DISENTANGLING THE RETICULATE EVOLUTIONARY HISTORY OF A NEOTROPICAL PLANT RADIATION

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ABSTRACT

Determining the causes and nature of speciation is important for understanding the origin and maintenance of the world's biodiversity. Hybridization—the exchange of genes between different species—is sometimes recognized as an important process in the evolution of animals and fungi, but seems to be a ubiquitous feature of the evolutionary history of plants where it is particularly prominent. Hybrid speciation can occur either by keeping the same genome size—homoploid hybrid speciation—or more frequently via hybridization and genome duplication—allopolyploidy. Hybridization, in the form of homoploid hybrid speciation and polyploidy has contributed extensively to angiosperm diversity, but also represents a great challenge for phylogenetic reconstruction due the incongruence patterns that a reticulate process produces within and between the nuclear and chloroplast genomes. Additional processes can also produce similar reticulate patterns, creating the necessity to disentangle these processes and model phylogenetic networks that best represent species relationships in the presence of reticulation, rather than a classic bifurcating tree.

This dissertation combines extensive natural history collections with a plurality of molecular phylogenetic methods to elucidate the reticulate evolutionary history of the plant genus *Lachemilla* in the rose family. This is a morphologically highly variable group of ca. 60 species that includes perennial herbs and shrubs, with a nearly ubiquitous presence in the Neotropical high-altitude grasslands and remarkable diversity in the Andes. First, I reconstructed the phylogeny of *Lachemilla* using nuclear ribosomal ITS and plastid *trnL-F* DNA sequences. I identified four major clades in *Lachemilla* that are in part congruent with previous morphological

classifications of the group. However, widespread patterns of cytonuclear discordance was detected that, in combination with chromosome counts and genome size estimates, provided evidence of the putative allopolyploid origin of multiple species. Finally, I established the date of origin of *Lachemilla* to be around 15 million years ago, with a rapid accumulation of lineages during the last five million years.

In my second chapter, I conducted a phylogenomic analysis of 396 loci from low-copy nuclear genes and complete chloroplast sequences to clarify the relationships among the major groups of *Lachemilla*, and explore a multiple of sources of conflict among gene trees and species trees. All phylogenetic approaches used recovered the four major groups previously proposed for *Lachemilla,* but these methods all recovered different topologies for the relationships of these four clades. Species network analyses revealed that one of the four major clades is actually of ancient hybrid origin, and represents one of the main sources of the incongruence among the species trees. Additionally, I found evidence for a whole genome duplication event shared by *Lachemilla* and allied genera.

In my third chapter, I used PCR target enrichment in combination with high-throughput sequencing to obtain allelic information for the nuclear ribosomal cistron and multiple regions of the plastid genome in 219 samples of 48 species of *Lachemilla*, to provide direct evidence of the allopolyploid speciation in this group. I found evidence of the allopolyploid origin of 30 species of *Lachemilla*, demonstrating that this condition is common and widespread in the genus. Additionally, based on a well-resolved chloroplast phylogeny, I identified that the monotypic genus *Farinopsis* is the sister group of *Lachemilla* and allied genera within subtribe Fragariinae. However, this result is discordant with the nrDNA phylogeny,

a pattern also observed between *Lachemilla* and allied genera, which corroborates the notion of widespread hybridization in Fragariinae.

Finally, with the extensive fieldwork that I conducted throughout the Neotropics, I discovered and described the new species *Lachemilla mexiquense,* which is endemic to central Mexico. This species is characterized by a unique combination of characters in the genus, and through the network-based analyses that I conducted, was later shown to be of hybrid origin.

With all this work, I have attempted to clarify the role that hybridization has played in the evolutionary history of *Lachemilla*. This work will not only facilitate continued research questions, including historical biogeography, diversification patterns, trait evolution, and future taxonomic treatments of the genus, but also broader questions regarding the evolutionary and ecological consequences of polyploidy and hybridization.

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DEDICATION

Para Rosario y Eduardo por su confianza y apoyo incondicional.

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Morales-Briones, D. F., K. Romoleroux, F. Kolář, and D. C. Tank. Phylogeny and Evolution of the Neotropical Radiation of *Lachemilla* (Rosaceae): Uncovering a History of Reticulate Evolution and Implications for Infrageneric Classification. Submitted to *Systematic Botany*

Abstract

Lachemilla Focke (Rydb.) is a highly morphologically variable group of perennial herbs and shrubs, and a nearly ubiquitous member of the diverse Neotropical high-altitude grasslands. Here we focus on reconstructing the phylogeny of *Lachemilla* using nuclear ribosomal ITS and plastid *trnL-F* DNA sequences, and explore widespread patterns of cytonuclear discordance in this group. Our analyses identified four major clades within *Lachemilla* that are in part congruent with previous morphological classifications of the group. However, using multiple sources of evidence, including a procrustean approach to cophylogeny estimation, coalescent-based simulations, phylogenetic networks, chromosome counts, and genome size estimations, we also revealed a surprisingly reticulate history of *Lachemilla*. Finally, we established the date of origin of *Lachemilla* to be around 15 mya, with a rapid accumulation of lineages during the last five mya.

Introduction

With 90,000 – 110,000 plant species, the Neotropics is one of the most diverse regions in the world, harboring ca. 37% of the world's total flora (Antonelli and Sanmartín 2011). A large part of this diversity is found in the high-altitude grasslands (between about 3,000 m and 5,000 m) that include the zacatonales in Mexico and Guatemala, the páramos in Central and northern South America, the jalca in northern Peru, and the drier puna from central Peru to northern Argentina. These regions all have similar vegetation (tussock grasses, rosette plants, cushion plants, and evergreen sclerophyllous shrubs) and ecological correspondence, and a large number of the genera are shared between them (Rzedowski 1978; Luteyn, 1999; García and Beck 2006; Mena and Hofstede 2006; Sánchez-Vega and Dillon 2006). Several lineages that span the páramo and puna ecosystems, most notably Espeletiinae, *Gentianella*, *Hypericum*, *Lupinus*, *Neobartsia*, *Puya*, and *Valeriana*, among others (Bell and Donoghue 2005; Cuatrecasas 1986; Drummond et al. 2012; Hughes and Eastwood 2006; Jabaily and Sytsma 2013; Nürk et al. 2013; Rauscher 2002; Uribe-Convers and Tank 2015; von Hagen and Kadereit 2001), have undergone an extraordinary diversification in these regions, and these remarkable radiations have been associated with the most recent uplift of the Northern and Central Andes in the last five to 10 millions years (reviewed in Sklenář et al. 2011; Madriñán et al. 2013; Luebert and Weigend 2014).

Lachemilla Focke (Rydb.), a morphologically highly variable group in the rose family, Rosaceae, that includes perennial rosette-forming herbs, stoloniferous herbs, trailing herbs, procumbent herbs, subshrubs, and dwarf shrubs (Fig. 1.1) is a nearly ubiquitous member of the high-altitude grasslands, comprising ca. 80 species that

are distributed from northern Mexico to northern Argentina and Chile between 2,200 m and 5,000 m in elevation (with one species occurring in the Dominican Republic; Romoleroux 1996, 2004; Gaviria 1997). *Lachemilla* is one of the main elements of the páramo and superpáramo floras, extending into the upper margins of high Andean forests (Gaviria 1997; Romoleroux 2004); it is also a very common element of the punas, and in Mexico and Guatemala it can be found in sub-alpine and alpine habitats from the mountain pine forest to high elevation zacatonales (Morales-Briones 2016). At least 36 species (60% of total diversity) representing most of the morphological variation within *Lachemilla* can be found between Venezuela and Ecuador, suggesting that the northern Andes are the center of diversification of the group (Romoleroux 2004). Furthermore, in the northern Andes, *Lachemilla* occurs in all ecological zones, ranging from the upper margins of the cloud forest to the dry grasslands and shrub-steppe habitats, and even occurs in aquatic to semi-aquatic regions of the páramo (Romoleroux 1996). This morphological and ecological diversity, combined with the relatively young age of the páramo ecosystem (ca. 2.7 mya; Gregory-Wodzicki, 2000), suggests that *Lachemilla* may be the result of a rapid ecological radiation concomitant with the most recent Andean orogeny.

Figure 1.1. Examples of morphological diversity in *Lachemilla.* A. *L. polylepis.* B. *L. pectinata.* C. *L. venusta.* D. *L. procumbens.* E. *L. pringlei.* F. *L. velutina.* G. *L. purdiei.* H. *L. ranunculoides.* I. *L. nivalis.* J. *L. pinnata.* K. *L. diplophylla.* L. *L. rupestris.*

Lachemilla is part of the subtribe Alchemillinae along with *Alchemilla* and *Aphanes* within the tribe Potentilleae (Rothmaler 1937; Notov and Kusnetzova 2004; Soják 2008). Alchemillinae is characterized by small, apetalous flowers, 1-4(–5) stamens that have anthers with one elliptic theca on the ventral side of the connective that opens by one transverse split, and achenes with subbasally to basally persistent styles (Perry 1929; Soják 2008). The taxonomic history of *Lachemilla* has been confusing, where it has been variously treated as a section (Focke 1888; Perry 1929) or subgenus (Lagerheim 1894; Rothmaler 1935a; Kalkman 2004) of *Alchemilla* (reviewed in Notov and Kusnetzova 2004; Gehrke et al. 2008), or as an independent genus (Rydberg 1908; Rothmaler 1937; Romoleroux 1996; Gaviria 1997; Notov and Kusnetzova 2004; Soják 2008; Barrie 2015). Gehrke et al. (2008) presented the first molecular phylogeny of Alchemillinae recognizing four clades, *Aphanes*, *Lachemilla,* and two paraphyletic clades of *Alchemilla* (Eurasian and African), and suggested treating these four groups as the single genus *Alchemilla* within the subtribe Fragariinae (see also Eriksson et al. 2003; Potter et al. 2007) based on nomenclatural stability and the lack of morphological characters to distinguish the African from the Eurasian clade of *Alchemilla.* Nonetheless, *Lachemilla* has a long history of being recognized as an independent genus (e.g., Rydberg 1908; Rothmaler 1937), and based on their Neotropical distribution, the presence of mainly two stamens inserted on the inner side of the disc opposite the sepals, and extrorse anthers, recent treatments of *Lachemilla* have maintained this distinction (Romoleroux 1996; Gaviria 1997; Notov and Kusnetzova 2004; Soják 2008; Barrie 2015).

Despite its nearly ubiquitous occurrence throughout the montane American tropics, *Lachemilla* remains a taxonomically complex group where species

boundaries are often unclear and the infrageneric taxonomy is poorly defined. Perry (1929) carried out the first comprehensive taxonomic revision of *Lachemilla* recognizing 41 species in six series and six subseries (Fig. 1.2; Table 1.1). Later, Rothmaler proposed a five-section, five-subsection classification (1935a), and completed the revision of *Lachemilla* from Colombia (1935b). Shortly thereafter, Rothmaler (1937) revised the entire genus recognizing 71 species in six sections and five subsections (Fig. 1.2, Table 1.2).

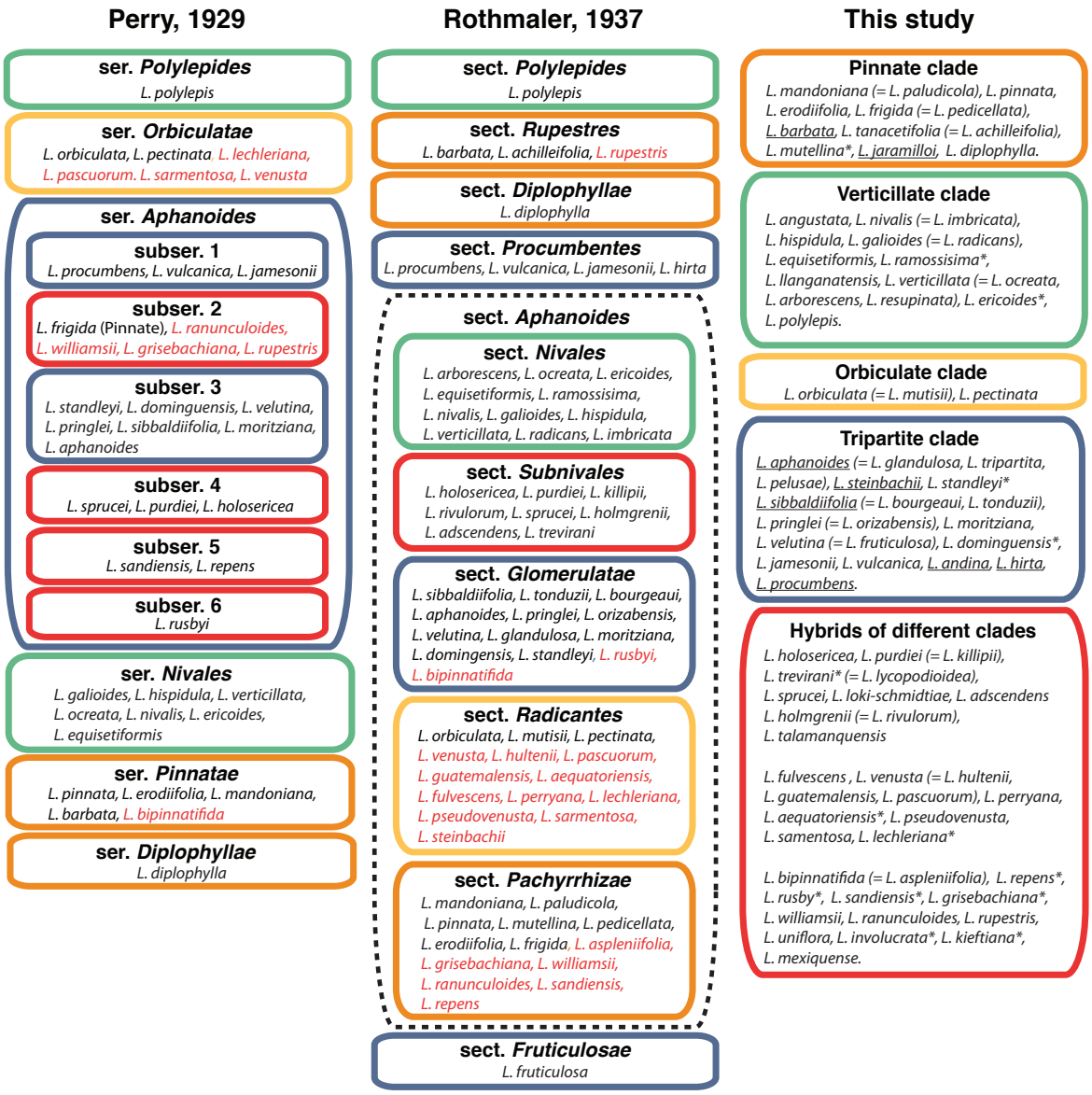

Figure 1.2. Classification systems of Perry (1929) and Rothmaler (1937), and summary of the four main clades of *Lachemilla* found in this study. Boxes are colored to match infrageneric groups of the two previous systems with their counterpart of the four clades proposed here. Groups and species in red in previous classifications represent putative hybrids between different major clades; underlined species represent putative hybrids of species found in the same major clade. Species with an asterisk (*) were not sampled for this study. Sect. *Aphanoides* of Rothmaler's system (dotted line) does not have a counterpart clade. Accepted taxa and synonymy used for this study follows in part Romoleroux (1996), Luteyn (1999), Romoleroux (2004), and Barrie (2015), with additional unpublished synonymy proposed here.

Table 1.1. Perry's infrageneric classification of *Lachemilla*. Species circumscription and synonymy as in Perry (1929). Country abbreviation: Argentina (ARG), Bolivia (BOL), Chile (CHI), Colombia (COL), Costa Rica (CR), Ecuador (ECU), Peru (PER), Mexico (MEX).

Series	Main characteristics	Distribution	Species
Polylepides	Low shrubs profusely branching, leaves sessile, stipule lobes connate in front of the leaf-blade	CR, VEN–COL	*L. polylepis*
Orbiculatae	Herbs with repent or decumbent stems, leaves 5–11-lobed or –cleft; flowers disposed in racemose cymes.	MEX–BOL	*L. orbiculata, L. pectinata, L. lechleriana, L. sarmentosa, L. pascuorum. L. venusta*
Aphanoides	Herbs with prostrate, decumbent or erects stems; leaves tripartite often appearing 5-parted on account of cleft lateral lobes: inflorescence loosely cymose or glomerulate	MEX -BOL	
Subseries 1	Flowers with hypanthium pubescent or villous within; inflorescence an open lax disposed cyme with flowers on pedicels 2–10 mm long	MEX–BOL	*L. procumbens, L. vulcanica, L. jamesonii*
Subseries 2	Stems prostrate, leaves tripartite with lateral segments various lobed	PER–ARG	*L. williamsii, L. frigida, L. ranunculoides, L. grisebachiana, L. rupestris*
Subseries 3	Stems decumbent or ascending; leaves palmately tripartite, lateral segments of the leaves entire of bifid; inflorescence glomerulate axilliary or terminal.	MEX–BOL	*L. standleyi, L. dominguensis, L. velutina, L. pringlei, L. sibbaldiifolia, L. moritziana, L. aphanoides.*
Subseries 4	Leaf blades of middle stem not conspicuously longer than the stipules, uppermost leaves with stipules forming sheaths with lobes ascending and equal as in the Nivales	COL–PER	*L. sprucei, L. purdiei, L. holosericea*
Subseries 5	Herbs with basal leaves subpinnately tripartite	PER, ARG, CHI	*L. sandiensis, L. repens*
Subseries 6	Herbs with hypanthium lobes uniseriate	BOL	*L. rusbyi*
Nivales	Herbs with erect or decumbent stems; basal leaves 3–5-lobed or -cleft; stem leaves reduced, with the adnate stipule forming verticillate sheaths with 4–15 lobes.	CR, VEN–BOL	*L. galioides, L. hispidula, L. verticillata, L. ocreata, L. nivalis, L. ericoides, L. equisetiformis*
Pinnatae	Herbs with repent or decumbent stems; basal leaves pinnate or bipinnatifid; inflorescence glomerulate and terminal or flowers solitary and axillary.	MEX–ARG/CHI	*L. bipinnatifida, L. pinnata, L. erodiifolia, L. mandoniana, L. barbata.*
Diplophyllae	Low herbs with creeping rhizomes; upper surface of the leaves appendaged on both sides of the midrib; flowers solitary and axillary.	ECU–BOL/CHI	*L. diplophylla*

Table 1.2. Rothmaler's infrageneric classification of *Lachemilla*. Species circumscription and synonymy as in Rothmaler (1937), sectional definitions as in Rothmaler (1935a; 1935b). Country abbreviations as in Table 1.1. The parenthesis next to a species name contains its position in Perry's system.

Sections	Main characteristics	Distribution	Species
Polylepides	Same as ser. *Polylepides*	CR, VEN–COL	*L. polylepis*
Rupestres	Low herbs, membranous stipule with free apex, leaves pinnate to tripartite.	VEN–BOL	*L. rupestris* (subser. 2), *L. barbata* (subseries V), *L. tanacetifolia.*
Diplophyllae	Same as ser. *Diplophyllae*	ECU–BOL/CHL	*L. diplophylla*
Procumbentes	Same as subser. 1	MEX–BOL	*L. procumbens, L. vulcanica, L. jamesonii, L. hirta*
Aphanoides	Small shrubs or herbs; leaves entire, lobed or pinnate, apex of basal stipules free and leafy, lobed or partite; inflorescence in dense cymes, pseudo racemes.		
Subsection *Nivales*	Same as ser. *Nivales*	CR, VEN–PER	*L. arborescens, L. ocreata, L. ericoides, L. equisetiformis, L. ramossisima, L. imbricata, L. nivalis, L. galioides, L. hispidula, L. verticillata, L. radicans.*
Subsection *Subnivales*	Same as subser. 4	COL–PER	*L. holosericea, L. purdiei, L. killipii, L. rivulorum, L. sprucei, L. holmgrenii, L. adscendens, L. trevirani.*
Subsection *Glomerulatae*	Same as subser. 3	MEX–BOL	*L. sibbaldiifolia, L. tonduzii, L. bourgeaui, L. aphanoides, L. pringlei, L. orizabensis, L. velutina, L. glandulosa, L. moritziana, L. domingensis, L. standleyi, L. rusbyi* (subser. 6), *L. bipinnatifida* (ser. Pinnatae).
Subsection *Radicantes*	Same as ser. *Orbiculatae*	MEX–BOL	*L. orbiculata, L. mutisii, L. pectinata, L. venusta, L. hultenii, L. pascuorum, L. guatemalensis, L. aequatoriensis, L. fulvescens, L. perryana, L. lechleriana, L. pseudovenusta, L. sarmentosa, L. steinbachii.*
Subsection *Pachyrrhizae*	Same as ser. *Pinnatae* + subser. 2 + subser. 5	MEX–ARG/CHI	*L. mandoniana, L. paludicola, L. sandiensis, L. repens, L. pinnata, L. aspleniifolia, L. erodiifolia, L. grisebachiana, L. williamsii, L. ranunculoides, L. frigida, L. mutellina, L. pedicellata.*
Fruticulosae	Erect herbs, lowermost leaves petiolated and 3-lobed, upper leaves sessile. Flowers lacking episepals.	MEX	*L. fruticulosa*

Most infrageneric classifications of *Lachemilla* have been based primarily on vegetative characters, like life form and leaf segmentation (Perry 1929; Gaviria 1997; Romoleroux 2004). The leaf blade can vary from hollowly lobed (*L. pectinata*, Fig. 1.1 B) to pinnately parted (*L. pinnata*, Fig. 1.1 J). The leaf blades can also be highly modified into whorls formed by simple elongate leaves fused to the stipules (*L. nivalis*, Fig. 1.1 I), and in an extreme case the leaves can even have two foliaceous appendages attached along the midrib that gives the appearance of a double leaf (*L. diplophylla*, Fig. 1.1 K). Although reproductive characters are not particularly useful for identifying major groups within *Lachemilla*, they are used extensively for species identification (Perry 1929; Gaviria 1997; Romoleroux 2004). The inflorescences are arranged in condensed to loose cymes, with few species having solitary flowers. The hypanthium shape varies from turbinate to urceolate and the outer surface may be pubescent, glabrate, or glabrous, while the inner part is mainly glabrous with few species having a pubescent inner hypanthium. The hypanthium lobes are arranged in two series, sepals and episepals; the latter can be absent in few species. The stamens are uniform throughout *Lachemilla* and lack of specific characters (Perry 1929). The achenes remain in the persistent calyx and vary from 1-6(–10); while this number can be useful to identify species, achene morphology does not provide informative characters for taxonomic purposes (Perry 1929). More recently, Notov and Kusnetzova (2004) reviewed the classification of Alchemillinae based on architectural analysis of plant body, but did not make any changes to the classification. Gehrke et al. (2008) found some congruence between the molecular phylogeny and the morphological classification of *Lachemilla,* but their sampling was insufficient to draw any conclusions regarding an infrageneric classification for *Lachemilla*.

In addition to these taxonomic difficulties, the molecular phylogeny of Alchemillinae by Gehrke et al. (2008) shows major incongruences between the nuclear and chloroplast phylogenies, suggesting hybridization and/or incomplete lineage sorting (ILS). This pattern has also been detected among several other members of the subtribe Fragariinae (including Euarasian *Alchemilla* and *Aphanes*) and Potentillinae, with allopolyploidy attributed as the main source of incongruence (Lundberg 2009; Töpel et al. 2011). Polyploidization is one of the most frequent mechanisms of speciation in plants (Otto and Whitton 2000; Levin 2002; Soltis et al. 2014). In the case of allopolyploids, species are produced by hybridization between two different species. These hybrids inherit the nuclear DNA from both parental species, while the organellar DNA is inherited only from the maternal parent. This reticulate process often leads to incongruence between nuclear and plastid phylogenies and discordance between independent nuclear loci and/or alleles (Rieseberg and Soltis 1991; Doyle 1992; Wendel and Doyle 1998; Linder and Rieseberg 2004). In plants, comparisons between nuclear and chloroplast phylogenies have been used to disentangle patterns of reticulation (e.g., Soltis and Kuzoff 1995; Sang et al. 1995; Sang et al. 1997). Although, incongruence is a necessary consequence of reticulate evolution, it is not sufficient evidence for hypothesizing hybridization (Doyle et al. 2003). Furthermore, incongruence can also be a product of phylogenetic error and incomplete lineage sorting (Rieseberg and Soltis 1991; Doyle 1992; Wendel and Doyle 1998). Consequently, the inconsistent placement of taxa between nuclear and plastid phylogenies is only an initial step to detect hybridization, and additional sources of evidence must be incorporated (Wendel and Doyle 1998; Guggisberg et al. 2009).

In addition to the phylogenetic incongruences detected by Gehrke et al. (2008), members of Alchemillinae are also known for high rates of polyploidy. The base chromosome number of the clade has been inferred to be $x = 8$ (Dickinson et al. 2007), and ploidy levels have been well documented in the Eurasian species of *Alchemilla* (e.g., Turesson 1957; Izmailow 1981; Wegener 1967; Hayirhoğlu -Ayaz et al. 2006), while very little is known about the other members of the clade. *Aphanes* has diploid ($2n$ =16) and tetraploid species (Montgomery et al. 1997), while only polyploid species have been documented in the Eurasian *Alchemilla* (octoploid to 24-ploid; Hayirhoğlu -Ayaz et al. 2006), African *Alchemilla* (octoploid to decaploid; Hjelmquist 1956; Morton 1993) and *Lachemilla* (octoploid to dodecaploid; Table 1.3). This suggests that allopolyploidy may also be a prominent source of gene tree incongruence in *Lachemilla*.

The aims of this work are to (i) estimate the phylogeny of *Lachemilla* using an expanded taxonomic sampling for the chloroplast region *trnL-F* and the nuclear ribosomal ITS region, (ii) determine the source of incongruence between the plastid and nuclear phylogenies, (iii) explore the variation in ploidy levels and genome sizes in species of *Lachemilla* using chromosome counts and flow cytometry, (iv) evaluate the taxonomic implications of the reticulate history of *Lachemilla*, and (v) provide a temporal framework for the origin of *Lachemilla* and its diversification in the Neotropics.

Materials and Methods

Taxon Sampling— We sampled 107 individuals of 46 species of *Lachemilla*, representing approximately 75% of the total diversity of the group (Fig 1.2). To

represent the morphological variation and geographic range of widespread species, when possible, more than one individual per species was included. This sampling includes all major groups within *Lachemilla* proposed by Perry (1929) and Rothmaler (1937). Additionally, GenBank sequences of *Alchemilla* (Eurasian and African clades), *Aphanes,* and the majority of genera in Fragariinae, as well as other member of Potentilleae and Rosoideae were included as outgroup taxa. Voucher information, GenBank accession numbers, and sources for published sequences are listed in Appendix 1.

DNA Extraction, Amplification and Sequencing— Total genomic DNA was isolated from fresh, silica-dried, or herbarium material using the DNeasy plant mini kit (Qiagen, Valencia, California) following the manufacturer's protocol, or with a modified 2 × CTAB method (Doyle and Doyle 1987). Polymerase chain reaction (PCR) was used to amplify the internal transcribed spacer region of nuclear ribosomal DNA (nrITS) with primers ITS4 (White et al. 1990) and ITS-I (Urbatsch et al. 2000), and the choloroplast (cpDNA) *trnL* intron, intergenic spacer between *trnL* and *trnF,* and *trnF* with primers trnL-c and trnL-f (*trnL-F*; Taberlet et al. 1991). The PCR was performed in a volume of 50 μl with final concentrations of 1× PCR buffer, dNTPs at 0.2 mM each, 3 mM $MgCl_2$, 1.0 unit of Platinum *Taq* DNA Polymerase (Invitrogen, Carlsbad,, California), forward and reverse primers at 0.2 mM each, 1–10 ng of genomic DNA, and ddH_2O to volume. Amplification conditions consisted of initial denaturation of 95°C for 2 min followed by 30 cycles of 94 °C for 45 s, 55 °C (ITS) or 50 °C (*trnL-F*) for 45 s and 72 °C for 45 s, followed by a final extension of 72 °C for 5 min. Amplification products were purified using QIAquick PCR purification kit (Qiagen, Valencia, California) following the manufacturer's protocol

or with a 20% polyethylene glycol 8000 (PEG) precipitation and washed in 70% ethanol. Cycle sequencing was performed on both strands using the same primers used for PCR amplification and BigDye Terminator v.3.1 (Applied Biosystems, Foster City, California). Sequencing PCR products were cleaned using a sodium acetate precipitation and washed in 70% ethanol. Sequence data were generated on ABI 3130*xl* analyzer.

***Phylogenetic Analyses*—** Sequences were assembled using Sequencher 4.5 (Gene Codes, Ann Arbor, Michigan) and aligned using MAFFT v7.037b (Katoh and Standley 2013). A first evaluation of topological congruence between the nuclear and chloroplast data sets was performed using Concatepillar 1.8a (Leigh et al. 2008). Concatepillar uses hierarchical clustering based on likelihood-ratio tests to identify congruent loci and evaluate if they can be combined by concatenation. Separate analyses were conducted for each dataset using maximum likelihood (ML) and Bayesian inferences (BI). The best-fit models of sequence evolution for each dataset were determined using a decision-theory (DT) approach (Minin et al. 2003) as implemented in PAUP* 4.0a147 (Swofford 2002). The ML analyses were conducted with RAxML v8.0.3 (Stamatakis 2014) using the GTR + G model for both the nrITS and *tnrL-F* data sets. One hundred searches for the best tree were performed and clade support was assessed with 1,000 non-parametric bootstrap (BS) replicates. BI analyses were conducted with MrBayes v3.2.6 (Ronquist et al. 2012) on the CIPRES portal (Miller et al. 2010). Analyses consisted of four independent runs with four Markov Chain Monte Carlo (MCMC) chains for 50 million generations with trees sampled every 1,000th generation, using the rate variation selected by the DT approach, and reversible-jump Markov Chain Monte Carlo (rjMCMC) to allow

sampling across the entire substitution rate model space (nst = mixed). Parameter estimate convergence of the four independent MCMC runs was assessed using Tracer 1.6 (Rambaut et al. 2014). A 50% majority rule consensus tree was generated and posterior probabilities (PP) were calculated after removing the first 10% of sampled trees.

An additional test of phylogenetic congruence and detection of outlier associations between the nuclear and plastid dataset were carried out using the Procrustean Approach to Cophylogeny (PACo) (Balbuena et al. 2013). To detect outliers, we followed the pipeline described in Pérez-Escobar et al. (2015), using 10,000 permutations for the global test of phylogeny congruence and 10,000 trees from the posterior distribution of each MrBayes analysis. Following Pirie et al. (2009), we constructed the 'excluded tree' matrix that excludes all potential outliers detected by the PACo analysis from the nuclear and plastid datasets before concatenation, and the 'duplicated tree' matrix that duplicates the outliers samples, with one copy represented by cpDNA sequence only (nrITS partition as missing data) and the other copy representing only the nrITS sequence (cpDNA partition as missing data). We carried out BI analyses on these matrices using the same parameters as in the individual gene tree inferences. The multilabeled phylogeny resulting from the 'duplicated tree' matrix was summarized in a phylogenetic network using the algorithm from Huber et al. (2006), as implemented in Dendroscope v3.5.7 (Huson and Scornavacca 2012).

Divergence Time Estimates— Dating analyses were carried out separately for the cpDNA and nrITS data sets using an uncorrelated lognormal (UCLN) relaxed-clock, as implemented in BEAST v1.8.3 (Drummond et al. 2012), and the same

models of sequence evolution as in the ML analyses. Condamine et at. (2015) found that the tree speciation prior can significantly influence age estimates, and suggested that dating analyses must involve a model selection step for this prior. Thus, we ran two analyses setting the tree speciation prior to Yule or the birth-death process. For each dataset, the best ML tree was supplied as the starting tree, and we ran three independent MCMC runs of 100 million generations, sampling every 10,000 generations. Convergence of the three independent MCMC runs was assessed using Tracer v1.6 (Rambaut et al. 2014), and the first 10% of sampled trees were discarded as burn-in. Trees were summarized with TreeAnnotator v1.8.3 (Drummond et al. 2012) in a maximum clade credibility chronogram with median divergence time estimates and 95% highest posterior density (HPD) intervals. To estimate support for the either of the two tree speciation priors we calculated Bayes factors (BF) from the marginal likelihood estimates (MLE) using stepping-stone sampling (SS) with 150 path steps each with a chain of one million generations.

To calibrate the tree, we used three fossils as minimum ages. For the split between *Rosa* and Potentilleae we used the oldest known fossil for *Rosa* (*R. germerensis*) from the Germer Tuffaceaus member of the Challis volcanic formation in Idaho, U. S. A. (55.8 – 48.6 mya; Edelman 1975). For the split between of *Potentilla* and Fragarinae, we used the oldest fossil of *Potentilla* from the Lusatian Brown Coal District in Lausitz, Germany (23.0 – 11.6 mya; Mai 2001). The final calibration point was for *Fragaria* using the age of macrofossils of this genus found in the Beaufort formation in Prince Patrick Island in Canada (2.5 – 1.8 mya; Matthews and Ovenden 1990). All fossils were assigned using a lognormal prior distribution and the parameters were set to incorporate ~10 Myr in the 95% tail of the distribution from the maximum age of each fossil calibration. Finally, we visualized the timing of

speciation events in *Lachemilla* using lineages-through-time (LTT) plots (Harvey et al. 1994) in both datasets trimming the phylogenies to randomly include a single individual per species.

***Assessment of Hybridization*—** To test mutational variance as the source of the discordance between the plastid and nuclear phylogenies, we used the Swofford-Olsen-Waddell-Hillis test (SOWH; Swofford et al. 1996). Following a similar approach to Reid et al. (2012), we conducted ML searches using the chloroplast dataset with the topology constrained to match the ML phylogeny of the nuclear dataset. The difference in likelihood scores between the constrained and unconstrained ML trees were calculated as the test statistic, and a null distribution for this test statistic was generated by simulating new datasets using the topology and parameter estimates from the constrained likelihood search. We carried out the SOWH test as implemented in SOWHAT v0.36 (Church et al. 2015). We then used coalescent simulations similar to Moureira-Butler et al. (2008) to test if incomplete lineage sorting (ILS) alone could explain plastid and nuclear incongruence. We used the R package Phybase v1.4 (Liu and Yu 2010) that uses the formula from Rannala and Yang (2003) to simulate gene trees using the time-calibrated gene trees as 'species tree' topologies. To incorporate phylogenetic uncertainty, we simulated 1,000 coalescent trees using a sample of 1,000 trees from the posterior distribution of each BEAST analysis. Then the tree-to-tree distances using Robinson and Foulds (1981) metric was calculated between the nuclear and chloroplast 'species trees' sampled from the posterior distribution and compared to each of the distributions of tree-to-tree distances between the simulated trees and the original nuclear and chloroplast 'species trees'. If the distances between the nuclear and chloroplast trees

were lager than 95% of the distribution of tree-to-tree distances of the simulated trees from their respective gene trees, then ILS alone is considered unlikely to explain the incongruences between the chloroplast and nuclear gene trees (Maureira-Butler et al. 2008). For coalescent simulations, the population mutation rate parameter (θ) was estimated using DNAsp v5.10.1 (Rozas et al. 2003) over 11 indivuduals of *Lachemilla mandoniana* from 11 different populations for the nrITS, and 12 individuals from 12 different populations for *trnL-F* chloroplast region. These values of θ might be underestimated due to the small sample size used for this calculation, therefore, we also explored a series of θ values to simulate the gene trees until we obtained an overlapping distribution of the distances between the nuclear and chloroplast trees and the tree-to-tree distances of the simulated trees from their respective gene trees.

***Chromosome Counts*—** Multiple living individuals of 22 species of *Lachemilla* (Appendix 2) were collected in the field, transplanted to garden soil, and maintained in a nursery. Counts were made from root tips following the protocol described in Hayirhoglu-Ayaz et al. (2006) with minor modifications. Briefly, root tips were pretreated in 0.05% colchicine for 3–3.5 h and then fixed in absolute ethanol – glacial acetic acid (3:1) for 24 h at °C. If necessary, root tips were transferred to 70% (v/v) ethanol and stored at –20 °C until used. Roots tips were hydrolyzed in 1 N HCl at 60 °C for 5-10 min and then rinsed with water for 5 min, stained with Feulgen or 1% aceto-carmine for 1.5 h, and finally squashed on a microscope slide in a drop of the same staining solution.

Flow Cytometry— Fresh leaf tissue from the same species collected for the chromosome counts, in addition to other tissues collected directly in the field (Appendix 2) were used to carry out the flow cytometry analyses. Genome size was inferred from nuclear DNA content determined by flow cytometry following the simplified two-step protocol of Doležel et al. (2007). One petiole per plant was chopped together with an appropriate volume of the internal reference standard (*Bellis perennis L.*, 2C = 3.38 pg; Schönswetter et al. 2007) using a sharp razor blade in a Petri-dish containing 0.5 mL of ice-cold Otto I buffer (0.1 M citric acid, 0.5 % Tween 20). The suspension was filtered through a 42-μm nylon mesh and incubated for 10 min at room temperature. Isolated nuclei were stained with 1 mL of Otto II buffer (0.4 M $Na_2HPO_4.12H_2O$) supplemented with propidium iodide and RNase (both in concentration 50 μg mL-1), and β mercaptoethanol in concentration 2 μg mL-1. After 1 min incubation the sample was run for 5,000 particles in CyFlow SL flow cytometer (Partec GmbH, Germany) equipped with green (532 nm) solid-state laser. We applied the following stringent criteria in order to get precise and stable genome size estimates: (i) only analyses with the coefficient of variation of the sample peak below 5 % were taken into account, (ii) each sample was measured at least three times on different days to minimize potential random instrumental drift (Doležel and Bartoš 2005), and (iii) the between-day variation was defined to not exceed the 4% threshold, otherwise the most remote value was discarded and the sample was re-analyzed.

Results

Phylogenetic Analyses— We generated 207 new sequences for this study, and all are available in GenBank (Appendix 1). Alignments will be deposited to the Dryad data repository. The first topological congruence analysis using Concatepillar detected significant conflict between the nuclear and plastid data sets ($p < 0.01$), thus phylogenetic inferences were carried out separately for each dataset. The chloroplast and nuclear phylogenies disagree significantly within *Lachemilla* (Fig. 1.3), but based on leaf morphology and habitat we recognize four major clades with moderate to high support that somewhat correspond to the groups previously described by Rothmaler (1937), and especially to the system proposed by Perry (1929). Nonetheless, these clades also include species with different morphologies than the majority of the species, and the relationships among these four clades remain unresolved. Below we describe the phylogenies using Perry's (1929) system as the primary taxonomic reference (for group equivalency between classification systems refer to Fig. 1.2 and Tables 1.1 and 1.2).

The ML and BI trees for the cpDNA dataset produced similar topologies (Fig. 1.3; Appendix 3). Together they are congruent with the four clades of Alchemillinae as recovered using chloroplast data by Gehrke et al. (2008; 2016). *Lachemilla* is monophyletic (BS = 84, PP = 0.95) and sister to the Afromilla clade (BS = 100, PP = 1.0), while the clade comprising *Aphanes* (BS = 100, PP = 1.00) and Eualchemilla (BS = 98, PP = 1.00) is sister to both. The first major group is the 'Pinnate clade' (BS = 82, PP = 1.00) that is composed of the species of the ser. *Pinnatae* and subser. 2 (ser. *Aphanoides*). This group is divided into two clades, the first one (BS = 88, PP = 1.00) comprises only the species of *Pinnatae* plus *L. diplophylla* that has been assigned to

its own monotypic group and does not have pinnate leaves. The second clade (BS = 93, PP = 1.00) comprises species from the subser. 2, along with several putative hybrid species like *L. sarmentosa, L. jaramilloi*, and *L. bipinnatifida* (*L. bipinnatifida* is also found in the 'Tripartite clade').

The second major group is the 'Verticillate clade' (BS = 98, PP = 1.00) that comprises species of the ser. *Nivales* and subser. 4 (ser. *Aphaniodes*) plus *L. rupestris* (subser. 2) nested within the verticillate species, and *L. uniflora* as the sister group to the entire clade. *Lachemilla uniflora* and *L. rupestris* do not share any obvious leaf or habitat characteristics with the other species of the clade.

The third major group is the 'Orbiculate clade' (BS = 95, PP = 1.00) that includes only species from the ser. *Orbiculatae*. As sister species of this clade we found *L. mexiquense* (BS = 80, PP = 0.86), which does not share the morphology of the rest of group.

The last major clade within *Lachemilla* is the 'Tripartite clade' (BS = 72, PP = 0.89). This clade includes mostly species from subser. 1 and 3 (ser. *Aphanoides*), but neither of these two clades where recovered as monophyletic. The 'Tripartite clade' is composed of four distinct clades. The first (BS = 97, PP = 1.00) comprises the species of the subser. 3 along with several putative hybrid species (*L. bipinnatifida, L. pseudovenusta, L. ranunculoides, L. steinbachi*). The second clade (BS = 56, PP = 0.90) includes additional samples of *L. aphanoides* (subser. 3) and species from subser. 1. The third clade (BS = 97, PP = 1.00) includes only species of subser. 3. Finally, the fourth clade (BS = 92, PP = 1.00) contains several species with distinctive morphologies when compared to the rest of the 'Triparite clade', including *L. hispidula* and *L. verticillata* (ser. *Nivales*) with verticillate leaves, and *L. polylepis*, which has been classified as a monotypic group

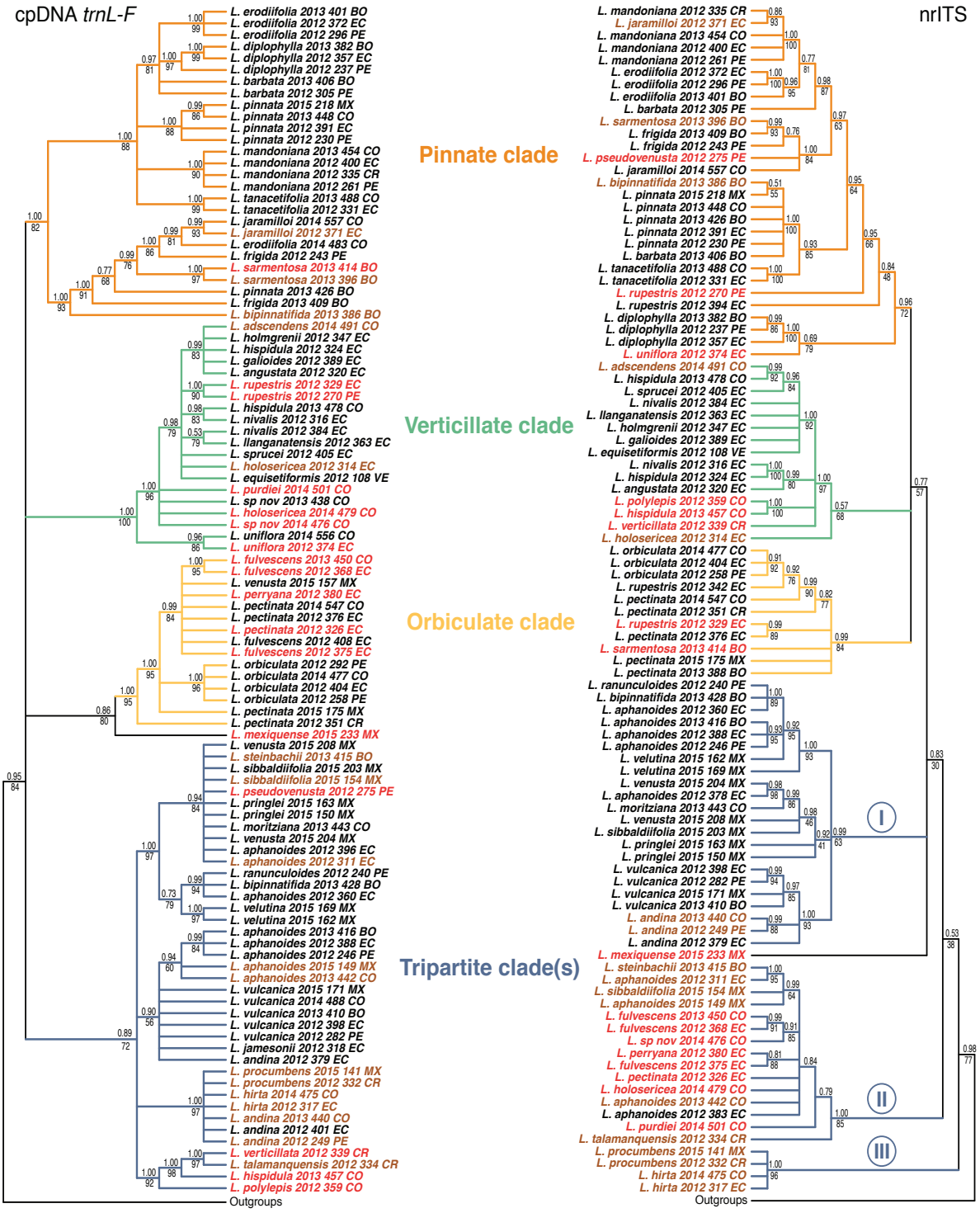

Figure 1.3. Bayesian 50% majority rule consensus tree of the *trnL-F* chloroplast region (left) and nuclear ribosomal ITS region (right). Posterior probabilities and maximum likelihood bootstrap support values are shown above and below the branches, respectively. Samples detected as outliers in the PACo analyses are highlighted in red when incongruencies span different major lineages of *Lachemilla*, and in brown when incongruencies were inferred within the same major clade in the two phylogenies.

The nuclear ribosomal ITS ML and BI trees produced topologies similar to the plastid phylogenies (Fig. 1.3; Appendix 3). The nuclear dataset recovered the same topology and major groups within Alchemillinae as in Gehrke et al. (2016) and Lundberg (2009), where *Lachemilla* (BS = 77, PP = 0.98) and Afromilla (BS = 100, PP = 1.00) are monophyletic groups and *Aphanes* (BS = 100, PP = 1.00) is nested within Eualchemilla (BS = 100, PP = 1.00). In this case the *Aphanes*/Eualchemilla clade is sister to *Lachemilla,* and Afromilla is sister to all of them. Within *Lachemilla* the topologies differ from the plastid phylogeny, especially in the position of putative hybrid species, but similar major groups can be identified based on the same morphological characters as in the plastid phylogeny.

The 'Pinnate clade' (BS = 72, PP = 0.96) includes the same species as in the plastid phylogeny, but also includes *L. rupestris* and *L. uniflora* that were both included in the 'Verticillate clade' in the plastid phylogeny. The 'Verticillate Clade' (BS = 97, PP = 1.00) includes most of the species included in the equivalent clade from the plastid phylogeny, but some species (e.g., *L. purdiei, L. holosericea*) are now part of the 'Tripartite' clades. The 'Verticillate Clade' also now includes the species with the verticillate morphology that were nested in the 'Tripartite Clade' in the plastid phylogeny (*L. hispidula, L. verticillata*), along with *L. polylepis*; this group also has *L. holosericea* as its sister species, albeit with low support (BS= 68, PP=0.57), which was also found in the 'Tripartite Clade II'. The 'Orbiculate clade' (BS = 84, PP = 0.99) comprises *L. orbiculata* and *L. pectinata*, as in the plastid phylogeny, but now also includes *L. rupestris* (subser. 2) and *L. sarmentosa* (ser. *Orbiculatae*) that were included in the 'Tripartite clade' in the plastid phylogeny. The other species of the 'Orbiculate clade' from the plastid phylogeny, *L. fulvescens*, *L. venusta,* and *L. perryana*, are located are variously partitioned among the three 'Tripartite' clades.

Finally, we identified three different 'Tripartite' clades that separately incorporate all the members of the 'Tripartite Clade' found in the plastid phylogeny, plus additional members of the other clades as noted above. The 'Tripartite Clade I' (BS =63, PP = 0.99) includes most of the species found in the single 'Tripartite clade' from the plastid phylogeny. The 'Tripartite Clade II' (BS = 85, PP = 1.00) encompasses several samples of the same species from the subser. 3 included in the 'Tripartite Clade I' plus several putative hybrids from the 'Orbiculate clade' (e.g., *L. fulvescens*, *L. perryana*) and most species of the subser. 4 included in the 'Verticillate Clade' of the plastid phylogeny. The 'Tripartite Clade III' (BS = 96, PP = 1.00) comprises only *L. hirta* and *L. procumbens* (subser. 3).

The PACo analysis of global congruence rejected ($p = 0.001$) the null hypothesis that the similarity between the trees is not higher than expected by chance, showing that despite the presence of outliers in the phylogenies, the chloroplast and nuclear datasets do reflect cophylogeny, to some extent. Also, this analysis detected 34 samples as outliers in *Lachemilla* (Fig. 1.3); these outliers correspond to putative hybrids and samples that have inconsistent placement in the nuclear and plastid phylogenies.

The 'excluded tree' concatenated analyses included only 27 species of *Lachemilla.* The ML and BI analyses recovered similar trees (Fig. 1.4; Appendix 4), and we can identify the four major clades that were recovered in the individual gene trees. The 'Triparite clade' (BS = 85, PP = 1.00) includes species of subser. 1 and 3, two putative hybrid species (*L. bipinnatifida* and *L. venusta*), and *L. ranunculoides* from the subser. 2. The 'Verticillate clade' (BS = 100, PP = 1.00) is composed mostly of the species of the ser. *Nivales* and some species of subser. 4 (e.g., *L. sprucei*). The 'Pinnate clade' (BS = 100, PP = 1.00) is composed of species of ser. *Pinnatae*, *L.*

diplophylla, *L. frigida* (subser. 2), and the presumed hybrid *L. jaramilloi*. The 'Orbiculate clade' (BS = 100, PP = 1.00) consists of only *L. pectinata* and *L. orbiculata* from ser. *Orbiculatae*. The relationships among these four groups remains unresolved even after the removal of the outliers.

The 'duplicated tree' matrix produced a multilabeled tree (Appendix 5) that was summarized in a phylogenetic network (Fig. 1.5) that showed 24 putative hybridization events. Furthermore, this network suggests that hybridization occurred between the four major clades of *Lachemilla.*

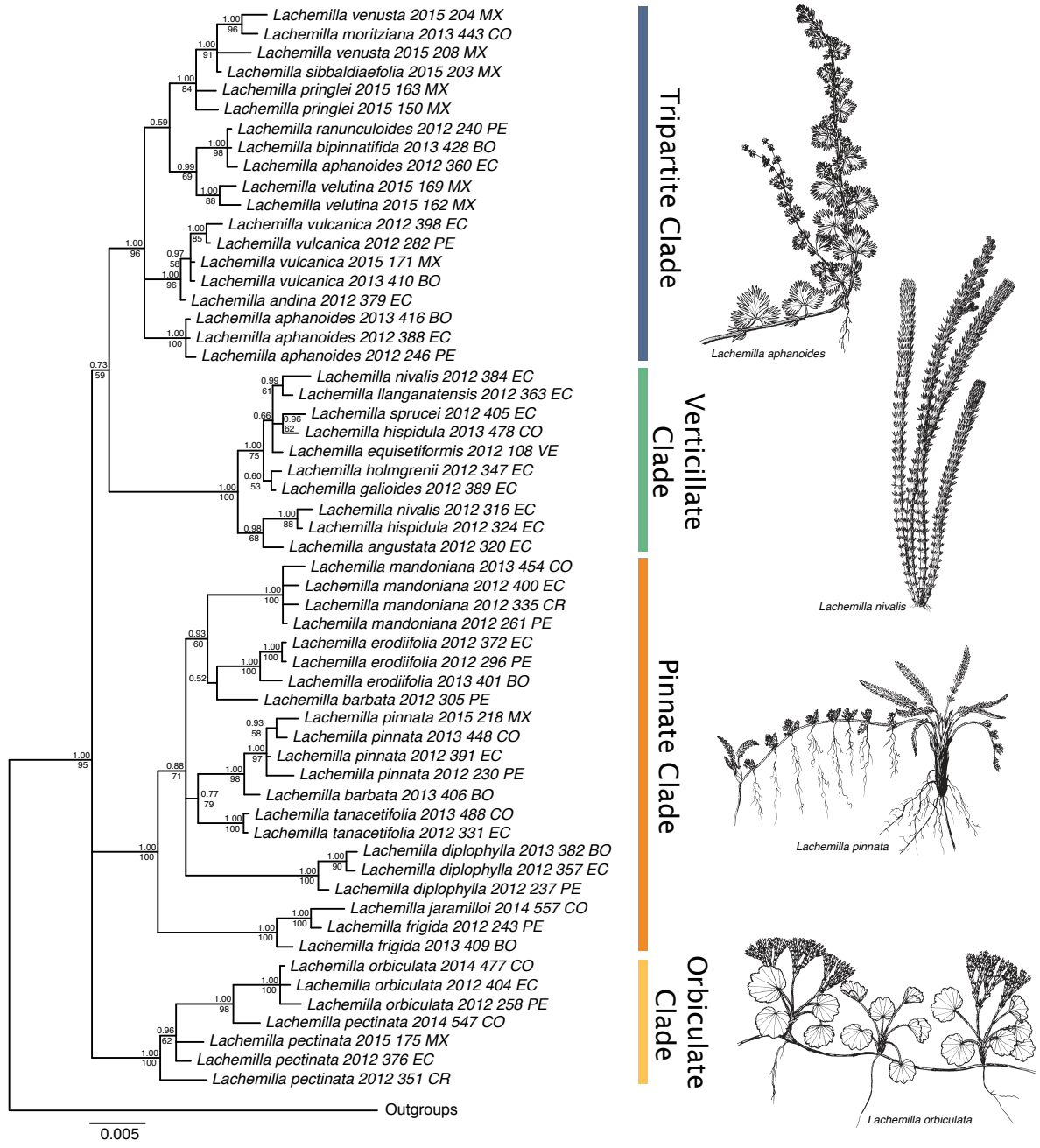

Figure 1.4. Bayesian 50% majority rule consensus tree of the concatenated *trnL-F* chloroplast and nuclear ribosomal ITS regions after removing outlier samples. Posterior probabilities and maximum likelihood bootstrap support values shown above and below the branches, respectively. Line drawings illustrate a representative species and morphology of each major clade. Illustrations modified from (Romoleroux, 1996).

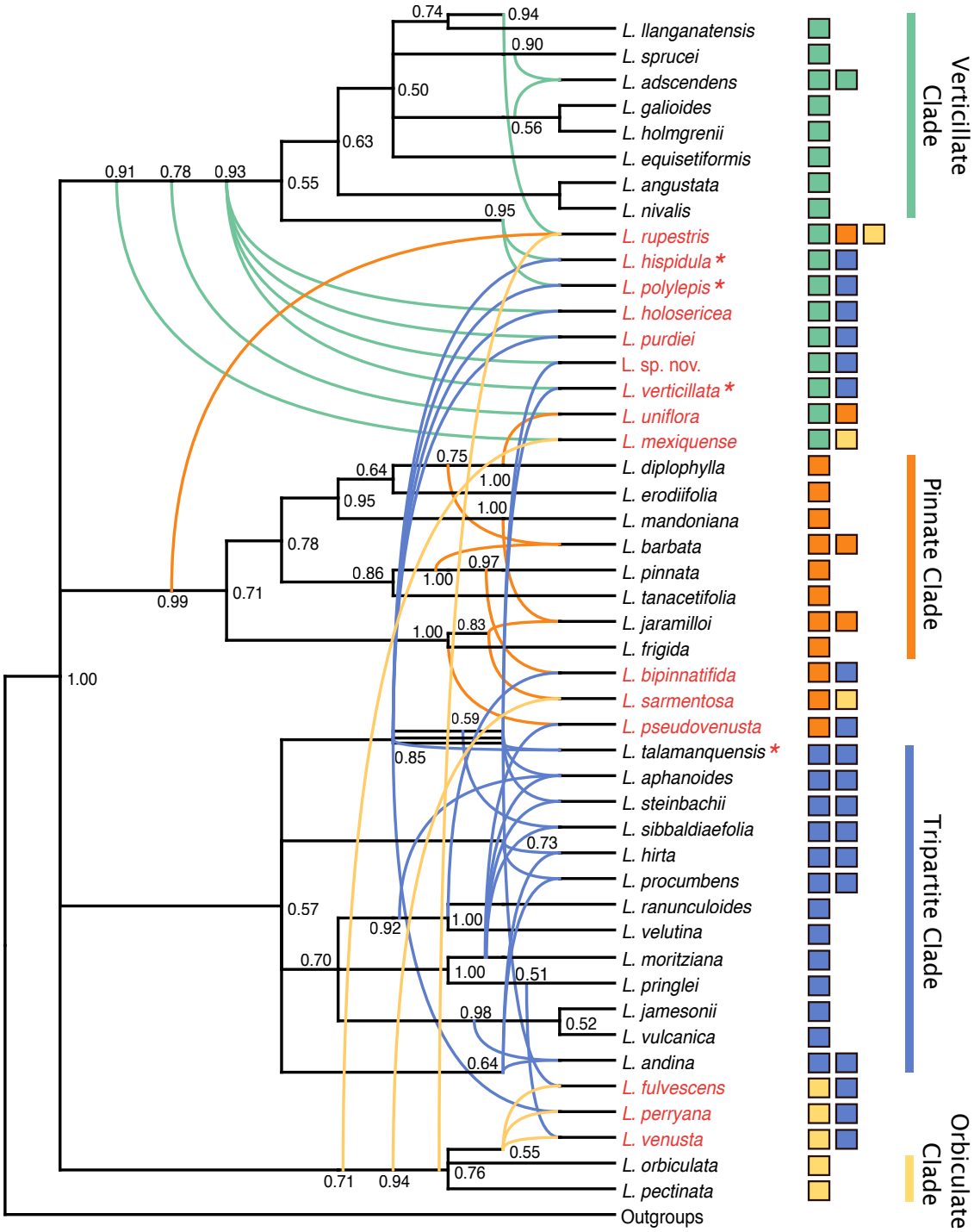

Figure 1.5. Phylogenetic network of the Bayesian 50% majority rule consensus multilabeled tree from the 'Duplicated tree' concatenated matrix of the *trnL-F* chloroplast and nuclear ribosomal ITS regions. Node labels show posterior probabilities. Species in red represent putative hybrids of two or more different clades, with colored lined connecting potential parental species of putative hybrids taxa. Colored boxes associated with tip labels represent the clades of origin, where multiple boxes imply a putative hybrid origin. Species with an asterisk (*) are involved in hypothesized chloroplast capture event (see text).

Chromosome Counts— Generally, it was very difficult to assess confidently the chromosome numbers of most species due to their small size and clustering (Fig. 1.6). We were able to obtain chromosome number for only six species of *Lachemilla* ranging from diploid ($2n = 16$) in the case of *L. mandoniana* to dodecaploid ($2n = 96$) in *L. jaramilloi* (Table 1.3). Additionally, several species showed multiple levels of ploidy and we obtained higher chromosome numbers for *L. tanacetifolia* than previously reported (Table 1.3).

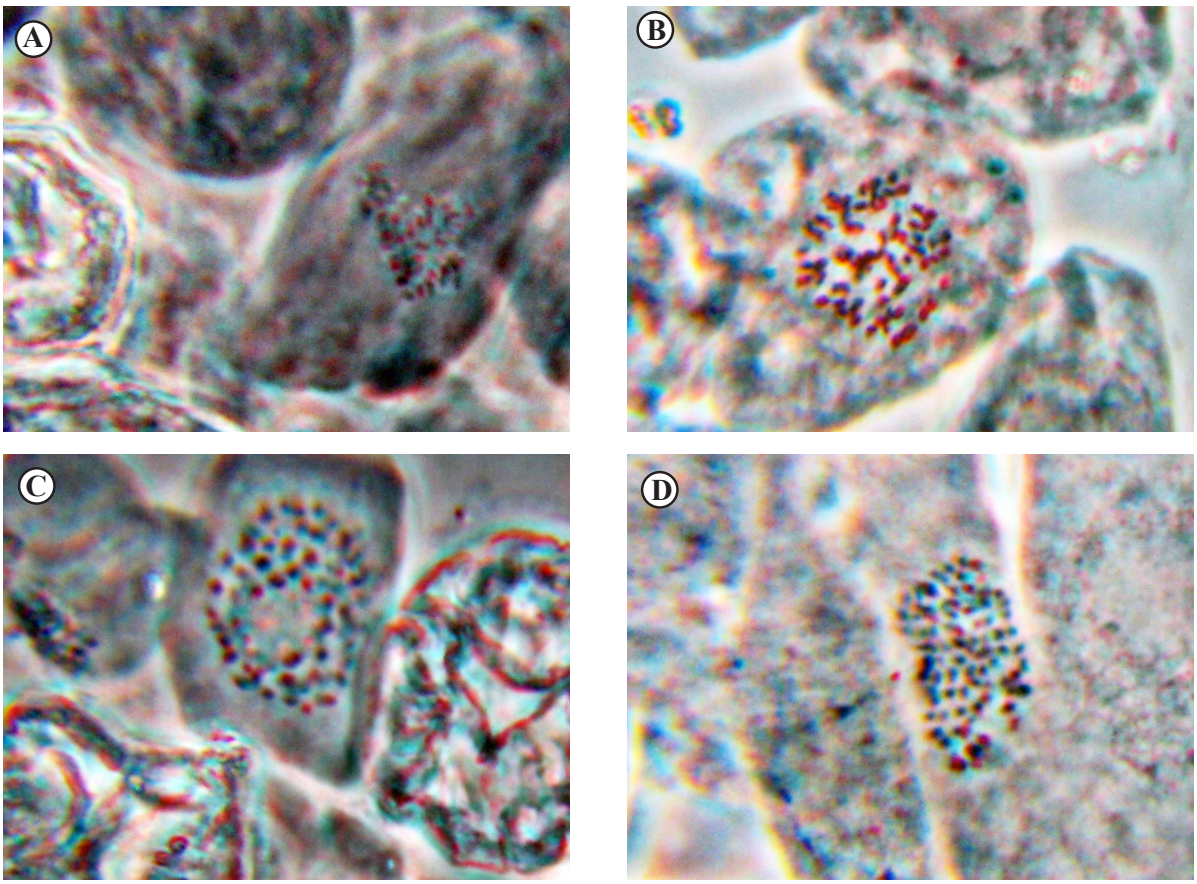

Figure 1.6. Examples of somatic metaphases in *Lachemilla.* A. *L. fulvescens* ($2n = 48$), B. *L. vulcanica* ($2n = 64$), C. *L. tanacetifolia* ($2n = 72$), D. *L. jaramilloi* ($2n = 96$).

Table 1.3. Chromosome numbers and genome size values estimated by flow cytometry in *Lachemilla*. New counts reported in this study are in bold. [b]B = Beaman et al. 1962; D = Diers 1961; F = Favarger 1965; H = Huynh 1965; T = This study. [c]Average value of genome size (in pg DNA); presented separately for each ploidy cytotype (sample(s) with at least 1.25-fold average difference from those of the other group, but with negligible, below 1.1-fold, variation within the same group). [d]Maximum difference among the individual measurements / Difference among average values of the two distinct cytotypes within the species, if present.

Species	Clade	Chromosome number (2*n*)[a]	Source[b]	N flow cytometry	average genome size (pg DNA)[c]	variation in genome size[d]
L. andina	Tripartite			3	1.76	1.02 / -
L. aphanoides	Tripartite	64, 96	D; F; H	8	1.34 / 1.96	1.58 / 1.36
L. diplophylla	Pinnate			1	1.37	-
L. erodiifolia	Pinnate			3	1.10 / 1.65	1.51 / 1.50
L. fulvescens	Hybrid/Outlier	**48**	T	4	1.65 / 2.10	1.32 / 1.27
L. galioides	Verticillate			1	1.99	-
L. hirta	Hybrid/Outlier			2	1.49	1.03 / -
L. hispidula	Verticillate	**24, 32**	T	5	1.40	1.04 / -
L. holmgreni	Verticillate			1	1.92	-
L. holosericea	Hybrid/Outlier			3	1.86	1.01 / -
L. jaramilloi	Hybrid/Outlier	**96**	T	1	2.41	-
L. llanganatensis	Verticillate			1	1.42	-
L. mandoniana	Pinnate	**16, 24**	T	6	1.12 / 1.85	1.71 / 1.64
L. nivalis	Verticillate			7	1.37 / 2.07	1.54 / 1.51
L. orbiculata	Orbiculate			5	1.41 / 1.84	1.33 / 1.30
L. pectinata	Orbiculate			4	1.91	1.08 / -
L. pinnata	Pinnate	64	D	2	1.42	1.01 / -
L. rupestris	Hybrid/Outlier			2	2.22	1.02 / -
L. sprucei	Verticillate			1	1.84	-
L. tanacetifolia	Pinnate	64, **72**	D; T	4	1.59 / 2.32	1.49 / 1.45
L. uniflora	Hybrid/Outlier			3	1.18 / 1.88	1.61 / 1.59
L. vulcanica	Tripartite	**64**	B; T	3	1.88	1.09 / -

Flow Cytometry—We estimated nuclear DNA content of 70 samples representing 22 species of *Lachemilla* (Appendix 2). The genome sizes varied 2.2–fold, ranging from ~1.10 pg in *L. mandoniana* and *L. erodiifolia*, to ~ 2.40 pg in *L. tanacetifolia* and *L. jaramilloi* (Table 1.3; Fig. 1.7). Eight species showed remarkable, 1.3 – 1.7–fold variation among individual samples, likely corresponding with intraspecific variation in ploidy level (Table 1.3). In each of these eight species, we were able to distinguish two groups of samples (interpreted as ploidy cytotypes)

which differed on average at least 1.25–fold from each other but showed little (< 1.1–fold) variation among individual samples.

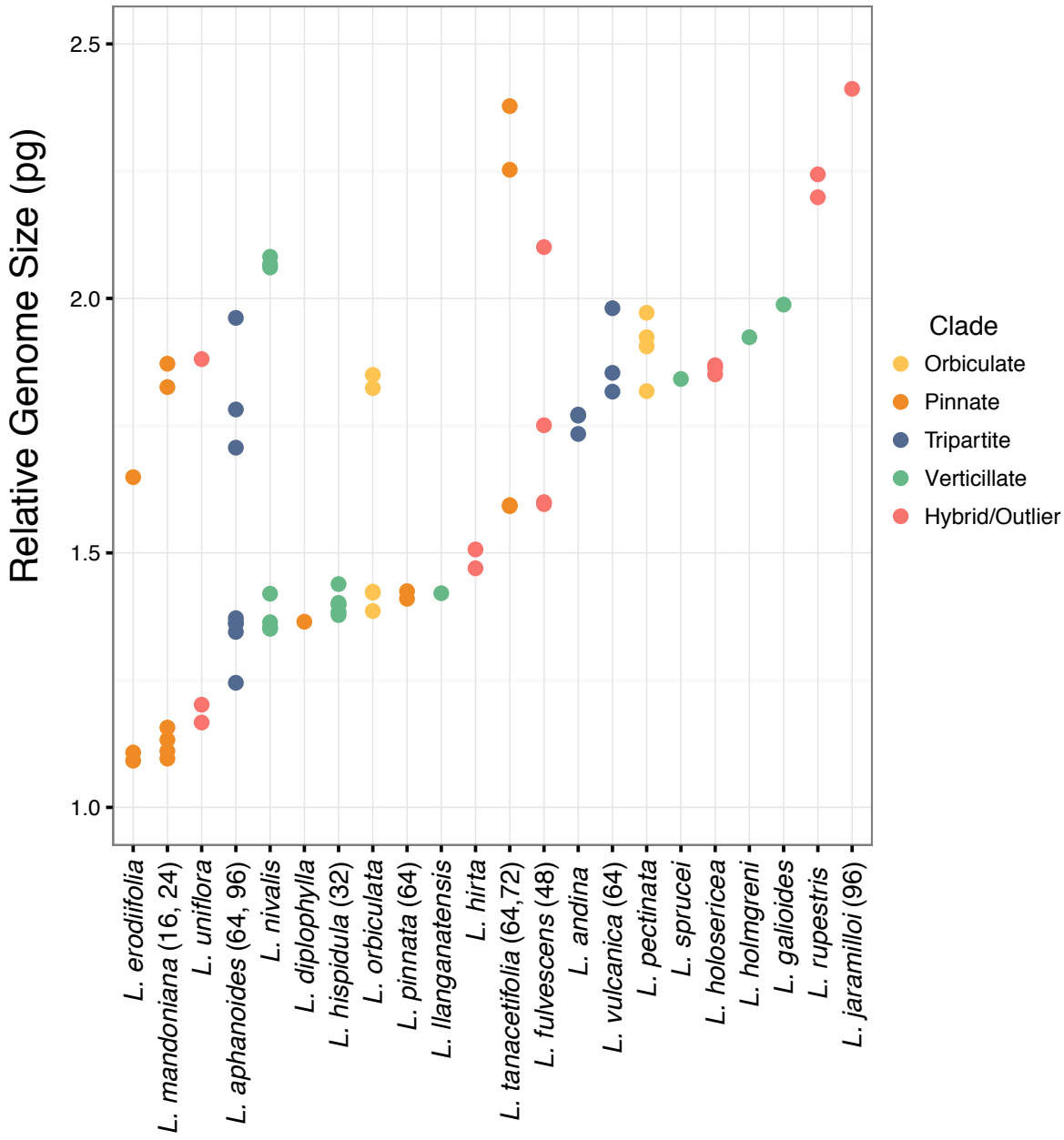

Figure 1.7. Relative genome sizes in various species of *Lachemilla*. Numbers next to species names are the known chromosome numbers for species with formal chromosome counts.

Assessment of Hybridization—The SOWH test (parametric bootstrap) after 1,000 simulations strongly rejected ($p = 0.001$) the null hypothesis that the incongruences between the chloroplast phylogeny and the nuclear phylogeny are due to only phylogenetic error produced by mutational variance. The distribution of tree-to-tree distances estimated from coalescent simulations, and the population rate parameters (θ) estimated from *L. mandoniana* for both the nuclear ($\theta = 0.01$) and the chloroplast ($\theta = 0.003$) lie outside the distribution between the two 'species trees', indicating that the incongruences seen between the two phylogenies cannot be explained solely by ILS (Fig. 1.8). Additionally, after increasing the value of θ, we estimated that the values needed to obtain overlapping distributions of tree-to-tree distances is $\theta \approx 10$, which is at least three orders of magnitude larger than our estimates of θ. This suggests that even if we grossly underestimated the value of θ (defined as $\theta = 4N\mu$) for our analyses, it is unlikely that species of *Lachemilla* could have an effective population size (N) or a mutation rate per site per generation (μ) large enough to produce a θ value as high as 10.

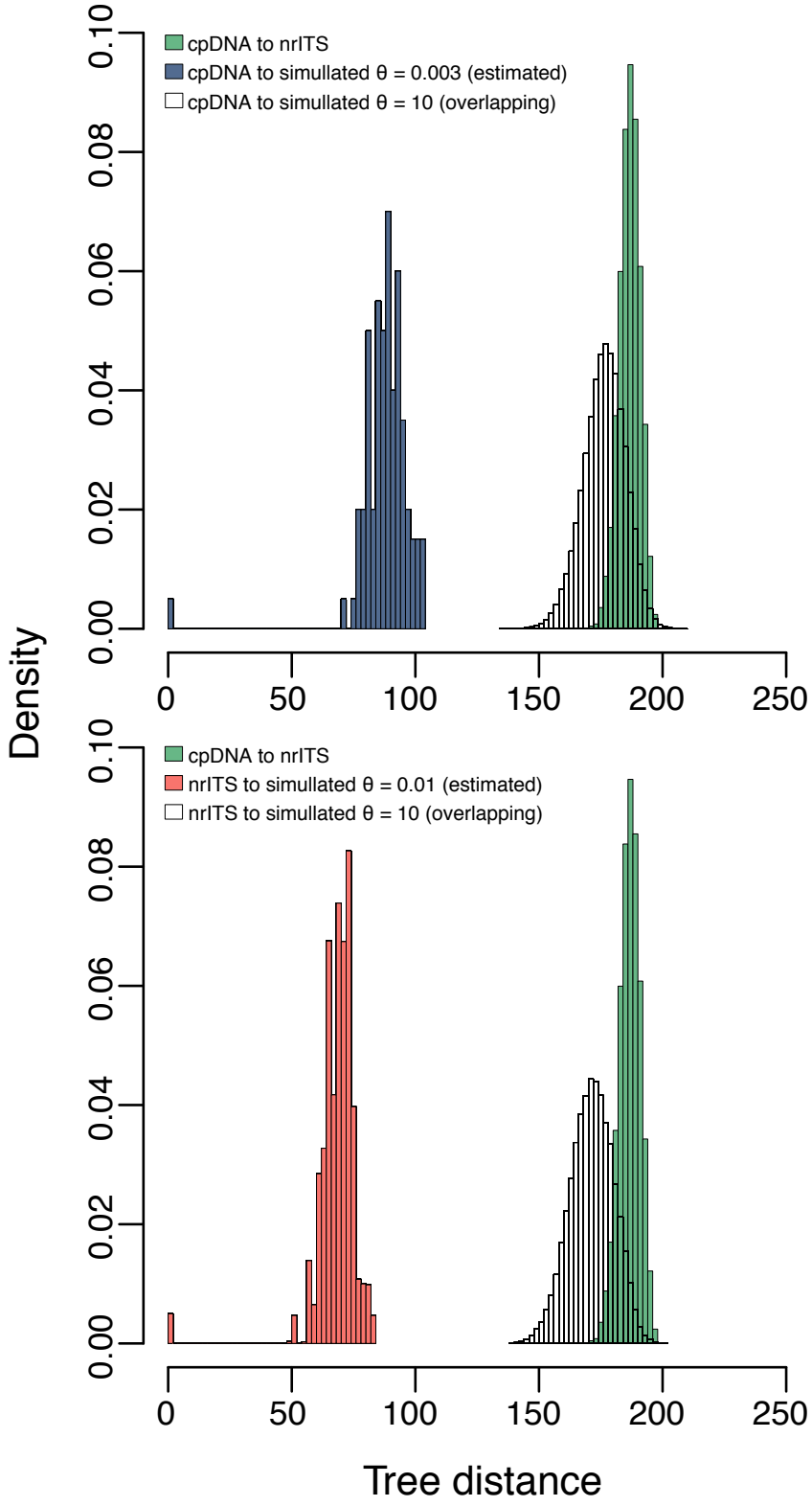

Figure 1.8. Observed and simulated tree distances. Distances simulated using cpDNA (top) and nrITS (bottom) time-calibrated trees with the estimated and overlapping θ.

Divergence Time Estimates—Topologies and PP values were similar to those obtained in the BI analyses for both datasets (Figs. 1.9, 1.10). The age estimates obtained with the Yule process as the tree speciation prior were older than the ages estimated using the birth-death process with most of the ages outside of the 95% HPD of the birth-death prior ages (Table 1.4). Bayes factors were calculated as the difference between the marginal likelihood estimates from stepping-stone searches of the birth-death and Yule prior. Following Kass and Raftery (1995), Bayes factor values greater than 10 indicate decisive evidence for the first model, in our case the birth-death model (Table 1.4). *Lachemilla* ages obtained with the nuclear and plastid datasets were similar and estimated a stem age of 14.67 mya (9.07 - 20.74) and 14.45 mya (9.02 - 20.44), respectively, while the crown ages were 11.03 mya (6.85 - 16.02) and 12.33 mya (7.90 - 17.73), respectively. Finally, we can detect a trend in the LTT plots (Fig. 1.11) where the biggest accumulation of lineages in *Lachemilla* starts around the last five mya.

