1- Environment: Taxonomy and morphology

Morphologic and genetic variability of *Chusquea fendleri* Munro (Bambusoideae: Poaceae) in Venezuela

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Abstract

Chusquea fendleri is a scandent woody bamboo, described originally by August Fendler, based on a single collection of the Colonia Tovar, located in the State of Aragua, in Northern central mountains of Venezuela. Further collections carried out in the last 30 years revealed that *C. fendleri* has a very broad geographic and altitudinal distribution in Venezuela (1,700-2,750 m a.s.l.), which we believe is the cause of the conspicuous morphological variability observed across the country and the source of local and regional phenotypes. The aim of our research was to determine whether these phenotypes should be included in *C. fendleri*, or considered new species. An extensive sampling was carried out, which included all of the voucher specimens available in national and regional herbaria, as well as fresh specimens, collected in field trips conducted in different cloud forests of the Venezuelan Andes that had not been sampled previously. Our study consisted of a comparative analysis, based on morphological characters and microsatellite markers. The morphological analysis included only vegetative characters, since over 80% of the study specimens collected were infertile. The genetic analysis was based on PCR amplifications of 7 SSR markers designed for *Guadua angustifolia*, given that no SSR markers have been developed for this genus. 43 morphological characters were analyzed, of which 18 varied consistently and considered informative. Of the 7 SSR markers assayed, only 3 detected polymorphic alleles of use to separate closely related populations. The morphological analysis revealed the existence of three distinctive phenotypes, whereas the genetic analysis only two. Based on the morphological and genetic analysis, we recognize two morphological phenotypes of *C. fendleri* and a potentially new, sister species.

Key words: cloud forests, scandent woody bamboos, SSR markers

Introduction

With approximately 174 species *Chusquea* Kunth is considered the most diverse genus of the American bamboos as well as Neotropical bamboos (Judziewicz et al. 1999; Clark 2001; Fisher et al. 2014; Ruiz-Sánchez et al. 2015; Clark et al. 2015). In Venezuela, 22 species of *Chusquea* have been described to date, with representatives growing from 500 - 4,010 m asl; although the greatest diversity occurs in the Andean cloud forests of the Cordillera de Mérida, followed by the Guiana Highlands (Clark and Ely 2011, 2013). The following research deals with the morphological and genetic variability of *Chusquea fendleri* Munro in Venezuela.

The species was originally described by Coronel Munro in 1868, based on a single collection made by August Fendler during the XIX century in the Colonia Tovar; in the central-northern mountains, covered by dense cloud forests, between 1,700-2,300 m asl. *C. fendleri* can be easily recognized due to the presence of conspicous adventitious roots, derived from the subsidiary buds of the culm nodes, an infrequent character, yet shared by a few central American species of this genus (Ruiz-Sánchez et al. 2014). *C. fendleri*, Subg. *Chusquea*, belongs to the section *Chusquea* Kunth, an heterogeneous group, with approximately 20 species described to date, that gathers many of the taxa of uncertain position occurring in mid and high-elevation Andean forests, characterized by scandent or climbing culms, terete, solid internodes, several to numerous triangular or circular central buds, subtended by several to numerous subsidiary buds, extravaginal and less frequently infravaginal branching patterns (Clark 1989; Fisher et al. 2009).

The original description of *Chusquea fendleri* by Munro (1868), concerning the vegetative aerial organs (translated from latin) states: "Culms solid with a diameter at the base of 1.3 cm; culm leaves large, abaxial surface tomentose, culm leaf with a conspicuous fusion at the base 3 cm long, culm sheath asperous, blades short, triangular, apex acuminate. Culm nodes slightly swollen, prophyll triangular, superimposed to numerous buds; internodes long, glabrous or slightly scabrous. Branch complements 30.5-40.0 cm long, geniculate at the base, subsidiary branches with slightly swollen nodes, bearing 5-6 separate leaves; these may eventually bear florets. Subsidiary branches frequently aborted, short, curved of thorny appearance. Foliage leaves up to 10.1 cm long, blade lineal-lanceolate, glabrous, with 6-8 conspicuous secondary nerves, base attenuate, apex long, setose-acuminate, margins serrate with long teeth. Sheath margins ciliate, sometimes glabrous, inner ligule conspicuous.

According to Judziewicz et al. (1999), *C. fendleri* is endemic of the Northern region of Venezuela. However, further herbaria revisions and repeated field trips across the country suggested that this species occurs across a much broader geographical and altitude range, which extends from the Northwestern cloud forests of the Venezuelan Andes, to the central and northeastern cloud forests between 1,600-2,750 m a.s.l. According to Fernández and Ely (2017), this broad distribution range has lead to consistent regional and local variations in foliage leaf traits. These authors, based on a comparative analysis of

morphological and anatomical of foliage leaves in specimens identified as *C. fendleri* and *C. aff. fendleri*, identified five different phenotypes: a first group constituted by the Northern central and Northeastern cloud forests, and four Northwestern Andean groups: the Andean proper group, distributed all across the Cordillera de Mérida and three Andean subgroups of restricted, local distribution: the Niquitao 3 group (National Monument of Niquitao), the Valle group (El Valle, National Park Sierra La Culata) and the Teleférico group (Parque Nacional Sierra Nevada).

Given that leaf traits are strongly influenced by local environmental conditions, further studies in this species were proposed, including additional morphological characters, as well as comparative DNA analysis, based on simple sequence repeat markers (SSR), with the objective of determining whether all of the specimens previously identified as *C. fendleri* and *C. aff. fendleri* where effectively the same species, sister species or just local and/or regional phenotypes. Microsatellite or SSR markers have proven very useful to separate closely related species and to assay genetic variability within and among populations of a same species. Due to their high mutation rates that originate allelic polymorphisms (Lewin 1990; Zhao et al. 2015), SSR markers have been successfully used in other American bamboos such as *Guadua angustifolia* Kunth to distinguish between local and regional phenotypes in Colombia (Marulanda et al. 2002; Muñoz et al. 2012).

Although population studies employing SSR are relatively scarce in Bambusoideae and the majority have been conducted in Asian bamboos, they have no doubt proved useful to discriminate between closely related species and assay population diversity (Nayak and Rout 2005; Ely 2009; Kitamura et al. 2009; Zhan et al. 2009; Dong et al. 2012; Attigala et al. 2017). To date, the only SSR markers developed for American woody bamboos were those designed by Pérez-Galindo et al. (2009) for *Guadua angustifolia* and by Abreu et al. (2011) for *Aulonemia aristulata* (Döll) McClure; yet no specific SSR markers have been developed for species of the genus *Chusquea*.

The aim of the following study was to assess the phenotypic variability of *C. fendleri* in Venezuela, examining specimens identified as *Chusquea fendleri* and as *C. aff. fendleri*, with the purpose of determining whether the morphological variability observed in the field and in national and regional herbaria collections correspond to local phenotypes of the species, or whether we are dealing in some cases with sympatric sister species of restricted distribution. In order to fulfill this objective, we included a comparative analysis based on culm and leaf traits; also, a comparative genotype analysis, based on SSR markers, using DNA samples from specimens of all of the localities sampled during the study.

Methods

Sampling material for morphological and genetic analysis

Study samples consisted of voucher specimens, including herbaria vouchers and specimens studied by Fernández and Ely (2017) (Table 1, Appendix 1). Only culm and foliage leaf characters were included in this study, given that less than a 20 % of the specimens examined were in fertile condition, and in all of the cases, corresponded to specimens collected in the North Central and Northeastern cloud forests. The

remaining 80% of the specimens, all in vegetative condition account for all of the Andean cloud forests specimens (Table 1).

Table 1. Localities sampled across the country included in this study for specimens of *Chusquea fendleri* and *C. aff. fendleri* Munro. NP: National Park.

