Phylogeny of *Chaetanthera* (Asteraceae: Mutisieae) reveals both ancient and recent origins of the high elevation lineages

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Abstract

Penalized likelihood analysis of previously published chloroplast DNA (cpDNA) *ndh*F sequences suggests that the central-southern Andean genus *Chaetanthera* diverged ca. 16.5 million years (my) ago, well before the uplift of the Andes to their present heights. Penalized likelihood analysis based on new nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) sequences indicates that the most relictual lineages occupy high elevation Andean habitats that did not exist until some 10 my later. This result is contrary to the expectation that younger habitats should be occupied by phylogenetically younger lineages. The results are interpreted with respect to the development of aridity in lowland habitats during the Miocene and Pliocene, which presumably extinguished the lowland relatives of the high elevation taxa or, in effect, forced them upwards in search of adequate moisture. As the more northerly lineages were being displaced upward, others diversified in the mediterranean-type climate area of central Chile, giving rise to additional high elevation taxa again, at an early date, as well as lowland taxa. Some species of *Chaetanthera* from lowland central Chile appear as the phylogenetically youngest taxa, suggesting secondary adaptation to lowland aridity. At the same time, at least two high elevation species, *Chaetanthera peruviana* and *Chaetanthera perpusilla*, appear to have been derived recently from a lower elevation ancestor, while some middle to low elevation taxa seem to have evolved recently out of a high elevation complex. The results suggest that the younger high elevation habitats have served as both "cradle" and "museum" for *Chaetanthera* lineages.

Keywords: *Chaetanthera*; nrITS; Calibration age; Molecular clock

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1. Introduction

The central and southern Andes comprise a continuous high mountain range spanning from central Peru and Bolivia south through Argentina and Chile to Tierra del Fuego. This region harbors a diverse high elevation flora of perhaps 3700 species (Arroyo, unpublished data) containing elements that are characteristic of, if not endemic to, South America, e.g. *Calceolaria* (Calceolariaceae; Erhart, 2000), *Tropaeolum* sect. *Chilensia* (Tropaeolaceae; Hershkovitz et al., 2006), *Alstroemeria* (Alstroemeriaceae; Muñoz Schick and Moriera Muñoz, 2003), and many genera of Asteraceae tribe Mutisieae (Bremer, 1994). The high elevation flora of the western slope of the Andes in Chile alone comprises an estimated 1700 species (Arroyo et al., 2004). For the present purposes, high elevation refers to the equivalent of above-treeline or alpine vegetation, a term that is difficult to apply in central and northern southern Andes because of lack of a well-defined treeline at the driest latitudes. In the northern part of the central Andes high elevation vegetation occurs between 3000 and 5000 m, descending to around 1800 and 3000 m heights at mediterranean latitudes in central Chile. Characterization as mid-elevation refers to plants below treeline or its equivalent along the slopes of the Andes, while lowland refers to coastal or low valleys below about 300–700 m elevation depending on latitude.

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Various lines of geological evidence indicate that uplifting of the central and southern Andes to their current elevations occurred between the Pliocene and Pleistocene (Arroyo et al., 1988; Farías et al., 2005; Gregory-Wodzicki, 2000; Giambiagi, 2003; Hartley, 2003; Irigoyen et al., 2000; Hinojosa and Villagrán, 1997; Simpson, 1983). Collectively, the data indicate that this region achieved far less to no more than one half of its current height by the end of the Miocene (Giambiagi, 2003; Gregory-Wodzicki, 2000). Assuming a correlation between the age of a habitat and the age of its biota, the geological data implicate a correspondingly recent origin of the central and southern high Andean flora. In terms of phylogenetic trees, the expectation is that the stems of high elevation lineages should be relatively shorter than and nested among those of lower elevation lineages. Indeed, upward migration of lowland elements (Arroyo et al., 1983), along with recent long distance dispersal of high elevation plants established elsewhere (e.g. Soltis et al., 2001), have been proposed as important sources of the high Andean flora.

Among the taxa endemic to southern South America and well represented in both higher and lower elevations is *Chaetanthera* Ruiz & Pav. (Asteraceae: Mutisieae). As currently conceived, *Chaetanthera* comprises 44 species in seven subgenera (Cabrera, 1937). Subgenus *Egania* (perennial herbs) is restricted to high elevation habitats ranging from central Chile and Argentina to Peru and Bolivia. Subgenus *Oriastrum* (annuals to short-lived perennials) comprises high and high to mid-elevation species of central Chile and adjacent Argentina. Subgenus *Carmelita* (perennial herbs) comprise mostly high elevation species of central Chile and Argentina with occasional populations of some species occurring at mid-elevations. Subgenus *Glandulosa* (one subshrub) occurs in central Chile at mid-elevation to treeline. Subgenera *Tylloma* (mostly annual) and *Euchaetanthera* (all annuals) may be found from sea level to high elevations in central Chile, in the Andes in adjacent Argentina, and less frequently in northern Chile, Peru, Bolivia, and southern Argentina. Subgenus *Proselia* (perennials) is mostly concentrated in low to mid-elevation habitats, including in *Araucaria* forest clearings in south-central Chile, just getting into Argentina. Here, as part of a more comprehensive study into the evolution of *Chaetanthera*, we undertake phylogenetic reconstruction and estimation of divergence dates in order to provide insights into the evolution of the autochthonous central-southern Andean flora in relation to the Andean uplift. We first calibrate the age of *Chaetanthera* by comparison to other Asteridae for which divergence dates have been estimated. We then develop an ITS phylogeny for *Chaetanthera* and use the preceding calibration to estimate the divergence dates of the individual subgenera and relate these to the ecological history of the central–southern Andes.

2. Materials and methods

Specimens of all but a few taxa of *Chaetanthera* were collected and portions preserved in silica gel for DNA analysis. Specimens were identified according to Cabrera (1937) and more recent publications on particular taxa. In addition, most identifications were verified by A. Davies (Munich, Germany), who is undertaking a revision of the genus in collaboration with the second author.

In order to estimate the divergence date of *Chaetanthera* (see below), chloroplast DNA (cpDNA) *ndh*F sequences from Asteridae and Asteraceae listed in Kim et al. (2005) and partial *ndh*F sequences (3′ end, ca. 1000 bp) listed in Kim et al. (2002) were obtained from GenBank and aligned manually. In addition, the sequence of *Cornus florida* (GenBank Accession No. AF130220) was obtained. The alignment was trimmed to include only the 3′ end used in the Kim et al. (2002) analysis. *Cornus* was selected as a calibration point based on an approximate fossil dating, as in Bremer et al. (2004).

Nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) region sequences were obtained from 82 samples of *Chaetanthera* following the extraction, amplification, and sequencing protocols described in Hershkovitz (2006). Eliminating 19 of the taxonomically duplicated samples, the present analysis is based on 63 samples (Table 1). Some samples did not amplify following the extraction protocol and were further purified using Chelex 100 resin (BioRad). 10 μl of genomic DNA is added to 250 μl of 5% aqueous Chelex, mixed, heated at 100 °C for 15 min, and the supernatant containing purified DNA removed to a new tube. This procedure removes heavy metal cations that apparently inhibited the PCR. Other purification methods (PEG precipitation and/or silica-NaI) were tried, but they did not alleviate this inhibition.

The resulting ITS sequences were aligned manually. The sequences included 56 apparently polymorphic sites, usually C/T, among the total 43,533 aligned sites. These sites were scored as ambiguities ("N") in the phylogenetic analysis. The polymorphisms are mainly restricted to taxonomically difficult species. Cloning of polymorphic samples is underway and the taxonomic and evolutionary implications will be considered in a future publication.

Maximum parsimony (MP) and maximum likelihood (ML) analysis of the *ndh*F and ITS sequences were undertaken using PAUP 4.0 (Swofford, 2002) version b10. The MP analysis of the ITS sequences included alignable gaps scored as separate characters. The MP analysis and bootstrap (500 replicates) were performed using the default heuristic search procedure. For the bootstrap analysis, maxtrees was set at 100. For the ML analysis, Modeltest (Posada and Crandall, 1998) was used to estimate the ML parameters. Tree rooting was based on Bremer et al. (2004) for Asteridae and on analysis of cpDNA *rpl*32-*trn*L intergenic spacer sequences for representative *Chaetanthera* species using *Mutisia* as the outgroup (data not shown).

A likelihood ratio test was used to test for rate constancy among lineages (Felsenstein, 1981). In all cases, the hypothesis of rate constancy among lineages was rejected. Divergence dates were estimated over the ML topology and branch lengths using penalized likelihood (PL) implemented in the r8s program (Sanderson, 2002a,b). This

Table 1
Taxa, vouchers, geographic origins, altitudes, and GenBank Accession numbers of specimens sampled for ITS. Vouchers are deposited in the Herbarium of the Universidad de Concepción (CONC)

Subgenus species	Voucher	Geographic origin	Altitude (m)	GenBank Accession
Carmelita				
C. lanata (Phil.) I. M. Johnst.	*Arroyo* et al. *25075*	CHILE: IV	2790	DQ355863
C. spathulifolia Cabrera	*Arroyo* et al. *25098*	ARGENTINA: Mendoza	3200	DQ355864
C. villosa D. Don	*Arroyo* et al. *210671*	CHILE: IX	1300	DQ355865
C. villosa	*Arroyo* et al. *20646*	CHILE: RM	2870	DQ355845
Egania				
C. acerosa (J. Rémy) Benth. & Hook. F. var. *acerosa*	*Arroyo* et al. *25087A*	CHILE: IV	3700	DQ355909
C. acerosa var. *dasycarpa* Cabrera	*Arroyo* et al. *25087B*	CHILE: IV	3700	DQ355914
C. acerosa var. indet.	*Arroyo* et al. *25077*	CHILE: IV	3210	DQ355905
C. apiculata (J. Rémy) F. Meigen	*Arroyo* et al. *25244*	CHILE: V	2700	DQ355910
C. aff. *boliviensis* J. Kost.	*Arroyo* et al. *25200*	BOLIVIA: La Paz	4811	DQ355911
C. aff. *cochlearifolia* (Gray) B. L. Robinson	*Arroyo* et al. *25111*	ARGENTINA: Tucumán	4220	DQ355895
C. dioica (J. Rémy) B. L. Robinson	*Arroyo* et al. *25102*	ARGENTINA: La Rioja	3340	DQ355898
C. pentacaenoides (Phil.) Hauman	*Arroyo* et al. *25099*	ARGENTINA: Mendoza	3180	DQ355893
C. pentacaenoides	*Arroyo* et al. *25168*	CHILE: RM	3310	DQ355904
C. pulvinata (Phil.) Hauman var. *pulvinata*	*Arroyo* et al. *25083*	CHILE: IV	4200	DQ355903
C. pulvinata var. *pulvinata*	*Arroyo* et al. *25100*	ARGENTINA: Mendoza	3030	DQ355909
C. revoluta (Phil.) Cabrera	*Arroyo* et al. *25126*	CHILE: II	4450	DQ355899
C. sphaeroidalis (Reiche) Hicken	*Arroyo* et al. *25082*	CHILE: IV	4360	DQ355897
C. aff. *sphaeroidalis*	*Arroyo* et al. *25104*	ARGENTINA: La Rioja	5100	DQ355913
C. steubelii Hieron. var. *abbreviata* Cabrera	*Arroyo* et al. *25110*	ARGENTINA: Tucumán	4220	DQ355900
C. steubelii var. *argentina* Cabrera	*Arroyo* et al. *25109*	ARGENTINA: Tucumán	3890	DQ355896
C. steubelii var. indet.	*Arroyo* et al. *25201*	BOLIVIA: Potosi	4300	DQ355912
C. sp. '25203'	*Arroyo* et al. *25203*	CHILE: I	4300	DQ355901
C. sp. '25204'	*Arroyo* et al. *25204*	CHILE: I	4850	DQ355913
Euchaetanthera				
C. australis Cabrera	*Arroyo* et al. *25177*	ARGENTINA: Río Negro	860	DQ355883
C. chiquianensis Ferreyra	*Arroyo 25252*	PERU: Ancash	3500	DQ355850
C. ciliata Ruiz & Pav.	*Arroyo* et al. *25157*	CHILE: VIII	100	DQ355888
C. euphrasioides (DC.) F. Meigen	*Arroyo* et al. *25176*	CHILE: RM	3315	DQ355868
C. euphrasioides	*Arroyo* et al. *25119*	CHILE: RM	2470	DQ355866
C. flabellata D. Don	*Arroyo* et al. *25161*	CHILE: RM	2200	DQ355867
C. incana Poepp. ex Less.	*Arroyo* et al. *25013*	CHILE: IV	960	DQ355885
C. leptocephala Cabrera	*Hershkovitz 02–109*	CHILE: III	2152	DQ355873
C. linearis Poepp. ex Less var. *albiflora* Phil.	*Arroyo* et al. *25012*	CHILE: IV	950	DQ355909
C. linearis var. *linearis*	*Arroyo* et al. *25033*	CHILE: V	760	DQ355869
C. linearis var *taltalensis* I. M. Johnst.	*Arroyo* et al. *25129*	CHILE: IV	ca. 50	DQ355870
C. linearis var *taltalensis*	*Arroyo* et al. *25019*	CHILE: IV	1050	DQ355909
C. microphylla (Cass.) Hook. & Arn.	*Arroyo* et al. *25007*	CHILE: RM	960	DQ355871
C. moenchioides Less.	*Arroyo* et al. *25122*	CHILE: IX	32	DQ355847
C. perpusilla (Wedd.) Anderb. & S. E. Freire	*Arroyo* et al. *25202*	CHILE: I	3370	DQ355880
C. peruviana Gray	*Arroyo 25254*	PERU: Tacna	3680	DQ355850
C. tenella Less. var. *taltalensis* Cabrera	*Arroyo* et al. *25055*	CHILE: IV	92	DQ355878
C. tenella var. *taltalensis*	*Arroyo* et al. *25128*	CHILE: II	175	DQ355879
C. tenella var. *tenella*	*Arroyo* et al. *25006*	CHILE: RM	910	DQ355882
Glandulosa				
C. glandulosa J. Rémy	*Arroyo* et al. *25181*	CHILE: RM	2430	DQ355881
Oriastrum				
C. aff. *gnaphalioides* (J. Rémy) I. M. Johnst.	*Arroyo* et al. *25086*	CHILE: IV	3500	DQ355908
C. aff. *gnaphalioides*	*Hershkovitz 02–154*	CHILE: IV	1056	DQ355906
C. lycopodioides (J. Rémy) Cabrera	*Arroyo* et al. *25169*	CHILE: RM	3140	DQ355920
C. minuta (Phil.) Cabrera	*Arroyo* et al. *25079*	CHILE: IV	3200	DQ355890
C. minuta	*Arroyo* et al. *25127*	CHILE: II	3500	DQ355907
C. planiseta Cabrera	*Arroyo* et al. *25120*	CHILE: RM	2460	DQ355892
C. pusilla (D. Don) Hook. and Arn.	*Arroyo* et al. *25180*	CHILE: RM	3360	DQ355916
Proselia				
C. brachylepis Phil.	*Arroyo 25250*	CHILE: IX	670	DQ355848
C. chilensis (Willd.) DC. var. *chilensis*	*Arroyo 25229*	CHILE: VII	1800	DQ355840
C. chilensis var. *tenuifolia* (D. Don) Cabrera	*Arroyo* et al. *25042*	CHILE: VII	1250	DQ355841
C. elegans (Phil.) var. *elegans*	*Arroyo* et al. *25069*	CHILE: VIII	1470	DQ355889
C. elegans var. *pratensis* (Phil.) Cabrera in M. N. Correa	*Arroyo* and *Humaña 26000*	CHILE: IX	1660	DQ355839
C. serrata Ruiz & Pav.	*Arroyo* et al. *25131*	CHILE: VIII	60	DQ355886