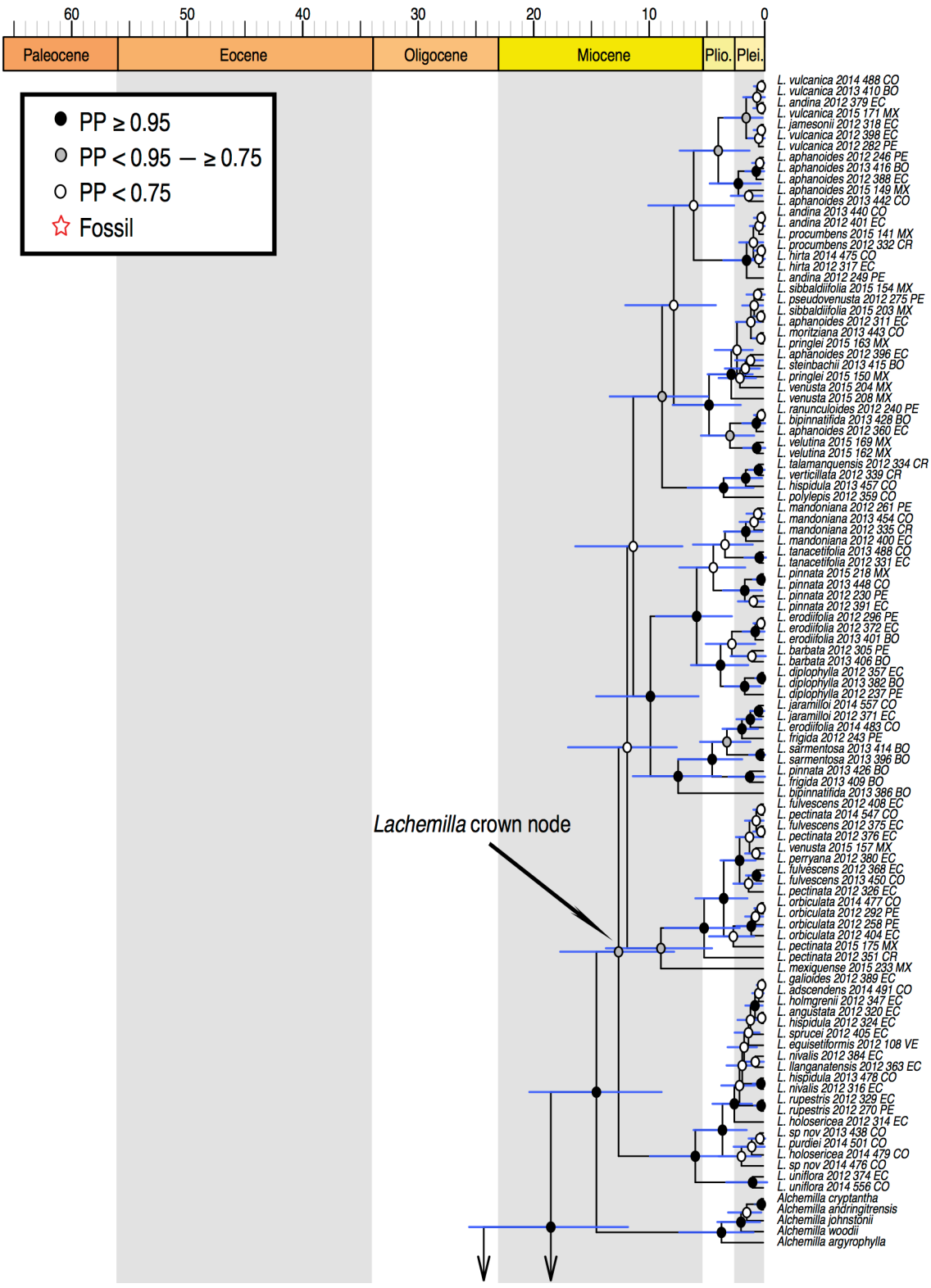

Figure 1.9. Maximum clade credibility chronogram of the *trnL-F* chloroplast region. Node labels represent median divergence time estimates, blue bars are the 95% highest posterior density (HPD) intervals and red stars denote fossil placements. Dots in the branches represent posterior probability values. Figure continues in next page.

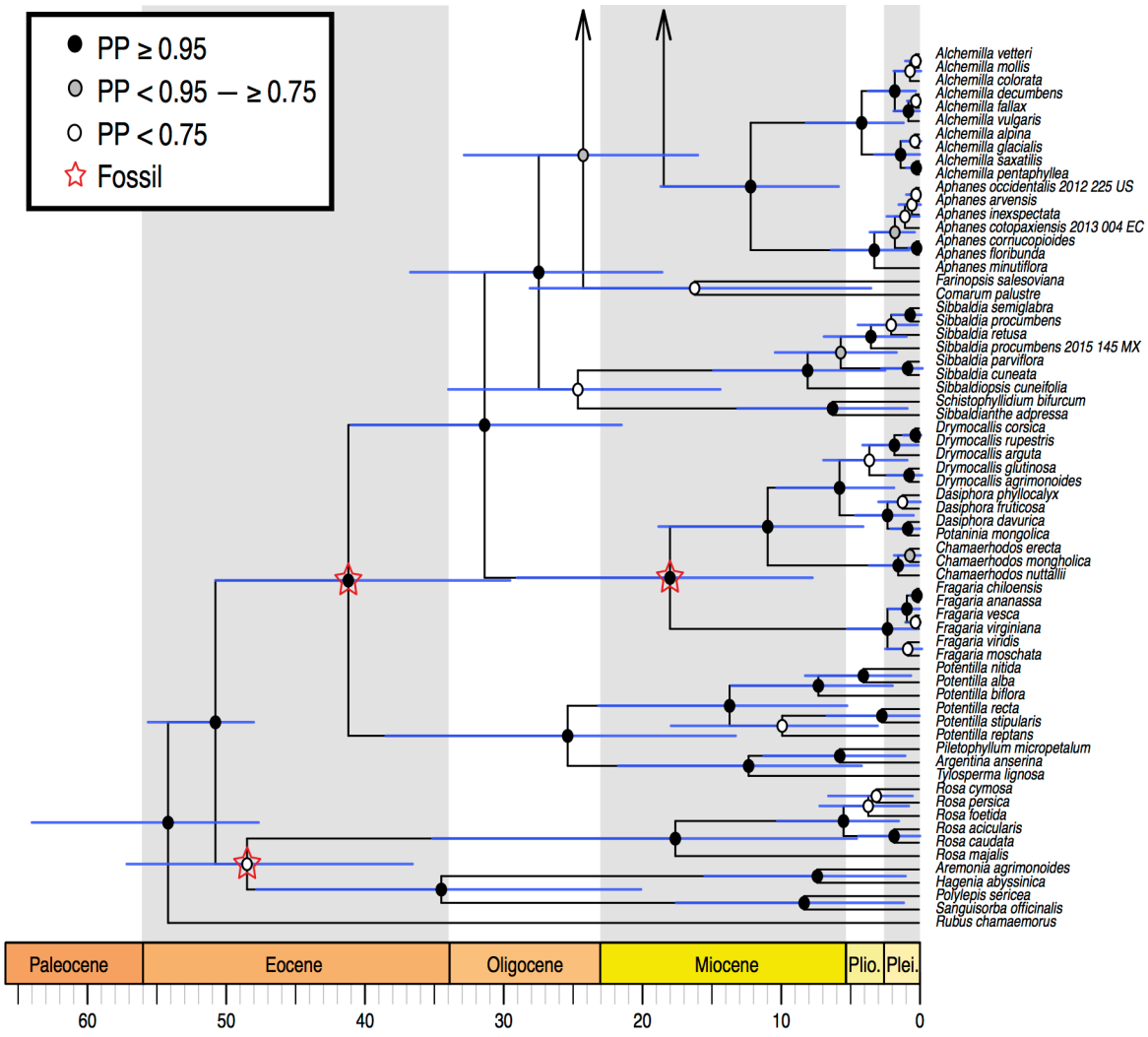

Figure 1.9 (Continued). Maximum clade credibility chronogram of the *trnL-F* chloroplast region. Node labels represent median divergence time estimates, blue bars are the 95% highest posterior density (HPD) intervals and red stars denote fossil placements. Dots in the branches represent posterior probability values.

Table 1.4. Age estimates of *Lachemilla*. Values are the median ages with the 95% Highest Posterior Density (HPD) in million years ago (Ma). MLE (SS): marginal likelihood estimates using stepping-stone sampling. BF: Bayes factors.

Dataset	Prior	*Lachemilla* (stem)	*Lachemilla* (crown)	MLE (SS)	BF
cpDNA trnL-F	Birth-death	14.45 (9.02 - 20.44)	12.33 (7.90 - 17.73)	-6610.68	18.56
	Yule	21.34 (15.69 - 27.64)	19.44 (14.12 - 25.22)	-6629.23	
nrITS	Birth-death	14.67 (9.07 - 20.74)	11.03 (6.85 - 16.02)	-9202.30	22.47
	Yule	20.94 (15.35 - 26.93)	17.76 (12.77 - 23.46)	-9224.77	

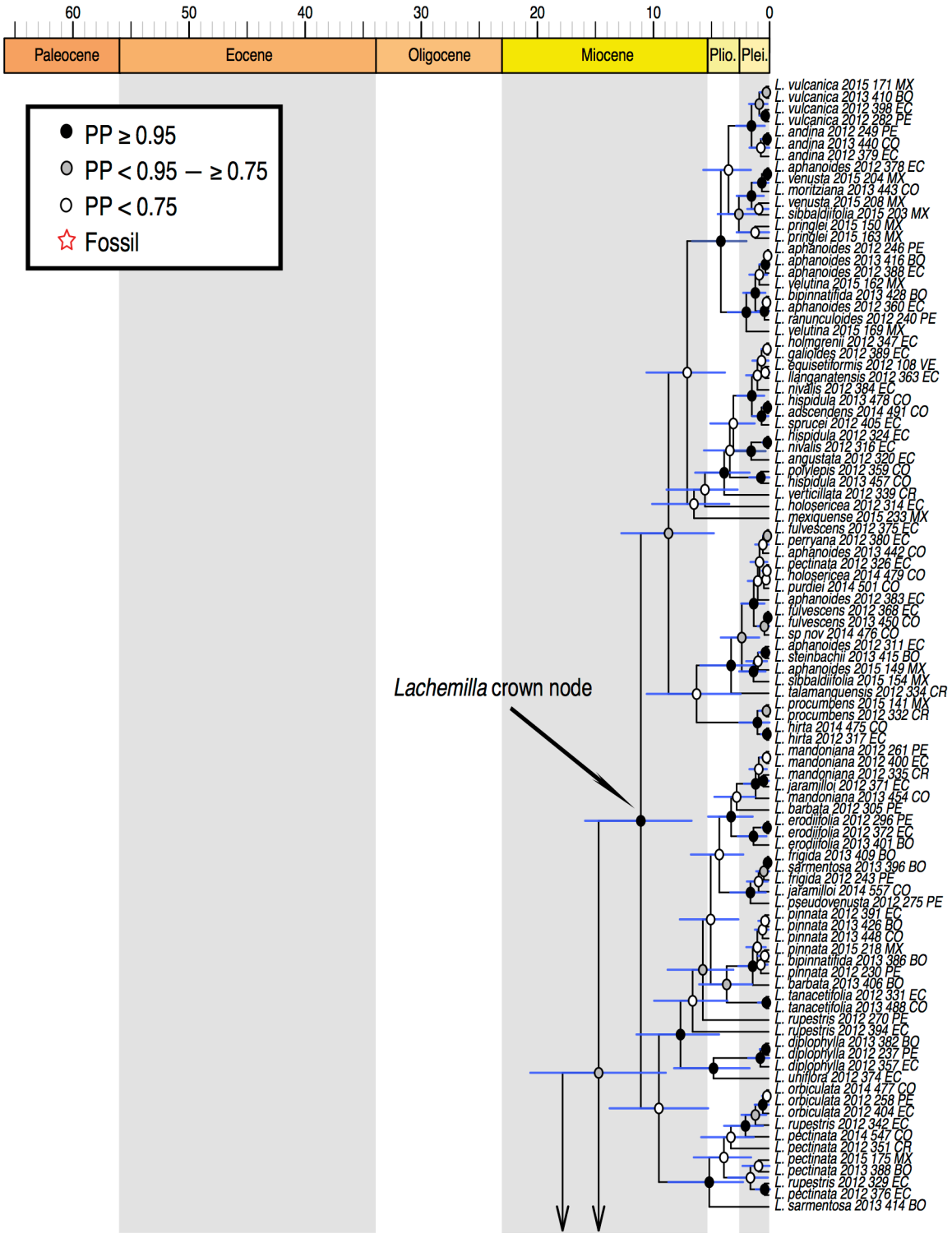

Figure 1.10. Maximum clade credibility chronogram of the nuclear ribosomal ITS region. Node labels represent median divergence time estimates, blue bars are the 95% highest posterior density (HPD) intervals and red stars denote fossil placements. Dots in the branches represent posterior probability values. Figure continues in next page.

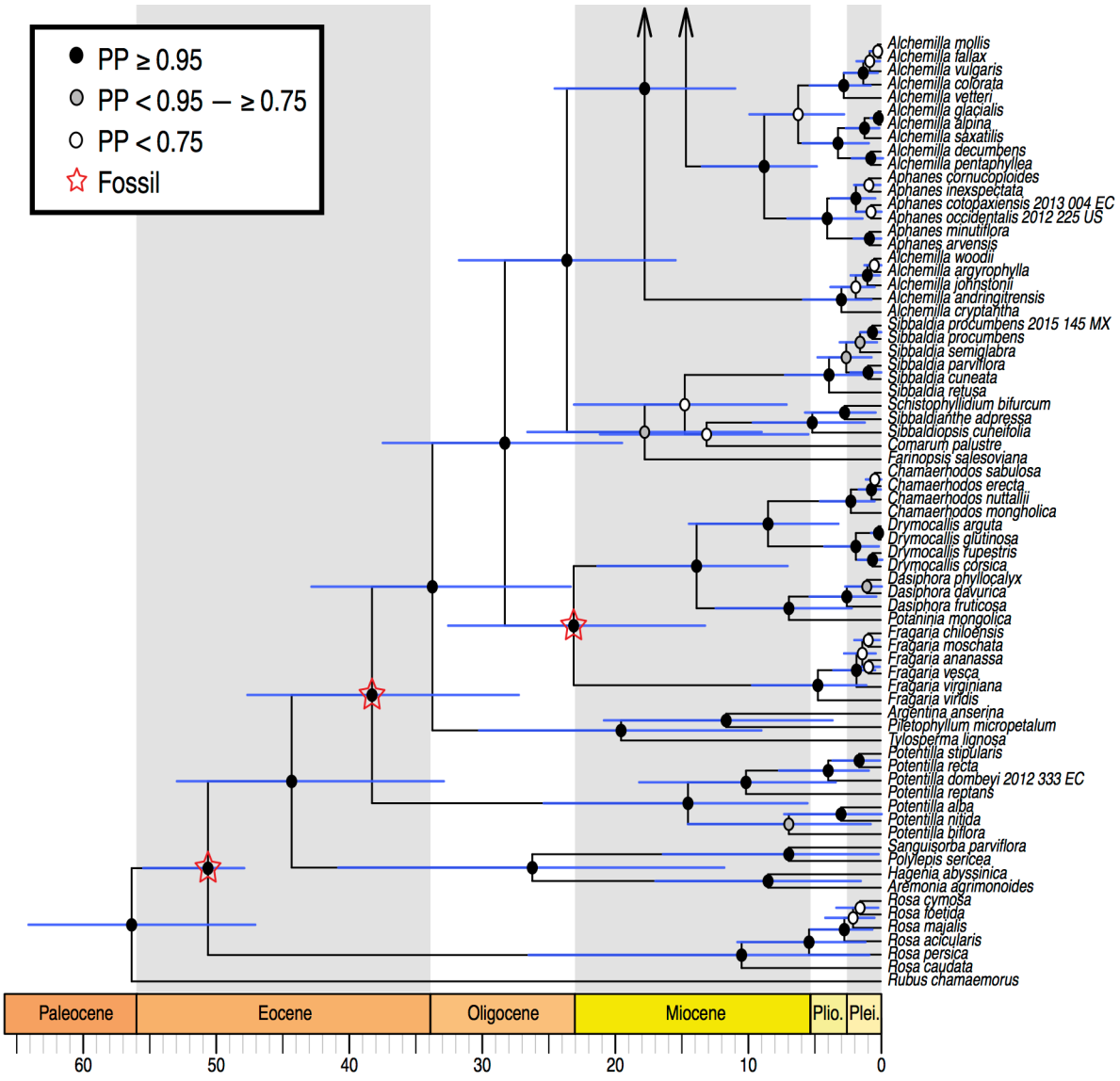

Figure 1.10 (Continued). Maximum clade credibility chronogram of the nuclear ribosomal ITS region. Node labels represent median divergence time estimates, blue bars are the 95% highest posterior density (HPD) intervals and red stars denote fossil placements. Dots in the branches represent posterior probability values.

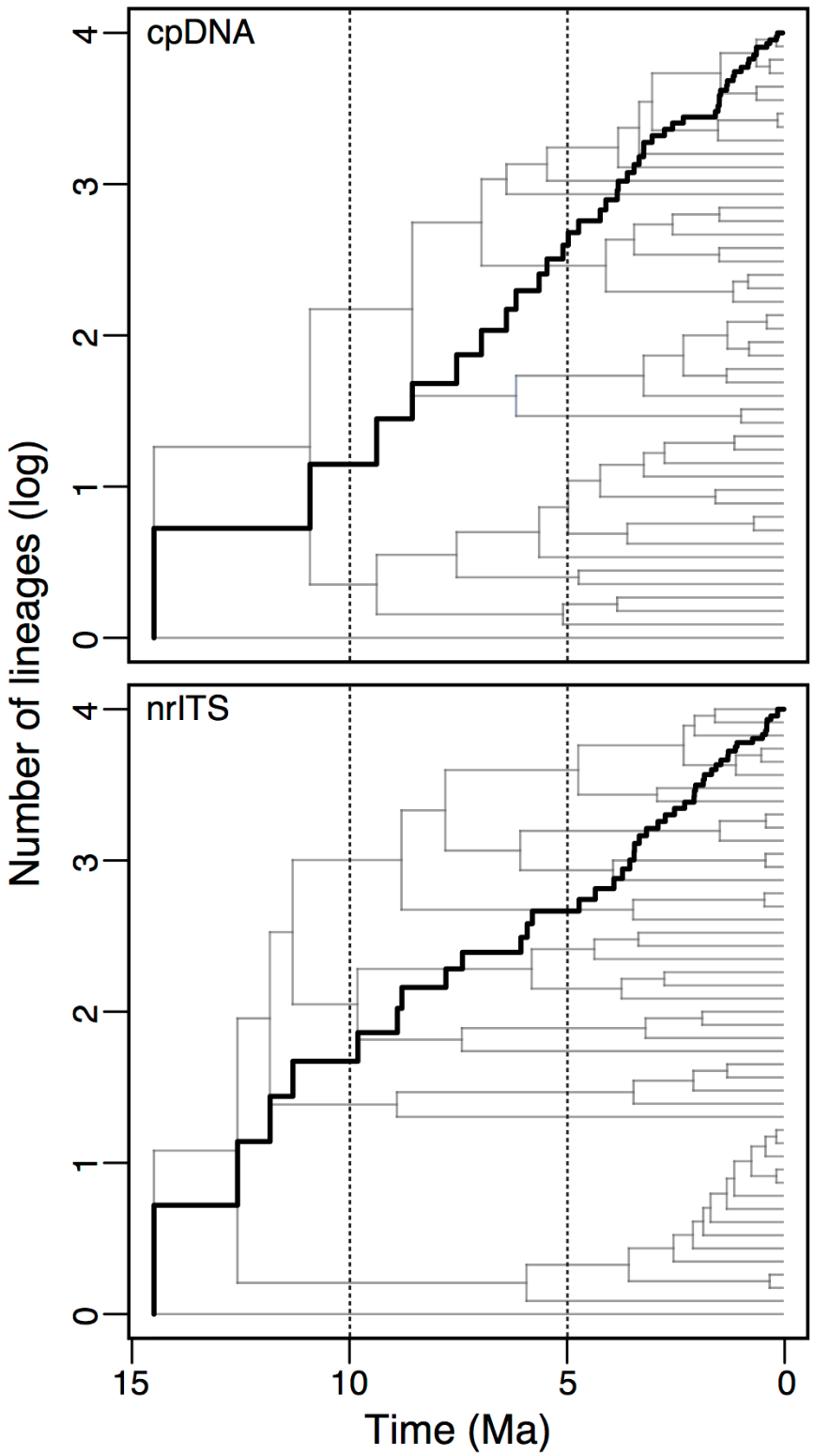

Figure 1.11. Lineage through time plots with the reduced (single sample per species) time-calibrated phylogenies from the cpDNA (top) and nrITS (bottom) datasets in the background. Dotted lines represent the time of the last major uplifts of the Central (10 Ma) and Northern (5 Ma) Andes.

Discussion

Incongruence between Nuclear and Plastid Phylogenies— A frequent pattern in plants is the incongruence between nuclear and plastid derived phylogenies and it has been and continues to be a good first approximation for the detection of reticulate evolution (e.g., Sang et al. 1995; Soltis and Kuzoff, 1995; Fehrer et al. 2007; Pirie et al. 2009; Lundberg et al. 2009; de Kuppler, 2015). Incongruence has also been associated with phylogenetic error and incomplete lineage sorting (Rieseberg and Soltis 1991; Doyle 1992, Wendel and Doyle 1998). Therefore, to establish hybridization as the main source of discordance we need to reject these two alternative scenarios (Sang and Zhong 2001; Maureira-Butler et al. 2008; Buckley et al. 2006; Joly et al. 2009; Reid et al. 2012) and explore additional sources of evidence to support this hypothesis (Wendel and Doyle 1998; Guggisberg et al. 2009).

We uncovered a clear pattern of incongruence between the plastid and nuclear phylogenies of *Lachemilla* (Fig. 1.3), and provide clear evidence for a complex, reticulate history of the group. Using two simulation approaches we were able to reject phylogenetic error and ILS as the only source of incongruence, leaving hybridization as the most likely explanation for the phylogenetic patterns that we observe. Using a statistical framework, the PACo analysis allowed us to detect 34 outlier samples with conflicting positions across the two gene trees, of which at least half of them correspond to species that have samples that were recovered in more than one of the four main clades of *Lachemilla.* In addition, the sampling of multiple specimens per species in combination with the phylogenetic network analysis allowed us to detect 24 instances of hybridization involving major lineages

of *Lachemilla*, not just individual species (for further discussion of hybridization see '*Taxonomic Implications'* section)**.**

Polyploidy— It is well known that polyploidy is common in Rosaceae (Dickinson et al. 2007), and allopolyploidy has been established as the main source for the cytonuclear discordance in members of *Pontentilla* and several members of subtribe Fragariinae (Rousseau-Gueutin et al. 2009; Lundberg et al. 2009; Töpel et al. 2011; Gehrke et al. 2016). Based on chromosome numbers and flow cytometry estimations of genome size (Table 1.3, Fig. 1.7), it is clear that polyploidy is also widespread in *Lachemilla.* Interestingly, *L. mandoniana* was determined to be diploid ($2n = 16$), and along with *L. erodiifolia*, has the smallest genome size. Even so, several populations of the two species have significantly bigger genome sizes (genome size corresponding to diploid and triploid cytotype), and in the case of *L. mandoniana*, a triploid ($2n = 24$) individual was also confirmed by chromosome count. This, along with congruence of these two species in the nuclear and plastid phylogeny, suggests that these two species likely have both diploid and autopolyploid populations. In contrast, for example *L. jaramilloi* ($2n = 96$) has one of the largest genome sizes, and has inconsistent positions in the phylogenies and even different copies of the nrITS region (Fig. 1.3), which suggests that this species has an allopolyploid origin. It worth noting that in some cases the chromosome number and the genome size do not match. For example *L. jaramilloi* and *L. tanacetifolia* have similar average genome sizes (2.41 and 2.32 pg respectively) but the latter has only 72 chromosomes in contrast of the 96 of the former. Also the 2.2–fold difference between the *L. mandoniana* ($2n= 16, 24$) and *L. jaramilloi* ($2n = 96$) does not correspond to the expected diploid-dodecaploid ratio. This incongruence may

reflect complex genomic rearrangements after genome duplication leading to reduction of genome size in polyploids (genome downsizing, Leitch and Bennet 2004). Based on the multiple cytotypes found with both the flow cytometry data and the chromosome counts, and the apparent disconnect between genome size and ploidy level within species (Table 1.3, Fig. 1.7), *Lachemilla* is clearly cytologically complex, and flow cytometry data alone may not be a good indicator of ploidy level in this clade. Reliable ploidy estimates may be restricted to chromosome squashes, which, unfortunately, proved to be difficult due to the small size and sometimes-large number of chromosomes in *Lachemilla*. In addition, it is possible that some species may contain mixed ploidy level populations, but we did not address this issue in this study. Moreover, we did not find any clear trend of chromosome number or genome size across the four mains group of *Lachemilla* (Fig. 1.7). It is possible that a sequence-based approach from multiple, independent single- or low-copy nuclear genes (e.g., isolating individual alleles via cloning and/or bioinformatically; e.g., Rothfels et al. 2017; Uribe-Convers et al. 2016) in combination with phylogenetic analyses (e.g., Sang and Zhang 1999; Smedmark et al. 2003; Kim et al. 2008; Tank and Olmstead 2009; Marcussen et al. 2012; Estep et al. 2014; Brassac and Blattner 2015) will give us a better picture of the polyploidy patterns across *Lachemilla.*

Taxonomic Implications— The taxonomy and infrageneric classification of *Lachemilla,* has been studied by several authors (Perry 1929; Rothmaler 1935a, 1935b, 1937; Frohner 1995; Romoleroux 1996; Gaviria 1997; Notov and Kusnetzova 2004), but species boundaries are often unclear and the infrageneric taxonomy is not well-defined. Perry (1929) proposed the first classification system for the group using

mostly foliar characteristics, and first noted that floral characters remain constant throughout the genus and determined these are not of particular use for this purpose, a condition also noted by Novot and Kusnetzova (2004). Later, Rothmaler (1937) proposed the most recent, and most species-inclusive system, using mostly vegetative characters (life form, shoot type, shape and dissection of the leaf blade, leaf sheath closeness). Most recently, Novot and Kusnetzova (2004) revised the classification of Alchemillinae and noted several problems with Rothmaler's classification. First, they noted the lack of a standard plan of taxon diagnosis and an incorrect determination of character homology, making some of Rothmaler's diagnoses incomplete with morphological fallacies. For example, Rothmaler's section *Aphanoides* was defined by possessing dense inflorescences, but those inflorescences are situated on the peduncles from non-homologous positions, making the diagnosis of the section invalid, by grouping species that are actually heterogeneous for that character (Novot and Kusnetzova 2004). Additionally, Rothmaler (1937) did not provide a formal description of the sections he proposed, only listing the taxa belonging to each group. Novot and Kusnetzova (2004) proposed the use of alternative characters like leaf and bud structure, and architectural models, to inform the classification of Alchemillinae, but made no changes to the classification of *Lachemilla.* The classification systems of Perry (1929) and Rothmaler (1937) are similar, with most of the series and subseries having a counterpart sections or subsections in the other system, with the exception of some specific taxa and the delimitation of the series/section *Aphanoides* (Fig. 1.2, Tables 1.1, 1.2).

Our phylogenetic analyses consistently identified four major groups within *Lachemilla* that are mostly congruent with these morphology-based classification

systems, especially with Perry's (1929) proposal. With a few exceptions, the composition of our four main clades and previous classifications differ only in placement of the hybrid species. Below we discuss the main characteristics of these groups, excluding species that were identified as hybrids, or potential hybrids, either by our phylogenetic analyses, or based on incongruent morphologies (final species composition of the clades can be found in Figure 1.2). It is worth noting that this is a first attempt to classify the species of *Lachemilla* in a phylogenetic context, and further studies that include all species, and a complete monographic revision of the genus are still needed.

PINNATE CLADE— This group is partially equivalent to ser. *Pinnatae*, and has species with repent or decumbent stems and pinnate or bipinnatifid basal leaves (e.g., *L. pinnata*; Fig. 1.1 J). The only species that we exclude from the ser. *Pinnatae* is *L. bipinnatifida*; this species is a putative hybrid of *L. pinnata* and *L. aphanoides* in the 'Tripartite clade'. Interestingly, Perry (1929) included *L. bipinnatifida* in ser. *Pinnatae* due to its pinnate leaves, while Rothmaler included it in the sect. *Glomerulate* (Tripartite clade), because of its glomerulate inflorescence. This is a result that we have found repeatedly; namely, that many of our identified hybrid species were placed in one taxonomic group based on the characteristics that the author relied upon most heavily (e.g., leaf morphology for Perry, inflorescence morphology in the case of Rothmaler), but these often have morphological anomalies in other characteristics that link them with putative parents in one of the other clades.

Lachemilla diplophylla (Fig. 1.1 K) is a semi-aquatic to aquatic herb that is characterized by its conspicuous appendaged leaves. As with many aquatic plants, *L. diplophylla* has morphological adaptations to the aquatic habit that result in

unique traits, and as a result, this species has been historically treated in its own section. Our results show that this species belongs to the 'Pinnate clade' even though it does not have pinnate leaves. In light of this result, closer examination of the reproductive morphology of *L. diplophylla* reveals a striking similarity to the flowers of *L. mandoniana* (Pinnate clade) both in overall shape and the lack of episepals, which are atypical characteristics in *Lachemilla*.

VERTICILLATE CLADE— This clade is completely equivalent to ser. *Nivales*, and is characterized by having species with erect or decumbent stems and reduced leaves that fuse with the stipules to form verticillate sheaths (e.g., *L. nivalis*; Fig. 1.1 I). Our network analyses (Fig. 1.5) reveal that this clade was heavily involved in repeated hybridization events. The resulting hybrid species comprise subser. 4 of ser. *Aphanoides* (e.g., *L. purdiei*; Fig. 1.1 G), and have tripartite basal and the distal leaves that form verticillate sheaths with the stipules as in the 'Verticillate clade', again expressing a combination of vegetative characters from multiple lineages of *Lachemilla*.

ORBICULATE CLADE— This group is partially equivalent to the ser. *Orbiculatae*, and is characterized by species with palmately lobed leaves and a stoloniferous habit. The only two species belonging to this group are *L. pectinata* (Fig. 1.1 B) and *L. orbiculata.* These two species, and/or an ancestral lineage, were involved in repeated hybridization events with the 'Tripartite', 'Pinnate', and 'Verticillate' clades, giving rise to species like *L. venusta* (Fig. 1.1 C), *L. sarmentosa,* and *L. mexiquense,* respectively. The main characteristic of these hybrids is having cleft leaves; with the exception of *L. mexiquense,* which was only recently described

(Morales-Briones 2016), these hybrid species were originally included in the ser. *Orbiculatae*.

TRIPARTITE CLADE— This clade corresponds only to subser. 1 (e.g., *L. procumbens*; Fig. 1.1 D) and subser. 3 (e.g., *L. pringlei*; Fig. 1.1 E) of ser. *Aphanoides*. The main characteristic of the group is the presence of tripartite leaves that often appear to have 5 divisions due to the bifid lateral segments of some species. The species assigned to the remaining subseries of sec. *Aphanoides* (subser. 2, 4-6; Fig. 1.2) were identified here as the result of extensive hybridization between core 'Tripartite clade' lineages and lineages of all of the other clades; Perry (1929) noticed that these species have similar characters to other series and suggested that those subseries may represent transitional forms that link ser. *Aphanoides* to ser. *Nivales* and ser. *Pinnatae*. The species of the subser. 2 (e.g., *L. ranunculoides,* Fig. 1.1 H), despite having glomerulate inflorescences as in subser. 3 and being nested in the 'Tripartite clade', have subpinnately tripartite leaves and a stoloniferous habit similar to ser. *Pinnatae*, while the rest of species of the 'Tripartite clade' have a decumbent or erect stem. Species of subser. 5, *L. sandiensis* and *L. repens*, have subpinnately tripartite leaves and creeping habits that resemble ser. *Pinnatae* more than ser. *Aphanoides,* but were included by Perry (1929) in the latter because of the presence of glomerulate inflorescences as in subser. 3. Although, we were unable to sample these series, the morphology suggests that these species are also potentially of hybrid origin. Interestingly, Rothmaler (1937) places the species of subser. 2 and 5 together with the species of ser. *Pinnatae* in subsect. *Pachyrrhizae* (Fig. 1.2, Table 1.2), based on their similar vegetative morphologies. The only species of subser. 6, *L. rusbyi*, was described as an anomalous species by Perry (1929) that has stem

leaves similar to ser. *Nivales*, tripartite basal leave similar to subser. 3, and flowers that resemble *L. mandoniana* (Pinnate clade). While ser. *Aphanoides* (Perry, 1929) contains the most species in Perry's classification (1929), our results suggests that the majority of these species are the result multiple hybrid origins from across the main lineages of *Lachemilla.*

ADDITIONAL PROCESSES— Forming part of the 'Tripartite clade' of the chloroplast phylogeny, we found a geographically restricted clade composed of four species that does not correspond to expected morphologies, and can not be explained solely by hybrid origins. *Lachemilla verticillata* from Costa Rica and one sample of *L. hispidula* from Northern Colombia show only morphological characteristics of the 'Verticillate clade', and the nuclear dataset place them securely with verticillate relatives (Fig. 1.3), suggesting that the location of these two species in the 'Tripartite clade' on the plastid phylogeny (Fig. 1.3) is likely the result of chloroplast capture events of these species in Northern Colombia and Costa Rica. The third species, *L. talamanquensis,* is also part of the 'Tripartite clade' in the plastid phylogeny, but is part of the same clade in the nuclear phylogeny. In this case this species shows characteristics of both groups. As pointed out in the original description of the species (Romoleroux and Morales-Briones 2012), *L. talamanquensis* resembles *L. verticillata* in having sericeous pubescence and whorled reduced distal leaves, while also resembling *L. standleyi* in having some tri-lobed distal leaves and similar flower shape and size. The three species occur in the Cordillera de Talamanca in Costa Rica and the combinations of characters suggest that *L. talamanquensis* might be a hybrid of *L. verticillata* and *L. standleyi.* This could explain the placement of *L. talamanquensis* as sister of *L. verticillata* in the 'Tripartite clade' in

the plastid phylogeny and in the 'Tripartite clade II' of the nuclear phylogeny along with other outlier samples. Unfortunately, *L. standleyi* was not sampled here, but its overall morphology, including tripartite leaves, suggests that this species belongs to the 'Tripartite clade'. The last species, *L. polylepis*, because of its very different vegetative morphology, has been traditionally treated as distinct from the rest of the species (ser. *Polylepides*; Fig. 1.1 A). This species is a small shrub with profuse branching that is restricted to Costa Rica, Venezuela, and northern Colombia, and is the only completely woody member of *Lachemilla. Lachemilla polylepis* shows similar phylogenetic patters as *L. verticillata* and *L. hispidula* of Northern Colombia, with the nuclear phylogeny placing this species within the 'Verticillate clade', while the chloroplast phylogeny places this species as a member of the 'Tripartite clade' along with three species discussed above. This could suggest that *L. polylepis* may have evolved from the 'Verticillate clade', and that its placement on the chloroplast phylogeny is also due to chloroplast capture, as hypothesized for *L. verticillata* and northern accessions of *L. hispidula*. However, because of the overall morphology of *L. polylepis* is so different than the rest of the 'Verticillate clade', additional data from multiple nuclear genes are still needed to confirm chloroplast capture in this species.

ADDITIONAL GROUPS— Rothmaler (1937) proposed two additional sections that do not have a counterpart series in Perry's system. The first is sect. *Rupestres* (Fig. 1.2, Table 1.2), with three species, two of which, *L. barbata* and *L. tanacetifolia*, have pinnate leaves and were recovered here as part of the 'Pinnate clade' in accordance with Perry (1929; Figs 1.2 and 1.3, Table 1.1), while the third species, *L. rupestris* (Fig. 1L), which was placed in subser. 2 of ser. *Aphanoides*, was identified in

our network analyses as a putative hybrid of the 'Pinnate', 'Orbiculate', and 'Verticillate' clades (Fig. 1.5). The second section, *Fruticulosae* (Fig. 1.2, Table 1.2), includes only *L. fruticulosa*. After detailed examination of the type material by Notov and Kusnetzova (2004) and the authors, has been determined to be a specimen of *L. velutina* (Fig. 1.1 F), which is a member of the 'Tripartite clade'.

Divergence Time Estimates— The greatest diversity of *Lachemilla* is found in the high elevation Andean grasslands (páramo, puna, and jalca), but it is especially diverse in the páramos of the Northern Andes with at least 36 species representing all of the morphological variation of the group (Romoleroux 2004). This has suggested to some that this group likely originated in this region, and that has undergone a rapid radiation after the last uplift of northern the Andes, like several other groups of plants in that region (reviewed in Sklenář et al. 2011; Madriñan et al. 2013; Luebert and Weigend 2014). Gehrke et al. (2015), using chloroplast DNA and the Yule model as the tree speciation prior, estimated the stem age of *Lachemilla* to be 17.2 mya (23.8–10.6), while our estimates using the *trnL-F* chloroplast region and nrITS independently were 14.45 mya (9.02 - 20.44) and 14.67 mya (9.07 - 20.74), respectively (Table 1.4, Figs. 1.9, 1.10). Although our age estimates were younger for the stem of *Lachemilla*, they fall squarely within the 95% HPD age estimates of Gehrke et al. (2016). Because we sampled *Lachemilla* extensively, we were able to also estimate the crown age of *Lachemilla* to be 12.33 (7.90 - 17.73) for the chloroplast dataset and 11.03 mya (6.85 - 16.02) for the nuclear dataset. These results suggest that origin of *Lachemilla* predates the last uplift of Northern and Central Andes, estimated around three to five mya and 6-10 mya, respectively (Gregory-Wodzicki 2000; Garzione et al. 2008; Leier et al. 2013). That said, our LTT plots (Fig. 1.11)

show that the majority of extant *Lachemilla* lineages appeared during the last ~5 million years, which agrees with Sklenář et al. (2011) that classify *Lachemilla* as a Neotropical element of the páramo flora that has evolved in the Neotropics and subsequently gave rise to species that were adapted to the high altitude environments that emerged during the last uplift of the Andes (Cleef 1979). A salient example of this within *Lachemilla* is the 'Verticillate clade' that is mainly restricted to the northern páramos, and has a crown age estimate between 3.5 and 3.8 mya for the chloroplast and nuclear datasets, respectively (Figs. 1.9, 1.10). Additionally, analyses of diversification rates in Fragariinae focused on the African species of *Alchemilla* (Gehrke et al. 2016) detected several shifts in net diversification rates in Alchemillinae, including one at the crown node of *Lachemilla* that could be associated with the formation of the páramos. However, based on our divergence time estimates and LTT plots (Fig 1.11), the montane Neotropical diversification of *Lachemilla* is more nuanced, and detailed analyses of diversification dynamics in the clade with a more completely sampled phylogeny will be necessary to tease apart the complex relationships between Andean orogeny and diversification rates.

Our results have shown that the large-scale patterns of incongruence between the plastid and nuclear phylogenies in *Lachemilla* are mainly the result of widespread hybridization and polyploidy. Our network analyses reveal a number of putative hybrid species, and given the limited molecular sampling presented here, and concerted evolution of the nuclear ribosomal repeat and maternal inheritance of the plastome, there is a distinct possibility that additional hybridization events have gone undetected. Despite the presence of extensive hybridization in *Lachemilla,* there is a surprising correspondence between major

lineages of *Lachemilla* and morphology-based classification systems. The four major groups recovered in our molecular phylogenies correspond well with Perry's (1929) infrageneric classification, while the differences between earlier classifications, and difficulties placing many taxa in distinct groups due to overlapping morphologies, can be largely explained by the presence of widespread hybridization among the species in *Lachemilla*. The relationship among these four major groups remains unresolved, and given observed ploidy levels, genome sizes, and frequency of hybridization in this clade, we do not discard the hypothesis of a hybrid origin of these major lineages, or even *Lachemilla* as a whole. Finally, we demonstrated that the origin of *Lachemilla* in South America (~14.5 mya) predates the "rapid-uplift" diversification model that has been used to explain the high species-richness and elevated diversification rates observed in other Andean plant clades. Rather, our analyses suggest that *Lachemilla* was present in South America prior to the latest Andean uplift events, and following the formation of the high-elevation Andean grasslands during the last 5 mya, a rapid accumulation of particular nested lineages occurred, contributing to it's ubiquitous presence in these biomes.

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CHAPTER 2: PHYLOGENOMIC ANALYSES REVEAL ANCIENT HYBRIDIZATION IN THE NEOTROPICAL GENUS *LACHEMILLA* (FOCKE) RYDB.

Abstract

Hybridization plays an important role in plant speciation, but also represents a great challenge for phylogenetic reconstruction, due the incongruence patterns that a reticulate process produces within and between the nuclear and chloroplast genomes. In addition, processes like incomplete lineage sorting and phylogenetic error also produce similar incongruence patterns, creating the necessity to distinguish and model these processes simultaneously while inferring phylogenies. Here we use sequence capture data and multiple species trees and species network approaches to resolve the backbone phylogeny of the Neotropical genus *Lachemilla* (Rosaceae). We used 396 loci from low-copy nuclear genes and complete chloroplast sequences of 27 species of *Lachemilla* to clarify the relationships among the major groups of *Lachemilla*, and explore multiple sources of conflict among gene tree and species tree topologies inferred with a plurality of approaches. All phylogenetic methods recover the four major groups previously proposed for *Lachemilla,* but species tree methods recover different topologies for the relationship of these four clades. Species network analyses reveal that one of the four major clades is of ancient hybrid origin, and represents one of the main sources of the incongruence among the species trees. Additionally, we found evidence for a whole genome duplication event share by *Lachemilla* and allied genera. *Lachemilla* show clear evidence of ancient and recent hybridization throughout the evolutionary history of the group. Also, is clear that phylogenetic approaches that can accommodate ILS

and hybridization simultaneously need to be applied when studying groups that show patterns of hybridization.

Introduction

Hybridization is now recognized as a fundamental process in the evolution of animals, plants and fungi (Giraud et al. 2008; Schwenk et al. 2008; Soltis and Soltis 2009; Payseur and Rieseberg 2016), but it seems to be particularly common in plants where hybrid speciation, especially through polyploidy, is a well-established mechanism (Linder and Rieseberg 2004; Mallet 2007). Many plant species might be of direct hybrid origin or descended from a hybrid species in the recent past (Soltis and Soltis 1995), and estimates reveal that 40–70% of all plant species are polyploids (Otto and Whitton 2000), suggesting that hybridization may indeed be a common mechanism for spurring adaptive radiations in plants.

Reticulate processes often lead to incongruence between nuclear and plastid phylogenies and discordance between independent nuclear loci and/or alleles (Rieseberg and Soltis 1991; Doyle 1992; Wendel and Doyle 1998; Linder and Rieseberg 2004). This pattern has been widely detected in plants, and continues to be a good first approximation for the detection of reticulate evolution (e.g., Sang et al. 1995; Soltis and Kuzoff 1995; Fehrer et al. 2007; Pirie et al. 2009; Lundberg et al. 2009; de Kuppler 2015; Scheunert and Heubl 2017). That said, incongruence may also be the product of several other processes, the most frequent being phylogenetic error and incomplete lineage sorting (ILS) (Pamilo and Nei 1988; Rieseberg and Soltis 1991; Doyle 1992; Maddison 1997; Wendel and Doyle 1998). Therefore, to establish hybridization as the main source of discordance, several approaches have

been used to identify and/or quantify phylogenetic error (e.g., Reid et al. 2012; Buddenhagen et al. 2016; Arcilla et al. 2017), and to distinguish ILS from hybridization (e.g., Buckley et al. 2006; Maureira-Butler et al. 2008; Joly et al. 2009; Konowalik et al. 2015; Meyer et al. 2016).

While several methods that model ILS using multilocus sequence data have been implemented and are now widely used (reviewed in Edwards et al. 2016; Mirarab et al. 2016; Xu and Yang 2016), approaches that can accommodate ILS and hybridization simultaneously need to be applied when studying groups that show patterns of hybridization. Recently, methods to estimate phylogenetic species networks from sequence data that incorporate gene tree uncertainty and discordance due to ILS and hybridization have been developed (e.g., Yu et al. 2014; Yu and Nakhelh 2015; Solís-Lemus and Ané 2016; Wen et al. 2016a). Although, these methods are computationally intensive and limited to a small number of species and hypothesized hybridization events (Hejase and Liu 2016), their usage to detect patterns of reticulation is increasing (e.g., Meyer et al. 2016; Wen et al. 2016b; Crowl et al. 2017).

The genus *Lachemilla* (Focke) Rydb. is a group of ca. 60 species that includes perennial rosette-forming herbs, stoloniferous herbs, trailing herbs, procumbent herbs, subshrubs, and dwarf shrubs (Romoleroux 1996, 2004; Gaviria 1997). *Lachemilla* is distributed between 2200-5000 m throughout the high mountains of the western American tropics from northern Mexico to northern Argentina and Chile (Gavira 1996; Romoleroux 2004), and is especially common and diverse in the high elevation ecosystems of the Northern Andes where the clade has undergone a rapid ecological radiation associated with the most recent Andean orogeny (Chapter 1). Previous phylogenetic analyses (Chapter 1) identified

four well-supported lineages within *Lachemilla* that correspond largely to traditional, morphologically defined sections. The first is the *Tripartite* clade that comprises ascending and procumbent herbs with tripartite leaves that often appear to have 5 divisions due to the bifid lateral segments of some species. The second group is the *Verticillate* clade that includes subshrubs with erect or decumbent stems and reduced leaves that fuse with the stipules to form verticillate sheaths. The third group is the *Orbiculate* clade that encompasses species with a stoloniferous habit and palmately lobed leaves. The last group is the *Pinnate* clade, which include species with repent or decumbent stems and pinnate or bipinnatifid basal leaves. These clades are in part congruent with previous morphological classifications of group (Perry 1929; Rothmaler 1937), but the relationships among these clades remain largely unresolved. Additionally, a widespread pattern of cytonuclear discordance due to hybridization has been identified, and evidence of at least 24 potential hybrid species involving all four of the major lineages of *Lachemilla* was established (Chapter 1).

Genomic data provide an excellent opportunity to detect hybridization (reviewed in Payseur and Rieseberg 2016), however, in groups where hybridization is widespread across the clade phylogenetic data from multiple independent single- or low-copy nuclear genes is required (Pamilo and Nei 1988; Sang & Zhang 1999). Targeted sequence capture and extensions of these methods allow for the sequencing of hundreds of low-copy nuclear loci and high-copy genomic targets like the chloroplast and/or mitochondrial genomes (Cronn et al. 2012; Lemmon and Lemmon 2013; Mandel et al. 2014; Weitemier et al. 2014; Folk et al. 2015), and have been used in multiple group of plants to resolve phylogenetic relationships (e.g., Heyduk et al 2015; Stephens et al. 2015; Sass et al. 2016) and to investigate patterns

of hybridization (e.g., Grover et al. 2015; Folk et al. 2016; Crowl et al. 2017; Mitchell et al. 2017). In this paper, we use a phylogenomic dataset of 396 nuclear loci and complete plastomes assembled via targeted sequence capture to (i) estimate the phylogeny of *Lachemilla* with a focus on relationships among the major clades, (ii) reexamine the source of incongruence between the plastid and nuclear phylogenies using genome-scale data, and (iii) investigate the sources of discordance among gene trees and species trees.

Materials and Methods

Taxon sampling — We sampled 29 individuals of 27 species of *Lachemilla*, representing approximately 50% of the total described diversity of the group, and most of the morphological variation within the four major clades of *Lachemilla* (Chapter 1). Additionally, two species of *Alchemilla*, representing the Eurasian and African clades, one species of *Aphanes*, and one species of *Fragaria* were included as outgroups. Voucher information is listed in Appendix 6.

DNA extraction, hybrid enrichment, and sequencing — Total genomic DNA was isolated from fresh, silica-dried, or herbarium material using the DNeasy Plant Mini Kit (Qiagen, Valencia, California) following the manufacturer's protocol, or with a modified 2 × CTAB method (Doyle and Doyle 1987). 257 putatively single-copy orthologous genes (1419 exons) were identified in Rosaceae via a comparison of the apple (*Malus*), peach (*Prunus*), and strawberry (*Fragaria*) genomes (see Liston et al. 2014 for details of bait design). Genomic DNAs were sheared by nebulization at 30 psi for 70 sec, yielding an average shear size of 500bp as measured by a

Bioanalyzer High-Sensitivity Chip (Agilent Technologies, Inc., Santa Clara, California, USA). Libraries were constructed using the Illumina TruSeq library preparation kit (Illumina Inc., San Diego, California, USA) and NEXTflex DNA barcodes (Bioo Scientific, Austin, Texas, USA). Libraries were standardized at 2nM and pooled in 16-plexes prior to hybrid enrichment. Library concentrations were determined using the KAPA qPCR kit (KK4835) (Kapa Biosystems, Woburn, Massachusetts, USA) on an ABI StepOnePlus Real-Time PCR System (Life Technologies, Grand Island, New York, USA). Solution-based hybridization with MYbaits biotinylated RNA baits (MYcroarray, Ann Arbor, Michigan, USA) and enrichment followed Weitemier et al. (2014). The target-enriched libraries were then sequenced on an Illumina HiSeq 2000 with 150bp paired-end reads at the Genomics CoreFacility at the University of Oregon. Raw Illumina data will be submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive

Read processing and assembly—De-multiplexed reads were cleaned with SeqyClean 1.8.10 (https://github.com/ibest/seqyclean) using defaults settings to remove sequencing adaptors and low quality reads (<20 Phred Quality Scores). Assemblies of nuclear loci were carried out with HybPiper (Jonhson et al. 2016) using *Fragaria vesca* exon sequences as references. Exons with an expected size ≥ 300 bp were assembled separately (400 exons of 225 genes), and paralog assessment was run for all samples and exons. Plastome assembly was carried out using Alignreads 2.5.2 (Straub et al. 2012) in an iterative process. First, all samples were assembled using the *Fragaria vesca* chloroplast genome as a reference (Genbank accession JF345175). Then, for all samples, a consensus sequence of this first assembly was used as final reference for a second assembly of each sample. The resulting

plastome assemblies were annotated using *Fragaria vesca* and *Dasiphora fruticosa* (Genbank accession KC507758) chloroplast genomes as references in Geneious v 7.1.9 (Kearse et al. 2012).

Nuclear data processing—HybPiper assemblies of nuclear loci resulted in multiple copies for most loci (Appendix 7 and 8). To choose the appropriate gene copy for downstream analyses, we aligned each exon with MAFFT v7.037b (Katoh and Standley 2013) using the automatic alignment strategy, and inferred gene trees for each alignment using FastTree2 (Price et al. 2010) with the '-slow', '-gtr', and '-gamma' options. Using the resulting individual gene trees and alignments, a tree-based approach implemented in PhlyoTreePruner (Kocot et al. 2013) was used to screen for evidence of paralogy. The maximal inclusive subtree with a minimum support of 0.75 and at least 25 taxa was selected where each taxon was represented by no more than one sequence. In cases where multiple sequences from the same taxon formed a clade, only the longest sequence was retained. Detected paralogs are likely the product of a whole genome duplication event (WGD) predating the diversification of *Lachemilla* (see Discussion), and as a result 351 exons have multiple subtrees that met the pruning criteria. Because the output of PhlyoTreePruner is a single alignment, we ran the paralog search in each exons after excluding the sequences of the subtrees that were pruned in the first search. When multiple paralog alignments were obtained for an exon, we kept the alignment with the most number of taxa. In some cases, multiple subtree alignments had the same number of taxa, and therefore, we kept all alignments and treated each gene copy as independent loci. Occasionally, multiple gene copies that did not form single clades were recovered from some species (likely the product of

the extensive hybridization and putative allopolyploidy present in *Lachemilla*; Chapter 1) preventing PhlyoTreePruner from pruning paralogs. In these cases, we randomly retained only one gene copy for those samples and reran the paralog pruning search. Finally, individual loci were realigned, and ambiguously aligned positions were removed with GBlocks V 0.91b using default parameters (Castresana 2000; Talavera and Castresana 2007).

Phylogenetic analyses—We used concatenation and coalescent-based methods to reconstruct the phylogeny of *Lachemilla.* We performed the nuclear phylogenetic analyses on four datasets: the COMPLETE dataset that includes all sampled species, the HYBRID-REDUCED dataset, which excludes the hybrid species identified previously by Morales-Briones et al. (Chapter 1), the ORBICULATE-REDUCED dataset that excludes the hybrid species and the *Orbiculate* clade, and the NO-RECOMBINATION dataset that excludes the hybrid species and loci that show evidence of recombination. For each dataset, we first estimated phylogenetic relationships using the concatenated matrix using RAxML v8.0.3 (Stamatakis 2014) with a partition-by-locus scheme selected using PartitionFinder V 2.1.1 (Lanfear et al. 2017). All partitions used the GTR + G model, 100 searches for the best tree were performed, and clade support was assessed with 1,000 bootstrap (BS) replicates. To estimate coalescent-based species trees, we used three different approaches. First, we used two summary statistic methods, ASTRAL-II (Mirarab and Warnow 2015) and MP-EST (Liu et al. 2010). Individual locus gene trees were estimated using RAxML with a GTR + G model, 10 searches for the best tree and 100 BS replicates to assess clade support. Individual gene trees and BS replicates were used to estimate species trees in ASTRAL-II and MP-EST

with 100 BS replicates. A third method, SVDquartets (Chifman and Kubatko 2014) as implemented in PAUP v. 4.0a152 (Swofford 2002), which utilizes the full data to estimate the species trees, was used on the concatenated matrix with 100 BS replicates to assess clade support.

For the chloroplast phylogenetic analyses complete plastome sequences (excluding one inverted repeat region) were aligned with MAFFT using the automatic alignment strategy. We used RAxML with a partition by coding and non-coding regions selected using PartitionFinder. All partitions used the GTR + G model and 100 searches for the best tree were performed and clade support was assessed with 1,000 bootstrap replicates.

Concordance analyses— To explore discordance between gene tree and species tree estimates, we first calculated the internode certainty (ICA), a measure that quantifies the degree of conflict on each node of a target tree (i.e., species tree estimates) given individual gene trees (Salichos et al. 2014). In addition, we identified the number of conflicting and concordant bipartitions on the species trees. We calculated ICA and the number of conflicting/concordant bipartitions with PhyParts (Smith et al. 2015) using the estimated species trees as the map tree and the individual gene trees with a BS support cutoff of 50%. We also summarized phylogenetic conflict across the genome using a Bayesian concordance analysis with BUCKy v.1.4.4 (Ane et al. 2007; Larget et al. 2010). First, we estimated posterior distributions of individual gene trees with MrBayes v3.2.6 (Ronquist et al. 2012). Analyses consisted of two independent runs with four Markov Chain Monte Carlo (MCMC) chains of 30 million generations each, sampling every 30,000th generation using a GTR + G model. Convergence of parameter estimates resulting from the

two independent MCMC runs was assessed using Tracer 1.6 (Rambaut et al. 2014). Only loci that had reached convergence by 30 million generations, and had complete taxon sampling (excluding *Aphanes occidentalis* to increase the number of loci) were used for the Bayesian concordance analysis. BUCKy was run using the posterior distribution of gene trees after discarding 10% as burin-in, and multiple values of the *a priori* discordance parameter (α = 2, 20, 200, 2000), to test for the impact of this parameter.

Assessment of recombination— Coalescent species trees methods assume that there is no recombination within loci and free recombination between loci. To determine the presence of recombination in our data set, we calculated the test for recombination, Φ (or pairwise homoplasy index, PHI; Bruen et al. 2006), using PhiPack (Bruen et al. 2006) with the default sliding window size of 100 bp.

Assessment of hybridization— We used coalescent simulations similar to Folk et al. (2016) to test if incomplete lineage sorting (ILS) alone could explain plastid and nuclear incongruence in the COMPLETE dataset. We simulated 10,000 species trees under ILS with DendroPy V 4.1.0 (Sukumaran and Holder, 2010) using a model of organellar inheritance using the MP-EST species trees from the COMPLETE dataset with branch lengths scaled by four. Gene tree clade frequencies were summarized on the chloroplast tree.

Gene genealogy interrogation analysis— To distinguish incompatible signals regarding the relationship of the four major clades of *Lachemilla*, we used gene genealogy interrogation (GGI; Arcilla et al. 2017), a recently described method that

discerns between estimation error and actual biological conflict explaining gene tree discordance. GGI identifies the best-supported hypothesis for each locus by enforcing monophyly of the clades of interest and performing constrained maximum likelihood searches for each hypothesis. Then, constrained gene trees are ranked based on their probabilities estimated using the approximately unbiased (AU) topology test (Shimodaira 2002). We performed the GGI analyses using the HYBRID-REDUCED and NON-RECOMBINATION datasets, and tested the four possible topologies obtained from the nuclear species trees, chloroplast tree, and concordance analysis (Fig. 2.5; Table 2.1).

***Species Network Analysis*—** We inferred species networks that models ILS and hybridization using a maximum pseudo-likelihood approach (Yu and Nakhelh 2015). Species network searches were carried out with PhyloNet v 3.6.1 **(**Than et al. 2008), using the individual gene trees from the HYBRID-REDUCED dataset as input with the 'InferNetwork_MPL' command allowing for up to 3 hybridization events, using only nodes in the gene trees that have bootstrap support of at least 75%, and optimizing the branch lengths and inheritance probabilities of the returned species networks under the full likelihood. To estimate the best number of hybridizations and test whether the species network fits our gene trees better than a strictly bifurcating species tree, we computed the likelihood score of the four tree topologies used in the GGI analyses given the individual gene trees, as implemented in Yu et al. (2012), using the command 'CalGTProb' in PhyloNet (again using individual gene trees and only nodes with bootstrap support of at least 75%). Finally, we performed model selection using three information criteria, AIC (Akaike 1974), AICc (Sugiura 1978), and BIC (Schwarz 1978), where number of

parameters equals the number of branch lengths being estimated, plus the number of hybridization probabilities being estimated and number of gene trees used to estimate the likelihood, to correct for finite sample size. We chose as the best model the one with the lowest information criteria value.

Results

***Exon assembly*—** The assembly resulted in sequences of up to 392 exons (≥ 300 bp) per species (Appendix 7). HybPiper identified paralogous gene copies for up to 284 exons per species (Appendix 7). After paralog pruning and removal of exons with poor coverage across samples (≤ 24 samples) we kept 333 exons from 196 different genes. Additionally, 63 of those exons showed the presence of two (60) and three (3) paralogs copies that met the pruning requirements giving us a total of 396 loci. The resulting concatenated matrix had an aligned length of 265,028 bp with 21,004 parsimony-informative sites, a minimum locus size of 277 bp, and a maximum locus size of 5739 bp. The chloroplast matrix (with one inverted repeat excluded) had an aligned length of 118,846 bp with 3,733 parsimony-informative sites.

***Nuclear phylogenetic analyses: COMPLETE dataset*—** All analyses recovered the four main clades of *Lachemilla* proposed by Morales-Briones et al. (Chapter 1), but the relationship among and within the four clades varied in each analysis (Table 2.1), with some species (e.g., *L. fulvescens* and *L. talamanquensis*) even recovered in different clades than in Morales-Briones et al. (Chapter 1). The concatenated analysis supports 'Topology 1' where the *Verticillate* and *Tripartite*

clades are monophyletic and sister to the clade formed by the *Orbiculate* and *Pinnate* clades (Fig. 2.1). ASTRAL-II, SVDquartets, and MP-EST analyses recovered 'Topology 2' where the *Verticillate* and *Tripartite* clades form a clade with the *Orbiculate* and *Pinnate* clades representing successive sister groups (Fig. 2.2 A). With the exception of the concatenated analyses, most of the major clades and relationships within and among them are well supported (BS ≥ 75%). However, the concordance analyses and ICA scores revealed that most gene trees are actually in conflict with the species trees (Fig. 2.2 B). All Bayesian concordance analyses with different *a priori* discordance parameter resulted in identical results. BUCKy recovered 'Topology 3' where the *Verticillate* and *Tripartite* clades are again monophyletic with the *Pinnate* clade and then *Orbiculate* clade as successive sister groups (Fig. 2.3 A). The Bayesian concordance factors are low for most clades, revealing a high degree of conflict among the gene trees (Fig. 2.3 A).

Table 2.1. Datasets used in this study, indicating the number of taxa, number of loci, and topology (following Fig. 2.5) recovered in each analysis.

Dataset	Number of taxa	Number of loci / Topology recovered					
		Concatenation (RAxML)	ASTRAL-II	MP-EST	SVDquartets	BUCKy	Chloroplast (RAxML)
COMPLETE	29	396 / 1	396 / 2	396 / 2	396 / 2	208 / 3	1 / 4
HYBRID-REDUCED	15	396 / 1	396 / 1	396 / 2	396 / 2	219 / 1	1 / 4
NO-RECOMBINATION	15	265 / 1	265 / 2	265 / 2	265 / 4	160 / 1	NA / NA
ORBICULATE-REDUCED	13	396 / NA	396 / NA	396 / NA	396 / NA	221 / NA	1 / NA

Table 2.2. Model selection between the different species trees and species networks recovered. The model with the lowest information criteria was selected as the best one (highlighted in bold). Topological hypotheses follow Fig. 2.5.

Topology	lnL	Parameters	Loci	Number of hybridizations	Information criteria		
					AIC	AICc	BIC
Tree topology 1	-6147.752	35	222	NA	12365.504	12379.052	12301.982
Tree topology 2	-6156.017	35	222	NA	12382.035	12395.583	12318.513
Tree topology 3	-6148.437	35	222	NA	12366.874	12380.423	12303.353
Tree topology 4	-6262.415	35	222	NA	12594.831	12608.379	12531.309
Network 1	-6083.621	36	222	1	12239.243	12253.643	12173.906
Network 2	**-5795.016**	**37**	**222**	**2**	**11664.032**	**11679.314**	**11596.88**
Network 3	-6092.135	39	222	3	12262.269	12279.412	12191.488

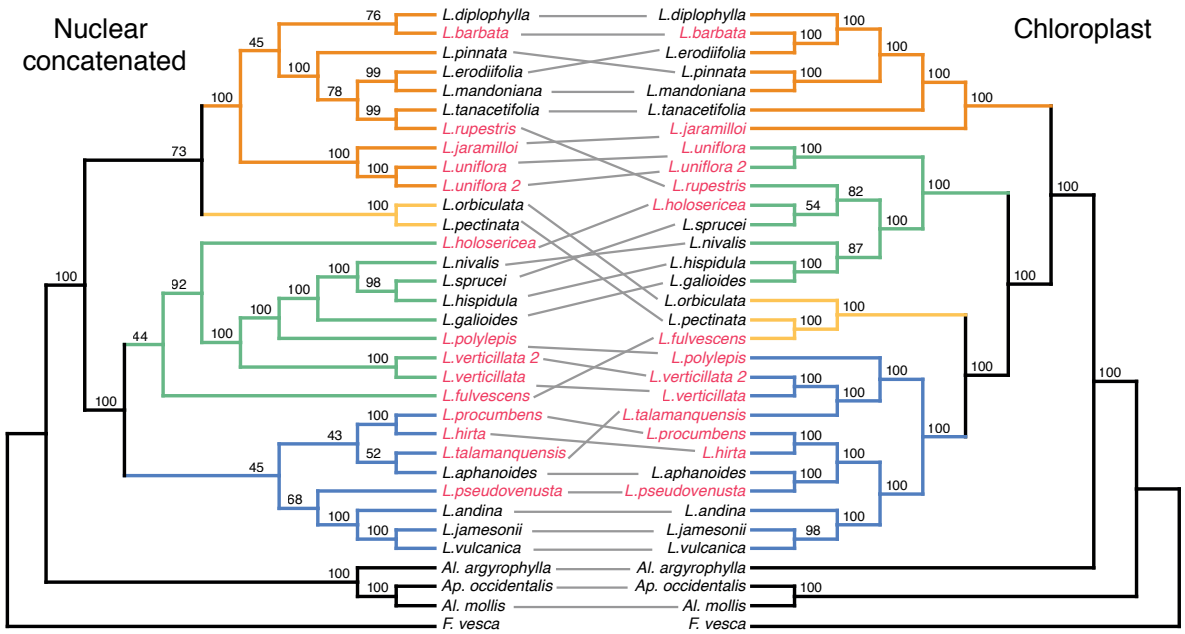

Figure 2.1. Tanglegram of the nuclear concatenated (left) and chloroplast (right) phylogenies of the COMPLETE dataset. Gray lines connect taxa between the phylogenies. Maximum likelihood bootstrap support values are shown above branches. Branches are colored by major clades within *Lachemilla:* orange – *Pinnate*, yellow – *Orbiculate*, green – *Verticillate*, and blue – *Tripartite*. Taxa previously identified as hybrids by Morales-Briones et al. (Chapter 1) are highlighted in red.

Chloroplast phylogenetic analyses and evidence of hybridization:

COMPLETE dataset—Phylogenetic analysis of the plastome dataset also recovered the four major clades in *Lachemilla*, but resulted in the fourth distinct topology with respect to relationships among these four lineages ('Topology 4') where the *Verticillate* clade is sister to a clade formed by the *Orbiculate* and *Tripartite* clades, with the *Pinnate* clade sister to all of them (Fig. 2.1, 2.3 B). Most of the relationships are supported with BS = 100, but the level of discordance between the nuclear and chloroplast trees is high (Fig. 2.1, 2.3 B), with multiple species (e.g., *L. uniflora*, *L. verticillata*, *L. fulvescens*) located in different clades, and with different relationships among the four groups. Coalescent simulations under the organellar model did not produce gene trees that resembled the observed chloroplast tree. When the

simulated gene trees were summarized on the observed chloroplast tree, most clade frequencies were 0% (Fig. 2.3 B), suggesting that ILS alone cannot explain the high level of cytonuclear discordance observed in *Lachemilla.*

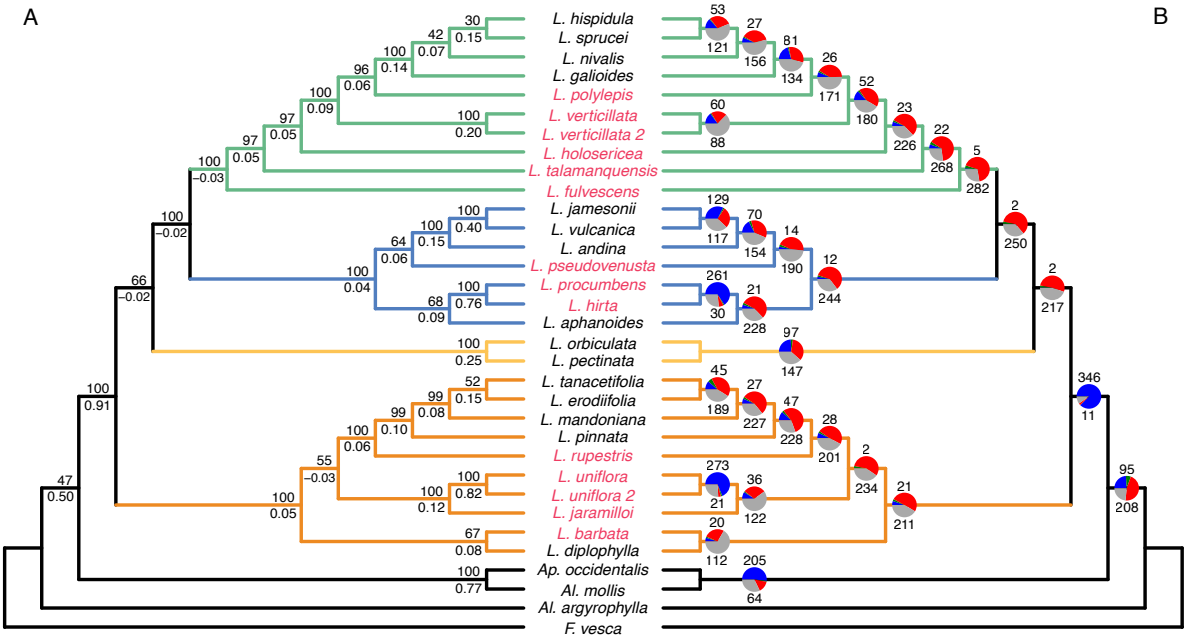

Figure 2.2. Species trees of the COMPLETE dataset inferred with ASTRAL-II. A. Maximum likelihood bootstrap support values and ICA scores are shown above and below branches, respectively. B. Pie charts next to the nodes present the proportion of gene trees that support that clade – blue, the proportion that support the main alternative for that clade – green, the proportion that support the remaining alternatives – red, and the proportion (conflict or support) that have less than 50% bootstrap support – gray. Numbers above the pie charts indicates the number of gene trees concordant with the species tree at that node, and the numbers below the pie charts indicate the number of gene trees in conflict with that node in the species tree. Hybrid taxa and branch colors as in Figure 2.1.

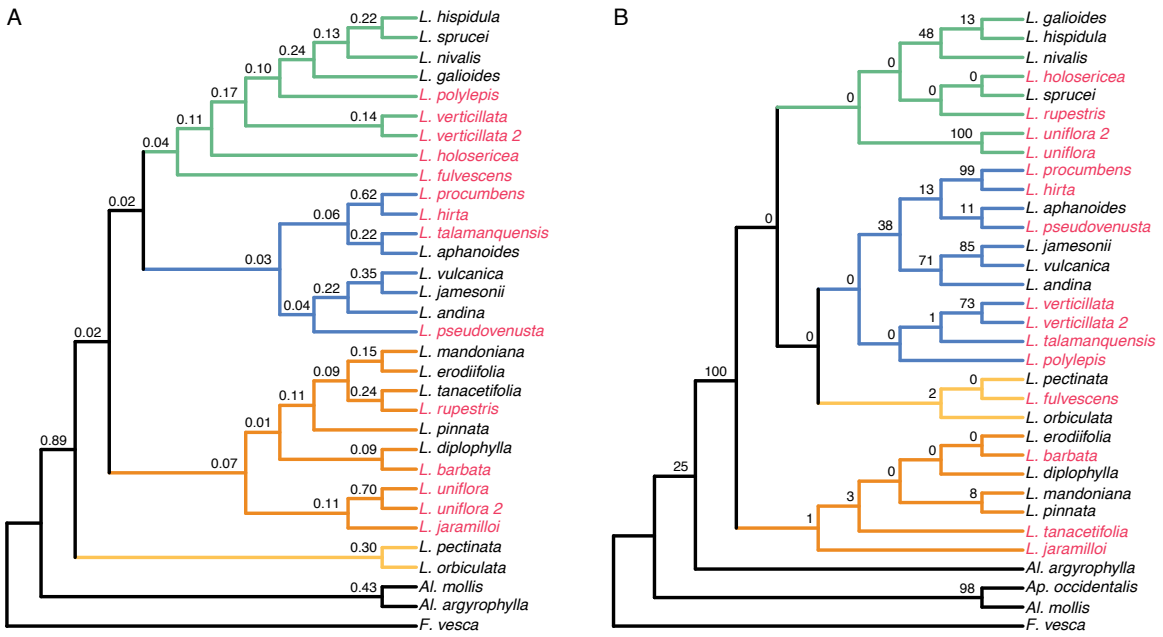

Figure 2.3. A. BUCKy concordance tree of the COMPLETE dataset; numbers above branches represent concordance factors. B. Chloroplast phylogeny of the COMPLETE dataset; numbers above branches represent clade frequencies of the simulated gene trees. Hybrid taxa and branch colors as in Figure 2.1.

Nuclear phylogenetic analyses: HYBRID-REDUCED dataset—With previously identified hybrid species (Chapter 1) removed, the concatenated and ASTRAL-II analyses both recovered 'Topology 1,' but BS support for the sister group relationship of the *Pinnate* and *Orbiculate* clades was low (63% and 21%, respectively; Fig. 2.4 A, Appendix 9). Although relationships within each major clade were identical in these analyses, the ASTRAL-II analysis recovered low support for relationships within the *Verticillate* clade (Fig 2.4 A). SVDquarters and MP-EST analyses both recovered 'Topology 2' with high BS for all clades (Fig 2.4 B, Appendix 9). Relationships within the major clades were constant in both analyses, but the position of *L. sprucei* in the *Verticillate* clade and *L. tanacetifolia* in the *Pinnate* clade varied with respect to the concatenated and ASTRAL-II topologies (Fig. 2.4). Concordance analyses and ICA scores continue to reveal a high level of

incongruence between individual gene trees and species tree estimations, even after the removal of the previously identified hybrids. As with the ASTRAL-II and concatenated analyses, BUCKy analyses of this dataset recovered 'Topology 1,' and concordance factors remain low for most clades (Appendix 9).

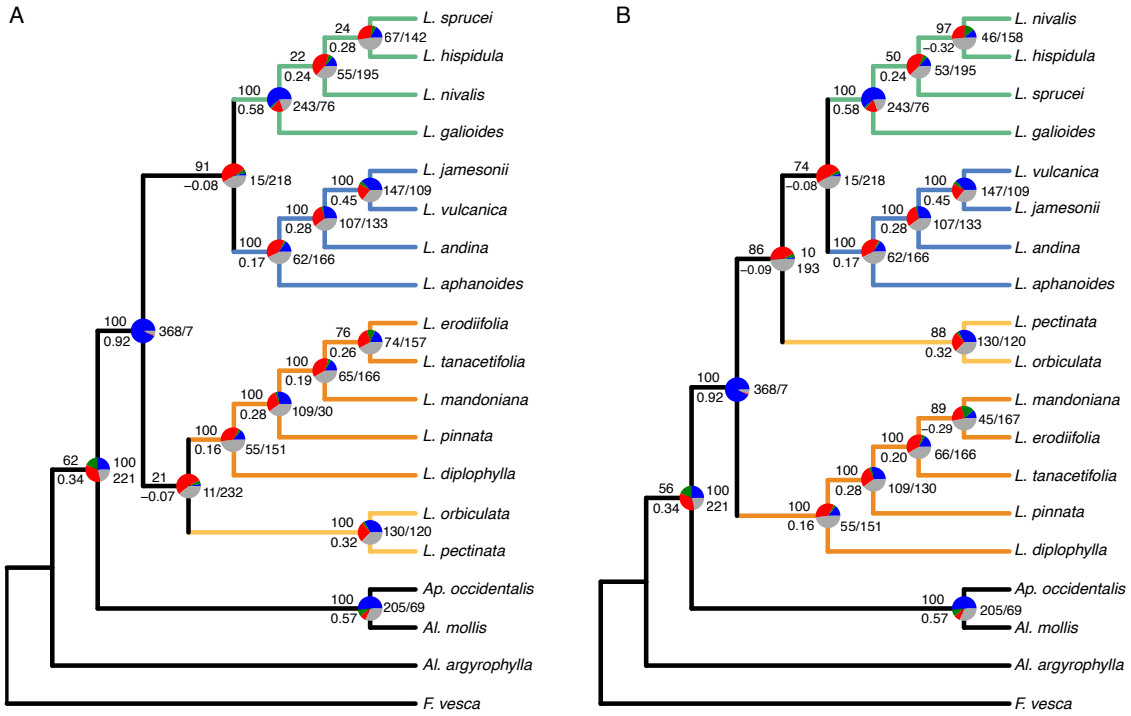

Figure 2.4. Species trees topologies recovered for the HYBRID-REDUCED dataset. A. Species trees inferred with ASTRAL-II. B. Species trees inferred with SVDquartets. Maximum likelihood bootstrap support values and ICA scores are shown above and below branches, respectively. Pie charts next to the nodes represent the proportion of gene trees that support that clade – blue, the proportion that support the main alternative for that clade – green, the proportion that support the remaining alternatives – red, and the proportion (conflict or support) that have less than 50% bootstrap support – gray. Numbers next to pie charts indicates the number of gene trees concordant/conflicting with that node in the species tree.

Recombination analyses—The test for recombination, Φ, identified 131 loci with a strong signal of recombination for the HYBRID-REDUCED dataset ($P < 0.05$; Appendix 10). Concatenated, MP-EST, SVDquartets, and BUCKy phylogenetic analyses of the NO-RECOMBINATION (after removal of recombinant loci)

recovered identical topologies to the analyses of the HYBRID-REDUCED dataset with all loci included. As in the HYBRID-REDUCED dataset, 'Topology 1' was inferred for all analyses, with the exception of the ASTRAL-II analysis where 'Topology 2' was recovered (Appendix 11).