Locality	Condition	Georeferentiation		Altitud
		N	W	(m asl)
N.P. Waraira Repano (Avi)	Fertile	10° 33' 54"	66° 52' 59"	1,680-2,000
Vía Colonia Tovar (Co To)	Fertile	10° 26' 23"	67° 09' 14"	1,879-2,095
Serrania Turimiquire (Tur)	Fertile	10° 05' 15"	64° 00' 09"	2,000-2,195
Vía Pregonero (Pre)	Infertile	07° 57' 36"	71° 49' 42'	2,420-2,610
Vía Capaz (Cap)	Infertile	08° 39' 13"	71° 21' 01"	2,098-2,190
N.P. Sierra Nevada, El Morro (Morro)	Infertile	08° 29' 42"	71° 12' 35"	1,970-2,340
N.P. Sierra Nevada, Trayecto Teleférico (Tele)	Infertile	08° 34' 47"	71° 07' 01"	2,400-2,450
N.P. Sierra Nevada, Páramo El Tusta (Tus1, Tus2)	Infertile	08° 23' 40"	71° 32 42"	2,480-2,700
N.P. Sierra de La Culata, El Valle (Valle)	Infertile	08° 42' 30"	71° 05' 6"	2,490-2,600
N.P. Sierra Nevada, Santo Domingo (Sto Do)	Infertile	08° 49' 57"	70° 44' 22"	2,560-2,700

A total of 43 morphological traits or characters were compared, which included foliage leaf characters analyzed previously by Fernández and Ely (2017). Both measurable and non-measurable characters were included in the analysis. Character states were defined and assigned a condition or character state, in the case of qualitative, non-measurable characters. Measurable characters, were assigned to interval ranges, according to their size. Both measurable and non-measurable characters were coded 0 or 1, according to character state or range of values. Coded character states were used to build a binary matrix (0/1). Data of specimens from a same locality were averaged; however, when consistent differences were observed in a same locality, these were treated as different phenotypes.

The following list indicates all of the characters included in this analysis (measurable and qualitative or non-measurable characters). Variation ranges for measurable characters are indicated in parenthesis.

Characters included in the study:

1- *Rhizomes* [amphimorph (0); pachymorph (1)]; 2- *culm height* [2-4 m (0); (>5 m) (1), 3- *internode contour* [terete (0), sulcate (1)], 4- *internode length* [10-20 cm (0); >20 cm (1)], 5- *internode surface* [smooth to scabrous (0); strigose or very asperous (1)], 6- *adventitious roots on basal and mid-culm nodes* [present (0); absent (1)], 7- *number of adventitious roots per node* [5-12 (0); >12 (1)], 8- *duration of culm leaves* [persistent (0); deciduous (1)], 9- *portion of the internode covered by the culm leaf* [1/2 (0); 2/3-3/4 (1)], 10- *culm leaf total length* [8-14 cm (0); >14≤24 cm (1)], 11- *fusion of the culm leaf sheath from the*

base [1.25-2.0 cm from the base (0); ≥3.0 cm from the base (1)], 12- *length of culm leaf sheath* [6-15 cm (0); >15 cm (1)], 13- *width of culm leaf at the base* [≥2≤4 (0); >4≤6 (1)], 14- *length of culm leaf blade* [1.5-4.0 cm (0); >5 cm (1)], 15- *sheath/blade ratio* [3-6 (0); 7-9 (1)], 16- *culm leaf sheath margins* [ciliate on non-overlapping margins and sheath summit (0); glabrous (1)], 17- *culm sheath apex* [continuous with the blade (0); not continuous, narrower at the summit (1)], 18- *length of the culm leaf inner ligule* [0.2-0.4 mm (0); >0.4 mm (1)], 19- *culm leaf abaxial surface* [antrorsely scabrous to hispid (0); densely strigose (1)], 20- *culm leaf base* [conspicuously fringed (0); not fringed or sparsely pilose (1)], 21- *girdle width* [0.2-0.3 mm (0); ≥ 0.5 cm (1)], 22- *contour of the central bud* [*triangular* (0); ovate or round (1)], 23- *number of the subsidiary buds flanking central bud* [14-35 per node (0); >35≤70 per node (1)], 24- *prophyll abaxial surface* [ciliate (0); glabrous (1)], 25- *branching pattern* [always extravaginal (0); combining more than one pattern (1)], 26- *branch complement position* [geniculate, (0); weakly geniculate (1)], 27- *number of branches per complement* [18-40 per node (0); >40≤70 per node (1)], 28- *length of the branch complements* [12-40 cm (0); >40≤65 cm (1)], 29- *number of foliage leaves per branch* [3-5 (0); >5 (1)], 30- *pseudopetiole length* [0.1-0.2 cm (0); >0.3 cm (1)], 31- length of foliage leaf inner ligule [0.2-0.3 cm (0); >0.3≤0.8 cm (1)], 32- *contour of foliage leaf blade* [lineal (0); lineal-lanceolate (1); broad lanceolate (2)], 33- foliage leaf length [7-12 cm (0); >12≤17 (1)], 34- foliage leaf blade width [0.5-1.2 cm (0); >1.2≤1.5 cm (1)], 35- *foliage leaf length : width ratio* (L:W) [6-8 (0); 7-15 (1)], 36- *number of abaxial veins in foliage leaves* [5-7 (0); >7 (1)], 37- foliage leaf blade margins [strong serrate (0); weakly serrate or serrulate (1)], 38- leaf blade apex [setose-acuminate (0); acuminate (1)], 39- *foliage leaf blade base* [cuneate (0); oblique (1)], 40- *abaxial surface of the foliage leaves* [glabrous to sparsely pilose (0); densely pilose forming sericeous indumentum (1)], 41- *type of microhairs* [simple (0); compound or multicell (1)], 42- *foliage leaf blade consistency* [cartaceous (0); membranaceous (1)], 43- *number of chlorenchyma layers* [3-4 (0); >4 (1)].

Morphological analysis

The results of the comparative morphological analysis based on the morphological characters were used to create a binary matrix, in which qualitative traits were coded (0/1) according to character states (presence/absence). Measurable characters, were assigned to intervals according to their size, and these coded according to the range of values. Non-measurable characters states were coded according to character state. Coded values were then included in a binary matrix, which was used to build a similarity dendrogram, based on morphological traits (UPGMA, Sokal & Michner 1958), according to phenetic distances of Dice (1945), employing 1,000 bootstrap replications, using PAST version 1.94B (Hammer et al. 2001).

DNA sampling and amplification

Total genomic DNA was isolated from fresh or dried leaf samples of herbaria vouchers, of a total of 28 leaf samples; since not all of the samples yielded positive PCR amplifications, especially in the case of

voucher specimens. However, we managed to obtain representatives of a 90% of the localities included in the study (Table 1). DNA samples consisted of 2-4 specimens/locality, which were collected at a minimal distance of 50 m between stands (Ely 2009). Extractions were made from fresh leaf tissues (when available) or voucher specimens deposited in VEN. DNA extractions and processing were conducted in the Laboratorio de Genética y Química Celular (GeQuimCel) of the University of the Andes, in Mérida, Venezuela. DNA extractions were conducted using a modified protocol of Dellaporta et al. (1983). Modifications included an increase in the amount of ground tissue from 0.5 suggested in the protocol to 0.7g and prolonging the precipitation of the DNA samples in isopropanol to 24 hours at -20ºC.

PCR amplifications were carried out using two types of molecular markers: the *rpl16* intron sequence of the ribosomal protein 16 of the Chloroplast (Table 2), previously used in phylogenetic studies of Bambusoideae and Chusqueinae (Kelchner and Clark 1997; Fisher et al. 2009, 2014). The intron sequence of the gene *rpl16* served the purpose of confirming that the DNA extractions corresponded effectively to a Chusqueinae, knowing that the amplified products yield a band of 1.030-1.050 Kb (Kelchner and Clark 1997, Ely 2009), easily visualized in agar gels at 0.8%. However, as previous phylogenetic studies in this group have proved, the use of this marker to separate taxa at an infrageneric level in *Chusquea* is limited, since chloroplast genes and intron sequences fail to separate closely related species in this genus (Kelchner and Clark 1997; Fisher *et al.* 2009, 2014). For these reasons, we selected SSR markers, with the purpose of distinguishing closely related species and determining whether consistent morphological differences, treated as different phenotypes were actually associated to specific genotypes, based on the presence or absence of specific alleles. The SSR markers selected for this study consisted of seven primers designed for *G. angustifolia* by Pérez-Galindo et al. (2009) (Table 2), which have proven very useful to distinguish between local phenotypes in *G. angustifolia* in Colombia (Muñoz et al. 2012).

Table 2. Sequence size and annealing temperatures of the SSR primers used in the PCR amplifications.

Locus	Primer sequence (5´- 3')	Size(pb)	Annealing temperature (°C)
FJ444930	R:CTTCACATGGTCTCACAAG F:GTCTAGCAATCAATTTGAAG	225-270	55
FJ444929	R:TAGATCTTCCTAATCAAAGTGG F:ACTAACCGATTGTCCCGTCTAG	240-260	48
FJ444934	R: CCCGACAGATAGATGGTCAAA F: CTCATTTCTCAATTGCGCAAGAG	170-190	50
FJ444931	R: GTCAATCACGCCAGCTCTAACA F: CTCTGACATGTATGGATCTTGCA	225-275	50
FJ444936	R: CCCAACAAAGATGGTCAGAT F: CAGGAGATGAGCCTGTTAGT	180-220	55
FJ444935	R: CTAGGCCCACTCCTATCCCA F: AGCTTCCTCAGAATGCCTAATTA	210-260	55
FJ476076	R: CCTTCAATTAGTACATAGATAG F: GTACAGAACCATCTCATCCT	230-255	55

Two taxa of American woody bamboos were included in the analysis as outgroups and as positive controls for the DNA amplification reactions; these were: *Guadua angustifolia* Kunth (Guaduinae) and *Chusquea aurea* LG Clark & F. Ely (Chusqueinae), with the purpose of testing the transferability of these SSR markers, designed originally for *G. angustifolia* to species of the genus *Chusquea*. If these SSR markers are indeed transferable, they should separate taxa of different genera (*Guadua* and *Chusquea*), as well as taxa of the same genus, amplifying specific alleles in each case. In the case of well defined local phenotypes, these should also be easily separated by specific SSR alleles.