Table 1 (*continued*)

Subgenus species	Voucher	Geographic origin	Altitude (m)	GenBank Accession
Tylloma				
C. flabellifolia Cabrera	*Arroyo* et al. *25078*	CHILE: IV	3200	DQ355852
C. glabrata (DC.) F. Meigen	*Arroyo* et al. *25130*	CHILE: II	145	DQ355858
C. glabrata (DC.) F. Meigen	*Arroyo* et al. *25163*	CHILE: RM	2190	DQ355856
C. glabrata (DC.) F. Meigen	*Arroyo* et al. *25065*	CHILE: IV	110	DQ355854
C. kalinae Davies	*Arroyo* et al. *25076*	CHILE: IV	2740	DQ355861
C. limbata (D. Don) Less.	*Arroyo* et al. *25150*	CHILE: III	828	DQ355843
C. renifolia (J. Rémy) Cabrera	*Arroyo* et al. *25175*	CHILE: RM	3410	DQ355860
C. splendens (J. Rémy) B. L. Robinson	*Arroyo* et al. *25084*	CHILE: IV	3250	DQ355853
C. sp. '02–96'	*Hershkovitz 02–96*	CHILE: III	2264	DQ355857

For geographic origin, roman numerals denote regions of Chile. RM refers to the Metropolitan Region.

method has been shown to perform relatively well under simulation (Sanderson, 2002a; Ho et al., 2005) and has been widely used to estimate divergence time in plant lineages, including divergence dates of angiosperms as a whole. (e.g. Schneider et al., 2004; Bell et al., 2005). The optimal smoothing level was chosen via the cross validation procedure described by Sanderson (2002a). Divergences were calibrated using a date of 128 my for the crown Asteridae (Bremer et al., 2004). This, in turn, generated a divergence date estimate for the crown of the four included *Chaetanthera* samples. Finally, the estimated divergence date of the *Chaetanthera* crown based on the *ndh*F data was used to calibrate the divergence dates of the ITS sequences.

3. Results

The MP bootstrap results of the Asteridae *ndh*F data are shown in Fig. 1. The consensus is poorly resolved relative to the Asteraceae phylogeny of Bremer et al. (2004) and with respect to Asteraceae tribal classification in general (cf. Bremer, 1994). Consistent with earlier results, the tribe Barnedesioideae diverges first from Asteraceae. The results for *Chaetanthera* are consistent with, but less resolved than those of Kim et al. (2002), who did not perform a bootstrap analysis. In particular, *Chaetanthera* is sister to *Duidaea*, although with only modest bootstrap support and keeping in mind that only 16 of the 76 genera of Mutisieae (Bremer, 1994) were sampled. *Duidaea* includes four shrubby species in Venezuela (Bremer, 1994).

The hierarchical likelihood ratio Test (hLRT) procedure in Modeltest selected the TVM+G model, whereas the Aikake Information Criterion (AIC) selected the TVM+G+I model with corresponding compensation in the gamma parameter to account for the invariant site proportion estimate. A model more general than that derived from hLRT, the GTR+G model with estimated base frequencies, was used for ML analysis. Initial parameters were those estimated from a neighbor-joining tree. Starting trees for ML analysis were those having the best ML score (18 trees) among the 3563 MP trees (length, L = 1704; rescaled consistency index, RC = 0.29; retention index, RI = 0.53). The final ML topology (Fig. 2) was among the MP trees. The ultrametric tree derived from PL analysis, along with approximate divergence dates, are shown in Fig. 5.

