Phylogeny and evolution of achenial trichomes in the *Lucilia*-group (Asteraceae: Gnaphalieae) and their systematic significance

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Abstract The Gnaphalieae (Asteraceae) are a cosmopolitan tribe with around 185 genera and 2000 species. The New World is one of the centers of diversity of the tribe with 24 genera and over 100 species, most of which form a clade called the *Lucilia*-group with 21 genera. However, the generic classification of the *Lucilia*-group has been controversial with no agreement on delimitation or circumscription of genera. Especially controversial has been the taxonomic value of achenial trichomes and molecular studies have shown equivocal results so far. The major aims of this paper are to provide a nearly complete phylogeny of the *Lucilia*-group at generic level and to discuss the evolutionary trends and taxonomic significance of achenial trichome morphology. We conducted a phylogenetic analysis of the New World Gnaphalieae with nrDNA (ETS, ITS) sequence data from a sampling of 18 genera of the *Lucilia*-group and utilized these results to examine morphological evolution of achenial trichome types and presence of apical myxogenic cells. Seven well-supported subclades can be recognized within the *Lucilia*-group (L1–L7). These results support Brazilian and Andean *Berroa*, *Facelis*, *Lucilia*, and *Micropsis* forming a clade (L1), the inclusion of Chilean *Lucilia* under *Belloa* (L2), the monophyly of *Stuckertiella*+*Gamochaeta*+*Gamochaetopsis* (L3), *Chevreulia*+*Cuatrecasasiella* (L4) and *Antennaria* (L5) excluding *Antennaria linearifolia*, which is resolved in a monophyletic group together with *Jalcophila*, *Loricaria* and *Mniodes* (L6), and the recognition of *Gnaphaliothamnus* (L7) removed from Brazilian taxa of *Chionolaena* (L2). Ancestral character state reconstruction of achenial trichome morphology suggests that clades are homogeneous in terms of trichome type, but with exceptions that make it highly homoplastic. Conversely, our results suggest that the presence of myxogenic apical cells is less homoplastic and that closely related species tend to resemble each other more than expected under random variation.

Keywords achenial trichome; *Lucilia*-group; morphology; nrDNA; phylogeny; South America

Supplementary Material Electronic Supplement (Fig. S1) and DNA sequence alignments are available in the Supplementary Data section of the online version of this article at http://ingentaconnect.com/content/iapt/tax

■ INTRODUCTION

The Gnaphalieae (Asteraceae) contains 180–190 genera and perhaps 2000 species with worldwide distribution (Anderberg, 1994), and centers of diversity are found in South Africa (Bayer & al., 2000), Australia (Bayer & al., 2002), New Zealand (Breitwieser & Ward, 2003), and South America (Dillon & Sagástegui Alva, 1991b). In the most recent taxonomic treatment of the whole Gnaphalieae, Anderberg (1991) prepared a morphological cladistic analysis that included 72 genera and utilized 82 morphological characters to establish five subtribes and many putatively monophyletic groups. Through adding taxa intuitively, his classification ultimately treated 146 genera.

Recent phylogenetic studies (e.g., Bergh & Linder, 2009; Ward & al., 2009; Blöch & al., 2010; Galbany-Casals & al., 2010, 2014; Bergh & al., 2011; Smissen & al., 2011; Nie & al., 2013, 2016; Freire & al., 2015) have advanced the understanding of the relationships between genera. These studies have shown that the subtribes of Gnaphalieae as proposed by Anderberg (1991) are all non-monophyletic, that most South African elements branch basally in the phylogeny of Gnaphalieae, and three additional major clades, collectively designated as the "crown radiation", can be identified: (i) the HAP-clade, with the genera *Anaphalis* DC., *Achyrocline* (Less.) DC., *Pseudognaphalium* Kirp. and Eurasian and African *Helichrysum* Mill.; (ii) the AUS-clade possibly including most of the genera native to Australasia excluding *Helichrysum luteoalbum* (L.) Rchb.; (iii) the FLAG-clade including *Filago* L. and allies, *Leontopodium* (Pers.) R.Br. and perhaps all remaining South American genera (i.e., including those species not belonging to *Pseudognaphalium* and *Achyrocline*). The species of *Gnaphalium* L. s.str. do not belong to any of these clades

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and their placement within the crown radiation is uncertain (Smissen & al., 2011; Nie & al., 2016).

The South American genera of the FLAG-clade are considered as members of the *Lucilia*-group, originally proposed to include *Lucilia* Cass., *Belloa* J.Rémy, *Chevreulia* Cass., *Jalcophila* M.O.Dillon & Sagást., *Cuatrecasasiella* H.Rob., *Berroa* Beauverd and *Facelis* Cass. (Anderberg, 1991). Anderberg & Freire (1991) and Anderberg (1994) expanded the *Lucilia*-group by including two newly described genera, *Gamochaetopsis* Anderb. & S.E.Freire and *Luciliocline* Anderb. & S.E.Freire. Freire & al. (2015) showed that *Antennaria* Gaertn., *Chionolaena* DC., *Gamochaeta* Wedd., *Gnaphaliothamnus* Kirp., *Loricaria* Wedd., *Micropsis* DC., *Mniodes* (A.Gray) Benth. and *Stuckertiella* Beauverd should also be included in the *Lucilia*-group. Finally, Nie & al. (2016) retrieved the position of *Diaperia* Nutt. and *Mexerion* G.L.Nesom within the *Lucilia*-group, along with an expansion of the sampling, which confirmed previous results. Among South American Gnaphalieae, the phylogenetic relationships of *Parachionolaena* M.O.Dillon & Sagást., *Pseudoligandra* M.O.Dillon & Sagást. and *Raouliopsis* S.F.Blake, remain to be clarified.

Generic limits within the *Lucilia*-group have been controversial. Freire & al. (2015) showed that *Luciliocline* cannot be phylogenetically distinguished from *Mniodes* and sunk the former into the latter. *Gamochaeta* has almost unanimously been considered to be closely related to *Stuckertiella* (e.g., Anderberg, 1991; Dillon, 2003), and the latter was sunk into the former by Urtubey & al. (2016), along with *Gamochaetopsis*. Likewise, Anderberg (1991) considered the relationships of *Micropsis* as obscure, but most likely within the genera of his *Filago*-group. Based on achenial trichome morphology, Dillon (2003) observed that *Berroa*, *Facelis*, and *Micropsis* are all similar and suggested that *Micropsis* was more closely related to those taxa than to any within the *Filago*-group, a suggestion that appears confirmed by Freire & al. (2015). *Chionolaena* was treated by Freire (1993) as accepted by Anderberg (1991), where several species of *Gnaphaliothamnus* were synonymized under *Chionolaena* along with the monospecific genera *Parachionolaena* and *Pseudoligandra* (Dillon & Sagástegui Alva, 1990, 1991b). Dillon & Sagástegui Alva (1991b), Nesom (1990a, b, 1994) and Dillon & Luebert (2015) treated *Gnaphaliothamnus* as a distinct genus composed of Mexican and Central American taxa. Nesom (2001) transferred the remaining Mexican and Central American species of *Gnaphaliothamnus* to *Chionolaena*, while maintaining the opinion that the northern hemispheric elements were monophyletic. Until the current study, no phylogenetic analysis has explicitly evaluated the affinities of *Gnaphaliothamnus* in the Gnaphalieae.

Generic limits of *Belloa* and *Lucilia* have also been controversial. The discussion has centered on the systematic value of morphological characters (see Ward & al., 2009 for a detailed account of this discussion). On one side Anderberg and Freire (Freire, 1987; Anderberg, 1991; Anderberg & Freire, 1991) argued for a more or less equal value of all morphological characters, while Dillon (Dillon & Sagástegui Alva, 1990, 1991b; Dillon, 2003) proposed that achenial trichomes are more important in the delimitation of genera.

The characters associated with the surface of the achenes have been of interest to various authors who have demonstrated their utility to circumscribe groups in the Asteraceae (Narayana, 1979; Pope, 1983; Hansen, 1990) including the Gnaphalieae (Ciccarelli & al., 2007; Abid & Qaiser, 2008a, b; Mukherjee & Nordenstam, 2012). Trichomes originate from a single protoderm initial within the epidermal tissue, regardless of the ultimate type formed. There are two primary modes of development of twin or duplex trichomes (*Zwillingshaare*) as discussed by Heß (1938), corresponding to 4-celled elongate or 4-celled clavate trichomes. These possess a distinctive, myxogenic basal cell (*Schwellpolster*) in an adaxial position at the base of the trichome, and this specialized cell appears to be homologous across the Asteraceae (Heß, 1938). With respect to morphology, Anderberg (1991) classified achenial trichomes of Gnaphalieae into six different types, four of them present in the *Lucilia*-group (Dillon & Sagástegui Alva, 1991b; Dillon, 2003). Andrés-Sánchez & al. (2015) conducted an analysis of the evolution of achenial trichomes in *Filago* and allies (FLAG-clade), finding that morphological types of achenial trichomes are highly homoplastic. However, the variability of achenial trichomes in the *Filago*-group (sensu Anderberg, 1991) is restricted to two of the six morphological types defined by Anderberg (1991). Presence of apical myxogenic cells on achenial trichomes was also included in the analysis of Anderberg (1991), but has otherwise not been examined. Trichomes with a myxogenic cell open apically and secrete mucilage, a character that is more common in globose trichomes, but also reported for some members of the *Lucilia*-group with clavate or elongate trichomes. The *Lucilia*-group therefore appears to be a suitable group to explore achenial trichome evolution in the Gnaphalieae.

We present a new analysis of plastid and nrDNA sequence data to assess phylogenetic relationships within the *Lucilia*-group and we analyze the evolution of achenial trichomes. Finally we discuss the systematic value of the latter characters.

■ MATERIALS AND METHODS

Taxon sampling and outgroup selection. — Material representing all genera of the *Lucilia*-group was selected from herbarium specimens at B, BONN, F, FB and SGO, or was collected in silica gel. Since several herbarium specimens were relatively old, only a fraction of these genera had DNA of enough quality to generate amplifications. No results were obtained for *Parachionolaena*, *Pseudoligandra* and *Raouliopsis*. Sequence data was obtained for 18 out of 21 genera of the *Lucilia*-group (86%). For each genus, we attempted to cover its morphological and geographical variation. The only exception is *Antennaria*, where our sampling includes only four species (out of ~40; Bayer & al., 2007). *Antennaria* is an exception within the *Lucilia*-group, with most species forming a monophyletic group (Bayer & al., 1996; Nie & al., 2016) ranging into the temperate and arctic regions, and with only three Andean species (Ward & al., 2009), two of which are included in the present analysis. Number of species sampled with respect to total number of species of each genus (according to Ward & al.,

2009) was as follows: *Antennaria* (4/~40), *Belloa* (1/1), *Berroa* (1/1), *Chevreulia* (2/~6), *Chionolaena* (3/9), *Cuatrecasasiella* (1/2), *Diaperia* (1/3), *Facelis* (2/4), *Gamochaeta* (13/~50–80), *Gamochaetopsis* (1/1), *Gnaphaliothamnus* (5/11), *Jalcophila* (2/3), *Loricaria* (4/19), *Lucilia* (8/12), *Mexerion* (1/2), *Micropsis* (3/4), *Mniodes* (10/~20), *Stuckertiella* (1/2). Fifteen species from the *Lucilia*-group are included for the first time in a phylogenetic analysis. A total of 107 sequences were newly generated for this study (Appendix 1). Outgroup taxa, mostly from the FLAG-clade, were selected based on previous phylogenetic studies in the Gnaphalieae, especially Ward & al. (2009), Galbany-Casals & al. (2010), Smissen & al. (2011), Freire & al. (2015), and Nie & al. (2016) in order to cover the generic diversity of the group and represent all previously described subclades. Accordingly, several sequences from previously published studies (Blöch & al., 2010; Galbany-Casals & al., 2004b, 2010; Pelser & al., 2010; Smissen & al., 2011; Nie & al., 2013, 2016; Freire & al., 2015) were downloaded from GenBank (Appendix 1).

