

This is to certify that the dissertation entitled

PHENOTYPES AND FLORAL VARIATION: A PHYLOGENETIC APPROACH

presented by

ANNA KIRSTEN MONFILS

has been accepted towards fulfillment of the requirements for the

Doctoral degree in Plant Biology

Alexandra Major Professor's Signature

20 August 7003

Date

MSU is an Affirmative Action/Equal Opportunity Institution

PHENOTYPES AND FLORAL VARIATION: A PHYLOGENETIC APPROACH

By

Anna Kirsten Monfils

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
For the degree of

DOCTOR OF PHILOSOPHY

Department of Plant Biology

2003

ABSTRACT

PHENOTYPES AND FLORAL VARIATION: A PHYLOGENETIC APPROACH

By

Anna Kirsten Monfils

Biological diversity is created by evolution. To adequately explore biodiversity and examine processes in evolution, scientists need perspective on past evolutionary events. In this study, phenotypic diversity was investigated at several taxonomic levels by examining floral morphological characters against a backdrop of phylogenetic hypotheses. Floral traits were measured and phylogenetic hypotheses were generated across the species' *Pedicularis densiflora* and *Pedicularis aurantiaca*, within the tropical genus *Cantua*, and throughout the family Brassicaceae.

Floral morphological variation among populations of *P. densiflora* s.l. was examined and two distinct floral morphological groups were determined. Phenotypic analysis was then combined with a phylogenetic hypothesis in order to examine the number of times different floral morphs arose, relationships among populations in different localities, and potential pollinator shifts. An investigation of the floral diversity and phylogentics among populations in the species *P. densiflora* s.l. warranted the splitting of the group into two distinct species, *P. densiflora* and *P. aurantiaca*.

Scanning electron microscopy was used to examine pollen grain morphology within the genus *Cantua*. Pollen characteristics were then assessed relative to a strongly supported

molecular phylogenetic hypothesis. Pollen grain morphology was found to be highly conserved within the genus and synapomorphies in pollen grain characteristics supported relationships hypothesized in the phylogenetic analysis. In addition, a potential correlation between pollen diameter and style length was examined using independent contrast and found to be insignificant. This investigation confirmed the phylogenetic utility of pollen characters and provided no support for the hypothesis that pollen grain diameter is functionally integrated with style length.

Floral morphological traits in the Brassicaceae were used to examine phenotypic variance-covariance matrix (P-matrix) stability relative to a comprehensive phylogenetic hypothesis. The P-matrix stability was highly conserved among some genera in the family and more labile among others. This examination of P-matrix stability within the Brassicaceae used a phylogenetic hypothesis to confirm that matrix stability did not follow a stepwise pattern of similarity corresponding to taxonomic rank.

Although each chapter of the dissertation used a different group of plants, and varied statistical techniques, they all showed the utility of combining both morphological and molecular analysis to examine patterns of floral diversity. The objectives of each study focused on different aspects of patterns of floral diversity. Ultimately, all of the investigations contained a phylogenetic hypothesis that provided a unique historical perspective to the questions being addressed.

ACKNOWLEDGEMENTS

This study was funded by the following sources: Botanical Society of America, California Native Plant Society, Department of Plant Biology (Michigan State University; MSU), Ecology, Evolutionary Biology and Behavior Program (MSU), The Graduate School (MSU), NSF Grant: Systematics of the Polemoniaceae Subfamily Cobaeoideae, Oregon Native Plant Society, Paul Taylor Funds (Department of Plant Biology, MSU), The Research Training Grant at Kellogg Biological Station (MSU) NSF DBI 9605168, and Sigma Xi. Special thanks to my major professor Alan Prather, for his guidance and assistance during this project. I would also like to thank my committee members Jeff Conner, Tao Sang, and Jim Smith. Thanks to Christy Stewart for assistance with the Brassicaceae project, Jan Szyren for her help with cultivating plants, and Shirley Owens for assistance with the scanning electron microscopy images. I would like to acknowledge and thank Jessie Keith and Chang Bao Li for their assistance in the laboratory, and Orlando Alvarez-Fuentes, Anita D'Avelos, and Eric Linton for statistical support. Thanks to Michael Monfils for editorial assistance and encouragement. Additional thanks to Tracey Barner, Jason Kilgore, and Anne Plavonich-Jones for helping me complete this work. I would also like to acknowledge my lab mates Amanda Posto, Nate Sammons, and Rachel Williams and the assistant curators of the MSC herbarium, Alan Fryday and Deb Trock.

TABLE OF CONTENTS

LIST OF TABLES	Vii
LIST OF FIGURES	ix
INTRODUCTION	1
CHAPTER 1	
Floral Morphological Variation, Molecular Systematics, and Distribution In Pedicularis densiflora Benth. ex Hook. and Pedicularis aurantiaca (E. F. Sprague) Monfils & Prather (Orobanchaceae)	9 11 22
CHAPTER 2	
Phylogeny and Pollen Evolution of Cantua (Polemoniaceae subfamily Cobaeoideae): Evidence from Chloroplast and Nuclear DNA Sequence Date Introduction. Materials & Methods. Results.	83 84 88
Conclusions	
CHAPTER 3	
Evolution of Variance-Covariance Structure of Floral Morphology Among	
Members of the Mustard Family (Brassicaceae)	
Introduction	
Materials & Methods	
Results	
Conclusions	
SUMMARY	157
APPENDICES	161
Appendix A. Aligned sequences of Pedicularis: nuclear ribosomal internal	
transcribed spacer regions and 5.8S ribosomal RNA gene	102
trnL3' and trnF coding regions	176

Table of Contents (Cont'd)

APPENDICES CONT'D. Appendix C. Aligned sequences of <i>Pedicularis</i> : intergenic region between	
psbA and trnH coding regions	
Appendix D. Aligned sequences of Cantua: intergenic region between	
trnT5' and trnL coding regions	200
Appendix E. Aligned sequences of Cantua: intergenic region between	
trnL3' and trnF coding regions	215
Appendix F. Aligned sequences of Cantua: 938 base pairs of the ndhF	
coding region	226
Appendix G. Aligned sequences of Cantua: nuclear ribosomal internal	
transcribed spacer regions and 5.8S ribosomal RNA gene	247
Appendix H. Aligned sequences of Brassicaceae: 771 base pairs of the	
ndhF coding region	263
Appendix I. Pollen data for Cantua (Polemoniaceae):Light microscopy	
pollen diameter and style length analysis	281
ITED ATLIDE CITED	282

LIST OF TABLES

Table		Page
1	List of populations and voucher specimens for DNA sequence analysis and floral morphological analysis of <i>Pedicularis densiflora</i> and <i>Pedicularis aurantiaca</i> .	12
2	List of the 17 floral morphological traits of ten populations of Pedicularis densiflora and Pedicularis aurantiaca analyzed using principal component analysis	22
3	Sample size, means, standard deviations, minima, maxima, and results from ANOVA and Bonferroni (Dunn) T-tests for characters studied in the morphological analysis of ten populations of <i>P. densiflora</i> and <i>P. aurantiaca</i> sampled from California and Oregon	30
4	Voucher information for material used in the molecular phylogenetic analyses of Cantua.	89
5	Voucher information for material used in palynological study of Cantua.	90
6	Pollen diameter and style length of Cantua species included in the analysis	112
7	Voucher information for material used in the <i>ndh</i> F sequencing analysis of Brassicaceae.	126
8	Brassicaceae species measured for floral morphological characters, with sample locality, cultivation site, sample size, and year measured	128
9	Example of the phenotypic variance-covariance matrix for the six variables in the Nasturtium officinale data set	132
10	The P-value results of the CPCR and analysis in the Brassicaceae	139
11	Results from the principal component analysis of populations in the Brassicaceae.	143
12	Results of a principal component analysis incorporating measurements from all populations measured in the Brassicaceae and Cleomaceae	151

List of Tables (cont'd)

Table		Page
13	Aligned sequences of the nuclear ribosomal internal transcribed spacer regions and the 5.8S subunit of ribosomal RNA from the taxa sampled in <i>Pedicularis</i>	163
14	Aligned sequences of intergenic region between trnL3' and trnF coding regions from the taxa sampled in Pedicularis	177
15	Aligned sequences of intergenic region between psbA and trnH coding regions from the taxa sampled in Pedicularis	187
16	Aligned sequences of the intergenic spacer region between the trnT5' and trnL coding regions for the taxa sampled in Cantua data set	201
17	Aligned sequences of the intergenic spacer region between the trnL3' and trnF coding regions for the taxa sampled in Cantua data set	216
18	Aligned sequences of 938 base pairs of the <i>ndh</i> F coding region from the taxa sampled in the <i>Cantua</i> data set	227
19	Aligned sequences of the nuclear ribosomal internal transcribed spacer regions and the 5.8S subunit of ribosomal RNA from the taxa sampled in <i>Cantua</i> data set	248
20	Aligned sequences of 771 base pairs of the <i>ndh</i> F coding region from the taxa sampled in the <i>Brassicaceae</i> data set	264
21	Pollen grain diameter and style length mean, sample size (N), standard deviation, and range for <i>Cantua</i> herbarium specimens used in the light microscopy pollen analysis	282

LIST OF FIGURES

Figure		Page
1	Map of Oregon and California representing distribution of 16 sampled populations of <i>P. densiflora</i> and <i>P. aurantiaca</i>	14
2	A Pedicularis densiflora flower X 6 and schematic representation of Pedicularis densiflora corolla	17
3	Plot of factor one by factor two from the principal component analysis of 17 floral morphological characters in ten populations of <i>Pedicularis densiflora</i> and <i>P. aurantiaca</i>	25
4	Plot of factor one by factor three from the principal component analysis of 17 floral morphological characters in ten populations of <i>Pedicularis densiflora</i> and <i>P. aurantiaca</i>	27
5	Plot of factor two by factor three from the principal component analysis of 17 floral morphological characters in ten populations of <i>Pedicularis densiflora</i> and <i>P. aurantiaca</i>	29
6	UPGMA phenogram of taxonomic distance based on 17 continuous floral morphological character traits in ten populations of <i>P. densiflora</i> and <i>P. aurantiaca</i>	40
7	County map of California. Map shows distribution of <i>P. densiflora</i> and <i>P. aurantiaca</i> based on morphological analysis of populations in the field and herbarium material cited	43
8	County map of Oregon. Map shows distribution of <i>P. densiflora</i> and <i>P. aurantiaca</i> based on morphological analysis of populations in the field and herbarium material cited	45
9	Strict consensus of most parsimonious trees based on nrITS sequences in populations of <i>P. densiflora</i> and <i>P. aurantiaca</i>	48
10	Strict consensus of most parsimonious trees based on psbA-trnH and trnL-trnF sequences in populations of P. densiflora and P. aurantiaca	52

List of Figures (cont'd)

Figure		Page
11	Strict consensus of most parsimonious trees based on <i>psbA-trnH</i> and <i>trnL-trnF</i> chloroplast sequences and nuclear ribosomal ITS sequences in populations of <i>P. densiflora</i> and <i>P. aurantiaca</i>	54
12	Strict consensus of 15 most parsimonious trees based on the <i>Cantua</i> total ITS data set including nucleotide substitutions and indels	98
13	Strict consensus of two most parsimonious trees based on the <i>Cantua</i> combined chloroplast sequences (<i>trn</i> T- <i>trn</i> L, <i>trn</i> L- <i>trn</i> F, partial <i>ndh</i> F) including nucleotide substitutions and indels	101
14	Strict consensus of four most parsimonious trees based on the <i>Cantua</i> combined chloroplast (<i>trn</i> T- <i>trn</i> L, <i>trn</i> L- <i>trn</i> F, partial <i>ndh</i> F) and nuclear ITS sequences including nucleotide substitutions and indels	104
15	Cantua tree estimate for comparative analysis	105
16	SEM photographs of pollen grains in Cantua species (Polemoniaceae): (A & B) C. flexuosa; (C & D) C. cuzcoensis; (E & F) C. bicolor; (G & H) C. candelilla	107
17	SEM photographs of pollen grains in Cantua species (Polemoniaceae): (A & B) C. buxifolia; (C & D) C. pyrifolia; (E & F) C. n. sp	108
18	SEM photographs of pollen grains in Cantua species (Polemoniaceae): (A & B) C. volcanica (C & D) C. quercifolia	109
19	Diagram representing phylogenetic relationships and pollen synapomorphies in Cantua	113
20	Schematic representation of a generic Brassicaceae flower viewed from the top.	130
21	Schematic representation of generic Brassicaceae flower in lateral cross-section.	131
22	A representation of the "Jump Up" approach to matrix comparisons using the Flury hierarchy.	134

Table of Figures (Cont'd)

Figure		Page
23	Strict consensus of 12 most parsimonious trees from the Brassicaceae based on <i>ndh</i> F sequences	137
24	Strict consensus of 12 most parsimonious trees from the Brassicaceae based on <i>ndh</i> F sequences with results from common principal component analysis	142

INTRODUCTION

PHENOTYPES AND FLORAL VARIATION: A PHYLOGENETIC APPROACH Biologists have long been interested in the origin, pattern, and maintenance of diversity among living organisms. Angiosperms, the dominant plant group, are of particular interest because they have highly variable floral morphologies among species. Theories have been proposed that explain flowering plant diversity as a product of the close relationships among floral form, plant pollinator interactions, and reproductive success. A comprehensive study of flowering plant evolution is central to understanding the pattern and diversity of floral morphological variation (Darwin 1862).

Even with the recent advances in statistics and molecular techniques, diversity among organisms is most often quantified using phenotypic characters. Phenotypes are the manifestation of heritable genetic variation and environmental influences. In order to study floral diversity, we need to consider morphology and variation in an evolutionary context (Felsenstein 1985, Harvey & Pagel 1991).

A phylogeny is a hypothesis of the evolutionary history of a group of organisms. A robust phylogenetic reconstruction can be used to assess patterns of evolutionary changes. Phylogenies are usually displayed as a bifurcating tree representing the evolutionary relationships among taxa, historical relationships between common ancestors, and speciation events. Although we can never know for certain the historical events leading to the diversity currently observed among organisms, phylogenies allow us to generate testable hypotheses of relationships (Hennig 1950). Without such an historical perspective, phenotypic diversity can be interpreted to support erroneous historical relationships and the polarity of floral character traits cannot be tested.

Phylogenetics has made great advances over the last twenty years with the advent of molecular methodologies. Data for cladistic analyses can be generated quickly and relatively cheaply across species, genera, families, and even kingdoms. Phenotypic characters can now be evaluated against an independent phylogenetic backdrop based on molecules.

Phylogenetic approaches have increasingly been applied to questions outside the realm of taxonomy. For instance, phylogenetic hypotheses are being used in the field of community ecology to explore community assemblages and niche structure (Webb et al. 2002). Conservation biologists use molecular systematics to define species, identify biologically important lineages, and set conservation priorities (Moritz 1995, Soltis and Gitzendanner 1999). Population biologists are starting to use comparative methods to explore quantitative genetic questions, thus initiating a new field of research, comparative quantitative genetics (Steppan et al. 2002). Phylogenies are currently used in comparative physiology and development to choose experimental systems, determine trait polarity, study the pace of physiological and developmental change, and establish independent contrasts of traits among related groups (Monson 1996). Plant ecologists and evolutionists alike are using phylogenetic techniques to explore the evolution of plant pollination systems (Armbruster 1993, Bruneau 1997, McDade 1992), breeding systems (Rieseberg et al. 1992, Sakai et al. 1997), hybridization (Riesberg & Ellstrad 1993, Sang et al. 1995), polyploid evolution (Soltis and Soltis 2000), coevolution (Futuyma 1995), and evolutionary development (Albert et al. 1998).

In this study, I used phylogenies to study floral morphology, functionally integrated floral characters, and variation and covariation among traits. I examined floral morphological variation in three groups: two closely related species of *Pedicularis*, among the species of *Cantua*, and across the family Brassicaceae. A molecular phylogenetic hypothesis was central to the analysis of each group.

Pedicularis densiflora and P. aurantiaca were examined to detect and explain patterns of variation among populations of the two species. UPGMA, ANOVA, and PCA analyses were conducted, over 1000 herbarium specimens were studied, and a phylogenetic analysis of psbA-trnH and trnL-trnF chloroplast sequences and nuclear ribosomal ITS sequences was undertaken. Examination of all three statistical morphological analyses and review of herbarium specimens verified two distinct species, P. densiflora and P. aurantiaca. Pedicularis aurantiaca has a large calyx, floral tubes not fully exserted at anthesis, reduced lower labium, and a large opening in the galea. Pedicularis densiflora has a short calyx with a fully exerted floral tube, an enlarged lower labium, and a smaller opening in the galea. Phylogenetic analysis of the two species confirmed they were closely related taxa, sister to a clade containing Pedicularis semibarbata. Comprehensive review of the floral variation coupled with a phylogenetic hypothesis justified elevating the subspecies P. aurantiaca subsp. aurantiaca to species status.

A study of *Cantua* (Polemoniaceae) was undertaken to estimate a phylogeny, examine pollen exine sculpturing and its utility in phylogenetic analysis, and investigate the

potential functional integration between pollen size and style length within the genus. A phylogenetic analysis of the genus using chloroplast (trnT-trnL, trnL-trnF, partial ndhF) and nuclear ITS sequences was generated, scanning electron and light microscopy was used to view pollen morphology, and independent contrasts were used for the pollen grain diameter and style length traits. Cantua was found to be monophyletic and several relationships within the genus were resolved. Cantua volcanica was shown to be most closely related to C. quercifolia. Cantua flexuosa, C. cuzcoensis, C. bicolor, C. candelilla, C. buxifolia, C. pyrifolia and C. nov. sp. were closely related and C. pyrifolia was sister to C. nov. sp. Analyses of the chloroplast and nuclear DNA sequences had conflicting results regarding the monophyly of C. buxifolia and its relationship within the clade. Pollen characteristics within *Cantua* were found to be highly conserved. Spheroidal pantoporate pollen with an insulous semitectate sexine is synapomorphic for Cantua in the Cobaeoideae, giving further support to the monophyly of the genus. The relationship between C. quercifolia and C. volcanica was supported by the synapomorphy of supratectal vertucae on the sexine of the pollen grains. Neither a significant regression slope nor correlation was found using independent contrasts of pollen diameter and style length in Cantua, which rejects the null hypothesis of a morphological integration between the two traits. To summarize the Cantua results, some phylogenetic relationships within the genus were resolved, a review of pollen morphology was completed and the utility of pollen morphology in phylogenetic analyses was confirmed, and pollen diameter and style length was found to be statistically unrelated in Cantua.

My research on the Brassicaceae was designed to test the hypothesis that variancecovariance matrices diverge and change following a step-wise structured pattern identical to the hierarchical family level phylogeny. Six floral morphological traits were measured in 24 Brassicaceae species to generate phenotypic variation-covariation matrices (Pmatrix) for each species. A phylogenetic hypothesis of the same 24 species was generated using sequences from the ndhF coding region. Common principal component analysis was used to compare matrices among groups of taxa which corresponded to clades in the phylogenetic hypothesis. Equality in matrices between different species was found between Barbarea verna and B. vulgaris and proportional matrices were accepted between Raphanus raphanistrum and Raphanus sativus, as well as Nasturtium officinale and Cardamine concatenata. Proportionality among matrices in five genera was found in the A. lyrata - Cardamine concatenata clade. Within Brassica, species had low levels of shared structure. In the Brassicae clade and its sister clade Sisymbrium altissimum /Stanleya pinnata low levels of matrix stability were found among genera. This was also true of the Hesperis matronalis / Matthiola clade and the Lobularia / Iberis clades. The results of this study confirm that P-matrix, and potentially G-matrix, stability is highly variable and the phylogenetic level of the matrix comparison did not appear to predict the level of stability found among matrices, although generally comparisons at higher taxonomic levels resulted in lower levels of shared structure.

Each chapter of my dissertation used a different group of plants to examine patterns of floral diversity using phenotypes, as well as different statistical techniques. The objectives of each study focused on different aspects of floral diversity and extended

from population-level analyses of variation to a family-wide analysis of floral evolution.

Ultimately all of the investigations were based on a phylogenetic hypothesis which provided an historical perspective.

CHAPTER 1

FLORAL MORPHOLOGICAL VARIATION, MOLECULAR SYSTEMATICS, AND DISTRIBUTION IN *PEDICULARIS DENSIFLORA* BENTH. EX HOOK. AND *PEDICULARIS AURANTIACA* (E. F. SPRAGUE) MONFILS & PRATHER (OROBANCHACEAE)

INTRODUCTION

Pedicularis L. is a large (600-800 species), monophyletic genus (Ree 2001) of hemiparasitic herbs, with a northern temperate distribution, and a tremendous amount of diversity in floral morphology. Previously, Pedicularis was placed in the family Scrophulariaceae, however recent taxonomic treatments have placed the genus in the Orobanchaceae (Olmstead et al. 2001). Pedicularis has undergone a relatively recent diversification, likely in the Pleistocene, and shares a center of diversity in eastern Asia with its primary pollinators, Bombus (De-Yaun 1983). Considerable research has been done to investigate the reproductive biology of Pedicularis and document the modes of plant-pollinator interaction (Sprague 1960, 1962, Grant 1966, 1967, Macior 1973, 1982, 1983, 1984, 1986a, 1986b, 1995a, 1995b, 1996, and references therein, Robart 2000, Ree 2001).

This study focused on *Pedicularis densiflora*, a species found only in California and southern Oregon, which was first described from a communication with D. Douglas by Bentham, and published in the <u>Flora Boreali-Americana</u> by J. W. Hooker (1838). Sprague (1958) divided the species into two subspecies, *P. densiflora* subsp. *densiflora* Benth. ex Hook. and *P. densiflora* subsp. *aurantiaca* E. F. Sprague. The circumscriptions of the subspecies were based on distribution, reproductive biology, phenology, host affinity, and floral morphology (Sprague 1958, 1960, 1961, and 1962).

Sprague's (1958) treatment indicated that *P. densiflora* subsp. *densiflora* is pollinated by both *Bombus* and hummingbirds while *P. densiflora* subsp. *aurantiaca* is pollinated

exclusively by hummingbirds. Macior (1986a) documented pollinator type, frequency and mode of pollination of both subspecies. Macior's (1986a) research supported Sprague's (1958, 1960, and 1962) conclusions of different pollination between subspecies. Sprague (1962) and Macior (1986a) suggested that hummingbird pollination was driving a change in morphology among populations and creating a shift within the species from a bumblebee pollination syndrome to a hummingbird syndrome.

Fundamental to their hypothesis was the concept that the species had diverged into two distinct subspecies *P. densiflora* subsp. *densiflora* and *P. densiflora* subsp. *aurantiaca* with unique floral morphologies and distinct pollinator regimes. Additionally, this hypothesis assumed that the ancestral pollination system in *P. densiflora* s.l. is *Bombus* pollination.

There have been no molecular or detailed quantitative morphological studies to either polarize character traits or definitely document either genetic or morphological divergence within the species. An extensive examination was undertaken to detect and explain patterns of infraspecific variation within the species *P. densiflora* s.l. and determine which pollination system is derived. This work revealed that *P. densiflora* should be more narrowly circumscribed, and that the subspecies *P. densiflora* subsp. aurantiaca should be elevated to species status as *P. aurantiaca* (E. F. Sprague) Monfils & Prather. The two taxa will be referred to by their species names for the remainder of this paper.

MATERIALS AND METHODS

Herbarium specimens – A total of 1069 herbarium specimens of Pedicularis densiflora, P. aurantiaca and P. semibarbata A. Gray from CAS, DS, JEPS, MSC, ORE, OSC, POM, RSA, UC, and WILLU were studied. Specimens were screened for both morphological variation and geographic distribution. A preliminary investigation of herbarium material was used to select population study sites and floral characters for measurement. Study populations were selected from throughout the range of both P. densiflora and P. aurantiaca, sampling a broad elevational, geographic, and environmental spectrum. Populations were representative of the full breadth of morphological diversity. Additional analyses of herbarium specimens were conducted to evaluate utility and reliability of morphological traits in distinguishing separate species and to comprehensively review the distribution of the two taxa.

Analysis of Floral Morphological Variation – During a preliminary field season in 1998 localities were screened and ten populations were chosen for the study of floral morphological divergence. Populations used in the morphological study were from the following ten localities: Bear Valley (Colusa/Lake Co., CA), Hurles Circle (Butte Co., CA), Inskip (Butte Co., CA), McBride Springs (Siskyou Co., CA), Missouri Flats Road (Josephine Co., OR), Mount Diablo (Contra Costa Co., CA), Paradise (Marin Co, CA), Pinehurst (Jackson Co., OR), Round Valley Historical Marker (Mendocino Co., CA), and Santa Margarita Lake (San Luis Obispo Co., CA) (Table 1 and Figure 1). Voucher specimens were deposited in the Michigan State University Herbarium (MSC).

Table 1. List of populations and voucher specimens for DNA sequence analysis and floral morphological analysis of *Pedicularis densiflora* and *Pedicularis aurantiaca*. All voucher specimens are deposited at Michigan State University Herbarium (MSC)

Species	Location (County, State)	Voucher	DNA Sequence Analysis	Floral Morphological Analysis
P. aurantiaca	Auberry	Monfils 4	X	
P. aurantiaca	(Fresno Co., CA) Hobo Gulch	Monfils 9	x	
P. aurantiaca	(Trinity Co., CA) Inskip (Butte Co., CA)	Monfils 37	X	x
P. aurantiaca	McBride Springs (Siskyou Co., CA)	Monfils 43	X	X
P. aurantiaca	Pinehurst (Jackson Co., OR)	Monfils 40	X	X
P. aurantiaca	Weaver Camp (Trinity Co., CA)	Monfils 8	X	
P. densiflora	Bear Valley (Colusa/Lake Co., CA)	Monfils 33	X	X
P. densiflora	Briceland (Humbolt Co., CA)	Monfils 14	X	
P. densiflora	Hurles Circle (Butte Co., CA)	Monfils 35	X	X
P. densiflora	Missouri Flat Road (Josephine Co., OR)	Monfils 36	X	X
P. densiflora	Mount Diablo (Contra Costa Co., CA)	Monfils 28	X	X
P. densiflora	Paradise (Marin Co., CA)	Monfils 34 Monfils 20	x x	Х
P. densiflora	Pinnacles National Monument (San Benito Co., CA)			
P. densiflora	Round Valley Historical Marker (Mendocino Co., CA)	Monfils 32	Х	X
P. densiflora	San Marcos Pass (Santa Barbara Co., CA)	Monfils 1	x	
P. densiflora	Santa Margarita Lake (San Luis Obispo Co., CA)	Monfils 21	x	X
P. semibarbata A	San Gorgonio Wilderness (San Bernardino Co., CA)	Holmgren 3591	Х	
P. semibarbata B	East Sirretta Pass (Tulare Co., CA)	Twisselman 15760	Х	
P. cystopteridifolia A	Red Lodge-Cook City Highway (Carbon Co., WY)	Beaman 1551	Х	
P. cystopteridifolia B	Cody (Park Co., WY)	Kirkpatrick 3491	x	
P. bracteosa A	Absaroka Mountains (Freemont Co., WY)	Nelson 10994	x	
P. bracteosa B	Mazama (Whatcom Co., WA)	Churchill 7671512	X	

Figure 1. Map of Oregon and California representing distribution of 16 sampled populations of *P. densiflora* and *P. aurantiaca*. Names refer to population localities. All populations sampled were used in the molecular analysis. Populations with astrix after the name were used in the morphological analysis. Circles represent populations of *P. densiflora* and triangles represent populations of *P. aurantiaca*. All populations sampled were used in the molecular analysis. Populations with asterix after the name were used in the morphological analysis.



Figure 1.

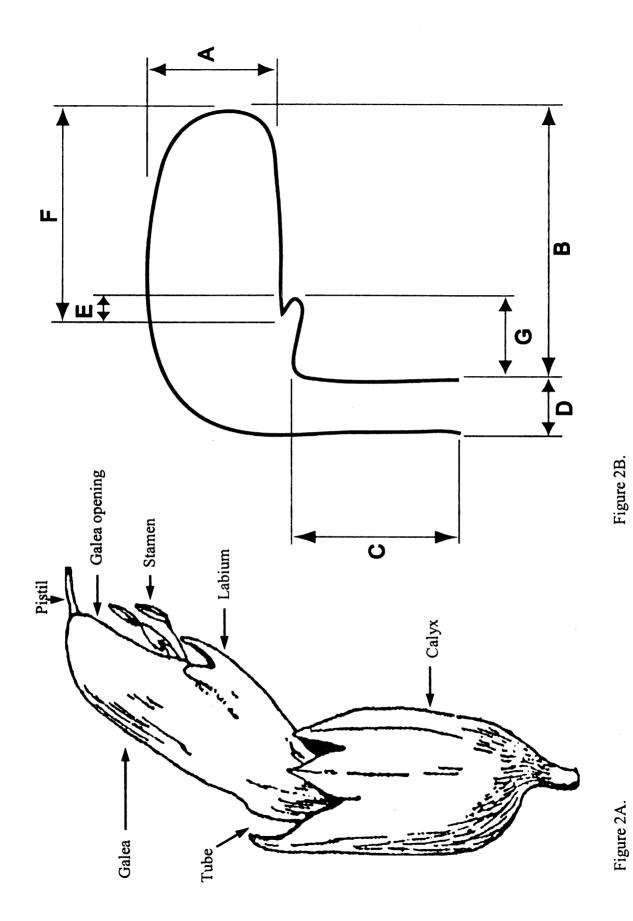
When available, fifty individuals per population were studied. A total of 466 flowers were measured. Within each population, one flower each was removed from 50 plants. Plants were selected by sampling at set intervals along transects encompassing the population range. Flower sampling was based on the most apical, fully developed, intact flower with an exserted stigma. Only flowers that were shedding pollen and had not yet begun to senesce were selected.

Measurements were made using digital calipers and recorded to the nearest 0.01 mm. Floral characters used in the analysis were chosen based on the characters used by Sprague (1958) to differentiate the subspecies, floral traits involved in effective pollination as described by Macior (1986), and floral traits associated with pollination syndromes of *Bombus* and Hummingbird pollination as described by (Citation)Thirteen quantitative characters were measured: galea height, tube height, galea length, tube length, abaxial margin of galea, calyx length, pistil length, stamen length, labium length, angle at the axis of galea and tube, tube exsertion (extension of tube above the calyx lobes), corolla length, and labium tip to galea bend (Figure 2). Four additional characters were derived from the initial measurements, treated as ratios, and used to quantify the relationship between shape and size: labium length/galea length, tube length/galea length, tube height/galea height, and abaxial margin of galea/galea length.

Statistical analysis -

Statistical Analysis System (SAS Institute, Inc. 1985) was used to perform a nonhierarchical analysis among population means for all 17 floral morphological characters

Figure 2: A. Pedicularis densiflora flower X 6. Reproduced from A Manual of the Flowering Plants of California (Jepson, 1925). B. Schematic representation of Pedicularis densiflora corolla. Lines represent parameters of quantitative measurements and letters correspond to the following measurements: A. galea height, B. galea length, C. tube length, D. tube height, E. labium length, F. abaxial margin of galea, and G. labium tip to galea bend.



using the principal component analysis (PCA). Before analysis, all mean measurements were standardized across the populations with a mean of zero and a variance of one.

Varimax rotation (Kaiser 1958) was conducted to maximize the variance of the variable loadings within a factor. The end result is a polarized loading for the variables in each factor closer to zero or one. Varimax rotation simplifies the interpretation of each factor in the PCA analysis (Johnson & Wichern 2002).

Analysis of variance (ANOVA) was performed on all 17 individual characters for the ten sampled populations. The PROC UNIVARIATE and HOVTEST=LEVENE tests were conducted to assure normality and homogeneity of variance, respectively. Where necessary, data were log, square root, or arccosine transformed to fulfill the assumptions of standard variance and normality. Bonferroni (Dunn) T-tests were conducted on each variable to examine differences between all possible pairs of means and significant groupings among means for all ten populations.

A symmetric dissimilarity matrix was created from a rectangular data matrix of the standardized 17 morphological character traits measured in the ten selected populations using the program NTSYSpc version 2.1 (Applied Biostatistics, Inc.). The average "taxonomic" distance coefficient was utilized as a measure of dissimilarity. The unweighted pair-group method of cluster analysis (UPGMA) was performed on the dissimilarity analysis, and a UPGMA phenogram was constructed.

Analysis of Population Level Molecular Variation -

Sampling - Leaf tissue was sampled from throughout the range of *P. densiflora* and *P. aurantiaca* for 16 populations. For the 10 populations used in the morphological analysis, floral tissue was collected and preserved in silica gel. Populations were used in the molecular study from the following sixteen localities: Auberry (Fresno Co., CA), Bear Valley (Colusa/Lake Co., CA), Briceland (Humbolt Co., CA), Hobo Gulch (Trinity Co., CA), Hurles Circle (Butte Co., CA), Inskip (Butte Co., CA), McBride Springs (Siskyou Co., CA), Missouri Flat Road (Josephine Co., OR), Mount Diablo (Contra Costa Co., CA), Paradise (Marin Co, CA), Pinehurst (Jackson Co., OR), Pinnacles National Monument (San Benito Co., CA), Round Valley Historical Marker (Mendocino Co., CA), Santa Margarita Lake (San Luis Obispo Co., CA), San Marcos Pass (Santa Barbara Co., CA), and Weaver Camp (Trinity Co., CA) (Table 1 and Figure 1). Additional tissue of *Pedicularis* taxa was collected from MSC specimens of the following species: *P. semibarbata, P. cystopteridifolia* Rydb., and *P. bracteosa* Benth. ex Hook. (Table 1).

Outgroup and Additional Taxa - Pedicularis cystopteridifolia, was chosen as an outgroup and P. semibarbata and P. bracteosa were sampled to explore potential relationships to P. densiflora and P. aurantiaca. Outgroup and taxon choice was based on Sprague's (1962) analysis of trait similarity among California species of Pedicularis, unpublished nuclear ribosomal internal transcribed spacer (ITS) phylogenies of North American Pedicularis (Robart 2000), and morphological studies of herbarium specimens.

