Revisiting monophyly in *Haworthia* Duval (Asphodelaceae): Incongruence, hybridization and contemporary speciation

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Abstract Molecular phylogenetic reconstructions have indicated that *Haworthia* is not monophyletic. Here we show, using considerably expanded datasets of both chloroplast (*trnL-trnF* and *psbA-trnH* intergenic spacers) and nuclear (ITS1) markers that the issue of a polyphyletic *Haworthia* is more complicated than previously reported. Both parsimony and bayesian analyses of cpDNA and ITS1 produced poorly resolved phylogenies, with little or no support for deeper nodes. Species of *Haworthia* are placed in three of the four main lineages in the cpDNA phylogeny, while in the ITS1 phylogeny *Haworthia* species were placed in two of the four main lineages retrieved. The cpDNA phylogeny was incongruent with that obtained from the ITS1 data, and the topology of the combined dataset reflected that obtained from the ITS1 dataset. As species of *H.* subg. *Hexangulares* and subg. *Robustipedunculares* have been reportedly involved in intergeneric hybridizations with other Alooideae, it is postulated that a history of ancient hybridization may account for a polyphyletic *Haworthia*. Hybridization is also ongoing between species within *H.* subg. *Haworthia*, and may be responsible for results indicating that some species in this subgenus are not monophyletic. This hybridization may be facilitated by the lack of effective reproductive barriers. We hypothesize that *Haworthia* is undergoing a contemporary and explosive radiation in southern Africa, driven in part by local adaptation to relatively fine-grained ecological heterogeneity of soils and microclimate.

Keywords contemporary radiation; *Haworthia*; hybridization; incongruence; ITS1; non-monophyly; *psbA-trnH*; *trnL-trnF*

INTRODUCTION

The succulent genus *Haworthia* Duval is endemic to South Africa, Swaziland, Namibia, and Mozambique (Maputaland) (Bayer, 1982, 1999; Scott, 1985). *Haworthia* is centred in the semi-arid parts of the bimodal rainfall region of South Africa where most species grow in subtropical thickets or mosaics of this vegetation type with renosterveld, karooid shrublands and grassy fynbos (Bayer, 1982, 1999). As such, it contributes endemic species to three centres of endemism: the eastern realms of the Succulent Karoo Region (Little Karoo) and Cape Floristic Region, and the Albany Centre (Van Wyk & Smith, 2001).

The genus includes approximately 61 described species and numerous infraspecific taxa (Bayer, 1999). Most species are habitat specialists that occur in small, scattered populations of limited geographical extent (Bayer, 1999, 2002, 2006, 2007, 2008). *Haworthia* is placed in the subfamily Alooideae (Asphodelaceae; Asparagales) which also includes *Aloe* L., *Astroloba* Uitew., *Chortolirion* A. Berger, *Gasteria* Duval, *Lomatophyllum* Willd., and *Poellnitzia* Uitew. (Chase & al., 2000; Treutlein & al., 2003a). Alooideae have a distinct southern African centre of diversification with outliers in the Arabian Peninsula, Madagascar, and the Mascarene Islands (Treutlein & al., 2003a). Generic concepts in this subfamily vary depending on authority (for details see Chase & al., 2000; Treutlein & al., 2003a) but five genera are widely recognized, viz., *Aloe*, *Astroloba*, *Chortolirion*, *Gasteria* and *Haworthia* (Treutlein & al., 2003a).

Haworthia species are invariably small rosette succulent herbs with relatively small, tubular white flowers. Differences in floral morphology have been used to delimit three subgenera, but there is a general paucity of differences in floral characters among species. *Haworthia* subg. *Haworthia* has a obclavate tube, upcurved style and triangular or rounded-triangular perianth base; *H.* subg. *Hexangulares* Uitewaal ex. M.B. Bayer has a curved tube, with the perianth gradually narrowing to the junction with the pedical (substipitate); and *H.* subg. *Robustipedunculares* Uitewaal ex. M.B. Bayer has a straight tube, with the perianth abruptly joined to the pedical (non-stipitate; Bayer, 1999). The leaves (and rosettes) are variable, even within species. The foliar diversity and variation within the genus has contributed to a large horticultural appeal (e.g., *H. attenuata* Haw. and *H. cymbiformis* (Haw.) Duval are fairly common house and garden plants).

The genus has a difficult and complicated taxonomy, with numerous, closely related taxa being recognized. Details are given in Bayer (1982, 1999), Scott (1985), Breuer (1998, 1999, 2002) and Treutlein & al. (2003b) and are not discussed here. Regular updates are made as more field populations are found and taxonomically assessed (e.g., Bayer, 2002, 2006, 2007, 2008). Bayer (1999) recognizes approximately 61 species and his species concepts are followed here unless otherwise stated. Bayer (1999) favoured photographic material and explanatory notes rather than complex and ambiguous keys. Correct identification relies on pictorial depiction of variation in species (based almost exclusively on leaf characteristics) in combination with

habitat and distribution data (Bayer, 1999). These are subjective and open to interpretation to the untrained eye. *Haworthia* has generated considerable interest amongst amateur botanists and succulent collectors. This collector and horticultural appeal has resulted in a plethora of names (at species-level and lower ranks), which has negatively impacted the taxonomic stability of the group. Consequently, species limits and relationships are often contentious, conflicting, and poorly understood.

Haworthia species have few discrete morphological characters suitable for phylogenetic reconstruction, and hence focus has been placed on molecular phylogenies. Chase & al. (2000) were the first to investigate phylogenetic relationships in Asphodelaceae using chloroplast DNA (cpDNA) sequence data. *Haworthia* (based on two species) was placed in Alooideae, and sister to *Gasteria* (Chase & al., 2000). Alooideae were recovered as monophyletic, while Asphodeloideae were paraphyletic (Chase & al., 2000). Treutlein & al. (2003a) concentrated on subfamily Alooideae, using cpDNA sequences and genomic fingerprinting (ISSRs). Increased sampling of Alooideae revealed that *Aloe* and *Haworthia* are both non-monophyletic (Treutlein & al., 2003a). Representatives of *H.* subg. *Haworthia* formed a monophyletic group with good support, but representatives of *H.* subg. *Hexangulares* (and supposedly *H.* subg. *Robustipedunculares*) formed a single lineage sister to *Gasteria* which was nested within a clade also containing *Poellnitzia*, *Astroloba*, ×*Astroworthia* G.D. Rowley, and *Aloe aristata* Haw. This clade was sister to a lineage comprising *Lomatophyllum*, *Chortolirion* and predominately *Aloe* samples. These two clades and *H.* subg. *Haworthia* were in turn sister to another clade of arborescent *Aloe* taxa ("tree Aloes"). Despite this result, the splitting of *Haworthia* into two genera was not advocated by Treutlein & al. (2003a) because the taxonomic rearrangement and rank changes would have complicated the taxonomy of *Haworthia* and related genera of Alooideae.

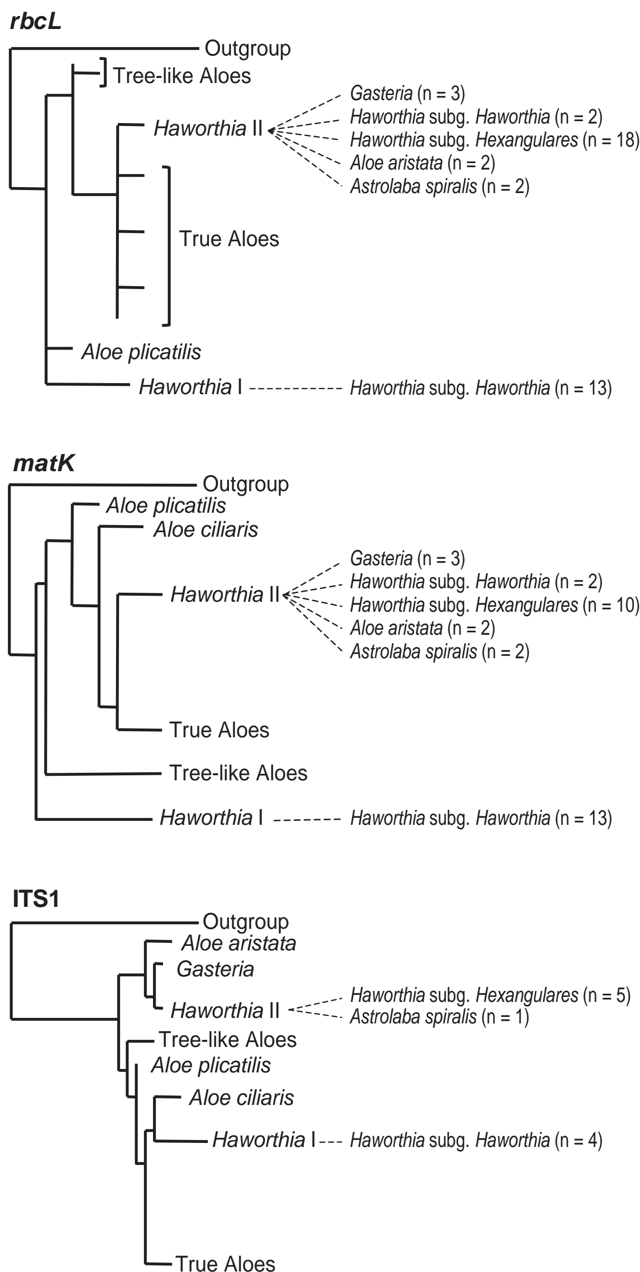

Fig. 1. Simplified summary tree topologies from the study of Treutlein & al. (2003b), showing the relationships among genera of Alooideae and *Haworthia* subgenera for the *rbcL*, *matK*, and ITS1 markers.

Treutlein & al. (2003b) examined *Haworthia* in greater detail using cpDNA markers (as in Treutlein & al., 2003a), a nuclear marker (ITS1) and genomic fingerprinting. Results again indicated that *Haworthia* was polyphyletic and found in two distinct lineages. In the cpDNA phylogenies five main clades were recovered: "Treelike Aloes", "Haworthia II", "True Aloes", "*Aloe plicatilis*", and "Haworthia I". Simplified summary topologies (and placement of *Haworthia* subgenera) for each marker are shown in Fig. 1. Treutlein & al. (2003b) sampled fewer *Haworthia* taxa for the ITS1 phylogeny and recovered two main lineages (Fig. 1) and the ISSR fingerprinting analyses supported the chloroplast analysis. Unfortunately, in all four analyses no samples of *H.* subg. *Robustipedunculares* were included.

The earlier studies used cpDNA *rbcL* and *matK* sequences, both of which are not entirely appropriate (lacking variable and informative characters) at species or subgeneric level (Gielly & Taberlet, 1994; Müller & al., 2006), although *matK* was found to be generally more variable than *rbcL* (Treutlein & al., 2003a). Here we investigate the relationships of the subgenera of *Haworthia* and the issue of possible non-monophyly using additional species (including the previously unsampled subg. *Robustipedunculares*) and faster evolving cpDNA markers (*trnL-trnF* spacer, *psbA-trnH* intergenic spacer). We compare the plastid phylogeny with a phylogeny derived from an expanded ITS1 dataset.