DNA extraction, amplification and sequencing. — DNA was extracted with a modified CTAB method (Doyle & Dickson, 1987) or using the NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany). Previous studies of the Gnaphalieae strongly suggest that traditional plastid markers used in the Asteraceae such as *trnL-trnF* and *trnL-rpl32* provide little phylogenetic resolution within clades of Gnaphalieae (Montes-Moreno & al., 2010, 2013; Smissen & al., 2011; Schmidt-Lebuhn & Constable, 2013). Our own preliminary analyses with these two plastid markers also provide little resolution (Electr. Suppl.: Fig S1A). In contrast, the nuclear ribosomal regions ITS and ETS contain more phylogenetically informative characters and are therefore of more phylogenetic utility than the plastid markers. Accordingly, we chose to work with the two nuclear markers (ITS, ETS) for the phylogenetic inference of Gnaphalieae. For ITS, primers P5 and P4 (White & al., 1990) were used for amplification and sequencing. For some samples, where P5 did not work, primer ITS1-leu (Urbatsch & al., 2000) was employed instead. The PCR amplifications were conducted in a TrioThermoblock thermal cycler (Biometra, Göttingen, Germany) in 25 µl volume containing 1.25 U *Taq* Polymerase, 3.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each primer and about 50 ng of genomic DNA. Amplification conditions were as follows: 4 min initial denaturation at 95°C, 35 cycles of 95°C for 1 min, 50°C for 45 s and 72°C for 1 min, and a final extension at 72°C for 10 min. For ETS, primers ETS-1F (Linder & al., 2000) and 18S-ETS (Baldwin & Markos, 1998) were employed and amplification conditions were the same as for ITS. PCR products were purified with the GeneJET PCR Purification Kit (Thermo Fisher Scientific Biosciences, St. Leon-Rot, Germany) following manufacturer's instructions. Cycle sequencing was performed using BigDye Terminator v.3.1 (Applied Biosystems, Foster City, California, U.S.A.). The resulting sequences were assembled using Geneious v.5.6.5 (Biomatters, Auckland, New Zealand) and aligned using the software MAFFT v.6.850b (Katoh & al., 2002), followed by manual adjustments using PhyDE v.0.9971 (available at http://www.phyde.de). Sequences generated in this study were deposited in GenBank (see Appendix 1).

Phylogenetic analyses. — Maximum likelihood (ML; Felsenstein, 1981) and Bayesian inference (BI; Mau & al., 1999) analyses were conducted for a combined ITS+ETS matrix. The Akaike information criterion implemented in jModelTest v.2.1.2 (Darriba & al., 2012) was used prior to the ML and BI to determine the best-fit nucleotide substitution model of each marker. Both ML and BI were conducted with unlinked partitions on the CIPRES Science Gateway (Miller & al., 2010). ML was carried out in RAxML v.8.2.4 (Stamatakis & al., 2008) using a Gamma model of substitution, and bootstrap support (MLB) was calculated based on 1000 replicates. BI was conducted in MrBayes v.3.2.6 (Ronquist & al., 2012) for 4×10^6 generations with a sampling frequency every 1000 generations with four chains in four independent runs. After inspection of convergence in Tracer v.1.5 (available at http://tree.bio.ed.ac.uk/software/tracer/) 25% of the trees were discarded as burn-in and Bayesian posterior probabilities (BPP) correspond to the frequency of the partitions in the majority-rule consensus tree calculated from the posterior tree samples. Trees were rooted in TreeGraph v.2.7.0-557 beta (Stöver & Müller, 2010) with *Relhania pungens* L'Hér. according to previous studies.

Morphological analysis. — A morphological study of achenial trichomes was conducted using light and scanning electron microscopy (SEM). Our morphological analysis was based on two characters reflecting variation in achenial trichomes: (i) general trichome morphology, according to Anderberg (1991) and (ii) presence of distal myxogenic cells.

Characterization of the achenial trichomes was carried out with a light microscope, using a ZEISS Axio Scope.A1 light microscope and a ZEISS AxioCam ERc5s camera (Carl Zeiss, Oberkochen, Germany). Achenes were mounted with one drop of Tween 20 and one drop of Hoyer's Solution was added. In order to characterize all species included in the phylogenetic analysis, we complemented the data with information from the literature, especially for the species outside the *Lucilia*-group, or where achenes were not available for study (Cabrera, 1932; Dillon & Sagástegui Alva, 1986, 1991a, b; Anderberg & Freire, 1990; Anderberg, 1991; Freire, 1993, 1995; Ascensão & al., 2001; Galbany-Casals & al., 2004a; Morefield, 2006; Abid & Qaiser, 2008b; Loeuille & al., 2011; Andrés-Sánchez & al., 2014, 2015; Urtubey & al., 2016). Appendix 2 contains a list of sources from which information on achenial trichome morphology was obtained.

Twenty-seven species of 15 genera of the *Lucilia*-group, mainly sampled from herbarium material (Appendix 2), were considered in the SEM morphological study. Achenes of all samples were carefully removed. Fresh material of *Antennaria* was dried one day before further preparation. A Balzers SCD 040 sputter coater (Bal Tec, Liechtenstein) was used for metal coating with silver (ca. 30 nm). Scanning electron microscopy was carried out using a LEO 1450 SEM (LEO, Oberkochen, Germany). Width of the elongated and short clavate twin trichomes was measured at the middle point of the trichomes. Diameter was used to measure the size of globose twin trichomes.

Achenial trichomes were classified into the following types according to Anderberg (1991): (1) elongated twin trichomes with a myxogenic basal cell, (2) short-clavate twin trichomes with a

myxogenic basal cell, (3) globose twin capitate trichomes with a myxogenic basal cell, (4) globose twin trichomes without an obvious basal cell, (5) achenes glabrous. In *Filago*, Andrés-Sánchez & al. (2015) reported that achenial trichomes present on external florets may differ from those found in inner florets. Such dimorphism has not been observed in New World Gnaphalieae. Two of the three types of achenial trichomes distinguished in the study of Andrés-Sánchez & al. (2015) on external florets (short-clavate, long-clavate) fall in character state (2) above, as well as those present on inner florets (short-clavate). "Baculate" trichomes of Andrés-Sánchez & al. (2015) correspond to character state (1) above (elongated), which in the *Filago*-group is present only in combination with short- or long-clavate trichomes as reported by Andrés-Sánchez & al. (2015). Since we recorded the presence of achenial trichomes, if a species reported by Andrés-Sánchez & al. (2015) does not have external florets, we obtained the information for the trichomes present in the inner florets (e.g., *Castroviejoa* Galbany & al.; see Galbany-Casals & al., 2004a). Likewise, we checked the presence of achenial trichomes of inner florets if achenes of external florets were reported as glabrous (e.g., *Logfia* Cass.; see Andrés-Sánchez & al., 2013). We also coded the presence/absence of myxogenic distal cells in the achenial trichomes as a separate character.

In order to evaluate the phylogenetic signal in the analyzed morphological characters, Blomberg's *K* and Blomberg's test (Blomberg & al., 2003; Münkemüller & al., 2012) were calculated using the R-package picante (Kembel & al., 2010). The former compares the observed phylogenetic signal present in a trait under a Brownian motion model of trait evolution versus its expected value. If $K > 1$, phylogenetically related species tend to resemble each other more than expected under Brownian motion. The latter compares the observed variance of phylogenetically independent contrasts (PICs) versus the variance of PICs obtained from randomly shuffling the tips of the tree. If the observed variance is lower than the random variance of PICs, the hypothesis of no phylogenetic signal can be rejected. We used 1000 randomizations to calculate the *P*-value of Blomberg's test.

We conducted ancestral character state reconstruction analyses using a stochastic character mapping (SIMMAP; Bollback, 2006). All analyses were based on the ML tree obtained from the phylogenetic analysis, which was made ultrametric using non-parametric rate smoothing (Sanderson, 1997) as implemented in the function chronopl of the R package APE (Paradis & al., 2004), with a lambda parameter set to 0 and a root age set to 1. Ancestral states were estimated with the R package phytools (Revell, 2012), using the function *make.simmap*. We assigned prior probabilities of 1 to each tip, except for polymorphic species, to which the prior was 1 / number of states present. Analyses were run with 1000 replicates for each character. Posterior probabilities were mapped onto the tree using the function *describe.simmap*, which averages the state frequencies across replicates. Phylogenetic uncertainty was taken into account by running the same analyses as described above on 1000 trees randomly selected from the posterior distribution of the Bayesian analysis. Ten replicates per tree were set for stochastic character mapping. The percentage of times each reconstructed character state was calculated integrating the results of the 1000 reconstructions for the node representing the most recent common ancestor of the same group of species.

■ RESULTS

Phylogenetic analyses. — Our matrix had a total of 2151 aligned positions (ETS: 1410, ITS: 741) and 1161 alignment patterns (ETS: 797, ITS: 364). Substitution model GTR+Γ was selected for ETS and GTR+I+Γ for ITS. ITS and ETS trees do not show significantly supported (bootstrap support > 70%) topological differences (Electr. Suppl.: Figs. S1B, C). ML and BI analyses yielded similar trees, with differences only in the support of some branches, these being generally higher in the BI tree. Figure 1 shows the topology of the BI analysis.

Our phylogenetic analyses suggest that most of the New World genera of the FLAG clade form a moderately well-supported monophyletic group (BPP: 1, MLB: 60). This monophyletic group includes all sampled genera of the *Lucilia*-group (Dillon, 2003; Freire & al., 2015: *Antennaria*, *Belloa*, *Berroa*, *Chevreulia*, *Chionolaena*, *Cuatrecasasiella*, *Diaperia*, *Facelis*, *Gamochaeta*, *Gamochaetopsis*, *Gnaphaliothamnus*, *Jalcophila*, *Loricaria*, *Lucilia*, *Mexerion*, *Micropsis*, *Mniodes*, *Stuckertiella*).

In the *Lucilia*-group, basal resolution is poor, but seven major clades (Fig. 1: L1–L7) can be recognized. *Lucilia* was recovered in two separate clades (L1, L2), one of which (L1) includes the type (*L. acutifolia* (Poir.) Cass.) and *Berroa*, *Facelis* and *Micropsis* (BPP: 1, MLB: 83). The species of *Lucilia* resolved as part of clade L1 are eastern South American and tropical Andean. Clade L2 comprises two southern Andean species of *Lucilia* plus *Belloa* and *Chionolaena* (BPP: 1, MLB: 88), the latter appearing as sister to the remainder of the clade.

Clade L3 is composed of *Gamochaeta*, *Gamochaetopsis* and *Stuckertiella* (BPP: 1, MLB: 78), with two well-supported subclades, one of which includes *Gamochaetopsis* (BPP: 1, MLB: 100), while *Stuckertiella* is in the other (BPP: 1, MLB: 62). Clade L4 comprises *Chevreulia* and *Cuatrecasasiella* (BPP: 1, MLB: 100), while clade L5 includes the Northern Hemisphere species of *Antennaria* (BPP: 1, MLB: 100). *Diaperia* appears as sister to Northern Hemisphere *Antennaria*, albeit with low support.

Clade L6 includes *Jalcophila*, *Loricaria*, *Mniodes* and *Antennaria linearifolia* Wedd. (BPP: 1, MLB: 84). *Jalcophila* is sister to a clade with *Antennaria linearifolia*, *Loricaria* and *Mniodes* (BPP: 1, MLB: 98). Relationships within the latter clade remained unresolved. *Loricaria* is well supported (BPP: 1, MLB: 99) and *Mniodes* forms a well-supported monophyletic group (BPP: 1, MLB: 90). The last clade within the *Lucilia*-group, L7, comprises *Gnaphaliothamnus* and *Mexerion* (BPP: 1, MLB: 74).

Achenial trichome morphology. — Table 1 and Figs. 2 & 3 show the trichome types and their distribution among the genera analyzed. After conducting SEM and light microscopy, the samples could be classified into five trichome types with clearly distinct characteristics. *Berroa* (Fig. 2E, F), *Chionolaena* (Fig.