PCR reaction mix consisted of Buffer Taq 1X, $MgCl_2$ 3.5 mM, dNTPs 0.2 mM, 1U Taq Polimerase enzyme, 2µM of primers and 10 ng of DNA and 0.4 mg/ml bovine serum albumin (BSA) recommended by Posso (2011). PCR reactions were all carried out in a thermal cycler *MJ Research*. PCR program for both types of primers consisted of an initial denaturation temperature of 94ºC during 2 minutes, followed by 35 cycles of initial temperature de 94ºC during, 1 minute of annealing temperature of 50ºC during 50 seconds, followed by an extension temperature of 72ºC during 2 minutes, and a final extension temperature of 72ºC during 5 minutes.

The PCR amplification products were electrophoresed on agarose gels containing 0.5 µg/ml ethidium bromide and TAE buffer at 1x. All of the gels were photographed on a UV transilluminator. Amplifications products using SSR markers were separated in 2.5% agarose gels. The size of all of the PCR amplification products were determined according to a DNA Promega ladder of 100-1500 bp, with a step of 100 bp. All of the electrophoresis were run at a average current of 70 v 4.5 h.

DNA data analysis

Although SSR markers are codominant, these were treated as dominant markers, since their purpose was to reveal the presence/absence of specific alleles and not their heritage. The size, pattern and number of bands (alleles) for each SSR locus were also used to create a binary matrix (Appendix 2), based on the presence/absence of alleles. The binary matrix was then used to create genetic distance dendrograms (Table 5). Software used for genetic analysis were POPGENE32 version.31 (Yeh et al. 1999) and PAST version.1.94B (Hammer et al. 2001). The genetic similarity dendrogram was based on UPGMA algorithms (Sokal and Michener 1958), using the distance of Dice (1945). The dendrogram based on genetic distances included *G. angustifolia* as an outgroup of the Euchusquea clade, since the Guaduinae subtribe is a sister to Chusqueinae (Wysocki et al. 2015), therefore it should segregate from the *Chusquea* taxa included in the analysis (*C. aurea*, *C. fendleri* and *C. aff. fendleri*). A third binary matrix was constructed combining both morphological and SSR matrixes and was used to create a consensus dendrogram, based on morphological and genetic similarities amongst specimens, using Neighbour-joining (Saitou and Nei 1987), with a total of 1000 bootstrap replications.

Results

Morphological analysis

The comparative morphological revealed that of the 43 morphological traits used to characterize woody bamboos, only 28 of these varied consistently and were treated as informative characters (tables 3 and 4); whereas the remaining 15 morphological traits were considered characteristic of *Chusquea fendleri* Munro *senso lato* (groups 1-4) as well as of specimens labeled under *C. aff. fendleri* (group 5).

The following characters where cataloged as common to *Chusquea fendleri* and *C. aff. fendleri* specimens, given that they shared the same character state in all groups, therefore they were not included in tables 3 and 4:

1- *Rhizome type* (always amphimorph, *character 1*), 2- *culm height* (2-6 m, *character 2*), 3- *internode contour* (always terete), *character 3*), 4- *adventitious roots* (always present, *character 6*), 5- *duration of culm leaves* (persistent, *character 8*), 6- *culm leaf sheath margins* (ciliate, *character 16*), 7- *culm leaf inner ligule* (0.2-0.4 mm, *character 18*), 8- *culm leaf base* (always conspicuously fringed, *character 20*), 9- *girdle width* (0.2-0.4 mm, *character 21*), 10- *contour of the central bud of the nodes* (always triangular, *character 22*), 11- *central bud prophyll margins* (always ciliate, *character 23*), 12- *branching pattern* (always extravaginal, *character 24*), *branch position* (always geniculate, *character 25*), 13- *foliage leaf margins* (strong serrate to serrulate on the same blade, *character 36*), 14- *foliage leaf blade apex* (always setose-acuminate, *character 37*), 15- foliage leaf base (always cuneate, *character 38*).

Based on this detailed morphological study of vegetative characters, we provide a description for specimens labeled as *Chusquea fendleri* Munro *senso lato* occurring in Venezuelan, which includes only

specimens of groups 1-4 (tables 3 and 4). Maximum, minimum and mean values of measurable characters are indicated in the description, minimum and maximum values are indicated in parenthesis. Group 5, labeled as *Chusquea aff. fendleri* (represented solely by the population Teleférico) was excluded from this description, due to significant differences regarding a both qualitative or non-measurable and measurable characters. Characters that differed consistently between group 5 and groups 1-4 were: internode surface, culm leaf abaxial surface and length of the fusion from the base of the sheath, number of subsidiary buds and branch complements, length of the branch complements, foliage leaf blade contour, consistency and mesophyll thickness. These differences segregate group 5 from the other four groups in the distance dendrogram based on morphological characters (Figure 1).

Table 3. Culm traits compared in specimens of *C. fendleri* collected in different localities of Venezuela.

Character	Group 1	Group 2	Group 3	Group 4	Group 5
Internode					
Length (cm)	(12)18.2(24)	(11)23.2(28)	(12)23(33)	(13)22(27)	(18)22(35)
Diameter at the base of the culm (cm)	(0.8)1.2(1.5)	(0.9)1.3(1.8)	(0.9)1.2(1.5)	(0.7)1.2(1.4)	(0.8)1.3(2.0)
Surface	Smooth to scabrous	Scabrous	Scabrous	Scabrous	Strigose
Culm leaf					
Total length (cm)	(9)13(20)	(8)14(23)	(10)12(20)	(10)14(16)	(11)15(20)
Width at base (cm)	(2.4)2.9(3.2)	(2.5)3.4(4.5)	(2.9)3.7(4.6)	(2.4)3(3.5)	(2.4)3.8(5.9)
Portion of the internode covered	1/2-2/3	1/2-2/3	1/2	1/2	2/3
Fusion at base (cm)	(1.2)2.5(3.0)	(1.6)2.4(2.7)	(1.4)2.2(2.8)	(1.6)2.3(2.5)	(0.8)1.4(2.2)
Sheath length (cm)	(6)10.8(16.5)	(9.6)12(18)	(9)13.2(15.4)	(7)12(13)	(8)11.7(14.5)
Blade length (cm)	(2.6)3.7(4.5)	(1.5)2.4(4.3)	(2.2)3(3.9)	(1.8)2.4(2.6)	(1.8)3.1(5)
S:B ratio	3.5-5.4	3.7-7.2	4.3-6.2	4.8-5.4	3-6.5
Constriction S:L	Evident to attenuate	Evident or attenuate	Absent	Evident	Evident
Abaxial surface	Scabrous to hispid	Scabrous to hispid	Scabrous to hispid	Scabrous to hispid	Strigose
Node					
N° of buds	(36)47(59)	(34)51(70)	(37)48(53)	(36)43(52)	(14)27(31)
Number of roots	(9)13(18)	(9)15(19)	(11)13(15)	(10)14(16)	(5)8(9)
Branches					
Length (cm)	(17)34(63)	(15)39(59)	(29)43(60)	(22)47(64)	(19)29(50)
N° branches	(28)43(54)	(26)47(70)	(32)39(48)	(29)45(50)	(15)24(33)
N° leaves x branch	4-6	4-6	4-7	4-6	4-5

Caption: *Group 1*: represented by specimens of the Cordillera de La Costa (Colonia Tovar and Waraira Repano) and Serranía de Turimiquire. *Group 2*: represented by the Andean specimens of the Cordillera de Mérida (Capaz, El Tusta, El Morro, Pregonero, Niquitao 1,2 and Santo Domingo). *Group 3*: El Valle. *Group 4*: Niquitao 3. *Group 5*: Teleférico. Mean values are indicated for measureable characters (central value), maximum and minimum values are indicated in parenthesis. S:B = Sheath: Blade ratio. S:L = Sheath summit: blade.

Table 4. Leaf traits measured in specimens of *C. fendleri* compared in specimens of *C. fendleri* collected in different localities of Venezuela.