The MP bootstrap results of the ITS data for *Chaetanthera* are shown in Fig. 3. The data strongly support a major division of taxa at the base of the tree, one comprising subgenera *Egania* and *Oriastrum* (Clade A) and one comprising the remaining taxa (Clade B). We reiterate that rooting was based on cpDNA *rpl*32-*trn*L intergenic spacer sequences for representative *Chaetanthera* species using *Mutisia* as the outgroup (Hershkovitz and Arroyo, unpublished data). ITS sequences of three Mutisieae (*Mutisia, Pachylaena*, and *Trichocline*) could not be confidently aligned with those of *Chaetanthera*, thus preventing outgroup rooting of the ITS data. However, midpoint rooting placed the root between the *Egania* and *Oriastrum* clades, reflecting the especially short branches of the former and long branches of the latter. This rooting would not impact the biogeographic or chronological interpretations. Both the hLRT and AIC procedures selected the TNM+G+I model. A more general GTR+G+I model with estimated base frequencies was used for ML analysis. Initial parameters were those estimated from a neighbor-joining tree. Starting trees for ML analysis were those having the best ML score (1 trees) among the 180 MP trees (L = 869, RC = 0.44, RI = 0.83). The ML analysis produced two similar topologies, the first of which is shown in Fig. 4. This topology had an MP score of 872. The ML topology suggests that some species, as traditionally circumscribed, are para- or polyphyletic, including *C. elegans*, *C. glabrata*, *C. tenella*, *C. minuta*, *C. steubelii*, *C. pulvinata*, and possibly *C. gnaphalioides* and *C. sphaeroidalis*. The divergence between the two collections of *C. villosa*, one from central and the other from southern Chile, is also notable. Additional ITS data (not shown) suggest that *C. moenchioides* is also unnatural. The finer details of *Chaetanthera* systematics will be the subject of a future paper.

The ML tree (Fig. 4) suggests that evolutionary rates of ITS have been heterogeneous in *Chaetanthera*. The LRT yielded $p = 0.002$ against the hypothesis of rate constancy (i.e. the clock). The strongest rate contrast is between *Egania* and *Oriastrum*. This is possibly related to life form, as species of *Egania* are long-lived perennials, whereas species of *Oriastrum* are annuals or short-lived perennials. The ITS divergence rate of *C. glandulosa*, a subshrub, appears slower than in related taxa.

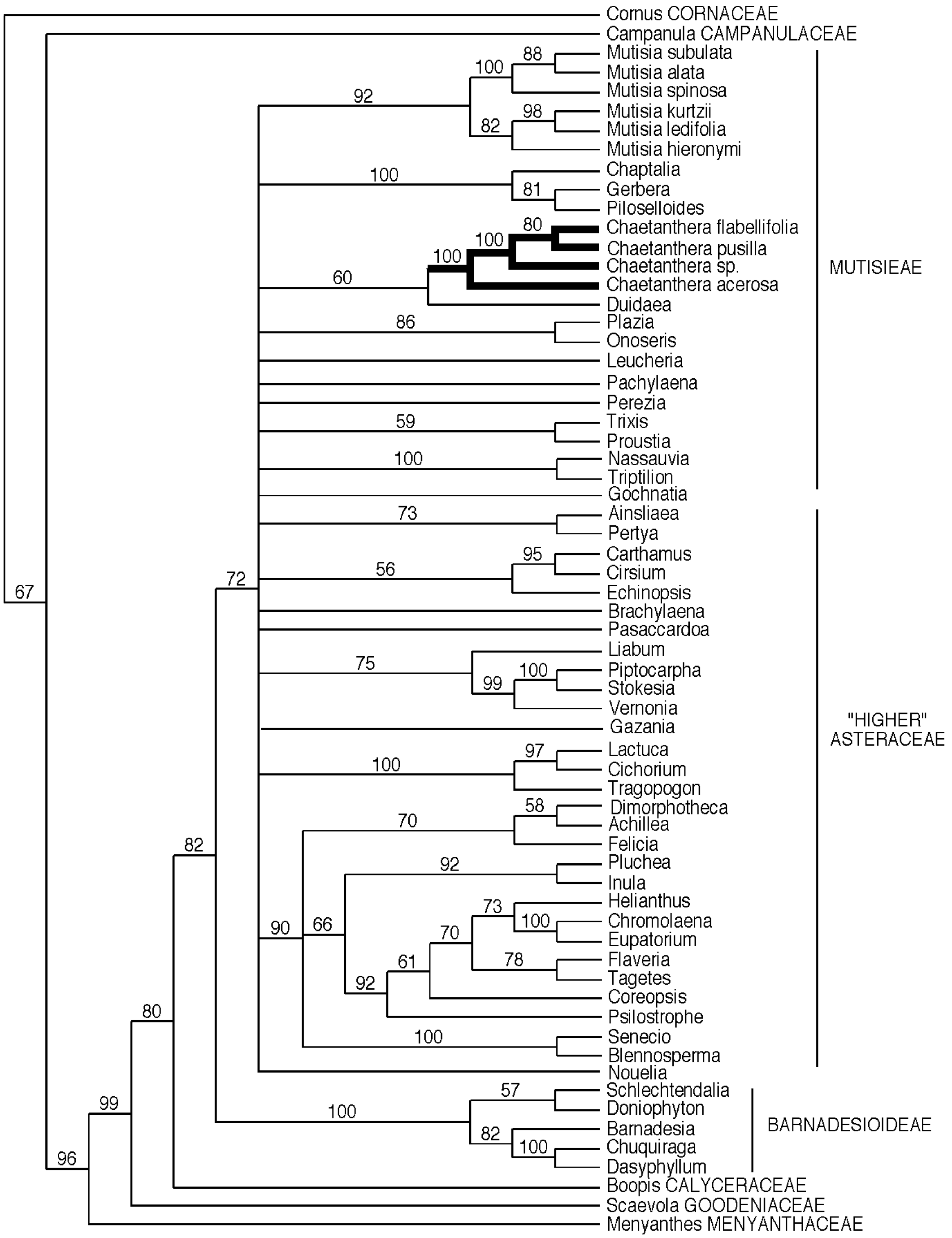

Fig. 1. Maximum parsimony bootstrap consensus of Asteridae partial *ndh*F sequences. Bootstrap proportions are indicated adjacent to the branches.

The divergence dating using PL (Fig. 5) suggests an origin of crown Asteraceae at ca. 36 my and an origin of crown *Chaetanthera* at ca. 16.5 my. This date separates subgenera Clades A and B and is in agreement with recent palynological evidence (Tellería and Katinas, 2004). *Egania* and *Oriastrum* subsequently diverge at ca. 13.5 my. The crown age of Clade B is ca. 10 my. The remaining subgenera, with the trivial exception of the monotypic subgenus *Glandulosa*, are nonmonophyletic, hence cannot be ascribed ages. With some exceptions, the higher elevation lineages appear to branch off relatively early, with the lowest elevation taxa diverging most recently. The most recently derived high elevation taxon appears to be *C. peruviana*, which diverged from *C. chiquianensis*, a mid-elevation species, within the past million years and is nested within a mid- to low elevation clade.