■ MATERIALS AND METHODS

Sampling. — Samples were taken primarily from living material in private collections, mostly collected by M.B. Bayer (the leading authority of *Haworthia*). These were originally collected in the wild, but subsequently housed at Sheilam

Nursery (Robertson), Shadowlands Nursery (Somerset West), and Selecta Succulenta Nursery (Cape Town). We opted to use material from the above horticultural collections because *Haworthia* taxa are usually rare and difficult to find in the field. Furthermore, as most species are under some degree of conservation protection (e.g., Witkowski & Liston, 1997; Victor & Dold, 2003), permission to collect wild populations may not have been forthcoming, and recollecting the material from the wild would have taken an inordinate amount of time. Only selected samples were collected from the field. Herbarium vouchers were deposited in KBG or GRA. However, some DNA samples with no herbarium voucher were used and tagged here as *sine numero* (*sn*) (Appendix). All *Haworthia* samples used in this study were identified by M.B. Bayer. A photographic library of these taxa and samples are available upon request from the first author (SR). We attempted to maximize sample representation from the three subgenera of *Haworthia*. Based on the findings of Treutlein & al. (2003a, b), we also attempted to include all genera of Alooideae with the exception of *Chortolirion* and *Lomatophyllum*.

DNA extraction, amplification, and sequencing. — DNA was extracted mostly from fresh leaves, occasionally from frozen leaves, or rarely from leaves dried in silica gel (Chase & Hills, 1991). Whole genomic DNA was extracted following a modified hot CTAB protocol of Doyle & Doyle (1987). Two non-coding cpDNA regions were used, viz., the *trnL-trnF* and *psbA-trnH* intergenic spacers (hereafter referred to as *trnL-F* and *psbA-trnH*, respectively). The *trnL-F* spacer is a widely used cpDNA marker in phylogenetic reconstruction at the species level (Shaw & al., 2005). Several studies on monocotyledons have used this marker at the infrageneric (subgenus, section) and species levels (e.g., Bytebier & al., 2007; Kocyan & al., 2008; Peterson & al., 2008). The *psbA-trnH* spacer is also a widely used cpDNA marker at the species level in monocotyledons (e.g., Devey & al., 2008; Meng & al., 2008; Dragon & Barrington, 2009). The *trnL-F* spacer was amplified and sequenced using primers "E" and "F" (Taberlet & al., 1991). The *psbA-trnH* spacer was initially amplified and sequenced using primers "psbA" and "trnH" (Sang & al., 1997). The "trnH" primer was problematic and an additional primer was designed slightly internal of the original "trnH" primer ("HawtrnH": 5′-TACATCCGCCCCTTATCTAGC-3′).

In addition, part of the ITS region (ITS1) was used to provide an independent source of sequence data. The ITS region as a whole is usually more variable than non-coding chloroplast regions, and has been used extensively in phylogenetic studies at the species level in monocotyledons (Kwembeya & al., 2007; Devey & al., 2008; Peterson & al., 2008; Snijman & Meerow, 2010). Adams & al. (2000) used the entire ITS region to study the phylogeny of *Aloe*, while Treutlein & al. (2003b) used the ITS1 spacer to study relationships in Alooideae.

Initial attempts to amplify the entire ITS region (ITS1, 5.8S, ITS2) failed despite numerous attempts using primers "ITS4" and "ITS5" (White & al., 1990) and conditions that have worked for *Aloe* (Adams & al., 2000). Additionally, primer combinations "ITS1"+"ITS4" (White & al., 1990), as well as "ITS18"+"ITS26" (Käss & Wink, 1997; Beyra-Matos & Lavin, 1999), which gave good results for *Kniphofia* (Asphodelaceae) (Ramdhani & al., 2009) did not routinely work. We thus focused on ITS1 as this marker had been used successfully before in Alooideae (Treutlein & al., 2003b). Amplification and sequencing was done with primers "ITS1" and "Chromo5.8R" (Barker & al., 2005).

PCR amplicons were purified using Promega Wizard or Invetec kits following the manufacturers' instructions. Cleaned products were sequenced using the BigDye Terminator sequencing kit v.3.1 (Applied Biosystems, Foster City, California, U.S.A.), in both forward and reverse directions. The cycle-sequencing products were precipitated using a sodium acetate/EDTA protocol. Sequencing was done on a ABI 3100 Genetic Analyzer (Applied Biosystems) at Rhodes University's Sequencing Unit.

Sequence editing and alignment. — Sequence trace files were assembled, checked and edited using Sequencher v.4.2.2. (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.). All sequences were deposited in GenBank (Appendix). Sequences were aligned manually in MacClade v.4.06 (Maddison & Maddison, 2000). In earlier studies (Chase & al., 2000; Treutlein & al., 2003a) the genus *Bulbine* (subfamily Asphodeliodeae) was used as the outgroup. In this study *Kniphofia* (subfamily Asphodeliodeae) was used as the outgroup because of sequence availability. Gaps were inserted intuitively based on visual inspection of the sequences to minimize the number of nucleotide differences among sequences.

Phylogenetic analyses. — Maximum parsimony (MP) and Bayesian inference (BI) methods were used in phylogenetic reconstruction. Maximum parsimony searches were performed with PAUP* v.4.0b10 (Swofford, 2002) in conjunction with PAUPRat v.1 (Sikes & Lewis, 2001). The parsimony ratchet procedure of Nixon (1999) was implemented. Each ratchet analysis consisted of 20 independent runs of 200 iterations. The trees found in the separate searches were pooled (n = 4000) and served as starting trees for heuristic searches in PAUP* employing TBR branch swapping. All trees were used to construct a strict consensus tree. Parsimony bootstrap analyses for each data partition consisted of 1000 heuristic bootstrap replicates with MAXTREES set at 4000. This proved to be computationally prohibitive and the MAXTREES were reduced to 500.

Bayesian inference was performed using MrBayes v.3.1.1 (Huelsenbeck & Ronquist, 2001). The most appropriate model of sequence evolution for each molecular marker partition was determined using the Akaike information criterion (AIC) as implemented in the program MrModeltest v.2.2. (Nylander, 2004). The analysis using the chosen model was conducted with four Monte Carlo Markov Chains (three heated and one cold). Chains were run for 5,000,000 generations and sampled every 100th generation. The log-likelihood scores were plotted to determine the point of stationarity, and all trees prior to stationarity were discarded as the "burn-in" phase. The subsequent trees were retained, and posterior probabilities (PP) were estimated by constructing a 50% majority-rule consensus tree in PAUP*. Trees were visualized using TreeView (Page, 1996).

Congruence of the cpDNA and nr ITS1 datasets was assessed using the partition homogeneity (ILD) test (Farris & al.,

1994). The ILD test was implemented in PAUP* using 1000 replicates, MAXTREES set at 100 and TBR branch swapping.

Phylogenetic networks. — Neighbor-Net (NN), a distance-based network construction method, allows for graphical representation of conflicting phylogenetic signals and interpretation of evolutionary histories which are not tree-like (Bryant & Moulton, 2004). NN splits graphs have been used with varying success to detect reticulate history (Carine & al., 2007; Frajman & Oxelman, 2007; Grimm & Denk, 2008; Weiss-Schneeweiss & al., 2008; Ramdhani & al., 2010; Schulte & al., 2010). Splits graphs were constructed using the NN algorithm with uncorrected p-distances in SplitsTree v.4.10 (Huson, 1998). As the plastid markers are inherited as a single unit they were combined prior to analysis. Both the cpDNA and ITS1 matrices were modified prior to analysis by excluding the outgroup, and subsequent removal of redundant gaps (mostly indels associated with the outgroup).

■ RESULTS

cpDNA phylogeny. — Details of the cpDNA datasets (individual and combined) are presented in Table 1. Prior to combining datasets, the alignment for individual markers had to be pruned to ensure that sample composition was uniform in the combined cpDNA dataset. An ILD test showed that the two cpDNA data partitions were significantly incongruent (P = 0.003). This incongruence may be caused by the limited signal in the *psbA-trnH* data, as the variability and informativeness were lower than in the *trnL-F* data (Table 1). Thus, despite the result of the ILD test, the cpDNA datasets were merged because the plastid genome is inherited as a single unit. Analysis of the combined cpDNA (hereafter termed cpDNA) dataset with the parsimony ratchet produced 4000 trees of 300 steps (CI = 0.620, RI = 0.772). In an independent MP analysis without the ratchet, the number of trees was in excess of 20,000. However, the tree length was higher, and the RI and CI values were lower than the ratchet analysis (results not shown). The overall cpDNA topology reflected that of the *trnL-F* marker (topologies of individual datasets are not shown). The topology from the BI analysis is better resolved, and presented here as Fig. 2a. Lineages with posterior probability (PP) values ≥0.95 and bootstrap (BS) values ≥70%, are considered well supported.

Four major clades were recovered (labelled A–D in Fig. 2a). Clade A comprised a single *Aloe dichotoma* sample (a "tree *Aloe*"). Clade B comprised *Poellnitzia rubiflora*, *Aloe tenuior* and *H. blackburniae* (subg. *Haworthia*), but this relationship has very weak PP support (0.55) and no BS support. Clade C also lacks support and comprises six clades (Ci–Cvi). Clade Cvi is composed of *H. venosa* (subg. *Hexangularis*) and *Gasteria brachyphylla* var. *brachyphylla*. This clade has poor PP support (0.60) and no BS support, and is sister to the remaining five clades (Ci–Cv). Clades Ci–Ciii are composed of representatives from *H.* subg. *Hexangularis*, and all are well supported in the BI analyses (PP ≥ 0.95). However, only clade Ci is well supported in the MP analysis (BS = 98). Clade Civ has good PP support (0.98) and no BS support and is divided into two subclades: one is composed exclusively of *H.* subg. *Robustipedunculares* samples, and the other is composed of *Astroloba bullata* samples. Both subclades have very good PP support (1.00), but weak BS support. Clade Cv is composed of *Aloe* samples (termed here the "*Aloe* group") and is well supported in the BI and MP analyses (PP = 1.00, BS = 88). Clade D has very good PP support (1.00) and weak BS support. It is composed of a single *H. nigra* sample (subg. *Hexangularis*, clade Di) which is sister to samples from *H.* subg. *Haworthia* (clade Dii).

Table 1. Summary of sequence characteristics of the *trnL-F*, *psbA-trnH*, and ITS1 markers.

	trnL-F	*psbA-trnH*	Combined cpDNA (*trnL-F*+*psbA-trnH*)	ITS1	Combined cpDNA+ITS1
Ingroup/s + *Haworthia*					
No. of samples	83	74	59	47	47
No. of bp in alignment	565	658	1192	284	1457
No. of variable characters (%)	113 (20.0)	93 (14.1)	173 (14.5)	114 (40.1)	278 (19.1)
No. of PI characters (%)	61 (10.8)	37 (5.6)	83 (7.0)	56 (19.7)	131 (8.3)
CI, RI, tree length	0.648, 0.795, 193	0.692, 0.860, 143	0.620, 0.772, 300	0.765, 0.910, 179	0.687, 0.834, 453
ILD test P value	–	–	P = 0.003	–	P = 0.001
BI Model	GRT+I+G	F81+I+G	*trnL-F*: GRT+I+G *psbA-trnH*: F81+I+G	GRT+G	*trnL-F*: GRT+G *psbA-trnH*: F81+I+G ITS1: GRT+G
Haworthia					
No. of samples	66	63	48	36	36
No. of bp in alignment	532	640	1164	275	1415
No. of variable characters (%)	67 (12.6)	55 (8.6)	115 (9.9)	71 (25.8)	171 (12.1)
No. of PI characters (%)	41 (7.7)	24 (3.8)	54 (4.6)	41(14.9)	86 (6.1)

CI, consistency index; PI, parsimony-informative; RI, retention index.

