Molecular phylogeny of the ‘Cape snow’ genus Syncarpha (Asteraceae: Gnaphalieae) reveals a need for generic re-delimitation

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A B S T R A C T
A phylogenetic hypothesis is presented for the charismatic but taxonomically poorly-known Cape daisy genus Syncarpha, based on near-complete ingroup sampling and good coverage of outgroup taxa. A combination of nuclear ribosomal and chloroplast spacer DNA sequence data gives a well-resolved phylogenetic hypothesis, the robustness of which is assessed via both parsimony bootstrap and Bayesian posterior probabilities based on the uncorrelated lognormal relaxed clock model. Syncarpha species fall into two well-supported and distantly-related clades that last shared a common ancestor in the mid-Miocene. The larger Syncarpha 1 grouping contains the type species and corresponds to African ‘Helipterum’; this clade is sister to Edmondia and belongs in a larger clade which also includes the Australian Gnaphalieae. The Syncarpha 2 clade contains the taxa associated with Syncarpha paniculata (formerly Helichrysum paniculatum) and is more closely related to Plecostachys and some species of Gnaphalium. Formal assessment of monophyly lays the groundwork for future revisionary taxonomic work.

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1. Introduction

The genus Syncarpha DC. (Asteraceae: Gnaphalieae) comprises 28 species as currently circumscribed (Nordenstam, 1989, 2003; Manning and Goldblatt, 2012). All species are sub-shrubs with homogamous flowerheads surrounded by conspicuous coloured involucral bracts, and almost all Syncarpha species are endemic to the Cape Floristic Region of South Africa (CFR; sensu Goldblatt, 1978) and confined to fynbos vegetation. Despite being a charismatic and conspicuous component of the Cape flora, the systematic genus remains poorly explored. Consequently, Syncarpha has recently been identified as requiring revision (Von Staden et al., 2013), the last comprehensive treatment being that of Harvey in Flora Capensis (Harvey and Sonder, 1894). Originally described in the Linnaean genera Xeranthemum, Staelhelina, Helichrysum or Gnaphalium (Linnaeus, 1760, 1763, 1767), many Syncarpha species were transferred by De Candolle (1838) to the genus Helipterum DC., which comprised both South African and Australian taxa and was distinguished from Helichrysum by its plumose pappus bristles, fused at the base into a smooth ring (Hilliard and Burtt, 1981). The name Helipterum is illegitimate (Nordenstam, 1989) and so all the South African members of the genus were transferred by Nordenstam (1989, 2003) to the genus Syncarpha DC. Nordenstam (1989, 2003) also transferred some Helichrysum and Gnaphalium species to Syncarpha (based on characters presented in Hilliard and Burtt, 1981), but did not provide a description of the genus. The morphological characters defining the genus have nowhere been systematically investigated, although Hilliard and Burtt (1981) discussed character variation in Syncarpha and related genera. Syncarpha species are all subshrubs with fealty leaves possessing flat margins; the involucral bracts comprise a basal, undivided stereome (although S. argyropsis is exceptional in having a fenestrated stereome) and a lanceolate, papery lamina; the capitula are homogamous and the receptacles fimbriiferous. Cypsela hairs in Syncarpha are duplex and either globose or very depressed-globose, while the pappus consists of bristles that vary from smooth through barbellate to plumose and are fused basally into a smooth ring (Hilliard and Burtt, 1981). Apart from the brief description in

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S. dregeana
S. eximia
Anderberg (1991), there is to date no examination of species concepts

colour varies from pale yellow (A, C, D, E) to dark pink or brown (H, I, J). A,
recurved (E, F, G). Involucral bracts vary in colour, pattern, shape and orientation. Flower
vary from small (J) to long and narrow (C) to very broad (B), being held erect (B, C) or
(B), low sub-shrubs (A, C, D, F, G, H and I) and low, creeping cushion-plants (E). Leaves
morphological variation, and the genus includes relatively tall, sparsely-branched shrubs
several new species (Nordenstam, 1989, 2003), and by the red-listing
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ry of geographic distributions. The need for a comprehensive and mod-
in light of all available specimens, no key to the species, and no summa-

