Species-level phylogenetic analysis in the Relhania clade of "everlastings" and a new generic treatment of species previously assigned to *Macowania* and *Arrowsmithia* (Asteraceae: Gnaphalieae)

Joanne Bentley,[1,2] G. Anthony Verboom[1] & Nicola G. Bergh[1,3]

1 *The Bolus Herbarium, Department of Biological Sciences, H.W. Pearson Building, University of Cape Town, Rhodes Gift 7707, Cape Town, South Africa*
2 *Department of Molecular and Cellular Biology, University of Cape Town, Rhodes Gift 7707, Cape Town, South Africa*
3 *The Compton Herbarium, Kirstenbosch National Botanical Gardens, Private Bag X7, Newlands, Cape Town, 7735 South Africa*
Author for correspondence: *Joanne Bentley, bntjoanne@gmail.com*
ORCID GAV, http://orcid.org/0000-0002-1363-9781

DOI https://doi.org/10.12705/666.9

Abstract The "Relhania clade" is a relatively species-poor lineage that is sister to the remaining members of the paper daisy tribe Gnaphalieae. Although the monophyly of the Relhania clade is well established, previous molecular studies are based on fairly sparse sampling. Here, we explore clade membership and generic circumscription using plastid and nuclear DNA sequence data, and near-complete species-level sampling of all putative member genera. Two subclades are recovered within the Relhania clade: (i) clade I housing the genera *Alatoseta*, *Athrixia*, *Pentatrichia* and *Phagnalon*, all monophyletic as currently circumscribed; and (ii) clade II, containing the genera *Antithirixia*, *Comborhiza*, *Oedera*, *Oreoleyesera*, *Relhania* and *Rosenia*; the annual or otherwise "short-lived" genera *Leysera*, *Nestlera* and *Rhynchopsidium*; as well as a subclade in which the monospecific *Arrowsmithia* is embedded within *Macowania*. A new taxonomic treatment involving 12 new combinations is proposed for the two latter genera. Only demonstrably monophyletic and diagnosable genera are recognised, involving the erection of a novel monospecific genus, *Fluminaria*, to house *Macowania pinifolia*, and the transfer of the remaining members of *Macowania* to *Arrowsmithia*. Updated descriptions and keys are provided.

Keywords everlastings; Gnaphalieae; phylogeny; Relhania clade; taxonomy

Supplementary Material The Electronic Supplement (Figs. S1, S2) and DNA sequence alignment are available in the Supplementary Data section of the online version of this article (http://www.ingentaconnect.com/content/iapt/tax)

■ INTRODUCTION

Tribe Gnaphalieae Benth. ("paper daisies" or "everlastings") houses well over 2000 species and exhibits a near-global distribution, although it likely originated in southern Africa (Bergh & Linder, 2009; Nie & al., 2015). The independence of Gnaphalieae from other tribes in the large daisy subfamily Asteroideae, particularly Inuleae Cass., was for a long time obscured by high levels of homoplasy in morphological characters, particularly features of the leaves, involucral bracts, pappus, style and pollen (e.g., Merxmüller & al., 1977). It was only the advent of cladistic methodology that allowed researchers to separate tribe Gnaphalieae from tribe Inuleae (Anderberg, 1989) and not until the use of DNA sequence data it was discovered that Inuleae and Gnaphalieae are not even particularly closely related (Kim & Jansen, 1995; Eldenäs & al., 1998). Key to this confusion is the morphology of a few, difficult-to-place genera from Africa, the Mediterranean region and Australasia. Anderberg (1991) could not place these genera in his subtribal scheme for Gnaphalieae that he recognised based on parsimony analyses of 84 characters in over 140 taxa. Instead, he acknowledged an additional, problematic group of "basal genera" outside of what he termed "Gnaphalieae sensu stricto". Although these "basal genera" shared features with genera that he considered to be firmly supported as members of "Gnaphalieae sensu stricto", they often also shared features with genera belonging to other tribes. Subsequent molecular phylogenetic analyses (Bayer & al., 2000; Bergh & Linder, 2009; Montes-Moreno & al., 2010) have considerably changed our understanding of Gnaphalieae relationships, and demonstrated that most of Anderberg's (1991) subtribes are not monophyletic. These studies have, however, demonstrated the existence of a lineage that is sister to "Gnaphalieae s.str.", hereafter referred to as the core Gnaphalieae radiation, and houses most of the southern African and Mediterranean members of Anderberg's "basal genera" (Bayer & al., 2000; Bergh & Linder, 2009). The sister relationship between the rest of Gnaphalieae and this lineage, called the "Relhania clade" after *Relhania* L'Hér., conforms to the conceptual relationship implied by Anderberg's (1991) term "basal groups of Gnaphalieae". However, we now know that the Relhania clade also houses part of Anderberg's (1991) subtribe Relhaniinae, and that the Australasian members of the "basal

Received: 15 Nov 2016 | returned for (first) revision: 2 Jan 2017 | (last) revision received: 3 Aug 2017 | accepted: 15 Aug 2017 || publication date(s): online fast track, n/a; in print and online issues, 22 Dec 2017 ||

genera" are placed elsewhere in Gnaphalieae (Bayer & al., 2002; Nie & al., 2015). Also, seven of Anderberg's (1991) "basal genera" have since been shown to belong in various tribes other than Gnaphalieae: *Printzia* Cass. and *Denekia* Thunb. in Astereae; *Oxylaena* Benth. in Calenduleae; *Anisochaeta* DC., *Artemisopsis* S.Moore and *Philyrophyllum* O.Hoffm. in Athroismeae; and *Callilepis* DC. unplaced in any tribe (Bayer & Cross, 2002; Anderberg & al., 2005; Bentley & al., 2015; Bengston & al., 2017). The confusion stems from the fact that some members of the Relhania clade share morphological features with members of other tribes of Asteroideae. Examples of these traits include dentate leaf margins, involute leaves, radiate capitula, obtuse or acute disc-floret style-arm apices, and disc-floret stigmatic surfaces that are apically confluent. The core Gnaphalieae radiation lacks these features, and as a consequence has obscured our understanding.

Although phylogenetic studies have tended to include fairly sparse sampling of the Relhania clade, its monophyly has been demonstrated in several independent studies (Bergh & Linder, 2009; Montes-Moreno & al., 2010; Klaassen & al., 2009; Bentley & al., 2014; Nie & al., 2015). The Relhania clade was first identified as a clade on the basis of plastid DNA, with no bootstrap support but with a decay index of three, by Bayer & al. (2000), who referred to it as "Relhaniinae sensu stricto" and demonstrated the inclusion of eight genera (*Anisothrix* O.Hoffm., *Arrowsmithia* DC., *Leysera* L., *Oedera* L., *Pentatrichia* Klatt., *Relhania*, *Rhynchopsidium* DC., *Rosenia* Thunb.). Almost all of these genera are endemic to southern Africa, and in particular to the Greater Cape Floristic Region (GCFR: Born & al., 2007). Subsequently, Bergh & Linder (2009) recovered the same clade with high statistical support, using plastid and nuclear DNA, and coined the name "Relhania clade" to distinguish this group from Anderberg's (1991) Relhaniinae, which also included *Stoebe*, *Metalasia* and smaller related genera from the core Gnaphalieae radiation. In the same year, Ward & al. (2009) published a plastid DNA phylogeny that included representatives of five of the genera of the Relhania clade, further supporting the monophyly of the clade, and also demonstrating the inclusion of *Athrixia* Ker-Gawl., incorrectly placed in Bayer & al. (2000; R. Bayer, pers. comm.). Other studies have since expanded and clarified membership of, and generic circumscription within, the Relhania clade. Forest & al. (2007) recovered *Oreoleysera* K.Bremer in a clade with *Oedera* and *Relhania* based on plastid *rbcL* gene sequences. Montes-Moreno & al. (2010) used plastid DNA to demonstrate, with good support, the inclusion of the Northern-Hemisphere genera *Aliella* Qaiser & Lack and *Phagnalon* Cass. Later, using both nuclear and plastid DNA sequences, Bentley & al. (2014) included *Comborhiza* A.Anderb. & K.Bremer, while Nie & al. (2015) included the monospecific *Nestlera* Spreng. on the basis of nuclear ribosomal DNA.

Current knowledge sets the membership of the Relhania clade at 14 genera (*Anisothrix*, *Arrowsmithia*, *Athrixia*, *Comborhiza*, *Leysera*, *Oedera*, *Oreoleysera*, *Macowania*, *Nestlera*, *Pentatrichia*, *Phagnalon*, *Relhania*, *Rhynchopsidium*, *Rosenia*) and about 115 species, with distributions in southern Africa, the Afrotemperate zones of tropical Africa and Madagascar, and the greater Mediterranean region, with a few species extending eastwards to Asia. Despite its small size relative to the Gnaphalieae core radiation, the Relhania clade is morphologically extremely variable, housing species that have annual or perennial life-histories; leaf-margins that are entire or variously dentate and either involute, revolute or unrolled; capitula that are discoid, disciform or radiate and, if radiate, with ray-ligules yellow, pink or white; various types of trichomes on various plant parts; and a pappus of (mostly barbellate) bristles and/or variously connate scales, as well as a range of variation in many other characters (Fig. 1). No investigation of the evolution of the trait variation that caused so much taxonomic confusion exists in the context of a well-sampled species-level phylogenetic hypothesis of relationships within the Relhania clade. Here we use plastid and nuclear sequence data to infer the relationships amongst species and examine the monophyly of the genera as currently circumscribed, and use character reconstructions to examine the ancestral states and degree of evolutionary lability in several key taxonomic traits.

■ MATERIALS AND METHODS

Taxon sampling. — Outgroups to the Relhania clade were selected from both outside and inside Gnaphalieae. The sister clade to tribe Gnaphalieae is not known with certainty, but it is likely to comprise one or more of the tribes Anthemideae, Calenduleae or Astereae (Panero & Funk, 2008). These tribes, as well as the more distantly related Senecioneae, are each represented in our analysis by one or several species. Trees were rooted on *Senecio vulgaris* L., likely the most distant outgroup in our study (Panero & Funk, 2008). Outgroups from the core Gnaphalieae radiation include other southern African genera such as *Gamochaeta* Wedd., *Ifloga* Cass., *Lasiopogon* Cass., *Metalasia* R.Br., *Stoebe* L. and *Syncarpha* DC. Within the *Relhania* clade, we aimed to include all species of the southern African genera known or suspected to belong to the clade, represented, where possible, by two to four accessions from across their geographic range. Moreover, as the monophyly of Mediterranean-centred *Phagnalon* has been previously addressed (Montes-Moreno & al., 2010), only a subset of species, represented by just a single accession each, and including the type, were included. Of the two genera in Anderberg's (1991) "basal groups" that have never been sequenced (*Alatoseta* Compton, *Lepidostephium* Oliv.), we were able to obtain DNA sequences only for *Alatoseta tenuis* Compton. For the remaining genera, we were able to include representatives of almost all species, except for two species of *Athrixia* that occur outside of South Africa (*A. debilis* DC. from Madagascar, *A. nyassana* S.Moore from Tanzania), and three very rare South African species (*Oedera laevis* DC., *O. foveolata* K.Bremer [Anderb. & K.Bremer], *Relhania decussata* L'Hér.). Plant material was collected onto silica gel during field trips throughout South Africa. Where fresh leaf material could not be obtained, herbarium material was sampled with permission, or sequences were obtained from public-access databases.

DNA amplification and sequencing. — Total genomic DNA was isolated from silica-dried, field-sampled material using the modified CTAB extraction protocol of Gawel & Jarrett (1991), while the Qiagen DNeasy plant extraction-kit (Qiagen Sciences, Valencia, California, U.S.A.) was used for herbarium material. Two nuclear and two plastid regions with proven phylogenetic utility in Gnaphalieae were utilised (Bayer & al., 2002; Galbany-Casals & al., 2004; Bergh & Linder, 2009). The external transcribed spacer (ETS) of nuclear ribosomal DNA was amplified using the reverse primer 18S-ETS (Baldwin & Markos, 1998) and the forward primers AST-1 (Markos & Baldwin, 2001) and ETS1-F (Starr & al., 2003) while the

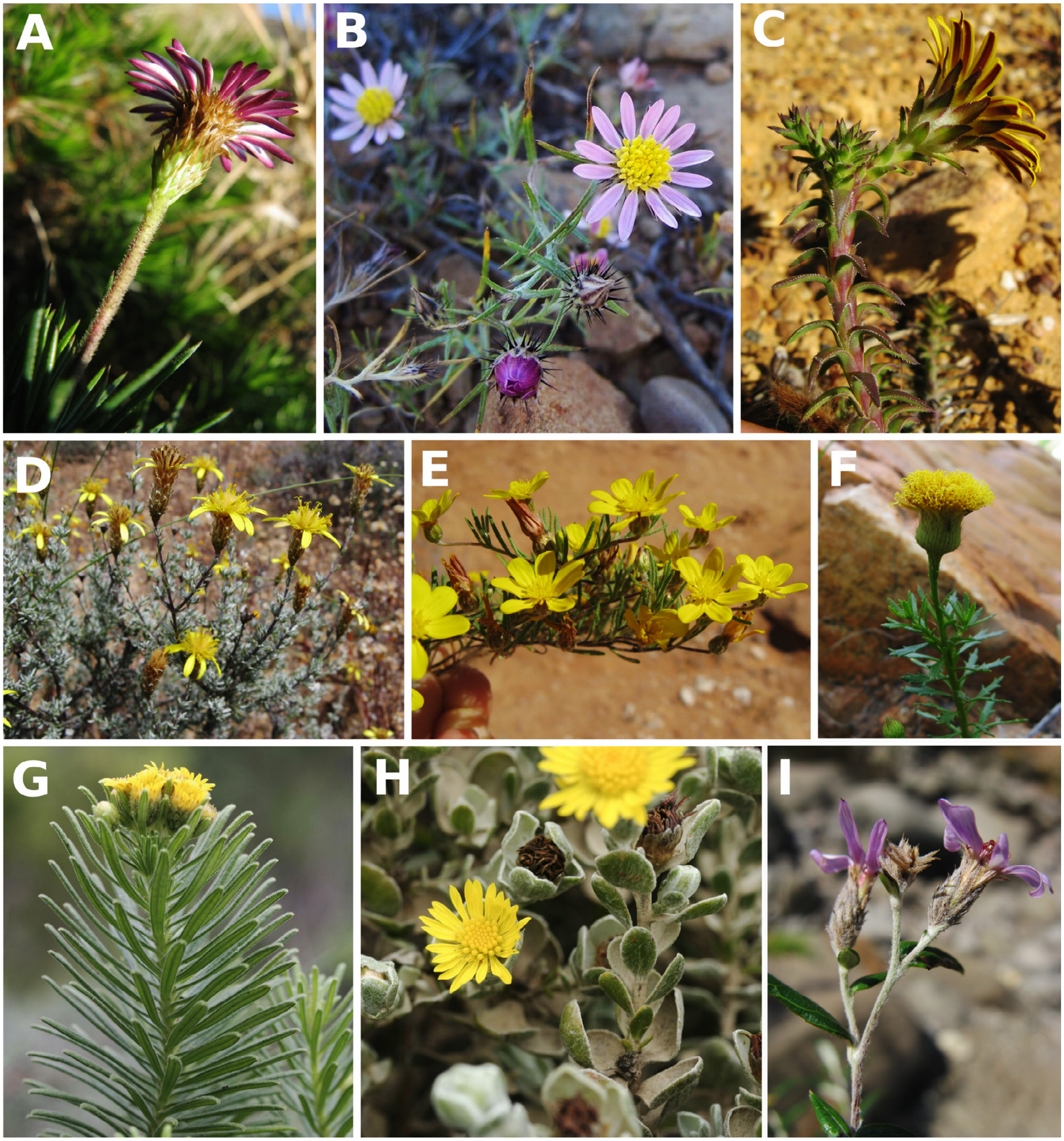


Fig. 1. A, *Macowania pinifolia* with needle-shaped leaves without revolute margins, and ray ligules that are white above and pink below; **B,** Narrowly linear leaves and pink-purple rays of *Alatoseta tenuis*; **C,** Synflorescence of *Oedera capensis* surrounded by a pseudo-involucre of leaves; **D,** *Antithrixia flavicoma* with glandular leaves borne in fascicles; **E,** The well-branched annual *Rhynchopsidium pumilim* with glandular-hispid stems, leaves and peduncles; **F,** Dentate leaves of *Pentatrichia kuntzei*; **G,** *Macowania corymbosa*: strongly revolute leaf margins with prominent abaxial midribs; **H,** Obtuse, involute leaves of *Oedera spathulifolia* showing white tomentum; **I,** Pink-purple rays and linear-lanceolate leaves of *Athrixia arachnoidea*. — All photos by N.G. Bergh except B, by V.R. Clark.

