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Disentangling the *Tillandsia capillaris* complex: phylogenetic relationships and taxon boundaries in Andean populations

LUCÍA V. CASTELLO[1]*, MICHAEL H. J. BARFUSS[2], WALTER TILL[2], LEONARDO GALETTO[1] and JORGE O. CHIAPELLA[1†]

[1]*Instituto Multidisciplinario de Biología Vegetal, CONICET, Universidad Nacional de Córdoba, Vélez Sarsfield 1611-X5016GCA, 5000 Córdoba, Argentina*
[2]*Faculty of Life Sciences, Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, A-1030 Vienna, Austria*
[†]*Current address: Faculty of Life Sciences, Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, A-1030 Vienna, Austria*

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We studied the genetic diversity and structure of the *Tillandsia capillaris* complex, a morphologically diverse group of highly specialized epiphytes, across the distribution range in arid mountain regions of central Peru, Chile, Bolivia and central Argentina. To elucidate the phylogenetic relationships in the complex and to explore the taxon boundaries among populations, we used three plastid markers (*rpoB-trnC-petN*, *trnK-matK-trnK*, *ycf1*, *c.* 8100 bp in total) and one single-copy nuclear gene (*PHYC*, *c.* 1200 bp) for 69 populations and 96 individuals of the *T. capillaris* complex plus 16 outgroup taxa. Bayesian inference of plastid DNA data indicates the existence of two evolutionary core lineages, which can be recognized as two distinct species: *T. capillaris* and *T. virescens* (as proposed previously on the basis of morphological characters). *Tillandsia capillaris* is a monophyletic and homogeneous group, widely distributed and less genetically variable, whereas *T. virescens* (including *T. kuehhasii*) is genetically more divergent with most of the forms growing at high elevation in arid areas, except for the small clade including *T. virescens s.s.* (= *T. cordobensis*), which grows in lower, more humid habitats. The nuclear analysis resulted in a polytomy with some individuals showing incongruent positions between plastid and nuclear topologies. The high haplotype diversity, consisting of 63 plastid DNA haplotypes in 69 populations, was resolved as two core lineages occurring from north to south, allowing us to establish a preliminary view of the genetic variation overlapping between the two taxa. The results suggest that the genetic differentiation into two main clades is consistent with morphological variation in this Andean complex. © 2016 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2016, **181**, 391–414

ADDITIONAL KEYWORDS: Bromeliaceae – genetic diversity – genetic structure – *PHYC* – phylogenetics – *rpoB-trnC-petN* – *trnK-matK-trnK* – *ycf1*.

INTRODUCTION

The Andes: a cradle of plant biodiversity

The Andes, the longest mountain system on Earth, is a magnificent laboratory for the study of biogeographical patterns in plants (Beck *et al.*, 2007) and contains a large number of biodiversity hot spots (Gentry, 1982; Killeen *et al.*, 2007; Pennington *et al.*, 2010; Sánchez-Baracaldo & Thomas, 2014). The Andes hosts many endangered species and forms an essential part of global ecosystems (Beniston, 2003; Diaz, Grosjean & Graumlich, 2003). From the Andes to the Himalayas, mountain areas are experiencing environmental degradation, and the impacts of climatic change are expected to be felt more drastically in arid regions of the continent, which tend to be associated with the rain shadow influence of the Andes (Beniston, 2003). Despite their crucial roles as a water reserve, sediment filter, carbon sink, source

*Corresponding author. E-mail: lvcastello@gmail.com

of energy, minerals, agricultural products and breeding areas for Andean fauna (Diaz *et al.*, 2003; Fagre, Peterson & Hessl, 2003), effective conservation of Andean habitats depends on a knowledge of the genetic structure and biogeography of the native organisms. In this regard, genetic studies, ideally when combined with ecological data, have contributed substantially to the resolution of taxonomic uncertainties and identification of genetic entities and conservation units (Manel *et al.*, 2003; Wee *et al.*, 2015).

Bromeliads in the Andes

Members of Tillandsioideae are predominantly epiphytic, lithophytic and, less often, terrestrial species in the Andes, which is one of the centres of diversity for the subfamily (Barfuss *et al.*, 2005; Givnish *et al.*, 2011, 2014). Species of *Tillandsia* L. are ecologically important epiphytes, retaining water and displaying specialized nutrient uptake, and providing a variety of microhabitats for invertebrates, amphibians and their predators (Benzing, 1990). Some *Tillandsia* spp. have even evolved a highly specialized growth habit, unrooted on sand, in the hyper-arid coastal zones of the Atacama Desert, in Chile and Peru, where they are dominant and provide essential biomass to these ecosystems (Rundel & Dillon, 1998). Nevertheless, the taxonomy of groups including *Tillandsia* is still provisional and requires substantial improvement. The main issues range from the realignment of species in genera to the establishment of additional higher taxa to accommodate separate lineages emerging from the phylogenetic relationships revealed by molecular data (Barfuss *et al.*, 2005; Givnish *et al.*, 2011). In recent years, numerous new *Tillandsia* spp. have been described, indicating a morphological diversity still not sufficiently explored (Ehlers, 2006a,b; Espejo-Serna, López-Ferrari & Till, 2007; Espejo-Serna & López-Ferrari, 2009; Pontes, 2012; Versieux, Nara Vasconcellos & Martinelli, 2013; Hernández-Cárdenas *et al.*, 2014; Büneker *et al.*, 2015).

The *Tillandsia capillaris* complex: epiphytic diversity in a montane environment

The *T. capillaris* Ruiz & Pav. complex stands out amongst the numerous bromeliad epiphytes growing in the Andes because of its extensive geographical range, extending from central Peru through Bolivia to central Argentina and Chile (Smith & Downs, 1977; Till, 1984, 1992), with a single occurrence in northern Ecuador. Across this range, the taxa of the *T. capillaris* complex form large populations on a number of different substrates in various environments, including vertical granite walls at high elevation, semi-arid forests in valleys and humid mountain forests on various hosts (Benzing, 1990) and in the hyper-arid coastal zone of Chile and Peru (Rundel & Dillon, 1998). The adaptation to environmental constraints with regard to growth and physiology results in a plastic response in morphology; changes in morphology may not necessarily correspond to evolutionary units defined by molecular data (Gavrilets, 2004). In wide-ranging species, it is likely that connectivity between populations is interrupted, a fact that contributes to the process of speciation (Gavrilets, 2004).

The wide phenotypic variability of the group offers the opportunity to study the influence of historical factors in shaping genetic variability and structure. Systematic work in the *T. capillaris* complex has resulted in a variable number of interrelated taxa (species or infraspecific taxa); more than 20 taxa were described or established between 1802 and 1945, whereas a single species with five forms (Smith & Downs, 1977) and two closely related species (Till, 1984, 1992; Castello & Galetto, 2013) have been recognized in the latest monographic revisions and morphological analyses. The cytology of *Tillandsia* subgenus *Diaphorantema* has been studied by Till (1984, 1992). Among 16 species analysed, nine were found to be only tetraploid, including the *T. capillaris* complex; other taxa, such as *T. aizoides* Mez, *T. gilliesii* Baker, *T. loliacea* Mart. ex Schult. & Schult.f. and *T. tricholepis* Baker, included diploid and tetraploid individuals. Previous phylogenetic analyses of Bromeliaceae, including *Tillandsia*, have generally shown good resolution at the generic level (Barfuss *et al.*, 2005; Givnish *et al.*, 2011, 2014). All efforts to date have been aimed at the resolution of the phylogenetic structure of the family and some large genera, but some morphologically diverse clades still require detailed study, especially in terms of taxon sampling and the inclusion of more individuals per species (Barfuss *et al.*, 2005; Givnish *et al.*, 2011). In *Tillandsia*, few plastid markers with informative sites at the infraspecific level have been identified (Barfuss *et al.*, 2005; Givnish *et al.*, 2011; Barfuss, 2012). Subsequently, several PCR primer pairs for nuclear DNA markers have been developed (Schulte, Barfuss & Zizka, 2009; Chew, De Luna & González, 2010); a combination of markers from the plastid and nuclear genomes is expected to offer better resolution in phylogenetic trees. Low-copy nuclear markers have been used in studies of tetraploid (Russell *et al.*, 2010, 2011) and diploid (Mathews & Donoghue, 2000; Samuel *et al.*, 2005) taxa. The present work is the first contribution using molecular data to verify taxonomic boundaries based on morphology in the *T. capillaris* complex and to establish whether they are real evolutionary units or environmentally

conditioned morphotypes. We aimed: (1) to determine the phylogenetic relationships within the complex; and (2) to explore the phylogeographical structure and the distribution of genetic variation in the whole distribution range of the *T. capillaris* complex, as a basis for future studies.