S. dykei
S. marlothii
S. sordescens

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Fig. 1. Syncarpha (as currently circumscribed) includes species with a wide range of
morphological variation, and the genus includes relatively tall, sparsely-branched shrubs
(B), low sub-shrubs (A, C, D, F, G, H and I) and low, creeping cushion-plants (E). Leaves vary
from small (J) to long and narrow (C) to very broad (B), being held erect (B, C) or
recurred (E, F, G). Involucral bracts vary in colour, pattern, shape and orientation. Flower
colour varies from pale yellow (A, C, D, E) to dark pink or brown (H, I, J). A, S. paniculata; B,
S. eximia (past flowering); C, S. gnaphaloides; D, S. ferruginea; E, S. sordescens; F, S. recurvata;
G, S. dregense; H, S. variegatum; I, S. vestita (‘Cape snow’); J, S. canescens. Photo credits: C, G:
Richard Adcock; A: Tony Rebelo; B, F, J: N. Bergh; D: Nicola van Berkel; E: Tony Verboom;
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Anderberg (1991), there is to date no examination of species concepts
in light of all available specimens, no key to the species, and no summary
of geographic distributions. The need for a comprehensive and modern
revision of the genus is underlined by the recent description of
several new species (Nordenstam, 1989, 2003), and by the red-listing
of several species, including S. aurea (threatened), S. chlororhylum
(near threatened), S. dykei (rare), S. lepidopodium (vulnerable),
S. marlothii (rare), S. montana (rare), S. recurvata (endangered),
S. sordescens (vulnerable) and S. zeyheri (rare; http://redlist.sanbi.org/
index.php; accessed January 2015).

Although phylogenetic research and revisionary taxonomy are
sometimes perceived as distinct and competing (for funding) branches of
systematics (e.g. see Wortley et al., 2002), these disciplines are, in
truth, highly complementary. Both seek to resolve evolutionarily
meaningful entities (species or monophyletic higher taxa) via a process
of hypothesis testing, and both have important roles to play in the description
of biodiversity (Wheeler, 2004). In fact, it is widely accepted in the
scientific community that phylogenetic results are indispensable in any
modern taxonomic treatment, and in a phylogenetic system of classification,
a formal assessment of monophyly constitutes an essential foundation
for revisionary work on a particular taxon.

As the basis of a revision currently in preparation, therefore, we generate
a phylogenetic hypothesis for Syncarpha with the principal aim of
evaluating its monophyly. Although Syncarpha has been included in a
number of studies exploring phylogenetic relationships within the
tribe Gnaphalieae (Bayer et al., 2000; Bergh and Linder, 2009;
Galbany-Casals et al., 2010, 2014; Bergh et al., 2011) these studies
have consistently each sampled only a single species of Syncarpha.
Thus, while it is now evident that Syncarpha is nested within the
“crown radiation” of Gnaphalieae (Ward et al., 2009), it remains
unknown whether the genus is monophyletic and, therefore, best treated
in a single revision. We address this gap, by presenting a well-
sampled phylogenetic hypothesis including all but two species of Syncarpha,
along with representatives of a large range of related genera.
Although our main phylogenetic output is a time-calibrated tree generated
in BEAST (Drummond and Rambaut, 2007) demonstrating the
times of divergence of clades of interest, it is not our goal to here discuss
the evolutionary history of the group in any depth. Also, while it is our intention to provide a solid foundation for revisionary work, it is not
our intention at this stage to implement formal taxonomic or nomenclatural
changes.

2. Materials and methods

2.1. Taxon sampling

Our analyses included most species currently accepted in Syncarpha
and outgroup sequences from a range of genera representing all major
lineages of Gnaphalieae, but especially the “crown radiation” clade of
Ward et al. (2009) (Table 1). The bulk of the sequences used were
generated de novo, either from field-sampled leaf material (vouchers
deposited in the Compton Herbarium, NBG or the National Herbarium,
PRE) or from herbarium specimen material, though some outgroup
sequences were taken from GenBank. Except for the widespread taxa
S. canescens (two accessions), S. paniculata (four accessions) and
S. staehelina (two accessions), species were represented by single
accessions.

2.2. DNA isolation, amplification and sequencing

Silica-dried leaf samples (about 15 mg) were ground using a mixer
mill (MM 400, Retsch, Haan, Germany) and total genomic DNA isolated
using a modified version of the CTAB protocol described by Doyle and
Doyle (1987). Extracted DNA was suspended in 100 μl Tris-EDTA buffer.
For herbarium material and problematic field-collected samples, DNA
was isolated using the DNeasy Plant Mini Kit (Qiagen GMBH, Hilden,
Germany).

For phylogenetic analysis, we sampled two nuclear and two chloro-
plast regions. The 3’ end of the external transcribed spacer (ETS) of
nuclear ribosomal DNA was amplified using the primers ETS-1F and
ETS-2R (Baldwin and Markos, 1998; Markos and Baldwin, 2001),
while the associated internal transcribed spacer (ITS) region (comprising
the ITS1 and ITS2 spacers and the intervening 5.8S ribosomal
gene) was amplified using the ITS4 and ITS5 primers of White et al.
(1990). The chloroplast trnL-intron and the trnL-trnf spacer were
amplified together using the ‘c’ and ‘f’ primers of Taberlet et al.
(1991), while the chloroplast trnT-trnL spacer was amplified using the
‘a’ and ‘b’ primers developed by the same authors.

