



Transworld Research Network  
37/661 (2), Fort P.O.  
Trivandrum-695 023  
Kerala, India

Recent Advances in Pharmaceutical Sciences III, 2013: 109-130 ISBN: 978-81-7895-605-3  
Editors: Diego Muñoz-Torrero, Amparo Cortés and Eduardo L. Mariño

## 7. Phylogenetic studies in Gnaphalieae (Compositae): The genera *Phagnalon* Cass. and *Aliella* Qaiser & Lack

Noemí Montes-Moreno<sup>1,3</sup>, Núria Garcia-Jacas<sup>1</sup>, Llorenç Sáez<sup>2</sup>  
and Carles Benedí<sup>3</sup>

<sup>1</sup>Botanic Institute of Barcelona (IBB-CSIC-ICUB). Passeig del Migdia s/n, 08038 Barcelona Spain; <sup>2</sup>Departament de Biologia Animal, Biologia Vegetal i Ecologia, Unitat de Botànica Facultat de Biociències, Universitat Autònoma de Barcelona. 08193, Bellaterra, Spain

<sup>3</sup>Departament de Productes Naturals, Biologia Vegetal i Edafologia, Unitat de Botànica Facultat de Farmàcia, Universitat de Barcelona. Avda. Joan XXIII s/n, 08028 Barcelona, Spain

**Abstract.** The precise generic delimitation of *Aliella* and *Phagnalon*, and their closest relatives within the Gnaphalieae are discussed in this review. Among the main results obtained, we have found that the genera *Aliella* and *Phagnalon* are nested within the “*Relhania* clade” and *Anisothrix*, *Athrixia* and *Pentatrichia* are their closest relatives. *Macowania* is also part of the “*Relhania* clade”, whereas the subtribal affinities of *Philyrophyllum* lie within the “crown radiation clade”. The monophyly of *Aliella* and *Phagnalon* is not supported statistically. In addition, *Aliella* appears to be paraphyletic in most of the analyses performed. The resulting phylogeny suggests an African origin for the ancestor of *Aliella* and *Phagnalon* and identifies three main clades within *Phagnalon* that constitute the following natural groups on a geographic basis: (1) the Irano-Turanian clade; (2) the Mediterranean-Macaronesian clade; and (3) the Yemen-Ethiopian

Correspondence/Reprint request: Dr. Noemí Montes-Moreno, Botanic Institute of Barcelona (IBB-CSIC-ICUB). Passeig del Migdia s/n, 08038 Barcelona, Spain. E-mail: n.montesmoreno@gmail.com

clade. Some endemics to Yemen and Ethiopia appeared merged in the Mediterranean-Macaronesian clade, providing new evidence of the phytogeographical links between Macaronesia, Eastern Africa and Southern Arabia. Incongruities 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 both *Phagnalon* and *Aliella*.

## 1. Historical overview, the Compositae family and the tribe Gnaphalieae

*Aliella* and *Phagnalon* belong to the Compositae family, which is the plant family with the largest number of described species, reaching 24,000–30,000 species distributed in 1,600–1,700 genera, with a cosmopolitan distribution except for Antarctica [1,2]. As it would be expected from its wide distribution, species of Compositae grow in a wide diversity of habitats: grasslands, wooded grasslands and montane vegetation, but they are comparatively scarce in humid tropical forests. In the same line, the morphological characters also exhibit wide variability. Species of Compositae exhibit most of the life forms, including annuals, pyrophytes, hemicryptophytes, trees, succulent plants, halophytes, lianas and also epiphytes and aquatics, although the majority of genera are subshrubs, shrubs or perennial herbs [3]. Another highly variable morphological trait is the number of florets per capitulum, which varies from one to 1,000 [1]. In spite of this high variability, the family has been traditionally considered a monophyletic group, morphologically well-defined by a combination of characters: (i) florets arranged on a receptacle, in centripetal heads surrounded by involucre bracts; (ii) syngenesious anthers, fused in a ring with the pollen pushed by the style; and (iii) fruits in achenes, usually with a pappus [2].

The tribe Gnaphalieae is one of the largest tribes of the Compositae, containing approximately 180–190 genera and 1,240 species. It is most diverse in the southern hemisphere, with three main centres of diversity: South America, South Africa, and Australia [4, 5, 6]. The current Gnaphalieae were initially classified under the Inuleae by Cassini [7] see Table 1. Later on, Bentham [8] split the Inuleae in nine subtribes based on the presence of homogamous or heterogamous capitula, and on other characters like the morphology of the receptacle, style and bracts. The majority of Gnaphalieae were included in the subtribes Filagineae, Gnaphalieae, Angianthiae, Relhanieae and Athrixieae. Merxmüller *et al.* [9] recognized three subtribes within the Inuleae: Inulinae, Gnaphaliinae and Athrixiinae, based on cytological, phytochemical, palinology and other traditionally used

morphological characters. These last authors placed the Gnaphalieae mainly in subtribe Gnaphaliinae, although some genera were also accommodated in the Athrixiinae. The Gnaphalieae were first recognized as independent from the Inuleae by Anderberg [4, 10], who divided the classic Inuleae [9] into three tribes: Inuleae, Plucheae and Gnaphalieae. Anderberg [4] suggested classifying the Gnaphalieae into five subtribes (Angianthinae, Cassiniinae, Gnaphaliinae, Loricariinae and Relhaniinae) based on cladistic analyses of morphological characters, except for some genera that were left in what he called the “basal group”. Several phylogenetic studies based on DNA sequences agreed in considering the Gnaphalieae as independent from the Inuleae [11, 12, 13].

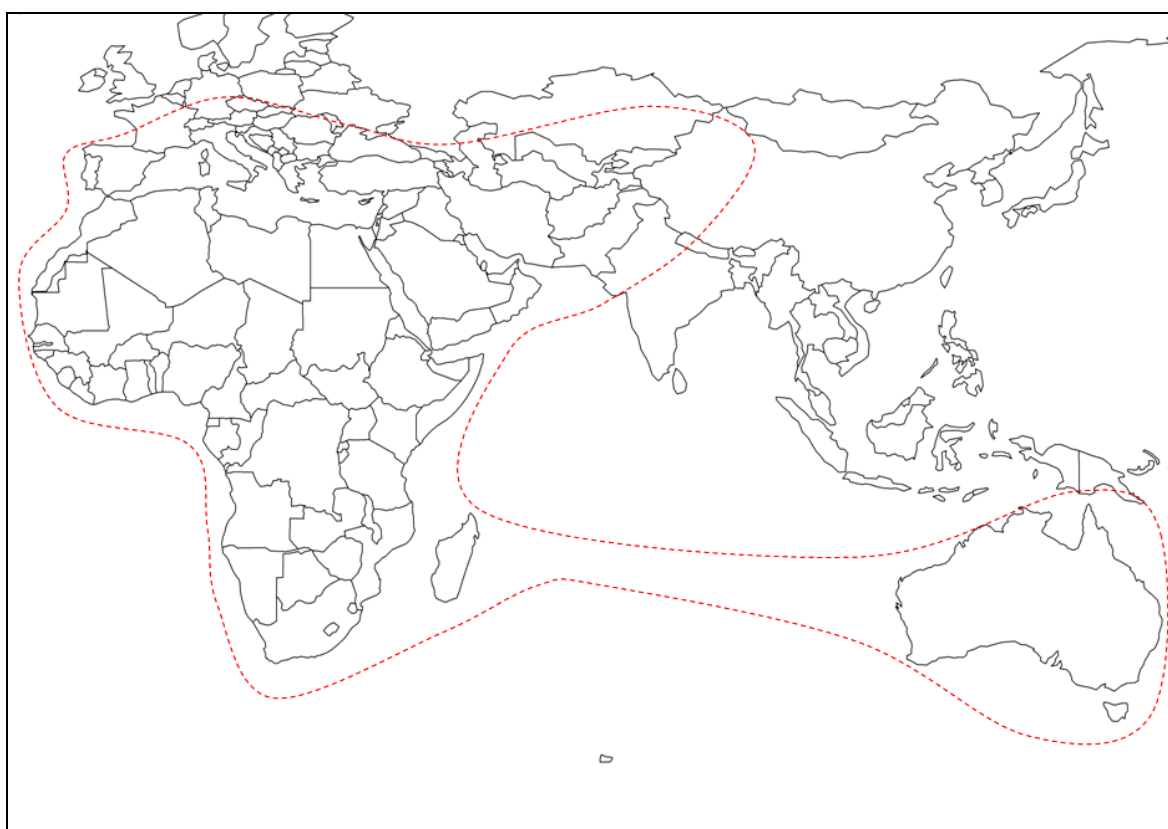
**Table 1.** Major taxonomic treatments of Gnaphalieae according to Ward *et al.* [6]. Asterisks indicate that the genera included in that sections or tribes currently belong to the modern tribe Gnaphalieae.

