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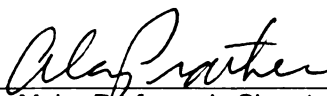
**MONARDA SECTION CHEILYCTIS: PATTERNS OF
SPECIATION AND ENDEMISM**

presented by

JESSIE ANNE KEITH

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Plant Biology/ EEBB


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MONARDA SECTION *CHEILYCTIS*: PATTERNS OF SPECIATION AND
ENDEMISM

By

Jessie Anne Keith

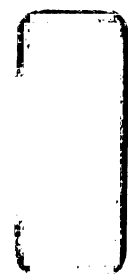
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Submitted to
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in partial fulfillment of the requirements
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ABSTRACT

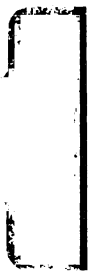
Monarda Section *Cheilyctis*: Patterns of Speciation and Endemism

By

Jessie Anne Keith

Monarda Section *Cheilyctis* (Lamiaceae) includes six species. Five are narrow endemics, with four (*M. viridissima*, *M. fruticulosa*, *M. maritima* and *M. stanfieldii*) present in unique edaphic conditions in Texas and the fifth (*M. humilis*) occurring in arid regions of New Mexico. The sixth species, *M. punctata*, is widespread, occurring across much of the United States. Phylogenies based on two *Adh* loci, *Adh* type 1 (*Adh1*) and *Adh* type 2 (*Adh2*), were used to investigate patterns of speciation, species relationships and endemic status (neo- versus paleoendemic) of these *Monarda* species. It was hypothesized that narrow endemic species were neoendemics that had speciated as peripheral isolates of *M. punctata*. Both *Adh1* and *Adh2* phylogenies had three primary clades that depicted similar relationships between taxa. *Monarda punctata* was the most ubiquitous taxon across both phylogenies. *Monarda fruticulosa*, *M. humilis*, *M. maritima* and *M. viridissima* sequences exhibited polyphyly and patterns consistent with lineage sorting across both *Adh1* and *Adh2* phylogenies. In both the *Adh1* and *Adh2* phylogeny sequences of *M. stanfieldii* were sister to a large clade including all samples of the other five species. Phylogenetic results suggest that *M. humilis*, *M. fruticulosa*, *M. maritima* and *M. viridissima* arose from their widespread congener, *M. punctata*, and that *M. stanfieldii* is a sister taxon to the other five species. Likewise, it was concluded that *M. fruticulosa*, *M. maritima* and *M. viridissima* and *M. humilis* are most likely neoendemics while *M. stanfieldii* is likely a paleoendemic species.

FOR THE TWO MEN THAT
INSPIRED ME TO PURSUE HIGHER EDUCATION
MY FATHER, DR. JAMES HILTON KEITH
AND
MY GRANDFATHER, DR. ARCHIE JUSTICE MACALPIN



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INTRODUCTION

1.1 Project Summary

Many historical events, geographic and genetic, have been used to explain potential modes of speciation. Allopatric and peripatric modes use geographic isolation of populations to explain species divergence. Under these modes of speciation, geographic isolation is expected to modify the genetic constitution of divided populations eventually resulting in their morphological, ecological and genetic separation.

Phylogenetic studies of closely related species can be combined with geographic patterns of distribution to infer mode of speciation (Harrison 1991). This study utilizes a small cohesive group of North American mints (Lamiaceae) in the genus *Monarda* to test peripatric speciation using phylogenies generated from *Adh* loci. These taxa appear well suited for this purpose because the pattern of distribution found among its members corresponded to those expected for peripatric speciation.

1.2 Peripatric Speciation

Speciation by peripheral isolation was first proposed by Mayr in 1942. Mayr believed that most species diverged in allopatry but that geographic isolation of small peripheral populations had the greatest potential for rapid speciation. He maintained that marginalized populations would be subjected to a higher degree of reduced gene flow and habitat dissimilarity resulting in founder effects (genetic drift in small populations), increased selection pressures and thus rapid speciation and morphological change (Mayr 1942, 1976, 1982). Founder populations are expected to have only a fraction of the

genetic composition found in parental populations (Mayr 1982). This genetic imbalance, when coupled with a change in the abiotic environment, has the potential to lead to heightened selection and ecological adaptation. This differs from the classic model of allopatric speciation where larger allopatric populations are expected to require longer periods of time to diverge phenotypically and genotypically due to larger more variable gene pools and more homogeneous habitats

Harrison (1991) predicted that ancestral population structure between sister taxa could be discerned using patterns of geographic dispersion and molecular phylogenetic data. He proposed a series of repeatable, testable patterns (geographic and phylogenetic) to model allopatric, peripatric and sympatric speciation. In the model of divergence of peripheral populations (peripatric speciation), he maintained that peripheral isolates would exist along the distribution margins of a more widespread sister taxon while appearing phylogenetically derived within a gene tree.

Narrowly endemic *Monarda* are located along the geographic periphery of their widespread congener, *M. punctata*. This distribution pattern suggests that they may have speciated as peripheral isolates from *M. punctata*. Likewise, ITS and cpDNA provided little phylogenetic signal for these taxa which suggests they diversified recently (Prather et al 2002, Monfils and Prather unpubl. data). Harrison's phylogenetic model for peripheral isolation was used to test this hypothesis. In concordance with this model *M. punctata* was expected to appear in a more basal position of the phylogeny and paraphyletic to the endemic species. This pattern would indicate that *M. punctata* is an



ancestral species. For instance, in the hypothetical phylogenies below (Fig 1), *M. punctata* has a more basal position in the tree, and is paraphyletic to *M. fruticulosa* and *M. viridissima*. Furthermore, *M. fruticulosa* and *M. viridissima* are more closely related to geographically proximate *M. punctata* samples in the phylogeny.

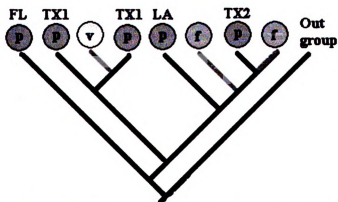
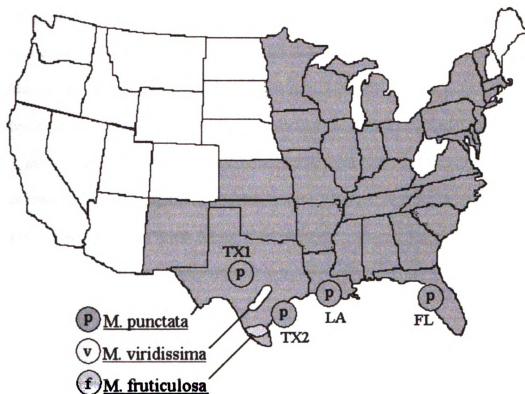


Figure 1: Geographic and hypothetical phylogenetic distributions given four samples of the widespread taxon *M. punctata* and two samples representing *M. viridissima* and *M. fruticulosa*.

1.3. Neoendemism versus Paleoendemism

Stebbins (1942 & 1942) defined two types of endemic species, paleoendemics and neoendemics. Paleoendemics were described as relict species whose once large distributions have been reduced to small disjunct populations that occur across a wide area (Mayer & Soltis 1994, Qian 2001). Due to their once widespread distribution, paleoendemics are expected to have highly heterozygous populations despite their small size (Macnair & Gardner 1993). In contrast, neoendemics are newer species that are expected to occur across very narrow range limits and are expected to have highly homozygous populations. The criteria used to distinguish paleoendemics from neoendemics include geographic distribution, genetic variation and placement in the phylogeny. The majority of North America's neoendemics are restricted to the southwest. This region is both a center for neoendemics in North America and the world (Qian, 2001).

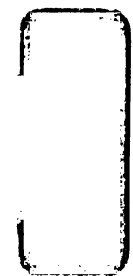
Narrow endemic *Monarda* taxa in Section *Cheilyctis* are hypothesized to have speciated as peripheral isolates, therefore they are believed to be neoendemic species. Phylogentic patterns that would support their neoendemic status would be consistent with those mentioned for speciation by peripheral isolation. Neoendemics are expected to be derived within *M. punctata* and paleoendemics are expected to exist on more basal branches separate to *M. punctata*.

1.4 Overview of Taxa

The genus *Monarda* contains 19 species that are split into two subgenera, one of which is divided into two sections (Table 1). Section *Cheilyctis* has five are narrow endemic species. *Monarda fruticulosa*, *M. maritima*, *M. stanfieldii* and *M. viridissima* are endemic to Texas, and *M. humilis*, is endemic to New Mexico.

Table 1: Species in *Monarda* (Turner, 1994, Prather et al.2002 and Prather and Keith 2003) with scientific and common names. Varieties and subspecies of *Monarda punctata* are included. Common names adapted from the National Plants Database.

Species	Common Names
SUBGENUS CHEILYCTIS	
SECTION ARISTATAE	
<i>M. citriodora</i> Cerv. ex Lag	Lemon Beebalm
<i>M. clinopodioides</i> A. Gray	Basil Beebalm
<i>M. pectinata</i> Nutt.	Pony Beebalm
SECTION CHEILYCTIS	
<i>M. fruticulosa</i> Epling	Shrubby Beebalm
<i>M. humilis</i> Prather & Keith	Spreading Beebalm
<i>M. maritima</i> (Cory) B.L. Turner	Seaside Beebalm
<i>M. punctata</i> L.	Spotted Beebalm/Horsemint
- var. <i>arkansana</i> (McClintock & Epling) Shinnars	Arkansas Spotted Beebalm
- var. <i>correllii</i> B.L. Turner	Correll's Spotted Beebalm
- var. <i>coryi</i> (McClintock & Epling) Cory	Cory's Spotted Beebalm
- var. <i>intermedia</i> (McClintock & Epling) Waterfall	Intermediate Spotted Beebalm
- var. <i>lasiodonta</i> Gray	Spotted Beebalm
- var. <i>occidentalis</i> (Epling) Palmer and Steyermark	Western Spotted Beebalm
- var. <i>villicaulis</i> (Pennell) Shinnars	Villous Spotted Beebalm
- subsp. <i>punctata</i> Epling	Spotted Beebalm
<i>M. stanfieldii</i> Small	Stanfield's Beebalm
<i>M. viridissima</i> Correll	Green Beebalm
SUBGENUS MONARDA	
<i>M. didyma</i> L.	Scarlet Beebalm
<i>M. bradburiana</i> Beck	Eastern Beebalm
<i>M. clinopodia</i> L.	White Beebalm
<i>M. eplingiana</i> Standl.	Epling's Beebalm
<i>M. fistulosa</i> L.	Wild Beebalm or Bergamot
<i>M. lindheimeri</i> Engelm. & A. Gray	Lindheimer's Beebalm
<i>M. media</i> Willd.	Purple Beebalm/ Bergamot
<i>M. pringlei</i> Fernald	Pringle's Beebalm
<i>M. russeliana</i> Nutt. ex. Sims	Redpurple Beebalm
<i>M. stipatoglandulosa</i> Waterf.	Wild Beebalm



In contrast, *M. punctata* is widely distributed across the central and eastern United States, southeast Canada, and northeast Mexico (Fig.2). These species also appear to have distinct edaphic constraints in their distribution.

No formal field studies have addressed interspecific hybridization between these species, but some field observations have been made. *Monarda maritima* is sympatric with *M. punctata* (Turner 1994, Prather pers comm., Keith pers. obs.), Turner indicated that it was found growing with other *Monarda* (unnamed taxa) and that no hybridization appeared to occur between them. *Monarda viridissima* is also sympatric with *M. punctata* populations (Turner 1994, Prather pers comm., Keith pers. Obs.). Turner found no evidence that these two taxa overlapped in distribution but Prather and Keith observed sympatric populations of *M. viridissima* and *M. punctata* in Bastrop County Texas, although there was no evidence that they bloomed at the same time. *Monarda fruticulosa* and *M. punctata* also grow together (Turner 1994, Prather pers comm., Keith pers. obs.). However, Turner did not find evidence that populations were confluent while Prather and Keith found evidence of hybrid zones between them. Finally, *M. stanfieldii* has not been found to intergrade with *M. punctata* (Turner 1994, Prather pers comm., Keith pers. obs.). Turner indicated that *M. stanfieldii* and *M. punctata* do not intergrade, and Keith (pers. obs) found no evidence that *M. stanfieldii* populations in Blanco, Burnet and Llano counties were sympatric with *M. punctata*. Lack of hybridization between these species suggests that they maintain unique gene-pools and helps substantiate their species designation.

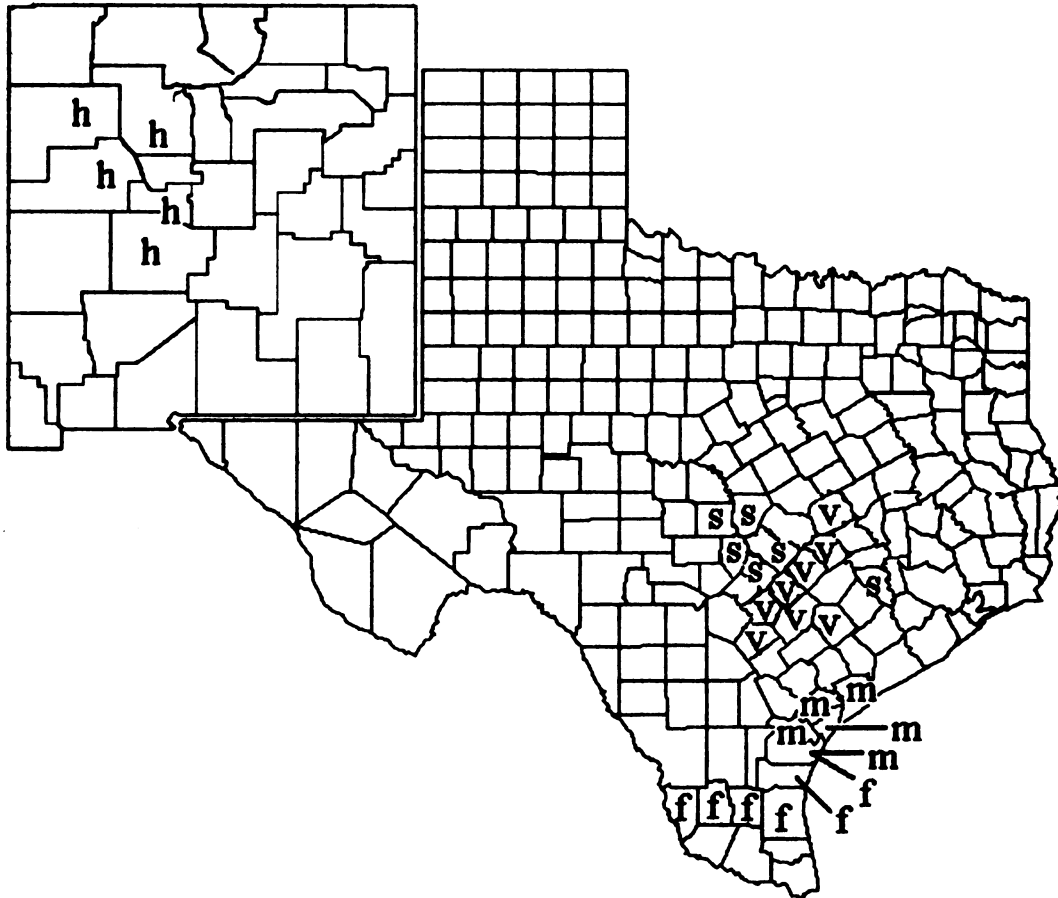


Figure 2: County distributions of Texas endemics, *Monarda viridissima* (v), *M. fruticulosa* (f), *M. maritima* (m), *M. stanfieldii* (s), and the New Mexico endemic, *M. humilis* (h). (based on Turner (1994), Johnston and Correll (1979) and herbarium material from the University of New Mexico and New Mexico State University)

1.5 Objectives

The purpose of this study was to test speciation by peripheral isolation and to determine the neo- and paleoendemic status of the six species in *Monarda* Section *Cheilyctis*. It was hypothesized that the widespread taxon, *M. punctata*, is the progenitor from which all narrow endemic species have derived. Narrow endemic *Monarda* taxa were also predicted to be neoendemic species. Geographic distributions and phylogenetic data were used to test this. The low copy nuclear gene, *Adh*, was used as the genetic marker for phylogenetic analyses. Maximum parsimony, maximum likelihood and Bayesian methods of inference were used to generate phylogenetic trees.

MATERIALS AND METHODS

2.1 Study System

i. Taxonomic Classification of *Monarda* Section *Cheilyctis*.

Three of the endemics have undergone conflicting taxonomic classifications (*M. fruticulosa*, *M. maritima* and *M. stanfieldii*). The taxonomic ranks of these species have been addressed in treatments by six primary authors (Table 2). Epling (1935), Epling and McClintock (1942) and Turner (1994) recognized *M. fruticulosa* as a species due to its distinct morphology and apparent lack of hybridization with *M. punctata*. Scora (1967) reduced its rank to a variety because he believed that it did intergrade with *M. punctata* var. *immaculata* (a variety not recognized in this study). Similarly, both Cory (1936) and Scora (1967) classified *M. maritima* as a variety of *M. punctata* because they believed it shared characteristics with *M. fruticulosa*. In contrast, both Turner (1994) and Correll (1968) treated it as a species due to its distinct morphology, habitat and distribution.

Monarda humilis was first described as a variety of *M. punctata* (Torrey 1853) but was recently recognized as a species by Prather and Keith (2003). This taxon was elevated to specific status due to its distinct floral morphology and geographic distribution.

Monarda stanfieldii was first described by Small (1903) and was later accepted as a species by Turner (1994). Epling (1935) reduced it to a subspecies, and Cory (1936) and Scora (1967) treated it as a variety of *M. punctata*.

Monarda viridissima was described as a species by Correll (1968) because of its unique morphological characters and temporal reproductive isolation from *M. punctata*. Correll observed little evidence of hybridization with *M. punctata* in the field. Turner (1994) and Correll and Johnston (1979) maintained *M. viridissima* at the species rank.

Turner's recent treatment of these *Monarda* was used for this study. He recognized the Texas endemics as species due to their distinct morphology, geographic distribution and apparent lack of gene introgression with *M. punctata*. He did not include the New Mexican taxon (*M. humilis*) in his treatment.

ii. Morphology and Life History Traits

Several life history traits and morphological characters have been fundamental in distinguishing these species. Key differences between them include life cycle and phenology as well as floral and vegetative traits (Table 3).

Table 2: Taxonomic ranks of endemic *Monarda* as recognized by six principal authors that have studied them

	Epling (1935)	McClintock & Epling (1942)	Scora (1967)	Correll & Johnston (1979)	Turner (1994)	Prather & Keith (2003)
<i>M. fruticulosa</i> Epling	<i>M. fruticulosa</i>	<i>M. fruticulosa</i>	<i>M. punctata</i> var. <i>fruticulosa</i>	<i>M. fruticulosa</i>	<i>M. fruticulosa</i>	<i>M. fruticulosa</i>
<i>M. humilis</i> Prather & Keith	not treated	not treated	not treated	not treated	not treated	<i>M. humilis</i>
<i>M. maritima</i> (Cory) B.L. Turner	not treated	not treated	<i>M. punctata</i> var. <i>maritima</i>	<i>M. maritima</i>	<i>M. maritima</i>	<i>M. maritima</i>
<i>M. stanfieldii</i> Small	<i>M. punctata</i> ssp. <i>stanfieldii</i>	<i>M. punctata</i> ssp. <i>stanfieldii</i>	<i>M. punctata</i> var. <i>stanfieldii</i>	<i>M. punctata</i> var. <i>stanfieldii</i>	<i>M. stanfieldii</i>	<i>M. stanfieldii</i>
<i>M. viridissima</i> Correll	not treated	not treated	not treated	<i>M. viridissima</i>	<i>M. viridissima</i>	<i>M. viridissima</i>

Table 3. Key differences in the morphological characters and life history traits between *Monarda* species in Section *Chelyoxis*. Morphological information obtained from McClintock & Epling (1942), Sorensen (1967), Correll & Johnston (1979) and Prather and Keith (2003).

Taxon	<i>Monarda pulchicosa</i>	<i>Monarda humilis</i>	<i>Monarda maritima</i>	<i>Monarda punctata</i>	<i>Monarda stanfieldii</i>	<i>Monarda viridissima</i>
Lifecycle	Perennial	Putative annual	Perennial	Annual/Short-lived	Short-lived Perennial	Short-lived Perennial
Bloom-time	Spring/early Summer	Spring/early Summer	Late Summer/ Fall	Spring to Fall	Spring/early Summer	Late Summer/early Fall
Habit	Shrub	Herbaceous	Sub-shrub	Herbaceous	Herbaceous	Sub-shrub
Height	30-40 (100) cm	40-60 cm	50-110 cm	25-100 cm	40-80 cm	80 cm
Stem surface	Canescent	Puberulent	Densely cottony tomentose	Pubescent	Hirsute	Puberulent
Leaf shape	Linear	Linear-lanceolate	Linear-lanceolate	Lanceolate	Broad-lanceolate	Narrowly Linear-lanceolate
Calyx teeth	Narrowly deltoid, acuminate	Narrowly triangular	Subulate	Acute, acuminate or subulate	Narrowly triangular	Sparsely hirsute-subulate
Calyx color	Green, or tinged pink	Purple-red to green	Green, or tinged pink	Green, or tinged pink	Green, or tinged pink	Purple-red
Calyx orifice	Long silky curled or spreading hairs	Pubescent	Green, or tinged pink	Pubescent	Densely hirsute/hispid	Short white hairs
Floral bract shape	Narrow, long-acuminate	Broadly ovate to elliptic-lanceolate, acuminate	Outer- rounded inner- narrow, long-acuminate	Lanceolate	Broadly ovate to elliptic-lanceolate, acuminate	Broadly ovate to elliptic-lanceolate, acuminate
Floral bract color	Light pink-cerise	Purple to green-white	Purple to green-white	Purple to green-white	Purple to green-white	Deep cerise pink to purple
Corolla color	White to pink	Pale lavender, white and purple	White or tinged pink, spots variable	Pale yellow, rarely with pink	Yellow	Creamy white or tinged purple
Corolla marks	Usually without spots	Purple, solid margin	Spots variable	Maroon spots	Maroon spots	Maroon spots

iii. Chromosome Numbers

All species in *Monarda* Section *Cheilyctis* have been found to be diploid. Scora (1967) found that several species and varieties of *M. punctata* in Section *Cheilyctis* (*M. punctata* vars. *arkansana*, *coryi* and *intermedia* and *M. fruticulosa*) had $n=11$ chromosomes, but *M. punctata* var. *villicaulis* had $n=12$ and $n=11$ chromosomes. This result was further confirmed in a study conducted by Bushnell (1936), which also showed the chromosome number in *M. punctata* var. *villicaulis* was $n=12$. *Monarda humilis* was also found to have $n=12$ chromosomes (Ward and Spellenberg 1984).

iv. Physiography and Vegetation

The Texas endemic *Monarda* seem to have distinct edaphic constraints on their distributions. Less is known about the edaphic conditions that may limit *M. humilis*.