Target regions were amplified using PCR, in reaction volumes of
25 μl. Each sample consisted of 2.5 μl of reaction buffer (Kapa
Biosystems Inc., Woburn, MA, USA), 0.5 μl DMSO, 1.25 μl of each primer
at 10 μM, 0.5 bovine serum albumin (BSA), 6.0 μl (trnL-trnF and trnT-
trnL) or 1.5 μl (ETS and ITS) of 25 μM MgCl2, 0.3 μl (trnL-trnF and trnT-
trnL) or 0.2 μl (ETS and ITS) Taq polymerase (Kapa Biosystems Inc.,
Woburn MA, USA), 1.2 μl (trnL-trnF and trnT-trnL) or 1.0 μl (ETS and
ITS) dNTP at 10 μM, and 9.5 μl (trnL-trnF and trnT-trnL) or 8.3 μl (ETS)
or 10.3 μl (ITS) nuclease-free water. Two microlitres (trnL-trnF and
trnT-trnL) or 7 μl (ETS) or 6 μl (ITS) of template DNA (at a dilution of
either 10⁻² or 10⁻³ relative to the raw isolate) was added to each
reaction mixture.
Amplification was performed using an Applied Biosystems 2720 thermal cycler (Foster City, CA, USA). The same thermal profile was applied to the two chloroplast loci, but different profiles were used for each nuclear region. All profiles started with an initial denaturation step of 2 min at 94 °C. This was followed by 30 cycles (trnL/trnF, trnL/trnF, trnL/F, trnL/F, and trnF/trnL) or 35 cycles (ITS) consisting of: 30 s (trnL/trnF and trnF/trnL), 30 s (trnL/trnF, trnL/trnF, and trnF/trnL), and 30 s (trnL/trnF and trnF/trnL) for each thermal cycle.
Chromatograms were checked and the bi-directional sequences assembled using Geneious Pro v. 5.4.3 (Biomatters Ltd., Auckland, New Zealand). The consensus sequence was aligned manually using BioEdit Sequence Alignment Editor (v. 7.0.9.0, Hall, 1999). Stretches of sequence that could not be unambiguously aligned were excluded from phylogenetic analyses.

2.3. Phylogenetic analysis

Trees were rooted on R. pungens (representing the Relhania clade of Bergh and Linder, 2009), this clade being recovered as sister to all remaining Gnaphalieae taxa by several earlier workers: Bayer et al., 2000; Bergh and Linder, 2009; Ward et al., 2009; Montes-Moreno et al., 2010). To identify incongruence among loci, parsimony bootstrap analyses were conducted on each locus (plastid DNA, ITS, ITS2), treatment of the two chloroplast regions as a single locus being justified by a preliminary comparison. These analyses were conducted in PAUP version 4.0b10 (Swofford, 2003) using 300 non-parametric bootstrap replicates, heuristic tree searches starting with a simple taxon addition tree and TBR swapping, under a maxtrees setting of 500. Separate bootstrap consensus trees for each gene region or locus were compared visually, with conflict considered to be well-supported when both competing nodes had BS ≥ 70%.

Combined analysis of the four DNA regions was performed using BEAST v1.5.4 (Drummond and Rambaut, 2007), with nodal support values also being assessed using the parsimony bootstrap as described above. The BEAST input file was generated using BEAUtil v1.5.4 (Drummond and Rambaut, 2007), implementing a separate model structure for each region, the optimal model in each case being determined under the AIC in MrModeltest v.2.3 (Nylander, 2004). The GTR + I + Γ model was selected for ITS, the GTR + Γ model for ETS and trnL-trnF and the HKY + Γ model for trnT-trnL. Nodal divergence times were estimated in the context of a Yule tree prior, and a lognormal relaxed clock model (Drummond et al., 2006). Calibration was achieved by applying age priors to two nodes, the Gnaphalieae crown node (Fig. 3, node A) and the ‘rest of Gnaphalieae’ clade crown node (Fig. 3, node B). We used Bergh and Linder’s (2009) posterior age estimates (median ± 95% HPD) to date these nodes, imposing these as normally-distributed age priors in such a way that the prior means and 95% confidence intervals matched the corresponding calibration posteriors as closely as possible. Three BEAST runs were performed, each comprising 20,000,000 iterations. Tracer v1.5 (Drummond and Rambaut, 2007) was used to assess the outputs of these runs for convergence and to determine the proportion of samples to be discarded as burn-in. LogCombiner v1.5.4 (Drummond and Rambaut, 2007) was then used to combine the post burn-in trees and parameter estimates from the three runs. Finally, a maximum clade credibility (MCC) tree, with median node heights, was generated in TreeAnnotator v1.5.4 (available for download from the BEAST website: http://beast.bio.ed.ac.uk/).