MATERIAL AND METHODS

Taxon sampling

Plant material was collected at locations in Argentina, Bolivia and Peru (Table 1), spanning most of the distribution of the complex. The localities were geo-referenced with a Garmin GPS, and herbarium specimens were deposited in CORD, CTES, SI and WU. In addition, material from Peru and Chile was taken from plants cultivated at the Botanical Garden of the University of Vienna collected by W. Till (1980–1990) and by H. and L. Hromadnik (1977–1997), which have subsequently been geo-referenced using Google Earth v.7.1.2.2041. The plant material was dried and stored in silica gel (Chase & Hills, 1991). Ninety-six individuals from 69 populations plus 16 outgroup taxa were investigated. We sampled numerous populations to obtain a complete geographical pattern of genetic variation (Acosta, Mathiasen & Premoli, 2014), with representatives of all the different morphotypes of the *T. capillaris* complex: *T. capillaris s.s.*, *T. capillaris s.l.* [= *T. capillaris* forma *hieronymi* (Mez) L.B.Sm., *T. capillaris* forma *incana* (Gillies ex Baker) L.B.Sm.], *T. virescens* Ruiz & Pav. *s.s.* [= *T. capillaris* forma *cordobensis* (Hieron.) L.B.Sm.], *T. virescens s.l.* [= *T. capillaris* forma *virescens* (Ruiz & Pav.) L.B.Sm. *sensu* Castello, *T. dependens* Hieron ex Mez *sensu* Till and *T. williamsii* Rusby] (Table 1, Fig. 1). The nomenclature follows a mix of Till (1984) and Castello & Galetto (2013) because the classification of Smith & Downs (1977) does not represent all of the morphological variation present in the complex (for a full synonymy, see Table 2). Some of these localities harbour individuals of the alleged different morphological forms. Individuals of *T. kuehhasii* and *T. lanuginosa*, believed to be closely related to the *T. capillaris* complex (Till, 1984, 1992, 1995), are included. Material from 16 additional *Tillandsia* spp. growing in the same area as the complex were used as outgroups: *T. cochabambae* Gross & Rauh and *T. lechneri* W.Till & Barfuss from subgenus *Allardtia*; *T. albertiana* Verv., *T. bermejoensis* H.Hrom ex Rauh, *T. esseriana* Rauh & L.B.Sm., *T. ixioides* Griseb. and *T. tenuifolia* L. from subgenus *Anoplophytum*; *T. duratii* Vis. and *T. kirschnekii* Rauh from subgenus *Phytarrhiza*; and *T. caliginosa* W.Till, *T. castellanii* L.B.Sm., *T. funebris* A.Cast., *T. landbeckii* Phil., *T. myosura* Griseb. ex Baker, *T. recurvata* (L.) L. and *T. usneoides* (L.) L. from the same subgenus as the *T. capillaris* complex, *Diaphoranthema*.

DNA extraction, amplification and sequencing

DNA extraction was performed using 50–75 mg of dehydrated leaf tissue and the sorbitol/cetyltrimethylammonium bromide (CTAB) method for difficult tissues (Tel-Zur *et al.*, 1999), with modifications according to Barfuss (2012). In some cases, to improve the purity of the extractions, we used a silica membrane-based DNA extraction kit (DNeasy Plant Mini Kit, QIAGEN, Hilden, Germany), which is more effective in removing secondary compounds and improves the precipitation of proteins and other cellular debris. Extracted DNA was electrophoresed on 1% agarose gels to assess the quantity and quality of the extract.

For PCR, we used 1.1 × ReddyMix PCR Master Mix (Thermo Scientific Inc., Waltham, MA, USA), which includes, in the final 1 × reaction, 0.625 units *Taq* DNA polymerase, 75 mM Tris-HCl (pH 8.8 at 25 °C), 20 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.01% Tween 20 and 0.2 mM each deoxynucleoside triphosphate (dNTP). For longer or difficult sequences, the 2 × ReddyMix Extensor PCR Master Mix (Thermo Scientific) was used, which includes PCR buffer 1, 1.25 units Extensor Hi-Fidelity polymerase, 2.25 mM $MgCl_2$ and 0.35 mM each dNTP. To each PCR, we added DNA template, 20 mM forward and reverse primers, and 20 mg ml^{-1} bovine serum albumin (BSA).

The *trnK-matK-trnK* marker was amplified in two overlapping fragments; this marker has been used extensively in previous studies (Barfuss *et al.*, 2005; Shaw *et al.*, 2007; Schulte *et al.*, 2009; Givnish *et al.*, 2011, 2014). *ycf1* (Drew & Sytsma, 2011) was amplified in three overlapping fragments. *rpoB-trnC-petN* (Demesure, Sodzi & Petit, 1995; Lee & Wen, 2004; Versieux *et al.*, 2012) was amplified in one fragment, but sequenced with two internal primers. The nuclear marker *PHYC* (Samuel *et al.*, 2005; Jabaily & Sytsma, 2010; Russell *et al.*, 2010; Barfuss, 2012; Louzada *et al.*, 2014) with *c.* 1200 bp was amplified in one fragment. In instances of low DNA quality, the PCR products were re-amplified. All the primer combinations used are provided in Table 3. All the primers from the plastid regions were developed by M. H. J. Barfuss for this study, and primers for *PHYC* were taken from Barfuss (2012). PCR parameters, such as temperature, extension time and cycle numbers, were adjusted for each marker according to the annealing temperatures of the primers and the fragment length. The PCR products were purified according to Werle *et al.* (1994), with slight modifications (Barfuss, 2012). Cycle sequencing was

Table 1. Voucher specimens, populations, geographical data and accession numbers of the *Tillandsia capillaris* complex individuals included. Population (Pop), individual identification (ID), Haplotype (Hapl.), accepted name (Taxa), Specimen voucher, Herbaria (Herb.), Locality, Coordinates, Elevation, GenBank Accession number. Country codes: PER, P (Peru); BOL, B (Bolivia); ARG, A (Argentina). Herbaria: 1 (WU), 2 (CORD)

Pop	ID	Hapl.	Taxa	Specimen voucher	Herb.	Locality	Latitude S	Longitude W	Elevation	GenBank Accession
1. PER	P1	H17	*T. capillaris*	*Hromadnik 4219a*	1	Ancash. San Marcos-Masin	9°26′46.14″	77°9′25.47″	2800	KU058733, KT935070, KU197089, KU285336
2. PER	P2	H13	*T. capillaris*	*Hromadnik 2039*[a]	1	Junín. Oberhalb von	11°25′38.98″	75°41′10.44″	3600	KU058734, KT935071, KU197090, KU285337
	P3	H40	*T. virescens*	*Hromadnik 18010*	1	Tarma und Unterhalb Tarma			3300	KU058735, KT935072, KU197091, KU285338
3. PER	P4	H5	*T. capillaris*	*Hromadnik 18002*	1	Lima. Oberhalb Matucana	11°49′58.64″	76°23′47.16″	3400	KU058736, KT935073, KU197092, KU285339
4. PER	P6	H19	*T. capillaris*	*Till WT 200*	1	Cuzco. R. Vilcanota	13°14′42.16″	72°17′23.10″	3110	KU058738, KT935075, KU197094, KU285339
5. PER	P7	H1	*T. capillaris*	*Till WT 207*	1	Cuzco. Sicuani	14°17′6.05″	71°13′29.01″	3020	KU058739, KT935076, KU197095, KU285342
6. PER	P9	H8	*T. capillaris*	*Till WT 212*	1	Puno. Pucara	15°2′46.38″	70°22′1.27″	3880	KU058741, KT935078, KU197097, KU285344
7. PER	P10	H30	*T. virescens*	*Till WT 215*	1	Puno. Sillustani	15°50′33.63″	70°2′11.80″	3730	KU058742, KT935079, KU197098, KU285345
8. PER	P11	H41	*T. virescens*	*Chiapella et al. 3006*	2	El Collao. Ilave	16°4′39.66″	69°39′30.03″	3845	KU058743, KT935080, KU197099, KU285346
9. PER	P13	H15	*T. capillaris*	*Till WT228*	1	Arequipa. Camana-R. Siguas	16°34′14.97″	72°15′54.46″		KU058744, KT935081, KU197100, KU285347
10. BOL	B1	H59	*T. virescens* (*cordobensis*)	*Chiapella et al. 3016*	2	La Paz. Sorata	15°47′29.1″	68°38′42.7″	2840	KU058745, KT935082, KU197101, KU285348

