SYSTEMATICS AND BIOGRAPHY OF MYRICACEAE

Jane Herbert

A Thesis Submitted for the Degree of PhD
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Systematics and biogeography of Myricaceae

Jane Herbert

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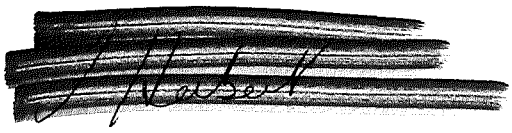
Abstract

Two molecular phylogenetic studies were undertaken to examine relationships within Myricaceae. Analyses of DNA sequences of the plastid *rbcL* gene, *trnL-F* region and nuclear ITS region showed the family to be monophyletic. In all analyses *Canacomyrica*, a monotypic genus endemic to New Caledonian that bears several distinctive features such as staminodes in the female flowers, fell into a well-supported clade sister to the rest of Myricaceae. Phylogenetic analyses of ITS and *trnL-F* sequence data, representing all genera and subgeneric groups, were undertaken using maximum parsimony and Bayesian methods. The following relationships were strongly supported: (*Canacomyrica* (*Comptonia* (*Myrica*, *Morella*))). The clade containing all species formerly considered to comprise *Myrica* s.l. was split into two strongly supported clades corresponding to *Myrica* s.s. and *Morella*; this finding strengthens the argument for recognition of these as separate genera. Within *Morella*, two clades corresponded to previously recognized subgenera. Molecular dating analyses were performed using Penalized Likelihood. Close correlations between lineage-specific diversification and major orogenic or climatic events were inferred. This study suggests that much of the diversity in *Morella* arose during the Neogene and seed-dispersal by birds has been a significant factor in determining the modern distribution. A study of the conservation status of *Canacomyrica* was conducted using field observations and data from herbarium specimens. This species was found to occur in just eleven fragmented localities: six outside protected areas and three threatened by mining or bush fires. IUCN Red List status of Endangered was recommended. The morphology and ecology of *Canacomyrica* was studied to enhance knowledge of this poorly known species and provide comparative data for use in a study of the morphology of the entire family. A new classification scheme with keys was presented including, for the first time, *Canacomyrica*. New combinations in *Morella* were recommended.

Declaration

I, Jane Herbert, hereby certify that this thesis, which is approximately 44,000 words in length, has been written by me, that it is a record of work carried out by me and that it has not been submitted in any previous application for a higher degree.



Jane Herbert

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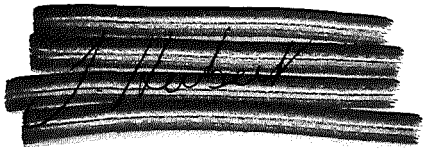

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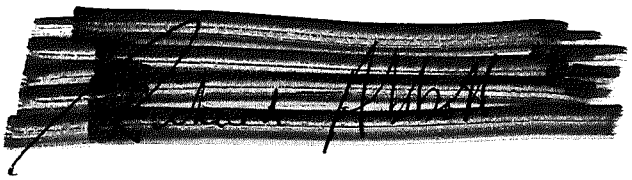

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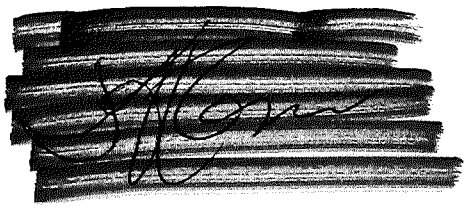

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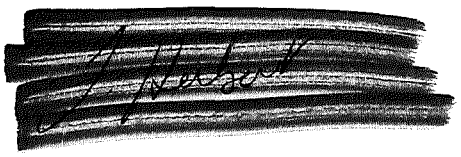

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Jane Herbert
November 2004

Dedicated to the memory of my grandmother

Iris Bradbury

1918 – 2002

Contents

Figure 1. Clockwise from top left: *Myrica gale* (Simon Gaskell), *Comptonia peregrina* (Kitty Kohout), *Canacomyrica monticola* (Pete Hollingsworth), *Morella faya* (Tom Meagher).

THESIS OVERVIEW

Five studies were carried out to investigate the systematics and biogeography of Myricaceae. The aims of these, and the methodologies used, are outlined below. Each of the experimental chapters is presented as a paper and the style followed is that of *American Journal of Botany*, except where stated otherwise.

Chapter 1 is a general introduction giving background to some of the key concepts presented in the thesis. Theoretical and methodological approaches are outlined and the study group is introduced.

Chapter 2 reports a molecular phylogenetic study of the placement of the New Caledonian endemic *Canacomyrica*. This monotypic genus is poorly known and its original placement in Myricaceae had been questioned by several authors. Phylogenetic analyses of DNA sequences from three different gene regions representing the plastid and nuclear genomes were carried out to assess the affinities of *Canacomyrica*.

Chapters 3 and 4 focus on aspects of the biology of *Canacomyrica* in an attempt to increase understanding of this unique species. Based on field observations and a survey of herbarium specimens, the distribution and conservation status of *Canacomyrica* is presented in Chapter 3.

In Chapter 4 the morphology and ecology of *Canacomyrica* is examined with particular attention given to features that were previously unknown (chromosome count) or poorly understood (breeding system, seed morphology).

Chapter 5 is a morphological study of Myricaceae using traditional taxonomic methods and phylogenetic analysis. This study was performed with the principal aim of identifying morphological characters that could be used, in conjunction with the molecular data, to define genera and subgeneric groups.

Chapter 6 presents the results of a molecular phylogenetic study of Myricaceae using data from the ITS region (nuclear DNA) and the *trnL-F* region (plastid DNA). Analyses were performed using Maximum Parsimony and Bayesian Likelihood. Inter-

and Intra-generic relationships were examined. Various biogeographic hypotheses for the family were tested using Penalized Likelihood methods.

Following on from the findings of Chapter 6, the new combinations found to be necessary in *Morella* are made in Chapter 7.

Chapter 8 is a synthesis of the major findings of the thesis and includes a revised taxonomic treatment of the family.

Chapter 1

Introduction

BIOGEOGRAPHIC PATTERNS

From Darwin's theory of evolution, to Wallace's Line and Wegener's theory of continental drift, biogeography has been central to some of the most important concepts in biology. Systematics - the study of taxonomic groups and their natural relationships - has played a key role in the development of these concepts by providing a framework for examining distribution patterns. By testing theories about the distribution patterns of individual groups in a systematic framework we can build a wider understanding of evolutionary processes.

Numerous patterns can be discerned among the distributions of the world's plants. Perhaps some of the most interesting and challenging to explain are major inter-continental disjunctions. In taxa with small, highly mobile propagules, long-distance sweepstakes dispersal may be the most likely explanation for modern intercontinental distribution patterns (e.g. *Brachypodium* [Poaceae] or *Senecio* [Asteraceae]; Catalan and Olmstead, 2000; Coleman et al., 2003). However, vicariance hypotheses involving major geological and/or climatic events (e.g. continental drift, mountain building) are often invoked to explain discontinuous distribution patterns exhibited by woody taxa with less mobile seeds.

Tertiary relict floras— Much botanical interest has focused on the eastern Asian-eastern North American disjunction found in a number of unrelated woody deciduous or evergreen (occasionally herbaceous) genera. The floristic association between these regions was first detailed by Asa Gray in the 1840s (Graham, 1972), when considering the similarities among species described for the Floras of North America and Japan. *Magnolia* and *Liriodendron* (Magnoliaceae) are perhaps the most well known taxa exhibiting this disjunction, which has come to be known as a Tertiary relict distribution. The concept of the Tertiary relict distribution has been widened to include taxa with representatives in western North America and eastern Eurasia, in

addition to eastern Asia and eastern North America. Wen (1999) lists 65 genera with this distribution pattern but other authors have listed as many as 120 (e.g. Wu, 1983); notable examples include *Styrax* (Styracaceae), *Carya* (Juglandaceae), *Hamamelis* and *Liquidambar* (Hamamelidaceae), *Lindera* and *Sassafras* (Lauraceae), and *Pachysandra* (Buxaceae). Plants exhibiting a Tertiary relict disjunction display a high level of morphological similarity. This has been interpreted to indicate a very close relationship arising from a former widespread distribution pattern.

The Tertiary relict distribution pattern is attributed to the fragmentation of a once continuous northern hemisphere boreotropical or mixed mesophytic flora (Wolfe, 1975). Migration of species between eastern North America and Eurasia, and between eastern Asia and western North America, is considered to have been possible via the North Atlantic land bridge and the Bering land bridge respectively. Such migration would have been aided by a warm, wet climate, especially during the Eocene (54-34 mya) and early to mid Miocene (25-10 mya) (Milne and Abbott, 2002).

Numerous recent molecular studies have examined Tertiary Relict disjunctions. Donoghue et al. (2001) analysed the phylogenetic histories of seven genera in five different families. They found relationships within these genera to conform either to a 'Pacific track' (i.e. via the Bering land bridge) or an 'Atlantic track' (i.e. via the North Atlantic land bridge). Various patterns of relationships and divergence times were estimated, leading to the conclusion that initial diversification in Asia was followed by repeated trans-Beringian dispersion and vicariance events at different times during the Tertiary. Whilst Xiang et al. (1998) found congruence of phylogenetic pattern among the taxa they examined, divergence times for some of the genera differed, again suggesting multiple dispersion/vicariance events during geological time. Dating a further ten genera, Xiang et al. (2000) showed that (in all but one genus) divergence times of species pairs spanned the mid Miocene to the Quaternary, with most estimates falling in the late Miocene and Pliocene.

Madrean-Tethyan vegetation— The distribution of some plant genera that are disjunct between the subhumid regions of Eurasia (i.e. the Mediterranean Basin) and North America (i.e. the Californian chaparral and adjoining areas) has been suggested to represent the fragmentation of a once continuous Madrean-Tethyan sclerophyllous

vegetation (Axelrod, 1975). Axelrod stated that a sclerophyllous flora was more or less continuous between northern America and Eurasia by the mid Eocene. He proposed that migration across the Atlantic (aided by a narrower sea than at present and the lower latitude of the east coast of America) was achieved via micro-continents (the Azores) and volcanic islands along the mid-Atlantic Ridge. By the end of the Tertiary, lower temperatures and decreased rainfall favoured grassland, steppe and desert, leading to the fragmentation of this flora and resulting in the present-day disjunct distribution of sclerophyllous genera.

Taxa that Axelrod considered to represent Madrean-Tethyan links include *Arbutus* (Ericaceae), *Cercis* (Fabaceae), *Ilex* (Aquifoliaceae), *Juniperus* (Cupressaceae), *Myrica* s.l. (Myricaceae), *Rhamnus* (Rhamnaceae) and *Styrax* (Styracaceae). These genera are characterised by small scleromorphic[1] evergreen leaves, hard wood, deep root systems and the capacity to resprout following fire. Axelrod considered these features to be preadaptations, allowing plants to persist in climates of reduced precipitiation and greater temperature ranges, i.e. the modern mediterranean regions. An interesting feature of Axelrod's thesis is the suggestion that scleromorphy may be an ancient adaptation to nutrient-poor soils, rather than a recent adaptation to limited water availability (Andrews, 1916; Beadle, 1966; Axelrod, 1975; Hill, 1998).

Whilst the mode of trans-Atlantic migration that Axelrod proposed for his Madrean-Tethyan flora has been questioned (see Milne and Abbott, 2002), several recent molecular phylogenetic studies have reported relationships consistent with the Madrean-Tethyan concept. In studies of *Styrax* (Fritsch, 2001) and Arbutoideae (Hileman et al., 2001) sister relationships between the North American and Mediterranean taxa were revealed. Likewise, in *Cercis* (Davis et al., 2002a) and the *Ocotea* complex (Lauraceae; Chanderbali et al., 2001) relationships and divergence times consistent with the Madrean-Tethyan concept were reported.

Long distance dispersal— Families or genera that are distributed in both the northern hemisphere and Africa have seldom been included in discussions of Tertiary relict or

[1] The term scleromorphy is often interchangeable in the literature with xeromorphy (an adaptation to dry conditions). Scleromorphy is used here in its strict sense to refer to plants showing an adaptation to low phosphorus soils (see Hill, 1998 for full discussion).

Madrean-Tethyan floras. A presence in Africa, particularly when combined with representatives in other southern continents, has traditionally been considered to suggest a southern hemisphere origin associated with the fragmentation of the ancient supercontinent Gondwana. For example, the modern Americas-Africa-Asia disjunction exhibited by Dalbergioid legumes and Malpighiaceae has been attributed to a Gondwanan origin (Raven and Axelrod, 1974). However, Lavin et al. (2000) and Davis et al. (2002b) respectively, proposed that the disjunct distribution of these groups was the result of migration through Laurasia via the North Atlantic land bridge during the Tertiary (Eocene).

Whilst a small number of studies have shown that plate tectonics have indeed played a role in the distribution of some families (e.g. Crypteroniaceae; Conti et al., 2002), an increasing number of studies using molecular dating techniques have rejected vicariance in favour of long-distance dispersal. Bird dispersal in the late Pliocene to Pleistocene has been invoked to explain the transatlantic distribution of *Corylus* (Betulaceae) species (Whitcher and Wen, 2001). In woody species of Hawaiian *Viola* (Violaceae), bird dispersal is strongly implied (in the mid Pliocene) for the close relationship with an amphi-Beringian species (Ballard and Sytsma, 2000). Renner et al. (2001) favoured long distance dispersal events in the Neogene for the distribution of Melastomataceae, a family with a widespread tropical distribution formerly interpreted as Gondwanan, but see Morley and Dick (2003) for an alternative view. Likewise, long distance dispersal appears to be the main factor in determining the intercontinental distributions of legumes and Annonaceae (R. T. Pennington, personal communication).

Biogeographic patterns in Myricaceae— Myricaceae are a family of predominantly evergreen shrubs and trees with drupaceous, mostly bird-dispersed fruits. The family is subcosmopolitan, exhibiting major disjunctions between Africa, the Americas and Asia (see Distribution Maps, Appendix 2). The family is absent from Australia and New Zealand and arid regions of the Old World; in South America it is mostly confined to the Andes; one species has an almost circumboreal distribution. In discussions of the biogeography of Myricaceae, previous authors have variously suggested a boreotropical origin (Macdonald, 1977), a Madrean-Tethyan origin (Axelrod, 1975), a Gondwanan origin (Bramwell, 1976; Macdonald, 1989) and a

Laurasian origin (Raven and Axelrod, 1974). In a review of major disjunctions in seed plants, Thorne (1972) stated that he was unable to place the family in any distribution category, but suggested that its present range is a relict of a once worldwide distribution. To date, a long-distance dispersal explanation for the distribution of Myricaceae has not been proposed.

Low morphological variation characterizes Myricaceae; this has resulted in poorly understood intra-familial relationships and uncertainty about the family's biogeographic history. The principal aim of this study was to use molecular tools to provide a new set of characters upon which to base our understanding of the relationships within Myricaceae; thus providing, for the first time, an opportunity to assess the relative importance of vicariance, migration and dispersal in the history of this family, and to assess, within a detailed systematic framework, biogeographic hypotheses previously suggested for it.

Study aims – The aims of this study were:

1. To use molecular markers to infer the phylogenetic history of Myricaceae and establish the relationship of *Canacomyrica* to the other genera;

2. To use phylogeny dating techniques and fossil evidence to investigate the biogeographic history of the family;

3. To combine inferences from molecular and morphological data to assess current generic concepts in Myricaceae and construct a classification scheme to include all genera;

4. To increase knowledge about aspects of the biology and ecology of *Canacomyrica*.

APPROACHES

Biogeographical approaches— The study of historical biogeography has undergone major advances over the past 20-30 years. Until the 1980s the prevailing concept was that of the centre of origin (Brown & Lomolino, 1998). Biologists of the early half of the 20th Century, such as Adams (1902) and Cain (1944), drew on the work of the 18th Century biologist Comte de Bouffon in formulating lists of criteria that could be used to identify areas as the birthplace, or centre of origin, for any taxon. The criteria used

did not make reference to fossil evidence, or geological or climatic events, yet the scenarios envisaged for individual taxa were often extrapolated to make generalizations about global patterns of diversity (Brown & Lomolino, 1998).

A move toward more rigorous approaches to the study of biogeography began with Croizat's concept of panbiogeography (e.g. Croizat, 1952). Croizat's major contribution was in studying and looking for patterns, not just within a single a taxon, but across a diversity of groups to arrive at a series of 'tracks' or dispersal routes. In the latter half of the 20th Century cladistic methods began to be applied to the study of biogeography, combining Croizat's multi-taxon approach with a clear evolutionary hypothesis provided by phylogenetic systematics. Derived from this combined approach, the contentious concept of vicariance biogeography (as distinct from dispersal biogeography) was coined by systematists from the American Museum of Natural History, who considered plate tectonics to be fundamental to the interpretation of distribution patterns within a phylogenetic framework (Wilson, 1988). In fact, their emphasis on continental drift was such that they rejected dispersal scenarios altogether. This group introduced the area cladogram, produced by replacing the taxon name in a given phylogeny with the area from which it originated and comparing with phylogenetic trees for other groups, in order to arrive at a picture of the common vicariance patterns shared by the groups (Wilson, 1988). Implicit in the vicariance biogeography theory are the very restrictive assumptions that all speciation occurs by geographic isolation and that the present distribution of organisms reflects past speciation events (Brown & Lomolino, 1998).

Although the area cladogram remains a feature of modern approaches to biogeography, most biogeographers now attempt to include fossil and geological evidence within their work (Tiffney & Manchester, 2001; Magallón, 2004). Molecular data provide a vast number of characters upon which to base phylogenetic hypotheses and the growth in this field of research has been accompanied by advances in techniques for analysing and interpreting this data (see below). It is becoming increasingly common for divergence times of lineages to be dated using molecular phylogenetic data, hypotheses about rates of molecular evolution, and known facts about fossils or geological dates (e.g. Richardson et al., 2001a; Richardson et al., 2001b; Berry et al., 2004; Klak et al., 2004). Such a multi-disciplinary approach was

chosen for this study, in order to use the maximum available evidence for investigating the history of Myricaceae.

Phylogeny reconstruction and dating— Phylogeny reconstruction can be achieved using either morphological or molecular data, or a combination of the two. The approaches chosen for this study are discussed below.

Molecular markers- Molecular markers can provide a valuable set of new characters upon which to base judgements about relationships, within and among species. Variation among the four DNA nucleotides (adenine, thymine, guanine and cytosine) provides a series of characters that may be compared, to evaluate the evolutionary proximity of different taxa. Automated sequencing and the widespread availability of universal primers has made the generation of sequence data relatively straightforward. The choice of region to be sequenced is guided by the level of variation exhibited by a given gene or region of non-coding DNA; at low taxonomic levels there may be insufficient variation, and at high taxonomic levels the degree of divergence may be such that there are difficulties in aligning sequences and determination of character homologies becomes problematic.

The chloroplast gene *rbcL* (ribulose bisphosphate carboxylase/oxygenase, large subunit) was selected for use in this study because its relatively slow rate of evolution is routinely found to be suitable for addressing questions at high taxonomic levels (e.g. Chase et al., 1993; Savolainen et al., 2000; Soltis et al., 2000). Alternative regions such as 18S rDNA, and *atpB* can also be useful at high taxonomic levels (e.g. Soltis et al., 2000), but the popularity of *rbcL* has resulted in a vast number of existing sequences that can be easily accessed. It was, therefore, the most appropriate choice for the investigation of the placement of *Canacomyrica*, where the aim was to compare a small number of novel sequences with a broad spectrum of taxa (see Chapter 2).

A second chloroplast marker, the *trnL-F* region (consisting of the *trnL* intron and the *trnL-trnF* intergenic spacer) and a nuclear marker, the ITS region (consisting of the internal transcribed spacer region of the 18S-5.8S-26S nuclear ribosomal cistron) were also selected for use in this thesis. The decision to use a combination of data

from different genomes was made because genomes have potentially different histories (the plastid genome is uniparentally inherited whilst the nuclear genome is biparentally inherited), thus a phylogenetic study based on more than one genome might be considered to yield more reliable results (i.e. more closely resemble the 'true' tree) than a study based on just one genome (Soltis & Soltis, 1998). Both *trnL-F* and ITS have higher rates of evolution than *rbcL* and were selected because they have been successfully used in many studies for determining relationships below the family level (e.g. Richardson et al., 2001a; Richardson et al., 2001b; Whitcher & Wen, 2001; Coleman et al., 2003; Berry et al., 2004; Renner et al., 2004).

Morphological data- In some studies, combining morphological data with molecular data, in a total evidence approach, has been found to produce greater resolution and/or greater support for phylogenetic relationships than molecular data alone (e.g. Pennington, 1996; Whiting et al., 1997). However, given the acknowledged paucity of informative morphological characters in Myricaceae, the aim of including a morphological investigation within this study was *not* to seek new morphological characters that could be analysed in conjunction with the molecular data. Instead, since a classification based on molecular data alone is of no use to the field botanist, the intention was to identify diagnostic characters that would permit the construction of a key, and formal descriptions, for groups resolved by analysis of the molecular data. Having established this aim as the principle purpose for carrying out a morphological investigation, the sampling for this part of the study was limited to a small number of species that were thought likely, on the basis of previous work (e.g. Chevalier, 1901), to represent natural groups within the family.

Analytical methods- There are two major discrete methods (as distinct from distance methods) that are commonly used to analyse sequence data: 1) maximum parsimony (MP), in which the tree (or trees) that requires the fewest evolutionary changes is sought; and 2) maximum likelihood (ML), in which the tree (or trees) that is most likely to have produced the observed data is sought (Page & Holmes, 1998). An alternative to the above is Bayesian inference (BI), which is a likelihood method that permits the inclusion of rate heterogeneity both in lineages and among data partitions by applying different models of molecular evolution (Hall, 2004). In contrast to ML, BI seeks the most likely tree given the observed data and model of evolution (Hall,

2004). The main advantage of BI is that it employs the Metropolis-coupled Markov Chain Monte Carlo algorithm, which is less likely to reach a suboptimal result than the algorithms used for MP and ML analysis, which perform point to point searches and may become 'trapped' on suboptimal trees (Hall, 2004).

Maximum parsimony has been criticized for being inconsistent under certain conditions (Page & Holmes, 1998), nonetheless, it continues to be widely used, usually in combination with ML and/or BI (e.g. Conti et al., 2002; Coleman et al., 2003; Albach & Chase, 2004). When results are available from more than one method of analysis it is possible to compare them for any incongruence, which can then be taken into account in any further analyses or discussion. Thus, for this study, both MP and BI methods were used. Furthermore, BI analyses produce results in a format that is compatible with the phylogeny dating technique that was used (see below).

Several recent studies have used a method called penalized likelihood (PL) to examine divergence times in various plant groups (Conti et al., 2002; Berry et al., 2004; Renner et al., 2004; Lavin et al., In press). PL is a semiparametric rate smoothing method that uses the data to find an optimal level of smoothing and which permits each lineage to have a separate rate, whilst penalizing rates that vary too much across a phylogeny (Sanderson, 2002). This method makes it possible to incorporate multiple calibration points into the calculations. Earlier methods based on nonparametric rate smoothing were not data-driven and did not permit the use of more than one calibration point (Sanderson, 2002). As the most comprehensive method for phylogeny dating currently available, PL was chosen for use in this study.

STUDY GROUP

Myricaceae are a small family of shrubs and trees comprising approximately 50[2] species (see Appendix 1 for list of species with authorities and distribution details). The family has a subcosmopolitan distribution with centres of diversity in Africa and the Americas (including the Caribbean). The family is characterised by unisexual flowers borne in catkins, peltate glands, simple, entire leaves, a unilucuclar ovary and single orthotropous ovule (see Chapter 8 for a formal description of the family). All species (except perhaps *Canacomyrica*) are actinorhizal, forming a nitrogen-fixing symbiosis with the actinomycete *Frankia* (see below).

The two species of *Myrica* L. are low-growing deciduous shrubs of north temperate regions with strongly aromatic leaves and dry fruits with spongiose, accrescent bracteoles, adapted for dispersal by water. The most widespread species is *Myrica gale* (bog myrtle) (Figure 1; Map A, Appendix 2) which is found throughout much of Britain and has an almost circumboreal distribution. Like *Myrica*, the monotypic *Comptonia* L'Hér. (Figure 1; Map C, Appendix 2) is a low-growing, aromatic shrub; it is found in eastern North America and Canada and has dry, possibly animal-dispersed fruits surrounded by spiny bracts. The c. 47 species of *Morella* Lour. (Figure 1; Map B, Appendix 2) are shrubs and trees disjunctly distributed throughout the Americas, Africa and Asia. Most species of *Morella* also possess aromatic oils; they are distinguished from *Myrica* by their fleshy and sometimes waxy fruits. Endemic to New Caledonia, the monotypic *Canacomyrica* Guillaumin (Figure 1; Map C, Appendix 2) is a small tree with odourless leaves, female flowers with staminodes and fruits that are enclosed in the enlarged perianth. Little is known about this species but, on the basis of its unusual features, several authors have expressed doubts about its placement in Myricaceae (see Chapter 2).

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[2] Species names in current use were taken from the most recent works available on each of the major geographical areas of distribution (Standley, 1937; Backer, 1951; Leon and Alain, 1951; Leroy, 1952; Standley and Steyermark, 1952; Burges, 1964; Ohwi, 1965; Baird, 1970; Kuzenev, 1970; Adams, 1972; Staples, 1988; Estigarribia, 1993; Liogier, 1996; Yang and Lu, 1996; Killick et al., 1998; Lu and Bornstein, 1999; Parra-O., 2003).

There is significant morphological similarity of leaf form and reproductive characters among the majority of Myricaceae species, particularly evident within *Morella* (see Chapter 5). Concomitantly, high infraspecific variability, particularly in leaf shape, can frequently be observed (Baird, 1968; Polhill and Verdcourt, 2000; Schatz, 2001). This has resulted in the publication of over 200 species names (International Plant Names Index; http://www.ipni.org/index.html) and the recognition of a large number of subspecies and varieties. Myricaceae provide the traditional taxonomist with a limited number of characters upon which to base taxonomic groups, leading to authors recognising widely varying numbers of species (see Chapter 5 for examples) and disagreement over generic delimitation (see below).

Taxonomic history of the genera— Most modern taxonomists recognize the three genera *Myrica*, *Morella* and *Comptonia*, with some also including *Canacomyrica* (see Chapter 2 for its taxonomic history). Authors have long disagreed on the delimitation of genera and subgeneric groups within the family and the use of *Morella* has only recently been generally accepted (see Chapters 6 and 7).

In the first edition of Species Plantarum, Linnaeus (1753) described the taxon now known as *Comptonia* under two names: *Liquidambar peregrina* (p. 999; for which a type specimen is present in the Linnaean herbarium) and *Myrica asplenifolia* (p. 1024; for which no type specimen is present in the Linnaean herbarium). He later combined the names under the single species *Liquidambar peregrina* (Linnaeus, 1759; p. 1273). Later, L'Héritier (in Aiton, 1789) placed the species in the monotypic genus *Comptonia*.

When Linnaeus (1753) named the genus *Myrica*, five species were known to him: *Myrica gale, M. cerifera, M. cordifolia, M. quercifolia* and *M. asplenifolia* (correctly *aspleniifolia* = *Comptonia peregrina*). There are significant morphological differences between *Myrica gale*, which has smooth dry fruits, and *M. cerifera*, *M. cordifolia* or *M. quercifolia*, all of which have papilose waxy fruits. These differences were not formally recognized, however, until Spach (1841) split the genus, adopting *Gale* Dumort. to accommodate *Myrica gale*, retaining *Comptonia*, and placing the remaining species in *Myrica*. Chevalier (1901) published a monographic study of the

family in which he followed Spach's classification, with the division of *Myrica* (*sensu* Spach) into three sections (Table 1). Neither Spach nor Chevalier cited types for the names of their genera and, as a result, their nomenclature was not widely adopted. The majority of literature published since 1901 has recognized only *Myrica* and *Comptonia*.

Engler (1888) gave recognition to the differences between *M. gale* (along with *M. hartwegii*) and the majority of other species, by recognising the subgenera *Gale* and *Morella* (Table 1). Some authors (Baird, 1968; Wilbur, 1994) expressed the opinion that *Myrica* (s.l.) should be split into two genera in order to distinguish between these two groups of species and stated that these should be named *Myrica* and *Morella*. However, this would entail transferring the majority of species to *Morella*. Verdcourt and Polhill (1997) made a proposal to conserve the generic names *Myrica* and *Gale,* in order to give these groups generic status whilst minimising the number of name changes that would be required. In line with nomenclatural rules their proposal would involve changing the type specimen of the genus *Myrica* from *M. gale* to *M. cerifera*. *Myrica gale* would then change its name to *Gale belgica* and become the type of the genus *Gale*. However, the proposal was rejected (Brummitt, 1999) and *M. gale* remains the type of the genus *Myrica*. Thus, the generic name *Morella* must be adopted for the majority of those species formerly treated as *Myrica*. Most of the new combinations have now been made (see Chapter 7). The nomenclature adopted herein follows Wilbur (1994) in recognising *Myrica* s.s., *Morella* and *Comptonia*, with the inclusion of *Canacomyrica*.

TABLE 1. The major generic classifications of Myricaceae (excluding *Canacomyrica*).

Spach (1841)	**Engler (1888)**	**Chevalier (1901)**	**Baird (1968), Wilbur (1994)**
Gale	*Myrica* Subgen. *Gale*	*Gale*	*Myrica*
Myrica	Subgen. *Morella*	*Myrica* Sect. *Morella*	*Morella* Subgen. *Morella**
		Sect. *Faya*	Subgen. *Cerothamnus** series *Faya**
		Sect. *Cerophora* Subsect. *Africanae* Subsect. *Americanae*	series *Cerothamnus**
Comptonia	*Comptonia*	*Comptonia*	*Comptonia*

*Subgenera recognized by Wilbur (1994)

Affinities— Until the early twentieth century, botanists placed Myricaceae near the beginning of their classification schemes implying that they considered the family to be primitive (e.g. Engler, 1888). Myricaceae, along with families sharing the character of much reduced flowers borne in catkins (or aments), such as Juglandaceae, Fagaceae, Betulaceae, Leitneriaceae, Urticaceae and Hamamelidaceae, were grouped together in the Hamamelidae (Takhtajan, 1980; Cronquist, 1981), the Amentiferae of some authors (for a discussion of the etymology and delimitation of this group, see Stern, 1973). Numerous morphological studies and, more recently, molecular phylogenetic studies have shown the Hamamelidae to be an artificial group (e.g. Hufford, 1992; Chase et al., 1993; Manos et al., 1993; Qiu et al., 1998).

Distinctive characters, such as the simple, entire leaves with resinous 'balloon' glands, the unilocular ovary and single orthotropous ovule, led some authors to place Myricaceae alone in the order Myricales (Engler, 1897; Thorne, 1973; Cronquist,

1981; Dahlgren, 1983; Takhtajan, 1997). However, the morphological similarities that Myricaceae share with Juglandaceae led other authors to classify the family within Juglandales (Hjelmqvist, 1948; Melchior, 1964; Kubitzki, 1993). On the basis of molecular phylogenetic studies (Chase et al., 1993; APG, 1998; Li et al., 2004), Myricaceae is now considered to belong to an expanded concept of Fagales that includes Casuarinaceae and Betulaceae (which, like Myricaceae, have actinorhizal members) and the economically important Juglandaceae (see Chapter 2).

Fossil record— The pollen of Myricaceae, Casuarinaceae and Betulaceae is very similar (Chourey, 1974; Sundberg, 1985) reflecting the close relationship among these families. Sundberg (1985) reported the diagnostic character of a *Myrica*-type aperture in the pollen grain of all Myricaceae genera but did not state whether this character was widely used by earlier palynologists. Therefore, it is not unreasonable to suggest that the pollen record for Myricaceae may, at least in part, be unreliable. Coetzee and Praglowski (1984) reported the presence of *Myrica* s.l. in South Africa in the lower Miocene. Microfossil evidence of *Myrica* s.l. from Mexico is present in upper Miocene deposits, where it is thought to have been a component of the 'needle-leaved forest' paleocommunity (Graham, 1987). Pollen attributed to *Canacomyrica* is recorded from sediments of the Eocene to Miocene in New Zealand (Mildenhall, 1980); there are no known macrofossils of *Canacomyrica*.

According to Chourey (1974), Myricaceae leaf macrofossils can be reliably identified on the basis of camptodromous venation (secondary veins curving toward the margin without forming loops), anomocytic stomata (lacking morphologically differentiated subsidiary cells) and peltate glands with a 2-(3) celled base; but her study showed that the majority of North American fossils previously attributed to *Myrica* s.l. had been assigned incorrectly. The nomenclatural problems associated with *Myrica* s.l. outlined above have a significant bearing on the interpretation of the fossil record. Leaves of *Myrica* s.s. can be distinguished from *Morella* species on the basis of the sunken stomata, whilst the structure of the fruits is sufficiently distinct to enable easy identification. However, the vast majority of literature uses *Myrica* in the wider sense, making no reference to these differences and thus providing us with a confusing record of the two genera. The detailed account of the middle Miocene flora of Jutland (Denmark; Friis, 1985) lists endocarps assigned by the author to *Myrica*; the

illustrations leave no doubt that these endocarps belong to *Morella* species. I am aware of no other fossil records that can be certainly attributed to *Morella*.

Leaves of *Comptonia* can be easily recognised by their pinnatifid shape and other leaf cuticular characters (Chourey, 1974), and their identification does not represent a significant problem. *Comptonia* is recorded from the Eocene of North America (Wolfe and Wehr, 1987) and has been recorded from Europe in middle Eocene (Wilde and Frankenhäuser, 1998), early Miocene (Kvacek, 1998) and Pliocene deposits (Ferguson and Knobloch, 1998). According to Manchester (1999), *Comptonia* has been recorded in eastern Asia from the Eocene of Yilan (Heilongjiang, China), the Miocene of Sikhote-Alin (eastern Russia) and the Middle Miocene of Japan.

Several authors consider Myricaceae to have a late Cretaceous origin (e.g. Muller, 1981; Ferguson, 1998) based on palynological evidence. As mentioned above, the correct assignment of pollen among the 'core Fagales' families is problematic, therefore this date should perhaps be viewed with caution. A recent wide-scale analysis of molecular data (Wikström et al., 2001) used fossil evidence to fix the date of the split between Fagales and Cucurbitales in the late Santonian (84 Ma). Based on this information, the authors calculated divergence times for many angiosperm families and suggested a divergence time of 36-38 Ma (late Eocene) between Myricaceae and Juglandaceae. This dramatically younger date is, however, inconsistent with multiple *Comptonia* fossils from the Eocene and Palaeocene fossils reported for Juglandaceae (Manchester, 1999; see Chapter 6).

Ecology— Myricaceae are commonly found in habitats such as sand-dune systems, areas of recent volcanism, water-logged areas and serpentine substrates where nutrients are limited. They are frequently components of early-successional communities (Côté et al., 1988; Dawson, 1990; Maggia and Bousquet, 1994; Swensen, 1996), but also occur in climax vegetation where environmental conditions are not suitable for taller trees (e.g. the fayal-brezal in Macaronesia; personal observations). Most, if not all, species appear to have a low tolerance of drought (personal observations in South Africa, Macaronesia and New Caledonia). Several species grow with their roots submerged in water: for example, *Myrica gale* is found in bogs and mires, *Morella faya* grows in the ever-wet cloud forests on the volcanic

soils of Macaronesia, and *M. serrata* occurs in riparian habitats in the Cape of South Africa. It has been recorded that *M. javanica* can grow as an epiphyte on other forest trees in Borneo (e.g. herbarium specimen: *Clemens & Clemens 32278* [BM]); this observation is illustrative of the high light requirements that the family has (see below) and its tolerance of nutrient-poor substrates.

Canacomyrica and most species of *Morella* occur in the tropics but they are found in montane habitats at altitudes where the climate is more temperate, the soil often thin and the cloud layer provides almost constant moisture. *Canacomyrica* grows on the serpentine soils of southern New Caledonia which are rich in toxic elements, such as iron and nickel, and notoriously poor in essential minerals. Several *Morella* species are also endemic to serpentine areas such as those found in Cuba and Malesia. Other species are endemic to the mediterranean regions of South Africa and California, where they are exposed to periods of drought. The requirements of Myricaceae species for plentiful water supply cannot, however, be over-emphasised and in these summer-drought regions *Morella* species are mostly confined to riparian habitats or altitudinal zones where a semi-constant cloud layer provides regular precipitation; two species that may have less stringent requirements for water are *M. cordifolia* and *M. quercifolia*, both South African Cape species.

Two notable species are found near sea level; *M. cordifolia* is an important dune-stabilising species on coastal sand dunes in the Cape region of South Africa and *M. cerifera* is frequently found in coastal habitats in North America. The latter species is the most wide-ranging *Morella* species, occurring throughout southern North America, Central America and some Caribbean islands. It is possible that this species has a higher tolerance to drought than its congeners, allowing it to populate a wider range of habitats. However, deep root systems are a feature of the family and permit species to tap deep ground-water.

Myricaceae have evolved two important adaptations for survival on nutrient poor soils: an actinorhizal association with a bacterium which provides the ability to fix nitrogen; and cluster roots which improve availability of other essential nutrients, especially phosphorus.

The actinorhizal association— Myricaceae are one of only nine angiosperm families known to form a symbiotic relationship with *Frankia* (filamentous, gram-positive, non-endospore forming, mycelial bacteria; Schwintzer and Tjepkema, 1990). Traditional classifications based upon morphological characters split actinorhizal plants among four subclasses of dicotyledons (Cronquist, 1981). However, recent molecular studies suggest that there is a common ancestor of all plants involved in nodular symbioses, including Fabaceae which form a nitrogen-fixing symbiosis with *Rhizobium* (e.g. Chase et al., 1993; Soltis et al., 1995; Swensen, 1996).

There are two ways in which *Frankia* can infect a host, either intercellular penetration or, as in Myricaceae, root hair infection (Callaham et al., 1978; Swensen, 1996). The bacterium induces curling of the root hair of the potential host, following which *Frankia* hyphae enter the cell through the deformed apical region of the root hair and become encapsulated by a cell wall deposit formed by the host plant. A prenodule is formed, in response to the infection, in the cortex near the root hair. The cells within the prenodule then become filled with the endophyte's hyphae and cell divisions are induced in the pericycle opposite the protoxylem which give rise to the nodule primordium (Franche et al., 1998). It is common for more than one strain of *Frankia* to infect a host root system and up to three strains have been shown to occupy a single nodule (Dobritsa and Stupar, 1989). Nitrogenase activity in the nodule allows *Frankia* to fix nitrogen in the form of ammonium. This is then exported to the plant cell cytoplasm where it is assimilated and metabolized to produce nitrogen transport compounds (Franche et al., 1998).

The host plant suffers a significant energetic cost in supplying the microsymbiont with photosynthate (Swensen, 1996). It has been documented that under conditions of abundant soil nitrogen or moisture stress, host plants of *Casuarina* and *Allocasuarina* (Casuarinaceae) do not form the actinorhizal association (Dommergues, 1984). Furthermore, in *Alnus glutinosa* (Betulaceae) it has been shown that leaves are dropped a month later than co-occurring individuals of *Tilia heterophylla* (Tiliaceae); this may provide the plant with a competitive advantage in early successional communities but may also indicate that prolonged carbon fixation prior to dormancy is necessary to balance the cost of nitrogen fixation (Neave et al., 1989).

Cluster roots— Cluster roots are the second adaptation that allows species of Myricaceae to exist on nutrient poor substrates. These structures are clusters of branched rootlets, the level of branching of which varies between species (Skene, 1998). Originally called Proteoid roots because of their prevalence in Proteaceae, cluster roots have also been recorded in Betulaceae, Casuarinaceae, Elaeagnaceae, Fabaceae, Moraceae, Cucurbitaceae, Restionaceae and Cyperaceae (Neumann and Martinoia, 2002). In Myricaceae, cluster roots have been found in species of *Myrica*, *Comptonia* and North American species of *Morella* (Skene, 1998) and it is likely that many more species in the family, if not all, possess this feature.

Formation of cluster roots occurs in response to low internal phosphorus levels. When the rootlets are fully formed they exude large amounts of malate, citrate (organic chelators) and acid phosphatase (an ectoenzyme) over a period of 2-3 days (Neumann and Martinoia, 2002). This burst of chemicals makes phosphorus in the substrate available for uptake by the plant. Cluster roots grow quickly and may be an alternative to mycorrihzal associations which take longer to establish and may not be as efficient in seasonally arid regions (Neumann and Martinoia, 2002). A nutritional lack of phosphorus has been shown to have a negative effect on nitrogen status and increased levels of phosphorus can stimulate nodulation in some actinorhizal plants (Huss-Danell, 1997).

Pollination and seed dispersal— All species of Myricaceae are considered to be anemophilous (but see Chapter 2) and bear their flowers on short catkin-like spikes which facilitate this mode of pollination (Cronquist, 1981; Kubitzki, 1993). After fertilization the female flower develops into a one-seeded fruit. In *Myrica*, the fruit is dry and the bracteoles adhering to it have a spongy texture which allows the fruit to float in water. The fruits of *Comptonia*, are also dry and are usually borne in an aggregation among the bracts which are feathery and somewhat burr-like, suggesting that the fruits may be animal-dispersed. The fruits of *Canacomyrica* are surrounded by a fleshy pericarp which is black and smooth at maturity and contains an apical pore through which the style continues to protrude (Kubitzki, 1993; personal observations); these fruits are likely to be bird-dispersed.

In *Morella* species, the fruits are covered by fleshy papillae which may be further covered (in the North American and South African species) by a waxy exudate composed of mono- and diglycerides of three saturated fatty acids (myristic, palmitic and stearic acids; Place and Stiles, 1992). Aronne and Wilcock (1994) have observed fleshy fruits of various shrubby taxa (e.g. *Rhamnus* [Rhamnaceae], *Pistacia* [Anacardiaceae] and *Phillyrea* [Oleaceae]) in the nests of ants in the Mediterranean Basin; although there is no documentary evidence available, it is possible that the waxy covering of *Morella* fruits may perhaps act as an elaiosome-type attractant to ants. Alternatively, the waxy coating may act to conserve water in the seed. The fruits of *Morella* species are dispersed by a variety of bird species (Ridley, 1930).

Seeds of all species of Myricaceae are very hard and the presence of a fatty, water-repellant covering in some species appears to suggest adaptation to long periods in the soil. Internal factors inhibiting germination have been recorded in *Comptonia* (Del Tredici and Torrey, 1976). Del Tredici (1996) observed that soil disturbance in a forest clearing initiated germination in *Comptonia* seed after an estimated 70 years of dormancy. Large temperature fluctuations are known to be important in eliciting germination in this temperate species (Dow and Schwintzer, 1999). It is tempting to speculate that adaptations to germination in open habitat and the observed intolerance of most Myricaceae species to an over-shadowing canopy (personal observations), may perhaps be linked to the high energy demands of the N-fixing process.

All Myricaceae species have the capacity to reproduce vegetatively, often producing large clonal stands. As a result, Myricaceae are able to cope with the fires that are a natural influence on the vegetation of mediterranean biomes. In these regions they are exposed to periodical burning but are able to resprout from the rootstock which survives below the soil (pers. obs. South Africa). It is unclear whether the seeds of Myricaceae species remain viable after burning but it is more likely that they fall into the category of obligate resprouters - those plants which are not reliant on fire to complete any part of their lifecycle and that are considered, in southern Africa, to be relicts of pre-mediterranean forest (Linder et al., 1992).

Breeding systems— Like many closely related families, Myricaceae are predominantly dioecious. At approximately 25%, the incidence of dioecy at the genus

level in Fagales is much greater than the average 7% among flowering plants (Renner and Ricklefs, 1995). The major contribution to this figure comes from Myricaceae, in which at least three out of four genera (including *Canacomyrica*) have some dioecious species; and from Casuarinaceae, in which all four genera include dioecious species.

Comptonia and c. 25% of *Morella* species are monoecious. There are suggestions that plants of *M. gale* can change sex from year to year (Davey and Gibson, 1917; Lloyd, 1981). In Scotland stands of *M. gale* observed by the author (in a single year) have been predominantly male (see also Skene et al., 2000), highlighting the importance of vegetative reproduction in these plants. In its native Macaronesia, *M. faya* is dioecious, yet in its introduced range in Hawaii it has been reported to be monoecious (Vitousek and Walker, 1989; see Appendix 6). Heterotopy, the occurrence of functional anthers on the fruit wall, has been observed in some *Morella* species (Macdonald, 1989). *Canacomyrica* is unique in the family in having male flowers and apparently hermaphrodite flowers on separate plants (see Chapter 2).

Economic importance— Although none is of great economic value, many species of the family have traditional uses in regions where they grow. The aromatic leaves of *M. gale* were traditionally used in Scotland as an insect repellent (Mabey, 1996); research is currently underway to grow and harvest *M. gale* on a commercial scale for its insect repellant and anti-bacterial properties (Smith, 2004). In both South Africa and North America, the fruits of wax-producing *Morella* species were used by settlers for candle-making and the fruits of *M. faya* were once used by the Portuguese in wine-making (Mabberley, 1997). Throughout much of Himalayan Asia, *M. rubra* is cultivated for its edible fruits that may be as large as 3 cm diameter; its bark is known to have medicinal properties and is also used to make fish poisons (Gardner et al., 2000). In North America, native species are cultivated as ornamentals, and in South Africa *M. cordifolia* is sold as a garden plant (personal observations).

Conservation status— The IUCN Red List of Threatened Plants (Walter and Gillett, 1998) lists five Myricaceae species. One of these is a misprint and will be ignored

hereafter[3]. All four of the threatened species have restricted distributions, three of them occurring on islands making them particularly vulnerable to habitat change. The most threatened species is *Morella holdridgeana* which occurs in Puerto Rico and is listed as Endangered. *Morella phanerodonta*, endemic to Costa Rica, is listed as Vulnerable. A variety of *Morella adenophora* from Taiwan and *Morella rivas-martinezii* from the Canary Islands are both listed as Rare.

[3] Walter and Gillett (1998) include the species *Myrica calcicola* based on a list of the threatened plants of Jamaica (Kelly 1980). However, the plant listed in that publication is *Myrcia calcicola* (Myrtaceae). This error has been reported to UNEP-WCMC.

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Chapter 2

Nuclear and plastid DNA sequences confirm the placement of the enigmatic *Canacomyrica monticola* in Myricaceae[1]

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ABSTRACT

Phylogenetic analyses of DNA sequences of the plastid *rbcL* gene were used to obtain a phylogenetic framework for the New Caledonian endemic genus *Canacomyrica* (monotypic). A further analysis of selected genera within Fagales, combining DNA sequences of the *rbcL* gene, plastid *trnL-F* region and nuclear ITS region, was also performed. In all analyses *Canacomyrica* fell into a well-supported clade in which it occupied a position as sister to the remaining genera of Myricaceae. A chromosome number of 2n=16, consistent with Myricaceae, is reported for *Canacomyrica* for the first time. On the basis of the phylogenetic data and numerous shared morphological features, the original placement of *Canacomyrica* in Myricaceae is accepted. *Canacomyrica* is distinguished from other members of the family by the presence of staminodes in the female flower, a six-lobed perianth, and a lamellular, laciniate style. The affinities of Myricaceae within Fagales are re-evaluated in the light of the unusual morphological features of *Canacomyrica*.

Key words: *Canacomyrica*, dioecy, Fagales, ITS, Juglandaceae, Myricaceae, New Caledonia, staminodes.

INTRODUCTION

The monotypic genus *Canacomyrica* is an endangered shrub or small tree, endemic to the ultramafic (serpentine) soils of New Caledonia (Herbert, submitted b). Since its description by Guillaumin (1940; see also Guillaumin, 1939), who placed it in Myricaceae, there has been confusion over its morphology and doubt about its

[1] This chapter has been submitted to *Taxon*. All experimental work and writing was carried out by J. Herbert; the contribution from M. W. Chase and M. Möller was in guiding the molecular and cytological work respectively.

affinity. Prior to this study, little research had been carried out into the biology of this species and basic information, such as chromosome number, was unknown.

Most modern taxonomists consider Myricaceae to comprise three genera: *Myrica* L. (ditypic); *Comptonia* L'Hérit. (monotypic); and *Morella* Lour. (c. 47 species) (Wilbur, 1994; Polhill and Verdcourt, 2000; Herbert, submitted a). All three genera share features such as a simple bifid style, no perianth, and an orthotropous ovule. There is little morphological variation among species, particularly within *Morella*, and as a result, inter- and intra-generic relationships are unclear. Molecular data may provide a clearer understanding of relationships within Myricaceae. The first author is currently sampling the family in this context (J. Herbert, unpublished data; Chapter 4).

In his original description, Guillaumin (1940) listed a number of anomalous features that distinguished *Canacomyrica monticola* from all other Myricaceae species: androdioecious breeding system (male flowers and hermaphrodite flowers on separate plants); lamellular, laciniate style; fleshy perianth; inferior ovary; and ovule hanging from a long funicle with an inferior micropyle. He later modified his description, stating that the inflorescences were co-sexual and the ovule anatropous (Guillaumin, 1948). In a study of the floral morphology of *Canacomyrica*, Leroy (1949) showed that Guillaumin had incorrectly described several characters. He stated that the flowers were unisexual with the female flowers bearing sterile anthers and that the ovule was erect, sessile, and orthotropous with a superior micropyle. On the basis of this re-evaluation of morphology, Leroy (1949) accepted the placement of *Canacomyrica* in Myricaceae. However, in recognition of the remaining unusual features he placed it in the new subfamily Canacomyricoideae. The work of Leroy (1949) appears to have been largely overlooked, resulting in continued misinterpretation of the morphology, particularly regarding the true nature of the flowers (e.g. Macdonald, 1977; Cronquist, 1981; Macdonald, 1989; but see Kubitzki, 1993).

Several authors (e.g. Elias, 1971; Raven and Axelrod, 1974) have questioned the inclusion of *Canacomyrica* in Myricaceae based on its distinctive morphology and distribution (Myricaceae as previously circumscribed do not extend into Australasia). Thorne (1973) placed it in his list of *Taxa Incertae Sedis*, later including it in

Myricaceae without comment (Thorne, 1992a, b, 2000). Others have accepted the original placement of *Canacomyrica* (Cronquist, 1981; Kubitzki, 1993; Takhtajan, 1997). The recent placement of *Canacomyrica* in a separate family (Canacomyricaceae; Doweld, 2000) added little insight into the problem of the systematic position of this taxon.

To date *Canacomyrica* has not been included in a phylogenetic study, but it has been suggested that its anomalous features may be primitive in Myricaceae (Macdonald, 1989; Carlquist, 2002). If this is the case, then a better understanding of the relationship of *Canacomyrica* to Myricaceae may shed light on the affinities of the family. Based on molecular data, Myricaceae are placed in Fagales (*sensu* APG, 1998, 2003) in the nitrogen-fixing clade, which are nested within the fabids (eurosid I clade) of the eudicots (Chase et al., 1993; Soltis et al., 1995, 2000; Swensen, 1996; APG, 1998, 2003). The expanded concept of Fagales comprises Nothofagaceae (sister to the rest of the order), Fagaceae, Ticodendraceae, Betulaceae, Casuarinaceae, Juglandaceae (including Rhoipteleaceae) and Myricaceae. With one exception (Maggia and Bousquet, 1994), all recent molecular studies of Fagales have reported Fagaceae (s.s.) as sister to the remaining five families (the core 'higher' hamamelids of Manos and Steele, 1997). However, relationships among these five families are not clear, especially with regard to the placement of Myricaceae. Manos and Steele (1997) showed Myricaceae to be sister to Betulaceae, Ticodendraceae and Casuarinaceae. Li et al. (2002) placed it sister to all other core 'higher' hamamelids, and Li et al. (2004) showed Myricaceae as sister to only Juglandaceae.

Many studies have employed phylogenetic analysis of DNA sequences of the plastid *rbcL* gene (ribulose bisphosphate carboxylase/oxygenase, large subunit) to assess the affinities of taxonomically enigmatic angiosperm taxa (Fay et al., 1997; Hibsch-Jetter et al., 1997; Morton et al., 1997; Fay et al., 1998; Chase et al., 2002; Längström and Chase, 2002; Sosa and Chase, 2003; Whitlock et al., 2003). A wide range of *rbcL* sequences are now available (Savolainen et al., 2000), permitting analyses across a diverse array of flowering plants.

In this study we used *rbcL* sequences to investigate the phylogenetic relationships of *Canacomyrica*. We chose to combine *rbcL* sequences with two more variable regions:

the plastid *trnL-F* region (consisting of the *trnL* intron and the *trnL-trnF* intergenic spacer); and the nuclear ITS region (consisting of the internal transcribed spacer region of the 18S-5.8S-26S nuclear ribosomal cistron). Many studies have shown the *trnL-F* region to be useful in resolving relationships above family level (e. g. Richardson et al., 2000; Li et al., 2002). Although ITS has been widely used for resolving sub-familial level relationships (Alvarez and Wendel, 2003), it is seldom used at higher taxonomic levels due to alignment difficulties. However, alternative sources of sequence data from the nuclear genome are limited. The availability of ITS sequence data for Fagales species motivated its use in this study as an additional source of potentially informative characters from a different genome.

MATERIALS AND METHODS

Plant material— Silica gel-dried leaf material (Chase and Hills, 1991) was obtained for *C. monticola*, *Myrica hartwegii* Watson, *Morella cordifolia* (L.) Killick and *Comptonia peregrina* (L.) L'Hérit. Voucher information and GenBank accession numbers for plant material used in this study are listed in Table 1. DNA was extracted using a 2X CTAB method adapted from Doyle and Doyle (1990), modified to include a wash with ammonium acetate (7.5 M NH_4AC; Weising et al., 1995) to remove impurities co-precipitated with the DNA.

TABLE 1. Accessions sampled. Voucher information and GenBank accession number are given for sequences reported here for the first time. Taxa for which sequences were previously published are listed with the original reference and GenBank accession number.

Taxon	*rbcL*		*trnL-F*		ITS	
	Voucher/Source	**Genbank**	**Voucher/Source**	**Genbank**	**Voucher/Source**	**Genbank**
Myricaceae						
Canacomyrica monticola Guillaumin	*Herbert 934* (E)	To be submitted	*Herbert 934* (E)	To be submitted	*Herbert 934* (E)	To be submitted
Myrica hartwegii S. Watson	*Edwards 93* (RPBG)	To be submitted	*Edwards 93* (RPBG)	To be submitted	*Edwards 93* (RPBG)	To be submitted
Morella cordifolia (L.) Killick	*Herbert 1007* (E)	To be submitted	*Herbert 1007* (E)	To be submitted	*Herbert 1007* (E)	To be submitted
Comptonia peregrina (L.) L'Hérit.	*Meagher sn* (E)	To be submitted	*Meagher sn* (E)	To be submitted	*Meagher* sn (E)	To be submitted
Juglandaceae						
Juglans nigra L.	Soltis et al., 1999	AF206785	Manos and Stone, 2001	AF303783	Potter et al., 2002	AF338491
Rhoiptelea chiliantha Diels & Hand.-Mazz.	Chen et al., 1998	AF017687	Manos and Stone, 2001	AF303773	Manos and Stone, 2001	AF303800
Betulaceae						
Alnus firma Siebold & Zucc.	Kamiya, unpublished	AB060562	Kamiya and Harada, unpublished	AB063524 and AB 063548	Navarro et al., 2003	AJ251684
Carpinus laxiflora (Siebold & Zucc.) Blume	Kamiya and Harada, unpublished	AB060585	Kamiya and Harada, unpublished	AB063571 and AB063541	Yoo and Wen, 2002	AF432038

Taxon	*rbcL*		*trnL-F*		ITS	
	Voucher/Source	**Genbank**	**Voucher/Source**	**Genbank**	**Voucher/Source**	**Genbank**
Casuarinaceae						
Casuarina equisetifolia L.	Sogo et al., 2001	AY033859	Li et al., 2002	AY147090	Steane, unpublished	To be submitted
Fagaceae						
Fagus crenata Blume	Martin and Dowd, 1993	L13339	Fuji et al., 2002	AB046508	Denk et al., 2002	AF456969
Quercus rubra L.	Bousquet et al., 1992	M58391	Gielly and Taberlet, 1994	X75707	Manos et al., 1999	AF098418

PCR amplification and sequencing— Amplification of the *rbcL* gene was carried out in two overlapping fragments using the forward primers 1F and 636F and the reverse primers 724R and 1460R (Fay et al., 1997). Amplification of the ITS1-5.8S-ITS2 region was carried out using the forward primer ITS5 and the reverse primer ITS4 (White et al., 1990). Amplification of the *trnL-F* region was carried out using the forward primer c and the reverse primer f (Taberlet et al., 1991).