Analysis - Total DNA was extracted from either leaf or flower material using the techniques of Loockerman and Jansen (1996) and purified using the Schleicher & Schuell Elu-quick DNA Purification Kit (Keene, NH). Two sets of chloroplast primers were used: trnL-trnF primers from Fujii et al. (1997), and psbA-trnH from Sang et al. (1997). ITS1, ITS2, and the 5.8s regions of nuclear ribosomal DNA were amplified using ITS-4 primers of White et al (1990) and the modified ITS-5 primer of Sang et al. (1995). Standard amplification protocols were used, including reaction mixture components and the PCR profile (e. g. Prather et al. 2002), and amplifications were performed on a MJ Research PTC-100 thermalcycler. PCR products were gel purified using the Schleicher & Schuell Elu-quick DNA Purification Kit, (Keene, NH) and sequenced in both directions using an ABI-373 automated sequencer. Sequencing reactions were conducted using the AmpliTaq DNA Dye Terminator Cycle Sequencing reagents (PE Applied Biosystems, Norwalk, CT).

Edited sequences were aligned using the Sequencher 3.0 software (Gene Codes Corporation, Ann Arbor, MI). Termini of the *trnL-trnF* intergenic spacer region and *psbA-trnH* intergenic spacer region were determined from Fujii (1997) and Ambrosini et al. (1992) respectively. Termini of ITS1, ITS2, and the 5.8s regions of nuclear ribosomal DNA were determined by comparison with published sequences of Beardsley and Olmstead (2002). In the aligned sequences of the *psbA-trnH* intergenic spacer region, 39 base pairs (bp237-bp276) were excluded from the analysis due to ambiguity in alignment. Parsimony methods were implemented using PAUP* (version 4.0b4; Swofford 2000). Heuristic searches were performed using the TREE BISECTION RECONNECTION, and

MULTREES options. All insertion/deletion events, displayed as gaps in the data set, were treated as missing data. Bootstrap analyses were conducted using 10,000 replicates with 100 random addition-sequence replicates per bootstrap. A partition homogeneity test was conducted to test for homogeneity in the distribution of phylogenetic information between the nuclear and chloroplast data sets.

RESULTS

Morphological Variation -

Based on the PCA analysis, 96% of the variance in the data set was explained by the first three factors. Factor one explained 45% of the variation and was weighted for characters of galea height, tube height, abaxial margin of galea, calyx length, tube exsertion, labium tip to galea bend, abaxial margin of galea/galea length. All these features corresponded to floral shape. Factor two explained 40% of the variation and was weighted by the following characters: tube length, pistil length, stamen length, corolla length, and tube length/galea length. These floral characteristics were predominantly attributed to flower size and exsertion of the galea. Factor three explained 11 % of the variation in the data set and was strongly weighted by tube height/galea height (Table 2).

Table 2. List of the 17 floral morphological traits of ten populations of *Pedicularis densiflora* and *P. aurantiaca* analyzed using principal component analysis. The first row shows proportion of variance explained by the first three factors. Varimax rotated factor patterns for the first three factors are listed next to the floral morphological trait

	Factor 1	Factor 2	Factor 3
Proportion of variance explained by factor	45%	40%	11%
Rotated factor pa	ittern for Varimax	rotation method	
GALEA HEIGHT	0.93716	0.15449	0.24154
TUBE HEIGHT	0.85129	0.31551	0.37159
GALEA LENGTH	0.46109	0.45989	0.53159
TUBE LENGTH	-0.00203	0.93353	0.21847
ABAXIAL MARGIN OF GALEA	0.89167	0.23682	0.19791
CALYX LENGTH	0.98270	0.07492	0.03785
PISTIL LENGTH	0.18670	0.93626	0.02864
STAMEN LENGTH	0.24642	0.89566	0.32723
LABIUM LENGTH	-0.84662	0.28518	0.15236
ANGLE AT AXIS OF GALEA AND TUBE	-0.40163	0.52962	0.26179
TUBE EXSERTION	-0.88867	0.42396	0.07335
COROLLA LENGTH	0.19754	0.84532	0.37367
LABIUM TIP TO GALEA BEND	0.93273	0.13294	0.21471
ABAXIAL MARGIN OF GALEA/GALEA	0.93363	0.15635	0.10176
LENGTH			
LABIUM LENGTH / GALEA LENGTH	-0.70018	0.24598	0.29567
TUBE LENGTH / GALEA LENGTH	-0.24703	0.93865	0.00712
TUBE HEIGHT / GALEA HEIGHT	0.19050	0.31215	0.91926

Factor one clearly separated two distinct clusters of populations: One group consisting of Inskip (J), McBride Springs (H), and Pinehurst (I) and the second of Bear Valley (D), Hurles Circle (F), Missouri Flats (G), Mount Diablo (C), Paradise (B), Round Valley (E), and Santa Margarita Lake (A) (Figure 3 & 4). Factor two did not separate the populations into distinct clusters; populations were broadly distributed with Bear Valley (D) and Santa Margarita Lake (A) representing the two extremes (Figure 3 & 5).

Santa Margarita Lake had the smallest size measurements for overall corolla size, galea length, stamen length, tube length, pistil length, abaxial margin of galea/galea length, tube length/galea length, and abaxial margin of galea. Bear Valley had the largest values for tube length and tube exsertion. Many of these variables were heavily weighted in the second factor of the PCA analysis and provide an explanation for the separation of Santa Margarita Lake and Bear Valley in the plots with factor two. Factor three displayed a continuum of values with Missouri Flats (G) and Inskip (J) having larger values. Factor three was heavily weighted for tube height/galea height. Missouri Flats (G) and Inskip (J) exhibited the highest ratio of all populations sampled (Figure 4 & 5).

Sixteen of the seventeen variables showed significant variation among population means (p value < 0.0001) in the ANOVA (Table 3). The only character that was not significantly different among populations (p = 0.0363) was the angle at axis of galea and tube. This substantiated and quantified the previous reports by Sprague (1958, 1960, and 1962) and Macior (1986a) of a broad spectrum of morphological diversity across the distribution of P. densiflora and P. aurantiaca.

Figure 3. Plot of factor one by factor two from the principal component analysis of 17 floral morphological characters in ten populations of *Pedicularis densiflora* and *P. aurantiaca*. Letters represent populations as follows: A. Santa Margarita Lake, B. Paradise, C. Mount Diablo, D. Bear Valley, E. Round Valley Historical Marker, F. Hurles Circle, G. Missouri Flat Road. H. McBride Springs, I. Pinehurst, and J. Inskip.

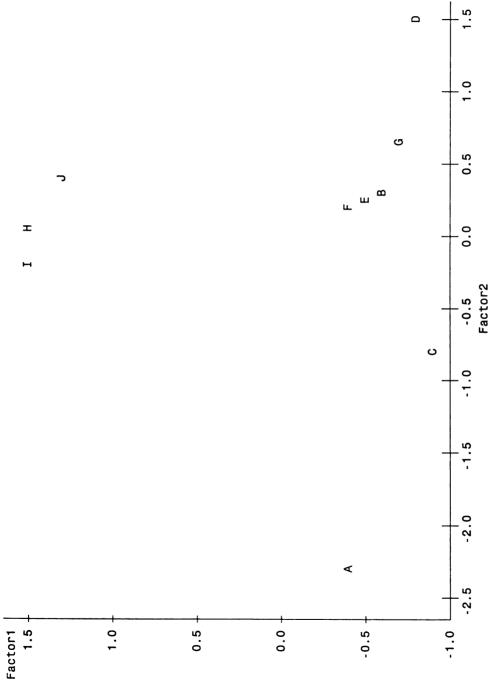


Figure 3.

Figure 4. Plot of factor one by factor three from the principal component analysis of 17 floral morphological characters in ten populations of *Pedicularis densiflora* and *P. aurantiaca*. Letters represent populations as follows: A. Santa Margarita Lake, B. Paradise, C. Mount Diablo, D. Bear Valley, E. Round Valley Historical Marker, F. Hurles Circle, G. Missouri Flat Road. H. McBride Springs, I. Pinehurst, and J. Inskip.

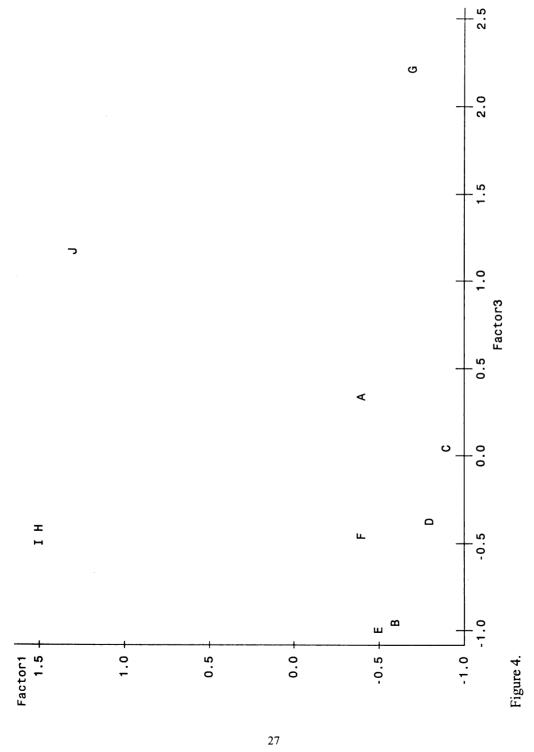


Figure 5. plot of factor two by factor three from the principal component analysis of 17 floral morphological characters in ten populations of *Pedicularis densiflora* and *P. aurantiaca*. Letters represent populations as follows: A. Santa Margarita Lake, B. Paradise, C. Mount Diablo, D. Bear Valley, E. Round Valley Historical Marker, F. Hurles Circle, G. Missouri Flat Road. H. McBride Springs, I. Pinehurst, and J. Inskip.

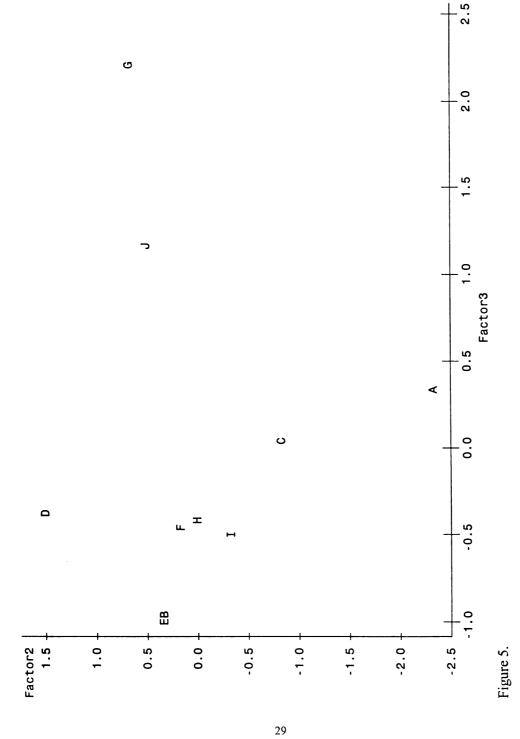


Table 3. Sample size, means, standard deviations, minima, maxima and results from ANOVA and Bonferroni (Dunn) T-tests for characters studied in the morphological analysis of ten populations of *Pedicularis densiflora* and *P. aurantiaca* sampled from California and Oregon. In Bonferroni (Dunn) T-tests means with same letter are not significantly different. Where necessary, data were log, square root, or arcos transformed, this is denoted in the ANOVA RESULTS column as LOG, SQUARE ROOT, or ARCOS respectively.

POPULATION (Sample size)	MEAN (SD)	MIN MAX.	ANOVA RESULTS (p-value)		FERRONI NN) T-TESTS
	1 GALE	A HEIGHT (mm)	(p-value)		
SANTA MARGARITA	5.27	4.43-6.56	(PR>F=0.0001)	D	С
LAKE (N=19)	(0.49)		(110 1 0.0001)		Ü
PARADISE	5.12	4.20-6.45	LOG	D	
(N=50)	(0.44)	1.20 0.15	200		
MOUNT DIABLO	5.10	3.99-6.33		D	
(N=46)	(0.46)	3.77 0.33			
BEAR VALLEY	5.31	4.20-6.53		D	С
(N=50)	(0.53)	1.20 0.33		2	Č
ROUND VALLEY	5.01	4.02-6.42		D	
HISTORICAL MARKER	(0.52)	1.02 0.12			
(N=51)	(0.52)				
HURLES CIRCLE	5.58	4.37-7.11		В	С
(N=51)	(0.59)	4.57-7.11		b	C
MISSOURI FLAT ROAD	5.68	4.60-6.64		В	
(N=50)	(0.48)	4.00-0.04		Ь	
McBRIDE SPRINGS	6.27	5.21-8.07		Α	
(N=50)	(0.64)	3.21-0.07		А	
PINEHURST	6.19	4.89-7.63		Α	
(N=50)	(0.61)	4.03-7.03		Λ.	
INSKIP	6.46	5.08-8.62		Α	
(N=50)	(0.76)	3.00-0.02		Λ.	
(14–30)		HEIGHT (mm)			
CANTA MADCADITA	3.18	2.75-3.91	(DD > E-0 0001)	D	Е
SANTA MARGARITA		2.73-3.91	(PR>F=0.0001)	D	E
LAKE (N=19)	(0.31)	254200	LOG	Е	
PARADISE	3.09	2.54-3.88	LOG	E	
(N=50)	(0.32)	2 41 2 76		E	
MOUNT DIABLO	2.99	2.41-3.76		E	
(N=46)	(0.31)	2 (7 4 2		n	
BEAR VALLEY	3.39	2.67-4.3		D	
(N=50)	(0.39)	2.54.2.60		ъ	-
ROUND VALLEY	3.16	2.54-3.69		D	E
HISTORICAL MARKER	(0.30)				
(N=51)	2.20	275 405		D	
HURLES CIRCLE	3.39	2.75-4.05		D	
(N=51)	(0.33)	2.00 4.55		_	
MISSOURI FLAT ROAD	3.70	2.89-4.55		С	
(N=50)	(0.35)	2 20 4 04			D
McBRIDE SPRINGS	4.00	3.30-4.94		Α	В
(N=50)	(0.44)	2.04.4.70		0	D
PINEHURST	3.81	3.04-4.79		C	В
(N=50)	(0.38)				

Table 3 (cont'd)

POPULATION (Sample size)	MEAN (SD)	MIN MAX.	ANOVA RESULTS		BONFERRONI (DUNN) T-TESTS		
•	` ,		(p-value)	`		•	
	2. TUBE	HEIGHT CONTI					
INSKIP	4.26	2.97-5.12		Α			
(N=50)	(0.48)						
•		A LENGTH (mm))				
SANTA MARGARITA	18.79	15.39-21.10	(PR>F=0.0001)	Ε			
LAKE (N=19)	(1.41)		,				
PARADISE	18.95	15.19-22.20	LOG	E			
(N=50)	(1.70)						
MOUNT DIABLO	18.98	15.45-22.88		Е			
(N=46)	(1.94)						
BEAR VALLEY	19.72	16.39-23.19		Е	С	D	В
(N=50)	(1.69)			_	-	_	
ROUND VALLEY	19.25	16.70-22.40		Е	С	D	
HISTORICAL MARKER	(1.38)			_	_	~	
(N=51)	(1.50)						
HURLES CIRCLE	19.07	16.03-22.83		Е		D	
(N=51)	(1.69)	10.05-22.05					
MISSOURI FLAT ROAD	21.35	18.00-24.75		Α			
(N=50)	(1.64)	18.00-24.73		Λ.			
McBRIDE SPRINGS	20.28	16.64-24.87		Α	С	D	В
		10.04-24.67		А	C	D	D
(N=50) PINEHURST	(1.85) 20.41	14.29-24.03		Α	С		В
		14.29-24.03		А	C		Б
(N=50)	(1.69)	16 50 25 52					n
INSKIP	20.60	16.50-25.53		Α			В
(N=50)	(2.08)	I ENIOTHY ()					
6.35m. 36.56.55m.		LENGTH (mm)	(DD: E 0 0001)	_			
SANTA MARGARITA	10.26	7.18-13.53	(PR>F=0.0001)	D			
LAKE (N=19)	(1.69)			_		_	
PARADISE	12.83	8.90-18.13	LOG	C		В	
(N=50)	(1.90)			_			
MOUNT DIABLO	11.90	7.53-15.27		C			
(N=46)	(1.52)						
BEAR VALLEY	15.29	10.51-20.70		Α			
(N=50)	(2.35)	0.00		_			
ROUND VALLEY	12.56	9.38-18.70		C			
HISTORICAL MARKER	(1.77)						
(N=51)				_		_	
HURLES CIRCLE	13.01	9.49-17.85		C		В	
(N=51)	(1.83)						
MISSOURI FLAT ROAD		9.49-20.65		Α			
(N=50)	(2.37)						
McBRIDE SPRINGS	12.81	8.60-16.93		С		В	
(N=50)	(1.77)						
PINEHURST	11.91	9.05-16.45		C			
(N=50)	(1.41)						
INSKIP	14.18	8.28-19.41		Α		В	
(N=50)	(2.22)						

Table 3 (cont'd)

POPULATION	MEAN	MIN MAX.	ANOVA	BONFERRONI
(Sample size)	(SD)		RESULTS	(DUNN) T-TESTS
• •	` ′		(p-value)	,
	5. ABAX	IAL MARGIN OF		
SANTA MARGARITA	11.36	10.05-13.01	(PR>F=0.0001)	D
LAKE (N=19)	(0.98)		,	
PARADISE	12.30	9.86-15.91	LOG	C
(N=50)	(1.37)			
MOUNT DIABLO	12.54	9.80-20.01		C
(N=46)	(1.63)			
BEAR VALLEY	12.66	9.98-15.71		C
(N=50)	(1.24)			
ROUND VALLEY	12.45	10.33-15.01		C
HISTORICAL MARKER	(1.13)			
(N=51)				
HURLES CIRCLE	12.87	10.36-15.99		C
(N=51)	(1.39)			
MISSOURI FLAT ROAD	14.86	12.18-17.63		В
(N=50)	(1.23)			
McBRIDE SPRINGS	17.52	14.57-22.07		Α
(N=50)	(1.86)			
PINEHURST	18.15	13.10-22.28		Α
(N=50)	(1.59)			
INSKIP	17.88	14.18-23.27		Α
(N=50)	(2.04)			
		X LENGTH (mm)		
SANTA MARGARITA	13.99	12.36-16.78	(PR>F=0.0001)	С
LAKE (N=19)	(1.20)			
PARADISE	12.67	10.13-15.11	LOG	D
(N=50)	(1.17)			
MOUNT DIABLO	12.70	10.54-16.78		D
(N=46)	(1.41)			_
BEAR VALLEY	14.17	10.72-17.75		С
(N=50)	(1.35)			_
ROUND VALLEY	12.39	9.83-16.09		D
HISTORICAL MARKER	(1.25)			
(N=51)		10 10 11 22		
HURLES CIRCLE	13.66	10.19-16.35		С
(N=51)	(1.45)	10.01.55.50		
MISSOURI FLAT ROAD	13.24	10.81-15.60		C D
(N=50)	(0.97)	10.00.00.00		
McBRIDE SPRINGS	18.83	13.08-24.03		Α
(N=50)	(2.31)			_
PINEHURST	17.14	11.98-21.10		В
(N=50)	(1.77)	16.68.00.00		
INSKIP	18.64	15.57-22.92		Α
(N=50)	(1.69)	I ENGTH (
CANTE ANA DO A DETE		LENGTH (mm)	(DD: E 0.0001)	
SANTA MARGARITA	25.48	22.47-29.61	(PR>F=0.0001)	E
LAKE (N=19)	(1.93)			

Table 3 (cont'd)

POPULATION (Sample size)	MEAN (SD)	MIN MAX.	ANOVA RESULTS (p-value)		BONFERRONI (DUNN) T-TESTS		
	7 PISTII	LENGTH CONT	CINITED (mm)				
PARADISE	7. FISTIL 35.44	24.67-44.08	INOED (IIIII)	С		D	D
		24.07-44.06		C		D	В
(N=50)	(4.79)	25 54 42 01		ъ			
MOUNT DIABLO	33.18	25.54-42.01		D			
(N=46)	(3.52)	20.50.46.00					
BEAR VALLEY	38.73	28.50-46.00		Α			
(N=50)	(3.83)	20 44 42 71		_		_	_
ROUND VALLEY	35.92	30.44-43.71		C	Α	D	В
HISTORICAL MARKER	(2.87)						
(N=51)						_	
HURLES CIRCLE	34.08	24.64-43.29		C		D	
(N=51)	(4.50)						
MISSOURI FLAT ROAD	37.99	29.33-48.64		Α		В	
(N=50)	(4.19)						
McBRIDE SPRINGS	36.62	29.74-44.42		C	Α		В
(N=50)	(3.30)						
PINEHURST	36.09	17.14-44.73		C	Α	D	В
(N=50)	(4.63)						
INSKIP	37.17	27.68-46.03		Α			В
(N=50)	(4.89)						
	8. STAM	EN LENGTH (mi	m)				
SANTA MARGARITA	26.42	16.91-30.95	(PR>F=0.0001)	Ε			
LAKE (N=19)	(3.11)						
PARADISE	31.09	23.19-38.63		C			
(N=50)	(3.45)						
MOUNT DIABLO	28.67	21.45-35.49		D			
(N=46)	(3.45)						
BEAR VALLEY	33.29	24.03-40.28		C	Α		
(N=50)	(3.32)						
ROUND VALLEY	31.16	24.39-39.42		С			
HISTORICAL MARKER	(3.01)			_			
(N=51)	(2.55)						
HURLES CIRCLE	31.06	25.18-39.67		С			
(N=51)	(3.54)	20110 07107		·			
MISSOURI FLAT ROAD	34.46	27.78-41.66		Α			
(N=50)	(3.14)	2		••			
McBRIDE SPRINGS	31.92	24.62-41.15		С		E	3
(N=50)	(3.02)	225		·		_	
PINEHURST	31.41	24.09-36.88		С			
(N=50)	(2.61)	21.09 30.00		Ŭ			
INSKIP	34.13	26.51-40.94		Α		В	
(N=50)	(3.19)	20.51 10.77				_	
(20)		M LENGTH (mr	n)				
SANTA MARGARITA	3.82	1.12-5.49	(PR>F=0.0001)	D			
LAKE (N=19)	(1.07)	1.12 J.T/	(110-1-0.0001)	ט			
PARADISE	4.09	1.84-7.01	SQUARE	С		D	
(N=50)	(0.95)	1.07-7.01	ROOT	C		J	
(14-30)	(0.73)		KOO I				

Table 3 (cont'd)

POPULATION (Sample size)	MEAN (SD)	MIN MAX.	ANOVA RESULTS		FERRONI NN) T-TESTS
	OIADII	JM LENGTH CO	(p-value)		
MOUNT DIABLO	5.20	3.72-12.23	NTINUED(IIIII)	Α	В
(N=46)	(1.32)	3.72-12.23		А	Ь
BEAR VALLEY	5.29	3.22-8.98		Α	В
		3.22-0.70		A	Б
(N=50) ROUND VALLEY	(1.19) 4.65	2.88-6.77		С	В
		2.00-0.77		C	Б
HISTORICAL MARKER	(0.98)				
(N=51)	4.20	2 (0 7 44		_	Ъ
HURLES CIRCLE	4.30	2.69-7.44		С	D
(N=51)	(0.91)	271270			
MISSOURI FLAT ROAD	5.85	3.7-12.68		Α	
(N=50)	(1.34)	1 20 4 21		_	
McBRIDE SPRINGS	2.87	1.30-4.71		E	
(N=50)	(0.75)	1 41 4 24		_	
PINEHURST	2.86	1.41-4.24		E	
(N=50)	(0.70)			_	
INSKIP	3.13	1.64-5.26		E	
(N=50)	(0.90)				
	10. ANG		GALEA AND TUE	BE (deg	grees)
SANTA MARGARITA	144.56	123.50-180	(PR>F=0.0363)	Α	
LAKE (N=19)	(12.47)				
PARADISE	147.32	122.76-180		Α	
(N=50)	(10.86)				
MOUNT DIABLO	145.63	125.60-180		Α	
(N=46)	(9.02)				
BEAR VALLEY	149.90	121.68-180		Α	
(N=50)	(10.09)				
ROUND VALLEY	143.73	99.37-180		Α	
HISTORICAL MARKER	(13.98)				
(N=51)	, ,				
HURLES CIRCLE	149.02	118.10-180		Α	
(N=51)	(13.63)				
MISSOURI FLAT ROAD	151.57	132.58-180		Α	
(N=50)	(10.14)				
McBRIDE SPRINGS	144.71	117.73-		Α	
(N=50)	(9.90)	163.88		• •	
PINEHURST	143.34	109.30-180		Α	
(N=50)	(15.45)	107.50 100		••	
INSKIP	145.90	105.92-		Α	
(N=50)	(11.64)	167.81		••	
(11 30)		E EXSERTION (1	mm)		
SANTA MARGARITA	-3.74	-7.14-0.16	(PR>F=0.0001)	С	
LAKE (N=19)	-3.74 (1.94)	-7.17-0.10	(11000.0-1.711)	C	
PARADISE	0.17	-4.12-5.26		Α	В
		-4.12-3.20		А	ь
(N=50)	(1.95)	476221		р	
MOUNT DIABLO	-0.80	-4.76-2.31		В	
(N=46)	(1.54)				

Table 3 (cont'd)

POPULATION	MEAN	MIN MAX.	ANOVA	BO	NFERRONI
(Sample size)	(SD)	MIN-MAX.	RESULTS		JNN) T-TESTS
(Sample size)	(3D)		(p-value)	(DC	JININ) 1-1E313
	11 TIR	F FYSERTION (CONTINUED (mm)		
BEAR VALLEY	1.12	-2.99-5.39	CONTINUED (IIIII)	Α	
(N=50)	(2.17)	-2.55-5.55		Λ	
ROUND VALLEY	0.16	-3.76-6.88		Α	В
HISTORICAL MARKER	(1.93)	-3.70-0.00		А	Ь
(N=51)	(1.75)				
HURLES CIRCLE	-0.65	-5.74-4.31		В	
(N=51)	(2.06)	-5.74-4.51		Ъ	
MISSOURI FLAT ROAD	1.38	-3.05-7.95		Α	
(N=50)	(2.31)	-3.03-1.73		Λ	
McBRIDE SPRINGS	-6.02	-10.33-		Е	
(N=50)	(2.00)	(-1.63)		L	
PINEHURST	-5.36	-9.62-12.19		Е	D
(N=50)	(1.76)	-9.02-12.19		L	D
INSKIP	-4.46	-11.25-2.05		С	D
(N=50)	(2.65)	-11.23-2.03		C	D
(14–30)		OLLA LENGTH	(mm)		
SANTA MARGARITA	29.04	24.80-32.93	(PR>F=0.0001)	Е	
LAKE (N=19)	(2.33)	24.60-32.93	(FK>F-0.0001)	E	
PARADISE	31.79	25.06-39.71		С	D
(N=50)	(3.19)	23.00-39.71		C	D
MOUNT DIABLO	30.88	23.24-37.05		Е	D
(N=46)	(2.95)	23.24-37.03		E	D
BEAR VALLEY	35.00	27.98-42.15		Α	В
(N=50)	(3.35)	27.90-42.13		А	ь
ROUND VALLEY	31.81	27.99-41.10		С	D
HISTORICAL MARKER	(2.70)	27.99-41.10		C	ט
(N=51)	(2.70)				
HURLES CIRCLE	32.08	26.62-39.99		С	D
(N=51)	(3.23)	20.02-39.99		C	ט
MISSOURI FLAT ROAD	36.18	28.60-42.24		Α	
(N=50)	(3.61)	20.00-42.24		A	
McBRIDE SPRINGS	33.09	25.54-41.80		С	В
(N=50)	(3.04)	23.34-41.60		C	Б
PINEHURST	32.32	23.97-38.18		С	D
(N=50)	(2.50)	23.97-30.10		C	D
INSKIP	34.78	29.33-42.15		Α	В
		29.33-42.13		A	Б
(N=50)	(3.02)	IIIM TID TO CA	LEA BEND (mm)		
SANTA MARGARITA	13. LAB			D	C
LAKE (N=19)		8.90-13.83	(PR>F=0.0001)	В	С
PARADISE	(1.41) 10.54	9.76.13.07		D	С
		8.76-13.07	LOG	В	C
(N=50) MOUNT DIABLO	(1.20)	0.20.14.69	LOG		D
	11.64	9.29-14.68		Α	В
(N=46) BEAR VALLEY	(1.35) 12.34	9.48-15.54			
		9.46-13.34		Α	
(N=50)	(1.28)				

Table 3 (cont'd)

POPULATION	MEAN	MIN MAX.	ANOVA	BC	NFERRONI
(Sample size)	(SD)	2.222.11	RESULTS		UNN) T-TESTS
, ,	` '		(p-value)	`	•
	13. LABI	UM TIP TO GA	LEA BEND CONT	INUE	ED(mm)
ROUND VALLEY	11.45	8.74-13.69		Α	BC
HISTORICAL MARKER	(1.08)				
(N=51)	, ,				
HURLES CIRCLE	10.51	8.28-13.56			
(N=51)	(1.36)				
MISSOURI FLAT ROAD	12.34	9.90-16.09		Α	
(N=50)	(1.28)				
McBRIDE SPRINGS	5.63	3.27-7.28		D	
(N=50)	(0.91)				
PINEHURST	5 .11	3.62-6.88		Ε	
(N=50)	(0.72)				
ÎNSKIP	5 .85	4.48-7.82		D	
(N=50)	(0.84)				
	` '	XIAL MARGIN	OF GALEA/GALE	A LE	NGTH
SANTA MARGARITA	0.60	0.53-0.67	(PR>F=0.0001)	Е	
LAKE (N=19)	(0.05)		,		
PARADISE ´	0.65	0.55-0.72	ARCCOS	С	D
(N=50)	(0.04)				
MOUNT DIABLO	0.66	0.57-1.07		C	D
(N=46)	(0.07)				
BEAR VALLEY	0.64	0.52-0.75		D	
(N=50)	(0.05)				
ROUND VALLEY	0.65	0.58-0.73		C	D
HISTORICAL MARKER	(0.04)				
(N=51)					
HURLES CIRCLE	0.68	0.53-0.79		В	C
(N=51)	(0.05)				
MISSOURI FLAT ROAD	0.70	0.62-0.96		В	
(N=50)	(0.05)				
McBRIDE SPRINGS	0.86	0.79-0.95		Α	
(N=50)	(0.03)				
PINEHURST	0.89	0.82-0.96		Α	
(N=50)	(0.03)				
INSKIP	0.87	0.78-0.94		Α	
(N=50)	0.03				
			GALEA LENGTH		
SANTA MARGARITA	0.205	0.060-0.297	(PR>F=0.0001)	В	
LAKE (N=19)	(0.058)				
PARADISE	0.216	0.097360		В	
(N=50)	(0.045)	_			
MOUNT DIABLO	0.276	0.166-0.655		Α	
(N=46)	(0.073)				
BEAR VALLEY	0.270	0.167-0.525		Α	
(N=50)	(0.063)				_
ROUND VALLEY	0.242	0.150-0.378		Α	В
HISTORICAL MARKER	(0.052)				
(N=51)					

Table 3 (cont'd)

POPULATION	MEAN	MIN	ANOVA	BC	NFER	RONI	
(Sample size)	(SD)	MAX.	RESULTS			T-TES	
(()		(p-value)		,		
	15. LABI	UM LENGTH /	GALEA LENGTH	CON	TINUI	ED	
HURLES CIRCLE	0.226	0.147-0.341		В			
(N=51)	(0.042)						
MISSOURI FLAT ROAD	0.275	0.188-0.690		Α			
(N=50)	(0.071)						
McBRIDE SPRINGS	Ò.141	0.069-0.214		C			
(N=50)	(0.034)						
PINEHURST	0.141	0.060-0.201		С			
(N=50)	(0.035)						
INSKIP	0.152	0.079-0.239		C			
(N=50)	(.044)						
	16. TUB	E LENGTH / GA	ALEA LENGTH				
SANTA MARGARITA	0.548	0.365-0.744	(PR>F=0.0001)	E			
LAKE (N=19)	(0.096)		·				
PARADISE	0.677	0.533-0.840		В	C		
(N=50)	(0.081)						
MOUNT DIABLO	0.629	0.479-0.824		C		D	
(N=46)	(0.079)						
BEAR VALLEY	0.777	0.548-1.106		Α			
(N=50)	(0.115)						
ROUND VALLEY	0.653	0.488-0.878		В	C		
HISTORICAL MARKER	(0.080)						
(N=51)							
HURLES CIRCLE	0.682	0.489-0.877		В	C		
(N=51)	(0.071)						
MISSOURI FLAT ROAD	0.682	0.497-0.977		В	C		
(N=50)	(0.111)			_	_	_	
McBRIDE SPRINGS	0.634	0.491-0.827		В	С	D	
(N=50)	(0.084)			_		_	
PINEHURST	0.586	0.437-0.757		E		D	
(N=50)	(0.072)	0.24.1.042		_			
INSKIP	0.696	0.364-1.042		В			
(N=50)	(0.127)		I E A HELOUTE				
CANTTANANCANTT		E HEIGHT / GA		~		г.	
SANTA MARGARITA	0.606	0.474-0.694	(PR>F=0.0001)	С		D	
LAKE (N=19)	(0.056)	0.445.0.730		_		-	
PARADISE	0.605	0.445-0.739		С		D	
(N=50)	(0.060)	0.400.0.712		_			
MOUNT DIABLO	0.589	0.489-0.713		D			
(N=46)	(0.056)	0.401.0036		~			D
BEAR VALLEY	0.642	0.481-0.835		С	Α		В
(N=50)	(0.072) 0.633	0.500.0.742		_		D	p
ROUND VALLEY HISTORICAL MARKER		0.509-0.762		C	Α	D	В
(N=51)	(0.063)						
HURLES CIRCLE	0.611	0.479-0.771		С		D	В
(N=51)	(0.059)	U.7/2-U.//I		C		U	D
(11-21)	(0.039)						

Table 3 (cont'd)

POPULATION	MEAN	MIN MAX.	ANOVA	ВО	BONFERRONI		
(Sample size)	(SD)		RESULTS	(DU	(DUNN) T-TEST		
			(p-value)				
	17. TUBI	E HEIGHT / GAI	LEA HEIGHT CO	DNTINU	JED		
MISSOURI FLAT ROAD	0.654	0.501-0.806		Α		В	
(N=50)	(0.070)						
McBRIDE SPRINGS	0.639	0.494-0.747		С	Α		В
(N=50)	(0.062)						
PINEHURST	0.618	0.504-0.733		С		D	В
(N=50)	(0.056)						
INSKIP	0.664	0.466-0.921		Α			
(N=50)	(0.078)						

Bonferroni (Dunn) T-tests indicated that the Inskip, Pinehurst, and McBride Springs populations showed means not significantly different from each other, but distinct from the remaining populations for the following five variables: galea height, abaxial margin of galea, labium length, abaxial margin of galea/galea length, and labium length/galea length. McBride Springs and Inskip had no significant difference in mean calyx length (18.83 mm and 18.64 mm, respectively) but were significantly different from the remaining eight populations. Pinehurst had a mean calyx length of 17.14 mm, which was also significantly different from all other populations (<14.17 mm). In the case of labium tip to galea bend, Pinehurst had the lowest mean at 5.11 mm and was significantly different from all other populations. McBride Springs and Inskip had similar means of 5.63 mm and 5.85 mm, respectively, which were significantly different from the remaining populations and represented the lowest means besides Pinehurst. All other mean measurements of labium tip to galea bend exceeded 10.51mm.