associated ITS1 and ITS2 introns and the intervening 5.8S ribosomal gene were amplified as a unit using the ITS5 and ITS4 primers of White & al. (1990). For the chloroplast genome, the *trnT-trnL* region was amplified using the primers "Tab A" and "Tab B" (also named "trna" and "trnb") of Taberlet & al. (1991). The entire *trnL-trnF* region, including the *trnL* intron and *trnL-F* spacer, was amplified with the "Tab C" and "Tab F" primers of Taberlet & al. (1991) and treated as a single region in the analyses.

PCR amplification was performed in an Applied Biosystems 2720 thermal cycler (Applied Biosystems, Carlsbad, California, U.S.A.) with the following thermal profile: initial denaturation of 2 min at 94°C; 35 cycles consisting of 94°C for 45 s, 52°C for 45 s (annealing) and 72°C for 2 min (extension); and a final extension step of 72°C for 8 min. Reaction mixtures consisted of 12.8 µl nuclease-free H_2O, 2.5 µl of 10× buffer (Kapa Biosystems, Wilmington, Massachusetts, U.S.A.), 1.5 µl of 25 µM $MgCl_2$, 1 µl dNTP mix at 0.2 µM each dNTP, 0.5 µl DMSO, 1.25 µl of each primer at 10 µM, 0.2 µl of *Taq* DNA polymerase (Kapa Biosystems) and 4 µl of template DNA at various dilutions. Successfully amplified target DNA was cleaned and sequenced either by Macrogen (http://www.macrogen.com/eng/sequencing/sequence_main.jsp) or by the Central Analytical Facility at Stellenbosch University (South Africa) using BigDye terminator cycling. The products were purified using ethanol precipitation and visualised on an ABI Automated Sequencer 3730XL (Life Technologies Corporation, Carlsbad, California, U.S.A.). Chromatograms were assembled, examined and corrected where necessary using Geneious Pro v.5.4.4 (Biomatters, Auckland, New Zealand) and manually aligned to minimise substitutions using BioEdit v.7.1.3.0 (Hall, 1999). In all analyses, indels were treated as missing data.

Assessment of congruence. — Phylogenetic congruence across independent gene trees was assessed using both parsimony bootstrap percentages (BS) and Bayesian inference posterior probabilities (PP). The parsimony bootstrap is considered a relatively conservative measure of phylogenetic support (Hillis & Bull, 2009) that is unlikely to retrieve spurious conflict. To check for topological incongruence, the four DNA regions (ETS, ITS, *trnL-F*, *trnT-L*) were first analysed individually and congruence evaluated using cutoff support values of 75% (BS) and 0.95 (PP). Since no supported conflict was found, the individual datasets were concatenated to form (i) a plastid (*trnL-F*+*trnT-L*) alignment, and (ii) a nuclear (ETS+ITS) alignment. These were again subjected to parsimony analysis and Bayesian inference, and topologies compared. Again, no supported conflict was found, and so a single concatenated alignment of both plastid and nuclear DNA was analysed as described below.

Parsimony bootstrap analysis. — PAUP v.4.0b10 (Swofford, 2002) was used to perform 1000 bootstrap replicates using only parsimony-informative sites. Each bootstrap replicate started with 100 random-addition sequences, and tree searching implemented tree bisection-reconnection (TBR) branch swapping with multiple trees saved per random-addition replicate, to a maximum of 500 trees per bootstrap replicate.

Bayesian inference of phylogeny. — MrBayes v.3.2.3 (Huelsenbeck & Ronquist, 2001) was used for Bayesian inference of phylogeny on the Cipres Science Gateway online platform (Miller & al., 2010). A mixed model approach was employed in which substitution model parameters were estimated separately for each of the four DNA data partitions. Optimal models were chosen under the Akaike information criterion (AIC; Akaike, 1973) using MrModeltest v.2.2 (Nylander, 2004) or jModelTest v.2 (Guindon & Gascuel, 2003; Darriba & al., 2012). The selected models were: GTR+G for ITS, GTR+I+G for ETS, and separate GTR+G models for each plastid region. Parameter estimation was achieved using a Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) sampling procedure (Gilks & al., 1996; Yang & Rannala, 1997) with analyses starting at different random trees and running for 10 million generations. Parameters were sampled every 1000 generations. In all analyses, the chain heating parameter was set at 0.3. Apart from the model settings, the default MrBayes settings were retained. Two concurrent runs were performed during each analysis, and each analysis was run twice, resulting in a total of four independent runs.

Examination of convergence. — Convergence of individual MCMCMC runs was assessed by examining the average standard deviation of split frequencies from the MrBayes output to determine when this value dropped below 0.01 (Ronquist & al., 2011). The tree topologies and posterior probability support values from the four independent runs were also compared to check that these converged on the same tree sample. Convergence was further tested using Tracer v.1.3 (Drummond & Rambaut, 2007) to examine parameter estimates, ESS scores and likelihood traces. After examining convergence, the first ca. 10% of samples from each run were discarded as burn-in, and the parameters were summarised on the 50% majority-rule consensus tree obtained from all runs using the Python implementation SumTrees v.3.3.1 (Sukumaran & Holder, 2010, 2015).

Ancestral trait reconstruction. — Ancestral traits were reconstructed on the combined data maximum clade credibility tree, which had been pruned to remove the non-Gnaphalieae outgroups as well as multiple accessions of ingroup species. Up to six discrete character states were reconstructed under likelihood with a one-parameter equal rates Markov model using the package "Phytools" (Revell, 2012) in the R v.3.3.1 environment (R Development Core Team, 2014). The reconstructed traits were selected to capture the variability across both the Relhania clade and the core Gnaphalieae radiation, and included (i) ray floret colour (white only; yellow only; pink or polymorphic for pink/white; polymorphic for yellow/white; white above and mauve below; ray florets not present), (ii) leaf tomentum (adaxially tomentose with different tomentum abaxially; abaxially tomentose with different tomentum adaxially; abaxial and adaxial surfaces not differentiated by hairiness), (iii) leaf margin (entire; incised/toothed), (iv) disc floret style branch stigmatic lines (apically confluent; apically separated), (v) disc floret style branch apices (acute-acuminate; obtuse; truncated; polymorphic for obtuse and truncated) and (vi) leaf margin (revolute [rolled towards the abaxial surface]; flat; involute [rolled towards the adaxial surface]).

■ RESULTS

Individual gene phylogenies. — The plastid *trnT-L* matrix consisted of 113 accessions and 569 aligned nucleotides, and 12% of the nucleotides of the ingroup taxa were parsimony informative (PI) as determined by PAUP. The plastid *trnL-F* matrix consisted of 77 accessions and 941 aligned nucleotides, of which 4% were PI in the ingroup. Independent analyses of the two separate plastid regions yielded poorly resolved but congruent trees, justifying their combination to form a matrix consisting of 144 accessions and 1,510 characters, of which 7% were PI in the ingroup (Electr. Suppl.: Fig. S1).

The nuclear ETS matrix consisted of 146 taxa and 1107 aligned sites, and the ITS matrix comprised 166 taxa and 708 aligned sites (38% PI characters in the ingroup for both matrices). The combined nuclear matrix consisted of 175 accessions and 1815 aligned nucleotides, of which 38% were PI in the ingroup. The nuclear gene tree is broadly congruent with the plastid tree but has a greater number of resolved and supported clades (Electr. Suppl.: Fig. S2).

The supported branches of the plastid and nuclear trees produced topologies that were completely congruent. Although lack of resolution in the plastid tree in particular might hide potential incongruence, the effect of this would generally be the reduction of support across conflicting nodes in a concatenated analysis, resulting in lowered confidence for the resulting clades. Given congruent topologies and lack of any supported conflict, we combined the nuclear and plastid regions for subsequent analyses.

Combined nuclear and plastid phylogeny. — The "*Relhania* generic group" of Anderberg & Bremer (1991) is recovered as monophyletic and is assessed in a parallel publication (Bergh & al., submitted). Therefore only a pruned subset of this group comprising single-species accessions is represented in the final combined region analysis (Fig. 2). The remaining sequences, represented by all accessions, are included in the analyses. The final matrix comprised 176 accessions and 3325 aligned nucleotides, of which 24% were PI for the ingroup. The combined plastid and nuclear DNA analysis (Fig. 2) recovers each of the outgroup tribes Anthemideae, Calenduleae and Astereae as monophyletic, but the sister group to Gnaphalieae cannot be elucidated. Tribe Gnaphalieae itself is monophyletic (BS = 99, PP = 1), as is the core Gnaphalieae radiation (BS = 89, PP = 1) and the Relhania clade (BS = 100, PP = 1).

Within the Relhania clade, 49 species are represented by more than one accession in the combined nuclear and plastid tree (Fig. 2), of which 38 were recovered as monophyletic with high clade support. The monophyly of the multiple accessions of a further six species is not contradicted, while there is no support for the monophyly of *Athrixia heterophylla* (Thunb.) Less., *A. rosmarinifolia* Oliv. & Hearn, *Leysera gnaphalodes* (L.) L., *Rosenia spinescens* DC. and *R. oppositifolia* (DC.) K.Bremer. The non-monophyly involves only one or a few closely related species, and is probably explicable with reference to incomplete lineage-sorting. There are two well-supported and reciprocally monophyletic sister groups in the Relhania clade. The clade comprising *Alatoseta*, *Athrixia*, *Pentatrichia* and *Phagnalon* we have termed clade I. Within this clade, one subclade ("B"; BS = 100, PP = 1) comprises the largely Mediterranean *Phagnalon* (monophyletic; BS = 97, PP = 1) and the southern African *Pentatrichia*. Unlike in the gene trees, *Pentatrichia* is here recovered as monophyletic, including *P. alata*, in the Bayesian analysis only (PP = 0.95). The other lineage within clade I recovered with Bayesian support ("A"; PP = 1) comprises the monospecific *Alatoseta* sister to a monophyletic *Athrixia* (BS = 100, PP = 1).

The other clade within the Relhania clade, clade II, comprises two smaller subclades, indicated by "C" and "D" in Fig. 2. The Drakensberg-centred representatives of *Macowania* and *Arrowsmithia*, excluding the East African members and *M. pinifolia*, are supported as a clade ("E"; BS = 100, PP = 1), as previously discussed by Bentley & al. (2014). While a sister relationship between *M. pinifolia* and the rest of *Macowania* and *Arrowsmithia* (clade C) is supported by the nuclear data (Electr. Suppl.: Fig. S2), this relationship is not supported in the combined analysis. Clade D (BS = 100, PP = 1) houses the remaining genera, most of which occur in the GCFR. This clade, which we term the "Cape clade", corresponds to the "*Relhania* generic group" of Anderberg & Bremer (1991). While several species within the Cape clade are united in well-supported clades, resolution is poor in this lineage.

Ancestral trait reconstruction. — The ancestor of the Relhania clade is likely to have had entire leaf margins (probability $P = 0.63$; Fig. 3A). The disc floret stigmatic apices are reconstructed to have been truncate, with apically separated stigmatic lines ($P = 0.63$; Fig. 3D), and the disc floret style branch apices are likely to have been truncated ($P = 0.85$; Fig. 3E). Highest probability lies with this ancestor having lacked ray florets ($P = 0.65$; Fig. 3F), but when present, they were most likely to have been yellow ($P = 0.15$). The reconstructed ancestral form for the degree of leaf tomentum on the adaxial and abaxial surfaces, as well as leaf margin rolling (flat/involute/revolute; Fig. 3B, C) is equivocal, with each option receiving more-or-less equal support.