Monarda fruticulosa grows in a region of South Texas called the Eolian Plain (Johnston 1963) that is divided into central and peripheral zones. The Central Eolian Plain has loose, fine sandy soils that originally sustained copses of live oak (*Quercus virginiana*) and some mesquite (*Prosopis sp.*). Many of the central plain soils are newer windblown beach deposits, but also have older soils such as Galveston deep sands (Clover 1937). The Peripheral Eolian Plain has loamy sand soils that once supported mesquite prairie. Combinations of soil types may also exist in areas such as Brooks County where thin layers of wind blown beach sands cover older soils. Suppression of natural fires, heavy grazing and agriculture have altered the vegetation of the Eolian Plain, which is now referred to as The Texas Brush Country.

Monarda humilis samples have been collected from the northwestern part of New Mexico within the Rio Grand Basin. These plants most likely inhabit Penistaja soils, which are deep, well drained, and comprised of alluvium from sandstone and shale. Penistaja soils are found primarily on upland regions such as plateaus and hills, and in drainageways (USDA National Cooperative Soil Survey 2003).

Monarda maritima inhabits the deep sandy soils of the Coastal Belt (Turner 1994) that extends across approximately a 100-mile strip from Nueces to Galveston Bays. Its deep coastal sands show little textural change with depth and are thought to be an old chain of barrier islands from the late Pleistocene or Holocene called the Ingleside Terrace (Price 1933, Shideler 1986, Otvos 1991, Blum et al. 2002). Live oak (*Quercus virginiana*) dominates these deep sands.

Monarda stanfieldii is primarily restricted to the middle course of the Colorado River (Turner 1994) within the Central Texas Uplift. This region makes up the eastern portion of The Texas Hill Country. *Monarda stanfieldii* only appears to inhabit granitic sands (Turner 1994). Original vegetation in the Central Texas Uplift consisted of grasslands and open savannah-like plains with small trees and brush scrub growing along rocky slopes and stream bottoms (Bray 1901 and Buechner 1944).

Monarda viridissima is confined to the Carrizo sand outcrop (Turner 1994) that runs adjacent to The Blackland Prairies of East central Texas. Carrizo sands are unusually

homogeneous and harbor many endemic plant species (McBryde 1933). The Carrizo sand belt originally harbored post oak (*Quercus drummondii*), blackjack oak (*Q. marilandica*) and hickory (*Carya texana*) (Johnson 1963). *Monarda viridissima* grows in open post oak or pine-oak forests

v. Edaphic Constraints

Vegetation and plant growth are largely reliant on the substrate in which a plant grows (Jenny 1941). Most plant species are able to inhabit a wide range of soils but some have adapted to become limited to specific soil types (Kruckeberg and Rabinowitz 1985).

These plants are referred to as edaphic endemics. Edaphic constraints can include chemical, physical and biological properties. Edaphic endemics have been identified on many substrates including soils composed of granite, limestone, Carrizo sand, oil shale, gypsum and serpentines (McBryde 1933, Kruckeberg and Rabinowitz 1985). Edaphic constraints may also be attributable to soil water holding capacity. Studies have found that landscapes with sandy soils can have soil water environments that vary widely from water permeated lowlands to arid dunes (Hoover and Parker 1991) and can sustain a highly variable landscape flora (Ramaley 1939).

Several modes of speciation have been used to explain the evolution behind edaphically restricted species including peripatric, parapatric and sympatric speciation (MacNair and Gardner 1998). The most well documented and extreme examples of edaphic endemism are in plants adapted to serpentine soils which are high in heavy metals and minerals such as magnesium and calcium (Brooks 1987). Obligate edaphic endemics inhabiting

serpentine soils are reported to have speciated very rapidly (Kruckeberg 1967) and often are parapatric to sister taxa. All Texas endemic *Monarda* are reported as being edaphic endemics (Turner 1994).

vi. Species Recognition

In this study it was assumed that the *Monarda* endemics were “good” species despite a hypothesized phylogenetic outcome that did not render them reciprocally monophyletic within the gene trees. This anticipated outcome conflicts with criteria set by some species concepts, such as evolutionary, cladistic and phylogenetic species concepts, that delimit species based on their phylogenetic relationships.

The lines that delimit species phenotypically, ecologically, genetically, and phylogenetically do not always run parallel. If *Monarda* do not appear phylogenetically distinct on the *Adh* gene trees it will not discredit their status as a biological species.

Based on their temporal and geographic separation they appear to be reproductively isolated. Likewise, their morphological distinction suggests that they maintain their own gene pools, and the putative edaphic constraints on their natural populations indicates that they are ecologically distinct. These characteristics conform to several species concepts, including Mayr’s (1942) biological species concept, and the phenetic species concept.

Other researchers have taken a comparable approach to recognizing species when faced with marked differences in their ecological, morphological and phylogenetic distinction. A phylogenetic study using ribosomal and cpDNA revealed that the widespread *Mimulus*

guttatus and several closely related serpentine endemic species were genetically indistinct despite being highly dissimilar morphologically and ecologically (Macnair and Gardener 1998). Despite the lack of genetic distinction between these *Mimulus*, Macnair and Gardener opted to recognize them as species because they maintained morphological and ecological differences despite the fact they existed in sympatry.

2.2 Sampling

All *Monarda* species in Section *Cheilyctis* (Table 4) were included in the *Adh* analyses. Three individuals were sampled, each from a different population of *M. fruticulosa*, *M. maritima*, *M. stanfieldii* and *M. viridissima*, one individual from a population of *M. humilis* (Fig 3), and *M. punctata* from 19 separate geographic locations across the United States (Table 4, Fig. 4).

Outgroup sequences included taxa of *Monarda* and *Pycnanthemum* (*M. clinopodioides*, *M. citriodora*, *P. loomisii*, *P. muticum* and *P. tenifolium*). The *Pycnanthemum* sequences were provided by Rachel Williams. Based on ITS phylogenies of *Monarda* and the Lamiaceae subfamily Nepetoideae, *Monarda* Section *Aristatae* (*M. citriodora* and *M. clinopodioides*) is sister to *Monarda* Section *Cheilyctis*, and the genus *Pycnanthemum* is closely related to *Monarda* (Prather et al. 2002 and Williams *pers. comm.*). I chose these outgroups based on this phylogenetic evidence.

In addition to *Adh*, some preliminary data was collected from a second molecular marker, *ncpGS*. The *ncpGS* preliminary analysis included three endemic species (*M. fruticulosa*, *M. maritima* and *M. viridissima*) and *M. punctata* collected from four different

geographic locations (Table 5). Two *Pycnanthemum* species were used as outgroups.

Samples came from greenhouse, herbarium or silica dried plant material.

Table 4: DNA collection numbers and locations for all *Monarda* species in *Adh* phylogenetic analyses.

Species	DNA #	State	County	Collector	Collection #
<i>M. fruticulosa</i>	1920	Texas	Kenedy	Prather, LA	1858
	2301	Texas	Jim Hogg	Prather, LA	1860
	2317	Texas	Kenedy	Keith, JA	TX30
	2420	Texas	Zapata	Prather, LA	2014
<i>M. humilis</i>	2176	New Mexico	Socorro	Tafoya, MA	94450 UNM
<i>M. maritima</i>	2181/2318	Texas	Refugio	Keith, JA	TX36
	2314	Texas	Aransas	Keith, JA	TX23
	2319	Texas	Aransas	Prather, LA	1940
<i>M. punctata</i>	2060	Arkansas	Calhoun	Thomas, DR Amanson, C	141, 500 (7296) UTH
	2303	Florida	Bay	Keith, JA	FL4
	2397	Florida	Franklin	Keith, JA	FL9
	2388	Florida	Gadsden	Keith, JA	FL1
	2398	Oklahoma	Marshall	Barclay, A	PI 326567
	2305	Louisiana	Iberville	Thomas, DR Davies, M	142, 284 (7296) UTH
	2401	S. Carolina	Florence	Churchill, JA	332325 MSU
	2373	Texas	Travis	Keith, JA	TX11
	2387	Texas	Zavala	Ertter, B	527083 RMH
	2393	Texas	Aransas	Keith, JA	TX25
	2396	Texas	Bastrop	Keith, JA	TX14
	2468	Texas	Angelina	Orzell, SL Bridges, EL	18952 TAMU
<i>M. p. var. intermedia</i>	2473	Texas	Callahan	Turner, BL, G	18900 TAMU
<i>M. p. var. villicaulis</i>	1937	Delaware	Sussex	Keith, JA	DE1
	2048	Michigan	Allegan	Keith, JA	MI1
	2172	Missouri	Madison	Ladd, D Yatskievych G	4236830 MBG
	1946/2189	New Jersey	Burlington	Keith, JA	NJ1
<i>M. p. var. occidentalis</i>	2307	New Mexico	Eddy	Martin, WC Schmidt, B	79100 UNM (1215)
<i>M. p. subsp. monarda</i>	2389	Florida	Alachua	Keith, JA	FL11
<i>M. stanfieldii</i>	2339	Texas	Llano	Keith, JA	TX7
	2367	Texas	Burnet	Keith, JA	TX2
	2369	Texas	Blanco	Keith, JA	TX9
<i>M. viridissima</i>	2055	Texas	Milam	Keith, JA	029007 Tamu
	2441/2442	Texas	Bastrop	Keith, JA	TX39
	2444	Texas	Milam	Keith, JA	TX40

Table 5: DNA collection numbers and locations for all *Monarda* species in *ncpGS* phylogenetic analyses.

Species	DNA #	State	County
<i>M. fruticulosa</i>	1910	Texas	Kenedy
<i>M. fruticulosa</i>	1920	Texas	Kenedy
<i>M. maritima</i>	1977	Texas	Aransas
<i>M. viridissima</i>	1989	Texas	Bastrop
<i>M. viridissima</i>	1908	Texas	Milam
<i>M. punctata</i>	1907	Texas	Kleburg
<i>M. punctata</i>	1909	Texas	Cass
<i>M. punctata</i>	1905	Michigan	Allegan
<i>M. punctata</i>	1946	New Jersey	Burlington

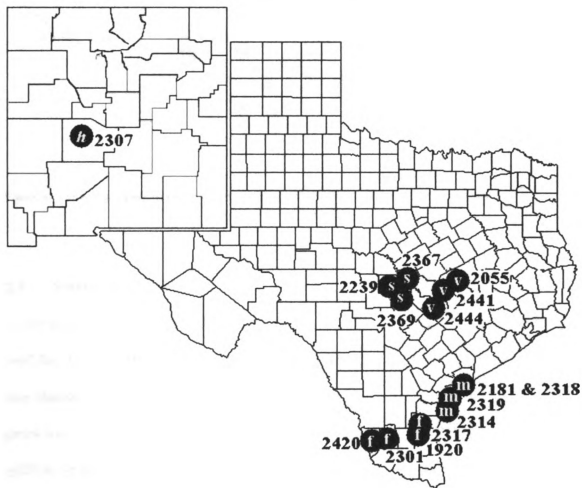


Figure 3: Collection locations of endemic species with their corresponding DNA numbers.

Texas samples include *M. fruticulosa* (1920, 2301, 2317 and 2420 (**f**)), *M. maritima* (2314, 2319 and 2318/2181 (**m**)), *M. stanfieldii* (2239, 2367 and 2369 (**s**)) and *M. viridissima* (2055, 2441 and 2444 (**v**)). The New Mexico sample is of *M. humilis* (2307 (**h**))



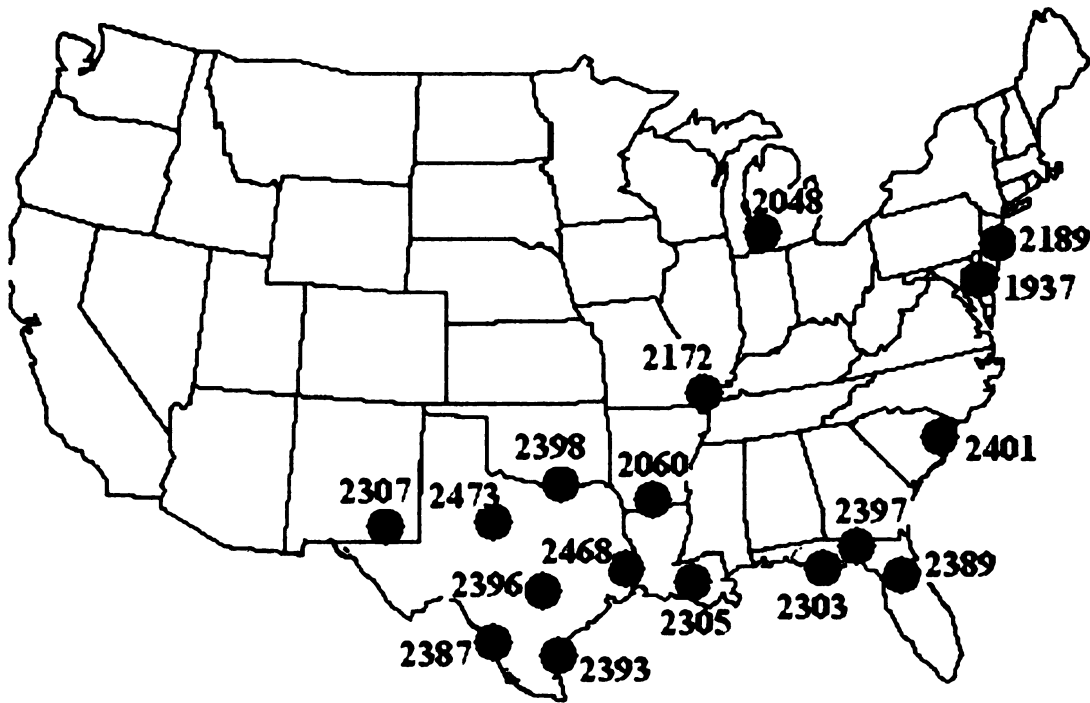


Figure 4: Collection locations of the 16 *M. punctata* samples with their corresponding DNA numbers.

2.3 Marker Choice

In this study, two homologs of the nuclear gene, Alcohol dehydrogenase (*Adh*), were used for phylogenetic analysis. In addition to *Adh* data, some preliminary results were also obtained for chloroplast expressed glutamine synthetase (*nepGS*). Low copy nuclear genes were chosen for this work because in previous studies ITS (Prather et al 2002) and cpDNA (Prather and Monfils unpub. data) provided no phylogenetic information for *Monarda* species in Section *Cheilyctis*.

Adh has been widely used as a molecular marker in plant phylogenetics. *Adh1* produced a more resolved phylogeny in *Paeonia* with sequence divergence one and one half times higher than ITS (Sang 1997). *Adh* and cpDNA sequences were also compared in

Gossypium and *Adh* was found to be far more informative (Small et al. 1998). Likewise, in additional phylogenetic studies of *Gossypium*, *Adh1* sequences were phylogenetically informative and produced well-resolved trees (Small and Wendel 2000). *Adh* has also been used to compare inter- and intraspecific relationships in DNA variation and codon bias in the closely related brassicas *Arabis* and *Arabidopsis* (Miyashita et al. 1996), so it can provide phylogenetic informative at the highest taxonomic levels.

Adh is referred to as “classical’ alcohol dehydrogenase. The alcohol dehydrogenase produced by *Adh* is NAD⁺ dependent and oxidizes ethanol (reviewed in Chase 1999). In most plant systems, there are two *Adh* loci. In Solanaceous plants, the loci differ in their tissue-specific expression. One *Adh* enzyme is expressed when the plants are subjected to anaerobic conditions (hypoxia) whereas the second is only expressed in anthers and seeds. Moreover, two additional *Adh* genes have been identified and described in *Lycopersicon esculentum* and *Solanum tuberosum* (Chase 1999).

Glutamine synthetase (*ncpGS*) encodes for an enzyme that functions in the assimilation of ammonium into glutamine (reviewed in Ford & Cullimore 1989). In most plants, two distinct classes of these genes exist. One class encodes for cytosolic enzymes and in most plants four to six genes have been described. The other class encodes for a plastidic protein and only one gene has been described in most plant species (Emshwiller & Doyle 1999). The chloroplast-targeted glutamine synthetase was used to determine relationships between eight *Oxalis* species and sequence divergence in intron regions was shown to be higher (8.63%) than that of ITS sequences (7.75%; Emshwiller & Doyle 1999).

2.4 DNA Extraction

DNA was extracted using the 2xCTAB method (Doyle & Doyle 1987) as modified by Zhang and Stewart (2000). DNA was extracted from living plants and herbarium material. Extracted genomic DNA was cleaned using Schleicher & Schuell Elu-quick DNA purification kit (Neese, NH).

2.5 Primers

Four sets of *Adh* primers, both general and specific, were used for PCR amplification of *Monarda*. The general primers, *Adh*-R and *Adh*-F (Sang et al. 1997), amplified ~1400 basepairs of *Adh* sequence in *Paeonia* (Table 6). *Adh*-R and *Adh*-F primers were used to amplify the first *Adh* sequences in these *Monarda*, but they provided poor amplification results. Consequently, it was necessary to design *Monarda* specific general primers that provided better *Adh* amplification in these plants.

Cloned *Adh* sequences were obtained from *Monarda* and *Pycnanthemum* using *Adh*-F and *Adh*-R. Twenty of these sequences were aligned using Sequencher 3.0. (Gene Codes, Ann Arbor, MI). General primers, labeled *Adhm*-F and *Adhm*-R (*Adh*-*Monarda*-forward and reverse; Table 6), were designed at conserved regions near the 5' and 3' ends of this alignment. The *Adhm*-F and *Adhm*-R primers amplified a ~1300 basepair region of *Adh* in *Monarda* and *Pycnanthemum*.

An additional primer set, *Adhmc*-F and *Adhmc*-R (*Adh*-*Monarda* cut-forward and reverse), was designed to differentially amplify a ~1200 basepair region believed to be specific to a single locus, named *Adh* type 1 (*Adh1*, see below). These primers were used



to test direct sequencing of the *Adh1* locus and allowed for more direct selection of these clones for sequencing. PCR product amplified with *Adhmc-F/Adhmc-R* primers was directly sequenced without cloning, but direct sequencing was problematic due to allelic variation in *Adh1* sequences so as an alternative these primers were used to amplify *Adh1* sequences for cloning. Finally, an additional primer *Adhi-F* (*Adh*-internal-forward) was designed to sequence an internal region in all *Adh* homologs in *Monarda*. This was a forward primer that amplified a region starting approximately 350 bases downstream from the *Adhm* and *Adhc* forward primers that was needed for obtaining a complete sequence of the entire amplified fragment.

One set of *nepGS* primers, GScp687-f and GScp994-r (designed by Emshwiller & Doyle 1999) (Table 6), were used to amplify a 700 basepair region of the glutamine synthetase gene in *Monarda*. This gene region was known to contain four intron regions in *Oxalis* species (Emshwiller & Doyle 1999).

Table 6: Primer sequences used for the amplification of *Adh* gene regions, and *nepGS* regions.

<i>Adh</i> -f:	5'-TAC TTCTGGGAAGCYAAG G-3'
<i>Adh</i> -r:	3'-CCTCGCATATTT GGTCA GAA G-5'
<i>Adhm</i> -f	5'-TATCTCAGGCGCAAGATTCA-3'
<i>Adhm</i> -r	3'-GCATCTTTGTGTGGAATCCT-5'
<i>Adhc</i> -f	5'- GAACATCCCAGGCTCAAGAT-3'
<i>Adhc</i> -r	3'- TAGAGCAGAATGCAGCCACA-5'
<i>Adhi</i> -f	5'- CCCGTTTACCATTTTCTT-3'
GScp687-f	5'-GATGCTCACTACAAGGCTTG-3'
GScp994-r	5'-AATGTGCTCTTTGTGGCGAAG-3'

2.6 DNA Amplification

PCR was conducted on a PTC-100 thermocycler. Three separate thermocycler programs were used (Table 7) depending on the ease at which the DNA could be amplified. DNA



extracted from herbarium tissue generally required the use of either a stepdown program or an annealing temperature of 55° C. Higher quality DNA could often be amplified at 60° C. Two Taq Polymerases were used for amplification. Platinum[®] Taq DNA Polymerase (Invitrogen[™] Life Technologies) was used to improve specificity and yield of PCR product for DNA samples that were either difficult to amplify or showed a high rate of PCR recombination. Standard Taq DNA Polymerase (New England Biolabs[®], Inc.) was used for all other PCR reactions. Amplification components follow those used by Prather and Jansen (1998) with the addition of 5% DMSO to each reaction and a 5 minute hotstart at 94°C for each thermocycler run.

Table 7: Three thermocycler programs used for PCR amplification

55° and 60° C annealing program	Stepdown program
1. 95° C for 5 minutes	1. 95° C for 5 minutes
2. 72° C for 2 minutes	2. 72° C for 2 minutes
3. 94° C for 1 minute	3. 94° C for 1 minute
4. 55° or 60° C for 1 minute	4. 56° C for 1 minute
5. 72° C for 3 minutes	5. 72° C for 3 minutes
6. 29 times to step 3	6. 2 times to step 3
7. 72° C for 5 minutes	7. Repeat steps 3 to 6 but reduce the annealing temperature by 3° C each time until an annealing temperature of 37° C is reached.
8. 15° C indefinitely	8. 72° C for 5 minutes
	9. 15° C indefinitely

2.7 Cloning

PCR products were gel purified from 2% agarose gels and cleaned using the Schleicher & Schuell Elu-quick DNA purification kit (Neese, NH). Purified DNA was cloned into *E.*

coli using TOPO™ TA Sequence Cloning® Kit (Invitrogen, Carlesbad, CA). Plasmid DNA was purified from bacteria by the use of either QIAGEN® QIAprep Spin Miniprep Kits (Qiagen™) or the manual DNA extraction method for bacteria (Sambrook et al. 1989).