Table 1. *Continued*

Pop	ID	Hapl.	Taxa	Specimen voucher	Herb.	Locality	Latitude S	Longitude W	Elevation	GenBank Accession
11. BOL	B2	H30	*T. virescens*	*Chiapella et al. 3008*	2	La Paz. Copacabana, Ruta 2, saliendo de Copacabana camino a La Paz	16°10′05″	69°3′42.5″	4016	KU058746, KT935083, KU197102, KU285349
12. BOL	B3	H38	*T. virescens*	*Chiapella et al. 3027*	2	La Paz. La Paz	16°32′18.59″	68°4′6.12″	3419	KU058747, KT935084, KU197103, KU285350
13. BOL	B4	H23	*T. capillaris* (*hieronymi*)	*Chiapella et al. 3028*	2	Cochabamba. Cochabamba	17°22′41.4″	66°8′24.5″	2747	KU058748, KT935085, KU197104, KU285351
14. BOL	B5	H49	*T. virescens*	*Chiapella et al. 3036*	2	Cochabamba. Entre Cochabamba y Tiraque, km41	17°23′42.2″	65°49′17.1″	3296	KU058749, KT935086, KU197105, KU285352
15. BOL	B6	H18	*T. capillaris*	*Hromadnik 5332*	1	Cochabamba. Quillacollo	17°24′29.91″	66°16′20.31″	2500	KU058750, KT935087, KU197106, KU285353
16. BOL	B7	H26	*T. virescens*	*Hromadnik 24081*	1	Cochabamba. Tiraque- Monte Puncu	17°28′39.39″	65°29′39.73″	3400	KU058751, KT935088, KU197107, KU285354
17. BOL	B8	H56	*T. virescens* (*cordobensis*)	*Hromadnik 24082*	1	Cochabamba. Chulchucani westl von Monte Puncu	17°28′47.20″	65°17′57.55″	3400	KU058752, KT935089, KU197108, KU285355
18. BOL	B9	H21	*T. capillaris* (*hieronymi*)	*Chiapella et al. 3044*	2	Cochabamba. Cochabamba	17°43′22.4″	64°54′27.4″	2251	KU058753, KT935090, KU197109, KU285356
19. BOL	B10	H45	*T. virescens*	*Hromadnik 24066*	1	Cochabamba. Pass nördl. Anzaldo	17°46′40.19″	65°55′48.81″	3500	KU058754, KT935091, KU197110, KU285357
20. BOL	B11 B12	H3	*T. capillaris*	*Chiapella et al. 3056*	2	Santa Cruz. Comarapa	17°55′16″	64°31′45″	1820	KU058755, KT935092, KU197111, KU285358

Table 1. *Continued*

Pop	ID	Hapl.	Taxa	Specimen voucher	Herb.	Locality	Latitude S	Longitude W	Elevation	GenBank Accession
21. BOL	B13 B14 B15	H59 H57 H7	*T. virescens* (*cordobensis*) *T. capillaris*	*Chiapella et al. 3079*	2	Santa Cruz. Valle Grande	18°28′37.9″	64°6′53.3″	2028	KU058757, KT935094, KU197113, KU285360 KU058758, KT935095, KU197114, KU285361 KU058759, KT935096, KU197115, KU285362
22. BOL	B17 B18	H20 H61	*T. capillaris* *T. virescens (cordobensis)*	*Chiapella et al. 3066*	2	Santa Cruz. Entre Valle Grande y Camiri	18°38′28.6″	63°58′58.6″	1749	KU058760, KT935097, KU197116, KU285363 KU058761, KT935098, KU197117, KU285364
23. BOL	B20	H39	*T. virescens*	*Chiapella et al. 2969*	2	Chuquisaca. Entre Ravelo y Ocurí	18°48′8.8″	65°37′21.7″	3507	KU058762, KT935099, KU197118, KU285365
24. BOL	B21 B22	H42	*T. virescens* (*fv*)	*Chiapella et al. 2986*	2	Oruro. Entre Ventilla y Pazña	18°51′45.9″	66°31′17.6″	3883	KU058763, KT935100, KU197119, KU285366 KU058764, KT935101, KU197120, KU285367
25. BOL	B23 B24	H42 H44	*T. virescens* (*dependens*)	*Chiapella et al. 2944*	2	Chuquisaca: Entre Sucre y Ravelo, 12 km antes de llegar a Ravelo	18°53′48″	65°26′10″	3259	KU058765, KT935102, KU197121, KU285368 KU058766, KT935103, KU197122, KU285369
26. BOL	B25	H36	*T. virescens*	*Hromadnik 19009*	2	Chuquisaca. Cerro Chataicha	18°59′1.71″	65°25′1.55″	3700	KU058767, KT935104, KU197123, KU285370

Table 1. *Continued*

Pop	ID	Hapl.	Taxa	Specimen voucher	Herb.	Locality	Latitude S	Longitude W	Elevation	GenBank Accession
27. BOL	B26 B27 B28 B29	H46 H60 H59 H22	*T. virescens* *T. virescens* (*cordobensis*) *T. virescens* (*cordobensis*) *T. capillaris* (*hieronymi*)	*Chiapella et al. 2939* *Chiapella et al. 2940* *Chiapella et al. 2938* *Chiapella et al. 2940b*	2	Sucre: Entre Sucre y Ravelo, 15 Km saliendo de Sucre	18°59′20.6″	65°20′47.8″	3275	KU058768, KT935105, KU197124, KU285371 KU058769, KT935106, KU197125, KU285372 KU058770, KT935107, KU197126. KU285373 KU058771, KT935108, KU197127, KU285374
28. BOL	B31	H3	*T. capillaris* (*hieronymi*)	*Chiapella et al. 2917b*	2	Sucre: 60 Km antes de llegar a Sucre	19°30′49.8″	65°18′19.2″	2526	KU058772, KT935109, KU197128, KU285375
29. BOL	B33 B34	H32 H35	*T. virescens* (*williamsii*)	*Chiapella et al. 2883*	2	Potosí: Saliendo desde Potosí a Sucre, a 5 km de Potosí	19°31′45.9″	65°42′35.2″	3894	KU058773, KT935110, KU197129, KU285376 KU058774, KT935111, KU197130, KU285377
30. BOL	B35	H16	*T. capillaris*	*Hromadnik 5061*	1	Chuquisaca. Abzw. Esquiri Häng nördl. Pilcomayo	19°42′49.58″	64°45′52.47″	3000	KU058775, KT935112, KU197131, KU285378
31. BOL	B36	H25	*T. virescens*	*Hromadnik 9212*[a]	1	Santa Cruz. Rio Bermejo-canon	19°46′16.21″	63°42′6.64″	1300	KU058776, KT935113, KU197132, KU285379
32. BOL	B37	H47	*T. virescens*	*Hromadnik 5088*	1	Potosí. Berge NÖ R. Tumusla	20°27′36.32″	65°37′4.88″	2800	KU058777, KT935114, KU197133, KU285380

Table 1. *Continued*

Pop	ID	Hapl.	Taxa	Specimen voucher	Herb.	Locality	Latitude S	Longitude W	Elevation	GenBank Accession
33. BOL	B38 B39	H33 H34	*T. virescens* (*williamsii*)	*Chiapella et al. 2846*	2	Potosí: Atocha, Atocha	21°6′26.6″	66°2′14.0″	3995	KU058778, KT935115, KU197134, KU285381 KU058779, KT935116, KU197135, KU285382
34. BOL	B41	H47	*T. virescens*	*Hromadnik 24042*	1	Chuquisaca. Cord. De Mochara westl. Impora	21°27′50.13″	65°21′1.04″	3300	KU058780, KT935117, KU197136, KU285383
35. BOL	B42	H43	*T. virescens*	*Hromadnik 19020*	1	Tarija. El puente Iscachayi	21°29′17.54″	64°57′40.36″	3160	KU058781, KT935118, KU197137, KU285384
36. BOL	B43	H45	*T. virescens*	*Hromadnik 19021c*	1	Tarija. El puente Iscachayi	21°29′17.54″	64°57′40.36″	3160	KU058782, KT935119, KU197138, KU285385
37. BOL	B44 B45	H48 H42	*T. virescens*	*Chiapella et al. 2806*	2	Tarija: Entre Villazón y Tupiza	21°40′5.9″	65°34′55.1″	3357	KU058783, KT935120, KU197139, KU285386 KU058784, KT935121, KU197140, KU285387
38. BOL	B47 B48	H24 H14	*T. capillaris*	*Hromadnik 5108*	1	Tarija. Tojo-Yunchara	21°49′19.29″	65°14′28.77″	3400	KU058785, KT935122, KU197141, KU285388 KU058786, KT935123, KU197142, KU285389
39. CHI	C1	H37	*T. virescens*	*Novak sn*	1	Atacama. Mina Algarrobo	28°50′0.37″	70°58′58.50″	894	KU058787, KT935124, KU197143, KU285390
40. CHI	C2	H5	*T. capillaris*	*Hromadnik 11002*	1	Coquimbo. Elqui, Nördl. Las Cardas km83	30°18′23.82″	71°15′27.16″	600	KU058788, KT935125, KU197144, KU285391

