

**An Intrageneric and Intraspecific Study of Morphological and Genetic
Variation in the Neotropical *Compsonaura* and *Viola* (Myristicaceae)**

by

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ABSTRACT

AN INTRAGENERIC AND INTRASPECIFIC STUDY OF MORPHOLOGICAL AND GENETIC VARIATION IN THE NEOTROPICAL *COMPSONEURA* AND *VIOLA* (MYRISTICACEAE)

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The Myristicaceae, or nutmeg family, consists of 21 genera and about 500 species of dioecious canopy to sub canopy trees that are distributed worldwide in tropical rainforests. The Myristicaceae are of considerable ecological and ethnobotanical significance as they are important food for many animals and are harvested by humans for timber, spices, dart/arrow poison, medicine, and a hallucinogenic snuff employed in medico-religious ceremonies.

Despite the importance of the Myristicaceae throughout the wet tropics, our taxonomic knowledge of these trees is primarily based on the last revision of the five neotropical genera completed in 1937. The objective of this thesis was to perform a molecular and morphological study of the neotropical genera *Compsonaura* and *Viola*. To this end, I generated phylogenetic hypotheses, surveyed morphological and genetic

diversity of focal species, and tested the ability of DNA barcodes to distinguish species of wild nutmegs.

Morphological and molecular analyses of *Compsoeura*. indicate a deep divergence between two monophyletic clades corresponding to informal sections *Hadrocarpa* and *Compsoeura*. Although 23 loci were tested for DNA variability, only the trnH-psbA intergenic spacer contained enough variation to delimit 11 of 13 species sequenced. A morphological and molecular investigation of *Compsoeura capitellata* showed little discrete morphological variation among populations but significant genetic structure among populations.

Phylogenetic analysis of *Virola* also revealed a deep molecular divergence between two clades having numerous contrasting morphologies. In contrast to *Compsoeura*, the trnH-psbA intergenic spacer failed to differentiate the majority of *Virola* species tested. An infraspecific morphological and molecular study of *V. sebifera* and *V. lorentensis* showed that each of these species contains morphologically and ecologically discrete sympatric morphotypes that likely represent new species.

In total, this investigation found 5 provisional new species from fewer than 600 collections at biological stations in Ecuador and Peru where these new species were among the most abundant trees in the forest. This suggests that much diversity likely remains to be described in the Myristicaceae and other tropical plant families.

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GENERAL INTRODUCTION

Taxonomy

Ethnotaxonomy

It is likely that the first profession was that of the shaman; the healer, guide to the realm of spirits and maker of medicines. The need for this profession was presumably born of the inherent difficulty and serious nature of plant identification. The shaman may have varying roles in numerous cultures but in most embodiments they are the authority on the use and implementation of the medicinal and religious application of plants. In many cultures these tasks are regarded with such great importance that individuals within traditional cultures may be relieved of the tasks of everyday life from a young age to devote their whole lives to the practice of identifying and implementing plants in medico-religious applications. The act and practice of naming and differentiating species, variants, or cultivars of plants often holds great cultural importance to traditional peoples likely because it has developed from the need to identify the nutritious and intoxicating plants available to them.

Humans have an inherent ability to recognize and classify the diversity of life around them (Newmaster et al. 2006, Diamond and Bishop 1999). Although many species of animals distinguish edible and inedible species from one another based on senses and/or innate aversions to particular foodstuffs, language and tradition have afforded humans the ability to pass on this knowledge to subsequent generations. A unifying trait among human cultures is the development of systems to name and describe the biodiversity in their realm. Although vernacular names may be different, it is interesting that a population of individuals with similar phenotypes is often recognized as

a 'species' both in aboriginal cultures and the scientific, or Linnaean, system and aboriginal classifications sometimes recognize more 'species' than current scientific nomenclature (Diamond and Bishop 1999).

Taxonomy and Species

Taxonomy is the scientific practice of classifying, identifying, and describing organisms. Although taxonomists are concerned with all organismal levels of classification, but of particular importance is the determination, discovery and description of species. Traditionally, plant taxonomists have used a wide range of characters to help them classify earth's botanical diversity. Until relatively recently, botanists were largely restricted to studies of gross morphology, anatomy and inter-fertility to classify diversity. Additional characters became available with the use of secondary metabolite analysis and with the advent of chromosome staining botanists have been able to determine and consider chromosome number as a taxonomic character . More recently flow cytometry, allozyme analysis, and DNA-DNA hybridization gave taxonomists more sensitive means of delimiting taxa. However, it was the advent of the polymerase chain reaction (PCR) by Kary Mullis (Mullis and Faloona 1987) that enabled the determination of DNA sequences in a quick, accurate and inexpensive manner, that has revolutionized the field of systematics as we know it today. The additional information afforded by DNA sequence analysis has not only given taxonomists a more characters to help classify taxa, but has allowed systematists to estimate their evolutionary histories with data that is independent of morphology.

There exists well over 20 species concepts in use today however, many biologists will agree that species are lineages of separately evolving populations (de Quieroz 2005).

Species concepts with respect to plants have troubled botanists as phenotypic plasticity, polyploidy, hybridization, and differing methods of reproduction and genetic inheritance makes the standardization of criteria for species recognition difficult and have led some to suggest that plant species do not exist as real entities like animal species (Levin 1979, Bachmann 1998). In spite of these assertions, a recent analysis of crossing data in more than 400 plant and animal genera found that 70% of plant species correspond to reproductively isolated lineages, compared to only 39% of animal species tested (Rieseberg et al. 2006). Although hybridization is known to occur frequently in plants (Golden and Bain 2000, Palme et al. 2003), and is expected to be an important in the generation of new species (Whitney et al 2010), there is emerging evidence that reproductive isolation, genetic and morphological integrity can be maintained in the face of hybridization (Palma-Silva et al. 2011). In a recent study of four morphologically distinct sympatric neotropical Bromeliads Palma-Silva and colleagues (2011) found that 7 haplotypes were shared among species, however, nuclear microsatellite markers showed significant differentiation between species. This differential permeability of chloroplast and nuclear genomes between freely hybridizing species is of prime interest to plant taxonomists as it demonstrates that any given species can largely maintain its nuclear genetic integrity, and therefore morphological integrity despite the capture, or introgression, or another species' chloroplast haplotype. Plant systematists must consider these data as it may result in strong chloroplast versus nuclear gene tree incongruence (Rieseberg and Soltis 1991), and there exists the strong possibility that different loci of the nuclear genome (such as neutral versus non-neutral loci) may exhibit differential permeability between species (i.e. asymmetrical introgression).

Species are the “measuring-stick” with which biologists measure biodiversity. Virtually all major fields of biology rely upon accurate species identification in order to test scientific hypotheses. Therefore, the power of biological inference relies largely upon the accuracy of the measuring stick used, as well as the skill with which it is applied. However, many taxa lack a modern systematic revision, many areas of the world are uncollected, many investigators lack the taxonomic expertise necessary to identify species of interest and most of the world biodiversity has yet to be described (Mora et al 2010). Additionally, taxonomic experts, if there are any for a particular group, are sometimes too occupied with their own studies to perform routine identifications for others. Also, barriers such as language, funding constraints, and manuscript availability may further hinder access to taxonomic information. The specimens themselves may be difficult to identify due to a lack of distinguishing features (such as fertile structures), specimen age, quality and quantity of material. This combination of factors often leads to numerous errors in the course of many investigations where the taxonomy of a group of species is not well studied. Despite its fundamental importance to biological sciences, the field of taxonomy has been in decline in recent years, with a reduction in both the number of investigators as well as financial support (Godfray 2002).

DNA Barcoding

A potential improvement for some of these problems is the implementation of DNA-based identification systems such as DNA barcoding. DNA barcoding is the use of one or several standardized genomic regions as the basis for identification of a species (Hebert et al. 2003a,b). The mitochondrial coding gene cytochrome oxidase I has proven useful in numerous animal groups and algae (Hebert et al. 2004, Hebert et al. 2006,

Saunders 2005) and various barcoding campaigns are quickly building sequence libraries for the world's flora and fauna (iBOL 2010). Once DNA region(s) suitable for delimitation of species has been found, agreed upon, and a sequence library has been compiled, DNA-based identification systems can be potentially revolutionary for species identification since assignments can be performed on specimens which may be degraded, lacking critical morphological characters, or from small or otherwise unidentifiable pieces of tissue. The ability to identify species from material lacking morphologically differentiating characters represents a drastic transformation in the types of ecological questions that can be addressed. Additional benefits are that it is rapid, largely automatable, accessible to anyone with access to a thermocycler and sequencing equipment (although this could potentially be miniaturized for public use). This automation may ultimately relieve taxonomic experts of the task of routine identifications, giving them more time to pursue questions of more theoretical nature.

Progress towards a universal plant DNA barcode has been largely impeded by the comparatively slow and heterogeneous rate of plastid nucleotide mutation in plants, the desire for universal primers, poor sequence quality in the most discriminating plastid loci (Chase et al. 2007, Fazekas et al. 2009), and the low rates of species discrimination of a single locus (Hollingsworth et al. 2009). Recently, plant taxonomists have proposed the multi-locus combination of plastid coding regions *matK* and *rbcL* as the proposed loci for a global campaign to barcode land plants (Hollingsworth et al. 2009). These more conserved loci could serve as core regions to identify samples to higher ranks (family and genus) while more variable markers (plastid spacers, nrDNA, and low copy nuclear loci)

will be needed for the identification of some taxa. DNA barcoding will undoubtedly be more difficult in plants as they are prone to interspecific hybridization, allopolyploidy, introgression, and many species are of relatively recent origin. The asymmetrical introgression of one species' chloroplast genome into another species, also known as chloroplast capture, is well documented by phylogenetic, phylogeographic, and population genetic literature (Rieseberg and Soltis 1991, Golden and Bain 2000, Palme et al. 2003, Palma-Silva et al 2011) and it is expected that this phenomena will confound cpDNA-based species delimitation (such as plant DNA barcoding) even where species limits are rather clear (Hollingsworth et al. 2011).

Phenetic versus Cladistic Analyses in Systematics

Phenetics is method of grouping taxa based upon the amount of similarity or difference, regardless of phylogeny. Phenetic approaches are generally based on molecular or morphological data and seek to group taxa according to overall similarity. When nucleotide sequence data is employed in phenetic approaches the nucleotide differences among two sequences are converted to a numerical value. Then a matrix of these pair-wise values is generated from the nucleotide sequence alignment and a phenetic tree is constructed to group sequences based on similarity.

Cladistic methodologies seek to construct relationships of taxa based on shared derived characters, or synapomorphies. Cladistic methods have largely replaced phenetic means of estimating ancestor-descendant relationships as phenetics is based on summary-statistics (overall similarity), which is widely regarded as inferior to character based cladistic methods of phylogeny estimation (Steel et al. 1988, Farris et al. 1996, Murphy

and Doyle 1998). Cladistic methods on the other hand generally require much more time (hours or days) and often recovers numerous equally probable trees.

This thesis will investigate two main questions with respect to *Compsoeura* and *Viola*: 1) What are the sister-species relationships within these genera and do these clades agree with past sub-generic taxonomic groupings? and 2) Do cryptic species exist in these genera and can DNA loci be used to discriminate species of these trees that are exceedingly difficult to identify? Separate molecular analyses will be used to help answer each of these questions. Bayesian and parsimony based cladistic analyses will be used to estimate evolutionary relationships among taxa (*Compsoeura*=Chapter 1, *Viola*=Chapter 3). Although the use of phenetic analyses with DNA barcoding data has been criticized by some taxonomists (Will and Rubinoff 2004), phenetic methods will be used to assess the ability of highly variable cpDNA loci to discriminate species of these two genera as I am concerned primarily with taxon distinctiveness rather than the reconstruction of sister group relationships in such analyses.

A benefit of analyzing large volumes of sequence data with phenetic methods is that many samples (hundreds if not thousands) can be included from within populations as well as across a species' range in order to survey for undetected speciation. Such dense taxon sampling patterns have revealed potential cryptic speciation events in butterflies, bats, fish, flies, algae, wasps, and amphipods that had gone unnoticed by traditional taxonomic characters (Hebert et al. 2004, Saunders 2005, Witt et al. 2006, Clare et al. 2007, Smith et al. 2007, Hubert et al. 2008, Smith et al. 2008). It is important to note, however, that many of these investigations complemented their molecular characters with additional natural history data such as plant or insect-host-specificity, and geography

(Hebert et al. 2004, Smith et al. 2004, Saunders 2005, Smith et al. 2008). These DNA barcoding studies, as well as population genetic, phylogeography and systemic studies are discovering units that may represent undescribed species faster than these units can be further tested and described. Padial and colleagues (2010) have proposed multiple protocols for recognizing and testing candidate species with integrative data from multiple character sets (molecular, morphology, geography, ecology, etc.) so that these putative taxa can be recognized in some form until they can be fully described. It appears that the sequence data generated by DNA barcoding efforts will only serve to aid systematist in identifying molecular operational taxonomic units (MOTU: as discussed by Blaxter et al. 2005) that can then be treated as candidate species hypotheses to be tested with integrative approaches.

Throughout their evolution as hunter-gatherers, humans have sustained themselves by being able to recognize the biotic elements that can nourish and heal us. Agriculture, a buttress and hallmark of large sedentary societies, has selected choice genetic variants as the basis of improvement for domesticated plants and animals. Without genetic variation such selection is impossible. Therefore, genetic diversity is critical to the welfare of human societies. In our continued search for biological treasures to improve the human condition we will need increasingly sophisticated means to survey the diversity surrounding us. After about 200 years of Linnaean taxonomic pursuits there exists about 1.2 million described species and an estimated 7 million more yet to be discovered, described, and classified (Mora et al. 2011), it can only be hoped that genetic systems of identification will expedite the taxonomic process so that we can more intelligently manage our organismal resources.

Human and geologic history of Central and South America

The neotropical region (the landmass situated between the tropics of Cancer and Capricorn in Central and South America) is home to an estimated 90,000 plant species (Raven 1976, Prance 1977, Thomas 1999), which represents a significant proportion of the world's estimated 235,000-420,000 seed plant species (Govaerts 2003, Scotland and Wortley 2003). Permanent plots and transects conducted in the Amazon basin have routinely found 200 to 300 species per hectare, making the Amazon the world's most diverse forest in terms of tree species richness (Gentry 1988, Pitman et al. 2002).

There are a number of noteworthy geological events that have had a significant effect on the evolution and composition of the neotropical flora. Approximately 130 million years before present (mybp) the African and South American continents began to separate, with complete separation occurring about 80-90 mybp (Raven and Axelrod 1974). The continents of Africa and South America continued to separate during the late Cretaceous (99-65 mybp) while volcanic islands formed between North and South America (Raven and Axelrod 1974). The uplift of the Central American landmass continued into the Miocene (23-5 mybp) culminating in the physical connection of the North and South America an estimated 3 mybp (Keigwin 1978, Marshall 1982). The Andean orogeny began its uplift around 23 mybp but the most intense activity occurred from the middle Miocene (12 mybp) to the early Pliocene (Hoorn et al. 2010). This uplift created the worlds longest current tropical mountain range as well as a large network of lakes and swamps that existed from about 25-10 mybp when the East-West drainage of

the continent was impeded by the forming mountains (Pons and Franceschi 2007, Hoorn et al 2010).

The uplift of the Andean orogeny has likely been a key factor in the generation of neotropical floristic diversity. Gentry (1982, 1988) hypothesized that the relatively recent uplift of the Andean cordillera caused explosive speciation in herbaceous plants and is responsible for the exceedingly high diversity of plant species in the neotropics. Rapid speciation has also been found in tree genera as a molecular analysis of the genus *Inga* found very low level of nucleotide substitutions among species despite morphological differentiation, suggesting recent and rapid speciation in the genus (Richardson et al. 2001). Contemporary analyses that have integrated molecular phylogenetic and geologic data appears to indicate the uplift of the Andes was crucial to the diversification of the neotropical flora primarily through the generation of topographic features, fertile and heterogeneous edaphic conditions, as well as varying precipitation regimes (Hoorn et al. 2010).

The diverse biota of South America has also been impacted in past and present times by its human inhabitants. The first humans are believed to have arrived in South America 12,000-15,000 years ago and they are thought to have contributed to the extinction of numerous large bodied vertebrates through hunting (Janzen 1983). The extinction of these large herbivores likely led to the extinction, or rarefaction, of many species of plants dependent on these megafauna for dispersal and/or germination of their seeds (Janzen and Martin 1981).

Shortly after they arrived in the Neotropics, humans made use of the rich flora around them by domesticating numerous species, making South and Central America an

independent origin of agriculture (Olsen and Schaal 1999, Smith 2006). When Gaspar de Carvajal sailed down the Amazon from 1541-1542 he wrote of large settlements in the region of modern day Manaus, Brazil where some communities stretched uninterrupted for 25 km. However, after studying cultures living at the periphery of the Northwest Amazon basin, anthropologists largely concluded that all pre-Colombian societies were small hunter-gatherer groups clinging to existence and sparsely scattered across the Amazon (Meggers 1971, Hames and Vickers 1982). This was largely based on the notion that great societies, such as those described by Carvajal, required large-scale agriculture that was regarded as impossible on the poor soils of the region. Relatively recently however, scientists came to realize that rich black soils, called terra preta (or black earth), scattered across the Amazon were of human origin (Smith 1980, Glaser et al. 2001), These soils appear to have been created by the generation and application of charcoal to highly oxidized, and therefore poor, soils. It appears that this soil amelioration technique was employed across the Amazon watershed as deposits of terra preta soils have been found from the rivers source to the sea and up many of its tributaries to the North and South (Glaser 2007). The application of charcoal to these soils appears to change the soil microbe community (Grossman et al. 2010) and increase the cation exchange capacity, and therefore nitrogen retention of the soils, leading to improved crop yields (Lehmann et al. 2005, Chan et al. 2007). Increased yields from these soils might have enabled the persistence of large societies (perhaps millions of people) such as those documented by Carvajal (Hackenberger 1999). Assessments of species richness done by Junquiera et al. (2010) found that domesticated and semi-domesticated woody plant diversity is higher on terra preta soils compared to adjacent non-anthropogenic soils. This could mean that the

very composition of the Amazon forest was changed by these societies and that the Amazon rainforest is not the wild “forest primeval” envisioned by many people.

Myristicaceae

The nutmeg family, or Myristicaceae, is composed of sub-canopy to canopy evergreen trees found in virtually any tropical rainforest of the world. The family is currently represented taxonomically by 21 genera and about 500 species (Janovec 2000). The Myristicaceae are ecologically important as species in this family are abundant in tropical rainforests and the family as a whole is often among the top 10 most abundant tree families in these forests (Gentry 1988, Pitman et al. 2001, Pitman et al. 2002).

General Morphology and Cytology

Members of the Myristicaceae have numerous morphological traits that make them easy to identify to the family level in the field. Most species have brown-red bark that is smooth and flakes off in thin slivers or large plates. Perhaps the most striking feature of the family is their characteristic branching pattern whereby the mostly horizontal branches radiate in whorls from the trunk. All leaves of the Myristicaceae are simple, entire, 2 ranked and alternately arranged on branches. Leaf veins are pinnate and free to anastomosing or brochidodromous. Leaf blades may be densely pubescent or totally glabrous. Members of the Myristicaceae are dioecious with the exception of a few monoecious members of the neotropical genus *Iryanthera*. Flowers are unisexual, inconspicuous, small in size (< 4 mm), composed of three sepals (rarely 4) that are fused to varying degrees, and exude a strong fragrance (Smith 1937, Armstrong 1997, Janovec

2000). Female flowers are uni-carpellate and uni-ovulate and they show very little morphological differentiation within the family. Male flowers are composed of 2-60 anthers which are fused to various degrees to a central column. This fused androecium is often referred to as a synandrium and has been of key interest to systematic studies of the family owing to the largely amorphous nature of the leaves and gynoecium (Smith 1937, Warburg 1987, Janovec 2000, Sauquet et al. 2003b). Flowers are borne on racemose or thrysoidal paniculate inflorescences, which themselves originate within leaf axils (Wilde 1991). Once fertilized, the unilocular ovary develops into an elliptical fruit called a follicle. The fruit is comprised of a single seed, typically with a ruminate endosperm, covered to various degrees by a fatty white-reddish aril. The arillate seed is encapsulated by a pericarp which dehisces along a single suture in most species at maturity to present the seed to animal dispersers.

Cytological investigations in the family Myristicaceae have largely been restricted to members of *Myristica*. The amount of DNA in an unreplicated haploid nucleus of plants ranges approximately 1000-fold, from 0.1 pg to approximately 125 pg (Bennett et al. 2000). This haploid nuclear complement of DNA, also known as the C-Value, has only been determined for *Myristica dactyloides* and *Myristica fragrans*, which were estimated to contain 1.4 pg and 1.2 pg (or 1,372 and 1176 Mbp) respectively in a haploid complement of their 24 chromosomes (Bennet et al. 2000). Chromosome counts in other taxa have revealed haploid chromosome numbers of 19, 21, 25 and 26 (Mehra and Bawa 1969, Raven 1975). The chromosomes of Myristicaceae are holocentric (Flach 1966), meaning that they do not possess a localized centromere, and consequently kinetochore activity is distributed over the length of the chromosome. Holocentric chromosomes are

rather unusual among angiosperms and have been reported in few families including Cyperaceae, Juncaceae, Melanthiaceae, Cuscutaceae, (Pazy and Plitman 1994, Luceno et al. 1998, Nagaki et al 2005, Hipp et al. 2009). A high degree of variation of in chromosome arrangement in species of *Carex*, which is afforded by holocentric chromosomes, has led to speculation that chromosome rearrangements may have played a significant role in the generation of the extraordinary number of species in *Carex* (Hipp et al. 2009). An additional chromosomal curiosity is that *Myristica fragrans* is one of relatively few angiosperms that have been found to have heterogametic sex chromosomes (Ganeshiah et al. 2000).

Ecology

Wild nutmeg trees are entirely dependent upon animals for both the pollination of their flowers and dispersal of their seeds. The flowers emit a strong sweet fragrance and individual flowers are open for only a few days (Armstrong and Irvine 1989a). Members of the nutmeg family are believed to be pollinated by small generalist pollinators such as beetles (Coleoptera), thrips (Thysanoptera), and flies (Diptera) that consume pollen (Armstrong and Drummond 1986, Armstrong and Irvine 1989b, Armstrong 1997). However, there has yet to be a comprehensive study of pollination agents of the family. Since the female flowers offer no apparent reward for pollinators, it is believed they attract pollinators by mimicking the fragrance of male flowers (Armstrong 1997). The seeds of Myristicaceae are highly prized by birds and mammals for their aril, or seed covering, which is particularly rich in energy-rich fats (Howe 1981). Seed-set in some species may be low; a study by Bullock (1982) found that most female trees of *Compsonoura sprucei* produce less than 10 fruits per fruiting cycle. The nutritious aril

and diurnal opening of the fruit is believed to encourage consumption of seeds by avian and mammalian dispersal vectors. Numerous species of birds such as trogons (*Trogon massena*), motmots (*Baryphthengus martii*), toucans (*Ramphastos sulfuratus*, *R. swainsonii*), and guans (*Penelope purpurascens*) consume the fruit but typically regurgitate or expel the seeds only minutes after consuming the nutritious aril (Howe and Vande Kerckhove 1981, Howe *et al.* 1985). However, spider monkeys (*Ateles fusciceps*) may act as one of the most important long distance dispersers of nutmeg seeds as they consume the entire seed-aril unit and the seed is excreted intact many hours later (Russo 2003). Additional studies of seed dispersal in *Virola flexuosa* have indicated that human hunting can have an impact on seed removal as nearly 90% of seeds were found to be removed from parent trees in unhunted plots versus only 67% removal in hunted areas (Holbrook and Voiselle 2009). Seed dispersal might be particularly important as Howe *et al.* (1985) found that 99.2% of *Virola surinamensis* seeds that fell within 45 m of the parent tree succumbed to predation but those which were dispersed to greater distances experienced a 44-fold survival advantage. Additional ecological studies are needed to ascertain whether bats or other large terrestrial mammals are significant dispersers of various species of nutmeg seeds. It is still unknown whether nutmeg trees are diphasic, exactly which species pollinate their flowers, and what effects pollinators and dispersers have on the genetic structure of populations.

Ethnobotany

In addition to their ecological importance, the Myristicaceae are also significant ethnobotanically. The hollowed branches of the neotropical nutmeg genus *Otoba* are used

to construct blowpipes used in hunting (Schultes and Raffauf 1990). The blood red latex of *Virola spp.* is used as part of the mixture for curare; an arrow and dart poison for immobilizing animals (Macrae and Towers 1984). *Virola* latex is also the primary source of tryptamine alkaloids included in yakee or ebena snuff which is employed widely across the Amazon by natives to produce powerful visions for shamanic, religious, and sometimes recreational purposes (Macrae and Towers 1984a and b, Macrae and Towers 1985, Schultes and Raffauf 1990, McKenna et al. 1984). Various preparations of the sap, leaves and bark are used to treat malaria, diarrhea, fungal skin infections, arthritis, and hemorrhoids (Schultes and Raffauf 1990, Lopes et al. 1999). Members of the genus *Virola* are harvested extensively in many South American countries as a source of wood for veneer and timber. In some neotropical countries, exports of *Virola spp.* are rivaled in economic importance only by big-leaf mahogany [*Swietenia macrophylla*, Meliaceae] (Macedo and Anderson 1993).

The best known member of the Myristicaceae is the spice bearing species *Myristica fragrans*, the common nutmeg tree. Although believed to have been domesticated only in the last 500 years, *M. fragrans*, has been employed ethnobotanically wherever it has been spread by humans in the world. *Myristica fragrans* is native to a small area known as the Banda Islands of the Maluku Province of modern day Indonesia. The nutmeg tree has been dispersed far beyond its endemic range in the Banda Islands to a pantropical range by people since the late 18th century (Hanna 1978). The nutmeg tree bears a fruit that dehisces when ripe to reveal a single seed covered by a bright red aril, which are known commercially as nutmeg and mace respectively. The primary use of *M. fragrans* is as a flavoring in cooking but many cultures around the world have found it

useful for a myriad of purposes. In its native range the pericarps of nutmeg are made into a sweet snack called 'pala manis' or 'pala gulu' by repeated soaking in a sugar solution (Gils and Cox 1994). Indigenous Malukans also topically apply nutmeg oil, which gives a warm strengthening feeling, to relieve headaches, stomach aches, diarrhea and flu symptoms (Gils and Cox 1994). Both nutmeg and mace have been used as an aphrodisiac, anti-rheumatoid, anti-malarial, stimulant, and post childbirth tonic (Burkill 1935).

Myristicaceae Systematics

Nutmeg trees made their first appearance in the scientific literature in 1742 when Linnaeus first described the genus *Myristica*. Myristicaceae was established in 1810 by Brown, but the first thorough monographic treatment of the Myristicaceae was not completed until 1856 by De Candolle (cited from Janovec 2000). De Candolle recognized about 90 species worldwide and organized them into 13 sections of the single genus *Myristica*. Bentham and Hooker (1880) retained the monogeneric concept for Myristicaceae and condensed the 13 sections of De Candolle into 7 sections. The most comprehensive work of the pantropical Myristicaceae was done by Warburg (1897, cited from Janovec 2000). Warburg (1897) recognized 240 species organized in 15 genera, the majority of which are still recognized. The last comprehensive treatment of the neotropical Myristicaceae was done by Smith (1937) who recognized the genera *Virola*, *Compsonaura* (split into sections *Coniostele* and *Compsonaura* based on degrees of anther fusion), *Osteophloeum*, *Iryanthera*, and *Dialyanthera* (currently synonymous with *Otoba*), all of which are endemic to South and Central America. Since Smith's (1937)

work, further collections of the Myristicaceae have led to descriptions of both new species and genera (Rodrigues 1989a and b, Janovec and Harrison 2002, Janovec 2002, Janovec and Neill 2002, Sauquet 2003, Sauquet 2004, de Wilde 1991). Taxonomic works on the neotropical Myristicaceae since 1937 have been largely limited to descriptions of new species. The most recent taxonomic work concerning neotropical nutmegs, a monograph of *Compsonaura*, by Janovec (2000) nearly doubled the number of species in the genus and divided the genus into informal sections *Compsonaura* and *Hadrocarpa*, the latter of which was further subdivided into informal sub-groups *Atopa* and *Capitellata*.

Myristicaceae are members of the eumagnoliid clade which are among the most primitive of extant angiosperms (Soltis et al. 2000, APG III). Although initial molecular analyses placed Myristicaceae at the base of order Magnoliales making it sister to all remaining families of the order (Soltis et al. 2000, Sauquet et al. 2003), the most recent phylogenetic analysis to date found Magnoliaceae to be sister to all other families including Myristicaceae (Soltis et al. 2011). Molecular phylogenetic analysis completed on the family by Sauquet et al. (2003) and Doyle et al. (2004) found insufficient molecular divergence within the Myristicaceae to make strong phylogenetic inferences of generic relationships. Despite this very weak consensus of intergeneric relationships within the Myristicaceae, there was some support among numerous loci for the placement of the neotropical genus *Compsonaura* as the sister to the rest of the family (Doyle et al. 2004, Sauquet et al. 2003).

Biogeography of the Myristicaceae

Very little is known about the phylogeographic and biogeographic history of the Myristicaceae despite being an ancient group of trees with a pantropical distribution. This paucity of information is largely due to the fact that there is very poor representation of the family in the fossil record compared to other angiosperm families (Doyle et al. 2004). The earliest fossil evidence of the Myristicaceae is from a fossilized wood sample of *Myristicoxylon princeps* from the Paleocene of the African Sahara region. It is unclear, however, as to whether this specimen belongs to a stem or crown group of the family (Boureau 1950). Doyle et al. (2008) document the identification of a Myristicaceous seed from England's London Clay which dates to the Early Eocene (~56-34mya) which is markedly older than the only seed found previously from Miocene (23-5mya) deposits of Germany (Gregor 1977). Additional fossils of seeds (Berry 1929), leaves (Wolfe 1977), flowers (Poinar and Poinar 1999), and pollen (Frederikson 1973, Jan du Chene et al. 1978) of likely Myristicaceae origin have been found but they cannot be reliably distinguished from morphologically similar families (Doyle et al. 2004). The rarity of nutmeg fossils prior to the Paleocene corroborates the molecular clock estimate of Doyle et al. (2004) predicting that the Myristicaceae crown group diversified in the last 15-20 million years. Such a recent diversification is rather surprising as the same markers estimated that the closely related Annonaceae radiated approximately 100-120mya, which correlated well with their rich fossil record (Doyle et al. 2004). However, this relatively young estimation of Myristicaceae radiation could be an artifact of poor fossil representation of the family and/or a marked decrease in the rate of molecular evolution compared to closely related families, leading to a mis-calibration of the molecular clock.

Therefore it is unclear as to whether this low level of molecular divergence is truly the result of a relatively recent diversification in the family or a marked slowdown in molecular evolution resulting in the conservation of DNA sequences. Given the Myristicaceae's pantropical distribution, basal position on the flowering plant phylogeny, and poor long distance dispersal ability, it is unlikely that they radiated in the last 20 million years when the continents were almost in the same positions as today. This leaves us with little knowledge of the evolutionary and phylogeographic patterns of one of the most abundant and diverse plant families of tropical rain forests.

Challenges to Molecular work with the Myristicaceae

A diverse assembly of metabolites produced in plant cells often co-purify during DNA isolation procedures leading to the degradation of genomic DNA and/or the inhibition of PCR. These inhibitors often include but are not restricted to phenols, tannins, polysaccharides, proteins, alkaloids, lignans, flavonoids, and RNA. The Myristicaceae, like many other plants, contain copious amounts of secondary metabolites that are troublesome for molecular investigations. The most notorious are perhaps flavans called myristinins that are found in numerous species of nutmegs and are the most potent inhibitors known of β DNA polymerases and have the added action of autolytic cleavage of dsDNA (Sawadjoon et al. 2002, Deng et al. 2005, Maloney et al. 2005). Additionally, numerous other flavonoids have been isolated and characterized from various genera of the Myristicaceae (Braz Filho et al. 1973, Gottlieb et al. 1973, Gottlieb et al. 1976) which include phenols and tannins, which are known inhibitors of PCR (Wilson 1999, Krause et al. 2001). The inhibitory effects of these contaminating chemicals may be minimized by

the inclusion of chemicals such as Polyvinylpyrillidone, phenacylthiazolium bromide, and sodium sulfite in lysis buffers during DNA extraction (Ivanova et al. 2008, Poinar et al. 1998, Whitlock et al. 2008). If inhibitory chemicals remain in purified DNA extracts then the inclusion of PCR facilitating chemicals such as trehalose, PVP, tween 20, PEG, and BSA may be necessary to achieve amplification of DNA sequences (Pierpont 1969, Sasaki et al. 1998, Horne et al. 2004, Speiss et al. 2004, Barabwal et al. 2003,). Thus, overcoming the challenges associated with nucleic acid manipulation in the Myristicaceae may contribute to enhancing molecular protocols for DNA extraction, amplification, and sequencing vital to studying many species of plants.

Why work with the Myristicaceae?

Biologists depend largely on morphological taxonomic descriptions to identify the species encountered in their studies. Examinations of the composition and diversity of tropical rainforests have been the focus of many contemporary tropical plant ecologists, however, they are faced with a flora that is exceedingly diverse and difficult to identify. Previous investigations of the Myristicaceae have revealed that the mis-identification rate of Myristicaceae species typically exceeds 50% in herbarium samples and 25% in ecological plots (J. Janovec personal communication, Steeves personal observations). A DNA based identification system would aid tropical plant identification, however there have been few tests of DNA barcoding regions in a diverse group with many pairs of morphologically similar and recently evolved sister species (Gao et al. 2009, Gonzales et al. 2009, Spooner et al. 2009). The Myristicaceae, and *Compsonera* and *Virola* in particular, provide a rigorous test of DNA-based identification for plants as they are

diverse, morphologically difficult to identify, contain many sister species, and the family is known to have exceedingly slow rates of molecular evolution (Sauquet et al. 2003).

The primary goal of this thesis was to explore morphological and molecular diversity among and within species of *Virola* and *Compsonaura*; two genera of the neotropical Myristicaceae endemic to Central and South America. This was accomplished by surveying DNA sequence variation of many species within each of these genera and by selecting species complexes from each genus to provide an assessment of genetic variation within these focal species. The objective of the generic level investigations was to generate phylogenetic estimates of relationships of the respective species in hopes of elucidating general trends of morphological evolution, biogeography, and illuminating cases of high genetic variability that may indicate cryptic and/or incipient speciation events. The main objective of surveying DNA sequence variability within the selected species was to see if the high morphological variability exhibited by these taxa was the result of phenotypic plasticity, cryptic/incipient speciation, or infraspecific genetic variation. This thesis reveals that there are many species yet to be described in one of the most common tree families of the neotropics and that the taxonomic understanding of the Amazon and other tropical forests is arguably in its infancy. If we are to attempt to understand this botanically diverse region from an ecological, evolutionary, biogeographical, or any other biological standpoint we must first finely dissect its most fundamental units: the species of which it is composed.

Literature cited

- Armstrong, J.E., & Drummond, B.A. 1986. Floral biology of *Myristica fragrans* Houtt. (Myristicaceae), the nutmeg of commerce. *Biotropica* **18**: 32-38.
- Armstrong, J. E., & Irvine, A. K. 1989a. Floral biology of *Myristica insipida* (Myristicaceae), a distinctive beetle pollination syndrome. *American Journal of Botany* **76**: 86–94.
- Armstrong, J.E. & Irvine, A.K. 1989b. Flowering, sex ratios, pollen-ovule ratios, fruit set, and reproductive effort of a dioecious tree, *Myristica insipida* (Myristicaceae), in two different rainforest communities. *American Journal of Botany* **76**: 74-85.
- Armstrong, J. E. 1997. Pollination by deceit in nutmeg (*Myristica insipida*, Myristicaceae): floral displays and beetle activity at male and female trees. *American journal of botany* **84**: 1266-1274.
- Bachmann, K. 1998. Species as units of diversity: an outdated concept. *Theory Bioscience* **117**: 213-230.
- Bennett, M.D., Bhandol, P., & Leitch, I. 2000. Nuclear DNA amounts in Angiosperms and their modern uses-807 new estimates. *Annals of Botany* **86**: 859-909.
- Bentham, G., & Hooker, J.D. 1880. *Genera plantarum*, London: Reeve and Co.
- Berry, E. 1929. Early Tertiary fruits and seeds from Belen, Peru. *Johns Hopkins University Studies in Geology* **10**: 137-180.
- Blaxter, M. L. 2004. The promise of a DNA taxonomy. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **359**: 669.
- Boureau, E. 1950. Étude paléoxylologique du Sahara (IX). Sur un Myristicoxylon princeps n. gen., n. sp., du Danién d'Asselar (Sahara soudanais). *Bulletin du Muséum National d'Histoire Naturelle* **2**: 523-528.
- Braz Filho, R., Frota Leite, M., & Gottlieb, O. 1973. Constitutions of diarylpropanoids from *Virola multinervia*. *Phytochemistry* **12**: 417-419.
- Bullock, S. H. 1982. Population structure and reproduction in the neotropical dioecious tree *Compsonaura sprucei*. *Oecologia* **55**: 238–242.
- Chan, K. Y., Van Zwieten, L., Meszaros, I., Downie, A., & Joseph, S. 2008. Using poultry litter biochars as soil amendments. *Australian Journal of Soil Research* **46**: 437–444.

- Clare, E. L., Lim, B. K., Engstrom, M. D., Eger, J. L., & Hebert, P. D. 2007. DNA barcoding of Neotropical bats: species identification and discovery within Guyana. *Molecular Ecology Notes* **7**: 184–190.
- Cole, R. J. 2009. Postdispersal seed fate of tropical montane trees in an agricultural landscape, Southern Costa Rica. *Biotropica* **41**: 319–327.
- Deng, J., Starck, S. R., Li, S., & Hecht, S. M. 2005. (+)-Myristinins A and D from *Knema elegans*, which Inhibit DNA Polymerase β and Cleave DNA. *Journal of Natural Products* **68**: 1625-1628.
- de Queiroz, K. 2005. A unified concept of species and its consequences for the future of taxonomy. *Proceedings of the California Academy of Sciences* **56**: 196.
- Diamond, J., & Bishop, K. D. 1999. Ethno-ornithology of the Ketengban people, Indonesian New Guinea. In *Folkbiology*. MIT Press (pp. 17-45).
- Doyle, J. A., Manchester, S. R., & Sauquet, H. 2008. A seed related to Myristicaceae in the early Eocene of Southern England. *Systematic Botany* **33**: 636–646.
- Doyle, J. A., Sauquet, H., Scharaschkin, T., & Le Thomas, A. 2004. Phylogeny, molecular and fossil dating, and biogeographic history of Annonaceae and Myristicaceae (Magnoliales). *International Journal of Plant Science* **165**: S55–S67.
- Farris, J. S., Albert, V. A., Källersjö, M., Lipscomb, D., & Kluge, A. G. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* **12**: 99–124.
- Fazekas, A. J., Kesanakurti, P. R., Burgess, K. S., Percy, D. M., Graham, S. W., Barrett, S. C., Newmaster, S. G., Hajibabael, M., & Husband, B. C. 2009. Are plant species inherently harder to discriminate than animal species using DNA barcoding markers? *Molecular Ecology Resources* **9**: 130–139.
- Felsenstein, J. 1984. Distance methods for inferring phylogenies: A justification. *Evolution* **38**: 16-24.
- Felsenstein, J. 1987. Estimation of hominoid phylogeny from a DNA hybridization data set. *Journal of molecular evolution* **26**: 123–131.
- Flach, M. 1966. Diffuse centromeres in a dicotyledenous plant. *Nature* **5030**:1369-1370.
- Frederiksen, N. 1973. New mid-Tertiary spores and pollen grains from Mississippi and Alabama. *Tulane Studies in Geology and Paleontology* **10**: 65–86.
- Ganeshiah, K.N., Ravishankar, K.V., Anand, L., Shibu, M.P., Shaanker, U. 2000. Identification of sex-specific DNA markers in the dioecious tree, nutmeg (*Myristica fragrans* Houtt.). *Plant Genetic Resources Newsletter* **121**:59–61

- Gao, T., Sun, Z., Yao, H., Song, J., Zhu, Y., Ma, X., & Chen, S. 2009. Identification of Fabaceae plants using the DNA barcode matK. *Planta Medica* **4**: 1-3.
- Gentry, A. H. 1982. Neotropical floristic diversity: phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden* **69**: 557–593.
- Gentry, A. H. 1988. Tree species richness of upper Amazonian forests. *Proceedings of the National Academy of Sciences of the United States of America* **85**: 156-159.
- Glaser, B. 2007. Prehistorically modified soils of central Amazonia: a model for sustainable agriculture in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences* **362**: 187.
- Glaser, B., Haumaier, L., Guggenberger, G., & Zech, W. 2001. The 'Terra Preta' phenomenon: a model for sustainable agriculture in the humid tropics. *Naturwissenschaften* **88**: 37-41.
- Godfray, H. C. 2002. Challenges for taxonomy. *Nature* **417**: 17–19.
- Golden, J. L., & Bain, J. F. 2000. Phylogeographic patterns and high levels of chloroplast DNA diversity in four *Packeria* (Asteraceae) species in southwestern Alberta. *Evolution* **54**: 1566–1579.
- Gonzalez, M. A., Baraloto, C., Engel, J., Mori, S. A., Pétronelli, P., Riéra, B., Roger, A., Thébaud, C., & Chave, J. 2009. Identification of Amazonian trees with DNA barcodes. *PLoS One* **4**: e7483.
- Gottlieb, O. 1976. Neolignans from *Virola carinata*. *Phytochemistry* **15**: 773-774.
- Gottlieb, O., Loureiro, A., Dos Santo Carneiro, M., & Da Rocha, A. 1973. Distribution of diarylpropanoids in Amazonian *Virola* species. *Phytochemistry* **12**: 1830.
- Govaerts, R. 2003. How many species of seed plants are there?-a response. *Taxon* **52**: 583-584.
- Gregor, H. 1977. Subtropische Elemente im europäischen Tertiär II (Fruktifikationen). *Paläontologische Zeitschrift* **51**: 199–226.
- Grossman, J. M., O'Neill, B. E., Tsai, S. M., Liang, B., Neves, E., Lehmann, J., & Thies, J. E. 2010. Amazonian anthrosols support similar microbial communities that differ distinctly from those extant in adjacent, unmodified soils of the same mineralogy. *Microbial Ecology* **60**: 192-205.

- Hackenberger, M. J., Petersen, J. B., & Neves, E. G. 1999. Village size and permanence in Amazonia: two archaeological examples from Brazil. *Latin American Antiquity* **10**: 353-376.
- Hames, R. B., & Vickers, W. T. 1982. *Adaptive responses of native americans*. New York: Academic press.
- Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* **270**: 313-321.
- Hebert, P. D. N., Ratnasingham, S., & de Waard, J. R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B: Biological Sciences* **270**: S96-S99.
- Hebert, P. D. N., Stoeckle, M. Y., Zemplak, T. S., & Francis, C. M. 2004. Identification of birds through DNA barcodes. *PLOS Biology* **2**: 1657-1663.
- Hebert, P. D., Penton, E. H., Burns, J. M., Janzen, D. H., & Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astrartes fulgerator*. *Proceedings of the National Academy of Sciences* **101**: 14812.
- Hipp, A.L., Rothrock, P.E., & Roalson, E.H. 2009. The evolution of Chromosome arrangements in *Carex* (Cyperaceae). *Botanical Review* **75**: 96-109.
- Holbrook, K. M., & Loiselle, B. A. 2009. Dispersal in a Neotropical tree, *Virola flexuosa* (Myristicaceae): Does hunting of large vertebrates limit seed removal? *Ecology* **90**: 1449–1455.
- Hollingsworth, P. M., Forrest, L. L., Spouge, J. L., Hajibabaei, M., Ratnasingham, S., Van Der Bank, M., Chase, M. W., Cowan, R. S., Erickson, D. L., Fazekas, A. J., & others. 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences* **106**: 12794-12797.
- Hollingsworth, P. M., Graham, S. W., & Little, D. P. 2011. Choosing and using a plant DNA barcode. *PloS one* **6**: e19254.
- Hoorn, C., Wesselingh, F. P., ter Steege, H., Bermudez, M. A., Mora, A., Sevink, J., Sanmartin, I., Sanchez-Meseguer, A., Anderson, C. L., Figueiredo, J. P., Jaramillo, C., Riff, D., Negri, F. R., Hooghiemstra, H., Lundberg, J., et al. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* **330**: 927-931.
- Howe, H. F. 1981. Dispersal of a neotropical nutmeg (*Virola sebifera*) by birds. *The Auk* **98**: 88–98.

- Howe, H. F., & Vande Kerckhove, G. A. 1981. Removal of wild nutmeg (*Virola surinamensis*) crops by birds. *Ecology* **62**: 1093–1106.
- Howe, H. F., Schupp, E. W., & Westley, L. C. 1985. Early consequences of seed dispersal for a neotropical tree (*Virola surinamensis*). *Ecology* **66**: 781–791.
- Hubert, N., Hanner, R., Holm, E., Mandrak, N. E., Taylor, E., Burrige, M., Watkinson, D., Dumont, P., Curry, A., Bentzen, P., & others. 2008. Identifying Canadian freshwater fishes through DNA barcodes. *PLoS One* **3**: e2490.
- Ivanova, N. V., Fazekas, A. J., & Hebert, P. D. N. 2008. Semi-automated, membrane-based protocol for DNA isolation from plants. *Plant Molecular Biology Reporter* **26**: 186-198.
- Jan du Chene, R., Onyike, M., & Sowunmi, M. 1978. Some new Eocene pollen of the Ogwashi-Asabe formation, South-Eastern Nigeria. *Rev Esp Micropaleontol* **10**: 285–322.
- Janovec, J. P. 2002. *Compsonaura diazii* (Myristicaceae), a new species from the Rio Cenepa area of Northwestern Peru. *Novon* **12**: 366–368.
- Janovec, J. P., & Harrison, J. S. 2002. A morphological analysis of the *Compsonaura sprucei* complex (Myristicaceae), with a new combination for the Central American species *Compsonaura mexicana*. *Systematic Botany* **27**: 662–673.
- Janovec, J. P., & Neill, A. K. 2002. Studies of the Myristicaceae: an overview of the *Compsonaura atopa* complex, with descriptions of new species from Colombia. *Brittonia* **54**: 251–261.
- Janovec, J. P. 2000. A systematic study of *Compsonaura* (A. DC.) Warb., A Neotropical member of the nutmeg family. Texas A&M University Dissertation: 1-359.
- Janzen, D. H. 1983. The Pleistocene hunters had help. *The American naturalist* **121**: 598–599.
- Junqueira, A. B., Shepard, G. H., & Clement, C. R. 2010. Secondary forests on anthropogenic soils in Brazilian Amazonia conserve agrobiodiversity. *Biodiversity and Conservation* **19**: 1933-1961.
- Keigwin, L. D. 1978. Pliocene closing of the Isthmus of Panama, based on biostratigraphic evidence from nearby Pacific Ocean and Caribbean Sea cores. *Geology* **6**: 630.
- Krause, D., Smith, W., & McSweeney, C. 2001. Extraction of microbial DNA from rumen contents containing plant tannins. *Biotechniques* **31**: 294-298.

- Lehmann, J., Pereira da Silva, J., Steiner, C., Nehls, T., Zech, W., & Glaser, B. 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant and Soil* **249**: 343–357.
- Levin, D. A. 1979. The nature of plant species. *Science* **204**: 381-384.
- Lopes, N. P., & Kato, M. J. 1999. Antimalarial use of volatile oil from leaves of *Virola surinamensis* (Rol.) Warb. by Waiãpi Amazon Indians. *Journal of ethnopharmacology* **67**: 313–319.
- Luceno, M., Vanzela, A.L.L., & Guerra, M. 1998. Cytotaxonomic studies in Brazilian *Rhynchospora* (Cyperaceae), a genus exhibiting holocentric chromosomes. *Canadian Journal of Botany* **76**:440-449.
- Macedo, D. S., & Anderson, A. B. 1993. Early ecological changes associated with logging in an Amazon floodplain. *Biotropica* **25**: 151-163.
- MacRae, W. D., & Towers, G. H. 1985. Non-alkaloidal constituents of *Virola elongata* bark. *Phytochemistry* **24**: 561–566.
- Macrae, W. D., & Towers, G. H. 1984a. *Justicia pectoralis*: a study of the basis for its use as a hallucinogenic snuff ingredient. *Journal of ethnopharmacology* **12**: 93–111.
- Macrae, W., & Towers, G. 1984b. An ethnopharmacological examination of *Virola elongata* bark: A South American arrow poison. *Journal of ethnopharmacology* **12**: 75–92.
- Maloney, D. J., Deng, J., Starck, S. R., Gao, Z., & Hecht, S. M. 2005. (+)-Myristinin A, a naturally occurring DNA polymerase β inhibitor and potent DNA-damaging agent. *Journal of the American Chemical Society* **127**: 4140-4141.
- Marshall, L. G., Webb, S. D., Sepkoski, J. J., & Raup, D. M. 1982. Mammalian evolution and the great American interchange. *Science* **215**: 1351-1357.
- McKenna, D. J., Towers, G. H. N., & Abbott, F. S. 1984. Monoamine oxidase inhibitors in South American hallucinogenic plants part 2: Constituents of orally-active Myristicaceous hallucinogens. *Journal of Ethnopharmacology* **12**: 179–211.
- Meggers, B. J. 1971. *Amazonia: Man and nature in a counterfeit paradise*. Chicago: Aldine.
- Mehra, P.H., & Bawa, K.S. 1969. Chromosomal evolution in tropical hardwoods. *Evolution* **23**: 466-481.

- Mora, C., Tittensor, D.P., Adl, S., Simpson, A.G.B., & Worm, B. 2011. How many species are there on the earth and in the oceans. *Public Library of Science-Biology* **9**: e1001127.
- Mullis, K. B., & Faloona, F. A. 1987. Specific synthesis of DNA in Vitro via a Polymerase-Catalyzed chain reaction. *Methods in Enzymology* **155**: 335-350.
- Nagaki, K., Kasjihara, K., & Murata, M. 2005. Visualization of diffuse centromeres with centromere-specific histone H3 in the holocentric plant *Luzula nivea*. *The plant cell* **17**:1886-1893.
- Olsen, K. M., & Schaal, B. A. 1999. Evidence on the origin of cassava: phylogeography of *Manihot esculenta*. *Proceedings of the National Academy of Sciences of the United States of America* **96**: 5586.
- Padial, J.M., Miralles, A., De la Riva, I., and Vences, M. 2010. The integrative future of taxonomy. *Frontiers in Zoology* **7**:16.
- Palma-Silva, C., Wendt, T., Pinheiro, F., Barbará, T., FAY, M. F., Cozzolino, S., & Lexer, C. Sympatric bromeliad species (*Pitcairnia spp.*) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. *Molecular Ecology* **20**: 3185–3201
- Palmé, A. E., Semerikov, V., & Lascoux, M. 2003. Absence of geographical structure of chloroplast DNA variation in sallow, *Salix caprea* L. *Heredity* **91**: 465–474.
- Pazy, B., & Plitmann, U. 1994. Holocentric chromosome behaviour in *Cuscuta* (Cuscutaceae). *Plant Systematics and Evolution* **191**:105-109.
- Pierpoint, W. S. 1969. o-Quinones formed in plant extracts. Their reaction with bovine serum albumin. *Biochemical Journal* **112**: 619.
- Pitman, N. C., Terborgh, J. W., Silman, M. R., Núñez V, P., Neill, D. A., Cerón, C. E., Palacios, W. A., & Aulestia, M. 2001. Dominance and distribution of tree species in upper Amazonian terra firme forests. *Ecology* **82**: 2101–2117.
- Pitman, N. C., Terborgh, J. W., Silman, M. R., Núñez V, P., Neill, D. A., Cerón, C. E., Palacios, W. A., & Aulestia, M. 2002. A comparison of tree species diversity in two upper Amazonian forests. *Ecology* **83**: 3210–3224.
- Poinar, G. J., & Poinar, R. 1999. *The amber forest: a reconstruction of a vanished world*. Princeton, N.J.: Princeton University Press.
- Poinar, H. N. 1998. Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* **281**: 402-406.

- Pons, D., & Franceschi, D. D. 2007. Neogene woods from western Peruvian Amazon and palaeoenvironmental interpretation. *Bulletin of Geosciences* **82**: 343-354.
- Prance, G. T. 1977. Floristic Inventory of the Tropics: Where Do We Stand? *Annals of the Missouri Botanical Garden* **64**: 659-684.
- Raven, P.H. 1975. The bases of Angiosperm phylogeny: Cytology. *Annals of the Missouri Botanical Garden* **62**: 724-764.
- Raven, P. H. 1976. Systematics and plant population biology. *Systematic Botany* **1**: 284-316.
- Raven, P. H., & Axelrod, D. I. 1974. Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden* **61**: 539-673.
- Richardson, J. E. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. *Science* **293**: 2242-2245.
- Rieseberg, L.H., & Soltis, D.E. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* **5**: 65-84.
- Rieseberg, L.H., Wood, T.E., & Baack, E.J. 2006. The nature of plant species. *Nature* **440**: 524-527.
- Roch, S. 2010. Toward extracting all phylogenetic information from matrices of evolutionary distances. *Science* **327**: 1376-1379.
- Rodrigues, W. A. 1989a. A new Venezuelan *Virola* (Myristicaceae). *Annals of the Missouri Botanical Garden* **76**: 1163-1164.
- Rodrigues, W. A. 1989b. Two new neotropical species of *Compsonaura* (Myristicaceae). *Brittonia* **4**: 160-163.
- Russo, S. E. 2003. Responses of dispersal agents to tree and fruit traits in *Virola calophylla* (Myristicaceae): implications for selection. *Oecologia* **136**: 80-87.
- Saunders, G. W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society B: Biological Sciences* **360**: 1879.
- Sauquet, H. 2003. Androecium diversity and evolution in Myristicaceae (Magnoliales), with a description of a new Malagasy genus, *Doyleanthus* gen. nov. *American Journal of Botany* **90**: 1293-1305.
- Sauquet, H. 2004. Systematic revision of Myristicaceae (Magnoliales) in Madagascar, with four new species of *Mauloutchia*. *Botanical Journal of the Linnean Society* **146**: 351-368.

- Sauquet, H., Doyle, J. A., Scharaschkin, T., Borsch, T., Hilu, K. W., Chatrou, L. W., & Le Thomas, A. 2003. Phylogenetic analysis of Magnoliales and Myristicaceae based on multiple data sets: implications for character evolution. *Botanical Journal of the Linnean Society* **142**: 125–186.
- Sawadjoon, S., Kittakoop, P., Kirtikara, K., Vichai, V., Tanticharoen, M., & Thebtaranonth, Y. 2002. Atropisomeric myristinins: Selective COX-2 inhibitors and antifungal agents from *Myristica cinnamomea*. *The Journal of Organic Chemistry* **67**: 5470-5475.
- Scotland, R. W., & Wortley, A. H. 2003. How many species of seed plants are there? *Taxon* **52**: 101–104.
- Smith, A. C. 1937. The American species of Myristicaceae. *Brittonia* **2**: 393-510.
- Smith, B. D. 2006. Eastern North America as an independent center of plant domestication. *Proceedings of the National Academy of Sciences* **103**: 12223.
- Smith, M. A., Rodriguez, J. J., Whitfield, J. B., Deans, A. R., Janzen, D. H., Hallwachs, W., & Hebert, P. D. 2008. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proceedings of the National Academy of Sciences* **105**: 12359.
- Smith, M. A., Wood, D. M., Janzen, D. H., Hallwachs, W., & Hebert, P. D. N. 2007. DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. *Proceedings of the National Academy of Sciences* **104**: 4967-4972.
- Smith, N. J. H. 1980. Anthrosols and human carrying capacity in Amazonia. *Annals of the Association of American Geographers* **70**: 553–566.
- Soltis, D. E., Soltis, P. S., Chase, M. W., Mort, M. E., Albach, D. C., Zanis, M., Savolainen, V., Hahn, W. H., Hoop, S. B., & Fay, M. F. 2000. Angiosperm phylogeny inferred from 18S rDNA, rbcL, and atpB sequences. *Botanical Journal of the Linnean Society* **133**: 381–461.
- Soltis, D. E., Smith, S. A., Cellinese, N., Wurdack, K. J., Tank, D. C., Brockington, S. F., Refulio-Rodriguez, N. F., Walker, J. B., Moore, M. J., Carlsward, B. S., & others. 2011. Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal of Botany* **98**: 704.
- Spooner, D. M. 2009. DNA barcoding will frequently fail in complicated groups: An example in wild potatoes. *American Journal of Botany* **96**: 1177-1189.
- Steel, M. A., Hendy, M. D., & Penny, D. 1988. Loss of information in genetic distances. *Nature* **336**: 118.

- Thomas, W. W. 1999. Conservation and monographic research on the flora of Tropical America. *Biodiversity and Conservation* **8**: 1007–1015.
- Trueman, J. W. 1998. Reverse successive weighting. *Systematic Biology* **47**: 733.
- Van Gils, C., & Cox, P. A. 1994. Ethnobotany of nutmeg in the Spice Islands. *Journal of Ethnopharmacology* **42**: 117–124.
- Warburg, O. 1897. Monographie der Myristicaceen. *Nova Acta Acad. Caes. Leop.-Carol* **68**: 1-680.
- Whitlock, R., Hipperson, H., Mannarelli, M., & Burke, T. 2008. A high-throughput protocol for extracting high-purity genomic DNA from plants and animals. *Molecular Ecology Resources* **8**: 736–741.
- Wilde, W. J. J. O. D. 1991. The genera of the Myristicaceae as distinguished by their inflorescences and the description of a new genus: *Bicuiba*. *Beitr. Biol. Pflanzen* **66**: 95-125.
- Will, K. W., & Rubinoff, D. 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics* **20**: 47–55.
- Will, K., Mishler, B., & Wheeler, Q. 2005. The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology* **54**: 844-851.
- Wilson, I. G. 1997. Inhibition and facilitation of nucleic acid amplification. *Applied and Environmental Microbiology* **63**: 3741-3751.
- Witt, J. D., Threlloff, D. L., & Hebert, P. D. 2006. DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation. *Molecular Ecology* **15**: 3073–3082.
- Wolfe, J. 1977. Paleogene floras from the Gulf of Alaska region. *U.S. Geological Survey Professional Paper* **997**: 1–108.

Chapter 1

A PHYLOGENETIC AND MORPHOLOGICAL ANALYSIS OF *COMPSONEURA*

Abstract

Compsonaura currently comprises 21 described species of canopy to sub-canopy Myristicaceae trees native to tropical rainforests below 1500 m elevation in South and Central America. *Compsonaura* species are difficult to identify due to their similar looking leaves and small (1-4mm), largely amorphous, flowers. In this chapter infrageneric relationships are estimated using molecular phylogenetic analyses employing one chloroplast (*trnH-psbA*) and two nuclear markers (*AT103* and *AGT1*) and both maximum parsimony and Bayesian inference. Phylogenetic analyses revealed a deep evolutionary rift within *Compsonaura* corresponding to informal sections *Compsonaura* and *Hadrocarpa* proposed by previous morphological and biogeographical studies. Phylogenetic analyses also revealed that mountain building has likely been an important generator of speciation in the taxa studied. A distance based molecular analysis was also conducted with increased taxon sampling to test DNA-based identification using the highly variable locus *trnH-psbA*. Distance analyses revealed that all but two of 13 *Compsonaura* species included can be identified by one or more nucleotide polymorphisms. The molecularly indistinguishable species do not share a common range, making molecular identification of all tested species possible with a combination of sequence and location data. Herein I present molecular and morphological evidence supporting the sub-generic classification of Janovec (2000) and discuss the results with respect to sub-generic classification, morphology, and biogeography.

Introduction

The Myristicaceae, or nutmeg family, consists of 21 genera and more than 500 species of dioecious, and rarely monoecious, canopy to sub canopy trees distributed worldwide in tropical rainforest environments (Smith 1937, Wilde 1991, Janovec 2000). The Myristicaceae diverged early in the radiation of the angiosperms and previous molecular studies has placed them as the sister to all members of the order Magnoliales (APG III 2003, Qiu et al. 2006). However, the most recent and comprehensive molecular phylogenetic analysis employing 17 genes places Magnoliaceae as sister to all other members of the Magnoliales, including Myristicaceae (Soltis et al 2011). Nutmeg trees are readily recognized in the field by their combination of some or all of the following diagnostic features: (1) Myristicaceous branching (branches originating in a whorled fashion in a single plane and at relatively regular intervals on the trunk); (2) frequent presence of pink to blood-red sap with a bitter, astringent taste; (3) tiny urceolate to cup-shaped flowers with three to four tepals; (4) reduced to elongated filament column terminated with free to fused anthers dehiscing to the outside; (5) a one-seeded fruit that typically dehisces longitudinally into two valves; and (6) the presence of an aril that is usually red, or white. Numerous studies have reported the significant ecological importance of the Myristicaceae in wet, lowland tropical forests of Central and South America, Africa, Madagascar, India, and Asia (Gentry 1982, Pascal and Pelissier 1996, Poulsen et al. 1996, Spichiger et al. 1996). The Myristicaceae also hold considerable economic and cultural importance as the source of the commercial nutmeg spice (*Myristica fragrans*) and DMT-containing hallucinogens derived from *Virola spp.* in the

Amazon basin (Chagnon 1971, Mckenna et al. 1984, Schultes 1984, Van Gils and Cox 1994). Many members of the family are also valued for their wood, which is used in rural house construction as well as fine carpentry that reaches an international market (Macedo and Anderson 1993). Additionally, plant extracts of members of the nutmeg family have been investigated for their pharmacological potential, including, but not limited to, aphrodisiac and anti-cancer effects (Sawadjoon et al. 2002, Maloney et al. 2005, Tajuddin et al. 2005, Nguyen et al. 2010, Patro et al. 2010, Pusztai et al. 2010).

Compsonaura is one of six neotropical genera of Myristicaceae, which comprises 21 described dioecious species that inhabit rainforest environments below 1500m elevation in South and Central America (Smith 1937, Wilde 1991, Janovec 2000).

Compsonaura is easily differentiated from other neotropical members of the family by virtual absence of pubescence on the leaf lamina and subparallel tertiary nerves (some taxa of Annonaceae and Icacinaceae [*Discophora* spp.] also have similarly arranged nerves and often confused with *Compsonaura* spp.). Since species of *Compsonaura* share similar leaf morphologies, identification relies heavily upon characteristics of the small flowers (1–4 mm), particularly characteristics of the androecium (Warburg 1897, Smith 1937, Armstrong and Tucker 1986, Janovec 2000, Janovec and Harrison 2002). Flowers are only present on adult trees for a few months every year (Bullock 1982, Armstrong 1997), which can make identification of female and vegetative specimens of these dioecious trees exceedingly difficult. Herbarium studies of the genus *Compsonaura* (Janovec 2000) and studies of the neotropical Myristicaceae in ecological plots by Steeves (2008, personal observations) encountered mis-identification rates of approximately 50% and 25% respectively.

Despite the cultural, ecological, and economic importance of the Myristicaceae throughout the wet tropics of the world, our systematic knowledge of the Myristicaceae is mostly based on the last, grand synthesis of the family by Warburg (1897). In *Monographie der Myristicaceen* (1897), which was rooted in prior studies by De Candolle (1856), Warburg described 240 species and 15 genera, including four of the six neotropical genera recognized today. Among the genera elevated by Warburg to the generic rank was *Compsonaura*, which was based on de Candolle's (1856) *Myristica* section *Compsonaura*. Both agreed that *Compsonaura* as a group of similar species was characterized by tertiary leaf venation parallel to the secondary veins, an orange fruit pericarp dehiscent longitudinally, an entire red aril, and mottled seeds. With the eight specimens he had available at the time, Warburg recognized five species of *Compsonaura* and divided them into two sections: (1) section *Eucompsonaura* (Warb.) with free anthers and (2) section *Coniostele* (Warb.) with fused anthers. Based on a study of 70 specimens available at the time from Central and South America, Smith (1937) described five additional species of *Compsonaura* and synonymized one (*C. capitellata* and *C. tessmannii* as the former), but adopted Warburg's sections *Eucompsonaura* and *Coniostele*. Since that time, the neotropical Myristicaceae has been the focus of only a few studies focused on new species and regional floristics. In 1956 Smith described an intriguing new species named *Virola atopa* from the Pacific coastal region of Colombia but later placed this species in *Compsonaura* once staminate specimens were made available by the explorations of Richard Evans Schultes (Smith 1956). Rodrigues (1989) described two new species of *Compsonaura*, bringing the total to 11 species. These preceding studies of the 19th and 20th centuries have been plagued by the availability of

few samples from across the geographical range of *Compsooneura* as well as a paucity of differentiating morphological characters.

In recent years, increased collecting efforts and the advent of molecular analyses has made more specimens and characters available for taxonomic investigation. Contemporary studies by Janovec and co-workers over the past 10 years have examined over 3000 specimens of the genus *Compsooneura* for studies concerning population genetics, morphological evolution and taxonomy (Janovec 2000, Janovec and Harrison 2002, Janovec and Neill 2002, Newmaster et al. 2008). Janovec and Neill (2002) described two new species in the *Compsooneura atopa* complex and differentiated this species group from other *Compsooneura* based on the following combination of characters: (1) Secondary venation conspicuously brochidodromous (the secondary veins strongly anastomosing near the laminar margins); (2) Trichomes stellate to dendritic, often forming a ferruginous tomentum on the young stem, leaf node, leaf lamina, petiole, external perianth surface, ovary, and pericarp surface; (3) Anthers free, their apices slightly incurved; (4) Oil cells abundant in the perianth and androecium, visible under low magnification and conspicuous when viewed in longitudinal or cross section; (5) Pericarp ligneous, strongly rostrate, and carinate with longitudinal ridges running from base to near apex; (6) Aril deeply lacinate or reduced, white to tan when fresh, tan to light brown when dry; (7) Testa brown, muricate, often with reticulate, vein-like impressions.

In the most recent systematic treatment of *Compsooneura*, Janovec (2000) opted to refer to informal sections *Compsooneura* and *Hadrocarpa*, which were based on multiple vegetative and reproductive characters, as opposed to Warburg's (1897) sectional

divisions based solely on free or fused anthers (hereafter non-italicized “Hadrocarpa” and “Compsonneura” will refer to these informal sections sensu Janovec 2000). Although morphological evidence indicated that Hadrocarpa and Compsonneura were distinct evolutionary lineages, Janovec (2000) and Janovec and Neill (2002) lacked molecular support. Such molecular evidence has been long in coming due to the difficulties of extracting, amplifying, and sequencing members of the Myristicaceae that possess potent DNA polymerase inhibitors (Sawadjoon et al. 2002, Maloney et al. 2005). These problems have been particularly difficult to overcome in *Compsonneura s.l.* as the vast majority of specimens are from old collections, which were not made with molecular investigations in mind. Additionally, molecular work of the family has been plagued by low rates of molecular evolution in loci traditionally used for phylogenetics (Sauquet et al. 2003).

The genus *Compsonneura* provides fertile ground for testing taxonomic hypotheses and DNA based identification methodologies as they are ecologically and ethnobotanically important, likely contains sister species of relatively recent origin (Janovec & Harrison 2002), and also has additional new cryptic species (Janovec et al. in preparation). The objectives of this research were (1) to test the sub-generic classifications of Warburg (*Eucompsonneura* and *Coniosteles*) and Janovec (*Compsonneura* and *Hadrocarpa*) with molecular phylogenetic analyses and morphological comparisons and (2) to assess the ability of DNA sequences to differentiate species of *Compsonneura sensu lato*. Molecular phylogenetic analyses were performed using trnH-psbA, the most rapidly evolving cpDNA loci surveyed in a DNA barcoding study of *Compsonneura* (Newmaster et al. 2008), as well as two low copy nuclear loci (*ATI03* and *AGT1*).

Materials and Methods

Taxon sampling and outgroup selection

Many species of neotropical *Compsooneura* are represented by one or very few herbarium specimens, most of which were collected over 20 years ago. It is well known that DNA extraction can be particularly difficult from such herbarium material (Savolainen et al. 1995, Lister et al. 2008, Telle and Thines 2008). This problem is exacerbated in members of the nutmeg family as they contain high levels of potent DNA polymerase inhibiting secondary metabolites and DNA degrading chemicals (Sawajadon et al. 2002, Maloney et al. 2005). Due to the rare nature and poor condition of many collections, it was not possible to include all species of *Compsooneura* in molecular analyses. Forty-six collections representing 13 species of *Compsooneura* were used in the molecular analyses. Some sequences used in this analysis were retrieved from Genbank from previous studies of *Compsooneura* (Newmaster et al. 2008). Since DNA sequences were not retrieved from all collections nor all loci, the number of collections and species used in the phylogenetic and distance analyses differ. All collections used in molecular analyses were identified according to the most current taxonomic treatment (Janovec 2000) and all taxa have accessions archived at one or more of the following herbaria: Botanical Research Institute of Texas (BRIT), Ontario Agricultural College (OAC), New York Botanical Gardens (NYBG), Missouri Botanical Gardens (MO), and the National Herbarium of the Ecuadorian Museum of Natural Sciences (QCNE)[Table 1.1].

Since phylogenetic relationships remain unresolved in the Myristicaceae (Sauquet et al. 2003), members of *Iryanthera*, *Otoba* and *Virola* were selected as outgroups for phylogenetic and distance-based analyses.

DNA extraction, Amplification and Sequencing

Total genomic DNA was extracted from leaf tissue of silica-dried or herbarium specimens using the Macherey-Nagel Nucleospin II plant Kit. Lysis buffer 1 was used according to the manufactures' instructions with the exception of an increase of the post homogenization incubation period to 1hr (from 10 min) and the addition of 20 mM N-Phenacylthiazolium Bromide, which has been found to improve amplification of recalcitrant samples (Poinar et al. 1998, Asif and Cannon 2005).

Initially a suite of chloroplast (*accD*, *atpF-H*, *matK*, *psbK-I*, *rbcL*, *rpoB*, *rpoC1*, *trnH-psbA*, *trnL-F*, *trnS-G*, *UPA*, and *ycf5*) and nuclear (*AGT1*, *APG1*, *AT103*, *EIF3E*, *GI*, *GS*, *ITS*, *IGS*, *PHYA*, *PHYC*, and *sqd1*) loci were amplified to investigate their utility for systemic investigations of *Compsooneura*. However, only a small subset (*trnH-psbA*, *AT103*, and *AGT1*) consistently produced a single banded PCR product with high quality sequence traces and were sufficiently variable for systematic investigations.