4. Discussion

The divergence dates among Asterales families based on partial *ndh*F sequences are in remarkably good agreement with those of Bremer et al. (2004) based on six chloroplast

Fig. 2. Penalized likelihood estimatence of divergence dates of Asteridae based on partial *ndh*F sequences.

sequences. The resulting estimate of ca. 16.5 my for the divergence of the crown group of *Chaetanthera* thus seems reasonably robust. The estimates for Asterales are somewhat different from those of Kim et al. (2005) using different methods. In particular, the latter shows Asterideae as much younger (according to their Fig. 6, apparently ca. 85 my), while Asteraceae are somewhat older (ca. 40 my) than in Bremer et al. (2004) and the present analysis. Thus, the discrepancy cannot be explained as a matter of scaling. It should be emphasized, however, that the Asteraceae datings generated by Kim et al. (2005) should yield an age estimate for *Chaetanthera* at least as old as that predicted by the Bremer et al. (2004) scaffold.

Our age estimate for *Chaetanthera* is further supported by the ITS divergences. In Hershkovitz and Zimmer (2000), we estimated (based on Baum et al., 1998) a rate of 5×10^{-9}

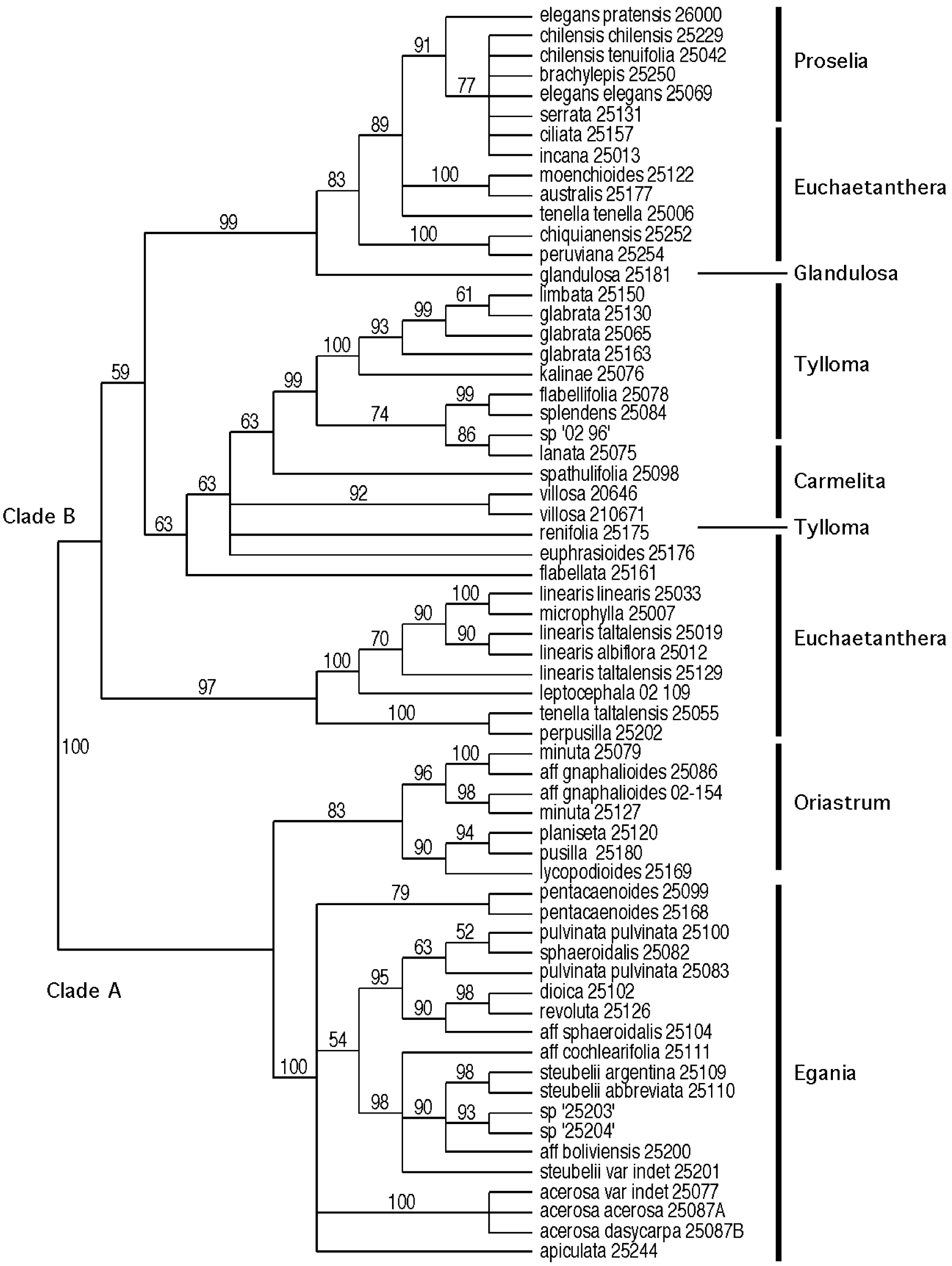

Fig. 3. Maximum parsimony bootstrap consensus of *Chaetanthera* ITS sequences. Bootstrap proportions are indicated adjacent to the branches. Numbers next to taxon names correspond to collection numbers, cf. Table 1. The traditional subgeneric classification (Cabrera, 1937) of the taxa is shown at right.

substitutions per site per year (ssy, Kimura 2-parameter, or K2P distances, unfortunately ignoring gamma for comparison to other published rates) for herbaceous plants. The *Chaetanthera* divergences would be at least slightly faster than this given our dates, which again points to the conservatism in our estimate for the age of the genus. For example, the *C. flabellata–C. elegans* var. *elegans* split at ca. 10.5 my yields a rate of 7.6×10^{-9} ssy. The *C. minuta–C. lycopodioides* split at ca. 14 my yields a rate of 1×10^{-8} ssy, thus double the typical rate.