PCR reactions of 50 µl contained: 2 µl DNA template, 0.2 mM of each dNTP, 0.3 µM of each primer, 2 units Taq polymerase (Bioline, London, UK), 2 mM $MgCl_2$, and 5 µl reaction buffer (160 mM $(NH_4)_2 SO_4$, 670 mM Tris HCl, 0.1% Tween 20, pH 8.8). The following PCR profile was used for both *rbcL* and *trnL-F*: 1 cycle at 94°C for 4 minutes; 30 cycles at 94°C for 45 seconds, 55°C for 45 seconds and 72°C for 3 minutes; 1 cycle at 72°C for 10 minutes. The PCR profile used for the ITS region was as follows: 1 cycle at 94°C for 3 minutes; 30 cycles at 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute 30 seconds; 1 cycle at 72°C for 5 minutes. The resulting PCR products were purified using QIAquick purification kits (QIAGEN Ltd., Crawley, UK) according to the manufacturer's instructions.

Sequencing reactions of 20 µl contained the CEQ™ DTCS Quick Start Kit (Beckman Coulter Ltd, High Wycombe, UK) and the following primers: the same primers as in the PCR for the *rbcL* gene; primers ITS2, ITS3, ITS4 and ITS5 (White et al., 1990) for the ITS region; and primers c, d, e and f (Taberlet et al., 1991) for the *trnL-F* region. Reactions were performed using the following PCR profile: 25 cycles at 96°C for 10 seconds, 50°C for 5 seconds, 60°C for 4 minutes. The sequence reaction products were cleaned according to manufacturer's instructions before being run on a CEQ™ 8000 Genetic Analysis System (Beckman Coulter Ltd, High Wycombe, UK). Forward and reverse sequences were manually assembled in Chromas version 2.12 (Technelysium Pty. Ltd., Helensvale, Australia).

Alignment and analyses— A preliminary study of over 500 eudicot taxa (Savolainen et al., 2000) was first performed to establish the broad-scale relationships of *Canacomyrica* (results not shown). Based on these results an *rbcL* data set of 35 taxa (the "narrow *rbcL*" data set) was assembled from the fabid (eurosid I) clade. Fabales

taxa were chosen as outgroup. New sequences included in the narrow *rbcL* data set are listed in Table 1; the remainder were previously published (Savolainen et al., 2000).

A combined data set of *rbcL*, ITS and *trnL-F* sequences focusing on 11 Fagales taxa (the "combined Fagales" data set) was assembled using new sequences produced in this study and previously published sequences (Table 1). For this data set, families are represented by just one or two genera because there was insufficient time to allow for additional sampling of ingroup and outgroup genera for all three gene regions. Following the approach of authors such as Wiens (1998) and Reeves et al. (2001) it was considered appropriate to combine these data sets after separate analyses showed there to be no strongly supported (>85%) incongruent clades among the individual data sets (results not shown). Fagaceae taxa were used as outgroup in this data set. Alignment of all sequences was carried out by eye. Alignment of *rbcL* sequences required no gaps. Alignment of the *trnL-F* sequences required the insertion of gaps (gaps were not coded). Alignment of the ITS sequences required the insertion of gaps (not coded) and the exclusion of some regions due to alignment ambiguity.

Phylogenetic analyses were performed using PAUP* Version 4.0b10 (Swofford, 1998). Maximum parsimony analysis was carried out with tree-bisection-reconnection (TBR) and saving multiple trees (MulTrees). Maximum parsimony trees were obtained using the heuristic search option for the narrow *rbcL* data set (Fitch parsimony). The small size of the combined Fagales data set made it possible to carry out a branch and bound search. Branch lengths were calculated using the delayed transformation (DELTRAN) optimization.

Support for the clades obtained was assessed using bootstrap analysis (Felsenstein, 1985) performed using the heuristic search option and 1000 replicates for the narrow *rbcL* data set, and using the branch and bound search option and 100 replicates for the combined Fagales data set. The following categories were used to describe bootstrap percentage (BP) results: 50-74, weak support; 75-84, moderate support; 85-100, strong support.

Chromosome count— Root tip preparations were made from living seedlings of *Canacomyrica* housed in the research collection at the Royal Botanic Garden, Edinburgh (RBGE). Root tips were treated, following the protocol of Jong and Möller (2000), in colchicine (0.05%) for 4 hours at room temperature, or in saturated aqueous 1-bromonapthalene for 2 to 8 hours at room temperature, or in 0.002 M 8-hydroxyquinoline for 4 to 8 hours at 12°C. They were variously stained in Feulgen's reagent (Fox, 1969), lacto-propionic orcein or aceto-carmine, but no method gave satisfactorily intense staining. After the root tips had been softened in an enzyme solution of 5% pectinase and 5% cellulase at 35°C for 20 minutes and mounted on slides, they were viewed using phase-contrast (Zeiss, Axiophot) at 40X1.6 or 100X magnification and images photographed digitally.

RESULTS

Sequencing— The narrow *rbcL* matrix contained 35 taxa and 1343 characters, of which 206 were potentially parsimony informative. Maximum parsimony analysis of the narrow *rbcL* data set produced 12 most parsimonious trees (tree length 869, CI=0.51, RI=0.58). One of the 12 trees is shown in Figure 1 with branch lengths above the branches (DELTRAN optimization) and bootstrap percentages (BP) equal to or greater than 50 shown below the branches.

In all trees, *Canacomyrica* is sister to Myricaceae taxa in a strongly supported clade (92 BP). In Figure 1 and in the strict consensus tree (not shown) Myricaceae forms a polytomy with two other clades, the first comprising Juglandaceae (including *Rhoiptelea*), the second comprising Betulaceae, Casuarinaceae and Ticodendraceae.

The combined Fagales matrix contained 11 taxa and 2728 characters, of which 210 were potentially parsimony informative. The *rbcL* data contributed 51 potentially parsimony informative characters (total 1286 characters), the *trnL-F* data contributed 75 potentially parsimony informative characters (total 1014 characters), and the ITS data contributed 84 potentially parsimony informative characters (total 428 characters). Maximum parsimony analysis of the combined Fagales data set produced a single most parsimonious tree (tree length 754, CI=0.82, RI=0.67). Figure 2 shows the single most parsimonious tree with branch lengths (DELTRAN optimization)

above the branches, and bootstrap percentages greater than or equal to 50 shown below the branches.

In the single most parsimonious tree, *Canacomyrica* is sister to a strongly supported clade (100 BP) comprising the rest of Myricaceae. Monophyly of Myricaceae s.l. (including *Canacomyrica*) is also strongly supported (100 BP). Myricaceae s.l. are sister to Juglandaceae, but this relationship received bootstrap support of less than 50%.

Chromosome count— The root material available for this study was of inferior quality due to the difficulty of cultivation for this rare material, which subsequently died. Under phase-contrast, chromosomes were clearly discernible in three metaphase or pro-metaphase cells. A total of 16 chromosomes (2n=16) were counted for *Canacomyrica monticola* (see Chapter 4).

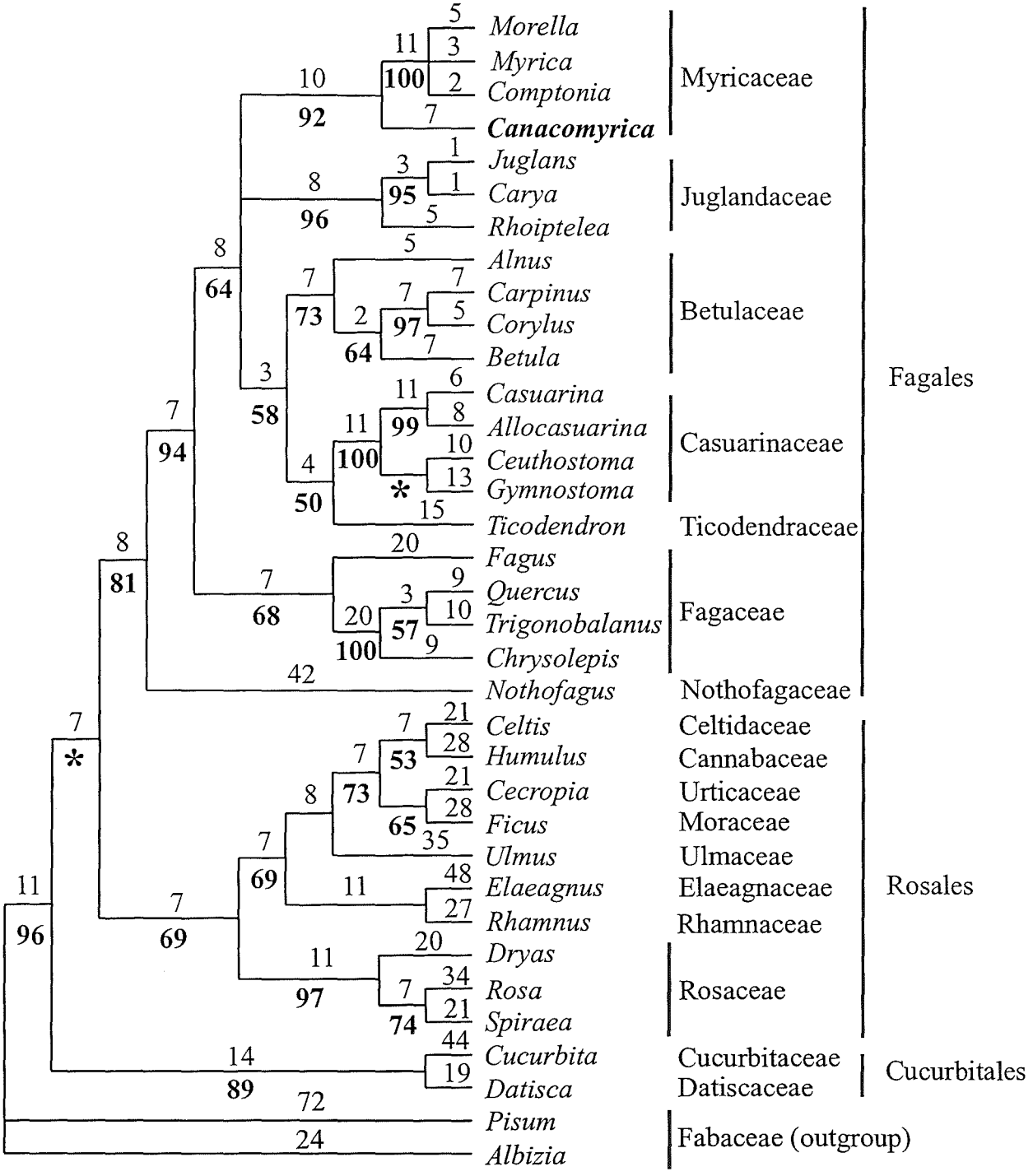

Figure 1: One of the 12 most parsimonious trees produced from analysis of the narrow *rbcL* data set. Branch lengths are shown above the branches (DELTRAN optimization), bootstrap percentages greater than or equal to 50 are shown in bold below the branches. Asterisks indicate groups not present in the strict consensus tree.

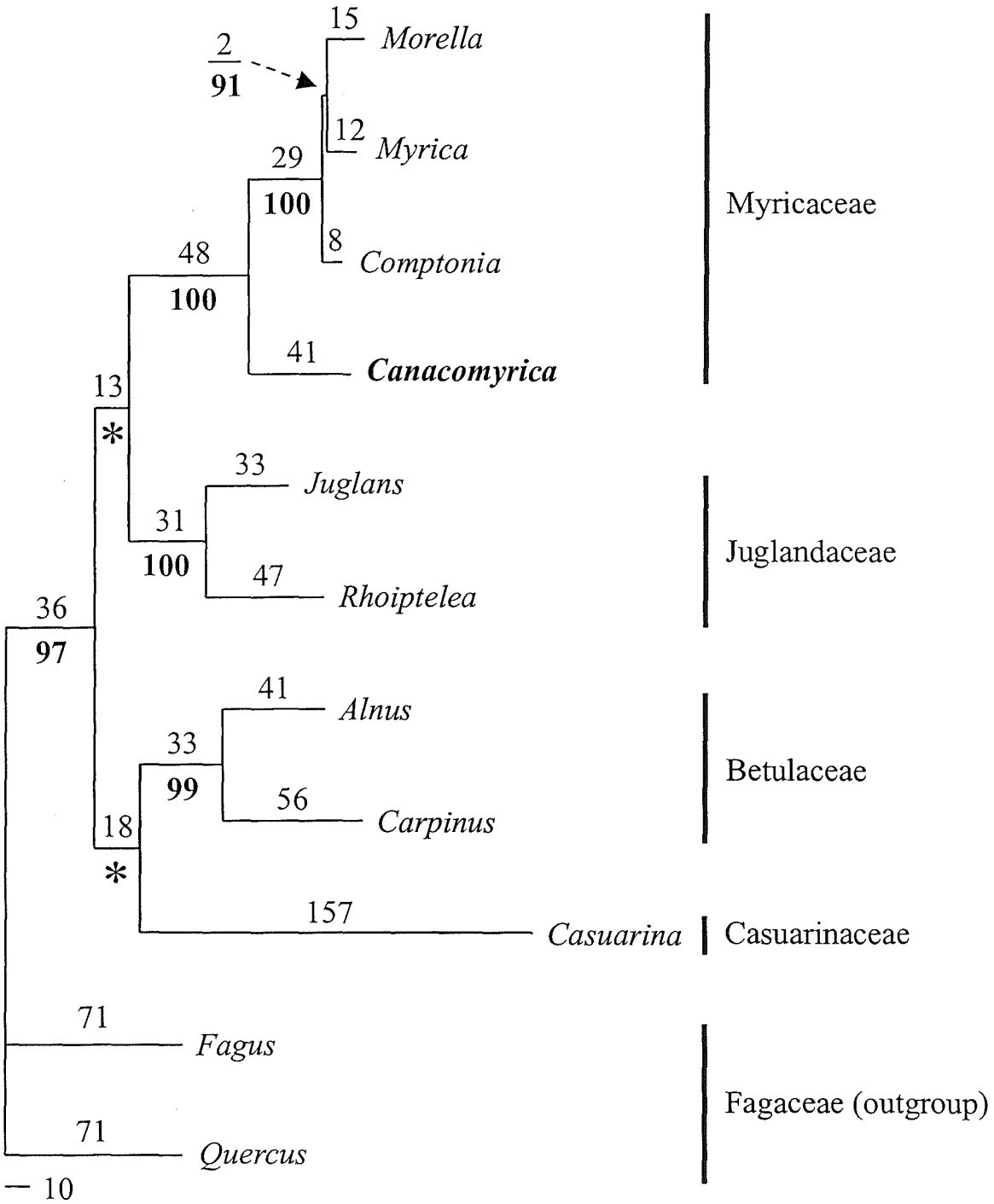

Figure 2: The single most parsimonious tree produced from analysis of the combined Fagales data set. Branch lengths are shown above the branches (DELTRAN optimization), bootstrap percentages greater than or equal to 50 are shown in bold below the branches. Asterisk indicates group with bootstrap percentage of less than 50.

DISCUSSION

Molecular evolution— In all our analyses, *Canacomyrica* formed a strongly supported monophyletic group with Myricaceae s.s. The combined use of DNA sequences from three different regions gave greater bootstrap support for this group than the analysis of *rbcL* alone (100 BP versus 92 BP). In our combined analysis, 40% of informative characters were from the ITS region. Of a total length of 664 aligned characters (including gaps) sequenced for the region, we were unable to use 236 characters (36%; 183 at the start of ITS1, 53 at the start of ITS2) due to alignment difficulties. Although the ITS region is seldom used above the genus level (but see Loockerman et al., 2003; Muellner et al., 2003), it appears that this region has some utility at higher taxonomic levels within Fagales.

Systematic implications— In common with most molecular studies of Fagales, there was insufficient variability within the DNA markers used to resolve the sister group of Myricaceae. In the single most parsimonious tree produced by the combined analysis Juglandaceae were sister to Myricaceae s.l. (including *Canacomyrica*), although this relationship received bootstrap support of less than 50%. It is possible that additional data may have improved resolution of the sister group of Myricaceae, but the findings of Li et al. (2004) suggest that even with a significantly increased amount of data, the relationships within the core 'higher' hamamelidae remain somewhat equivocal. In a phylogenetic study of Fagales using data from five regions, representing three genomes, Li et al. (2004) recovered a sister group relationship between Myricaceae and Juglandaceae with weak bootstrap support (66 BP) and high Bayesian posterior probability (0.95 PP; probability ranges from 0-1). They did not include *Canacomyrica*. A morphological phylogenetic study by Hufford (1992) also supported a sister group relationship between Myricaceae and Juglandaceae (but not *Rhoiptelea*), and a close relationship between these families has been recognized previously by several authors (e. g. Hjelmqvist, 1948; Leroy, 1949; Melchior, 1964; Cronquist, 1981; Macdonald, 1989; Thorne, 1992a, b, 2000; Kubitzki, 1993).

TABLE 2. Comparison of morphological characters of Fagales (excluding *Nothofagus*) (Goldberg, 1986; Kubitzki, 1993; Wilson and Johnson, 1989; Zheng-yi and Raven 1999)

Character	Fagaceae	Myricaceae	*Canacomyrica*	Juglandaceae	Betulaceae	Casuarinaceae
N-fixing root nodules	Absent	Present	Unknown	Absent	Present	Present
Stipules	Present	Absent or foliaceous	Absent	Absent (present in *Rhoiptelea*)	Present	Absent
Style	Linear, short	Linear, elongate	Lamellular, laciniate	Plumose or lamellular	Linear, elongate	Linear, elongate
Inflorescence structure	Usually lax	Erect, spicate	Erect, spicate	Lax	Lax	Erect, spicate
Ovule	Anatropous	Orthotropous	Orthotropous	Orthotropous (hemitropous in *Rhoiptelea*)	Anatropous	Orthotropous
Fruit size and structure	Nut (>1 cm long) borne in cupule	Drupe (to 3 cm diam.) usually covered with fleshy papillae	Drupe (to 5 mm diam.) with a smooth fleshy pericarp	Winged nutlet (to 9 mm diam.) or large nut (to 6 cm diam.) with much lobed cotyledons	Winged nutlet (to 5 mm diam.) or nut (to 2 cm diam.) enclosed in enlarged bracts	Samara (2-12 mm long) enclosed in woody bracteoles
Leaf form	Simple	Simple or pinnatifid	Simple	Pinnately or imparipinnately compound	Simple	Simple, reduced
Tepals/perianth	6 tepals	Absent	6 lobed perianth	0-4 tepals	(0-)1-4(-6) tepals	Absent
Integuments	2 (1)	1	2	1 (2)	1, 2	2
Chromosomes	x=12, 11	x=8	x=8	x=16	x=8,14	x=8,14

The principal differences between Myricaceae s.l. and Juglandaceae are leaf form, inflorescence structure, and fruit morphology and size (Table 2). The two families share the synapomorphies of chains of cuboidal crystal-containing cells in the wood (Carlquist, 2002) and aromatic resin glands. The lamellular, laciniate style and fleshy perianth that differentiate *Canacomyrica* from the rest of Myricaceae are characters that can be found in Juglandaceae (Table 2). The ovule in *Canacomyrica* is bitegmic (A. Doweld, personal communication; J. Herbert, unpublished data), which is shared homoplasiously with *Rhoiptelea*.

The sister relationship of *Canacomyrica* to the other Myricaceae genera, indicated in this study, is consistent with the original proposal to include it in the family (Guillaumin, 1939, 1940). Myricaceae are generally defined by simple, entire (pinnatifid in *Comptonia*) leaves with resinous (usually aromatic) 'balloon' glands (Chourey, 1974), short, erect inflorescences, solitary ovules and small drupaceous fruits with small (typically <10 mm diameter) seeds. *Canacomyrica* shares all these features with other genera of Myricaceae. Furthermore, studies have shown that *Canacomyrica* has pollen with a *Myrica*-type aperture (Sundberg, 1985) and myricaceous wood anatomy (Carlquist, 2002). The chromosome count reported here for *Canacomyrica* (2n=16) is consistent with counts reported for other members of Myricaceae, e.g. *Myrica* 2n= 16, 48, 96 (Löve, 1980, 1982; Morawetz and Samuel, 1989; Al-Bermani et al., 1993), *Comptonia* 2n=32 (Löve, 1982), and *Morella* 2n=16 (Oginuma and Tanaka, 1987).

The sister relationship between *Canacomyrica* and Myricaceae s.s. is also consistent with the opinion of Leroy (1949), who argued for subfamily status for *Canacomyrica*. The presence of a long terminal branch for *Canacomyrica* (Figure 2) indicates significant divergence between the two lineages as might be expected given its unique morphological features. However, on the basis of the strongly supported monophyly of Myricaceae s.l. in our analyses, and the numerous morphological features shared between *Canacomyrica* and Myricaceae, described above, we accept the original placement of *Canacomyrica* in Myricaceae.

***Dioecy in* Canacomyrica**— Many authors (e.g. Macdonald, 1977; Cronquist, 1981; Macdonald, 1989; Zomlefer, 1994) have continued to follow Guillaumin's (1940)

description of *Canacomyrica* in which he stated that there are both hermaphrodite and male flowers, despite later studies showing that the flowers are unisexual with female flowers bearing six sterile anthers or staminodes (Leroy, 1949; Kubitzki, 1993). Based on observations in the field and examination of a total of 50 flowers from 16 individual plants (J. Herbert, unpublished data; Chapter 4), we confirm that male flowers and functionally female flowers are found on separate plants in *Canacomyrica*.

A dioecious breeding system is consistent with Myricaceae (at least 75% of species are dioecious), but the presence of staminodes is unknown in the rest of the family. Staminodes have been recorded in *Lithocarpus* Blume and *Quercus* subg. *Cyclobalanopsis* (Oersted) C. K. Schneider (Fagaceae); the former is known to be entomophilous (Kaul, 1985). In *Canacomyrica*, the catkin-like inflorescence, the lax attachment of the anthers and the laciniate stigma indicate that it is wind pollinated. It could be argued, however, that the staminodes in *Canacomyrica* function as attractants for generalist pollinators. Although Myricaceae are generally considered to be an anemophilous family, in Hawaii it has been observed that introduced honey bees (*Apis mellifera*) visit the flowers of *Morella faya* (Aiton) Wilbur (Vitousek and Walker 1989). Insect pollination has also been observed in *Platycarya* Siebold & Zucc. (Juglandaceae; Endress, 1986). This raises the possibility that *Canacomyrica* is, at least in part, insect pollinated.

In the case of species in which monoecy or dioecy are common, the occurrence of staminodes may be due to incomplete suppression of male function (Walker-Larsen and Harder 2000). It appears that incomplete suppression of male function is frequently found in Myricaceae: within a single population of *Myrica gale* L. both dioecy and monoecy can occur, and sex expression within a single stem has been reported to be unstable from year to year (Lloyd 1981); androgynous or mixed inflorescences have been observed in several species of *Myrica* and *Morella*; and, perhaps most significantly of all, occasional functional stamens are often observed on the fruit wall of *Morella* species (Macdonald 1989; J. Herbert personal observations). In *Canacomyrica* the presence of staminodes could be viewed as an extreme example of the incomplete suppression of male function found throughout Myricaceae.

CONCLUSION

In conclusion, we accept the placement of *Canacomyrica* in Myricaceae on the basis of the molecular data presented here. This decision is supported by the confirmation of a dioecious breeding system and a chromosome count of 2n=16. Although the data are ambiguous with regards to the sister group of Myricaceae, the positive placement of *Canacomyrica* enhances our understanding of both the morphological characters within the family, and the characters shared by Myricaceae and Juglandaceae.

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Chapter 3

Distribution, habitat and Red List status of the New Caledonian endemic tree *Canacomyrica monticola* (Myricaceae)[1]

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Key words: bush fire, Endangered, *in situ* conservation, mining, primary forest, serpentine, ultramafic

Abstract. The monotypic genus *Canacomyrica* Guillaumin is a small tree endemic to the rare remaining fragments of primary forest growing on ultramafic geology in New Caledonia. In the rich flora of this island it is one of many endemics to be threatened by habitat loss due to a variety of factors, most significantly open-cast mining for nickel. Using field observations and data from herbarium specimens the extent of occurrence of *Canacomyrica monticola* is established to be approximately 1,510 km^2. Within this area the distribution of *C. monticola* is very fragmented and limited to just eleven known localities. Six localities are outside protected areas; two of these may be imminently threatened by mining activity and another may be threatened by bush fires. It is recommended that the IUCN Red List status of Endangered (EN B1ab(i,ii,iv,v)) is assigned to this species.

Introduction

Canacomyrica is a monotypic genus endemic to the Pacific island group of New Caledonia, a territory renowned for its rich flora (Jaffré et al. 1998). Grande Terre, the main island has an area of just 19,000 km^2 yet it is home to five endemic families, 110 endemic genera and more than 3,000 species of vascular plants, a remarkable 77% of which are endemic (WWF and IUCN 1995). Among these endemics are representatives of ancient lineages, such as the taxon considered to be sister to all other extant flowering plants, *Amborella trichopoda* (Zanis et al. 2002). The combination of high endemism and the presence of 'relict' taxa has lead to the recognition of New Caledonia as a distinct phytogeographic region (Takhtajan 1986).

[1] This chapter has been submitted to *Biodiversity and Conservation* and is presented in the style of that journal

The unique flora of New Caledonia is under threat from a number of factors, including deforestation, introduced species, fire, agriculture and livestock grazing (Olson et al. 2000). However, the greatest single threat posed to the island's plants is the practice of open-cast mining for metals. New Caledonia has one third of the world's reserves of nickel ore, found mainly in the ultramafic geology of the southern region of Grande Terre (Brooks 1987). It is to these ultramafic outcrops, with their economically valuable, nutrient-deficient soils, that many endemic plant taxa are restricted (e.g. Herbert et al. 2002; Jaffré et al. 2001; Pintaud and Jaffré 2001; Whitlock et al. 2003).

According to IUCN criteria (IUCN 1997), 14.4% of plant species in New Caledonia are Red Listed. However, this figure is likely to be much higher for plants endemic to, or growing predominantly on, ultramafic substrate. The lack of sufficient data on many species is likely to be a further factor contributing to a misrepresentation of the true number of plants threatened with extinction. Where specific groups have been examined in detail, the number of threatened species is found to be high. For example, the 43 species of conifer in the Territory are all endemic and of these, 67% are Red Listed (Farjon and Page 1999); similarly, of the 37 endemic palms, 35% are considered to be threatened (Pintaud and Jaffré 2001).

Regrettably, only 5,000 km^2 of primary vegetation (28% of the original extent) remains in New Caledonia (Myers et al. 2000) and less than 10% of the land area has protected status (Jaffré et al. 1998; Pintaud and Jaffré 2001; WWF and IUCN 1995). It has become clear in recent years that there is an urgent need for understanding and protection of the flora of this island, along with its unique fauna and marine biota (Bouchet et al. 1995; Jaffré et al. 1998; Mittermeier et al. 1996; Proctor 2003). New Caledonia has been acknowledged as one of the world's 25 biodiversity 'hotspots' (Myers et al. 2000) and has been identified as one of the WWF's 'Global 200' ecoregions, singled out as priority targets for conservation action (Olson et al. 2000).

Canacomyrica monticola is an evergreen shrub or small tree (up to 7m) with coriaceous leaves, flowers borne in spikes and black drupaceous fruits. It is entirely restricted in its distribution to primary forest on the ultramafic soils in the south of

Grande Terre. Since its description (Guillaumin 1940) *Canacomyrica* has been largely neglected, almost nothing is known of its ecology, the plant is not in cultivation anywhere in the world, and its conservation status remains unknown.

As the sole member of a geographically isolated genus, *Canacomyrica* has an important bearing on our understanding of evolutionary processes in Myricaceae (see Chapter 2). Basic data about its ecology and distribution are much needed to further knowledge about the entire family. Such information will also contribute to a better appreciation of the ecology of the rare remaining fragments of primary vegetation in New Caledonia. The aim of this study is to determine the distribution and habitat of *Canacomyrica* in New Caledonia and to assess its conservation status.

Materials and Methods

A list of all known localities for *Canacomyrica* was compiled from herbarium specimens held in three collections: Royal Botanic Garden Edinburgh (E) (16 specimens); Institut de Recherche pour le Développement, Noumea (NOU) (35 specimens); the Muséum National D'Histoire Naturelle, Paris (P) (47 specimens). The latter two institutions hold large numbers of collections of the New Caledonian flora. Herbarium specimens have been acknowledged as suitable data sources for assessing plant distributions in the absence of other information (Willis et al. 2003).

During a three-week expedition to New Caledonia (RBGE expedition, May 2001) populations of *Canacomyrica* were sought from throughout the ultramafic region of Grande Terre. Field observations, collections of herbarium specimens, seed and seedling collections were made by the author. Two populations were located and at each field site, data were recorded on substrate, elevation, habitat features and threats to habitat. An attempt was made in each case to assess the extent and demography of the population.

The conservation status of *Canacomyrica* was determined using the IUCN Red List criteria (IUCN 2001). Measurement of extent of occurrence is the first step in estimating the geographic distribution of a species. The known localities of

Canacomyrica were plotted on a map and a boundary drawn between them, the extent of occurrence was estimated by manual measurement of the area within this boundary.

Results

A survey of the herbarium specimens held in Edinburgh (E), Paris (P) and Noumea (NOU) revealed nine localities for *Canacomyrica*, a further two were reported by T. Jaffré (personal communication). The eleven localities are shown in Figure 1 and details are given in Table 1. All eleven localities were in the south of the island, on ultramafic substrate. Manual measurement of the area between the eleven known localities gave an extent of occurrence of 1,510 km^2.

Canacomyrica was examined in the field at Mont Bouo and Mont Mamié (Table 1, Figure 1). Plants at both sites were highly localised, occurring in almost monospecific stands with few or no outlying individuals. The local distribution of the populations appeared to be limited to continually wet habitat, however it is possible that there is seasonal variation in water availability at these sites.

At Mont Bouo, plants of *Canacomyrica* were found only between altitudes of 1,050 m and approximately 1,150 m, growing in a rainforest community on ultramafic substrate. Mature individuals were 3-4 m in height. The habitat at Mont Bouo appeared to be undisturbed and access was difficult.

At Mont Mamié, plants of *Canacomyrica* were found at 500 m, the lowest recorded altitude for the plant (most collections have been made above 800 m). *Canacomyrica* was growing in a low scrub community co-dominated by Cyperaceae species on ultramafic substrate, inundated with water from abundant natural springs. Mature individuals were up to 1 m in height; many seedlings were observed in this population. The habitat at Mont Mamié was disturbed and access was relatively easy due to the presence of mining prospecting tracks.

At both sites there were more than 30 mature individuals, but it was not possible to estimate the total number of mature plants at either site due to time constraints. The

total area occupied by the population at Mont Bouo was estimated to be approximately 100 m^2. Time constraints prevented estimation of the total area occupied by the population at Mont Mamié.

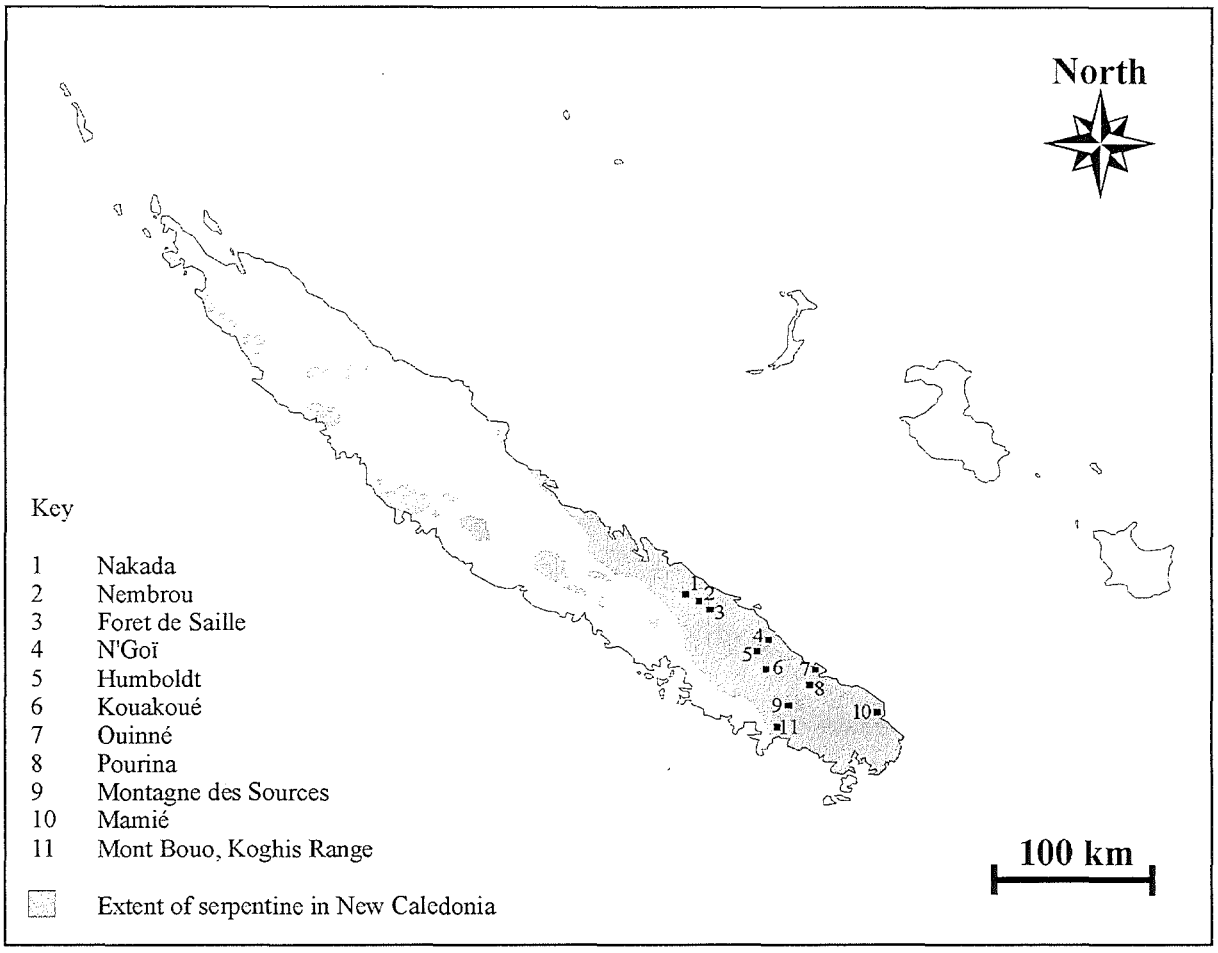

Figure 1. Map of New Caledonia showing the extent of ultramafic geology and the position of the eleven known localities for *Canacomyrica*

It was expected that further populations would be found at Mont Mou and Rivière Bleue but plants of *Canacomyrica* were not found at these localities during the expedition. All suitable habitat for *Canacomyrica* was searched at Mont Mou. At Rivière Bleue it was not possible to search all suitable habitat due to the difficulty of the terrain. On the basis of these observations, it is considered that a single collection from Mont Mou (*Baumann-Bodenheim* 15679, P) is a doubtful locality for *Canacomyrica*. It is thought likely that the specimen was collected elsewhere and incorrectly labelled, alternatively (but less likely) this collection may represent an extinct population. It was not possible to gain access to populations within the strictly protected Nature Reserve of Montagne des Sources. Other locations were not visited due to the time constraints of the field study.

Discussion

Canacomyrica is known to occur in eleven localities in the south of Grande Terre, New Caledonia where it grows exclusively on ultramafic substrates. Of these localities, six are afforded no official protection and disturbance has been observed at the Mont Mamié site. There is evidence of disturbance caused by mining prospecting at the Ouinné site and the Nembrou site is potentially threatened by bush fire (J. Manaute and T. Jaffré, personal communication). *Canacomyrica* is estimated to have an extent of occurrence of 1,510 km^2 and within this area its distribution is severely fragmented. If the population at Mt Bouo is typical, where *Canacomyrica* occurs in an area of approximately 100 m^2, then it is tempting to speculate that the total area of occurrence for the species is significantly smaller than its area of occupancy. Continuing decline is projected in both the area and extent of occurrence, and number of locations. Also, continuing decline in number of mature individuals is inferred at the Mont Mamié, Ouinné and Nembrou sites. It is therefore recommended that *Canacomyrica monticola* is given the IUCN (2001) Red List status of Endangered (EN B1ab (i,ii,iv,v)).

Table 1. Known localities for *Canacomyrica*, with coordinates and protected status details

Locality	Coordinates	Protected status and notes[1]
Mont Bouo (Koghis range)[2]	22°10'S 166°30'E	**Protected** - amenity protected area, adjacent to the Strict Nature Reserve of Montagne des Sources
Mont Mamié[2]	22°06'S 166°53'E	**Unprotected** - mining activity observed
N'Goï[3]	21° 49'S 166°30'E	**Unprotected**[A] - adjacent to existing Special Botanical Reserve of Mt Humboldt (5 kms)
Montagnes des Sources[3]	22°07'S 166°33'E	**Protected** - Strict Nature Reserve of Montagne des Sources
Pourina[3]	22° 01'S 166°44'E	**Unprotected** - adjacent to Special Botanical Reserve of Haute Pourina (3 kms) and Natural park of Rivière Bleue (5 kms)
Ouinné[3]	21° 57'S 166°42'E	**Unprotected** [A]
Kouakoué[3]	21° 57'S 166°32'E	**Protected** [B] - Special Fauna and Flora Reserve of Mt Kouakoué
Humboldt[3]	21° 53'S 166°25'E	**Protected** [B] - Special Botanical Reserve of Mt Humboldt
Nembrou[3]	21° 40'S 166°09'E	**Unprotected** - adjacent to Special Botanical Reserve of Forêt de Saille (5 kms)
Nakada[4]	21° 38'S 166°03'E	**Unprotected** - adjacent to Special Botanical Reserve of Forêt de Saille (16 kms)
Forêt de Saille[4]	21° 40'S 166°13'E	**Protected** - Special Fauna and Flora Reserve of Forêt de Saille

[1]Sources of information on protected status: Pintaud and Jaffré (2001), J. Manaute (personal communication); [2]Locality visited by the author; [3]Locality determined from herbarium specimens; [4]Locality according to T. Jaffré (personal communication); [A] site adjacent to proposed "Ni-Kouakoué-Ouinné" reserve; [B] site expected to be included in the proposed "Ni-Kouakoué-Ouinné" reserve.

The principal threats to *Canacomyrica* are likely to be open-cast mining and bush fires in areas of its occurrence lacking protected status. The most imminently threatened populations are at Mont Mamié, Ouinné and Nembrou. Habitat destruction in an area where *Canacomyrica* occurs may entirely destroy localised and fragmentary populations that are characteristic of this species. It is encouraging that age structure, indicative of recent regeneration, was observed at Mont Mamié although it is stressed that this was only observed on undisturbed substrate. In experimental work, approximately 90% of fruits examined were sterile and *ex situ* germination of seeds and cultivation of seedlings was unsuccessful (J. Herbert, unpublished data; see Chapter 4). This suggests that *in situ* measures are likely to be the best if not the only approach for the conservation of this species.

Conservation achieved through a network of protected areas is considered to be the most effective way to preserve biodiversity (Primack 2000). This is never more appropriate than in the case of species with highly specialized ecological requirements that are unlikely to thrive anywhere but in their natural habitat, such as *Canacomyrica*. Whilst it is desirable for additional data to be collected on numbers of individuals, area of occurrence and threats to localities other than those detailed here, the data presented are sufficient to permit the following recommendations to be made: 1) expansion of the Special Botanical Reserves of Haute Pourina and the Forêt de Saille should be undertaken to protect the populations at Pourina and Nembrou; 2) the proposed "Ni-Kouakoué-Ouinné" reserve (project under consideration; J. Manaute personal communication) should include the sites at N'Goï and Ouinné; 3) special attention should be given to *Canacomyrica* when botanical surveys or inventories are carried out at sites on ultramafic to enhance knowledge of the distribution and demography of this species; 4) an investigation to assess the level of genetic diversity, both within and among populations, should be carried out to act as a guide for the prioritisation of populations in future conservation management.

Protection of the above mentioned populations would raise the number of protected localities for *Canacomyrica* from five to nine, or 82% of all known sites. These measures represent the first steps towards ensuring the continued survival of *Canacomyrica*. Conservation measures targeted at *Canacomyrica* will help to raise the

profile and survival prospects of some of the last remaining fragments of New Caledonia's primary forest.

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Chapter 4

Aspects of the morphology and ecology of *Canacomyrica*

ABSTRACT

Plant material from two natural populations of *Canacomyrica monticola* was investigated. Contentious aspects of the floral and seed morphology were clarified and aspects of the biology of this rare species were examined. A chromosome count of 2n=16 was determined for *Canacomyrica* and the seed was found to have two integuments. In addition to these new findings, it was confirmed that *Canacomyrica* has a dioecious breeding system and that the female flowers have six sterile anthers or staminodes. Seed viability and germination tests appear to suggest that low seed set is a feature of this species. It was not possible to distinguish between low seed viability or the presence of germination-inhibition factors. Despite being endemic to ultramafic (serpentine) substrates, it was shown experimentally that *Canacomyrica* is not an accumulator of nickel. It was not possible to determine whether nitrogen-fixing root nodules are present in this species.

INTRODUCTION

The confirmation of the placement of *Canacomyrica* in Myricaceae (Chapter 2) makes it possible, for the first time, to examine the morphology of this poorly known species within a taxonomic framework. In the original description of *Canacomyrica*, Guillaumin (1940) noted several unique features: an androdioecious breeding system; a lamellar, laciniate style; a fleshy perianth; an inferior ovary; and an ovule hanging from a long funicle[1] with an inferior micropyle. Guillaumin (1939) considered these characteristics to be of sufficient significance to suggest that *Canacomyrica* should be given the rank of tribe or subfamily. Subsequently, Guillaumin (1948) altered his description of the taxon, stating that the inflorescences were co-sexual and the ovule anatropous with the micropyle at the end of a long funicle[2]. This description was rejected, in part, by Leroy (1949) who considered the ovule to be erect, sessile and orthotropous with a superior micropyle. He also asserted that the stamens of the female flower were non-functional. Further work by Leroy (1957) regarding the anatomy of the fruit of *Canacomyrica* was

[1] Funicle is apparently used here in its conventional sense to mean the placental attachment of the ovule to the fruit wall but see note 2.

[2] Funicle is used here in a different sense than previously, apparently to mean the micropylar tube.

inconclusive. The confusion surrounding the description of this taxon suggested that its reproductive morphology required re-evaluation.

Canacomyrica is endemic to serpentine outcrops on Grande Terre, New Caledonia. Among the plants growing on the serpentine of this island, some have responded to the challenging edaphic conditions (high levels of toxic metals, low level of essential nutrients; Brooks, 1987) by accumulating high levels of metals in the stem and leaf. New Caledonia has 47 species of hyperaccumulators (plants containing levels of nickel in excess of 0.1% in the leaves), the highest number of all the serpentine regions of the world (Brooks, 1987). There is no published information regarding the status of *Canacomyrica* as a nickel accumulating species.

A feature of Myricaceae is the actinorhizal association that species of *Myrica*, *Morella* and *Comptonia* have with the nitrogen-fixing actinomycete *Frankia*. This symbiosis is closely linked with the ability of these plants to colonise nutrient-poor substrates. It is not known whether *C. monticola* bears root nodules.

The following aspects of the morphology and biology of *Canacomyrica* were studied in order to improve our understanding of this species: (1) chromosome number; (2) floral morphology and seed anatomy; (3) seed viability and germination rates; (4) nickel accumulation; and (5) nodulation. The results of the investigations into the cytology and floral morphology of *Canacomyrica* are those referred to in Chapter 2.

MATERIALS AND METHODS

Plant material— Samples of *Canacomyrica* were collected in New Caledonia (May 2001) by the author (for details see Chapter 3). Prior to the start of the study, *Canacomyrica* seeds were stored at 4 °C in the Harold Mitchell Laboratories, University of St Andrews. Living specimens were housed in the tropical glasshouses at the Royal Botanic Garden Edinburgh and herbarium specimens were lodged in the herbarium of

RBGE (E). Additional herbarium specimens were obtained on loan from Muséum National D'Histoire Naturelle, Paris (P; see Chapter 3).

Chromosome count— Root tips were harvested from seedlings of *Canacomyrica* growing in bark compost in tropical glasshouse conditions. Root tips were harvested in tap water and adhering soil was removed before transferring to pre-treatment solutions (spindle-fibre inhibitors). To maximise the number of cells in metaphase, three pre-treatment chemicals were used: colchicine; 1-bromonaphthaline; 8-hydroxyquinoline (Table 1). Newly harvested root tips were used for each study. Following pre-treatment, root tips were rinsed in tap water and placed in a fixative solution of 3 parts ethanol to one part glacial acetic acid (Farmer's Fluid) for a minimum of 20 minutes.

Root tips were rinsed in distilled water and hydrolysed in 5N HCl for varying lengths of time (see Table 1). They were then briefly rinsed in distilled water and placed in Feulgen's Reagent for 3 hours to stain the chromosomes. To intensify the stain the root tips were placed in several changes of tap water for 10 minutes. At this point it was necessary to soften the root tips by incubating them at 37 °C in an aqueous enzyme solution (1 part 4% pectinase and 1 part 4% cellulase; see Table 1), in order to make them easier to squash.

After the enzyme treatment, a root tip was rinsed in distilled water and placed on a microscope slide in a drop of 45% acetic acid and the apical region dissected. This region was then macerated using a metal tapper, before applying a cover slip. Pressure was applied to the coverslip through 2 layers of folded filter paper which absorbed any excess liquid. Finally the slide was sealed with rubber solution to prevent it from drying out too quickly.

Slides were viewed using bright-field and phase-contrast (Zeiss, Axiophot) at 40x1.6 or 100x magnification. Images were captured using a high resolution digital camera and the software package OPTIMAS 6.0.

TABLE 1. Treatments carried out on root tips

Pre-treatment chemical	Length of pre-treatment[1]			Temperature[2]
	#1	#2	#3	
Colchicine (0.05%)	4 hrs 30 mins	-	-	RT
1-bromonaphthaline (saturated aqueous solution)	8 hrs	2 hrs	7 hrs	RT
8-hydroxyquinoline (0.002M aqueous solution)	8 hrs	4 hrs	7 hrs	12°C
None	-	-	-	-

[1] Details of pre-treatments: #1 No hydrolysis, enzyme softening for 2 hours 30 minutes; #2 Hydrolysis in HCl for 30 minutes, enzyme softening for 30 minutes; #3 Hydrolysis in HCl for 1 hour, enzyme softening for 2 hours 30 minutes.

[2] RT=standard room temperature

Investigation of floral morphology— Ten herbarium specimens (Table 2) of *Canacomyrica* were examined under a light microscope (Zeiss Stemi 2000-C; Royal Botanic Garden Edinburgh). A total of 50 flowers (morphologically male and morphologically hermaphrodite flowers) from 16 plants originating from two geographically separate populations (Table 2) were dissected. A list of characters was scored (Table 3). These specimens were chosen because they had been recently collected and had been pressed without the use of heat. Therefore, the quality of the floral organs was expected to be higher than in any of the older specimens that were available for examination. Photographs were taken with a manual camera using Fujichrome 64T slide film. Further investigation of anthers was undertaken using a scanning electron microscope (FEG SEM Supra 55VP) at the Royal Botanic Garden, Edinburgh.

TABLE 2. Herbarium specimens consulted for floral morphology data (all specimens held at RBGE Edinburgh (E)

Locality	Date of collection	Collector and collection number	Herbarium barcode
New Caledonia:	1st June 2001	*Herbert* 929, 935, 931*	E00137005
Province Sud:		*Herbert* 932	E00137023
Dumbéa: Monts		*Herbert* 927	E00137022
Koghis: Mt. Bouo		*Herbert* 934, 901, 925, 926, 936*	E00137011
New Caledonia:	9th June 2001	*Herbert* 1807	E00137013
Province Sud: Yaté:		*Herbert* 1823	E00137017
Mont Mamié		*Herbert* 1832	E00137015
		Herbert 1816	E00137016
		Herbert 1803	E00137021
		Herbert 1814	E00137018

* denotes single herbarium specimen bearing several separate collections

TABLE 3. Floral characters scored in this investigation

Flower part	Character	Character states
Androecium	Anthers	Present (1) Absent (0)
	Anther dehiscence	Dehiscent (1) Indehiscent (0)
	Pollen	Present (1) Absent (0)
Gynoecium	Stigma	Fully developed (1) Vestigial or lacking (0)

Investigation of reproductive ecology— Fruits collected in New Caledonia were stored at 4 °C prior to commencement of tests. The bony endocarp of 100 fruits was opened using a small vice and the seed removed. The number of fruits containing apparently healthy seed was recorded.

As previously mentioned, a comparison of the work of Guillaumin (1939, 1940, 1948) and Leroy (1949, 1957) reveals a number of inconsistencies in the description of seed morphology in this taxon, particularly regarding the presence of a 'funicle'. For this reason fruits were examined under a light microscope (Zeiss Stemi 2000-C; Royal Botanic Garden Edinburgh) for evidence of the 'funicle' and to ascertain the number of integuments present. Both fully developed seed and apparently sterile testa were examined and photographs taken using a manual camera as above.

Seed viability— The TTC (2,3,5- Triphenyl tetrazolium chloride: $C_{19}H_{15}N_4Cl$) test was used to investigate seed viability (Hartmann and Kester, 1983). The action of dehydrogenase enzymes, present only in living seeds, causes TTC to become hydrogenated producing a red stain that can be easily seen with the naked eye. Fruits were soaked in distilled water for approximately 48 hours before commencement of the experiment. The endocarp was cracked open using a small vice and the seed removed. The seed was divided in half and placed, cut face down in a 1% solution of TTC. After 30 minutes had elapsed the sections of endosperm were examined for any colour change. Pea (*Pisum sativum* L.) seeds, treated as above, were used as a positive control in this investigation.

Germination tests— The conditions required for germination of seed vary widely depending on a number of factors including the temperature and daylight regime of the region of origin, dispersal mechanism and internal factors. It has been documented that *Comptonia peregrina* contains an inhibitory factor, possibly abscisic acid, that causes fruit dormancy (Del Tredici and Torrey, 1976). Del Tredici and Torrey (1976) were able to overcome seed dormancy in *C. peregrina* by using gibberellic acid (GA_3); this was most effective at a concentration of 500 ppm. An investigation was undertaken to establish the best conditions for germination and to test whether gibberellic acid would promote germination in *Canacomyrica*. A subset of fruits was exposed to a variety of pre-treatments and germination conditions (Table 4).

Dimethylglyoxime test for nickel (Ni)— The dimethylglyoxime test is a simple colour-change test for the presence of nickel. Presence of the metal is indicated by a change from white to pink. Whatman No. 1 filter paper was soaked in a saturated solution of dimethylglyoxime (2,3-Butanedione diozime: $C_4H_8N_2O_2$) in ethanol. The filter paper was then allowed to dry in a fume cupboard and was stored in an airtight container prior to use. Silica dried leaf material of *Canacomyrica* was ground to a powder and re-hydrated using hand-warm distilled water. The leaf pulp was then applied to the centre of a prepared filter paper and any colour change was recorded. Four leaf samples from each of the two populations of *Canacomyrica* were tested.

Ability of* Canacomyrica *to develop root nodules— The presence of root nodules in all members of the Myricaceae indicates the presence of an actinorhizal association with the nitrogen-fixing actinomycete *Frankia*. *Canacomyrica* was observed in its natural habitat growing in a hard substrate that made it impossible to determine presence or absence of root nodules. Living seedling, not bearing root nodules at the time of collection, were brought back to the UK and housed in quarantine in the tropical greenhouse at RBGE. By inoculating the growing medium of these seedlings with free living *Frankia* sourced from the growing medium of another Myricaceae species, it was expected that nodulation could be induced in *Canacomyrica*.

TABLE 4. Treatments to which fruits were subjected

Pre-treatment 1	Pre-treatment 2	Planting medium	Temperature
None	None	Levington potting compost (F1)	20 °C constant
		Levington ericaceous compost (M1A)	
None	None	Nylon gauze over distilled water	30°C day temp. /
		Nylon gauze over distilled water containing fragments of rock[1]	25°C night temp.
Abrasion with sandpaper	Soaking in H_2O (24hours)	Levington potting compost (F1)	20 °C constant
		Levington ericaceous compost (M1A)	
Abrasion with sandpaper	Soaking in ethanol (1-10	Levington potting compost (F1)	20 °C constant
	days)	Levington ericaceous compost (M1A)	
Abrasion with sandpaper	Soaking in 1M HCl (1-10	Levington potting compost (F1)	20 °C constant
	days)	Levington ericaceous compost (M1A)	
Deep abrasion (endocarp cut with blade)	Soaking in GA_3 (500ppm)	Agar growth medium	RT
Excision from endocarp	None	Agar growth medium	RT
Excision from endocarp and testa	None	Agar growth medium	RT

[1]collected from vicinity of natural population in New Caledonia

RESULTS

Chromosome count— Experimental conditions #1 and #2 yielded no results, the chromatin appearing unstained and the roots moribund. Experimental conditions #3 yielded slides that, when viewed using bright-field, showed the chromatin to be unstained. The root material available for this study was of inferior quality due to the difficulty in the cultivation of this rare material. Under phase-contrast, however, the root tip preparations showed three countable metaphase or pro-metaphase cells in which sixteen chromosomes could be counted (Figure 1), indicating a chromosome number of 2n=16.

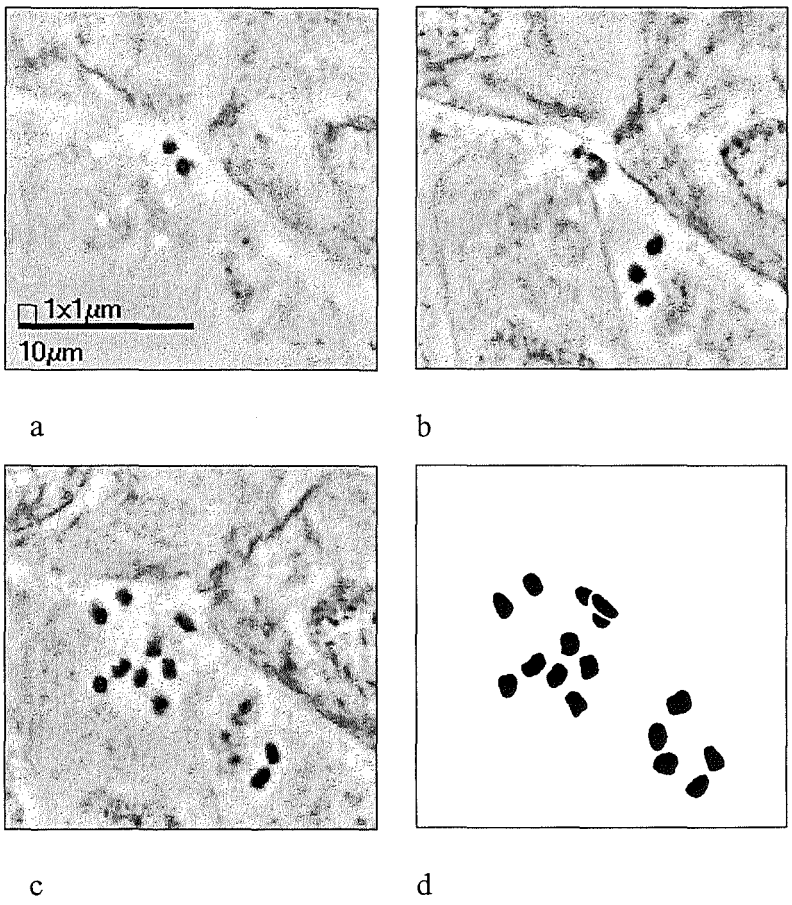

Figure 1 (a-d). Chromosomes of *C. monticola*. Photographs a-c show the same cell taken at different depths of field to show all the chromosomes present. A composite drawing (d) shows the 16 chromosomes, based on images a-c.