2.8 *Adh* Clone Isolation

In several species it was necessary to pre-select sequences from a specific locus prior to cloning. Potential loci were preselected by digesting the *Adhm*-F/*Adhm*-R PCR product with two restriction enzymes. *AccI* was used for *Adh1* selection, and *EcoRI* was used for *Adh2* and *Adh3* selection (see below). Digested DNA was gel purified and undigested bands were isolated. *AccI*-selected and purified PCR product was cleaned and directly cloned. *Adh2* and *Adh3* selection (see below) involved additional steps due to the presence of an *EcoRI* cutting site located 43 basepairs downstream from the 5' end of the amplified sequence. It was necessary to fill this *EcoRI* overhang and adjoin an adenine-tail for insertion into the Topo® vector. Purified PCR products were cleaned and precipitated. Precipitated product was then vacuum dried for 10 minutes in a Labnet DyNAVap vacuum microfuge and rehydrated in 10 microliters of sterile distilled deionized water. *EcoRI* overhangs were filled using Klenow DNA Polymerase I Large Fragment (Tabor and Struhl, 1990) resulting in a blunt-ended product. Klenow reactions were stopped by placement on a 75° Centigrade heating block for 10 minutes. For the addition 3' adenine overhangs, the heating block was lowered to 72° C, Taq polymerase was added and, reactions were held at this temperature for 10 minutes. A-tailed DNA

samples were then precipitated, rehydrated in sterile distilled deionized water and directly cloned using Topo® TA kits.

2.9 Restriction Digest Analysis of *Adh*

Prior to sequencing, *Adh* clones were digested with the restriction enzyme *EcoR*I.

Digested clones exhibited three digest patterns when gel purified: no digestion, digestion at base 900 and digestion at base 400 and 900 (Fig 5). It was discovered that digested clones (both those that were digested at bases 400 and at 900) had sequences that represented one locus (*Adh1*) while undigested clones had sequences that represented the second and third locus (*Adh2* and *Adh3*). Restriction site cutting locations on sequences were confirmed using Webcutter 2.0 (Heiman 1997).

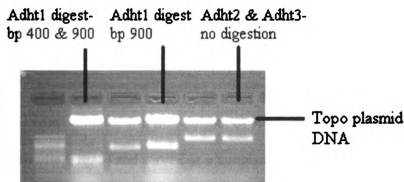


Figure 5: Cloned samples of *M. punctata* DNA (2048) digested from the Topo plasmid vector using *EcoRI*. Digested bands show three digestion patterns that were found to correspond to the three putative *Adh* loci: *Adh1* with single and double-digested bands and *Adh2* and *Adh3* with undigested bands.

2.10 Sequencing

Each *Adh* DNA insert was digested from the TOPO™ plasmid vector using *EcoRI* and quantified against a 100 basepair DNA ladder of known concentration (New England Biolabs®, Inc.). Sequence reactions were prepared using ABI PRISM® BigDye™

Terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase (Applied Biosystems©) and thermocycler parameters were set to those specified in the kit. Each *Adh* DNA insert was sequenced from the 5' and 3' ends and internally. All sequences were obtained using an ABI-373 automated sequencer.

2.11 DNA Alignment

Sequences were aligned and edited manually using Sequencer 3.0. (Gene Codes, Ann Arbor, MI). Aligned sequences were truncated so that they were all approximately 1200

basepairs in length. Complete datasets representing each marker were aligned first followed by subsets that represented an individual locus.

2.12 Identification of Putative *Adh* and *ncpGS* Loci

A tree containing the full *Adh* dataset of 99 sequences was used to identify putative loci (see results, Fig. 8) and served as a template to identify potential PCR recombinant sequences. These sequences were manually aligned and analyzed by maximum parsimony using Paup* version 4.0bv (Swofford 2000) (see results, Fig. 6) and parsimony settings were consistent with those used for all other analyses (see below). Trees were viewed as unrooted phylograms. Phylogenetically divergent sequence sets revealed three putative loci, labeled *Adh* type 1 (*Adh1*), *Adh* type 2 (*Adh2*) and *Adh* type 3 (*Adh3*). A second parsimony tree was used to identify suitable outgroup sequences for the same three putative loci. An unrooted phylogram was generated using 22 outgroup sequences (*Pycnanthemum* and *Monarda* spp.) and nine ingroup sequences representing *Adh1*, *Adh2* and *Adh3*. The phylogeny was generated by maximum parsimony using the same methods, and two equally parsimonious trees were created and tree one was used for analysis (see results, Fig 9).

2.13 Outgroup Selection and Rooting

Outgroup sequences that appeared at the base of the ingroup sequences representing *Adh1*, *Adh2* and *Adh3* were used for analysis (see results, Fig 8). The *Adh1* sequence set was rooted with two sequences of *M. clinopodioides* and sequences of *P. muticum*, *P. loomisii* and *P. tenuifolium*. The *Adh2* and *Adh3* sequence set was rooted with *P. muticum*, *P. loomisii*, three sequences of *M. citriodora*, and three sequences of *P.*

tenuifolium. The *Adh2* dataset was rooted with two sequences of *M. citriodora* and two sequences *P. tenuifolium*. *Adh1* and *Adh2* datasets were reduced to contain the same samples. The reduced *Adh1* dataset was rooted with *P. muticum*, *P. tenuifolium*, and two sequences of *M. clinopodioides* and the reduced *Adh2* dataset was rooted with the same sequences used for the full *Adh2* dataset.

nepGS loci were identified using a phylogeny of nineteen ingroup sequences (twelve taxa) and two outgroup sequences (*Pycnanthemum*). Phylogenetic analyses were the same as those used for *Adh*. Results showed that there were two putative *nepGS* loci that were divided by 24 base changes (see results, Fig 20). These putative loci were labeled *GSt1* and *GSt2*.

2.14 Phylogenetic Analyses

For ease in interpreting the phylogenetic results, analyses completed for each dataset are indexed in Table 9. Sequences were analyzed using Paup* version 4.0bv (Swofford 2000). Maximum parsimony analyses were performed using Tree Bisection Reconnection, Collapse, and Multrees options and Steepest Decent was inactive. Strict consensus trees were obtained and bootstrap support was measured using 1000 bootstrap replicates (Felsenstein 1985) with 100 addition-sequence replicates per bootstrap. The sequences were also analyzed using maximum likelihood methods. Optimal likelihood analyses were determined using Modeltest version 3.6 (Posada and Crandall 1998). The TrN+I+G model was selected for *Adh2* and the TrNef+G model was selected for *Adh1* (Table 8). The Shimodaira-Hasegawa (S-H) test was used to determine whether

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maximum parsimony trees were significantly worse than the maximum likelihood trees (Goldman et al. 2000).

Table 8: Likelihood model parameters for TrN+I+G and Trnef+G

Likelihood Model	TrN+I+G	Trnef+G
Base Frequencies	Freq A= 0.2766 Freq C= 0.1752 Freq G= 0.2228 Freq T= 0.3254	Empirical frequencies
Substitution Model	[A-C]= 1.0000 [A-G]= 2.3130 [A-T]= 1.0000 [C-G]= 1.0000 [C-T]= 3.3248 [G-T]= 1.0000	[A-C]= 1.0000 [A-G]= 6.1615 [A-T]= 1.0000 [C-G]= 1.0000 [C-T]= 11.7499 [G-T]= 1.0000
Proportion if invariable sites	=0.3488	= 0
Gamma distribution	= 0.7773	= 0.1897

Maximum likelihood methods were used because data contained imbalanced base frequencies (see below) and sites that have evolved quickly (intron regions) and slowly (exon regions). When data trends show these types of anomalies, it is useful to include likelihood-based methods of analysis (Collins et al. 1994).

The base frequencies for all *Adh* datasets were calculated on PAUP * version 4.0bv (Swofford 2000) and histograms were made using Microsoft Excel (Fig. 6 and 7). Results were used to identify biases in the datasets that may potentially skew parsimony results. The nucleotide frequencies among taxa showed a character state bias in the dataset..



Table 9: List of all phylogenetic analyses completed for each *Adh* dataset. Check marks indicate a particular test was completed for that dataset.

Analysis	Full <i>Adh</i>	Outgroup	<i>Adh1</i>	<i>Adh2</i> & <i>Adh3</i>	<i>Adh2</i>	<i>Adh3</i>	<i>Reduced Adh1 & Adh2</i>
Maximum parsimony	✓	✓	✓	✓	✓	✓	✓
Parsimony strict consensus		✓	✓	✓	✓		✓
Parsimony bootstrap			✓	✓	✓		✓
Maximum likelihood			✓		✓		
Shimodaira-Hasegawa test			✓		✓		
Bayesian analysis			✓	✓	✓		✓

Bayesian analyses were run using Mr. Bayes 3.0 (Huelsenbeck, 2001). Bayesian inference is a more recent tool for phylogenetic analysis that is just beginning to gain popularity (Alfaro et al. 2003). This phylogenetic method uses Baye's theorem of inverse probability and likelihood parameters to measure tree topologies and branch lengths. Bayesian methods apply probabilities that a given tree is correct given a particular dataset and maximum likelihood parameters. In Bayesian analyses Bayesian confidence values are also applied to the tree nodes. These tree node probabilities are determined using Bayesian Markov Chain Monte Carlo (BMCMC) sampling method inference (Alfaro et al. 2003). Probabilities are determined from multiple phylogenies generated by Bayesian inference. The Markov chain samples the branch lengths and substitution parameters of a given set of Bayesian trees to generate node probabilities. Node values above 90 are considered to be well supported.

2.15 Phylogenetic Interpretation

Neoendemic species were distinguished from paleoendemic species based on their phylogenetic position. Paleoendemics were expected to appear as the sister group to *M. punctata* and to have longer branch lengths, and neoendemics were expected to be derived within *M. punctata* and have shorter branches. In addition, species that speciated as peripheral isolates of *M. punctata* are expected to show tree topologies similar to those shown in the hypothetical gene tree depicting peripatric speciation in Figure 1.

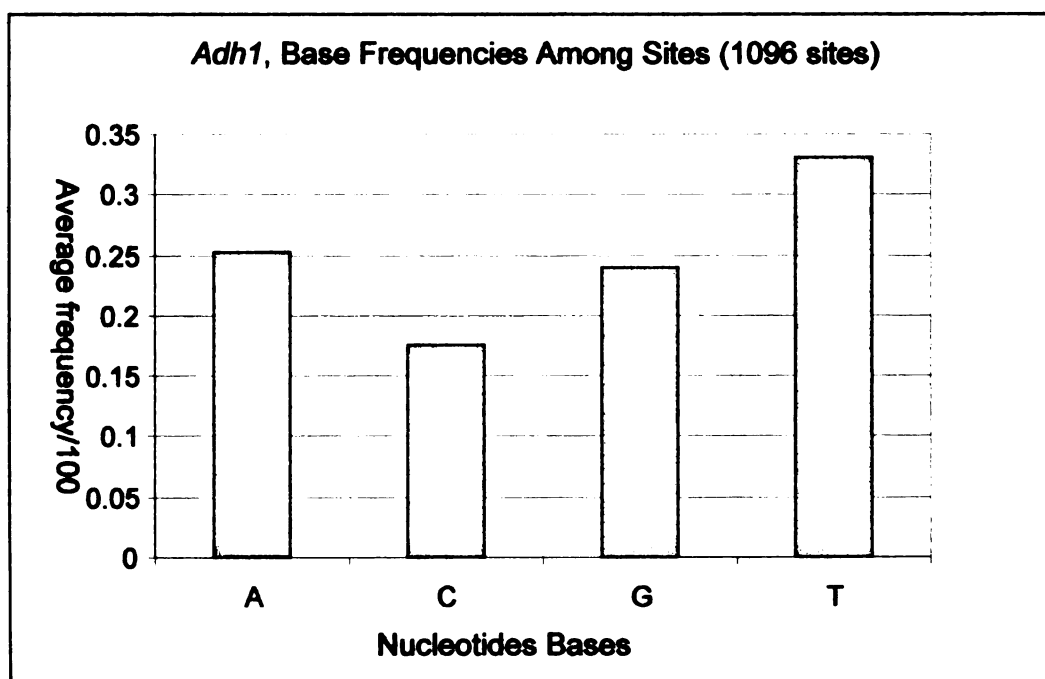


Fig 6: Histogram of the base frequencies (Average frequency/100) in the *Adh1* dataset. These results are based on 1096 base sites.

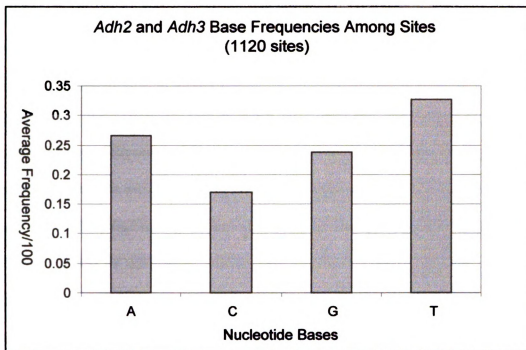
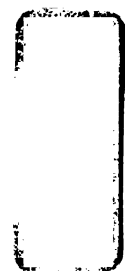


Fig 7: Histogram of the base frequencies (Average frequency/100) in the *Adh2* and *Adh3* datasets. These results are based on 1120 base sites.



RESULTS

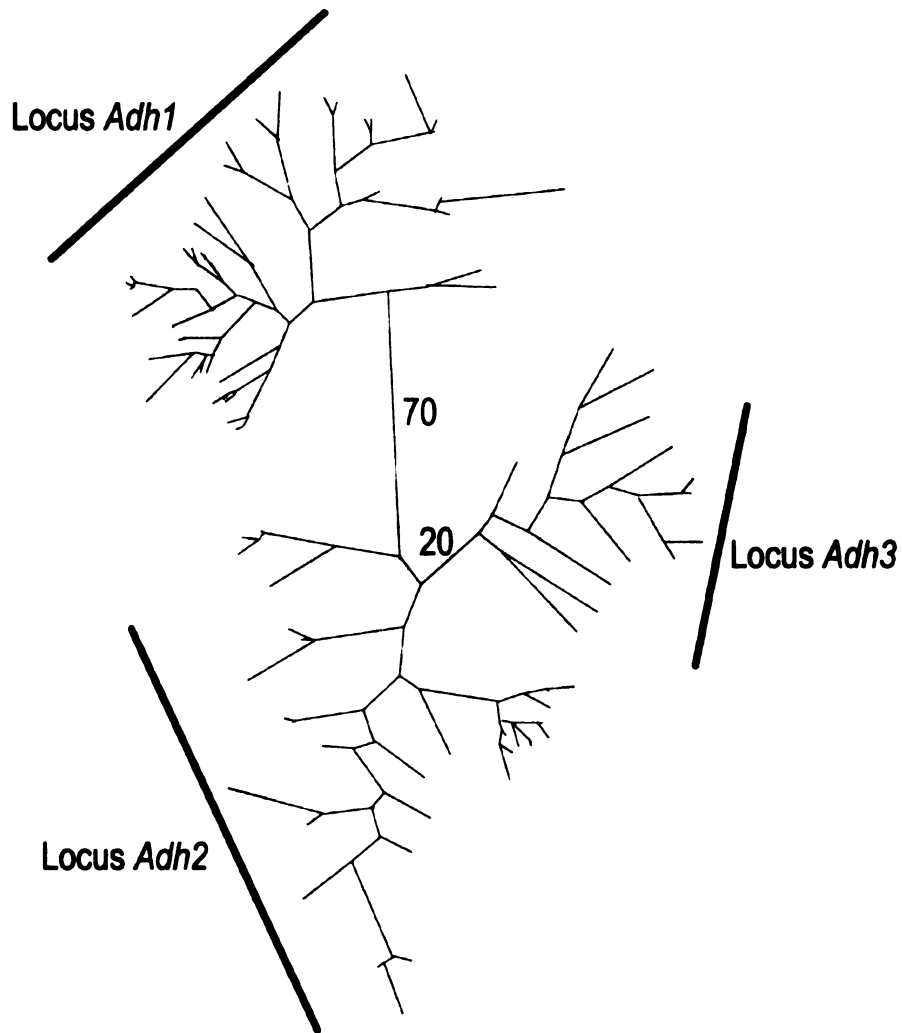
3.1 Identification of Putative Loci

Ninety-nine *Adh* sequences, approximately 1,200 basepairs in length were obtained from the six ingroup *Monarda* species. Two separate phylogenetic analyses were used to delineate potential loci. The first phylogeny was generated by maximum parsimony and contained all 99 ingroup *Monarda* sequences. One hundred equally parsimonious trees were generated for this dataset, tree length was 1663, the consistency index (CI) was 0.518 and the retention index (RI) was 0.870. In an unrooted phylogram (Fig. 8), *Adh1* and *Adh2* were divided by 70 base changes and *Adh2* and *Adh3* were divided by 20 base changes. This phylogeny was used to determine how and where ingroup sequences parsed, and allowed for better ingroup sequence selection for the second analysis.

The second analysis contained 23 potential outgroup sequences (seven species) and nine ingroup sequences (five species) representing *Adh1*, *Adh2* and *Adh3*. The ingroup sequences were selected from several points across the each putative locus. Data were analyzed by maximum parsimony and two equally parsimonious trees were created with a length of 797, a CI of 0.649 and a RI of 0.806. In an unrooted phylogram (Fig. 9), *Adh1* and *Adh2* were separated by 43 base changes and *Adh2* and *Adh3* were separated by 12 changes. A strict consensus tree was generated from the two most parsimonious trees (Fig.10), and compared to the unrooted phylogram. Branch length disparity between the full *Adh* tree and outgroup tree was attributed to the increased sample number in the full *Adh* tree and the inclusion of outgroup samples. *Pycnanthemum* samples appear to have a

fourth putative locus adjacent to *Adh1* that is not found in *Monarda*, and no *Pycnanthemum* samples appear to branch within *Adh2*.

The *Adh1* dataset was rooted with two *M. clinopodioides* samples and *P. muticum*, *P. loomisii* and *P. tenuifolium* samples. The *Adh2/Adh3* dataset was rooted with *P.-muticum*, *P. loomisii*, three *M. citriodora* samples, and three *P. tenuifolium* samples. The *Adh2* dataset was rooted with two *M. citriodora* and *P. tenuifolium* samples.



— 5 changes

Figure 8: An unrooted phylogram of ninety-nine *Adh* sequences representing all ingroup *Monarda* samples. The three putative loci, *Adh1*, *Adh2* and *Adh3*, are labeled. Seventy steps separate *Adh1* and *Adh2* and 20 steps separate *Adh2* and *Adh3*. Several sequences branch between *Adh1* and *Adh3* but were found to fall in the *Adh2* dataset when *Adh2* and *Adh3* were separated and outgroup sequences were incorporated.

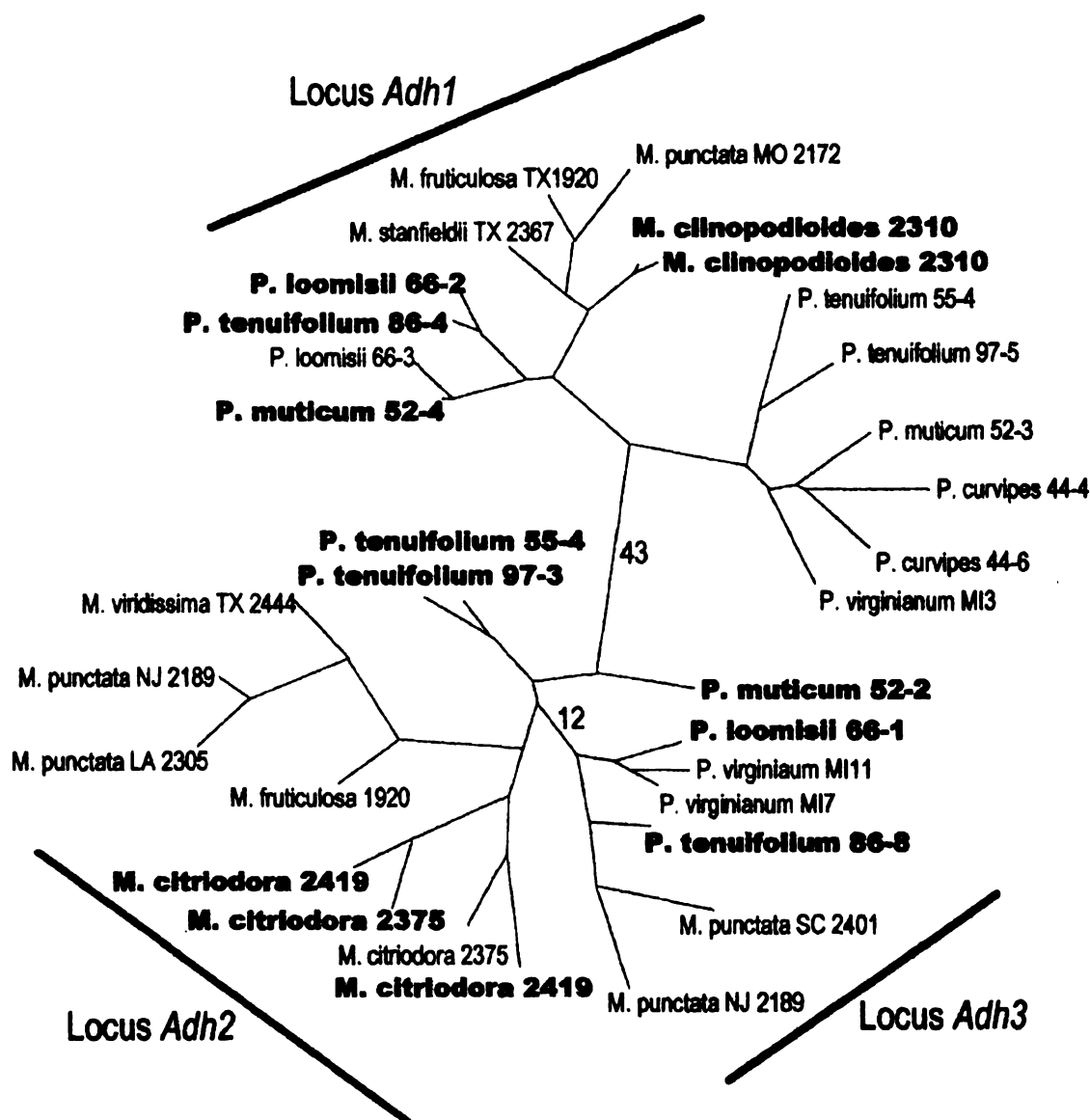


Figure 9: Phylogeny of 23 outgroup sequences and nine ingroup sequences representing all three putative loci identified (*Adh1*, *Adh2* and *Adh3*). The branch dividing *Adh1* and *Adh2* has 43 changes and the branch dividing *Adh2* and *Adh3* has 12 changes. Taxon labels include species and DNA numbers. Samples in bold were chosen as outgroups for later analyses.