GGI and network analysis: HYBRID-REDUCED dataset — The GGI analysis indicated the largest support for 'Topology 3,' with 73 gene trees supporting this topology; however, this analysis also shows that the majority of gene trees do not provide significant support for any of the four alternative topologies ($P < 0.05$; Fig 2.5, Appendix 12). GGI analysis of the NO-RECOMBINATION dataset was similar (Appendix 13 and 14).

Species network analyses recovered topologies with up to three hybridization events. All networks recovered the four major clades of *Lachemilla* (Appendix 15) with the *Orbiculate* clade always identified as a hybrid node. All three information criteria indicated that the species networks with hybridization events involving the *Orbiculate* clade provided a better fit for our data than any of the four strictly bifurcating hypotheses (Table 2.2; Fig. 2.5). The network with two hybridization events (Fig. 2.6 A) had the best support for the three information criteria. With this best species network, the first hybridization event involves *L. aphanoides* and an extinct or unsampled lineage that gave rise to the rest of the *Tripartite* clade species (inheritance probabilities of 0.612 from the extinct/unsampled lineage and 0.388 from *L. aphanoides*). The second hybridization event reveals the hybrid origin of the *Orbiculate* clade. Inferred inheritance probabilities for this event indicate that the largest genomic contribution comes

from the *Tripartite* clade (0.863), and only a small portion comes from the *Pinnate* clade (0.137).

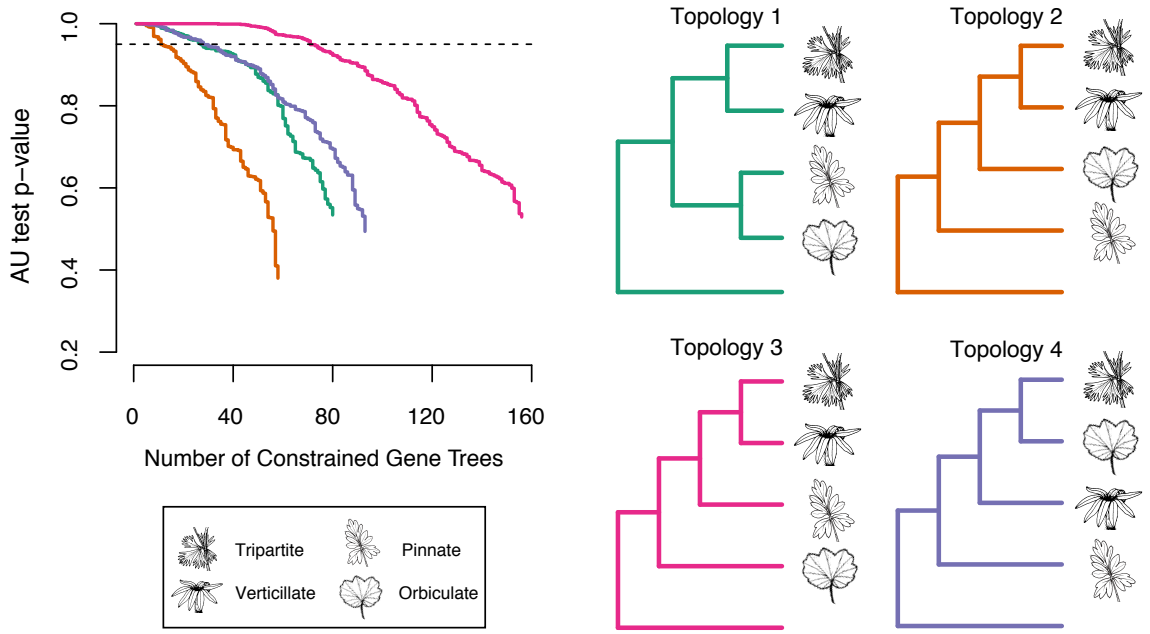

Figure 2.5. Gene genealogy interrogation results testing the four topologies inferred for the four major clades of *Lachemilla*. Embedded plot represents the cumulative number of genes supporting each topology with highest probability and their P-values from the AU tests. Values above the dashed line indicate topologies that are significantly better than the alternatives ($P \leq 0.05$). Line drawings illustrate representative leaf morphologies of each major clade; illustrations modified from Romoleroux (1996).

Phylogenetic analyses: ORBICULATE-REDUCED dataset—After the removal of the *Orbiculate* clade, all phylogenetic analyses recovered the same well supported topology with the *Verticillate* and *Tripartite* clades sister to each other, and the *Pinnate* clade sister to that clade (Fig. 2.6 B, Appendix 16). Despite this consistent result, the levels of gene tree discordance with this topology were still high, especially with respect to relationships within the *Verticillate* and *Pinnate* clades.

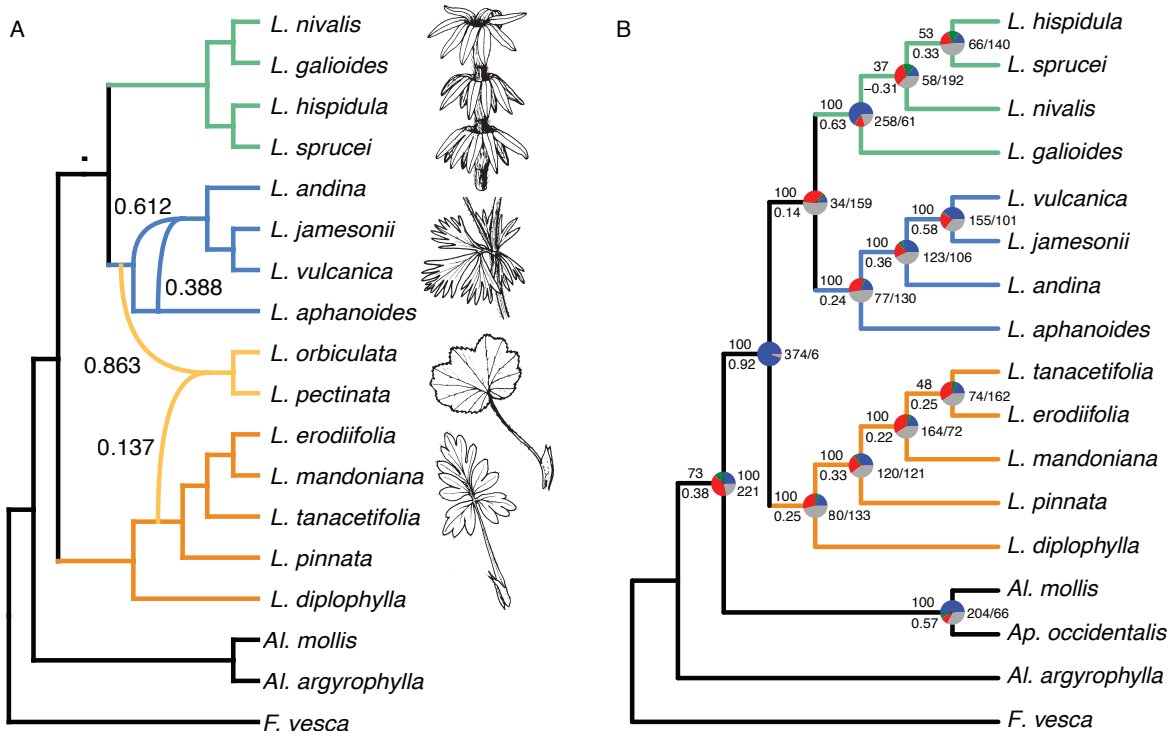

Figure 2.6. Best species network of the HYBRID-REDUCED dataset inferred with PhyloNet. Numbers next to the hybrid branches indicate inheritance probabilities. Line drawings illustrate representative leaf morphologies of each major clade; illustrations modified from Romoleroux (1996). B. Species tree of the ORBICULATE-REDUCED dataset inferred with ASTRAL-II. Maximum likelihood bootstrap support values and ICA scores are shown above and below branches, respectively. Pie charts next to the nodes represent the proportion of gene trees that support that clade – blue, the proportion that support the main alternative for that clade – green, the proportion that support the remaining alternatives – red, and the proportion (conflict or support) that have less than 50% bootstrap support – gray. Numbers next to pie charts indicate the number of gene trees concordant and conflicting with that node in the species tree.

Discussion

Cytonuclear discordance and evidence of hybridization—Evidence of extensive hybridization has been previously detected in *Lachemilla* with at least 24 species identified as putative hybrids (Chapter 1). The extensive analyses performed here revealed a similar pattern of cytonuclear discordance, where several species are recovered in different positions between the nuclear and chloroplast phylogenies (Fig. 2.1). Additionally, the Bayesian concordance analysis

and ICA scores revealed a large amount of conflict between individual gene trees and the species tree estimates. Although these patterns may also be attributable to other processes, like ILS and phylogenetic error (Pamilo and Nei 1988; Rieseberg and Soltis 1991; Doyle 1992, Maddison 1997; Wendel and Doyle 1998), as in Morales-Briones et al. (Chapter 1), our coalescent simulations showed that the observed cytonuclear discordance cannot be explained by ILS alone, and furthermore, this is emerging as a common pattern in plant systems (e.g., Maureira-Butler et al. 2008; Blanco-Pastor et al. 2012; Folk et al. 2016; Reginato and Michelangeli 2016; Vargas et al. 2017).

Although removal of identified hybrid lineages reduces conflicting signals across gene trees, ICA values and concordance factors indicate that discordant signals are still persistent for some clades, suggesting that ILS and/or unidentified hybrid lineages continue to muddle our understanding of relationships in *Lachemilla*. For example, species like *L. diplophylla, L. sprucei, and L. tanacetifolia* that have not previously been identified as hybrid taxa show conflicting positions between species tree estimates and the chloroplast tree, suggesting that these species may be of hybrid origin. Additional work identifying parental lineages of putative hybrid species using allelic information from single-copy nuclear genes – e.g., statistical phasing of alleles from sequence capture data and/or isolating individual alleles via molecular cloning and/or bioinformatically from high throughput amplicon datasets (e.g., Pyron et al. 2016; Uribe-Convers et al. 2016; Motazedi et al. 2017; Rothfels et al. 2017;) – remain to be done in *Lachemilla.*

Discordance among individual gene trees and species trees— Our analysis of concordance also reveals that a significant number of bipartitions on individual

gene trees are not well supported, implying low phylogenetic information in the sampled loci. However, low support values can also be the product of the inclusion of hybrid lineages, and the removal of these taxa from our analyses does result in a general improvement of support measures (although a significant amount of weakly supported bipartitions are still recovered; Fig. 2.3). Our species trees analyses produced well-supported and congruent trees after the removal of hybrid taxa, suggesting that the low phylogenetic signal in the individual gene trees is not necessarily negatively affecting our species trees estimations, as has been seen in other studies that use capture data (e.g., Bloom et al. 2016; Mitchell et al. 2017).

Although the four main well-supported clades within *Lachemilla* have been previously recognized, relationships among these clades have remained largely unresolved (Chapter 1). Our phylogenetic analyses recover the same four major lineages, but depending on the dataset used and the phylogenetic approaches employed, these relationships vary considerably. Phylogenetic analyses of the COMPLETE dataset recovered four distinct topologies, and after removal of previously identified hybrid species, three of those topologies were consistently recovered (Table 1; Figure 2.5). The major difference between these hypotheses is with respect to the placement of the *Orbiculate* clade that, with the exception of chloroplast tree, is associated with low concordance and support values, suggesting that the *Orbiculate* clade might be involved in a hybridization event.

Although recombination was detected for more than 30% of the loci, our analyses with these loci removed (NO-RECOMBINATION dataset) were largely the same as with them included. Some studies (e.g., Gatesy and Springer 2013; Springer and Gatesy 2016) claim that recombination might be problematic for coalescent-based phylogenetic analyses, but simulation studies have shown that methods for

species tree inference may be largely robust to intra-locus recombination (Lanier and Knowles 2012; Wang and Liu 2016), and a recent empirical study showed that despite a large amount of recombinant loci (~42%), ASTRAL-II still recovered the same topology with these loci included or excluded from species tree analyses (Folk et al. 2016). With respect to our ASTRAL-II analyses, the only difference with and without recombinant loci is again in the placement of the ORBICULATE clade. The varying placements of the *Orbiculate* clade when analyzing different datasets and/or using different approaches to estimate the species tree seems to be primarily the product of the inconsistency of species tree estimation when hybrid taxa are included (Solís-Lemus and Ané 2016). Our network analysis of the HYBRID-REDUCED dataset using PhyloNet revealed that the *Orbiculate* clade is of hybrid origin, and additionally, all models involving hybridization events fit our data better that any model with strict bifurcating trees (Table 2). These empirical results corroborate simulation studies that have shown that phylogenetic species network methods that simultaneously model discordance due to ILS and hybridization should be the preferred approach for investigating phylogenetic relationships in groups where gene flow is prominent (Solís-Lemus and Ané 2016).

Because of the large amount of conflict between gene trees, we also used gene genealogy interrogation (GGI; Arcilla et al. 2017) to assess the potential for gene tree estimation error as the reason for the pattern of incongruence among species tree topologies. Although this method can be useful for distinguishing between estimation error and actual biological conflict in explaining gene tree discordance, as pointed out by the authors, additional analyses are necessary to correctly interpret the signal of gene tree discordance when other processes like ILS or hybridization might also contribute to the observed conflict. In our case, GGI

selects 'Topology 4' as the hypothesis with the highest support from individual gene trees (Fig. 2.5), but it is likely that this topology is chosen over the alternative hypotheses, because by placing the *Orbiculate* clade sister to the rest of *Lachemilla,* it removes the source of conflict between the other three clades. This interpretation is corroborated by the convergence on the same topology by all phylogenetic methods using the dataset with *Orbiculate* clade removed (Fig. 2.6).

Relationships among major clades of Lachemilla and systematic implications— Based mainly on foliar characters, Perry (1929) divided *Lachemilla* in to six groups, and recent phylogenetic analyses recover four main clades of *Lachemilla* that have a partial correspondence with four of Perry's groups (Chapter 1). This partial correspondence is the product of the inclusion of a number of species, now recognized to be of hybrid origin from taxa in distinct groups that have incongruent positions in molecular phylogenies. The other two groups (both monotypic – *L. polylepis* and *L. diplophylla*) were found to be distinctive members of two of the major clades, where *L. polylepis* belongs to the *Verticillate* clade and *L. diplophylla* to the *Pinnate* clade, although in both cases these species have different overall morphologies when compared the rest of the clade. Although, these major clades were identified with strong support by Morales-Briones et al. (Chapter 1), relationships between them remained unresolved, probably due to the limited amount of DNA sequence data used, as well as the hybrid origin of the *Orbiculate* clade identified here.

Our analyses strongly support the sister group relationship of the *Verticillate* and *Tripartite* clades (Fig. 2.6). The *Verticillate* clade, mainly characterized by the highly modified lead-blades that fuse with the stipules to simulate a whorl of

simple, elongate leaves, was considered by Perry (1929) as transition from the *Tripartite* clade, which has tripartite leaves that often appear to have 5 divisions due to the bifid lateral segments of some species and usually bifid, leaf-like stipules. Gaviria (1997) also recognized this leaf transition, although it is worth noting that some of the species used to identify this transition correspond to hybrid species between the two groups (Chapter 1).

The *Tripartite* clade as defined by Perry (1929; series *Aphanoides*) was subdivided into six subgroups, where four of them are actually composed of only hybrid species between this group and the other three major clades, while and the other two correspond to the *Tripartite* clade (Chapter 1). Here we identified an additional hybridization event between *L. aphanoides* and an extinct or unsampled lineage that led to a clade of three species. Interestingly, *L. aphanoides* belongs to one of the two clades of *Tripartite* that is characterized by glomerulate inflorescences, while the other three species belong to the second *Tripartite* clade that is characterized by loose inflorescences and pubescence in the inner part of the hypanthium; several hybridization events between species of these two clades within the *Tripartite* clade were also identified by Morales-Briones et al. (Chapter 1), and Notov and Kusnetzova (2004) found the distinction of these two groups rather ambiguous, likely due to the promiscuity of members of this clade with respect to interspecific hybridization.

Perry (1929) interpreted the *Orbiculate* clade (her series *Orbiculatae*), characterized by species with a stoloniferous habit and palmately lobed leaves, as most closely related to series *Aphanoides*, again probably due to the presence of numerous hybrid species between the *Tripartite* and *Orbiculate* clades. Here we find strong support for the hybrid origin of the *Orbiculate* clade, with genomic

contributions from taxa of the *Pinnate* and *Tripartite* clades (Fig. 2.6). Multiple regional treatments (e.g., Gaviria 1997) have proposed infrageneric groups within *Lachemilla* that do not reflect phylogenetic relationships, and often several hybrid species and/or species belonging to the *Orbiculate* clade are used as transitional states for these groups. Thus, it is significant that our analyses have clarified the role that hybridization has played in the morphological complexity of *Lachemilla*, and especially in future taxonomic treatments of the clade.

Multiple gene copies and evidence of whole genome duplication— All loci targeted in this study appear to be single-copy genes in *Fragaria* and across Rosaceae (Liston et al. 2014), however, our results show that more that 70% of these loci have multiple copies in *Lachemilla, Alchemilla*, and *Aphanes* (Appendix 7 and 8). A similar pattern of multi-copy genes recovered from exon capture data has been reported in *Artocarpus* (Moraceae; Johnson et al. 2016), which is known to have undergone at least one whole genome duplication (Gardner et al. 2016). This suggests that the pattern detected in *Lachemilla, Alchemilla*, and *Aphanes* might also be the result of a whole genome duplication that predates the diversification of the clade. Additionally, in a recent transcriptome-based phylogenomic analysis of Rosaceae, Xiang et al. (2017) identified multiple whole genome duplication events across the family, and more than 33% of genes used in their analysis showed evidence of duplications in the two species of *Alchemilla* sampled in their study. The precise phylogenetic position of this duplication remains unresolved until additional members of the subtribe Fragarinae are sampled, and statistical methods to detect whole genome duplications are applied (e.g., Jiao et al. 2011; Rabier et al. 2014; Huang et al. 2016; Tiley et al. 2016).

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CHAPTER 3: EXTENSIVE ALLOPOLYPLOIDY IN THE NEOTROPICAL GENUS *LACHEMILLA* (FOCKE) RYDB. REVEALED BY INCOMPLETE CONCERTED EVOLUTION OF THE NUCLEAR RIBOSOMAL DNA CISTRON AND PLASTID PHYLOGENOMICS.

Abstract

Polyploidy has been long recognized as an importance force in plant evolution. New allopolyploid species are produced by hybridization of two different species, inheriting the nuclear genome of both parental species and the plastid genome from the maternal lineage. Allopolyploid species can be identified when the multiple homeologs present in the genome are placed in different clades in the phylogeny that represent each of the parental lineages. Here we used PCR target enrichment in combination with high throughput sequencing to obtain multiple copies of the nuclear ribosomal nrDNA cistron and 45 regions of the plastid genome (cpDNA) from 219 samples of 48 species of the Neotropical genus *Lachemilla* (Focke) Rydb., and explore the allopolyploid origin of species of this group. We were able to identify multiple copies of nrDNA and establish clear evidence of the allopolyploid origin of 30 species of *Lachemilla*, showing that this condition is common and widespread in the genus. Additionally, based solely on the cpDNA phylogeny we identified that the monotypic genus *Faripnosis* Chrtek & Soják is the sister group of *Lachemilla* and allied genera within subtribe Fragariinae. However, this result is discordant with the nrDNA phylogeny, a pattern also observed between *Lachemilla* and allied genera, which corroborates the notion of widespread hybridization in Fragariinae.

Introduction

Polyploidy, the presence of more than one set of homologous chromosomes within an organism, is recognized as a key process in plant evolution (for some recent reviews see Moghe and Shiu 2014; Mason and Pires 2015; Soltis et al. 2016). All seed and flowering plants descend from repeated ancestral polyploidy events (Jiao et al. 2011), and between 40–70% of vascular plant species are estimated to be have a recent polyploidy origin (Otto and Whitton 2000). In allopolyploids, polyploid species are produced via hybridization between two different species. These hybrids inherit the complete nuclear genome of both parental species, while the organellar DNA is inherited only from the maternal parent. The use of low-copy nuclear gene sequences to reconstruct allopolypoid speciation allows for the direct reconstruction of phylogenetic relationships of both parental lineages of the hybrid species (Sang & Zhang 1999). In allopolyploids there are multiple homeologous loci of a specific gene, and each is expected to be sister to the homologous locus in the parental taxa, rather than to each other (Smedmark et al. 2003). Strong support for allopolyploid hybridization – both at shallow and deep time scales – is found when individual homeologs from polyploids are resolved within different strongly supported clades (Lo et al. 2010; Marcussen et al. 2012). This approach can be straightforward, and has been used to detect allopolyploid events and hybrid speciation in many groups of plants (e.g., Sang and Zhang 1999; Smedmark et al. 2003; Kim et al. 2008; Tank and Olmstead 2009; Marcussen et al. 2012; Estep et al. 2014; Brassac and Blattner 2015).

Despite their clear utility for reconstructing allopolyploidy in a phylogenetic context, there are some important limitations to using single- or low-copy nuclear

genes for these types of questions. First, nuclear genes often exist in gene families that are the result of complex evolutionary dynamics (e.g., gene duplication and deletion) that confound the ability of disentangle orthology and paralogy (Tank and Sang 2001; Sang 2002), and this may be especially problematic in groups where there is evidence of ancient whole genome duplications. Second, identifying and isolating sequences from these regions – either via cloning or bioinformatically – often requires additional steps that are not necessarily straightforward (Sang 2002; Small et al. 2004). Third, low-copy nuclear loci that have been developed for a specific taxonomic group are often not universally applicable across divergent lineages (Hughes 2006; Li et al. 2008; Zimmer and Wen 2012). Furthermore, to develop single- or low-copy nuclear genes for phylogenetic analyses, it is necessary to have some existing genomics resources, like an expressed sequence tag library (e.g., Fulton 2002), transcriptome sequences (e.g., Rothfels et al. 2013), or genomic sequence data (e.g., Han et al. 2014), which can be limited or absent for non-model organisms. Although data from reduced representation genomes (e.g., Uribe-Convers et al. 2016) or exon capture studies (e.g., Chapter 2) are useful for developing single- or low-copy nuclear genes in non-model organisms, common problems, including the failure of primers to amplify in some species, differences in intron presence and/or length, or differences in nucleotide substitution rates within and among genera (Hughes 2006), make this process difficult to accomplish on a large scale.

The nuclear ribosomal DNA (nrDNA) cistron, especially the internal transcribed spacer (ITS) and external transcribed spacer (ETS) regions have been widely used for lower-level phylogenetic reconstructions in flowering plants (reviewed in Baldwin et al. 1995; Álvarez and Wendel 2003; Soltis et al. 2008). This

widespread use likely corresponds to the high copy number of the cistron in the genome, making it relatively easy to sequence, as well as several genomic processes (e.g., concerted evolution) that result in a single nrDNA type among the thousands of tandemly repeated copies at a locus, making the cistron behave like a single-copy gene (Soltis et al. 2008; but see Álvarez and Wendel 2003). In allopolyploids, mechanisms of homogenization, like the preferential expression of one homeolog (e.g., Joly et al. 2004) or concerted evolution of copies from one parent to those from the other parent (e.g., Rauscher et al. 2004; Kovarik et al. 2005), can remove direct evidence of allopolyploidy from nrDNA ITS and ETS sequences (Soltis et al. 2008); if this homogenization acts towards the maternal genotype, incongruence between the chloroplast and nuclear phylogenies will be removed and the hybridization event will go undetected (Töpel et al. 2011). Nevertheless, homogenization of the nrDNA cistron can be incomplete (reviewed in Bailey et al. 2003), and both parental copies may be present in allopolyploid species, making this region still useful for detecting reticulate evolution in flowering plants (e.g. Sang et al. 1995, Rauscher et al. 200;, Rauscher et al. 2004; Wan et al. 2014; Zarrei et al. 2014; Kosachev et al. 2016; Xu et al. 2017).

Due to its small size, relatively slow substitution rates, and absence of paralogy issues (Clegg et al. 1994), the chloroplast genome (cpDNA) has also been widely used for phylogenetic reconstruction in plants, and has been particularly useful for resolving several recalcitrant relationships in flowering plants (e.g., Moore et al. 2007; Soltis et al. 2011; Xi 2012; Barret el al. 2014). Despite the fact that cpDNA only contains information from a single locus, it has been useful for resolving phylogenetic relationships among major clades and genera in Rosaceae, which is well known for its multiple rapid radiations and numerous reticulation

events, as well as allowing researchers to track the direction of gene flow during hybridization (e.g. Campbell et al. 2007; Dobeš and Paule 2010; Zang et al. 2017).

The genus *Lachemilla* (Focke) Rydb. in the Rosaceae is a morphologically highly variable group that includes perennial rosette-forming herbs, stoloniferous herbs, trailing herbs, procumbent herbs, subshrubs, and dwarf shrubs (Romoleroux 1996, 2004; Gaviria 1997). The genus comprises ca. 60 species that are distributed from northern Mexico to northern Argentina and Chile, between 2,200 m and 5,000 m in elevation (with one species occurring in the Dominican Republic; Romoleroux 1996, 2004; Gaviria 1997; Fig. 3.1). Previous phylogenetic analyses of *Lachemilla* have found clear evidence of reticulate evolution in the group. Morales-Briones et al. (Chapter 1) detected a widespread pattern of cytonuclear discordance due to hybridization and evidence of at least 24 potential hybrid species. Furthermore, it has been established that one of the four major clades of *Lachemilla* is of ancient hybrid origin, and there is evidence of a whole genome duplication event involving the entire genus, as well as several allied genera (Chapter 2). Based on chromosome numbers and flow cytometry estimations of genome size, it has also been established that polyploidy is common in the group, ranging from diploid species like *L. mandoniana* ($2n = 16$) to docecaploid species like *L. jaramilloi* ($2n = 96$), but there is also an apparent disconnect between genome size and ploidy level within species (Chapter 1). Although the utility of detecting reticulate speciation based on incongruence between nuclear and plastid phylogenies in *Lachemilla* has been demonstrated (Chapter 1), direct evidence of phylogenetic relationships of parental lineages of allopolyploid species is still needed.

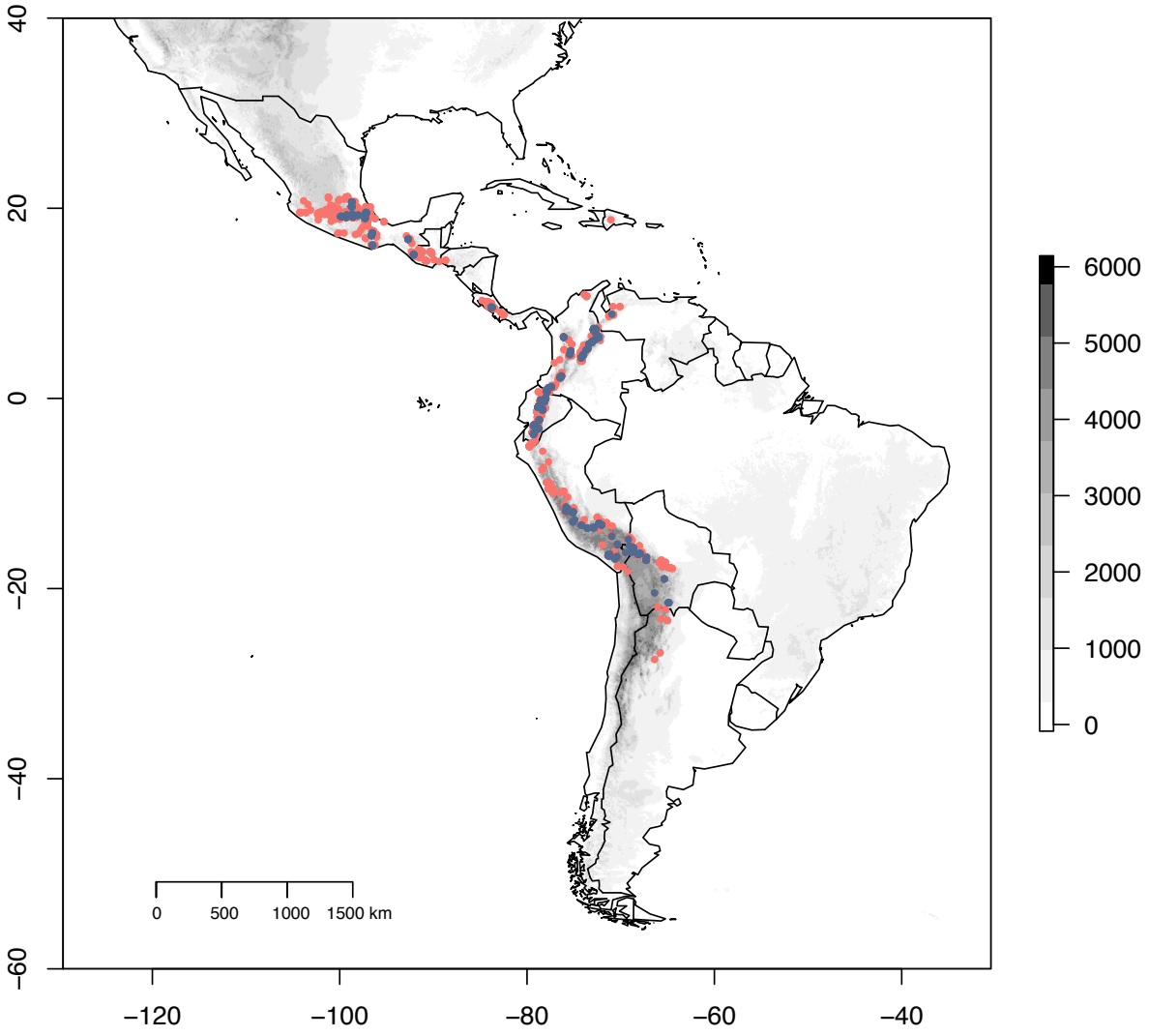

Figure 3.1. Distribution and sampling of *Lachemilla* in the Neotropics. Red dots represent the distribution of *Lachemilla* in the Neotropics based on GBIF (2017) occurrences, and blue dots represent the sampling of *Lachemilla* used in this study.

Lachemilla is most often treated as part of subtribe Alchemillinae, along with *Alchemilla* and *Aphanes* within tribe Potentilleae (Rothmaler 1937; Notov and Kusnetzova 2004; Soják 2008). Molecular phylogenetic studies of Alchemillinae (Gehrke et al. 2008, 2016) have shown that the subtribe is comprised of four clades, *Aphanes*, *Lachemilla,* and two distinct clades of the paraphyletic *Alchemilla* (Eurasian and African). It has been suggested that these four groups be treated within subtribe Fragariinae as the single genus *Alchemilla* (see also Eriksson et al. 2003;

Potter et al. 2007), but this convention has not been widely adopted, primarily due to the lack of agreement between various phylogenetic studies. For example, within Alchemillinae, Gehrke et al. (2008) found major cytonuclear discordance regarding the relationships among the four major clades. Likewise, similar patterns, often attributed to hybridization and allopolyploidy, have been detected among most genera in Fragariinae and Potentillinae (Lundberg 2009; Töpel et al. 2011; Eriksson et al. 2015; Gehrke et al. 2016; Feng 2017), and phylogenetic relationships between Alchemillinae and the members of Fragariinae are still largely unresolved.

Recently developed phylogenomic approaches based on PCR target enrichment in combination with high throughput sequencing (Uribe-Convers et al. 2016), have been shown to be an ideal and cost-effective method to sequence multiple chloroplast and nuclear regions simultaneously from hundreds of samples of a specific group. Moreover, in the case of the nuclear loci, allelic information can be obtained directly from the pool of PCR products without the necessity of cloning, which is essential for the study of plant groups with the presence of allopolyploidy. Additionally, this approach and group-specific PCR primers have been successfully transferred to work across entire families (e.g., Latvis et al. in press) and orders (e.g., Collins et al. 2016).

In the present study we aim to estimate the phylogeny of *Lachemilla* using a taxonomically and geographically comprehensive sampling, in combination with PCR-based target enrichment of the chloroplast genome and the nrDNA ETS and ITS regions to, (i) identify allopolyploid species using the allelic information of the nuclear ribosomal repeat in combination with the chloroplast phylogeny, and (ii) investigate the relationship of subtribe Alchemillinae and its relatives to subtribe Fragariinae.

Material and Methods

Taxon Sampling— We sampled 219 individuals of 48 species of *Lachemilla*, representing 80% of the total diversity of the group. The sampling represents the complete morphological variation and geographic range of widespread species (Fig. 3.1), as well as the four major groups of *Lachemilla* proposed by Morales-Briones et al. (Chapter 1 and Chapter 2). Additionally, we sampled 34 individuals of *Alchemilla* (Eualchemilla and Afromilla) and *Aphanes*, several genera representing subtribe Fragariinae, and one species of *Potentilla* as an outgroup. The complete list of species and voucher information is provided in Appendix 17.

Chloroplast primer design and validation— Complete chloroplast sequences (excluding one inverted repeat) for 27 species of *Lachemilla,* two of *Alchemilla*, and one of *Aphanes* generated in Chapter 2 were aligned with MAFFT v7.037b (Katoh and Standley 2013) using the automatic alignment strategy. We identified the most variable regions of the alignment using an R script from Uribe-Convers et al. (2016). These regions spanned between 400 bp and 1000 bp, and were flanked by conserved regions appropriate for primer design. Primer design and validation followed Uribe-Convers et al. (2016) and the Access Array System protocol (Fluidigm, San Francisco, CA, USA). Briefly, we used Primer3 (Untergasser et al. 2012) to design primer pairs with an annealing temperature of 60°C (+/- 1°C) for all primers, and no more than three continuous nucleotides of the same base were allowed (Max Poly-X=3). A conserved sequence (CS) tag was added to the 5'end of the forward and reverse primers to provide an annealing site for the Illumina sequencing adapters and sample-specific barcodes.

Validation reactions were carried out on two species of *Lachemilla* (*L. pinnata* and *L. polylepis*), *Aphanes cotopaxiensis*, and a negative control. Validation reactions simulate the four-primer reaction of the Fluidigm microfluidic PCR system using a standard thermocycler. Reactions were carried out in a final volume of 10 μL with final concentrations of 1X FastStart High Fidelity Reaction Buffer without MgCl2 (Roche), 4.5 mM MgCl2 (Roche), 5% DMSO (Roche), 200 μM ea PCR Grade Nucleotide Mix (Roche), 0.05 U/μL, FastStart High Fidelity Enzyme Blend (Roche), 1× Access Array Loading Reagent (Fluidigm), 400 nM Access Array Barcode Primers for Illumina, 30-70 ng/μL genomic DNA, 50 mM ea of target specific primer and PCR Certified Water (Teknova, Hollister, California, USA) to volume. Amplification conditions consisted of 50ºC for 2 minutes, 70ºC for 20 minutes, 95ºC for 10 minutes, ten cycles of 95ºC 15 for seconds, 60ºC for 30 seconds, and 72ºC for 1 minute, two cycles of 95ºC for 15 seconds, 80ºC for 30 seconds, 60ºC for 30 seconds, and 72ºC for 1 minute, eight cycles of 95ºC for 15 seconds, 60ºC for 30 seconds, and 72º for 1 minute, two cycles of 95ºC for 15 seconds, 80ºC for 30 seconds, 60ºC for 30 seconds, and 72ºC for 1 minute, eight cycles of 95ºC for 15 seconds, 60ºC for 30 seconds, and 72ºC for 1 minute and 5 cycles of 95ºC for 15 seconds, 80ºC for 30 seconds, 60ºC for 30 seconds, and 72ºC for 1 minute. Amplicons from these reactions were analyzed in a QIAxcel Advance System (Qiagen, Valencia, California, USA), and primer pairs that produced a single amplicon and had no (or minimal) primer dimers were selected.

Nuclear ribosomal ETS and ITS primer design and validation— The nuclear ribosomal repeat (18S-ITS1-5.8S-ITS2-26S) was assemble from raw reads of the exon capture data of 27 species of *Lachemilla*, two of *Alchemilla*, and one of *Aphanes* from

Chapter 2, using Bowtie2 (Langmead and Salzberg 2012) and a combination of references from Rosaceae that were obtained from GenBank (*Sanguisorba sitchensis* JF317394 and JF317375, *Lachemilla vulcanica* HM453917, and *Alchemilla mollis* FJ422345). Contigs were then imported into Geneious R7 v.7.0.6 (Kearse et al. 2012) and a consensus sequence was obtained by calling regions with less than 5x coverage as 'N' and using the 'Highest Quality' as a threshold. Primers for the ETS, ITS1, and ITS2 regions were designed and validated as in the chloroplast primer design.

DNA Extraction, amplification and sequencing— Total genomic DNA was isolated from fresh, silica-dried, or herbarium material using the DNeasy Plant Mini Kit (Qiagen, Valencia, California) following the manufacturer's protocol, or with a modified 2 × CTAB method (Doyle and Doyle 1987). Microfluidic PCRs were carried out either in an Access Array or Juno system (Fluidigm) following the manufacturer's protocols. To remove unused reagents and/or undetected primer dimers smaller than 350 bp, each pool was purified with 0.6X AMPure XP beads (AgencourtT, Beverly, Massachusetts, USA). PCR pools were analyzed in a Bioanalyzer High-Sensitivity Chip (Agilent Technologies, Santa Clara, California, USA), and standardized to 13 pM using the KAPA qPCR kit (KK4835; Kapa Biosystems, Woburn, Massachusetts, USA) on an ABI StepOnePlus Real-Time PCR System (Life Technologies, Grand Island, New York, USA). The resulting pools were multiplexed and sequenced in an Illumina MiSeq with 300 bp paired-end reads. Microfluidic PCR, downstream quality control and assurance, and sequencing were carried out at the University of Idaho Institute for Bioinformatics and Evolutionary Studies Genomics Resources Core facility.

***Data processing*—** All Raw reads were cleaned and demultiplexed by barcode and target-specific primer using dbcAmplicons (https://github.com/msettles/dbcAmplicons; Uribe-Convers et al. 2016) and merged using FLASH (Magoč and Salzberg, 2011). For the chloroplast regions, consensus sequences for each sample in all amplicons were generated using the reduce_amplicons R script (https://github.com/msettles/dbcAmplicons/blob/master/scripts/R/reduce_amplicons.R; Uribe-Convers et al. 2016). To investigate the potential for multiple nrDNA copies, merged reads from the nrDNA amplicons were processed with PURC (https://bitbucket.org/crothfels/purc; Rothfels et al., 2017). PURC uses an iterative read clustering/consensus approach using USEARCH (Edgar 2010), and identifies PCR recombinants with UCHIME (Edgar et al 2011), to produce a final alignment of sequence clusters that represent potential alleles or paralogs identified in each sample. We used 0.997, 0.995, 0.990, and 0.997 as cluster thresholds in each of the four clustering iterations, respectively, and four as the minimum number of sequences/cluster necessary for a cluster to be retained.

Chloroplast phylogenetic analyses —Each chloroplast region was aligned with MAFFT using the automatic alignment strategy, and concatenated into a single alignment. Maximum likelihood (ML) analyses were conducted on the concatenated matrix using RAxML v8.0.3 (Stamatakis 2014) with a 'partition by locus' scheme selected using PartitionFinder V 2.1.1 (Lanfear et al. 2017). All partitions used the GTR + G model, 100 searches for the best tree were performed, and clade support was assessed with 1,000 non-parametric bootstrap (BS) replicates. Bayesian analyses were performed with MrBayes v3.2.6 (Ronquist et al. 2012) on the

CIPRES portal (Miller et al. 2010). Analyses consisted of two independent runs with four Markov Chain Monte Carlo (MCMC) chains for 20 million generations with trees sampled every 20,000th generation. The concatenated dataset also ran using a 'partition by locus' scheme, and to allow sampling across the entire substitution rate model space for each partition, reversible-jump Markov Chain Monte Carlo (rjMCMC) was employed (nst = mixed). Convergence of parameter estimates for the two independent MCMC runs was assessed using Tracer v1.6 (Rambaut et al. 2014). A 50% majority rule consensus tree was generated and posterior probabilities (PP) were calculated after removing the first 10% of sampled trees.

Nuclear phylogenetic analyses —Final selection of ribotypes for each sample was done based on cluster size and visual inspection of the alignment of all clusters produced by PURC, as well as expected number of alleles based in ploidy levels and hybridization patterns previously reported for *Lachemilla* (Chapter 1). Approximate ML trees for each alignment (ITS1, ITS2, and ETS) were inferred using FastTree2 (Price et al. 2010) with the –slow, –gtr, and –gamma options. When multiple ribotype for a sample formed a monophyletic group, only the ribotype with the largest cluster was retained. Because the nrDNA cistron behaves as a single locus, concatentation of the separate ITS1, ITS2, and ETS alignments is appropriate, and desirable. However, given the presence of multiple nrDNA copies in many accessions, it is first necessary to assess the orthology of these copies with respect to each region sequenced. To do this, the approximate ML trees from FastTree (Appendix 18) were used as a guide to track the position of each copy from each gene region based on its relative position with other species, and the their placement among the four main clades of the *Lachemilla*. For example, in the ITS1

tree *L. jaramilloi*_2012_006_EC_A was resolved in a clade with *L. uniflora*_2012_363_EC and *L. uniflora*_2012_385_EC_B. Likewise, in the ITS2 and ETS trees, this accession has a copy that is resolved in a clade with these same *L. uniflora* accessions. Therefore, these three sequences from this accession of *L. jaramilloi* were identified as belonging to the same nrDNA ribotype, and were concatenated. As with the chloroplast data, final phylogenetic analyses of the nrDNA data were conducted on the concatenated matrix.

***ITS2 Secondary structure analyses*—** To investigate the presence of pseudogenes in the nrDNA region, we used the predicted secondary structure of the ITS2 region as an approximation. Sequences were annotated and secondary structure predicted using a Hidden Markov model-based method (Keller et al. 2009) and the ITS2 Database III (Koetschan et al. 2012). We investigated the presence of the four common helix structures characterized for Rosaceae (Mai and Coleman, 1997), and the three major motifs (UGGU, triple-A, and the U–U mismatch motifs) characterized for plants (Schultz et al. 2005). Secondary structures of the ITS2 were plotted using PseudoViewer v.3 (Han et al. 2002).

Results

***Primer design and validation*—** For the chloroplast genome we designed 82 pairs of primers, from which 66 pairs where successfully validated, and 48 chosen for final amplification and sequencing in all samples (Appendix 18). For the nrDNA one primer pair for each spacer region (ETS, ITS1 and ITS2) was designed and successfully validated (Appendix 18).

nrDNA amplification and ribotype calling— The cluster-based sequence processing resulted from 1 to 56 unique clusters per sample (Appendix 20), with small clusters being the most numerous. Although cluster size and number can vary significantly based on the clustering thresholds used in PURC (Rothfels et al. 2017), we noticed a clear pattern that species previously reported as putative hybrids (Chapter 1) tended to have several large clusters, while non-hybrid species had a single large cluster that included the large majority of reads. After final selection of ribotypes (or nrDNA copies), we found that of the 212 total samples of *Lachemilla* used here, 143 showed the presence of only one, 65 had two ribotypes, and four samples had three copies (a total of 285 sequences, where the same species can have variable number of copies; Table 3.1).

Phylogenetic analyses: cpDNA— The final chloroplast concatenated alignment included 212 samples with a length of 22,017 bp. The ML and BI analyses recovered the same overall topology (Figs. 3.2, 3.3). *Lachemilla* is recovered as monophyletic (PP=1.00, BS=100) with Afromilla (PP=1.00, BS=100) sister to *Lachemilla* (PP=1.00, BS=100), and a clade (PP=1.00, BS=100) formed by *Aphanes* (PP=1.00, BS=100) and Eualchemilla (PP=1.00, BS=100) as the sister group to the former two. All members of Alchemillinae comprise a monophyletic group (PP=1.00, BS=100) with *Farinopsis salesoviana* as the sister group of Alchemillinae (PP=1.00, BS=94), and two additional members of Fragariinae as successive sister groups, *Comarum palustre* (PP=1.00, BS=100) and *Sibbaldia procumbens* (PP=1.00, BS=100). The remaining species of Fragariinae, *Dasiphora, Drymocallis, Chamaerhodos,* and *Fragaria* form a clade (PP=0.99, BS=58). Within *Lachemilla* we recovered the four major clades of the group, where the *Tripartite* clade (PP=1.00, BS=98) and *Orbiculate* clade (PP=1.00,

BS=100) form a clade (PP=1.00, BS=100), and the *Verticillate* clade (PP=1.00, BS=100) and *Pinnate* clade (PP=1.00, BS=100) are successive sister groups with strong support.

To describe each major clade and make comparisons between the nrDNA and cpDNA trees, it was necessary to divide the major clades into several subclades based on species composition and morphology. The *Tripartite* clade is composed of eight subclades. The first subclade, 'Glomerulatae I' (PP=1.00, BS=93), is composed of species with tripartite leaves and glomerulate inflorescence (*L. sibbaldiifolia*, *L. moritziana*, *L. aphanoides*, and *L. pringlei*), and the hybrid *L. pseudovenusta.* The subclade 'Glomerulatae II' (PP=1.00, BS=93) includes species with characteristics similar to 'Glomerulatae I' (*L. velutina* and *L. aphanoides*), in addition to the hybrid species *L. ranunculoides* and *L. bipinnatifida.* The next subclade, 'Procumbentes I' (PP=1.00, BS=100), is comprised of species with tripartite leaves, a lax inflorescence, and pubescence in the inner wall of the hypanthium (*L. vulcanica*, *L. procumbens*, *L. hirta*, and *L. andina*). The 'Procumbentes II' subclade (PP=1.00, BS=100) is similar to 'Procumbentes I,' and includes *L. vulcanica*, *L. jamesonii,* and *L. andina.* The 'Glomerulatae III' subclade (PP=1.00, BS=100) includes only samples of *L. aphanoides.* The next three subclades included in the *Tripartite* clade are composed of species that morphologically seem to have more affinity to other major clades. The 'Polylepis' subclade (PP=1.00, BS=99), comprising only samples of the uniquely woody species of the genus, *L. polylepis.* Subclade 'A' (PP=1.00, BS=98) is composed by species with verticillate leaves (*L. talamanquensis*, *L. verticillata,* and *L. hispidula*), and subclade 'B' (PP=1.00, BS=99) includes *L. repens* and *L. williamsii* with pinnate leaves, and *L. lechleriana* with orbiculate 5—7-cleft leaves.

The *Orbiculate* clade (PP=1.00, BS=100) includes the subclade 'Orbiculata' (PP=1.00, BS=100) comprised only by *L. orbiculata,* the subclade 'Pectinata' (PP=1.00, BS=100), which includes *L. pectinata* and the hybrid species *L. perryana*, *L. fulvescens*, and *L. venusta*, and *L. mexiquense*, which has tripartite leaves, as the sister species to the rest of the clade.

The *Verticillate* clade does not have much structure within the clade, but some patterns can be highlighted. The subclade 'Nivales' (PP=1.00, BS=64) includes mostly species with verticillate leaves along the entire stem (*L. hispidula*, *L. galioides*, *L. nivalis*); only *L. holmgrenii* has cleft leaves on the lower part of the stem and verticillate leaves on the upper part. All samples of the hybrid species *L. rupestris* were found to be monophyletic (subclade 'Rupestris'; PP=1.00, BS=87). Primarily to facilitate comparison to the nrDNA tree, we have designated a grade of several hybrid species characterized by having cleft leaves on the lower part of the stem and verticillate leaves above, similar to *L. holmgrenii,* as 'Subnivales' (*L. sprucei*, *L. purdiei*, *L. adscendens, L. holosericea*). Finally, the hybrid species *L. uniflora* is found as the sister group to all remaining species in the *Verticillate* clade.

The last major clade, the *Pinnate* clade, is composed mainly of highly supported (i.e., PP = 1.00 and BS ≥ 80) monophyletic species clades, and is the only clade where the relationships among the species are completely resolved (i.e., PP = 1.00 and BS ≥ 88). *Lachemilla pinnata* is sister to *L. mandoniana*, and these two species are sister to a clade composed of *L. erodiifolia* and *L. diplophylla*, with *L. tanacetifolia* sister to these four species. The only clade containing non-monophyletic species is the 'Frigida' clade, which is composed of two distinct clades of *L. frigida* and the two hybrid species *L. jaramilloi* and *L. sarmentosa*. This clade is the sister group of the rest of species in the *Pinnate* clade.

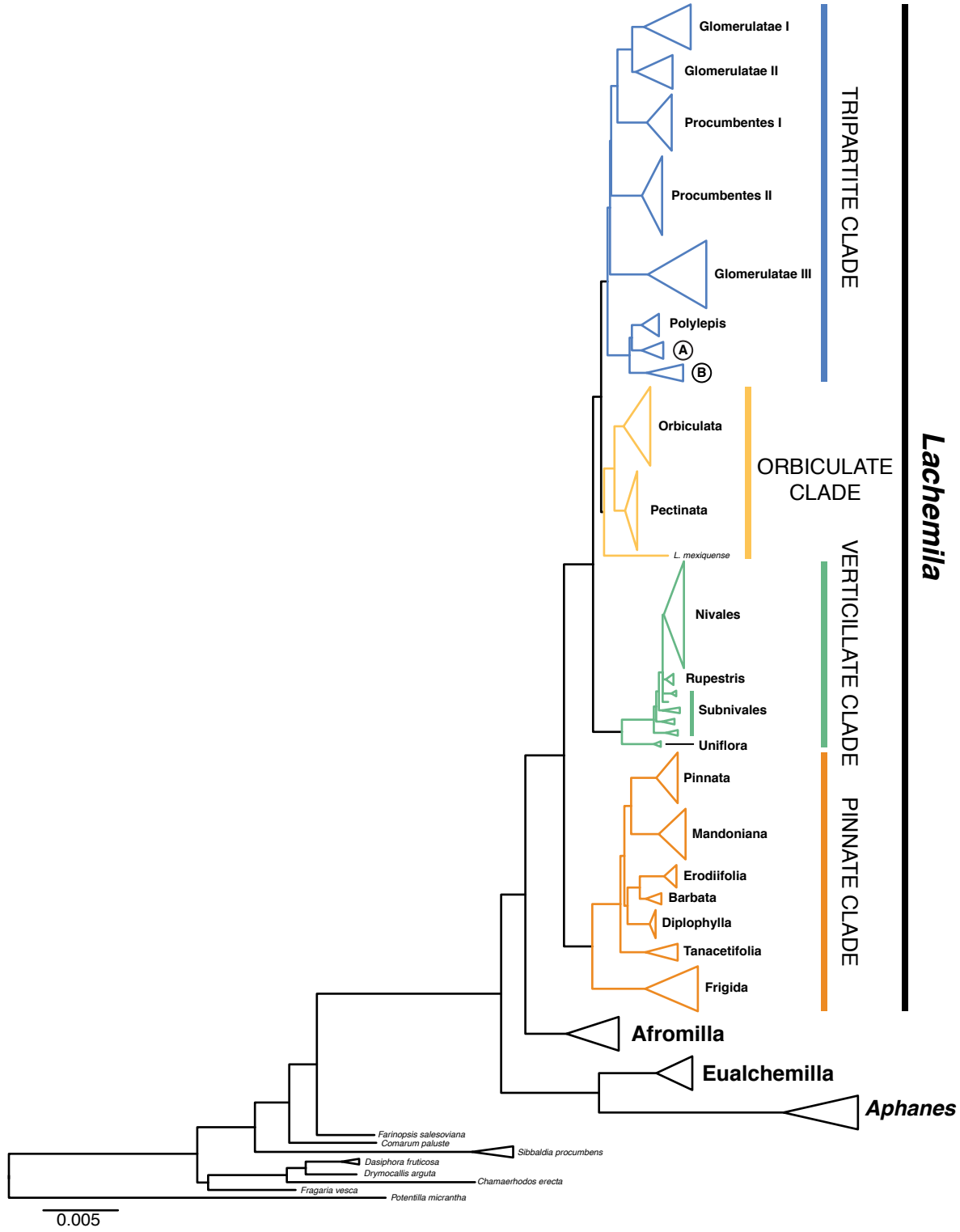

Figure 3.2. Bayesian 50% majority rule consensus tree of the cpDNA dataset. Clades discussed in the main text are collapsed, and branch lengths are shown proportional to the scale bar (substitutions/site).

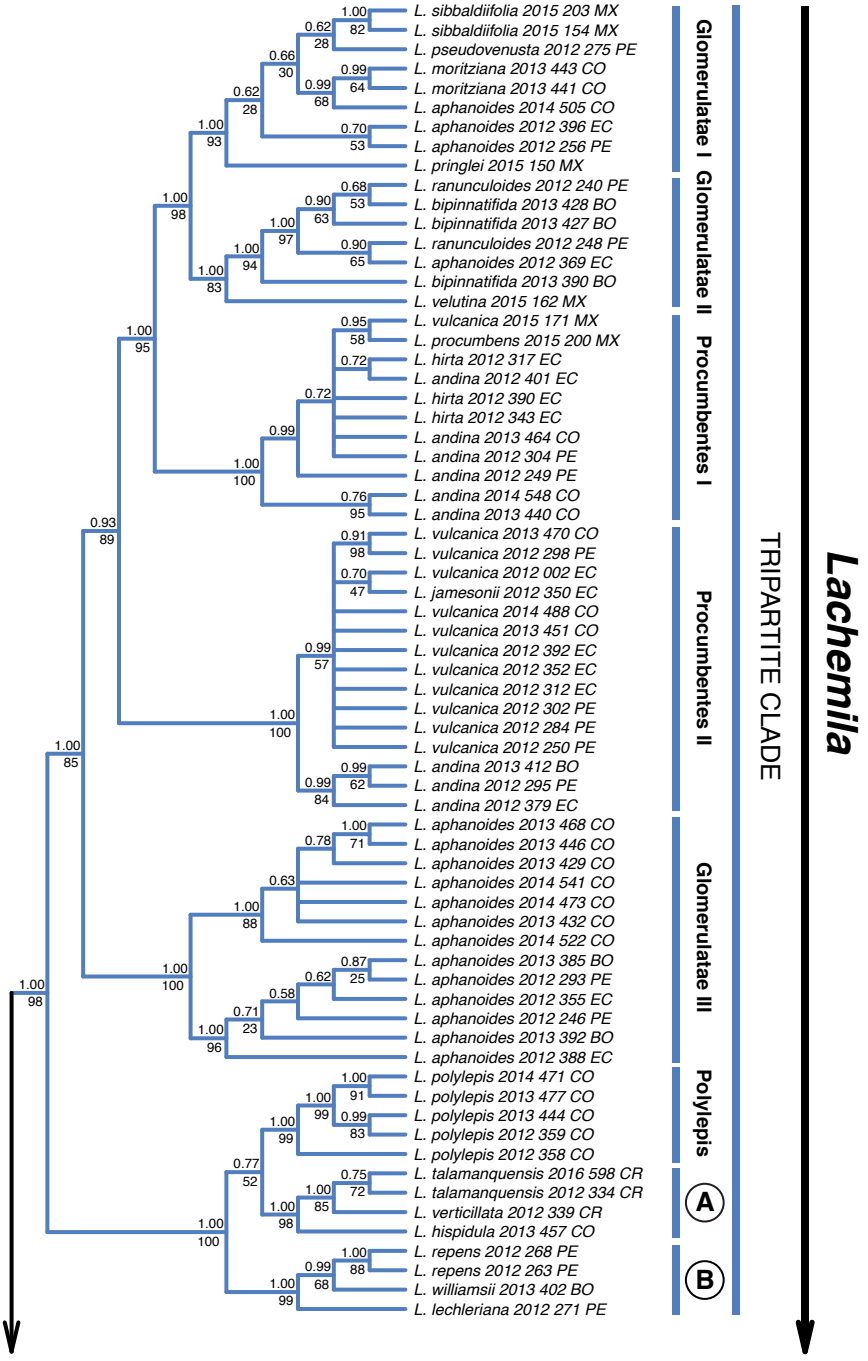

Figure 3.3. Expanded Bayesian 50% majority rule consensus tree of the cpDNA dataset showing all samples. Posterior probabilities and maximum likelihood bootstrap support values are shown above and below the branches, respectively. Figure continues in next page.

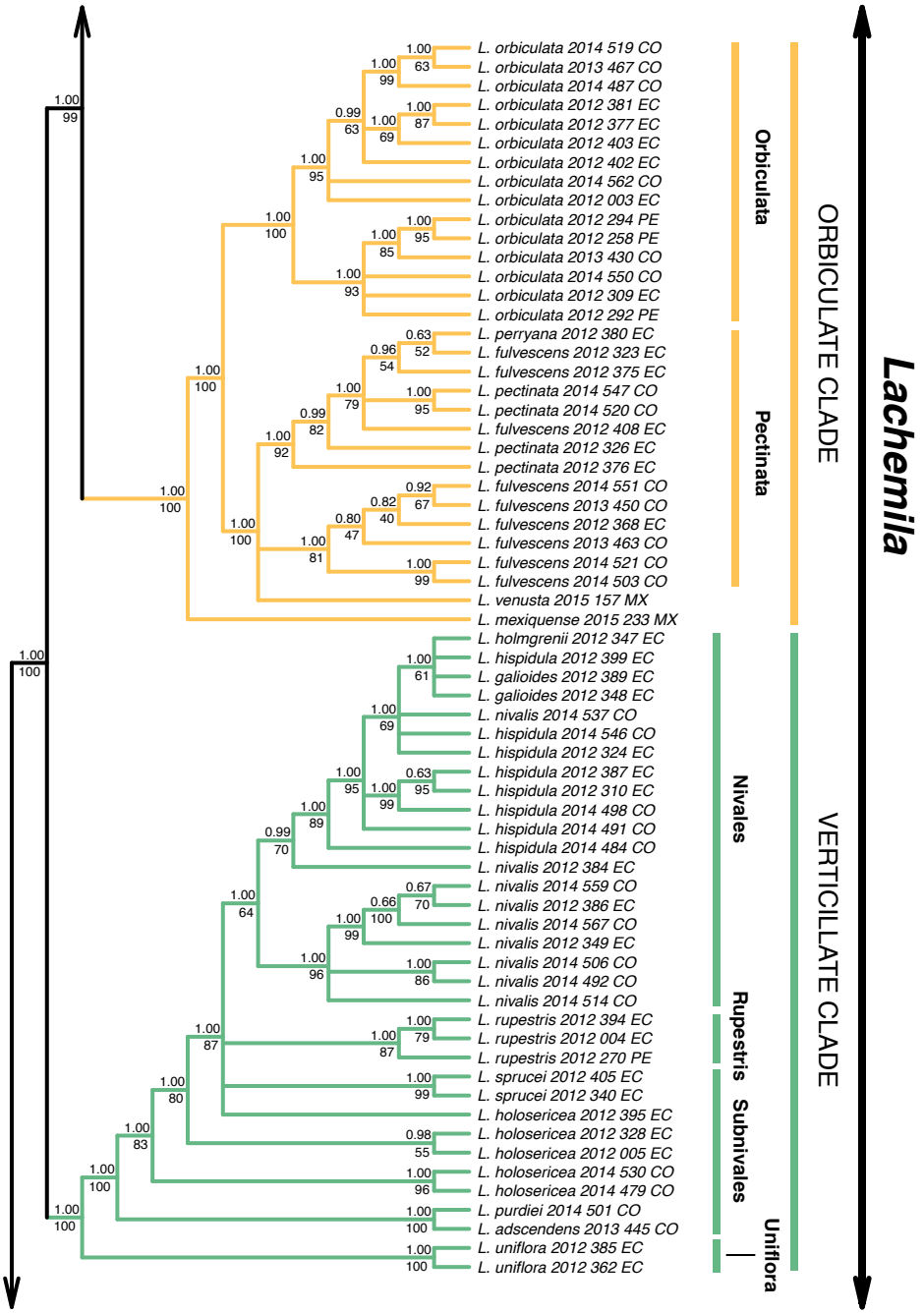

Fig. 3.3 (Continued). Expanded Bayesian 50% majority rule consensus tree of the cpDNA dataset showing all samples. Posterior probabilities and maximum likelihood bootstrap support values are shown above and below the branches, respectively. Figure continues in next page.

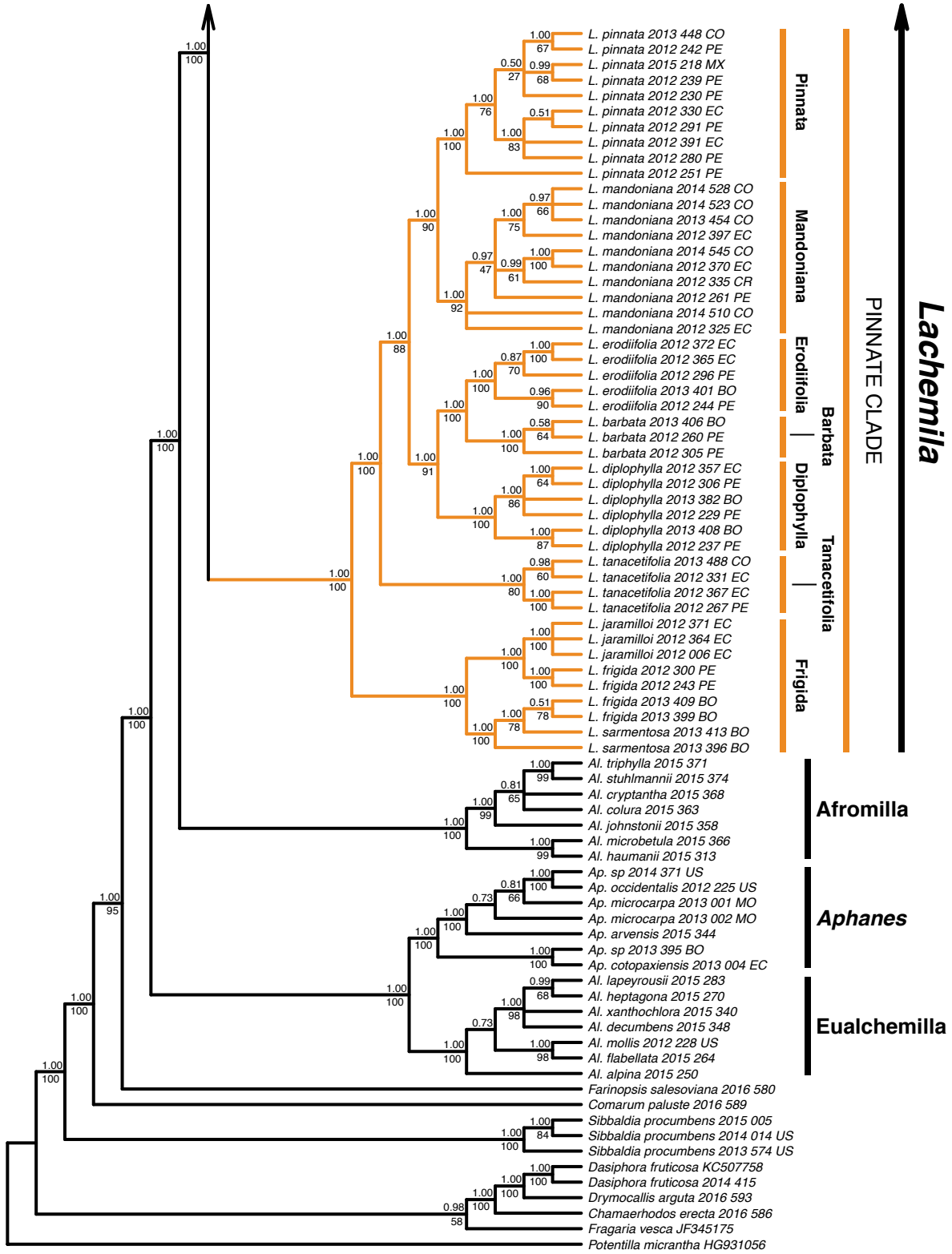

Fig. 3.3 (Continued). Expanded Bayesian 50% majority rule consensus tree of the cpDNA dataset showing all samples. Posterior probabilities and maximum likelihood bootstrap support values are shown above and below the branches, respectively.

***Phylogenetic analyses: nrDNA*—**The final nrDNA matrix included 353 sequences with a length of 1057 bp, and as with the cpDNA tree, the ML and BI analyses recovered the same overall topology (Figs. 3.4, 3.5). *Lachemilla* is recovered as monophyletic (PP=1.00, BS=98), with a well-supported clade (PP=1.00, BS=1.00) formed by *Aphanes* (PP=1.00, BS=100), Eualchemilla I (PP=1.00, BS=99), and Eualchemilla II (PP=1.00, BS=100) sister to *Lachemilla*. All members of Alchemillinae form a clade (PP=1.00, BS=100) that is sister (PP=1.00, BS=95) to a weakly supported clade (PP=0.65, BS=44) formed by the clade (PP=1.00, BS=90) of *Sibbaldianthe, Sibbaldiopsis, Sibbaldia,* and the weakly supported clade (PP=0.78, BS=55) of *Farinopsis salesoviana* and *Comarum palustre.* The remaining Fragariinae genera form an additional weakly supported clade (PP=0.79, BS=59). Within *Lachemilla* we recovered the same the four major clades, but relationships among them are not strongly supported.

Following the cpDNA tree, we again subdivided each major clade into several subclades based on species composition and morphology, using the same subclade names for similar clades between the two trees. The *Tripartite* clade (PP=100, BS=100) is composed of six subclades. We identified the 'Procumbentes I' and 'Procumbentes II' subclades that have similar species and specimen composition as in the cpDNA tree. Most species have two nrDNA copies distributed between the two 'Procumbentes' clades; importantly, both copies are never found in the same clade. We also recovered the 'Glomerulatae I' (PP=100, BS=95) and 'Glomerulatae II' (PP=100, BS=98) clades that comprise mostly one of two copies of the same species and samples of these subclades in the cpDNA tree, including one of the two copies of the hybrid species *L. bipinnatifida* and *L. ranunculoides* in the 'Glomerulatae II' clade. All of the second ribotypes from

accessions in these two subclades are found in the 'Glomerulatae III' clade, which also includes all of the same samples of *L. aphanoides* found in its chloroplast counterpart. Some of these *L. aphanoides* samples also have a second nrDNA copy in the 'Glomerulatae II' clade. In addition, the 'Glomerulatae III' clade includes one copy from the hybrid species *L. fulvescens, L. pseudovenusta, L. perryana, L. purdiei,* and *L. venusta*, and a unique copy of *L. standleyi* and *L. steinbachii* that are not present in the cpDNA tree. The former species is morphologically similar to *L. aphanoides* (tripartite leaves and glomerulate inflorescence; Perry 1929), while the latter has been identified has a potential hybrid related to the *Orbiculate* clade, based on its orbiculate basal leaves and glomerulate inflorescence (Morales-Briones pers. obs). As in the cpDNA tree, subclade 'A' was recovered, including *L. talamanquensis* and *L. verticillata,* but not *L. hispidula*. Finally, *L. mexiquense* was not part of any of the subclades, but rather on a lone branch, this time placed sister to a large part of the *Tripartite* clade.

The *Pinnate* clade includes highly supported clades (i.e. PP = 1.00 and BS ≥ 98) of the same seven species as in the cpDNA tree (*L. pinnata, L. tanacetifolia, L. mandoniana, L. barbata, L. erodiifolia, L. frigida,* and *L. diplophylla*), but in the case of *L. pinnata*, *L. mandoniana*, *L. tanacetifolia*, and *L. frigida,* these species clades include nrDNA copies of hybrid species. The 'Pinnata' subclade includes *L. bipinnatifida,* the 'Tanacetifolia' subclade includes *L. rupestris* and *L. lechleriana*, the 'Mandoniana' subclade includes *L. jaramilloi*, *L. galioides*, and *L. holmgrenii*, and the 'Frigida' subclade includes *L. ranunculoides, L. jaramilloi, L. bipinnatifida,* and *L. sarmentosa.* Also within the *Pinnate* clade we identified subclade 'B' with a similar composition to subclade 'B' from the *Tripartite* clade in the cpDNA tree. An identical subclade 'B' can also be found in the *Verticillate* clade. The novel subclade 'C' comprising *L.*

uniflora and additional nrDNA copies of *L. jaramilloi* was also recovered. Within the *Pinnate* clade, the species relationships also are different than in the cpDNA, with varying levels of support.

As in the cpDNA tree, the *Orbiculate* clade is again divided into the 'Pectinata' and 'Orbiculata' subclades. The former includes *L. pectinata*, and one copy of the hybrids *L. sprucei, L. fulvescens, L. rupestris, L. venusta, L. sarmentosa, L. pseudovenusta, L. perryana,* and *L. lechleriana*, and the latter includes *L. orbiculata*, two samples of *L. pectinata*, and one sample of *L. rupestris.*

The *Verticillate* clade, as in the cpDNA tree, does not have good internal resolution. The same species found in the 'Nivales' and 'Subnivales' subclades of the cpDNA tree are found here, in addition to *L. jaramilloi*, *L. polylepis*, one of the two 'B' subclades, and *L. llanganatensis* and *L. equisetiformis* that were not included in the cpDNA tree, but are morphologically similar to 'Nivales' species with verticillate leaves throughout the stem. Finally, a clade of several samples of *L. holosericea* is sister group of the rest of species in the *Verticillate* clade, rather than *L. uniflora* as in the cpDNA tree, which is placed along with *L. nivalis* here.

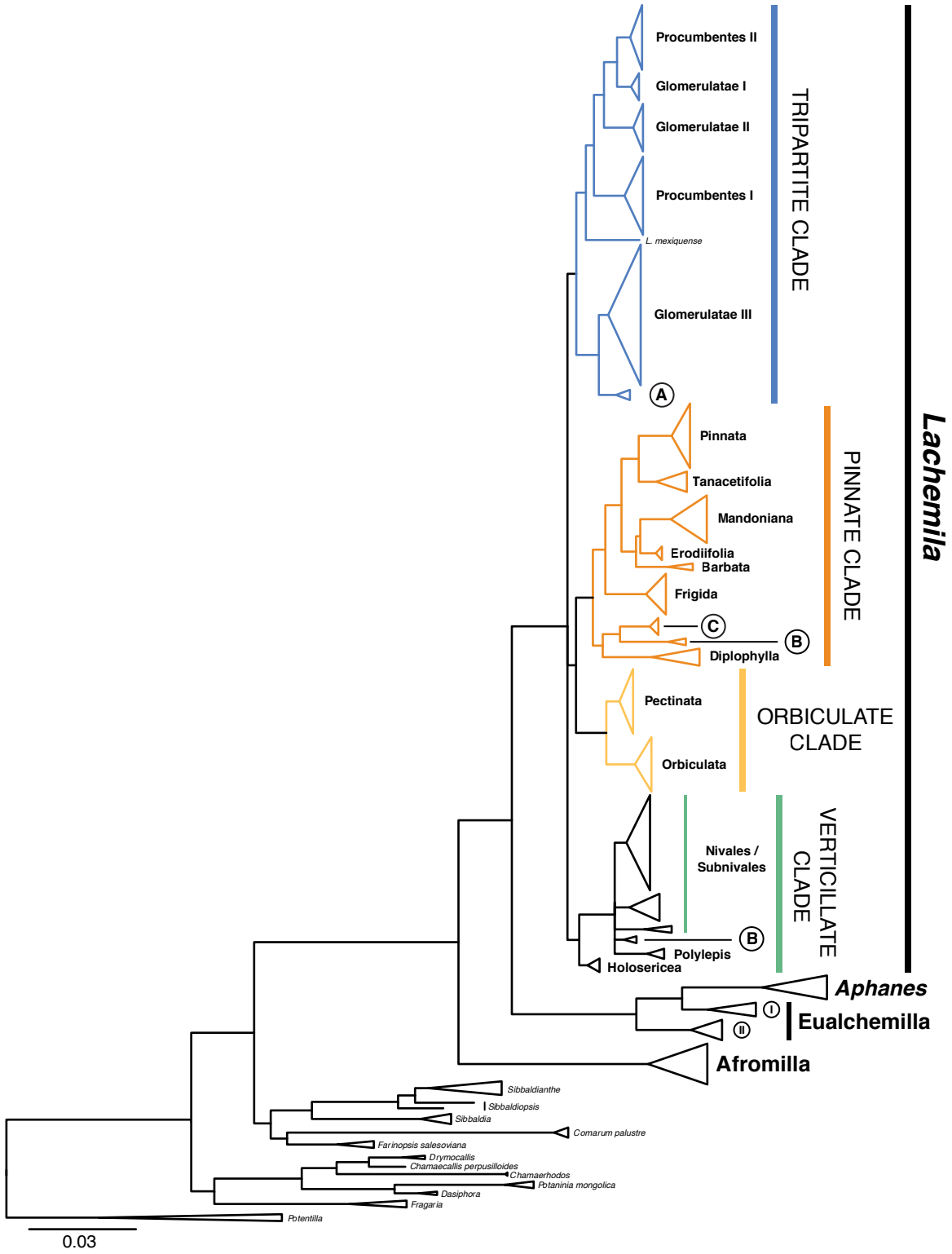

Figure 3.4. Bayesian 50% majority rule consensus tree of the nrDNA dataset. Clades discussed in the main text are collapsed and branch lengths are shown proportional to the scale bar (substitutions / site).

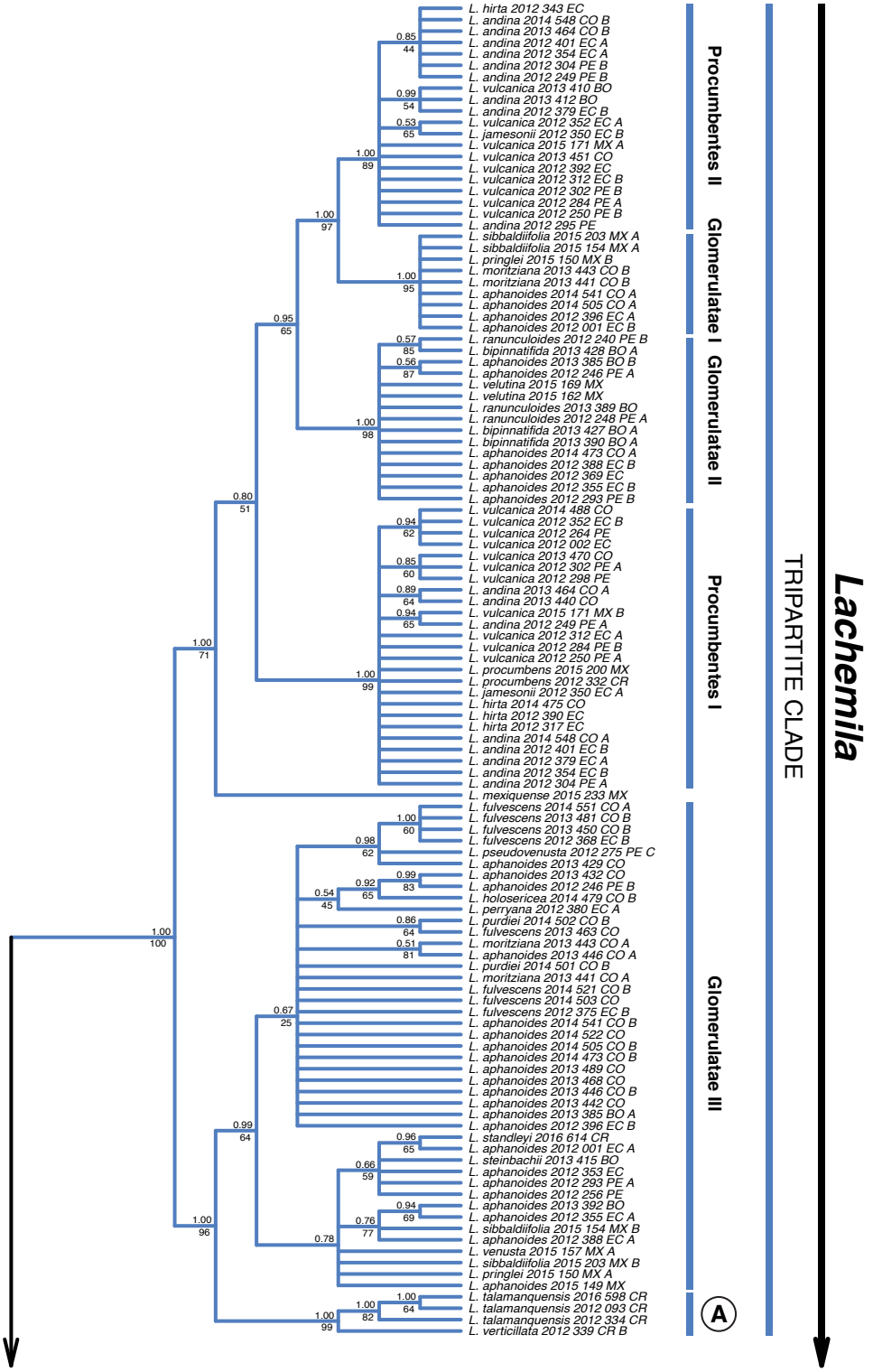

Figure 3.5. Expanded Bayesian 50% majority rule consensus tree of the nrDNA dataset. showing all samples. Posterior probabilities and maximum likelihood bootstrap support values are shown above and below the branches, respectively. Figure continues in next page.