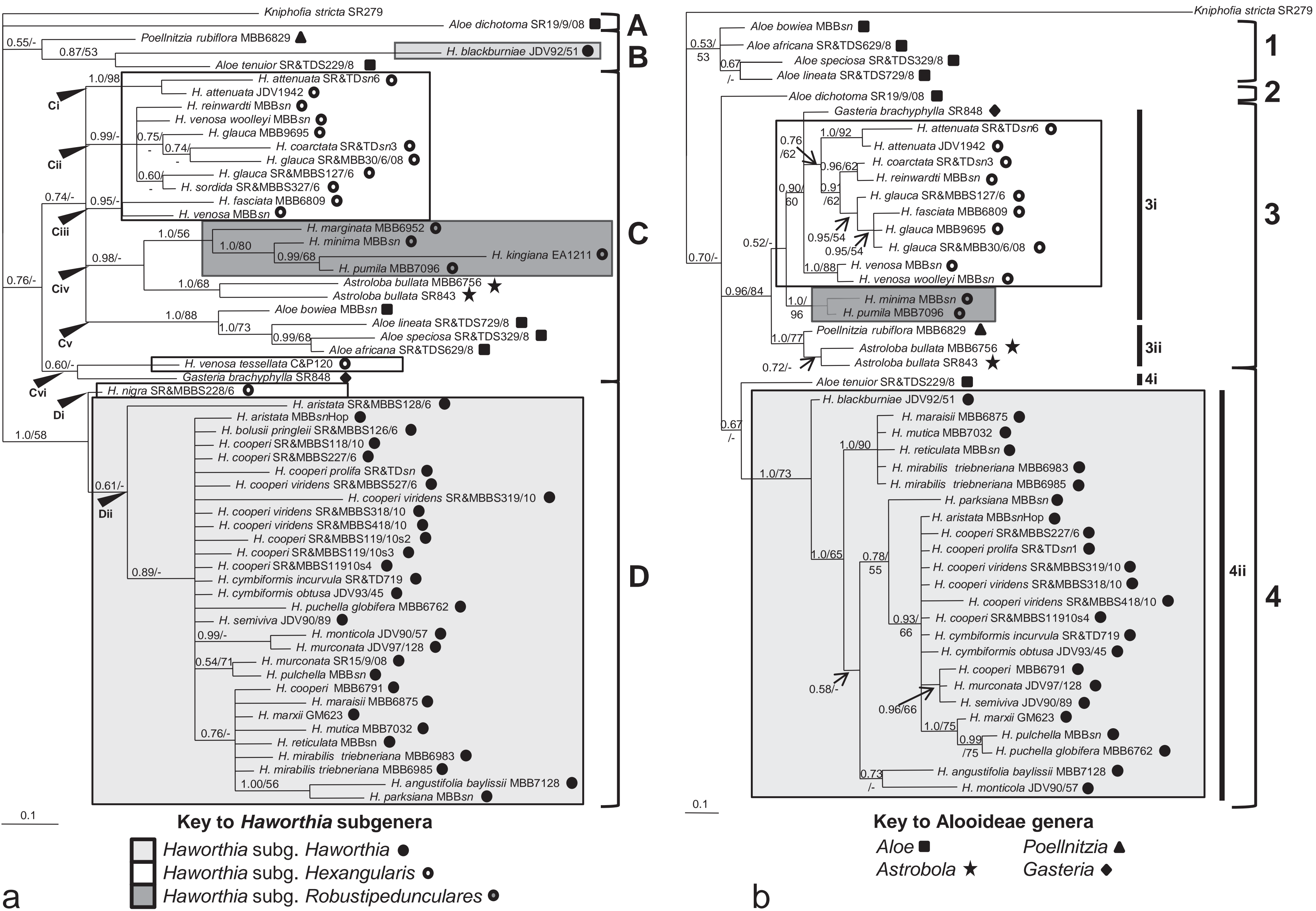

Fig. 2. a, Bayesian inference tree of the combined cpDNA dataset estimated using the following models: GRT+I+R (*trnL-F*) and F81+I+G (*psbA-trnH*) (determined by the Akaike information criterion [AIC]) **b,** Bayesian inference tree of the ITS1 dataset estimated using the GRT+G model (determined by the AIC). All main lineages and clades are discussed in the text for both topologies. Numbers above the branches or arrowed indicate posterior probability (BI)/bootstrap (MP) values.

ITS1 phylogeny. — Details of the ITS dataset are presented in Table 1. Analysis of ITS1 dataset with MP produced 4000 trees of 179 steps (CI = 0.765, RI = 0.910). In an independent MP analysis without the ratchet, the number of trees was in excess of 20,000. However, the tree length was higher, and the RI and CI values were lower than the ratchet analysis (results not shown). The BI tree is shown in Fig. 2b. Four major clades were recovered (labelled 1–4 in Fig. 2b). Clade 1 is unsupported and composed of *Aloe* samples (identical to the cpDNA "*Aloe* group"; Clade Cv in Fig. 2a). Clade 2 is composed of a single *Aloe dichotoma* sample. Clade 3 is well supported in the BI and MP analyses (PP = 0.96, BS = 84), and is further subdivided into two clades. Clade 3i is composed of *Gasteria brachyphylla* var. *brachyphylla*, a clade of *H.* subg. *Hexangularis* representatives (corresponding to cpDNA lineages Ci–Ciii), and two samples from *H.* subg. *Robustipedunclares*, but receives no support. Clade 3ii is composed of *Astroloba bullata* samples, and *Poellnitzia rubiflora*. It is well supported in both the BI and MP analyses (PP = 1.00, BS = 77). Clade 4 has weak support and is subdivided into two clades. Clade 4i is composed of a single *Aloe tenuior* sample and is sister to clade 4ii which comprises samples from *H.* subg. *Haworthia*. This latter clade has very good support (PP = 1.00, BS = 73) and corresponds to cpDNA clade Dii. There is thus only limited congruence between the two topologies, viz.: ITS1 clade 1 and cpDNA clade Cv ("*Aloe* group"), and ITS1 clade 4ii and cpDNA clade Dii (subg. *Haworthia*).

Combined cpDNA and ITS1 phylogeny. — An ILD test showed that the cpDNA and ITS1 data partitions were significantly incongruent (*P* = 0.001). Despite this result we opted to combine individual alignments to explore the data. A summary of aligned sequence characteristics of the combined cpDNA and ITS1 (hereafter termed combined DNA) dataset is presented in Table 1. The combined DNA tree topology (not shown) had the same general structure as the ITS1 phylogeny (discussed above). The topological structure recovered suggested that the signal provided by the ITS1 marker dominated that of the chloroplast markers. This is a surprising result considering the relatively small size of this marker and the fact that it has fewer variable and parsimony-informative sites (Table 1, although this might in part be attributed to the smaller sample size of this dataset). The retention index for the trees derived from MP analysis of the ITS dataset is very high (Table 1), indicating that the ITS dataset comprises many non-homoplastic synapomorphies, which may be reflected in the observation that the ITS1 phylogeny generally had higher PP and BS values.

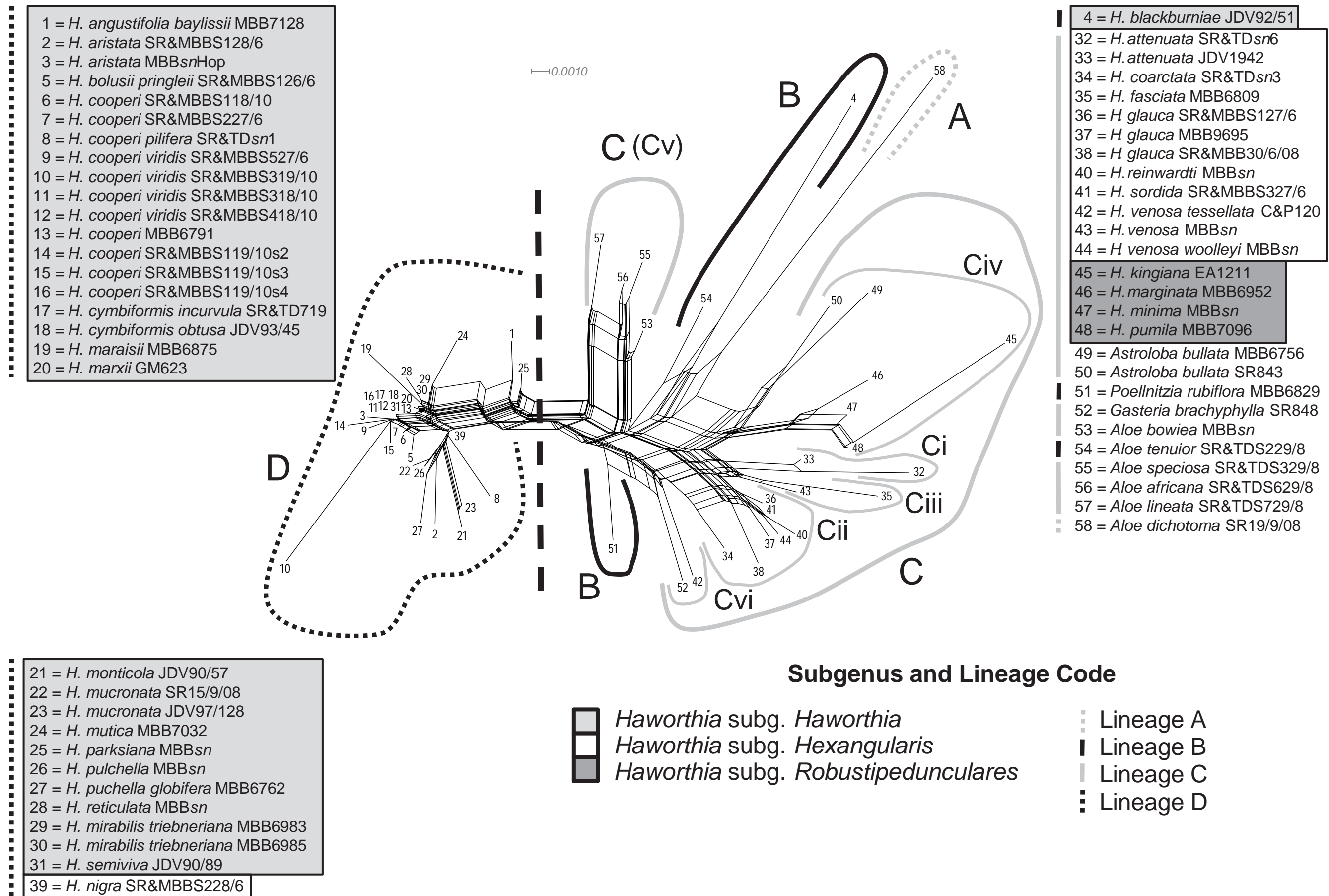

Fig. 3. Splits graph for chloroplast DNA (cpDNA) sequences of Alooideae. Two major branches were recovered (i.e., lineage D and other lineages [A–C]). The major lineages recovered are more or less congruent with the major cpDNA lineages (see Fig. 2a).