Means for tube exsertion were extreme for Inskip (-4.46 mm), Pinehurst (-5.36 mm), McBride Springs (-6.02 mm), and Santa Margarita Lake (-3.74 mm); pair-wise

comparisons of the population means were significantly different from the remaining populations. Three separate groupings of the above populations were assessed as not significantly different: Pinehurst/Inskip, Santa Margarita Lake/Inskip, and McBride Springs/Pinehurst. These taxa were clearly larger and separated from the remaining populations, whose means for tube exsertion from -.0880 mm to 1.38 mm. No distinguishable patterns were observed for the remaining variables; however, the Bear Valley, Missouri Flat, and Inskip populations showed overall larger measurements.

ANOVA results corroborated the reports by Sprague (1958, 1960, 1962) of substantial morphological variation within the species *P. densiflora* and *P. aurantiaca*. Sixteen of the 17 traits measured were significantly different among the ten populations in this study. Examination of population means using the Bonferroni (Dunn) T-tests indicated a cohesive morphology among the McBride Springs, Inskip, and Pinehurst populations.

The UPGMA phenogram summarized potential relationships indicated by the metric of taxonomic distance. Division of the populations into two distinct clusters was substantiated by the phenogram which showed high similarity among the Inskip, McBride Springs, and Pinehurst populations. These three populations formed a cluster while the remaining seven populations, Bear Valley, Hurles Circle, Missouri Flats, Mount Diablo, Paradise, Round Valley, and Santa Margarita Lake formed a second distinct grouping (Figure 6).

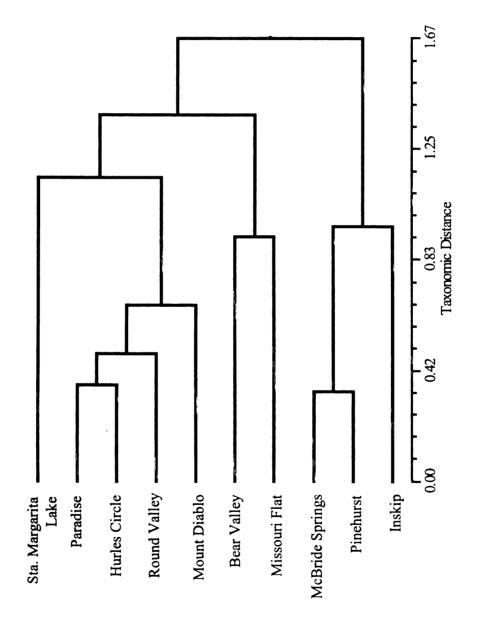


Figure 6. UPGMA phenogram of taxonomic distance based on 17 continuous floral morphological character traits in populations of *P. densiflora* and *P. aurantiaca*. Population names, locations, and voucher information described in Table 1 and Figure 1.

Examination of all three morphological analyses, PCA, ANOVA, and UPGMA, suggested two distinct morphological groups of populations. *Pedicularis aurantiaca* was represented by Inskip, McBride Springs, and Pinehurst populations which formed a well-defined group of populations with similar floral morphology. *Pedicularis densiflora* was represented by seven populations which formed a second distinct group, these included Bear Valley, Hurles Circle, Missouri Flats, Mount Diablo, Paradise, Round Valley, and Santa Margarita Lake populations.

Observations on the ten populations combined with a review of herbarium specimens helped characterize floral traits, phenology and distribution in the two species.

Pedicularis aurantiaca had long calyces (12-24 mm), a reduced ratio of the labium to galea (approx. 1:7), a short labium (1-5 mm), a short region from the labium tip to corolla bend (3.5-7.5 mm), an enlarged abaxial margin of the galea about 7/8 as long as the galea, and a tube that is included in the calyx. Pedicularis aurantiaca flowered late April through June and grew at elevations between 100 and 7000 feet. Pedicularis aurantiaca was distributed in southeast Oregon in Klamath and Jackson Counties, northern

California in the Trinity and Siskiyou Alps, and in the Sierra Nevadas as far south as Kern County (Figure 7 & 8).

Pedicularis densiflora had short calyces (10-18mm), a larger labium to galea ratio (approx. 1:4), a long labium (2-12 mm), a long region from the labium tip to corolla bend (8.5 – 16 mm), an truncated abaxial margin of galea approximately 2/3 the length of the

Figure 7. County map of California. Map shows distribution of *P. densiflora* and *P. aurantiaca* based on morphological analysis of populations in the field and herbarium material cited. Circle represents presence of *P. densiflora* herbarium material in the county. Triangle represents presence of *P. aurantiaca* herbarium material in the county.

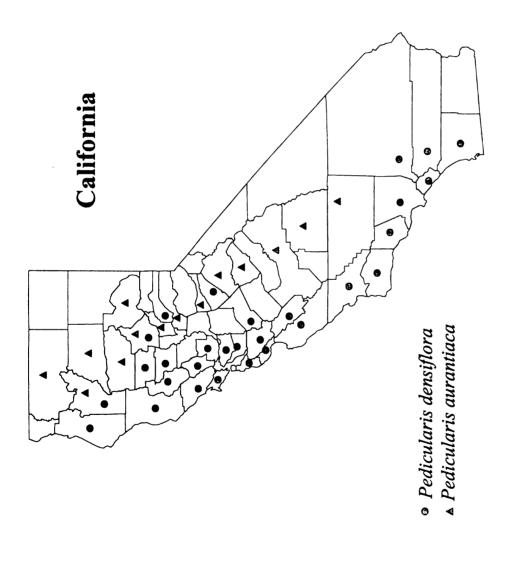


Figure 7.

Figure 8. County map of Oregon. Map shows distribution of *P. densiflora* and *P. aurantiaca* based on morphological analysis of populations in the field and herbarium material cited. Circle represents presence of *P. densiflora* herbarium material in the county. Triangle represents presence of *P. aurantiaca* herbarium material in the county.

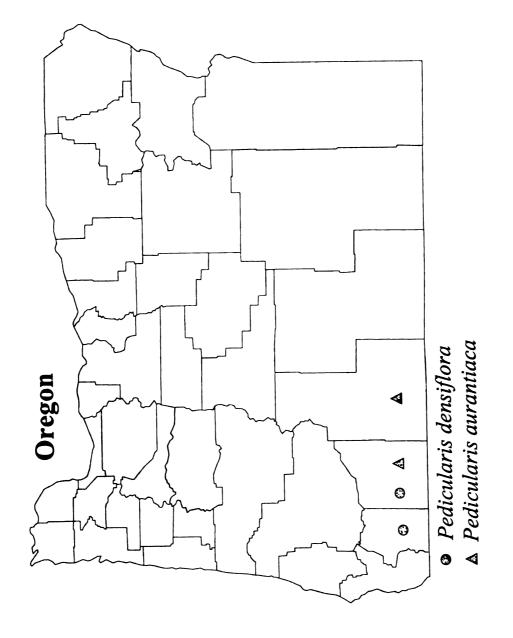


Figure 8.

galea, and a tube that is exserted from the calyx. The species flowered between February and April and grew at elevations ranging from 100-4000 feet. *Pedicularis densiflora* was distributed along the coast from southern California into southern Oregon, with occurrences as far south as Baja, California. Populations occur in the foothills of the Sierra Nevadas in Butte, Calavaras, and Nevada counties. Field observations and a review of herbarium specimens indicate that the distribution of *Pedicularis aurantiaca* is contiguous with that of *P. densiflora* in Jackson County in Oregon and Butte, Calavaras, Colusa, Lake, Nevada and Trinity Counties in California (Figure 7 & 8).

Molecular Variation -

Six of the 16 populations examined in the molecular phylogenetic analysis were representative of *P. aurantiaca*: Auberry, Hobo Gulch. Inskip, McBride Springs, Pinehurst, and Weaver Camp. *Pedicularis densiflora* was represented by ten of the sixteen populations examined: Bear Valley, Briceland Bridge, Hurles Circle, Missouri Flat, Mount Diablo, Paradise, Pinnacles National Monument, Round Valley Historical Marker, San Marcos Pass, and Santa Margarita Lake.

The ITS sequences were 603 bp long, with 74 parsimony-informative characters. There were 16 most parsimonious trees with a length of 114, C.I. excluding uninformative characters (C.I.°) = 0.9032, and R.I. = 0.9543. Bootstrap support, as shown on the strict consensus tree (Figure 9), for a clade including McBride Springs, Inskip, and Pinehurst, as well as two geographically proximate sites not measured in our morphological analysis formed a monophyletic group, albeit with weak bootstrap support (bootstrap value of

Figure 9. Figure represents the strict consensus tree of 16 most parsimonious trees and is based on nuclear ribosomal ITS sequences 603 bp long, with 74 parsimony-informative characters. Phylogeny has a tree length of 114, C.I. = 0.9032, and R.I. = 0.9543. Numbers above the branches represent bootstrap percentages based on 10,000 replicates. Names represent locations of populations of *P. densiflora/P. aurantiaca*. Populations of *P. aurantiaca* denoted with an asterix. Location and voucher information is available in Table 1 and Figure 1.

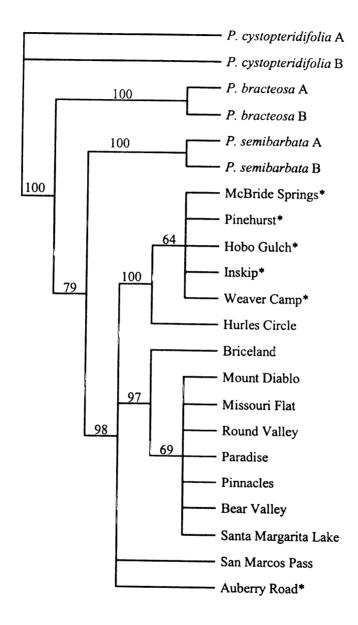


Figure 9.

64%). Inclusion of Hurles circle into the clade McBride Springs – Hurles Circle had 100 % bootstrap support. The clade Briceland- Santa Margarita Lake had strong bootstrap support (97%). San Marcos Pass and Auberry Road populations were part of a polytomy at the base of the *P. densiflora/P. aurantiaca* clade. The phylogeny showed 98% bootstrap support for the monophyly of the *P. densiflora/P. aurantiaca* clade.

Independent phylogenetic analyses were initially conducted on the two chloroplast regions. The aligned sequences of *psb*A-*trn*H were 605 base pairs long with 566 base pairs included in the analysis, 20 of which were phylogenetically informative. Thirty nine base pairs (bp238 through bp276) were excluded from the analysis due to ambiguity in alignment. Parsimony analysis of *psb*A-*trn*H intergenic spacer resulted in 42 most parsimonious trees, with a length of 41, a C.I.^e = 1.00, and a R.I. = 1.00. The aligned sequences of *trn*L-*trn*F were 404 base pairs long with 12 substitutions, nine of which were phylogenetically informative. Parsimony analysis resulted in six most parsimonious trees, with a length of 12, a C.I.^e = 1, and a R.I. = 1. The *trn*L-*trn*F base pair data analysis showed no resolution among the populations of the *P. densiflora* and *P. aurantiaca* species. Five *P. densiflora* populations (Briceland, Mount Diablo, Missouri Flat, Round Valley, and Paradise) and one *P. aurantiaca* population (Inskip) share a 51 bp insertion/deletion event (Indel). This indel was treated as missing data in the parsimony analysis.

Data from the chloroplast region was combined for a total of 970 base pairs with 29 parsimony informative characters (Figure 10). Twenty eight most parsimonious trees were generated, with a length of 53, C.I.^e = 1.00, and R. I. = 1.00. Two clades with low support were resolved. Briceland, Missouri flats, and Round Valley formed a polytomy with 63% bootstrap support. The clade that includes a more inclusive clade of those three populations and Mount Diablo had a bootstrap support of 64%. The *P. densiflora/P. aurantiaca* clade has 94% bootstrap support

I did not reject the null hypothesis of homogeneity in distribution of phylogenetic information between the data sets based on the partition homogeneity test (p=0.59) and the data were combined for an analysis of relationships among populations of P.

densiflora and P. aurantiaca (Figure 11).

The combined data set had 1573 base pairs with 103 parsimony informative characters. Eight most parsimonious trees resulted from the analysis, with a length of 168, C.I. = 0.9194, and R. I. = 0.9600. The clade McBride Springs – Hurles Circle had 100% bootstrap support. The Briceland-Santa Margarita Lake clade formed a monophyletic group with 99% bootstrap support. The San Marcos Pass and Auberry Road populations were part of an unresolved polytomy at the base of the *P. densiflora/P. aurantiaca* clade. The phylogeny had 100% bootstrap support for the monophyly of the *P. densiflora/P. aurantiaca* clade.

Figure 10: Figure represents the strict consensus tree of 28 most parsimonious trees and is based on psbA-trnH and trnL-trnF sequences 970 bp long, with 29 parsimony-informative characters. Phylogeny has a tree length of 53, C.I. = 1.00, and R. I. = 1.00. Numbers above branches are bootstrap percentages based on 10,000 replicates. Names represent locations of populations of P. densiflora/P. aurantiaca. Populations of P. aurantiaca denoted with an asterix. Location and voucher information is available in Table 1 and Figure 1.

11.

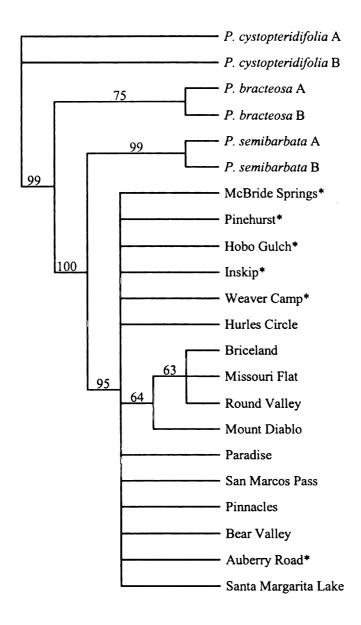


Figure 10.

Figure 11: Figure represents a strict consensus of 8 most parsimonious trees and is based on combined analysis of psbA-trnH and trnL-trnF chloroplast sequences and nuclear ribosomal ITS sequences 1573 bp long, with 103 parsimony-informative characters. Phylogeny has a tree length of 168, C.I. = 0.940, and R. I. = 0.960. Numbers above branches represent bootstrap percentages based on 10,000 replicates. Names represent locations of populations of P. densiflora/P. aurantiaca. Populations of P. aurantiaca denoted with an asterix. Location and voucher information is available in Table 1 and Figure 1.

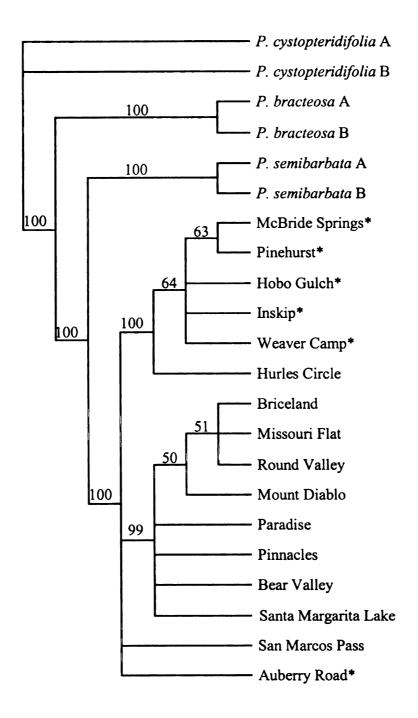


Figure 11.

The chloroplast and ITS primers were used to amplify regions of DNA to assess some inter-specific relationships among closely related *Pedicularis* species. Our combined analysis showed the *P. semibarbata* and *P. bracteosa* clades were monophyletic (100% bootstrap support). *Pedicularis semibarbata* was sister to the *P. densiflora/P.* aurantiaca clade (100%) and *P. bracteosa* was sister to the *P. densiflora/P. semibarbata* clade with 100% bootstrap support.

CONCLUSION

Morphological Variation -

Quantitative traits measured on ten populations, field observations, and an examination of herbarium specimens showed considerable variation in floral morphological measurements among the populations studied. These results indicated two points: the *P. densiflora/P. aurantiaca* group had considerable floral morphological variation among populations, and some of this variation could be attributable to distinct groups, which herein are treated as two species, *P. densiflora* and *P. aurantiaca*.

Molecular Variation -

Pedicularis densiflora and P. aurantiaca are closely related sister taxa. The phylogenetic analysis indicated the McBride Springs – Weaver Camp clade of P. aurantiaca populations was closely related, genetically distinct and derived from within P. densiflora/P. aurantiaca clade. However the Hurles Circle (P. densiflora) population had a shared ancestry with this clade and the P. aurantiaca population of Auberry road and a P. densiflora population of San Marcos Pass were part of a polytomy at the base of the P. densiflora/P aurantiaca clade. In addition, in the trnL-trnF spacer region, Inskip (P. aurantiaca) shared a 51 bp indel with Briceland, Mount Diablo, Missouri Flat, Round Valley, and Paradise (P. densiflora) and this provides some evidence for a shared chloroplast ancestry among these six populations. Therefore no conclusions regarding the monophyly or ancestry of P. aurantiaca could be made. More informative markers may provide additional resolution for discerning the relationships among the species.

Sequence data from the sampled species indicated a common ancestor between *P*. *densiflora*, *P*. *aurantiaca* and *P*. *semibarbata*. This relationship was proposed by Li (cited as a personal communication by Sprague) and Sprague (1962). Robart's (2000) ITS sequence phylogeny included the narrow California endemic *P*. *howellii* A. Gray. This taxon was sister to *P*. *semibarbata*, in a clade sister to the clade of *P*. *densiflora* and *P*. *aurantiaca*. This result was not surprising, *P*. *densiflora*, *P*. *aurantiaca*, *P*. *semibarbata* and *P*. *howellii* have similar floral morphologies, inhabit similar habitats, are geographically proximate, and parasitize similar woody host plants.

Ancestral Pollination State-

Many studies of reproductive biology of *Pedicularis* in the United States were conducted by Macior (1973, 1977, 1982, 1983, 1984, 1986a, 1986b, 1995a, 1995b, 1996, 1997 and references therein) who noted the close association between *Pedicularis* and *Bombus* pollinators. With some rare exceptions noted in this study, there appeared to be a "virtually exclusive *Bombus/Pedicularis* reproductive association" in the genus (Macior 1997; 1). *Pedicularis cystopteridifolia* (Macior 1983), *P. howellii* (Macior 1986b), and *P. bracteosa* (Macior 1973, Robart 2001) are *Bombus* pollinated. *Pedicularis semibarbata* is pollinated exclusively by small bees, *Osmia tristella* (Macior 1977). *Pedicularis densiflora* is pollinated by both *Bombus* and hummingbird pollinators and *P. aurantiaca* is pollinated exclusively by hummingbirds (based on overlapping populations studied by Macior 1986a, and Sprague 1960, 1962).

Long corolla tubes, abundant nectar, lack of floral scent, and red coloration are floral traits present in both *P. densiflora* and *P. aurantiaca* which have an association with hummingbird pollinators (Sprague 1960, Grant & Grant 1968, Faegri & van der Pijl 1966). In *P. densiflora* young flowers are visited by *Bombus* pollinators before the floral tubes are fully exserted and while the nectar is accessible to the short-tongued pollinators (Macior 1986). The lower labium of *P. densiflora* is larger and capable of serving as a landing platform for *Bombus* pollinators. In *P. aurantiaca* the calyx is larger and supports the corolla tube. This trait can serve to protect the flower during the nectar probing of hummingbird visitation (Faegri & van der Pijl 1966). The labium is also significantly smaller and the abaxial margin larger in the *P. aurantiaca* vs. *P. densiflora* flowers. The labium reduction eliminates the *Bombus* landing platform and the enlarged abaxial margin can accommodate the larger head of the hummingbird pollinators.

Detailed pollination ecology studies (Macior 1973, 1977, 1983, 1986 a and b, Robart 2001, and Sprague 1960, 1962).were coupled with phylogenetic sequence data from the chloroplast and nuclear genome and the ITS sequence phylogeny of Robart (2000). This work supported the hypothesis of a major pollination shift in *P. semibarbata* with a transition from *Bombus* to small bee pollination. In addition, there are two potential evolutionary scenarios for pollinator shifts within *P. densiflora* and *P. aurantiaca*: A. The ancestral state of *Bombus* pollination in the outgroup of *P. cystopteridifolia* and *P. bracteosa*, with a partial switch to a shared pollination biology of *Bombus* and hummingbird pollinators in *P. densiflora* and a complete transition to exclusive hummingbird pollination in *P. aurantiaca*, or B. The ancestral pollination state of

Bombus pollination in the outgroup of P. cystopteridifolia and P. bracteosa, with a complete loss of Bombus pollination and a switch to hummingbird pollination in the P. aurantiaca/P. densiflora clade, and a second shift to shared pollination biology of regained Bombus pollination in addition to hummingbird pollination in P. densiflora.

Due to the partially unresolved phylogeny among populations of P. densiflora and P. aurantiaca and the difficulty of mapping polymorphic pollination states, it is not possible to distinguish between the two evolutionary scenarios described.

Taxonomic treatment -

Review of herbarium specimens, statistical analysis including ANOVA, PCA, and UPGMA, show a clear distinction between the species *P. aurantiaca* and *P. densiflora*. *Pedicularis aurantiaca* has a large calyx, floral tubes not fully exerted at anthesis, reduced lower labium and a large opening in the galea. *Pedicularis densiflora* has a short calyx with a fully exerted floral tube, an enlarged lower labium and a smaller opening in the galea. Flowering times and locality are different between the species. *Pedicularis aurantiaca* flowers later in the season, at higher elevations and grows in southern Oregon, and northern California into the Sierra Nevadas. *Pedicularis densiflora* flowers early in the spring at lower elevations, and grows primarily along the coastal mountain ranges of California. A comprehensive review of over 1000 herbarium specimens confirms the morphological distinctions and diagnosability of the two species. Previous work by Sprague (1958, 1960, and 1962) and Macior (1986a) on variation among pollinator regimes indicated exclusive pollination by hummingbirds in *P. aurantiaca* and a shared pollination by *Bombus* and hummingbirds in *P. densiflora*.

Pedicularis aurantiaca is morphologically distinct, has a later growing season, grows at higher elevations, and has a different suite of pollinators than P. densiflora. Pedicularis aurantiaca is prezygotically isolated from P. densiflora. Support for the elevation of P. aurantiaca to species status is unambiguous. The appropriate combinations for the species are below.

Pedicularis aurantiaca (E. F. Sprague) Monfils & Prather, comb. et stat. nov. Pedicularis densiflora subsp. aurantiaca E. F. Sprague, Aliso, 4:130. 1962. Type: USA: from a burn in manzanita brush, Yellow Pine Forest, between the campground and the forest, Lake Almanor, Plumas County, California, at 4300 feet, 30 May 1957, E. F. Sprague 1202 (holotype: RSA; Isotype RSA!)

Description: Perennial herb with woody root. Leaves petiolate, in basal rosette, oblanceolate, bipinnate to pinnate. Inflorescence a raceme, capitate with few open flowers. Bracts of the inflorescence oblanceolate and partially laciniate. Calyx tube 12-24 mm long, hirsutulous, 5 equal, acuminate to acute, erose lobes. Corolla galeate, 23-43 mm, deep red to purple to orange-yellow, galea 14-25.5 mm long, 4.5-8.5 mm deep, abaxial margin 13-23 mm long, lower labium petals 3.5-7.5 mm long from bend in corolla to petal tips. Corolla tube not fully exserted at anthesis. Fruit a capsule.

1000-7000 feet. Flowers April through June. Pine forests.

Note: Plants in diffuse patches, haustoria preferentially parasitize woody plants

Additional material seen: UNITED STATES. Oregon: Jackson County: 1-1.5 miles past Pinehurst Inn. N 42° 07.452', W 122° 20.783', 1167 m, 24 May 1998, Monfils 12 (MSC): 1-1.5 miles east of Pinehurst Inn. N 42° 07.461', W 122° 20.756', 1131 m. 11 May 2000, Monfils 40 (MSC); Pinehurst, 20 Jun 1927, Peck 15044 (DS, WILLU); Pinehurst. 27 May 1948, Peck 24973 (WILLU, UC); Near Pinehurst, 1219 m, 13 Apr 1934, Thompson 10347 (CAS, DS, POM, WILLU). Klamath County: Cascade Mnts. Near Long Prairie, along old Klamath Falls-Ashland road, 15 Jun 1895, Applegate 421a (DS); Southeast of Topsy, 1372 m, 12 May 1898, Applegate 2059 (DS); Top of Spencer Mt., Cascade Mts., 11 May 1924, Applegate 4046 (DS, WILLU); Eastern Cascades, 30 Jun 1902, Cusick 2851 (DS, ORE, POM); Klamath Falls Road "East of Mt.", 2 Aug 1925, Henderson s.n. (ORE); Along Ashland-Klamath Falls road, 18 miles W of Keno, May 1932, Peck 9302 (DS, WILLU); Mts. Below Klamath Falls near Ore-Calif line, on Klamath river, May 1932, Sprague s.n. (OSC). County Unknown: Southern Oregon, 13 May 1907, Henderson s.n. (ORE); Location and date unknown, Bellinger s.n., (WILLU). California: Amador County: Jackson, 12 Apr 1933, Ball 18269, (RSA). Butte County, Colby, May 1890, Austin 111 (UC); Jonesville, 16 Jun 1923, Bassett s.n. (CAS); Paradise, May 1898, Bruce 2419 (DS, POM); Brush Creek, 1907, Conger 453 (CAS, POM); Chico Meadows in the Sierra Nevada, 1219 m, 11 Jun 1915, Heller s. n. (DS); Stirling, 1073 m, 18 May 1919, Heller 13170 (CAS, DS); Pentz Road, 3 miles below Paradise, 23 Mar 1939, Heller 15358 (DS); DeSabla, 853 m, 18 Apr 1978, Howell 52852 (CAS); Above the road between Paradise and Butte Meadows .1 mi. s. of Inskip. T25N, R.4E, Sec. 33, 10 May 1981, McNeal 2487 (OSC); Inskip on Skyway Road. Elevation N

39° 59.541', W 121° 32.389', 1309 m, 22 May 1998, Monfils 6 (MSC); Inskip on Skyway Road. N 39° 59.527', W 121° 32.461', 1309 m, 9 May 2000, *Monfils 37* (MSC); Durham, 17 Apr 1932, Morrison s. n. (CAS). Fresno County: 5 miles east of Auberry, Big Sandy Bluff, 945 m. 19 Mar 1969, Ahner s. n. (CAS); Big Sandy Valley near the foot of ne. slope of Black Mnt. Big Sandy Bluff beyond Ridge View Ranch which is across the road from my place, 610 m, 20 Mar 1953, Carter 157 (CAS, UC); About 2 miles north of Kerckhoff Resevoir, 610 m, 29 May 1967, McClintock, Roderick & Johnson s. n. (CAS); Old fire trail road off Auberry Road. N 37° 05.144', W 119° 26.859', 1147 m, 20 May 1998, Monfils 4 (MSC); R. R. Grade Rd. 1+ m. w. of jct. Hiway 168 at Shaver Xing between Shaver L. & Big Crk. 1st live crk. – small intake with pipe heading down crk. – Big Creek pentstocks in view. Heavy yellow pine for. 4500'- 1000' up s. wall Big Crk. Canyon; 6830' Music Mt. Just sw.; 8107' Black Pt. N. across canyon, San Joaquin R. at 2000' 4 map miles w., 17 Jun 1955, Quibell 5102 (RSA); 5 miles east of Auberry, Big Sandy Bluff range, 945 m, 19 Mar 1969, Shannon s. n. (RSA); Scattered along Old Railroad Grade Road. R.23E., T9S., SW1/4 section 34, 853 m, 22 Mar 1980, Shevlock 6819 (CAS, MSC); Taken near Fish Camp, 14 Apr 1938, Whilton s. n. (RSA); Along road from Shaver Lake to Big Creek Power House. W. slope of Sierra Nevada, 1829 m, 5 May 1929, Wolf 3682 (RSA); 6 mi. above Auberry on road to Pineridge. W. slope of Sierra Nevada, 18 May 1933, Wolf 4786 (CAS, RSA, UC). Kern County: Poso Creek narrows, below Poso Flat, 762 m, 9 Mar 1963, Record 82-1 (CAS-2); Near mouth of South Fork Kern River Canyon, trail along Bartolas Creek, Domeland Wilderness, north of California highway 178 and NW of the Bloomfield Ranch. R35E, T25S, section 23. Lat/Long: 35 degrees 44'45" N, 118 degrees 11'15" W., 1006 m, 20 Apr 1991, Shevock

12026 (CAS); Back canyon at the cypress grove, 1265 m, 26 May 1964, Twisselmann 950 (CAS-2). Mariposa County; Mariposa, 610 m, 10 Apr 1959, Ballantyne 236 (CAS); Awahnee, 9-16 Apr 1926, Shank 17471, (RSA). Placer County: Tahoe Forest, Rebel ridge Range, 701 m, 12 Apr 1926, Smith 53916 (CAS-2). Plumas County: Sw. shore Lake Almanor, 2 mi. s. of jct. of State Hwys. 89/36, 1402 m, 23 May 1957, Balls 22519 (RSA, UC); 1 3/10 s. of Drakesbad, 3 Jul 1938, Cantelow 2323 (CAS); Prattville, sSummer 1906, Coombs s. n. (CAS); Woodleaf, 3000-4000', 14 Apr 1931, Rose s. n. (CAS, DS, POM, UC); Lake Almanor, 4300', 30 May 1957, Sprague 1214 (RSA); Growing in dry loam in shade of *Libodedrus decurrens* A low cool draw 6 miles southwest of Viola, 2800', 30 May 1957, Sprague 1231 (RSA). Shasta County: Montgomery Creek, 18 Apr 1923, Beltiel s. n. (CAS); Near McBride Springs. On banks and under chaparral, 24 Jun 1938, Cooke 11098, (DS, OSC); Squaw Creek Ranger Station, Jun 1916, Drew s. n. (DS); Mt. Shasta, 21 Jun 1893, Dudley s. n. (DS); Burney Butte, 12 Jul 1912, Eastwood 1034 (CAS); Shasta Springs, 20 May 1923, Eastwood 11854 (CAS-2); Shasta Springs, 15 May 1918, Herrin s. n. (CAS); Shasta Springs, May 1922, Herrin s. n. (CAS); Open manzanita-oak association3 miles east of Redding, 500', 17 May 1940, Hitchcock 6466 (DS, POM); Highway 44, 5 miles west of Lassen National Park, 4600', 9 May 1974, Keller 1301 (CAS); Mt. Shasta, McBride Springs. N 41° 20.687', W 122° 16.506', 4922', 11 May 2000, *Monfils 43* (MSC). Siskiyou County, Bare serpentine gravel hillside, Scott Mnt. 8 miles above Callahan, 5150', 17 May 1954, Barneby 11537 (CAS); Near Mt. Shasta, 6000-7000', 1-15 Jun 1897, Brown 356 (DS); Alpina Mnts. Goosenest foothills, 5000', 10 May 1910, Butler 1324 (DS, POM, UC); N. slope of Scott Mnt.,4000', 20 May 1936, Cantelow 1435 (CAS); Scott Mountain campground, 10 miles

S. of Callahan, 5350', 21 May 1949, Constance, Bonar, Holm & Wood 3288 (UC); 0.8 miles N of Scott Mnt. Summit on Hwy 3. South Slope, 5200', 5 Jun 1975, Davidson 2684, (RSA); Bald mountain in road from Mt. Hebron to Montague, 5500', 15 May 1940. Gould 1242 (DS); Mi. Eddy in open gravelly places in the forest, 6800', 15 Jul 1915, Heller 12085 (CAS, DS, UC); Sugar Creek, Salomon Mountain Range, 5500', 26 May 1949, Parker 257 (UC); Weed, 10 Apr 1913, Smith 66 (CAS); Dunsmuir, 29 Apr 1913, Smith 151 (CAS); Shady hillside near Weed, 28 Mar 1930, Tebbe 61 (UC); Salmon Mnts., Klamath National Forest. Near South Fork of Salmon River, 5 to 9 mile southeast of Cecilville, between Lat. 41°03' and 41°06'N., and Long 122°58' and 123°03'W. Vicinity of Blind Horse Creek, 3000-3500', 13 May 1954, Thomas 4129 (DS-2); Salmon-Trinity Mnts. about 6 miles SE of Cecilville. West side of Rush Creek, 3800-4000', 21 Jul 1954, Thomas & Thomas 4425 (DS). Tehama County: Along road from Viola to Mineral, 9.5 miles south of Viola, 5000', 12 Jun 1962, Breedlove 3423 (CAS, DS); Deer Creek Rd. (Rt. 32) at milepost 12, E side of Deer Creek ca 20 airmiles SW of Chester, 3200', 3 May 1989, Ertter 8435 (RSA); Mill Crk. Meadows, 6 Jun 1951, Quick 51-93 (CAS); Northern Sierra Nevada. Red Bluff – Susanville Road. 3 6/10 mi. below Mineral, 4800', 17 May 1937, Wolf 8712 (RSA). Trinity County: T35N, R11W, Sec. 6. North fork of Trinity River, Hobo Gulch Camp and Vicinity. (18 miles NW of Weaverville) Along East Fork Trail, just over on the east side of Backbone Ridge, 4000', 25 Apr 1972, Carter 374 (CAS-2); T36N, R11W, Sec. 31. North fork of Trinity River, Hobo Gulch Camp and Vicinity. (18 miles NW of Weaverville) Along trail 0.1 miles north of Hobo Gulch. (Same location as 399-27 Apr. 72), 3100', 8 Jun 1972, Carter 399.01, (CAS-2); Scott Mnts. N. of Carrville, 25 Jun 1937, Eastwood & Howell 5013 (CAS); 3 miles from Douglas City on Redding Road, 26 Apr 1954, Howell 29164 (CAS); Dry open coniferous forest in Canadian Zone, Scott Mt. Summit on road from Carrville to Callahan, 5400', 20 May 1980, Howell, Fuller & Barbe 53541 (CAS); Weaverville, Spring 1915, Junkans s. n. (CAS); Trinity Center, 30 Apr 1928, Kildale 4605 (DS); Weaverville, 30 May 1931, Kildale 10812 (DS); Foothills, Weaverville, 4000', 11 Apr 1880, Kleiberger s. n. (CAS); East Weaver Campground on East Weaver Creek Road. N 40° 46.399', W 122° 55.371'. 4248', 23 May 1998, Monfils 8 (MSC); Hobo Gulch. N 40° 55.723', W 123° 09.398', 4249', 23 May 1998, Monfils 9 (MSC); East Weaver Public Camp, East Weaver Creek, 3000', 16 May 1949, Munz 13256 (RSA); T32N, R9W, Secs. 28 & 33; Mt. Diablo Meridian; Southeast of confluence of Panwauket Gulch and Reading Creek; blue oakpine woodland, 610 m, 18 May 1975, Sullivan 88 (RSA); Little East Weaver Creek, 3000', 21 May 1914, Yates 19370 (CAS). Tulare County: Mineral King, 2000', 11 Apr 1958, Pawek 418 (DS); Occasional in recently disturbed road bank along Blue ridge, section 10, R.29E., T. 19S. South facing slope, 4900', 12 May 1979, Shevock 6186 (CAS); Uncommon along Calif. Hwy 245, about one mile west from the junction with Dry Creek Road. R.27E., T.15S. section 15, 2800', 11 Mar 1980, Shevock 6775 (CAS); Lone Pine Spring, White River, 3350', 8 Mar 1940, Smith 51, (CAS). Tuolumne County: Priest Grade, near Big Oak Flat, Yosemite National Park, 20 Feb 1982, Botti 1489 (CAS). Groveland. Woodland edge of route 120 about 4 miles east of town, 23 Apr 1974, Churchill 744231 (MSC). Yuba County: Between Dobbins and Bullard Bar Dam, Watershed of North Fork of the Yuba River, 3.1 mi. e. of Dobbins (or 5.5 miles s.w. of Bullard Bar Dam), 2570', 19 Apr 1956, Bacigalupi, Robbins & Chisaki 5622 (RSA);

Comptonville, 7 Apr 1918, Eastwood 6795 (CAS). County Unknown: Sterling City, 18 May 1935, Whitaker s. n. (OSC).