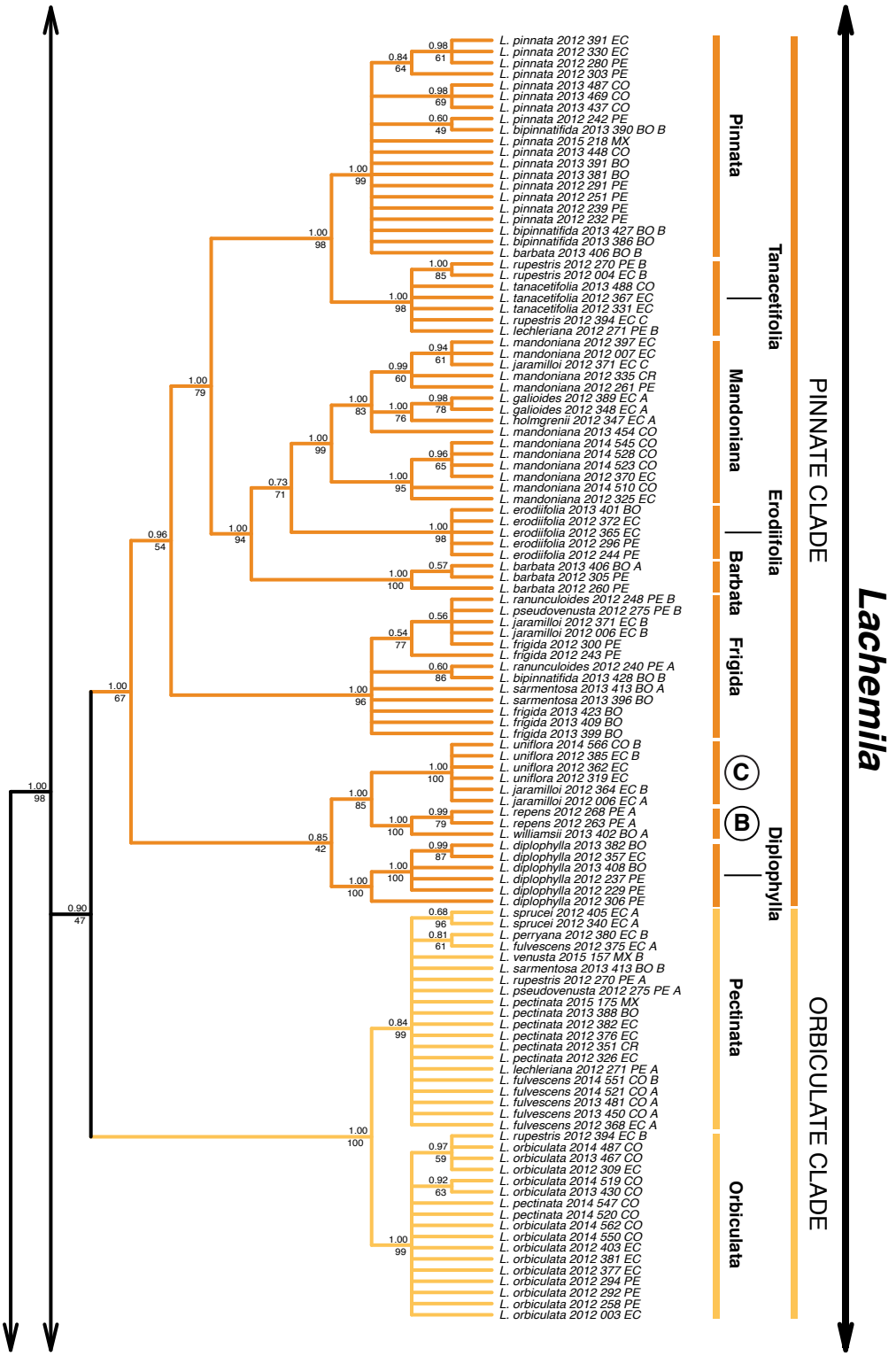

Fig. 3.5 (Continued). Expanded Bayesian 50% majority rule consensus tree of the nrDNA dataset. showing all samples. Posterior probabilities and maximum likelihood bootstrap support values are shown above and below the branches, respectively. Figure continues in next page.

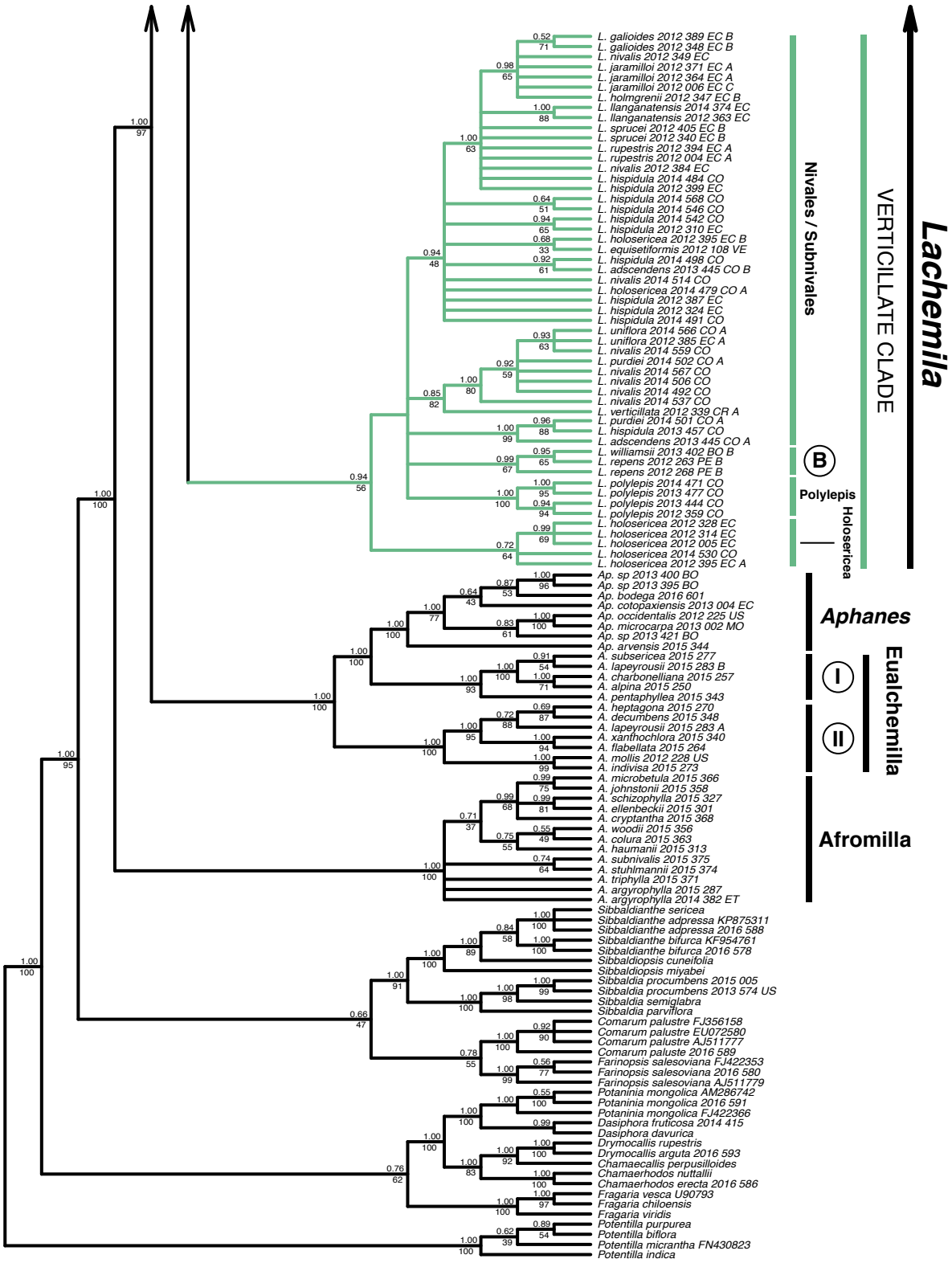

Fig. 3.5 (Continued). Expanded Bayesian 50% majority rule consensus tree of the nrDNA dataset. showing all samples. Posterior probabilities and maximum likelihood bootstrap support values are shown above and below the branches, respectively. Figure continues in next page.

Table 3.1. Samples of *Lachemilla* used in this study with their corresponding chloroplast and nuclear ribotype clades. Allopolyploid species are shown in bold. Country abbreviations in the sample names are as follows: BO – Bolivia, CO – Colombia, CR – Costa Rica, EC – Ecuador, MX – Mexico, PE – Peru, VE – Venezuela.

Species	Sample	cpDNA clade	nrDNA clade
L. adscendens	2013_445_CO	Subnivales - VERTCILLATE	Nivales/Subnivales - VERTICILLATE Nivales/Subnivales - VERTICILLATE
L. andina	2012_249_PE	Procumbentes I - TRIPARTITE	Procumbentes I - TRIPARTITE Procumbentes II - TRIPARTITE
	2012_295_PE	Procumbentes II - TRIPARTITE	Procumbentes II - TRIPARTITE
	2012_304_PE	Procumbentes I - TRIPARTITE	Procumbentes I - TRIPARTITE Procumbentes II - TRIPARTITE
	2012_354_EC	-----	Procumbentes II - TRIPARTITE Procumbentes I - TRIPARTITE
	2012_379_EC	Procumbentes II - TRIPARTITE	Procumbentes I - TRIPARTITE Procumbentes II - TRIPARTITE
	2012_401_EC	Procumbentes I - TRIPARTITE	Procumbentes II - TRIPARTITE Procumbentes I - TRIPARTITE
	2013_412_BO	Procumbentes II - TRIPARTITE	Procumbentes II - TRIPARTITE
	2013_440_CO	Procumbentes I - TRIPARTITE	Procumbentes I - TRIPARTITE
	2013_464_CO	Procumbentes I - TRIPARTITE	Procumbentes I - TRIPARTITE Procumbentes II - TRIPARTITE
	2014_548_CO	Procumbentes I - TRIPARTITE	Procumbentes I - TRIPARTITE Procumbentes II - TRIPARTITE
L. aphanoides	2012_001_EC	-----	Glomerulate III - TRIPARTITE Glomerulate II - TRIPARTITE
	2012_246_PE	Glomerulate III - TRIPARTITE	Glomerulate II - TRIPARTITE Glomerulate III - TRIPARTITE
	2012_256_PE	Glomerulate I - TRIPARTITE	Glomerulate III - TRIPARTITE
	2012_293_PE	Glomerulate III - TRIPARTITE	Glomerulate III - TRIPARTITE Glomerulate II - TRIPARTITE
	2012_353_EC	-----	Glomerulate III - TRIPARTITE
	2012_355_EC	Glomerulate III - TRIPARTITE	Glomerulate III - TRIPARTITE Glomerulate II - TRIPARTITE
	2012_369_EC	Glomerulate II - TRIPARTITE	Glomerulate II - TRIPARTITE
	2012_388_EC	Glomerulate III - TRIPARTITE	Glomerulate III - TRIPARTITE Glomerulate II - TRIPARTITE
	2012_396_EC	Glomerulate I - TRIPARTITE	Glomerulate I - TRIPARTITE Glomerulate III - TRIPARTITE
	2013_385_BO	Glomerulate III - TRIPARTITE	Glomerulate III - TRIPARTITE Glomerulate II - TRIPARTITE

Species	Sample	cpDNA clade	nrDNA clade
	2013_392_BO	Glomerulate III - TRIPARTITE	Glomerulate III - TRIPARTITE
	2013_429_CO	Glomerulate III - TRIPARTITE	Glomerulate III - TRIPARTITE
	2013_432_CO	Glomerulate III - TRIPARTITE	Glomerulate III - TRIPARTITE
	2013_442_CO	-----	Glomerulate III - TRIPARTITE
	2013_446_CO	Glomerulate III - TRIPARTITE	Glomerulate III - TRIPARTITE Glomerulate III - TRIPARTITE
	2013_468_CO	Glomerulate III - TRIPARTITE	Glomerulate III - TRIPARTITE
	2013_489_CO	-----	Glomerulate III - TRIPARTITE
	2014_473_CO	Glomerulate III - TRIPARTITE	Glomerulate II - TRIPARTITE Glomerulate III - TRIPARTITE
	2014_505_CO	Glomerulate I - TRIPARTITE	Glomerulate I - TRIPARTITE Glomerulate III - TRIPARTITE
	2014_522_CO	Glomerulate III - TRIPARTITE	Glomerulate III - TRIPARTITE
	2014_541_CO	Glomerulate III - TRIPARTITE	Glomerulate I - TRIPARTITE Glomerulate III - TRIPARTITE
	2015_149_MX	-----	Glomerulate III - TRIPARTITE
L. barbata	2012_260_PE	Barbata - PINNATE	Barbata - PINNATE
	2012_305_PE	Barbata - PINNATE	Barbata - PINNATE
	2013_406_BO	Barbata - PINNATE	Barbata - PINNATE Pinnata - PINNATE
L. bipinnatifida	2013_386_BO	-----	Pinnata - PINNATE
	2013_390_BO	Glomerulate II - TRIPARTITE	Glomerulate II - TRIPARTITE Pinnata - PINNATE
	2013_427_BO	Glomerulate II - TRIPARTITE	Glomerulate II - TRIPARTITE Pinnata - PINNATE
	2013_428_BO	Glomerulate II - TRIPARTITE	Glomerulate II - TRIPARTITE Frigida - PINNATE
L. diplophylla	2012_229_PE	Diplophylla - PINNATE	Diplophylla - PINNATE
	2012_237_PE	Diplophylla - PINNATE	Diplophylla - PINNATE
	2012_306_PE	Diplophylla - PINNATE	Diplophylla - PINNATE
	2012_357_EC	Diplophylla - PINNATE	Diplophylla - PINNATE
	2013_382_BO	Diplophylla - PINNATE	Diplophylla - PINNATE
	2013_408_BO	Diplophylla - PINNATE	Diplophylla - PINNATE
L. equisetiformis	2012_108_VE	-----	Nivales/Subnivales - VERTICILLATE
L. erodiifolia	2012_244_PE	Erodiifolia - PINNATE	Erodiifolia - PINNATE
	2012_296_PE	Erodiifolia - PINNATE	Erodiifolia - PINNATE
	2012_365_EC	Erodiifolia - PINNATE	Erodiifolia - PINNATE
	2012_372_EC	Erodiifolia - PINNATE	Erodiifolia - PINNATE
	2013_401_BO	Erodiifolia - PINNATE	Erodiifolia - PINNATE
L. frigida	2012_243_PE	Frigida - PINNATE	Frigida - PINNATE

Species	Sample	cpDNA clade	nrDNA clade
	2012_300_PE	Frigida - PINNATE	Frigida - PINNATE
	2013_399_BO	Frigida - PINNATE	Frigida - PINNATE
	2013_409_BO	Frigida - PINNATE	Frigida - PINNATE
	2013_423_BO	-----	Frigida - PINNATE
L. fulvescens	2012_323_EC	Pectinata - ORBICULATE	-----
	2012_368_EC	Pectinata - ORBICULATE	Pectinata - ORBICULATE Glomerulate III - TRIPARTITE
	2012_375_EC	Pectinata - ORBICULATE	Pectinata - ORBICULATE Glomerulate III - TRIPARTITE
	2012_408_EC	Pectinata - ORBICULATE	-----
	2013_450_CO	Pectinata - ORBICULATE	Pectinata - ORBICULATE Glomerulate III - TRIPARTITE
	2013_463_CO	Pectinata - ORBICULATE	Glomerulate III - TRIPARTITE
	2013_481_CO	-----	Pectinata - ORBICULATE Glomerulate III - TRIPARTITE
	2014_503_CO	Pectinata - ORBICULATE	Glomerulate III - TRIPARTITE
	2014_521_CO	Pectinata - ORBICULATE	Pectinata - ORBICULATE Glomerulate III - TRIPARTITE
	2014_551_CO	Pectinata - ORBICULATE	Glomerulate III - TRIPARTITE Pectinata - ORBICULATE
L. galioides	2012_348_EC	Nivales - VERTICILLATE	Mandoniana - PINNATE Nivales/Subnivales - VERTICILLATE
	2012_389_EC	Nivales - VERTICILLATE	Mandoniana - PINNATE Nivales/Subnivales - VERTICILLATE
L. hirta	2012_317_EC	Procumbentes I - TRIPARTITE	Procumbentes I - TRIPARTITE
	2012_343_EC	Procumbentes I - TRIPARTITE	Procumbentes II - TRIPARTITE
	2012_390_EC	Procumbentes I - TRIPARTITE	Procumbentes I - TRIPARTITE
	2014_475_CO	-----	Procumbentes I - TRIPARTITE
L. hispidula	2012_310_EC	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2012_324_EC	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2012_387_EC	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2012_399_EC	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2013_457_CO	A - TRIPARTITE	Nivales/Subnivales - VERTICILLATE
	2014_484_CO	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2014_491_CO	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2014_498_CO	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2014_542_CO		Nivales/Subnivales - VERTICILLATE
	2014_546_CO	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2014_568_CO		Nivales/Subnivales - VERTICILLATE
L. holmgrenii	2012_347_EC	Nivales - VERTICILLATE	Mandoniana - PINNATE

Species	Sample	cpDNA clade	nrDNA clade
			Nivales/Subnivales - VERTICILLATE
L. holosericea	2012_005_EC	Subnivales - VERTCILLATE	Holosericea - VERTICILLATE
	2012_314_EC		Holosericea - VERTICILLATE
	2012_328_EC	Subnivales - VERTCILLATE	Holosericea - VERTICILLATE
	2012_395_EC	Subnivales - VERTCILLATE	Holosericea - VERTICILLATE
			Nivales/Subnivales - VERTICILLATE
	2014_479_CO	Subnivales - VERTCILLATE	Nivales/Subnivales - VERTICILLATE
			Glomerulate III - TRIPARTITE
	2014_530_CO	Subnivales - VERTCILLATE	Holosericea - VERTICILLATE
L. jamesonii	2012_350_EC	Procumbentes II - TRIPARTITE	Procumbentes I - TRIPARTITE
			Procumbentes II - TRIPARTITE
L. jaramilloi	2012_006_EC	Frigida - PINNATE	Nivales/Subnivales - VERTICILLATE
			C - PINNATE
			Frigida - PINNATE
	2012_364_EC	Frigida - PINNATE	Nivales/Subnivales - VERTICILLATE
			C - PINNATE
	2012_371_EC	Frigida - PINNATE	Nivales/Subnivales - VERTICILLATE
			Frigida - PINNATE
			Mandoniana - PINNATE
L. lechleriana	2012_271_PE	B - TRIPARTITE	Pectinata - ORBICULATE
			Tanacetifolia - PINNATE
L. llanganatensis	2012_363_EC	-----	Nivales/Subnivales - VERTICILLATE
	2014_374_EC	-----	Nivales/Subnivales - VERTICILLATE
L. mandoniana	2012_007_EC	-----	Mandoniana - PINNATE
	2012_261_PE	Mandoniana - PINNATE	Mandoniana - PINNATE
	2012_325_EC	Mandoniana - PINNATE	Mandoniana - PINNATE
	2012_335_CR	Mandoniana - PINNATE	Mandoniana - PINNATE
	2012_370_EC	Mandoniana - PINNATE	Mandoniana - PINNATE
	2012_397_EC	Mandoniana - PINNATE	Mandoniana - PINNATE
	2013_454_CO	Mandoniana - PINNATE	Mandoniana - PINNATE
	2014_510_CO	Mandoniana - PINNATE	Mandoniana - PINNATE
	2014_523_CO	Mandoniana - PINNATE	Mandoniana - PINNATE
	2014_528_CO	Mandoniana - PINNATE	Mandoniana - PINNATE
	2014_545_CO	Mandoniana - PINNATE	Mandoniana - PINNATE
L. mexiquense	2015_233_MX	ORBICULATE	TRIPARTITE
L. moritziana	2013_441_CO	Glomerulate I - TRIPARTITE	Glomerulate III - TRIPARTITE
			Glomerulate I - TRIPARTITE
	2013_443_CO	Glomerulate I - TRIPARTITE	Glomerulate III - TRIPARTITE
			Glomerulate I - TRIPARTITE
L. nivalis	2012_349_EC	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE

Species	Sample	cpDNA clade	nrDNA clade
	2012_384_EC	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2012_386_EC	Nivales - VERTICILLATE	-----
	2014_492_CO	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2014_506_CO	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2014_514_CO	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2014_537_CO	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2014_559_CO	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
	2014_567_CO	Nivales - VERTICILLATE	Nivales/Subnivales - VERTICILLATE
L. orbiculata	2012_003_EC	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2012_258_PE	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2012_292_PE	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2012_294_PE	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2012_309_EC	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2012_377_EC	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2012_381_EC	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2012_402_EC	Orbiculata - ORBICULATE	-----
	2012_403_EC	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2013_430_CO	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2013_467_CO	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2014_487_CO	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2014_519_CO	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2014_550_CO	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
	2014_562_CO	Orbiculata - ORBICULATE	Orbiculata - ORBICULATE
L. pectinata	2012_326_EC	Pectinata - ORBICULATE	Pectinata - ORBICULATE
	2012_351_CR	-----	Pectinata - ORBICULATE
	2012_376_EC	Pectinata - ORBICULATE	Pectinata - ORBICULATE
	2012_382_EC	-----	Pectinata - ORBICULATE
	2013_388_BO	-----	Pectinata - ORBICULATE
	2014_520_CO	Pectinata - ORBICULATE	Orbiculata - ORBICULATE
	2014_547_CO	Pectinata - ORBICULATE	Orbiculata - ORBICULATE
	2015_175_MX		Pectinata - ORBICULATE
L. perryana	2012_380_EC	Pectinata - ORBICULATE	Glomerulate III - TRIPARTITE Pectinata - ORBICULATE
L. pinnata	2012_230_PE	Pinnata - PINNATE	-----
	2012_232_PE	-----	Pinnata - PINNATE
	2012_239_PE	Pinnata - PINNATE	Pinnata - PINNATE
	2012_242_PE	Pinnata - PINNATE	Pinnata - PINNATE
	2012_251_PE	Pinnata - PINNATE	Pinnata - PINNATE
	2012_280_PE	Pinnata - PINNATE	Pinnata - PINNATE
	2012_291_PE	Pinnata - PINNATE	Pinnata - PINNATE

Species	Sample	cpDNA clade	nrDNA clade
	2012_330_EC	Pinnata - PINNATE	Pinnata - PINNATE
	2012_330_EC	-----	Pinnata - PINNATE
	2012_391_EC	Pinnata - PINNATE	Pinnata - PINNATE
	2013_381_BO	-----	Pinnata - PINNATE
	2013_391_BO	-----	Pinnata - PINNATE
	2013_437_CO	-----	Pinnata - PINNATE
	2013_448_CO	Pinnata - PINNATE	Pinnata - PINNATE
	2013_469_CO	-----	Pinnata - PINNATE
	2013_487_CO	-----	Pinnata - PINNATE
	2015_218_MX	Pinnata - PINNATE	Pinnata - PINNATE
L. polylepis	2012_358_CO	Polylepis - TRIPARTITE	-----
	2012_359_CO	Polylepis - TRIPARTITE	Polylepis - VERTICILLATE
	2013_444_CO	Polylepis - TRIPARTITE	Polylepis - VERTICILLATE
	2013_477_CO	Polylepis - TRIPARTITE	Polylepis - VERTICILLATE
	2014_471_CO	Polylepis - TRIPARTITE	Polylepis - VERTICILLATE
L. pringlei	2015_150_MX	Glomerulate I - TRIPARTITE	Glomerulate III - TRIPARTITE Glomerulate I - TRIPARTITE
L. procumbens	2012_332_CR	-----	Procumbentes I - TRIPARTITE
	2015_200_MX	Procumbentes I - TRIPARTITE	Procumbentes I - TRIPARTITE
L. pseudovenusta	2012_275_PE	Glomerulate I - TRIPARTITE	Pectinata - ORBICULATE Frigida - PINNATE Glomerulate III - TRIPARTITE
L. purdiei	2014_501_CO	Subnivales - VERTCILLATE	Nivales/Subnivales - VERTICILLATE Glomerulate III - TRIPARTITE
	2014_502_CO	-----	Nivales/Subnivales - VERTICILLATE Glomerulate III - TRIPARTITE
L. ***ranunculoides***	2012_240_PE	Glomerulate II - TRIPARTITE	Frigida - PINNATE Glomerulate II - TRIPARTITE
	2012_248_PE	Glomerulate II - TRIPARTITE	Glomerulate II - TRIPARTITE Frigida - PINNATE
	2013_389_BO	-----	Glomerulate II - TRIPARTITE
L. repens	2012_263_PE	B - TRIPARTITE	B - PINNATE B - VERTICILLATE
	2012_268_PE	B - TRIPARTITE	B - PINNATE B - VERTICILLATE
L. rupestris	2012_004_EC	Rupestris - VERTCILLATE	Nivales/Subnivales - VERTICILLATE Tanacetifolia - PINNATE
	2012_270_PE	Rupestris - VERTCILLATE	Pectinata - ORBICULATE Tanacetifolia - PINNATE
	2012_394_EC	Rupestris - VERTCILLATE	Nivales/Subnivales - VERTICILLATE

Species	Sample	cpDNA clade	nrDNA clade
			Orbiculata - ORBICULATE
			Tanacetifolia - PINNATE
L. sarmentosa	2013_396_BO	Frigida - PINNATE	Frigida - PINNATE
	2013_413_BO	Frigida - PINNATE	Frigida - PINNATE
			Pectinata - ORBICULATE
L. sibbaldiifolia	2015_154_MX	Glomerulate I - TRIPARTITE	Glomerulate I - TRIPARTITE
			Glomerulate III - TRIPARTITE
	2015_203_MX	Glomerulate I - TRIPARTITE	Glomerulate I - TRIPARTITE
			Glomerulate III - TRIPARTITE
L. sprucei	2012_340_EC	Subnivales - VERTCILLATE	Pectinata - ORBICULATE
			Nivales/Subnivales - VERTICILLATE
	2012_405_EC	Subnivales - VERTCILLATE	Pectinata - ORBICULATE
			Nivales/Subnivales - VERTICILLATE
L. standleyi	2016_614_CR	-----	Glomerulate III - TRIPARTITE
L. steinbachii	2013_415_BO	-----	Glomerulate III - TRIPARTITE
L. talamanquensis	2012_093_CR	-----	A - TRIPARTITE
	2012_334_CR	A - TRIPARTITE	A - TRIPARTITE
	2016_598_CR	A - TRIPARTITE	A - TRIPARTITE
L. tanacetifolia	2012_267_PE	Tanacetifolia - PINNATE	-----
	2012_331_EC	Tanacetifolia - PINNATE	Tanacetifolia - PINNATE
	2012_367_EC	Tanacetifolia - PINNATE	Tanacetifolia - PINNATE
	2013_488_CO	Tanacetifolia - PINNATE	Tanacetifolia - PINNATE
L. uniflora	2012_319_EC	-----	C - PINNATE
	2012_362_EC	Uniflora - VERTCILLATE	C - PINNATE
	2012_385_EC	Uniflora - VERTCILLATE	Nivales/Subnivales - VERTICILLATE
	2012_385_EC	-----	C - PINNATE
	2014_566_CO	-----	Nivales/Subnivales - VERTICILLATE
	2014_566_CO	-----	C - PINNATE
L. velutina	2015_162_MX	Glomerulate II - TRIPARTITE	Glomerulate II - TRIPARTITE
	2015_169_MX	-----	Glomerulate II - TRIPARTITE
L. venusta	2015_157_MX	Pectinata - ORBICULATE	Glomerulate III - TRIPARTITE
			Pectinata - ORBICULATE
L. verticillata	2012_339_CR	A - TRIPARTITE	Nivales/Subnivales - VERTICILLATE
			A - TRIPARTITE
L. vulcanica	2012_002_EC	Procumbentes II - TRIPARTITE	Procumbentes I - TRIPARTITE
	2012_250_PE	Procumbentes II - TRIPARTITE	Procumbentes I - TRIPARTITE
			Procumbentes II - TRIPARTITE
	2012_264_PE	-----	Procumbentes I - TRIPARTITE
	2012_284_PE	Procumbentes II - TRIPARTITE	Procumbentes II - TRIPARTITE
			Procumbentes I - TRIPARTITE

Species	Sample	cpDNA clade	nrDNA clade
	2012_298_PE	Procumbentes II - TRIPARTITE	Procumbentes I - TRIPARTITE
	2012_302_PE	Procumbentes II - TRIPARTITE	Procumbentes I - TRIPARTITE Procumbentes II - TRIPARTITE
	2012_312_EC	Procumbentes II - TRIPARTITE	Procumbentes I - TRIPARTITE Procumbentes II - TRIPARTITE
	2012_352_EC	Procumbentes II - TRIPARTITE	Procumbentes II - TRIPARTITE Procumbentes I - TRIPARTITE
	2012_392_EC	Procumbentes II - TRIPARTITE	Procumbentes II - TRIPARTITE
	2013_410_BO	-----	Procumbentes II - TRIPARTITE
	2013_451_CO	Procumbentes II - TRIPARTITE	Procumbentes II - TRIPARTITE
	2013_470_CO	Procumbentes II - TRIPARTITE	Procumbentes I - TRIPARTITE
	2014_488_CO	Procumbentes II - TRIPARTITE	Procumbentes I - TRIPARTITE
	2015_171_MX	Procumbentes I - TRIPARTITE	Procumbentes II - TRIPARTITE Procumbentes I - TRIPARTITE
L. williamsii	2013_402_BO	B - TRIPARTITE	B - PINNATE B - VERTICILLATE

***ITS2 secondary structure analyses*—** All sequences resulted in the same expected secondary structure with the four common helix structures characterized for Rosaceae (Mai and Coleman, 1997) (Fig. 3.6), reason why we considered that all copies obtain here are functional. Motif annotation showed that all genera in Fragariinae (including Alchemillinae) have a one 1 bp insertion in the U–U mismatch (Fig. 3.6B), while all species of *Potentilla* have the expected motif (Fig 3.6A). Additionally, we identified that all ITS2 sequences belonging to the 'Procumbentes II' subclade have a mutation in the Triple-A motif (Fig. 3.6C), while the second copy of those species in the 'Procumbentes I' have the expected motif as in *Potentilla.*

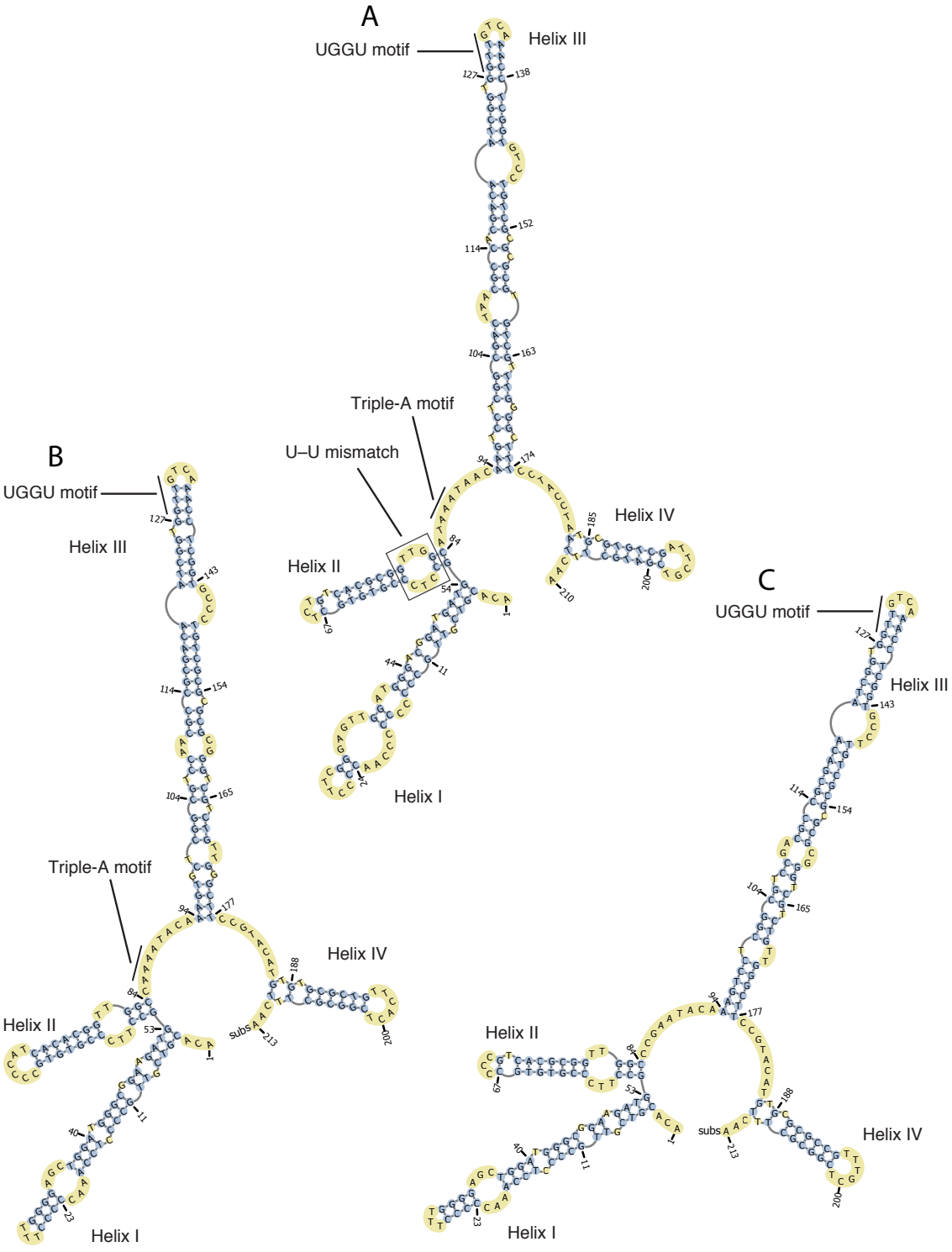

Figure 3.6. Predicted secondary structure of the ITS2 region. The four common helix structures are shown for all samples. A. *Potentilla micrantha* has the UGGU, triple-A, and the U–U mismatch motifs. B. *Lachemilla vulcanica* 2012_302_PE_A ('Procumbentes I' – *Tripartite*) is missing the U–U mismatch, and C. *Lachemilla vulcanica* 2012_302_PE_A ('Procumbentes II' – *Tripartite*) is missing the U–U mismatch and triple-A motifs.

Discussion

Incomplete concerted evolution of the nrDNA cistron— Concerted evolution is common in the nrDNA cistron of plants (Álvarez and Wendel 2003), but the incomplete homogenization of this region has also been widely reported (e.g., Sang et al. 1995; Rauscher et al. 2002; Rauscher et al. 2004; Wan et al. 2014; Zarrei et al. 2014, Kosachev et al. 2016, Xu et al. 2017). Here we were able to obtain polymorphic nrDNA copies from individuals of 33 of the 48 species of *Lachemilla* sampled (Table 3.1). However, we did not obtain the same number of nrDNA copies in all individuals of the same species, possibly due to preferential expression of one homeolog (e.g., Joly et al. 2004), PCR amplification bias (e.g., Bellamin et al. 2010), or omission of rare copies during bioinformatic processing of the PCR amplicon pools. The disruption or delay of concerted evolution processes has also been reported in several other genera of Rosaceae where it may be linked to other genomic processes, including polyploidy, asexual reproduction, and apomixis (e.g., Campbell et al 1997; Feng et al. 2007). Additionally, higher ribotype diversity has been suggested for genera that have an ancient polyploidy origin (Evans and Campbell 2002; Campbell et al. 2007; Zarrei et al. 2014). Based on our detailed phylogenetic analyses, we showed that the presence of multiple nrDNA copies in *Lachemilla* is primarily due to allopolyploidy, as hypothesized is Chapter 1(see below). However, an ancient hybrid origin of the clade that likely involved polyploidy has also been suggested (Chapter 2), and apomixis (Samaniego 2014) has been reported for *Lachemilla*, which may also contribute to the large diversity of nrDNA copies recovered here.

***Allopolyploidy in Lachemilla*—** Evidence for the putative allopolyploid origin of 24 species of *Lachemilla*, based on cytonuclear discordance, phylogenetic network analyses, cytology, and genome size estimates has been established (Chapter 1). Here, direct evidence of allopolyploidy of 30 species, explicitly based both on 1) the phylogenetic position of the multiple nrDNA copies recovered from individual accessions, and 2) the robust, phylogenomic reconstruction of the chloroplast phylogeny (Table 3.1). Individual hybridization events occur among species spanning all four major groups of *Lachemilla*, and some species (e.g., *L. aphanoides* and *L. pectinata*) seem to be involved in the origin of multiple allopolyploid species.

Ploidy levels in *Lachemilla* range from diploid ($2n = 16$) in *L. mandoniana* to dodecaploid ($2n = 96$) in *L. jaramilloi* and *L. aphanoides* (Chapter 1). Although we obtained one nrDNA copy for *L. mandoniana,* there is not a perfect correlation between ploidy-level and the number of ribotypes recovered; we isolated only two copies from *L. aphanoides*, and three from *L. jaramilloi,* which would correspond to an allotetraploid and an allohexaploid, respectively. Many factors may influence this including, for example, partial homogenization of the nrDNA cistron between homeologous chromosomes, mixed allo- and autopolyploidy within the origin of individual species and/or clades, preferential PCR amplification, and/or the failure to identify all copies in the sequence processing steps. Furthermore, in known octoploid species like *L. pinnata* and *L. tanacetifolia* ($2n = 64$), we detected only one nrDNA copy (as in all species in the *Pinnate* clade), which could suggest that these species are autopolyploids. Alternatively, given the observed discordance between species-level relationships in the nrDNA and cpDNA trees (Figs. 3.2, 3.4), a hybrid origin of these species involving members of the *Pinnate* clade, is also possible.

Additional data, e.g., from multiple, independent, and informative nuclear loci, will be necessary to discern between these two alternatives.

Within the *Tripartite* clade, nrDNA analyses revealed a pattern where the species of the 'Procumbentes' clades have two distinct ribotypes nested within the 'Glomerulate' subclades, demonstrating that species belonging to the 'Procumbentes' clades have a polyploid origin. In addition, these species form multiple clades in the cpDNA phylogeny, where most are paraphyletic (e.g., *L. aphanoides, L. andina, L. vulcanica*). Together, these results suggest a complicated pattern of hybridization and polyploidy within the *Tripartite* clade, where it is likely that there have been multiple origins of the same allopolyploid species (Soltis and Soltis 1993, 1999). Morales-Briones et al. (Chapter 1) showed that all 'Procumbentes' species are likely hybrids involving *L. aphanoides,* which could also explain the pattern seen here. *Lachemilla aphanoides* is the most widespread species of *Lachemilla*, and shows a wide range of morphological variation across its distribution, and the presence of *L. aphanoides* in all 'Glomerulate' subclades in both the nrDNA and cpDNA phylogenies could be the result of cryptic diversity within this species. Also, the species of the *Tripartite* clade are involved in the origin of allopolyploid species with other clades. For example *L. aphanoides* seems to be the maternal lineage of *L. bipinnatifida* and *L. ranunculoides* (*Pinnate* clade) and paternal lineage of *L. fulvescens* (*Orbiculate* clade) and *L. purdiei* (*Verticillate* clade).

Included in the *Tripartite* clade in the cpDNA and nrDNA trees are a group of species and samples that have morphological affinity with other clades (subclade 'A'). Based on limited molecular and taxonomic sampling, Morales-Briones et al. (Chapter 1) suggested the recovery of *L. verticillata* in this clade was likely the product of introgression and organellar capture. Here we found that *L. verticillata*

actually has two distinct nrDNA copies – one recovered in the *Verticillate* clade, and one in subclade 'A' of the *Tripartite* clade, suggesting that despite its morphological integrity, this species might actually be an allopolyploid. That said, these results are based on the inclusion of a single sample of *L. verticillata* from Costa Rica, and the inclusion of more samples from different populations is necessary to confirm this hypothesis. The other species from subclade 'A', *L. talamanquensis*, was previously suggested to be a hybrid (Romoleroux and Morales-Briones et al. 2012), but we did not found any evidence for this here. Similarly, the cpDNA subclade 'B' contains species with morphological affinities to other clades and forms a clade with along with *L. polylepis* and subclade 'A'. The species of the subclade 'B' are shown to be allopolyploids in the nrDNA tree, with ribotypes in the *Pinnate* and *Verticillate* clades. Although, additional work is needed to discern between introgression and hybridization in these species, it is clear all of them share a similar chloroplast haplotype that is closely related to the *Tripartite* clade, but no 'true' *Tripartite* species are found among these species.

The *Orbiculate* clade is involved in multiple allopolyploid events, with *L. pectinata* contributing the maternal lineage to several species (e.g., *L. fulvescens, L. venusta*, and *L. perryana*) when hybridizing with species of the *Tripartite* clade, but when hybridizing with members of the *Verticillate* and *Pinnate* clades, *L. pectinata* serves as the paternal lineage (e.g., *L. sprucei* and *L. sarmentosa*). We found several samples of *L. pectinata* from Colombia that only contained a single ribotype nested among the *L. orbiculata* ribotypes, while in the cpDNA tree, these same accessions are recovered in a clade with the remaining samples of *L. pectinata*. This result could be due to introgression between these two species, although a general lack of resolution in the nrDNA tree prevents determining if these Colombian samples of

L. pectinata are more closely related to samples of *L. orbiculata* from geographically proximate populations.

Within the *Pinnate* clade, most species are monophyletic and well resolved in both phylogenies, which allow us to assign specific parental lineages to hybrid species involving members of this clade (Table 3.1). With the exception of *L. frigida*, species of this group only serve as the paternal lineage of hybrid species. The subclades 'C' and 'B' in the nrDNA tree contain only polyploid species, suggesting that these orphan ribotypes may correspond to missing diploids (either extinct or unsampled), a result commonly seen in studies involving complex allopolyploid origins (e.g., Kim et al. 2008; Beck et al. 2010; Marcussen et al. 2015).

As with the other major clades, members of the *Verticillate* clade hybridize widely across *Lachemilla*, and are most often the maternal donor (e.g., *L. sprucei*, with the paternal lineage from subclade 'Pectinata,' and *L. purdiei*, with the paternal lineage from the 'Glomerulate III' subclade). In the nrDNA tree, we recovered only one ribotype for most samples of *L. holosericea*, and these were monophyletic. However, we also found one sample (*L. holosericea 2012 395 EC*) with two distinct ribotypes, one in the main 'Holosericea' clade and the other nested with the core species of the *Verticillate* clade, and another sample (*L. holosericea 2014 479 CO*) that has one allele nested also within *Verticillate* clade and the other in the *Tripartite* clade. Based on morphology, *L. holosericea* is believed to be a hybrid, but we found evidence for this in only the one sample. Interestingly, the 'Holosericea' clade in the nrDNA tree is resolved sister to the rest of the *Verticillate* clade, which could be an effect of recombination between the two possible nrDNA copies in this species (Álvarez and Wendel 2003; Soltis et al. 2008).

Although, most allopolyploid species here show the presence of only two nrDNA copies, and can be assumed to be tetraploid, we also identified some species that show evidence of higher ploidy levels based on the number of ribotypes recovered. Species like *L. pseudovenusta* and *L. rupestris* have nrDNA copies found in three different major clades. We can also infer a similar pattern in several other species where only two nrDNA ribotypes were recovered. In those species the two nrDNA copies are found in two different clades, while in the cpDNA phylogeny this species belongs to a third clade (e.g., *L. repens*, *L. williamsii*, and *L. lechleriana*).

On the other hand, *L. mexiquense* shows a clearly distinct position in different major clades on both phylogenies, but only one nrDNA copy was detected. In this case, it is likely that concerted evolution of the nrDNA cistron is complete (or nearly so), and has homogenized the nrDNA copies to a single parental homeolog. *Lachemilla mexiquense* shows morphological affinity to both parental lineages, including tripartite leaves and a glomerulate inflorescence (characteristics of the *Tripartite* clade), and a stoloniferous habit (a characteristic of the *Orbiculate* clade), which is a unique combination in the genus that is in agreement with its hybrid origin.

Another interesting case can be seen in *L. rupestris*, which was previously thought to be endemic to northern Ecuador, but that we collected in southern Peru. Our analyses show that the samples from northern Ecuador and southern Peru share the same chloroplast haplotype (in the *Verticillate* clade). However, while one of the two nuclear ribotypes from these samples is resolved in the *Pinnate* clade (in subclade 'Tanacetifolia'), the second ribotype of the Ecuadorian samples is nested within subclade 'Orbiculata', while the second nrDNA copy in the Peruvian sample is in subclade 'Pectinata'. These suggest multiple origins of these morphologically

identical populations, and these may represent cryptic allopolyploid species that share one parent, but not the other (e.g., Holloway et al. 2006). A similar case can be seen in *L. bipinnatifida*, where all maternal haplotypes come from the same subclade ('Glomerulatae II'), and while most accessions have the same paternal ribotype from subclade 'Pinnata', one sample has a 'Frigida' ribotype. As with *L. rupestris*, this could represent cryptic diversity, but may also be the result of a failure to detect all species involved in the hybridization event, which is a limitation of our two-locus approach.

In summary, using microfluidic PCR and high throughput sequencing, we were able to obtain allelic information from the nrDNA cistron for hundreds of samples that allowed us to show direct evidence of allopolyploidy in *Lachemilla*. Although nrDNA data is clearly useful for studying allopolyploid origins (e.g., Zarrei et al. 2014; Kosachev et al. 2016; Xu et al. 2017), especially where distinct ribotypes can be recovered, it is important to note the problems and limitations inherent to the nrDNA cistron for phylogenetic analysis (reviewed in Álvarez and Wendel 2003; Bailey et al. 2003), and the study of allopolyploidy (Soltis et al. 2008). In *Lachemilla*, the use of multiple single- or low-copy nuclear genes to confirm and refine these results continues to be an important goal.

***Phylogenetic relationships of Alchemillinae*—** While phylogenetic relationships within Potentilleae has been well-studied, the placement of several groups and/or species have been challenging due to hybridization, allopolyploidy, and apomixis (Eriksson et al. 1998; Eriksson et al. 2003; Gehrke et al. 2008; Lundberg 2009; Dobeš and Paule 2010; Dobeš et al. 2015; Eriksson et al. 2015; Gehrke et al. 2016; Feng 2017). This is the case with subtribe Alchemillinae (Rothmaler 1937; Notov and

Kusnetzova 2004; Soják 2008), which have been shown to be a monophyletic group nested within subtribe Fragariinae, but its exact placement has remained unclear (Gehrke et al. 2008; Gehrke et al. 2016). Based on a limited sampling of cpDNA data, Alchemillinae was places, with weak support, with the monotypic genera *Comarum* and *Farinopsis* (sensu Soják 2008). This clade was sister to a highly support clade formed by *Sibbaldia, Sibbaldianthe*, and *Sibbaldiopsis* (Lundberg 2009; Eriksson et al. 2015; Gehrke et al. 2016; Feng 2017). Here using a large cpDNA dataset of 45 variable regions (22,017 bp) and a broad sampling of the relevant lineages of subtribe Fragariinae, we able to clearly establish that Alchemillinae form a clade with *Farinopsis salesoviana*, and these two have *Comarum palustre* as their sister group (Fig. 3.2, 3.3). This finding is supported by a recent complete chloroplast phylogeny of Rosaceae (Zhang 2017), although their sampling included only two species sampled of Alchemillinae, and *Comarum palustre* was not sampled.

Using the same nrDNA regions as in Gehrke et al. (2016), but a much larger sampling of Alchemillinae, we found the same results where Alchemillinae is sister to a clade to a weakly supported clade formed by *Comarum*, *Farinopis*, *Sibbaldia, Sibbaldianthe*, and *Sibbaldiopsis.* This incongruence between the two phylogenies has been attributed to widespread hybridization and allopolyploidy in Fragariinae (Lundberg 2009; Gehrke et al. 2016). In a recent phylogenetic analysis of transciptomes from across Rosaceae, Xiang et al. (2017) found that the two sampled species of *Alchemilla* formed a monophyletic group along with the clade comprised by *Sibbaldia* and *Sibbaldiante*, leaving *Comarum palustre* as the sister group of the *Alchemilla*/*Sibbaldia*/*Sibbaldianthe* clade. It is also worth noting these relationships were highly sensitive to the amount of dataset filtering and phylogenetic method

used (i.e., number of genes, and ML vs. species-trees analyses), corroborating the notion of the widespread hybridization in Fragariinae.

Within Alchemillinae we found the same well supported topologies and cytonuclear discordance with respect to the four major clades (*Lachemilla, Aphanes*, Eualchemilla, and Afromilla) as reported by Gherke et al. (2008, 2016). This incongruence has also been attributed to hybridization, but so far only one nuclear locus (nrDNA) has been used to study these relationships. A multilocus nuclear analysis that combines species trees and network estimations, similar to Chapter 2, is still needed to resolve relationships in this clade, and investigate evolutionary processes leading to the continued observation of cytonuclear discordance. This will provide us with a better perspective on the role of reticulate evolution in the origin of these groups, and allow for the investigation of remaining questions like historical biogeography, diversification dynamics, and trait evolution, among others, as well to clarify the taxonomy and classification of this complex group.

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CHAPTER 4: *LACHEMILLA MEXIQUENSE* (ROSACEAE), A NEW SPECIES FROM MEXICO

Morales-Briones DF (2016) *Lachemilla mexiquense* (Rosaceae), a new species from Mexico. PhytoKeys 62: 25–32.

Abstract

A new species of *Lachemilla* (Rosaceae), *Lachemilla mexiquense* D.F. Morales-B., from Mexico is described and illustrated. This species is similar to *Lachemilla aphanoides* by its tripartite leaves and glomerulate inflorescence with entirely glabrous flowers, but it differs by its stonoliferous habit, persistent basal leaves and basal stipules, and smaller flowers with a campanulate-elongate hypanthium and single carpel. A key to the species of *Lachemilla* in Mexico is provided.

Introduction

Lachemilla Focke (Rydb.) is a morphologically highly variable group that includes perennial herbs, sub-shrubs, and shrubs. It comprises ca. 80 species and occurs between 2200 and 5000 m in elevation in the high mountains of the Neotropics, from northern Mexico to northern Argentina and Chile (Romoleroux 1996; Gaviria 1997), where it is one of the main elements of the páramo and superpáramo flora in South America. In Mexico the genus is represented by at least 10 species that can be found in sub-alpine and alpine habitats from the mountain pine forest to the high elevation zacotanales. *Lachemilla* has a nearly ubiquitous occurrence throughout the montane American tropics and remains a taxonomically

complex group where species boundaries are often unclear and the infrageneric taxonomy is poorly defined. Since the only comprehensive revision of *Lachemilla* (Perry 1929), several works have tried to clarify its taxonomy (Rothmaler 1935, 1937; Notov and Kusnetzova 2004) and recently several regional treatments have been published (Romoleroux 2004; Barrie 2015), but a complete revision of the group is still needed. Recent taxonomic work aiming to produce a monographic treatment of *Lachemilla* has resulted in the description of several new species (Romoleroux 2009; Romoleroux and Morales-Briones 2012).

Here, I describe and illustrate a new species of *Lachemilla* from Mexico. Material of the new species was collected in June 2015 during an expedition focusing solely on the genus *Lachemilla*. After detailed examination of the specimen, revision of species descriptions, and comparison with specimens at CAS, F, MEXU, MO, NY, TEX, and UC, it was established that the specimen collected in central Mexico represents a new species. Below I provide the taxonomic treatment of this new species, including a key to species of *Lachemilla* in Mexico.

Taxonomic treatment

***Lachemilla mexiquense* D.F. Morales-B. sp. nov.** (Figures 4.1, 4.2 A)

Diagnosis. *Lachemilla mexiquense* differs from *Lachemilla aphanoides* (Mutis ex L. f.) Rothm. by its caespitose and stoloniferous habit, creeping stems, basal leaves and basal stipule persistent, campanulate-elongate hypanthium and the presence of a single carpel.

Type. MEXICO, Estado de Mexico: Municipio Ocuilan, 4 km NE of Santa Martha on road Santa Martha– Huitzilac, 19.07567°N, 99.36215°W, alt. 3,050 m, 30 June, 2015, *Morales-Briones D. F. & Tenorio-Lezama P. 683.* (holotype: ID!; isotype: MEXU!, QCA!).

Description. Caespitose herbs, stoloniferous; stems creeping, mat-forming, branches sometimes rooting, pilose. Basal leaves 3-parted, 6 – 20 × 5 – 15 mm, chartaceous, lateral segments bifid, segments obovate to cuneate, margin incised-dentate, lower surface pilose, upper surface sparsely pilose to glabrescent; petioles 12 – 35 mm long; stipules 5 – 15 mm long, adnate to the petiole at base, free, entire and acute at apex, membranaceous, greenish-white. Stem leaves 3-parted, 7 – 12 × 4 – 7 mm, chartaceous, lateral lobes entire or bifid, segments obovate to cuneate, margin deeply cleft, lower surface pilose, upper surface sparsely pilose to glabrescent; petioles 3 – 5 mm long; stipules 3 – 8 mm long, adnate to the petiole at base, free at apex, 6-lobed at apex, membranaceous and greenish-white at base, chartaceous and green at apex. Inflorescences axillary or terminal, glomerulate, 6 – 10 flowered cymes; floral bracts lobed, spreading; pedicels 1 – 1.5 mm long, pilose at apex. Flowers 1.2 – 1.5 mm long; hypanthium campanulate-elongate 1 – 1.2 × 0.6 – 0.8 mm, glabrous outside, glabrous inside, green when young, reddish at maturity; episepals 4, ovate, 0.6 – 0.7 × 0.5 – 0.7 mm, glabrous, apex acute; sepals 4, lanceolate, 0.5 – 0.6 × 0.2 – 0.3 mm, glabrous, apex acute; stamens 2, filaments 0.2 – 0.3 mm long; carpels 1, stigma clavate. Achenes ovoid-globose, 0.9 – 1.1 × 0.6 – 0.8 mm, glabrous, one-seeded. Seeds ovate, 0.7 – 0.8 × 0.4 – 0.6 mm, pink, glabrous.

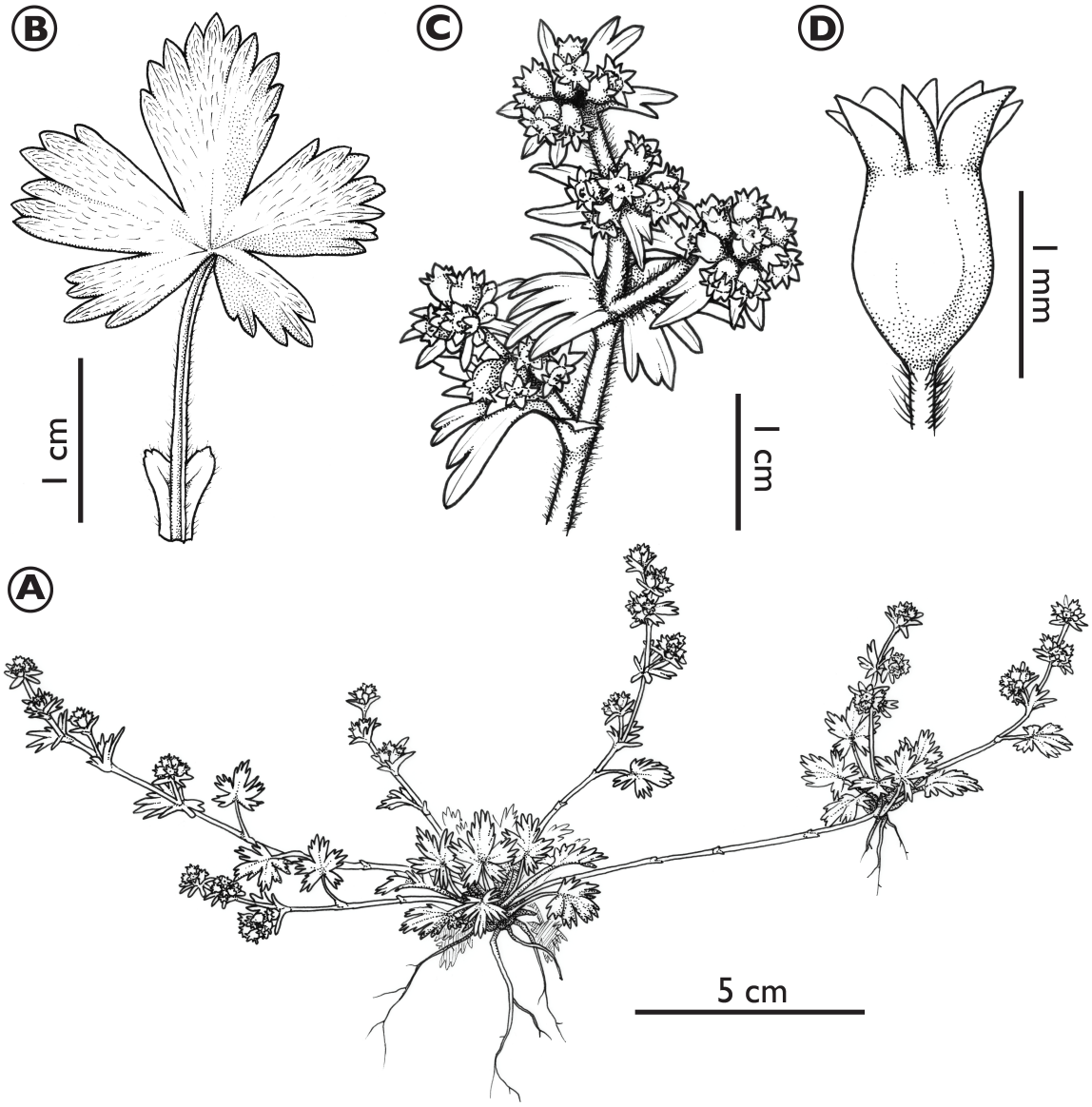

Figure 4.1. *Lachemilla mexiquense.* **A.** Habit **B.** Basal leaf and stipule **C.** Flowering branch **D.** Flower.

Distribution and Ecology. *Lachemilla mexiquense* is only known from the State of Mexico, municipality of Ocuilan, at ca. 3050 m altitude (Figure 4.2 B, 4.3). *Lachemilla mexiquense* grows at the border of dense forest of various species of *Pinus.* This species lives in sympatry with *Lachemilla procumbens* (Rose) Rydb., *L. vulcanica* (Schltdl. & Cham.) Rydb., and *L. aphanoides* (Mutis ex L. f.) Rothm. It was collected in flower and fruit in late June.

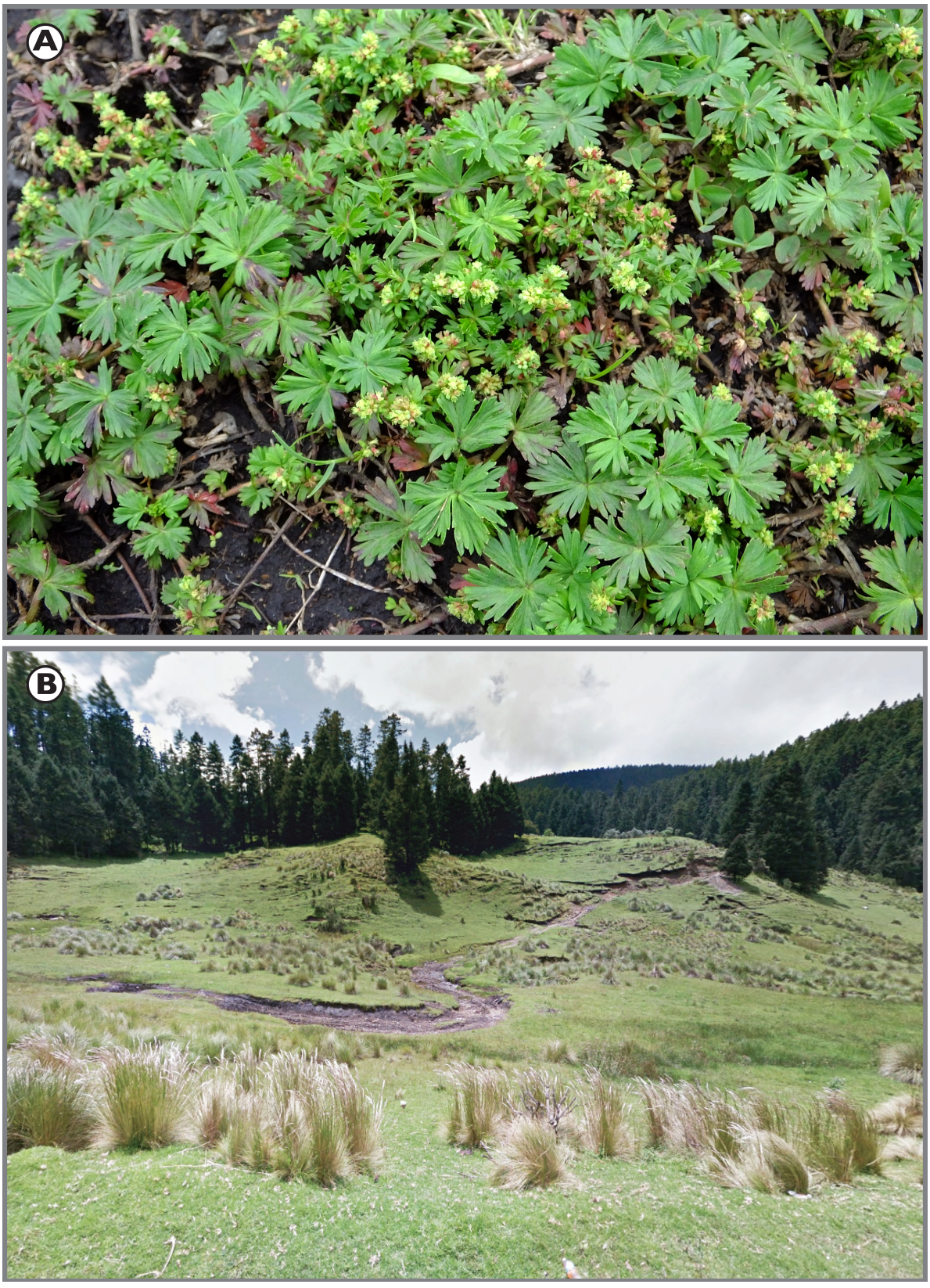

Figure 4.2. *Lachemilla mexiquense.* **A.** Habit **B.** Type locality.

Etymology. The specific epithet refers to the demonym for State of México where the type specimen was collected.

Conservation Status. *Lachemilla mexiquense* has a very limited geographic distribution, and is only known from the type locality (Figures 4.2 B, 4.3). It occurs right outside the limits of the Cumbres del Ajusco National Park and Lagunas de Zempoala National Park. The type locality has been severely impacted by human activities, including conversion to agriculture (sheep and cow grazing). Following the IUCN (2014) guidelines, based on the reduced geographic distribution and altered land use at the type locality, this species should be categorized as endangered (EN), at least until other populations are discovered.

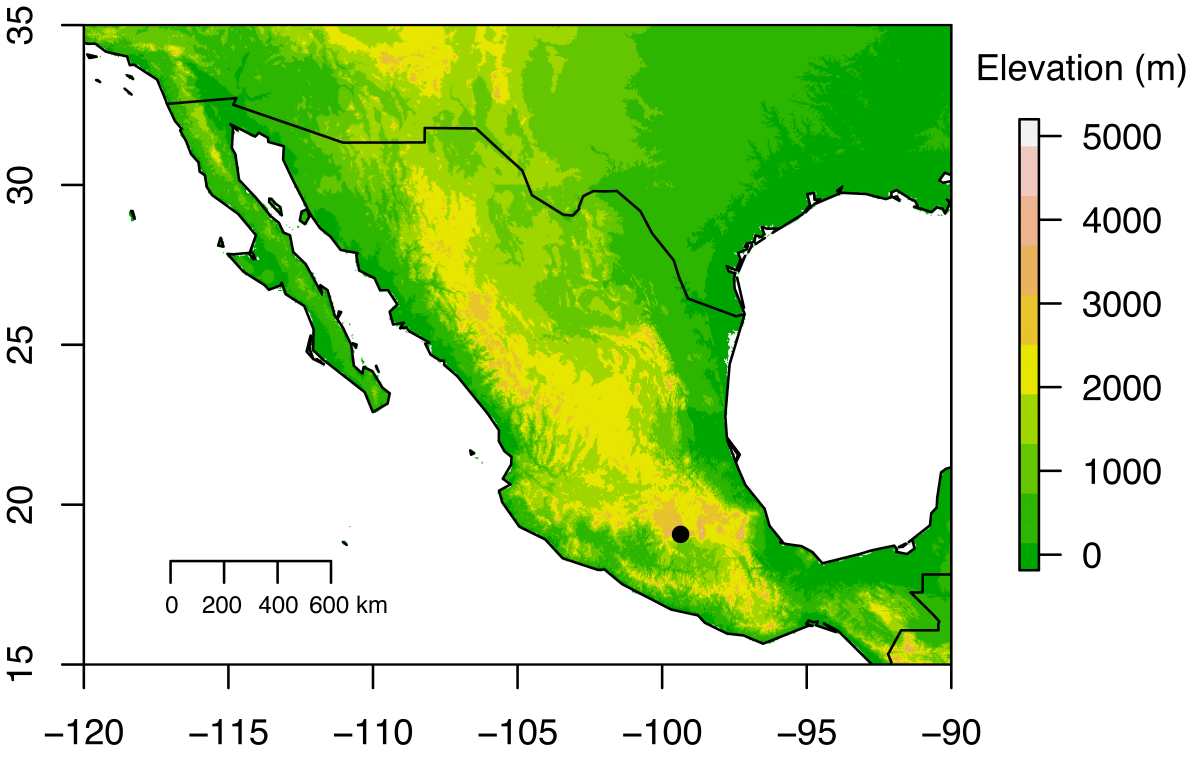

Figure 4.3. Geographic distribution of *Lachemilla mexiquense*.

Notes. *Lachemilla mexiquense* resembles *L. aphanoides* by having tripartite leaves with bifid lateral segments and glomerulate inflorescence with entirely glabrous flowers. Nevertheless, *L. mexiquense* differs from *L. aphanoides* by its caespitose habit, creeping stems, and stolons that form dense mats. Also, the basal leaves and basal stipules are persistent, and flowers are smaller (1.2 – 1.5 mm long) with a campanulate-elongate hypanthium and single carpel. *Lachemilla rupestris* (Kunth) Rothm, a species from Andean South America with similar habit, differs from *L. mexiquense* by having entire lateral segments of the leaves, yellow-brown membranaceuos basal stipules, and larger flowers (2.5 – 3 mm long) with a turbinate-campanulate hypanthium, sericeous-hirsute pubescence, and 2 – 4 carpels.

In addition, phylogenetic analyses of chloroplast and nuclear DNA clearly separate *L. mexiquense* from *L. aphanoides* and *L. rupestris* (Morales-Briones et al. unpubl. data). The chloroplast phylogeny place it as sister species of the 'Orbiculate group,' which encompasses species with stoloniferous habit, palmately lobed or cleft leaves, and flowers disposed in profuse terminal cymes, like *Lachemilla pectinata* (Kunth) Rothm. The nuclear phylogeny fails to confidently resolve the phylogenetic position of *L. mexiquense*, suggesting that it may be of hybrid origin, a common pattern seen throughout *Lachemilla*.

Key to the species of *Lachemilla* in Mexico

Notes. Adapted from Standley and Steyermark (1946), Romoleroux (2004), and Barrie (2015). Accepted taxa and synonymy follows the regional revisions of Romoleroux (2004) and Barrie (2015), with the exception of *L. sibbaldiifolia* (Kunth)

Rydb. and *L. pringlei* Rydb., which based on extensive field observations and the examination of herbarium material, are considered here as two distinct taxa.

1 Leaves pinnately divided.....***L. pinnata***

– Leaves simple or palmately divided or cleft.....**2**

2 Basal leaves 5 – 11-lobed or 5 – 7-cleft.....**3**

– Basal leaves 3 – 5-cleft or 3 – 5-parted.....**4**

3 Leaves shallowly 5 – 11-lobed, lobes triangular.....***L. pectinata***

– Leaves deeply 5 – 7-cleft, lobes elliptical to ovate.....***L. venusta***

4 Inflorescence of loose cymes; hypanthium pubescent within.....**5**

– Inflorescence glomerulate, forming dense cymes; hypanthium glabrous within.....**6**

5 Leaves 3-parted with bifid lateral segments, appearing 5-parted; stipules lobed or incised-dentate.....***L. procumbens***

– Leaves 3-parted with entire lateral segments, not appearing 5-parted; stipules bifid.....***L. vulcanica***

6 Plants pilose to glabrate; flowers glabrous.....**7**

– Plants hirsute to sericeous; flowers pubescent, sometimes glabrate with age.....**8**

7 Stems decumbent, ascending or erect; basal leaves and basal stipules often caduceus; flowers 1.5 – 3.0 mm; 1 – 3 carpels.....***L. aphanoides***

– Stems creeping, stoloniferous; basal leaves and basal stipules persistent; flowers 1.2 – 1.5 mm; 1 carpel.....***L. mexiquense***

8 Hypanthium densely pubescent with very short hairs; lower leaves short-petioled, the upper leaves sessile.....***L. velutina***

– Hypanthium sparingly pubescent with appressed hairs, lower and upper leaves petioled.....**9**

9 Leaves appearing 5-lobed, the lateral lobes bifid; achenes subacute or subobtuse.....***L. sibbaldiifolia***

– Leaves appearing 3-lobed, the lateral lobes not bifid; achenes tapering to an acute apex.....***L. pringlei***

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APPENDICES

Appendix 1

Voucher information for Chapter 1.

Arranged by: Species name and authority, DNA code, collector & number (herbarium code), nrITS, and *trnL-trnF* GenBank accession numbers. An asterisk (*) indicates sequence for the subsequent region was not obtained. For sequences obtained directly from GenBank only GenBank accession numbers is provided.

Aphanes cotopaxiensis Romol. & Frost-Olsen: 2013_004_EC, D.F. *Morales-Briones et al. 276* (ID), KY351933, KY351828; *Aphanes occidentalis* (Nutt.) Rydb.: 2012_225_US, *D.C. Tank 1107* (ID), KY351934, KY351829; *Lachemilla adscendens* Rothm.: 2014_491_CO, *D.F. Morales-Briones & S. Uribe-Convers 497* (ID), KY351935, KY351830; *Lachemilla andina* (L.M. Perry) Rothm.: 2012_249_PE, *D.F. Morales-Briones & S. Uribe-Convers 218* (ID), KY351936, KY351831, 2012_379_EC, *D.F. Morales-Briones & K. Romoleroux 162* (QCA), KY351937, KY351832, 2012_401_EC, *D.F. Morales-Briones et al 197* (QCA), *, KY351833, 2013_440_CO, *D.F. Morales-Briones et al. 424* (ID), KY351938, KY351834; *Lachemilla angustata* Romol.: 2012_320_EC, *K. Romoleroux et al. 4697* (QCA), KY351939, KY351835; *Lachemilla aphanoides* (Mutis ex L. f.) Rothm.: 2012_246_PE, *D.F. Morales-Briones & S. Uribe-Convers 215* (ID), KY351940, KY351836, 2012_311_EC, *K. Romoleroux et al. 4471* (QCA), KY351941, KY351837, 2012_360_EC, *K. Romoleroux et al. 5298A* (QCA), KY351942, KY351838, 2012_378_EC, *D.F. Morales-Briones & K. Romoleroux 159* (QCA), KY351943, *, 2012_383_EC, *D.F. Morales-Briones & K.*

Romoleroux 170 (QCA), KY351944, *, 2012_388_EC, *D.F. Morales-Briones et al. 130* (QCA), KY351945, KY351839, 2012_396_EC, *D.F. Morales-Briones et al. 115* (QCA), *, KY351840, 2013_416_BO, *D.F. Morales-Briones et al. 311* (ID), KY351946, KY351841, 2013_442_CO, *D.F. Morales-Briones et al. 426* (ID), KY351947, KY351842; 2015_149_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 594* (ID), KY351948, KY351843, *Lachemilla barbata* (C. Presl) Rothm.: 2012_305_PE, *D.F. Morales-Briones & S. Uribe-Convers 274* (ID), KY351949, KY351844, 2013_406_BO, *D.F. Morales-Briones et al. 302* (ID), KY351950, KY351845; *Lachemilla bipinnatifida* (L.M. Perry) Rothm.: 2013_428_BO, *D.F. Morales-Briones et al. 326* (ID), KY351952, KY351847, 2013_386_BO, *D.F. Morales-Briones et al. 282* (ID), KY351951, KY351846; *Lachemilla diplophylla* (Diels) Rothm.: 2012_237_PE, *D.F. Morales-Briones & S. Uribe-Convers 206* (ID), KY351953, KY351848, 2012_357_EC, D.F. *Morales-Briones & E. Morales-Checa 30* (QCA), KY351954, KY351849, 2013_382_BO, *D.F. Morales-Briones et al. 278* (ID), KY351955, KY351850; *Lachemilla equisetiformis* (Trevis.) Rothm.: 2012_108_VE, *A.J.P Martin & M. Lind van Wijngaarden 740* (QCA), KY351956, KY351851; *Lachemilla erodiifolia* (Wedd.) Rothm.: 2012_296_PE, *D.F. Morales-Briones & S. Uribe-Convers 265* (ID), KY351957, KY351852, 2012_372_EC, *D.F. Morales-Briones & K. Romoleroux 119* (QCA), KY351958, KY351853, 2013_401_BO, *D.F. Morales-Briones et al. 297* (ID), KY351959, KY351854, 2014_483_CO, *D.F. Morales-Briones et al. 489* (ID), *, KY351855; *Lachemilla frigida* (Wedd.) Rothm.: 2013_409_BO, *D.F. Morales-Briones et al. 305* (ID), KY351961, KY351857, 2012_243_PE, *D.F. Morales-Briones & S. Uribe-Convers 212* (ID), KY351960, KY351856; *Lachemilla fulvescens* (L.M. Perry) Rothm.: 2012_368_EC, *D.F. Morales-Briones et al. 128* (QCA), KY351962, KY351858, 2012_375_EC, *D.F. Morales-Briones & K. Romoleroux 164* (QCA), KY351963, KY351859, 2012_408_EC, *D.F. Morales-Briones et al. 151* (QCA), *, KY351860, 2013_450_CO, *D.F. Morales-Briones et*

al. 434 (ID), KY351964, KY351861; *Lachemilla galioides* (Benth.) Rothm.: 2012_389_EC, *D.F. Morales-Briones et al. 109* (QCA), KY351965, KY351862; *Lachemilla hirta* (L.M. Perry) Rothm.: 2012_317_EC, *K. Romoleroux et al. 4588* (QCA), KY351966, KY351863, 2014_475_CO, *D.F. Morales-Briones et al. 481* (ID), KY351967, KY351864; *Lachemilla hispidula* (L.M. Perry) Rothm.: 2012_324_EC, *K. Romoleroux et al. 4703* (QCA), KY351968, KY351865, 2013_457_CO, *D.F. Morales-Briones et al. 441* (ID), KY351969, KY351866, 2013_478_CO, *D.F. Morales-Briones et al. 462* (ID), KY351970, KY351867; *Lachemilla holmgrenii* Rothm.: 2012_347_EC, *K. Romoleroux et al. 4397* (QCA), KY351971, KY351868; *Lachemilla holosericea* (L.M. Perry) Rothm.: 2012_314_EC, *K. Romoleroux et al. 4528* (QCA), KY351972, KY351869, 2014_479_CO, *D.F. Morales-Briones et al. 485* (ID), KY351973, KY351870; *Lachemilla jamesonii* (L.M. Perry) Rothm.: 2012_318_EC, *K. Romoleroux et al. 4684* (QCA), *, KY351871; *Lachemilla jaramilloi* Romol. & D.F. Morales-B.: 2012_371_EC, *D.F. Morales-Briones et al. 126* (QCA), KY351974, KY351872, 2014_557_CO, *D.F. Morales-Briones & S. Uribe-Convers 566* (ID), KY351975, KY351873; *Lachemilla llanganatensis* Romol.: 2012_363_EC, *D.F. Morales-Briones et al. 63* (QCA), KY351976, KY351874; *Lachemilla mandoniana* (Wedd.) Rothm.: 2012_261_PE, *D.F. Morales-Briones & S. Uribe-Convers 230* (ID), KY351977, KY351875, 2012_335_CR, *K. Romoleroux et al. 5010* (QCA), KY351978, KY351876, 2012_400_EC, *D.F. Morales-Briones et al. 103* (QCA), KY351979, KY351877, 2013_454_CO, *D.F. Morales-Briones et al. 438* (ID), KY351980, KY351878; *Lachemilla mexiquense* D.F. Morales-B.: 2015_233_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 683* (ID), KY351981, KY351879; *Lachemilla moritziana* Dammer : 2013_443_CO, *D.F. Morales-Briones et al. 427* (ID), KY351982, KY351880; *Lachemilla nivalis* (Kunth) Rothm.: 2012_316_EC, *K. Romoleroux et al. 4580* (QCA), KY351983, KY351881, 2012_384_EC, *D.F. Morales-Briones et al. 138* (QCA), KY351984, KY351882; *Lachemilla*

orbiculata (Ruiz & Pav.) Rydb.: 2012_258_PE, *D.F. Morales-Briones & S. Uribe-Convers 227* (ID), KY351985, KY351883, 2012_404_EC, *D.F. Morales-Briones et al. 111* (QCA), KY351986, KY351885, 2014_477_CO, *D.F. Morales-Briones et al. 483* (ID), KY351987, KY351886, 2012_292_PE, *D.F. Morales-Briones & S. Uribe-Convers 261* (ID), *, KY351884; *Lachemilla pectinata* (Kunth) Rothm.: 2012_326_EC, K. *Romoleroux et al. 4706* (QCA), KY351988, KY351887, 2012_351_CR, *K. Romoleroux et al. 5020* (QCA), KY351989, KY351888, 2012_376_EC, *D.F. Morales-Briones & K. Romoleroux 161* (QCA), KY351990, KY351889, 2013_388_BO, *D.F. Morales-Briones et al. 284* (ID), KY351991, *, 2014_547_CO, *D.F. Morales-Briones & S. Uribe-Convers 556* (ID), KY351992, KY351890, 2015_175_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 621* (ID), KY351993, KY351891; *Lachemilla perryana* (Rothm.) Rothm.: 2012_380_EC, *D.F. Morales-Briones & K. Romoleroux 167* (QCA), KY351994, KY351892; *Lachemilla pinnata* (Ruiz & Pav.) Rothm.: 2012_230_PE, *D.F. Morales-Briones & S. Uribe-Convers 199* (ID), KY351995, KY351893, 2012_391_EC, *D.F. Morales-Briones et al. 113* (QCA), KY351996, KY351894, 2013_426_BO, *D.F. Morales-Briones et al. 324* (ID), KY351997, KY351895, 2013_448_CO, *D.F. Morales-Briones et al. 432* (ID), KY351998, KY351896, 2015_218_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 666* (ID), KY351999, KY351897; *Lachemilla polylepis* (Wedd.) Rothm.: 2012_359_CO, *P. Sklenář 12207* (QCA), KY352000, KY351898; *Lachemilla pringlei* Rydb.: 2015_150_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 595* (ID), KY352001, KY351899, 2015_163_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 608* (ID), KY352002, KY351900; *Lachemilla procumbens* (Rose) Rydb.: 2012_332_CR, *K. Romoleroux et al. 5011* (QCA), KY352003, KY351901, 2015_141_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 583* (ID), KY352004, KY351902; *Lachemilla pseudovenusta* Rothm.: 2012_275_PE, *D.F. Morales-Briones & S. Uribe-Convers 244* (ID), KY352005, KY351903; *Lachemilla purdiei* (L.M.