Table 1. *Continued*

Pop	ID	Hapl.	Taxa	Specimen voucher	Herb.	Locality	Latitude S	Longitude W	Elevation	GenBank Accession
41. CHI	C3	H1	*T. capillaris*	*Hromadnik 11001*	1	Coquimbo. Km 39 nördl. Ovalle-Las Cardas	30°29′19.41″	71°9′26.25″		KU058789, KT935126, KU197145, KU285392
42. ARG	A1	H52	*T. virescens* (*fv*)	*Castello 315*	2	Jujuy. Tumbaya. Purmamarca	23°44′11.0″	65°30′38.6″	2420	KU058790, KT935127, KU197146, KU285393
43. ARG	A4	H6	*T. capillaris*	*Chiapella et al. 3087b*	2	Jujuy. Huajara, antes de Purmamarca	23°52′40.9″	65°27′26.9″	2101	KU058791, KT935128, KU197147, KU285394
44. ARG	A6 A7 A8	H31	*T. virescens* (*fv*)	*Castello 300*	2	Jujuy. Susques. Tuzgle, RN40 entre Sey y San Antonio de los Cobres	24°00′03.3″	66°30′30.3″	4121	KU058792, KT935129, KU197148, KU285395 KU058793, KT935130, KU197149, KU285396 KU058794, KT935131, KU197150, KU285397
45. ARG	A9	H59	*T. virescens* (*cordobensis*)	*Castello 317*	2	Jujuy. Capital. RN9, León	24°2′58.0″	65°25′24.0″	1640	KU058795, KT935132, KU197151, KU285398
46. ARG	A10	H51	*T. virescens* (*fv*)	*Castello 291*	2	Salta. Rosario de Lerma. Ingeniero Maury, en Cerro Golgota	24°40′44.4″	65°46′23.3″	2400 –3500	KU058796, KT935133, KU197152, KU285399
47. ARG	A11	H59	*T. virescens* (*cordobensis*)	*Castello 282*	1, 2	Salta. Rosario de Lerma: Lugar: entre Campo Quijano y Chorrillos	24°51′48.2″	65°42′06.6″	1760	KU058797, KT935134, KU197153, KU285400
48. ARG	A12	H3	*T. capillaris*	*Castello 279*	1, 2	Salta. Rosario de Lerma. Campo Quijano	24°54′17.6″	65°37′44.1″	1217	KU058798, KT935135, KU197154, KU285401

Table 1. *Continued*

Pop	ID	Hapl.	Taxa	Specimen voucher	Herb.	Locality	Latitude S	Longitude W	Elevation	GenBank Accession
49. ARG	A13	H2	*T. capillaris*	*Castello 275*	1, 2	Salta. Guachipas. Alrededores de Alemania	25°38′21.7″	65°39′25.1″	1244	KU058799, KT935136, KU197155, KU285402
50. ARG	A14	H2	*T. capillaris*	*Castello 319*	1, 2	Salta. Rosario de la Frontera. RN9, Km 1382	26°6′42.3″	65°10′17.3″	892	KU058800, KT935137, KU197156, KU285403
51. ARG	A15	H2	*T. capillaris*	*Castello 322*	1, 2	Tucumán. Trancas. RN9, cerca de Los Nogales	26°41′21.2″	65°13′51.1″	636	KU058801, KT935138, KU197157, KU285404
52. ARG	A17 A18	H2 H59	*T. capillaris* *T. virescens* (*cordobensis*)	*Castello 328* *Castello 328b*	2	Tucumán. Yerba Buena	26°48′36.1″	65°18′37.5″	533	KU058802, KT935139, KU197158, KU285405 KU058803, KT935140, KU197159, KU285406
53. ARG	A20	H63	*T. virescens* (*cordobensis*)	*Castello 329*	2	Tucumán. Durazno Blanco	26°58′5.6″	65°39′26.3″	1502	KU058804, KT935141, KU197160, KU285407
54. ARG	A21	H1	*T. capillaris*	*Castello 268*	1, 2	Catamarca. Belén. Londres	27°42′37″	67°9′24.3″	1242	KU058805, KT935142, KU197161, KU285408
55. ARG	A22 A23	H2 H11	*T. capillaris*	*Chiapella et al. 3095*	2	Santiago del Estero. Santiago del Estero	27°52′10.3″	64°14′38.2″	190	KU058806, KT935143, KU197162, KU285409 KU058807, KT935144, KU197163, KU285410
56. ARG	A24	H12	*T. capillaris*	*Castello 107*	2	La Rioja. San Blas. Amuschina	28°34′23.2″	67°11′15.7″	1167	KU058808, KT935145, KU197164, KU285411
57. ARG	A25	H9	*T. capillaris*	*Castello 254*	1, 2	Catamarca. Capayán. Miraflores	28°36′49.3″	65°54′07.4″	505	KU058809, KT935146, KU197165, KU285412

Table 1. *Continued*

Pop	ID	Hapl.	Taxa	Specimen voucher	Herb.	Locality	Latitude S	Longitude W	Elevation	GenBank Accession
58. ARG	A26	H1	*T. capillaris* (*incana*)	*Castello 111*	2	La Rioja. Chilecito. Cuesta de Miranda, a km de Sañogasta, RN 40	29°20′34.5″	67°45′47.3″	1945	KU058810, KT935147, KU197166, KU285413
59. ARG	A29	H2	*T. capillaris*	*Castello 93*	2	Córdoba. Cruz del Eje. Dique Cruz del Eje, a orillas del Río Cruz del Eje	30°45′49.3″	64°45′34.1″	503	KU058811, KT935148, KU197167, KU285414
60. ARG	A32	H1	*T. capillaris* (*incana*)	*Castello 90*	2	Córdoba. Punilla. Ruta E 34, Km 63, entrada da a Parque Nacional Quebrada del Condorito	31°36′41.1″	64°41′33.3″	1670	KU058812, KT935149, KU197168, KU285415

Table 1. *Continued*

Pop	ID	Hapl.	Taxa	Specimen voucher	Herb.	Locality	Latitude S	Longitude W	Elevation	GenBank Accession
61. ARG	A33 A34 A35 A36 A37 A38	H54 H47 H47 H55 H53	*T. virescens* (*dependens*) *T. virescens* (*fv*)	*Castello 88* *Castello 86* *Castello 87* *Castello 81* *Castello 81b* *Castello 82*	2	Córdoba. San Alberto. Ruta E 34, a 5 km de La Posta, cruce de caminos de Ea. La Trinidad y Colonia José M. Liqueno	31°36′37.2″	64°49′36.7″	2241	KU058813, KT935150, KU197169, KU285416 KU058814, KT935151, KU197170, KU285417 KU058815, KT935152, KU197171, KU285418 KU058816, KT935153, KU197172, KU285419 KU058817, KT935154, KU197173, KU285420 KU058818, KT935155, KU197174, KU285421
62. ARG	A39	H58	*T. virescens* (*cordobensis*)	*Castello 137*	2	Córdoba. San Javier. Los Hornillos, a orillas del arroyo los Hornillos	31°54′05.3″	64°58′29.9″	1111	KU058819, KT935156, KU197175, KU285422
63. ARG	A41	H50	*T. virescens* (*fv*)	*Castello 140*	2	Mendoza. Hotel Villavicencio, RP 52	32°31′37.8″	69°0′59.96″	1807	KU058820, KT935157, KU197176, KU285423
64. ARG	A43	H62	*T. virescens* (*cordobensis*)	*Castello 66*	2	Córdoba. Río Cuarto. Elena, sobre R.N. 36	32°34′26.6″	64°23′24.6″	601	KU058821, KT935158, KU197177, KU285424

Table 1. *Continued*

Pop	ID	Hapl.	Taxa	Specimen voucher	Herb.	Locality	Latitude S	Longitude W	Elevation	GenBank Accession
65. ARG	A44 A45	H4 H4	*T. capillaris* (*incana*)	*Chiapella et al. 3210*	2	Mendoza: entrada a Potrerillos	32°57′47″	69°11′47.2″	1432	KU058822, KT935159, KU197178, KU285425 KU058823, KT935160, KU197179, KU285426
66. ARG	A46	H10	*T. capillaris*	*Castello 71*	2	Córdoba. Río Cuarto. R.N. 35, entre Rio Cuarto y Santa Catalina	33°11′48.1″	64°25′12.8″	603	KU058824, KT935161, KU197180, KU285427
67. ARG	A47	H1	*T. capillaris*	*Chiapella et al. 3378*	2	San Luis. RN148, entre La Angelina y Río Quinto	33°52′12.8″	65°19′50.9″	443	KU058825, KT935162, KU197181, KU285428
68. BOL	O18 O20	H28 O20	*T. kuehhasii*	*Hromadnik 19009*	1	Chuquisaca. Sucre - Ravelo, Cerro Chatajlla	19°1′41.0″	65°21′19.0″	3610	KU058730, KT935067, KU197086, KU285333 KU058732, KT935069, KU197088, KU285335
69. BOL	O19	H29	*T. kuehhasii*	*Hromadnik 238*	1	Chuquisaca, W Sucre, Cristal Mayo	18°57′38″	65°22′33″	3400	KU058731, KT935068, KU197087, KU285334y