Pedicularis densiflora Benth. ex. Hook. Fl. Bor. Am. ii. 110. 1838. Type unknown.

Description: Perennial herb with woody root. Leaves petiolate in basal rosette, oblanceolate, bipinnate to pinnate. Inflorescence a raceme, elongated with many open flowers. Bracts of the inflorescence oblanceolate with serrate margins. Calyx tube 10-18 mm long, hirsutulous, 5 equal, acuminate to acute, erose lobes. Corolla galeate, 23-43 mm, deep red to purple to orange-yellow, sometimes white, galea 15-25 mm long, 4-7 mm deep, abaxial margin 9.5-20 mm long, lower labium petals 8-16 mm long from bend in corolla to petal tips. Corolla tube exserted at anthesis. Fruit a capsule.

100-4000 feet. Flowers February through April. Chaparral to pine forest.

Note: Plants in diffuse patches, haustoria preferentially parasitize woody plants,

Additional material seen: UNITED STATES. Oregon: Jackson County: Thompson Creek, near Applegate, steep hill slope. Sec. 21 T.38S R.4W, 1400', 19 Mar 1940, Detling 3864 (ORE); Fls. Deep purple-red open pine woods on alluvium, along Applegate River, 11/2 mi. s.e. of Provolt, 28 Apr 1948, Glowenke 11113 (UC); 5 miles S of Applegate, under madronas, 1 Jun 1951, Hitchcock 19395 (RSA); Applegate Creek, Mar 1921, Leiberg s. n. (ORE); Applegate R., Jacksonville, 27 Mar 1936, Lund s. n.

(OSC); Pilot Rock, 20 Apr 1932, Neiman s. n. (WILLU); Woods along Thompson Cr. 5 mi. S. of Applegate P.O., 26 Jun 1931, Peck 16423 (WILLU-2); Along the Applegate River, 12 Apr 1927, Thompson 2225 (DS,ORE). Josephine County: 4 mi. N.W. of Provost, 25 Apr 1943, Bellinger s. n. (WILLU); 3 miles south of Grants Pass on New Hope Road, 9 May 1963, Curtis s. n. (OSC); Applegate Valley T37S R5W sec. 34, 21 Apr 1942, Detling 5138 (ORE); In open woods, Fruitvale, 21 Apr 1930, Henderson 12513 (ORE); Roadside in mixed Oak-Pseudotsuga woods; S. facing; on old river bench. 2 mi E of Murphy R5W; T37S; SE1/4 OF NW1/4, 345 m, 20 Apr 1968, Lillico 426 (ORE); Missouri Flat Road. N 42° 19.288', W 123° 13.871', 3900', 24 May 1998, Monfils 13 (MSC); Missouri Flat Road. N 42° 19.288', W 123° 13.871', 3900', 10 Apr 1999, Monfils 36 (MSC); Applegate River, North Bank Road c. I mi. from Redwood Highway; S. of Grants Pass, 1300', 23 Apr 1967, Pike 111 (ORE); Hillside near Grants Pass, 19 Mar 1918, Prescott s. n. (WILLU); Near Williams Creek highway between Provolt and Williams; also on N. side of Applegate River on Missouri Flat. Clayey soil in woods, 13 Apr 1924, Savage s. n. (ORE); Fruit Dale, Murphy Road. In open woods, 24 Mar 1926, Savage s. n. (ORE); Mixed woods near Takilma, 21 Apr 1930, White s. n. (ORE). California: Alameda County: Laundry Harm, 29 Jan 1895, Cannon s.n. (CAS); Oakland Hills, 28 Feb 1936, Covel 371 (CAS); Laundry Harm, 26 Apr 1891, Eastwood s.n. (ORE); Mines Road s. w. of Livermore, near entrance to Rancho Los Mochos Boy Scout Camp, 1500', 3 Mar 1968, Gagné s.n. (CAS). Butte County: Near Hurleton, 1800', 26 Mar 1980, Ahart 2060 (CAS); Cherokee mine, 29 Mar 1919, Heller 13097 (CAS, DS, POM); Hurles Circle, in center island. N 39° 29.764', W 121° 22.632', 2050', 22 May 1998, Monfils 5 (MSC); Hurles Circle, in center island. N 39° 29.764', W 121° 22.632',

2050', 7 Apr 1999, Monfils 35 (MSC). Calavaras County: Comanche, 16 Apr 1939, Hoover 4032 (CAS). Colusa County: Wilbur Springs. T15N, R6W, sec. 35, 3500', 14 Apr 1979, Roth 9 (RSA); Along Brim Rd., 2.9 mi. W jct. Bear Valley Rd., 30 Mar 1996, Vincent & Rhode 7304 (RSA), Contra Costa County: Martinez, 300', 18 Mar 1931, Benson 2662 (POM); Mount Diablo, 17 Mar 1922, Eastwood 11084 (CAS); Rocky Point, Mount Diablo. N 37° 51.814, W 121° 55.770', 2550', 22 Mar 1999, Monfils 28 (MSC); Rocky Point, Mount Diablo, 2000', 5 Apr 1956, Sprague 1096 (RSA); Mount Diablo, 2000', 11 Apr 1957, Sprague 1124 (RSA); Rocky Point, Mount Diablo, 2000', 30 May 1957, Sprague 1134 (RSA). Glenn County: 2 miles north of Alder Springs, 3900', 19 May 1949, Munz 13330 (RSA). Humbolt County: T7N, R5E, Sec. 29. Along highway 96, 1.9 miles north of Willow Creek, 1 May 1965, Anderson 3556 (RSA); In damp fir woods near creek, Boise Creek and Willow Creek, Trinity River, 1 Apr 1947, Brown 25 (DS); Philabiumsville, south fork of El River, 20 Mar 1927, Kildale 2909 (DS); Briceland Bridge near Garberville, 375', 17 Apr 1925, Kildale 3096 (DS, RSA); Briceland Road. N 40° 05.678', W 123° 51.222', 1874', 25 May 1998, Monfils 14 (MSC); Round Valley Historical Marker. N 39° 43.749', W 123° 15.112', 2160', 25 May 1998, Monfils 16 (MSC); Kneeland Prarie, in woods in ravine, 2500', 8 Jun 1908, Tracy 2637 (UC); Vicinity of Garberville, 400', 17 Mar 1923, Tracy 6160 (UC); Trinity River Valley, near the south fork, 600', 28 Feb 1926, Tracy 7369 (UC); Hoopa Mnt., near summit on road west from Hoopa to Bair's, 3500', 15 May 1927, Tracy 8060 (UC); Willow Creek Canyon, 2000', 26 Apr 1931, Tracy 9340 (UC); Trinity River Valley, at Willow Creek, 500', 3 Apr 1937, Tracy 15251 (UC); Trinity River Valley, at Willow Creek, 500', 26 Mar 1941, Tracy 16818 (UC); Harris, in woods, 2500', 31 May 1948,

Tracy 18006 (UC). Lake County: Lakeport, 20 Apr 1917, Bentley s. n. (DS); Mt. Kelseyville, Middle N. Coast, Near Cold Creek Canyon River, 1700', 30 Mar 1928, Benson 87 (POM); Bogg's Lake, Mt. Hanna, Middle N. Coast, Clear Lake watershed, 3500', 18 May 1935, Benson 6636 (POM); Hannah, 3000', 8 Apr 1923, Blankinship s. n. (CAS); Mt. Konocti, 2000', 27 Mar 1926, Blankinship s.n. (RSA); Sulphur Banks, Apr 1902, Bowman s. n. (DS); E. of Middleton, 7 Apr 1940, Cantelow 4346 (CAS); 4 mi. below Tollhouse on Middleton Road, 22 Feb 1924, Duncan s. n. (DS); Dasheills Mt., Sanhedrin, 23 May 1925, *Eastwood 12912* (CAS); Elk Mt., 17 May 1938, *Eastwood &*. Howell 5703 (CAS); Clear Lake, 14 Apr 1928, Galloway s.n. (CAS), Glenbrook, near Jordan Park, 30 Mar 1931, Jussel 29 (CAS); Cobb Mt., 30 Mar 1931, Jussel s. n. (CAS); Cobb Mt., 31 Mar 1931, Jussel s. n. (CAS); Mt. St. Helena, 1 Apr 1933, La Motte s. n.(POM); Cobb Mt., 4 Jul 1893, Leitholt s. n. (DS); Bear Valley on Brim Road. N 39° 09.504', W 122° 28.778', 2665', 26 May 1998, Monfils 17 (MSC); Bear Valley on Brim Road. N 39° 09.431', W 122° 28.798', 2180', 26 May 1998, Monfils 33 (MSC); Butt's Cyn. Rd. near Middleton, 1000', 25 Mar 1972, Shevock 1437 (RSA); Northeast facing slope, 0.4 mile southeast of Black Oak Villa in Butts Canyon, 1.1 miles from Lake-Napa county line on Pope Valley road, 800', 7 Mar 1953, Sweeney 971 (UC); Chaparral 11 mi. S. of Lower Lake, 13 Mar 1932, Wiggins 5769 (DS); 21/2 miles S. W. of Lakeport. Sec. 34, T.14N, R.10W, 1850', 14 May 1937, Wilson 376 (UC). Los Angeles County: Mts. above Claremont, Johnson Pasture, 15 Feb, Bragg s. n. (POM); North slope Sta. Monica Hills, Feb 1903, Braunton 809 (DS, ORE-2); Topanga Canyon, 28 Mar 1929, Clare s.n. (RSA-2); Laurel Canyon, 24 Feb 1929, Detruers s.n. (RSA); Mulholland Drive, 6 Mar 1935, MacFadden 13246 (CAS); Laurel Canon, Mar 1943, Merritt s.n. (RSA); Franklyn

Canyon, Santa Monica Mts., 21 Feb, *Peirson 1168* (RSA); Glendora, Little Dalton Trail, 16 Feb 1916, Perkins s.n. (RSA); 0.7 mile from Triunfo Canyon, 2 miles west of Cornell, Lobo Canyon, 800', 31 Mar 1959, Thompson 1010 (CAS, RSA); Hwy, 23 south of Lake Elanor, Santa Monica Mtns., 1000', no date, Wallace & Wilkin 150 (RSA), Marin County: East side of the Tiburon Peninsula just below the summit, 31 Mar 1981, Best s. n. (CAS); On trail 0.5 mi. above Phoenix Lake near jct. of Mt. Tamalpais trail, 1200', 5 Apr 1956, Campbell 8 (RSA); Mount Tamalpais, 22 Feb 1901, Chandler 760 (POM, UC); Old hardpacked fire road, 100', 9 Feb 1975, Edelbrock 4, (CAS); Summit Alpine Lake Trail above Deer Park, 8 Mar 1936, Ewan 9408 (RSA); Summit Alpine Lake trail above Deer Park, 8 Mar 1936, Ewan 9409 (UC); Mt. Tamalpais, 22 Apr 1930, Forest s. n. (RSA); Tamalpais T1N R6W, 600', 28 Mar 1935, French 619 (UC); South side of Mt. Tamalpais, 7 Mar 1902, Heller & Brown 5008 (DS, MSC, POM); Tiburon, 15 Feb 1938, Hoover 2739 (UC); San Geronimo Ridge, 25 Feb 1940, Howell 15388 (CAS); Carson Ridge, 19 Apr 1942, Howell 16949A (CAS-2); San Arseloro Canyon, 25 Feb 1940. Howell s. n. (CAS); Above Blythedale Canyon, east side of Mt. Tamalpais, 2 Mar 1947 Howell s. n. (CAS); Mill Valley, 29 Mar 1891, Jepson s.n. (UC); Alpine Dam Road, about 1 mile from Alpine Gulch on Fairfax side, 1040', 10 Mar 1968, Kawahara 29 (CAS); Marin County, 1868-1869, Kellogg & Harford 713 (CAS); Ross Valley, Apr 1892, Michener & Bioletti s. n. (MSC); Tamalpais, Apr 1892, Michener & Bioletti s. n. (MSC); Fire road, south side of Mount Tamalpais, 12 Apr 1969, Mitchell 4 (OSC); Paradise Beach Park, Tiburin Uplands Nature Preserve. N 37° 53.329', W 122° 26.954', 109', 26 May 1998, Monfils 18 (MSC); Paradise Beach Park, Tiburin Uplands Nature Preserve. N 37° 53.309', W 122° 27.055', 137', 3 Apr 1999, Monfils 34 (MSC); Mt.

Tamalpais, 3 Mar. 1930, Morris s. n. (RSA); Roky and clayey bank by Paradise Dr., 0.7 mi. N. Paradise Beach Park, Tiburon Peninsula, 10 Apr 1975, Norris 2305 (RSA); Tiburon Peninsula, 200', 15 Mar 1930, Parks 402 (POM, UC); Tiburon, Spring 1926, Parks (UC); Alpine Dam Road above Fairfax, 800', 9 Mar 1963, Sharsmith 5194 (UC); Corte Madera, 1 Mar 1903, Sheldon 11563 (ORE); Phoenix Lake, Ross, 1000', 4 Apr 1956, Sprague 1096 (RSA); Carson Ridge, 4 Apr 1957, Sprague 1120 (RSA); Fairfax, Mar 1928, Suttliffe s. n. (RSA); North slope of hill near Forest Knolls, 21 Mar 1936, True s. n. (RSA); Phoenix Lake, Ross, 1000', no date, no collector (RSA); Mt. Tamalpais, Apr 1898, no collector (CAS). Mendocino County: Abt. 12 mi. e. of U. S. 101, along Calif. 20. In shade above road, 6 Apr 1954, Clarkson 300 (OSC); Red Mnt., n. Mendocino Co., 21 Jun 1937, Eastwood & Howell 4663 (CAS); Near Woodville, May 1889, Howell s. n. (UC); Ukiah, 25 Apr 1924, Jones s. n. (DS-2, POM); Kaiser District, Mar 1903, McMurphy 306 (DS); 8 miles north of Ukiah, 6 Apr 1938, Meyer 1384 (UC); Round Valley Historical Marker. N 39° 43.749', W 123° 15.112', 2160', 25 May 1998, Monfils 16 (MSC); Round Valley Historical Marker. N 39° 43.760', W 123° 15.093', 1966', 29 Mar 1999, Monfils 32 (MSC); Mad River, 6 Jul 1890, Price s. n. (UC); Potter Valley, Apr 1894, Purpus 1009 (UC); Shady Hills, n. Potter Vall., Mar 1894, Purpus 3089 (UC); Along rte. 128 at MP24.26, 400', 5 Mar 1979, Smith & Wheeler 5128 (CAS); Red Flat, ¼ mi. from wooden gate btwn, BLM & Coombs property. Red Mnt. N., 2350', 6 Jul 1981, Smith et al. 6855 (CAS); Summit area of Red Mountain North, 7 Jul 1981, Smith et al. 6868 (CAS); Seven miles north of Laytonville near highway 101, 20 Apr 1968, Thomas 14331 (DS); Rte. 253. South of Robinson Creek Rd., Ukiah, 650', 11 Mar 1978, Wheeler 60 (CAS); South of Leggett on the Old Redwood Hwy, 1000', 1

Feb 1980, Wheeler 1298 (CAS); Round Valley Historical Marker, overlooking Covelo on Hwy. 162, 2000', 12 Apr 1979, Wheeler & Smith 905 (CAS); Little River - Albion Road. Near Little River Airport, 600', 23 May 1979, Wheeler & Smith 1026 (CAS); On Northwesterly slope, 4 miles east of Laytonville, along road to Dos Rios, 20 Mar 1948, Wiggins 11587 (DS, RSA). Monterey County: Gravel Pitt hill, 27 Jun 1905, Dudley s. n. (DS); Monterey, 1874, Abbott s. n. (CAS-2); Carmel Highlands, Peter Pan Rd., 200', 21 Feb 1948, Balls 7834 (RSA); Monterey, 29 Mar 1933, Detling 1108 (ORE); Pine Cañon, 1500', 27 Mar 1920, Duncan 86 (DS); Near Cypress Pt., 28 May 1912, Eastwood 94a (CAS); Pacific Grove, 8 Mar 1923, Eastwood 2471 (CAS); Monterey, 9 Mar 1913, Eastwood 2489 (CAS); Pacific Grove, Apr 1902, Elmer 3543 (DS-2, POM, UC); Two miles south of Jolon on hilltop, 10 Mar 1952, Evans s. n. (CAS); Sand Stone Cliffs near north fork of San Antonio River, 1500', 27 Mar 1920, Ferris 1810 (DS); Landels-Hill Big Creek Reserve, Gamboa Point Section, Santa Lucia Mountains, sect.4, 2 Apr 1982, Genetti & Engles 48 (CAS); Del Monte Forest, Pacific Grove, 1 Apr 1955, Howitt 132 (CAS); Pacific Grove, 12 Apr 1933, Jussel s. n. (DS); Summit of Hesperia Mountain, north of Bryson, southern Monterey County, 1550', 2 May 1933, Keck 2093 (DS-2); Near the school. Francis Simes Hastings Natural History Reservation, Santa Lucia Mts., 2 Apr 1944, Linsdale 71 (CAS); Santa Lucia Mountains, Apr 1898, Plaskett 75 (UC) Carmel-by-the-Sea, 3 Mar 1910, Randell 53 (DS); By trail from Carmel to Monterey, 30 Dec 1909, Randall s. n. (DS); Parkfield Road, 1.5 miles east of Vineyard Canyon Summit, 2200', 4 Apr 1956, Twisselmann 2618 (CAS-2); Del Mouh ur. Salinias Road, 15 Apr 1912, Woodcock s. n. (POM). Napa County: Mt. St. Helena Trail, 4 May 1928, Abrams 12265 (DS); Summit of St. Helena Grade, 3 May 1928, Abrams s. n. (DS);

Howell Mt., 22 Mar 1936, Cantelow 1142 (CAS); East slope in the Howell Mountains, 5 miles east of Napa, North Coast Range, 1500', 27 Mar 1938, Constance 2036 (DS, UC); Northern exposure above Putah Creek, along State Hwy. 128, about 6 miles east of Monticello, 26 Feb 1953, Crampton 989 (UC); 7.2 miles drom St. Helena on road to Pope Valley, 2 Apr 1950, Finfrock 17 (UC); Wooded slope in the oak belt about 5 miles south of Calistoga, 12 Apr 1924, Heller 13840 (DS, POM); Near the summit of the ridges east of Napa on the Monticello road on a clay bank among shrubs, 12 Mar 1940, Heller 15514 (DS); Wooden Valley road, east side of Napa Range, 8 miles from Napa, 2 Apr 1931, Keck 1030 (DS, POM); Calistoga, 13 Apr 1929, Linsdale 257 (UC); Old Howell Mountain Road, 1500', 31 Mar 1967, Muth 596 (RSA); 1 mile from Pacific Union College, beside road to Pope Valley, 1 May 1949, *Popenoe 18* (OSC); Hills just n. of White Sulphur Ck., w. of Saint Helena, 500', 22 Feb 1954, Raven s. n. (CAS); Base of Mt. St. Helena, 3 May 1928, Wolf 1845 (DS); Summit of Mt. St. Helena Grade, north of Calistoga, Wolf 1845 (RSA); Upper slopes of Mt. St. Helena, 4 May 1928, Wolf 1856 (RSA). Nevada County: American Ranch Hills, 5 miles south-west of Grass Valley, on McCourtney Rd., 2200', 8 Apr 1962, True 356 (CAS). Orange County: 14.3 miles east of San Jaun Capistrano on State 74, 1100', 9 Mar 1962, Breedlove 1789 (DS); Santa Ana Mountains, Cleveland Nat. Forest. Pleasants Peak (on Orange-Riverside Co. line), 4000', 18 May 1977, Davidson 5601 (RSA); Sitton Peak Truck Trail: 0.3 mi W of ranger station near hot springs, on Ortega H'way (SR 74), 25 Apr 1990, Jaroslow B36 (RSA); Sierra Peak Trail, Santa Ana Mts, 11 Apr 1929, Johnson 1259 (RSA); 5 miles east of Trabuco Oaks, Trabuco Canyon, Cleveland National Forest, 1300', 6 Apr 1966, Lathrop 6142 (RSA). Riverside County: Santa Ana Mountains, 3 miles above De Luz on dirt road,

3000', 30 Apr 1966, Adams s. n. (RSA); Vail Lake area, summit of "Big" Oak Mtn, N. of lake. T7S, R1W, SW ¼ sec. 34. Saddle between summits, N slope along rd heading N to Black Hills, 2600', 30 Mar 1990, Boyd, Ross & Arnseth 3944 (RSA); Vail Lake area, saddle at N base of "Big" Oak Mtn, S of Black Hills. T7S, R1W NE ¼, SW ¼ sec. 34, 2400', 30 Mar 1990, Boyd, Ross & Arnseth 3953 (RSA); Santa Ana Mountains, San Mateo Cyn. Wilderness Area. Tenaja Trail from jtn. W/ Morgan Tr. S to Pigeon Spring area. T6S, R5W sec.32; T7S, R5W sec. 5, 2200-2600', 31 Mar 1992, Boyd, Ross & Arnseth. 6763 (RSA); Aguate, So. Calif., 7 Apr 1929, Clarkk 2035 (RSA); Corona Skyline Drive, Santa Ana Mts. So. Calif., 7 Apr 1929, Crow 304 (RSA); Vicinity of Beaumont, 17 Apr 1897, Hall 476 (UC); Aguanga, 22 Dec 1925, Jaeger s. n. (POM); Corona, sec. 22, T4S, R7W, 2800', Mar 1934, Jensen 318 (UC); 18 miles S. W. of Elsinore. South slope of Tenaja Canyon, 2 Apr 1959, Lathrop 4408 (RSA); 4 miles west of Corona between Tin Mine Canyon & Santiago Peak, Skyline Drive, 4000', 9 Apr 1969, Lathrop 6968 (RSA); Beaumont, 2000', 25 Mar 1919, Munz, Street & Williams 2327 (DS, POM); 4 mi. SE of Oak Flat near west county line on Santiago Pk. Fire road near top of peak west of Santiago Pk., Santa Ana Mts., 4200', 14 Apr 1959, Olmsted 374 (RSA); Along roadside in red clay soil, about 4 miles west of Beaumont, on road to Redlands, 19 Mar 1921, Peirson 2741 (RSA); Rancho Calif. area, ca. 8 mi. (airline) NW of Temecula, between Bruce Lane & Via View Dr. (T 7S, R 1W SBBM NW /4 of SW /4, sec.27), 2400', 22 Feb 1988, Pendelton s. n. (RSA); Santa Ana Canyon, Santa Ana Mountains, 15 Apr 1922, Pierce s. n. (POM); 1 mile south of Aguangam, Hwy 79, Agua Tibia Mts., 1940', 12 Apr 1951, Rush 169 (POM-2); In herbosis, Lambs Canyon proper, Banning, 2300', 25 Apr 1922, Spencer 1910 (RSA). San Benito County: Eastern

exposure on Peak Trail. Pinnacles National Monument, Paicines, 1500', 22 Mar 1955, Burgess 84 (UC); Pinnacles Nat. Mon., 550 m, 6 Jun 1931, Fosberg 35251 (RSA); North slope, the Pinnacles, 29 Mar 1930, Howell 4611 (CAS); Pinnacles National Monument, High Peaks Trail. N 37° 53.386', W 122° 26.991', 107', 27 May 1998, Monfils 20 (MSC): Pinnacles National Monument, 1600-1700', 11 May 1940, Pennell & Powell 25370 (UC); Pinnacles National Monument. Pinnacles Loop Trail, 1200-2500', 27 April 1975, Thomas 17828 (DS). San Bernardino County: Devore near San Bernardino, 1900', 12 Mar 1928, Feudge 1960 (POM); Foothills San Bernardino Mts., Apr 1882, Parish 707 (DS, ORE, UC), San Diego County: Santa Ana Mtns, San Mateo Wilderness Area, "Miller Canyon" on the south base and flank of Miller Mtn from 8S02 upstream to the eastern summit area. T8S,R5W, sec. 10.15, 2100-2900', 3 Mar 1992, Boyd & Ross 6717 (RSA); W. of Warner Hot Springs, 6 Apr 1929, Clark 2007 (RSA); South side of Gonzales Canyon, east of Del Mar, 30 Mar 1969, Copp 69-1 (CAS); Mt. Soledad, 3 Jan 1935, Gander 103 (RSA, UC); North side of San Miguel Mountain, 32°42'N 116°55 3/4'W, 900', 24 April 1957, Moran 6000 (DS); Beaumont, 2000', 25 Mar 1919, Munz. Street & Williams 2327 (ORE); Colorado Desert, 1500', 9 Apr 1921, Spencer 231 (POM); Potrero Grade, 18 Mar 1917, Spencer s.n., 9 Apr. 1921 (POM); Grape Vine Canyon, 1200 m, 21 May 1930, Templeton 1625 (RSA-2); Near Santee, Feb 1915, Valentien s.n. (UC). San Louis Obispo County: Atascadero, 22 Mar 1926, Abrams 10942 (DS); 4.4 miles east of Santa Margarita; La Panza Range, 1200', 29 Mar 1962, Breedlove 2029 (DS); 3 miles E. of Pozo, sec. 13, T.30S, R.15E, 1800', 27 Mar 1937, Gifford 801 (UC); 1 mile S. of Bee Rock, Bradley, Sec 7, T.25S, R.10E, 1200', 11 Apr 1938, Graham 305 (UC); 7X Ranch, Santa Lucia Mountains, 2200', 2 Mar 1956, 2200', Hardman 143

(CAS); 7X Ranch, Santa Lucia Mountains, 2200', 2 Mar 1956, 2200', Hardman 144 (CAS); Santa Rita Canyon, Santa Lucia Mountains, 13 Apr 1956, Hardman 404 (CAS); L. Delagenna Ranch, Santa Lucia Mountains, 26 Apr 1956, Hardman 533 (CAS); Calf Canyon, 5 Apr 1967, Hoover 10334 (CAS); E. side of Santa Margarita Lake, 8 Apr 1986. Keil 19136 (RSA); 3 miles N. E. of Templeton, Paso Robles, T. 27S, R. 12E, 900', 31 Mar 1937, Lee 806 (UC); Growing in a disturbed area on a road bank beside Hwy. #229, 5.9 mi. sw. of Hwy. #41 at Creston, 29 Mar 1981, McNeal 2433 (OSC); Riconda Trail Head off Pozo Road. N 35° 17.214, W 120° 28.659', 2200', 18 May 1998, Monfils 2 (MSC); Riconda Trail Head off Pozo Road. N 35° 17.267, W 120° 28.677', 2070', 27 May 1998, Monfils 20, (MSC); Santa Rita Canyon, 1000', 17 Apr 1957, Sprague 1136 (RSA); On Cayucos Rd. to Cambira on Jack Mt. Nick Marquat Ranch, 16 Apr 1957, Sprague 1137 (RSA); Hill by a spring near San Louis Obispo, May 1889, Summers 527 (CAS); 2 miles north Cuesta Pass, Santa Lucia Mountains, 2000', 4 Apr 1963, Toschi 63:97 (CAS); Santa Margarita, Eldorado School, 20 Apr 1933, Wall s.n. (CAS-2, RSA); Roadside and hills near Adelaide, W. of Paso Robles, 24 Mar 1932, Wiggins 5847 (CAS, DS, POM); Prefumo Canyon, no collector (DS). San Mateo County: Woodside, 16 Mar 1902, Abrams 2278 (DS); Kings Mountain Road, Santa Cruz Mountains, 500-700', 1 Mar 1914, Abrams 5058 (POM); Woodside, 3 Mar 1895, Applegate 421 (DS); Woods on Coal Mine Ridge, 23 May 1937, Barry 155 (DS); Hill east of Lake Searsville, North Slope, 2 Mar 1929, Benson 987 (POM); Woodside, Santa Cruz Mtns, near San Fransquito Cr., 600', 28 Feb 1931, Benson 2610 (POM); Santa Cruz Mountain Peninsula, Crystal Spring Lake, 28 Apr 1920, Borthwick 97 (DS); Near the intersection of Canada and Edgewood roads. About 3 mi. westward from Redwood City, 13 May 1974, Cahill 248 (DS); La