The ML topology based on partial *ndh*F sequences is in remarkably good agreement with the tree based on combined complete *ndh*F and *rbc*L sequences (Kim et al., 2005). A notable exception is with respect to relations among the tribe Barnedesioideae, in particular *Chuquiraga*. This genus is sister to *Dasyphyllum* in the present analysis but sister to *Doniophyon* in Kim et al. (2005). This possibly represents a sequence submission error. A BLAST search (Altschul et al., 1990) indicated that the complete *Chuquiraga* (GenBank No. L39393) and *Dasyphyllum* (L39392) *ndh*F sequences are identical, whereas the *rbc*L sequence of the former (AY874427) is more similar to that of *Doniophytum* (AY874430). Yet, *ndh*F sequences evolve more rapidly than *rbc*L. We leave this

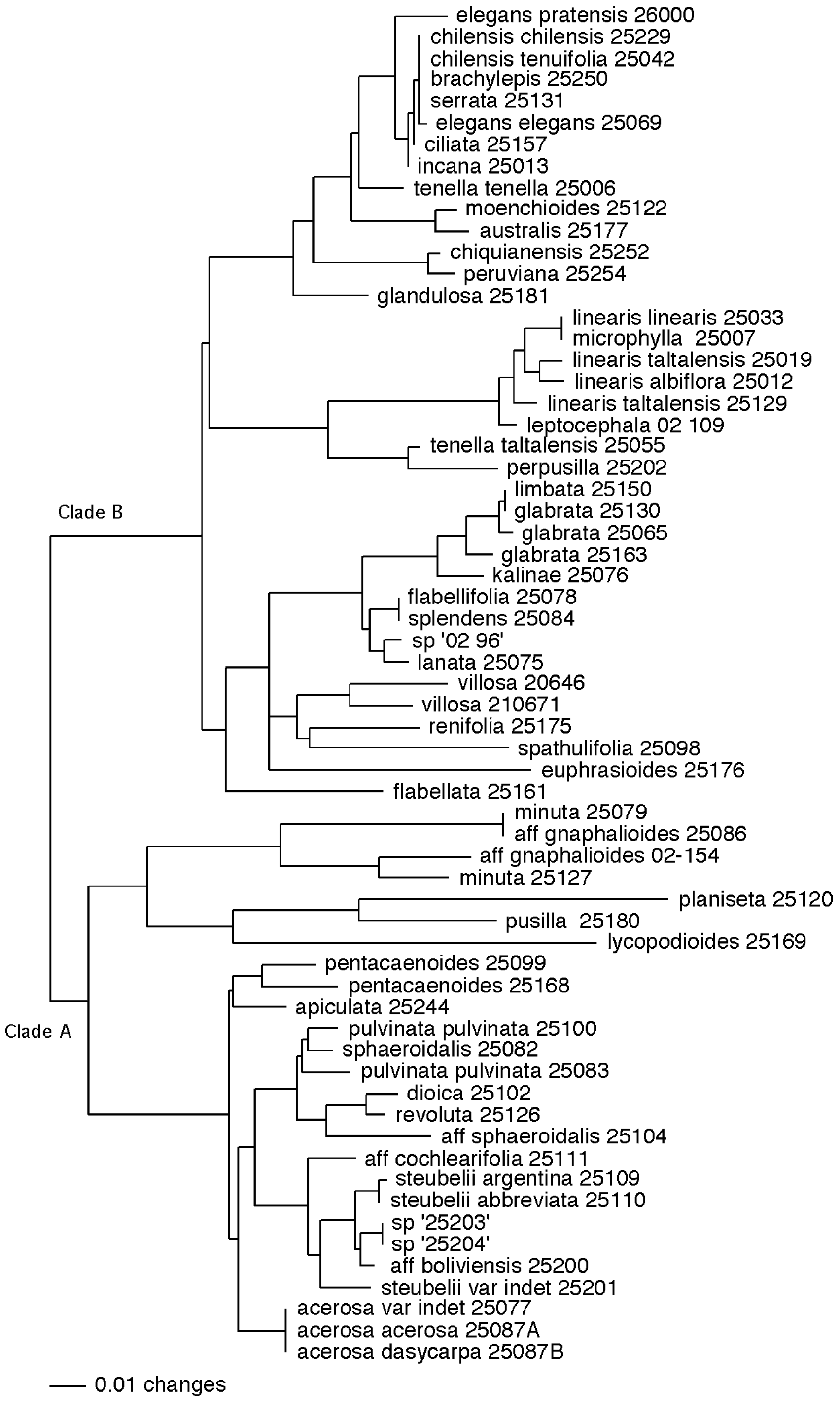

Fig. 4. Maximum likelihood phylogram of *Chaetanthera* ITS sequences. One of two topologies of equal likelihood. Numbers next to taxon names correspond to collection numbers, cf. Table 1.

problem unresolved, however, because it does not affect our general conclusions.

There appears to be some discrepancy between the *ndh*F (Figs. 1 and 2) and ITS data (Figs. 3–5) concerning the relations among *Chaetanthera* species. In particular, the former shows strong bootstrap support for a clade comprising *C. pusilla* and *C. flabellifolia* relative to *C. acerosa*. The ITS bootstrap (Fig. 3) and our cpDNA *rpl*32-*trn*L intergenic spacer sequences (data not shown) support the latter two as closer relatives. This makes better sense considering the respective subgeneric placements of the three species. *Chaetanthera pusilla* and *C. acerosa* belongs to Clade A without any doubt, whereas *C. flabellifolia* belongs to Clade B. Assuming that the specimens are correctly determined in both cases, the source of the discrepancy is not clear. It is possible that poor sampling for *ndh*F yields a branch attraction artifact, or it is possible that the ITS tree is misrooted. If indeed the relations shown in the *ndh*F tree are