Strict

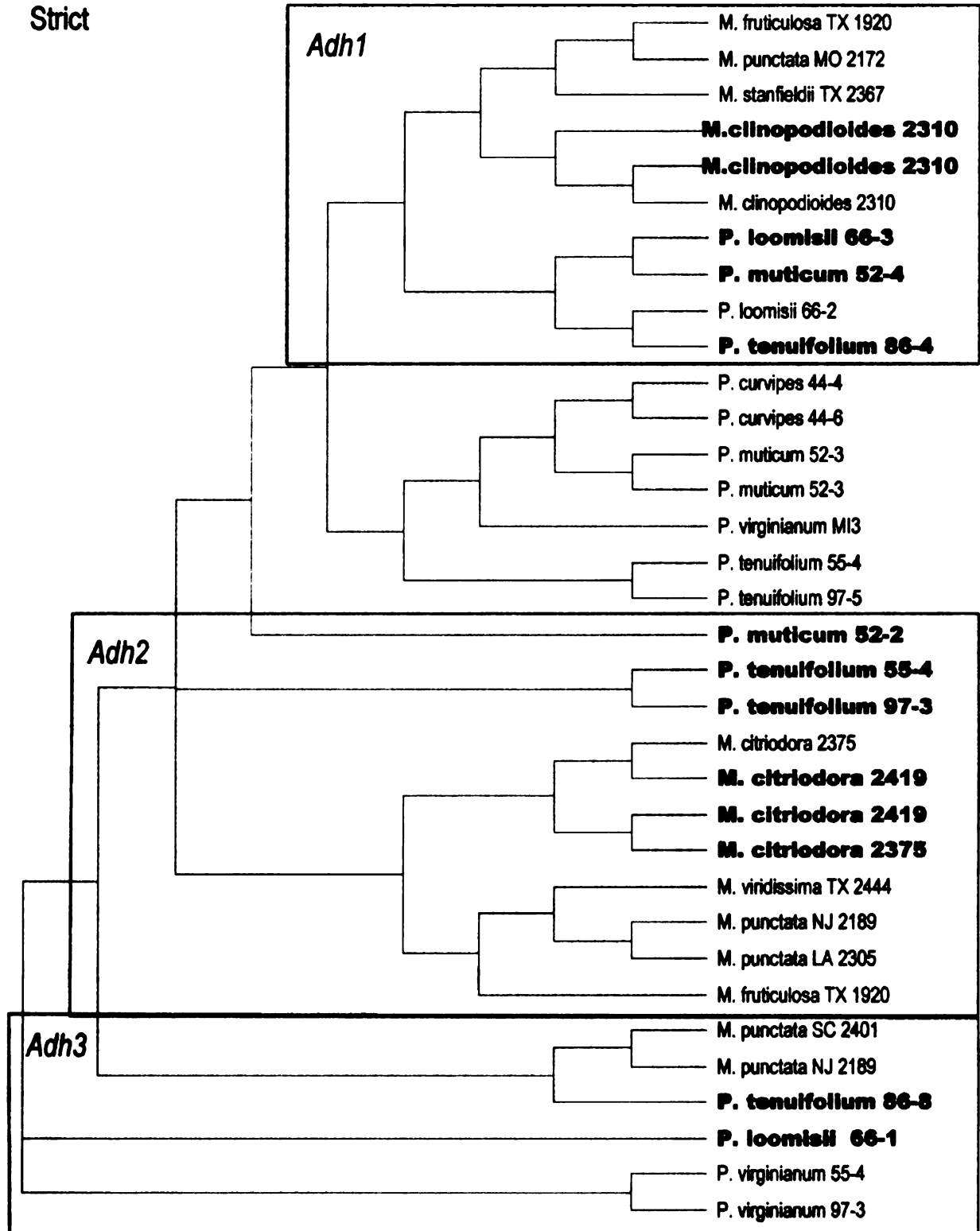


Figure 10: Strict consensus tree of 2 equally parsimonious trees representing 23 potential outgroup sequences and nine ingroup sequences from all three loci identified (Adh1, Adh2 and Adh3). The Pycnanthemum clade between Adh1 and Adh2 represents a fourth putative locus that is unique to Pycnanthemum. Sequences that are in bold were chosen as outgroups. Taxon labels include species and DNA numbers.

3.2 *Adh1*

The *Adh1* dataset had 35 ingroup sequences of all six species collected from 24 populations. Data were analyzed by maximum parsimony, parsimony bootstrap, maximum likelihood, the S-H test and Bayesian analyses. Thirty-seven most-parsimonious trees were generated and each had a length of 480, a (CI) of 0.503 and a (RI) of 0.746. All uninformative characters were removed from the datasets before CI values were obtained. The S-H test was run on all 37 most parsimonious trees and one maximum likelihood tree (TrNef+G). The best maximum parsimony tree identified (Fig 11) had several topological differences relative to the TrNef+G tree (Fig. 12) including the placement of *M. stanfieldii*, *M. maritima* and *M. fruticulosa* (2301 and 2317) samples. The parsimony strict consensus tree (Fig. 13) and Bayesian consensus tree shared the same topology, and bootstrap values and Bayesian node confidence values were applied to this phylogeny.

The strict consensus tree had one major *M. punctata* clade with *M. fruticulosa*, *M. humilis*, *M. maritima* and *M. viridissima* sequences derived within it (Fig 13). The *M. punctata* clade had a high Bayesian confidence value of 99 (NC99), but had bootstrap support less than 50 (BS-). Most of the *M. punctata* samples were together in two smaller clades: One contained nine *M. punctata* samples (2303, 2397, 2398, 2305, 2048, 2172, 2307 and 2396) and had strong support (BS81/NC100), while the second had four samples (1937, 2048, and 2189) and higher support (99BS/100NC). *Monarda fruticulosa* (2301 and 2317) and *M. maritima* (2314, 2318 and 2319) samples shared two moderately well-supported clades (-BS/99NC and 59BS/100NC) within the larger *M. punctata* clade.

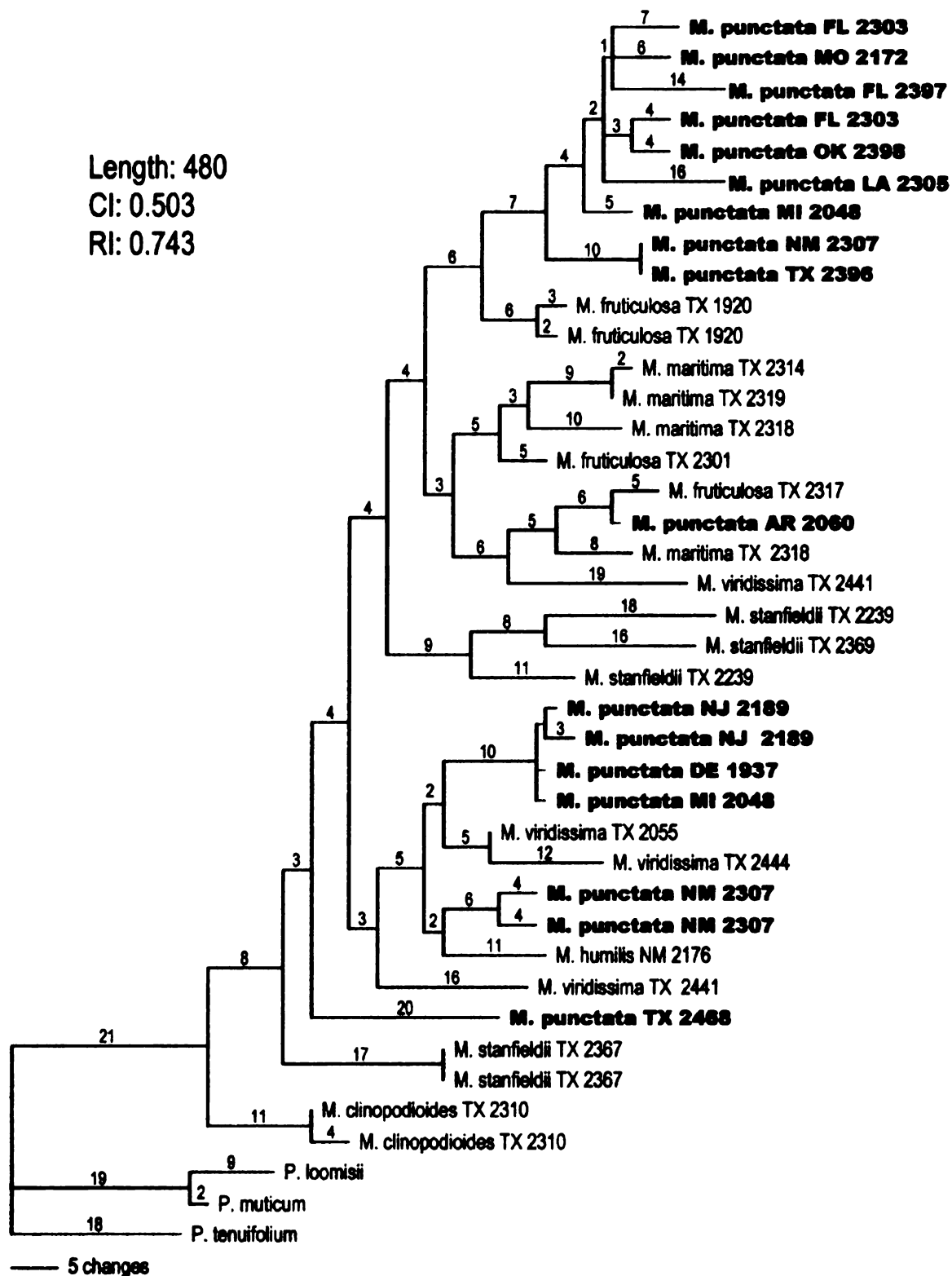


Figure 11: The *Adh1* parsimony phylogram selected by the S-H test. Branches are labeled with steps. Taxon labels include species, state of origin and DNA number. The larger *M. punctata* clade is labeled by the bar along the right margin. Tree length, CI and RI values are shown.

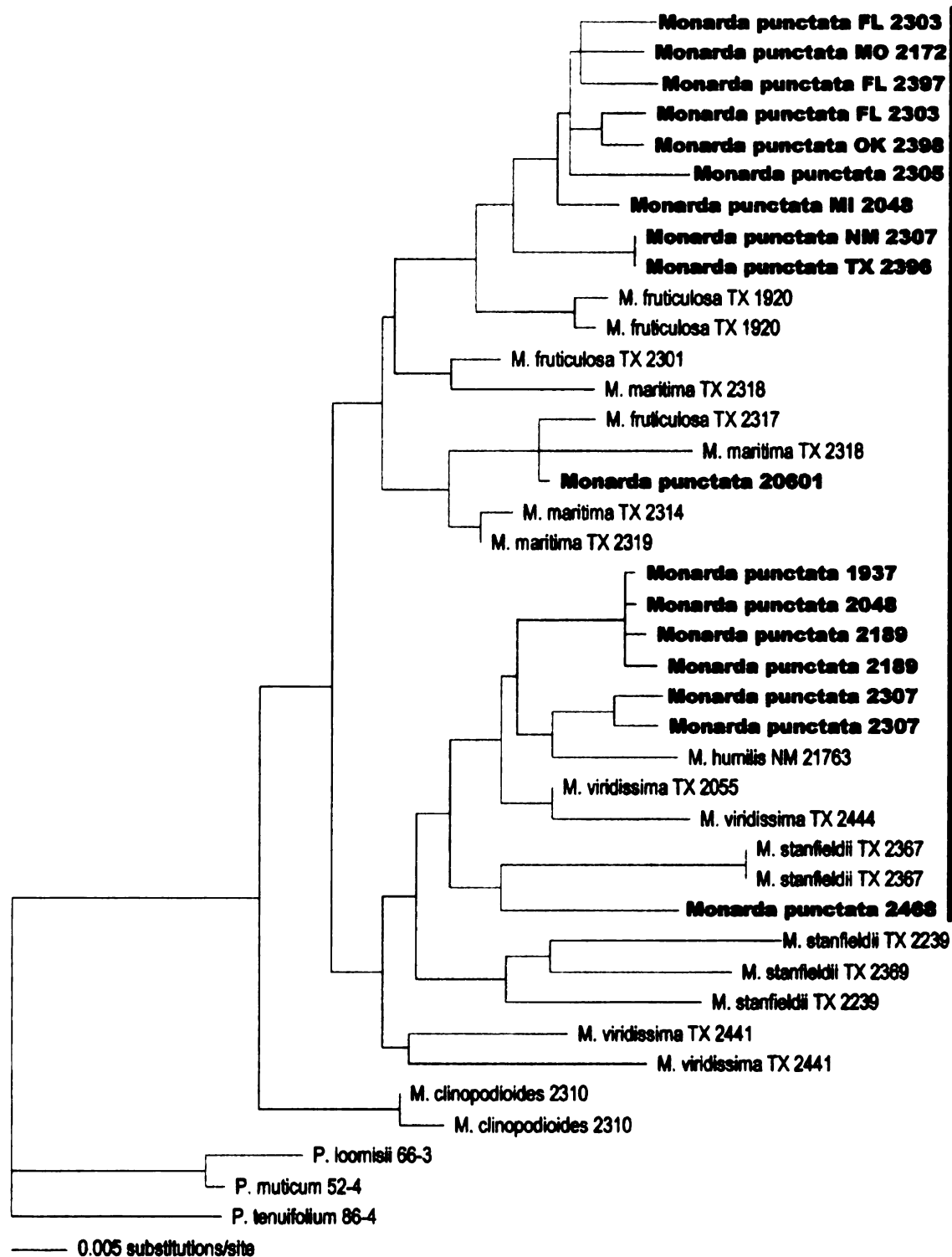


Figure 12: The *Adh1* likelihood phylogram using the TrNef+G model of substitution. Branches are labeled with values denoting substitutions per site. Taxon labels include species, state of origin and DNA number.

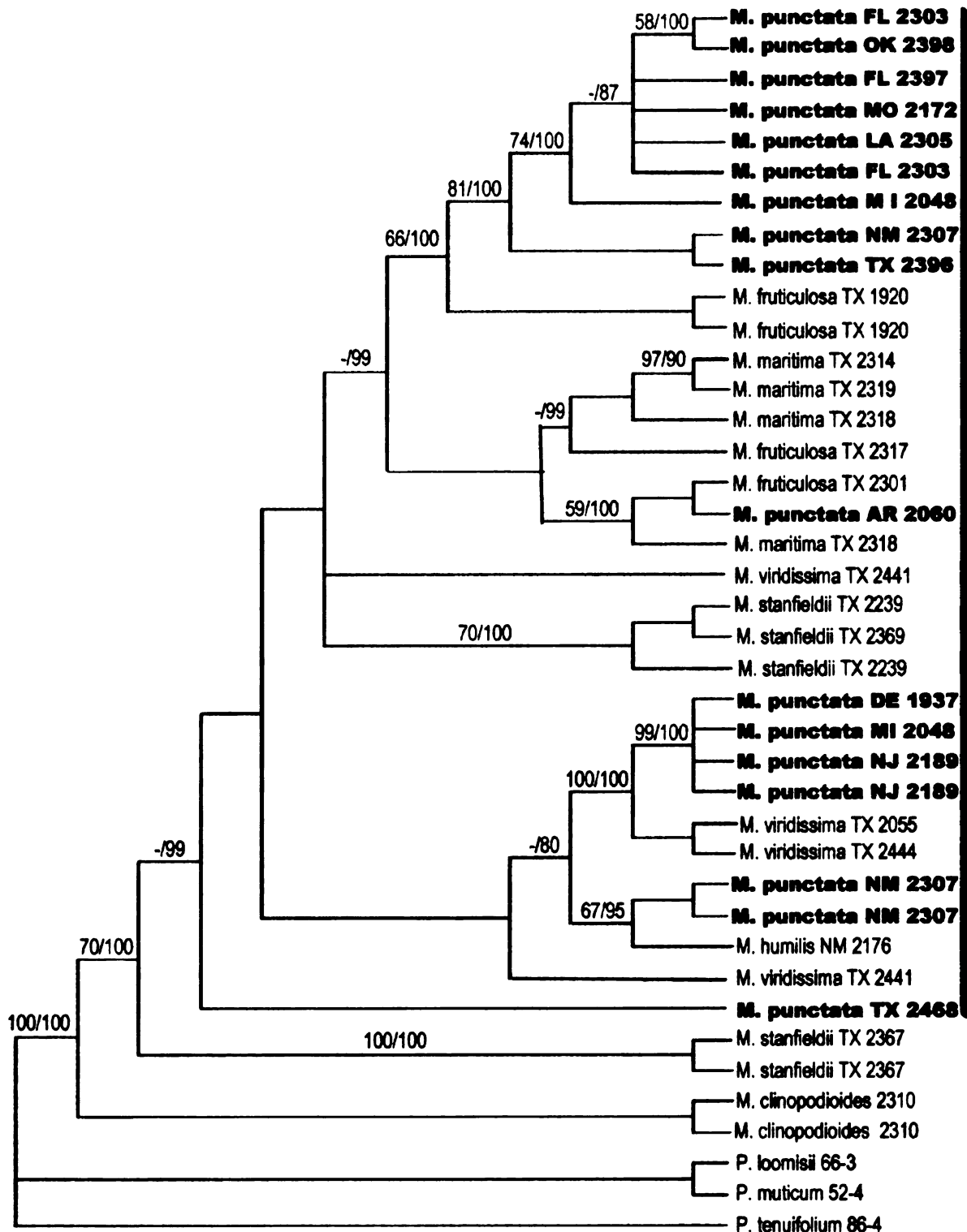


Figure 13: Strict consensus of 37 maximum parsimony trees from the full *Adh1* dataset. Taxon labels include species, state of origin and DNA number. Branches are labeled with bootstrap (left) and Bayesian node confidence values (right); only values above 50 are shown and values below 50 are labeled with dashes. The larger *M. punctata* clade is labeled by the bar along the right margin.

This was the only narrow endemic species that was not within the *M. punctata* clade. Two *M. stanfieldii* clones (2367) appeared on the most basal branch sister to the *M. punctata* clade and two additional *M. stanfieldii* samples (2239 and 2369) were derived within the larger *M. punctata* clade but with no support. *Monarda viridissima* was more widely dispersed in the phylogeny. Two samples (2055 and 2444) branched together with *M. punctata* and two clones of the third sample (2441) were not supported in the larger *M. punctata* clade. The single *M. humilis* sample (2176) had good support (-BS/95 NC) being in a clade with two *M. punctata* from New Mexico (2307).

3.3 *Adh2* and *Adh3*

These data were analyzed by maximum parsimony, parsimony bootstrap and Bayesian analyses. The *Adh2* and *Adh3* dataset included 38 sequences and generated 6 most parsimonious trees with a length of 571 steps, a CI of 0.434 and RI of 0.743. The parsimony and Bayesian consensus trees were identical and both parsimony bootstrap values and Bayesian node confidence values were applied to this tree (Fig. 14).

Many sequences were replicated in both *Adh2* and *Adh3* trees. Outgroup sample, *M. citriodora* had two sequences at the base of each putative locus (Fig. 14). The branch that delineated *Adh2* and *Adh3* had a high Bayesian node confidence value of 100 but no bootstrap support. Nearly all the samples in *Adh3* were of geographically separate *M. punctata* except for two *M. stanfieldii* sequences (2369) in the clade with *M. citriodora* (2419). Clades in *Adh3* had several high Bayesian node confidence values but little to no bootstrap support.

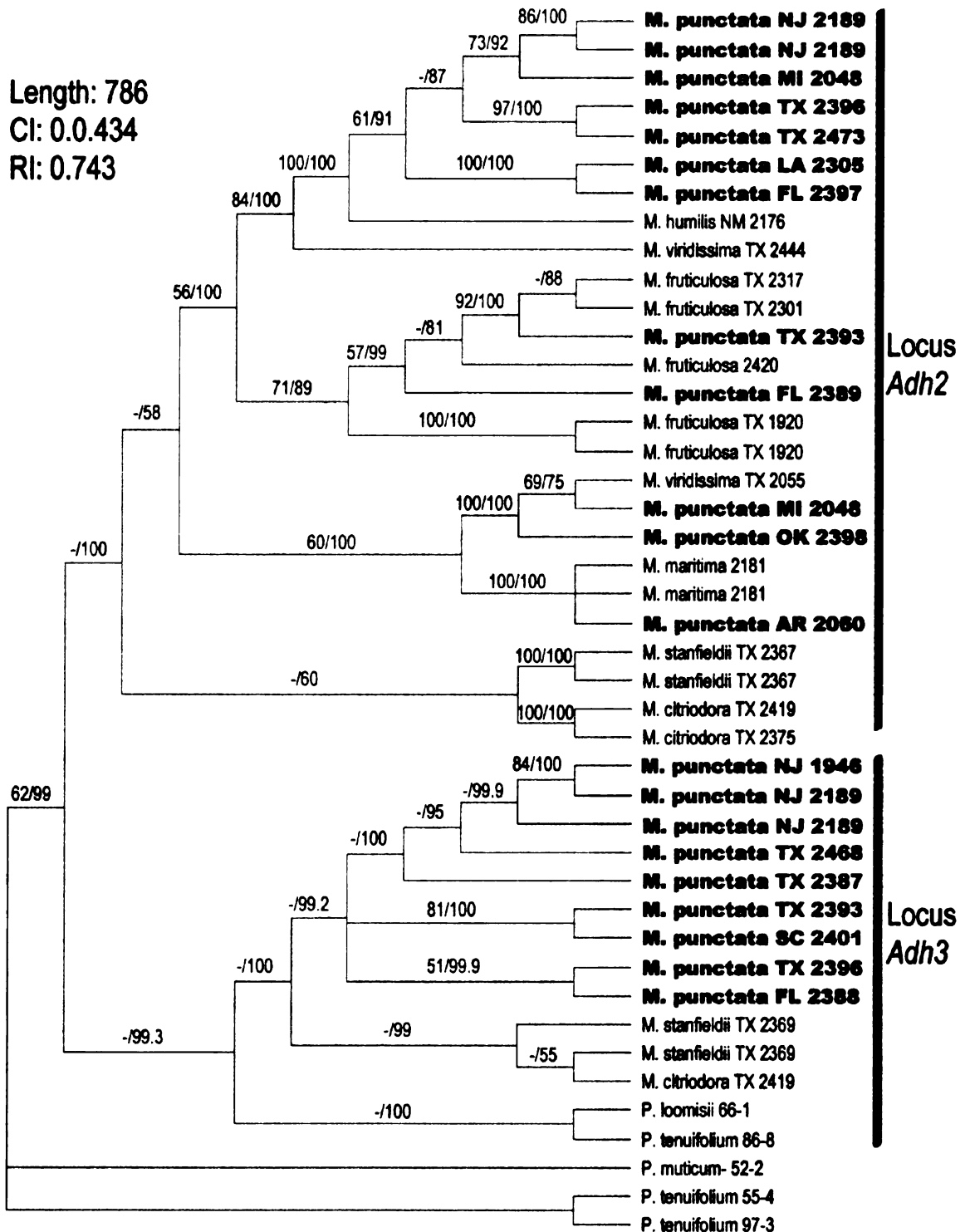


Figure 14: Strict consensus of 6 maximum parsimony trees from the full *Adh2* and *Adh3* dataset. Taxon labels include species, state of origin and DNA number. Branches are labeled with bootstrap (left) and Bayesian node confidence values (right) and only values above 50 are shown. The *Adh1* and *Adh3* loci are labeled by the bar along the right margin. Tree length, CI and RI values are shown.

