## MOLECULAR PHYLOGENETICS AND BIOGEOGRAPHY

# Generic delineation, phylogeny and subtribal affinities of *Phagnalon* and *Aliella* (Compositae, Gnaphalieae) based on nuclear and chloroplast sequences

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**Abstract**  The precise generic delimitation of *Aliella* and *Phagnalon* and their tribal affinities are at present unresolved. The main goals of our study were to verify the monophyly of these two genera and to determine their closest affinity group within Gnaphalieae. We analysed sequences of the *trnL* intron and *trnL-trnF* spacer of Gnaphalieae and other Compositae tribes, in order to elucidate the tribal position of *Aliella*, *Macowania*, *Phagnalon* and *Philyrophyllum*. In addition, we analysed ribosomal nrDNA together with the *ycf3-trnS* and *trnT-trnL* spacers of cpDNA to elucidate the relationships within *Aliella* and *Phagnalon*. The genera *Anisothrix*, *Athrixia* and *Pentatrichia* are closely related to *Aliella* and *Phagnalon*. *Aliella*, *Macowania* and *Phagnalon* are nested within the "*Relhania* clade", and the subtribal affinities of *Philyrophyllum* lie within the "crown radiation clade". The monophyly of *Aliella* and *Phagnalon* is not supported statistically and *Aliella* is paraphylethic in most of the analyses. The resulting phylogeny suggests an African origin for *Aliella* and *Phagnalon* and identifies three main clades in *Phagnalon*, the Irano-Turanian clade, the Mediterranean-Macaronesian clade and the Yemenite-Ethiopian clade. Some endemics to Yemen and Ethiopia are resolved in the Mediterranean-Macaronesian clade, providing new evidence of phytogeographical links between Macaronesia, Eastern Africa and Southern Arabia. Incongruence between the chloroplast and nuclear molecular data and the lack of resolution in some clades may indicate that hybridization could have played an important role in the evolution and diversification of *Phagnalon* and *Aliella*.

**Keywords**  *Aliella*; *Anisothrix*; *Athrixia*; ETS; ITS; *Phagnalon*; *Pentatrichia*; *trnL* intron; *trnL-trnF* intergenic spacer; *trnT-trnL*; *ycf3-trnS*

## ■ INTRODUCTION

The tribe Gnaphalieae is one of the largest of Compositae, with 185 genera and 1240 species worldwide but being most diverse in South America, South Africa and Australia (Bayer & al., 2007). The tribe was first recognized as independent from Inuleae by Anderberg (1989, 1991), who divided the classic Inuleae (sensu Merxmüller & al., 1977) into the three tribes Inuleae, Plucheeae and Gnaphalieae. Anderberg (1991) proposed classifying Gnaphalieae into five subtribes (Angianthinae, Cassiniinae, Gnaphaliinae, Loricariinae, Relhaniinae) based on cladistic analyses of morphological characters. However, phylogenetic studies of DNA sequences have shown that it is not only the sister relationships that are unclear but also the subtribal circumscription (Bayer & Starr, 1998; Bayer & al., 2000, 2002, 2007; Panero & Funk, 2008; Ward & al., 2009). The results of these studies also showed that the subtribes delimited by Anderberg (1991) are not natural groups, implying that a recircumscription was necessary.

The tribal and infratribal positions of some genera of Gnaphalieae, mainly those that belong to the "basal group taxa" of Anderberg (1991), are another persisting problem. The "basal group taxa" are an assemblage of genera previously included in Inuleae-Athrixiinae or Inuleae-Gnaphaliinae by Merxmüller & al. (1977), together with other genera considered plesiomorphic relatives of Gnaphalieae (Anderberg, 1989). In fact, according to the latest phylogenethic studies on Gnaphalieae, some of those genera are nested in the "*Relhania* clade" (Ward & al., 2009). The members of the "*Relhania* clade" and most of the genera of the "basal group taxa" are mainly distributed throughout Southern and tropical Africa and Australia, with several representatives in Macaronesia and the Mediterranean, North Africa, Arabia, the Middle East and the Irano-Turanian regions (Anderberg, 1991). As reported by Ward & al. (2009), the three main clades within Gnaphalieae are the "*Relhania* clade", which is sister to the rest of Gnaphalieae s.str. (as previously noted by Bayer & al., 2000); the "*Dolichothrix-Phaenocoma* clade", which is next to diverge from the "*Relhania* clade"; and the "crown radiation clade", which comprises the rest of the genera included in the phylogeny of Gnaphalieae. The "crown radiation clade" included genera previously assigned to the different subtribes delimited by Anderberg (1991) and some of the genera included in the "basal group taxa". However, the infratribal affinities of some of the members of the "basal group taxa" are unknown from the molecular point of view.

**The genera *Aliella* and *Phagnalon*.** — Two genera of the "basal group taxa" are *Aliella* Qaiser & Lack and *Phagnalon* Cass. Morphological analysis failed to resolve the affinities of the two genera (Anderberg, 1989). *Phagnalon* was placed in the tribe Inuleae, subtribe Gnaphaliinae, series Eugnaphalieae (Bentham, 1873). Later, it was accommodated in an informal group in the treatment of Inuleae by Merxmüller & al. (1977) because it has some intermediate characters between Gnaphaliinae, Inuliinae and Athrixiinae; these features include filiform female florets, cartilaginous involucral bracts, apically confluent stigmatic areas, tailless anthers, geographic distribution and a high basic chromosome number. Anderberg (1989) placed *Aliella* and *Phagnalon* in Gnaphalieae based on morphological data. However, the results did not clearly indicate the subtribal affinities of the two genera but showed that *Phagnalon* was nested within a clade with *Anisothrix* O. Hoffm., whereas *Aliella* was more related to other taxa of Gnaphalieae. Therefore, Anderberg (1991) included *Aliella* and *Phagnalon* in an informal group in Gnaphalieae together with *Anisothrix*, *Pentatrichia*, *Philyrophyllum* and others.

*Phagnalon* comprises about 36 species (Qaiser & Abid, 2003) and is distributed throughout Northeastern tropical Africa, the Macaronesian region, the Mediterranean basin, the Irano-Turanian region and the Saharo-Arabian region, but its greatest diversity is found in the Arabian Peninsula. All species are suffruticose chamaephytes and grow mainly in rocky areas in a wide range of habitats, ranging from sea level to 3800 m.

*Aliella* comprises four endemic species from the Atlas Mountains of Morocco: *Aliella ballii* (≡ *A. helichrysoides*), *A. embergeri*, *A. iminouakensis* and *A. platyphylla*. The four species are caespitose chamaephytes and grow in calcareous or siliceous rock crevices from 1800 to 3600 m in altitude.

Maire (1928) recognized two sections within *Phagnalon*, *Ph.* sect. *Gnaphaliopsis* and sect. *Euphagnalon*. *Phagnalon* sect. *Gnaphaliopsis* was characterized by caudate anthers and encompasses *Ph. embergeri*, *Ph. helichrysoides* and *Ph. platyphyllum*. The latter group of taxa was previously classified into *Helichrysum* Mill. and *Gnaphalium* L. (Ball, 1873; Klatt, 1896) and was later transferred to the genus *Aliella* by Qaiser & Lack (1986b) based on the presence of bracts on the peduncle and waxy cushions on the corolla lobes, tubular female florets, caudate anthers and pappus setae barbellate from the base to the apex. *Aliella iminouakensis* was combined under *Aliella* by Dobignard (1997). The genus *Aliella* has been widely accepted as an independent entity (Anderberg, 1991; Dobignard, 1997; Bayer & al., 2007; Ward & al., 2009).

All reports on the chromosome number of *Phagnalon* have shown that $2n = 18$. In *Aliella*, all the reports have also indicated that $2n = 18$ (Quézel, 1957; Humphries & al., 1978; Galland & Favarger, 1985), although there is one conflicting report on *A. ballii*, with $2n = 14$ (Humphries & al., 1978).

Despite several regional studies (Qaiser & Lack, 1985, 1986a; Qaiser & Abid, 2003), there is no complete revision of *Phagnalon* and the precise delimitation of the genus is still unclear. Because non-homologous morphological similarities are quite frequent in Gnaphalieae (Bayer & al., 2000), we have explored the relationships among *Phagnalon* and *Aliella* using nuclear and chloroplast phylogeny sequences. We also tested possible phylogenetic incongruities, as reported in other phylogenetic studies in Gnaphalieae (Smissen & al., 2004). Combined nuclear and chloroplast phylogenies have been successfully used in Compositae and Gnaphalieae at the generic and species levels (Bayer & al., 2000, 2002; Konishi & al., 2000; Álvarez & al., 2001; Susanna & al., 2003; Liu & al., 2004; Lee & al., 2005; Sonnante & al., 2007). We gathered information from several datasets of non-coding sequences, including ITS and ETS sequences of nrDNA, together with the *ycf3-trnS* and *trnL* introns and *trnL-trnF* and *trnT-trnL* spacers of cpDNA. Our objectives were: (1) to shed light on the position of *Aliella*, *Macowania*, *Phagnalon* and *Philyrophyllum* within Gnaphalieae; (2) to elucidate the relationships between the early-branching genera of the "*Relhania* clade" and *Aliella* and *Phagnalon*; (3) to check the hypothesized monophyly of *Aliella* and *Phagnalon*; (4) to contrast the sequence data with both the traditional classification and morphology; and (5) to infer the biogeographic patterns within *Aliella* and *Phagnalon*.

## ■ MATERIALS AND METHODS

**Plant material.** — The sample included 29 of 36 species of the genus *Phagnalon* together with four *Aliella* species. Representatives of the "*Relhania* clade" and the "basal group taxa" comprising *Leysera*, *Macowania*, *Philyrophyllum* and *Relhania*, with emphasis on those that are nested in the "*Relhania* clade", were also included. The affinities of *Aliella* and *Phagnalon* could not be determined from the molecular phylogenetic data; therefore, the selection of an appropriate outgroup for our phylogenetic reconstruction was difficult. Outgroups were chosen from Heliantheae, Inuleae, Calenduleae, Anthemideae and Astereae for the analysis of the *trnL* intron and the *trnL-trnF* intergenic spacer. To analyze the combined cpDNA dataset (*ycf3-trnS* + *trnT-trnL*), nrDNA dataset (ETS + ITS) and nr-cpDNA dataset (ETS + ITS + *ycf3-trnS* + *trnT-trnL*), outgroups were species of *Anisothrix*, *Pentatrichia* and *Athrixia* because they were placed as the closest genera to *Aliella* and *Phagnalon* in our previous *trnL* intron and *trnL-trnF* intergenic spacer analyses.

The analyses used 172 new sequences (40 ETS, 40 ITS, 34 *ycf3-trnS*, 34 *trnT-trnL* and 24 *trnL* intron and the *trnL-trnF* intergenic spacer) together with 47 sequences from published EMBL/GenBank accessions as documented in Bayer & Starr (1998), Bayer & al. (2000, 2002), Panero & Funk (2008), Bergh & Linder (2009), Galbany-Casals & al. (in press), Boelch & al. (unpub.), Panero & al. (unpub.). Sources of published sequences, voucher data and GenBank sequence accession numbers are given in the Appendix.

**DNA extraction.** — Total genomic DNA was extracted following the CTAB method of Doyle & Doyle (1987) as modified by Cullings (1992) from silica-gel–dried leaves collected in the field, herbarium material, or fresh leaves from plants cultivated in the Botanic Institute of Barcelona. DNA from old herbarium specimens was extracted using the Nucleospin Plant Kit (Macherey Nagel Inc., Pennsylvania, U.S.A.) or with the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, California, U.S.A.).