Cassini (1822)	Bentham (1873)	Mermüller <i>et al.</i> (1977)	Anderberg (1991)
Tribe Inuleae	Tribe Inuleae	Tribe Inuleae	Tribes:
Sections:	Subtribes:	Subtribes:	-Inuleae -Plucheae -Gnaphalieae
-Inuleae- Gnaphalieae *	-Filagineae* -Gnaphalieae*	-Inulinae -Gnaphaliinae*	Gnaphalieae subtribes:
-Inulae-Archetypae* -Inuleae- Bupthalthmeae	-Angianthieae* -Relhanieae* -Athrixieae*	-Athrixiinae*	-Angianthinae -Cassiniinae -Gnaphaliinae -Loricariinae -Relhaniinae

## 2. Phylogenetic relationships within the Gnaphalieae. The “*Relhania* clade”

As reported by Ward *et al.* [6], there are three main clades within the Gnaphalieae: (1) the “*Relhania* clade”, which is sister to the rest of the Gnaphalieae s. str., as previously noted by Bayer *et al.* [14]; (2) the “*Dolichothrix-Phaenocoma* clade”, which is the next one to diverge; and (3) the “crown radiation clade”, which comprises the rest of the genera included,

up to now, in the Gnaphalieae phylogenies. According to Bayer *et al.* [14, 15] the five subtribes recognized by Anderberg [4] are not monophyletic, implying that a recircumscription was necessary. The tribal and infratribal position of some genera of the Gnaphalieae, mainly those that belong to Anderberg's [4] “basal group”, are another taxonomic problem. The “basal group” comprises an assemblage of genera previously included in the Inuleae-Athrixiinae or Inuleae-Gnaphaliinae by Merxmüller *et al.* [9], together with other genera considered plesiomorphic relatives of the Gnaphalieae [10]. In fact, according to the latest phylogenetic studies on the Gnaphalieae, some of those genera are nested in the “*Relhania* clade” [6]. The members of the “*Relhania* clade” and most of the genera of the “basal group” are mainly distributed throughout Southern and tropical Africa and Australia, with several representatives in Macaronesia and the Mediterranean region, North Africa, Arabia, Middle East and the Irano-Turanian region [4], see Fig. 1. The “crown radiation clade” included genera previously assigned to the different subtribes delimited by Anderberg [4] and some of the genera included in the “basal group”. However, the infratribal affinities of some of the members of the “basal group” are unknown from a molecular point of view.



**Figure 1.** Distribution area of “*Relhania* clade” genera.

### 3. The genera *Aliella* and *Phagnalon*

The genus *Phagnalon* 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; it comprises about 36 species [16]. *Phagnalon* species are suffruticose shrubs or subshrubs and grow mainly in rocky areas, in a wide range of habitats: from sea level to 4.140 m altitude (Fig. 2). Species are morphologically characterized as having heterogamous disciform capitula;



**Figure 2.** Habit and morphology of capitula in *Phagnalon* taxa. a and b) *Ph. saxatile* (L.) Cass. (N. Montes-Moreno); c and d) *Ph. bicolor* Ball (M. Galbany-Casals); e) *Ph. rupestre* (L.) DC. (N. Montes-Moreno) and f) *Ph. rupestre* (L.) DC. (L. Sáez); g and h) *Ph. melanoleucum* Webb (M. Galbany-Casals).

female florets outnumbering the hermaphrodite ones; involucre bracts with undivided stereome; receptacle flat; anthers provided with long or medium tails; style bifid with obtuse or acute sweeping hairs; achenes with duplex hairs and monomorphic and uniseriate pappus composed by barbellate bristles. As regards to chromosome number, all the cytologically known species of *Phagnalon* are diploids with  $2n = 18$ .

Cassini [17a,b] separated *Phagnalon* from *Gnaphalium* based on the morphology of the involucre bracts and the ecaudate anthers. The only infrageneric classification of *Phagnalon* was proposed by Maire [18] who recognized two sections, *Phagnalon* sect. *Gnaphaliopsis* and sect. *Euphagnalon* (Table 1). Section *Euphagnalon* was characterized by ecaudate anthers and comprised *Ph. bicolor* Ball, *Ph. calycinum* (Brouss. ex Cav.) DC., *Ph. iminouakense* Emb., *Ph. latifolium* Maire, *Ph. rupestre* (L.) DC., *Ph. saxatile* (L.) Cass., and *Ph. sordidum* (L.) Rchb. *Gnaphaliopsis* section was characterized by caudate anthers and encompassed *Ph. embergeri* Humbert & Maire, *Ph. helichrysoides* Coss. & Maire and *Ph. platyphyllum* Maire. Subsequently, these three species were transferred to the genus *Aliella* by Qaiser & Lack [19]. *Phagnalon iminouakense* was combined under *Aliella* by Dobignard [20].

The genus *Aliella* (Compositae, Gnaphalieae) was described as a segregate from *Phagnalon* [19]. It is currently accepted as an independent genus [4, 20, 5, 6]. *Aliella* comprises four species and two subspecies: *A. ballii* (Klatt) Greuter [= *A. helichrysoides* (Ball) Qaiser & Lack]; *A. ballii* subsp. *ballii*; *A. ballii* subsp. *nitida* (Emb.) Qaiser & Lack; *A. embergeri* (Humbert & Maire) Qaiser & Lack; *A. iminouakensis* (Emb.) Dobignard & Jeanm. and *A. platyphylla* (Maire) Qaiser & Lack (Table 2).

**Table 2a.** Major taxonomic treatments of species included in *Phagnalon* sect. *Gnaphaliopsis* and *Aliella*.

Maire (1928)	Emberger (1932, 1935)	(Quézel, 1951)
<i>Ph. helichrysoides</i> (Ball) Coss. ex Maire	<i>Ph. helichrysoides</i>	<i>Ph. helichrysoides</i>
	<i>Ph. helichrysoides</i> var. <i>nitidum</i> Emb.	—
<i>Ph. platyphyllum</i> (Maire) Maire	<i>Ph. platyphyllum</i>	<i>Ph. platyphyllum</i>
<i>Ph. embergeri</i> Humbert & Maire	<i>Ph. embergeri</i>	<i>Ph. embergeri</i>
	<i>Ph. lepineyi</i> Emb.	—
		<i>Ph. iminouakense</i> Emb.