3.4 *Adh2*

As with *Adh1*, these data were analyzed by maximum parsimony, parsimony bootstrap, maximum likelihood, the S-H test and Bayesian analyses. The *Adh2* dataset contained only 24 ingroup samples. This was due in part to the presence of *Adh3* and difficulties in obtaining *M. maritima* and *M. viridissima* sequences for this locus. Many of the sequences that were selected for the *Adh2* dataset fell within *Adh3* because both duplicates had the same restriction digest pattern and it was impossible to differentiate between them prior to sequencing. Likewise, I had much difficulty in obtaining *Adh2* sequences from *M. maritima* and *M. viridissima*.

Parsimony analysis of *Adh2* data generated six trees with 446 steps, CI value of (0.588) and RI value (0.830) than the *Adh2* and *Adh3* combined tree. The topology of the best tree (Fig. 15) was identical to an additional tree generated by likelihood (Trn+I+G, Fig. 16) with the exception to its placement of *M. stanfieldii* sequences relative to *M. citriodora*.

The parsimony strict consensus tree and the Bayesian tree shared the same topology, and parsimony bootstrap values and Bayesian node confidence values were applied to its branches/nodes. This tree also contained one major clade of *M. punctata* that had *M. fruticulosa*, *M. humilis*, *M. maritima* and *M. viridissima* sequences nested within it (Fig. 14), but the clade had poor Bayesian and bootstrap support (55BS/64NC). Like the *Adh1* tree, two clones of *M. stanfieldii* (2367) were on a basal branch adjacent to the outgroup and were not derived within *M. punctata*.

Most of the *M. punctata* samples were together on one supported branch (74BS/89NC) that contained seven *M. punctata* samples (2189, 2048, 2305, 2397, 2396 and 2473) with one *M. humilis* (2176) and one *M. viridissima* (2444) sample nested within. All three *M. fruticulosa* samples were on one well-supported branch (68BS/93NC) with two *M. punctata* samples.

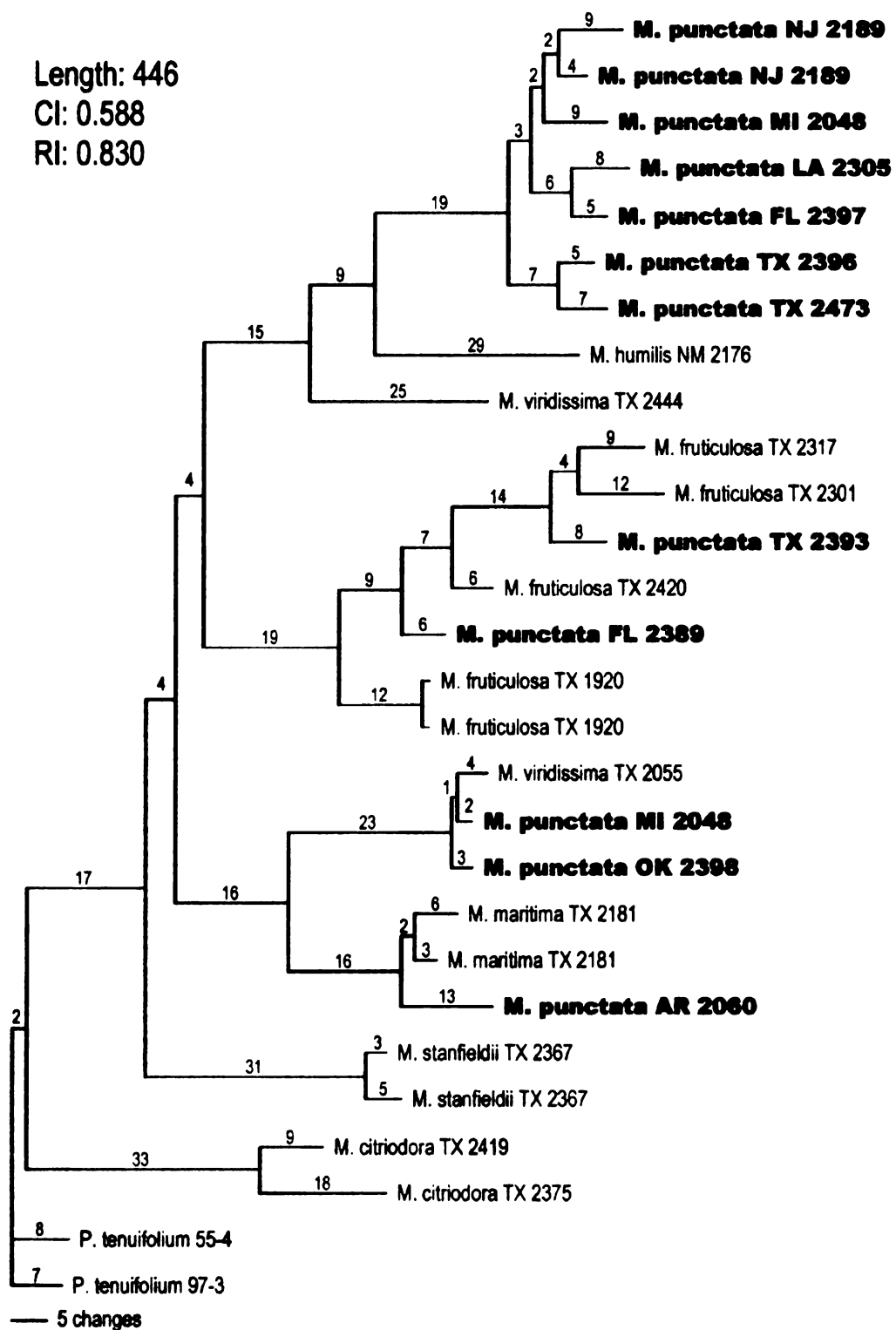


Figure 15: A phylogram of the full *Adh2* tree. Taxon labels include species, state of origin, DNA number. Branches are labeled with base change values. The larger *M. punctata* clade is labeled with a bar along the right margin.

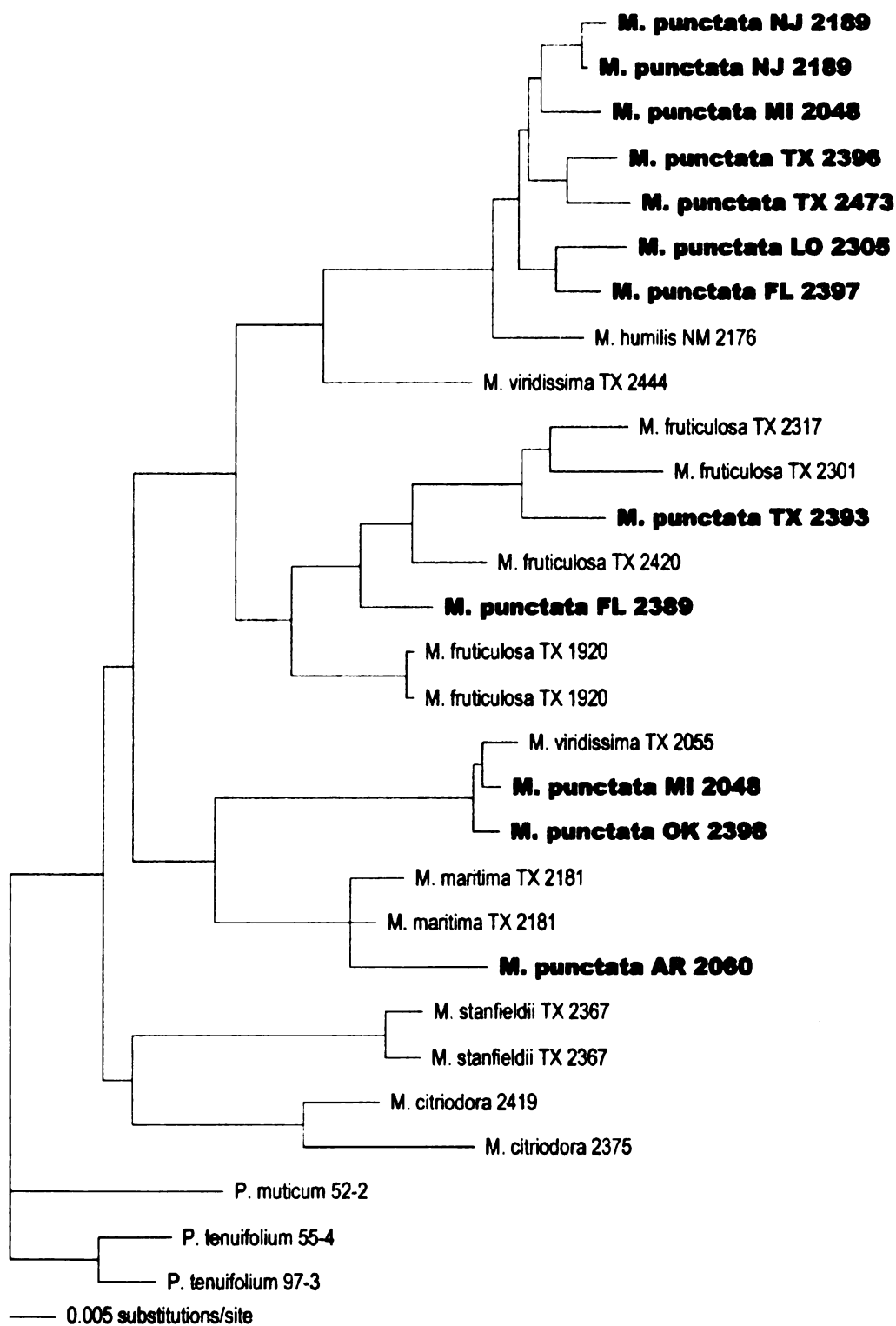


Figure 16: The *Adh2* likelihood phylogram using the Trn+I+G model of substitution. Branches are labeled with values denoting substitutions per site. Taxon labels include species, state of origin and DNA number.

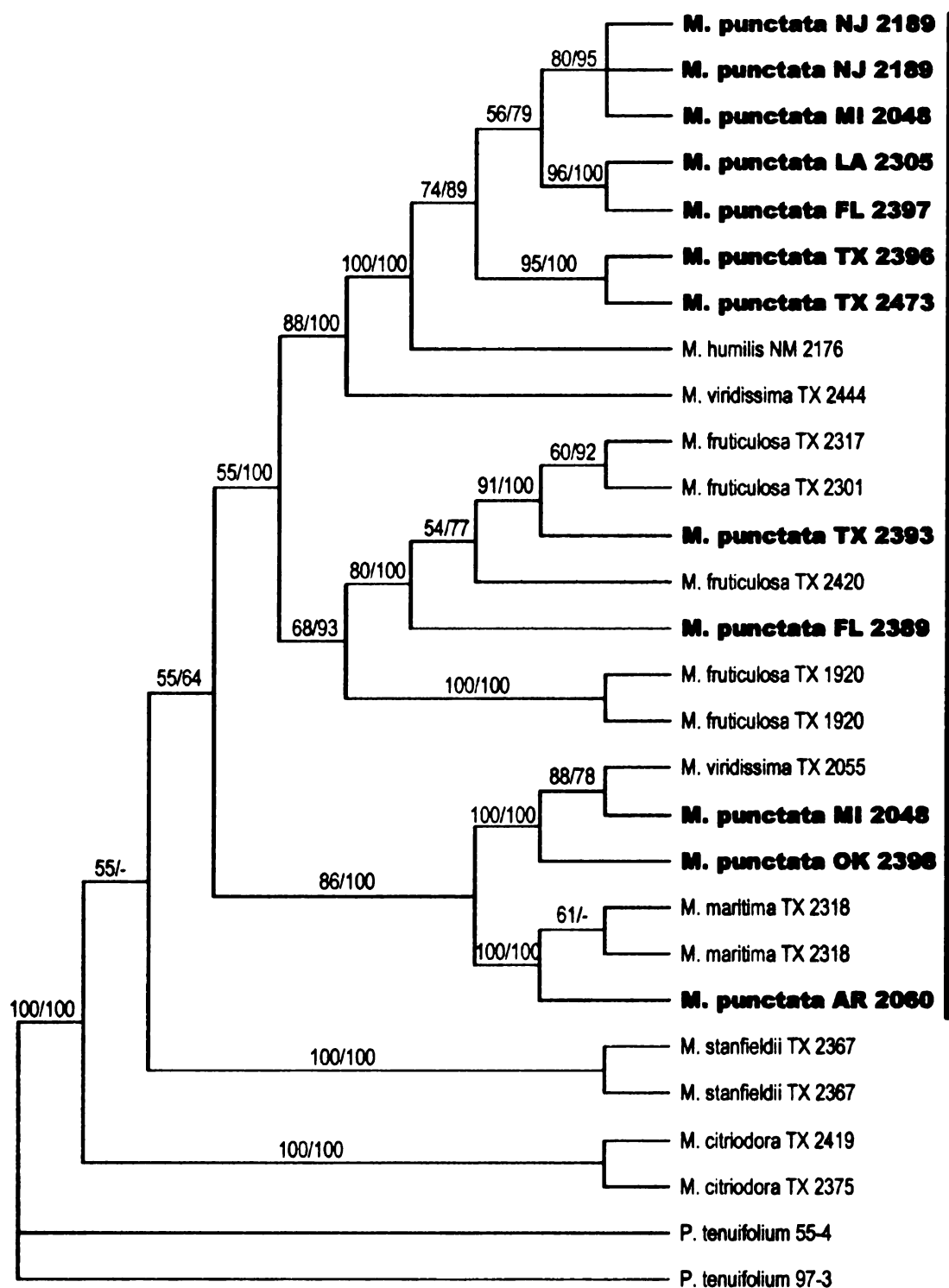


Figure 17: Strict consensus of the six most parsimonious trees of *Adh2* with 20 ingroup sequences. Taxon labels include species, state of origin, and DNA number. Branches are labeled with bootstrap (left) and Bayesian node confidence values (right) and only values above 50 are shown. The larger *M. punctata* clade is labeled by the bar along the right margin.

3.5 *Adh3*

This dataset contained eight *M. punctata* samples and two *M. stanfieldii* samples and was analyzed by parsimony analysis only. One maximum parsimony tree was generated and it had a length of 358, a CI of 0.676 and a RI of 0.408. *Monarda stanfieldii* samples were at the base of the tree with a *M. citriodora* outgroup sequence. *Monarda punctata* samples were in two clades. One had *M. punctata* samples only and the second included the *M. stanfieldii* and *M. citriodora* samples.

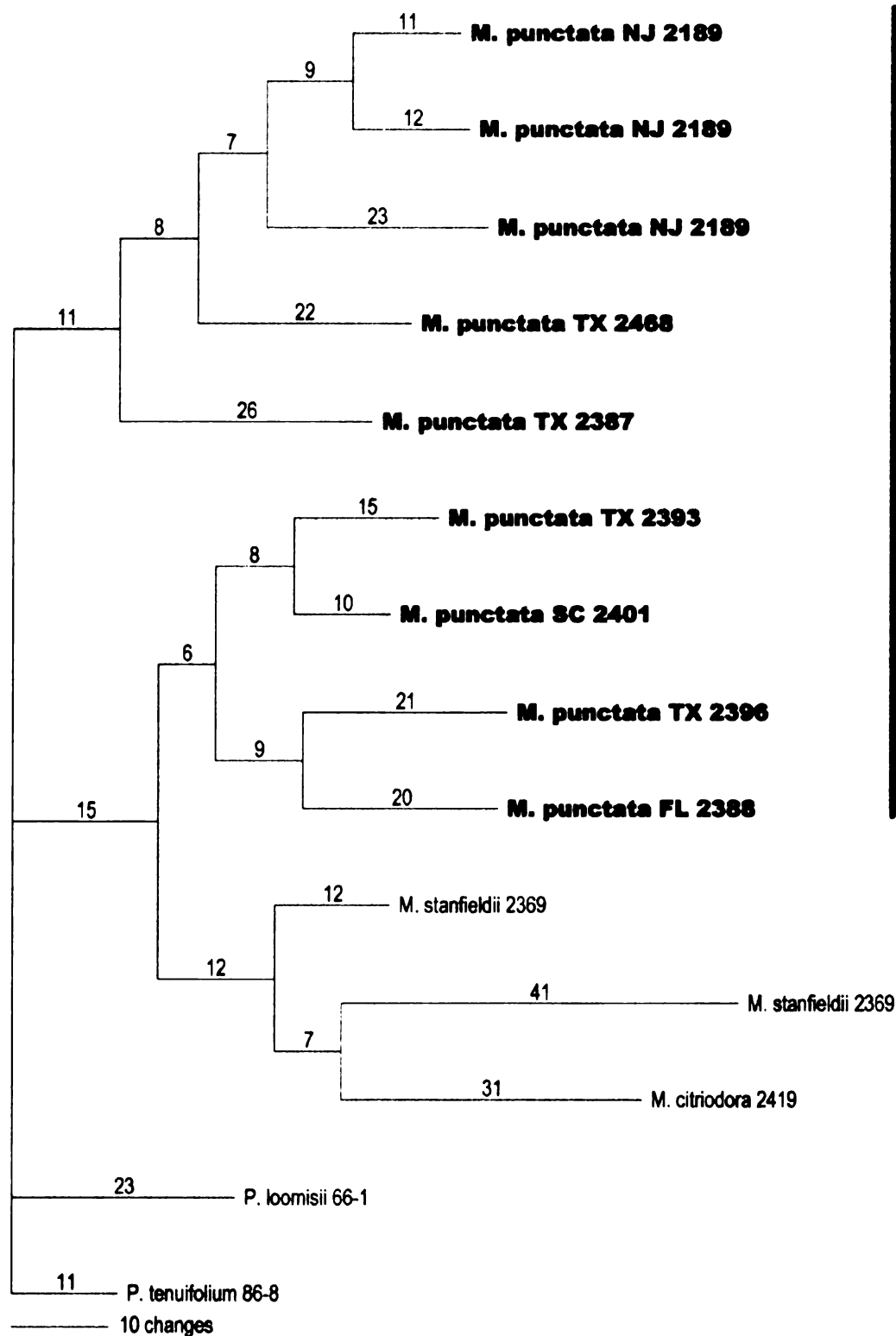


Figure 18: Adh3 parsimony tree with 11 ingroup sequences. Branches are labeled with steps. Taxon labels include species, state of origin and DNA number. The *M. punctata* clades are labeled by the bar along the right margin.

3.6 Combined and Reduced *Adh1* and *Adh2*

As with full *Adh1* and *Adh2* datasets, these data were analyzed by maximum parsimony, parsimony bootstrap and Bayesian analyses. *Adh1* and *Adh2* datasets were reduced so they shared the same samples (Fig 19). Eighteen trees were generated in the maximum parsimony analysis of the reduced *Adh1* dataset (length: 200, CI: 0.585, RI: 0.771). The parsimony strict consensus tree and the Bayesian tree shared the same tree topology (Fig. 15). Two trees were generated in the maximum parsimony analysis of the reduced *Adh2* dataset (length: 275, CI: 0.618, RI: 0.835) and the parsimony strict consensus tree and Bayesian tree were also topologically the same.

Both trees placed *M. stanfieldii* (2367) sequences at the most basal branch of the tree. *Monarda punctata* was in two primary clades in both trees. Endemic species (*M. fruticulosa*, *M. humilis*, *M. maritima* and *M. viridissima*) showed a higher degree of variability regarding their placement in the trees, although each phylogeny had a clade that consistently contained the majority of the *M. fruticulosa* sequences included in the study.