Floral morphology— The investigation of floral morphology showed that all flowers bore anthers. The results are collated for each individual plant (Table 5). Seven of the 16 individual plants examined bore flowers with fully developed styles, and nine bore flowers with vestigial styles (Figure 2 and 3). The lamellular and laciniate character of the style is clearly illustrated in Figure 3. All flowers with vestigial styles had dehiscent anthers (Figure 4) and, in all but one flower, pollen grains were observed. All flowers with fully developed styles had indehiscent anthers (Figure 5) within which no pollen grains were observed. A further 50 herbarium specimens were examined. No specimen was observed to differ from the pattern stated here.

TABLE 5. Results of investigation of floral morphology

Specimen (*Herbert* #)	**No. flowers dissected**	**Anther presence**	**Anther dehiscence**	**Pollen presence**	**Style development**
901	4	Present	Dehiscent	Present	Vestigial
925	3	Present	Dehiscent	Present	Vestigial
926	3	Present	Dehiscent	Present	Vestigial
927	4	Present	Indehiscent	Absent	Developed
929	3	Present	Indehiscent	Absent	Developed
931	3	Present	Indehiscent	Absent	Developed
932	3	Present	Dehiscent	Present	Vestigial
934	3	Present	Dehiscent	Present	Vestigial
935	3	Present	Indehiscent	Absent	Developed
936	3	Present	Dehiscent	Present	Vestigial
1803	3	Present	Indehiscent	Absent	Developed
1807	3	Present	Dehiscent	Absent*	Vestigial
1814	3	Present	Indehiscent	Absent	Developed
1816	3	Present	Dehiscent	Present	Vestigial
1823	3	Present	Indehiscent	Absent	Developed
1832	3	Present	Dehiscent	Present	Vestigial

* The lack of pollen in this specimen is considered to be the result of complete dehiscence of the anthers observed and not due to sterility

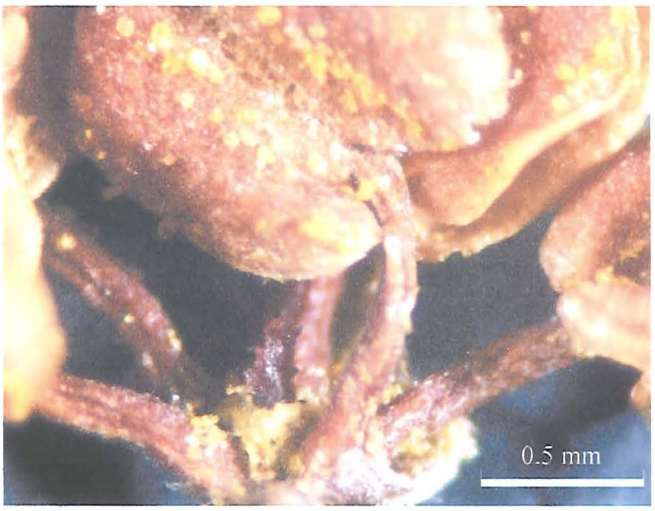

Figure 2. Male flower showing developed anthers and vestigial style.

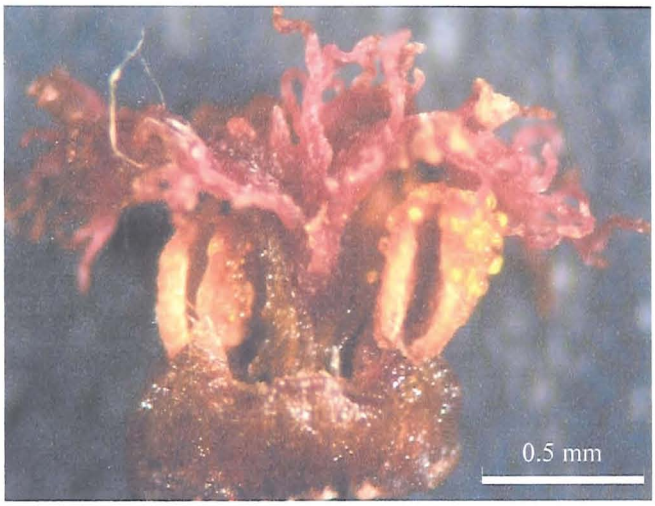

Figure 3. Female flower showing lamellular, laciniate style and staminodes.

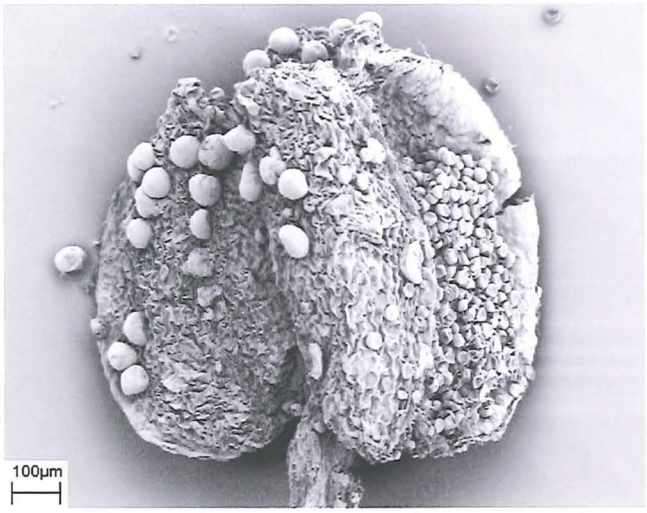

Figure 4. Scanning electron micrograph of dehiscent anther showing pollen grains on right (larger spherical objects are the resinous balloon glands found in all Myricaceae species).

Figure 5. Scanning electron micrograph of indehiscent anther.

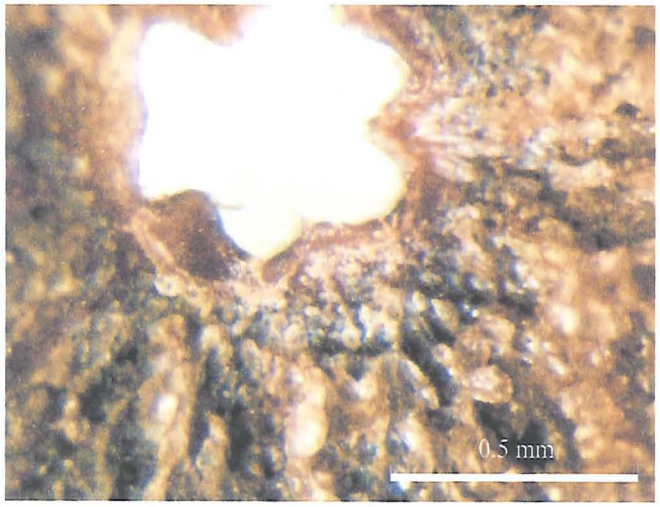

Figure 6. Inner surface of the mesocarp showing its spongey (papillose) texture and trichomes at the apical edge.

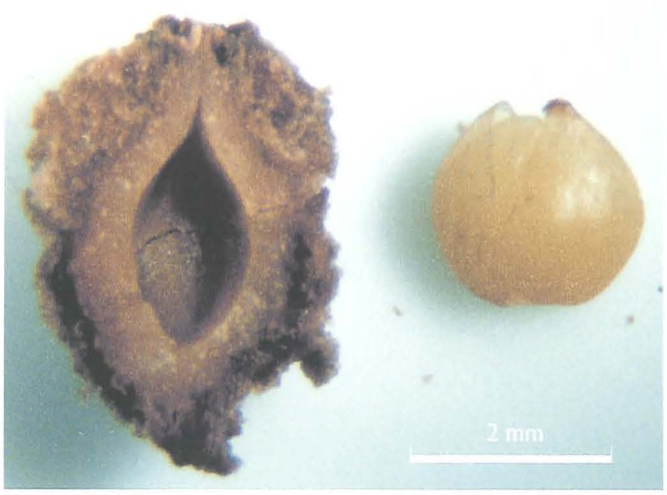

Figure 7. Longitudinal section of fruit (smooth pericarp removed) showing the empty nut on the left and the seed with transparent testa (split) on the right.

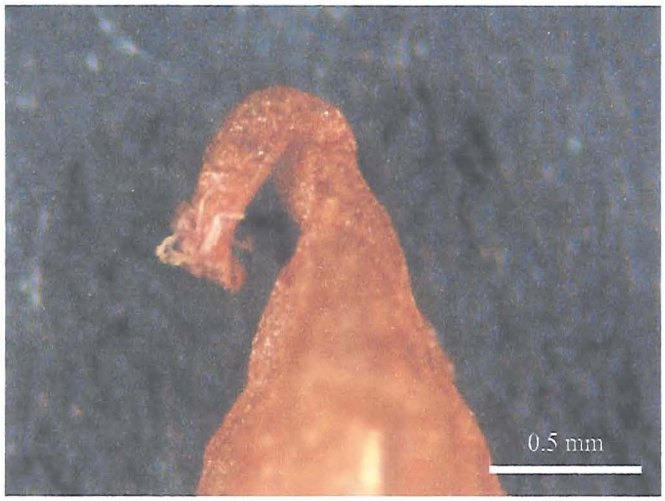

Figure 8. Sterile testa showing a long, apical tube, laciniate at its end.

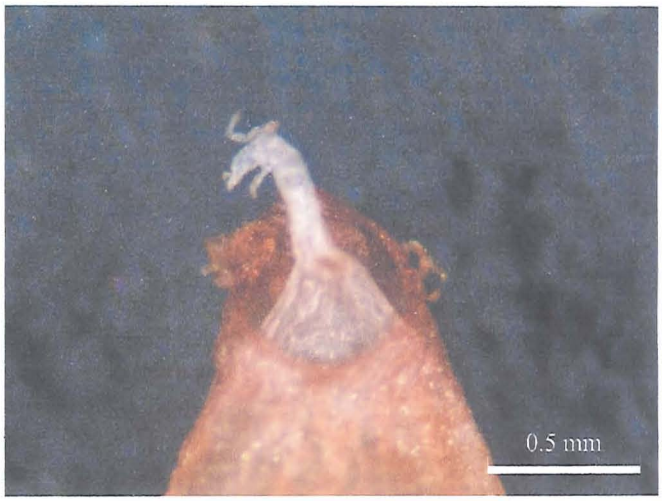

Figure 9. Sterile testa appearing to show the presence of two integuments

Seed morphology— The fruit of *Canacomyrica* is a drupe with a smooth black pericarp (see Figure 1, Chapter 1), spongy mesocarp (Figure 6) and a bony endocarp. From a total of 100 fruits that were examined, only 27% of fruits were found to contain a seed (Figure 7). The majority of fruits contained a sterile testa (Figure 8) which may represent an unfertilized ovule or an aborted seed. The apex of a sterile testa is shown in Figure 8, clearly showing a long apical tube, the end of which is laciniate. Figure 9 appears to show the presence of two integuments in the sterile testa.

Seed viability and germination tests—The TTC test for seed viability produced no staining in *Canacomyrica* seeds. Deep red staining was observed in the peas. Of the pre-treatment and planting conditions tested, the only conditions to result in germination were complete excision from the endocarp and testa followed by planting on agar growth medium. This resulted in the germination and production of green cotyledons in a single seed. However, this plantlet did not produce roots or develop any further.

Dimethylglyoxime test for nickel (Ni) — No colour change was observed for any of the leaf samples of *Canacomyrica* tested.

Ability of C. monticola seedlings to develop root nodules— It was not possible to test for nodulation in seedlings because all individuals had died before the test could be performed.

DISCUSSION

Several aspects of the morphology of *Canacomyrica monticola* were investigated. The chromosome count of 2n=16 for *Canacomyrica* agrees with the base number of Myricaceae (x=8). The investigation of floral morphology suggests that plants of *Canacomyrica* bear either male inflorescences or inflorescences of flowers composed of staminodes and a functional gynoecium. Thus, this investigation appears to confirm that *Canacomyrica* is dioecious. Both chromosome number and floral morphology provide support for a close relationship between *Canacomyrica* and the remainder of Myricaceae.

It appears that the seed of *Canacomyrica* has two integuments (confirmed by A. Doweld, personal communication), a character linking it to *Rhoiptelea* (Juglandaceae). These characters are discussed, in the light of analyses of molecular data, in Chapter 2.

It can be confirmed that Leroy (1949) was correct in stating that *Canacomyrica* has an erect sessile and orthotropous ovule with a superior micropyle. His use of the word funicle is misleading as it is generally used to refer to the placental attachment of the ovule. Guillaumin's (1940) original description can be confirmed to be correct with respect to the two other unique aspects of the plant (the lamellular, laciniate style and the fleshy perianth).

The other morphological character investigated was nodulation. Unfortunately, it was not possible to determine whether *Canacomyrica* bears nitrogen-fixing root nodules, nor whether it has cluster roots, an adaptation to low phosphorus observed in a variety of plant species including *Myrica gale* (Skene et al., 2000). In its natural habitat *Canacomyrica* is entirely restricted to continuously wet conditions on serpentine substrates that are very low in basic nutrients. Under such conditions, it might be expected that *Canacomyrica* would have nitrogen-fixing root nodules and cluster roots to enhance nutrient-uptake. Due to the death of the seedlings, which coincided with the application of nutrient feed, field studies remain the only way to establish whether these features are present in *Canacomyrica*.

The remainder of this investigation concerned aspects of the ecology of *Canacomyrica*. The results of the dimethylglyoxime test suggest that *Canacomyrica* does not accumulate nickel. This result is consistent with the fact that, even in New Caledonia where there is a high proportion of hyperaccumulators, the vast majority of plants growing on serpentine substrates do not accumulate metals (R. Reeves, pers. comm.)

A low ratio of seed to fruit was observed in the sample of fruits available (from two different populations). Both the seed viability and germination tests suggest that none of the seeds examined were viable. It is possible that the seed was not fully developed when

collected but it is also possible that low seed set is a feature of the biology of this species. The negative result for the TTC test appears to suggest that the seeds were not viable. However, inhibitory factors such as those observed in *Comptonia* may have been present in the testa of the seeds. If an inhibitory factor, such as abscisic acid (as suggested by Del Tredici and Torrey, 1976), was acting to suppress the enzymes involved in germination then it might be possible to obtain a false negative result in the TTC test. A further study has suggested that temperature fluctuations with an amplitude of 10 °C are required to break the dormancy of *Comptonia* (Dow and Schwintzer, 1999). This finding raises the possibility that a variety of factors are working to inhibit germination in this genus which is a component of open habitat in temperate North America. Given that *Canacomyrica* is a tropical species, its germination biology might be expected to be more similar to species of *Morella* (also occurring in tropical or subtropical regions and with fleshy fruits). Seed viability has been observed to decline after approximately nine months in *Morella cerifera* (Erickson and Hamrick, 2003). Therefore, it is possible that the period in storage between collection of the seed and the start of the study (approximately 12 months) may have severely impaired seed viability. Finally, it is noteworthy that the flora of the southern region of Grande Terre, New Caledonia, is exposed to natural fires. In other regions of the world where fires are a regular feature of the local ecology (e.g. the South African fynbos), the seeds of many plants require exposure to fire for germination (Le Maitre & Midgley, 1992). This possibility was not investigated for *Canacomyrica*.

All aspects of the biology of *Canacomyrica* are intrinsically interesting. Given that *Canacomyrica* is of conservation concern (see Chapter 3) investigation of its reproductive ecology, especially pollination biology and dispersal agents, would be particularly useful in developing a conservation strategy for the species and enhancing the chances of its preservation. The presence of staminodes in *Canacomyrica* is unique within Myricaceae. Future studies of the genetic and/or developmental control of staminodes in *Canacomyrica* may shed light on the processes involved in sexual variability throughout Myricaceae.

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Chapter 5

Myricaceae: a morphological study

ABSTRACT

A survey of 21 taxa representing *Canacomyrica*, *Comptonia*, *Myrica* and all previously recognised sections of *Morella* was carried out to identify potentially phylogenetically informative characters. Thirteen parsimony informative characters were identified but phylogenetic analysis revealed high homoplasy within the data set and the resulting trees were poorly resolved. Characters defining genera and some subgeneric groups were identified for use in a subsequent classification scheme, based on molecular data.

INTRODUCTION

It has long been recognized that the majority of Myricaceae species are morphologically similar and that species delimitation, particularly within *Morella* (*Myrica* auctt.), is difficult based on morphology alone (eg. Polhill and Verdcourt, 2000). A variety of approaches has been employed to attempt to elucidate the patterns and relationships within Myricaceae. These include: palynology (Sundberg, 1985); chemistry (Halim and Collins, 1973); leaf cuticular morphology (Chourey, 1974); floral morphology and development (Hjelmqvist, 1948; Abbe, 1974; Macdonald, 1977); stem anatomy (Baird, 1968); and cytology (Stokes, 1937; Oginuma and Tanaka, 1987). Working on just a few representative species, some authors have proposed evolutionary hypotheses, principally with respect to inter-generic relationships (Hjelmqvist 1948, Baird 1968, MacDonald 1977). With the exception of Sundberg (1985), none of these studies included *Canacomyrica*.

Over a century after its publication, Chevalier's (1901) monograph of Myricaceae remains the most complete survey of the morphology of the family. The first part of the monograph was devoted to an examination of aspects of wood, leaf and floral anatomy. In the second part, Chevalier presented his classification of the family, listing morphological characters to support his genera and sections. He recognized three genera, dividing the largest of these into three sections (Tables 1 and 2), and recognized a total of 55 species and numerous varieties. His largest section

(*Cerophora*) comprised 41 species and was divided into an African subsection and a subsection from the Americas. It is indicative of the difficulties associated with this family that Chevalier was apparently unable to offer morphological characters for differentiating between these two subsections.

Since Chevalier's time, few authors have attempted a thorough examination of the range of variation within the largest genus, *Morella* (*Myrica* sensu Chevalier). In an unpublished Ph.D. thesis, Baird (1968) examined the morphology of all the North American taxa, including all three of Chevalier's (1901) genera and representatives of two of his sections. Baird (1968) looked at stem and leaf anatomy, chromosome number and reproductive morphology, including pollen. He appears to have accepted the subgeneric groups of Chevalier (1901), providing additional morphological characters to support two of them (Table 2).

In a study of leaf cuticular morphology, in both extant and fossil species, Chourey (1974) sampled comprehensively from throughout Chevalier's (1901) genera and sections. She concluded that *Comptonia* and *Gale* (=*Myrica* s.s.) could be differentiated from the remaining species on the basis of characters of the stomata and glandular hairs (Table 1) but was unable to find characters to support Chevalier's (1901) sections. Her study also indicated that the vast majority of fossil leaves attributed to Myricaceae were incorrectly assigned (see Chapter 1).

The floral morphology of some *Morella* species has been investigated by several authors although never in a phylogenetic context (Hjelmqvist 1948, Abbe 1974). Both Hjelmqvist (1948) and Abbe (1974) examined representatives of all Chevalier's (1901) genera and sections but neither commented on delimitation of these groups. Abbe (1974) remarked upon the uniformity of the reproductive structures in the family.

In regional Floras, particularly in areas where the family is species-rich, the most commonly used characters to differentiate between species are those of leaf shape (e.g. leaf apex shape, venation) and breeding system (monoecy versus dioecy) (Hutchinson, 1925; Adamson, 1950; Killick, 1969; Polhill and Verdcourt, 2000). Among the

African species, the use of such characters resulted in Hutchinson (1925) recognising 15 species, Killick *et al.* (1999) recognising 13, with four subspecies and two varieties, and White (1993), who had a wide field experience in eastern Africa, recognising a mere five species.

Whilst the characters listed in Table 1 indicate significant morphological differences between the three genera recognized by Chevalier (1901), the characters used to differentiate between his sections (listed in Table 2) are far fewer and mostly non-discrete. It is beyond the scope of this study to carry out a revision of Myricaceae. Instead, the aim of this study was to identify a set of meaningful diagnostic morphological characters that could ultimately be used to support a classification based on the results of the molecular analyses (Chapter 6). In the process of determining a set of characters to define genera and subgeneric groups, some of the characters listed above were evaluated, new morphological characters were sought, and an analysis of the data was carried out to investigate the phylogenetic utility, if any, of the morphological data.

TABLE 1. Characters distinguishing three genera of Myricaceae (Chevalier 1901; Baird 1968; Halim and Collins 1973; Chourey 1974)

Character	*Comptonia*	*Myrica* (=*Gale* sensu Chevalier)	*Morella* (=*Myrica* s.l. sensu Chevalier)
Leaves	Thin, deciduous, pinnatifid	Thin, deciduous, entire or feebly dentate	Coriaceous, usually persistent, entire or dentate
	Stipules present	Without stipules	Without stipules
	Stomata sunken	Stomata sunken	Stomata not sunken
Glands	Capitate glands with unicellular base	? [a]	Balloon glands with 2-celled stalk base
Essential oils			
cineole	17.8%	trace	1.4-11.3%
γ-terpinene	14.7%	absent	2.0-7.8%
Inflorescences	Catkins inserted on the deciduous branches	Catkins inserted on the deciduous branches	Catkins inserted on the growing branches
	Usually 4 stamens	Usually 4 stamens	From 4 – 8 (-20) stamens
Fruit	Smooth, subtended by 2 laciniate bracteoles, emergences at the base developing into a cupule	Smooth, subtended by 2 spongiose bracteoles	Covered with waxy or fleshy emergences, bracteoles absent or if present non-accrescent
	Fruits in a spherical spike	Fruits in a dense subcylindric spike	Fruits in a loose cluster
	Bracteoles not adnate with fruit wall	Bracteoles strongly adnate with fruit wall	Bracteoles not adnate with fruit wall
Ectocarp			
Relative thickness	Thin	Thin	Thick
Cell type	Sclerenchyma	Parenchyma	Parenchyma
Papillae	Absent	Absent	Present
Trichomes	Absent	Absent	Absent to dense
Epidermis	Sclerified	Not sclerous or waxy	Not sclerous, usually waxy
Mesocarp cell type	Parenchyma	Sclerenchyma	Parenchyma

Character	*Comptonia*	*Myrica* (=*Gale* sensu Chevalier)	*Morella* (=*Myrica* s.l. sensu Chevalier)
Terminal buds	Lacking	Lacking	Present
Wood			
Vessel elements	Diffuse porous	Ring porous	Diffuse porous
Distribution	Mostly solitary (moderate clustering)	Mostly solitary (slightly clustering)	Clusters or bands
Outline	Very slightly angular	Circular (very slightly angular)	Angular
Perforation plate	Simple (few scalariform)	Mostly scalariform (6-50% simple)	Scalariform (none simple)
Imperforate elements			
Type	Fibre-Tracheid or Fibre	Fibre-tracheid	Tracheid
Wood rays			
Predominant type	Uniseriate	Multiseriate	Uniseriate
Cellular composition	Homocellular	Heterocellular	Homocellular
Pericycle	Complete ring	Complete ring	Interupted ring
Chromosome number	2n=32	2n=16, 48, 96	2n=16

[a] The status of this character in *Myrica* is not clear (Chourey, 1974)

TABLE 2. Characters used by Chevalier (1901) and Baird (1968) to differentiate between subgeneric groups in *Morella*, taxonomy follows Chevalier

Character	sect. *Morella*	sect. *Faya*	sect. *Cerophora*
Inflorescences	Branched	Simple or branched	Usually simple
Female flowers	Several ovaries produced (in the sexual bud) from which only one develops	Several ovaries produced (in the sexual bud), not all developing into fruit	Several ovaries produced (in the sexual bud), never more than one developing into fruit
	-[a]	Pistils 1-3 in axil of each bract[a]	Pistils solitary in axil of each bract[a]
Fruits	Large (6-8 mm)	Medium (4-6 mm diam.), often forming a syncarpium	Small (1-5 mm diam.)
	Covered at maturity with a number of small fleshy overlapping emergences	Emergences waxy or not, never fleshy	Emergences usually waxy, never fleshy
		Fruit wall densely pubescent, papillae glabrous[a]	Fruit wall glabrous or pubescent, if pubesncent then the papillae also pubsecent[a]
Male flowers	-[a]	8 or more stamens[a]	Less than 7 stamens[a]
		2 anthers per staminal column branch[a]	1 anther per staminal collumn branch[a]
		2-6 bracteoles[a]	0-3 bracteoles[a]

[a]Baird (1968) worked on the North America taxa and did not include any species from *Myrica* sect. *Morella* in his study

MATERIALS AND METHODS

Selection of study material— Herbarium specimens representative of all genera and the entire geographical range of the family were examined. Herbarium specimens were obtained from the following collections: Edinburgh (E), Kew (K), the Natural History Museum (BM), Paris (P), Noumea, New Caledonia (NOU) and the Compton herbarium, South Africa (NBG). A data set of morphological characters was compiled for 20 ingroup taxa (including *Canacomyrica*) and one outgroup taxon (see Table 3). The ingroup taxa were selected to reflect all Chevalier's (1901) genera and subgeneric groups. Where possible, taxon sampling reflected the differing species numbers in the various regions of distribution. Several herbarium specimens were examined for each species so that, where possible, at least one example of a mature fruit, a female inflorescence and a male inflorescence were observed (herbarium specimens consulted are listed in Table 3).

Character choice— Based on existing studies of the morphology of Myricaceae (e.g. Chevalier 1901, Baird 1968), a list of potentially useful characters was compiled. This list was employed in an examination of a range of herbarium specimens from which a short-list of characters could be selected (see below). The characters were chosen on the basis that they should be straightforward to score (i.e. no special equipment other than a dissecting microscope was necessary for their evaluation), that they could be reliably scored in more than one individual of a given taxon, and that they showed sufficient variation among species to be of taxonomic value. Several continuous characters, such as stamen number, floral bract number and leaf dimensions have been used in previous studies of Myricaceae (Baird 1968), but including such characters would entail subjective and ultimately arbitrary definition of character states. Continuous data have been considered less powerful in resolving species relationships than discrete data (Pimentel and Riggins, 1987). It was intended that the morphological data generated would be analysed in PAUP* (see below), thus requiring that they should be readily converted into a binary matrix. For the above reasons, continuous or descriptive characters were avoided.

TABLE 3. Taxa included in the morphological study

Species	Taxonomic group (Chevalier, 1901)	Distribution	Herbarium specimens consulted
Myrica gale	*Gale*	Northern Hemisphere	UK, Scotland: Killean, *Macalister Hall* 1734 (E); Orkney, *Johnston* 1098 (E); Balquhidder, *Edgar Evans* s.n. (E). Canada, Quebec: O Hawa district, *Dore and Breitung* 47-1270 (E); Vancouver Island: Shawnigan Lake, *Macoun* 85712 (E); USA, Oregon: Portland, *Suksdorf* s.n. (E). Portugal: Douro Litoral, Carregal, *Sales & Hedge* 03/108 (E). Japan: Kushiro Province, Hokkaido, *Furuse* 8809 (K); Benten, Tomakomai-shi, *Furuse* 6004 (K). Russia: cult. in Japan: Nikko Botanical Garden, Tokyo, *Kenicer* s.n. (E).
Myrica hartwegii	*Gale*	North America	USA: California, Mariposa County, *Congdon* s.n. (2 sheets) (E); California, Sonora, *Sutton* s.n. (K); California: Mariposa County, *Mason* 11208 and 11209 (BM); California: Cosumnes River, *Bacigalupi* 7329 (BM).
Comptonia peregrina	*Comptonia*	North America	USA: Michigan, Port Huron, *Dodge* 1895 (E); North Carolina, Henderson County, *Boufford and Wood* 16417 (E); Virginia, Southampton County, *Franklin et al.* 1042 (E); Massachusetts, Middlesex County, *Smith et al.* 1041 (E). Canada: Ontario, Brockville, *Marie-Victoria et al.* 56846 (E).
Canacomyrica monticola	-	New Caledonia	New Caledonia: Mt Kouakoue, *Schmid* 4298 (P); Piste du Nekando, *Veillon* 3791 (P); Montages des Sources, *Jaffré* 2414 (P); Monte Mamié, *Herbert* 1803 (E); Monts Koghi, *Herbert* 901 (E).
Morella pavonis	*Myrica* sect. *Cerophora* subsect. *Americanae*	South America	Chile: Tarapacá, Arica, *Gardner & Knees* 6304 and 6222 (E). Peru: Moquegua Province, above Moquegua, *Weberbauer* 7391 (BM).

Species	Taxonomic group (Chevalier, 1901)	Distribution	Herbarium specimens consulted
Morella cerifera	*Myrica* sect. *Cerophora* subsect. *Americanae*	North America	USA: Texas, Polk County, *Lewis* 6937 (E); South Carolina, Orangeburg County, *Radford et al.* 11462 (E); Florida, Jacksonville, *Curtiss* 4627 (E); Virginia, Princess Anne County, *Heller* 859 (E); Georgia, Richmond County, *Harper* 2064 (E); Mississippi, Jackson County, *Demaree* 36039 (E); Bermuda, *Moseley* s.n. (E)
Morella inodora	*Myrica* sect. *Faya*	North America	USA: Florida, Okaloosa County, *Ford* 4002 (BM); Florida, Walton County, *Harper* 1191 (BM); Mississippi, Biloxi, *Tracey* 5/35 (BM).
Morella californica	*Myrica* sect. *Faya*	North America	USA: California, Mendocino County, *McMurphy* 191 (E); California, Monterey, *Balls* 11743; California, San Fransisco, Lake Merced, *Rose* 65091 (E); Washington, Chehalis County, *Heller & Heller* 3941 (E).
Morella faya	*Myrica* sect. *Faya*	Macaronesia	Portugal: Algarve, Monchique mountains, *Sales & Hedge* 94/23 (E); Estremadura, Nazaré, *Sales & Hedge* 01/16 (E); Azores, Sao Miguel, *Herbert* SM201 (E); Azores: Terceira, *Herbert* TE204 (E).
Morella rivas-martinezii	-	Macaronesia	Spain: Canary Islands, La Gomera, *Herbert* F233 and F239 (E); Canary Islands, El Hierro, *Herbert* F101 (E).
Morella salicifolia	*Myrica* sect. *Cerophora* subsect. *Africanae*	Africa	Saudi Arabia: Khamis Mushayt, 82-236S (E). Yemen: Between Saddah and Nadirah, Wadi Banna, *Wood* 3121 (two sheets) (BM); Above Dhi Sufal, on road to Waraf, *Wood* 1560 (two sheets) (BM).
Morella cordifolia	*Myrica* sect. *Cerophora* subsect. *Africanae*	Southern Africa	South Africa: Western Cape: cult. in Kirstenbosch Botanic Garden, *Herbert* 1003 (E); Knysna, Buffelsbaai, *Herbert* 1010 (E); Western Cape, Kleinmond, *Herbert* 1007 (E); Paardeneiland, *Dümmeri* 1367 (E); Cape of Good Hope, *Ecklon* s.n.

Species	Taxonomic group (Chevalier, 1901)	Distribution	Herbarium specimens consulted
			(E); Port Elizabeth, *ESCA* 221 (E).
Morella quercifolia	*Myrica* sect. *Cerophora* subsect. *Africanae*	Southern Africa	South Africa: Cape Town, Cape flats, *Rehmann* 2046, 2047, 2048 (one sheet) (BM); Cape of Good Hope, Wynberg, *Wallich* 592 (BM); Cape Peninsula, *Dümmeri* 1175 (E); Knysna, The Heads, *Mande* s.n. (BM); Western Cape, Cape Peninsula, *Herbert* 1001 (E); Western Cape, Kleinmond, *Herbert* 1006 (E).
Morella serrata	*Myrica* sect. *Cerophora* subsect. *Africanae*	Southern Africa	South Africa: Western Cape, Kleinmond, *Herbert* 1008 (E); Western Cape, Kleinmond, *Herbert* 1009 (E); Transvaal, Songimvelo Game Reserve, 9191 (E); Natal, Enyati Ridge, *Hilliard & Burtt* 5876 (E).
Morella integra	*Myrica* sect. *Cerophora* subsect. *Africanae*	Southern Africa	South Africa: Western Cape, Limietberg Conservation Area, *Herbert* 1014 (E); Cape, Bainskloof, *Marsh et al.* 3 (NBG); Clanwilliam, Boontjiesrivier, *Wagener* 56 (NBG); Tulbagh, New Kloof, *Gillet* 372 (NBG).
Morella javanica	*Myrica* sect. *Morella*	Asia	Celebes: Kelelonde, Soputan Mountains, *Alston* 15844 (BM). Indonesia: Bali, Karangasem, *McDonald & Ismail* 4733 (E). Sarawak: G. Murut, Lawas, *Anderson & Ilias* S26477 (E). Sabah: Penampang, *Sumbing* 127808 (E). Philippines: Mindanao, *Elmer* 11380 and 11475 (one sheet) (BM). Sumatra: Korinchi Peak, *Robinson & Kloss* s.n. (BM). Borneo: Mt. Kinabalu, *Clemens & Clemens* 32278 (BM).
Morella adenophora	*Myrica* sect. *Morella*	Asia	China: Hainan, Nodoa, *McClure* 8970 (E); Hainan, *Liang* 64074 (E).Taiwan: Ping Tung Hsian, Kenting National Park, *Clarke et al.* 516 (E); Ping Tung County, Hsuhai, *Cheng* 4157, 4158 and 4159 (E).

Species	Taxonomic group (Chevalier, 1901)	Distribution	Herbarium specimens consulted
Morella nana	*Myrica* sect. *Morella*	Asia	China: Yunnan, Kunming, *Murata* 10568 (E); Yunnan, Sungkwei, *Forrest* 13781 (E); Yunnan, Dali Xian, *Bartholomew et al* 844 (E).
Morella rubra	*Myrica* sect. *Morella*	Asia	Japan: Honshu Prov., Idzumi, *Tamura* 24663 (E). China: Kwangtung, Jen-hwa district, *Tsang* 62083 (E); Fukien, Chuanchow, *Chung* 3147 (E); Yunnan, Tengchong valley, *Forrest* 7833 (E); Yunnan, Shweli-Salween divide, *Forrest* 24375 (E).
Morella esculenta	*Myrica* sect. *Morella*	Asia	Burma: Kanpetlet, Chin Hills, *Dickason* 8452 (E). Nepal: Kaski District, Kande (Kaare), *Suzuki et al* 81798 (E). China: Yunnan, Shweli Valley, *Forrest* 12098 (E).
Juglans cinerea (Outgroup)	-	North America	USA: Michigan, near Port Huron, *Dodge* s.n. (E); Missouri, Stone County, Galena, *Palmer* 4615 (E); Pennsylvania, Lancaster County, *Heller* s.n. (E); Washington D.C., Steele s.n. (E); Vermont, *Beck* s.n. (E).

The characters chosen for this study are described below:

1. **Stipules:**

   0. Absent

   1. Present

   This character is found in *Comptonia* in which the stipules are foliaceous. The remainder of Myricaceae are generally considered not to possess stipules but they have apparently been noted in young specimens of *M. javanica* (Backer, 1951).

2. **Leaf persistence:**

   0. Evergreen

   1. Deciduous

   Comptonia and *Myrica* species are deciduous whilst the rest of the family is evergreen. In the North American species of *Morella*, foliage occurring on flowering branches is shed once the flowers have appeared. New foliage develops in the distal portion of the flowering branch, thus giving the false impression that the plants are deciduous.

3. **Male inflorescence branching:**

   0. branched

   1. unbranched

   In a simple, unbranched male inflorescence the flowers (one or several stamens subtended by a bract) are borne on a single floral stem. In more complex inflorescences the floral stem may be branched. In the Asian *M. esculenta* the elongation of the branches of the floral stem gives the inflorescence a very loose character, whilst in the other Asian species the branches are quite short giving the inflorescence a condensed character, but the same degree of branching is present in both cases.

4. **Stamen branching:**

   0. Unbranched

   1. Branched

The arrangement of stamens in the male flower can be simple or unbranched i.e. each filament is free from the others. Alternatively, there may be a branched staminal column. Several authors have suggested that there may be up to 20 stamens in a single male flower (see Table 1), however, no more than 8 were ever observed in the course of this study.

5. Fruit type:

0. Dry
1. Fleshy

6. Wax on fruit:

0. Absent
1. Present

Both characters 5 and 6 are important in clarifying the puzzling distinction that Chevalier (1901) made between those taxa that he considered to have only fleshy fruits, and those he considered to have only waxy fruits. Wax is here defined as the whitish coating found on the exterior of the fruits; it is dry, crystalline in appearance, and flaky in dried specimens. Examination of the material in which papillae cover the nut shows that whilst the degree of fleshiness varies, all fruits with a waxy coating are also fleshy. Although Chevalier (1901) states that the papillae are overlapping, they are instead densely crowded together. Fruit size is not included in this analysis but it is noteworthy that Chevalier gives the maximum size of fruits in *Myrica* sect. *Morella* as 8 mm diam. (Table 2). This differs dramatically from the size of fruits observed on several specimens collected by George Forrest (e.g. *Forrest* 7833 and *Forrest* 24375, E) from the Yunnan region of China that are as large as 30 mm diam. The fruit of *Canacomyrica* was coded as fleshy because the mesocarp appears papillose (see Figure 6). The homology of this character, however, may be doubtful.

7. **Retention of bract on fruit:**

0. Not adnate to fruit wall

1. Adnate to fruit wall

In *Myrica* species the bracteoles surrounding the female flower become adnate with the fruit wall and develop into spongiose appendages that have been proposed to play a role in water dispersal (Macdonald, 1977). In the remainder of species the bracts and bracteoles are not adnate to the fruit although they may be incorporated in the syncarpium of some species such as *M. faya*.

8. **Style base:**

0. United

1. Free

The style comprises two stigmatic branches which may be free at their point of attachment with the ovary, or fused together at the base.

9. **Style shape:**

0. Round

1. Flattened

The stigmatic branches may be narrow and round in cross section or flattened.

10. **Trichomes on leaves:**

0. Absent

1. Present

Simple, unicellular hairs or trichomes are present in most species, of those surveyed, and are distinct from the 'balloon glands' that characterise the family. The degree of vestiture varies continuously among, and within, species.

11. **Trichomes on endocarp:**

0. Absent

1. Present

Simple trichomes may be present in species of *Morella* on the endocarp; at the apex of the fruit or covering the entire fruit wall. They are observed by removing some of the papillae and in some species such as *M. esculenta* they

are so numerous and dense that they may be observed protruding between the papillae. Baird (1968) suggested that, in the American species, if the endocarp was pubescent then the papillae were also pubescent (Table 2). However, trichomes on the papillae were so seldom encountered in the specimens consulted that this character was not included in the study.

12. Trichomes on floral stems:

0. Absent
1. Present

Trichomes are present on the floral stem in almost all species. They differ in the density of their occurrence within *Myrica*. The degree of vestiture may be ecologically determined.

13. Sexual morphs:

0. Monoecious
1. Dioecious
2. Subdioecious

Monoecious individuals were those in which all flowering specimens exhibited both male and female inflorescences on the same plant. Dioecious individuals were those in which male and female inflorescences occurred on separate plants. Where specimens exhibited heterotopy (apparently functional stamens positioned on the fruit wall; Macdonald, 1989) the species was classed as subdioecious.

14. Plant scented:

0. Unscented
1. Scented

Most species are strongly scented as a result of the numerous essential oils they contain (Halim and Collins, 1973). In addition to *Morella inodora*, there are some notable exceptions including *Morella faya* and *Morella californica*.

Several characters that were included at the outset were abandoned during the course of data collection for various reasons as listed in Table 4. In general, characters that

were present in only one taxon (autapomorphies) were excluded from the analysis since the aim of this study was to identify characters that would support groupings within the family, specifically within *Morella*.

TABLE 4. Morphological characters excluded during the course of the study

Character	Character states	Reason for excluding
Floral bud position	Present year's growth/ Previous year's growth	Difficult to determine in *Morella* species
Position of inflorescence	Leaf axil/Not leaf axil	Difficult to determine in *Morella* species
Number of ovaries within a sexual bud developing into fruit		Difficult to determine without fresh material
Nitrogen fixing root nodules	Presence/Absence	Insufficient material
Trichomes on papillae	Presence/Absence	Deemed to be insufficiently variable
Number of bracts and bracteoles surrounding female flower		Continuous variation
Anther attachment	Dorsifixed/Basifixed	Invariable (dorsifixed)
Number of anthers		Continuous variation
Leaf texture	Coriaceous/Leathery/Thin	Continuous variation, difficult to assess in herbarium material

Analysis— A morphological data matrix was created using NEXUS Data Editor (NDE) (http://taxonomy.zoology.gla.ac.uk/rod/NDE/nde.html) which produces an output compatible with PAUP* Version 4.0b10 (Swofford, 1998). Maximum parsimony analysis was carried out using PAUP* with tree bisection-reconnection (TBR) and saving multiple trees (MulTrees). Maximum parsimony trees were obtained using the heuristic search option (Fitch parsimony). Branch lengths were calculated using the delayed transformation (DELTRAN) optimisation. Support for the tree topologies obtained was assessed using bootstrap analysis (Felsenstein, 1985) performed using the heuristic search option and 1000 replicates. The following categories were used to describe bootstrap percentage (BP) results: 50-74, weak support; 75-84, moderate; 85-100, strong support.

RESULTS

The morphology data set contained 21 taxa and 14 characters (Table 5) of which 13 were parsimony informative. Fruiting and floral material was unavailable for *M. rivas-martinezii* hence the high proportion of missing data for this taxon. Several characters applied to the ingroup were not applicable to the outgroup taxon (*Juglans cinerea*) and were coded as such.

Maximum parsimony analysis of the morphology data set produced 344 most parsimonious trees (tree length 28, CI=0.54, RI=0.79). The strict consensus tree is shown in Figure 1. Bootstrap support for all internal nodes was less than 50%; three monophyletic groups were weakly supported. The clade comprising *Myrica* species and *Comptonia* (73 BP) is united by the sympleisomorphy of deciduous leaves and synapomorphy of dry fruits (characters 2 and 5). The clade comprising all the Asian taxa (*Morella javanica, M. adenophora, M. nana, M. rubra, M. esculenta*) is united by the synapomorphy of branched male inflorescences (character 3) and the sympleisomorphy of unbranched stamens (character 4). The species pair *M. faya* and *M. rivas-martinezii* (63 BP) is united by the synapomorphy of leaves lacking trichomes (character 10).

Whilst no further patterns were resolved in the analysis of the morphological data set the following comments can be made about the remaining characters. Character 1 (stipules): this character was parsimony uninformative, being an autapomorphy for *Comptonia peregrina*; stipules were not observed in any other material. Character 6 (wax on the fruit): this character unites the non-Asian *Morella* species although it is not constant throughout the group. Character 7 (retention of bract on fruit): this character is a synapomorphy for the two *Myrica* species. Character 8 (style base): this character is very variable among taxa showing no discernable pattern among taxa. Character 9 (style shape): this character is also very variable showing no discernable pattern among taxa. Character 11 (trichomes on the endocarp): this character unites *Morella* species but is not constant throughout the group, it is however constant in the Asian group. Character 12 (trichomes on the floral stem): this character unites *Morella californica* and *Morella inodora* but is also found in *Morella faya*. Character 13 (sexual morph): this character is very variable, most taxa appear to be dioecious but there is significant variation within species and determination of the character from herbarium specimens was difficult. Character 14 (scent): determination of this character from herbarium specimens was not always possible and the character is seldom noted in Flora accounts.

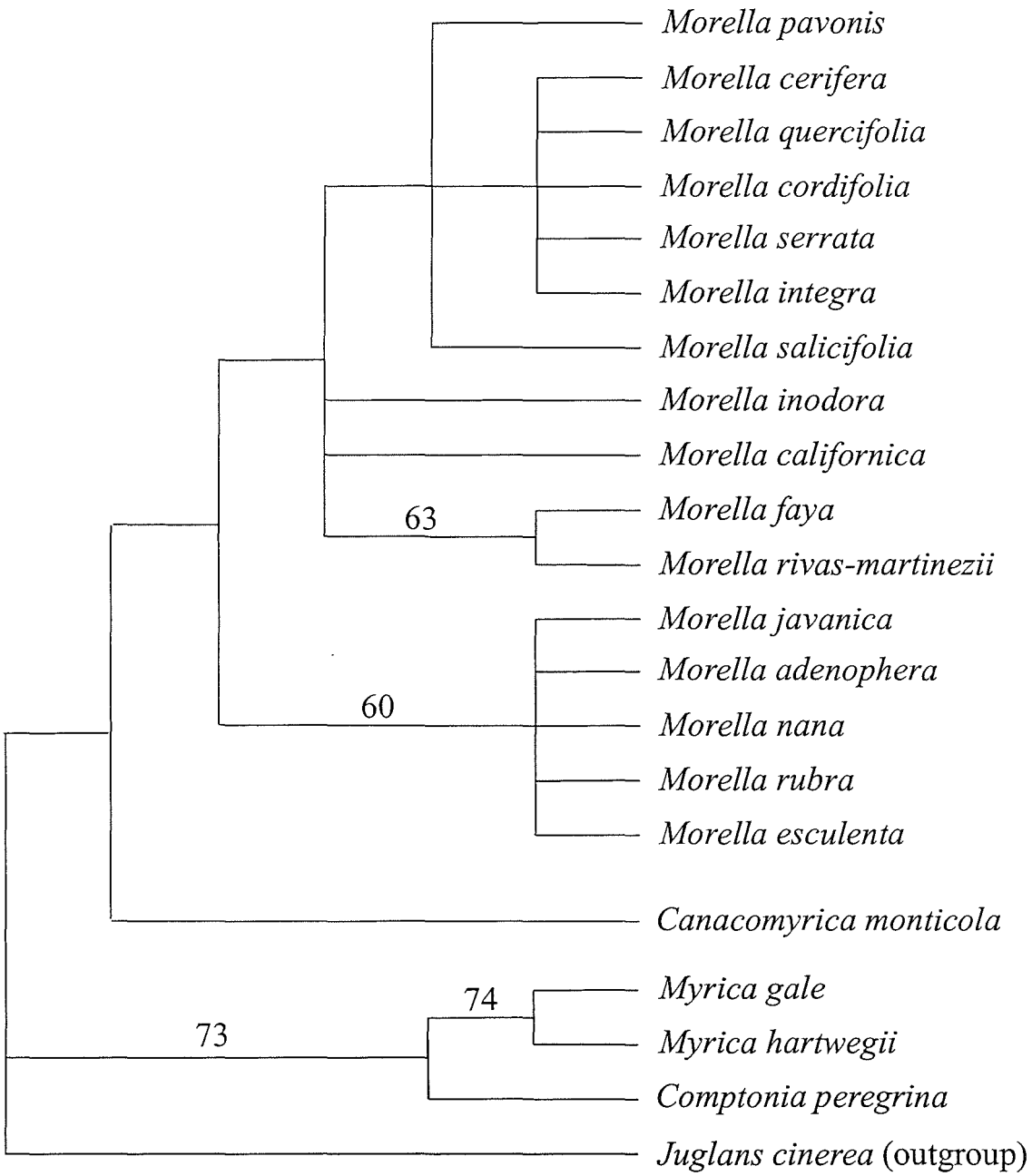

Figure 1: Strict consensus of the 344 trees produced from the morphological data set. Bootstrap percentages >50% shown above the branches.

TABLE 5. Matrix of morphological data for Myricaceae.

	Character number													
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Canacomyrica monticola	0	0	1	0	1	0	0	0	1	1	0	1	1	0
Myrica gale	0	1	1	0	0	0	1	0	0	1	0	1	1	1
Myrica hartwegii	0	1	1	?	0	0	1	0	0	1	0	1	1	1
Comptonia peregrina	1	1	1	0	0	0	0	1	0	1	0	1	2	1
Morella pavonis	0	0	0	1	1	1	0	0	1	1	1	1	2	1
Morella inodora	0	0	1	?	1	1	0	0	1	1	1	0	1	0
Morella californica	0	0	1	1	1	1	0	1	1	1	1	0	0	0
Morella cerifera	0	0	1	1	1	1	0	1	0	1	0	1	1	1
Morella salicifolia	0	0	1	1	1	1	0	1	1	1	1	1	1	1
Morella faya	0	0	1	1	1	0	0	1	1	0	1	0	2	0
Morella rivas-martinezii	0	0	1	1	?	?	?	?	?	0	?	?	?	0
Morella quercifolia	0	0	1	1	1	1	0	1	1	1	0	1	1	1
Morella cordifolia	0	0	1	1	1	1	0	1	0	1	0	1	0	1
Morella serrata	0	0	1	1	1	1	0	1	1	1	0	1	0	1
Morella integra	0	0	1	1	1	1	0	1	1	1	0	1	1	1
Morella javanica	0	0	0	0	1	0	0	1	1	1	1	1	1	?
Morella adenophora	0	0	0	0	1	0	0	?	?	1	1	1	1	0
Morella nana	0	0	0	0	1	0	0	1	0	1	1	1	1	0
Morella rubra	0	0	0	0	1	0	0	?	?	1	1	1	1	0
Morella esculenta	0	0	0	0	1	0	0	1	0	1	1	1	1	0
Juglans cinerea	0	1	1	0	1	0	-	0	1	-	-	-	?	?

? = missing data

- = inapplicable

DISCUSSION

Thirteen parsimony informative morphological characters were identified in this study. Most of these characters had been applied to at least some of the species by other authors but none had been previously applied to *Canacomyrica*. Characters 9 (style shape), and 14 (scent) were used for the first time. Some characters showed genus-specific variation (see below) whilst others were highly variable at the genus level. The character of scent (character 14) is highly subjective and is not likely to be useful in diagnostic keys. The sexual morph character (character 13) is frequently used in the literature to distinguish between species but was found, during the course of this study, to be particularly variable. Differentiation between dioecy, monoecy and subdioecy or heterotopy was difficult using herbarium material. Furthermore, sex expression is highly unstable within species and appears to be dictated by environmental conditions (e.g. Lloyd, 1981). Additional characters of gross morphology used by previous authors for Myricaceae were mostly found to be continuous. Others were found to display high levels of intra-specific variation, for example leaf shape characters are frequently used in taxonomic accounts of Myricaceae but were not included in this study because they are highly variable and tend to be of limited use in defining generic or subgeneric groups; they are however routinely used to define species (e.g. Polhill & Verdcourt, 2000)

Phylogenetic analysis of the morphological data produced low resolution of relationships within and among genera, suggesting a high level of homoplasy within the data set. The results of the morphological analyses were expected and confirm the difficulties associated with attempting a classification of Myricaceae based on morphological characters alone. The phylogenetic analysis of the morphological data set recovered a monophyletic group containing both *Myrica* species and *Comptonia* (Figure 1), united by dry fruits and deciduous leaves. This relationship conflicts with all other analyses of molecular data (see Chapters 2 and 6) and is therefore considered spurious. No synapomorphic characters were identified for *Morella*, which shared several characters with *Canacomyrica* (eg. fleshy fruits, evergreen leaves). Within *Morella* however, the Asian species formed a monophyletic group united by branched male inflorescences and unbranched stamens. This group corresponds to Chevalier's

(1901) *Myrica* section *Morella*. Some patterns not resolved by the analysis were observed within the data set. For instance, the taxa Chevalier treated as *Myrica* subg. *Faya*, were united by lack of scent and lack of trichomes on the floral stem.

Diagnostic morphological characters for the four currently recognized genera can be identified from those characters included in the study (Table 6). During the course of the study a large number of herbarium specimens was examined, observations were made and characters noted that were not included in the above analysis. These observations are included in detailed descriptions of genera and subgeneric groups that are given, along with keys to the main groups, in Chapter 8.

TABLE 6. Diagnostic morphological characters for the genera of Myricaceae.

Character	***Canacomyrica***	***Comptonia***	***Myrica***	***Morella***
Stipules	Absent	Present	Absent	Absent
Leaf persistence	Evergreen	Deciduous	Deciduous	Evergreen
Fruit	Fleshy Lacking wax	Dry Lacking wax	Dry Lacking wax	Fleshy Mostly waxy
Retention of bract on fruit	Not adnate	Not adnate	Adnate	Not adnate
Trichomes on the endocarp	Absent	Absent	Absent	Usually present
Style	Flattened and laciniate	Round, never laciniate	Round, never laciniate	Flattened or round, never laciniate
Staminodes*	Present	Absent	Absent	Absent
Stamens*	Six	Usually four	Usually four	Four - 20

* The characters marked with an asterisk concern the definition of *Canacomyrica* only and were not included in the analysis of the morphological data set

CONCLUSIONS

Whilst the utility of the morphological data set alone to resolve relationships is low, some of the characters included in this study may be helpful for defining genera and subgeneric groups identified by the molecular data. In Chapter 6, genera, subgeneric groups and the morphological characters that define them, are discussed in the light of a taxonomic framework based on the analyses of molecular data.

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Chapter 6

Phylogenetic relationships and biogeography of Myricaceae based on nuclear and plastid DNA sequences

ABSTRACT

To determine relationships within Myricaceae, phylogenetic analyses of nuclear ITS and plastid *trnL-F* sequence data for 31 taxa, representing all genera and subgeneric groups of Myricaceae, and four outgroup taxa, were carried out using maximum parsimony and Bayesian inference methods. The monophyly of Myricaceae was strongly supported as were the relationships among the four genera: (*Canacomyrica* (*Comptonia* (*Myrica*, *Morella*))). The clade containing all species formerly considered to comprise *Myrica* s.l. is split into two strongly supported clades. The results of this study strengthen the argument for the formal recognition of these two groups as separate genera. The clade comprising *Myrica* s.s. can be defined by a deciduous habit, dry fruits with accrescent spongiose bracteoles and leaves with sunken stomata. The clade comprising *Morella* can be defined by a typically evergreen habit, fleshy, papillose fruits frequently with a covering of wax, and leaves with stomata not sunken. Within *Morella*, two clades can be recognized that correspond to previously recognized subgenera. Molecular dating analyses were carried out using the Penalized Likelihood method to date key divergence times within Myricaceae. Close correlations between lineage-specific diversification and major orogenic or climatic events were inferred. The results of this study suggest that much of the present diversity in *Morella* has arisen during the Neogene and that dispersal of seed by birds is likely to have been the most significant factor in determining the modern distribution of the genus.

INTRODUCTION

Vicariance hypotheses involving major geological and/or climatic events (e.g. continental drift, mountain building) have often been invoked to explain intercontinental disjunct distribution patterns for woody plant taxa with fleshy fruits (e.g. Raven and Axelrod, 1974). A presence in Africa, particularly when combined with representatives in other southern continents, has been considered to suggest a southern hemisphere origin associated with the fragmentation of the ancient supercontinent Gondwana (see numerous examples in Raven and Axelrod, 1974). However, an increasing number of studies suggest alternative explanations for disjunct distributions that include Africa. For example, Laurasian migration via land bridges (Dalbergioid Legumes, Lavin et al., 2000; Malpighiaceae, Davis et al., 2002)

or relatively recent (Neogene) long distance dispersal (Melastomataceae, Renner et al., 2001; Annonaceae and Rhamnaceae, Richardson et al., 2004) have been proposed.

Myricaceae are a family of predominantly evergreen shrubs and trees exhibiting major disjunctions between Africa, the Americas and Asia. The discontinuous distribution of the family has been variously attributed to a Gondwanan origin (Bramwell, 1976; Macdonald, 1989), a boreotropical origin (Macdonald, 1977), a Madrean-Tethyan origin (Axelrod, 1975), and a Laurasian origin (Raven and Axelrod, 1974). In a review of major disjunctions in seed plants, Thorne (1972) stated that he was unable to place the family in any distribution category, suggesting that its present range could be interpreted as a relict of a once worldwide distribution.