Species not represented by *trnH-psbA* accessions in GenBank from Newmaster et al. (2008) were PCR amplified and sequenced using the primers *trnH2* (5'-CGCATGGTGGATTCAATCC-3'; Tate and Simpson 2003) and *psbAF* (5'GTTATGCATGAACGTAATGCTC-3'; Sang et al. 1997). PCR was performed in a 20 µl volume using 0.4 µl of Phire II polymerase (Finnzymes) with 1X Phire II reaction buffer (with 1.5mM MgCl), 0.2 mM of each DNTP, 0.2 µM of each primer and 2.0 µg of BSA (Kreader 1996). Cycling conditions entailed an initial denaturation step of 1 min at 98°; 35 cycles of 98° for 5 s, 64° for 5 s, 72° for 10 s: and a final elongation step of 72°

for 1 min followed by a 4° hold. Phire II was used to amplify trnH-psbA as it is robust to the inhibitors contained in nutmeg extracts and as it is a fusion-based polymerase which has been found to reduce stuttering in sequences containing homopolymer regions such as the trnH-psbA intergenic spacer (Fazekas et al. 2010).

Nuclear loci were amplified using the primers AT103F (5'-CTTCAAGCCMAAGTTCATCTTCTA-3'; Li et al. 2008), AT103R (5'-TTGGCAATCATTGAGGTACATNGTMACATA-3');), AGT1-MYR-F (5'-GGGCATTGACGTAGCTTTGACAGG-3'; this thesis), and AGT1-MYR-R (5'-GTGCAGTTCTTCAAGCCCCAAGC-3'; this thesis). Nuclear loci were amplified with 0.5U of AmpliTaq Gold (Applied biosystems) DNA polymerase in a 20 µl reaction containing 1X reaction buffer, 2.5 mM MgCl, 8% W/V Polyethylene glycol (Zimmerman and Harrison 1987, Sasaki et al. 2006) , 0.2 M trehalose (Speiss et al. 2004), 2 µg BSA, 0.2 mM each DNTP, and 0.2 µM of each primer.

Amplification products were sequenced directly using the same primers employed in PCR. Cycle sequencing reactions were performed in a 10.5 µL reaction volume containing 0.5 µL of BigDye terminator mix v3.1, 1.88 µL of 5x sequencing buffer (Applied Biosystems), 1.0 µM of primer and 0.5 µL of PCR product. Thermal cycling parameters were 96° for 2 min; 30 cycles of 96° for 30s, 56-60° (primer dependant) for 15s, and 72° for 4 min; and a 4° hold. Cycle sequencing reactions were cleaned using sephadex columns (Cat.no. S5897; Sigma-Aldrich, St. Louis, MO, USA) and the samples were run on an ABI 3730 sequencer (Applied Biosystems).

Alignment, phylogenetic and distance analysis

Sequence contigs were assembled and edited using Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA). DNA sequences were aligned using the default settings of the ClustalW algorithm (Thompson et al. 1994) in Bioedit (Hall 1999) and adjusted manually. Alignments can be found in Appendix 1 at the end of this thesis. A 4bp inversion believed to belong to the loop structure of the 3'UTR (untranscribed region) of the *psbA* gene (Storchova and Olsen 2007) was omitted from data matrices due to the high likelihood of homoplasious inversions. Gaps in the alignments were coded using Simple Indel Coding (SIC) (Simmons and Ochoterena 2000) for Bayesian analyses and Modified Complex Indel Coding for distance-based and parsimony analyses using IndelCoder (Muller 2006).

A previous study of *Compsooneura* by Newmaster et al. (2008) found the *trnH-psbA* intergenic spacer to be the most useful of numerous proposed chloroplast barcode loci. I amplified and sequenced 5 additional species of rarely collected *Compsooneura* from herbarium material and generated a Neighbour-Joining (NJ) uncorrected p-distance tree using PAUP 4.0 (Swafford 2002) to further explore the ability of this locus to discriminate species of *Compsooneura s.l.* with increased taxon sampling. Additionally, intra and interspecific distances were calculated using pair-wise uncorrected p-distance in Mega 3.1 (Kumar et al. 2008) for species with multiple sequences per taxon to investigate whether there exists a barcoding gap (Hebert et al. 2004a, Hebert et al. 2004b) in these taxa. Uncorrected p-distances were calculated as there existed a very low level of nucleotide substitution among taxa and therefore it was not deemed necessary to employ a substitution model in calculating distance estimates.

An incongruence length difference test (ILD: Farris et al. 1995) was executed in PAUP (Swafford 2002) to determine whether the three loci employed in this study contained significant incongruence. The ILD was performed using 100 replicates of a heuristic search strategy, MAXTREES set at 100, 10 random addition sequence replicates holding 5 trees at each step, MULTREES option in effect, and tree bisection-reconnection (TBR) branch swapping. No significant incongruence was detected (p-value=0.17) and individual gene trees generated by Bayesian inference showed no significant topological discrepancies among the major clades, therefore all three loci were concatenated in a total evidence approach for Bayesian inference and parsimony analyses.

Maximum parsimony analyses were performed in PAUP 4.0b10 (Swafford 2002) and each nucleotide position was treated as an unordered character and all positions were equally weighted. The single heuristic search was performed with 1000 replicates of random addition sequence, holding 10 trees at each step, TBR branch swapping with the MULTREES option not in effect and MAXTREES set to 5000. Branch support was estimated using a heuristic search, 5000 bootstrap (BS) replicates, 10 random addition sequence replicates holding 1 tree at each step, TBR branch swapping, and a maximum number of trees set at 50,000 with MULTREES not in effect.

Phylogenetic trees were also generated using Bayesian inference with the program MrBayes (Ronquist and Huelsenbeck 2003). Nucleotide substitution models were selected using Mrmodeltest (Nylander 2004) using the Akaike Information Criterion. Mrmodeltest selected the following substitution models for the three loci: *trnH-psbA*=HKY+G, *AT103*=GTR+G, *AGT1*=GTR+G. Ten million generations were performed

using 4 chains and 2 runs with trees being sampled every 100 generations. Log-likelihood values stabilized after 2.5 million generations; therefore the consensus tree and posterior probabilities (PP) were estimated using a burn-in of 25,000 trees. Since posterior probabilities are largely considered to be overestimations of support for clades (Douady et al. 2003, Alfaro and Holder 2006, Yang and Rannala 2010), especially when employing concatenated data sets (Suzuki et al. 2002), any nodes with less than 0.75 posterior probability were collapsed on the phylogram using treegraph2 (Stover and Muller 2010).

Additionally, gross morphological differences and similarities between the informal sections *Hadrocarpa* and *Compsonera* were observed in the field, herbaria and literature (Janovec 2000, Janovec and Harrison 2002). Differentiating traits of the leaves, flowers, inflorescence, fruit and indument of all these parts were synthesized from the most recent taxonomic treatment (Janovec 2000) and subsequent species descriptions (Janovec and Harrison 2002, Janovec and Neil 2002).

Results

DNA sequencing and alignment

Table 1.1 presents specimens included in various genetic analyses that were successfully amplified for one or more loci along with their Barcode of Life Database (BOLD) process identification numbers that can be used to access sequence and specimen info online at www.barcodinglife.org. Genbank accession numbers are included in Table 1.1 for specimens whose trnH-psbA sequences were retrieved from Newmaster et al. (2008). The trnH-psbA spacer alignment for phylogenetic analyses consisted of 342

nucleotides, 39 variable characters and 18 parsimony informative characters. The AGT1 gene alignment consisted of 710 nucleotides, 59 variable and 34 parsimony informative sites while the AT103 gene alignment consisted of 412 nucleotides, 31 variable characters and 22 parsimony informative sites. The concatenated alignment contained 1464 characters of which 129 were variable and 74 parsimony informative nucleotide positions. The outgroups contributed 89 variable and 29 parsimony informative characters. The MCIC algorithm employed in indelcoder coded 14 separate indel events.

Phylogenetic Analyses

A total of 14 collections representing 8 species of *Compsooneura* were included in the phylogenetic analyses as high-quality DNA was not extracted from all samples. Trees constructed from individual loci varied slightly in their overall topology, mostly due to the markers varying information content, but in all cases the monophyly of informal sections *Hadrocarpa* and *Compsooneura* was recovered and supported by high (1.0) posterior probabilities (data not shown). Additionally, no significant incongruence was detected with an ILD test, therefore the loci were combined in an all evidence approach. The 50% majority-rule Bayesian consensus tree with posterior probabilities is shown in Figure 1.1. The most parsimonious phylogram estimated by PAUP recovered a nearly identical topology (Figure 1.2). A single most parsimonious tree was found with a length of 187, a consistency index (CI) of 0.85, a homoplasy index (HI) of 0.15, and a retention index (RI) of 0.88. Both Bayesian and parsimony analyses of the three regions revealed strong support of the monophyly of *Compsooneura* s.l. as measured by posterior probabilities (1.0) and bootstrap support (99). There was also strong support (PP=1.0,

BS=99) for the nodes separating informal sections *Hadrocarpa* and *Compsooneura*. However, it appears that fused anthers have evolved multiple times in *Compsooneura* (Figures 1.1 and 1.2), making Warburg's sections *Eucompsooneura* and *Coniosteles* paraphyletic in nature. Additionally, there was a considerable branch length separating *Hadrocarpa* and *Compsooneura* compared to the relatively short branches within each group, indicating a significant amount of evolutionary divergence between these two informal sections.

Morphology

Species of the two sections share a few morphological similarities such as tertiary veins growing more or less perpendicular to the midvein, an entire endosperm, and generally glabrous leaves. However, thirteen contrasting morphological characters were found to differentiate *Hadrocarpa* from *Compsooneura* (Table 1.2).

Distance analysis

This study was able to obtain sequences from 5 additional species of *Compsooneura* than were analysed by Newmaster et al. (2008). These 5 species are relatively rare and poorly collected members of section *Hadrocarpa* (sensu Janovec 2000), which were collected between 1972 and 1996. All species with the exception of *C. cuatrecasasi* and *C. carchifolia* can be identified by one or more single nucleotide polymorphisms (Figure 1.3). It is also interesting to note that *C. capitellata*, *C. sprucei*, and *C. mexicana* exhibit a high degree of molecular variation relative to other species. Intraspecific distances were found to sometimes be larger than interspecific distances and

therefore there was no barcoding gap found in the taxa tested (Figure 1.3). Additionally, both sections *Hadrocarpa* and *Compsooneura* were found to be monophyletic in this analysis and within section *Hadrocarpa*, species from Western Colombia and Panama formed their own clade due to a 9-bp indel as well as a shared single nucleotide polymorphism.

Discussion

Taxonomy is most biologically relevant when it seeks to classify and identify organisms with respect to their evolutionary history. This study represents the most comprehensive molecular analysis of the genus *Compsooneura* to date. Previous work by Newmaster et al. (2008) assessed levels of molecular variation of different cpDNA markers in the genus on a more restricted suite of taxa but did not conduct a phylogenetic analysis. Janovec (2000) investigated the microsatellite allele variation amongst populations of South and Central American *Compsooneura sprucei* which, together with a morphometric analysis, led to the resurrection of *C. mexicana* (Janovec and Harrison 2002). With the use of markers from the chloroplast and nuclear genomes, my analyses revealed a significant molecular divergence that is mirrored by morphological adaptations of sections *Hadrocarpa* and *Compsooneura* (sensu Janovec 2000) that I feel are sufficient to warrant the recognition of these sections as separate respective genera. The following discussion will address this as well as implications of the distance analysis.

Phylogenetic Analyses

Previous studies of *Compsoeura* have highlighted numerous morphological and biogeographical differences between informal sections *Hadrocarpa* and *Compsoeura* but did not include molecular analyses (Janovec 2000). In this study I present the first phylogenetic estimation of a genus of the Myristicaceae, thus providing some illumination of the relationships between members of *Compsoeura*. Very high posterior probabilities (1.0) and bootstrap support (99) was found on the nodes that separate sections *Compsoeura* and *Hadrocarpa*, indicating they are monophyletic. It is also interesting to note that both phylogenetic analyses indicated that Warburg's sections *Eucompsoeura* (free anthers) and *Coniostele* (fused anthers) are paraphyletic, as fused anthers appear to have evolved multiple times within *Compsoeura*. Additionally, similar branch lengths were recovered separating the two sections of *Compsoeura* as were found between *Otoba-Compsoeura* and *Otoba-Iryanthera*. This suggests that the two sections have been on diverging evolutionary paths for a substantial amount of time. The two sections also have distinct biogeographic patterns. Members of *Compsoeura* are widespread in the Amazon basin, the Western coast of the Andes as well as Central America. Members of *Hadrocarpa* on the other hand primarily reside on the Western side of the Andes in Ecuador, Colombia and Panama with the vast majority of species being endemic to Western Colombia.

It thus appears that the informal sections *Hadrocarpa* and *Compsoeura* represent a more natural sub-generic classification of the genus compared to Warburg's (1897) sections *Eucompsoeura* and *Coniostele* when both molecular and morphological data are considered. There exists so many dichotomous morphological and molecular characters among section *Hadrocarpa* and *Compsoeura*, that I suggest that the former be

elevated to the generic rank so as to recognize the morphological and molecular distinctiveness of these clades. I believe that this is important as end-users of taxonomy use field characters not only to recognize species, but also to help group collections and as an aid to memory. If recognized formally, I proposed that the genus be named *Hadrocarpa* (derived from the greek *hadros*, or thick, and *carpa*, or seed/fruit) as large fruits with thick ligneous pericarps are characteristic of these trees.

The Myristicaceae remain poorly understood from a phylogeographic perspective due to a paucity of data and low rates of nucleotide substitution that has plagued previous investigators (Sauquet et al. 2003). The only molecular phylogenetic analysis of the family conducted by Sauquet et al. (2003) was unable to recover well supported relationships among genera of the family despite using some of the most variable loci commonly used in low-level angiosperm phylogenetics (*trnL-trnF*, *ndhF*, *trnK*, and *matk*). I elected to use *Iryanthera* as an outgroup to my analyses as they are the only dioecious (assumed ancestral state) members of the family and *Iryanthera* leaves appear to be the most similar of any Myristicaceae to the sister of the family, *Idiospermum australiense* (Calycanthaceae). Initial efforts were made to use *Idiospermum australiense* as an outgroup, however, difficulty in amplification and alignment of sequences prevented its inclusion. Despite this uncertainty of rooting and therefore character polarity, there remain numerous interesting and well supported relationships that have been revealed with this analysis.

The well supported *C. mutisii*-*C. mexicana*-*C. excelsa* clade was recovered in all trees made from the three individual loci (data not shown) and is of particular phylogeographic interest. It appears that the species of this clade arose through a series of

allopatric speciation events initiated by the uprise of the Andean Orogeny. These speciation events would likely have been initiated by the rise of the Andean range resulting in the separation of current *C. mutisii* from its Amazonian progenitor populations (likely *C. ulei* or *C. sprucei*). This event was followed by dispersal of an ancestor of *C. mutisii* to Central America (likely via birds, their known dispersal vector) to a forming Central American archipelago or after the formation of the Panamanian Isthmus (Raven and Axelrod 1974). After this event, the up-rise of a mountain range in present day Costa Rica separated populations on the East and West side of the forming cordillera. This vicariance event likely led to the differentiation of the species we know as *C. excelsa* and *C. mexicana* respectively. It is also interesting to note that a morphological cladistic analysis of *Compsooneura* by Janovec (2000) found that *C. ulei* consistently grouped within the *mutisii-excelsa-mexicana clade*, a group which shares the trait of fused or partially fused anthers on the filament column. The somewhat low support (PP=0.81, BS=51) on the node separating *C. ulei* and *C. debilis* from the other members of the genus may be due to the fact that the most variable locus used in this study, *AGTI*, was not successfully sequenced for *C. ulei*. Recovery of *AGTI* or other variable low copy nuclear markers may provide increased resolution and support of infrageneric relationships within section *Compsooneura*. Although the position of *C. ulei* is not well supported, it has a fused anther column like *C. mutisii* and *C. excelsa* but the rest of the *Compsooneura* and *Hadrocarpa* clades have free anthers. The phylogenetic estimation created here requires multiple conversions from the state of free to fused anthers. Great importance has been placed on the androecium in morphological cladistics since it is one of the few variable characters of *Compsooneura* (Janovec 2000) as well as

other genera (Sauquet et al. 2003). If the phylogenetic estimation presented here is found to be robust with future data, it may mean that fusion of the androecium is potentially homoplasious.

Although there were relatively few species included from section *Hadrocarpa*, due to the old age of most collections and difficulty in extracting high quality DNA for the amplification of low copy nuclear sequences, there remains a few interesting trends to note. The first is the apparent paraphyletic nature of the cpDNA of *C. capitellata*. Due to difficulties in amplifying herbarium material, the *C. atopa* specimen used in this analysis came from the most recent collection which was found growing in a coffee plantation in the vicinity of Comuna 24 de Mayo, Ecuador, in 2000. This tree also constitutes one of only a few collections of *C. atopa* in the Amazonian basin as it is endemic to Colombia West of the Andes. Despite numerous attempts to locate additional individuals, there have been only two collections made of this species east of the Andes to date. Therefore it is possible that this tree might be one of the last remnants of a refugial Amazonian population of *C. atopa* and may have hybridized to some extent with *C. capitellata*. Additional data from chloroplast and nuclear markers will be required to test this hypothesis as the most variable marker (AGT1) was not recovered for *C. atopa* in this study. Additionally there appears to be a great deal of molecular divergence within the species *C. capitellata* which will be discussed below and in a subsequent chapter.

A robust phylogenetic analysis will require known outgroups, however, this is difficult due to the unresolved phylogeny of the Myristicaceae (Sauquet et al. 2003) and the fact that the family likely diverged from sister taxa about 100 mybp. This large amount of evolutionary time may lead to a great deal of homoplasy and long stem

lengths, potentially leading to long branch attraction in the highly variable markers necessary to resolve intergeneric relationships of the Myristicaceae. Although it cannot be assured that this is a properly rooted phylogeny, the topology recovered here, however, makes intuitive sense based on the apparent pattern of trans-andean speciation of some taxa of *Compsooneura*. Additionally, the 3 markers employed in this study had substantially more variation than others used by previous studies and may be informative for future studies of Myristicaceae phylogeny.

Distance Analysis

The second objective of this study was to perform an analysis of the ability of a chloroplast locus to distinguish members of *Compsooneura*, including rare collections and samples already sequenced by Newmaster et al. (2008). The morphological uniformity, slow rates of molecular evolution, and dearth of molecular markers from previous investigators has made the taxonomy of this group particularly difficult. The inclusion of 5 additional taxa to the 8 taxa studied by Newmaster et al. (2008) resulted in a trnH-psbA NJ tree where all but one species pair (*C. carchifolia* and *C. cuatrecasasi*) are differentiated by unique haplotypes. These two species species are easily distinguished morphologically by dissimilar leaf and flower morphologies and also have disjunct distributions.

Although most species are rather morphologically coherent, there appears to be a relatively large divergence among the sequences of different populations of some species (*C. sprucei*, *C. mutisii*, *C. mexicana*, and *C. capitellata* for example). This pattern may be due to a number of non-mutual exclusive phenomena. The first possibility is that these

divergent populations may represent cryptic species. This is very possible as recent molecular studies of tropical insects (Hebert et al. 2004b, Smith et al. 2007, Smith et al. 2008) have revealed previously unnoticed alpha diversity. It is highly possible that there exists undescribed species of neotropical Myristicaceae considering the cryptic morphological nature of these trees and the wide range of climatological and edaphic regimes present across the Amazon basin. If these populations are not continuous it is also possible that these geographically isolated haplotypes represent restricted gene flow between discontinuous populations. Since the chloroplast genome is inherited from the maternal parent and spread via seed, this pattern could be generated by restricted seed dispersal and/or restricted seed establishment between populations. If these genetic polymorphisms are indeed an indicator of intra-specific haplotype diversity and not cryptic speciation it will prove difficult to unambiguously identify new collections by means of DNA barcodes as the genetic distance within a species may be equal or greater than that between currently recognized species. Despite these problems, trnH-psbA will be a very useful tool to biologists to help identify sterile, herbarium and fragmentary material of *Compsooneura* since morphological means of identification may lead to mis-identification rates of 25-60% (J. Janovec personal communication, R. Steeves personal observations). Additional studies with increased population-level sampling are recommended to sample haplotype diversity across the geographic range of these species; of particular interest will be the study of the more hypervariable taxa such as *C. sprucei*, *C. mexicana*, and *C. capitellata*.

Although the primary function of the distance analysis was to assess the ability of the trnH-psbA locus to discriminate species of *Compsooneura*, there are also some

relationships on the tree (Figure 1.3) of biogeographical significance worthy of discussion. Two members of Janovec's (2000) informal subgroup Atopa (*C. rigidifolia* and *C. sp1*) form a clade (Choco-Panama clade Figure 1.3) with the exception of the groups namesake, *C. atopa*. Likewise, members of Janovec's (2000) informal subgroup Capitellata included in this analysis (*C. capitellata*, *C. cuatrecasii*, *C. diazii*, and *C. sp2*) are also paraphyletic in this tree. Members of subgroup Atopa are endemic to Western Colombia and Panama and those of subgroup Capitellata are found in both the East and Western slopes of the Andes. Members of subgroup Atopa and Capitellata have a number of vegetative and reproductive morphological traits which distinguish each respective subgroup (Janovec 2000) but it appears that the similarity of their trnH-psbA haplotypes is primarily correlated to geographic region (Amazonian or Choco-Panama) rather than morphological subgroupings (sensu Janovec 2000). This apparent incongruence of morphological affinities of taxa and their haplotypes similarities is possibly due to convergent morphological evolution but is more likely a result of cpDNA paraphyly due to introgression of the maternally inherited cpDNA. Chloroplast haplotype introgression between species, also known as chloroplast sharing or chloroplast capture, is well documented in plants by phylogenetic and phylogeographic studies and appears to be relatively common (Rieseberg and Soltis 1991, Golden and Bain 2000, Palme et al. 2003, Palma-Silva et al. 2011). This phenomena may also account for high levels of intraspecific genetic distance found in plant plastid regions (Hollingsworth et al 2011). In the future it would be desirable to compare nuclear and plastid phylogenies of *Compsoneura* (subgroups Atopa and Capitellata in particular) to establish whether

introgression or convergent morphological evolution are likely the cause of discrepancy between morphological and molecular data.

Tables

Table 1.1 *Compsonera* and outgroup taxa used in all analyses. Collector(s), Collections number (Coll. #), code of Herbaria storing (Herb.), Country of origin, decimal degrees latitude (Lat.), decimal degrees longitude (Long.), Barcode of Life Database sequence process ID (BOLD #), and genbank accession numbers are listed if applicable.

Species	Collector (s)	Coll. #	Herb.	Country	Lat.	Long.	BOLD #	Genbank #
<i>C. atopa</i>	J.P. Janovec and W. Quizhpe	1374	NY	Ecuador	-0.47	-77.26	RSMYR002-11	EU090622.1
<i>C. capitellata</i>	R.A.D. Steeves	527	OAC	Peru	-13.23	-70.78	RSMYR003-11	
<i>C. capitellata</i>	R.A.D. Steeves	551	OAC	Peru	-13.23	-70.78	RSMYR004-11	
<i>C. capitellata</i>	J.P. Janovec	835		Peru	-6.15	-76.17	RSMYR005-11	EU090623.1
<i>C. capitellata</i>	J.P. Janovec	855		Peru	-3.52	-73.15	RSMYR006-11	EU090624.1
<i>C. capitellata</i>	J.P. Janovec	872		Peru	-3.52	-73.15	RSMYR007-11	EU090625.1
<i>C. capitellata</i>	J.P. Janovec	875		Peru	-3.52	-73.15	RSMYR008-11	EU090626.1
<i>C. capitellata</i>	J.P. Janovec and W. Quizhpe	889		Ecuador	-1.04	-77.37	RSMYR009-11	EU090627.1
<i>C. sp.1</i>	C. Aulestia et al.	848	QCNE	Ecuador	0.55	-78.32	RSMYR010-11	
<i>C. cuatrecasasi</i>	A.H. Gentry and A. Juncosa	40682	MO	Colombia	3.15	-77.25	RSMYR011-11	
<i>C. diazii</i>	C. Diaz et al.	7644	MO	Peru	-5.03	-78.22	RSMYR012-11	
<i>C. rigidifolia</i>	G. Mcpherson	10008	Duke	Panama	9.15	-79.30	RSMYR013-11	
<i>C. sp.2</i>	A.H. Gentry and E. Renteria	23826	MO	Colombia	5.30	-76.33	RSMYR014-11	
<i>C. debilis</i>	M.L. Kawasaki	190	CAS	Brazil	0.18	-68.40	RSMYR015-11	EU090628.1
<i>C. debilis</i>	P.E. Berry et al.	6172	MO	Venezuela	2.52	-67.18	RSMYR016-11	EU090629.1
<i>C. debilis</i>	P.E. Berry	7209		Venezuela			RSMYR017-11	EU090630.1
<i>C. debilis</i>	R.L. Liesner and G. Carnevali	22972	MO	Venezuela	1.51	-67.03	RSMYR018-11	EU090631.1
<i>C. excelsa</i>	J.P. Janovec and R. Aguilar	636	NY	Costa Rica	8.43	-83.12	RSMYR019-11	EU090632.1
<i>C. excelsa</i>	J.P. Janovec and R. Aguilar	666	NY	Costa Rica	8.44	-83.28	RSMYR020-11	EU090633.1
<i>C. excelsa</i>	J.P. Janovec	668	NY	Costa Rica	8.26	-83.22	RSMYR021-11	EU090634.1
<i>C. excelsa</i>	J.P. Janovec	669	NY	Costa Rica	8.26	-83.22	RSMYR022-11	EU090635.1
<i>C. excelsa</i>	J.P. Janovec	671	NY	Costa Rica	8.26	-83.22	RSMYR023-11	EU090636.1
<i>C. mexicana</i>	V. Tzub	007					RSMYR024-11	EU090637.1
<i>C. mexicana</i>	J.P. Janovec	354	TAMU	Costa Rica	10.25	-83.29	RSMYR025-11	EU090638.1
<i>C. mexicana</i>	J.P. Janovec	362	TAMU	Costa Rica	10.25	-83.29	RSMYR026-11	EU090639.1

Species	Collector (s)	Coll. #	Herb.	Country	Lat.	Long.	BOLD	GB
<i>C. mexicana</i>	J.P. Janovec and A. Neill	696	NY	Belize	16.23	-89.08	RSMYR027-11	EU090640.1
<i>C. mexicana</i>	J.P. Janovec and A. Neill	701	NY	Belize	16.23	-89.08	RSMYR028-11	EU090641.1
<i>C. mexicana</i>	J.P. Janovec and A. Neill	719	NY	Belize	16.12	-89.03	RSMYR029-11	EU090642.1
<i>C. mexicana</i>	J.P. Janovec and A. Neill	720	NY	Belize	16.12	-89.03	RSMYR030-11	EU090643.1
<i>C. mexicana</i>	J.P. Janovec and J.A. Janovec	757	NY	Belize	16.20	-89.10	RSMYR031-11	EU090644.1
<i>C. mexicana</i>	J.P. Janovec	1283					RSMYR032-11	EU090645.1
<i>C. mutisii</i>	J.P. Janovec and Quizhpe	911	NY	Ecuador	1.03	-78.32	RSMYR033-11	EU090646.1
<i>C. mutisii</i>	J.P. Janovec and Quizhpe	913	NY	Ecuador	1.03	-78.32	RSMYR034-11	EU090647.1
<i>C. mutisii</i>	J.P. Janovec and Quizhpe	914	NY	Ecuador	1.03	-78.32	RSMYR035-11	EU090648.1
<i>C. mutisii</i>	J.P. Janovec	1290					RSMYR036-11	EU090649.1
<i>C. mutisii</i>	J.P. Janovec	1295					RSMYR037-11	EU090650.1
<i>C. sprucei</i>	J.P. Janovec and A. Pena Cruz	812	NY	Peru	-6.06	-76.11	RSMYR038-11	EU090651.1
<i>C. sprucei</i>	J.P. Janovec and A. Pena Cruz	817	NY	Peru	-6.06	-76.11	RSMYR039-11	EU090652.1
<i>C. sprucei</i>	J.P. Janovec and A. Pena Cruz	821	NY	Peru	-6.06	-76.11	RSMYR040-11	EU090653.1
<i>C. sprucei</i>	J.P. Janovec and W. Quizhpe	884	NY	Ecuador	-1.04	-77.37	RSMYR041-11	EU090654.1
<i>C. sprucei</i>	J.P. Janovec and W. Quizhpe	887	NY	Ecuador	-1.04	-77.37	RSMYR042-11	EU090655.1
<i>C. sprucei</i>	J.P. Janovec and W. Quizhpe	903	NY	Ecuador	-1.04	-77.37	RSMYR043-11	EU090656.1
<i>C. ulei</i>	E. Lima and A. Silva	088	NY	Brazil	-3.50	-49.42	RSMYR044-11	EU090657.1
<i>C. ulei</i>	C.R. Sperling et al.	6192	US	Brazil	-5.49	-50.32	RSMYR045-11	EU090658.1
<i>C. ulei</i>	M. Nee	42644	NY	Brazil	-2.25	-59.54	RSMYR046-11	EU090659.1
<i>O. glycyarpa</i>	R.A.D. Steeves	546	OAC	Peru	-13.23	-70.78	RSMYR047-11	
<i>O. parvifolia</i>	R.A.D. Steeves	598	OAC	Peru	-13.23	-70.78	RSMYR048-11	
<i>V. surinamensis</i>	R.A.D. Steeves	078	OAC	Peru	-13.23	-70.78	RSMYR049-11	
<i>I. juruensis</i>	R.A.D. Steeves	451	OAC	Peru	-13.23	-70.78	RSMYR050-11	
<i>I. laevis</i>	R.A.D. Steeves	460	OAC	Peru	-13.23	-70.78	RSMYR051-11	

Table 1.2 Unambiguous morphological character transformations that differentiate informal sections *Compsonaura* and *Hadrocarpa*.

Compsonaura	Morphological Character	Hadrocarpa
Drying green, sometimes brown	Leaves	Drying brown, rarely olive or brownish-green
Free, arching (venation eucamptodromous)	Secondary leaf veins	Anastomosing marginally, at least in part (venation weakly to strongly brochidodromous)
Free from above the base, semi-connate, or connate	Anthers	Free from the base
Present	Glandular trichomes on inner flower perianth, filaments, and filament columns	Absent or rarely present in one species (<i>C. diazii</i>)
Absent	T-shaped or stellate trichomes on vegetative and reproductive organs	Present
Absent, or few and inconspicuous	Floral oil cells	Abundant and conspicuous
Elliptic to ovate-elliptic	Fruit shape	Sub-globose, elliptic-ovate, to strongly rostrate
Orange, thin, fleshy	Fruit pericarp at maturity	Green to brown, thick, fortified, ligneous
Always smooth	Fruit pericarp surface	Sulcate to strongly furrowed, or carinate to strongly ridged
Strongly dehiscent along one longitudinal line	Fruit pericarp dehiscence	Indehiscent to partially or "forced" dehiscent
Glabrous	Fruit pericarp pubescence	Pubescent throughout or at least in part; hairs t-shaped to irregularly stellate
Thick, entire, somewhat fleshy, bright red to scarlet when mature	Aril	Thin, entire to deeply lacinate from base, or rudimentary, reduced, and sometimes seemingly absent; white when mature but rarely seen because the fruit pericarps are largely indehiscent

Smooth, gray, black to purple
mottled

Seed testa

Rough to rugose, weakly furrowed to veined, brown, non-
mottled

Figures

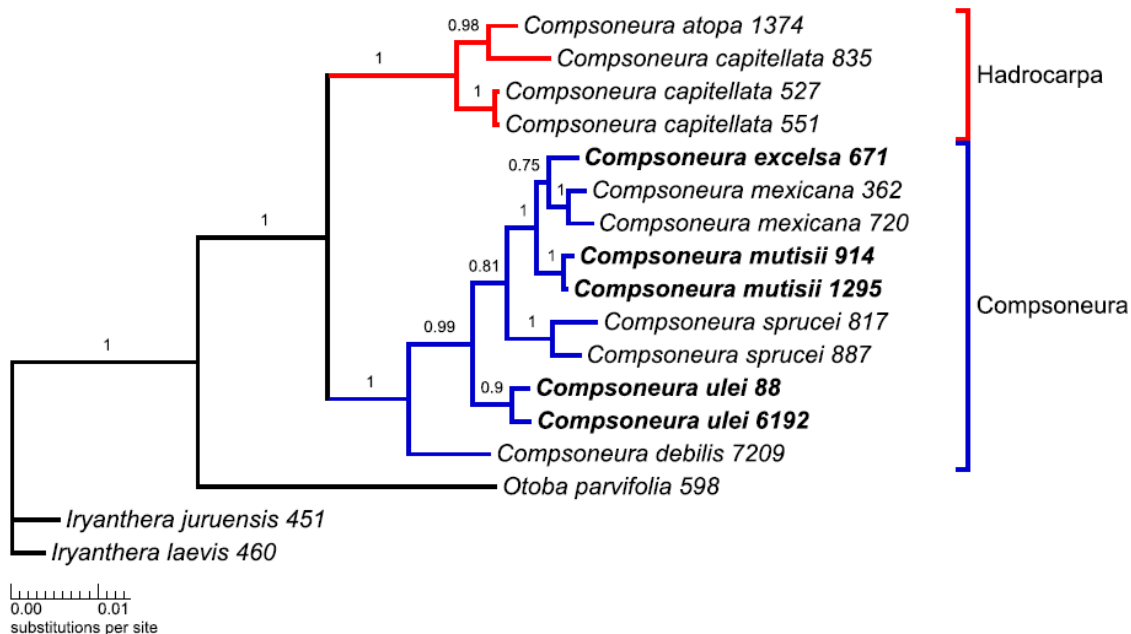


Figure 1.1 Phylogram of consensus tree created with sequences of trnH-psbA, AT103 and AGT1 sequences of 8 species of *Componeura*. Numbers on branches indicate Bayesian posterior probabilities of nodes and numbers following taxon names are the collection numbers of herbarium samples from which DNA was extracted (see table 1.1 for collection information). Branch colours indicate sections Hadrocarpa (red) and *Componeura* (blue). Bolded taxa possess fused anthers. Branch lengths are proportional to distance.

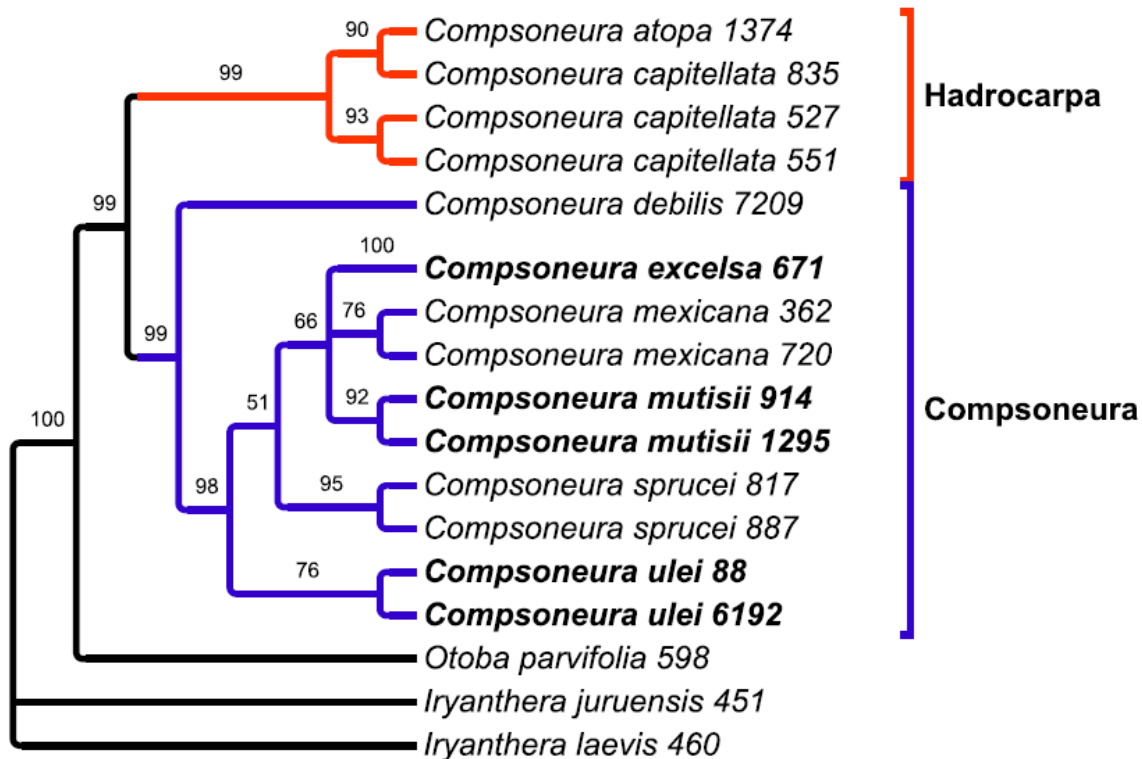


Figure 1.2 Phylogram of the single most parsimonious tree (Length=187) constructed from a concatenated alignment of trnH-psbA, AGT1 and AT103 data sets (CI=0.85, RI=0.88, HI=0.15). Numbers on branches indicate bootstrap values and numbers following taxon names are the collection numbers of herbarium samples from which DNA was extracted (see table 1.1 for collection information). Branch colours indicate sections Hadrocarpa (red) and Compsonaura (blue). *Compsonaura* taxa in bold font possess fused anthers and non-bolded taxa possess free anthers.

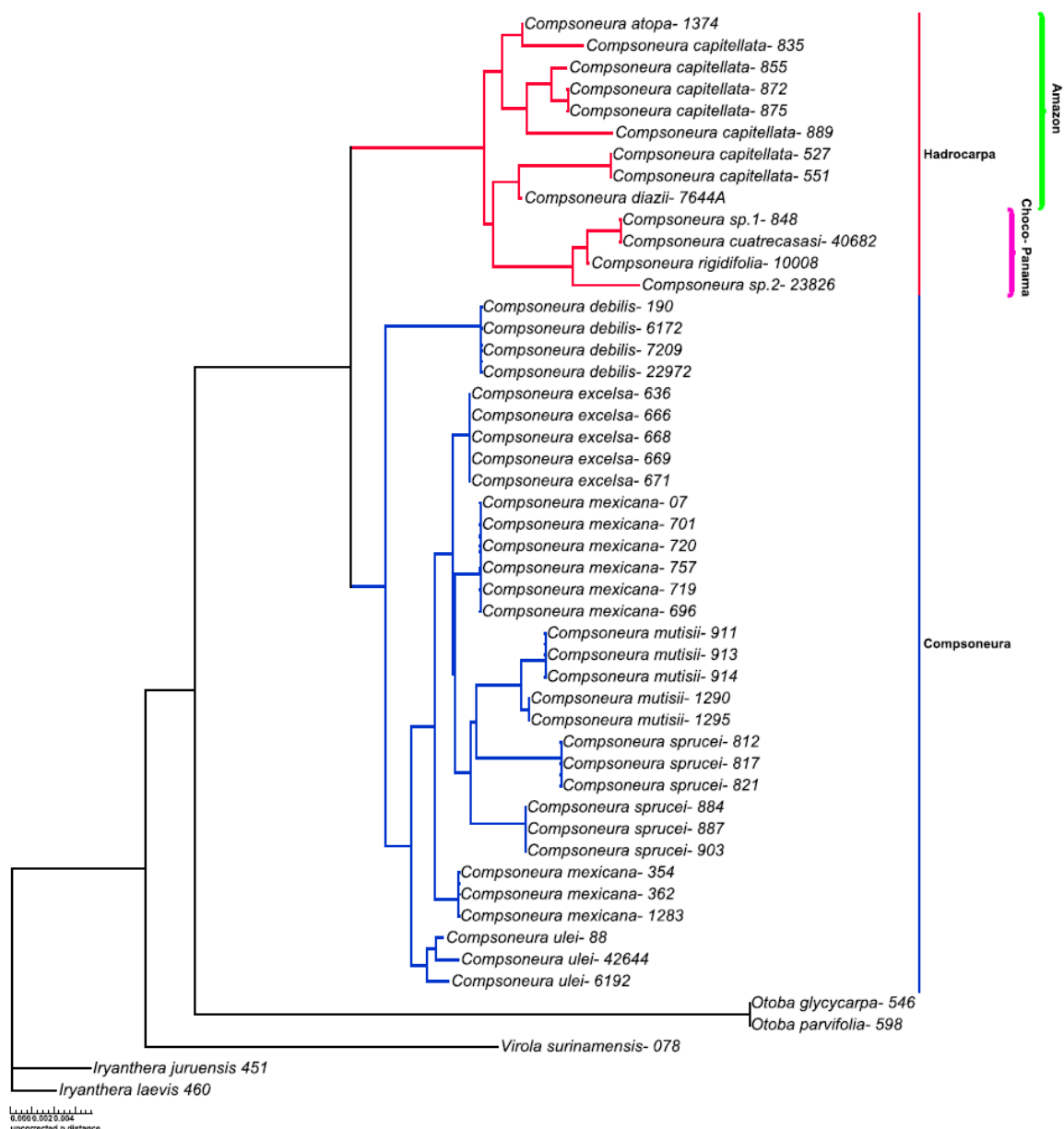


Figure 1.3 Neighbour joining (uncorrected p-distance) tree constructed using trnH-psbA data. Red branches indicate members of section *Hadrocarpa* and blue branches indicate section *Compsonneura*. Numbers indicate collection numbers of herbarium samples from which DNA was extracted (also see table 1.1). Geographical zones (Amazonian or Choco-Panama) of various species of *Hadrocarpa* is indicated by lines on the right. Branch lengths are proportional to distance.

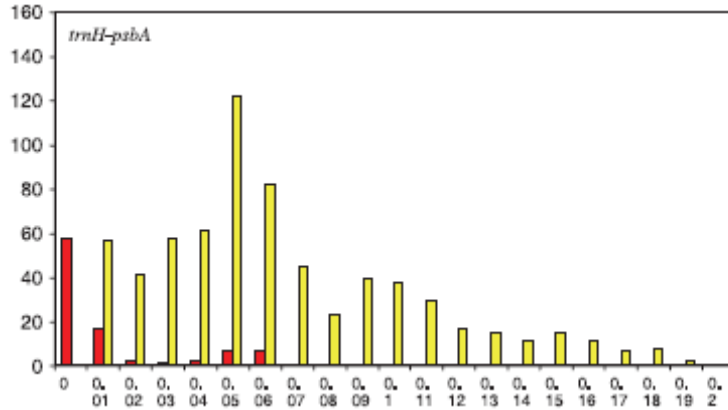


Figure 1.4 Histograms of the number of pairwise intraspecific (red bars) and interspecific divergence distances estimates (x-axes) among all *Compsooneura* samples included in the neighbour joining analysis. The number of pairwise comparisons is on the y-axis and the pairwise p-distance for the trnH-psbA region is on the x-axis.

Literature Cited

- Alfaro, M. E., & Holder, M. T. 2006. The posterior and the prior in Bayesian phylogenetics. *Annual review of ecology, evolution, and systematics* **37**: 19-42.
- APG III. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II.
- Armstrong, J. E., & Tucker, S. C. 1986. Floral development in *Myristica* (Myristicaceae). *American Journal of Botany* **73**: 1131–1143.
- Armstrong, J. E. 1997. Pollination by deceit in nutmeg (*Myristica insipida*, Myristicaceae): floral displays and beetle activity at male and female trees. *American journal of botany* **84**: 1266-1274.
- Asif, M. J., & Cannon, C. H. 2005. DNA extraction from processed wood: a case study for the identification of an endangered timber species (*Gonystylus bancanus*). *Plant Molecular Biology Reporter* **23**: 185–192.
- Bullock, S. H. 1982. Population structure and reproduction in the neotropical dioecious tree *Compsonaura sprucei*. *Oecologia* **55**: 238–242.
- Candolle, A. D. 1856. Myristicaceae. *Prodromus Systematis Naturali Vegetabilis* **14**: 187-208.
- Chagnon, N. A., Le Quesne, P., & Cook, J. M. 1971. Yanomamó Hallucinogens: Anthropological, Botanical, and Chemical Findings. *Current Anthropology* **12**: 72–74.
- Douady, C. J., Delsuc, F., Boucher, Y., Doolittle, W. F., & Douzery, E. J. 2003. Comparison of Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability. *Molecular Biology and Evolution* **20**: 248-254.
- Farris, J. S., Källersjö, M., Kluge, A. G., & Bult, C. 1995. Testing significance of incongruence. *Cladistics* **10**: 315-319.
- Fazekas, A. J., Steeves, R., & Newmaster, S. G. 2010. Improving sequencing quality from PCR products containing long mononucleotide repeats. *Biotechniques* **48**: 277–285.
- Gentry, A. H. 1982. Neotropical floristic diversity: phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden* **69**: 557–593.

- Golden, J. L., & Bain, J. F. 2000. Phylogeographic patterns and high levels of chloroplast DNA diversity in four *Packera* (Asteraceae) species in southwestern Alberta. *Evolution* **54**: 1566–1579.
- Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series*. (pp. 95–98).
- Hebert, P. D. N., Stoeckle, M. Y., Zemplack, T. S., & Francis, C. M. 2004. Identification of birds through DNA barcodes. *PLOS Biology* **2**: 1657-1663.
- Hebert, P. D., Penton, E. H., Burns, J. M., Janzen, D. H., & Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astrartes fulgerator*. *Proceedings of the National Academy of Sciences* **101**: 14812-14817.
- Hollingsworth, P. M., Graham, S. W., & Little, D. P. 2011. Choosing and using a plant DNA barcode. *PloS one* **6**: e19254.
- Janovec, J. P., & Harrison, J. S. 2002. A morphological analysis of the *Compsoeura sprucei* complex (Myristicaceae), with a new combination for the Central American species *Compsoeura mexicana*. *Systematic Botany* **27**: 662–673.
- Janovec, J. P., & Neill, A. K. 2002. Studies of the Myristicaceae: an overview of the *Compsoeura atopa* complex, with descriptions of new species from Colombia. *Brittonia* **54**: 251–261.
- Janovec, J. P. 2000. A systematic study of *Compsoeura* (A. DC.) Warb., A Neotropical member of the nutmeg family. Texas A&M University Dissertation: 1-359.
- Kreader, C. A. 1996. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Applied and Environmental Microbiology* **62**: 1102-1106.
- Kumar, S., Nei, M., Dudley, J., & Tamura, K. 2008. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in bioinformatics* **9**: 299-306.
- Li, M., Wunder, J., Bissoli, G., Scarponi, E., Gazzani, S., Barbaro, E., Saedler, H., & Varotto, C. 2008. Development of COS genes as universally amplifiable markers for phylogenetic reconstructions of closely related plant species. *Cladistics* **24**: 727–745.
- Lister, D. L., Bower, M. A., Howe, C. J., & Jones, M. K. 2008. Extraction and amplification of nuclear DNA from herbarium specimens of emmer wheat: a

- method for assessing DNA preservation by maximum amplicon length recovery. *Taxon* **57**: 254–258.
- Macedo, D. S., & Anderson, A. B. 1993. Early ecological changes associated with logging in an Amazon floodplain. *Biotropica* **25**: 151–163.
- Maloney, D. J., Deng, J., Starck, S. R., Gao, Z., & Hecht, S. M. 2005. (+)-Myristinin A, a naturally occurring DNA polymerase β inhibitor and potent DNA-damaging agent. *Journal of the American Chemical Society* **127**: 4140–4141.
- McKenna, D. J., Towers, G. H. N., & Abbott, F. S. 1984. Monoamine oxidase inhibitors in South American hallucinogenic plants part 2: Constituents of orally-active Myristicaceous hallucinogens. *Journal of Ethnopharmacology* **12**: 179–211.
- Müller, K. 2006. Incorporating information from length-mutational events into phylogenetic analysis. *Molecular phylogenetics and evolution* **38**: 667–676.
- Newmaster, S. G., Fazekas, A. J., Steeves, R. A. D., & Janovec, J. 2008. Testing candidate plant barcode regions in the Myristicaceae. *Molecular Ecology Resources* **8**: 480–490.
- Nguyen, P. H., Le, T. V. T., Kang, H. W., Chae, J., Kim, S. K., Kwon, K., Seo, D. B., Lee, S. J., & Oh, W. K. 2010. AMP-activated protein kinase (AMPK) activators from *Myristica fragrans* (nutmeg) and their anti-obesity effect. *Bioorganic and Medicinal Chemistry Letters* **20**: 4128–4131.
- Nylander, J. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Palma-Silva, C., Wendt, T., Pinheiro, F., Barbará, T., FAY, M. F., Cozzolino, S., & Lexer, C. Sympatric bromeliad species (*Pitcairnia spp.*) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. *Molecular Ecology* **20**: 3185–3201
- Palmé, A. E., Semerikov, V., & Lascoux, M. 2003. Absence of geographical structure of chloroplast DNA variation in sallow, *Salix caprea* L. *Heredity* **91**: 465–474.
- Pascal, J. P., & Pelissier, R. 1996. Structure and floristic composition of a tropical evergreen forest in south-west India. *Journal of Tropical Ecology* **12**: 191–214.
- Patro, S. P., Tyagi, M., Saha, J., & Chattopadhyay, S. 2010. Comparative nuclease and anti-cancer properties of the naturally occurring malabaricones. *Bioorganic and Medicinal Chemistry* **18**: 7043–7051.
- Poinar, H. N. 1998. Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* **281**: 402–406.

- Poulsen, A., Nelson, I., Tan, S., & Balsev, H. 1996. A quantitative inventory of trees in one hectare of mixed dipterocarp forest in Temburong, Borneo Darussalam. In *Tropical rainforest research: current issues*. London: Kluwer Academic Publishers (pp. 139-150).
- Pusztai, R., Abrantes, M., Sherly, J., Duarte, N., Molnar, J., & Ferreira, M. J. 2010. Antitumor-promoting Activity of Lignans: Inhibition of Human Cytomegalovirus IE Gene Expression. *Anticancer research* **30**: 451-454.
- Qiu, Y-L., Li, L., Hendry, T.A., Li, R., Taylor, D.W., Issa, M.J., Ronen, A.J., Vekaria, M.L., & White, A.M. 2006. Reconstructing the basal angiosperm phylogeny: evaluating information content of mitochondrial genes. *Taxon* **55**:837-856.
- Raven, P. H., & Axelrod, D. I. 1974. Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden* **61**: 539-673.
- Rieseberg, L.H., & Soltis, D.E. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* **5**: 65-84.
- Rodrigues, W. A. 1989. Two new neotropical species of *Compsonera* (Myristicaceae). *Brittonia* **4**: 160-163.
- Ronquist, F., & Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574.
- Sang, T., Crawford, D. J., & Stuessy, T. F. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* **84**: 1120-1136.
- Sasaki, Y., Miyoshi, D., & Sugimoto, N. 2006. Effect of molecular crowding on DNA polymerase activity. *Biotechnology Journal* **1**: 440-446.
- Sauquet, H., Doyle, J. A., Scharaschkin, T., Borsch, T., Hilu, K. W., Chatrou, L. W., & Le Thomas, A. 2003. Phylogenetic analysis of Magnoliales and Myristicaceae based on multiple data sets: implications for character evolution. *Botanical Journal of the Linnean Society* **142**: 125-186.
- Savolainen, V., Cuénoud, P., Spichiger, R., Martinez, M. D., Crèvecoeur, M., & Manen, J. F. 1995. The use of herbarium specimens in DNA phylogenetics: evaluation and improvement. *Plant Systematics and Evolution* **197**: 87-98.
- Sawadjoon, S., Kittakoop, P., Kirtikara, K., Vichai, V., Tanticharoen, M., & Thebtaranonth, Y. 2002. Atropisomeric myristinins: Selective COX-2 inhibitors and antifungal agents from *Myristica cinnamomea*. *The Journal of Organic Chemistry* **67**: 5470-5475.
- Schultes, R. 1981. Iconography of the New World Hallucinogens. *Arnoldia* **41**: 80-125.

- Simmons, M., & Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analysis. *Systematic Botany* **49**: 369-381.
- Smith, A. C. Studies of South American plants: XV. *American Journal of Botany* **43**: 573-577.
- Smith, A. C. 1937. The American species of Myristicaceae. *Brittonia* **2**: 393-510.
- Smith, M. A., Rodriguez, J. J., Whitfield, J. B., Deans, A. R., Janzen, D. H., Hallwachs, W., & Hebert, P. D. 2008. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proceedings of the National Academy of Sciences* **105**: 12359-12364.
- Smith, M. A., Wood, D. M., Janzen, D. H., Hallwachs, W., & Hebert, P. D. N. 2007. DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. *Proceedings of the National Academy of Sciences* **104**: 4967-4972.
- Soltis, D. E., Smith, S. A., Cellinese, N., Wurdack, K. J., Tank, D. C., Brockington, S. F., Refulio-Rodriguez, N. F., Walker, J. B., Moore, M. J., Carlswald, B. S., & others. 2011. Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal of Botany* **98**: 704.
- Speiss, A., T. Mueller, & Ivell, R. 2004. Trehalose is a potent PCR enhancer: lowering of DNA melting temperature and thermal stabilization of Taq polymerase by the disaccharide trehalose. *Clinical chemistry* **50**: 1256-1259.
- Spichiger, R., Loizeau, P., Latour, C., & Barriera, G. 1996. Tree species richness in a southwestern Amazonian forest. *Candollea* **51**: 559-577.
- Štorchová, H., & Olson, M. S. 2007. The architecture of the chloroplast psbA-trnH non-coding region in angiosperms. *Plant Systematics and Evolution* **268**: 235-256.
- Stöver, B. C., & Müller, K. F. 2010. TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC bioinformatics* **11**: 1-9.
- Suzuki, Y., Glazko, G. V., & Nei, M. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proceedings of the National Academy of Sciences* **99**: 16138-16143.
- Swafford, D. 1999. PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods). Version 4. Sunderland: Sinauer Associates.
- Tajuddin, S. A., Latif, A., Qasmi, I., & Amin, K. 2005. An experimental study of sexual function improving effect of *Myristica fragrans* Houtt. (nutmeg). *BMC Complementary and Alternative Medicine* **5**: 16-22.

- Tate, J. A., & Simpson, B. B. 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Systematic Botany* **28**: 723–737.
- Telle, S., & Thines, M. 2008. Amplification of *cox2* (~620 bp) from 2 mg of Up to 129 Years Old Herbarium Specimens, Comparing 19 Extraction Methods and 15 Polymerases (R. DeSalle, Ed.). *PLoS ONE* **3**: e3584.
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research* **22**: 4673-4680.
- Van Gils, C., & Cox, P. A. 1994. Ethnobotany of nutmeg in the Spice Islands. *Journal of Ethnopharmacology* **42**: 117–124.
- Warburg, O. 1897. Monographie der Myristicaceen. *Nova Acta Acad. Caes. Leop.-Carol* **68**: 1-680.
- Wilde, W. J. J. O. D. 1991. The genera of the Myristicaceae as distinguished by their inflorescences and the description of a new genus: *Bicuiba*. *Beitr. Biol. Pflanzen* **66**: 95-125.
- Yang, Z., & Rannala, B. 2010. Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences* **107**: 9264-9269.
- Zimmerman, S. B., & Harrison, B. 1987. Macromolecular crowding increases binding of DNA polymerase to DNA: an adaptive effect. *Proceedings of the National Academy of Sciences of the United States of America* **84**: 1871-1875.

Chapter 2

A MORPHOLOGICAL AND MOLECULAR INVESTIGATION OF THE *COMPSONEURA CAPITELLATA* (A. DC) Warb. COMPLEX

Abstract

Members of *Compsonneura* are canopy-subcanopy trees native to tropical rainforests of Central and South America. *Compsonneura capitellata* is a common and widespread member of the Myristicaceae family found in both cloud and lowland rainforests of Northwestern Amazonia. The flowers, fruits and leaves of members of the *Compsonneura capitellata* complex exhibit a high degree of morphological variation but the species has not received careful study since 1937 when few specimens were available and even the fruits were unknown. Many specimens of *C. capitellata* have since been collected from the entire species' range. The aim of this study was to investigate patterns of morphological and genetic diversity in the *C. capitellata* complex. Morphological analyses of leaf, perianth and androecial characters revealed little differentiation among populations, however, leaf characters exhibited some differentiation according to habitat (montane versus lowland populations). In contrast, DNA sequencing of 60 *C. capitellata* individuals, from 7 populations, with the trnH-psbA intergenic spacer revealed 9 haplotypes, with each population containing only haplotypes unique to that locale. I speculate that the apparent morphological continuum and contrasting genetic sub-division across the geographic landscape is potentially a result of restricted seed dispersal, historic anthropogenic use, cryptic speciation, or a combination of these phenomena. Herein I

present *C. morona-santiagoensis*, a provisional new species endemic to the sandstone substrates of Cutucu and the Cordillera del Condor of Ecuador and suggest the resurrection of *C. tessmannii* (Markgraf), a large-leaved taxa from the mountainous cloud forests of Peru.

Introduction

The Myristicaceae, or nutmeg family, is a pantropical assemblage of canopy to subcanopy trees comprised of 21 genera and about 500 species. These trees are native to cloud and lowland tropical rainforests where they are often among the most diverse and abundant tree families of those environments (Gentry 1988, Pitman et al. 2001, Pitman et al. 2002). Six genera, *Bicuiba*, *Compsoeura*, *Iryanthera*, *Ostephloem*, *Otoba*, and *Virola* are native to the Central and South America. A recent investigation of *Compsoeura* (chapter 1 this thesis) revealed evidence of a deep evolutionary divergence between informal sections *Hadrocarpa* and *Compsoeura* (sensu Janovec 2000) supported by numerous morphological and molecular characters. Members of *Compsoeura* are typified by having mostly glabrous leaves with brochidodromous or eucamptodromous secondary veins, tertiary venation perpendicular to the midvein and are endemic to Central and South America.

Compsoeura capitellata is a common and widespread dioecious tree species that inhabits old growth cloud and lowland rainforests of Northwest Amazonia (Brazil, Colombia, Ecuador, and Peru) from 100 m-1500 m elevation above sea level (asl). *Compsoeura capitellata* was one of the first species of *Compsoeura* described by de Candolle (1856), however it has been studied little since that time. The flowers, fruits and leaves of *Compsoeura capitellata* exhibit a high degree of morphological diversity but

the species has not received careful study since the last monographic treatment by Smith (1937). At that time very few specimens were available for study (n=11) from only a few localities and the only fruit sample available (Ducke 1957, B, K, S, US, Utr) was deemed so atypical (i.e. a thick woody pericarp and large non-arillate seed) that it couldn't belong to Myristicaceae (Smith 1937), although the fruit in question was that of *C. capitellata* (Janovec 2000). Smith had very few fertile specimens to study and elected to ascribe vegetatively diverse collections from the Northwest Amazon as *C. capitellata* and synonymized the previously described *C. tessmannii* (Markgraf), following the monospecific concept of Warburg (1897).

Many collections of *C. capitellata* have been made since the last taxonomic treatment, which has expanded the species' known range and revealed a considerable degree of morphological variation. The chartaceous to thick coriaceous, simple, and alternate leaves of this species are elliptic-ovate in shape, being 2-13 cm wide (at half length) and 4-50 cm long with the smallest leaves generally occurring in the lowlands and the larger-leaved varieties generally occurring at higher elevations. The bark exhibits a wide range of colours and textures; from pale grey to brown to red and peeling off in large brittle plates or long fibrous slivers. The staminate flower is typically composed of 3-4 tepals forming a globose to cupuliform flower 1.4-2.5 mm long and 1.1-1.8 mm wide with an androecium composed of 5-12 anthers which are 0.4-0.7 mm in length. Fruits of this species are typically 2-6 cm long and 2-5 cm wide. The fruit is composed of a greenish, woody, indehiscent or forced-dehiscent pericarp which is 3-7 mm thick, glabrous to ferruginous-tomentellous, green to reddish brown in colour, and smooth, muricate, or linearly to irregularly sulcate in texture. A thin white aril, which is

sometimes greatly reduced, typically covers the large dull brown seed (1.5-4 cm), which is comprised of an entire, white, endosperm. *Compsonaura capitellata* is often confused with *C. sprucei* collections as *C. capitellata* is only reliably differentiated by its thick, woody pericarp, white aril, and sometimes by the thickness of the leaves and more strongly arcuate secondary veins compared to *C. sprucei*.

While other members of Myristicaceae have been the subject of ecological investigations (Howe and Vande Kerkhove 1981, Bullock 1982, Cole 2009, Holbrook and Loiselle 2009), members of *Compsonaura* have been the subject of few ecological studies (Bullock 1982). *C. capitellata* has yet to be the focus of any detailed ecological studies and thus mechanisms of pollination, vectors of dispersal, and habitat preference are virtually unknown (Janovec personal communication). The large geographic and altitudinal range that this species inhabits translates into a wide range of light regimes, temperatures, precipitation, and substratum encountered by populations. Although the pollinators of *C. capitellata* are not known, the small (1-4mm) size of the yellow flowers and casual observations suggests that small generalist pollinators such as thrips (Thysanoptera) and beetles (Coleoptera) pollinate these dioecious trees (personal observation) as has been found in the nutmeg of commerce, *Myristica fragrans* (Armstrong and Irvine 1989). Informal questioning of local foresters has failed to identify a known disperser of the large, indehiscent, white arillate fruits.

More intensive collection and specimen-based studies of herbarium material are needed to improve our understanding of tropical species of plants (Tobler et al. 2007, Thomas 1999). Examination of field and herbarium specimens by J. Janovec and R. Steeves revealed a relatively high degree of leaf and fruit morphological variation in *C. capitellata* compared to other congeners. Such specimen studies quickly lead to the realization that our

understanding of the *C. capitellata* complex is hampered by lack of both morphological and nucleotide sequence data. In fact, no intensive survey of floral morphological patterns and genetic variation had ever been conducted in this group, or *Compsooneura* in general.

Although the tiny flowers of *C. capitellata* have been broadly characterized by a syndrome of free anthers and presence of a filament column, variation in relative size, shape, and positioning of anthers and filament columns was observed in field and herbaria. However, these androecial characteristics have never been studied with quantitative techniques.

Levels of gene flow, inbreeding, and genetic differentiation among tropical plants are of prime interest to tropical ecologists, conservationists and taxonomists. Although the Northwest Amazon, to which *Compsooneura capitellata* is endemic, is considered to be the most botanically diverse region of the world (Prance 1977, Gentry 1982) it is rather poorly understood from a genetic perspective, especially with respect to the Myristicaceae.

Previous molecular investigations of *Compsooneura* have revealed considerable nucleotide sequence variation in *C. capitellata* (Newmaster et al. 2008, Chapter 1 of this thesis). The aim of this study was to examine the morphological and molecular variation of this species in order to investigate whether genetic isolation exists between populations. If genetic diversity is greater among than within populations then it is more likely that reproductive isolation has occurred and morphological diversity may represent cryptic speciation.

Materials and Methods

Morphological data sampling

In order to quantify morphological differences, measures of 10 leaf, 7 androecium and 9 perianth dimensions were made (Figure 2.1) from collections for many locations

across *C. capitellata*'s range. Leaf measurements were made by measuring two leaves of herbarium samples with a ruler or by measuring photographs of pressed samples with a digital ruler in the program TPSdig2 (Rohlf 2006). Likewise, perianth and androecial metrics were measured using the Miniscale (Bioquip Inc., 2000), a miniature 5 mm ruler demarcated in 0.10 mm increments that can be used to gather quantitative data under a microscope, or from high resolution digital images of androecia which were captured using a Hirox microscope and associated software (Hirox-USA, Hackensack, NJ). Minimum and maximum measurements of mature structures on each specimen for the 26 metrics were recorded to document the range of leaf sizes within a population. Since species of *Compsooneura* are dioecious, only a subset of the total number of specimens possessed perianths and/or staminate flowers. Of the 268 specimens available for this study (Table 2.1), only 41 possessed staminate flowers. SEM micrographs and light microscope images were also compiled to demonstrate variation in androecial characters.

Morphological data analysis

A principal component analysis (PCA) was performed on leaf morphometric data using Canoco 4.5 (ter Braak, 1998) to identify the length of the ordination axis and unimodal ordination model was applied (Correspondence Analysis, CA). The relationship between quantitative leaf characters was analysed via nonmetric multi-dimensional scaling (NMS; Kruskal 1964; Primer 2002). In NMS, the Bray-Curtis distance measure was used because of its robustness for both large and small scales on the axes (Minchin 1987). Data were standardized by species maxima and two-dimensional solutions were appropriately chosen based on plotting a measure of fit ('stress') to the number of dimensions. Stress represents distortion in the data and a stress value over 0.2 is high

enough that the results are invalidated (Primer 2002). One thousand iterations were used for each NMS run, using random start coordinates. The first two ordination axes were rotated to enhance interpretability with the different axes. As an independent check, detrended correspondence analysis (DCA; ter Braak 1998) was used to evaluate the NMS classification. A Pearson-Correlation analysis was performed to investigate which characters contributed the most to the differentiation of the samples.

DNA extraction, amplification, sequencing and alignment

Like many members of the Myristicaceae, PCR-amenable genomic DNA of *C. capitellata* is very difficult to obtain from old collections or even fresh herbarium material that hasn't been rapidly dried and maintained in a desiccated state (personal observations). Almost all species of *Compsonaura* dry a dark brown colour, presumably due to the oxidation of secondary metabolites released from vacuoles as the leaves dry. Therefore DNA was sampled mostly from recent collections (< ~10 years old) and/or had been immediately placed in silica gel upon collection in the field. In attempts to capture the inherent morphological and genetic variability encountered in this species, seven populations were sampled from a wide area of the species' range in Ecuador (n=4) and Peru (n=3) spanning 1550 km and 1200 m of elevation. One specimen each of *C. atopa* and *C. diazii* were included in analyses as a previous genetic study (Chapter 1 of this thesis) has found a close relationship with *C. capitellata*. Figure 2.2 indicates collection locations and elevations of sampled populations.

Total genomic DNA was extracted from leaf tissue of silica-dried or herbarium specimens using the Macherey-Nagel Nucleospin II plant Kit. Lysis buffer 1 was used

according to the manufactures' instructions with the exception of the elongation of the post homogenization incubation period to 1hr (from 10 minutes) and the addition of 20mM N-Phenacylthiazolium Bromide which has been found to improve amplification of recalcitrant samples (Poinar et al. 1998, Asif and Cannon 2005).

Initially a suite of chloroplast (*matK*, *psbK-I*, *rbcL*, *rpoB*, *rpoC1*, *trnH-psbA*, *UPA*, and *ycf5*) and nuclear (*AGT1*, *AT103*, *ITS*, *IGS*, *PHYA*, *PHYC*, and *sqd1*) loci were amplified to investigate their utility for infra-specific studies of *C. capitellata*. However, only *trnH-psbA* was both consistently amplified and contained numerous polymorphisms. Five *C. capitellata* *trnH-psbA* accessions were retrieved via GenBank from Newmaster et al. (2008). An additional 55 samples were PCR amplified and sequenced using the primers *trnH2* (5'-CGCATGGTGGATTCAACAATCC-3'; Tate and Simpson 2003) and *psbAF* (5'GTTATGCATGAACGTAATGCTC-3'; Sang et al. 1997) or the custom designed primer pair *trnH-Myrist* (5'-TTGATCCACTTGGCTACATCC-3'; this thesis) and *psbA-Myrist* (5'-GACCTAGCTGCTGTTGAAGC-3':this thesis). PCR was performed in a 20 µl volume using 0.4 µl of Phire II polymerase (Finnzymes) with 1X Phire II reaction buffer (with 1.5mM MgCl), 0.2 mM of each DNTP, 0.2 µM of each primer and 2.0 µg of BSA (Kreader 1996). Cycling conditions entailed an initial denaturation step of 1 min at 98°; 35 cycles of 98° for 5 s, 64° for 5 s, 72° for 10 s: and a final elongation step of 72° for 1 min followed by a 4° hold. Phire II was used to amplify *trnH-psbA* as it has been found to be robust to the inhibitors contained in nutmeg extracts and as it is a fusion-based polymerase which has been found to reduce stuttering in regions containing homopolymer sequences such as the *trnH-psbA* intergenic spacer (Fazekas et al. 2010).

Amplification products were sequenced directly using the primers trnH-Myrist and psbA-Myrist. Cycle sequencing reactions were performed in a 10.5 μ L reaction volume containing 0.5 μ L of BigDye terminator mix v3.1, 1.88 μ L of 5x sequencing buffer (Applied Biosystems), 1.0 μ M of primer and 0.5 μ L of PCR product. Thermal cycling parameters were 96° for 2min; 30 cycles of 96° for 30s, 56° for 15s, and 72° for 4min; and a 4° hold. Cycle sequencing reactions were cleaned using sephadex columns (Cat.no. S5897; Sigma-Aldrich, St. Louis, MO, USA) and the samples were run on an ABI 3730 sequencer (Applied Biosystems).

Sequence contigs were assembled and edited using Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA). DNA sequences were aligned using the default settings of the ClustalW algorithm (Thompson et al. 1994) in Bioedit (Hall 1999) and adjusted manually. Three of four nucleotides of a loop structure believed to be the 3' untranslated region (UTR) of the psbA gene (Storchova and Olsen 2007) was omitted from the data matrix. Stem-loop secondary structures of DNA have a high likelihood of homoplasious inversions, even at low taxonomic ranks (Golenberg et al. 1993, Graham et al. 2000, Shaw et al. 2005). DNA sequence alignments can be found in appendix 2. Gaps in the alignments were coded using Modified Complex Indel Coding for distance analyses using Indelcoder (Muller 2006). All sequences generated for the molecular analyses have been deposited in the Barcode of Life Database (BOLD) [www.barcodinglife.org] and their BOLD process identification numbers (which can be used online to access sequence data, images, as well as additional specimen information) are found in Table 2.3.

Statistical Parsimony analyses

In attempts to estimate the phylogeographic history of *C. capitellata*, haplotype networks were constructed by statistical parsimony (Templeton et al. 1992, Templeton 1998) using the program TCS (Clement et al. 2000), which calculates the number of differences between haplotypes that are due to a sequence of single mutations at each site. Networks were calculated with TCS as implemented by ANeCA (Panchal 2007) by treating gaps as a 5th character state and with a 95% confidence interval. Haplotype networks may be preferable to traditional phylogenetic methods at the intraspecific level because population level data often violate many of the assumptions made by traditional tree-building methods, such as Maximum Parsimony, Maximum Likelihood and Bayesian Analyses (Posada and Crandall 2001). In comparison, networks are able to utilize haplotypic data that contains low levels of genetic divergence, ancestral haplotypes, multiple mutational variants from a given haplotype within the population, and reticulation that may be caused by recombination or hybridization (Templeton et al. 1992; Posada and Crandall 2001). Since loops are not theoretically possible in haplotype networks of non-recombining regions such as plastid DNA, the connection deemed the most unlikely connecting haplotypes of a loop was removed.

