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# Molecular Phylogeny Estimation of the Bamboo Genus *Chusquea* (Poaceae: Bambusoideae: Bambuseae) and Description of Two New Subgenera

**Amanda E. Fisher,[1,4] Lynn G. Clark,[2] and Scot A. Kelchner[3]**

[1]Rancho Santa Ana Botanic Garden, Claremont Graduate University, Claremont, California 91711-3157, U. S. A.
[2]Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, Iowa 50011-1020, U. S. A.
[3]Department of Biological Sciences, Idaho State University, Pocatello, Idaho 83209-8007 U. S. A.
[4]Author for correspondence (afisher@rsabg.org)

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***Abstract*—***Chusquea* is a diverse genus of American woody bamboos, accounting for almost half of the woody bamboo species in the Neotropics. Previous analyses of molecular data have recovered four major lineages within *Chusquea*, but morphological synapomorphies have been identified only for subgenus *Rettbergia*. This study estimates a chloroplast phylogeny of *Chusquea* with a focus on relationships within the large and intractable Euchusquea clade. Phylogenetic analyses were conducted on 40% of the described species in *Chusquea*, with data from five chloroplast regions and a preliminary survey of the nuclear internal transcribed spacer complex. Several results from previous studies were corroborated, including the presence of two clades formerly comprising the genus *Neurolepis* and monophyly of subgenus *Rettbergia*. The clades formerly in *Neurolepis* are named as ***Chusquea*** subgenus ***Platonia*** and ***Chusquea*** subgenus ***Magnifoliae*** based on molecular support and potential morphological synapomorphies. We recovered two strongly supported and five weakly supported clades within Euchusquea, but relationships among these lineages were not resolved and species composition of the clades conflicts strongly with current taxonomic groupings based on morphology. Low resolution of the chloroplast phylogeny estimation, low variability in nuclear data, character conflict, and geographical distribution of chloroplast lineages all suggest a recent radiation of the Euchusquea clade. Given the present weak molecular support for relationships within Euchusquea and the lack of synapomorphic morphological characters to define clades, we recommend the use of the current morphology-based taxonomy as a practical means of assessing and describing diversity in the Euchusquea clade.

***Keywords*—**Bamboo, Neotropical radiation, chloroplast phylogeny, grass evolution, Shimodaira-Hasegawa test.

*Chusquea* Kunth is the most diverse genus of Neotropical woody bamboos, with 169 described species and estimates of as many as 220 species in total (Judziewicz et al. 1999; Fisher et al. 2009). It is the sole genus in subtribe Chusqueinae Soderstr. & Ellis and is well supported by molecular data as monophyletic (Kelchner and Clark 1997; Clark et al. 2007; Fisher et al. 2009; Kelchner and Bamboo Phylogeny Group 2013). Detailed reviews of the nomenclatural and taxonomic history of *Chusquea* can be found in Fisher et al. (2009) and Clark (1989). Following the recent taxonomic submergence of *Neurolepis* Meisn. within *Chusquea* (Fisher et al. 2009), diagnosable morphological characters for *Chusquea* are (i) spikelets with four glumes and a single, terminal, fertile floret lacking a rachilla extension, and (ii) the presence of two papillae on subsidiary cells of the foliar stomatal complex. Lack of aerial branching, a single bud per node, and strongly nerved ligules appear to be plesiomorphic in the genus.

*Chusquea* species are found in mountainous regions of the Neotropics and austral temperate zone (21°N-47°S), with centers of diversity in Mexico, Central America, the northern Andes, and southeastern Brazil. These bamboos primarily occur in montane forests and high-elevation grasslands and tend to occupy specialized habitats such as Andean páramos, Brazilian campos de altitude, *Araucaria* forests, *Nothofagus* forests, and Mexico's pine-oak forests (McClure 1973; Soderstrom and Calderón 1978b; Veblen 1982; Clark 1992; Londoño 1996; Clark 1997; Judziewicz et al. 1999; Safford 1999). The dominant vegetation in these high-elevation grasslands often consists of *Chusquea* species such as *C. pinifolia* (Nees) Nees and *C. heterophylla* Nees in southeastern Brazil, *C. subtessellata* Hitchc. in Costa Rica's Talamanca range (Tol and Cleef 1994), *C. spencei* Ernst and *C. angustifolia* (Soderstr. & C. E. Calderón) L. G. Clark in Venezuelan páramos, *C. tessellata* Munro in Colombian 'bamboo páramos,' and *C. stuebelii* (Pilg.) L. G. Clark, *C. aristata* Munro, *C. rigida* (L. G. Clark) L. G. Clark, and *C. nana* (L. G. Clark) L. G. Clark in Ecuador (Laegaard 1992; Clark 1996). Many *Chusquea* species are able to aggressively colonize areas disturbed naturally or by humans (Judziewicz et al. 1999) and some species can form dense thickets in montane forests. Once established, *Chusquea* bamboos may inhibit germination of surrounding plants by creating a thick leaf litter (Tol and Cleef 1994) or by limiting understory light (Widmer 1997; Holz and Veblen 2006; Giordano et al. 2009). In some cases, *Chusquea* species are primary drivers of tree regeneration cycles. For example, mature stands of *C. montana* Phil., *C. uliginosa* Phil. (as *C. tenuiflora* Phil.), and *C. culeou* E. Desv. in austral Andean beech forests can out-compete shade intolerant *Nothofagus* seedlings (Veblen 1982). Eventually, the monocarpic *Chusquea* flower and senesce, allowing a new cohort of *Nothofagus* to grow at an accelerated rate (Holz and Veblen 2006).

Excluding subg. *Platonia* and subg. *Magnifoliae* (*Neurolepis* I and II clades), *Chusquea* characteristically have abundant vegetative branching derived from multiple buds per node and solid culms (McClure 1973; Clark 1989, 1997), but otherwise exhibit considerable morphological variation (Fig. 1). Species in montane forests are typically scandent to clambering on surrounding vegetation, but species in high-elevation grasslands have an erect and shrubby habit. *Chusquea* have unusually complex vegetative branch complements due to the presence of up to 200 buds at each stem node. These buds are commonly dimorphic, with distinctive arrangements of a single large central bud and two to many smaller subsidiary buds (Soderstrom and Calderón 1978a; Clark 1989).

Previous work identified the sister group to *Chusquea* (Clark et al. 2007; Fisher et al. 2009) and provided a preliminary molecular survey of the genus (Kelchner and Clark 1997). *Chusquea* collections were first described as *Nastus* Juss. and were viewed by later authors as being closely

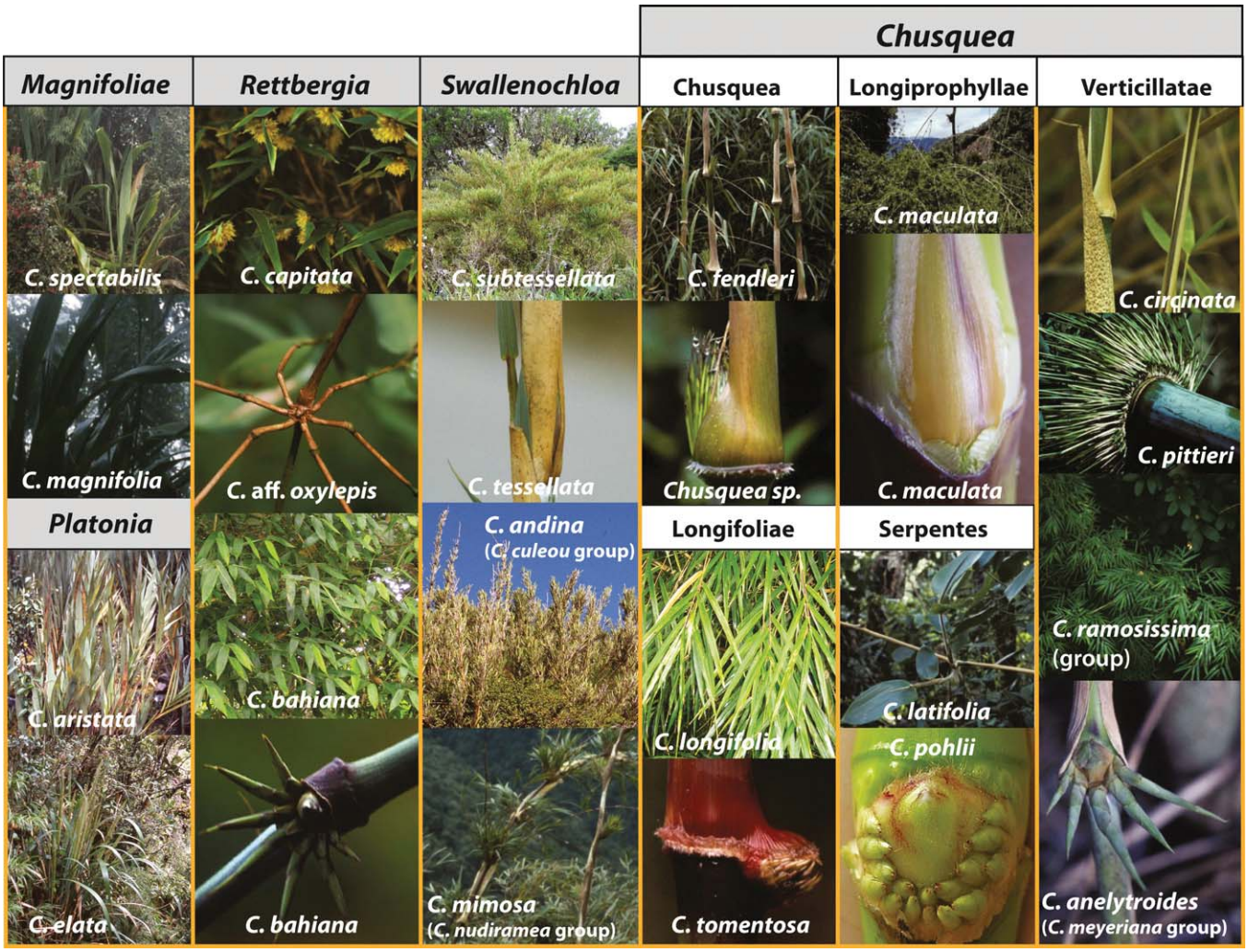


FIG. 1. *Chusquea* classification and morphological variation. Photographs of characteristic species for each group are arranged below each heading, within the orange outline. Five subgenera (gray boxes) are listed, including the newly described subg. *Platonia* (*Neurolepis* I) and subg. *Magnifoliae* (*Neurolepis* II). Within subg. *Chusquea* there are five sections (white boxes). With the exception of subg. *Magnifoliae* and subg. *Platonia*, *Chusquea* species tend to have abundant vegetative branching and they exhibit considerable variation in their bud complement, branch complement, and overall habit. Photographs by L. G. Clark except *C. subtessellata* and *C. pohlii* by A. E. Fisher.

aligned with *Nastus* based on the shared character of a single floret per spikelet (Kunth 1816; Desvaux 1831; Nees von Esenbeck 1835; Munro 1868). Although Kelchner and Clark (1997) found evidence that one sampled *Neurolepis* species is sister to *Chusquea* s. s., morphological analyses suggested a sister relationship between *Chusquea* and *Nastus* + *Hickelia* A. Camus (Clark 1997). Clark et al. (2007) explicitly tested these two hypotheses and found that *Chusquea* + *Neurolepis* (Chusqueinae) is sister to an Arthrostylidiinae + Guaduinae clade with chloroplast *rpl16* intron data, even though morphological data resolve Chusqueinae as sister to the Ehrhartoideae (rice) subfamily (Clark et al. 2007). Phylogenetic estimation based on a five region chloroplast dataset by Kelchner and Bamboo Phylogeny Group (2013) corroborated the *rpl16* intron data of Clark et al. (2007), but refuted analyses based on morphology in that there is a moderately supported sister relationship between the Chusqueinae and an Arthrostylidiinae + Guaduinae clade. Importantly, Clark et al.'s (2007) molecular analysis of the one-flowered bamboos unexpectedly recovered two distinct lineages of *Neurolepis* paraphyletic to *Chusquea*. Fisher et al. (2009) verified the paraphyletic chloroplast relationship of *Neurolepis* and *Chusquea* with increased taxon sampling and statistical testing of alternative hypotheses. The two *Neurolepis* lineages are now submerged into *Chusquea* as subg. *Platonia* and subg. *Magnifoliae*.