Wind-pollinated unisexual flowers, aromatic resin-filled balloon[1] glands, and drupaceous, mostly bird-dispersed fruits characterize Myricaceae. Many of the c. 60 species are actinorhizal, forming a nitrogen-fixing symbiosis with the actinomycete *Frankia*, and are therefore able to thrive in nutrient-poor habitats such as sand dune systems, volcanically derived substrates or ultramafic (serpentine) geology. Species are generally found in moist habitats like cloud forests, bogs or riparian zones.

Myricaceae comprise four genera, *Canacomyrica*, *Comptonia*, *Myrica* and *Morella*. Two species of *Myrica* are recognized, both are low-growing deciduous shrubs of north-temperate regions with strongly aromatic leaves and dry fruits with spongiose, accrescent bracteoles adapted for dispersal by water. The most widespread species is *Myrica gale* (bog myrtle) which has an almost circumboreal distribution. Like *Myrica*, the monotypic *Comptonia* (sweet fern) is a low-growing, aromatic shrub; it is found in eastern North America and Canada and has dry, possibly animal-dispersed fruits surrounded by spiny bracts. The c. 56 species of *Morella* (wax myrtle, bay berry) are evergreen shrubs and trees (up to 20 m in Asia), disjunctly distributed throughout the Americas, Africa and Asia. Centres of endemism of the genus are in tropical East Africa, the Cape region of South Africa, the Caribbean and Andean South America. Members of *Morella* tend to have narrow distributions, occurring on islands or within

[1] Balloon gland is the term coined by Chourey (1974) to describe the resinous mass that forms when a multicellular peltate gland matures. They are found in slight depressions in the epidermis of all Myricaceae species.

specific altitudinal ranges on mountains. Most species are aromatic and have drupaceous fruits covered in fleshy papillae, and sometimes also wax, that are bird-dispersed. Finally, endemic to New Caledonia, the monotypic *Canacomyrica* is a shrub or small tree with odourless leaves, bearing male and morphologically perfect (functionally female) flowers and fleshy fruits that may be bird-dispersed.

The majority of Myricaceae species are morphologically similar (Adamson, 1950; Abbe, 1974; White, 1993; Polhill and Verdcourt, 2000). Concomitantly, high infraspecific variability, particularly in leaf shape, can frequently be observed (Baird, 1968; Schatz, 2001). As a result, authors have long disagreed on the delimitation of genera and subgeneric groups within the family and the use of *Morella* has only recently been generally accepted (Chapter 7). Some authors have recognized subgenera within *Morella* (*Myrica* auctt.; e.g. Chevalier, 1901; Baird, 1968; Wilbur, 1994), but relationships are poorly understood. Molecular phylogenetic data has shown *Canacomyrica* to occupy a position sister to the other genera of Myricaceae (Chapter 2). However, the relationships among and within the remaining three genera have not previously been examined in a phylogenetic context. The taxonomic uncertainty surrounding Myricaceae contributes to the problem of understanding the distribution of the family, since a well-established systematic framework is a prerequisite for examining biogeographical scenarios.

In this study all genera of Myricaceae were sampled, using sequence data from both nuclear (ITS) and plastid (*trnL-F*) genomes to reconstruct the phylogenetic history of the family and to attempt to clarify generic and subgeneric relationships. Molecular dating analyses based on PL (Penalized Likelihood; Sanderson, 2002) were used to test some of the various biogeographic scenarios that have been proposed to explain the present distribution of the family. Several recent studies have used PL to examine divergence times in various plant groups (Conti et al., 2002; Berry et al., 2004; Renner et al., 2004; Lavin et al., in press). PL is a semiparametric rate smoothing method that uses the data to find an optimal level of smoothing and which permits each lineage to have a separate rate, whilst penalizing rates that vary too much across a phylogeny (Sanderson, 2002). Furthermore, this method makes it possible to incorporate multiple calibration points into the calculations.

MATERIALS AND METHODS

Plant material— Silica gel-dried leaf material (Chase and Hills, 1991) was collected for this study in New Caledonia, South Africa and Macaronesia; further leaf material was obtained from cultivated plants and herbarium specimens. Voucher information and Genbank accession numbers for plant material used in this study are listed in Table 1. All genera and sections of Myricaceae identified by Chevalier (1901) and all major areas of distribution were sampled. Where possible, multiple sampling was carried out to reflect the wide distribution of some taxa (e.g. *Morella cerifera*) or those taxa with commonly recognized varieties (e.g. *Morella adenophora*; see Table 1). However, multiple sampling was limited by time and resources. Based on the results of previous analyses (see Chapter 2), Juglandaceae species were chosen as out-group and sequences for these taxa were obtained from GenBank (Table 1). DNA was extracted using a 2X CTAB method adapted from Doyle & Doyle (1990), modified to include a wash with ammonium acetate (7.5 M NH_4AC; Weising et al., 1995) to remove impurities co-isolated with the DNA.

PCR amplification and sequencing— Amplification of the *trnL-F* region was carried out using the forward primer c and the reverse primer f (Taberlet et al., 1991). PCR reactions of 50 µl contained: 2 µl DNA template, 0.2 mM of each dNTP, 0.3 µM of each primer, 2 units Taq polymerase (Bioline, London, UK), 2 mM $MgCl_2$, and 5 µl reaction buffer (160 mM $(NH_4)_2 SO_4$, 670 mM Tris HCl, 0.1% Tween 20, pH 8.8). The following PCR profile was used: 1 cycle at 94°C for 4 minutes; 30 cycles at 94°C for 45 seconds, 55°C for 45 seconds and 72°C for 3 minutes; 1 cycle at 72°C for 10 minutes. The resulting PCR products were purified using QIAquick purification kits (QIAGEN Ltd., Crawley, UK) according to the manufacturer's instructions.

TABLE 1. Myricaceae material sequenced in this study

Taxon	Code [1]	Voucher/Source	Geographical origin	GenBank No. [2]
Ingroup (Myricaceae)				
Myrica gale	GSCO	*Hedge* s.n. (E)	UK, Scotland, S. Uist	
Myrica gale	GJAP	*Kenicer* s.n. (E)	Japan, Tokyo University Nikko Botanical Garden	
Myrica gale var. tomentosum	TOM	*Pirie* 87BL00481(E)	East Siberia (cultivated)	
Myrica hartwegii	HART	*Edwards* 93 (RPBG)	California	
Comptonia peregrina	COM3	*Meagher* s.n. (E)	Massachusetts, Nantucket	
Canacomyrica monticola	905	*Herbert* 934 (E)	New Caledonia	
Morella punctata	PUNC1	*Gardner* 25 (E)	Cuba	
Morella pavonis	PV	*Gardner & Knees* 6304 (E)	Chile	
Morella parvifolia	PARV	*Laegaard* 55225 (BM)	Ecuador, Loja, Saraguro	
Morella pubescens	T2	*Pennington, Reynel and Daza* 1093 (E)	Peru	
Morella esculenta	ESC7	*Moeller* 01183 (E)	China	
Morella nana	N1	*Gardner* 523 (E)	China (cultivated)	
Morella javanica	JAV	*Radhiah & Cronk* 135 (E)	Sumatra	

Taxon	Code [1]	Voucher/Source	Geographical origin	GenBank No. [2]
Morella adenophora	AD7758	*Ye Hua-gu* 7758 (IBSC)	China	
Morella adenophora	AD4157	*Cheng* 4157 (E)	Taiwan	
Morella rubra	RUB1	*Ge Xhu-jun* s.n. (IBSC)	China	
Morella rubra	R4160	*Cheng* 4160 (E)	Taiwan	
Morella rubra	R1	*Gardner* 522 (E)	Japan (cultivated)	
Morella spathulata	SPATH	*Lowry & Randrianasolo* 4546 (P)	Madagascar	
Morella pilulifera	PIL	*Balkwill* 7889 (E)	Transvaal, South Africa	
Morella serrata	SER	*Herbert* 1009 (E)	Cape, South Africa	
Morella integra	INT14	*Herbert* 1014 (E)	Cape, South Africa	
Morella salicifolia	SA	*Wood* 3121 (BM)	Yemen	
Morella brevifolia	BR	*Balkwill* 7575 (E)	Transvaal, South Africa	
Morella diversifolia	DIV5	*Herbert* 1005 (E)	Cape, South Africa	
Morella quercifolia	QUER6	*Herbert* 1006 (E)	Cape, South Africa	
Morella cordifolia	CORD3	*Herbert* 1003 (E)	Cape, South Africa	
Morella humilis	HUM11	*Herbert* 1011 (E)	Cape, South Africa	
Morella kraussiana	KRAU4	*Herbert* 1004 (E)	Cape, South Africa	
Morella faya	SM204	*Herbert* SM201 (E)	Azores	
Morella rivas-martinezii	F118	*Herbert* F101 (E)	Canaries	

Taxon	Code [1]	Voucher/Source	Geographical origin	GenBank No. [2]
Morella carolinensis	HET	*Anderson* A20055 (FSU)	Florida, USA	
Morella californica	CA	*Herbert* V1 (E)	California, USA	
Morella inodora	I70	*Anderson* A19870 (FSU)	Florida, USA	
Morella cerifera	CER60	*Anderson* A20060 (FSU)	Florida, USA	
Morella cerifera	CERB	*Ibanez et al.* 128A1(E)	Belize	
Outgroup (Juglandaceae)	-			
Juglans nigra	-	Potter et al. 2002 (ITS)	North America	AF338491
		Manos & Stone, 2001 (*trnL-F*)		AF303783
Juglans cinerea	-	Stanford et al. 2000 (ITS)	North America	AF179572
		Manos & Stone, 2001 (*trnL-F*)		AF303786
Engelhardia roxburgiana	-	Manos & Stone, 2001 (ITS)	China	AF303801
		Manos & Stone, 2001 (*trnL-F*)		AF303774
Rhoiptelea chiliantha	-	Manos & Stone, 2001 (ITS)	China	AF303800
		Manos & Stone, 2001 (*trnL-F*)		AF303773

[1] Sequencing reference code.
[2] GenBank accession numbers not yet available for sequences produced in this study.

Amplification of the ITS1-5.8S-ITS2 region was carried out using the forward primer ITS5 and the reverse primer ITS4; in some cases it was necessary to amplify the region in two pieces using the two internal primers ITS2 and ITS3, in addition to ITS5 and ITS4 (White et al., 1990). PCR reactions were as for *trnL-F* with the addition of 1% DMSO to improve amplification (Frackman et al., 1998). The following PCR profile was used: 1 cycle at 94°C for 3 minutes; 30 cycles at 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute 30 seconds; 1 cycle at 72°C for 5 minutes.

For some taxa the ITS PCR product contained multiple bands of varying length. Cloning was carried out on a number of such samples using pGEM-T Easy Vector kit (Promega UK, Southampton, UK) according to the manufacturers instructions, followed by extraction and purification using Perfectprep Plasmid Mini kit (Eppendorf A.G., Hamburg, Germany). Plasmids were sequenced as above using either the ITS primers or the pUC primers supplied with the pGEM-T Easy Vector kit. This method was carried out to confirm that the additional bands were present as a result of fungal contamination of the sample and not as a result of the presence of multiple (paralogous) copies of ITS. Subsequently, for samples with multiple bands, the sample was run on low melting temperature agarose, the target band was excised and extracted from the gel using QIAquick gel extraction kit (QIAGEN Ltd., Crawley, UK), then the PCR was performed again on the gel-extracted product.

Sequencing reactions of 20µl contained the CEQ™ DTCS Quick Start Kit (Beckman Coulter Ltd., High Wycombe, UK). For the ITS region the primers ITS2, ITS3, ITS4 and ITS5 (White et al., 1990) were used and 1M Betaine was added to some samples to improve sequencing read-through (Frackman et al., 1998). For the *trnL-F* region the primers c, d, e and f (Taberlet et al., 1991) were used and in some cases it was necessary to use a further internal primer 'm' which was designed to amplify the middle section of the *trnL-F* region. The internal primer (m) is previously unpublished and matches bases 317-336 of the *trnL* gene; the sequence of this primer is GAGTCCCATTCTACATGTCA. Sequencing reactions were performed using the following PCR profile: 25 cycles at 96°C for 10 seconds, 50°C for 5 seconds, 60°C for 4 minutes. The sequence reaction products were cleaned according to manufacturer's instructions before being run on a CEQ™ 8000 Genetic Analysis System (Beckman

Coulter Ltd., High Wycombe, UK). Forward and reverse sequences were manually assembled in Chromas version 2.12 (Technelysium Pty. Ltd., Helensvale, Australia).

Alignment and analyses— Alignment of *trnL-F* sequences required the insertion of gaps. Alignment of ITS sequences required the insertion of gaps and the exclusion of some regions due to alignment ambiguity. Gaps were coded following the simple method of Simmons and Ochoterena (2000; see alignment, Appendix 4). Initial analyses of these data sets (results not shown) indicated that there were no strongly supported (>85%) incongruent clades among them. Therefore, a combined *trnL-F* and ITS data set (data set I) was compiled. All sequence alignment was carried out by eye.

The phylogeny of Myricaceae was reconstructed using both maximum parsimony (MP) and Bayesian inference (BI). Maximum parsimony analyses were performed on data set I using PAUP* Version 4.0b10 (Swofford, 1998). Heuristic searches were carried out with tree-bisection-reconnection (TBR) and saving multiple trees (MulTrees). Branch lengths were calculated using the delayed transformation (DELTRAN) optimisation. Support for the tree topologies obtained was assessed using bootstrap analysis (Felsenstein, 1985) performed using the heuristic search option and 1000 replicates. The following categories were used to describe bootstrap percentage (BP) results: 50-74, weak support; 75-84, moderate support; 85-100, strong support.

Bayesian inference was conducted using MrBayes version 3.0 (Huelsenbeck and Ronquist, 2001). Modeltest 3.5 (Posada and Crandall, 1998) was used to determine the most appropriate substitution model for the data (ITS and *trnL-F* assessed separately). The Bayesian analysis was performed on the combined data set (data set I with gap coding removed). Four incrementally heated Markov chains were run simultaneously for different numbers of generations and the likelihood values per generation were plotted to examine at what point the tree scores reached a plateau. The effects of varying the heating parameter (default T=0.2) were also investigated. A maximum number of 500,000 generations and a heating parameter of T=0.08 were selected and the analyses run several times with a tree being saved every 100 generations. A burn-in value of 100 (=10,000 generations) was used to discard the trees generated up to the point of convergence. A majority-rule consensus tree

showing the posterior probability values was generated in PAUP*. Bayesian posterior probability values range from zero to one, with zero being low and one being high.

Molecular dating— To evaluate whether the sequences evolved in a clocklike fashion, a likelihood ratio test (Felsenstein, 1988) was performed on the combined ITS and *trnL-F* data set. A molecular clock was rejected (LR=50.7866, df=24, p=0.01) indicating significant variation in the rate of molecular evolution in different branches of the tree. The penalized likelihood (PL) method implemented in r8s (Sanderson, 2002; http://ginger.ucdavis.edu/r8s/) was used to estimate nucleotide substitution rates and ages of selected clades. For the purposes of this analysis it was necessary to compile a reduced data set (data set II) of combined ITS and *trnL-F* sequences that minimized the number of polytomies in the data set and included a more distant outgroup (*Fagus crenata* [Fagaceae]) that would eventually be pruned out of the tree. Data set II was analyzed in MrBayes (as above) and the resulting consensus tree was then used in the PL analysis. Cross-validation was first performed in r8s to obtain the optimum rate-smoothing parameter for the transition of substitution rate between ancestor and descendants based on the given data. The BI tree was converted to an ultrametric tree in PL using the recommended algorithm (TN=truncated Newton method), collapsing branches of zero length. Analyses were performed using various combinations of fossil calibration points and fixing the root node at two different ages (see below). Trees were viewed in TreeView X (http://darwin.zoology.gla.ac.uk/%7Erpage/treeviewx/).

Age constraints were derived from the fossil record for Myricaceae and Juglandaceae. The ages of geological epochs follow Harland et al. (1990). The *Comptonia* node was set to a minimum of 49 Ma (Million years) based on the presence of leaves of the fossil species *Comptonia columbiana* in the 'Republic' flora, NE Washington (Wolfe and Wehr, 1987); this fossil appears to be the oldest known Myricaceae record. *Comptonia* leaves are easily recognized in the fossil record (Manchester, 1999) and the Republic flora was dated using radiometric techniques (Wolfe and Wehr, 1987). The root of the tree could be fixed at two possible dates. Firstly, the oldest known fossil fruits that can be attributed to extant tribes of Juglandaceae are Palaeocene (Manchester, 1987), thus a date of 61 Ma (corresponding to mid-Palaeocene) was chosen for the root. Secondly, Normapolles flowers in the Middle Senonian of

Sweden (Friis, 1983) have been suggested to have affinity with Juglandaceae or *Canacomyrica*, thus a date of 83 Ma (corresponding to the Santonian-Campanian boundary) was selected for the root. It was deemed necessary to take the conservative approach of including this older date because the sister group of Myricaceae is equivocal (see Chapter 2).

RESULTS

Combined analyses— Data set I (combined ITS and *trnL-F*) contained 40 taxa (36 ingroup taxa) and 1511 characters (including gap coding), of which 170 (11%) were potentially parsimony informative. The ITS data contributed 532 characters, including three coded gaps, and 111 potentially parsimony informative characters (21%) whilst the *trnL-F* data contributed 979 characters, including two coded gaps, and 54 potentially parsimony informative characters (6%). A single indel (length=9 bases) in the *trnL-F* data (see Appendix 4) was shared by the African and Malagasy species. MP analysis of data set I produced 232 most parsimonious trees (tree length 438, CI=0.80, RI=0.85). The strict consensus of the 232 most parsimonious trees is shown in Figure 1 with bootstrap percentages (BP) equal to or greater than 50 shown above the branches. The strict consensus tree produced in this analysis of combined data is more fully resolved than the strict consensus trees produced by analysis of either ITS or *trnL-F* separately (results not shown). One of the 232 most parsimonious trees is shown in Figure 2 with branch lengths above the branches (DELTRAN optimization).

Pairwise sequence divergences among species of Myricaceae were calculated in PAUP* (Appendix 5) using the GTR (General Time Reversible; Rodriguez et al., 1990) model of molecular evolution. Within Myricaceae pairwise distances ranged from zero between samples of *Morella rubra* (from Taiwan and China) to 0.069 between *Canacomyrica* and *Myrica gale* (UK sample). Pairwise distances between *Myrica* species and *Morella* species ranged from 0.017 between *Myrica gale* (Japanese sample) and *Morella punctata*, to 0.027 between *M. gale* (UK sample) and *Morella pubescens* (South America) (mean between all *Myrica* species and *Morella* species=0.022). The greatest pairwise distance within *Morella* was 0.019 between *Morella adenophora* (Taiwan) and *M. pubescens* (mean among all *Morella* species=0.010).

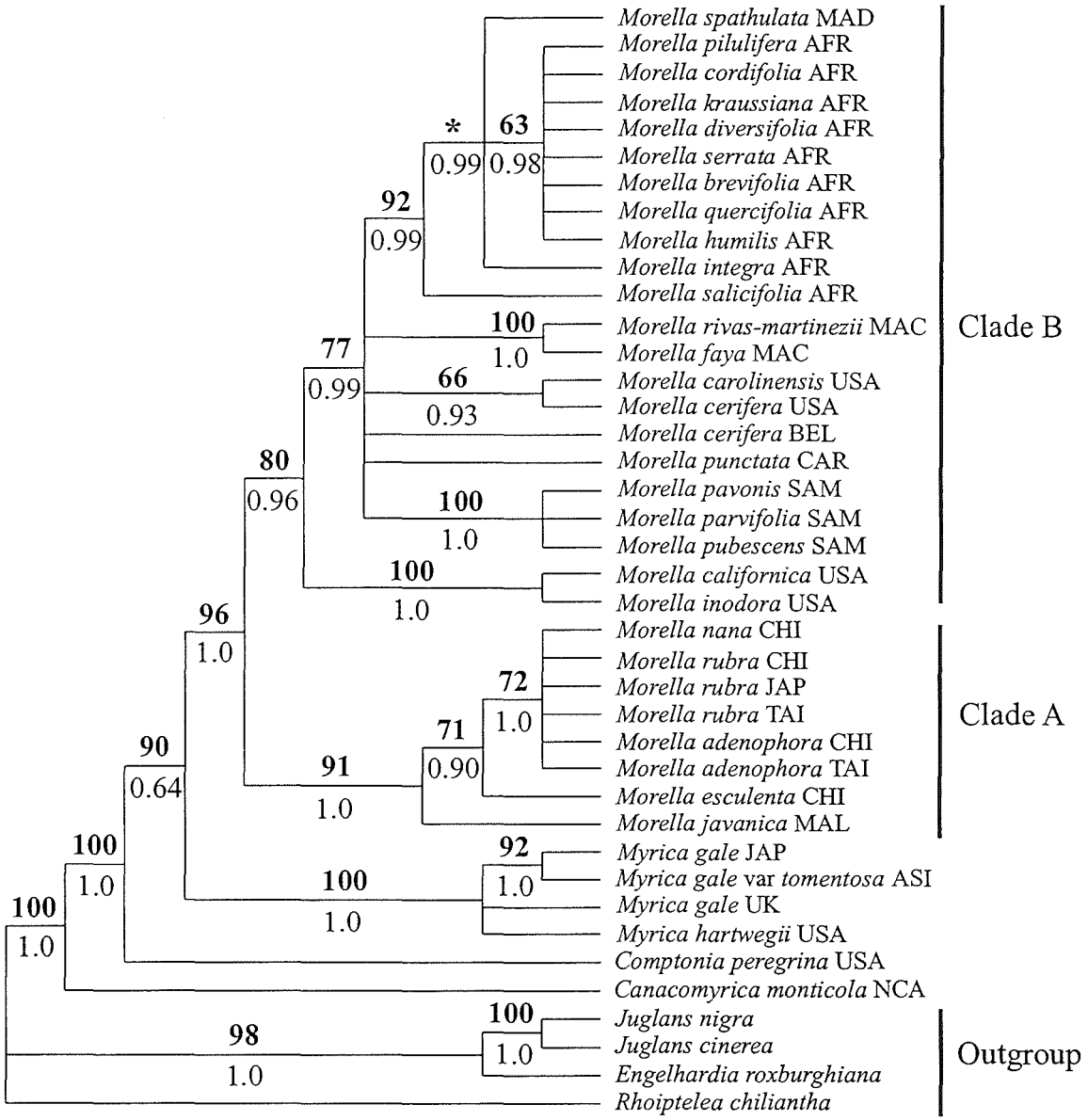

Figure 1: Strict consensus tree of the 232 equally most parsimonious trees produced from analysis of dataset I, combined ITS and *trnL-F*. Bootstrap percentages greater than or equal to 50 are shown in bold above the branches, Bayesian posterior probabilities are shown below the branches. Asterisk indicates group with bootstrap percentage of less than 50. Codes used for the geographical origin of the taxa are as follows: AFR= Africa; MAD=Madagascar;MAC=Macaronesia; USA=North America; BEL=Belize; CAR=Caribbean; SAM=SouthAmerica; CHI=China; TAI=Taiwan; MAL=Malesia; JAP=Japan; ASI=Northern Asia;UK=United Kingdom; NCA=New Caledonia.

The model indicated by Modeltest 3.06 for the ITS data was TrNef+G (Tamura and Nei, 1993) with equal base frequencies; substitution rates A-C=1.00, A-G=1.43, A-T=1.00, C-G=1.00, C-T=3.29, G-T=1.00; and gamma shape parameter =0.2657. The model indicated for the *trnL-F* data was K81uf+G (Kimura, 1981) with base frequencies A=0.35, C=0.16, G=0.15, T=0.33; substitution rates A-C=1.00, A-G=1.10, A-T=0.17, C-G=0.17, C-T=1.10, G-T=1.00; and gamma shape parameter =0.1648. Bayesian analysis of data set I was carried out with partitioning of the data so that the appropriate models could be applied to the different regions. Bayesian analysis produced a topologically identical strict consensus tree to that produced in the MP analysis. Bayesian posterior probabilities (PP) are shown in Figure 1 below the branches and are largely congruent with the Bootstrap support percentages (see below).

In all trees the monophyly of Myricaceae is strongly supported (100 BP, 1.00 PP). *Canacomyrica* is sister to a strongly supported clade (100 BP, 1.00 PP) comprising the rest of Myricaceae. *Comptonia* is sister to a clade comprising *Myrica* and *Morella* species; this clade receives strong bootstrap support (90 BP) although the Bayesian posterior probability is somewhat low (0.64 PP). *Myrica hartwegii* and the varieties of *Myrica gale* included in the analysis form a strongly supported clade (100 BP, 1.00 PP), which is itself sister to a strongly supported clade (96 BP, 1.00 PP) comprising all species of *Morella*. Within *Morella* two major clades can be identified (Figure 1). Clade A comprises all Asian species, the monophyly of which is strongly supported (91 BP, 1.00 PP). Within Clade A, *M. javanica* is sister to the rest of the species and *M. esculenta* is sister to the remaining species; these relationships receive weak bootstrap support (71 and 72 BP respectively) but high Bayesian posterior probabilities (0.90 and 1.00 respectively). Clade A is sister to the moderately supported Clade B (80 BP, 96 PP) containing all non-Asian species. Within Clade B, the two North American taxa, *Morella californica* and *Morella inodora*, form a strongly supported clade (100 BP, 1.00 PP) sister to a large polytomy comprising the remaining species. Within this polytomy there are several strongly supported relationships: the three South American taxa form a terminal trichotomy (100 BP, 1.00 PP); the Macaronesian taxa form a clade (100 BP, 1.00 PP); the African and Malagasy taxa form a clade (92 BP, 0.99 PP) within which the crown group of southern African (mostly Cape) taxa is unresolved.

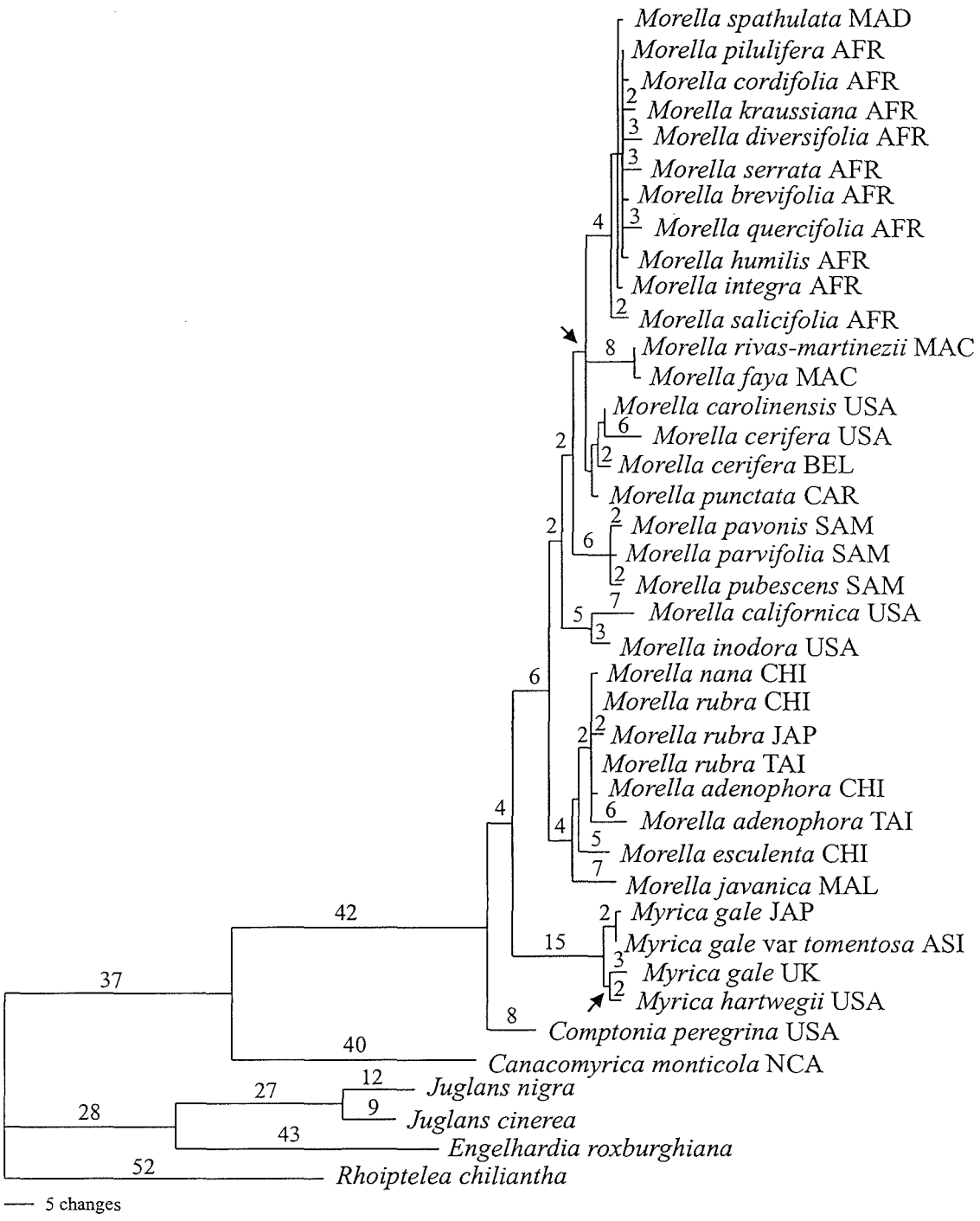

Figure 2: One of the 232 equally most parsimonious trees produced from analysis of dataset I, combined ITS and *trnL-F*. Branch lengths >1 are shown above the branches (DELTRAN optimization). Arrows indicate groups not present in the strict consensus tree. Codes used for the geographical origin of the taxa are as follows: AFR= Africa; MAD=Madagascar; MAC=Macaronesia; USA=North America; BEL=Belize; CAR=Caribbean; SAM=South America; CHI=China; TAI=Taiwan; MAL=Malesia; JAP=Japan; ASI=Northern Asia; UK=United Kingdom; NCA=New Caledonia.

Molecular dating analysis— Data set II contained 26 taxa (23 ingroup taxa, two outgroup taxa retained, one pruned out) and 1524 characters (including gaps). The topology of the 50% majority rule consensus tree produced in the Bayesian analysis of data set II did not differ significantly from that produced in the wider analyses (above). This tree formed the basis of the molecular dating analysis. Table 2 shows the estimated ages (all dates given are minimal ages) of various nodes in the tree based on the different combinations of root ages and fossil calibration point. Each node is defined as the most recent common ancestor (MRCA) of a pair of taxa (see Table 2). Figure 3 shows the result of the PL analysis with a smoothing rate of 50.12, the root fixed at 61 Ma and the *Comptonia* node (node X) fixed at 49 Ma.

DISCUSSION

Phylogeny and taxonomic implications— In all analyses the monophyly of Myricaceae is strongly supported. This result is consistent with the findings of a wider survey of Fabid taxa based on *rbcL* (Chapter 2). The placement of *Canacomyrica* as sister to the remaining species is also consistent with previous findings (Chapter 2). The other monotypic genus of the family, *Comptonia*, is sister to a strongly supported clade containing the two remaining genera, *Myrica* and *Morella*. The position of *Comptonia* and its several unique (within the family) morphological characters, such as pinnatifid leaves with stipules and fruits surrounded by laciniate bracteoles forming a cupule, support its generic status.

The clade containing all species formerly considered to comprise *Myrica* s.l. is split into two strongly supported clades. The clade comprising *Myrica* s.s. can be defined by a deciduous habit, dry fruits with accrescent spongiose bracteoles and leaves with sunken stomata. The clade comprising *Morella* can be defined by a typically evergreen habit, fleshy, papillose fruits frequently with a covering of wax, and leaves with stomata not sunken. The molecular data, therefore, lend convincing support to the argument for the formal recognition of these two groups as separate genera.

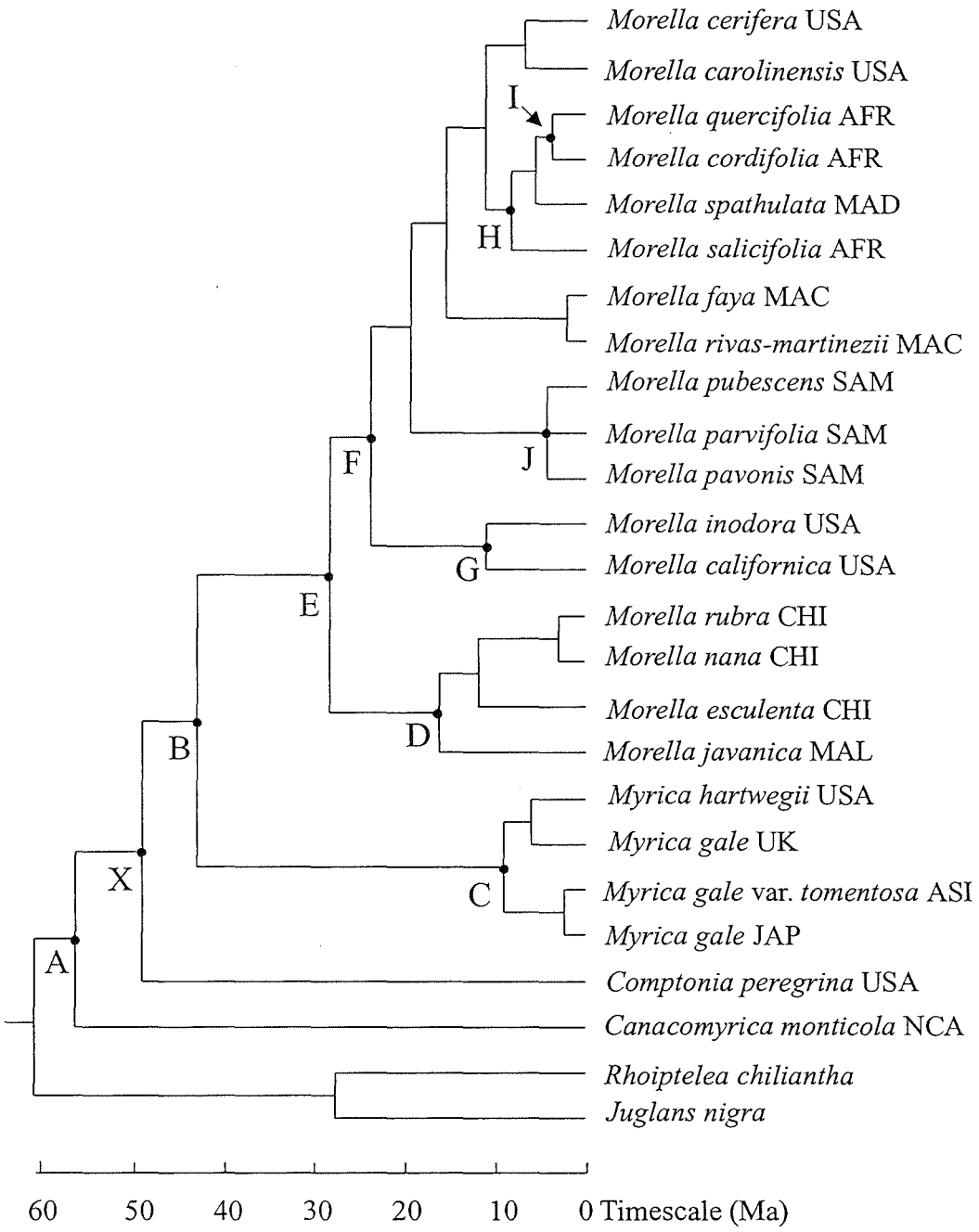

Figure 3: Maximum likelihood tree of Myricaceae based on dataset II, combined ITS and *trnL-F* with the root set at 61 Ma, subjected to penalized likelihood rate smoothing. Nodes identified by letter are discussed in the text.

TABLE 2. Results of the Penalized Likelihood analyses

Node (defined as MRCA[1] of:) and statistics	Age of Node	
	Root fixed at 61 Ma	**Root fixed at 83 Ma**
A (*Canacomyrica, Comptonia*)	56.31	69.82
X Fixed (*Comptonia, Myrica hartwegii*)	49	49
B (*Myrica hartwegii, Morella javanica*)	43.02	43.17
C (*Myrica gale* var. *tomentosa, Myrica hartwegii*)	8.92	9.30
D (*Morella javanica, Morella esculenta*)	16.15	16.41
E (*Morella javanica, Morella californica*)	28.31	28.66
F (*Morella californica, Morella pavonis*)	23.77	24.13
G (*Morella californica, Morella inodora*)	10.99	11.20
H (*Morella salicifolia, Morella quercifolia*)	8.28	8.45
I (*Morella cordifolia, Morella quercifolia*)	3.84	3.93
J (*Morella pavonis, Morella parvifolia*)	4.23	4.32
Smoothing factor	50.12	63.10
Mean rate of variation (substitutions/site/year)	5.392×10^{-10}	4.910×10^{-10}
SD of rate of variation	0.00030	0.00021

[1]MRCA = most recent common ancestor

Within *Morella*, two moderately to strongly supported clades can be identified. The Asian taxa, Clade A (Figure 1), are defined by fruits lacking wax, branched male inflorescences and unbranched stamens. Within this Asian group *M. javanica*, a species found throughout Malesia, is sister to the other species. *M. esculenta* is sister to a poorly resolved group of species comprising *M. nana* (found at high altitudes in Yunnan, China), *M. adenophora* (occurring from Taiwan in the east to Yunnan in the west) and *M. rubra* (occurring in Japan and Taiwan in the east to Yunnan in the west). *M. esculenta* is clearly differentiated from this crown group, hereafter referred to as the '*Rubra* complex', on the basis of its fruit morphology which is more similar to that of *M. javanica* (see Chapter 8). Within the '*Rubra* compex' it seems likely that *M. nana* is not separate from *M. rubra* or the Chinese *M. adenophora*, based on the low morphological and molecular divergence between them. However, the Taiwanese sample of *M. adenophora*, often referred to var. *kusanoi*, has a number of molecular autapomorphies (see Figure 2) suggesting that it may be genetically isolated from the rest of the '*Rubra* complex'. This species group clearly requires more detailed study of both molecular and morphological variation at population level.

Within the non-Asian Clade B (Figure 1), which is defined by male flowers with branched stamens and mostly waxy fruits, there are several interesting patterns. The North American taxa are polyphyletic with *M. californica* and *M. inodora* forming a well-supported group sister to the rest of the clade, whilst *M. cerifera* (a wide-ranging species of North and Central America and the Caribbean) and *M. carolinensis* together form a weakly supported group embedded in the Clade B polytomy (see below). The relationship of the Belize sample, attributed to *M. cerifera*, to the *M. cerifera-M. carolinensis* clade perhaps suggests that its status warrants reappraisal in the light of these results.

The African taxa are the most derived group with very little molecular divergence among the species (see Figure 2). *M. salicifolia*, a wide-ranging African species (also in Saudi Arabia and Yemen) is sister to the rest of this group, which includes the Malagasy species *M. spathulata*. The sampling of the African taxa is not complete due to under-representation of tropical species of which a number of subspecies and varieties are recognized in the Flora of Tropical east Africa (Polhill and Verdcourt, 2000). However, sampling from the Cape region of southern Africa is complete. A

crown group comprising all the species of the Cape (including *M. brevifolia* and *M. pilulifera* which are not Cape species but are found further east in southern Africa) forms a trichotomy with *M. spathulata* and *M. integra*. The position of *M. integra* is somewhat surprising, given its previous status as a subspecies of *M. serrata*, although support for this relationship is low (Figure 1).

There is low molecular variation among the Cape crown group yet significant morphological and ecological variation can be observed among at least some of them: *M. quercifolia* has distinctive pinnatifid leaves and a low habit; *M. cordata* has distinctive cordate leaves and is entirely restricted to sand dune habitats in the western Cape. This degree of morphological variation, albeit only in leaf shape, is remarkable given the lack of morphological variation among the rest of *Morella* species (particularly in Clade B). Among some of the other species, however, there is almost continuous morphological variation in leaf shape and character (e.g. *M. humilis*, *M. kraussiana*, *M. diversifolia*) indicative of recent (perhaps ongoing) speciation within this group. The solution chosen by White (1993) to synonomize most of these species does little to resolve the problem. Some species (e.g. *M. quercifolia* and *M. cordifolia*) are easily recognized in the field and are unlikely to be confused with any other species, but it is clear that further investigation at population level is required to establish relationships among all the Cape taxa.

Within *Morella*, the recognition of an Asian group is consistent with the work of Chevalier (1901), who placed these species in *Myrica* subg. *Morella*. The results of this study suggest that Chevalier's subgenera *Faya* (*M. faya*, *M. inodora* and *M. californica*) and *Cerophora* are polyphyletic with respect to one another. The recognition of an Asian and a non-Asian group is, however, consistent with the classification proposed by Wilbur (1994) who recognized Chevalier's *Morella* subg. *Morella* but placed the remainder of *Morella* species in a new subgenus *Cerothamnus* (Tidstr.) Wilbur. Within subgenus *Cerothamnus*, Wilbur recognised series *Faya* (equivalent to Chevalier's subgenus of the same name) and series *Cerothamnus* (for the remainder of species), but the results of this study provide no support for the recognition of these subdivisions of the group. The results of this study suggest that there are similarities between the African species and *M. cerifera*, which (with the exception of *M. salicifolia*) share the synapomorphy of a lack of trichomes on the

endocarp. However, the recognition of this group to the exclusion of the other non-Asian taxa would render that group paraphyletic. It seems appropriate therefore to recognise only two groups: *Morella* subg. *Morella* and *Morella* subg. *Cerothamnus* (see Chapter 8 for keys and descriptions).

Substitution rate estimates and the fossil record— The substitution rates calculated in this study, based on combined analysis of ITS and *trnL-F*, were 5.392 $x10^{-10}$ (substitutions per site per year; based on a root age of 61 Ma) and 4.910 $x10^{-10}$ (substitutions per site per year; based on a root age of 83 Ma). These rates are somewhat higher than those reported for ITS for other woody plants such as *Corylus* (Betulaceae; 2.27-4.31 $x10^{-10}$ substitutions per site per year; Whitcher and Wen, 2001) or *Hamamelis* (Hamamelidaceae; 3.5-4.1 $x10^{-10}$ substitutions per site per year; Wen and Shi, 1999) but lower than those reported for *Phylica* (Rhamnaceae; 2.44 $x10^{-9}$ substitutions per site per year; Richardson et al., 2001a); they are broadly congruent with the rate published for *trnL-F* for the woody *Phylica* (4.87 $x10^{-10}$ substitutions per site per year; Richardson et al., 2001a). Direct comparison of substitution rates with those from other studies is difficult because the rates calculated here are based on combined use of ITS and *trnL-F* data with differing modes of molecular evolution.

The fossil record for Myricaceae requires re-examination in the light of recent taxonomic changes and the molecular data presented here. Those fossils attributed to *Myrica* s.l. may in fact represent *Morella*, but their potential for informing our understanding of the history of the family is obscured until they are correctly identified. The differences in stomata and fruit morphology between the two genera mean that it is possible to make such reassessments of macrofossils. Friis (1985) described endocarps, which she attributed to *Myrica*, from the late Middle Miocene (c. 14.2 Ma) Fasterholt flora, Denmark. In one illustration (Friis, 1985, Figure 7) the presence of papillae on the outer surface of the endocarp is clear, thus the material can be attributed to *Morella* without the need to examine the actual fossils. Conversely, the pollen of Myricaceae is frequently mistaken for that of closely related families (especially Casuarinaceae and Betulaceae; Chourey, 1974; Sundberg, 1985) and there is little intra-generic differentiation within Myricaceae (Sundberg, 1985).

The age of the Fasterholt fossils does not conflict with the divergence time estimates (Table 2) calculated for *Morella*. Since it is not possible to assign these fossils to any particular lineage within *Morella* they must be placed at the stem of the *Morella* clade which diverged from the *Myrica* lineage at least 43.17 Ma ago. All the divergence time estimates should be viewed with caution given that they are based on inexact calibration points.

Biogeography— Due to its presence in New Caledonia, the confirmation of *Canacomyrica* as a member of Myricaceae significantly alters our understanding of the distribution of the family. The PL analyses performed here suggest that the *Canacomyrica* lineage diverged between 56 Ma ago and 70 Ma ago (all calculated divergence date estimates are rounded to the nearest integer for the purposes of discussion). The older date is derived from calculations in which the root of the tree (Figure 3) was set at 83 Ma based on Normapolles fossil evidence (i.e. ancestral to both Juglandaceae and Myricaceae, if not all 'core' higher hamamelidae) and as such may be artificially old for the true divergence between Juglandaceae and Myricaceae. The younger date, however (based on oldest known Juglandaceae fossils), is consistent with estimates of the age of New Caledonia which, although an island of Gondwanan continental derivation, was submerged from the Late Cretaceous to the Early Eocene and is thought to have re-emerged, coincident with undergoing the orogeny that put in place its large ultramafic outcrops, in the mid to late Eocene (from approx. 56 Ma; McLoughlin, 2001).

That the ancestors of *Canacomyrica*, and indeed Myricaceae, may have been among the earliest colonizers of new, volcanically derived, substrates is consistent with our knowledge of the ecology of the modern species (see below). But an early presence in the South Pacific region is not. Some authors have considered Myricaceae as a family of Laurasian origin (Raven and Axelrod, 1974). The placement of *Canacomyrica* may be interpreted as conflicting with this northern hemisphere concept of the family. Whilst it is possible that *Canacomyrica* may have been dispersed by migratory birds to New Caledonia from ancestral populations in southeast Asia, there is no fossil evidence to support this route nor are there any extant members of the genus on the abundant ultramafic outcrops in southeast Asia. Fossil pollen attributed to *Canacomyrica* has, however, been recorded from Eocene-Miocene deposits in New

Zealand. Within Fagales both Nothofagaceae and Casuarinaceae are entirely restricted to the southern hemisphere; the former has often been interpreted as a classic Gondwanan disjunct (e.g. McLoughlin, 2001). In the light of this study and other phylogenetic data now available for all other families in Fagales, it may be timely to re-examine the southern hemisphere history of the order.

Based on the PL analyses the *Myrica* and *Morella* lineages diverged 43 Ma ago (mid Eocene). Ancestors of both *Comptonia*, for which numerous Eurasian fossils (Eocene-Pliocene; Manchester, 1999) are known, and *Myrica* may have made use of the Bering land bridge (present throughout much of the Paleogene and Neogene; Tiffney and Manchester, 2001) and the North Atlantic land bridge (present during the Eocene-Oligocene; Tiffney and Manchester, 2001). The deciduous habit and nitrogen-fixing ability of extant members of these two genera suggest that their ancestors are likely to have been able to tolerate 1) the low light availability postulated for the higher latitude occurrences of these land bridges (Tiffney and Manchester, 2001; Milne and Abbott, 2002), and 2) the new, and therefore nutrient-impoverished soils that would have characterized them.

The cooling climate of the Pliocene, and glaciation events in the Pleistocene, are likely to have significantly impacted the distribution of these two genera. The extinction of *Comptonia* throughout Eurasia is likely to be attributable to the east-west tending mountain chains that are considered to have caused the demise of many plant genera (e.g. *Liriodendron* [Magnoliaceae], Parks and Wendel, 1990; *Hamamelis*, Tiffney and Manchester, 2001). *Myrica* seeds are water dispersed so it is not vital to invoke land connections to explain the present distribution of this genus and this feature of the seeds may explain how the genus was able to retain an almost circumboreal distribution, despite repeated local extinctions during the Pleistocene, whilst *Comptonia* was not.

Within *Myrica* there is relatively little divergence among the British sample (*Myrica gale*), the North American (Sierra Nevada) *M. hartwegii*, and a group comprising the Japanese and northeast Asian samples (pairwise distances range from 0.002 to 0.008). The low differentiation (similar to that seen among the Cape species of *Morella*; see Figure 2) between these geographically distant populations (see Map 1, Appendix 2)

may be due to recent genetic communication. The presence of *Myrica hartwegii* in the Sierra Nevada may be interpreted as a relict of a more southerly distribution of the genus during colder climatic conditions. According to the results of the PL analyses these taxa diverged 9 Ma ago (Figure 3, node C; i.e. late Miocene). It seems more likely however, that the taxa included in this study represent lineages that have been isolated by the repeated glaciation events of the Pleistocene. It appears that an elevated rate of molecular evolution is in effect in the *Myrica* lineage (the mean pairwise distance between *Myrica* and *Morella* is more than double the mean pairwise distance within *Morella*), perhaps associated with the polyploidy that has been recorded in this genus. As a result of this elevated rate, it is possible that the dating method used has significantly overestimated the age of the divergence of the *Myrica* species. The recommended reappraisal of fossil evidence from Eurasia may help to elucidate the history of this genus.

Within *Morella*, the largest genus of the family, diversification appears to have begun with the split between the Asian (Clade A) and non-Asian (Clade B) species at 28-29 Ma ago (Figure 3, node E; Table 2). The earliest diverging group within the non-Asian clade is a strongly supported clade comprising *M. californica* from western North America and *M. inodora* from eastern North America. Taxa exhibiting this kind of Asian-north American disjunction (such as *Styrax* [Styracaceae], *Carya* [Juglandaceae], *Hamamelis*, *Pachysandra* [Buxaceae]) have been termed Tertiary relicts and have been the subject of a number of molecular phylogenetic studies (e.g. Xiang et al., 1998; Xiang et al., 2000; Donoghue et al., 2001). The distribution of Tertiary relict taxa is considered to reflect a once widespread boreotropical flora the distribution of which contracted to the present locations in response to globally cooling temperatures (beginning in the Early Eocene; Tiffney and Manchester, 2001). The life form of *Morella* species (woody, evergreen, mesophytic trees) fits with the typical description of a Tertiary relict or boreotropical element, and led MacDonald (1977) to postulate a boreotropical origin for the family. However, dated phylogenies indicate that the timing of these disjunctions is much more recent (clustering around 10 Ma ago or 5 Ma ago; Milne and Abbott, 2002) than the late Oligocene date suggested by this study for *Morella*. The Bering land bridge would certainly have been available for migration of *Morella* species during the Oligocene and the

temperatures and water availability prevailing during that period are unlikely to have been limiting (Tiffney and Manchester, 2001).

The date for the split between the early branching north American taxa and the rest of non-Asian *Morella* is 24 Ma ago (Figure 3, node F). The lack of resolution among these remaining non-Asian lineages suggests a rapid radiation at this point in the history of the group. Axelrod (1975) suggested a Madrean-Tethyan origin for *Morella* citing *M. californica* and *M. faya* as examples of the link between dry-adapted floras in the northern hemisphere. Whilst the date of the split is consistent with a Madrean-Tethyan hypothesis, it is not possible to determine whether there is a sister group relationship between these two lineages based on the available data and, perhaps more importantly, the ecology of Myricaceae does not fit well with the concept. It is not appropriate to consider members of this family as dry-adapted although they bear some of the features common to plants of mediterranean-type regions (i.e. evergreen, sclerophyllous leaves, hard wood, deep tap roots and the ability to resprout following fire) which permit them to withstand periodic drought. Sclerophylly (or scleromorphy) and xeromorphy share many common features (Andrews, 1916; Beadle, 1966; Hill, 1998). Hill (1998) defines scleromorphy as an adaptive response to soils low in phosphorus (i.e. not an adaptation to dry climatic conditions). Bearing in mind the ubiquitous (except perhaps in *Canacomyrica*) presence of nitrogen-fixing root nodules and phosphorus assimilating cluster roots in Myricaceae, it becomes clear that these plants have evolved sclerophylly as an adaptation to nutrient-poor soils, not climate. Furthermore, actinorhizal plants are known to require a reliable supply of water for normal functioning of the root nodules (Dommergues et al., 1984) which may account for the deep tap roots and the occurrence of Myricaceae in wet habitats like bogs or riparian zones (personal observations). Tree species growing in stressful or disturbed sites (i.e. young soils with low nutrient status or areas of orogenic activity of the kind that Myricaceae are often found in) are considered more likely to engage in resprouting than those in less stressful sites (Del Tredici, 2001). Thus, the attributes that Axelrod (1975) recognized as indicating adaptation to dry climatic conditions can, in Myricaceae, equally be attributed to the narrow ecological tolerances of these plants.

Although the lack of resolution among lineages in South America, the Caribbean, North and Central America, Macaronesia and Africa precludes discussion of routes of colonization within the non-Asian group, some clades within this group are well supported and deserve discussion. Rapid recent diversification approximately 4 Ma ago (Figure 3, node J) can be inferred in the South American species. As already mentioned, the ecology of these plants makes them excellent colonizers of open ground, especially volcanic or otherwise new substrates. Therefore, it is perhaps not surprising that the date for diversification corresponds well with the date of uplift of the northwestern Andes that commenced about 5 Ma ago (Gentry, 1982).

The evidence for the role of birds in dispersal of *Morella* seed between North America, Central and South America appears compelling (Ridley, 1930; Place and Stiles, 1992; Borgmann et al., 2004). Due to the waxy coating (consisting of mono- and diglycerides of myristic, palmitic and stearic fatty acids) that most *Morella* fruits have, the seed has been postulated to stay in birds' guts longer than equivalent sized non-waxy fruits, making them particularly likely to be transported over large distances (Place and Stiles, 1992). The presence of these plants on isolated islands such as the Azores (*M. faya*) is testament to just how far it is possible for seed to be transported by birds (nearest source area is the Iberian Peninsula at about 1000 miles away). It seems highly probable that birds have been instrumental in determining the past and modern distribution of *Morella*, not just those species of the Americas. For instance, the fossil evidence for *Morella* in Europe in the Miocene (Friis, 1985) suggests that this genus had a wider distribution although it is unlikely ever to have been globally widespread. More data would be required to resolve the relationships within the non-Asian clade and permit inferences to be made about the routes of dispersal and/or migration.

Rapid recent diversification in *Morella* has occurred in the Cape Floristic Region of southern Africa. The age of the African group is estimated at 8 Ma (Figure 3, node H) with the radiation of the Cape species estimated to have occurred just 4 Ma ago (Figure 3, node I). The concept of *Morella* as a group that has radiated in response to Pleistocene climate change is contrary to the opinion of a number of authors that the plants are relicts of a tropical forest flora (e.g. Linder et al., 1992; R. Cowling, pers. comm.). In the Mid-Miocene the Cape supported a tropical forest vegetation under

tropical to subtropical climatic conditions (Linder and Hardy, 2004). However, climatic conditions began to change approximately 8-10 Ma ago as a result of changing global climate and the upwelling of cold waters along the Atlantic seaboard of southern Africa, leading to a much drier climate with little summer rain (Linder and Hardy, 2004). These climatic changes are considered to have caused the demise of the tropical forests, permitting radiation of the taxa that form the modern fynbos vegetation (characteristically shrubby, sclerophyllous and fire-prone). The dates calculated for the diversification of *Morella* in the Cape region fit with the suggested climatic changes and the older date for the African clade is congruent with dates of diversification calculated for another woody southern African genus, *Phylica*, at 7-8 Ma ago (Richardson et al., 2001b).

Several unique features of the Cape region are likely to have had an influence on *Morella* species. Both the extremely nutrient-poor (especially low in N and P) soils, and the summer rain that is a limited but important feature of the Cape, distinguish it from the other four regions of the world where mediterranean-type climates dominate (Cody and Mooney, 1978). These two factors are likely to favour *Morella* species relative to other mediterranean regions where *Morella* are present with low diversity (e.g. Californian chaparral; Munz, 1974), or have become extinct (e.g. Mediterranean basin; Axelrod, 1975). In addition, large topographical variation and sea level fluctuations appear to have played an important role in speciation in the western Cape (Linder, 2003) and the latter would clearly have had a significant impact on the distribution of the coastal species *M. cordifolia*.

CONCLUSIONS

The results of this study provide the first phylogenetic evidence for the following relationships within Myricaceae (*Canacomyrica* (*Comptonia* (*Myrica*, *Morella*))); and strongly support the formal recognition of *Morella* for the papillose, fleshy fruited, evergreen species of the family. Within *Morella*, the Asian and non-Asian species form two strongly supported clades for which a number of diagnostic morphological characters can be identified. The subgeneric status of these two groups is recognized following Wilbur (1994): *Morella* subg. *Morella*; *Morella* subg. *Cerothamnus*.