Phylogenetic networks. — The splits graph shows extensive internal network structure, indicating reticulation. The groups formed in the splits graph are (with minor exceptions) readily correlated to the clades recovered in the phylogenies, especially where these received good support. In the discussion below, we use the term "lineage" to refer to groups of specimens in the NN trees (Figs. 3 & 4), and "clade" to refer to groups in the phylogenies (Fig. 2).

The cpDNA splits graph revealed two main lineages (Fig. 3). One of these, lineage D (Fig. 3), correlates to clade D in Fig. 2a and is composed of a single *H. nigra* sample (subg. *Hexangularis*) and samples from *H.* subg. *Haworthia*. The rest of the NN tree is composed of three lineages (labelled A–C in Fig. 3). Lineage A is composed only of *Aloe dichotoma*. Clade B (Fig. 2a) is not recovered in the splits graph. Instead, *Aloe tenuior* and *H. blackburniae* (subg. *Haworthia*) are shown as related, while *Poellnitzia rubiflora* is placed on the opposite side of the splits graph (Fig. 3). This is not unexpected, as there is very weak support for clade B in the cpDNA phylogeny (Fig. 2a). Lineage C in the splits graph is largely congruent with Clade C of the cpDNA phylogeny. However, the *Aloe* clade (clade Cv in Fig. 2a) is distinct and separate from the remaining samples of NN lineage C. The other subclades of clade C (cpDNA phylogeny) are also retrieved as sublineages in the splits graph (Fig. 3).

The ITS1 splits graph revealed two main groups separated by splits with long branches (Fig. 4). One of the main lineages is Lineage 4ii, while the other main lineage is composed of several clusters (labelled 1–4 in Fig. 4). Lineage 4ii is largely congruent with clade 4 of the ITS1 phylogeny (Fig. 2b), with the exception of *Aloe tenuior* (lineage 4i), which is placed on the other side of the splits graph with lineages 1, 2 and 3. Lineage 1 is composed of the *Aloe* group, and is identical to clade 1 in the ITS1 phylogeny (Fig. 2b). Lineage 2 is composed of a single *Aloe dichotoma* sample (= clade 2 in Fig. 2b). It is interesting to note that in the splits graph, all *Aloe* samples cluster together (lineages 1, 2 and 4i). Lineage 3 (including lineages 3i and 3ii) is largely congruent with clade 3 of the ITS phylogeny (Fig. 2b). Lineage 3i comprises *H.* subg. *Hexangularis* and subg. *Robustipedunclares* samples, and a single *Gasteria* sample. Lineage 3ii comprises *Astroloba* and *Poellnitzia* taxa.

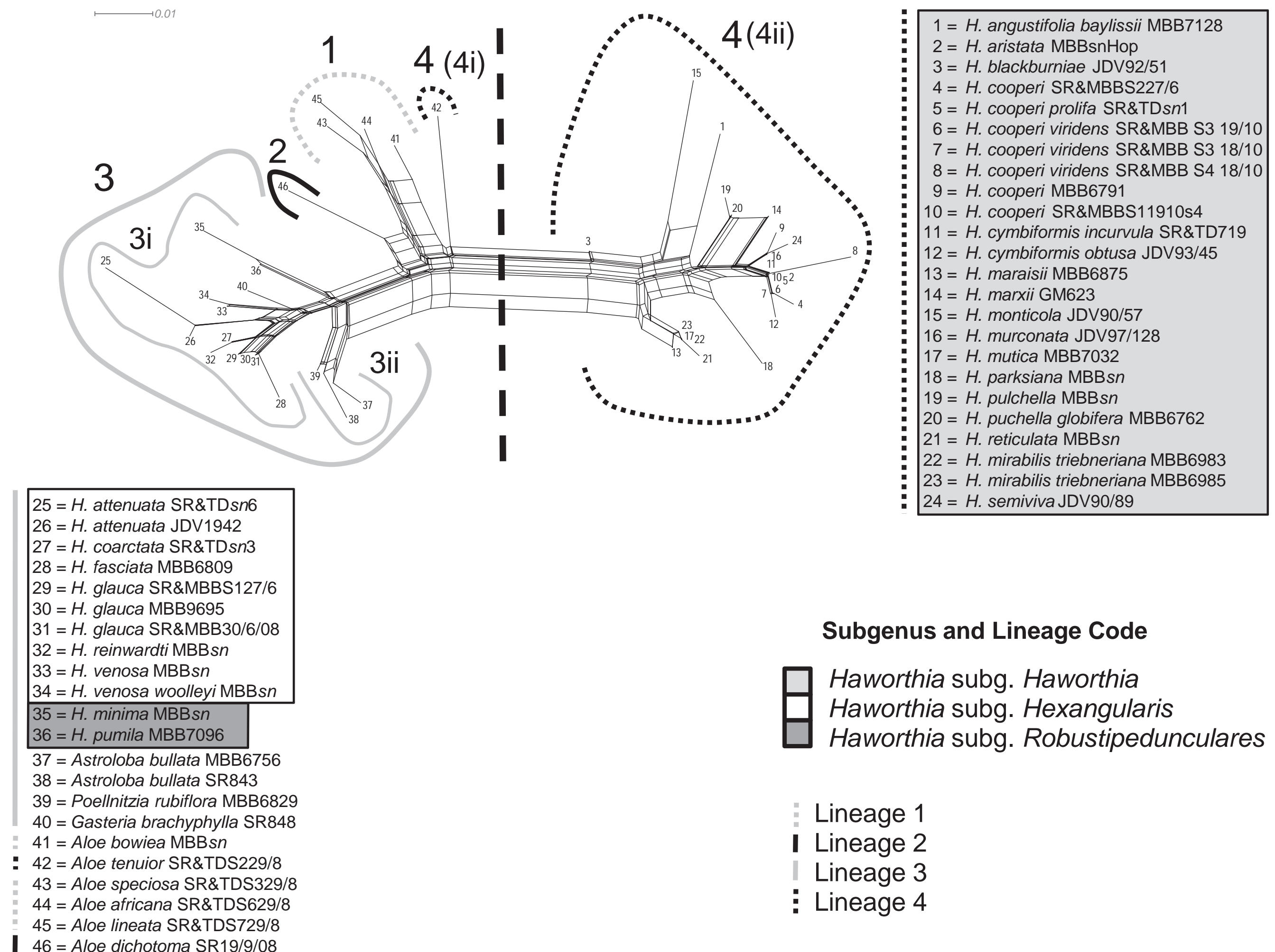

Fig. 4. Splits graph for ITS1 sequences of Alooideae. Two major branches were recovered (i.e., lineage 4 and other lineages [1–4]). The major lineages recovered are more or less congruent with the major combined DNA clades (see Fig. 2b).

DISCUSSION

This study demonstrates that, irrespective of whether nrDNA or cpDNA is used, *Haworthia* is not monophyletic and reiterates that *Haworthia* s.l. (and indeed all the currently recognized genera of Alooideae) cannot be studied in isolation, but require a subfamily level contextualization.

The issue of monophyly (or the lack thereof) needs to be assessed critically in all molecular-based phylogenetic studies, especially when considering the weight currently being placed on phylogenies (viz., gene trees) and the consequent implications for evolution, ecology, biogeography and more importantly taxonomic adjustments. Monophyly based on molecular data is becoming an increasingly important facet of generic conceptual delimitation (Humphreys & Linder, 2009), but there has been much debate regarding the treatment of non-monophyletic (viz., paraphyletic) taxa. Brummitt (1996, 1997, 2002, 2003), Rieseberg & Brouillet (1994), Hörandl (2006, 2010), Hörandl & Stuessy (2010) and Nordal & Stedje (2005) have argued that to operate within a taxonomic framework, paraphyletic taxa (irrespective of rank) must be accommodated. Zander (2008) has even suggested that non-monophyly may be evolutionarily informative. Other authors (e.g., De Queiroz, 1988; De Queiroz & Gauthier, 1990, 1992, 1994; Freudenstein, 1998; Scopece & al., 2010) maintain the need for monophyletic taxa. According to Nordal & Stedje (2005) and Van Wyk (2007) the dismissal of paraphyletic groups will cause unnecessary chaos in plant taxonomy for pragmatic reasons. In addition, molecular phylogenetic studies rely on a few markers and many conclusions can be erroneous for various reasons (see Van Welzen, 1998). Although the three currently circumscribed subgenera of *Haworthia* are distinguishable from each other morphologically using floral characters, the molecular data supports generic non-monophyly (like Treutlein & al., 2003a, b). Thus, we feel it is premature to undertake any taxonomic and nomenclatural rearrangements at generic or subgeneric level.

Additionally, our results also indicate numerous instances of non-monophyletic species (sometimes with good support). In the cpDNA phylogeny *H. glauca*, *H. venosa* (both subg. *Hexangularis*), *H. aristata*, *H. cooperi*, *H. cymbiformis*, *H. pulchella*, *H. mucronata*, and *H. mirabilis* (all subg. *Haworthia*) are resolved as non-monophyletic. In the ITS1 phylogeny a subset of these species were also recovered as non-monophyletic: *H. glauca* (subg. *Hexangularis*); *H. cooperi*, *H. cymbiformis*, and *H. mirabilis* (subg. *Haworthia*; Fig. 2a & b).

Monophyly (or the lack thereof) from a molecular perspective is receiving increasing attention, and several studies have recovered extensive species and/or subgeneric non-monophyly (Kizirian & Donnelly, 2004; Kamiya & al., 2005; Shaw & Small, 2005; Syring & al., 2007; Flagel & al., 2008; Barker & al., 2009; Dragon & Barrington, 2009; Howis & al., 2009; Ramdhani & al., 2009; Carrió & al., 2010; Gurushidze & al., 2010). Syring & al. (2007) and Ramdhani & al. (2009) have suggested that single exemplar phylogenetics can be misleading. We suspect that non-monophyly is common in the southern Africa flora (e.g., Ramdhani & al., 2009, 2010; Howis & al., 2009; Barker & al., 2009) and deserves further research.

Isolating the factors that account for non-monophyly in gene trees irrespective of rank can be challenging. However, it is possible to make causal inferences from topological patterns (Syring & al., 2007). Several factors could account for the lack of monophyly, including unrecognized amplification of a paralogous locus, recombination of divergent alleles, inadequate phylogenetic signal, poor or imperfect alpha taxonomy, introgressive hybridization and incomplete lineage sorting (Draper & al., 2007; Syring & al., 2007; Flagel & al., 2008; Dragon & Barrington, 2009; Ramdhani & al., 2009). These causes can be placed into three non-mutually exclusive categories, viz., human error, erroneously interpretation of data and actual evolutionary processes. Human error due to a poor or imperfect alpha taxonomy could be reflected as non-monophyly. Inadequate phylogenetic signal and amplification of a paralogous locus may result in erroneous interpretation of data. Lastly, actual evolutionary processes such as recombination of divergent alleles, incomplete lineage sorting and introgressive hybridization all result in non-monophyly.

Human error. — *Haworthia* is known for its complex taxonomy, and this could explain non-monophyly at the species level (but not at higher levels). Taxonomic issues in *Haworthia* range from typification to additional fieldwork needed to understand species variation over geographic distribution ranges. Species concepts differ depending on authority. Species are considered to "intergrade" across geographic barriers and vary across ecological barriers. In very simplistic terms important ecological factors include vegetation, geology and soil type. This intergradation is not continuous but manifests itself in isolated populations creating an irregular pattern difficult to unravel (Bayer, 1982). However, outstanding taxonomic issues at the species level are being resolved with ongoing field and herbarium work (Bayer, 1999, 2002, 2006, 2007, 2008). The only way this can ever be resolved using molecular techniques is to sample many populations and adopt a phylogeographic approach. In this way, perhaps "evolutionarily significant units" (ESUs) sensu Ryder (1986) can be detected, and explained by processes such as those hypothesized below.