Results

Morphology

Leaf length in *C. capitellata* varied between 3.6 and 51.7 cm long and the width at half length ranged from 1.7-13.5 cm. The ordination analyses of quantitative leaf metrics did not reveal that any *C. capitellata* regional populations were distinct when all regions are considered. However, there are many specimens from Amazonas, and Cusco, Peru

that were found to cluster together on the NMS ordination (Figure 2.3). Pearson correlation analyses revealed that the X-axis is strongly correlated with 8 measures of leaf length and width (Figure 2.3; Table 2.2) and the y-axis was mostly correlated to leaf vein number and space (Figure 2.3; Table 2.2). In order to investigate whether populations isolated within Andean valleys and at high elevations exhibit morphological affinities of their leaves, collections were coded as either Montane or Lowland and plotted using the NMS ordination values (Figure 2.4). It was evident from this plot that there is a general trend for montane populations to have larger leaves compared to lowland populations (Figure 2.5).

Anther number ranged from 4-9 per androecium and the individual mature anthers were 0.4-1.6 mm long. Much of this variation in androecial characters is demonstrated in the SEM and light microscope images of Figure 2.6. Ordination analyses revealed three distinct clusters that were due to variation in minimum and maximum anther number, which was variable within almost all regions sampled. Pearson correlation analyses showed that the x-axis was strongly correlated to max/min anther number, length and cluster width while the y-axis was mostly correlated to those same measures as well as anther sac length (Figure 2.7; Table 2.2).

Perianths of *C. capitellata* were 0.4-2.0 mm at their widest, 1.1-3.2 mm long and were on petioles 0.5-3.0 mm long and 0.1-1.0 mm wide. No particular groupings were evident from the ordination, but two peripheral points (from Peru-Huanuco and Ecuador-Napo populations) belonged to the only female flowers included in the analysis (Figure 2.8). Pistillate perianths also had the thickest pedicels at 1.0 mm, presumably owing to the mechanical stresses placed upon the pedicel during fruit development.

The fruits of the *C. capitellata* complex were found to be relatively consistent in size but highly variable in pericarp texture. Pericarps ranged from green to ferruginous, glabrous to tomentellous, and smooth to sulcate or warty in appearance (Figure 2.9). Although few fruits were available for study, pericarp morphology appeared to be consistent within populations.

Molecular analyses

Bi-directional trnH-psbA intergenic spacer sequences were obtained for 59 *Compsooneura capitellata* individuals representing 7 populations and single specimens of *C. atopa* and *C. diazii*. Aligned sequences were 302 bp in length and contained 13 variable sites including one indel originating from an A/T homopolymer run 9-12 bp in length. Table 2.3 documents the species determinations, collection numbers, locations and Barcode of Life Database process ID numbers of collections used in the molecular analyses.

The haplotype network estimated by TCS at a 95% confidence level revealed 10 haplotypes (Figure 2.10). Each haplotype was restricted to a particular sampling location and no evidence was found of haplotypes being shared among two populations. The only locality that showed intra-population variability, Loreto, had three haplotypes that varied by only by either a single nucleotide polymorphism or a single base insertion in the longest homopolymer tract. A branch connecting the Zamora-Chinchi and Morona-Santiago populations was removed as the sole difference between these three populations is a GAAG, GAAA, or CTTC (5'-3') sequence of the apical loop of the 3' UTR (untranslated region) and the most probable mutational pattern for this region is for GAAG↔CTTC through an inversion and GAAG↔GAAA through a single transition.

Likewise a branch connecting the Morona-Santiago population to an unsampled/ancestral haplotype was removed as it was more likely to have originated from an extant and nearby population than an unsampled haplotype.

Discussion

This study represents the first intraspecific morphological and molecular investigation of the genus *Compsooneura*, representing 266 and 60 specimens examined for each respective analysis. Although the findings of this investigation raise many questions about the taxonomic status of the *C. capitellata* complex, I will hypothesize causes of the patterns of morphological and molecular variation observed across the species' range and provide an account of a provisional new and resurrected species from the *Compsooneura capitellata* complex.

Morphology

Since leaves are always present on male, female and juvenile trees they should be very important taxonomically, however, they are notoriously similar in morphology among species and even genera of Myristicaceae. Despite great differences in leaf size among some populations of *C. capitellata*, the ordination analyses indicate that leaf metrics alone cannot unambiguously differentiate populations when specimens from the entire range of the species are included. However, there was a general trend observed where montane populations showed larger leaves and fewer veins compared to their lowland conspecifics. Many of these montane specimens are from the Peruvian Andean provinces of Cusco, Amazonas and Huanuco and Junin. These populations are not only at higher altitudes (500-1000 m asl), but are typically separated from the lowland Amazon

basin by small mountain ranges. Even relatively small mountain ranges and associated changes in altitude are considered to be important generators of speciation in the tropics (Janzen 1967) so the contrasting sizes of leaves in montane and lowland may be of taxonomic significance. The leaves of trees from Morona-Santiago, Ecuador were not distinctive in size from other populations of *C. capitellata* but were distinct in the fact that their laminae were domed between the secondary veins rather than plane as in all other specimens examined.

Characteristics of the androecium have traditionally been regarded as the most important taxonomic characters of *Compsooneura* (Smith 1937, Janovec 2000). Although the ordination of androecial characters has three distinct groups, these groupings are caused mainly by anther number differences, with each cluster containing most populations. It is possible that these clusters are a result of infraspecific variation in androecial characters rather than incipient speciation but anther number is rarely characteristic of *Compsooneura* species (Janovec 2000).

Characteristics of the perianth have been given little taxonomic attention with respect to Myristicaceae species but multivariate analyses have never been performed to verify their taxonomic utility in the family. Although this analysis was primarily infraspecific, it appears to confirm that quantitative perianth characters show little variation and form a continuum and are unlikely to be of great taxonomic utility.

Even though many additional specimens have been collected since Smith's (1937) monograph of *Compsooneura*, few populations of *C. capitellata* have mature staminate/pistillate flowers as well as fruits available for analysis. The relatively few fruits available for study were wildly divergent in pericarp morphologies. Fruit specimens

from the San Martin population were smooth, green and glabrescent, those of Loreto were Ferruginous-tomerntellous and longitudinally sulcate, while a single fruit found in the Pastaza population was ferruginous-tomentellous and reticulated-furrowed with a warted appearance. Although it appears that fruit morphology is conserved within populations, so few fruits are available for study that it is not evident whether this holds true for all populations. Future collections of *C. capitellata* should make deliberate efforts to collect and document multiple fruits within populations

Genetic analyses

Compsoeura atopa* and *Compsoeura diazii

C. atopa and *C. diazii* were included in this investigation since previous phylogenetic investigations of *Compsoeura* had found these taxa to be similar to *C. capitellata* with respect to their trnH-psbA haplotypes. Perhaps one of the most surprising revelations of the molecular sequence data was that this *C. atopa* specimen differed by only a single nucleotide polymorphism (SNP) when compared to three populations of Ecuadorian *C. capitellata* and by two SNP's from a Peruvian *C. capitellata* population. *C. atopa* is a large tree (35 m or more tall) that has been collected extensively on the Western slopes of the Andes in Colombia and Ecuador but is known from only two collections east of the Ecuadorian Andes. *C. atopa* differs from *C. capitellata* in a number of morphological traits including the possession of brochidodromous venation, persistent abaxial leaf pubescence, secondary vein number (12-27 versus 4-12), a dense indument of the perianth, apical anther spurs, and the presence of bracteoles on inflorescences. The differences between these two species are so numerous and pronounced that Janovec (2000) subdivided the informal section *Hadrocarpa* into

subgroups *Atopa* and *Capitellata*, which were named after these species. Therefore, it was truly surprising to find that *C. atopa* differed by only 1 or 2 SNP's from 4 different populations of *C. capitellata*, especially considering that these SNP's are primarily due to hairpin-loop inversions or homopolymer run indels. It is also interesting that this specimen's haplotype is most similar to *C. capitellata* from southeastern Ecuador (120-608 km distant) rather than the nearby *C. capitellata* population (only 38 km distant). A previous investigation (Chapter 1 of this thesis) that employed the *trnH-psbA* spacer for some members of subgroup *Atopa* (*C. atopa*, *C. rigidifolia*, and *C. sp1*) found that *C. atopa* (J. J. Janovec 1374), *C. cuatrecasasi* (AHG 40682), and *C. sp 2* (AHG 23826) are paraphyletic with respect to other members of their subgroups. The two *C. atopa* individuals that have been found east of the Andean Cordillera may either represent a population that predates the uplift of the Andes or a more contemporary migration event. The seed source for such a dispersal event could have come from the western slopes of Colombia or the 20 km wide, 1874 m asl, Las Cruces mountain pass which connects the Choco region of Colombia with the lowland Amazon via the Magdalena valley. Although other members of subgroup *Atopa* have been collected in the Magdalena Valley, this latter suggestion seems unlikely as *C. atopa* has yet to be collected there. It is also interesting to note that the seeds of *C. atopa* are boiled and roasted by indigenous peoples of the Baudo region of Colombia (La Rotta 1985) so it is conceivable that humans may have played a role in the dispersal of this species across the Andes. Plastid haplotype sharing, or chloroplast capture (Hollingsworth et al 2011), is well document in many plant taxa (Rieseberg and Soltis 1991, Golden and Bain 2000, Palma et al 2003) even in the presence strong morphological dissimilarity among species (Palma-Silva et al. 2011).

It is therefore possible that haplotype similarity among the morphologically dissimilar *C. capitellata*, *C. atopa*, and *C. diazii* observed in this study is due to relatively recent hybridization between these species resulting in plastid introgression. If the low collection rates of *C. atopa* east of the Andes are an indicator of its population density then hybridization with the more numerous *C. capitellata* trees may have been more likely. In light of these findings, I plotted the collection locations of *C. atopa* on a map of precipitation regimes in South America (Figure 2.11) to see if there was any obvious trend. It appears that *C. atopa* is restricted to areas of high precipitation (>4000mm/year). Therefore future collections in unexplored regions of Amazonian Ecuador and Colombia may find additional populations of this species. Future investigations should seek additional collections of *C. atopa* from throughout its western and eastern Andean range as well as more variable chloroplast and multiple bi-parentally inherited markers in order to investigate the origin of Amazonian *C. atopa*.

Compsoeura diazii was separated from the *C. capitellata* complex as a new species by Janovec (2002) so it is of little surprise that *C. diazii* is nested well within the haplotype network. It is somewhat surprising, however, that the *C. diazii* haplotype was most similar to the Pastaza, Ecuador population of *C. capitellata* rather than the less geographically distant populations of *C. capitellata* from San Martin, Peru, and South Western Ecuador (Zamora-Chinchipec and Morona-Santiago).

Compsoeura capitellata

All sampled populations of *C. capitellata* appear to be fixed for one or very few haplotypes. Only one sampled population, Loreto, exhibited SNP's within its population but each of these other haplotypes consisted of a single SNP relative to the most common

haplotype. This is somewhat surprising given that relatively high levels of within population diversity has been found in previous studies of tropical tree cpDNA using restriction polymorphisms (Caron et al. 2000, Latouche-Halle et al. 2003). However, the low variability found in this study was not unexpected given that Sauquet et al. (2003) found very low levels of nucleotide substitution in cpDNA coding and non-coding regions amongst genera of Myristicaceae. Once the loops in the haplotype network were removed it is evident that the most northerly populations (Napo, Pastaza, Morona-Santiago, Zamora-Chinchiipe and San Martin) have the fewest nucleotide differences between them as compared to the more isolated populations (at least with respect to sampling patterns) of Loreto and Cusco. Given the apparent fixation of haplotypes for each population it is difficult to tell if these populations represent a large panmictic assemblage of populations of a single morphologically and molecularly diverse species or numerous isolated populations and/or distinct undescribed species. These questions are particularly difficult given the low levels of molecular variation available and missing morphological characters for many populations, and few distinguishing characters when present.

Of central importance to understanding and interpreting patterns of molecular and morphological variation is the natural history of the species of study. Such high levels of population differentiation can be easily generated in maternally inherited DNA, such as plastid DNA, if propagule dispersal is restricted. The dispersal agents of these large-seeded trees remains unknown to date but it is possible that these trees were dispersed by now extinct large mammals such as the giant ground sloths or gomphotheres (Janzen and Martin 1982). The fruits of *C. capitellata* are covered by a thick, woody, and indehiscent

to forced-dehiscent pericarp that protects a single large seed (2-4 cm in diameter) covered by a thin, white and fatty aril (Janovec 2000). These fruits fit many of the criteria of the Pleistocene megafaunal dispersal syndrome outlined by Janzen and Martin (1982). The largest mammals currently found in the range of *C. capitellata* that could act as potential dispersal agents are white-tailed and Brouette's deer (*Mazama spp.*), tapir (*Tapirus spp.*), as well as spider (*Ateles spp.*), howler (*Alouatta spp.*) and woolly monkeys (*Lagothrix spp.*) but it seems highly unlikely that any of these species would act as effective long distance dispersers as it would necessitate them swallowing the 2-4 cm seeds, which is likely too large for them to consume intact. This hypothesis could be tested with greenhouse studies to see whether scarification and the application of stomach-like acids have a positive effect on the germination potential of *C. capitellata* as well as field studies to investigate the rate of seed removal, and predation versus dispersal.

There remains one other extant indigenous mammal capable of dispersing the seeds of these trees: *Homo sapiens*. Humans have also likely had a great impact on the Amazonian flora and fauna for the last 10,000 years (Janzen 1983, Roosevelt et al. 1996, Paz-Riviera and Putz 2009). It has been found relatively recently that a charcoal enriched soil called *Terra Preta*, which is found in large areas (perhaps 20% of the Brazilian Amazon) of Brazil adjacent Amazon river, was created by humans between 600-8700 years BP (Smith 1980, Liang et al. 2008). *Terra Preta* vastly changes the soil microbiota (Kim et al. 2007, Grossman et al. 2010) and increases a soils cation-exchange capacity which helps to trap nitrogen within the soil and release it and other nutrients to plants (Liang et al. 2006, Chan et al. 2007). It is believed that the advent of *Terra Preta* may have enabled the development of large human populations on the relatively poor oxisols

of the Amazon via a pre-Colombian agricultural revolution (Smith 1980, Hackenberger et al. 1999) but these societies may have collapsed due to introduced diseases. The rich endosperm of *Compsonaura mexicana* seeds is commonly eaten by children in Central America (Janovec 2000). It is therefore plausible that such ancient societies may have actively cultivated and spread *C. capitellata* seeds for their fatty arils and rich endosperm which is reported to taste similar to Brazil nuts (*Bertholettia excelsa*). If this occurred it is possible that the cultivation of these trees has impacted its distribution and genetic structure.

A large degree of population subdivision may be expected in a non-recombining genome of a shade tolerant, old growth forest species such as *C. capitellata*. Previous studies of tropical (Hamilton 1999) and temperate broadleaved trees (Demesure et al. 1996, Petit et al. 2002) have reported a large degree of fixation of cpDNA haplotypes within populations even at small spatial scales. Demesure et al.(1996) found a large degree of population differentiation in European Beech (*Fagus sylvatica*) which was attributed to the last glaciation but there was also a widespread haplotype that connected all populations. Most analyses of cpDNA structure in the Neotropics have focused on investigating the effects of forest fragmentation on population genetic structure and have therefore been concerned with relatively small spatial scales. Additionally, population subdivision may be easily achieved with cpDNA as its effective population is a quarter that of nuclear markers and selection may be especially strong in non-recombining genomes dense in mRNA and protein-coding genes.

It is also possible that these populations are exchanging genes via pollen but this would not be evident using maternally inherited markers. Although possible, I think this

is highly unlikely as the flowers are likely visited only by small thrips and beetles which are unlikely to travel great distances. More field work is needed to ascertain whether *C. capitellata* exists as discrete or continuous breeding populations across their range. Additional and more variable molecular markers will also be needed in the future to help elucidate whether seed dispersal, pollen flow, or other factors are restricting gene flow between these populations. It would also be of great interest to investigate whether similar genetic patterns exist for other taxa as this may indicate that such patterns are the result of ecological speciation, human cultivation and/or phylogeographic processes.

Compsoneura capitellata inhabits the largest geographical and altitudinal range of any species in the genus, spanning 1700 km of latitude, 1200 km longitude, and 1200 m elevation. These trees can be found growing in white sands (San Martin), sandstone ridges (Morona-Santiago), and red oxisols (much of its range). These studies have revealed a large degree of variation in leaf, flower, and fruit morphologies but most of these values form a continuum with respect to geographical populations. The highest degree of morphological and genetic diversity was found in the topographically and edaphically diverse Southwestern region of Ecuador.

Conclusion

With these data it appears that the *C. capitellata* complex exists as a morphologically diverse group with pronounced genetic discontinuities between populations. Perhaps more variable markers and increased sampling of the Andes to the Amazon region will help answer whether these populations are actively exchanging genes via seed and/or pollen dispersal. It would also be of considerable interest to determine whether the larger leaved montane populations are more genetically similar to

local lowland populations or more distant populations of similar morphology and altitude (i.e. have montane varieties arisen multiple times in the Andes). Given the large number of nucleotide differences of *C. capitellata* in the mountains of Cusco, Peru and this population's atypical large leaf morphologies and affinities to Markgraf's *C. tessmannii*, I propose the provisional resurrection of *C. tessmannii*. Further genetic evidence would be desirable to investigate whether these two large-leaved trees are indeed genetically related. Additionally, collections from the sandstone ridges of the Cordillera del Condor and Cutucu formations of Ecuador appear to represent a new taxon that is provisionally described below as *Componeura morona-santiagoensis*. *C. morona-santiagoensis* is unique within the *C. capitellata* complex in having domed leaf lamina in between the secondary veins but in other characters of the leaves, flowers and fruits it is similar to other members of the *C. capitellata* complex. It is possible that some of the other taxa with morphological oddities such as the Pastaza, Ecuador population that possesses a warted pericarp and distinct haplotype represents new species, however the description of these taxa as new species requires additional morphological and molecular data in order to establish the degree to which these populations are related.

Provisional Taxonomic descriptions:

The following information summarizes a provisional combination (*C. morona-santiago*) and provisional resurrection (*C. tessmannii*) of taxa from the *C. capitellata* complex.

Componeura morona-santiago J. Janovec & R. Steeves, sp. nov. -TYPE: ECUADOR. Morona-Santiago: Taisha, 2°225' S, 77°31 W, 500 m, 31 Jan 1962, *P.C. D. Cazalet and T. D. Pennington 7602* (holotype: NY; isotype: US).

Tree to 15 m. **Bark** unknown. **Sap** red from inner bark. **Branches** teret to subterete, 0.2-0.6 cm diam., rugose to longitudinally striate, brown, densely to sparsely ferrugineous-tomentellous throughout when young, glabrescent to minutely tomentellous in leaf axils and throughout with age, the hairs short-stalked to sessile, 2-branched, the upper branch with a concave surface. **Leaves** simple, alternate, spirally arranged on upper trunk, distichous on branches, **Petioles** stout, subterete, slightly canaliculate, 0.9-1.5 x 0.1-0.2 cm, brown to nearly black, glabrous to glabrescent, the hairs short stalked to sessile, 2-branched; **Blades** elliptic to slightly obovate-elliptic, 7.5-21.1 cm long, 2.2-6.5 cm wide at $\frac{1}{2}$ length, 2.5-7.6 cm wide at $\frac{1}{2}$ the length, 2.3-6.8 cm at $\frac{3}{4}$ length, thick chartaceous to thick coriaceous, the adaxial surface drying olive-brown to dark brown, dull to glossy, glabrescent to sparsely ferrugineous-tomentellous when young, especially at the base, glabrous to glabrescent throughout with age, the abaxial surface drying light brown to brown, dull, glabrescent to sparsely ferrugineous-tomentellous when young, specially at base, glabrous to glabrescent throughout with age, the hairs minute, sessile, short-stalked, 2-branched, the base acute to broadly to weakly acute, the margins conspicuously revolute, the apex weakly to strongly caudate, the acumen 0.7-1.1 cm long; **Venation** with costa raised and glabrous to glabrescent at base adaxially, prominently keeled and glabrescent or sparsely tomentellous abaxially, especially toward base, the secondary nerves acute-ascending, eucamptodromous, distinct near margins, 7-12 per side, spaced 0.6-3.5cm, slightly impressed, same color as lamina glabrous adaxially, prominent, same color as lamina, glabrous to glabrescent abaxially, the tertiary veins conspicuous, semi-parallel, and semi-perpendicular to secondary veins. **Staminate**

inflorescence axillary, paired, paniculate, 5.5-8.0 x 0.8-1.2 cm, sparsely surface drying olive-brown to dark brown, dull to glossy, glabrescent to sparsely ferruginous-tomentellous to glabrescent with age, the hairs 2-branched; **Rachis** densely alternate clusters of about 6-9 per branchlet, arising slightly unilaterally from branchlet pedicels, up to 0.15-0.28 x 0.02 x 0.03 cm; **Pistillate inflorescence** unknown. **Staminate flower buds** long ovate to long elliptic-ovate. **Staminate buds** unknown. **Staminate perianth** elongate vasiform, 0.23-0.32 x 0.15-0.2 cm, coriaceous to thick-coriaceous, yellow to orange when fresh, drying brown, glabrous adaxially, densely to minutely ferruginous-tomentellous abaxially, the hairs short-stalked or sessile, 2-branched, tube 0.6-0.10 cm long, lobes 3-4, lanceolate to ovate-lanceolate to narrowly deltoid, 0.16-0.24 x 0.13- 0.18 cm, erect from base to apex, the apices acute. **Androecium** 0.07-0.15 x 0.12 cm long, the filament column 0.02-0.04 cm long, 0.01-0.05 cm at base. 0.01-0.04 cm at apex, dark, solid, oily, glabrous, the anthers 6-7, distinct, erect from base, 0.05-0.07cm long. The bases rounded, oil cells conspicuous in the anther connective, the apices strongly acute. **Pistillate flowers**: known only from persistent perianth subtending fruit, thin coriaceous, drying brown, glabrous adaxially, densely to thinly ferruginous-tomentellous abaxially, the hairs 2-branched. **Mature Fruits** 1-5 per infructescence, sub-globose to widely transversely elliptic, 2.9-4.5 x 3.0-5.0 cm, minutely to densely, muricate, slightly rugose and green when fresh, drying brown, glabrous to glabrescent; **Pericarp** minutely to densely muricate, glabrescent to sparsely ferruginous-tomentellous, strongly ligneous, 0.2-0.3 cm thick, appearing indehiscent or partially deshiscent from base; **Seed** with an entire, thin aril, white when fresh, yellowish when dry; **Testa** dull, dark brown, somewhat warty to slightly muricate; **Endosperm** white, entire.

Distribution. This species is a member of the Capitellata subgroup (sensu Janovec 2000), with brown drying leaves, weakly anastomosing secondary veins, and sub-globose, liginous fruit with a softly sulcate or furrowed pericarp. Elongate, narrow, densely flowered paniculate inflorescences characterize this species. Based on anthers which are strongly erect-ascending from base to apex, this species is distinct from *C. capitellata* and may be related to *C. diazii*. In leaf morphology it is similar to *C. capitellata*, with brown lamina and weakly anastomosing veins. *Componeura diazii* is easily differentiated on the basis of strongly anastomosing veins. This species is endemic to southeastern Ecuador where it has been collected on numerous occasions from moist premontane to cloud forest exclusively on soils of sandstone origin. In Morona-Santiago it was collected from around the municipality of Taisha and the in the Cordillera de Cutucu on the road between Mendez and Morona. In Zamora-Chinchipe it was collected along the road between Nudo de Sabanillo and Vallodolid as well as near the town of Quimi. Sandstone substrate is prevalent in this region of southeastern Ecuador and, with future field work, may prove to be the limiting biogeographic factor for this species.

Etymology: This species name is derived from the province of Morona-Santiago, Ecuador, where this species was first collected.

Componeura tessmannii (Markgraf) , Notizbl. Bot. Gart. Berlin 9: 964. 1926. TYPE: PERU. Loreto: basin of Rio Marañon from Iquitos upstream on the way to the mouth of Rio Santiago at Pongo de Manseriche, ca. 3° 50' S, 77° 40' W, 23 Sep 1924, *Tessmann 4108* (holotype: B; fragment of holotype: F; photos of holotype: F, H, MO, NY; isotype fragment: F, NY).

Tree to 18 m tall and 30 cm dbh. **Bark** reddish in colour, furrowed and peels off in long thin fibrous slivers. **Sap** profuse and clear-red. **Branchlets** terete to subterete, 0.3-0.8 cm wide, rugose to longitudinally striate when dried, brown-green, lenticellate, glabrescent to minutely tomentellous in leaf axils and glabrous throughout with age, the hairs short-stalked to sessile, 2-branched. **Leaves** simple, alternate, thick-coriaceous, distichous on branches, leaf buds ferruginous but soon glabrous upon leaf expansion; **petioles** stout, subterete, canaliculate, 0.8-2.6 cm long and 0.3-0.6 cm wide, brown to nearly black when dry, glabrous to glabrescent, the hairs short stalked to sessile, 2-branched; **blades** elliptic-oblong, lamina 14.8-30.0 cm long, 4.5-11.5 cm wide at $\frac{1}{4}$ length, 3.5-13.6 cm wide at $\frac{1}{2}$ width, 4.9-12.0 cm wide at $\frac{3}{4}$ length, base broadly cuneate to acute and mucronate to cuspidate at apex, conspicuously revolute at margins, drying light to dark brown or infrequently greenish brown, glabrescent when young but soon glabrous, adaxial surface deep green and glossy, abaxial surface lighter green with a dull shine; **venation** costa glabrous and raised above, prominently keeled and glabrous-glabrescent below; secondary nerves 6-11 per side, spaced by 1.1-2.5 cm, arcuate ascending, eucamptodromous, partially anastomosing near margin. **Staminate inflorescences** axillary, paniculate 3-12 cm long, 2-4 cm wide, rachis 4-25 alternate branched, 7-15 cm long, vestiture of staminate inflorescences densely ferruginous-tomentellous, hairs 2-branched. **Staminate flower buds** green and globose. **Staminate flowers** subglobose to globose, arranged in clusters of 4-12 per inflorescence branch, pedicels slender, 0.1-0.13 cm wide and 0.29-0.42 cm long; **perianth** coriaceous, yellow fresh, drying dark brown, 0.56-0.61 cm long, 0.44-0.5 cm wide; **tepal lobes** 3 (rarely 2-4), ovate-deltoid, 0.43-0.45

long, 0.32-0.37 wide at base, **perianth tube** 0.14-0.17 cm long; perianth staminate vestiture glabrous within, densely ferruginous-tomentellous outside, hairs 2-branched. Androecium 0.2-0.22 cm long, filament column 0.09-0.15 cm long, 0.1-0.11 cm wide at apex, 0.1-0.12 cm wide at base, the anthers 6-10, free and spreading from the base, slightly incurved above, 0.2-0.22 cm long, acute-obtuse at apex, rounded at base.

Pistillate inflorescences unknown. **Fruits** (immature) 1-3 per infructescence, globose, tepals slightly accrescent; **Pericarp** red tomentose to glabrescent with age, sulcate to rugose with a warty appearance, presumably indehiscent or forced dehiscent; **Seed** unknown; **Endosperm** unknown

Distribution: Recently collected from the vicinity of Quincemil, Peru in the mountains of the Madre de Dios watershed. The dried leaf lamina of the Quincemil population has a strong resemblance to type specimens of *C. tessmannii* (Markgraf) which was described in 1926 as a new species of with affinities to *capitellata* from Pongo de Manseriche, Iquitos, Peru but was synonymized as *C. capitellata* by Janovec (2000). Further morphological and genetic studies are desirable to determine whether *C. tessmannii* populations of Amazonas and Cusco provinces represent disjunct conspecific taxa.

Additional specimens examined: **Peru. CUSCO:** Quispicanchi Province, District of Camanti, Community of Quincemil, -13.23, -70.78, 500-1200 m, 06/26-07/04, 2008, R. Steeves and P. Centeno 527(OAC); R. Steeves and P. Centeno 531 (OAC); R. Steeves and P. Centeno 535 (OAC); R. Steeves and P. Centeno 538 (OAC); R. Steeves and P. Centeno 548 (OAC); R. Steeves and P. Centeno 550 (OAC); R. Steeves and P. Centeno 551 (OAC); R. Steeves and P. Centeno 556 (OAC); R. Steeves and P. Centeno 558

(OAC); R. Steeves and P. Centeno 562 (OAC); R. Steeves and P. Centeno 563 (OAC); R. Steeves and P. Centeno 568 (OAC); R. Steeves and P. Centeno 569 (OAC); R. Steeves and P. Centeno 570 (OAC); R. Steeves and P. Centeno 571 (OAC); R. Steeves and P. Centeno 572 (OAC); R. Steeves and P. Centeno 577 (OAC); R. Steeves and P. Centeno 591 (OAC); R. Steeves and P. Centeno 593 (OAC); and R. Steeves and P. Centeno 600 (OAC).

Tables

Table 2.1 Specimens used in vegetative and floral ordinations. Herbarium code and accession number, collectors, collection number (Coll.#), country, decimal degrees latitude (DD Lat.), decimal degrees longitude (DD Long.), and elevation (Elev.) are listed.

Herbarium	Collector(s)	Coll. #	Country	DD		Elev.
				DD Lat.	Long.	
QCNE-88196	Aulestia, M.	2260	Ecuador	0.65	-76.43	250
	Schultes, R. E., and I. Cabrera	14144	Colombia	0.12	-71.25	250
US 2171356	Schultes, R. E., and I. Cabrera	14144	Colombia	0.12	-71.25	250
QCNE-64227	Palacios, W., et al.	9197	Ecuador	0.08	-76.18	230
	Schultes, R. E., and I.					
US 2171510		15107	Colombia	0.07	-71.12	300
	Schultes, R. E., and I.					
US 2171510	Cabrera	15107	Colombia	0.07	-71.12	300
	Schultes, R. E., and I.					
US 2220060	Cabrera	15107	Colombia	0.07	-71.12	300
	Schultes, R. E., and I.					
H	Cabrera	15107	Colombia	0.07	-71.12	300
	Schultes, R. E., and I.					
US 2220060	Cabrera	15107	Colombia	0.07	-71.12	300
	Schultes, R. E., and I.					
H	Cabrera	15107	Colombia	0.07	-71.12	300
	Schultes, R. E., and I.					
NY	Cabrera	15107	Colombia	0.07	-71.12	300
QCNE-64522	Palacios, W., et al.	9226	Ecuador	0.00	-76.18	230
QCNE-63507	Palacios, W., et al.	8989	Ecuador	0.00	-76.18	230
QCNE-64198	Palacios, W., et al.	9311	Ecuador	0.00	-76.18	230
NY	Cerçn, C., and F. Hurtado	4138	Ecuador	-0.10	-76.18	200
QCNE-44552	Cerçn, C., and J. Ayala	9497	Ecuador	-0.13	-76.37	240
MO-5020318	Gudi, O, E.	118	Ecuador	-0.33	-77.08	250
QCNE-45495	Rubio, D.	280	Ecuador	-0.33	-77.08	250
MO3806254	Espinoza, S.	83	Ecuador	-0.33	-77.08	250
QCNE 39714	Gudi, O, E.	118	Ecuador	-0.33	-77.08	250
NY	Rubio, D.	280	Ecuador	-0.33	-77.00	250
MO 3794978	Rubio, D.	280	Ecuador	-0.33	-77.00	250
MO						
05030983	Aulestia, M.	1651	Ecuador	-0.42	-76.58	250
QCNE 84139	Aulestia, M.	1651	Ecuador	-0.42	-76.58	250
MO 4063871	Palacios, W.	2310	Ecuador	-0.43	-77.10	300
NY	Palacios, W.	2310	Ecuador	-0.43	-77.10	300
QCNE 19906	Palacios, W.	2310	Ecuador	-0.43	-77.10	300
NY	Neill, D.	7150	Ecuador	-0.43	-77.02	250
MO 3482889	Neill, D.	7150	Ecuador	-0.43	-77.02	250
QCNE 55032	Palacios, W., et al.	7586	Ecuador	-0.48	-75.53	230
MO 4063877	Cerçn, C., and W. Palacios	3029	Ecuador	-0.50	-77.02	250

NY	Ollgaard, B., et al.	57050	Ecuador	-0.53	-76.37	300
QCNE 87119	Dik, A., and C. Aulestia	1436	Ecuador	-0.55	-76.52	250
QCNE-75762	Aulestia, M., and G. Grefa	269	Ecuador	-0.55	-76.50	250
QCNE-53357	Korning, J., and K. Thomsen	47562	Ecuador	-0.55	-76.37	370
QCNE-53352	Korning, J., and K. Thomsen	47556	Ecuador	-0.55	-76.37	250
F 2612343	Palacios, W., et al.	7798	Ecuador	-0.55	-75.53	230
F 2162341	Palacios, W., et al.	7812	Ecuador	-0.55	-75.53	230
QCNE 54636	Palacios, W., et al.	7798	Ecuador	-0.55	-75.53	230
QCNE 54624	Palacios, W., et al.	7812	Ecuador	-0.55	-75.53	230
QCNE/MO	Freire, E., P. Cerda	23	Ecuador	-0.63	-77.45	690
MO						
05030981	Aulestia, M.	2746	Ecuador	-0.65	-76.43	250
QCNE-88049	Aulestia, M.	2446	Ecuador	-0.65	-76.43	250
MO						
05030982	Aulestia, M.	2713	Ecuador	-0.65	-76.43	250
QCNE-88239	Aulestia, M.	2497	Ecuador	-0.65	-76.43	250
QCNE-88319	Aulestia, M.	2497	Ecuador	-0.65	-76.43	250
QCNE-88464	Aulestia, M.	2746	Ecuador	-0.65	-76.43	250
QCNE-88658	Aulestia, M., and T. Ahue	2909	Ecuador	-0.68	-76.45	260
QCNE/MO	Alvarado, A.	390	Ecuador	-0.70	-77.33	810
Mo 05030980	Aulestia, M., and T. Ahue	2909	Ecuador	-0.78	-76.45	260
QCNE-8642	Dik, A., and J. Andi	953	Ecuador	-0.83	-76.35	270
MO						
05030962	Dik, A., and J. Andi	953	Ecuador	-0.83	-76.35	270
QCNE-86816	Dik, A., and R. Enomenga	1145	Ecuador	-0.83	-76.30	250
QCNE-86694	Dik, A.	1232	Ecuador	-0.85	-76.25	260
MO 3654088	Cerçn, C., et al.	4123	Ecuador	-0.92	-76.18	200
MO 3654089	Cerçn, C., et al.	4138	Ecuador	-0.92	-76.18	200
US 3129061	Cerçn, C., and F. Hurtado	4140	Ecuador	-0.92	-76.18	200
QCNE-17872	Cerçn, C., and F. Hurtado	4140	Ecuador	-0.92	-76.18	200
QCNE 28641	Cerçn, C., and F. Hurtado	4123	Ecuador	-0.92	-76.18	200
MO 3654090	Cerçn, C., and F. Hurtado	4140	Ecuador	-0.92	-76.18	200
QCNE-28645	Cerçn, C., and F. Hurtado	4138	Ecuador	-0.92	-76.18	200
MO 4063873	Cerçn, C., and F. Hurtado	3876	Ecuador	-0.92	-76.18	200
QCNE-84478	Aulestia, M., and O. Gonti	1983	Ecuador	-0.92	-76.15	250
QCNE 84462	Aulestia, M.	1859	Ecuador	-0.92	-76.15	250
QCNE 86988	Dik, A.	1455	Ecuador	-0.93	-76.22	248
QCNE-88822	Pitman, N.	587	Ecuador	-0.93	-76.22	250
QCNE-86996	Dik, A.	1447	Ecuador	-0.93	-76.22	248
NY	Palacios, W.	2409	Ecuador	-0.95	-76.22	230
QCNE-28524	Palacios, W.	2409	Ecuador	-0.95	-76.22	230
MO 3597372	Palacios, W.	2409	Ecuador	-0.95	-76.22	230
QCNE/MO	Aulestia, M., and A. Omehuat	3246	Ecuador	-0.98	-76.20	235
QCNE/MO	Aulestia, M., and B.					
QCNE/MO	Quihuinamo	3111	Ecuador	-0.98	-76.20	237
MO	Aulestia, M., and B.					
QCNE	Quihuinamo	3207	Ecuador	-0.98	-76.20	235
096476	Aulestia, M., and B.					
QCNE	Quihuinamo	3111	Ecuador	-0.98	-76.20	237
QCNE	Aulestia, M., and B.					
096452	Quihuinamo	3207	Ecuador	-0.98	-76.20	235

QCNE 87099	Dik, A., and T. Ahue	1563	Ecuador	-1.00	-76.18	250
Harvard	Schultes, R. E., and F. Lopez	10200	Brazil	-1.00	-69.50	150
QCNE-87276	Dik, A., and T. Ahue	1598	Ecuador	-1.03	-76.18	249
MO 4221156	Cerçon, C., and C. Iguago	5599	Ecuador	-1.07	-77.60	450
QCNE 46430	Cerçon, C., et al.	8728	Ecuador	-1.07	-77.60	400
MO 4066077	Cerçon, C., et al.	8728	Ecuador	-1.07	-77.60	400
QCNE-46737	Cerçon, C., et al.	8728	Ecuador	-1.07	-77.60	400
NY	Zak, V.	4161	Ecuador	-1.25	-76.92	320
MO 4210196	Zak, V.	4161	Ecuador	-1.25	-76.92	320
QCNE-21871	Zak, V.	4161	Ecuador	-1.25	-76.92	320
Harvard	Garcia-Barriga, H.	14769	Colombia	-1.25	-69.63	240
NY	Tipaz, G., et al.	571	Ecuador	-1.42	-77.33	400
F 2157840	Hurtado, F.	2987	Ecuador	-1.42	-77.33	400
QCNE/MO	Palacios, W.	12164	Ecuador	-1.47	-77.43	500
QCNE-49315	Gudi, O, E.	930	Ecuador	-1.57	-77.42	580
QCNE 80470	Palacios, W.	11379	Ecuador	-1.77	-78.00	900
Mo 2820305	Diaz, C.	1380	Peru	-2.50	-75.75	200
F 668926	Klug, G.	2130	Peru	-2.87	-75.25	180
Harvard	Klug, G.	2130	Peru	-2.87	-75.25	180
Harvard	Klug, G.	2130	Peru	-2.88	-75.25	180
US 1456778	Klug, G.	2130	Peru	-2.88	-75.25	180
NY	Klug, G.	2130	Peru	-2.88	-75.25	180
MO1039604	Klug, G.	2130	Peru	-2.88	-75.25	180
NY	Cid Ferreira, C. A., et al.	8462	Brazil	-2.88	-67.75	180
H	Schultes, R. E., and G. A.	8166	Brazil	-3.00	-69.00	100
US 1989313	Black	8166	Brazil	-3.00	-69.00	100
MO 4257892	Pipoly, J.	13212	Peru	-3.33	-72.92	400
MO 3826656	Vasquez, R.	11810	Peru	-3.33	-72.92	130
MO 4229666	Vasquez, R., and N. Jaramillo	16072	Peru	-3.33	-72.92	140
F1868820	Rimachi Y., M.	3043	Peru	-3.50	-73.07	100
NY	Rimachi Y., M.	3043	Peru	-3.50	-73.07	100
MO 2825596	Rimachi Y., M.	3043	Peru	-3.50	-73.07	100
MO 3630620	Vasquez, R., et al.	5164	Peru	-3.50	-72.83	106
NY	Vasquez, R., et al.	5164	Peru	-3.50	-72.83	106
F 2034668	Vasquez, R., et al.	5164	Peru	-3.50	-72.83	106
F 1312427	Frøes, R. L. de	20820	Brazil	-3.50	-68.95	200
US 2343268	Frøes, R. L. de	34864	Brazil	-3.50	-68.95	200
MO 2188259	Ducke, A.	19576	Brazil	-3.50	-68.95	200
NY	Ducke, A.	314	Brazil	-3.50	-68.95	200
NY 3097	Frøes, R. L. de	20813	Brazil	-3.50	-68.95	200
MO 2188260	Ducke, A.	23693	Brazil	-3.50	-68.95	200
F 1230229	Ducke, A.	561	Brazil	-3.50	-68.95	200
H	Ducke, A.	1486	Brazil	-3.50	-68.95	100
NY	Ducke, A.	23693	Brazil	-3.50	-68.95	200
MO 1255784	Ducke, A.	561	Brazil	-3.50	-68.95	200
US 1516503	Ducke, A.	23693	Brazil	-3.50	-68.95	200
Harvard	Ducke, A.	143	Brazil	-3.50	-68.95	200
NY-2728	Ducke, A.	1486	Brazil	-3.50	-68.95	100
NY	Ducke, A.	19576	Brazil	-3.50	-68.95	200

F 1486643	Ducke, A.	1486	Brazil	-3.50	-68.95	200
NY	Ducke, A.	561	Brazil	-3.50	-68.95	200
NY	Frøes, R. L. de	20820	Brazil	-3.50	-68.95	200
MO 4257890	Pipoly, J., et al.	12632	Peru	-3.58	-73.25	110
NY	Klug, G.	180	Peru	-3.58	-73.25	100
F 6184000	Williams, L.	3786	Peru	-3.58	-73.25	120
NY	Tessman, G.	5101	Peru	-3.58	-73.25	120
F 624285	Klug, G.	180	Peru	-3.58	-73.25	100
MO 3869563	Vasquez, R., and N. Jaramillo	9676	Peru	-3.80	-73.42	122
MO 3042849	Tunqui, S.	189	Peru	-3.83	-77.67	200
MO 2892231	Huashikat, V.	1786	Peru	-3.83	-77.67	200
MO 2813382	Huashikat, V.	1035	Peru	-3.83	-77.67	180
MO 2892223	Huashikat, V.	2012	Peru	-3.83	-77.67	200
MO 3042848	Tunqui, S.	301	Peru	-3.83	-77.67	200
MO 4257893	Vasquez, R., et al.	15809	Peru	-3.87	-73.25	180
MO 4257891	Vasquez, R.	14517	Peru	-3.87	-73.25	200
MO 3630613	Vasquez, R., et al.	5880	Peru	-3.87	-73.25	200
MO 2734836	Rimachi Y., M.	3303	Peru	-3.88	-73.63	170
FLAS 186349	Rimachi Y., M.	4532	Peru	-3.88	-73.63	170
NY	McDaniel, S., and M. Rimachi	21069	Peru	-3.88	-73.63	160
NY	Rimachi Y., M.	3303	Peru	-3.88	-73.63	170
MO 2427881	Rimachi Y., M.	2849	Peru	-3.88	-73.63	160
MO 2734836	Rimachi Y., M.	3303	Peru	-3.88	-73.63	160
NY	Rimachi Y., M.	4532	Peru	-3.88	-73.63	160
NY	Rimachi Y., M.	165	Peru	-3.88	-73.63	150
US 3177354	Rimachi Y., M.	4532	Peru	-3.88	-73.63	160
NY	Vasquez, R., et al.	5354	Peru	-3.92	-73.92	0
MO 3842988	Vasquez, R., et al.	12195	Peru	-3.92	-73.67	150
Tex	Vasquez, R., et al.	5354	Peru	-3.92	-73.58	130
MO 3584403	Vasquez, R., et al.	5354	Peru	-3.92	-73.58	130
MO 2204538	Kayap, R.	662	Peru	-4.00	-78.00	375
NY	McDaniel, S.	29641	Peru	-4.00	-73.25	200
F2027320	Vasquez, R., et al.	7296	Peru	-4.17	-72.00	116
NY	Vasquez, R., et al.	7296	Peru	-4.17	-72.00	116
MO 3628224	Vasquez, R., et al.	7296	Peru	-4.17	-72.00	116
QCNE 54324	Palacios, W., et al.	8654	Ecuador	-4.27	-78.70	930
NY	Prance, G. T., et al.	23868	Brazil	-4.47	-71.68	300
MO 2725124	Prance, G. T., et al.	23868	Brazil	-4.47	-71.68	200
MO 2323593	Ancuash, E.	218	Peru	-4.50	-78.12	333
MO 2205012	Ancuash, E.	142	Peru	-4.50	-78.12	33
NY-00066801	Tessmann, G.	4108	Peru	-4.50	-77.45	500
F-1022586	Tessmann, G.	4108	Peru	-4.50	-77.42	500
MO -4929828	Vasquez, R., et al.	24158	Peru	-4.55	-78.20	600
MO	Vasquez, R.	24158	Peru	-4.55	-78.20	600
F 2157471	Cid Ferreira, C. A., et al.	9949	Brazil	-4.55	-71.67	100
NY	Cid Ferreira, C. A., et al.	9949	Brazil	-4.55	-71.67	100
US 3290254	Cid Ferreira, C. A., et al.	9949	Brazil	-4.55	-71.67	200
MO 2435914	Ancuash, E.	424	Peru	-4.63	-78.13	440
NY	Ancuash, E.	424	Peru	-4.63	-78.13	440
MO 3032654	Ancuash, E.	424	Peru	-4.63	-78.13	440

MO-5096929	Jaramillo, N., and K. Katip	797	Peru	-4.92	-78.32	320
MO-5096932	Vasquez, R., et al.	19932	Peru	-4.92	-78.32	550
MO-5096933	Vasquez, R., et al.	19956	Peru	-4.92	-78.32	550
MO-5096931	Vasquez, R., et al.	20016	Peru	-4.92	-78.32	550
MO-4929838	Vasquez, R., et al.	20363	Peru	-4.92	-78.32	320
MO-5096930	Jaramillo, N.	869	Peru	-4.92	-78.32	320
MO-4929829	Vasquez, R., et al.	23886	Peru	-5.05	-78.33	600
MO-4929833	Vasquez, R., et al.	21416	Peru	-5.05	-78.33	380
MO-429825	Diaz, C., et al.	4172	Peru	-5.05	-78.33	310
MO	Diaz, C.	4128	Peru	-5.05	-78.33	310
MO-4929827	Diaz, C., et al.	4128	Peru	-5.05	-78.33	310
MO	Diaz, C., et al.	4172	Peru	-5.05	-78.33	310
MO-4929832	Vasquez, R., et al.	24895	Peru	-5.05	-78.33	450
	Vasquez, R., et al.	24895	Peru	-5.07	-78.33	450
MO0429834	Vasquez, R., et al.	24643	Peru	-5.25	-78.37	900
MO	Vasquez, R., et al.	24643	Peru	-5.25	-78.37	900
F-1706096	Vigo, J. S.	6439	Peru	-6.03	-75.88	540
MO 5103767	Vigo, J. S.	6519	Peru	-6.03	-75.88	532
US 2703971	Vigo, J. S.	6519	Peru	-6.03	-75.88	532
MO 5103768	Vigo, J. S.	6439	Peru	-6.03	-75.88	540
F 1706102	Vigo, J. S.	6519	Peru	-6.03	-75.88	532
US 3260273	Knapp, S., and J. Mallet	8466	Peru	-6.25	-76.28	200
MO 3632210	Knapp, S., and J. Mallet	8466	Peru	-6.25	-76.28	200
NY	Vigo, J. S.	4596	Peru	-8.00	-76.62	500
H	Vigo, J. S.	4596	Peru	-8.00	-76.62	500
F 1871036	Vigo, J. S.	4596	Peru	-8.00	-76.62	500
Tex	Vigo, J. S.	5566	Peru	-8.12	-76.52	600
F 1874381	Vigo, J. S.	5566	Peru	-8.12	-76.52	600
Duke 311051	Vigo, J. S.	5566	Peru	-8.12	-76.52	600
NY	Vigo, J. S.	5566	Peru	-8.12	-76.52	600
F 1898708	Rodriguez, L. T.	03	Peru	-9.00	-76.00	650
NY	MacBride, J. F.	5065	Peru	-9.55	-75.90	1170
F 536109	MacBride, J. F.	5065	Peru	-9.55	-75.90	1170
NY	Killip, E. P., and A. C. Smith	26053	Peru	-10.87	-73.75	1100
F 616725	Killip, E. P., and A. C. Smith	26053	Peru	-10.87	-73.75	1100
MO-4657673	Betancur, J., et al.	4316	Colombia	1.30	-78.13	1325
MO-2637120	Renteria, E., et al.	28	Colombia	7.38	-73.83	320
MO-2665544	Renteria, E., et al.	14	Colombia	5.58	-73.50	320
H	Soejarto, D.	2782	Colombia	7.50	-74.92	700
H	Soejarto, D.	2782	Colombia	7.50	-74.92	700
H	Soejarto, D.	2782	Colombia	7.50	-74.92	700
MO 2716725	Gentry, A. H., and E. Renteria	23826	Colombia	5.50	-76.55	50
NY	Gentry, A. H., and E. Renteria	23826	Colombia	5.50	-76.55	50
	Cazalet, P. C. D., and T. D.					
NY	Pennington	7602	Ecuador	-2.38	-77.52	500
	Cazalet, P. C. D., and T. D.					
US 2406117	Pennington	7602	Ecuador	-2.38	-77.52	500
	van der Werff, H., and W.					
NY	Palacios	10331	Ecuador	-2.78	-77.83	800
	van der Werff, H., and W.					
MO 4066528	Palacios	10331	Ecuador	-2.78	-77.83	800

	van der Werff, H., and W.					
NY	Palacios	9393	Ecuador	-4.25	-79.20	2000
OAC	Steeves, R. et al	RS 328	Ecuador	-3.57	-78.45	1200
OAC	Steeves, R. et al	RS 328	Ecuador	-3.57	-78.45	1200
OAC	Steeves, R. et al	RS 332	Ecuador	-3.57	-78.45	1200
OAC	Steeves, R. et al	RS 334	Ecuador	-3.57	-78.45	1200
OAC	Steeves, R. et al	RS 334	Ecuador	-3.57	-78.45	1200
OAC	Steeves, R. et al	RS 336	Ecuador	-3.57	-78.45	1200
OAC	Steeves, R. et al	RS 338	Ecuador	-3.57	-78.45	1200
OAC	Steeves, R. et al	RS 346	Ecuador	-3.57	-78.45	1200
OAC	Steeves, R. et al	RS 271	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 271	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 274	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 278	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 278	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 280	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 280	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 284	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 284	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 293	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 294	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 295	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 295	Ecuador	-1.04	-77.37	450
OAC	Steeves, R. et al	RS 364	Ecuador	-1.78	-77.83	900
OAC	Steeves, R. et al	RS 364	Ecuador	-1.78	-77.83	900
OAC	Steeves, R. et al	RS 370	Ecuador	-1.78	-77.83	900
OAC	Steeves, R. et al	RS 406	Ecuador	-1.78	-77.83	900
OAC	Steeves, R. et al	RS 418	Ecuador	-1.78	-77.83	900
OAC	Steeves, R. et al	RS 406	Ecuador	-1.78	-77.83	900
OAC	Steeves, R. et al	RS 527	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 531	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 535	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 535	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 538	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 538	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 538	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 538	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 551	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 562	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 566	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 568	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 570	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 571	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 571	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 572	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 572	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 577	Peru	-13.24	-70.78	1000
OAC	Steeves, R. et al	RS 600	Peru	-13.24	-70.78	1000

Table 2.2 CA analysis of 48 quantitative morphological metrics for *C. capitellata* taxa. Bolded Pearson correlation (P Corr.) values indicate the metrics most significant to the ordination (** = p value < 0.01).

Metric	X-axis		Y-axis	
	P Corr.	Sig. (2-tailed)	P Corr.	Sig.(2-tailed)
Stem and leaf				
Stem width-min.	0.261	0.000	0.060	0.329
Stem width-max	0.127	0.038	-0.032	0.607
Petiole length-min	0.713**	0.000	0.083	0.175
Petiole length-max	0.671	0.000	0.141	0.021
Leaf Petiole width-min	0.585	0.000	-0.080	0.194
Leaf Petiole width-max	0.653	0.000	-0.036	0.558
Lamina Length-min	0.919**	0.000	-0.136	0.026
Lamina Length-max	0.857**	0.000	0.081	0.187
Leaf width ¼ length-min	0.924**	0.000	-0.046	0.454
Leaf width ¼ length-max	0.918**	0.000	0.126	0.040
Leaf width ½ length-min	0.941**	0.000	-0.046	0.459
Leaf width ½ length-max	0.904**	0.000	0.135	0.027
Leaf width ¾ length-min	0.945**	0.000	-0.030	0.624
Leaf width ¾ length-max	0.883**	0.000	0.140	0.023
Leaf vein number-min	0.604	0.000	-0.454**	0.000
Leaf vein number-max	0.544	0.000	-0.464**	0.000
Leaf vein space-min	0.463	0.000	0.475**	0.000
Leaf vein space-max	0.325	0.000	0.487**	0.000
Leaf acumen length-min	0.467	0.000	0.004	0.951
Leaf acumen length-max	0.443	0.000	-0.043	0.487
Androecial				
Anther number min	-0.790**	0.000	0.502	0.000
Anther number max	-0.736**	0.000	0.556**	0.000
Anther length min	-0.593**	0.000	-0.684**	0.000
Anther length max	-0.593**	0.000	-0.684**	0.000
Anther sac length min	-0.419	0.000	-0.608**	0.000
Anther sac length max	-0.496	0.000	-0.517**	0.000
Anther cluster width min	-0.576**	0.000	-0.555**	0.000
Anther cluster width max	-0.590**	0.000	-0.535**	0.000
Filament column length min	-0.545**	0.000	-0.609**	0.000
Filament column length max	-0.573**	0.000	-0.566	0.000
Filament column apical width min	-0.440	0.000	-0.251	0.030
Filament column apical width max	-0.408	0.000	-0.238	0.040
Filament column basal width min	-0.482	0.000	-0.195	0.093
Filament column basal width max	-0.515	0.000	-0.144	0.217
Perianth				
Flower pedicel length min	0.572	0.000	0.700**	0.000
Flower pedicel length max	0.659	0.000	0.638**	0.000
Flower pedicel width min	0.329	0.036	-0.337	0.031
Flower pedicel width max	0.345	0.027	-0.391	0.011
Perianth thickness min	0.174	0.277	-0.374	0.016
Perianth thickness max	0.230	0.148	-0.405**	0.009
Tepal lobe length min	0.881**	0.000	-0.235	0.139
Tepal lobe length max	0.881**	0.000	-0.236	0.138
Perianth tube length min	0.529	0.000	-0.028	0.863
Perianth tube length max	0.737	0.000	-0.014	0.929
Perianth total length min	0.915**	0.000	-0.211	0.184
Perianth total length max	0.948**	0.000	-0.183	0.253

Perianth width at widest point min	0.878**	0.000	0.049	0.760
Perianth width at widest point max	0.856**	0.000	0.026	0.872

Table 2.3 Collection numbers (Coll. #), Country, Population name, decimal degrees latitude (DD Lat.), decimal degrees longitude (DD long.), elevation above sea level (Elev a.s.l.), and Barcode of Life Database process identification numbers (BOLD. process ID) for specimens used in molecular analyses. Collectors of samples were J. Janovec (JPJ), Camila Diaz (CDiaz), and R. Steeves (RADS). Specimens of *C. atopa* and *C. diazii* are indicated by a “-Ca” and “Cd” preceding the collection number. All other collections represent *C. capitellata*.

Coll. #	Country	Population name	DD Lat.	DD Long.	Elev. a.s.l.	BOLD process ID
JPJ1374-Ca	Ecuador	C. atopa-Orellana	-0.47	-77.26	400m	RSMYR002-11
RADS332	Ecuador	Morona-Santiagoensis	-3.57	-78.45	1200m	RSMYR053-11
RADS334	Ecuador	Morona-Santiagoensis	-3.57	-78.45	1200m	RSMYR054-11
RADS336	Ecuador	Morona-Santiagoensis	-3.57	-78.45	1200m	RSMYR055-11
RADS338	Ecuador	Morona-Santiagoensis	-3.57	-78.45	1200m	RSMYR056-11
RADS346	Ecuador	Santiagoensis	-3.57	-78.45	1200m	RSMYR057-11
JPJ893	Ecuador	Napo	-1.04	-77.37	450m	RSMYR058-11
JPJ894	Ecuador	Napo	-1.04	-77.37	450m	RSMYR059-11
JPJ899	Ecuador	Napo	-1.04	-77.37	450m	RSMYR009-11
JPJ900	Ecuador	Napo	-1.04	-77.37	450m	RSMYR060-11
JPJ902	Ecuador	Napo	-1.04	-77.37	450m	RSMYR061-11
RADS271	Ecuador	Napo	-1.04	-77.37	450m	RSMYR062-11
RADS274	Ecuador	Napo	-1.04	-77.37	450m	RSMYR063-11
RADS278	Ecuador	Napo	-1.04	-77.37	450m	RSMYR064-11
RADS280	Ecuador	Napo	-1.04	-77.37	450m	RSMYR065-11
RADS284	Ecuador	Napo	-1.04	-77.37	450m	RSMYR066-11
RADS293	Ecuador	Napo	-1.04	-77.37	450m	RSMYR067-11
RADS294	Ecuador	Napo	-1.04	-77.37	450m	RSMYR068-11
RADS295	Ecuador	Napo	-1.04	-77.37	450m	RSMYR069-11
RADS364	Ecuador	Pastaza	-1.78	-77.83	1000m	RSMYR070-11
RADS370	Ecuador	Pastaza	-1.78	-77.83	1000m	RSMYR071-11
RADS406	Ecuador	Pastaza	-1.78	-77.83	1000m	RSMYR072-11
RADS409	Ecuador	Pastaza	-1.78	-77.83	1000m	RSMYR073-11
RADS418	Ecuador	Pastaza	-1.78	-77.83	1000m	RSMYR074-11
RADS427	Ecuador	Pastaza	-1.78	-77.83	1000m	RSMYR075-11
JPJ1542	Ecuador	Zamora-Chinchipe	-4.29	-78.63	1000m	RSMYR076-11
JPJ1543	Ecuador	Zamora-Chinchipe	-4.29	-78.63	1000m	RSMYR077-11
JPJ1544	Ecuador	Zamora-Chinchipe	-4.29	-78.63	1000m	RSMYR078-11
JPJ1545	Ecuador	Zamora-Chinchipe	-4.29	-78.63	1000m	RSMYR079-11
RADS527	Peru	Cusco	-13.24	-70.78	1000m	RSMYR003-11

RADS531	Peru	Cusco	-13.24	-70.78	1000m	RSMYR080-11
RADS535	Peru	Cusco	-13.24	-70.78	1000m	RSMYR081-11
RADS538	Peru	Cusco	-13.24	-70.78	1000m	RSMYR082-11
RADS550	Peru	Cusco	-13.24	-70.78	1000m	RSMYR083-11
RADS551	Peru	Cusco	-13.24	-70.78	1000m	RSMYR004-11
RADS562	Peru	Cusco	-13.24	-70.78	1000m	RSMYR084-11
RADS566	Peru	Cusco	-13.24	-70.78	1000m	RSMYR085-11
RADS568	Peru	Cusco	-13.24	-70.78	1000m	RSMYR086-11
RADS569	Peru	Cusco	-13.24	-70.78	1000m	RSMYR087-11
RADS570	Peru	Cusco	-13.24	-70.78	1000m	RSMYR088-11
RADS571	Peru	Cusco	-13.24	-70.78	1000m	RSMYR089-11
RADS572	Peru	Cusco	-13.24	-70.78	1000m	RSMYR090-11
JPJ843	Peru	Loreto	-3.48	-74.25	100m	RSMYR093-11
JPJ844	Peru	Loreto	-3.48	-74.25	100m	RSMYR094-11
JPJ855	Peru	Loreto	-3.52	-73.15	100m	RSMYR006-11
JPJ860	Peru	Loreto	-3.52	-73.15	100m	RSMYR095-11
JPJ862	Peru	Loreto	-3.52	-73.15	100m	RSMYR096-11
JPJ863	Peru	Loreto	-3.52	-73.15	100m	RSMYR097-11
JPJ872	Peru	Loreto	-3.52	-73.15	100m	RSMYR007-11
JPJ873	Peru	Loreto	-3.52	-73.15	100m	RSMYR098-11
JPJ874	Peru	Loreto	-3.52	-73.15	100m	RSMYR099-11
JPJ875	Peru	Loreto	-3.52	-73.15	100m	RSMYR008-11
JPJ827	Peru	San Martin	-6.15	-76.17	250m	RSMYR101-11
JPJ829	Peru	San Martin	-6.15	-76.17	250m	RSMYR103-11
JPJ830	Peru	San Martin	-6.15	-76.17	250m	RSMYR104-11
JPJ831	Peru	San Martin	-6.15	-76.17	250m	RSMYR105-11
JPJ832	Peru	San Martin	-6.15	-76.17	250m	RSMYR106-11
JPJ833	Peru	San Martin	-6.15	-76.17	250m	RSMYR107-11
JPJ834	Peru	San Martin	-6.15	-76.17	250m	RSMYR108-11
JPJ835	Peru	San Martin	-6.15	-76.17	250m	RSMYR005-11
CDiaz7644-Cd	Peru	Bagua	-5.03	-78.22	800m	RSMYR012-11

Figures

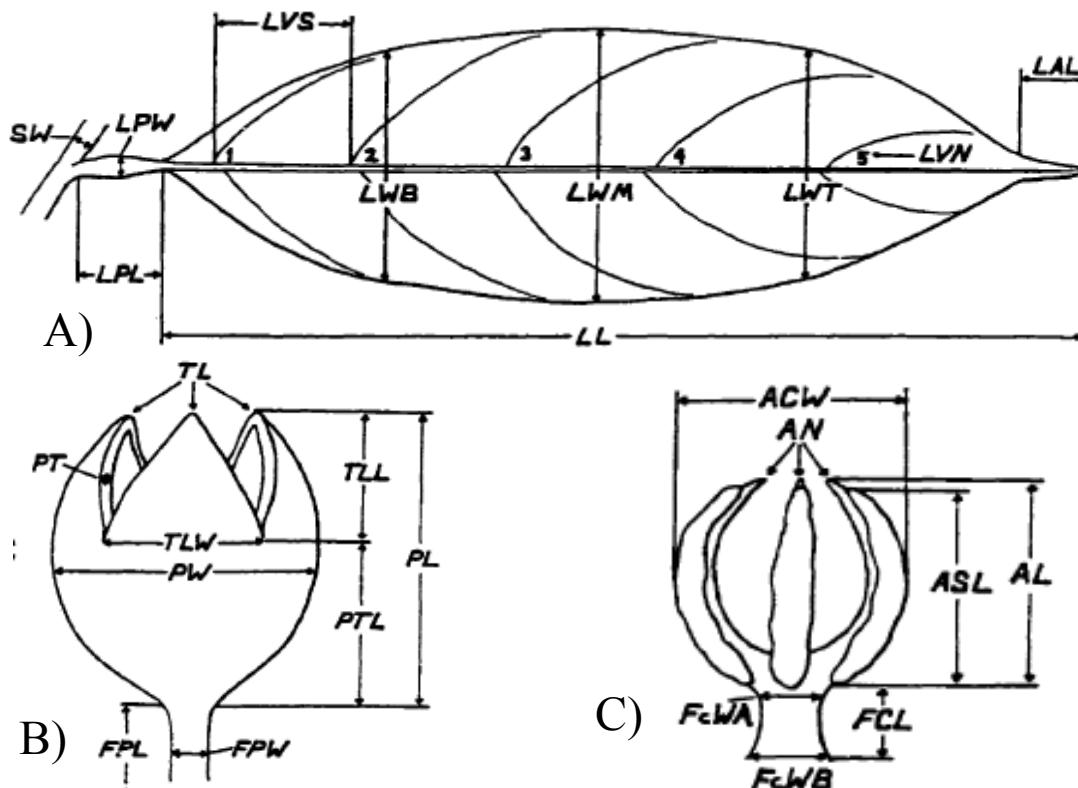


Figure 2.1 Illustration of morphological metrics: A) Vegetative data points: SW=Stem Width, LPL=Petiole Length, LPW=Petiole Width, LVS=space between secondary veins, LVN=Vein Number, LL=Lamina Length, LWB= Leaf Width at $\frac{1}{4}$ length, LWM=Leaf Width at $\frac{1}{2}$ length, LWT=Leaf Width at $\frac{3}{4}$ length, LVN=Leaf Vein Number, LAL=Leaf Acumen Length. B) Perianth data points: TL=Tepal Lobe number, PT=Perianth Thickness, TLW=Tepal Lobe Basal Width, PW=Perianth Width at widest point, FPL=Flower Pedicel Length, FPW=Flower Pedicel Width, TLL=Perianth Tube Length, PTL= Perianth tube length, PL=Perianth total Length. C) Androecium data points: AN=Anther Number, AL=Anther Length, ASL=Anther Sac Length, ACW=Anther Cluster Width, FCL=Filament Column Length, FCWA=Filament Column Apical Width, FCWB= Filament Column Basal Width. (modified from Janovec 2000).



Figure 2.2 Sampling localities of *Compsoeura* taxa for genetic analyses: 1) *C. atopa*, Ecuador, Orellana. 2) *C. capitellata*, Ecuador, Napo. 3) *C. capitellata*, Ecuador, Pastaza. 4) *C. capitellata*, Ecuador, Morona-Santiago. 5) *C. capitellata*, Ecuador, Zamora-Chinchipec. 6) *C. capitellata*, Peru, Loreto. 7) *C. capitellata*, Peru, San Martin. 8) *C. capitellata*, Peru, Cusco. 9) *C. diazii*, Peru, Bagua. The area inside the blue line indicates the known range of *C. capitellata*.

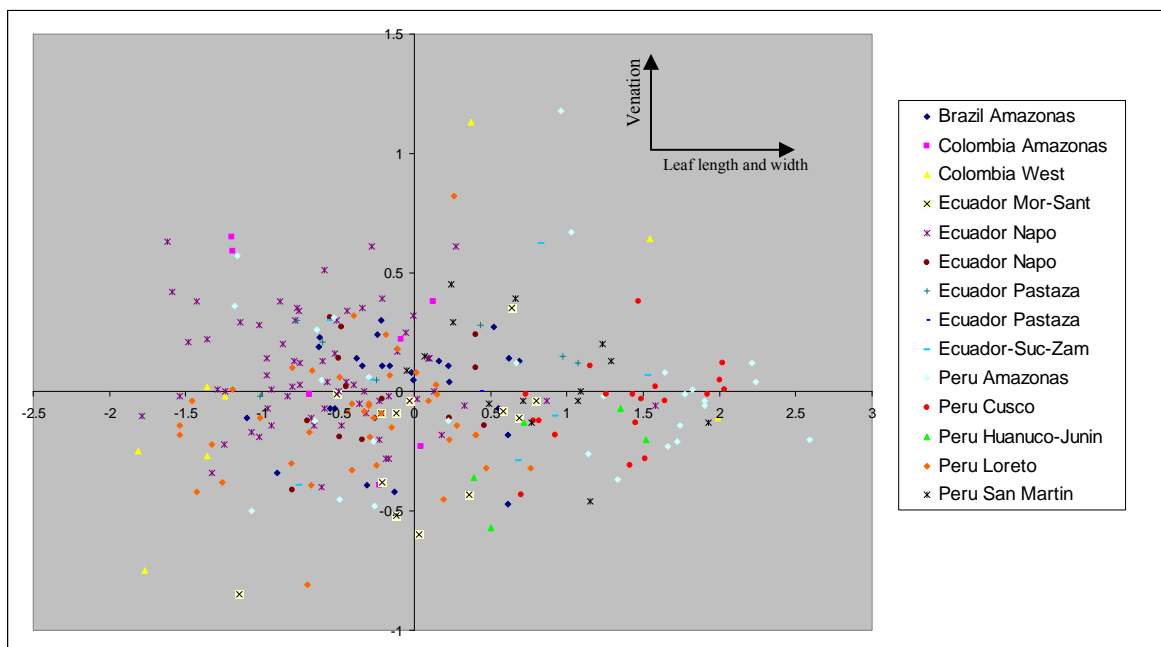


Figure 2.3 NMS ordination of quantitative leaf data for 268 specimens of *C. capitellata*. Stress value 0.09. Legend indicates the country and province or region the specimens originated from. Arrows show the morphological characters most significant to the axes as revealed by the Pearson correlation analysis.

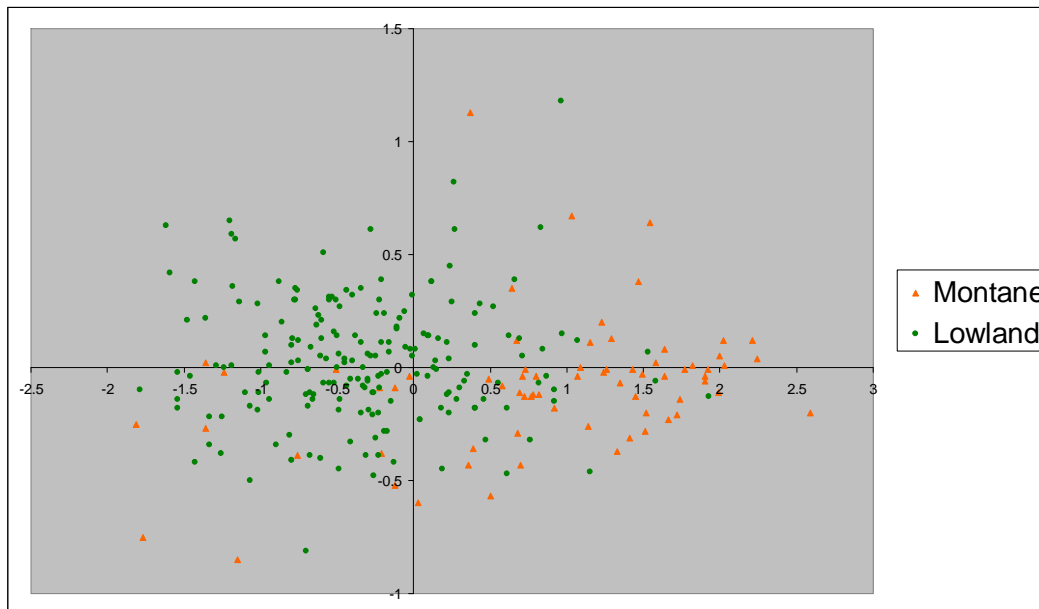


Figure 2.4 NMS ordination of quantitative leaf data for 268 specimens of the *C. capitellata* complex with specimens classified as montane and lowland. Stress value 0.09.