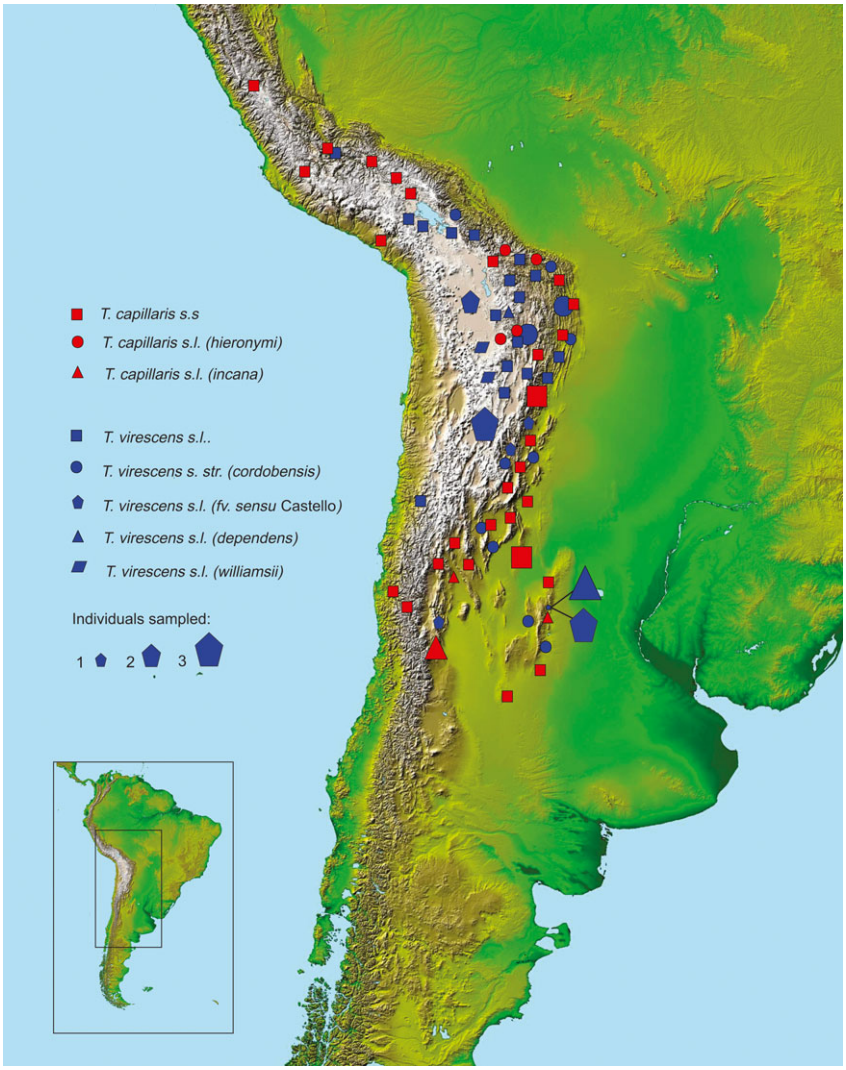

Figure 1. Andean region map showing all the morphotypes of the *Tillandsia capillaris* complex sampled: blue, *T. virescens*; red, *T. capillaris*. Different morphotypes are indicated with symbols. The size of the symbols represents the frequencies of the individuals sampled for each population. Overlapping symbols mean that different morphotypes are presented in the same location.

performed using the BigDye Terminator Cycle Sequencing Kit v.3.1 (Thermo Scientific Inc.), and the same PCR primers were generally used, except in some cases in which additional internal primers were designed to obtain clear and unambiguous sequences (Barfuss, 2012).

Sequence alignment and indel coding

The sequences were manually edited and assembled into a consensus sequence using SeqMan V7.1.0 (DNASTAR Inc., Madison, WI, USA, 2006). Multiple alignments were first performed using ClustalX (Larkin *et al.*, 2007) and these were then manually edited with the software Bioedit v.7.1.3.0 (Hall, 1999). In total, two matrices were generated; one comprising the three plastid markers (*rpoB-trnC-petN*, *trnK-matK-trnK*, *ycf1*) with *c.* 8100 bp, and another for the nuclear marker (*PHYC*) with 1161 bp. Gaps were added according to the parsimony criterion, which attempts to minimize the number of gaps and sequence variation (Wiley & Lieberman, 2011). In *rpoB-trnC-petN* (*c.* 2600 bp), an unambiguous poly-A region near the *petN* locus was excluded from the analyses. Gaps were coded as binary characters using simple indel coding (Simmons & Ochoterena, 2000) and appended to the matrices. Sequences of *PHYC* were generally clean with only a few polymorphic sites and no length differences; therefore cloning was not performed (Jabaily &

Table 2. Common synonyms for the morphotypes in the *Tillandsia capillaris* complex

Names used in figures and tables	Common names used in the literature
Tillandsia capillaris	*T. capillaris s.s.* *T. capillaris* Ruiz & Pav. forma typica L.B.Sm.
T. capillaris (*hieronymi*)	*Tillandsia hieronymi* Mez *T. capillaris* Ruiz & Pav. forma *hieronymi* (Mez) L.B.Sm.
T. capillaris (*incana*)	*Tillandsia incana* Gillies ex Baker *T. capillaris* Ruiz & Pav. forma *incana* (Gillies ex Baker) L.B.Sm.
T. virescens	*T. virescens s.l.* *T. capillaris* Ruiz & Pav. forma *virescens* (Ruiz & Pav.) L.B.Sm.
T. virescens (*cordobensis*)	*T. virescens s.s.* *Tillandsia cordobensis* Hieron. *T. capillaris* Ruiz & Pav. forma *cordobensis* (Hieron.) L.B.Sm.
T. virescens (*fv*)	*T. capillaris* forma *virescens sensu* Castello
T. virescens (*dependens*)	*Tillandsia dependens* Hieron. ex Mez
T. virescens (*williamsii*)	*Tillandsia williamsii* Rusby
T. virescens (*kuehhasii*)	*Tillandsia kuehhasii* Till

Sytsma, 2010; Barfuss, 2012). In some cases, *PHYC* PCR products containing two or more alleles were visualized as double peaks at certain positions; these single nucleotide polymorphisms (SNPs) were coded using the IUPAC-IUB ambiguity symbols for nucleotide nomenclature (Barfuss, 2012). All sequences were deposited in GenBank (Table 1).

Phylogenetic and phylogeographical analysis

To explore the variability of each marker, we calculated the number of variable and potentially parsimony informative sites (Table 4) using DNAsp v.5 (Librado & Rozas, 2009). To infer phylogenetic relationships, we constructed phylogenetic trees using Bayesian inference (BI) with MrBayes 3.2.2 (Ronquist *et al.*, 2012) for the two matrices. We first calculated the evolutionary substitution model for each marker using the Akaike information criterion (AIC) implemented in jModelTest v.2.1.5 (Darriba *et al.*, 2012). We then chose the most generalist substitution model, GTR + G + I, $N = 6$, to build the phylogenetic trees using both matrices. Two independent runs were performed for 10 million generations

Table 3. Primer combinations used in this study. The primers for the plastid regions were developed by M. H. J. Barfuss for this study. Primers for *PHYC* were taken from Barfuss (2012)

Marker	Primer	Primer sequence
ycf1 (part 1)	ycf1-1113f-br	5′-TTTYGATTATAAAMGATGGAATCGYCCA-3′
	ycf1-2660r-br	5′-TCTTTATCTGAATACCGTCTGYTAACCAAT-3′
ycf1 (part 2)	ycf1-2567f-br	5′-CGTATGTTATTATTCCAATTCCCCGA-3′
	ycf1-4104r-br	5′-CTTCTTCTAGATAATCACTAATAGCTGTACTTACC-3′
ycf1 (part 3)	ycf1-3872f-br	5′-AARTGGCGAAATAGAATCAATCAACG-3′
	ycf1-5440r-br	5′-TGAAACGACTGCCATTATTGGTATC-3′
rpoB-trnC-petN	rpob-3165f-br	5′-TTAATGAACCTACAAAATCCCTCAAATTG-3′
	petn-5337r-br	5′-CAGCCCAAGCGAGACTTACTATATCCA-3′
rpoB-trnC-petN (internal primers)	rpob-3950f-br	5′-ATTCAATACAATGAAAGATTAAAGCACG-3′
	trnc-4127r-dp	5′-CGGTTCTTCTATTTTGTATTTAATATCAATG-3′
trnK-matK-trnK (part 1)	trnk-799f-br	5′-CCCTGTTCTGACCATATTGCACTATGTAT-3′
	matk-1769r-br	5′-GCCAGAGATTGACAAGGTAACATTTCC-3′
trnK-matK-trnK (part 2)	matk-1610f-br	5′-AACATCTTCTGGAACCTTTCTTGAGCG-3′
	trnk-2662r-an	5′-CTCGAACCCGGAACTAGTCGG-3′
PHYC	phyc-515f-br	5′-AAGCCCTTYTACGCTATCCTGCACCG-3′
	phyc1699r-br	5′-ATWGCATCCATTTCAACATCTTCCCA-3′
PHYC (re-amplification)	phyc-524f-br	5′-GCTATCCTGCACCGGATCGAYGT-3′
	phyc-1690r-br	5′-TCAACATCTTCCCAYGGGAGGCT-3′

Table 4. Information on plastid DNA and nuclear gene regions used for complete study

Molecular marker	Total size (bp)	PS	PIS	PIS/TS	MEV	Weight	nst
ycf1	4157	271	156	0.037	TPM1uf+I+G	0.293	6
rpoB-trnC-petN	2117	103	46	0.022	TPM1uf+G	0.232	6
trnK-matK-trnK	1794	77	41	0.022	TVM+I	0.235	6
PHYC	1161	65	34	0.029	TIM3 + I	0.226	6

The last three columns were calculated using JModelTest.
PS, polymorphic sites; PIS, potentially parsimony informative sites; PIS/TS, potentially parsimony informative sites vs. total size; MEV, molecular evolutionary model, calculated with the Akaike criterion.