■ DISCUSSION

Our analyses of plastid and nuclear data, extensively sampled at the species level, confirm the monophyly of the Relhania clade and its position as sister to the core Gnaphalieae radiation. Several previously unsampled species, as well as the monospecific genera *Alatoseta* and *Antithrixia* are here demonstrated to be members of the clade. On the basis of the present study, we consider the Relhania clade to be sufficiently well known and distinct to warrant formal recognition as a subtribe. However, such formal recognition may be premature given the lack of a subtribal classification for the rest of the Gnaphalieae, and so we do not establish a subtribe here. We note, however, that a subtribe corresponding to the Relhania clade would differ from subtribe Relhaniinae of Anderberg (1991), in that it would incorporate several genera placed by Anderberg (1991) in his "basal group of genera, unassigned to any subtribe" (*Alatoseta*, *Arrowsmithia*, *Athrixia*, *Macowania*, *Pentatrichia*, *Phagnalon*),

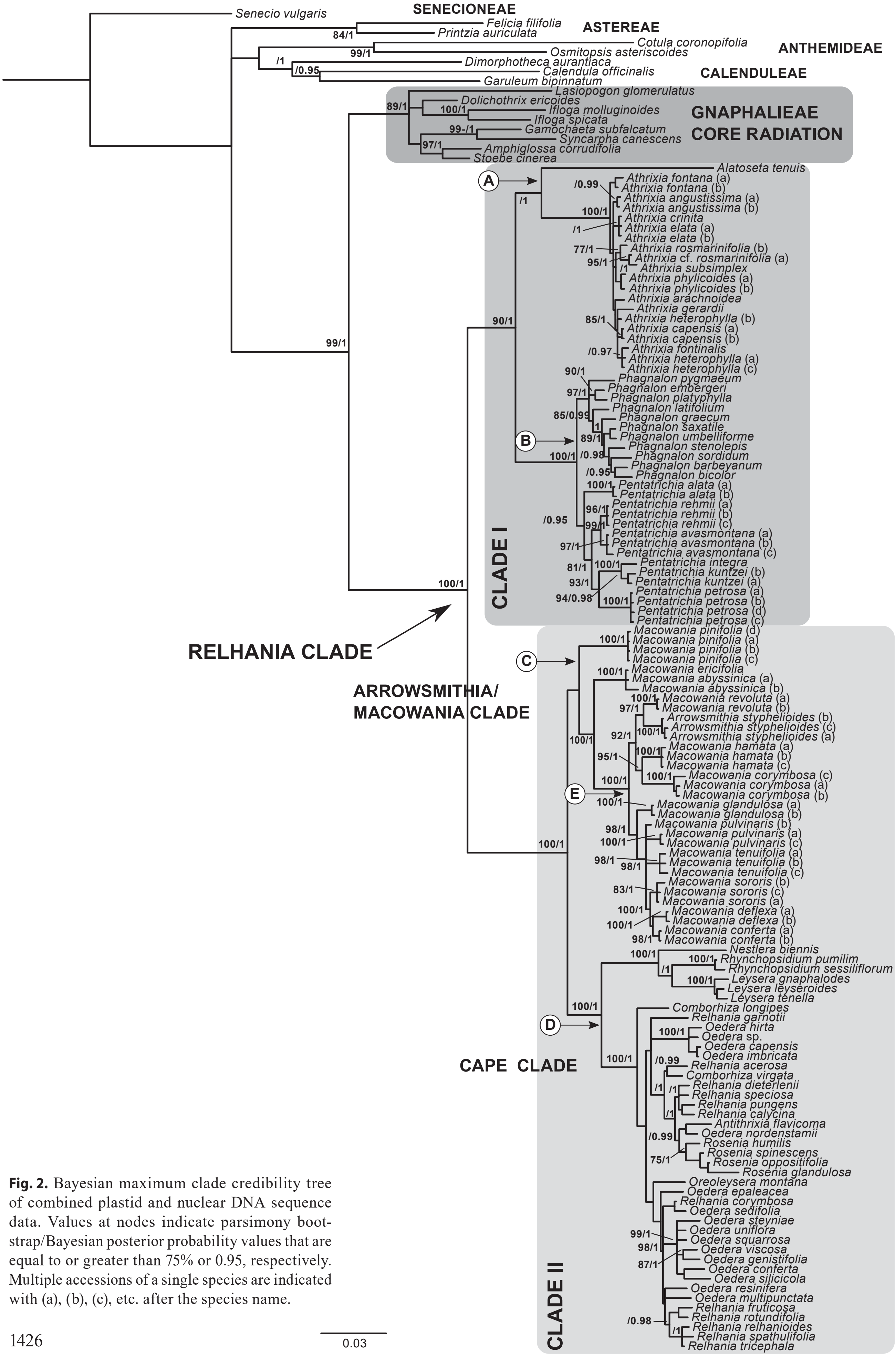


Fig. 2. Bayesian maximum clade credibility tree of combined plastid and nuclear DNA sequence data. Values at nodes indicate parsimony bootstrap/Bayesian posterior probability values that are equal to or greater than 75% or 0.95, respectively. Multiple accessions of a single species are indicated with (a), (b), (c), etc. after the species name.

while also excluding those genera which Anderberg treated as the "*Metalasia* group" of his subtribe Relhaniinae.

The Relhania clade comprises two reciprocally-monophyletic and morphologically distinct subclades, also recovered in previous, more sparsely sampled studies (Montes-Moreno & al., 2010; Bentley & al., 2014). Within clade I, all genera are monophyletic as currently circumscribed, but within clade II there is extensive generic non-monophyly.

Members of the Relhania clade possess several features that are anomalous within the context of the core Gnaphalieae radiation, and have caused much confusion as to the delimitation of the tribe (Merxmüller & al., 1977; Anderberg, 1991). Most of these features appear to arise on the branch leading to clade I, whereas clade II members share more traits that are in line with those prevalent in the core Gnaphalieae radiation (Fig. 3). One of the most confusing characters is the apical stylar morphology of the disc florets. In the core Gnaphalieae radiation, the typical disc floret style apex is of the "Senecioneae type" in which the style branches are generally truncate and penicillate, with the receptive stigmatic surfaces forming two marginal lines on the adaxial surface; these lines remain parallel and do not merge at the style apex. Although there are exceptions to this arrangement, particularly in the "FLAG clade" and in the large Australasian radiation, apically separate stigmatic surfaces are a general feature of the core Gnaphalieae radiation. In contrast, all members of clade I as well as *Arrowsmithia* and *Macowania* have obtuse to rounded (seldom acute) disc floret stylar arm apices. In clade I, the stigmatic surfaces are confluent at the tips of these stylar arms, but in *Macowania* they are separated, giving this genus a unique combination of stylar arm features. In *Phagnalon*, the stigmatic lines are either convergent or even fusing (Merxmüller & al., 1977), while in *Alatoseta* the style branches are even more unusual, having acuminate ends and unique apical appendages. Converging disc style stigmatic lines characterise the "*Inula*-type" style that these genera share with members of Inuleae. These genera were formerly placed in subtribe Athrixiinae of Inuleae by Merxmüller & al. (1977), due in some part to this apparent synapomorphy. Current evidence shows that Inuleae is only distantly related to Gnaphalieae, implying that this trait is homoplasious. The ancestral trait reconstruction (Fig. 3) supports separated stigmatic surfaces as the ancestral condition of Gnaphalieae. If this reconstruction is accurate, it suggests that the features that appear to unite members of the Relhania clade (and particularly members of clade I) with taxa from other tribes arose independently within this clade. Although our sampling of the Gnaphalieae core radiation is extremely sparse, some inferences about the character states of the ancestor of the tribe as a whole are nevertheless possible.

The incised leaf margins in clade I are distinctive in the context of Gnaphalieae where they are almost universally entire. Clade II has more typical gnaphalioid leaf margins, although there are a few species, including the type of *Oedera*, *O. capensis*, where the leaf margins bear tooth-like trichomes. The ancestral trait reconstruction is consistent with entire margins as a potential ancestral trait for the group, with a relatively high probability. Radiate capitula are also rare in the core Gnaphalieae radiation (with a few exceptions in *Disparago* Gaertn. [pink/white], *Asteridia* Lindl. [white/yellow] and *Podolepis* Labill. [yellow/pink]; Anderberg, 1991), but are prevalent in the Relhania clade and also occur in some members of *Pentatrichia* and *Phagnalon*. Where rays are present, the ligule colour almost exactly corresponds with membership of clade I (pink or white), or clade II (yellow). There is a single exception in *M. pinifolia*; the only member of clade II to have rays that are both pink (mauve) and white (Figs. 1A). The involucral bracts of clade II are not very different from many in the core Gnaphalieae radiation, being imbricate in several series, chaffy or cartilaginous, and with the apex obtuse to rounded-acute. The involucre in clade I is distinctive, being made up of a greater number of individual bracts in more series, and with the bract apices acute or attenuate into an acuminate, almost aristate and darker tip which gives a spiny, almost thistle-like appearance to the heads in *Alatoseta*, *Athrixia* and *Pentatrichia* (Fig. 1B, F, I).

It is unclear why some members of the Relhania clade have features that are so unusual relative to those of the core radiation. The traits discussed above are relatively uniform within the core Gnaphalieae radiation, but show much higher levels of variation, particularly in clade I, despite its smaller size.

Clade I

***Alatoseta.* —** Compton (1931) erected *Alatoseta* to house a single peculiar species, *A. tenuis*. This is the only known annual member of clade I, and also has several other unique features, such as winged pappus scales and the unusually acute apical appendage to the disc floret styles. Anderberg (1991) was unsure of the placement of *Alatoseta* in his "basal group of genera", but our analyses place it sister to *Athrixia*, which is supported by numerous morphological characters. Although all *Athrixia* species are perennial, both genera have pink ray florets, glandular, revolute leaves, and involucral bracts with dry scarious margins and a brown, long acuminate apex. An argument could be made for the inclusion of *Alatoseta* in *Athrixia*, but their reciprocal monophyly makes it straightforward to retain the existing taxonomy, which is supported by the unique morphological and life-history features in *Alatoseta*.

***Athrixia.* —** Although we were unable to include material of two of the 14 *Athrixia* species, the genus is well defined morphologically (Kroner, 1980) as well as phylogenetically, and we retain its current circumscription. It is unfortunate that we have no representative of *Lepidostephium* in our analysis, since Anderberg (1991) considered it likely that the two species of this genus would be nested within *Athrixia*. *Lepidostephium* comprises rosulate, perennial herbs from the summer-rainfall parts of South Africa, and in these features is similar to *Athrixia fontana*, which is resolved as sister to the rest of *Athrixia*, albeit without support.

***Pentatrichia.* —** The two species of the GCFR-endemic genus *Anisothrix* were previously thought to be closely related to *Pentatrichia* (Anderberg, 1991; Montes-Moreno & al., 2010). Subsequently, Klaassen & Bergh (2012) used nuclear and plastid DNA sequence data to show that *Anisothrix* is embedded within *Pentatrichia* and synonymised the former with the latter. The new circumscription of *Pentatrichia* incorporates five species from southern Africa. Klaassen & Bergh (2012) used as outgroups only three *Athrixia* species, obtaining high support for a monophyletic *Pentatrichia* with *P. alata* sister to the remaining species. In the current, more extensively sampled molecular analysis, the former *Anisothrix* species (*P. integra* (Compton) Klaassen & N.G.Bergh, *P. kuntzei* (O. Hoffm.) Klaassen & N.G.Bergh) are clearly nested within *Pentatrichia*. However, only the Bayesian analysis supports the inclusion of *P. alata* with the remaining species of *Pentatrichia*, though this is supported by multiple morphological characters that indicate a close relationship between *P. alata* and the rest of *Pentatrichia*. These include capitula that are either radiate with female-fertile rays and white ray laminas, or discoid; style-branch apices of both rays and discs that are obtuse to acute, with obtuse dorsal sweeping-hairs and the stigmatic surfaces basally separated but apically confluent. The pappus is also identical, comprising long bristle-like scales alternating with

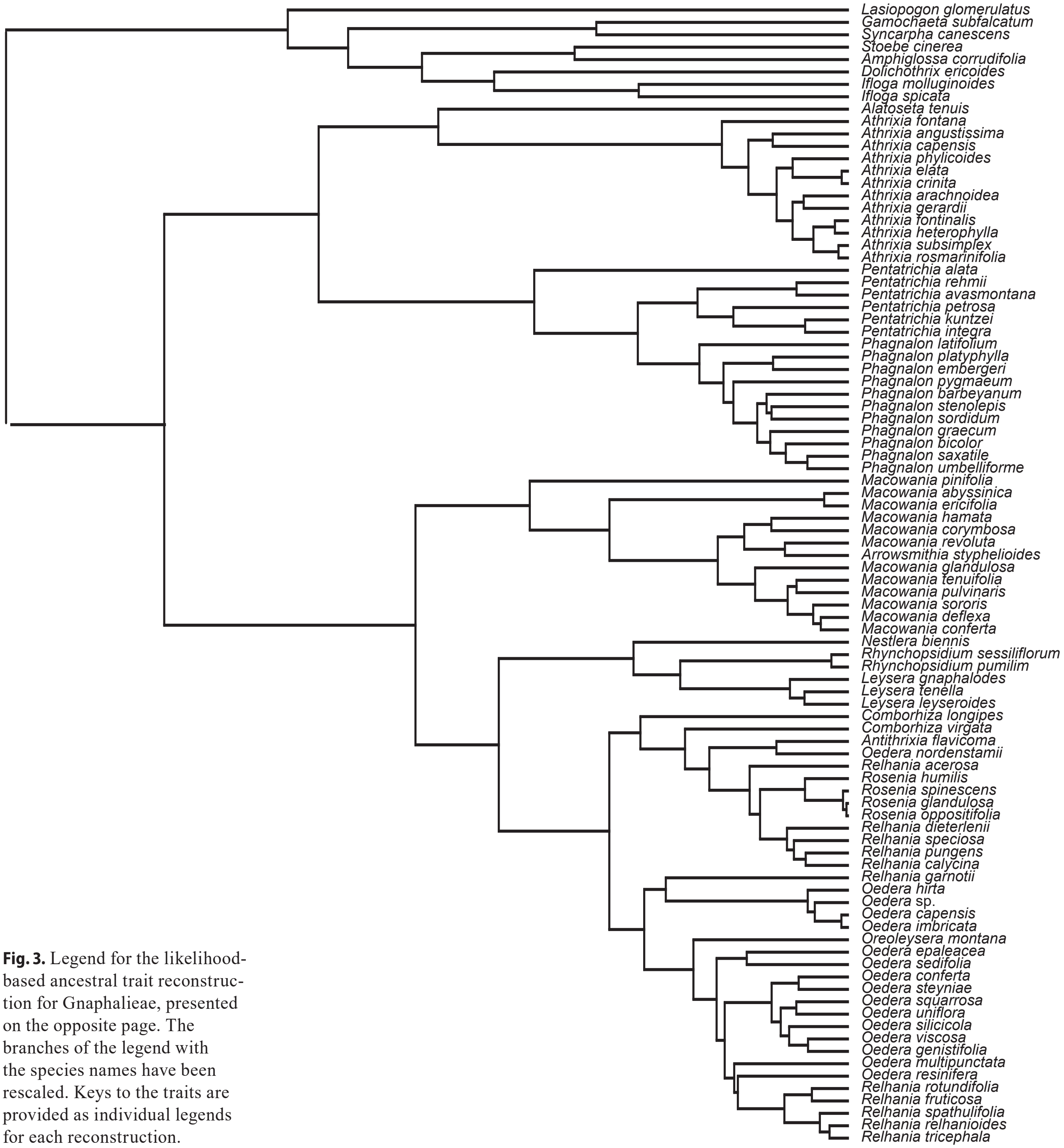
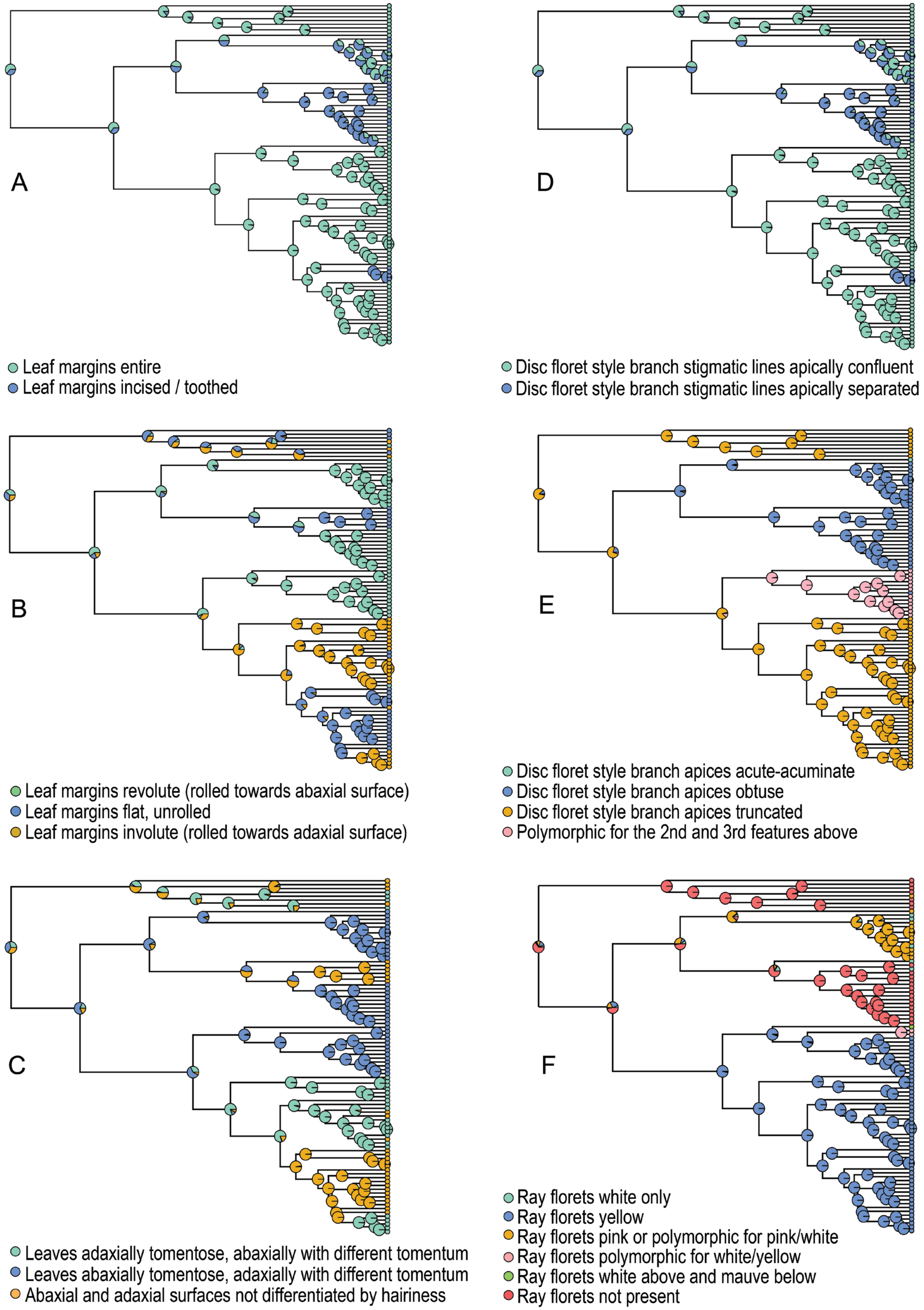