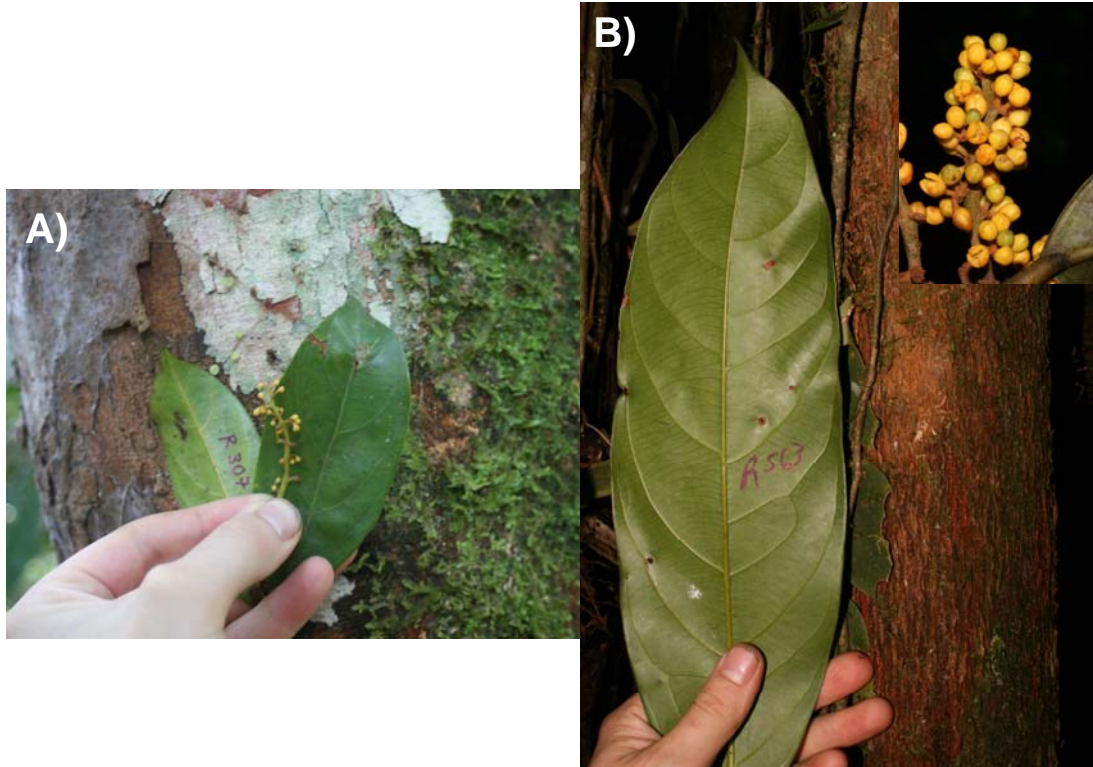


Figure 2.5 An example of bark, leaf and flower morphological trait variation in *C. capitellata* among (A) lowland populations (Ecuador, Napo) and (B) montane populations (Peru, Cusco).

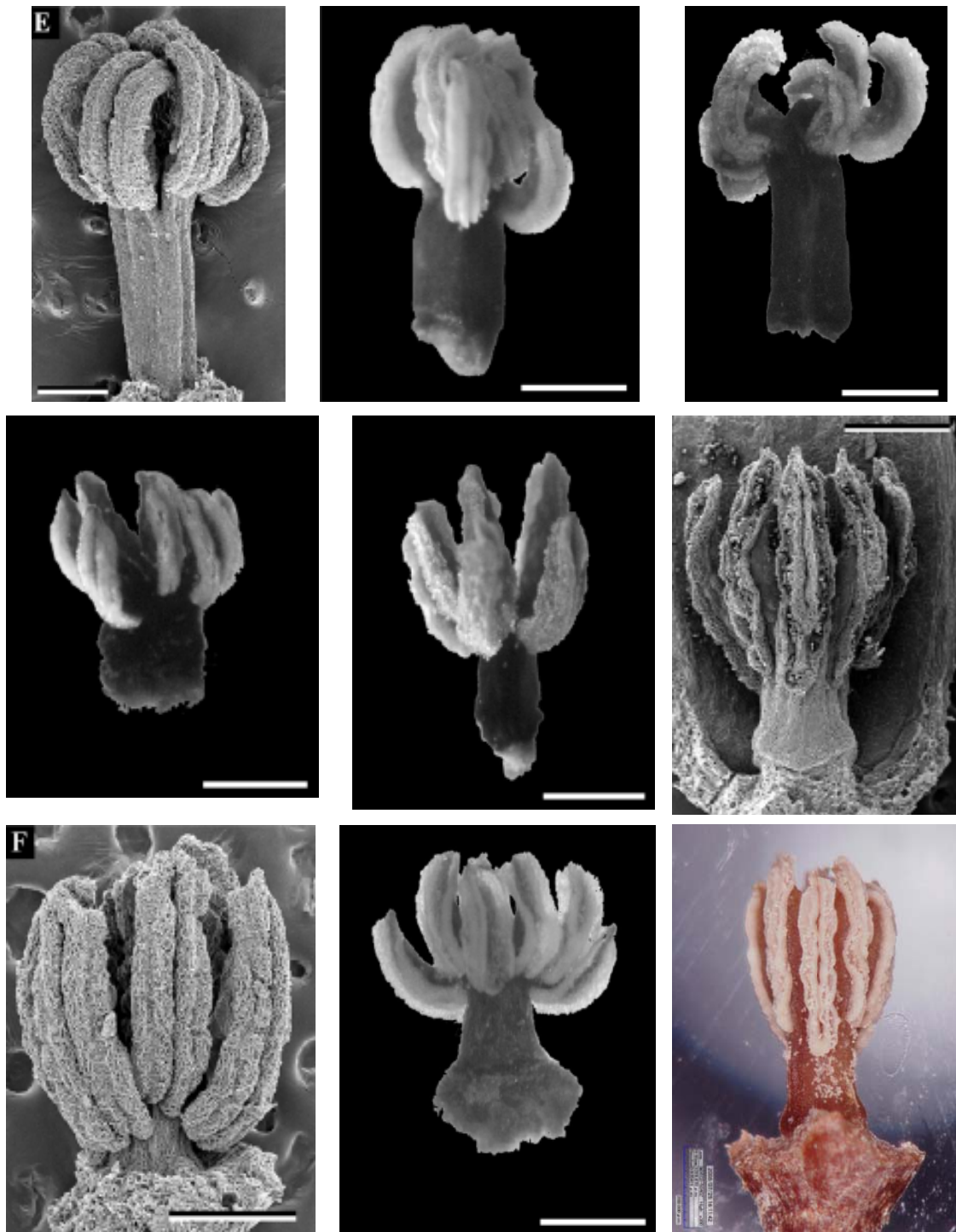


Figure 2.6 S.E.M. and light micrographs of androecial morphology of 9 staminate collections from the *C. capitellata* complex of Brazil, Ecuador and Peru. A) R. L. Froes 34648, Brazil (scale bar= 250 μ m) B) A. Ducke, 561, Brazil (scale bar= 500 μ m) C) A. Ducke 23693, Brazil (scale bar= 500 μ m) D) M. Aulestia 2713, Ecuador (scale bar= 500 μ m) E) M. Aulestia 1651, Ecuador (scale bar= 500 μ m) F) P. C. D. Cazalet and T. D. Pennington 7602, Ecuador (scale bar= 500 μ m) G) E. P. Killip and A. C. Smith 26053,

Peru (scale bar= 500 μm) H) E. Ancuash 424, Peru (scale bar= 500 μm) I) R. S. Steeves 600, Peru (scale bar= 200 μm).

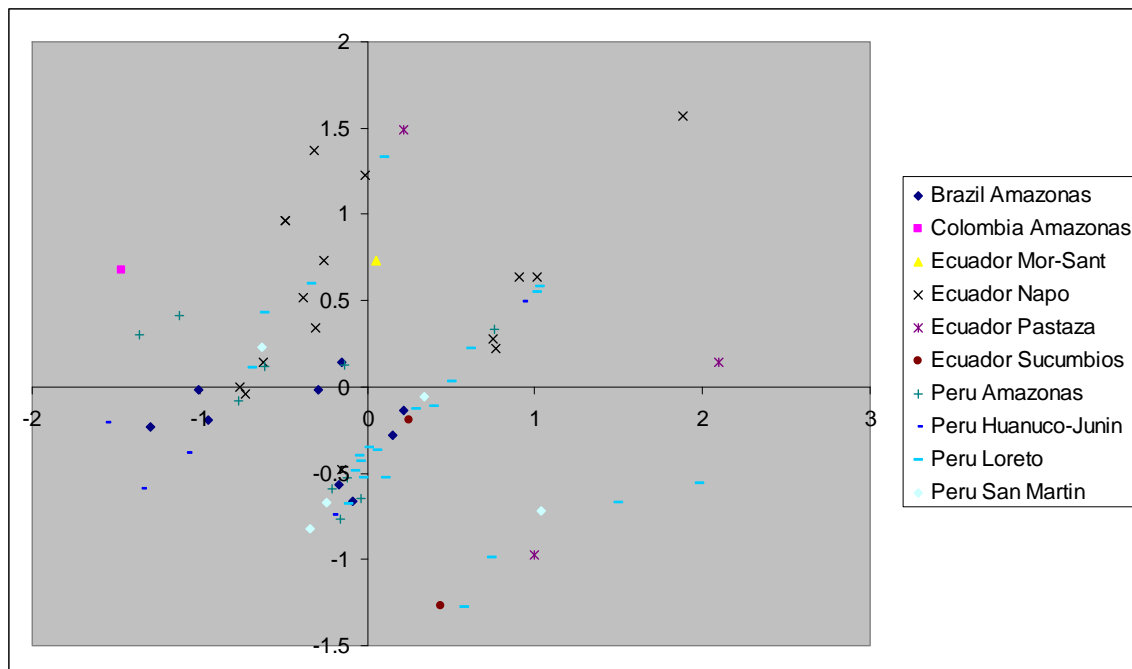


Figure 2.7 NMS ordination of 14 androecial characters for 75 samples. The legend indicates the country and region of origin for the samples. Stress=0.13.

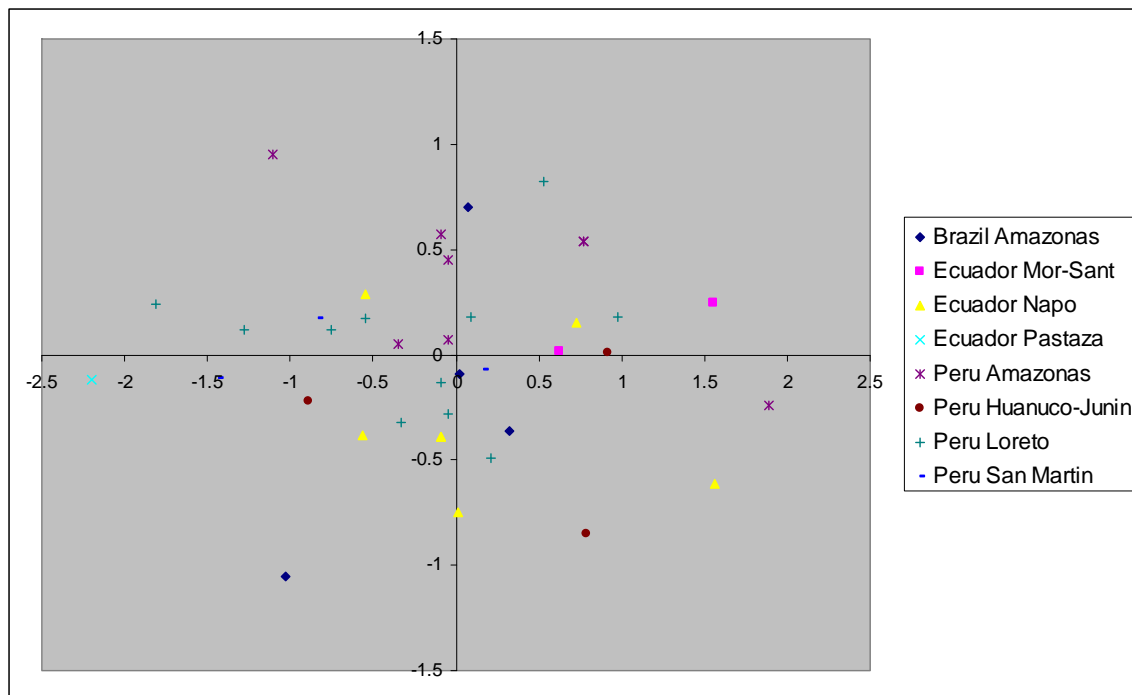


Figure 2.8 NMS ordination of 7 perianth characters for 41 samples. Legend indicates the country and region of origin for the samples used. Stress=0.10.



Figure 2.9 Fruit variation in *C. capitellata*: A) Intact glabrous fruit type found in San Martin, Peru (Top: J. Janovec 836) and intact sulcate fruit of Loreto, Peru (bottom: J. Janovec 843) B) Dissected fruit from Pastaza province, Ecuador (R. Steeves 427); interior of pericarp on left, seed exterior and interior in middle, and warty external surface of pericarp on the right.

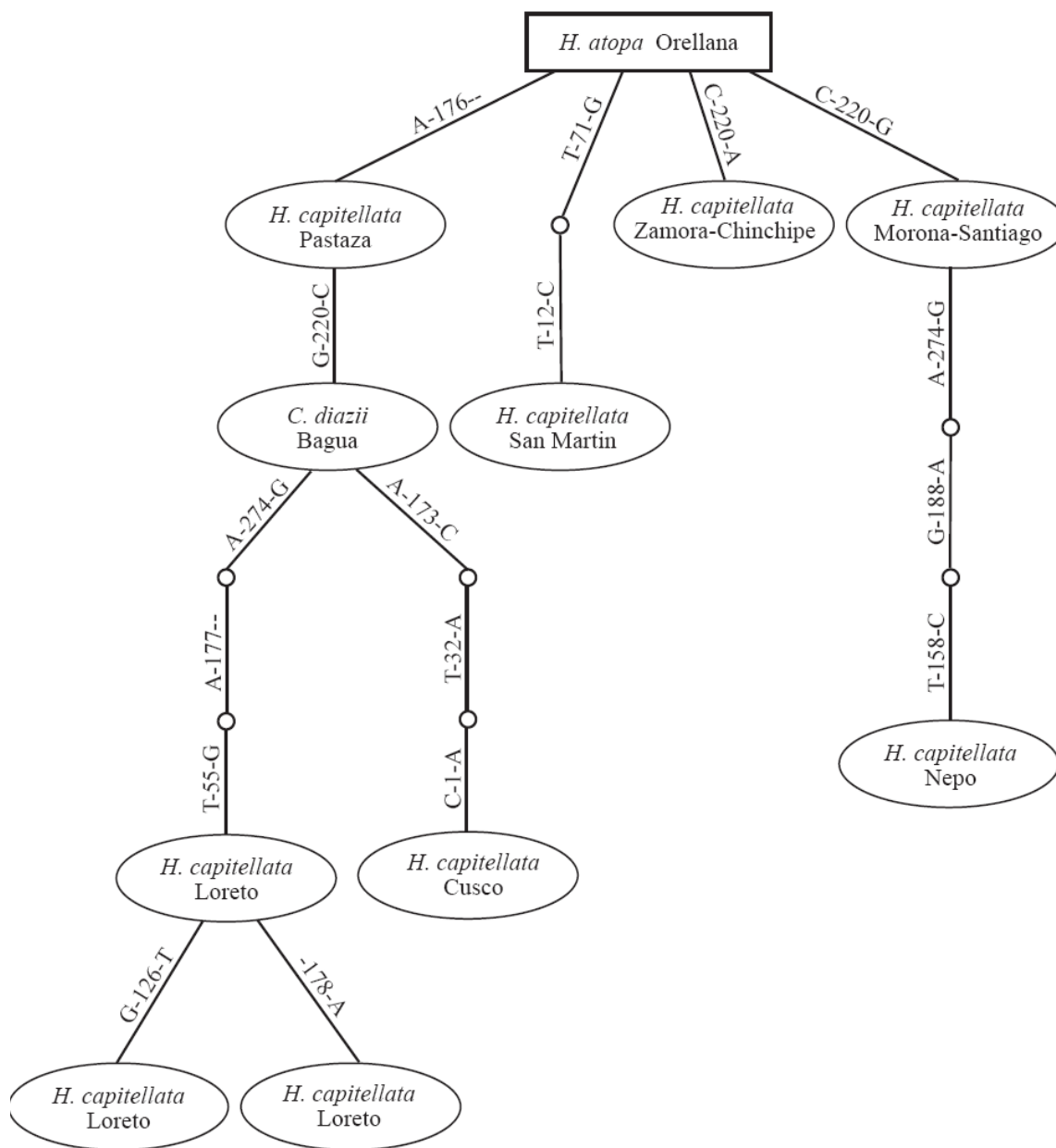


Figure 2.10 trnH-psbA haplotype network for 7 populations of *C. capitellata* and 1 sample each of *C. atopa* and *C. diazii*. Nucleotide changes, and their positions are shown on branches between nodes.

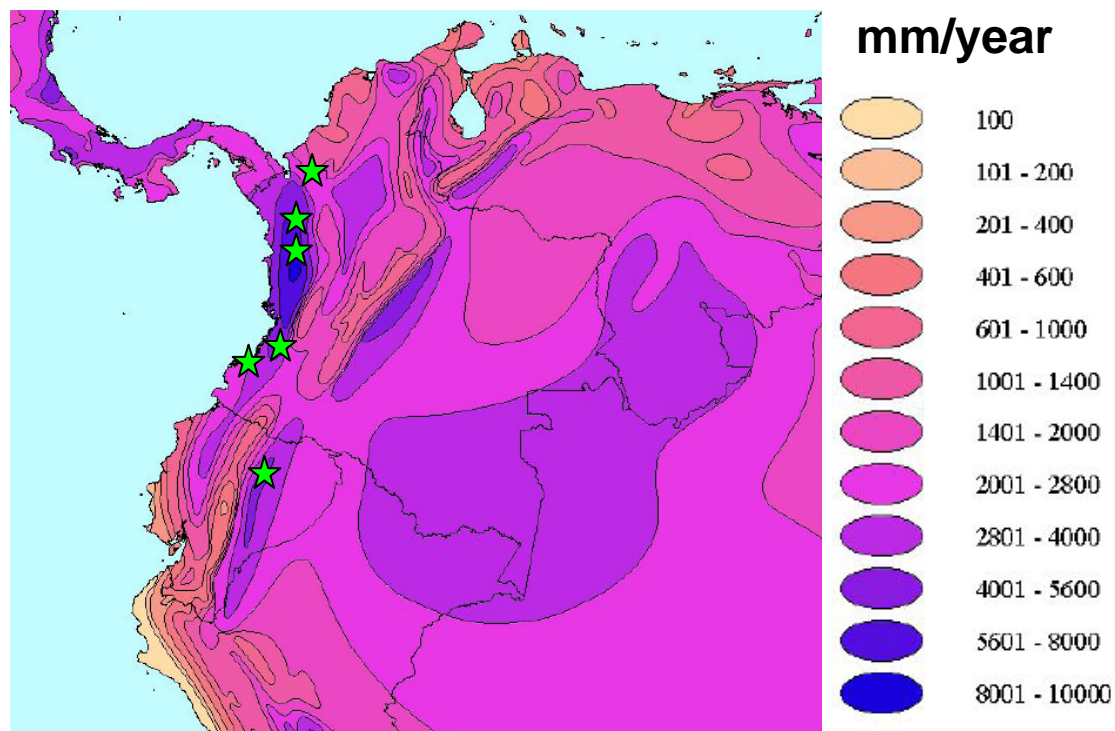


Figure 2.11 Precipitation regimes in South America and collection localities of *C. atopa*.

Literature Cited

- Armstrong, J. E., & Irvine, A. K. 1989. Floral biology of *Myristica insipida* (Myristicaceae), a distinctive beetle pollination syndrome. *American Journal of Botany* **76**: 86–94.
- Asif, M. J., & Cannon, C. H. 2005. DNA extraction from processed wood: a case study for the identification of an endangered timber species (*Gonystylus bancanus*). *Plant Molecular Biology Reporter* **23**: 185–192.
- Bullock, S. H. 1982. Population structure and reproduction in the neotropical dioecious tree *Compsonera sprucei*. *Oecologia* **55**: 238–242.
- Candolle, A. D. 1856. Myristicaceae. *Prodromus Systematis Naturali Vegetabilis* **14**: 187–208.
- Caron, H., Dumas, S., Marque, G., Messier, C., Bandou, E., Petit, R. J., & Kremer, A. 2000. Spatial and temporal distribution of chloroplast DNA polymorphism in a tropical tree species. *Molecular Ecology* **9**: 1089–1098.
- Chan, K. Y., Zwieten, L., Meszaros, I., Downie, A., & Joseph, S. 2007. Agronomic values of greenwaste biochar as a soil amendment. *Australian Journal of Soil Research* **45**: 629–634.
- Clement, M., Posada, D., & Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657–1659.
- Cole, R. J. 2009. Postdispersal seed fate of tropical montane trees in an agricultural landscape, Southern Costa Rica. *Biotropica* **41**: 319–327.
- Demesure, B., Comps, B., & Petit, R. J. 1996. Chloroplast DNA phylogeography of the common beech (*Fagus sylvatica* L.) in Europe. *Evolution* **50**: 2515–2520.
- Fazekas, A. J., Steeves, R., & Newmaster, S. G. 2010. Improving sequencing quality from PCR products containing long mononucleotide repeats. *Biotechniques* **48**: 277–285.
- Gentry, A. H. 1982. Neotropical floristic diversity: phylogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden* **69**: 557–593.
- Gentry, A. H. 1988. Tree species richness of upper Amazonian forests. *Proceedings of the National Academy of Sciences of the United States of America* **85**: 156–159.

- Golden, J. L., & Bain, J. F. 2000. Phylogeographic patterns and high levels of chloroplast DNA diversity in four *Packeria* (Asteraceae) species in southwestern Alberta. *Evolution* **54**: 1566–1579.
- Golenberg, E. M., Clegg, M. T., Durbin, M. L., Doebley, J., & Pow Ma, D. 1993. Evolution of a noncoding region of the chloroplast genome. *Molecular phylogenetics and evolution* **2**: 52-64.
- Graham, S. W., Reeves, P. A., Burns, A. C., & Olmstead, R. G. 2000. Microstructural changes in noncoding chloroplast DNA: interpretation, evolution, and utility of indels and inversions in basal angiosperm phylogenetic inference. *International Journal of Plant Science* **161**: S83–S96.
- Grossman, J. M., O'Neill, B. E., Tsai, S. M., Liang, B., Neves, E., Lehmann, J., & Thies, J. E. 2010. Amazonian anthrosols support similar microbial communities that differ distinctly from those extant in adjacent, unmodified soils of the same mineralogy. *Microbial Ecology* **60**: 192-205.
- Hackenberger, M. J., Petersen, J. B., & Neves, E. G. 1999. Village size and permanence in Amazonia: two archaeological examples from Brazil. *Latin American Antiquity* **10**: 353-376.
- Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series*. (pp. 95–98).
- Hamilton, M. B. 1999. Tropical tree gene flow and seed dispersal-deforestation affects the genetic structure of the surviving forest fragments. *Nature* **401**: 129-130.
- Holbrook, K. M., & Loiselle, B. A. 2009. Dispersal in a Neotropical tree, *Virola flexuosa* (Myristicaceae): Does hunting of large vertebrates limit seed removal? *Ecology* **90**: 1449–1455.
- Hollingsworth, P. M., Graham, S. W., & Little, D. P. 2011. Choosing and using a plant DNA barcode. *PloS one* **6**: e19254.
- Howe, H. F., & Vande Kerckhove, G. A. 1981. Removal of wild nutmeg (*Virola surinamensis*) crops by birds. *Ecology* **62**: 1093–1106.
- Janovec, J. P. 2000. A systematic study of *Compsoeura* (A. DC.) Warb., A Neotropical member of the nutmeg family. Texas A&M University Dissertation: 1-359.
- Janovec, J. P. 2002. *Compsoeura diazii* (Myristicaceae), a new species from the Rio Cenepa area of Northwestern Peru. *Novon* **12**: 366–368.
- Janzen, D. H. 1983. The Pleistocene hunters had help. *The American naturalist* **121**: 598–599.

- Janzen, D. H. 1967. Why mountain passes are higher in the tropics. *The American Naturalist* **101**: 233-249.
- Janzen, D. H., & Martin, P. S. 1982. Neotropical anachronisms: the fruits the gomphotheres ate. *Science* **215**: 19-27.
- Kim, J. S., Sparovek, G., Longo, R. M., De Melo, W. J., & Crowley, D. 2007. Bacterial diversity of terra preta and pristine forest soil from the Western Amazon. *Soil Biology and Biochemistry* **39**: 684–690.
- Kreader, C. A. 1996. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Applied and Environmental Microbiology* **62**: 1102-1106.
- Kruskal, J. B. 1964. Nonmetric multidimensional scaling: a numerical method. *Psychometrika* **29**: 115–129.
- La Rotta, C. 1985. *Estudio etnobotánico de las especies utilizadas por la comunidad indígena Emera del alto río Baudo. Mecanografado. Fundación Segunda Expedición Botánica. Bogota, Colombia.* Bogota Colombia: Mecanografado. Fundación Segunda Expedición Botánica.
- Latouche-Hallé, C., Ramboer, A., Bandou, E., Caron, H., & Kremer, A. 2003. Nuclear and chloroplast genetic structure indicate fine-scale spatial dynamics in a neotropical tree population. *Heredity* **91**: 181–190.
- Liang, B., Lehmann, J., Solomon, D., Sohi, S., Thies, J. E., Skjemstad, J. O., Luizao, F. J., Engelhard, M. H., Neves, E. G., & Wirrick, S. 2008. Stability of biomass-derived black carbon in soils. *Geochimica et Cosmochimica Acta* **72**: 6069–6078.
- Liang, B., Solomon, D., Grossman, J. M., O'Neill, B., Skjemstad, J. O., Thies, J., Luizao, F. J., J. Petersen, & Neves, E. G. 2006. Black carbon increases cation exchange capacity in soils. *Soil Science Society of America Journal* **70**: 1719-1730.
- Minchin, P. R. 1987. An evaluation of the relative robustness of techniques for ecological ordination. *Plant Ecology* **69**: 89–107.
- Müller, K. 2006. Incorporating information from length-mutational events into phylogenetic analysis. *Molecular phylogenetics and evolution* **38**: 667–676.
- Newmaster, S. G., Fazekas, A. J., Steeves, R. A. D., & Janovec, J. 2008. Testing candidate plant barcode regions in the Myristicaceae. *Molecular Ecology Resources* **8**: 480–490.
- Palma-Silva, C., Wendt, T., Pinheiro, F., Barbará, T., FAY, M. F., Cozzolino, S., & Lexer, C. Sympatric bromeliad species (*Pitcairnia spp.*) facilitate tests of

- mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. *Molecular Ecology* **20**: 3185–3201
- Palmé, A. E., Semerikov, V., & Lascoux, M. 2003. Absence of geographical structure of chloroplast DNA variation in sallow, *Salix caprea* L. *Heredity* **91**: 465–474.
- Panchal, M. 2007. The automation of nested clade phylogeographic analysis. *Bioinformatics* **23**: 509-510.
- Paz-Rivera, C., & Putz, F. E. 2009. Anthropogenic soils and tree distributions in a lowland forest in Bolivia. *Biotropica* **41**: 665–675.
- Petit R.J., Csaikl, U.M., Bordacs, S., Burg, K., Coart, E., et al. 2002. Chloroplast DNA variation in European white oaks - Phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecology and Management* **156**: 5–26.
- Pitman, N. C., Terborgh, J. W., Silman, M. R., Núñez V, P., Neill, D. A., Cerón, C. E., Palacios, W. A., & Aulestia, M. 2002. A comparison of tree species diversity in two upper Amazonian forests. *Ecology* **83**: 3210–3224.
- Pitman, N. C., Terborgh, J. W., Silman, M. R., Núñez V, P., Neill, D. A., Cerón, C. E., Palacios, W. A., & Aulestia, M. 2001. Dominance and distribution of tree species in upper Amazonian terra firme forests. *Ecology* **82**: 2101–2117.
- Poinar, H. N. 1998. Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* **281**: 402-406.
- Posada, D., & Crandall, K. A. 2001. Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology & Evolution* **16**: 37–45.
- Prance, G. T. 1977. Floristic inventory of the tropics: Where do we stand? *Annals of the Missouri Botanical Garden* **64**: 659–684.
- Rieseberg, L.H., & Soltis, D.E. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* **5**: 65-84.
- Rohlf, F. J. 2006. *tpsDig version 2.10*, Department of Ecology and Evolution. State University of New York at Stony Brook, New York.
- Roosevelt, A. C., Lima da Costa, M., Lopes Machado, C., Michab, M., Mercier, N., Valladas, H., Feathers, J., Barnett, W., Imazio da Silveira, M., Henderson, A., Silva, J., Chernoff, B., Reese, D. S., Holman, J. A., Toth, N., et al. 1996. Paleoindian cave dwellers in the Amazon: The peopling of the Americas. *Science* **272**: 373-384.

- Sang, T., Crawford, D. J., & Stuessy, T. F. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* **84**: 1120-1136.
- Sauquet, H., Doyle, J. A., Scharaschkin, T., Borsch, T., Hilu, K. W., Chatrou, L. W., & Le Thomas, A. 2003. Phylogenetic analysis of Magnoliales and Myristicaceae based on multiple data sets: implications for character evolution. *Botanical Journal of the Linnean Society* **142**: 125–186.
- Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Liu, W., Miller, J., Siripun, K. C., Winder, C. T., Schilling, E. E., & Small, R. L. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* **92**: 142-166.
- Smith, A. C. 1956. Studies of South American plants: XV. *American Journal of Botany* **43**: 573-577.
- Smith, A. C. 1937. The American species of Myristicaceae. *Brittonia* **2**: 393-510.
- Smith, N. J. H. 1980. Anthrosols and human carrying capacity in Amazonia. *Annals of the Association of American Geographers* **70**: 553–566.
- Štorchová, H., & Olson, M. S. 2007. The architecture of the chloroplast psbA-trnH non-coding region in angiosperms. *Plant Systematics and Evolution* **268**: 235-256.
- Tate, J. A., & Simpson, B. B. 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Systematic Botany* **28**: 723–737.
- Templeton, A. R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology* **7**: 381–397.
- Templeton, A. R., Crandall, K. A., & Sing, C. F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**: 619-633.
- ter Braak, C. 1998. *Canoco 4 Centre for Biometry*. Wageningen, The Netherlands.
- Thomas, W. W. 1999. Conservation and monographic research on the flora of Tropical America. *Biodiversity and Conservation* **8**: 1007–1015.
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research* **22**: 4673-4680.

- Tobler, M., Honorio, E., Janovec, J., & Reynel, C. 2007. Implications of collection patterns of botanical specimens on their usefulness for conservation planning: an example of two neotropical plant families (Moraceae and Myristicaceae) in Peru. *Biodiversity and Conservation* **16**: 659-677.
- Warburg, O. 1897. Monographie der Myristicaceen. *Nova Acta Acad. Caes. Leop.-Carol* **68**: 1-680.

Chapter 3

A MOLECULAR PHYLOGENETIC INVESTIGATION OF VIROLA Aublet

Abstract

The Myristicaceae are a widespread and specios family of tropical trees with great ethnobotanical and ecological importance, yet they remain to be understood from a phylogenetic perspective. *Virola* is a genus comprised of about 60 species of canopy and sub-canopy trees endemic to the neotropics whose fruits are important food for many vertebrates and sap is revered by numerous native tribes as a source of medicine and hallucinogenic snuff. The objective of this study was to estimate a phylogenetic tree for to illuminate infrageneric relationships among *Virola* taxa from Northwestern South America. An additional objective of this study was to test whether the trnH-psbA spacer could differentiate species of *Virola* and whether DNA sequence data indicates undescribed species. Although levels of DNA sequence divergence were low, phylogenetic hypotheses generated using both Bayesian and Parsimony methodologies supported similar topologies and showed a great deal of divergence amongst two groups in the genus (Multinervae and Sebiferae). Phylogenetic analyses also supported the recognition of 3 new provisional species. The most variable plant DNA barcode known for the Myristicaceae, the trnH-psbA intergenic spacer, failed to differentiate the majority of species included in this study.

Introduction

The Myristicaceae are a family of flowering plants consisting of 21 genera and about 500 species found worldwide in tropical rainforests. The family is best known as the source of the spices nutmeg and mace, which are produced from the fruit of the Asian species *Myristica fragrans*. Myristicaceae species are small to large trees (5-55 m tall) that hold significant ecological importance throughout most of the lowland wet tropical forests of the world (Gentry 1982, Pascal and Pelissier 1996, Pitman et al. 2002, Pitman et al. 2008). The family has been represented in molecular systematics studies (Chase et al. 1993, Qiu et al. 1993, Sauquet et al. 2003, Soltis et al. 2011), which have confirmed traditional hypotheses of classification of the Myristicaceae as belonging to order Magnoliales of subclass Magnoliidae. Unfortunately, few studies have addressed species diversity and distribution of the Myristicaceae within genera (Sauquet et al. 2003, Sauquet 2004), at the level of species (Janovec and Harrison 2002), populations (Degen et al. 2004), and individual specimens. Because of their abundance and diversity in tropical moist forests of the world, the Myristicaceae are an ideal family for studying speciation, evolution, and biogeography of lowland tropical forests but such investigations are difficult without well resolved phylogenies.

Virola is a genus of approximately 60 described species that are endemic to the lowland and cloud rainforests of Central and South America. Species of *Virola* are vegetatively characterized by having profuse red latex (rarely green-yellow), simple, entire, alternate, and regularly spaced linear-ovate to obovate leaves that differ from other neotropical genera by the presence of dendritic or stalked-sessile stellate hairs on their

abaxial surface. Secondary veins are prominent, pinnate, and arcuate-ascending but sometimes diverge towards the apex. Like all other Myristicaceae their panicle-raceme-like inflorescences are born in leaf axils. The flowers of these dioecious trees are very small (1-4mm in diameter) and are composed of 3-4 tepals which are often covered in a dendritic or stellate pubescence. The fruits of *Virola* are composed of a dehiscent pubescent-glabrous pericarp which opens at maturity to reveal a single globose-elliptic seed which is covered in a bright orange-red fat-rich aril that is attractive to numerous large vertebrate species.

Ecology

Due to their ubiquitous nature and position as one of the 5-10 most abundant fruit-producing tree genera of South American rainforests (Gentry 1988, Pitman et al. 2001, Pitman et al. 2002, Pitman 2008), *Virola spp.* have been extensively studied from an ecological perspective. For example, investigation of sex ratios in *Virola* trees have revealed a male bias within smaller age classes (< 30 cm DBH) of *Virola* trees (Ackerly et al. 1990, Queenborough et al. 2003), which is typical of dioecious tropical trees (Armstrong and Irvine 1989, Opler and Bawa 1978). The arils of *Virola spp.* are energy-dense and highly nutritious. Analyses of *Virola surinamensis* fruit arils have found a 63% lipid content, 3% protein and 9% carbohydrate (Howe and Vande Kerkhove 1981) while a similar study of *V. sebifera* found comparable percentages of lipid (53.7%), protein (7.1%) and carbohydrates (8.4%) (Howe 1981). Numerous studies have found that birds are by far the most speciose and abundant agents of seed dispersal for this genus (*V. sebifera*; Howe 1981, *V. surinamensis*; Howe and Vande Kerkhove 1981, *V. surinamensis*; Howe et al. 1985, *V. flexuosa*; Holbrook and Loiselle 2009). Although

birds are undeniably important dispersers of *Virola* seeds, they often regurgitate seeds directly under the parent tree after stripping the nutritious aril from the seeds with their gizzards (Howe 1981, Howe et al. 1985). It is therefore notable that two studies by Russo (1992, 2003) found that even though *V. calophylla* fruits were consumed by 17 species of birds, 82% (1992) and 92% (2003) of all seeds dispersed by vertebrates were removed by spider monkeys (*Ateles paniscus*). These primates typically consume the seed and defecate it many hours later, thus acting to more efficiently disperse the seeds away from the parent tree. This may be especially important as it has been found that 99.96% of *V. surinamensis* and 30-35% of *V. nobilis* seeds that fell within 45 m of a parent tree were consumed by invertebrate pre-dispersal seed predators (Howe et al. 1985, Howe 1993) and studies of *V. sebifera* found low to high rates (5-96%) of seed predation in Panama (Howe 1981). These studies seem to support the conformity of *Virola* species to the Janzen-Connell model (Janzen 1970, Connell 1971). However, investigations of *V. michellii*, *V. bicuhyba* and *V. kwatae* have found high seed survival with little to no seed predation by invertebrates or no decrease in seed predation with increasing distance from the mother tree (Forget et al. 2000, Zipparo and Morellato 2005, Forget and Cuijpers 2008). Additionally, it has been found that fruit removal in *V. flexuosa* by dispersal agents is more efficient in non-hunted versus hunted forests (89.4% versus 66.8%) in the forests of Amazonian Ecuador (Holbrook and Loiselle 2009). Despite these extensive investigations of seed dispersal/predation in various species of *Virola*, little is known of their pollination biology, what may act as herbivores/parasites, or whether these ecologically important trees are truly dioecious or diphasic in nature (alternating male and female depending on life stage).

Ethnobotany

Virola spp. also hold considerable ethnobotanical importance to both industrial and traditional societies of Central and South America. *Virola surinamensis*, known as ucuuba in the Amazonian basin, grows in the lowland floodplains of the Amazon basin from the Foothills of the Andes to the Amazonian delta in near monospecific stands and are valued for their lumber, which is in such abundant supply that it is rivaled in economic importance only by big-leafed mahogany [*Swietenia macrophylla*] (Macedo and Anderson 1993). The fatty seeds of *V. surinamensis* and *V. sebifera* are also harvested for their abundant and aromatic waxes which are used in the production of candle waxes (Williams 1960). Members of the genus are also widely employed by natives across their range for a multitude of uses. Perhaps one of the most widespread applications of *Virola* is as a hallucinogenic snuff. Traditional cultures of the Northwest Amazon purify the red latex derived from the bark of various *Virola* species to make a powdered snuff rich in various tryptamine alkaloids (primarily N,N-Dimethyltryptamine and 5-Methoxydimethyltryptamine), which purportedly produces powerful visions to aid in the teaching of medicine, divination of evil spirits, communication with other shamen, and healing of the sick (Macrae and Towers 1984a and b, Chagnon 1971, McKenna et al. 1984, Bennett and Alarcon 1994, Schultes and Holmstedt 1968, Schultes 1981, Schultes and Raffauf 1990, Prance 1972). Interestingly, similar preparations of the sap can be used as arrow/dart poisons to immobilize animals (McKenna et al. 1984, Macrae and Towers 1984b). Although these tryptamines are rendered inert by ingestion via the enzyme monoamine oxidase (MAO), some tribes have combined potent MAO inhibitors with the resin to make it orally active as an entheogen (McKenna et al. 1984). *Virola* sap is also

used to decrease pain and inflammation, as an anti-fungal skin treatment, to prevent and treat dental caries, bladder/stomach ailments, thrush, malaria, and is given as a snuff to hunting dogs to increase their ability to smell (Davis and Yost 1983, Schultes and Raffauf 1991, Beloz 1992, Bennett and Alarcon 1994, Roumy et al. 1997). The limited pharmacological investigations performed on *Virola* species have found them efficacious as oral hallucinogens as well as anti-malarial and anti-microbial agents (McKenna et al. 1984, Roumy et al. 1997, Lopes et al. 1999).

Taxonomy

Despite being the most species-rich genus of neotropical Myristicaceae, *Virola* has received little taxonomic attention. *Virola* was first described by Aublet (1775) as a genus endemic to Central and South America. The first comprehensive treatment of the Myristicaceae was performed by De Candolle (1856) who organized 90 species into 13 sections of the single genus *Myristica*. De Candolle grouped taxa we today recognize as *Virola* into sections *Virola* and *Sychoneura* based upon length of the filament column, anther apiculation, and leaf venation. Warburg (1897) relegitimated the generic concept of *Virola* in his thorough study of the Myristicaceae where he proposed its subdivision into sections *Oxyanthera* and *Amblyanthera* on the basis of the ratio of filament column to anther ratios. The genus was further divided into 6 unranked species groups (*Mollissimae*, *Sebiferae*, *Calophyllae*, *Rugulosae*, *Surinamenses*, and *Subsessilis*) in Smith's (1937) monograph of the neotropical Myristicaceae. Although these sections were arguably more natural and utilitarian owing to the inclusion of vegetative traits as well as characters of the pistillate inflorescences, some species belonged to multiple groups. The last comprehensive treatment of *Virola* was performed by Rodrigues (1980)

but is somewhat incomplete due to its restriction to Brazilian members of the genus. Walker and Walker (1979) examined pollen morphology in 30 species of *Virola* which were found to be highly similar in terms of size and shape but divided them into tree groups based upon differences in exine sculpturing patterns. Additional palynological comparisons among genera of Myristicaceae have shown that *Virola* along with the other American, Asian and African genera possess monosulcate boat-shaped pollen with a reticulate-rugulate tectum and a columnate infratectum (Sauquet and le Thomas 2003). Since 1980, taxonomic investigations of *Virola* have been primarily alpha-taxonomic in nature and about 60 species are currently recognized in the genus (Rodrigues 1989, Sabatier 1997, Jaramillo et al. 2000, Rodrigues 2002). Paleobotanical studies of *Virola* have been difficult as the genus is represented in the fossil record by a single cluster of flowers from the Dominican amber (Poinar and Poinar 1999).

In spite of its abundance and diversity in the Neotropics, *Virola* has been underrepresented in modern molecular studies of the Myristicaceae. *Virola* was represented by three species in the only molecular systematic study of Myristicaceae to date, which used chloroplast coding and non-coding intergenic spacers to estimate the evolutionary relationships among the 21 genera. This investigation failed to find well supported relationships among genera, largely due to low levels of nucleotide substitution among loci sampled (Sauquet et al. 2003). This phylogenetic uncertainty has hindered further studies of character evolution and biogeography of this pantropical family. Using RAPD's, Degen et al.(2001) studied *V. michellii*'s spatial genetic structure in comparison to 7 other tropical tree species and found significant genetic structure at small spatial scales (50-300m), which they hypothesized was due to aggregated seed dispersal from

monkeys excreting ingested seeds. Additionally, microsatellite markers have also been developed for *V. flexuosa* and *V. surinamensis* (Holbrook et al. 2006, Draheim et al. 2009) but their application in wide-ranging studies have yet to be published.

The objectives of this investigation were to 1) reconstruct infrageneric evolutionary relationships in order to test previous sub-generic classifications and 2) to test the ability of chloroplast DNA to discriminate species of *Viola*. Although poor in terms of resolution in some clades, a phylogenetic tree was generated for the genus and relationships are discussed with respect to ecology, morphology, and biogeography. Additionally, three new provisional species of *Viola* from the neotropics are inferred from molecular phylogenetic analysis and morphological distinctiveness.

Materials and Methods

Taxon Sampling

Viola is well represented in herbarium collections owing to their abundance in neotropical rainforests and intensive ecological investigations. These samples are often soaked in alcohol upon collection or dried immediately in the field. Most *Viola spp.* dry dark brown upon collection and DNA from samples more than a year old are generally not amenable to DNA extraction and amplification (personal observation), likely due to abundant secondary metabolites that have the ability to degrade DNA and inhibit PCR through direct enzymatic inhibition and/or the generation of non-bypassable crosslinking via maillard reactions (Poinar 1998, Sawadjoon 2002). Initial efforts to extract whole genomic DNA from *Viola* herbarium specimens were largely ineffectual. Therefore, new herbarium and DNA collections were made in Peru and Ecuador so that DNA of

sufficient quality and quantity could be extracted from specimens. Fresh DNA collections were made by John Janovec or the author by putting young and clean (i.e. visibly free of epiphytes) leaf tissue immediately on silica gel upon specimen collection in order to minimize DNA degradation. Conventional herbarium vouchers were also made of all collections. In all, 45 new collections representing 14 species of *Virola* were included in phylogenetic analyses. Greater taxon sampling was not possible due to the poor condition of DNA retrieved from herbarium specimens. Since this is the first phylogenetic investigation of the genus, it is unclear as to whether this particular suite of species represents the phylogenetic breadth of the genus. Additional sequences and species were included in distance analyses from tropical DNA barcoding campaigns (Gonzalez et al. 2009, Kress et al. 2009). All collections used in molecular analyses were identified according to the most current taxonomic treatment (Smith 1937) and all specimens employed have accessions archived at one or more of the following herbaria: Botanical Research Institute of Texas (BRIT), Ontario Agricultural College (OAC), and the National Herbarium of the Ecuadorian Museum of Natural Sciences (QCNE). Figures 3.1-3.14 show vegetative and reproductive structures of the 14 taxa collected. It was observed in the field that there were big-leafed and small-leafed morphotypes of *V. sebifera* and *V. lorentensis*. Consequently, collections have been annotated with a BL (Big-Leaf) or SL (Small-Leaf) to indicate to which of the morphotypes they belong. A more thorough analysis and discussion of the taxonomic implications of these morphotypes is provided in chapter 4 of this thesis. Collection information of the specimens used in this study, including Barcode Of Life Database (BOLD) process identification numbers, can be found in Table 3.1. Since phylogenetic relationships

remain largely unresolved in the Myristicaceae (Sauquet et al. 2003), 2 members of *Iryanthera*, and *Compsonaura* were selected as outgroups for phylogenetic analysis as they are hypothesized to have close relationships to *Virola* (Sauquet et al. 2003).

Morphology

In addition to sterile material (that was photographed, pressed, and dried) fruits and flowers were dried and/or preserved in FAA (Formalin-Acetic Acid-Alcohol) for morphological investigations when they were available. A data matrix of quantitative vegetative and reproductive morphological metrics was assembled for 8 species available from Smith (1937) and other species were not included due to a lack of data on leaf, flower and fruit metrics. Measurements of 10 quantitative vegetative metrics (Figure 3.15) were made from digital photographs using tpsDig 2.0 (Rohlf 2006) for use in species descriptions when provisional new species were collected.

DNA extraction, Amplification, Sequencing and Alignment

Total genomic DNA was extracted from leaf tissue of silica-dried or herbarium specimens using the Macherey-Nagel Nucleospin II Plant Kit (Macherey-Nagel, Duren Germany). Lysis buffer 1 was used according to the manufactures' instructions with the exception of an increase of the post homogenization incubation period from 10 minutes to 1hr and the addition of 20 mM N-Phenacylthiazolium Bromide, which has been found to result in improved PCR amplification of recalcitrant samples (Poinar et al. 1998, Asif and Cannon 2005).

Due to the low levels of nucleotide substitution in the Myristicaceae (Sauquet et al. 2003), the most variable *Virola* loci available were targeted by screening available chloroplast sequences from genbank (matK, psbK-I, rbcL, rpoB, rpoC1, trnH-psbA, trnL-F) and from a preliminary screening of the most variable nuclear markers from studies of *Compsonaura* (Chapter 2 of this thesis)[AGT1 and AT103]. Only trnH-psbA, AT103, and AGT1 consistently produced a single PCR product that yielded high quality sequence traces and were sufficiently variable for systematic investigations.

Taxa not represented by trnH-psbA accessions in GenBank from Newmaster et al. (2008) were PCR amplified and sequenced using the primers trnH2 (5'-CGCATGGTGGATTCACAATCC-3'; Tate and Simpson 2003) and psbAF (5'GTTATGCATGAACGTAATGCTC-3'; Sang et al. 1997). PCR was performed in a 20 µl volume using 0.4 µl of Phire II polymerase (Finnzymes) with 1X Phire II reaction buffer (with 1.5mM MgCl), 0.2 mM of each DNTP, 0.2 µM of each primer and 2.0 µg of BSA (Kreader 1996). Cycling conditions entailed an initial denaturation step of 1 min at 98°C; 35 cycles of 98°C for 5 s, 64°C for 5 s, 72°C for 10 s; and a final elongation step of 72°C for 1 min followed by a 4° hold. Phire II was used to amplify trnH-psbA as it is robust to the inhibitors contained in nutmeg extracts (personal observations) and as it is a fusion-based polymerase which has been found to reduce stuttering in sequences containing homopolymer regions such as the trnH-psbA intergenic spacer (Fazekas et al. 2010).

Nuclear loci were amplified using the primers AT103F (5'-CTTCAAGCCMAAGTTCATCTTCTA-3'; Li et al. 2008), AT103R (5'-TTGGCAATCATTGAGGTACATNGTMACATA-3'; this thesis), AGT1-MYR-F (5'-

GGGCATTGACGTAGCTTTGACAGG-3'; this thesis), and AGT1-MYR-R (5'-GTGCAGTTCTTCAAGCCCCAAGC-3'; this thesis). Nuclear loci were amplified with 0.5U of *AmpliTaq* Gold (Applied biosystems) DNA polymerase in a 20 μ l reaction containing 1X reaction buffer, 2.5 mM MgCl, 8% W/V Polyethylene glycol (Zimmerman and Harrison 1987, Sasaki et al. 2006), 0.2 M trehalose (Speiss et al. 2004), 2 μ g BSA, 0.2 mM each DNTP, and 0.2 μ M of each primer.

Amplification products were sequenced directly using the same primers employed in PCR. Cycle sequencing reactions were performed in a 10.5 μ L reaction volume containing 0.5 μ L of BigDye terminator mix v3.1, 1.88 μ L of 5x sequencing buffer (Applied Biosystems), 1.0 μ M of primer and 0.5 μ L of PCR product. Thermal cycling parameters were 96° for 2 min; 30 cycles of 96° for 30s, 56-60° (primer dependant) for 15s, and 72° for 4 min; and a 4° hold. Cycle sequencing reactions were cleaned using sephadex columns (Cat.no. S5897; Sigma-Aldrich, St. Louis, MO, USA) and the samples were run on an ABI 3730 sequencer (Applied Biosystems).

Sequence contigs were assembled and edited using Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA). DNA sequences were aligned using the default settings of the ClustalW algorithm (Thompson et al. 1994) in Bioedit (Hall 1999) and adjusted manually. Alignments can be found in Appendix 3. Gaps in the alignments were coded using Simple Indel Coding (SIC) (Simmons and Ochoterena 2000) for Bayesian analyses and Modified Complex Indel Coding for distance analyses using Indelcoder (Muller 2006).

Cladistic analyses

An incongruence length difference test (ILD: Farris et al. 1994) was executed in PAUP (Swofford 2002) to determine whether the three loci employed in this study contained significant incongruence. The ILD was performed using 100 replicates of a heuristic search strategy, MAXTREES set at 100, 10 random addition sequence replicates holding 5 trees at each step, MULTREES in effect, and tree bisection-reconnection (TBR) branch swapping. No significant incongruence was detected (p-value=0.98) therefore all three loci were concatenated in a total evidence approach in Bayesian and Parsimony analyses.

Phylogenetic trees were generated using Bayesian inference with the program MrBayes (Ronquist and Huelsenbeck 2003). Nucleotide substitution models were first selected using Mrmodeltest (Nylander 2004) which uses the Akaike Information Criterion to assess the significance of adding parameters to the different models of evolution employed by MrBayes 3.1 (Ronquist and Huelsenbeck 2003). Mrmodeltest selected the following substitution models for the three loci: trnH-psbA= F81, AT103=GTR, and AGT1=HKY+I. The three genetic loci were assigned to separate partitions with their corresponding models of nucleotide substitution and the indel data was given a fourth partition and assigned the model “restriction data” as recommended by Ronquist and Huelsenbeck (2003). Ten million generations were performed using 4 chains and 2 runs with trees being sampled every 100 generations. Log-likelihood values stabilized after 2.5 million generations; therefore the consensus tree and posterior probabilities were estimated using a burn-in of 25,000 trees. Since posterior probabilities are largely considered to be overestimations of clade support (Douady et al. 2003, Alfaro and Holder 2006, Yang and Rannala 2010), especially when employing concatenated

data sets (Suzuki et al. 2002), all nodes with less than 0.75 posterior probability were collapsed on the cladogram using treegraph2 (Stover and Muller 2010).

Maximum parsimony analyses were performed in PAUP 4.0b10 (Swafford 2002) and each nucleotide position was treated as an unordered character and all positions were equally weighted. A “two step” analysis was performed as it may permit the investigation of more optimal tree topologies compared to a “one step” analysis (Davis et al. 2004). The first heuristic search was performed with 1000 replicates of random addition sequence holding 10 trees at each step, TBR branch swapping with the MULTREES option not in effect with MAXTREES set to 5000. Using these trees in memory from the initial analysis, a second heuristic search was performed using the same parameters with a MAXTREES of 50 000 and the MULTREES option in effect. Branch support was estimated using a heuristic search, 5000 bootstrap replicates, 10 random addition sequence replicates holding 1 tree at each step, TBR branch swapping, and a maximum number of trees set at 50,000 with MULTREES not in effect.

Distance analyses

Full length trnH-psbA sequences of 13 species of *Viola* were amplified from whole-genomic DNA extracts and 5 species' sequences were retrieved from Genbank accessions (*Viola kwatae*: FJ039018.1; *Viola michelli*: GQ428669.1, FJ039021.1, FJ039020.1, FJ039019.1; *Viola multcostata*: GQ428671.1, GQ428670.1; *Viola multiflora*: GQ982401.1; *Viola nobilis*: GQ982402.1). These sequences were then used to generate a Neighbour-Joining uncorrected p-distance tree using PAUP 4.0 (Swafford 2002) to explore the ability of this locus to discriminate species of *Viola*. Uncorrected p-

distance was used as there existed very low levels of nucleotide substitution among taxa and it was therefore not thought to be beneficial to include a model of molecular substitution patterns, which is employed by other distance calculation methods. An 11 bp tract of homopolymer A/T repeat was omitted from distance analyses due to the high likelihood of homoplasious indels.

Results

Morphology

Morphological trait data was retrieved from Smith (1937) for a total of 8 of the 14 species included in the phylogenetic analyses. These data are presented in Table 3.2 and the species are broken up into informal groups Sebiferae and Multinervae (see “Cladistic Analysis” below) in order to show contrasting morphological traits of two clades from phylogenetic analyses. Many values were very comparable amongst the two clades, however, leaf vein number was greater in members of Multinervae (20-60 veins) than Sebiferae (9-28), Multinervae also had thicker pericarps (1-7 mm) than did members of Sebiferae (0.3-1.3 mm) and the arils of Multinervae taxa are generally lacinate to nearly the base while those of Sebiferae taxa are only lacinate for half the length of the seed.

DNA sequencing and Alignment

Bi-directional sequence pherograms were obtained for 41 *Virola* samples and 4 outgroup members for the trnH-psbA intergenic spacer. The trnH-psbA alignment for the distance analysis included 9 additional sequences retrieved from Genbank and was 363 base pairs (bp) long with 49 variable sites, 23 of which were provided from outgroup

taxa. These sequences varied from 162-339 bp in length. The trnH-psbA alignment for phylogenetic analysis was 357 bp in length and contained 42 variable and 30 parsimony informative sites, with outgroups responsible for 22 and 11 of these sites respectively.

AGT1 bi-directional sequences were recovered from 42 *Virola* and all 4 outgroup taxa. The aligned AGT1 data matrix was 728 bp in length with 122 variable sites of which 89 were parsimony informative. Outgroups provided 38 variable and 21 parsimony informative sites. The short coding section (264 bp) of AGT1 contained no stop codons.

AT103 sequences were recovered from all 49 samples (45 *Virola* taxa and 4 outgroup members). The aligned AT103 data matrix was 438 bp in length with 42 variable sites of which 27 were parsimony informative. Outgroup taxa were responsible for 16 variable and 8 parsimony informative sites. AT103 Sequences varied from 428-438 bp in length.

The concatenation and alignment of all three loci for phylogenetic analyses was 1517 bp in length and contained 206 (13.6%) variable sites, of which 146 (9.6%) were parsimony informative. The 4 outgroup taxa contributed 76 variable and 40 parsimony informative characters.

Cladistic Analysis

The first heuristic search of the concatenated data retrieved 2856 equally parsimonious trees (Length=245 steps). The second heuristic search yielded 50,000 equally parsimonious trees (Length=245 steps, consistency index [CI]=0.910, retention index [RI]=0.979, and rescaled retention index [RC]=0.891). The semi-strict consensus tree of the 50,000 most parsimonious trees was almost identical in topology to that of the

Bayesian analysis. Therefore, parametric bootstrap support measures were plotted on the Bayesian cladogram.

The Bayesian consensus tree and posterior probabilities of the two MCMC runs estimated in MrBayes with proportional branch lengths is presented in Figure 3.16 along with bootstrap support values. Since the Bayesian inference and parsimony topologies are virtually the same, the results of the two will be discussed together. Since two large clades were evident on the resulting tree, the clades were informally named Multinervae and Sebiferae for the sake of discussion.

Distance analyses

Four outgroup and 41 full length *Virola* trnh-psbA sequences (the same sequences as used in phylogenetic analyses) were recovered from fresh, silica gel dried, tissue and an additional 9 sequences were retrieved from Genbank. An uncorrected p-distance phylogram is presented in Figure 3.17. Of the 18 aligned species of *Virola*, only 6 (*V. sp. RADS1*, *V. caducifolia*, *V. multcostata*, *V. michellii*, *V. pavonis*, and *V. kwatae*) form distinct monophyletic clades on the phylogram. All but one sample of *V. multinervia* formed a clade. All 8 species of the Sebiferae clade possessed a single haplotype.

Discussion

Cladistic analysis and Morphology

This study is the first molecular systematic investigation of *Virola* and the first systematic study of this species-rich and widely distributed genus since Rodrigues'

investigation of its Brazilian members (1980). Otto Warburg (1897) presented the first cladistic hypothesis of the Myristicaceae based on morphological characters but even contemporary attempts to hypothesize relationships within the family using both morphological and molecular data have failed to find well supported relationships, mainly due to the conservative nature of morphological and molecular characters and/or recent diversification of the family (Sauquet et al 2003). Smith (1937) noted that previous attempts by De Candolle, Warburg and Ducke to form groups within *Virola* have relied mainly on characteristics of the androecium that were, at times, continuous. This heavy reliance on characters of the male flower also made classification of female or otherwise sterile specimens difficult. Therefore, Smith (1937) sought groupings supported by vegetative as well as male and female reproductive characters so that specimens without flowers and fruits could be more easily keyed to groups with major morphological affinities. Since Smith's primary objective was identification and not estimating ancestor-descendant relationships, some species were placed in multiple groups.

Although there are few species that form well supported monophyletic clades, this investigation recovered numerous clades above the species-level within *Virola* supported by high bootstrap and posterior probability values. Since node support inferred from Bayesian posterior probabilities are generally viewed as overestimations (Suzuki et al. 2002, Douady et al. 2003), a conservative approach was taken in this analysis by collapsing nodes with relatively low support (<0.75); the resulting tree is nearly identical to relationships inferred from the more conservative approach of parametric bootstrapping.

Both tree building approaches revealed cladistic support for Smith's (1937) sub-generic groups that were included in this analysis. The first is Smith's Rugulosae, containing *V. multinervia* and *V. flexuosa*, which was recovered in my phylogenetic analysis, Smith also included *V. lorentensis* which is not placed in this clade on the phylogeny so it appears that this clade is paraphyletic with respect to molecular data. Surinameses, *sensu* Smith (1937), encompasses *V. surinamensis* and *V. pavonis*, also formed a monophyletic clade. Although there is relatively limited taxon sampling in this analysis, it appears that there is considerable degree of support for the groupings of Smith (1937).

The genus *Virola* is divided into two well supported clades that I have elected to informally name "Multinervae" and "Sebiferae". These two clades have numerous contrasting morphological traits as well as some ecological and ethnobotanical distinctions. Multinervae is composed of tall emergent canopy trees that typically have more numerous secondary leaf veins compared to the sub-canopy to canopy species of Sebiferae (15-60 versus 9-28 respectively). The pericarps of Multinervae (1-7 mm) are also typically thicker than those of Sebiferae (0.3-1.3 mm). Furthermore, taxa of Multinervae have an aril that is laciniate nearly to its base while those of Sebiferae are laciniate for half the length of the seed. Although incomplete quantitative data exists, it has also been observed in the field that members of Multinervae generally have larger, globose seeds while those of Sebiferae are generally smaller and more ellipsoid in shape. Additionally, 4 species (*V. flexuosa*, *V. multinervia*, *V. pavonis*, and *V. surinamensis*) of Multinervae included in this analysis were studied by Walker and Walker (1979) and classified as having type II pollen while 5 species (*V. calophylla*, *V. elongata*, *V.*

loretensis, *V. peruviana*, and *V. sebifera*) of the Sebiferae clade were classified as having type I pollen.

Although some species in this analysis formed well supported clades, there was little to no phylogenetic signal in members of the Sebiferae group, although a previous investigation of the neotropical genus *Compsooneura* (Chapter 1 of this thesis) found relatively well resolved relationships among many species with the same three loci. It is unclear as to whether the differing levels of phylogenetic signal between these informal subgroups of *Virola* are due to incomplete taxon sampling, nucleotide substitution rate heterogeneity, incomplete lineage sorting, a recent origin of Sebiferae species, or some combination of these. Since the sub-generic classification of *Virola* has been rather unstable due to a low degree of morphological trait variation, it is hoped that increased sampling of taxa, loci, and morphological characters in the future will enable a more stable classification rooted in the evolutionary histories of these species.

The Multinervae and Sebiferae clades exhibit numerous contrasting traits that likely have ecological and evolutionary origins. The Multinervae clade, as circumscribed here, is composed of tall canopy trees with globose seeds and relatively thick pericarps. Although speculative, it is possible that these adaptations are a result of co-evolution with primate and large bird species that act as dispersal agents as numerous studies have found members of this clade to be primarily dispersed by toucans and large primates (Julliot 1996, Juliotte and Sabatier 1993, Holbrook and Loiselle 2009). Their thick, astringent, pericarps may be adapted to prevent pre-dispersal seed consumption by these larger dispersers and/or discourage ovipositioning of invertebrate seed predators that have been found to infect large percentages of seeds of some species in this clade (Howe et al. 1985,

Howe 1993). The leaves of these canopy trees generally have longer and thinner leaves with more secondary veins/unit of length than members of Sebiferae (see Table 3.2). These characters may possibly be an adaptation to deal with the increased stresses of wind and rain experienced in the canopy. Another dichotomy previously mentioned is the type II pollen of Multinervae (sensu Walker and Walker 1979) opposed to the type I pollen of Sebiferae (Walker and Walker 1979), however it is unclear as to what implications, if any, this difference in pollen morphology has to pollination ecology and evolution of *Virola*. One more anecdotal difference between these two clades is that members of Sebiferae are often employed as hallucinogenic snuffs by natives while members of Multinervae are rarely used (Schultes and Raffauf 1990), possibly due to differences in the quality and/or quantity of their alkaloidal constituents or perhaps the increased ease with which the bark is removed from the smaller sub-canopy trees of Sebiferae.

After careful investigation of *Virola spp. in situ* in Ecuador and Peru as well as extensive herbarium investigations (BRIT, KEW, QCNE, LOJA, QAP, and USM), it was suspected that the taxa included in this study contained a minimum of three new species. This phylogenetic analysis gives further support for the distinction of these species but it is difficult to justify formal description with such incomplete taxon sampling. Additionally, reproductive material is lacking from these 3 putative new species, therefore their morphological characters are incomplete and their descriptions are provisional. These putative new species will be referred to in the following discussion according to their taxon names currently registered on the Neotropical Myristicaceae

project of the Barcode of Life Database (www.barcodinglife.org). The “RADS” portion of the species name refers to the initials of the author.

Working outwards from the base of the phylogeny, the first novel species is *V. sp.RADS4*; a species found west of the Andes near Camarones, Ecuador on the coastal plain in a seasonally dry forest. *Virola sp.RADS4* is a tall emergent tree (50 m, 30 cm dbh) that usually possesses stilt roots when mature and was found growing near streams in an otherwise dry forest that contained cacti, an unusual habitat for *Virola*. It appears to have affinities to *V. duckei* but differs in having fewer secondary veins (10-17 versus 25-37) and less pubescence (essentially glabrous versus ferruginous-tomentose). This species also differs from *V. aequatorialis* (Jaramillo et al. 2000), another species of the coastal plains of Ecuador, in not having a winged petiole and having less than 20 veins per leaf. The red sap of this species was also self-administered for a recalcitrant ringworm (*Tinea sp.*) infection of the skin and found to be highly efficacious.

Virola sp.RADS3 is also proposed as a new species with affinities to *V. calophylloidea*. *Virola sp.RADS3* has only been found in the Cordillera del Condor of Ecuador growing at an elevation of 1200 m asl. Due to its deep reddish sessile pubescence, *V. sp.RADS3* was initially identified in a permanent ecological plot as *Otoba parvifolia*. It appears that this taxon does not represent a range extension of *V. calophylloidea* of the Rio Negro of Brazil, owing to its rich reddish pubescence and the fact that it was growing on a rare sandstone substrate home to many highly endemic species (Rogers 2002, Ulloa and Neil 2006, Clarke et al. 2010, Janovec et al. in prep.).

The third novel species discovered in the course of this analysis is *V. sp.RADS1*. This species has been collected solely in the Madre de Dios watershed of Peru in terra

firme forests from the foothills of the Andes (750 m asl) and the lowland Amazon (200 m asl). This species had been identified in ecological plots as *V. pavonis* due to its whitish abaxial lamina colour and superficially similar leaf venation. However, these molecular analyses clearly show that it forms a well supported monophyletic clade and has no close genetic affinity to *V. pavonis*. *Virola sp.RADS1* can be easily differentiated vegetatively from *V. pavonis* in having fewer leaf veins (11-15 versus 15-20) and conspicuously undulating edges of the leaf lamina.

Distance analysis

Of the 18 species of *Virola* for which trnH-psbA sequences were retrieved, 6 (*V. sp.RADS4*, *V. caducifolia*, *V. multcostata*, *V. michelii*, *V. pavonis*, and *V. kwatae*) had haplotypes distinct from other species (i.e. had diagnostic characters). All members of the Sebiferae clade had a large deletion in the most variable region of the trnH-psbA spacer and exhibited identical haplotypes with their homopolymer runs omitted, making species delimitation impossible within this clade with this loci. However, only 4 species pairs in the Multinervae clade shared haplotypes. One specimen of *V. multinervia* had an identical haplotype to *V. flexuosa*; *V. multiflora* and *V. nobilis* also shared haplotypes. Of the remaining 7 species, *V. surinamensis* had two paraphyletic haplotypes. This apparent paraphyly of conspecific sequences may be due to undetected speciation or population divergence as the two *V. surinamensis* sequence clusters on the distance tree represent populations in both Peru and Ecuador. However, chloroplast haplotype introgression, or chloroplast capture, is expected to occur frequently in plant species (Rieseberg and Soltis 1991, Hollingsworth et al 2011) and it is important to keep this in mind before delimiting species based solely on haplotype data. Since many species were represented by fewer

than 4 specimens from restricted geographical areas, it remains unclear whether chloroplast haplotype introgression is commonplace in this genus but given the emerging evidence of widespread plastid introgression in other plant families (Golden and Bain 2000, Palme et al. 2003, Palma-Silva et al. 2011) it is likely that plastid-based DNA identification will only be confounded by increased taxon sampling of *Virola*. Despite this possibility, the trnH-psbA spacer may represent an alternative means of identifying collections (particularly juveniles or difficult material such as pollen or roots) of the Multinervae clade as they are often mis-identified in ecological plots and there are usually few species at any one locale. Since numerous other proposed DNA barcoding loci have exhibited little variation in initial trials and introgression and paraphyly are likely to plague species discrimination, efforts should be focused on finding variable low-copy nuclear loci that can more effectively discriminate species of these ecologically dominant trees.

Descriptions of provisional species

Virola sp. RADS1 R. Steeves sp. nov.-TYPE: Peru. Madre de dios: Los Amigos Biological Research Station -12.57 DD Lat, -70.10 DD Long, 250 m, 17 Jun 2008, R. Steeves and A. Balarezo 500 (holotype: OAC)

Tree to 20 m tall and 40 cm dbh. **Bark** grey in colour, tough and ridged on young trees but growing smoother with age. **Sap** profuse and clear-red. **Branchlets** terete to subterete, 1.5-4 mm wide, bark smooth, green and may be covered in an evanescent

pubescence, the hairs short-stalked to sessile, stellate. **Leaves** simple, alternate, thin-chartaceous, leaf buds ferruginous but soon glabrous upon leaf expansion; **petioles** stout, subterete, canaliculate, 0.5-1.2 cm long and 1-2.5 mm wide, brown when dry, glabrous to glabrescent, the hairs short stalked to sessile, stellate; **blades** elliptic-oblong, lamina 12.7-17.0 cm long, 3.25-4.8 cm wide at $\frac{1}{4}$ length, 3.3-5.5 cm wide at $\frac{1}{2}$ width, 1.9-4.1 cm wide at $\frac{3}{4}$ length, base attenuate to rounded and acuminate at apex, drying dark brown, glabrescent, adaxial surface whitish with a waxy appearance, abaxial surface dark green with a glossy appearance; **venation** costa glabrous and raised above, prominently keeled and glabrescent below; secondary nerves 11-16 per side, spaced by 7-9 mm, arcuate ascending, eucamptodromous, partially anastomosing near margin. **Staminate inflorescences** unknown **Pistillate inflorescences** unknown. **Fruits** (nearly mature) 1-3 per infructescence, globose, pedicel 0.9-1.0 cm long and 2.5-3 mm wide; **Pericarp** green and glabrescent with age, smooth and ferruginous-tomentose, 1.2-1.5 cm wide and 1.5-2 cm long and 0.8-1.2 mm thick, presumably dehiscent when mature; **Seed** 1-1.2cm wide, 1.3-1.5cm long; **Testa** striated by aril; **Aril** lacinate to base; **Endosperm** unknown.

Distribution and notes: Collected only from the Madre de Dios watershed in Peru from elevations of 150-750 m. *V. sp.RADSI*'s leaf colour, indument and venation has many affinities to *V. pavonis* and *V. surinamensis* but differs in having an acuminate apex, fewer than 16 secondary veins and a comparatively thinner pericarp. When formalized this species will be named after the forester and conservationist Aldo Leopold whose early 20th century essay "The River of the Mother of God" about the Madre de Dios River has inspired me and many others to explore wild places.

Additional specimens examined: Peru. CUSCO: Quispicanchi Province, District of Camanti, Community of Quincemil, -13.23, -70.78, 500-1200 m, 06/26-07/04, 2008, R. Steeves, P. Centeno, D. White, and K. Ward 561 (OAC); **Madre de Dios:** Manu province, Los amigos biological station, Confluence of Los Amigos and Madre de Dios rivers, -12.57, -70.1, 200-250 m, 06/7-17/2008, R. Steeves and A. Balarezo 431 (OAC), R. Steeves and A. Balarezo 432 (OAC), R. Steeves and A. Balarezo 447 (OAC), R. Steeves and A. Balarezo 450 (OAC), R. Steeves and A. Balarezo 470 (OAC), R. Steeves and A. Balarezo 500 (OAC).

