Geography shapes the phylogeny of frailejones (Espeletiinae Cuatrec., Asteraceae): a remarkable example of recent rapid radiation in sky islands

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Background. The páramo ecosystem, located above the timberline in the tropical Andes, has been the setting for some of the most dramatic plant radiations, and it is one of the world's fastest evolving and most diverse high-altitude ecosystems. Today 144+ species of frailejones (subtribe Espeletiinae Cuatrec., Asteraceae) dominate the páramo. Frailejones have intrigued naturalists and botanists, not just for their appealing beauty and impressive morphological diversity, but also for their remarkable adaptations to the extremely harsh environmental conditions of the páramo. Previous attempts to reconstruct the evolutionary history of this group failed to resolve relationships among genera and species, and there is no agreement regarding the classification of the group. Thus, our goal was to reconstruct the phylogeny of the frailejones and to test the influence of the geography on it as a first step to understand the patterns of radiation of these plants. **Methods.** Field expeditions in 70 páramos of Colombia and Venezuela resulted in 555 collected samples from 110 species. Additional material was obtained from herbarium specimens. Sequence data included nrDNA (ITS and ETS) and cpDNA (rpl16), for an aligned total of 2954 bp. Fragment analysis was performed with AFLP data using 28 primer combinations and yielding 1665 fragments. Phylogenies based on sequence data were reconstructed under maximum parsimony, maximum likelihood and Bayesian inference. The AFLP dataset employed minimum evolution analyses. A Monte Carlo permutation test was used to infer the influence of the geography on the phylogeny. **Results.** Phylogenies reconstructed suggest that most genera are paraphyletic, but the phylogenetic signal may be misled by hybridization and incomplete lineage sorting. A tree with all the available molecular data shows two large clades: one of primarily Venezuelan species that includes a few neighboring Colombian species; and a second clade of only Colombian species. Results from the Monte Carlo permutation test suggests a very strong influence of the geography

on the phylogenetic relationships. Venezuelan páramos tend to hold taxa that are more distantly-related to each other than Colombian páramos, where taxa are more closely-related to each other. **Conclusions.** Our data suggest the presence of two independent radiations: one in Venezuela and the other in Colombia. In addition, the current generic classification will need to be deeply revised. Analyses show a strong geographic structure in the phylogeny, with large clades grouped in hotspots of diversity at a regional scale, and in páramo localities at a local scale. Differences in the degrees of relatedness between sympatric species of Venezuelan and Colombian páramos may be explained because of the younger age of the latter páramos, and the lesser time for speciation of Espeletiinae in them.

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10   **ABSTRACT**

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18   of this group failed to resolve relationships among genera and species, and there is no agreement

19   regarding the classification of the group. Thus, our goal was to reconstruct the phylogeny of the

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21   of radiation of these plants.

22   **Methods.** Field expeditions in 70 páramos of Colombia and Venezuela resulted in 555 collected

23   samples from 110 species. Additional material was obtained from herbarium specimens.

24   Sequence data included nrDNA (ITS and ETS) and cpDNA (rpl16), for an aligned total of 2954

25   bp. Fragment analysis was performed with AFLP data using 28 primer combinations and yielding

26   1665 fragments. Phylogenies based on sequence data were reconstructed under maximum

27   parsimony, maximum likelihood and Bayesian inference. The AFLP dataset employed minimum

28   evolution analyses. A Monte Carlo permutation test was used to infer the influence of the

29   geography on the phylogeny.

30   **Results.** Phylogenies reconstructed suggest that most genera are paraphyletic, but the

31   phylogenetic signal may be misled by hybridization and incomplete lineage sorting. A tree with

32   all the available molecular data shows two large clades: one of primarily Venezuelan species that

33   includes a few neighboring Colombian species; and a second clade of only Colombian species.

34   Results from the Monte Carlo permutation test suggests a very strong influence of the geography

35   on the phylogenetic relationships. Venezuelan páramos tend to hold taxa that are more distantly-

36   related to each other than Colombian páramos, where taxa are more closely-related to each other.

37   **Conclusions.** Our data suggest the presence of two independent radiations: one in Venezuela and

38   the other in Colombia. In addition, the current generic classification will need to be deeply

39   revised. Analyses show a strong geographic structure in the phylogeny, with large clades grouped

40   in hotspots of diversity at a regional scale, and in páramo localities at a local scale. Differences in

41   the degrees of relatedness between sympatric species of Venezuelan and Colombian páramos may

42   be explained because of the younger age of the latter páramos, and the lesser time for speciation

43   of Espeletiinae in them.

44   **INTRODUCTION**

45   The mechanisms of evolution and drivers of diversity in tropical ecosystems are not well

46   understood, and most existing studies have focused on lowland taxa and ecosystems. The Andes

47   are one of the most topographically and climatically complex orographic systems (Killeen et al.

48   2007; Särkinen et al. 2012) and they are a renowned hotspot for biodiversity (Brooks et al. 2006;

49   Young et al. 2015). Above the timberline in the tropical Andes, the páramos dominate the

50   landscape. They are considered the world's most diverse high-altitude ecosystem (Luteyn 1999;

51   Rangel-Ch. 2000; Sklenár et al. 2005), and one of the fastest evolving ecosystems (Madriñán et

52   al. 2013). With an estimated age of 2–4 million-years (Hooghiemstra & Van der Hammen 2004),

53   the páramos emerge on top of the Andean cordilleras and massifs, rising in some instances to the

54   snow line above 4700 m. In a few cases, they appear in small humid mountain refugia with

55   poorly drained soils surrounded by high Andean forest. Whether they are isolated by deep

56   Andean valleys or by dense forest, biogeographically páramos function like islands and are often

57   referred to as "sky islands".

58   Cuatrecasas (1934) classified the páramos in three zones based on the type of vegetation

59   and the altitudinal gradient: 1) subpáramos or low páramo, have thickets and shrubby vegetation

60   (~3000–3600 m); 2) páramo proper, are open grasslands of heliophilous plants (~3600–4200 m);

61   and 3) superpáramo, the highest plant-life zone of these neotropical mountains, with soil affected

62   by frequent frost and scarce psychrophilic vegetation (~4200–4800 m). The páramos have been

63   the stage for great diversification of several groups of organisms, including amphibians, mosses,

64   and vascular plants (Diazgranados 2015; Fernández-Alonso 2002; Madriñán et al. 2013; Rangel-

65   Ch. 2000), in a short period of time (less than 4 my BP; Cuatrecasas 1986; Hooghiemstra & Van

66   der Hammen 2004; Madriñán et al. 2013; Van der Hammen & Cleef 1986). Thus, they are an

67   ideal system to understand rapid adaptive radiations and speciation mechanisms in sky islands.

68   Frailejones (the name used in this work to refer to all species within subtribe Espeletiinae

69   Cuatrec. (Asteraceae: Millerieae Lindl.)) are the most representative and iconic plants of the

70   páramo. They are all 144+ species distributed in eight genera (Fig. 1): *Carramboa* Cuatrec. (4

71   spp.), *Coespeletia* Cuatrec. (8), *Espeletia* Mutis ex Humb. & Bonpl. (72), *Espeletiopsis* Cuatrec.

72   (23), *Libanothamnus* Ernst (11), *Paramiflos* Cuatrec. (1), *Ruilopezia* Cuatrec. (24) and *Tamania*

73   Cuatrec. (1) (Diazgranados 2012a; Diazgranados & Morillo 2013; Diazgranados & Sanchez

74   2013). In reality there are many undescribed taxa, for instance the first author is currently

75   describing 14 new species discovered during the course of his field and herbarium work. Despite

76   the interest in the frailejones, there is considerable controversy about the classification within the

77   subtribe, and many of the genera were not resolved as monophyletic in earlier studies (Panero

78   2007; Rauscher 2002; Sklenár et al. 2005). The subtribe has enormous morphological variation

79   (Fig. 1), including numerous synapomorphies: spiral leaf phyllotaxis; obpyramidal to prismatic

80   shape of the glabrous and epappose cypselae; fertile female ray flowers and functionally male

81   disc flowers; pluriseriate involucre and persistent pales of the receptacle; thick and woody stems;

82   xeromorphic structure; specialized life-forms; and a static chromosome number (n=19)

83   (Cuatrecasas 1976; Cuatrecasas 2013; Robinson 1981).

84   Frailejones are widely distributed and abundant in the high Andean forest and páramos of

85   Colombia (88 spp.) and Venezuela (68 spp.); only one species occur in northern Ecuador, with an

86   isolated population in the Sierra de Llanganates (Diazgranados 2012a; Diazgranados 2013).

87   Although there are some species endemic to the high Andean forests, and a few that can succeed

88   at altitudes as low as 1300 m or as high as 4780 m, most of the taxa (104 spp.) are found between

89   3200–3400 m of altitude (Diazgranados 2012a). There are three apparent centers of species

90   diversity: Mérida (with 44 spp.) in Venezuela, Santander–Norte de Santander (41 spp. combined)

91   and Boyacá (45 spp.) in Colombia (Cuatrecasas 1986; Diazgranados 2012a).

92   Local endemism at the specific level is extremely high (ca. 90%), possibly as a result of

93   island-like radiation on a continental scale (Hughes & Eastwood 2006). Most of the species are

94   gregarious and often represent more than 40% of plant cover (Luteyn 1999; Rangel-Ch. 2000).

95   Seeds lack a pappus or other disseminating device and are dispersed mainly by gravity.

96   According to Cuatrecasas (2013), wind or animals may disperse the cypselae short distances but

97   never more than 1–3 m from the parent plant, although light rains and small streams may disperse

98   them longer distances. Bees (principally species of *Bombus*, *Colletes* and *Apis*) are the most

99   frequent pollinators of Espeletiinae and no long-distance pollinators are known (Berry & Calvo

100   1989; Berry & Calvo 1994; Fagua & Gonzalez 2007; Sobrevila 1988). Hence, both pollination

101   and seed dispersal suggest that there is a strong isolation by distance among different páramos,

102   which are normally separated by several kilometers of areas unsuitable for frailejones species.

103   The subtribe has been recently circumscribed within the tribe Millerieae Lindl., as part of

104   the Heliantheae Alliance (Baldwin 2009; Baldwin et al. 2002; Panero 2007). There is no

105   agreement regarding Cuatrecasas' classification within the subtribe. The monophyly of the

106   subtribe was confirmed based on nrDNA ITS with 14 frailejones species and 51 outgroups

107   (Rauscher 2002). According to this work the closest relatives are *Rumfordia* DC., *Ichthyothere*

108   Mart. and *Smallanthus* Mack.

109   Speciation of the group started very recently, most likely during the late Pliocene or early

110   Pleistocene (2–4 my BP) (Cuatrecasas 1986; Hooghiemstra & Van der Hammen 2004; Torres et

111   al. 2013; Van der Hammen & Cleef 1986), and it is likely an ongoing process. Due to this

112   remarkable diversity, which appears to have evolved over a relatively short period of time, the

113   group has been considered a classic example of rapid radiation in the tropics (Cuatrecasas 1986;

114   Monasterio & Sarmiento 1991; Rauscher 2002), although this hypothesis has never been

115   rigorously tested. Expansions (with reconnection) and contractions (with isolation) of the páramo

116   ecosystem during Pleistocene glaciations and inter-glaciations could have played a major role in

117   the radiation and dispersion of these taxa (Cuatrecasas 2013).

118   Two general hypotheses for the radiation of frailejones can be proposed:

119   1) Upward migration → horizontal migration/expansion → speciation

120   Ancestors colonized the high altitudes of the mountains finding available niches and

121   subsequently expanded their distributions occupying different areas of their fundamental

122   ecological niche. In this scenario, distant high-altitude species would be more closely related to

123   each other than to geographically closer lower altitude species and overall morphology would

124   reflect synapomorphies rather than convergence.

125   2) Horizontal migration/expansion → upward migration → speciation

126   Ancestors of the different genera migrated horizontally (i.e. at the same altitude), probably during

127   the Pleistocene glaciations, followed by vertical migrations (upward in altitude) with subsequent

128   allopatric speciation events. In this scenario, phylogenetic clustering (with closely distributed

129   species more related to each other) would be more common; morphology with frequent

130   homoplasy would be the dominant mode of evolution.