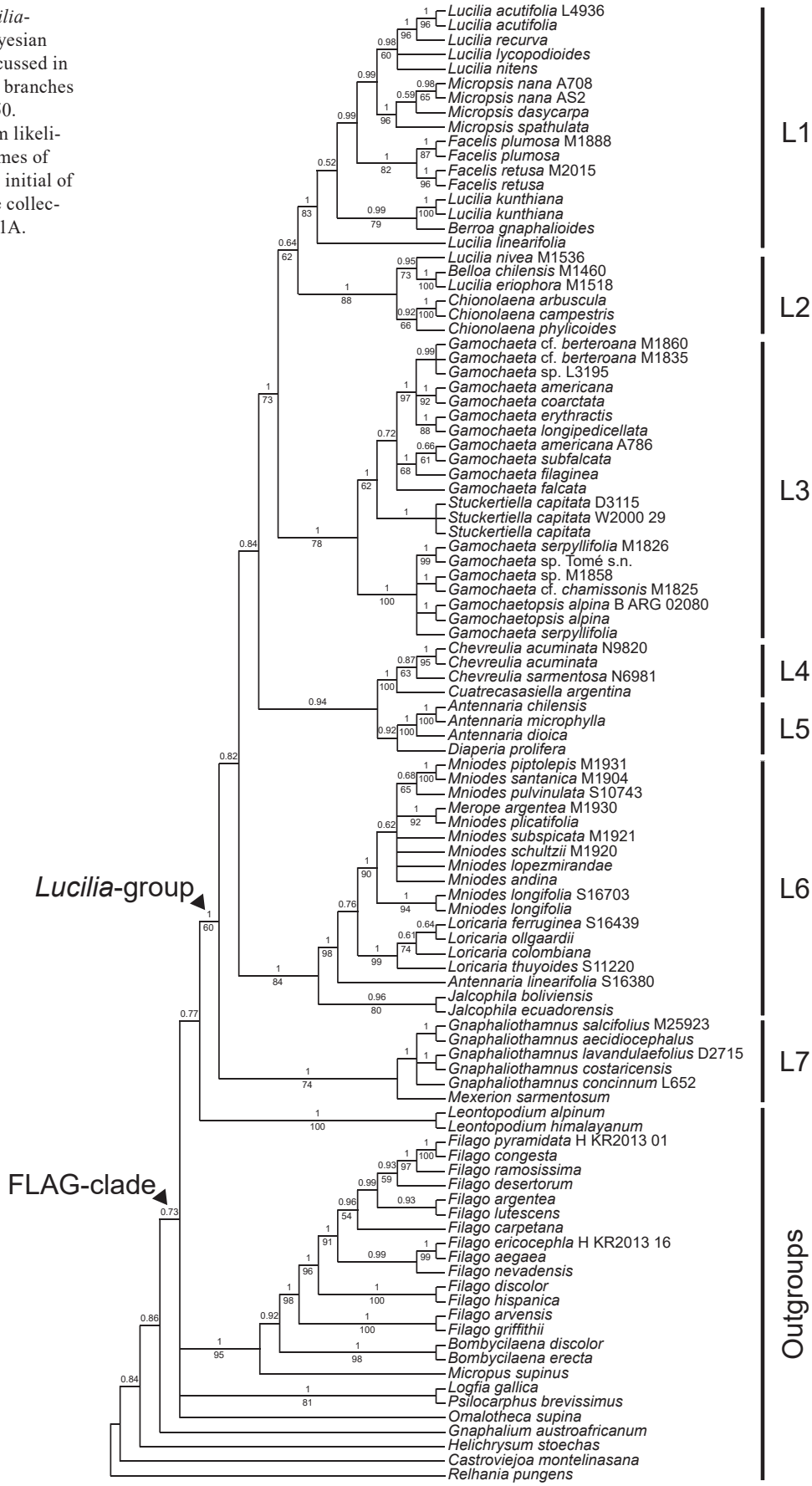


Fig. 1. Phylogenetic analysis of the *Lucilia*-group. Topology obtained from the Bayesian analysis in MrBayes. Major clades discussed in the text are annotated. Numbers above branches are Bayesian posterior probabilities ≥50. Numbers below branches are maximum likelihood bootstrap values ≥50. Next to names of species sequenced in this study are the initial of the senior collector's last name and the collection number as indicated in Appendix 1A.

Table 1. Morphology and size of achenes and achenial trichomes in the *Lucilia*-group for the samples analysed with SEM.

Species (voucher)	Trichome size [µm]	Trichome form	Basal cell	Trichome type	Tip of hair divided	Twisted hairs	Cypsela size	Cypsela shape
Antennaria microphylla Rydb. (*Wilke 1*)	39.82 × 14.59	sc	yes	2			1063,97 × 229,88	o
A. parvifolia Nutt. (*Wilke 2*)	41.85 × 15.24	sc	yes	2			1091,71 × 220,50	o
A. plantaginifolia (L.) Hook. (*Wilke 3*)	40.38 × 16.25	sc	yes	2			1080,90 × 255,39	o
Belloa chilensis (Hook. & Arn.) J.Rémy (*Moreira 1460*)	53.58 × 14.91	sc	yes	2			1786,31 × 279,94	e
Berroa gnaphalioides (Less.) Beauverd (*Cabrera 3184*)	633.94 × 24.53	e	yes	1		x	1267,07 × 623,16	o-e
Chevreulia sarmentosa (Pers.) S.F. Blake (*Novara 8166*)	43.12 × 12.65	sc	yes	2			1741,22 × 313,59	e
Chionolaena capitata (Baker) S.E.Freire (*Joly 6790*)	176.3 × 10.35	e	yes	1	x		815,45 × 175,86	o
Ch. jeffreyi H.Rob. (*Harley & al. 24387*)	259.31 × 15.73	e	yes	1		x	422,33 × 232,79	e
Facelis plumosa (Wedd.) Sch.Bip. (*Moreira 1888*)	218.07 × 13.82	e	no	1			1672,32 × 633,79	e
F. plumosa (*Moreira 1948*)	315.91 × 14.79	e	no	1			1536,03 × 647,47	e
F. retusa (Lam.) Sch.Bip. (*Moreira 2051*)	622.36 × 12.58	e	no	1			2904,22 × 883,12	e
Gamochaeta americana (Mill.) Wedd. (*Álvarez 739*)	12.27 (diam.)	g	no	4			518,2 × 156,45	o
G. americana (*Álvarez 786*)	19.15 (diam.)	g	no	4			197,24 × 76,74	o
G. americana (*Álvarez 849*)	9.67 (diam.)	g	no	4			333,26 × 111,61	o
G. cf. *chamissonis* (DC.) Cabrera (*Moreira 1825*)	12.27 (diam.)	g	no	4			494,28 × 221,18	o
Gamochaetopsis alpina (Poepp.) Anderb. & S.E.Freire (*Montero 2717*)	50.20 × 8.93	sc	yes	2			681,78 × 181,96	o
G. alpina (*Neumeyer 509*)	57.28 × 12.68	sc	yes	2			959,69 × 324,89	o
G. alpina (*De la Sota 2179*)	68.09 × 13.56	sc	yes	2			1157,16 × 352,58	o
G. alpina (*Boelcke & Correa 6952*)	43.95 × 15.26	sc	yes	2			734,53 × 227,81	o
G. alpina (*Bayer & Chandler ARG-02080*)	54.03 × 10.23	sc	yes	2			424,17 × 109,83	o
Gnaphaliothamnus concinnus (A.Gray) G.L.Nesom (*Lorance 652*)	56.62 × 15.08	sc	yes	2			759,39 × 205,97	o
Loricaria ferruginea (Ruiz & Pav.) Wedd. (*Sagástegui & al. 16439*)	N/A	nh	N/A	5			875,70 × 175,86	e
Lucilia acutifolia (Poir.) Cass. (*Anderson 1657*)	468.74 × 15.78	e	yes	1			1130,15 × 522,05	o
L. conoidea Wedd. (*Dillon & al. 1082*)	253.09 × 10.68	e	yes	1		x	841,39 × 372,46	o-e
L. eriophora J.Rémy (*Moreira 1465*)	261.33 × 13.99	e	yes	1		x	962,83 × 313,51	o
L. eriophora (*Teiller 4269*)	182.69 × 13.26	e	yes	1		x	1031,47 × 266,27	o
L. kunthiana (DC.) Zardini (*Dillon & Tunner 1392*)	168.82 × 8.18	e	yes	1		x	549,18 × 188,88	o
L. kunthiana (*Smith & al. 11218*)	182.32 × 10.96	e	yes	1		x	461,53 × 168,80	o
L. kunthiana (*Smith & al. 11590*)	461.41 × 8.47	e	yes	1		x	761,59 × 225,72	o
L. kunthiana (*Steyermark & Koyama 102370*)	309.84 × 10.26	e	yes	1		x	548,79 × 297,38	o
L. kunthiana (*Cabrera 9424*)	228.97 × 7.99	e	yes	1			720,37 × 237,40	o
Merope argentea Wedd. (*Moreira 1930*)	19.32 (diam.)	g	yes	3			579,45 × 183,53	e-o
Micropsis nana DC. (*Álvarez S2*)	147.21 × 10.57	e	yes	1	x		1420,48 × 412,63	o
M. nana (*Álvarez 708*)	237.52 × 9.62	e	yes	1	x		1110,53 × 356,08	o
Mniodes aretioides (Wedd.) Cuatrec. (*Dillon & al. 1083*)	25.30 (diam.)	g	yes	3			1024,49 × 396,23	e
M. longifolia (Cuatrec. & Aristeg.) S.E.Freire & al. (*Sagástegui 12853*)	33.14 (diam.)	g	yes	3			1225,56 × 331,71	e-o
M. longifolia (*Sagástegui & al. 12841*)	33.40 (diam.)	g	yes	3			1306,13 × 282,77	e-o
M. longifolia (*Sagástegui & al. 16703*)	35.66 (diam.)	g	yes	3			1246,51 × 298,12	e-o
M. longifolia (*Sagástegui & al. 11981*)	35.82 (diam.)	g	yes	3			1232,26 × 344,74	e-o
M. piptolepis (Wedd.) S.E.Freire & al. (*Moreira 1931*)	23.88 (diam.)	g	yes	3			1022,56 × 406,44	e-o
M. piptolepis (*Moscatero & al. 1911*)	30.40 (diam.)	g	yes	3			779,94 × 207,93	e-o
M. pulvinulata Cuatrec. (*Sánchez & al. 10743*)	41.71 (diam.)	g	yes	3			685,21 × 199,20	e
Stuckertiella capitata Beauverd (*Weigend 2000/29*)	13.81 (diam.)	g	no	4			475,61 × 177,15	o
St. capitata (*Dillon & al. 3115*)	13.30 (diam.)	g	no	4			433,49 × 145,22	o

Trichome form: e, elongated; g, globose; nh: no hairs; sc, short-clavate. — cypsela shape: e, ellipsoid; o, oblong

2I, J), *Facelis* (Fig. 2K, L), *Lucilia* (Fig. 3E–H) and *Micropsis* (Fig. 3I–J) have elongated twin trichomes with a myxogenic basal cell (type 1). Type 2 is found in *Antennaria* (Fig. 2A, B), *Belloa* (Fig. 2C, D), *Chevreulia* (Fig. 2G, H), *Gamochaetopsis* (Fig. 2O, P) and *Gnaphaliothamnus* (Fig. 3A, B); all with short clavate twin trichomes with a myxogenic basal cell. Globose twin trichomes with a myxogenic basal cell appear in *Mniodes* (Fig. 3K–N) (type 3). *Gamochaeta* (Fig. 2M, N) and *Stuckertiella* (Fig. 3O, P) have globose twin trichomes without an obvious myxogenic basal cell (type 4). *Loricaria* (Fig. 3C, D) and *Cuatrecasasiella* generally have glabrous achenes (type 5).

Morphological analysis. — Blomberg's *K* was 0.095 for achenial trichome type and 1.108 for presence of distal myxogenic cells. Blomberg's test was not significant for achenial trichome type ($P = 0.212$) and significant for presence of distal myxogenic cells ($P = 0.001$). These results suggest that the null hypothesis of no phylogenetic signal can only be rejected for presence of distal myxogenic cells. For achenial trichome type homoplasy (convergence, reversals) can be invoked to explain the results.

Ancestral state reconstructions suggest that the ancestral achenial trichome type of the *Lucilia*-group was clavate with basal myxogenic cell with apical myxogenic cells (Fig. 4). Further, these results indicate that achenial trichome type has changed several times during the evolution of the *Lucilia*-group (Fig. 4A).