Erroneous interpretation of data. — The most common measure of phylogenetic signal is sequence divergence, and lack of sequence divergence could manifest itself as species non-monophyly (e.g., Shaw & Small, 2005; Flagel & al., 2008; Ramdhani & al., 2009; Kellner & al., 2010). Markers used for phylogenetic reconstruction in plants evolve at different rates (Hörandl, 2010). However, if the mode and tempo of sequence divergence has lagged behind morphological evolution, or if the group studied is of recent origin, then no matter which and how many markers are used, non-monophyly may still be evident as a consequence of low sequence divergence. As noted in Table 1, the amount of variation and informativeness of markers used in this study are reasonable, but it could certainly be argued that additional data could improve resolution. Another universal measure of phylogenetic signal is the support value of nodes (bootstrap or posterior probability; Syring & al., 2007). In our datasets we do have weak support values for most of the main lineages and clades. The retention index (RI) is an additional measure of support in the data (synapomorphies). As

noted earlier, the RI value of the ITS1 data is very high (0.910; Table 1), indicating that many of the characters are not homoplasious. Nonetheless, the ITS1 data could still be "positively misleading" (Felsenstein, 1978). Both our result and that of Treutlein (2003a, b) indicate that there is reasonable sequence divergence in *Haworthia*, but generally poor support for many lineages. However, inadequate phylogenetic signal cannot exclusively explain the patterns we recovered.

Paralogy versus orthology. — Paralogy is not a problem usually associated with cpDNA markers because most cp genes are single copy (for an exception see Pirie & al., 2007). This is not usually the case for nuclear markers (Oxelman & Bremer, 2000). In the present study we utilized the ITS1 marker to determine relationships. The ITS region is now widely known to be unsuited for phylogeny reconstruction (Alvarez & Wendel, 2003; Bailey & al., 2003; Small & al., 2004). Despite this, it is widely used, and as long as its limitations are understood it can provide meaningful insights into evolutionary history (Feliner & Rosselló, 2007). Non-monophyly could be the result of ITS paralogy (e.g., Roalson & Friar, 2004; Devey & al., 2008, King & Roalson, 2008). Initial attempts to amplify the entire ITS region (ITS1, 5.8S, ITS2) failed using conditions that have worked for *Aloe* (Adams & al., 2000). Additionally, primer combinations that worked for the related genus *Kniphofia* (Ramdhani & al., 2009) did not routinely work for our study system. Consequently, we used only the ITS1 marker, which has been successfully employed in Alooideae (Treutlein & al., 2003b). The failure to amplify the 5.8S and ITS2 regions does raise some concerns and could suggest that paralogues for this marker may exist. A more detailed investigation (PCR amplification and subsequent cloning) will be needed to assess ITS paralogy in Alooideae.

Evolutionary processes: Recombination. — The effect of recombination can have profound consequences on reconstructing genealogies (Posada & Crandall, 2002; Popp & Oxelman, 2007). Recombination results in sequence segments that have different genealogical histories. Consequently, organismal history cannot be accurately depicted by a single phylogenetic tree, but as a set of correlated trees across recombinant segments (Syring & al., 2007). In the present study the effect of recombination cannot be properly assessed because of the limited sampling. However, we cannot rule out the possibility of recombination, which can lead to evolutionary histories that are non-tree-like with reticulate patterns (Bryant & al., 2007), such as those found in the NN analysis (Figs. 3 & 4).

Evolutionary processes: Lineage sorting. — Several studies have shown that incomplete lineage sorting (the persistence and retention of ancestral polymorphisms through speciation events; Syring & al., 2007) may confound phylogenetic reconstruction (Chiang & al., 2004; Roalson & Friar, 2004; Bouillé & Bousquet, 2005; Knowles & Carstens, 2007; Gurushidze & al., 2010; Ramdhani & al., 2010). Paraphyly should be expected as a direct result of incomplete lineage sorting. Over time monophyly may be achieved via the sorting and extinction of lineages. However, mode of speciation and the time needed for sorting of lineages are important factors to consider (Rieseberg & Brouillet, 1994). In *Haworthia,* incomplete lineage sorting could explain all cases of species non-monophyly within and/or among each of the subgenera. In some studies it is difficult to determine whether hybridization and/or incomplete lineage sorting are driving the pattern of non-monophyly (e.g., Kamiya & al., 2005; Flagel & al., 2008). Non-monophyly of *Haworthia* could be due to both processes, as these are not mutually exclusive (Church & Taylor, 2005; King & Roalson, 2008; Maki & al., 2010). In this scenario, lineage sorting is ongoing while interbreeding (hybridizing) species may exchange genetic material because reproductive barriers are weak or absent.

Evolutionary processes: Hybridization. — It is very difficult to predict the behaviour of hybrids in phylogenetic reconstruction, and hybrids can cause loss of resolution resulting in topological changes in weakly supported regions (McDade, 1992). Hybridization and introgression can result in non-monophyly at various ranks (Roalson & Friar, 2004; Lee & al., 2005; Shaw & Small, 2005; Wang & al., 2005; Mason-Gamer 2008; Yi & al., 2008; Howis & al., 2009; Jabaily & Sytsma, 2010; Ramdhani & al., 2010; Valcárcel & Vargas, 2010). The ability for *Haworthia* species to hybridize under artificial and field conditions has been well documented (Bayer, 1982, 1999). Bayer (1982) listed nine interspecific hybrids and a single hybrid between *Haworthia pumila* (subg. *Robustipedunculares*) and *Astroloba muricata*. Bayer (1999) added five more intrageneric hybrids to this list. Six hybrids are within *Haworthia* subg. *Haworthia* (*H. turgida* × *H. floribunda*, *H. blackburniae* var. *graminifolia* × *H. arachnoidea*, *H. herbacea* × *H. reticulata*, *H. cymbiformis* × *H. angustifolia*, *H. truncata* × *H. arachnoidea*, *H. blackburniae* × *H. arachnoidea*), six within subg. *Hexangulares* (*H. scabra* × *H. viscosa*, *H. viscosa* × *H. longiana*, *H. sordida* × *H. venosa* var. *woolleyi*, *H. viscosa* × *H. fasciata*, *H. viscosa* × *H. glauca* var. *herrei*, *H. monticola* × *H. cooperi* var. *gordoniana*) and two are within subg. *Robustipedunculares* (*H. pumila* × *H. marginata*, *H. minima* × *H. marginata*). None of these natural hybrids are between *Haworthia* subgenera, suggesting that the floral morphologies or other reproductive barriers are sufficient to ensure isolation.

Several hybrids between *Gasteria* and *Haworthia*, viz., *H. venosa* var. *tessellata* (subg. *Hexangulares*) and other unspecified taxa exist, and *H.* subg. *Hexangulares* and subg. *Robustipedunculares* form intergeneric hybrids (Riley & Majumdar, 1979; Bayer, 1982, 1999; Treutlein & al., 2003a). Thus, (relatively) ancient hybridization between these two *Haworthia* subgenera and other genera of Alooideae is probably the main cause of non-monophyly.

Treutlein & al. (2003a) included the reputed hybrid *H. kewensis* (considered to belong to subg. *Hexangulares*) and ×*Astroloba bicarinata* (considered to be an intergeneric hybrid between a member of subg. *Robustipedunculare*s and an *Astroloba*). Treutlein & al. (2003b) included two hybrid taxa (*Haworthia* × *resendiana* and *Haworthia* × *ryderiana*) which have doubtful origins and could not be assigned to a subgenus. As far as we are aware, no hybrid taxa (neither intergeneric nor interspecific) were included in our study.

Bayer (1982) did not consider field hybridization to be a serious problem and acknowledged that hybridization is

expected in a relatively young genus until isolation becomes established. Problems associated with species-level variability and identification were not attributed to hybridization (Bayer, 1982). Despite this, it seems plausible that hybridization (old and recent) may have some bearing on the evolutionary history of Alooideae (Treutlein & al., 2003a). Bayer (1999) considers *Haworthia* (subg. *Haworthia*) to be actively speciating, a process driven by factors discussed below. If this is the case, then the species non-monophyly that we observed would not be surprising.

Is *Haworthia* undergoing contemporary speciation? — The morphological rate and tempo of evolution can outpace the molecular rate and the sensitivity of molecular markers, and non-monophyly may be a consequence of this. This raises concerns about our capacity to resolve the phylogenetic history and relationships using molecular sequences and gene trees (Rokas & al., 2003). Some studies have made the link between non-monophyly and ancient radiations (e.g., Fishbein & al., 2001). Even more studies have documented non-monophyly with strong morphological divergence and attribute this to recent or rapid radiations (Knox & Palmer, 1995; Moore & al., 2002; Klak & al., 2004; Lee & al., 2005; Wang & al., 2005; Popp & Oxelman, 2007; Ramdhani & al., 2009; Kellner & al., 2010). Few studies have considered a contemporary radiation scenario (e.g., Rymer & al., 2010). Contemporary speciation presents difficult challenges in molecular phylogenetics as molecular evolution can lag behind morphological evolution. We hypothesize that the patterns we recovered indicate that *Haworthia* subg. *Haworthia* is undergoing rapid and contemporary speciation.

A combination of Early Pliocene geomorphic uplift and rapid climatic aridification produced habitat heterogeneity (Cowling & al., 2009) and triggered the radiation of many southern African lineages, both plant (e.g., Linder, 2005; Howis & al., 2009; Verboom & al., 2009) and animals (e.g., Tolley & al., 2008; Russo & al., 2010). The glacial-interglacial cycles in the Pleistocene would have promoted ongoing speciation, but relative climatic stability, at least in comparison to other mid-latitude areas, reduced extinction rates (Jannson & Dynesius, 2002). Consequently, large numbers of young species have accumulated over much of southern Africa (Cowling & Hilton-Taylor, 1994).

As is the case with many other rapidly diverging lineages in southern Africa (e.g., Ellis & Weis, 2006; Ellis & al., 2006; Kellner & al., 2010), speciation in *Haworthia* is associated with fine-scale habitat, especially edaphic differentiation (Bayer, 1999, 2002, 2006, 2007, 2008). However, at this stage it is uncertain as to which biological traits predispose *Haworthia* to such rapid speciation. Incipient speciation in many plant taxa is reinforced by pollinator specialization, an extremely well documented phenomenon in the southern African flora (Van der Niet & al., 2006; Johnson, 2010). However, this is unlikely to be important in *Haworthia* owing to the similarity in flower structure among taxa (a feature that could facilitate hybridization). Pollination and reproduction biology studies for *Haworthia* are needed to confirm this. We thus hypothesize that contemporary speciation in *Haworthia* (along with ongoing hybridization) can best account for the high degree of non-monophyly, complex taxonomy and high numbers of range-restricted and habitat specialist species.

Further research along the lines of that conducted for *Argyroderma* (Aizoaceae) by Ellis & al. (2006) are required to untangle the effects of habitat on plant form and function within species complexes of *Haworthia*. Preliminary data suggest complex patterns of morphological variation with regard to edaphic factors and habitat (Bayer, 1999, 2002, 2006, 2007, 2008).