Virola sp.RADS4 R. Steeves-TYPE: Ecuador. Manabi: Diez de Augusto, Camarones, -0.083 DD Lat, -80.16 DD Long, 80 m, 28 April 2008, R. Steeves, A. Reid and D. Simba 205 (holotype: OAC; QCNE)

Tree to 50 m tall and 30 cm dbh. **Bark** grey in colour, tough and ridged. **Sap** profuse and clear-red. **Branchlets** terete to subterete, 0.27-0.42 cm wide, bark smooth, green and may be covered in an evanescent pubescence, the hairs short-stalked, stellate. **Leaves** simple, alternate, thin-chartaceous, leaf buds ferruginous but soon glabrescent upon leaf expansion; **petioles** stout, subterete, canaliculate, 0.48-1.22 cm long and 0.26-0.44 cm wide, brown when dry, glabrous to glabrescent, the hairs short stalked, stellate; **blades** elliptic-oblong, lamina 11.5-19.7 cm long, 3.0-7.0 cm wide at ¼ length, 3.4-7.6 cm wide at ½ width, 3.2-6.9 cm wide at ¾ length, base attenuate to rounded and acuminate at apex, drying dark brown, glabrescent, adaxial surface dull green when fresh, abaxial surface dark green with a glossy appearance; **venation** costa glabrous and raised above, prominently keeled and glabrescent below; secondary nerves 11-17 per side, spaced by

0.35-0.7 cm, arcuate ascending, eucamptodromous, partially anastomosing near margin.

Staminate inflorescences unknown **Pistillate inflorescences** unknown. **Fruits**

(immature) 1-3 per infructescence, elliptic, pedicel 0.9-1.0 cm long and 0.2-3 mm wide;

Pericarp green and glabrescent with age, smooth and ferruginous-tomentose, 1.2-1.5 cm

wide and 1.5-2 cm long and 0.8-1.2 mm thick, presumably dehiscent when mature; **Seed**

1-1.2 cm wide, 1.3-1.5 cm long; **Testa** striated by aril; **Aril** lacinate to base; **Endosperm**

unknown.

Distribution and notes: Collected only from dry coastal forests 20 km south of Pedernales, Ecuador at the Lalo Llor research station near Camarones where it was one of the most common canopy tree species. This species has morphological affinities to *V. duckei* from which it differs in having only 10-17 secondary nerves compared to the 25-37 typical of *V. duckei* (Smith 1937).

Additional specimens examined: Ecuador. Manabi: Diez de Augusto, -0.083 DD Lat, -80.16 DD Long, 80 m, 04/26-29/2008, R. Steeves, A. Reid and D. Simba 205 (holotype: OAC! ; QCNE!), 2008, R. Steeves, A. Reid and D. Larco 188 (OAC), R. Steeves, A. Reid and D. Larco 189 (OAC), R. Steeves, A. Reid and D. Larco 190 (OAC), R. Steeves, A. Reid and D. Larco 191 (OAC), R. Steeves, A. Reid and D. Larco 192 (OAC), R. Steeves, A. Reid and D. Larco 193 (OAC), R. Steeves, A. Reid and D. Larco 194 (OAC), R. Steeves, A. Reid and D. Larco 195 (OAC), R. Steeves, A. Reid and D. Larco 196 (OAC), R. Steeves, A. Reid and D. Larco 197 (OAC), R. Steeves, A. Reid and D. Larco 198 (OAC), R. Steeves, A. Reid and D. Larco 199 (OAC), R. Steeves, A. Reid and D. Larco 200 (OAC), R. Steeves, A. Reid and D. Larco 201 (OAC), R. Steeves, A.

Reid and D. Larco 202 (OAC), R. Steeves, A. Reid and D. Larco 203 (OAC), R. Steeves, A. Reid and D. Larco 204 (OAC), R. Steeves, A. Reid and D. Larco 206 (OAC), R. Steeves, A. Reid and D. Larco 207 (OAC), R. Steeves, A. Reid and D. Larco 208 (OAC), R. Steeves, A. Reid and D. Larco 209 (OAC), R. Steeves, A. Reid and D. Larco 210 (OAC), R. Steeves, A. Reid and D. Larco 211 (OAC), R. Steeves, A. Reid and D. Larco 212 (OAC), R. Steeves, A. Reid and D. Larco 213 (OAC), R. Steeves, A. Reid and D. Larco 214 (OAC), R. Steeves, A. Reid and D. Larco 215 (OAC).

Viola sp. RADS3 R. Steeves-TYPE: Ecuador. Zamora-Chinchipe, Quimi, -3.59 DD Lat, -78.50 DD Long, 1200 m, 17 May 2008, R. Steeves, W. Quizhpe and D. Simba 335 (holotype: OAC; QCNE)

Tree to 30 m tall and 25 cm dbh. **Bark** grey in colour, tough and ridged-smooth. **Sap** profuse and clear-red. **Branchlets** terete to subterete, 4-7 mm wide, bark smooth, grey and may be covered in an evanescent pubescence, the hairs short-stalked sessile, stellate. **Leaves** simple, alternate, thin-chartaceous, leaf buds ferruginous, persistently ferruginous-tomentellous; **petioles** stout, subterete, canaliculate, 1.27-1.74 cm long and 3-5 mm wide, brown when dry, glabrescent, the hairs sessile, stellate; **blades** lanceolate-oblong, lamina 12.3-19.2 cm long, 4.7-5.9 cm wide at $\frac{1}{4}$ length, 5.3-6.6 cm wide at $\frac{1}{2}$ width, 4.4-5.56 cm wide at $\frac{3}{4}$ length, base rounded and acuminate at apex, drying dark brown, glabrescent, adaxial surface reddish-white when fresh, abaxial surface dark green with a glossy appearance when fresh, dark brown when dry; **venation** costa glabrous and raised above, prominently keeled and glabrescent below; secondary nerves 9-12 per side, spaced by 6.4-

7.8 mm, arcuate ascending, eucamptodromous, partially anastomosing near margin.

Staminate inflorescences unknown **Pistillate inflorescences** unknown. **Fruits** unknown.

Distribution and notes: Collected only from the slopes of the Cordillera del Condor near Quimi, Ecuador on the site of a proposed Canadian gold mine. Two trees were found growing at 1200 m elevation in a permanent plot situated on soil of sandstone origin. This species was originally identified as *Otoba parvifolia* in a permanent plot owing to its faint secondary veins and reddish adaxial leaf colour. This species has morphological affinities to *V. calophylloidea* but differs in its reddish pubescence, distribution, and its unique habitat where many highly endemic species are found. When formalized, this species will be named after the native Shuar people that inhabit the area where these trees are found.

Additional specimens examined: Ecuador. Zamora-Chinchipec: -3.59 DD Lat, -78.50 DD Long, 1200 m, 17 May 2008, R. Steeves, W. Quizhpe and D. Simba 339 (OAC).

Tables

Table 3.1 Species[SL and BL indicate small leaf and big leaf morphotypes of *V. loretensis* and *V. Sebifera*], collectors, collection numbers (Coll. #), Barcode of Life Database process ID number (BOLD ID), and decimal degree co-ordinates (latitude= DD Lat., longitude= DD long.) of taxa collected for this study.

Species	Collector(s)	Coll. #	BOLD ID	DD Lat.	DD Long.
<i>C. capitellata</i>	Steeves, R. et al	RS 551	RSMYR004-11	-13.24	-70.78
<i>C. debilis</i>	Berry, P	PB 7209	RSMYR017-11		
<i>I. juruensis</i>	Steeves, R. et al	RS 451	RSMYR050-11	-12.57	-70.10
<i>I. laevis</i>	Steeves, R. et al	RS 460	RSMYR051-11	-12.57	-70.10
<i>V. caducifolia</i>	Janovec, J.	JJ 847	RSMYR109-11		
<i>V. calophylla</i>	Steeves, R. et al	RS 430	RSMYR110-11	-12.57	-70.10
<i>V. calophylla</i>	Steeves, R. et al	RS 454	RSMYR111-11	-12.57	-70.10
<i>V. calophylla</i>	Steeves, R. et al	RS 481	RSMYR112-11	-12.57	-70.10
<i>V. calopylla</i>	Steeves, R. et al	RS 511	RSMYR113-11	-12.61	-69.20
<i>V. dixonii</i>	Steeves, R. et al	RS 225	RSMYR114-11	-1.04	-77.37
<i>V. elongata</i>	Steeves, R. et al	RS 502	RSMYR115-11	-12.61	-69.20
<i>V. elongata</i>	Steeves, R. et al	RS 437	RSMYR116-11	-12.57	-70.10
<i>V. elongata</i>	Steeves, R. et al	RS 494	RSMYR117-11	-12.57	-70.10
<i>V. flexuosa</i>	Steeves, R. et al	RS 522	RSMYR118-11	-12.61	-69.20
<i>V. flexuosa</i>	Steeves, R. et al	RS 595	RSMYR119-11	-13.24	-70.78
<i>V. flexuosa</i>	Steeves, R. et al	RS 442	RSMYR120-11	-12.57	-70.10
<i>V. sp.RADS1</i>	Steeves, R. et al	RS 510	RSMYR121-11	-12.61	-69.20
<i>V. sp.RADS1</i>	Steeves, R. et al	RS 561	RSMYR122-11	-13.24	-70.78
<i>V. sp.RADS1</i>	Steeves, R. et al	RS 432	RSMYR123-11	-12.57	-70.10
<i>V. sp.RADS1</i>	Steeves, R. et al	RS 500	RSMYR124-11	-12.57	-70.10
<i>V. loretensis</i>	Steeves, R. et al	RS 516	RSMYR125-11	-12.61	-69.20
<i>V. loretensis</i>	Steeves, R. et al	RS 526	RSMYR126-11	-12.61	-69.20
<i>V. loretensis</i>	Steeves, R. et al	RS 480	RSMYR127-11	-12.57	-70.10
<i>V. multinervia</i>	Steeves, R. et al	RS 107	RSMYR128-11	-12.57	-70.10
<i>V. multinervia</i>	Steeves, R. et al	RS 108	RSMYR129-11	-12.57	-70.10
<i>V. multinervia</i>	Steeves, R. et al	RS 543	RSMYR130-11	-13.24	-70.78
<i>V. multinervia</i>	Steeves, R. et al	RS 549	RSMYR131-11	-13.24	-70.78
<i>V. multinervia</i>	Steeves, R. et al	RS 429	RSMYR132-11	-12.57	-70.10
<i>V. multinervia</i>	Steeves, R. et al	RS 466	RSMYR133-11	-12.57	-70.10
<i>V. multinervia</i>	Steeves, R. et al	RS 350	RSMYR134-11	-3.57	-78.45
<i>V. pavonis</i>	Steeves, R. et al	RS 216	RSMYR135-11	-1.04	-77.37
<i>V. peruviana</i>	Janovec, J.	JJ 772	RSMYR136-11		
<i>V. sebifera-BL</i>	Steeves, R. et al	RS 584	RSMYR137-11	-13.24	-70.78
<i>V. sebifera-SL</i>	Steeves, R. et al	RS 507	RSMYR138-11	-12.61	-69.20
<i>V. sebifera-SL</i>	Steeves, R. et al	RS 534	RSMYR139-11	-13.24	-70.78
<i>V. sebifera-SL</i>	Steeves, R. et al	RS 552	RSMYR140-11	-13.24	-70.78
<i>V. loretensis-SL</i>	Steeves, R. et al	RS 483	RSMYR141-11	-12.57	-70.10

<i>V. sp.RADS3</i>	Steeves, R. et al	RS 335	RSMYR142-11	-3.57	-78.45
<i>V. sp.RADS3</i>	Steeves, R. et al	RS 339	RSMYR143-11	-3.57	-78.45
<i>V. sp.RADS4</i>	Steeves, R. et al	RS 213	RSMYR144-11	-0.08	-80.17
<i>V. sp.RADS4</i>	Steeves, R. et al	RS 214	RSMYR145-11	-0.08	-80.17
<i>V. surinamensis</i>	Steeves, R. et al	RS 501	RSMYR146-11	-12.61	-69.20
<i>V. surinamensis</i>	Steeves, R. et al	RS 428	RSMYR147-11	-12.57	-70.10
<i>V. surinamensis</i>	Steeves, R. et al	RS 489	RSMYR148-11	-12.57	-70.10
<i>V. surinamensis</i>	Steeves, R. et al	RS 82	RSMYR149-11	-12.57	-70.10
<i>V. surinamensis</i>	Steeves, R. et al	RS 83	RSMYR150-11	-12.57	-70.10
<i>V. surinamensis</i>	Steeves, R. et al	RS 84	RSMYR151-11	-12.57	-70.10
<i>V. surinamensis</i>	Steeves, R. et al	RS 248	RSMYR152-11	-1.04	-77.37
<i>V. surinamensis</i>	Steeves, R. et al	RS 324	RSMYR153-11	-3.57	-78.45

Table 3.2 Morphological trait values of 8 species of *Virola* taken from species descriptions of Smith (1937). Minimum and maximum trait values are given as ranges. NDA indicates that there was no data available.

	Sebiferae				Multinervae			
	<i>V. elongata</i>	<i>V. sebifera</i>	<i>V. calophylla</i>	<i>V. lorentensis</i>	<i>V. multinervia</i>	<i>V. flexuosa</i>	<i>V. surinamensis</i>	<i>V. pavonis</i>
Height (m)	25	40	4 to 10	4 to 10	30	30	25	23
Leaf petiole length (mm)	5-16	10-25	7-20	3-12	4-15	2-7	2-9	4-13
Leaf lamina length (cm)	12-32	15-47	20-55	15-35	25-45	5-11	10-22	8-21
Leaf width at half length (cm)	4-11	6-15	7-24	4-10	8-16	1.7-4	2-5	2-6.5
Leaf vein number	9-20	10-28	12-27	15-26	40-60	40-50	16-30	15-20
Inflorescence length (cm)	4-18	8-23	12-30	to 25	15-20	4-9	7-17	7-15
Flowers per branch (#)	2-8	3-10	4-10	5-10	20-50	10-15	5-20	3-8
Anther length (mm)	0.6-1.6	0.9-2	1-1.7	1-1.4	to 0.9	0.8-0.9	1.3-1.9	1.1-1.5
Anther number	3(4-6)	3(4-5)	3	3	3	3	3	3
Fruit length (mm)	11-16	10-19	NDA	13-22	20-30	NDA	13-21	25-50
Fruit width (mm)	8-12	7-14	NDA	13-22	15-25	NDA	11-18	15-23
Pericarp thickness (mm)	0.3-1.3	0.5-1	NDA	<.5mm	1.5-4 mm	NDA	1 to 2	2 to 7
Aril	1/2	1/2	1/2	1/2	lacinate to	lacinate to	lacinate to	lacinate

lacinata

lacinata

lacinata

lacinata

base

base

base

to base

Figures

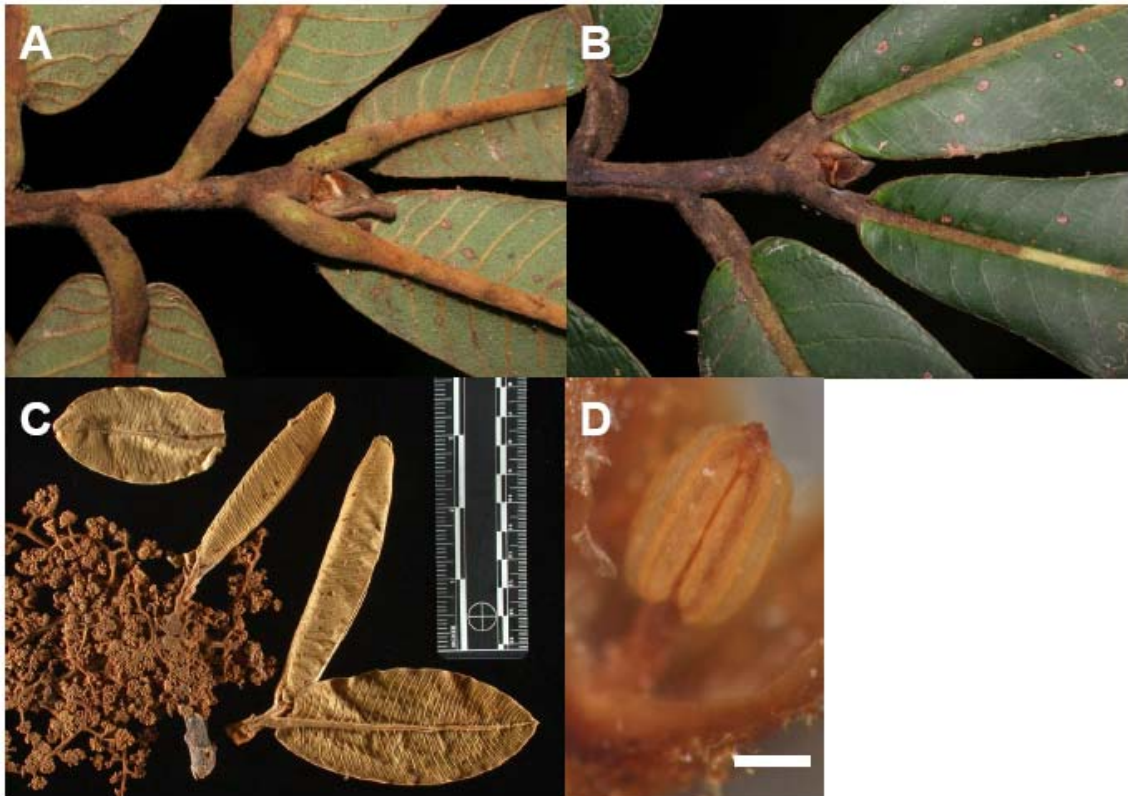


Figure 3.1 *Virola flexuosa*: A) Abaxial leaf surfaces. B) Adaxial leaf surfaces. C) Herbarium specimen with inflorescence. D) Staminate flower showing androecium (scale bar= 0.5 mm).

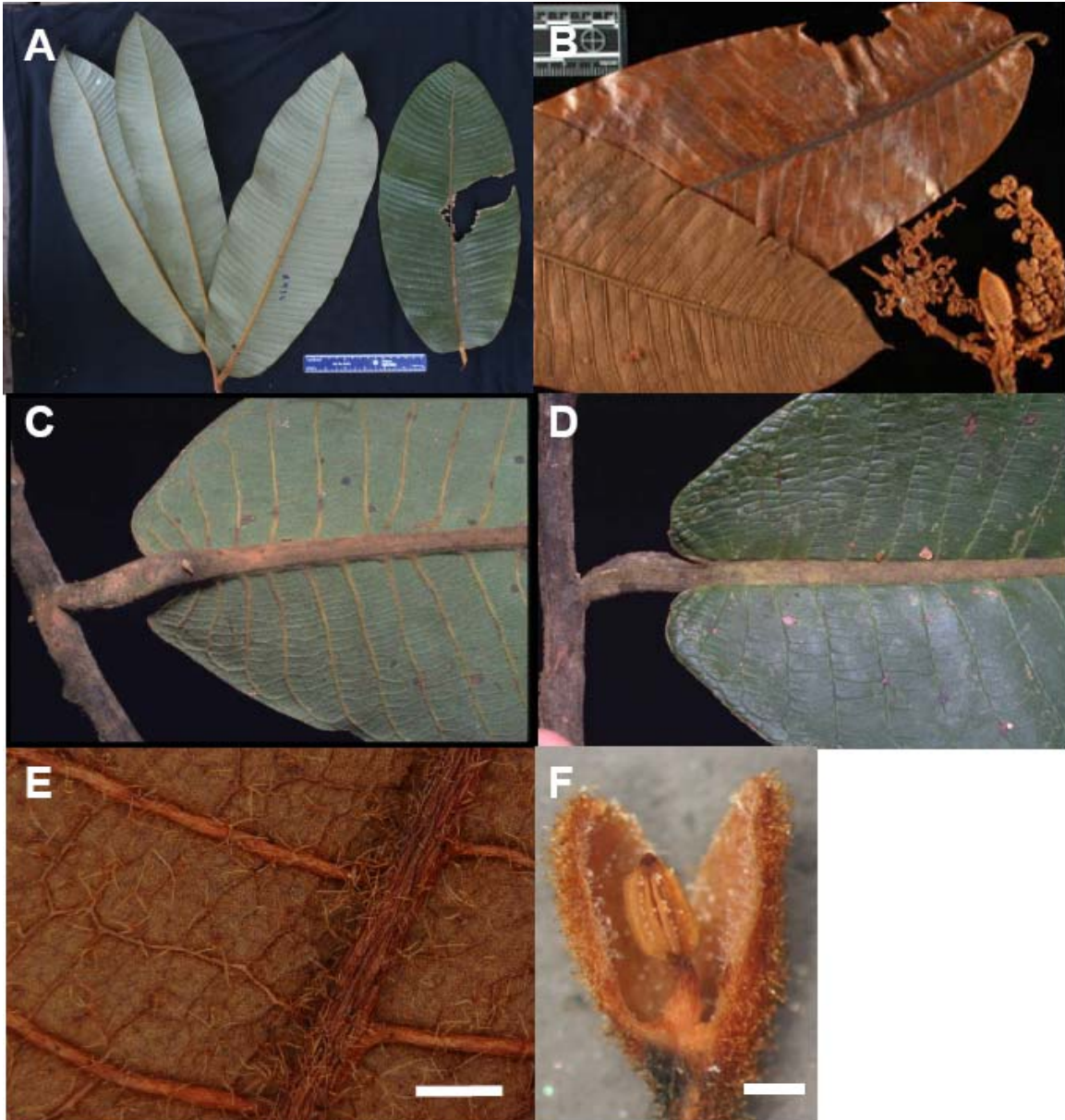


Figure 3.2 *Virola multinervia*: A) Abaxial and adaxial leaf surfaces from juvenile tree (15 cm ruler). B) Herbarium specimen with immature inflorescence. C) Close-up of adaxial surface of mature tree. D) Close-up of adaxial surface of mature tree. E) Abaxial leaf pubescence (scale bar= 1 mm). F) Flower with one tepal dissected to show androecium (scale bar= 0.5 mm).

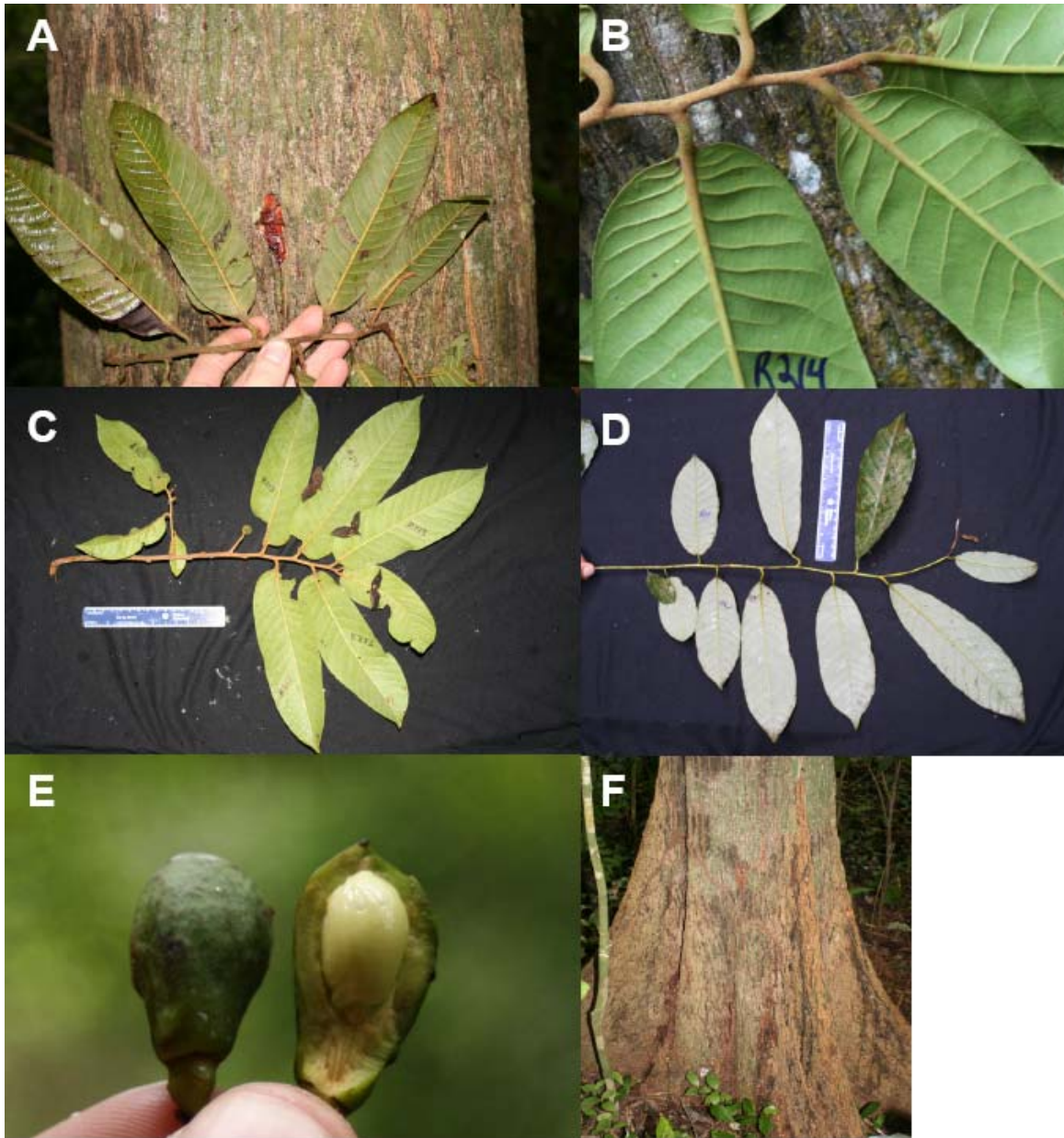


Figure 3.3 *Virola sp. RADS4* A) Bark, leaves and red sap. B) Abaxial leaf surface showing glabrescent surface and secondary veins. C) Branchlet of mature tree (15 cm ruler). D) Branchlet of immature tree (15 cm ruler). E) Nearly mature fruit dissected to show outer glabrescent pericarp and nearly entire aril. F) Buttressed base of mature tree.

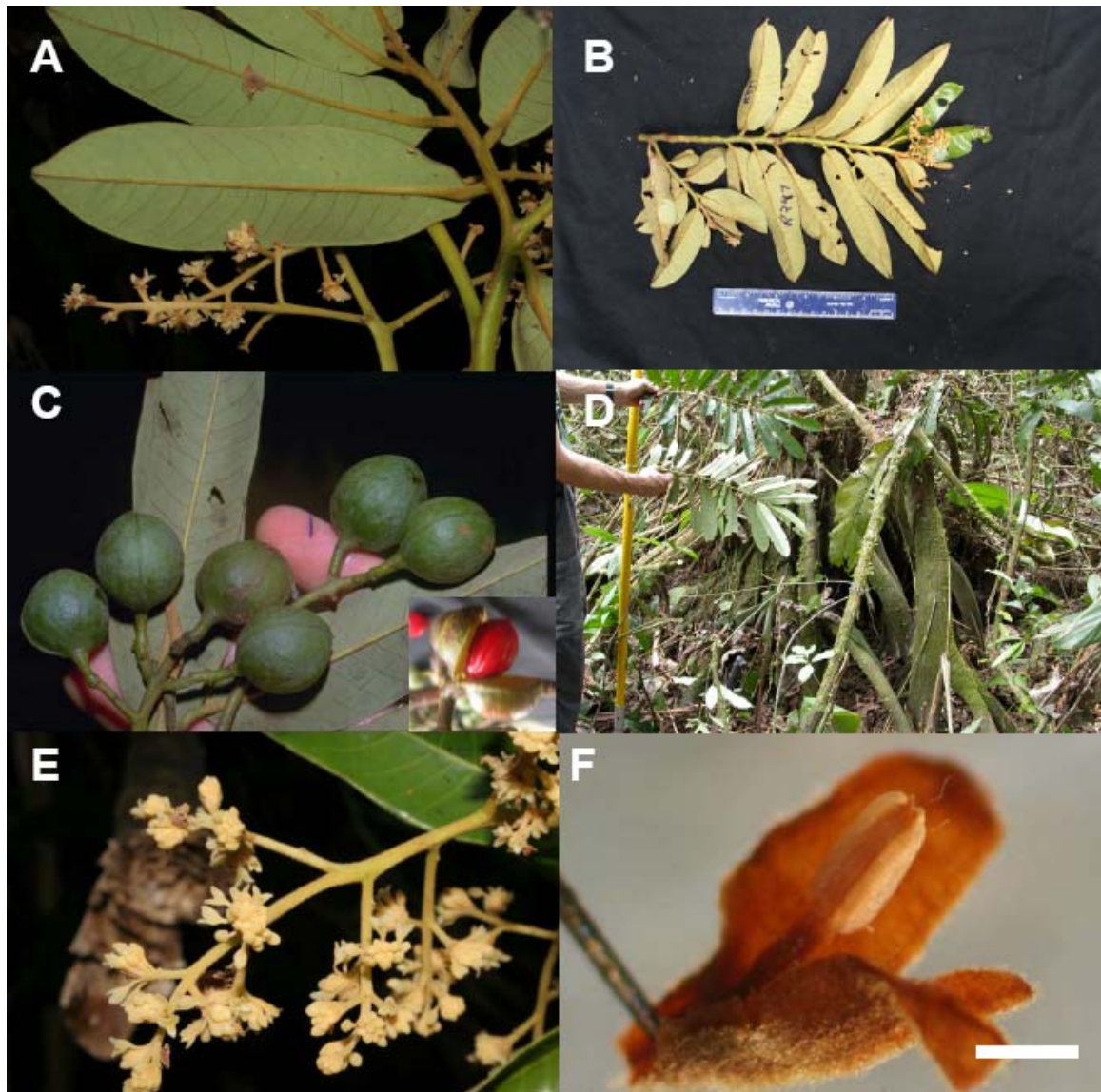


Figure 3.4 *Virola surinamensis*: A) Branch with inflorescence and abaxial leaf surface. B) Branch of mature tree with inflorescences. C) Undehiscent, nearly mature fruits and dehiscent fruit showing red aril (inset). D) Branches and stilt roots typical of this species. E) Close-up of inflorescence. F) Staminate flower with petal dissected to show androecium (scale bar= 0.5 mm).

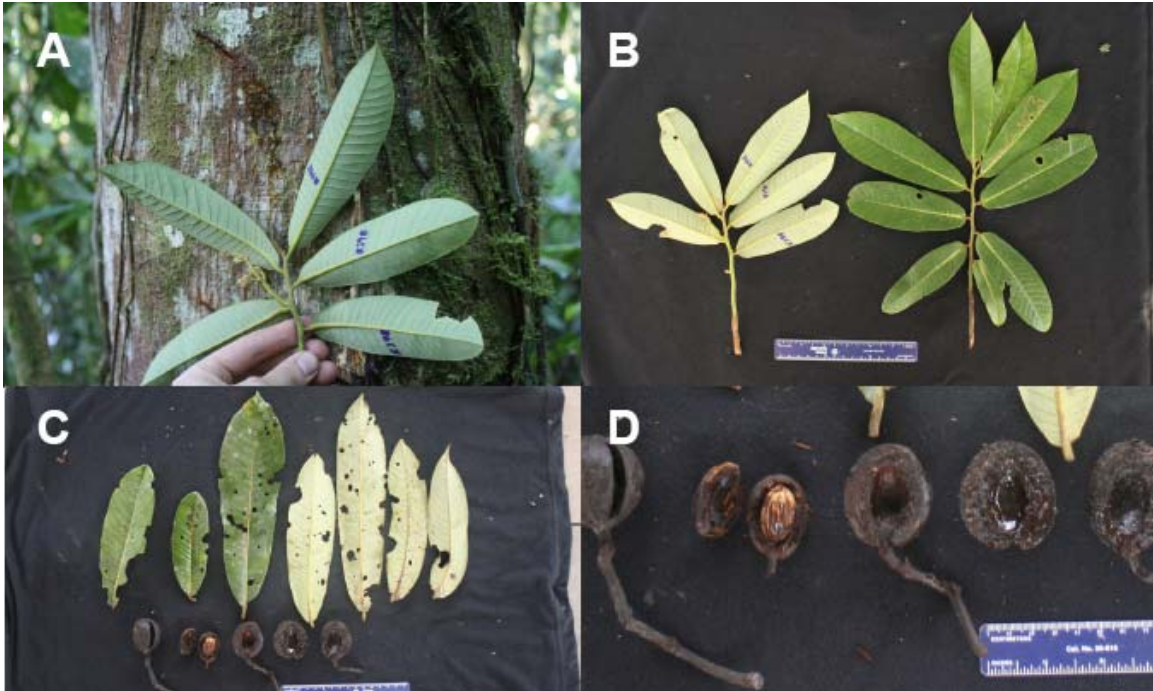


Figure 3.5 *Virola pavonis*: A) Bark, leaves, inflorescence, and greenish latex. B) Specimens showing adaxial and abaxial leaf surfaces (15 cm ruler). C) Leaves and pericarps (15 cm ruler). D) Close-up of pericarps and seed testa.



Figure 3.6 *Virola sp. RADS3* specimen showing reddish abaxial pubescence and glabrous glossy green adaxial surface (15 cm ruler).



Figure 3.7 *Virola calophylla*: A) Leaves with inflorescence. B) Bark and leaves of mature tree. C) Nearly mature florescence. D) Close-up of inflorescence with ants. E) Abaxial leaf pubescence (scale bar= 2 mm). F) Indehiscent and dehiscent mature fruit with laciniate aril.

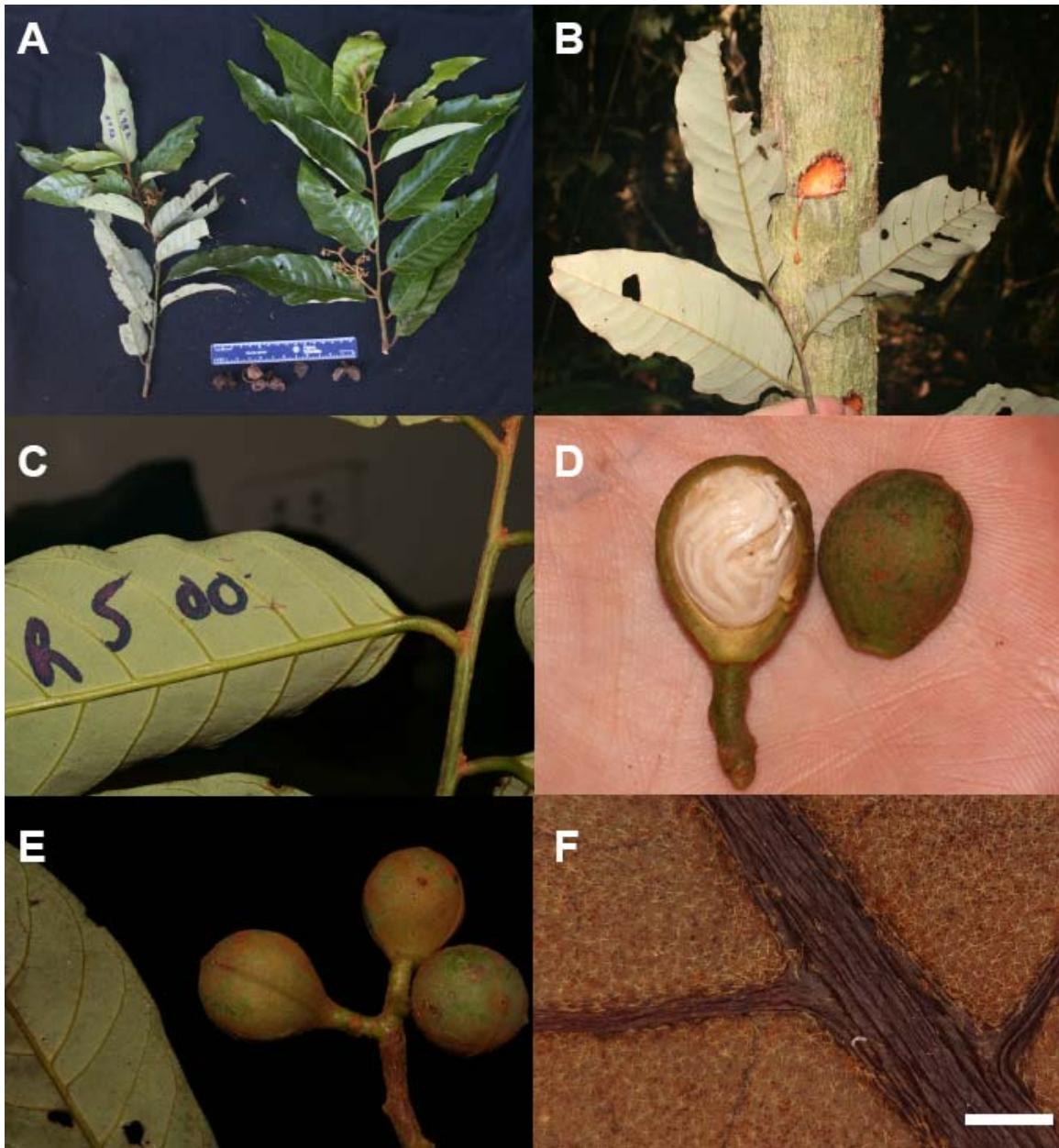


Figure 3.8 *Virola* sp. *RADSI*: A) Leaves with inflorescence and pericarps (15 cm ruler). B) Bark showing latex and leaves. C) Close-up of leaf and branch showing undulating leaf margins. D) Dissected fruit showing deeply lacinate aril and red-pubescent pericarp. E) Leaf and nearly mature un-dehiscent fruits. F) Adaxial leaf pubescence, primary and secondary veins (Scale bar= 1 mm).

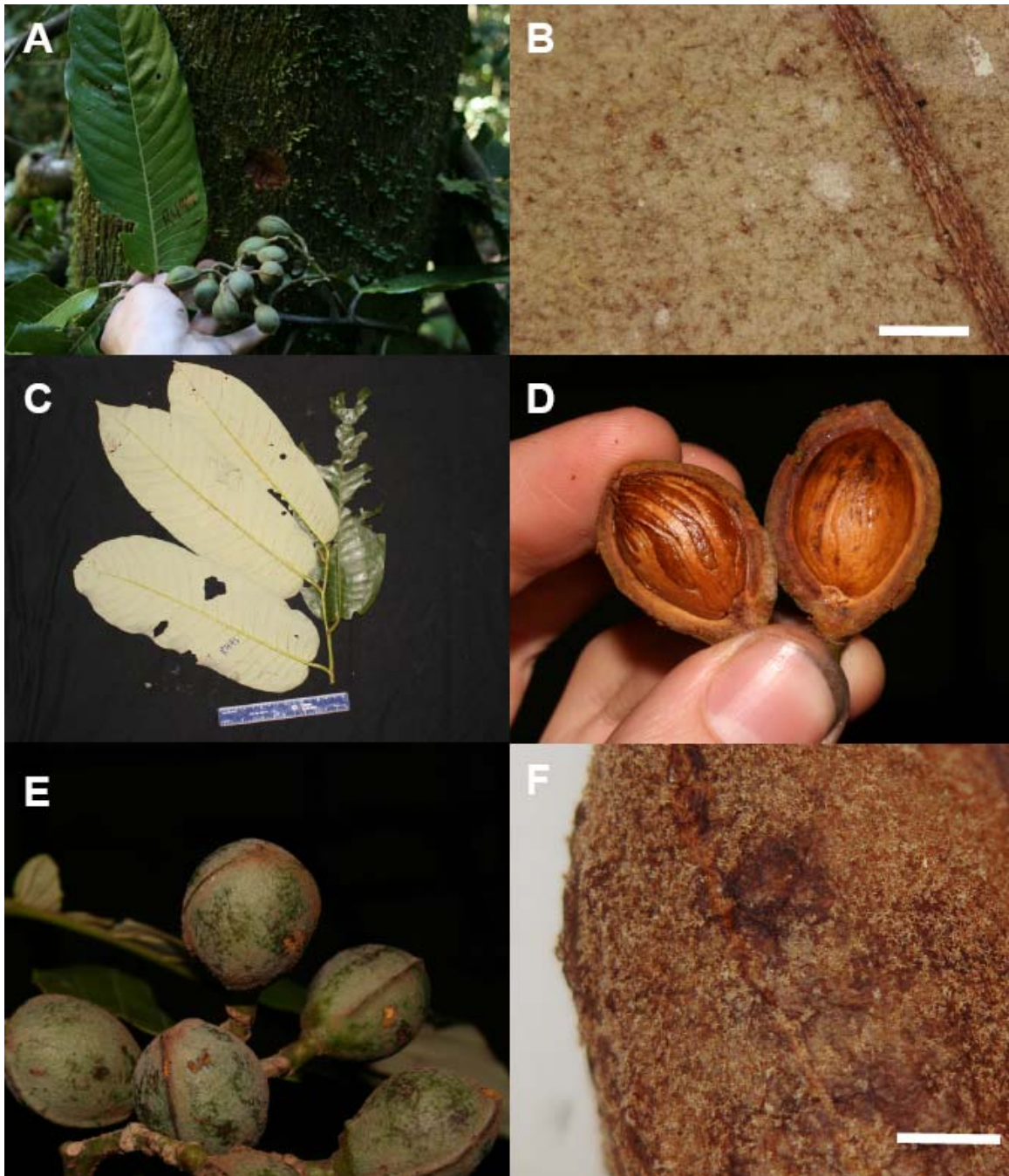


Figure 3.9 *Virola elongata*: A) Leaves, infructescence and bark. B) Adaxial leaf pubescence and secondary vein (scalebar= 1 mm) C) Abaxial and abaxial leaf surfaces (15 cm ruler). D) Dissected mature fruit with deeply lacinated aril (semi-dried). E) Undehiscent nearly mature fruits. F) Close-up of fruit pubescence (scale bar= 1 mm).

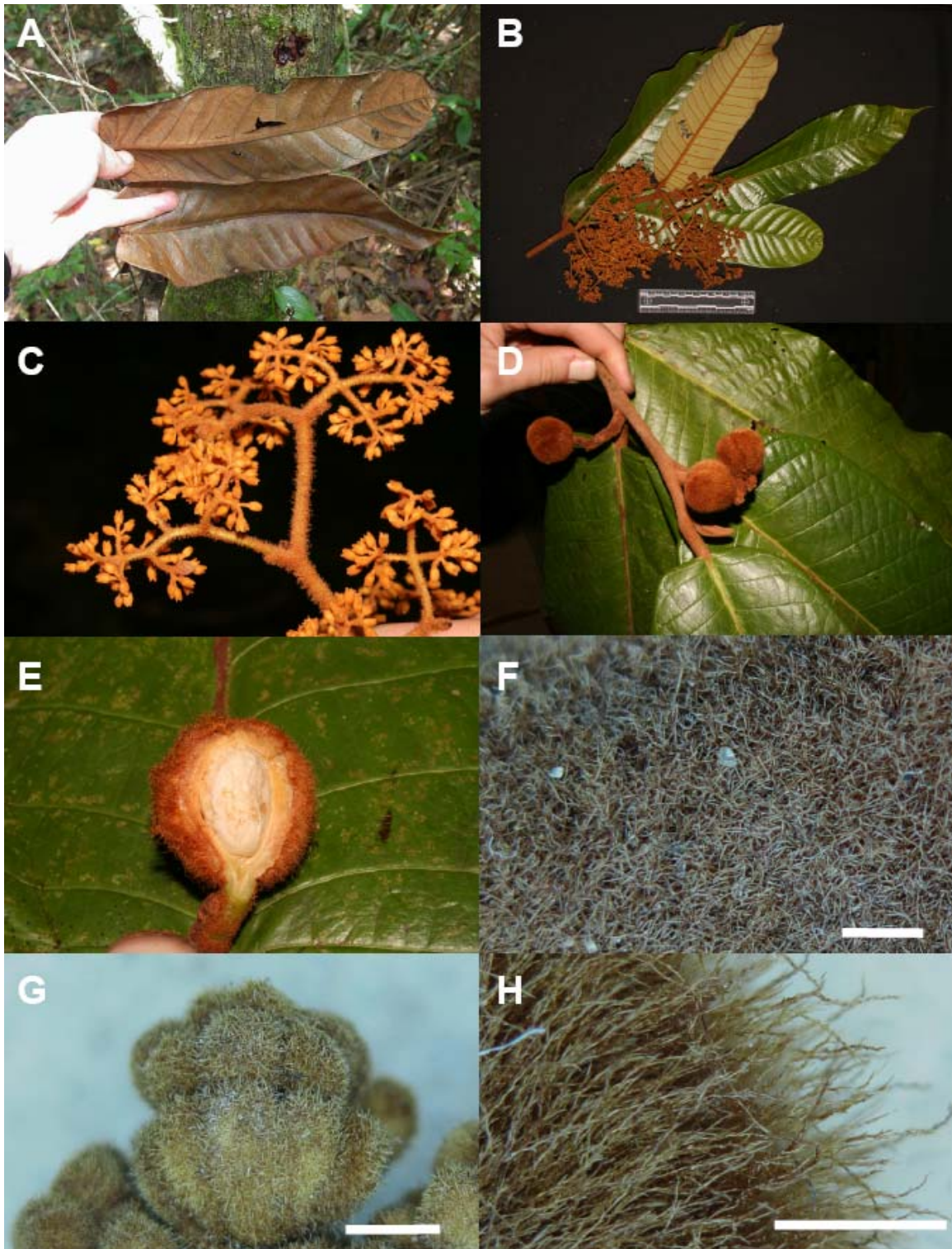


Figure 3.10 *Virola lorentensis*-BL (Big Leaf morphotype): A) Bark, deep red latex and dried (dropped) leaves. B) Abaxial, adaxial leaf surfaces of sample with inflorescences (15 cm ruler). C) Close-up of inflorescence. D) Branch with mature fruits. E) Dissected

immature fruit showing dense pubescence. F) Adaxial leaf pubescence. G) Developing flower buds (scale bar=1 mm). H) Close-up of fruit indument (scale bar= 1 mm).

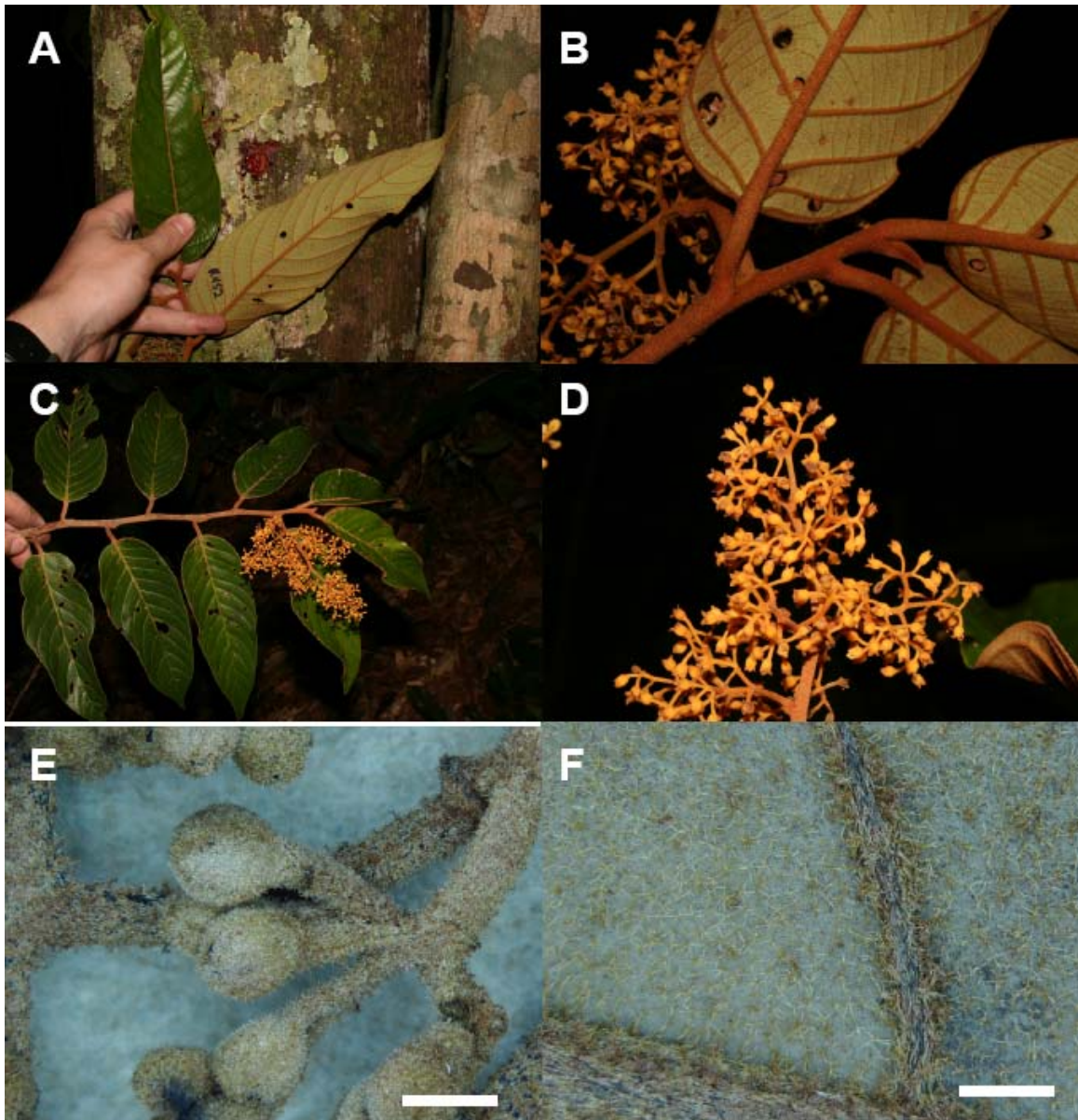


Figure 3.11 *Virola lorentensis*-SL (Small Leaf morphotype): A) Bark, leaves and red latex. B) Adaxial leaf surfaces with inflorescence. C) Branch with inflorescence. D) Close-up of mature inflorescence. E) Flower pubescence (scale bar= 1 mm). F) Adaxial leaf pubescence (scale bar= 1 mm).

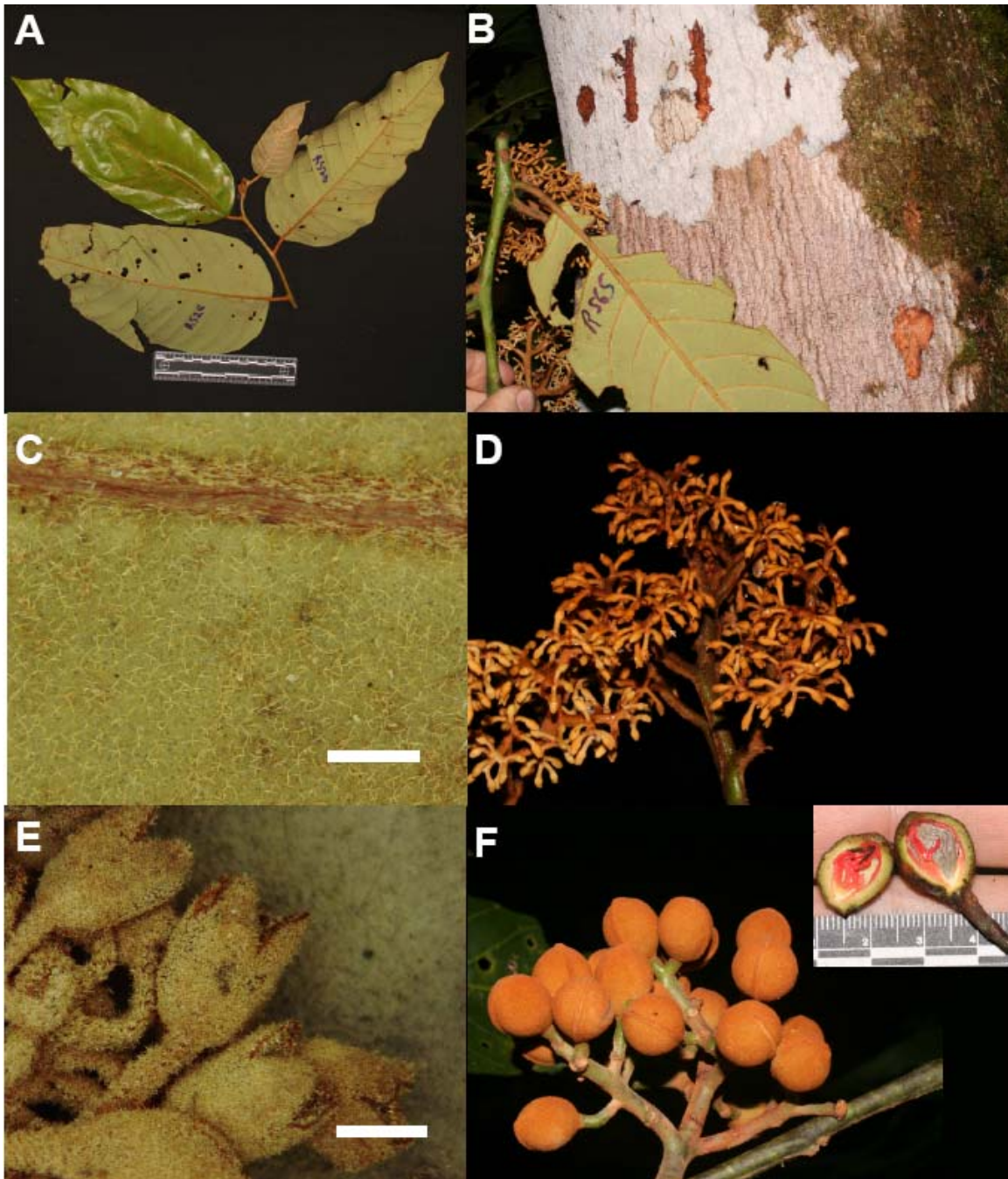


Figure 3.12 *Virola sebifera*-SL (Small Leaf morphotype): A) Leaves with orange-red pubescence (15 cm ruler) B) Bark, leaves and inflorescence of mature tree. C) Abaxial leaf pubescence (scale bar= 1 mm). D) Inflorescence. E) Flowers (scale bar= 1 mm). F) Nearly mature fruits with dissected fruit showing seed testa and aril (inset).

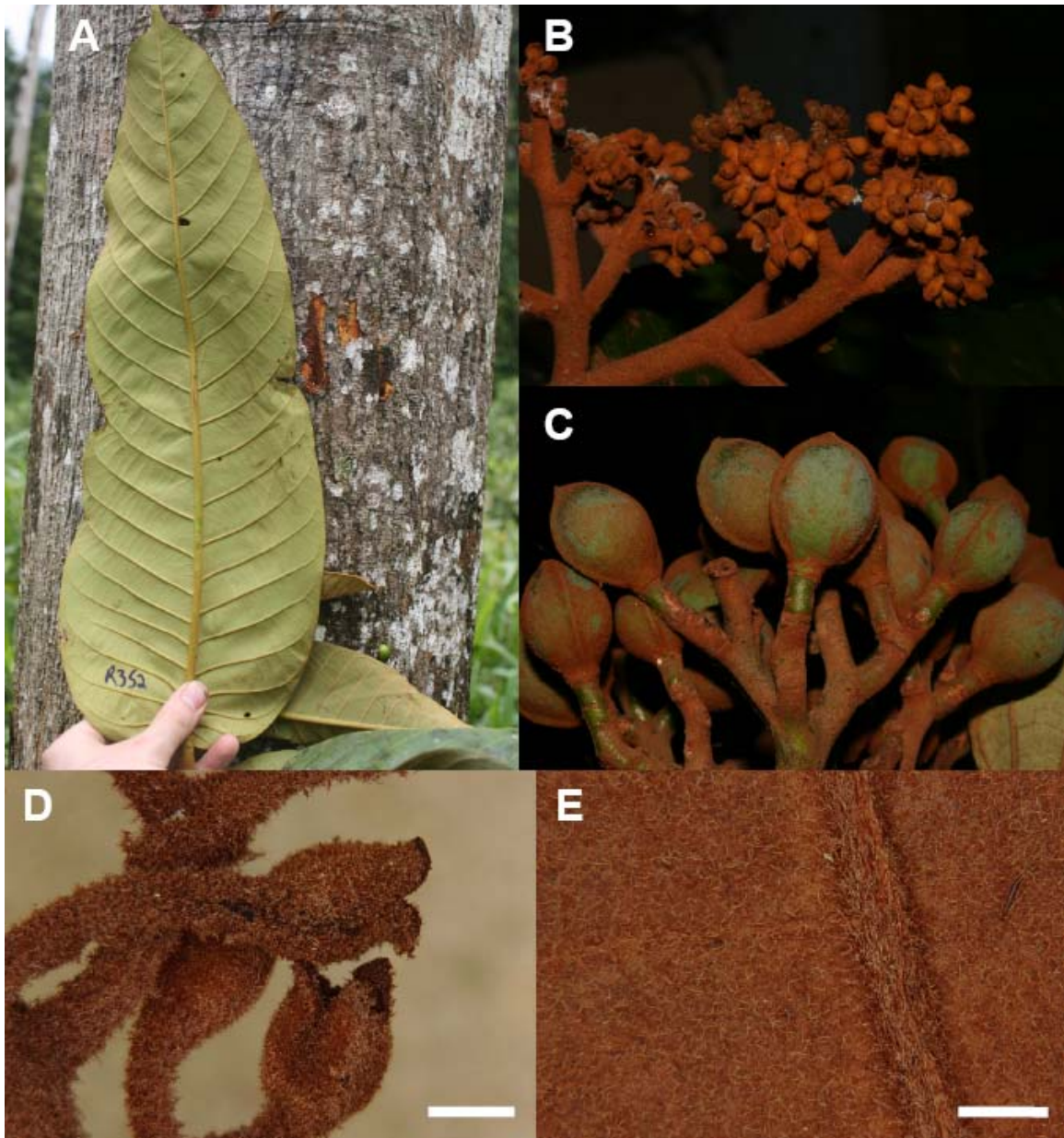


Figure 3.13 *Virola sebifera*-BL (Big Leaf morphotype) : A) Bark with leaf showing abaxial surface. B) Mature pistillate inflorescence. C) Nearly mature infructescence showing deciduous red pubescence. D) Close-up of staminate flowers (scale bar= 1 mm). E) Abaxial leaf pubescence and secondary vein (scale bar= 1 mm).



Figure 3.14 *Virola dixonii* branch showing adaxial and abaxial leaf surfaces (15 cm ruler).

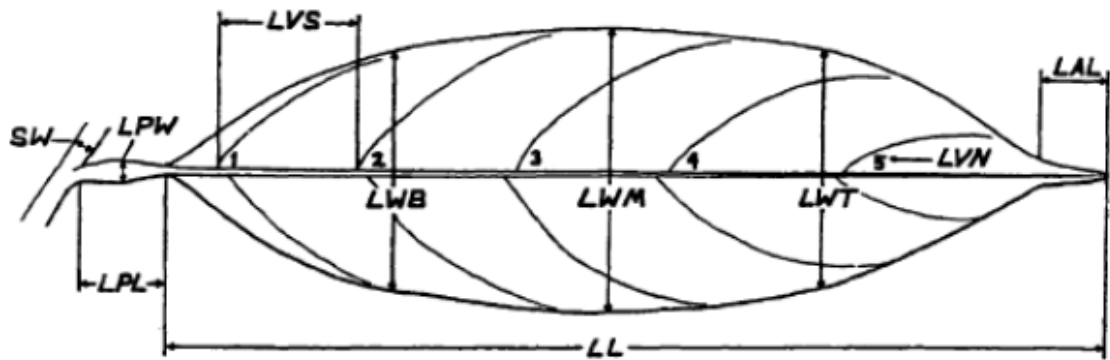


Figure 3.15 Illustration of morphological metrics measured on provisional new species: SW=Stem Width, LPL=Petiole Length, LPW=Petiole Width, LVS=space between secondary veins, LVN=Vein Number, LL=Lamina Length, LWB= Leaf Width at $\frac{1}{4}$ length, LWM=Leaf Width at $\frac{1}{2}$ length, LWT=Leaf Width at $\frac{3}{4}$ length, LVN=Leaf Vein Number, LAL=Leaf Acumen Length.

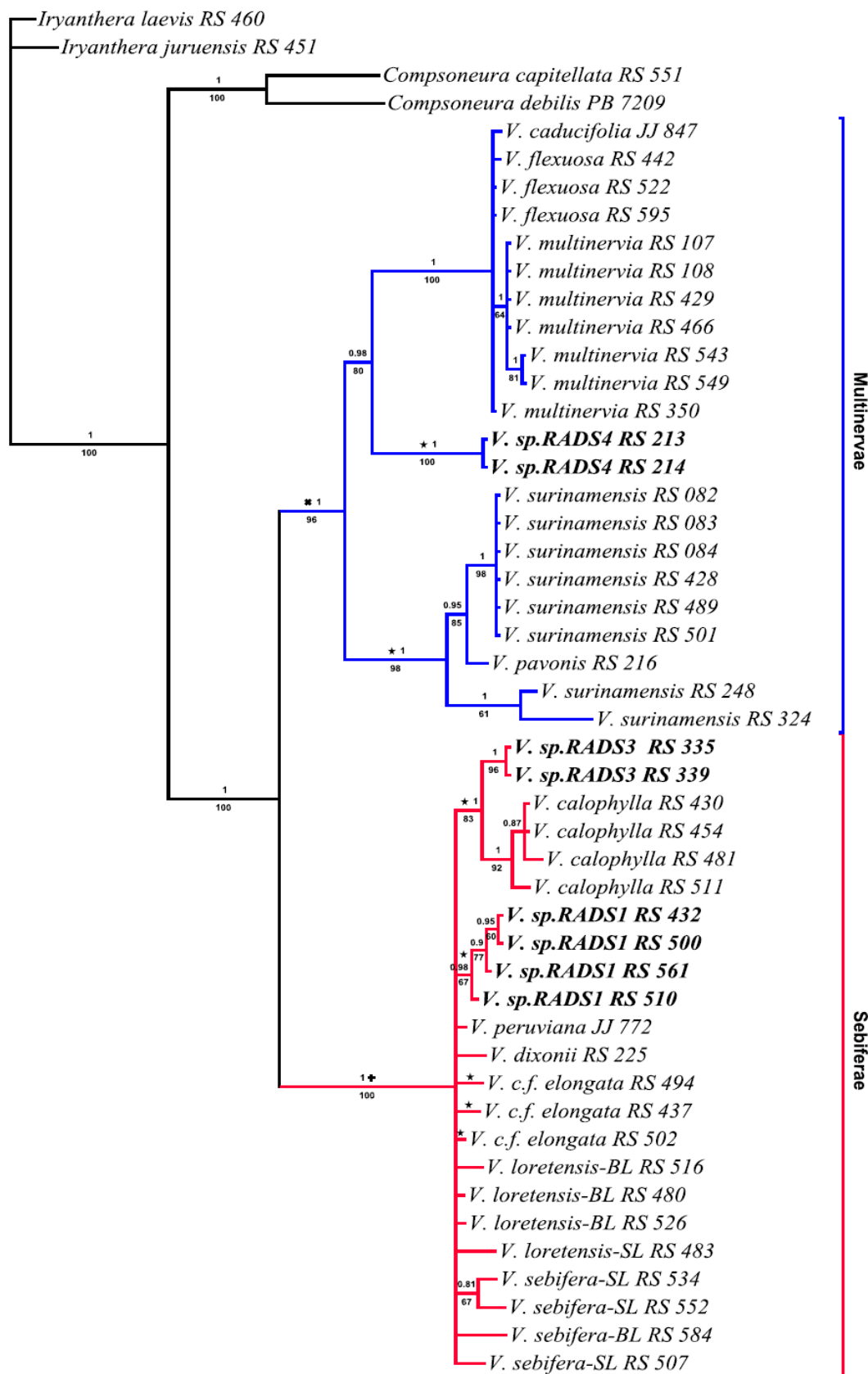


Figure 3.16 Bayesian majority rule consensus tree of 45 *Virola* taxa with proportional branch lengths and Bayesian posterior probabilities (PP) above, and bootstrap support measures below branches. Stars (★) indicate clades with sessile leaf hairs (compared to stalked leaf hairs), the “✖” indicates a clade with type II pollen and the “+” indicates type I pollen (*sensu* Walker and Walker 1979). “SL” and “BL” following *Virola sebifera* and *Virola lorentensis* collections indicate small-leaf and big-leaf morphotypes respectively. Additional letters following taxa names indicate collectors names and numbers (RS=Royce Steeves, JJ=John Janovec, and PB=Paul Berry). Additional information can be found about these specimens in table 3.1 as well as the barcode of life database. Taxa in bold are provisional new species.

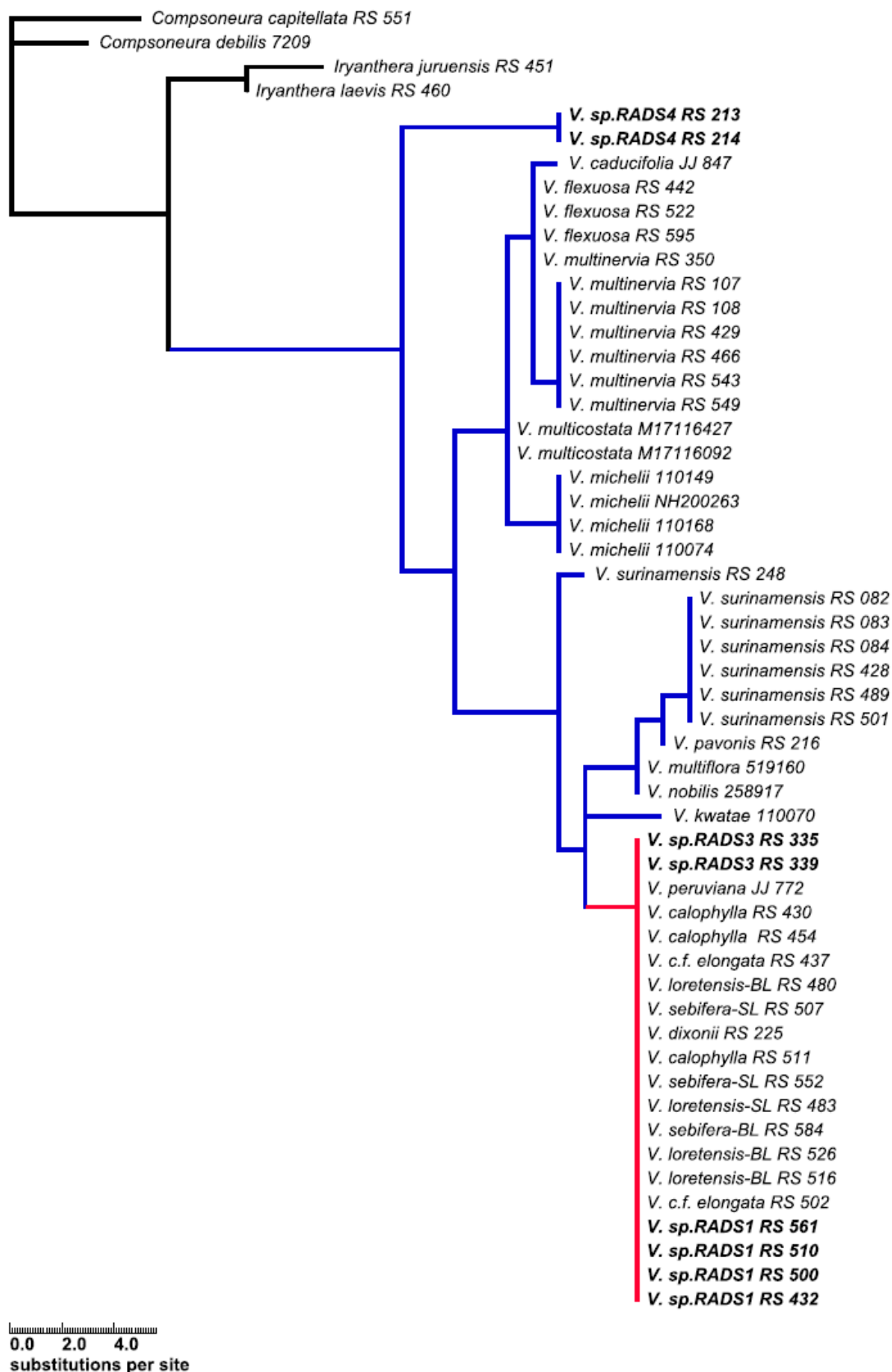


Figure 3.17 Neighbour-joining uncorrected p-distance gene tree of 17 *Viola* species. Taxa in bold are proposed new species. Blue branches indicate taxa of the Multinervae clade and red those of the Sebiferae clade. “SL” and “BL” following *Viola sebifera* taxa indicate whether they are of the small-leaf or big-leaf morphotypes. Letters and numbers preceding species names indicate the collector and collection number.

Literature Cited

- Ackerly, D. D., Rankin-De-Merona, J. M., & Rodrigues, W. A. 1990. Tree densities and sex ratios in breeding populations of dioecious central Amazonian Myristicaceae. *Journal of Tropical Ecology* **6**: 239-348.
- Alfaro, M. E., & Holder, M. T. 2006. The posterior and the prior in Bayesian phylogenetics. *Annual review of ecology, evolution, and systematics* **37**: 19-42.
- Armstrong, J. E., & Irvine, A. K. 1989. Floral biology of *Myristica insipida* (Myristicaceae), a distinctive beetle pollination syndrome. *American Journal of Botany* **76**: 86-94.
- Asif, M. J., & Cannon, C. H. 2005. DNA extraction from processed wood: a case study for the identification of an endangered timber species (*Gonystylus bancanus*). *Plant Molecular Biology Reporter* **23**: 185-192.
- Beloz, A. 1992. Brine shrimp bioassay screening of two medicinal plants used by the Warao: *Solanum straminifolium* and *Virola surinamensis*. *Journal of Ethnopharmacology* **37**: 225-227.
- Bennett, B. C., & Alarcón, R. 1994. *Osteophloeum platyspermum* and *Virola duckei* (Myristicaceae): newly reported as hallucinogens from Amazonian Ecuador. *Economic Botany* **48**: 152-158.
- Candolle, A. D. 1856. Myristicaceae. *Prodromus Systematis Naturali Vegetabilis* **14**: 187-208.
- Chagnon, N. A., Le Quesne, P., & Cook, J. M. 1971. Yanomamö Hallucinogens: Anthropological, Botanical, and Chemical Findings. *Current Anthropology* **12**: 72-74.
- Chase, M. W., Soltis, D. E., Olmstead, R. G., Morgan, D., Les, D. H., Mishler, B. D., Duvall, M. R., Price, R. A., Hills, H. G., Qiu, Y. L., & others. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden* **80**: 528-580.
- Clark, J. L., Neill, D. A., Weber, A., Gruhn, J. A., & Katan, T. 2010. *Shuaria* (Gesneriaceae), an Arborescent New Genus from the Cordillera del Cóndor and Amazonian Ecuador. *Systematic Botany* **35**: 662-674.
- Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In *Dynamics of populations*. Proceedings of the Advanced Study Institute on Dynamics of numbers in populations. Wageningen: Oosterbeek (pp. 298-310).