Perry) Rothm. : 2014_501_CO, *D.F. Morales-Briones & S. Uribe-Convers 508* (ID), KY352006, KY351904; *Lachemilla ranunculoides* (L.M. Perry) Rothm.: 2012_240_PE, *D.F. Morales-Briones & S. Uribe-Convers 209* (ID), KY352007, KY351905; *Lachemilla rupestris* (Kunth) Rothm.: 2012_270_PE, *D.F. Morales-Briones & S. Uribe-Convers 239* (ID), KY352008, KY351906, 2012_329_EC, *K. Romoleroux et al. 4714* (QCA), KY352009, KY351907, 2012_342_EC, *D.F. Morales-Briones & M.F. Latorre-Barragán. 8* (QCA), KY352010, *, 2012_394_EC, *D.F. Morales-Briones et al. 105* (ID), KY352011, *; *Lachemilla sarmentosa* (L.M. Perry) Rothm.: 2013_396_BO, *D.F. Morales-Briones et al. 292* (ID), KY352012, KY351908, 2013_414_BO, *D.F. Morales-Briones et al. 310* (ID), KY352013, KY351909; *Lachemilla sibbaldiifolia* (Kunth) Rydb.: 2015_154_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 599* (ID), KY352014, KY351910, 2015_203_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 651* (ID), KY352015, KY351911; *Lachemilla sp.*: 2014_476_CO, *D.F. Morales-Briones et al. 482* (ID), KY352016, KY351913, 2013_438_CO, *D.F. Morales-Briones et al. 422* (ID), *, KY351912; *Lachemilla sprucei* (L.M. Perry) Rothm.: 2012_405_EC, *D.F. Morales-Briones et al. 141* (QCA), KY352017, KY351914; *Lachemilla steinbachii* Rothm.: 2013_415_BO, *D.F. Morales-Briones et al. 312* (ID), KY352018, KY351915; *Lachemilla talamanquensis* Romol. & D.F. Morales-B.: 2012_334_CR, *K. Romoleroux et al. 5008* (QCA), KY352019, KY351916; *Lachemilla tanacetifolia* Rothm. : 2012_331_EC, *K. Romoleroux et al. 4396* (QCA), KY352020, KY351917, 2013_488_CO, *D.F. Morales-Briones et al. 472* (ID), KY352021, KY351918; *Lachemilla uniflora* Maguire: 2012_374_EC, *D.F. Morales-Briones & K. Romoleroux 163* (QCA), KY352022, KY351919, 2014_556_CO, *D.F. Morales-Briones & S. Uribe-Convers 565* (ID), *, KY351920; *Lachemilla velutina* (S. Watson) Rydb.: 2015_162_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 607* (ID), KY352023, KY351921, 2015_169_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 615* (ID), KY352024, KY351922; *Lachemilla*

venusta (Schltdl. & Cham.) Rydb.: 2015_157_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 602* (ID), *, KY351923, 2015_204_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 652* (ID), KY352025, KY351924, 2015_208_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 656* (ID), KY352026, KY351925; *Lachemilla verticillata* (Fielding & Gardner) Rothm.: 2012_339_CR, *K. Romoleroux et al. 5007* (QCA), KY352027, KY351926; *Lachemilla vulcanica* (Schltdl. & Cham.) Rydb.: 2012_282_PE, *D.F. Morales-Briones & S. Uribe-Convers 251* (ID), KY352028, KY351927, 2012_398_EC, *D.F. Morales-Briones et al. 106* (QCA), KY352029, KY351928, 2013_410_BO, *D.F. Morales-Briones et al. 306* (ID), KY352030, KY351929, 2014_488_CO, *D.F. Morales-Briones & S. Uribe-Convers 494* (ID), *, KY351930, 2015_171_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 617* (ID), KY352031, KY351931; *Potentilla dombeyi* Nestl.: 2012_333_EC, *K. Romoleroux et al. 4579* (QCA), HM453948, *; *Sibbaldia procumbens* L.: 2015_145_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 590* (ID), KY352032, KY351932; *Alchemilla alpina* L.: EU072508, EU072595; *Alchemilla andringitrensis* R. Vig. & De Wild.: EU072509, EU072596; *Alchemilla argyrophylla* Oliv.: EU072512, EU072599; *Alchemilla colorata* Buser: EU072516, EU072603; *Alchemilla cryptantha* Steud. ex A. Rich.: EU072520, EU072607; *Alchemilla decumbens* Buser: EU072521, EU072608; *Alchemilla fallax* Buser ex Besse: EU072528, EU072616; *Alchemilla glacialis* Buser: EU072562, EU072619; *Alchemilla johnstonii* Oliv.: EU072544, EU072632; *Alchemilla mollis* (Buser) Rothm.: EU072550, EU072638; *Alchemilla pentaphyllea* L.: EU072554, EU072643; *Alchemilla saxatilis* Buser: EU072559, EU072645; *Alchemilla vetteri* Buser: EU072571, EU072656; *Alchemilla vulgaris* L.: EU072573, EU072657; *Alchemilla woodii* Kuntze: EU072574, EU072658; *Aphanes arvensis* L.: AJ511770, AJ512234; Aphanes cornucopioides Lag.: EU072576, EU072660; Aphanes floribunda Rothm.: *, EU072661; *Aphanes inexspectata* W. Lippert: EU072577, EU072662; *Aphanes*

minutiflora (Azn.) Holub: EU072578, EU072663; *Aremonia agrimonoides* (L.) DC.: U90799, AJ512230; *Argentina anserina* (L.) Rydb.: AJ511773, AJ512238; *Chamaerhodos erecta* (L.) Bunge: U90794, AJ512219; *Chamaerhodos mongholica* Bunge: FJ356155, FJ422285; *Chamaerhodos nuttallii* Pickering ex Rydb.: FJ356156, FJ422286; *Chamaerhodos sabulosa* Bunge: FJ356157, *; *Comarum palustre* L.: EU072580, EU072665; *Dasiphora davurica* Kom.: FJ356159, FJ422287; *Dasiphora fruticosa* (L.) Rydb.: U90808, AJ512233; *Dasiphora phyllocalyx* Juz.: FJ356160, FJ422288; *Drymocallis agrimonoides* (Pursh) Rydb.: *, FJ422289; *Drymocallis arguta* (Pursh) Rydb.: U90787, AJ512223; *Drymocallis corsica* (Soleirol ex Lehm.) Kurtto: FJ356161, FJ422290; *Drymocallis glutinosa* Rydb.: FJ356162, FJ422291; *Drymocallis rupestris* (L.) Soják : FJ356163, FJ422292; *Farinopsis salesoviana* (Stephan) Chrtek & Soják: AJ511779, AJ512228; *Fragaria* × *ananassa* Duchesne ex Rozier: FJ356167, FJ422296; *Fragaria chiloensis* (L.) Mill.: FJ356164, FJ422293; *Fragaria moschata* Weston : FJ356165, FJ422294; *Fragaria vesca* L.: AJ511771, AJ512232; *Fragaria virginiana* Mill.: AJ511772, AJ512220; *Fragaria viridis* Weston: FJ356166, FJ422295; *Hagenia abyssinica* J.F. Gmel.: U90800, AY634727; *Piletophyllum micropetalum* (D.Don) Soják: KF954771, KJ020641; *Polylepis sericea* Wedd.: AY635002, AY634756; *Potaninia mongolica* Maxim.: AM286742, AM286743; *Potentilla alba* L.: FN430774, FN556397; *Potentilla biflora* D.F.K. Schltdl.: KJ396292, KJ396304; *Potentilla nitida* L.: KJ396295, KJ396307; *Potentilla recta* L.: FN430784, FN556419; *Potentilla reptans* L.: U90784, AJ512241; *Potentilla stipularis* L.: KJ396296, KJ396308; *Rosa acicularis* Lindl.: HM593902, DQ778822; *Rosa caudata* Baker: HM593912, DQ778841; *Rosa cymosa* Tratt.: HM593924, DQ778846; *Rosa foetida* Herrm.: AB035653, DQ778850; *Rosa majalis* Herrm.: U90801, AJ512229; *Rosa persica* Michx.: AB035657, DQ778878; *Rubus chamaemorus* L.: U90803, AJ416464; *Sanguisorba officinalis* L.: *, AJ416465; *Sanguisorba parviflora* (Maxim.) Takeda: U90797, *;

Schistophyllidium bifurcum (L.) Ikonn.: U90786, AJ512224; *Sibbaldia cuneata* Hornem. ex Kuntze: FJ356173, FJ422301; *Sibbaldia parviflora* Willd.: FJ356174, FJ422302; *Sibbaldia procumbens* L.: EU072593, EU072680; *Sibbaldia retusa* (O.F. Müll.) T. Erikss.: U90791, AJ512236; *Sibbaldia semiglabra* C.A. Mey.: FJ356175, FJ422303; *Sibbaldianthe adpressa* (Bunge) Juz.: FJ356176, FJ422304; *Sibbaldiopsis cuneifolia* (Bertol.) Soják: FJ356169, FJ422298; *Tylosperma lignosa* Botsch.: FJ356171, FJ422299.

Appendix 2

Flow cytometry genome size estimates of *Lachemilla* from Chapter 1.

Arranged by: Species name and authority, collector and number (herbarium code), and genome size in picograms (μg).

Lachemilla andina (L.M. Perry) Rothm.: *D.F. Morales-Briones & K. Romoleroux 162* (QCA), 1.77, *D.F. Morales-Briones & K. Romoleroux 169* (QCA), 1.73, *D.F. Morales-Briones et al 197* (QCA), 1.77; *Lachemilla aphanoides* (Mutis ex L. f.) Rothm.: *D.F. Morales-Briones 46* (QCA), 1.34, *D.F. Morales-Briones et al. 114* (QCA), 1.25, *D.F. Morales-Briones et al. 115* (QCA), 1.36, *D.F. Morales-Briones et al. 130* (QCA), 1.96, *D.F. Morales-Briones et al. 131* (QCA), 1.78, *D.F. Morales-Briones et al. 155* (QCA), 1.37, *D.F. Morales-Briones & K. Romoleroux 159* (QCA), 1.36, *D.F. Morales-Briones & K. Romoleroux 170* (QCA), 1.71; *Lachemilla diplophylla* (Diels) Rothm.: *D.F. Morales-Briones & E. Morales-Checa. 40* (QCA), 1.36; *Lachemilla erodiifolia* (Wedd.) Rothm.: *D.F. Morales-Briones et al. 116* (QCA), 1.11, *D.F. Morales-Briones & K. Romoleroux 119* (QCA), 1.09, *D.F. Morales-Briones et al. 153* (QCA), 1.65; *Lachemilla fulvescens* (L.M. Perry) Rothm.: *D.F. Morales-Briones et al. 128* (QCA), 1.60, *D.F. Morales-Briones et al. 129* (QCA), 1.60, *D.F. Morales-Briones et al. 151* (QCA), 1.75; *D.F. Morales-Briones & K. Romoleroux 164* (QCA), 2.10, *Lachemilla galioides* (Benth.) Rothm.: *D.F. Morales-Briones et al. 109* (QCA), 1.99; *Lachemilla hirta* (L.M. Perry) Rothm.: *D.F. Morales-Briones et al. 118* (QCA), 1.51, *D.F. Morales-Briones et al. 148* (QCA), 1.47; *Lachemilla hispidula* (L.M. Perry) Rothm.: *D.F. Morales-Briones et al. 110* (QCA), 1.38, *D.F. Morales-Briones et al. 133* (QCA), 1.40, *D.F. Morales-Briones et al. 136* (QCA), 1.38, *D.F. Morales-Briones et al.*

139 (QCA), 1.40, *D.F. Morales-Briones et al. 144* (QCA), 1.44; *Lachemilla holmgrenii* Rothm.: *D.F. Morales-Briones et al. 105* (QCA), 1.92; *Lachemilla holosericea* (L.M. Perry) Rothm.: *D.F. Morales-Briones et al. 123* (QCA), 1.87, *D.F. Morales-Briones et al. 124* (QCA), 1.86, *D.F. Morales-Briones 149* (QCA), 1.85; *Lachemilla jaramilloi* Romol. & D.F. Morales-B.: *D.F. Morales-Briones et al. 126* (QCA), 2.41; *Lachemilla llanganatensis* Romol.: *P. Sklenář 13120* (PRC), 1.42; *Lachemilla mandoniana* (Wedd.) Rothm.: *D.F. Morales-Briones et al. 103* (QCA), 1.11, *D.F. Morales-Briones et al. 112* (QCA), 1.10, *D.F. Morales-Briones et al. 120* (QCA), 1.87, *D.F. Morales-Briones et al. 125* (QCA), 1.16, *D.F. Morales-Briones et al. 127* (QCA), 1.83, *D.F. Morales-Briones 150* (QCA), 1.13; *Lachemilla nivalis* (Kunth) Rothm.: *D.F. Morales-Briones et al. 134* (QCA), 1.42, *D.F. Morales-Briones et al. 135* (QCA), 1.35, *D.F. Morales-Briones et al. 137* (QCA), 2.06, *D.F. Morales-Briones et al. 138* (QCA), 2.07, *D.F. Morales-Briones et al. 140* (QCA), 2.08, *D.F. Morales-Briones et al. 152* (QCA), 1.36 *P. Sklenář 13124* (PRC), 1.35; *Lachemilla orbiculata* (Ruiz & Pav.) Rydb.: *D.F. Morales-Briones et al. 108* (QCA), 1.42, *D.F. Morales-Briones et al. 111* (QCA), 1.42, *D.F. Morales-Briones & K. Romoleroux 143* (QCA), 1.39, *D.F. Morales-Briones et al. 156* (QCA), 1.85, *D.F. Morales-Briones et al. 157* (QCA), 1.82; *Lachemilla pectinata* (Kunth) Rothm.: *D.F. Morales-Briones et al. 147* (QCA), 1.82, *D.F. Morales-Briones et al. 158* (QCA), 1.97, *D.F. Morales-Briones & K. Romoleroux 161* (QCA), 1.92, *D.F. Morales-Briones & K. Romoleroux 171* (QCA), 1.91; *Lachemilla pinnata* (Ruiz & Pav.) Rothm.: *D.F. Morales-Briones et al. 113* (QCA), 1.41, D.F. *Morales-Briones & E. Morales-Checa. 145* (QCA), 1.42; *Lachemilla rupestris* (Kunth) Rothm.: *D.F. Morales-Briones et al. 105* (QCA), 2.24, *D.F. Morales-Briones et al. 146* (QCA), 2.20; *Lachemilla sprucei* (L.M. Perry) Rothm.: *D.F. Morales-Briones et al. 141* (QCA), 1.84; *Lachemilla tanacetifolia* Rothm.: *D.F. Morales-Briones et al. 107* (QCA), 1.59, *D.F. Morales-Briones et al. 122* (QCA), 2.38, P. *Sklenář 13147* (PRC), 2.25, *P.*

Sklenář 13171 (PRC), 1.59; *Lachemilla uniflora* Maguire: D.F. *Morales-Briones et al. 121* (QCA), 1.17, *D.F. Morales-Briones 142* (QCA), 1.88, *D.F. Morales-Briones & K. Romoleroux 163* (QCA), 1.20; *Lachemilla vulcanica* (Schltdl. & Cham.) Rydb.: *D.F. Morales-Briones et al. 106* (QCA), 1.98, *D.F. Morales-Briones et al. 117* (QCA), 1.85, *D.F. Morales-Briones et al. 154* (QCA), 1.82.

Appendix 3

Expanded Figure 1.3 from Chapter 1.

A. Bayesian 50% majority rule consensus tree of the *trnL-F* chloroplast region. Node labels represent posterior probabilities.

B. Maximum likelihood tree of the *trnL-F* chloroplast region. Node labels represent bootstrap support values.

C. Bayesian 50% majority rule consensus tree of the nuclear ribosomal ITS region. Node labels represent posterior probabilities.

D. Maximum likelihood tree of the nuclear ribosomal ITS region. Node labels represent bootstrap support values.

A


Lachemilla venusta 2015 208 MX
Lachemilla steinbachii 2013 415 BO
Lachemilla sibbaldiaefolia 2015 203 MX
Lachemilla sibbaldiaefolia 2015 154 MX
Lachemilla pseudovenusta 2012 275 PE
Lachemilla pringlei 2015 163 MX
Lachemilla pringlei 2015 150 MX
Lachemilla moritziana 2013 443 CO
Lachemilla venusta 2015 204 MX
Lachemilla aphanoides 2012 396 EC
Lachemilla aphanoides 2012 311 EC
Lachemilla ranunculoides 2012 240 PE
Lachemilla bipinnatifida 2013 428 BO
Lachemilla aphanoides 2012 360 EC
Lachemilla velutina 2015 159 MX
Lachemilla velutina 2015 162 MX
Lachemilla aphanoides 2013 416 BO
Lachemilla aphanoides 2012 388 EC
Lachemilla aphanoides 2012 246 PE
Lachemilla aphanoides 2015 149 MX
Lachemilla aphanoides 2013 442 CO
Lachemilla vulcanica 2015 171 MX
Lachemilla vulcanica 2014 468 CO
Lachemilla vulcanica 2013 410 BO
Lachemilla vulcanica 2012 398 EC
Lachemilla vulcanica 2012 282 PE
Lachemilla jamesonii 2012 318 EC
Lachemilla andina 2012 379 EC
Lachemilla procumbens 2015 141 MX
Lachemilla procumbens 2012 332 CR
Lachemilla hirta 2014 475 CO
Lachemilla hirta 2012 317 EC
Lachemilla andina 2013 440 CO
Lachemilla andina 2012 401 EC
Lachemilla andina 2012 249 PE
Lachemilla vertiginata 2012 339 CR
Lachemilla talamancaensis 2012 334 CR
Lachemilla hispidula 2013 457 CO
Lachemilla polylepis 2012 359 CO
Lachemilla erodiifolia 2013 401 BO
Lachemilla erodiifolia 2012 372 EC
Lachemilla erodiifolia 2012 296 PE
Lachemilla diplophylla 2013 382 BO
Lachemilla diplophylla 2012 357 EC
Lachemilla diplophylla 2012 237 PE
Lachemilla barbata 2013 406 BO
Lachemilla barbata 2012 305 PE
Lachemilla pinnata 2015 218 MX
Lachemilla pinnata 2013 448 CO
Lachemilla pinnata 2012 391 EC
Lachemilla pinnata 2012 233 PE
Lachemilla mandoniana 2013 454 CO
Lachemilla mandoniana 2012 400 EC
Lachemilla mandoniana 2012 335 CR
Lachemilla mandoniana 2012 261 PE
Lachemilla tanacetifolia 2013 448 CO
Lachemilla tanacetifolia 2012 391 EC
Lachemilla jaramilloi 2014 557 CO
Lachemilla jaramilloi 2012 371 EC
Lachemilla erodiifolia 2014 483 CO
Lachemilla frigida 2012 243 PE
Lachemilla sarmentosa 2013 414 BO
Lachemilla sarmentosa 2013 396 BO
Lachemilla pinnata 2013 426 BO
Lachemilla frigida 2013 409 BO
Lachemilla bipinnatifida 2013 386 BO
Lachemilla adscendens 2014 491 CO
Lachemilla holmgrenii 2012 347 EC
Lachemilla hispidula 2012 324 EC
Lachemilla galioides 2012 388 EC
Lachemilla angustata 2012 320 EC
Lachemilla rupestris 2012 329 EC
Lachemilla rupestris 2012 270 PE
Lachemilla hispidula 2013 478 CO
Lachemilla rivalis 2012 316 EC
Lachemilla rivalis 2012 364 EC
Lachemilla llanganatensis 2012 363 EC
Lachemilla spruceli 2012 405 EC
Lachemilla holosericea 2012 314 EC
Lachemilla aequiseticrinis 2012 108 VE
Lachemilla purdiei 2014 501 CO
Lachemilla sp nov 2013 438 CO
Lachemilla holosericea 2014 479 CO
Lachemilla sp nov 2014 476 CO
Lachemilla uniflora 2014 556 CO
Lachemilla uniflora 2012 374 EC
Lachemilla tulvescens 2013 450 CO
Lachemilla fulvescens 2012 368 EC
Lachemilla venusta 2015 175 MX
Lachemilla perryana 2012 380 EC
Lachemilla pectinata 2014 547 CO
Lachemilla pectinata 2012 376 EC
Lachemilla pectinata 2012 326 EC
Lachemilla fulvescens 2012 408 EC
Lachemilla fulvescens 2012 375 EC
Lachemilla orbiculata 2012 282 PE
Lachemilla orbiculata 2014 477 CO
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Lachemilla mexicuense 2015 233 MX
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Alchemilla cryptantha
Alchemilla johnstonii
Alchemilla woodii
Alchemilla argyrophylla
Alchemilla decumbens
Alchemilla fallax
Alchemilla vulgaris
Alchemilla colorata
Alchemilla vetteri
Alchemilla mollis
Alchemilla saxatilis
Alchemilla pentaphyllea
Alchemilla glacialis
Alchemilla alpina
Aphanes floribunda
Aphanes cornucopioides
Aphanes occidentalis 2012 225 US
Aphanes inexspectata
Aphanes cotopaxiensis 2013 004 EC
Aphanes arvensis
Aphanes minutiflora
Comarum palustre
Farinopsis salesoviana
Sibbaldia semiglabra
Sibbaldia procumbens
Sibbaldia retusa
Sibbaldia procumbens 2015 145 MX
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Sibbaldia cuneata
Sibbaldiopsis cuneifolia
Schistophyllidium bifurcum
Sibbaldianthe adpressa
Potaninia mongolica
Dasiphora davurica
Dasiphora phylocalyx
Dasiphora fruticosa
Drymocallis rupestris
Drymocallis corsica
Drymocallis arguta
Drymocallis glandulosa
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Chamaerhodos mongholica
Chamaerhodos erecta
Chamaerhodos nuttallii
Fragaria chiloensis
Fragaria ananassa
Fragaria virginiana
Fragaria vesca
Fragaria viridis
Fragaria moschata
Potentilla nitida
Potentilla alba
Potentilla biflora
Potentilla reptans
Potentilla stipularis
Potentilla recta
Piletophyllum micropetalum
Argentina anserina
Tylosperma lignosa
Rosa persica
Rosa foetida
Rosa cymosa
Rosa caudata
Rosa acicularis
Rosa majalis
Sanguisorba officinalis
Polylepis sericea
Hagenia abyssinica
Aremonia agrimonoides
Rubus chamaemorus
0.01

B


Lachemilla pringlei 2015 150 MX
Lachemilla aphanoides 2012 396 EC
Lachemilla pringlei 2015 183 MX
Lachemilla venusta 2015 208 MX
Lachemilla sibbaldiaefolia 2015 154 MX
Lachemilla pseudovenusta 2012 276 PE
Lachemilla sibbaldiaefolia 2015 203 MX
Lachemilla aphanoides 2012 311 EC
Lachemilla montziana 2013 443 CO
Lachemilla venusta 2015 204 MX
Lachemilla steinbachii 2013 415 BO
Lachemilla ranunculoides 2012 240 PE
Lachemilla bipinnatifida 2013 428 BO
Lachemilla aphanoides 2012 360 EC
Lachemilla velutina 2015 159 MX
Lachemilla velutina 2015 162 MX
Lachemilla vulcanica 2013 410 BO
Lachemilla vulcanica 2015 171 MX
Lachemilla vulcanica 2012 282 PE
Lachemilla vulcanica 2014 468 CO
Lachemilla andina 2012 370 EC
Lachemilla jamesonii 2012 318 EC
Lachemilla vulcanica 2012 398 EC
Lachemilla aphanoides 2012 388 EC
Lachemilla aphanoides 2013 416 BO
Lachemilla aphanoides 2012 246 PE
Lachemilla aphanoides 2013 442 CO
Lachemilla aphanoides 2015 149 MX
Lachemilla talamanquensis 2012 334 CR
Lachemilla verticillata 2012 339 CR
Lachemilla hispidula 2013 457 CO
Lachemilla polylepis 2012 359 CO
Lachemilla andina 2012 401 EC
Lachemilla hirta 2012 317 EC
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Lachemilla andina 2012 249 PE
Lachemilla hirta 2014 475 CO
Lachemilla andina 2013 440 CO
Lachemilla tulvescens 2012 368 EC
Lachemilla tulvescens 2013 450 CO
Lachemilla pectinata 2012 328 EC
Lachemilla pectinata 2014 542 CO
Lachemilla pectinata 2012 376 EC
Lachemilla perryana 2012 380 EC
Lachemilla fulvescens 2012 375 EC
Lachemilla fulvescens 2012 408 EC
Lachemilla venusta 2015 157 MX
Lachemilla orbiculata 2012 404 EC
Lachemilla orbiculata 2012 292 PE
Lachemilla orbiculata 2014 477 CO
Lachemilla orbiculata 2012 258 PE
Lachemilla pectinata 2015 176 MX
Lachemilla pectinata 2012 351 CR
Lachemilla mexiquense 2015 233 MX
Lachemilla erodiifolia 2012 296 PE
Lachemilla erodiifolia 2012 372 EC
Lachemilla erodiifolia 2013 401 BO
Lachemilla diplophylla 2013 382 BO
Lachemilla diplophylla 2012 357 EC
Lachemilla diplophylla 2012 237 PE
Lachemilla barbata 2012 305 PE
Lachemilla barbata 2013 406 BO
Lachemilla tanacetifolia 2013 488 CO
Lachemilla tanacetifolia 2012 331 EC
Lachemilla mandoniana 2013 464 CO
Lachemilla mandoniana 2012 400 EC
Lachemilla mandoniana 2012 345 CR
Lachemilla mandoniana 2012 261 PE
Lachemilla pinnata 2015 216 MX
Lachemilla pinnata 2013 448 CO
Lachemilla pinnata 2012 381 EC
Lachemilla pinnata 2012 230 PE
Lachemilla jaramilloi 2012 371 EC
Lachemilla jaramilloi 2014 557 CO
Lachemilla erodiifolia 2014 483 CO
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Lachemilla sarmentosa 2013 414 BO
Lachemilla sarmentosa 2013 396 BO
Lachemilla pinnata 2013 426 BO
Lachemilla frigida 2013 409 BO
Lachemilla bipinnatifida 2013 386 BO
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Lachemilla sprucei 2012 405 EC
Lachemilla nivalis 2012 384 EC
Lachemilla llanganatensis 2012 363 EC
Lachemilla hispidula 2013 478 CO
Lachemilla rivalis 2012 316 EC
Lachemilla rupestris 2012 329 EC
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Lachemilla holosericea 2012 314 EC
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Lachemilla sp nov 2013 438 CO
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Lachemilla uniflora 2012 374 EC
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Alchemilla andringitrensis
Alchemilla argyrophylla
Alchemilla mollis
Alchemilla vetteri
Alchemilla colorata
Alchemilla decumbens
Alchemilla vulgaris
Alchemilla fallax
Alchemilla glacialis
Alchemilla alpina
Alchemilla pentaphyllea
Alchemilla saxatilis
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Farinopsis salesoviana
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Drymocallis agrimonioides
Potaninia mongolica
Dasiphora davurica
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Dasiphora fruticosa
Chamaerhodos mongholica
Chamaerhodos erecta
Chamaerhodos nuttallii
Fragaria chiloensis
Fragaria ananassa
Fragaria virginiana
Fragaria vesca
Fragaria moschata
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Potentilla nitida
Potentilla bifora
Potentilla reptans
Potentilla recta
Potentilla supina
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Argentina anserina
Tylosperma lignosa
Rosa cymosa
Rosa persica
Rosa foetida
Rosa acicularis
Rosa caudata
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Hagenia abyssinica
Polylepis sericea
Sanguisorba officinalis
Rubus chamaemorus
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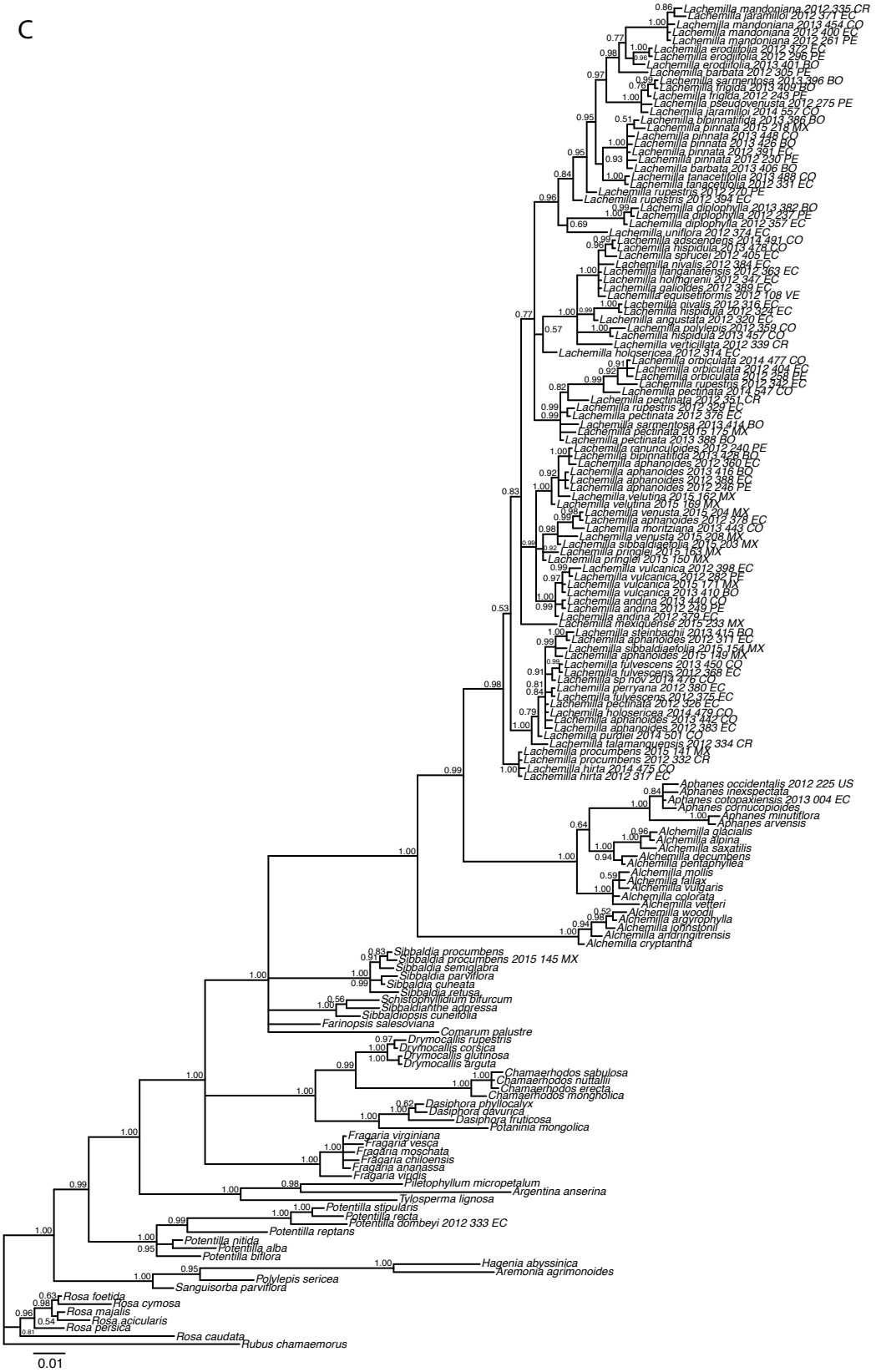
C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Lachemilla sprucei 2012 405 EC
Lachemilla nivalis 2012 384 EC
Lachemilla llanganatensis 2012 363 EC
Lachemilla holmgreni 2012 347 EC
Lachemilla galioides 2012 389 EC
Lachemilla equisetiformis 2012 108 VE
Lachemilla nivalis 2012 316 EC
Lachemilla hispidula 2012 324 EC
Lachemilla angustata 2012 320 EC
Lachemilla polylepis 2012 358 CO
Lachemilla hispidula 2013 457 CO
Lachemilla verticillata 2012 339 CR
Lachemilla holosericea 2012 314 EC
Lachemilla orbiculata 2014 477 CO
Lachemilla orbiculata 2012 404 EC
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Lachemilla vulcanica 2013 171 MX
Lachemilla vulcanica 2013 410 BO
Lachemilla andina 2013 440 CO
Lachemilla andina 2012 249 PE
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Lachemilla sibbaldiaefolia 2015 154 MX
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Aphanes minutiflora
Aphanes arvensis
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Alchemilla alpina
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Alchemilla pentaphyllea
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Alchemilla fallax
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Alchemilla colorata
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Alchemilla argyrophylla
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Alchemilla andringitrensis
Alchemilla cryptantha
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Schistophyllidium bifurcum
Sibbaldianthe adpressa
Sibbaldiopsis cuneifolia
Farinopsis salesoviana
Comarum palustre
Drymocallis rupestris
Drymocallis corsica
Drymocallis glutinosa
Drymocallis arguta
Chamaerhodos sabulosa
Chamaerhodos nuttallii
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Chamaerhodos mongholica
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Fragaria viridis
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Argentina anserina
Tylosperma lignosa
Potentilla stipularis
Potentilla recta
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Potentilla reptans
Potentilla nitida
Potentilla alba
Potentilla biflora
Hagenia abyssinica
Aremonia agrimonoides
Polylepis sericea
Sanguisorba parviflora
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Rosa acicularis
Rosa persica
Rosa caudata
Rubus chamaemorus
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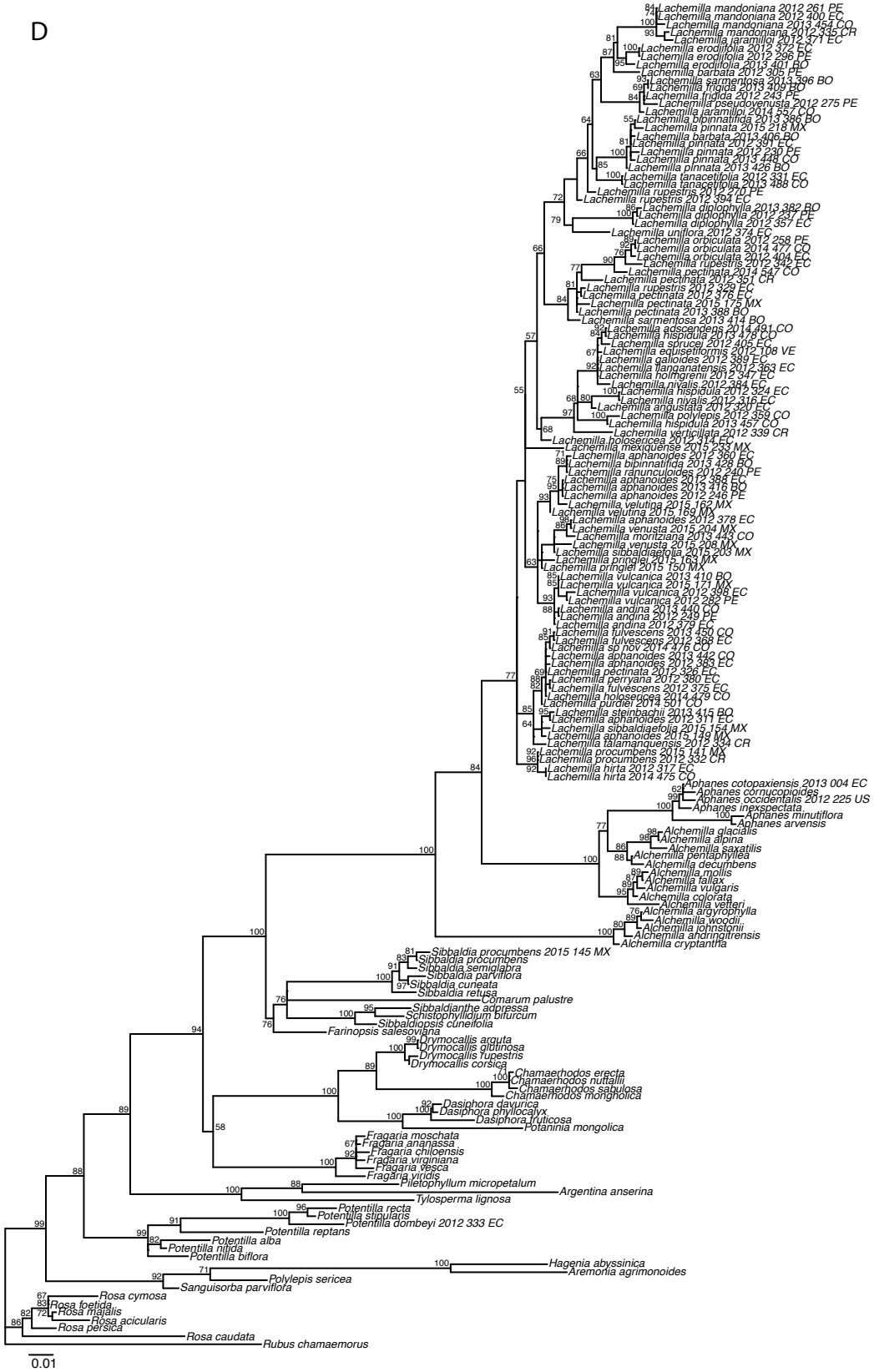
D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Lachemilla erodiifolia 2012 372 EC
Lachemilla erodiifolia 2012 296 PE
Lachemilla erodiifolia 2013 401 BO
Lachemilla barbata 2012 305 PE
Lachemilla sarmentosa 2013 396 BO
Lachemilla frigida 2013 409 BO
Lachemilla frigida 2012 243 PE
Lachemilla pseudovenusta 2012 275 PE
Lachemilla jaramillii 2014 557 CO
Lachemilla bipinnatifida 2013 396 BO
Lachemilla pinnata 2015 218 MX
Lachemilla barbata 2013 406 BO
Lachemilla pinnata 2012 391 EC
Lachemilla pinnata 2012 230 PE
Lachemilla pinnata 2013 448 CO
Lachemilla pinnata 2013 426 BO
Lachemilla tanacetifolia 2012 331 EC
Lachemilla tanacetifolia 2013 488 CO
Lachemilla rupestris 2012 270 PE
Lachemilla rupestris 2012 394 EC
Lachemilla diplophylla 2013 382 BO
Lachemilla diplophylla 2012 237 PE
Lachemilla diplophylla 2012 357 EC
Lachemilla uniflora 2012 374 EC
Lachemilla orbiculata 2012 258 PE
Lachemilla orbiculata 2014 477 CO
Lachemilla orbiculata 2012 404 EC
Lachemilla rupestris 2012 342 EC
Lachemilla pectinata 2014 547 CO
Lachemilla pectinata 2012 351 CR
Lachemilla rupestris 2012 329 EC
Lachemilla pectinata 2012 378 EC
Lachemilla pectinata 2015 175 MX
Lachemilla pectinata 2013 388 BO
Lachemilla sarmentosa 2013 414 BO
Lachemilla adscendens 2014 481 CO
Lachemilla hispidula 2013 478 CO
Lachemilla sprucei 2012 405 EC
Lachemilla aequisetiformis 2012 108 VE
Lachemilla galioides 2012 389 EC
Lachemilla tlanganatensis 2012 363 EC
Lachemilla hoffmgreni 2012 347 EC
Lachemilla nivalis 2012 384 EC
Lachemilla hispidula 2012 324 EC
Lachemilla nivalis 2012 316 EC
Lachemilla angustata 2015 220 EC
Lachemilla polylepis 2012 359 CO
Lachemilla hispidula 2013 457 CO
Lachemilla verticillata 2012 339 CR
Lachemilla holosericea 2012 414 EC
Lachemilla mexiquense 2015 233 MX
Lachemilla aphanoides 2012 360 EC
Lachemilla bipinnatifida 2013 428 BO
Lachemilla ranunculoides 2012 240 PE
Lachemilla aphanoides 2012 388 EC
Lachemilla aphanoides 2013 416 BO
Lachemilla aphanoides 2012 246 PE
Lachemilla velutina 2015 162 MX
Lachemilla velutina 2015 169 MX
Lachemilla aphanoides 2012 378 EC
Lachemilla venusta 2015 204 MX
Lachemilla moritziana 2013 443 CO
Lachemilla venusta 2015 206 MX
Lachemilla sibbaldiaefolia 2015 203 MX
Lachemilla pringlei 2015 153 MX
Lachemilla pringlei 2015 150 MX
Lachemilla vulcanica 2013 410 BO
Lachemilla vulcanica 2015 171 MX
Lachemilla vulcanica 2012 398 EC
Lachemilla vulcanica 2012 282 PE
Lachemilla andina 2013 440 CO
Lachemilla andina 2012 249 PE
Lachemilla andina 2012 379 EC
Lachemilla fulvescens 2013 450 CO
Lachemilla fulvescens 2012 368 EC
Lachemilla sp nov 2014 476 CO
Lachemilla aphanoides 2013 442 CO
Lachemilla aphanoides 2012 383 EC
Lachemilla pectinata 2012 326 EC
Lachemilla perryana 2012 380 EC
Lachemilla fulvescens 2012 375 EC
Lachemilla holosericea 2014 479 CO
Lachemilla purdiei 2014 501 CO
Lachemilla steinbachii 2013 415 BO
Lachemilla aphanoides 2012 311 EC
Lachemilla sibbaldiaefolia 2015 154 MX
Lachemilla aphanoides 2015 149 MX
Lachemilla talamanquensis 2012 334 CR
Lachemilla procumbens 2015 141 MX
Lachemilla procumbens 2012 332 CR
Lachemilla hirta 2012 317 EC
Lachemilla hirta 2014 475 CO
Aphanes cotopaxiensis 2013 004 EC
Aphanes cornucopioides
Aphanes occidentalis 2012 225 US
Aphanes inexpectata
Aphanes minutiflora
Aphanes arvensis
Alchemilla glacialis
Alchemilla alpina
Alchemilla saxatilis
Alchemilla pentaphyllea
Alchemilla decumbens
Alchemilla mollis
Alchemilla fallax
Alchemilla vulgaris
Alchemilla colorata
Alchemilla vetteri
Alchemilla argyrophylla
Alchemilla woodii
Alchemilla lohnstonii
Alchemilla andringitrensis
Alchemilla cryptantha
Sibbaldia procumbens 2015 145 MX
Sibbaldia procumbens
Sibbaldia semiglabra
Sibbaldia parviflora
Sibbaldia cuneata
Sibbaldia retusa
Comarum palustre
Sibbaldianthe adpressa
Schistophyllidium bifurcum
Sibbaldiopsis cuneifolia
Farinopsis salesoviana
Drymocallis arguta
Drymocallis glutinosa
Drymocallis rupestris
Drymocallis corsica
Chamaerhodos erecta
Chamaerhodos nuttallii
Chamaerhodos sabulosa
Chamaerhodos mongholica
Dasiphora davurica
Dasiphora phylocalyx
Dasiphora fruticosa
Potaninia mongolica
Fragaria moschata
Fragaria ananassa
Fragaria chiloensis
Fragaria virginiana
Fragaria vesca
Fragaria viridis
Filetophyllum micropetalum
Argentina anserina
Tylosperma lignosa
Potentilla recta
Potentilla sto ularis
Potentilla dombeyi 2012 333 EC
Potentilla reptans
Potentilla alba
Potentilla nitida
Potentilla biflora
Hagenia abyssinica
Aremoria agrimonoides
Polylepis sericea
Sanguisorba parviflora
Rosa cymosa
Rosa foetida
Rosa majalis
Rosa acicularis
Rosa persica
Rosa caudata
Rubus chamaemorus
0.01

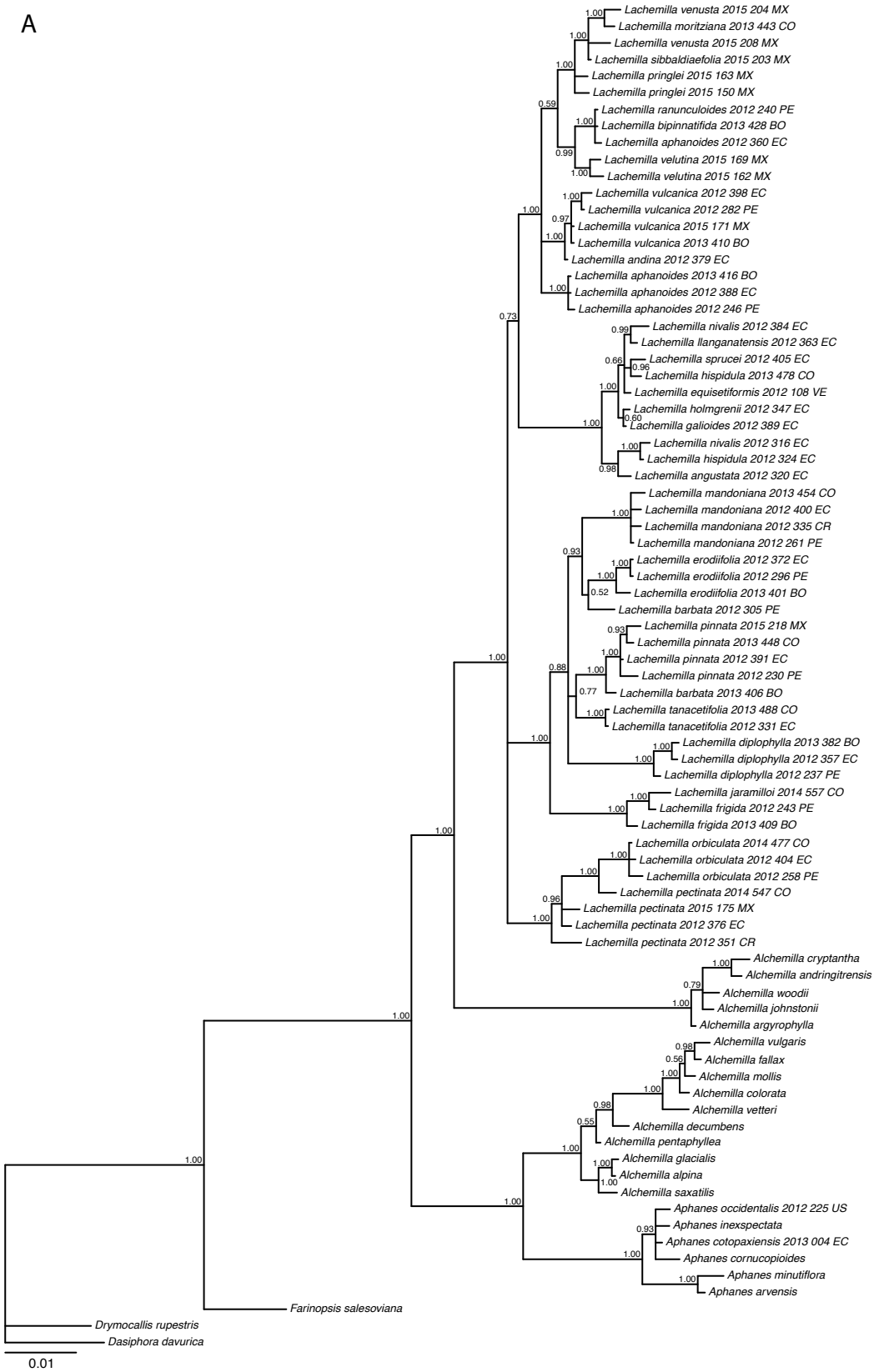
Appendix 4

Expanded Figure 1.4 from Chapter 1.

A. Maximum likelihood tree of the concatenated *trnL-F* chloroplast and nuclear ribosomal ITS regions after removing outlier samples identified by PACo analyses. Node labels represent bootstrap support values.

B. Maximum likelihood tree of the concatenated *trnL-F* chloroplast and nuclear ribosomal ITS regions after removing outlier samples identified by PACo analyses. Node labels represent bootstrap support values.

A


Lachemilla venusta 2015 204 MX
Lachemilla moritziana 2013 443 CO
Lachemilla venusta 2015 208 MX
Lachemilla sibbaldiaefolia 2015 203 MX
Lachemilla pringlei 2015 163 MX
Lachemilla pringlei 2015 150 MX
Lachemilla ranunculoides 2012 240 PE
Lachemilla bipinnatifida 2013 428 BO
Lachemilla aphanoides 2012 360 EC
Lachemilla velutina 2015 169 MX
Lachemilla velutina 2015 162 MX
Lachemilla vulcanica 2012 398 EC
Lachemilla vulcanica 2012 282 PE
Lachemilla vulcanica 2015 171 MX
Lachemilla vulcanica 2013 410 BO
Lachemilla andina 2012 379 EC
Lachemilla aphanoides 2013 416 BO
Lachemilla aphanoides 2012 388 EC
Lachemilla aphanoides 2012 246 PE
Lachemilla nivalis 2012 384 EC
Lachemilla llanganatensis 2012 363 EC
Lachemilla sprucei 2012 405 EC
Lachemilla hispidula 2013 478 CO
Lachemilla equisetiformis 2012 108 VE
Lachemilla holmgrenii 2012 347 EC
Lachemilla galioides 2012 389 EC
Lachemilla nivalis 2012 316 EC
Lachemilla hispidula 2012 324 EC
Lachemilla angustata 2012 320 EC
Lachemilla mandoniana 2013 454 CO
Lachemilla mandoniana 2012 400 EC
Lachemilla mandoniana 2012 335 CR
Lachemilla mandoniana 2012 261 PE
Lachemilla erodiifolia 2012 372 EC
Lachemilla erodiifolia 2012 296 PE
Lachemilla erodiifolia 2013 401 BO
Lachemilla barbata 2012 305 PE
Lachemilla pinnata 2015 218 MX
Lachemilla pinnata 2013 448 CO
Lachemilla pinnata 2012 391 EC
Lachemilla pinnata 2012 230 PE
Lachemilla barbata 2013 406 BO
Lachemilla tanacetifolia 2013 488 CO
Lachemilla tanacetifolia 2012 331 EC
Lachemilla diplophylla 2013 382 BO
Lachemilla diplophylla 2012 357 EC
Lachemilla diplophylla 2012 237 PE
Lachemilla jaramilloi 2014 557 CO
Lachemilla frigida 2012 243 PE
Lachemilla frigida 2013 409 BO
Lachemilla orbiculata 2014 477 CO
Lachemilla orbiculata 2012 404 EC
Lachemilla orbiculata 2012 258 PE
Lachemilla pectinata 2014 547 CO
Lachemilla pectinata 2015 175 MX
Lachemilla pectinata 2012 376 EC
Lachemilla pectinata 2012 351 CR
Alchemilla cryptantha
Alchemilla andringitrensis
Alchemilla woodii
Alchemilla johnstonii
Alchemilla argyrophylla
Alchemilla vulgaris
Alchemilla fallax
Alchemilla mollis
Alchemilla colorata
Alchemilla vetteri
Alchemilla decumbens
Alchemilla pentaphyllea
Alchemilla glacialis
Alchemilla alpina
Alchemilla saxatilis
Aphanes occidentalis 2012 225 US
Aphanes inexspectata
Aphanes cotopaxiensis 2013 004 EC
Aphanes cornucopioides
Aphanes minutiflora
Aphanes arvensis
Farinopsis salesoviana
Drymocallis rupestris
Dasiphora davurica
0.01

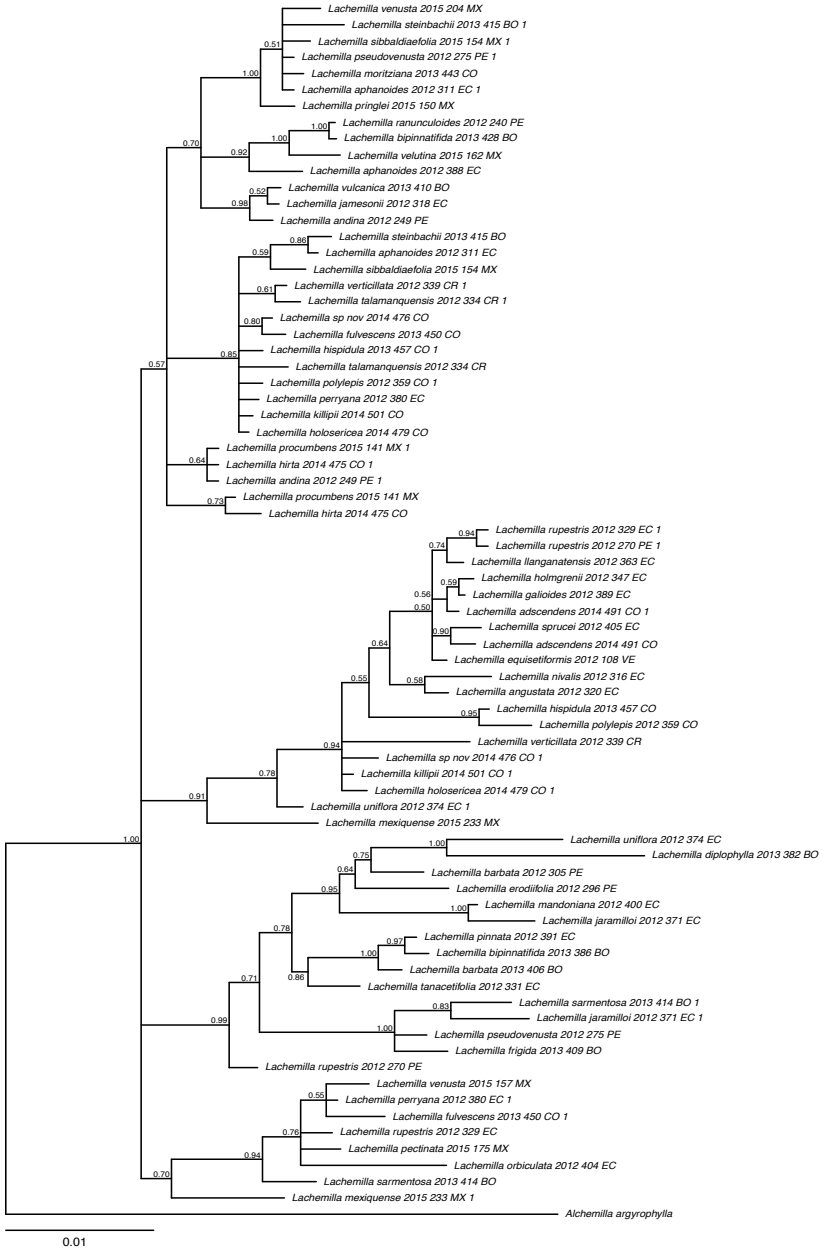
B


Lachemilla aphanoides 2012 360 EC
Lachemilla bipinnatifida 2013 428 BO
Lachemilla ranunculoides 2012 240 PE
Lachemilla velutina 2015 162 MX
Lachemilla velutina 2015 169 MX
Lachemilla aphanoides 2013 416 BO
Lachemilla aphanoides 2012 388 EC
Lachemilla aphanoides 2012 246 PE
Lachemilla venusta 2015 204 MX
Lachemilla moritziana 2013 443 CO
Lachemilla venusta 2015 208 MX
Lachemilla sibbaldiaefolia 2015 203 MX
Lachemilla pringlei 2015 150 MX
Lachemilla pringlei 2015 163 MX
Lachemilla vulcanica 2015 171 MX
Lachemilla vulcanica 2013 410 BO
Lachemilla vulcanica 2012 282 PE
Lachemilla vulcanica 2012 398 EC
Lachemilla andina 2012 379 EC
Lachemilla llanganatensis 2012 363 EC
Lachemilla nivalis 2012 384 EC
Lachemilla sprucei 2012 405 EC
Lachemilla hispidula 2013 478 CO
Lachemilla equisetiformis 2012 108 VE
Lachemilla holmgrenii 2012 347 EC
Lachemilla galioides 2012 389 EC
Lachemilla nivalis 2012 316 EC
Lachemilla hispidula 2012 324 EC
Lachemilla angustata 2012 320 EC
Lachemilla orbiculata 2012 258 PE
Lachemilla orbiculata 2014 477 CO
Lachemilla orbiculata 2012 404 EC
Lachemilla pectinata 2014 547 CO
Lachemilla pectinata 2012 376 EC
Lachemilla pectinata 2015 175 MX
Lachemilla pectinata 2012 351 CR
Lachemilla mandoniana 2012 400 EC
Lachemilla mandoniana 2012 261 PE
Lachemilla mandoniana 2013 454 CO
Lachemilla mandoniana 2012 335 CR
Lachemilla erodiifolia 2012 372 EC
Lachemilla erodiifolia 2012 296 PE
Lachemilla erodiifolia 2013 401 BO
Lachemilla barbata 2012 305 PE
Lachemilla diplophylla 2013 382 BO
Lachemilla diplophylla 2012 357 EC
Lachemilla diplophylla 2012 237 PE
Lachemilla pinnata 2015 218 MX
Lachemilla pinnata 2013 448 CO
Lachemilla pinnata 2012 230 PE
Lachemilla pinnata 2012 391 EC
Lachemilla barbata 2013 406 BO
Lachemilla tanacetifolia 2012 331 EC
Lachemilla tanacetifolia 2013 488 CO
Lachemilla frigida 2012 243 PE
Lachemilla jaramilloi 2014 557 CO
Lachemilla frigida 2013 409 BO
Alchemilla cryptantha
Alchemilla andringitrensis
Alchemilla johnstonii
Alchemilla woodii
Alchemilla argyrophylla
Alchemilla vulgaris
Alchemilla fallax
Alchemilla mollis
Alchemilla colorata
Alchemilla vetteri
Alchemilla decumbens
Alchemilla pentaphyllea
Alchemilla glacialis
Alchemilla alpina
Alchemilla saxatilis
Aphanes occidentalis 2012 225 US
Aphanes cotopaxiensis 2013 004 EC
Aphanes cornucopioides
Aphanes inexspectata
Aphanes minutiflora
Aphanes arvensis
Farinopsis salesoviana
Dasiphora davurica
Drymocallis rupestris
0.01

Appendix 5

Expanded Figure 1.5 from Chapter 1.

Bayesian 50% majority rule consensus multilabel tree from the 'Duplicated tree' concatenated matrix of the *trnL-F* chloroplast and nuclear ribosomal ITS regions. Node labels represent posterior probabilities.



Appendix 6

Voucher information for Chapter 2.

Arranged by: Species name and authority, collector and number (herbarium code).

Alchemilla argyrophylla Oliv., Rault 139 (MSB); *Alchemilla mollis* (Buser) Rothm., *D.F. Morales-Briones 687* (ID); *Aphanes occidentalis* (Nutt.) Rydb., *D.C. Tank 1107* (ID); *Lachemilla andina* (L.M. Perry) Rothm., *D.F. Morales-Briones & K. Romoleroux 162* (QCA); *Lachemilla aphanoides* (Mutis ex L. f.) Rothm., *D.F. Morales-Briones et al. 115* (QCA); *Lachemilla barbata* (C. Presl) Rothm., *D.F. Morales-Briones & S. Uribe-Convers 229* (ID); *Lachemilla diplophylla* (Diels) Rothm, *D.F. Morales-Briones & E. Morales-Checa. 30* (QCA); *Lachemilla erodiifolia* (Wedd.) Rothm, *D.F. Morales-Briones & K. Romoleroux 119* (QCA); *Lachemilla fulvescens* (L.M. Perry) Rothm, *D.F. Morales-Briones et al. 128* (QCA); *Lachemilla galioides* (Benth.) Rothm., *K. Romoleroux et al. 4699* (QCA); *Lachemilla hirta* (L.M. Perry) Rothm., *D.F. Morales-Briones & K. Romoleroux 118* (QCA); *Lachemilla hispidula* (L.M. Perry) Rothm., *D.F. Morales-Briones & K. Romoleroux 110* (QCA); *Lachemilla holosericea* (L.M. Perry) Rothm., *D.F. Morales-Briones & K. Romoleroux 123* (QCA); *Lachemilla jamesonii* (L.M. Perry) Rothm., *K. Romoleroux et al. 4684* (QCA); *Lachemilla jaramilloi* Romol. & D.F. Morales-B., *D.F. Morales-Briones et al. 126* (QCA); *Lachemilla mandoniana* (Wedd.) Rothm., *D.F. Morales-Briones et al. 112* (QCA); *Lachemilla nivalis* (Kunth) Rothm., *K. Romoleroux et al. 4580* (QCA); *Lachemilla orbiculata* (Ruiz & Pav.) Rydb., *D.F. Morales-Briones et al. 108* (QCA); *Lachemilla pectinata* (Kunth) Rothm., *D.F. Morales-Briones & K. Romoleroux 161* (QCA); *Lachemilla pinnata* (Ruiz & Pav.) Rothm., *D.F. Morales-Briones*

et al. 113 (QCA); *Lachemilla polylepis* (Wedd.) Rothm., *P. Sklenář 12207* (QCA); *Lachemilla procumbens* (Rose) Rydb., *K. Romoleroux et al. 5011* (QCA); *Lachemilla pseudovenusta* Rothm., *D.F. Morales-Briones & S. Uribe-Convers 244* (ID); *Lachemilla rupestris* (Kunth) Rothm., *D.F. Morales-Briones et al. 105* (QCA); *Lachemilla sprucei* (L.M. Perry) Rothm., *D.F. Morales-Briones et al. 141* (QCA); *Lachemilla talamanquensis* Romol. & D.F. Morales-B., *K. Romoleroux et al. 5008* (QCA); *Lachemilla tanacetifolia* Rothm.., *D.F. Morales-Briones et al. 122* (QCA); *Lachemilla uniflora* Maguire, D.F. *Morales-Briones & K. Romoleroux 163* (QCA), *D.F. Morales-Briones et al. 75* (QCA); *Lachemilla verticillata* (Fielding & Gardner) Rothm., *K. Romoleroux et al. 5007* (QCA), *K. Romoleroux et al. 5027* (QCA); *Lachemilla vulcanica* (Schltdl. & Cham.) Rydb., D.F. *Morales-Briones et al. 117* (QCA).

Appendix 7

HybPiper assembly statistics from Chapter 2

Hybpiper statistics for assembly exons of 300 bp or greater (400 targets). [a]Total number of reads of capure of 1419 exons per species. [c]Percentage of target reads out of total number of reads. [c]Number of target exons mapped. [d]Number of target exons with contigs. [e]Number of target exons with sequences. [f]Number of target exons with sequences of at least 50% of the length of the target. [g]Number of target exons with more than one paralog copy

Name	Total reads[a]	Reads mapped[b]	Percentage on target[c]	Exons mapped[d]	Exons with contigs[e]	Exons with sequences[f]	Paralog warnings[g]
Alchemilla argyrophylla	11892439	1786112	0.151	398	389	386	262
Alchemilla mollis	10038792	2788558	0.278	397	394	390	284
Aphanes occidentalis	14522552	2435232	0.168	395	367	360	47
Lachemilla andina	10173514	2953757	0.291	396	391	388	253
Lachemilla aphanoides	16979140	5277913	0.311	400	395	391	269
Lachemilla barbata	22003534	6292101	0.286	399	395	392	258
Lachemilla diplophylla	12660647	4015779	0.317	397	392	389	254
Lachemilla erodiifolia	7748031	2313294	0.299	398	389	387	254
Lachemilla fulvescens	8768450	2697466	0.307	398	391	389	255
Lachemilla galioides	7461262	2214454	0.297	397	388	386	258
Lachemilla hirta	13413961	4269780	0.319	399	394	389	263
Lachemilla hispidula	7605197	2273606	0.299	399	390	388	245
Lachemilla holosericea	8086994	2669418	0.33	397	391	388	264
Lachemilla jamesonii	10017380	2935590	0.293	399	389	387	256
Lachemilla jaramilloi	8418559	2497205	0.296	397	392	390	260

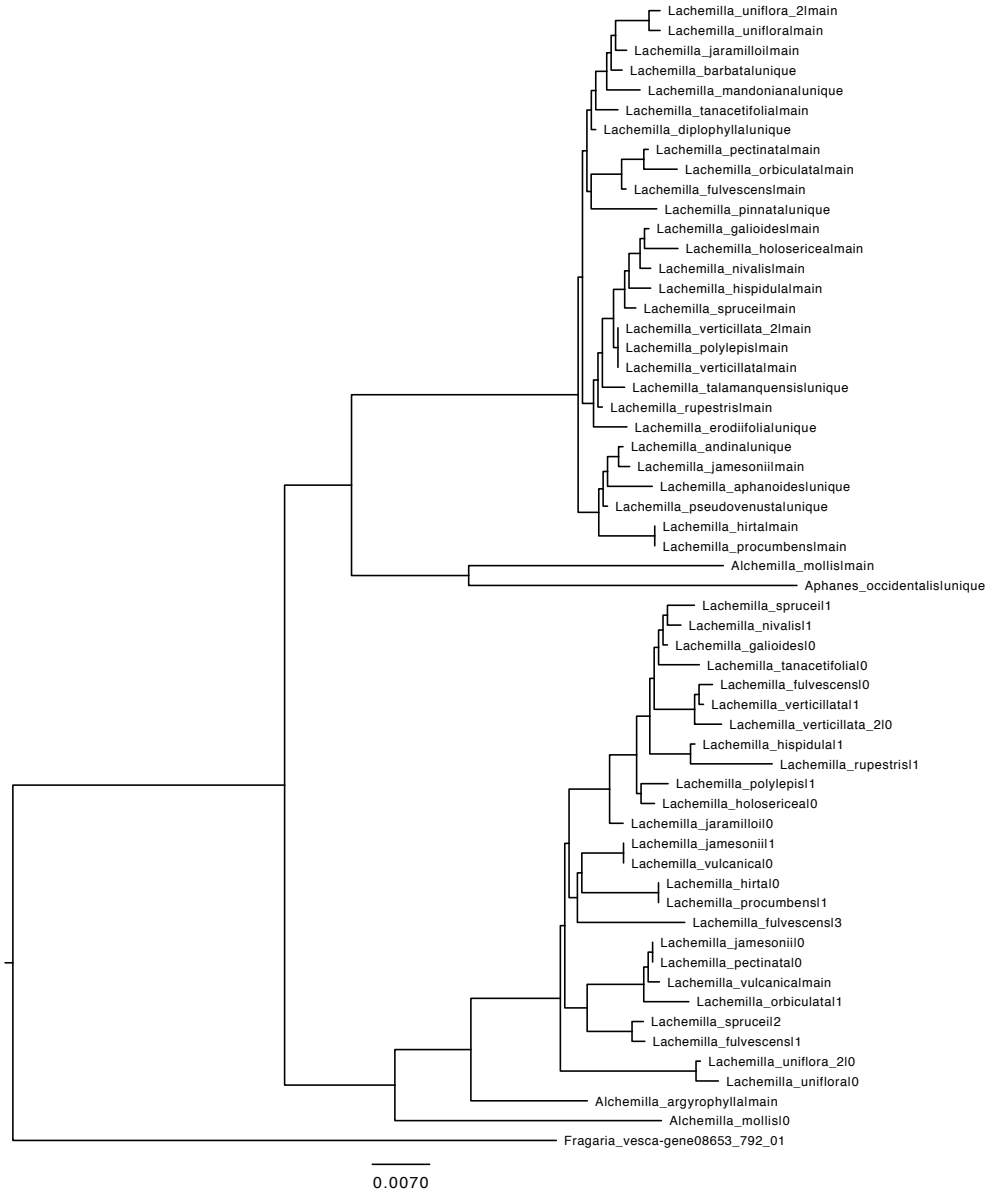
Name	Total reads[a]	Reads mapped[b]	Percentage on target[c]	Exons mapped[d]	Exons with contigs[e]	Exons with sequences[f]	Paralog warnings[g]
Lachemilla mandoniana	6530864	1666621	0.255	398	389	388	249
Lachemilla nivalis	7020723	1761494	0.251	395	389	386	248
Lachemilla orbiculata	8196435	2350768	0.287	396	389	387	252
Lachemilla pectinata	12434363	3946864	0.318	397	392	390	269
Lachemilla pinnata	19499617	4917364	0.252	398	393	390	244
Lachemilla polylepis	12900557	3137962	0.243	399	391	390	258
Lachemilla procumbens	8005351	2173715	0.272	398	388	385	252
Lachemilla pseudovenusta	6236227	1860456	0.299	397	389	386	258
Lachemilla rupestris	8095894	2146138	0.265	398	390	388	247
Lachemilla sprucei	7170736	1973946	0.275	399	387	386	251
Lachemilla talamanquensis	10067232	2912757	0.29	396	389	384	258
Lachemilla tanacetifolia	4721575	1550959	0.328	397	388	384	261
Lachemilla uniflora	5257166	1336547	0.254	397	386	384	261
Lachemilla uniflora_2	5579547	1423193	0.255	396	385	384	264
Lachemilla verticillata	8388269	2671653	0.319	399	392	390	245
Lachemilla verticillata_2	6476513	1648264	0.255	398	389	387	245
Lachemilla vulcanica	8338652	2668779	0.32	400	390	388	251

Appendix 8

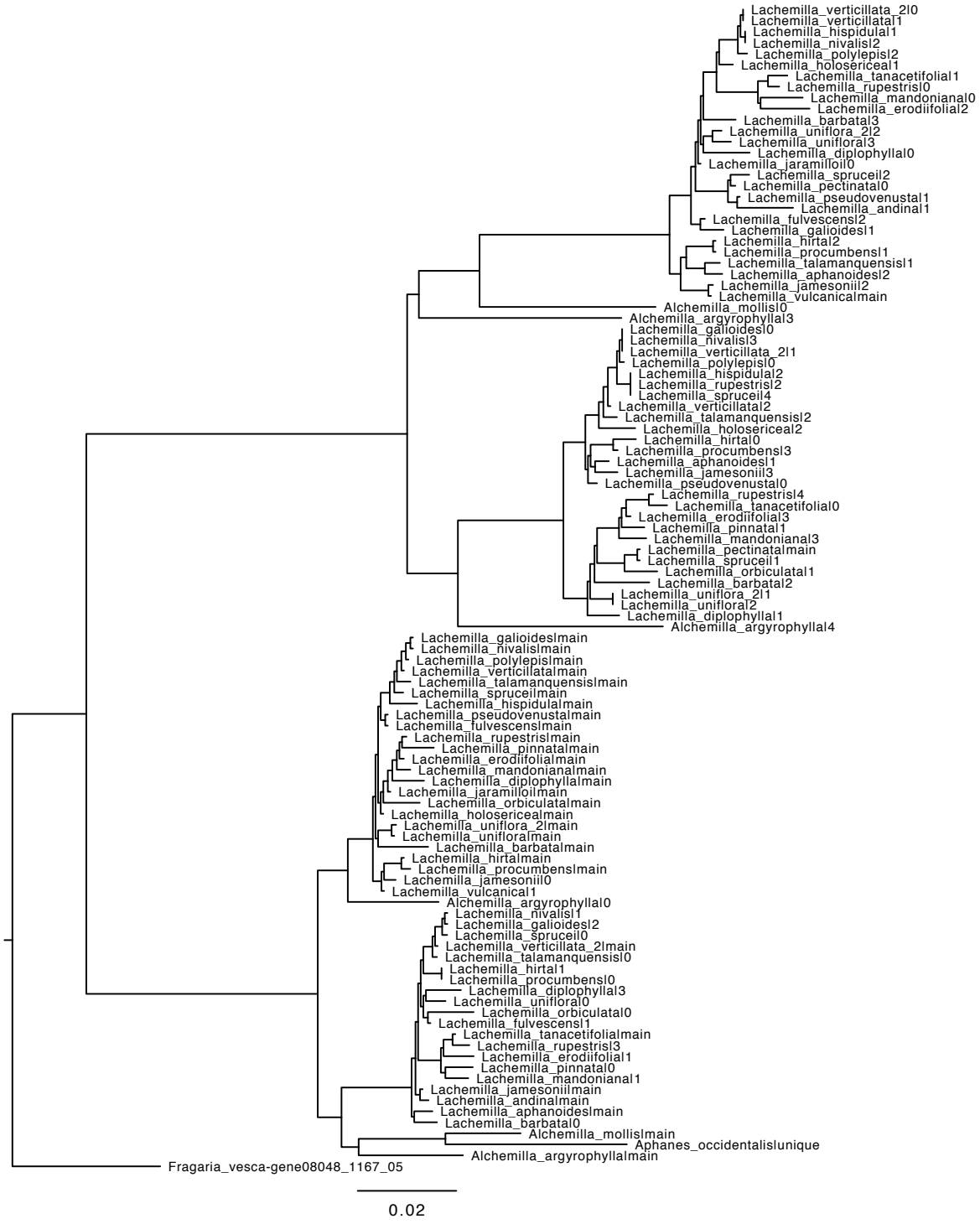
Examples of gene family trees from Chapter 2.