**Table 2b.** Major taxonomic treatments of species included in *Phagnalon* sect. *Gnaphaliopsis* and *Aliella* (cont.).

<b>Qaiser &amp; Lack (1986a)</b>	<b>Anderberg (1991)</b>	<b>Dobignard (1997)</b>	<b>Greuter (2008)</b>
<i>A. helichrysoides</i> (Ball) Qaiser & Lack	<i>A. bracteata</i> Anderb.	<i>A. helichrysoides</i>	<i>A. ballii</i> (Klatt) Greuter
<i>A. helichrysoides</i> subsp. <i>nitida</i> (Emb.) Qaiser & Lack	–	<i>A. helichrysoides</i> subsp. <i>nitida</i>	<i>A. ballii</i> subsp. <i>nitida</i> (Emb.) Greuter
<i>A. platyphylla</i> (Maire) Qaiser & Lack	<i>A. platyphylla</i>	<i>A. platyphylla</i>	<i>A. platyphylla</i>
<i>A. embergeri</i> (Humbert & Maire) Qaiser & Lack	<i>A. embergeri</i>	<i>A. embergeri</i>	–
–	–	–	–
–	–	<i>A. iminouakensis</i> (Emb.) Dobignard & Jeanm.	<i>A. iminouakensis</i>

The generic diagnosis was based on the presence of bracts on the peduncle similar in shape and size to the involucre bracts, the presence of waxy cushions on the corolla lobes, tubular female florets, caudate anthers, and pappus bristles barbellate from the base to the apex. The species of *Aliella* are caespitose or decumbent chasmophytic endemics and grow in calcareous or siliceous rock crevices in the Atlas Mountains of Morocco, at altitudes from 1.800 to 3.630 m (Fig. 3).

Regarding chromosome number, all the reports for *Aliella* show  $2n = 18$  [21, 22, 23], although there is a conflicting report of  $2n = 14$  for *A. ballii* [22].

According to the results of an analysis of morphological characters, the affinities of *Aliella* and *Phagnalon* are unknown [10]. *Phagnalon* was first placed in the tribe Inuleae, subtribe Gnaphaliinae, series Eugnaphalieae [8]. Later, it was accommodated in an informal group in the treatment of Inuleae by Merxmüller *et al.* [9], because it has some intermediate characters between the Gnaphaliinae, Inuliinae and Athriixinae; these features include filiform female florets, cartilaginous involucre bracts, apically confluent stigmatic areas, tailless anthers, a particular geographic distribution and a high basic chromosome number. Anderberg [10] placed *Aliella* and *Phagnalon*



**Figure 3.** Habit of *Aliella* taxa: a) *Aliella platyphylla* (Maire) Qaiser & Lack (L. Sáez); b, c and d) *Aliella ballii* subsp. *ballii* (N. Montes-Moreno); e) *Aliella ballii* subsp. *nitida* (Emb.) Greuter (N. Montes-Moreno); f) *Aliella iminouakensis* (Emb.) Dobignard & Jeanm. (N. Montes-Moreno).

in the Gnaphalieae based on morphological data. However, the results did not clearly indicate the subtribal affinities of *Aliella* and *Phagnalon* but pointed out that *Phagnalon* was nested within a clade with *Anisothrix* O. Hoffm., whereas *Aliella* was more related to the majority of the taxa of the Gnaphalieae than to *Phagnalon*. Therefore, Anderberg [4] included *Aliella* and *Phagnalon* in “the basal group” of the Gnaphalieae, together with the genera *Anisothrix*, *Pentatrachia* Klatt and *Philyrophyllum* O. Hoffm., among others. Up to date, tribal affinities of *Aliella* and *Phagnalon* have not been investigated with molecular markers.



#### 4. Applications in the field of pharmacology and medicine

Some *Phagnalon* species have economic importance as they are a source of compounds with several applications in the field of pharmacology and medicine. According to Góngora [24a] and Góngora *et al.* [24b], different compounds were isolated from *Ph. rupestre* (L.) Cass. Some of them, mainly flavonoids and hydroquinone glucosides, produced reduction of inflammatory reactions. Ali-Shtayeh *et al.* [25] investigated the antimicrobial activities of 20 Palestinian plants against five bacterial species (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) and one yeast (*Candida albicans*). The results showed that the most antimicrobial active plants were *Ph. rupestre* and *Micromeria nervosa* (Desf.) Benth. Other phytochemical studies have isolated phenolic compounds with antioxidant activity from *Ph. rupestre* [26]. Similarly, other compounds were isolated from other *Phagnalon* species. For example, thymol derivatives were isolated from *Ph. sinaicum* Bornm. & Kneuck. [27] and 1-chiro-inositol has been isolated from *Ph. sordidum* (L.) Rchb. [28]. According to Nuissier *et al.* [29] the chiro-inositol can be used in managing the diabetes. Other phenolic compounds were isolated from *Ph. saxatile* (L.) Cass. with applications in the treatment of Alzheimer's disease according to Conforti *et al.* [30]. In the Balearic Islands, *Ph. sordidum* is used alone or mixed with *Lippia citrodora* (Gómez Ortega & Palau) Kunth or *Malva sylvestris* L. to treat renal calculosis [28].

#### 5. Material and methods of the phylogenetic study

Given the lack of phylogenetic studies on *Aliella* and *Phagnalon* in the literature, Montes-Moreno *et al.* [31] performed a combined nuclear and chloroplast phylogeny to check the hypothesized monophyly of *Aliella* and *Phagnalon* to provide more information on the position of both genera within the tribe Gnaphalieae, and on their interspecific relationships. This approach was chosen because non-homologous morphological similarities are frequent in the Gnaphalieae [14]. Moreover, combined nuclear and chloroplast phylogenies have been successfully used in the Compositae and the Gnaphalieae at the generic and specific levels [14, 15, 32, 33, 34, 35, 36, 37].

Montes-Moreno *et al.* [31] sampled 29 of the 36 species of the genus *Phagnalon* together with four *Aliella* species. Representatives of the “*Dolicho-thrix-Phaenocoma* clade”, “crown radiation clade”, “*Relhania* clade” and the “basal group”, with emphasis on those that were already known to be placed within the “*Relhania* clade”, were also included. Outgroups were chosen from the Heliantheae, Inuleae, Calenduleae,

Anthemideae and Astereae for the analysis of the *trnL* intron and the *trnL/F* intergenic spacer.

The phylogenetic analyses were performed with the five following data sets: (1) *trnL* intron and *trnL/F* intergenic spacer regions (Fig. 4) to: (i) elucidate the tribal position 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”(2) ETS+ITS+ycf3-trnS+trnT-trnL (Fig. 5) to study the generic limits of *Aliella* and *Phagnalon*, and to elucidate the relationships within the *Aliella* and *Phagnalon* taxa (3) ETS+ITS (Fig. 6), in order to examine the position of some taxa not included in previous analyses and for which we could not amplify any chloroplast region (4) ycf3-trnS+trnT-trnL (Fig. 7) to verify the relationships between *Aliella* and *Phagnalon* and to elucidate possible incongruities with the nrDNA data and (5) ETS+ITS, performed with the exclusion of *Ph. latifolium* and *A. iminouakensis* (Fig. 8).