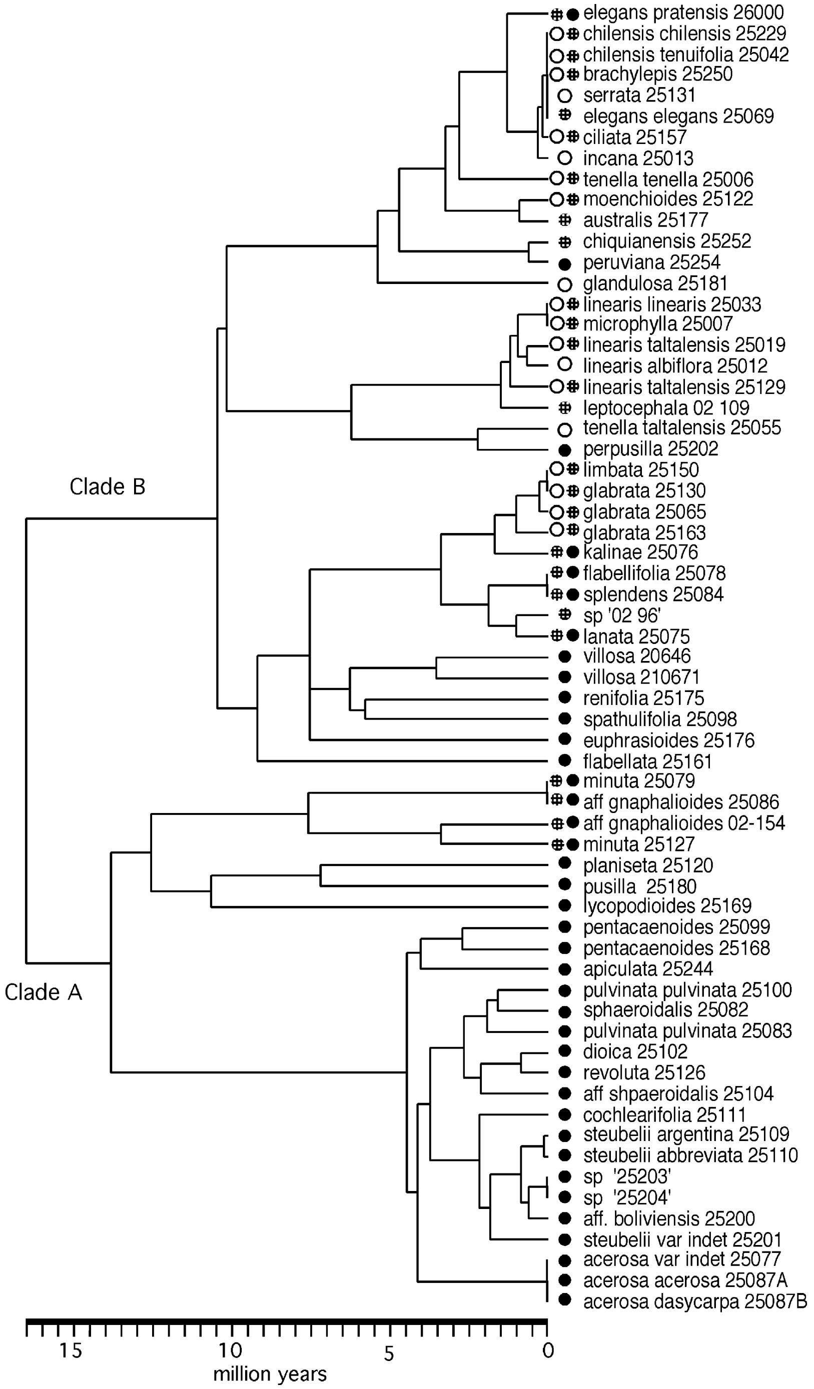

Fig. 5. Penalized likelihood estimate of divergence dates of *Chaetanthera* based on ITS sequences. Numbers next to taxon names correspond to collection numbers, cf. Table 1. Altitudinal ranges are indicated as follows: white dots, low elevation; hatched dots, mid-elevation; black dots, high elevation.

correct, the divergence date of *Egania* would increase to 16.5 my.

The ultrametric tree with estimated divergence dates indicate that the high elevation subgenus *Egania*, centered in the central Andes and northern part of the southern Andes, diverged from the remainder of *Chaetanthera* at least 13.5 my ago, at which time the Andes had uplifted to perhaps half of their current height (Gregory-Wodzicki, 2000). However, Fig. 5 illustrates that the diversification of modern taxa of *Egania* occurred during the past 5 my, in agreement with the age of the highest modern surfaces of the Andes. This pattern might have been produced by high extinction rates between 13.5 and 5 my ago of lower-altitude adapted plants in this lineage. Alternatively, this lineage may have been represented by a single species prior to a Pliocene radiation. This situation contrasts with that of subgenus *Oriastrum*, for which the lineages giving rise to the modern high-elevation taxa diversified prior to the Pliocene.

The apparently early divergence of the lineages giving rise to *Egania* and *Oriastrum* begs the question as to what habitat the ancestor could have occupied, considering that the modern habitats of these taxa did not exist at that time. For that matter, what was the habitat of the ancestor of *Chaetanthera* and what circumstances account for the initial split between the *Egania* plus *Oriastrum* and the remainder of the genus?

Existing geological evidence seems to rule out the possibility that the ancestors referred to above were already adapted to the high elevation environment. Although the sampling for Mutisieae remains sparse, there is no indication that *Chaetanthera* is closely related to any of the other genera with high elevation species, e.g. *Nassauvia*, *Perezia*, or *Leucheria*. As a whole, and notwithstanding the high incidence in the Andes, the tribe is considered to be pantropical and low elevation (Bremer, 1994). That the genus did not migrate from high elevation habitats in the northern Andes is ruled out by the apparently younger age of the northern relative to the central and southern Andes (Gregory-Wodzicki, 2000). The distribution of Mutisieae also seems to rule out the possibility that *Chaetanthera* migrated from a high elevation zone outside of South America.

A more plausible explanation for apparent relictuality of *Egania* and *Oriastrum* is the increasing aridity from coastal Peru through the Atacama region to north-central Chile during the Pliocene (Hartley and Chong, 2002). Existing evidence suggests that aridity emerged in the Atacama region as early as the Jurassic, making it perhaps the world's oldest desert (Hartley et al., 2005). However, hyperaridity did not develop until the late Pliocene. Thus, the Atacama region could have supported the existence of the *Chaetanthera* lineages prior to the Pliocene. These ancestors apparently escaped hyperaridity by elevating their habitat as the Andean uplift gradually proceeded. According to Kœrner (1999), higher elevation habitats are less water stressed than lower elevation habitats, in part because of lower evapotranspiration resulting from a shorter growing season and colder temperatures. One consequence is that soil moisture, especially deeper in the profile, remains higher than at low elevation locations with comparable precipitation. In the case of the central and southern Andes to around 38 °S, precipitation is always higher at the highest elevations. This is the case for moisture derived from both the westerlies in central and southern Chile and the "invierno boliviano" easterlies that characterize the Andes of northern Chile, Bolivia, and Peru (Schwerdtfeger, 1976). Moreover, precipitation at high elevations is largely in the form of snow, which allows release of available water for an extended period. The principal innovation required for the evolution of the high elevation *Chaetanthera* species would be not for drought, but for cold tolerance (Kœrner, 1999). A variety of morphological and physiological mechanisms could result in this tolerance, among these the formation clusters of subterranean perennating buds and extensive fine roots (cf. Kœrner, 1999), as seen in all species of *Egania*. This lineage subsequently diversified to occupy the high elevation zones receiving both winter precipitation (central Chile) and summer precipitation (the altiplano). Mechanisms of cold tolerance in the annual species of *Oriastrum* are less clear. However, the arid Andes in Chile stand out for their large number of annual species derived from many different genera, with some (e.g. *Chaetanthera pusilla*) reaching the upper limits of the vegetation. The long, sunny growing season in the arid Andes of central Chile does not exclude annuals from successfully completing their life cycle (Arroyo et al., 1981). In any case, physiological mechanisms for high elevation cold tolerance in *Chaetanthera* have not been examined.