3. Results

3.1. Sequence characteristics and parsimony analyses

The alignments and resulting trees are available from TreeBase at the following address: http://purl.org/phylo/treebase/phylows/study/TB2:S168477x?access-code=178d776a86900d6b1fe8dfebe99f973a&format=html. The ETS, ITS and combined chloroplast alignments used in analyses were 1311, 669 and 1470 base pairs long, containing 287, 173 and 97 parsimony informative characters, respectively. All three loci yielded 70% majority rule bootstrap consensus trees that were poorly resolved, containing large polytomies (Fig. 2). The available resolution, however, indicated no supported incongruence amongst the three gene trees (except for one localised example discussed below) and several congruent groupings. The plastid data, for example, recovers a clade (S1) containing 18 Syncarpha species, including the type S. gnaphaloides. This clade is also recovered in the ETS gene tree, although here three of the species fall outside of the clade, being instead unplaced on a backbone polytomy (Fig. 2). Another clade, comprising S. sordescens, S. striata, S. recurvata, S. chlorochrysum, S. mucronata and S. paniculata (S2) was consistently retrieved, as was a monophyletic Edmondia. Also, several sister pairs (e.g. S. flavia + S. ferruginea, S. gnaphaloides + S. argyropsis, and S. speciosissima + S. vestita) were retrieved in more than one tree. One instance of supported incongruence was noted, but this was confined to relationships within the S1 clade: where the chloroplast data retrieved a well-supported subclade (BS = 89%) comprising S. aurea, S. dregeana, S. ferruginea, S. flavia and S. variegata, the ETS data resolved S. dregeana as sister to S. dykel (BS = 71%) and S. variegata as sister to S. loganiiana + S. montana (BS = 97%), while S. aurea was placed in a well-supported polytomy with S. affinis, S. staelhelina and S. virgata (BS = 96%).

3.2. Combined analysis

The relatively localised nature of gene tree incongruence justified combined analysis of the four DNA regions. In contrast to the topologies yielded by the individual data partitions, the combined data yielded a tree (BEAST maximum clade credibility tree) that is generally well resolved and supported (Fig. 3). In this tree, a clade comprising the Stoebe, Ilflags and Metalasia lineages [hereafter termed the SIM clade; Bayesian posterior probability, PP = 1.00] is resolved as sister to a clade (PP = 1.00) corresponding to the ‘rest of Gnaphalieae’ clade resolved by Bergh and Linder (2009). Within the latter clade, a monophyletic group is recovered comprising Helichrysum, Anaphalis and Pseudognaphalium, as well as Galeomma aculus-cati (PP = 1.00; BS = 99%: this corresponds to the HAP clade sensu Smissen et al., 2011). Within this clade, Galeomma is sister to Helichrysum stoloniferum (PP = 1.00; BS = 97%). The HAP clade is placed sister (PP = 1.00) to a clade (PP = 0.98; BS = 81%) containing all species of Syncarpha plus the included species of Plecostachys, Gnaphalium, Leontopodium, Antennaria, Filago, Vellereophyton, Edmondia, Lasiopogon debilis, and the two representatives of the Australasian radiation, Helichrysum lanceolatum and Ozothamnus diosmifolius. Significantly, Syncarpha is not retrieved as monophyletic, its species segregating into two well-supported clades. The Syncarpha 1 clade (PP = 1.00; BS = 92%), contains the type species, S. gnaphaloides, and corresponds to the South African members of De Candolle’s (1838) genus Helipterum. The Syncarpha 2 clade contains species that were more recently transferred to Syncarpha from Helichrysum, by Nordenstam (1989). Syncarpha 1 is sister to Edmondia (PP = 1.00; BS = 84%), this pair being sister to the sampled Australasian taxa (PP = 1.00; BS = 82%). Sister to these (PP = 1.00, BS = 73%) is a clade comprising Vellereophyton dealbatum and Lasiopogon debilis (PP = 1.00; BS = 90%). The sampled representatives of the Filago-Leontopodium-Antennaria-Gamochaeta (FLAG) clade (sensu Galbany-Casals et al., 2010) are monophyletic in our analysis (PP = 1.00; BS = 82) and placed sister to the Vellereophyton-Lasiopogon + Australasian + Edmondia + Syncarpha 1 clade in the MCC tree, but with no support. The Syncarpha 2 clade (PP = 1.00; BS = 100%), comprising S. sordescens, S. striata, S. recurvata, S. chlorochrysum, S. mucronata and S. paniculata is sister to Gnaphalium austroafricanum (PP = 0.95; BS = 75%), the whole in turn being resolved as sister to Plecostachys (node C in Fig. 3; PP = 1.00; BS = 77%).
Divergence estimates indicate a substantial interval between the time at which the two Syncarpha clades last shared a common ancestor (13.2 [7.9, 19.5] Ma) and the times at which they differentiated from their sister clades (Syncarpha 1: 7.0 [3.8, 10.7] Ma; Syncarpha 2: 8.3 [4.3, 12.8] Ma). The radiation of both Syncarpha clades took place after the Miocene–Pliocene boundary, the crown nodes of these two clades are dated to 7.0 [3.8, 10.7] Ma (SYNCARPHA 1) and 3.7 [1.8, 6.2] Ma (SYNCARPHA 2). Notwithstanding the recent timing of their diversification events, relationships within both Syncarpha clades are fairly well resolved. Within Syncarpha 1, in both cases in which species are represented by two accessions (S. canescens and S. staehelina), the species are recovered as monophyletic, although with low support for the latter. Within Syncarpha 1, relationships are resolved according to the ETS version of the alternative topologies shown in Fig. 2, and this is probably due to the greater phylogenetic information content of the ETS relative to the chloroplast data. Nevertheless, the ETS topology corresponds better with morphology than the plastid one, for example grouping S. paniculata in the plastid tree. The ITS and combined trees also recover a clad of species with yellow bracts (the ‘citrine clade’). The ITS tree corroborates earlier phylogenetic studies on Gnaphalieae (Bergh and Linder, 2009; Ward et al., 2009) in retrieving a monophyletic ‘rest of Gnaphalieae’ clade (sensu Bergh and Linder, 2009) with good support. The ‘rest of Gnaphalieae’ corresponds with the more elegantly named ‘Gnaphalieae crown radiation’ of Ward et al. (2009), except that the latter authors included the Stoebe clade. Most earlier studies, including Ward et al. (2009) identified a topology where the Stoebe clade is sister to the ‘rest of Gnaphalieae’ clade, and the Metalasia and then the Iflaga clades are successively sister to these (Bayer et al., 2000; Bergh and Linder, 2009; Montes-Moreno et al., 2010 [although they did not include any members of the Iflaga clade]; Bengston et al., 2014). However, in all these studies the branch lengths subtending these nodes are short and in some cases support values are low, indicating that speciation events occurred in rapid succession and that the true branching order may be difficult to discern. Our analysis supports an alternative arrangement, also retrieved by Galbany-Casals et al. (2010; although they did not include any members of the Metalasia clade) and Bengston et al. (2014).
Our crown radiation is thus the same as the clade so named by Galbany-Casals et al., 2010, and comprises all of Gnaphalieae, excluding the Relhania clade and also excluding the SIM lineages. There are several morphological synapomorphies that support the hypothesis that the SIM clade shares a common ancestor. Chief amongst these is an unusual leaf morphology, in which the leaf margins are involute, curling over the adaxial leaf surface, which is also usually densely white-felted. Frequently, the leaves are twisted so that parts of this white-felted adaxial surface face downwards. Leaves are also frequently borne on fascicles, most often subtended by a longer leaf. Although a detailed study has not been performed on all species in the SIM clade, several members of the Stoebe and Metalasia clades also share an anomalous form of secondary thickening (Lachnospermum and Phaenocoma from the Metalasia clade; Elytropappus, Disparago, and Stoebe from the Stoebe clade; Adamson, 1934) that may represent an additional, and complex, synapomorphy.