**nrDNA ITS and ETS region amplification strategies. —** Double-stranded DNA of the ITS region was obtained by PCR amplification and was sequenced using 17 SE (Sun & al., 1994) and ITS1 (White & al., 1990) as the forward primers, together with 26 SE (Sun & al., 1994) and ITS4 (White & al., 1990) as the reverse primers. In some cases, ITS1 and ITS2 were amplified and sequenced separately using 5.8 I2 as the forward primer and 5.8 I1 as the reverse primer (Sun & al., 1994). The PCR profile used for the amplification followed the protocol of Galbany-Casals & al. (2004). The ETS region was amplified by PCR and sequenced using ETS1F (Linder & al., 2000) and AST1F (Markos & Baldwin, 2001) as the forward primers and AST1R (Markos & Baldwin, 2001), 18S2L (Linder & al., 2000) and 18S-ETS (Baldwin & Markos, 1998) as the reverse primers. The PCR profile followed the protocol of Galbany-Casals & al. (2009).

In some cases, to obtain readable sequences, the PCR products were cloned using TOPO TA Cloning Kit (Invitrogen, Carlsbad, California, U.S.A.) following the manufacturer's instructions and using half reactions. When possible, ten positive colonies were screened via PCR using the T7 and M13 universal primers, using the PCR procedure described in Vilatersana & al. (2007). Five to ten PCR products were selected for sequencing in both directions using the same primers.

**cpDNA *ycf3-trnS*, *trnL* intron, *trnL-trnF* intergenic spacer and *trnT-trnL* region amplification strategies. —** Double-stranded DNA of the *ycf3-trnS* spacer was amplified by PCR and sequenced using SP43122F and SP44097R as the forward and reverse primers, respectively (Hershkovitz, 2006). Double-stranded DNA of the *trnT-trnL* spacer was amplified and sequenced using trnT-A2F (Cronn & al., 2002) as the forward primer and trnL-b as the reverse primer (Taberlet & al., 1991). The PCR profile used for the amplification of both spacers included a start at 95°C for 1 min 35 s, followed by 35 cycles of denaturation at 95°C for 1 min 30 s, annealing at 52°C for 1 min 30 s, extension at 72°C for 2 min and a final extension at 72°C for 10 min. Double-stranded DNA of the *trnL*(UAA) intron, the *trnL*(UAA) 3′ exon and the intergenic spacer between the *trnL*(UAA) 3′ exon and the *trnF*(GAA) 5′ exon was amplified and sequenced using the primers c, d, e and f (Taberlet & al., 1991). The PCR profile used for the amplification followed the protocol of Susanna & al. (2006).

**Purification of the PCR products and sequencing. —** The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc.). PCR products of recalcitrant samples were purified according to the manufacturer's instructions included with the DNA Clean and Concentrator Kit (Zymo Research Inc., California, U.S.A.). Direct sequencing of the amplified DNA segments was performed at the "Serveis Cientificotècnics" of the University of Barcelona using a BigDye Terminator Cycle Sequencing v.3.1 (Applied Biosystems, Foster City, California, U.S.A.) following the manufacturer's protocol and analyzed on an ABI PRISM 3730 DNA analyzer (Applied Biosystems).

**Phylogenetic analysis. —** The nucleotide sequences were edited using Chromas v.1.56 (Technelysium Pty, Tewantin, Australia). The DNA sequences were aligned visually by sequential pairwise comparison (Swofford & Olsen, 1990). The phylogenetic analyses were performed with the five following datasets: (1) *trnL* intron and *trnL-trnF* intergenic spacer regions to (i) elucidate the tribal positions of *Aliella*, *Macowania*, *Phagnalon* and *Philyrophyllum* and to (ii) determine the relationships between *Aliella* and *Phagnalon* and the early branching genera of the "*Relhania* clade"; this dataset included 59 taxa; (2) ETS+ITS+*ycf3-trnS*+*trnT-trnL* to study the generic limits of *Aliella* and *Phagnalon* and to elucidate the relationships within the *Aliella* and *Phagnalon* taxa; this dataset included 34 taxa; (3) ETS+ITS to examine the position of some taxa not included in previous analyses and for which we could not amplify any chloroplast region; this dataset included 40 taxa; (4) *ycf3-trnS*+*trnT-trnL* to verify the relationships between *Aliella* and *Phagnalon* and to elucidate possible incongruencies with the nrDNA data; this dataset included 34 taxa; and (5) ETS+ITS, performed with the exclusion of *Ph. latifolium* and *A. iminouakensis*; this dataset included 38 taxa. Phylogenetic studies have sometimes been performed with the exclusion of hybrid taxa from the analysis with the aim of testing its putative hybrid origin. The analyses are usually performed after the detection of incongruencies between different datasets to avoid disruptive effects on the topology produced by introgression effect in the data (Vriesendorp & Bakker, 2005). Similarly, the low support levels of the internal nodes in a tree have been previously considered characteristic of hybridization and introgression (Wendel & Doyle, 1998; Font & al., 2002). Moreover, better resolution and an increase in the bootstrap in the topology of phylogenetic trees was also reported in other plant groups after excluding putative hybrid taxa (e.g., in *Armeria* Willd. and *Hippophae* L.), where fewer most parsimonious trees (MPTs) with a higher consistency index were found (Sun & al., 2002; Fuertes Aguilar & Nieto Feliner, 2003). We used the combined nrDNA dataset to test the behaviour and possible origin of *Ph. latifolium* and *A. iminouakensis* and to assess their effects on the topology of the MPTs. Congruence in the phylogenetic signal between the combined nrDNA datasets, nrDNA and cpDNA and combined cpDNA datasets was examined by a visual comparison of the tree topologies and by conducting an ILD test (ILD; Farris & al., 1995a,b). The ILD significance values were calculated using 1000 replicates with TNT (Goloboff & al., 2003–2005) using the INCTST script, which was kindly provided by the authors of the program.

**Parsimony analysis. —** Parsimony analysis involved heuristic searches and was conducted with PAUP* v.4.0b10 (Swofford, 2002) using tree bisection reconnection (TBR) branch swapping with the character states unordered and unweighted. All most parsimonious trees were saved. To locate islands of MPTs (Maddison, 1991), 100 replications were carried out with random taxon addition and with TBR branch swapping. The insertion/deletion events (indels) were not excluded from the analysis and were coded as missing data. Tree lengths, the consistency index (CI), retention index (RI) and homoplasy index (HI) were always given after excluding uninformative characters. Bootstrap (BS; Felsenstein, 1985) analyses were performed using 100 replicates of heuristic search with simple

taxon addition and TBR branch swapping to obtain support estimates for the nodes in the consensus trees. In the analyses of the *trnL* intron region, *trnL-trnF* intergenic spacer region and *ycf3-trnS+trnT-trnL* datasets, we followed the approach of Lidén & al. (1997) using 1000 replicates, random taxon addition with 20 and 10 replicates, respectively, and no branch swapping.

**Bayesian analyses. —** Three partitions were defined (two cpDNA, one nrDNA) to analyze the ETS, ITS, *ycf3-trnS* and *trnT-trnL* datasets prior to the Bayesian analyses. Two partitions were also defined to analyze the *ycf3-trnS+trnT-trnL* dataset. The *trnL* intron and *trnL-trnF* intergenic spacer datasets were analyzed as a single dataset without defining partitions. The best fit model of nucleotide substitution for each partition was selected by means of hierarchical likelihood ratio tests (hLRTs) and Akaike information criterion (AIC) as implemented in MrModeltest v.2.2 (Nylander, 2004). The optimal nucleotide-substitution model *trnL* intron and *trnL-trnF* intergenic spacer was general time reversible (Rodríguez & al., 1990) with variable sites assumed to follow a discrete gamma distribution (GTR+G). However, the Felsenstein Model (Felsenstein, 1981) using a gamma distribution to account for rate variation among sites (F81+G) was selected as the best-fit model for *trnT-trnL*, while the Felsenstein Model (Felsenstein, 1981) was used for the *ycf3-trnS* with some sites assumed to be invariable and variable sites assumed to follow a discrete gamma distribution (F81+I+G). Bayesian analysis was carried out using MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Two analyses (each with four Markov chains), initiated with random starting trees, were run for $2 \times 10^6$ generations and sampled every 100 generations. The first 2000 trees, sampled prior to the stabilization of the log-likelihood value, were discarded as the burn-in samples. The posterior probabilities (PPs) were estimated for the post–burn-in trees by constructing a 50% majority-rule consensus tree in PAUP* v.4.0b10 (Swofford, 2002).

## ■ RESULTS

**Lengths and alignment of the ETS. —** Due to the different number of repeats located in the 5′ end, the three following types of ETS sequences could be distinguished: 1038 bp, 1342 bp and 1483 bp. However, the alignment was unambiguous, as in other Compositae genera (Linder & al., 2000), and required only small (11 bp) gaps outside of the subrepeats with the exception of a 21 bp deletion in *Ph. harazianum*. Interestingly, the 5′ ETS sequences showed homogeneity within *Aliella* and *Phagnalon* and also across the *Anisothrix* and *Pentatrichia* genera, as reported in other families (Volkov & al., 2003). In contrast, homology was difficult to establish with regards to other members of the "*Relhania* clade" like *Athrixia*, *Leysera*, *Macowania*, *Philyrophyllum* and *Relhania*.

**Comparison of the divergence and phylogenetic utility of chloroplast and nuclear DNA datasets. —** The main characteristics of the five combined datasets, along with the corresponding tree statistics, are summarized in Table 1. The ETS sequences were 3.47 times more informative regarding absolute numbers than the ITS sequences, and the average ingroup pairwise divergence in the complete ETS region was 1.36 times greater than ITS. Therefore, using ETS sequences seemed an appropriate strategy for phylogenetic reconstruction in terms of the number of informative characters, as suggested previously by other authors (Linder & al., 2000). Regarding chloroplast data, the cpDNA sequences were 3.32 times less variable according to average pairwise divergences and 6.83 times less informative than nrDNA sequences with regards to the absolute number of informative characters.

**Congruence assessment. —** The two methods of phylogenetic reconstruction led to congruent results for the majority of the strongly supported clades. However, some incongruent relationships were found among the nrDNA and cpDNA datasets.

**Table 1.** Comparison of results obtained from the ITS, ETS, ETS+ITS, ETS+ITS *A. iminouakensis* and *Ph. latifolium* excluded, *trnL* intron+*trnL-trnF* intergenic spacer, *ycf3-trnS+trnT-trnL* and ETS+ITS+*ycf3-trnS+trnT-trnL* datasets. The consistency and homoplasy indexes are calculated excluding uninformative characters.

| | ITS | ETS | ETS+ITS | ETS+ITS *A. iminouakensis* and *Ph. latifolium* excluded | *trnL* intron + *trnL-trnF* | *ycf3-trnS* +*trnT-trnL* | ETS+ITS +*ycf3-trnS* +*trnT-trnL* |
|---|---|---|---|---|---|---|---|
| Number of taxa | 40 | 40 | 40 | 38 | 59 | 34 | 34 |
| Total characters | 645 | 1652 | 2297 | 2297 | 861 | 1193 | 3478 |
| Informative characters | 53 (8.22%) | 184 (11.14%) | 237 (10.56%) | 220 (9.57%) | 112 (13.01%) | 30 (2.51%) | 235 (6.75%) |
| Number of MPTs | 141,317 | 2735 | 757 | 1759 | 1516 | 851,436 | 73 |
| Number of steps | 103 | 361 | 474 | 431 | 246 | 42 | 471 |
| Islands | 1 | 1 | 2 | 2 | 3 | 1 | 3 |
| Consistency index (CI) | 0.4422 | 0.5791 | 0.5782 | 0.5919 | 0.6260 | 0.7442 | 0.5714 |
| Retention index (RI) | 0.6095 | 0.7840 | 0.7819 | 0.4084 | 0.8521 | 0.8854 | 0.7434 |
| Range divergence, ingroup (%) | 0–4.94 | 0–4.75 | 0–4.66 | 0–4.66 | 0–8.46 | 0–1.12 | 0–2.93 |
| Divergence mean, ingroup (%) | 2.23 | 3.03 | 2.74 | 2.70 | 2.83 | 0.43 | 1.86 |
| Range divergence, ingroup-outgroup (%) | 1.61–6.13 | 5.73–8.25 | 4.61–7.22 | 4.60–7.21 | 2.66–10.23 | 0.80–1.64 | 3.57–5.06 |
| Divergence mean, ingroup-outgroup (%) | 3.63 | 7.13 | 5.91 | 5.92 | 5.61 | 1.28 | 4.37 |