131   Of course it is possible that it was not one or the other but rather a combination of the two

132   previous hypotheses. With more than 22–24 known Pleistocene glaciations and inter-glaciations it

133   is plausible that radiation could have followed this pattern, with downward migration,

134   hybridization and introgression during the glaciations, and upward migration and allopatric

135   speciation during the inter-glaciations.

136   A well-resolved phylogeny can provide information to test these hypotheses. Although

137   there have been some previous attempts to reconstruct the evolutionary history of this group

138   based on morphological (Cuatrecasas 1976; Cuatrecasas 2013) and molecular data (Rauscher

139   2002; Rauscher 2000; Sánchez 2005b), relationships among genera and species remain largely

140   unresolved. We present here the most complete phylogeny to date for frailejones and discuss

141   Cuatrecasas' generic classification and the influence of geography in shaping the evolution of this

142   group, in a first attempt to understand the processes underlying this spectacular radiation.

143   MATERIALS & METHODS

144   **Taxon sampling**

145   During major expeditions beginning in 2007, ca. 70 páramo locations of Colombia and Venezuela

146   were visited to photograph, collect and geo-reference frailejones species. A total of 555 samples

147   (MDC 3537–4092) from ca. 110 species were collected (Table 1), following standard protocols

148   for herbarium and molecular analyses (voucher are in ANDES, COL, HECASA and to be

149   distributed (Thiers [continuously updated])). Collections were made under permits No. 2698 of

150   09/23/2009 and No. 2 of 02/03/2010 (Ministerio de Ambiente, Colombia), and IE-126

151   (Venezuela, authorized by Petr Sklenář). Notable collections included 14 new taxa (two already

152   published: *Coespeletia palustris* Diazgr. & Morillo and *Espeletiopsis diazii* Diazgr. &

153   L.R.Sánchez), the first report of the genus *Ruilopezia* for Colombia (with *R. cardonae* (Cuatrec.)

154   Cuatrec.), several new reports for localities, numerous putative hybrids, a few species previously

155   thought to be extinct, and the identification of several critically endangered species

156   (Diazgranados 2012a; Diazgranados 2013; Diazgranados 2015; Diazgranados & Morillo 2013;

157   Diazgranados & Sanchez 2013). Additional material was obtained from specimens at MO, US

158   and F. *Espeletia pycnophylla* Cuatrec., the sole species found in Ecuador, was collected in the

159   south of Colombia near the border with Ecuador, eliminating the need for field work in Ecuador.

160   **DNA purification**

161   DNA extraction from frailejones is particularly complex because of the abundant indumentum of

162   the leaves and the high concentration of terpenes and other metabolites. Extractions from

163   pubescent tissue yield degraded DNA. Adult leaves with old indumentum are sometimes

164   contaminated by fungi. Therefore, young developing leaves from the center of the rosette were

165   use for this purpose, shaving the tissue part of interest. We highly recommend shaving the leaves

166   *in situ*. An initial set of 16 species was used for high quality large scale extractions with the

167   CTAB protocol (Doyle & Doyle 1987) followed by purification via cesium chloride gradients.

168   Subsequently the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) was used,

169   optimizing the Qiagen protocol (2006) to obtain comparable quality. Modifications to the

170   manufacturer's protocol included the following: 1) after shaving and grinding the leaf fragments

171   under liquid nitrogen, ground tissue is incubated for 24 hours with 400 μl of buffer AP1, 4 μl of

172   RNase, 60 μl of 2-β-mercaptoethanol, 60 μl of proteinase-k and 5 μl of sodium dodecyl sulfate

173   (SDS) at 20% (w/v), inverting the tubes a few times during incubation; 2) after addition of buffer

174   AP2, the mix is incubated on ice for one hour; and 3) when indument was not totally removed, a

175   double amount of reagents was used. After checking for DNA quality and protein content through

176   spectrophotometry, extracted DNA was cleaned using the following procedure: 1) incubation for

177   30 minutes at 37ºC with 0.1% (w/v) SDS, 20 μl/ml of proteinase-k; 2) precipitation by incubation

178   for 30 minutes at –20ºC with 5 μl 3M of NaAc and 100 μl of EtOH 95%; 3) centrifugation at

179   14000 rpm for 15 min; 4) removal of the upper phase by inversion and lyophilization; and 5)

180   resuspension with 50 µl of AE buffer. The ingroup included DNA extracted from 240 samples,

181   representing all eight genera and 140 species (including various new taxa being described, and

182   *Tamananthus crinitus* V.M.Badillo); the outgroup comprised 40 samples from 26 species of the

183   following genera: *Ichthyothere, Polymnia, Rumfordia* and *Smallanthus*. Available Genbank

184   sequences (14 ingroup and 51 outgroup sequences for ITS generated by Rauscher; 2002) were

185   compared to the sequences obtained here.

186   **PCR amplifications for sequence data**

187   A combined approach of using DNA sequence data from three lines of evidence (cpDNA, nrDNA

188   and AFLPs) was used in this project. Preliminary screening of chloroplast and nuclear regions

189   was performed with 24 samples from species covering the entire geographic range of the

190   subtribe. Sánchez (2005b) explored 18 chloroplast regions based on Shaw's recommendations

191   (2005; 1998) for 24 frailejones species, but none of the amplified regions was of utility to

192   establish well-supported relationships at generic and species levels. We screened seven additional

193   regions reported as highly variable (Shaw et al. 2005; Shaw et al. 2007; Timme et al. 2007a):

194   rpoB-trnY, ndhC-trnV, trnL-rpl32, rpl16, rps16, ycf6-psbM and trnG. The variable region trnS–

195   trnG has an inversion in Asteraceae that prevents amplification (Jansen & Palmer 1987). Initial

196   screenings showed rpl16 as promising, with 29 haplotypes in 47 sequences. Therefore this region

197   was amplified for phylogenetic reconstructions, using the primers by Small et al. (1998): F71 (5'-

198   GCTATGCTTAGTGTGTGACTCGTTG-3') and R1516 (5'-

199   CCCTTCATTCTTCCTCTATGTTG-3').

200   The nuclear ribosomal internal transcribed spacer (nrITS) has been used widely to

201   reconstruct phylogenies in several groups of Asteraceae (Blöch 2010; Friar et al. 2008;

202   Gruenstaeudl et al. 2009; Keeley et al. 2007; Morgan et al. 2009; Schilling & Panero 2011; Vaezi

203   & Brouillet 2009; Zhang et al. 2011; and others). Rauscher (2000) conducted extensive work in

204   the Espeletiinae with nrITS, using 169 accessions from 15 ingroup and 51 outgroup species. He

205   found a level of variation between 0–4.5%, with no detected intraspecific variation in numerous

206   species. He cloned all accessions and found that 20% of these had only one polymorphic

207   nucleotide position (two haplotypes), while 25% had nucleotide polymorphisms at more sites,

208   suggesting a certain level of within-individual variation. However, he reported that variants

209   typically coalesced within the population and/or species, with no effect on phylogenetic

210   inference; furthermore, several species yielded exactly the same sequences. To build upon

211   Rauscher's work, ITS1–5.8S–ITS2 (ITS, hereafter) was also used, with the universal primers:

212   ITS-1AF (5'-TCCTTCCGCTTATTGATATGC-3') and ITS-4R (5'-

213   GGAAGTAAAAGTCGTAACAAGG-3') (White et al. 1990). We tested for the presence of

214   pseudogenic ITS regions as a consequence of incomplete concerted evolution based on the

215   identification of the conserved 5.8S motifs (CGATGAAGAACGTAGC, GAATTGCAGAATCC

216   and TTTGAACGCA) and a GC% comparison across all the sequences (Harpke & Peterson

217   2008a; Harpke & Peterson 2008b). No pseudogenic copies were found.

218   Additional nuclear DNA regions, including single copy genes, were screened without

219   success, either because of very low variability (e.g. gapC), high complexity and multiple bands

220   (e.g. *leafy*, *pepC*), or unsuccessful amplifications (e.g. *waxy*). However, the external transcribed

221   spacer region (ETS) of the nuclear 18S-26S ribosomal repeat showed fairly good phylogenetic

222   resolution power. Numerous studies in related groups have used ETS for reconstructing

223   phylogenies (Baldwin & Markos 1998; Clevinger & Panero 2000; Ekenäs et al. 2007; Garcia et

224   al. 2011; Masuda et al. 2009; Mavrodiev et al. 2008; Moore et al. 2012; Morgan et al. 2009;

225   Schilling & Panero 2011; Soltis et al. 2008; Timme et al. 2007b; and others; Wahrmund et al.

226   2010). Timme et al. (2007b) reported a large region of one to five subrepeats (each of ~250 bp) in

227   the ETS for *Helianthus* L., with intraspecific variation, evidenced by multiple bands during gel

228   electrophoresis. Although we did not find multiple bands in frailejones, we did find interspecific

229   variation in the number of subrepeats. The length of this region in Espeletiinae is between

230   ~1450–2500 bp. Therefore, in addition to external primers, internal primers were used: ETS1f

231   (5'-CTTTTTGTGCATAATGTATATATAGGGGG-3'), ETS2f (5'-

232   CTGAGCCCCACTTCGGTAGTTTGGC-3'), 11r (5'-CAAACCAAACACCACTCATGCACC-

233   3'), and 18S2l (5'-TGACTACTGGCAGGATCAACCAG-3') (Timme et al. 2007b). Three

234   additional internal primers were developed for this study: ETS3f (5'-

235   GASCTGACGAAGTACCCATGA-3'), ETS4f (5'-CTCAATGGGCCACAACATC-3') and 10r

236   (5'-CGGGTGGCTAATTGTTGG-3').

237   All polymerase chain reactions (PCR) were carried out in a final volume of 25 µl.

238   Reactions were prepared with reagents from the GoTaq DNA polymerase kit (Promega, Madison,

239   WI, USA), with 0.5 µl of template DNA (~10–100 ng), 5 µl of 5X Green GoTaq Reaction Buffer,

240   2.5 µl of MgCl$_2$ (25 Mm), 1.5 µl dNTPs (10 mM), 0.5 µl of each primer (10 mM), 0.25 µl of

241   GoTaq DNA polymerase, 1.5 µl of DMSO and 0.5 µl of bovine serum albumin (BSA; 0.1 µg/µl).

242   Amplifications were performed using Eppendorf (Westbury, New York, USA) Mastercycler

243   gradient ep thermal cyclers. Cycler programs were: for rpl16: denaturation at 94ºC for 2 min, 30

244   cycles of denaturation at 95ºC for 1 min, annealing at 52ºC for 1 min, and extension at 65ºC for 4

245   min, followed by a final extension at 65ºC for 10 min; for ITS: denaturation at 94ºC for 2 min, 35

246   cycles of denaturation at 94ºC for 20 s, annealing at 54ºC for 35 s, and extension at 72ºC for 45 s,

247   followed by a final extension at 72ºC for 7 min; and for ETS: denaturation at 94ºC for 5 min, 30

248   cycles of denaturation at 94ºC for 30 s, annealing at 50ºC for 30 s, and extension at 72ºC for 1.5

249   min, followed by a final extension at 72ºC for 5 min PCR products were checked on 1% agarose

250   gels before being cleaned with the QIAquick PCR purification kit (Qiagen, Valencia, California,

251   USA). PCR products were sent for sequencing to Macrogen Inc. (Seoul, South Korea).

252   Consensus sequences were assembled in Sequencher™ 4.2, and aligned manually using Se-Al

253   v2.0a11 (Rambaut 1996). Concatenation of matrices and final editing of nexus files were

254   conducted in Mesquite 2.75 (Maddison & Maddison 2011). Sequences are available in GenBank,

255   with accession numbers: ETS: KY231675–KY231818, ITS: KY231383–KY231532 and rpl16:

256   KY231533–KY231674. GenBank accession numbers and voucher information per sample are

257   provided in Table S1.