Fig. 3. Legend for the likelihood-based ancestral trait reconstruction for Gnaphalieae, presented on the opposite page. The branches of the legend with the species names have been rescaled. Keys to the traits are provided as individual legends for each reconstruction.


A
● Leaf margins entire
● Leaf margins incised / toothed
D
● Disc floret style branch stigmatic lines apically confluent
● Disc floret style branch stigmatic lines apically separated
B
● Leaf margins revolute (rolled towards abaxial surface)
● Leaf margins flat, unrolled
● Leaf margins involute (rolled towards adaxial surface)
E
● Disc floret style branch apices acute-acuminate
● Disc floret style branch apices obtuse
● Disc floret style branch apices truncated
● Polymorphic for the 2nd and 3rd features above
C
● Leaves adaxially tomentose, abaxially with different tomentum
● Leaves abaxially tomentose, adaxially with different tomentum
● Abaxial and adaxial surfaces not differentiated by hairiness
F
● Ray florets white only
● Ray florets yellow
● Ray florets pink or polymorphic for pink/white
● Ray florets polymorphic for white/yellow
● Ray florets white above and mauve below
● Ray florets not present

short scales. While *Pentatrichia* and *Phagnalon* do share some synapomorphies, such as long caudate anthers, waxy cushions on the outside of the corolla lobes, absence of myxogenic filiform twin hairs and the phloem not being concealed in fibres (Montes-Moreno & al., 2010), the recovery of *Pentatrichia* as the sister genus to *Phagnalon* is nevertheless an interesting biogeographical finding since the former is endemic to southern Africa, and the latter to North Africa and the greater Mediterranean region.

***Phagnalon*. —** *Phagnalon* is a genus of about 40 species (Montes-Moreno & al., 2010, 2013) distributed in North Africa, Macaronesia, the Mediterranean basin, the Irano-Turanian region and the Saharo-Arabian region, with its greatest diversity on the Arabian Peninsula. *Phagnalon* is strongly supported as monophyletic in the context of our well-sampled phylogeny. The genus is characterised by capitula that lack rays, being disciform with filiform female, and tubular hermaphrodite, florets. The disc floret style branches are apically obtuse, with stigmatic surfaces that are basally separated but apically confluent, and the pappus comprises a single series of barbellate bristles (Montes-Moreno & al., 2013).

Clade II

This subclade comprises two quite different smaller clades: clade C (*Arrowsmithia* and *Macowania*) and clade D (the Cape clade). In all members of clade II, leaves are entire and lack teeth, except for a small lineage of four species (*Oedera capensis*, *O. hirta*, *O. imbricata*,*O. laevis*) with compound secondary heads, but here the small marginal teeth appear to be made of trichomes rather than leaf laminar tissue (Anderberg, 1991: 15). Leaves are either revolute and then usually white-felted abaxially (clade C), or involute (rarely with flat margins) and usually white-felted adaxially in clade D. In this regard, *M. pinifolia* is unusual in having glabrous leaves whose margins are neither revolute nor involute, although the abaxial leaf surface is concave. Involucral bracts in clade II are usually yellow-brown and scarious, although the outer bracts are often leaf-like, especially in *Macowania*; the receptacle can be paleate or epaleate; and the capitula are always heterogamous and radiate. Ray florets are female-fertile (sterile in *M. corymbosa*) with a yellow lamina, often with brown streaks. *Macowania pinifolia* is again the single exception, the ray laminas in this species being white above and pink below. Ray floret style branches in clade II are linear and apically rounded or subacute with the stigmatic surfaces basally separated but apically confluent. The ray floret pappus comprises scales, or both scales and bristles. The disc florets are yellow, tubular and hermaphrodite (rarely functionally male); when fertile, the style branches are truncate to obtuse, furnished with an apical fringe of sweeping-hairs; and the stigmatic surfaces are separated along their entire length. Again, *M. pinifolia* is an exception, since the disc floret stigmatic surfaces reportedly fuse apically in this species (Merxmüller & al., 1977). Anthers in clade II have an acute or blunt apical appendage and are ecalcarate with short, often slightly branched, tails. Disc floret pappus elements in clade II consist of free capillary bristles in one row, sometimes also with small scales, or scales only.

***Arrowsmithia* and *Macowania* (clade C). —** *Macowania pinifolia*. – Kroner (1980), on revising *Athrixia*, argued that *A. pinifolia* did not belong in the genus since the leaves are not revolute, are not marginally dentate nor adaxially convex, and lack the white abaxial tomentum typical of *Athrixia*. *Macowania pinifolia* also differs from *Athrixia* in having involucral bracts that are partially leaf-like, and not apically attenuate into an acuminate, spreading-reflexed point. Kroner (1980) proposed transferring this species to *Macowania*, but Hilliard & Burtt (1985) asserted that it did not belong there either since its leaves lack the abaxial groove present in all other species, and it also differs in ray floret colour (white above and pink below, while all *Macowania* species have yellow or whitish/pale yellow ray ligules) and cypsela rib number (3 in *M. pinifolia*, 10–15 in *Macowania*). Anderberg (1991) retained the species in *Macowania* as an interim measure but agreed with both these sets of authors, stating that *M. pinifolia* might be better placed in its own, monospecific genus. While molecular phylogenetic results show that *M. pinifolia* has a closer affinity to *Macowania* than to *Athrixia*, different studies disagree on its position within this subclade. Bentley & al. (2014) recovered *M. pinifolia* sister to the Cape clade, while our nuclear data supports its placement as sister to [*Macowani*a+ *Arrowsmithia*] (Electr. Suppl.: Fig. S1). Our combined analysis (Fig. 2), however, does not resolve the branching order for the three main lineages of clade II (*M. pinifolia*; [*Arrowsmithia* +*Macowania*]; and the Cape clade). While the inclusion of *M. pinifolia* in *Macowania* is not contradicted by our molecular phylogenetic results, there are strong morphological arguments for keeping *M. pinifolia* separate from *Macowania*. This species differs from both *Macowania* and *Arrowsmithia* by: its possession of leaves without revolute margins, that are abaxially glabrous without any grooves, and adaxially convex; very long peduncles; a greater number of involucral bracts; narrower corolla tubes and finer pappus bristles; rays that are white above and pink below instead of uniformly yellow or whitish; and cypselas with only three ribs, where *Macowania* and *Arrowsmithia* have ten or more (Hilliard & Burtt, 1985). Even though we present an expanded morphological concept for *Arrowsmithia* that encompasses *Macowania* (see below), the inclusion of *M. pinifolia* would result in a loss of cohesion and a poor morphological definition of *Arrowsmithia*. Therefore, we choose to recognise *Macowania pinifolia* as a monospecific genus, using the name *Fluminaria* to reflect its mountain-streamside habitat.

Arrowsmithia styphelioides. – Prior to molecular phylogenetic analysis, *Arrowsmithia* was considered a monospecific genus of uncertain affinities, separated from *Macowania* by its distinctive pungent leaves, by the presence of bristles on the receptacle, and by the lack of a pappus (Hoffmann, 1894). However, both Kroner (1980) and Hilliard & Burtt (1985) pointed out that the latter two observations are incorrect, and that *Arrowsmithia* shares with *Macowania* a naked receptacle and a pappus of distinct bristles. In addition, the overall appearance and many features of the leaves, involucre, capitula and florets are very similar in the two genera. Kroner (1980) used these similarities to argue that *Arrowsmithia* should be congeneric with *Macowania*, but did not formalise the new combination. Hilliard & Burtt (1985) acknowledged the closeness of these two genera, but considered that they should be kept separate owing to a suite of differences involving the leaf veins on the leaf adaxial surface (three to five raised veins in *Arrowsmithia*, and one, usually depressed, vein in *Macowania*); the involucral bract veins (not reaching the bract tip in *Arrowsmithia*, extending to the bract tip in *Macowania*); the anther apical appendages (acute in *Arrowsmithia*; deltoid or oblong-obtuse in *Macowania*); indumentum of the floret tube (long woolly hairs in *Arrowsmithia*; glabrous or with short glandular hairs in *Macowania*); the number of ribs on the ray cypsela ribs (20 in *Arrowsmithia*; 10–15 in *Macowania*); and the number of ribs on the sterile disc-floret cypselas (12 ribs in *Arrowsmithia*, 10 in *Macowania*). Within *Macowania* however, the amount of variation in many morphological characters is large, perhaps unusually so for such a small genus. For example, species of *Macowania* vary in leaf orientation, size, shape and indumentum; in the arrangement of the leaf palisade tissue; in the positioning of the stomata (on both or only one leaf surface); in involucral bract colour and indumentum; in having female-fertile or sterile rays; in having hermaphrodite or functionally male discs; and in the number of ribs on the ray cypselas. Pappus bristles can be short and caducous or long and persistent, and anther apical appendages can be deltoid or oblong-obtuse. These differences have led previous authors to consider subdividing *Macowania*, suggesting that there are at least two distinct morphological groups within the genus (Hilliard & Burtt, 1976). Anderson & al. (1960) presented a case, on technical grounds (particularly the sterile rays), for *M. corymbosa* to be segregated into a monospecific genus, and for *M. conferta* and the similar species *M. glandulosa* and *M. pulvinaris* to be partitioned into *Homochaete* Benth., which is distinguished by the production of fertile cypselas in both rays and discs, and persistent pappus bristles. Anderson & al. (1960) also argued, however, that *Macowania* did not bear subdivision based on similarities in habit. The later addition of the two Northern Hemisphere species *M. abyssinica* and *M. ericifolia* further broadened the range of morphological variation encompassed by the genus. While the species presently in *Macowania* vary in features of the florets, arrangement of heads, involucral bracts and leaf indumentum, *A. styphelioides* possesses a strikingly different leaf shape and venation; otherwise it shares the typical capitula features of *Macowania.* We consider the molecular data to show convincingly that *Arrowsmithia* is in fact a novel member of *Macowania*. Its placement as sister to the type, *M. revoluta*, gives two options for a generic recircumscription. The first option would be to synonymise the two genera under the name *Arrowsmithia* (Candolle, 1838), which takes priority over *Macowania* (described by Oliver, 1870). Sinking *Macowania* into *Arrowsmithia* is potentially unpalatable because it requires 12 new combinations. However, there are insufficient grounds to support a proposal to conserve *Macowania* over *Arrowsmithia*, given that *Macowania* is a small, little-known genus with a relatively circumscribed geographic range and little economic value. It would be a pity to lose one of the generic names commemorating the renowned Cape botanist and professor at the South African College, Peter MacOwan (1830–1909). So, a second taxonomic option, aimed at preserving the name *Macowania*, would be to erect several small genera for the subclades within *Macowania*. However, because of the sister relationship between *Arrowsmithia* and the type of *Macowania*, we would need to erect at least five such small genera (two monospecific, one each for *M. revoluta* and *A. styphelioides*; two ditypic, one each for *M. abyssinica* + *M. ericifolia*, and *M. hamata*+*M. corymbosa*; and an additional genus, *Homochaete* Benth., for the remaining six species). This option is clearly undesirable, and there is insufficient morphological distinctness to support five separate genera. We instead formally combine the existing species of *Macowania* (except *M. pinifolia*) under the name *Arrowsmithia* (see below).