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LITERATURE CITED

Adams, S.P., Leitch, I.J., Bennett, M.D., Chase, M.W. & Leitch, A.R. 2000. Ribosomal DNA evolution and phylogeny in *Aloe* (Asphodelaceae). *Amer. J. Bot.* 87: 1578– 1583.

Alvarez, I. & Wendel, J.F. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molec. Phylog. Evol.* 29: 417–434.

Bailey, C.D., Carr, T.G., Harris, S.A. & Hughes, C.E. 2003. Characterization of angiosperm nrDNA polymorphism, paralogy and pseudogenes. *Molec. Phylog. Evol.* 29: 435–455.

Barker, N.P., von Senger, I., Howis, S., Zachariades, C. & Ripley, B.S. 2005. Plant phylogeography based on nrDNA ITS sequence data: Two examples from the Asteraceae. Pp. 217–244 in: Bakker, F.T., Chatrou, L.W., Gravendeel, B. & Pelser, P.B. (eds.), *Plant species level systematics: New perspectives on pattern and process.* Regnum Vegetabile 143. Ruggell, Liechtenstein: Gantner.

Barker, N.P., Howis, S., Nordenstam, B., Källersjö, M., Eldenäs, P., Griffioen, C. & Linder, H.P. 2009. Nuclear and chloroplast DNA-based phylogenies of *Chrysanthemoides* Tourn. ex Medik. (Calenduleae; Asteraceae) reveal extensive incongruence and generic paraphyly, but support the recognition of infraspecific taxa in *C. monilifera*. *S. African J. Bot.* 75: 560–572.

Bayer, B. 1982. *The new Haworthia handbook*. Kirstenbosch: National Botanic Gardens of South Africa.

Bayer, B. 1999. *Haworthia revisited: A revision of the genus*. Hatfield: Umdaus Press.

Bayer, B. 2002. *Haworthia update, essays on Haworthia*, vol. 1. Hatfield: Umdaus Press.

Bayer, B. 2006. *Haworthia update, essays on Haworthia*, vol. 2. Preston: Alsterworthia International.

Bayer, B. 2007. *Haworthia update, essays on Haworthia*, vol. 3. Preston: Alsterworthia International.

Bayer, B. 2008. *Haworthia update, essays on Haworthia*, vol. 4. Preston: Alsterworthia International.

Beyra-Matos, A. & Lavin, M. 1999. A monograph of *Pictetia* (Papilionoideae, Leguminosae) and review of the Aeschynomeneae. *Syst. Bot. Monogr.* 56: 1–93.

Bouillé, M. & Bousquet, J. 2005. Trans-species shared polymorphisms at orthologous nuclear gene loci among distant species in the conifer *Picea* (Pinaceae): Implications for the long-term maintenance of the genetic diversity in trees. *Amer. J. Bot.* 92: 63–73.

Breuer, I. 1998. *The world of Haworthias*, vol. 1. Niederzeier: Breuer & AfM.

Breuer, I. 1999. *Haworthia photographs used to typify taxa described by Dr Karl von Poellnitz*. Niederzeier: Breuer & AfM.

Breuer, I. 2002. *The world of Haworthias*, vol. 2. Niederzeier: Breuer & AfM.

Brummitt, R.K. 1996. In defence of paraphyletic taxa. Pp. 371–384 in: Van der Maesen, L.J.G., Van der Burgt, X.M. & Van Medenbach de Rooy, J.M. (eds.), *The biodiversity of African plants: Proceedings, XIVth AETFAT Congress, 22–27 August 1994, Wageningen, The Netherlands*. Dordrecht: Kluwer Academic Press.

Brummitt, R.K. 1997. Taxonomy versus cladonomy, a fundamental controversy in biological systematics. *Taxon* 46: 723–734.

Brummitt, R.K. 2002. How to chop up a tree. *Taxon* 51: 31–41.

Brummitt, R.K. 2003. Further dogged defense of paraphyletic taxa. *Taxon* 52: 803–804.

Bryant, D. & Moulton, V. 2004. Neighbor-Net: An agglomerative method for the construction of phylogenetic networks. *Molec. Biol. Evol.* 21: 255–265.

Bryant, D., Moulton, V. & Spillner, A. 2007. Consistency of the Neighbor-Net Algorithm. *Algorithms Molec. Biol.* 2: 8. DOI: 10.1186/1748-7188-2-8.

Bytebier, B., Bellstedt, D.U. & Linder, H.P. 2007. A molecular phylogeny for the large African orchid genus *Disa*. *Molec. Phylog. Evol.* 43: 75–90.

Carine, M.A., Robba, L., Little, R., Russel, S. & Guerra A.S. 2007. Molecular and morphological evidence for hybridization between endemic Canary Island *Convolvulus*. *Bot. J. Linn. Soc.* 154: 187–204.

Carrió, E., Forrest, A.D., Güemes, J. & Vargas, P. 2010. Evaluating species nonmonophyly as a trait affecting genetic diversity: A case study of three endangered species of *Antirrhinum* L. (Scrophulariaceae). *Pl. Syst. Evol.* 288: 43– 58.

Chase, M.W. & Hills, H.H. 1991. Silica gel: An ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215–220.

Chase, M.W., De Bruijn, A.Y., Reeves, G., Cox, A.V., Rudall, P.J., Johnson, M.A.T. & Eguiarte, L.E. 2000. Phylogenetics of Asphodelaceae (Asparagales): An analysis of plastid *rbcL* and *trnL-trnF* DNA sequences. *Ann. Bot.* 86: 935–956.

Chiang, T.-Y., Hung, K.-H., Hsu, T.-W. & Wu, W.-L. 2004. Lineage sorting and phylogeography in *Lithocarpus formosanus* and *L. dodonaeifolius* (Fagaceae) from Taiwan. *Ann. Missouri Bot. Gard.* 91: 207–222.

Church, S.A. & Taylor, D.R. 2005. Speciation and hybridisation among *Houstonia* (Rubiaceae) species: The influence of polyploidy on reticulate evolution. *Amer. J. Bot.* 92: 1372–1380.

Cowling, R.M. & Hilton-Taylor, C. 1994. Patterns of plant diversity and endemism in southern Africa: An overview. Pp. 31–52 in: Huntley, B.J. (ed.), *Botanical diversity in southern Africa*. Pretoria: National Botanical Institute.

Cowling, R.M., Procheş, Ş. & Partridge, T.C. 2009. Explaining the uniqueness of the Cape flora: Incorporating geomorphic evolution as a factor for explaining its diversification. *Molec. Phylog. Evol.* 51: 64–74.

De Queiroz, K. 1988. Systematics and the Darwinian revolution. *Philos. Sci.* 55: 238– 259.

De Queiroz, K. & Gauthier, J. 1990. Phylogeny as a central principle in taxonomy: Phylogenetic definitions of taxon names. *Syst. Zool.* 39: 307–322.

De Queiroz, K. & Gauthier, J. 1992. Phylogenetic taxonomy. *Annual Rev. Ecol. Syst.* 23: 449–480.

De Queiroz, K. & Gauthier, J. 1994. Towards a phylogenetic system of biological nomenclature. *Trends Ecol. Evol.* 9: 27–31.

Devey, D.S., Bateman, R.M., Fay, M.F. & Hawkins, J.A. 2008. Friends or Relatives? Phylogenetics and species delimitation in the controversial european orchid genus *Ophrys*. *Ann. Bot.* 101: 385–402.

Doyle, J.J. & Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11–15.

Dragon, J.A. & Barrington, D.S. 2009. Systematics of the *Carex aquatilis* and *C. lenticularis* lineages: Geographically and ecologically divergent sister clades of *Carex* section *Phacocytis*. *Amer. J. Bot.* 96: 1896–1906.

Draper, I., Hedenäs, L. & Grimm, G.W. 2007. Molecular and morphological incongruence in European species of *Isothecium* (Bryophyta). *Molec. Phylog. Evol.* 42: 700–716.

Ellis, A.G. & Weis, A.E. 2006. Coexistence and differentiation of 'flowering stones': The role of local adaptation to soil microenvironment. *J. Ecol.* 94: 322–335.

Ellis, A.G., Weis, A.E. & Gaut, B. 2006. Evolutionary radiation of "stone plants" in the genus *Argyroderma*: Unravelling the effects of landscape, habitat and flowering time. *Evolution 60:* 39–55.

Farris, J.S., Källersjö, M., Kluge, A.G. & Bult, C. 1994. Testing significance of congruence. *Cladistics* 10: 315–319.

Feliner, G.N. & Rosselló, J.A. 2007. Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. *Molec. Phylog. Evol.* 44: 911–919.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. *Syst. Zool.* 27: 401–410.

Fishbein, M., Hibsch-Jetter, C., Soltis, D.E. & Hufford, L. 2001. Phylogeny of Saxifragales (angiosperms, eudicots): Analysis of a rapid, ancient radiation. *Syst. Biol.* 50: 817–849.

Flagel, L.E., Rapp, R.A., Grover, C.E., Widrlechner, M.P., Hawkins, J., Grafenberg, J.L., Álvarez, I., Chung, G.Y. & Wendel, J.F. 2008. Phylogenetic, morphological, and chemotaxonomic incongruence in the North American endemic genus *Echinacea*. *Amer. J. Bot.* 95: 756–765.

Frajman, B. & Oxelman, B. 2007. Reticulate phylogenetics and phytogeographical structure of *Heliosperma* (Sileneae, Caryophyllaceae) inferred from chloroplast and nuclear DNA sequences. *Molec. Phylog. Evol.* 43: 140–155.

Freudenstein, J.V. 1998. Paraphyly, ancestors, and classification—a response to Sosef and Brummitt. *Taxon* 47: 95–104.

Gielly, L. & Taberlet, P. 1994. The use of chloroplast DNA to resolve plant phylogenies: Noncoding versus *rbcL* sequences. *Molec. Biol. Evol.* 11: 769–777.

Grimm, G.W. & Denk, T. 2008. ITS evolution in *Platanus* (Platanaceae): Homoeologues, pseudogenes and ancient hybridization. *Ann. Bot.* 101: 403–419

Gurushidze, M., Fritsch, R.M. & Blattner, F.R. 2010. Species level phylogeny of *Allium* subgenus *Melanocrommyum*: Incomplete lineage sorting, hybridization and *trnF* gene duplication. *Taxon* 59: 829–840.

Hörandl, E. 2006. Paraphyletic versus monophyletic taxa—evolutionary versus cladistic classifications. *Taxon* 55: 564–570.

Hörandl, E. 2010. Beyond cladistics: Extending evolutionary classifications into deeper time levels. *Taxon* 59: 345–350.

Hörandl, E. & Stuessy, T. 2010. Paraphyletic groups as natural units of biological classification. *Taxon* 59: 1641–1653.

Howis, S., Barker, N.P. & Mucina, L. 2009. Globally grown, but poorly known: Species limits and biogeography of *Gazania* Gaertn.

(Asteraceae) inferred from chloroplast and nuclear DNA sequence data. *Taxon* 58: 871–882.

Huelsenbeck, J.P. & Ronquist, F. 2001. MR BAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Humphreys, A.M. & Linder, H.P. 2009. Concept versus data in delimitation of plant genera. *Taxon* 58: 1054–1074.