258   **AFLP amplifications and genotyping**

259   In addition to sequence data, amplified fragment-length polymorphisms (AFLPs) were used to

260   infer relationships among the species, and particularly to resolve polytomies or nodes with low or

261   no support by sequence data. AFLPs are variable markers typically used for fingerprinting,

262   estimating relatedness, genetic mapping and more recently for reconstructing phylogenies (Avise

263   2004; McKinnon et al. 2008; Meudt & Clarke 2007; Vos et al. 1995). Numerous phylogenetic

264   studies in recently radiated groups have used AFLPs (Jabaily 2009; Koopman et al. 2008;

265   McKinnon et al. 2008; Schmidt-Lebuhn 2007; Schmidt-Lebuhn et al. 2009; Worley et al. 2009).

266   These markers have also been used successfully in phylogenetic reconstructions in Asteraceae

267   (Koopman 2005; Koopman et al. 2001). Acquisition of AFLP data followed a modified version of

268   the protocol by Trybush et al. (2006). DNA was digested for 1h at 37ºC in 30 μL of total volume

269   with 0.25 μl of EcoRI, 0.5 μl of MseI, 3 μl of 1x NEB buffer 4 (New England Biolabs, Ipswich,

270   MA), 1.5 μg of BSA and 2 μl of template DNA. Each pair of EcoRI and MseI adaptors was

271   annealed after incubation for 10 min at 65ºC, and allowed to cool very slowly for 4 h. Ligation

272   included the 30 μl digestion mix plus a 10 μl mix containing 1.5 μl of T4 DNA ligase (60 U), 1 μl

273   of ATP, 1 μl of 1x NEB buffer 4, 0.5 μl of the annealed EcoRI adaptors, 5.0 μl of the annealed

274   MseI adaptors and 1 μl of ddH$_2$O. The ligation reaction was incubated at 37ºC for 4 h 15 min.

275   The digested-ligated mix was 10-fold diluted. Pre-amplifications were prepared in a total volume

276   of 10 μl, with 2 μl of 5X Green GoTaq Reaction Buffer, 1 μl of MgCl$_2$, 0.25 μl of dNTPs (10

277   mM), 0.05 μl of each pre-selective primer (*Eco*RI primer +A and *Mse*I primer +C), 0.6 μl of

278   DMSO, 0.2 μl of BSA 1X, 2.5 μl of the digested-ligated template DNA, 0.1 μl of GoTaq DNA

279   polymerase and 2,35 μl of ddH$_2$O. Thermocycling was performed with a first step of enzyme

280   denaturation at 65ºC for 5 min, followed by 30 cycles of 30 s at 94ºC, 30 s at 56ºC and 1 min at

281   72ºC. PCR products with pre-amplified DNA were 20-fold diluted. Selective amplifications were

282   performed in 10 μl reactions with the same quantities and reagents as for the pre-selective PCR,

283   except for the addition of 0.07 μl of each selective primer (*Eco*RI primer +3b and *Mse*I primer

284   +3b) and only 1 μl of pre-amplified DNA. Selective reactions included denaturation at 95ºC for

285   15 min, 13 cycles of 30 s at 94ºC, 1 min at 65ºC with a ramp temperature of –0.7ºC/cycle, and 1

286   min at 72ºC; then 25 cycles of 30 s at 94ºC, 30 s at 55ºC and 1 min at 72ºC; and a final extension

287   of 10 min at 72ºC.

288   WellRED D2-, D3- and D4-PA fluorescent primers (Sigma-Aldrich, St. Louis, MO) were

289   used for selective amplifications. In total, 36 primer combinations were tested following

290   recommendations for *Helianthus* (Applied Biosystems 2007). Five combinations failed to

291   amplify (selective 3b for EcoRI-selective 3b for MseI: ACT-CAC, ACC-CAG, ACT-CAG, AAC-

292   CAT and ACT-CAT), and three primer combinations showed only weak amplification (ACG-

293   CAA, ACG-CAC and ACT-CAA). The remaining 28 primer combinations were used for

294   genotyping (Table 2). Results from each step were checked by electrophoresis on 1.5% agarose

295   gels. Each 96-well plate contained 81 samples, of which 12 (~15%) were replicated. In addition,

296   12 samples were repeated between plates and where possible, multiple samples were included per

297   species. Genotyping was perfomed on a Beckman-Coulter CEQ8000 sequencer, multiplexing the

298   reactions with the three WellRED dyes, in a final volume of 10 μl. Scoring was conservative,

299   with only the strongest allele peaks scored. Alleles not consistent between replicated samples or

300   between different runs for the same sample were discarded. Minimum bin (peak) width was set to

301   1 base (b). Bins of < 60 b were eliminated. To determine minimum fragment size and minimum

302   intensity threshold, multiple partitions were tested by measuring homoplasy and other tree

303   descriptors (see Table 3). Since a preponderance of phylogenetic signal was found in sizes

304   between 60 and 100 b, the minimum size of 60 b was retained. The minimum intensity threshold

305   with phylogenetic signal was established at 1000 relative fluorescent units (RFU). From an initial

306   total of 5551 fragments, only 1665 were retained. Samples with poor amplifications were

307   discarded. In total, 118 ingroup and 20 outgroup taxa were genotyped.

308   **Phylogenetic reconstruction**

309   Phylogenies based on sequence data were reconstructed under maximum parsimony (MP),

310   maximum likelihood (ML) and Bayesian inference (BI). The AFLP dataset employed minimum

311   evolution (ME) analyses. Trees were rooted with *Smallanthus*, *Ichthyothere* and *Rumfordia*

312   species, based on Rauscher (2002). MP analyses were performed in PAUP* version 4.0b10

313   (Swofford 2002). For all analyses, gaps were treated as missing data and ambiguous positions

314   were excluded. Full heuristic searches were configured with MAXTREES set to 100,000, with

315   the tree-bisection-and-reconnection (TBR) branch swapping algorithm, 10 random additions, and

316   holding 1 tree at each step. Non-parametric boostrap searches were estimated with 100,000

317   replicates and MAXTREES set to 1. For ML and BI reconstructions, evolutionary models were

318   selected using the AIC criterion in JModelTest 0.1.1 (Posada 2008), which uses the Phyml

319   algorithm to test 88 models in large phylogenies by maximum likelihood (Guindon S 2003). ML

320   reconstructions were performed in Garli 2.0.1019 (Zwickl 2006) at the the CIPRES Science

321   Gateway V. 3.1 (http://www.phylo.org/sub_sections/portal/). The candidate model of evolution

322   selected via AIC by JModelTest was established for the ML searches without optimization,

323   allowing Garli to infer parameter values for each model. Each data set was analyzed in eight

324   independent runs. Boostrap analyses were performed with 120 replicates and 8 independent runs.

325   The CIPRES portal allows a maximum of 100 replications×runs, therefore each bootstrap

326 analysis was performed in 10 tasks of 12×8. Bayesian inference was obtained in MrBayes v3.1.2

327 implemented also at the CIPRES portal. As with ML analyses, models of evolution were tested

328 for each partition. Settings included 2 runs, 8 independent chains, temperature factor of 0.05,

329 50,000,000 generations sampling every 5,000 trees, and discarding the first 8,000 trees as burn-

330 in. Data sets containing AFLPs were run for 150,000,000 generations with a temperature factor of

331 0.005 and a stopping rule when convergence reached 0.01, sampling every 5,000 trees and

332 discarding the first 17,500 trees. ME analyses were performed in PAUP* with the Nei-Li distance

333 (fragments), a starting tree by neighbor joining, TBR branch swapping and MulTrees on.

334 Bootstrap searches had 10,000 replicates. Partitions (ETS, ITS, rpl16 and AFLPs) were analyzed

335 both separately and combined. One-tailed Shimodaira-Hasegawa (SH) tests (Shimodaira &

336 Hasegawa 1999) were conducted in PAUP* to compare between topologies, and incongruence

337 length difference (ILD) tests (Farris et al. 1995), implemented as partition homogeneity in

338 PAUP*, were used to assess combinability of partitions. The ILD test was run with 100

339 replications and 10 random additions. Bootstrap support values and posterior probabilities were

340 summarized with the function Sumtrees implemented in DendroPy (Sukumaran & Holder 2010).

341 Concatenation of support values was performed with the function Sumlabels in the same

342 program. FigTree v1.3.1 (Rambaut 2009) was used to edit the trees. A Monte Carlo permutation

343 test with 1000 permutations, as implemented in GenGIS (Parks et al. 2009), was used to test for

344 the influence of the geography on the phylogeny. Two additional analyses were run to further

345 verify the resulting topologies: an unrooted phylogenetic network (split network) on the ETS-

346 ITS-rpl16 data set, with the NeighborNet algorithm as implemented in SplitsTree4 (Huson &

347 Bryant 2006); and a haplotype network based on rpl16, with equal weighting on

348 transversions/transitions, the *median-joining* (MJ) network algorithm, and "Connection cost"

349 distance method, built with Network 4.6.1.1. (Fluxus Technology Ltd. 2012). The split network

350 displays simultaneously all the possible networks, without giving any hierarchy. Haplotype

351   networks, despite phylogenetic approaches, use autapomorphic characters (unique mutations) for

352   the calculations.

353   **RESULTS**

354   Field collections included ~75% of the recognized taxa. During the development of this project

355   17 new species were published by other researchers (Díaz-Piedrahita & Rodriguez-Cabeza 2008;

356   Díaz-Piedrahita & Rodriguez-Cabeza 2010; Díaz-Piedrahita & Rodriguez-Cabeza 2011; Díaz-

357   Piedrahita et al. 2006; Diazgranados & Morillo 2013; Diazgranados & Sanchez 2013). Most of

358   these newer taxa remain unsampled in the field by us, and fragments from herbarium specimens

359   could not be amplified due to the preservation techniques used when they were collected. A few

360   species known from only a single collection have not been found in decades (e.g. *Espeletia*

361   *canescens* A.C.Sm., *E. marnixiana* S.Díaz & Pedraza, *E. miradorensis* (Cuatrec.) Cuatrec., *E.*

362   *tapirophila* Cuatrec., *E. tillettii* Cuatrec., *Espeletiopsis trianae* (Cuatrec.) Cuatrec., *Ruilopezia*

363   *usubillagae* Cuatrec., etc.); furthermore, those species were collected in areas that are currently

364   politically unstable or with very difficult access. *Tamananthus crinitus*, included in the subtribe

365   by Panero (2007), is known from only one poorly preserved specimen, glued to the herbarium

366   sheet (US). DNA amplifications for *T. crinitus* were unsuccessful, but amplifications for AFLPs

367   were fruitful. Two recognized hybrids (Diazgranados 2012a) with clean sequences were included

368   in most of the data sets: *Espeletiopsis ×bogotensis* (Cuatrec.) Cuatrec. (= *Espeletiopsis*

369   *corymbosa* Bonpl. × *Espeletia grandiflora* Bonpl.), and *Espeletiopsis ×cristalinensis* (Cuatrec.)

370   Cuatrec.

371   Success in DNA purification varied from taxon to taxon. In general, we found that

372   extractions are particularly difficult from species with leaves that are highly tomentose (e.g.

373   *Espeletia paipana* S.Díaz & Pedraza) or extremely coriaceous (*Espeletiopsis jimenez-quesadae*

374   (Cuatrec.) Cuatrec.). Sequences were not successfully obtained from all samples for all markers.

375   Therefore, combined data sets vary in the number of the species to secure the maximum possible

376   coverage. Statistics for all data sets are shown in Table 3 and sequences used for reconstructions

377   are shown in Table 1S. The chloroplast region showed very little interspecific and intergeneric

378   variation within the subtribe. Only 35 (3.6%) characters were informative, including outgroups.

379   Within the subtribe (after excluding uninformative characters), many species even from different

380   genera share exactly the same sequence in the alignment. Trees from rpl16 (not shown) had little

381   resolution, with only a few clades recovered in the strict consensus tree: one clade including the

382   Venezuelan species and one clade comprising the Colombian species (including *Espeletia*

383   *pycnophylla*, present in both Colombia and Ecuador).