Three additional subgenera have been recognized (subg. *Chusquea*, subg. *Rettbergia* (Raddi) L. G. Clark, subg. *Swallenochloa* (McClure) L. G. Clark, Table 1) and are mainly defined by differences in habit, branching pattern, and bud arrangement (Clark 1989, 1992, 1997). Using sequence data from the chloroplast *rpl16* intron, Kelchner and Clark (1997) found that the sampled species of subg. *Rettbergia* are monophyletic, whereas subg. *Swallenochloa* and subg. *Chusquea* are polyphyletic within a larger, well-supported clade informally referred to as the Euchusquea clade.

Despite these earlier analyses of the genus based on molecular data and recent work across the Bambusoideae (Sungkaew et al. 2009; Kelchner and Bamboo Phylogeny Group 2013), little is known about evolutionary relationships within *Chusquea*, especially within the species-rich Euchusquea. The current study was devised to test the monophyly of taxonomic groups within Euchusquea and to provide a more complete phylogenetic framework for *Chusquea*. To that end,

TABLE 1. A summary of the genus *Chusquea*. Species are classified into five subgenera and then into sections or informal groups. For each taxon the table lists the number of species, the morphological characteristics, and known distribution, habitat, and elevation preferences.

| | Species # | Characteristics | Distribution and Habitat |
|---|---|---|---|
| subg. *Chusquea* | 88 | 2–∞ subsidiary buds; triangular or circular central bud | Widespread |
| sect. *Chusquea* | 20 | Extravaginal branching; several to many subsidiary buds per node | Mid- and high-elevation forests of Andes |
| sect. *Longifoliae* | 11 | Infravaginal branching; triangular central bud; 18–80 subsidiary buds per node; long, narrow foliage leaf blades | Montane forests of southern Mexico, Central America, and northern Andes |
| sect. *Longiprophyllae* | 6 | Viny to arching or pendant habit; scabrid culm leaves; infravaginal branching; subsidiary buds in a usually tight circular cluster; elongated central bud prophylls; glume II twice as long as glume I | Montane forests of northern Andes, with five spp. restricted to the cordilleras of Colombia |
| sect. *Serpentes* | 6 | Viny with long, scabrid internodes; infravaginal branching; 2–12 subsidiary buds per node; foliage leaf blades relatively long and wide | Montane forests of Mexico, Central America, and northern Andes |
| sect. *Verticillatae* | 18 | Crescent or fully encircling verticil of subsidiary buds; thin foliage leaf blades; dorsally compressed spikelets; reduced glumes I and II | Mid- to low-elevation montane forests of Mexico, Central America, and northern Andes |
| *C. ramosissima* group | 3 | Pseudopetiolate culm leaf blade that remains green | Atlantic and lowland forests of Misiones, Argentina, Paraguay, and Brazil |
| *C. meyeriana* group | 5 | Spatheate bracts often subtending the synflorescence; reflexed lower inflorescence branches; reduced glumes I and II | Brazil, especially Atlantic forest |
| subg. *Rettbergia* | 11 | Clambering habit; circular central bud; infravaginal branching; arachnoid subsidiary branches; spatheate bracts usually subtending the synflorescence; connate lemma apices | Brazilian montane and Atlantic forests, except *C. arachniformis*, which is endemic to montane forests of northwestern Colombia |
| subg. *Swallenochloa* | 39 | Shrubby habit; erect culms; shortened waxy internodes; triangular central bud; subsidiary buds forming a crescent below or linearly flanking the central bud; intravaginal or extravaginal branching; tessellate leaf blades | Usually open habitats, often at high elevation; widespread, but most diverse in subpáramos and páramos of the northern Andes, Central America, and campos de altitude of eastern Brazil |
| sect. *Swallenochloa* | 39 | Subsidiary buds forming a crescent below or linearly flanking the central bud; intravaginal branching; short, waxy internodes; thick, stiff foliage leaf blades; narrowly paniculate synflorescences; irregular flowering or short cycles. | Widespread distribution and habitat preferences, as in subg. *Swallenochloa* |
| *C. culeou* group | 3 | Usually subequal, linearly arranged buds lacking an obvious dominant central bud | Open meadows and *Nothofagus* forest understory in the austral Andes (0–2,000m) |
| *C. heterophylla* group | 2 | Erect, fastigiate culms; subsidiary buds forming a crescent below the dominant central bud; extravaginal branching; small leaves (< 15 cm) | Brazilian campos de altitude |
| *C. mimosa* group | 4 | Erect culms that arch distally; deciduous culm and foliage leaf sheaths; scarious culm and foliage leaf sheath margins; dimorphic or trimorphic bud complements | Mid- to high-elevation forests in Brazil; *C. nudiramea* is also found in low-elevation Atlantic forests |
| subg. *Platonia* (*Neurolepis* I) | 11 | Lacking aerial branching; internodes 1–14 cm long (relatively elongated); foliage leaf blades (8-)16–180 cm L, 1–6.3(–8.3) cm W (except for *N. nobilis*); absent to short pseudopetioles; glumes usually with well developed awns | Upper montane forest and páramos of Ecuador and Colombia; *C. fimbriligulata* extends into northern Peru |
| subg. *Magnifoliae* (*Neurolepis* II) | 10 | Lacking aerial branching; internodes 1–3 cm long (relatively compressed); foliage leaf blades (20–)30–300(–377) cm L, 1.2–24(–30) cm W; pseudopetioles well developed; glumes usually lacking awns | Central America, northern and central Andes, Trinidad, Guyana, and northern Brazil |

65 *Chusquea* species (40% of the total number of described species) were sampled for five chloroplast regions and the nuclear region ITS. Species sampling included representatives from all of the described taxonomic groups, spanned the geographic distribution of the genus, and encompassed prominent features of morphological diversity in *Chusquea* s. l. (Fisher et al. 2009).

## Materials and Methods

***Taxon and Character Sampling*—**Leaf material for 65 species of *Chusquea* and three outgroup taxa (Appendix 1) was collected from young branches and immediately dried in silica gel. Vouchers are deposited at ISC, US, INB, and CR. Three outgroups were chosen from the Arthrostylidiinae + Guaduinae Neotropical woody bamboo clade, the lineage that is estimated to be sister to the Chusqueinae in recent molecular phylogenetic studies (Clark et al. 2007; Sungkaew et al. 2009; Kelchner and Bamboo Phylogeny Group 2013).

Five chloroplast regions were used in the analysis: *rpl16* intron, *ndhA* intron, *trnD-trnT* intergenic spacer, *trnT-trnL* intergenic spacer, and *ndhF* gene. The nuclear internal transcribed spacer complex (ITS1, 5.8S, ITS2) was also surveyed for potential information. The individual chloroplast regions were chosen based on prior use in bamboos (Kelchner and Clark 1997; Zhang 2000; Clark et al. 2007; Triplett and Clark 2010) and variation of these regions across seed plants (Shaw et al. 2005; Shaw et al. 2007).

Although there can be serious issues with paralogy determination when using ITS in phylogenetic studies (Alvarez and Wendel 2003), the region was sampled in this study after repeated failure to amplify more desirable low-copy nuclear loci (*GBSSI* and *rpb2*), presumably due to degraded DNA in our dried leaf tissue. The ITS region was sequenced with the goal of assessing its variation in *Chusquea* and as a potential means of corroborating the topology derived from chloroplast data. Because evidence indicates there are multiple copies of ITS present in *Chusquea*'s genome and we did not clone PCR amplicons, we take a cautious approach to the use and interpretation of our ITS data.

***DNA Isolation, Amplification, and Sequencing*—**Total genomic DNA was isolated from silica-gel-dried leaf samples with either Extract-N-Amp kits (Sigma-Aldrich, St. Louis, Missouri) or Qiagen DNEasy plant mini kits (Qiagen, Inc., Valencia, California) using the following modifications to the Qiagen kit protocol: 35–50 mg of dry leaf material, 500 µl of lysis buffer, and a final wash with cold EtOH. Samples extracted with the Extract-N-Amp kit were subsequently cleaned with Qiagen PCR purification columns. Cleaned total genomic DNA served as the template for PCR amplification and sequencing reactions for each molecular region (protocols and primers as in Table 2). Three primers were trialed for ITS (Table 2) in two pairwise combinations (Prince and Kress 2006). Direct sequencing of 18SF-26SR amplicons resulted in multiple sequences in the same chromatogram and this primer pair was abandoned owing to inadequate resources for cloning. However, direct sequencing of ITSL-26SR amplicons resulted in a single sequence except that, in a few cases, ITSL-26SR amplicons were of two lengths. In these cases, the band of length consistent with this ITS copy in other *Chusquea* species was excised with a Wizard SV gel and PCR clean-up kit (Promega, Madison, Wisconsin) according to the manufacturer's instructions. Forty-nine *Chusquea* species and one outgroup were sampled for the ITS nuclear region.

Sequencing was carried out on an Applied Biosystems 3130XL genetic analyzer (Applied Biosystems, Foster City, California) at the Idaho State University Molecular Research Core Facility or on an Automated 3730XL DNA Analyzer (Applied Biosystems) at the Iowa State University DNA Sequencing and Synthesis Facility. Sequence files were manually checked for base-calling errors using the program 4Peaks 1.7.2 (Griekspoor and Groothuis 2006).

***Alignment and Data Exploration*—**Edited sequences were aligned by hand in Se-Al 2.0 (Rambaut 2001). The criterion-based manual alignment of gaps followed Kelchner (2000), Graham et al. (2000), and Borsch et al. (2003). Shared microstructural changes, including insertions, deletions, and hairpin inversion events were coded as absent or present in a 0/1 matrix after probable biological origins for the events were inferred from the surrounding sequence. Nucleotide characters of insertion-deletion regions were then excluded from the dataset in phylogenetic analyses. Areas with ambiguous alignments, such as variation between sequences due to mononucleotide repeats, were also excluded during analyses. Sequences were searched against the National Center for Biotechnology Information (NCBI) nucleotide collection database (nr/nt) to evaluate their similarity to the target regions in grasses.

Indices of nucleotide base frequencies, uncorrected-p distances, and number of parsimony informative characters were assessed using PAUP* v4b10 (Swofford 2003). Neighbor-net analyses of uncorrected-p distances were performed in SplitsTree 4 (Huson and Bryant 2006) to assess character conflict in individual and combined datasets (Morrison 2010). In addition, secondary structures of ITS sequences were modeled on the Mfold server (http://mfold.rna.albany.edu; Zuker 2003) in order to reduce the likelihood of including paralogous sequences (including pseudogenes) or sequences of fungal origin (Feliner and Rosselló 2007).

***Model Selection*—**Models of nucleotide evolution for individual data sets and the combined chloroplast data were evaluated using dynamic likelihood ratio tests (dLRT) and the Akaike information criterion (AIC) method as implemented in jModelTest (Posada 2008). Likelihood scores were computed within jModelTest using PhyML (Guindon and Gascuel 2003) with 88 candidate models, 11 substitution schemes, and options for unequal base frequencies, proportion of invariant sites (I), and rate variation among sites. Multiple models were considered best-fit for the data if AIC differences were $\Delta \leq 2$.

***Phylogenetic Tree Estimation*—**Phylogenetic analyses were performed on the chloroplast and ITS nuclear data separately, to account for the possibility that the organellar and nuclear genomes track separate evolutionary histories. Tree topologies and support values resulting from different estimation frameworks (Bayesian inference, maximum likelihood, and maximum parsimony) were compared for each dataset to assess robustness of tree topology to changes in model assumptions. Time intensive analyses that could be facilitated by parallel processing were run on the Idaho State University EGG Bioinformatics computing cluster.