To analyze the combined cpDNA data set (*ycf3-trnS+trnT-trnL*), nrDNA data set (ETS+ITS) and nr-cpDNA data set (ETS+ITS+ycf3-trnS+trnT-trnL), *Anisothrix*, *Pentatrachia* and *Athrixia* were selected as outgroups, because they were the closest genera to *Aliella* and *Phagnalon* according to the results obtained from the *trnL* intron and the *trnL/F* intergenic spacer analyses carried out (Fig. 4).

The analyses used 221 new sequences (40 ETS, 49 3’ETS, 40 ITS, 34 *ycf3-trnS*, 34 *trnT-trnL* and 24 *trnL* intron and the *trnL/F* intergenic spacer) together with 47 sequences from published EMBL/GenBank accessions [12, 14, 15, 38, 39, 40]; and also unpublished ones [Boelch *et al.* (unpublished) and Panero *et al.* (unpublished)]. Sources of published sequences, voucher data, and GenBank sequence accession numbers are given in Montes-Moreno *et al.* [31].

The protocols for DNA extraction, DNA amplification strategies, sequencing and phylogenetic analyses were described in Montes-Moreno *et al.* [31].

## 6. Phylogenetic relationships in the “*Relhania* clade”

Here we present the main relationships in the “*Relhania* clade” according to Montes-Moreno *et al.* [31].

The results from the combined *trnL* intron and *trnL-trnF* intergenic spacer pointed out that *Macowania* belongs to the “*Relhania* clade”, given that *Macowania tenuifolia* forms a monophyletic group with *Arrowsmithia* (Fig. 4) within that clade. *Macowania* Oliv. consists of 12 species from South Africa, Arabia and Ethiopia [4], and it was already stated to be close to the “*Relhania* clade” on the basis of morphological data [4, 41, 42].

Our results strongly supported that *Philyrophyllum* O. Hoffm., a genus with two species endemic to Namibia and Botswana [43], belongs to the “crown radiation clade”, in contrast with evidences from morphological characters which led Anderberg [4, 44] to consider it part of the “basal group”, pointing out a close relationship with *Anisothrix* and *Pentatrichia*.

*Athrixia* Ker.-Gawl. comprises about 14 species from South Africa, tropical Africa and Madagascar [41]. Some authors pointed out that the phylogenetic affinities of this genus were outside of “*Relhania* clade” [14], while other authors argued that it was close to the “*Relhania* clade” [6].

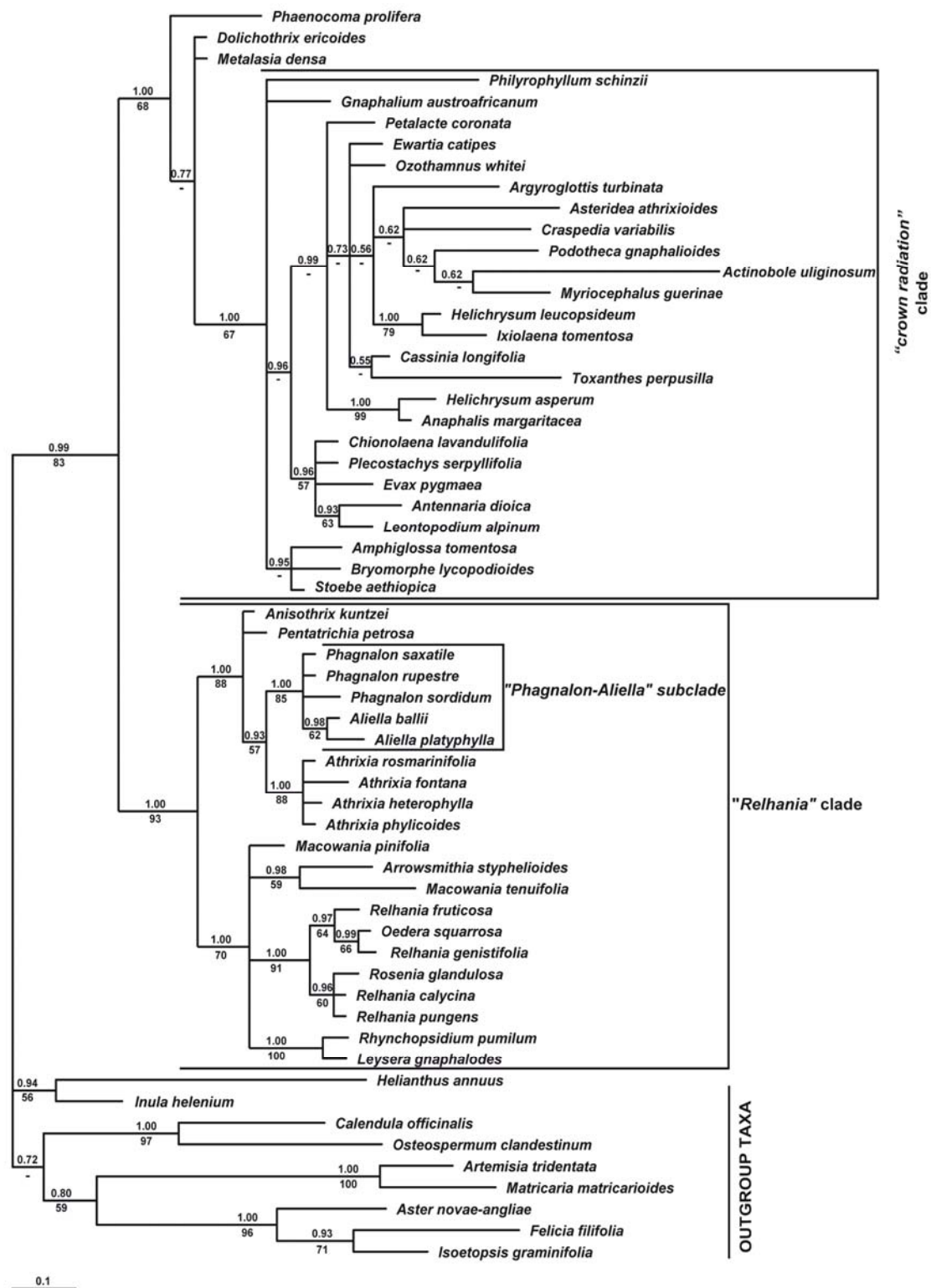
Our results were in accordance with Ward *et al.* [6], because the four *Athrixia* species sampled formed a strongly supported monophyletic group within the *Relhania* clade (Fig. 4). These incongruent results between the different studies could be due to the different species sampled and indicate a possible problem of generic delimitation in *Athrixia*.

According to Montes-Moreno *et al.* [31], *Phagnalon* and *Aliella* are nested within the “*Relhania* clade”, as they formed a monophyletic group together with *Anisothrix*, *Athrixia*, and *Pentatrichia*. An African origin for the ancestor of *Aliella* and *Phagnalon* was suggested because the genera more closely related to them are restricted to South Africa, tropical Africa and Madagascar, as it is the case of other Gnaphalieae with Mediterranean representatives (e.g., *Helichrysum* Mill., [40]; *Ifloga* Cass. and *Leysera* L., [39]).

Although the lack of informative characters did not allow to establish their sister group, the unambiguous alignment of 5'ETS sequences of *Aliella*, *Anisothrix*, *Pentatrichia* and *Phagnalon* suggested that *Anisothrix* and *Pentatrichia* are the most closely related to *Aliella* and *Phagnalon*, while the rest of representatives from the “*Relhania* clade” are more distantly related. This hypothetically sister relationship agreed with morphological and phytochemical data, as *Anisothrix*, *Pentatrichia* share with at least some *Aliella* and *Phagnalon* several morphological features: long caudate anthers, waxy cushions on the outside of the corolla lobes, non myxogenic filiform twin hairs on the achene surface, acute sweeping hairs arranged apically on the stigmatic surface (Montes-Moreno, pers. obs.), leaves with dentate margins [14], phloem not concealed in fibers as well, and presence of six derivatives of leysseral compounds among *Anisothrix* and *Phagnalon* [10,45].