Example of approximately-maximum-likelihood phylogenetic trees inferred with FastTree2 from gene families in *Lachemilla.* A.

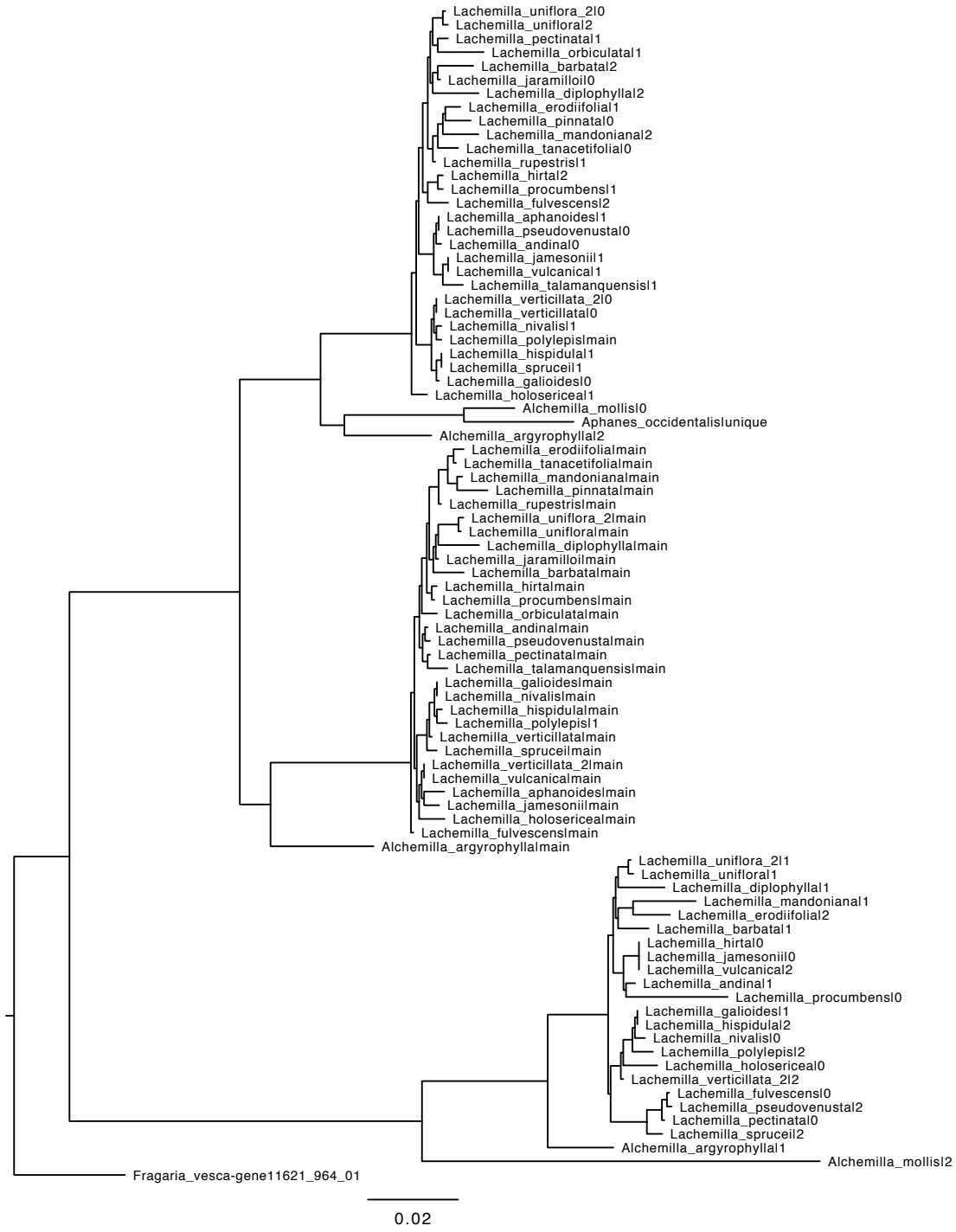
A. Exon 08048_1167_05.



B. Exon 08653_792_01.



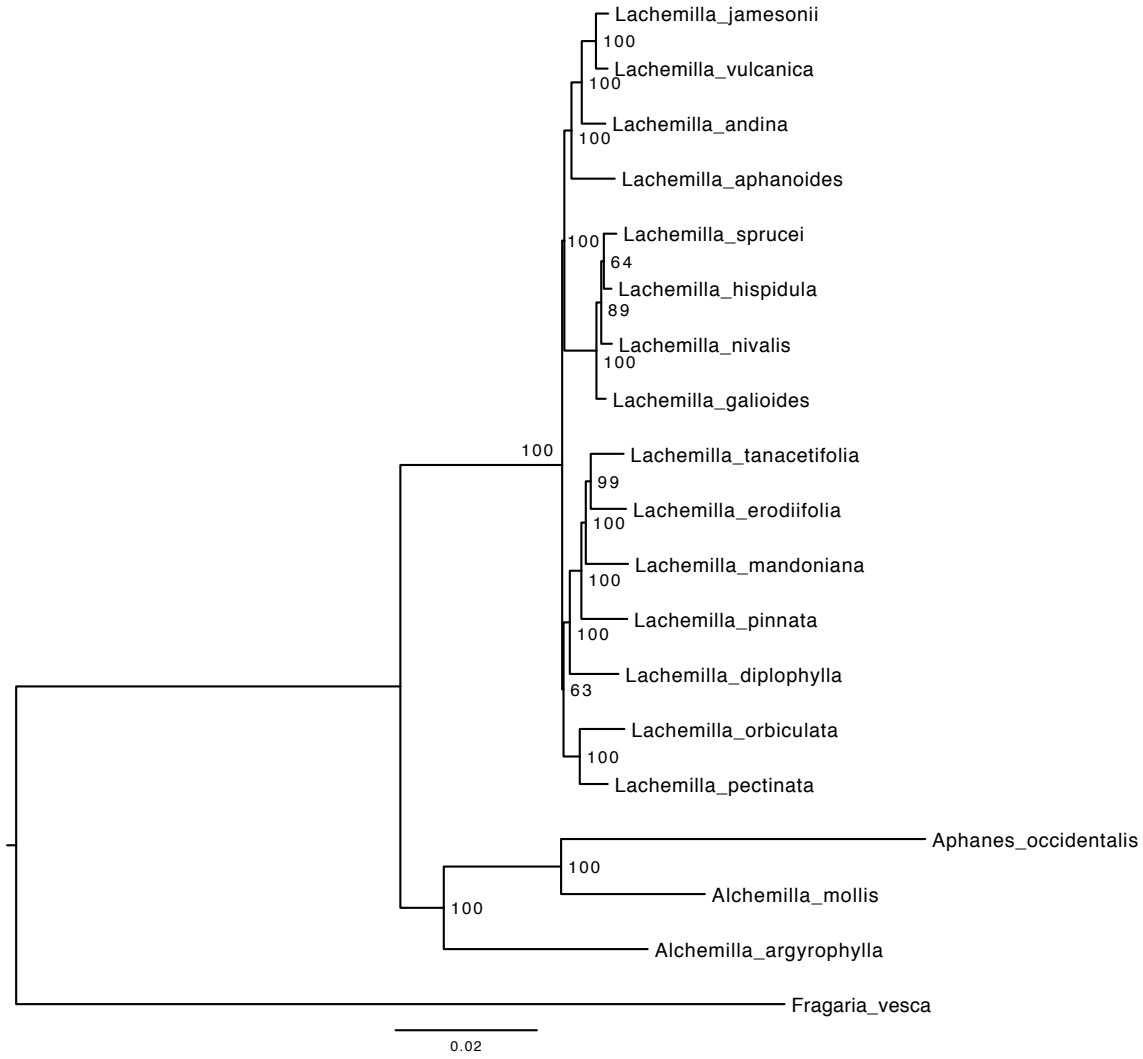
C. Exon 11621_964_01.



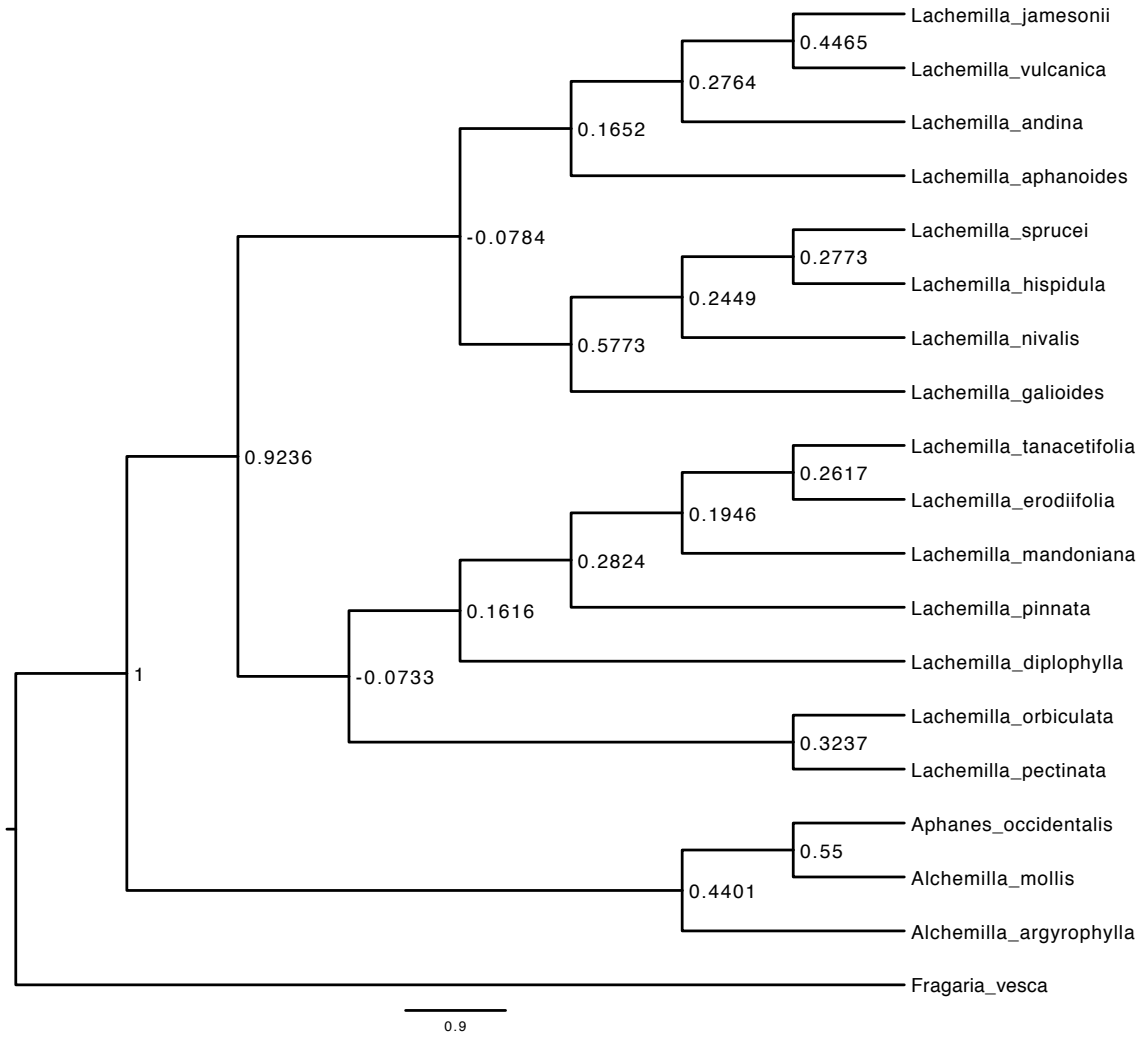
Appendix 9

Additional phylogenetic analysis of the REDUCED-HYBIRD dataset from Chapter 2.

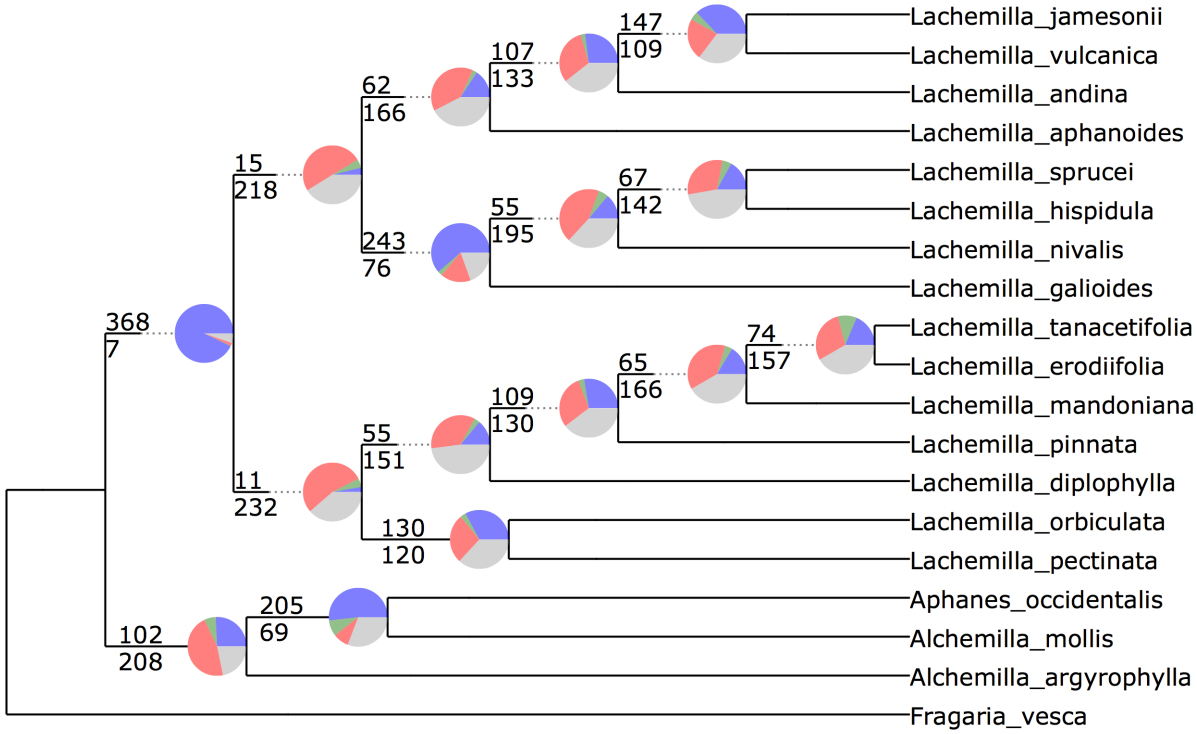
A. RAxML phylogeny of the concatenated matrix; node labels indicate bootstrap values.



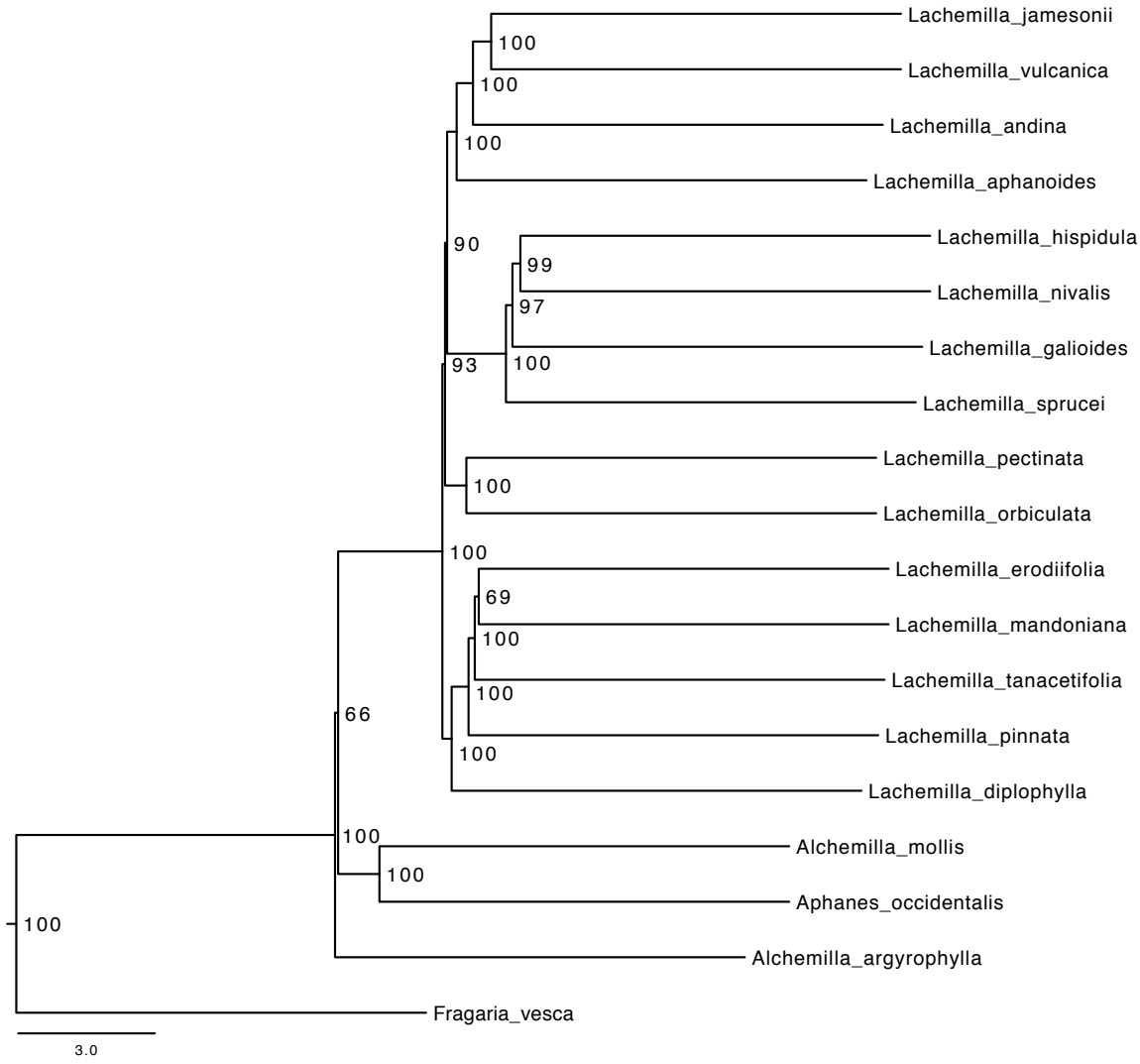
B. RAxML phylogeny of the concatenated matrix; node labels indicate ICA scores.



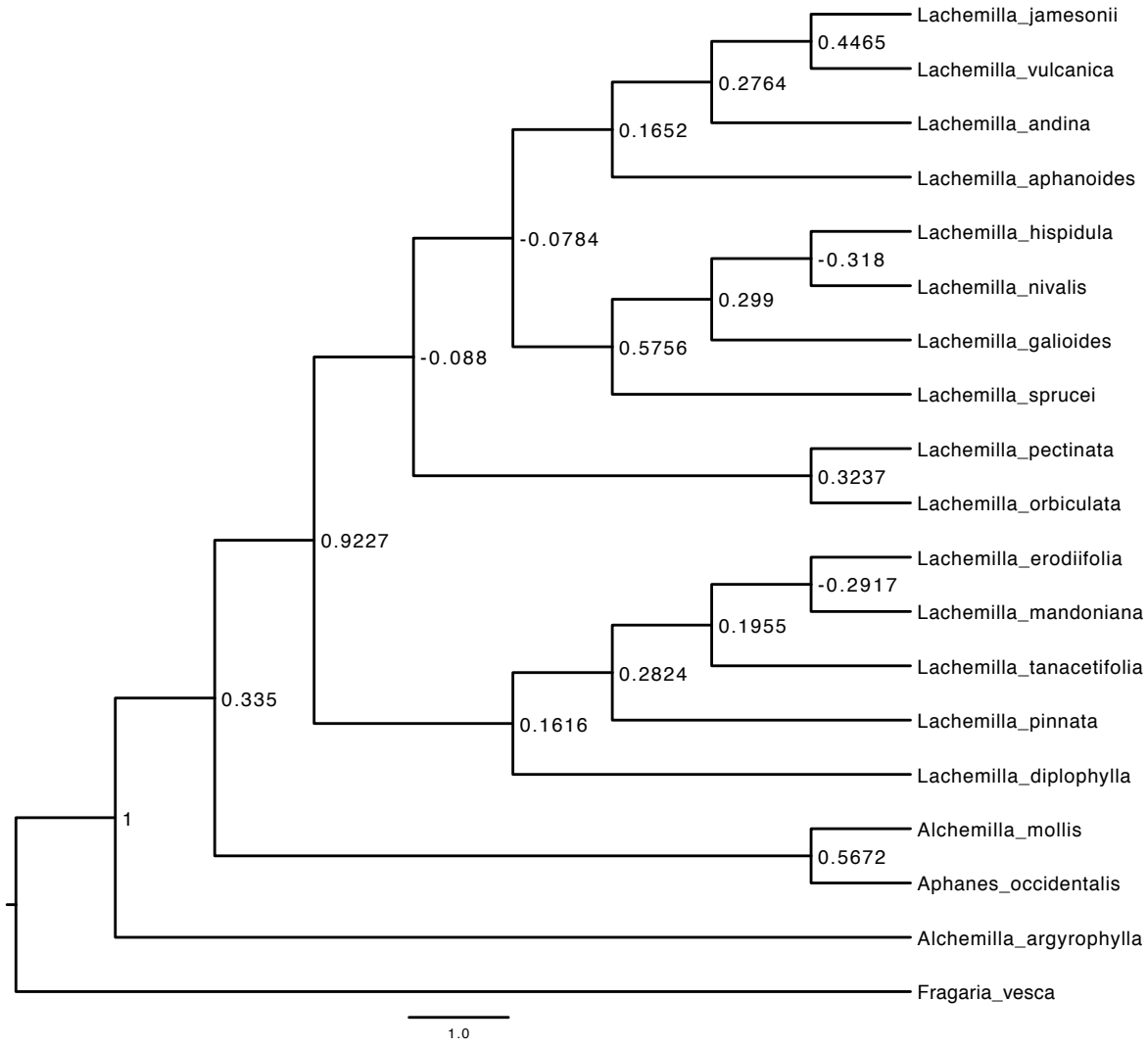
C. RAxML phylogeny of the concatenated matrix; numbers above and below branches indicate the number of individual gene trees in agreement and in conflict, respectively Pie charts next to the nodes represent the proportion of gene trees that support that clade – blue, the proportion that support the main alternative for that clade – green, the proportion that support the remaining alternatives – red, and the proportion (conflict or support) that have less than 50% bootstrap support – gray.



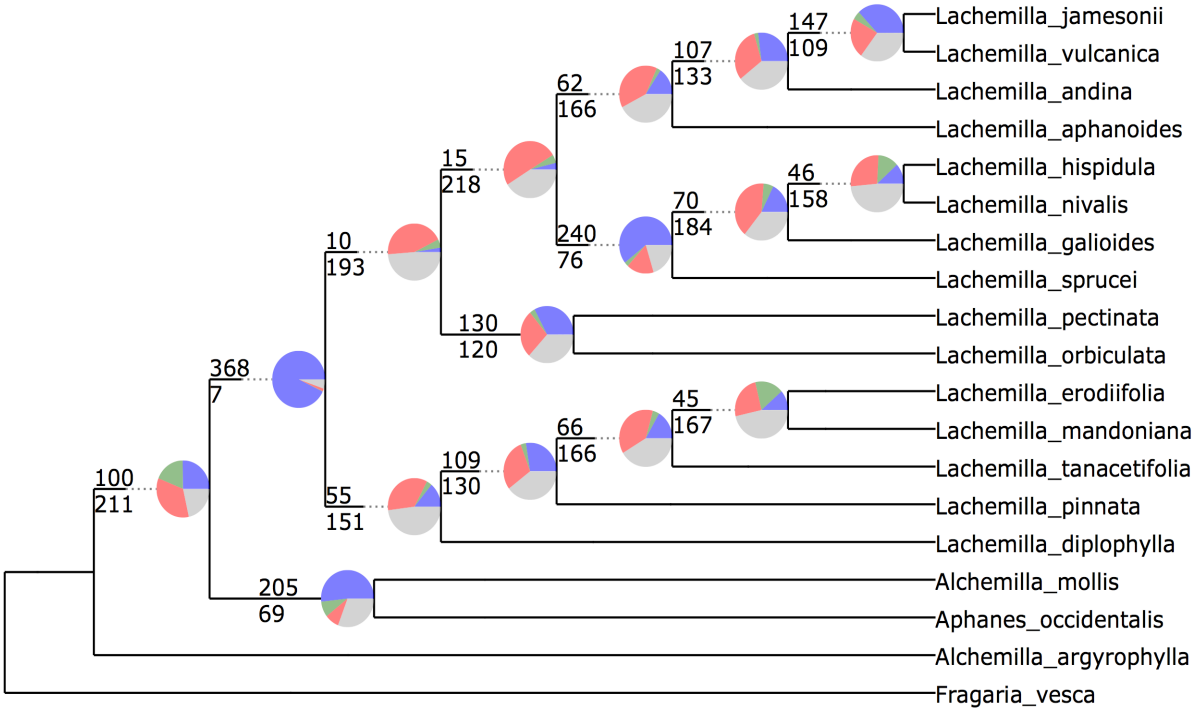
D. MP-EST species trees of the concatenated matrix; node label indicate bootstrap values.



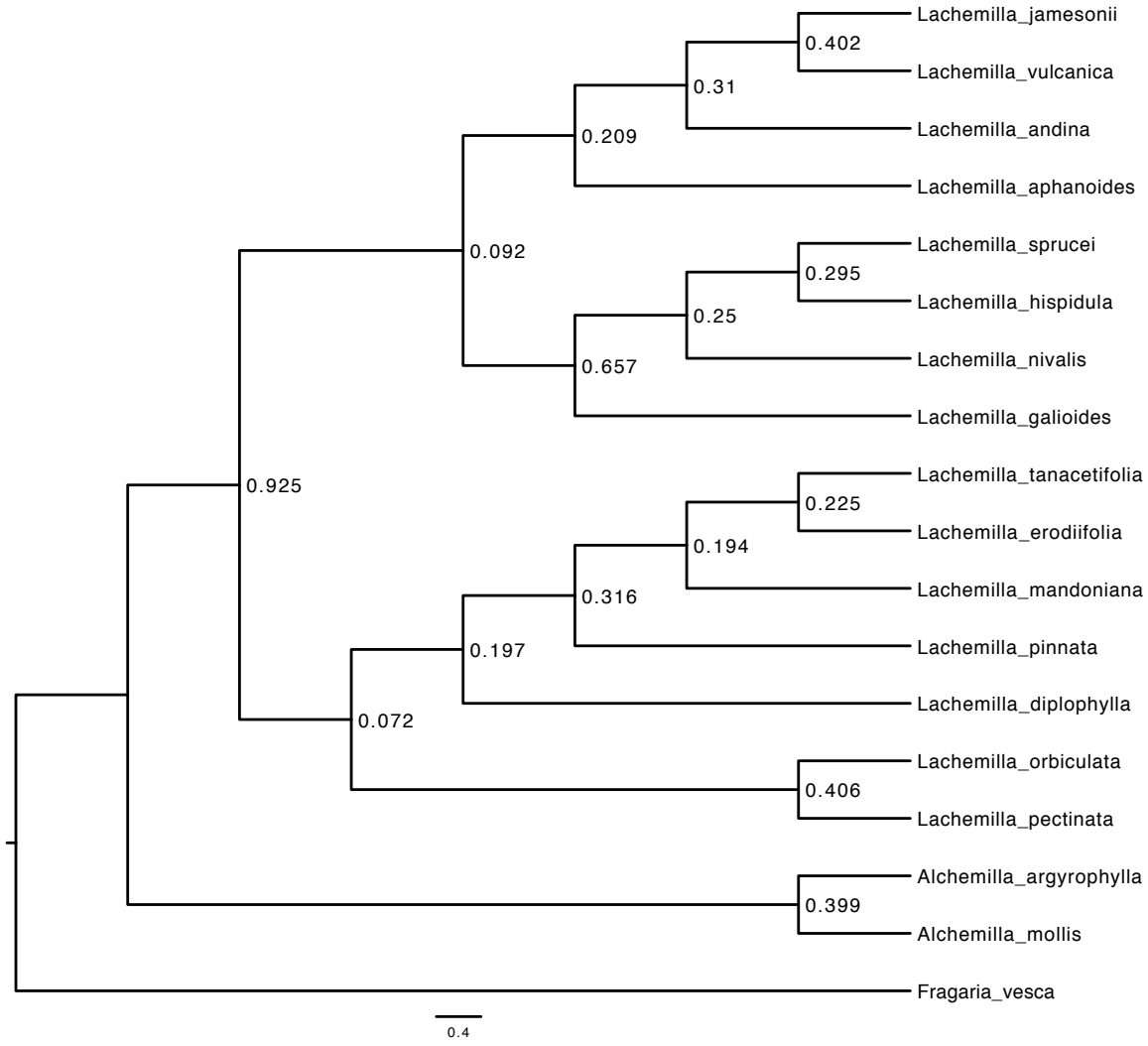
E. MP-EST species trees of the concatenated matrix node label indicate ICA scores.



F. MP-EST species trees of the concatenated matrix; numbers above and below the branches indicate the number of gene trees in agreement and in conflict, respectively. Pie charts next to the nodes represent the proportion of gene trees that support that clade – blue, the proportion that support the main alternative for that clade – green, the proportion that support the remaining alternatives – red, and the proportion (conflict or support) that have less than 50% bootstrap support – gray.



G. Bayesian concordance analysis with BUCKy; node label indicate concordance factors.



Appendix 10

Recombination test of nuclear loci used in Chapter 2.

Results of test of recombination Φ. Loci with strong signal of recombination (P-value ≤ 0.05). Notes: Recombinant loci = 131, Non-recombinant loci = 265

Loci	P-value	Recombination
gene00141_364_01	0.8910	FALSE
gene00817_1511_10	0.1920	FALSE
gene00817_3028_06	0.0058	TRUE
gene00817_356_14	1.0000	FALSE
gene00817_524_04	0.2650	FALSE
gene00817_5739_12	0.3630	FALSE
gene00817_727_13	0.0001	TRUE
gene00817_912_03	0.0109	TRUE
gene01049_659_04	0.0048	TRUE
gene01049_659_04_2	0.2490	FALSE
gene01053_1189_02	0.8180	FALSE
gene01053_595_01	0.7500	FALSE
gene01831_360_16	1.0000	FALSE
gene01831_390_19	0.0437	TRUE
gene01874_1056_05	0.2270	FALSE
gene01874_348_01	0.1190	FALSE
gene01874_349_03	0.9130	FALSE
gene01874_868_07	0.0357	TRUE
gene02115_602_06	0.0004	TRUE
gene02215_322_08	0.9450	FALSE
gene02215_374_06	0.3730	FALSE
gene02215_413_02	0.0569	FALSE
gene02215_413_05	0.1930	FALSE
gene02215_545_01	0.2400	FALSE
gene02335_382_03	0.5930	FALSE
gene02335_382_03_2	0.2960	FALSE
gene02335_405_02	0.0446	TRUE
gene02475_1110_03	0.3650	FALSE
gene02475_349_06	0.3340	FALSE
gene02540_348_02	0.0001	TRUE
gene02540_438_01	0.0925	FALSE
gene02540_633_03	0.0064	TRUE

Loci	P-value	Recombination
gene02540_633_03_2	0.6250	FALSE
gene03141_457_09	0.2070	FALSE
gene03141_677_10	0.0014	TRUE
gene03310_370_01	0.6560	FALSE
gene03310_423_05	0.6320	FALSE
gene03310_423_05_2	0.2070	FALSE
gene03310_462_06	0.4680	FALSE
gene03310_834_03	0.1110	FALSE
gene03310_834_03_2	0.0909	FALSE
gene03653_348_01	0.9350	FALSE
gene03874_429_01	0.5970	FALSE
gene03874_429_01_2	0.0738	FALSE
gene03965_2019_04	0.0118	TRUE
gene03965_543_02	0.9770	FALSE
gene03965_852_01	0.1750	FALSE
gene04205_1920_01	0.0074	TRUE
gene04871_372_02	0.8630	FALSE
gene04871_451_05	0.5550	FALSE
gene05003_772_01	0.0283	TRUE
gene05024_361_02	0.2070	FALSE
gene05461_526_04	0.0026	TRUE
gene05461_526_04_2	0.5510	FALSE
gene05461_526_04_3	0.0182	TRUE
gene05461_544_03	0.1630	FALSE
gene05461_544_03_2	1.0000	FALSE
gene05491_305_07	0.0331	TRUE
gene05539_313_07	0.8600	FALSE
gene05539_567_01	0.0154	TRUE
gene05983_339_07	0.0608	FALSE
gene06030_990_01	0.0011	TRUE
gene06334_1953_01	0.0855	FALSE
gene06392_433_01	0.7490	FALSE
gene06392_846_06	0.0000	TRUE
gene06545_1466_04	0.2720	FALSE
gene06545_441_02	0.5650	FALSE
gene06545_532_01	0.1480	FALSE
gene06545_532_01_2	0.0192	TRUE
gene06563_391_03	1.0000	FALSE
gene06563_678_04	0.3530	FALSE
gene06778_351_08	0.0246	TRUE
gene06778_510_09	0.7240	FALSE

Loci	P-value	Recombination
gene07060_504_11	0.0163	TRUE
gene07331_492_01	0.1120	FALSE
gene07331_492_01_2	0.0238	TRUE
gene07367_318_01	0.0602	FALSE
gene07367_318_01_2	0.3720	FALSE
gene07464_3449_14	0.0000	TRUE
gene07464_418_21	0.1860	FALSE
gene07464_418_21_2	0.0639	FALSE
gene07645_426_01	0.0001	TRUE
gene07792_372_04	0.5800	FALSE
gene07792_432_03	0.4320	FALSE
gene07792_432_03_2	0.8900	FALSE
gene07792_559_02	0.7050	FALSE
gene07892_400_15	0.0070	TRUE
gene07892_478_07	0.1890	FALSE
gene07892_684_05	0.0001	TRUE
gene07926_444_08	0.2510	FALSE
gene07942_779_06	0.0000	TRUE
gene07947_315_01	0.1830	FALSE
gene07947_315_01_2	0.4050	FALSE
gene07947_701_02	0.1430	FALSE
gene08027_486_09	0.1180	FALSE
gene08027_528_11	0.2470	FALSE
gene08027_657_10	0.2780	FALSE
gene08048_1167_05	0.0000	TRUE
gene08624_1311_01	0.1860	FALSE
gene08653_330_02	0.8300	FALSE
gene08653_792_01	0.2850	FALSE
gene08786_474_02	0.0359	TRUE
gene08972_811_03	0.0052	TRUE
gene09551_765_02	0.1450	FALSE
gene09580_2232_01	0.0000	TRUE
gene09610_465_05	1.0000	FALSE
gene09610_929_07	0.0000	TRUE
gene09622_344_05	0.1140	FALSE
gene09668_1173_01	0.0000	TRUE
gene09896_1321_02	0.5410	FALSE
gene09896_824_01	0.6620	FALSE
gene10536_2170_01	0.1470	FALSE
gene11116_567_01	0.4220	FALSE
gene11116_992_04	0.0005	TRUE

Loci	P-value	Recombination
gene11116_992_04_2	0.0308	TRUE
gene11230_635_03	0.1210	FALSE
gene11263_1854_06	0.0000	TRUE
gene11478_322_07	0.3550	FALSE
gene11478_595_04	0.6880	FALSE
gene11621_381_04	0.6700	FALSE
gene11621_474_02	0.0003	TRUE
gene11621_474_02_2	0.2640	FALSE
gene11621_964_01	0.5460	FALSE
gene12090_1647_02	0.6420	FALSE
gene12320_679_01	0.0413	TRUE
gene12546_1076_01	0.2180	FALSE
gene12558_454_01	0.0179	TRUE
gene12671_447_07	0.0511	FALSE
gene12685_429_06	0.0785	FALSE
gene12685_753_07	0.4150	FALSE
gene12945_361_07	0.8580	FALSE
gene13202_314_01	0.0667	FALSE
gene13202_324_06	0.8370	FALSE
gene13339_465_01	0.0000	TRUE
gene13582_1167_02	0.0525	FALSE
gene13582_525_01	0.5090	FALSE
gene13675_316_02	0.0770	FALSE
gene14077_448_01	0.0305	TRUE
gene14077_495_04	0.1010	FALSE
gene14077_495_04_2	0.2140	FALSE
gene14097_1107_01	0.8850	FALSE
gene14155_352_05	0.1630	FALSE
gene14166_2715_01	0.0000	TRUE
gene14467_340_03	0.1100	FALSE
gene14467_470_06	0.0432	TRUE
gene14467_487_01	0.9160	FALSE
gene14575_311_04	0.5360	FALSE
gene14575_370_05	0.3800	FALSE
gene14696_411_05	0.0004	TRUE
gene14696_411_05_2	0.1310	FALSE
gene14696_492_01	0.3730	FALSE
gene14696_492_01_2	0.1420	FALSE
gene14783_325_06	0.6870	FALSE
gene14886_564_03	0.0005	TRUE
gene15177_327_01	0.4560	FALSE

Loci	P-value	Recombination
gene15177_457_05	0.0500	TRUE
gene15204_592_04	0.0001	TRUE
gene15520_1299_01	0.0003	TRUE
gene15520_1299_01_2	0.0305	TRUE
gene15629_340_12	0.2050	FALSE
gene15629_340_12_2	0.6110	FALSE
gene15629_394_10	0.1770	FALSE
gene15773_489_02	0.0112	TRUE
gene15844_327_11	0.0872	FALSE
gene15966_1458_01	0.1340	FALSE
gene15991_967_02	0.0000	TRUE
gene15996_1557_01	0.0009	TRUE
gene15996_505_12	0.0656	FALSE
gene15996_505_12_2	0.0145	TRUE
gene15996_528_14	0.2680	FALSE
gene16000_641_03	0.0001	TRUE
gene16098_399_03	0.0008	TRUE
gene16098_575_04	0.4280	FALSE
gene16115_306_03	0.5000	FALSE
gene16115_495_01	0.5000	FALSE
gene16123_556_01	0.2560	FALSE
gene16123_556_01_2	0.2170	FALSE
gene16123_675_06	0.4380	FALSE
gene16123_675_06_2	0.6500	FALSE
gene16206_829_01	0.4640	FALSE
gene16220_353_02	0.8430	FALSE
gene16311_902_02	0.0030	TRUE
gene16900_708_06	0.5400	FALSE
gene16900_708_06_2	0.1680	FALSE
gene16900_708_06_3	0.3930	FALSE
gene16939_386_06	1.0000	FALSE
gene16959_703_01	0.1130	FALSE
gene16959_703_01_2	0.1330	FALSE
gene17026_407_01	0.0020	TRUE
gene17026_407_01_2	0.4720	FALSE
gene17112_1026_02	0.5180	FALSE
gene17112_981_01	0.0479	TRUE
gene17249_536_01	0.0021	TRUE
gene17942_354_06	0.7670	FALSE
gene17942_354_06_2	0.0418	TRUE
gene17942_535_01	0.0984	FALSE

Loci	P-value	Recombination
gene17942_535_01_2	0.9340	FALSE
gene17946_606_04	0.1100	FALSE
gene17946_606_04_2	0.6510	FALSE
gene18143_316_06	0.6450	FALSE
gene18143_347_12	0.0231	TRUE
gene18143_559_01	0.0179	TRUE
gene18499_1623_01	0.0119	TRUE
gene18567_303_02	0.3980	FALSE
gene18567_874_04	0.3910	FALSE
gene18567_874_04_2	0.6630	FALSE
gene18567_930_01	0.3760	FALSE
gene18567_930_01_2	0.0121	TRUE
gene18678_1468_07	0.3130	FALSE
gene18678_384_04	1.0000	FALSE
gene18678_564_01	0.6850	FALSE
gene18685_1014_05	0.0139	TRUE
gene18685_330_01	0.0159	TRUE
gene18685_359_03	0.1900	FALSE
gene18685_399_07	0.3980	FALSE
gene18685_734_06	0.1390	FALSE
gene18949_312_02	0.9100	FALSE
gene18949_452_04	0.2850	FALSE
gene19156_751_05	0.0052	TRUE
gene19378_314_01	1.0000	FALSE
gene19378_314_01_2	0.1710	FALSE
gene19390_1123_10	0.1960	FALSE
gene19390_1123_10_2	0.0075	TRUE
gene19618_849_02	0.0001	TRUE
gene20081_1457_02	0.5020	FALSE
gene20081_1457_02_2	0.5600	FALSE
gene20081_414_01	0.0122	TRUE
gene20081_729_11	0.0639	FALSE
gene20081_729_11_2	0.2980	FALSE
gene20812_917_02	0.0120	TRUE
gene20992_402_01	0.1760	FALSE
gene20992_460_19	0.0154	TRUE
gene20992_488_20	0.2180	FALSE
gene21604_1821_05	0.0013	TRUE
gene21604_337_02	1.0000	FALSE
gene21604_549_01	0.6850	FALSE
gene21620_349_04	0.9490	FALSE

Loci	P-value	Recombination
gene21620_466_01	1.0000	FALSE
gene21620_751_02	0.0092	TRUE
gene21678_2745_01	0.2300	FALSE
gene21878_333_01	0.1760	FALSE
gene21878_398_03	0.0912	FALSE
gene21878_794_08	0.6450	FALSE
gene22056_2034_01	0.0292	TRUE
gene22085_353_06	0.9020	FALSE
gene22185_324_04	0.2990	FALSE
gene22185_402_06	0.1010	FALSE
gene22419_336_04	0.1100	FALSE
gene22419_651_01	0.0875	FALSE
gene22828_592_02	0.2390	FALSE
gene22838_1258_04	0.0117	TRUE
gene22838_320_06	0.0388	TRUE
gene22926_314_06	0.0402	TRUE
gene22926_422_01	0.5940	FALSE
gene22963_1183_05	0.0052	TRUE
gene22963_522_03	0.2570	FALSE
gene23026_590_03	1.0000	FALSE
gene23026_848_02	0.0427	TRUE
gene23026_848_02_2	0.2650	FALSE
gene23128_513_02	0.0201	TRUE
gene23128_513_02_2	0.8960	FALSE
gene23128_639_01	0.5880	FALSE
gene23345_1894_03	0.0209	TRUE
gene23345_318_01	1.0000	FALSE
gene23345_362_04	0.2690	FALSE
gene23601_1209_01	0.0075	TRUE
gene23731_416_03	0.0001	TRUE
gene23736_1383_01	0.0621	FALSE
gene23736_1383_01_2	0.0118	TRUE
gene23780_385_02	0.8090	FALSE
gene23780_385_02_2	0.5000	FALSE
gene23780_499_03	0.6270	FALSE
gene23780_503_04	0.2770	FALSE
gene24025_440_02	0.4110	FALSE
gene24141_315_03	0.0529	FALSE
gene24141_327_01	0.0353	TRUE
gene24141_401_05	0.1050	FALSE
gene24244_438_01	0.0080	TRUE

Loci	P-value	Recombination
gene24244_750_04	0.0004	TRUE
gene24244_750_04_2	0.0101	TRUE
gene24250_1005_03	0.0104	TRUE
gene24275_354_05	0.3130	FALSE
gene24275_356_03	1.0000	FALSE
gene24275_492_01	0.0676	FALSE
gene24275_492_01_2	0.8340	FALSE
gene24302_409_02	0.0163	TRUE
gene24302_478_14	0.0515	FALSE
gene24407_1710_01	0.0015	TRUE
gene24475_384_01	0.0106	TRUE
gene24477_339_01	0.7840	FALSE
gene24477_339_01_2	0.2730	FALSE
gene24477_668_04	0.1050	FALSE
gene24477_668_04_2	0.0089	TRUE
gene24511_1110_01	0.0197	TRUE
gene24511_1110_01_2	0.0187	TRUE
gene24640_650_07	0.3080	FALSE
gene24646_354_03	0.6180	FALSE
gene24653_459_03	1.0000	FALSE
gene24834_1341_09	0.0058	TRUE
gene24834_327_06	0.0483	TRUE
gene24834_327_06_2	0.6180	FALSE
gene24834_375_14	0.8710	FALSE
gene24834_614_15	0.0244	TRUE
gene24860_389_03	0.1100	FALSE
gene24860_522_16	0.1770	FALSE
gene24860_522_16_2	0.6200	FALSE
gene25161_469_02	0.1370	FALSE
gene25463_381_12	0.0526	FALSE
gene25463_412_08	0.0975	FALSE
gene25463_460_07	0.1550	FALSE
gene25633_710_10	0.0029	TRUE
gene25925_1115_02	0.0246	TRUE
gene25925_1115_02_2	0.0313	TRUE
gene26791_363_01	0.4660	FALSE
gene26791_443_05	0.1250	FALSE
gene26791_728_02	0.3800	FALSE
gene26791_728_02_2	0.2460	FALSE
gene27696_396_01	0.0000	TRUE
gene27750_465_02	0.1990	FALSE

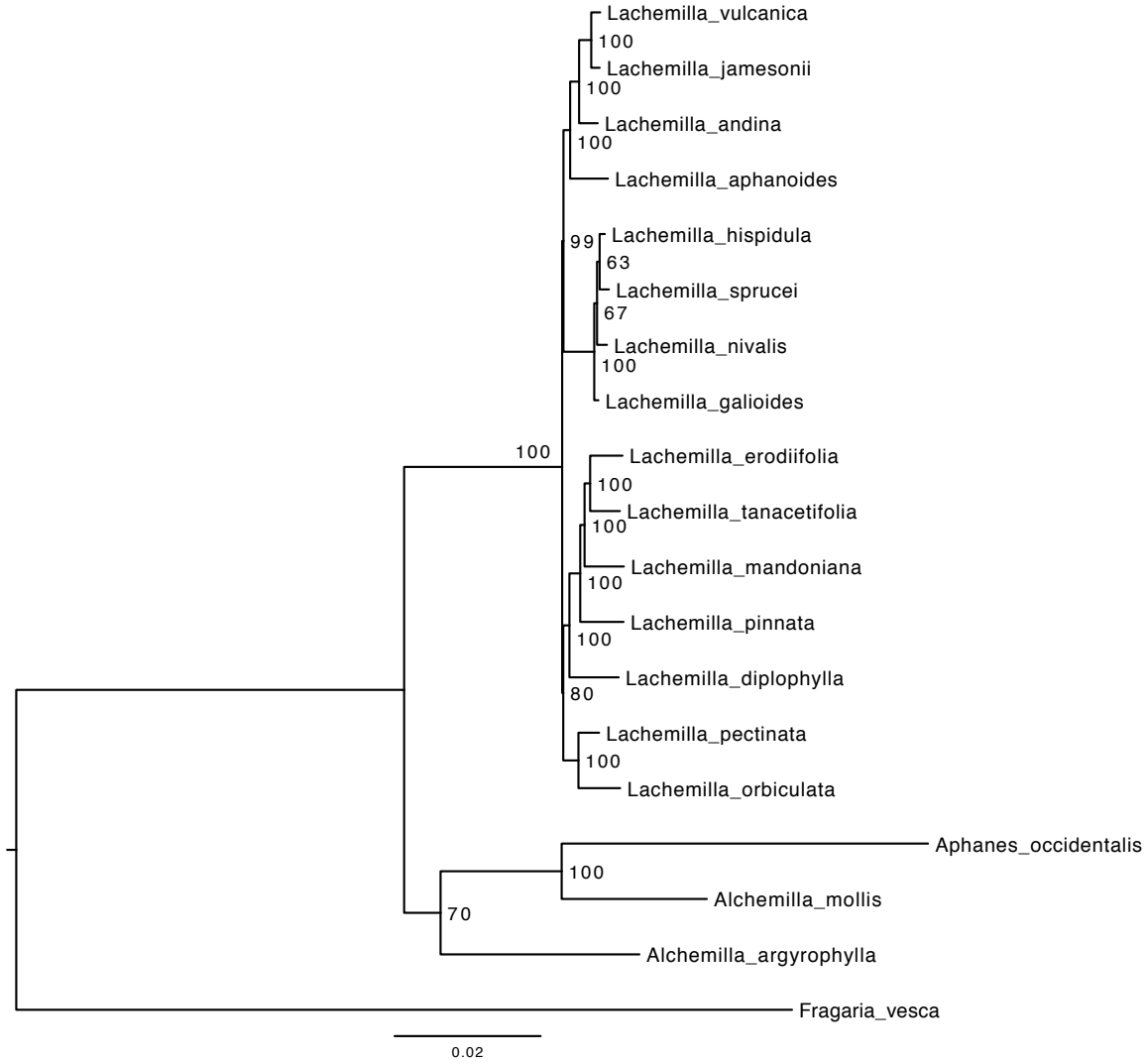
Loci	P-value	Recombination
gene27750_569_08	0.3730	FALSE
gene27813_450_06	0.1060	FALSE
gene27943_546_09	0.8370	FALSE
gene27943_546_09_2	0.0473	TRUE
gene27943_762_08	0.0026	TRUE
gene27943_762_08_2	0.0340	TRUE
gene28151_414_06	1.0000	FALSE
gene28151_450_05	0.1940	FALSE
gene28218_597_06	0.1270	FALSE
gene28218_597_06_2	0.6350	FALSE
gene28218_795_03	0.0340	TRUE
gene28218_869_04	0.0000	TRUE
gene28243_539_06	0.4880	FALSE
gene28245_1581_01	0.0004	TRUE
gene28247_435_07	0.1770	FALSE
gene28260_355_02	0.0121	TRUE
gene28260_741_04	0.0000	TRUE
gene28260_741_04_2	0.0000	TRUE
gene28281_723_18	0.0124	TRUE
gene28317_1002_06	0.0623	FALSE
gene28330_2739_03	0.0280	TRUE
gene28330_561_01	0.0582	FALSE
gene28559_376_04	0.1470	FALSE
gene28559_462_03	0.0000	TRUE
gene28569_331_02	0.0094	TRUE
gene28629_309_07	1.0000	FALSE
gene28629_423_02	0.9940	FALSE
gene28629_446_03	0.1990	FALSE
gene28686_1040_01	0.1520	FALSE
gene28686_1040_01_2	0.0300	TRUE
gene28689_952_01	0.9350	FALSE
gene29299_354_07	0.2790	FALSE
gene29299_367_02	1.0000	FALSE
gene29878_522_07	0.0003	TRUE
gene29895_1211_02	0.0000	TRUE
gene29895_430_03	0.0639	FALSE
gene29895_590_04	0.3080	FALSE
gene29895_590_04_2	0.0026	TRUE
gene29895_961_01	0.3820	FALSE
gene30048_349_02	0.0189	TRUE
gene30118_348_04	0.9390	FALSE

Loci	P-value	Recombination
gene30190_1191_01	0.1170	FALSE
gene30214_453_02	0.4080	FALSE
gene30214_523_03	0.1100	FALSE
gene30392_448_03	0.1420	FALSE
gene30392_624_02	0.7040	FALSE
gene30405_1896_01	0.0000	TRUE
gene30425_1367_01	0.0637	FALSE
gene30526_327_01	0.8200	FALSE
gene30526_663_04	0.0003	TRUE
gene30622_973_01	0.9420	FALSE
gene30829_324_04	0.0511	FALSE
gene30829_352_01	0.8900	FALSE
gene30829_729_03	0.0388	TRUE
gene30829_729_03_2	0.1810	FALSE
gene31237_438_06	0.2260	FALSE
gene31237_643_08	0.1980	FALSE
gene31269_342_06	1.0000	FALSE
gene31269_369_04	1.0000	FALSE
gene31345_439_03	0.0948	FALSE
gene31345_463_04	0.0000	TRUE
gene31345_463_04_2	0.9680	FALSE
gene31345_499_11	0.1700	FALSE
gene31345_499_11_2	0.8650	FALSE
gene31484_635_02	0.1450	FALSE
gene31508_1455_01	0.0638	FALSE
gene31508_1455_01_2	0.6900	FALSE
gene32082_324_03	0.7930	FALSE
gene32082_641_01	0.7080	FALSE
gene32117_403_01	0.0017	TRUE
gene32117_420_04	0.5000	FALSE
gene32182_498_05	0.3180	FALSE
gene32182_498_05_2	0.0005	TRUE
gene32182_498_05_3	0.0058	TRUE
gene32260_1611_01	0.0028	TRUE
gene32640_319_02	0.2640	FALSE
gene34105_796_02	0.0710	FALSE

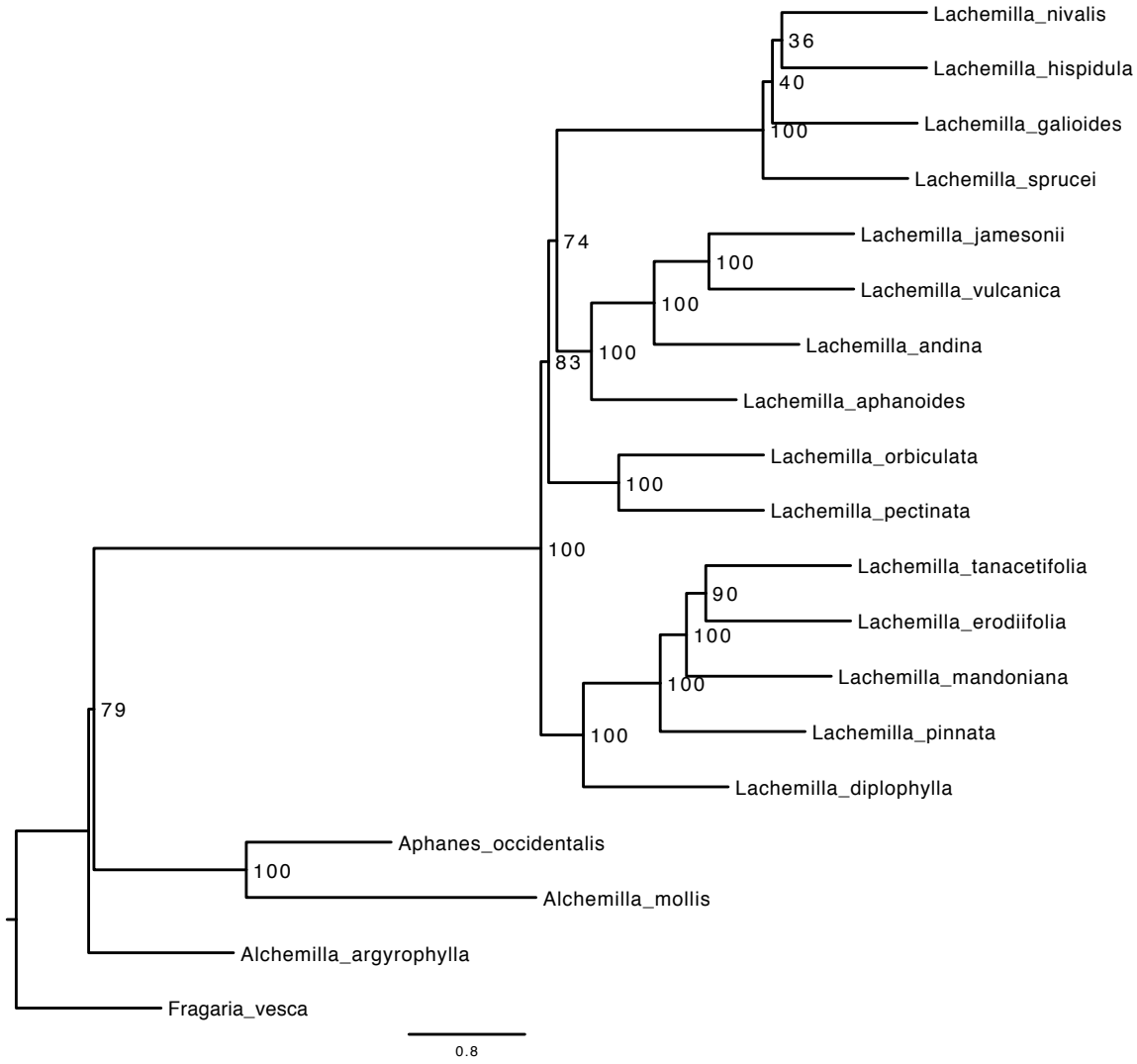
Appendix 11

Phylogenetic analyses of the NO-RECOMBINATION dataset from Chapter 2.

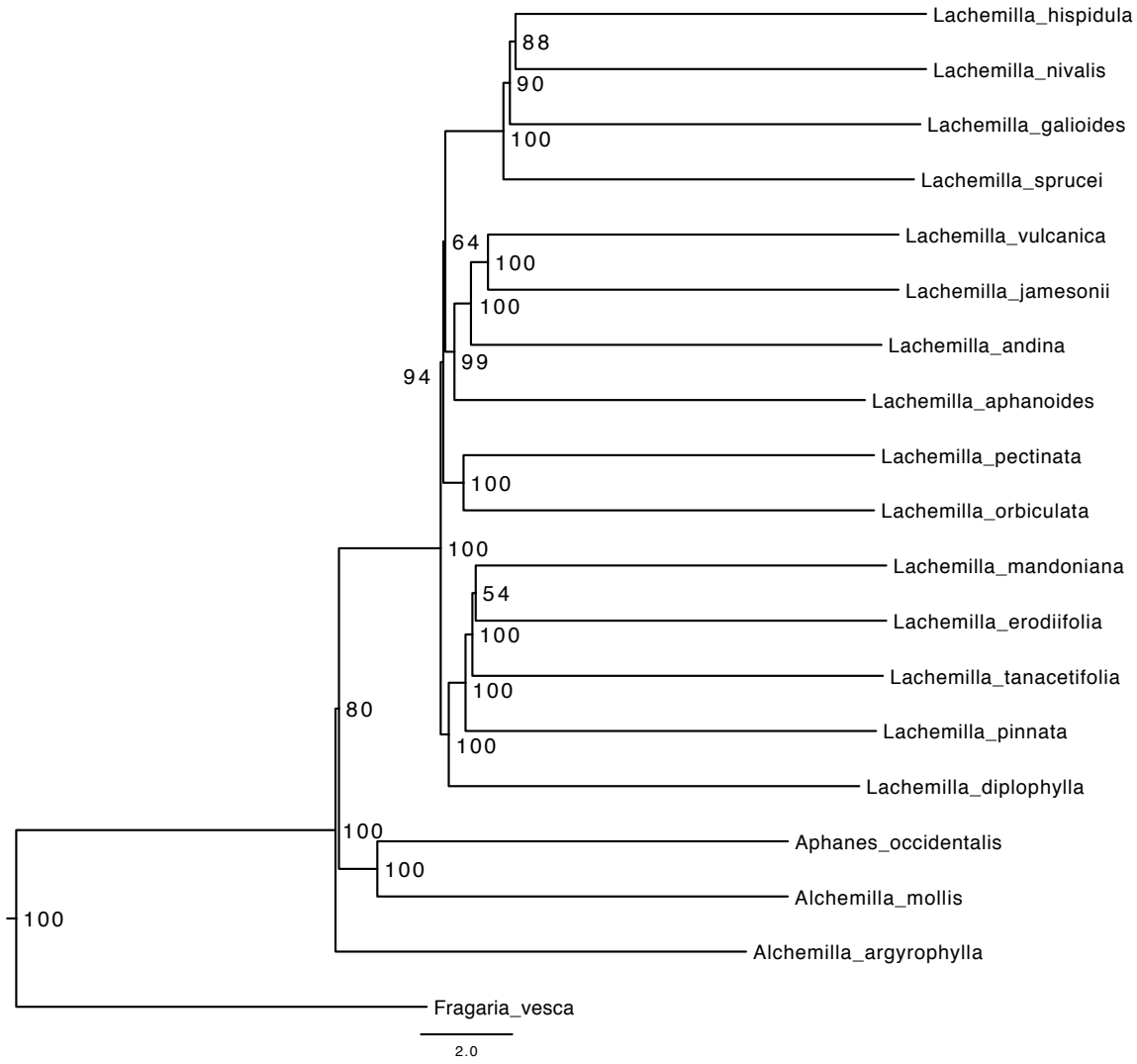
A. RAxML phylogeny of the concatenated matrix; node label indicate bootstrap values.



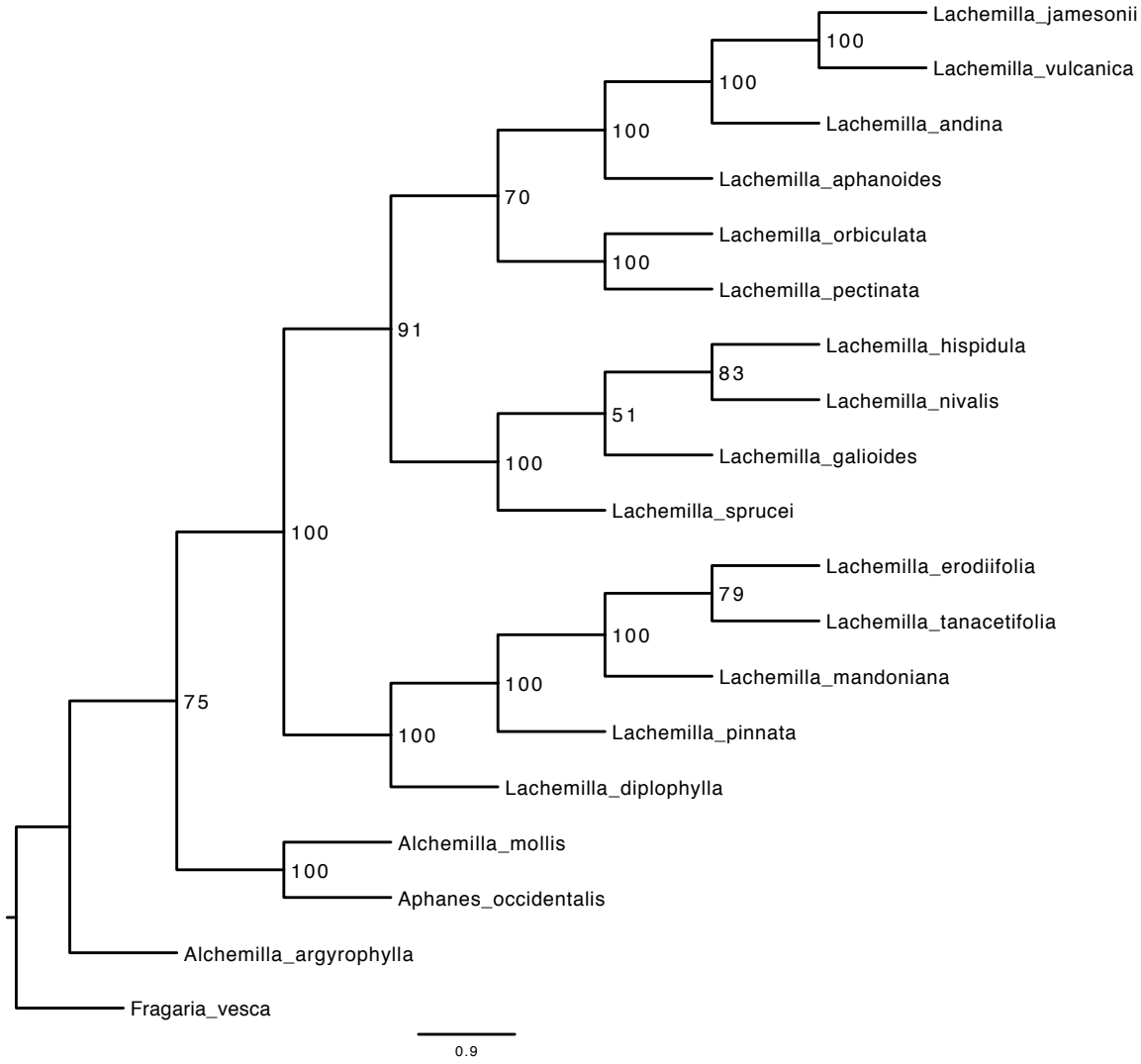
B. ASTRAL-II species tree; node labels indicate bootstrap values.



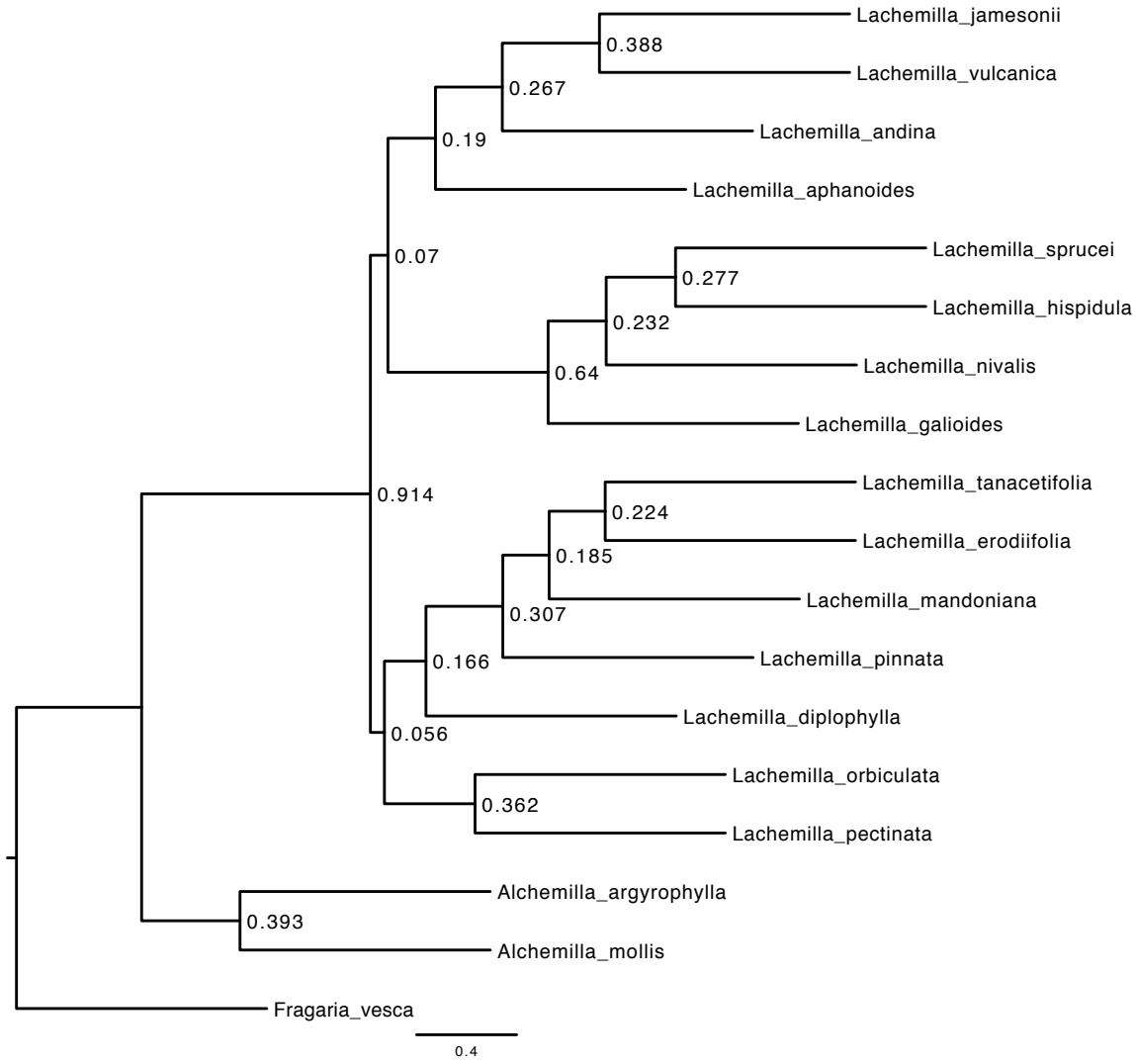
C. MP-EST species tree; node labels indicate bootstrap values.



D. SVDquartets species tree; node label indicate bootstrap values.



E. Bayesian concordance analysis with BUCKy; node labels indicate concordance factors.



Appendix 12

Approximately Unbiased test results from Gene Genealogy Interrogation of the HYBRID-REDUCED dataset from Chapter 2.