Within the Gnaphalieae crown radiation we recover both the HAP and FLAG clades identified by previous authors (Smissen et al., 2011; Galbany-Casals et al., 2010). Despite sparse sampling of the HAP clade, our data corroborates that of Bergh et al. (2011) in indicating that Galeomma is a member of this well-defined and very large clade (Nie et al., 2012; Smissen et al., 2011; Galbany-Casals et al., 2014), a finding supported by the fact that Galeomma, like all members of the HAP clade, has a divided stereome on the involucral bracts (Hilliard and Burtt, 1981; Hilliard, 1983; Anderberg, 1991). All other major groupings of Gnaphalieae lack the divided stereome.

The southern African genus Lasiopogon was demonstrated to be non-monophyletic by Bergh et al. (2011), who found L. debilis to be...
supported as sister to *Gnaphalium declinatum*, nested within the crown radiation, while *L. glomerulatus*, *L. muscoïdes* and *L. micropoides* were placed outside of the crown radiation, findings corroborated in the placement of single species by Bayer et al. (2000) and Galbany-Casals et al. (2010). Full species sampling of *Lasiopogon*, and phylogenetic analysis incorporating a wide range of crown radiation taxa, is required to determine the generic affinities of species currently assigned to *Lasiopogon*.

4.2. Polyphyly of Syncarpha

Strong evidence for the polyphyly of *Syncarpha* indicates a need for a realignment of generic boundaries. Our data separate *Syncarpha* into two distinct, well supported clades, *Syncarpha 1* and *Syncarpha 2*, each of which is embedded within a broader clade containing representatives of genera previously thought to be distantly-related (Australasian taxa for *Syncarpha 1*; *Gnaphalium* and *Plecostachys* for *Syncarpha 2*). The two clades of *Syncarpha* were previously united (Hilliard and Burtt, 1981; Nordenstam, 1989) because they both contained species that are sub-shrubby with grey-felted leaves, have homogamous capitula borne singly or in paniculate synflorescences, and with the capitula surrounded by many series of shiny, white, yellow or pink involucral bracts. The involucral bracts in most species have stereomes which are undivided or only partially fenestrated, a character which was influential in segregating these taxa from *Helichrysum* (where most were originally described). A stronger uniting feature was the presence of a unique type of cypsela hair (Hilliard and Burtt, 1981) that is very large, remarkably myxogenous, two-celled (lacking a swelling cushion) and depressed-globose in shape.