Huson, D.H. 1998. SplitsTree: A program for analyzing and visualising evolutionary data. *Bioinformatics* 14: 68–73.

Jabaily, R.C. & Sytsma, K.J. 2010. Phylogenetics of *Puya* (Bromeliaceae): Placement, major lineages, and evolution of the Chilean species. *Amer. J. Bot.* 97: 337–356.

Jansson, R. & Dynesius, M. 2002. The fate of clades in a world of recurrent climate change: Milankovitch oscillations and evolution. *Annual Rev. Ecol. Syst.* 33: 741– 777.

Johnson, S.D. 2010. The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philos. Trans., Ser. B* 365: 499–516.

Kamiya, K., Harada, K., Tachida, H. & Ashton, P.S. 2005. Phylogeny of *pgiC* gene in *Shorea* and its closely related genera (Dipterocarpaceae), the dominant trees in southeast Asian tropical rain forests. *Amer. J. Bot.* 92: 775–788.

Käss, E. & Wink, M. 1997. Phylogenetic relationships in the Papilionideae (family Leguminosae) based on nucleotide sequences of cpDNA (*rbcL*) and nrDNA (ITS 1 and 2). *Molec. Phylog. Evol.* 8: 65–88.

Kellner, A., Ritz, C.M., Schlittenhardt. P. & Hellwig, F.H. 2010. Genetic differentiation in the genus *Lithops* L. (Ruschioideae, Aizoaceae) reveals a high level of convergent evolution and reflects geographic distribution. *Pl. Biol.* 13: 368–380.

King, M.G. & Roalson, E.H. 2008. Exploring evolutionary dynamics of nrDNA in *Carex* subgenus *Vignea* (Cyperaceae). *Syst. Bot.* 33: 514–524.

Kizirian, D. & Donnelly, M.A. 2004. The criterion of reciprocal monophyly and classification of nested diversity at the species level. *Molec. Phylog. Evol.* 32: 1072–1076.

Klak, C., Reeves, G. & Hedderson, T. 2004. Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature* 427: 63–65.

Knowles, L.L. & Carstens, B.C. 2007. Delimiting species without monophyletic gene trees. *Syst. Biol.* 56: 887–895.

Knox, E.B. & Palmer, J.D. 1995. Chloroplast DNA variation and the recent radiation of the gaint senecios (Asteraceae) on the tall mountains of eastern Africa. *Proc. Natl. Acad. Sci. U.S.A.* 92: 10349–10353.

Kocyan, A., De Vogel, E.F., Conti, E. & Gravendeel, B. 2008. Molecular phylogeny of *Aerides* (Orchidaceae) based on one nuclear and two plastid markers: A step forward in understanding the evolution of the Aeridinae. *Molec. Phylog. Evol.* 48: 422–443.

Kwembeya, E.G., Bjorå, C.S., Stedje, B. & Nordal, I. 2007. Phylogenetic relationships in the genus *Crinum* (Amaryllidaceae) with emphasis on tropical African species: Evidence from *trnL-F* and nuclear ITS DNA sequence data. *Taxon* 56: 801–810.

Lee, C., Kim, S.-C., Lundy, K. & Santos-Guerra, A. 2005. Chloroplast DNA phylogeny of the woody *Sonchus* alliance (Asteraceae: Sonchinae) in the Macaronesian Islands. *Amer. J. Bot.* 92: 2072–2085.

Linder, H.P. 2005. Evolution of diversity: The Cape flora. *Trends Pl. Sci.* 10: 536–541.

Maddison, W.P. & Maddison, D.R. 2000. MacClade 4: Analysis of phylogeny and character evolution. Sunderland, Massachusetts: Sinauer.

Maki, M., Yamashiro, T., Dohzono, I. & Suzuki, K. 2010. Molecular phylogeny of *Isodon* (Lamiaceae) in Japan using chloroplast DNA sequences: Recent rapid radiations or ancient introgressive hybridization? *Pl. Spec. Biol.* 25: 240–248.

Mason-Gamer, R.J. 2008. Allohexaploidy, introgression, and the complex phylogenetic history of *Elymus repens* (Poaceae). *Molec. Phylog. Evol.* 47: 598–611.

McDade, L.A. 1992. Hybrids and phylogenetic systematics II. The impacts of hybrids on cladistic analysis. *Evolution* 46: 1329–1346.

Meng, Y., Wen, J., Nie, Z-L., Sun, H. & Yang, Y-P. 2008. Phylogeny and biogeographic diversification of *Maianthemum* (Ruscaceae: Polygonatae). *Molec. Phylog. Evol.* 49: 424–434.

Moore, M.J., Francisco-Ortega, J., Santos-Guerra, A. & Jansen, R.K. 2002. Chloroplast DNA evidence for the roles of island colonization and extinction in *Tolpis* (Asteraceae: Lactuceae). *Amer. J. Bot.* 89: 518–526.

Müller, K.F., Borsch, T. & Hilu, K.W. 2006. Phylogenetic utility of rapidly evolving DNA at high taxonomical levels: Contrasting *matK*, *trnT-F*, and *rbcL* in basal angiosperms. *Molec. Phylog. Evol.* 41: 99–117.

Nixon, K.C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407–414.

Nordal, I. & Stedje, B. 2005. Paraphyletic taxa should be accepted. *Taxon* 54: 5–6.

Nylander, J.A. 2004. MrModeltest, version 2.2. Distributed by the author. Evolutionary Biology Centre, Uppsala University. http://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html.

Oxelman, B. & Bremer, B. 2000. Discovery of paralogous nuclear gene sequences coding for the second-largest subunit of RNA polymerase II (*RPB2*) and their phylogenetic utility in Gentianales of the asterids. *Molec. Biol. Evol.* 17: 1131–1145.

Page, R.D.M. 1996. TreeView: An application to display phylogenetic trees on personal computers. *Computer Applic. Biosci.* 12: 357–358.

Peterson, A., Levichev, I.G. & Peterson, J. 2008. Systematics of *Gagea* and *Lloydia* (Liliaceae) and infrageneric classification of *Gagea* based on molecular and morphological data. *Molec. Phylog. Evol.* 46: 446–465.

Pirie, M.D., Vargas, M.P.B., Botermans, M., Bakker, F.T. & Chatrou, L.W. 2007. Ancient paralogy in the cpDNA *trnL-F* region in Annonaceae: Implications for plant molecular systematics. *Amer. J. Bot.* 94: 1003–1016.

Popp, M. & Oxelman, B. 2007. Origin and evolution of North American polyploid *Silene* (Caryophyllaceae). *Amer. J. Bot.* 94: 330–349.

Posada, D. & Crandall, K.A. 2002. The effect of recombination on the accuracy of phylogeny estimation. *J. Molec. Evol.* 54: 396–402.

Ramdhani, S., Barker, N.P. & Baijnath, H. 2009. Rampant non-monophyly of species in *Kniphofia* Moench (Asphodelaceae) suggests a recent Afromontane radiation. *Taxon* 58: 1141–1152.

Ramdhani, S., Cowling, R.M. & Barker, N.P. 2010. Phylogeography of *Schotia* (Fabaceae): Recent evolutionary processes in an ancient Thicket biome lineage. *Int. J. Pl. Sci.* 171: 626–640.

Rieseberg, L.H. & Broulliet, L. 1994. Are many plant species paraphyletic? *Taxon* 43: 21–32.

Riley, H.P. & Majumdar, S.K. 1979. *The Aloineae: A biosystematic survey*. Kentucky: The University Press of Kentucky.

Roalson, E.H. & Friar, E.A. 2004. Phylogenetic relationships and biogeographic patterns in North American members of *Carex* section *Acrocystis* (Cyperaceae) using nrDNA ITS and ETS sequence data. *Pl. Syst. Evol.* 243: 175–187.

Rokas, A., King, N., Finnerty, J. & Carroll, S.B. 2003. Conflicting phylogenetic signals at the base of the metazoan tree. *Evol. & Developm.* 5: 346–359.

Russo, I.M., Chimimba, C.T. & Bloomer, P. 2010. Bioregion heterogeneity correlates with extensive mitochondrial DNA diversity in the Namaqua rock mouse, *Micaelamys namaquensis* (Rodentia: Muridae) from southern Africa—evidence for a species complex. *B.M.C. Evol. Biol.* 10: 307.

Ryder, O.A. 1986. Species conservation and systematics: The dilemma of subspecies. *Trends Ecol. Evol.* 1: 9–10.

Rymer, P.D., Manning, J.C., Goldblatt, P., Powell, M.P. & Savolainen, V. 2010. Evidence of recent and continuous speciation in a biodiversity hotspot: A population genetic approach in southern African gladioli (*Gladiolus*; Iridaceae) *Molec. Ecol.* 19: 4765–4782.

Sang, T., Crawford, D.J. & Stuessy, T.F. 1997. Chloroplast DNA

phylogeny, reticulate evolution and biogeography of *Paeonia* (Paeoniaceae). *Amer. J. Bot.* 84: 1120–1136.

Schulte, K., Silvestro, D., Kiehlmann, E., Vesely, S., Novoa, P. & Zizka, G. 2010. Detection of recent hybridization between sympatric Chilean *Puya* species (Bromeliaceae) using AFLP markers and reconstruction of complex relationships. *Molec. Phylog. Evol.* 57: 1105–1119.

Scopece, G., Cozzolini, S. & Bateman, R.M. 2010. Just what is a genus? *Taxon* 59: 1754–1764.

Scott, C.L. 1985. *The genus Haworthia: A taxonomic revision*. Johannesberg: Aloe Books.

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Lui, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E. & Small, R.L. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Amer. J. Bot.* 92: 142–166.

Shaw, J. & Small, R.L. 2005. Chloroplast DNA phylogeny and phylogeography of the North American plums (*Prunus* subgenus *Prunus* Section *Prunocerasus*, Rosaceae). *Amer. J. Bot.* 92: 2011–2030.

Sikes, D.S. & Lewis, P.O. 2001. PAUPRat, version 1: PAUP* implementation of the parsimony ratchet. Distributed by the authors. http://users.iab.uaf.edu/~derek_sikes/software2.htm.

Small, R.L., Cronn, R.C. & Wendel, J.F. 2004. Use of nuclear genes for phylogeny reconstruction in plants. *Austral. Syst. Bot.* 17: 145–170.

Snijman, D.A. & Meerow, A.W. 2010. Floral and macroecological evolution within *Cyrtanthus* (Amaryllidaceae): Inferences from combined analyses of plastid *ndhF* and nrDNA ITS sequences. *S. African J. Bot.* 76: 217–238.

Swofford, D.L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sunderland, Massachusetts: Sinauer.

Syring, J., Farrell, K., Businskỳ, R., Cronn, R. & Liston, A. 2007. Widespread genealogical nonmonophyly in species of *Pinus* subgenus *Strobus*. *Syst. Biol.* 56: 163–181.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.

Tolley, K.A., Chase, B.M. & Forest, F. 2008. Speciation and radiations track climate transitions since the Miocene climatic optimum: A case study of southern African chameleons. *J. Biogeogr.* 35: 1402–1414.

Treutlein, J., Smith, G.F., Van Wyk, B-E. & Wink, M. 2003a. Phylogenetic relationships in Asphodelaceae (subfamily Alooideae) inferred from chloroplast DNA sequences (*rbcL*, *matK*) and from genomic fingerprinting (ISSR). *Taxon* 52: 193–207.