First, *Ph. stenolepis* forms a monophyletic group together with the Atlas endemics (*Aliella iminouakensis*, *Ph. bicolor*) and other Mediterranean and Irano-Turanian taxa according to the combined cpDNA tree (PP = 0.97, BS = 66%; Fig. 4). In contrast, with regards to nrDNA data, *Ph. stenolepis* clustered in a highly supported clade only with the Yemenite and Ethiopian endemics *Ph. abyssinicum*, *Ph. harazianum*, *Ph. phagnaloides* and *Ph. woodii* (PP = 1.00, BS = 100%; Figs. 2 and 3). Secondly, from to the cpDNA tree, *Ph. niveum* forms a monophyletic group together with *Ph. sinaicum* and *Ph. sordidum* (PP = 1.00, BS = 70%; Fig. 4). Conversely and in accordance with nrDNA trees, *Ph. niveum* forms a highly supported monophyletic group together with other Irano-Turanian taxa (PP = 1.00, BS = 100%; Figs. 2 and 3), and *Ph. sinaicum* appears to be a sister to *Ph. schweinfurthii* (PP = 1.00, BS = 98/100% and PP = 1.00, BS = 100%, respectively; Figs. 2 and 3). However, because the reported incongruencies affected only some taxa from the Saharo-Arabian and Irano-Turanian clades, most of which were of unresolved phylogenetic position, we combined the data to enhance the resolution. Results from the ILD test also found significant incongruencies between the nrDNA datasets (ITS+ETS) and the cpDNA datasets (*ycf3-trnS*+*trnT-trnL*) (*P* = 0.001) and also between the *ycf3-trnS* and *trnT-trnL* datasets (*P* = 0.002). In addition, combining the different chloroplast regions is justified because the chloroplast genome is inherited as a single unit without recombinations (Soltis & Soltis, 1998). However, no significant incongruencies were found between the ETS dataset and the ITS dataset (*P* = 0.318).

**Phylogenetic analyses. —** The two methods of phylogenetic reconstruction yielded trees that were almost equal; hereon, our figures will only show the Bayesian majority-rule consensus trees with the addition of the parsimony bootstrap percentages. Figure 1 is the tree generated from the combined *trnL* intron and *trnL-trnF* intergenic spacer; Fig. 2 was produced by the combined nr-cpDNA dataset; Fig. 3 by the ETS+ITS datasets; Fig. 4 by the *ycf3-trnS*+*trnT-trnL* datasets, while Fig. 5 was the tree produced from ETS+ITS sequences performed with the exclusion of *Ph. latifolium* and *A. iminouakensis*.

The results obtained from the *trnL* intron–*trnL-trnF* intergenic spacer showed that the three main clades of Gnaphalieae found in previous phylogenetic studies (Bayer & al., 2000; Ward & al., 2009) are recovered, i.e., the "*Relhania* clade", the "*Dolichothrix-Phaenocoma* clade" and the "crown radiation clade". The "*Relhania* clade" is divided into two subclades: the first includes *Aliella*, *Anisothrix*, *Athrixia*, *Phagnalon* and *Pentatrichia* (PP = 1.00, BS = 88%) and the second is formed by *Arrowsmithia*, *Leysera*, *Macowania*, *Oedera*, *Relhania*, *Rhynchopsidium* and *Rosenia* (PP = 1.00, BS = 70%). Therefore, *Aliella*, *Macowania* and *Phagnalon* belong to the "*Relhania* clade". In contrast, *Philyrophyllum* appears nested in the "crown radiation clade" (PP = 1.00, BS = 68%). Species of *Athrixia* form a highly supported monophyletic group (PP = 1.00, BS = 88%). In the "*Dolichothrix-Phaenocoma* clade", *Metalasia* and *Dolichothrix* appeared as unresolved.

*Aliella* and *Phagnalon* form a strongly supported monophyletic group according to all the datasets (PP = 1.00, BS = 100/85; Figs. 1–5). The phylogenetic position of *Aliella* remains unresolved according to the results obtained from combined nr-cpDNA datasets because only three species of *Aliella* form a statistically supported monophyletic group (PP = 1.00, BS = 74%; Fig. 2). In addition, this clade is poorly supported according to the analyses of combined nrDNA dataset (PP = 0.91, BS = 53%; Fig. 3). From the results of the Bayesian analyses of the cpDNA dataset, *Aliella* appears paraphylethic because *A. iminouakensis* is firmly nested in the *Phagnalon* clade with significant Bayesian support (PP = 0.97, BS = 66%; Fig. 4). However, once the analysis was performed while excluding *Ph. latifolium* and *A. iminouakensis*, the bootstrap support of *Aliella* also increased slightly (from BS = 53% to BS = 65%) or maintained its value (from PP = 0.91 to PP = 0.90) (Fig. 5). Despite the paraphyly of *Aliella* and due to the position of *A. iminouakensis*, there is no nomenclatural impact as this species was described as *Phagnalon* and *A. platyphylla* was designated as the type species.

The monophyly of *Phagnalon* is not supported statistically according to both nr-cpDNA datasets (Figs. 1–4). However, if *Ph. latifolium* and *A. iminouakensis* are removed from the ingroup, the bootstrap support of *Phagnalon* clade increases from no bootstrap support to BS = 90% and from PP = 0.72 up to PP = 1.00, according to the combined nr-cp DNA dataset (Fig. 5).

Based on the combined nrDNA and nr-cpDNA datasets, the *Phagnalon* clade consists of two main clades. The first encompasses mainly the Irano-Turanian taxa (PP = 1.00, BS = 100%; Figs. 2 and 3). The second clade encompasses two branches: the first branch consists of the two subclades, the Eritreo-Arabian subclade (PP = 1.00; BS = 100%; Figs. 2 and 3) and the Saharo-Arabian clade (PP = 1.00; BS = 98%; Fig. 2) while the second evolutionary branch consists mainly of the Mediterranean and Macaronesian taxa (PP = 1.00, BS = 65; Figs. 2 and 3).

# ■ DISCUSSION

**Congruence between the cpDNA and ITS datasets. —** The ILD test results and inspection of the combined tree topologies from the separate analyses of the nrDNA and cpDNA datasets indicated some incongruencies. Reticulation and chloroplast capture are considered the most likely reasons behind the incongruencies between biparentally and maternally inherited genes at lower taxonomic levels (Rieseberg & Soltis, 1991). However, the different evolutionary histories of the nrDNA and cpDNA data could also be the cause of the disagreement between the topologies. Several processes have been indicated as sources of incongruence between nrDNA and cpDNA; some examples are insufficient phylogenetic signal (McKenzie & al., 2006), sampling error, convergence, long-branch attraction or incomplete linage sorting (Soltis & Kuzoff, 1995; Sang & Zong, 2000). Given the high Bayesian support of the incongruent relationships of *Ph. niveum* and *Ph. stenolepis* between the cpDNA tree (PP = 1.00 and PP = 0.97, respectively; Fig. 4) and the nrDNA tree (PP = 1.00 and PP = 1.00, respectively; Fig. 2 and 3), the hypothesis of an insufficient signal in the datasets seems less plausible. The differences in rate variation among the lineages indicate that the hypothesis of long-branch attraction as the main
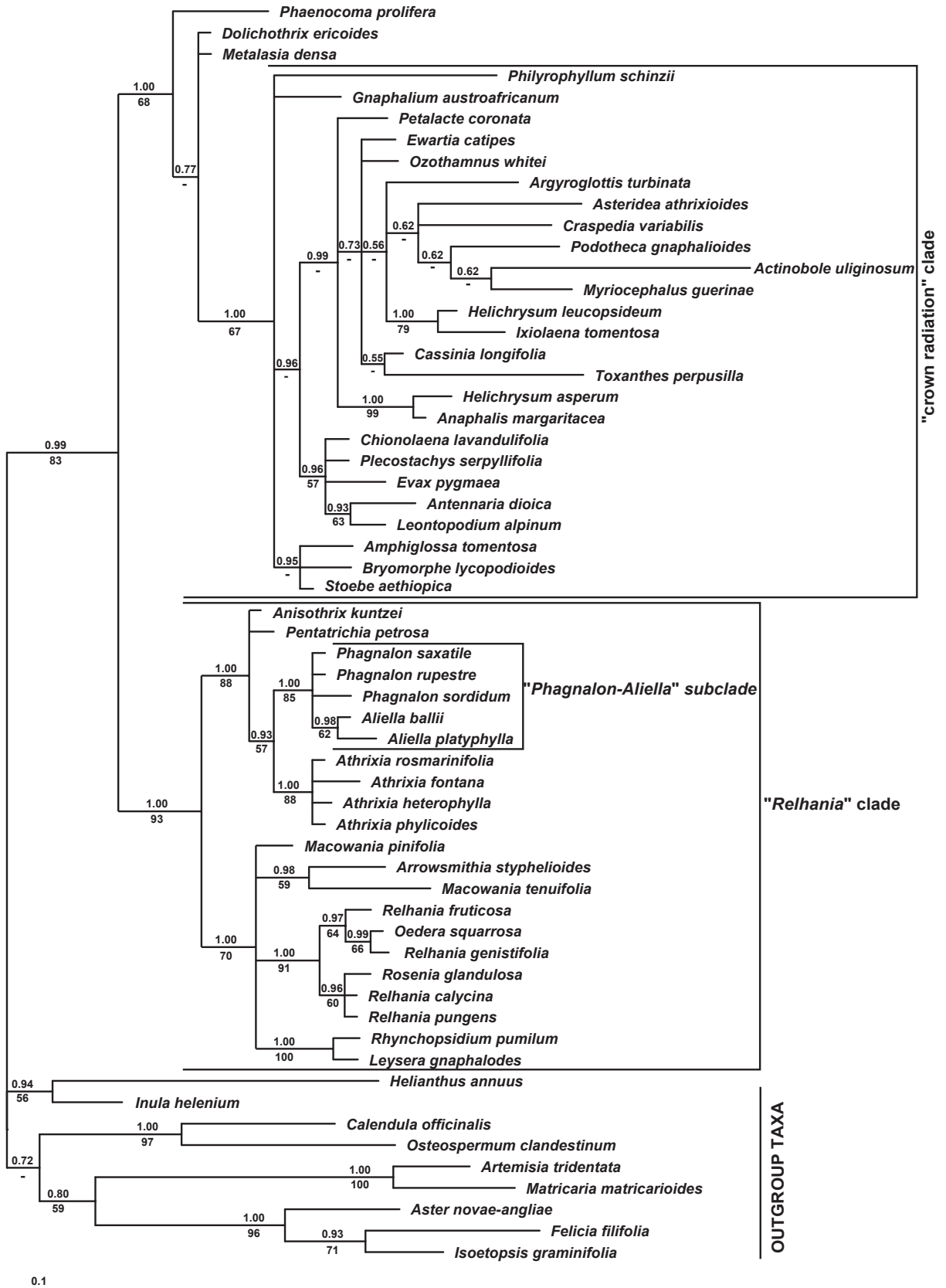
**Fig. 1.** Strict consensus tree of the 1514 equally parsimonious trees obtained in the heuristic search from the *trnL* intron and *trnL-trnF* intergenic spacer sequence data. Numbers below the branches indicate parsimony bootstrap percentages (BS). Numbers above the branches are Bayesian clade-credibility values (posterior probability, PP).

source of disagreement should be ruled out. Therefore, these results could indicate the presence of reticulation or ancient hybridization, which will be discussed later.