The two clades were historically recognised under different genera, with *Syncarpha 1* species corresponding to the South African taxa of *De Candolle*’s (1838) *Helipterum*. *Helipterum* was segregated from *Helichrysum* on the basis of plumose pappus bristles (De Candolle, 1838), Hilliard and Burtt (1981) examined the morphology of several *Helichrysum* and ‘*Helipterum*’ species and concluded that the plumose pappus character does not hold for all *Helipterum* species. Instead, they considered *Helipterum* to be separated from *Helichrysum* due to the former having an undivided or only partially fenestrated stereome (with one exception, *H. argyropsis*). The finding by Hilliard and Burtt (1981) that the pappus bristles in South African *Helichrysum* vary from being smooth with apically clavate cells, to barbellate becoming apically plumose, to plumose throughout, resulted in an acknowledgement that *Helipterum* cannot be defined by plumose pappus bristles. All species in *Syncarpha 1* and *Syncarpha 2*, however, share the feature of pappus elements being fused basally into a smooth ring (Hilliard and Burtt, 1981).

*Syncarpha 2* taxa (the former ‘*Helichrysum paniculatum* group’) were all housed in *Helichrysum* at the time that *Helipterum* was erected, having non-plumose pappus elements. However, their pappus elements are fused at the base, they have non-fenestrated stereomes in the involucral bracts, and their cypsela hairs are flattened-globose without a swelling cushion. This led Hilliard and Burtt (1981) to (rightly) suggest that they are erroneously housed in *Helichrysum*, and to suggest that they were instead more closely related to the South African species of *Helipterum*. When Nordenstam (1989) transferred all the South African species housed in the illegitimate *Helipterum* to *Syncarpha*, he agreed with Hilliard and Burtt (1981) and combined the ‘*Helichrysum paniculatum*’ group under *Syncarpha*.

Differences between the two groups *Syncarpha 1* (the South African species formerly in *Helipterum*) and *Syncarpha 2* (the species termed the ‘*Helichrysum paniculatum*’ group by Hilliard and Burtt, 1981) are evident in the leaves (which are erect with flat margins and obtuse tips in *Syncarpha 1*, but often apically hooked, weakly to strongly involute, and with acute tips in *Syncarpha 2*); the pappus bristles (which are scabrid throughout in *Syncarpha 2*) and the anthers, which are apically caudicid or mucronate in *Syncarpha 2*.

Although there are morphological differences between the two clades of *Syncarpha*, each difference has an apparent exception and *S. argyropsis* from *Syncarpha 1*, for example, has a very similar appearance to a typical *Syncarpha 2* species (Fig. 1). Thus, homoplasy within each of the two clades, and across *Gnaphalieae* in general, serves to obscure true relationships. High levels of morphological homoplasy are well known across *Gnaphalieae* (e.g. Anderberg, 1991; Bayer et al., 2000), making generic circumscription extremely difficult on the basis of morphology alone.

4.3. *Syncarpha 1* clade

The *Syncarpha 1* clade + *Edmondia* is recovered as sister to two Australasian taxa, corroborating the study of Bergh and Linder (2009) which found, on the basis of a more sparsely-sampled *Syncarpha 1* + *Edmondia* (only one species of each) but a much denser sampling of Australasian taxa (24 species), the same sister relationship. This is an interesting result considering that the *Syncarpha 1* clade corresponds in its membership to the former genus *Helipterum* which originally comprised both Cape and Australian members. *Helipterum* was once the largest genus of Australian *Gnaphalieae*, but after its illegitimacy and polyphyly were recognised, almost all Australasian *Helipterum* species were transferred to other genera (Wilson, 1989a, 1989b, 1992a, 1992b, 1992c). Most were transferred to *Rhodanthe* Lindl. but the genera *Leucocorynium* (A.Cunn. ex DC.) Paul G.Wilson, *Hyalosperma* Steetz., *Gilberta* Turcz. and *Eryophyllum* Paul C.Wilson are also involved. The monophyly of the Australasian members of *Gnaphalieae* has never been examined with exhaustive sampling, and although several broadly-sampled analyses of the tribe appeared to recover monophyletic or near-monophyletic Australasian radiations (Bayer et al., 2002; Bergh and Linder, 2009; Ward et al., 2009; Smissen et al., 2011), all are based on sparse sampling and none has a majority of well-supported branches. The Australasian gnaphaloid floras is characterised by a large number of small or monotypic genera (Bayer et al., 2002), and by many genera of doubtful monophyly (Schmidt-Lebuhn and Constable, 2013) making interpretation of broadly-sampled phylogenetic studies more difficult. Nevertheless, a close relationship between members of *Syncarpha 1* and at least some Australasian taxa is clearly indicated by the results of the present, and other studies (e.g. Bergh and Linder, 2009). The Australian gnaphaloid flora is extremely diverse, harbouring ca. 475 species (Bayer et al., 2002), but preliminary phylogenetic analyses (N. Bergh, unpubl.) indicate that the clade *Syncarpha 1* + *Edmondia* remains monophyletic with increased sampling of Australasian taxa (representatives of the genera *Anaphaloides*, *Anemocarpa*, *Chrysoccephalum*, *Craspedia*, *Ewartia*, *Gilberta*, *Hyalosperma*, *Leucocorynium*, *Ozothamnus*, *Parantennaria*, *Pterygopappus*, *Pycnosorus*, *Raoulia*, *Rhodanthe* and *Stuartiana*). The morphological characters previously thought to unite the South African and Australasian species of *Helipterum* (De Candolle, 1838) are clearly homoplasious.