Table 2. Molecular regions used in this study, PCR amplification primers, and thermocycler protocols. Primers labeled SEQ were favored for sequencing, but were also used for amplification. Primer sequences were obtained from the following publications: ITS nuclear region 18SF, 26SR (Prince and Kress 2006); ITS nuclear region ITSL (Hsiao et al. 1995); *ndhA* intron SAK26 & SAK28 (Watts et al. 2008); *ndhF* gene 972F, 2110R, 1318F and 1603R (Olmstead and Sweere 1994); *rpl16* intron F71, R1516 (Kelchner and Clark 1997); *trnD-trnT* intergenic spacer trnDF & trnTR (Demesure et al. 1995); *trnT-trnL* intergenic spacer TABA, TABB (Taberlet et al. 1991). Touchdown PCR was used to amplify *ndhF* and *trnD-trnT* intergenic spacer by reducing the annealing temperature of each cycle -1°C, until a target temperature is reached, followed with additional cycles at this annealing temperature (Don et al. 1991).

| | | | |
|---|---|---|---|
| **ITS** | 800 bp | 18SF: CGATTGAATGGTCCGGTGAAG (54.4°C)<br>26SR: AGGACGCTTCTACAGACTACAA (53°C)<br>ITSL: TCGTAACAAGGTTTCCGTAGGTG(55.3°C) | 30× (94°C, 1m 30s; 58°C, 2m; 72°C, 1m); 72°C, 7m. |
| ***ndhA* intron** | 800 bp | SAK26: CAATATCTCTACGTGYGATTCG (°C)<br>SAK28: AACTGTTRGATAATCATAGTCG (47.4–49.2°C)<br>SAK43: TCTTTTTCAGGTGGTCTACGAG (53°C)<br>SAK44: ACTGTGCTTCAACTATATCAAC (49.9°C) | 80°C, 5m; 35× (95°C, 1m; 50°C, 1m; +15°C, 0.3°C/s; 65°C, 5m); 65°C, 4m. |
| ***ndhF* gene (3′ end)** | 1,140 bp | 927F: GTCTCAATTGGGTTATATGATG (48.9°C)<br>2110R: CCCCCTAYATATTTGATACCTTCTCC (55.2°C)<br>SEQ:1318F: GGATTAACTGCGTTTTATATGTTTCG (55.2°C)<br>1603R: GCATAGTATTTCCCGTTTCATGAGG (56°C) | 94°C, 1m; 10× (94°C, 1m 30s; touchdown 53–43°C, 2m; 72°C, 3m); 20× (94°C, 1m 30s; 43°C, 2m; 72°C, 3m); 72°C 10m. |
| ***rpl16* intron** | 1,000 bp | F71: GCTATGCTTAGTGTGTGACTCGTTG (57.7°C)<br>SAK7: GAACGACAGAACCTATGA (45.8°C)<br>SAK8: CCATCCCACCCAATGAAG (53.2°C)<br>R1516: CCCTTCATTCTTCCTCTATGTTG (53°C)<br>R1661: CGTACCCATATTTTTCCACCACGAC (57.9°C) | 80°C, 5m; 35× (95°C, 1m; 50°C, 1m; +15°C, 0.3°C/s; 65°C, 5m); 65°C, 4m. |
| ***trnD-trnT* intergenic spacer** | 1,100 bp | TrnDF: ACCAATTGAACTACAATCCC (48.5°C)<br>SAK9F: ACCAATTGAACTACAATCCC (50.2°C)<br>SAK10R: GCATAAGTCATCGGTTCAAATC (51.7°C)<br>trnTR: CCCTTTTAACTCAGTGGTA (48.8°C)<br>SEQ: SAK2R: TGCCCCTATCGTCTAGTGGT (53.8°C)<br>SAK1F: GGATTTGAACCAGCGTATACA (50.5°C) | 94°C, 2m; 35× (94°C, 45s; touchdown 58–48.5°C, 1m; 72°C, 1m 15s); 72°C, 5m. |
| ***trnT-trnL* intergenic spacer** | 880 bp | TABA: CATTACAAATGCGATGCTCT (48.5°C)<br>TABB: TCTACCGATTTCGCCATATC (49.7°C)<br>AFIP1: TAAGGAGAACATAGAATCATAGC (50.5°C)<br>AFIP2: GCTATGATTCTATGTTCTCCT (49°C) | 80°C, 5m; 35× (95°C, 1m; 50°C, 1m; +15°C, 0.3°C/s; 65°C, 5m); 65°C, 4m. |

Bayesian inference (BI) estimation was performed in MrBayes 3.1 (Huelsenbeck and Ronquist 2001) using the general time reversible (GTR) model, with estimates of invariant characters (I) and substitution rate variation (G) parameters as recommended by Huelsenbeck and Rannala (2004). Analysis of the combined chloroplast data included partitions for each region plus coded microstructural changes (with a standard discrete model specified for the microstructural data). In separate analyses of the partitioned chloroplast and ITS nuclear datasets the following settings were used: 40 million generations, two runs, four chains per run, sampling every 2,000 generations, heating temperature of 0.2, and a burn-in of 10,000 trees. Priors were set to Dirichlet on base frequencies and the rate matrix, exponential on branch lengths, and uniform on the gamma shape parameter ($\alpha$), proportion of invariable sites (I), and topology. MrBayes output files for each run were combined to estimate sample size scores in Tracer 1.4.1 (Rambaut and Drummond 2007). Convergence of the posterior distribution was considered to have occurred when the average standard deviation of split frequencies fell below 0.01 and likelihood values appeared to be stable over several thousand generations.

Maximum likelihood (ML) analyses were performed in PhyML ver. 2.4.4 (Guindon and Gascuel 2003) using one of the best-fit models determined in jModelTest. Nonparametric bootstrap (BS) analyses were conducted with 1,000 replicates to evaluate support for branches.

Maximum parsimony (MP) analyses were conducted in PAUP* 4b10 using a two-step "long-thin" search process (Kelchner 2003). This method was used to estimate a robust topology across frameworks and facilitate extensive BS analyses and was not intended for recovery of the most parsimonious tree(s) from the data set. Sequences were added randomly for 1,000 repetitions of heuristic search, with no more than 100 trees greater than or equal to length 1 kept in memory for each repetition. The second step of searching used the subsequent pool of 100,000 trees as starting trees and swapped branches to completion. Trees generated in the second step were combined in a strict consensus topology.

Support for branches was evaluated in PAUP* v4b10 using two non-optimal, heuristic approaches after a conventional BS search (search = heuristic addseq = random nreps = 10,000) ran for more than two months on an iMac G5 (Apple Inc., Cupertino, California). A lack of phylogenetic information may have overwhelmed tree-bisection-reconnection branch swapping during a conventional exhaustive PAUP* bootstrap search (Sullivan 2005). Instead, we trialed a parsimony fast-step BS analysis and an analysis using a single optimal tree held in memory with tree-bisection-reconnection branch swapping (parsimony single tree; Mort et al. 2000).

***Alternative Hypothesis Testing***—Relationships in the chloroplast phylogeny estimation and the morphological classification of *Chusquea* present competing hypotheses of evolution. Shimodaira-Hasegawa tests (SH tests; 1999) were conducted to compare several sets of hypotheses.

The unconstrained maximized likelihood estimate (MLE) tree of combined chloroplast data was tested in a series of two-tree comparisons against MLE trees with monophyletic constraints on each subgenus or section previously described for *Chusquea* (three subgenera and seven sections). Trees were considered significantly different given the chloroplast data and best-fit model if $p < 0.05$ for the test distribution. After the initial phylogenetic analyses suggested that a geographic signal might be present in the data, a second series of SH tests compared the chloroplast unconstrained MLE tree to a number of alternative hypotheses based on geographical distribution. Species occurring in five separate geographic regions were constrained to be monophyletic in individual MLE analyses: austral Andes, Brazil, Brazilian species excluding subg. *Rettbergia*, northern Andes, and central American + Mexican species. In addition, a single SH test compared the ITS MLE tree to a topology with a constraint on a monophyletic subg. *Swallenochloa* sect. *Swallenochloa*.

***Isolation By Distance***—To explore scenarios of evolution in subg. *Rettbergia* and Euchusquea, genetic distances were compared with physical distances (Slatkin 1993). An isolation by distance analysis was conducted on a dataset that sampled across subg. *Rettbergia* and Euchusquea and a dataset that sampled only within Euchusquea. Uncorrected-p distances of the chloroplast data were calculated in PAUP* v4b10 and latitude and longitude values were recorded from herbarium labels for the accessions sampled in this study. A distance matrix was calculated with the software Geographic Distance Matrix Generator v. 1.2.3 (Ersts 2009).

## Results

***Chloroplast and Nuclear Sequence Data***—The combined chloroplast alignment contains 5,278 nucleotide characters for 68 species (0.71% missing data) and a binary matrix of 88 inferred microstructural characters (Appendix S1, TreeBASE study number TB2:S14424). BLAST searches returned sequences of the expected regions from Bambusoideae as top matches. The combined chloroplast alignment includes 446 variable characters and 260 parsimonious informative characters (dataset characteristics available in Appendix S2), and uncorrected-p distances were small for both the chloroplast and nuclear regions (0.0064–0.0167). No region contains a unique sequence for each species, although the combined chloroplast data set does.

In *Chusquea*, the *ndhA* intron is the least variable region, with 114 taxon pairs sharing identical sequences. The *trnD-trnT* region has the highest combined number of variable nucleotides and microstructural changes in the chloroplast dataset (144), but many of these changes are not informative (70). The *trnT-trnL* region is one of the shorter alignments, but contains a moderate number of variable characters (78), a high percentage of which are informative (79%). Although it adds informative data to the analysis, *trnT-trnL* was difficult to amplify and sequence due to five regions of mononucleotide repeats over six bases long within a 250 bp section. Two internal primers (AFIP1, AFIP2) were designed to overcome polymerase failure during *trnT-trnL* amplification, requiring four sequencing runs to obtain the entire region for most samples. The *ndhF* gene showed variation comparable to the non-coding regions and, in this study, it contained more parsimony informative nucleotide characters (52) than the other chloroplast regions, except *trnD-trnT* (58). Most of the parsimony informative characters supplied by *ndhF* provide support for branches deep in the tree topology, particularly those leading to the outgroups and subg. *Platonia* and subg. *Magnifoliae*.

Nucleotide sequences of the ITS region were generated for 50 taxa. Because we were not able to clone amplicons, these data are of limited value and we use them here only as a preliminary survey of nuclear sequence variation in *Chusquea* and to check for broad patterns of hierarchical signal at the nuclear level. We do not consider the data sufficient for phylogeny estimation of *Chusquea* nuclear relationships.

BLAST searches of ITS sequences generated in this study returned bamboo ITS accessions as top hits. Alignment length was 162 bp for ITS1, 221 bp (including a 15 bp indel in some species) for 5.8S, and 356 bp for ITS2. Many Euchusquea clade ITS sequences are identical and the variation that is present in the ITS dataset is concentrated in deep branches of the tree. Five species (*C. andina* Phil., *C. culeou*, *C. leonardiorum* L. G. Clark, *C. liebmannii* E. Fourn., and *C. pinifolia*) have large deletions in ITS (spanning all three regions) ranging from 121 bp in *C. andina* and *C. culeou* to 613 bp in *C. liebmannii*. These five sequences were removed from the alignment before phylogenetic analyses to reduce potential error associated with homology uncertainty.

Several characteristics of the *Chusquea* ITS sequences analyzed in this study suggest that they are not pseudogenes (Feliner and Rosselló 2007). Specifically, i) the *Chusquea* ITS1 spacer was found to contain a conserved sequence motif with AAGGAA 3′ of a hairpin structure, ii) an AAGAA loop and an *EcoRV* site (GATATC; 283–287 bp) are conserved in the 5.8S gene, and iii) several ITS2 RNA sequence folding structures estimated in Mfold show universally conserved pyrimidine mismatches (Zuker 2003). There is no obvious indication that paralogous or pseudogenized ITS sequences

are present in the alignment, particularly because of the overall lack of observed sequence variation.