Character	Group				
	1	2	3	4	5
Pseudopetiole length (mm)	1-3	1-3	2-3	2-3	2-3
Blade contour	Lineal to lineal-lanceolate	Lineal-lanceolate	Lineal-lanceolate	Lineal-lanceolate	Broad lanceolate
Blade length (cm)	(7.7)10.4(13.7)	(7)11(17)	(9)12(14)	(8)12(14)	(9)11(16)
Blade width (cm)	(0.6)0.8(0.9)	(0.7)0.9(1.7)	(0.7)1.2(1.4)	(0.8)1.2(1.5)	(1.1)1.6(2.0)
L:W relation	7(12)17	7(10)15	7(9)12	8(11)13	(6)7.4(8)
Inner ligule length (mm)	1-3	1-3	5-8	1-2	1-3
Abaxial surface	Glabrous, exceptionally sparsely pilose	Sparse to dense sericeous indumentum	Sparse to dense sericeous indumentum	Glabrous	Glabrous
N° veins	5-7	6-7	6-7	6-7	7-9
Layers of chlorenchyma	4-5	4-5	3-5	4-5	3-4
Blade consistency	Cartaceous	Cartaceous	Cartaceous	Cartaceous	Membranaceous
Type of microhair	Simple	Simple	Simple	Multicell	Simple

Caption: *Group 1*: represented by specimens of the Cordillera de La Costa (Colonia Tovar and Waraira Repano) and Serranía de Turimiquire. *Group 2*: represented by the Andean specimens of the Cordillera de Mérida (Capaz, El Tusta, El Morro, Pregonero, Niquitao 1,2 and Santo Domingo). *Group 3*: El Valle. *Group 4*: Niquitao 3. *Group 5*: Teleférico. Mean values are indicated for measureable characters (central value), maximum and minimum values are indicated in parenthesis. L:W = Length: Width ratio.

Description of *Chusquea fendleri* Munro *senso lato (groups 1-4)*

Rhizomes amphimorph. Culms 2-6 m tall, diameter at the base of the culm of (0.8)1.5(1.8) cm, internodes (8) 22.2(33) cm long, terete, solid, smooth to scabrous. Basal and midculm nodes always bearing (9)14 (19) adventitious roots. Culm leaves persistent, disintegrating on the culms, extending 1/2 - 2/3 along the internodes, (8)13.5(23) long, fused (1.2)2.4(2.8) cm from the base; sheaths (6)12.3(18) cm long and (2.4)3.25 (4.6) cm wide at the base, continuous with the blade or narrowing at the sheath summit, blades triangular, (1.5)2.9(4.5) cm long, S:B ratio = 3.3-7.2, sheath margins and apex always ciliate, outer ligule inconspicuous, inner ligule straw colored, 0.2-0.4 mm, culm leaf adaxially glabrous and abaxially antrorsely scabrous to hispid, base conspicuously fringed, with cream to straw colored trichomes, girdle conspicuous, corky, 0.2-0.4 mm. Nodes at mid culm bearing a large, triangular central bud, subtended by (34)46(70) smaller, subequal, subsidiary buds, prophyll margins ciliate. Branching always extravaginal; branch complements always geniculate at the base, with (28)44(70) branches per complement, branches (17)38(64) cm long, with (4)5(7) leaves per complement. Foliage leaf pseudopetiole 2.0-3.0 mm long, sheaths glabrous, margins ciliate at the apex and sheath summit, leaf blades cartaceous, lineal or lineal-lanceolate, (7.7)11(17) cm long and (0.7)1.0(1.7) cm wide, L:W = (7)12(17); outer ligule inconspicuous, inner ligule membranaceous, glabrous, (1)3.5(8) mm long, blades abaxially non-tessellate, with 6-8 veins, margins serrate to serrulate on the same blade, blade apex always setose-acuminate and base cuneate; blades abaxially glabrous to sparse or densely pilose, indumentum sericeous, microhairs typically simple, less frequently compound or multicell. Mesophyll chlorenchyma 3-5 layers thick.

Variations observed in culm and leaf traits are summarized in tables 3 and 4. Groups mentioned in and may be appreciated better in the distance dendrogram (Figure 1). Various conspicuous characters mentioned in this description may be appreciated in Figure 2.

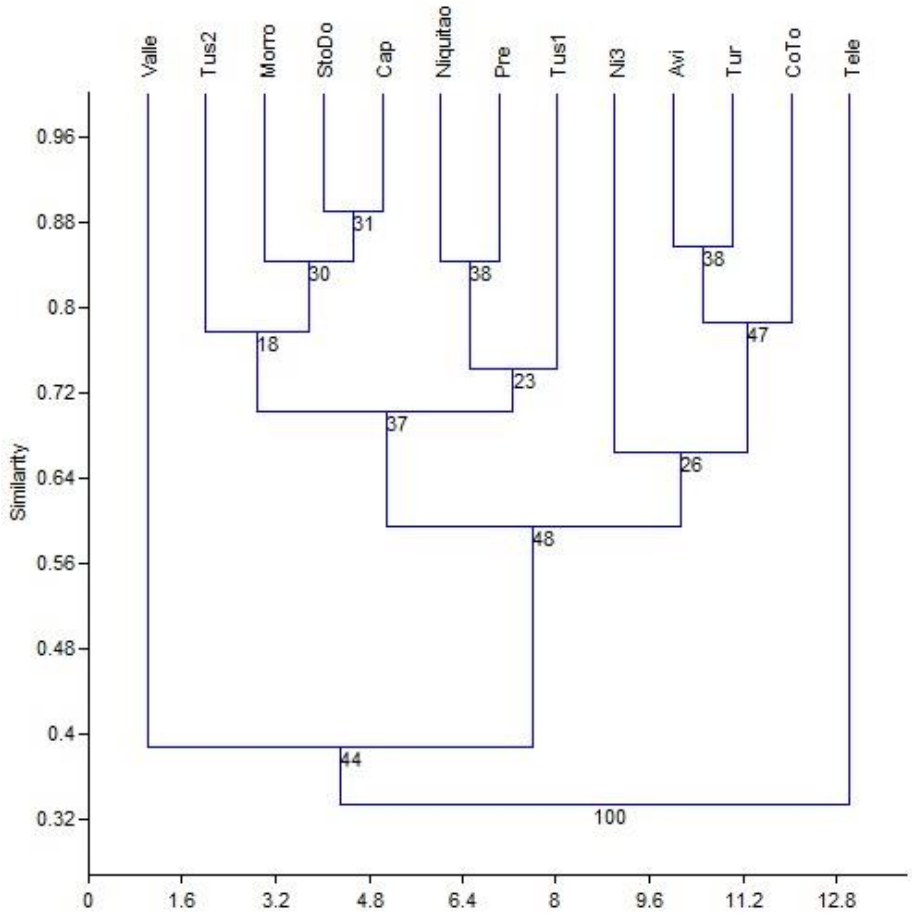

Figure 1. Similarity dendrogram based the UPGMA algorithm (Sokal and Michener 1958), using the distance coefficient of Dice (1945), constructed with the morphological data of Venezuelan specimens of *C. fendleri* and *C. aff. fendleri. Abbreviations of the localities included in this study: Avi: Waraira Repano (El Avila), Cap: Capaz; CoTo: Colonia Tovar; Morro: El Morro; Niquitao: Niquitao (samples 1 and 2), Niq3: Niquitao 3; Pre: Pregonero; Sto Do: Santo Domingo; Tele: Teleférico; Tur: Serranía de Turimiqire; Tus1 and Tus2: El Tusta; Valle: El Valle.*

Figure 1. A: Culm with numerous branches in specimens of *Chusquea fendleri* collected in Waraira Repano (El Ávila). **B:** Extravaginal branching in *C. aff. fendleri* (Teleférico population), nodal area swollen due to adventicious roots bellow the culm leaf sheath. **C**, **D** and **E**: Andean specimens collected under *C. fendleri*. **C:** Nodal area and collar, with short, triangular prophyll and central bud, surrounded by numerous subsidiary buds, laterally flanked by short adventicious roots**. D:** Detail of the nodal area showing elongated, triangular prophyll and central bud, surrounded by numerous subsidiary buds, laterally flanked by adventitious roots. **E:** Detail of the nodal line and collar showing relatively long adventitious roots at mid culm and dip of the infranodal line. **F:** Developing adventitious roots on the basal nodes of the culm in *C. aff. fendleri*.