- Davis, E. W., & Yost, J. A. 1983. The ethnomedicine of the Waorani of Amazonian Ecuador. *Journal of Ethnopharmacology* **9**: 273–297.
- Davis, J. I., Stevenson, D. W., Petersen, G., Seberg, O., Campbell, L. M., Freudenstein, J. V., Goldman, D. H., Hardy, C. R., Michelangeli, F. A., Simmons, M. P., & others. 2004. A phylogeny of the monocots, as inferred from *rbcL* and *atpA* sequence variation, and a comparison of methods for calculating jackknife and bootstrap values. *Systematic Botany* **29**: 467–510.
- Degen, B., Bandou, E., & Caron, H. 2004. Limited pollen dispersal and biparental inbreeding in *Symphonia globulifera* in French Guiana. *Heredity* **93**: 585–591.
- Douady, C. J., Delsuc, F., Boucher, Y., Doolittle, W. F., & Douzery, E. J. 2003. Comparison of Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability. *Molecular Biology and Evolution* **20**: 248–254.
- Draheim, H., Cui, M., & Dick, C. W. 2009. Characterization of 14 microsatellite DNA markers for the tropical forest tree *Virola surinamensis* (Rol.) Warb.(Myristicaceae). *Molecular Ecology Resources* **9**: 1386–1388.
- Farris, J. S., Källersjö, M., Kluge, A. G., & Bult, C. 1995. Testing significance of incongruence. *Cladistics* **10**: 315–319.
- Fazekas, A. J., Steeves, R., & Newmaster, S. G. 2010. Improving sequencing quality from PCR products containing long mononucleotide repeats. *Biotechniques* **48**: 277–285.
- Forget, P. M., & Cuijpers, L. 2008. Survival and scatterhoarding of frugivores-dispersed seeds as a function of forest disturbance. *Biotropica* **40**: 380–385.
- Forget, P. M., Milleron, T., Feer, F., Henry, O., & Dubost, G. 2000. Effects of dispersal pattern and mammalian herbivores on seedling recruitment for *Virola michelii* (Myristicaceae) in French Guiana. *Biotropica* **32**: 452–462.
- Gentry, A. H. 1988. Tree species richness of upper Amazonian forests. *Proceedings of the National Academy of Sciences of the United States of America* **85**: 156–159.
- Golden, J. L., & Bain, J. F. 2000. Phylogeographic patterns and high levels of chloroplast DNA diversity in four *Packera* (Asteraceae) species in southwestern Alberta. *Evolution* **54**: 1566–1579.
- Gonzalez, M. A., Baraloto, C., Engel, J., Mori, S. A., Pétronelli, P., Riéra, B., Roger, A., Thébaud, C., & Chave, J. 2009. Identification of Amazonian trees with DNA barcodes. *PLoS One* **4**: e7483.

- Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series*. (pp. 95–98).
- Holbrook, K. M., & Loiselle, B. A. 2009. Dispersal in a Neotropical tree, *Virola flexuosa* (Myristicaceae): Does hunting of large vertebrates limit seed removal? *Ecology* **90**: 1449–1455.
- Holbrook, K. M., Loiselle, B. A., & Clark, A. M. 2007. Eight polymorphic microsatellite loci for a Neotropical nutmeg, *Virola flexuosa* (Myristicaceae). *Molecular ecology notes* **7**: 248–250.
- Hollingsworth, P. M., Graham, S. W., & Little, D. P. 2011. Choosing and using a plant DNA barcode. *PloS one* **6**: e19254.
- Howe, H. F. 1993. Aspects of variation in a neotropical seed dispersal system. *Plant Ecology* **107**: 149–162.
- Howe, H. F. 1981. Dispersal of a neotropical nutmeg (*Virola sebifera*) by birds. *The Auk* **98**: 88–98.
- Howe, H. F., & Vande Kerckhove, G. A. 1981. Removal of wild nutmeg (*Virola surinamensis*) crops by birds. *Ecology* **62**: 1093–1106.
- Howe, H. F., Schupp, E. W., & Westley, L. C. 1985. Early consequences of seed dispersal for a neotropical tree (*Virola surinamensis*). *Ecology* **66**: 781–791.
- Janovec, J. P. 2000. A systematic study of *Compsonera* (A. DC.) Warb., A Neotropical member of the nutmeg family. Texas A&M University Dissertation: 1-359.
- Janovec, J. P., & Harrison, J. S. 2002. A morphological analysis of the *Compsonera sprucei* complex (Myristicaceae), with a new combination for the Central American species *Compsonera mexicana*. *Systematic Botany* **27**: 662–673.
- Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forests. *The American Naturalist* **104**: 501-528.
- Jaramillo, T. S., Muriel, P., Rodrigues, W. A., & Balslev, H. 2000. Myristicaceae novelties from Ecuador. *Nordic Journal of Botany* **20**: 443–447.
- Julliot, C. 1996. Fruit choice by red howler monkeys (*Alouatta seniculus*) in a tropical rain forest. *American Journal of Primatology* **40**: 261–282.
- Julliot, C., & Sabatier, D. 1993. Diet of the red howler monkey (*Alouatta seniculus*) in French Guiana. *International Journal of Primatology* **14**: 527–550.

- Kreader, C. A. 1996. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Applied and Environmental Microbiology* **62**: 1102-1106.
- Kress, W. J., Erickson, D. L., Jones, F. A., Swenson, N. G., Perez, R., Sanjur, O., & Bermingham, E. 2009. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proceedings of the National Academy of Sciences* **106**: 18621-18626.
- Li, M., Wunder, J., Bissoli, G., Scarponi, E., Gazzani, S., Barbaro, E., Saedler, H., & Varotto, C. 2008. Development of COS genes as universally amplifiable markers for phylogenetic reconstructions of closely related plant species. *Cladistics* **24**: 727-745.
- Lopes, N. P., Kato, M. J., & others. 1999. Antimalarial use of volatile oil from leaves of *Virola surinamensis* (Rol.) Warb. by Waiãpi Amazon Indians. *Journal of ethnopharmacology* **67**: 313-319.
- Macedo, D. S., & Anderson, A. B. 1993. Early ecological changes associated with logging in an Amazon floodplain. *Biotropica* **25**: 151-163.
- Macrae, W. D., & Towers, G. H. 1984a. *Justicia pectoralis*: a study of the basis for its use as a hallucinogenic snuff ingredient. *Journal of ethnopharmacology* **12**: 93-111.
- Macrae, W., & Towers, G. 1984b. An ethnopharmacological examination of *Virola elongata* bark: A South American arrow poison. *Journal of ethnopharmacology* **12**: 75-92.
- McKenna, D. J., Towers, G. H. N., & Abbott, F. S. 1984. Monoamine oxidase inhibitors in South American hallucinogenic plants part 2: Constituents of orally-active Myristicaceous hallucinogens. *Journal of Ethnopharmacology* **12**: 179-211.
- Müller, K. 2006. Incorporating information from length-mutational events into phylogenetic analysis. *Molecular phylogenetics and evolution* **38**: 667-676.
- Nylander, J. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Opler, P. A., & Bawa, K. S. 1978. Sex ratios in tropical forest trees. *Evolution* **32**: 812-821.
- Palma-Silva, C., Wendt, T., Pinheiro, F., Barbará, T., FAY, M. F., Cozzolino, S., & Lexer, C. Sympatric bromeliad species (*Pitcairnia spp.*) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. *Molecular Ecology* **20**: 3185-3201

- Palmé, A. E., Semerikov, V., & Lascoux, M. 2003. Absence of geographical structure of chloroplast DNA variation in willow, *Salix caprea* L. *Heredity* **91**: 465–474.
- Pascal, J. P., & Pelissier, R. 1996. Structure and floristic composition of a tropical evergreen forest in south-west India. *Journal of Tropical Ecology* **12**: 191–214.
- Pitman, N. C., Mogollón, H., Dávila, N., Ríos, M., García-Villacorta, R., Guevara, J., Baker, T. R., Monteagudo, A., Phillips, O. L., Vásquez-Martínez, R., & others. 2008. Tree community change across 700 km of lowland Amazonian forest from the Andean foothills to Brazil. *Biotropica* **40**: 525–535.
- Pitman, N. C., Terborgh, J. W., Silman, M. R., Núñez V, P., Neill, D. A., Cerón, C. E., Palacios, W. A., & Aulestia, M. 2002. A comparison of tree species diversity in two upper Amazonian forests. *Ecology* **83**: 3210–3224.
- Pitman, N. C., Terborgh, J. W., Silman, M. R., Núñez V, P., Neill, D. A., Cerón, C. E., Palacios, W. A., & Aulestia, M. 2001. Dominance and distribution of tree species in upper Amazonian terra firme forests. *Ecology* **82**: 2101–2117.
- Poinar, G. J., & Poinar, R. 1999. *The amber forest: a reconstruction of a vanished world*. Princeton, N.J.: Princeton University Press.
- Poinar, H. N. 1998. Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* **281**: 402–406.
- Prance, G. T. 1972. Ethnobotanical notes from Amazonian Brazil. *Economic Botany* **26**: 221–237.
- Qiu, Y. L., Chase, M. W., Les, D. H., & Parks, C. R. 1993. Molecular phylogenetics of the Magnoliidae: cladistic analyses of nucleotide sequences of the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden* **80**: 587–606.
- Rieseberg, L.H., & Soltis, D.E. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* **5**: 65–84.
- Rodrigues, W. A. 1980. Revisão taxonômica das espécies de *Virola* Aublet (Myristicaceae) do Brasil. *Acta Amazonica (suplemento)*: 1–127.
- Rodrigues, W. A. 1989. A new Venezuelan *Virola* (Myristicaceae). *Annals of the Missouri Botanical Garden* **76**: 1163–1164.
- Rodrigues, W. A. 2002. Notas taxonômicas sobre Myristicaceae neotropicais. *Acta Biológica Paranaense* **31**: 71–77.
- Rogers, Z. S. 2002. A new species of *Weinmannia* (Cunoniaceae: Cunoniaceae) from southern Ecuador. *Novon* **12**: 249–252.

- Rohlf, F. J. 2006. *tpsDig version 2.10, Department of Ecology and Evolution*. State University of New York at Stony Brook, New York.
- Ronquist, F., & Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574.
- Roumy, V., Garcia-Pizango, G., Gutierrez-Choquevilca, A. L., Ruiz, L., Jullian, V., Winterton, P., Fabre, N., Moulis, C., & Valentin, A. 2007. Amazonian plants from Peru used by Quechua and Mestizo to treat malaria with evaluation of their activity. *Journal of ethnopharmacology* **112**: 482–489.
- Russo, S.E. 1992. Linking spatial patterns of seed dispersal and plant recruitment in a neotropical tree, *Virola calophylla* (Myristicaceae). Harvard University. PhD dissertation: 1-148.
- Russo, S. E. 2003. Responses of dispersal agents to tree and fruit traits in *Virola calophylla* (Myristicaceae): implications for selection. *Oecologia* **136**: 80-87.
- Sabatier, D. 1997. Description et biologie d'une nouvelle espèce de *Virola* (Myristicaceae) de Guyane. *Adansonia, Série 3*: 273–278.
- Sang, T., Crawford, D. J., & Stuessy, T. F. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* **84**: 1120-1136.
- Sasaki, Y., Miyoshi, D., & Sugimoto, N. 2006. Effect of molecular crowding on DNA polymerase activity. *Biotechnology Journal* **1**: 440–446.
- Sauquet, H. 2004. Systematic revision of Myristicaceae (Magnoliales) in Madagascar, with four new species of Mauloutchia. *Botanical Journal of the Linnean Society* **146**: 351–368.
- Sauquet, H., Doyle, J. A., Scharaschkin, T., Borsch, T., Hilu, K. W., Chatrou, L. W., & Le Thomas, A. 2003. Phylogenetic analysis of Magnoliales and Myristicaceae based on multiple data sets: implications for character evolution. *Botanical Journal of the Linnean Society* **142**: 125–186.
- Sauquet, H., & Le Thomas, A. 2003. Pollen diversity and evolution in Myristicaceae (Magnoliales). *International Journal of Plant Sciences* **164**: 613–628.
- Sawadjoon, S., Kittakoop, P., Kirtikara, K., Vichai, V., Tanticharoen, M., & Thebtaranonth, Y. 2002. Atropisomeric myristinins: Selective COX-2 inhibitors and antifungal agents from *Myristica cinnamomea*. *The Journal of Organic Chemistry* **67**: 5470-5475.
- Schultes, R. E., & Holmstedt, B. 1968. The vegetal ingredients of the Myristicaceous snuffs of the Northwest Amazon. *Rhodora* **70**: 113-160.

- Schultes, R. E., & Raffauf, R. F. 1990. *The Healing Forest*. Portland, Oregon: Dioscorides Press.
- Schultes, R. 1981. Iconography of the New World Hallucinogens. *Arnoldia* **41**: 80-125.
- Simmons, M., & Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analysis. *Systematic Botany* **49**: 369-381.
- Smith, A. C. 1937. The American species of Myristicaceae. *Brittonia* **2**: 393-510.
- Soltis, D. E., Smith, S. A., Cellinese, N., Wurdack, K. J., Tank, D. C., Brockington, S. F., Refulio-Rodriguez, N. F., Walker, J. B., Moore, M. J., Carlswald, B. S., & others. 2011. Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal of Botany* **98**: 704.
- Speiss, A., T. Mueller, & Ivell, R. 2004. Trehalose is a potent PCR enhancer: lowering of DNA melting temperature and thermal stabilization of Taq polymerase by the disaccharide trehalose. *Clinical chemistry* **50**: 1256-1259.
- Stöver, B. C., & Müller, K. F. 2010. TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC bioinformatics* **11**: 1-9.
- Suzuki, Y., Glazko, G. V., & Nei, M. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proceedings of the National Academy of Sciences of the United States of America* **99**: 16138-16143.
- Swafford, D. 1999. PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods). Version 4 . Sunderland : Sinauer Associates.
- Tate, J. A., & Simpson, B. B. 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Systematic Botany* **28**: 723-737.
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research* **22**: 4673-4680.
- Ulloa Ulloa, C., & Neill, D. A. 2006. *Phainantha shuariorum* (Melastomataceae), una especie nueva de la Cordillera del Condor, Ecuador, Disyunta de un genero Guyanes. *Novon* **16**: 281-285.
- Warburg, O. 1897. Monographie der Myristicaceen. *Nova Acta Acad. Caes. Leop.-Carol* **68**: 1-680.
- Walker, J.W. & A.G. Walker. 1979. Comparative pollen morphology of the American Myristicaceous genera *Compsonaura* and *Virola*. *Annals of the Missouri Botanical Garden* **66**: 731-755.

- Williams, L. O. 1960. Ucuuba and related wax-like vegetable tallows. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum)* **7**: 295–296.
- Yang, Z., & Rannala, B. 2010. Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences* **107**: 9264-9269.
- Zimmerman, S. B., & Harrison, B. 1987. Macromolecular crowding increases binding of DNA polymerase to DNA: an adaptive effect. *Proceedings of the National Academy of Sciences of the United States of America* **84**: 1871-1875.
- Zipparro, V. B., & Morellato, L. P. 2005. Seed predation of *Virola bicuhyba* (Schott) Warb.(Myristicaceae) in the Atlantic forest of south-eastern Brazil. *Revista Brasileira de Botânica* **28**: 515–522.

Chapter 4

A MORPHOLOGICAL AND MOLECULAR INVESTIGATION OF *VIROLA* *SEBIFERA* Aublet AND *VIROLA LORETENSIS* A. C. Sm.

Abstract

Virola is a widespread and species-rich genus of neotropical Myristicaceae. Although *Virola* is an ecologically dominant and ethnobotanically significant group of trees, they have received little contemporary taxonomic study. In this study I test whether ecologically separate and polymorphic morphotypes of *V. sebifera* and *V. lorentensis* represent undescribed cryptic species using morphological and molecular data. Species hypotheses were tested using a multivariate analysis of morphological characters as well as a statistical parsimony analysis using a low-copy nuclear locus. Morphological analyses show discrete variation in morphotypes of both *V. sebifera* and *V. lorentensis* that corresponds to upland and floodplain forest habitats. Haplotype networks constructed from molecular data reveal some distinct clades as well as a high degree of apparent incomplete lineage sorting in these taxa. It is concluded that these data suggest that these ecologically separated morphotypes of each species represent distinct undescribed species.

Introduction

There are an estimated 235,000-420,000 (Govaerts 2003, Scotland and Wortley 2003) seed plant species in the world. The vast majority (about 200,000) of these plant species reside in tropical areas, with the neotropical region contributing about 90,000 species (Thomas 1999). It is now widely recognized that tropical moist forests of the world are being rapidly destroyed by human activity (Dodson and Gentry 1991, Gentry 1988, 1992). The sheer organismal diversity and short history of intensive study in tropical regions are demonstrated by the meager understanding we have of these fragile ecosystems and their astonishing number of species. Many species remain to be discovered, described, and classified; many more remain to be understood from ecological and evolutionarily perspectives. Examinations of the composition and diversity of tropical rainforests have been the focus of many contemporary plant ecologists, however, they are faced with a flora that is exceedingly diverse and difficult to identify.

Myristicaceae is composed of 21 genera and approximately 500 species of trees that have significant ecological and ethnobotanical importance worldwide in wet lowland tropical forests. Floristic and ecological studies have revealed that Myristicaceae ranks among the ten, and often five, most diverse and prominent tree families in these ecosystems (Gentry 1982, 1988, Pascal and Pelissier 1996, Pitman et al. 2001, 2002, 2008). Worldwide, the most important species economically is the Asiatic *Myristica fragrans*, which is widely cultivated in tropical areas for the spices nutmeg and mace. In the American tropics, numerous species are valued as sources of food, medicine, narcotics, and timber (Gottlieb 1973, Prance 1972, Schultes and Raffaf 1990). The most

widely employed Myristicaceae genus in the Neotropics for both commercial and traditional ethnobotanical uses is *Virola*.

Virola is a genus of canopy to sub-canopy trees endemic to South and Central America. They are the most speciose nutmeg genus in the new world tropics and often rank amongst the most abundant trees in this region (Pitman et al. 2001, 2002, 2008). Despite being the most species-rich genus of neotropical Myristicaceae, *Virola* has received relatively little recent taxonomic attention. *Virola* was first described by Aublet (1775) as a genus endemic to Central and South America. The genus was divided into 6 groups (*Mollissimae*, *Sebiferae*, *Calophyllae*, *Rugulosae*, *Surinamenses*, and *Subsessilis*) by Smith's (1937) monograph of the neotropical Myristicaceae where he recognized 38 species. The last comprehensive treatment of *Virola* was performed by Rodrigues (1980) and was largely restricted to the species residing within the political boundaries of Brazil. Since 1980, taxonomic investigations of *Virola* have been primarily alpha-taxonomic in nature and about 60 species are currently recognized in the genus (Rodrigues 1989, Sabatier 1997, Jaramillo et al. 2000, Rodrigues 2002).

Virola sebifera is a common and widespread canopy tree that is found in tropical rainforests from 1700 m a.s.l. in the Andes to near sea level in Eastern Brazil. This species also ranges across a wide latitudinal gradient as it can be found from Costa Rica to Southern parts of Brazil (Provinces of São Paulo and Matto Grosso del Sul). Across its range, and even within populations, members of *V. sebifera* exhibit a great deal of morphological diversity. Smith (1937) described *V. sebifera* leaves as being ovate, oblong, deltoid, or elliptic in shape with cordate, rounded, or truncate bases and ranging from 15-47 cm in length and 6-15 cm in width. In spite of this great variation in leaf

morphologies, Smith (1937) united 9 species previously described species (*V. macoa*, *V. boliviensis*, *V. panamensis*, *V. cordifolia*, *V. fulva*, *V. warburgii*, *V. peruviana*, *V. mycetis*, and *V. venezuelensis*) into *V. sebifera*, citing numerous connecting morphological forms for this monospecific concept.

Virola loretensis is a small tree (3-10 m according to Smith 1937) of floodplain forests of the Northwestern Amazon basin (Ecuador, Peru and Colombia). It was first described by Smith (1937) from 12 collections from the Peruvian province of Loreto and Acre, Brazil. The leaves of *V. loretensis* are distinguished by having a ferruginous-tomentose pubescence which is particularly prominent on abaxial veins, large size (15-37 cm long and 4-10 cm broad) and deciduous habit during pronounced dry seasons. The inflorescences of this species are also easily distinguished by a dense ferruginous-tomentose pubescence which is also present on the fruits (Smith 1937).

In the course of field and herbarium investigations, it became apparent that both *V. sebifera* and *V. loretensis* possess a great deal of morphological variation within each species, often within the same locality. The objective of this investigation was to test whether this morphological diversity is indicative of within-species phenotypic plasticity or cryptic speciation. This was tested by multivariate analysis of morphological data as well as the construction of a haplotype network from a low-copy nuclear gene.

Materials and Methods

Collection sites

Since members of *Virola* are particularly recalcitrant with respect to DNA extraction and sequencing, previously collected herbarium specimens were of no utility to

this investigation. Consequently, only recent collections made where leaf tissue was promptly preserved in silica dessicant could be employed in this study, thereby greatly limiting the geographic and morphological breadth of sampling. In attempts to capture the inherent morphological and genetic variability encountered in these species, five populations were sampled from Southern Peru (n=4) and Brazil (n=1) spanning 2560 km in distance and 1600 m of elevation (Figure 4.1). *Virola sebifera* samples were collected in Peru from two cloud forests (Manu Paradise Lodge and Quincemil) and two lowland rainforests (Centro de Investigación y Capacitación Río Los Amigos [CICRA] and Puerto Maldonado). *Virola sebifera* samples were also collected from a single Brazilian population by Mark Leithead and Lucas Silva at the Reserva Ecologica do IBGE near the city of Brasilia. *Virola lorentensis* samples were collected from CICRA and Puerto Maldonado, Peru. All Peruvian samples were collected by the author and associated collectors. Herbarium vouchers were made for all collections and have been deposited at the OAC herbarium (Table 4.1). Leaf tissue was placed in silica gel immediately upon collection to minimize oxidation as members of this genus typically do not yield DNA amenable to PCR unless tissue is desiccated immediately.

Study species

Two relatively common morphotypes of *Virola sebifera* were collected. A big-leaved form (hereafter *V. sebifera*-BL) was found growing at all locations in either seasonal flooded forests of the Madre de Dios river or in well drained upland habitats in cloud forests (750-1700 m asl). A second and comparatively smaller-leaved form (*V.*

sebifera-SL) was found to grow exclusively in terra firme forests (upland, non-flooded, forests) of the Madre de Dios watershed and near streams in cloud forest habitats.

Large and small-leafed forms of *V. lorentensis* were also collected from the Madre de Dios watershed. The big-leafed variety (*V. lorentensis*-BL) was relatively rare (only 3 trees encountered in the course of 2 months) and was collected from floodplain forests of two locations. The small-leafed variety (*V. lorentensis*-SL) was rarely collected (only 3 trees encountered) in terra firme forests at the Los Amigos Biological Station. Pictures were taken of vegetative and reproductive material (when available) to document the 4 different morphotypes (Figures 4.2-4.5).

Morphological analysis

After sterile material was photographed, pressed, and dried, measurements of 10 quantitative vegetative metrics (Figure 4.6) were made from digital photographs using tpsDig 2.0 (Rohlf 2006). A principal component analysis (PCA) was performed on leaf morphometric data using Canoco 4.5 (ter Braak, 1998) to identify the length of the ordination axis and unimodal ordination model was applied (Correspondence Analysis, CA). The relationship between quantitative leaf characters was analysed via nonmetric multi-dimensional scaling (NMS; Kruskal 1964, Primer 2002). In NMS, the Bray-Curtis distance measure was used because of its robustness for both large and small scales on the axes (Minchin 1987). Data were standardized by species maxima and two-dimensional solutions were appropriately chosen based on plotting a measure of fit (‘stress’) to the number of dimensions. Stress represents distortion in the data and a stress value over 0.2 is high enough that the results are invalidated (Primer 2002). One thousand

iterations were used for each NMS run, using random start coordinates. The first two ordination axes were rotated to enhance interpretability with the different axes. As an independent check, detrended correspondence analysis (DCA; ter Braak 1998) was used to evaluate the NMS classification. A Pearson-Correlation analysis was performed to investigate which metrics contributed the most to the differentiation of taxa.

DNA extraction, amplification, molecular cloning, sequencing and alignment

A total of 16 samples including 5 *V. sebifera*-BL, 5 *V. sebifera*-SL, 3 *V. lorentensis*-BL, and 3 *V. lorentensis*-SL were used in genetic analyses. Collection information for samples used in genetic analyses can be found in Table 4.1. Total genomic DNA was extracted from leaf tissue of silica-dried specimens using the Macherey-Nagel Nucleospin II plant Kit. Lysis buffer 1 was used according to the manufactures' instructions with the exception of the elongation of the post homogenization incubation period to 1hr (from 10 minutes) and the addition of 20mM N-Phenacylthiazolium Bromide which has been found to improve amplification of recalcitrant samples (Poinar et al. 1998, Asif and Cannon 2005).

A phylogenetic investigation of *Virola* (chapter 3 of this thesis) found the AGT1 gene and associated exons (Li et al. 2008) to be the most variable loci in *Virola* and was therefore employed in this study. This locus was amplified using the primers AGT1-MYR-F (5'-GGGCATTGACGTAGCTTTGACAGG-3'; this thesis), and AGT1-MYR-R (5'-GTGCAGTTCTTCAAGCCCCAAGC-3'; this thesis). PCR was performed with 0.5U of Platinum*Taq*® (Invotrogen, Burlington, Ontario) DNA polymerase in a 20 µl reaction containing 1X reaction buffer, 2.5 mM MgCl₂, 8% W/V Polyethylene glycol

(Zimmerman and Harrison 1987, Sasaki et al. 2006) , 0.2 M trehalose (Speiss et al. 2004), 2 µg BSA, 0.2 mM each DNTP, and 0.2 µM of each primer. Cycling conditions entailed an initial denaturation step of 4 min at 95°; 30 cycles of 95° for 15 s, 67° for 10 s, 72° for 30 s; and a final elongation step of 72° for 2 min followed by a 4° hold. Platinum*Taq*® was used as it was found to work well with the AGT1 primer set and be more robust than two high fidelity polymerases (Kapa HIFI and Finnzymes Phusion) to the inhibiting substances present in these samples.

Since there exists the possibility of multiple alleles with low-copy nuclear genes, PCR amplicons were cloned to sequence individual alleles. PCR product was diluted 10X and then cloned using the StrataClone™ PCR Cloning Kit (VWR, Mississauga, Ontario). PCR fragments were ligated to the StrataClone™ PCR cloning vector in a reaction mixture containing 1.5 µl of StrataClone™ Cloning Buffer, 1 µl of diluted PCR product and 0.5 µl of StrataClone™ Vector Mix amp/kan. The reaction mixture was then incubated at room temperature for 10 minutes and transformed into SoloPack competent *E.coli* cells according to the manufacturer's instructions. The transformed cells were incubated in Luria-Bertani (LB) Medium with agitation at 37°C for 2 hours to allow the cells to recover. Transformed cell were then spread onto 1% agar LB plates containing 100mg/L ampicillin (Sigma, Oakville, Ontario) and 64mg/L X-Gal (Fisher Scientific, Ottawa, Ontario, Canada). Plates were then incubated at 37°C for 20 hours.

Colonies are white in color if they fail to express the β-Galactosidase gene, which is interrupted with the successfully insertion of a sequence into the vector. These white colonies were selected and transferred into a buffer containing low TE and 2% V/V tween-20 and then subjected to 95 °C for 10 min to lyse the cells. This lysate was then

used as template DNA for a subsequent PCR reaction using the same primers and cycling conditions employed for the initial amplification reaction.

In case multiple copies of this locus were present in any individuals, 16 colonies were sequenced in one direction from each individual tree. This number of colonies was selected as it gives a 0.96 probability of detecting all alleles present in a sample with 4 alleles according to the formula:

$$P = [1 - (1/t)^n]^t$$

where “t” is the number of alleles in an individual and “n” is the number of colonies sequenced (Joly et al. 2006).

Amplification products were sequenced directly using the AGT1-MYR-F primer used for PCR. Cycle sequencing reactions were performed in a 10.5 µL reaction volume containing 0.5 µL of BigDye terminator mix v3.1, 1.88 µL of 5x sequencing buffer (Applied Biosystems), 1.0 µM of primer and 0.5 µL of PCR product. Thermal cycling parameters were 96° for 2 min; 30 cycles of 96° for 30s, and 60° for 4 min; and a 4° hold. Cycle sequencing reactions were cleaned using sephadex columns (Cat. no. S5897; Sigma-Aldrich, St. Louis, MO, USA) and the samples were run on an ABI 3730 sequencer (Applied Biosystems).

Sequences were edited using Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA). DNA sequences were aligned using the default settings of the ClustalW algorithm (Thompson et al. 1994) in Bioedit (Hall 1999) and adjusted manually. Alignments can be found in appendix 4. Single nucleotide polymorphisms (SNP's) found in only one of the cloned sequences of a specimen were assumed to be due to polymerase error and were therefore omitted from the alignment (i.e. the allele was

retained but suspected erroneous base pairs were changed to the nucleotide used at that position by the other alleles of that individual). If two or more clones of an individual specimen contained the same alleles they were retained but it was kept in mind that these may be artifacts of PCR. All unique alleles from each individual were then aligned for haplotype network analysis.

Haplotype networks were constructed by statistical parsimony (Templeton et al. 1992, Templeton 1998) using the program TCS (Clement et al. 2000) which calculates the number of differences between haplotypes that are due to a sequence of single mutations at each site. Networks were calculated with TCS as implemented by ANeCA (Panchal 2007) by treating gaps as a 5th character state and with a 95% confidence interval. Haplotype networks may be preferable to traditional phylogenetic methods at low taxonomic levels because population level data often violate many of the assumptions made by traditional tree-building methods, such as Maximum Parsimony, Maximum Likelihood and Bayesian Analyses (Posada and Crandall 2001). In comparison, networks are able to utilize haplotypic data that contains low levels of genetic divergence, ancestral haplotypes, multiple mutational variants from a given haplotype within the population, and reticulation that may be caused by recombination or hybridization (Templeton et al. 1992, Posada and Crandall 2001).

Results

Morphological Analyses

Morphological measurements were recorded for 3 specimens each of *V. lorentensis*-BL and *V. lorentensis*-SL, 11 *V. sebifera*-BL and 9 *V. sebifera*-SL. Leaf length of *V. sebifera*-BL ranged from 24-49 cm while *V. sebifera*-SL leaves were 13-32 cm

long; *V. lorentensis*-BL was 24.5-42 cm and *V. lorentensis*-SL ranged from 16-22 cm in length. The ordination analyses of quantitative leaf characters showed a pronounced distinction between big-leaf and small-leaf varieties of the two species but both *V. sebifera* and *V. lorentensis* clustered together with respect to big or small leaf-type (Figure 4.7). One sample of *V. lorentensis*-BL (RS 480) was positioned approximately mid-way between big-leaf and small-leaf varieties. *Virola lorentensis*-BL is deciduous during the dry season in South-Eastern Peru and this sample was in the process of regenerating its leaves and therefore this sample may not represent its fully developed size. Additionally, one sample of *V. sebifera*-SL was grouped with *V. sebifera*-BL and this may be due to the fact that this sample was the only juvenile included in the analysis and it was observed that juveniles growing under the canopy tend to have larger leaves than adult trees that have emerged from the sub-canopy. Pearson correlation analyses revealed that the X-axis is strongly correlated with numerous measures of leaf length and width (Figure 4.7; Table 4.2) and the y-axis was relatively weakly correlated to leaf acumen length and stem width (Fig 4.7; Table 4.2).

Although the fruits of these species were not available for quantitative analysis they were qualitatively observed in the field (Figures 4.2-4.5). The fruits of *V. sebifera*-BL are generally elliptic in shape, strongly carinate at suture of the pericarps, and ferruginous-tomentose but quickly deciduous. The fruits of *V. sebifera*-SL are more globose in shape, weakly carinate at pericarp suture, and persistently ferruginous-tomentose. Individuals of *V. sebifera*-BL were typically found growing in the floodplain forests or cloud forests; *V. sebifera*-SL were typically found inhabiting terra firme forests.

The fruits of *V. lorentensis*-BL are globose in shape but are readily distinguished from other taxa in having long (6-9 mm) persistent ferruginous-tomentose pubescence on the pericarp surface. The fruits of *V. lorentensis*-SL are covered in a comparatively shorter indument (<1 mm) and their size, shape and color are similar to the fruits of *V. sebifera*-SL. In addition to fruit characters, these *V. lorentensis* taxa can be distinguished based on their habitats (flood plain versus terra firme forest) and height (*V. lorentensis*-BL = 3-5 m, *V. lorentensis*-SL = 20-40 m).

Molecular Analyses

In total, 233 *AGT1* clones were successfully sequenced for the 16 individuals used in these analyses. The alignment consisted of 686 bp, 20 of which were parsimony informative. There were 30 unique alleles found and only four alleles were shared by multiple individuals. Two alleles were shared by members of *V. sebifera*-SL, two amongst *V. lorentensis*-BL, and one was shared amongst *V. lorentensis*-BL and *V. lorentensis*-SL. The number of alleles per individual varied from 1-4 but most individuals (n=8) contained only 2 alleles. Of the 233 *AGT1* sequences recovered from clones, 90 (38.6%) contained one or more single nucleotide polymorphisms not found in any other cloned sequence of their respective individual specimens. These SNP's were assumed to be due to PCR error and the nucleotide(s) was/were changed in the alignment to the same nucleotide used in equivalent alleles of that individual.

The haplotype network (Figure 4.8) shows 30 unique alleles and contains only a single loop. The node representing *V. sebifera*-BL allele "RS 636-AL-R" connects primarily to other *V. sebifera*-BL alleles and one *V. lorentensis*-SL allele (RS 465-AL-X), which itself connects solely to other *V. lorentensis* individuals. The one loop recovered in

the haplotype network (between AL-N and AL-O) analysis may be due to an ancient recombination event or may be an artifact of PCR mediated recombination (Jumpponen and Johnson 2005, Lahr and Katz 2009).

Discussion

This investigation represents one of the first assessments of infraspecific genetic variation in Myristicaceae (Chapter 1 of this thesis, Degen et al. 2001). To date, investigations of infraspecific genetic variation in neotropical trees have mainly been restricted to questions concerning population genetics (Hamrick et al. 1993, Dick et al. 2003), with few investigations testing taxonomic hypotheses with molecular methods (Duminil et al. 2006). The Myristicaceae, like many other diverse tropical plant families, have received relatively little taxonomic study since the advent of molecular methods. The following discourse outlines the implications of the morphological and molecular investigations with respect to the taxonomic status of *V. sebifera* and *V. lorentensis*.

Morphological Analyses

Observations of *V. sebifera* and *V. lorentensis* taxa in the field suggested that both of these species were either polymorphic, morphologically plastic depending on which habitat they were found in, or contain undescribed species. The multivariate analysis indicates that both *V. sebifera* small and big-leaf morphotypes form practically discrete clusters on each side of the y-axis of the ordination. This suggests that leaf size variation is not continuous between these two morphotypes. Although sample sizes were relatively small, the two morphotypes of *V. lorentensis* also form two discrete clusters on each side of the y-axis. Big-leaf and small-leaf morphotypes of *V. sebifera* and *V. lorentensis* also

grouped together in the ordination of leaf characters. This clustering is of interest since both small-leafed varieties were found growing in terra firme forests and big-leafed varieties were found growing primarily in floodplain forests. The two small-leafed morphotypes were both elliptic-lanceolate in shape with rounded bases and acuminate tips, while the big-leafed morphotypes were oblong in shape and had cordate bases and acute tips. Increased morphological sampling from additional trees and geographic regions would be beneficial to explore whether these morphological patterns between the two habitats are maintained with increased taxon and geographic sampling.

Molecular Analyses

In spite of relatively clear vegetative and reproductive morphological differences among *V. sebifera* and *V. lorentensis* and their respective morphotypes, there was little structure in terms of species and morphotypes in the haplotype network. Six alleles of *V. sebifera*-BL (alleles R-W, Figure 4.8) formed a clade as did 9 *V. lorentensis*-BL and SL alleles (alleles X-AC, Figure 4.8). Aside from these two clades, alleles from both species and leaf types were distributed throughout the remaining network. This may indicate that contemporary gene flow and/or incomplete lineage sorting may be responsible for the lack of resolution between *V. sebifera* and *V. lorentensis* and their respective morphotypes. Gene flow resulting in introgression of alleles among these taxa is probably as interspecific hybridization is thought to be a commonplace among closely related angiosperms (Golden and Bain 2000, Palme et al. 2003 Palma-Silva et al. 2001) and is likely an important mechanism in plant speciation (Whitney et al. 2010, Southcott et al. 2011). Incomplete lineage sorting and/or low nucleotide substitution levels could also be responsible for the lack of resolution in this haplotype network as a previous

phylogenetic analysis of *Virola spp.* found little to no resolution among *V. sebifera*, *V. lorentensis* and closely related species (see Sebiferae clade, Figure 3.16 in Chapter 3 of this thesis).

Hybridization in tropical trees is presumed to be rare (Ashton 1969) but has been observed with morphological and molecular data in paleotropical Dipterocarpaceae (Kamiya et al. 2010) and neotropical Meliaceae (Duminil et al. 2006). In the course of 4 months of field work no apparent big-leaf x small-leaf hybrids were observed in either *V. sebifera* or *V. lorentensis*. It was noted in the field that *Virola lorentensis*-SL had morphological affinities to both *V. sebifera* and *V. lorentensis*. *Virola lorentensis*-SL has leaf size and shape (rounded leaf bases) comparable to *V. sebifera*-SL in addition to similar inflorescences, fruit indument, and tree height, however, *V. lorentensis*-SL has a rich reddish leaf indument and orange aril typical of *V. lorentensis*-BL. Furthermore, both *V. lorentensis* varieties and *V. sebifera*-SL flower at the same time where they were found in South-Eastern Peru so it is conceivable that hybridization could occur. However, of 7 *V. lorentensis*-SL alleles sampled only one (AL-Q) was apparently derived from a *V. sebifera*-SL allele. Although it is possible that *V. lorentensis*-SL is a contemporary hybrid derived from *V. lorentensis*-BL and *V. sebifera*-SL, this may be unlikely given the fact that no sampled alleles were shared between them and few alleles were similar to *V. sebifera*-SL. These potential hybrid collections may merit further investigation as hybridization in tropical trees may be underestimated due to the difficulty in collecting fertile specimens, few morphological differences between species, and general paucity of rigorous taxonomic investigations employing both morphological and molecular data.

It also appears that AGT1 may potentially exist in multiple copies in these *Virola* taxa. Of the 16 trees sampled for molecular analyses, 5 individuals had clones with 3 or 4 alleles (Table 4.1). If alleles differing by only a single nucleotide polymorphism from another allele within the same individual are deleted, then only 3 individuals potentially have multiple copies (RS 480, RS 503, and ML 013). Two of these collections (RS 480 and RS 503) contain alleles that differ by only two polymorphisms from other alleles of the same individual and therefore may be due to PCR error as well. This leaves the single collection from Brazil (ML 013) as an individual with 3 or more alleles that differ by more than two polymorphisms from other alleles in the same individual. It is well known that DNA polymerase error can introduce erroneous bases into PCR amplicons (Ashelford et al. 2005, Dickie 2010, Fazekas et al 2010) and 38.6% of sequenced clones in this study contained suspected erroneous single nucleotide polymorphisms. Since the error rate of Platinum*Taq*® is not advertised, it cannot be ascertained whether this percentage of errors is to be expected. However, a PCR reaction performed using comparable parameters to this study (i.e. 30 cycles, 700 bp fragment, and a *Taq*-based polymerase with an error rate of 2.28×10^{-5}) is expected to produce errors in about 48% of PCR-generated amplicons (Finnzymes PCR fidelity calculator- http://finnzymes.com/pcr/fidelity_calc.php). Although attempts made in this study to use more accurate DNA polymerases were largely ineffectual, it would be preferable to perform additional PCR reactions with a higher fidelity DNA polymerase for subsequent cloning reactions to determine whether the multiple alleles detected are an artifact of polymerase error or due to the existence of multiple copies.

Conclusions

This investigation endeavored to elucidate taxonomic hypotheses in *V. sebifera* and *V. lorentensis* by integrating morphological and molecular data. Although the haplotype analysis was somewhat ambiguous in terms of delimiting the 4 morphotypes studied, the combination of morphological discontinuity among conspecific morphotypes and separation of morphotypes among different edaphic habitats is indicative of multiple unrecognized species. These species are not provisionally described in this study as increased sample sizes, more variable molecular markers, and the study of more type specimens are desired prior to the recognition of putative new species.

The discovery of two novel taxa would be highly significant as both were found at one of the most active research stations in the lowland Amazon (Los Amigos Biological Research Station). These two cryptic taxa differed morphologically and ecologically from their larger-leaved conspecifics yet they were not recognized in ecological plots as distinct taxa. A recent study of *Inga spp.* in South-Eastern Peru found that error rates in the identification of *Inga* taxa were around 7% and significantly impacted the accuracy of some, but not all, ecological conclusions from such data (Dexter et al. 2010). After observing permanent ecological plots in Ecuador and Peru, I estimated that mis-identification rates often exceed 20% for Myristicaceae species if they are identified as the correct family, although high, this is still lower than the estimated 50% mis-identification rate estimated for herbarium material (J. Janovec, personal communication).

This study demonstrates the utility of field observation and integrative taxonomic approaches, both of which will be essential to help discover new species in the highly diverse neotropical region. Identification of Myristicaceae taxa solely by molecular

means will likely prove exceedingly difficult as their plastid DNA exhibits low rates of molecular evolution (Sauquet et al. 2003, chapter 1,2, and 3 of this thesis), more variable nuclear loci may be prone to incomplete lineage sorting, and these species may have rapidly diversified relatively recently as has been found in the speciose genus *Inga* (Richardson et al. 2001). The need for comprehensive studies of tropical flowering plants precludes knowledgeable conservation decisions and sustainable utilization of tropical forests. This study shows that our taxonomic understanding of one of the most widespread and abundant genera of the neotropics is rudimentary at best and that much work remains to be done if we wish to explore the ecological and evolutionary history of this important floristic region.

Tables

Table 4.1 Species collected, collection numbers (Coll. #), Morphotype (type)[BL=Big Leaf, SL=Small Leaf], location (CICRA=Los Amigos Biological Station), decimal degrees latitude (Lat.) and longitude (Long.), herbarium accession numbers (Herb. Acc.), and data set sample was used in (M=molecular, m=morphology, # indicates how many alleles were recovered) for 29 collections employed in this study.

Species	Coll. #	type	Location	Lat.	Long.	Herb. Acc.	Data Set
<i>V. loretensis</i>	RS 480	BL	Peru-CICRA	-12.56	-70.09	OAC 94718	Mm-3
			Peru-Puerto				
<i>V. loretensis</i>	RS 516	BL	Maldonado	-12.72	-69.24	OAC 94754	Mm-2
			Peru-Puerto				
<i>V. loretensis</i>	RS 526	BL	Maldonado	-12.72	-69.24	OAC 94764	Mm-2
<i>V. loretensis</i>	RS 452	SL	Peru-CICRA	-12.56	-70.09	OAC 94690	Mm-3
<i>V. loretensis</i>	RS 465	SL	Peru-CICRA	-12.56	-70.09	OAC 94703	Mm-4
<i>V. loretensis</i>	RS 483	SL	Peru-CICRA	-12.56	-70.09	OAC 94721	Mm-2
<i>V. sebifera</i>	ML 013	BL	Brazil-Brasilia	-15.89	-47.86		M-3
<i>V. sebifera</i>	RS 435	BL	Peru-CICRA	-12.56	-70.09	OAC 94673	M-2
<i>V. sebifera</i>	RS 472	BL	Peru-CICRA	-12.56	-70.09	OAC 94710	m
<i>V. sebifera</i>	RS 496	BL	Peru-CICRA	-12.56	-70.09	OAC 94734	m
			Peru-Puerto				
<i>V. sebifera</i>	RS 503	BL	Maldonado	-12.72	-69.24	OAC 94741	Mm-3
			Peru-Puerto				
<i>V. sebifera</i>	RS 506	BL	Maldonado	-12.72	-69.24	OAC 94744	m
<i>V. sebifera</i>	RS 578	BL	Peru-Quincemil	-13.24	-70.78	OAC 94816	m
<i>V. sebifera</i>	RS 582	BL	Peru-Quincemil	-13.24	-70.78	OAC 94820	Mm-2
<i>V. sebifera</i>	RS 587	BL	Peru-Quincemil	-13.24	-70.78	OAC 94825	m
<i>V. sebifera</i>	RS 611	BL	Peru-Manu	-13.05	-71.53	OAC 94849	m
<i>V. sebifera</i>	RS 618	BL	Peru-Manu	-13.05	-71.53	OAC 94856	m
<i>V. sebifera</i>	RS 624	BL	Peru-Manu	-13.05	-71.53	OAC 94862	m
<i>V. sebifera</i>	RS 636	BL	Peru-Manu	-13.05	-71.53		Mm-2
<i>V. sebifera</i>	RS 434	SL	Peru-CICRA	-12.56	-70.09	OAC 94672	m
<i>V. sebifera</i>	RS 443	SL	Peru-CICRA	-12.56	-70.09	OAC 94681	Mm-2
<i>V. sebifera</i>	RS 444	SL	Peru-CICRA	-12.56	-70.09	OAC 94682	Mm-1
<i>V. sebifera</i>	RS 464	SL	Peru-CICRA	-12.56	-70.09	OAC 94702	Mm-1
			Peru-Puerto				
<i>V. sebifera</i>	RS 513	SL	Maldonado	-12.72	-69.24	OAC 94751	m
<i>V. sebifera</i>	RS 529	SL	Peru-Quincemil	-13.24	-70.78	OAC 94767	Mm-2
<i>V. sebifera</i>	RS 533	SL	Peru-Quincemil	-13.24	-70.78	OAC 94771	M-2
<i>V. sebifera</i>	RS 553	SL	Peru-Quincemil	-13.24	-70.78	OAC 94791	m
<i>V. sebifera</i>	RS 565	SL	Peru-Quincemil	-13.24	-70.78	OAC 94803	m
<i>V. sebifera</i>	RS 602	SL	Peru-Quincemil	-13.24	-70.78	OAC 94840	m

Table 4.2 Correspondence analysis of 10 morphological metrics (minimum and maximum) measured for *V. sebifera* and *V. lorentensis* taxa. Bolded Pearson correlations (P Corr.) indicate the 4 metrics most significant to the differentiation of taxa (p value < 0.01).

Metric	X-axis		Y-axis	
	P Corr.	Sig. (2-tailed)	P Corr.	Sig.(2-tailed)
Stem width-min.	-0.577	0.002	0.496**	0.010
Stem width-max	-0.494	0.010	0.565**	0.003
Petiole length-min	-0.574	0.002	0.126	0.540
Petiole length-max	-0.683	0.000	0.238	0.242
Leaf Petiole width-min	-0.792	0.000	0.334	0.096
Leaf Petiole width-max	-0.792	0.000	0.354	0.076
Lamina Length-min	-0.966**	0.000	-0.074	0.718
Lamina Length-max	-0.959**	0.000	-0.098	0.635
Leaf width ¼ length-min	-0.841	0.000	0.020	0.922
Leaf width ¼ length-max	-0.871	0.000	0.197	0.336
Leaf width ½ length-min	-0.914	0.000	-0.134	0.515
Leaf width ½ length-max	-0.940**	0.000	-0.064	0.756
Leaf width ¾ length-min	-0.873	0.000	-0.287	0.155
Leaf width ¾ length-max	-0.921	0.000	-0.264	0.193
Leaf vein number-min	-0.917	0.000	0.282	0.163
Leaf vein number-max	-0.927**	0.000	0.220	0.280
Leaf vein space-min	-0.458	0.019	0.067	0.745
Leaf vein space-max	-0.360	0.071	-0.090	0.661
Leaf acumen length-min	-0.133	0.517	-0.574**	0.002
Leaf acumen length-max	-0.134	0.515	-0.703**	0.000

Figures

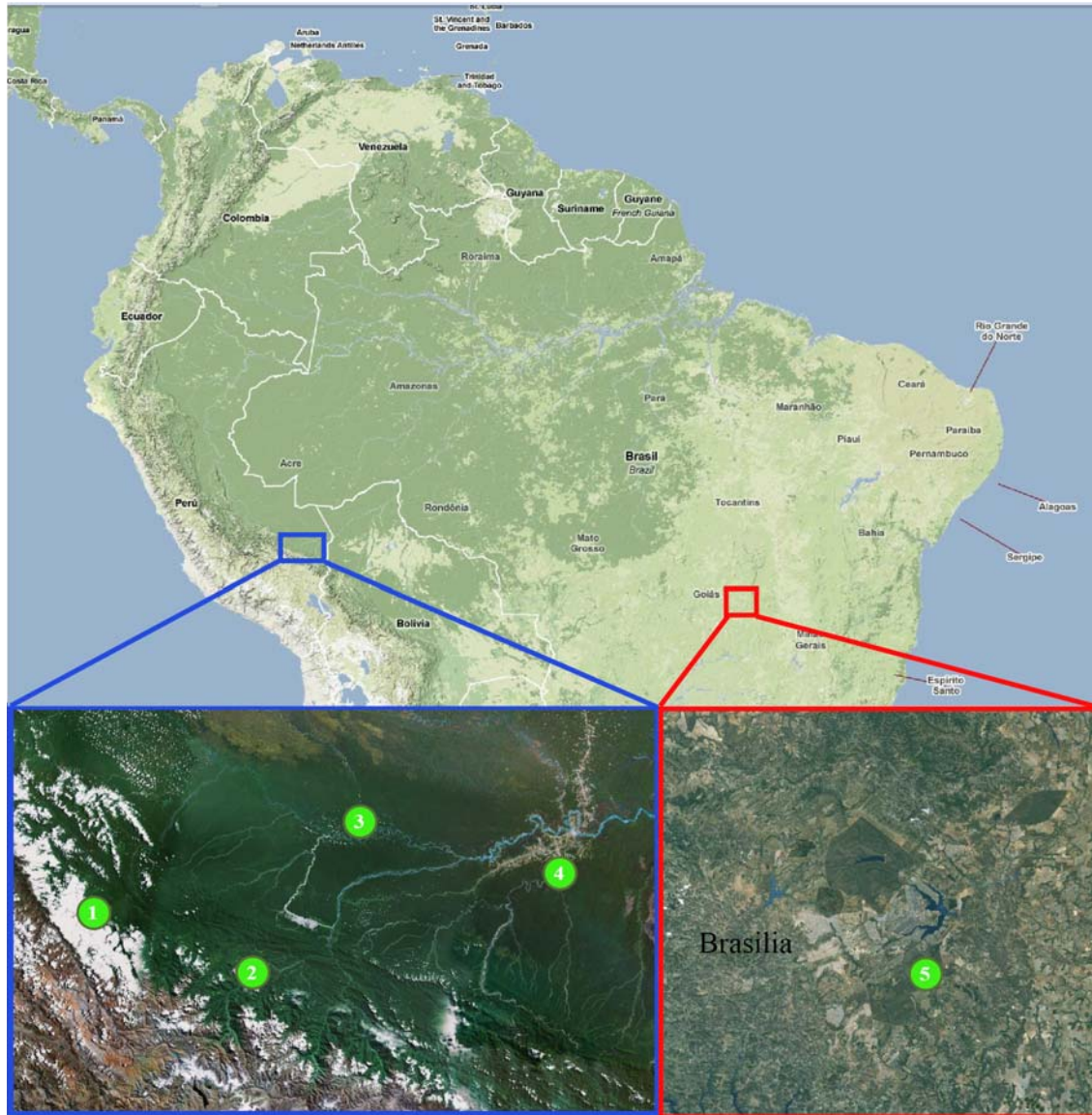


Figure 4.1 Collection locations in Peru ($n=4$) and Brazil ($n=1$): 1) Manu Paradise Lodge, 2) Quincemil, 3) CICRA-Los Amigos Biological Reserve, 4) Puerto Maldonado, 5) Brasilia-Reserva Ecologia do IBGE.

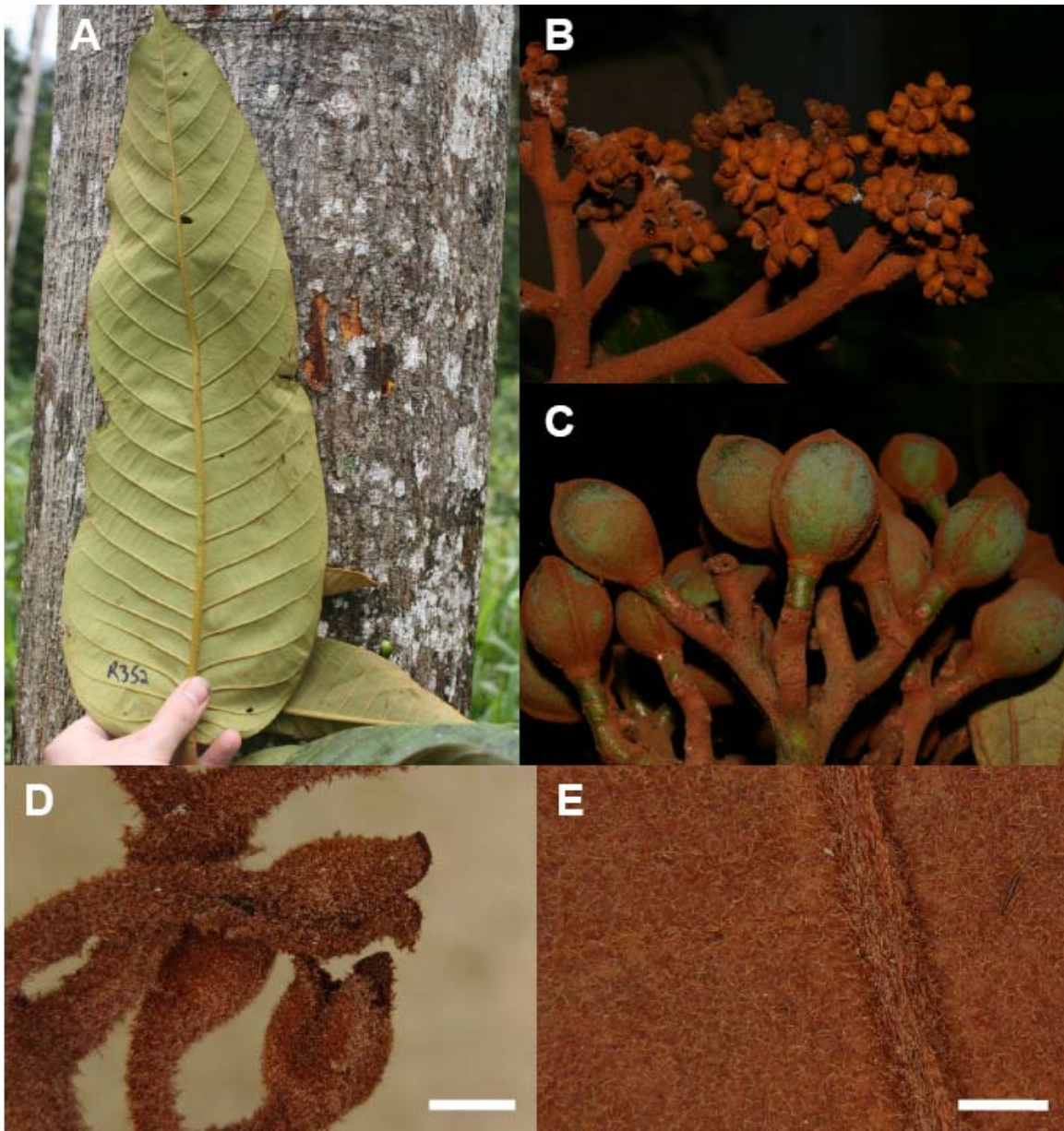


Figure 4.2 *Virola sebifera*-BL (Big Leaf morphotype): A) Bark with leaf showing abaxial surface. B) Mature pistillate inflorescence. C) Nearly mature infructescence showing deciduous red pubescence. D) Close-up of staminate flowers (scale bar= 1 mm). E) Abaxial leaf pubescence and secondary vein (scale bar= 1 mm).

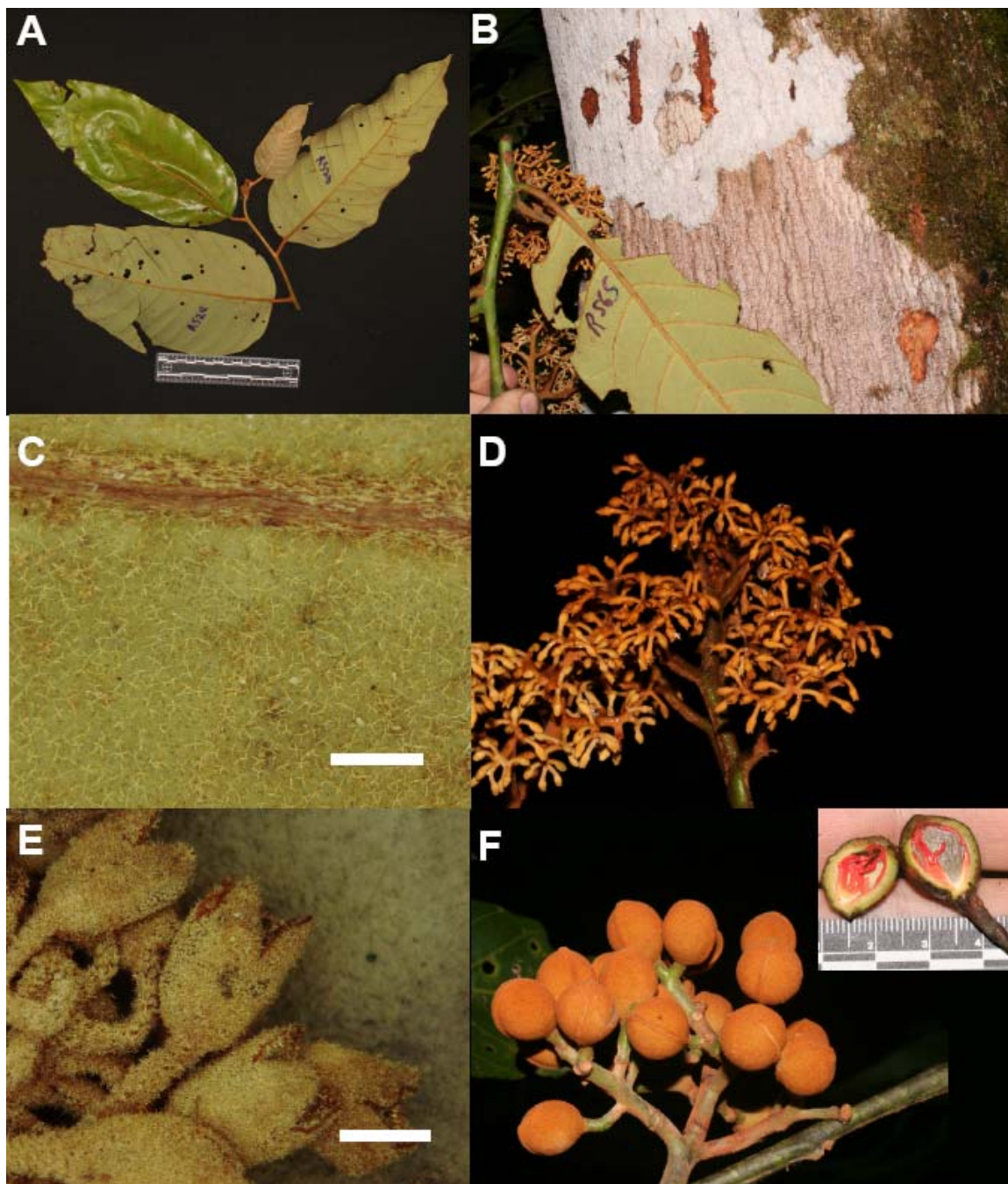


Figure 4.3 *Virola sebifera*-SL (Small Leaf morphotype): A) Leaves with orange-red pubescence (15 cm ruler) B) Bark, leaves and inflorescence of mature tree. C) Abaxial leaf pubescence (scale bar= 1 mm). D) Inflorescence. E) Flowers (scale bar= 1 mm). F) Nearly mature fruits with dissected fruit showing seed testa and aril (inset).

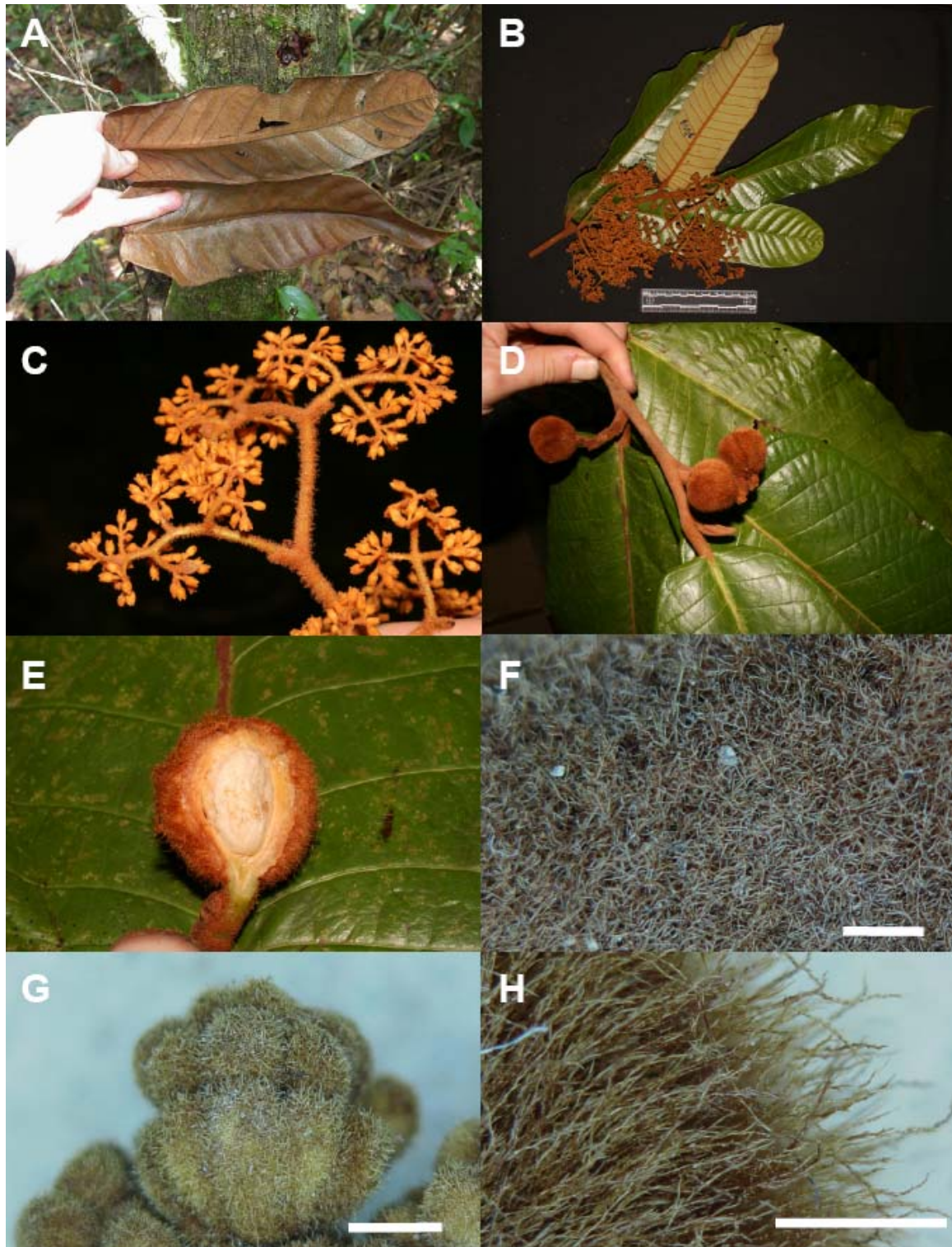


Figure 4.4 *Virola lorentensis*-BL (Big Leaf morphotype): A) Bark, deep red latex and dried (dropped) leaves. B) Abaxial, adaxial leaf surfaces of sample with inflorescences (15 cm ruler). C) Close-up of inflorescence. D) Branch with mature fruits. E) Dissected

immature fruit showing dense pubescence. F) Adaxial leaf pubescence. G) Developing flower buds (scale bar=1 mm). H) Close-up of fruit indument (scale bar= 1 mm).

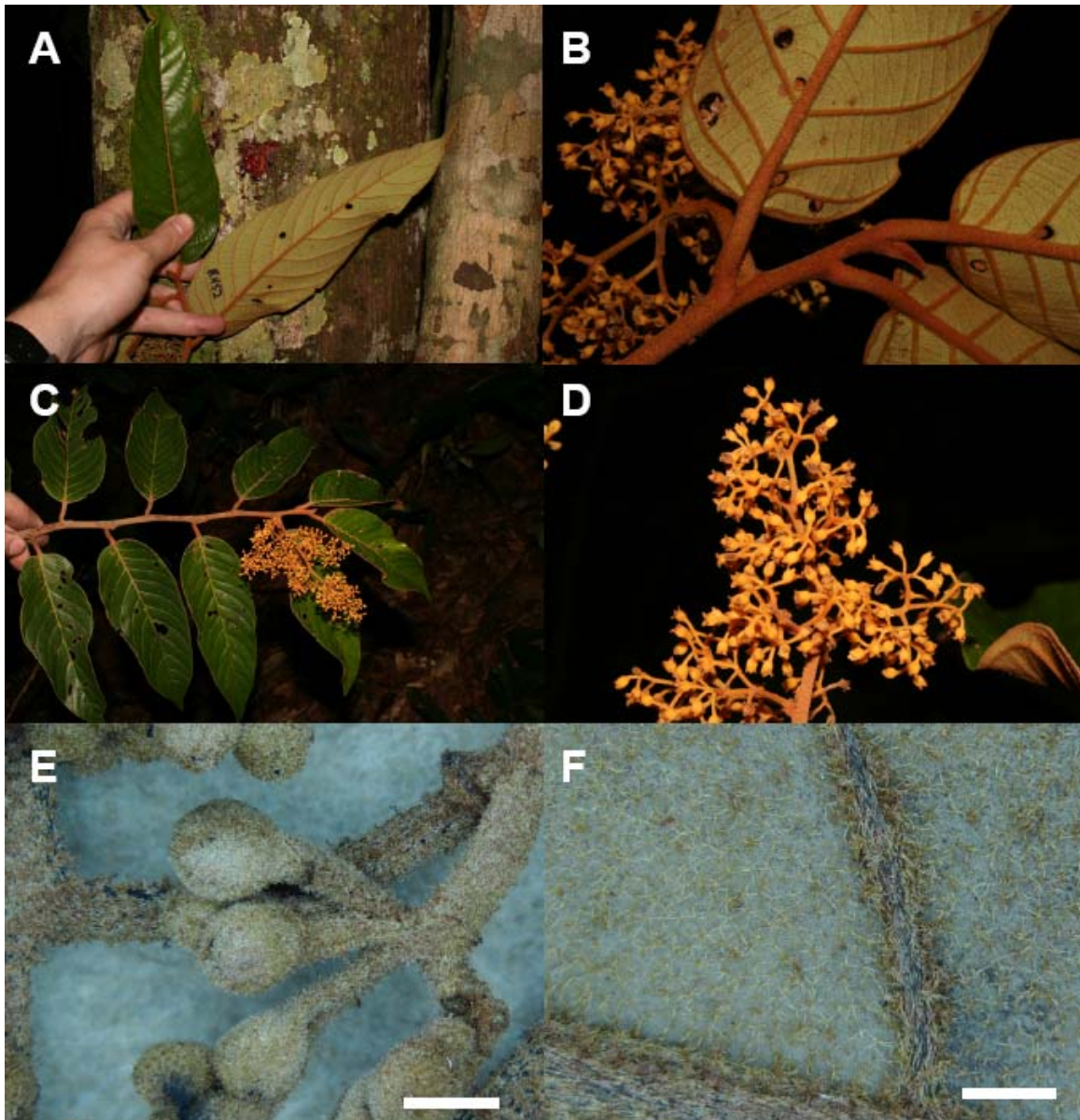


Figure 4.5 *Virola lorentensis*-SL (Small Leaf morphotype): A) Bark, leaves and red latex. B) Adaxial leaf surfaces with inflorescence. C) Branch with inflorescence. D) Close-up of mature inflorescence. E) Flower pubescence (scale bar= 1 mm). F) Adaxial leaf pubescence (scale bar= 1 mm).

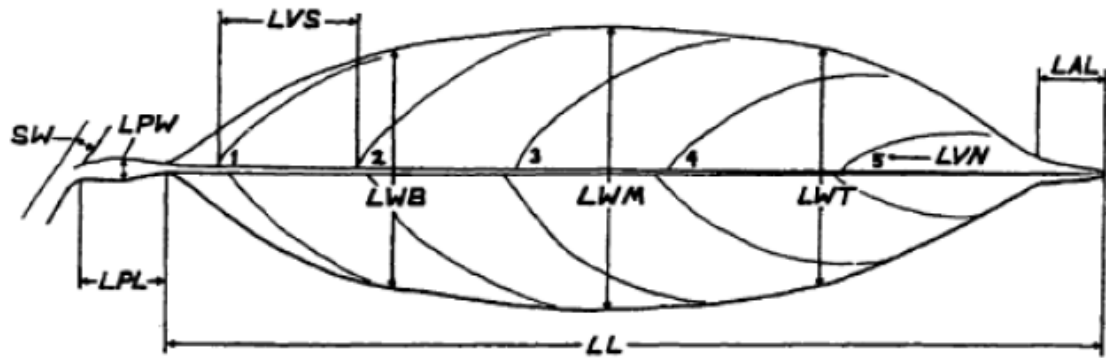


Figure 4.6 Illustration of 10 vegetative morphological metrics measured: SW=Stem Width, LPL=Petiole Length, LPW=Petiole Width, LVS=space between secondary veins, LVN=Vein Number, LL=Lamina Length, LWB= Leaf Width at $\frac{1}{4}$ length, LWM=Leaf Width at $\frac{1}{2}$ length, LWT=Leaf Width at $\frac{3}{4}$ length, LVN=Leaf Vein Number, LAL=Leaf Acumen Length. A maximum and minimum value of each metric was recorded for every specimen measured.