384   Most ITS sequences were clean and easily readable, showing only one band in gels and

385   none or only a few double peaks; these positions were coded using IUPAC notations. Noisy

386   sequences were re-amplified, and subsequently discarded if the problem persisted. For our data

387   sets, including outgroups (13 species), 173 (26.7%) characters were informative; excluding

388   outgroups, only 90 (13.9%) characters were informative. The ingroup for ITS included 119

389   species of frailejones (84.4%). Resolution based on ITS was better than rpl16 but still insufficient

390   to resolve the phylogeny (tree not shown). The subtribe was recovered as monophyletic, in

391   agreement with Rauscher (2002). Clades comprising Colombian and Venezuelan species were

392   also recovered, but with low support. In the ML tree the following genera were recovered in

393   clades with low support: *Carramboa* species, *Libanothamnus* species including *E.*

394   *×cristalinensis*, most of the *Ruilopezia* species including the monotypic *Tamania*, and two clades

395   of Colombian *Espeletiopsis* nested within a big clade of Colombian *Espeletia*.

396   ETS proved to be much more informative than ITS. However, amplification was difficult

397   due to the internal repeats. Outgroups have a very large indel (~700 bp) of repeats in the 5' end.

398   In *Carramboa* species, this indel is reduced to ~560 bp, whereas in most of the remaining

399   Venezuelan species (except for the *Libanothamnus* taxa) the indel is interrupted by four or five

400   conserved regions of ~28 bp, so that the large indel is replaced by 5-6 smaller indels of ~84 bp.

401   These indels disappear in most of the Colombian species and *Libanothamnus*. A second section

402   (~300 bp) downstream with two additional large indels extends from there. This second section is

403   very difficult to amplify due to the previous repeats. Therefore, this entire section was excluded

404   from the analyses. Sequences in the 3' end are, by contrast, highly conserved. The complete ETS

405   region varies from ~1450–2500 bp. The final alignment did not include the far 3' end because of

406   low variability. Instead, the region between primers ETS1f and 11r (including internal primers)

407   was used (1324 characters in alignment). Coverage included 151 sequences comprising 119

408   species. As with ITS, samples with multiple bands or noisy sequences were discarded. Contig

409   assemblage included as many as 12 single stranded sequences, and samples were often re-

410   amplified to verify sequences. Bootstrap analyses for the ML tree for ETS produced 64 nodes

411   with support >60%, and 25 nodes with support >90%.

412   The best ME tree for the AFLP data had a score of 3.39528, and the length of the shortest

413   ME tree was 10,490 steps. The data set showed a very large amount of homoplasy (HI: 0.841),

414   but phylogenetic signal was evident. A phylogeny based on AFLPs resolves the subtribe as

415   monophyletic, although with support values <50 (Fig. 2). *Tamanathus crinitus*, included as part

416   of the ingroup, is placed within the outgroup, supporting the exclusion of this genus from the

417   subtribe (Diazgranados 2012a). A number of clades have good support; however, there is no

418   support for the backbone of the tree. An unsupported clade of four species, two from Colombia

419   and two from Venezuela, is sister to the rest of the Espeletiinae. This result could be a product of

420   homoplasy or of incomplete amplifications for those samples due to DNA quality. The tree shows

421   two large reciprocally monophyletic clades with distinct geographic structure. One clade contains

422   two smaller clades: one with primarily Venezuelan species and a few neighboring Colombian

423   species; and other containing northern Colombian species (Fig. 2). Within the Venezuelan clade,

424 *Carramboa badilloi* (Cuatrec.) Cuatrec. (three samples of two varieties) appears to be

425 monophyletic. *Carramboa rodriguezii* (Cuatrec.) Cuatrec. falls in a sister clade but with no

426 support. *Carramboa trujillensis* (Cuatrec.) Cuatrec. is grouped with a sympatric species,

427 *Libanothamnus griffini* (Ruiz-Terán & López-Fig.) Cuatrec. with high posterior probability (PP).

428 *Tamania* appears to be sister to a few *Ruilopezia* species. Most of the *Libanothamnus* species

429 form a clade containing some nodes with fair support. The smaller clade of northern Colombian

430 taxa comprises species restricted to Santander and Norte de Santander, two states bordering

431 Venezuela, with the exception of two species (*Espeletia frontinoensis* Cuatrec. and *E.*

432 *praefrontina* Cuatrec.) from the extreme northwest of Colombia. The second large clade within

433 the subtribe contains only Colombian species, from Boyacá down to the limits with Ecuador.

434 Most of the species within this clade belong to *Espeletia*, except for seven species of

435 *Espeletiopsis* and the monotypic *Paramiflos*. Some species (e.g. *Espeletia lopezii* Cuatrec., *E.*

436 *argentea* Bonpl., *Espeletiopsis pleiochasia* (Cuatrec.) Cuatrec.) show strong support for their

437 monophyly.

438   The partition homogeneity test (ILD test) suggested some incongruence between the

439 nuclear ribosomal regions (p=0.01), and between nuclear ribosomal and chloroplast regions

440 (p=0.14). However, topologically these trees are almost identical, with only a few conflicting

441 positions (Fig. 3 and 4). In both trees combining sequence data, the monophyly of the subtribe is

442 strongly supported with a clear geographic structure, in general agreement with the phylogeny

443 based on AFLPs. The tree based on the three regions (ETS-ITS-rpl16) showed increased support

444 for most nodes, and more resolution than the trees based solely on nrDNA data or on individual

445 partitions. In this tree, numerous clades are well supported. *Coespeletia* species (except for *C.*

446 *moritziana* (Sch.Bip. ex Wedd.) Cuatrec.) fall in the same clade along with the sympatric

447 *Espeletia semiglobulata* Cuatrec. and *E. cuniculorum* Cuatrec., all from the superpáramos of

448 Mérida (Venezuela). The tree recovers a clade containing *Espeletiopsis pannosa* (Standl.)

449   Cuatrec. and *E. angustifolia* (Cuatrec.) Cuatrec., two Venezuelan species with sericeous silvery

450   indumentum and white-purplish flowers, and *Ruilopezia floccose* (Standl.) Cuatrec., another

451   species with silvery indumentum. Three similar *Ruilopezia* species (*R. marcescens* (S.F.Blake)

452   Cuatrec., *R. lindenii* (Sch.Bip. ex Wedd.) Cuatrec. and *R. leucactina* (Cuatrec.) Cuatrec.) are

453   grouped in a clade separated from the rest of the species of the genus. *Espeletia schultzii* Wedd.

454   forms a well-supported clade with *E. aristeguietana* Cuatrec. and *E. jajoensis* Aristeg. Another

455   clade of very similar species is recovered: *Espeletia ulotricha* Cuatrec., *E. nana* Cuatrec. and *E.*

456   *marthae* Cuatrec. The latter species are all very small plants from rocky páramos, with

457   simplification of synflorescence, small leaves and rectangular sheaths. Most of the *Ruilopezia*

458   species form a clade, with *Tamania* nested within. The monophyly of *Libanothamnus* is

459   supported, although the clade contains *R. cardonae*, the southernmost species of the genus, and

460   *Espeletiopsis ×cristalinensis*. The Colombian clade is only well-supported by posterior

461   probability. A clade with most of the species of *Espeletiopsis* is recovered. *Paramiflos* is nested

462   within a small *Espeletiopsis* clade. Several small clades geographically meaningful are also

463   supported.

464   A tree reconstructed with all the available molecular data (sequence data and AFLPs)

465   loses some resolution, and some nodes with very low support collapse (Fig. 5). However, the

466   base of the Espeletiinae clade is more resolved, showing two large clades: one of primarily

467   Venezuelan species that includes a few neighboring Colombian species; and a second clade of

468   only Colombian species, depicting a shallower resolution. Despite some loss of resolution, the

469   geographic structure is still obvious even in the Colombian clade. Furthermore, the topology is

470   congruent with trees generated only with sequence data, and all the small clades described

471   previously are retained. Both the split network based on ITS-ETS-rpl16 (Fig. S1) and the

472   haplotype network with rpl16 (Fig. S2) show this striking geographic structure. It is particularly

473   interesting to note in the haplotype network that one cluster of haplotypes show only Colombian

474   taxa, while the other cluster includes all the Venezuelan species, a few Colombian species and

475   some haplotypes shared by taxa from the two countries. Lastly, a Monte Carlo permutation test

476   performed with the ML reconstruction suggests a very strong influence of the geography on the

477   phylogenetic relationships (p=0.00, Fig. 6).

478   **DISCUSSION**

479   **Molecular markers**

480   Evolutionary relationships within numerous recent rapid radiations in animals and plants are still

481   obscure because of the low phylogenetic signal of conventional markers. An exhaustive screening

482   of 25 chloroplast regions for frailejones has been carried out in this work and by Sánchez

483   (2005a), with unsatisfactory results. The only chloroplast region to show usable variation was

484   rpl16 and therefore it was selected to investigate sequence variability throughout the entire

485   subtribe. With a length of 962 bases when aligned, only 3.5% were informative characters, which

486   was insufficient to resolve the phylogeny. ITS was more variable, but only large clades were

487   recovered but with low support. ETS turned out to be a much more informative region. According

488   to Baldwin and Markos (1998), ETS is part of the same transcription unit as the ITS region and

489   consequently should not be regarded as an independent line of phylogenetic evidence for

490   comparison with ITS results. These authors affirmed that ETS can fulfill the need for additional

491   nucleotide characters to augment the phylogenetic signal of ITS in young angiosperm clades.

492   The partition homogeneity test (ILD test) suggested some levels of incongruence between

493   these ITS and ETS, and between the nuclear ribosomal and chloroplast markers used (p=0.01 and

494   0.14, respectively). Results from this test, however, can be affected by the disparity in levels of

495   homoplasy between the data sets, as reported by Dolphin et al. (2000). Numerous papers have

496   discussed the limitations of the ILD to measure incongruence among data partitions (Barker &

497   Lutzoni 2002; Dowton & Austin 2002; Planet 2006; Quicke et al. 2007). Imbalance between

498   partitions is evident in this case (Table 3). Excessive type I error for the ILD test as a measure of

499   combinability has been reported and a critical value of 0.001 has been proposed (Cunningham

500   1997). Therefore, the ILD test should not be used as a "hard" method to decide about

501   combinability, but as an approach to explore congruence. On the other hand, the utility of

502   topological tests is questionable when trees from some partitions recover only a few clades with

503   low support and no support whatever for the remaining groups (e.g. rpl16).

504   The tree based on all available molecular evidence preserves the geographic structure for

505   the most part, retaining nodes with high support. Moreover, it resolves a split at the base of the

506   subtribe, showing two clear clades containing Venezuelan and Colombian species. Both the split

507   network (Fig. S1) and the haplotype network (Fig. S2) show similar results. Results of this

508   project indicate that increasing the amount of data can help to increase the phylogenetic signal, at

509   least for shallow phylogenies of rapid radiated groups. Data from the latest high-throughput

510   sequencing technologies should facilitate deeper exploration into the origin and evolution of such

511   groups.

512   **Hybridization and evolution of frailejones**

513   Hybrid speciation and reticulate evolution are common processes in plants, and have been

514   reported widely for Asteraceae (Mallet 2007; Moodya & Rieseberg 2012; Nolte & Tautz 2010). It

515   may take several million years after the split of a species pair before the capacity to hybridize is

516   completely lost (Nolte & Tautz 2010). Thus, species of hybrid origin in young groups may be

517   more common than previously thought. A species of hybrid origin maintains allelic combinations

518   that contribute to the spread and stabilization of the hybrid lineage, and it is generally recognized

519   as a species through time (Mallet 2007). Hybridization does not necessarily involve polyploidy in

520   closely related taxa; there are other mechanisms that facilitate rapid hybrid speciation in

521   sympatric or parapatric species, such as transgressive segregation and ecological hybrid

522   speciation (e.g., sexual selection; (Seehausen 2004).

523   Hybridization is a frequent natural process across the Espeletiinae and it has been reported

524   in a number of sources. Diazgranados (2012a) states that when two or more sympatric or

525   parapatric species of frailejones occur, hybridization usually happens and he has documented this

526   with a listing of the 33 published hybrids. In addition, individuals that hypothetically have

527   introgressed with three species have been documented and numerous hybrid zones have been

528   reported for the páramos of Mérida (Morillo & Briceño 2007; Rauscher 2000). It is also possible

529   that a few putative species with unknown populations are hybrids. Cuatrecasas (2013) recognized

530   the putative hybrid origin of a few taxa, and Rauscher (2002) confirmed eight different natural

531   hybrid crosses involving 12 species. Hybridization has been proposed as an important mechanism

532   in adaptive radiations (Seehausen 2004) and it appears that it is important in the Expeletiinae as

533   well.