4.4. *Syncarpha 2* clade

*Gnaphalium* appears to be non-monophyletic, since Galbany-Casals et al. (2010) recovered *G. supinum* as a member of the FLAG clade, while Smissen et al. (2011) recovered the clade (*G. austroafricanum* + *G. uliginosum*) *Syncarpha mucronata* as a lineage separate from the FLAG clade. Although we did not include *G. uliginosum* in our analysis, the above studies indicate that this species is likely to group with *G. austroafricanum* and *Syncarpha 1* in the clade marked ‘C’ in Fig. 3, distant from the FLAG clade. *Gnaphalium uliginosum* is the type of the genus, and so the findings of Smissen et al. (2011) support the conclusion that our *Syncarpha 2* clade and Plecostychys *serrifolifolia* are members of a clade (‘C’ in Fig. 3) that also includes, according to taxa sampled so far, the type species of the genus *Gnaphalium* as well as mainly southern African members of the genus. Both
Plecostachys and the 13 southern African members of Gnaphalium are characterised by grey-felted leaves and involucral bracts with undivided stereoemes and white-tipped involucral bracts. The heads in these taxa are heterogamous (comprising both female and hermaphrodite florets) and the Syncarpha 2 clade is thus a lineage of taxa with homogamous capitula contained within this ‘true Gnaphalium’ clade. The southern African Gnaphalium species vary in the degree of basal fusion of the pappus bristles, but nearly half of the species have the bristles fused basally into a smooth ring, supporting their affinity with Syncarpha 2 species. This character, which was influential in the decision of Hilliard and Burtt (1981) and Nordenstam (1989) to sink the Helichrysum paniculatum group into Syncarpha, is here identified as being homoplous.

At present, there is still considerable uncertainty surrounding the relationships of the major lineages within Gnaphalaeae, especially with regards to the larger genera such as Gnaphalium, and this is likely be resolved only with additional sampling of both taxa and markers.

4.5. Morphological characters differentiating Syncarpha 1 and Syncarpha 2

Full identification of the characters that distinguish the two clades of Syncarpha will require detailed examination of morphological features, and good candidates include the leaf margins, pappus bristles, style apex, anther apical appendage and cypsela hairs (Hilliard and Burtt, 1981). This work will form part of a revision of the two clades, but several differences can be enumerated here, despite that fact that most characters exhibit some overlap between the two groups. Leaves in the Syncarpha 1 clade are generally held erect and are usually apically rounded or with a weakly mucronate tip; leaf margins are flat and the hairs on the apical margins are usually distinctly tinged a rust-brown colour. In contrast, leaves in the Syncarpha 2 clade are generally smaller and more rigid, often recurved, with the tips acute to acuminate and often hooked. The leaves in this clade are never characterised by rust-coloured hairs on the margins. In Syncarpha 1 taxa, only the midvein is apparent on the lower (abaxial) leaf surface, if any, while the leaves in Syncarpha 2 are characterised abaxially by three or more very distinct parallel veins originating from the base of the leaf (a feature shared with Plecostachys). The heads in Syncarpha 1 clade species are in many cases distinctly cylindrical in shape and usually at least 2.0 cm in diameter, although some taxa have heads only about 1.5–2.0 cm wide (S. gnaphaloides, S. argyropsis). The heads in Syncarpha 2 taxa are always fairly small (up to 1.5 but usually 1.0 cm or less in diameter) and frequently globose in outline, at least as wide as they are long, with a rounded apex. Syncarpha argyropsis, in Syncarpha 1, has rounded heads but they are generally larger (to about 2 cm) than in Syncarpha 2 taxa.