In two of the Andean localities, consistent variations were observed between stands of a same locality and were treated as different phenotypes, such was the case of the specimens collected in El Valle and Niquitao (Niquitao 1,2 and Niquitao 3*), which were treated as two separate groups (tables 3 and 4). The dendrogram based exclusively on morphological characters revealed three main groups (Figure 1). The first node separates the samples of the locality Teleférico (first group at the right, Figure 1) with a bootstrap value of 100 %, which shares only a 32 % of similarity with the other locality specimens. The second group (lower left-hand node, Figure 1), is represented by the Andean specimens of the locality El Valle (Valle), which shares only a 38% of similarity with the remaining seven localities, due to the consistently longer inner ligules of the foliage leaves (Table 4). However, this separation is weakly supported (bootstrap values of 44 %). The separation of third node from the first two nodes is also weakly

supported (bootstrap value of 48 %) and gathers all of the remaining localities, which share a 58% of similarities.

Although bootstrap values are weakly supported after the second node, two large groups derive from the third node; the right-hand group, which share a 68% of similarity, represented by all of the remaining Andean populations (Capaz, El Morro, Pregonero, Niquitao, Santo Domingo, Tusta 1, 2 and 4), which share scabrous internodes, lineal-lanceolate foliage leaves, short inner ligules, sparsely pilose to densely pilose blades and simple microhairs, with exception of the Andean specimens labeled as Niq3* (Niquitao 3). The population labeled as Niq3* (Niquitao, Trujillo, Cordillera de Mérida) differs from the rest of the Andean specimens, as well as with the other specimens of the same locality, due to the abaxially glabrous foliage leaf blades and multicell microhairs. The remaining right-hand group derived from the this third node group, also weakly supported is constituted by the three non-Andean populations of the country, which include the locality where the original type specimens was collected, Colonia Tovar (Cordillera de La Costa, State of Aragua), the population of Waraira Repano (originally known as Cerro El Ávila, Cordillera de La Costa, Distrito Capital), and the most northern locality, represented by the population located on the Serranía de Turimiquire (State of Anzoátegui), characterized by smooth to scabrous internodes, glabrous lineal to lineal-lanceolate foliage leaves and simple microhairs

All of the mentioned groups may be considered local variations of *Chusquea fendleri*, with exception of the Andean group of the locality Teleférico, located on the Northern slope the Sierra Nevada de Mérida, which we have identified as *Chusquea aff. fendleri* and may be distinguished from true *C. fendleri* specimens, due to the consistently lower number of branches per node, internodes with a rough, strigose, almost warty surface. Other distinctive characters of specimens belonging to the *C. aff. fendleri* group (group 5, tables 3 and 4) are its shorter, lanceolate foliage leaves (inferior L:W ratio) and abaxially glabrous blades of membranaceous consistency.

DNA amplifications and SSR analysis

Positive DNA extractions (30 in total) were confirmed for all of the specimens collected under *C. fendleri* and *C. aff. fendleri* of the different localities in this study, using the intron sequence of the gene *rpl16*, which was used as a positive control for Bambosideae DNA producing clear, reproducible bands of 1.030-1.060 Kp in all of the samples, including the two outgroups, *Guadua angustifolia* and *Chusquea aurea*.

SSR FJ444930 failed to amplify in repeated tests, despite modifications of annealing temperatures and $MgCl_2$ concentrations. The remaining six markers amplified clear, reproducible bands, ranging between 125-850 pb (Table 5). The results of the amplifications were tallied and used to construct the binary matrix mentioned previously (Appendix 2). Absent bands were treated where treated as missing alleles in the analysis.

Table 5. Number and size of the alleles obtained through PCR amplification using SSR markers designed by Pérez-Galindo *et al.* (2009).

Marker	Study					
	Guadua		*Chusquea fendleri*		*C. aff. fendleri*	
	Size (pb)	N°Alleles	Size (pb)	N°Alleles	Size (pb)	N°Alleles
FJ444929	240-260	8	290	1	290	1
FJ444931	225-275	9	185	1	-	0
FJ444934	170-190	8	125-140	2	140-460	2
FJ444935	210-260	3	-	0	-	0
FJ444936	180-220	9	170-530	7	170-380	3
FJ444076	230-255	4	450	1	450	1

Of the six SSR markers that gave positive amplifications, not all amplified DNA of *C. fendleri* or *C. aff. fendleri*; such was the case of the marker FJ444935, which yielded positive amplifications only for the two outgroups, producing a single band of 670 bp in the case of *C. aurea* and of 512 bp in *G. angustifolia* (Appendix 2). The markers FJ444929 and FJ444076 were monomorphic in all of the specimens of *C. fendleri* and *C. aff. fendleri* that yielded positive amplifications, producing a single allele of 290 bp and of 450 bp, respectively (Table 5). The marker FJ444929 amplified a single allele of 290 bp in the outgroup specimen *G. angustifolia*, yet none in *C. aurea*. No positive amplifications where observed for either of these outgroups with the marker FJ444076 (Appendix 2), although the reference study carried out by Pérez-Galindo et al. (2009) mentions 4 different alleles for this marker (Table 5).

The remaining markers were polymorphic for all of the specimens assayed (Table 5); two of these markers resulted useful to separate taxa at the level of genus as well as species, separating the two outgroups from the study specimens. FJ444931 amplified an allele of 460 bp exclusive of *Guadua angustifolia*, whereas in the species of the genus *Chusquea* the same marker yielded a single allele of 400 bp in *C. aurea* and of 185 bp in all of the specimens of *C. fendler* yet none in the specimens labeled as *C. aff fendleri* of the locality Teleférico (Table 5, Appendix 2). The marker FJ444934 also resulted useful to differentiate amongst species of *Chusquea* from *Guadua*, as well as amongst species of *Chusquea*, producing a single band of 155 bp in *G. angustifolia*, segregating it from the remaining the species of *Chusquea* (Appendix 2), in which it amplified a single band 460 bp in the specimens identified as *C. aff. fendleri* (Teleférico group), two bands in the specimens labeled as C. *fendleri* of the locality Niquitao, one of 125 bp and another of 140 bp and a single band of 140 bp for all of the remaining specimens labeled as *C. fendleri* (Table 5), yet none for the outgroup *C. aurea* (Appendix 2).

FJ444936 resulted the most polymorphic of all; in terms of allele diversity, yielding a total of 7 different alleles in specimens labeled as *C. fendleri*, ranging between 170-530 bp and 3 alleles in the specimens labeld as *C. aff. fendleri* ranging between 170-380 bp (Table 5). This marker proved also very useful for separating species of a same genus, as well as specimens of different populations, segregating *C. aurea* from the rest of the samples with a band of 850 bp; Tusta 3 from the rest of the Andean populations, due to a band of 710 bp, and Niquitao 3 (Niq 3*) with a band of 220 pb (Appendix 2). Other examples of unique

alleles, also in a low frequency and restricted to a single stand within a same locality were the allele 485 bp in the specimens labeled Pre1(Pregonero, State of Táchira), the allele of of 380 bp associated exclusively to the stand labeled Tus* (El Tusta, Mérida), and the allele and of 370 bp exclusive of Valle (El Valle, Mérida) (Appendix 2).

This distance dendrogram based solely on the presence/absence of alleles (Figure 3) evidences the early separation of *Guadua angustifolia* as the first outgroup, followed by a second node, which separates *Chusquea aurea* from the *C. fendleri* and alike group (bootsrap value of 55 %), with which it shares only a 50% similarity. The third node includes all of the specimens identified as *C. fendleri* that share a that share a 75% of a similarity (bootsrap value of 82 %). The fourth node separates on the left the specimens labelled as *C. aff. fendleri* collected of the locality Teleférico (Parque Nacional Sierra Nevada), with a bootsrap value of a 66%. The remaining groups are very weakly supported, as may be inferred from the low bootstrap values (Figure 3), and the remaining nodes combine specimens from the Cordillera de la Costa, Serranía de Turimiquire with the Andean specimens of the Cordillera de Mérida.