534   The rapid radiation of frailejones could be explained by hypotheses of both allopatric

535   speciation and hybrid swarm origin. The high altitudes of the tropical Andes provided multiple

536   underutilized niches (i.e. fitness peaks) for colonization. If a colonizing species contained

537   sufficient variation at functional loci, it could express multiple fitness peaks simultaneously. If

538   only subsets of these peaks were effectively utilized, different populations of functional

539   genotypes could have emerged rapidly by multiple events of ecological speciation (Schluter

540   2000). Glaciations would have increased the probability of secondary contact between related

541   species or divergent populations, generating a 'syngameon' scenario. Under these conditions, if

542   hybrids had no ecological disadvantage and more functional gene combinations than the parental

543   species, niche partitioning could have favored rapid speciation in sympatric species.

544   Interglaciations would later segregate populations, favoring allopatric speciation.

545   Intermediacy or conflicting positions in the phylogenies can be explained by hybridization

546   events or incomplete lineage sorting in recent radiations (Knowles & Chan 2009; Knowles &

547   Carstens 2007). The very large amount of homoplasy found in the AFLPs data (HI=0.841) can be

548   explained as well by the homogenization of hybrid parental genomes at each AFLP locus

549   (Seehausen 2004).

550   **Generic relationships**

551   Most of the genera proposed by Cuatrecasas (1976; 1995) are at least partially supported in all

552   trees. *Carramboa* is monophyletic in most trees (Figs. 3–5) except the AFLP tree (Fig. 2), where

553   *C. rodriguezii* is placed (without support) in a different clade that includes a sympatric species, *R.*

554   *marcescens*. Hybridization occurs frequently between *Carramboa* and other sympatric species

555   (Morillo & Briceño 2007; Rauscher 2000) and it is possible that the very small and patchy

556   populations of this species are hybridizing with *R. marcescens*.

557   The *Carramboa* clade is subtended by *Ruilopezia josephensis* and *Espeletia weddellii*,

558   both placements with no support (Figs. 3–4). The first species is likely a hybrid (Mavárez, com.

559   pers., based on new genomic evidence), with scattered individuals in only one locality of the

560   Páramo de San José, in sympatry with *Carramboa badilloi* and various species of *Ruilopezia*.

561   *Espeletia weddellii* is a species with a somehow variable morphology that hybridizes frequently

562   (e.g. with *E. marthae* or *E. schultzii*). Therefore its conflicting position with lack of support might

563   be explained by the effect of introgression.

564   In the trees based on sequence data (Figs. 3–4) and all the available molecular evidence

565   (AME; Fig. 5), *Libanothamnus* is paraphyletic because it includes *Espeletiopsis ×cristalinensis*

566   (not included in the total evidence analysis) and *Ruilopezia cardonae*. However, the former

567   species is a hybrid between *Libanothamnus neriifolius* and *Espeletia aristeguietana*

568   *(Diazgranados 2012a)*. With respect to the latter, *R. cardonae* is the southernmost of the

569   *Ruilopezia* species, found in an area of less than 1 km² in the Tamá massif. This páramo is

570   separated from the closest páramo in Venezuela by a distance of ca. ~40 km. In between, the

571   Táchira depression forms a deep valley of unsuitable conditions for any Espeletiinae. It is

572   plausible that *Ruilopezia cardonae*, along with two or three *Libanothamnus* species, could have

573   crossed this depression during a cold Pleistocene period. Since then, *R. cardonae* has been

574   evolving isolated from its congeners, and probably hybridizing with *L. tamanus* (Cuatrec.)

575   Cuatrec. Interestingly, both species share some morphological characteristics, such as very

576   similar leaf tomentum and venation. Ongoing or past hybridization between these species can

577   explain the position of *R. cardonae* within the *Libanothamnus* clade.

578   In the AME reconstruction (Fig. 5), three *Coespeletia* species form a clade with *Espeletia*

579   *cuniculorum,* whereas in the nrDNA (Fig. 3) and sequence data (Fig. 4) trees, *E. cuniculorum* and

580   *E. semiglobulata* (not included in Fig. 5) are sister to the *Coespeletia* clade. Both *Espeletia*

581   species grow in sympatry with species of *Coespeletia* and are restricted to the superpáramos over

582   4000 m. *Espeletia cuniculorum* is a rare species, known only from a few collections from the

583   Páramo de los Conejos (Mérida), and it may be a hybrid species. *Espeletia semiglobulata* grows

584   in the same massif, and its epithet refers to the semiglobular capitula, similar to the capitula of all

585   *Coespeletia* species. Furthermore, *E. semiglobulata* shares with *C. moritziana*, *C. spicata* and *C.*

586   *timotensis* characters such as large pendulous capitula, pluri- or multi-(6-7)-seriate short rays, and

587   reduced length of pollen spines. It was originally assigned to *Espeletia* because of its thyrsoid

588   synflorescence, but placed in its own section (sect. Badilloa Cuatrec., in Cuatrecasas 2013); the

589   molecular evidence suggests that the delimitation of *Coespeletia* must be revised.

590   *Coespeletia thyrsiformis* (A.C.Sm.) Cuatrec. is at the base of the clade with two

591   *Coespeletia* species and *E. cuniculorum* in the AME tree (Fig. 5), but the sequence data

592   reconstruction (Fig. 4) shows it in a clade with other four *Ruilopezia* species and the two

593   *Espeletiopsis* with white-purplish flowers and thin leaves. It grows in sympatry with species of

594   *Ruilopezia* (e.g. *R. leucactina*) in the Páramo del Batallón, ca. 100 km southwest from the

595   páramos in Mérida, where the other species of *Coespeletia* are found. While *C. thyrsiformis*

596   grows at ca. 3230 m (2500–3510 m) of altitude, in the páramo proper, the other species of the

597   genus grow at ca. 4000 m., in the superpáramos. Because of its characteristic thyrsoid

598   capitulescence, with polycephalous peduncles becoming monocephalous, Cuatrecasas (1986,

599   2013) suggested that *C. thyrsiformis* could be more related to the ancestor of the genus that was

600   presumably adapted to lower altitudes. Later migration upward with adaptation to extreme cold

601   habitats would have produced the superpáramo species that are known today. However, an

602   alternative explanation is that the species exhibits introgression with the sympatric *Ruilopezia*

603   species, explaining its confusing position in the phylogeny.

604   A fifth species, *Coespeletia moritziana*, is also sympatric with the superpáramo species of

605   Mérida. However, it appears at the base of a larger clade containing five different genera (Figs.

606   3–5). This conflicting position was also reported by Rauscher (2000) for ITS. He suggested two

607   possible explanations: parallel evolution or hybridization. *Coespeletia moritziana* shares

608   numerous morphological and phenological characteristics that place it without doubt in the genus

609   *Coespeletia*. However, it hybridizes frequently, at least with *C. timotensis* and *E. schultzii*, and

610   exhibits a plastic morphology. *Coespeletia moritziana* forms large well-established populations

611   and could be a species of hybrid origin.

612   Sister to the *Coespeletia* clade (minus *C. moritziana*) is a small clade containing

613   *Espeletiopsis angustifolia, E. pannosa* and *Ruilopezia floccosa*. These three species share silvery

614   sericeous pubescence and have common names such as 'frailejón plateado' (i.e. silvery frailejón).

615   There is no doubt about the relatedness of two of the *Espeletiopsis* species, both of which have

616   white-purplish flowers and thin leaves. These two species show no close relationship with other

617   species of *Espeletiopsis*, and a re-classification could be suggested for them. *Ruilopezia floccosa*

618   grows in sympatry with *E. angustifolia*, but no large populations are currently known. It is

619   peculiar that whereas most of the *Ruilopezia* species live in subpáramo areas at the limit of the

620   forest, *R. floccosa* is adapted to open grass páramos. Further analyses are needed to determine the

621   origin of this taxon.

622   The remaining species of *Ruilopezia* are grouped in a clade that includes *Tamania* (Figs.

623   3–5). Both genera have terminal synflorescences. The life form of *Ruilopezia* was defined as a

624   monocarpic caulirosula, i.e. a rosette of imbricated leaves at the end of a straight stem, with a

625   terminal synflorescence (Cuatrecasas 1933; Cuatrecasas 1934; Cuatrecasas 2013). After

626   flowering, the rosette (or the ramet) dies. *Tamania* is a tree with a monopodial trunk but

627   sympodial (pseudodichotomous) branching, and terminal monochasial synflorescences as well.

628   Cuatrecasas (1986) hypothesized a relationship between these two genera. According to him,

629   these two genera and *Libanothamnus*, which also has terminal synflorescences, share a common

630   ancestor. Interestingly, a clade of most of the *Ruilopezia* species + *Tamania* + *Libanothamnus* is

631   well supported in the tree based on total evidence (Fig. 5).

632   Three segregated species of *Ruilopezia* (*R. leucactina*, *R. lindenii* and *R. marcescens*)

633   form a separate well-supported clade (Figs. 3–5). *Ruilopezia lindenii* and *R. marcescens* share

634   numerous synapomorphies: large broad herbaceous sterile phyllaries (≥20 mm long), densely

635   glanduliferous floral tube with sparse hairs, and very long disk corollas (6–8 mm long). The three

636   species share creamy-white (sometimes light greenish) ray flowers.

637   The Colombian clade contains only species of *Espeletia*, *Espeletiopsis* and *Paramiflos*

638   (Fig. 5). *Espeletiopsis diazii*, a new species recently discovered (Diazgranados & Sanchez 2013)

639   is at the base of this clade. This is a unique species, particularly interesting because it shares with

640   some Venezuelan species of *Espeletia* the oblong shape of the sheaths at the base of linear

641   tomentose leaves, among other characteristics. However, the synflorescence is a typical

642   monochasium that places this species under *Espeletiopsis*. Currently found in a remote páramo in

643   Norte de Santander, it may be a descendant of one of the first species that migrated from

644   Venezuela.

645   A clade containing twelve *Espeletiopsis* species suggests the monophyly of the

646   Colombian *Espeletiopsis*. In addition to *E. diazii*, five species (*E. colombiana* (Cuatrec.) Cuatrec.,

647   *E. funckii* (Sch. Bip. ex Wedd.) Cuatrec., *E. guacharaca* (S. Díaz) Cuatrec., *E. petiolata*

648   (Cuatrec.) Cuatrec., and *E. pleiochasia*) and one sample of *E. garciae* (Cuatrec.) Cuatrec. fall

649   outside this clade. The positions of these species, however, are either not supported or represent

650   relationships with sympatric species. Rauscher (2000) found two different ITS haplotypes for *E.*

651   *garciae* and *E. pleiochasia*, suggesting either hybridization or two possible lineages for ITS.

652   Sister to the large clade of Colombian *Espeletiopsis*, is a large clade of Colombian *Espeletia*,

653   although support for this clade is very low. Species within this last clade are grouped mainly by

654   their geographic location.

655   One sample of *Espeletia congestiflora* (Coll. number 3651) seems to be nested with

656   *Espeletiopsis diazii* and related to other *Espeletiopsis* taxa sister to the larger clade containing

657   mainly *Espeletia* species (Figs. 3–4). Diazgranados (2012b) initially treated this collection as a

658   new species to be described (tentatively named *Espeletia multicongestiflora* sp. nov. in his

659   dissertation). However, the species has not been described yet, and can fit in the broad description

660   of *E. congestiflora* sensu lato, which will need to be adjusted. Population of *E. congestiflora* 3651

661   differs mainly from the typical *E. congestiflora* morphology in having capitulescences with 9–12

662   congested capitula, rather than 3–7, and alternate sterile bracts along the scape, which is typical

663   of *Espeletiopsis* and not *Espeletia*. This putative new species has yet to be studied, and for the

664   moment the collection was placed within the conservative definition of *E. congestiflora*.

665 **Evolution and geography**

666 Our results support a recent rapid radiation of the Espeletiinae, based on a shallow phylogeny

667 with respect to other sister species, a recent origin (less than 2-4 my BP), and a great number of

668 species. Frequent hybridization is unmistakable, and most species may exhibit incomplete lineage

669 sorting. Trees based on sequence data clearly support an origin of the subtribe in Venezuela, as

670 hypothesized originally by Cuatrecasas (1986).