(sampling every 1000 generations and 25% discarded as burn-in) and were summarized in a 50% majority-rule consensus tree. Phylogenetic trees and phylogenetic networks were built for the nuclear DNA matrix. The network analysis was performed using Splitstree (Huson & Bryant, 2006) employing standard Neighbor-Net distances (a distance based method based on the Neighbor-Joining algorithm implemented in Splitstree for constructing phylogenetic networks) (Bryant & Moulton, 2004).

To investigate the genetic population structure, we constructed a haplotype network for the plastid markers based on statistical parsimony using TCS v.1.21 (Clement, Posada & Crandall, 2000). After identification of the haplotypes, we constructed a phylogenetic tree also using BI with MrBayes v.3.2.2 (Ronquist *et al.*, 2012). The frequency and distribution of haplotypes were plotted on maps using the geo-references obtained for the collected samples with DIVA-GIS 7.5.0.0. (Hijmans, 2012).

RESULTS

Molecular marker attributes

Within the complex, we achieved good resolution at the infrageneric level using the plastid DNA markers *trnK-matK-trnK*, *rpoB-trnC-petN* and *ycf1,* but low resolution with the nuclear DNA marker *PHYC*.

The characteristics of the aligned matrices, with the different substitution models for each marker, are given in Table 4. The most informative marker was *ycf1* with 0.037% potentially parsimony informative sites, distributed equally along the entire sequence, followed by *PHYC* with 0.029%, and *trnK-matK-trnK* and *rpoB-trnC-petN* with 0.022%. The substitution models were different for each marker (Table 4), but they formed part of the same substitution type nst = 6. Therefore, we used the General Time Reversible (GTR) model, which is one of the three main types of model used by MrBayes and has six substitution types (lset nst = 6), one for each pair of nucleotides.

Phylogenetic analyses of the plastid markers

The Bayesian majority-rule consensus tree using the three plastid markers was well resolved (Fig. 2). The *T. capillaris* complex was distinguished from the outgroup species as a weakly supported monophyletic group [posterior probability (PP) 0.61]. The complex splits into two well-supported branches (PP 1), which are highlighted in blue and red in the figures. The 'red clade' includes the samples identified morphologically as *T. capillaris*; this group comprises the taxa classified as *T. capillaris s.s.*, *T. capillaris* forma *incana* and *T. capillaris* forma *hieronymi* in Smith & Downs (1977). In this 'red clade', one early-diverging branch is well supported (PP 1) and corresponds to four individuals classified as *T. capillaris* forma *hieronymii* (Smith & Downs, 1977), with a distribution in southernmost Bolivia.

Most of the studied accessions of the '*virescens* clade' belonged to *T. virescens s.l.* This group comprises more taxa. On the one hand, nested in one subclade of the '*virescens* clade', we can identify a well-supported small clade '*cordobensis*' (PP 1) of accessions classified as *T. virescens s.s.* (= *T. cordobensis* or *T. capillaris* forma *cordobensis* of Smith & Downs, 1977; see Castello & Galetto, 2013). On the other hand, the individuals of *T. kuehhasii* were nested in a different subclade '*kuehhasii*' inside the '*virescens* clade' (PP 1). In addition to these two lineages, inside the '*virescens* clade' that corresponds to *T. virescens s.l.*, all remaining accessions are intermingled and not separated into groups according to the morphological classification [samples identified as *T. virescens s.l.* (= *T. williamsii*) and *T. virescens s.l.* (= *T. dependens*)].

Phylogenetic analyses of the nuclear marker

Monophyly for the *T. capillaris* complex using the nuclear marker in the phylogenetic tree was weakly supported (PP 0.81), including the individuals of *T. kuehhasii* (Supporting Information, Fig. S1). The Bayesian phylogenetic tree from the nuclear marker

Figure 2. Bayesian gene tree reconstruction with the combination of three plastid markers (*rpoB-trnC-petN*, *trnK-matK-trnK*, *ycf1*, *c.* 8100 bp). Different colours of the branches reflect major groups in the *Tillandsia capillaris* complex: blue, *T. virescens*; red, *T. capillaris*. Posterior probabilities on branch nodes are given as decimal numbers.

showed a large polytomy in the complex (PP 1). However, the network showed that some individuals identified as *T. capillaris* (B29, A4, A12, A15, A17, A13, A45) were included in *T. virescens* (Supporting Information, Fig. S2).

Haplotype network analysis for the complex

The analysis of the parsimony network with the plastid markers yielded one network including 63 haplotypes with internal connections and a star-like pattern (Fig. 3). Overall, the most frequent haplotypes occupy marginal positions in the network, whereas the haplotypes with more internal positions show low frequencies. We identified two large groups of haplotypes, suggesting a non-random distribution. In particular, a group of haplotypes identified as *T. capillaris*, called the 'red group' (highlighted with red, yellow and orange), were separated by 18 mutational steps from the samples identified as

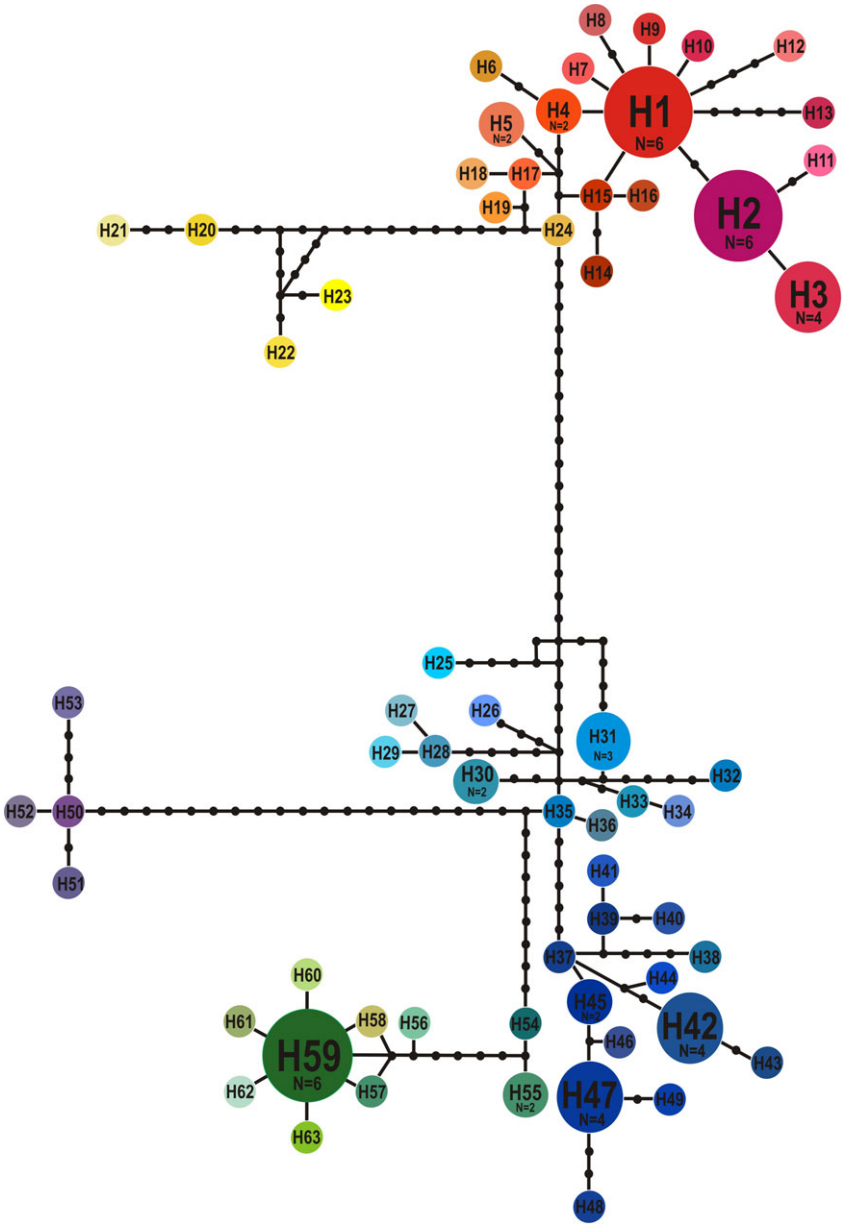

Figure 3. Parsimony haplotype network reconstruction of the three plastid markers (*rpoB-trnC-petN*, *trnK-matK-trnK*, *ycf1*) in the *Tillandsia capillaris* complex. The frequency of each haplotype is represented by the size of the circles. Each colored circle represents a different haplotype

T. virescens, called the 'blue group' (highlighted with blue, green and violet). In the 'red group', a small subset of four haplotypes (H20, H21, H22 and H23, marked with yellow) was separated by ten mutational steps from the central positions of the group. In the 'blue group', four haplotypes (H50, H51, H52 and H53, marked in violet) were separated from the central region of the group by 19 mutational steps. Finally, a subset of ten haplotypes (H54–H63, marked with green) was separated from the central region of the group by eight mutational steps and comprised the accessions identified as *T. virescens s.s.* (= *T. cordobensis* or *T. capillaris* forma *cordobensis* of Smith & Downs, 1977; see Castello & Galetto, 2013). The central part of the 'blue group' comprised 25 different haplotypes, including *T. kuehhasii* (H27, H28, H29) and other *T. virescens s.l.* The phylogenetic Bayesian analysis using haplotypes showed the same pattern as the parsimony network, resolving two clades with branch support (PP 1; Fig. 4).