Achenial trichomes have been lost four times within the *Lucilia*-group (*Antennaria* [L4], *Cuatrecasasiella* [L5], *Loricaria* [L6], *Mniodes* [L6]). In the *Lucilia*-group, at least seven transitions in achenial trichome morphology can be inferred: from clavate into elongate in the clade formed by *Berroa*, *Chionolaena*, *Facelis*, *Lucilia* and *Micropsis* (L1, L2); from elongate into clavate in *Belloa* (L2); from clavate into globose without basal cell in *Gamochaeta* and *Stuckertiella* (L3), *Diaperia* (L4) and *Jalcophila boliviensis* (L6); from globose without basal cell into clavate in *Gamochaetopsis* (L3); from clavate into globose with basal cell in *Antennaria linearifolia* and *Mniodes* (L6). The transitions in *Gamochaetopsis* and *Belloa* can be interpreted as reversals into the ancestral clavate trichomes (Fig. 4A).

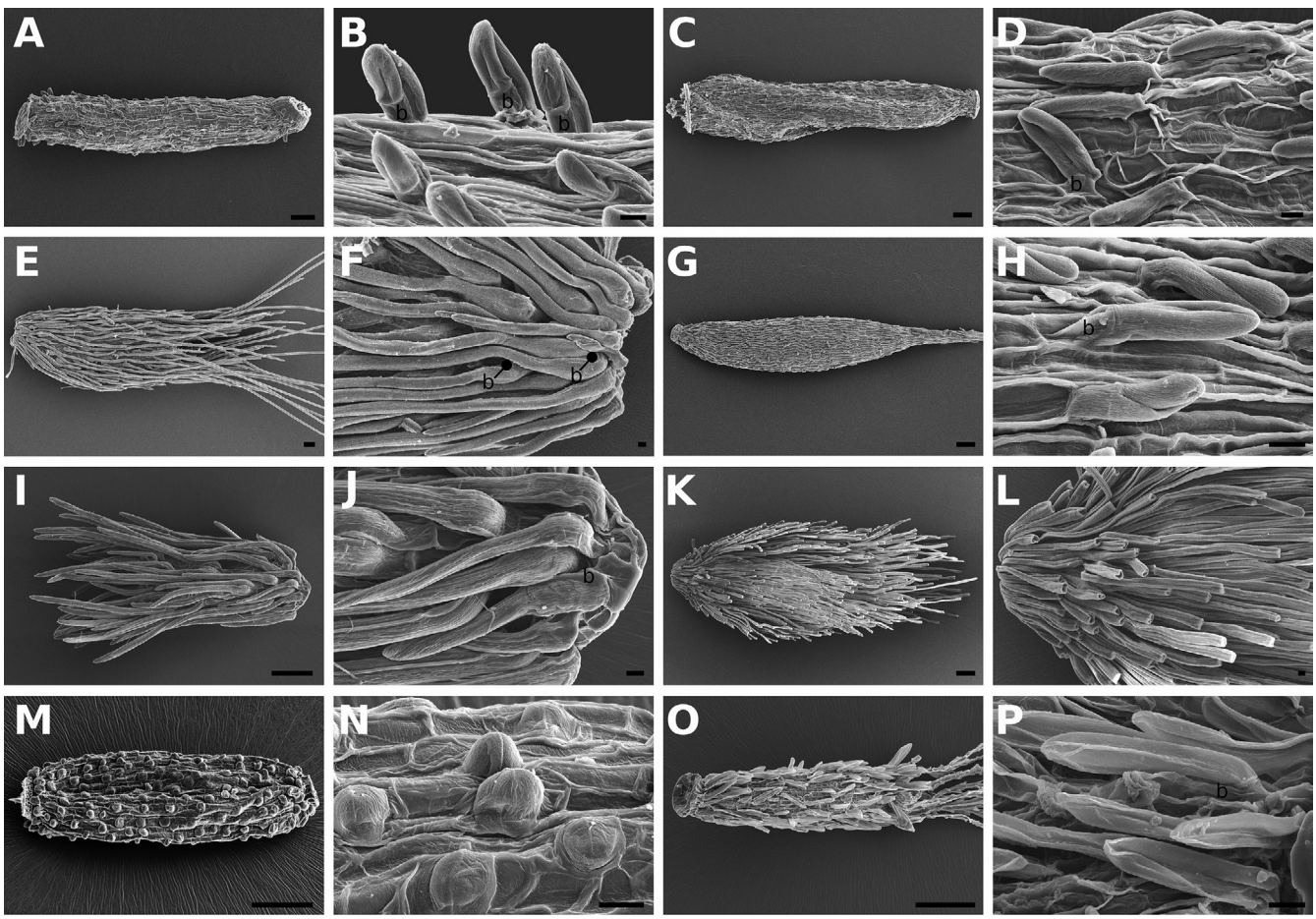


Fig. 2. SEM pictures of achene **(A, C, E, G, I, K, M, O)** and achenial trichome morphology **(B, D, F, H, J, L, N, P)** of selected New World Gnaphalieae. Collector data as in Appendix 2. **A,** *Antennaria parvifolia* (*Wilke 2*); **B,** *Antennaria microphylla* (*Wilke 1*); **C & D,** *Belloa chilensis* (*Moreira 1460*); **E & F,** *Berroa gnaphaloides* (*Cabrera 3184*); **G & H,** *Chevreulia sarmentosa* (*Novara 8166*); **I & J,** *Chionolaena jeffreyi* (*Harley 24387*); **K & L,** *Facelis plumosa* (*Moreira 1888*); **M & N,** *Gamochaeta americana* (*Alvarez 739*); **O & P,** *Gamochaetopsis alpina* (*Bayer ARG-02080*). — When visible and present, basal cells are indicated (b) on achenial trichome pictures. Scale bar for whole achenes (A, C, E, G, I, K, M, O) = 100 μm; scale bar for achenial trichomes (B, D, F, H, J, L, N, P) = 10 μm.

Uncertainty in ancestral character state reconstruction is greatest at the crown node of clades L1–L3, where clavate trichomes are suggested for the single ML tree, while elongated trichomes become more probable if phylogenetic uncertainty is taken into account (Fig. 4C). The former scenario suggests two independent transitions from clavate into elongate (L1, L2) and globose without basal cell (L3), respectively. The latter scenario would imply a transition from clavate into elongate trichomes followed by a reduction from elongate into globose without basal cell in L3. Within clade L6, the origin of globose trichomes with basal cell would be suggested from an ancestor that lost trichomes. Here, transition from clavate into globose trichomes with basal cell followed by loss of trichomes in *Loricaria* cannot be ruled out.

Presence of distal myxogenic cells in the *Lucilia*-group is followed by independent losses in the clade formed by *Belloa*, *Berroa*, *Chionolaena*, *Lucilia* and *Micropsis* (with one reversal in *Facelis*), and in *Gamochaetopsis*, *Loricaria* and *Mniodes* (Fig. 4B). The reconstruction at the crown node of L1–L3 with the single ML tree shows that distal myxogenic cells would be present at this node (Fig. 4B), but this becomes uncertain when phylogenetic uncertainty is taken into account (Fig. 4C). Absence of distal myxogenic cells at that node would imply a loss followed by a gain of distal myxogenic cells in clade L3.

■ DISCUSSION

Phylogenetic relationships of the *Lucilia*-group. — Different studies in past years have contributed to clarifying the phylogenetic relationships in the Gnaphalieae, confirming the placement of the *Lucilia*-group within the FLAG-clade (see Introduction). Relationships of the genera within the *Lucilia*-group have already been evaluated in previous papers (Freire & al., 2015; Nie & al., 2016; Urtubey & al., 2016), but our study adds species of the *Lucilia*-group not included in earlier studies.

Based on the results of Freire & al. (2015) and this work, the *Lucilia*-group (sensu Anderberg & Freire, 1991) is expanded to include *Loricaria* and *Mniodes* of the Loricariinae, *Antennaria*, *Chionolaena*, and *Gnaphaliothamnus* of the Cassiniinae,

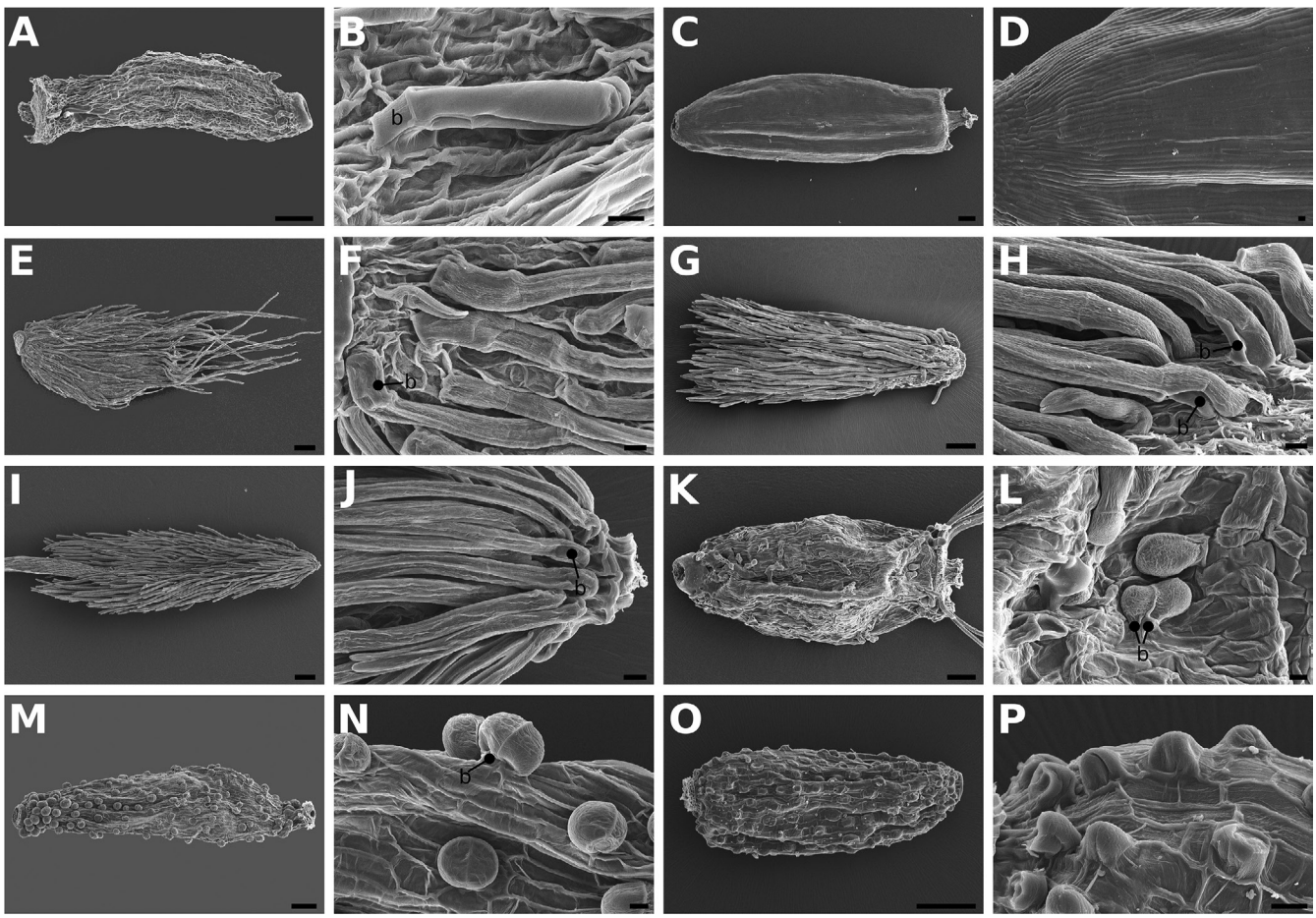


Fig. 3. SEM pictures of achene **(A, C, E, G, I, K, M, O)** and achenial trichome morphology **(B, D, F, H, J, L, N, P)** of selected New World Gnaphalieae. Collector data as in Appendix 2. **A & B,** *Gnaphaliothamnus concinnus* (*Lorance 652*); **C & D,** *Loricaria ferruginea* (*Sagástegui 16439*); **E & F,** *Lucilia conoidea* (*Dillon 1082*); **G & H,** *Lucilia eriophora* (*Moreira 1465*); **I & J,** *Micropsis nana* (*Alvarez S2*); **K & L,** *Mniodes aretioides* (*Dillon 1083*); **M & N,** *Mniodes longifolia* (*Sagástegui 16703*); **O & P,** *Stuckertiella capitata* (*Weigend 2000/29*). — When visible and present, basal cells are indicated (b) on achenial trichome pictures. Scale bar for whole achenes (A, C, E, G, I, K, M, O) = 100 µm; scale bar for achenial trichomes (B, D, F, H, J, L, N, P) = 10 µm.