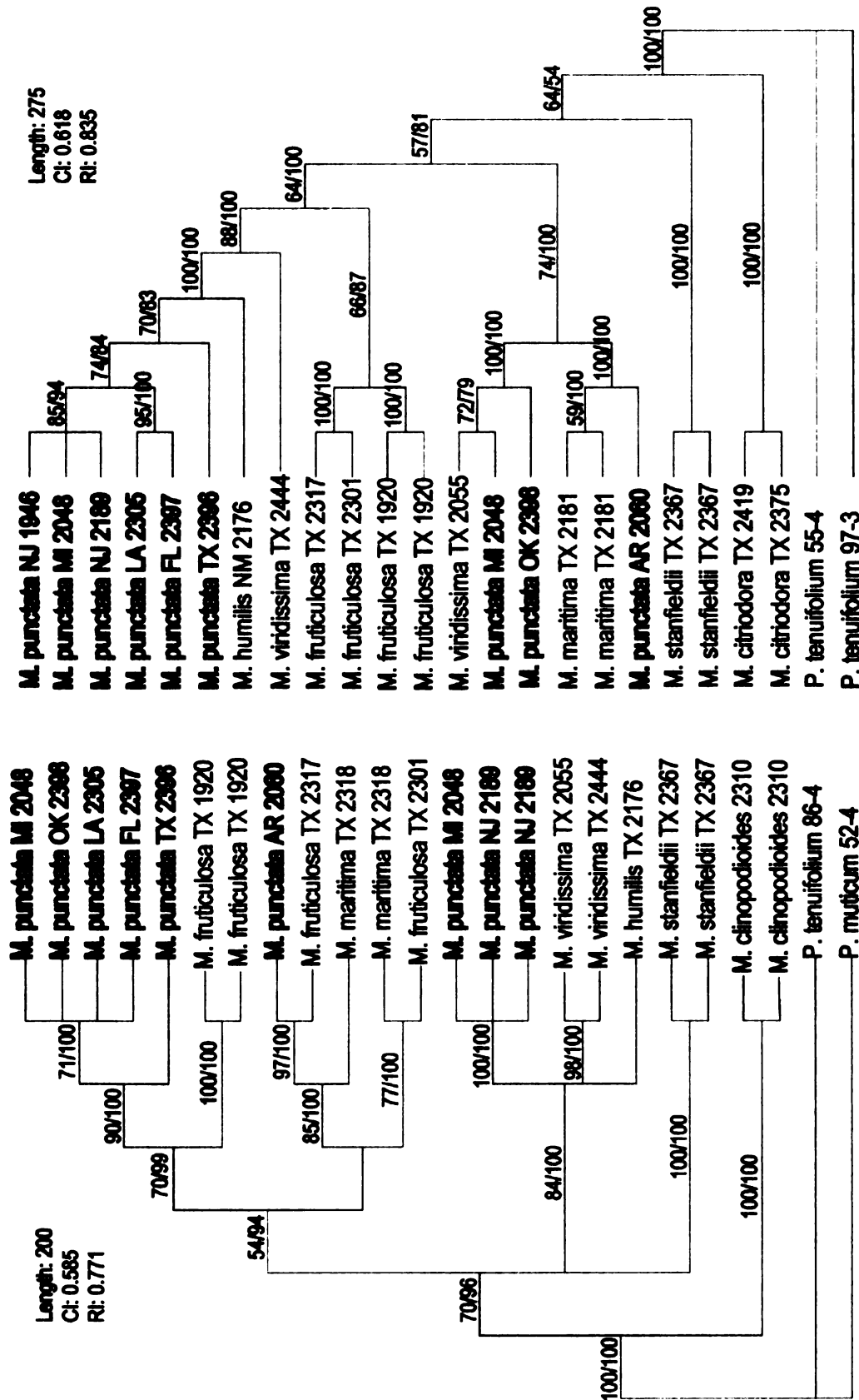


Figure 19: Reduced *Adh1* and *Adh2* phylogenies. The larger *M. punctata* clade is labeled by the bar along the right margin. Branches are labeled with bootstrap (left) and Bayesian node confidence values (right) and only values above 50 are shown.

3.7 *ncpGS*

Nineteen *ncpGS* sequences, approximately 600 basepairs in length were obtained from four *Monarda* species (*M. fruticulosa*, *M. maritima*, *M. punctata* and *M. viridissima*) with two *Pycnanthemum* sequences set as outgroups. Results showed that there were two distinct *ncpGS* loci (Fig.16) with 264 steps, a CI of 0.586 and a RI of 0.842. The two putative loci were separated by 24 base changes. Due to the limited number of taxa in this dataset, no further analyses were run on these trees.

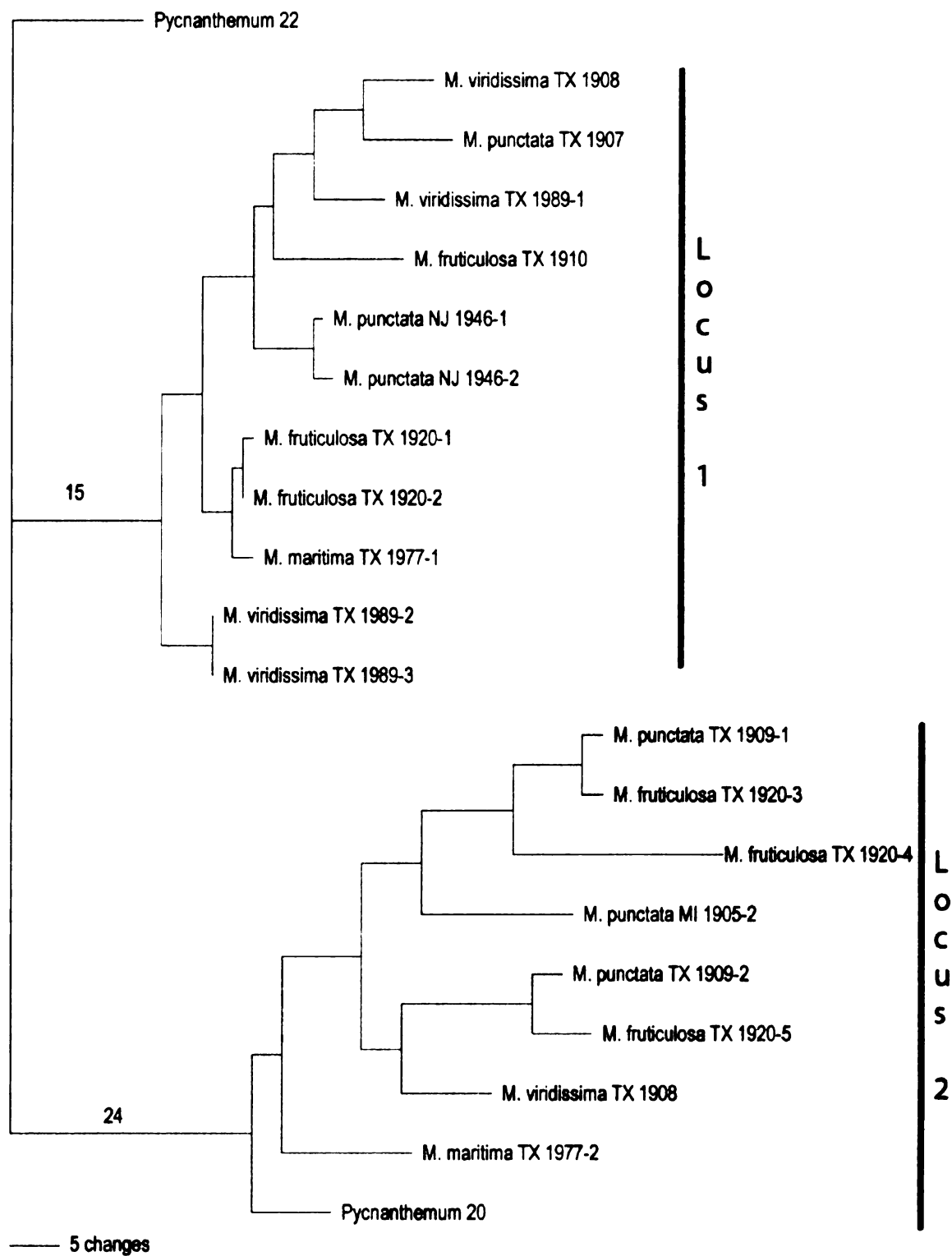


Figure 20: Full *ncpGS* sequence data representing ten different taxa. Taxon labels include genus/species, state of origin and DNA number. Locus 1 and 2 are listed along the right margin.

DISCUSSION

4.1 General Overview of Results

The objectives of this project were to use the species in *Monarda* Section *Cheilyctis* to test whether speciation by peripheral isolation had occurred among the species of the section, and to determine whether the endemic species were paleo- or neoendemics. Data showed a high degree of *Adh* allelic variability within and between *Monarda* species. However, the phylogenetic results provided by *Adh* loci offered insight in answering these questions.

In each dataset all of the methods employed, maximum parsimony, maximum likelihood, and Bayesian analyses consistently produced trees with similar topologies. Consistency in their phylogenetic results increased the chances that the best trees were found for interpretation. The *Adh1* phylogeny had the most complete representation of samples. All the endemic species were represented in the *Adh2* phylogeny samples. Both phylogenies were used for interpretation. The *Adh3* phylogeny had only *M. punctata* and *M. stanfieldii* samples. It is unknown whether this putative *Adh* locus is a pseudogene or functional duplicate, but its distinction from *Adh2* suggests that there was enough variation in this duplicate to enable it to be distinguished as a separate type.

Phylogenetic data of *ncpGS* were not complete enough to be included in the analysis, although *Monarda* to have two loci for the chloroplast-expressed *ncpGS* gene as opposed a single locus, as documented in other plant systems (Emshwiller and Doyle 1999).

4.2 Gene Trees and Species Trees

There are limits that are inherent to the use of low copy nuclear genes for phylogenetic analysis. Phylogenies are hypotheses that can be useful tools for the inference of species relationships but there are caveats to those based on DNA markers. It is important to recognize that gene trees, particularly those based on nuclear genes, may not clearly reflect the same relationships as a true species tree (Doyle 1992, Schaal and Olsen 2000). Coding and noncoding gene regions can accrue genetic variation from point mutations, insertions/deletions and meiotic recombination that can disseminate or be disseminated within and among populations independent of speciation events. Genetic changes such as these can convolute gene trees when species have not undergone complete lineage sorting. Homoplasy can also be a source of incongruence between gene trees and species trees (Doyle 1992). Because of these problems, it is prudent to obtain molecular phylogenies from at least two different genetic markers. In this study only *Adh* sequences were used for analysis. Nonetheless, *Adh* has been widely used in phylogenetic studies (Sang 2002) and has gene regions that are highly conserved between distant organisms therefore greater confidence may be placed in its results.

Additional complications can arise when using low copy nuclear genes for phylogenetic inference depending on whether gene homology is paralogous or orthologous.

Orthologous homologs precede speciation and are followed by divergence of an ancestral sequence (Gogarten and Olendzenski 1999, Sang 2002). Therefore, orthologous sequences can be used for phylogenetic reconstruction of species. Paralogous homologs result from either a duplication or deletion event after that occurs after speciation.



Paralogous sequences have been found to confound phylogenetic results, although some studies have shown them to be phylogenetically useful for both rooting purposes and to test comparative rates of divergence (Sang 2002). *Adh1* and *Adh2* are believed to be orthologous because they duplicated prior to the divergence of monocotyledonous and dicotyledonous plants (Sang *pers. corr.*). These orthologs may be synonymous to *Adh1* and *Adh2* loci identified in this study, but this cannot be confirmed because much information about these genes is lacking. This conjecture was drawn from the fact that *Adh1* and *Adh2* sequences were found in all six species while *Adh3* sequences were only found in *M. punctata* and *M. stanfieldii*. There is no evidence to suggest that *Adh3* is orthologous.

4.3 Gene Trees as Tools for Macroevolutionary Inference

It is important to draw a clear distinction between population level research and studies like this where species-level gene trees are used to infer population level processes. Coalescent theory has supplied much of the theoretical foundation for predicted gene tree topologies given different demographic histories of the populations they represent (Maddison 1995, Avise 2000). In the absence of gene flow, inter-specific gene trees can depict shared evolutionary histories and allelic variation that may coalesce to a common ancestral gene (Harrison 1991). Population structure and genetic structure are key in understanding how gene trees can be used to infer speciation events. If discontinuity in population structure reflects historic barriers to geneflow then one might expect comparable phylogenetic discontinuity in the genetic variation of neutral genetic markers.

✓ 1000 1000 1000 1000 1000

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This provides the underlying theory used by Harrison (1991) for his gene tree models of speciation.

4.4 Peripatric Speciation

The hypothesis that narrow endemic *Monarda* taxa speciated as peripheral isolates of *M. punctata* had phylogenetic support for some species but not all. In addition to being the most morphologically variable and geographically widespread, *M. punctata* was the most ubiquitous taxon throughout both *Adh1* and *Adh2* phylogenies. All narrow endemic species were derived within the large *M. punctata* clade with the exception of *M. stanfieldii*. This suggests that *M. punctata* is the progenitor of *M. fruticulosa*, *M. humilis*, *M. maritima* and *M. viridissima* and a sister taxon to *M. stanfieldii*. The *Adh3* phylogeny included few samples, but was consistent with the result that *M. stanfieldii* is the sister group to *M. punctata*.

It was unexpected that *M. punctata* samples did not tend to exist on longer branches closer to outgroup samples. Factors that might explain why most *M. punctata* appeared on shallow terminal tree branches include recent divergence of species resulting in shared ancestral polymorphisms, inter-specific gene introgression (Schaal et al. 1998, Templeton et al. 1995) or inadequate sampling. If allopatric species are sexually compatible and their populations expand resulting in a sympatric or parapatric distribution genetic distinction between them can be blurred (Macnair and Gardener 1998). In addition, shared ancestral polymorphisms that are maintained in sister species may also convolute gene tree results and can be misinterpreted as evidence for recent gene flow (Schaal and Olsen 2000).

Based on what is known about the current geography, morphology, and life cycles of

these species these results are likely due to either low sampling or shared ancestral polymorphisms. Species are either geographically or temporally separated and they maintain unique morphological characteristics. It is likely that they speciated recently and rapidly, and it would have been prudent to include more *M. punctata* samples from Texas populations.

In the *Adh1* parsimony trees, *M. fruticulosa* and *M. maritima* sequences were consistently on the same clades. Similarly, in the *Adh2* parsimony trees all *M. fruticulosa* sequences shared the same clade, although the only *M. maritima* sample in the phylogeny existed in a different clade. Based on their placement in the *Adh1* phylogeny results suggest that *M. fruticulosa* and *M. maritima* share more allelic variation with each other than any of the other narrow endemic species. This might suggest that there has been recent gene flow between these species, or that they diverged recently from a common ancestor. However, the results do not rule out the possibility that some of the endemics are sister taxa that derived independently from *M. punctata*.

4.5 Neoendemic and Paleoendemic Species

Adh phylogenies provided useful information that could be used to infer the paleo- and neoendemic status of these species. Most *M. stanfieldii* (2367, 2369) samples were not derived within the larger *M. punctata* clade and had long branch lengths. This short-lived herbaceous perennial is thought to be completely geographically isolated from *M. punctata* and inhabits a unique substrate (granitic sands) relative to the other narrow endemic species. Overall, the phylogenies provided strong evidence that *M. stanfieldii* is a paleoendemic



In both *Adh1* and *Adh2* parsimony trees, one clade consisted almost entirely of *M. maritima* and *M. fruticulosa* sequences as well as *M. punctata* samples from southeastern Arkansas (2060), southeastern Texas (2393) and northcentral Florida (2389) to name a few. The close phylogenetic relationship between *M. fruticulosa* and *M. maritima* is not surprising considering they share a woody habit, perennial life cycle and they are the most geographically proximate of the narrow endemics. These results suggest these species are neoendemics, but also may indicate they may share a common ancestor.

Monarda viridissima sequences are found in different positions across both phylogenies which suggests they have a high degree of allelic variability. Regardless, most sample sequences were on branches that were close to *M. punctata* samples, which suggests they are closely related. Their close genetic relationships were most apparent in the *Adh1* tree where *M. viridissima* sequences (2055 and 2444) clustered with *M. punctata* sequences. Geographic and phylogenetic data support that *M. viridissima* is a neoendemic species.

Monarda humilis was not well represented in either dataset but some inferences can be made regarding its placement in the phylogenies. This species is the most geographically disjunct relative to the other southwestern narrow endemics, and like *M. viridissima* it was found within the larger *M. punctata* clade. Even though this species was represented by only one sample its apparently close genetic relationship suggests that it is also a neoendemic that speciated from *M. punctata*.

4.6 Lineage Sorting and Noncoalescence

Several samples in the phylogeny appear to have allelic variation at given locus. In the *Adh1* tree, two *M. punctata* (2307 and 2048) and one *M. maritima* (2318) had alleles that consistently fell within the two separate clades. Likewise, two *M. viridissima* sequences (2055, 2444) were within one clade, while the two remaining samples (2441) were in two separate locations in the phylogeny. Allelic variation in a phylogenetic marker leads to incongruence between gene trees and species trees. Such allelic variation may be caused by several phenomena such as lineage sorting, noncoalescence and gene flow between species.

Lineage sorting refers to the fixation of different ancestral alleles for a given marker into a daughter species at speciation (Small and Wendel 2000). In cases of lineage sorting fixed alleles should be present throughout the species. If samples of a species from multiple populations had the same patterns of allelic variation in a phylogeny it may provide evidence for lineage sorting. In addition, shared ancestral polymorphisms caused by lineage sorting should be divided by many steps in a phylogeny, while newer alleles that have arisen after speciation should have fewer steps dividing them. Lineage sorting is a more common problem in species that have diverged recently (Sang 2002).

Noncoalescence refers to the introgression of alleles that are maintained as ancestral polymorphisms in a multi-gene family (Small and Wendel 2000). The events that lead to lineage sorting and noncoalescence both involve the maintenance of ancient allelic

polymorphisms and can produce phylogenetic results that show widely disparate taxa closely related on a gene tree.

Introgression and hybridization both involve the transfer of genes from one species to another (Small and Wendel 2000). Introgression results in the permanent fixation of genes between species while species still maintain phenotypic distinction. In contrast, hybridization results in phenotypic intermediates and progeny with novel gene combinations which can either lead to a merging of species or create a platform for speciation.

It is not certain which phenomena are the cause of allelic variation in the *Adh* phylogenies. Phylogenetic data alone is not sufficient to distinguish between lineage sorting, noncoalescence and gene flow between species. Further research is needed to determine this.

4.7 Species Protection and Conservation

The Endangered Species Act does not use biological terms alone to define species. Instead species are defined based on the most up to date knowledge of the genetics, evolution and speciation of a given taxon. There are several considerations that may warrant species protection for some of the *Monarda* taxa used in this study.

Monarda maritima has the most limited distribution of the Texas endemics. It is only known to exist within the Ingleside Terrace sand body that extends approximately 100

miles along the Texas coast. The fact that it is a coastal endemic indicates that it has likely suffered much habitat loss due to ranching and development associated with the oil industry. This plant is challenging to find in the field and has not been extensively collected, which suggests that it is not common in its range. All evidence indicates that this species is a good candidate for conservation.

Monarda viridissima is limited to the Carrizo sands of the Blackland Prairies that extend in a discrete belt from northeastern to southwestern Texas. Populations have been found in only the central region of this belt. *Monarda viridissima* is not common in its range, but I was able to find it in moderate sized populations (>25 individuals). It is also noted as being rare in a list of southern U. S. endemics (Estill and Cruzan, 2001). Field observations suggested that it inhabits more established plant communities rather than newly disturbed sites, which concurs with its tendency to grow in established post-oak and pine-oak forests. Like *M. maritima*, it may be in need of protection due to its limited range and propensity to exist in more established plant communities.

Monarda fruticulosa is the most abundant of the Texas endemic *Monarda* species. It occurs across the southern tip of the state in the coastal prairies and interior coastal plains. Within its range it can commonly be found in large populations that are often distributed along roadsides. Therefore, *M. fruticulosa* is the least likely to be in immediate need of conservation, despite its narrow endemic status.

Monarda stanfieldii has a narrow range across the Central Texas Uplift. Like *M. viridissima*, it only appears to grow in more established plant communities, and tends to prefer open woodlands (*pers. obs.*). This species was also difficult to find in the field, although one of the populations had a relatively good size (>50). *Monarda stanfieldii* is also likely to be in need of protection particularly because of its apparent paleoendemic status. It exists in more in established plant communities, its range is very small and it grows in very specific soils.

4.8 Prospects for Future Studies of *Monarda* Section *Cheilyctis*

Several changes in our experimental design might have helped to better resolve phylogenetic relationships between these species. An increase in sampling, particularly of Texas *M. punctata*, might have added useful information. Five varieties of *M. punctata* were not included in this study. A more complete sampling of these taxa may have provided a better understanding of the origins of the Texas endemics. Additional samples could also better resolve patterns due to incomplete lineage sorting and noncoalescence.

It would also would have been advantageous to include data from an additional low copy nuclear gene. Molecular phylogenies from distinct genetic markers can be used to compare and hopefully strengthen phylogenetic hypotheses, thus providing more tools for distinguishing gene trees and species trees (Schaal and Olsen 2000)

A population-level study using Amplified Fragment Length Polymorphisms (AFLPs) for analysis may have resolved complicated reticulating patterns between these taxa. In

several studies of Cichlid fishes, various genetic markers were used to determine the relationships between species, but all showed a high level of DNA polymorphisms among species (Klein et al. 1998, Moran and Kornfield 1995). Relationships between these Cichlids were not fully resolved until AFLPs were used for analysis (Albertson et al. 1999). AFLPs are advantageous because thousands of genetic regions can be used to generate an organism's genetic profile, and no preexisting knowledge of its genome is required before use.

Some individual relationships between species may also spur additional investigation regarding the history of these plants and their speciation. *Monarda fruticulosa* and *M. maritima* share more allelic variation with each other than any of the other endemics. This may suggest that there has been recent gene flow between them, or that they diverged recently from either a common ancestor. Our current knowledge of these species show that they do not overlap geographically or phenologically, which better supports the latter, although they exist in such close proximity that their geographic distributions may have overlapped recently. These species inhabit comparable substrates (homogenous beach sands), they are the most geographically proximate and they are both woody perennials. Geologic and physiographic history suggests that *M. maritima* may have been separated from mainland species when the Ingleside Terrace was a barrier island. If *M. fruticulosa*, or a shared *M. fruticulosa*/*M. maritima* ancestor had become isolated on the barrier-island this may have given it a window of time to diverge.

Questions also arise regarding the speciation mechanism acting to maintain distinction between *M. punctata* and *M. fruticulosa*. These are the only two species in the study that

grow in sympatry, bloom at the same time and are thought to have hybrid zones (*pers. obs*). They have been observed growing together along hilly sandy landscapes with *M. fruticulosa* growing at the sandy hilltops, *M. punctata* at the base of the hills, and possible hybrids along the cline between have been observed at one locality (Prather *pers. comm.*). This distribution better fits a parapatric species distribution with disruptive selection acting to maintain genetic distinction between species. In a study by Leuth and Prather (unpubl.), soils were collected from some of these sites and reciprocal seed plantings of *M. punctata* and *M. fruticulosa* were conducted. Results showed that *M. fruticulosa* had better survivorship on its own soil than it did on *M. punctata* soil. Other factors that may be involved include differences in water availability in high and low areas of the sandy soils or subsoil component, such as high salt content.