The Cape clade (clade D). — The monophyly of the clade is strongly supported. Members of the clade share the following morphological features: entire leaf margins (with the exception of tooth-like trichomes in four species); involute margins (rarely with flat margins); leaf surfaces which are frequently white-felted above, and often glandular, with stalked or sessile glands; yellow-brown, scarious involucral bracts, often with an apical membranous portion and membranous margins; paleate or epaleate receptacles, and capitula that are always heterogamous and radiate; female-fertile ray florets with a yellow lamina, often with brown streaks; apically acute to obtuse style branches with stigmatic surfaces that are basally separated but apically confluent; style arms that bear an apical tuft of blunt sweeping-hairs; yellow, tubular disc florets that are hermaphrodite or rarely male-fertile only; a pappus present on both types of florets (usually) and comprising a row of small scales, sometimes with an additional whorl of (usually only a few) free barbellate or plumose capillary bristles, rarely of bristles only; and truncate to obtuse disc floret style branches with a tuft of sweeping hairs and the stigmatic surfaces are separated along their entire length. Apart from these shared characters, the members of this clade are morphologically extremely heterogeneous, so it is not surprising that they currently are classified as nine genera, many of which are monospecific or very small. Despite the presence of several well-supported clades within the Cape clade, the backbone remains poorly resolved, with several species or small groups of species whose phylogenetic positions are not conclusively supported. Generic circumscription of this group is dealt with in Bergh & al. (submitted).

■ TAXONOMY

This section provides a key to the genera of the Relhania clade. Twelve members of *Macowania* are transferred to *Arrowsmithia*; we provide an updated description for this expanded concept of *Arrowsmithia*, and a key to the species. A new monospecific genus, *Fluminaria* gen. nov., is erected to house *Macowania pinifolia*. All types housed in herbaria outside of South Africa were seen only as digital images (JSTOR Global Plants, https://plants.jstor.org/) indicated by [image!].

Key to the genera of the Relhania clade:

1. Capitula lacking ray florets 2
1. Capitula radiate 3
2. Capitula disciform, known only from the Northern Hemisphere .... ***Phagnalon***
2. Capitula discoid, known only from southern Africa .... ***Pentatrichia***
3. Ray florets white, pale to deep pink, or white above and pink/reddish below 4
3. Ray florets pale to bright yellow 6
4. Leaf margins revolute; leaves discolorous, adaxially green and ± glabrous, abaxially white-tomentose 5
4. Leaf margins flat; leaves not discolorous, green on both surfaces 7
5. Annual; pappus of slender nude setae alternating with very broad, wing-like scales; receptacular paleae acuminate, apex dark brown .... ***Alatoseta***
5. Perennials; pappus of slender serrate bristle-like scales alternating with short narrow scales; receptacular paleae absent (present in one species) .... ***Athrixia***
6. Leaf margins strongly revolute, white-woolly covering denser on the abaxial (lower) leaf surface; either abaxial midrib broad, glabrous and prominent, or, when abaxial midrib indistinct, five prominent raised veins visible on the adaxial surface .... ***Arrowsmithia***
6. Leaf margins flat or involute; leaves glabrous or, if hairy, white-woolly covering denser on the adaxial (upper) leaf surface; leaf veins not as above .... **the Cape clade**
7. Leaves flat, cordate, lanceolate or ovate, >4 mm broad at widest point; margins incised (at least in young leaves); rays white above and below .... ***Pentatrichia***
7. Leaves narrow, linear-subulate, ≤2.5 mm broad at widest point (the base); leaf margins entire but bearing fine bristles; rays white above and pink below .... ***Fluminaria***

Arrowsmithia DC., Prodr. 7: 254. 1838 – Type: *A. styphelioides* DC.
= *Macowania* Oliv. in Hooker's Icon. Pl. 11: 49, t. 1062. 1870, **syn. nov.** – Type: *M. revoluta* Oliv.
= *Homochaete* Benth. in Hooker's Icon. Pl. 12: 9, t. 1110. 1872 – Type: *H. conferta* Benth.

Leaves linear or linear-lanceolate with revolute or thickened margins and a usually dense white abaxial tomentum, midrib usually glabrous, abaxially expanded and prominent; adaxial midrib sunken and leaves canaliculate (rarely adaxial surface with several raised parallel veins). Capitula large (both length and width ≥8 mm); involucral bracts frequently green and leaf-like, at least on the central portion, scarious, brown or hyaline on margins and apex of at least the innermost bracts; receptacle epaleate; ray florets female, fertile (rarely sterile), ligules pale to bright yellow; cypselas with 10–20 longitudinal ribs.

Shrublets, erect or dwarf, and then often well branched, sometimes resprouting, often with glandular trichomes that are stalked and/or sessile. *Leaves* alternate, crowded, linear or linear-lanceolate, sessile, apex mucronate, margins revolute or thickened, midrib usually prominent below, abaxial surface usually white-woolly; adaxial surface glandular or glabrous, frequently canaliculate, in one species with several parallel veins. *Heads* solitary or clustered in small groups, terminal, sessile or very shortly pedunculate, heterogamous-radiate. *Involucre* campanulate or turbinate, at least 8 mm long by 8 mm broad at anthesis, involucral bracts imbricate in several series, becoming progressively longer inwards, at least the margins and apices scarious, brown or hyaline, generally lightly woolly outside, often glandular, sometimes glabrous. *Receptacle* flat, smooth or honeycombed, epaleate. *Ray florets* bright yellow (South Africa) or whitish or pale yellow (Afrotemperate), uniseriate, generally female-fertile, rarely sterile; corolla tubular below, glabrous or bearing trichomes, lamina narrowly elliptic, three-toothed; style branches linear, apex subacute; stigmatic papillae confined to marginal bands on the adaxial surfaces and confluent at the branch tips; ovary with 10–20 ribs; generally pubescent, rarely glabrous; pappus of long scabrid bristles alternating with short bristle-like scales; caducous or persistent. *Disc florets* bright yellow, hermaphrodite or rarely functionally male; corolla narrowly cylindric below, glabrous or hairy, widening gradually or abruptly campanulate above, five-lobed; anthers with a deltoid, oblong-obtuse or acute apical appendage, ecalcarate, bases tailed, tails free or adjacent ones cohering, smooth or fimbriate; style branches (when fertile) oblong, slightly broadened at the obtuse tips, minute pollen-sweeping papillae on outer faces; stigmatic bands marginal on the adaxial surfaces, not meeting at the tips; ovary with ca. 10–20 ribs, generally pubescent, rarely glabrous; pappus of acute scabrid bristles alternating with short bristle-like scales, caducous or persistent. Cypselas (when fertile) cylindric or fusiform, ribbed, glabrous or hairy. Chromosome number unknown. Twelve species.

Key to the species of *Arrowsmithia*

1. Ray laminas white to pale yellow 2
1. Rays laminas bright yellow 3
2. Leaves linear, at least some exceeding 2 cm in length, subglabrous adaxially when fully grown, usually overtopping the heads .... ***A. ericifolia***
2. Leaves narrowly elliptic, not exceeding 2 cm in length, thinly woolly adaxially throughout, not overtopping the heads .... ***A. abyssinica***
3. Involucral bracts with brown margins, abaxially with glandular trichomes on thick stalks 4
3. Involucral bracts with hyaline margins, tomentose at least when young, abaxially with glandular trichomes with slender stalks or with sessile glands 5
4. Ray flowers female, fertile; disc flowers functionally male; involucral bracts tipped with a fine mucro .. ***A. revoluta***
4. Ray flowers sterile, with or without styles; disc flowers hermaphrodite, producing fertile cypselas; involucral bracts with acuminate membranous tips . ***A. corymbosa***
5. Leaf abaxial midrib indistinct or obscured by the revolute leaf margins, leaf margins abaxially prominent and obscuring the midrib where they unite at the leaf tip and merge into the apiculus 6
5. Leaf abaxial midrib prominent, running out to the leaf

tip and merging into the apiculus, midrib apically more prominent than the margins 7
6. Leaves coarsely glandular-hispid, never with punctate glands 8
6. Leaves not coarsely glandular hispid, or when so then also conspicuously glandular punctate 9
7. Dwarf, spreading, intricately branched shrublet; leaf tip broadly acute, apiculus short; heads on short ebracteate peduncles .... ***A. pulvinaris***
7. Erect shrublet; leaf tip gradually narrowed to the relatively long apiculus; heads sessile or on short, heavily bracteate peduncles .... ***A. tenuifolia***
8. Leaves not woolly on their abaxial surfaces ***A. conferta***
8. Leaves woolly on their abaxial surfaces 10
9. Leaf adaxial surface lacking glands; leaves triangular, narrowing to a prominent and pungent apiculus; several parallel longitudinal leaf veins clearly visible on leaf adaxial surface in addition to the midrib.. ***A. styphelioides***
9. Leaf adaxial leaf surface glandular; leaves linear; leaf apex sometimes acute but apiculus small or rounded; only the midrib visible or no veins discernable on the leaf adaxial surface 11
10. Leaves with or without stalked glands but always glandular-punctate; ascending or spreading, large, oblong-linear, ca. 15–20 mm long .... ***A. glandulosa***
10. Leaves never glandular-punctate; at first tomentose then glabrous above, with small inconspicuous glandular hairs near the margins; soon deflexed, slender, linear, ca. 8 mm long .... ***A. deflexa***
11. Leaf apiculus strongly developed, deflexed to form a sharp hook; heads sessile .... ***A. hamata***
11. Leaf apiculus short, straight and rather blunt; heads usually stalked .... ***A. sororis***

Arrowsmithia abyssinica (Sch.Bip. ex Walp.) N.G.Bergh, **comb. nov.** ≡ *Klenzea abyssinica* Sch.Bip. ex Walp., Repert. Bot. Syst. 2: 973. 1843 ≡ *Macowania abyssinica* (Sch.Bip. ex Walp.) B.L.Burtt in Notes Roy. Bot. Gard. Edinburgh 31: 376. 1972 – **Lectotype (designated here):** Ethiopia, In monte Semajata [in rupibus nudis regionis superioris montis Scholoda / Mt. Scholada near Adua], 22 Dec 1837, *G.W. Schimper 227* (P barcode P00053730 [image!]; isolectotypes: BM barcode BM000924256 [image!], BR barcodes 0000008361455 & 0000008876485 [images!], HOH, K barcode K000415215 [image!], LG, M barcodes M-0105450 & M-0105451 [images!], P barcodes P00053731, P00053732 & P00053733 [images!], S No. S07-13231 [image!], TUB).

Note. – Schultz Bipontinus's Asteraceae herbarium was brought to the herbarium at P through E. Cosson. P53730 is marked with a ticket stamped "Herb. E. Cosson, 18". The other P specimens that I have viewed are marked "Herbier E. Drake". Although the capitula of the *Schimper 227* collection are immature, the leaf features are distinctive, as are the involucral bracts.

Arrowsmithia conferta (Benth.) N.G.Bergh, **comb. nov.** ≡ *Homochaete conferta* Benth. in Hooker's Icon. Pl. 12: t. 1110. 1872 ≡ *Macowania conferta* (Benth.) E.Phillips in J. S. African Bot. 16: 21. 1950 – **Lectotype (designated here):** Faku's Territory [Pondoland], *Sutherland s.n.* (K barcode K000415217 [image!]; isolectotypes: E barcode E00531202 [fragment; image!], PRE barcode PRE0180739-0! [fragment]).

Arrowsmithia corymbosa (M.D.Hend.) N.G.Bergh, **comb. nov.** ≡ *Macowania corymbosa* M.D.Hend. in Kirkia 1: 114. 1961 – Holotype: Natal, Bergville Distr., Umlambonja River, Jul 1936, *Marriot s.n.* (PRE barcode PRE0180740-0!).

Arrowsmithia deflexa (Hilliard & B.L.Burtt) N.G.Bergh, **comb. nov.** ≡ *Macowania deflexa* Hilliard & B.L.Burtt in Notes Roy. Bot. Gard. Edinburgh 42: 231. 1985 – Holotype: Natal, Estcourt Distr., Monk's Cowl Forest Station, valley below Ship's Prow Pass, 2100 m, 7 Dec 1983, *Balkwill, Manning & Meyer 1038* (NU barcode NU0014354-0 [image!]; isotypes: E barcode E00239188 [image!], K [image!], PRE barcode PRE0654858-0!, PRF).

Arrowsmithia ericifolia (Forssk.) N.G.Bergh, **comb. nov.** ≡ *Aster ericifolius* Forssk., Fl. Aegypt.-Arab.: 150. 1775 ≡ *Macowania ericifolia* (Forssk.) B.L.Burtt & Grau in Notes Roy. Bot. Gard. Edinburgh 31: 376. 1972 – Syntypes: Yemen, Kurmae, *Forsskål s.n.* (C barcode C10001814 [image!], BM barcode BM000945877 [image!], M, S Nos. S04-1155 & S-G-707 [images!]).

Note. – Although many of Forsskål's types are at C, some are also lodged at other herbaria such as B and BM (Stafleu & Cowan, 1976). The specimen at C is annotated indicating that it was at one time in the herbarium of Schumacher. With no evidence directly linking Forsskål to this specimen, we have not designated a type here.

Arrowsmithia glandulosa (N.E.Br.) N.G.Bergh, **comb. nov.** ≡ *Macowania glandulosa* N.E.Br. in Bull. Misc. Inform. Kew 1901: 124. 1901 – **Lectotype (designated here):** Natal, top of Tabamhlope Mt, 6–7000 ft, Feb 1895, *Evans 412* (K barcode K000415218 [image!]; isolectotypes: NH barcodes NH0019585-0 & NH0008811-0 [images!], PRE barcode PRE0180723-0!).

Paratype. – Natal, Langalibalele's location, Jun 1874, *J.S. Fannin 2013* (K barcode K000415219 [image!]).

Note. – It is likely for several reasons that Brown used the Kew specimens. Firstly, Brown's types are most likely at Kew (Desmond & Ellwood, 1994). Secondly, Brown lists both the Evans and the Fannin collection, and the Kew specimen of each collector is mounted on the same sheet. Thirdly, Evans's label on specimen K000415218 states "round shrub", and Brown (1901) wrote that "Mr Evans describes this as a 'round shrub' on his label". Evans wrote various descriptions, but only on this particular specimen are the words "round shrub" written on the label. This provides strong evidence that Brown saw this particular specimen, here designated as the lectotype.