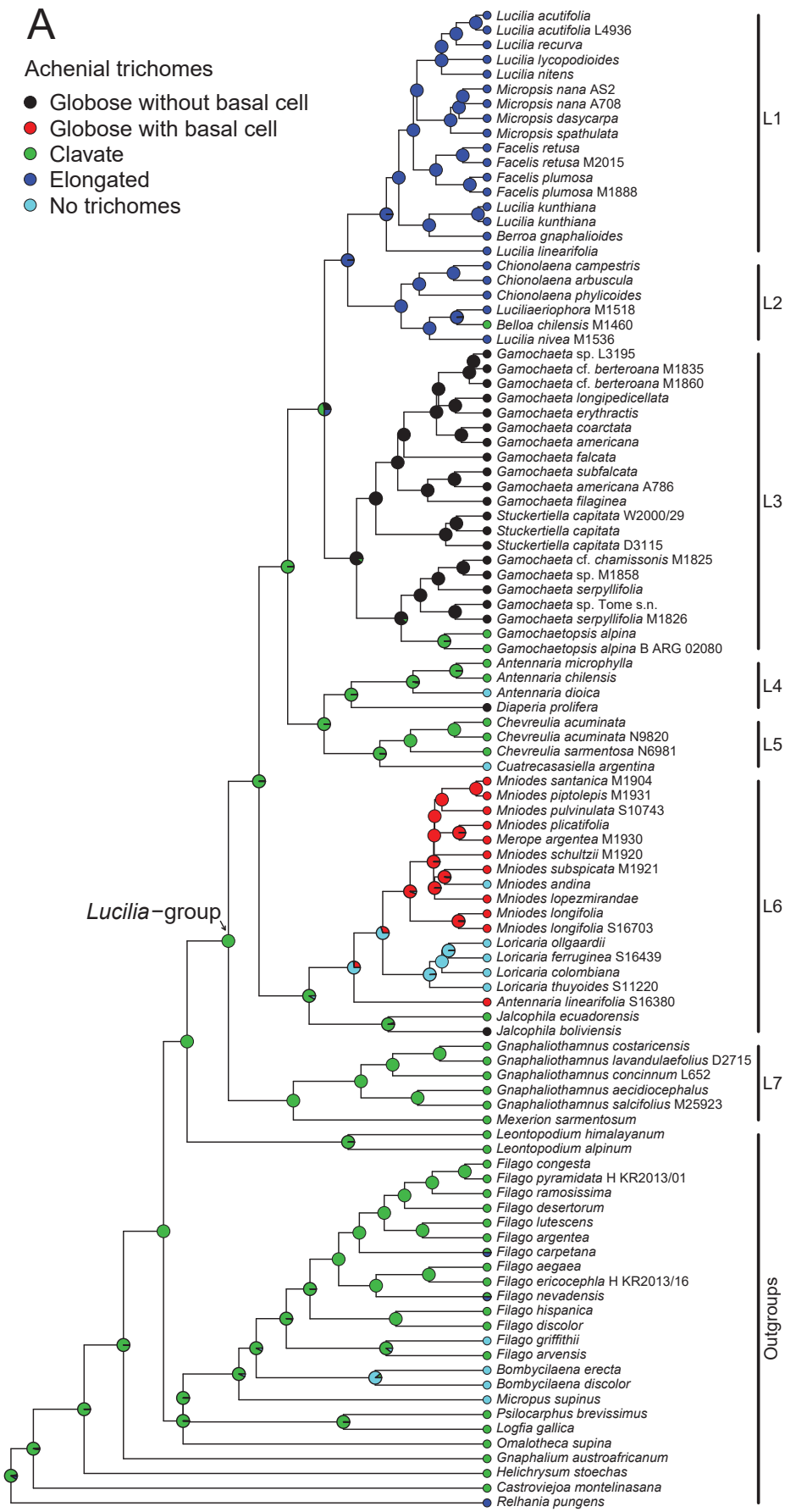
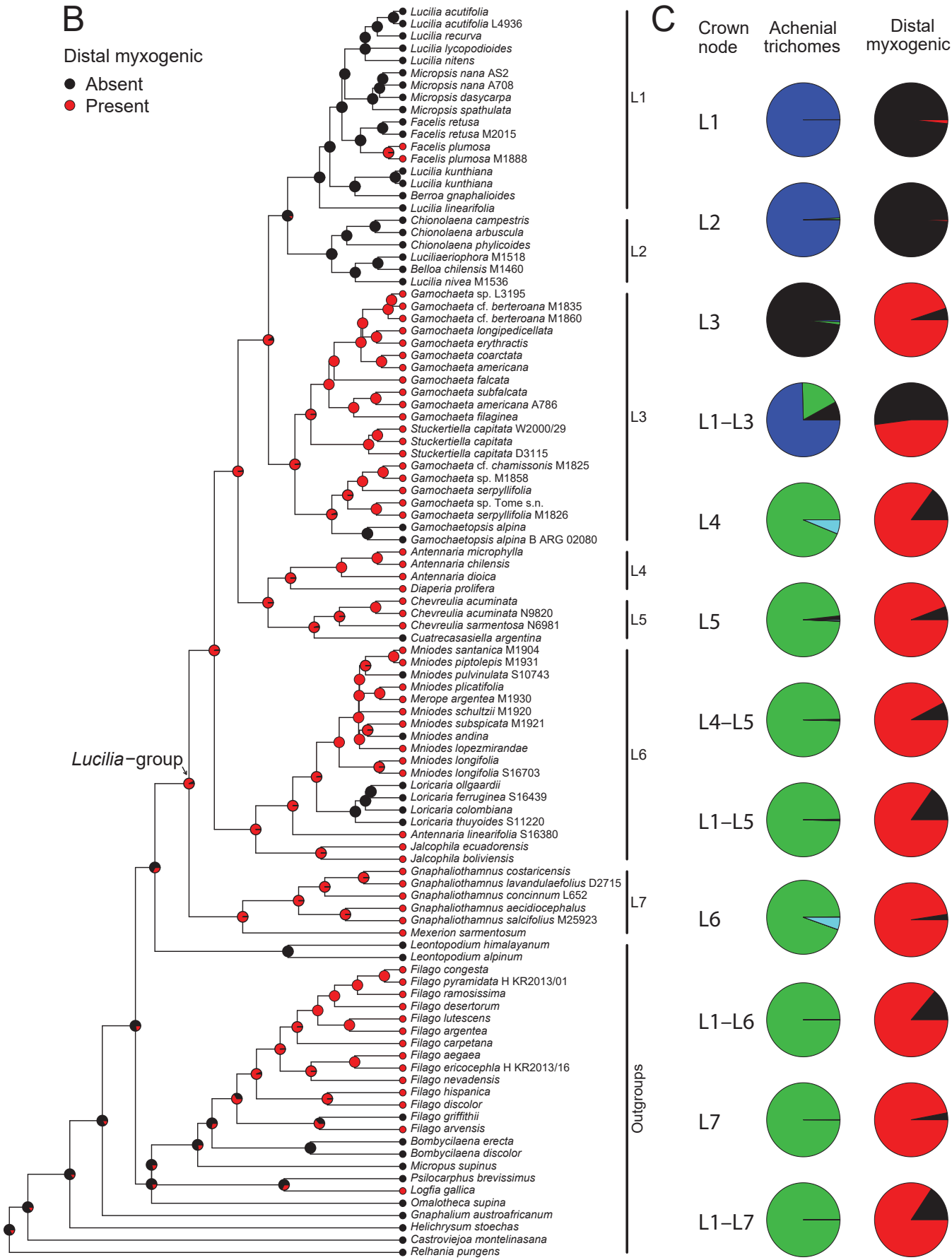


Fig. 4. Ancestral character state reconstruction of achenial trichome type **(A)** and presence of apical myxogenic cells **(B)** with SIMMAP on the ML phylogeny of Gnaphalieae. Pie charts for selected nodes are also shown **(C)** with the ancestral character state reconstruction with SIMMAP taking into account phylogenetic uncertainty (see Methods). Pie charts at nodes represent relative probability of ancestral states given the phylogeny. Major clades are indicated as in Fig. 1. Next to names of species sequenced in this study are the initial of the senior collector's last name and the collection number as indicated in Appendix 1A.


B
Distal myxogenic
Absent
Present
Lucilia acutifolia
Lucilia acutifolia L4936
Lucilia recurva
Lucilia lycopodioides
Lucilia nitens
Micropsis nana AS2
Micropsis nana A708
Micropsis dasycarpa
Micropsis spathulata
Facelis retusa
Facelis retusa M2015
Facelis plumosa
Facelis plumosa M1888
Lucilia kunthiana
Lucilia kunthiana
Berroa gnaphalioides
Lucilia linearifolia
Chionolaena campestris
Chionolaena arbuscula
Chionolaena phylicoides
Luciliaeriophora M1518
Belloa chilensis M1460
Lucilia nivea M1536
Gamochaeta sp. L3195
Gamochaeta cf. berteroana M1835
Gamochaeta cf. berteroana M1860
Gamochaeta longipedicellata
Gamochaeta erythractis
Gamochaeta coarctata
Gamochaeta americana
Gamochaeta falcata
Gamochaeta subfalcata
Gamochaeta americana A786
Gamochaeta filaginea
Stuckertiella capitata W2000/29
Stuckertiella capitata
Stuckertiella capitata D3115
Gamochaeta cf. chamissonis M1825
Gamochaeta sp. M1858
Gamochaeta serpyllifolia
Gamochaeta sp. Tome s.n.
Gamochaeta serpyllifolia M1826
Gamochaetopsis alpina
Gamochaetopsis alpina B ARG 02080
Antennaria microphylla
Antennaria chilensis
Antennaria dioica
Diaperia prolifera
Chevreulia acuminata
Chevreulia acuminata N9820
Chevreulia sarmentosa N6981
Cuatrecasasiella argentina
Mniodes santanica M1904
Mniodes piptolepis M1931
Mniodes pulvinulata S10743
Mniodes plicatifolia
Merope argentea M1930
Mniodes schultzii M1920
Mniodes subspicata M1921
Mniodes andina
Mniodes lopezmirandae
Mniodes longifolia
Mniodes longifolia S16703
Loricaria ollgaardii
Loricaria ferruginea S16439
Loricaria colombiana
Loricaria thuyoides S11220
Antennaria linearifolia S16380
Jalcophila ecuadorensis
Jalcophila boliviensis
Gnaphaliothamnus costaricensis
Gnaphaliothamnus lavandulaefolius D2715
Gnaphaliothamnus concinnum L652
Gnaphaliothamnus aecidiocephalus
Gnaphaliothamnus salcifolius M25923
Mexerion sarmentosum
Leontopodium himalayanum
Leontopodium alpinum
Filago congesta
Filago pyramidata H KR2013/01
Filago ramosissima
Filago desertorum
Filago lutescens
Filago argentea
Filago carpetana
Filago aegaea
Filago ericocephla H KR2013/16
Filago nevadensis
Filago hispanica
Filago discolor
Filago griffithii
Filago arvensis
Bombycilaena erecta
Bombycilaena discolor
Micropus supinus
Psilocarphus brevissimus
Logfia gallica
Omalotheca supina
Gnaphalium austroafricanum
Helichrysum stoechas
Castroviejoa montelinasana
Relhania pungens
Lucilia–group
L1
L2
L3
L4
L5
L6
L7
Outgroups
C
Crown node
Achenial trichomes
Distal myxogenic
L1
L2
L3
L1–L3
L4
L5
L4–L5
L1–L5
L6
L1–L6
L7
L1–L7

Gamochaeta, *Diaperia*, *Micropsis*, and *Stuckertiella* of the Gnaphaliinae. Furthermore, *Luciliocline* forms a clade together with *Mniodes.* Therefore, the species of the former genus have been transferred to the latter (Freire & al., 2015). Our results are basically in agreement with Freire & al. (2015), but add three major findings regarding the phylogeny of the *Lucilia*-group. The first refers to the relationships between *Chionolaena* and *Gnaphaliothamnus. Gnaphaliothamnus*, a genus from Mexico and Central America, had been subsumed into *Chionolaena* (Anderberg & Freire, 1991; Freire, 1993; Nesom, 1994). Based upon differences in achenial trichomes, Dillon (2003) suggested that *Gnaphaliothamnus* was a monophyletic group not necessarily close to *Chionolaena* of Brazil and austral South America. Our results suggest that any similarity between the two genera may be convergent.