4.9 Conclusions

Few studies have used phylogenies based on low copy nuclear genes to infer population-level processes (Schaal 2000) such as speciation by peripheral isolation.

This research revealed a high degree of variation between alleles, loci and individuals in both *Adh1* and *Adh2* datasets. *Monarda punctata* sequences were widely distributed across both phylogenies and *M. humilis*, *M. fruticulosa*, *M. maritima* and *M. viridissima* sequences derived within this larger *M. punctata* clade. These results support the hypothesis that these narrow endemic *Monarda* speciated as peripheral isolates of *M. punctata*. In contrast, phylogenies suggest that *M. stanfieldii* speciated as sister to of *M. punctata*. The data suggest that *Monarda fruticulosa*, *M. humilis*, *M. maritima* and *M. viridissima* are neoendemics while *M. stanfieldii* is a paleoendemic. Likewise, the high

degree of lineage sorting and noncoalescence in the dataset suggests that these species diverged recently and rapidly. Despite the lack of phylogenetic distinction between these species they have remained morphologically, geographically and phenologically distinct.

Adh1 Sequence Alignment
~1200 nucleotides in length

P. tenuifolium 86-4	GCAGGGTATGCA-NCATCAATCTTTTCCAAAAAATCTTGAATTTTGTGAT	TTAGCATTTCTGAGTATATAT-NACAAAATTTGTAATACACATTACAGAAAT
P. loomisii 66-3	GCAGGGTATGCA-NCATCAATCTTTTCTCAAGAAATCTTGAATTTTGTAT	TTAACATTTCTGAGTATATCT-NACAAAATTTGTAATACACATACAGAAAT
P. muticum 52-4	GCAGGGTATGCA-NCATCAGTCTTTTCTCAAGAAATCTTGAATTTTGTAT	TTAACATTTCTGAGTATATCT-NACAAAATTTGTAATACACATACAGAAAT
M. clinopodioides 2310-1	TCAGGGTATGCA-NGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGT-NNNNNNNNAAATTTGTAATACACATACAGAAAT
M. clinopodioides 2310-2	TCAGGGTATGCA-NGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGT-NNNNNNNNAAATTTGTAATACACATACAGAAAT
M. fruticulosa TX 1920-1	TCAGGGTATGCACAGATCAATCATCTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. fruticulosa TX 1920-2	TCAGGGTATGCACAGATCAATCATCTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. fruticulosa TX 2301	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. fruticulosa TX 2317	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata NM 2307-1	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata NM 2307-2	NNNNNTATGCG-NCATCAATCTTTTCTCAAGATCTTGAATTTTGTGAT	T-AGCAATTTCTGAGTATAT-NACAAAATTTGCAATACACATACAGAAAT
M. punctata NM 2307-3	NNNNNNNNNNNNNNAAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TAAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. humilis NM 2176	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	T-AGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. maritima TX 2314	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. maritima TX 2319	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. maritima TX 2318-1	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. maritima TX 2318-2	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. stanfieldii TX 2367-1	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. stanfieldii TX 2367-2	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. stanfieldii TX 2239-1	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. stanfieldii TX 2239-2	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. stanfieldii TX 2369-1	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. viridissima TX 2055	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. viridissima TX 2441-1	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. viridissima TX 2444	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. viridissima TX 2441-2	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata AR 2060	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata TX 2468	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata FL 2303-1	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata FL 2303-2	NNNNNNNNNNNNNNAAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata MO 2172	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata OK 2398	TCAGGGTATGCA-NGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAA	T-AGCAATTTCTGAGTATAT-NACAAAATTTGCAATACACATACAGAAAT
M. punctata TX 2396	CCAGGGTATGCG-NCATCAATCTTTTCTCAAGATCTTGAATTTTGTGAT	TAAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata LA 2305	GCAGGGTATGCA-NCATCAATCTTTTCTCAAGATCTTGAATTTTGTGAT	TAAGCAATTTCTGAGTATAT-N7CAAAATTTGTAATACACATACAGAAAT
M. punctata FL 2397	TCAGGGTATGCA-NGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata DE 1937	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata MI 2048-1	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata MI 2048-2	TCAGGGTATGCA-NGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAA	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata NJ 2189-1	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT
M. punctata NJ 2189-2	TCAGGGTATGCACAGATCAATCTTTTCTCAAGATCTTGAATTCCTTGAT	TTAGCAATTTCTGAGTATAT-NACAAAATTTGTAATACACATACAGAAAT



1. The first part of the document is a letter from the President of the United States to the Congress, dated January 1, 1861. It is a very important document, as it is the first official communication from the President to the Congress since the inauguration of Abraham Lincoln. The letter is written in a formal, dignified style, and it contains a great deal of information about the state of the Union at that time.

2. The second part of the document is a report from the Secretary of the Treasury, dated January 1, 1861. It is a very important document, as it is the first official communication from the Secretary to the Congress since the inauguration of Abraham Lincoln. The report is written in a formal, dignified style, and it contains a great deal of information about the state of the Treasury at that time.

P. tenuifolium 86-4	TAAAGTCTGCATCTCGAGCTCGGGAATTTCCACTGGTATTTCATCGTTT	CCTTTGATGATTT-CTTTTC-NNNN-NNNNNNNNNTTGATGAGGA-
P. loomisii 66-3	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTTGATGATTTCTCTTTTC-NNNN-NNNNNNNNNTTGATGAGGA-
P. muticum 52-4	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTTGATGATTTCTCTTTTC-NNNN-NNNNNNNNNTTGATGAGGA-
M. clinopodioides 2310-1	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CATTTAAAGATTTCTTTTCTTTTCTTTTCTTTT-CTTGATG-GGGA-
M. clinopodioides 2310-2	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CATTTAAAGATTTCTTTTCTTTTCTTTTCTTTT-CTTGATG-GGGA-
M. fruticulosa TX 1920-1	TAAAGCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CATCT-AAAGATTTCTTTTC-NNNGTCTCTTTCTTTCTTGATG-GGGA-
M. fruticulosa TX 1920-2	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CATTT-AAAGATTTCTTTTC-NNNGTCTCTTTCTTTCTTGATG-GGGA-
M. fruticulosa TX 2301	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. fruticulosa TX 2317	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CATTTAAAGATTTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata NM 2307-1	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTTGATGATTTCTTTTTC-NNNN-NNNNNNNNNTTGATG-GGGA-
M. punctata NM 2307-2	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTTGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata NM 2307-3	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTTGATGATTTCTTTTTC-NNNN-NNNNNNNNNTTGATG-GGGA-
M. humilis NM 2176	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTTGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. maritima TX 2314	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNN-NNNNNNNNNTTGATG-GGGA-
M. maritima TX 2319	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	C?TTC?A?GATTCCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. maritima TX 2318-1	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CATTTAAAGATTTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. maritima TX 2318-2	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. stanfieldii TX 2367-1	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTTGATGATTTCTTTTTC-NNNN-NNNNNNNNNTTGATG-GGGA-
M. stanfieldii TX 2367-2	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTTGATGATTTCTTTTTC-NNNN-NNNNNNNNNTTGATG-GGGA-
M. stanfieldii TX 2239-1	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CATTTGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. stanfieldii TX 2239-2	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CATTTAAAGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. stanfieldii TX 2369-1	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CATTTAAAGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
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M. viridissima TX 2441-1	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. viridissima TX 2444	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. viridissima TX 2441-2	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGATT	CATTTAAAGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata AR 2060	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CATTTAAAGATTTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata TX 2468	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTTGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata FL 2303-1	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata FL 2303-2	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata MO 2172	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata OK 2398	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata TX 2396	CAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata LA 2305	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
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M. punctata DE 1937	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata MI 2048-1	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata MI 2048-2	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata NJ 2189-1	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-
M. punctata NJ 2189-2	TAAAGTCTGCATTTCTGAGCTCGGGAATCTCCACTGGTATTTCATCGTTT	CCTTCGATGATTTCTTTTTC-NNNGTCTCTTTT-CTTGATG-GGGA-



P. tenuifolium 86-4
NNNNGGTGTGATTTTAAAGCTGCAGAAAGAGCAAGATTGGCAGNTGCT
NNNGGCTGTGATT-TTAAAGCTGCAGAAAGAGCCAGATTGGCTGGTCT
TT-NGGTGTGATT-TTAAAGCTGCAGAAAGAGCCAGATTGGCTGGTCT
NNGGGGTGTGATTTTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT
M. clinopodioides 2310-1
NNNGGCTGTGATTTTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT
M. clinopodioides 2310-2
NNNGGCTGTGATT-TTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT
M. fruticulosa TX 1920-1
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M. fruticulosa TX 1920-2
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M. punctata NM 2307-3
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M. humilis NM 2176
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M. maritima TX 2319
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M. maritima TX 2318-2
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M. stanfieldii TX 2367-1
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M. stanfieldii TX 2367-2
NNNGGCTGTGATTTTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT
M. stanfieldii TX 2239-1
NNNGGCTGTGATTTTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT
M. stanfieldii TX 2239-2
NNNGGCTGTGATTTTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT
M. stanfieldii TX 2369-1
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M. viridissima TX 2055
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M. viridissima TX 2441-1
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M. viridissima TX 2444
NNNGGCTGTGATTTTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT
M. viridissima TX 2441-2
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M. punctata TX 2468
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M. punctata FL 2303-1
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M. punctata FL 2303-2
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M. punctata MO 2172
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M. punctata OK 2398
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M. punctata TX 2396
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M. punctata LA 2305
NNNGGCTGTGATTTTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT
M. punctata FL 2397
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M. punctata DE 1937
NNNGGCTGTGATT-TTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT
M. punctata MI 2048-1
NNNGGCTGTGATT-TTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT
M. punctata MI 2048-2
NNNGGCTGTGATT-TTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT
M. punctata NJ 2189-1
NNNGGCTGTGATT-TTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT
M. punctata NJ 2189-2
NNNGGCTGTGATT-TTAAAGCTGCAGAAAGAGCAAGATTGGCTGGTCT



Adh2 Sequence Alignment
~1200 nucleotides in length

M. punctata NJ 1946-27	CAGGTATGCGCA-?TAAATCTTTCTCAAGATTCCTTGAATTTTGGATT	TAGCATAT??GAGTATATATACAAATT-????????GCAATACACATA
M. punctata MI 2048-1	CAGGTATGCGCA-?TAAATCTTTCTCAAGATTCCTTGAATTTTGGATT	TAGCATATCTGATTATATATACAAATT-????????GCAATACACATA
M. punctata NJ 2189-7	CAGGTATGCGCA-?TAAATCTTTCTCAAGATTCCTTGAATTTTGGATT	TAGCAT?TCTGATTATATATACAAATT-????????GCAATACACATA
M. punctata LA 2305-2	CAGGTATGCGCA-?TAAATCTTTCTCAAGATTCCTTGAATTTTGGATT	TAGCATTTCTGAGTATATACACAAATT-????????GCAATACACATA
M. punctata FL 2397-1	CAGGTATGCGCA-?TAAATCTTTCTCAAGATTCCTTGAATTTTGGATT	TAGCATTTCTGAGTATATATACAAATT-????????GCAATACACATA
M. punctata TX 2396-3	CAGGTATGCGCA-?TAAATCTTTCTCAAGATTCCTTGAATTTTGGATT	AAGCATTTCTGAGTATATATACAAATT-????????GCAATACACATA
M. punctata TX 2473-3	CAGGTATGCGCA-?TAAATCTTTCTCAAGATTCCTTGAATTTTGGATT	AAGCATTTCTGAGTATATATACAAATT-????????GCAATACACATA
M. punctata TX 2393-3	CAGGTATGCGCA-?TAAATCTTTCTCATGATTCCTGAAATTTTGGATT	TAGCATTTCTGAGTATGTATACAAATT-????????GAAATACACATA
M. punctata FL 2048-9	CAGGTATGCGCA-?ACAAATCTTTCTCATGATTCCTTGAATTTTGGATT	AAGCAT?TCTGAGTATGTATACAAATT-????????GCAATACACATA
M. punctata OK 2398-2	CAGGTATGCGCA-?ACAAATCTTTCTCATGATTCCTTGAATTTTGGATT	AAGCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. punctata FL 2389-1	CAGGTATGCGCA-?TAAATCTTTCTCATGATTCCTTGAATTTTGGATT	TAGCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. punctata AR 2060-1	CAGGTATGCGCA-?TAAATCTTTCTCATGATTCCTTGAATTTTGGATT	AAGCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. humilis 2176-3	CAGGTATGCGCA-?TAAATCTTTCTCAAGATTCCTTGAATTTTGGATT	AACCATTTCTGA-TATATAT?CAAAATT-????????GCAATACACATA
M. viridissima 2055	CAGGTATGCGCA-?ACAAATCTTTCTCATGATTCCTTGAATTTTGGATT	AAGCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. viridissima 2444-3	CAGGTATGCGCA-?TAAATCTTTCTCATGATTCCTTGAATTTTGGATT	TAGCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. fruticulosa 2317-3	CAGGTATGCGCA-?TAAATCTTTCTCATGATTCCTTGAATTTTGGATT	TAGCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. fruticulosa 2420-1	CAGGTATGCGCA-?TAAATCTTTCTCATGATTCCTTGAATTTTGGATT	TAGCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. fruticulosa 2301-1	CAGGTATGCGCA-?TAAATCTTTCTCATGATTCCTTGAATTTTGGATT	TAGCATTTCTGAGTATGTATACAAATT-????????GAAATACACATA
M. fruticulosa 1920-4	CAGGTATGCGCA-?TAAATCTTTCTCATGATTCCTTGAATTTTGGATT	AAGCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. fruticulosa 1920-10	CAGGTATGCGCA-?TAAATCTTTCTCATGATTCCTTGAATTTTGGATT	AAGCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. maritima 2181-3	CAGGTATGCGCA-?TAAATCTTTCTCATGATTCCTTGAATTTTGGATT	TAGCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. maritima 2181-4	CAGGTATGCGCA-?TAAATCTTTCTCATGATTCCTTGAATTTTGGATT	TAGCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. stanfieldii 2367-2	CAGGTATGCGCA-?TAAATCTTTTTCGTGATTCCTTGAATCCTTGGATT	AAGCATTTCTGAGTATGTATACCAATT-????????GCAATACACATA
M. stanfieldii 2367-1	CAGGTATGCGCA-?TAAATCTTTTTCGTGATTCCTTGAATCCTTGGATT	TAGCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. citriodora 2419-1	CAGGTATGCGCA-?ACAGTCTTTCTCAAGATTCCTTGAATTTTGGATT	TATCATTTCTGAGTATGTATACAAATT-????????GCAATACACATA
M. citriodora 2375-4	CAGGTATGCGCA-?ACAGTCTTTCTCAAGATTCCTTGAATTTTGGATT	AATCATTTCTGAGTATGAATACAAATT-????????GCAATACACATA
P. tenuifolium 55-4	CAGGTATGCGCA-?T?A-TCTCTCTCATGATTCCTTGAATTTTGGATT	CAGCATTTCTAAGTATATATACAAATT-????????GCAATACACATA
P. tenuifolium 97-3	CAGGTATGCGCA-?T?A-TCTCTCTCATGATTCCTTGAATTTTGGATT	CAGCATTTCTAAGTATATATACAAATT-????????GCAATACACATA



M. punctata NJ 1946-27	CAGAAATCGTGGAGAGCGGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. punctata MI 2048-1	CAGAAATCGTGGAG?GCGTGGGAGAAAGCGGTGACGGAGCTGGTCTTAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTG?TCA
M. punctata NJ 2189-7	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. punctata LA 2305-2	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. punctata FL 2397-1	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. punctata TX 2396-3	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. punctata TX 2473-3	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTG?TCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. punctata TX 2393-3	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. punctata FL 2048-9	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. punctata OK 2398-2	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. punctata FL 2389-1	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. punctata AR 2060-1	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. humilis 2176-3	CAGAAATCGTGGAGAGCGTGGGAGAAAGCG?TGACGGAGCTG?TCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. viridissima 2055	CAGAGTCTGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. viridissima 2444-3	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. fruticulosa 2317-3	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. fruticulosa 2420-1	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. fruticulosa 2301-1	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. fruticulosa 1920-4	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. fruticulosa 1920-10	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. maritima 2181-3	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. maritima 2181-4	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. stanfieldii 2367-2	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. stanfieldii 2367-1	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. citriodora 2419-1	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
M. citriodora 2375-4	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGTGCTCA
P. tenuifolium 55-4	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGCCGCTCA
P. tenuifolium 97-3	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACGGAGCTGGTCCCAGG	CGATCACGTTCTTCCGGTGTTCACCGGAGAAATGCAGAGAATGCCGCTCA

M. punctata NJ 1946-27	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTGTTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. punctata MI 2048-1	CAGAAATCGTGGAG?GCGTGGGAGAAAGCGGTGACCGAGCTGGTCCTAGG	CGATCAGGTTCTTCCGGTGTTCACCCGGAGAAATGCAGAGAAATGTG?TCA
M. punctata NJ 2189-7	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTGTTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. punctata LA 2305-2	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTGTTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. punctata FL 2397-1	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTGTTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. punctata TX 2396-3	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTGTTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. punctata TX 2473-3	CAGAAATCGTGGAGAGCGTGGGAGAAAGCGGTGACCGAGCTG?TCCCAGG	CGATCAGGTTCTTCCGGTGTTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. punctata TX 2393-3	CAGAAATCGTGGAGAGCGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. punctata FL 2048-9	CAGAAATCGTGGAGAGCGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGCGAAATGCAGAGAAATGTGCTCA
M. punctata OK 2398-2	CAGAAATCGTGGAGGCGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGCGAAATGCAGAGAAATGTGCTCA
M. punctata FL 2389-1	CAGAAATCGTGGAGAGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCTGGAGAAATGCAGAGAAATGTGCTCA
M. punctata AR 2060-1	CAGAAATCGTGGAGAGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. humilis 2176-3	CAGAAATCGTGGAGAGCGTGGGAGAAAGCG?TGACCGAGCTG?TCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. viridissima 2055	CAGAGTCGTGGAGAGCGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGCGAAATGCAGAGAAATGTGCTCA
M. viridissima 2444-3	CAGAAATCGTGGAGAGTGTG?CGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. fruticulosa 2317-3	CAGAAATCGTGGAGAGCGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. fruticulosa 2420-1	CAGAAATCGTGGAGAGTGTG?CGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCTGGAGAAATGCAGAGAAATGTGCTCA
M. fruticulosa 2301-1	CAGAAATCGTGGAGAGCGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. fruticulosa 1920-4	CAGAAATCGTGGAGAGTGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCTGGAGAAATGCAGAGAAATGTGCTCA
M. fruticulosa 1920-10	CAGAAATCGTGGAGAGTGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCTGGAGAAATGCAGAGAAATGTGCTCA
M. maritima 2181-3	CAGAAATCGTGGAGAGTGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGGAAATGCAGAGAAATGTGCTCA
M. maritima 2181-4	CAGAAATCGTGGAGAGTGTGGGCGGAGGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. stanfieldii 2367-2	CAGAAATCGTGGAGAGCGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. stanfieldii 2367-1	CAGAAATCGTGGAGAGCGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCCGGAGAAATGCAGAGAAATGTGCTCA
M. citriodora 2419-1	CAGAAATCGTGGAGAGTGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTGTTCACCTGGAGAAATGCAGAGAAATGTGCTCA
M. citriodora 2375-4	CAGAAATCGTGGAGAGTGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTGTTCACCTGGAGAAATGCAGAGAAATGTGCTCA
P. tenuifolium 55-4	CAGAAATCGTGGAGAGTGTGGGCGAAAGCGGTGAAGGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCTGGAGAAATGCAGAGAAATGCGGCTCA
P. tenuifolium 97-3	CAGAAATCGTGGAGAGTGTGGGCGAAAGCGGTGACCGAGCTGGTCCCAGG	CGATCAGGTTCTTCCGGTATTCACCTGGAGAAATGCAGAGAAATGCGGCTCA



M. punctata NJ 1946-27	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. punctata MI 2048-1	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. punctata NJ 2189-7	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. punctata LA 2305-2	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. punctata FL 2397-1	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. punctata TX 2396-3	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. punctata TX 2473-3	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. punctata TX 2393-3	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. punctata FL 2048-9	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. punctata OK 2398-2	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. punctata FL 2389-1	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. punctata AR 2060-1	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. humilis 2176-3	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. viridissima 2055	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. viridissima 2444-3	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. fruticulosa 2317-3	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. fruticulosa 2420-1	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. fruticulosa 2301-1	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. fruticulosa 1920-4	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. fruticulosa 1920-10	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. maritima 2181-3	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. maritima 2181-4	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. stanfieldii 2367-2	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. stanfieldii 2367-1	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. citriodora 2419-1	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
M. citriodora 2375-4	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
P. tenuifolium 55-4	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC
P. tenuifolium 97-3	GGGAAAAACCCGTTTACCAATTTCTTGGAACTTCCACATTCAGTGAGTA	CACTGTTCTTCATGTTGGCTGTGTTGCTAAGATCAATCCACTGGCCCC

M. punctata NJ 1946-27 AAT-??-?????????TAATGTTGCAATGGGTTGTTGTTTAAAGG
M. punctata MI 2048-1 AAT-??-?????????TAATGTTTGCATTTGGGTTGTTGTTT-AGGG
M. punctata NJ 2189-7 AAT-??-?????????TAATGTTTGCATTTGGGTTGTTGTTT-AGGG
M. punctata LA 2305-2 AAT-??-?????????TAATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. punctata FL 2397-1 AAT-??-?????????TAATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. punctata TX 2396-3 AAT-??-?????????TAATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. punctata TX 2473-3 AAT-??-?????????TAATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. punctata TX 2393-3 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. punctata FL 2048-9 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. punctata OK 2398-2 ?AT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. punctata FL 2389-1 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. punctata AR 2060-1 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. humilis 2176-3 AAT-??-?????????TAATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. viridissima 2055 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. viridissima 2444-3 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. fruticulosa 2317-3 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. fruticulosa 2420-1 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. fruticulosa 2301-1 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. fruticulosa 1920-4 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. fruticulosa 1920-10 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. maritima 2181-3 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. maritima 2181-4 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. stanfieldii 2367-2 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. stanfieldii 2367-1 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. citriodora 2419-1 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
M. citriodora 2375-4 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
P. tenuifolium 55-4 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG
P. tenuifolium 97-3 AAT-??GAA-??GTAATATGTTTGCATTTGGGTTGTTGTTTAAAGG