Treutlein, J., Smith, G.F., Van Wyk, B-E. & Wink, M. 2003b. Evidence for the polyphyly of *Haworthia* (Asphodelaceae subfamily Alooideae: Asparagales) inferred from nucleotide sequences of *rbcL*, *matK*, ITS1 and genomic fingerprinting with ISSR-PCR. *Pl. Biol.* 5: 513–521.

Valcárcel, V. & Vargas, P. 2010. Quantitative morphology and species delimitation under the general lineage concept: Optimization for *Hedera* (Araliaceae). *Amer. J. Bot.* 97: 1555–1573.

Van der Niet, T., Johnson, S.D. & Linder, H.P. 2006. Macroevolutionary data suggests a role for reinforcement in pollination system shifts. *Evolution* 60: 1596–1601.

Van Welzen, P.C. 1998. Phylogenetic versus Linnaean taxonomy, the continuing story. *Taxon* 47: 413–423.

Van Wyk, A.E. 2007. The end justifies the means. *Taxon* 56: 645–648.

Van Wyk, A.E. & Smith, G.F. 2001. *Regions of floristic endemism in southern Africa: A review with emphasis on succulents*. Pretoria: Umdaus Press.

Verboom, G.A., Archibald, J.K., Bakker, F.T., Bellstedt, D.U., Conrad, F., Dreyer, L.L., Forest, F., Galley, C., Goldblatt, P., Henning, J.F., Mummenhoff, K., Linder, H.P., Muasya, A.M., Oberlander, K.C., Savolainen, V., Snijman, D.A., Van der Niet, T. & Nowell, T.L. 2009. Origin and diversification of the Greater Cape flora: Ancient species repository, hot-bed of recent radiation, or both? *Molec. Phylog. Evol.* 51: 44–53.

Victor, J.E. & Dold, A.P. 2003. Threatened plants of the Albany Centre of Floristic Endemism, South Africa. *S. African J. Sci.* 99: 437–446.

Wang, A., Yang, M. & Liu, J. 2005. Molecular phylogeny, recent radiation and evolution of gross morphology of the Rhubarb genus *Rheum* (Polygonaceae) inferred from chloroplast DNA *trnL-F* sequences. *Ann. Bot.* 96: 489–498.

Weiss-Schneeweiss, H., Tremetsberger, K., Schneeweiss, G.M., Parker, J.S. & Stuessy T.F. 2008. Karyotype diversification and evolution in diploid and polyploid South American *Hypochaeris* (Asteraceae) inferred from rDNA localization and genetic fingerprint data. *Ann. Bot.* 101: 909–918.

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–324 in: Innis, M.A., Gelfand, D.H., Sninsky. J.J. & White, T.J. (eds.), *PCR protocols: A guide to methods and applications*. San Diego: Academic Press.

Witkowski, E.T.F. & Liston, R.J. 1997. Population structure, habitat profile and regeneration of *Haworthia koelmaniorum*, a vulnerable dwarf succulent, endemic to Mpumalanga, South Africa. *S. African J. Bot.* 63: 363–370.

Yi, T., Wen, J., Golan-Goldhirsh, A. & Parfitt, D.E. 2008. Phylogenetics and reticulate evolution in *Pistacia* (Anacardiaceae). *Amer. J. Bot.* 95: 241–251.

Zander, R.H. 2008. Evolutionary inferences from non-monophyly on molecular trees. *Taxon* 57: 1182–1188.

Appendix. Voucher information and GenBank accession numbers included in this study.

Taxa, authorities, collector plus number (herbarium acronym), and GenBank accession numbers (*trnL-F*, *psbA-trnH*, ITS1). Abbreviations used in voucher information: *C&P*, *Clark & Pienaar*; *EA*, *E. Aslander*; *GM*, *G. Marx*; *JDV*, *J.D. Venter*; *MBB*, *M.B. Bayer*; *sn* = unnumbered collections with no herbarium voucher; *SR*, = *S. Ramdhani*; *TD*, *T. Dold*.

Outgroup: ***Kniphofia stricta*** Codd, *SR279* (GRA), HQ646848, HQ646907, HQ646954**. Ingroups:** ***Aloe*: *Aloe africana*** Mill., *SR&TDS629/8* (–), HQ646845, HQ646904, HQ646951; ***Aloe bowiea*** Schult. & J.H Schult., *MBBsn* (–), HQ646842, HQ646901, HQ646948; ***Aloe dichotoma*** Masson, *SR19/9/08* (–), HQ646847, HQ646906, HQ646953; ***Aloe lineata*** (Aiton) Haw., *SR&TDS729/8* (–), HQ646846, HQ646905, HQ646952; ***Aloe speciosa*** Baker, *SR&TDS329/8* (–), HQ646844, HQ646903, HQ646950; ***Aloe tenuior*** Haw., *SR&TDS229/8* (–), HQ646843, HQ646902, HQ646949. ***Astroloba*: *Astroloba bullata*** (Jacq.) Uitew., *MBB6756* (KBG), HQ646838, HQ646897, HQ646944; *SR843* (GRA), HQ646839, HQ646898, HQ646945. ***Gasteria*: *Gasteria brachyphylla*** var. ***brachyphylla*** (Salm-Dyck) E. van Jaarsveld, *SR848* (GRA), HQ646841, HQ646900, HQ646947. ***Poellnitzia*: *Poellnitzia rubiflora*** Uitew., *MBB6829* (KBG), HQ646840, HQ646899, HQ646946. ***Haworthia*** subg. ***Haworthia*: *Haworthia angustifolia*** var. ***baylissii*** (C.L. Scott) M.B. Bayer, *MBB7128* (KBG), HQ646790, HQ646849, HQ646908; ***Haworthia aristata*** Haw., SR&MBBS128/6 (–), HQ646791, HQ646850, –; *MBBsn*Hop (–), HQ646792, HQ646851, HQ646909; ***Haworthia blackburniae*** var. ***derustensis*** M.B. Bayer, *JDV92/51* (–), HQ646793, HQ646852, HQ646910; ***Haworthia bolusii*** var. ***pringleii*** Baker, *SR&MBBS126/6* (–), HQ646794, HQ646853, –; ***Haworthia cooperi*** var. ***cooperi*** Baker, *SR&MBBS118/10* (–), HQ646795, HQ646854, –; *MBB6791* (KBG), HQ646802, HQ646861, HQ646916; *SR&MBBS119/10s2* (–), HQ646803, HQ646862, –; *SR&MBBS119/10s3* (–), HQ646804, HQ646863, –; *SR&MBBS119/10s4* (–), Kaboega, HQ646805, HQ646864, HQ646917; *SR&MBBS227/6* (–), HQ646796, HQ646855, HQ646911; ***Haworthia cooperi*** var. ***pilifera*** (Baker) M.B. Bayer, *SR&TDsn* (–), HQ646797, HQ646856,

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Appendix. Continued.

HQ646912**;** ***Haworthia cooperi*** var. ***viridis*** (Baker) M.B. Bayer, *SR&MBBS527/6* (–), HQ646798, HQ646857, –; *SR&MBBS319/10* (–), HQ646799, HQ646858, HQ646913; *SR&MBBS318/10* (–), HQ646800, HQ646859, HQ646914; *SR&MBBS418/10* (–), HQ646801, HQ646860, HQ646915; ***Haworthia cymbiformis* var. *incurvula*** (V. Poelln.) M.B. Bayer, *SR&TD719* (GRA), HQ646806, HQ646865, HQ646918; ***Haworthia cymbiformis*** var. ***obtusa*** (Haw.) Baker, *JDV93/45* (–), HQ646807, HQ646866, HQ646919; ***Haworthia maraisii*** V. Poelln., *MBB6875* (KBG), HQ646808, HQ646867, HQ646920; ***Haworthia marxii*** M.B. Bayer, *GM623* (–), HQ646809, HQ646868, HQ646921; ***Haworthia monticola*** var. ***asema*** M.B. Bayer, *JDV90/57* (–), HQ646810, HQ646869, HQ646922; ***Haworthia mucronata*** Haw. *SRS115/09/08* (–), HQ646811, HQ646870, –; *JDV97/128* (–), HQ646812, HQ646871, HQ646923; ***Haworthia mutica*** Haw., *MBB7032* (KBG), HQ646813, HQ646872, HQ646924; ***Haworthia parksiana*** V. Poelln., *MBBsn* (–), HQ646814, HQ646873, HQ646925; ***Haworthia pulchella*** var. ***pulchella*** M.B. Bayer, *MBBsn* (–), HQ646815, HQ646874, HQ646926; ***H. pulchella*** var. ***globifera*** M.B. Bayer, *MBB6762* (KBG), HQ646816, HQ646875, HQ646927; ***Haworthia reticulata*** Haw., *MBBsn* (–), HQ646817, HQ646876, HQ646928; ***Haworthia mirabilis*** var. ***triebneriana*** (V. Poelln.) M.B. Bayer**,** *MBB6983* (KBG), HQ646818, HQ646877, HQ646929; *MBB6985* (KBG), HQ646819, HQ646878, HQ646930; ***Haworthia*** var. ***semiviva*** (V. Poelln.) M. B. Bayer, *JDV90/89* (–), HQ646820, HQ646879, HQ646931. ***Haworthia*** subg. ***Hexangulares*****:** ***Haworthia attenuata*** Haw., *SR&TDsn*6 (–), HQ646821, HQ646880, HQ646932; *JDV1942* (–), HQ646822, HQ646881, HQ646933; ***Haworthia coarctata*** Haw., SR&TD*sn*3 (–), HQ646823, HQ646882, HQ646934; ***Haworthia fasciata*** (Willd.) Haw., *MBB6809* (–), HQ646824, HQ646883, HQ646935; ***Haworthia glauca*** Baker, *SR&MBBS12/76* (–), HQ646825, HQ646884, HQ646936; *MBB9695* (KBG), HQ646826, HQ646885, HQ646937; *SR30/06/08* (–), HQ646827, HQ646886, HQ646938; ***Haworthia nigra*** (Haw.) Baker, *SR&MBBS228/6* (–), HQ646828, HQ646887, –; ***Haworthia reinwardtii*** (Salm-Dyck) Haw., *MBBsn* (–), HQ646829, HQ646888, HQ646939; ***Haworthia sordida*** Haw., *SR&MBBS327/6* (–), HQ646830, HQ646889; –; ***Haworthia venosa*** (Lam.) Haw., *MBBsn* (–), HQ646832, HQ646891, HQ646940; ***Haworthia venosa*** subsp. ***tessellata*** (Haw.) M. B. Bayer, *C&P120* (GRA), HQ646831, HQ646890, –; ***Haworthia venosa*** var. ***woolleyi*** (V. Poelln.) M.B. Bayer, *MBBsn* (–), HQ646833, HQ646892, HQ646941. ***Haworthia*** subg. ***Robustipedunculares*****:** ***Haworthia kingiana*** V. Poelln., *EA1211* (–), HQ646834, HQ646893, –; ***Haworthia marginata*** (Lam.) Stearn, *MBB6952* (KBG), HQ646835, HQ646894, –; ***Haworthia minima*** (Ait.) Haw., *MBBsn* (–), HQ646836, HQ646895, HQ646942; ***Haworthia*** *pumila* (L.) M.B. Bayer, *MBB7096* (KBG), HQ646837, HQ646896, HQ646943.