The second major finding relates to *Lucilia*, which in our results is recovered in two different clades. The first clade includes the type (*L. acutifolia*), other eastern South American and Andean species (*L. kunthiana* (DC.) Zardini, *L. linearifolia* Baker, *L. lycopodioides* (Less.) S.E.Freire, *L. nitens* Less., *L. recurva* Wedd.), plus *Berroa*, *Facelis* and *Micropsis*. Dillon (2003) considered *Belloa* as monospecific (i.e., *Belloa chilensis* (Hook. & Arn.) J.Rémy) and transferred the other species of *Belloa* to *Luciliocline*, with the exception of *Belloa kunthiana* (DC.) Anderb. & S.E.Freire, which was treated by Dillon (2003) as *Lucilia kunthiana*. The latter was excluded by Dillon (2003) largely due to its elongated achenial trichomes (see Table 1). Freire & al. (2015) transferred all *Luciliocline* to *Mniodes*, including *Belloa kunthiana* and suggested the resemblance to species of *Lucilia* was due to parallel evolution. Our placement of *Lucilia kunthiana* is more parsimonious and one also supported by Nie & al. (2016). Re-examination of the material assigned by Freire & al. (2015) to *Belloa kunthiana* may result in a corrected determination. The second clade includes the southern Andean species of *Lucilia* accepted by Dillon & Sagástegui Alva (1991b) (*L. eriophora* J.Rémy, *L. nivea* (Phil.) Cabrera) along with *Belloa chilensis*, which are together sister to *Chionolaena*. If *Berroa*, *Chionolaena*, *Facelis* and *Micropsis* are to be maintained as accepted genera, the southern Andean species of *Lucilia* need to be included in *Belloa*. Still, *Lucilia* appears paraphyletic with respect to *Berroa*, *Facelis* and *Micropsis* and further studies are needed to clarify the phylogenetic relationships within clade L1.

The third major finding of our analysis is that *Antennaria linearifolia* was recovered in a clade together with *Loricaria* and *Mniodes*, while the remainder of *Antennaria* species form a separate clade. This result was not apparent in the work of Bayer & al. (1996), because material of *A. linearifolia* was not available for that study. This may be a case of convergence since there are a number of morphological characters that distinguish *A. linearifolia* from its suggested congeners. This position is being investigated in greater detail and a larger selection of *Antennaria* taxa.

Our phylogenetic analysis suggests that some further taxonomic rearrangements are required in the *Lucilia*-group in addition to those proposed by Freire & al. (2015), who basically united *Luciliocline* and *Mniodes*. *Stuckertiella*, which shares many morphological characters with *Gamochaeta*, is nested in the latter genus and has been treated as synonymous by Urtubey & al. (2016). *Gamochaetopsis* was also resolved as nested in a clade also including *Gamochaeta* and *Stuckertiella*, confirming the results of Urtubey & al. (2016). Cabrera (1961) considered *Laennecia alpina* Poepp. to be congeneric with *Lucilia* (making the combination *Lucilia alpina* (Poepp.) Cabrera) and stated that it could be confused vegetatively with *Gamochaeta nivalis* (Phil.) Cabrera, but was easily distinguished from the latter taxon by its pubescent achenes. Anderberg & Freire (1991) removed the species to the new monospecific genus *Gamochaetopsis.* We have examined the type collection at W. We have observed that the achenial trichomes of *Gamochaetopsis* are essentially reduced clavate trichomes (ca. 60 µm long) and quite unlike the sessile, biseriate trichomes found in all true *Gamochaeta* (<20 µm). *Gamochaetopsis* has been transferred to *Gamochaeta* by Urtubey & al. (2016).

Lucilia is polyphyletic, with one clade (incl. type) grouping together with *Facelis* and *Micropsis* and a second clade (excl. type) grouping with *Belloa*. The latter clade includes all southern Andean species of *Lucilia*. These should then be transferred to *Belloa* if the genera *Chionolaena*, *Facelis* and *Micropsis* are to be retained.

Chionolaena and *Gnaphaliothamnus*, considered as synonyms by Anderberg (1991), Freire (1993) and Nesom (2001), are here resolved as separate clades. They have different geographical ranges (the former in South America and the latter in Central America and Mexico). Moreover, there are a number of morphological characters separating them, as discussed above. These two genera should be considered separate taxonomic entities as suggested by Nesom (1990a, 1994) and Dillon & Luebert (2015). The status of *Parachionolaena* and *Pseudoligandra*, also considered synonyms of *Chionolaena* (Freire, 1993), still needs to be assessed in the light of molecular data.

Evolution of achenial trichomes in the *Lucilia*-group. — The seven major and well-supported clades of the *Lucilia*-group (L1–L7) have each one predominant trichome morphology, but in almost all of them there are exceptions. In the second clade (L2: *Belloa*+*Chionolaena*+*Lucilia* p.p.), elongate trichomes are present in all species except *Belloa chilensis.* Our analysis suggests that an evolutionary transition to elongate trichomes would have occurred at the origin of this clade or perhaps earlier, with a reversal to clavate trichomes in *Belloa.* A similar pattern is observed in clade L3 (*Gamochaeta*+*Gamochaetopsis*+*Stuckertiella*), but in this case globose trichomes without basal cell is the plesiomorphic state, with a reversal to clavate trichomes in *Gamochaetopsis.* In clade L6, composed of *Antennaria linearifolia*, *Mniodes*, *Loricaria* and *Jalcophila*, the plesiomorphic state is again clavate. A transition to globose trichomes with basal cell is inferred at the origin of *Loricaria* and *Mniodes*, with a further loss of achenial trichomes in *Loricaria* and some *Mniodes*, but the sequence of events is uncertain here. In *Loricaria*, material assigned to *Loricaria graveolens* (Sch. Bip.) Wedd. (not included in the current analysis) from Peru (*Schmidt s.n.*, F1223404) possesses clavate achenial trichomes with basal myxogenic cells, which may indicate that the loss

of trichomes occurred during the evolution of some species of this genus. Inclusion of *Loricaria graveolens* in phylogenetic analyses may result in changes in the sequence of events within clade L6. Achenial trichome morphology lend support to the molecular data for the placement of *Gnaphaliothamnus* and *Chionolaena* in different clades. The achenial trichomes in *Gnaphaliothamnus* are specialized with apical cells 120–140 µm long and clearly myxogenic in character. Achenial trichomes in *Chionolaena* are not myxogenic and have terminal cells 150–450 µm long (Dillon & Sagástegui Alva, 1991b).

In agreement with Heß (1938), our results suggest that at least three different processes are involved in the evolution of achenial trichome types in the *Lucilia*-group.

(1) Elongation of apical cells. – This is evident in clades L1 and L2, leading to taxa with elongate trichomes originating from an ancestor with clavate trichomes. The myxogenic basal cell remains unaltered here. A reversal of this process could be inferred in *Belloa* (L2).

(2) Reduction of both basal and apical cells. – This appears in L3, where most species possess globose trichomes without obvious basal cells. It is not clear from our analysis whether this type of reduction started from an ancestor with clavate or elongate cells, though the former option seems more plausible, as also inferred for *Diaperia* (L4) and *Jalcophila boliviensis* (L6). A reversal of this process could be inferred in *Gamochaetopsis* (L3). Heß (1938) proposed that the origin of a myxogenic basal cell occurs along with a thickening of the cell wall, a common phenomenon in trichome development (Werker, 2000; Mathur, 2006). In cases where that basal cell has been reduced, a rudimentary thickening of the cell wall could still be observed. Unfortunately, the reduction of the basal cells is such that the basal cells cannot be observed with the techniques we employed for the characterization of trichomes.

(3) Reduction of apical cells without reduction of basal cells. – This process is inferred for clade L6 from an ancestor with clavate trichomes, though with uncertainty as to whether loss of trichomes would have preceded the appearance of globose trichomes. In addition to these processes, our results suggest that loss of trichomes occurred at least three times independently in the *Lucilia*-group.

The results presented here regarding the morphological evolution of achenial trichomes might be limited by problems associated with the markers used to infer phylogenetic relationships. Even if we took into account possible effects of phylogenetic uncertainty, hybrid speciation suggested to have occurred in the Gnaphalieae (Breitwieser & Ward, 2003; Smissen & al., 2011) may weaken conclusions about homoplasy in achenial trichome morphology. Hybrid speciation in the *Lucilia*-group has not been suggested outside *Antennaria* (e.g., Bayer, 1991), and chromosome counts available so far (Ward & al., 2009) do not provide evidence of polyploidy.

Overall, molecular studies carried out in recent years (e.g., Bayer & al., 1996, 2000; Breitwieser & Ward, 2003; Bergh & Linder, 2009; Ward & al., 2009; Blöch & al., 2010; Galbany-Casals & al., 2010, 2014; Smissen & al., 2011; Nie & al., 2013, 2016; Freire & al., 2015) have substantially contributed to clarifying phylogenetic relations in the Gnaphalieae in general and in the *Lucilia*-group in particular. In spite of the controversy about the systematic significance of achenial trichome morphology and the lack of phylogenetic signal, our results suggest that some predictive value regarding assignment to clades can be given to it, at least within the *Lucilia*-group. If so, other genera not included in our analysis (i.e., *Parachionolaena*, *Pseudoligandra*, *Raouliopsis*) may also be recovered as members of the *Lucilia*-group should they be included in a molecular phylogenetic analysis.

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Appendix 1. Voucher details and GenBank accession numbers. GenBank numbers for each specimen appear in the order ITS, ETS, *trnL-trnF*, *trnL-rpl32*. A dash (–) indicates that the sequence was not included in the study.

A. Sequences generated in this study. Indicated are species name, isolate number, voucher information and GenBank accession numbers.