Neighbor-net networks were used to evaluate character conflict and the combinability of chloroplast data (Suppl. 2). Except for the *trnT-trnL* and *trnD-trnT* intergenic spacers, networks for each region suggest a similar pattern in the data, which is largely tree-like but lacking general resolution. The *trnT-trnL* network includes a large reticulation caused by a 13 base sequence of undetermined origin that is shared by *C. gracilis* McClure & L. B. Sm. and *C. aristata*. The region in the alignment containing this ambiguous sequence was excluded in phylogenetic analyses. The *trnD-trnT* network found a well-defined split between two groups of *Chusquea* species (Suppl. 2) although this split is supported by only six characters.

***Tree Estimation***—The AIC and dLRT model choice methods agree on adequate models for each dataset from our candidate pool (Suppl. 3). GTR + I + G was estimated as one of the best-fit models ($\Delta \leq 2$) for all regions except ITS. Less complex nucleotide substitution rate categories are adequate for the ITS dataset (TrN or TIM + I + G).

A BI consensus tree was summarized from 10,001 trees after a burn-in of 20 million generations (Fig. 2) and effective sample sizes were found to be adequate in Tracer for the combined BI runs (> 200 samples, as recommended by Drummond and Rambaut (2007)). The posterior distribution appeared to reach convergence after 4.4 million MCMC iterations as indicated by the average standard deviation of split frequencies reaching a value < 0.01 and the stability of likelihood values. ML analysis of the combined chloroplast data resulted in a topology with -lnL = 14,152.99. ML and MP bootstrap support (BS) values are lower than BI posterior probability values on all branches (Fig. 2). Reports of support

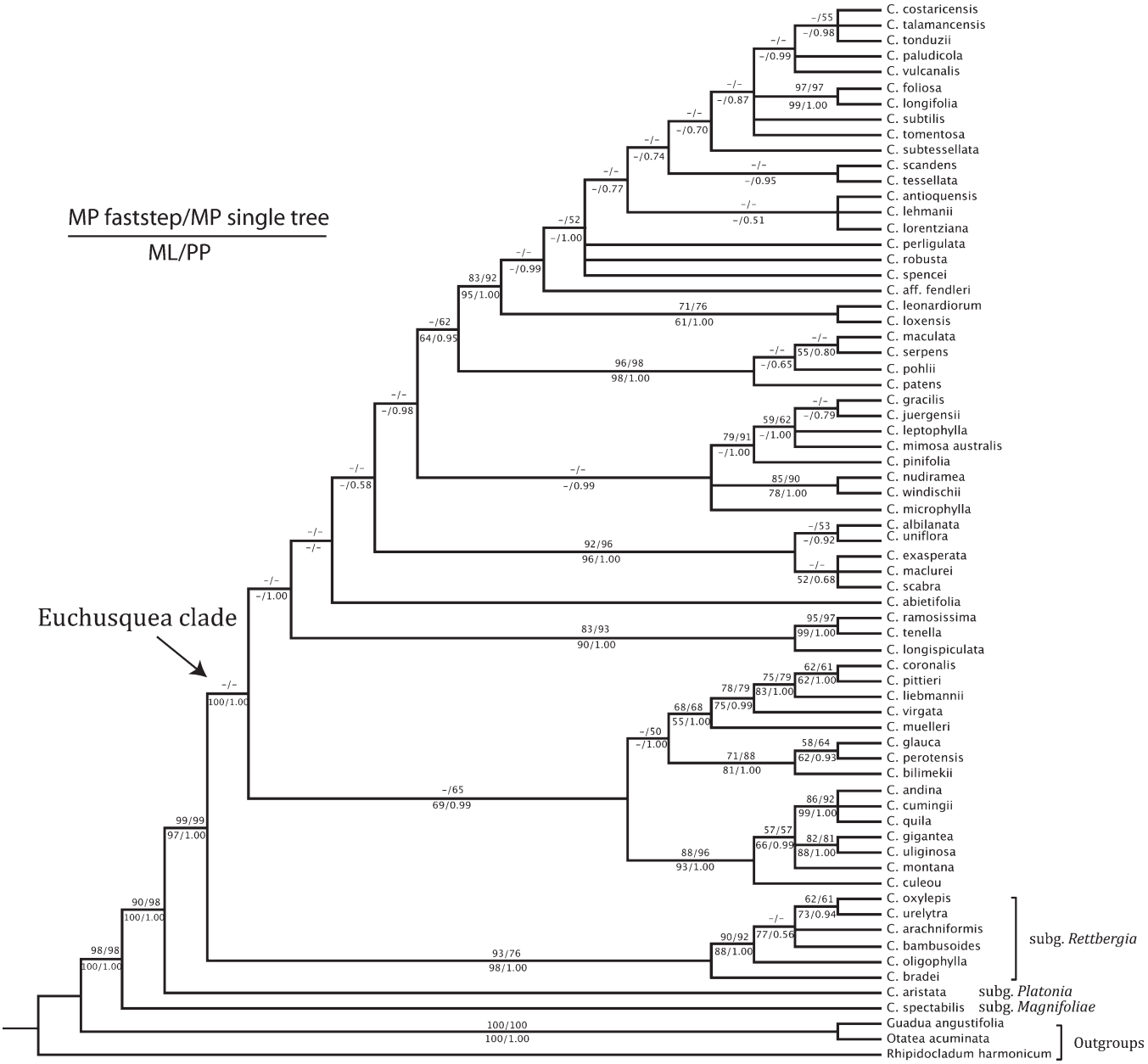


FIG. 2. Bayesian inference cladogram of combined chloroplast data including support values (maximum parsimony faststep bootstrap/maximum parsimony single tree bootstrap above the branch and maximum likelihood bootstrap/Bayesian posterior probability below the branch).

for relationships in the following paragraphs are not meant to reflect total evidence for branches (i.e. branches reported as strongly supported may not be clear in network analyses or supported by SH tests), but are based on BI posterior probability (PP) and MP/ML BS values.

The combined chloroplast data recovered many clades with consistent species compositions in BI, ML, and MP phylogeny estimations (Fig. 2). The tree is rooted with *Rhipidocladum harmonicum*, making *Guadua angustifolia* sister to *Otatea acuminata*. Subgenus *Magnifoliae* and subg. *Platonia* are the two earliest-diverging clades. Subgenus *Rettbergia* is monophyletic and sister to a large Euchusquea clade. Branches leading to some clades within Euchusquea change position along the tree backbone depending upon phylogenetic estimation method (Fig. 3).

The major clades in a BI analysis of the ITS nuclear region (Fig. 4) correspond to those found in the chloroplast analyses (Fig. 3). The ITS topology is not as resolved as the chloroplast estimate, but it does contain several small groupings that are also found in the chloroplast tree.

Sampled Euchusquea species form a monophyletic group in chloroplast phylogeny estimations (Fig. 3). Delineating relationships within Euchusquea is difficult, however, due to low variation among nucleotide sequences. Short branches and poorly supported branching order among Euchusquea lineages creates a polytomy at the base of the clade (Fig. 3). Five Euchusquea clades appear in all BI, ML, and MP topologies with relatively high branch support. These five clades do not agree with the taxonomic groupings within *Chusquea*. Instead, the chloroplast signal suggests strong geographic partitioning of Euchusquea species relationships. The five supported Euchusquea clades are described here in terms of their species compositions as well as their distributions.

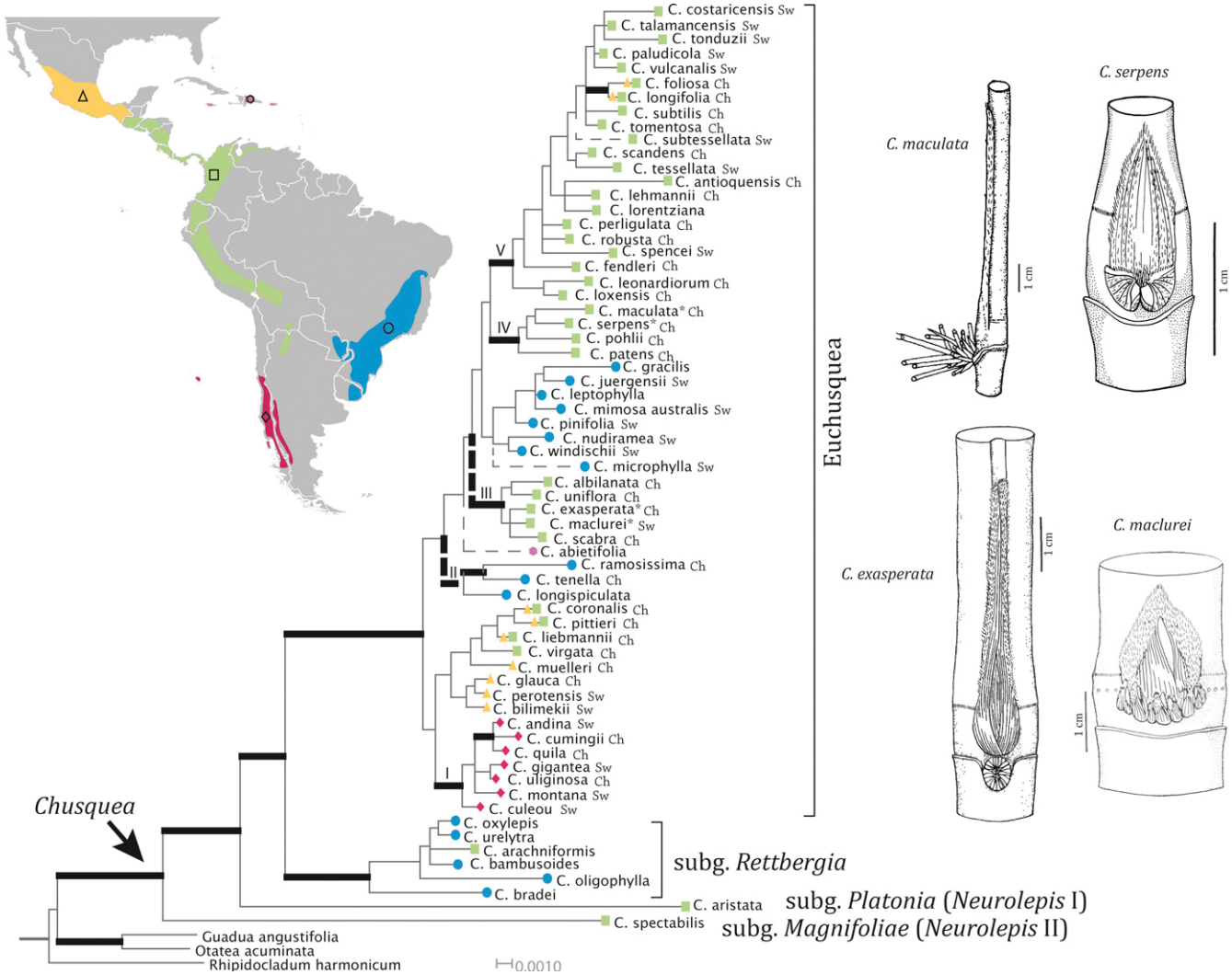


FIG. 3. Bayesian inference phylogram of a partitioned analysis of the chloroplast data. Weighted branches indicate maximum likelihood bootstrap support > 90 and Bayesian inference posterior probability support > 0.95. Dashed-line branches change position in maximum likelihood or maximum parsimony analyses. Sw and Ch indicate the species is classified as subg. *Swallenochloa* or subg. *Chusquea*, respectively. *Chusquea longispiculata*, *C. leptophylla*, *C. lorentziana*, *C. gracilis*, and *C. abietifolia* are not currently placed in a subgenus. Numbered clades are referenced in the text. *Chusquea* contains four major lineages: subg. *Platonia* (*Neurolepis* I), subg. *Magnifoliae* (*Neurolepis* II), subg. *Rettbergia*, and a Euchusquea clade. Within Euchusquea there are five well supported lineages, I-V. Shapes at the end of branches correspond to geographic distribution areas as indicated on the map. Illustrated species are indicated with asterisks. *Chusquea maculata* and *C. exasperata* are members of subg. *Chusquea* sect. *Longiprophyllae* and are morphologically similar, yet they are not closely related in the tree topology. Their closest relatives according to chloroplast data are shown for comparison. Line drawing of *C. serpens* is reproduced from Clark 1985; *C. maclurei* is from Clark 1986; *C. maculata* and *C. exasperata* are from Clark 1990.