671 The apparent mixing of genera in the Venezuelan clade can potentially be explained by a

672 much longer history of introgressive hybridization. The relatively longer branches of the

673 Venezuelan species in comparison with the Colombian species suggest older ages of those taxa.

674 Branch length differences between putatively older Venezuelan and younger Colombian taxa

675 (most pronounced in the combined sequence tree; Fig. 4), could perhaps reflect a progression of

676 species migrations in time and space. Longer branches are more common among the species from

677 the massif of the Venezuelan Mérida páramos, while species at the extremes of the geographic

678 range of the subtribe tend to have shorter branches. Thus, the massif of the Mérida páramos could

679 be hypothesized as a putative center of origin for the subtribe. From there, it followed mainly

680 southward migrations along the Andes cordilleras (Cuatrecasas 1986; Cuatrecasas 2013).

681 The Colombian clade of *Espeletiopsis* (including *Paramiflos*, Fig. 5) shows numerous

682 relationships between sympatric or parapatric species: *E. guacharaca* and *P. glandulosus*

683 (Cuatrec.) Cuatrec. in the páramo de la Rusia (Boyacá); *E. insignis* (Cuatrec.) Cuatrec. and *E.*

684 *sclerophylla* (Cuatrec.) Cuatrec. in the páramo de Almorzadero-Chitagá (Norte de Santander); *E.*

685 *caldasii* (Cuatrec.) Cuatrec. and *E. santanderensis* (A. C. Sm.) Cuatrec. in the páramo de Berlín-

686 Almorzadero (Norte de Santander); *E. corymbosa*, *E. garciae* (col. 3715) and *E. rabanalensis* S.

687 Díaz & Rodr.-Cabeza in the páramos of Cundinamarca-Boyacá; and *E. sanchezii* S. Díaz &

688 Obando and *E. purpurascens* (Cuatrec.) Cuatrec in the páramo complex of Tierranegra–Tamá

689 (Norte de Santander).

690   The Colombian clade of *Espeletia* species in the AME tree (Fig. 5) does not show

691   resolution at the base; however, numerous small clades reflecting geographic distributions are

692   recovered. Colombian species suggest two important centers of radiation for Espeletiinae: the

693   páramos of Santander and Norte de Santander, close to Venezuela; and the páramos of Boyacá,

694   where the Eastern Cordillera reaches its maximum width and topographic complexity. A smaller

695   center of diversification is the complex of páramos in Cundinamarca, around the 'Sabana de

696   Bogotá'. The AME phylogeny suggests that Colombian species of *Espeletia* from these areas are

697   more related to each other, in disregard of their altitude or niche specialization, than with other

698   vicariant species. As an example, *E. lopezii* appears to be related to *E. cleefii* Cuatrec. These are

699   two parapatric species that can be found in the Sierra Nevada del Cocuy (Boyacá–Arauca).

700   *Espeletia lopezii* prefers swampy, very wet meadows, while *E. cleefii* thrives better in scarped

701   ridges of the superpáramo. The former has long robust naked scapes with a simple 3-cephalous

702   cyme, while the latter has scapes with multiple pairs of sterile leaves and 15–27 capitula. There

703   are numerous morphological differences that would classify these two species in totally different

704   groups within *Espeletia*. Similarly, several clades containing morphologically divergent species,

705   found in the same páramo massifs, are recognized as closely related species (i.e. clades for the

706   páramos of Pisba, Frontino, Chingaza-Sumapáz-Tablazo, Tota, Iguaque-La Rusia, Nariño, etc.). It

707   is likely that these clades represent the most recent colonization events that likely occurred after

708   the Last Glacial. Additional glaciations would have enabled secondary crosses between vicariant

709   species, with a diffusion of the geographic structure.

710   Geographically, the phylogeny suggests that the radiation of frailejones is an ongoing and

711   highly dynamic process. None of the proposed two hypotheses describes entirely this radiation,

712   but rather a combination of the two, with numerous horizontal and vertical migrations, isolations

713   and reconnections, and a strong geographic structure. Frequent hybridization is likely prolonging

714   the radiation momentum.

715   Based on morphology and his knowledge of the group, Cuatrecasas (2013) proposed a

716   schematic phylogeny of the subtribe (Fig. 4), in which he highlighted two main clades. One

717   contains *Libanothamnus*, *Ruilopezia* and *Tamania*, the group with terminal capitulescences. Our

718   results (Figs. 3–5) support this hypothesis, with *Tamania* clearly nested with *Ruilopezia*. The

719   other clade proposed by Cuatrecasas (2013) includes the rest of the genera, with *Paramiflos*

720   closely related to *Espeletiopsis*, as well as *Coespeletia* with *Espeletia*, and *Carramboa* as sister of

721   the latter clade. Our phylogenetic reconstructions support the position of *Paramiflos* within

722   *Espeletiopsis* (Figs. 3–5). However, it does not support the relationship of *Carramboa* and

723   *Coespeletia* with the large clade of *Espeletia* (with the Colombian species of the genus).

724   Moreover, the result of two large clades (mostly Venezuelan species and Colombian species)

725   deeply challenges Cuatrecasas' generic classification, with two possible outcomes: a splitting

726   approach or a lumping approach. The former would imply: 1) preserving the monophyletic

727   *Carramboa*; 2) redefining generic limits of *Coespeletia*, likely including *Espeletia semiglobulata*

728   and *E. cuniculorum*; 3) proposing a new generic combination for *Espeletiopsis pannosa* and *E.*

729   *angustifolia*; 4) splitting *Ruilopezia* in two genera, one of them including *Tamania*, and the other

730   one the mainly glandulous *Ruilopezia* species; 5) creating a new genus (or two) for the

731   Venezuelan species of *Espeletia* (considered by Cuatrecasas (2013) as section *Weddellia*

732   Cuatrec.); 6) splitting Colombian *Espeletiopsis* in three genera; and 7) keeping Colombian

733   *Espeletia* species as the proper genus *Espeletia*. The lumping approach would imply creating two

734   groups (or three if keeping the monophyletic *Carramboa*): one with the mainly Venezuelan

735   species, in which case should be named *Libanothamnus*; and one group with the Colombian taxa,

736   named *Espeletia*. In any case, it seems premature to address these profound changes in the

737   classification without further analysis (e.g. genomic data using population sampling) to fully

738   understand conflicting inter-specific relationships.

739   Finally, our conclusions strongly support that relationships between species cannot be

740   established solely in the light of morphology. Behind every species there is an evolutionary

741   hypothesis to test, and frailejones represent a fertile field for studying migration, speciation and

742   hybridization mechanisms.

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1058   **Table 1** Number of species collected and documented during fieldwork for this project. In addition to these
1059   collections, samples from other species were obtained from different sources.

Genus	No. of species	No. collected (%)*	New species**
Carramboa	4	3 (75)	1
Coespeletia	8	6 (86)	1
Espeletia	72	56 (79)	6
Espeletiopsis	23	19 (86)	4
Libanothamnus	11	9 (82)	1
Paramiflos	1	1 (100)	0
Ruilopezia	24	15 (63)	0
Tamania	1	1 (100)	1
TOTAL	**144**	**110 (78)**	**14**

1060   * Putative new species not counted in this column.
1061   ** New species being described

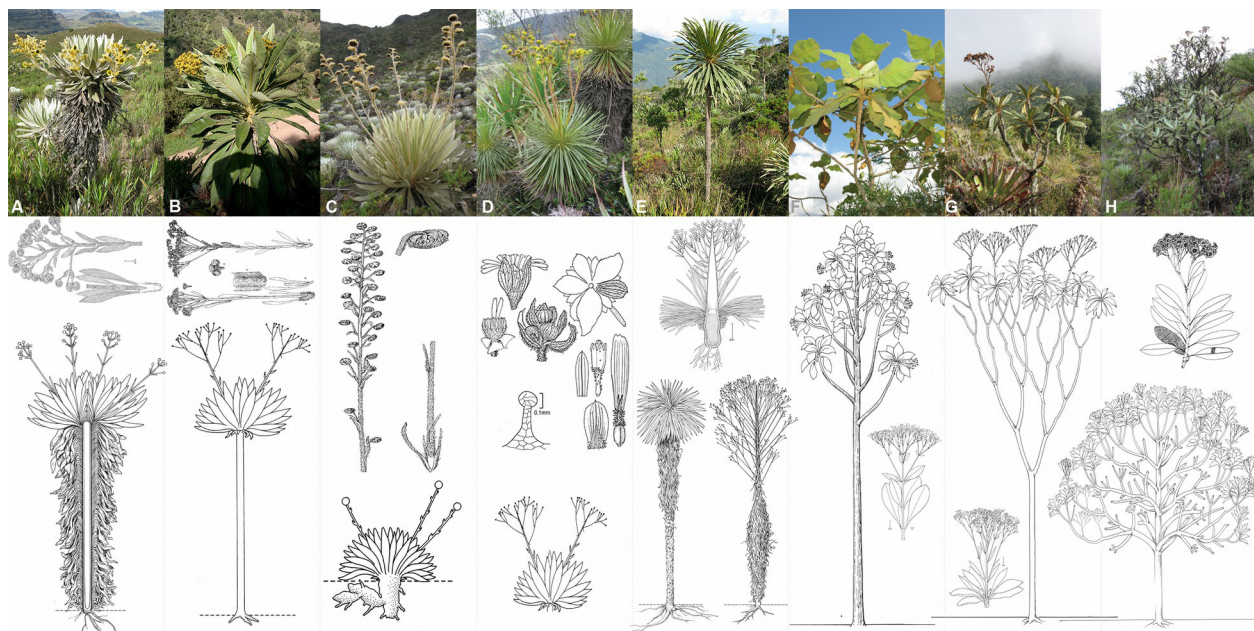
1062 **Table 2** AFLP primer combinations used for genotyping. First triplet for each primer combination corresponds to the
1063 selective anchor for EcoRI; second triplet corresponds to the selective anchor for MseI. Mean Y threshold
1064 corresponds to the intensity of each band (peak height), in relative fluorescence units (RFU).

Primer combination	Number of fragments	Minimum size (b)	Maximum size (b)	Mean size (b)	Variance of size (b²)	Mean Y threshold (RFU)
AAC-CAA	39	61.7	417.5	135.5	0.0	4603.9
AAC-CAC	43	65.9	231.5	130.0	0.1	4296.3
AAC-CAG	41	61.7	348.5	116.2	0.0	9722.0
AAC-CAT	51	59.8	376.6	141.9	0.0	4027.3
AAG-CAA	39	62.5	294.0	144.6	0.0	14775.7
AAG-CAC	56	59.6	417.8	160.3	0.1	9138.6
AAG-CAG	39	59.8	259.3	158.6	0.1	10322.9
AAG-CAT	69	60.0	311.0	128.5	0.1	7942.1
ACA-CAA	47	63.1	307.0	155.7	0.0	8447.5
ACA-CAC	65	64.9	310.4	160.3	0.1	8102.3
ACA-CAG	58	63.8	334.5	159.8	0.1	10157.2
ACA-CAT	62	63.5	351.1	170.6	0.0	4192.8
ACG-CAA	56	66.7	412.5	174.5	0.0	4644.9
ACG-CAC	51	60.4	267.0	139.4	0.0	6245.9
ACG-CAG	58	59.5	307.2	148.4	0.0	6970.9
ACG-CAT	51	64.9	348.0	158.3	0.1	3253.5
ACT-CAA	91	62.6	443.8	169.9	0.0	15551.5
ACT-CAC	59	60.7	383.9	219.0	0.1	8792.9
ACT-CAG	58	65.9	378.5	181.1	0.1	11644.2
ACT-CAT	83	59.0	360.8	201.9	0.1	10579.9
AGC-CAA	54	61.4	318.2	164.8	0.0	3303.6
AGC-CAC	40	59.2	281.8	137.8	0.1	5745.0
AGC-CAG	57	62.9	363.1	143.6	0.0	7534.2
AGC-CAT	40	62.0	320.3	158.4	0.0	3997.4
AGG-CAA	75	58.2	360.5	182.6	0.0	10933.4
AGG-CAC	109	59.6	436.4	185.5	0.2	12275.0
AGG-CAG	86	63.8	381.6	190.8	0.1	16182.5
AGG-CAT	88	59.7	369.7	193.8	0.1	9577.2
Total	**1665**	**58.2**	**443.8**	**165.5**	**0.1**	**8892.5**

1065 **Table 3** Sequence characteristics and tree statistics for all data sets. Tree scores were estimated from the MP
1066 consensus tree.