Antennaria linearifolia Wedd., L3212, Peru, *Sagástegui 16380 & al.* (F), MF118846, MF118814, MF118792, MF118775; ***Belloa chilensis*** (Hook. & Arn.) J.Rémy, L3172, Chile, *Moreira 1460* (SGO), MF118847, MF118815, MF118793, MF118776; ***Chevreulia acuminata*** Less., L3236, Argentina, *Novara & Bruno 9820* (B), MF118848, –, –, –; ***Chevreulia sarmentosa*** (Pers.) S.F.Blake, L3249, Argentina, *Novara 6981* (B), MF118849, MF118816, –, –; ***Facelis plumosa*** (Wedd.) Sch.Bip., L3265, Chile, *Moreira 1888* (SGO), MF118850, MF118817, MF118794, MF118777; ***Facelis retusa*** (Lam.) Sch.Bip., L3438, Chile, *Moreira 2051* (BONN), MF118851, MF118818, MF118795, –; ***Filago eriocephala*** Guss., L3393, Greece, *Hilger KR 2013/16* (BONN), MF118852, MF118819, –, –; ***Filago pyramidata*** L., Greece, L3391, *Hilger KR 2013/01* (BONN), MF118853, MF118820, MF118796, –; ***Gamochaeta americana*** (Mill.) Wedd., L3386, Chile, *Álvarez 786* (FB), MF118854, MF118821, –, –; ***Gamochaeta*** cf. ***berteroana*** (DC.) Cabrera, L3181, Chile, *Moreira 1835* (SGO), MF118855, MF118822, MF118797, MF118778; ***Gamochaeta*** cf. ***berteroana*** (DC.) Cabrera, L3179, Chile, *Moreira 1860* (SGO), MF118856, MF118823, MF118798, MF118779; ***Gamochaeta*** cf. ***chamissonis*** (DC.) Cabrera, L3183, Chile, *Moreira 1825* (SGO), MF118857, MF118824, MF118799, MF118780; ***Gamochaeta serpyllifolia*** Wedd., L3180, Chile, *Moreira 1826* (SGO), MF118858, MF118825, –, –; ***Gamochaeta* sp.,** W4208, Chile, *Luebert 3195* (BONN), MF118861, MF118826, –, –; ***Gamochaeta* sp.,** L3182, Chile, *Moreira 1858* (SGO), MF118859, MF118827, MF118800, MF118781; ***Gamochaeta* sp.,** L3259, Chile, *Tomé s.n.* (F), MF118860, MF118828, MF118801, MF118782; ***Gamochaetopsis alpina*** (Poepp.) Anderb. & S.E.Freire, L3218, Argentina, *Bayer & Chandler ARG-02080* (F), MF118862, MF118829, MF118802, –; ***Gnaphaliothamnus concinnus*** (A.Gray) G.L.Nesom, L3374, Mexico, *Cedillo & Lorence 652* (F), MF118863, –, –, –; ***Gnaphaliothamnus lavandulifolius*** (Kunth) G.L.Nesom, L3372, Mexico, *Dovz 2715 & al.* (F), MF118864, MF118830, –, –; ***Gnaphaliothamnus salicifolius*** (Bertol.) G.L.Nesom, L3213, Mexico, *McVaugh 25923* (F), MF118865, MF118831, –, MF118783; ***Loricaria ferruginea*** (Ruiz & Pav.) Wedd., L3194, Peru, *Sagástegui 16439 & al.* (F), MF118866, MF118832, –, –; ***Loricaria thuyoides*** (Lam.) Sch.Bip., L3189, Peru, *Sánchez 11220 & al.* (F), MF118867, MF118833, MF118803, MF118784; ***Lucilia acutifolia*** (Poir.) Cass., L3360, Argentina, *Leuenberger 4936 & al.* (B), MF118868, MF118834, –, –; ***Lucilia eriophora*** J.Rémy, L3175, Chile, *Moreira 1518* (SGO), MF118869, MF118835, MF118804, –; ***Lucilia nivea*** (Phil.) Cabrera, L3170, Chile, *Moreira 1536* (SGO), MF118870, MF118836, MF118805, MF118785; ***Merope argentea*** Wedd., L3269, Chile, *Moreira 1930* (SGO), MF118873, MF118837, MF118806, MF118787; ***Micropsis nana*** DC., L3382, Chile, *Álvarez 708* (FB), MF118871, MF118838, MF118807, MF118786; ***Micropsis nana*** DC., L3385, Chile, *Álvarez S2* (FB), MF118872, MF118839, –, –; ***Mniodes longifolia*** (Cuatrec. & Aristeg.) S.E.Freire, Chemisquy, Anderb. & Urtubey, L3367, Peru, *Sagástegui 16703 & al.* (F), MF118874, MF118840, –, –; ***Mniodes piptolepis*** (Wedd.) S.E.Freire, Chemisquy, Anderb. & Urtubey, L3267, Chile, *Moreira 1931* (SGO), MF118875, MF118841, MF118808, –; ***Mniodes pulvinulata*** Cuatrec., L3210, Peru, *Sánchez 10743 & al.* (F), MF118876, MF118842, MF118809, MF118788; ***Mniodes santanica*** (Cabrera) S.E.Freire, Chemisquy, Anderb. & Urtubey, L3270, Chile, *Moreira 1904* (SGO), MF118877, MF118843, –, –; ***Mniodes schultzii*** (Wedd.) S.E.Freire, Chemisquy, Anderb. & Urtubey, L3271, Chile, *Moreira 1920* (SGO), MF118878, MF118844, MF118810, MF118789; ***Mniodes subspicata*** (Wedd.) S.E.Freire, Chemisquy, Anderb. & Urtubey, L3268, Chile, *Moreira 1921* (SGO), MF118879, MF118845, MF118811, –; ***Stuckertiella capitata*** (Wedd.) Beauverd, L3184, Peru, *Dillon 3115 & al.* (F), MF118880, –, MF118812, MF118790; ***Stuckertiella capitata*** (Wedd.) Beauverd, L3211, Peru, *Weigend 2000/29* (F), MF118881, –, MF118813, MF118791.

B. Sequences obtained from GenBank. Indicated are species name, references and GenBank accession numbers.

Antennaria chilensis J.Rémy, Freire & al. (2015), KM091395, KM091373, –, –; ***Antennaria dioica*** (L.) Gaertn., Galbany-Casals & al. (2010), FN645886, FN645610, FN645790, FN649336; ***Antennaria microphylla*** Rydb., Smissen & al. (2011), HM244731, HM450870, HM364534, –; ***Berroa gnaphalioides*** (Less.) Beauverd, Freire & al. (2015), KM091386, KM091355, KM091418, KM091325; ***Bombycilaena discolor*** (Pers.) M.Laínz, Galbany-Casals & al. (2010), FN645844, FN645562, HM364536, FN649365; ***Bombycilaena erecta*** (L.) Smoljan., Galbany-Casals & al. (2010), FN645842, FN645561, FN645770, FN649366; ***Castroviejoa montelinasana*** (Em.Schmid) Galbany, L.Sáez & Benedí, Galbany-Casals & al. (2004b, 2010), AY445229, FN645559, –, –; ***Chevreulia acuminata*** Less., Freire & al. (2015), KM091392, KM091361, KM091421, KM091322; ***Chionolaena arbuscula*** DC., Freire & al. (2015), KM091406, KM091376, –, KM091334; ***Chionolaena campestris*** Deble, Freire & al. (2015), KM091407, KM091377, KM091436, KM091338; ***Chionolaena phylicoides*** (Gardner) Baker, Nie & al. (2013), JQ895510, JQ895373, –, –; ***Cuatrecasasiella argentina*** (Cabrera) H.Rob., Freire & al. (2015), KM091401, KM091366, KM091427, KM091337; ***Diaperia prolifera*** (Nutt. ex DC.) Nutt., Galbany-Casals & al. (2010), FN645835, FN645611, FN645798, –; ***Facelis plumosa*** (Wedd.) Sch.Bip., Freire & al. (2015), KM091394, KM091372, KM091428, KM091345; ***Facelis retusa*** (Lam.) Sch.Bip., Freire & al. (2015), KM091385, KM091352, KM091413, KM091321; ***Filago aegaea*** Wagenitz, Galbany-Casals & al. (2010), FN645865, FN645602, FN645809, FN649404; ***Filago argentea*** (Pomel) Chrtek & Holub, Galbany-Casals & al. (2010), FN645860, FN645570, FN645785, FN649373; ***Filago arvensis*** L., Galbany-Casals & al. (2010), FN645886, FN645606, FN645795, FN649361; ***Filago carpetana*** (Lange) Chrtek & Holub, Galbany-Casals & al. (2010), FN645858, FN645568, FN645781, FN649372; ***Filago congesta*** DC., Galbany-Casals & al. (2010), FN645871, FN645578, FN645768, FN649382; ***Filago desertorum*** Pomel, Galbany-Casals & al. (2010), FN645875, FN645592, FN645766, FN649391; ***Filago discolor*** (DC.) Andrés-Sánchez & Galbany, Galbany-Casals & al. (2010), FN645853, FN645564, FN645773, FN649368; ***Filago griffithii*** (A.Gray) Andrés-Sánchez & Galbany, Galbany-Casals & al. (2010), FN645888, FN645608, FN645796, FN649405; ***Filago hispanica*** (Degen & Hervier) Chrtek & Holub, Galbany-Casals & al. (2010), FN645855, FN645566, FN645775, FN649370; ***Filago lutescens*** Jord., Galbany-Casals & al. (2010), FN645883, FN645581, FN645779, FN649397; ***Filago nevadensis*** (Boiss.) Wagenitz & Greuter, Galbany-Casals & al. (2010), FN645863, FN645600, FN645776, FN649401; ***Filago ramosissima*** Lange, Galbany-Casals & al. (2010), FN645880, FN645563, FN645811, FN649367; ***Gamochaeta americana*** (Mill.) Wedd., Freire & al. (2015), KM091411, KM091382, KM091437, KM091348; ***Gamochaeta coarctata*** (Willd.) Kerg., Smissen & al. (2011), HM244732, HM450871, HM364533, –; ***Gamochaeta coarctata*** (Willd.) Kerg., Nie & al. (2016), KT865473, KT865271, –, –; ***Gamochaeta erythractis*** (Wedd.) Cabrera, Nie & al. (2016), KT865474, KT865272, –, –; ***Gamochaeta falcata*** (Lam.) Cabrera, Nie & al. (2016), KT865475, KT865273, –, –; ***Gamochaeta filaginea*** (DC.) Cabrera, Nie & al. (2016), KT865476, KT865274, –, –; ***Gamochaeta longipedicellata*** Cabrera, Freire & al. (2015), KM091410, KM091381, –, KM091347; ***Gamochaeta serpyllifolia*** Wedd., Freire & al. (2015), KM091409, KM091380, –, KM091346; ***Gamochaeta subfalcata*** (Urban) Anderb., Galbany-Casals & al. (2010), FN645834, FN645557, –, –; ***Gamochaetopsis alpina*** (Poepp.) Anderb. & S.E.Freire, Freire & al. (2015), KM091390, KM091356, KM091417, KM091326; ***Gnaphaliothamnus aecidiocephalus*** (Grierson) G.L.Nesom, Nie & al. (2016), KT865466, KT865261, –, –; ***Gnaphaliothamnus costaricensis*** G.L.Nesom, Nie & al. (2013), JQ895509, JQ895372, –, –; ***Gnaphalium austroafricanum*** Hilliard, Galbany-Casals & al. (2010), FN645830, FN645630, FN645756, FN649353; ***Helichrysum stoechas*** DC., Galbany-Casals & al. (2010), Pelser & al. (2010), HE611495, GU818178, GU818009, FN649351; ***Jalcophila boliviensis*** Anderb. & S.E.Freire, Freire & al. (2015), KM091402, KM091370, KM091429, KM091432; ***Jalcophila ecuadorensis*** M.O.Dillon & Sagást., Freire & al. (2015), –, KM091383, –, –; ***Leontopodium alpinum*** Cass., Galbany-Casals & al. (2010), FN645824, FN645625, FN645794, FN649348; ***Leontopodium himalayanum*** DC., Blöch & al. (2010), FJ639938, FJ640004, –, –; ***Logfia gallica*** (L.) Coss. & Germ., Galbany-Casals & al. (2010), FN645838, FN645556, FN645799, FN649339; ***Loricaria colombiana*** Cuatrec., Freire & al. (2015), KM091404, KM091371, KM091435, KM091330; ***Loricaria ollgaardii*** M.O.Dillon & Sagást., Nie & al. (2016), KT865550, KT865370, –, –; ***Lucilia acutifolia*** (Poir.) Cass., Freire & al. (2015), KM091396, KM091374, KM091432, KM091332; ***Lucilia kunthiana*** (DC.) Zardini, Nie & al. (2016), KT865557, –, –, –; ***Lucilia kunthiana*** (DC.) Zardini, Nie & al. (2016), KT865555, KT865375, –, –; ***Lucilia linearifolia*** Baker, Freire & al. (2015), –, KM091351, KM091419, KM091324; ***Lucilia lycopodioides*** (Less.) S.E.Freire, Freire & al. (2015), KM091384, KM091357, KM091414, KM091323; ***Lucilia nitens*** Less., Freire & al. (2015), KM091399, KM091353, KM091419, KM091331; ***Lucilia recurva*** Wedd., Nie & al. (2016), KT865558, KT865377, –, –; ***Mexerion sarmentosum*** (Klatt) G.L.Nesom, Nie & al. (2016), KT865561, KT865380, –, –; ***Micropsis dasycarpa*** (Griseb.) Beauverd, Freire & al. (2015), KM091408, KM091378, KM091339, KM091434; ***Micropsis spathulata*** (Pers.) Cabrera, Nie & al. (2016), KT865562, –, –, –; ***Micropus supinus*** L., Galbany-Casals & al. (2010), FN645818, FN645609, FN645805, FN649335; ***Mniodes andina*** (A.Gray) A.Gray ex Cuatrec., Freire & al. (2015), –, KM091367, –, –; ***Mniodes longifolia*** (Cuatrec. & Aristeg.) S.E.Freire, Chemisquy, Anderb. & Urtubey, Freire & al. (2015), KM091400, KM091364, KM091425, KM091335;

Appendix 1. Continued.