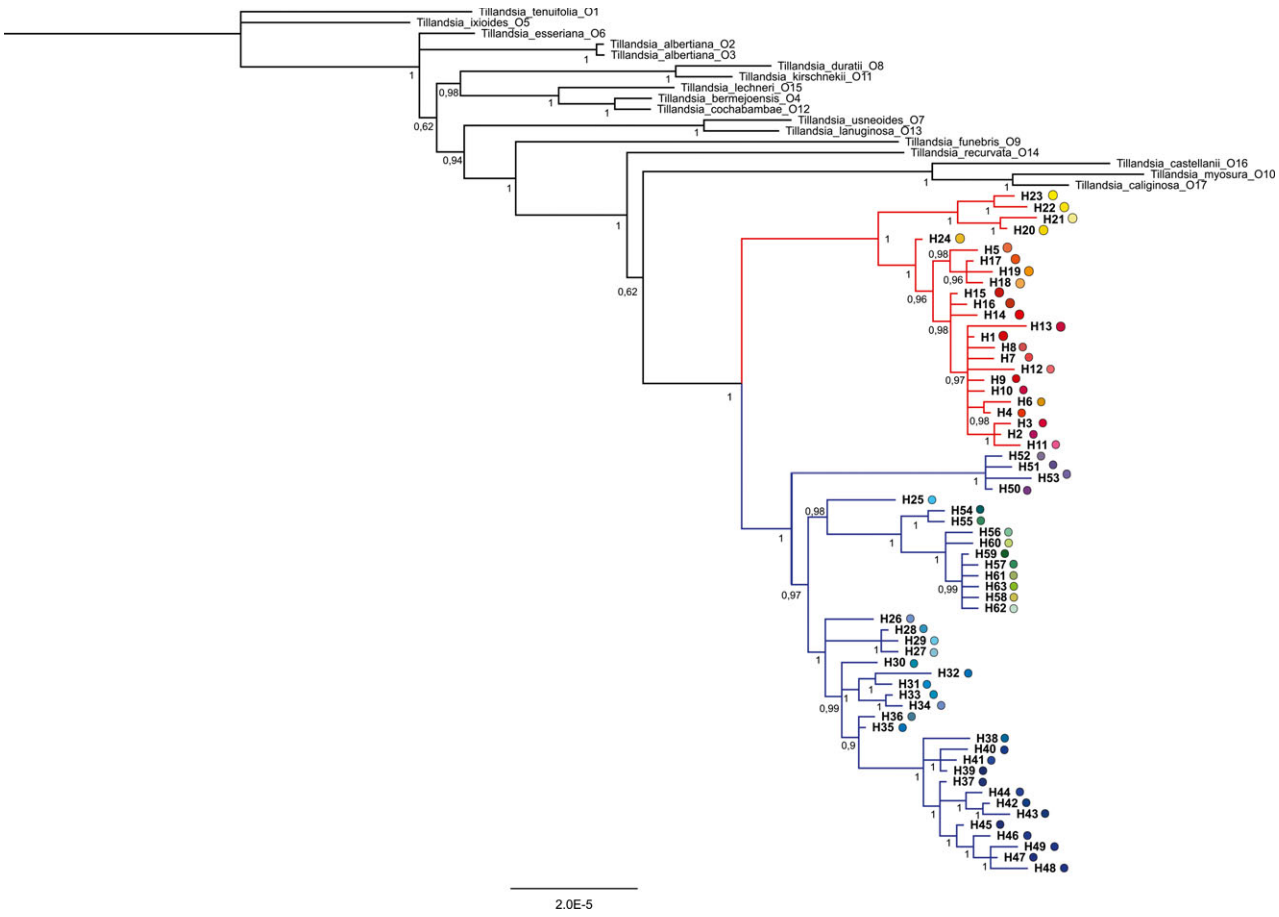

Figure 4. Bayesian haplotype tree reconstruction for the three plastid markers (*rpoB-trnC-petN*, *trnK-matK-trnK*, *ycf1*). Different colours of the branches reflect the different groups for the *Tillandsia capillaris* complex: blue, *T. virescens*; red, *T. capillaris*. Posterior probabilities on branch nodes are given as decimal numbers. Each coloured circle represents a different haplotype in the network.

Geographical distribution of genetic variation

The sequence analysis of 69 populations yielded 63 different geographically structured plastid DNA haplotypes (Figs 5, 6). The most frequent haplotypes have overlapping distributions along the latitudinal range of the *T. capillaris* complex. Most of the internal haplotypes are less frequent and have a central Andean distribution. The three most frequent haplotypes (H1, H2, H59; $N = 6$) occupy different ranges, but have overlapping latitudinal distributions in geographically contiguous regions, e.g. H1 is widely distributed, whereas the areas of H2 and H59 are less extended. Less frequent are the H3, H4 and H5 haplotypes from the 'red group' (*T. capillaris*) and the H30, H31, H42, H45 and H47 haplotypes from the 'blue group' (*T. virescens*). These are geographically scattered and latitudinal not contingent, except for H4 and H31, respectively ($N = 2$, $N = 3$); these haplotypes appear repeatedly in the same population. The remaining haplotypes represent a high molecular diversity and are unique.

High haplotype diversity appears in Bolivia in the 'blue group' (*T. virescens*), whereas, in the 'red group' (*T. capillaris*), the diversity is more equally spread along the Andean range from Peru to Chile. Populations with more than one individual sampled, in general, have haplotypes from neighbouring populations (for population names and locations, see Table 1). In the 'red group', the most frequent haplotypes H1 and H2 occur in the southern Andes (Argentina and Chile), whereas haplotype H24 occurs in Bolivia (Fig. 5). Haplotype H40 of the 'blue group' is scarcely represented in the central Andes (Fig. 6).

DISCUSSION

Phylogenetic relationships in the complex

The analysis of the three plastid markers resolved the phylogenetic structure of the complex (Fig. 2) and identified two separate clades in the complex:

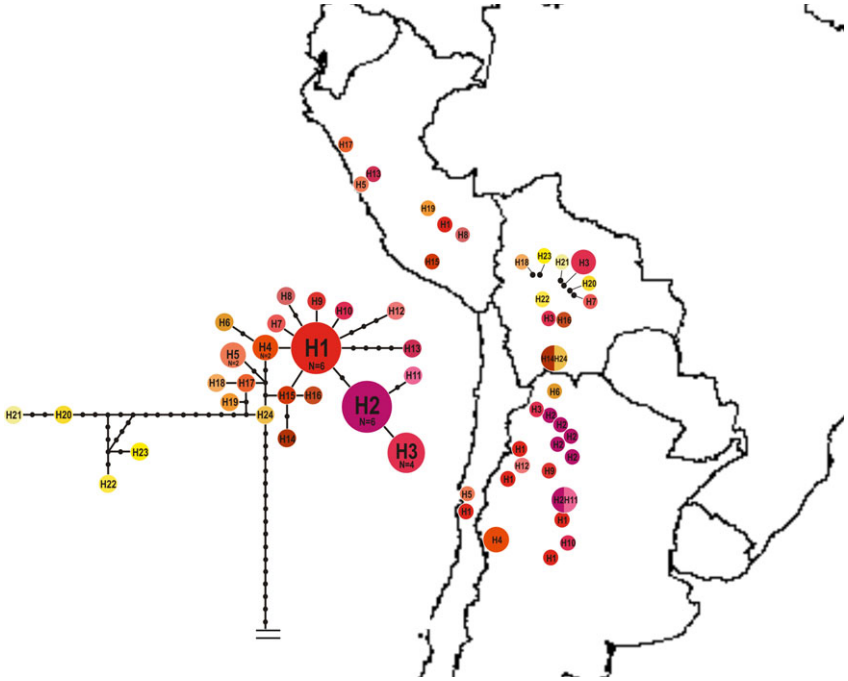

Figure 5. Distribution of plastid DNA (*rpoB-trnC-petN*, *trnK-matK-trnK*, *ycf1*) haplotypes previously identified as *Tillandsia capillaris*. The frequency of each haplotype is represented by the size of the circles.