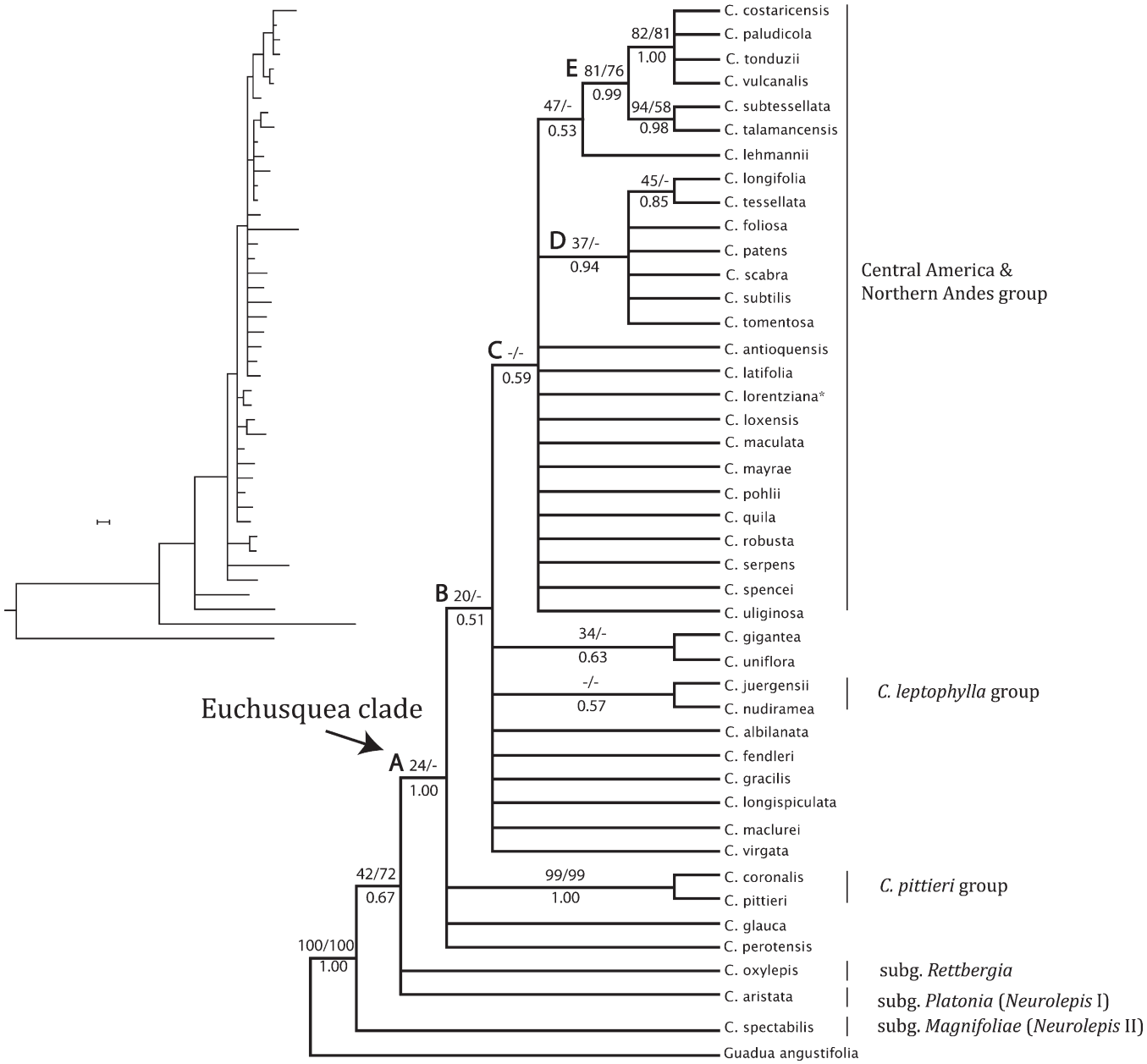


FIG. 4. ITS Bayesian phylogram (left) and cladogram (right). Scale-bar on phylogram is 0.01 changes. Support values are listed as maximum parsimony bootstrap above the branch; maximum likelihood bootstrap/Bayesian posterior probability below the branch. Lettered nodes are referenced in the text.

I. CHUSQUEA CULEOU CLADE: AUSTRAL ANDES—A *C. culeou* clade was recovered in chloroplast analyses with strong support (I, Fig. 3; 93% ML BS, 1.00 PP) and contains species restricted to austral Andean montane forests (Triplett and Clark 2003). *C. andina*, *C. cumingii* Nees, and *C. quila* Kunth form a well-supported group and *C. gigantea* Demoly + *C. uliginosa* Phil. is also supported (88% ML BS, 1.00 PP). The widely distributed *C. culeou* appears to be sister to the rest of this clade.

II. CHUSQUEA RAMOSISSIMA CLADE: PARAGUAY, URUGUAY, BRAZIL, ARGENTINA—The *C. ramosissima* clade consists of *C. ramosissima* Lindm., *C. tenella* Nees, and *C. longispiculata* L. G. Clark and is moderately supported as monophyletic (II, Fig. 3; 90% ML BS, 1.00 PP). *Chusquea ramosissima* is sister to *C. tenella* with strong support. *Chusquea ramosissima* and *C. tenella* are widespread species in low elevation areas of Paraguay, Uruguay, eastern Brazil, and the state of Misiones, Argentina, while *C. longispiculata* is restricted to southeastern Brazil.

III. CHUSQUEA UNIFLORA CLADE: NORTHERN ANDES AND CENTRAL AMERICA—The *C. uniflora* clade is composed of species found in low to middle elevation montane and cloud forests in the northern Andes, along with *C. scabra* Soderstr. & C. E. Calderón, a low elevation species from Costa Rica. There is strong support for monophyly (III, Fig. 3; 96% ML BS, 1.00 PP), but relationships within the clade are unclear, with BI, ML, and MP bootstrap topologies recovering distinct relationships.

IV. CHUSQUEA SERPENS CLADE: NORTHERN AND CENTRAL ANDES, CENTRAL AMERICA—The *C. serpens* clade is well supported

(IV, Fig. 3; 98% ML BS, 1.00 PP) and contains four species, *C. patens* L. G. Clark, *C. pohlii* L. G. Clark, *C. serpens* L. G. Clark, and *C. maculata* L. G. Clark. *Chusquea pohlii* and *C. patens* are restricted to Costa Rica and Panama. *Chusquea maculata* is found only in Colombia and Venezuela, while *C. serpens* is distributed through those countries, as well as Ecuador.

V. Chusquea scandens Clade: northern and Central Andes, Central America—The *C. scandens* clade is strongly supported with chloroplast data (V, Fig. 3; 95% ML BS, 1.00 PP) and includes 21 species in BI, ML, and MP analyses. There are several relationships within the *C. scandens* clade with some branch support in chloroplast analyses. The only strongly supported sister relationship exists between *C. foliosa* L. G. Clark + *C. longifolia* Swallen. Many species in the *C. scandens* clade (e.g. *C. leonardiorum* and *C. subtessellata*) are found in high-elevation habitats such as cloud forest, subpáramo, and páramo (Soderstrom and Calderón 1978b; Clark 1992). This group is mainly northern Andean, with a more recently diverging group of Central American species. Several of the Central American species are also found in southern Mexico (*C. foliosa* and *C. longifolia*), and *C. lorentziana* Griseb. from northern Argentina and Bolivia also appears in this clade in all chloroplast analyses (although an SH test cannot reject the possibility that it is a member of the *C. culeou* clade; see below). In the ITS topology a group of six species from the chloroplast *C. scandens* clade are recovered as monophyletic with weak support (E, Fig. 4). Within this clade there are supported relationships between *C. subtessellata* + *C. talamancensis* and *C. costaricensis*, *C. paludicola*, *C. tonduzii* and *C. vulcanalis*.

***Alternative Hypothesis Testing***—The possibility that morphologically defined taxonomic groups might be supported by the chloroplast data given the best-fit model was investigated with a series of two-tree comparison SH tests. The SH test rejected most alternative hypotheses with three exceptions: monophyletic constraint of subg. *Chusquea* sect. *Longiprophyllae,* subg. *Swallenochloa C. culeou* group, and subg. *Swallenochloa C. nudiramea* group (Table 3). These taxonomic groups contain a small number of species or were sparsely sampled in this study, as in the case of sect. *Longiprophyllae*.

An SH test rejected a constrained topology containing a monophyletic subg. *Swallenochloa* sect. *Swallenochloa* as an alternative to the unconstrained MLE topology ($p = 0.000$*). The sampled species of subg. *Swallenochloa* sect. *Swallenochloa* are not monophyletic in any of the chloroplast optimal topologies. However, an SH test with the ITS data and the best-fit model failed to reject a topology constraining a group of ten sect. *Swallenochloa* species as monophyletic ($p = 0.06$), although we note again the low level of character variation present in the ITS data set.

Hypotheses of relationships predicted by geographic proximity were also tested. The *C. culeou* clade is composed of all sampled taxa from high-elevation habitats and western slopes of the austral Andes. *C. ramosissima* and *C. tenella* are mainly found in southeastern Brazil, but extend slightly into Argentina and Paraguay. An SH test failed to reject a topology containing a monophyletic *C. culeou* clade + *C. lorentziana* + *C. ramosissima* + *C. tenella* ($p = 0.057$). Another SH test assessed the three Brazilian clades in the chloroplast topology: subg. *Rettbergia*, except *C. arachniformis* (known only from the northern Andes), the *C. leptophylla* clade, and the *C. ramosissima* clade. Given the chloroplast data and the model, an SH test rejected a topology in which all of the Brazilian species are monophyletic ($p = 0.000$*). A second SH test of Brazilian species that excluded subg. *Rettbergia* failed to reject the possibility of a topology containing a monophyletic Brazilian clade ($p = 0.675$). An SH test of a topology constraining northern Andean taxa to monophyly (species present in subg. *Rettbergia*, *C. uniflora*, *C. serpens*, and *C. scandens* clades in the chloroplast topology) is rejected given the data and the model ($p = 0.000$*). Finally, nineteen Central American species are present in the *C. pittieri*, *C. uniflora*, *C. serpens*, and *C. scandens* clades in the chloroplast topology and an SH test rejected a topology that constrained these groups to be monophyletic ($p = 0.000$*).

***Isolation by distance***—Comparisons of genetic distances to the proximity of collected individuals were conducted on two groups of *Chusquea* species: (i) subg. *Rettbergia* + Euchusquea (Fig. 5A) and (ii) the Euchusquea clade (Fig. 5B). In both analyses there is no apparent relationship between genetic distance and the location of any two individuals. Genetically similar species might be found in the same location (*C. talamancensis* and *C. paludicola*, p distance = 0, physical distance = 0) or > 6,000 km apart (*C. ramosissima* and *C. bilimekii*, p distance = 0.001, physical distance = 6,943 km). Species that are genetically less similar to one another might also be in the same location (*C. virgata* and *C. longifolia*, p distance = 0.01, physical distance = 67 km) or they might be distant (*C. nudiramea* and *C. muelleri*, p distance = 0.012, physical distance = 7,350 km). Across the complete dataset, two distinct patterns are seen: large genetic distances between the Euchusquea and subg. *Rettbergia* species and small genetic distances within Euchusquea.