Mniodes lopezmirandae (Cabrera) S.E.Freire, Chemisquy, Anderb. & Urtubey, Freire & al. (2015), –, KM091354, KM091416, –; ***Mniodes plicatifolia*** (Sagást. & M.O.Dillon) S.E.Freire, Chemisquy, Anderb. & Urtubey, Freire & al. (2015), –, KM091368, KM091431, –; ***Omalotheca supina*** (L.) Cass., Galbany-Casals & al. (2004b, 2010), AY445230, FN645558, FN645789, FN649354; ***Psilocarphus brevissimus*** Nutt., Galbany-Casals & al. (2010), FN645822, FN645620, –, –; ***Relhania pungens*** L'Hér., Galbany-Casals & al. (2010), FN645814, FN645635, FN645749, FN649331; ***Stuckertiella capitata*** (Wedd.) Beauverd, Freire & al. (2015), KM091398, KM091369, KM091412, KM091341.

Appendix 2. Material used for achenial trichome morphology. Species, voucher information and origin or reference are indicated. "SEM" indicates whether the information was obtained from SEM pictures. Otherwise, information was obtained from light microscopic observations.

Antennaria chilensis J.Rémy, *Bayer 2073* (F), Argentina; ***Antennaria dioica*** (L.) Gaertn., *Jordal 73* (F), Norway; ***Antennaria linearifolia*** Wedd., Dillon & Sagástegui Alva (1991b), SEM; ***Antennaria microphylla*** Rydb., *Wilke 1* (BONN), cultivated at Bonn Botanical Gardens, SEM; ***Antennaria parvifolia*** Nutt., *Wilke 2* (BONN), cultivated at Bonn Botanical Gardens, SEM; ***Antennaria plantaginifolia*** (L.) Richardson, *Wilke 3* (BONN), cultivated at Bonn Botanical Gardens, SEM; ***Belloa chilensis*** (Hook. & Arn.) J.Rémy, *Moreira 1460* (SGO), Chile, SEM; ***Berroa gnaphalioides*** (Less.) Beauverd, *Cabrera 3184* (LP), Argentina, SEM; ***Bombycilaena discolor*** (Pers.) M.Laínz, Andrés-Sánchez & al. (2014), SEM; ***Bombycilaena erecta*** (L.) Smoljan., Andrés-Sánchez & al. (2014), SEM; ***Castroviejoa montelinasana*** (Em.Schmid) Galbany, L.Sáez & Benedí, Galbany-Casals & al. (2004), SEM; ***Chevreulia acuminata*** Less., *Novara & Bruno 9820* (B), Argentina; ***Chevreulia sarmentosa*** (Pers.) S.F.Blake, *Novara 8166* (B), Argentina, SEM; ***Chionolaena arbuscula*** DC., Dillon & Sagástegui Alva (1991b), SEM; ***Chionolaena campestris*** Deble, Loeuille & al. (2011); ***Chionolaena capitata*** (Baker) Freire, *Joly 6790* (F), Brazil, SEM; ***Chionolaena jeffreyi*** H.Rob., *Harley & al. 24387* (F), Brazil, SEM; ***Chionolaena phylicoides*** (Gardner) Baker, Freire (1993); ***Cuatrecasasiella argentina*** (Cabrera) H.Rob., Freire (1995); ***Diaperia prolifera*** (Nutt. ex DC.) Nutt., *Tracy 8088* (F131092), U.S.A.; ***Facelis plumosa*** (Wedd.) Sch.Bip., *Moreira 1888* (SGO), Chile; *Moreira 1948* (SGO), Chile, SEM; ***Facelis retusa*** (Lam.) Sch.Bip., *Moreira 2051* (BONN), Chile, SEM; ***Filago aegaea*** Wagenitz, Andrés-Sánchez & al. (2015), SEM; ***Filago argentea*** (Pomel) Chrtek & Holub, Andrés-Sánchez & al. (2015), SEM; ***Filago arvensis*** L., Andrés-Sánchez & al. (2015), SEM; ***Filago carpetana*** (Lange) Chrtek & Holub, Andrés-Sánchez & al. (2015), SEM; ***Filago congesta*** DC., Andrés-Sánchez & al. (2015), SEM; ***Filago desertorum*** Pomel, Andrés-Sánchez & al. (2015), SEM; ***Filago discolor*** (DC.) Andrés-Sánchez & Galbany, Andrés-Sánchez & al. (2015), SEM; ***Filago eriocephala*** Guss., Andrés-Sánchez & al. (2015), SEM; ***Filago griffithii*** (A.Gray) Andrés-Sánchez & Galbany, Abid & Qaiser (2008b); Andrés-Sánchez & al. (2015), SEM; ***Filago hispanica*** (Degen & Hervier) Chrtek & Holub, Andrés-Sánchez & al. (2015), SEM; ***Filago lutescens*** Jord., Andrés-Sánchez & al. (2015), SEM; ***Filago nevadensis*** (Boiss.) Wagenitz & Greuter, Andrés-Sánchez & al. (2015), SEM; ***Filago pyramidata*** L., Andrés-Sánchez & al. (2015), SEM; ***Filago ramosissima*** Lange, Andrés-Sánchez & al. (2015), SEM; ***Gamochaeta americana*** (Mill.) Wedd., *Álvarez 739* (FB), Chile; *Álvarez 786* (FB), Chile; *Álvarez 849* (FB), Chile, SEM; ***Gamochaeta*** cf. ***berteroana*** (DC.) Cabrera, *Moreira 1835* (SGO), Chile; *Moreira 1860* (SGO), Chile; ***Gamochaeta*** cf. ***chamissonis*** (Phil.) Cabrera, *Moreira 1825* (SGO), Chile, SEM; ***Gamochaeta coarctata*** (Willd.) Kerg., *Cabrera 2226* (F670313), Argentina; ***Gamochaeta erythractis*** (Wedd.) Cabrera, *d'Orbigny 1370* (F97446), Bolivia; ***Gamochaeta falcata*** (Lam.) Cabrera, *Cabrera 11611* (F1549533), Argentina; ***Gamochaeta filaginea*** (DC.) Cabrera, *Bayer 2066* (F2244908), Argentina; ***Gamochaeta longipedicellata*** Cabrera, Urtubey & al. (2016); ***Gamochaeta serpyllifolia*** Wedd., *Moreira 1826* (SGO), Chile; *Gay s.n.* (F28720), Chile; *Marticorena & al. 52* (F1811328), Chile; ***Gamochaeta* sp.,** *Luebert 3195* (BONN); ***Gamochaeta* sp.,** *Moreira 1858* (SGO), Chile; ***Gamochaeta* sp.,** *Tomé s.n.* (F), Chile; ***Gamochaeta subfalcata*** (Urban) Anderb., *Cabrera 17003* (F1644189), Argentina, SEM; ***Gamochaetopsis alpina*** (Poepp. & Endl.) Anderb. & S.E.Freire, *Montero 2717* (LP), Chile; *Neumeyer 509* (LP), Argentina; *de la Sota 2179* (LP), Argentina; *Boelcke & Correa 6952* (LP), Argentina; *Bayer & Chandler ARG-02080* (F), Argentina; *Poeppig 889* (W), Chile, SEM; ***Gnaphaliothamnus aecidiocephalus*** (Grierson) G.L.Nesom, Freire (1993); ***Gnaphaliothamnus concinnus*** (A.Gray) G.L.Nesom, *Cedillo & Lorence 652* (F), Mexico, SEM; ***Gnaphaliothamnus costaricensis*** G.L.Nesom, *Davidse et al. 26003* (F); ***Gnaphaliothamnus lavandulifolius*** (Kunth) G.L.Nesom, *Dovz 2715 & al.* (F); ***Gnaphaliothamnus salicifolius*** (Bertol.) G.L.Nesom, *McVaugh 25923* (F); ***Gnaphalium austroafricanum*** Hilliard, *Seegeler 2336* (F), Ethiopia; ***Helichrysum stoechas*** DC., Ascensão & al. (2001), SEM; ***Jalcophila boliviensis*** Anderb. & S.E.Freire, Anderberg & Freire (1990); ***Jalcophila ecuadorensis*** M.O.Dillon & Sagást., Dillon & Sagástegui Alva (1991b), SEM; ***Leontopodium alpinum*** Cass., Heß (1938); ***Leontopodium himalayanum*** DC., Abid & Qaiser (2008b), SEM; ***Logfia gallica*** (L.) Coss. & Germ., Anderberg (1991); ***Loricaria colombiana*** Cuatrec., *Cuatrecasas 2947* (F843036), Colombia; ***Loricaria ferruginea*** (Ruiz & Pav.) Wedd., *Sagástegui & al. 16439* (F), Peru, SEM; ***Loricaria graveolens*** (Sch.Bip.) Wedd., *Schmidt s.n.* (F1223404), Peru; ***Loricaria ollgaardii*** M.O.Dillon & Sagást., Dillon & Sagástegui Alva (1986); ***Loricaria thuyoides*** (Lam.) Sch.Bip., *Sagástegui & al. 11220* (F), Peru; ***Lucilia acutifolia*** (Poir.) Cass., *Anderson 1657* (LP), Argentina, SEM; ***Lucilia conoidea*** Wedd., *Dillon & al. 1082* (F) Peru, SEM; ***Lucilia eriophora*** J.Rémy, *Moreira 1465* (SGO), Chile; *Teiller 4269* (F), Chile; *Poeppig 888* (F8778548), Chile, SEM; ***Lucilia kunthiana*** (DC.) Zardini, *Dillon & Tunner 1392* (F), Peru; *Smith & al. 11218* (F), Peru; *Smith & al. 11590* (F), Peru; *Steyermark & Koyama 102370* (B), Venezuela; *Cabrera 9424* (BAB), Argentina, SEM; ***Lucilia linearifolia*** Baker, *Glaziou 8142* (F1023233), Brazil; ***Lucilia lycopodioides*** (Less.) S.E.Freire, *Barret 8155* (F1004384), Brazil; ***Lucilia nitens*** Less., *Wasum 816* (B); ***Lucilia nivea*** (Phil.) Cabrera, *Moreira 1536* (SGO), Chile; *Cabrera 6248* (F1549506), Chile; ***Lucilia recurva*** Wedd., *Lewis s.n.* (F2015133), Bolivia; ***Merope argentea*** Wedd., *Moreira 1930* (SGO), Chile, SEM; ***Mexerion sarmentosum*** (Klatt) G.L.Nesom, *Purpus 1520* (F), Mexico; ***Micropsis dasycarpa*** (Griseb.) Beauverd, Morefield (2006); ***Micropsis nana*** DC., *Álvarez 708* (FB), Chile; *Álvarez S2* (FB), Chile, SEM; ***Micropsis spathulata*** (Pers.) Cabrera, Cabrera (1932); ***Micropus supinus*** L., Anderberg (1991), SEM; ***Mniodes andina*** (A.Gray) A.Gray ex Cuatrec., Dillon & Sagástegui Alva (1991a); ***Mniodes aretioides*** (Sch.Bip.) Cuatrec., *Dillon & al. 1083* (F), Peru, SEM; ***Mniodes longifolia*** (Cuatrec. & Aristeg.) S.E.Freire, *Sagástegui 12853* (F), Peru; *Sagástegui & al. 12841* (F), Peru; *Sagástegui & al. 16703* (F), Peru; *Sagástegui & al. 11981* (F), Peru, SEM; ***Mniodes lopezmirandae*** (Cabrera) S.E.Freire, *López 858* (HUT), Peru; ***Mniodes piptolepis*** (Wedd.) S.E.Freire, *Moreira 1931* (SGO), Chile, SEM; *Moscatero & al. 1911* (F), Peru, SEM; ***Mniodes plicatifolia*** (Sagást. & M.O.Dillon) S.E.Freire, Dillon & Sagástegui Alva (1991b), SEM; ***Mniodes pulvinulata*** Cuatrec., *Sánchez & al. 10743* (F), Peru, SEM; ***Mniodes santanica*** (Cabrera) S.E.Freire, *Moreira 1904* (SGO), Chile; ***Mniodes schultzii*** (Wedd.) S.E.Freire, *Moreira 1920* (SGO), Chile; ***Mniodes subspicata*** (Wedd.) S.E.Freire, *Moreira 1921* (SGO), Chile; ***Omalotheca supina*** (L.) Cass., Anderberg (1991), SEM; ***Psilocarphus brevissimus*** Nutt., Anderberg (1991); ***Relhania pungens*** L'Hér., *Bayer & Puttock 96226* (F); ***Stuckertiella capitata*** (Wedd.) Beauverd, *K. & M. Weigend 2000/29* (F), Peru; *Dillon & al. 3115* (F), Peru, SEM.