Results of the Approximately Unbiased (AU) test from GGI for the HYBRID-REDUCED dataset. Values above 0.95 indicate topologies (following Fig. 2.5) that are significantly better than the alternatives ($P \leq 0.05$)

Locus	Tree topology with Rank 1	AU test P-value
gene00141_364_01	4	0.753
gene00817_1511_10	4	0.889
gene00817_3028_06	2	0.605
gene00817_356_14	4	0.986
gene00817_524_04	4	0.829
gene00817_5739_12	4	0.805
gene00817_727_13	3	0.483
gene00817_912_03	4	0.987
gene01049_659_04_2	1	0.938
gene01049_659_04	1	0.959
gene01053_1189_02	3	0.751
gene01053_595_01	4	1.000
gene01831_360_16	4	0.870
gene01831_390_19	4	0.687
gene01874_1056_05	1	0.936
gene01874_348_01	4	0.844
gene01874_349_03	4	0.619
gene01874_868_07	4	1.000
gene02115_602_06	3	0.982
gene02215_322_08	3	0.607
gene02215_374_06	1	0.948
gene02215_413_02	1	0.758
gene02215_413_05	4	0.879
gene02215_545_01	4	0.953
gene02335_382_03_2	1	0.866
gene02335_382_03	3	0.999
gene02335_405_02	4	1.000
gene02475_1110_03	3	0.882
gene02475_349_06	2	0.623

Locus	Tree topology with Rank 1	AU test P-value
gene02540_348_02	3	0.805
gene02540_438_01	2	0.951
gene02540_633_03_2	4	0.935
gene02540_633_03	4	0.997
gene03141_457_09	4	0.915
gene03141_677_10	3	0.668
gene03310_370_01	1	0.942
gene03310_423_05_2	4	1.000
gene03310_423_05	3	0.824
gene03310_462_06	4	0.969
gene03310_834_03_2	3	0.497
gene03310_834_03	3	0.830
gene03653_348_01	2	0.998
gene03874_429_01_2	4	0.713
gene03874_429_01	4	1.000
gene03965_2019_04	2	0.435
gene03965_543_02	3	0.721
gene03965_852_01	3	0.802
gene04205_1920_01	4	0.872
gene04871_372_02	3	0.980
gene04871_451_05	4	0.715
gene05003_772_01	4	0.684
gene05024_361_02	2	0.308
gene05461_526_04_2	3	0.956
gene05461_526_04_3	3	0.642
gene05461_526_04	1	0.908
gene05461_544_03_2	4	0.970
gene05461_544_03	1	0.890
gene05491_305_07	4	0.922
gene05539_313_07	4	0.898
gene05539_567_01	2	0.629
gene05983_339_07	2	0.922
gene06030_990_01	3	0.571
gene06334_1953_01	1	0.798
gene06392_433_01	1	0.735
gene06392_846_06	4	0.582
gene06545_1466_04	3	0.909
gene06545_441_02	4	0.991
gene06545_532_01_2	4	0.742
gene06545_532_01	3	0.758
gene06563_391_03	3	0.925

Locus	Tree topology with Rank 1	AU test P-value
gene06563_678_04	3	0.884
gene06778_351_08	4	0.996
gene06778_510_09	4	1.000
gene07060_504_11	4	0.636
gene07331_492_01_2	4	0.962
gene07331_492_01	3	0.782
gene07367_318_01_2	4	0.675
gene07367_318_01	1	0.986
gene07464_3449_14	2	0.574
gene07464_418_21_2	1	0.752
gene07464_418_21	4	1.000
gene07645_426_01	2	0.998
gene07792_372_04	1	0.937
gene07792_432_03_2	4	0.943
gene07792_432_03	4	0.936
gene07792_559_02	3	0.988
gene07892_400_15	3	0.785
gene07892_478_07	3	1.000
gene07892_684_05	1	0.797
gene07926_444_08	2	0.474
gene07942_779_06	4	0.680
gene07947_315_01_2	2	0.939
gene07947_315_01	4	0.587
gene07947_701_02	3	0.894
gene08027_486_09	2	0.996
gene08027_528_11	2	1.000
gene08027_657_10	4	0.991
gene08048_1167_05	4	0.657
gene08624_1311_01	4	0.977
gene08653_330_02	3	0.975
gene08653_792_01	4	0.633
gene08786_474_02	4	0.923
gene08972_811_03	4	0.761
gene09551_765_02	3	0.966
gene09580_2232_01	4	0.656
gene09610_465_05	1	0.885
gene09610_929_07	4	0.786
gene09622_344_05	3	0.937
gene09668_1173_01	3	0.583
gene09896_1321_02	1	0.863
gene09896_824_01	1	0.967

Locus	Tree topology with Rank 1	AU test P-value
gene10536_2170_01	3	0.683
gene11116_567_01	3	0.842
gene11116_992_04_2	4	0.761
gene11116_992_04	4	0.988
gene11230_635_03	4	0.718
gene11263_1854_06	3	0.817
gene11478_322_07	2	0.654
gene11478_595_04	1	1.000
gene11621_381_04	4	0.533
gene11621_474_02_2	1	0.766
gene11621_474_02	3	0.935
gene11621_964_01	1	0.960
gene12090_1647_02	2	0.554
gene12320_679_01	4	0.991
gene12546_1076_01	4	0.968
gene12558_454_01	1	0.993
gene12671_447_07	4	0.774
gene12685_429_06	4	0.968
gene12685_753_07	3	0.817
gene12945_361_07	4	0.635
gene13202_314_01	4	0.931
gene13202_324_06	2	0.998
gene13339_465_01	1	0.874
gene13582_1167_02	4	0.655
gene13582_525_01	4	0.992
gene13675_316_02	4	0.830
gene14077_448_01	1	0.640
gene14077_495_04_2	2	1.000
gene14077_495_04	3	0.611
gene14097_1107_01	2	0.828
gene14155_352_05	3	0.957
gene14166_2715_01	3	0.790
gene14467_340_03	2	0.000
gene14467_470_06	1	0.638
gene14467_487_01	4	0.964
gene14575_311_04	3	0.703
gene14575_370_05	1	1.000
gene14696_411_05_2	4	0.717
gene14696_411_05	3	0.933
gene14696_492_01_2	4	1.000
gene14696_492_01	4	1.000

Locus	Tree topology with Rank 1	AU test P-value
gene14783_325_06	3	0.869
gene14886_564_03	1	0.953
gene15177_327_01	1	0.000
gene15177_457_05	1	0.962
gene15204_592_04	3	0.940
gene15520_1299_01_2	4	0.980
gene15520_1299_01	4	0.993
gene15629_340_12_2	4	0.816
gene15629_340_12	4	0.577
gene15629_394_10	3	0.933
gene15773_489_02	1	0.980
gene15844_327_11	4	0.558
gene15966_1458_01	3	0.788
gene15991_967_02	3	0.633
gene15996_1557_01	3	0.988
gene15996_505_12_2	3	0.741
gene15996_505_12	1	0.930
gene15996_528_14	2	0.904
gene16000_641_03	4	0.982
gene16098_399_03	4	0.817
gene16098_575_04	3	0.677
gene16115_306_03	1	0.921
gene16115_495_01	4	1.000
gene16123_556_01_2	3	0.697
gene16123_556_01	1	0.000
gene16123_675_06_2	2	0.976
gene16123_675_06	2	0.927
gene16206_829_01	3	0.831
gene16220_353_02	4	0.851
gene16311_902_02	1	0.854
gene16900_708_06_2	4	0.674
gene16900_708_06_3	2	0.885
gene16900_708_06	4	0.708
gene16939_386_06	4	0.963
gene16959_703_01_2	4	1.000
gene16959_703_01	4	0.718
gene17026_407_01_2	1	0.599
gene17026_407_01	4	1.000
gene17112_1026_02	4	0.811
gene17112_981_01	4	0.677
gene17249_536_01	3	0.605

Locus	Tree topology with Rank 1	AU test P-value
gene17942_354_06_2	2	0.994
gene17942_354_06	2	1.000
gene17942_535_01_2	4	0.691
gene17942_535_01	3	0.906
gene17946_606_04_2	4	0.999
gene17946_606_04	4	0.783
gene18143_316_06	1	1.000
gene18143_347_12	1	0.997
gene18143_559_01	1	0.729
gene18499_1623_01	3	0.762
gene18567_303_02	4	0.909
gene18567_874_04_2	3	0.568
gene18567_874_04	4	1.000
gene18567_930_01_2	1	0.920
gene18567_930_01	1	0.543
gene18678_1468_07	1	0.971
gene18678_384_04	4	0.973
gene18678_564_01	3	0.621
gene18685_1014_05	2	0.991
gene18685_330_01	2	0.936
gene18685_359_03	2	0.604
gene18685_399_07	2	0.645
gene18685_734_06	4	0.833
gene18949_312_02	4	0.987
gene18949_452_04	2	0.946
gene19156_751_05	3	0.899
gene19378_314_01_2	1	0.804
gene19378_314_01	4	0.966
gene19390_1123_10_2	3	0.931
gene19390_1123_10	2	0.945
gene19618_849_02	1	0.991
gene20081_1457_02_2	3	0.730
gene20081_1457_02	4	0.863
gene20081_414_01	4	0.845
gene20081_729_11_2	3	0.966
gene20081_729_11	1	0.915
gene20812_917_02	4	0.623
gene20992_402_01	1	0.817
gene20992_460_19	2	0.718
gene20992_488_20	4	0.818
gene21604_1821_05	1	0.828

Locus	Tree topology with Rank 1	AU test P-value
gene21604_337_02	1	0.997
gene21604_549_01	3	0.608
gene21620_349_04	3	0.854
gene21620_466_01	1	0.925
gene21620_751_02	4	0.897
gene21678_2745_01	2	0.917
gene21878_333_01	1	0.993
gene21878_398_03	4	0.909
gene21878_794_08	4	0.846
gene22056_2034_01	3	0.633
gene22085_353_06	1	0.922
gene22185_324_04	4	0.703
gene22185_402_06	4	0.997
gene22419_336_04	2	0.986
gene22419_651_01	4	0.850
gene22828_592_02	3	0.795
gene22838_1258_04	3	0.784
gene22838_320_06	1	0.879
gene22926_314_06	3	0.924
gene22926_422_01	3	0.886
gene22963_522_03	4	0.976
gene23026_590_03	2	0.999
gene23026_848_02_2	2	1.000
gene23026_848_02	1	1.000
gene23128_513_02_2	2	0.786
gene23128_513_02	3	0.876
gene23128_639_01	4	0.752
gene23345_1894_03	2	0.717
gene23345_318_01	1	0.942
gene23345_362_04	1	1.000
gene23601_1209_01	3	0.882
gene23731_416_03	3	0.914
gene23736_1383_01_2	4	0.758
gene23736_1383_01	3	0.805
gene23780_385_02_2	1	0.933
gene23780_385_02	4	0.791
gene23780_499_03	3	0.742
gene23780_503_04	2	0.825
gene24025_440_02	4	0.963
gene24141_315_03	2	0.951
gene24141_327_01	4	0.841

Locus	Tree topology with Rank 1	AU test P-value
gene24141_401_05	4	0.878
gene24244_438_01	4	0.968
gene24244_750_04_2	2	0.823
gene24244_750_04	2	0.963
gene24250_1005_03	4	0.908
gene24275_354_05	4	0.934
gene24275_356_03	4	0.963
gene24275_492_01_2	4	0.883
gene24275_492_01	4	0.999
gene24302_409_02	1	0.819
gene24302_478_14	2	0.972
gene24407_1710_01	1	0.904
gene24475_384_01	2	0.759
gene24477_339_01_2	4	0.945
gene24477_339_01	2	0.611
gene24477_668_04_2	4	0.940
gene24477_668_04	3	0.977
gene24511_1110_01_2	4	0.995
gene24511_1110_01	4	0.593
gene24640_650_07	4	0.879
gene24646_354_03	1	0.000
gene24653_459_03	4	0.765
gene24834_327_06_2	1	0.472
gene24834_327_06	2	0.857
gene24834_375_14	4	0.913
gene24834_614_15	2	0.828
gene24860_389_03	4	1.000
gene24860_522_16_2	3	0.754
gene24860_522_16	4	1.000
gene25161_469_02	3	0.999
gene25463_381_12	1	0.985
gene25463_412_08	1	0.694
gene25463_460_07	2	0.602
gene25633_710_10	2	0.826
gene25925_1115_02_2	3	0.558
gene25925_1115_02	2	0.555
gene26791_363_01	1	0.933
gene26791_443_05	2	0.852
gene26791_728_02_2	4	0.916
gene26791_728_02	4	0.901
gene27696_396_01	4	0.821

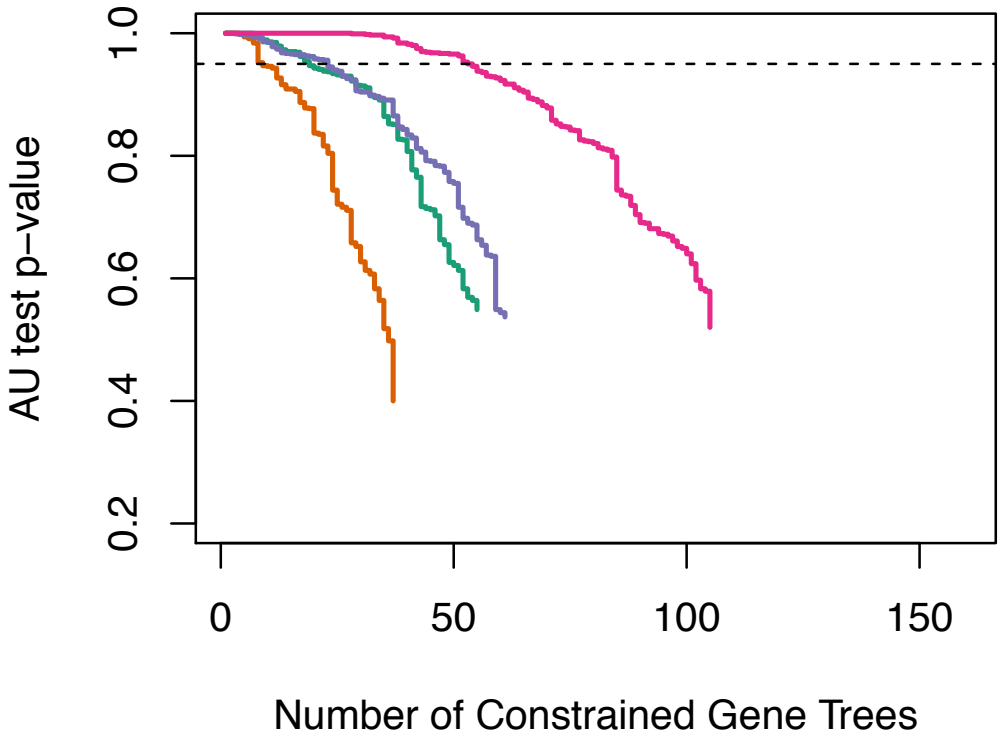
Locus	Tree topology with Rank 1	AU test P-value
gene27750_465_02	2	0.883
gene27750_569_08	1	0.583
gene27813_450_06	4	0.993
gene27943_546_09_2	4	0.624
gene27943_546_09	3	0.924
gene27943_762_08_2	4	0.629
gene27943_762_08	4	1.000
gene28151_414_06	2	0.000
gene28151_450_05	3	0.905
gene28218_597_06_2	1	0.946
gene28218_597_06	3	0.782
gene28218_795_03	3	0.939
gene28218_869_04	2	0.797
gene28243_539_06	1	0.962
gene28245_1581_01	3	0.938
gene28247_435_07	2	0.963
gene28260_355_02	4	0.635
gene28260_741_04_2	3	0.931
gene28260_741_04	1	0.628
gene28281_723_18	4	0.913
gene28317_1002_06	2	0.956
gene28330_2739_03	4	0.686
gene28330_561_01	4	1.000
gene28559_376_04	4	0.983
gene28559_462_03	2	0.812
gene28569_331_02	2	0.870
gene28629_309_07	1	0.617
gene28629_423_02	2	0.880
gene28629_446_03	2	0.662
gene28686_1040_01_2	2	0.740
gene28686_1040_01	3	0.904
gene28689_952_01	3	0.970
gene29299_354_07	4	0.866
gene29299_367_02	4	1.000
gene29878_522_07	4	0.829
gene29895_1211_02	2	0.631
gene29895_430_03	2	0.496
gene29895_590_04_2	3	1.000
gene29895_961_01	2	0.617
gene30048_349_02	1	0.719
gene30118_348_04	1	0.905

Locus	Tree topology with Rank 1	AU test P-value
gene30190_1191_01	4	1.000
gene30214_453_02	4	0.633
gene30214_523_03	1	0.582
gene30392_448_03	4	0.953
gene30392_624_02	1	0.590
gene30405_1896_01	2	0.759
gene30425_1367_01	3	0.997
gene30526_327_01	4	0.604
gene30526_663_04	3	0.582
gene30622_973_01	1	0.632
gene30829_324_04	4	1.000
gene30829_352_01	4	0.673
gene30829_729_03_2	4	0.981
gene30829_729_03	3	0.638
gene31237_438_06	4	0.953
gene31237_643_08	4	0.690
gene31269_342_06	2	0.512
gene31269_369_04	3	0.736
gene31345_439_03	1	0.864
gene31345_463_04_2	2	0.957
gene31345_463_04	3	0.658
gene31345_499_11_2	3	0.848
gene31345_499_11	2	0.887
gene31484_635_02	4	1.000
gene31508_1455_01_2	2	0.992
gene31508_1455_01	1	0.915
gene32082_324_03	4	1.000
gene32082_641_01	4	0.947
gene32117_403_01	3	0.830
gene32117_420_04	2	0.879
gene32182_498_05_2	4	0.901
gene32182_498_05_3	2	0.626
gene32182_498_05	4	0.721
gene32260_1611_01	1	0.936
gene32640_319_02	4	1.000
gene34105_796_02	3	0.964

Appendix 13

Gene Genealogy Interrogation results of the NO-RECOMBINATION dataset from Chapter 2.

Gene genealogy interrogation results of the NO-RECOMBINATION dataset testing the four topologies inferred for the four major clades of *Lachemilla* (following Fig. 2.5) Lines represent the cumulative number of genes supporting each topology with highest probability and their P-values. Values above the dashed line indicate topologies that are significantly better than the alternatives ($P \leq 0.05$). Line colors as in Figure 2.5.



Appendix 14

Approximately Unbiased test results from Gene Genealogy Interrogation of the NO-RECOMBINATION dataset from Chapter 2.

Results of the Approximately Unbiased (AU) test from GGI for the NO-RECOMBINATION dataset. Values above 0.95 indicate topologies (following Fig. 2.5) that are significantly better than the alternatives ($P \leq 0.05$)

Locus	Tree topology with Rank 1	AU test P-value
gene00141_364_01	3	0.679
gene00817_1511_10	4	0.858
gene00817_356_14	4	0.904
gene00817_524_04	4	0.861
gene00817_5739_12	1	0.973
gene01049_659_04_2	1	0.798
gene01053_1189_02	2	0.804
gene01053_595_01	4	0.757
gene01831_360_16	4	0.672
gene01874_1056_05	1	0.971
gene01874_348_01	1	0.603
gene01874_349_03	4	0.978
gene02215_322_08	4	0.550
gene02215_374_06	1	0.951
gene02215_413_02	3	0.921
gene02215_413_05	2	0.727
gene02215_545_01	4	0.965
gene02335_382_03	1	0.948
gene02335_382_03_2	1	0.929
gene02475_1110_03	3	0.909
gene02475_349_06	2	0.582
gene02540_438_01	3	0.808
gene02540_633_03_2	4	0.774
gene03141_457_09	4	0.701
gene03310_370_01	1	0.967
gene03310_423_05	2	0.977
gene03310_423_05_2	1	0.863
gene03310_462_06	3	0.827
gene03310_834_03	3	0.820

Locus	Tree topology with Rank 1	AU test P-value
gene03310_834_03_2	1	0.963
gene03653_348_01	1	0.643
gene03874_429_01	3	0.764
gene03874_429_01_2	3	0.495
gene03965_543_02	1	0.812
gene03965_852_01	3	0.662
gene04871_372_02	2	0.704
gene04871_451_05	2	0.625
gene05024_361_02	1	0.724
gene05461_526_04_2	3	0.955
gene05461_544_03	4	0.908
gene05461_544_03_2	1	0.765
gene05539_313_07	4	0.753
gene05983_339_07	4	0.485
gene06334_1953_01	1	0.541
gene06392_433_01	1	0.608
gene06545_1466_04	3	0.906
gene06545_441_02	4	0.715
gene06545_532_01	3	0.637
gene06563_391_03	3	0.676
gene06563_678_04	3	0.405
gene06778_510_09	3	0.686
gene07331_492_01	3	0.615
gene07367_318_01	1	0.968
gene07367_318_01_2	4	0.918
gene07464_418_21	4	0.566
gene07464_418_21_2	2	0.525
gene07792_372_04	1	1.000
gene07792_432_03	3	0.908
gene07792_432_03_2	1	0.777
gene07792_559_02	3	0.914
gene07892_478_07	2	0.885
gene07926_444_08	1	0.689
gene07947_315_01	4	0.989
gene07947_315_01_2	2	0.903
gene07947_701_02	4	0.693
gene08027_486_09	1	0.998
gene08027_528_11	1	0.549
gene08027_657_10	3	0.685
gene08624_1311_01	1	0.861
gene08653_330_02	3	0.992

Locus	Tree topology with Rank 1	AU test P-value
gene08653_792_01	4	0.888
gene09551_765_02	4	0.580
gene09610_465_05	1	0.901
gene09622_344_05	1	0.557
gene09896_1321_02	1	0.918
gene09896_824_01	1	0.943
gene10536_2170_01	3	0.896
gene11116_567_01	3	0.871
gene11230_635_03	4	0.585
gene11478_322_07	1	0.625
gene11478_595_04	3	0.996
gene11621_381_04	1	0.600
gene11621_474_02_2	1	0.781
gene11621_964_01	1	0.967
gene12090_1647_02	2	0.682
gene12546_1076_01	4	0.804
gene12671_447_07	4	0.843
gene12685_429_06	1	0.568
gene12685_753_07	1	0.623
gene12945_361_07	1	0.746
gene13202_314_01	1	0.582
gene13202_324_06	1	0.777
gene13582_1167_02	4	0.757
gene13582_525_01	1	0.862
gene13675_316_02	4	0.779
gene14077_495_04	2	0.728
gene14077_495_04_2	2	0.932
gene14097_1107_01	2	0.979
gene14155_352_05	3	0.947
gene14467_340_03	1	0.677
gene14467_487_01	1	0.543
gene14575_311_04	3	0.927
gene14575_370_05	1	1.000
gene14696_411_05_2	4	0.629
gene14696_492_01	2	0.732
gene14696_492_01_2	3	0.603
gene14783_325_06	4	0.958
gene15177_327_01	3	0.704
gene15629_340_12	4	0.799
gene15629_340_12_2	4	0.641
gene15629_394_10	1	0.980

Locus	Tree topology with Rank 1	AU test P-value
gene15844_327_11	1	0.992
gene15966_1458_01	3	0.786
gene15996_505_12	1	0.803
gene15996_528_14	3	0.944
gene16098_575_04	3	0.881
gene16115_306_03	1	0.801
gene16115_495_01	4	0.551
gene16123_556_01	1	0.701
gene16123_556_01_2	2	0.762
gene16123_675_06	2	0.540
gene16123_675_06_2	4	0.825
gene16206_829_01	1	0.791
gene16220_353_02	4	0.945
gene16900_708_06	4	0.716
gene16900_708_06_2	1	0.850
gene16900_708_06_3	3	0.726
gene16939_386_06	1	0.586
gene16959_703_01	2	0.725
gene16959_703_01_2	4	0.866
gene17026_407_01_2	3	0.854
gene17112_1026_02	4	0.992
gene17942_354_06	4	0.949
gene17942_535_01	3	0.592
gene17942_535_01_2	4	0.542
gene17946_606_04	3	0.942
gene17946_606_04_2	2	0.891
gene18143_316_06	1	0.616
gene18567_303_02	3	0.668
gene18567_874_04	4	0.867
gene18567_874_04_2	4	0.688
gene18567_930_01	1	0.589
gene18678_1468_07	1	0.970
gene18678_384_04	4	0.585
gene18678_564_01	4	0.701
gene18685_359_03	2	0.596
gene18685_399_07	3	0.605
gene18685_734_06	1	0.685
gene18949_312_02	4	0.581
gene18949_452_04	1	0.687
gene19378_314_01	1	0.893
gene19378_314_01_2	1	0.690

Locus	Tree topology with Rank 1	AU test P-value
gene19390_1123_10	3	0.829
gene20081_1457_02	4	0.749
gene20081_1457_02_2	1	0.524
gene20081_729_11	1	0.883
gene20081_729_11_2	3	0.971
gene20992_402_01	2	0.756
gene20992_488_20	2	0.805
gene21604_337_02	2	0.577
gene21604_549_01	4	0.721
gene21620_349_04	2	0.626
gene21620_466_01	3	0.692
gene21678_2745_01	2	0.761
gene21878_333_01	1	0.560
gene21878_398_03	4	0.905
gene21878_794_08	4	0.595
gene22085_353_06	4	0.874
gene22185_324_04	2	0.482
gene22185_402_06	1	0.848
gene22419_336_04	3	0.627
gene22419_651_01	3	0.659
gene22828_592_02	4	0.761
gene22926_422_01	2	0.697
gene22963_522_03	4	0.721
gene23026_590_03	1	0.531
gene23026_848_02_2	4	0.974
gene23128_513_02_2	2	0.791
gene23128_639_01	4	0.628
gene23345_318_01	2	0.548
gene23345_362_04	4	0.660
gene23736_1383_01	2	0.828
gene23780_385_02	4	0.519
gene23780_385_02_2	4	0.668
gene23780_499_03	3	0.903
gene23780_503_04	1	0.914
gene24025_440_02	4	0.873
gene24141_315_03	1	0.616
gene24141_401_05	4	0.754
gene24275_354_05	1	0.968
gene24275_356_03	1	1.000
gene24275_492_01	4	0.766
gene24302_478_14	3	0.512

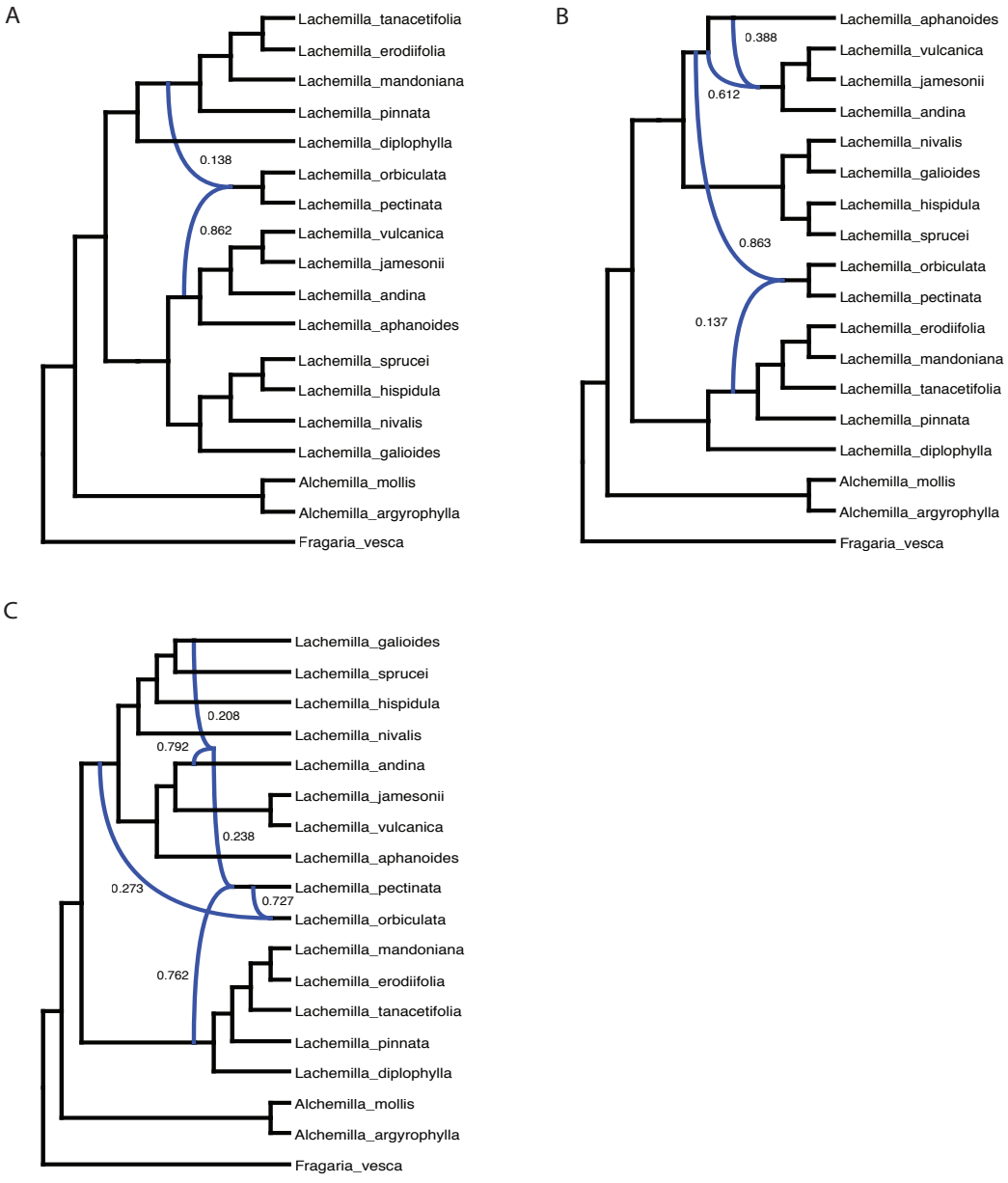
Locus	Tree topology with Rank 1	AU test P-value
gene24477_339_01	1	0.789
gene24477_339_01_2	3	0.558
gene24477_668_04	2	0.711
gene24640_650_07	1	0.890
gene24653_459_03	2	1.000
gene24834_327_06_2	4	0.529
gene24834_375_14	3	0.586
gene24860_389_03	3	0.907
gene24860_522_16	1	0.587
gene24860_522_16_2	1	0.895
gene25161_469_02	1	0.743
gene25463_381_12	2	0.640
gene25463_412_08	1	0.813
gene25463_460_07	4	0.642
gene26791_363_01	4	0.766
gene26791_443_05	1	0.530
gene26791_728_02	4	0.952
gene26791_728_02_2	1	0.915
gene27750_465_02	3	0.703
gene27750_569_08	1	0.786
gene27813_450_06	4	0.948
gene27943_546_09	3	0.883
gene28151_414_06	2	0.798
gene28151_450_05	3	0.928
gene28218_597_06	3	0.827
gene28218_597_06_2	1	0.943
gene28243_539_06	2	0.823
gene28247_435_07	4	0.905
gene28317_1002_06	3	0.936
gene28330_561_01	4	0.997
gene28559_376_04	1	0.593
gene28629_309_07	1	0.596
gene28629_423_02	3	0.741
gene28629_446_03	4	0.828
gene28686_1040_01	3	0.914
gene28689_952_01	3	0.564
gene29299_354_07	2	0.593
gene29299_367_02	3	0.821
gene29895_430_03	2	0.793
gene29895_590_04	3	0.879
gene29895_961_01	3	0.604

Locus	Tree topology with Rank 1	AU test P-value
gene30118_348_04	1	0.792
gene30190_1191_01	1	0.520
gene30214_453_02	4	0.995
gene30214_523_03	1	0.597
gene30392_448_03	4	0.795
gene30425_1367_01	4	0.968
gene30526_327_01	4	0.703
gene30622_973_01	4	0.879
gene30829_352_01	2	0.586
gene30829_729_03_2	2	0.861
gene31237_438_06	1	0.877
gene31237_643_08	2	0.812
gene31269_342_06	3	0.733
gene31269_369_04	4	0.561
gene31345_439_03	3	0.973
gene31345_463_04_2	3	0.741
gene31345_499_11	4	0.996
gene31345_499_11_2	3	0.814
gene31484_635_02	1	0.614
gene31508_1455_01	1	0.927
gene31508_1455_01_2	2	0.831
gene32082_324_03	4	0.685
gene32082_641_01	4	0.667
gene32117_420_04	3	0.850
gene32182_498_05	1	0.865
gene32640_319_02	4	0.652
gene34105_796_02	3	0.961

Appendix 15

Best species networks of the HYBRID-REDUCED dataset from Chapter 2.

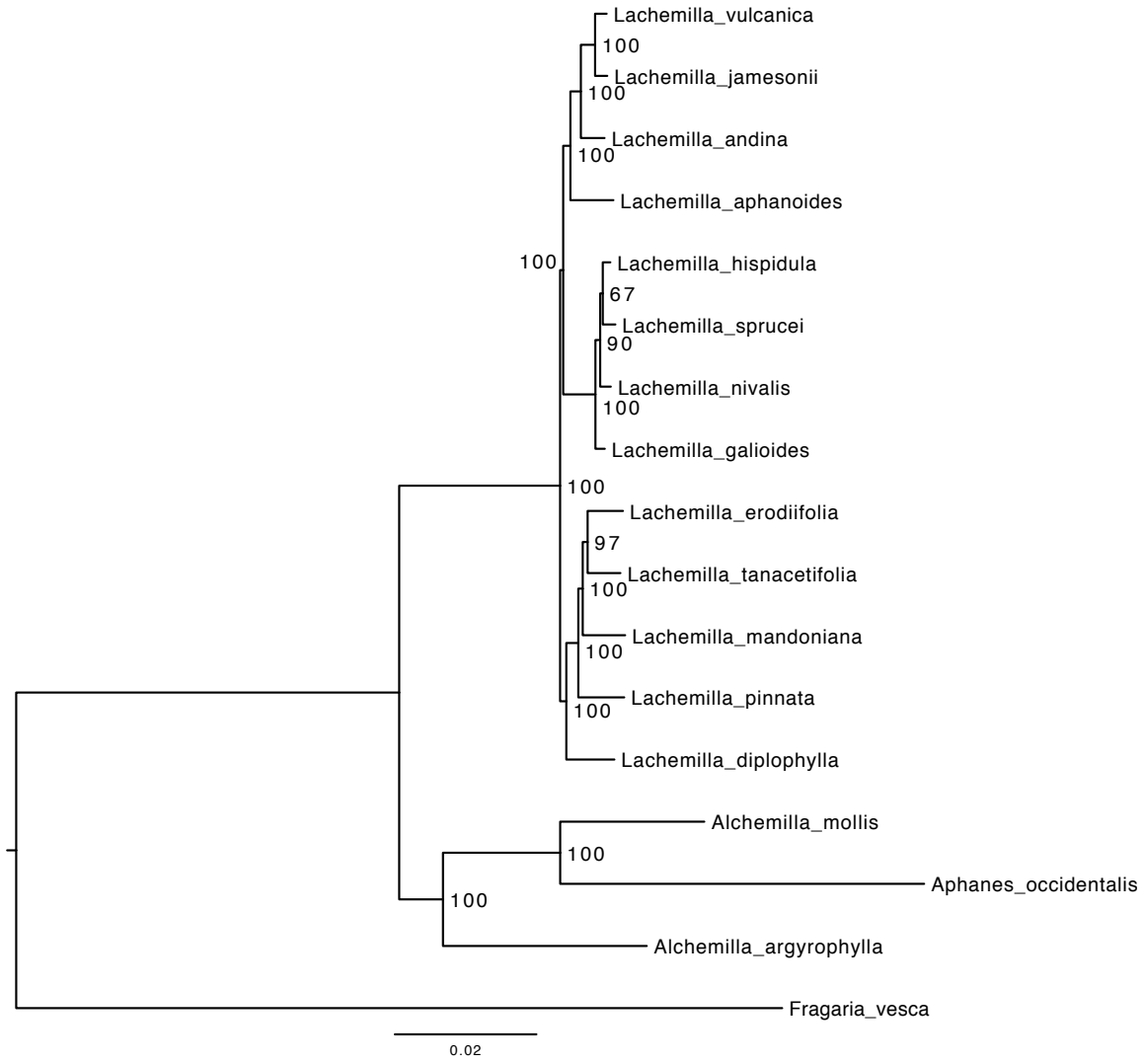
Best species networks of the HYBRID-REDUCED dataset estimated with PhyloNet with one (A), two (B), and three (C) hybridization events. Blue branches connect the hybrid nodes. Numbers next to blue branches indicate inheritance probabilities.



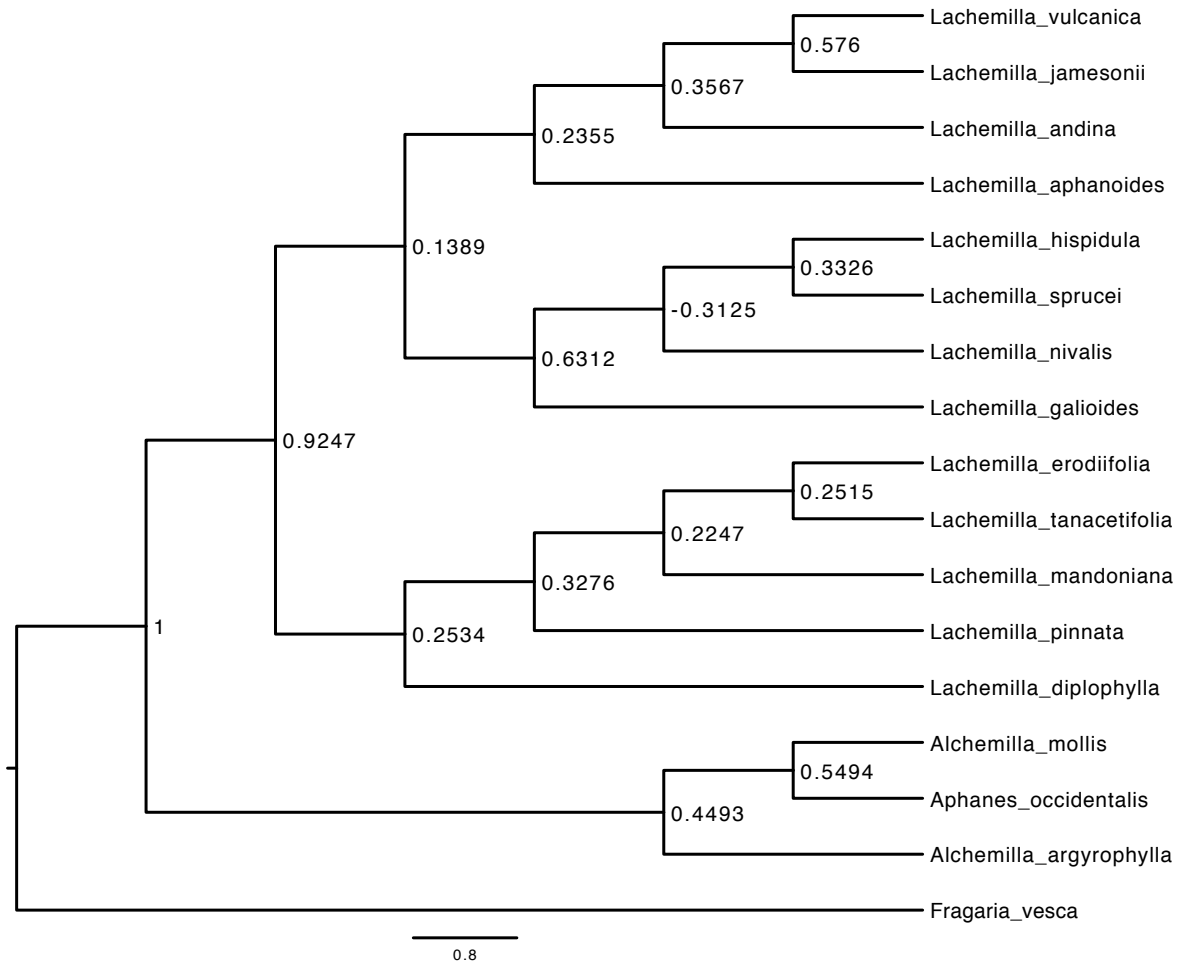
Appendix 16

Additional phylogenetic analysis of the ORBICULATE-REDUCED dataset from Chapter 2.

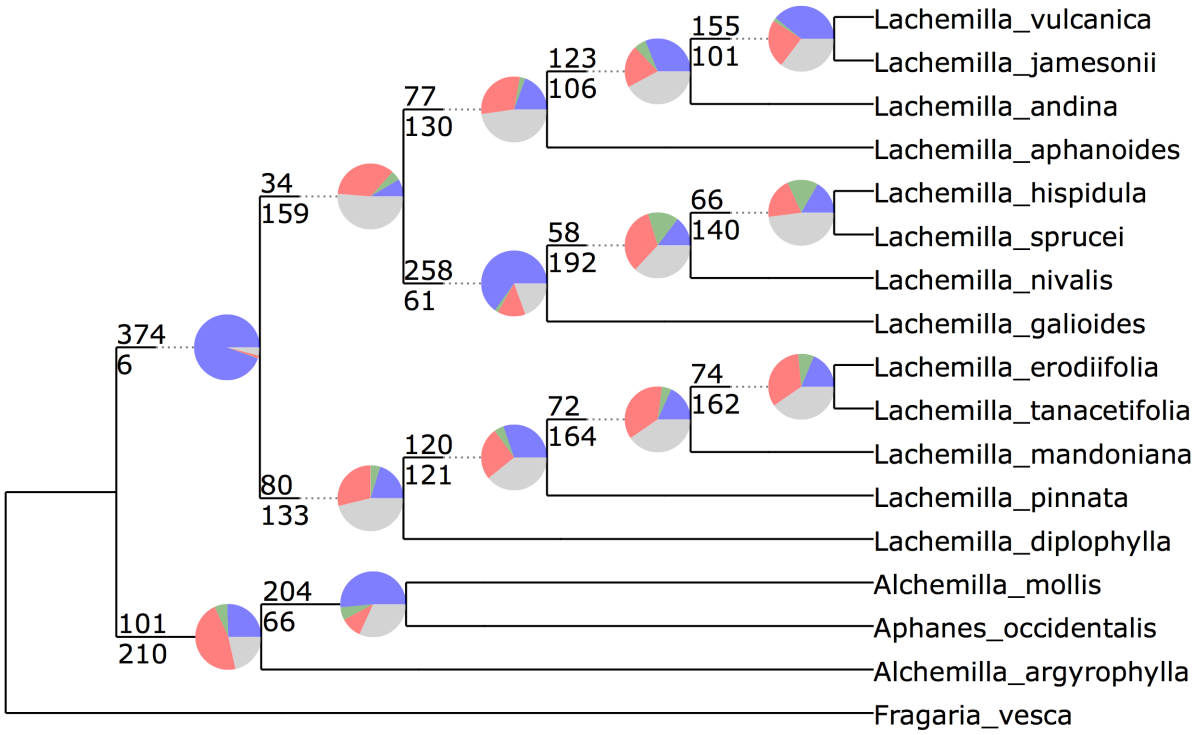
A. RAxML phylogeny of the concatenated matrix; node labels indicate bootstrap values.



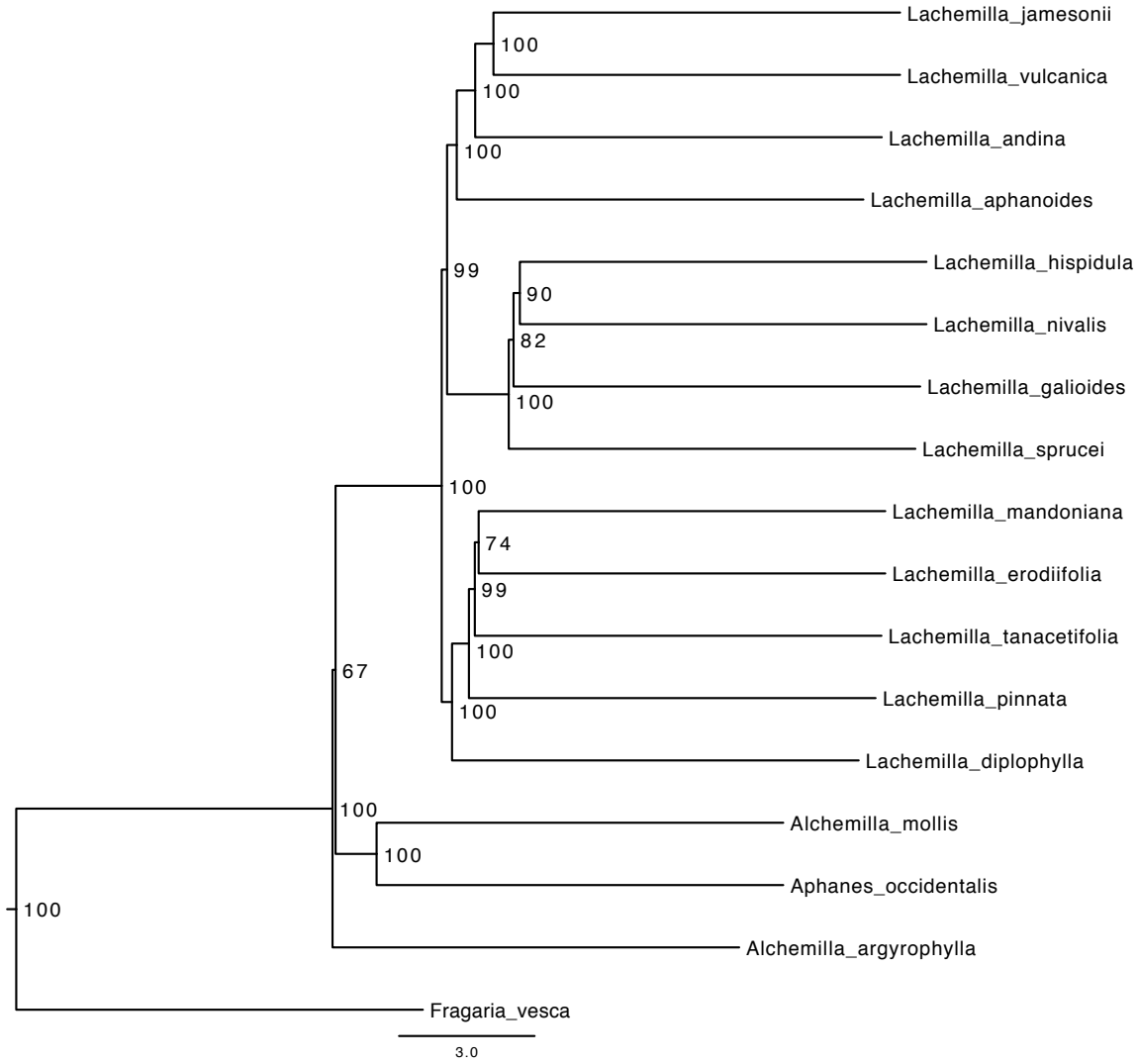
B. RAxML phylogeny of the concatenated matrix; node labels indicate ICA scores.



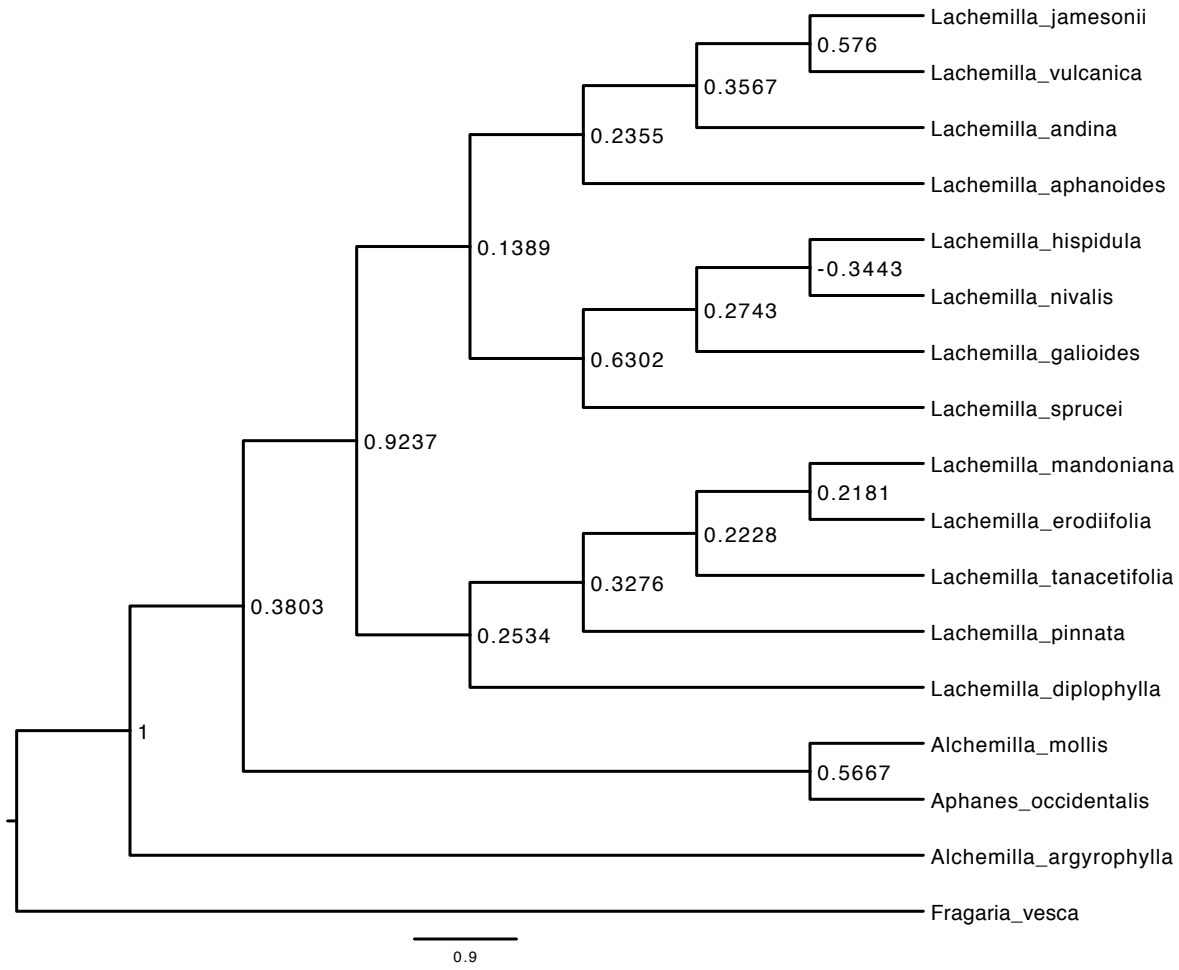
C. RAxML phylogeny of the concatenated matrix; numbers above and below the branches indicate the number of gene trees and in conflict respectively. Pie charts next to the nodes represent the proportion of gene trees that support that clade – blue, the proportion that support the main alternative for that clade – green, the proportion that support the remaining alternatives – red, and the proportion (conflict or support) that have less than 50% bootstrap support – gray.



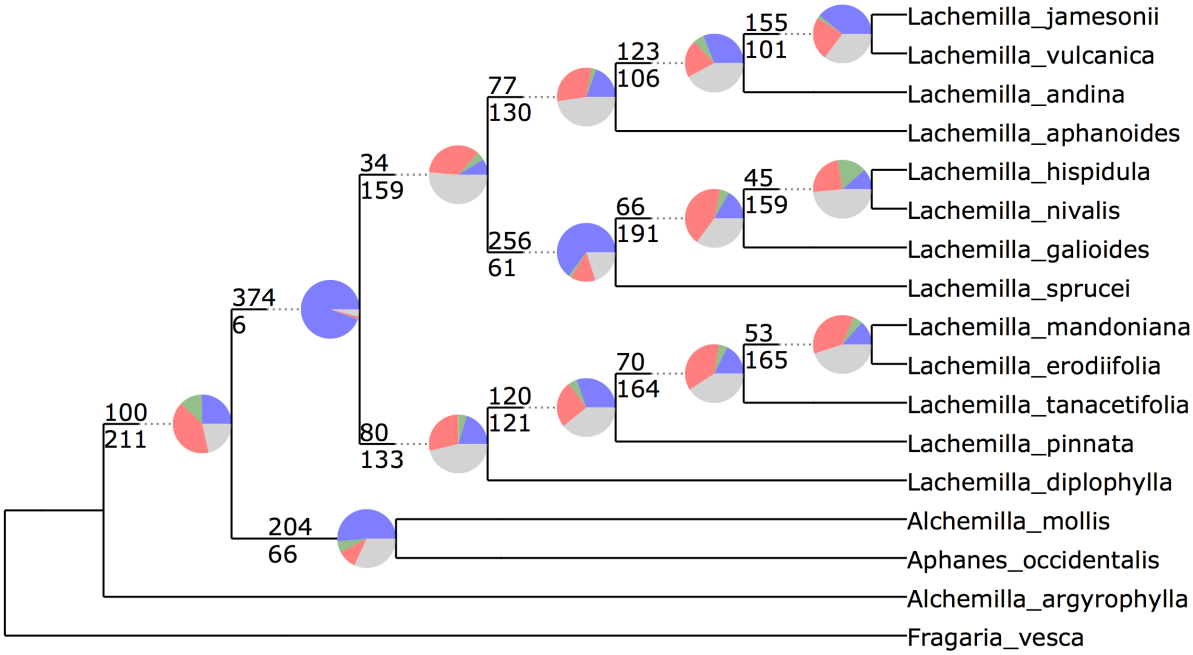
D. MP-EST species trees; node labels indicate bootstrap values.



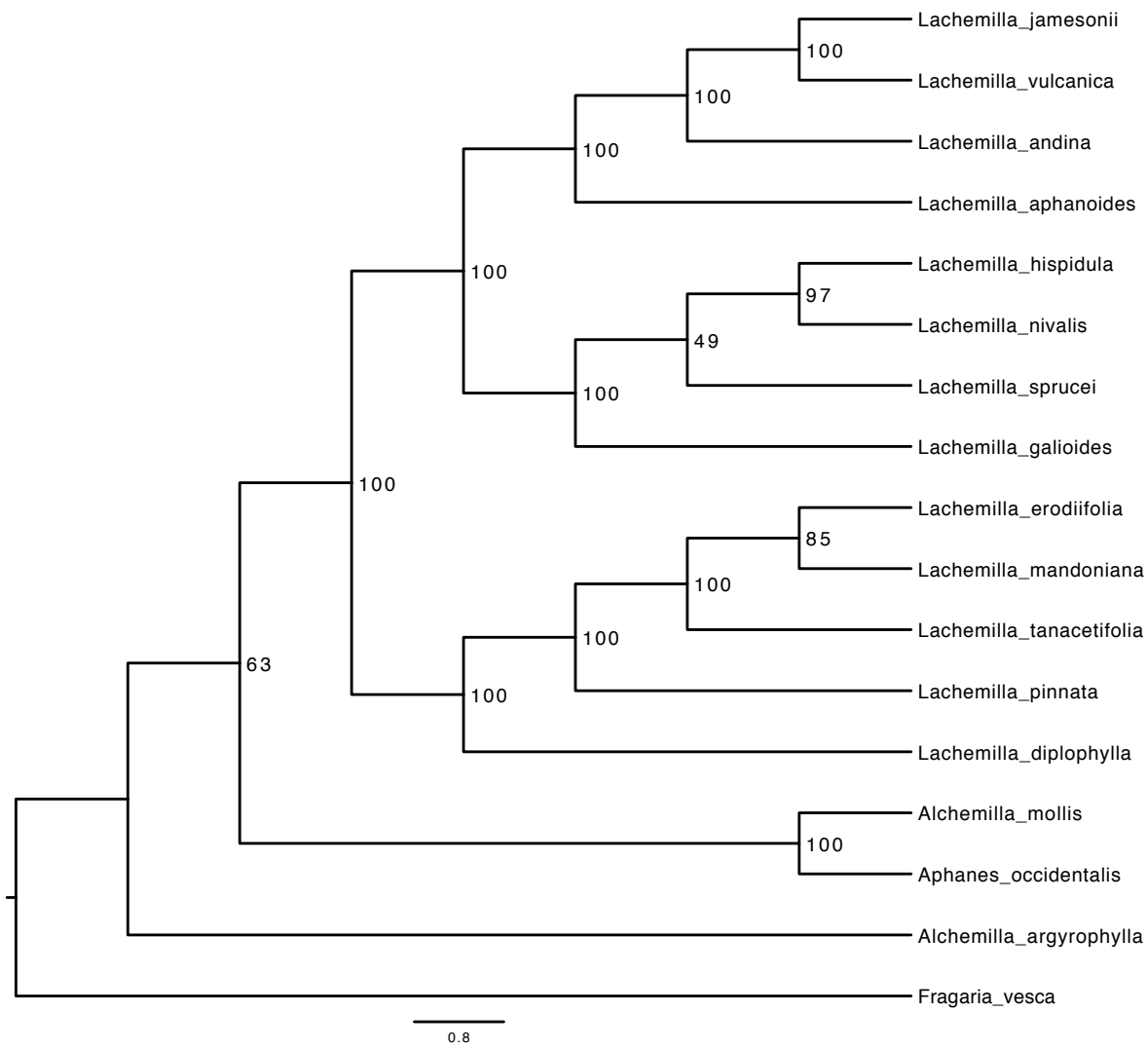
E. MP-EST species trees; node labels indicate ICA scores.



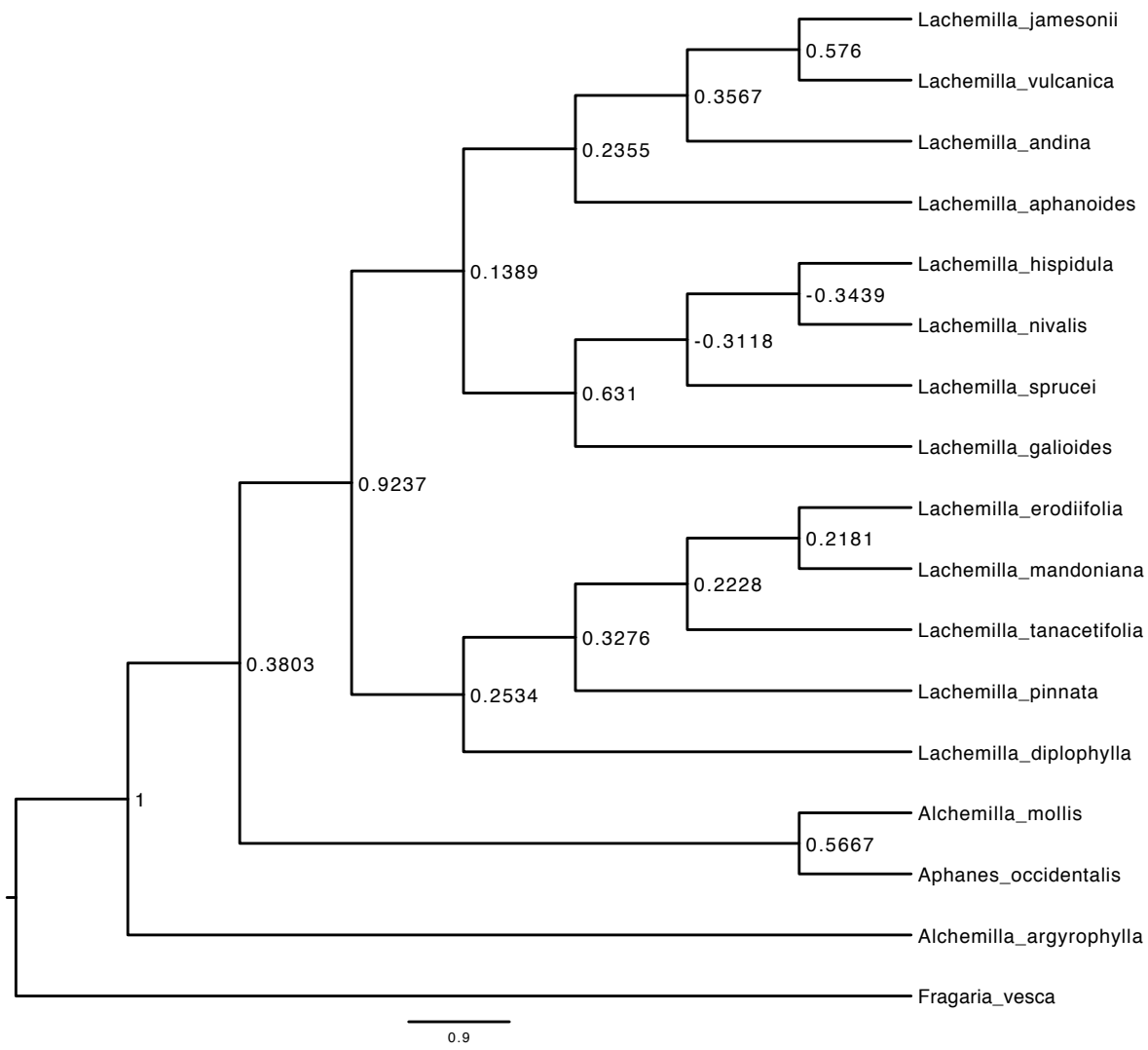
F. MP-EST species trees; numbers above and below the branches indicate the number of gene trees and in conflict respectively. Pie charts next to the nodes represent the proportion of gene trees that support that clade – blue, the proportion that support the main alternative for that clade – green, the proportion that support the remaining alternatives – red, and the proportion (conflict or support) that have less than 50% bootstrap support – gray.



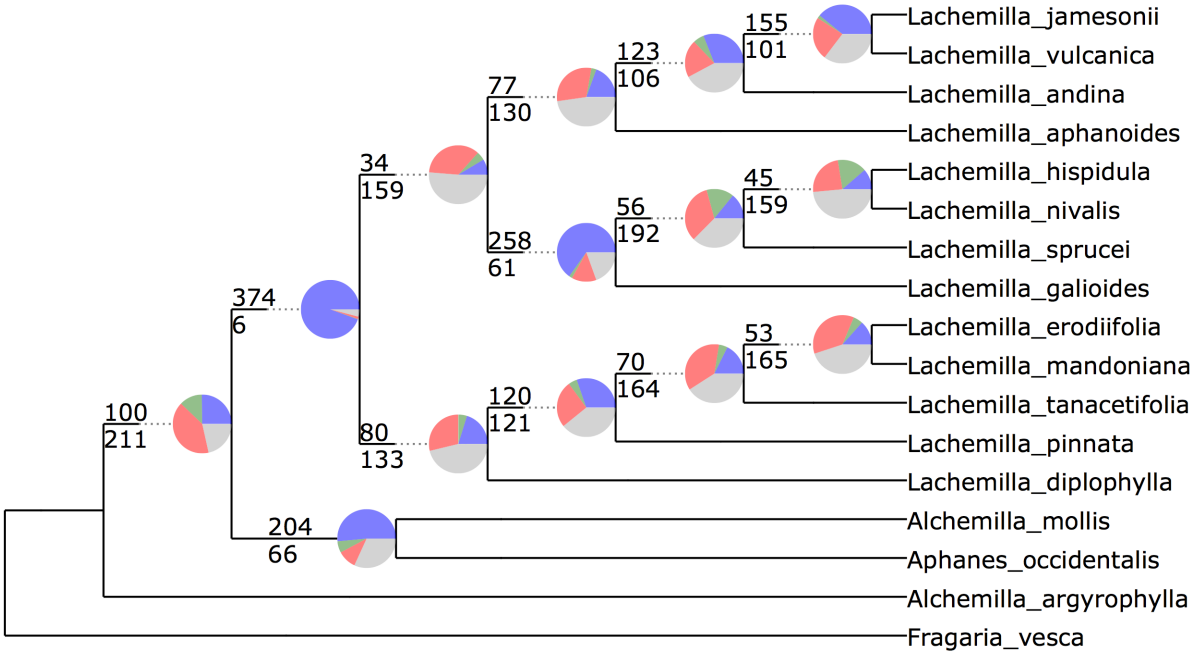
G. SVDquartets species trees; node labels indicate bootstrap values.



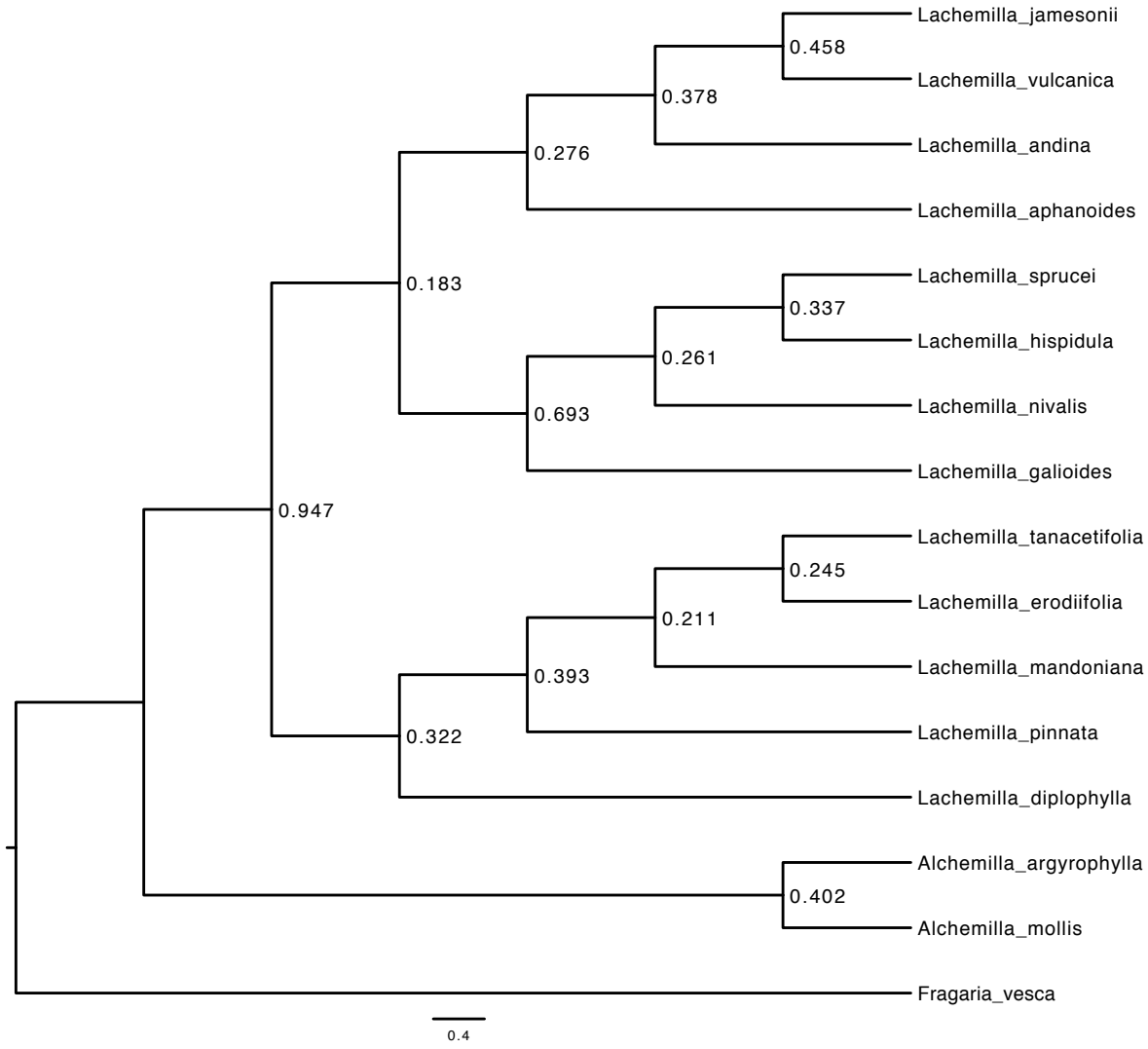
H. SVDquartets species trees; node labels indicate ICA scores.



I. SVDquartets species trees; numbers above and below the branches indicate the number of gene trees and in conflict respectively. Pie charts next to the nodes represent the proportion of gene trees that support that clade – blue, the proportion that support the main alternative for that clade – green, the proportion that support the remaining alternatives – red, and the proportion (conflict or support) that have less than 50% bootstrap support – gray.



J. Bayesian concordance analysis with BUCKy. Node label indicate concordance factors.



Appendix 17

Voucher information for Chapter 3.

Arranged by: Species name and authority, collector and number (herbarium code).

Alchemilla alpina L.: 2015_250, *SL 11498* (UZ); *Alchemilla argyrophylla* Oliv.: 2014_382_ET, *B. Gehrke 354* (UZH), 2015_287, *B. Gehrke 016* (UZH); *Alchemilla charbonelliana* Buser: 2015_257, *P. Frost-Olsen 12912* (AAU); *Alchemilla colura* Hill.: 2015_363, *B. Gehrke 464* (UZH); *Alchemilla cryptantha* Steud. ex A. Rich.: 2015_368, *B. Gehrke 306* (UZH); *Alchemilla decumbens* Buser: 2015_348, *B. Gehrke 662* (UZH); *Alchemilla ellenbeckii* Engl.: 2015_301, *B. Gehrke 104* (UZH); *Alchemilla flabellata* Buser: 2015_264, *P. Frost-Olsen 11859* (AAU); *Alchemilla haumanii* Engl.: 2015_313, *B. Gehrke 204* (UZH); *Alchemilla heptagona* Juz.: 2015_270, *SF 6999* (UZH); *Alchemilla indivisa* (Ruser) Rothm.: 2015_273, *P. Frost-Olsen 3383* (AAU); *Alchemilla johnstonii* Oliv.: 2015_358, *B. Gehrke 364* (UZH); *Alchemilla lapeyrousii* Buser: 2015_283, *P. Frost-Olsen 10029* (AAU); *Alchemilla microbetula* T.C.E. Fr.: 2015_366, *B. Gehrke 360* (UZH); *Alchemilla mollis* (Buser) Rothm.: 2012_228_US, *D.F. Morales-Briones 687* (ID); *Alchemilla pentaphyllea* L.: 2015_343, *J. Kadereit & M. Lauterbach 201252* (JGU); *Alchemilla schizophylla* Bak.: 2015_327, *B. Gehrke 282* (UZH); *Alchemilla stuhlmannii* Engl.: 2015_374, *B. Gehrke 363* (UZH); *Alchemilla subnivalis* Bak.: 2015_375, *B. Gehrke 362* (UZH); *Alchemilla subsericea* Reut.: 2015_277, *P. Frost-Olsen 11984* (AAU); *Alchemilla triphylla* Rothm.: 2015_371, *B. Gehrke 361* (UZH); *Alchemilla woodii* Kuntze: 2015_356, *B. Gehrke 453* (UZH); *Aphanes cotopaxiensis* Romol. & Frost-Olsen: 2013_004_EC, *D.F. Morales-Briones 276* (ID); *Aphanes microcarpa* (Boiss. & Reut.)

Rothm.: 2013_001_MO, *F. Schuhwer 90-43* (QCA); *Aphanes microcarpa* (Boiss. & Reut.) Rothm.: 2013_002_MO, *L. Koepke 201000524-1* (OSU), 2012_225_US, *D.C. Tank 1107* (ID); *Aphanes* sp.: 2013_395_BO, *D.F. Morales-Briones et al.* 291 (ID), 2013_400_BO, *D.F. Morales-Briones et al.* 296 (ID), 2013_421_BO, D.F. *Morales-Briones et al.* 317 (ID), 2014_371_US, *D.F. Morales-Briones et al. 580* (ID), 2016_601_US, *D.F. Morales-Briones 579* (ID); *Chamaerhodos erecta* (L.) Bunge: 2016_586, *R.L. Hartman 54641* (ID); *Comarum palustre* L.: 2016_589, *J. Duemmel 29-08* (ID); *Dasiphora fruticosa* (L.) Rydb.: 2014_415, *H.E. Marx 2013-209* (ID); *Drymocallis arguta* (Pursh) Rydb.: 2016_593, *J.F. Smith 8736* (ID); *Farinopsis salesoviana* (Stephan) Chrtek & Soják, 2016_580, *B. Bartholomew 8364* (MO); *Lachemilla adscendens* Rothm.: 2013_445_CO, *D.F. Morales-Briones & S. Uribe-Convers 429A* (ID); *Lachemilla andina* (L.M. Perry) Rothm.: 2012_249_PE, *D.F. Morales-Briones & S. Uribe-Convers 218* (ID), 2012_295_PE, *D.F. Morales-Briones & S. Uribe-Convers 264* (ID), 2012_304_PE, *D.F. Morales-Briones & S. Uribe-Convers 273* (ID), 2012_354_EC, *K. Romoleroux & D.F. Morales-Briones 5215* (QCA), 2012_379_EC, *D.F. Morales-Briones et al. 122* (QCA), 2012_401_EC, *D.F. Morales-Briones & K. Romoleroux 167* (QCA), 2013_412_BO, *D.F. Morales-Briones et al. 308* (ID), 2013_440_CO, *D.F. Morales-Briones & S. Uribe-Convers 424* (ID), 2013_464_CO, *D.F. Morales-Briones & S. Uribe-Convers 448* (ID), 2014_548_CO, *D.F. Morales-Briones & S. Uribe-Convers 557* (ID); *Lachemilla aphanoides* (Mutis ex L. f.) Rothm.: 2012_001_EC, *D.F. Morales-Briones & S. Uribe-Convers 189* (ID), 2012_246_PE, *D.F. Morales-Briones & S. Uribe-Convers 215* (ID), _256_PE, *D.F. Morales-Briones & S. Uribe-Convers 225* (ID), 2012_293_PE, *D.F. Morales-Briones & S. Uribe-Convers 262* (ID), 2012_353_EC, *K. Romoleroux et al. 4478* (QCA), 2012_355_EC, *K. Romoleroux et al. 4685* (QCA), 2012_369_EC, *D.F. Morales-Briones et al. 113* (QCA), 2012_388_EC, *D.F. Morales-Briones et al. 138* (QCA), 2012_396_EC, *D.F. Morales-*

Briones & K. Romoleroux 162 (QCA), 2013_385_BO, *D.F. Morales-Briones et al. 281* (ID), 2013_392_BO, *D.F. Morales-Briones et al. 288* (ID), 2013_429_CO, *D.F. Morales-Briones & S. Uribe-Convers 413* (ID), 2013_432_CO, *D.F. Morales-Briones & S. Uribe-Convers 416* (ID), 2013_442_CO, *D.F. Morales-Briones & S. Uribe-Convers 426* (ID), 2013_446_CO, *D.F. Morales-Briones & S. Uribe-Convers 430* (ID), 2013_468_CO, *D.F. Morales-Briones & S. Uribe-Convers 452* (ID), 2013_489_CO, *D.F. Morales-Briones & S. Uribe-Convers 473A* (ID); 2014_473_CO, *D.F. Morales-Briones et al. 479* (ID), 2014_505_CO, *D.F. Morales-Briones & S. Uribe-Convers 512* (ID), 2014_522_CO, *D.F. Morales-Briones et al. 530* (ID), 2014_541_CO, *D.F. Morales-Briones & S. Uribe-Convers 550* (ID), 2015_149_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 594* (ID); *Lachemilla barbata* (C. Presl) Rothm.: 2012_260_PE, *D.F. Morales-Briones & S. Uribe-Convers 229* (ID), 2012_305_PE, *D.F. Morales-Briones & S. Uribe-Convers 274* (ID), 2013_406_BO, *D.F. Morales-Briones et al. 302* (ID); *Lachemilla bipinnatifida* (L.M. Perry) Rothm.: 2013_386_BO, *D.F. Morales-Briones et al. 282* (ID), 2013_390_BO, *D.F. Morales-Briones et al. 286* (ID), 2013_427_BO, *D.F. Morales-Briones et al. 325* (ID), 2013_428_BO, *D.F. Morales-Briones et al. 326* (ID); *Lachemilla diplophylla* (Diels) Rothm., 2012_229_PE, *D.F. Morales-Briones & S. Uribe-Convers 198* (ID), 2012_237_PE, *D.F. Morales-Briones & S. Uribe-Convers 206* (ID), 2012_306_PE, *D.F. Morales-Briones & S. Uribe-Convers 275* (ID), 2012_357_EC, *D.F. Morales-Briones & E. Morales-Checa 30* (QCA), 2013_382_BO, *D.F. Morales-Briones et al. 278* (ID), 2013_408_BO, *D.F. Morales-Briones et al. 304* (ID); *Lachemilla equisetiformis* (Trevis.) Rothm.: 2012_108_VE, *A.J.P. Martin 740* (QCA); *Lachemilla erodiifolia* (Wedd.) Rothm.: 2012_244_PE, *D.F. Morales-Briones & S. Uribe-Convers 213* (ID), 2012_296_PE, *D.F. Morales-Briones & S. Uribe-Convers 265* (ID), 2012_365_EC, *D.F. Morales-Briones et al. 109* (QCA), 2012_372_EC, *D.F. Morales-Briones et al. 116* (QCA), 2013_401_BO, *D.F. Morales-Briones et al. 297* (ID);

Lachemilla frigida (Wedd.) Rothm.: 2012_243_PE, *D.F. Morales-Briones & S. Uribe-Convers 212* (ID), 2012_300_PE, *D.F. Morales-Briones & S. Uribe-Convers 269* (ID), 2013_399_BO, *D.F. Morales-Briones et al. 295* (ID), 2013_409_BO, *D.F. Morales-Briones et al. 305* (ID), 2013_423_BO, *D.F. Morales-Briones et al. 319* (ID); *Lachemilla fulvescens* (L.M. Perry) Rothm.: 2012_323_EC, *K. Romoleroux et al. 4702* (QCA), 2012_368_EC, *D.F. Morales-Briones et al. 112* (QCA), 2012_375_EC, *D.F. Morales-Briones et al. 118* (QCA), 2012_408_EC, *D.F. Morales-Briones et al. 75* (QCA), 2013_450_CO, *D.F. Morales-Briones & S. Uribe-Convers 434* (ID), 2013_463_CO, *D.F. Morales-Briones & S. Uribe-Convers 447* (ID), 2013_481_CO, *D.F. Morales-Briones & S. Uribe-Convers 465* (ID), 2014_503_CO, *D.F. Morales-Briones & S. Uribe-Convers 510* (ID), 2014_521_CO, *D.F. Morales-Briones et al. 529* (ID), 2014_551_CO, *D.F. Morales-Briones & S. Uribe-Convers 560* (ID); *Lachemilla galioides* (Benth.) Rothm.: 2012_348_EC, *K. Romoleroux et al. 4403* (QCA), 2012_389_EC, *D.F. Morales-Briones et al. 138* (QCA); *Lachemilla hirta* (L.M. Perry) Rothm.: 2012_317_EC, *K. Romoleroux et al. 4588* (QCA), 2012_343_EC, *K. Romoleroux et al. 4389* (QCA), 2012_390_EC, *D.F. Morales-Briones et al. 141* (QCA); *Lachemilla hispidula* (L.M. Perry) Rothm.:, 2012_310_EC, *K. Romoleroux et al. 4470* (QCA), 2012_324_EC, *K. Romoleroux et al. 4703* (QCA), 2012_387_EC, *D.F. Morales-Briones et al. 133* (QCA), 2012_399_EC, *D.F. Morales-Briones & K. Romoleroux 165* (QCA), 2013_457_CO, *D.F. Morales-Briones & S. Uribe-Convers 441* (ID), 2014_484_CO, *D.F. Morales-Briones et al. 490* (ID), 2014_491_CO, *D.F. Morales-Briones & S. Uribe-Convers 497* (ID), 2014_498_CO, *D.F. Morales-Briones & S. Uribe-Convers 505* (ID), 2014_542_CO, *D.F. Morales-Briones & S. Uribe-Convers 551* (ID), 2014_546_CO, *D.F. Morales-Briones & S. Uribe-Convers 555* (ID), 2014_568_CO, *D.F. Morales-Briones & S. Uribe-Convers 577* (ID); *Lachemilla holmgrenii* Rothm.: 2012_347_EC, *K. Romoleroux et al. 4397* (QCA); *Lachemilla holosericea* (L.M. Perry)

Rothm.: 2012_005_EC, *D.F. Morales-Briones & S. Uribe-Convers 192* (ID), 2012_314_EC, *K. Romoleroux et al. 4528* (QCA), 2012_328_EC, *K. Romoleroux et al. 4710* (QCA), 2012_395_EC, *D.F. Morales-Briones & K. Romoleroux 161* (QCA), 2014_479_CO, *D.F. Morales-Briones et al. 485* (ID), 2014_530_CO, *D.F. Morales-Briones et al. 538* (ID); *Lachemilla jamesonii* (L.M. Perry) Rothm.: 2012_350_EC, *K. Romoleroux et al. 4387* (QCA); *Lachemilla jaramilloi* Romol. & D.F. Morales-B.: 2012_006_EC, *D.F. Morales-Briones & S. Uribe-Convers 193* (ID), 2012_364_EC, *D.F. Morales-Briones et al. 101* (QCA), 2012_371_EC, *D.F. Morales-Briones et al. 115* (QCA); *Lachemilla lechleriana* (Griseb.) Rothm.: 2012_271_PE, *D.F. Morales-Briones & S. Uribe-Convers 240* (ID); *Lachemilla llanganatensis* Romol.: 2012_363_EC, *D.F. Morales-Briones et al. 105* (QCA), 2014_374_EC, *D.M. Morales-Briones et al. 64* (QCA); *Lachemilla mandoniana* (Wedd.) Rothm.: 2012_007_EC, *D.F. Morales-Briones & S. Uribe-Convers 194* (ID), 2012_261_PE, *D.F. Morales-Briones & S. Uribe-Convers 230* (ID), 2012_325_EC, *K. Romoleroux et al. 4705* (QCA), 2012_335_CR, *K. Romoleroux et al. 5010* (QCA), 2012_370_EC, *D.F. Morales-Briones et al. 114* (QCA), 2012_397_EC, *D.F. Morales-Briones & K. Romoleroux 164* (QCA), 2013_454_CO, *D.F. Morales-Briones & S. Uribe-Convers 438* (ID), 2014_510_CO, *D.F. Morales-Briones & S. Uribe-Convers 517* (ID), 2014_523_CO, *D.F. Morales-Briones et al. 531* (ID), 2014_528_CO, *D.F. Morales-Briones et al. 536* (ID), 2014_545_CO, *D.F. Morales-Briones & S. Uribe-Convers 554* (ID); *Lachemilla mexiquense* D.F. Morales-B.: 2015_233_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 683* (ID); *Lachemilla moritziana* Dammer: 2013_441_CO, *D.F. Morales-Briones & S. Uribe-Convers 425* (ID), 2013_443_CO, *D.F. Morales-Briones & S. Uribe-Convers 427* (ID); *Lachemilla nivalis* (Kunth) Rothm.: 2012_349_EC, *D.F. Morales-Briones & M.F. Latorre-Barragán 11* (QCA), 2012_384_EC, *D.F. Morales-Briones et al. 128* (QCA), 2012_386_EC, *D.F. Morales-Briones et al. 132* (QCA), 2014_492_CO, *D.F.*

Morales-Briones & S. Uribe-Convers 498 (ID), 2014_506_CO, *D.F. Morales-Briones & S. Uribe-Convers 513* (ID), 2014_514_CO, *D.F. Morales-Briones & S. Uribe-Convers 521* (ID), 2014_537_CO, *D.F. Morales-Briones et al. 546* (ID), 2014_559_CO, *D.F. Morales-Briones & S. Uribe-Convers 568* (ID), 2014_567_CO, *D.F. Morales-Briones & S. Uribe-Convers 576* (ID); *Lachemilla orbiculata* (Ruiz & Pav.) Rydb.: 2012_003_EC, *D.F. Morales-Briones & S. Uribe-Convers 186* (ID), 2012_258_PE, *D.F. Morales-Briones & S. Uribe-Convers 227* (ID), 2012_292_PE, *D.F. Morales-Briones & S. Uribe-Convers 261* (ID), 2012_294_PE, *D.F. Morales-Briones & S. Uribe-Convers 263* (ID), 2012_309_EC, *K. Romoleroux et al. 4469* (QCA), 2012_377_EC, *D.F. Morales-Briones et al. 121* (QCA), 2012_381_EC, *D.F. Morales-Briones et al. 126* (QCA), 2012_402_EC, *D.F. Morales-Briones & K. Romoleroux 171* (QCA), 2012_403_EC, *D.F. Morales-Briones et al 197* (QCA), 2013_430_CO, *D.F. Morales-Briones & S. Uribe-Convers 414* (ID), 2013_467_CO, *D.F. Morales-Briones & S. Uribe-Convers 451* (ID), 2014_487_CO, *D.F. Morales-Briones & S. Uribe-Convers 493* (ID), 2014_519_CO, *D.F. Morales-Briones et al. 527* (ID), 2014_550_CO, *D.F. Morales-Briones & S. Uribe-Convers 559* (ID), 2014_562_CO*D.F. Morales-Briones & S. Uribe-Convers 571* (ID); *Lachemilla pectinata* (Kunth) Rothm.:, 2012_326_EC, *K. Romoleroux et al. 4706* (QCA), 2012_376_EC, *D.F. Morales-Briones & K. Romoleroux 119* (QCA), 2012_382_EC, *D.F. Morales-Briones et al. 127* (QCA), 2013_388_BO, *D.F. Morales-Briones et al. 284* (ID), 2014_520_CO , *D.F. Morales-Briones et al. 528* (ID), 2014_547_CO, *D.F. Morales-Briones & S. Uribe-Convers 556* (ID); *Lachemilla perryana* (Rothm.) Rothm.: 2012_380_EC, *D.F. Morales-Briones et al. 123* (QCA); *Lachemilla pinnata* (Ruiz & Pav.) Rothm.: 2012_230_PE, *D.F. Morales-Briones & S. Uribe-Convers 199* (ID, 2012_232_PE, *D.F. Morales-Briones & S. Uribe-Convers 201* (ID), _239_PE, *D.F. Morales-Briones & S. Uribe-Convers 208* (ID), 2012_242_PE, *D.F. Morales-Briones & S. Uribe-Convers 211* (ID), 2012_251_PE, *D.F.*

Morales-Briones & S. Uribe-Convers 220 (ID), 2012_280_PE, *D.F. Morales-Briones & S. Uribe-Convers 249* (ID), 2012_291_PE, *D.F. Morales-Briones & S. Uribe-Convers 260* (ID), 2012_303_PE, *D.F. Morales-Briones & S. Uribe-Convers 272* (ID), 2012_330_EC, *D. Minga 2538* (QCA), 2012_391_EC, *D.F. Morales-Briones et al. 151* (QCA), 2013_381_BO, *D.F. Morales-Briones et al. 277 (*ID), 2013_391_BO, *D.F. Morales-Briones et al. 287* (ID), 2013_437_CO, *D.F. Morales-Briones & S. Uribe-Convers 421* (ID), 2013_448_CO, *D.F. Morales-Briones & S. Uribe-Convers 432* (ID), 2013_469_CO, *D.F. Morales-Briones & S. Uribe-Convers 453* (ID), 2013_487_CO, *D.F. Morales-Briones & S. Uribe-Convers 471* (ID), 2015_218_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 666* (ID); *Lachemilla polylepis* (Wedd.) Rothm.: 2012_358_CO, *P. Sklenář 12207* (QCA), 2012_359_CO, *P. Sklenář FAA652* (PRC), 2013_444_CO, *D.F. Morales-Briones & S. Uribe-Convers 428* (ID), 2013_477_CO, *D.F. Morales-Briones & S. Uribe-Convers 461* (ID), 2014_471_CO, *D.F. Morales-Briones et al. 477* (ID); *Lachemilla pringlei* Rydb.: 2015_150_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 595* (ID); *Lachemilla procumbens* (Rose) Rydb.: 2015_141_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 583* (ID), 2015_200_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 648* (ID); *Lachemilla pseudovenusta* Rothm.: 2012_275_PE, *D.F. Morales-Briones & S. Uribe-Convers 244* (ID); *Lachemilla purdiei* (L.M. Perry) Rothm.: 2014_501_CO, *D.F. Morales-Briones & S. Uribe-Convers 508* (ID), 2014_502_CO, *D.F. Morales-Briones & S. Uribe-Convers 509* (ID); *Lachemilla ranunculoides* (L.M. Perry) Rothm.:, 2012_240_PE, *D.F. Morales-Briones & S. Uribe-Convers 209* (ID), 2012_248_PE, *D.F. Morales-Briones & S. Uribe-Convers 217* (ID), 2013_389_BO, *D.F. Morales-Briones et al. 285* (ID); *Lachemilla repens* (C. Presl) Rothm.: 2012_263_PE, *D.F. Morales-Briones & S. Uribe-Convers 232* (ID), 2012_268_PE, *D.F. Morales-Briones & S. Uribe-Convers 237* (ID); *Lachemilla rupestris* (Kunth) Rothm.: 2012_004_EC, *D.F. Morales-Briones & S. Uribe-Convers 191* (ID),

2012_270_PE, *D.F. Morales-Briones & S. Uribe-Convers 239* (ID), 2012_394_EC, *D.F. Morales-Briones et al. 157* (QCA); *Lachemilla sarmentosa* (L.M. Perry) Rothm.: 2013_396_BO, *D.F. Morales-Briones et al. 292* (ID), 2013_413_BO, *D.F. Morales-Briones et al. 309* (ID); *Lachemilla sibbaldiifolia* (Kunth) Rydb.: 2015_154_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 599* (ID), 2015_203_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 651* (ID); *Lachemilla sprucei* (L.M. Perry) Rothm.: 2012_340_EC, *K. Romoleroux et al. 4474* (QCA), 2012_405_EC, *D.F. Morales-Briones et al. 63* (QCA); *Lachemilla standleyi* (L.M. Perry) Rothm.: 2016_614_CR, *K. Romoleroux et al. 5023* (QCA); *Lachemilla steinbachii* Rothm.: 2013_415_BO, *D.F. Morales-Briones et al. 311* (ID); *Lachemilla talamanquensis* Romol. & D.F. Morales-B.: 2012_093_CR, *H. Hertel 35556* (BSM); 2012_334_CR, *K. Romoleroux et al. 5008* (QCA), 2016_598_CR, L. *Frost 5059* (WTU); *Lachemilla tanacetifolia* Rothm.: 2012_267_PE, *D.F. Morales-Briones & S. Uribe-Convers 236* (ID), 2012_331_EC, *K. Romoleroux et al. 4396* (QCA), 2012_367_EC, *D.F. Morales-Briones et al. 110* (QCA), 2013_488_CO, *D.F. Morales-Briones & S. Uribe-Convers 472* (ID); *Lachemilla uniflora* Maguire: 2012_319_EC, *K. Romoleroux et al. 4696* (QCA), 2012_362_EC, *D.F. Morales-Briones et al. 75* (QCA), 2012_385_EC, *D.F. Morales-Briones et al. 130* (QCA), 2014_566_CO, *D.F. Morales-Briones & S. Uribe-Convers 575* (ID); *Lachemilla velutina* (S. Watson) Rydb.: 2015_162_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 607* (ID), 2015_169_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 615* (ID); *Lachemilla venusta* (Schltdl. & Cham.) Rydb.: 2015_157_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 602* (ID); *Lachemilla verticillata* (Fielding & Gardner) Rothm.; 2012_339_CR, *K. Romoleroux et al. 5007* (QCA); *Lachemilla vulcanica* (Schltdl. & Cham.) Rydb.: 2012_002_EC, *D.F. Morales-Briones & S. Uribe-Convers 184* (ID), 2012_250_PE, *D.F. Morales-Briones & S. Uribe-Convers 219* (ID), 2012_264_PE, *D.F. Morales-Briones & S. Uribe-Convers 233* (ID), 2012_284_PE, *D.F. Morales-Briones*

& S. Uribe-Convers 253 (ID), 2012_298_PE, *D.F. Morales-Briones & S. Uribe-Convers 267* (ID), 2012_302_PE, *D.F. Morales-Briones & S. Uribe-Convers 271* (ID), 2012_312_EC, *K. Romoleroux et al. 4472* (QCA), 2012_352_EC, *K. Romoleroux et al. 5212* (QCA), 2012_392_EC, *D.F. Morales-Briones et al. 156* (QCA), 2013_410_BO, *D.F. Morales-Briones et al. 306* (ID), 2013_451_CO, *D.F. Morales-Briones & S. Uribe-Convers 435* (ID), 2013_470_CO, *D.F. Morales-Briones & S. Uribe-Convers 454* (ID), 2014_488_CO, *D.F. Morales-Briones & S. Uribe-Convers 494* (ID), 2015_171_MX, *D.F. Morales-Briones & P. Tenorio-Lezama 617* (ID); *Lachemilla williamsii* (L.M. Perry) Rothm.: 2013_402_BO, *D.F. Morales-Briones et al. 298* (ID); *Potaninia mongolica* Maxim.: 2016_591, *L. Yingxin & Z. Xiufu 93039* (MO); *Sibbaldianthe bifurca* (L.) Kurtto & T. Erikss.: 2016_578, *D.E. Boufford et al. 29796* (MO); *Sibbaldia procumbens* L.: 2013_574_US, *H.E. Marx 2012-041* (ID), 2014_014_US, *B. Clevenger 2012-005* (ID), 2015_005, *H.E. Marx 2013-292* (ID); *Sibbaldianthe adpressa* (Bunge) Juz.: 2016_588, *И.М. Красноборов 320* (MO).