Table 3. Results of two-tree SH tests comparing the maximum likelihood estimate tree with alternative topology hypotheses as described in the text. Chloroplast data was used, unless ITS data is indicated. The sampling column refers to the number of species sampled in this study out of the total number of species in the group.

| Hypothesis of monophyly | Species sampled | SH test *p* value |
|---|---|---|
| subg. *Rettbergia* + *C. ramosissima* | 7/13 | 0.024* |
| subg. *Swallenochloa* | 21/38 | 0.000* |
| subg. *Swallenochloa* sect. *Swallenochloa* | 14/29 | 0.000* |
| subg. *Chusquea* sect. *Longifoliae* | 8/11 | 0.000* |
| subg. *Chusquea* sect. *Serpentes* | 4/6 | 0.028* |
| subg. *Chusquea* sect. *Verticillatae* | 7/18 | 0.007* |
| subg. *Chusquea* sect. *Chusquea* | 8/19 | 0.000* |
| subg. *Swallenochloa C. culeou* group | 3/3 | 0.378 |
| subg. *Swallenochloa C. nudiramea* group | 3/4 | 0.303 |
| subg. *Chusquea* sect. *Longiprophyllae* | 2/6 | 0.182 |
| Brazilian Euchusquea species | NA | 0.675 |
| ITS data subg. *Swallenochloa* sect. *Swallenochloa* | 10/29 | 0.060 |
| austral Andean species | NA | 0.057 |
| Brazilian species | NA | 0.000* |
| northern Andean species | NA | 0.000* |
| Central American species | NA | 0.000* |

## Discussion

Our study recovered four major lineages in *Chusquea* with strong support (Fig. 3). A monophyletic *Chusquea* includes subg. *Magnifoliae* (*Neurolepis* II) sister to subg. *Platonia*

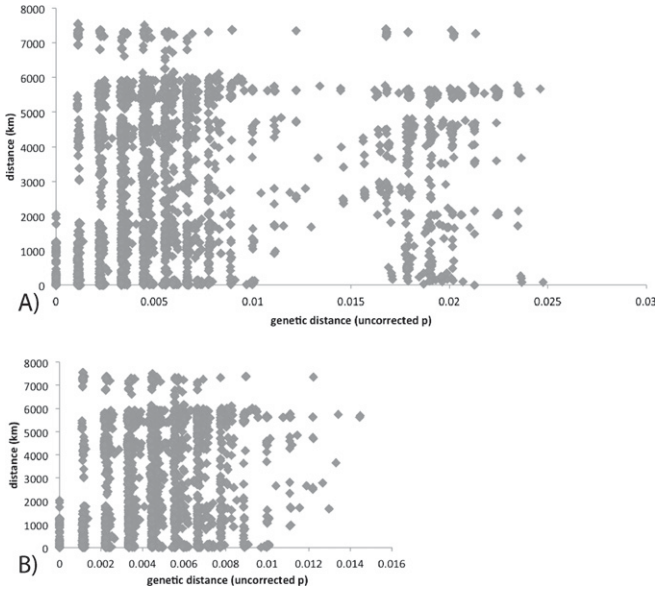


FIG. 5. Isolation by distance analyses comparing chloroplast genetic distance on the x axis with physical distance of accessions sampled in this study on the y axis for A) species in Euchusquea and subg. *Rettbergia* and B) Euchusquea species only.

(*Neurolepis* I) + (subg. *Rettbergia* + Euchusquea). Relationships among the four lineages corroborate those recovered by Kelchner and Clark (1997), Clark et al. (2007), and Fisher et al. (2009). The ITS nuclear dataset shows little variation (Fig. 4), but is consistent with the chloroplast dataset in resolving the four major *Chusquea* lineages.

We recovered five supported lineages in the Euchusquea clade (Fig. 3) and chloroplast species relationships within Euchusquea correspond to some degree with geographic areas, although the pattern is complicated by more than one *Chusquea* lineage present in most geographic regions (i.e. northern Andes, Central America, Brazil, possibly the austral Andes). Given geographic patterns, low variation in Euchusquea, and conflicts between molecular and morphological hypotheses of relationships, the data suggest that *Chusquea* may be a young and actively radiating group of bamboos.

Several limitations of the study prevent unambiguous interpretation of the data and leave many relationships unresolved. The chloroplast data contains little variation compared to similar sized datasets for other grass genera, a common problem in bamboo molecular phylogeny studies (Triplett and Clark 2010; Yang et al. 2010). The limited molecular variation may be causing problems with analyses (i.e. tree topology estimation, branch support values, SH tests, isolation by distance) by introducing sampling error. Additionally, the ITS data should be viewed as exploratory because amplicons were not cloned and the sequences exhibit low levels of sequence variation.

***Supported Lineages***—Subgenus *Rettbergia* is a well-supported lineage that is primarily distributed in Brazil but also includes the northern Andean *C. arachniformis*. This species was only recently described from Colombia (Clark and Londoño 1998) and exhibits the infravaginal branching, spatheate capitate inflorescences, and connate lemma tips characteristic of subg. *Rettbergia* species. Kelchner and Clark (1997) sampled the *rpl16* intron for this species (as *Chusquea* sp. A) and placed it well within the subg. *Rettbergia* clade. Several other *Chusquea* species were included in subg. *Rettbergia* until recently: *C. anelythra* Nees, *C. anelytroides* Rupr. ex Döll, *C. barbata* L. G. Clark, *C. pallida* Munro, *C. ramosissima*, *C. tenuiglumis* Döll, and *C. wilkesii* Munro (Clark 1993; Judziewicz et al. 1999). This study sampled *C. ramosissima* and found no support for its inclusion in subg. *Rettbergia* in combined chloroplast MP, MLE, or BI trees, and an SH test rejected inclusion of this taxon in the subgenus. *Chusquea ramosissima* was also found to be more closely related to Euchusquea than subg. *Rettbergia* in a parsimony analysis of *rpl16* intron data (Kelchner and Clark 1997, Clark et al. 2007), but this result is contradicted by results of a morphological analysis (Clark et al. 2007) and also of analysis of the *trnD-trnT* intergenic spacer (data not shown).

In this study, Euchusquea is monophyletic, but expands to include members of the polyphyletic subg. *Swallenochloa* and subg. *Chusquea*. This finding corroborates other evidence for Euchusquea monophyly (Kelchner and Clark 1997; Clark et al. 2007; Fisher et al. 2009), supports Clark's (1989) inclusion of *Swallenochloa* within *Chusquea*, and supports Clark's (1997) hypothesis of a close relationship between subg. *Chusquea* and subg. *Swallenochloa*. Potentially, the Euchusquea clade could be designated a subgenus as it is supported as monophyletic by molecular data; there are, however, no obvious morphological synapomorphies for the clade (Clark 1997).

Relatively dense species sampling allowed us to detect a number of clades within the Euchusquea clade that were robust to changes in methodological framework and corroborate relationships found in other studies.

Chloroplast data supports the *C. culeou* clade (I, Fig. 3) as a monophyletic lineage in *Chusquea* although species in this clade are morphologically different. Most of the species of *Chusquea* found in Chile have extravaginal branching and are classified in subg. *Chusquea* sect. *Chusquea*. They are represented here by *C. quila* and *C. uliginosa*. A single species in Argentina (*C. deficiens*) exhibits characteristics that place it in sect. *Verticillatae*, but it was not sampled in this study. The high-elevation *C. montana* is found in both Argentina and Chile and is a shrubby member of subg. *Swallenochloa* sect. *Swallenochloa*. Other austral Andean members of subg. *Swallenochloa* are *C. culeou*, *C. gigantea,* and *C. andina* (the *C. culeou* group); they exhibit overlapping variation in overall height and branch dimorphism, which is considered to be a result of elevation, latitude, and shade effects (Veblen 1982; Pearson et al. 1994; Triplett and Clark 2003).

Both Kelchner and Clark (1997) and Clark et al. (2007) sampled *C. ramosissima*, but neither study was able to resolve its placement beyond inclusion in the Euchusquea clade. Our results corroborate the exclusion of *C. ramosissima* from subg. *Rettbergia* and instead suggest a close relationship with the sympatric *C. tenella* (II, Fig. 3). Both *C. tenella* and *C. ramosissima* exhibit a pseudopetiolate culm leaf blade that often remains green after the plant has reached maturity. They share this trait with *C. tenuiglumis* (not sampled).

Species in the *C. uniflora* clade (III, Fig. 3) are from low-elevation montane or cloud forest habitats in the northern Andes and Central America and are classified in subg. *Chusquea* sect. *Verticillatae*, sect. *Longifoliae*, sect. *Longiprophyllae*, and subg. *Swallenochloa* sect. *Swallenochloa*. This clade has been sampled in previous studies (represented by *C. exasperata*;

Kelchner and Clark 1997; Clark et al. 2007), but its position within Euchusquea has not been resolved.

The *C. serpens* clade (IV, Fig. 3) contains species from the northern Andes and Central America that are classified in subg. *Chusquea* sect. *Longiprophyllae*, sect. *Longifoliae*, and sect. *Serpentes*. There is strong support for monophyly of the clade, but no apparent morphological synapomorphy. *Chusquea patens* and *C. pohlii* are both found only in Costa Rica and Panama, while *C. serpens* extends into Colombia, Ecuador, and Venezuela and *C. maculata* is found in Colombia and Venezuela. Given the relationships of these species in the phylogeny estimation and their distributions, this clade may represent a dispersal of *Chusquea* from Central America to the Andes.

Species in the C. *scandens* clade (V, Fig. 3) are drawn from subg. *Swallenochloa* sect. *Swallenochloa*, subg. *Chusquea* sect. *Chusquea*, sect. *Longifoliae*, sect. *Verticillatae*, and sect. *Serpentes*. These species are found in the northern Andes and Central America, with the exception of *C. lorentziana* (Argentina and Bolivia) and the extended distribution of *C. foliosa* and *C. longifolia* into Mexico. The close relationship between *C. foliosa* and *C. longifolia* is interesting in that both species are classified in subg. *Chusquea* sect. *Longifoliae* and are found in oak cloud forests. The *C. scandens* clade also contains a group of closely related species with a center of diversity in Costa Rica: *C. costaricensis, C. talamancensis, C. tonduzii, C. paludicola, C. vulcanalis, C. foliosa, C. longifolia, C. subtilis, C. tomentosa,* and *C. subtessellata*.

Several other interesting relationships are worth discussing, but are in need of further study. This is the first molecular evidence for the placement of *C. abietifolia* within Euchusquea (Fig. 3). *Chusquea abietifolia* is the only *Chusquea* species found in the West Indies (with collections from Jamaica, Hispaniola, Cuba, and Puerto Rico; Tropicos). The BI and ML analyses place it (unsupported) as sister to the *C. uniflora* group + the remainder of Euchusquea, while the MP analysis places it as sister to the *C. uniflora* group (unsupported). *Chusquea abietifolia*'s relationship within Euchusquea might be difficult to estimate due to a long branch (13 changes in this dataset) distinguishing it from the Euchusquea backbone.

Our analysis also recovered an unsupported group of *Chusquea* species that are primarily found in Brazil (Fig. 3). Resolution within this group is also unsupported and is limited to a sister relationship between *C. nudiramea* L. G. Clark and *C. windischii* L. G. Clark and a lineage containing *C. gracilis*, *C. juergensii* Hack., *C. leptophylla* Nees, *C. mimosa* McClure & L. B. Sm., and *C. pinifolia*. The majority of these species are restricted to the South Atlantic forest (Espírito Santo to southern Santa Catarina), although *C. leptophylla* also occurs in Minas Gerais (Tropicos). *C. juergensii*, *C. mimosa*, and *C. pinifolia* are fairly widely distributed, while *C. nudiramea*, *C. windischii*, and *C. microphylla* are narrow endemics (Clark 1997). Three species in this group (*C. pinifolia*, *C. windischii*, and *C. microphylla*) are found in campos de altitude habitats (Clark 1992). *Chusquea juergensii* and *C. mimosa australis* are often associated with *Araucaria* formations.

***Chusquea Evolution***—One explanation for the small amount of variation found in Euchusquea chloroplast and nuclear sequences is that *Chusquea* exhibits slow evolutionary rates, possibly compounded by long generation times (Wilson et al. 1990; Gaut et al. 1997). Yet, chloroplast substitution rates in *Chusquea* are faster than those seen in most bamboo lineages, with the exception of the herbaceous bamboos (Kelchner and Bamboo Phylogeny Group 2013). Low sequence variation within Euchusquea may also stem from recent origin of the clade and a lack of time to incur substitutions (Ruiz-Sanchez 2011). We prefer a recent origin explanation based on the small amounts of variation found in both the chloroplast and ITS datasets and the geographic patterning of Euchusquea species.