## 7. Generic delineation of *Aliella* and *Phagnalon*

Montes-Moreno *et al.* [31] found that *Phagnalon* and *Aliella* form a strongly supported monophyletic group (Figs. 4–8), but the generic boundaries between them were difficult to establish because the results showed unresolved phylogenetic relationships. Firstly, only three *Aliella*



**Figure 4.** Strict consensus tree of the 1.516 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).

species (*A. ballii*, *A. embergeri* and *A. platyphylla*) formed a monophyletic group; (Figs. 5 and 6). Secondly, there were incongruities between the molecular and morphological data because *A. iminouakensis* was firmly nested within the *Phagnalon* clade, even though presented all the diagnostic characters of *Aliella* (Fig. 7). Finally, the monophyly of *Phagnalon* was only statistically supported by the analysis carried out excluding *A. iminouakensis* and *Ph. latifolium* (Fig. 8).

These results suggested that *A. iminouakensis* and *Ph. latifolium* have a complex phylogenetic history and could be derived from an ancient hybridization event for the following reasons: a) the noteworthy increase in the bootstrap value of the *Phagnalon* clade, after the exclusion of both taxa; b) the incongruities between nrDNA and cpDNA data sets indicated by the ILD test and the tree topologies; c) the unresolved position of these two taxa; and d) the morphological intermediacy of *Ph. latifolium* between *Aliella* and *Phagnalon*.

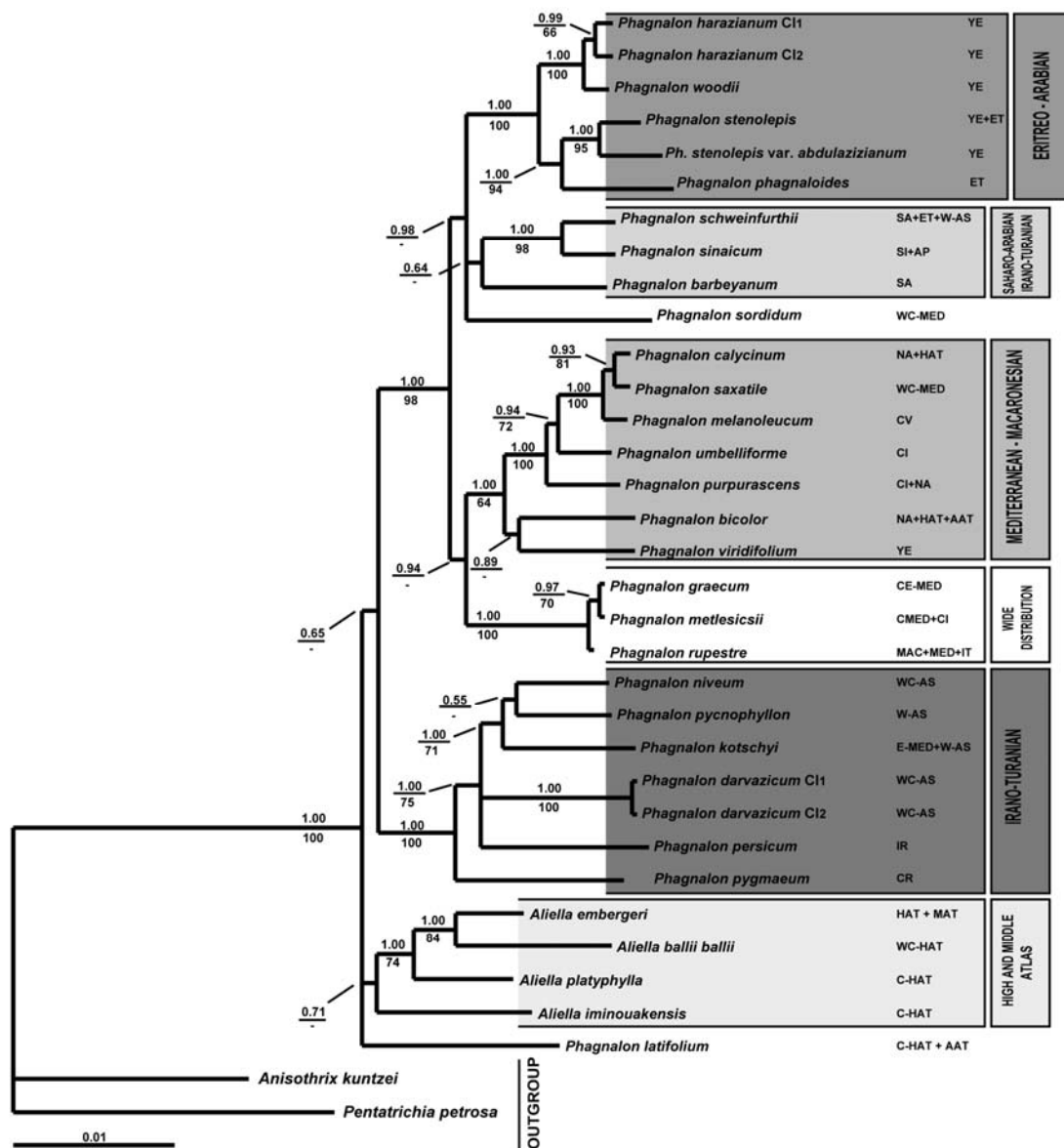
Hybridization has been documented among several *Phagnalon* species (*Ph. saxatile*, *Ph. sordidum* and *Ph. rupestre*, cf. [46]; and *Ph. niveum* Edgew. and *Ph. pycnophyllum* Rech. f., cf. [16]; Montes-Moreno, pers. obs.), from the observation of several intermediate specimens in the field and in herbarium material. The geographical distribution of *A. iminouakensis* does not currently overlap with the putative *Phagnalon* maternal (cpDNA) donors (Fig. 6), but the geographical distribution of *Ph. latifolium* overlaps with *A. ballii* and *A. platyphylla*, since vouchers of *A. ballii*, *A. platyphylla* and *Ph. latifolium* have been collected in the same locality. Moreover, other hybridization or introgression events have also been reported between taxa included in different sections or genera in the Compositae (*Andryala* L., *Hieracium* L., and *Pilosella* Hill., cf. [47]), and particularly in the Gnaphalieae [*Anaphalioides* (Benth.) Kirp. and *Ewartia* Beauverd, cf. [48]; *Anaphalioides* and *Helichrysum*, cf. [49]].

Further studies involving genetic markers are required to confirm the putative hybridization hypothesized for *Phagnalon* and *Aliella*.

## 8. Phylogenetic relationships in *Phagnalon*

Results of the combined nuclear-chloroplast phylogeny indicated that the diversification in *Phagnalon* took place in three main areas [31]: the Irano-Turanian region, the Mediterranean and Macaronesian regions, and the Saharo-Arabian region. The acronyms used in all the phylogenetic trees are: AS Asian, AAT AntiAtlas, AP Arabian Peninsula, 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.

The Irano-Turanian Clade included species from the Irano-Turanian region and *Ph. pygmaeum* (Sieber) Greuter, a Crete endemic which is sister to the rest of the Irano-Turanian species (Figs. 5, 6 and 8). This relationship suggested that a common ancestor of the whole Irano-Turanian clade reached Crete and, from there, colonized the mainland, where it radiated and diversified in the Irano-Turanian region by wind dispersal of the achenes, as Qaiser & Lack (1986a) reported for other *Phagnalon* species.