The pappus setae in both Syncarpha clades are fused at the base into a smooth ring. The two groups differ in that the pappus bristle shaft in Syncarpha 1 taxa is smooth, barbellate or plumose, with the setae towards the apex of the pappus bristle always becoming longer and ending in clavate hairs. In contrast, the pappus bristles in the Syncarpha 2 clade are uniformly scabrid or barbellate. Two species of Syncarpha for which we were unable to obtain DNA sequence data, S. argentea and S. virgata, can confidently be placed into one of the two clades based on morphological features and affinities with sampled species. Syncarpha argentea with small heads, a southern Cape coastal distribution (see Section 4.7, below) and recurved leaves is most probably a member of Syncarpha 2, while S. virgata most likely belongs in the Syncarpha 1 clade, where its yellow involucral bracts place it in the ‘citrine’ clade, probably close to S. staeheilina with which it has been previously synonymised.

4.6. Edmondia

Edmondia comprises three species, all included in our analysis, that form the sister clade to the Syncarpha 1 clade. Edmondia was erected by Cassini (1818), with type species Helichrysum sesamoides (L.) Willd. De Candolle (1838) included Edmondia as a section within the genus Helipterum, but it was later returned to Helichrysum by Harvey (Harvey and Sonder, 1894) where it remained until Hilliard and Burtt (1981) reinstated Edmondia on the basis of (mainly) its distinctive foliage. Our analysis supports the separation of Edmondia and the Syncarpha 2 clade, but the placement of Edmondia sister to Syncarpha 1 raises the question of merging the two genera. Hilliard and Burtt (1981) and Hilliard (1983) used morphological grounds to keep Edmondia separate from Helipterum; since the Syncarpha 1 clade corresponds closely with the concept of Helipterum used by these authors, we feel that the morphological arguments retain their weight and we support maintaining a separate Edmondia and Syncarpha 1. Edmondia species are distinguished from those in Syncarpha 1 by having leaves that are adaxially glabrous but abaxially involute and white-tomentose; by scaly peduncles; by stereomes that are at least partially fenestrated; and by heterogamous heads (although the last character is not present in all species).

4.7. Origin and diversification

Based on the calibration employed here, the radiation of the two Syncarpha clades are very recent compared with most other dated fynbos clades, but they are nested within a greater Cape-centred radiation: that of tribe Gnaphalaeae (Verboom et al., 2014). Gnaphalaeae is an unusual lineage of the Asteraceae in that it houses several radiations of fynbos-vegetation taxa, i.e. those endemic to the oligotrophic, quartzitic environments of the CFR. The recent ages of the Syncarpha radiations closely match the radiations of two other dated fynbos gnaphalioid lineages (Metalasia clade: Bengston et al., 2014; Stoebke clade: Bergh et al. in prep.) in being very recent. The parallels with Metalasia are particularly striking. Like the Metalasia A clade, which is also centred in the eastern CFR, the radiation of the Syncarpha 2 clade appears to have been initiated in the Late Pliocene (Syncarpha 2: 3.7 [1.8, 6.2] Ma; Metalasia A: 3.3 [1.3, 6.7] Ma). By contrast, the Syncarpha 1 clade more closely resembles the Metalasia B clade both in being more widespread, and in having its diversification initiated at the Miocene-Pliocene boundary (Syncarpha 1: 5.6 [3.0, 8.5] Ma; Metalasia B: 6.4 [2.4, 12.7] Ma). The Stoebke clade resembles the first pair of clades more closely in that the radiation of its core clade is more recent, dating to 3.21 [1.5, 5.04] Ma. The reasons for the recentness of these Cape gnaphalioid radiations, which contrasts with a general trend of older radiations in other fynbos vegetation lineages (Linder, 2005, 2008; Verboom et al., 2014), remains something of a mystery, but a possible explanation is parallel radiation into more recently-exposed and environmentally diverse lowland habitats, that were to a large extent produced by late-Miocene uplift and the resulting rejuvenation of erosion in the Cape lowlands (Cowling et al., 2009).

4.7. Conclusions

The two Syncarpha clades last shared a common ancestor 13.2 [7.9, 19.5] Ma, so their evolutionary independence is well-established. Under a phylogenetic system of classification, therefore, the case for treating these Syncarpha lineages as distinct genera is persuasive. Ecological and morphological data provide further support, with Syncarpha 1 species being associated mostly with mid- to high-elevation habitats (and the S. dregeanum–S. staeheilina subclade is generally associated with high-elevation habitats); typically underlain by Table Mountain Group quartzites, while Syncarpha 2 species occur predominantly at low elevations, often along the southern Cape coast, where they inhabit a variety of geological substrates (e.g. limestones, coastal dunes and alluvial gravels). Also, in contrast to Syncarpha 1 which is widespread across both the western and eastern CFR, Syncarpha 2 has its diversity concentrated in the eastern half of the region. Syncarpha 1 species are characterised by plumose to barbellate pappus bristles, flat leaf margins and rounded leaf tips, whereas Syncarpha 2 species have generally
scabrid pappus bristles, involute leaf margins and acute or acuminate, hooked leaf tips. Further morphological investigations are required to determine differences in characters that apparently unite the two clades (such as depressed-gloseus duplex achenial hairs).

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