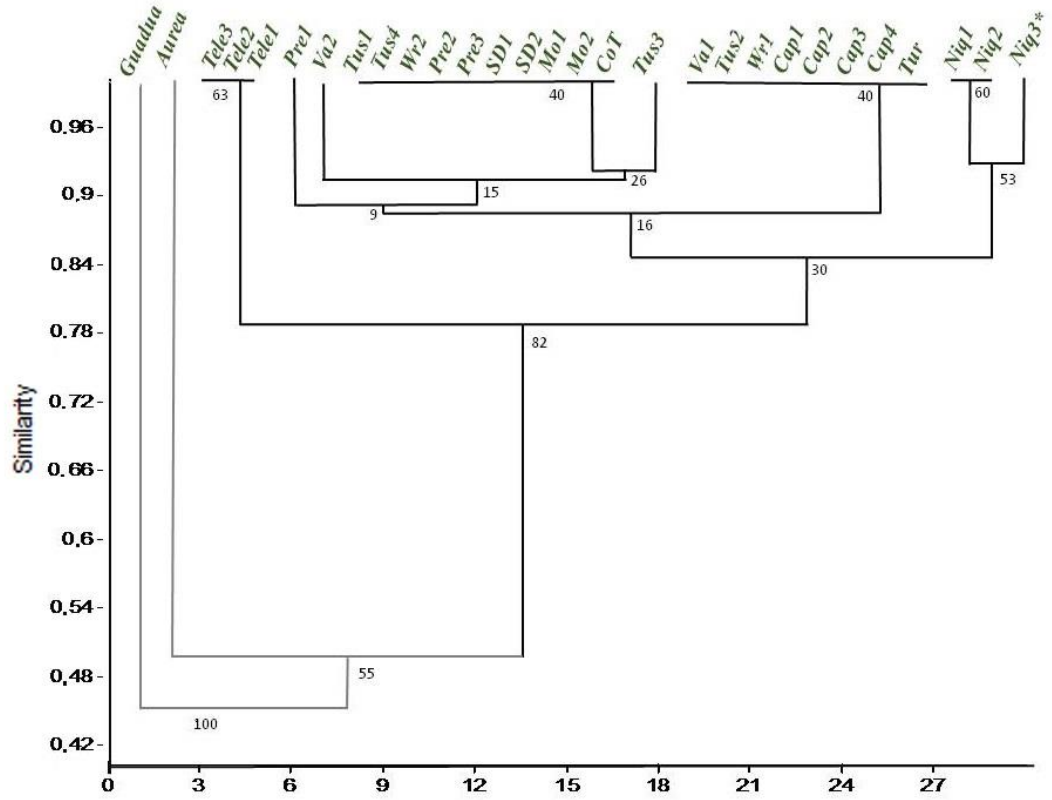

Figure 3. Similarity dendrogram based the UPGMA algorithm (Sokal and Michener 1958), using the distance coefficient of Dice (1945), based on the SSR allele data of Venezuelan specimens of *C. fendleri* and *C. aff. fendleri*. *Abbreviations of the localities included in this study: Wr 1,2: Waraira Repano (El Avila), Cap: Capaz; CoT: Colonia Tovar; Mo 1,2: El Morro; Niq 1,2: Niquitao; Niq3*: Niquitao 3; Pre 2,3: Pregonero; SD 1,2: Santo Domingo; Tele 1,2,3: Teleférico; Tur: Serranía de Turimiqire; Tus 1,2,3,4: El Tusta; Va 1,2: El Valle.*

The dendrogram based solely on genetic distances (Figure 3) did not segregate in the same manner or with the same resolution the Andean groups from the Northern Central and Northeastern regions as the

dendrogram based solely on morphological traits (Figure 1). Although the resolution groups derived from the fourth node is a very poor, the group represented by the specimens of Niquitao (Bootstrap value of 53 %) maintained the same tendency observed in the dendrogram based on morphological traits, which separates the specimens Niquitao 3 (Niq3*) from the two remaining samples of this locality (bootstrap value 60 %).

Based on the genetic distances obtained with the six SSR markers, we can only distinguish only two major groups: the *C. aff. fendleri* Andean group of the locality Teleférico and the *C. fendleri* proper, represented by the populations occurring in the Cordillera de La Costa, Serranía de Turimiquire and all of the Andean populations with exception of Teleférico. The left-hand group is constituted by those populations characterized by the presence of unique alleles or infrequent alleles, which was the case of the specimens from Pregonero 1 (Pre1*), Valle 2 (Va2*) and Tusta 3 (Tus3*) (Figure 3).

Combined dendrogram

The binary matrix combining morphological and genetic data (Appendix 3) was used to create a consensus dendrogram, using Neighbour-joining method (Figure 4).

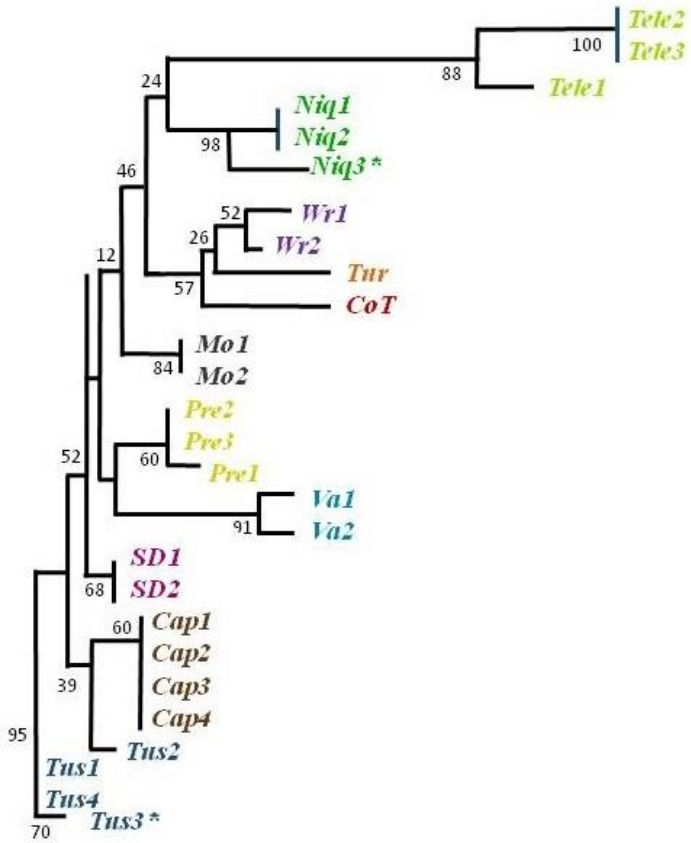

Figure 4. Similarity dendrogram based the Neighbour-joining algorithm (Saitou and Nei 1987), using the distance coefficient of Dice, combinig SSR allele and morphological data of Venezuelan specimens of *C. fendleri* and *C. aff. fendleri*.

Based on this dendrogram, only two groups are well supported:

a- The *C.aff. fendleri* group of the locality Teleférico, which segregates with a high bootstrap value (88 %), characterized by rough, asperous internodes, due to the strigose, stiff indumentum; culm leaf sheaths abaxially strigose; fewer branch complements; membranaceous foliage leaves, 7-9 veins, blades always broad lanceolate, abaxially glabrous, simple microhairs and unique alleles for the SSR markers FJ444934 and FJ444936 (Appendix 2).

b- The remaining groups, which based on these findings, we may now consider *C. fendleri* proper *senso lato*, constituted by all of the remaining nodes, based on the low bootstrap value (24 %), with which they separate from this first group (Figure 4).

Nevertheless, even when we must consider this large, morphologically as a single species, it becomes evident that it is a very heterogenous group, due to the existence of local and/or regional phenotypes, which may be placed in three large groups:

1. 1.- The non-Andean representatives of the Northern Central and Nothern Eastern cloud forests facing the coast; represented by the populations of *C. fendleri* proper, described by August Fendler, of the locality Colonia Tovar (State of Aragua), the populations of the Waraira Repano (formerly known as Cerro El Ávila (Capital District, Distrito Capital) and Serranía de Turimiquire (State of Aragua), characterized by smooth to scabrous internodes, culm leaf sheaths abaxially scabrous to hispid, numerous branch complements per node; foliage leaves lineal to lineal-lanceolate, cartaceous, abaxially glabrous, rarely sparsely pilose, with short inner ligules (1-3 mm), 5-7 veins, simple microhairs, and a similar allele profile for the SSR markers.
2. 2- The Andean group constituted by the specimens of *C. fendleri* occurring the upper montane Andean cloud forests of the Cordillera de Mérida, represented by the populations of the localities Capaz, El Morro, El Tusta, El Valle Santo Domingo, (State of Mérida) and Pregonero (State of Táchira), by scabrous internodes, culm leaf sheaths abaxially scabrous to hispid, numerous branch complements per node, foliage leaves lineal-lanceolate blades, abaxially pilose or sparsely pilose, cartaceous, with short inner ligules (1-3), 5-7 veins, simple microhairs and a similar allele profile, with exception of the specimens Pre*1 and Tus*3.
3. 3- Within the Andean group (Cordillera de Mérida) of representatives of *C. fendleri*, the local variation of the species, which may be considered a local phenotype, represented by the populations of El Valle (Valle) and Niquitao 3 (Niq.*3). All of the specimens collected in the locality known as El Valle (State of Mérida) were characterized by a longer inner ligule in the foliage leaves (5-8 mm) and a allele of 380 bp for the allele marker FJ444936 (Appendix 2, Figure 4). The other local variation or local phenotype is Niquitao 3 (Niq*3, State of Trujillo) that differs from the other Andean specimens as including specimens collected in the same locality, due to its culm leaf sheaths with short scabrous trichomes (instead of scabrous or hispid)

and glabrous foliage leaves (tables 3 and 4) with multicell microhairs on the abaxial surface of the blade (Fernández and Ely 2017), and the allele of 530 bp with the SSR marker FJ444936.