If species in Euchusquea are actively radiating, then our molecular datasets might be complicated by processes that are common in recently diverged lineages, including incomplete lineage sorting and hybridization that result in chloroplast sharing. Hybridization and incomplete lineage sorting have been discussed in many phylogenetic studies (Maddison and Knowles 2006; Rokas and Carroll 2006; Holland et al. 2008; Degnan and Rosenberg 2009; King and Roalson 2009; Polihronakis 2010) because these non-treelike processes of genetic inheritance can result in erroneous inferences about the evolution of a group when tree diagrams are used for such data in phylogenetic analyses (Huson and Bryant 2006; Bapteste et al. 2013). These processes could lead to a case where tree-based estimates of DNA phylogeny do not predict morphology. This might be occurring in Euchusquea, where the chloroplast topology contradicts the current taxonomic classification based on hypotheses of morphological evolution (although morphological characters may also evolve in a manner that leads to a homoplasious interpretation of phylogeny). For example, the sampled species in subg. *Chusquea* sect. *Longiprophyllae* have prophylls as long as 10 cm, buds arranged in a tight circle at the node, relatively narrow, lanceolate leaf blades, and a first glume that is twice as long as the second glume (Fig. 3, Clark 1990); but the two species of sect. *Longiprophyllae* sampled in this study (*C. maculata* and *C. exasperata*) are not sister. Instead, our chloroplast DNA topology places *C. maculata* as sister to *C. serpens*, a species with no elongation of the prophyll, buds arranged in a crescent at the node, wide leaves, and subequal lower glumes. *Chusquea exasperata* is closely related to *C. maclurei* L. G. Clark and *C. scabra* according to our chloroplast estimate, although the three species are different from each other in branching pattern and leaf shape.

In another example of conflict between the chloroplast topology and the taxonomic classification of *Chusquea*, 16 species of subg. *Chusquea* sect. *Verticillatae* exhibit distinctive thin leaf blades, dorsally compressed spikelets, reduced first and second glumes, and often a verticillate arrangement of buds (Fig. 1, Soderstrom and Calderón 1978a; Clark 1989). In our analysis of chloroplast data, the sampled members of sect. *Verticillatae* are recovered in several different Euchusquea clades and an SH test rejects a topology that constrains these species to monophyly.

Hybridization has not often been implicated as an important process in bamboo evolution because of the infrequent flowering of most bamboo species (Clark et al. 1989). Bamboos exhibit gregarious monocarpy, a reproductive strategy known as semelparous, hapaxanthic, or plietesial in other plant groups. Bamboos with this life cycle might remain vegetative for decades before a flowering event occurs (Janzen 1974) and the asynchronous nature of flowering among bamboo species is expected to decrease the potential for hybridization. Recent molecular work, however, has shown that hybridization was involved in the formation

of temperate bamboo species in the genera *Hibanobambusa* Maruy. & H. Okamura, *Sasa* Makino & Shibata, *Sasamorpha* Nakai, and *Pleioblastus* Nakai (Triplett and Clark 2010). Hybridization also seems to occur among species in *Arundinaria* Michx. (Triplett et al. 2010) and *Chimonocalamus* (Yang et al. 2013) and likely occurs among the Paleotropical bamboos as well (Goh et al. 2011; Wong and Low 2011).

Within *Chusquea*, hybridization appears to have occurred between frequently flowering species of subg. *Swallenochloa* sect. *Swallenochloa* (Clark et al. 1989; Pohl 1991). Clark et al. (1989) identified morphologically intermediate plants hypothesized to represent *C. amistadensis* L. G. Clark, Davidse & R. P. Ellis x *C. subtessellata* and *C. vulcanalis* x *C. subtessellata* in several populations in Panama and Costa Rica and *C. spencei* x *C. tessellata* in Colombian populations. Based on low nucleotide sequence variation within Euchusquea, the presence of morphological intermediates, a large number of sympatric species in Central America and the northern Andes, frequent flowering of some high-elevation species, and the conflict between molecular and morphological based estimates of relatedness, hybridization between Euchusquea species is likely to occur more than previously thought, and merits further investigation. The *Chusquea* ITS tree topology (Fig. 4) is not based on cloned sequences which unfortunately restricts our ability to test hypotheses of hybridization in Euchusquea in this study.

Although hybridization probably occurs between some Euchusquea species, incomplete lineage sorting might also affect tree-based chloroplast and nuclear phylogenetic estimates in *Chusquea*. Incomplete lineage sorting occurs during the divergence of nascent species when descendent populations retain multiple ancestral haplotypes before alleles coalesce to a single haplotype (Maddison 1997). Although several studies have identified coalescent-based approaches to investigate the effects of incomplete lineage sorting on phylogeny estimations of closely related species (Maddison and Knowles 2006; Carstens and Knowles 2007; King and Roalson 2009; Polihronakis 2010), the current *Chusquea* dataset does not include adequate independent loci nor the population level sampling necessary to use these approaches effectively. If the common ancestor of Euchusquea contained polymorphisms that have not yet had time to fix in diverging lineages, then one would expect to see a random pattern of ancestral haplotypes in progeny species (Comes and Abbott 2001; Maddison and Knowles 2006), with no morphological or geographic signal (Morando et al. 2004; Jabaily and Sytsma 2010). Chloroplast genetic relationships among Euchusquea species seem to be largely unlinked from morphological traits and a test of isolation by distance did not uncover a simple pattern between chloroplast genetic relatedness and geographic distance (Fig. 5). There are apparent geographic patterns in Euchusquea chloroplast relationships, but those patterns are complicated by multiple lineages co-occurring geographically. Since Euchusquea may have recently undergone a species radiation or may be radiating at present, it is reasonable to infer that incomplete lineage sorting can partly explain the observed incongruence between chloroplast and morphological hypotheses of relationships.

Contrasting with results of the isolation by distance analysis, the phylogeny estimation for Euchusquea shows a pattern of chloroplast lineages that correspond to geographic distribution. Considering the pattern present in the chloroplast analysis, it is possible that gene flow among Euchusquea species within a geographical region led to chloroplast sharing and development of a regional chloroplast signal. There are few supported relationships within clades (Fig. 3) and it is therefore possible that chloroplasts in extant Euchusquea have not completed lineage sorting at the level of morphologically distinguishable species, but instead retain polymorphisms that are shared with other species in their geographic area. The geographic signal in the Euchusquea is most likely due to a combination of the effects of a recent radiation of Euchusquea species, incomplete lineage sorting, and hybridization-mediated chloroplast sharing. Euchusquea is probably a young group that is actively radiating in several geographic areas, particularly the northern Andes, Central America, and southeastern Brazil and reproductive isolation barriers may be weak or non-existent.

***Implications for Taxonomy***—Species in *Chusquea* have previously been placed in three subgenera, numerous sections, and informal groups based on unique combinations of morphological characters. Molecular evidence supports the existence of a monophyletic subg. *Rettbergia*, a Euchusquea clade containing polyphyletic subg. *Swallenochloa* and subg. *Chusquea*, and two early-diverging clades comprised of species formerly in the genus *Neurolepis* that have been informally referred to as *Neurolepis* I and *Neurolepis* II.

Chloroplast data suggest a consistent pattern of lineages within Euchusquea, but most of these lineages are not strongly supported and alternative sets of relationships, including morphologically defined taxonomic groups, cannot be ruled out by SH tests. In addition, Euchusquea chloroplast lineages lack obvious morphological synapomorphies that would serve in a new subgeneric or sectional classification. There remains a need to organize and reference the large amount of morphological variation present in Euchusquea for floristic, systematic, ecological, and physiological studies (Bortolus 2008). Until there is compelling evidence for the existence of evolutionary lineages that are also morphologically definable, we propose that maintaining the current subgeneric classification is the most practical and biologically relevant course in Euchusquea, with the caveat that subg. *Chusquea* and subg. *Swallenochloa* appear to be polyphyletic with chloroplast data.

In contrast to the lineages of the Euchusquea clade, early-diverging lineages of *Chusquea* represent well-supported primary divisions within the genus and several potential morphological synapomorphies have been identified. We propose elevating the *Neurolepis* I and *Neurolepis* II clades to subgeneric rank. Symplesiomorphies for the *Neurolepis* clades are a lack of aerial branching (an unusual character shared by only two other woody bamboo genera, *Glaziophyton* Franchet and *Greslania* Balansa) and inner ligules with evident nerves (Judziewicz et al. 1999). Morphological differences distinguishing the *Neurolepis* clades were identified in Fisher et al. (2009), but the large-leaved *C. nobilis* was in a seemingly incongruous placement in the otherwise small-leaved *Neurolepis* I clade. After re-examination, *C. nobilis* has been found to exhibit characters such as awned glumes that are consistent with *Neurolepis* I species, with the exception of its large leaves.

The only previously published subgeneric name for *Neurolepis* is subg. *Platonia* (Kunth) Nees. Nees von Esenbeck (1835) created subg. *Platonia* on the occasion of submerging *Platonia* Kunth (a basionym of *Neurolepis* and a later homonym of *Platonia* Mart.) and other bamboo genera with one-flowered spikelets in *Chusquea*. Following Kunth's concept, the type

of subg. *Platonia* (Kunth) Nees is *C. elata*, a species resolved within the *Neurolepis* I clade in the analyses of Fisher et al. (2009). Therefore, subg. *Platonia* follows *C. elata* and is the correct name of the *Neurolepis* I clade at the subgenus level. As there are no additional subgeneric names for these clades, *Chusquea* subg. *Magnifoliae* is published here as a subgenus novum. The epithet describes the large leaves characteristic of species in the *Neurolepis* II clade and correspondingly, *C. magnifolia* L. G. Clark is the type species. The descriptions below follow Fisher et al. (2009), with the addition of a distinguishing reproductive character (presence or absence of awned glumes).

**Chusquea** subgenus **Platonia** Fisher & L. G. Clark, nom. et stat. nov. *Platonia* Kunth, Rev. Gram 1: 139. 1829., non Raf. (1810), nec Mart. (1832).—TYPE: *P. elata* Kunth. *Neurolepis* Meisner, Pl. Vasc. Gen. 1: 426. 1843.—TYPE: *N. elata* (Kunth) Pilg.

Internodes 1–14 cm long, solid or hollow. Culm leaves poorly or not differentiated. Bud complement, if developed, consisting of a single bud triangular in outline and oriented vertically. Aerial branching absent. Foliage leaf blades usually 8–164(-180) cm long and (0.6-)1–6.3(-8.3) cm wide but 162–300(-400) cm long and 3.5–10(-12) cm wide in *C. nobilis*; pseudopetioles absent to short (1–6 mm long), but 1.5–17.5 cm in *C. asymmetrica*. Glumes usually with well developed awns, sometimes acuminate to subulate. Lemma with apex free.

*Chusquea acuminatissima* (Munro) L. G. Clark, *C. aristata* Munro, *C. asymmetrica* (L. G. Clark) L. G. Clark, *C. elata* (Kunth) L. G. Clark, *C. fimbriligulata* (L. G. Clark) L. G. Clark, *C. laegaardii* (L. G. Clark) L. G. Clark, *C. nana* (L. G. Clark), L. G. Clark, *C. nobilis* (Munro) L. G. Clark, *C. rigida* (L. G. Clark) L. G. Clark, *C. stuebelii* (Pilg.) L. G. Clark, *C. villosa* (L. G. Clark) L. G. Clark.

**Chusquea** subgenus **Magnifoliae** L. G. Clark & Fisher, subg. nov.—TYPE: *Chusquea magnifolia* L. G. Clark.

Internodes 1–3 cm long, solid. Culm leaves poorly or not differentiated. Bud complement, if developed, consisting of a single bud triangular in outline and oriented vertically. Aerial branching absent. Foliage leaf blades (20-)30–300 (-377) cm long and 1.2–24(-30) cm wide; pseudopetioles well developed; lacking blade tissue, (1-)3–50 cm long. Glumes usually lacking awns. Lemma with apex free.