Arrowsmithia hamata (Hilliard & B.L.Burtt) N.G.Bergh, **comb. nov.** ≡ *Macowania hamata* Hilliard & B.L.Burtt in Notes Roy. Bot. Gard. Edinburgh 34: 269. 1976 – **Lectotype (designated here):** Natal, Underberg Distr., Garden Castle Nature Reserve, main stream, valley W of forester's house, ca. 1800 m, 28 Jan 1975, *Hilliard & Burtt 7816* (E barcode E00239186 [image!]; isolectotypes: E barcode E00239187 [image!], M barcode M-0105448 [image!], MO barcode MO-2071220 [image!], NBG barcode NBG0108544-0!, NH barcode NH0084660-0 [image!], NU barcodes NU0014355-1! & NU0014355-2!, P barcode P00138947 [image!], PRE barcode PRE0359128-0!, S No. S-G-3879 [image!]).

Note. – The protologue refers to a specimen in E as the holotype, but there are two specimens from this gathering in that herbarium, requiring that one be designated as lectotype. The two specimens have identical labels, and neither has any annotation, so in this instance the choice of a lectotype has been arbitrary.

Arrowsmithia pulvinaris (N.E.Br.) N.G.Bergh, **comb. nov.** ≡ *Macowania pulvinaris* N.E.Br. in Bull. Misc. Inform. Kew 1901: 124. 1901 – **Lectotype (designated here):** Cape, Queenstown Division, on the summit of Andriesberg, near Bailey, 6800 ft., 17 Jan 1897, *Galpin 2258* (K barcode K000415220 [image!]; isolectotypes: BOL barcode BOL139004!, E barcode E00570004 [fragment; image!], GRA barcodes GRA0003095-1 & GRA0003095-2 [images!], NBG barcode NBG0027641-0!, P barcode P00053767 [image!], PRE barcodes PRE0180735-0! & PRE0180736-0!).

Note. – There is only a single specimen at K, where Brown worked, which is why this has been designated as the lectotype.

Arrowsmithia revoluta (Oliv.) N.G.Bergh, **comb. nov.** ≡ *Macowania revoluta* Oliv. in Hooker's Icon. Pl. 11: 49, t. 1062. 1870 – **Lectotype (designated here):** Cape, British Kaffraria, Mts of the Buffalo River near Pirie, 3500 ft., fl. Oct–Nov, *P. Macowan 2013* (K barcode K000415221 [image!]; isolectotypes: BOL barcode BOL139003!, E barcode E00531204 [fragment; image!], GH barcodes 00009964 & 00009965 [images!], GRA barcode GRA0003096-0!, K barcode K000415222 [image!], NY barcodes 00214952 & 00214953 [images!], P barcode P00138946 [image!], SAM barcode SAM0038595-0!)

Note. – K000415221 was selected as lectotype because attached to the specimen is a set of pencil sketches matching the protologue illustrations, clearly by W.H. Fitch. The PRE specimen with barcode PRE0180712-0 listed on JSTOR as *Macowan 2013* is a Leighton collection.

Arrowsmithia sororis (Compton) N.G.Bergh, **comb. nov.** ≡ *Macowania sororis* Compton in J. S. African Bot. 19: 114. 1953 – Holotype: East Griqualand, Mount Currie, 7000 ft, Apr 1938, *Sister Mildred 8* (NBG barcode NBG0027640-0!).

Arrowsmithia styphelioides DC., Prodr. 7: 254. 1838 ≡ *Macowania styphelioides* (DC.) Kroner in Mitt. Bot. Staatssamml. München 16: 33. 1980 – Holotype: In Africa Capensi ad Katriviersberg alt. 4000–5000 ped., *Drege 3762* (G-DC barcode G00493676 [image!]; isotypes: HAL barcode HAL0113255 [image!], P barcodes P00053770, P00053771 & P00053772 [images!]).

Arrowsmithia tenuifolia (M.D.Hend.) N.G.Bergh, **comb. nov.** ≡ *Macowania tenuifolia* M.D.Hend. in Kirkia 1: 116. 1961 – Holotype: Transvaal, 8 miles SE of Nigel, Vrisgewaag 337, 10 Oct 1950, *Mogg 20116* (PRE barcode PRE0183477-0!; isotype [fragment]: E barcode E00531203 [image!]).

Fluminaria N.G.Bergh, **gen. nov.** – Type: *Fluminaria pinifolia* (N.E.Br.) N.G.Bergh (≡ *Macowania pinifolia* (N.E.Br.) Kroner).

Shrubs with bright green, narrowly linear-subulate leaves; leaves abaxially concave but margins not revolute, glabrous and green on all surfaces, bearing small teethlike bristles on the margins; capitula solitary, borne above the leaves on nude, greenish, pubescent peduncles; ray lamina white above and pink or purplish below.

Erect, sparsely branched shrublets to 1 m, stems nude towards the base, marked by circular leaf scars. Leaves alternate, crowded towards the stem apices, sessile, linear-subulate, very acute, up to 30 mm long, 2 mm broad at the base, somewhat clasping, abaxially concave but margins not revolute, hispid on the margins, otherwise glabrous, mid-rib distinct. Heads turbinate, terminal, radiate, solitary on long, slender, pubescent peduncles, 15–100 mm long. Involucre ca. 10 × 6 mm, bracts in many series, with a green, glabrous stereome, middle to inner bracts also with an acute, yellow-brown scarious lamina, somewhat spreading, receptacle epaleate. Ray florets female fertile, corolla tube sparsely glandular, lamina linear-oblong, spreading, white above, pink or purplish below; pappus and cypsela as in disc florets. Disc florets hermaphrodite, perfect, corolla tube sparsely glandular, narrowly campanulate, anthers with slender tails, apical appendage obtuse, cypselas narrowly oblong with three slender ribs, to 3 mm long, sparsely pilose with a basal coma; pappus of about 12 delicate barbellate bristles, subplumose in an area below the apex, alternating with very short, delicate scales.

Species included. – *Fluminaria pinifolia* (N.E.Br.) N.G.Bergh.

Chromosome number. – Unknown.

Distribution. – Near high-altitude (>1200 m) streams of the KwaZulu-Natal Drakensberg mountains and Lesotho eastern escarpment. Disjunct in Utrecht in northern KwaZulu-Natal Province, and Naude's Nek in the Eastern Cape Province.

Etymology. – The generic name *Fluminaria* refers to the occurrence of this plant mainly in or near alpine streams.

Fluminaria pinifolia (N.E.Br.) N.G.Bergh, **comb. nov.** ≡ *Athrixia pinifolia* N.E.Br. in Bull. Misc. Inform. Kew 98: 26. 1895 ≡ *Macowania pinifolia* (N.E.Br.) Kroner in Mitt. Bot. Staatssamml. München 16: 33. 1980 – **Lectotype (designated here):** Drakensberg, among stones in bed of Bushman's River near Old Bushman's Caves, 6–7000 ft, Jul 1894, *M.S. Evans 59* (K barcode K000415247 [image!]; isolectotypes: NH barcodes NH0019241 & NH0006712 [images!]).

Note. – The lectotype represents the only specimen of the cited type in Brown's home herbarium at K, and is annotated by Brown.

■ ACKNOWLEDGEMENTS

The authors gratefully acknowledge the BOL, GRA, NBG, NU, PRE and PRU herbaria for the loan of specimens and leaf material; N. Hind and L. Csiba of K for providing DNA for several rare and under-collected species; T.A. Abbott, P. Armour and B. Armour for their assistance in locating *M. conferta*; A. Miya and several other Ezemvelo-KwaZulu-Natal reserve staff who were extremely helpful during fieldwork; C. Appleby, M. Britton, C. Cozien, V. Hoffmann, T. Lane, T. Moore, C. McKune, M. Stehr and T. Trinder-Smith for their field assistance, and J. Manning and the nomenclature editors of the journal for advice on nomenclature. Collecting permits were issued by the Cape Nature authorities, Ezemvelo-KwaZulu-Natal Parks board, Mpumalanga Parks board, and the South African National Parks board, with access permission by several individual park managers. This study was funded by the NRF Knowledge Fields Development SABI (South African Biosystematics Initiative) grant 71072 awarded to N.G. Bergh and NRF M.Sc. bursaries awarded to J. Bentley.

■ LITERATURE CITED

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. Pp. 267–281 in: Petrov, B.N. & Csaki, F. (eds.), *Proceedings of the 2nd International Symposium on Information Theory*. Budapest: Akademiai Kiado.

Anderberg, A.A. 1989. Phylogeny and reclassification of the tribe Inuleae (Asteraceae). *Canad. J. Bot.* 67: 2277–2296. https://doi.org/10.1139/b89-292

Anderberg, A.A. 1991. Taxonomy and phylogeny of the tribe Gnaphalieae (Asteraceae). *Opera Bot.* 104: 1–195.

Anderberg, A.A. & Bremer, K. 1991. Parsimony analysis and cladistic reclassification of the Relhania generic group (Asteraceae–Gnaphalieae). *Ann. Missouri Bot. Gard.* 1061–1072. https://doi.org/10.2307/2399743

Anderberg, A.A., Eldenäs, P., Bayer, R.J. & Englund, M. 2005. Evolutionary relationships in the Asteraceae tribe Inuleae (incl. Plucheeae) evidenced by DNA sequences of *ndhF*; with Notes on the systematic positions of some aberrant genera. *Organisms Diversity Evol.* 5: 135–146. https://doi.org/10.1016/j.ode.2004.10.015

Anderson, J.G., Codd, L.E., Dyer, R.A., Henderson, M.D., Killick, D.J.B. & De Winter, B. 1960. New and interesting taxa from southern Africa. *Kirkia* 1: 100–118.

Baldwin, B.G. & Markos, S. 1998. Phylogenetic utility of the external transcribed spacer ETS of 18S-26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molec. Phylogen. Evol.* 10: 449–463. https://doi.org/10.1006/mpev.1998.0545

Bayer, R.J. & Cross, E.W. 2002. A reassessment of tribal affinities of the enigmatic genera *Printzia* and *Isoetopsis* (Asteraceae), based on three chloroplast sequences. *Austral. J. Bot.* 50: 677–686. https://doi.org/10.1071/BT01108

Bayer, R.J., Puttock, C.F. & Kelchner, S.A. 2000. Phylogeny of South African Gnaphalieae (Asteraceae) based on two noncoding chloroplast sequences. *Amer. J. Bot.* 87: 259–272.

Bayer, R.J., Greber, D.G. & Bagnall, N.H. 2002. Phylogeny of Australian Gnaphalieae (Asteraceae) based on chloroplast and nuclear sequences, the *trnL* intron, *trnL/trnF* intergenic spacer, *matK*, and ETS. *Syst. Bot.* 27*:* 801–814. https://doi.org/10.1043/0363-6445-27.4.801

Bengtson, A., Englund, M., Pruski, J.F. & Anderberg, A.A. 2017. Phylogeny of the Athroismeae (Asteraceae), with a new circumscription of the tribe. *Taxon* 66: 408–420. https://doi.org/10.12705/662.8

Bentley, J., Verboom, G.A. & Bergh, N.G. 2014. Erosive processes after tectonic uplift stimulate vicariant and adaptive speciation: Evolution in an Afrotemperate-endemic paper daisy genus. *B. M. C. Evol. Biol.* 14: 27. https://doi.org/10.1186/1471-2148-14-27

Bentley, J., Klaassen, E.S. & Bergh, N.G. 2015. *Philyrophyllum* (Asteraceae) transferred from Gnaphalieae to Athroismeae based on phylogenetic analysis of nuclear and plastid DNA sequence data. *Taxon* 64: 975–986. https://doi-org.ezproxy.uct.ac.za/10.12705/645.7

Bergh, N.G. & Linder, H.P. 2009. Cape diversification and repeated out-of-southern-Africa dispersal in paper daisies (Asteraceae–Gnaphalieae). *Molec. Phylogen. Evol.* 51: 5–18. https://doi.org/10.1016/j.ympev.2008.09.001

Born, J., Linder, H.P. & Desmet, P. 2007. The Greater Cape Floristic Region. *J. Biogeogr.* 34*:* 147–162. https://doi.org/10.1111/j.1365-2699.2006.01595.x

Brown, N.E. 1901. Diagnoses Africanae: XIII. Bull. Misc. Inform. Kew 1901: 119–138.

Candolle, A.D. de 1838 ("1837"). *Prodromus systematis naturalis regni vegetabilis*, vol. 6. Parisiis [Paris]: sumptibus sociorum Treuttel et Wurtz. https://doi.org/10.5962/bhl.title.286

Compton, R.H. 1931. The flora of the Whitehill District. *Trans. Roy. Soc. South Africa* 19: 269–326.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. jModelTest 2: More models, new heuristics and parallel computing. *Nature, Meth.* 9: 772–772. https://doi.org/10.1038/nmeth.2109

Desmond, R. & Ellwood, C. 1994. *Dictionary of British and Irish botanists and horticulturalists.* London: Taylor & Francis; The Natural History Museum of London.

Drummond, A.J. & Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *B. M. C. Evol. Biol.* 7: 214. https://doi.org/10.1186/1471-2148-7-214

Eldenäs, P., Anderberg, A.A. & Källersjö, M. 1998. Molecular phylogenetics of the tribeInuleae s.str. (Asteraceae), based on ITS sequences of nuclear ribosomal DNA. *Pl. Syst. Evol.* 210: 159–173. https://doi.org/10.1007/BF00985666

Forest, F., Grenyer, R., Rouget, M., Davies, T.J., Cowling, R.M., Faith, D.P., Balmford, A., Manning, J.C., Procheş, Ş., Van der Bank, M., Reeves, G., Hedderson. T.A.J. & Savolainen, V. 2007. Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature* 445: 757–760. https://doi.org/10.1038/nature05587

Galbany-Casals, M., Garcia-Jacas, N., Susanna, A., Sáez, L. & Benedí, C. 2004. Phylogenetic relationships in the Mediterranean *Helichrysum* (Asteraceae, Gnaphalieae) based on nuclear rDNA ITS sequence data. *Austral. Syst. Bot.* 17: 241–253. https://doi.org/10.1071/SB03031

Gawel, N.J. & Jarrett R.L. 1991. A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. *Pl. Molec. Biol. Reporter* 9: 262–266. https://doi.org/10.1007/BF02672076

Gilks, W.R., Richardson, S. & Spiegelhalter, D.J. 1996. Introducing Markov chain Monte Carlo. Pp. 1–19 in: Gilks, W.R., Richardson, S. & Spiegelhalter, D.J. (eds.), *Markov chain Monte Carlo in practice*. Boca Raton: CRC Press.

Guindon, S. & Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52: 696–704. https://doi.org/10.1080/10635150390235520

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41: 95–98.

Hilliard, O.M. & Burtt, B. 1976. Notes on some plants of southern Africa chiefly from Natal: V. *Notes Roy. Bot. Gard.* 34: 260–279.