Honda Road (from Hwy. 5) to Woodside, 3 mi. from Mt. Home-Portola road, 4 Apr 1956, Campbell I (RSA); Big Basin of Pescadero creek, 9 May 1903, Copeland 3050 (POM); Santa Cruz Peninsula, near Belmont, no date, Dudley s.n. (DS); Woodside, 1903 May, Elmer 4497 (CAS-2, DS, ORE, OSC, POM, UC); Near Belmont, Mar 1886, Greene 9 (ORE); San Carlos. Chaparral area north of Malabar Rd. and Melendy Dr. Lat N. 37° 29' 38", Long. 122° 16' 41" W, 600-680', 22 Apr 1973, Hemphill 737 (DS); Woods near Spring Valley Lakes, Santa Cruz Mountain Peninsula, 29 Apr 1920, Hickborn s.n. (DS); Los Trancos, 30 Apr 1908, Lewis s.n. (RSA); Kings Mt., May 1902, McMurphy s.n. (DS); Mt. above Woodside, 9 Mar 1906, McMurphy s.n. (DS); Sawyer's Road, near Crystal Springs lake above Burlingame and Millbrae, 15 Apr 1956, O'Bannon s.n. (DS); Sawyer Ridge, 17 Apr 1949, Oberlander 86 (DS); Santa Cruz Peninsula, Kings Mt., 27 Apr 1907, Patterson s.n. (RSA); Santa Cruz Peninsula, Kings Mt. Road, 27 Apr 1907, Randall 394 (DS); Santa Cruz Peninsula, Kings Mt. Road, 28 Mar 1908, Randall s.n. (DS, RSA); Belmont, 24 Feb 1935, Rose 35016 (RSA); Crystal Springs lake, 500', 12 Apr 1939, Rose 39061 (RSA, UC); Millbrae Highlands, 300', 31 Mar 1948, Rose 48031 (RSA); Emerald Lake, w. of Redwood City, 400', 23 Mar 1964, Rose 64013 (DS, RSA); 2 mi. nw of Woodside, 600', 27 Mar 1969, Rose 69009 (MSC); King's Mountain, 27 Apr 1907, Rust 135 (RSA); King's Mountain Road, 29 Mar 1949, Thomas 195 (DS); Jasper Ridge, about 5 miles southwest of Palo Alto. Lat N. 37° 24.5', Long. 122° 14' W, 500', 5 Apr 1959, Thomas 7663 (MSC); ½ way up east slope of La Honda Grade, Santa Cruz Mts., 24 Jan 1927, Wolf 190 (RSA); East side of La Honda Grade, 26 Feb 1927, Wolf 221 (RSA); Above Searsville Lake, 2 Mar 1927, Wolf 235 (RSA). Santa Barbara County: Purissima Hills, on road to Mission, 27 Mar 1938, Abrams 13755, (DS); North

side of the Point Sal Ridge, 3 ½ mi. west of Corralillos Canyon. Point Sal, 3 Mar 1958, Blakley & Muller B-2695 (CAS-2, RSA); Beside Refugio Pass Road, 5.4 miles north of U. S. 101, 24 Feb 1962, Breedlove 1778 (DS); San Marcos Pass, Old Stagecoach Road. N 34° 31.790', W 119° 50.070', 1402', 17 May 1998, Monfils I (MSC); Old Freemont Trail and Coach Rd., Santa Ynez Mts., - Area of Refugio Pass, 2200', 18 Mar 1956, Pollard s.n. (CAS); Near summit of Refugio Pass, Santa Ynez Mts, 2200', 19 Apr 1968, Pollard s.n. (CAS); Stagecoach Road, 1650', 5 Apr 1970, Shevock 106 (RSA); Entrance to Foster Glen Park, along highway in San Marcos Pass, south side of Santa Ynez Mountains near summit, 11 Mar 1955, Smith 3918 (RSA, UC); Three miles north of summit of San Marcos Pass, 19 Mar 1961, Turner, Sphon & Ball C-494 (RSA). Santa Clara County: Santa Cruz Peninsula, charcoal burners, Page Mill Road, Black Mt., 6 Jul 1903, Dudley s.n. (DS); Santa Cruz Peninsula, Page Mill Road, Black Mt., 23 Apr 1904, *Dudley s.n.* (DS); Adelante Villa, Palo Alto, 25 Feb 1894, *A. M. K. s.n.* (RSA); Foothills near Stanford University, 9 Mar and 15 May 1902, Baker 283 (CAS, DS, MSC-2, POM, UC); Black Mt., 9 Feb 1895, Burnham 9 Feb. 1895 (MSC); Eastern slope of Mount Hamilton, 3000', 31 Mar 1933, Chambers 144 (UC); Los Gatos, 19 Mar 1897, Davy s.n. (UC); North-facing bank, south side of page Mill road, ca. 1 mile west of entrance to Palo Alto Foothills Park, Palo Alto, 1200', 25 Mar 1969, Doty 593 (RSA); Foothills west of Los Gatos, 25 Mar 1904, Heller 7281 (DS, MSC, UC); Alpine Creek Road, northeast side of Santa Cruz Mountains, 200', 22 Feb 1932, Keck 1387 (DS, RSA); Foothills of the eastern side of the Santa Cruz Mountains, 5 miles south of the center of Palo Alto. Los Tracos Trail above Los Tracos Creek, 600-1800', 3 Apr 1974, Martineau 16 (DS); 23.1 mi. w. of Interstate #5, Patterson exit, in Del Puerto canyon (2.5 mi. e. of

the Mt. Hamilton-Livermore Rd.), 15 Mar 1970, McNeal 472 (OSC); Fire trail near Loma base above spring, 3400', 21 Mar 1940, Nelson 71 (UC); Hills 1 ½ miles south od Saratoga, Western Santa Clara County, 800', 1 Mar 1906, Pendleton 290 (POM); 10 miles south of Black Mountain, s. of Palo Alto, 1200-1300', 16 May 1940, Pennell & Abrams 25429 (CAS, UC); Seeboy Ridge, Mount Hamilton Range, 2400', 10 Feb 1934. Sharsmith 537 (UC); South end of Mount Day Ridge above Santa Isabella Creek, Mount Hamilton Range, 1800', 25 Mar 1935, Sharsmith 1520 (UC); Northwest slope of Black Mountain, 1100', 31 Jan 1948, Silva 2616 (RSA); Vicinity of Mt. Umunhum, Lat. N. 37° 09.5', Long. 121° 54.2' W, 3400', 23 Apr 1954, *Thomas 3963* (DS). Santa Cruz County: Ben Lomond Mountain area; on Eagle Rock; T9S, R3W, Sec. 16, 2200', 23 Mar 1974. Halse 986 (OSC); Big Basin, Santa Cruz Mts., Reed s.n. (CAS); Santa Cruz Peninsula, Swanton, Spring 1912, Rich s.n. (DS); La Honda Rd. Sta. Cruz Mts., 2000', 3 Apr 1956, Sprague 1095 (RSA); Between Eagle Rock and Locatelli Ranch. Lat. N. 37° 08.7', Long. 122° 12' W, 2400', 27 Mar 1950, Thomas 1346 (DS); East side of Mill Creek Drainage about 2 miles S-SW of Eagle Rock at Lat. N. 37° 07.3', Long. 122° 12.4' W, 1800-1900', 30 Apr 1954, Thomas 3035 (DS); Between Eagle Rock and Locatelli Ranch. Lat. N. 37° 08.7', Long. 122° 12' W, 2200', 15 Mar 1954, Thomas 3776 (DS); Vicinity of Eagle Rock. Lat N. 37 08.8, Long. 122 11.7 W. 2200-2500', 30 Mar 1961, *Thomas 9041* (DS); Halfway down La Honda Rd. Sta. Cruz Mts., 2000', 13 Apr 1957, no collector (RSA);. Solano County: Gates Canyon, northwest of Vacaville, 3 Mar 1951, Kehlor s. n. (OSC); 3 ½ miles west of Rockville, 25 Mar 1966, Olson & Gorelick (RSA-2). Sonoma County: Pepperwood Ranch, Oak woodland between the 2 fir-Redwood canyons, 25 Mar 1981, Denevers 565 (CAS); Calistoga, 25 Mar 1922, Eastwood 11097 (CAS); Camp Meeker, 22 Mar 1924, Howell 287 (CAS); Near Sonoma, Mar 1962, Menzius s.n. (CAS); 2 miles from juncture of Highway 12 and Triniti Road, 750', 27 Feb 1960, Ruckert 5 (CAS). Sonoma County, 10 Mar 1903, Rattan s.n. (DS); 0.5 mi. south of Calistoga, 400', 29 Mar 1953, Raven 5207 (CAS); Santa Rosa Creek Canyon, 8-10 miles east of Santa Rosa, 25 Mar 1937, Robbins 10 (UC); N. side of Vine Hill School Road ½ miles from Vine Hill Road, 250', 19 Apr 1965, Thorne 34319 (RSA); Parker Hill Rd. ca. 2 ½ mi. N of Santa Rosa and 1 mi. N of Sonoma Co. Hosp., 350', 20 Apr 1965, *Thorne 34328* (RSA). Stanislaus County: Near head of Del Puerto Canyon, 20 Apr 1941, *Hoover 4886* (UC). Trinity County: Vic. Dam at Ruth Resevoir on Mad River, 2700', 21 May 1979, Mattoon 121 (RSA, UC); Road along Coffee Creek, above Coffee Creek Ranch, 16 Jun 1956, McClintock s. n. (CAS); T6N, R6E, Sec. 34, Found in Grays Falls Campground, 21 Apr 1979, Miller 2-87 (RSA); Sec. 10, T4S, R7E, West slope of Salt Cr., 2400', 28 May 1933, Sack & Iverson 70393 (CAS); New River Trail from Grays Falls Campground on the North side of the Trinity River and west side of the New River, 800-1000', 7 Apr 1973, Smith 6006 (RSA); Under scattered yellow pine; along Van Duzen River, about 3 miles southeast of Kuntz, 20 Apr 1950, Tracy 18633 (RSA, UC). Ventura County: Las Turas Lake, Santa Monica Mts., 14 Feb 1931, Ewan 4027 (POM); Upper Ojai, Ojai Valley and vicinity, 7 Mar 1895, Pettibone & Hubby s.n. (CAS); Hills north of Ojai, Upper Ojai, Ventura River Basin, 16 Apr 1949, Pollard s. n. (CAS); Hills north of Ojai, Upper Ojai, Ventura River Basin, 21 Apr 1949, *Pollard s. n.* (CAS). County Unknown: Belmont (MSC); California, 1853-1854, Bigelow s.n. (DS); S. Oakland Hills, Mar 1900, Carruth s.n. (CAS); Mt. Hamilton, 3000', 3 Apr 1906, Chaualer 6014 (UC); Cuesta Summit, 2600', 2 Apr 1908, Condit s.n. (UC); California, Coulter s.n. (UC); Mt. St.

Helena, 1 May 1918, Eastwood 6811 (CAS, UC); Near Woodville, May 1889, Howell 1387 (ORE); Northern California, 7 Apr 1937, Javete s.n. (OSC); Napa River Basin, Trail to beaux's Cabin, 25 Apr 1893, Jepson s.n. (UC); Near San Jaun Hot Springs, 6 Mar 1913, Perkins s.n. (RSA); Oakland, 1903, Rattan s.n. (DS); California, 1889, Wright s.n. (UC). MEXICO: Baja California: Elev. Of peak with microwave towers at 1275m, 32°19'N-116°40'W, collections made from peak to base (ca. 600m)., Thorne et al. 62130 (CAS, RSA).

Key:

Key to the two species, *Pedicularis densiflora* and *P. aurantiaca*, adapted in part from the <u>Jepson Manual</u> (Vorobik 1993): Key to *Pedicularis* by Linda Ann Vorobik. Steps 4' is directly transcribed from the Jepson Key. It is intended that 7,7', 7a, and 7b fit directly into the existing key;

- 4'. Corolla ± club-like, upper labium hooded, not beaked; lower labium not fan-like
 7. Corolla deep red to red-purple (or yellow to orange, occasionally white), lower labium < 1/2 upper

7' Corolla yellowish or purplish, lower labium gen >1/2 upper

Pedicularis densiflora and P. aurantiaca remain an interesting and dynamic group for the evolutionary study of character evolution, pollinator mediated selection, host specificity for parasitism, and habitat preference. Future research should focus on pollination of the two species, and the potential adaptation of floral morphology to different pollination regimes. Additional genetic work in the form of more informative sequence data (perhaps a low copy nuclear gene) or AFLPs would help resolve relationships among populations of P. densiflora and P. aurantiaca. The inferred phylogeny could be used to investigate the number of times floral morphs arose, relationships among populations in different localities, and the evolution of pollinator shifts. Pedicularis densiflora and P. aurantiaca could be used to investigate theories of speciation, pollinator mediated selection, and the rapid radiation of angiosperms.

CHAPTER 2

PHYLOGENY AND POLLEN EVOLUTION OF *CANTUA* (POLEMONIACEAE SUBFAMILY COBAEOIDEAE): EVIDENCE FROM CHLOROPLAST AND NUCLEAR DNA SEQUENCE DATA

INTRODUCTION

Cantua Juss. is a member of the Polemoniaceae subfamily Cobaeoideae. It has approximately ten species distributed at mid to high elevations in the Andes of Bolivia, Peru, and Ecuador. Most of the species of Cantua are narrow endemics. The relatively small genus has a tremendous amount of floral diversity, with flowers varying in size, form, and color, and a variety of pollination syndromes. Bee, bird, and moth pollination are known or suspected within the genus. Cantua is one of the few genera of the Polemoniaceae that includes woody species, and the only genus that includes species with a tree habit. The pollen exine of Cantua species is pantoporate, with large tectal insulae, and is distinct from all other pollen grains in the family. The genus is the only member of the Polemoniaceae endemic to South America. One taxon, Cantua buxifolia, has the distinction of being the national flower of both Bolivia and Peru and was cultivated by the Incas and used to adorn temples.

One or two species of *Cantua* have been included in several molecular phylogenetic analyses in an attempt to understand the relationships within the Polemoniaceae and elucidate the relationships among lineages within the angiosperms (*mat*K phylogeny in Steele & Vilgalys 1994, *mat*K phylogeny in Johnson et al. 1996, nrITS phylogeny in Porter 1997, *NAD1B* phylogeny in Porter and Johnson 1998, 18S phylogeny in Johnson et al. 1999, and *ndh*F phylogeny in Prather et al. 2000). These molecular analyses have resolved the Core Cobaeoideae (*Bonplandia*, *Cantua* and *Cobaea*) as a monophyletic clade distinct from the Polemonoideae with the exception of two studies: the *mat*K phylogeny in Steele & Vilgalys (1994) and the nrITS phylogeny in Porter (1997). The

matK phylogeny had an unresolved polytomy at the base of the Polemoniaceae that included members of the Cobaeoideae. This polytomy was resolved in a later phylogenetic analysis based on a longer region of the matK gene and more extensive taxonomic sampling (Johnson et al. 1996). Porter's (1997) nrITS phylogeny lacked support in the basal nodes of the Core Cobaeoideae and relationships among genera changed depending upon taxon inclusion, character weighting, and alignments. Porter (1997; pg 67) commented that "it is difficult to argue that the ITS phylogeny is error free" in the relationships among the taxa that make up the Core Cobaeoideae. Overall, the molecular analyses provide sufficient evidence to accept that Cantua is a member of the Core Cobaeoideae and has a recent shared ancestry with Bonplandia and Cobaea. However, the relationships among the species of Cantua have not been explored.

In the most recent classification of the Polemoniaceae, based on both morphology and molecular phylogenetics, *Cantua* was placed in its own tribe in the subfamily Cobaeoideae, Tribe Cantueae. The authors subsumed the small genus *Huthia* into *Cantua* (Porter & Johnson 2000). The two species previously in *Huthia* have been renamed *C. volcanica* (Brand.) J. M. Porter & Prather (=*Huthia coerulea* Brand), and *C. mediamnis* (Brand) J. M. Porter & Prather (=*H. longiflora* Brand). There have been no molecular phylogenetic analyses to test the monophyletic status of the genus.

Previous work on pollen exine sculpturing in the Polemoniaceae indicated a congruent relationship between pollen morphology and inferred evolutionary history (Stuchlik 1967a, 1967b, and Taylor and Levin 1975). Several studies have examined pollen across

the family Polemoniaceae, including the Core Cobaeoideae (Stuchlik 1967a, 1967b, and Taylor and Levin 1975). Additional generic level studies have been conducted on members of the Cobaeoideae: Day and Moran (1986) described the pollen in *Acanthogilia gloriosa* (Brand.) Day & Moran; Prather (1999) did an extensive review of pollen in *Cobaea*, and a review of *Bonplandia geminiflora* pollen exine sculpturing is underway (Dickman and Prather, unpublished data). Until now, few species of *Cantua* pollen have been described (Stuchlik 1967a, 1967b, and Taylor and Levin 1975).

Pollen grains in flowering plants give rise to pollen tubes, which grow through the transmitting tissue of the style and into the ovary. Once the pollen tube reaches the ovary, male gametes are released and fertilization takes place. Delpino (see Darwin 1896; 250) hypothesized pollen grain diameter is a product of the length of the style and the energy storage requirements of the pollen tube to reach the ovary. Several researchers have investigated this potential connection between style length and pollen diameter (Lee 1978, Baker & Baker 1979, Cruden and Lyon 1985, Elisens 1986, Lee 1989, Cruzan 1990, Stroo 2000, Aguilar et al 2002). Plitmann and Levin (1983) tested the correlation between pollen grain diameter and style length in the Polemoniaceae. They measured mean style length and pollen diameter for 140 species in 18 genera across the family. Using Pearson's pair-wise correlations, they found a strong significant correlation within and among genera across the family. However, these results could be an effect of non-independence due to shared phylogenetic history instead of independent evolution (Cruden and Lyon 1985, Felsenstein 1985).

Plitmann and Levin (1983) sampled five *Cantua* specimens in their analysis of the Polemoniaceae. While they found a strong correlation between pollen diameter and style length within the family, and within other genera, they did not find a significant correlation within the genus *Cantua* (r = 0.39, p-value > 0.05). A broader sampling of the genus, a morphological review of the pollen, and a comprehensive phylogenetic hypothesis would add insight into the potential correlation between pollen diameter and style length among species of *Cantua*.

This study had three primary goals: Estimate a phylogeny of the genus *Cantua*, examine the pollen exine sculpturing of the species, and investigate the relationship between pollen diameter and style length in *Cantua* in a phylogenetic context. Sequences from the nuclear ribosomal ITS gene and the chloroplast spacers *trnT-trnL. trnL-trnF*, and partial *ndhF* coding region were used to generate a phylogenetic hypothesis of the genus. A comprehensive review of pollen grains across *Cantua* completed the pollen grain sampling within the Core Cobacids. The pollen grain study was combined with the phylogenetic analysis to address evolution of exine sculpturing within the genus and directly test the phylogenetic utility of pollen morphology. Comparative analyses were conducted to further test the potential correlation between pollen diameter and style length in *Cantua*. To our knowledge this was the first attempt to examine the correlation between pollen diameter and style length within a phylogenetic context.

MATERIALS AND METHODS

Herbarium Specimens and sampling - A total of 903 herbarium specimens of Cantua were examined from the following herbaria: AAU, BM, CAS, DAV, DES, DS, F, G, GB, GH,H, HUT, JEPS, K, LL, LPB, MO, MSB, MSC, PH, QCNE, S, TEX, U, UC, UPS, and US. Permission was requested and granted to sample herbarium material for DNA extraction and/or scanning electron microscopy (SEM) and light microscopy of pollen grains from the following herbaria: F, HUH, LL, MO, MSB, MSC, NY, UC, and US. Specimens were chosen to represent the morphological and geographical variation of the species.

Individuals were used in the phylogenetic and pollen analysis from the following nine species: *C. bicolor, C. buxifolia, C. candelilla, C. cuzcoensis, C. flexuosa, C. pyrifolia, C. quercifolia, C. volcanica,* and an as yet undescribed species (Table 4 & 5). The tenth species, *Cantua mediamnis,* is known from only one collection and the material was unavailable for sampling.

Phylogenetic Analysis -

Sampling – Fresh leaf or floral material from either field-collected or greenhouse-grown material was sampled whenever possible. When fresh samples were unavailable, tissue was sampled, with permission, from herbarium material. For species with broad

Table 4: Voucher information for material used in the molecular phylogenetic analyses of Cantua.

Species	Voucher	Location
Bonplandia geminiflora Cav.	Crespo 255 (MSC)	Mexico: Estado Morelos
Bonplandia geminiflora	Cultivated	San Francisco State Univ.
Cantua bicolor Lem.	Beck, Kiesling & Metzing 22154 (MSC)	Bolivia: Tarija
Cantua buxifolia Lam. (A)	Weigand 214 (MSB)	unknown
Cantua buxifolia (B)	Porter 12228 (MSC)	Peru: Junin
Cantua buxifolia (C)	Weigand & Skrabal 5883 (MSC)	Peru: Lima
Cantua candelilla Brand (A)	Cultivated	San Francisco State Univ.
Cantua candelilla (B)	Porter 12192 (RSA)	Peru: Arequipa
Cantua cuzcoensis Infantes	Porter 12227 (MSC)	Peru: Junin
Cantua flexuosa Pers. (A)	Porter 12224 (MSC)	Peru: Huancavelica
Cantua flexuosa (B)	Weigand & Skrabal 5862 (MSB)	Peru: Huancavelica
Cantua n. sp.	Weigand, Rodriguez, Förther & Dostert 915 (MSC)	Peru: Amazonas
Cantua pyrifolia Juss.	Porter 12168 (MSC)	Peru: Amazonas
Cantua quercifolia Juss (A)	Boeke 2206 (MSC)	Ecuador: Azuay
Cantua quercifolia (B)	Wrigley 2 (MSC)	Ecuador: Loja
Cantua quercifolia (C)	Weigand 808 (MSC)	Peru: La Libertad
Cantua volcanica (Brand) J. M. Porter & Prather (A)	Hodgsen 7924 (F)	Peru: Arequipa
Cantua volcanica (B)	Porter 12199 (MSC)	Peru: Arequipa
Cobaea minor M. Martens & Galeotti	Prather 1575 (TEX)	Panama: Chiriqui
Cobaea pachysepala Standl.	Prather 1522 (TEX)	Guatemala: Quezaltenango

Analysis. In voucher column, * denotes specimens examined with SEM, * denotes specimens using light microscopy.

Species	Voucher	Location	Figure
Cantua bicolor Lem.	Cardenas 5479 (HUH)*‡	Bolivia: Cochabamba	Fig. 16 F
Cantua bicolor t	Beck, Kiesling & Metzing 22154 (MSC) *#	Bolivia: Tarija	Fig. 16 E
Cantua buxifolia Lam.	Gentry, Smith & Tredwell 37515 (MO) *‡	Peru: Pasco	
Cantua buxifolia	Lopez & Sagastegui 8023 (LL) * #	Peru: La Libertad	
Cantua buxifolia	Plowman & Davis 4653 (HUH) *‡	Peru: Ayachuco	
Cantua buxifolia t	Porter 12228 (MSC)*	Peru: Junin	
Cantua buxifolia	Bohs 2053A(HUH) * #	Bolivia: La Paz	Fig. 17A & B
Cantua candelilla Brand	Hutchison 1252 (UC) *#	Peru: Ayacucho	
Cantua candelilla	Hutchison & Wright 7235 (UC) *#	Peru: Arequipa	Fig. 16 D & E
Cantua cuzcoensis Infantes	Vargas 9785 (MO) * \$	Peru: Cuzco	
Cantua cuzcoensis †	Porter 12227 (MSC)*	Peru: Junin	Fig. 16 C & D
Cantua cuzcoensis	Balls B6903 (UC) *#	Peru: Apurimac	
Cantua flexuosa Pers.	Gentry, Smith & Tredwell 37518 (NY) *#	Peru: Pasco	
Cantua flexuosa †	Porter 12224 (MSC)*	Peru: Huancavelica	Fig. 16 A
Cantua flexuosa	Beck 1349 (MSC) *#	Bolivia: La Paz	Fig. 16 B
Cantua n. sp.	Knapp & Alcom 7556 (NY) * #	Peru: Amazonas	
Cantua n. sp.	Leiva, Chuna & Cadle 1419 (F) *#	Peru: Cajamarca	Fig. 17 E & F
Cantua pyrifolia Juss.	Hitchcock 21580 (HUH) **	Ecuador: Azuay	
Cantua pyrifolia	Sagástegui & Cabanillas 8560 (MO) * ‡	Peru: Piura	
Cantua pyrifolia	Van der Werff & Palacios 8891 (NY) *#	Ecuador: Loja	
Cantua pyrifolia	Woytkowski 7670 (HUH) **	Peru: Amazonas	Fig. 17 C & D
Cantua pyrifolia t	Porter 12168 (MSC)*	Peru: Amazonas	
Cantua pyrifolia	Camp 4280 (NY) * #	Ecuador: Azuay	
Cantua quercifolia Juss.t	Wrigley 2 (MSC) **	Ecuador: Loja	Fig. 18 C
Cantua quercifolia	Hutchison & Wright 3482 (HUH) * #	Peru: Lambayaque	Fig. 18 D
Cantua volcanica (Brand) J. M. Porter & Prather	West 7139 (MO) * #	Peru: Arequipa	
Cantua volcanica	Vargas 19918 (US) * #	Peru: Arequipa	
Courses volconice +	Porter 12109 (MSC)*	Peru: Arequina	Fig 18 A & B

distributions and/or high levels of intraspecific morphological variation, several geographically and/or morphologically distinct individuals were sampled (Table 4). Nineteen samples were included in the phylogenetic analyses. I used two sequences of the internal transcribed spacer region of nuclear ribosomal DNA (ITS) from outgroup species that were previously published. The taxa and their GenBank accession numbers are as follows: *Cobaea minor* (AH005678) and *Cobaea pachysepala* (AH005690). Additional sequences were made available for *Bonplandia geminiflora*, *Cantua volcanica*, *Cobaea minor*, and *Cobaea pachysepala* by David C. Jarrell and L. Alan Prather (unpublished data).

Outgroup - Bonplandia geminiflora and two species of Cobaea: C. minor and C.

pachysepala were chosen as outgroups based on the ndhF phylogeny of Prather et al.

(2000).

Phylogenetic Analysis - DNA was extracted using the techniques of Loockerman and

Jansen (1996) and purified using the Schleicher & Schuell Elu-quick DNA Purification

Kit (Keene, NH). Three sets of chloroplast primers were used: trnT - trnL 5' primers

m Fujii et al. (1997), trnL 3' - trnF primers from Taberlet et al. (1991), and primers

a partial ndhF coding region using the 1201 forward primer of Jansen (1992) and a

erse primer designed for this analysis. The sequence of the ndhF reverse primer is as

1 ows: 5'-GTAGTAAATTACTAAAAAGATTRATAC-3'. Additional internal primers

e designed to amplify the trnT - trnL intergenic spacer. The forward primer was 5'
CTAAGCGGGCTCACATAA-3', and the reverse primer sequence was 5'-

TGTGATGTCCTTTCCCCTTT-3'. ITS1, ITS2, and the 5.8S regions of nuclear ribosomal DNA were amplified using ITS-4 primers of White et al (1990) and the modified ITS-5 primer of Sang et al. (1995).

Standard amplification protocols were used, including reaction mixture components and the PCR profile (Prather et al. 2002), and amplifications were performed on a MJ Research PTC-100 thermalcycler. PCR products were gel purified using the Schleicher & Schuell Elu-quick DNA Purification Kit, (Keene, NH) and then sequenced in both directions using an ABI-373 automated sequencer. Sequencing reactions were conducted using the AmpliTaq DNA Dye Terminator Cycle Sequencing reagents (PE Applied Biosystems, Norwalk, CT).

Edited sequences were aligned using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, MI). Termini of the *trn*T-*trn*L intergenic spacer region and *trn*L-*trn*F intergenic spacer region were determined from Fujii et al. (1995) and Morrell et al. (1998),

Sepectively. Termini of ITS1, ITS2, and the 5.8S regions of nuclear ribosomal DNA

Sere determined by comparison with published sequences of Prather and Jansen (1998).

The segment of *ndh*F used for the analysis began at the 1244th base pair of tobacco

simony methods were implemented using PAUP* (Version 4.0b4; Swofford 2000).

Paristic searches were performed using the TREE BISECTION RECONNECTION, and LTREES options. All insertion/deletion events, displayed as gaps in the data set,

were treated as characters using the "Simple Indel Coding" method outlined by Simmons and Ochoterena (2000). Bootstrap analyses were conducted using 10,000 replicates with 200 random addition-sequence replicates per bootstrap replicate. A partition homogeneity test, using 10,000 replicates, was conducted to test for homogeneity in the distribution of phylogenetic information between the nuclear and chloroplast data sets.

A second analysis was conducted to determine branch lengths for the independent contrast of pollen grain diameter and style length. This analysis included only one sample of each taxon. The sample was chosen by selecting the species with the most complete sequences for both the chloroplast and nuclear molecular data sets. All insertion/deletion events, displayed as gaps in the data set, were treated as missing data. Branch lengths were determined using the MinF option in PAUP* (version 4.0b4; Swofford 2000, Swofford and Maddison 1987). Sequence termini, parsimony methods, heuristic searches, and bootstrap analysis were identical to the analysis described above.

Pollen Analysis -

Pollen Grain Morphology and Style Length - For the SEM study, I examined the pollen orphology of 28 individuals of nine species of Cantua. For each taxon, at least two dividuals were sampled from different parts of their geographical range (Table 5).

Ilen preparation for the SEM was conducted using the methods of Prather (1999).

opened anthers were removed from herbarium specimens, boiled in water until the len was released, and acetolyzed using the methods of Adams and Morton (1972).

coated with 60% palladium and 40% gold, and examined using a JEOL 6400V scanning electron microscope at the Center for Electron Optics at Michigan State University.

Light microscopy was used to measure pollen diameter and style length on a minimum of two populations for each species. Twenty three populations were sampled for a total of 555 pollen grains and 90 individual styles (Table 5, Appendix I). Whenever possible, 30 pollen grains from each population were sampled. Number of styles measured per collection varied from 1-8.

Pollen grains were removed from two dehiscing anthers on herbarium specimens and stained in Cotton-blue with lactophenol for at least 24 hours prior to measurement. This assured that all pollen grains had absorbed the stain and were fully hydrated. Pollen grains were measured with a calibrated eyepiece micrometer under 400X magnification.

Only fully stained, intact pollen grains were measured. All available styles, from fully developed, non-senescing flowers were measured on all duplicates of the same collection than the same collection makes the same were made using digital calipers and recorded to the nearest 0.01

Length and Pollen Diameter Correlation—Traits in related species are not dependent. Closely related species may share common traits due to shared ancestry ther than independent evolution. To remove the issue of non-independence in data sets imparative methods have been developed to examine relationships among pairs of tinuous traits while accounting for potential effects of shared evolutionary history. To

assess the relationship between style length and pollen diameter, the independent contrasts method of Felsenstein (1985) was implemented using the Comparative Analysis of Independent Contrasts (CAIC; Purvis and Rambaut 1995). CAIC is suitable for analyzing comparative data on continuous characters and can accept polytomies in a phylogeny.

Mean values were calculated for pollen diameter and style length for each species, and log-transformed before analysis. In the independent contrasts analysis, two types of data were used. The raw data set assumed every branch in the phylogeny was of equal length indicating equal amounts of evolutionary change between speciation events. The raw data analysis assumed a puntuational view of evolution with evolutionary change occurring only at the nodes. The transformed data set utilized branch lengths from the phylogenetic analysis as expected units of evolutionary change between speciation events; this data analysis assumed a gradualistic view of evolution with evolutionary change occurring along the branches between speciation events. CAIC does not accept branch lengths less than 2, so all branch lengths were multiplied by 2 to fulfill the program requirements

Purvis and Rambaut 1995). Both the raw and transformed data sets were checked to

the output, CAIC computes the standardized linear contrasts for the traits in the

alysis and forces the regression of the standardized linear contrasts through the origin.

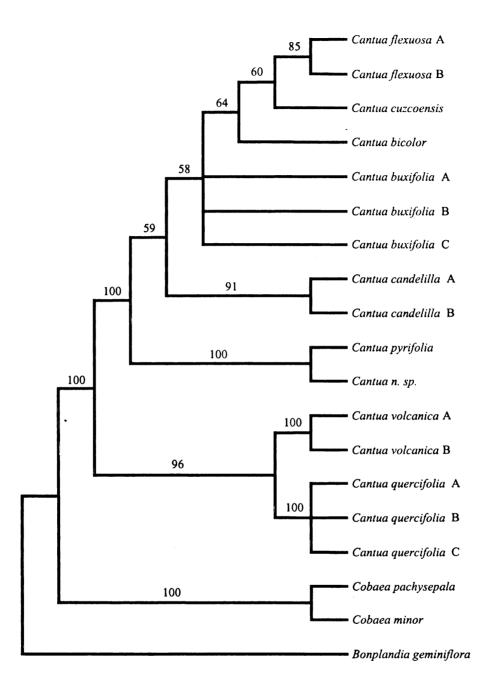
IC computes a slope and the significance of the linear contrasts. The value of the

slope describes the relationship between pollen diameter and style length based on independent contrasts (Purvis and Rambaut 1995). An additional analysis of the correlation of standardized linear contrasts was computed using the JMP statistical package (SAS Institute Inc., Cary, NC) for a direct comparison to the results reported by Plitmann and Levin (1983) who performed a Pearson pair-wise correlations correlation on non-independent data. The resultant correlation, like the slope in the regression analysis, is representative of the relationship between pollen grain diameter and style length minus phylogenetic effects (Pagel 1993).

RESULTS

Phylogenetic Analysis – In the data set excluding insertion/deletion events (indels), the aligned ITS sequences were 660 bp long, with a total of 201 potentially phylogenetically informative characters. Phylogenetic analyses of the nucleotide data resulted in 15 most parsimonious trees with a length of 437, C.I. = 0.7889, and R.I. = 0.8783. Forty-five indels were scored and appended to the ITS nucleotide data, 25 of those characters were potentially phylogenetically informative. The analysis of the data set including indels resulted in 15 most parsimonious trees with a length of 489, C.I. = 0.7882, and R.I. = 0.8783 (Figure 12). Tree topologies were identical for the analyses including and excluding the indel characters.

A clade comprising all members of the genus (Clade A, Fig. 12), including C. volcanica (formerly Huthia), had strong (100%) bootstrap support. Likewise, two major clades within Cantua were strongly supported: One clade consisting of Cantua volcanica and C. quercifolia (96%) and a second clade which encompasses the remaining Cantua species (100% bootstrap support). Relationships within the second clade had weak bootstrap support with the exception of the Cantua pyrifolia / C. n. sp. clade which had strong otstrap support (100%).



gure 12: Strict consensus of 15 most parsimonious trees based on the *Cantua* total ITS a set including nucleotide substitutions and indels. Tree length = 489, with a C.I.^e = 882 and R.I. = 0.8783. Numbers above the branches represent bootstrap support based 10,000 replicates. Location and voucher information for each sample is available in ble 4.

Multiple taxa from the same species were in exclusive clades with strong bootstrap support (*C. flexuosa* 85%, *C. candelilla* 91%, *C. volcanica* 100%, and *C. quercifolia* 100%) with the exception of the three individuals of *C. buxifolia*, which had unresolved relationships at the base of a clade including *C. bicolor*, *C. cuzcoensis* and *C. flexuosa*.

In the data set excluding indels, the aligned sequences from the *trn*T-*trn*L intergenic spacer were 642 bp long with 18 potentially phylogenetically informative characters.

Parsimony analysis resulted in 207 most parsimonious trees with a length of 63, a C.I.^e. = 0.9524, and a R.I. = 0.9697. The data set had five insertion-deletion events, one of which was potentially phylogenetically informative. Phylogenetic analysis of the data set including indels resulted in 441 most parsimonious trees with a length of 68, a C.I.^e. = 0.9545 and a R.I. = 0.9714. I was unable to amplify *Cantua flexuosa* B (Weigand 5862, see Table 4) and it was not included in *trn*T-*trn*L analyses. Analyses of the data, both including and excluding the indels, yielded a consensus trees with no resolution among

the data set excluding indels, the *trnL-trnF* intergenic spacer was 448 bp long with 13 tentially phylogenetically informative characters. The parsimony analysis of the cleotide data set resulted in three most parsimonious trees, with a length of 48, a C.I.^c.

1.0, and a R.I. = 1.0. There were 14 insertion-deletion events, 9 of which were tentially phylogenetically informative. Analyses of the data set including indels found most parsimonious trees, with a length of 65, C.I.^c = 0.8846 and a R.I = 0.9348.

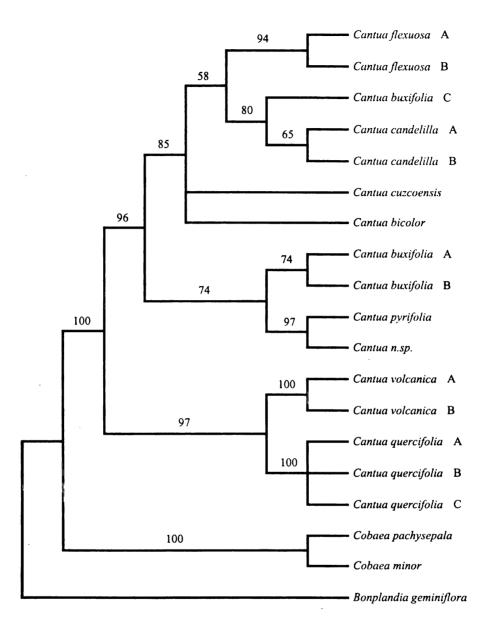
alyses of the data set both including and excluding indels, had some resolution and

concordant topologies. The analysis of the data set including indels had more resolution within *Cantua*.

The aligned sequences from the partial ndhF-coding region were 938 bp long, with 59 potentially phylogenetically informative characters. Two most parsimonious trees resulted from the analysis and each had a length of 119, a C.I. e =0.9444 and a R. I. = 0.9690. No indels were present and the strict consensus tree showed some inter- and intraspecific resolution.

The chloroplast data sets including indels were combined for a total of 2047 bp and 19 indels with 100 parsimony informative characters (Figure 13). Two most parsimonious trees were generated, with a length of 256, a C.I. = 0.9032, and a R.I. = 0.9429. The monophyly of Cantua, including C. volcanica, had strong support (100%). Cantua volcanica and C. quercifolia were strongly supported (97%) as sister taxa, and together they were sister to the remainder of the genus (96% bootstrap support). A clade comprising Cantua buxifolia (A & B), C. pyrifolia and C. n. sp. had weak bootstrap poport (73%). The clade containing C. buxifolia A & B was moderately supported 75%) and sister to a well supported clade containing C. pyrifolia and C. n.sp. (97%).