The divergence time estimates calculated for Myricaceae make it possible to evaluate some of the previously posited hypotheses for the origin of the family. Several close correlations between lineage-specific diversification and major orogenic or climatic events are inferred from the molecular dating analyses. The nitrogen-fixing and phosphorus-assimilating capacity of most members of Myricaceae is acknowledged to be closely linked with the ability of the family to colonize new substrates throughout the world. The results of this study suggest that much of the present diversity in *Morella* has arisen during the Neogene and that dispersal of seed by birds is likely to have been the most significant factor in determining the modern distribution of the genus. This is in stark contrast to the notion that the distribution of Myricaceae is a relictual one formed by continental drift (eg. Thorne, 1972; Bramwell, 1976; Macdonald, 1989). However, the relationship of the New Caledonian endemic *Canacomyrica* to the rest of the family, and the presence of other members of Fagales in the southern hemisphere, suggests that the role of the break-up of Gondwana is likely to be highly significant to the history of the entire order.

It is tempting to speculate that the ancestors of Myricaceae arose in Australasia in montane and/or marginal habitats with migration north for the *Comptonia* and *Myrica* lineages. Evolution of the *Morella* lineage in the vicinity of the Pacific would then have been followed by migration (possibly across the Bering land bridge), then subsequent widespread dispersal by birds.

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Chapter 7

New combinations and a new species in *Morella* (Myricaceae)[1]

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ABSTRACT

The molecular support for current generic concepts in Myricaceae has made it necessary to make new combinations in *Morella*. *Morella* comprises approximately 47 species found in the Old and New World tropics. Three new combinations are made here: *Morella adenophora, M. nana,* and *M. punctata. M. nana* is lectotypified, and a new species, *M. rivas-martinezii*, is described.

Key words: Canary Islands, China, Cuba, Myricaceae, *Morella*, new combinations, Taiwan.

With the exception of the monotypic genera *Comptonia* L'Héritier ex Aiton (northeastern USA) and *Canacomyrica* Guillaumin (New Caledonia), the species of Myricaceae have traditionally been referred to the Linnaean genus *Myrica* (Cronquist, 1981; Kubitzki, 1993; Zomlefer, 1994; Takhtajan, 1997). There are, however, significant differences between the two deciduous species with dry fruits of the temperate northern hemisphere (*Myrica gale* L. and *M. hartwegii* S. Watson), and the remaining c. 47 evergreen species with papillose, fleshy fruits that are disjunctly distributed in the Old and New World tropics. Some authors have recognized these two groups at subgeneric level (Engler, 1888; Melchior, 1964; Elias, 1971) while others have given them generic status (Spach, 1841; Chevalier, 1901; Baird, 1969, unpubl. thesis, Univ. of NC, Chapel Hill; Wilbur, 1994; Judd et al., 1999; Polhill & Verdcourt, 2000). Previous authors have used both *Myrica* L. and *Morella* Loureiro to refer to the evergreen species and a long history of nomenclatural difficulties is associated with the typification of these two genera.

[1] This chapter has been accepted for publication in *Novon* and is presented in the style of that journal

In the only monograph of the family, Chevalier (1901) placed the dry-fruited species in the genus *Gale* Duhamel. It can be assumed that he considered one of the fleshy-fruited species to typify the genus *Myrica*, in which he placed the majority of species. However, he did not cite types for these genera. Subsequent authors (e.g., Baird, 1969, unpubl.; Wilbur, 1994) have retained *Myrica* for the dry-fruited species, adopting *Morella* for the remaining taxa.

Recently, Verdcourt & Polhill (1997) made a proposal to conserve the name *Myrica* with the conserved type of *M. cerifera* L. (one of the fleshy-fruited species) in an attempt to minimize the number of necessary name changes. However, the Committee for Spermatophyta (Brummitt, 1999) rejected the proposal. Thus *Myrica gale* is the lectotype of *Myrica* (Herb. Linn. No. 373 [Inst. France]; lectotype designated by Jonsell & Jarvis (Jarvis et al., 1993)) and the majority of species previously known as *Myrica* must be transferred to *Morella*. The following key separates the two genera:

KEY TO DIFFERENTIATE *MYRICA* AND *MORELLA*

1a. Shrubs deciduous; leaves with sunken stomata; inflorescences borne on the previous year's growth; fruits dry with adherent spongiose bracteoles, water-dispersed ..... *Myrica*

1b. Shrubs or trees evergreen; leaves with stomata not sunken; inflorescences borne on the present year's growth; fruits papillose, fleshy, sometimes with a waxy covering, bird-dispersed ..... *Morella*

New combinations in *Morella* have been made for species in North America (Wilbur, 1994), Africa (Killick et al., 1999), Malesia (Turner, 2001), South America (Parra-O., 2002) and some in central America and the Caribbean (Wilbur, 2001; Knapp, 2002). In the course of the preparation of a PhD thesis on Myricaceae, in which new molecular data support the recognition of *Morella* (Herbert, 2004 unpubl. thesis, Univ. of St. Andrews, Scotland), it became apparent that some new combinations in *Morella* were necessary. A new species is described and three new combinations are made here, to provide valid names for species recognized in a forthcoming molecular phylogenetic paper.

Morella adenophora (Hance) J. Herb., comb. nov. Basionym: *Myrica adenophora* Hance, Journ. Bot. 21: 357. 1883. TYPE: [China.] "In dicione Ting-on ins. Hai-nan, m. Nov. 1882", *B. C. Henry 22159* (holotype, BM).

This species occurs in Hainan, Guangdong and Guangxi in China (Lu & Bornstein, 1999); var. *kusanoi* Hayata is recognized in the Flora of Taiwan (Yang & Lu, 1996). *M. adenophora* is a shrub (up to 3 m in height) and is closely related to *Morella nana* which occurs in the neighboring provinces of Yunnan and Guizhou (see below). *M. adenophora* is also closely related to *Morella rubra* Loureiro which is widely cultivated in China and also occurs in Japan. The fruits of *M. rubra* (up to 3 cm diam.) are significantly larger than those of *M. adenophora* (up to 1 cm diam.). All three species share the character of a single fruit developing at the apex of the infructescence, thus differing from the other Asian species, such as *M. javanica* (Blume) I. M. Turner, in which more than one fruit per infructescence develops.

Morella nana (A. Chevalier) J. Herb., comb. nov. Basionym: *Myrica nana* A. Chev., Mém. Soc. Sci. Nat. Cherbourg 32: 202. 1901. TYPE: China. Yunnan province: Bois de Mao-Kou-Tchang, au dessus du Tapintz. *P. J. M. Delavay 148* (lectotype, P, here designated).

This species is found in central and north Yunnan and west Guizhou in China. At up to 3 m in height it is much smaller than the more widespread *M. rubra* (up to 15 m). When Chevalier (1901) described this species he had not seen specimens of *M. adenophora*; the ranges of the two species are contiguous, but detailed population-level studies of morphology and/or genetics would be required to confirm their status. *M. nana* is recognized in the Flora of China (Lu & Bornstein, 1999).

Morella punctata (Grisebach) J. Herb., comb. nov. Basionym: *Myrica punctata* Griseb., Mem. Acad. Am. Sci. Art N. S. 8 [Plantae Wrightianae, pt. I]: 177. 1860. TYPE: [Cuba.] "in saxosis prope Monte Verde, Feb", *C. Wright 1460* (holotype, GH).

This species is endemic to Cuba. It has entire oblong-oblanceolate leaves (3--5 cm long) and ovoid fruits (Leon & Alain, 1951), distinguishing it from the widespread

Morella cerifera (L.) Small, which is found throughout the Caribbean (also in the United States and Central America) and which has longer leaves (to 8 cm) and globose fruits.

Morella rivas-martinezii A. Santos & J. Herb., sp. nov.: descr. by A. Santos in Fund. Juan March, ser. univ., Cienc. Agrar. (Contrib. conocim. fl. veg. Hierro), 114: 45. 1980, sub "*Myrica rivas-martinezii*". TYPE: Spain. Canary Islands: Isla de Hierro, La Dehesa, 22 Feb. 1976, *A. Santos 24699* (holotype, ORT).

A. Santos (in Fund. Juan March, ser. univ., Cienc. Agrar. 114: 45–46. 1980) intended to publish "*Myrica rivas-martinez*" as a new species. Because three different gatherings were cited as "Holotypi atque isotypi" (holotypes and isotypes), the name was not validly published (Art. 37.1 & 37.2, with Art. 8.1 & 8.2). Dr Santos has accepted my invitation to publish the taxon here as a new species of *Morella* (on the basis of his 1980 description), and this we do above.

This species is sympatric with *Morella faya* (Aiton) Wilbur in the laurel forests of La Gomera, El Hierro and La Palma in the Canary Islands. The mature leaves, which are small (up to 20 mm) and spathulate, are distinct from the much larger and oblanceolate leaves of *M. faya*.

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Chapter 8

Conclusions and Classification

Conclusions— The two molecular phylogenetic studies presented here provide, for the first time, an insight into the relationships within Myricaceae. The family was found to be monophyletic, with *Canacomyrica* (a monotypic genus endemic to New Caledonia that bears a number of distinctive morphological features including staminodes in the female flower) occupying a position sister to the other three genera. Based on this finding, in conjunction with reports of sexual instability in *Myrica* and observed heterotopy (functional stamens on the fruit wall) in *Morella*, it is possible to view the staminodes of *Canacomyrica* as an extreme, perhaps ancestral, form of the incomplete suppression of male function found throughout the family. It is also possible to view the presence of staminodes in *Canacomyrica* as attractants for generalist insect pollinators, as has been observed in other members of Fagales. Future investigations of breeding system evolution in Fagales should certainly include *Canacomyrica* since the morphological features that it shares with species of Juglandaceae suggest a close link between these two families, despite the somewhat weak support for this relationship revealed by the molecular study. The importance of *Canacomyrica* to the understanding of relationships and patterns of evolution within Fagales highlights the importance of conserving this species, which was identified as Endangered under IUCN Red List criteria in this study.

A detailed molecular phylogenetic analysis of the relationships within Myricaceae revealed the following relationships: (*Canacomyrica* (*Comptonia* (*Myrica*, *Morella*))). The molecular data collected were augmented by two investigations of morphology, one focussing solely on *Canacomyrica*, the other treating representatives of all the other genera and subgeneric groups, to gain an understanding of the variation within the family. The position of *Comptonia* and its several unique (within the family) morphological features support its traditional generic status. These results are the first to confirm that *Myrica* s.l. is split into two strongly supported clades that correspond to *Myrica* s.s. and *Morella*. These results add strength to the argument for the recent formal recognition of *Morella* for the mostly tropical, evergreen species with fleshy

papillose fruits (frequently with a covering of wax). In line with these results a number of new combinations in *Morella* were proposed. Furthermore, two clades within *Morella* were identified that correspond to previously recognized subgenera: *Morella* subgen. *Morella*, which comprises all the Asian species; and *Morella* subgen. *Cerothamnus*, which comprises all the other species (i.e. African and North and South American). These groups had been recognized by previous authors but other lower taxonomic groupings, mostly within subgen. *Cerothamnus*, received no support in this study, nor were any diagnostic morphological characters found to support them.

Some of the most interesting findings of the molecular study of Myricaceae were the recent rapid radiations that could be observed in the African lineage, in particular. Divergence times estimated using Penalized Likelihood methods revealed several close correlations between lineage-specific diversification and major orogenic or climatic events. For instance, the *Canacomyrica* lineage is estimated to have split from the rest of Myricaceae approximately 56 Ma ago (based on oldest known Juglandaceae fossils). This date is consistent with estimates of the age of New Caledonia which, although an island of Gondwanan continental derivation, was submerged from the Late Cretaceous to the Early Eocene and is thought to have re-emerged, coincident with undergoing the orogeny that put in place its large ultramafic outcrops (to which *Canacomyrica* is endemic), in the mid to late Eocene (from approx. 56 Ma). Furthermore, the radiation in the South American species was found to have occurred at approximately 4 Ma ago, corresponding to the date inferred for the commencement of uplift of the northwestern Andes (about 5 Ma ago). A close correlation was also observed between diversification in the southern African Cape species (approximately 4 Ma ago), and the changes associated with the onset of a mediterranean-type climate in that region. This finding contrasts with the opinion of several authors that *Morella* species are relicts of a tropical forest flora in the Cape.

It has become clear during the course of these investigations that the nitrogen-fixing and phosphorus-assimilating capacity of most members of Myricaceae is closely linked with the ability of the family to colonize new substrates throughout the world. Significantly the results of this study also suggest that much of the present diversity in *Morella* has arisen during the Neogene and that dispersal of seed by birds has been the most important factor in determining the modern distribution of the genus. This is in

stark contrast to the common notion that the distribution of Myricaceae is a relictual one formed by continental drift. However, the relationship of the New Caledonian endemic *Canacomyrica* to the rest of the family, and the presence of other members of Fagales in the southern hemisphere, suggests that the role of the break-up of Gondwana is likely to be highly significant to the history of the entire order. It is perhaps timely to re-examine the southern hemisphere history of Fagales in the light of these findings.

Classification—The following section is a classification for Myricaceae based upon the results presented above. Keys to the genera and subgenera are given. Descriptions are given for the family, genera and subgenera. Species are listed under the relevant genus or subgenus.

MYRICACEAE Blume, Fl. Javae 17-18: 3. 1829. *nom. cons.*

TYPE: *Myrica* L.

Mostly wind-pollinated shrubs or trees, usually dioecious, possessing nitrogen-fixing root nodules (except, perhaps, *Canacomyrica*). Leaves deciduous or evergreen, alternate, simple, entire to serrate, occasionally pinnatifid, thin to leathery or coriaceous; covered in numerous resinous balloon glands, usually strongly scented; simple trichomes present (except in *Canacomyrica*); stomata anomocytic, confined to lower surface, sunken in *Myrica* and *Comptonia*; estipulate (except in *Comptonia*). Inflorescence an erect spike, somewhat lax in some *Morella* species, usually unisexual flowers (staminodes present in *Canacomyrica*), female flowers beneath male in mixed inflorescences. Flowers spirally arranged, perianth lacking (except in *Canacomyrica*), surrounded by 1 (-3) bracts and 2-6 bracteoles. Male flowers with 2-8 stamens; filaments short, free or united into a staminal column, sometimes branched; anthers erect, dorsifixed, 2-thecous, opening length-wise; vestigial style present in *Canacomyrica*; pollen grains with '*Myrica*-type' aperture. Female flowers with pink-red style, bifid, lamellular or linear (laciniate in *Canacomyrica*); ovary sessile, 1-locular; single ovule, erect, orthotropous. Fruit a drupe, pericarp papillose, red-black, sometimes with a waxy covering, or smooth and fleshy(*Canacomyrica*), or fruit a nut (*Comptonia* and *Myrica*). Seed with membranous testa and little endosperm. Chromosome number, n=8, *Comptonia* tetraploid, *Myrica* hexaploid or higher.

Key to the genera

1a. Deciduous shrubs with dry fruits; leaves with sunken stomata; inflorescences borne on the previous year's growth.....**2**

1b. Evergreen shrubs or trees with fleshy fruits; leaves with stomata not sunken; inflorescences borne on the present year's growth.....**3**

2a. Fruits with adherent spongiose bracteoles; leaves serrate at apex, lacking stipules .....**1 *Myrica***

2b. Fruits surrounded by laciniate bracteoles forming a cupule, leaves pinnatifid with stipules.....**2 *Comptonia***

3a. Fruits papillose, sometimes with a waxy covering (widespread in Old & New World tropics).....**3 *Morella***

3b. Fruits smooth without wax; female flowers with staminodes (New Caledonia) ....**4 *Canacomyrica***

1. *MYRICA* L., Syst. Nat. ed. 1. 1735.; Sp. Pl. 1024. 1753.; Gen. Pl. ed. 5. 1754.

Syn.: *Gale* Tourn. ex Adans. Fam. 2: 345. 1763.

TYPE: *Myrica gale* L.

> Deciduous, dioecious, scented shrubs. Leaves thin, oblanceolate, serrate at apex. Inflorescences short and compact. Male flowers surrounded by 1 bract, 4 bracteoles; 3-6 stamens, filaments free. Female flowers surrounded by 1 bract and 2-3 bracteoles; style linear. Fruit a nut, the bracteoles becoming spongiose and accrescent.

Two species, one variety:

Myrica gale

Myrica gale var. tomentosa

Myrica hartwegii

Myrica gale and *M. hartwegii* are very similar: the only clear difference is that the bracteoles subtending the fruit are glabrous in the former and densely hairy in the latter. Whilst var. *tomentosa* has been widely accepted, the distinguishing character of leaf vestiture is highly variable in *Myrica* and likely to be environmentally, rather than genetically, determined. Nonetheless, the varietal name is retained here in order to recognise the genetic divergence (albeit small) of the Japanese and northern Asian (i.e. Kamchatka and neighbouring regions) populations from the European and North American species.

2. *COMPTONIA* L'Hérit. in Aiton, Hort. Kew. 3:334. 1789.

Syn.: *Myrica* L., Syst. Nat. ed 1. 1735. pro parte.
Liquidambar L., Hort. Cliff. 486. 1737 pro parte.
Comptonia Banks in Gaertn., Fruct. 2: 90. 1791.

TYPE: *Comptonia asplenifolia* [sic] L'Hérit. (*aspleniifolia*)

Deciduous, monoecious or dioecious, scented shrub. Leaves thin, deeply pinnatifid. Inflorescences short and compact, male inflorescences somewhat lax. Male flowers surrounded by 1 bract; up to 8 stamens, filaments free. Female flowers surrounded by 1 bract and 2 bracteoles; style linear the two stigmatic arms united at base. Fruit a smooth nut, the bracteoles becoming much elongated with elongated scales together forming a burr or cupule surrounding the nut.

One species:

Comptonia peregrina (L.) L'Hérit. in Aiton, Hort. Kew. 3: 334. 1789.

Syn.: *Liquidambar peregrina* L., Sp. Pl. 2: 999. 1753.
Myrica asplenifolia L., Sp. Pl. 2: 1024. 1753.
Liquidambar peregrina L., Syst. Nat. ed. 10. 2: 1273. 1759.
Myrica comptonia C. DC., Prodr. 16: 151. 1864.
Myrica peregina Kunze Revis. Gen. Pl. 2: 638. 1891.
Comptonia peregrina (L.) Coult., Mem. Torr. Bot. Club 5: 127. 1893-1894.

TYPE: Lectotype: LINN 1134.3. Designated here.

Full synonomy for both the genus and species names are given for clarity. The citation *Comptonia peregrina* (L.) Coult. is widely used, but incorrect, since L'Héritier's use of the generic name in reference to this species predates Coulter by over 100 years.

3. *MORELLA* Lour., Fl. Cochinch. 548. 1790.

TYPE: *Morella rubra* Lour.

Evergreen, dioecious or occasionally monoecious, shrubs or trees. Leaves leathery to coriaceous, oblanceolate or spathulate, occasionally pinnatifid or cordate, margin serrate to dentate or sometimes entire, frequently recurved. Inflorescences short and compact or relatively elongate and lax, sometimes branched. Male flowers surrounded by 1 bract, bracteoles sometimes present; up to 8 stamens, filaments fused into a staminal column or free. Female flowers surrounded by 1 (-3) bracts and 2-6 bracteoles; style linear or lamellular. Fruit a drupe, covered in red-black fleshy papillae, sometimes also numerous dense trichomes or a waxy covering.

Key to the subgenera

1a. Shrubs or trees with unbranched stamens, fruits lacking wax (Asia) .... **3a subgen. *Morella***

1b. Shrubs or trees with branched stamens, fruits usually with a waxy covering (North America, Caribbean, South America, Africa)..... **3b subgen. *Cerothamnus***

3a. *MORELLA* subgen. *MORELLA* Lour.

Syn.: *Myrica* sect. *Morella* (Lour.) Benth. & Hook..f., Gen. Pl. 3: 401. 1880.
Myrica subgen. *Morella* (Lour.) Engler, Nat. Pflanzenfam. II. 1: 27. 1893.

TYPE: *Morella rubra* Lour.

Evergreen, dioecious or occasionally monoecious, shrubs or trees. Leaves leathery to coriaceous, oblanceolate or spathulate, margin serrate to dentate or sometimes entire, frequently recurved. Male flowers with filaments free and unbranched. Female flowers surrounded by 1 (-3) bracts and 2-6 bracteoles; style linear or lamellular. Fruit never waxy.

Two species and a species complex comprising three putative species and a variety:
Morella javanica
Morella esculenta

The field notes of *Clemens & Clemens* 32278 (BM) state that *M. javanica* is epiphytic on large trees on Mt. Kinabalu. A number of specimens from the Philippines differ significantly from the description given above in having much thinner textured, more elongate leaves, crowded together on the stem, e.g. *Ramos* 1333 (BM) and *Mendoza* 18201 (BM). However, where fruiting specimens were observed the fruit and inflorescence morphology matched the description given above. *M. esculenta* is much like *M. javanica* except that the leaves are more clearly elliptical in shape and leathery in texture. Confusingly, despite the name (which means edible), this is not the species that is cultivated throughout China for its fruits (*M. rubra*).

The *rubra* complex (*Morella rubra, Morella nana, Morella adenophora, M. adenophora* var. *kusanoi*)

M. rubra is undoubtedly the widely cultivated tree of China and is readily distinguished from *M. esculenta* on the basis of the form of the inflorescence which is the same as that of *M. nana* and *M. adenophora* (see below) in being short and condensed with only a single fruit developing at the apex of the inflorescence. The fruits can be as large as 3 cm diam. in cultivated specimens. However, the low genetic

divergence (see Chapter 6) and the lack of morphological variation between *M. rubra*, *M. nana* and *M. adenophora* suggests that all these species should be treated as a species complex, the '*Rubra* complex'. It may not be appropriate to include the Taiwanese variety *kusanoi* in the 'Rubra complex'; more detailed morphological and population genetic studies would be required to elucidate the relationships of these taxa. It appears that the Asian species are not scented although they all possess the 'balloon' glands typical of all Myricaceae species; fresh material would be required to confirm this.

3b. *MORELLA* subgen. *CEROTHAMNUS* (Tidestr.) Wilbur, Sida 16: 99. 1994.

Basionym: *Cerothamnus* Tidestr., Elys. Marian., Ferns 41. 1910.

Syn.: *Myrica* sect. *Faya* (Webb & Berthel.) C. DC., Prodr. 16: 151. 1864.
Myrica sect. *Cerophora* (Raf.) A. Chev., Mem. Soc. Sci. Nat. & Math. Cherbourg 32: 223. 1901.
Morella series *Faya* (P. Webb & Berthel.) Wilbur, Sida 16: 103. 1994.
Morella series *Cerothamnus* (Tidestr.) Wilbur, Sida 16: 100. 1994.

TYPE: *Myrica cerifera* L.

Evergreen, dioecious or occasionally monoecious, shrubs or trees. Leaves leathery to coriaceous, oblanceolate, occasionally pinnatifid or cordate, margin serrate to dentate or sometimes entire, frequently recurved. Inflorescences short and compact or relatively elongate and lax, sometimes branched. Male flowers with filaments fused into a branched staminal column. Female flowers surrounded by 1 (-3) bracts and 2-6 bracteoles; style linear or lamellular. Fruit with a waxy covering, and sometimes with sparse trichomes.

Forty-two species, four subspecies and three varieties:

Morella apiculata
Morella arborea
Morella arguta
Morella bojeriana
Morella brevifolia
Morella cacuminis
Morella californica
Morella carolinensis
Morella cerifera
Morella chevalieri
Morella cordifolia
Morella dentulata
Morella dentulata* var. *comorensis
Morella diversifolia
Morella faya
Morella microcarpa
Morella parvifolia
Morella pavonis
Morella phanerodonta
Morella phillyreifolia
Morella picardae
Morella pilulifera
Morella pubescens
Morella punctata
Morella quercifolia
Morella reticulata
Morella rivas-martinezii
Morella rugulosa
Morella salicifolia
Morella salicifolia* subsp. *mildbraedii

Morella funckii
Morella holdridgeana
Morella humilis
Morella inodora
Morella integra
Morella kandtiana
Morella kraussiana
Morella lindeniana
Morella madagascariensis
Morella microbracteata
Morella salicifolia* subsp. *kilimandscharica* var. *kilimandscharica
Morella salicifolia* subsp. *kilimandscharica* var. *goetzei
Morella salicifolia* subsp. *meyeri-johannis
Morella serrata
Morella singularis
Morella shaferi
Morella spathulata

Morella cerifera, the type species of the subgenus is one of the most widespread of all *Morella* species. Several subspecies and varieties have been named but the plant is known to be dispersed by migratory bird species (such as warblers and swallows, see Chapter 6), so it is likely that most populations are not genetically isolated.

Morella californica, *M. inodora* and *M. faya* are all unscented and share glabrous inflorescence stems. They have previously been treated in a separate section or series but their relationships remain unclear so they are not treated separately here. Despite the geographical isolation of *M. californica* and *M. inodora* on opposite sides of the continent, they differ little except that *M. inodora* has a lamellular style with the two stigmatic branches fused towards the point of attachment, and entire leaves. *M. rivas-martinezii* is sympatric with *M. faya* in the laurel forests of La Gomera, El Hierro and La Palma in the Canary Islands. The mature leaves, which are small (up to 20 mm) and spathulate, are distinct from the much larger and oblanceolate leaves of *M. faya*. Whether these taxa are truly distinct and, if so, whether this distinction warrants species status requires further morphological and genetic study.

Morella salicifolia occurs throughout tropical Africa and in the southern tip of the Arabian peninsula. The Ethiopian type specimen (Lectotype to be designated, *Schimper* 1135, E) closely resembles the Arabian specimens, but more southerly and tropical specimens appear somewhat different, reflecting the differences that have led to the recognition of numerous subspecies and varieties of this species. The validity of

these subspecific taxa, and the true relationships among them, requires extensive morphological and population-level genetic study.

The reference by Linnaeus to "Aethiopia" for the type locality of *M. cordifolia* and *M. quercifolia* is clearly incorrect, they are both endemic to the Cape region of South Africa. The relatively high level of morphological variation among the southern African species, particularly those of the Cape is epitomised by *M. cordifolia* and *M. quercifolia*, both of which have a leaf shape significantly different from the typical Myricaceae elliptical-oblanceolate shape. The leaf shape in combination with their low habit and the limited number of localities in which they occur, means that these species are readily recognisable and unlikely to be confused with either each other or any other species of the region.

Morella serrata is quite distinctive in the Cape, where it is the largest species and confined to very moist habitat, such as river banks. *M. integra* has, in the past, been treated as a subspecies of *M. serrata* but it seems that it is quite distinct, having leaves with few or no serrations and distinctly small balloon glands (so small that they are difficult to see with a hand lens), and being geographically isolated further north and at much higher altitudes than *M. serrata* is typically found. These two species seem much more typical of Myricaceae in their ecological preferences than *M. cordifolia* and *M. quercifolia* both of which have reduced, coriaceous leaves and a low habit that appears more suitable to the seasonal drought of the Cape region. An additional complex of at least three putative species is present in the Cape: *Morella kraussiana*, *M. humilis* and *M. diversifolia*. The leaf morphology of these three species intergrades completely and the descriptions of them given by Killick are difficult to apply in the field. Identification is further complicated by the fact that there is significant variation between young and mature leaf characteristics (the leaves of most of the South African species are tomentose when the plant is young or has resprouted following fire). Based on currently available information it is not possible to satisfactorily circumscribe these species.

4. *CANACOMYRICA* Guillaumin, Bull. Soc. Bot. France 87: 300. 1941.

Syn.: Canacomyricaceae Baum.-Bod. ex Doweld, Bulletin of Moscow Society of Naturalists 105: 59. 1992.

TYPE: *Canacomyrica monticola* Guillaumin

Dioecious shrub or small tree. Leaves evergreen, coriaceous, oblanceolate, serrate at apex, dark green above, whitish below, unscented resinous glands, trichomes absent; stomata anomocytic; stipules absent. Inflorescences compact; flowers spirally arranged and widely spaced on floral stem, trichomes sparse on floral stem. Male flowers surrounded by 3 bracts, lacking bracteoles; 6 stamens, filaments free; vestigial style and perianth present. Female flowers surrounded by 3 bracts, lacking bracteoles; 6 staminodes present; style pink-red, bifid, lamellular and laciniate; 6-lobed perianth present; ovary sessile, 1-locular; ovule 1, erect, orthotropous. Fruit a drupe, fleshy, pericarp white or pink becoming black, very hard endocarp.

One species:

Canacomyrica monticola

APPENDIX 1

Species List

Species names accepted in this study (including authorities and distribution details).

Species	Distribution	Region
Canacomyrica monticola Guillaumin	Southern Grande Terre, New Caledonia	Pacific
Myrica gale L.	Canada, Alaska, northern USA, Scandinavia, Central & Atlantic Europe	Circumboreal
Myrica gale var. *tomentosa* (DC.) Asch. & Graebn.	Western Russia, Central & Atlantic Europe, Kamchatka, Japan	Northern Hemisphere
Myrica hartwegii Watson	California	USA
Comptonia peregrina (L.) L'Hérit.	East USA	USA
Morella faya (Aiton) Wilbur	Canary Islands, Azores, Portugal	Macaronesia
Morella rivas-martinezii (A. S. Guerra) J. Herbert ined.	Canary Islands	Macaronesia
Morella cerifera (L.) Small	Southern and Eastern USA, Mexico, Guatemala, Nicaragua, Belize, Costa Rica, Bahamas, Dominican republic, Caymans, Puerto Rico, Jamaica, Lesser Antilles, Colombia	USA, South and central America, Caribbean
Morella caroliniensis (Mill.) Small	Eastern USA	USA
Morella inodora (Bartram) Small	South-eastern USA	USA
Morella californica (Cham. & Schltdl.) Wilbur	Western USA	USA

Species	Distribution	Region
Morella lindeniana (C. DC.) S. Knapp	Mexico, Guatemala, Honduras	Central America
Morella phanerodonta (Standl.) Wilbur	Costa Rica	Central America
Morella apiculata (Urb. & Ekm.) J. Herbert ined.	Dominican Republic	Caribbean
Morella microcarpa Bentham (combination not yet made)	Jamaica, Dominican republic	Caribbean
Morella picardae (Krug & Urb.) Wilbur	Dominican Republic	Caribbean
Morella reticulata (Krug & Urban) J. Herbert ined.	Dominican Republic	Caribbean
Morella punctata (Griseb.) J. Herbert ined.	Cuba	Caribbean
Morella shaferi (Urb. & Britt.) J. Herbert ined.	Cuba	Caribbean
Morella cacuminis (Britt. & Wils.) J. Herbert ined.	Cuba	Caribbean
Morella holdridgeana (Lundell) Wilbur	Puerto Rico	Caribbean
Morella arguta HBK (combination not yet made)	Bolivia	South America
Morella chevalieri C. Parra-O.	Bolivia, northern Argentina	South America
Morella funckii (A. Chev.) C. Parra-O.	Venezuela, Colombia	South America
Morella parvifolia (Benth.) C. Parra-O.	Ecuador, Colombia, Venezuela, Peru	South America
Morella pavonis (DC.) C. Parra-O.	Bolivia, Peru, Chile	South America
Morella pubescens (Humb. & Bonpl. ex Willd.) Wilbur	Ecuador, Colombia, Cost Rica, Venezuela, Peru, Bolivia	South America
Morella singularis (C. Parra-O.) C. Parra-O.	Colombia, Ecuador	South America

Species	Distribution	Region
Morella arborea (Hutch.) Cheek	Mount Cameroon	Trop. W. Africa
Morella integra (A. Chev.) Killick	SW Cape	Southern Africa
Morella microbracteata (Weim.) Verdc. & Polhill	Zimbabwe	Southern Africa
Morella quercifolia (L.) Killick	Cape	Southern Africa
Morella diversifolia (Adamson) Killick	Cape peninsula	Southern Africa
Morella pilulifera (Rendle) Killick	Rhodesia, Malawi, Swaziland and South Africa	Southern Africa
Morella brevifolia (E. Mey. ex C. DC.) Killick	Natal and east Cape	Southern Africa
Morella cordifolia (L.) Killick	Cape	Southern Africa
Morella kraussiana (Buchinger ex Meisn.) Killick	Cape	Southern Africa
Morella humilis (Cham. & Schltdl.) Killick	Cape	Southern Africa
Morella serrata (Lam.) Killick	South Africa, SW Tropical Africa,Tanzania	S./Trop Africa
Morella kandtiana (Engl.) Verdc. & Polhill	Uganda, Kenya, Tanzania, Congo, Rwanda, Burundi	Trop. E. Africa
Morella salicifolia (A. Rich.) Verdc. & Polhill	Sudan, Uganda, Congo, Rwanda, Burundi, Ethiopia, Saudi Arabia, Yemen	Trop. E. Africa
Morella salicifolia subsp. *mildbraedii* (Engl.) Verdc. & Polhill	Uganda, Kenya, Congo, Rwanda, Burundi	Trop. E. Africa
Morella salicifolia subsp. *kilimandscharica* var. *kilimandscharica* (Engl.) Verdc. & Polhill	Kenya, Tanzania, Zambia, Malawi	Trop. E. Africa

Species	Distribution	Region
Morella salicifolia subsp. *kilimandscharica* var. *goetzei* (Engl.) Verdc. & Polhill	Tanzania	Trop. E. Africa
Morella salicifolia subsp. *meyeri-johannis* (Engl.) Verdc. & Polhill	Kenya , Tanzania	Trop. E. Africa
Morella spathulata (Mirb.) Verdc. & Polhill	Madagascar (east coast), Tanzania (doubtful)	Africa/Madagascar
Morella rugulosa (Baill.) J. Herbert ined.	Madagascar	Madagascar
Morella phillyreifolia (Baker) J. Herbert ined.	Madagascar	Madagascar
Morella bojeriana (Baker) J. Herbert ined.	Madagascar	Madagascar
Morella dentulata (Baill.) J. Herbert ined.	Madagascar	Madagascar
Morella dentulata var. *comorensis* (A. Chev.) Leroy (combination not yet made)	Comores	Madagascar
Morella madagascariensis (Leroy) J. Herbert ined.	Madagascar	Madagascar
Morella esculenta (Buch.-Ham. ex D. Don) I. M. Turner	India, Nepal, Bhutan, China, Indo-China, Malesia inc. New Guinea	China/Malesia
Morella adenophora (Hance) J. Herbert ined.	China, Taiwan	China
Morella rubra Loureiro	Japan, Taiwan, Phillipines, China	China/Japan
Morella nana (A. Chevalier) J. Herbert ined.	China	China
Morella javanica (Blume) I. M. Turner	Malesia except Malay peninsula	Malesia

APPENDIX 2

Distribution Maps

Maps are based on data from herbarium specimens consulted during this study and published floras (see Chapter 1 for references)

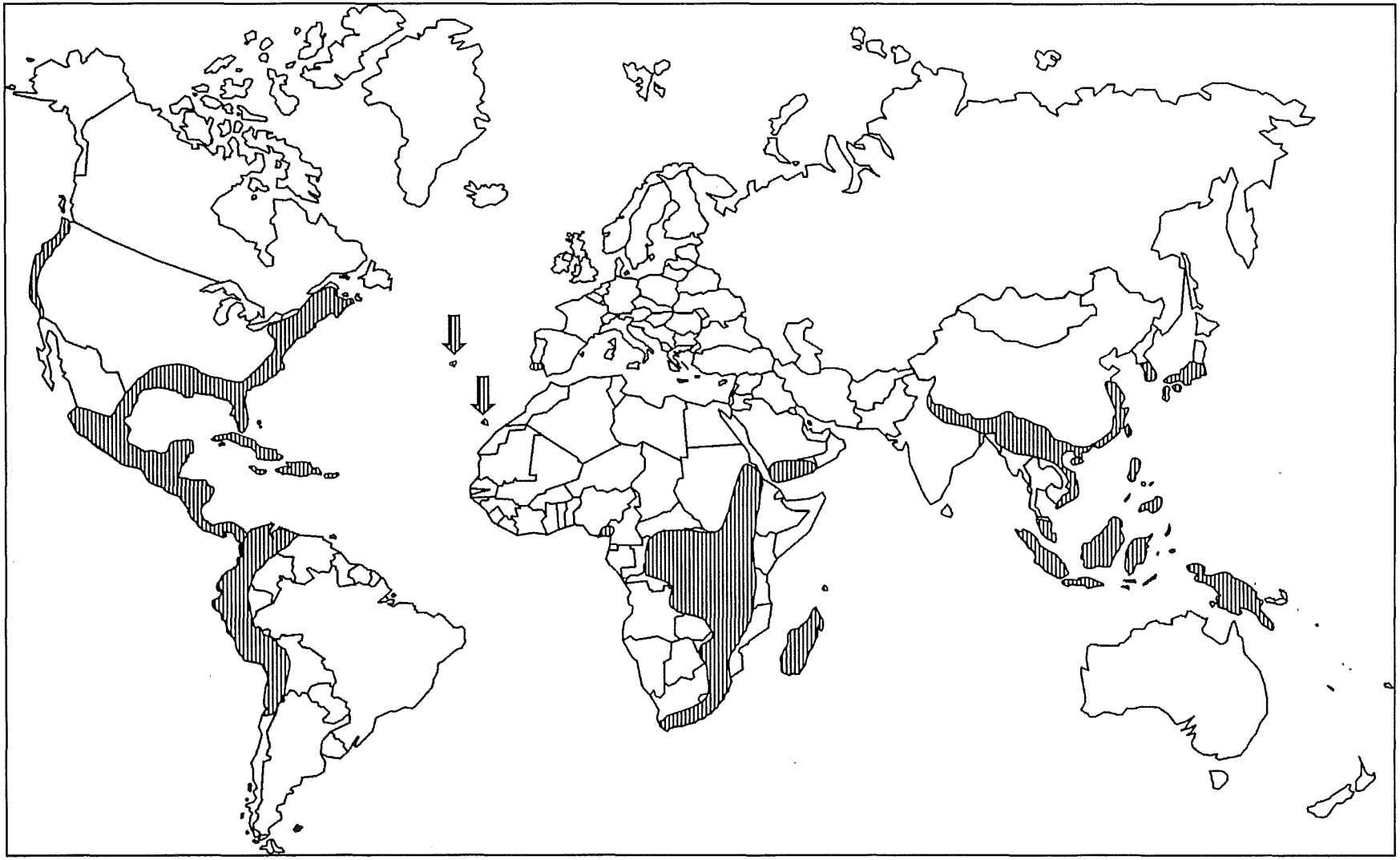
MAP C. Worldwide distribution of *Comptonia* and *Canacomyrica*

Key:

Comptonia

Canacomyrica

MAP B. Worldwide distribution of *Morella*



Key:

Morella

MAP A. Worldwide distribution of *Myrica*

Key:

Myrica

APPENDIX 3

Sequence alignments for Chapter 2

DAMAGED TEXT IN ORIGINAL

quence alignment for the Combined Fagales Data set

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                *[ITS start]
                         1         2         3         4         5         6
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rica            CAACGAACCCCGGCGCGGACTGCGCCAAGGAATTTCAACAAAAGAGTGCCTCTGATCGCC
nacomyrica      CAACGAACCCCGGCGCGGAATGCGCCAAGGAACATGAACATAAGAGTGCCTCCAGCCGCC
mptonia         CAACGAACCCCGGCGCGGACTGCGCCAAGGAACTTCAACAAAAGAGTGCCTCCGGTCGCC
nus             CAACGTACCCCGGCGCGGTCTGCGCCAAGGAACATGAACGAAAGAGTGCCTCCGGTAGCC
rpinus          CAACGAACCCCGGCGCGGTCTGCGCCAAGGAACTTCAATTAAAGAGTGCCTCCGGTCGCC
glans           CAATGAACCCCGGCGCGGTCTGCGCCAAGGAACTTAAACAA-GGAGTAACCACGGGCGCC
piptelea        CAACGAACCCCGGCGCGGACCGCGCCAAGGAA-TTAAAACGAAAGAGTACCTGCGGCCGC
suarina         ???????????????GGCGCGTCCGCGCCAAGGAAACCAAAAAACGCGAGGGCCTCGGGCCC-
gus             AACCGAACCCCGGCGCGGAATGTGCCAAGGAACTGAAACCAAAGAGCGTCGCCGGCCGCC
ercus           AACCGAACCCCAGCGCGGAACGCGCCAAGGAAATCTAACCAAGAGAGCCACGCTGGAGGC

                                                                  1
                         7         8         9         0         1         2
                1234567890123456789012345678901234567890123456789012345678901234567890
rella           C-CGGAAACGGTGTGCGT--CGGATGGGACGTCTTGAC-TGTTATACAAAAACGACTCTC
rica            C-CGGAAACGGTGTGCGT--CGGTTGGGACGTCTTGAC-TGTTATACAAAA-CGACTCTC
nacomyrica      C-CGGAAACGGTGTGCGCGCCGGTTGGGACGTCTTGACTTGTATTACAAAA-CGACTCTC
mptonia         C-CGGAAACGGTGTGCGT--CGGTTGGGACGTCTTGAC-TGTTATACAAAA-CGACTCTC
nus             T-CGGAAACGTTGCGCTTGCCGGAGGCGAA-TCTTGTC---TAAAACCATAACGACTCTC
rpinus          T-CGGAAACGGTGCGCGTGTCGGAGGCGAA-TCTTGTA---CAAAACCACAACGACTCTC
glans           C-CGGAAACGGTGTGCGTGTCGTTGGTGACGTCTT--TACCATGATACATAACGACTCTC
piptelea        CCCGGAGACGGTGGCGAGTCGTCGGTGATGTCTTT----TCTTGATA-CATACGACTCTC
suarina         ---GGAC-------------------------GAAAAGAGTATATTCAAAA-CCGATCTC
gus             T-CGGACACGATGTGCGT-GCCAGCGTCGACGTCTTGTATTTATC-CAAAA-CGACTCTC
ercus           CCCGGACACGGTGTGCC--CCCGACGTCGGCGCTTTACGAATTATTCAAAA-CGACTCTC

                         3         4         5         6         7         8
                1234567890123456789012345678901234567890123456789012345678901234567890
rella           GGCAACGGATATCTCGGCTCTCGCA-TCGATGAAGAACGTAGCGAAATGCGATACTTGGT
rica            GGCAACGGATATCTCGGCTCTCGCA-TCGATGAAGAACGTAGCGAAATGCGATACTTGGT
nacomyrica      GGCAACGGATATCTCGGCTCTCGCA-TCGATGAAGAACGTAGCGAAATGCGATACTTGGT
mptonia         GGCAACGGATATCTCGGCTCTCGCA-TCGATGAAGAACGTAGCGAAATGCGATACTTGGT
nus             GGCAACGGATATCTCGGCTCTCGCA-TCGATGAAGAACGTAGCGAAATGCGATACTTGGT
rpinus          GGCAACGGATATCTCGGCTCTCGCA-TCGATGAAGAACGTAGCGAAATGCGATACTTGGT
glans           GGCAACGGATATCTCGGCTCTCGCA-TCGATGAAGAACGTAGCGAAATGCGATACTTGGT
piptelea        GC-AACGGATATCTCGGCTCTCGCA-TCGATGAAGAACGTAGCGAAATGCGATACTTGGT
suarina         GGCAACGGATATCTCGGCTCTCGCAATCGATGAAGAACGTAGCGAAATGCGATACTTGGT
gus             GGCAACGGATATCTCGGCTCTCGCA-TCGATGAAGAACGTAGCGAAATGCGATACTTGGT
ercus           GGCAACGGATATCTAGGCTCTCGCA-TCGATGAAGAACGTAGCGAAATGCGATACTTGAT
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                                 2
                       9         0         1         2         3         4
              1234567890123456789012345678901234567890123456789012345678901234567890
rella         GTGAATTGCAGAATCCCGCGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCAT
rica          GTGAATTGCAGAATCCCGCGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCGT
nacomyrica    GTGAATTGCAGAATCCCGCGAATCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCGT
mptonia       GTGAATTGCAGAATCCCGCGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCGT
nus           GTGAATTGCAGAATCCCGCGAATCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCAC
rpinus        GTGAATTGCAGAATCCCGCGAATCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCAT
glans         GTGAATTGCAGAATCCCGCGAATCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCAT
oiptelea      GTGAATTGCAGAATCCCGCGAATCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCAT
suarina       GTGAATTGCAGAATCCCGCGAATCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCAC
gus           GTGAATTGCAGAATCCCGTGAATCATCGAGTCTTTGAACGCAAGTTGCGCCCGACGCCAT
ercus         GTGAATTGCAGAATCCCGCGAATCATCGAGTTTTTGAACGCAAGTTGCGCCTGAAGCCAT

                                                                 3
                       5         6         7         8         9         0
              123456789012345678901234567890123456789012345678901234567890
rella         TTGGCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCTTGGCCTCCCGTGAGC
rica          TTGGCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCTTGGCCTCCCGTGAGG
nacomyrica    TTGGCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCTTGGCCTCCCGTGCGC
mptonia       TTGGCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCTTGGCCTCCCGTGAGC
nus           CTGGCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCTTGGCCTCCCGTGGGC
rpinus        CTGGTCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTCGCCCCTTGGCCTCCCGTGAGC
glans         TCGGCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCTTGGCCTCCCGTGCGC
oiptelea      TCGGCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCTTGGCCTCCCGTGCGC
suarina       CCGGCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCTTCGGGGTGCGGACG
gus           TCGGCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCTTGGCCTCCCGTGGGC
ercus         TCGGCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCTTGGCCTCCCGTGCGT

                       1         2         3         4         5         6
              123456789012345678901234567890123456789012345678901234567890
rella         CAGTTCTCGC------------------GGTTAGCCTAAATACG--AGT-CCTCGG-CGA
rica          TAGTTCTCGC------------------GGTTAGCCTAAATACG--AGT-CCTCGG-CGA
nacomyrica    TATTGCTCGC------------------GGTTAGCCTAAACATG--AGT-CCTCGG-CGA
mptonia       TAGTTCTCGC------------------GGTTAGCCTAAATACG--AGT-CCTCGG-CGA
nus           TGATGCCTGC------------------GGCTGGCCTAAAAACG--AGT-CCTCGG-CGA
rpinus        TTCCACTTGC------------------GGTTGGCCTAAAAGCG--AGT-CCTAGG-CGA
glans         TTKTGCTCGC------------------GGTTAGCCTAAAAGTG--AGT-CCTAGG-CGA
oiptelea      TGTAGCTCGC------------------GGTTAGCCTAAAAGTG--AGT-CTTAGG-CGA
suarina       CTGGCCTCCCGTGCGCGGTCCCGCGCGGTTGGCCCAAATGCCGCAGTCCCTCGGGCGA
gus           CTGTGCTCGC------------------GGTTAGCCTAAAAAGG--AGT-CCTCGG-CGA
ercus         GCCTGCGCGC----------------GCGGTTAGCCCAAAGCG--AGT-CCTCGG-CGA
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                                            4
                          7         8         9         0         1         2
                 123456789012345678901234567890123456789012345678901234567890
rella            C--GAGCGCCACGACAATCGGTGGTTGATAAAGCCCTCGTTTCCCGTCGTGCGTGCCTCG
rica             C--GAGCGCCACAACAATCGGTGGTTGATAAAGCCCTCGTTTCCAGTCGTGCGCGCCTCG
nacomyrica       T--GAACGCCACGACAATCGGTGGTTGTCAA-GCCCTCGTGTCCCGTCGTGAGCGTCGCG
mptonia          C--GAGCGCCACGACAATCGGTGGTTGATAAAGCCCTCGTTTCCCGTCGTGCGCGCCTTG
nus              C--GAGCGCCACGACAATCGGTGGTTGACAAA-CCCTCGTGACCCGTCGTGCGTGCATCG
rpinus           C--GAGCGCCACGACAATCGGTGGTTGCCAAAACCCTCGTGTCCCGTCGTGCGTGCCTCG
glans            C--GAGCGCCACGACAATCGGTGGTTGAGAA-ACCCTCGTGACCCGTCGTGTGTCGCCCG
oiptelea         C--GAGCGCCACGACAATCGGTGGTCGAAAAAACCCTCGTTTCCCGTCGTGTGTGTCCCG
suarina          ACCGAGCGCCACGACAATCGGTGTGGTTGAGAGACCTCGCGTCCCGTCGTGCGCGCCTCG
gus              C--GAGCGCCACGACAATCGGTGGTTGATTA-GACCTCGGTCCCCGTCGTGCGCGTCTGG
ercus            C--GAGCGCCACGACAATCGGTGGTTTTCTTG-CCCTCGTTCCTCGTCGTGCGCGCCCCG


                       *[trnL-F starts]
                          3         4         5         6         7         8
                 123456789012345678901234567890123456789012345678901234567890
rella            TCTCCCTTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTC
rica             TCGCCCTTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTC
nacomyrica       TCGTCTTTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCTGTTTTC
mptonia          TCGCCCTTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTC
nus              TCGCTCTTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCTGTTTTC
rpinus           TTGCTCTTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCTGTTTTC
glans            TCGCTG??????????????????????????????????????????????????????
oiptelea         TCGCTC??????????????????????????????????????????????????????
suarina          TCGCCCTTCAGAGAAACCCGGGAATTCTAAATGGGCAATCCTGAGCCAAATCCTGTTTTC
gus              TCGCCATTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCTATTTTC
ercus            TCGCCCTTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCTATTTTA


                                    5
                          9         0         1         2         3         4
                 123456789012345678901234567890123456789012345678901234567890
rella            TGAAAA-CAAATAA-----------GGGTTCATAAA---GCGAGAATCAAAAA-------
rica             TGAAAA-CAAATAA-----------GGGTTCATAAA---GCGAGAATCAAAAA-------
nacomyrica       CGAAAA-CAAATTAA----------GGGTTCAGAAA---GCGAGAATCAAAAA-------
mptonia          TGAAAA-CAAATAA-----------GGGTTCATAAA---GCGAGAATCAAAAA-------
nus              CGAAAA-CAAATAAAACAAATTGAAGGGTTCATAAA---GCGAGACTAAAAA--------
rpinus           CCAAAA-CAAATAAAACAAATTTAAGGGTTCATAAA---GCGAGAATAAAAAA-------
glans            ????????????????????????????????????????????????????????????
oiptelea         ????????????????????????????????????????????????????????????
suarina          CGAAAA-CAAATTAA----------GGGTTCATAAA---GCCAAAATAAAAAA-------
gus              CGAAAA-CAAATAA-----------GGGTTGAGAAGAAAGCAAGAATAAAATAAAATAAA
ercus            CGAAAAACAAATAA-----------GGGTTCAGAAGAAAGCAAGAATAAAAAAA------
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                                                                           6
                          5         6         7         8         9         0
                 123456789012345678901234567890123456789012345678901234567890
rella            -GGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAA-----TGGGGTTGACTTCGT
rica             -GGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAA-----TGGGGTTGACTTCGT
nacomyrica       -GGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAAACAAATGGGGTTAACTTCGT
mptonia          -GGATAGGTGCAGAGACTCAATGGAAGTTGTTCTAACAAA-----TGGGGTTGACTTCGT
nus              -GGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAA-----TTGGGGTTGACTTCGT
rpinus           -GGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAA-----TTGGAGTTGACTTCGT
glans            ????????????????????????????????????????????????????????????
oiptelea         ????????????????????????????????????????????????????????????
suarina          -GGATAGGTGCAGAGACTCAATGGAAGGTGTTCTAACAAA-----TGGGGTTTACTTCTT
gus              AGGATAGGTGCAGAGACTCGATGGAAGCTGTTCTAACAAA-----TGGGGTTGACTTCGT
ercus            -GGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACA-G-----TGGGGTTGACTTCTT


                          1         2         3         4         5         6
                 123456789012345678901234567890123456789012345678901234567890
rella            TCCGTTATT-------------AAAGTAATCCTTCTATCAAAACTACAGAAAGGATGAAG
rica             TCCGTTATT-------------AAAGTAATCCTTCTATCAAAACTACAGAAAGGATGAAG
nacomyrica       TCCGTTATTA----------------AAATCCTTCTATCAAAACTACAGAAAGGATGAAG
mptonia          TCCGTTATT-AAATGAAAGTATAAAGTAATCCTTCTATCAAAACTACAGAAAGGATGAAG
nus              TCCGTTATT-------------AAAGTAATCCTTCTATCAAAACTACAGAAAGGATGAAG
rpinus           TCCGTTAGT-------------AAAGTAATCCTTCTATCAAAACTACAGAAAGGATGAAG
glans            ????????????????????????????????????????????????????????????
oiptelea         ????????????????????????????????????????????????????????????
suarina          TCCGTTATTAATATTTAATATTAAAGCCACCCTTCTATCAAAACTACAGAAAGGAT-AAC
gus              TACGTTATTAAAT--------TCAAGTAATCCTTCTATCAAAACTACAGAAAGCATGAAG
ercus            TACGTTATTACAT--------TAACGTAATCCTTATATCAAAACTACAGAAAGGA-----


                                                         7
                          7         8         9         0         1         2
                 123456789012345678901234567890123456789012345678901234567890
rella            GATAAACCTATATACATACGTATACG----------TACTGAAATACTATC-CCAACTAA
rica             GATAAACCTATATACATACGTATACG----------TACTGAAATACTATC-CCAACTAA
nacomyrica       GATAAACCTATATACATACGTATACG----------TACTGAAATACTATC-CCAACTAA
mptonia          GATAAACCTATATACATACGTATACG----------TACTGAAATACTATC-CCAACTAA
nus              GATAACCGTATATACATACGTATACG----------TACTGAAATACTATC-TCAAAAAA
rpinus           GATAACCGTATATACATACGTATACG----------TCCTGAAATACTATC-TCAAAAAA
glans            ????????????????????????????????????????????????????????????
oiptelea         ????????????????????????????????????????????????????????????
suarina          TATATCTACATATGTATACATCTACATATGTATACGTACTGAAATATTATCGTCAAATAA
gus              GATAAACCTATATACATACGTATATG----------TACTGAAATCCTATC-TCAAATGA
ercus            --TAAACCTATATACATACGTATATG----------TACTGAAATCCTATC-TCAAATGA
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                    3         4         5         6         7         8
           12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella    TTAATAACGACCTGAATCTTTATTTCTTTTTTTCTATTTCATATTTATA--------TAA
Myrica     TTAATAACGACCTGAATCTTTATTTCTTTTTTTCTATTTCATATTTATA--------TAA
Canacomyrica TTAATAACGACCTGAATCTTTATTTCTTTTTTTCTATTTCATATTTATA--------TAA
Comptonia  TTAATAACGACCTGAATCTTTATTTCTTTTTTTCTATTTCATATTTATA--------TAA
Alnus      TTAATGACGACCTGAATCTTTATTTCTTTTTTTTATATTCTTTTTTTTATATTTAAATAA
Carpinus   TGAATGACGACCCGAATCTTTATTTCTTTTTTTATATTTATA---------------TAA
Juglans    ??????????????????????????????????????????????????????????????
Rhoiptelea ??????????????????????????????????????????????????????????????
Casuarina  TTAATGACGACCCGACTCTTTTCTTTTTTTTTTTTTTTTTTTNGGGTTTATTTATATAAA
Fagus      TTAATGACGACCGGAATCTTTATTTATTTATATTTCTATAAA--------------TAAA
Quercus    TTAATGACGACCCGAATCTTTATTTATTTATATTTCTATAAATAAGAAA-------TAAA

                              8
                    9         0         1         2         3         4
           12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella    AAACT----GAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAAGAATCGAATTT----
Myrica     AAACG----GAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAAGAATCGAATTT----
Canacomyrica AAACT----AAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAAGAATCGAATCGAATA
Comptonia  AAACT----GAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAAGAATCGAATTT----
Alnus      AAACTGAATGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAAGAATCGAATATTTAT
Carpinus   AAACTGAATGAAAGAGTTGGTGTGAATCGATCCAAATTGAAGAAAGAATCGAATATTTAT
Juglans    ??????????????????????????????????????????????????????????????
Rhoiptelea ??????????????????????????????????????????????????????????????
Casuarina  AACC-----GAAAGAGTTGTTGTGAATCGATCCAAATTGAAAAAAGAATCGAATATTTAT
Fagus      TACC-----GAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAAGAATCGAATATTTAT
Quercus    AATC-----GAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAAGAATCGAATATTTAT

                                                                      9
                    5         6         7         8         9         0
           12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella    ------AATCAAATTATTTACTCCA-CATAGTCTGATAGATCTTTTGAAGAACTAATTAA
Myrica     ------AATCAAATTATTTACTCCA-CATAGTCTGATAGATCTTTTGAAGAACTAATTAA
Canacomyrica TTTATTAATCAAATTATTTACTCCA-CATAGTCTGATAGATCTTTTGAAGAACTAATTAA
Comptonia  ------AATCAAATTATTTACTCCA-CATAGTCTGATAGATCTTTTGAAGAACTAATTAA
Alnus      T-----AATCAAATTATTTACTCCATCATAGTCTGATAGATCTTTTGAAGAACTAATTAA
Carpinus   T-----AATCAAATTAGTTACTCCATCATAGTCTGATAGATCTTTTGAAGAACTAATTAA
Juglans    ??????????????????????????????????????????????????????????????
Rhoiptelea ??????????????????????????????????????????????????????????????
Casuarina  T-----AATCAAATTCTTTACTCCATCATAGTCTGATAGATCTTTTGAAGAACTAATTAA
Fagus      T-----AATTCAATTATTTACTCCATCATAGTCTGATAGATCTTTTGAAGAACTAATTTA
Quercus    T-----AATAAAATTTA-------------------------------------------
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                    1         2         3         4         5         6
           12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella    TCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAACAATGAAATTT
Myrica     TCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAACAATGAAATTT
Canacomyrica TCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCAACAACAATGAAATTT
Comptonia  TCGGATGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAACAATGAAATTT
Alnus      TCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAACAATGAAATTT
Carpinus   TCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAACAATGAAATTT
Juglans    ??????????????????????????????????????????????????????????????????
Rhoiptelea ??????????????????????????????????????????????????????????????????
Casuarina  TCGGACGAGAATAAAGATAGAGTCCCATTTTACATGTCAATATCGACAACAATGAAATTT
Fagus      TCGTACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAAGAATGAAATTT
Quercus    TCGTACGAGAATAAAGATAGAGTCCCATTCCACACGTCAATACCGACAAGAATGAAATTT

                                                                  1
                                                                  0
                    7         8         9         0         1         2
           12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella    ATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAAGTCCCTCTATCCCC
Myrica     ATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAAGTCCCTCTATCCCC
Canacomyrica ATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAAGTCCCTCTATCCCC
Comptonia  ATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAAGTCCCTCTATCCCC
Alnus      ATAGTAAGAGG????????????????????????????????????????????????????
Carpinus   ATAGTAAGAGG????????????????????????????????????????????????????
Juglans    ??????????????????????????????????????????????????????????????????
Rhoiptelea ??????????????????????????????????????????????????????????????????
Casuarina  ATAGTAAGAGGAAAATCCGTCGATTTTAGAAATCGTGAGGGTTCAAGTCCCTCTATCCCC
Fagus      ATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAAGTCCCTCTATCCCC
Quercus    ATAGGAAGAGGAAAATCCGTCGACTTTA??????????????????????????????????