**Figure 5.** Consensus phylogram of the post-burn-in trees resulting from Bayesian MCMC analysis of the combined ETS, ITS, *ycf3-trnS* and *trnT-trnL* data sets. 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.

However, a second possible scenario may have involved a common ancestor, distributed and diversified throughout the mainland that colonized Crete, via land connections or long distance dispersal, before becoming extinct. In addition, a combination of both scenarios was also possible.

The interspecific relationships among these Irano-Turanian taxa could be explained by considering some morphological affinities: *Ph. acuminatum* Boiss., *Ph. kotschyi* Sch. Bip. ex Boiss. and *Ph. pygmaeum* have linear to subulate bracts in the capitulum; *Phagnalon kotschyi*, *Ph. pygmaeum* and *Ph. persicum* Boiss. have waxy cushions on the outside of the corolla lobes. The lack of resolution of the Irano-Turanian clade was mainly attributed to recent diversification or to the putative hybridization between *Ph. niveum* and *Ph. pycnophyllum* [16].

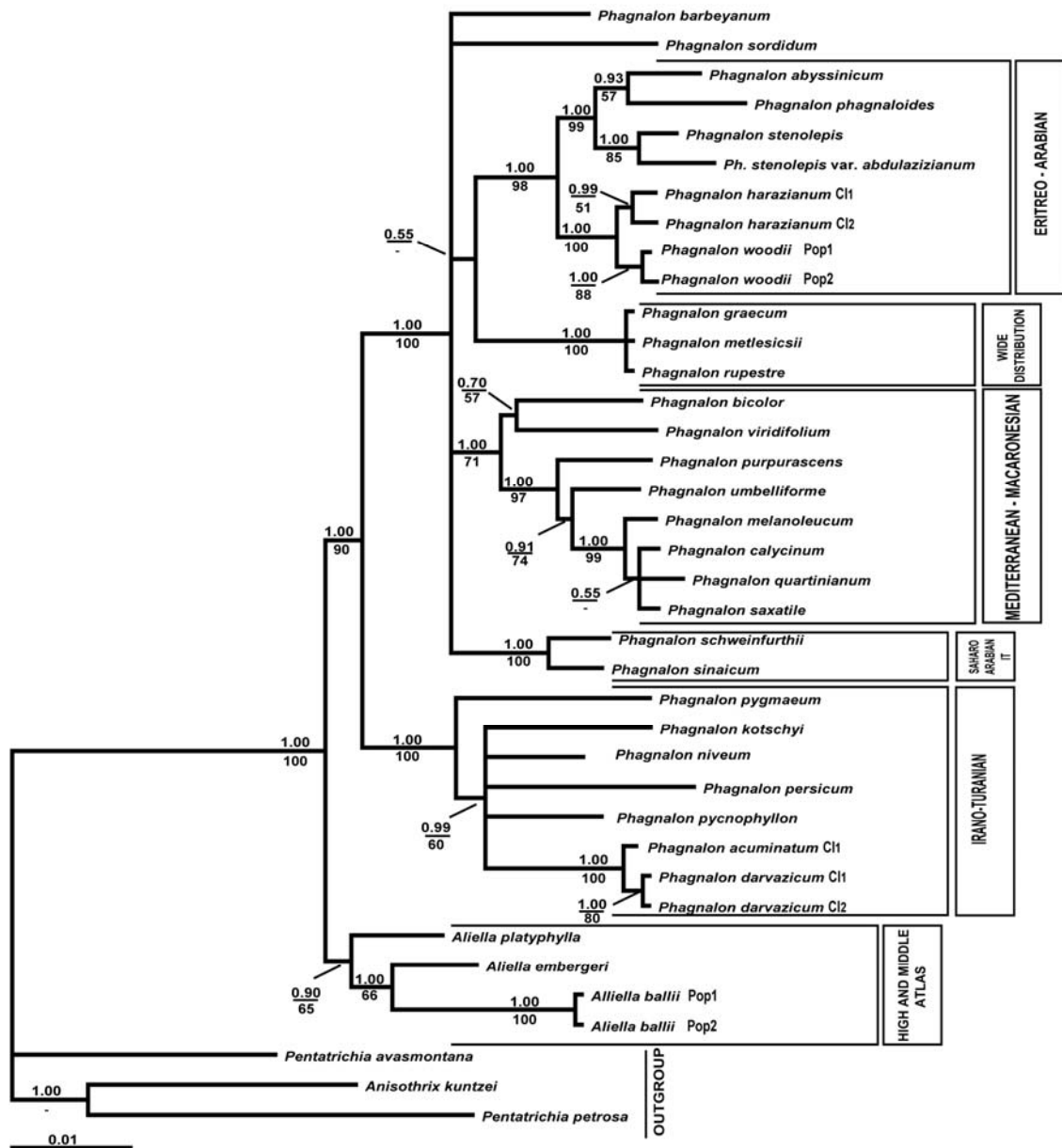


**Figure 6.** Consensus phylogram of the post-burn-in trees resulting from Bayesian MCMC analysis of the combined ETS and ITS data sets. Numbers above the branches are Bayesian clade credibilities (posterior probability, PP). Numbers below the branches indicate parsimony bootstrap percentages (BS). Other abbreviations: Cl = clone; Pop = population.



**Figure 7.** 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). Other abbreviations: CI = clone; Pop = population.





**Figure 8.** 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.

The Mediterranean and Macaronesian Clade encompassed mostly Mediterranean and Macaronesian taxa. This clade included some East African and Saudi Arabian-Omani endemics (*Ph. quartinianum* A. Rich. and *Ph. viridifolium* Decne. ex Boiss. respectively; Fig. 6 and 8) and a subclade involving *Ph. purpurascens* Sch. Bip., *Ph. umbelliforme* DC., *Ph. melanoleucum* Webb, *Ph. calycinum* (Brouss. ex Cav.) DC. and *Ph. saxatile* (Figs. 5, 6 and 8).

This pattern is found in phylogenetic studies of other genera: *Aeonium* Webb & Berthel., *Pulicaria* Gaertn. [50], *Euphorbia* L. [51], and *Helichrysum* [45]. Traditionally, these disjunctions have been considered evidences of a continuous flora that existed in the late Miocene [52, 53]. 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 [52, 54, 55]. Some morphological affinities between several representatives of this clade were also reported: *Ph. bicolor* Ball, *Ph. quartinianum* and *Ph. viridifolium* share the transparent scarious margins of the bracts.

The subclade involving *Ph. purpurascens* (North Africa and Canary Islands), *Ph. umbelliforme* (Canary Islands), *Ph. melanoleucum* (Cape Verde), *Ph. calycinum* (North Africa) and *Ph. saxatile* (Western Mediterranean and Macaronesian region) suggested the two following hypotheses: (1) The Canary Islands were colonized by a North African ancestor by a long-distance dispersal event from North Africa, as has been hypothesized for other plant groups [45, 56]. A Canary Islands ancestor would have colonized the Cape Verde Islands, followed by a back colonization to the mainland, where *Ph. calycinum* and *Ph. saxatile* would have originated (Fig. 5, 6 and 8); (2) The second scenario involved an extinct ancestor distributed in 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 [45, 57, 58]. In addition, the species distributed in the Canary Islands do not form a monophyletic group. Therefore, the Canary Islands colonization by *Phagnalon* would have been produced by, at least, two colonization events, as other phylogenetic studies have pointed out for some Macaronesian plant groups [45, 59].