**Phylogenetic relationships in the "*Relhania* clade".** — According to the results from the combined *trnL* intron and *trnL-trnF* intergenic spacer, *Macowania* belongs to the "*Relhania* clade" (Fig. 1). *Macowania* consists of twelve species from South Africa, Arabia and Ethiopia (Anderberg, 1991). The proximity of *Macowania* to the "*Relhania* clade" has been

reported in previous morphologic studies, although its closest relative has not been clearly defined. Kroner (1980) first found a close relationship between *Macowania* and *Athrixia* and then transferred *Athrixia pinifolia* to *Macowania* based on revolute leaves, bracts, habit and floret color. In contrast, Hilliard & Burt (1985) argued that it should be maintained under *Athrixia* due to its pink ray-florets, three-ribbed achenes and pappus structure. Anderberg (1991) argued that *Macowania* is sister to *Arrowsmithia* based on the cladistic analysis
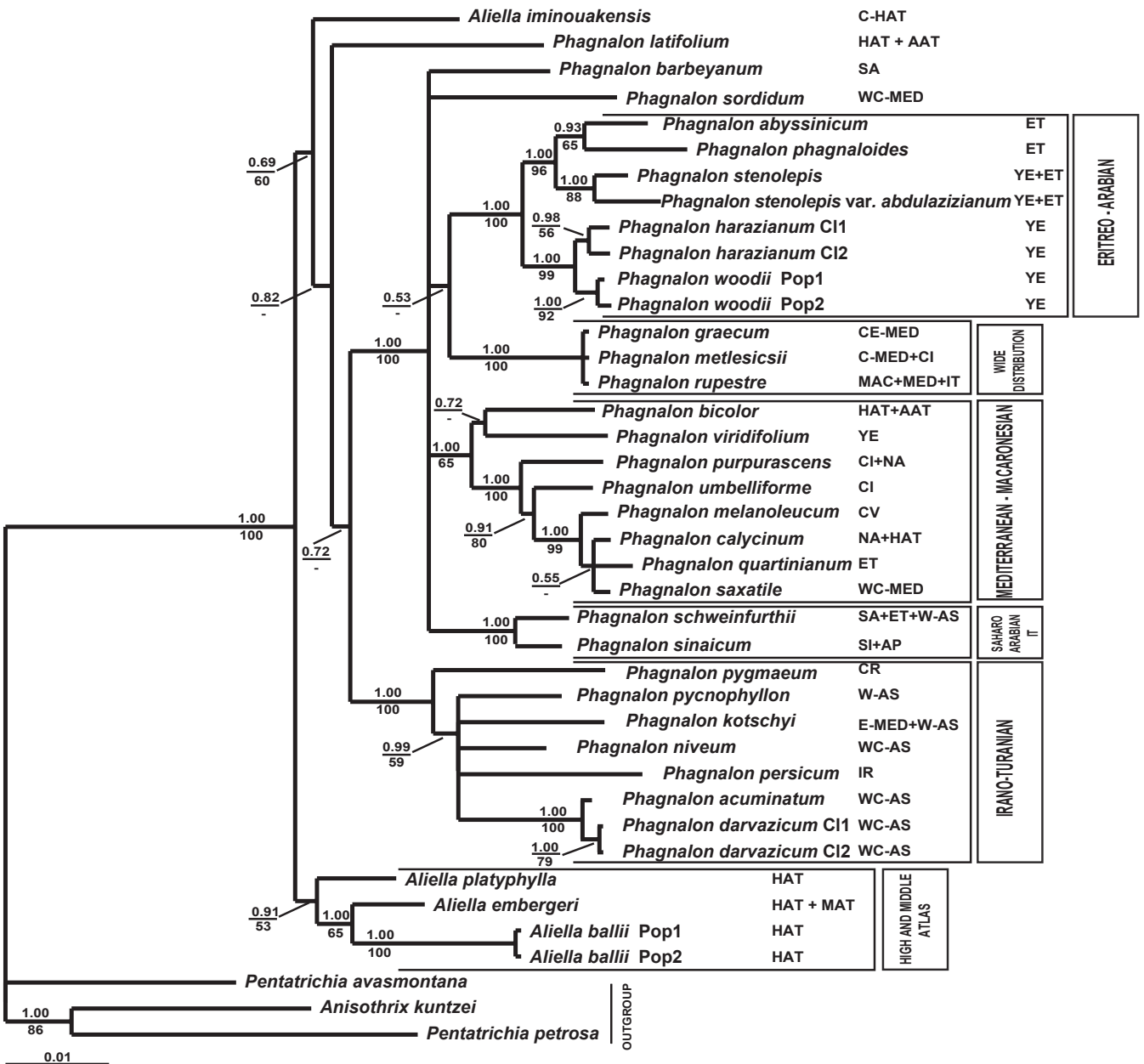


**Fig. 2.** Consensus phylogram of the post–burn-in trees resulting from Bayesian MCMC analysis of the combined ETS, ITS, *ycf3-trnS* and *trnT-trnL* datasets. Numbers above the branches are Bayesian clade credibilities (posterior probability, PP). Numbers below the branches indicate parsimony bootstrap percentages (BS). Regional acronyms: AAT Anti Atlas, AP Arabian Peninsula, AS Asian, C central, CI Canary Islands, CR Crete, CV Cape Verde, E Eastern, ET Ethiopia, HAT High Atlas, IR Iran, IT Irano-Turanian, MAC Macaronesian, MAT Middle Atlas, MED Mediterranean, NA North African, SA Saharo-Arabian, SI Sinai, W Western, YE Yemen. Other abbreviations: Cl=clone.
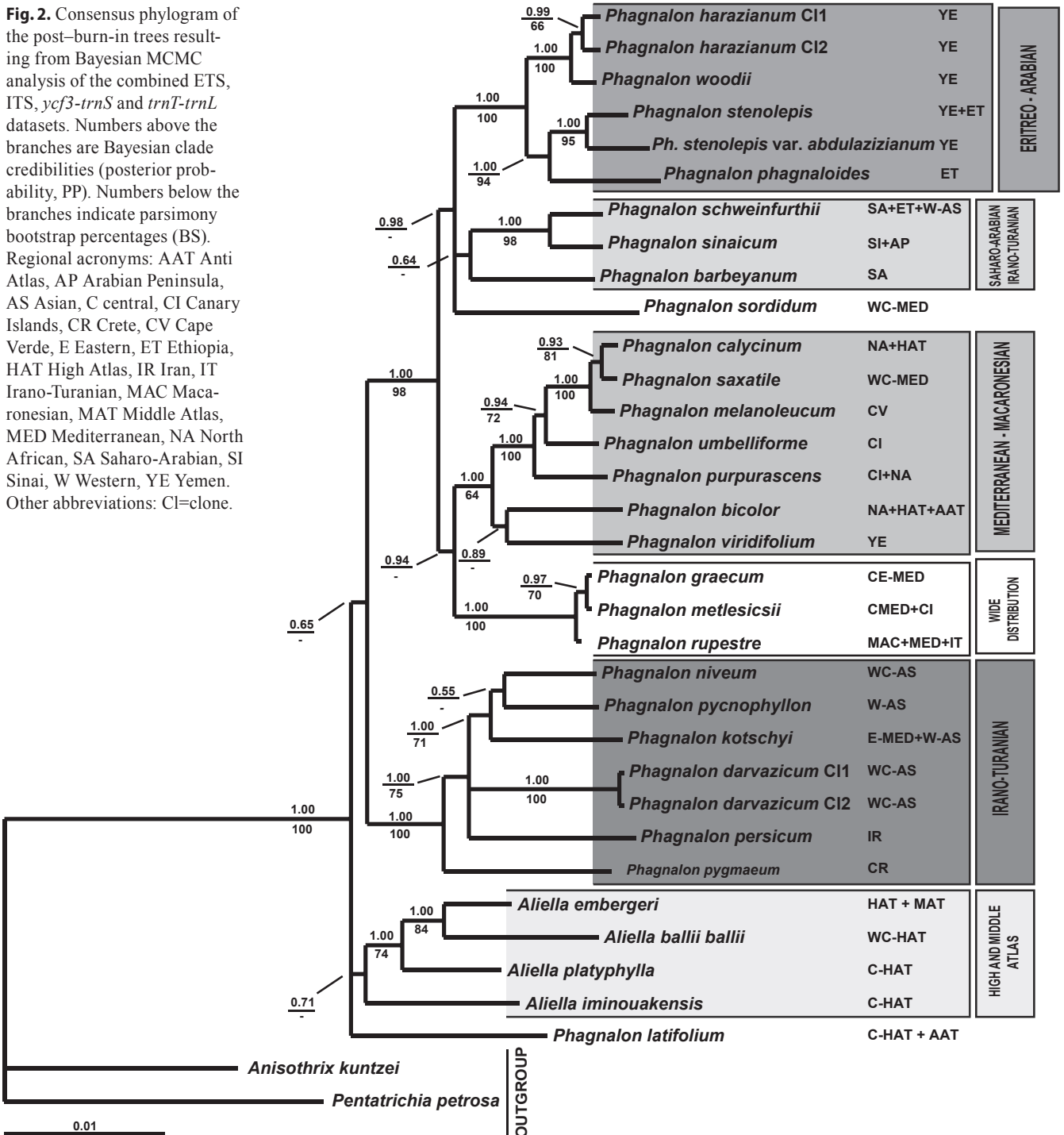
of morphological characters. In agreement with Kroner (1980), Anderberg (1991) also suggested transferring *A. pinifolia* to *Macowania*, pointing out that *A. pinifolia* is probably an early-branching representative of the *Macowania* clade. Our results are consistent with Anderberg (1991) because *Macowania tenuifolia* forms a monophylethic group with *Arrowsmithia* (PP = 0.98, BS = 59%; Fig. 1); our results are also consistent with those of Kroner (1980) because *A. pinifolia* does not form a monophylethic group with *Athrixia*, but it does form a group with *Arrowsmithia*, *Leysera*, *Macowania*, *Oedera*, *Relhania*, *Rhynchopsidium* and *Rosenia* (PP = 1.00, BS = 70%; Fig. 1).

However, the affinities of *A. pinifolia* among the "*Relhania* clade" are not resolved due to the lack of informative characters. Anderberg (1991) also indicated that the cladistic analysis of *A. pinifolia*, *Arrowsmithia*, *Macowania* and *Oxylaena* might show that *A. pinifolia* would be better treated as a separate, monotypic genus. Further phylogenetic studies are needed to confirm whether *A. pinifolia* should be transferred to *Macowania* or should be treated as a separated genus.

*Philyrophyllum* is a monotypic genus endemic to Namibia and Botswana, the infratribal affinities of which were previously unknown based on molecular data. The present results



**Fig. 3.** Consensus phylogram of the post–burn-in trees resulting from Bayesian MCMC analysis of the combined ETS and ITS datasets. Numbers above the branches are Bayesian clade credibilities (posterior probability, PP). Numbers below the branches indicate parsimony bootstrap percentages (BS). Regional acronyms as in Fig. 2. Other abbreviations: Cl =clone; Pop = population.

strongly support the hypothesis that *Philyrophyllum* belongs to the "crown radiation clade". In contrast, Anderberg (1991, 1988; Leins, 1971) assigned it to the "basal group taxa" and pointed out a close relationship between *Anisothrix*, *Philyrophyllum* and *Pentatrichia* based on cladistic analyses of morphological characters and palynology. However, other molecular phylogenies (Bergh & Linder, 2009) have shown that other genera of the "basal group taxa" belong to the "crown radiation clade" (e.g., *Millotia* and *Ixiolaena*).