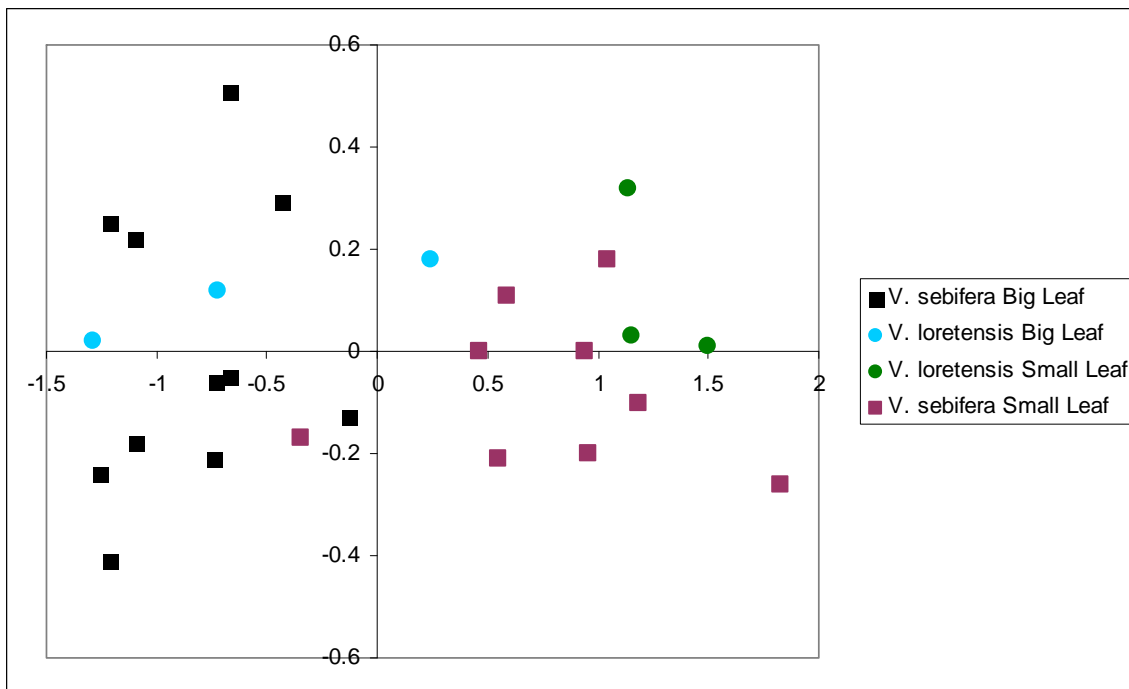


Figure 4.7 NMS ordination of quantitative leaf metrics of *V. sebifera* and *V. lorentensis* morphotypes.

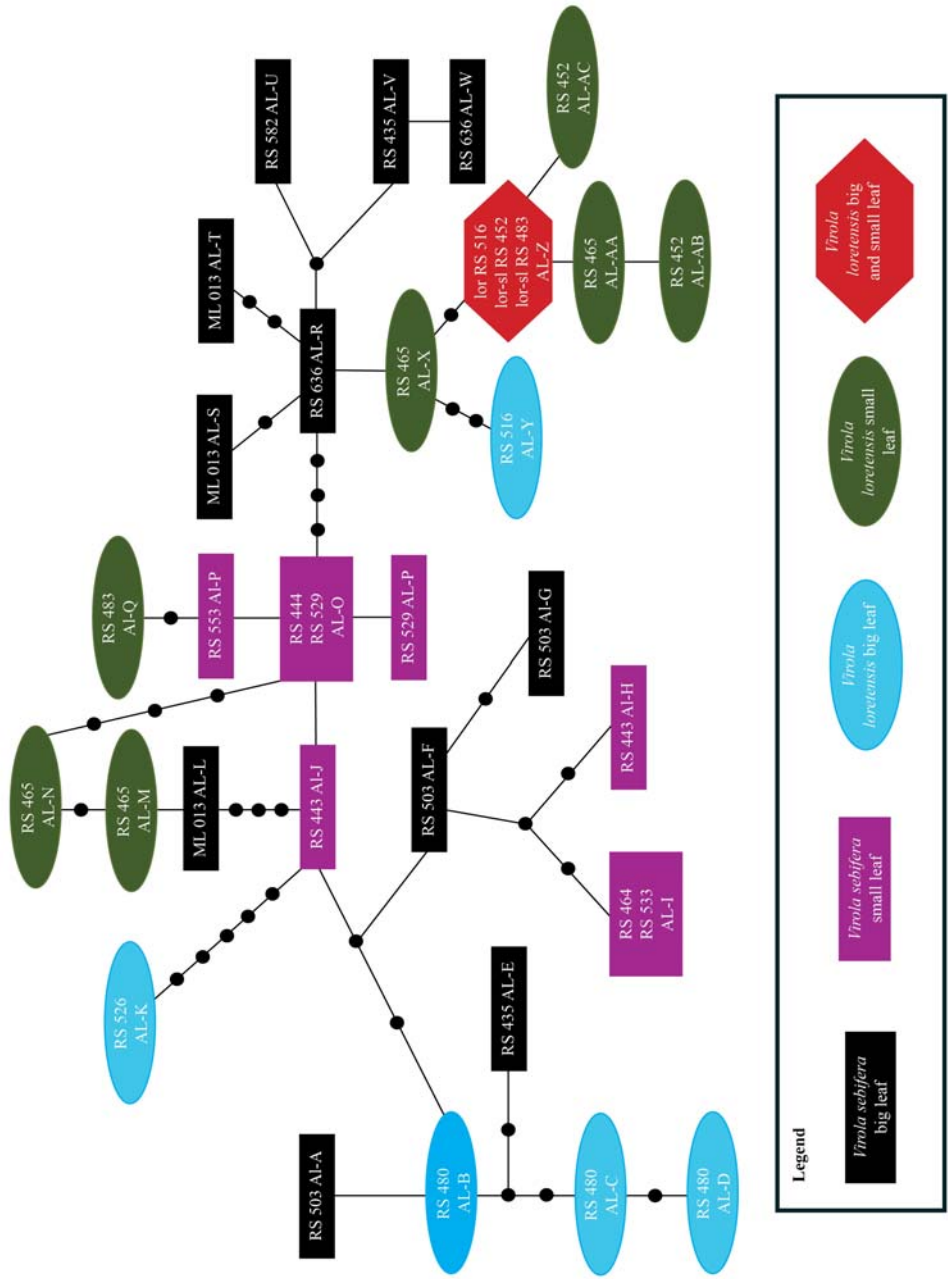


Figure 4.8 Haplotype network of 35 sequences from *V. sebifera* and *V. loretensis* clones. Legend indicates the shapes and colours used for each of the morphotypes and the one shared allele.

Literature Cited

- Ashelford, K. E., Chuzhanova, N. A., Fry, J. C., Jones, A. J., & Weightman, A. J. 2005. At least 1 in 20 16S rRNA sequence records currently held in public repositories is estimated to contain substantial anomalies. *Applied and Environmental Microbiology* **71**: 7724-7736.
- Ashton, P. S. 1969. Speciation among tropical forest trees: some deductions in the light of recent evidence. *Biological Journal of the Linnean Society* **1**: 155–196.
- Asif, M. J., & Cannon, C. H. 2005. DNA extraction from processed wood: a case study for the identification of an endangered timber species (*Gonystylus bancanus*). *Plant Molecular Biology Reporter* **23**: 185–192.
- Clement, M., Posada, D., & Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657–1659.
- Degen, B., Caron, H., Bandou, E., Maggia, L., Chevallier, M. H., Leveau, A., & Kremer, A. 2001. Fine-scale spatial genetic structure of eight tropical tree species as analysed by RAPDs. *Heredity* **87**: 497–507.
- Dexter, K. G., Pennington, T. D., & Cunningham, C. W. 2010. Using DNA to assess errors in tropical tree identifications: How often are ecologists wrong and when does it matter? *Ecological Monographs* **80**: 267–286.
- Dick, C. W., Abdul-Salim, K., & Bermingham, E. 2003. Molecular systematic analysis reveals cryptic tertiary diversification of a widespread tropical rain forest tree. *American Naturalist* **162**: 691–703.
- Dickie, I. A. 2010. Insidious effects of sequencing errors on perceived diversity in molecular surveys. *New Phytologist* **188**: 916-918.
- Dodson, C. H., & Gentry, A. H. 1991. Biological extinction in Western Ecuador. *Annals of the Missouri Botanical Garden* **78**: 273-295.
- Duminil, J., Caron, H., Scotti, I., CAZAL, S. O., & Petit, R. J. 2006. Blind population genetics survey of tropical rainforest trees. *Molecular Ecology* **15**: 3505–3513.
- Gentry, A. H. 1982. Neotropical floristic diversity: phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden* **69**: 557–593.
- Gentry, A. H. 1988. Tree species richness of upper Amazonian forests. *Proceedings of the National Academy of Sciences of the United States of America* **85**: 156-159.

- Gentry, A. H. 1992. Tropical forest biodiversity: distributional patterns and their conservational significance. *Oikos* **63**: 19–28.
- Gottlieb, O., Loureiro, A., Dos Santo Carneiro, M., & Da Rocha, A. 1973. Distribution of diarylpropanoids in Amazonian *Virola* species. *Phytochemistry* **12**: 1830.
- Golden, J. L., & Bain, J. F. 2000. Phylogeographic patterns and high levels of chloroplast DNA diversity in four *Packeria* (Asteraceae) species in southwestern Alberta. *Evolution* **54**: 1566–1579.
- Govaerts, R. 2003. How many species of seed plants are there?-a response. *Taxon* **52**: 583-584.
- Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series*. (pp. 95–98).
- Hamrick, J. L., Murawski, D. A., & Nason, J. D. 1993. The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. *Plant Ecology* **107**: 281–297.
- Jaramillo, T. S., Muriel, P., Rodrigues, W. A., & Balslev, H. 2000. Myristicaceae novelties from Ecuador. *Nordic Journal of Botany* **20**: 443–447.
- Joly, S., Starr, J. R., Lewis, W. H., & Bruneau, A. 2006. Polyploid and hybrid evolution in roses east of the Rocky Mountains. *American Journal of Botany* **93**: 412-425.
- Jumpponen, A., & Johnson, L. C. 2005. Can rDNA analyses of diverse fungal communities in soil and roots detect effects of environmental manipulations—a case study from tallgrass prairie. *Mycologia* **97**: 1177-1194.
- Kamiya, K., Gan, Y. Y., Lum, S. K. Y., Khoo, M. S., Chua, S. C., & Faizu, N. N. H. 2010. Morphological and molecular evidence of natural hybridization in *Shorea* (Dipterocarpaceae). *Tree Genetics & Genomes*. Retrieved from <http://www.springerlink.com/index/10.1007/s11295-010-0332-8>
- Kruskal, J. B. 1964. Nonmetric multidimensional scaling: a numerical method. *Psychometrika* **29**: 115–129.
- Lahr, D. J., & Katz, L. A. 2009. Reducing the impact of PCR-mediated recombination in molecular evolution and environmental studies using a new-generation high-fidelity DNA polymerase. *BioTechniques* **47**: 351-357.
- Li, M., Wunder, J., Bissoli, G., Scarponi, E., Gazzani, S., Barbaro, E., Saedler, H., & Varotto, C. 2008. Development of COS genes as universally amplifiable markers for phylogenetic reconstructions of closely related plant species. *Cladistics* **24**: 727–745.

- Minchin, P. R. 1987. An evaluation of the relative robustness of techniques for ecological ordination. *Plant Ecology* **69**: 89–107.
- Panchal, M. 2007. The automation of nested clade phylogeographic analysis. *Bioinformatics* **23**: 509-510.
- Pascal, J. P., & Pelissier, R. 1996. Structure and floristic composition of a tropical evergreen forest in south-west India. *Journal of Tropical Ecology* **12**: 191–214.
- Pitman, N. C., Mogollón, H., Dávila, N., Ríos, M., García-Villacorta, R., Guevara, J., Baker, T. R., Monteagudo, A., Phillips, O. L., Vásquez-Martínez, R., & others. 2008. Tree community change across 700 km of lowland Amazonian forest from the Andean foothills to Brazil. *Biotropica* **40**: 525–535.
- Pitman, N. C., Terborgh, J. W., Silman, M. R., Núñez V, P., Neill, D. A., Cerón, C. E., Palacios, W. A., & Aulestia, M. 2002. A comparison of tree species diversity in two upper Amazonian forests. *Ecology* **83**: 3210–3224.
- Pitman, N. C., Terborgh, J. W., Silman, M. R., Núñez V, P., Neill, D. A., Cerón, C. E., Palacios, W. A., & Aulestia, M. 2001. Dominance and distribution of tree species in upper Amazonian terra firme forests. *Ecology* **82**: 2101–2117.
- Poinar, H. N. 1998. Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* **281**: 402-406.
- Posada, D., & Crandall, K. A. 2001. Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology & Evolution* **16**: 37–45.
- Prance, G. T. 1972. Ethnobotanical notes from Amazonian Brazil. *Economic Botany* **26**: 221–237.
- ter Braak, C. 1998. *Canoco 4 Centre for Biometry*. Wageningen, The Netherlands
- Richardson, J. E. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. *Science* **293**: 2242-2245.
- Rodrigues, W. A. 2002. Notas taxonômicas sobre Myristicaceae neotropicais. *Acta Biológica Paranaense* **31**: 71-77.
- Rodrigues, W. A. 1980. Revisão taxonômica das espécies de *Virola* Aublet (Myristicaceae) do Brasil. *Acta Amazonica (suplemento)*: 1-127.
- Rodrigues, W. A. 1989. Two new neotropical species of *Compsonaura* (Myristicaceae). *Brittonia* **4**: 160-163.
- Rohlf, F. J. 2006. *tpsDig version 2.10, Department of Ecology and Evolution*. State University of New York at Stony Brook, New York.

- Sabatier, D. 1997. Description et biologie d'une nouvelle espèce de *Viola* (Myristicaceae) de Guyane. *Adansonia, Série 3*: 273–278.
- Sasaki, Y., Miyoshi, D., & Sugimoto, N. 2006. Effect of molecular crowding on DNA polymerase activity. *Biotechnology Journal* **1**: 440–446.
- Sauquet, H., Doyle, J. A., Scharaschkin, T., Borsch, T., Hilu, K. W., Chatrou, L. W., & Le Thomas, A. 2003. Phylogenetic analysis of Magnoliales and Myristicaceae based on multiple data sets: implications for character evolution. *Botanical Journal of the Linnean Society* **142**: 125–186.
- Schultes, R. E., & Raffauf, R. F. 1990. *The Healing Forest*. Portland, Oregon: Dioscorides Press.
- Scotland, R. W., & Wortley, A. H. 2003. How many species of seed plants are there? *Taxon* **52**: 101–104.
- Southcott, L., & K.L. Ostevic. 2011. Bromeliad population genetics reveals species cohesion against the odds. *Molecular Ecology* **20**: 3081-3083
- Smith, A. C. 1937. The American species of Myristicaceae. *Brittonia* **2**: 393-510.
- Speiss, A., T. Mueller, & Ivell, R. 2004. Trehalose is a potent PCR enhancer: lowering of DNA melting temperature and thermal stabilization of Taq polymerase by the disaccharide trehalose. *Clinical chemistry* **50**: 1256-1259.
- Templeton, A. R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology* **7**: 381–397.
- Templeton, A. R., Crandall, K. A., & Sing, C. F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**: 619-633.
- ter Braak, C. 1998. *Canoco 4 Centre for Biometry*. Wageningenm, The Netherlands.
- Thomas, W. W. 1999. Conservation and monographic research on the flora of Tropical America. *Biodiversity and Conservation* **8**: 1007–1015.
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673-4680.
- Whitney, K.D., Randell, R.A., & L.H. Rieseberg. 2010. Adaptive introgression of abiotic tolerance traits in the sunflower *Helianthus annuus*. *New Phytologist* **187**: 230-239.

Zimmerman, S. B., & Harrison, B. 1987. Macromolecular crowding increases binding of DNA polymerase to DNA: an adaptive effect. *Proceedings of the National Academy of Sciences of the United States of America* **84**: 1871-1875.

CONCLUDING STATEMENTS

This research represents a long overdue molecular and morphological revision of two of the most elemental genera of neotropical forests. The following remarks summarize the implications of my findings as they relate to future research and conservation efforts in the tropics.

This work is also among the first tests of DNA barcoding in a diverse group of plants (Spooner 2009). Most plant DNA barcoding projects to date have focused on local/regional floras and have included few congeneric species and have rarely included multiple collections from across a species' range (Lahaye et al. 2008, Kress et al. 2008). This approach could be likened to that of assembling a flora, or regional checklist, as compared to that of a monograph that focuses on taxonomy. There are a number of reasons why a "flora-like" approach has been applied with plants. Firstly, plant DNA barcoders have been somewhat hindered by low mitochondrial and chloroplast DNA sequence divergence and have spent much time converging on a set of loci that are to be employed as the core plant DNA barcodes (Hollingsworth et al 2011). Furthermore, sister-species are often not sympatric so little emphasis has been put on thorough taxon sampling.

Although most members of *Compsooneura* could be indentified by the trnH-psbA region (Chapter 2 of this thesis), the low levels of plastid sequence variability in *Virola* currently makes molecular identification difficult (Chapter 3 of this thesis) and plant DNA barcoding efforts are likely to be hindered by haplotype sharing among species (Hollingsworth et al. 2011). Consequently, species rich groups such as *Virola* may require multiple rapidly evolving loci for molecular discrimination. Similar levels of

variation within and among described species in Myristicaceae also confounds molecular species delimitation via barcodes. In my opinion, future plant DNA barcoding efforts should take on a molecular-monographic approach where described taxa are tested with dense taxon sampling across a species' known range and rigorously combined with additional data sources (morphological, edaphic, environmental, ethnobotanical, etc) for an integrative taxonomic approach.

Thomas (1999) found that 29% of species included in recent monographs were newly described, causing him to estimate that the South American flora contains approximately 90,000 species of seed plants. A total of 4 provision new species and one resurrection are presented within this thesis entirely from collections made by myself. I find this highly significant as I collected in only 7 locations, most of which were amongst the most active research stations in the Amazon. Furthermore, these new species were often among the most abundant trees in these forests (R. Steeves and J. Janovec, unpublished data). In my investigations of the infraspecific morphological and molecular variation in *Compsonera capitellata*, *Virola sebifera*, and *V. lorentensis* two provisional new species and possibly four or more, were found within these widespread species. It is likely that a great deal of additional species remain to be documented in these and other genera of Myristicaceae, as well as other tropical plant families. If future investigations of infraspecific molecular diversity discover a similar rate of cryptic species we may have to recalculate our estimates of neotropical plant diversity. It is likely that we may find significantly more than Thomas' (1999) estimate as a study of herbarium collection patterns in Peru done by Tobler et al.(2007) found that the vast majority of collections were made near very few localities that were close to roads and cities, leaving much of

the Peruvian rainforest unexplored by botanists.

According to most estimates, well over half of our medicinal drugs come either directly or indirectly from plants. With roughly a quarter to a third of the world's plant diversity, the neotropics offers enormous prospects for the discovery of new drugs for implementation in Western medicinal practice. However, this great diversity can also be a hindrance to bio-prospecting in the region. Species, genera and even families are often mis-identified and taxon identification are often inconsistent even with the same collector (R. Steeves, personal observations). This means that even if desirable properties are found in a particular plant extract, subsequent expeditions often collect a different species with correspondingly dissimilar, and potentially ineffective, chemical constitution. When DNA identification is possible in tropical plants it will undoubtedly help collectors ensure their identifications are consistent.

My research forms a much needed foundation for future investigations aimed at understanding the neotropical Myristicaceae and the Amazon as a whole. This work represents only the beginning of a modern systematic revision of the genera *Compsonaura* and *Virola*. To these ends, this investigation generated about 600 novel collections of Myristicaceae from the Northwest Amazon and produced about as many DNA nucleotide sequences from coding and non-coding regions. Future research should attempt to resolve evolutionary relationships amongst the genera of the Myristicaceae, which has remained largely unresolved due to low levels of nucleotide variation and the difficulty of extracting and amplifying DNA from samples. Although low levels of sequence divergence were found in this study, the low copy nuclear and non-coding chloroplast regions employed in this study show increased sequence divergence

compared to those used in previous investigations (Sauquet et al 2003). Additionally, the difficulty of extracting DNA from both fresh and archived (Herbarium) specimens was found to be greatly alleviated by the use of PTB (Phenacylthiazolium bromide) and the use of PCR enhancers such as trehalose and polypropylene glycol. Future investigations should seek to determine the causes of chloroplast haplotype sharing among species and illuminate phylogeographic patterns in medicinal and edible members of the Myristicaceae as well as other families as the possibility exists that humans played a pivotal role in the determination of tree distributions in the Amazon basin.

Literature Cited

- Hollingsworth, P. M., Graham, S. W., & Little, D. P. 2011. Choosing and using a plant DNA barcode. *PloS one* **6**: e19254.
- Kress, W. J., Erickson, D. L., Jones, F. A., Swenson, N. G., Perez, R., Sanjur, O., & Bermingham, E. 2009. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proceedings of the National Academy of Sciences* **106**: 18621-18626.
- Lahaye, R., Van Der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T. G., & Savolainen, V. 2008. DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences* **105**: 2923-2928.
- Spooner, D. M. 2009. DNA barcoding will frequently fail in complicated groups: An example in wild potatoes. *American Journal of Botany* **96**: 1177-1189.
- Thomas, W. W. 1999. Conservation and monographic research on the flora of Tropical America. *Biodiversity and Conservation* **8**: 1007-1015.
- Tobler, M., Honorio, E., Janovec, J., & Reynel, C. 2007. Implications of collection patterns of botanical specimens on their usefulness for conservation planning: an example of two neotropical plant families (Moraceae and Myristicaceae) in Peru. *Biodiversity and Conservation* **16**: 659-677.

APPENDICES

Appendix 1

Alignment 1.1

Concatenated DNA nucleotide sequence alignment 3-loci, 1464 characters, and 17 taxa used in Bayesian and Maximum parsimony analyses in chapter 1. 50 base pairs per line.

trnH-psba 1-342, AGT1 343-1052, AT103 1053-1464

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C_atopa_1374   TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTACTAGT
C_cap_RS_527   TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTCGATTACTAGT
C_cap_RS_551   TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTCGATTACTAGT
C_cap_835      TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTACTAGT
C_debilis_7209 TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTACTAGT
C_excelsa_671  TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTACTAGT
C_mexicana_362 TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTACTAGT
C_mexicana_720 TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTACTAGT
C_mutisii_914  TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTACTAGT
C_mutisii_1295 TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTACTAGT
C_sprucei_817  TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTACTAGT
C_sprucei_887  TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTATAGATTACTAGT
C_ulei_88      TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTACTAGT
C_ulei_6192    TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTACTAGT
I_juru_451     TCCGCCCTTGTCTTTCTTTTCTAAAGACAAAAATTTTAGATTACTAGT
I_laev_460     TCCGCCCTTGTCTTTCTTTTCTAAAGACAAAAATTTTAGATTACTAGT
O_parvi_RS_598 TCCGCCCTTGTCTTTCTG-----AAAGACTAAAATTTTAGATTACTAGT

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C_atopa_1374   CTTTCTTATTTTTTTCATACAAATTTCTACCCTTTAGAAAATCGACAATA
C_cap_RS_527   CTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATCGACAATA
C_cap_RS_551   CTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATCGACAATA
C_cap_835      TTTTCTTATTTTTTTCATACAAATTTCTACCCTTTAGAAAATCGACAATA
C_debilis_7209 CTTTCTTATTTTTTTCATACAAATTTCTATCCTTTAGAAAA----CAATA
C_excelsa_671  CTTTCTTATTTTTTTCATACAAATTTCTACCCTTTAGAAAA----CAATA
C_mexicana_362 CTTTCTTATTTTTTTCATACAAATTTCTACCCTTTAGAAAA----CAATA
C_mexicana_720 CTTTCTTATTTTTTTCATACAAATTTCTACCCTTTAGAAAA----CAATA
C_mutisii_914  CTTTCTTATTTTTTTCATACAAATTTTATACCCTTTAGAAAA----CAATA
C_mutisii_1295 CTTTCTTATTTTTTTCATACAAATTTTATACCCTTTAGAAAA----CAATA
C_sprucei_817  CTTTCTTATTTTTTTCATACAAATTTCTACCCTTTGAAAA----CAATA
C_sprucei_887  CTTTCTTATTTTTTTCATACAAATTTCTACCCTTTAGAAAA----CAATA
C_ulei_88      CTTTCTTATTTTTTTCATACAAATTTCTACCCTTTAGAAAA----CAATA
C_ulei_6192    CTTTCTTATTTTTTTCATACAAATTTCTACCCTTTAGAAAA----CAATA
I_juru_451     CTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATTGACAATA
I_laev_460     CTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATTGACAATA
O_parvi_RS_598 CTTTCTTATTTTTTTCATACTAATTTCTACCCTTTCTAAAATTTACAATA

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C_atopa_1374   GGAAAAAATGCATTTTAGGAATGTACATGAACTGAAGATCAGTTCAAATC
C_cap_RS_527   GGAAAAAATGCATTTTAGGAATGTACATGAACTGAAGATCAGTTCAAATC
C_cap_RS_551   GGAAAAAATGCATTTTAGGAATGTACATGAACTGAAGATCAGTTCAAATC
C_cap_835      GGAAAAAATTCATTTTAGGAATGTACATGAACTGAAGATCAGTTCAAATC
C_debilis_7209 GGAAAAAATGCATTTTAGGAATGTACATAAACTGAAGATCCGTTAAAATC
C_excelsa_671  GGAAAAAATGCATTTTAGGAATGTACATAAACTGAAGATCAGTTAAAATA
C_mexicana_362 GGAAAAAATGCATTTTAGGAATGTACATAAACTGAAGATCAGTTAAAATA

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C_mexicana_720 GGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGTTAAAATA
 C_mutisii_914 GGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGTTAAAATA
 C_mutisii_1295 GGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGTTAAAATA
 C_sprucei_817 GGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGTTAAAATA
 C_sprucei_887 GGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGTTAAAATA
 C_ulei_88 GGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGTTAAAATA
 C_ulei_6192 GGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGTTAAAATA
 I_juru_451 GGAAAAAATGCATTTT TGGGAATGTACATAAACTGAAGATCAGTTAAAATA
 I_laev_460 GGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGTTAAAATA
 O_parvi_RS_598 GGAAAAAAT----T TTTTGGGAATGTACATAAAACAGAAGATCAGTTAAAAC

C_atopa_1374 AAAAAAA-----GGTATGATGTTTCGATTATGAACCAAATAATTCATA
 C_cap_RS_527 AAAAAAA-----GGTATGATGTTTCGATTATGAACCAAATAATTCATA
 C_cap_RS_551 AAAAAAA-----GGTATGATGTTTCGATTATGAACCAAATAATTCATA
 C_cap_835 AAAAAAA-----GGTATGATGTTTCGATTATGAACCAAATAATTCATA
 C_debilis_7209 AAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATTAATA
 C_excelsa_671 AAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATTAATA
 C_mexicana_362 AAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATTAATA
 C_mexicana_720 AAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATTAATA
 C_mutisii_914 AAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATTCATA
 C_mutisii_1295 AAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATTAATA
 C_sprucei_817 AAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATGAATA
 C_sprucei_887 AAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATTAATA
 C_ulei_88 AAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATTAATA
 C_ulei_6192 AAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATTAATA
 I_juru_451 AAAATAAAAAAAAAAAGGTATGATGTTTCGATCATGAA-CAAA-AATGAATA
 I_laev_460 AAAAAAAA-----GGTATGATGTTTCGATCATGAA-CAAA-AATGAATA
 O_parvi_RS_598 AAAAAAA-----GGTATGATGTTTCGATCCTGAACCAACTAATTAATA

C_atopa_1374 TTTTCTGAAATTGAAAAAAAAA-TCTTATGTGAGTAAACCACTACTGAAC
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 C_cap_RS_551 TTTTCTGAAATTGAAAAAAAAA-CAAATCTTATGTGAGTAAACCACTACTGAAC
 C_cap_835 TTTTCTGAAATTGAAAAAAAAA-TCTTATGTGAGTAAACCACTACTGAAC
 C_debilis_7209 TTTTCTGAAATTGAAAAAAAAA-TCTTATGTGAGTAAACCACTACTGAAC
 C_excelsa_671 TTTTCTGAAATGGAAAAAAAAA--TCTTATGTGAGTAAACCACTACTGAAC
 C_mexicana_362 TTTTCTGAAATGGAAAAAAAAA-TCTTATGTGAGTAAACCACTACTGAAC
 C_mexicana_720 TTTTCTGAAATGGAAAAAAAAA--TCTTATGTGAGTAAACCACTACTGAAC
 C_mutisii_914 TTTTCTTAAATGGAAAAAAAAA--TCTTATGTGAGTAAACCACTACTGAAC
 C_mutisii_1295 TTTTCTTAAATGGAAAAAAAAA--TCTTATGTGAGTAAACCACTACTGAAC
 C_sprucei_817 TTTTCTTAAATGGAAAAAAAAA--TCTTATGTGAGTAAACCACTACTGAAC
 C_sprucei_887 TTTTCTTAAATGGAAAAAAAAA--TCTTATGTGAGTAAACCACTACTGAAC
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 I_juru_451 TTTTCTTAAATTGAAAAAAAAAATCTTATGTGAGTAAACCACTACTGAAC
 I_laev_460 TTTTCTTAAATTGAAAAACAAATCTTATGTGAGTAAACCACTACTGAAC
 O_parvi_RS_598 TTTTTTAAAATTGAAAAAAAAAATCTTATGTGAGTAAACCACTACTGAAA

C_atopa_1374 CAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCGTATACA
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 C_cap_RS_551 CAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCGTATACA
 C_cap_835 CAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCGTATACA
 C_debilis_7209 CAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCGTATACA
 C_excelsa_671 CAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCGTATACA
 C_mexicana_362 CAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCGTATACA
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 C_mutisii_914 CAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCGTATACA
 C_mutisii_1295 CAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCGTATACA

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 I_laev_460 TCCAAGACTGCTAAATCAGTCAGAGTATTCTTTGACTGGAAAGACTACCT
 O_parvi_RS_598 TCCAAGACTGCTAAATCAGTCAGAGTATTCTTTGACTGGAAAGACTACCT

C_atopa_1374 NNN
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 C_cap_RS_551 GAAGTTCTATAANTTGGGAACATATTGGCCCTACACACCTTCTATAACAAC
 C_cap_835 NNN
 C_debilis_7209 GAAGTTCTATAAGCTGGGAACATATTGGCCCTACACACCTTCTATAACAAC
 C_excelsa_671 NNN
 C_mexicana_362 GAAGTTCTATAAGTTGGGAACATATTGGCCCTACACACCTTCTATAACAAC
 C_mexicana_720 GAAGTTCTATAAGTTGGGAACATATTGGCCCTACACACCTTCTATAACAAC
 C_mutisii_914 GAAGTTCTATAAGTTGGGAACATATTGGCCCTACACACCTTCTATAACAAC
 C_mutisii_1295 GAAGTTCTATAAGTTGGGAACATATTGGCCCTACACACCTTCTATAACAAC
 C_sprucei_817 GAAGTTCTATAAGTTGGGAACATATTGGCCCTACACACCTTCTATAACAAC
 C_sprucei_887 GAAGTTCTATAAGTTGGGAACATATTGGCCCTACACACCTTCTATAACAAC
 C_ulei_88 NNN
 C_ulei_6192 NNN
 I_juru_451 GAAGTTCTATAAGTTGGGAACATATTGGCCCTACACACCTTCTATAACAAC
 I_laev_460 GAAGTTCTATAAGTTGGGAACATATTGGCCCTACACACCTTCTATAACAAC
 O_parvi_RS_598 GAAGTTCTATAAGTTGGGAACATATTGGCCCTACACACCTTCTATAACAAC

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 C_cap_RS_551 TTTTGTATGGCCTGAGAGCAGCTTTGGATCTCATATTTGTGGAAGGACTT
 C_cap_835 NNN
 C_debilis_7209 TTTTGTATGGCCTGAGAGCAGCTTTGGATCTCATATTTTGTGGAAGGACTT
 C_excelsa_671 NNN
 C_mexicana_362 TTTTGTATGGCCTGAGAGCAGCTTTGGATCTCATATTTTGTGGAAGGACTT
 C_mexicana_720 TTTTGTATGGCCTGAGAGCAGCTTTGGATCTCATATTTTGTGGAAGGACTT
 C_mutisii_914 TTTTGTATGGCCTGAGAGCAGCTTTGGATCTCATATTTTGTGGAAGGACTT
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 C_sprucei_817 TTTTGTATGGCCTGAGAGCAGCTTTGGATCTCATATTTTGTGGAAGGACTT
 C_sprucei_887 TTTTGTATGGCCTGAGAGCAGCTTTGGATCTCATATTTTGTGGAAGGACTT
 C_ulei_88 NNN
 C_ulei_6192 NNN
 I_juru_451 TTTTGTATGGCCTGAGAGCAGCTTTGGATCTCATATTTGTGGAAGGACTT
 I_laev_460 TTTTGTATGGCCTGAGAGCAGCTTTGGATCTCATATTTGTGGAAGGACTT
 O_parvi_RS_598 TTTTGTATGGCCTGAGAGCAGCTTTGGATCTCATATTTGTGGAAGGACTT

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 C_cap_RS_551 GAAAATGTGTTTGACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATA
 C_cap_835 NNN
 C_debilis_7209 GAAAATGTGTTTGACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATA
 C_excelsa_671 NNN
 C_mexicana_362 GAAAATGTGTTTGNCAGACACAAACGTTTGGGCAAAGCAACAAGGTAATN
 C_mexicana_720 GAAAATGTGTTTGACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATA
 C_mutisii_914 GAAAATGTGTTTGACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATA
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 C_sprucei_817 GAAAATGTGTTTGACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATA
 C_sprucei_887 GAAAATGTGTTTGACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATA
 C_ulei_88 NNN
 C_ulei_6192 NNN
 I_juru_451 GAAAATGTGTTTGACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATA
 I_laev_460 GAAAATGTGTTTGACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATA

O_parvi_RS_598 GAAAATGTGTTTGACAGACACAAACGTTTGGGCAAAGCAACAAGGTAA--

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C_cap_RS_551 GATTTTGGAAAGATGTAGTGCTGAATGCTAATATTGAGCACTCGATGTCC
C_cap_835 NNN
C_debilis_7209 GATTTTGGAAAGATGCAGTGCTGAATGCTAATATTGAGCACTCGATGTCC
C_excelsa_671 NNN
C_mexicana_362 GATTTTGGAAAGATGCAGTGCTGAATGCTAATATTGAGCACTCGATGTCC
C_mexicana_720 GATTTTGGAAAGATGCAGTGCTGAATGCTAATATTGAGCACTCGATGTCC
C_mutisii_914 GATTTTGGGAAGATGCAGTGCTGAATGCTAATATTGAGCACTCGATGTCC
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C_sprucei_887 GATTTTGGAAAGATGCAGTGCTGAATGCTAATATTGAGCACTCGATGTCC
C_ulei_88 NNN
C_ulei_6192 NNN
I_juru_451 GATTTTGGAAATATGTAGTGCTGAATGCTAATATTGAGCACTTGTATGTCC
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O_parvi_RS_598 -----AGATGTAGTACTGAATGCTAATATTGAGCACTCGATGTCC

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C_cap_835 NNN
C_debilis_7209 ATAGTAAGAGAGGCACATCATTATTTATTGGGAACTACCCTGGATGGGA
C_excelsa_671 NNN
C_mexicana_362 ATAGTAAGAGAGGCACACCATTTATTTATTGGGAACTAACCTGGATGGGA
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C_sprucei_817 ATAGTAAGAGAGGCACACCATTTATTTATTGGGAACTAACCTGGATGGGA
C_sprucei_887 ATAGTAAGAGAGGCACACCATTTATTTATTGGGAACTAACCTGGATGGGA
C_ulei_88 NNN
C_ulei_6192 NNN
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I_laev_460 ATAGTAAGAAAGGCACACCATTTATTTATTGGGAACTGCCCTGGATGGGT
O_parvi_RS_598 ATAGTAAGAGAGGCACACCATTTATTTATTGGGAACTACACTGGATGAGC

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C_cap_RS_551 GGGGAATATT-CCAAAATTTTCAGCAGTGGGACAATCCTAGACACTGATA
C_cap_835 NNN
C_debilis_7209 GGGGAATATT-CCAAAATTTTCAGCAGTGGGACAATCCTAGACACTGATG
C_excelsa_671 NNN
C_mexicana_362 GGGGAATATC-CCAAAATTTTCAGCAGTGGGACAATCCTAGACACTGATA
C_mexicana_720 GGGGCATATT-CCAAAATTTTCAGCAGTGGGACAATCCTAGACACTGATA
C_mutisii_914 GGGGAATATT-CCAAAATTTTCAGCAGTGGGACAATCCTAGACACTGATA
C_mutisii_1295 GGGGAATATT-CCAAAATTTTCAGCAGTGGGACAATCCTAGACACTGATA
C_sprucei_817 GGGGAATATT-CCAAAATTTTCAGCAGTGGGACAATCCTAGACACTGATA
C_sprucei_887 GGGGAATATT-CCAAAATTTTCAGCAGTGGGACAATCCTNGACACTGATA
C_ulei_88 NNN
C_ulei_6192 NNN
I_juru_451 GGGGAATATT-CAAAAATTTTCAGCAGTGGGACAATCCTAGACACTGATT
I_laev_460 GGGGAATATT-CAAAAATTTTCAGCAGTGGGACAATCCTAGACACTGATT
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 C_mexicana_720 CTCATGAAGGTGTGACATTTGTAAGATGATAA-GAGTGCCTGAGATCTAA
 C_mutisii_914 CTCATGAAGGTGTGACATTTGTAAGATGATAA-GAGTGCCTGAGATCTAA
 C_mutisii_1295 CTCATGAAGGTGTGACATTTGTAAGATGATAA-GAGTGCCTGAGATCTAA
 C_sprucei_817 CTCATGAAGGTGTGACATTTGTAAGATGATAA-GAGTGCCTGAGATCTAA
 C_sprucei_887 CTCATGAAGGTGTGACATTTGTAAGATGATAA-GAGTGCCTGAGATCTAA
 C_ulei_88 NNN
 C_ulei_6192 NNN
 I_juru_451 CTCATGAAGGTGTGACATTTGTAAGATGATAAAGAGTGCCTGAGATCTAA
 I_laev_460 CTCATGAAGGTGTGACATTTGTAAGATGATAAAGAGTGCCTGAGATCTAA
 O_parvi_RS_598 CTCATGAAGGTGTGACATTTGTAAGATGATAA-GAGTGCCTGAGATCTAA

C_atopa_1374 NNN
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 C_cap_RS_551 CAAATAAGGACTGCAGGAAAATTTTCTCATATAAAT----GACTTGGAAT
 C_cap_835 NNN
 C_debilis_7209 CAAATAAGGACTGCAGGAAAATTTTCTCATATACAT----GACTTGGAAT
 C_excelsa_671 NNN
 C_mexicana_362 CAAATAAGGACTGCAGGAAAATTTTCTCATATA-----
 C_mexicana_720 CAAATAAGGACTGCAGGAAAATTTTCTCATATA-----
 C_mutisii_914 CAAATGAGGACTGCAGGAAAATTTTCTCATATATATATATGACTTGGAAT
 C_mutisii_1295 CAAATGAGGACTGCAGGAAAATTTTCTCATATATATATATGACTTGGAAT
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 C_sprucei_887 CAAATAAGGACTGCAGGAAAATTTTCTCATNTATATAT--GACTTGGAAT
 C_ulei_88 NNN
 C_ulei_6192 NNN
 I_juru_451 CAAATAAGGACTGCAGGAAAATTTTCTCATATATATAT--GATTTGAAAT
 I_laev_460 CAAATAAGGACTGCAGGAAAATTTTCTCATATATATAT--GATTTGAAAT
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 C_cap_835 NNN
 C_debilis_7209 GTCTATAGACAGAGAAATGGCCTTTTCACTAATGTTCTTTTGTCTTTTTTAA
 C_excelsa_671 NNN
 C_mexicana_362 -----GACAGAGAAATGGCCTTTTCACTAATGCTCTTTTGTCTTTTTTAA
 C_mexicana_720 -----GACAGAGAAATGGCCTTTTCACTAATGCTCTTTTGTCTTTTTTAA
 C_mutisii_914 GTCTATAGACAGAGAAATGGCCTTTTCACTAATGCTCTTTTGTCTTTTTTAA
 C_mutisii_1295 GTCTATAGACAGAGAAATGGCCTTTTCACTAATGCTCTTTTGTCTTTTTTAA
 C_sprucei_817 GTCTATAGACAGAGAAATGGCCTTTTCACTAATGCTCTTTTGT-----
 C_sprucei_887 GTCTATAGACAGAGAAATGGCCTTTTCACTAATGCTCTTTTGTCTTTGTTAA
 C_ulei_88 NNN
 C_ulei_6192 NNN
 I_juru_451 GTCTATCGACAGA--AATGGCCTTTTCACTAATGTTCTTTTGTCTTTTTTAA
 I_laev_460 GTCTATCGACAGA--AATGGCCTTTTCACTAATGTTCTTTTGTCTTTTTTAA
 O_parvi_RS_598 GTCTATAGACAGAGAAATGGCCTTTTCACTAATGTTCTCTGTCTTTTTTAA

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 C_cap_RS_551 ATATCTTCTACGCCACATACTTGTCTGNGAAAATTGGGTATTGGAGGTAC
 C_cap_835 NNATCTTCTACGCCACATACTTGTCTGAGAAAATTGGGTATTGGAGGTAC
 C_debilis_7209 ATATCTTCTATGCCACATANTTGTCTGAGAAAATTGGGTATTGGAGGTAC
 C_excelsa_671 NNATCTTCTATGCCACATATTTGTCTGAGAAAATTGGGTATTGGAGGTAC
 C_mexicana_362 ATATCTTCTATGCCACATATTTGTCTGAGAAAATTGGGTATTGGAGGTAC

C_mexicana_720 ATATCTTCTATGCCACATATTTGTCTGAGAAAATTGGGTATTGGAGGTAC
 C_mutisii_914 ATATCTTCTATGCCACATATTTGTCTGAGAAAATTGGGTATTGGAGGTAC
 C_mutisii_1295 ATATCTTCTATGCCACATATTTGTCTGAGAAAATTGGGTATTGGAGGTAC
 C_sprucei_817 --ATCTTCTATGCCACATATTTNTCTGAGAAAATTGGGTATTGGAGGTAC
 C_sprucei_887 ATATCTTCTATGCCACATATTTGTCTGAGAAAATTGGGTATTGGAGGTAC
 C_ulei_88 NNN
 C_ulei_6192 NNATCTTCTATGCCACATATTTGTCTGAGAAAATTGGGTATTGGAGGTAC
 I_juru_451 ATATCTTCTATGCCACATACCTGTCTGAGAAAATTGGGTATTGGAGGTAC
 I_laev_460 ATATCTTCTATGCCACGTACTTGTCTGAGAAAATTGGGTATTGGAGGTAC
 O_parvi_RS_598 AAATCTTCTACGCCACATACCTGTCTGAGAAAATTGGGTATTGGAGGTAC

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 C_cap_RS_551 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
 C_cap_835 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
 C_debilis_7209 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
 C_excelsa_671 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
 C_mexicana_362 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
 C_mexicana_720 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
 C_mutisii_914 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
 C_mutisii_1295 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
 C_sprucei_817 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
 C_sprucei_887 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
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 C_ulei_6192 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
 I_juru_451 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
 I_laev_460 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC
 O_parvi_RS_598 ATAAC TATTTACAGGCATTTGAAGGCCAACCCAGAGTACCAACTTTACCC

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 C_cap_835 AATCTTCAAGTATTTTCGAGA ACTGGTGCCAGGATGAGAATAGGCATGGAG
 C_debilis_7209 AATCTTCAAGTATTTTCGAGA ACTGGTGCCAGGATGAGAATAGGCATGGAG
 C_excelsa_671 GATCTTCAAGTATTTTCGAGA ACTGGTGCCAGGATGAGAATAGGCATGGAG
 C_mexicana_362 GATCTTCAAGTATTTTCGAGA ACTGGTGCCAGGATGAGAATAGGCATGGAG
 C_mexicana_720 GATCTTCAAGTATTTTCGAGA ACTGGTGCCAGGATGAGAATAGGCATGGAG
 C_mutisii_914 GATCTTCAAGTATTTTCGAGA ACTGGTGCCAGGATGAGAATAGGCATGGAG
 C_mutisii_1295 GATCTTCAAGTATTTTCGAGA ACTGGTGCCAGGATGAGAATAGGCATGGAG
 C_sprucei_817 AATCTTCAAGTATTTTGAAA ACTGGTGCCAGGATGAGAATAGGCANGGAG
 C_sprucei_887 AATCTTCAAGTATTTTGAAA ACTGGTGCCAGGATGAGAATAGGCATGGAG
 C_ulei_88 NNN
 C_ulei_6192 GATCTTCAAGTATTTTCGAGA ACTGGTGCCAGGATGAGAATAGGCATGGAG
 I_juru_451 AATCTTCAAGTATTTTGAGA ACTGGTGCCAGGATGAGAACAGGCATGGAG
 I_laev_460 AATCTTCAAGTATTTTGAGA ACTGGTGCCAGGATGAGAACAGGCATGGAG
 O_parvi_RS_598 AATCTTCAAGTATTTTCGAGA ACTGGTGCCAGGATGAGAACAGGCATGGAG

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 C_cap_RS_551 ATTTCTTCTCTGCATTGATGAAGGCGCAGCCTCAATTCCTCAATGACTGG
 C_cap_835 ATTTCTTCTCTGCATTGATGAAGGCGCAGCCTCAATTCCTCAATGACTGG
 C_debilis_7209 ANTTCTTCTCTGCATTGATGAAGGCNCAGCCTCAATTCCTCAATGACTGG
 C_excelsa_671 ATTTCTTCTCTGCATTGATGAAGGCGCAGCCTCAATTCCTCAATGACTGG
 C_mexicana_362 ATTTCTTCTCTGCATTGATGAAGGCGCAGCCTCAATTCCTCAATGACTGG
 C_mexicana_720 ATTTCTTCTCTGCATTGATGAAGGCGCAGCCTCAATTCCTCAATGACTGG
 C_mutisii_914 ATTTCTTCTCTGCATTGATGAAGGCGCAGCCTCAATTCCTCAATGACTGG
 C_mutisii_1295 ATTTCTTCTCTGCATTGATGAAGGCGCAGCCTCAATTCCTCAATGACTGG

C_ulei_6192 TGATTATTGTAGACTCACAATCTGTTGCTTCAAGCTTTTGATTTTTTCATG
 I_juru_451 TGATTATTGGATACTCACAATCTGTTGCTTCCAGCTTTTGATTTTTTCATG
 I_laev_460 TGATTATTGGATACTCACAATCTGTNGCTTCCAGCTTTTGATTTTTTCATG
 O_parvi_RS_598 TGATTATTGGAGACTCACAATCTGTTGCTTCCAGCTTTTGATTTTTTCATG

C_atopa_1374 GATGAAGAGGTTTTCAAAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 C_cap_RS_527 GATGAAGTGGTTTTCAAAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 C_cap_RS_551 GATGAAGTGGTTTTCAAAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 C_cap_835 GATGAAGAGGTTTTCAAAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 C_debilis_7209 GATGAAGNNGTTTTCAAAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 C_excelsa_671 GATGAAGTGGTTTTCAAGAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 C_mexicana_362 GATGAAGTGGTTTTCAAGAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 C_mexicana_720 GATGAAGTGGTTTTCAAGAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 C_mutisii_914 GATGAAGTGGTTTTCAAGAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 C_mutisii_1295 GATGAAGTGGTTTTCAAGAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 C_sprucei_817 GATGAAGTGGTTTTCAAAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 C_sprucei_887 GATGAAGTAGTTTTCAAAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 C_ulei_88 NNN
 C_ulei_6192 GATGAAGTGGTTTTCAAAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA
 I_juru_451 GATGAAGTAGTTTTCAAAGTGGATTTTGTGGTCATCTGTTCTCTTTTTCA
 I_laev_460 GATGAAGTAGTTTTCAAAGTGGATTTTGTGGTCATCTGTTCTCTTTTTCA
 O_parvi_RS_598 GATGAAGTGGTTNCAAAGTGGATTTTGTGGTCATCTGTTCTCTTTTTTA

C_atopa_1374 CATTGCTTATGCCT
 C_cap_RS_527 CGTTGCTTATGCCT
 C_cap_RS_551 CGTTGCTTATGCCT
 C_cap_835 CGTTGCTTATGCCT
 C_debilis_7209 TGTNGCTTATGCTT
 C_excelsa_671 TGTTGCTNANGCTT
 C_mexicana_362 TGTTGCTTATGCTT
 C_mexicana_720 TGTNGCTTATGCTT
 C_mutisii_914 TGTTGCTTATGCTT
 C_mutisii_1295 TGTNGCTNANGCTT
 C_sprucei_817 TGTNGCTTATGCTT
 C_sprucei_887 TGTTGCTTATGCTT
 C_ulei_88 NNNNNNNNNNNNNNN
 C_ulei_6192 TNNNGNTNANNNTT
 I_juru_451 TNTNNCTNANNNTT
 I_laev_460 TGTNGCTNATGCTT
 O_parvi_RS_598 TGTTGCTTATGCTT

Alignment 1.2

DNA nucleotide sequence alignment of trnH-psbA for 50 taxa and 348 characters used to construct NJ tree in chapter 1. 50 base pairs per line.

```

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C_cap_835         TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_cap_855         TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_cap_872         TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_cap_875         TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_cap_RS_551      TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTCGATTAC
C_cap_RS_527      TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTCGATTAC
C_diaz_7644A      TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_cap_889         TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_sp1_A848        TCCGCCCTTGTCTTTCTAAACTTTCTAAAGAAAATAATTTTAGATTAC
C_cuatre_40682    TCCGCCCTTGTCTTTCTAAACTTTCTAAAGAAAATAATTTTAGATTAC
C_rigid_10008     TCCGCCCTTGTCTTTCTAAACTTTCTAAAGAAAATAATTTTAGATTAC
C_sp2_23826       TCCGCCCTTGTCTTTCTAAACTTTCTAAAGAAAATAATTTTAGATTAC
C_debilis_190     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_debilis_6172    TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_debilis_7209    TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_debilis_22972   TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_excelsa_636     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_excelsa_666     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_excelsa_668     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_excelsa_669     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_excelsa_671     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_mexicana_07     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_mexicana_354    TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
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C_mexicana_757    TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_mexicana_1283   TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_mutisii_911     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_mutisii_913     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_mutisii_914     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_mutisii_1290    TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_mutisii_1295    TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_sprucei_812     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_sprucei_817     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_sprucei_821     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_sprucei_884     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_sprucei_887     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_sprucei_903     TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_ulei_88         TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_ulei_6192       TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
C_ulei_42644      TCCGCCCTTGTCTTTCT-----AAAGAAAAAATTTTAGATTAC
O_gly_RS_546      TCCGCCCTTGTCTTTTCG-----AAAGACTAAAATTTTAGATTAC
O_parvi_RS_598    TCCGCCCTTGTCTTTTCG-----AAAGACTAAAATTTTAGATTAC
V_sur_RS_078      ?CCG?CCCTTGTCTTTTCT---TTTTCTAAAGAAAAAATTTTAGATTAC
I_juru_RS_451     TCCGCCCTTGTCTTTTCT---TTTCTAAAGACAAAAATTTTAGATTAC
I_laev_RS_460     TCCGCCCTTGTCTTTTCT---TTTCTAAAGACAAAAATTTTAGATTAC

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C_cap_855 TAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAAAATCTAC
C_cap_872 TAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAAAATCTAC
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C_cap_RS_551 TAGTCTTTCTTATTTTTTTTCATACTAATTTCTACCCTTTAGAAAATCGAC
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C_diaz_7644A TAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAAAATCGAC
C_cap_889 TAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAAAATCGAC
C_spl_A848 TAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAAAATCGAC
C_cuatre_40682 TAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAAAATCGAC
C_rigid_10008 TAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAAAATCGAC
C_sp2_23826 TAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAAAATCGAC
C_debilis_190 TAGTCTTTCTTATTTTTTTTCATACAAATTTCTATCCTTTAGAAAA----C
C_debilis_6172 TAGTCTTTCTTATTTTTTTTCATACAAATTTCTATCCTTTAGAAAA----C
C_debilis_7209 TAGTCTTTCTTATTTTTTTTCATACAAATTTCTATCCTTTAGAAAA----C
C_debilis_22972 TAGTCTTTCTTATTTTTTTTCATACAAATTTCTATCCTTTAGAAAA----C
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V_sur_RS_078 TAGTCTTTCTTATTTTTTTTCATACTAATTTCTACCCTTTAGAAAATTGAC
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 V_sur_RS_078 AATAGGAAAAAATTCATTTT TGG AATGTACATAAACTGAAGATCAGTTAA
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C_debilis_7209 AATCAAAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATT
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C_ulei_88 AATAAAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATT
C_ulei_6192 AATAAAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATT
C_ulei_42644 AATAAAAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATT
O_gly_RS_546 AAACAAAAAAAA-----GGTATGATGTTTCGATCCTGAACCAACTAATT
O_parvi_RS_598 AAACAAAAAAAA-----GGTATGATGTTTCGATCCTGAACCAACTAATT
V_sur_RS_078 AATAAAAAATAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATG
I_juru_RS_451 AATAAAAAATAAAAAAAGGTATGATGTTTCGATCATGAA-CAAA-AATG
I_laev_RS_460 AATAAAAAAAAA-----GGTATGATGTTTCGATCATGAA-CAAA-AATG

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C_cap_872 CATATTTTCTGAAATTGAAAAAAAAAATCTTATGTGAGTAAACCACTA
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C_spl_A848 AATATTTTCTGAAATTGAAAAAAAA---TCTTATGTGAGTAAACCACTA
C_cuatre_40682 AATATTTTCTGAAATTGAAAAAAAA---TCTTATGTGAGTAAACCACTA
C_rigid_10008 AATATTTTCTGAAATTGAAAAAAAA--????????????????????
C_sp2_23826 CATATTTTCTGAAATTGAAAAAACAAA--TCTTATGTGAGTAAACCACTA
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C_debilis_6172 AATATTTTCTGAAATTGAAAAAAAA---TCTTATGTGAGTAAACCACTA
C_debilis_7209 AATATTTTCTGAAATTGAAAAAAAA---TCTTATGTGAGTAAACCACTA
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 C_mexicana_354 AATATTTTCTGAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_mexicana_362 AATATTTTCTGAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_mexicana_696 AATATTTTCTGAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_mexicana_701 AATATTTTCTGAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_mexicana_719 AATATTTTCTGAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_mexicana_720 AATATTTTCTGAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_mexicana_757 AATATTTTCTGAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_mexicana_1283 AATATTTTCTGAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_mutisii_911 CATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_mutisii_913 CATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_mutisii_914 CATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_mutisii_1290 AATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_mutisii_1295 AATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_sprucei_812 AATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_sprucei_817 AATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_sprucei_821 AATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_sprucei_884 AATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_sprucei_887 AATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_sprucei_903 AATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_ulei_88 AATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_ulei_6192 AATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 C_ulei_42644 CATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 O_gly_RS_546 AATATTTTTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 O_parvi_RS_598 AATATTTTTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 V_sur_RS_078 AATATTTTCTTAAAAAAAAAAAAAAAAAGAAA--TCTTATGTGAGTAAACCACTA
 I_juru_RS_451 AATATTTTCTTAAATGGAAAAAAAA---TCTTATGTGAGTAAACCACTA
 I_laev_RS_460 AATATTTTCTTAAATGGAAAAACAAAA--TCTTATGTGAGTAAACCACTA

C_atopa_1374 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_cap_835 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_cap_855 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_cap_872 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_cap_875 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_cap_RS_551 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_cap_RS_527 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_diaz_7644A CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_cap_889 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_sp1_A848 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_cuatre_40682 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_rigid_10008 ???
 C_sp2_23826 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_debilis_190 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_debilis_6172 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_debilis_7209 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_debilis_22972 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_excelsa_636 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_excelsa_666 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_excelsa_668 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_excelsa_669 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_excelsa_671 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_mexicana_07 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG

C_mexicana_354 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_mexicana_362 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_mexicana_696 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_mexicana_701 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_mexicana_719 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_mexicana_720 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_mexicana_757 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_mexicana_1283 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_mutisii_911 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_mutisii_913 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
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 C_sprucei_812 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_sprucei_817 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_sprucei_821 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_sprucei_884 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_sprucei_887 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_sprucei_903 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_ulei_88 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_ulei_6192 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 C_ulei_42644 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 O_gly_RS_546 CTGAAACAGATCAATACCCATGGGTATTGATCTGATCTTTCAATGACTCG
 O_parvi_RS_598 CTGAAACAGATCAATACCCATGGGTATTGATCTGATCTTTCAATGACTCG
 V_sur_RS_078 CTGAACCAGATCAATACCCATGGGTATTGATCTGATCTTTCAATGACTCG
 I_juru_RS_451 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG
 I_laev_RS_460 CTGAACCAGATCAATACCCATGGGTATTGATCTGGTCCTTCAATGACTCG

C_atopa_1374 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_cap_835 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_cap_855 TATACACTAATACCGAAATATTAACCATTTATTGATAGAGCTTCAACA
 C_cap_872 TATACACTAATACCGAAATATTAACCATTTATTGATAGAGCTTCAACA
 C_cap_875 TATACACTAATACCGAAATATTAACCATTTATTGATAGAGCTTCAACA
 C_cap_RS_551 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_cap_RS_527 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_diaz_7644A TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_cap_889 TATACACTAATACCGAAATATTAACCATTTATTGATAGAGCTTCAACA
 C_sp1_A848 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_cuatre_40682 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_rigid_10008 ???
 C_sp2_23826 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_debilis_190 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_debilis_6172 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_debilis_7209 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
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 C_excelsa_666 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
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 C_excelsa_671 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_mexicana_07 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_mexicana_354 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_mexicana_362 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_mexicana_696 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_mexicana_701 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_mexicana_719 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
 C_mexicana_720 TATACACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA

C_mexicana_757 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
C_mexicana_1283 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
C_mutisii_911 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
C_mutisii_913 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
C_mutisii_914 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
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C_mutisii_1295 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
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C_sprucei_817 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
C_sprucei_821 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
C_sprucei_884 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
C_sprucei_887 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
C_sprucei_903 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
C_ulei_88 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
C_ulei_6192 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
C_ulei_42644 TATACTAATACCGAAATATTAGCCATTTATTGATAGAGCTTCAACA
O_gly_RS_546 TATACTAATACCGAAGCATTAGCCATTTGTTGATAGA?CTTCA???
O_parvi_RS_598 TATACTAATACCGAAGCATT?GCCATTTGTTGATAGAGCTTCAACA
V_sur_RS_078 TATACTAATACCGAAATATTAGCCATTTGTTGATGGAGCTTCAACA
I_juru_RS_451 TATACTAATACCGAAGTATTAGCCATTTGTTGATAGAGCTTCAACA
I_laev_RS_460 TATACTAATACCGAAGTATTAGCCATTTGTTGATAGAGCTTCAACA

Appendix 2

Alignment 2.1

The trnH-psbA DNA nucleotide sequence alignment of 61 taxa and 302 characters used in haplotype network analysis in chapter 2. 50 base pairs per line.

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C_cap_JJ_1542 AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_1543 AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_1544 AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_1545 AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_827  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_829  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_830  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_831  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_832  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_833  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_834  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_835  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_843  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_844  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_855  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_860  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_862  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_863  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_872  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_873  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_874  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_875  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_893  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_894  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_899  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_900  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_JJ_902  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_271  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_274  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_278  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_280  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_284  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_293  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_294  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_295  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_332  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_334  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_336  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_338  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_346  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_364  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_370  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_406  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_409  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_418  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_427  AGATTACTAGTCTTTCTTATTTTTTTTCATACAAATTTCTACCCTTTAGAA
C_cap_RS_527  CGATTACTAGTCTTTCTTATTTTTTTTCATACTAATTTCTACCCTTTAGAA
C_cap_RS_531  CGATTACTAGTCTTTCTTATTTTTTTTCATACTAATTTCTACCCTTTAGAA
C_cap_RS_535  CGATTACTAGTCTTTCTTATTTTTTTTCATACTAATTTCTACCCTTTAGAA

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Appendix 3

Alignment 3.1

Concatenated DNA nucleotide sequence alignment of 3-loci, 1517 characters, and 49 taxa used in Bayesian and Maximum parsimony analyses in chapter 3. 50 base pairs per line.

loci order and positions in alignment:

trnH-psba 1-357, AGT1 358-1078, AT103 1079-1517

```

C_cap_RS_551      TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAAAA--TTTTCGATTAC
C_debilis_7209   TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAAAA--TTTTAGATTAC
I_juru_RS_451    TCCGCCCCTTGTCTTTTCTTTT--CTAAAGACAAAAA--TTTTAGATTAC
I_laev_RS_460    TCCGCCCCTTGTCTTTTCTTTT--CTAAAGACAAAAA--TTTTAGATTAC
V_cadu_JJ_847    TCCGCCCCTTGTCTTTTCTTTT-----T-CTAAAGAAAAAAAAAATTTAGATTAC
V_spRADS3_RS_335 TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_spRADS3_RS_339 TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_peru_JJ_772    TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_calor_RS_430   TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_calor_RS_454   ??????CCTTGTCTTTTCTTTTCT-----CT-----
V_calor_RS_481   ?????????????????????????????????-----
V_calor_RS_511   TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_spRADS1_RS_432 TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_spRADS1_RS_500 TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_spRADS1_R1_510 TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_spRADS1_RS_561 TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_dix_RS_225     TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_elon_RS_437    ??????CCTTGTCTTTTCTTTTCT-----CT-----
V_elon_RS_494    ?????????????????????????????????-----
V_elon_RS_502    TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_flex_RS_442    TCCGCCCCTTGTCTTTT-----T-CTAAAGAAAAAAAAAATTTAGATTAC
V_flex_RS_522    TCCGCCCCTTGTCTTTT-----T-CTAAAGAAAAAAAAAATTTAGATTAC
V_flex_RS_595    TCCGCCCCTTGTCTTTT-----T-CTAAAGAAAAAAAAAATTTAGATTAC
V_multin_RS_107  TCCGCCCCTTGTCTTTT-----T-CTAAAGAAAAAGAAAATTTAGATTAC
V_multin_RS_108  TCCGCCCCTTGTCTTTT-----T-CTAAAGAAAAAGAAAATTTAGATTAC
V_loret_RS_480   TCC?CCCCTTGTCTTTTCTTTTCT-----CT-----
V_loret_RS_516   TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_loret_RS_526   TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_multi_RS_350   TCCGCCCCTTGTCTTTT-----T-CTAAAGAAAAAAAAAATTTAGATTAC
V_multi_RS_429   TCCGCCCCTTGTCTTTT-----T-CTAAAGAAAAAGAAAATTTAGATTAC
V_multi_RS_466   TCCGCCCCTTGTCTTTT-----T-CTAAAGAAAAAGAAAATTTAGATTAC
V_multi_RS_543   TCCGCCCCTTGTCTTTT-----T-CTAAAGAAAAAGAAAATTTAGATTAC
V_multi_RS_549   TCCGCCCCTTGTCTTTT-----T-CTAAAGAAAAAGAAAATTTAGATTAC
V_sebBL_RS_584   TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_lorSL_RS_483   TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_lorSL_RS_507   TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_sebSL_RS_534   ?????????????????????????????????-----
V_sebSL_RS_552   TCCGCCCCTTGTCTTTTCTTTTCT-----CT-----
V_spRADS4_RS_213 TCCGCCCCTTGTCTTTTCTTTTCTTTTGTACAGAAAAA--TTTTGGATTTTC
V_spRADS4_RS_214 TCCGCCCCTTGTCTTTTCTTTTCTTTTGTACAGAAAAA--TTTTGGATTTTC
V_sur_RS_082     TCCGCCCCTTGTCTTTTCTTTTCT-----CTAAAGAAAAA--TTTTAGATTAC
V_sur_RS_083     TCCGCCCCTTGTCTTTTCTTTTCT-----CTAAAGAAAAA--TTTTAGATTAC

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V_sur_RS_084 TCCGCCCTTGTCTTTTCTTTTT-CTAAAGAAAAAAA--TTTTAGATTAC
V_sur_RS_216 TCCGCCCTTGTCTTTTCTTTTT-CTAAAGAAAAAAA--TTTTAGATTAC
V_sur_RS_248 ?????????????????????????????????????GAAAAAAA--TTTTAGATTAC
V_sur_RS_324 ???
V_sur_RS_428 TCCGCCCTTGTCTTTTCTTTTT-CTAAAGAAAAAAA--TTTTAGATTAC
V_sur_RS_489 TCCGCCCTTGTCTTTTCTTTTT-CTAAAGAAAAAAA--TTTTAGATTAC
V_sur_RS_501 TCCGCCCTTGTCTTTTCTTTTT-CTAAAGAAAAAAA--TTTTAGATTAC

C_cap_RS_551 TAGTCTTTCTTATTTTTTTTCATACTAATTTCTACCCTTTAGAAAATCGAC
C_debilis_7209 TAGTCTTTCTTATTTTTTTTCATACAAATTTCTATCCTTTAGAAAA---C
I_juru_RS_451 TAGTCTTTCTTATTTTTTTTCATACTAATTTCTACCCTTTAGAAAATTGAC
I_laev_RS_460 TAGTCTTTCTTATTTTTTTTCATACTAATTTCTACCCTTTAGAAAATTGAC
V_cadu_JJ_847 TAGTCTTTCTTATTTTTTTTCATACTAATTTCTACCCTTTAGAAAATTGAC
V_spRADS3_RS_335 -----
V_spRADS3_RS_339 -----
V_peru_JJ_772 -----
V_calor_RS_430 -----
V_calor_RS_454 -----
V_calor_RS_481 -----
V_calor_RS_511 -----
V_spRADS1_RS_432 -----
V_spRADS1_RS_500 -----
V_spRADS1_R1_510 -----
V_spRADS1_RS_561 -----
V_dix_RS_225 -----
V_elon_RS_437 -----
V_elon_RS_494 -----
V_elon_RS_502 -----
V_flex_RS_442 TAGTCTTTCTTCTTTTTTTTCATACTAATTTCTACCCTTTAGAAAATTGAC
V_flex_RS_522 TAGTCTTTCTTCTTTTTTTTCATACTAATTTCTACCCTTTAGAAAATTGAC
V_flex_RS_595 TAGTCTTTCTTCTTTTTTTTCATACTAATTTCTACCCTTTAGAAAATTGAC
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V_multin_RS_108 TAGTCTTTCTTCTTTTTTTTCATACTAATTTCTACCCTTTAGAAAATTGAC
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V_loret_RS_526 -----
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V_lorSL_RS_483 -----
V_lorSL_RS_507 -----
V_sebSL_RS_534 -----
V_sebSL_RS_552 -----
V_spRADS4_RS_213 TAGTCTTTCTTATTTTTTTTCATACTAA-----
V_spRADS4_RS_214 TAGTCTTTCTTATTTTTTTTCATACTAA-----
V_sur_RS_082 TAGTCTTTCTTATTTTTTT-CATACTAATTTCTACCCTTTAGAAAATTGAC
V_sur_RS_083 TAGTCTTTCTTATTTTTTT-CATACTAATTTCTACCCTTTAGAAAATTGAC
V_sur_RS_084 TAGTCTTTCTTATTTTTTT-CATACTAATTTCTACCCTTTAGAAAATTGAC
V_sur_RS_216 TAGTCTTTCTTATTTTTTT-CATACTAATTTCTACCCTTTAGAAAATTGAC
V_sur_RS_248 TAGTCTTTCTTATTTTTTT-CATACTAATTTCTACCCTTTAGAAAATTGAC
V_sur_RS_324 ???
V_sur_RS_428 TAGTCTTTCTTATTTTTTT-CATACTAATTTCTACCCTTTAGAAAATTGAC
V_sur_RS_489 TAGTCTTTCTTATTTTTTT-CATACTAATTTCTACCCTTTAGAAAATTGAC
V_sur_RS_501 TAGTCTTTCTTATTTTTTT-CATACTAATTTCTACCCTTTAGAAAATTGAC

C_cap_RS_551 AATAGGAAAAAATGCATTTT TAGGAATGTACATGAACTGAAGATCAGTTCA
C_debilis_7209 AATAGGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCCGTTAA
I_juru_RS_451 AATAGGAAAAAATGCATTTT TGGGAATGTACATAAACTGAAGATCAGTTAA
I_laev_RS_460 AATAGGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGTTAA
V_cadu_JJ_847 AATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGTTAA
V_spRADS3_RS_335 -----
V_spRADS3_RS_339 -----
V_peru_JJ_772 -----
V_calor_RS_430 -----
V_calor_RS_454 -----
V_calor_RS_481 -----
V_calor_RS_511 -----
V_spRADS1_RS_432 -----
V_spRADS1_RS_500 -----
V_spRADS1_R1_510 -----
V_spRADS1_RS_561 -----
V_dix_RS_225 -----
V_elon_RS_437 -----
V_elon_RS_494 -----
V_elon_RS_502 -----
V_flex_RS_442 AATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGTTAA
V_flex_RS_522 AATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGTTAA
V_flex_RS_595 AATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGTTAA
V_multin_RS_107 AATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGTTAA
V_multin_RS_108 AATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGTTAA
V_loret_RS_480 -----
V_loret_RS_516 -----
V_loret_RS_526 -----
V_multi_RS_350 AATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGTTAA
V_multi_RS_429 AATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGTTAA
V_multi_RS_466 AATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGTTAA
V_multi_RS_543 AATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGTTAA
V_multi_RS_549 AATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGTTAA
V_sebBL_RS_584 -----
V_lorSL_RS_483 -----
V_lorSL_RS_507 -----
V_sebSL_RS_534 -----
V_sebSL_RS_552 -----
V_spRADS4_RS_213 ---AGGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGTTAA
V_spRADS4_RS_214 ---AGGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGTTAA
V_sur_RS_082 AATAGGAAAAAATTCATTTT TGGGAATGTACATAAACTGAAGATCAGTTAA
V_sur_RS_083 AATAGGAAAAAATTCATTTT TGGGAATGTACATAAACTGAAGATCAGTTAA
V_sur_RS_084 AATAGGAAAAAATTCATTTT TGGGAATGTACATAAACTGAAGATCAGTTAA
V_sur_RS_216 AATAGGAAAAAATTCATTTT TGGGAATGTACATAAACTGAAGATCAGTTAA
V_sur_RS_248 AATAGGAAAAAATTCATTTT TGGGAATGTACAGAACTGAAGATCAGTTAA
V_sur_RS_324 ???
V_sur_RS_428 AATAGGAAAAAATTCATTTT TGGGAATGTACATAAACTGAAGATCAGTTAA
V_sur_RS_489 AATAGGAAAAAATTCATTTT TGGGAATGTACATAAACTGAAGATCAGTTAA
V_sur_RS_501 AATAGGAAAAAATTCATTTT TGGGAATGTACATAAACTGAAGATCAGTTAA