The Eritreo-Arabian Clade included a second subclade encompassed mainly endemics to Yemen, and included *Ph. harazianum* Deflers and *Ph. woodii* Qaiser & Lack (Figs. 5, 6 and 8). This Eritreo-Arabian Clade also included *Ph. abyssinicum* Sch. Bip. ex A. Rich. and *Ph. phagnaloides* (Sch. Bip. ex A. Rich.) Cufod. (both endemic from Ethiopia) and *Ph. stenolepis* Chiov., which has a disjoint distribution between Sudan (Jebel Marra), Chad (Tibesti), Ethiopia, Saudi Arabia and Yemen (Fig. 5, 6 and 8). 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, as previously indicated by Wickens [60]. This dispersal event likely followed a West-to-East route, which is in accordance to the African migration routes hypothesized for the Pliocene and Pleistocene floras [52]. From a systematic point of view, our results are the first in confirming that *Ph. phagnaloides* belongs to *Phagnalon*, as this species is

very different from the rest of *Phagnalon* species in having the capitulum arranged in leafy racemes, and was considered by other authors [A. Richard and Schultz Bipontinus] under the genus *Blumea* and *Pluchea* respectively.

Another endemic from adjacent Saudi Arabian-Oman area (*Ph. quartinianum*) was clustered in the Mediterranean-Macaronesian clade together with East African *Ph. viridifolium*. This phylogenetic relationship suggested that the East African and Yemen-Oman areas underwent, at least, two colonization events, because all the remaining endemics to Arabia and Ethiopia are nested within the Eritreo-Arabian subclade.

Other highly supported monophyletic groups were not nested within the three main clades. One of them included *Ph. graecum* Boiss. & Heldr., *Ph. metlesicsii* Pignatti, and *Ph. rupestre* (Figs. 5, 6 and 8). *Phagnalon metlesicsii* was described from Sicily based on glabrous leaves with dentate margins, and some other glabrous populations of *Ph. rupestre* have been located in the Canary Islands [61]. Based on the molecular, morphological and chorological data, *Ph. metlesicsii* was concluded to fall into the morphological variability of *Ph. rupestre*.

However, there were some incongruities between the morphological and molecular data: *Ph. stenolepis* and *Ph. acuminatum* have linear to lanceolate bracts and lanceolate leaves with dentate margins but did not form a monophyletic group. Likewise, *Ph. quartinianum*, *Ph. schweinfurthii* Sch. Bip. ex Schweinf., *Ph. sinaicum* Bornm. & Kneuck. and *Ph. viridifolium* have oblong to spatulate bracts, with broadly scarious transparent margins, but they did not form a monophyletic group. This result indicated that these morphological traits appeared more than once in *Phagnalon* and should be interpreted as morphological convergence.

## 9. Conclusion

The study of Montes-Moreno *et al.* [31] discussed in this review was the first to define, on a molecular basis, the phylogenetic position of *Aliella*, *Macowania* and *Phagnalon* in the “*Relhania* clade”. In addition, the *Philyrophyllum* was found to be placed within the “crown radiation clade”. Moreover, *Anisothrix*, *Athrixia* and *Pentatrachia* are the most closely related genera to *Aliella* and *Phagnalon*. The diversification in *Phagnalon* took place in three main areas: Western and Central Asia, the Mediterranean and Macaronesian region, and the Eritreo-Arabian region. Some incongruities between the chloroplast and nuclear molecular data, as well as the lack of resolution in some clades, suggested that hybridization could have played an important role in the evolution and diversification of both *Aliella* and *Phagnalon*.

## Acknowledgements

The authors thank the curators of all herbaria that provided material (B, BC, BCN, BM, E, LE, MA, SAF, TFC, UPS, W and WU), and the “Viera & Clavijo” and Mediterranean Agronomic Institute of Chania Botanical Gardens for providing seeds. We also acknowledge S. Arrabal, R. Bayer, N. Bergh, M. Galbany-Casals, F. Gómiz, O. Hidalgo, M. Koekemoer, J. Molero, R. Rodríguez-Gómez, A. Romo, K. Romashchenko, Jaume X. Soler and J. Vicens who provided material and/or field collections, and M. Veny for keeping the collections of living plants. We also want to thank M. Galbany-Casals, M. Sanz, R. Vilatersana, an anonymous reviewer, and J. Lundberg for technical assistance and/or helpful comments. In addition, we acknowledge M. Galbany-Casals for reviewing this manuscript. This work has been partly financed by the Spanish government (REN2002-04634-C05-01, CGL2004-04563-C02-01/BOS) and the Catalan government (“Ajuts a grups de recerca consolidats” 2009/SGR/00439).

## References

1. Funk, V.A., Bayer, R.J., Keeley, S., Chan, R., Watson, L., Gemeinholzer, B., Schilling, E., Panero, J.L., Baldwin, B.G., Garcia-Jacas, N., Susanna, A. & Jansen, R.K. 2005, *Biol. Skr.*, 55, 343.
2. Funk, V.A., Susanna, A., Stuessy, T.F. & Robinson, H. 2009, Classification of Compositae. In: Funk, V.A., Susanna, A., Stuessy, T.F. & Bayer, R.J. (Eds.). Systematics, evolution and biogeography of Compositae. Vienna: IAPT, 171.
3. Jeffrey, C. 2007, Compositae. Introduction with key to tribes. In: Kadereit, J.W., Jeffrey, C. (Eds.). The families and genera of vascular plants. Flowering plants. Eudicots. Asterales, vol. VIII. Springer-Verlag Press: Berlin and Heidelberg, 61.
4. Anderberg, A.A. 1991, *Opera Bot.*, 104, 1.
5. Bayer, R.J., Breitwieser, I., Ward, J.M. & Puttock, C.F. 2007, Tribe Gnaphalieae. In: Kadereit, J.W., Jeffrey, C. (Eds.). The families and genera of vascular plants. Flowering plants. Eudicots. Asterales, vol. VIII. Springer-Verlag Press: Berlin and Heidelberg, 246.
6. Ward, J.M., Bayer, R.J., Breitwieser, I., Smitsen, R.D., Galbany-Casals, M. & Unwin, M. 2009, Gnaphalieae – Systematic and phylogenetic review. In: Funk, V.A., Susanna, A., Stuessy, T.F. & Bayer, R.J. (Eds.). Systematics, evolution, and biogeography of Compositae. Vienna: IAPT, 537.
7. Cassini, H. 1822, Inulées. In: Cuvier, F. (Ed.). Dictionnaire des Sciences Naturelles, ed. 2, vol. XXIII. Paris: Le Normant, 559.
8. Bentham, G. 1873, Compositae. In: Bentham, G., Hooker, J.D. (Eds.). Genera Plantarum, vol. II. London: Reeve, 163.
9. Merxmüller, H., Leins, P. & Roessler, H. 1977. Inuleae – systematic review. In: Heywood, V.H., Harbone, J.B. & Turner, B.L. (Eds.). The biology and chemistry of the Compositae, vol. I. London: Academic Press, 577.

