogenetic analyses, but are only represented by one accession each . The accessions of *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 United Republic of Tanzania is sister to all other accessions in the group except *C. macrosporum*. Since there are only two accessions of *C. viridescens* in this study, we cannot conclude on the phylogenetic position of this species. The two accessions of *C. viridescens* are morphologically very similar indicating no obvious splitting of the taxon. In addition, one accession from Benin determined as *C. andongense* had fruit characters like *C. viridescens* but found very far from the latter species' known distribution (Sinsin 2978, O, WAG). This might suggest that there is unknown variation within the *C. andongense* complex. More collections are needed to get a full understanding of *C. viridescens*.

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 distinctly broader leaves with margins that are not undulate, but 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.

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 × 1.4–2.5 cm	25–80.5 × 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

When collecting the two species in the field, they were found 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. Among the taxa in this group, *C. andongense* has by far the widest distribution (Fig. 2), and it is not surprising that there is genetic structure within this clade. In the nuclear tree, the *C. andongense* accessions 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 United Republic of 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. However, it should be taken into consideration that the position of the South Sudanese accession is based on one plastid region only.

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 Tanzanian 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.

Conclusion

Based on both molecular and morphological evidence, "the paniculate group" is a well-supported monophyletic clade, however *C. hirsutum* does not belong to this group. *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. *Chlorophytum viridescens* did not resolve as monophyletic and more accessions should be added throughout its distribution area to fully understand this taxon.

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