Appendix 18

Supplemental figures from Chapter 3.

Fig. S1. Approximately-maximum-likelihood phylogenetic tree of the ITS1 regions. Main clades of *Lachemilla* are colored following Fig. 3.1.

Fig. S2. Approximately-maximum-likelihood phylogenetic tree of the ITS2 regions. Main clades of *Lachemilla* are colored following Fig. 3.1.

Fig. S3. Approximately-maximum-likelihood phylogenetic tree of the ETS regions. Main clades of *Lachemilla* are colored following Fig. 3.1.

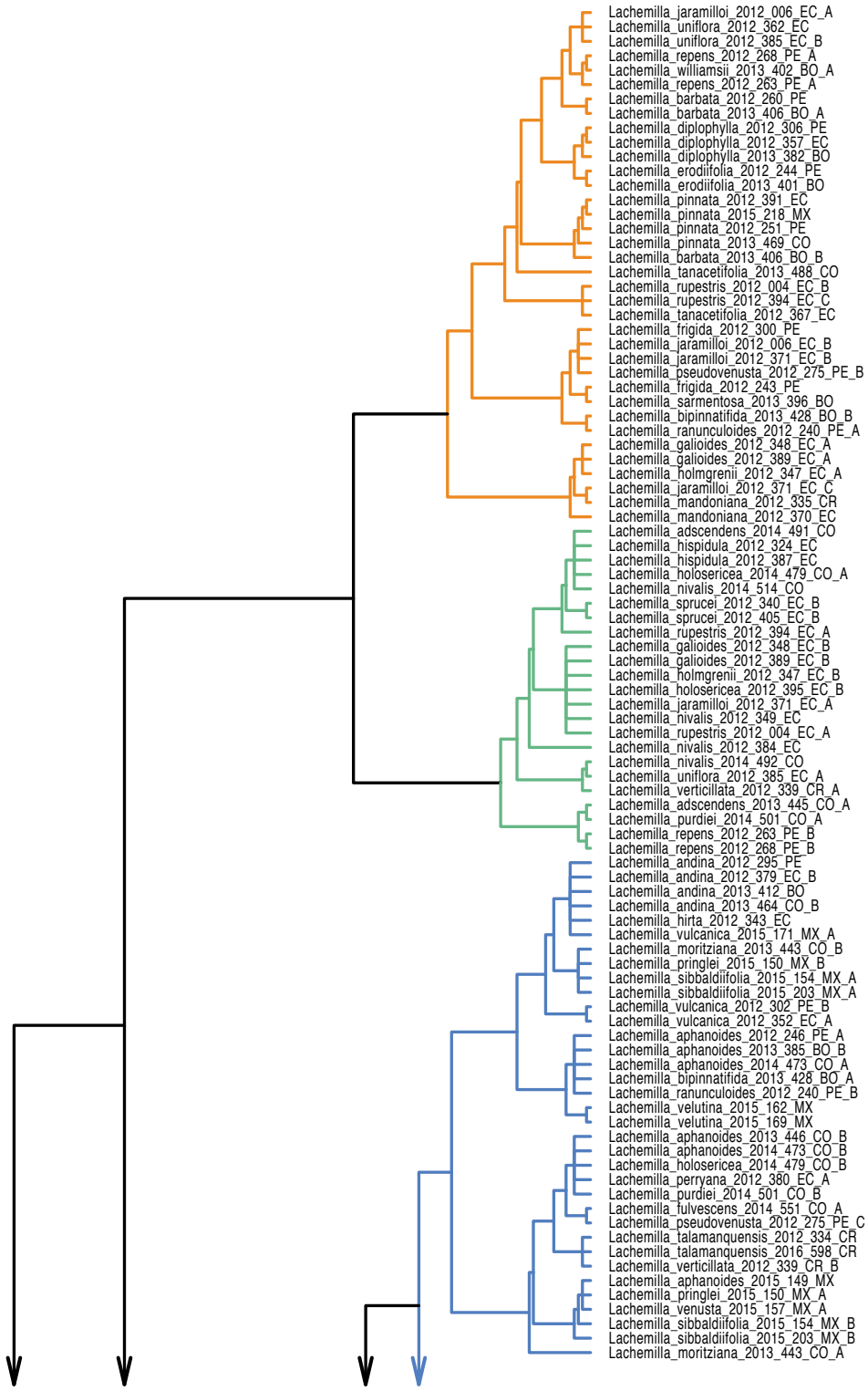


Fig. S1. Approximately-maximum-likelihood phylogenetic tree of the ITS1 region. Branches are colored by major clades within *Lachemilla:* orange – *Pinnate*, yellow – *Orbiculate*, green – *Verticillate*, and blue – *Tripartite*. Figure continues on next page.

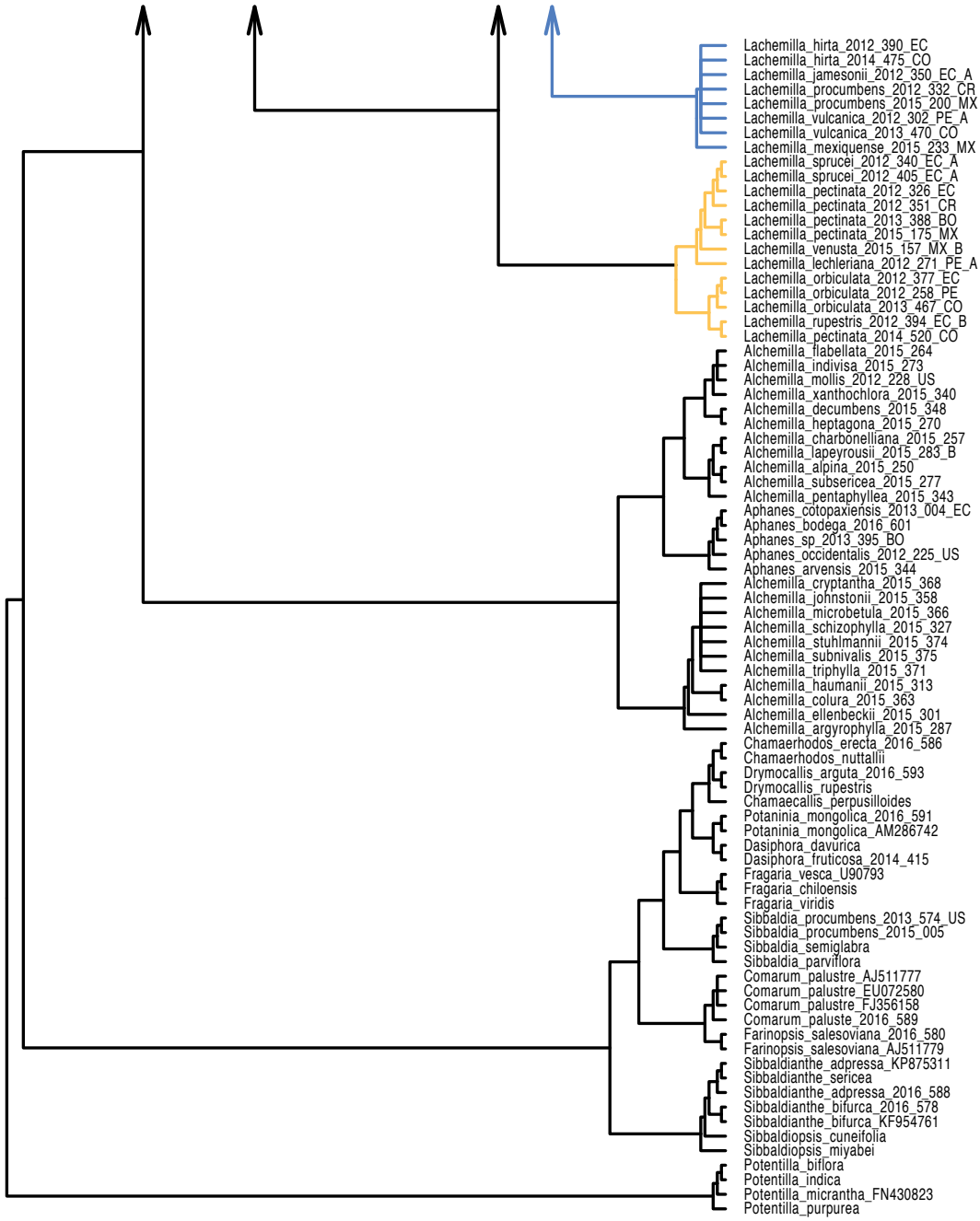


Fig. S1 (Continued). Approximately-maximum-likelihood phylogenetic tree of the ITS1 region. Branches are colored by major clades within *Lachemilla:* orange – *Pinnate*, yellow – *Orbiculate*, green – *Verticillate*, and blue – *Tripartite*.

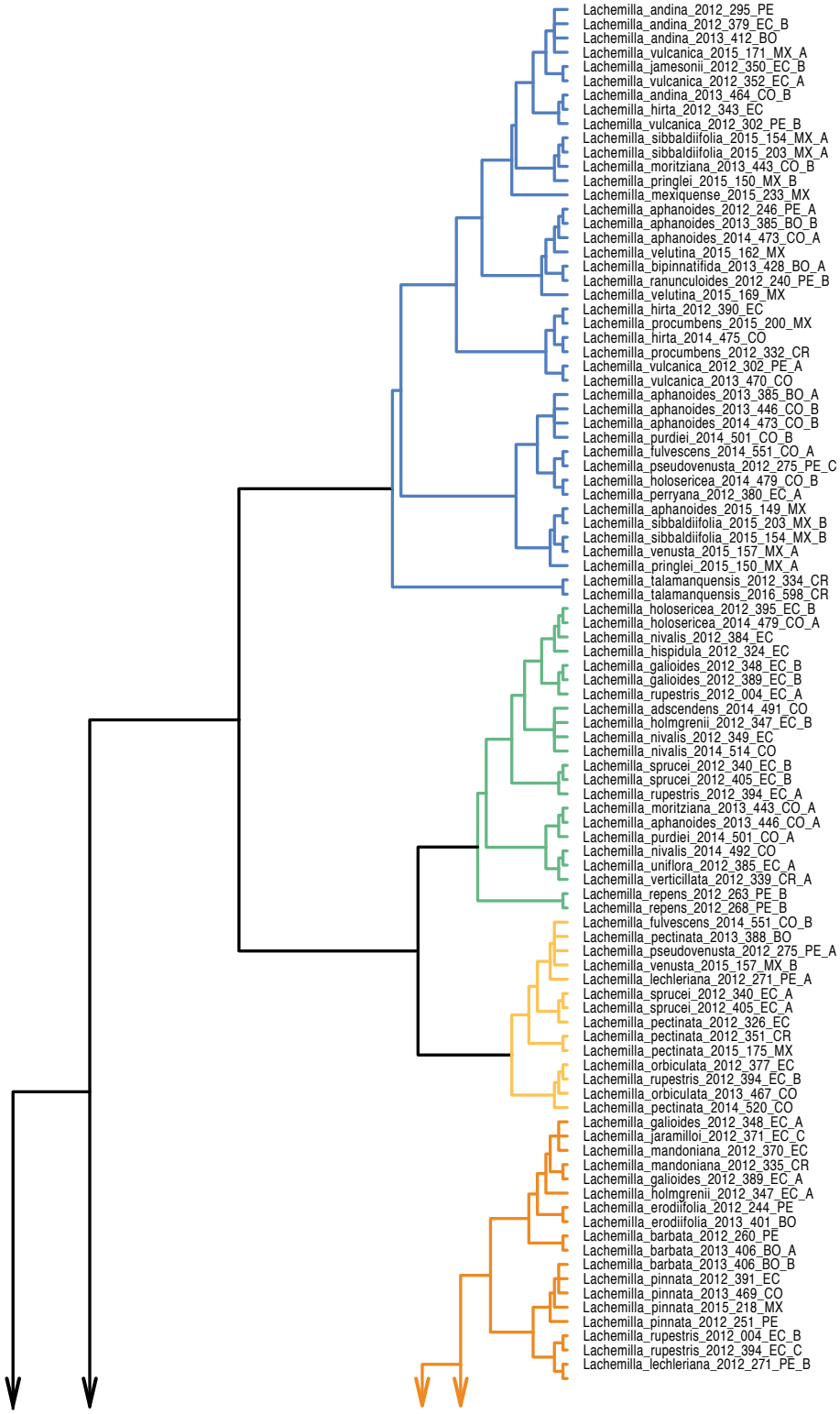


Fig. S2. Approximately-maximum-likelihood phylogenetic tree of the ITS2 region. Main clades of *Lachemilla* are colored following Fig. S1. Figure continues on next page.

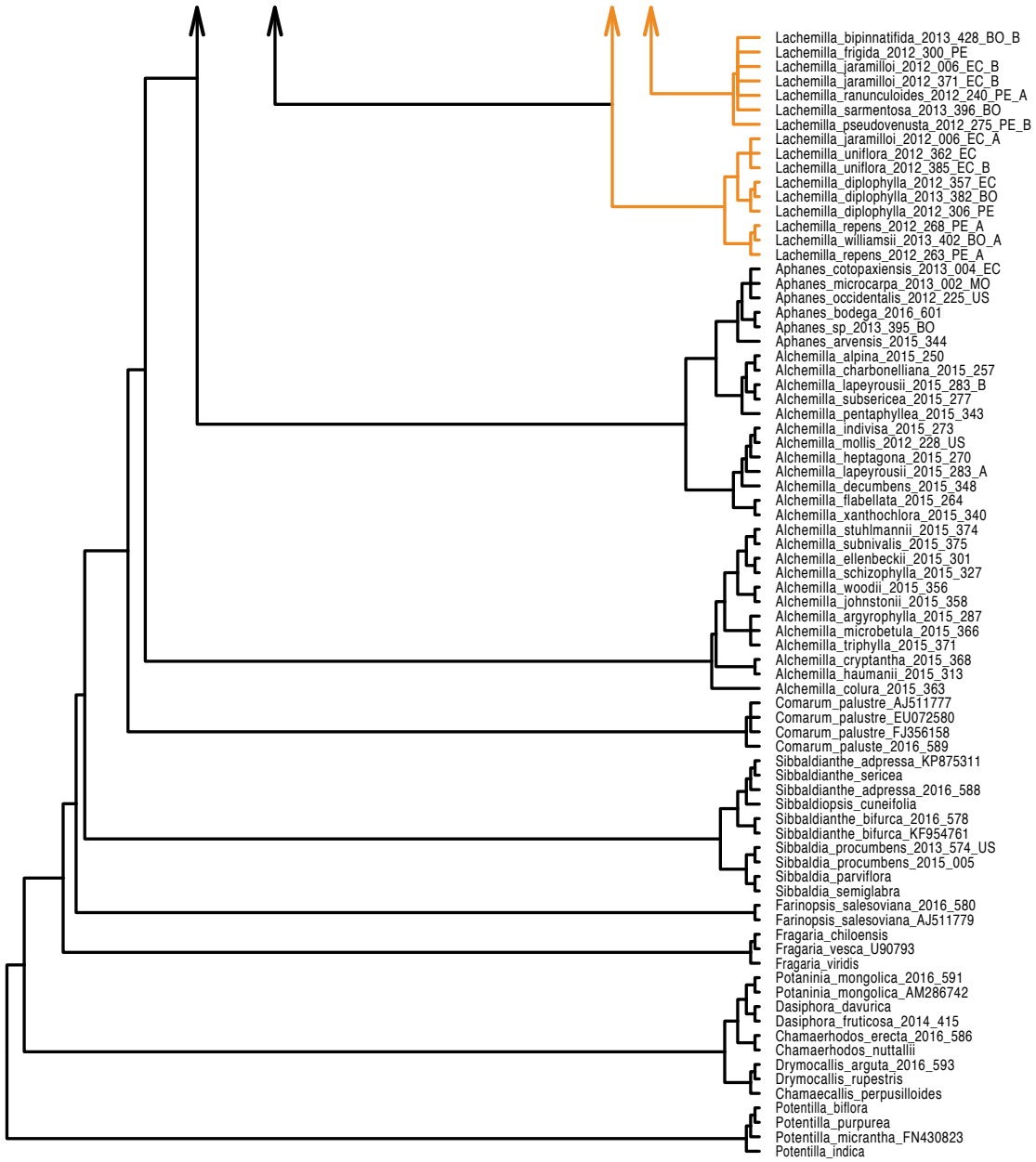


Fig. S2 (Continued). Approximately-maximum-likelihood phylogenetic tree of the ITS2 region. Main clades of *Lachemilla* are colored following Fig. S1.

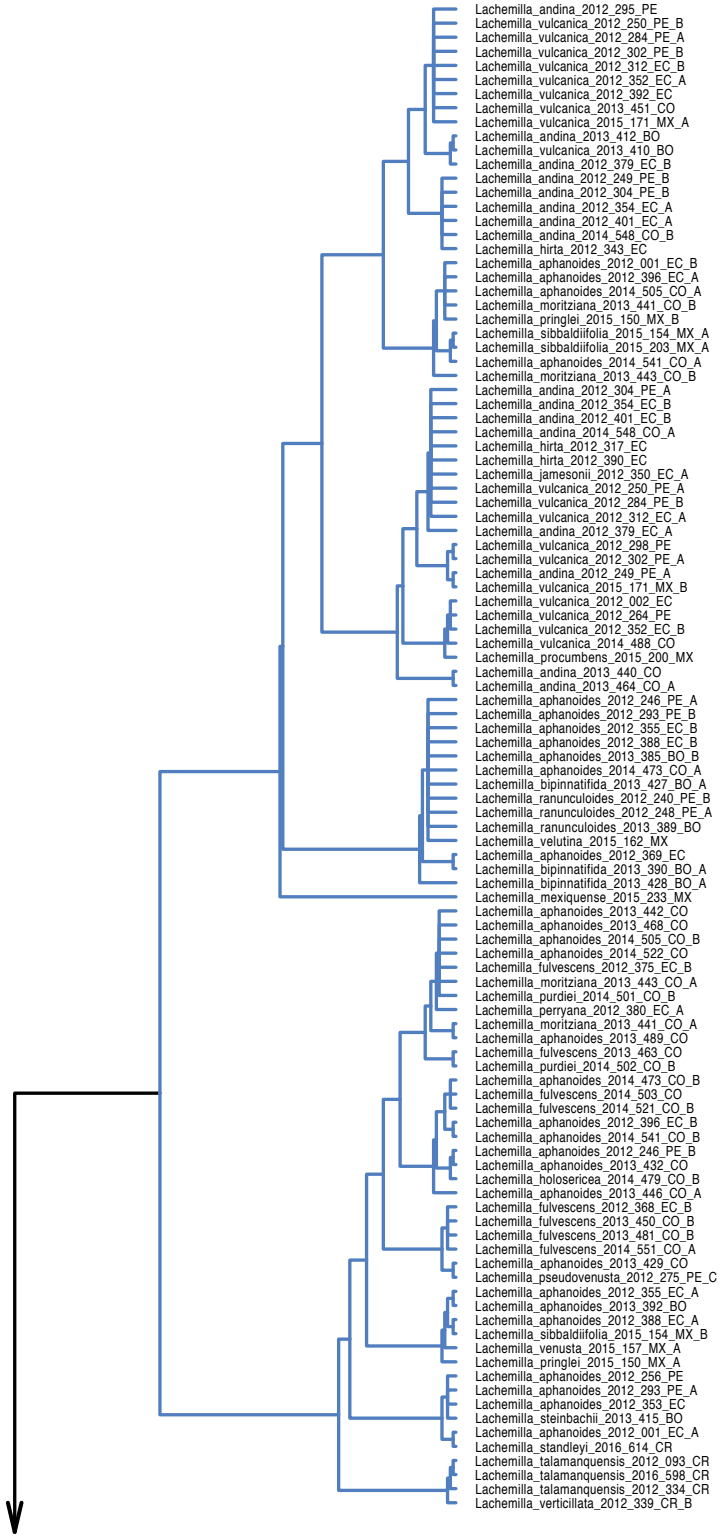


Fig. S3. Approximately-maximum-likelihood phylogenetic tree of the ETS region. Main clades of *Lachemilla* are colored following Fig. S1. Figure continues on next page.

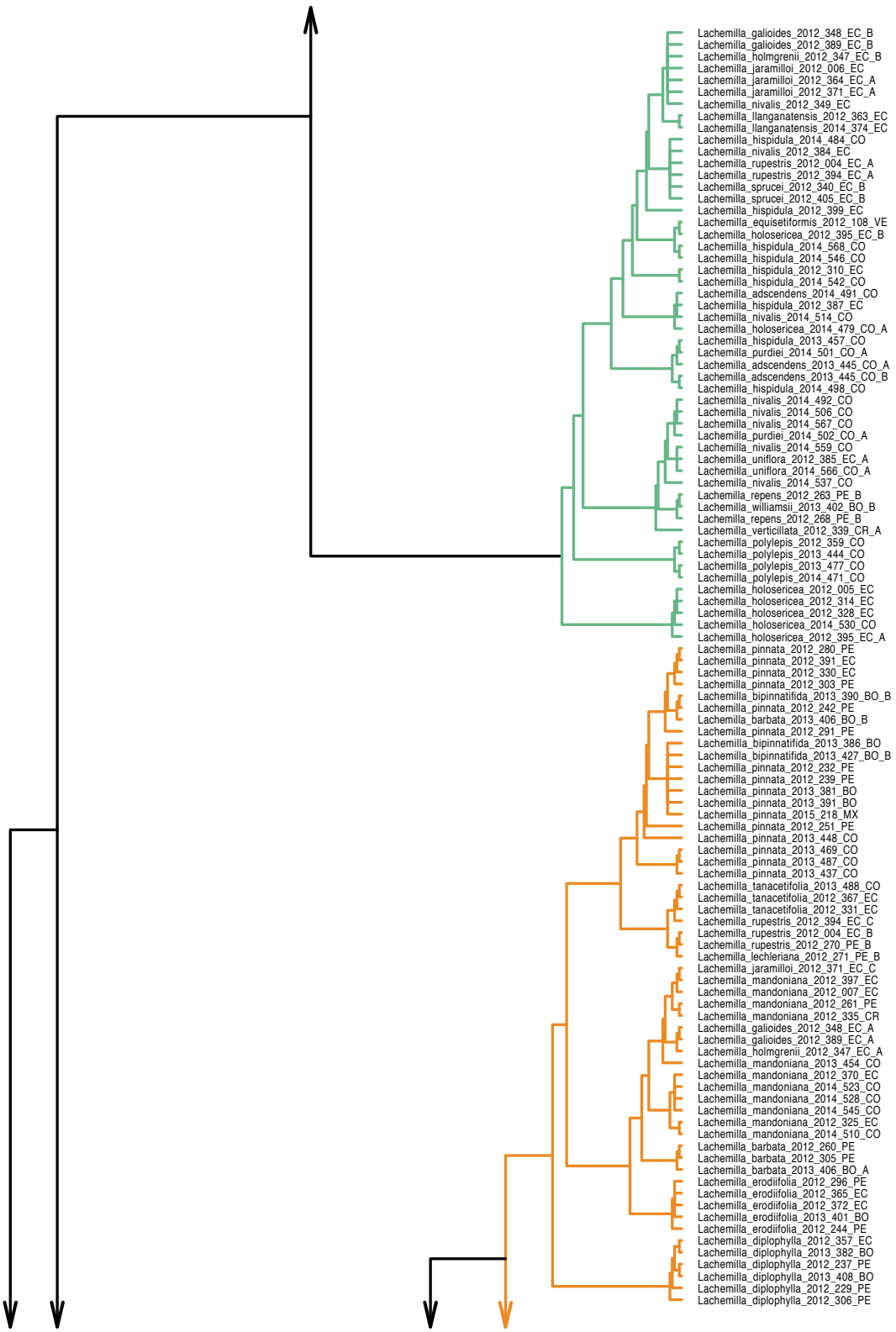


Fig. S3 (Continued). Approximately-maximum-likelihood phylogenetic tree of the ETS region. Main clades of *Lachemilla* are colored following Fig. S1. Figure continues on next page.

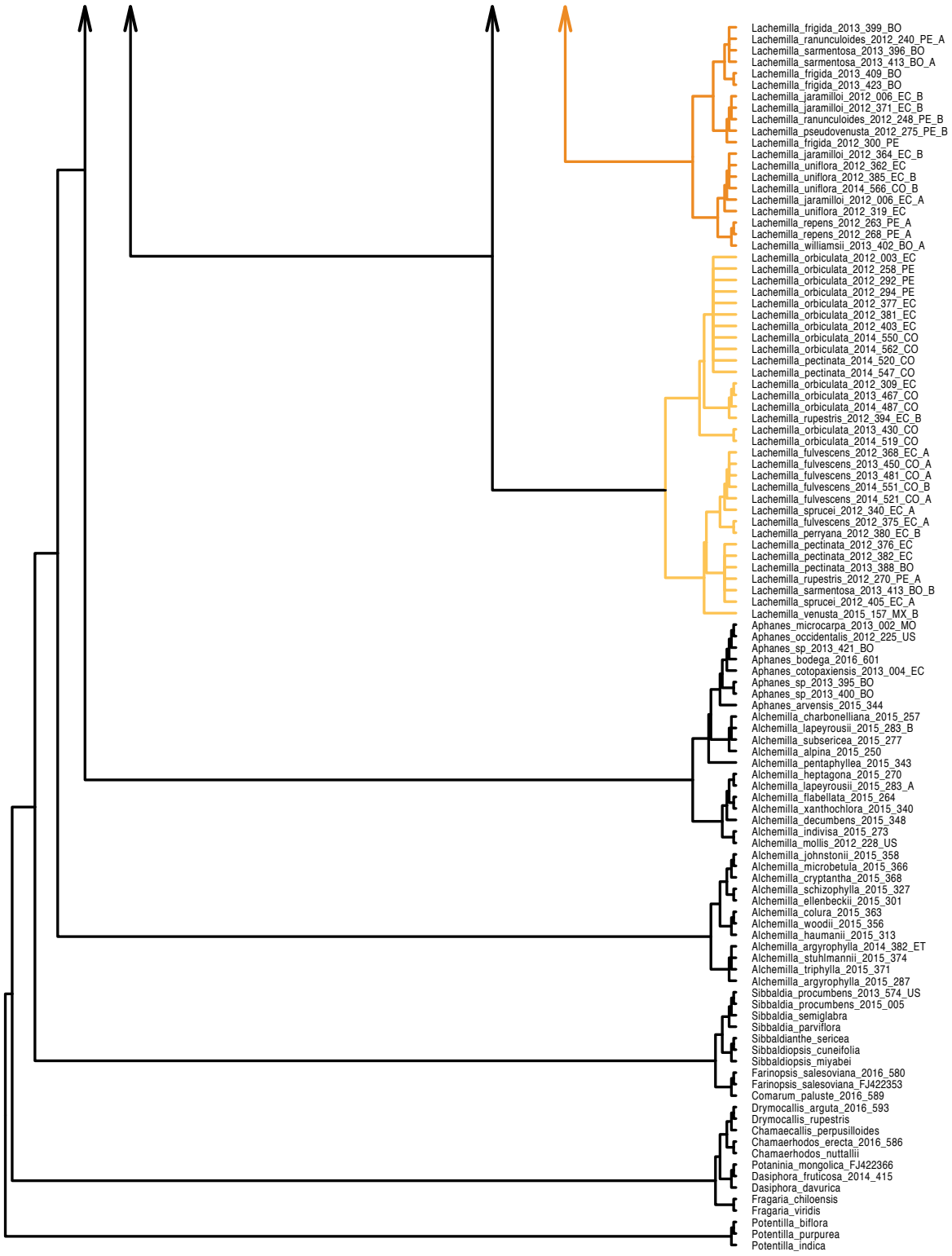


Fig. S3 (Continued). Approximately-maximum-likelihood phylogenetic tree of the ETS region. Main clades of *Lachemilla* are colored following Fig. S1.

Appendix 19

Primer pairs used in Chapter 3.

List of primers used microfluidic PCR in Chapter 3.

Primer Name	Region	Forward 5' – 3'	Reverse 5' – 3'	Expected amplification length (bp)
002-Lach-cp	*matK* partial	TTGAAGATCCGCTATAATAATGAGAA	ACGGCTCTTTCTTCACGAGT	673
007-Lach-cp	*rpoC2* partial	AAGGCTTCGCCGATCTTATT	AAGTGCAAGTGGTCTGATTCAA	704
008-Lach-cp	*rpoC2* partial	AAATTATCAAAGATTGGGATTCCTT	TGATACCGCCAGGAACAAAT	552
012-Lach-cp	*rpoB* partial	CCGGAGCATATGACGATTCT	CGTCAAGCAGTTCCACTCTCT	575
013-Lach-cp	*rpoA* partial - IGS - *rps11* partial	CAACGATTTCTATAGAGGGTGGTAA	TGCCACATAATGGCTGTAGG	449
016-Lach-cp	*psbA* partial - IGS - *trnK*-UUU	ACAGGAGCGGAATATGCAAC	CGACTAGTTCCGGGTTCG	700
021-Lach-cp	*psbI* partial -IGS - *trnS*-GCU - IGS - *trnG*-GCC partial	ATCCCGGACGTGAAGACTAA	TTACCACTAAACTATACCCGCTACAA	563
022-Lach-cp	*trnG*-GCC partial	TTGAATCCTTTACCTCTCAATGAA	CGTTAGCTTGGAAGGCTAGG	573
024-Lach-cp	*atpA* partial	GGAGAGATCGTCATAAATGATTAAAGT	GGTGACGGTTTGATGATACAAG	515
026-Lach-cp	*rpoC1*partial	CGTTGATCTAGTTGCCATCG	TCGTGGTTTAATTAGGCAGCA	559
027-Lach-cp	*ycf4* partial	GCTGGTTGATGAGAGTTACTTCG	TAGGCCAATTCAGCAGCTTT	582
028-Lach-cp	*accD* partial	TTTGAAATTGAAACCAACCATTC	GGAAGCACACACTAGAATAAGAGGT	750
029-Lach-cp	*atpI* partial	ATTGCGTCAGGCTAAGCTAAA	GCTGTTCGTAATCCGCAAAC	582
031-Lach-cp	*rps2* partial	AGCTAGAACGACCCTCACAAAT	GCAAAGCGAAAGGGTATTCA	544
032-Lach-cp	*rpoC2* partial	CGAATCCTTTGAATCCGGTA	TGTTCATGGACATTATGGAGAAA	610
042-Lach-cp	*psaB* partial	GCCCTTTACTAAGATCAATGTAGTTGT	GGCCTTGCTCTAGCCTCTTT	562
044-Lach-cp	*ycf1* partial	CCATTCGGGAAGTTGAAACA	TTAATAATACTGATCAACCAGACGAAG	459

Primer Name	Region	Forward 5' – 3'	Reverse 5' – 3'	Expected amplification length (bp)
046-Lach-cp	*ndhD* partial	GCCATTGATAAGATACTGAAAGTCG	GCAATGTATAGCGGTCAAATAGG	746
047-Lach-cp	*petD* partial	CTGAGACGACCCATAAAGCAC	CCAAAGAGCTACTACAGTACCAATCA	661
053-Lach-cp	*cemA* partial	TGGTGGGATATTAAGCAATCG	CACGATTTAAATAACGAAAGATCCA	489
054-Lach-cp	*rps4* partial	CAAACCCACCCATTTACTATCTATTAT	TAGAGCCGGAAGCGATCTT	435
055-Lach-cp	*petB* partial	CAGGCAATTGCAGATGATATAACTA	CATTGGGAAGTGCATTAACATAAA	524
068-Lach-cp	*rpoC2* partial - IGS - *rpoC1* partial	CGACATCATATGGACTGGGTTA	CGAGAAATCGAAGAAGCTATCC	591
069-Lach-cp	*rps2* partial - IGS - *rpoC2* partial	TGAATACCCTTTCGCTTTGC	TACCGGATTCAAAGGATTCG	482
076-Lach-cp	*psbC* partial	CCGAAGCTTCTCAAGCTCAA	TTTAAATAGGACCTCATCCACCA	557
078-Lach-cp	*trnG*-UCC partial - IGS - *trnfM*-CAU gene - IGS - *rps14* partial	GGGTTCGATTCCCGCTAT	GAAATGGGAAATTCATGGAAAG	603
079-Lach-cp	*rps14* partial - IGS - *psaB* partial	TTTCCCATTTCTCGCTCAAC	GGAATGAATAGTTTATCGGTCTGG	504
082-Lach-cp	*ycf3* partial	AGAAATCTCTAGCCAACCTTCTTG	TGTCTAGATCTGGGATAAATGGAA	620
084-Lach-cp	*ndhJ* partial - IGS - *ndhK* partial	GATACGTTTCAGGCGTGGAT	GATCAAGGATTGCTCTATGAACC	576
085-Lach-cp	*trnV*-UAC partial	TTGCATTGGGCTCTTTCATT	TCAGTTAGGTAGAGCACCTCGTT	517
089-Lach-cp	*accD* partial	TTACCCATCCTGTATATTGTCCTTT	AAGAATGGTTGGTTTCAATTTCA	544
090-Lach-cp	*ycf4* partial - IGS - *cemA* partial	TGCGCGTACCAATTGAAGT	CGATTGCTTAATATCCCACCA	567
091-Lach-cp	*cemA* partial - IGS - *petA* partial	TTGGATCTTTCGTTATTTAAATCGT	ATCCGGAAGTACCGTTTGTG	463
093-Lach-cp	*rps18* partial - IGS - *rpl20* partial	ACCGTTAGAACTCCCGGTCT	CGAGGGTATATTGCTCGAAGA	626
095-Lach-cp	IGS partial *rpl20 - clpP*	TAACCTTCCCGACCACGAT	AGGGTGTATGTGCGACTCGT	600
097-Lach-cp	*clpP* partial	GTTTGTGACGCTGAAATAGGC	TATCCCAACCAACCGACTTT	478
099-Lach-cp	*clpP* partial - IGS - *psbB* partial	CGTCAATCCACACTGCATCT	CAAACGACCTGGATCATTCAA	535
103-Lach-cp	*petD* partial - IGS - *rpoA* partial	GCTCTTTGGTTGGGTATTGG	CGGAAGCTTAACTCCGAAAG	603
108-Lach-cp	*rpl16* partial	GTTCGTTCCGCCATCCTAC	GCTTAGTGTGTGACTCGTTGGT	545
115-Lach-cp	*trnL*-UAG partial - IGS - *ccsA* partial	AGAGCATCTCGGTTCGAGTC	CACCATCATACTTACGTGCATCA	517

Primer Name	Region	Forward 5' – 3'	Reverse 5' – 3'	Expected amplification length (bp)
117-Lach-cp	*ndhD* partial - IGS - *psaC* partial	GCCGCTAAAGTAGCTAAAGTTGTAA	TGTACTCAATGTGTGCGAGCTT	595
118-Lach-cp	*psaC* partial - IGS - *ndhE* partial	CAAGCTCGCACACATTGAGTA	TGATCACGAGCCGAAATATG	495
127-Lach-cp	*trnF*-GAA partial - IGS - *ndhJ* partial	CGGGATAGCTCAGCTGGTAG	CCACGCCTGAAACGTATCTT	669
145-Lach-cp	*psbK* partial - IGS - *psbI* partial	ACCTTAATTCTGCTCTTTATTCCAGT	CGTGTAAACAAATAGTTTGAGGGTAA	365
147-Lach-cp	*rpoC2* partial	CGATTGGAATGGAATGATGA	GACCAATACGTAGAATCAGAGCAA	466
159-Lach-cp	*rps3* partial - IGS - *rpl22* partial	ATACGTGCAATTCCCTCGAC	CGTTCCTATGAAGAAACACTTATGAT	577
164-Lach-cp	*ccsA* partial	TGATGCACGTAAGTATGATGGTG	CCATGCTTCATTAGCCCATAC	291
171-Lach-cp	*atpF* partial	GAGTATCCAGTCATTCGAAACTGAT	ACCCGTATAAGACCTGGTACCTAA	482
ETS-Lach-nu	ETS	GCTTAAGTAACGGTGTATGAGTTGTG	TGGCAGGATCAACCAGGTA	404
ITS1-Lach-nu	ITS1	CGGAAGGATCATTGTCGAA	TGCAATTCACACCAAGTATCG	313
ITS2-Lach-nu	ITS2	GAAATGCGATACTTGGTGTGAA	TCCTCCGCTTATTGATATGCTT	353

Appendix 20

Supplemental tables from Chapter 3.

Supplemental Table S1. Number of raw reads and final number of clusters after running PURC on merged reads of the ITS1 region.

Sample	Raw reads per accession	Final number of clusters
2012_004_EC_Lachemilla_rupestris	2191	35
2012_006_EC_Lachemilla_jaramilloi	858	17
2012_225_US_Aphanes_occidentalis	344	1
2012_228_US_Alchemilla_mollis	1143	8
2012_240_PE_Lachemilla_ranunculoides	1119	13
2012_243_PE_Lachemilla_frigida	13	1
2012_244_PE_Lachemilla_erodiifolia	1019	11
2012_246_PE_Lachemilla_aphanoides	46	5
2012_251_PE_Lachemilla_pinnata	1931	20
2012_258_PE_Lachemilla_orbiculata	941	12
2012_260_PE_Lachemilla_barbata	1918	21
2012_263_PE_Lachemilla_repens	590	15
2012_268_PE_Lachemilla_repens	2099	47
2012_271_PE_Lachemilla_lechleriana	954	26
2012_275_PE_Lachemilla_pseudovenusta	1655	32
2012_295_PE_Lachemilla_andina	2065	28
2012_300_PE_Lachemilla_frigida	999	7
2012_302_PE_Lachemilla_vulcanica	2560	36
2012_306_PE_Lachemilla_diplophylla	2079	29
2012_324_EC_Lachemilla_hispidula	2619	21
2012_326_EC_Lachemilla_pectinata	193	2
2012_334_CR_Lachemilla_talamanquensis	1007	6
2012_335_CR_Lachemilla_mandoniana	1613	17
2012_339_CR_Lachemilla_verticillata	644	11
2012_340_EC_Lachemilla_sprucei	1892	23
2012_343_EC_Lachemilla_hirta	2130	31
2012_347_EC_Lachemilla_holmgrenii	2458	24
2012_348_EC_Lachemilla_galioides	2190	28
2012_349_EC_Lachemilla_nivalis	2231	18
2012_350_EC_Lachemilla_jamesonii	2927	38
2012_352_EC_Lachemilla_vulcanica	2372	11
2012_357_EC_Lachemilla_diplophylla	3267	38

Sample	Raw reads per accession	Final number of clusters
2012_362_EC_Lachemilla_uniflora	1209	10
2012_367_EC_Lachemilla_tanacetifolia	2657	34
2012_370_EC_Lachemilla_mandoniana	3041	34
2012_371_EC_Lachemilla_jaramilloi	2616	36
2012_377_EC_Lachemilla_orbiculata	2216	31
2012_379_EC_Lachemilla_andina	2815	34
2012_380_EC_Lachemilla_perryana	1592	22
2012_384_EC_Lachemilla_nivalis	2867	18
2012_385_EC_Lachemilla_uniflora	3286	34
2012_387_EC_Lachemilla_hispidula	2337	17
2012_389_EC_Lachemilla_galioides	2590	27
2012_390_EC_Lachemilla_hirta	2356	30
2012_391_EC_Lachemilla_pinnata	2059	16
2012_394_EC_Lachemilla_rupestris	1702	26
2012_395_EC_Lachemilla_holosericea	2758	24
2012_405_EC_Lachemilla_sprucei	2471	40
2013_004_EC_Aphanes_cotopaxiensis	1421	12
2013_382_BO_Lachemilla_diplophylla	1207	15
2013_385_BO_Lachemilla_aphanoides	1505	16
2013_388_BO_Lachemilla_pectinata	2284	37
2013_395_BO_Aphanes_sp	1900	13
2013_396_BO_Lachemilla_sarmentosa	1112	17
2013_401_BO_Lachemilla_erodiifolia	1426	27
2013_402_BO_Lachemilla_williamsii	2298	44
2013_406_BO_Lachemilla_barbata	2213	34
2013_412_BO_Lachemilla_andina	2236	30
2013_428_BO_Lachemilla_bipinnatifida	88	2
2013_443_CO_Lachemilla_moritziana	1600	24
2013_445_CO_Lachemilla_adscendens	1804	29
2013_446_CO_Lachemilla_aphanoides	1339	25
2013_464_CO_Lachemilla_andina	1160	23
2013_467_CO_Lachemilla_orbiculata	2059	21
2013_469_CO_Lachemilla_pinnata	2503	28
2013_470_CO_Lachemilla_vulcanica	1698	18
2013_488_CO_Lachemilla_tanacetifolia	124	5
2013_574_US_Sibbaldia_procumbens	2709	56
2014_415_Dasiphora_fruticosa	2888	26
2014_473_CO_Lachemilla_aphanoides	1915	21
2014_479_CO_Lachemilla_holosericea	2013	24
2014_491_CO_Lachemilla_adscendens	2181	28
2014_492_CO_Lachemilla_nivalis	1463	10

Sample	Raw reads per accession	Final number of clusters
2014_501_CO_Lachemilla_purdiei	1739	28
2014_514_CO_Lachemilla_nivalis	2316	16
2014_520_CO_Lachemilla_pectinata	1570	15
2014_551_CO_Lachemilla_fulvescens	1280	23
2015_005_Sibbaldia_procumbens	3119	51
2015_149_MX_Lachemilla_aphanoides	134	2
2015_150_MX_Lachemilla_pringlei	1482	18
2015_154_MX_Lachemilla_sibbaldiifolia	2560	40
2015_157_MX_Lachemilla_venusta	1909	38
2015_162_MX_Lachemilla_velutina	1856	21
2015_169_MX_Lachemilla_velutina	14	1
2015_171_MX_Lachemilla_vulcanica	1739	13
2015_200_MX_Lachemilla_procumbens	1845	15
2015_203_MX_Lachemilla_sibbaldiifolia	3010	25
2015_218_MX_Lachemilla_pinnata	1004	6
2015_233_MX_Lachemilla_mexiquense	858	4
2015_250_Alchemilla_alpina	1945	21
2015_257_Alchemilla_charbonelliana	134	3
2015_264_Alchemilla_flabellata	940	9
2015_270_Alchemilla_heptagona	233	8
2015_273_Alchemilla_indivisa	199	3
2015_277_Alchemilla_subsericea	11	1
2015_283_Alchemilla_lapeyrousii	369	8
2015_287_Alchemilla_argyrophylla	597	2
2015_301_Alchemilla_ellenbeckii	167	1
2015_313_Alchemilla_haumanii	1836	18
2015_327_Alchemilla_schizophylla	263	3
2015_340_Alchemilla_xanthochlora	2419	25
2015_343_Alchemilla_pentaphyllea	13	1
2015_344_Aphanes_arvensis	1816	16
2015_348_Alchemilla_decumbens	53	4
2015_358_Alchemilla_johnstonii	2294	11
2015_363_Alchemilla_colura	1327	9
2015_366_Alchemilla_microbetula	3030	21
2015_368_Alchemilla_cryptantha	1136	4
2015_371_Alchemilla_triphylla	451	1
2015_374_Alchemilla_stuhlmannii	930	7
2015_375_Alchemilla_subnivalis	11	1
2016_578_Sibbaldianthe_bifurca	898	9
2016_580_Farinopsis_salesoviana	1429	11
2016_586_Chamaerhodos_erecta	1694	7

Sample	Raw reads per accession	Final number of clusters
2016_588_Sibbaldianthe_adpressa	22	1
2016_589_Comarum_paluste	3194	31
2016_591_Potaninia_mongolica	423	2
2016_593_Drymocallis_arguta	2246	28
2016_598_CR_Lachemilla_talamanquensis	2245	20
2016_601_Aphanes_sp	809	6

Supplemental Table S2. Number of raw reads and final number of clusters after running PURC on merged reads of the ITS2 region.

Sample	Raw reads per accession	Final number of clusters
2012_004_EC_Lachemilla_rupestris	3466	42
2012_006_EC_Lachemilla_jaramilloi	608	5
2012_225_US_Aphanes_occidentalis	108	1
2012_228_US_Alchemilla_mollis	825	7
2012_240_PE_Lachemilla_ranunculoides	446	4
2012_244_PE_Lachemilla_erodiifolia	1078	4
2012_246_PE_Lachemilla_aphanoides	10	1
2012_251_PE_Lachemilla_pinnata	1687	12
2012_260_PE_Lachemilla_barbata	410	3
2012_263_PE_Lachemilla_repens	252	3
2012_268_PE_Lachemilla_repens	469	2
2012_271_PE_Lachemilla_lechleriana	78	3
2012_275_PE_Lachemilla_pseudovenusta	1758	23
2012_295_PE_Lachemilla_andina	2770	34
2012_300_PE_Lachemilla_frigida	1097	2
2012_302_PE_Lachemilla_vulcanica	2854	36
2012_306_PE_Lachemilla_diplophylla	2028	24
2012_324_EC_Lachemilla_hispidula	3263	26
2012_326_EC_Lachemilla_pectinata	114	2
2012_334_CR_Lachemilla_talamanquensis	1135	6
2012_335_CR_Lachemilla_mandoniana	3674	26
2012_339_CR_Lachemilla_verticillata	584	9
2012_340_EC_Lachemilla_sprucei	3025	32
2012_343_EC_Lachemilla_hirta	2433	33
2012_347_EC_Lachemilla_holmgrenii	2906	24
2012_348_EC_Lachemilla_galioides	2700	24
2012_349_EC_Lachemilla_nivalis	2814	13
2012_350_EC_Lachemilla_jamesonii	3059	46
2012_352_EC_Lachemilla_vulcanica	2336	23
2012_357_EC_Lachemilla_diplophylla	3747	49
2012_362_EC_Lachemilla_uniflora	922	3
2012_367_EC_Lachemilla_tanacetifolia	3418	39
2012_370_EC_Lachemilla_mandoniana	3584	44
2012_371_EC_Lachemilla_jaramilloi	3873	34
2012_377_EC_Lachemilla_orbiculata	3700	32
2012_379_EC_Lachemilla_andina	3414	43
2012_380_EC_Lachemilla_perryana	1975	17
2012_384_EC_Lachemilla_nivalis	3363	19
2012_385_EC_Lachemilla_uniflora	3517	27

Sample	Raw reads per accession	Final number of clusters
2012_389_EC_Lachemilla_galioides	3412	29
2012_390_EC_Lachemilla_hirta	3620	32
2012_391_EC_Lachemilla_pinnata	4403	35
2012_394_EC_Lachemilla_rupestris	2542	28
2012_395_EC_Lachemilla_holosericea	3732	28
2012_405_EC_Lachemilla_sprucei	3836	43
2013_002_MO_Aphanes_microcarpa	1954	4
2013_004_EC_Aphanes_cotopaxiensis	2148	2
2013_382_BO_Lachemilla_diplophylla	88	3
2013_385_BO_Lachemilla_aphanoides	863	8
2013_388_BO_Lachemilla_pectinata	3319	37
2013_395_BO_Aphanes_sp	2500	23
2013_396_BO_Lachemilla_sarmentosa	2005	14
2013_401_BO_Lachemilla_erodiifolia	1295	7
2013_402_BO_Lachemilla_williamsii	2231	8
2013_406_BO_Lachemilla_barbata	3099	25
2013_412_BO_Lachemilla_andina	3210	50
2013_428_BO_Lachemilla_bipinnatifida	94	2
2013_443_CO_Lachemilla_moritziana	1832	28
2013_446_CO_Lachemilla_aphanoides	1687	11
2013_464_CO_Lachemilla_andina	2185	52
2013_467_CO_Lachemilla_orbiculata	1852	11
2013_469_CO_Lachemilla_pinnata	2392	23
2013_470_CO_Lachemilla_vulcanica	1152	10
2013_574_US_Sibbaldia_procumbens	3548	51
2014_415_Dasiphora_fruticosa	3570	39
2014_473_CO_Lachemilla_aphanoides	1510	9
2014_479_CO_Lachemilla_holosericea	2895	25
2014_491_CO_Lachemilla_adscendens	3264	32
2014_492_CO_Lachemilla_nivalis	1379	7
2014_501_CO_Lachemilla_purdiei	2168	22
2014_514_CO_Lachemilla_nivalis	3313	34
2014_520_CO_Lachemilla_pectinata	2023	12
2014_551_CO_Lachemilla_fulvescens	1480	30
2015_005_Potentilla_glaucophylla	3458	50
2015_149_MX_Lachemilla_aphanoides	59	1
2015_150_MX_Lachemilla_pringlei	1426	16
2015_154_MX_Lachemilla_sibbaldiaefolia	3946	43
2015_157_MX_Lachemilla_venusta	1977	22
2015_162_MX_Lachemilla_velutina	2861	28
2015_169_MX_Lachemilla_velutina	11	1

Sample	Raw reads per accession	Final number of clusters
2015_171_MX_Lachemilla_vulcanica	1774	21
2015_200_MX_Lachemilla_procumbens	1548	20
2015_203_MX_Lachemilla_sibbaldiifolia	2846	23
2015_218_MX_Lachemilla_pinnata	537	1
2015_233_MX_Lachemilla_mexiquense	630	2
2015_250_Alchemilla_alpina	2864	22
2015_257_Alchemilla_charbonelliana	123	3
2015_264_Alchemilla_flabellata	674	8
2015_270_Alchemilla_heptagona	1196	13
2015_273_Alchemilla_indivisa	145	1
2015_277_Alchemilla_subsericea	20	2
2015_283_Alchemilla_lapeyrousii	535	10
2015_287_Alchemilla_argyrophylla	522	3
2015_301_Alchemilla_ellenbeckii	89	1
2015_313_Alchemilla_haumanii	2169	44
2015_327_Alchemilla_schizophylla	66	1
2015_340_Alchemilla_xanthochlora	3520	36
2015_343_Alchemilla_pentaphyllea	10	1
2015_344_Aphanes_arvensis	2395	6
2015_348_Alchemilla_decumbens	748	3
2015_356_Alchemilla_woodii	16	1
2015_358_Alchemilla_johnstonii	3348	49
2015_363_Alchemilla_colura	1144	14
2015_366_Alchemilla_microbetula	2594	26
2015_368_Alchemilla_cryptantha	1577	26
2015_371_Alchemilla_triphylla	305	3
2015_374_Alchemilla_stuhlmannii	1011	7
2015_375_Alchemilla_subnivalis	10	1
2016_578_Sibbaldianthe_bifurca	979	9
2016_580_Farinopsis_salesoviana	1676	25
2016_586_Chamaerhodos_erecta	2625	28
2016_588_Sibbaldianthe_adpressa	48	2
2016_589_Comarum_paluste	3515	40
2016_591_Potaninia_mongolica	506	4
2016_593_Drymocallis_arguta	2642	31
2016_598_CR_Lachemilla_talamanquensis	2800	23
2016_601_Aphanes_sp	823	8

Supplemental Table S3. Number of raw reads and final number of clusters after running PURC on merged reads of the ETS region.

Sample	Raw reads per accession	Final number of clusters
2012_001_EC_Lachemilla_aphanoides	246	3
2012_002_EC_Lachemilla_vulcanica	760	11
2012_003_EC_Lachemilla_orbiculata	811	6
2012_004_EC_Lachemilla_rupestris	2590	21
2012_005_EC_Lachemilla_holosericea	2673	6
2012_006_EC_Lachemilla_jaramilloi	1610	18
2012_007_EC_Lachemilla_mandoniana	703	10
2012_093_CR_Lachemilla_talamanquensis	376	2
2012_108_VE_Lachemilla_equisetiformis	251	1
2012_225_US_Aphanes_occidentalis	486	1
2012_228_US_Alchemilla_mollis	2052	10
2012_229_PE_Lachemilla_diplophylla	420	2
2012_232_PE_Lachemilla_pinnata	533	6
2012_237_PE_Lachemilla_diplophylla	566	2
2012_239_PE_Lachemilla_pinnata	450	1
2012_240_PE_Lachemilla_ranunculoides	2479	22
2012_242_PE_Lachemilla_pinnata	605	9
2012_244_PE_Lachemilla_erodiifolia	1990	6
2012_246_PE_Lachemilla_aphanoides	926	11
2012_248_PE_Lachemilla_ranunculoides	951	5
2012_249_PE_Lachemilla_andina	954	5
2012_250_PE_Lachemilla_vulcanica	1050	8
2012_251_PE_Lachemilla_pinnata	3504	21
2012_256_PE_Lachemilla_aphanoides	986	2
2012_258_PE_Lachemilla_orbiculata	2112	14
2012_260_PE_Lachemilla_barbata	2938	15
2012_261_PE_Lachemilla_mandoniana	836	5
2012_263_PE_Lachemilla_repens	1214	15
2012_264_PE_Lachemilla_vulcanica	87	2
2012_268_PE_Lachemilla_tanacetifolia	2876	36
2012_270_PE_Lachemilla_rupestris	1595	28
2012_271_PE_Lachemilla_lechleriana	1913	16
2012_275_PE_Lachemilla_pseudovenusta	2817	27
2012_280_PE_Lachemilla_pinnata	240	3
2012_284_PE_Lachemilla_vulcanica	161	2
2012_291_PE_Lachemilla_pinnata	207	3
2012_292_PE_Lachemilla_orbiculata	176	2
2012_293_PE_Lachemilla_aphanoides	303	5
2012_294_PE_Lachemilla_orbiculata	167	3

Sample	Raw reads per accession	Final number of clusters
2012_295_PE_Lachemilla_andina	2263	15
2012_296_PE_Lachemilla_erodiifolia	80	1
2012_298_PE_Lachemilla_vulcanica	89	5
2012_300_PE_Lachemilla_frigida	1875	7
2012_302_PE_Lachemilla_vulcanica	3071	27
2012_303_PE_Lachemilla_pinnata	224	4
2012_304_PE_Lachemilla_andina	397	7
2012_305_PE_Lachemilla_barbata	38	1
2012_306_PE_Lachemilla_diplophylla	2915	25
2012_309_EC_Lachemilla_orbiculata	42	1
2012_310_EC_Lachemilla_hispidula	30	2
2012_312_EC_Lachemilla_vulcanica	68	3
2012_314_EC_Lachemilla_holosericea	134	2
2012_317_EC_Lachemilla_hirta	199	1
2012_319_EC_Lachemilla_uniflora	178	2
2012_325_EC_Lachemilla_mandoniana	171	2
2012_328_EC_Lachemilla_holosericea	268	1
2012_330_EC_Lachemilla_pinnata	125	5
2012_331_EC_Lachemilla_tanacetifolia	99	3
2012_334_CR_Lachemilla_talamanquensis	916	3
2012_335_CR_Lachemilla_mandoniana	2498	11
2012_339_CR_Lachemilla_verticillata	359	5
2012_340_EC_Lachemilla_sprucei	2227	16
2012_343_EC_Lachemilla_hirta	1386	13
2012_347_EC_Lachemilla_holmgrenii	2307	10
2012_348_EC_Lachemilla_galioides	2231	11
2012_349_EC_Lachemilla_nivalis	2193	15
2012_350_EC_Lachemilla_jamesonii	2754	35
2012_352_EC_Lachemilla_vulcanica	2380	24
2012_353_EC_Lachemilla_aphanoides	108	1
2012_354_EC_Lachemilla_andina	1686	30
2012_355_EC_Lachemilla_aphanoides	1841	12
2012_357_EC_Lachemilla_diplophylla	3458	15
2012_359_CO_Lachemilla_polylepis	1575	18
2012_362_EC_Lachemilla_uniflora	866	2
2012_363_EC_Lachemilla_llanganatensis	47	1
2012_364_EC_Lachemilla_jaramilloi	1731	6
2012_365_EC_Lachemilla_erodiifolia	1181	2
2012_367_EC_Lachemilla_tanacetifolia	2795	23
2012_368_EC_Lachemilla_fulvescens	1189	8
2012_369_EC_Lachemilla_aphanoides	1265	7

Sample	Raw reads per accession	Final number of clusters
2012_370_EC_Lachemilla_mandoniana	2916	25
2012_371_EC_Lachemilla_jaramilloi	2736	27
2012_372_EC_Lachemilla_erodiifolia	1334	3
2012_376_EC_Lachemilla_pectinata	1266	14
2012_377_EC_Lachemilla_orbiculata	2739	13
2012_379_EC_Lachemilla_andina	2890	30
2012_380_EC_Lachemilla_perryana	2100	29
2012_381_EC_Lachemilla_orbiculata	1065	11
2012_382_EC_Lachemilla_pectinata	357	8
2012_384_EC_Lachemilla_nivalis	2937	11
2012_385_EC_Lachemilla_uniflora	3217	17
2012_387_EC_Lachemilla_hispidula	2873	14
2012_388_EC_Lachemilla_aphanoides	1623	14
2012_389_EC_Lachemilla_galioides	2727	16
2012_390_EC_Lachemilla_hirta	3117	16
2012_391_EC_Lachemilla_pinnata	2309	15
2012_392_EC_Lachemilla_vulcanica	1997	25
2012_394_EC_Lachemilla_rupestris	1732	15
2012_395_EC_Lachemilla_holosericea	2966	15
2012_396_EC_Lachemilla_aphanoides	1646	18
2012_397_EC_Lachemilla_mandoniana	1893	12
2012_399_EC_Lachemilla_hispidula	1469	7
2012_401_EC_Lachemilla_andina	2239	19
2012_403_EC_Lachemilla_orbiculata	1168	4
2012_405_EC_Lachemilla_sprucei	2682	21
2013_002_MO_Aphanes_microcarpa	2676	3
2013_004_EC_Aphanes_cotopaxiensis	3082	8
2013_381_BO_Lachemilla_pinnata	45	1
2013_382_BO_Lachemilla_diplophylla	2372	11
2013_385_BO_Lachemilla_aphanoides	2723	30
2013_386_BO_Lachemilla_bipinnatifida	14	1
2013_388_BO_Lachemilla_pectinata	2815	9
2013_389_BO_Lachemilla_ranunculoides	736	18
2013_390_BO_Lachemilla_bipinnatifida	2668	22
2013_391_BO_Lachemilla_pinnata	2098	12
2013_392_BO_Lachemilla_aphanoides	1972	21
2013_395_BO_Aphanes_sp	2586	15
2013_396_BO_Lachemilla_sarmentosa	2510	30
2013_399_BO_Lachemilla_frigida	1144	3
2013_400_BO_Aphanes_sp	1086	5
2013_401_BO_Lachemilla_erodiifolia	2170	10

Sample	Raw reads per accession	Final number of clusters
2013_402_BO_Lachemilla_williamsii	2521	16
2013_406_BO_Lachemilla_barbata	2536	27
2013_408_BO_Lachemilla_diplophylla	2266	6
2013_409_BO_Lachemilla_frigida	3078	27
2013_410_BO_Lachemilla_vulcanica	1049	15
2013_412_BO_Lachemilla_andina	3227	48
2013_413_BO_Lachemilla_sarmentosa	2022	21
2013_415_BO_Lachemilla_steinbachii	231	3
2013_421_BO_Aphanes_sp	910	3
2013_423_BO_Lachemilla_frigida	1415	9
2013_427_BO_Lachemilla_bipinnatifida	2592	19
2013_428_BO_Lachemilla_bipinnatifida	78	1
2013_429_CO_Lachemilla_aphanoides	1450	6
2013_430_CO_Lachemilla_orbiculata	2070	13
2013_432_CO_Lachemilla_aphanoides	2561	12
2013_437_CO_Lachemilla_pinnata	822	10
2013_440_CO_Lachemilla_andina	3118	28
2013_441_CO_Lachemilla_moritziana	4222	47
2013_442_CO_Lachemilla_aphanoides	410	1
2013_443_CO_Lachemilla_moritziana	2670	40
2013_444_CO_Lachemilla_polylepis	2417	22
2013_445_CO_Lachemilla_adscendens	2465	18
2013_446_CO_Lachemilla_aphanoides	2759	21
2013_448_CO_Lachemilla_pinnata	3714	34
2013_450_CO_Lachemilla_fulvescens	2230	23
2013_451_CO_Lachemilla_vulcanica	2254	8
2013_454_CO_Lachemilla_mandoniana	2281	12
2013_457_CO_Lachemilla_hispidula	33	2
2013_463_CO_Lachemilla_fulvescens	2059	18
2013_464_CO_Lachemilla_andina	1735	17
2013_467_CO_Lachemilla_orbiculata	2892	19
2013_468_CO_Lachemilla_aphanoides	2792	33
2013_469_CO_Lachemilla_pinnata	3352	29
2013_477_CO_Lachemilla_polylepis	2401	19
2013_481_CO_Lachemilla_fulvescens	1356	13
2013_487_CO_Lachemilla_pinnata	2125	9
2013_488_CO_Lachemilla_tanacetifolia	812	7
2013_489_CO_Lachemilla_aphanoides	1010	17
2013_574_US_Sibbaldia_procumbens	1492	6
2014_014_US_Sibbaldia_procumbens	1023	10
2014_371_US_Aphanes_sp	878	6

Sample	Raw reads per accession	Final number of clusters
2014_372_US_Aphanes_sp	1860	7
2014_374_EC_Lachemilla_llanganatensis	416	2
2014_382_ET_Alchemilla_argyrophylla	818	12
2014_415_Dasiphora_fruticosa	911	3
2014_471_CO_Lachemilla_polylepis	2095	10
2014_473_CO_Lachemilla_aphanoides	2510	17
2014_479_CO_Lachemilla_holosericea	3265	23
2014_484_CO_Lachemilla_hispidula	2435	9
2014_487_CO_Lachemilla_orbiculata	3356	36
2014_488_CO_Lachemilla_vulcanica	4648	44
2014_491_CO_Lachemilla_adscendens	2919	12
2014_492_CO_Lachemilla_nivalis	1797	9
2014_498_CO_Lachemilla_hispidula	2768	27
2014_501_CO_Lachemilla_purdiei	2128	28
2014_502_CO_Lachemilla_purdiei	3917	22
2014_503_CO_Lachemilla_fulvescens	3049	26
2014_505_CO_Lachemilla_aphanoides	3690	48
2014_506_CO_Lachemilla_nivalis	2506	22
2014_510_CO_Lachemilla_mandoniana	3390	22
2014_514_CO_Lachemilla_nivalis	2728	6
2014_519_CO_Lachemilla_orbiculata	2501	17
2014_520_CO_Lachemilla_pectinata	2090	16
2014_521_CO_Lachemilla_fulvescens	2697	28
2014_522_CO_Lachemilla_aphanoides	3184	23
2014_523_CO_Lachemilla_mandoniana	2944	22
2014_528_CO_Lachemilla_mandoniana	4514	47
2014_530_CO_Lachemilla_holosericea	107	1
2014_537_CO_Lachemilla_nivalis	2979	17
2014_541_CO_Lachemilla_aphanoides	2201	22
2014_542_CO_Lachemilla_hispidula	3903	33
2014_545_CO_Lachemilla_mandoniana	3241	29
2014_546_CO_Lachemilla_hispidula	4070	21
2014_547_CO_Lachemilla_pectinata	3380	28
2014_548_CO_Lachemilla_andina	7145	87
2014_550_CO_Lachemilla_orbiculata	3610	23
2014_551_CO_Lachemilla_fulvescens	2529	16
2014_559_CO_Lachemilla_nivalis	3934	37
2014_562_CO_Lachemilla_orbiculata	3099	23
2014_566_CO_Lachemilla_uniflora	438	7
2014_567_CO_Lachemilla_nivalis	3765	36
2014_568_CO_Lachemilla_hispidula	2675	13

Sample	Raw reads per accession	Final number of clusters
2015_150_MX_Lachemilla_pringlei	1870	19
2015_154_MX_Lachemilla_sibbaldiifolia	3476	52
2015_157_MX_Lachemilla_venusta	2628	29
2015_162_MX_Lachemilla_velutina	2996	17
2015_171_MX_Lachemilla_vulcanica	2234	20
2015_200_MX_Lachemilla_procumbens	2267	10
2015_203_MX_Lachemilla_sibbaldiaefolia	2942	50
2015_218_MX_Lachemilla_pinnata	1471	6
2015_233_MX_Lachemilla_mexiquense	978	5
2015_250_Alchemilla_alpina	2098	18
2015_257_Alchemilla_charbonelliana	117	1
2015_264_Alchemilla_flabellata	1445	4
2015_270_Alchemilla_heptagona	1330	8
2015_273_Alchemilla_indivisa	69	2
2015_277_Alchemilla_subsericea	85	3
2015_283_Alchemilla_lapeyrousii	693	6
2015_287_Alchemilla_argyrophylla	841	1
2015_301_Alchemilla_ellenbeckii	463	2
2015_313_Alchemilla_haumanii	3403	16
2015_327_Alchemilla_schizophylla	776	5
2015_340_Alchemilla_xanthochlora	3414	15
2015_343_Alchemilla_pentaphyllea	18	1
2015_344_Aphanes_arvensis	2573	33
2015_348_Alchemilla_decumbens	964	9
2015_356_Alchemilla_woodii	21	1
2015_358_Alchemilla_johnstonii	3432	18
2015_363_Alchemilla_colura	1755	13
2015_366_Alchemilla_microbetula	3599	17
2015_368_Alchemilla_cryptantha	2462	14
2015_371_Alchemilla_triphylla	917	4
2015_374_Alchemilla_stuhlmannii	1526	9
2016_580_Farinopsis_salesoviana	61	1
2016_586_Chamaerhodos_erecta	559	2
2016_589_Comarum_paluste	499	4
2016_593_Drymocallis_arguta	813	5
2016_598_CR_Lachemilla_talamanquensis	3192	25
2016_601_Aphanes_sp	898	2
2016_614_CR_Lachemilla_standleyi	39	2

Appendix 21

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RESEARCH ARTICLE

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Lachemilla mexiquense (Rosaceae), a new species from Mexico

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Abstract

A new species of *Lachemilla* (Rosaceae), *Lachemilla mexiquense* D.F. Morales-B., from Mexico is described and illustrated. This species is similar to *Lachemilla aphanoides* by its tripartite leaves and glomerulate inflorescence with entirely glabrous flowers, but it differs by its stonoliferous habit, persistent basal leaves and basal stipules, and smaller flowers with a campanulate-elongate hypanthium and single carpel. A key to the species of *Lachemilla* in Mexico is provided.

Resumen

Una nueva especie de *Lachemilla* (Rosaceae), *Lachemilla mexiquense* D.F. Morales-B., de México se describe e ilustra. Esta especie es similar a *Lachemilla aphanoides* por sus hojas tripartitas e inflorescencias glomeruladas con flores completamente glabras, pero difiere por su hábito estolonífero, hojas basales y estipulas basales persistentes y flores de menor tamaño con hipantio campanulado-elongado con un solo carpelo. Se provee una clave para las especies de *Lachemilla* en México.

Keywords

Lachemilla mexiquense, *Lachemilla*, Rosaceae, Mexico, new species