Cantua flexuosa A & B, C. buxifolia C, both samples of C. candelilla A & B, C.



rule 13: Strict consensus of two most parsimonious trees based on the *Cantua* rule chloroplast sequences (trnT-trnL, trnL-trnF, partial ndhF) for the data sets luding indels. Tree length = 256, C.I. = 0.9032 and a R.I. = 0.9429. Numbers above branches represent bootstrap support based on 10,000 replicates.

(81%). With the exception of *C. buxifolia* which is paraphyletic in the phylogeny, multiple taxa from the same species were in exclusive clades with strong bootstrap support (*C. flexuosa* 77%, *C. candelilla* 66%, *C. volcanica* 100%, and *C. quercifolia* 100%).

When the data sets including indels from the nuclear and chloroplast genomes were combined, the null hypothesis of homogeneity in distribution of phylogenetic information between the data sets based on the partition homogeneity test was rejected (p = 0.0127). The data set was reviewed and the relationships of C. buxifolia in the nuclear and chloroplast phylogenies were not concordant. In the ITS phylogeny (Fig 12), Cantua buxifolia was part of an unresolved polytomy at the base of a clade including C. bicolor, C. cuzcoensis and C. flexuosa. In the chloroplast phylogeny (Fig. 13), C. buxifolia C was sister to a clade with C. candelilla, but C. buxifolia A & B were sister to the clade containing C. pyrifolia and the C. n. sp. Cantua buxifolia has a high level of intraspecific **Proorphological** variation and has been cultivated throughout much of Andean South merica for centuries. The likelihood of hybridization is high, and is a likely explanation For the lack of congruence between the chloroplast and nuclear phylogenies. The pecimen of C. buxifolia C had different relationships within Cantua in the chloroplast NA and nuclear DNA phylogenies. This sample was removed from both data sets and a second partition homogeneity test was run. This test did not reject the null hypothesis of mogeneity among data sets (p = 0.2529).

The total combined data set including indels had 2752 total characters: 2688 nucleotides and 64 indels. The data set had 324 potentially phylogenetically informative characters. Four most parsimonious trees resulted from the analysis, each with a tree length of 741, a C.I.^e = 0.8133, and a R.I. = 0.8903. A strict consensus tree was generated (Figure 14). The monophyly of *Cantua* was strongly supported (100%). The sister relationship between C. quercifolia and C. volcanica had strong bootstrap support (99%). All remaining Cantua taxa are included in a strongly supported clade (100% bootstrap support). Cantua pyrifolia and C. n. sp. are strongly supported as sister taxa (100%) bootstrap support) in a polytomy at the base of the clade including the remaining Cantua species. Cantua candelilla, C. bicolor, and C. cuzcoensis, had weak bootstrap support (71%). A monophyletic group of Cantua bicolor, C. cuzcoensis, and C. flexuosa, had moderate support (81%). In this phylogenetic analysis, with the removal of C. buxifolia C, multiple taxa from the same species were in exclusive clades with strong bootstrap support (C. quercifolia 100%, C. volcanica 100%, C. buxifolia 79%, C. candelilla 100%, and C. flexuosa 97%).

The tree estimated for the independent contrasts, with only one sample per species, had

688 total characters (Figure 15). The data set had 210 potentially phylogenetically

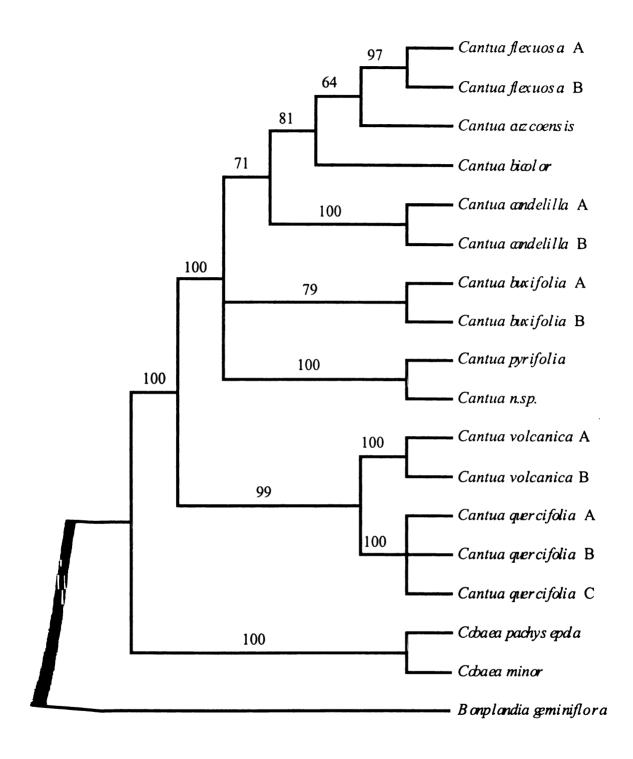
formative characters. A single most parsimonious tree resulted from the analysis, with a

gth of 650, a C.I. = 0.7771, and a R.I. = 0.7869. The tree topology did not conflict

ween the tree from the total combined data set and the tree estimated for the

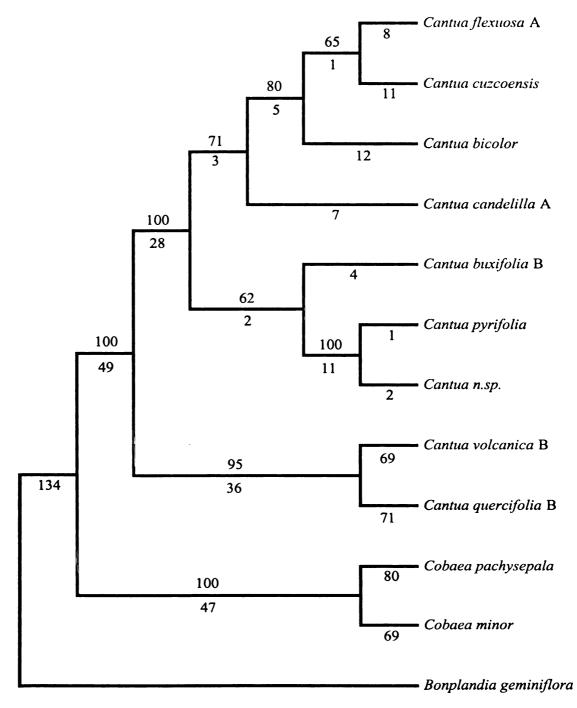
nparative analysis, with one exception, in the tree estimated for the comparative

alysis a sister relationship was resolved between C.



ure 14: Strict consensus of four most parsimonious trees based on the *Cantua* bined chloroplast (*trnT-trnL*, *trnL-trnF*, and partial *ndhF*) and nuclear ITS sequences the data set including indels. Tree length = 747, C.I. = 0.8133 and a R.I. = 0.8903.

There above the branches represent bootstrap support based on 10,000 replicates.



gure 15: Cantua tree estimated for comparative analysis. Single most parsimonious tree sed on the modified data set of chloroplast (trnT-trnL, trnL-trnF, partial ndhF) and clear ITS sequences excluding indels. Tree length is 650, with a C.I. = 0.7771 and a 1. = 0.7869. Numbers above the branches represent bootstrap support based on 10,000 clicates, numbers below the branches indicate branch lengths based on the MINF tion in Paup*.

buxifolia and C. pyrifolia/C. n.sp. This relationship, however, was weakly supported (bootstrap support of 62%).

Pollen Morphology

Description of Cantua Pollen – Cantua grains were large (49-93μm), spheroidal and pantoporate with mean pore size ranging from 3.78-14.4μm. The sexine was semitectate with irregularly shaped insulae, and insulae were evenly distributed over the surface of the pollen grain, with the exception of *C. flexuosa* where they were more sparsely and haphazardly distributed. Bacula were reticulate and the supratectal surfaces striated in all species. *Cantua quercifolia* and *C. volcanica* shared a synapomorphy of supratectal verrucae. A pollen diagnosis for each of the species of *Cantua* is given below (Figures 16, 17, & 18).

Cantua flexuosa (Figure 16, A & B). Pantoporate. Pollen grain spheroidal, 58 – 83 µm in diameter. Pores circular, 4.66-6.29 µm in diameter. Sexine semitectate (insulous).

Insulae irregular in shape, haphazardly distributed with considerable, irregular intratectal space. Bacula reticulate. Supratectal surface deeply striated.

Cantua cuzcoensis (Figure 16, C & D). Pantoporate. Pollen grain spheroidal, 63-81 µm diameter. Pores circular, 4.62-7.94 µm in diameter. Sexine semitectate (insulous).

Sulae irregular in shape, evenly distributed. Bacula reticulate. Supratectal surface eply striated.

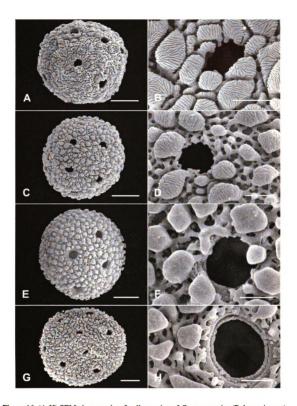


Figure 16. (A-H) SEM photographs of pollen grains of *Cantua* species (Polemoniaceae): (A & B) C. *flexuosa*; (C & D) C. *cuzcoensis*; (E & F) C. *bicolor*; (G & H) C. *candelilla*. Scale bars = 25 μ m (A, C, E, & G) & 5 μ m (B, D, F, & H). Voucher information available in Table 5.

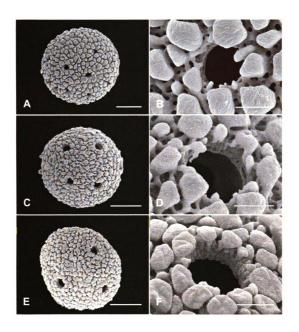


Figure 17. (A-F) SEM photographs of pollen grains of *Cantua* species (Polemoniaceae): (A & B) C. buxifolia; (C & D) C. pyrifolia; (E & F) C. n. sp.. Scale bars = 25 μ m (A, C, & E) & 5 μ m (B, D, & F).

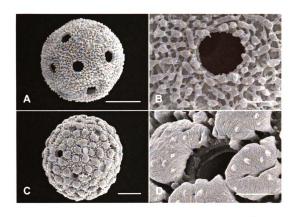


Figure 18. (A-D) SEM photographs of pollen grains of Cantua species (Polemoniaceae): (A & B) C. volcanica (C & D) C. quercifolia. Scale bars = 25 μm (A & C) & 5 μm (B & D).

Cantua bicolor (Figure 16, D & E). Pantoporate. Pollen grain spheroidal, 51.5-78.5 μm in diameter. Pores circular, 7.40-9.32 μm in diameter. Sexine semitectate (insulous). Insulae irregular in shape, evenly distributed. Bacula reticulate. Supratectal surface faintly striated.

Cantua candelilla (Figure 16, F & G). Pantoporate. Pollen grain spheroidal, 75.5-90.5 µm in diameter. Pores circular., 3.90-9.00 µm in diameter. Sexine semitectate (insulous). Insulae irregular in shape, evenly distributed. Bacula reticulate. Supratectal surface faintly striated.

Cantua buxifolia (Figure 17, A & B). Pantoporate. Pollen grain spheroidal, 49-89 µm in diameter. Pores circular, 3.78-11.11 µm in diameter. Sexine semitectate (insulous). Insulae irregular in shape, evenly distributed to randomly distributed with considerable, irregular intratectal spaces. Bacula reticulate. Supratectal surface faintly striated.

Cantua pyrifolia (Figure 17, C & D). Pantoporate. Pollen grain spheroidal, 64-86 µm in diameter. Pores circular, 4.86-14.4 µm in diameter, considerable variation in pore size among individual samples. Sexine semitectate (insulous). Insulae irregular in shape, evenly distributed. Bacula reticulate, density of bacula variable among individuals sampled. Supratectal surface faintly striated.

Cantua n. sp. (Figure 17, E & F). Pantoporate. Pollen grain spheroidal, 67-81 μm in diameter. Pores circular, 6.30-7.56 μm in diameter. Sexine semitectate (insulous).

Insulae irregular in shape, evenly distributed. Bacula reticulate with occasional stray free bacula. Supratectal surface faintly striated.

Cantua volcanica (Figure 18, A & B). Pantoporate. Pollen grain spheroidal, 54-69 µm in diameter. Pores circular, 4.10-8.22 µm in diameter, considerable variation in pore size among individual samples. Sexine semitectate (insulous). Insulae elongated, evenly distributed. Bacula reticulate with occasional stray free bacula. Supratectal surface faintly striated with supratectal verrucae.

Cantua quercifolia (Figure 18, C & D). Pantoporate. Pollen grain spheroidal, 78.5-93 µm in diameter. Pores circular, 5.38-8.62 µm in diameter, considerable variation in pore size among individual samples. Sexine semitectate (insulous). Insulae circular, evenly distributed. Stray free bacula. Supratectal surface faintly striated with supratectal verrucae.

Pollen Trait Evolution-Pollen grains in Cobaea have been extensively reviewed (Prather 1999) and an analysis of pollen in Bonplandia geminiflora is currently underway (Dickman and Prather, unpublished data). Cobaea pollen grains are pantoporate and spheroidal. The grains are large (112-195 μm) and have a reticulate sexine. The grains of the monotypic genus Bonplandia are also spheroidal and pantoporate. The grains are smaller in Bonplandia (60μm) and the sexine is striato reticulate.

Cantua grains were large (49-93µm), spheroidal and pantoporate, these traits are shared with the two outgroup taxa (Cobaea and Bonplandia). Cantua pollen grains share synapomorphic characters not present in the outgoup taxa: semitectate sexine with irregularly shaped insulae and reticulate bacula. Cantua quercifolia and C. volcanica shared a synapomorphy of supratectal verrucae (Figure 19).

Style Length and Pollen Grain Diameter Correlation—Raw data (Table 6) did not conform to the assumption for regression analysis of equal variance of residuals (p-value < 0.05). When the data were transformed, and branch length data were utilized, data conformed to all the assumptions of the analysis. The transformed analyses did not suggest a significant relationship between pollen grain diameter and style length based on the regression of standardized linear contrasts through the origin ($r^2 = 0.07$, slope = -0.05, p-value = 0.48). Standardized linear contrasts were analyzed on the transformed data using Pearson pair-wise correlations and the resultant correlation values were insignificant (-0.23 Pearson's pair-wise correlation, p-value = 0.58).

Table 6. Pollen diameter and style length of *Cantua* taxa included in the analysis. Whenever possible, 30 pollen grains from each population were sampled. Total number of styles per collection varied from 1-10.

Caraina	Number of	Style length (mm)		Pollen diameter (µm)	
Species	populations measured	Mean	Range	Mean	Range
Cantua bicolor	2	39.24	35.21-42.69	67.31	51.45-78.40
Cantua buxifolia	4	68.98	35.02-80.30	74.31	49.00-85.75
Cantua candelilla	2	65.69	56.86-73.95	81.96	73.50-90.65
Cantua cuzcoensis	2	53.54	48.50-57.96	72.80	63.70-80.85
Cantua flexuosa	2	34.10	30.92-39.67	71.34	58.80-83.30
Cantua n. sp.	2	42.78	38.27-45.95	75.91	68.60-80.85
Cantua pyrifolia	5	32.98	24.96-46.77	77.23	63.70-85.75
Cantua quercifolia	2	42.49	39.87-52.13	85.94	78.40-93.10
Cantua volcanica	2	21.16	16.42-24.62	62.45	53.90-68.60

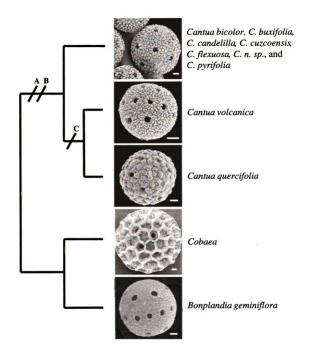


Figure 19. Diagram representing phylogenetic relationships found in the analysis of the Cantua combined chloroplast (tmT-tmL, tmL-tmF, partial ndhF) and nuclear ITS sequences and represented in Figure 14. Slash marks on the tree branches represent synapomorphic characters: A. semitectate sexine with irregularly shaped insulae, B. reticulate bacula, C. supratectal verrucae. Representative pollen grains are shown at the tree tips: Bonplandia geminiflora (Rzedowski 18144), Cobaea (C. trianae; Panero & Clark 3044), Cantua quercifolia (Wrigley 2), Cantua volcanica (West 7139), and seven remaining Cantua (Cantua bicolor; Cardenas 5479). Scale bar = 10 μm

CONCLUSIONS

The phylogenetic analysis of the nuclear ribosomal ITS and chloroplast trnT-trnL, trnL-trnF and ndhF regions in Cantua strongly supported the inclusion of the two species formerly placed in Huthia and verified the monophyly of the genus Cantua. Cantua volcanica (=Huthia coerulea) was most closely related to C. quercifolia. These two species were the sister group to the rest of Cantua. Cantua flexuosa, C. cuzcoensis, C. bicolor, C. candelilla, C. buxifolia, C. pyrifolia and C. n. sp were closely related and C. pyrifolia was sister to C. n. sp. Relationships between C. buxifolia and the other species in the clade were unresolved. The monophyly of C. buxifolia and its relationship to the other members of the genus Cantua could be investigated further with additional sampling within C. buxifolia and more informative molecular sequences. The work outlined here is an important link into research on the evolution of the Cobaeoideae and part of a large ongoing project to monograph the subfamily.

Pollen morphological work in *Cantua* supported the findings from the molecular phylogenetic analysis. Pollen characteristics within *Cantua* were highly conserved, pollen morphology examined was consistent within populations and among species. All nine species of *Cantua* have spheroidal pantoporate pollen with a semitectate sexine and insulae. This is a shared derived characteristic in the Polemoniaceae, giving further support to the subsumption of *Huthia* and the monophyly of the genus. The relationship between *C. quercifolia* and *C. volcanica* was supported by the synapomorphy of supratectal verrucae on the sexine of the pollen grains. The pollen work in *Cantua* confirms that pollen grain morphology is highly conserved and a valuable morphological

character for cladistic analysis. In addition, this research completes a comprehensive review of pollen grain morphology within the subfamily Cobaeoideae.

Plitmann and Levin (1983) addressed the potential relationship between pollen diameter and style length in a correlation analysis across the Polemoniaceae. They found a strong significant correlation between the traits within and among genera in the family. The results from their analysis supported the hypothesis of Delpino (Darwin 1896); pollen grain diameter is functionally integrated with style length. Lack of independence in the data set may have confounded the results of Plitmann and Levin's research. Data were collected from several groups of related species within the Polemoniaceae, and could be affected by non-independence due to shared evolutionary history (Cruden and Lyon 1985, Felsenstein 1985, and Harvey and Pagel 1991).

Plitmann & Levin (1983) found a non-significant correlation between pollen diameter and style length in five of the ten species of *Cantua*. My research was designed to address the correlation question using a comprehensive sample of the *Cantua* species and pairwise independent comparisons of the traits. I almost doubled the representative species used in the previous study, included the recently subsumed *C. volcanica*, and used independent contrasts to control for the effects of shared ancestry. Independent contrasts would not result in a correlation which was not present in the non-independent data. However, the increased, more inclusive sampling could result in a correlation and the independent contrasts would be necessary to address the non-independence of data in the *Cantua* data set. The results from my analysis of *Cantua* and the analysis of Plitmann

and Levin (1983) are consistent. Neither significant regression nor correlation was found in the genus *Cantua*, which failed to find a relationship between the diameter of the pollen grain and the length of the style in *Cantua*.

This comprehensive phylogenetic analysis of *Cantua* provided insight into relationships within the genus and confirmed the monophyly of the group. The investigation into pollen grain morphology substantiated the subsumption of Huthia into Cantua and provided further evidence for the monophyly of the genus. Both the pollen grain morphology and the phylogenetic analyses supported the relationship between C. volcanica and C. quercifolia. This pollen work also confirmed that pollen grain morphology is highly conserved and a valuable morphological character for cladistic analysis in Cantua. Despite comprehensive sampling and a well-supported phylogenetic hypothesis, pollen diameter and style length were found to be unrelated in Cantua. The work on pollen grain diameter and style length is the first step in a family-wide comparison of this correlation using independent contrasts to control for the effects of shared ancestry. The study outlined here is an important link into research on the evolution of the Cobaeoideae, molecular analysis using the same chloroplast and nuclear sequences and identical pollen grain preparation for SEM and light microscopy will be used in a large ongoing project to monograph this sub-family.

CHAPTER 3

EVOLUTION OF VARIANCE-COVARIANCE STRUCTURE OF FLORAL MORPHOLOGY AMONG MEMBERS OF THE MUSTARD FAMILY (BRASSICACEAE)

INTRODUCTION

Individual floral traits may not evolve independently. Flowers are complex structures made up of several functionally integrated traits which work together to aid in successful plant reproduction (Darwin 1862). Often groups of traits will evolve together due to genetically, developmentally, and functionally integrated constraints (Arnold 1992). Consequently, how diverse floral traits evolve as a unit is of tremendous interest to botanists and evolutionists alike.

The matrix of genetic variance and covariance (G-matrix) represents the amount of heritable variation in a trait and its genetic covariation with other traits. It is an important concept because selective forces acting on one trait will cause evolution of a second trait even if the change infers no specific selective advantage. The G-matrix represents the type and quantity of genetic variation available to natural selection. While the G-matrix may confer a selective advantage, over time the amount of genetic variation and covariation in a population could constrain the direction of genetic drift and dictate response to selection. Genetic variation-covariation can affect both the rate and direction of evolutionary change (Lande 1976, 1979, Lande and Arnold 1983, Conner and Via 1993, Steppan 1997a, 1997b, Arnold and Phillips 1999, Phillips and Arnold 1999, Badyaev and Hill 2000, Arnold et al. 2001, and Steppan et al. 2002).

The potential constraint imposed by the G-matrix has been an issue under intense scrutiny. Futuyma (1995) studied the evolution of the host affiliation in the leaf beetle genus *Ophraella* and found that evolution of ecologically important traits were

influenced by genetic constraints. Schluter (1996) examined several vertebrate taxa to determine the long term effects of genetic variation on morphological divergence. He found that among the species he studied, morphological variation had been biased in the multivariate direction of greatest additive genetic variance for the last four million years. Both studies support the theory that quantitative genetic variance and covariance can constrain evolution over time.

The degree to which the G-matrix is conserved is an important unsolved problem in evolutionary biology. Lande (1979) pointed out how consistency in the G-matrix would allow us to make inferences about patterns of selection. Lande (1979) developed an important equation for the measuring changes in traits in evolutionary time:

$$\Delta \bar{z} = G\beta$$

 $\Delta \bar{z}$ is the change in phenotypic traits across one generation. G is the matrix of genetic variances and covariances among traits. β is the selection gradient. So the rate of $\Delta \bar{z}$ is the product of additive genetic variance-covariance and the strength of selection and can be used to predict the response to selection over time, including the effects on correlated traits. Lande (1979) predicted that G-matrices would remain constant over time and could therefore be used to predict the response of natural populations to selection and drift. If the G-matrix is stable over time, then scientists can predict the response of a population to selection and reconstruct ancestral events that have lead to speciation (Arnold 1992, Arnold et al. 2001, and Steppan et al. 2002).

Several empirical studies have been conducted using both selection and drift experiments and comparative analyses to investigate the stability and evolution of the G-matrix (Arnold and Phillips 1999, Phillips and Arnold 1999, Roff and Mousseau 1999, Roff 2000, and Steppan et al. 2002 and references therein). Both matrix similarity and dissimilarity have been found when comparing G-matrices between populations, subspecies and species and the closer the taxonomic relationship the more stable the G-matrixes have been. Additional studies, with similar results, have been conducted using the phenotypic variance-covariance matrix (P-matrix; Steppan 1997a, 1997b, Badayeav & Hill 2000, Ackermann and Cheverud 2000, Dodd at al. 2000, and Baker and Wilkinson 2003). While the P-matrix is not equivalent to the G-matrix, phenotypic variance-covariance has genetic variance-covariance as a component and can give insight into the heritable genetic variation-covariation available (Lofsvold 1986, Cheverud 1988, Arnold 1992, Steppan 1997a, 1997b, Wait and Levin 1988, Badyaev & Hill 2000, Arnold et al. 2001, and Steppan et al. 2002).

Baker and Wilkinson (2003) and was the first to look at the evolution of the P-matrix using a phylogenetic hypothesis at the species level or higher. This study showed equality of the P-matrices among three species of *Diasemopsis* (stalk-eyed flies). In reviewing the findings from studies of G and P-matrices, only the Baker and Wilkinson (2003) study found proportionality or equality in the shared structure among matrices when comparing taxa at the subspecies or higher taxonomic level (see review in Steppan et al. 2002).

The evolution of G-matrix structure is still not well understood, particularly in an historical context (Badyaev and Hill 2000). The question of whether the G-matrix remains stable over evolutionary time remains an unresolved issue (Arnold et al. 2001, Steppan et al. 2002). Additional investigations into the nature, stability, and historical pattern of change in the G-matrix are required. Understanding how variance-covariance structures evolve over time will provide insight into how populations evolve and species diverge (Lande 1979, 1985; Price and Grant 1985, Grant and Grant 1995, Cheverud 1996, Arnold and Phillips 1999, Camara and Pigliucci 1999, Roff et al. 1999, Badyaev and Foresman 2000, Badyaev and Hill 2000, Arnold et al. 2001, and Steppan et al. 2002).

This study examined the relationship among P-matrices across the family Brassicaceae using a strong phylogenetic hypothesis. The Brassicaceae study system was chosen for several practical reasons: investigative work on G-matrices, genetic correlations, and phenotypic correlations have already been conducted in several members of the family (Conner and Via 1993, Conner and Sterling 1995, 1996, Kercher and Conner 1996, and Conner 2003 and references therein), preliminary research on phylogentic relationships in the Brassicaceae had been conducted by Galloway et al. (1998) and were available for analysis, species in the family were widely distributed and easily collectable, and the floral characters were conserved and straightforward to measure across the family.

The Brassicaceae includes several economically and agronomically important plants, most notably several *Brassica* species, which include such diverse crops as canola

(Brassica napus), broccoli (Brassica oleraceae var. italica), cabbage (Brassica oleraceae var. capitata), and turnips (Brassica rapa var. rapa). Several species are cultivated as ornamentals including Lobularia maritima (sweet alyssum), Iberis (candy tuft), and Matthiola (stock). The Brassicaceae also contains the model species Arabidopsis thaliana and the problematic invasive plant Alliaria petiolata (garlic mustard). The family has approximately 340 genera and as many as 3350 species (Al-Shehbaz 1984). Brassicaceae are widely distributed, grow predominantly in the temperate northern hemisphere, and are most prevalent in arid regions. The largest concentrations of Brassicaceae species occur in southwestern/central Asia and the Mediterranean (Al-Shehbaz 1984).

In 1998 the Angiosperm Phylogeny Group advocated lumping the Capparaceae and Brassicaceae sensu stricto into one family, the Brassicaceae. This nomenclatural change was based on the paraphyly of the Capparaceae in the morphological cladistic analysis of Judd et al. (1994) and the chloroplast *rbc*l molecular phylogenetic analysis of Rodman et al (1996). Recent nuclear 18S molecular phylogenetic analysis (Rodman et al 1998) and chloroplast *ndh*F and *trnL-trn*F molecular phylogenetic analyses (Hall et al. 2002) have revisited the issue of relationship and monophyly in the Capparaceae and Brassicaceae sensu stricto. Hall et al. (2002) advocated the recognition of the three families:

Capparaceae, Cleomaceae, and Brassicaceae, with the Cleomaceae most closely related to the Brassicaceae. The most recent update by the Angiosperm Phylogeny Group (2003), recognizes these studies, and advocates dissolving the Brassicaceae sensu APG 1998, and recognizing the three families as suggested by Hall et al. (2002). In addition to the

molecular phylogenetic evidence for the narrowly, and traditionally, circumscribed Brassicaceae, the family has some distinct floral characteristics that distinguish the flowers from its close relatives in the Capparaceae and Cleomaceae (Hall et al. 2002). These features include four alternating clawed petals arranged in the form of a cross and tetradynamous stamens.

Several attempts have been made to establish a tribal classification of the Brassicaceae (Hayek 1911, Schulz 1936, Janchen 1942, and Al-Shehbaz 1984). Most of this work has been based on morphological characters, most notably fruit morphology, pubescence, and cotyledon orientation (Al-Shehbaz 1984). In his morphological treatment of the Brassicaceae in the southeastern United States, Al-Shehbaz (1984), used a modification of the Janchen (1942) system and recognized seven major tribes, though he suspected that not all seven tribes were naturally monophyletic (Al-Shehbaz 1984, Price et al. 1994, Galloway et al. 1998). Koch et al. (2001) did the most comprehensive molecular systematic analysis of the Brassicaceae to date, using chloroplast matK and nuclear Chs sequences, and reviewed the tribal classification systems of Hayek (1911), Schulz (1936), and Janchen (1942). The study concluded that many of the tribal and subtribal classifications were artificial and "a new classification of crucifers based on molecular data would be desirable" (Koch et al. 2001; 541). No consensus has been reached on relationships among the major clades within the Brassicaceae and only a handful of studies have been conducted to investigate relationships across the family (Price et al. 1994, Galloway et al. 1998, Koch et al. 2001, and Hall et al. 2002).

This study incorporates a phylogenetic hypothesis of the family Brassicaceae and a study of floral morphological traits to test Lande's (1979) theories about stability in variance - covariance structure over time. The goal of this research was to test the hypothesis that P-matrix stability within the Brassicaceae followed a stepwise pattern of decrease in similarity with decreased relatedness corresponding to taxonomic rank within the phylogeny. This was the first attempt to investigate P-matrix relationships across a family-level phylogeny.

ΓĊ

Sta

the

Res

Sch

direc

MATERIALS AND METHODS

Phylogenetic Analysis-

Sampling – Fresh leaf or floral material from field collections or greenhouse material was collected, dried on silica gel, and stored at -80 degrees. A total of 25 individual specimens were used in the phylogenetic analysis. Eight DNA sequences of the partial ndhF coding region were previously published in Galloway et al. (1998) and available on GENBANK (Table 7).

Outgroup -Cleome spinosa was chosen as the outgroup based on the ndhF phylogeny of Galloway et al. (1998) and evidence from the chloroplast ndhF gene and trnL-trnF spacer sequences of Hall et al. (2002).

Analysis - DNA was extracted using the techniques of Loockerman and Jansen (1996) and purified using the Schleicher & Schuell Elu-quick DNA Purification Kit (Keene, NH). The *ndh*F coding region was amplified using the forward primer ndhF 1318 and the reverse primer ndhF 2110R of Olmstead and Sweere (1994).

Standard amplification protocols were used, including reaction mixture components and the PCR profile (Prather et al. 2002), and amplifications were performed on a MJ Research PTC-100 thermalcycler. PCR products were gel purified using the Schleicher & Schuell Elu-quick DNA Purification Kit, (Keene, NH) and then sequenced in both directions using an ABI-373 automated sequencer. Sequencing reactions were conducted

Table 7. Voucher and location information for material used in the ndhF sequencing analysis of Brassicaceae. In location column, * denotes specimens grown in greenhouses at Michigan State University.

Species	DNA	Location
Aethionema arabicum (L.) Rothm.	Monfils & Conner 66 (MSC)	Cultivated seed from Jardin Botanique de Nancy (France)*
Alliaria petiolata Cavara & Grande	Conner, Sahli & Fant 15 May 2002 (MSC)	Michigan: Gull Lake, Kellogg Biological Station
Arabidopsis lyrata (L.) O'Kane & AL-Shehbaz	Monfils & Conner 68 (MSC)	Michigan: Saugatuk Dunes State Park
Arabidopsis thaliana (L.) Heynh.	AF064654	GenBank submission from Galloway et al. 1998
Arabis hirsuta DC.	Conner & Stewart May 2000 (MSC)	Cultivated seed from Munster Botanical Garden*
Barbarea verna (Mill.) Asch.	Conner, Monfils & Stewart summer 2000 (MSC)	Michigan: Gull Lake, Kellogg Biological Station
Barbarea vulgaris R.Br.	AF064652	GenBank submission from Galloway et al. 1998
Brassica napus L.	Monfils et al.70 (MSC)	Cultivated from Cascade Rape Seed *
Brassica nigra (L.) Koch	Monfils & Conner 56 (MSC)	Illinnois: Urbana Junkyard*
Brassica oleraceae L.	AF064647	GenBank submission from Galloway et al. 1998
Brassica rapa L.	Monfils & Conner 82 (MSC)	Crucifer Genetics Cooperative*
Cakile edentula (Bigelow) Hook.	Conner & Stewart Aug 2000 (MSC)	Michigan: Saugatuk Dunes State Park
Capsella bursa-pastoris (L.) Medik.	AF2064653	GenBank submission from Galloway et al. 1998
Cardamine concatenata (Michx.) O. Schwarz	Conner, Sahli & Fant 24 Apr 2002 (MSC)	Michigan: East Lansing, Hudson Woodlot, MSU
Cleome spinosa Jacq.	Monfils & Conner 67 (MSC)	Cultivated seed from Thompson & Morgan *
Hesperis matronalis L.	Monfils & Conner 48 (MSC)	Michigan: Gull Lake, Kellogg Biological Station
Iberis L.	Monfils & Conner 61 (MSC)	Cultivated seed from Thompson & Morgan *
Lobularia maritima (L.) Desv.	Monfils & Conner 71 (MSC)	Cultivated seed from Thompson & Morgan *
Matthiola R.Br.	Monfils & Conner 54 (MSC)	Cultivated seed from Thompson & Morgan *
Nasturtium officinale R.Br.	AF064659	GenBank submission from Galloway et al. 1998
Raphanus raphanistrum L.	Conner & Stewart 15 May 2002 (MSC)	New York: Binghamton *
Raphanus sativus L.	Monfils & Conner 49 (MSC)	California*
Sisymbrium altissimum L.	AF064648	GenBank submission from Galloway et al. 1998
Stanleya pinnata Britton	AF064655	GenBank submission from Galloway et al. 1998

:

b

W

 F_{i0}

c0[]

using the AmpliTaq DNA Dye Terminator Cycle Sequencing reagents (PE Applied Biosystems, Norwalk, CT).