Hilliard, O.M. & Burtt, B.L. 1985. Notes on some plants of southern Africa chiefly from Natal: 11. *Notes Roy. Bot. Gard. Edinburgh* 42: 227–260.

Hillis, D.M. & Bull, J.J. 2009. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* 42: 182–192. https://doi.org/10.1093/sysbio/42.2.182

Hoffmann, O. 1894. Compositae. Pp. 87–391 in: Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien*, IV(5). Leipzig: Engelmann. https://doi.org/10.5962/bhl.title.4635

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 178: 754–755.

Kim, K.J. & Jansen, R.K. 1995. *ndhF* sequence evolution and the major clades in the sunflower family. *Proc. Natl. Acad. Sci. U.S.A.* 92: 10379–10383.

Klaassen, E.S. & Bergh, N.G. 2012. Inclusion of the Cape genus *Anisothrix* in the Namibian-centred genus *Pentatrichia* (Asteraceae, Gnaphalieae) based on a molecular phylogenetic analysis. *S. African J. Bot.* 78*:* 2–11. https://doi.org/10.1016/j.sajb.2011.04.005

Klaassen, E.S., Kwembeya, E.G. & Maass, E. 2009. A taxonomic revision of the genus *Pentatrichia*. *S. African J. Bot*. 75*:* 153–164. https://doi.org/10.1016/j.sajb.2008.10.002

Kroner, G. 1980. Systematische Studien im Umkreis von *Athrixia* Ker-Gawler (Asteraceae). *Mitt. Bot. Staatssamml München* 16: 1–267.

Markos, S. & Baldwin, B.G. 2001. Higher-level relationships and major lineages of *Lessingia* (Compositae, Astereae) based on nuclear rDNA internal and external transcribed spacer (ITS and ETS) sequences. *Syst. Bot.* 26: 168–183. https://doi.org/10.1043/0363-6445-26.1.168

Merxmüller, H., Leins, P. & Roessler, H. 1977. Inuleae—Systematic review. Pp. 577–601 in: Heywood, V.H., Harbourne, J.B. & Turner, B.L. (eds), *The biology and chemistry of the Compositae*. London: Academic Press.

Miller, M.A., Pfeiffer, W. & Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 45–52 in: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, Louisiana, 14 Nov 2010. Piscataway: IEEE.

Miller, M.A., Schwartz, T., Hoover, P., Yoshimoto, K., Sivagnanam, S. & Majumdar, A. 2015. The CIPRES workbench: A flexible framework for creating science gateways. Art. no. 39 in: *Proceedings of the 2015 XSEDE Conference: Scientific Advancements Enabled by Enhanced Cyberinfrastructure*. New York: ACM.

Montes-Moreno, N., Sáez, L., Benedí, C., Susanna, A. & Garcia-Jacas, N. 2010. Generic delineation, phylogeny and subtribal affinities of *Phagnalon* and *Aliella* (Compositae, Gnaphalieae) based on nuclear and chloroplast sequences. *Taxon* 59: 1654–1670. http://www.jstor.org/stable/41059864

Montes-Moreno, N., Garcia-Jacas, N., Benedí, C. & Sáez, L. 2013. Evaluation of the taxonomic status of the genus *Aliella* Compositae, Gnaphalieae: A recircumscription of the genus *Phagnalon*. *Phytotaxa* 148: 1–31. https://doi.org/10.11646/phytotaxa.148.1.1

Nie, Z.L., Funk, V.A., Meng, Y., Deng, T., Sun, H. & Wen, J. 2015. Recent assembly of the global herbaceous flora: Evidence from the paper daisies (Asteraceae: Gnaphalieae). *New Phytol.* 209: 1795–1806. https://doi.org/10.1111/nph.13740

Nylander, J.A.A. 2004. MrModeltest, version 2.4. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. http://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html

Oliver, D. 1870 ("1867–71"). *Macowania revoluta* Oliv. *Hooker's Icon. Pl.* 11: 49–50, pl. 1062. https://doi.org/10.5962/bhl.title.16059

Panero, J.L. & Funk, V.A. 2008. The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. *Molec. Phylogen. Evol.* 47: 757–782. https://doi.org/10.1016/j.ympev.2008.02.011

R Development Core Team 2014. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. http://www.R-project.org

Revel, L.J. 2012. Phytools: An R package for phylogenetic comparative biology (and other things). *Meth. Ecol. Evol.* 3: 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Ronquist, F., Huelsenbeck, J.P. & Teslenko, M. 2011. *MrBayes version 3.2 manual: Tutorials and model summaries.* http://mrbayes.sourceforge.net/mb3.2_manual.pdf

Stafleu, F.A. & Cowan, R.S. 1976. *Taxonomic literature*, ed. 2, vol. 1. Utrecht: Bohn, Scheltema and Holkema. https://doi.org/10.5962/bhl.title.48631

Starr, J.R., Harris, S.A. & Simpson, D.A. 2003. Potential of the 5′ and 3′ ends of the intergenic spacer (IGS) of rDNA in Cyperaceae: New sequences for lower-level phylogenies in sedges with an example from *Uncinia* Pers. *Int. J. Pl. Sci.* 164: 213–227. https://doi.org/10.1086/346168

Sukumaran, J. & Holder, M.T. 2010. DendroPy: A Python library for phylogenetic computing. *Bioinformatics* 26: 1569–1571. https://doi.org/10.1093/bioinformatics/btq228

Sukumaran, J. & Holder, M.T. 2015. SumTrees: Phylogenetic Tree Summarization, version 4.0.0. https://github.com/jeetsukumaran/DendroPy

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

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. https://doi.org/10.1007/BF00037152

Ward, J., Bayer, R.J., Breitwieser, I., Smissen, R., Galbany-Casals, M. & Unwin, M. 2009. Gnaphalieae. Pp. 539–588 in: Funk V.A., Susanna A., Stuessy T.F. & Bayer R.J. (eds.), *Systematics, evolution and biogeography of Compositae*. Vienna: International Association for Plant Taxonomy.

White, T.J., Bruns, T., Lee, S. & Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in: Innis M.A., Gelfand D.H., Sninsky J.J. & White T.J. (eds.), *PCR protocols: A guide to methods and applications*. New York: Academic Press.

Yang, Z. & Rannala, B. 1997. Bayesian phylogenetic inference using DNA sequences: A Markov Chain Monte Carlo Method. *Molec. Biol. Evol.* 14: 717–724.

Appendix 1. List of taxa [for sequences generated in this study: geographic locality (collected in South Africa unless otherwise indicated), voucher and the herbarium] and the GenBank accession numbers for each gene region (ETS, ITS, *trnL-F*, *trnT-L*). A dash (–) indicates missing gene regions; an asterisk (*) indicates new sequences.

Alatoseta tenuis Compton, Tankwa Karoo, *J.C. Manning 3187* (NBG), MF382026*, MF382010*, MF401580*, –; ***Amphiglossa corrudifolia*** DC., KR559455, KR559468, KR559445, –; ***Antithrixia flavicoma*** DC., Kamiesberg, *N.G. Bergh 2095* (NBG), KY694349*, KY694165*, –, –; ***Athrixia angustissima*** **(a)** DC., –, KF997172, –, KF997131; ***A. angustissima*** **(b)**, Drakensberg, *N.G. Bergh 1499* (NBG), MF382027*, MF382011*, MF382040*, –; ***A. arachnoidea*** J.M.Wood & M.S.Evans ex J.M.Wood, Drakensberg, *N.G. Bergh 2198* (NBG), MF382034*, KF997173, MF382043*, KF997132; ***A. capensis*** **(a)** Ker Gawl., KR559454, KR559467, –, –; ***A. capensis*** **(b)**, Tygerberg, *CREW & H. Stummer 3597* (NBG), –, MF382013*, MF382041*, –; ***A. crinita*** (L.) Druce, Tulbagh Valley,

Appendix 1. Continued.