*C. angusta* (Swallen) L. G. Clark, *C. cylindrica* L. G. Clark, *C. diversiglumis* (Soderstr.) L. G. Clark, *C. glomerata* (Swallen) Dorr, *C. magnifolia* L. G. Clark, *C. mollis* (Swallen) L. G. Clark, *C. petiolata* (Davidse & L. G. Clark) L. G. Clark, *C. silverstonei* (Davidse & L. G. Clark) L. G. Clark, *C. spectabilis* L. G. Clark, *C. tovari* L. G. Clark.

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Appendix 1. Taxon sampling, voucher information, and GenBank accession information for specimens used in this study. All vouchers are deposited at ISC. The information is listed as: taxon, voucher collection number; GenBank accessions: *ndhF* gene, rpl16 intron, ndhA intron, trnD-trnT intergenic spacer, trnT-trnL intergenic spacer, and ITS complex (AC = Andre Mauricio Carvalho; AF = Amanda Fisher; EG = Elizabeth Gordon; ER = Eduardo Ruiz; GSK = Gabriel Sánchez-Ken; FZ = Fernando Zuloaga; IM = I. Montecinos; JT = Jimmy Triplett; LC = Lynn Clark; LSS= Luis Sergio Sarahyba; MS = Margaret Stern; PA = Patricio Asimbaya; PT = Pedro Tenorio; PW = Paulo Windisch; SK = Scot Kelchner; SL = Simon Laegaard; WO = Walter de Oliviera; WZ = Weiping Zhang; XL = Ximena Londoño).

***Rhipidocladum harmonicum*** (Parodi) McClure. *LC & WO 1103*; KF945324, KF945624, KF945453, KF945223, KF945387,–. ***Otatea acuminata*** (Munro) C. Calderón & Soderstr. *LC et al. 1312*; AF182350, AF133473, KF945452, FJ751732, KC020559, KF945270. ***Guadua angustifolia*** Kunth. *LC & XL 931*; FJ751641, FJ751664, KF945451, FJ751734, FJ644154, AY993946. ***Chusquea aristata*** Munro. *LC et al. 1084*; FJ751654, AY912196, KF945450, FJ751747, KF945386, KF945268. ***Chusquea spectabilis*** L. G. Clark. *XL & LC 905*; AF182355, U62793, KF945449, FJ751752, KC020550, KF945269. ***Chusquea abietifolia*** Griesebach. *LC et al. 1509*; KF945271, KF945591, KF945388, KF945170, KF945325,–. ***Chusquea albilanata*** L. G. Clark & Londoño. *LC et al. 1125*; KF945272, KF945592, KF945389, KF945171, KF945326, KF945224. ***Chusquea andina*** Philippi. *LC & ER 980*; KF945273, KF945593, KF945390, KF945172, KF945327, KF945225. ***Chusquea antioquensis*** L. G. Clark & Londoño. *LC & XL 1222*; KF945274, U62783, KF945391, KF945173, KF945328, KF945226. ***Chusquea arachniformis*** L. G. Clark & Londoño. *LC & XL 1228*; FJ751646, U62787, KF945392, KF945329, FJ751739,–. ***Chusquea bambusoides*** (Raddi) Hack. *LC 1029*; FJ751649, AY912194, KF945393, FJ751742, KC020548,–. ***Chusquea bilimekii*** Fournier. *LC et al. 1311*; KF945275, U54757, KF945394, KF945174, KF945330,–. ***Chusquea bradei*** L. G.Clark. *AC 4377*; FJ751650, FJ751666, KF945395, FJ751743, KF945331,–. ***Chusquea coronalis*** Soderstr. & C. Calderón. *SK 19*; FJ751642, U54759, KF945396, FJ751735, KF945332, KF945227. ***Chusquea costaricensis*** L. G. Clark & R. March. *SK 15*; KF945276, JQ352318, KF945397, KF945175, KF945333, KF945228. ***Chusquea culeou*** Desvaux. *LC & ER 976*; KF945277, AY912195, KF945398, KF945176, KF945334, KF945229. ***Chusquea cumingii*** Nees.— s. n. dried sample; KF945278, KF945596, KF945399, KF945177, KF945335,–. ***Chusquea exasperata*** L. G. Clark. *LC et al. 1093*; KF945279, U62784, KF945597, KF945400, KF945178, KF945336,–. ***Chusquea*** aff. ***fendleri*** Munro. *LC & XL 1220*; KF945280, U62780, KF945401, KF945179, KF945337, KF945230. ***Chusquea foliosa*** L. G. Clark. *AF 31*; KF945281, JQ352306, KF945402, KF945180, KF945338, KF945231. ***Chusquea gigantea*** Demoly. s. n. Brest, France; KF945282, KF945598, KF945403, KF945181, KF945339, KF945232. ***Chusquea glauca*** L. G. Clark. *LC et al. 1310*; KF945283, KF945599, KF945404, KF945182, KF945340, KF945233. ***Chusquea gracilis*** McClure & Smith. *LC 1034*; KF945284, KF945600, KF945405, KF945183, KF945341, KF945234. ***Chusquea juergensii*** Hackel. *LC & PW 1067*; KF945285, U62792, KF945406, KF945184, KF945342, KF945235. ***Chusquea latifolia*** L. G. Clark. *LC & XL 417;*–,–,–,–,–, AF019788. ***Chusquea lehmannii*** Pilger. *LC & PA 1386*; KF945286, JQ352307, KF945407, KF945185, KF945343, KF945236. ***Chusquea leonardiorum*** L. G. Clark. *LC et al. 1081*; KF945287, JQ352308,–, KF945186, KF945344, KF945237. ***Chusquea leptophylla*** Nees. *LC & XL 1043*; KF945288, KF945602, available on request (<200b), KF945187, KF945345,–. ***Chusquea liebmannii*** Fournier. *AF 1*; KF945289, KF945603, KF945408, KF945188, KF945346, available on request (<200b). ***Chusquea longifolia*** Swallen. *AF 41*; KF945290, JQ352309, KF945409, KF945189, KF945347, KF945238. ***Chusquea longispiculata*** L. G. Clark. *LC & XL 1026*; KF945291, KF945604, KF945410, KF945190, KF945348, KF945239. ***Chusquea lorentziana*** Grisebach. *LC & FZ 1017*; KF945292, JQ352310, KF945411, KF945191, KF945349, KF945240. ***Chusquea loxensis*** L. G. Clark. *LC et al. 1114*; KF945293, JQ352311, KF945412, KF945192, KF945350, KF945241. ***Chusquea maclurei*** L. G. Clark. *LC & SL 1127*; KF945294, KF945605, KF945413, KF945193, KF945351, KF945242. ***Chusquea maculata*** L. G. Clark. *LC & XL 1226*; KF945295, JQ352312, KF945414, KF945194, KF945352, KF945243. ***Chusquea mayrae*** Fisher, Tyrrell & L. G. Clark. *AF 32;*–, JQ352313,–,–,–, KF945244. ***Chusquea*** *microphylla* (Doell in Martius) L. G. Clark. s. n. airdried; KF945296, KF945606, KF945415, KF945195, KF945353,–. ***Chusquea mimosa*** *subsp.* **australis** L. G. Clark. *LC & XL 1040*; KF945297, KF945607, KF945416, KF945196, KF945354,–. ***Chusquea montana*** R.A. Philippi. *LC & ER 993*; KF945298, KF945608, KF945417, KF945197, KF945355,–. ***Chusquea muelleri*** Munro. *LC et al. 1140*; KF945299, KF945609, KF945418, KF945198, KF945356,–. ***Chusquea nudiramea*** L. G. Clark. *LC & PW 1068*; KF945300, U62792, KF945419, KF945199, KF945357, KF945245. ***Chusquea oligophylla*** Ruprecht. *LC & XL 1031*; FJ751651, U62785, KF945420, FJ751744, KF945358,–. ***Chusquea oxylepis*** (Hack.) Ekman. *LC & PW 1069*; FJ751647, U62786, KF945421, FJ751740, KF945359, KF945246. ***Chusquea paludicola*** L. G. Clark. *SK 11*; KF945301, JQ352320, KF945422, KF945200, KF945360, KF945247. ***Chusquea patens*** L. G. Clark. *AF 2*; KF945302, JQ352314, KF945423, KF945201, KF945361, KF945248. ***Chusquea perligulata*** (Pilger) McClure. *LC et al. 1074;* KF945303, JQ352315, KF945424, KF945202, KF945362,–. ***Chusquea perotensis*** L. G. Clark, Cortés & Cházaro. *LC & PT 952*; KF945304, KF945611, KF945425, JQ352304, KF945363, KF945249. ***Chusquea pinifolia*** (Nees) Nees. *LC & PW 1056*; KF945305, U54756, KF945426, KF945204, KF945364, KF945250. ***Chusquea pittieri*** Hackel. *AF 35*; KF945306, KF945613, KF945427, KF945205, KF945365, KF945251. ***Chusquea pohlii*** L. G. Clark. *AF 6*; KF945307, JQ352316, KF945428, KF945206, KF945366, KF945252. ***Chusquea quila*** Kunth. *LC & IM 970*; KF945308, KF945614, KF945429, KF945207, KF945367, KF945253. ***Chusquea ramosissima*** Lindman. *AC 4358*; KF945309, AF133472, KF945430, KF945208, KF945368,–. ***Chusquea robusta*** L. G. Clark & Losure. *LC et al. 1132*; KF945310, JQ352317, KF945431, KF945209, KF945369, KF945254. ***Chusquea scabra*** Soderstrom & Calderón. *AF 10*; KF945311, KF945615, KF945432, KF945210, KF945370, KF945255. ***Chusquea scandens*** Kunth. *LC & XL 1235*; FJ751643, U62781, KF945433, FJ751736, KC020549,–. ***Chusquea serpens*** L. G.Clark. *LC & XL 1253*; FJ751645, U54754, KF945434, FJ751738, KF945371, KF945256. ***Chusquea spencei*** Ernst. *LC et al. 1269*; KF945312, U62788, KF945435, KF945211, KF945372, KF945257. ***Chusquea subtessellata*** Hitchcock. *SK 14*; KF945313, U54753, KF945436, KF945212, KF945373, KF945258. ***Chusquea subtilis*** Widmer & L. G. Clark.*AF 19*; KF945314, JQ352322, KF945437, KF945213, KF945374, KF945259. ***Chusquea***

***talamancensis*** Widmer & L. G. Clark. *SK 16*; KF945315, U62789, KF945438, KF945214, KF945375, KF945260. ***Chusquea tenella*** Nees. *LC & XL 1032*; KF945316, KF945618, KF945439, KF945215, KF945376,–. ***Chusquea tessellata*** Munro. *LC et al. 1267*; FJ751644, U54752, KF945440, FJ751737, KF945377, KF945261. ***Chusquea tomentosa*** Widmer & L. G. Clark. *AF 20*; KF945317, JQ352321, KF945441, KF945216, KF945378, KF945262. ***Chusquea tonduzii*** Hackel. *SK 13*; KF945318, JQ352319, KF945442, KF945217, KF945379, KF945263. ***Chusquea uliginosa*** R.A. Philippi. *LC & ER 992*; KF945319, KF945621, KF945443, KF945218, KF945380, KF945264. ***Chusquea uniflora*** Steudel. *LC & PA 1387*; KF945320, KF945622, KF945444, KF945219, KF945381, KF945265. ***Chusquea urelytra*** Hackel. *LC & XL 1052*; FJ751648, FJ751665, KF945445, FJ751741, KF945382,–. ***Chusquea virgata*** Hackel.*AF 34*; KF945321, KF945623, KF945446, KF945220, KF945383, KF945266. ***Chusquea vulcanalis*** (Soderstrom & Calderón) L. G. Clark. *SK 12*; KF945322, U62790, KF945447, KF945221, KF945384, KF945267. ***Chusquea windischii*** L. G. Clark. *LC & XL 1047*; KF945323, U54755, KF945448, KF945222, KF945385,–.