C_cap_RS_551 AATCAAAAAA-----GGTATGATGTTTCGATTATGAACCAAATAATT
C_debilis_7209 AATCAAAAAA-----GGTATGATGTTTCGATCATGAACCAAATAATT
I_juru_RS_451 AATAAAAAATAAAAAAAAAAAGGTATGATGTTTCGATCATGAA-CAAA-AATG
I_laev_RS_460 AATAAAAAA-----GGTATGATGTTTCGATCATGAA-CAAA-AATG
V_cadu_JJ_847 AATAAAAAAGAAAA-----GGTATGATGTTTCGATCATGAAACAAATAATT
V_spRADS3_RS_335 -----

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V_spRADS3_RS_339 -----
V_peru_JJ_772 -----
V_calor_RS_430 -----
V_calor_RS_454 -----
V_calor_RS_481 -----
V_calor_RS_511 -----
V_spRADS1_RS_432 -----
V_spRADS1_RS_500 -----
V_spRADS1_R1_510 -----
V_spRADS1_RS_561 -----
V_dix_RS_225 -----
V_elon_RS_437 -----
V_elon_RS_494 -----
V_elon_RS_502 -----
V_flex_RS_442 AATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATT
V_flex_RS_522 AATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATT
V_flex_RS_595 AATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATT
V_multin_RS_107 AATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATT
V_multin_RS_108 AATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATT
V_loret_RS_480 -----
V_loret_RS_516 -----
V_loret_RS_526 -----
V_multi_RS_350 AATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATT
V_multi_RS_429 AATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATT
V_multi_RS_466 AATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATT
V_multi_RS_543 AATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATT
V_multi_RS_549 AATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATT
V_sebBL_RS_584 -----
V_lorSL_RS_483 -----
V_lorSL_RS_507 -----
V_sebSL_RS_534 -----
V_sebSL_RS_552 -----
V_spRADS4_RS_213 AATAAAAAAGAAAAA----GGTATAATGTTTCGATCATTAAACAAATAATT
V_spRADS4_RS_214 AATAAAAAAGAAAAA----GGTATAATGTTTCGATCATTAAACAAATAATT
V_sur_RS_082 AATAAAAAATAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATG
V_sur_RS_083 AATAAAAAATAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATG
V_sur_RS_084 AATAAAAAATAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATG
V_sur_RS_216 AATAAAAAATAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATT
V_sur_RS_248 AATAAAAAATAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATT
V_sur_RS_324 ??????????????????-----????????????????????????????????????
V_sur_RS_428 AATAAAAAATAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATG
V_sur_RS_489 AATAAAAAATAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATG
V_sur_RS_501 AATAAAAAATAAAAA----GGTATGATGTTTCGATCATGAAACAAATAATG

C_cap_RS_551 CATATTTTCTGAAATTGAAAAAACAAA-----TCTTATG-----TGAGTA
C_debilis_7209 AATATTTTCTGAAATTGAAAAAACAAA-----TCTTATG-----TGAGTA
I_juru_RS_451 AATATTTTCTTAAATTGAAAAAACAAA-----TCTTATG-----TGAGTA
I_laev_RS_460 AATATTTTCTTAAATTGAAAAAACAAA-----TCTTATG-----TGAGTA
V_cadu_JJ_847 AATATTTTAT-AAAAAAAA-----GAAATCTTATG-----TGAGTA
V_spRADS3_RS_335 -----AAAAAAAA-----GAAATCTTATGTTATGTGAGTA
V_spRADS3_RS_339 -----AAAAAAAA-----GAAATCTTATGTTATGTGAGTA
V_peru_JJ_772 -----AAAAAAAA-----TCTTATGTTATGTGAGTA
V_calor_RS_430 -----AAAAAAAA-----TCTTATGTTATGTGAGTA
V_calor_RS_454 -----AAAAAAAA-----TCTTATGTTATGTGAGTA
V_calor_RS_481 -----????????????????????????????????????????????????????????
V_calor_RS_511 -----AAAAAAAA-----TCTTATGTTATGTGAGTA
V_spRADS1_RS_432 -----AAAAAAAA-----TCTTATGTTATGTGAGTA

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V_spRADS1_RS_500 -----AAAAAAAAAAAAA-----TCTTATGTTATGTGAGTA
V_spRADS1_R1_510 -----AAAAAAAAAAAAA-----TCTTATGTTATGTGAGTA
V_spRADS1_RS_561 -----AAAAAAAAAAAAA-----TCTTATGTTATGTGAGTA
V_dix_RS_225 -----AAAAAAAAAAAAA-----TCTTATGTTATGTGAGTA
V_elon_RS_437 -----AAAAAAAAAAAAA-----TCTTATGTTATGTGAGTA
V_elon_RS_494 -----??
V_elon_RS_502 -----AAAAAAAAAAAAA-----TCTTATGTTATGTGAGTA
V_flex_RS_442 AATATTTTCT-AAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_flex_RS_522 AATATTTTCT-AAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_flex_RS_595 AATATTTTCT-AAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_multin_RS_107 AATATTTTCT-AAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_multin_RS_108 AATATTTTCT-AAAAAAAAA-----GAAATCTTATG-----TGAGTA
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V_loret_RS_516 -----AAAAAAAAAAAAA-----TCTTATGTTATGTGAGTA
V_loret_RS_526 -----AAAAAAAAAAAAA-----TCTTATGTTATGTGAGTA
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V_multi_RS_429 AATATTTTCT-AAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_multi_RS_466 AATATTTTCT-AAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_multi_RS_543 AATATTTTCT-AAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_multi_RS_549 AATATTTTCT-AAAAAAAAA-----GAAATCTTATG-----TGAGTA
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V_lorSL_RS_483 -----AAAAAAAAAAAAA-----TCTTATGTTATGTGAGTA
V_lorSL_RS_507 -----AAAAAAAAAAAAA-----TCTTATGTTATGTGAGTA
V_sebSL_RS_534 -----??
V_sebSL_RS_552 -----AAAAAAAAAAAAA-----TCTTATGTTATGTGAGTA
V_spRADS4_RS_213 AATATTTTCTTAAAAAAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_spRADS4_RS_214 AATATTTTCTTAAAAAAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_sur_RS_082 AATATTTTCTTAAAAAAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_sur_RS_083 AATATTTTCTTAAAAAAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_sur_RS_084 AATATTTTCTTAAAAAAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_sur_RS_216 AATATTTTCTTAAAAAAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_sur_RS_248 AATATTTTCTTAAAAAAAAAAAAAAGAAATCTTATG-----TGAGTA
V_sur_RS_324 ???
V_sur_RS_428 AATATTTTCTTAAAAAAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_sur_RS_489 AATATTTTCTTAAAAAAAAAAAAA-----GAAATCTTATG-----TGAGTA
V_sur_RS_501 AATATTTTCTTAAAAAAAAAAAAA-----GAAATCTTATG-----TGAGTA

C_cap_RS_551 AACCCTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGGTCCTTC
C_debilis_7209 AACCCTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGGTCCTTC
I_juru_RS_451 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGGTCCTTC
I_laev_RS_460 AACCCTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGGTCCTTC
V_cadu_JJ_847 AACCCTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGATCCTTC
V_spRADS3_RS_335 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
V_spRADS3_RS_339 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
V_peru_JJ_772 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
V_calor_RS_430 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
V_calor_RS_454 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
V_calor_RS_481 ???
V_calor_RS_511 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
V_spRADS1_RS_432 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
V_spRADS1_RS_500 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
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V_spRADS1_RS_561 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
V_dix_RS_225 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
V_elon_RS_437 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
V_elon_RS_494 ???
V_elon_RS_502 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC

V_flex_RS_442 AACCCTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGATCCTTC
 V_flex_RS_522 AACCCTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGATCCTTC
 V_flex_RS_595 AACCCTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGATCCTTC
 V_multin_RS_107 AACCCTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGATCCTTC
 V_multin_RS_108 AACCCTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGATCCTTC
 V_loret_RS_480 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
 V_loret_RS_516 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
 V_loret_RS_526 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
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 V_multi_RS_549 AACCCTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGATCCTTC
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 V_lorSL_RS_507 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
 V_sebSL_RS_534 ???
 V_sebSL_RS_552 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
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 V_spRADS4_RS_214 AACCCTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGATCCTTC
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 V_sur_RS_248 AACCCTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGATCCTTC
 V_sur_RS_324 ???
 V_sur_RS_428 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
 V_sur_RS_489 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC
 V_sur_RS_501 AACCCTACTGAACCAGATCAATACCCACTGGGTATTGATCTGATCCTTC

 C_cap_RS_551 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTATTGATAGAGC
 C_debilis_7209 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTATTGATAGAGC
 I_juru_RS_451 AATGACTCGTATACACTAATACCGAAGTATTAGCCATTTGTTGATAGAGC
 I_laev_RS_460 AATGACTCGTATACACTAATACCGAAGTATTAGCCATTTGTTGATAGAGC
 V_cadu_JJ_847 AATTACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATGGAGC
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 V_spRADS3_RS_339 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATAGAGC
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 V_calor_RS_430 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATAGAGC
 V_calor_RS_454 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATAGAGC
 V_calor_RS_481 ???
 V_calor_RS_511 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATAGAGC
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 V_spRADS1_RS_500 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATAGAGC
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 V_spRADS1_RS_561 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATAGAGC
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 V_elon_RS_437 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATAGAGC
 V_elon_RS_494 ???
 V_elon_RS_502 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATAGAGC
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V_loret_RS_526 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATAGAGC
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 V_lorSL_RS_483 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATAGAGC
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 V_sebSL_RS_534 ???
 V_sebSL_RS_552 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATAGAGC
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 V_sur_RS_216 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATGGAGC
 V_sur_RS_248 AATGACTCGTATACACTAATACC????????????????????????????
 V_sur_RS_324 ???
 V_sur_RS_428 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATGGAGC
 V_sur_RS_489 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATGGAGC
 V_sur_RS_501 AATGACTCGTATACACTAATACCGAAATATTAGCCATTTGTTGATGGAGC

 C_cap_RS_551 TTCAACA????????????CTCTTTTCTATGCCTACTGGCATGGGA?TCGTAT
 C_debilis_7209 TTCAACA????????????CTCTTTTCTATGCCTACTGG?ATGGGAATCGTAT
 I_juru_RS_451 TTCAACA????????????CTCTTTTCTATGCCTACTGGTATGGGAATCGTAT
 I_laev_RS_460 TTCAACA????????????CTCTTTTCTATGCCTACTGGTATGGGA?TCGTAT
 V_cadu_JJ_847 TTCAACATCCCAAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_spRADS3_RS_335 TTCAACTTCTCAAAGGCTCTTTTCTATGCCTACTGGCTTGGGAATCGTAT
 V_spRADS3_RS_339 TTCAACTTCTCAAAGGCTCTTTTCTATGCCTACTGGCTTGGGAATCGTAT
 V_peru_JJ_772 TTCAACT????????????????????????GCCTAC?GGCATGGGAATCGTAT
 V_calor_RS_430 TTCAACTTCTCAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_calor_RS_454 TTCAACTTCTCAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_calor_RS_481 ????????TCTCAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_calor_RS_511 TTCAACTTCTCAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_spRADS1_RS_432 TTCAACTTCTCAAAGGCTCTTTTCTATGCCTACCGGCATGGGAATCGTAT
 V_spRADS1_RS_500 TTCAACTTCTCAAAGGCTCTTTTCTATGCCTACCGGCATGGGAATCGTAT
 V_spRADS1_R1_510 TTCAACT??GGGAATCGTAT
 V_spRADS1_RS_561 TTCAACTTCTCAAAGGCTCTTTTCTATGCCTACCGGCATGGGAATCGTAT
 V_dix_RS_225 TTCAACT????????????????????TCTATGCCTACTGGCATGGGAATCGTAT
 V_elon_RS_437 TTCAACT????????????????????????????????TACCGGCATGGGAATCGTAT
 V_elon_RS_494 ????????TCTCAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_elon_RS_502 TTCAACT??AT
 V_flex_RS_442 TTCAACATCCCAAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_flex_RS_522 TTCAACATCCCAAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_flex_RS_595 TTCAACATCCCAAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_multin_RS_107 TTCAACATCCCAAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_multin_RS_108 TTCAACATCCCAAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_loret_RS_480 TTCAACTTCTCAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_loret_RS_516 TTCAACT??ATCGTAT
 V_loret_RS_526 TTCAACTTCTCAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_multi_RS_350 TTCAACATCCCAAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_multi_RS_429 TTCAACATCCCAAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_multi_RS_466 TTCAACATCCCAAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_multi_RS_543 TTCAACATCCCAAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_multi_RS_549 TTCAACATCCCAAAAGGCTCTTTTCTATGCCTACTGGCATGGGAATCGTAT
 V_sebBL_RS_584 TTCAACTTTGACAGGGGCTCTTTTCTATGCCTACCGGCATGGGAATCGTAT

V_lorSL_RS_483 TTCAACT??
V_lorSL_RS_507 TTCAACTTCTCAAAAGGCTCTTTCTATGCCTACCGGCATGGGAATCGTAT
V_sebSL_RS_534 ???????TCTCAAAAGGCTCTTTCTATGCCTACCGGCATGGGAATCGTAT
V_sebSL_RS_552 TTCAACTTTTGACAGGGCTCTTTCTATGCCTACCGGCATGGGAATCGTAT
V_spRADS4_RS_213 TTCAACATCCCAAAAGGCTCTTTCTATGCCTACTGGCATGGGAATCGTAT
V_spRADS4_RS_214 TTCAACA????????GGCTCTTTCTATGCCTACTGGCATGGGAATCGTAT
V_sur_RS_082 TTCAACATCCCAAAAGGCTCTTTCTATGCCTACTGGCATGGGAATCGTAT
V_sur_RS_083 TTCAACATCCCAAAAGGCTCTTTCTATGCCTACTGGCATGGGAATCGTAT
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V_sur_RS_216 TTCAACA??CcAAAAGGCTCTTTCTATGCCTACTGGCATGGGAATCGTAT
V_sur_RS_248 ???
V_sur_RS_324 ???
V_sur_RS_428 TTCAACATCCCAAAAGGCTCTTTCTATGCCTACTGGCATGGGAATCGTAT
V_sur_RS_489 TTCAACATCCCAAAAGGCTCTTTCTATGCCTACTGGCATGGGAATCGTAT
V_sur_RS_501 TTCAACA??C AAAAGGCTCTTTCTATGCCTACTGGCATGGGAATCGTAT

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I_laev_RS_460 GCGCAAGTCCAAAAGCAATAGAAGCATCCAAGACTGCTAAATCAGTCAGA
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 V_sur_RS_248 ???
 V_sur_RS_324 ???
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 V_cadu_JJ_847 TTTATTGGGAACTACCCTGGATGGGCGGGGAATATTCCAAAAA-TTTCAA
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 V_spRADS1_R1_510 TTTATTGGGAACT??
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 V_sur_RS_248 ???
 V_sur_RS_324 ???
 V_sur_RS_428 TTTATTGGGAACTACCCTGGATGGGCGGGGAATATTCCAAAAA-TTTCAG
 V_sur_RS_489 TTTATTGGGAACTACCCTGGATGGGCGGGGAATATTCCAAAAA-TTTCAG
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 V_sur_RS_501 CAGTGGGACAATCATAGACACTGATATTGACAACCTTTTGCCCCAGTGAAT

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 C_debilis_7209 GCTGACCATATATGTTTTTT-G--AGCTTTCTATTTATAATCCTCAAAAT
 I_juru_RS_451 GTTGACCATATATGTTTTTT-G--AACTTCCTATTTATAATCCTCAAAAT
 I_laev_RS_460 GTTGACCATATATGTTTTTT-G--AACTTCCTATTTATAATCCTCAAAAT
 V_cadu_JJ_847 GTTGACCATATRTATTTTTCTG--AACTTTCCATTTATAATCCTCAAAAT
 V_spRADS3_RS_335 GTTGACCATATGTGTTTTTTTTG--AACTTTCCATTTATAATCCTCAAAAT
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 V_dix_RS_225 GTTGACCATATGTGTTTTTTTTT-AACTTTCCATTTATAATCCTCAAAAT
 V_elon_RS_437 GTTGACCATATGTGTTTTTTTT????????????????????????????????
 V_elon_RS_494 GTTGACCATATGTGTTTTTTTTG--AACTTTCCATTTATAATCCTTAAAT
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 V_spRADS4_RS_214 GTTGACCACATATGTTTTTTTTG--AACTT-----TCCTCAAAAT

V_sur_RS_489 TCCTCCAAACAGCAGCTAAAATCATCATATTCGTTTACTTTTAGGCTGTG
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 C_debilis_7209 ACCTGCCCAATGGATGAATGGTTCAATTCTCATGAAGGTGTGACATT-GT
 I_juru_RS_451 ACCCGCCCAATGGATGAATGGTTCAATTCTCATGAAGGTGTGACATTTGT
 I_laev_RS_460 ACCCGCCCAATGGATGAATGGTTCAATTCTCATGAAGGTGTGACATTTGT
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 V_spRADS3_RS_335 ACCCACCCAATGGATGAATGGTTCAATTCTCATGAAGGTGTGACATTTGT
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 V_calor_RS_430 ACCCACCCAATGGATGAATGGTTCAATTCTCATGAAGGTGTGACATTTGT
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 V_elon_RS_437 ???ACATTTGT
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 V_lorSL_RS_483 ???
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 V_sur_RS_501 ACCCACCCAATGGATGAATAGTTTCAGTTCTCATGAAGGTGTGACATTTGT

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 C_debilis_7209 AAGATGATAAGA-GTGCC?GAGATCTAACAAATAAGGACTGCAGGAAAAT
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V_elon_RS_437 AAGATGATATGA-GTGCCTGAGACGTAACAATAAGGAGTGCAGGAAAAA
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V_elon_RS_502 AAGATGATATGA-GTGCCTGAGACGTAACAATAAGGAGTGCAGGAAAAA
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V_flex_RS_522 AAGATGATAAGA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAT
V_flex_RS_595 AAGATGATAAGA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAT
V_multin_RS_107 AAGATGATAAGA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAT
V_multin_RS_108 AAGATGATAAGA-GTRCCTGAGATCTAACAAATAAGGACTGCAGGAAAAT
V_loret_RS_480 AAGATGATAWGA-GTGCCTGAGACGTAACAATAAGGAGTGCAGGAAAGA
V_loret_RS_516 AAGATGATAKGA-GTGCCTGAGACGTAACAATAAGGAGTGCAGGAAARA
V_loret_RS_526 AAGATGATATGA-GTGCCTGAGACGTAACAATAAGGAGTGCAGGAAAAA
V_multi_RS_350 AAGATGATAAGA-GTGYCTGAGATCTAACAAATAAGGACTGCAGGAAAAT
V_multi_RS_429 AAGATGATAAGA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAT
V_multi_RS_466 AAGATGATAAGA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAT
V_multi_RS_543 AAGATGATAAGA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAT
V_multi_RS_549 AAGATGATAAGA-GTRCCTGAGATCTAACAAATAAGGACTGCAGGAAAAT
V_sebBL_RS_584 AAGATGATATGA-GTGCCTGAGACGTAACAATAAGGAGTGCAGGAAAAA
V_lorSL_RS_483 ??
V_lorSL_RS_507 AAGATGATATGA-GTGCCTGAGACGTAACAATAAGGAGTGCAGGAAAGA
V_sebSL_RS_534 AAGATGATATGA-GTGCCTGAGACGTAACAATAAGGAGTGCAGGAAAAA
V_sebSL_RS_552 AAGATGATATGA-GTGCCTGAGACGTAACAATAAGGAGTGCAGGAAAAA
V_spRADS4_RS_213 AAGATGATAAGA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAT
V_spRADS4_RS_214 AAGATGATAAGA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAT
V_sur_RS_082 AAGATGATAARA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAA
V_sur_RS_083 AAGATGATAAAA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAA
V_sur_RS_084 AAGATGATAAAA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAA
V_sur_RS_216 AAGATGATAAGA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAA
V_sur_RS_248 ??
V_sur_RS_324 ??
V_sur_RS_428 AAGATGATAARA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAA
V_sur_RS_489 AAGATGATAAAA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAA
V_sur_RS_501 AAGATGATAAAA-GTGCCTGAGATCTAACAAATAAGGACTGCAGGAAAAA

C_cap_RS_551 TTTCTCATATAAAT--GACTTGGAATGTCTATAGACAGAGAAATGGCCTT
C_debilis_7209 TTTCTCATATACAT--GACTTGGAATGTCTATAGACAGAGAAATGGCCTT
I_juru_RS_451 TTTCTCATATATATATGATTTGAAATGTCTATCGACAGA--AATGGCCTT
I_laev_RS_460 TTTCTCATATATATATGATTTGAAATGTCTATCGACAGA--AATGGCCTT
V_cadu_JJ_847 TTTCTCTTATATAT--GATTTGAGATGTCCATAGACAAAGAAATGGCCTT
V_spRADS3_RS_335 TTTCTCATATTTAT--GATTTGAGATGTCTATAGACAGAGAAATGGCCTT
V_spRADS3_RS_339 TTTCTCATATTTAT--GATTTGAGATGTCTATAGACAGAGAAATGGCCTT
V_peru_JJ_772 TTTCTCATATATAT--GATTTGAGATGTCTATAGACAGAGAAATGGCCTT
V_calor_RS_430 TTTCTCATATATAT--GATTTGAGATGTCTATAGACAGAGAAATGGCCTT
V_calor_RS_454 TTTCTCATATATAT--GATTTGAGATGTCTATAGACAGAGAAATGGCCTT
V_calor_RS_481 TTTCTCATATATAT--GATTTGAGATGTCTATAGACAGAGAAATGGCCTT

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 V_elon_RS_502 -CACTAACGTTCTT?????????????ATCTTCTATGCCACATACTTGT
 V_flex_RS_442 TCACTAATGTTCTTTGTCCTTTTATACATATCTTCTACGCCACATACTTGT
 V_flex_RS_522 TCACTAATGTTCTTTGTCCTTTTATACAT????????????????????
 V_flex_RS_595 TCACTAATGTTCTTTGTCCTTTTATACAT????????????????????
 V_multin_RS_107 TCACTAATGTTCTTTGTCCTTTTATACATAT?TTCTACGCCACATACTTGT
 V_multin_RS_108 TCACTAATGTTCTTTGYCCTTTTATACATATSTTCTACGCCACATACTTGT
 V_loret_RS_480 -CACTAATGTTCTTTGTCCTTTTAAATATCTTCTATGCCACATACTTGT
 V_loret_RS_516 -CACTAA?????????????????????ATCTTCTATGCCACATACTTGT
 V_loret_RS_526 -CACTA?GTTCTTTGYCCTTTTAAATATCTTCTATGCCACATACTTGT
 V_multi_RS_350 TCACTAATGTTCTTTGTCCTTTTATACATATCTTCTACGCCACATACTTGT
 V_multi_RS_429 TCACTAATGTTCTTTGTCCTTTTATACATATCTTCT?YGCCACATACTTGT
 V_multi_RS_466 TCACTAATGTTCTTTGTCCTTTTATACATATCTTCTACGCCACATACTTGT
 V_multi_RS_543 TCACTAATGTTCTTTGTCCTTTTATACATATCTTCTATGCCACATACTTGT
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 V_sebSL_RS_552 -CACTAATGTTCTTTGTCCTTTTAAATATCTTCTATGCCACATACTTGT
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 V_sur_RS_083 TCACTAATGTTCTTTGTCCTTTATGAATATCTTCTACGCCACATACTTGT
 V_sur_RS_084 TCACTAATGTTCTTTGTCCTTTATGAATAT?TTCTACGCCACATACTTGT
 V_sur_RS_216 TCACTAATGTTCTTTGTCCTTTTAAATATCTTCTACGCCACATACTTGT
 V_sur_RS_248 ??????????????????????????????????ATCTTCTACGCCACATACTTGT
 V_sur_RS_324 ??????????????????????????????????ATCTTCTACGCCACATACTTGT
 V_sur_RS_428 TCACTAATGTTCTTTGTCCTTTATGAATATCTTCTACGCCACATACTTGT
 V_sur_RS_489 TCACTAATGTTCTTTGTCCTTTATGAATATCTTCTACGCCACATACTTGT
 V_sur_RS_501 TCACTAATGTTCTTTGTCCTTTATGAATATCTTCTACGCCACATACTTGT

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 C_debilis_7209 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACAGGCATTTGAAG
 I_juru_RS_451 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACAGGCATTTGAAG
 I_laev_RS_460 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACAGGCATTTGAAG
 V_cadu_JJ_847 CTGAGAAAATTGGGTATTGGAGGTACATAACCATTTACAGGCATTTGAAG
 V_spRADS3_RS_335 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_spRADS3_RS_339 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_peru_JJ_772 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_calor_RS_430 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_calor_RS_454 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_calor_RS_481 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_calor_RS_511 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
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 V_dix_RS_225 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_elon_RS_437 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_elon_RS_494 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_elon_RS_502 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_flex_RS_442 CTGAGAAAATTGGGTATTGGAGGTACATAACCATTTACAGGCATTTGAAG
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 V_flex_RS_595 ??????????????????????GAGGTACATAACCATTTACAGGCATTTGAAG
 V_multin_RS_107 CTGAGAAAATTGGGTATTGGAGGTACATAACCATTTACAGGCATTTGAAG
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V_loret_RS_480 CTGAGAAAATTGGGTATTGGMGGTACATAACTATTTACCGGCATTTGAAG
 V_loret_RS_516 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_loret_RS_526 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_multi_RS_350 CTGAGAAAATTGGGTATTGGAGGTACATAACCATTTACAGGCATTTGAAG
 V_multi_RS_429 CTGAGAAAATTGGGTATTGGAGGTACATAACCATTTACAGGCATTTGAAG
 V_multi_RS_466 CTGAGAAAATTGGGTATTGGAGGTACATAACCATTTACAGGCATTTGAAG
 V_multi_RS_543 CTGAGAAAATTGGGTATTGGAGGTACATAACCATTTACAGGCATTTGAAG
 V_multi_RS_549 CTGAGAAAATTGGGTATTGGAGGTACATAACCATTTACAGGCATTTGAAG
 V_sebBL_RS_584 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_lorSL_RS_483 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
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 V_sebSL_RS_534 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_sebSL_RS_552 CTGAGAAAATTGGGTATTGGAGGTACATAACTATTTACCGGCATTTGAAG
 V_spRADS4_RS_213 CTGAGAAAATTGGGTATTGGAGGTACATAACCATTTACAGGCATTTGAAG
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 V_sur_RS_428 CTGAGAAAATTGGGTATTGGAGGTACATCACTATTTACAGGCATTTGAAG
 V_sur_RS_489 CTGAGAAAATTGGGTATTGGAGGTACATCACTATTTACAGGCATTTGAAG
 V_sur_RS_501 CTGAGAAAATTGGGTATTGGAGGTACATCACTATTTACAGGCATTTGAAG

 C_cap_RS_551 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 C_debilis_7209 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 I_juru_RS_451 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 I_laev_RS_460 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 V_cadu_JJ_847 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 V_spRADS3_RS_335 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 V_spRADS3_RS_339 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 V_peru_JJ_772 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 V_calor_RS_430 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 V_calor_RS_454 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
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 V_spRADS1_RS_432 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
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 V_loret_RS_516 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 V_loret_RS_526 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 V_multi_RS_350 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 V_multi_RS_429 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
 V_multi_RS_466 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAAGCTG
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V_multi_RS_549 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAACTG
V_sebBL_RS_584 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAACTG
V_lorSL_RS_483 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAACTG
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V_spRADS4_RS_213 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAACTG
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V_sur_RS_428 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAACTG
V_sur_RS_489 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAACTG
V_sur_RS_501 GCCAACCCAGAGTACCAACTTTACCCAATCTTCAAGTATTTGAGAACTG

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I_juru_RS_451 GTGCCAGGATGAGAACAGGCATGGAGATTTCTTCTCTGCATTGATGAAGG
I_laev_RS_460 GTGCCAGGATGAGAACAGGCATGGAGATTTCTTCTCTGCATTGATGAAGG
V_cadu_JJ_847 GTGCCAGGATGAGAACAGGCAYGGAGATTTCTTCTCTGCATTGATGAAGG
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V_peru_JJ_772 GTGCCAGGATGAGAACAGGCATGGAGATTTCTTCTCTGCATTGATGAAGG
V_calor_RS_430 GTGCCAGGATGAGAACAGGCATGGAGATTTCTTCTCTGCATTGATGAAGG
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 C_cap_RS_551 CGCAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTT
 C_debilis_7209 C?CAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTT
 I_juru_RS_451 CACAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTT
 I_laev_RS_460 CACAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTT
 V_cadu_JJ_847 CGCAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTT
 V_spRADS3_RS_335 CGCAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTT
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 V_lorSL_RS_507 CGCAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTT
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 V_sur_RS_084 CRCAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTT
 V_sur_RS_216 CGCAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTT
 V_sur_RS_248 CGCAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTT
 V_sur_RS_324 CGCAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTT

V_sur_RS_428 CGCAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTC
 V_sur_RS_489 CGCAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTC
 V_sur_RS_501 CRCAGCCTCAATTCCTCAATGACTGGAAAGCGAAGCTGTGGGCTCGTTTC

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 C_debilis_7209 TTCTGTCTATCGGTAAATGTTATTCAATTTTATTATA-----TTT
 I_juru_RS_451 TTCTGTCTATCGGTAAATGTTATTCAATTTTATTATA-----TTT
 I_laev_RS_460 TTCTGTCTATCGGTAAATGTTATTCAATTTTATTATA-----TTT
 V_cadu_JJ_847 TTCTGTCTATCGGTAAATGTTATTCAATTTTCTTAT-----ATTT
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 V_sur_RS_501 TTCTGTCTATCGGTAAATATTATTCAATTTTATTATTACTATTATTATTT

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 C_debilis_7209 AAAAGTGGATTTTTGTGGGTCATCTGTTCTCTTTTTATGT?GCTTATGCTT
 I_juru_RS_451 AAAAGTGGATTTTTGTGGGTCATCTGTTCTCTTTTTCATGT?T??CT?A??TT
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 V_flex_RS_522 AA??
 V_flex_RS_595 ???
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 V_sur_RS_428 AAAAGTGGATTTTGTGGGTCATCTGTTCTCTTTTTATGTTGCTTATGCTT
 V_sur_RS_489 AAAAGTGGATTTTGTGGGTCATCTGTTCTCTTTTTATGTTGCTTATGCTT
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V_multi_RS_466 CATTTCCTTGCAGGTAT
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V_sur_RS_216 C?????????????????
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V_sur_RS_489 CATTCTCTTGCAGGTAT
V_sur_RS_501 CATTCTCTTGCAGGTAT

Alignment 3.2

The trnH-psbA DNA nucleotide sequence alignment of 54 taxa and 363 characters used in NJ analyses in chapter 3. 50 base pairs per line.

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C_cap_RS_551      TCCGCCCCTTGTCTTTT-CT-----AAAGAAAAAAAA----TTTTCGAT
C_debilis_7209   TCCGCCCCTTGTCTTTT-CT-----AAAGAAAAAAAA----TTTTAGAT
I_juru_RS_451    TCCGCCCCTTGTCTTTT-CTTTTCT--AAAGACAAAAA----TTTTAGAT
I_laev_RS_460    TCCGCCCCTTGTCTTTT-CTTTTCT--AAAGACAAAAA----TTTTAGAT
V_cadu_JJ_847    TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAAAAAAAA--TTTAGAT
V_flex_RS_442    TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAAAAAAAA--TTTAGAT
V_flex_RS_522    TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAAAAAAAA--TTTAGAT
V_flex_RS_595    TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAAAAAAAA--TTTAGAT
V_multi_RS_107   TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAGAAAA--TTTAGAT
V_multi_RS_108   TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAGAAAA--TTTAGAT
V_multi_RS_350   TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAGAAAA--TTTAGAT
V_multi_RS_429   TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAGAAAA--TTTAGAT
V_multi_RS_466   TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAGAAAA--TTTAGAT
V_multi_RS_543   TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAGAAAA--TTTAGAT
V_multi_RS_549   TCCGCCCCTTGTCTTTTCT-----AAAGAAAAAGAAAA--TTTAGAT
V_spRADS4_RS_213 TCCGCCCCTTGTCTTTT-CCTTTTTGTACAGAAAAAAAA----TTTTGGAT
V_spRADS4_RS_214 TCCGCCCCTTGTCTTTT-CCTTTTTGTACAGAAAAAAAA----TTTTGGAT
V_surRS_082      TCCGCCCCTTGTCTTTT-CTTTTCT-AAAGAAAAAAAA----TTTTAGAT
V_sur_RS_083     TCCGCCCCTTGTCTTTT-CTTTTCT-AAAGAAAAAAAA----TTTTAGAT
V_sur_RS_084     TCCGCCCCTTGTCTTTT-CTTTTCT-AAAGAAAAAAAA----TTTTAGAT
V_pav_RS_216     TCCGCCCCTTGTCTTTT-CTTTTCT-AAAGAAAAAAAA----TTTTAGAT
V_sur_RS_248     ?????????????????-????????-??GAAAAAAAA----TTTTAGAT
V_sur_RS_428     TCCGCCCCTTGTCTTTT-CTTTTCT-AAAGAAAAAAAA----TTTTAGAT
V_sur_RS_489     TCCGCCCCTTGTCTTTT-CTTTTCT-AAAGAAAAAAAA----TTTTAGAT
V_sur_RS_501     TCCGCCCCTTGTCTTTT-CTTTTCT-AAAGAAAAAAAA----TTTTAGAT
V_multicost_1    ?????????????????????????????T-AAAGAAAAAAAAAATTTTAGAT
V_multicost_2    ?????????????????????????TTTTTTT-AAAGAAAAAAAAAATTTTAGAT
V_nobilis        TCCGCCCCTTGTCTTTT-CTTTTCT-AAAGAAAAAAAA----TTTTAGAT
V_multiflora     ???GCCCTTGTCTTTT-CTTTTCT-AAAGAAAAAAAA----TTTTAGAT
V_michelii_110149 ?????CCCTTGTCTTTTCT-----AAAGAAAAAAAAAAAA-TTTTAGAT
V_michelii_200263 ?????????????????????????????-?AAGAAAAAAAAAAAA-TTTTAGAT
V_michelii_110168 ?????CCCTTGTCTTTTCT-----AAAGAAAAAAAAAAAA-TTTTAGAT
V_michelii_110074 ?????CCCTTGTCTTTTCT-----AAAGAAAAAAAAAAAA-TTTTAGAT
V_kwatae_110070 ?????CCCTTGTCTTTT-CTTTTCT-AAAGAAAAAAAA----TTTTAGAT
V_spRADS3_RS_335 TCCGCCCCTTGTCTTTT-CTTTTCT-----
V_RADS3_RS_339   TCCGCCCCTTGTCTTTT-CTTTTCT-----
V_peru_JJ_772    TCCGCCCCTTGTCTTTT-CTTTTCT-----
V_calor_RS_430   TCCGCCCCTTGTCTTTT-CTTTTCT-----
V_calor_RS_454   ?????CCTTGTCTTTT-CTTTTCT-----
V_calor_RS_511   TCCGCCCCTTGTCTTTT-CTTTTCT-----
V_spRADS1_RS_432 TCCGCCCCTTGTCTTTT-CTTTTCT-----
V_spRADS1_RS_500 TCCGCCCCTTGTCTTTT-CTTTTCT-----
V_spRADS1_RS_510 TCCGCCCCTTGTCTTTT-CTTTTCT-----
V_spRADS1_RS_561 TCCGCCCCTTGTCTTTT-CTTTTCT-----
V_dix_RS_225     TCCGCCCCTTGTCTTTT-CTTTTCT-----
V_elon_RS_437    ?????CCTTGTCTTTT-CTTTTCT-----
V_elonRS_502     TCCGCCCCTTGTCTTTT-CTTTTCT-----
V_lorBL_RS_480   TCC?CCCCTTGTCTTTT-CTTTTCT-----
V_lorBL_RS_526   TCCGCCCCTTGTCTTTT-CTTTTCT-----

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V_sebBL_RS_584	TCCGCCCTTGTCTTTT-CTTTTTCT-----
V_lorBL_RS_516	TCCGCCCTTGTCTTTT-CTTTTTCT-----
V_lorSL_RS_483	TCCGCCCTTGTCTTTT-CTTTTTCT-----
V_sebSL_RS_507	TCCGCCCTTGTCTTTT-CTTTTTCT-----
V_sebSL_RS_552	TCCGCCCTTGTCTTTT-CTTTTTCT-----
C_cap_RS_551	TACTAGTCTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATC
C_debilis_7209	TACTAGTCTTTCTTATTTTTTTCATACAAATTTCTATCCTTTAGAAA--
I_juru_RS_451	TACTAGTCTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
I_laev_RS_460	TACTAGTCTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_cadu_JJ_847	TACTAGTCTTTCTTCTTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_flex_RS_442	TACTAGTCTTTCTTCTTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_flex_RS_522	TACTAGTCTTTCTTCTTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_flex_RS_595	TACTAGTCTTTCTTCTTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_multi_RS_107	TACTAGTCTTTCTTCTTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_multi_RS_108	TACTAGTCTTTCTTCTTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_multi_RS_350	TACTAGTCTTTCTTCTTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_multi_RS_429	TACTAGTCTTTCTTCTTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_multi_RS_466	TACTAGTCTTTCTTCTTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_multi_RS_543	TACTAGTCTTTCTTCTTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_multi_RS_549	TACTAGTCTTTCTTCTTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_spRADS4_RS_213	TTCTAGTCTTTCTTATTTTTTTCATACTAA-----
V_spRADS4_RS_214	TTCTAGTCTTTCTTATTTTTTTCATACTAA-----
V_surRS_082	TACTAGTCTTTCTTATTTTTT-CATACTAATTTCTACCCTTTAGAAAATT
V_sur_RS_083	TACTAGTCTTTCTTATTTTTT-CATACTAATTTCTACCCTTTAGAAAATT
V_sur_RS_084	TACTAGTCTTTCTTATTTTTT-CATACTAATTTCTACCCTTTAGAAAATT
V_pav_RS_216	TACTAGTCTTTCTTATTTTTT-CATACTAATTTCTACCCTTTAGAAAATT
V_sur_RS_248	TACTAGTCTTTCTTATTTTTT-CATACTAATTTCTACCCTTTAGAAAATT
V_sur_RS_428	TACTAGTCTTTCTTATTTTTT-CATACTAATTTCTACCCTTTAGAAAATT
V_sur_RS_489	TACTAGTCTTTCTTATTTTTT-CATACTAATTTCTACCCTTTAGAAAATT
V_sur_RS_501	TACTAGTCTTTCTTATTTTTT-CATACTAATTTCTACCCTTTAGAAAATT
V_multicost_1	TACTAGTCTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_multicost_2	TACTAGTCTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_nobilis	TACTAGTCTTTCTTATTTTTT-CATACTAATTTCTACCCTTTAGAAAATT
V_multiflora	TACTAGTCTTTCTTATTTTTT-CATACTAATTTCTACCCTTTAGAAAATT
V_michelii_110149	TACTAGTCTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_michelii_200263	TACTAGTCTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_michelii_110168	TACTAGTCTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_michelii_110074	TACTAGTCTTTCTTATTTTTTTCATACTAATTTCTACCCTTTAGAAAATT
V_kwatae_110070	TACTAGTCTTTCTTATTTTTT-CATACTAATTTCTACCCTTTAGAAAATT
V_spRADS3_RS_335	-----
V_RADS3_RS_339	-----
V_peru_JJ_772	-----
V_calor_RS_430	-----
V_calor_RS_454	-----
V_calor_RS_511	-----
V_spRADS1_RS_432	-----
V_spRADS1_RS_500	-----
V_spRADS1_RS_510	-----
V_spRADS1_RS_561	-----
V_dix_RS_225	-----
V_elon_RS_437	-----
V_elonRS_502	-----
V_lorBL_RS_480	-----
V_lorBL_RS_526	-----
V_sebBL_RS_584	-----
V_lorBL_RS_516	-----

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V_lorSL_RS_483 -----
V_sebSL_RS_507 -----
V_sebSL_RS_552 -----

C_cap_RS_551 GACAATAGGAAAAAATGCATTTT TAGGAATGTACATGAACTGAAGATCAGT
C_debilis_7209 --CAATAGGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCCGT
I_juru_RS_451 GACAATAGGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGT
I_laev_RS_460 GACAATAGGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_cadu_JJ_847 GACAATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGT
V_flex_RS_442 GACAATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGT
V_flex_RS_522 GACAATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGT
V_flex_RS_595 GACAATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGT
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V_multi_RS_350 GACAATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGT
V_multi_RS_429 GACAATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGT
V_multi_RS_466 GACAATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGT
V_multi_RS_543 GACAATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGT
V_multi_RS_549 GACAATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGT
V_spRADS4_RS_213 -----AGGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_spRADS4_RS_214 -----AGGAAAAAATGCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_surRS_082 GACAATAGGAAAAAATTCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_sur_RS_083 GACAATAGGAAAAAATTCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_sur_RS_084 GACAATAGGAAAAAATTCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_pav_RS_216 GACAATAGGAAAAAATTCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_sur_RS_248 GACAATAGGAAAAAATTCATTTT TAGGAATGTACAGAACTGAAGATCAGT
V_sur_RS_428 GACAATAGGAAAAAATTCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_sur_RS_489 GACAATAGGAAAAAATTCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_sur_RS_501 GACAATAGGAAAAAATTCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_multicost_1 GACAATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGT
V_multicost_2 GACAATAGGAAAAAATTCATTTT TAGGAATGAACATAAACTGAAGATCAGT
V_nobilis GACAATAGGAAAAAATTCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_multiflora GACAATAGGAAAAAATTCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_michelii_110149 GACAATAGGAAAAAATGCATTTT TAGGAATGAACATAAACTGAAGATCAGT
V_michelii_200263 GACAATAGGAAAAAATGCATTTT TAGGAATGAACATAAACTGAAGATCAGT
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V_michelii_110074 GACAATAGGAAAAAATGCATTTT TAGGAATGAACATAAACTGAAGATCAGT
V_kwatae_110070 GACAATAGGAAAAAATTCATTTT TAGGAATGTACATAAACTGAAGATCAGT
V_spRADS3_RS_335 -----
V_RADS3_RS_339 -----
V_peru_JJ_772 -----
V_calor_RS_430 -----
V_calor_RS_454 -----
V_calor_RS_511 -----
V_spRADS1_RS_432 -----
V_spRADS1_RS_500 -----
V_spRADS1_RS_510 -----
V_spRADS1_RS_561 -----
V_dix_RS_225 -----
V_elon_RS_437 -----
V_elonRS_502 -----
V_lorBL_RS_480 -----
V_lorBL_RS_526 -----
V_sebBL_RS_584 -----
V_lorBL_RS_516 -----
V_lorSL_RS_483 -----
V_sebSL_RS_507 -----

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V_sebSL_RS_552 -----
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C_debilis_7209 T-AAAATCAAAAAA-----GGTATGATGTTTCGATCATGAACCAAAT
I_juru_RS_451 T-AAAATAAAAAATAAAAAAAGGTATGATGTTTCGATCATGAA-CAAA-
I_laev_RS_460 T-AAAATAAAAAA-----GGTATGATGTTTCGATCATGAA-CAAA-
V_cadu_JJ_847 T-AAAATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
V_flex_RS_442 T-AAAATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
V_flex_RS_522 T-AAAATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
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V_spRADS4_RS_214 T-AAAATAAAAAAGAAAAA----GGTATAATGTTTCGATCATTAAACAAAT
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V_sur_RS_084 T-AAAATAAAAAATAAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
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V_sur_RS_501 T-AAAATAAAAAATAAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
V_multicost_1 T-AAAATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
V_multicost_2 T-AAAATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
V_nobilis T-AAAATAAAAAATAAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
V_multiflora T-AAAATAAAAAATAAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
V_michelii_110149 T-AAAATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
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V_michelii_110168 T-AAAATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
V_michelii_110074 T-AAAATAAAAAAGAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
V_kwatae_110070 T-AAAATAAAAAATAAAAAA----GGTATGATGTTTCGATCATGAAACAAAT
V_spRADS3_RS_335 -----
V_RADS3_RS_339 -----
V_peru_JJ_772 -----
V_calor_RS_430 -----
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V_spRADS1_RS_561 -----
V_dix_RS_225 -----
V_elon_RS_437 -----
V_elonRS_502 -----
V_lorBL_RS_480 -----
V_lorBL_RS_526 -----
V_sebBL_RS_584 -----
V_lorBL_RS_516 -----
V_lorSL_RS_483 -----
V_sebSL_RS_507 -----
V_sebSL_RS_552 -----

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C_debilis_7209 AATTAATATTTTCTGAAATTGAAAAA-----TCTTATG-----
I_juru_RS_451 AATGAATATTTTCTTAAATTGAAAAA-----TCTTATG-----
I_laev_RS_460 AATGAATATTTTCTTAAATTGAAAAA-----CAAAATCTTATG-----
V_cadu_JJ_847 AATTAATATTTTAT-AAAAAAAA-----GAAA-TCTTATG-----
V_flex_RS_442 AATTAATATTTTCT-AAAAAAAA-----GAAA-TCTTATG-----
V_flex_RS_522 AATTAATATTTTCT-AAAAAAAA-----GAAA-TCTTATG-----
V_flex_RS_595 AATTAATATTTTCT-AAAAAAAA-----GAAA-TCTTATG-----
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V_multi_RS_549 AATTAATATTTTCT-AAAAAAAA-----GAAA-TCTTATG-----
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V_spRADS4_RS_214 AATTAATATTTTCTTAAAAAAAA-----GAAA-TCTTATG-----
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V_sur_RS_083 AATGAATATTTTCTTAAAAAAAA-----GAAA-TCTTATG-----
V_sur_RS_084 AATGAATATTTTCTTAAAAAAAA-----GAAA-TCTTATG-----
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V_sur_RS_248 AATTAATATTTTCTTAAAAAAAAAAAAAAAA-----GAAA-TCTTATG-----
V_sur_RS_428 AATGAATATTTTCTTAAAAAAAA-----GAAA-TCTTATG-----
V_sur_RS_489 AATGAATATTTTCTTAAAAAAAA-----GAAA-TCTTATG-----
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V_multicost_2 AATTAATATTTTCT-AAAAAAAA-----GAAA-TCTTATG-----
V_nobilis AATTAATATTTTCTTAAAAAAAA-----GAAA-TCTTATG-----
V_multiflora AATTAATATTTTCTTAAAAAAAA-----GAAA-TCTTATG-----
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V_michelii_110074 AATGAATATTTTCT-AAAAAAAA-----GAAA-TCTTATG-----
V_kwatae_110070 AATTAATATTTTCTTAAAAAAAAAAAAAAAA-----CTTTTAGG-----
V_spRADS3_RS_335 -----AAAAAAAA-----GAAA-TCTTATGTTATG
V_RADS3_RS_339 -----AAAAAAAA-----GAAA-TCTTATGTTATG
V_peru_JJ_772 -----AAAAAAAA-----TCTTATGTTATG
V_calor_RS_430 -----AAAAAAAA-----TCTTATGTTATG
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V_lorBL_RS_516 -----AAAAAAAA-----TCTTATGTTATG
V_lorSL_RS_483 -----AAAAAAAA-----TCTTATGTTATG
V_sebSL_RS_507 -----AAAAAAAA-----TCTTATGTTATG
V_sebSL_RS_552 -----AAAAAAAA-----TCTTATGTTATG

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C_debilis_7209 TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGG

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V_cadu_JJ_847	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_flex_RS_442	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
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V_flex_RS_595	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_multi_RS_107	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_multi_RS_108	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_multi_RS_350	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_multi_RS_429	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_multi_RS_466	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
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V_multi_RS_549	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_spRADS4_RS_213	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_spRADS4_RS_214	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_surRS_082	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_sur_RS_083	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_sur_RS_084	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_pav_RS_216	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_sur_RS_248	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_sur_RS_428	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_sur_RS_489	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_sur_RS_501	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
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V_multicost_2	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_nobilis	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_multiflora	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_michelii_110149	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_michelii_200263	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_michelii_110168	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_michelii_110074	TGAGTAAACCACTACTGAACCAGATCAATACCCAGTGGGTATTGATCTGA
V_kwatae_110070	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_spRADS3_RS_335	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_RADS3_RS_339	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_peru_JJ_772	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_calor_RS_430	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_calor_RS_454	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_calor_RS_511	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_spRADS1_RS_432	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_spRADS1_RS_500	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_spRADS1_RS_510	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_spRADS1_RS_561	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_dix_RS_225	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_elon_RS_437	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_elonRS_502	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_lorBL_RS_480	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_lorBL_RS_526	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_sebBL_RS_584	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_lorBL_RS_516	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_lorSL_RS_483	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_sebSL_RS_507	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
V_sebSL_RS_552	TGAGTAAACCACTACTGAACCAGATCAATACCCACTGGGTATTGATCTGA
C_cap_RS_551	TCCTTCAATGACTCGTATACTAATAACCGAAATATTAGCCATTTATTGA
C_debilis_7209	TCCTTCAATGACTCGTATACTAATAACCGAAATATTAGCCATTTATTGA
I_juru_RS_451	TCCTTCAATGACTCGTATACTAATAACCGAAGTATTAGCCATTTGTTGA
I_laev_RS_460	TCCTTCAATGACTCGTATACTAATAACCGAAGTATTAGCCATTTGTTGA

V_cadu_JJ_847	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_flex_RS_442	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_flex_RS_522	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_flex_RS_595	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_multi_RS_107	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_multi_RS_108	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_multi_RS_350	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_multi_RS_429	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_multi_RS_466	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_multi_RS_543	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_multi_RS_549	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_spRADS4_RS_213	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_spRADS4_RS_214	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_surRS_082	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_sur_RS_083	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_sur_RS_084	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_pav_RS_216	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_sur_RS_248	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_sur_RS_428	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_sur_RS_489	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_sur_RS_501	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_multicost_1	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_multicost_2	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_nobilis	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_multiflora	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_michelii_110149	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_michelii_200263	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_michelii_110168	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_michelii_110074	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_kwatae_110070	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_spRADS3_RS_335	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_RADS3_RS_339	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_peru_JJ_772	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_calor_RS_430	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_calor_RS_454	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_calor_RS_511	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_spRADS1_RS_432	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_spRADS1_RS_500	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_spRADS1_RS_510	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_spRADS1_RS_561	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_dix_RS_225	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_elon_RS_437	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_elonRS_502	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_lorBL_RS_480	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_lorBL_RS_526	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_sebBL_RS_584	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_lorBL_RS_516	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_lorSL_RS_483	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_sebSL_RS_507	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
V_sebSL_RS_552	TCCTTCAATTACTCGTATACTAATAACCGAAATATTAGCCATTTGTTGA
C_cap_RS_551	TAGAGCTTCAACA
C_debilis_7209	TAGAGCTTCAACA
I_juru_RS_451	TAGAGCTTCAACA
I_laev_RS_460	TAGAGCTTCAACA
V_cadu_JJ_847	TGGAGCTTCAACA
V_flex_RS_442	TGGAGCTTCAACA

V_flex_RS_522	TGGAGCTTCAACA
V_flex_RS_595	TGGAGCTTCAACA
V_multi_RS_107	TGGAGCTTCAACA
V_multi_RS_108	TGGAGCTTCAACA
V_multi_RS_350	TGGAGCTTCAACA
V_multi_RS_429	TGGAGCTTCAACA
V_multi_RS_466	TGGAGCTTCAACA
V_multi_RS_543	TGGAGCTTCAACA
V_multi_RS_549	TGGAGCTTCAACA
V_spRADS4_RS_213	TGGAGCTTCAACA
V_spRADS4_RS_214	TGGAGCTTCAACA
V_surRS_082	TGGAGCTTCAACA
V_sur_RS_083	TGGAGCTTCAACA
V_sur_RS_084	TGGAGCTTCAACA
V_pav_RS_216	TGGAGCTTCAACA
V_sur_RS_248	??????????????
V_sur_RS_428	TGGAGCTTCAACA
V_sur_RS_489	TGGAGCTTCAACA
V_sur_RS_501	TGGAGCTTCAACA
V_multicost_1	TGGAGC????????
V_multicost_2	????????????????
V_nobilis	????????????????
V_multiflora	????????????????
V_michelii_110149	????????????????
V_michelii_200263	????????????????
V_michelii_110168	????????????????
V_michelii_110074	????????????????
V_kwatae_110070	????????????????
V_spRADS3_RS_335	TAGAGCTTCAACT
V_RADS3_RS_339	TAGAGCTTCAACT
V_peru_JJ_772	TAGAGCTTCAACT
V_calor_RS_430	TAGAGCTTCAACT
V_calor_RS_454	TAGAGCTTCAACT
V_calor_RS_511	TAGAGCTTCAACT
V_spRADS1_RS_432	TAGAGCTTCAACT
V_spRADS1_RS_500	TAGAGCTTCAACT
V_spRADS1_RS_510	TAGAGCTTCAACT
V_spRADS1_RS_561	TAGAGCTTCAACT
V_dix_RS_225	TAGAGCTTCAACT
V_elon_RS_437	TAGAGCTTCAACT
V_elonRS_502	TAGAGCTTCAACT
V_lorBL_RS_480	TAGAGCTTCAACT
V_lorBL_RS_526	TAGAGCTTCAACT
V_sebBL_RS_584	TAGAGCTTCAACT
V_lorBL_RS_516	TAGAGCTTCAACT
V_lorSL_RS_483	TAGAGCTTCAACT
V_sebSL_RS_507	TAGAGCTTCAACT
V_sebSL_RS_552	TAGAGCTTCAACT

Appendix 4

Alignment 4.1

DNA nucleotide sequence alignment for AGT1 containing 683 characters and 36 unique alleles from cloned sequences employed in haplotype analyses in chapter 4. 50 base pairs per line.

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V_lorBL_RS516      TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorBL_RS516_2    TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorBL_RS526      TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorBL_RS526_2    TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorBL_RS480      TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorBL_RS480_2    TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorBL_RS480_2_2  TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorSL_RS452      TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorSL_RS452_2    TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorSL_RS452_3    TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorSL_RS465      TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorSL_RS465_2    TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorSL_RS465_3    TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorSL_RS465_4    TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebBL_ML13       TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebBL_ML13_2     TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebBL_ML13_3     TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebBL_RS435      TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebBL_RS435_2    TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebBL_RS503      TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebBL_RS503_2    TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebBL_RS503_3    TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebBL_RS582      TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebBL_RS582_2    TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebBL_RS636      TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebBL_RS636_2    TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorSL_RS483      TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_lorSL_RS483_2    TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebSL_RS443      TATGTGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebSL_RS443_2    TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebSL_RS444      TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebSL_RS464      TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebSL_RS529      TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebSL_RS529_2    TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebSL_RS533      TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG
V_sebSL_RS533_2    TATGCGCAAGTCCAAAAGCATTGGAAGCATCCAAGACTGCTAAATCAG

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V_lorBL_RS516      TCAGAGTATTCTTTGACTGGAAAGACTACCTCAAGTTCTACAAGTTGG
V_lorBL_RS516_2    TCAGAGTATTCTTTGACTGGAAAGACTACCTCAAGTTCTACAAGTTGG
V_lorBL_RS526      TCAGAGTATTCTTTGACTGGAAAGACTACCTCAAGTTCTACAAGTTGG
V_lorBL_RS526_2    TCAGAGTATTCTTTGACTGGAAAGACTACCTCAAGTTCTACAAGTTGG
V_lorBL_RS480      TCAGAGTATTCTTTGACTGGAAAGACTACCTCAAGTTCTACAAGTTGG
V_lorBL_RS480_2    TCAGAGTATTCTTTGACTGGAAAGACTACCTCAAGTTCTACAAGTTGG
V_lorBL_RS480_2_2  TCAGAGTATTCTTTGACTGGAAAGACTACCTCAAGTTCTACAAGTTGG
V_lorSL_RS452      TCAGAGTATTCTTTGACTGGAAAGACTACCTCAAGTTCTACAAGTTGG
V_lorSL_RS452_2    TCAGAGTATTCTTTGACTGGAAAGACTACCTCAAGTTCTACAAGTTGG
V_lorSL_RS452_3    TCAGAGTATTCTTTGACTGGAAAGACTACCTCAAGTTCTACAAGTTGG
V_lorSL_RS465      TCAGAGTATTCTTTGACTGGAAAGACTACCTCAAGTTCTACAAGTTGG

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V_sebSL_RS464 GAACATATTGGCCCTACACACCTTCTATACATCTTTTGTATGGCCTGA
 V_sebSL_RS529 GAACATATTGGCCCTACACACCTTCTATACATCTTTTGTATGGCCTGA
 V_sebSL_RS529_2 GAACATATTGGCCCTACACACCTTCTATACATCTTTTGTATGGCCTGA
 V_sebSL_RS533 GAACATATTGGCCCTACACACCTTCTATACATCTTTTGTATGGCCTGA
 V_sebSL_RS533_2 GAACATATTGGCCCTACACACCTTCTATACATCTTTTGTATGGCCTGA

 V_lorBL_RS516 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorBL_RS516_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorBL_RS526 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorBL_RS526_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorBL_RS480 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorBL_RS480_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorBL_RS480_2_ GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorSL_RS452 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorSL_RS452_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorSL_RS452_3 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorSL_RS465 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorSL_RS465_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorSL_RS465_3 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorSL_RS465_4 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebBL_ML13 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebBL_ML13_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebBL_ML13_3 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebBL_RS435 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebBL_RS435_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebBL_RS503 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebBL_RS503_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebBL_RS503_3 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebBL_RS582 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebBL_RS582_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebBL_RS636 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebBL_RS636_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorSL_RS483 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_lorSL_RS483_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebSL_RS443 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebSL_RS443_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebSL_RS444 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebSL_RS464 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebSL_RS529 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebSL_RS529_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebSL_RS533 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG
 V_sebSL_RS533_2 GAGCAGCTTTGGATCTCATATTTGTGGAAGGACTTGAAAATGTGTTTG

 V_lorBL_RS516 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorBL_RS516_2 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorBL_RS526 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorBL_RS526_2 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorBL_RS480 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorBL_RS480_2 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorBL_RS480_2_ ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorSL_RS452 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorSL_RS452_2 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorSL_RS452_3 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorSL_RS465 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorSL_RS465_2 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorSL_RS465_3 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA
 V_lorSL_RS465_4 ACAGACACAAACGTTTGGGCAAAGCAACAAGGTAATAGATTTTGGAAA

V_sebSL_RS533 GATGTAGTGATAAATGCTAATATTGAGCACTCGATGTCCATAGTAAGA
V_sebSL_RS533_2 GATGTAGTGATGAATGCTAATATTGAGCACTCGATGTCCATAGTAAGA

V_lorBL_RS516 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorBL_RS516_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorBL_RS526 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorBL_RS526_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorBL_RS480 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorBL_RS480_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorBL_RS480_3 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorSL_RS452 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorSL_RS452_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorSL_RS452_3 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorSL_RS465 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorSL_RS465_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorSL_RS465_3 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorSL_RS465_4 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebBL_ML13 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebBL_ML13_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebBL_ML13_3 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebBL_RS435 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebBL_RS435_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebBL_RS503 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebBL_RS503_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebBL_RS503_3 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebBL_RS582 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebBL_RS582_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebBL_RS636 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebBL_RS636_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorSL_RS483 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_lorSL_RS483_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebSL_RS443 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebSL_RS443_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebSL_RS444 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebSL_RS464 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebSL_RS529 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebSL_RS529_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebSL_RS533 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT
V_sebSL_RS533_2 GAGGCACACCATTTATTTATTGGGAACTACCCTGGATGGGCGGGGAAT

V_lorBL_RS516 ATTGCAAAAAATTTTCAGCAGTGGGACTGTCCTAGACACTGATATTGAC
V_lorBL_RS516_2 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_lorBL_RS526 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_lorBL_RS526_2 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_lorBL_RS480 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_lorBL_RS480_2 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_lorBL_RS480_3 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_lorSL_RS452 ATTGCAAAAAATTTTCAGCAGTGGGACTGTCCTAGACACTGATATTGAC
V_lorSL_RS452_2 ATTGCAAAAAATTTTCAGCAGTGGGACTGTCCTAGACACTGATATTGAC
V_lorSL_RS452_3 ATTGCAAAAAATTTTCAGCAGTGGGACTGTCCTAGACACTGATATTGAC
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V_lorSL_RS465_4 ATTGCAAAAA-TTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_sebBL_ML13 ATTGCAAAAAATTTTCAGCAGTGGGACTGTCCTAGACACTGATATTGAC
V_sebBL_ML13_2 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_sebBL_ML13_3 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC

V_sebBL_RS435 ATTGCAAAAAATTTTCAGCAGTCGGACTATCCTAGACACTGATATTGAC
V_sebBL_RS435_2 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_sebBL_RS503 ATTGCAAAAAATTTTCAGCAGTCGGACTATCCTAGACACTGATATTGAC
V_sebBL_RS503_2 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_sebBL_RS503_3 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_sebBL_RS582 ATTGCAAAAAATTTTCAGCAGTCGGACTATCCTAGACACTGATATTGAC
V_sebBL_RS582_2 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_sebBL_RS636 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
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V_lorSL_RS483 ATTGCAAAAAATTTTCAGCAGTGGGACTGTCTAGACACTGATATTGAC
V_lorSL_RS483_2 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_sebSL_RS443 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
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V_sebSL_RS529_2 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_sebSL_RS533 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC
V_sebSL_RS533_2 ATTGCAAAAAATTTTCAGCAGTGGGACTATCCTAGACACTGATATTGAC

V_lorBL_RS516 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTT---GAACT
V_lorBL_RS516_2 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTT---GAACT
V_lorBL_RS526 AACTTTTGCCCCAGTGAATGTTGACCATATATGTGTTTTTTTT-GAACT
V_lorBL_RS526_2 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTT---GAACT
V_lorBL_RS480 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTTTTT-AACT
V_lorBL_RS480_2 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTTTTT-AACT
V_lorBL_RS480_3 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTTTTT-AACT
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V_lorSL_RS465_3 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTT---AACT
V_lorSL_RS465_4 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTT---AACT
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V_sebBL_ML13_2 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTT---GAACT
V_sebBL_ML13_3 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTT---AACT
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V_sebBL_RS435_2 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTT---GAACT
V_sebBL_RS503 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTTTTT-AACT
V_sebBL_RS503_2 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTCTT-AACT
V_sebBL_RS503_3 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTCTT-AACT
V_sebBL_RS582 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTTTTT-AACT
V_sebBL_RS582_2 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTT---GAACT
V_sebBL_RS636 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTT---GAACT
V_sebBL_RS636_2 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTT---GAACT
V_lorSL_RS483 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTT---GAACT
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V_sebSL_RS444 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTTTTT-AACT
V_sebSL_RS464 TACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTCTT-AACT
V_sebSL_RS529 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTTTTT-AACT
V_sebSL_RS529_2 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTTTTT-AACT
V_sebSL_RS533 AACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTTTTT-AACT
V_sebSL_RS533_2 TACTTTTGCCCCAGTGAATGTTGACCATATGTGTTTTTTTTCTT-AACT

V_lorBL_RS516 TTCCATTTATAATCCTCAAAATTCCTCCAAACAGTAGCTAAAATCGTT
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V_lorBL_RS526 TTCCATTTATAATCCTCAAAATTCCTCCAAACAGTAGCTAAAATCATT
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V_sebBL_RS636_2 TTCCATTTATAATCCTCAAAATTCCTCCAAACAGTAGCTAAAATCATT
V_lorSL_RS483 TTCCATTTATAATCCTCAAAATTCCTCCAAACAGTAGCTAAAATCGTT
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V_sebSL_RS529_2 TTCCATTTATAATCCTCAAAATTCCTCCAAACAGTAGCTAAAATCATT
V_sebSL_RS533 TTCCATTTATAATCCTCAAAATTCCTCCAAACAGTAGCTAAAATCATT
V_sebSL_RS533_2 TTCCATTTATAATCCTCAAAATTCCTCCAAACAGTAGCTAAAATCATT

V_lorBL_RS516 ATATTCTTTTACTTTAAGGCTGTGACCCACCCAATGGATGAATGGTTC
V_lorBL_RS516_2 ATATTCTTTTACTTTAAGGCTGTGACCCACCCAATGGATGAATGGTTC
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V_lorBL_RS526_2 ATATTCTTTTACTTTAAGGCTGTGACCCACCCAATGGATGAATGGTTC
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V_lorBL_RS480_3 ATAGTCTTTTACTTTAAGGCTGTGACCCACCCAATGGATAAATGGTTC
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V_lorSL_RS465_4 ATATTCTTTTACTTTAAGGCTGTGACCCACCCAATGGATATATGGTTC
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V_sebBL_ML13_2 ATATTCTTTTACTTTAAGGCTGTGACCCACCCAATGGATGAATGGTTC
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V_sebBL_RS503_2 ATATTCTTTTACTTTAAGGCTGTGACCCACCCAATGGATAAATGGCTC
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V_sebBL_RS582_2 ATATTCTTTTACTTTAAGGCTGTGACCCACCCAATGGATGAATGGTTC
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V_sebBL_RS636_2 ATATTCTTTTACTTTAAGGCTGTGACCCACCCAATGGATGAATGGTTC
V_lorSL_RS483 ATATTCTTTTACTTTAAGGCTGTGACCCACCCAATGGATGAATGGTTC
V_lorSL_RS483_2 ATATTCTTTTACTTTAAGGCTGTGACCCACCCAATGGATAAATGGTTC
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V_sebSL_RS533 ATATTCTTTTACTTTAAGGCTGTGACCCACCCAATGGATAAATGGTTC
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V_lorBL_RS516 AATTCTCATGAAGGTGTGACATTTGTAAGATGATATGAGTGCCTGAGA
V_lorBL_RS516_2 AATTCTCATGAAGGTGTGACATTTGTAAGATGATAAGAGTGCCTGAGA
V_lorBL_RS526 AATTCTCATGAAGGTGTGACATTTGTAAGATGATATGAGTGCCTGAGA
V_lorBL_RS526_2 AATTCTCATGAAGGTGTGACATTTGTAAGATGATAAGAGTGCCTGAGA
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V_lorBL_RS480_2_2 AATTCTCATGAAGGTGTGACATTTGTAAGATGATATGAGTGCCTGAGA
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V_lorSL_RS452_3 AATTCTCATGAAGGTGTGACATTTGTAAGATGATATGAGTGCCTGAGG
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V_lorSL_RS465_3 AATTCTCATGAAGGTGTGACATTTGTAAGATGATATGAGTGCCTGAGA
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V_sebBL_ML13_2 AATTCTCATGAAGGTGTGACATTTGTAAGATGATATGTGTGCCTGAGA
V_sebBL_ML13_3 AATTCTCATGAAGGTGTGACATTTGTAAGATGATATGAGTGCCTGAGA
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V_lorBL_RS516_2 CGTAACAAATAAGGAGTGCAGGAAAGATTTCTCATATATATGATTTGA
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 V_sebBL_RS435_2 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT
 V_sebBL_RS503 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT
 V_sebBL_RS503_2 GATGCCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT
 V_sebBL_RS503_3 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT
 V_sebBL_RS582 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT

V_sebBL_RS582_2 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT
V_sebBL_RS636 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCGTTT
V_sebBL_RS636_2 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT
V_lorSL_RS483 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT
V_lorSL_RS483_2 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT
V_sebSL_RS443 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT
V_sebSL_RS443_2 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT
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V_sebSL_RS464 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT
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V_sebSL_RS533 GATGTCTATAGACAGAGAAATGGCCTTCACTAATGTTCTTTGTCCTTT
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V_lorBL_RS516 TTAAATGTAGG
V_lorBL_RS516_2 TTAAATGTAGG
V_lorBL_RS526 TTAAATGTAGG
V_lorBL_RS526_2 TTAAATGTAGG
V_lorBL_RS480 TTAAATGTAGG
V_lorBL_RS480_2 TTAAATGTAGG
V_lorBL_RS480_2_ TTAAATGTAGG
V_lorSL_RS452 TTAAATGTAGG
V_lorSL_RS452_2 TTAAATGTAGG
V_lorSL_RS452_3 TTAAATGTAGG
V_lorSL_RS465 TTAAATGTAGG
V_lorSL_RS465_2 TTAAATGTAGG
V_lorSL_RS465_3 TTAAATGTAGG
V_lorSL_RS465_4 TTAAATGTAGG
V_sebBL_ML13 TTAAATGTAGG
V_sebBL_ML13_2 TTAAATGTAGG
V_sebBL_ML13_3 TTAAATGTAGG
V_sebBL_RS435 TTAAATGTAGG
V_sebBL_RS435_2 TTAAATGTAGG
V_sebBL_RS503 TTAAATGTAGG
V_sebBL_RS503_2 TTAAATGTAGG
V_sebBL_RS503_3 TTAAATGTAGG
V_sebBL_RS582 TTAAATGTAGG
V_sebBL_RS582_2 TTAAATGTAGG
V_sebBL_RS636 TTAAATGTAGG
V_sebBL_RS636_2 TTAAATGTAGG
V_lorSL_RS483 TTAAATGTAGG
V_lorSL_RS483_2 TTAAATGTAGG
V_sebSL_RS443 TTAAATGTAGG
V_sebSL_RS443_2 TTAAATGTAGG
V_sebSL_RS444 TTAAATGTAGG
V_sebSL_RS464 TTAAATGTAGG
V_sebSL_RS529 TTAAATGTAGG
V_sebSL_RS529_2 TTAAATGTAGG
V_sebSL_RS533 TTAAATGTAGG
V_sebSL_RS533_2 TTAAATGTAGG