E. Esterhuysen 29942 (BOL), –, MF382016*, –, –; ***A. elata*** **(a)** Sond., Pretoria, *S.P. Bester 6919* (PRE), MF382035*, MF382017*, MF382042*, MF382050*; ***A. elata*** **(b)**, Mashishing, *N.G. Bergh 2203* (NBG), MF382036*, KF997174, MF382044*, KF997133; ***A. fontana*** **(a)** (MacOwan), Lesotho, *M. Koekemoer 3554* (PRE), MF382037*, KF997175, KF997134, –; ***A. fontana*** **(b)**, Drakensberg, *N.G. Bergh 1508* (NBG), MF382038*, –, –, MF382051*; ***A. fontinalis*** Wild, Mozambique, *Grosvenor 191* (K), –, MF382018*, –, –; ***A. gerardii*** Harv., Utrecht, *N.G. Bergh 1523* (NBG), MF382029*, MF382019*, –, –; ***A. heterophylla*** **(a)** (Thunb.) Less., Kouga, *D. Euston-Brown s.n.* (BOL), MF382030*, MF382020*, –, –; ***A. heterophylla*** **(b)**, Caledon, *P. Goldblatt 11634* (NBG), –, MF382021*, MF382045*, –; ***A. heterophylla*** **(c)**, Joubertina, *P. Goldblatt & L.J. Porter 12493* (NBG), –, MF382022* MF382046*,–; ***A. phylicoides*** **(a)** DC., Rhodes, *Romo & al. 14395* (PRE), FN645634.1*, FN645816.1*, FN645751.1*, –; ***A. phylicoides*** **(b)**, KF997220.1, KF997176.1, –, –; ***A.*** cf. ***rosmarinifolia*** **(a)** (Sch.Bip. ex Walp.) Oliv. & Hiern, Nyika National Park, *C.K. Willis & J.M. Luhanga 78* (PRE), MF382028*, MF382014*, –, –; ***A. rosmarinifolia*** **(b)**, Ethiopia near Geddo, *W.J.J.O. de Wilde 10436* (K), MF382031*, MF382015*, –, –; ***A. subsimplex*** Brenan, Malawi, *Pócs 6752/C* (K), –, MF382023*, –, –; ***Arrowsmithia styphelioides*** **(a)** DC., –, KF997179, –, –; ***A. styphelioides*** **(b)**, –, KF997178, –, KF997135; ***A. styphelioides*** **(c)**, KF997221, KF997177, KF997136, –; ***Calendula officinalis*** L., JN315917.1, –, –, JN315965.1; ***Comborhiza longipes*** (K.Bremer) Anderb. & K.Bremer, Tulbagh Valley, *E. Esterhuysen 30022* (BOL), –, KY694166*, –, –; ***C. virgata*** **(a)** (N.E.Br.) Anderb. & K.Bremer, KF997224, KF997180, KF997137, –; ***C. virgata*** **(b)**, Drakensberg, *N.G. Bergh 2179* (NBG), KY694310*, KY694167*, KY694229*, KY694269*; ***C. virgata*** **(c)**, Drakensberg, *N.G. Bergh 2195* (NBG), KY694311*, KY694168*, KY694230*, KY694270*; ***Cotula coronopifolia*** L., –, AF422118.1, –, HQ439873.1; ***Dimorphotheca aurantiaca*** (Thunb.) Less., South Africa, *N.G. Bergh 1722B* (NBG), MF382032*, MF382024*, MF382047*, MF382052*; ***Felicia filifolia*** Burtt Davey, AF319703.1, –, –, AF318120.1; ***Dolichothrix ericoides*** (Lam.) Hilliard & B.L.Burtt, JF893776.1, JF893871.1, –, JF893839.1; ***Gamochaeta subfalcata*** (Cabrera) Cabrera, FN645557.1, FN645834.1, –, –; ***Garuleum bipinnatum*** Less., KR611332, KR611331, –, KR611333; ***Ifloga molluginoides*** (DC.) Hilliard, FR821627.1, –, –, FR822639.1; ***Ifloga spicata*** (Forrsk.) Sch.Bip., –, KR559465, –, FR822640.1; ***Lasiopogon glomerulatus*** (Harv.) Hilliard, KP294102.1, –, –, KP294181.1; ***Leysera gnaphalodes*** **(a)** (L.) L., FN645636.1, FN645815.1, FN645750.1, –; ***L. gnaphalodes*** **(b)**, Namaqua National Park, *M. Koekemoer 3133* (PRE), –, KY694169*, KY694231*, –; ***L. leyseroides*** (Desf.) Maire, –, KF997181, –, KR559442; ***L. tenella*** DC., Kamieskroon, *N.G. Bergh 1554* (NBG), KY694350*, KY694170*, KY694232*, –; ***Macowania abyssinica*** **(a)** (Sch.Bip. ex Sch.Bip.) B.L.Burtt, –, KF997183, –, –; ***M. abyssinica*** **(b)**, –, KF997182, KF997139, –; ***M. conferta*** **(a)** (Benth.) E.Phillips, KF997225, KF997184, KF997140, –; ***M. conferta*** **(b)**, KF997226, KF997185, KF997141, –; ***M. corymbosa*** **(a)** M.D.Hend., KF997227, KF997186, KF997142, –; ***M. corymbosa*** **(b)**, –, KF997188, –, –; ***M. corymbosa*** **(c)**, KF997228, KF997187, KF997143, –; ***M. deflexa*** **(a)** Hilliard & B.L.Burtt, KF997229, KF997189, KF997144, –; ***M. deflexa*** **(b)**, KF997230, KF997190, KF997145, –; ***M. ericifolia*** (Forssk.) B.L.Burtt & Grau, –, KF997191, –, KF997146; ***M. glandulosa*** **(a)** N.E.Br., KF997232, KF997193, –, –; ***M. glandulosa*** **(b)**, KF997231, KF997192, KF997147, –; ***M. hamata*** **(a)** Hilliard & Burtt, KF997233, KF997194, KF997148, –; ***M. hamata*** **(b)**, KF997234, KF997195, KF997149, –; ***M. hamata*** **(c)**, KF997235, KF997196, KF997150, –; ***M. pinifolia*** **(a)** (N.E.Br.) Kroner, Drakensberg, *J. Bentley 003* (NBG), KF997236, KF997197, MF382048*, KF997151; ***M. pinifolia*** **(b)**, KF997237, KF997198, –, –; ***M. pinifolia*** **(c)**, KF997238, KF997199, KF997152, –; ***M. pinifolia*** **(d)**, – KF997200, –, KF997153; ***M. pulvinaris*** **(a)** N.E.Br. KF997240, KF997201, KF997154, –; ***M. pulvinaris*** **(b)**, KF997241, KF997202, KF997155, –; ***M. pulvinaris*** **(c)**, KF997242, KF997203, KF997156, –; ***M. revoluta*** **(a)** Oliv., KF997243, KF997204, –, –; ***M. revoluta*** **(b)**, KF997244, KF997205, KF997157, –; ***M. sororis*** **(a)** Compton, KF997245, KF997206, KF997158, –; ***M. sororis*** **(b)**, –, KF997207, –, –; ***M. sororis*** **(c)**, KF997246, KF997208, KF997159, –; ***M. tenuifolia*** **(a)** M.D.Hend., –, KF997209, –, KF997160; ***M. tenuifolia*** **(b)**, KF997247, KF997210, KF997161, –; ***M. tenuifolia*** **(c)**, Mount Sheba, *N.G. Bergh 2211* (NBG), KF997248, KF997211, MF382049*, KF997162; ***Nestlera biennis*** (Jacq.) Spreng., Elandsbaai, *N.G. Bergh 1819* (NBG), KY694351*, KY694171*, KY694233*, KY694271*; ***Oedera capensis*** **(a)** (L.) Druce, Gansbaai, *M. Koekmoer 3731* (PRE), KY694312*, KY694172*, –, KY694274 *; ***O. capensis*** **(b)**, Bontebok National Park, *N.G. Bergh 1771* (NBG), KY694352*, KY694174*, –, –; ***O. capensis*** **(c)**, De Hoop Nature Reserve, *M. Koekemoer 4051* (PRE), KY694313*, KY694173*, KY694234*, KY694275*; ***O. conferta*** (Hutch.) Anderb. & K. Bremer, Kamieskroon, *N.G. Bergh 2103* (NBG), KY694353*, KY694175*, –, KY694276*; ***O. epaleacea*** **(a)** Beyers, Ceres, *J.B.P. Beyers 272* (PRE), KY694314*, KY694176*, KY694235*, KY694277*; ***O. epaleacea*** **(b)**, Swartruggens, *N.G. Bergh 1748* (NBG), KY694354*, KY694177*, KY694236*, KY694282*; ***O. genistifolia*** **(a)** (L.) Anderb. & K.Bremer, Hessequa Reserve, *J. Bentley 264* (BOL), KY694315*, KY694178*, KY694237*, KY694277*; ***O. genistifolia*** **(b)**, Gifberg, *J. Bentley 295* (BOL), KY694316*, KY694179*, KY694238*, KY694278*; ***O. genistifolia*** **(c)**, Grahamstown, *M. Koekemoer 2336* (PRE), KY694317*, KY694180*, –, KY694279*; ***O. genistifolia*** **(d)**, KF997249, KF997212, KF997163, –; ***O. hirta*** Thunb., Grootwinterhoek, *H.C. Taylor 9732* (PRE), KY694318*, KY694181*, KY694239*, KY694283*; ***O. imbricata*** **(a)** Lam., Calitzdorp, *M. Koekemoer 1436* (PRE), KY694319*, KY694182*, –, –; ***O. imbricata*** **(b)**, De Hoop Reserve, *N.G. Bergh 1754* (NBG), –, KY694183*, KY694240*, KY694284*; ***O. multipunctata*** **(a)** (DC.) Anderb. & K.Bremer, Botterkloof Pass, *C.L. Bellamy 17* (PRE), KY694320*, KY694184*, –, –; ***O, multipunctata*** **(b)**, Cederberg, *N.G. Bergh 1808* (NBG), KY694356*, KY694185*, –, –; ***O. nordenstamii*** (K.Bremer) Anderb. & K.Bremer, Richtersveld, *N.G. Bergh 2120* (NBG), KY694321*, KY694186*, KY694241*, KY694285*; ***O. resinifera*** **(a)** (K.Bremer) Anderb. & K.Bremer, Montagu, *M.B. Bayer 6102* (PRE), KY694322*, KY694187*, –, KY694286*; ***O. resinifera*** **(b)**, Riversdal, *N.G. Bergh 2044* (NBG), KY694323*, KY694188*, KY694242*, – ; ***O. sedifolia*** **(a)** (DC.) Anderb. & K.Bremer, Swartberg Pass, *J. Bentley 015* (NBG), KY694324*, KY694189*, –, –; ***O. sedifolia*** **(c)**, Tankwa Karoo, *N.G. Bergh 1745* (NGB), KY694357*, KY694190*, –, KY694287*; ***O. silicicola*** (K.Bremer) Anderb. & K.Bremer, Vredendal, *N.G. Bergh 2088* (NBG), –, KY694191*, –, –; ***O.* sp.**, Kwadousberg, *N.A. Helme 4887* (NBG), KY694325*, KY694192*, KY694243*, –; ***O. steyniae*** (L.Bolus) Anderb. & K.Bremer, KF997250, KF997213, KF997164, –; ***O. squarrosa*** **(a)** (L.) Anderb. & K.Bremer, Villiersdorp, *J. Bentley 193* (BOL), KY694326*, KY694193*, KY694244*, KY694288*; ***O. squarrosa*** **(b)**, Pakhuis Pass, *M. Koekemoer 3634* (PRE), KY694328*, KY694195*, KY694246*, KY694291*; ***O. squarrosa*** **(c)**, Gifberg, *J. Bentley 282* (BOL), KY694327*, KY694194*, KY694245*, KY694289*; ***O. squarrosa*** **(d)**, Karoopoort, *N.G. Bergh 1065* (NBG), KY694358*, KY694196*, KY694247*, KY694290*; ***O. uniflora*** **(a)** (L.f.) Anderb. & K.Bremer, De Hoop Nature Reserve, *M. Koekemoer 4050* (PRE), KY694329*, KY694197*, KY694248*, KY694294*; ***O. uniflora*** **(b)**, Bredasdorp, *J.J. Meyer 1787* (PRE), KY694330*, KY694198*, –, KY694292*; ***O. uniflora*** **(c)**, KF997251, KF997214, KF997166, –; ***O. viscosa*** **(a)** (L'Hér.) Anderb. & K.Bremer, Malmesbury, *N.A. Helme 3151* (NBG), KY694331*, KY694199*, KY694249*, –; ***O. viscosa*** **(b)**, Gifberg, *N.G. Bergh 1813* (NBG), KY694332*, KY694200*, –, KY694295*; ***Oreoleysera montana*** (Bolus) K.Bremer, Hex River, *N.A. Helme 2863* (NBG), –, KY694201*, –, –; ***Osmitopsis asteriscoides*** (L.) Less., KR559453, KR559463, –, –; ***Pentatrichia alata*** **(a)** S.Moore, Pilgrims Rest, *N.G. Bergh 2209* (NBG), FR823344.1, FR832504.1 –, FR832577.1; ***P. alata*** **(b)**, Pilgrims Rest, *H. Burn 9016*, FR823345.1, FR832506.1, –, –; ***P. integra*** (Compton) Klaassen & N.G.Bergh, –, FR832499.1, –, FR832573.1; ***P. kuntzei*** **(a)** (O.Hoffm.) Klaassen & N.G.Bergh, FR823340.1, FR832500.1, FR832574.1, –; ***P. kuntzei*** **(b)**, Cogmanskloof, *R.J. Bayer & C.F. Puttock s.n.* (NBG), MF382039*, MF382025*, –, –; ***P. petrosa*** **(a)** Klatt, FR823346.1, FR832507.1, –, FR832578.1; ***P. petrosa*** **(b)**, FR823347.1, FR832508.1, –, –; ***P. petrosa*** **(c)**, FR823348, FR832509, FR832580, –; ***P. petrosa*** **(d)**, HM245973.1, HM246013.1, –, HM246075.1; ***P. rehmii*** subsp. ***rehmii*** **(a)** (Merxm.) Merxm., FR823352.1, FR832513.1, FR832584.1, –; ***P. rehmii*** subsp. ***rehmii*** **(b)**, FR823353.1, FR832514.1, FR832585.1, –; ***P. rehmii*** subsp. ***rehmii*** **(c)**, –, FR832515.1, FR832586.1, –; ***P. rehmii*** subsp. ***avasmontana*** **(a)** (Merxm.) Klaasen & Kwembeya, FR823350.1, FR832511.1, FR832582.1, –; ***P. rehmii*** subsp. ***avasmontana*** **(b)**, FR823351.1, FR832512.1, FR832583.1, –; ***P. rehmii*** subsp. ***avasmontana*** **(c)**, HM245972.1, HM246012.1, –, –; ***Phagnalon barbeyanum*** Asch. & Schweinf., HM245976.1, HM246016.1, HM246076.1, –; ***P. bicolor*** Ball, HM245977.1, HM246017.1, HM246077.1, –; ***P. embergeri*** (Humbert & Maire) Qaiser & Lack, HM245968.1, HM246008.1, HM246069.1, –; ***P. graecum*** Boiss. & Heldr., HM245981.1, HM246021.1, HM246080.1, –; ***P. latifolium*** Maire, HM245985.1, HM246025.1, HM246083.1, –; ***P. platyphyllus*** (Maire) Qaiser & Lack, HM245970.1, HM246010.1, HM246071.1, –; ***P. pygmaeum*** (Sieber) Greuter, HM245993.1, HM246033.1, HM246091.1, –; ***P. saxatile*** (L.) Cass., HM245996.1, HM246036.1, HM246093.1, HM246053.1 HM246064.1; ***P. sordidum*** (L.) Rchb., HM245999.1, HM246039.1, HM246096.1, HM246065.1 HM246054.1; ***P. stenolepis*** Chiov., HM246001.1, HM246041.1, HM246098.1, –; ***P. umbelliforme*** DC., HM246002.1, HM246042.1, HM246099.1, –; ***Printzia auriculata*** Harv., –, KR559458, –, KR559440; ***Relhania acerosa*** **(a)** (DC.) K.Bremer, Naudesnek, *M. Koekemoer 2067* (PRE), KR559448, KR559457, KR559439, –; ***R. acerosa*** **(b)**, KF997252, KF997215, –, KF997167; ***R. acerosa*** **(c)**, Qwa Qwa National Park, *P.C. Zietsman 3536* (PRE), KY694333*, KY694202*, KY694250*, –; ***R. calycina*** **(a)** (L.f.) L'Hér, Swartberg, *J. Bentley 10* (NBG), KY694334*, KY694203*, KY694251*, KY694296*;

Appendix 1. Continued.

R. calycina* (b)**, Swartberg, *M. Koekemoer 3471* (PRE), KY694335*, KY694204*, –, KY694297*; ***R. corymbosa (Bolus) K.Bremer, Laingsburg, *N.G. Bergh 1795* (NBG), KY694359*, KY694205*, KY694252*, KY694298*; ***R. dieterlenii* (a)** (E.Phillips) K.Bremer, Rhodes, *M.S. Mothogoane 127* (PRE), KY694336*, KY694206*, KY694253*, –; ***R. dieterlenii* (b)**, KF997253, KF997216, –, KF997168; ***R. fruticosa* (a)** (L.) K.Bremer, Romansrivier, *J. Bentley 471* (NBG), KY694337*, KY694207*, KY694254*, –; ***R. fruticosa* (b)**, Elandsberg, *J. Manning 3365A* (NBG), –, KY694208*, KY694255*, –; ***R. garnotii* (a)** (Less.) K.Bremer, Bredasdorp, *P. Goldblatt 5617* (PRE), KY694338*, KY694209*, –, KY694300*; ***R. garnotii* (b)**, De Hoop Nature Reserve, *N.G. Bergh 1755* (NBG), KY694360*, KY694210*, KY694256*, KY694299*; ***R. pungens* (a)** L'Hér, Mpofu Game Reserve, *C.L. Bredenkamp 3353* (PRE), KY694339*, KY694211*, KY694257*, KY694301*; ***R. pungens* (b)**, Garcias Pass, *M. Koekemoer 3430* (PRE), KY694340*, KY694212*, –, –; ***R. pungens* (c)**, Bontebok National Park, *N.G. Bergh 1772* (NBG), KY694341*, KY694213*, KY694258*, KY694302*; ***R. relhanioides* (a)** (Schltr.) K.Bremer, Laingsburg, *P. Goldblatt 4176* (PRE), KY694342*, KY694214*, KY694260*, KY694303*; ***R. relhanioides* (b)**, Touwsriver, *N.G. Bergh 2067* (NBG), KY694343*, KY694215*, KY694259*, –; ***R. rotundifolia* (a)** Less., Hopefield, *N.A. Helme 5585* (NBG), KY694344*, KY694216*, KY694261*, –; ***R. rotundifolia* (b)**, KF997254, KF997217, KF997169, –; ***R. spathulifolia*** K.Bremer, Elim, *N.G. Bergh 1777* (NBG), KY694361*, KY694217*, –, –; ***R. speciosa* (a)** (DC.) Harv., Touwsberg, *C.L. Bredenkamp 841* (PRE), KY694345*, KY694218*, KY694262*, KY694304*; ***R. speciosa* (b)**, Swartberg, *J. Bentley 11* (NBG), KY694346*, KY694219*, –, –; ***R. speciosa* (c)**, Laingsburg, *N.G. Bergh 1797* (NBG), KY817317*, –, KY694263*, KY694305*; ***R. tricephala* (a)** (DC.) K.Bremer, Waboomsberge, *Koekemoer 489* (PRE), KY694347*, KY694220*, –, –; ***R. tricephala* (b)**, Ceres, *N.G. Bergh 1790* (NBG), KY694362*, KY694221*, KY694264*, KY694306*; ***Rhynchopsidium pumilim*** (L.f.) DC., Namaqua National Park, *H.M. Steyn 1328* (PRE), –, KY694222*, KY694267*, –; ***R. sessiliflorum* (a)** (L.f.) DC., Blinkberg Pass, *M. Koekemoer 2404* (PRE), –, KY694223*, KY694268*, –; ***R. sessiliflorum* (b)**, KF997255, KF997218, KF997170, –; ***Rosenia glandulosa*** Thunb., Calvinia, *N.G. Bergh 1729* (NBG), KY694363*, KY694224*, –, –; ***R. humilis* (a)** (Less.) K.Bremer, Middelburg, *E.P. Nienaber 427* (PRE), KY694348*, –, KY694265*, KY694307*; ***R. humilis* (b)**, Victoria West, *M. Koekemoer 2865* (PRE), KY745899*, KF997219*, KF997171*, –; ***R. oppositifolia* (a)** (DC.) K.Bremer, Sutherland, *M. Koekemoer 2771* (PRE), KY694364*, KY694225*, –, –; ***R. oppositifolia* (b)**, Roggeveld, *N.G. Bergh 1705* (NBG), KY694365*, KY694226*, –, KY694308*; ***R. spinescens* (a)** DC., South Africa, *B s.n.* (PRE), KY694366*, KY694227*, –,KY694309*; ***R. spinescens* (b)**, Roggeveld, *N.G. Bergh 1740* (NBG), KY694367*, KY694228*, KY694266*, –; ***Stoebe cinerea*** (L.). Thunb., KR559447, –, –, KR559438; ***Syncarpha canescens*** (L.) B.Nord., KR559446, KR559456, –, KR559437; ***Senecio vulgaris*** L., HQ43955.1, AF422136.1, –, HQ439900.1.