*Athrixia* comprises about 14 species from South Africa, tropical Africa and Madagascar (Kroner, 1980). The phylogenetic position of *Athrixia* is controversial. Bayer & al. (2000) nested *Athrixia* (*A. capensis*) outside the "*Relhania* clade" in Gnaphalieae s.str. However, Ward & al. (2009) found *A. phylicoides* to be part of the "*Relhania* clade". Our results are in accordance with Ward & al. (2009) because the four *Athrixia* species sampled form a strongly supported monophyletic group within the *Aliella-Phagnalon* subclade, in the "*Relhania* clade"

**Fig. 4.** Consensus phylogram of the post–burn-in trees resulting from Bayesian MCMC analysis of the combined chloroplast *ycf3-trnS* and *trnT-trnL* sequence data. Numbers above the branches are Bayesian clade credibilities (posterior probability, PP). Numbers below the branches indicate parsimony bootstrap percentages (BS). Regional acronyms as in Fig. 2.
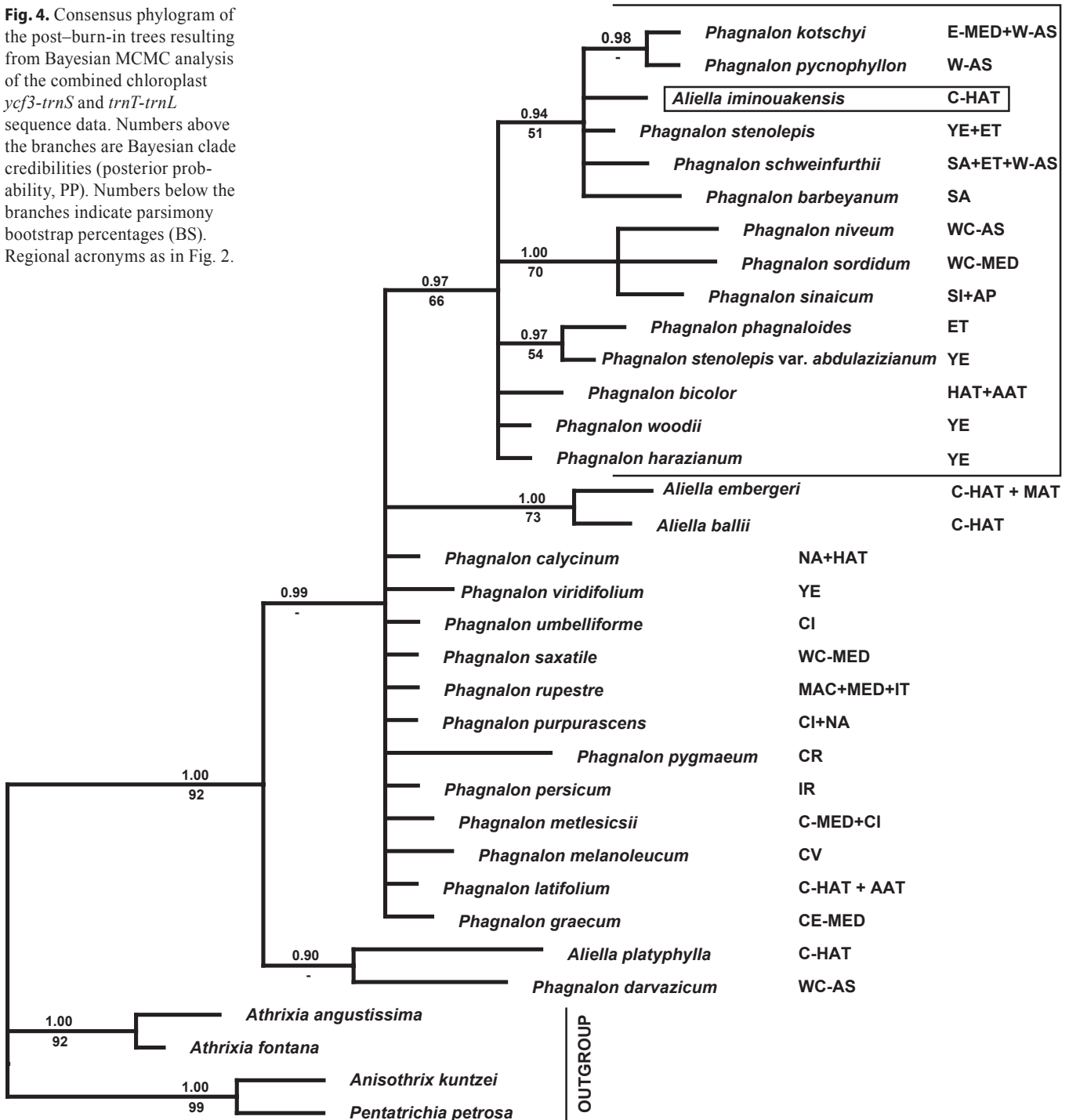
(Fig. 1). These results indicate a possible problem of generic delimitation in *Athrixia*. Therefore, more species should be added to phylogenetic studies to test whether *Athrixia* is monophyletic.

**The genera closest to *Aliella* and *Phagnalon*. —** Based on the *trnL* intron and *trnL-trnF* phylogeny, the monophyletic group consisting of *Aliella*, *Anisothrix*, *Athrixia*, *Phagnalon* and *Pentatrichia* is highly supported and the affinities of *Aliella* and *Phagnalon*, nested within the "*Relhania* clade", are resolved for the first time. This monophylethic group suggests an African origin for *Aliella* and *Phagnalon*, as the representatives of the

"*Relhania* clade" more closely related to them are restricted to South Africa, Tropical Africa and Madagascar. This African ancestor could have followed the African migration routes indicated by Quézel (1978) for the Pliocene and Pleistocene flora and then colonized some point on the Mediterranean, as indicated for other Gnaphalieae with representatives of Mediterranean distribution of African origin (e.g., *Helichrysum*, Galbany-Casals & al., 2009; *Ifloga* and *Leysera*, Bergh & Linder, 2009).

However, the lack of informative characters does not allow us to establish their sister group. The alignment of 5′ ETS



**Fig. 5.** Consensus phylogram of the post–burn-in trees resulting from Bayesian MCMC analysis of the combined ETS and ITS sequence data performed with the exclusion of *Aliella iminouakensis* and *Phagnalon latifolium*. Numbers above the branches are Bayesian clade credibilities (posterior probability, PP). Numbers below the branches indicate parsimony bootstrap percentages (BS). Other abbreviations: Cl = clone; Pop = population.
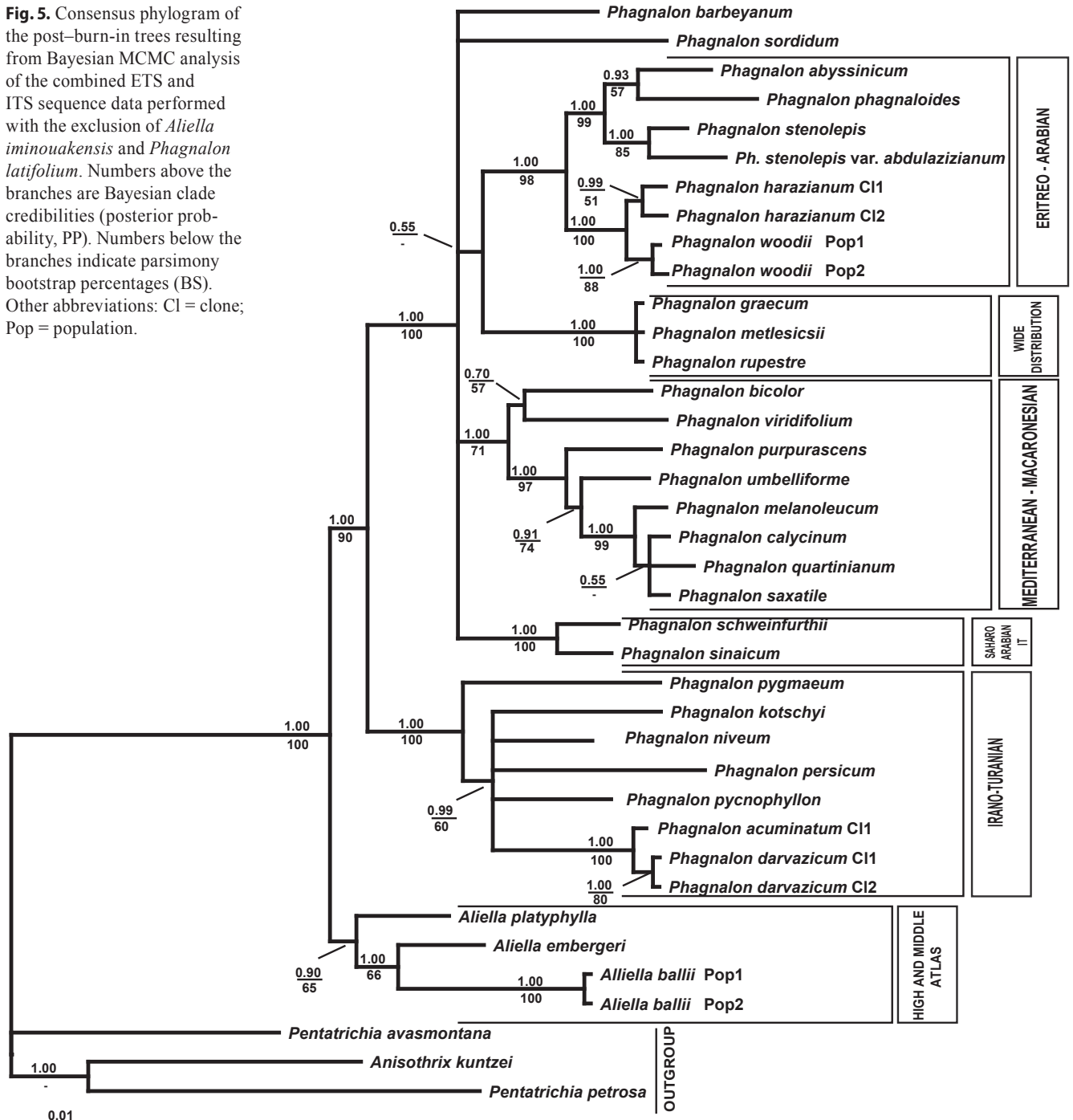
sequences was unambiguous among *Aliella*, *Anisothrix*, *Pentatrichia* and *Phagnalon*, which supports a close relationship among these genera. In contrast, it was impossible to align the 5′ ETS sequences among *Athrixia* and the rest of the "*Relhania* clade" representatives, which suggests that *Anisothrix* and *Pentatrichia* are closely related to *Aliella* and *Phagnalon*, while the rest of "*Relhania* clade" representatives are more distantly related. Moreover, *Aliella*, *Anisothrix*, *Pentatrichia* and *Phagnalon* share several morphological features including long caudate anthers, waxy cushions on the outside of the corolla lobes, no myxogenic filiform twin hairs on the achene surface and acute sweeping hairs arranged apically on the stigmatic surface (Montes-Moreno, pers. obs.). In addition, these genera share some unusual morphological characteristics with Gnaphalieae such as leaves with dentate margins (Bayer & al., 2000). Furthermore, the proximity between *Anisothrix* and *Phagnalon* is supported by cladistic analyses of morphological characters because the two genera formed a monophyletic group; anatomical evidence also reveals that the phloem is not concealed in fibers in both genera (Anderberg, 1989). The two genera are also related from a phytochemical point of view, as six derivatives of leysseral compounds have been isolated from the aerial parts of *Anisothrix integra* and *Phagnalon purpurascens* (Zdero & al., 1991).

The molecular phylogenetic position of *Aliella* and *Phagnalon* agrees with the results of El Ghazaly & Anderberg (1995), who found that the pollen morphology of *Aliella* and *Phagnalon* was similar to that of some genera from Gnaphalieae "basal group taxa".

**Generic delineation between *Aliella* and *Phagnalon*. —** The generic boundaries between *Aliella* and *Phagnalon* are difficult to establish on a molecular basis because the results obtained from all datasets showed unresolved phylogenetic relationships. Nevertheless, both *Aliella* and *Phagnalon* form two separate monophyletic groups when the analysis was performed without *A. iminouakensis* and *Ph. latifolium* (Fig. 5).