                    3         4         5         6         7         8
           12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella    AAAAA---GGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTGCTCTTTTCATT
Myrica     AAAAA---GGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTGCTCTTTTCATT
Canacomyrica AAAAA---GGCCCGTTTCCGTTTGACTCCGTAATTCTTTATCCAATCTGCTCTTTTCGTT
Comptonia  AAAAA---GGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTGCTCTTTTCATT
Alnus      ??????????CCCGTTT------GACTCCGTAATTATTTACCCGATCTGCTCTTTTCGTT
Carpinus   ??????????CCCGTTT------GCCTCCGTAATTATTTACCCGAGCTGCTCTTTTCGTT
Juglans    ????????????????TT------GACTCCGTAATTATTTATCCGATCTGCTCTTTTCTTT
Rhoiptelea ????????????????TT------GA-TCCGTAATTATTTATCCGATCTGCTCTTTTATTT
Casuarina  AAACAAAAGGCCCCTTC------GACTCCGTAATTATTTATCCGATCTGTTCTTTTATTT
Fagus      AAAAA---GGCCCGTTT------GACTCCCTAATTATTTATCCGATCTTCTCTTTTCGTT
Quercus    ??????????????????????????????????????????????????????????????????
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                          1
                 9        0         1         2         3         4
                1234567890123456789012345678901234567890123456789012345678901234567890
Morella         ATTTTCATTAGCGGTTTCAAATT------CGTTATGTTTCTCGCTGATTCTACTCTTTTA
Myrica          ---------AGCGGTTTCAAATT------CGTTATGTTTCTCGCTGATTCTACTCTTTTA
Canacomyrica    ---------AGCGGTTTCAAATT------CGTTATGTTTCTCGCTGATTCTACTCTTTTA
Comptonia       ---------AGCGGTTTCAAATT------CGTTATGTTTCTCGCTGATTCTACTCTTTTA
Alnus           ---------AGTGGTTTAAAATT------CGTTATGTTTCTCACAGATTCTACTCTTTTC
Carpinus        ---------AGCAGTTTAAAATT------CGTTATGTTTCTCACAGATTCTACTCTTTTA
Juglans         TCGTT----AGCGGTTTCAAATT------TGTTATGTTTCTCACTGATTTGACTCTTTTA
Rhoiptelea      TCGTT----AGCGGTTTAAAATT------CGTTATGTTTCTCACTGATTTTACTCTTTTA
Casuarina       TCGTT----AGCGGTTTAAAAAA--AATTCGTTATGTTTTTCGCGGATTCTACTCTTTTA
Fagus           TTGTT----AGCGTTTTCAAATTGGAATTCGTTATGTTTTTCAATCATTCTAATCTTTTA
Quercus         ??????????????????????????????????????????????????????????

                                                                          2
                 5         6         7         8         9         0
                1234567890123456789012345678901234567890123456789012345678901234567890
Morella         CAAATGGATC-GATTGTAAATCGTTTTTTCTTATTA---------ACAAGATTTGTGATA
Myrica          CAAATGGATC-GATTGTAAATCGTTTTTTCTTATTA----------CAAGATTTGTGATA
Canacomyrica    CAAATGGATC-GATTGTCAATTGTTTTTTCTTATTA----------CAAGATTTGTGATA
Comptonia       CAAATGGATC-GATTGTAAATTGTTTTTTCTTATTA----------CAAGATTTGTGATA
Alnus           CAAATGGATCCGATTGGAAATTGTTTTTTCTTATTA----------CAATATTTGTGATA
Carpinus        CAAATGGATCCGATTGGAAATTGTTCTTTCTTATTA----------CAATATTTGGATAT
Juglans         CAAATGGAGCCAATTGGGAA-TTTTTTTTCTTATTTTTTCTTCTTACAATATTTGTGATA
Rhoiptelea      CAAATGGAGCCGATTGGAATTTTTTTTTTCTTATTTTTTCTTATTACAATATTTGTGATA
Casuarina       CAAATGGATCCGATTGGAAATTGTTTTTTATTATCA----------CAATATTTGTGATA
Fagus           CAAATGGATCTGATTGTAAAT----------------------------------------
Quercus         ??????????????????????????????????????????????????????????

                         1         2         3         4         5         6
                1234567890123456789012345678901234567890123456789012345678901234567890
Morella         TATATGA-------TACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
Myrica          TATATGA-------TACACGTACAAATGAACATCCTTGAAGAAGGTATCCCTACTTCAAA
Canacomyrica    TATATGA-------TACACGTACAAATGAACATCCTTGAATAAGGTACCCCTACTTCAAA
Comptonia       TATATGA-------TACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
Alnus           TATATGA-------TACACGTACAATTGAACATCTTTGAGTAAGGTATCCCCACTTCAAA
Carpinus        ATTGTAATATATAATACACGTACAATTGAACATCTTTGAGTAAGGTATCCCCGCTTCAAA
Juglans         TATATGA-------TACAAGTACAAATAAACATCTTTGAGTAAGGTATCCCCACTTCAAA
Rhoiptelea      TATATGA-------TACAAGTACAAATAAACATCTTTGAGTAAGGTATCCCCACTTCAAA
Casuarina       TATATGA-------TACACCCACAAATGAACATCTTTGAGTAAGGTATCCCCACTTCAAA
Fagus           ------------------------------------------------------------
Quercus         ??????????????????????????????????????????????????????????
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                                                       3
                             7         8         9         0         1         2
                    123456789012345678901234567890123456789012345678901234567890
Morella             TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
Myrica              TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
Canacomyrica        TTTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
Comptonia           TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATACAGT
Alnus               TTTTAATGATTAACAATA-----CATATCATCTCTGATACTGTACTAAAACTTATAAAGT
Carpinus            TTTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
Juglans             TTTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATCAAGT
Rhoiptelea          TTTTAATGATTAACAATACAATACATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
Casuarina           TATTAATGATTAACAATA-----TATATCATCTCCGGTACTGTACTAAAACTTATAAAGT
Fagus               ------------------------------------------------------------
Quercus             ????????????????????????????????????????????????????????????

                             3         4         5         6         7         8
                    123456789012345678901234567890123456789012345678901234567890
Morella             TTTCTTTTT----GAAAATACAAGAAATTCCAGGGTCTAGATAAGACTTTGTAA--TTTT
Myrica              TTTCTTTTT----AAAAACCCAAGAAATTCCAG---------AAGACTTTGTAA--TTTT
Canacomyrica        TTTCTTTTT----GAAAATCCAAGAAATTCCAGGGTCTAGATAAGACTTTGTCA--TTTT
Comptonia           TTTCTTTTT----GAAAATCTAAGAAATTCCAGGGTCTAGATAAGACTTTGTCA--TTTT
Alnus               TTTCTTTTTTTTTGAAGATACAAGAAATCTCAGGGTCTAGATAAAACTTTGTAATATTTT
Carpinus            CTTTTTT------GAAGATACAAGAAATTTCAGGGTCTAGATAAAACTTTGTAATATTTT
Juglans             TTTCTTTTT----AAAGATCCAAGAAATTCCAGGATCTAGATAAGATTTTGTAATATTTA
Rhoiptelea          TTTCTTTTT----GAAGATCCAAGAAATTCCAGGATCTAGATAAGACTTTGTAATATTTT
Casuarina           TTTCTTTTTTTT-GAAGATACAAGAAATTTCAGGGTCTAGGTTTGTGATAT-----CTTT
Fagus               --------------------------------------------------------CTTT
Quercus             ????????????????????????????????????????????????????????????

                                       4
                             9         0         1         2         3         4
                    123456789012345678901234567890123456789012345678901234567890
Morella             TTTT-GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTG
Myrica              TTTT-GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTG
Canacomyrica        TTTT-GTCTTTTTAATTGACATAAACCCAAGTCATCTATTAAAATAAGGATGATGCGCTG
Comptonia           TTTT-GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGC????
Alnus               TTTT-GTCTTTTTAATTGACATAAACCCAAGTCATCTATTAAAATAAGGACGATGTGCCG
Carpinus            TTTT-GTCTTTTTAATTGACATAAACCCAAGTCATCTATTAAGATAAGGATGATGCGCCG
Juglans             TTTT-GTCTTTTTAATTGACATAAACCCAAGTCATCTATTAAAATAAGGATGATACGCCA
Rhoiptelea          TTTTGGTCTTTTTAATTGACATAAACCCAAGTCATCTATTAAAATAAGGATGATACGCCA
Casuarina           TTTATGTCTTTTTAATTGACATAAACCTAAGTCATC????????????????????????
Fagus               TTTT---CTTATTAATTGACATAAACTCAAGTTATCTATTAAAATAAGGATGATGCACCG
Quercus             ????????????????????????????????????????????????????????????
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                    *[rbcL starts]
                                                                                5
                              5         6         7         8         9         0
                    123456789012345678901234567890123456789012345678901234567890
Morella             GT??????????????????????????????????????????????????????????
Myrica              GT??????????????????????????????????????????????????????????
Canacomyrica        GTGATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAGGATACGGATATCTTGG
Comptonia           ??GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAGGATACTGATATCTTGG
Alnus               GTGATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGG
Carpinus            GTGATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACAGATATCTTGG
Juglans             GAGATTATAAATTGACTTATTATACTCCTGAATATCAAACCAAAGATACTGATATCTTGG
Rhoiptelea          GAGATTATAAATTGACTTATTATACTCCTGAATATCAAACCAAAGATACTGATATCTTGG
Casuarina           ??GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGG
Fagus               GTGATTATAAATTGACTTATTATACTCCTGACTATGAAACTAAAGATACTGATATCTTGG
Quercus             ??GATTATAAATTGACTTATCATACTCCTGACTATCAAACCAAAGATACTGATATCTTGG


                             1         2         3         4         5         6
                    123456789012345678901234567890123456789012345678901234567890
Morella             ?????TTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGGGCAGCAGTAG
Myrica              ???????????????CTCCTCTACCTGGTGTTCCGCCTGAGGAAGCAGGGGCAGCAGTAG
Canacomyrica        CAGCGTTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAGGAAGCAGGGGCAGCAGTAG
Comptonia           CAGCGTTCCGAGTAACTCCTCAACCTGGAGTGCCGCCTGAGGAAGCAGGGGCAGCAGTAG
Alnus               CAGCGTTCCGAGTAACTCCTCAACCTGGAGTGCCGCCTGAGGAAGCAGGGGCAGCAGTAG
Carpinus            CAGCGTTCCGAGTAACTCCTCAACCTGGAGTTCCGCCAGAGGAAGCAGGGGCAGCAGTAG
Juglans             CAGCGTTCCGAGTAAGCCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGGGCAGCAGTAG
Rhoiptelea          CAGCGTTCCGAGTAAGTCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGGGCAGCAGTAG
Casuarina           CAGCGTTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAGGAAGCAGGGGCGGCAGTAG
Fagus               CAGCCTTCCGAGTAACTCCTCAACCTGGGGTTCCGCCTGAAGAAGCGGGGGCCGCGGTAG
Quercus             CAGCCTTCCGAGTAACTCCTCAACCTGGAGTTCCGCCCGAGGAAGCAGGGGCCGCGGTAG


                                                          6
                             7         8         9        0         1         2
                    123456789012345678901234567890123456789012345678901234567890
Morella             CTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTG
Myrica              CTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTG
Canacomyrica        CTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACGAGTCTTG
Comptonia           CTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTG
Alnus               CTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACTAGTCTTG
Carpinus            CTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACTAGTCTTG
Juglans             CTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTG
Rhoiptelea          CTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTG
Casuarina           CTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTG
Fagus               CTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACTGATGGGCTTACCAGTCTTG
Quercus             CTGCTGAATCTTCCACTGGGACATGGACAACTGTGTGGACTGACGGGCTTACCAGTCTTG
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                    3         4         5         6         7         8
           12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella    ATCGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTA
Myrica     ATCGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTA
Canacomyrica ATCGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAATCAATATA
Comptonia  ATCGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTA
Alnus      ATCGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTA
Carpinus   ATCGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTA
Juglans    ATCGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAATCAATTTA
Rhoiptelea ATCGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAATCAATATA
Casuarina  ATCGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTA
Fagus      ATCGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTA
Quercus    ATCGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAATCAATTTA

                              7
                    9         0         1         2         3         4
           12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella    TTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTA
Myrica     TTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTA
Canacomyrica TTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTA
Comptonia  TTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTA
Alnus      TTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTA
Carpinus   TTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTA
Juglans    TTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTA
Rhoiptelea TTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTA
Casuarina  TTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTA
Fagus      TTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTA
Quercus    TTGCTTATGTAGCTTACCCCTTAGACCTCTTTGAAGAAGGTTCTGTTACTAACATGTTTA

                                                                      8
                    5         6         7         8         9         0
           12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella    CTTCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATT
Myrica     CTTCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATT
Canacomyrica CTTCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATT
Comptonia  CTTCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATT
Alnus      CTTCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATT
Carpinus   CTTCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATT
Juglans    CTTCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATT
Rhoiptelea CTTCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATT
Casuarina  CTTCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATT
Fagus      CTTCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATT
Quercus    CTTCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATT
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                    1         2         3         4         5         6
           1234567890123456789012345678901234567890123456789012345678901234567890
Morella    TGCGAATCCCTACTTCTTATGTTAAAACTTTCCAAGGCCCGCCGCATGGCATCCAAGTTG
Myrica     TGCGAATCCCTACTTCTTATGTTAAAACTTTCCAAGGCCCGCCGCATGGCATCCAAGTTG
Canacomyrica TGCGAATCCCTCCTTCTTATTCTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAAGTTG
Comptonia  TGCGAATCCCTACTTCTTATGTTAAAACTTTCCAAGGCCCGCCGCATGGCATCCAAGTTG
Alnus      TGCGAATCCCTCCTGCTTATTCTAAAACTTTCCAAGGCCCGCCTCACGGCATCCAAGTTG
Carpinus   TGCGAATCCCCACTGCTTATACTAAAACTTTCCAAGGCCCGCCTCACGGCATCCAAGTTG
Juglans    TGCGAATCCCTCCTGCTTATTCTAAAACTTTCCAAGGCCCGCCTCATGGAATCCAAGTTG
Rhoiptelea TGCGAATCCCTCCTGCTTATTCTAAAACTTTCCAAGGCCCGCCTCATGGAATCCAAGTTG
Casuarina  TGCGAATCCCTACTGCTTATTCTAAAACTTTCCAAGGTCCGCCTCATGGCATTCAAGTTG
Fagus      TGCGAATCCCTACTTCTTACGCTAAAACTTTCCAAGGCCCGCCTCACGGCATCCAAGTTG
Quercus    TGCGAATCCCTACTTCTTATTCTAAAACTTTCCAAGGTCCGCCTCATGGCATCCAAGTTG

                                                  9
                    7         8         9         0         1         2
           1234567890123456789012345678901234567890123456789012345678901234567890
Morella    AGAGAGATAAATTAAACAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCCAAAT
Myrica     AGAGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCCAAAT
Canacomyrica AGAGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCCAAAT
Comptonia  AGAGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCCAAAT
Alnus      AGAGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAGCCTAAAT
Carpinus   AGAGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAAT
Juglans    AGAGAGATAAATTAAACAAGTATGGCCGCCCTCTATTGGGATGTACTATTAAACCTAAAT
Rhoiptelea AGAGAGATAAATTAAACAAGTATGGCCGCCCTCTATTGGGATGTACTATTAAACCTAAAT
Casuarina  AGAGAGATAAATTAAACAAGTATGGCCGACCCCTATTAGGATGTACTATTAAACCTAAAT
Fagus      AGAGAGATAAATTAAACAAGTATGCCCGCCCCCTATTGGGATGTACTATTAAACCTAAAT
Quercus    AGAGGGATAAATTAAACAAGTATGGCCGCCCCCTATTAGGATGTACTATTAAACCTAAAT

                    3         4         5         6         7         8
           1234567890123456789012345678901234567890123456789012345678901234567890
Morella    TGGGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTG
Myrica     TGGGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTG
Canacomyrica TGGGATTATCTGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTG
Comptonia  TGGGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTG
Alnus      TGGGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGAGGTGGGCTTG
Carpinus   TGGGATTATCCGCTAAGAATTATGGTAGAGCGGTTTATGAATGTCTCCGCGGTGGGCTTG
Juglans    TGGGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTG
Rhoiptelea TGGGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTG
Casuarina  TGGGATTATCTGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTG
Fagus      TGGGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTG
Quercus    TGGGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTG
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                                  2
                                  0
                          9       0       1       2       3       4
                 123456789012345678901234567890123456789012345678901234567890
Morella          ATTTTACCAAAGATGATGAGAACGTGAATTCCCAACCGTTTATGCGTTGGAGAGATCGTT
Myrica           ATTTTACCAAAGATGATGAGAACGTGAATTCCCAACCGTTTATGCGTTGGAGAGATCGTT
Canacomyrica     ATTTTACCAAAGATGATGAGAACGTGAATTCCCAACCGTTTATGCGTTGGAGAGACCGTT
Comptonia        ATTTTACCAAAGATGATGAGAACGTGAATTCCCAACCGTTTATGCGTTGGAGAGATCGTT
Alnus            ATTTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTT
Carpinus         ATTTTACCAAAGATGATGAAAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTT
Juglans          ATTTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTT
Rhoiptelea       ATTTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTT
Casuarina        ATTTTACCAAAGATGATGAAAATGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTT
Fagus            ATTTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTT
Quercus          ATTTTACCAAAGATGATGAGAACGTTAATTCCCAACCATTTATGCGTTGGAGAGACCGTT

                                                                          1
                          5       6       7       8       9       0
                 123456789012345678901234567890123456789012345678901234567890
Morella          TCCTATTTTGTGCCGAAGCTCTTTATAAAGCGCAGACTGAAACAGGTGAAATCAAAGGAC
Myrica           TCCTATTTTGTGCCGAAGCTCTTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGAC
Canacomyrica     TCGTATTTTGTGCCGAAGCTCTTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGC
Comptonia        TCCTATTTTGTGCCGAAGCTCTTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGAC
Alnus            TCCTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGC
Carpinus         TCCTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGC
Juglans          TCCTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACCGGTGAAATCAAAGGGC
Rhoiptelea       TCCTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACCGGTGAAATCAAAGGGC
Casuarina        TCGTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCCGAAACAGGTGAAATCAAAGGGC
Fagus            TCCTGTTTTGTGCCGAAGCTCTTTATAAAGCGCAGGCGGAAACCGGTGAAATCAAAGGGC
Quercus          TCGTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGC

                          1       2       3       4       5       6
                 123456789012345678901234567890123456789012345678901234567890
Morella          ATTACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATGAAAAGGGCTGTATTTGCCA
Myrica           ATTACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATTAAAAGGGCTGTATTTGCCA
Canacomyrica     ATTACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATTAAAAGGGCTGTATTTGCCA
Comptonia        ATTACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATTAAAAGGGCTGTATTTGCCA
Alnus            ATTACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATGAAAAGGGCTGTATTTGCCA
Carpinus         ATTACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATGAAAAGGGCTGTATTTGCCA
Juglans          ATTACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCA
Rhoiptelea       ATTACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCA
Casuarina        ATTACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATGAAAAGGGCTATATTTGCCA
Fagus            ATTACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCA
Quercus          ATTACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCA
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                                                                  2
                          7         8         9         0         1         2
                 123456789012345678901234567890123456789012345678901234567890
Morella          GAGAATTGGGAGTTCCTATCGTAATGCACGACTACTTAACCGGGGGATTCACGGCAAATA
Myrica           GAGAATTGGGAGTTCCTATCGTAATGCACGACTACTTAACCGGGGGATTCACGGCAAATA
Canacomyrica     GAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATA
Comptonia        GAGAATTGGGAGTTCCTATCGTAATGCACGACTACTTAACCGGGGGATTCACGGCAAATA
Alnus            GAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATA
Carpinus         GAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATA
Juglans          GAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTTACTGCAAATA
Rhoiptelea       GAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATA
Casuarina        GAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATA
Fagus            GAGAATTGGGAGTTCCTATCGTAATGCACGACTACTTAACCGGGGGATTCACTGCAAGTA
Quercus          GAGAACTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGGTTCACTGCAAATA


                          3         4         5         6         7         8
                 123456789012345678901234567890123456789012345678901234567890
Morella          CTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGC
Myrica           CTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGC
Canacomyrica     CTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGC
Comptonia        CTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGC
Alnus            CTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGC
Carpinus         CTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGC
Juglans          CTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGC
Rhoiptelea       CTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGC
Casuarina        CTAGTTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGC
Fagus            CTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCACCGTGCAATGC
Quercus          CTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCCATGC


                                   3
                          9         0         1         2         3         4
                 123456789012345678901234567890123456789012345678901234567890
Morella          ATGCAGTTATTGATAGACAGAAAAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGT
Myrica           ATGCAGTTATTGATAGACAGAAAAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGT
Canacomyrica     ATGCAGTTATTGATAGACAGAAAAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGT
Comptonia        ATGCAGTTATTGATAGACAGAAAAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGT
Alnus            ATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGT
Carpinus         ATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGTGTACTAGCTAAAGCGT
Juglans          ATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGT
Rhoiptelea       ATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGT
Casuarina        ATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGTGTATTAGCTAAAGCGT
Fagus            ATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGT
Quercus          ATGCAGTTATTGATCGACAGAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCAT
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                                                                      4
                5         6         7         8         9         0
           12345678901234567890123456789012345678901234567890123456789
Morella    TACGTATGTCTGGTGGGGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGG
Myrica     TACGTATGTCTGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGG
Canacomyrica TACGTATGTCTGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGG
Comptonia  TACGTATGTCTGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGG
Alnus      TACGTATGTCTGGTGGAGATCATATTCACGCGGGTACCGTAGTAGGTAAACTTGAAGGGG
Carpinus   TACGCATGTCTGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGG
Juglans    TACGTATGTCTGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGG
Rhoiptelea TACGTATGTCTGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGG
Casuarina  TACGTCTATCCGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGG
Fagus      TACGTATGTCTGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGG
Quercus    TACGTATGTCTGGTGGAGATCATATTCATGCCGGTACCGTAGTAGGTAAACTTGAAGGGG

                1         2         3         4         5         6
           12345678901234567890123456789012345678901234567890123456789
Morella    AAAGAGACATCACTTTAGGCTTTGTTGATTTACTACGCGATGATTTTATTGAAAAAGATC
Myrica     AAAGAGACATCACTTTAGGCTTTGTTGATTTACTACGCGATGATTTTATTGAAAAAGATC
Canacomyrica AAAGAGACATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTATTGAAAAAGATC
Comptonia  AAAGAGACATCACTTTAGGCTTTGTTGATTTACTACGCGATGATTTTATTGAAAAAGATC
Alnus      AAAGAGAGATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATC
Carpinus   AAAGAGAGATCACTTTAGGCTTTGTTGATTTACTGCGTGATGATTATATTGAAAAAGATC
Juglans    AAAGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTGTTGAAAAAGATC
Rhoiptelea AAAGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTGTTGAAAAAGATA
Casuarina  AAAGAGATATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATC
Fagus      AAAGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATC
Quercus    AAAGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATC

                                                  5
                7         8         9         0         1         2
           12345678901234567890123456789012345678901234567890123456789
Morella    GAAGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGG
Myrica     GAAGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGG
Canacomyrica GAAGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTTTGCCCGTGG
Comptonia  GAAGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGG
Alnus      GAAGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGG
Carpinus   GAAGCCGCGGTATTTATTTTACTCAAGATTGGGTCTCTCTACCAGGTGTTCTGCCCGTGG
Juglans    GAAGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGG
Rhoiptelea GAAGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGG
Casuarina  GAAGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCAGGCGTTCTGCCCGTGG
Fagus      GAAGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCAGGTGTTCTGCCTGTGG
Quercus    GAAGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTTTACCAGGTGTTCTGCCCGTGG
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                    3         4         5         6         7         8
           12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella    CTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATT
Myrica     CTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATT
Canacomyrica CTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATT
Comptonia  CTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATT
Alnus      CTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATT
Carpinus   CTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATT
Juglans    CTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATT
Rhoiptelea CTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATT
Casuarina  CTTCAGGAGGTATTCATGTTTGGCATATGCCCGCTCTGACCGAAATCTTTGGAGATGATT
Fagus      CTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATT
Quercus    CTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTTTGACCGAAATCTTTGGAGATGATT

                              6
                    9         0         1         2         3         4
           12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella    CCGTACTACAATTCGGCGGAGGAACTTTAGGACACCCTTGGGGAAATGCACCTGGTGCCG
Myrica     CCGTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCTGGTGCTG
Canacomyrica CCGTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCTGGTGCCG
Comptonia  CCGTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCTGGTGCCG
Alnus      CCGTACTACAATTCGGTGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCTGGTGCTG
Carpinus   CCGTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCGGGTGCTG
Juglans    CCGTACTACAATTCGGTGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCCGGTGCCG
Rhoiptelea CCGTACTACAATTCGGCGGAGGAACTTTGGGGCACCCTTGGGGAAATGCACCTGGTGCCG
Casuarina  CCGTACTACAATTCGGCGGAGGAACTTTAGGGCATCCTTGGGGAAATGCACCCGGTGCTG
Fagus      CCGTACTACAATTTGGTGGAGGAACTTTGGGGCACCCTTGGCGAAATGCACCTGGTGCCG
Quercus    CCGTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCCGGTGCTG

                                                                      7
                    5         6         7         8         9         0
           12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella    TAGCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTG
Myrica     TAGCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTG
Canacomyrica TAGCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTG
Comptonia  TAGCTAATCGAGTAGCTCTCGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTG
Alnus      TAGCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTG
Carpinus   TAGCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTG
Juglans    TAGCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTG
Rhoiptelea TAGCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTG
Casuarina  TAGCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGGCGCGATCTTG
Fagus      TAGCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTG
Quercus    TAGCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTG
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                    1         2
           12345678901234567890123456789
Morella    CTCGTGAGGGTAATGAAATTATTCGTGA
Myrica     CTCGTGAGGGTAATGAAATTATTCGTGA
Canacomyrica CTCGTGAGGGTAATGAAATTATTCGTGA
Comptonia  CTCGTGAGGGTAATGAAATTATTCGTGA
Alnus      CTCGTGAGGGTAATGAAATTATTCGTGA
Carpinus   CTCGTGAGGGTAATGAAATTATTCGTGC
Juglans    CTCGTGAGGGTAATGAAATTATTCGTGA
Rhoiptelea CTCGTGAGGGTAATGAAATTATTCGCGA
Casuarina  CTCGTGAGGGTAATGAAATTATTCGTGA
Fagus      CTCGTGAGGGTAATGAAATTATTCGTGA
Quercus    CTCGTGAGGGTAATGAAATTATCCGTGA
```

Sequence alignment for the *rbcL* narrow Data set

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                          1         2         3         4         5         6
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Morella          ??????????????????????????????????????????????????????????????????????
Myrica           ??????????????????????????????????????????????????????????????????????
Canacomyrica     GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAGGATACGGATATCTTGGCA
Comptonia        GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAGGATACTGATATCTTGGCA
Alnus            GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCA
Carpinus         GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACAGATATCTTGGCA
Juglans          GATTATAAATTGACTTATTATACTCCTGAATATCAAACCAAAGATACTGATATCTTGGCA
Carya            GATTATAAATTGACTTATTATACTCCTGAATATCAAACCAAAGATACTGATATCTTGGCA
Rhoiptelea       GATTATAAATTGACTTATTATACTCCTGAATATCAAACCAAAGATACTGATATCTTGGCA
Casuarina        GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCA
Fagus            GATTATAAATTGACTTATTATACTCCTGACTATGAAACTAAAGATACTGATATCTTGGCA
Quercus          GATTATAAATTGACTTATCATACTCCTGACTATCAAACCAAAGATACTGATATCTTGGCA
Trigonoba        GATTATAAATTGACCTATTATACTCCTGACTATCAAACCAAAGATACTGATATCTTGGCA
Ticodendron      GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCA
Allocasuarina    GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCA
Ceuthostoma      GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCA
Gymnostoma       GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCA
Nothofagus       GATTATAAATTGACTTATTATACTCCTGACTACGAAACCAAAGATACTGATATGTTGGCA
Betula           GAGTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCA
Celtis           GATTACAAATTGACTTATTACACTCCTGAATATGAAACCAAAGATACTGATATCTTGGCA
Corylus          GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCA
Chrysolepis      GATTATAAATTGACTTATTATACTCCTGACTATCAAACCAAAGATACTGATATCTTGGCA
Cecropia         GATTATAAATTGACTTATTACACTCCTGAATATGAAACCAAAGATACTGATATCTTAGCA
Cucurbita        GATTATAAATTGACTTATTATACTCCTGAATATGAAACCAAAGATACTGATATCTTGGCA
Datisca          GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACGGATATCTTGGCA
Ficus            ??????????????????????????????????????????????????????????????????????
Eleagnus         GATTATAAATTGACTTATTACACTCCTGACTATCAAACCAAAGATACTGATATCTTAGCA
Dryas            GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATACTGATATCTTGGCA
Humulus          GATTATAAATTGACTTATTACACTCCGGACTATGAAACCAAAGATACTGATATCTTGGCA
Rhamnus          GATTATAAATTGACTTATTATACTCCTGAATATGAAACCAAAGATACCGATATCTTGGCA
Ulmus            GATTAT?AATTGACTTATTACACTCCTGAATATGAAACCAAAGATACTGATATCTTGGCA
Rosa             GATTATAAATTGACTTATTATACTCCGGATTATGAAACCAAAGATACTGATATCTTGGCA
Spirea           GAGTATAAATTGACTTATTATACTCCGGAATATGAAACCAAAGATACTGATATCTTGGCA
Pisum            GATTATAAATTGACTTATTATACTCCTGACTATCAAACCAAAGATACTGATATCTTGGCA
Albizia          GATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAAGATAGTGATATCTTGGCA
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                                                                1
                        7         8         9         0         1         2
                        12345678901234567890123456789012345678901234567890123456789
Morella                 ???TTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGGGCAGCAGTAGCT
Myrica                  ???????????????CTCCTCTACCTGGTGTTCCGCCTGAGGAAGCAGGGGCAGCAGTAGCT
Canacomyrica            GCGTTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAGGAAGCAGGGGCAGCAGTAGCT
Comptonia               GCGTTCCGAGTAACTCCTCAACCTGGAGTGCCGCCTGAGGAAGCAGGGGCAGCAGTAGCT
Alnus                   GCGTTCCGAGTAACTCCTCAACCTGGAGTGCCGCCTGAGGAAGCAGGGGCAGCAGTAGCT
Carpinus                GCGTTCCGAGTAACTCCTCAACCTGGAGTTCCGCCAGAGGAAGCAGGGGCAGCAGTAGCT
Juglans                 GCGTTCCGAGTAAGCCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGGGCAGCAGTAGCT
Carya                   GCGTTCCGAGTAAGCCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGGGCAGCAGTAGCT
Rhoiptelea              GCGTTCCGAGTAAGTCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGGGCAGCAGTAGCT
Casuarina               GCGTTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAGGAAGCAGGGGCGGCAGTAGCT
Fagus                   GCCTTCCGAGTAACTCCTCAACCTGGGGTTCCGCCTGAAGAAGCGGGGGCCGCGGTAGCT
Quercus                 GCCTTCCGAGTAACTCCTCAACCTGGAGTTCCGCCCGAGGAAGCAGGGGCCGCGGTAGCT
Trigonoba               GCCTTCCGAGTAACTCCTCAACCTGGAGTTCCGCCCGAGGAAGCAGGGGCCGCGGTAGCT
Ticodendron             GCGTTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGGGCAGCAGTAGCT
Allocasuarina           GCGTTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAGGAAGCAGGGGCGGCAGTAGCT
Ceuthostoma             GCGTTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAGGAAGCAGGGGCGGCAGTAGCT
Gymnostoma              GCGTTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAGGAAGCAGGGGCGGCAGTAGCT
Nothofagus              GCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCCTGAGGAAGGGGGGGCTGCGGTAGCT
Betula                  GCGTTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGGGCAGCAGTAGCT
Celtis                  GCATTTAGAGTAACTCCTCAACCTGGAGTTCCCCCTGAAGAAGCAGGGGCTGCGGTAGCT
Corylus                 GCGTTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGGGCAGCAGTAGCT
Chrysolepis             GCCTTCCGAGTAACTCCTCAACCTGGAGTTCCGCCCGAGGAAGCAGGGGCCGCGGTAGCT
Cecropia                GCATTTCGAGTAACTCCTCAACCTGGAGTTCCCCCTGAAGAAGCAGGGGCTGCGGTAGCT
Cucurbita               GCATTCCGAGTAACTCCTCAACCGGGAGTTCCACCCGAGGAAGCGGGGGCCGCCGTAGCT
Datisca                 GCATTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAGGAAGCAGGTGCCGCTGTAGCT
Ficus                   ????????????????????????????????????????????????????????????
Eleagnus                GCATTTCGAGTAACTCCTCAACCAGGAGTTCCACCCGAAGAAGCAGGGGCAGCGGTAGCT
Dryas                   GCATTTCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGGGCTGCGGTAGCT
Humulus                 GCATTTCGAGTAACTCCTCAACCTGGAGTTCCCCCTGAAGAAGCTGGGGCTGCGGTAGCT
Rhamnus                 GCATTTCGAGTAACTCCTCAACCCGGAGTTCCACCTGAGGAAGCAGGGGCCGCGGTAGCT
Ulmus                   GCATTTCGAGTAACTCCTCAACCCGGAGTTCCACCTGAGGAAGCAGGAGCTGCGGTAGCT
Rosa                    GCATTTCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAGGAAGCAGGGGCAGCGGTAGCT
Spirea                  GCATTTCGAGTAACTCCTCAACCTGGAGTTCCACCTGAGGAAGCAGGGGCCGCGGTAGCT
Pisum                   GCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGTGCCGCGGTAGCT
Albizia                 GCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGTGCCGCGGTAGCT
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                    3         4         5         6         7         8
                    12345678901234567890123456789012345678901234567890123456789012345678901234567890
Morella             GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTGAT
Myrica              GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTGAT
Canacomyrica        GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACGAGTCTTGAT
Comptonia           GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTGAT
Alnus               GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACTAGTCTTGAT
Carpinus            GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACTAGTCTTGAT
Juglans             GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTGAT
Carya               GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTGAT
Rhoiptelea          GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTGAT
Casuarina           GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTGAT
Fagus               GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACTGATGGGCTTACCAGTCTTGAT
Quercus             GCTGAATCTTCCACTGGGACATGGACAACTGTGTGGACTGACGGGCTTACCAGTCTTGAT
Trigonoba           GCTGAATCTTCCACTGGGACATGGACAACTGTGTGGACTGACGGGCTTACCAGTCTTGAT
Ticodendron         GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACTAGTCTTGAT
Allocasuarina       GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTGAT
Ceuthostoma         GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTGAT
Gymnostoma          GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGTCTTGAT
Nothofagus          GCGGAATCCTCTACTGGTACATGGACAACGGTGTGGACCGATGGACTTACCAGTCTTGAT
Betula              GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACTAGTCTTGAT
Celtis              GCTGAATCTTCTACTGGTACATGGACAACTGTATGGACTGACGGGCTTACCAGCCTTGAT
Corylus             GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACTAGTCTTGAT
Chrysolepis         GCTGAATCTTCTACTGGGACATGGACAACTGTGTGGACCTATCGAGTTACCAGTCTTGAT
Cecropia            GCTGAATCTTCTACTGGTACATGGACAACTGTATGGACTGACGGGCTTACCAGTCTTGAT
Cucurbita           GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGAT
Datisca             GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGTCTTACCAGTCTTGAT
Ficus               ????????????????????????????????????????????????????????????
Eleagnus            GCTGAATCTTCTACTGGTACATGGACAACTGTATGGACTGACGGGCTTACCAGTCTTGAT
Dryas               GCTGAATCTTCTACTGGTACATGGACAACTGTATGGACTGACGGGCTTACCAGTCTTGAT
Humulus             GCTGAATCTTCTACTGGTACATGGACAACTGTATGGACTGACGGGCTTACCAGCCTTGAT
Rhamnus             GCTGAATCTTCTACTGGTACATGGACAACTGTATGGACTGACGGGCTTACCAGTCTTGAT
Ulmus               GCTGAATCTTCTACTGGTACATGGACAACTGTATGGACTGACGGGCTTACTAGTCTTGAT
Rosa                GCGGAATCTTCTACTGGTACATGGACAACTGTATGGACTGATGGGCTTACCAGTCTTGAT
Spirea              GCTGAATCTTCTACGGGTACATGGACAACTGTATGGACTGACGGGCTTACCAGTCTTGAT
Pisum               GCAGAATCTTCCACTGGTACATGGACAACTGTGTGGACCGATGGACTTACGAGCCTCGAT
Albizia             GCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGAT
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                                  2
                        9         0         1         2         3         4
                        123456789012345678901234567890123456789012345678901234567890
Morella                 CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTATT
Myrica                  CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTATT
Canacomyrica            CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAATCAATATATT
Comptonia               CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTATT
Alnus                   CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTATT
Carpinus                CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTATT
Juglans                 CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAATCAATTTATT
Carya                   CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAATCAATTTATT
Rhoiptelea              CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAATCAATATATT
Casuarina               CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTATT
Fagus                   CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTATT
Quercus                 CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAATCAATTTATT
Trigonoba               CGTTACAAAGGACGATGCTACCACATCGAGCCGGTTGCTGGAGAAGAAAATCAATTTATT
Ticodendron             CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAATCAATTTATT
Allocasuarina           CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTATT
Ceuthostoma             CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAATCAATATATT
Gymnostoma              CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGCGAAGAAAATCAATATATT
Nothofagus              CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAATCAATTTATT
Betula                  CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTATT
Celtis                  CCTTACAAAGGTCGATGCTACCACATCGAGCCCGTTGCTGGAGAAGAAAGTCAATTTATT
Corylus                 CGTTACAAAGGACGATGCTACCACATCGAGCCAGTTGCTGGAGAAGAAAGTCAATTTATT
Chrysolepis             CGTTACAAAGGACGATGCTACCACATCGAGCCGGTTGCTGGAGAAGAAAATCAATTTATT
Cecropia                CGCTACAAAGGTCGATGCTACCACATCGAGCCTGTTGCTGGAGAAGAAAATCAATTTATT
Cucurbita               CGTTACAAAGGACGATGCTATGGAATCGAGCCTGTTCCTGGAGAAGAAAATCAATATATT
Datisca                 CGTTACAAAGGACGATGCTACGGCATCGAGCCTGTTGCTGGAGGAGAAAATCAATATATT
Ficus                   ????????????????????????????????????????????????????????????
Eleagnus                CGTTACAAGGGTCGATGCTACGGCCTTGAGCCCGTTGCTGGAGAAGAAAATCAATATATT
Dryas                   CGTTACAAAGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAGAAAGTCAATTTATT
Humulus                 CGCTACAAAGGTCGATGCTACCACATCGAGCCCGTTGCTGGAGAAGAAAATCAATATATT
Rhamnus                 CGTTACAAAGGTCGATGCTACCACATCGAGCCCGTTGCTGGAGAAGAAAGTCAATTTATT
Ulmus                   CGCTACAAAGGTCGATGCTACCACATTGAGCCTGTTGCTGGAGAAGAAAGTCAATATATA
Rosa                    CGTTACAAAGGGCGATGCTACCACATTGAACCTGTTGCTGGAGAAGAAAGTCAATTTATT
Spirea                  CGTTACAAAGGTCGATGCTACCACATTGAGCCCGTTGCTGGAGAAGAAAATCAATTTATT
Pisum                   CGTTATAAAGGACGCTGCTACGAGATCGAGCCTGTTCCTGGAGAAGATAATCAATTTATT
Albizia                 CGTTACAAAGGACGATGCTACCACATCGAGTCCGTTGCTGGAGAAGAAAATCAATATATT
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                                                                      3
                          5         6         7         8         9         0
                 12345678901234567890123456789012345678901234567890123456789
Morella          GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Myrica           GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Canacomyrica     GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Comptonia        GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Alnus            GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Carpinus         GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Juglans          GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Carya            GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Rhoiptelea       GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Casuarina        GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Fagus            GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Quercus          GCTTATGTAGCTTACCCCTTAGACCTCTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Trigonoba        GCTTATGTAGCTTACCCCTTAGACCTCTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Ticodendron      GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Allocasuarina    GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Ceuthostoma      GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Gymnostoma       GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Nothofagus       GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Betula           GCTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Celtis           GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Corylus          GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Chrysolepis      GCTTATGTAGCTTACCCCTTAGACCTCTTTGAAGAGGGTTCTGTTACTAACATGTTTACT
Cecropia         GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Cucurbita        GCTTATGTAGCTTATCCCCTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Datisca          GCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Ficus            ??????GTAGCTTACCCTTTAGACCTTTTTGAAGAGGGTTCTGTTACTAATCTGTTTACT
Eleagnus         GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGCTCTGTTACTAACATGTTTACT
Dryas            GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Humulus          GCTTATGTAGCTTATCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Rhamnus          GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Ulmus            TGTTATGTAGCTTACCCTTTAGACCTTTTCGAAGAAGGTTCTGTTACTAACATGTTTACT
Rosa             GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAGGGTTCGGTTACTAACATGTTTACT
Spirea           GCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
Pisum            GCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACC
Albizia          GCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACT
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                    1         2         3         4         5         6
                    12345678901234567890123456789012345678901234567890123456789
Morella             TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Myrica              TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Canacomyrica        TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Comptonia           TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Alnus               TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATTTG
Carpinus            TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATTTG
Juglans             TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Carya               TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Rhoiptelea          TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Casuarina           TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATTTG
Fagus               TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Quercus             TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Trigonoba           TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Ticodendron         TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGTGCTCTACGTCTGGAAGATTTG
Allocasuarina       TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATTTG
Ceuthostoma         TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATTTG
Gymnostoma          TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATTTG
Nothofagus          TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Betula              TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATTTG
Celtis              TCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Corylus             TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATTTG
Chrysolepis         TCCATTGTGGGTAATGTATTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Cecropia            TCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Cucurbita           TCCATTGTGGGTAATGTATTTGGATTCAAGGCTCTGCGTGCTCTACGTCTGGAGGATTTG
Datisca             TCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAAGATTTG
Ficus               TCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGTGCGCTACGTCTGGAAGATTTG
Eleagnus            TCCATTGTGGGTAATGTGTTTGGATTCAAGGCCCTGCGCGCTCTACGTCTGGAAGATTTG
Dryas               TCCATTGTAGGTAATGTGTTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTA
Humulus             TCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTGGAAGATTTG
Rhamnus             TCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTG
Ulmus               TCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTAGAGGATTTA
Rosa                TCCATTGTAGGTAATGTGTTTGGGTTCAAGGCCTTGCGCGCTCTACGTCTGGAGGATTTA
Spirea              TCCATTGTAGGTAATGTGTTTGGGTTCAAGGCCCTGCGTGCTCTACGTCTGGAGGATTTG
Pisum               TCCATTGTAGGTAATGTATTTGGGTTCAAGGCCTTGCGTGCTCTACGTCTGGAAGATTTG
Albizia             TCGATTGTGGGTAATGTATTTGGGTTCAAGGCCCTTCGTGCTCTACGTCTGGAAGATTTG
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Morella             CGAATCCCTACTTCTTATGTTAAAACTTTCCAAGGCCCGCCGCATGGCATCCAAGTTGAG
Myrica              CGAATCCCTACTTCTTATGTTAAAACTTTCCAAGGCCCGCCGCATGGCATCCAAGTTGAG
Canacomyrica        CGAATCCCTCCTTCTTATTCTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAAGTTGAG
Comptonia           CGAATCCCTACTTCTTATGTTAAAACTTTCCAAGGCCCGCCGCATGGCATCCAAGTTGAG
Alnus               CGAATCCCTCCTGCTTATTCTAAAACTTTCCAAGGCCCGCCTCACGGCATCCAAGTTGAG
Carpinus            CGAATCCCCACTGCTTATACTAAAACTTTCCAAGGCCCGCCTCACGGCATCCAAGTTGAG
Juglans             CGAATCCCTCCTGCTTATTCTAAAACTTTCCAAGGCCCGCCTCATGGAATCCAAGTTGAG
Carya               CGAATCCCTCCTGCTTATTCTAAAACTTTCCAAGGCCCGCCTCATGGAATCCAAGTTGAG
Rhoiptelea          CGAATCCCTCCTGCTTATTCTAAAACTTTCCAAGGCCCGCCTCATGGAATCCAAGTTGAG
Casuarina           CGAATCCCTACTGCTTATTCTAAAACTTTCCAAGGTCCGCCTCATGGCATTCAAGTTGAG
Fagus               CGAATCCCTACTTCTTACGCTAAAACTTTCCAAGGCCCGCCTCACGGCATCCAAGTTGAG
Quercus             CGAATCCCTACTTCTTATTCTAAAACTTTCCAAGGTCCGCCTCATGGCATCCAAGTTGAG
Trigonoba           CGAATCCCTCCTTCTTATTCTAAAACTTTCCAAGGTCCGCCTCATGGCATCCAAGTTGAG
Ticodendron         CGAATCCCTACTTCTTATGTTAAAACTTTCCAAGGTCCGCCCCATGGCATCCAAGTTGAG
Allocasuarina       CGAATCCCTACTGCTTATTCTAAAACTTTCCAAGGCCCGCCTCACGGCATCCAAGTTGAG
Ceuthostoma         CGAATCCCTACTGCTTATTCTAAAACTTTCCAAGGTCCGCCTCATGGCATCCAAGTTGAG
Gymnostoma          CGAATCCCTACTTCTTATACTAAAACTTTCCAAGGTCCGCCTCATGGCATCCAAGTTGAG
Nothofagus          CGAATCCCTCCTGCTTATGTTAAAACTTTTCAAGGTCCGCCTCATGGCATCCAAGTTGAG
Betula              CGAATCCCTCCTGCTTATTCTAAAACTTTCCAAGGCCCGCCTCACGGCATCCAAGTTGAG
Celtis              AGAATCCCTACTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAAGTTGAG
Corylus             CGAATCCCTCCCGCTTATTCTAAGACTTTCCAAGGCCCGCCTCACGGCATCCAAGTTGAG
Chrysolepis         CGAATCCCTACTTCTTATTCTAAAACTTTCCAAGGTCCGCCTCATGGCATCCAAGTTGAG
Cecropia            CGAATCCCCACTTCTTACACTAAAACTTTCCAAGGACCGCCTCATGGCATCCAAGTTGAG
Cucurbita           CGAATCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCATGGTATCCAGGTTGAA
Datisca             CGAATCCCTCCTGCTTATGTTAAAACTTTCCAAGGGCCGCCTCATGGTATCCAAGTTGAG
Ficus               CGAATCCCTCCTTCTTATTCTAAAACTTTCCAAGGACCACCTCATGGTATCCAAGTTGAG
Eleagnus            CGAATCCCTACTGCTTATACTAAAACTTTCCAAGGCCCGCCTCATGGTATCCAAGTTGAG
Dryas               CGAATCCCTACTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCATGGGATCCAAGTTGAG
Humulus             AGAATCCCTACTTCTTATACTAAAACTTTCCAAGGTCCGCCTCATGGGATCCAAGTTGAG
Rhamnus             CGAATCCCCCCTGCTTATACTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAAGTTGAG
Ulmus               CGAATCCCTCCTGCTTATACTAAAACTTTCCAAGGTCCGCCTCATGGCATCCAAGTTGAG
Rosa                CGAATCCCTACTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCACGGGATCCAAGTTGAA
Spirea              CGAATCCCTACTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCATGGGATCCAAGTTGAG
Pisum               CGAATCCCTTATGCTTATGTTAAAACTTTCCAAGGTCCTCCTCACGGAATCCAAGTTGAG
Albizia             CGAATCCCTCCTTCTTATTCTAAAACTTTCCAAGGTCCGCCTCACGGCATCCAAGTTGAG
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Morella             AGAGATAAATTAAACAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCCAAATTG
Myrica              AGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCCAAATTG
Canacomyrica        AGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCCAAATTG
Comptonia           AGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCCAAATTG
Alnus               AGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAGCCTAAATTG
Carpinus            AGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTG
Juglans             AGAGATAAATTAAACAAGTATGGCCGCCCTCTATTGGGATGTACTATTAAACCTAAATTG
Carya               AGAGATAAATTAAACAAGTATGGCCGCCCTCTATTGGGATGTACTATTAAACCTAAATTG
Rhoiptelea          AGAGATAAATTAAACAAGTATGGCCGCCCTCTATTGGGATGTACTATTAAACCTAAATTG
Casuarina           AGAGATAAATTAAACAAGTATGGCCGACCCCTATTAGGATGTACTATTAAACCTAAATTG
Fagus               AGAGATAAATTAAACAAGTATGCCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTG
Quercus             AGGGATAAATTAAACAAGTATGGCCGCCCCCTATTAGGATGTACTATTAAACCTAAATTG
Trigonoba           AGGGATAAATTAAACAAGTATGGCCGCCCCCTATTAGGATGTACTATTAAACCTAAATTG
Ticodendron         AGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTG
Allocasuarina       AGAGATAAATTAAACAAGTATGGCCGACCCCTATTAGGATGTACTATTAAACCTAAATTG
Ceuthostoma         AGAGATAAATTAAACAAGTATGGCCGACCCCTATTAGGATGTACTATTAAACCTAAATTG
Gymnostoma          AGAGATAAATTAAACAAGTATGGCCGACCCCTATTAGGATGTACTATTAAACCTAAATTG
Nothofagus          AGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTAGTAAACCAAAAGTG
Betula              AGAGATAAATTAAACAAATATGGCCGCCCCCTATTAGGATGTACTATTAAACCTAAATTG
Celtis              AGAGATAAATTGAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTG
Corylus             AGAGATAAATTAAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTG
Chrysolepis         AGGGATAAATTAAACAAGTATGGCCGCCCCCTATTAGGATGTACTATTAAACCTAAATTG
Cecropia            AGAGATAAATTGAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTG
Cucurbita           AGAGATAAATTGAACAAGTATGGTCGCCCTCTATTGGGATGTACTATTAAACCAAAATTG
Datisca             AGAGATAAATTGAACAAGTATGGCCGCCCTCTATTGGGATGTACTATTAAACCTAAATTG
Ficus               AGAGATAAATTGAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTG
Eleagnus            AGAGATAAATTGAACAAGTATGGCCGTCCCCTATTAGGATGTACTATTAAACCTAAATTG
Dryas               AGAGATAAATTGAACAAGTACGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTG
Humulus             AGAGATAAATTGAACAAGTATGGCCGCCCACTATTGGGATGTACTATTAAACCTAAATTG
Rhamnus             AGAGATAAGTTGAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTG
Ulmus               AGAGATAAATTGAACAAGTATGGCCGCCCCCTATTAGGATGTACTATTAAACCTAAATTG
Rosa                AGAGATAAATTGAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTG
Spirea              AGAGATAAATTAAACAAGTATGGACGCCCCCTATTGGGATGTACTATTAAACCTAAATTG
Pisum               AGAGATAAATTGAACAAGTATGGACGTCCCCTATTGGGATGTACTATTAAACCAAAATTG
Albizia             AGAGATAAATTGAACAAGTACGGCCGTCCCCTATTGGGATGTACTATTAAACCAAAATTG
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Myrica          GGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTGAT
Canacomyrica    GGATTATCTGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Comptonia       GGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTGAT
Alnus           GGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGAGGTGGGCTTGAT
Carpinus        GGATTATCCGCTAAGAATTATGGTAGAGCGGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Juglans         GGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Carya           GGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Rhoiptelea      GGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Casuarina       GGATTATCTGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Fagus           GGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Quercus         GGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Trigonoba       GGATTATCCGCTAAGAATTACGGTAGAGCGGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Ticodendron     GGATTATCCGCTAAGAATTACGGGAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTGAT
Allocasuarina   GGATTATCTGCTAAGAATTATGGCAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Ceuthostoma     GGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGTGGTGGGCTTGAT
Gymnostoma      GGATTATCCGCTAAGAATTATGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Nothofagus      GGGTTATCCGCTAAAAATTGCGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTGAT
Betula          GGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Celtis          GGGTTATCCGCTAAGAATTACGGTAGAGCTGTTTATGAATGTCTTCGCGGTGGACTTGAT
Corylus         GGATTATCCGCTAAGAATTACGGTAGAGCGGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Chrysolepis     GGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGGCTTGAT
Cecropia        GGGTTATCCGCTAAGAATTATGGTAGAGCGGTTTATGAATGTCTTCGTGGTGGACTTGAT
Cucurbita       GGATTATCCGCTAAGAATTATGGTAGAGCAGTTTATGAATGTCTACGCGGTGGACTTGAT
Datisca         GGATTATCCGCTAAGAATTACGGTAGAGCTGTTTATGAATGTCTACGTGGTGGACTTGAT
Ficus           GGATTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGGCTTGAT
Eleagnus        GGGTTATCCGCTAAGAATTATGGTAGAGCTGTTTATGAATGTCTTCGCGGTGGGCTTGAT
Dryas           GGGTTATCTGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTGAT
Humulus         GGGTTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTTCGTGGTGGACTTGAT
Rhamnus         GGGTTATCCGCTAAGAATTACGGTAGAGCCGTTTATGAATGTCTTCGCGGTGGACTTGAT
Ulmus           GGGTTATCTGCTAAGAATTATGGTAGAGCAGTTTAT?AATGTCTTCGCGGTGGACTTGAT
Rosa            GGGTTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTGAT
Spirea          GGGTTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAGTGTCTCCGCGGTGGACTTGAT
Pisum           GGTTTATCCGCTAAGAATTATGGTAGAGCAGTTTATGAATGTCTCCGCGGGGGACTTGAT
Albizia         GGGTTATCCGCGAAGAATTACGGTAGAGCGGTTTATGAATGTCTCCGTGGTGGACTTGAT
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Morella             TTTACCAAAGATGATGAGAACGTGAATTCCCAACCGTTTATGCGTTGGAGAGATCGTTTC
Myrica              TTTACCAAAGATGATGAGAACGTGAATTCCCAACCGTTTATGCGTTGGAGAGATCGTTTC
Canacomyrica        TTTACCAAAGATGATGAGAACGTGAATTCCCAACCGTTTATGCGTTGGAGAGACCGTTTC
Comptonia           TTTACCAAAGATGATGAGAACGTGAATTCCCAACCGTTTATGCGTTGGAGAGATCGTTTC
Alnus               TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Carpinus            TTTACCAAAGATGATGAAAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Juglans             TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Carya               TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Rhoiptelea          TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Casuarina           TTTACCAAAGATGATGAAAATGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Fagus               TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Quercus             TTTACCAAAGATGATGAGAACGTTAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Trigonoba           TTTACCAAAGATGATGAGAACGTTAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Ticodendron         TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Allocasuarina       TTTACCAAAGATGATGAAAATGTGAATTCCCAACCTTTTATGCGTTGGAGAGACCGTTTC
Ceuthostoma         TTTACCAAAGATGATGAAAATGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Gymnostoma          TTTACCAAAGATGATGAGAATGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Nothofagus          TTTACCAAAGATGATGAGAACGTAAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Betula              TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Celtis              TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Corylus             TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGATCGTTTC
Chrysolepis         TTTACCAAAGATGATGAGAACGTTAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Cecropia            TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Cucurbita           TTTACCAAAGATGATGAAAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Datisca             TTTACCAAAGATGATGAAAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Ficus               TTTACCAAAGATGATGAAAACGTGAACTCTCAACCATTTATGCGTTGGAGAGACCGTTTC
Eleagnus            TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Dryas               TTTACCAAAGATGATGAGAATGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGGTTC
Humulus             TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Rhamnus             TTTACCAAAGATGATGAGAACGTGAATTCCCAACCGTTTATGCGTTGGAGAGACCGGTTC
Ulmus               TTTACCAAAGATGATGAGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Rosa                TTTACCAAAGATGATGAGAATGTTAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Spirea              TTTACCAAAGATGATGAGAATGTTAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Pisum               TTTACCAAAGATGATGAAAATGTGAACTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
Albizia             TTTACCAAAGATGATGAGAATGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTC
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Myrica              CTATTTTGTGCCGAAGCTCTTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGACAT
Canacomyrica        GTATTTTGTGCCGAAGCTCTTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGCAT
Comptonia           CTATTTTGTGCCGAAGCTCTTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGACAT
Alnus               CTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGCAT
Carpinus            CTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGCAT
Juglans             CTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACCGGTGAAATCAAAGGGCAT
Carya               CTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACCGGTGAAATCAAAGGGCAT
Rhoiptelea          CTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACCGGTGAAATCAAAGGGCAT
Casuarina           GTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCCGAAACAGGTGAAATCAAAGGGCAT
Fagus               CTGTTTTGTGCCGAAGCTCTTTATAAAGCGCAGGCGGAAACCGGTGAAATCAAAGGGCAT
Quercus             GTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGCAT
Trigonoba           ATATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGCAT
Ticodendron         CTATTTTGTACCGAAGCAATTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGCAT
Allocasuarina       CTATTTTGTGCCGAAGCACTTTATAAAGCGCAGGCCGAAACAGGTGAAATCAAAGGGCAT
Ceuthostoma         CTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGCAT
Gymnostoma          CTATTTTGTGCCGAAGCACTTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGCAT
Nothofagus          CTATTTTGTGCCGAAGCAATTTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGACAT
Betula              CTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGCAT
Celtis              TTATTTTGTGCCGAAGCGATTTATAAATCACAGGCTGAAACAGGTGAAATCAAAGGACAT
Corylus             CTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGCAT
Chrysolepis         CTATTTTGTGCCGAAGCAATTTATAAAGCGCAGGCTGAAACAGGTGAAATCAAAGGGCAT
Cecropia            TTATTTTGTGCCGAAGCAATTTTTAAAGCACAGGCTGAAACAGGTGAAATCAAAGGACAT
Cucurbita           CTATTTTGTGCGGAAGCCATTTATAAATCACAGGCTGAAACAGGTGAAATCAAGGGACAT
Datisca             CTATTTTGTGCGGAACGAATTTATAAATCACAGGCTGAAACAGGTGAAATCAAGGGGCAT
Ficus               TTATTTTGTGCCGAAGCAATTTATAAAGCACAAGCTGAAACAGGTGAAATCAAAGGACAT
Eleagnus            TTATTTTGTGCCGAAGCCCTTTTTAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCAT
Dryas               TTATTTTGTGCCGAAGCACTTTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCAT
Humulus             TTATTTTGTGCAGAAGCAATTTATAAATCACAGTCTGAAACAGGGGAAATCAAAGGACAT
Rhamnus             TTATTTTGTGCCGAAGCAATTTATAAAGCACAGGCCGAAACTGGTGAAATCAAAGGGCAT
Ulmus               TTATTTTGTGCCGAAGCTATTTATAAATCACAGGCTGAAACAGGTGAAATCAAAGGACAT
Rosa                TTATTTTGTGCCGAAGCAATTTATAAATCGCAGGCTGAAACAGGTGAAATCAAAGGGCAT
Spirea              GTATTTTGTGCCGAAGCAATTTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCAT
Pisum               TTATTTTGTGCCGAAGCAATTTATAAATCACAGGCCGAAACAGGTGAAATCAAAGGACAT
Albizia             TTATTTTGTGCCGAAGCACTTTATAAAGCACAGGCCGAAACAGGTGAAATCAAAGGGCAT
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Myrica            TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATTAAAAGGGCTGTATTTGCCAGA
Canacomyrica      TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATTAAAAGGGCTGTATTTGCCAGA
Comptonia         TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATTAAAAGGGCTGTATTTGCCAGA
Alnus             TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATGAAAAGGGCTGTATTTGCCAGA
Carpinus          TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATGAAAAGGGCTGTATTTGCCAGA
Juglans           TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCAGA
Carya             TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCAGA
Rhoiptelea        TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCAGA
Casuarina         TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATGAAAAGGGCTATATTTGCCAGA
Fagus             TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCAGA
Quercus           TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCAGA
Trigonoba         TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCAGA
Ticodendron       TACTTGAATGCTACGGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCAGA
Allocasuarina     TACTTGAATGCTACCGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCAGA
Ceuthostoma       TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATAAAAAGGGCTGTATTTGCCAGA
Gymnostoma        TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATAAAAAGGGCTGTATTTGCCAGA
Nothofagus        TACTTGAATGCTACTGCGGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCGAGA
Betula            TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATGAAAAGGGCTATATTTGCCAGA
Celtis            TACTTGAATGCTACTGCAGGTACATGTGAAGACATGATGAAAAGGGCTGTATTTGCTAGA
Corylus           TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCAGA
Chrysolepis       TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCAGA
Cecropia          TACTTGAATGCTACTGCAGGTACATGTGAAGAAATGATGAAAAGGGCCGTATGTGCCAGA
Cucurbita         TACTTGAATGCTACTGCGGGTACATGCGAAGAAATGATGAAAAGGGCTATATTTGCTCGA
Datisca           TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCAGA
Ficus             TACTTGAATGCTACTGCAGGTACATGTGAAGAAATGATCAAAAGAGCTGTATGTGCCAGA
Eleagnus          TATTTGAATGCTACGGCAGGTACATGCGAAGAAATGATGAAAAGGGCTATATTTGCCAGA
Dryas             TACTTGAACGCTACTGCAGGTACATGTGAAGAGATGATCAAAAGAGCTGTATTTGCCAGA
Humulus           TACTTGAATGCTACTGCAGGTACATGTGAAGAAATGATGAAAAGGGCTGTATTTGCCAGA
Rhamnus           TACTTGAATGCTACGGCAGGTACATGCGAAGAAATGATGAAAAGGGCTGCATGTGCCAGA
Ulmus             TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCCAGA
Rosa              TACTTGAATGCTACTGCAGGTACATGCGAAGATATGATGAAAAGAGCCGTATTTGCCAGA
Spirea            TACTTGAACGCTACTGCAGGTACATGCGAAGAGATGATGAAAAGAGCTGCATTTGCCAGA
Pisum             TATTTGAATGCTACTGCGGGTACATGTGAAGAAATGCTAAAAAGAGCTGTATTTGCTAGA
Albizia           TACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGAGCTGTATTTGCCCGA
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Myrica     GAATTGGGAGTTCCTATCGTAATGCACGACTACTTAACCGGGGGATTCACGGCAAATACT
Canacomyrica GAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATACT
Comptonia  GAATTGGGAGTTCCTATCGTAATGCACGACTACTTAACCGGGGGATTCACGGCAAATACT
Alnus      GAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATACT
Carpinus   GAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATACT
Juglans    GAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTTACTGCAAATACT
Carya      GAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTTACTGCAAATACT
Rhoiptelea GAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATACT
Casuarina  GAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATACT
Fagus      GAATTGGGAGTTCCTATCGTAATGCACGACTACTTAACCGGGGGATTCACTGCAAGTACT
Quercus    GAACTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGGTTCACTGCAAATACT
Trigonoba  GAACTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGGTTCACTGCAAATACT
Ticodendron GAATTGGGCGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATACT
Allocasuarina GAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATACT
Ceuthostoma GAATTGGGAGCTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATACT
Gymnostoma GAATTGGGAACTCCTATCGTAATGCACGACTACTTAACTGGGGGATTCACTGCAAATACT
Nothofagus GAATTGGGAGTTCCTATCGTAATGCATGACTACATAACAGGGGGATTCACTGCAAATACT
Betula     GAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATACT
Celtis     GAATTGGGAGTTCCTATCGTAATGCACGATTACTTAACAGGAGGATTCACTGCAAATACT
Corylus    GAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGATTCACTGCAAATACT
Chrysolepis GAACTGGGAGTTCCTATCGTAATGCATGACTACTTAACCGGGGGGTTCACTGCAAATACT
Cecropia   GAATTGGGAGTTCCTATCGTAATGCATGATTACTTAACAGGAGGATTCACTGCAAATACC
Cucurbita  GAATTGGGAGCTCCTATCGTAATGCATGACTACTTAACAGGTGGATTCACTGCAAATACT
Datisca    GAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGGGGCTTCACTGCAAATACT
Ficus      GAATTGGGAGCTCCTATCGTAATGCATGACTACTTAACAGGAGGATTCACTGCAAATACT
Eleagnus   GAATTGGGAGCTCCTATCGTAATGCACGATTACTTAACTGGGGGATTCACTGCAAACACT
Dryas      GAATTGGGAGTTCCTATCGTAATGCATGATTACTTAACAGGGGGATTCACTGCAAATACC
Humulus    GAATTGGGAGTTCCTATTGTAATGCATGATTACTTAACAGGAGGATTCACTGCAAATACT
Rhamnus    GAATTGGGAGTTCCTATCGTAATGCACGATTACTTAACGGGGGGATTCACTGCAAATACT
Ulmus      GAATTAGGAGTTCCTATCGTAATGCATGATTACTTAACAGGGGGATTCACTGCAAATACT
Rosa       GAATTGGGAGTTCCTATCGTAATGCACGATTACTTAACGGGGGGATTCACTGCAAATACT
Spirea     GAATTGGGAGTTCCTATCGTAATGCATGATTACTTAACAGGGGGATTCACTGCAAATACT
Pisum      GAATTGGGCGTTCCTATCGTAATGCATGACTACTTAACAGGTGGATTCACTGCAAATACT
Albizia    GAATTGGGCGTTCCTATCGTAATGCATGACTACTTAACAGGGGGATTCACTGCAAATACT
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Morella         AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Myrica          AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Canacomyrica    AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Comptonia       AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Alnus           AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Carpinus        AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Juglans         AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Carya           AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Rhoiptelea      AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Casuarina       AGTTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Fagus           AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCACCGTGCAATGCAT
Quercus         AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCCATGCAT
Trigonoba       AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Ticodendron     AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Allocasuarina   AGTTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Ceuthostoma     AGTTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Gymnostoma      AGTTTAGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Nothofagus      AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACAGTCATCGTGCAATGCAT
Betula          AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Celtis          ACTCTAGCTCATTATTGTCGAGATAATGGTTTACTTCTTCACATCCATCGTGCAATGCAT
Corylus         AGCTTGGCTCATTATTGCCGGGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Chrysolepis     AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Cecropia        AGCCTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Cucurbita       AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCACCGTGCAATGCAT
Datisca         AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCACCGTGCAATGCAT
Ficus           AGCCTGGCTCATTATTGTCGAGATAACGGTCTACTTCTTCACATCCATCGTGCAATGCAT
Eleagnus        AGCTTGGCTCATTATTGCCGAGATAATGGTTTACTTCTTCACATCCACCGTGCAATGCAT
Dryas           AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCACCGTGCAATGCAT
Humulus         AGCCTGGCTCATTATTGTCGAGATAATGGTCTACTTCTTCACATCCACCGTGCAATGCAT
Rhamnus         ACCTTGGCTCATTATTGCCGAGATAATGGTCTGCTTCTTCACATCCACCGTGCAATGCAT
Ulmus           AGCCTGGCTCATTATTGTAGAGATAATGGTTTACTTCTTCACATCCACCGTGCAATGCAT
Rosa            ACCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCACCGTGCAATGCAT
Spirea          ACCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCACCGTGCAATGCAT
Pisum           ACCTTGTCTCACTATTGCCGGGATAATGGTCTACTTCTTCATATCCACCGTGCAATGCAT
Albizia         AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCATATCCATCGTGCAATGCAT
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Morella         GCAGTTATTGATAGACAGAAAAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGTTA
Myrica          GCAGTTATTGATAGACAGAAAAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGTTA
Canacomyrica    GCAGTTATTGATAGACAGAAAAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGTTA
Comptonia       GCAGTTATTGATAGACAGAAAAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGTTA
Alnus           GCAGTTATTGATAGACAGAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGTTA
Carpinus        GCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGTGTACTAGCTAAAGCGTTA
Juglans         GCAGTTATTGATAGACAGAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGTTA
Carya           GCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGTGTACTAGCTAAAGCGTTA
Rhoiptelea      GCAGTTATTGATAGACAGAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGTTA
Casuarina       GCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGTGTATTAGCTAAAGCGTTA
Fagus           GCAGTTATTGATAGACAGAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGTTA
Quercus         GCAGTTATTGATCGACAGAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCATTA
Trigonoba       GCAGTTATTGATCGACAGAAGAATCATGGTATACCCTTTCGTGTACTAGCTAAAGCATTA
Ticodendron     GCAGTTATTGATAGACAGAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGTTA
Allocasuarina   GCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGTGTATTAGCTAAAGCGTTA
Ceuthostoma     GCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGTGTATTAGCTAAAGCGTTA
Gymnostoma      GCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGTGTATTAGCTAAAGCGTTA
Nothofagus      GCAGTTATTGATAGACAGAATGATCATGGTATGCACTTTCGTGTACTAGCTGAAGCGTTA
Betula          GCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGTGTACTAGCTAAAGCGTTA
Celtis          GCAGTTATTGATAGACAAAAGAATCATGGTATGCACTTTCGTGTACTAGCTAAAGCGTTA
Corylus         GCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGTGTACTAGCTAAAGCGTTA
Chrysolepis     GCAGTTATTGATCGCCAGAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCATTA
Cecropia        GCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGCGTGCTAGCTAAAGCCTTA
Cucurbita       GCCGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGTTA
Datisca         GCAGTTATTGATAGACAGAAGAATCACGGTATACACTTTCGTGTACTAGCTAAAGCGTTA
Ficus           GCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGCGTACTAGCTAAAGCTTTA
Eleagnus        GCAGTTATTGATAGACAGAAAAATCATGGTATGCACTTCCGTGTACTGGCTAAAGGGTTA
Dryas           GCAGTTATCGATAGACAGAAGAATCATGGCATACACTTTCGTGTACTAGCTAAAGCGTTA
Humulus         GCAGTTATTGATAGACAAAAGAATCATGGTATACACTTTCGTGTACTAGCTAAAGCGTTA
Rhamnus         GCGGTTATTGATAGACAGAAAAATCATGGTATGCACTTTCGTGTACTAGCTAAAGCGTTA
Ulmus           GCAGTTATTGATAGACAGAAGAATCATGGTATGCAT????GTGTACTAGCTAAAGCGTTA
Rosa            GCTGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGTGTACTAGCTAAAGCATTA
Spirea          GCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTTCGTGTACTAGCTAAAGCGTTA
Pisum           GCAGTTATCGATAGACAAAAAAATCATGGTATGCACTTTCGTGTATTAGCTAAAGCCTTA
Albizia         GCAGTTATCGATAGACAGAAGAATCATGGTATGCACTTTCGTGTACTAGCTAAAGCGTTA
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Morella    CGTATGTCTGGTGGGGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Myrica     CGTATGTCTGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Canacomyrica CGTATGTCTGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Comptonia  CGTATGTCTGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Alnus      CGTATGTCTGGTGGAGATCATATTCACGCGGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Carpinus   CGCATGTCTGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Juglans    CGTATGTCTGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Carya      CGTATGTCTGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Rhoiptelea CGTATGTCTGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Casuarina  CGTCTATCCGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Fagus      CGTATGTCTGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Quercus    CGTATGTCTGGTGGAGATCATATTCATGCCGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Trigonoba  CGTATGTCTGGTGGAGATCATATTCATTCCGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Ticodendron CGTATGTCTGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Allocasuarina CGTCTGTCCGGTGGAGATCATATTCACGCCGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Ceuthostoma CGTCTGTCTGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Gymnostoma CGTCTGTCTGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Nothofagus CGTATGTCTGGTGGAGATCATATTCACGCCGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Betula     CGTATGTCTGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Celtis     CGTATGTCTGGTGGAGATCATATTCACGCTGGTACTGTAGTAGGTAAACTTGAAGGGGAA
Corylus    CGCATGTCTGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Chrysolepis CGTATGTCCGGTGGAGATCATATTCATTCCGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Cecropia   CGTATGTCTGGTGGAGATCATATTCACGCTGGTACTGTAGTAGGTAAACTTGAAGGGGAA
Cucurbita  CGTCTGTCTGGTGGAGACCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Datisca    CGTATGTCTGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
Ficus      CGTCTGTCTGGTGGAGATCATATTCACGCAGGTACTGTAGTAGGTAAACTTGAAGGGGAA
Eleagnus   CGTATGTCTGGTGGAGATCATATTCACGCTCGTACTGTAGTAGGTAAGCTTGAAGGGGAA
Dryas      CGTATGTCTGGTGGAGATCATATACACTCTGGTACCGTAGTAGGTAAACTTGAGGGGGAA
Humulus    CGTATGTCTGGTGGAGATCATATCCATGCGGGTACTGTAGTAGGTAAACTTGAAGGGGAA
Rhamnus    CGTATGTCTGGTGGAGATCATATTCACGCTGGTACTGTAGTGGGTAAACTTGAAGGGGAA
Ulmus      CGTCTGTCTGGTGGAGATCATATTCACGCTGGTACTGTAGTAGGTAAACTTGAAGGAGAA
Rosa       CGTATGTCTGGTGGAGATCATATCCACGCTGGTACCGTAGTAGGTAAACTTGAGGGGGAA
Spirea     CGTATGTCTGGTGGAGATCATATACACGCTGGTACCGTAGTAGGTAAACTTGAGGGGGAA
Pisum      CGTTTGTCTGGTGGAGATCATATTCACGCTGGTACTGTAGTAGGTAAACTTGAAGGAGAA
Albizia    CGTTTGTCTGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGGGAA
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Myrica                  AGAGACATCACTTTAGGCTTTGTTGATTTACTACGCGATGATTTTATTGAAAAAGATCGA
Canacomyrica            AGAGACATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTATTGAAAAAGATCGA
Comptonia               AGAGACATCACTTTAGGCTTTGTTGATTTACTACGCGATGATTTTATTGAAAAAGATCGA
Alnus                   AGAGAGATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATCGA
Carpinus                AGAGAGATCACTTTAGGCTTTGTTGATTTACTGCGTGATGATTATATTGAAAAAGATCGA
Juglans                 AGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTGTTGAAAAAGATCGA
Carya                   AGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTGTTGAAAAAGATCGA
Rhoiptelea              AGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTGTTGAAAAAGATAGA
Casuarina               AGAGATATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATCGA
Fagus                   AGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATCGA
Quercus                 AGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATCGA
Trigonoba               AGAGAAATCACTTTAGGCTTTGTTGATTTACTACGCGATGATTATATTGAAAAAGATCGA
Ticodendron             AGAGAGATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTATTGAAAAAGATCGA
Allocasuarina           AGAGATATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATCGA
Ceuthostoma             AGAGATATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATGTTGAAAAAGATCGA
Gymnostoma              AGAGAGATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATCGA
Nothofagus              AGAGACATCACTTTAGACTTTGTTGATTTACTACGCGATGATTTTATTGAAAAAGATCGA
Betula                  AGAGAGATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATCGA
Celtis                  AGAGAAATCACTTTAGGCTTTGTTGATTTACCACGTGATGATTTTATTGAAAAAGATCGA
Corylus                 AGAGAGATCACTTTAGGCTTTGTTGATTTACTGCGTGATGATTATATTGAAAAAGATCGA
Chrysolepis             AGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATCGA
Cecropia                AGAGAAATCACTTTAGGATTTGTTGATTTACTACGTGATGATTTTGTTGAAAAAGATCGA
Cucurbita               AGAGACATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTGTTGAAAAAGATCGA
Datisca                 AGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTATTGAAAAAGATCGA
Ficus                   AGAGAAATAACTTTAGGATTTGTTGATTTACTACGTGATGATTTTATTGAAAAAGATCGA
Eleagnus                AGAGAAATTACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGATCGA
Dryas                   AGAGAAATAACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTATTGAAAAAGATCGA
Humulus                 AGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTATTGAAAAAGATCGA
Rhamnus                 AGAGAGATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTGTTGATAAAGATCGA
Ulmus                   AGAGAGATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTGTTGAAAAAGATCGA
Rosa                    AGAGAAATCACTTTAGGCTTTGTTGATTTACTACGTGATGATTATATTGAAAAAGACCGA
Spirea                  AGAGAGATAACTTTAGGCTTTGTTGATTTACTACGTGATGATTTTATTGAAAAAGACAGA
Pisum                   AGGGAGATAACTTTAGGTTTTGTTGATTTACTACGTGATGATTATATTAAAAAAGATAGA
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Myrica     AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGGCT
Canacomyrica AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTTTGCCCGTGGCT
Comptonia  AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGGCT
Alnus      AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGGCT
Carpinus   AGCCGCGGTATTTATTTTACTCAAGATTGGGTCTCTCTACCAGGTGTTCTGCCCGTGGCT
Juglans    AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGGCT
Carya      AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGGCT
Rhoiptelea AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGGCT
Casuarina  AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCAGGCGTTCTGCCCGTGGCT
Fagus      AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCAGGTGTTCTGCCTGTGGCT
Quercus    AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTTTACCAGGTGTTCTGCCCGTGGCT
Trigonoba  AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTTTGCCAGGTGTTCTGCCCGTGGCT
Ticodendron AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGGCT
Allocasuarina AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCAGGTGTTCTGCCCGTGGCT
Ceuthostoma AGCCGTGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTATACCCGTGGCT
Gymnostoma AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGTGTTCTGCCCGTGGCT
Nothofagus AGCCGTGGTATTTATTTCACTCAAGATTGGGTTTCTCTACCAGGTGTTCTGCCCGTGGCT
Betula     AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCTGGCGTTCTGCCCGTGGCT
Celtis     AGCCGTGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCAGGTGTTCTGCCCGTGGCT
Corylus    AGCCGCGGTATTTATTTTACTCAAGATTGGGTCTCTCTACCAGGTGTTCTGCCCGTGGCT
Chrysolepis AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTTTACCAGGTGTTCTGCCCGTGGCT
Cecropia   AGCCGTGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCAGGTGTTCTGCCCGTG??T
Cucurbita  AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTTTACCAGGTGTTCTGCCAGTGGCT
Datisca    AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCAGGTGTTCTGCCTGTGGCT
Ficus      AGTCGGGGTATTTATTTCACTCAAGATTGGGTTTCTCTACCAGGTGTTCTGCCCGTGGCT
Eleagnus   AGCCGTGGTATTTATTTCACCCAAGATTGGGTCTCTCTACCGGGTGTTCTTCCTGTGGCT
Dryas      AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCAGGTGTTTTGCCTGTGGCT
Humulus    AGCCGTGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCAGGTGTTCTGCCCGTGGCT
Rhamnus    AGCCGTGGTATTTATTTCACTCAAGATTGGGTCTCTTTACCGGGTGTTCTGCCCGTGGCT
Ulmus      AGCCGTGGTATTTATTTCACTCAAGATTGGGTCTCTCTACCAGGTGTTCTACCCGTGGCT
Rosa       AGCCGCGGTATTTATTTTACTCAAGATTGGGTTTCTTTACCGGGTGTTTTACCTGTGGCT
Spirea     AGCCGCGGTATTTATTTCACTCAAGATTGGGTCTCTCTCCCAGGTGTTTTGCCTGTGGCT
Pisum      AGTCGCGGTATTTATTTCACTCAGGATTGGGTTTCTTTACCAGGTGTTATCCCTGTTGCT
Albizia    AGCCGCGGTATTTATTTCACTCAGGATTGGGTCTCTCTACCGGGTGTTCTGCCCGTTGCT
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Myrica            TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Canacomyrica      TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Comptonia         TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Alnus             TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Carpinus          TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Juglans           TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Carya             TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Rhoiptelea        TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Casuarina         TCAGGAGGTATTCATGTTTGGCATATGCCCGCTCTGACCGAAATCTTTGGAGATGATTCC
Fagus             TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Quercus           TCAGGGGGTATTCACGTTTGGCATATGCCTGCTTTGACCGAAATCTTTGGAGATGATTCC
Trigonoba         TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Ticodendron       TCAGGAGGTATTCATGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Allocasuarina     TCAGGAGGTATTCATGTTTGGCATATGCCCGCTCTGACCGAAATCTTTGGAGATGATTCC
Ceuthostoma       TCAGGAGGTATTCATGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Gymnostoma        TCAGGAGGTATTCATGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCT
Nothofagus        TCAGGGGGTATTCACGTTTGGCATATGCCCGCTCTGACCGAAATCTTTGGGGATGATTCC
Betula            TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Celtis            TCAGGGGGTATTCATGTTTGGCATATGCCTGCTTTGACCGAGATCTTTGGAGATGATTCC
Corylus           TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGATGATTCC
Chrysolepis       TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAAATCTTTGGAGACGATTCC
Cecropia          TCAGGGGGTATTCATGTTTGGCATATGCTG?CTTTGACCGAGATCTTTGGAGATGATTCC
Cucurbita         TCCGGTGGTATTCACGTTTGGCATATGCCTGCTCTAACCGAGATTTTTGGAGATGATTCT
Datisca           TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGATCTTTGGAGATGATTCC
Ficus             TCAGGGGGTATTCACGTTTGGCATATGCCTGCTTTGACCGAGATCTTTGGAGATGACTCC
Eleagnus          TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGATCTTTGGAGATGATGCC
Dryas             TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGATCTTTGGAGATGATTCT
Humulus           TCAGGGGGTATTCACGTTTGGCATATGCCCGCTTTGACCGAGATCTTTGGAGATGATTCC
Rhamnus           TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGATCTTTGGAGACGATTCC
Ulmus             TCAGGGGGTATTCATGTTTGGCATATGCCTGCTCTGACCGAGATCTTTGGAGATGATTCC
Rosa              TCCGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGATCTTTGGAGATGATTCT
Spirea            TCCGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGATCTTTGGAGATGATTCT
Pisum             TCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGATATTTGGAGATGATTCT
Albizia           TCGGGGGGTATTCACGTTTGGCATATGCCTGCTCTTACCGAGATCTTTGGAGATGATTCC
```

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                                                                      2
                        5         6         7         8         9         0
                        12345678901234567890123456789012345678901234567890
Morella                 GTACTACAATTCGGCGGAGGAACTTTAGGACACCCTTGGGGAAATGCACCTGGTGCCGTA
Myrica                  GTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCTGGTGCTGTA
Canacomyrica            GTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCTGGTGCCGTA
Comptonia               GTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCTGGTGCCGTA
Alnus                   GTACTACAATTCGGTGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCTGGTGCTGTA
Carpinus                GTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCGGGTGCTGTA
Juglans                 GTACTACAATTCGGTGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCCGGTGCCGTA
Carya                   GTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCCGGTGCCGTA
Rhoiptelea              GTACTACAATTCGGCGGAGGAACTTTGGGGCACCCTTGGGGAAATGCACCTGGTGCCGTA
Casuarina               GTACTACAATTCGGCGGAGGAACTTTAGGGCATCCTTGGGGAAATGCACCCGGTGCTGTA
Fagus                   GTACTACAATTTGGTGGAGGAACTTTGGGGCACCCTTGGCGAAATGCACCTGGTGCCGTA
Quercus                 GTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCCGGTGCTGTA
Trigonoba               GTACTACAATTTGGCGGAGGAACTTTGGGGCACCCTTGGGGAAATGCACCTGGTGCCGTA
Ticodendron             GTATTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCCGGTGCTGTA
Allocasuarina           GTACTACAATTCGGCGGAGGAACTTTAGGGCATCCTTGGGGAAATGCACCCGGTGCTGTA
Ceuthostoma             GTACTACAATTCGGCGGAGGAACTTTAGGGCATCCTTGGGGAAATGCACCTGGTGCTGTA
Gymnostoma              GTACTACAATTCGGCGGAGGAACTTTAGGGCATCCTTGGGGAAATGCACCTGGTGCTGTA
Nothofagus              GTATTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCTGGCGCCGTG
Betula                  GTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCCGGTGCTGTA
Celtis                  GTACTACAATTCGGCGGAGGAACTTTAGGACATCCTTGGGGAAATGCACCCGGTGCCGTA
Corylus                 GTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCGGGTGCTGTA
Chrysolepis             GTACTACAATTTGGCGGAGGAACTTTGGGGCACCCTTGGGGAAATGCACCTGGTGCCGTA
Cecropia                GTACTACAATTCGGTGGAGGGACTTTAGGACATCCTTGGGGAAATGCACCCGG????GTA
Cucurbita               GTACTACAATTCGGCGGAGGAACTTTGGGGCACCCTTGGGGTAATGCACCAGGTGCCGTA
Datisca                 GTACTACAATTCGGTGGAGGAACTTTAGGGCACCCTTGGGGTAATGCACCCGGTGCCGTA
Ficus                   GTACTACAA??????????????????????????????????????????????????
Eleagnus                GTACTACAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCTGGTGCTGTA
Dryas                   GTACTACAATTCGGCGGCGGAACTTTAGGACACCCATGGGGAAATGCACCTGGTGCCGTA
Humulus                 GTACTACAATTTGGTGGAGGAACTTTAGGACATCCTTGGGGAAATGCACCCGGTGCTGTC
Rhamnus                 GTACTACAATTCGGCGGAGGAACTATAGGGCACCCTTGGGGAAATGCACCCGGTGCCGTA
Ulmus                   GTACTCCAATTCGGCGGAGGAACTTTAGGGCACCCTTGGGGAAATGCACCCGGTGCTGTA
Rosa                    GTACTACAATTCGGTGGAGGAACTTTAGGACACCCTTGGGGAAATGCACCAGGTGCCGTA
Spirea                  GTACTACAATTTGGCGGCGGAACTTTAGGACACCCTTGGGGAAATGCACCCGGTGCCGTA
Pisum                   GTACTCCAATTCGGTGGAGGAACTTTAGGACACCCTTGGGGAAATGCACCTGGTGCCGTA
Albizia                 GTACTACAATTCGGGGGGGGAACTTTAGGGCACCCTTGGGGAAATGCACCCGGTGCCGTA
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                1         2         3         4         5         6
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Myrica GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Canacomyrica GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Comptonia GCTAATCGAGTAGCTCTCGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Alnus GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Carpinus GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Juglans GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Carya GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Rhoiptelea GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Casuarina GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGGCGCGATCTTGCT
Fagus GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Quercus GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Trigonoba GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Ticodendron GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Allocasuarina GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGGCGCGATCTTGCT
Ceuthostoma GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGGCGTGATCTTGCT
Gymnostoma GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCCCGTAATGAGGGACGCAATCTTGCT
Nothofagus GCTAATCGAGTCGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGCGACCTTGCT
Betula GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Celtis GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCT
Corylus GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Chrysolepis GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Cecropia GCTAATCGAGTAGCTCTAGAAGCATGTGTMCAA?CTCGTAATGAGGGACGTGATCTTGCT
Cucurbita GCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Datisca GCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Ficus ????????????????????????????????????????????????????????????
Eleagnus GCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGGCGTGATCTTGCT
Dryas GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Humulus GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Rhamnus GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Ulmus GCTAATCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCT
Rosa GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTCGCT
Spirea GCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGTGATCTTGCT
Pisum GCGAATCGAGTAGCTCTGGAAGCATGTGTACAAGCTCGGAATGAGGGACGTGATCTTGCT
Albizia GCTAACCGAGTAGCTCTAGAAGCATGTGTACAGGCTCGTAATGAGGGACGTGATCTTGCT
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                                                                3
                          7         8         9         0         1         2
                1234567890123456789012345678901234567890123456789012345678901234567890
Morella         CGTGAGGGTAATGAAATTATTCGTGAGGCTAGTAAATGGAGTCCTGAGCTAGCTGCTGCT
Myrica          CGTGAGGGTAATGAAATTATTCGTGAGGCTAGTAAATGGAGTCCTGAGCTAGCTGCTGCT
Canacomyrica    CGTGAGGGTAATGAAATTATTCGTGAGGCTAGTAAATGGAGTCCTGAGCTAGCTGCTGCT
Comptonia       CGTGAGGGTAATGAAATTATTCGTGAGGCTAGTAAATGGAGTCCTGAGCTAGCTGCTGCT
Alnus           CGTGAGGGTAATGAAATTATTCGTGAGGCTGGTAAATGGAGTCCTGAGCTAGCTGCCGCT
Carpinus        CGTGAGGGTAATGAAATTATTCGTGCGGCTGCTAAATGGAGTCCTGAGCTAGCTGCCGCT
Juglans         CGTGAGGGTAATGAAATTATTCGTGAGGCTAGTAAATGGAGTCCTGAGCTAGCGGCTGCT
Carya           CGTGAGGGTAATGAAATTATTCGTGAGGCTAGTAAATGGAGTCCTGAGCTAGCGGCTGCT
Rhoiptelea      CGTGAGGGTAATGAAATTATTCGCGAGGCTAGTAAATGGAGTCCTGAGCTAGCGGCTGCT
Casuarina       CGTGAGGGTAATGAAATTATTCGTGA????????????????????????????????????
Fagus           CGTGAGGGTAATGAAATTATTCGTGAGGCTAGTAAATGGAGTCCTGAGCTAGCTGCTGCT
Quercus         CGTGAGGGTAATGAAATTATCCGTGAGGCTGCTAAATGGAGTCCTGAGCTAGCTGCTGCT
Trigonoba       CGTGAGGGTAATGAAATTATTCGTCAGGCTGCTAAATGGAGTCCTGAGCTAGCTGCTGCT
Ticodendron     CGTGAGGGTAATGAAATTATTCGTGAGGCTAGTAAG????????????????????????
Allocasuarina   CGTGAGGGTAATGAAATTATTCGTGA????????????????????????????????????
Ceuthostoma     CGCGAGGGTAATGAAATTATTCGTGA????????????????????????????????????
Gymnostoma      CGTGAGGGTAATGAAATTATTCGTGA????????????????????????????????????
Nothofagus      CGTGAGGGTAATGAAATTATACGTGAGGCTAGTAAATGGAGTCCTGAGCTAGCTGCTGCT
Betula          CGTGAGGGTAATGAAATTATTCGTGAGGCTGCTAAATGGAGTCCTGAGCTAGCTGCCGCT
Celtis          CGTGAGGGTAATGAAATTATTCGTGAGGCTAGTAAATGGAGTCCTGAACTAGCTGCTGCT
Corylus         CGTGAGGGTAATGAAATTATTCGTGCGGCTGCTAAATGGAGTCCTGAGCTAGCTGCCGCT
Chrysolepis     CGTGAGGGTAATGAAATTATCCGTGAGGCTGCTAAATGGAGTCCTGAGCTAGCTGCTGCT
Cecropia        CGTGAGGGTAATGAAATTATTCGTGAGGCTAGTAAATGGAGTCCTGAACTAGCTGCTGCT
Cucurbita       CGTGAGGGTAATGAAATTATCCGTGAGGCTAGTAAATGGAGTCCTGAACTAGCTGCTGCT
Datisca         CGTGAGGGTAATGAAATTAACCGTGAGGCTACTAAATGGAGTCCAGAACTAGCTGCTGCT
Ficus           ????????????????????????????????????????????????????????????
Eleagnus        CGTGAGGGTAATGRAATTATTCGTGAGGCTTGTAAATGGAGTCCTGAACTAGCTGCTGCT
Dryas           CGTGAGGGTAATGAAATTATTCGTGAGGCTACTAAATGGAGTCCTGAACTAGCTGCTGCT
Humulus         CGTGAGGGTAATGAAATTATTCGTGAGGCTTGTAAATGGAGTCCTGAACTAGCTGCTGCT
Rhamnus         CGTGAAGGTAATGAAATTATTCGTGAGGCTAGTAAATGGAGTCCTGAACTAGCTGCTGCT
Ulmus           CGTGAGGGTAATSAAATTATTCGTGAGGCTAGTAAATGGAGTCCTGAACTAGCTGCTGCT
Rosa            CGTGAGGGTAATGAAATTATTCGTGAGGCTAGTAAATGGAGTCCTGAACTAGCTGCCGCT
Spirea          CGTGAGGGTAATGAAATTATTCGTGAGGCTTGTAAATGGAGTCCTGAACTAGCGGCTGCG
Pisum           CGCGAGGGTAATGCAATTATCCGTGAAGCTTGCAAATGGAGTCCTGAATTAGCTGCTGCT
Albizia         CGTGAGGGTAATGAAATTATTCGTGAGGCTAGCAAATGGAGTCCTGAATTAGCTGCTGCT
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                          3         4
                 12345678901234567890123
Morella          TGTGAAGTATGGAAGGAGATCAA
Myrica           TGTGAAGTATGGAAGGAGATCAA
Canacomyrica     TGTGAAGTATGGAAGGAGATCAA
Comptonia        TGTGAAGTATGGAAGGAGATCAA
Alnus            TGTGAAGTATGGAAGGAGATCAA
Carpinus         TGTGAAGTATGGAAGGAGATCAA
Juglans          TGTGAAGTATGGAAGGAGATCAA
Carya            TGTGAAGTATGGAAGGAGATCAA
Rhoiptelea       TGTGAAGTATGGAAGGAGATCAA
Casuarina        ???????????????????????
Fagus            T??????????????????????
Quercus          TGTGAAGTATGGAAAGAGATCAA
Trigonoba        TGTGAAGTATGGAAAGAGATCAA
Ticodendron      ???????????????????????
Allocasuarina    ???????????????????????
Ceuthostoma      ???????????????????????
Gymnostoma       ???????????????????????
Nothofagus       T??????????????????????
Betula           TGTGAAGTATGGAAGGAGATCAA
Celtis           TGTGAAGTGTGGAAGGAAATCAA
Corylus          TGTGAAGTATGGAAGGAGATCAA
Chrysolepis      TGTGAAGTATGGAAAGAGATCAA
Cecropia         TGTGAAGTATGGAAGGAGATCAA
Cucurbita        TGTGAAGTATGGAAGGCGATCAA
Datisca          TGTGAAGTATGGAAGGAGATCAA
Ficus            ???????????????????????
Eleagnus         TGTGAAGTATGGAAGGAGATCAA
Dryas            TGTGAAGTATGGAAGGAGATCAA
Humulus          TGTGAAGTTTGGAAGGAAATCAA
Rhamnus          TGTGAAGTATGGAAGGAGATTAA
Ulmus            TGTGAAGTATGGAAGGAGATTAA
Rosa             TGTGAGGTATGGAAAGAGATCAA
Spirea           TGTGAAGTATGGAAGGAGATCAA
Pisum            TGTGAAGTCTGGAAGGAAATCAA
Albizia          TGTGAAGTATGGAAAGAAAT???
```

APPENDIX 4

Sequence alignment for Chapter 6

Sequence alignment for Data set I

```
                          *[ITS starts]
                                   1         2         3         4         5         6
                          12345678901234567890123456789012345678901234567890123456789012345678890
M. spathulata             CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. pilulifera             CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. integra                CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. cordifolia             CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. kraussiana             CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. diversifolia           ??????????????????????TAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. serrata                CAGAACGACCCGCGAACATGTTAATAACTATCGGGGGCAGGCGGCGATCAAAAGCCTCCG
M. salicifolia            CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. brevifolia             CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. quercifolia            CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. humilis                CAGAACGACCCGCGAACATGTCAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. rivas-martinezii       CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. faya                   ?????????CCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. carolinensis           CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. californica            CAGAACGACCCGCGAACATGTTAATAACTTTCGGGGGCAGGGGGCGATCAAAAGCCTCCT
M. inodora                CAGAACGACCCGCGAACATGTTAATAACTTCCGGGGGCAGGGGGCGATCAAAAGCCTCCT
M. cerifera (USA)         CAGAACGACCCGCGAACATGTTAATAACTACTGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. cerifera (Belize)      CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCG
M. punctata               ????????????????????????????????????????????????CAAAAGCCTCCG
M. pavonis                CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATATAAAGCCTCAT
M. parvifolia             CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATAAAAAGCCTCAT
M. pubescens              ??????????????????????AATAACTACCGGGGGCAGGGGGCGATAAAAAGCCTCAT
M. nana                   CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCC
M. javanica               ????????????????????TGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCC
M. rubra (China)          ???AACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCC
M. rubra (Japan)          CAGAACGACCCGCGAACATGTTAATAACTACTGGGGGCAGGGGGCGATCAAAAGCCTCCC
M. rubra (Taiwan)         CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCC
M. adenophera (China)     CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGAGGGCGATCAAAAGCCTCCC
M. adenophera (Taiwan)    CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCAGGGGGCGATCAAAAGCCTCCC
M. esculenta              CAGAACGACCCGTGAACATGTTAATAACTACCGGGGGCGGGGGGCGATCAAAAGCCTCCT
M. gale (Japan)           CAGAACGACCCGCGAACATGTTAATAACTACCGCGGGCGGGGGGCGATCAAAAGCCTCCC
M. gale (UK)              ???AACGACCCGCGAACATGTTAATAACTACCGGGGGCGGGGGGCGATCAAAAGCCTCCC
M. gale var. tomentosa    CAGAACGACCCGCGAACATGTTAATAACTACCGCGGGCGGGGGGCGATCAAAAGCCTCCC
M. hartwegii              CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCGGGGGGCGATGAAAAGCCTCCC
C. peregrina              CAGAACGACCCGCGAACATGTTAATAACTACCGGGGGCGGGCGGCGATCAAAAGCCTCCC
C. monticola              ????????????????????TGT-CATAAC-ACCCGAGGAAGGGGGCGATCACAAGCCTCCC
J. nigra                  CAGAACGACCTGTGAACATGTAATAATAACCTTCTGGGTGGGGGTGTAATGCCCCC----
J. cinerea                CAGAACGACCCGTGAACATGTAATAA---CCTTCTGGGTGGGTGTGTAATGCCCCC----
E. roxburghiana           CAGAACGACCCGTGAACTTGTGTAACAACCTCCGGGCGGGGGTGTCATGCCCCCTC----
R. chiliantha             CAGAACGACCCGTGAACCTGTCACAACAATCGGGGCGGGGGCATGATGTCCCTCG-----
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                          7         8         9         0         1         2
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M. pilulifera    GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. integra       GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. cordifolia    GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. kraussiana    GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. diversifolia  GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. serrata       GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. salicifolia   GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. brevifolia    GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. quercifolia   GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. humilis       GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. rivas-martinezii GTCCCC-AAAACGGTTGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. faya          GTCCCC-AAAACGGTTGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. carolinensis  GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. californica   GTCCCC-AAAACGGTTGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. inodora       GTCCCC-AAAACGGATGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. cerifera (USA) GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. cerifera (Belize) GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. punctata      GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. pavonis       GTCCCC-AAAACGGTCGGGCCCAACCGAACCGAACAACGAACCCCGGCGCGGACTGCGCC
M. parvifolia    GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. pubescens     GTCCCC-AAAACGGTCGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. nana          GTCCCC-AAAACGGTTGGGCCCANCCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. javanica      GTCCCC-AAAACGGTTGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. rubra (China) GTCCCC-AAAACGGTTGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. rubra (Japan) GTCCCC-AAAACGGTTGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. rubra (Taiwan) GTCCCC-AAAACGGTTGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. adenophera (China) GTCCCC-AAAACGGTTGGGCCCAACCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. adenophera (Taiwan) GTCCCC-AAAACGGTTGGGCCCAACCAAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. esculenta     GTCCCC-AAAACGGTTGGGCCCAATCAAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. gale (Japan)  GTCCCC-AAAACGGTTGGGCCCAGCCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. gale (UK)     GTCCCC-AAAACGGTTGGGCCCAGCCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. gale var. tomentosa GTCCCC-AAAACGGTTGGGCCCAGCCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
M. hartwegii     GTCCCC-AAAACGGTTGGGCCCAGCCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
C. peregrina     GTCCCC-AAAACGGTTGGGCCCAGCCGAA-----CAACGAACCCCGGCGCGGACTGCGCC
C. monticola     GTCCTC-GACACGGTTGGGCCCCGCCGAA-----CAACGAACCCCGGCGCGGAATGCGCC
J. nigra         -TCCCARAAAACGGTTGGGCCCGATCGAA-----CAATGAACCCCGGCGCGGTCTGCGCC
J. cinerea       -TCCC-AAAAACGGTTGGCCCCAATCGAA-----CAACGAACCCCGGCG--GTCTGCGCC
E. roxburghiana  -TCG---AAAACGGATGGGCCCAGCCGAA-----CAACGAACCCCGGCGCGGTCTGCGCC
R. chiliantha    -TCCCC-AAAACGGTCGGGCCCGGCCGAA-----CAACGAACCCCGGCGCGGACCGCGCC
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                          3         4         5         6         7         8
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M. spathulata    AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. pilulifera    AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. integra       AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. cordifolia    AAGGAACTTCAACAAAAGAGTGCCTCCGATTGCCCTGTTATACAAAAACGACTCTCGGCA
M. kraussiana    AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. diversifolia  AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. serrata       AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. salicifolia   AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. brevifolia    AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. quercifolia   AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCT
M. humilis       AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. rivas-martinezii AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. faya          AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. carolinensis  AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. californica   AAGGAACTTCAACAAAAGAGTGCCTCTGATTGCCCTGTTATACAAAA-CGACTCTCGGCA
M. inodora       AAGGAACTTCAACAAAAGAGTGCCTCTGATTGCCCTGTTATACAAAA-CGACTCTCGGCA
M. cerifera (USA) AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. cerifera (Belize) AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. punctata      AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. pavonis       AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. parvifolia    AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. pubescens     AAGGAACTTCAACAAAAGAGTGCCTCCGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
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M. gale var. tomentosa AAGGAATTTCAACAAAAGAGTGCCTCTGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
M. hartwegii     AAGGAATTTCAACAAAAGAGTGCCTCTGATCGCCCTGTTATACAAAA-CGACTCTCGGCA
C. peregrina     AAGGAACTTCAACAAAAGAGTGCCTCCGGTCGCCCTGTTATACAAAA-CGACTCTCGGCA
C. monticola     AAGGAACATGAACATAAGAGTGCCTCCAGCCGCCCTGTATTACAAAA-CGACTCTCGGCA
J. nigra         AAGGAACTTAAACAA-GGAGTAACCACGGGCGCCCCATGATACATAA-CGACTCTCGGCA
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R. chiliantha    AAGGAATTAAAACGAAAGAGTACCTGCGGCCGCCC--TGATACATA--CGACTCTCGC-A
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C. peregrina	ACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAA
C. monticola	ACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAA
J. nigra	ACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAA
J. cinerea	ACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAA
E. roxburghiana	ACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAA
R. chiliantha	ACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAA

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J. nigra                  TTGCAGAATCCCGCGAATCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGC
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M. rivas-martinezii CGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCAACCCC-AAACACCTCGCAAG
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M. carolinensis  CGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCAACCCC-AAACACCTCGCAAG
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J. nigra         CGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCAACCCC-AAACACTTCTTACG
J. cinerea       CGAGG-CACGTCTGCCTGGGTGTCACGCATCGTTGCCCCAACCCC-AGACACTTCTCATG
E. roxburghiana  CGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCAACCCC-ATCACCT-CTCACG
R. chiliantha    CGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCAAACCC--ACCGCATCCCAAT
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M. integra                      AGGGAGTTC1-GGGGAATA1-T-CGGGGCGGACATTGGCCTCCCGTGAGCCAGTTCTCGC
M. cordifolia                   AGGGAGTTC1-GGGGAATA1-T-CGGGGCGGACATTGGCCTCCCGTGAGCCAGTTCTCGC
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M. diversifolia                 AGGGAGTTC1-GGGGAATA1-T-CGGGGCGGACATTGGCCTCCCGTGAGCCAGTTCTCGC
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J. cinerea                      ATTTGT-----GGGG------TGCGGGGCAGACATTGGCCTCCCGTGTGCTTCTGCTCGC
E. roxburghiana                 CGGCAT-----GGGG------TGCGGGGCAGACATTGGCCTCCCGTGCGCTTTCGCTCGC
R. chiliantha                   GGATGC-----GGGCGGC--TTGTTGGGCGGACATTGGCCTCCCGTGCGCTGTAGCTCGC
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M. gale (Japan)           GGTTAGCCTAAATACGAGTCCTCGGCGACGAGCGCCACGACAATCGGTGGTTGATAAA0G
M. gale (UK)              GGTTAGCCTAAATACGAGTCCTCGGCGACGAGCGCCACAACAATCGGTGGTTGATAAA0G
M. gale var. tomentosa    GGTTAGCCTAAATACGAGTCCTCGGCGACGAGCGCCACGACAATCGGTGGTTGATAAA0G
M. hartwegii              GGTTAGCCTAAATACGAGTCCTCGGCGACGAGCGCCACAACAATCGGTGGTTGATAAA0G
C. peregrina              GGTTAGCCTAAATACGAGTCCTCGGCGACGAGCGCCACGACAATCGGTGGTTGATAAA0G
C. monticola              GGTTAGCCTAAACATGAGTCCTCGGCGATGAACGCCACGACAATCGGTGGTTGTCAAG1-
J. nigra                  GGTTAGCCTAAAAGTGAGTCCTAGGCGACGAGCGCCACGACAATCGGTGGTTGAGAAA1-
J. cinerea                GGTTAGCCTAAAAGCGAGTCCTGGGCGACGAGCGCCACGACAATCGGTGGTTGAGAAA1-
E. roxburghiana           GGTTAGCCTAAAAACGAGTCCTAGGCGGCGAGCGCCACGACAATCGGTGGTTGTCAAG0A
R. chiliantha             GGTTAGCCTAAAAGTGAGTCTTAGGCGACGAGCGCCACGACAATCGGTGGTCGAAAAA0A
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                                                            [trnL-F starts]*
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                                   9        0         1         2         3         4
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M. spathulata            CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGTTCTCTGTGA-CCAAATTCAG
M. pilulifera            CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGTTCTCTGTGA-CC????????
M. integra               CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGTTCTCTGTGA-CCAAATTCAG
M. cordifolia            CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGTTCTCTGTGA-CCAAATTCAG
M. kraussiana            CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGTTCTCTGTGA-CCAAATTCAG
M. diversifolia          CCCTCGTTTCCCGTCG????????????????????????????????????AAATTCAG
M. serrata               CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGTTCTCTGTGA-CCAAATTCAG
M. salicifolia           CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGTTCTCTGTGA-CCAAATTCAG
M. brevifolia            CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGTTCTCTGTGA-CCAAATTCAG
M. quercifolia           CCCTCGTTTCCCGTCGTGCGAGCCTCGTCTCCCTATGCGTTCTCTGTGATCCAAATTCAG
M. humilis               CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGTTCTCTGTGA-CCAAATTCAG
M. rivas-martinezii      CCCTCGTTTCCCGTCGTGGGTGCGTCGTCTCCATATGCGTTCTCTGTGA-CCAAATTCAG
M. faya                  CCCTCGTTTCCCGTCGTGGGTGCGTCGTCTCCATATGCGTTCTCTGTGA-CCAAATTCAG
M. carolinensis          CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGTTCTCTGTGA-CCAAATTCAG
M. californica           CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTA?????????????????AAATTCAG
M. inodora               CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTACGTGTTCTCTGTGA-CCAAATTCAG
M. cerifera (USA)        CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGTTCTCTGTGA???AAATTCAA
M. cerifera (Belize)     CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGTTCTCTGTGA-CCAAATTCAG
M. punctata              CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGCGCTCTCTGTGA-CC?????CAG
M. pavonis               CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCTTATGTGTTCTCTGTGA-CCAAATTCAG
M. parvifolia            CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCTTATGTGTTCTCTGTGA-CC????????
M. pubescens             CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCTTATGTGTTCTCTGTGA-CCAAATTCAG
M. nana                  CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGTGCGCTCTGTGA-CCAAATTCAG
M. javanica              CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGTGCGCTCTGTGA-CCAAATTCAG
M. rubra (China)         CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGTGCGCTCTGTGA-CCAAATTCAG
M. rubra (Japan)         CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGTGCGCTCTGTGA-CC???TTCAG
M. rubra (Taiwan)        CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGTGCGCTCTGTGA-CCAAATTCAG
M. adenophera (China)    CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGTGCGCTCTGTGA-CCAAATTCAG
M. adenophera (Taiwan)   CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGTGCGCTCTGTGA-CCAAATTCAG
M. esculenta             CCCTCGTTTCCCGTCGTGCGTGCCTCGTCTCCCTATGTGCGCTCTGTGA-CCAAATTCAG
M. gale (Japan)          CCCTCGTTTCCAGT??????????????????????????????????????AAATTCAG
M. gale (UK)             CCCTCGTTTCCAGTCGTGCGCGCCTCATCGCCCTAAGTGTGCTCCGTGATCC???TTCAG
M. gale var. tomentosa   CCCTCGTTTCCAGTCGTGCGCGCCTCGTCGCCCTACGTGTGCTCCGTGA-CCAAATTCAG
M. hartwegii             CCCTCGTTTCCAGTCGTGCGCGCCTCGTCGCCCTACGTGTGCTCTGTGA-CCAAATTCAG
C. peregrina             CCCTCGTTTCCCGTCGTGCGCGCCTTGTCGCCCTATGTGCGCTCCGTGA-CCAAATTCAG
C. monticola             CCCTCGTGTCCCGTCGTGAGCGTCGCGTCGTCTATTTTGTGCTCTTTGA-CCAAATTCAG
J. nigra                 CCCTCGTGACCCGTCGTGTGTCGCCCGTCGCTGTGAAGGTGCTCCTCGA-CC????????
J. cinerea               CCCTCGTGACCCGTCGTGTGTTGCCCGTCGCTGTGAAGGTGCTCCTAGA-CC????????
E. roxburghiana          CCCTCGTGACCCGTCGTGCGCTGCCCGTCGCCGTGAAGGCGCTCCTCGG-CC????????
R. chiliantha            CCCTCGTTTCCCGTCGTGTGTGTCCCGTCGCTCATTTTGTGCTCTGCGA-CC????????
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	123456789012345678901234567890123456789012345678901234567890
M. spathulata	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. pilulifera	???????????????????????GCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. integra	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. cordifolia	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. kraussiana	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. diversifolia	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCAGTTTTCTGAAAACAAAT
M. serrata	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. salicifolia	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. brevifolia	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. quercifolia	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. humilis	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. rivas-martinezii	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. faya	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. carolinensis	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCCGAAAACAAAT
M. californica	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. inodora	AGAAACCCTGGAATGAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. cerifera (USA)	AAAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCCGAAAACAAAT
M. cerifera (Belize)	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCCGAAAACAAAT
M. punctata	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCCGAAAACAAAT
M. pavonis	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCCGAAAACAAAT
M. parvifolia	??AAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCCGAAAACAAAT
M. pubescens	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCCGAAAACAAAT
M. nana	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. javanica	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. rubra (China)	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. rubra (Japan)	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. rubra (Taiwan)	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. adenophera (China)	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. adenophera (Taiwan)	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. esculenta	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. gale (Japan)	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. gale (UK)	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. gale var. tomentosa	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
M. hartwegii	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
C. peregrina	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCGGTTTTCTGAAAACAAAT
C. monticola	AGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCTGTTTTCCGAAAACAAAT
J. nigra	??
J. cinerea	??
E. roxburghiana	??
R. chiliantha	??