10. Anderberg, A.A. 1989, *Canad. J. Bot.*, 67, 2277.
11. Kim, K.-J. & Jansen, R.K. 1995, *Proc. Natl. Acad. Sci. U.S.A.*, 92, 10379.
12. Bayer, R.J. & Starr, J.R. 1998, *Ann. Missouri Bot. Gard.*, 85, 242.
13. Wagstaff, S.J. & Breitwieser, I. 2002, *Pl. Syst. Evol.*, 231, 203.
14. Bayer, R.J., Puttock, C.F. & Kelchner, S.A. 2000, *Amer. J. Bot.*, 87, 259.
15. Bayer, R.J., Greber, D.G. & Bagnall, N.H. 2002, *Syst. Bot.*, 27, 801.
16. Qaiser, M. & Abid, R. 2003, *Phagnalon* Cass. In: Ali, S.I. & Qaiser, M. (Eds.). Flora of Pakistan. Asteraceae (II) Inuleae, Plucheeae & Gnaphalieae, vol. 210. Department of Botany, University of Karachi and Missouri Press, Missouri Botanical Garden, St. Louis.
17. a) Cassini, H. 1819, *J. Phys. Chim. Hist. Nat. Arts*, 88, 189., b) Cassini, H. 1819, *Bull. Sci. Soc. Philom. Paris*, 1819, 172.
18. Maire, R. 1928, *Bull. Soc. Hist. Nat. Afrique N.*, 19, 29.
19. Qaiser, M. & Lack, H.W. 1986, *Bot. Jahrb. Syst.*, 106, 487.
20. Dobignard, A. 1997, *Candollea*, 52, 119.
21. Quézel, P. 1957, *Peuplements végétaux des hautes montagnes de l'Afrique du Nord*. Paris: Editions P. Lechevalier.
22. Humphries, C.J., Murray, B.G., Bocquet, G. & Vasudevan, K. 1978, *Bot. Not.*, 131, 391.
23. Galland, N. & Favarger, C. 1985, *Candollea*, 40, 231.
24. a) Góngora, L. 2002, PhD Thesis dissertation, Universitat de València, València, Spain., b) Góngora, L., Giner, R.M., Máñez, S., Recio, M. del C. & Ríos, J.L. 2002, *Planta Med.*, 68, 561.
25. Ali-Shtayeh, M.S., Yaghmour, R.M.-R., Faidi, Y.R., Salem, K. & Al-Nuri, M.A. 1998, *J. Ethnopharmacol.*, 60, 265.
26. Olmos, A., Máñez, S., Giner, R.M., Recio, M. del C. & Ríos, J.L. 2005, *Nitric Oxide-Biol. Ch.*, 12, 54.
27. El-Dahmy, S.I., Abdel Aal, M., Abd el-Fatah, H. & Fid, F. 1994, *Acta Pharm. Hung.*, 64, 115.
28. Epifano, F., Marcotullio, M.C. & Menghini, L. 2002, *Chem. Nat. Compd.*, 38, 204.
29. Nuissier, G., Diaba, F. & Grignon-Dubois, M. 2008, *Innov. Food Sci. Emerg.*, 9, 396.
30. Conforti, F., Rigano, D., Formisano, C., Bruno, M., Loizzo, M.R., Menichini, F. & Senatore, F. 2010, *J. Enzyme Inhib. Med. Chem.*, 25, 97.
31. Montes-Moreno, N., Sáez, L. Benedí, C, Susanna, A. & Garcia-Jacas, N. 2010, *Taxon*, 59, 1654.
32. Konishi, N., Watanabe, K. & Kosuge, K. 2000, *Austral. Syst. Bot.*, 13, 709.
33. Álvarez Fernández, I., Fuertes Aguilar, J., Panero, J.L. & Nieto Feliner, G. 2001, *Molec. Phylogen. Evol.*, 20, 41.
34. Susanna, A., Garcia-Jacas, N., Vilatersana, R. & Garnatje, T. 2003, *Collect. Bot. (Barcelona)*, 26, 101.
35. Liu, H., Trusty, J., Oviedo, R., Anderberg, A.A. & Francisco-Ortega, J. 2004, *Int. J. Pl. Sci.*, 165, 209.
36. Lee, C., Kim, S.C., Lundy, K. & Santos-Guerra, A. 2005, *Amer. J. Bot.*, 92, 2072.

37. Sonnante, G., Carluccio, A.V., Vilatersana, R. & Pignone, D. 2007, *Genet. Resources Crop Evol.*, 54, 483.
38. Panero, J.L. & Funk, V.A. 2008, *Molec. Phylogen. Evol.*, 47, 757.
39. Bergh, N.G. & Linder, H.P. 2009, *Molec. Phylogen. Evol.*, 44, 5.
40. Galbany-Casals, M., Garcia-Jacas, N., Sáez, L., Benedí, C. & Susanna, A. 2009, *Int. J. Pl. Sci.*, 170, 365.
41. Kroner, G. 1980, *Mitt. Bot. Staatssamml. München*, 16, 1.
42. Hilliard, O. & Burt, B.L. 1985, *Notes Roy. Bot. Gard. Edinburgh*, 42, 227.
43. Herman, P.P.J. 2003, *Bothalia*, 33, 118.
44. Anderberg, A.A. 1988, *Bot. Jahrb. Syst.*, 109, 363.
45. Zdero, C., Bohlmann, F. & Anderberg, A.A. 1991, *Phytochemistry*, 30, 3009.
46. Faure, A. 1923, *Bull. Soc. Hist. Nat. Afrique N.*, 14, 233 et 293.
47. Fehrer, J., Gemeinholzer, B., Chrtek, J. & Brautigam, S. 2007, *Molec. Phylogen. Evol.*, 42, 347.
48. McKenzie, R.J., Ward, J.M. & Breitwieser, I. 2008, Hybridization beyond the F1 generation between the New Zealand endemic everlastings *Anaphalioides bellidioides* and *Ewartia sinclairii* (Asteraceae, Gnaphalieae). *Pl. Syst. Evol.*, 273: 13-24.
49. Smissen, R.D., Breitwieser, I. & Ward, J.M. 2007, *Bot. J. Linn. Soc.*, 154, 89.
50. Andrus, N., Trusty, J., Santos-Guerra, A., Jansen, R.K. & Francisco-Ortega, J. 2004, *Taxon*, 53, 333.
51. Molero, J., Garnatje, T., Rovira, A., Garcia-Jacas, N. & Susanna, A. 2002, *Pl. Syst. Evol.*, 231, 109.
52. Quézel, P. 1978, *Ann. Missouri Bot. Gard.*, 65, 479.
53. Bramwell, D. 1985, *Bot. Macaronés.*, 14, 3.
54. Axelrod, D.I. 1975, *Ann. Missouri Bot. Gard.*, 62, 280.
55. Sunding, P. 1979. Origins of the Macaronesian flora. In: Bramwell, D. (Ed.). *Plants and islands*. London: Academic Press, 13.
56. Francisco-Ortega, J., Goertzen, L.R., Santos-Guerra, A., Benabid, A. & Jansen, R.K. 1999, *Syst. Bot.*, 24, 249.
57. Allan, G.J., Francisco-Ortega, J., Santos-Guerra, A., Boerner, E. & Zimmer, E.A. 2004, *Molec. Phylogen. Evol.*, 32, 123.
58. Font, M., Garcia-Jacas, N., Vilatersana, R., Roquet, C. & Susanna, A. 2009, *Ann. Bot. (Oxford)*, 103, 985.
59. Martín-Bravo, S., Meimberg, H., Luceño, M., Märkl, W., Valcárcel, V., Bräuchler, C., Vargas, P. & Heubl, G. 2007, *Molec. Phylogen. Evol.*, 44, 1105.
60. Wickens, G.E. 1976, *Kew Bull.*, 31, 105.
61. Reyes-Betancort, J.A., León Arencibia, M.C. & Wildpret de la Torre, W. 1996, *Bot. Macaronés.*, 23, 297.