Edited sequences were aligned using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, MI). Insertion deletion events were treated as missing data. Parsimony methods were implemented using PAUP* (Version 4.0b4; Swofford 2000). Heuristic searches were performed using the TREE BISECTION RECONNECTION, and MULTREES options. Bootstrap analysis was conducted using 10,000 replicates with 200 random addition-sequence replicates per bootstrap replicate.

Floral Morphological Trait Measurements-

Sampling-Floral morphological measurements were made on populations from twenty-four species within the Brassicaceae and one species from the Cleomaceae. Taxon selection was based on the phylogeny of Galloway et al. (1997) and discussions with Robert Price (personal communication). Two species, *Brassica nigra* and *Raphanus raphanistrum*, were sampled from two populations each. Study populations were selected based on availability of seeds and natural populations (Table 8).

Floral measurements- Flowers were measured from wild populations whenever possible.

When wild populations were unavailable, seeds from wild populations, research

collections, or commercial seed companies were acquired and grown to flowering. If

Table 8. Brassicaceae species measured for floral morphological characters, with sample locality, cultivation site, sample size and year measured. Species measured on cultivated seed are noted in sample locality column. Species with no cultivation information were measured in the field on natural populations.

sapado	Sample Locality	Cultivation Site	Z	Year
Aethionema arabicum (L.) Rothm.	France: Jardin Botanique de Nancy	Greenhouse - Michigan State U.	50	2000
Alliaria petiolata Cavara & Grande	Michigan: Kellogg Biological Station	1	20	2002
Arabidopsis lyrata (L.) O'Kane & AL-Shehbaz	Michigan: Saugatuck Dunes State Park	•	48	1997
Arabidopsis thaliana (L.) Heynh.	Michigan: Kellogg Biological Station		47	1997
Arabis hirsuta DC.	Munster Botanical Gardens	Greenhouse - Michigan State U.	21	1999
Barbarea verna (Mill.) Asch.	Michigan: Kellogg Biological Station	•	20	2000
Barbarea vulgaris R.Br.	Michigan: Kellogg Biological Station	1	49	1997
Brassica napus L.	Commercial Seed - Cascade Rape Seed	Phillips Tract - U. of Illinois	20	1991
Brassica nigra (L.) Koch	Illinois: Urbana, Junkyard	1	50	1661
Brassica nigra (L.) Koch	Illinois: Urbana, Yard Waste Disposal Site		48	1992
Brassica oleraceae L.	Crucifer Genetics Cooperative	Greenhouse - Michigan State U.	20	1999
Brassica rapa L.	Crucifer Genetics Cooperative	Greenhouse - Michigan State U.	50	1999
Cakile edentula (Bigelow) Hook.	Michigan: Saugatuck Dunes State Park	•	20	1998
Capsella bursa-pastoris (L.) Medik.	Michigan: Kellogg Biological Station	•	20	1997
Cardamine concatenata (Michx.) O. Schwarz	Michigan: Michigan State University	•	45	2002
Cleome spinosa Jacq.	Commercial Seed - Thompson & Morgan	Greenhouse - Michigan State U.	22	1997
Hesperis matronalis L.	Illinois: Urbana, Phillips Tract	•	50	1661
Iberis L.	Commercial Seed - Thompson & Morgan	Greenhouse - Michigan State U.	4 4	1997
Lobularia maritima (L.) Desv.	Commercial Seed - Thompson & Morgan	Greenhouse - Michigan State U.	40	1661
Matthiola R.Br.	Commercial Seed - Thompson & Morgan	Greenhouse - Michigan State U.	41	1997
Nasturtium officinale R.Br.	Michigan: Kalamazoo Nature Center	•	20	1998
Raphanus raphanistrum L.	New York: Binghamton	Greenhouse – U. of Illinois	340	1989
Raphanus raphanistrum L.	New York: Binghamton	Phillips Tract – U. of Illinois	65	1992
Raphanus sativus L.	California:	Greenhouse - Michigan State U.	28	1998
Sisymbrium altissimum L.	Michigan: Mackinac City	•	41	1998
Stanleya pinnata Britton	Utah: Lake Powell	Greenhouse - Michigan State U.	35	1999

available, measurements were made on a minimum of 50 plants per population. A range of 22-340 plants per population were measured for a total of 1539 plants. One to three flowers per plant were measured. If more than one flower per plant was measured, the mean value was calculated and used in the analysis. Measurements were made using digital calipers and recorded to the nearest 0.01 mm. Six quantitative characters were measured: claw length, limb length, limb width, short filament length, long filament length, and pistil length (Figure 20 and 21)

Populations of Raphanus raphanistrum, Brassica napus, B. nigra, and Hesperis matronalis were measured according to the methods outlined in Conner & Sterling (1995). For all other populations, measurements were taken on the most apical, fully developed, intact flower. Only flowers that had not begun to senesce were measured. In wild populations, plants and seeds were sampled at set intervals along transects encompassing the population range. In the greenhouse, when the number of flowering plants exceeded 50, plants were selected for measurement using a random number table.

P-matrix construction – The Phenotypic variance-covariance matrix was constructed from the morphological data sets of six floral measurements recorded for the plants measured in the 26 populations. Data was imported into the JMP statistical package (SAS Institute Inc., Cary, NC) to construct a variance-covariance matrix using Multivariate Analysis-Covariance Matrix. The resultant matrix has variances for the six variables on the diagonal and covariances among the six variable above and below the diagonal (Table 9).

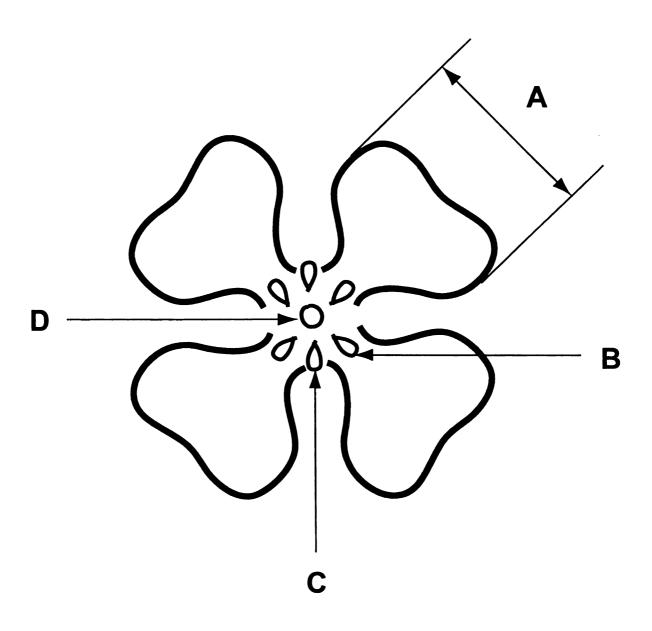


Figure 20. Schematic of generic Brassicaceae flower viewed from the top. Figure illustrates the cruciform petal arrangement. Letter A shows the floral measurement for limb width. The six tetradynamous stamens are shown: B shows one of four long antepetalous stamens located opposite each petal, C shows one of two outer short stamens borne between the petals. Letter D shows the central location of the pistil.

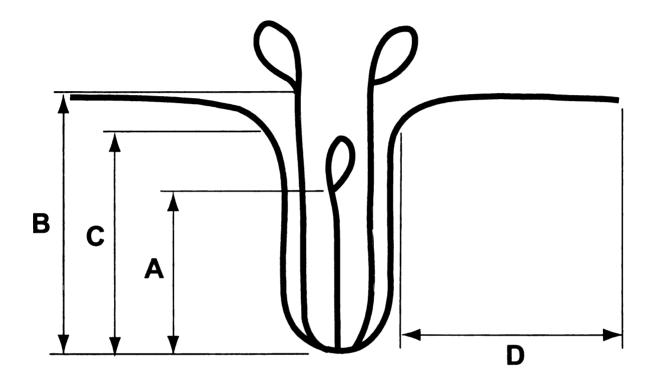


Figure 21. Schematic representation of a generic Brassicaceae flower in lateral cross-section. Pistil is not shown and would be located in the flower center. Only three stamens are shown (two long and one short) to illustrate the tetradynamous stamens. Letters in the figure refer to floral measurements: A=short filament, B=long filament, C=claw length of the petal, D=Limb length.

Table 9. Example of the Phenotypic variance-covariance matrix for the six variables in the *Nasturtium officinale* data set. The variance for each variable is along the diagonal and the covariances are on the upper and lower triangle off the diagonal.

	Limb length	Limb Width	Claw length	Short filament	Long filament	Pistil
Limb length	0.11440	0.06869	0.07314	0.03818	0.05610	0.06642
Limb Width	0.06869	0.06361	0.05202	0.02987	0.04101	0.05925
Claw length	0.07314	0.05202	0.13396	0.06672	0.06858	0.07521
Short filament	0.03818	0.02987	0.06672	0.12914	0.06953	0.06974
Long filament	0.05610	0.04101	0.06858	0.06953	0.08878	0.06358
Pistil	0.06642	0.05925	0.07521	0.06974	0.06358	0.16584

Hierarchical Matrix Comparisons - Comparisons of phenotypic variance-covariance matrices were made using the common principal component (CPC) analysis described by Flury (1988) and implemented using CPCRand (Phillips 1998, Phillips and Arnold 1999). CPCRand allows the simultaneous comparison of multiple matrices and uses randomization procedures to circumvent problems in the data set associated with the assumption of multivariate normality and degrees of freedom. Matrices were compared among related taxa in progressively more inclusive clades based on the phylogeny of the Brassicaceae. CPCRand could not accommodate comparisons of more than ten matrices and clades including more than ten populations were not included in this analysis. A total of sixteen matrix comparisons were made.

The Flury hierarchy (1988) goes beyond the determination of whether matrices are equal or unequal. The "Jump-Up" approach described by Phillips and Arnold (1999) starts by testing the matrices for related structure in the partial common principal components (PCPC) and then the common principal components for all the eigenvectors (CPC). If related structure in all the eigenvectors is not rejected, then the matrices are compared for proportional eigenvalues (proportional). The final step in the hierarchy is equality of eigenvectors and eigenvalues (equality). Each level in the hierarchy is inclusive of all the lower levels and each level is tested against the model of unrelated structure. Significance testing starts at the level of PCPC1 and proceeds up the hierarchy until a significant p-value is obtained. Once a significant p-value is determined, that level of the hierarchy is rejected, and the next lower level is accepted (Figure 22; Phillips & Arnold 1999).

The "Jump Up" approach is dependent on acceptance of PCPC1 in order to test any higher level in the hierarchy. In the analysis using CPCRand, eigenvectors are initially ordered by decreasing eigenvalues. A significant P-value for the first eigenvector discontinues the analysis and may preclude any investigation into matrix similarity among other eigenvectors with smaller eigenvalues. The CPCRand program has a reordering option which allows the user to change the order of the PCPCs and analyze additional eigenvectors for similarity. Houle et al. (2002) recommend reordering PCPCs to examine the possible similarity among the eigenvectors and this method was used by Baker and Wilkinson (2003) when comparing stalk-eyed flies.

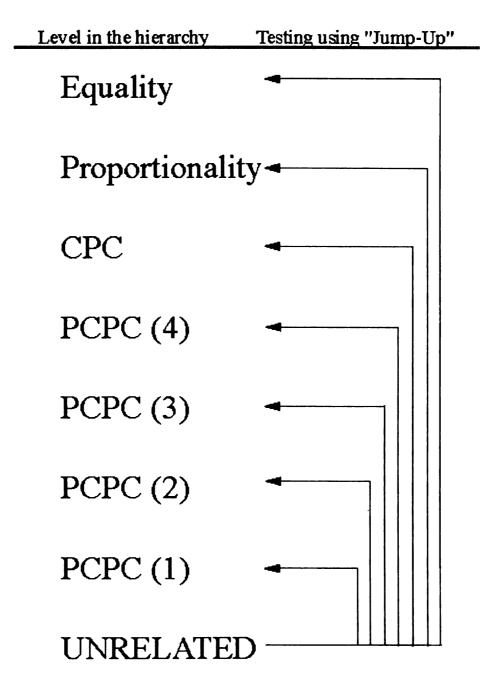


Figure 22. A representation of the "Jump Up" approach to matrix comparisons using the Flury hierarchy. This hierarchy represents the steps in the Brassicaceae six trait by six trait matrix comparison. Each level in the hierarchy is inclusive of all the lower levels and each level is measured against the model of unrelated structure. Testing starts with PCPC1 and moves up the hierarchy. PCPC is the partial common principal component model for successively higher numbers of eigenvectors from one to four, CPC is common principal components for all six eigenvectors, proportionality indicates that the eigenvectors are identical and the eigenvalues are proportional, and equality has equal eigenvectors and eigenvalues (Phillips & Arnold 1999).

Reordering of the PCPC was done on all sixteen matrix comparisons in the Brassicaceae data set. In the reordered analysis, instead of proceeding through the PC in the order of highest eigenvalue to lowest, the hierarchy of matrix comparisons skipped the first PC (the PC with the largest eigenvalue) started with the 2nd PC (the PC with the second largest eigenvalue) and proceeded through the remaining smaller PCs in order of largest to smallest eigenvalue. The first PC was the final component and included in the CPC level in the hierarchy. PCA analysis was completed for each individual population and eigenvectors were compared among populations where reordering had considerable effect on the matrix comparisons in the CPC analysis.

In phenotypic matrix comparisons the first PC is most often associated with size. In this analysis there was a great disparity in overall flower size across the Brassicaceae. To investigate the potential size disparity I ran a principal component analysis on the data set including all measurements in each population. The average first principal component was tabulated for each population based on the individual first PC values for measured flowers in each population. These values can be compared to examine the overall size difference among flowers assuming the first principal component is size.

RESULTS

Phylogenetic hypothesis – The aligned ndhF region was 771 bp long, with 113 parsimony informative-characters. The analysis resulted in 12 most parsimonious trees with a length of 362, C.I. = 0.5975, and a R.I. = 0.7328 (Figure 23). The Aethionema clade was strongly supported (100%) and basal to the well supported clade of the remaining Brassicaceae (99%). Three additional clades of interest were resolved in the strict consensus tree: Hesperis matronalis - Matthiola (87% bootstrap support), Arabidopsis lyrata – Cardamine concatenata (63% bootstrap support), and Brassica nigra - Stanleya pinnata (99% bootstrap support).

Within the Arabidopsis lyrata – Cardamine concatenata clade, Arabidopsis lyrata was sister to A. thaliana (95%) and these taxa formed a clade sister to C. bursa-pastoris (99% bootstrap support). The clade containing Barbarea verna and B. vulgaris was strongly supported (96%) and the clade of N. officinale and C. concatenata had moderate support (78%). Support for the clade including B. verna, B. vulgaris, N. officinale and C. concatenata was strong (89%).

The clade of *Brassica nigra - Stanleya pinnata* was comprised of two smaller clades: The *S. altissimum* and *S. pinnata* clade had strong support (95%), and the Brassiceae clade (*Brassica nigra - Brassica rapa*) had weak support (71%). The *B. nigra* and *C. edentula* clade had weak bootstrap support (52%). The clade of the two *Raphanus* species had moderate support (83 %) and the clade containing, *B. oleraceae*, *B. napus*, and *B. rapa*, was unresolved and strongly supported (94%). The clade consisting of *Raphanus* and the unresolved *Brassica* was strongly supported (93%).

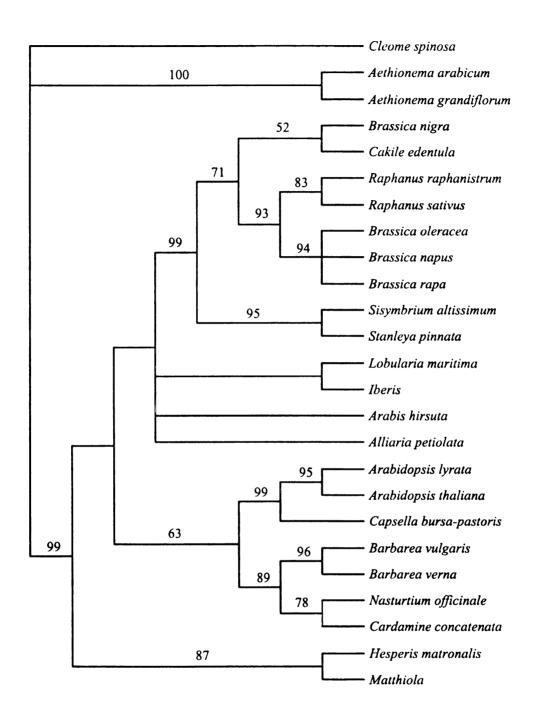


Figure 23. Strict consensus of 12 most parsimonious trees from the Brassicaceae based on parsimony analyses of 771 base pairs of *ndh*F coding region. Phylogeny has a tree length of 362, a C.I.^e. = 0.732, and a R.I. = 0.733. Numbers above the branches represent bootstrap percentages based on 10,000 replicates.

Matrix comparisons –Sixteen comparisons were made among matrices. Nine comparisons showed some level of matrix similarity without reordering. After reordering the first principal component, all 16 matrix comparisons had common structure at some level in the hierarchy of matrix association. Comparisons of matrix similarity were equal for two intraspecific assessments, and two of the four within genera comparisons had equal or proportional matrices. One of seven pair-wise comparisons among genera had a level of shared structure above CPC. Of the three comparisons done among more than two genera, two were proportional and one shared a common structure for only one PC (Table 10 and Figure 24).

The two populations of *Brassica nigra*, Junkyard and Yard Waste Disposal Site, shared two principal components in common in the unordered analysis. The hypothesis of three PC in common was rejected (P=0.014). The trait loadings from the first PCs from the individual population level analysis were reviewed; *Brassica nigra* – Junkyard had a lower claw length weight (0.28599) on the first PC than *B. nigra* - Yard Waste Disposal Site (0.38854; Table 11). Principal components were reordered in the analysis and equality was found between the two P-matrices. When both populations of *Raphanus raphanistrum* were compared the matrices were found to be equal matrices whether the principal components were reordered or not.

Table 10. The P-value results of the CPCRand analysis in the Brassicaceae. P-value indicated without reordering principal components, followed by the value with reordering. Species correspond to populations listed in Table 8.

	Partial	Partial	Partial	Partial			
	Common	Common	Common	Common	Common		
Matrixes compared	Principal	Principal	Principal	Principal	Principal	Proportional	Equal
	Component	Component	Component	Component	component		
	Ξ	(2)	(3)	(4)			
Brassica nigra - two populations Junkyard and Yard	0.10560/	0.16620/	0.01400/	0.04520/	0.06390	0.20870	0.25150
Waste Disposal Site	0.24000	0.44670	0.46050	0.06860			
B. nigra populations and Cakile edentula	0.00010/	0.00010/	0.00010/	/0900000	0.00000	0.01060	0.00000
	0.21320	0.15750	0.05320	0.01120			
Raphanus raphanistrum - two populations Greenhouse	0.37740/	0.61210/	/0009910	0.78340/	0.80970	0.36140	0.10230
and Field	0.55440	0.71290	0.88360	0.75030			
R. raphanistrum populations and Raphanus sativus	0.05070/	0.17650/	0.33800/	0.54240/	0.56400	0.33690	0.00020
	0.79320	0.93450	0.99270	0.88860			
Brassica oleraceae, Brassica napus, and Brassica rapa	0.35190/	0.05300/	0.00310/	0.00560/	0.00680	0.00000	0.00000
	0.39930	0.000050	0.01000	0.00150			
B. oleraceae, B. napus, B rapa, R raphanistrum, and R	/0060000	0.00300/	/0/00000	/0800000	0.00180	0.00000	0.00000
sativus	0.27890	0.01130	0.01000	0.00440			
B. oleraceae, B. napus, B rapa, R raphanistrum, R	/0000000	0.00010/	0.00000	/000000	0.00000	0.00000	0.00000.0
sativus, B. nigra populations, and C. edentula	0.08600	0.01750	0.00010	0.00000			
Sisymbrium altissimum and Stanleya pinnata	0.01070/	0.02100/	0.11210/	0.20260/	0.19880	0.00030	0.00000
	0.13270	0.64970	0.08930	0.12650			
Lobularia maritima and Iberis	/0000000	/000000	/0000000	/000000	0.00000	0.00000	0.00000
	0.30450	0.18100	0.15820	0.02690			
Arabidopsis lyrata and Arabidopsis thaliana	0.11000/	0.11570/	0.06110/	0.02480/	0.03000	0.02230	0.00210
	0.18680	0.25290	0.02580	0.01640			
A. Iyrata, A. thaliana, and Capsella bursa-pastoris	0.01240/	0.02010/	0.02700/	0.02390/	0.00920	0.00160	0.00000.0
	0.06760	0.07320	0.00400	0.00410			

Table 10 (cont'd)

	Partial	Partial	Partial	Partial			
	Common	Common	Common	Common	Common		
Matrixes compared	Principal	Principal	Principal	Principal	Principal	Proportional	Equal
	Component	Component	Component	Component	component		
	(1)	(2)	(3)	(4)			
Barbarea vulgaris and Barbarea verna	0.25830/	0.09820/	0.24020/	0.28450/	09208:0	0.22660	0.10610
	0.53490	0.50880	0.59780	0.39700			
Nasturtium officinale and Cardamine concatenata	0.18860/	0.37410/	0.69460/	0.56340/	09290.0	0.36980	0.00000
	0.67100	0.06290	0.11970	0.04840			
B. vulgaris, B. verna, N. officinale and C. concatenata	0.29780/	0.64470/	0.74630/	0.85240/	0.54000	0.76710	0.00000
	0.96120	0.60460	0.71630	0.46640			
A. lyrata, A. thaliana, C. bursa-pastoris, B. vulgaris, B.	0.35220/	0.40440/	0.55600/	0.58010/	06898.0	0.33730	0.00000
verna, N. officinale and C. concatenata	0.61420	0.35930	0.30040	0.25690			
Hesperis matronalis and Matthiola	/000000	0.00010/	0900000	0.00100/	0.00190	0.00000	0.00000
	0.11830	0.24930	0.37300	0.00140			

Figure 24. Strict consensus of tree for the Brassicaceae (see figure 23) Numbers above the branches represent bootstrap percentages based on 10,000 replicates. Notations below the branches indicate level of matrix similarity among species in the clade above the node. Matrix similarity is indicated without reordering principal components, followed by the value with reordering. Notations for similarity among matrices are as follows: U = unrelated matrix structure, PCPC # = partial common principal component followed by the number of principal components in common between the matrices, CPC = all principal components have shared structure among matrices, P = matrices are proportional and E = equality among matrices.

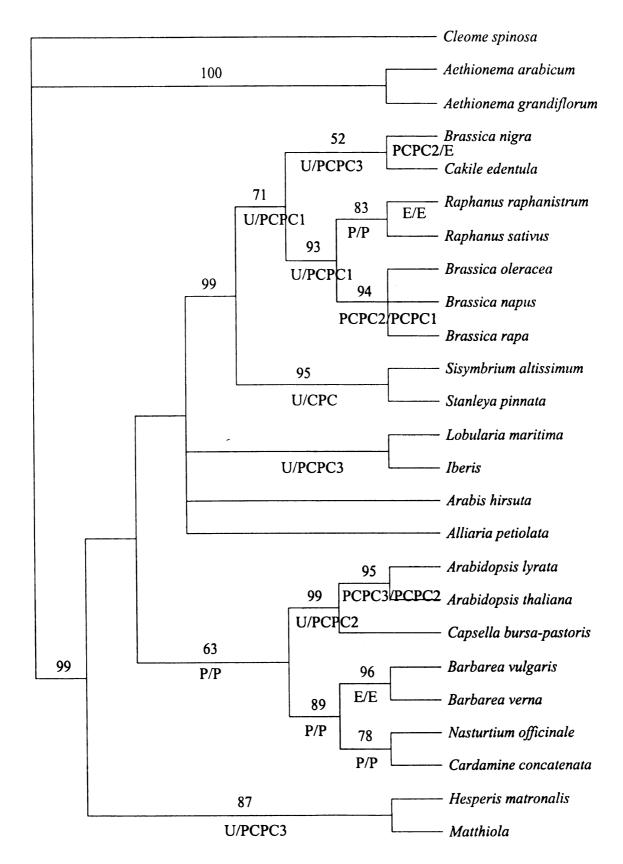


Figure 24.

Table 11. Results from the principal component analysis of populations in the Brassicaceae. Eigenvalues with percent variation explained, and eigenvectors are given. Principal components are denoted as "PC" and ordered by decreasing eigenvalues.

Aethionema arab	icum					
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	2.4429	1.4401	0.8811	0.6824	0.3579	0.0955
Percent	40.7150	24.0022	14.6849	11.3738	5.9654	3.2587
Eigenvectors						
Claw Length	0.42235	-0.51436	0.30469	-0.09881	0.19075	0.64658
Limb Width	0.29559	0.37414	0.69189	-0.45631	-0.16321	-0.24308
Limb Length	0.12228	0.77109	-0.10780	0.18014	0.20898	0.55021
Anther (S)	0.48071	0.02203	-0.51354	-0.26786	-0.65086	0.09659
Anther (L)	0.54013	0.01448	-0.32011	-0.14104	0.65011	-0.40379
Pistil	0.44325	-0.01364	0.22503	0.81113	-0.21689	-0.21849
Alliaria petiolata	•					•
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	4.5930	0.6266	0.2830	0.2303	0.1464	0.1208
Percent	76.5497	10.4438	4.7159	3.8379	2.4402	2.0126
Eigenvectors	1					
Claw Length	0.35009	0.75867	0.46052	0.19729	0.22509	0.01405
Limb Width	0.42932	-0.15763	0.37834	-0.28156	-0.63868	-0.40064
Limb Length	0.41948	-0.36886	0.27756	-0.29492	0.23880	0.68333
Anther (S)	0.41641	0.25999	-0.60996	0.18511	-0.46159	0.37367
Anther (L)	0.42726	0.05789	-0.44232	-0.46369	0.47783	-0.41848
Pistil	0.40151	-0.43883	0.00723	0.73861	0.20742	-0.2399
Arabidopsis lyrat		<u>L </u>	<u> </u>	<u> </u>	L	
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.3577	0.9194	0.8266	0.4163	0.3097	0.1704
Percent	55.9611	15.3239	13.7759	6.9375	5.1617	2.8399
Eigenvectors	+					
Claw Length	0.32558	0.66184	0.46898	0.26566	0.11861	0.38906
Limb Width	0.36274	-0.50309	0.53501	0.21701	0.39048	-0.35988
Limb Length	0.44662	-0.47636	0.00780	-0.18331	-0.33439	0.65432
Anther (S)	0.38146	0.01362	-0.68088	0.28295	0.54191	0.11564
Anther (L)	0.47243	0.13370	-0.17244	0.34133	-0.63800	-0.45349
Pistil	0.44073	0.25272	-0.02054	-0.80533	0.14469	-0.26825
Arabidopsis thali		0.20272	3.0200	0.00000	0.105	0.20020
- = uo.copo.o u.u	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.5445	1.2464	0.5987	0.2588	0.2013	0.1503
Percent	59.0754	20.7731	9.9778	4.3138	3.3557	2.5043
Eigenvectors	1,			1	2.233,	
Claw Length	0.23853	0.71138	0.42324	0.39072	0.31381	0.08235
Limb Width	0.46103	0.28745	0.01912	0.21490	-0.79829	0.14494
Limb Length	0.47159	-0.14632	0.33119	-0.42528	0.22512	-0.64418
Anther (S)	0.33116	-0.59067	0.33680	0.62752	-0.16839	0.07663
Anther (L)	0.48235	-0.17037	-0.14102	-0.30868	0.41846	0.66935
Pistil	0.4073	0.10949	-0.75993	0.36225	0.10052	-0.32148
Arabis hirsuta	1 0.7073	0.10272	-0.73773	1 0.50225	0.10032	-0.34140
ruavis illisuta	PC1	PC2	PC3	PC4	PC5	DC6
Eigenvalue	· · · · · · · · · · · · · · · · · · ·					PC6
	2.6684	1.2457	0.8039	0.7038	0.3268	0.2515
Percent	44.4728	20.7622	13.3975	11.7294	5.4465	4.1916

Table 11 (cont'd)

rabis hirsuta cont'e	d				100	711-
igenvectors			2.16007	0.27006	0.35058	-0.15680
Claw Length	0.14121	0.7510.	0.40077	PC4	PC5	PC6
law Length	PC1	PC2	PC3	0.27672	-0.54323	0.56182
Limb Width	0.52667	0.00320	-0.17691	0.62981	0,44672	-0.09862
Limb Length	0.39905	-0.29906	0.38128	-0.62766	0.48935	0.36118
	0.47523	0.04191	0.09237	-0.23544	-0.36911	-0.67343
Anther (S)	0.52018	0.16940	0.23565	0.06662	-0.08202	0.25707
Anther (L)	-0.21682	0.58000	0.73439	0.06662	-0.00202	to contract
Pistil	-0.21002			PC4	PC5	PC6
Babarea verna	PC1	PC2	PC3	0.3925	0.2710	0.13335
	3,4760	0.9761	0.7508		4.5174	2.2254
Eigenvalue	57.9335	16.2684	12.5137	6.5406	4.3174	1444
Percent	31.9333				-0.27918	0.42825
Eigenvectors	0.44198	-0.05373	0.41064	-0.60976	0.00868	-0.70551
Claw Length		0.29467	-0.26014	-0.36491		0.52565
Limb Width	0.46317	0.32583	-0.65083	0.20300	-0.04192	-0.20003
Limb Length	0.3885	-0.46225	0.04606	0.49431	-0.57207	0.04934
Anther (S)	0.41543	-0.55370	-0.04178	0.01202	0.72750	-0.01035
Anther (L)	0.39980	0.53278	0.57987	0.45754	0.25240	-0.01055
Pistil	0.32670	0.33276	0.0			PC6
Barbarea vulgaris	S	PC2	PC3	PC4	PC5	0.1214
	PCI		0.7133	0.5854	0.2286	2.0234
Eigenvalue	3.0987	1.2526	11.8882	9.7559	3.8105	2.0234
Percent	51.6453	20.8764	11.0002		-	- 27700
Eigenvectors			-0.18349	0.54073	0.54165	0.27798
Claw Length	0.43446	-0.33843	-0.18349	0.52709	-0.46739	-0.00327
Limb Width	0.31173	0.60337	-0.25860	-0.56948	0.36962	0.28694
Limb Length	0.35518	0.51354	0.13570	-0.25660	-0.56134	0.48217
Anther (S)	0.45779	-0.39822		-0.19257	-0.05559	-0.76233
Anther (L)	0.50496	-0.23057	-0.26557	0.05051	0.18262	-0.16357
Pistil	0.35122	0.21436	0.87638	0.05051		
Brassica napus				PC4	PC5	PC6
Brassica napus	PC1	PC2	PC3	0,4569	0.3619	0.3165
1	2,6892	1.3756	0.7998	7,6157	6.0319	5.2755
Eigenvalue	44.8198	22.9264	13.3306	7.0137	0.051	1506
Percent	41.015			0.50240	0.48427	-0.43390
Eigenvectors	0,47275	-0.31788	-0.01312	-0.50249 -0.01004	0.54740	0.36867
Claw Length	0.24031		-0.07985		-0.33322	-0.57719
Limb Width	0.44321		-0.29018	0.36107	-0.33446	0.53198
Limb Length	0.44321		-0.56018	-0.20321	0.24921	0.20422
Anther (S)	0.41348	0.00	0.28482	0.69704		- 12100
Anther (L)	0.41346		0.71716	-0.29977	-0.42323	
Pistil		0.20100			PC5	PC6
Brassica nigra	a - Junkyard PC1	PC2	PC3	PC4	0.3987	0.3067
1000	2.7011		0.9392	0.6106		
Eigenvalue			15.6528	10.1773	6.6430	
Percent	45.018	1,10711			-0.2138	2 0.20286
Eigenvectors	0.2050	0.28913	0.78873			2
Claw Length	0.2859			-0.1206		1
Limb Width		2		-0.1393		- 10015
Limb Length	h 0.4474				0.4831	1 0.49242

Table 11 (cont'd)

Brassica nigra – J	unkyard cont'c	<u> </u>				
	PC1	PC2	PC3	PC4	PC5	PC6
Anther (L)	0.43565	0.35152	0.08586	-0.68018	-0.16555	-0.43499
Pistil	0.46294	-0.03073	-0.31878	0.61320	-0.09904	-0.54526
Brassica nigra - Y	ard Waste Dis	posal Site				
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.3141	1.0112	0.5855	0.4439	0.3658	0.2796
Percent	55.2344	16.8527	9.7585	7.3983	6.0962	4.6599
Eigenvectors						
Claw Length	0.38854	0.11397	0.90879	0.02315	-0.02752	0.09413
Limb Width	0.34617	0.63997	-0.28826	0.41597	-0.19557	0.41980
Limb Length	0.41096	0.43530	-0.16308	-0.52524	0.42846	-0.39442
Anther (S)	0.41653	-0.47873	-0.16687	-0.17933	0.38034	0.62663
Anther (L)	0.43425	-0.31595	-0.10518	0.64494	0.19970	-0.49468
Pistil	0.44524	-0.24281	-0.15972	-0.32007	-0.76999	-0.14824
Brassica napus					<u> </u>	
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	2.6892	1.3756	0.7998	0.4569	0.3619	0.3165
Percent	44.8198	22.9264	13.3306	7.6157	6.0319	5.2755
Eigenvectors						
Claw Length	0.47275	-0.31788	-0.01312	-0.50249	0.48427	-0.43390
Limb Width	0.24031	0.070726	-0.07985	-0.01004	0.54740	0.36867
Limb Length	0.44321	0.38054	-0.29018	0.36107	-0.33322	-0.57719
Anther (S)	0.44157	-0.23462	-0.56018	-0.20321	-0.33446	0.53198
Anther (L)	0.41348	-0.39778	0.28482	0.69704	0.24921	0.20422
Pistil	0.39544	0.20163	0.71716	-0.2997	-0.42525	0.13400
Barbarea oleracea	1		<u> </u>	•		
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	4.1835	0.9090	.4340	0.2404	.1604	0.0726
Percent	69.7250	15.1512	7.2329	4.0073	2.6732	1.2103
Eigenvectors						
Claw Length	0.43622	-0.10026	-0.51300	-0.02993	0.68741	0.25113
Limb Width	0.34290	0.64996	0.27229	0.61234	0.10386	0.00880
Limb Length	0.39056	0.48987	0.08736	-0.74889	-0.17634	0.08907
Anther (S)	0.44262	-0.28520	-0.17312	0.23054	-0.62427	0.49991
Anther (L)	0.46392	-0.16488	-0.21505	0.05252	-0.17591	-0.82319
Pistil	0.35842	-0.46797	0.76081	-0.08604	0.25481	0.03702
Brassica rapa	*			•		
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.1750	0.9990	0.8323	0.4101	0.3296	0.2540
Percent	52.9163	16.6498	13.8721	6.8350	5.4927	4.2341
Eigenvectors						
Claw Length	0.31273	0.41859	0.73278	0.28260	0.29946	0.14308
Limb Width	0.41588	-0.50822	0.28734	-0.00759	-0.089849	-0.69147
Limb Length	0.40253	-0.60106	-0.00059	-0.08143	0.18229	0.66093
Anther (S)	0.40653	0.17204	-0.58846	0.34037	0.54994	-0.20139
Anther (L)	0.46660	0.20465	-0.17225	0.34775	-0.75274	0.15224
Pistil	0.42922	0.36570	-0.06716	-0.82260	-0.00501	-0.02886
Cakile edentula	<u> </u>		•		!	
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	2.7187	1.3946	0.7914	0.5300	0.3270	0.2383