Discussion

Our aim in this comparative analysis of the species described as *Chusquea fendleri* in Venezuela was to determine whether the notable phenotypic variability observed in the field and in herbaria voucher specimens collected under this name, were the result of its adaptation to different environmental conditions across the country, taking in account that local soil and microclimate conditions may vary considerably within relatively small geographic distances, on this mountain range, depending on elevation, slope orientation and geological origin. Taking in account the strong influence of the environmental factors, we included not only morphological but molecular data as well, with purpose of obtaining a better resolution of what should be considered as the species proper and separating from sister species, keeping in mind the difficulties of differentiating between closely related species in this complex section.

According to Fisher et al. (2009, 2014), the Euchusquea clade is of very recent origin in the region and in active species radiation; therefore, limits between *Chusquea* species, especially those of Andean origin remain unclear. Extensive phylogenetic analysis carried out by these authors using on DNA chloroplast genes have not proportioned yet an adequate resolution for closely related taxa. For these reasons, Fisher et al. (2009, 2014) recommend the use of the current morphology-based taxonomy defined for the subtribe to assess the phenotypic diversity observed in this group.
Taking in account the challenge of obtaining a clear separation between closely related species, we recurred to the use SSR markers, since they have proven useful to separate closely related species, populations and individuals of a same population (Ely 2009), which may be tricky in the case of clonal organisms, such as bamboos. This preliminary study was limited to Venezuela, where the species was described as endemic, and may limit the scope of our analysis, due to the relatively limited geographic extension of the mountain areas in country, and we are aware that our results could change, if this species is reaches Colombia and Ecuador, and we would have to broaden our study to a regional scale.

Based on our findings, we consider the following morphological traits taxonomically informative in this group: internode surface, type of abaxial indumentum on the culm leaf sheath, number of subsidiary buds subtending the central bud, number of adventitious roots at the nodes, number and length of the branches, foliage leaf blade contour, length, L:W ratio, inner ligule length, number of veins, consistency (related with mesophyll thickness), presence and density of an abaxial indumentum on the foliage leaf blades and the type of microhairs. Variations in the culm diameter at the base, internode length, shape of the central bud, girdle width, length of the branches and pseudopetioles did not follow any consistent pattern, varying within a same stand and locality. Another character that varied inconsistently was the base of the culm leaf blade, which could be continuous with the sheath or become narrower at the sheath summit.

Based on these morphological traits we acknowledge three regional phenotypes:

1- *Chusquea fendleri* proper of the Northern Central and Eastern mountains (Cordillera de La Costa and Serranía de Turimiquire, 1,600-2,000 m sal).
2- The Andean *C. fendleri* group (Cordillera de Mérida 1,700-2,700 m asl).
3- The Andean *C. aff. fendleri* group (Cordillera de Mérida, 2,400-2,450 m asl), represented by the specimens of the locality Teleférico, which could potentially be a new, sister species.

The condition of scabrous internodes is fairly common in *Chusquea* species and is frequently included in various systematic studies in this group (Clark 1993, Viana et al. 2011, Da Mota et al. 2013, Clark and Ely 2013, Ruiz-Sánchez et al. 2014, 2015). In contrast, the presence of adventitious roots is not as frequent in this genus, although they have been describe for some of the species, mainly Central American representatives with verticillate branches, such as *C. circinata* Soderstrom & C. Calderón, *C. coronalis* Soderstrom & C. Calderón, *C. pittieri* and *C. liebmannii* E. Fournier Hackel (Soderstrom and Calderón 1978, Ruiz-Sánchez et al. 2014, 2015). To date, *Chusquea fendleri* appears to be the only South American species of the genus in which they have been described as a consistent character with taxonomic value. The number and length of adventitious roots varies locally and regionally in this species and are undoubtedly related with the number of subsidiary buds of the nodes, which have taxonomic value, yet the length of roots is more likely influenced by the humidity conditions in which the culms develop (Ely personal observation), therefore, the length should not be considered of taxonomic value.

The presence of trichomes on the forming a sericeous indumentum on the abaxial surface of the foliage leaves is mentioned for the first time in *C. fendleri* and qualified as a morphological character subject to regional variations within the species; which are not uncommon in the Andean cloud forest representative of this species, particularly those growing at higher altitudes like the populations of the localities Santo Domingo and El Tusta (2,500-2,700 m a.s.l). The dense, sericeous indumentum observed in many of the Andean representatives of this species very likely developed as an adaptation to higher radiation levels and lower air temperatures, observed also in other species of the genus, mainly those associated to high altitude grasslands of the subgenus *Swallenochloa* (Kiyota 2011). The presence of macrohairs forming an indumentum on the abaxial surface of the foliage leaves has been treated as a trait of taxonomic value in this genus (Vieira et al. 2002, Kiyota 2011, Viana et al. 2011, Da Mota et al. 2013, Clark and Ely 2013, Ruiz-Sánchez et al. 2014). Our findings suggest that the presence and density of abaxial trichomes or macrohairs is a variable character in *C. fendleri*, that appears to be associated the Andean representatives; therefore it should not be considered a stable character in this species.
When we compare the allele diversity obtained with these SSR markers of *C. fendleri* with the studies using these markers conducted in for *Guadua angustifolia* in Colombia reported by Pérez-Galindo et al. (2009), we can appreciate a significant reduction in the number of alleles per locus in all of the markers assayed (Table 5). Recent population studies using these SSR markers, conducted also in *G. angustifolia*

by Posso (2011) and Muñoz et al. (2012) in the same region also indicate a relatively low allele diversity per locus, despite the larger number of samples included in their studies (32), yet in a much more reduced study site, in terms of geographic extension. The relatively low SSR allele diversity accounted for in this study could be related with deletions of repetitive sequences or recombination errors during recombination events, which are likely to occur during species evolution (Chenuil 2005, Zhao et al. 2015). Other explanations are that the repetitive sequences amplified by these markers are in a lower frequency in species of the genus *Chusquea*, due to mutations that generate replication errors during DNA recombination. However, mutation events these should occur at a relatively low rate in woody bamboos compared to other groups of plants, particularly in the monocarpic, semelparous species of the genus, due to the length of their flowering cycles, reducing the incidence of DNA polymorphisms in microsatellite loci due to slower evolution rates (Abreu et al. 2011).

Despite the low frequency for the majority of these SSR markers, it is worth stressing that this study revealed the existence of alleles exclusive of the genus *Chusquea* for the SSR markers FJ444934 and FJ444936, which yielded unique alleles for *C. fendleri* and *C. aff. fendleri*. The higher allelic diversity observed in FJ444936 and the appearance of local allelic variations in the species were also mentioned by Posso (2011) and Muñoz et al. (2012) in their population studies in *G. angustifolia.*

The molecular analysis conducted with these six SSR markers supported the results of the morphological analysis, allowing us to distinguish two major groups, *Chusquea fendleri* Munro and *C. aff. fendleri*, which could be potentially a sister species of *C. fendleri*. Future herbaria revisions are also necessary, in order to determine whether *C. fendleri* is endemic of the Venezuelan Andes or also reaches Colombia. Further studies could proportion fertile specimens of the group labeled in this study as *C. aff. fendleri*, which may be compared with the type specimens of *C. fendleri* deposited in VEN and confirm whether these differences also include reproductive characters, which are generally more stable, compared to vegetative organs like culms and leaves, in which case we could confirm the existence of a new, sister species. Future studies in this group including morphological and a higher number of molecular markers will contribute to clarify species diversity and occurrence in the tropical Andes, where the distinction between species may result complex to the recent origin of this group in the region (Fisher et al. 2009, 2014).