Statistics	rpl16	ETS	ITS	AFLPs	ETS-ITS	ETS-ITS-rpl16	All combined
Aligned length (characters)	962	1,324	649	1,665	1,973	2,935	4,600
Number of samples	144[1]	150[2]	142[3]	134	149	145	111
Number of species	127	119	130	115	117	116	95
Variable characters	93	560	245	1,665	750	831	2,294
Informative characters	35	368	173	1,583	507	551	1,818
Number of MP trees	100,000	100,000	100,000	20	100,000	100,000	24
Tree length (best MP tree)	84	1,155	527	10,200	1,545	1,760	9,797
CI*	0.464	0.113	0.454	0.159	0.338	0.405	0.200
RI*	0.930	0.061	0.841	0.159	0.747	0.813	0.456
RC*	0.432	0.007	0.381	0.057	0.252	0.329	0.091
HI*	0.536	0.887	0.546	0.841	0.662	0.595	0.800
Model of evolution	TVM+I+G	TrN+G	TIM2+G	Mkv	N/A	N/A	N/A
-ln L	-2288.46	-9409.64	-3944.67	-44743.38	-14066.11	-17054.85	-54659.98

1067 [1] Genbank accession numbers KY231675–KY231818
1068 [2] Genbank accession numbers KY231383–KY231532
1069 [3] Genbank accession numbers KY231533–KY231674

1070 *Statistics based on one of the shortest maximum parsimony trees: CI, consistency index; RI, retention index; RC,
1071 rescaled consistency index; HI: homoplasy index.

1072 **Figure 1** Morphological diversity in the genera of Espeletiinae. (A) *Espeletia* (72 species); (B) *Espeletiopsis* (23);
1073 (C) *Coespeletia* (8); (D) *Paramiflos* (1); (E) *Ruilopezia* (24); (F) *Carramboa* (4); (G) *Tamania* (1); and (H)
1074 *Libanothamnus* (11). Illustrations from Cuatrecasas (2013), made by Alice Tangerini and Florence Lambeth
1075 (Department of Botany, US National Herbarium, Smithsonian Institution); photographs: Diazgranados.

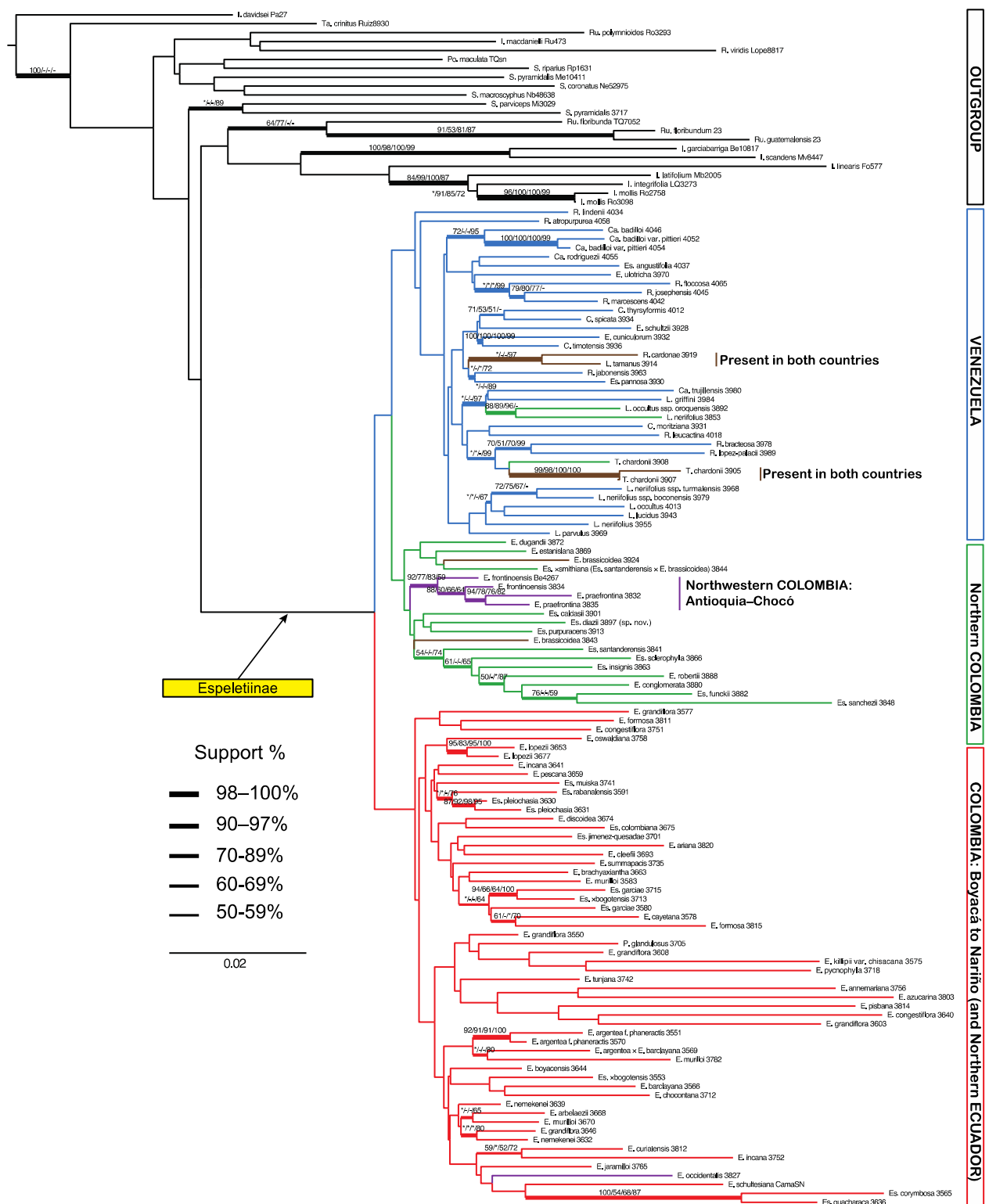



Figure 2 Minimum evolution (ME) tree for AFLPs. Branch support is indicated in the following order: ME bootstrap value/ML bootstrap value/MP bootstrap value/BI posterior probability (×100); *, value below 50; -, missing value. Support values shown only for nodes with at least one support metric ≥ 50. Genera abbreviations: I., *Ichthyothere*; Ru., *Rumfordia*; Po., *Polymnia*; S., *Smallanthus*; Ca., *Carramboa*; C., *Coespeletia*; E., *Espeletia*; Es., *Espeletiopsis*; L. *Libanothamnus*; P., *Paramiflos*; R., *Ruilopezia*; T., *Tamania*; Ta., *Tamananthus*.

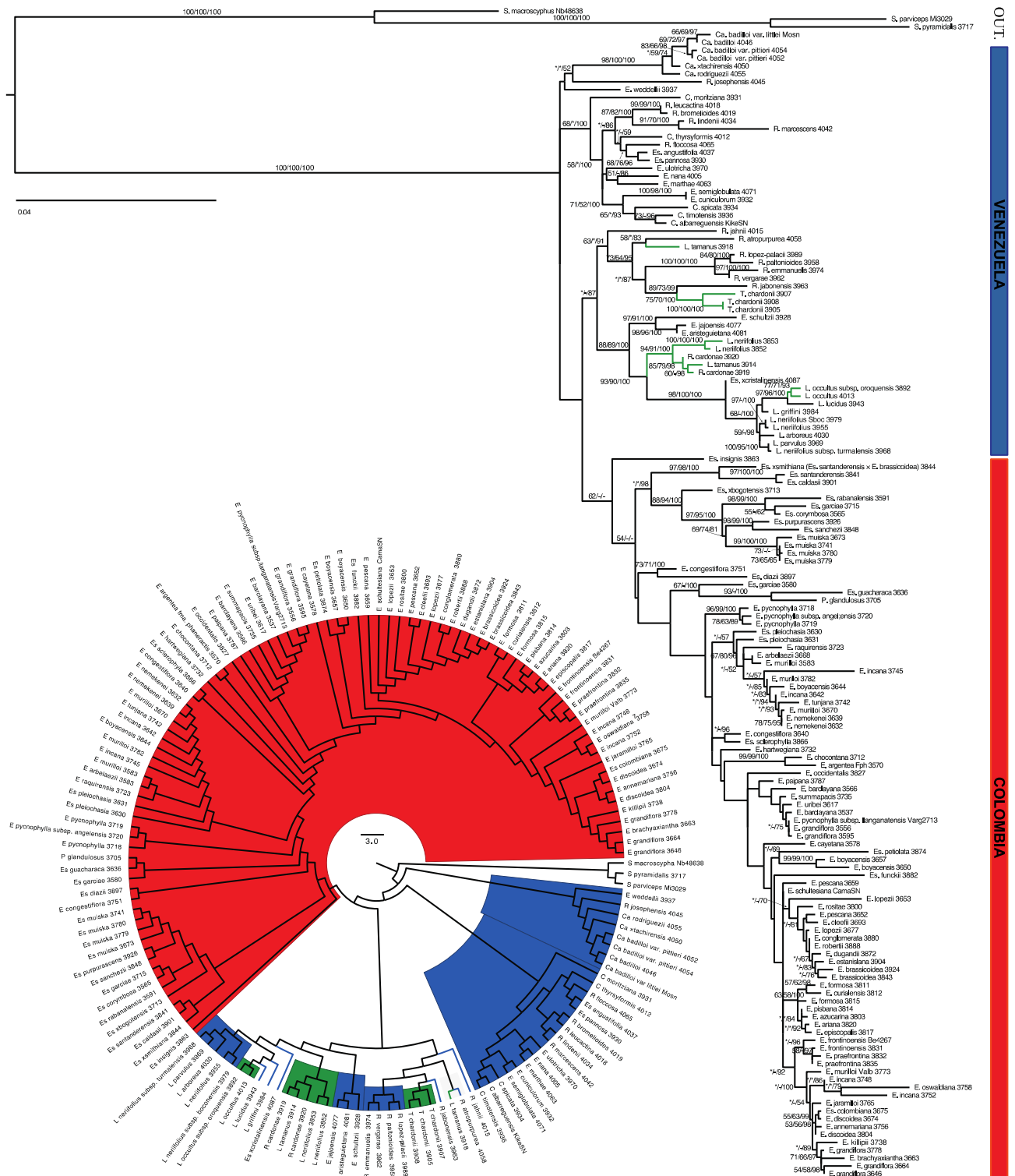



Figure 3 Maximum likelihood tree based on ITS and ETS. Branch support is indicated in the following order: ML bootstrap value/MP bootstrap value/BI posterior probability (×100); *, value below 50; -, missing value. Support values shown only for nodes with at least one support metric ≥ 50. In green: taxa present in the border between the two countries. Genera abbreviations: S., *Smallanthus*; Ca., *Carramboa*; C., *Coespeletia*; E., *Espeletia*; Es., *Espeletiopsis*; L. *Libanothamnus*; P., *Paramiflos*; R., *Ruilopezia*; T., *Tamania*.