Of equal importance is the biogeographic explanation for the divergence pattern of Clade B. These taxa are more decidedly central Chilean compared to *Egania*, with the exception of two closely related annual species (*C. chiquianensis*, *C. peruviana*) that occur in Peru and one (*C. perpusilla*) that occurs in Bolivia and Chile. The ancestor of this group is dated at ca. 11 my, which is still older than the high elevation habitats that some of the species occupy today, e.g. *C. flabellata*, *C. euphrasioides*, *C. spathulifolia and C. villosa* are high elevation species. Thus, it seems that a mechanism similar to that proposed for *Egania* and *Oriastrum* is again required to explain the age of the high elevation lineages, i.e. late Miocene aridity in central Chile driving some plants upwards. Geological evidence shows that between the latitudes 33°S and 35 °S, processes of Andes deformation began between 15 and 16 my, followed by uplift beginning at ca. 9 my (Irigoyen et al., 2000; Giambiagi, 2003; Farías et al., 2005). Uplift would have affected the circulation of humid air masses, coming from the Atlantic and the Pacific. This would have resulted in a major reduction in summer rain on the Chilean side of the Andes, along with a reduction in winter rain on the Argentinian side. Consequently, a subtropical xeric flora developed on opposite sides of the Andes (Hinojosa and Villagrán, 1997; Hinojosa, 2005; Villagrán and Hinojosa, 2005), the precursor to the present mediterranean matorral on the western side of the Andes and the Monte on the eastern side. The change from C3 to C4 grass domination that occurred between 7 and 4 my (Latorre et al., 1997, cf. Hartley and Chong, 2002) would be indirect evidence for the establishment of arid conditions at the end of the Miocene and beginning of the Pliocene as a result of the rain shadow produced by the Andes.

The only clear exceptions to the biogeographic scenario proposed above are the high elevation species *C. peruviana*, and *C. perpusilla*, the first of which is closely related and probably recently diverged from *C. chiquianensis*, a lower elevation species, and is nested in a predominantly mid- to low elevation clade. Although Table 1 shows the elevation of the collection of *C. peruviana* as only slightly higher than that of *C. chiquianensis*, the latter was collected further north. As we have noted, the limit of southern hemisphere alpine vegetation increases with decreasing latitude. In any case, *C. chiquianensis* was collected in a columnar cactus-shrubland vegetation, whereas *C. peruviana* was collected ca. 300 m above the lower limit of the alpine puna vegetation. *Chaetanthera perpusilla* is a high elevation species whose closest relative is the coastal desert species *C. tenella var. taltalensis*, which is found in disjunct populations along the coast, like many other arid mediterranean-type climate species. Because this clade is strongly central Chilean, it seems that long distance dispersal is the most likely explanation for the occurrence of these species in high elevation habitats. This seems all the more likely given the relatively easy dispersal of the hairy achenes of *Chaetanthera* and in view of apparently frequent longer-distance amphitropical dispersal of taxa with no evident dispersal mechanisms, e.g., Portulacaceae (Hershkovitz and Zimmer, 2000). In any case, *C. peruviana* and *C. perpusilla*, both small annuals, are notable in that they are some of the few species of *Chaetanthera* whose phylogenetic relations correspond to the conventional wisdom that the younger high elevation habitat is occupied by a species derived from a lower elevation older habitat.

Another striking aspect of Fig. 5 is that the generally lowest elevation species of *Chaetanthera* of Clade B appear to be the most evolutionarily derived, contrary to intuition. The above historical biogeographic scenario may provide some insight as to why this is so. The lower elevation taxa would have required a secondarily evolved tolerance to the increased aridity developing on the western slope of the Andes that became intense from the Pliocene onwards, giving rise to open mediterranean-type climate shrublands, which at the same time provided new niches for the establishment of an herbaceous flora. It is noteworthy in this regard that the low-mid elevation species classified by Cabrera (1937) in subgenus *Proselia* found in south-central Chile often occupy open sites in volcanic soils. The annual species classified by Cabrera in *Tylloma* and *Euchaetanthera* generally occur in washes, gravelly slopes and flats, and in well-drained open spaces between woody matorral shrubs, all of which become exceedingly dry in summer.

The degree to which the evolution of high-elevation *Chaetanthera* proves to be the exception or the rule among other Andean taxa remains to be determined, Apparent relictuality of high elevation lineages appears to be the case in Portulacaceae and *Tropaeolum* sect. *Chilensia*. In the former, divergence of western American Portulacaceae genera appears to have occurred rather abruptly (Hershkovitz, 2006). Among the lineages that diverged are the high elevation or likely ancestrally high elevation taxa *Montiopsis*, *Lenzia*, and *Calandrinia*. Based on minimal ITS divergence of 0.08% (K2P distance) between western American Portulacaceae and the outgroup *Phemeranthus*, and using a substitution rate of 5×10^{-9} substitutions per site per year, an age of 16 my also emerges. In *Tropaeolum* sect. *Chilensia* the high-elevation clade including *T. polyphyllum* diverges immediately following separation of the clade including *T. speciosum* and the Argentinian disjunct, *T. pentaphyllum*, a disjunction probably late Eocene to Oligocene in age (Hershkovitz et al., 2006). ITS sequence divergence of the high-altitude clade from the more derived lowland taxa is on the order of 0.06%, but species of *Tropaeolum* are more longer-lived perennials than most taxa of *Chaetanthera* and Portulacaceae, hence might have a slower rate of ITS divergence.

In addition to the cases above, Fig. 2 also shows relictuality of another high-elevation genus, *Pachylaena*, as well as early divergence of other genera that include high elevation species, e.g. *Perezia* and *Leucheria*. These results cannot be considered as conclusive regarding high-elevation plant divergence time as the results for *Chaetanthera*. In particular, the genera of Mutisieae remain poorly sampled at the molecular level (see Bremer, 1994). Likwise, *Perezia* and *Leucheria* include many low elevation species. Comparison of K.-J. Kim et al. (2005) and the present results for the high elevation genus *Nassauvia* reveals how poor sampling can be misleading. The former suggest a divergence of ca. 30 my for this genus, whereas, with the inclusion of the *Triptilion* sample, the divergence is relatively recent. However, the present sampling cannot discriminate between a recent divergence of the high elevation *Nassauvia* relative to the lowland *Triptilion* versus the reverse scenario.

The above discussion presents a new angle on the infamous "cradle versus museum" controversy regarding angiosperm origins (Stebbins, 1974), viz. whether angiosperms originated in tropical lowland forests where relictual species are overrepresented versus whether they originated in harsher habitats and evolved subsequently into habitats more favorable for luxurient growth. In the present case, the relatively young high altitude habitat appears to be a "museum" for some early derived lineages of *Chaetanthera*. In this particular case, the emerging high elevation habitat was hydrologically more favorable for growth than the increasingly arid lower elevation habitats in which the genus must have originated. However, as Stebbins (1974, p. 14), noted, a given habitat is probably an evolutionary laboratory for some taxa and a museum for others. This appears to be the case, as *C. peruviana* and *C. perpusilla* appear to have become adapted the high elevation habitat more directly and recently.

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