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M. punctata NJ 1946-27 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACGATTAATGATACTAATTGAGAAAAG-??ATGATA-??
M. punctata MI 2048-1 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACGATTAATGATAAC-ATTGAGAAAAG-??ATGATA-??
M. punctata NJ 2189-7 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACGATTAATGATAAC-ATTGAGAAAAG-??ATGATA-??
M. punctata LA 2305-2 GCTGTTTTGGGCTTGAGCAGTTGGGCTTGCTGATGTTT-CTGAAT CTTT-?ACTGATTAATGATACTAATTGAGAAAAG-??ATGATA-??
M. punctata FL 2397-1 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT CTTT-?ACTGATTAATGATACTAATTGAGAAAAG-??ATGATA-??
M. punctata TX 2396-3 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACGATTAATGATACTAATTGAGAAAAG-??ATGATA-??
M. punctata TX 2473-3 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACGATTAATGATACTAATTGAGAAAAG-??ATGATA-??
M. punctata TX 2393-3 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGAT-???AAAACATTGAGAAAAG-??ATGATA-??
M. punctata FL 2048-9 GCTGTTTTGGCTTGAGCAGTTGCACTTGCTGATGTTT-CT-AAT CTTT-?ACTGATTAATGATACTA-??GAAAAG-??ATGATA-??
M. punctata OK 2398-2 GCTGTTTTGGCTTGAGCAGTTGCACTTGCTGATGTTT-CT-AAT CTTT-?ACTGATTAATGATACTA-??GAAAAG-??ATGATA-??
M. punctata FL 2389-1 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CT-AAT TTTT-?ACTGAT-???AAAACATTGAGAAAAG-??ATGATA-??
M. punctata AR 2060-1 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT CTTT-?ACTGATTAATGATACTAATTGAGAAAAG-??ATGATA-??
M. humilis 2176-3 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?AC?GATTAATGATACTAATTGAGAAAAG-??ATGATA-??
M. viridissima 2055 GCTGTTTTGGCTTGAGCAGTTGCACTTGCTGATGTTT-CT-AAT CTTT-?ACTGATTAATGATACTAATTGAGAAAAG-??ATGATA-??
M. viridissima 2444-3 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGATTAATGATACTAATTGAGAAAAG-??ATGATA-??
M. fruticulosa 2317-3 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGAT-???AAAACATTGAGAAAAG-??ATGATA-??
M. fruticulosa 2420-1 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGAT-???AAAACATTGAGAAAAG-??ATGATA-??
M. fruticulosa 2301-1 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGAT-???AAAACATTGAGAAAAG-??ATGATA-??
M. fruticulosa 1920-4 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGAT-???AAAACATTGAGAAAAG-??ATGATA-??
M. fruticulosa 1920-10 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGAT-???AAAACATTGAGAAAAG-??ATGATA-??
M. maritima 2181-3 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CT-AAT CTTT-?ACTGATTAATGATA-CTAATGAGAAAAG-??ATGATA-??
M. maritima 2181-4 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CT-AAT CTTT-?ACTGATTAATGATA-CTAATGAGAAAAG-??ATGATA-??
M. stanfieldii 2367-2 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGATTAATGATA-CTAATGAGAAAAG-??ATGATA-??
M. stanfieldii 2367-1 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGATTAATGATA-CTAATGAGAAAAG-??ATGATA-??
M. citriodora 2419-1 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGATTAATGATA-CTAATGAGAAAAG-??ATGATA-??
M. citriodora 2375-4 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGATTAATGATA-CTAATGAGAAAAG-??ATGATA-??
P. tenuifolium 55-4 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGAT-???AAAACATTGAGAAAAG-??ATGATA-??
P. tenuifolium 97-3 GCTGTTTTGGGCTTGAGCAGTTGCACTTGCTGATGTTT-CTGAAT TTTT-?ACTGAT-???AAAACATTGAGAAAAG-??ATGATA-??

M. punctata NJ 1946-27 ??GAAGGATTTTAATAATTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. punctata MI 2048-1 ??GAAGGATTTTAATAATTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. punctata NJ 2189-7 ??GAAGGATTTTAATAATTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. punctata LA 2305-2 ??GAAGGATTTTAATAATTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. punctata FL 2397-1 ??GAAGGATTTCTGATAATTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. punctata TX 2396-3 ??GAAGGATTTCTGATAATTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. punctata TX 2473-3 ??GAAGGATTTTAATAATTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. punctata TX 2393-3 ??GAAGGATTTTGATTTT-??????-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. punctata FL 2048-9 ??GAAGGATTTTGATTTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. punctata OK 2398-2 ??GAAGGATTTTGATTTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. punctata FL 2389-1 ??GAAGGATTTTGATTTT-??????-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. punctata AR 2060-1 ??GAAGGATTTTGATTTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. humilis 2176-3 ??GAAGGATTTTAATAATTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. viridissima 2055 ??GAAGGATTTTGATTTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. viridissima 2444-3 ??GAAGGATTTTAATAATTTCTTGA-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. fruticulosa 2317-3 ??GAAGGATTTTGATTTT-??????-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. fruticulosa 2420-1 ??GAAGGATTTTGATTTT-??????-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. fruticulosa 2301-1 ??GAAGGATTTTGATTTT-??????-????????????????TGG TGTGATGTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. fruticulosa 1920-4 TAGAAGGATTTTGATTTTCTTGA-????????????????TGG CGTGATTTGTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. fruticulosa 1920-10 TAGAAGGATTTTGATTTTCTTGA-????????????????TGG CGTGATTTGTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. maritima 2181-3 ??GAAGGATTTTGATTTTCTTGA-????????????????TGG TGTGATTTGTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. maritima 2181-4 ??GAAGGATTTTGATTTTCTTGA-????????????????TGG TGTGATTTGTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. stanfieldii 2367-2 ??GAAGGATTTTGATTTTCTTGA-????????????????TGG TGTGATTTGTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. citriodora 2419-1 ??GAAGGATTTTGATTTTCTTGA-????????????????TGG TGTGATTTGTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
M. citriodora 2375-4 ??GAAGGATTTTGATTTTCTTGA-????????????????TGG TGTGATTTGTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
P. tenuifolium 55-4 ??GAGGATTTTGATTTTCTTGAATTTTCTTGAATTTTCTTGGGGG TGTGATTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG
P. tenuifolium 97-3 ??GAGGATTTTGATTTTCTTGAATTTTCTTGAATTTTCTTGGGGG TGTGATTTTAGGCTGCAGAGGAGCAAGATTGGCTGCTTCAAG

M. punctata NJ 1946-27 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. punctata MI 2048-1 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC ?TGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATA-TAGTGTA
M. punctata NJ 2189-7 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATA-TAGTCTA
M. punctata LA 2305-2 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. punctata FL 2397-1 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. punctata TX 2396-3 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. punctata TX 2473-3 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. punctata TX 2393-3 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. punctata FL 2048-9 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. punctata FL 2048-9 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. punctata OK 2398-2 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. punctata FL 2389-1 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. punctata AR 2060-1 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. humilis 2176-3 AGT-ATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. viridissima 2055 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. viridissima 2444-3 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. fruticulosa 2317-3 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. fruticulosa 2420-1 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. fruticulosa 2301-1 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. fruticulosa 1920-4 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. fruticulosa 1920-10 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. maritima 2181-3 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. maritima 2181-4 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. stanfieldii 2367-2 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. stanfieldii 2367-1 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. citriodora 2419-1 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
M. citriodora 2375-4 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
P. tenuifolium 55-4 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA
P. tenuifolium 97-3 AGTTATTGG-1GTGGACTTGAACCTCTGCTAGGTTTGAAGAAGGTATAC TTGTAACCTGAATATATATATTGCCACCCTCCGAGGTGCAAAATATATAGTCTA

M. punctata NU 1946-27 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAACCC-AAAAGACTACAA
M. punctata MI 2048-1 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTT-GCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAACCC-AAAAGACTACAA
M. punctata NJ 2189-7 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAACCC-AAAAGACTACAA
M. punctata LA 2305-2 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAACCC-AAAAGACTACAA
M. punctata FL 2397-1 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAACCC-AAAAGACTACAA
M. punctata TX 2396-3 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAACCC-AAAAGACTACAA
M. punctata TX 2473-3 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAACCC-AAAAGACTACAA
M. punctata TX 2393-3 CA-??TAGATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. punctata FL 2048-9 AT-??CAGATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. punctata OK 2398-2 AT-??CAGATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. punctata FL 2389-1 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. punctata AR 2060-1 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. humilis 2176-3 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. viridissima 2055 AT-??CAGATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. viridissima 2444-3 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. fruticulosa 2317-3 CA-??TAGATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. fruticulosa 2420-1 CA-??TAGATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. fruticulosa 2301-1 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. fruticulosa 1920-4 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. fruticulosa 1920-10 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. maritima 2181-3 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. maritima 2181-4 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. stanfieldii 2367-2 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. citriodora 2419-1 AT-??TAGATGTAATAGTAATTCGATTCCAATGGTG-?AATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
M. citriodora 2375-4 CT-??CAGATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
P. tenuifolium 55-4 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA
P. tenuifolium 97-3 CT-??CAAAATGTAATAGTAATTCGATTCCAATGGCGGTGAATATTTTGGCA GCAAAAGAAATTTGGTGTCACTGAATTTGTGAATCC-AAAAGACTACAA

M. punctata NJ 1946-27 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. punctata MI 2048-1 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. punctata NJ 2189-7 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. punctata LA 2305-2 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. punctata FL 2397-1 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. punctata TX 2396-3 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. punctata TX 2473-3 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. punctata TX 2393-3 GAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. punctata FL 2048-9 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. punctata OK 2398-2 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. punctata FL 2389-1 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. punctata AR 2060-1 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. humilis 2176-3 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. viridissima 2055 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. viridissima 2444-3 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. fruticulosa 2317-3 GAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. fruticulosa 2420-1 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. fruticulosa 2301-1 GAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. fruticulosa 1920-4 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. fruticulosa 1920-10 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. maritima 2181-3 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. maritima 2181-4 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. stanfieldii 2367-2 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. stanfieldii 2367-1 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. citriodora 2419-1 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
M. citriodora 2375-4 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
P. tenuifolium 55-4 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA
P. tenuifolium 97-3 CAAGCCAGTTCAGAGGTTGCT-??CT-?ACCTATTCCTCAATTCTCT GTGACTAT-??GATGCATCAACCA

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M. punctata NJ 1946-27 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAGCCG
M. punctata MI 2048-1 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGT?GATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. punctata NJ 2189-7 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAGCCG
M. punctata LA 2305-2 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. punctata FL 2397-1 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. punctata TX 2396-3 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. punctata TX 2473-3 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. punctata TX 2393-3 GATTTAAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC TGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. punctata FL 2048-9 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. punctata OK 2398-2 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. punctata FL 2389-1 GATTTAAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. punctata AR 2060-1 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. humilis 2176-3 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. viridissima 2055 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. viridissima 2444-3 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. fruticulosa 2317-3 GATTTAAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC TGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. fruticulosa 2420-1 GATTTAAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC TGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. fruticulosa 2301-1 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. fruticulosa 1920-4 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. fruticulosa 1920-10 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. maritima 2181-3 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. maritima 2181-4 AATT-2AAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. stanfieldii 2367-2 TATTTAAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC TGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. stanfieldii 2367-1 TATTTAAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC TGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. citriodora 2419-1 GATTTAAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC TGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
M. citriodora 2375-4 GATTTAAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC TGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
P. tenuifolium 55-4 GATTTAAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC
P. tenuifolium 97-3 GATTTAAGTTGTACTTATTGTGTGTATAGGTGATAGCGGAGATGAC CGATGAGGAGTTGATCGAAGCGGTGCAAGTGCACCCGAAACATCAAAGC

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M. punctata NJ 1946-27 CATGATCTCAGCAATTGGAATGTTGTCAGGATGTATATGCTCTTTTGAT CACCAAACTCACC
M. punctata MI 2048-1 CATGATCTCAGCAATTGGAATGTTGTCATGATGTATATGCTCTTTTGAT CACCAAACTCACC
M. punctata NJ 2189-7 CATGATCTCAGCAATTGGAATGTTGTCAGGATGTATATGCTCTTTTGAT CACCAAACTCACC
M. punctata LA 2305-2 CATGATCTCAGCAATTGGAATGTTGTCATGATGTATATGCTCTTTTGAT CACCAAACTCACC
M. punctata FL 2397-1 CATGATCTCAGCAATTGGAATGTTGTCATGATGTATATGCTCTTTTGAT CACCAAACTCACC
M. punctata TX 2396-3 CATGATCTCAGCAATTGGAATGTTGTCATGACGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. punctata TX 2473-3 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. punctata TX 2393-3 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. punctata FL 2048-9 CATGATCTCAGCAATTGGAATGTTGTCATGATGTATATGCTCTTTTGAT CACCAAACTCACC
M. punctata OK 2398-2 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GCTCTTTTGAT CACCAAACTCACC
M. punctata FL 2389-1 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GCTCTTTTGAT CACCAAACTCACC
M. punctata AR 2060-1 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GCTCTTTTGAT CACCAAACTCACC
M. humilis 2176-3 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GCTCTTTTGAT CACCAAACTCACC
M. viridissima 2055 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GCTCTTTTGAT CACCAAACTCACC
M. viridissima 2444-3 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. fruticulosa 2317-3 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. fruticulosa 2420-1 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GCTCTTTTGAT CACCAAACTCACC
M. fruticulosa 2301-1 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. fruticulosa 1920-4 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. fruticulosa 1920-10 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. maritima 2181-3 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. maritima 2181-4 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. stanfieldii 2367-2 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. stanfieldii 2367-1 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. citriodora 2419-1 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
M. citriodora 2375-4 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
P. tenuifolium 55-4 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC
P. tenuifolium 97-3 CATGATCTCAGCAATTGGAATGTTGTCATGATGTAT-?GTTCTTTTGAT CACCAAACTCACC

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Monarda Field Collection Data

Field Collected *Monarda*, Jessie Keith

Species	Coll. #	Date	County	Location	GPS Coordinates	Flowering
<i>M. punctata</i>	FL-1	7/15/01	Godspen	1 mile East of Lake Tallawarra Housing	N-30°38.481', W-84°27.793'	no
<i>M. punctata</i>	FL-4	7/16/01	Bay	Off of 98 on the East side of the road southeast of Tyndell Air Force Base	N-29°59.663', W-84°58.615'	no
<i>M. punctata</i>	FL-9	7/17/01	Franklin	2 miles South of Sumatra in Applachicola State Forest off hwy. 85, near ditch	N-29°57.086', W-82°48.529'	no
<i>M. punctata</i>	FL-10	7/18/01	Columbia	Across road from the Ichetucknee Springs State Park entrance off of hwy. 27	N-29°34.499', W-82°23.565'	no
<i>M. punctata</i>	FL-11	7/19/01	Alachua	3 miles South of Gainesville off of hwy. 121	N-29°58.283', W-85°27.479'	no
<i>M. punctata</i>	DE 1	8/31/2001	Sussex	Abbotts Mill Nature Area near Millford, DE.	N/A	no
<i>M. punctata</i>	NJ 1	7/1/01	Burlington	In New Jersey Pine Barrens Cemetery	N/A	no
<i>M. stanfieldii</i>	TX-2	5/8/02	Blanco	Pemales River State Park, in front of "picnic" sign along the path to beach area	N-30°18.704', W-98°14.384'	yes
<i>M. citriodora</i>	TX-3	5/8/02	Burnet	2 miles from hwy P-4 junction	N-30°43.005', W-98°23.857'	yes
<i>M. punctata</i>	TX-4	5/10/02	Burnet	Behind Galloway Hammond Recreation Center along fenceline, Marble Falls	N-30°44.629', W-98°13.686'	yes
<i>M. citriodora</i>	TX-4(2)	5/10/02	Burnet	Behind Galloway Hammond Recreation Center along fenceline, Marble Falls	N-30°44.629', W-98°13.686'	yes
<i>M. citriodora</i>	TX-5	5/10/02	Burnet	2 miles North of Buchanan off of hwy. 4	N-30°45.650', W-98°17.907'	yes
<i>M. stanfieldii</i>	TX-7	5/10/02	Burnet	In front of the Buchanan Dam in a residential area, along a wooded hillside	N-30°45.008', W-98°24.661'	yes
<i>M. citriodora</i>	TX-8	5/10/02	Burnet	Off of hwy. 2341 just above hwy. 690	N/A	yes
<i>M. stanfieldii</i>	TX-9	5/10/02	Llano	Across the road from the Black Rock State Park entrance	N-30°45.828', W-98°27.367'	yes
<i>M. citriodora</i>	TX-10	5/11/02	Blanco	At the junction of hwy. 71 and the Pemales River, northeast corner	N/A	yes
<i>M. citriodora</i>	TX-11	5/11/02	Travis	Off of hwy. 290 just northeast of Austin along the South side of the Road	N-30°12.809', W-97°43.346'	yes
<i>M. citriodora</i>	TX-12	5/11/02	Travis	Off of 290 near the junction at Royster Ave., southeast corner	N-30°12.193', W-97°38.433'	yes
<i>M. punctata</i>	TX-14	5/11/02	Bastrop	Corner of FM 141 and Bluebonnet Drive, near Bastrop	N-30°10.373', W-97°15.321'	yes
<i>M. punctata</i>	TX-15	5/11/02	Bastrop	Junction of 21 and Old Potato Road, northwest corner	N-30°10.735', W-97°10.462'	yes
<i>M. punctata</i>	TX-16	5/11/02	Bastrop	Off Old Potato Road 1.2 miles from hwy. 21 junction	N-30°11.247', W-97°10.593'	yes
<i>M. viridissima?</i>	TX-17	5/11/02	Bastrop	Off Old Potato Road 1.8 miles from hwy. 21 junction	N-30°11.549', W-97°10.898'	no



Species	Coll. #	Date	County	Location	GPS Coordinates	Flowering
<i>M. citropodoides</i>	TX-18	5/11/02	Bastrop	Off Old Potato Road 2 miles from hwy. 21 junction	N-30°11.557', W-07°11.003'	yes
<i>M. citrodora</i>	TX-19	5/12/02	Bastrop	1.4 miles West of McDade on the Old McDade Hwy.	N-30°17.793', W-07°14.902'	yes
<i>M. punctata</i>	TX-20	5/12/02	Bastrop	East of Elgin on the Old McDade hwy. about 0.5 miles from 280 crossing	N/A	yes
<i>M. punctata</i>	TX-21	5/13/02	Victoria	Off of hwy. 77 on the way to Victoria South of Hallettsville	N-28°10.515', W-08°59.151'	yes
<i>M. citrodora</i>	TX-22	5/13/02	Aransas	Approximately 5 miles from the AWR, 1 mile from the junction at hwy. 35	N/A	yes
<i>M. merittina</i>	TX-23	5/13/02	Aransas	Near the junction of hwy. 35 and 1761, 100 ft. up 35 across from oil tanks	N-28°08.547', W-07°01.795'	no
<i>M. merittina</i>	TX-24	5/14/02	Aransas	Off of hwy. 13 1/4 of a mile from the junction of hwy. 35 and 13	N-28°08.321', W-07°00.199'	no
<i>M. punctata</i>	TX-25	5/14/02	Aransas	Right next to the Goose Island State Park gate house	N-28°07.999', W-08°59.049'	yes
<i>M. punctata</i>	TX-26	5/14/02	Aransas	0.75 miles from Lamar Beach off of hwy. 13 in Goose Island State Park	N-28°08.917', W-08°58.381'	yes
<i>M. merittina</i>	TX-27	5/14/02	Aransas	Behind the "Big Tree", the largest live oak in Texas.	N/A	no
<i>M. punctata</i>	TX-28	5/14/02	Nueces	South Corpus Christi, at the corner of Yorktown Road near the airport	N/A	yes
<i>M. punctata</i>	TX-29	5/14/02	Kenedy	East of Riviera, about 1.5 miles East of the junction between 77 and 701	N/A	yes
<i>M. fruticulosa</i>	TX-30	5/14/02	Kenedy	Off of hwy. 77 on the West side of the road about 5 miles South of Sarita	N/A	yes
<i>M. fruticulosa</i>	TX-31	5/14/02	Kenedy	1 mi South of Sarita along the West side of road	N/A	yes
<i>M. fruticulosa</i>	TX-32	5/14/02	Kenedy	7 miles South of Sarita along the road median	N/A	yes
<i>M. fruticulosa ?</i>	TX-33	5/14/02	Kenedy	3 miles North of Sarita along East side of road	N/A	yes
<i>M. punctata</i>	TX-34	5/15/02	Kenedy	Off of 188 on the South side of the road near the junction of 83, growing in loam	N/A	yes
<i>M. punctata</i>	TX-35	5/15/02	Refugio	Off Copano Retreat road 2.5 miles from 188 junction, 11 miles North of Rockport	N/A	yes
<i>M. merittina</i>	TX-36	5/15/02	Refugio	Off of 776 about 0.5 miles from the AWR entrance on the West side of the road	N/A	no
<i>M. merittina</i>	TX-37	5/15/02	Refugio	outside of the AWR entrance on the East side of the road ~200 ft from the shore	N/A	no
<i>M. punctata</i>	DE 2	7/7/02	Sussex	Abbotts Mill Nature Area near Milford, DE. Next to AMVA Center and field circle	N/A	no
<i>M. viridissima</i>	TX-38	10/17/02	Bastrop	Old Potato Road about 3/4 mile from hwy. 21 junction	N-30°11.238', W-07°10.547'	yes
<i>M. viridissima</i>	TX-39	10/18/02	Millam	Off of hwy. 38 between Caldwell and Millam on fence row on West side of road	N-30°40.374', W-08°49.408'	yes
<i>M. punctata</i>	TX-40	10/18/02	Millam	Off of hwy. 38 on East side of the road 0.1 miles North of Cameron, had senesced	N/A	no
<i>M. viridissima</i>	TX-41	10/18/02	Bastrop	Off of hwy. 21 North of Bastrop, at the entrance of Pine View Estates	N-30°07.659', W-07°16.173'	yes

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