From the combined nr-cpDNA dataset, only three *Aliella* species form a monophyletic group (*A. platyphylla*, *A. embergeri*, *A. balli*; Fig. 3). In addition, there is incongruence between the molecular and morphological data, as *Aliella iminouakensis* does not group with the rest of the *Aliella* species even though *A. iminouakensis* shares all the diagnostic characters with the remaining species. Emberger (in Quézel, 1951) suggested including this taxon in *Phagnalon* sect. *Gnaphaliopsis*, but it was not included in *Aliella* until the suggestion forwarded by Dobignard (1997). This taxon is also endemic to the High Central Atlas mountains (highly restricted to two localities) and grows in rock crevices, although it is found at lower altitudes than the rest of the *Aliella* species. Conversely, the results obtained from the cpDNA dataset indicate that *A. iminouakensis* is firmly nested within the *Phagnalon* clade, which would suggest the paraphyly of the two genera.

The monophyly of *Phagnalon* is only statistically supported by the analysis carried out excluding *A. iminouakensis* and *Ph. latifolium* (Fig. 5). The two taxa *A. iminouakensis* and *Ph. latifolium* have a complex phylogenetic history and could be derived from an ancient hybridization event for the following reasons: (1)

the noteworthy increase in the bootstrap value of the *Phagnalon* clade after the exclusion of both taxa; (2) the incongruencies between nrDNA and cpDNA datasets concerning the position of *A. iminouakensis*, firmly nested within the Irano-Turanian and Saharo-Arabian *Phagnalon* taxa according to the cpDNA; (3) the unresolved position of these two taxa; and (4) the intermediate morphology of *Ph. latifolium* (dense caespitose perennial habit and presence of scapus similar to *Aliella* taxa, but the absence of waxy cushions together with long-tailed anthers like most *Phagnalon* taxa). A close relationship between *Ph. latifolium* and *Aliella* was already suggested by Maire (1928). Hybridization has been documented among several *Phagnalon* species (*Ph. saxatile*, *Ph. sordidum*, *Ph. rupestre*; cf. Faure, 1923), and several intermediate specimens have been observed in the field and in herbarium specimens; the same observation was reported for some Irano-Turanian taxa (Qaiser & Abid, 2003; Montes-Moreno, pers. obs.). The geographical distribution area of *A. iminouakensis* does not currently overlap with the putative *Phagnalon* maternal (cpDNA) donors suggested by the cpDNA dataset (Fig. 4). The putative ancient hybridization event in *Aliella iminouakensis* could have taken place after the diversification of an ancestor of African origin. However, there are several documented examples of ancient hybridization between lineages (the modern descendants of which are geographically very isolated) without evidence of reticulation detected before molecular investigation in other plant groups, e.g., *Gossypium* L. (Wendel & al., 1991; Cronn & Wendel, 2004). Moreover, other hybridization or introgression events have also been reported between highly divergent taxa, for instance, between taxa included in different sections or genera in Salicaceae (Hamzeh & Dayanandan, 2004); Saxifragaceae (*Heuchera* L., cf. Soltis & Kuzoff, 1995), Compositae (*Andryala* L., *Hieracium* L., and *Pilosella* Hill., cf. Fehrer & al., 2007), and particularly in Gnaphalieae (*Anaphalioides* (Benth.) Kirp. and *Ewartia* Beauverd, cf. McKenzie & al., 2008; *Anaphalioides* and *Helichrysum* Mill., cf. Smissen & al., 2007). *Phagnalon latifolium* was described from the Aouljdid Mountain in the High Central Atlas (Maire, 1928); it is noteworthy that we have studied vouchers of *A. platyphylla* and *A. ballii* collected in the same locality (Montes-Moreno, pers. obs.). Further studies involving genetic markers with higher evolutionary rates are required.

**Phylogenetic relationships in *Phagnalon*. —** Results of the combined nuclear-chloroplast phylogeny indicate that the diversification in *Phagnalon* took place in three main areas: the Irano-Turanian region, the Mediterranean and Macaronesian regions and the Saharo-Arabian region.

*Irano-Turanian clade.* – This clade includes species from the Irano-Turanian region and *Ph. pygmaeum*, which is endemic to Crete and is the only Mediterranean taxon that does not group with the remaining Mediterranean taxa (Figs. 2 and 3). *Phagnalon pygmaeum* is sister to the Irano-Turanian species (PP = 1.00, BS = 75% in Fig. 2; PP = 1.00, BS = 59% in Fig. 3), which suggests that a common ancestor of the whole Irano-Turanian clade reached Crete and from there colonized the mainland, where it radiated and diversified into the Irano-Turanian region. In view of these results, the first possible scenario involves wind dispersal of the tiny and light achenes (~1 mm), as reported for

other *Phagnalon* in other geographic areas, specifically between Tibesti, Jebel Marra and Yemen (Qaiser & Lack, 1986a). Some dispersal events have been inferred using a Bayesian approach for Campanulaceae endemic to Crete (Cellinese & al., 2009). However, a second possible scenario for the Irano-Turanian clade diversification may have involved a common ancestor distributed throughout the mainland that colonized Crete via land connections before becoming extinct because the mountains of Crete are considered remnants of an ancient mountain system that connected the Balkans with Southern Anatolia (Greuter, 1972). This putative ancestor also radiated to the Irano-Turanian region, where it generated *Ph. acuminatum*, *Ph. kotshcyi*, *Ph. niveum*, *Ph. persicum* and *Ph. pycnophyllon*. In addition, a combination of both scenarios is also possible.

The phylogenetic position of *Ph. pygmaeum* and the interspecific relationships among these Irano-Turanian taxa could be explained by considering some morphological affinities: *Ph. acuminatum*, *Ph. kotschyi* and *Ph. pygmaeum* have linear to subulate bracts in the capitulum; *Ph. kotschyi*, *Ph. pygmaeum* and *Ph. persicum* all have waxy cushions on the outside of the corolla lobes, which are present in some *Phagnalon* and in all *Aliella* species. In addition, the high phylogenetic divergence between *Ph. pygmaeum* and the other Mediterranean taxa could suggest that this taxon is a relict element from an older Aegean or Mediterranean flora that went extinct elsewhere. In fact, some authors reached the same conclusion for the majority of the endemics of Crete, which was isolated from the mainland for 5 million years during the Pliocene (Greuter, 1979). Although within the Irano-Turanian clade the resolution is poor, the morphological and molecular evidence indicates that *Ph. acuminatum* and *Ph. darvazicum* are sister taxa (PP = 1.00, BS = 100%; Fig. 3). Both species are small shrubs distributed throughout Afghanistan, Pakistan, Iran, Tajikistan and Turkmenistan, and they overlap in Eastern and Northeastern Afghanistan and in Western Pakistan. They have triangular to linear bracts, a similar-sized capitulum, and the main difference lies in the indumentum of the leaves and bracts, which is tomentose in *Ph. acuminatum* and glabrous in *Ph. darvazicum*. This morphological feature is very variable within *Phagnalon*, and many taxa have been described based on this character (Boissier, 1875; Pignatti, 1969; Chaudhary, 2000). However, both taxa cannot be separated by other qualitative morphological characters that are important in *Phagnalon*, namely the leaf and bract morphology, lack of waxy cushions and acaudate anthers. Based on the molecular and morphological data, we conclude that *Ph. darvazicum* falls within the morphological variability of *Ph. acuminatum*. Our results do not agree with the taxonomic conclusions of Qaiser & Abid (2003) who found a close relationship between *Ph. acuminatum* and *Ph. niveum* based on the habit, size of the capitulum and indumentum of the bracts, in addition to several local floras in which *Ph. darvazicum* is recognized (Lack, 1980; Borissova, 1990; Qaiser & Abid, 2003).

Another subclade includes *Ph. kotschyi*, *Ph. niveum* and *Ph. pycnophyllon* (PP = 1.00, BS = 71%; Fig. 2). *Phagnalon kotschyi* is distributed throughout the East Mediterranean (Lebanon, Syria, Turkey) and Irano-Turanian regions (Iran and Iraq); *Ph. niveum* and *Ph. pycnophyllon* are found mainly in Afghanistan

and Pakistan, although *Ph. pycnophyllon* is also found in India and Nepal. However, even though *Ph. kotschyi* shows close geographic affinity to both taxa, it is morphologically closer to *Ph. pygmaeum* because it has linear to subulate bracts and waxy cushions on the outside of the corolla lobes. *Phagnalon niveum* and *Ph. pycnophyllon* could be distinguished on the basis of quantitative differences like the width of the bracts, capitulum and leaves but are morphologically closer to the Iranian *Ph. persicum* than to *Ph. pygmaeum* or *Ph. kotschyi*. Besides this, the three species *Ph. niveum*, *Ph. persicum* and *Ph. pycnophyllon* are small, woody perennials that form small cushions, sometimes with decumbent stems. *Phagnalon niveum* and *Ph. pycnophyllon* show some morphological synapomorphies, which include the presence of triangular to lanceolate bracts and lanceolate to spatulate leaves. Some morphological intermediate specimens were found by Qaiser & Abid (2003) and Montes-Moreno (pers. obs.) in Kurram Valley on the border between Pakistan and Afghanistan. Some specimens could be putative hybrids, considering the lack of resolution found in the rest of the phylogenetic analyses. Besides gene flow, another factor that could contribute to the lack of resolution of this subclade is the recent diversification in this group of taxa.

*Mediterranean and Macaronesian clade.* – This clade encompasses mostly Mediterranean and Macaronesian taxa, although it also includes some East African and Saudi Arabian-Omani endemics (*Ph. quartinianum* and *Ph. viridifolium* respectively; Fig. 3). The East African and Saudi Arabian-Omani species, together with Mediterranean-Macaronesian taxa, constitute a monophylethic group in other genera: *Aeonium* Webb & Berthel. and *Pulicaria* Gaertn. (Andrus & al., 2004), *Euphorbia* L. (Molero & al., 2002), and *Helichrysum* (Galbany-Casals & al., 2009). These disjunctions have been considered evidence of a continuous flora that existed in the late Miocene (Quézel, 1978; Bramwell, 1985). This flora was widespread across the Sahara, North Africa and Mediterranean regions and disappeared in the major climatic changes of the late Tertiary and Quaternary (Axelrod, 1975; Quézel, 1978; Sunding, 1979). There are also some morphological affinities between some of the representatives of this clade: The African *Ph. bicolor* and *Ph. quartinianum* and the Arabian *Ph. viridifolium* share the transparent scariose margins of the bracts. However, *Ph. quartinianum* is also morphologically closer to *Ph. schweinfurthii* because it also shares transparent scariose margins in the bracts.

Similarly, *Ph. purpurascens* from North Africa and the Canary Islands is sister to a clade encompassing representatives from the Canary Islands (*Ph. umbelliforme*) and Cape Verde (*Ph. melanoleucum*), North Africa (*Ph. calycinum*) and the Western Mediterranean and Macaronesian regions (*Ph. saxatile*) (Figs. 2. and 3). *Phagnalon melanoleucum* is sister to a clade that includes *Ph. calycinum* and *Ph. saxatile* (Fig. 2). However, this relationship is not supported by the ETS + ITS phylogeny because all these taxa are clustered in a polytomy (Fig. 3). In addition, our results suggest the two following hypotheses. (1) The Canary Islands were colonized by a North African ancestor, as has been hypothesized in other plant groups (Galbany-Casals & al., 2009), whose dispersal would have been facilitated by a long-distance route from North Africa, as suggested by

Francisco-Ortega (1999). A Canary Islands ancestor colonized the Cape Verde Islands, and there might have been back colonization to the mainland, where it would have originated *Ph. calycinum* and *Ph. saxatile* (Fig. 2). A colonization route from the Canary Islands to Cape Verde has been indicated for *Echium* L. (Böhle & al., 1996) and *Sonchus* L. (Lee & al., 2005). Likewise, a back colonization route from the Canary Islands to North Africa has also been indicated by other authors (Allan & al., 2004; Carine & al., 2004). (2) The second scenario involves an extinct ancestor distributed to North Africa that radiated to the Canary Islands, Cape Verde Islands and the Mediterranean area. The colonization of the Cape Verde Islands and Mediterranean region by North African floristic elements has been postulated by other authors (Allan & al., 2004; Font & al., 2009, Galbany-Casals & al., 2009). In addition, the species distributed in the Canary Islands do not form a monophylethic group. Therefore, colonization of the Canary Islands by *Phagnalon* took place by at least two colonization events, like in other Macaronesian taxa (Galbany-Casals, 2009).