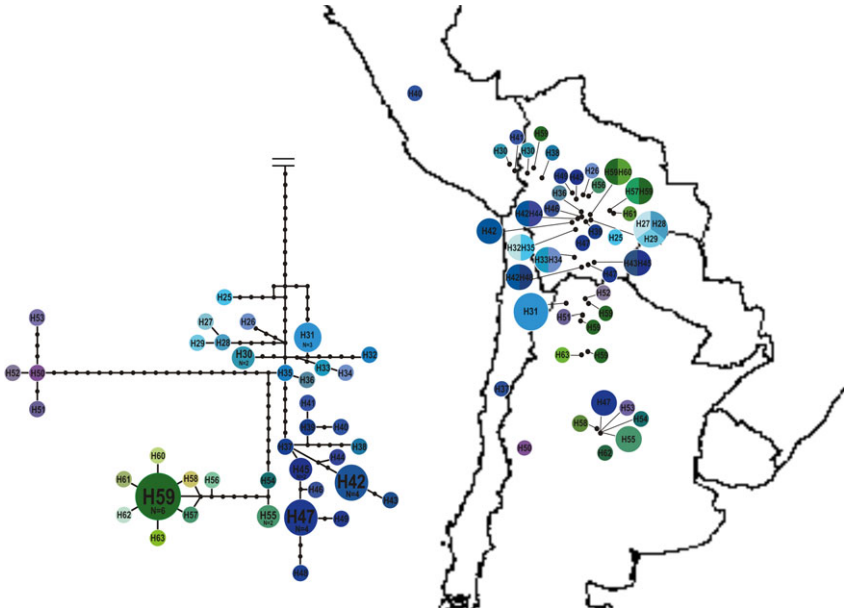

Figure 6. Distribution of plastid DNA (*rpoB-trnC-petN*, *trnK-matK-trnK*, *ycf1*) haplotypes previously identified as *Tillandsia virescens*. The frequency of each haplotype is represented by the size of the circles.

T. capillaris s.l. and *T. virescens s.l.* These clades correspond to the classification suggested by Till (1984) using morphological characters. However, the internal structure of the *T. virescens* clade requires further attention because of the high variability of morphotypes growing in Bolivia. The individuals of *T. kuehhasii* and *T. virescens s.l.* (*T. williamsii* and *T. dependens*) are positioned inside the '*virescens* clade', with *T. kuehhasii* forming a distinct clade. Clear delimitation of infraspecific taxa in this clade

requires further study using morphological characters and increased sampling; for example, *T. williamsii*, as described by Rusby (1910), comprises larger plants growing especially at high elevations in Bolivia, whereas another taxon which is morphologically and environmentally well defined, *T. dependens*, is nested in different positions in the '*virescens* clade'. For wide-ranging species in which connectivity between populations is interrupted, the different environmental conditions across the distribution could lead to changes in morphology (Gavrilets, 2004). In the present study, populations growing in the Andean regions of Bolivia and northern Argentina have high environmental variability which results in a clear genetic structure (this work) and high morphological differentiation (Till, 1992); this might have contributed to the process of divergence.

The nuclear marker *PHYC* yielded a less resolved topology than the plastid markers; most individuals of *T. virescens* resulted in a polytomy with some *T. capillaris* (Figs S1 and S2). Some individuals morphologically identified as *T. capillaris* (B29, A4, A12, A13, A15, A17 and A45) were included in *T. virescens*, hinting at possible hybridization events. These individuals belong to populations in northern Argentina and central Bolivia growing in sympatry (see localities in Table 1). Instead, most *T. capillaris* samples with some genetic structuring grow at opposite ends of the distribution area, e.g. Peru and north-western Argentina (La Rioja and Catamarca provinces) (Figs S1 and S2).

Barfuss (2012) stressed that the *PHYC* marker provides fairly good resolution in phylogenetic trees of Tillandsioideae, but our preliminary results indicated that *PHYC* is probably not well suited for use in the *T. capillaris* complex. The unrooted phylogenetic network obtained from *PHYC* showed some incongruence with the plastid DNA. This could be interpreted as a sign of hybridization (Russell *et al.*, 2010). The populations of *T. capillaris* and *T. virescens* growing in close proximity, even in the same locality, could support a hybridization hypothesis. However, the existence of recent hybridization or reticulation events was not specifically addressed by our study.

The *T. capillaris* complex: distribution in a montane environment

The results of the haplotype networks of the plastid markers support the hypothesis that the extant lineages have undergone recent and rapid radiation with subsequent diversification into newly formed ecological niches. Niches such as western Bolivian dry valleys and northern-central Argentinian low and humid mountains were created by the orography of the Andes. With regard to phylogeographical patterns, the distribution of 63 plastid DNA haplotypes in 69 populations also resulted in two main lineages co-occurring from north to south, with high haplotype diversity (Figs 5 and 6). The most frequent haplotypes (H1, H2, H3, H42, H47 and H59) were considered to be derived, occupied marginal positions in the network and were geographically widespread, whereas the haplotypes with the most internal positions, H24 and H35, were less frequent and closer to the ancestor between the two species *T. capillaris* and *T. virescens*. These haplotypes appeared geographically at close quarters in the mid-Andean region (Bolivia), indicating this zone as a centre of diversity.

The haplotype patterns were correlated with morphotypes. We identified two haplogroups with a non-random distribution based on morphology, but ambiguous geographical structuring or patterns corresponding to environment. Nevertheless, the phylogenetic study discriminated the two species in sympatric populations, e.g. in populations 21 (Bolivia, Valle Grande), 27 (Sucre) and 52 (Argentina, Yerba Buena). This suggests a certain level of reproductive isolation and genetic distinctiveness between them, and has been reported in other studies of taxonomically difficult species complexes (Wee *et al.*, 2015). The analysis of each species in greater detail is required to clarify geographical patterns.

Tillandsia capillaris is molecularly and morphologically more homogeneous than *T. virescens*, but comprises populations with sufficient genetic variation to expand into new habitats and, at the same time, to maintain certain cohesion. *Tillandsia capillaris* forms large populations in a number of different environments, including vertical granite walls at high elevations, semi-arid forests in valleys and humid mountain forests with native and exotic hosts (Benzing, 1990), and the hyper-arid coastal zone of Chile and Peru (Rundel & Dillon, 1998). Reproductive biology and mating systems in this species have not been studied in detail. A single study by Gilmartin & Brown (1985) reported cleistogamy in the small flowers of *T. capillaris s.l.* (= *T. capillaris* forma *hieronymii*).

Tillandsia virescens is genetically more divergent than *T. capillaris*, with most of the morphotypes growing in high and arid mountains. This diversity is still not sufficiently understood and could be attributed to evolution in isolated microsites along the species range (Till, 1984, 1992). Elevation heterogeneity and life on extensive, fertile cordilleras are also major drivers of epiphytic plant diversity (Givnish *et al.*, 2014). However, a well-distinguished clade corresponding to specimens classified as *T. virescens*

s.s. (see Table 2) was well differentiated, growing in low and humid habitats.

Tillandsia capillaris and *T. virescens* are widespread epiphytes in the Andes. In line with other bromeliads, this confirms that the central Andes is one of the centres of diversity, not only for the family, but also for many species groups (Till, 1992; Givnish *et al.*, 2011, 2014). The wide phenotypic variability of these species make it desirable to deepen studies on the potential influence of historical factors in shaping genetic variability and structure.

CONCLUSIONS

Our results revealed the overall genetic differentiation between *T. capillaris* and *T. virescens*, with plastid markers defining two main clades comprising high haplotype diversity and hinting at a complex evolutionary history involving geographical expansion in mountainous habitats. Our findings have implications for the assessment and definition of evolutionarily significant units in *T. capillaris* and *T. virescens* in the central and southern Andes, and therefore in conservation strategies. Effective Andean mountain conservation depends on an accurate knowledge of the genetic structure, phylogeny and geographical distribution of all taxa, especially those with extended distributions, to define taxon delimitation clearly. Traditionally, conservation biology used the number of species to measure biodiversity and considered the taxonomic category and geographical distribution patterns of taxa to establish priority sites (Stuessy, 2009). Different criteria may change the number of species of a genus, and therefore the geographical boundaries of the entities. The establishment of conservation strategies is the task that follows the critical definition and naming of organisms to be conserved.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Bayesian gene tree reconstruction for one single-copy nuclear gene (*PHYC*, 1161 bp). The different lineages identified previously with the plastid marker for the *Tillandsia capillaris* complex are indicated by different colours: blue, *T. virescens*; red, *T. capillaris*. Posterior probabilities on branch nodes are given as decimal numbers.

Figure S2. Unrooted phylogenetic networks (splitstree) for the single-copy nuclear gene (*PHYC*, 1161 bp). The different evolutionary core lineages in the *Tillandsia capillaris* complex are indicated by different colours: blue, *T. virescens*; red, *T. capillaris*.