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Appendix 1. Vouchers examined in this study:
Chusquea fendleri VENEZUELA: **Aragua**: Via el Junquito - Colonia Tovar, Municipio Tovar, 2095m snm, 07/01/2015, *Nosawa s/n* (MERC); Colonia Tovar, road to El Junquito, 1818-2121 m, *A. Fendler 1627* GH, MO (Lectotype); Mun. Ricaurte, ca 5 Km W of Colonia Tovar, road to El Junquito 2000 m, *G. Gonzalez & G. Davidse 16226* (MO VEN); **Anzoátegui:** *J. Steyermark 61635* (US VEN) Anzoátegui: Cerro Peonía (Los Pajaritos), Serranía de Turimiquire, above Sta Cruz, Río Mantiales of Bergantín. **Distrito Capital**: P.N. P. N. Waraira Repano, 1680 m snm, 30/10/2013, *F. Ely, J. Mostacero & Torres 346* (MERC); El Ávila (Waraira Repano) cerca del Hotel Humboldt, 2000 m, *B. J. Manara sn* (VEN US). **Mérida:** *F. Ely, Márquez & J. Fernández 356* (MERC); P. N. Sierra Nevada. Vía Páramo El Tusta Municipio Sucre, 2480m snm, 11/04/2013 *F. Ely, J. Márquez & J. Fernández 358* (MERC); P. N. Sierra Nevada. Vía Páramo El Tusta Municipio Sucre, 2750 m snm, 11/04/2013 *F. Ely, J. Márquez & J. Fernández 359* (MERC); P. N. Sierra Nevada. Santo Domingo. Municipio Rangel, 2560 m snm, *F. Ely, J. Márquez, J. Fernández & A. Aranguren 368* (MERC); P.N. Sierra de La Culata, Valle-La Caña. Municipio Libertador, 17/10/2014, *F. Ely, J. Márquez & J. Fernández 369* (MERC); P. N. Sierra Nevada. Santo Domingo. Municipio Rangel, 2560 m snm, *F. Ely, J. Márquez, J. Fernández & Aranguren* 368 (MERC); Via Capaz, Municipio Andrés Bello, 04/11/2014, *F. Ely, J. Márquez & J. Fernández 370* (MERC); P.N. Sierra Nevada. El Morro. Município Libertador, 2340m snm, 10/11/2014, *F. Ely, J. Márquez & J. Fernández 372* (MERC); **Táchira**: Vía Pregonero. Municipio Rivas Dávila, 1610m snm, 29/05/2014 *F. Ely, O. Araque & D.* Castillo 366 (MERC). **Trujillo**: Monumento Natural Niquitao. Município Niquitao, 2220m snm, 10/11/2014, *F. Ely, J. Márquez & J. Fernández* 379 (MERC). *Chusquea aff. fendleri* Munro VENEZUELA: **Mérida:** P. N. Sierra Nevada, Trayecto Teleférico. Municipio Libertador, 2400-2450 m snm, 10/12/2014, *F. Ely, J. Fernández & J. Parra 389 and 390* (MERC); Sierra La Culata, Valle-La Caña. Municipio Libertador, 17/10/2014, *F. Ely & J. Fernández 369* (MERC); P. N. Sierra Nevada. Vía Páramo El Tusta Municipio Sucre, 2750 m snm, 11/04/2013 *F. Ely, J. Márquez & J. Fernández 359* (MERC). **Trujillo:** Monumento Natural Niquitao. Município Niquitao, 2420m snm, 10/11/2014, *F. Ely, J. Márquez & J. Fernández 381* (MERC).

Appendix 2. Binary matrix based on the results of the PCR amplifications using SSR markers designed by Pérez-Galindo *et al* (2009) in the specimens *Chusquea fendleri* and *C. aff. fendleri* of collected in Venezuela.

DNA (bp)	SSR marker																			
	929		931			934			935					936						076
	290	150	185	400	125	140	155	460	512	670	170	220	248	370	380	485	530	710	850	450
Tus1	1	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	?
Tus2	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	?
Tus3*	1	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	1	0	?
Tus4	1	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	?
WR1	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	?
WR2	1	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1
Niq1	1	0	1	0	1	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1
Niq2	?	0	1	0	1	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1
Niq3*	1	0	1	0	1	1	0	0	0	0	1	1	1	0	1	0	1	0	0	1
Pre1	1	0	?	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	0	?
Pre2	1	0	?	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1
Pre3	1	0	?	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1
Va1	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1
Va2	1	0	1	0	0	1	0	0	0	0	1	0	1	1	1	0	0	0	0	1
Tele1	1	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	1
Tele2	1	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	1
Tele3	1	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	?
Ca1	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1
Ca2	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1
Ca3	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1
Ca4	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1
SD1	1	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	?
SD2	1	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	?
Mo1	1	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1
Mo2	1	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1
CoT	?	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1
Tum	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1
Aur	?	0	0	1	0	0	0	0		1	1	0	1	0	1	0	0	0	1	?
Guadua	1	1	0	0	0	0	1	1	1	0	1	0	1	0	0	0	1	0	0	?

Appendix 3. Binomial similarity matrix based on SSR polymorphism data and morphological traits assayed in the specimens included in this study.

Sample	SSR marker										Morphological trait																		
	1					2	3	4	5																				
	A	B	C	D	E				A	B	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Tus1	0	0	1	0	0	?	1	1	0	0	1	1	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0
Tus2	0	0	0	0	0	?	1	1	0	0	1	1	1	1	0	0	1	0	1	0	0	0	0	0	1	0	0	1	0
Tus3	1	0	1	0	0	?	1	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0
Tus4	0	0	1	0	0	?	1	1	0	0	1	1	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0
WR1	0	0	0	0	0	?	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0
WR2	0	0	1	0	0	1	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0
Niq1	0	0	1	0	0	1	1	1	1	0	1	1	1	1	0	0	0	1	1	0	1	0	0	1	1	0	0	1	0
Niq2	0	0	1	0	0	1	1	?	1	0	1	1	1	1	0	0	1	1	1	0	1	0	0	1	1	0	0	1	0
Niq3	0	0	1	0	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	0	0	0	1	0	0	0	0	1
Pre1	0	1	1	0	0	?	?	1	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	1	1	0	0	1	0
Pre2	0	0	1	0	0	1	?	1	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	1	1	0	0	1	0
Pre3	0	0	1	0	0	?	?	1	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	1	1	0	0	1	0
Va1	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	0	0
Va2	0	0	1	1	0	1	1	1	0	0	1	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1	0	0	0
Te1	0	0	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	0	0	1	0	1	1	0	0	0	1	0	0
Te2	0	0	1	0	0	1	0	1	0	1	1	1	1	1	1	1	0	0	0	1	0	1	1	0	0	0	1	0	0
Te3	0	0	1	0	0	?	0	1	0	1	1	1	1	1	1	1	0	0	0	1	0	1	1	0	0	0	1	0	0
Ca1	0	0	0	0	0	1	1	1	0	0	0	1	1	0	0	0	0	1	1	1	0	0	0	1	1	0	0	1	0
Ca2	0	0	0	0	0	1	1	1	0	0	0	1	1	0	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0
Ca3	0	0	0	0	0	1	1	1	0	0	0	1	1	0	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0
Ca4	0	0	0	0	0	1	1	1	0	0	0	1	1	0	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0
SD1	0	0	1	0	0	?	1	1	0	0	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	0	0	1	0
SD2	0	0	1	0	0	?	1	1	0	0	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	0	0	1	0
Mo1	0	0	1	0	0	1	1	1	0	0	0	1	1	1	0	0	1	1	1	1	1	0	0	0	1	0	0	1	0
Mo2	0	0	1	0	0	1	1	1	0	0	0	1	1	1	0	0	1	1	1	1	1	0	0	0	1	0	0	1	0
CT	0	0	1	0	0	1	1	?	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0
WR1	0	0	0	0	0	?	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0
WR2	0	0	1	0	0	1	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0
Tur	0	0	0	0	0	1	1	1	0	0	0	1	1	1	?	0	0	1	1	0	0	0	0	1	0	0	0	0	0

Captions:

Left-hand column: Abbreviations of specimen's locality of origin. *Tus*: El Tusta, *Niq*: Niquitao, *Pre*: Pregonero, *Va*: El Valle, *Te*: Teleférico-Sierra Nevada, *Ca*: Capaz, *SD*: Santo Domingo, *Mo*: El Morro, *CT*: Colonia Tovar, *WR*: Waraira Repano, *Tur*: Serranía de Turimiquire.

Columns 1-5, SSR markers: **1(A-E):** SSR_{936} alleles: 710, 485, 380, 370 and 220 bp, respectively. **2:** SSR_{076} alleles: 450 bp, **3**: SSR_{931} alleles: 185 bp, **4**: SSR_{929} alleles: 290 bp; **5(A-B):** SSR_{934} alleles: 125 and 460, respectively. *Columns 6-23, Morphological traits:* **6:** internode surface; **7:** portion internode covered by culm leaf; **8:** culm-leaf constriction; **9:** sheath abaxial surface, **10:** sheath length, **11:** lamina length; **12:** culm leaf length/width ratio; **13:** number of subsidiary buds; **14:** number of adventitious roots; **15:** length of branch complements; **16:** number of branches; **17:** foliage lamina contour; **18:** lamina consistency, **19:** foliage leaves length/width ratio; **20:** abaxial surface of the lamina; **21:** foliage leaf inner ligule length; **22:** number of veins; **23:** Macrohairs; **24:** type of microhairs.