The molecular data indicate that *Ph. purpurascens* should be accepted at the specific rank. This taxonomic decision is in accordance with Kunkel (1991) and Reyes-Betancort & al. (1996) and is supported by morphological data. *Phagnalon purpurascens* is morphologically characterized by lanceolate bracts with a flat margin and linear leaves. Our results do not agree with Battandier & Trabut (1889) and Ozenda (1983), who considered this taxon at a subspecific rank under *Ph. saxatile*.

*Eritreo-Arabian clade.* – This clade includes endemics from Ethiopia and Yemen (Fig. 3) and encompasses two subclades: the first includes *Ph. abyssinicum* and *Ph. phagnaloides*, which are endemics to Ethiopia, and the second includes *Ph. stenolepis*, which shows a remarkable disjunct distribution between Sudan (Jebel Marra), Chad (Tibesti), Ethiopia, Saudi Arabia and Yemen (PP = 1.00, BS = 94% in Fig. 2; PP = 1.00, BS = 96% in Fig. 3).

This result may suggest that a common ancestor of these three taxa reached either Yemen or Ethiopia and colonized these areas, probably by long-distance dispersal of the light achenes, with wind as the most probable agent, as previously indicated by Wickens (1976). However, based on our data, it is not possible to infer whether the ancestor reached East Africa and colonized Yemen by long-distance dispersal or vice versa. It seems likely that the colonization followed a West-to-East route, which is in accordance to the African migration routes of the Pliocene and Pleistocene floras (Quézel, 1978).

Regarding *Ph. phagnaloides*, this species shows an unusual trait within *Phagnalon*: the capitula grouped in leafy racemes. It was described as *Blumea* and combined under *Phagnalon* by Cufodontis (1966). Our study is the first to confirm, on a molecular basis, that *Ph. phagnaloides* belongs to *Phagnalon*. *Phagnalon phagnaloides* and *Ph. stenolepis* are sister taxa and share the presence of waxy cushions on the outside of the corolla lobes. However, there are some incongruencies between the morphological and molecular data: several morphological traits diagnostic of *Ph. stenolepis* (linear to lanceolate bracts and lanceolate leaves with dentate margins) are also present in *Ph. acuminatum*. In spite of this similarity, *Ph. stenolepis* and

*Ph. acuminatum* do not form a monophyletic group. This result indicates that these morphological traits appeared more than once in *Phagnalon* and should be interpreted as morphological convergence.

The second subclade encompasses mainly endemics to Yemen and includes *Ph. harazianum* and *Ph. woodii* (PP = 1.00, BS = 100% in Fig. 2; PP = 1.00, BS = 99% in Fig. 3). There is one Egyptian locality from a Forsskål sheet for *Ph. woodii* (Appendix; Montes-Moreno, pers. obs.); however, taking into account that Forsskål also visited Yemen, this could be interpreted as a labeling mistake. The sister relationship resolved for *Ph. harazianum* and *Ph. woodii* agrees with morphology as both taxa are small woody perennials that form dense cushions with lanceolate leaves (Qaiser & Lack, 1985).

In addition, two East African and Saudi Arabian-Omani endemics (*Ph. quartinianum*, *Ph. viridifolium*) are clustered in the Mediterranean-Macaronesian clade. This result suggests that the East African and Yemenite-Omani areas underwent at least two colonization events because all remaining endemics to Arabia and Ethiopia are nested within the Eritreo-Arabian subclade.

**Position of certain widely distributed species and related taxa. —** Other highly supported monophyletic groups were not nested within the three main clades. One of them includes *Ph. graecum*, *Ph. metlesicsii* and *Ph. rupestre*, (PP = 1.00, BS = 100%; Figs. 2 and 3). *Phagnalon rupestre* is distributed throughout the Canary Islands, the Mediterranean basin and the Irano-Turanian area. *Phagnalon metlesicsii* was described from Sicily based on glabrous leaves with dentate margins, and some other glabrous populations have been located in the Canary Islands (Reyes-Betancort & al., 1996). Based on the molecular, morphological and chorological data, we conclude that *Ph. metlesicsii* falls into the morphological variability of *Ph. rupestre*. *Phagnalon graecum* is found in the central Mediterranean area and is defined by triangular to lanceolate bracts with subacute margins, whereas *Ph. rupestre* has oblong and wider, obtuse bracts. Due to the variability found in bracts, *Ph. graecum* has been combined at the subspecific rank of *Ph. rupestre* or at the specific rank (Boissier, 1849; Battandier; 1889; Pignatti, 1982; Greuter & Raab-Straube, 2008). Unfortunately, the regions sequenced did not provide enough parsimony-informative characters to resolve the phylogenetic relationship between the two taxa.

*Phagnalon schweinfurthii* is distributed widely throughout the Irano-Turanian area, the Arabian Peninsula and tropical Africa. This taxon appeared as a sister of *Ph. sinaicum* (PP = 1.00, BS = 98% in Fig. 2; PP = 1.00, BS = 100% in Fig. 3), but its phylogenetic position remains unclear. *Phagnalon schweinfurthii* and *Ph. sinaicum* share morphological synapomorphies such as the presence of a broadly scariose membranous margin and oblanceolate to spatulate medium bracts. They can be distinguished by their leaf morphology and the presence of multicellular biseriate glandular trichomes with a wide base on the stem and leaf surface found in *Ph. sinaicum*, but not in *Ph. schweinfurthii*. Oblong to spatulate bracts with broadly scariose transparent margins, furthermore occur in *Ph. viridifolium* which, however is not a member of this clade and has biogeographical affinities to the Mediterranean-Macaronesian taxa.

## ■ CONCLUSIONS

The present study is the first to define, on a molecular basis, the tribal positions of *Aliella*, *Macowania* and *Phagnalon* in the "*Relhania*" clade". In addition, our results elucidate the phylogenetic position of *Philyrophyllum* in the "crown radiation clade" and suggest that *Anisothrix*, *Athrixia* and *Pentatrichia* are closely related to *Aliella* and *Phagnalon*. The results also indicate that diversification in *Phagnalon* took place in three main areas: Western and Central Asia, the Mediterranean and Macaronesia regions, and the Eritreo-Arabian region. Some incongruencies between the chloroplast and nuclear molecular data, as well as the lack of resolution in some clades, may indicate that hybridization could have played an important role in the evolution and diversification of both *Aliella* and *Phagnalon*.

## ■ ACKNOWLEDGMENTS

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**Appendix.** Species included in the molecular analysis, voucher information and GenBank accession numbers (ETS, ITS, *ycf3-trnS*, *trnT-trnL*, *trnL* intron and *trnL-trnF* intergenic spacer ). An en-dash indicates that a region was not sequenced for this taxon. An asterisk indicates a sequence previously published by Bayer & Starr (1998), Bayer & al. (2000, 2002), Panero & Funk (2008), Bergh & Linder (2009) or Galbany-Casals & al. (in press), or taken from Boelch & al. (unpub.), Panero & al. (unpub.).

*Actinobole uliginosum* (A. Gray) H. Eichler, –; –; –; –; AF141736*; AF141824*. *Aliella ballii* (Klatt) Greuter, Morocco: High Central Atlas, c. Tizi n'Ou Addi, 2900 m, 22-VII-2004, *Sáez 6186* (Sáez pers. herb.), Population 1, HM245966; HM246006; HM246102; HM246068; HM246046; HM246056. *Aliella ballii* (Klatt) Greuter, Morocco: High Central Atlas, Jbel Angour, 3450 m, *Sáez 6191* (Sáez pers. herb.), Population 2, HM245967; HM246007; –; –; –; –. *Aliella embergeri* (Humbert & Maire) Qaiser & Lack, Morocco: High Central Atlas, gorge at the entrance of the Cirque of Jaffar (Midelt), 09-V-2000, *Gómiz 5650* (Gómiz pers. herb.), HM245968;