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                 1234567890123456789012345678901234567890123456789012345678890
M. spathulata    AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. pilulifera    AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. integra       AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. cordifolia    AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. kraussiana    AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. diversifolia  AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. serrata       AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. salicifolia   AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. brevifolia    AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. quercifolia   AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. humilis       AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. rivas-martinezii AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. faya          AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. carolinensis  AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. californica   AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. inodora       AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. cerifera (USA) AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. cerifera (Belize) AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. punctata      AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. pavonis       AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. parvifolia    AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGT-GTTC
M. pubescens     AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. nana          AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. javanica      AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. rubra (China) AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. rubra (Japan) AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. rubra (Taiwan) AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. adenophera (China) AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. adenophera (Taiwan) AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. esculenta     AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. gale (Japan)  AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. gale (UK)     AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. gale var. tomentosa AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
M. hartwegii     AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
C. peregrina     AA-GGGTTCATAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
C. monticola     TAAGGGTTCAGAAAGCGAGAATCAAAAAGGATAGGTGCAGAGACTCAATGGAAGTTGTTC
J. nigra         ??????????????????????????????????????????????????????????
J. cinerea       ??????????????????????????????????????????????????????????
E. roxburghiana  ??????????????????????????????????????????????????????????
R. chiliantha    ??????????????????????????????????????????????????????????
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                                 7         8         9         0         1         2
                        123456789012345678901234567890123456789012345678901234567890
M. spathulata           TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. pilulifera           TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. integra              TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. cordifolia           TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. kraussiana           TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. diversifolia         TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. serrata              TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. salicifolia          TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. brevifolia           TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. quercifolia          TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. humilis              TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. rivas-martinezii     TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. faya                 TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. carolinensis         TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. californica          TAACAAA-----TGGGGTTGACTTCGTGCCGTTATT------------AAAGTAATCCTT
M. inodora              TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. cerifera (USA)       TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. cerifera (Belize)    TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. punctata             TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. pavonis              TAACAAA-----TGGGGTTGACTTCGTTCCTTTATT------------AAAGTAATCCTT
M. parvifolia           TAACAAA-----TGGGGTTGACTTCGTTCCTTTATT------------AAAGTAATCCTT
M. pubescens            TAACAAA-----TGGGGTTGACTTCGTTCCTTTATT------------AAAGTAATCCTT
M. nana                 TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. javanica             TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. rubra (China)        TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. rubra (Japan)        TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. rubra (Taiwan)       TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. adenophera (China)   TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. adenophera (Taiwan)  TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. esculenta            TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. gale (Japan)         TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. gale (UK)            TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. gale var. tomentosa  TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
M. hartwegii            TAACAAA-----TGGGGTTGACTTCGTTCCGTTATT------------AAAGTAATCCTT
C. peregrina            TAACAAA-----TGGGGTTGACTTCGTTCCGTTATTAAATGAAAGTATAAAGTAATCCTT
C. monticola            TAACAAAACAAATGGGGTTAACTTCGTTCCGTTATT---------------AAAATCCTT
J. nigra                ????????????????????????????????????????????????????????????
J. cinerea              ????????????????????????????????????????????????????????????
E. roxburghiana         ????????????????????????????????????????????????????????????
R. chiliantha           ????????????????????????????????????????????????????????????
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M. spathulata    CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. pilulifera    CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. integra       CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACGTATACGTA
M. cordifolia    CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. kraussiana    CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. diversifolia  CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. serrata       CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. salicifolia   CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. brevifolia    CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. quercifolia   CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. humilis       CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. rivas-martinezii CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. faya          CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. carolinensis  CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. californica   CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. inodora       CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. cerifera (USA) CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. cerifera (Belize) CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. punctata      CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. pavonis       CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. parvifolia    CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. pubescens     CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. nana          CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. javanica      CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. rubra (China) CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. rubra (Japan) CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. rubra (Taiwan) CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. adenophera (China) CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. adenophera (Taiwan) CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. esculenta     CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. gale (Japan)  CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. gale (UK)     CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. gale var. tomentosa CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
M. hartwegii     CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
C. peregrina     CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
C. monticola     CTATCAAAACTACAGAAAGGATGAAGGATAAACCTATATACATACGTATACG------TA
J. nigra         ????????????????????????????????????????????????????????????
J. cinerea       ????????????????????????????????????????????????????????????
E. roxburghiana  ????????????????????????????????????????????????????????????
R. chiliantha    ????????????????????????????????????????????????????????????
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                                         8
                               9         0         1         2         3         4
                          123456789012345678901234567890123456789012345678901234567890
M. spathulata             CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. pilulifera             CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. integra                CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. cordifolia             CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. kraussiana             CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. diversifolia           CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. serrata                CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. salicifolia            CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. brevifolia             CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. quercifolia            CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. humilis                CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. rivas-martinezii       CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. faya                   CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. carolinensis           CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. californica            CTGAAATACTATCCCAGAGCTAATTAATAACGACCTGAATCTTTATTGCTTTGTTTCTAT
M. inodora                CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. cerifera (USA)         CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTATTTTACTAT
M. cerifera (Belize)      CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. punctata               CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. pavonis                CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. parvifolia             CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. pubescens              CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. nana                   CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. javanica               CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. rubra (China)          CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. rubra (Japan)          CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. rubra (Taiwan)         CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. adenophera (China)     CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. adenophera (Taiwan)    CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. esculenta              CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. gale (Japan)           CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. gale (UK)              CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. gale var. tomentosa    CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
M. hartwegii              CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
C. peregrina              CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
C. monticola              CTGAAATACTATCCCAAC--TAATTAATAACGACCTGAATCTTTATTTCTTTTTTTCTAT
J. nigra                  ????????????????????????????????????????????????????????????
J. cinerea                ????????????????????????????????????????????????????????????
E. roxburghiana           ????????????????????????????????????????????????????????????
R. chiliantha             ????????????????????????????????????????????????????????????
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                                  5         6         7         8         9         0
                         123456789012345678901234567890123456789012345678901234567890
M. spathulata            TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. pilulifera            TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. integra               TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. cordifolia            TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. kraussiana            TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. diversifolia          TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. serrata               TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. salicifolia           TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. brevifolia            TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. quercifolia           TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. humilis               TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. rivas-martinezii      TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. faya                  TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. carolinensis          TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. californica           TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. inodora               TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. cerifera (USA)        TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. cerifera (Belize)     TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. punctata              TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. pavonis               TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. parvifolia            TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. pubescens             TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. nana                  TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. javanica              TTCATATTTATATAAATAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. rubra (China)         TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. rubra (Japan)         TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. rubra (Taiwan)        TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. adenophera (China)    TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. adenophera (Taiwan)   TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. esculenta             TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. gale (Japan)          TTCATATTTATA----TAAAAACGGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. gale (UK)             TTCATATTTATA----TAAAAACGGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. gale var. tomentosa   TTCATATTTATA----TAAAAACGGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
M. hartwegii             TTCATATTTATA----TAAAAACGGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
C. peregrina             TTCATATTTATA----TAAAAACTGAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
C. monticola             TTCATATTTATA----TAAAAACTAAAAGAGTTGTTGTGAATCGATCCAAATTGAAGAAA
J. nigra                 ????????????????????????????????????????????????????????????
J. cinerea               ????????????????????????????????????????????????????????????
E. roxburghiana          ????????????????????????????????????????????????????????????
R. chiliantha            ????????????????????????????????????????????????????????????
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                          1         2         3         4         5         6
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M. spathulata    GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. pilulifera    GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. integra       GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. cordifolia    GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. kraussiana    GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. diversifolia  GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. serrata       GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. salicifolia   GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. brevifolia    GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. quercifolia   GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. humilis       GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. rivas-martinezii GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. faya          GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. carolinensis  GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. californica   GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. inodora       GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. cerifera (USA) GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. cerifera (Belize) GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. punctata      GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. pavonis       GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. parvifolia    GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. pubescens     GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. nana          GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. javanica      GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. rubra (China) GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. rubra (Japan) GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. rubra (Taiwan) GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. adenophera (China) GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. adenophera (Taiwan) GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. esculenta     GAATCGA-TTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. gale (Japan)  GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. gale (UK)     GAATCGAATTG----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. gale var. tomentosa GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
M. hartwegii     GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
C. peregrina     GAATCGAATTT----------AATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
C. monticola     GAATCGAATCGAATATTTATTAATCAAATTATTTACTCCACATAGTCTGATAGATCTTTT
J. nigra         ???????????????????????????????????????????????????????????
J. cinerea       ???????????????????????????????????????????????????????????
E. roxburghiana  ???????????????????????????????????????????????????????????
R. chiliantha    ???????????????????????????????????????????????????????????
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                                                               0
                                 7         8         9         0         1         2
                        123456789012345678901234567890123456789012345678901234567890
M. spathulata           GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. pilulifera           GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. integra              GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. cordifolia           GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. kraussiana           GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. diversifolia         GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. serrata              GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. salicifolia          GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. brevifolia           GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. quercifolia          GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. humilis              GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. rivas-martinezii     GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. faya                 GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. carolinensis         GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. californica          GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. inodora              GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. cerifera (USA)       GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. cerifera (Belize)    GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. punctata             GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. pavonis              GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. parvifolia           GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. pubescens            GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. nana                 GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. javanica             GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. rubra (China)        GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. rubra (Japan)        GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. rubra (Taiwan)       GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. adenophera (China)   GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. adenophera (Taiwan)  GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. esculenta            GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. gale (Japan)         GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. gale (UK)            GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. gale var. tomentosa  GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
M. hartwegii            GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
C. peregrina            GAAGAACTAATTAATCGGATGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCGA
C. monticola            GAAGAACTAATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACCAA
J. nigra                ????????????????????????????????????????????????????????????
J. cinerea              ????????????????????????????????????????????????????????????
E. roxburghiana         ????????????????????????????????????????????????????????????
R. chiliantha           ????????????????????????????????????????????????????????????
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                          3         4         5         6         7         8
                 12345678901234567890123456789012345678901234567890123456789012345678890
M. spathulata    CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. pilulifera    CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. integra       CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. cordifolia    CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. kraussiana    CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. diversifolia  CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. serrata       CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. salicifolia   CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAAAAATCGTGAGGGTTCAA
M. brevifolia    CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. quercifolia   CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. humilis       CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. rivas-martinezii CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. faya          CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. carolinensis  CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. californica   CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. inodora       CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. cerifera (USA) CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. cerifera (Belize) CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. punctata      CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. pavonis       CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. parvifolia    CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTNAGAAATCGTGAGGGTTCAA
M. pubescens     CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. nana          CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCA-
M. javanica      CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. rubra (China) CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. rubra (Japan) CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. rubra (Taiwan) CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. adenophera (China) CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. adenophera (Taiwan) CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. esculenta     CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. gale (Japan)  CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. gale (UK)     CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. gale var. tomentosa CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
M. hartwegii     CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
C. peregrina     CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
C. monticola     CAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAA
J. nigra         ????????????????????????????????????????????????????????????
J. cinerea       ????????????????????????????????????????????????????????????
E. roxburghiana  ????????????????????????????????????????????????????????????
R. chiliantha    ????????????????????????????????????????????????????????????
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                                      1
                            9         0         1         2         3         4
                            123456789012345678901234567890123456789012345678901234567890
M. spathulata               GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. pilulifera               GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. integra                  GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. cordifolia               GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. kraussiana               GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. diversifolia             GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. serrata                  GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. salicifolia              GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. brevifolia               GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. quercifolia              GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. humilis                  GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. rivas-martinezii         GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTATTTATCCAATCTG
M. faya                     GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTATTTATCCAATCTG
M. carolinensis             GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. californica              GTCCCTCTATCCCCAAAAAGGCCGGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. inodora                  GTCCCTCTATCCCCAAAAAGGCCGGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. cerifera (USA)           GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. cerifera (Belize)        GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. punctata                 GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. pavonis                  GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. parvifolia               GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. pubescens                GTACCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. nana                     GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. javanica                 GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. rubra (China)            GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. rubra (Japan)            GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. rubra (Taiwan)           GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. adenophera (China)       GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. adenophera (Taiwan)      GTCCCTCTATCCCCCAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. esculenta                GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. gale (Japan)             GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. gale (UK)                GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. gale var. tomentosa      GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
M. hartwegii                GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
C. peregrina                GTCCCTCTATCCCCAAAAAGGCCCGTTT------GACTCCGTAATTCTTTATCCAATCTG
C. monticola                GTCCCTCTATCCCCAAAAAGGCCCGTTTCCGTTTGACTCCGTAATTCTTTATCCAATCTG
J. nigra                    ??????????????????????????TT------GACTCCGTAATTATTTATCCGATCTG
J. cinerea                  ??????????????????????????TT------GACTCCGTAATTATTTATCCGATCTG
E. roxburghiana             ??????????????????????????TT------GACTCCGTAATTATTTATCCGATCTG
R. chiliantha               ??????????????????????????TT------GA-TCCGTAATTATTTATCCGATCTG
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	5 6 7 8 9 0
	123456789012345678901234567890123456789012345678901234567890
M. spathulata	CTCTTTTCATT0ATTTTCATTAGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. pilulifera	CTCTTTTCATT0ATTTTCATTAGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. integra	CTCTTTTCATT0ATTTTCATTAGCGGTTTAAAATTCGTTATGTTTCTCGCTGATTCTACT
M. cordifolia	CTCTTTTCATT0ATTTTCATTAGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. kraussiana	CTCTTTTCATT0ATTTTCATTAGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTTTACT
M. diversifolia	CTCTTTTCATT0ATTTTCATTAGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. serrata	CTCTTTTCATT0ATTTTCATTAGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. salicifolia	CCCTTTTCATT0ATTTTCATTAGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. brevifolia	CTCTTTTCATT0ATTTTCATTAGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. quercifolia	CTCTTTTCATT0ATTTTCATTAGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. humilis	CTCTTTTCATT0ATTTTCATTAGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. rivas-martinezii	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. faya	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTTTACT
M. carolinensis	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. californica	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. inodora	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. cerifera (USA)	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. cerifera (Belize)	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. punctata	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. pavonis	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. parvifolia	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. pubescens	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. nana	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. javanica	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. rubra (China)	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. rubra (Japan)	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. rubra (Taiwan)	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. adenophera (China)	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. adenophera (Taiwan)	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. esculenta	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. gale (Japan)	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. gale (UK)	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. gale var. tomentosa	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
M. hartwegii	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
C. peregrina	CTCTTTTCATT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
C. monticola	CTCTTTTCGTT1---------AGCGGTTTCAAATTCGTTATGTTTCTCGCTGATTCTACT
J. nigra	CTCTTTTCTTT-----TCGTTAGCGGTTTCAAATTTGTTATGTTTCTCACTGATTTGACT
J. cinerea	CTCTTTTCTTT-----TCGTTAGCGGTTTCAAATTTGTTATGTTTCTCACTGATTTGACT
E. roxburghiana	CTCTTTTCTTT-----TCGTTAGTGGTTTAAAATTCGTTATATTTCTCACTGATTTGACT
R. chiliantha	CTCTTTTATTT-----TCGTTAGCGGTTTAAAATTCGTTATGTTTCTCACTGATTTTACT

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M. spathulata    CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. pilulifera    CTTTTACAAATGGATC-GATTGTAAATCGTTTTTTTCTTATTA----------CAA-GATT
M. integra       CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. cordifolia    CTTTTACAAATGGATC-GATTGTAAATCGTTTTTTTCTTATTA----------CAA-GATT
M. kraussiana    CTTTTACAAATGGATC-GATTGTAAATCGTTTTTTTCTTATTA----------CAA-GATT
M. diversifolia  CTTTTACAAATGGATC-GATTGTAAATCGTTTTTTTCTTATTA----------CAA-GATT
M. serrata       CTTTTACAAATGGATC-GATTGTAAATCGTTTTTTTCTTATTA----------CAATGATT
M. salicifolia   CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. brevifolia    CTTTTACAAATGGATC-GATTGTAAATCGTTTTTTTCTTATTA----------CAA-GATT
M. quercifolia   CTTTTACAAATGGATC-GATTGTAAATCGTTTTTTTCTTATTA----------CAA-GATT
M. humilis       CTTTTACAAATGGATC-GATTGTAAATCGTTTTTTTCTTATTA----------CAA-GATT
M. rivas-martinezii CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. faya          CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. carolinensis  CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. californica   CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. inodora       CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. cerifera (USA) CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. cerifera (Belize) CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. punctata      CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. pavonis       CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. parvifolia    CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. pubescens     CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. nana          CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. javanica      CTTTTCCAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. rubra (China) CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA---------ACAA-GATT
M. rubra (Japan) CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. rubra (Taiwan) CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. adenophera (China) CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. adenophera (Taiwan) CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. esculenta     CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
M. gale (Japan)  CTTTTACAAATGGATC-GATTGTAAATCGTTTTTTTCTTATTA----------CAA-GATT
M. gale (UK)     CTTTTACAAATGGATC-GATTGTAAATCGTTTTTTTCTTATTA----------CAA-GATT
M. gale var. tomentosa CTTTTACAAATGGATC-GATTGTAAATCGTTTTTTTCTTATTA----------CAA-GATT
M. hartwegii     CTTTTACAAATGGATC-GATTGTAAATCGTTTTTTTCTTATTA----------CAA-GATT
C. peregrina     CTTTTACAAATGGATC-GATTGTAAATTGTTTTTTTCTTATTA----------CAA-GATT
C. monticola     CTTTTACAAATGGATC-GATTGTCAATTGTTTTTTTCTTATTA----------CAA-GATT
J. nigra         CTTTTACAAATGGAGCCAATTGGGAA-TTTTTTTTCTTATTTTTTCTTCTTACAA-TATT
J. cinerea       CTTTTACAAATGGAGCCAATTGGGAATTTTTTTTTCTTATTTTTTCTTCTTACAA-TATT
E. roxburghiana  CTTTTACAAATGGAACCGATTGGGAGTTTTTTTTTCTTATTTTTTCTTATTACAA-TATT
R. chiliantha    CTTTTACAAATGGAGCCGATTGGAATTTTTTTTTTCTTATTTTTTCTTATTACAA-TATT
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M. pilulifera             TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. integra                TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. cordifolia             TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. kraussiana             TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. diversifolia           TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. serrata                TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. salicifolia            TGTGATATATATGATACATGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
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M. quercifolia            TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. humilis                TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. rivas-martinezii       TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. faya                   TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. carolinensis           TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. californica            TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. inodora                TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. cerifera (USA)         TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. cerifera (Belize)      TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. punctata               TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. pavonis                TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. parvifolia             TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. pubescens              TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. nana                   TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
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M. rubra (China)          TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. rubra (Japan)          TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. rubra (Taiwan)         TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. adenophera (China)     TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
M. adenophera (Taiwan)    TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
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M. gale (Japan)           TGTGATATATATGATACACGTACAAATGAACATCCTTGAAGAAGGTATCCCTACTTCAAA
M. gale (UK)              TGTGATATATATGATACACGTACAAATGAACATCCTTGAAGAAGGTATCCCTACTTCAAA
M. gale var. tomentosa    TGTGATATATATGATACACGTACAAATGAACATCCTTGAAGAAGGTATCCCTACTTCAAA
M. hartwegii              TGTGATATATATGATACACGTACAAATGAACATCCTTGAAGAAGGTATCCCTACTTCAAA
C. peregrina              TGTGATATATATGATACACGTACAAATGAACATCCTTGAATAAGGTATCCCTACTTCAAA
C. monticola              TGTGATATATATGATACA-GTACAAATGAACATCCTTGAATAAGGTACCCCTACTTCAAA
J. nigra                  TGTGATATATATGATACAAGTACAAATAAACATCTTTGAGTAAGGTATCCCCACTTCAAA
J. cinerea                TGTGATATATATGATACAAGTACAAATAAACATCTTTGAGTAAGGTATCCCCACTTCAAA
E. roxburghiana           TGTGATATATATGATACAAGTACAAATAAACATCTTTGAGTAAGGTATCCCTACTTCAAA
R. chiliantha             TGTGATATATATGATACAAGTACAAATAAACATCTTTGAGTAAGGTATCCCCACTTCAAA
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M. pilulifera    TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
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M. cordifolia    TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. kraussiana    TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. diversifolia  TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. serrata       TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. salicifolia   TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. brevifolia    TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. quercifolia   TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. humilis       TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. rivas-martinezii TGTTAATGATTAACAGTA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. faya          TGTTAATGATTAACAGTA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. carolinensis  TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. californica   TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. inodora       TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. cerifera (USA) TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. cerifera (Belize) TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. punctata      TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. pavonis       TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. parvifolia    TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. pubescens     TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. nana          TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. javanica      TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
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M. rubra (Japan) TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. rubra (Taiwan) TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. adenophera (China) TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. adenophera (Taiwan) TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. esculenta     TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. gale (Japan)  TGTTAATGATTAACAATA-----CATATCATCTTTGGTACTGTACTAAAACTTATAAAGT
M. gale (UK)     TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
M. gale var. tomentosa TGTTAATGATTAACAATA-----CATATCATCTTTGGTACTGTACTAAAACTTATAAAGT
M. hartwegii     TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
C. peregrina     TGTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATACAGT
C. monticola     TTTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
J. nigra         TTTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATCAAGT
J. cinerea       TTTTAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTTATCAAGT
E. roxburghiana  TTTGAATGATTAACAATA-----CATATCATCTCTGGTACTGTACTAAAACTGATCCAGT
R. chiliantha    TTTTAATGATTAACAATACAATACATATCATCTCTGGTACTGTACTAAAACTTATAAAGT
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M. pilulifera           TTTCTTTTTGAAAATACAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. integra              TTTCTTTTTGAAAATACAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. cordifolia           TTTCTTTTTGAAAATACAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. kraussiana           TTTATTTTTGAAAATACAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. diversifolia         TTTCTTTTTGAAAAT-CAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. serrata              TTTCTTTTTGAAAATACAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. salicifolia          TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. brevifolia           TTTCTTTTTGAAAATACAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. quercifolia          TTTCTTTTTGAAAATACAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. humilis              TTTCTTTTTGAAAATACAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. rivas-martinezii     TTTCTTTTTGAAAATCCAAGACATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. faya                 TTTCTTTTTGAAAATCCAAGACATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. carolinensis         TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. californica          TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. inodora              TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. cerifera (USA)       TTTTTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. cerifera (Belize)    TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. punctata             TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. pavonis              TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. parvifolia           TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. pubescens            TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTGTAA--TTTTTTT
M. nana                 TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTTTCA--TTTTTTT
M. javanica             TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTTTAA--TTTTTTT
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M. rubra (Taiwan)       TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTTTAA--TTTTTTT
M. adenophera (China)   TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTTTAA--TTTTTTT
M. adenophera (Taiwan)  TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTT0TAGATAAGACTTTTTAA--TTTTTTT
M. esculenta            TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTTTAA--TTTTTTT
M. gale (Japan)         TTTCTTTTTAAAAACCCAAGAAATTCCAGAAGA1---------CTTTGTAA--TTTTTTT
M. gale (UK)            TTTCTTTTTAAAAACCCAAGAAATTCCAGAAGA1---------CTTTGTAA--TTTTTTT
M. gale var. tomentosa  TTTCTTTTTAAAAACCCAAGAAATTCCAGAAGA1---------CTTTGTAA--TTTTTTT
M. hartwegii            TTTCTTTTTAAAAACCCAAGAAATTCCAGAAGA1---------CTTTGTAA--TTTTTTT
C. peregrina            TTTCTTTTTGAAAATCTAAGAAATTCCAGGGTC0TAGATAAGACTTTGTCA--TTTTTTT
C. monticola            TTTCTTTTTGAAAATCCAAGAAATTCCAGGGTC0TAGATAAGACTTTGTCA--TTTTTTT
J. nigra                TTTCTTTTTAAAGATCCAAGAAATTCCAGGATC0TAGATAAGATTTTGTAATATTTATTT
J. cinerea              TTTCTTTTTAAAGATCCAAGAAATTCCAGGATC0TAGATAAGATTTTGTAATATTTATTT
E. roxburghiana         TTTCTTTTTAAAGATCCAAGAAATTCCAGGATT0TAGATAAGATTTTTTAATATTTTTTT
R. chiliantha           TTTCTTTTTGAAGATCCAAGAAATTCCAGGATC0TAGATAAGACTTTGTAATATTTTTTT
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M. pilulifera            T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. integra               T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. cordifolia            T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. kraussiana            T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. diversifolia          T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. serrata               T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. salicifolia           T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. brevifolia            T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. quercifolia           T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. humilis               T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. rivas-martinezii      T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATT??????????????????????
M. faya                  T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. carolinensis          TT-GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. californica           TT-GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. inodora               T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCT???
M. cerifera (USA)        TT-GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. cerifera (Belize)     TTTGTCTTTTTAATTGACTTAAATTCAAGTCATCTATTAAAATAAGGATGATGCCCTGGT
M. punctata              T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. pavonis               T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. parvifolia            T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. pubescens             T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. nana                  T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGTATGATGCGCTGGG
M. javanica              T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGTATGATGCGCTGGG
M. rubra (China)         T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGTATGATGCGCTGGG
M. rubra (Japan)         T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGTATGATGCGCTGGG
M. rubra (Taiwan)        T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGTATGATGCGCTGGG
M. adenophera (China)    T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGTATGATGCGCTGGG
M. adenophera (Taiwan)   T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGTATGATGCGCTGGG
M. esculenta             T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGTATGATGCGCTGGG
M. gale (Japan)          T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. gale (UK)             T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGAT???????????
M. gale var. tomentosa   T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
M. hartwegii             T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
C. peregrina             T--GTCTTTTTAATTGACATAAATTCAAGTCATCTATTAAAATAAGGATGATGC??????
C. monticola             T--GTCTTTTTAATTGACATAAACCCAAGTCATCTATTAAAATAAGGATGATGCGCTGGT
J. nigra                 T--GTCTTTTTAATTGACATAAACCCAAGTCATCTATTAAAATAAGGATGATACGCCAGA
J. cinerea               T--GTCTTTTTAATTGACATAAACCCAAGTCATCTATTAAAATAAGGATGATACGCCAGA
E. roxburghiana          TT-GTCTTTTTAATTGACATAAACCCAAGTCATCTATTAAAATAAGGATGATACGCCAGA
R. chiliantha            TG-GTCTTTTTAATTGACATAAACCCAAGTCATCTATTAAAATAAGGATGATACGCCAGA
```

	1
	12345678901
M. spathulata	??????????
M. pilulifera	??????????
M. integra	AATGGTCGGGA
M. cordifolia	AATGGTCGGGA
M. kraussiana	AATGGTCGGGA
M. diversifolia	AATGGTCGGGA
M. serrata	AATGGTCGGGA
M. salicifolia	AATGGTCGGGA
M. brevifolia	AATGGTCGGGA
M. quercifolia	AATGGTCGGGA
M. humilis	AATGGTCGGGA
M. rivas-martinezii	??????????
M. faya	??????????
M. carolinensis	AATGGTCGGGA
M. californica	AATGGTCGGGA
M. inodora	??????????
M. cerifera (USA)	AATGGTCGGGA
M. cerifera (Belize)	AATGGTCGGGA
M. punctata	AATGGTCGGGA
M. pavonis	AATGGTCGGGA
M. parvifolia	AATGGTCGGGA
M. pubescens	AATGGTCGGGA
M. nana	AATGGTCGGGA
M. javanica	AATGGTCGGGA
M. rubra (China)	AATGGTCGGGA
M. rubra (Japan)	??????????
M. rubra (Taiwan)	AATGGTCGGGA
M. adenophera (China)	AATGGTCGGGA
M. adenophera (Taiwan)	AATGGTCGGGA
M. esculenta	AATGGTCGGGA
M. gale (Japan)	AATGGTCGGGA
M. gale (UK)	??????????
M. gale var. tomentosa	AATGGTCGGGA
M. hartwegii	AATGGTCGGGA
C. peregrina	??????????
C. monticola	AATGGTCGGGA
J. nigra	AATGGTCGGGA
J. cinerea	AATGGTCGGGA
E. roxburghiana	AATGGTCGGGA
R. chiliantha	AATGGTCGGGA

APPENDIX 5

Distance matrix for Chapter 6

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	-																																							
2	0.00146	-																																						
3	0.00142	0.00145	-																																					
4	0.00213	0.00072	0.00212	-																																				
5	0.00285	0.00145	0.00283	0.00212	-																																			
6	0.00368	0.00225	0.00365	0.00292	0.00365	-																																		
7	0.00356	0.00217	0.00353	0.00282	0.00353	0.00441	-																																	
8	0.00356	0.00362	0.00353	0.00425	0.00497	0.00516	0.00568	-																																
9	0.00213	0.00073	0.00212	0.00141	0.00211	0.00292	0.00282	0.00424	-																															
10	0.00357	0.00220	0.00354	0.00283	0.00353	0.00366	0.00424	0.00567	0.00284	-																														
11	0.00213	0.00071	0.00212	0.00141	0.00212	0.00295	0.00282	0.00425	0.00141	0.00283	-																													
12	0.00881	0.00897	0.00878	0.00952	0.01028	0.00684	0.01100	0.00950	0.00950	0.01098	0.00951	-																												
13	0.00955	0.00971	0.00949	0.01024	0.00950	0.00751	0.01172	0.01022	0.01022	0.01168	0.01024	0.00073	-																											
14	0.00359	0.00363	0.00357	0.00430	0.00502	0.00524	0.00572	0.00429	0.00428	0.00572	0.00429	0.00802	0.00875	-																										
15	0.01412	0.01433	0.01390	0.01316	0.01544	0.01584	0.01464	0.01470	0.01466	0.01614	0.01469	0.01584	0.01643	0.01322	-																									
16	0.01167	0.01109	0.01160	0.01087	0.01312	0.01208	0.01385	0.01237	0.01235	0.01383	0.01237	0.01329	0.01397	0.01092	0.00662	-																								
17	0.00796	0.00665	0.00789	0.00864	0.00863	0.00972	0.01009	0.00864	0.00863	0.01010	0.00864	0.01252	0.01322	0.00427	0.01768	0.01540	-																								
18	0.00430	0.00434	0.00427	0.00500	0.00572	0.00599	0.00642	0.00499	0.00498	0.00643	0.00499	0.00801	0.00947	0.00212	0.01394	0.01163	0.00644	-																							
19	0.00447	0.00452	0.00444	0.00520	0.00595	0.00534	0.00519	0.00519	0.00519	0.00668	0.00445	0.00758	0.00826	0.00220	0.01070	0.00979	0.00592	0.00293	-																						
20	0.01013	0.01029	0.01004	0.01079	0.01153	0.01046	0.01224	0.01078	0.01077	0.01223	0.01078	0.01248	0.01314	0.00787	0.01618	0.01309	0.01226	0.00858	0.00735	-																				
21	0.00946	0.00957	0.00938	0.01013	0.01088	0.00976	0.01159	0.01012	0.01011	0.01158	0.01012	0.01181	0.01249	0.00719	0.01555	0.01242	0.01015	0.00790	0.00663	0.00214	-																			
22	0.01036	0.01055	0.01028	0.01105	0.01181	0.01050	0.01252	0.01103	0.01103	0.01250	0.01031	0.01274	0.01332	0.00806	0.01647	0.01332	0.01251	0.00878	0.00740	0.00289	0.00219	-																	
23	0.01165	0.01259	0.01227	0.01229	0.01376	0.01203	0.01449	0.01302	0.01300	0.01447	0.01302	0.01253	0.01470	0.01157	0.01398	0.01165	0.01603	0.01228	0.00970	0.01450	0.01387	0.01479	-																
24	0.01403	0.01506	0.01463	0.01539	0.01614	0.01277	0.01689	0.01539	0.01539	0.01688	0.01540	0.01500	0.01702	0.01390	0.01706	0.01471	0.01844	0.01465	0.01198	0.01686	0.01624	0.01694	0.00801	-															
25	0.01092	0.01186	0.01154	0.01157	0.01303	0.01124	0.01377	0.01230	0.01228	0.01375	0.01230	0.01180	0.01393	0.01086	0.01326	0.01093	0.01530	0.01158	0.00895	0.01377	0.01314	0.01400	0.00071	0.00724	-														
26	0.01241	0.01337	0.01311	0.01314	0.01463	0.01290	0.01537	0.01389	0.01386	0.01534	0.01387	0.01331	0.01547	0.01242	0.01491	0.01243	0.01541	0.01314	0.00984	0.01538	0.01475	0.01566	0.00214	0.00880	0.00143	-													
27	0.01090	0.01184	0.01152	0.01155	0.01301	0.01124	0.01375	0.01228	0.01226	0.01372	0.01227	0.01177	0.01394	0.01084	0.01324	0.01092	0.01528	0.01156	0.00895	0.01375	0.01312	0.01402	0.00071	0.00724	0.00000	0.00143	-												
28	0.01162	0.01259	0.01224	0.01227	0.01373	0.01198	0.01448	0.01301	0.01298	0.01445	0.01300	0.01253	0.01469	0.01156	0.01397	0.01165	0.01602	0.01228	0.00894	0.01449	0.01386	0.01476	0.00142	0.00798	0.00071	0.00215	0.00071	-											
29	0.01537	0.01640	0.01596	0.01600	0.01749	0.01588	0.01823	0.01675	0.01672	0.01820	0.01674	0.01630	0.01847	0.01377	0.01776	0.01538	0.01826	0.01600	0.01349	0.01824	0.01763	0.01858	0.00500	0.01165	0.00428	0.00575	0.00427	0.00499	-										
30	0.01240	0.01337	0.01301	0.01378	0.01452	0.01200	0.01525	0.01379	0.01375	0.01522	0.01378	0.01329	0.01545	0.01235	0.01473	0.01238	0.01682	0.01305	0.00896	0.01452	0.01390	0.01401	0.00571	0.00871	0.00500	0.00649	0.00499	0.00571	0.00791	-									
31	0.01994	0.01870	0.01968	0.01891	0.01968	0.01991	0.02048	0.02053	0.01811	0.01963	0.01891	0.01945	0.02004	0.01742	0.02285	0.01914	0.02203	0.01977	0.01657	0.02207	0.02145	0.02247	0.01902	0.02231	0.01902	0.02079	0.01898	0.01975	0.02130	0.01982	-								
32	0.02413	0.02310	0.02414	0.02335	0.02414	0.01946	0.02493	0.02497	0.02259	0.02407	0.02335	0.02590	0.02666	0.02181	0.02459	0.02254	0.02656	0.02337	0.02273	0.02648	0.02590	0.02693	0.02179	0.02355	0.02178	0.02340	0.02178	0.02257	0.02412	0.02259	0.00377	-							
33	0.02326	0.02206	0.02296	0.02219	0.02296	0.01912	0.02373	0.02379	0.02141	0.02287	0.02218	0.02509	0.02568	0.02067	0.02326	0.02084	0.02533	0.02297	0.02078	0.02526	0.02470	0.02579	0.02143	0.02475	0.02145	0.02320	0.02141	0.02217	0.02369	0.02226	0.00074	0.00439	-						
34	0.02245	0.02128	0.02214	0.02138	0.02214	0.01910	0.02292	0.02296	0.02061	0.02207	0.02137	0.02432	0.02488	0.01987	0.02322	0.02007	0.02450	0.02215	0.02076	0.02370	0.02314	0.02417	0.02063	0.02238	0.02066	0.02242	0.02062	0.02139	0.02291	0.02145	0.00371	0.00366	0.00358	-					
35	0.01689	0.01721	0.01687	0.01768	0.01843	0.01374	0.01766	0.01767	0.01763	0.01834	0.01767	0.01859	0.01934	0.01464	0.01875	0.01768	0.01919	0.01616	0.01289	0.01916	0.01849	0.01952	0.01163	0.01630	0.01311	0.01461	0.01308	0.01382	0.01608	0.01386	0.01684	0.01854	0.01773	0.01849	-				
36	0.06348	0.06563	0.06357	0.06453	0.06541	0.05320	0.06536	0.06364	0.06451	0.06530	0.06361	0.06326	0.06309	0.05916	0.06529	0.06421	0.06452	0.06176	0.05821	0.06167	0.06222	0.06101	0.05748	0.06355	0.05911	0.06148	0.05910	0.05997	0.06171	0.06184	0.06142	0.06886	0.06845	0.06749	0.05991	-			
37	0.16710	0.17325	0.17075	0.17245	0.17075	0.15296	0.17506	0.16868	0.17062	0.17646	0.17029	0.16707	0.16931	0.16582	0.16447	0.17158	0.16642	0.16920	0.14459	0.16316	0.16716	0.16696	0.17218	0.17914	0.17069	0.17316	0.17024	0.17245	0.17617	0.16680	0.16714	0.18136	0.18009	0.18405	0.16875	0.17499	-		
38	0.15846	0.16447	0.16185	0.16354	0.16185	0.14535	0.16538	0.16022	0.16183	0.16768	0.16142	0.15859	0.16090	0.15725	0.15557	0.16292	0.15763	0.16066	0.14047	0.15491	0.15899	0.16012	0.16143	0.17192	0.16008	0.16270	0.15965	0.16192	0.16544	0.15661	0.15756	0.17224	0.17054	0.17470	0.15980	0.17409	0.02250	-	
39	0.19533	0.20146	0.19462	0.20052	0.19870	0.17678	0.19842	0.19630	0.19847	0.20251	0.19815	0.19577	0.19784	0.19311	0.18393	0.19595	0.19802	0.19671	0.16989	0.19471	0.19683	0.19729	0.18978	0.19842	0.19049	0.19713	0.18994	0.19225	0.19442	0.18635	0.18861	0.19793	0.19987	0.19862	0.17956	0.17882	0.10223	0.09947	-
40	0.16695	0.17283	0.16672	0.17207	0.17044	0.16013	0.17064	0.16828	0.17013	0.17588	0.16825	0.17172	0.17403	0.16640	0.17058	0.17054	0.16898	0.16984	0.13954	0.16317	0.16745	0.16699	0.17048	0.17581	0.17125	0.17572	0.17075	0.17301	0.17483	0.16904	0.17496	0.18409	0.18414	0.18057	0.16618	0.15786	0.11244	0.11822	0.14621	-

Key

1 *Morella spathulata*
2 *Morella pilulifera*
3 *Morella integra*
4 *Morella cordifolia*
5 *Morella kraussiana*
6 *Morella diversifolia*
7 *Morella serrata*
8 *Morella salicifolia*
9 *Morella brevifolia*
10 *Morella quercifolia*
11 *Morella humilis*
12 *Morella rivas-martinezii*
13 *Morella faya*
14 *Morella carolinensis*
15 *Morella californica*
16 *Morella inodora*
17 *Morella cerifera* (USA)
18 *Morella cerifera* (Belize)
19 *Morella punctata*
20 *Morella pavonis*
21 *Morella parvifolia*
22 *Morella pubescens*
23 *Morella nana*
24 *Morella javanica*
25 *Morella rubra* (China)
26 *Morella rubra* (Japan)
27 *Morella rubra* (Taiwan)
28 *Morella adenophora* (China)
29 *Morella adenophora* (Taiwan)
30 *Morella esculenta*
31 *Myrica gale* (Japan)
32 *Myrica gale* (UK)
33 *Myrica gale* var. *tomentosa* (USA)
34 *Myrica hartwegii*
35 *Comptonia peregrina*
36 *Canacomyrica monticola*
37 *Juglans nigra*
38 *Juglans cinerea*
39 *Engelhardia roxburghiana*
40 *Rhoiptelea chiliantha*

APPENDIX 6

Population genetic study of *Morella faya* using RAPDs
(pilot study)

INTRODUCTION

Morella faya (Aiton) Wilbur (Myricaceae) is a small tree considered to be endemic to Macaronesia. The Macaronesian region is an Atlantic archipelago of widely separated island groups comprising the Cape Verde Islands, the Canary Islands, the Salvage Islands, Madeira and the Azores. Of these predominantly volcanic islands, the Azores are the most northerly and most distant from a continental landmass (1600 km from the Iberian Peninsula), whilst the Canary Islands are the closest (115 km from the coast of north Africa). The Canary Islands, in particular, are renowned for their rich plant life with 520 endemic vascular species (28% of the total flora; Sunding, 1979).

Madeira, the Azores and the volcanic isles of the Canary Islands are home to a community known as laurel forest or 'laurisilva' (González Henríquez et al., 1986). Dominant tree species in the laurel forest include *Laurus azorica* (Seub.) Franco, *Apollonias barbusana* A. Braun, *Persea indica* Spreng., *Ocotea foetens* Benth. & Hook. (Lauraceae), *Picconia excelsa* DC. (Oleaceae) and *Ilex canariensis* Poir. (Aquifoliaceae). All these species are broadleaf evergreens restricted on Madeira and the Canaries to a high altitudinal zone where the semi-permanent cloud layer provides them with the high levels of precipitation that they require. On the Azores where the climate is wetter there is a less pronounced zonation of vegetation (pers. obs.).

Morella faya occurs throughout Macaronesia, except the low lying Cape Verde islands and the arid easterly Canary Islands (Chevalier, 1901). Throughout its natural range, the distribution of *M. faya* appears to be restricted by its low tolerance of drought. The species occurs in laurel forest (Schmid, 1976) and is co-dominant with *Erica arborea* L. (Ericaceae) in the fayal-brezal community type which is found adjacent to the laurel forest at higher altitudes (Schmid, 1976; González Henríquez et al., 1986). A number of populations of *M. faya* are found in central and southwest mainland Portugal (Pinto da Silva, 1972). Several authors (eg. Burges 1964, Chevalier, 1901) have considered these populations as naturalized. However, Pinto da Silva (1972) treated some of the southern populations as native.

In Hawaii, where *M. faya* was introduced by Portuguese settlers in the 19th Century and planted for reforestation in the early 20th Century, the plant is well-suited to the moist climate. This, in combination with its nitrogen-fixing ability and the abundance of suitable open habitat, has led to *M. faya* becoming an invasive weed on several of the Hawaiian islands (Smathers and Gardner, 1979; Vitousek and Walker, 1989). *Morella rivas-martinezii* (A. S. Guerra) J. Herbert is a rare close relative of *M. faya* found on the Canary Islands of La Gomera, El Hierro and La Palma. Whilst the two species differ somewhat in leaf morphology they are otherwise very similar and grow in sympatry (pers. obs.).

Since Engler (1879), some authors have considered elements of the Macaronesian flora, and the laurel forest in particular, to be relicts of a laurophyllous Tertiary flora (e.g. Takhtajan, 1969; Axelrod, 1975; Bramwell, 1976; Sunding, 1979). Several laurel forest taxa have widely disjunct floristic links, for example, with South America and Asia (*Ilex;* Bramwell, 1976), India (*Apollonias;* Mabberley, 1998), southern Africa and North America (*Morella;* Chapter 1), and even Australia (*Picconia;* Sunding 1972). These disjunctions led Bramwell (1976) to propose an ancient Gondwanan (i.e. southern hemisphere) origin for such groups. Axelrod (1975), however, proposed a Madrean-Tethyan (i.e. northern hemisphere) origin for the laurel forest which he considered to be a 'remnant' of the laurophyllous flora that was once widespread in Europe during the late Tertiary. There is fossil evidence to suggest that a number of present-day Macaronesian endemics including *Picconia excelsa*, *Laurus azorica* and *M. faya* were once widespread throughout Europe (Axelrod, 1975; Sunding, 1979).

Axelrod (1975) also proposed that migration during the Palaeogene (early Tertiary) across the Atlantic (aided by a narrower sea than at present and the lower latitude of the east coast of America) was achieved via micro-continents (the Azores) and volcanic islands along the mid-Atlantic Ridge. As an explanation for the present disjunction of Tertiary relict floras, Milne and Abbott (2002) rejected this theory on the basis that 'island hopping' is contrary to the hypothesis of morphological stasis by stabilising selection (in which climatic stability and the continual presence of a taxon in a given geographical area combine to create no opportunities for morphological novelties to spread in the population). However, the Macaronesian laurel forest would seem to contradict their suggestion since it represents the repeated colonisation, by

several unrelated plant species, of widely spaced islands without significant morphological change.

Colonisation history has been examined in molecular studies of several Macaronesian endemics (reviewed by Carine et al., 2004). Unfortunately, there have been few molecular studies of the history of laurel forest species. The only study I am aware of is that of Valcárcel et al. (2003) in which they concluded that the three Macaronesian species of *Hedera* L. (Araliaceae) originated from three separate colonisation events with no inter-island dispersal. The occurrence of morphologically static populations of *M. faya* throughout Macaronesia and in mainland Portugal provides an excellent opportunity to examine the colonisation history of a laurel forest species in the light of putative source populations.

The aim of this investigation was to use RAPDs (randomly amplified polymorphic DNA) to: (1) elucidate the colonisation process leading to the current distribution of *Morella faya*; (2) clarify the relationship between *Morella faya* and *Morella rivas-martinezii*; and (3) compare the genetic composition of native and introduced populations of *Morella faya*.

MATERIALS AND METHODS

Leaf material of *M. faya* was collected by the author from populations in Macaronesia (Canary Islands, Madeira and the Azores); further leaf material was obtained from Hawaii (kindly donated by Forest Starr, Maui, Hawaii) and mainland Portugal. Leaf material of the endangered *M. rivas-martinezii* was collected from protected populations in the Canary Islands. All leaf material was stored at -20°C in the Sir Harold Mitchell Laboratory, University of St Andrews. DNA was extracted from 12 individuals per population using a 2X CTAB method adapted from Doyle and Doyle (1990), modified to include a washing step with ammonium acetate (7.5 *M* NH_4AC; Weising et al., 1995) to remove impurities co-isolated with the DNA. The DNA was then diluted as appropriate to give a standard quantity of approximately 5ng per sample. DNA concentrations were determined by comparison with uncut lambda DNA on 1% agarose gels.

TABLE 1. Plant material used in this study. [a]Locality details for *M. rivas-martinezii* are with-held in compliance with the conditions of collection permits (issued by Ministerio de Medio Ambiente, Santa Cruz, Tenerife)

Species/Population locality details	No. samples collected	No. samples screened	Voucher
M. rivas-martinezii[a]			
Canary Islands, El Hierro	12	12	Herbert F101 (E)
Canary Islands, La Gomera	12	12	Herbert F233, F239 (E)
M. faya			
Canary Islands, E. Tenerife, Anaga, between Las Mercedes and Pico del Ingles	32	12	Herbert F001 (E)
Canary Islands, W. Tenerife, Ruigoméz, foothills of the Teno range	15	12	Herbert F064 (E)
Canary Islands, El Hierro, Fuente la Llania	30	12	Herbert F270 (E)
Canary Islands, La Gomera, La Laguna Grande	29	12	Herbert F240 (E)
Canary Islands, La Palma, above Barlovento	32	12	Herbert F201 (E)
Madeira, Ribiero Frio, between Ribiero Frio and Portela	20	12	Herbert & Gaskell MA01, MA2002(E)
Azores, Terceira, Monte Brasil	40	12	Herbert & Gaskell TE204 (E)
Azores, Sao Miguel, between Caldeiras and Lombadas	20	12	Herbert & Gaskell SM201 (E)
Azores, Faial, Reserva Florestal Natural Parcial do Cabeço do Fogo	20	12	Herbert & Gaskell FA17 (E)
Portugal, Algarve, Monchique, Foia	12	12	Sales & Hedge 94/23 (E)
Hawaii, Maui, Polipoli and Crater Road	30	24	Starr & Starr 020911-1, 020911-2 (HI)

RAPD reactions of 25µl contained: 2 µl DNA template, 2mM of each dNTP, 0.2 µM of each primer, 1 unit Biotaq polymerase (Bioline, London, UK), 2 µM $MgCl_2$, and 5 µl reaction buffer (160 mM $(NH_4)_2 SO_4$, 670 mM Tris HCl, 0.1% Tween 20, pH 8.8). The following PCR profile was used: 1 cycle at 94°C for 3 minutes; 45 cycles at 94°C for 30 seconds, 40°C for 45 seconds and 72°C for 1 minute 30 seconds; 1 cycle at 72°C for 4 minutes.

The PCR products were separated on 1% agarose gels containing 0.5 µg μl^{-1} ethidium bromide and visualized by UV transillumination. A total of 83 primers (RAPD sets #1 and #3; UBC, Vancouver, Canada) were screened using 11 individuals representing *M. rivas-martinezii* and *M. faya* populations from throughout the range of the species. Each screening PCR included a negative sample (containing water in place of DNA). A sub-set of primers were found to be variable and were used to screen all 168 samples. It was not possible to run all 168 samples in a single PCR plate, but all PCR reactions were carried out on the same PCR machine in order to replicate conditions as closely as possible.

RESULTS

RAPD analysis— From a total of 83 primers screened for variability, 13 appeared to show reproducible variability and were chosen for the study (Table 2). Of these 13 primers it was possible to score only 2 bands for a single primer (primer #102) with any certainty. For the remaining primers reproducibility was poor (see Figure 1). In most cases the only strong bands that gave a consistently reproducible pattern were invariable in all samples.

TABLE 2. Results of the initial screening of 83 primers

No. of primers producing no discernible pattern	No. of primers producing monomorphic bands	No. of primers producing a variable banding pattern
44	26	13

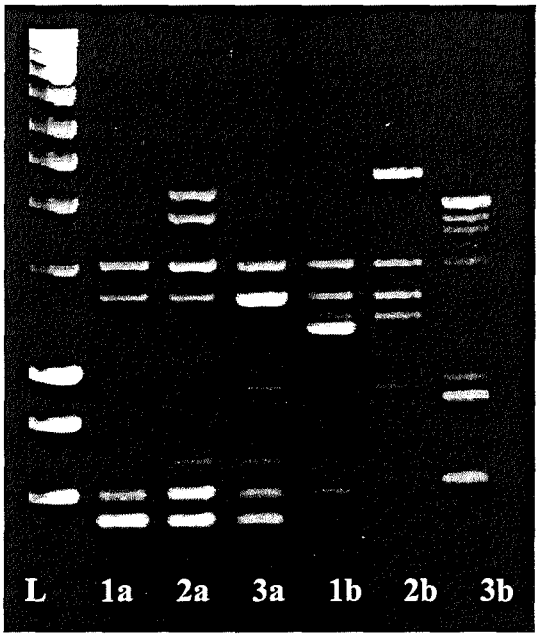

Figure 1. Results for primer #305 using 3 DNA samples (1,2 and 3); lanes marked 'a' show the results of the first PCR, lanes marked 'b' show the results of the second PCR. Reactions a and b were carried out under identical conditions.

DISCUSSION

RAPD analysis— RAPDs are routinely used by plant biologists (Gomes et al., 2004; Randell et al., 2004; Reisch, 2004; Viccini et al., 2004) to investigate population-level genetic variation. However, the reproducibility of the technique has been called into question (Jones et al., 1998). RAPDs were chosen for this study as an inexpensive method for gathering population genetic data. In this case, however, the method failed to produce reliable results. The reason for the failure of the technique is difficult to identify. It is possible that the quality of the primers had degraded due to age and

excessive freeze-thawing (the primers used were not newly ordered for the study). A further factor, noted in the sequencing of the plant material used, was the presence of leaf endophyte fungal contaminants. The presence of contaminant DNA in the reactions may have contributed to some of the artifacts observed. Previous population-level analyses of Myricaceae species have employed allozymes (Cheng et al., 2000) or microsatellites (Erickson et al., 2004).

For future studies of *M. faya*, an alternative method for investigating population-level variation, such as AFLP or microsatellites, might prove more successful. Microsatellites are highly polymorphic, single-locus markers that produce codominant data and have been successful in providing information on, among other things, dispersal patterns in plants (Ouborg et al., 1999).

Field observations— During collecting trips to the Canary Islands (October 2001), the Azores and Madeira (May 2002) and Portugal (December 2001), observations of the ecology of *M. faya* and *M. rivas-martinezii* were made. The sex expression of *M. faya* was predominantly dioecious in the Canaries and the Azores, but in Madeira specimens were observed to exhibit heterotopy (MacDonald, 1989), a condition in which stamens are found on the fruit wall. This condition is found in many Myricaceae species and is likely to be due to incomplete suppression of male function (see Chapter 2). The literature regarding *M. faya* in Hawaii includes reference to the monoecious breeding system of the plant there. It is possible that heterotopy may have been confused with monoecy by those unfamiliar with the flowers of Myricaceae species. If this is the case, then it might suggest an origin in Madeira for the Hawaiian populations.

The author has observed *M. faya* in Portugal at St Bartholomew's Hill near Nazaré (Estremadura), and at Foia and other nearby localities in the Serra de Monchique (Algarve). The habitat at Foia was apparently undisturbed and the plants were growing in a native community, with *Rhododendron ponticum* L. (Ericaceae), in an isolated locality at the summit of the mountain. At St Bartholomew's Hill the vegetation was similarly native in character. Based on these observations I agree with the opinion of I. C. Hedge and F. Sales that the populations in the Algarve are certainly native, and those at Nazaré are probably so (I. C. Hedge, pers. comm.).

Material from the mainland populations should be included in any future studies of this species.

Small populations of *M. rivas-martinezii* were visited during trips to El Hierro and La Gomera. In both cases cultivated seedlings were being re-introduced to the sites and represented the vast majority of specimens seen. The molecular phylogenetic analysis of the family shows that there is little divergence between *M. faya* and *M. rivas-martinezii* (see Chapter 6), perhaps suggesting recent speciation, as inferred for some of the South African species which are morphologically distinct but showed little or no molecular divergence. However, *M. faya* and *M. rivas-martinezii* do not appear, to me, to be significantly morphologically distinct, the only character that separates them being leaf shape. This, in combination with the low occurrence of individual plants and the lack of ecological separation between the two taxa, suggests that *M. rivas-martinezii* is a doubtful species.

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