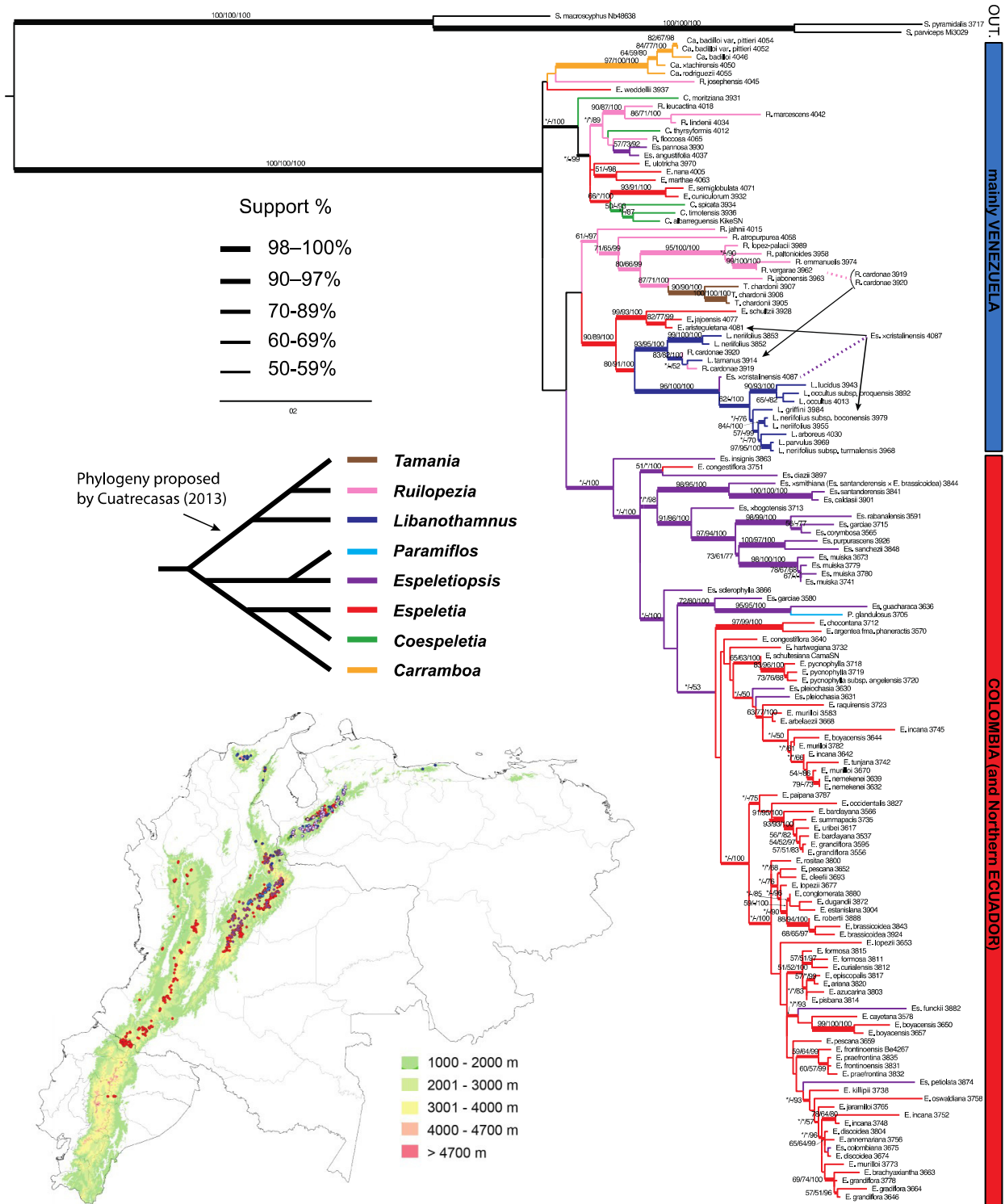



Figure 4 Maximum likelihood tree based on ITS, ETS and rpl16. Branch support is indicated over the branch or close to the angle of the branch in the following order: ML bootstrap value/MP bootstrap value/BI posterior probability (×100); *, value below 50; -, missing value. Support values shown only for nodes with at least one support metric ≥ 50. Genera abbreviations: S., *Smallanthus*; Ca., *Carramboa*; C., *Coespeletia*; E., *Espeletia*; Es., *Espeletiopsis*; L. *Libanothamnus*; P., *Paramiflos*; R., *Ruilopezia*; T., *Tamania*. Schematic phylogeny proposed by Cuatrecasas (2013) displayed with black lines. Geographic distribution of genera (colored dots) shown on map. Background color denotes mountains over 1000 m of altitude.

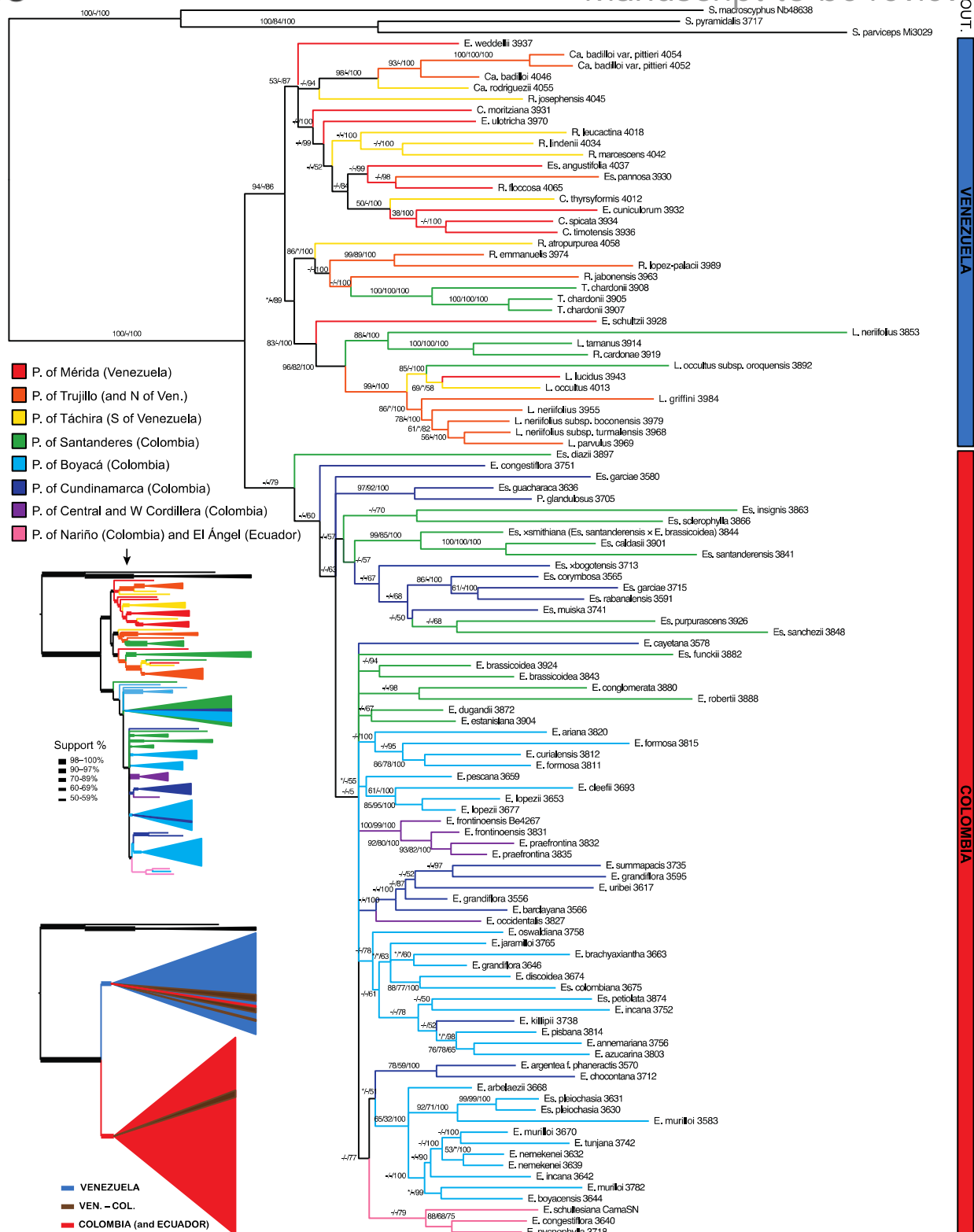



Figure 5 Bayesian tree estimated with all the available molecular evidence (ETS, ITS, rpl16 and AFLPs). Branch support is indicated over the branch or close to the angle of the branch in the following order: ML bootstrap value/MP bootstrap value/BI posterior probability (×100); *, value below 50; -, missing value. Support values shown only for nodes with at least one support metric ≥ 50. Genera abbreviations: S., *Smallanthus*; Ca., *Carramboa*; C., *Coespeletia*; E., *Espeletia*; Es., *Espeletiopsis*; L. *Libanothamnus*; P., *Paramiflos*; R., *Ruilopezia*; T., *Tamania*. Main páramo massifs indicated in colors.

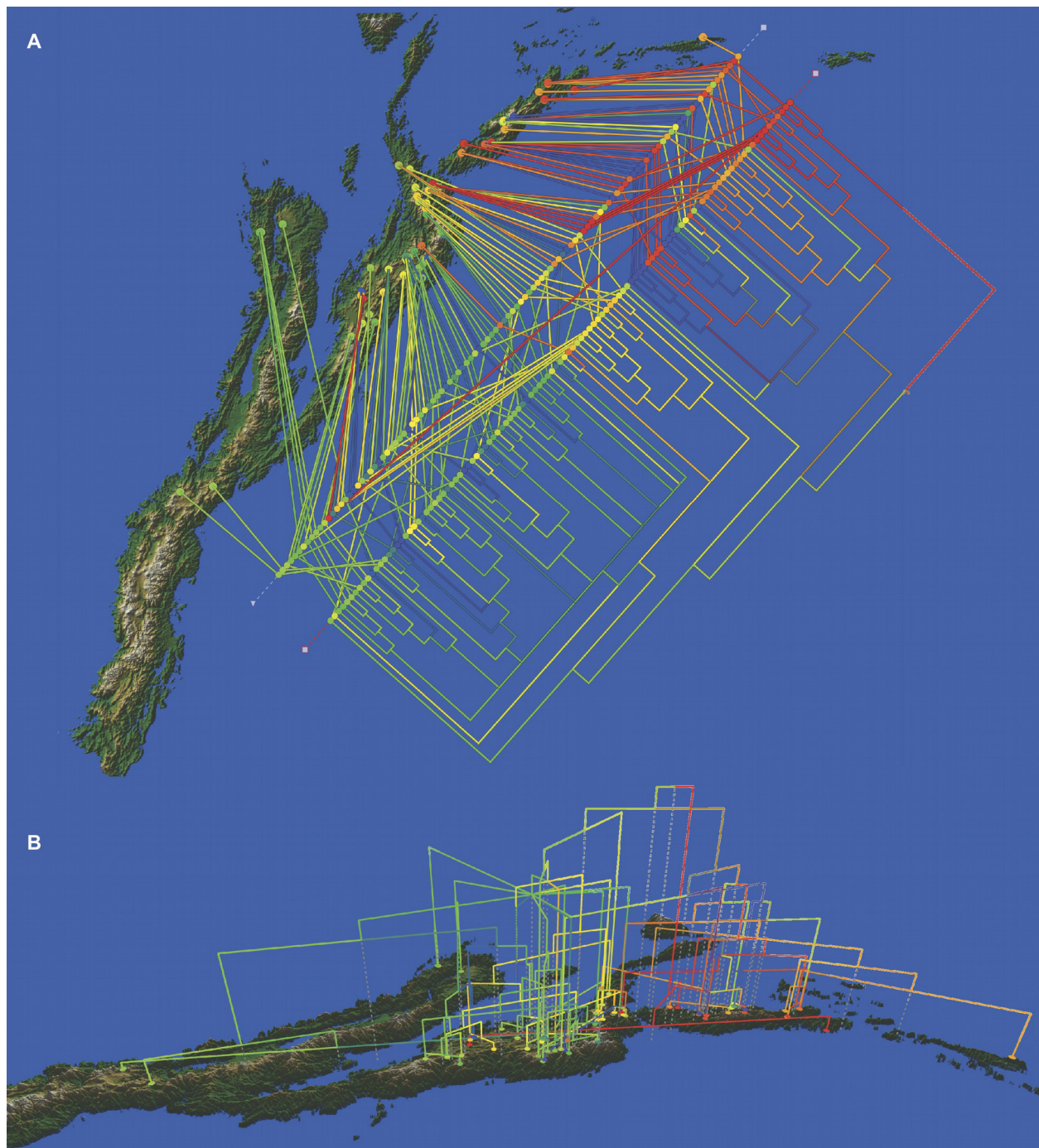



Figure 6 Cladogram of the Bayesian tree based on the available molecular evidence (ETS, ITS, rpl16 and AFLPs), mapped on the geographic range of the subtribe. (A) top view of the cladogram; (B) side view of the cladogram. Additional line of colored dots in the top view shows the samples arranged by geographic proximity.