**Appendix.** Continued.

HM246008; HM246103; HM246069; –; –. **Aliella iminouakensis** (Emb.) Dobignard & Jeanm., Morocco: between Imi n'Ouaqqa and Tirsal, 21-07-2004, *Sáez 6182* (Sáez pers. herb.), HM245969; HM246009; HM246104; HM246070; –; –. **Aliella platyphylla** (Maire) Qaiser & Lack, Morocco: C Tizi n'Ou Addi, 22-VII-2004, *Sáez 6188* (Sáez pers. herb.), HM245970; HM246010; HM246105; HM246071; HM246047; HM246057. **Amphiglossa tomentosa** Harv., –; –; –; –; FM173184*; FM173184*. **Anaphalis margaritacea** (L.) Benth. & Hook.f., –; –; –; –; FN645762*; FN645762*. **Anisothrix kuntzei** O. Hoffm., South Africa: Ladismith District, longe berg Range Kogmons, Kloof Pass, cliffs on Side of road near old English fort, 22-IX-1996, *Bayer & Puttock s.n.* (CSIRO, SAF), HM245971; HM246011; HM246106; HM246072; AF098859*; AF100522*. **Antennaria dioica** (L.) Gaertn., –; –; –; –; FJ640050*; FJ639980*. **Argyroglottis turbinata** Turcz., –; –; –; –; AF141692*; AF141780*. **Artemisia tridentata** Nutt., –; –; –; –; U82016*; U82017*. **Arrowsmithia styphelioides** DC., –; –; –; –; AF098809*; AF100472*. **Aster novae-angliae** L., –; –; –; –; U82018*; U82019*. **Asteridea athrixioides** (Sonder & Mueller) Kroner, –; –; –; –; AF318114*; AF318925*. **Athrixia angustissima** DC., South Africa, E Cape Province: between Rhodes and Naudesnek, 02-II-2007, *Arrabal, Galbany-Casals, Koekemoer & Romo 14419* (BC),  –; –; HM246107; HM246073; –; –. **Athrixia fontana** MacOwan, South Africa, Mpumalanga Province: Lydenburg district, Mokobulaan plantations, 25°12.349′S; 30°34.533′E, 16-II-2007, *Arrabal, Burrows, Galbany-Casals, Koekemoer, Romo 14587 & Unarine*, (BC), –; –; HM246108; HM246074; HM246048; HM246058. **Athrixia heterophylla** Less., South Africa: NW of Port Elizabeth on the road between the Groot Winterhoek and Elandsberg, top of Elandsberg just past the turn-off to Antoniesberg, 7-IX-99, *Koekemoer 2350* (PRE), –; –; –; –; –; HM246059. **Athrixia phylicoides** DC.; –; –; –; –; FN645751*; FN645751*. **Athrixia rosmarinifolia**, Tanzania: Southern Highlands, Uwemba, bush, 2150 m, 25-VII-1958, *Gilli 571* (W); –; –; –; HM246049; HM246060. **Bryomorphe lycopodioides** Levyns, –; –; –; –; AF098820*; AF100483*. **Calendula officinalis** L., –; –; –; –; U82020*; U82021*. **Chionolaena lavandulifolia** (Kunth) Benth. & Hook. f. ex B.D. Jacks., –; –; –; –; AY143593*; AY143593*. **Cassinia longifolia** R. Br.,  –; –; –; –; AF141686*; AF141774*. **Craspedia variabilis** J. Everett & Joy Thomps., –; –; –; –; AF141713*; AF141801*. **Dolichothrix ericoides** Hilliard & B.L. Burtt, –; –; –; –; AF098822*; AF100485*. **Evax pygmaea** (L.) Brot. subsp. **pygmaea,** –; –; –; –; FN645787*; FN645787*. **Ewartia catipes** Beauverd, –; –; –; –; AF141698*; AF141786*. **Felicia filifolia** Burtt Davy, –; –; –; –; AF318120*; AF318929*. **Gnaphalium austroafricanum** Hilliard, –; –; –; –; FN645756*; FN645756*. **Helianthus annuus** L., –; –; –; –; AY216058*; U82039*. **Helichrysum asperum** Hilliard & B.L. Burtt, –; –; –; –; FM173186*; FM173186*. **Helichrysum leucopsideum** DC., –; –; –; –; AF318122*; AF318931*. **Inula helenium** L.,  –; –; –; –; U82040*; U82041*. **Isoetopsis graminifolia** Turcz., –; –; –; –; AF141734*; AY069925*. **Ixiolaena tomentosa** Sond. & F. Muell., –; –; –; –; AF141704*; AF141792*. **Leontopodium alpinum** Cass., –; –; –; –; AF141733*; AF141821*. **Leysera gnaphalodes** (L.) L., Republic of South Africa: Western Cape Province, Worcester, NE of Over Hex, Romo 14546 & al. (BC), –; –; –; –; FN645750*; FN645750*. **Macowania pinifolia** (N.E. Br.) Kroner, South Africa: Mpendhle District, Loteni Nature Reserve, streamside rocks, 24-XII-1978, *Hilliard & Burtt s.n.* (E), –; –; –; –; HM246050; HM246061. **Macowania tenuifolia** M.D. Henders., South Africa: Mariepskop, among rocks on summit of mountain, 23-X-1962, *van der Schyff 6196* (W), –; –; –; –; HM246051; HM246062. **Matricaria matricarioides** (Less.) Porter, –; –; –; –; U82046*; U82047*. **Osteospermum clandestinum** (Less.) Norl., –; –; –; –; U82048*; U82049*. **Metalasia densa** (Lam.) P.O. Karis, –; –; –; –; AF098848*; AF100511*. **Myriocephalus guerinae** F. Muell., –; –; –; –; AF141751*; AF141839*. **Oedera squarrosa** (L.) Anderb. & K. Bremer, –; –; –; –; AF098812*; AF100475*. **Ozothamnus whitei** (Burb.) Anderb., –; –; –; –; AF141748*; AF141836*. **Pentatrichia avasmontana** Merxm., Namibia: Windhoek Bergland, foothills of Ausberge on reefs and rocks, Halbstrauch, Klein, 1600 m, 21-IX-1964, *Seydel s.n.* (B), HM245972; HM246012; –; –. **Pentatrichia petrosa** Klatt., South Africa: Klein-Windhoek, mica schist slope, Foothills of the Erosberge, 23-V-1962, *Giess 3949* (W), HM245973; HM246013; HM246109; HM246075; AF098817*; AF100480*. **Petalacte coronata** D. Don, –; –; –; –; AF098843*; AF100506*.**Phaenocoma prolifera** D. Don, –; –; –; –; AF098825*; AF100488*. **Phagnalon abyssinicum** Sch. Bip. ex A. Rich, Ethiopia, Prov. Begemden: Larger Sabra into Tällak Valley of Rocks, 3140 m, 16-III-1966, *Sebald 1277* (WU), HM245974; HM246014; –; –; –; –. **Phagnalon acuminatum** Boiss., Afghanistan: East Afghanistan, Kabul, 13-V-1967, *Rechinger 34484* (MA), HM245975; HM246015; –; –; –; –. **Phagnalon barbeyanum** Asch. & Schweinf., Saudi Arabia: Harrat al Harrah S of Turayf, Jabal Liss, 13-III-1988, *Collenette 6561* (E), HM245976; HM246016; HM246110; HM246076; –; –. **Phagnalon bicolor** Ball, Morocco: About 10 km south of Skhour-Rehamna (Marrakech), 03-IV-1999, *Gómiz s.n.* (Gómiz pers. herb.), HM245977; HM246011; HM246077; –; –. **Phagnalon calycinum** (Cav.) DC., Morocco: About 3 km south of Jorf Lasfar (El Jadida), 30-IV-1995, *Gómiz s.n.* (Gómiz pers. herb.), HM245978; HM246018; HM246112; HM246078; –; –. **Phagnalon darvazicum** Krasch., Tajikistan: Daphaz., 31-V-1986, *Kamelin, Kasparov &. Xanvinov s.n.* (LE), HM245979-HM245980; HM246020; HM246113; HM246079; –; –. **Phagnalon graecum** Boiss. & Heldr., Italy: Puglia, Foggia, Gargano, Monte San Angelo, 07-VII-2002, *Aldasoro 3308* (MA), HM245981; HM246021; HM246114; HM246080; –; –. **Phagnalon harazianum** Deflers, Yemen: Wadi Bana, Qal haqul, 19-VI-1979, *Wood Y1277* (E), HM245982-HM245983; HM246022; HM246115; HM246081; –; –. **Phagnalon kotschyi** Sch. Bip. ex Boiss**.**, Iraq: distr. Mosul (Kurdistan) near Turkey, prov. Hakari, Sharanish in calcareous mountain A Zakho, 04-09-VII-1957, *Rechinger 11524* (MA), HM245984; HM246024; HM246116; HM246082; –; –. **Phagnalon latifolium** Maire, Morocco: W Tizi n'Tagounit (Tiznit), 04-V-2000, *Gómiz s.n.* (Gómiz pers. herb.), HM245985; HM246025; HM246117; HM246083; –; –. **Phagnalon melanoleucum** Webb, Cape Verde Islands: São Nicolau Upper Rib de Prata path from Fregatta over W ridge to Praia Branca, 550-560 m, 18-I-1994, *Kilian 3248 & Leyens* (B), HM245986; HM246026; HM246118; HM246084; –; –. **Phagnalon metlesicsii** Pignatti, Spain, Lanzarote: San Bartolomé Tomaren, 24-II-1995, *Reyes Betancort & León-Arencibia s.n.* (TFC), HM245987; HM246027; HM246119; HM246085; –; –. **Phagnalon niveum** Edgew., Pakistan: Western Pakistan, Dera Ismail Khan, 21-V-1965, *Rechinger 30136* (MA), HM245988; HM246028; HM246120; HM246086; –; –. **Phagnalon persicum** Boiss., Iran, Prov Kerman: Kuh-e Kabr., 08-VI-1977, *Assadi, Edmonson & Miller 1763* (E), HM245989 ; HM246029; HM246121 ; HM246087; –; –. **Phagnalon phagnaloides** (Sch. Bip. ex A. Rich.) Cufod., Ethiopia: In Abyssinia, *W. Schimper s.n.* (UPS-s.n.), HM245990; HM246030; HM246122; HM246088; –; –. **Phagnalon purpurascens** Sch. Bip**.**, Spain, Gran Canaria: San Nicolás de Tolentino, cliff of Pino Gordo, 31-III-1998, *A. Marrero & González Martín s.n.* (MA), HM245991; HM246031; HM246123; HM246089; –; –. **Phagnalon pycnophyllon** Rech. f., Pakistan: Baluchistan: Quetta: Loralai to Harnai, Torkhan Pass, top section of pass above Dil Kuna Village, 1965, *Lamond s.n.* (E), HM245992; HM246032; HM246124; HM246090; –; –. **Phagnalon pygmaeum** (Sieber) Greuter, Greece, Creta: Kakovoli, IX-2000, ex Mediterranean Agronomic Institute of Chania, HM245993; HM246033; HM246125; HM246091; –; –. **Phagnalon quartinianum** A. Rich., Ethiopia: Asmara to Arbaroba, 12-X-1952, *H. Scott 200* (BM), HM245994; HM246034–; –; –. **Phagnalon rupestre** (L.) DC., Spain: Gata de Gorgos, path to Font de la Mata, 16-V-2004, *Montes-Moreno s.n.* (BCN), HM245995; HM246035; HM246126; HM246092; HM246052; HM246063. **Phagnalon saxatile** (L.) Cass., Spain: Collserola, Carretera de les Aigües to St. Pere Màrtir, 02-V-2004, *Montes-Moreno s.n. & R. Rodríguez* (BCN), HM245996; HM246036; HM246127; HM246093; HM246053; HM246064. **Phagnalon schweinfurthii** Sch. Bip. ex Schweinf., Turkmenistán: Badjyz, 6-V-1977, *Botchantzev s.n.* (LE), HM245997; HM246037; HM246128; HM246094; –; –. **Phagnalon sinaicum** Bornm. & Kneuck., Saudi Arabia: Hema Figra, 60 Km W of Madinah near Hublag's house, 22-IV-1989, *Collenette 7138* (E), HM245998; HM246038; HM246129; HM246095; –; –. **Phagnalon sordidum** (L.) Rchb., Spain: Montserrat, path from station to Sta. Cova church, 10-VII-2004, *Montes-Moreno s.n. & R. Rodríguez* (BCN), HM245999; HM246039; HM246130; HM246096; HM246054; HM246065. **Phagnalon stenolepis** Chiov. var. **abdulazizianum** Chaudhary, Saudi Arabia: Mindala & Jabal Ibrahim, 10-V-1994, *Collenette 9135* (E), HM246000; HM246040; HM246131; HM246097; –; –. **Phagnalon stenolepis** Chiov., Yemen: Amran to Huth road, 5 km. N of Khamir, 21-V-1983, *Miller 3155* (E), HM246001; HM246041; HM246132; HM246098; –; –. **Phagnalon umbelliforme** DC., Spain, El Hierro: El Pinar a Restinga, ex Hort. Bot. Viera & Clavijo, HM246002; HM246042; HM246133; HM246099; –; –. **Phagnalon viridifolium** Decne. ex Boiss., Oman: Northern Oman, Jabal Akhdar, 12-IV-1993, *Mc Leish 1724* (E), HM246003; HM246043; HM246134; HM246100; –; –. **Phagnalon woodii** Qaiser & Lack, Yemen: Jabal Sabir, near Taizz, amphibole granite, 23-IX-1977, *Lavranos & Newton 15959* (E), Population 1, HM246004; HM246044; –; –; –. **Phagnalon woodii** Qaiser & Lack, Yemen: Ibb, 45 km from Taizz along road to Ibb, 1995, *Thulin, Gjebrehiwet & Gifri s.n.* (E), Population 2, HM246005; HM246045; HM246135; HM246101; –; –. **Phagnalon woodii** Qaiser & Lack, Egypt: Egypt, 1761-1762, *P. Forsskål 1146* (K). **Philyrophyllum schinzii** O. Hoffm., Namibia: Otjiwarongo, Farm Hohensee, in sandstone gorge, climb to the Waterberg Plateau, 8-V-1967, *Giess 10152* (W), –; –; –; –; HM246055; HM246066. **Plecostachys serpyllifolia** (P.J. Bergius) Hilliard & B.L. Burtt, –; –; –; –; AF098849*; AF100512*. **Podotheca gnaphalioides** Graham, –; –; –; –; AF141710*; AF141798*. **Relhania calycina** L'Hér.,  –; –; –; –; EU385107*; EU385107*. **Relhania fruticosa** (L.) K. Bremer, –; –; –; –; AF098813*; AF100476*. **Relhania genistifolia** (L.) L'Hérit., South Africa, E Cape Province: Mountain Ludtrea Port Elizabeth, 17-IX-1975, *Dahlstrand 3246* (W), –; –; –; –; –; HM246067. **Relhania pungens** L'Hérit., –; –; –; –; FN645749*; FN645749*. **Rhynchopsidium pumilum** (L.f.) DC., –; –; –; –; AF098811*; AF100474*. **Rosenia glandulosa** Thunb., –; –; –; –; AF098815*; AF100478*. **Stoebe aethiopica** L., –; –; –; –; AF098845*; AF100508*. **Toxanthes perpusilla** Turcz., –; –; –; –; AF141757*; AF141845*.