Table 11 (cont'd)

Cakile edentula c	ont'd					
<u> </u>	PC1	PC2	PC3	PC4	PC5	PC6
Percent	45.3109	23.2440	13.1899	8.8339	5.4495	3.9718
Eigenvectors						
Claw Length	0.43903	-0.34189	-0.07112	0.69218	-0.25749	-0.37401
Limb Width	0.24453	0.62036	-0.46854	0.29079	0.50126	0.00213
Limb Length	0.41320	0.47387	-0.10425	-0.31329	-0.70365	-0.02370
Anther (S)	0.49178	-0.22837	0.03065	-0.54749	0.39113	-0.50232
Anther (L)	0.48425	-0.36785	-0.19795	-0.07067	0.07176	0.76214
Pistil	0.31682	0.29365	0.85113	0.18283	0.17084	0.16235
Capsella bursa-pa				·		
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.0733	1.0457	0.7345	0.5918	0.3327	0.2220
Percent	51.2210	17.4283	12.2422	9.8629	5.5452	3.7004
Eigenvectors						
Claw Length	0.44290	-0.13394	-0.43139	0.54102	0.05110	0.55181
Limb Width	0.47978	-0.09174	0.29296	0.34603	0.48889	-0.56286
Limb Length	0.43523	-0.13384	0.46441	-0.53915	0.21513	0.48993
Anther (S)	0.05346	0.94966	0.18169	0.15589	0.09947	0.16758
Anther (L)	0.37709	0.22618	-0.66730	-0.52092	0.12931	-0.27069
Pistil	0.48905	0.05220	0.18465	0.03500	-0.82794	-0.19315
Cardamine conca		0.00220	0.10.00	1 0.0000	0.02.7.7.	
Cur Guilline Collec	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	2.5952	1.1680	0.9131	0.6576	0.5127	0.1534
Percent	43.2542	19.4660	15.2189	10.9594	8.5445	2.5570
Eigenvectors	73.2372	12.4000	13.2107	10.2321	0.5115	2.3370
Claw Length	0.47256	-0.01118	-0.54336	-0.18276	0.408880	-0.52992
Limb Width	0.45417	-0.55907	-0.12199	-0.15391	0.08420	0.65993
Limb Length	0.36976	-0.33704	0.53540	0.58789	0.12343	-0.31966
Anther (S)	0.32369	0.29743	0.61720	-0.63702	0.13962	-0.02308
Anther (L)	0.31974	0.68100	-0.08764	0.43747	0.25911	0.40964
Pistil	0.47612	0.14661	-0.12088	0.01093	-0.85084	-0.11471
Cleome spinosa	0.47012	0.14001	-0.12000	0.01073	-0.03004	-0.111771
Cleonie spinosa	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	4.9859	0.5018	0.2854	0.1667	0.0457	0.0146
Percent	83.0977	8.3637	4.7564	2.7780	0.7612	0.2431
Eigenvectors	83.0977	8.3037	7.7304	2.7780	0.7012	0.2431
Claw Length	0.39963	0.16972	-0.75731	0.34991	0.33711	0.04353
	0.39903	0.10972	0.55650	0.53054	-0.01172	-0.00723
Limb Width			-0.07493	-0.71591	-0.01172	0.03278
Limb Length	0.39152	0.53792	0.16720	-0.71391	0.29477	-0.74465
Anther (S)	0.42794				0.29477	0.66458
Anther (L)	0.41997	-0.41523	0.25266	-0.15159	-0.80801	0.02850
Pistil	0.42473	-0.34880	-0.13921	0.15773	-0.80801	0.02830
Hesperis matron		DC2	DC2	DC4	DCs	PC6
T'	PC1	PC2	PC3	PC4	PC5	
Eigenvalue	3.6211	1.0398	0.7011	0.2753	0.2133	0.1494
Percent	60.3512	17.3300	11.6853	4.5887	3.5550	2.4898
Eigenvectors						0.000
Claw Length	0.28425	0.65318	0.57058	-0.01086	0.40681	0.03714
Limb Width	0.35238	-0.44962	0.62727	0.30532	-0.40882	0.14087
Limb Length	0.43914	-0.32499	0.02773	-0.82205	0.15632	-0.02392

Table 11 (cont'd)

Hesperis matronal	lis cont'd					
	PC1	PC2	PC3	PC4	PC5	PC6
Anther (S)	0.45043	0.24117	-0.40684	0.07313	-0.19583	0.72782
Anther (L)	0.45463	0.32402	-0.21086	0.03446	-0.48282	-0.63997
Pistil	0.43887	-0.32003	-0.26497	0.47367	0.60949	-0.19728
Iberis						
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.7128	1.0840	0.4946	0.3817	0.2274	0.0994
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvectors						
Claw Length	0.44567	0.23196	-0.28428	-0.29278	-0.75919	0.06836
Limb Width	0.41338	-0.52935	0.13132	0.02447	-0.04319	-0.72746
Limb Length	0.38609	-0.59096	0.02085	0.25203	0.00067	0.66162
Anther (S)	0.35526	0.45835	0.67535	0.44912	-0.07666	0.00987
Anther (L)	0.40220	0.29397	-0.64070	0.41534	0.39571	-0.11055
Pistil	0.43992	0.14227	0.18684	-0.68989	0.50922	0.12675
Lobularia maritim	na					
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.3567	0.9711	0.8468	0.3443	0.3105	0.1706
Percent	55.9444	16.1858	14.1128	5.7390	5.1745	2.8435
Eigenvectors						
Claw Length	0.37676	-0.00471	0.68480	0.44200	-0.43194	-0.08452
Limb Width	0.40421	0.47083	0.25824	-0.70473	-0.00871	0.22701
Limb Length	0.35009	0.62958	-0.29076	0.51517	0.36181	0.01479
Anther (S)	0.47148	-0.13317	-0.37219	-0.18774	-0.22638	-0.73141
Anther (L)	0.43532	-0.33120	-0.42386	0.08245	-0.32898	0.63727
Pistil	0.40038	-0.50448	0.24828	-0.02349	0.72316	0.00581
Matthiola						
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	2.4225	1.3719	0.8461	0.5957	0.4628	0.3010
Percent	40.3750	22.8656	14.1017	9.9282	7.7127	5.0168
Eigenvectors						
Claw Length	0.46963	-0.20275	-0.48369	-0.41711	0.22627	0.52840
Limb Width	0.33143	-0.52353	0.34559	0.59416	-0.14275	0.35104
Limb Length	0.47950	-0.39637	0.17814	-0.27302	0.15142	-0.69554
Anther (S)	0.38200	0.47353	-0.18816	0.52911	0.54189	-0.14442
Anther (L)	0.46220	0.33204	-0.23010	0.05210	-0.77858	-0.11947
Pistil	0.28309	0.43957	0.72563	-0.34027	0.07527	0.28045
Nasturtium officia						
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.6993	0.8981	0.5373	0.3954	0.2890	0.1810
Percent	61.6547	14.9679	8.9544	6.5906	4.8159	3.0166
Eigenvectors						
Claw Length	0.42110	0.05808	-0.36783	-0.81852	0.08776	-0.07950
Limb Width	0.42713	-0.47130	0.06193	0.24630	0.17950	-0.70620
Limb Length	0.41962	-0.48572	-0.21338	0.22875	0.16382	0.68067
Anther (S)	0.35307	0.67307	-0.02982	0.30304	0.57395	0.01332
Anther (L)	0.43186	0.28871	-0.25496	0.28019	-0.76426	-0.05037
Pistil	0.39115	0.04689	0.86570	-0.21606	-0.14049	0.17013

Table 11 (cont'd)

	trum Field					
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.4581	0.9490	0.7675	0.3710	0.3204	0.1341
Percent	57.6351	15.8161	12.7917	6.1828	5.3400	2.2343
Eigenvectors						
Claw Length	0.45968	-0.18612	-0.23824	0.02283	-0.72975	0.40526
Limb Width	0.33444	0.68345	-0.20880	0.59998	0.05708	-0.11923
Limb Length	0.39374	0.50443	0.11157	-0.75095	0.09736	0.06826
Anther (S)	0.44456	-0.34563	-0.19896	0.09729	0.67268	0.42585
Anther (L)	0.47764	-0.34439	-0.12325	-0.07345	-0.01499	-0.79526
Pistil	0.30989	-0.07577	0.91237	0.24640	-0.04473	0.05562
Raphanus raphanis	trum Greenho	use		·····		
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.5331	1.0431	0.6983	0.3916	0.2424	0.0917
Percent	58.884	17.3842	11.6379	6.5263	4.0395	1.5277
Eigenvectors						
Claw Length	0.45013	-0.34368	-0.11313	0.10218	0.76324	0.27111
Limb Width	0.31601	0.62771	-0.35510	0.61443	-0.02284	-0.04438
Limb Length	0.36638	0.53812	-0.03202	-0.74964	0.10669	0.04269
Anther (S)	0.47064	-0.28173	-0.14683	-0.02268	-0.61666	0.54477
Anther (L)	0.48205	-0.31726	-0.11705	-0.05442	-0.15620	-0.79115
Pistil	0.33076	0.13516	0.90820	0.21586	-0.02993	0.00403
Raphanus sativus	· · · · · · · · · · · · · · · · · · ·			L		
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.8613	0.8472	0.6630	0.3836	0.1853	0.0595
Percent	64.3557	14.1206	11.0507	6.3936	3.0885	0.9910
Eigenvectors						
Claw Length	0.45994	-0.00215	-0.28430	0.14226	-0.80194	-0.21043
Limb Width	0.32226	-0.67762	0.41630	0.49126	0.09617	0.11446
Limb Length	0.38139	0.07085	0.63053	-0.66139	-0.12022	-0.00799
Anther (S)	0.46508	-0.11848	-0.33148	-0.14733	0.51800	-0.60807
Anther (L)	0.46909	0.03430	-0.39107	-0.14125	0.18407	0.75631
Pistil	0.32064	0.72153	0.29236	0.50925	0.17603	-0.02815
Sisymbrium altissii	mum				<u> </u>	
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.2417	0.9487	1.6088	0.4861	0.4446	0.2700
Percent	54.0284	15.8117	10.1475	8.1022	7.4102	4.5001
Eigenvectors						
Claw Length	0.42668	0.26772	0.20615	0.30022	-0.78087	-0.06240
Limb Width	0.41056	-0.54283	0.28431	0.05238	0.08001	0.66843
Limb Length	0.43123	-0.46600	0.14554	-0.32248	0.04507	-0.68533
Anther (S)	0.36577	0.53586	0.47000	0.15638	0.57339	-0.07028
Anther (L)	0.40556	0.35236	-0.38379	-0.70236	-0.04983	0.26130
Pistil	0.40642	-0.07212	-0.69807	0.53420	0.22483	-0.08005
Stanleya pinnata						
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.6190	0.8382	0.6331	0.5579	0.2883	0.0635
Percent	60.3159	13.9697	10.5515	9.2987	4.8058	1.0584
Eigenvectors						
	0.41849	0.05914	-0.30768	0.59016	-0.61482	-0.02014

Table 11 (cont'd)

Stanleya pinnata	cont'd					
	PC1	PC2	PC3	PC4	PC5	PC6
Limb Width	0.33750	0.76137	0.05573	0.22972	0.49268	0.08831
Limb Length	0.39069	0.27976	0.46484	-0.56540	-0.48107	-0.04384
Anther (S)	0.46912	-0.19875	-0.36687	-0.24951	0.26681	-0.68731
Anther (L)	0.46874	-0.29893	-0.28838	-0.25679	0.16462	0.71743
Pistil	0.34428	-0.45792	0.68439	0.38867	0.22260	-0.05260

Four matrix comparisons were made between among species within a genus. The analysis of *Barbarea vulgaris* and *B. verna* showed equality of matrices and comparison *R* raphanistrum to the *R. sativus* found proportionality among matrices. The comparison of *Brassica oleraceae*, *B. napus*, and *B. rapa* found matrices with two PC in common. Arabidopsis lyrata and A. thaliana shared a common structure in the first three PC. Reordering reduced the number of PC with shared structure by one in both the Arabidopsis clade and the *Brassica* polytomy (PCPC2 and PCPC1 respectively).

The matrices in the clade including A. lyrata, A. thaliana, and Capsella bursa-pastoris were unrelated without reordering the PCs. Reordering showed a related structure in two PC. In the first PC for the individual population analysis of PCA, C. bursa-pastoris had an extremely low weight (0.05346) for the short anther length. This could explain why related structure in the first eigenvector was rejected.

Nasturtium officinale and Cardamine concatenata had proportional matrices and, when included in an analysis with the Barbarea species, all four matrices were proportional.

An even more inclusive clade of A. lyrata / Cardamine concatenata also was proportional. None of these values were affected by reordering PCs.

Several pair-wise comparisons showed significant structure only after reordering.

Sisymbrium altissimum and Stanleya pinnata had shared structure among all their principal components. Lobularia maritima and Iberis, Hesperis matronalis and Matthiola, and B. nigra and Cakile edentula had shared structure in three PCPC.

The PCA analysis for the combined data from all populations had a first PC, which when averaged within populations, was closely associated with overall size of the populations' flowers (Table 12). The first PC explained over 85% of the variation in the data set.

Larger flowers had larger average PCs and the inverse was true for smaller flowers.

Table 12. Results of a Principal Component Analysis incorporating measurements from all 26 populations measured in the Brassicaceae and Cleomaceae including eignvalues with percent variation explained, and eigenvectors. Principal components are denoted as "PC" and ordered by decreasing eigenvalues. Species listed correspond to species listed in Table 8 followed by the average first principal component for individuals in the population.

							
Principal Con	mponent Analys	sis of combined	l data from 26 p Cleomaceae	opulations in th	e family Brassio	caceae and	
	PC1	PC2	PC3	PC4	PC5	PC6	
Eigenvalue	5.0142	0.7472	0.1095	0.0928	0.0314	0.0050	
Percent	85.5698	12.4531	1.8253	1.5460	0.5233	0.0825	
1 Grount	03.5070	12551	1.0255	1.5 100	0.3255	0.0025	
			Eigenvectors				
Claw Length	0.39230	0.47053	0.67419	-0.36030	-0.18095	0.08726	
Limb Width	0.39454	0.46965	-0.08967	0.75023	0.22438	-0.05044	
Limb Length	0.41376	0.29390	-0.71195	-0.48161	-0.00344	0.05998	
Anther (S)	0.40872	-0.45219	0.10880	-0.00816	0.44415	0.64753	
Anther (L)	0.42242	-0.35926	0.11803	-0.12671	0.31094	-0.75222	
Pistil	0.41683	-0.37163	-0.06923	0.24347	-0.78927	0.03344	
					irst principal co		
	Spe	<u>cies</u>		<u>indivi</u>	duals in each po	<u>pulation</u>	
4 .1 . 1	* * * * * *				0.500		
Aethionema arab				-2.599			
Alliaria petiolata					-1.581		
Arabidopsis lyra			2	-1.371 -2.3208			
Arabidopsis thal		1.					
Arabis hirsuta D				-1.855 1.303			
Barbarea verna (-1.393 -1.443			
Barbarea vulgari Brassica napus I					-0.421		
Brassica nigra (I					-0.928		
Brassica nigra (I	•				-0.974		
Brassica olerace		J			1.699		
Brassica rapa L.	uc L.				-1.074		
Cakile edentula	(Bigelow) Hoo	k			-1.758		
Capsella bursa-p					-2.570		
Cardamine conc					0.257		
Cleome spinosa	•	., .,			12.361		
Hesperis matron	•				0.737		
Iberis L.					-1.871		
Lobularia mariti	ma (L.) Desv.				-2.429		
Matthiola R.Br.	(,				0.422		
Nasturtium offic	inale R.Br.				-1.918		
Raphanus rapha	nistrum L. FLD	92			0.996		
Raphanus rapha					1.896		
Raphanus sativu					1.481		
Sisymbrium alti	ssimum L.				-1.425		
Stanleya pinnata	Britton				1.062		

CONCLUSION

The phylogenetic analysis of the *ndh*F coding region was consistent with other molecular phylogenetic analyses within the family Brassicaceae (*rbc*L, Price et al. 1994; *ndh*F and *Adc*, Galloway et al. 1998; ITS, Mitchell & Heenan 2000; *mat*K and *Chs*, Koch et al. 2001; and *ndh*F and *trnL-trn*F, Hall et al. 2002). The genus *Aethionema* was basal within the family Brassicaceae and a clade including *Hesperis matronalis* and *Matthiola* was basal to the remainder of the genera sampled. Good support existed for the *Arabidopsis lyrata* – *Cardamine concatenata* clade and relationships within the clade were resolved; this was the first molecular support for a clade including *Nasturtium officinale* and *Cardamine concatenata*. *S. altissimum* and *S. pinnata* were in a clade sister to the tribe Brassiceae.

The Brassiceae tribe is one of the few natural tribes described in the Brassicaceae (Al-Shehbaz 1984, Warwick & Black 1997, Gomez-Campo 1999, Koch et al 2001). Previous phylogenetic and phenetic analyses of RFLP data have been conducted in the Brassiceae (Pradham et al 1992, Thorman et al. 1994, Song et al. 1988, 1990, 1992, Warwick & Black 1991, 1993, 1997 and references therein) and a few molecular phylogenetic analyses have studied relationships in this group (Yang et al. 1999, Wroblewski et al. 2000, and Inaba & Nishio 2002). The findings of the *ndh*F phylogenetic study were consistent with the previous investigations in finding two lineages of *Brassica*: one including B. *oleraceae*, B. napus, and B. rapa, and second including B. nigra.

The comparisons of the P-matrix across the Brassicaceae found matrix proportionality at higher taxonomic levels than any study conducted to date (Steppan et al. 2002 and references therein, Baker and Wilkinson 2003). Equality in matrices between different species was found between Barbarea verna and B. vulgaris and proportional matrices were not rejected between Raphanus raphanistrum and R. sativus, and Nasturtium officinale and Cardamine concatenata. Proportionality among matrices in five genera was found in the A. lyrata - Cardamine concatenata clade. The presence of matrix similarity at such high taxonomic levels was noteworthy. The study tested the hypothesis that matrix structure deteriorates at the sub-species level and higher (Steppan et al. 2002), and found evidence for shared matrix structure among different genera in Brassicaceae. The results indicate, that in at least some cases, the P-matrix remains proportional (eigenvectors are equal and the eigenvalues change proportionally among matrixes) over long spans of evolutionary time and if the P-matrix is a good proxy for the G-matrix, the G-matrix can be a useful tool for reconstructing past evolutionary events (Lande 1979). This supports the findings of Schluter (1996) and Futuyma (1995) regarding the potential for long term, sustained constraints of the G-matrix.

Within *Brassica*, species had low levels of shared structure. In the Brassicae clade and its sister clade *Sisymbrium altissimum /Stanleya pinnata* low levels of matrix stability were found among genera. This was also true of the *Hesperis matronalis / Matthiola* and the *Lobularia / Iberis* clades. This supports the premise of Steppan (1997a, 1997b, and 2002 and references therein) that variance-covariance matrix structure is not always constant.

The results of this study confirm that the stability of the P-matrix, and potentially the G-matrix, varies among lineages. Assumptions can not be made about either the stability or lack of stability in covariance structure at different taxonomic levels within a family. Within the Brassicaceae matrices were proportional among distantly related taxa, and completely unrelated among other closely related taxa. In this study the phylogenetic level of the matrix comparison did not dictate the level of stability found among matrices. More empirical studies of matrix structure are needed to investigate variance-covariance stability within a phylogenetic context.

An important caveat - Measurements of the G-matrix in multiple species are not practical. Phenotypic measurements are much easier to obtain when taxa are sampled across a phylogeny. Phenotypic matrices are not identical to genetic matrices, though they may shed light on their genetic counterparts. Several studies have found that homogeneity in P-matrices may imply homogeneity in G-matrices (Cheverud 1988, Arnold 1992, Waitt & Levin 1998, Arnold et al. 2001, and Steppan et al. 2002). The P-matrix can be a conservative estimator of G-matrix stability. P-matrices, when directly compared to G-matrices, tend to deviate more readily from shared matrix structure among more closely related taxa (Steppan et al. 2002). Both Cheverud (1988) and Waitt & Levin (1998) reviewed the literature and found phenotypic correlations were good indicators of genetic correlations. Willis et al (1991) questioned the findings of Cheverud (1988) and refuted the utility of phenotypic variation in determining genetic variation (for a review of the P-matrix / G-matrix debate see Steppan et al. 2002).

In the reordering process, the first PC was moved to the end of the PC hierarchy, and the five PCs with smaller eigenvalues were assessed for eigenvector similarity. In several cases, reordering the principal components increased the level of matrix similarity among taxa. Even though the first principal component was responsible for a large portion of the variation in the individual data sets (average 55.45%, range 40.38-83.10), the remaining PCs still account for approximately half the variance. The remaining PCs could account for significant shape differences between the populations and represent important principal components affecting floral morphology. It makes intuitive sense that even though populations have a high proportion of variation in their first vector the remaining vectors may share commonalities of biological significance (Houle 2002, Steppan et al. 2002, Baker and Wilkinson 2003).

Analysis of the first PC for the combined data set of all 26 populations showed a strong relationship between the first factor and overall flower size. In addition, in the individual PC analysis of the populations – most populations had positive, relatively equally weighted variables. It is likely that the first vector is predominantly a size factor. If size was the only biological explanation of the first PC, and the only variance found between the populations was proportional, all the first PCs eigenvectors would be identical among populations. There were, however subtle differences in the weighting of the variables in the first eigenvector which resulted in a finding of unrelated matrix structure in the Flury Hierarchy. These subtle differences in the variable weights could be attributed to differences unrelated to proportionality, but rather shape differences in the floral morphology. This is particularly important since small differences in flower structure can

have a large impact on pollen deposit, pollinator attraction, and pollen removal, all key factors in reproductive success (Conner 1997).

Empirical studies of patterns of P- and G-matrix similarity are important. The G-matrix plays a key role in understanding evolutionary processes (Lande 1988). Understanding how the G-matrix changes through evolutionary time helps to explain multivariate evolution (Phillips and Arnold 1999). Selection can only act on the amount of genetic variation-covariation available in a population. How this G-matrix changes dictates the potential response of the population to selection and drift. An understanding of how matrix stability changes over the course of evolutionary time may help us to understand the potential constraints caused by the G-matrix and the factors that can overcome these constraints. In understanding the leap from microevolutionary processes to macroevolutionary events, the G-matrix serves as the key component of change and evolution.

SUMMARY

This dissertation examined phenotypic diversity at several different hierarchical levels. In all studies, a phylogeny was used as the framework to interpret floral morphology. Floral traits were measured on individuals across the species *Pedicularis densiflora* and *Pedicularis aurantiaca*, within the tropical genus *Cantua*, and throughout the family Brassicaceae.

The species *Pedicularis densiflora* and *P. aurantiaca* were previously described as subspecies of *Pedicularis densiflora* s.l. A close examination of floral traits among populations revealed two distinct morphologies among the populations. This morphological variation was examined intensively using ANOVA tests, principal component analysis, UPGMA cluster analysis and a comprehensive review of over 1000 herbarium specimens. Phylogenetic analysis of the two species confirmed they were closely related taxa, sister to a clade containing *Pedicularis semibarbata*, but the relationships among taxa within the *P. densiflora | P. aurantiaca* clade were unresolved. Without resolution phylogenetic information can sometimes be of limited help. Lack of resolution within the phylogeny prevented conclusions regarding the number of times the different floral morphs arose, relationships among populations in different localities, and potential pollinator shifts.

Past research on diversity in pollen morphology showed that pollen exine sculpturing was helpful in exploring phylogenetic relationships in the Polemoniaceae (Stuchlik 1967a, 1967b, Taylor and Levin 1965). Additional work examined the potential functional relationship between pollen grain size and style length (Plitmann and Levin 1983). While

past research had been restricted to phenotypic data, this *Cantua* research was able to integrate pollen and floral morphological data and a molecular phylogeny. Pollen grain morphology was highly conserved in *Cantua*, which supported the subsumption of *Huthia* into *Cantua*. The synapomorphy of supratectal verrucae united the *C. volcanica* / *C. quercifolia* clade and provided additional support for the hypothesized relationship. The molecular phylogeny of *Cantua* allowed me to confirm the phylogenetic utility of pollen morphological characters and remove the issue of non-independence when examining the relationship between pollen diameter and style length. Neither a significant regression slope nor correlation was found using independent contrasts of pollen diameter and style length in *Cantua* and the hypothesis for a morphological integration of these two traits in *Cantua* was not supported.

The evolution of phenotypic variance-covariance structure is still not well understood, particularly in an historical context (Badyaev and Hill 2000). The question of whether the P-matrix remains stable over evolutionary time remains an unresolved issue (Arnold et al. 2001, Steppan et al. 2002). I studied six floral traits in the Brassicaceae to examine P-matrix stability within a phylogenetic context. The comparisons of the P-matrix across the Brassicaceae found matrix equality and proportionality at higher taxonomic levels than any study conducted to date (Steppan 2001 and references therein, Baker and Wilkinson 2003). Unrelated matrix structure also was found among species and genera in the family. The phylogenetic level of the matrix comparison did not appear to affect the level of stability found among matrices. A phylogenetic hypothesis in the Brassicaceae

was used to conclude that P-matrix stability does not follow a stepwise pattern of similarity corresponding to taxonomic rank within the phylogeny.

Biological diversity is created by evolution. When attempting to explore biodiversity and examine processes in evolution, we need perspective on past evolutionary events. At present one of the most effective methods to investigate past evolutionary events is phylogenetics. My work on floral diversity and phenotypes in the genus *Cantua* and the family Brassicaceae exemplifies how phylogenetic history can clarify our understanding of biological phenomena. In contrast, phylogenetic relationships in the *Pedicularis* study were unresolved and consequently I was unable to add insight into past evolutionary events. Investigations of evolution benefit from the perspective of an historical context.

APPENDICES

APPENDIX A

Aligned sequences of *Pedicularis*:
Nuclear ribosomal internal transcribed spacer regions and 5.8S ribosomal RNA gene

Table 13. Aligned sequences of the nuclear ribosomal internal transcribed spacer regions correspond to vouchers listed in Table 1. Gaps indicated with ":", Ambiguities indicated and the 5.8S subunit of ribosomal RNA from the taxa sampled in Pedicularis. Names with "N", missing data indicated with "-"

VITS 1

4 8

3GG GAG	3GG GAG	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	rgg grg	_	ree gre
0	TTG	_	_	_	_	-	•	-	-	-	-	-	-	•	-	-	•	-	•	•	
	GIC																				
CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG
\mathbf{TCG}	\mathbf{TCG}	\mathbf{T}^{CG}	\mathbf{T}^{CG}	\mathbf{T}^{CG}	\mathbf{T}^{CG}	\mathbf{T}^{CG}	\mathbf{TCG}	\mathbf{T}^{CG}	\mathbf{TCG}	\mathbf{T}^{CG}	\mathbf{TCG}	$\mathbf{T}^{\mathbf{G}}$	$\mathbf{T}^{\mathbf{G}}$	\mathbf{TCG}	TCG						
GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA
ATG	ATG	TTG	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	${ m TTG}$	TTG	TTG	${ m TTG}$	TTG	TTG	TTG	TIG	TIG	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	TIG	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG
•	ACA		•		•	•	•				. •		. •							. •	
ACC	ACC	ACC	ACC																		ACC
٠.	TAA	٠.	ι.					_	_	_	_	_	_	-	_	_	_	_	TAA	_	
CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGI	CGT	CGT	CGT	CGT	CGT	CGI	CGT	CGT	CGT	CGT	CGT
ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA
_	CGA	~	_	_	_	_	_	_	_	_		$\overline{}$	_	$\overline{}$	0	01	_	$\mathbf{\circ}$	$\overline{}$	$\overline{}$	_
SCG	CCG		CCG			CCG	CCG	CCG	CCG	CCG	CCG	CCG	CCG	CCG	CCG	CCG	CCG	CCG	CCG	CCG	CCG
AAA	AAA	AGA	AGA	AGA	AGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA
Pcystopteridifolia_A	P. cystopteridifolia_B	Pbracteosa_A	Pbracteosa_B	P. semibarbata A	P. semibarbata B	McBride_Springs	Pinehurst	Hobo Gulch	Inskip	Weaver Camp	Hurles Circle	Briceland	Mount Diablo	Missouri Flat	Round Valley	Paradise	San Marcos Pass	Pinnacles _	Bear_Valley	Auberry_Road	Lake Santa Margarita

Pcystopteridifolia_A	ACC	CTT	TGC	CCC CCC CCC CCC CCC CCC CCC CCC CCC CC	_	_				_				•	_	_
Pcystopteridifolia_B	ACC	CTT	\mathbf{TGC}	ဗ္ဗဘ	CIC	CCA	_			_				•	SGC	_
P. bracteosa A	ACC	CTA	TGA	S	_		•							•	_	
P. bracteosa B	ACC	CTA	TGA	SSS	_		•			_				•	_	_
P. semibarbata A	ACC	CTT	\mathbf{TGC}	CCG	CGC	CCA	CCT	CTC	GCA	A CGG	GGT	r GCG	CCT	r AGG	CGC	\mathtt{TCT}
P. semibarbata B	ACC	CTT	TGC	SSS	_					_				-	_	
McBride_Springs	ACC	CTT	TGC	SCC	_		•							-	_	
Pinehurst	ACC	CTT	${ m TGC}$	ဗ္ဗဘ္ဘ	_		•			_				-	_	
Hobo_Gulch	ACC	CTT	TGC	SCC	_		•			_				-	_	
Inskip	ACC	CTT	\mathbf{TGC}	SCC	_		•			_					_	
Weaver_Camp	ACC	CTT	TGC	ညည	_		•			_				•	_	
Hurles_Circle	ACC	CTT	TGC	ဗ္ဗဘ္ဘ	_		•			_				-	_	
Briceland	ACC	CTT	\mathbf{IGC}	S	_	_	•			_					_	
Mount_Diablo	ACC	CTT	TGC	ညည	_					_				•	_	
Missouri_Flat	ACC	CTT	\mathbf{TGC}	SCC	_	_				_				•	_	_
Round_Valley	ACC	CTT	TGC	SCG	_	_	•			_				•	_	_
Paradise	ACC	CTT	TGC	ညည	_	_	. •			_				•	_	
San_Marcos_Pass	ACC	CTT	\mathbf{TGC}	ညည	_	-	•			_				•	_	_
Pinnacles	ACC	CTT	TGC	ဗ္ဗဘ	_	_				_				•	_	_
Bear_Valley	ACC	CTT	TGC	CCG	SGC	_				_	_				CGC	_
Auberry_Road	ACC	CTT	TGC	SCG	Sec	_	•			_	_			•	_	GCG
Lake_Santa_Margarita	ACC C	CTT	IGC	၅၁၁	CGC	_				_		•			CGC	_

Pcystopteridifolia_A					AAC	GAA	ပ္ပ	C C C	ပ္ပ	C C C C	ATG	CGC	CAA	GGA	AAA	U
Pcystopteridifolia_B	TTG	_			AAC	GAA	CCC	CGG	CGC	GGC	ATG	CGC	CAA	GGA	AAA	Ĕ
Pbracteosa_A		_			AAC	GAA	CCC	CGG	CGC	ggc	CTG	CGT	CAA	GGA	AAA	E E
Pbracteosa_B	TTG 1				AAC	GAA	CCC	CGG	CGC	ggG	CTG	CGT	CAA	GGA	AAA	CIC
P. semibarbata_A	TTG				AAC	GAA	CCC	CGG	CGC	ggG	ATG	CGC	CAA	GGA	AAA	CTC
P. semibarbata B					AAC	GAA	CCC	CGG	CGC	GGC	ATG	CGC	CAA	GGA	AAA	CIO
McBride_Springs		_			AAC	GAA	CCC	CGG	CGT	ggG	ATG	CGC	CAA	GGA	AAA	r F J
Pinehurst	TTG	_			AAC	GAA	CCC	CGG	CGT	ggG	ATG	CGC	CAA	GGA	AAA	CTC
Hobo_Gulch		_			AAC	GAA	CCC	CGG	CGT	ggc	ATG	CGC	CAA	GGA	AAA	CTC
Inskip		_			AAC	GAA	CCC	CGG	CGT	ggG	ATG	CGC	CAA	GGA	AAA	CTC
Weaver_Camp		_			AAC	GAA	CCC	CGG	CGT	ggG	ATG	CGC	CAA	GGA	AAA	CTC
Hurles_Circle		_			AAC	GAA	ညည	CGG	CGT	GGG	ATG	CGC	CAA	GGA	AAA	CTC
Briceland		_			AAC	GAA	ညည	CGG	CGT	ggc	ATG	CGC	CAA	GGA	AAA	CTC
Mount_Diablo		_	999		AAC	GAA	ညည	CGG	CGT	GGG	ATG	CGC	CAA	GGA	AAA	CTC
Missouri_Flat		_			AAC	GAA	CCC	CGG	CGT	ggG	ATG	CGC	CAA	GGA	AAA	CIC
Round_Valley		_	_		AAC	GAA	CCC	CGG	CGT	GGG	ATG	CGC	CAA	GGA	AAA	CTC
Paradise		_	_		AAC	GAA	CCC	CGG	CGT	ggc	ATG	CGC	CAA	GGA	AAA	CTC
San_Marcos_Pass		_	_		AAC	GAA	CCC	CGG	CGT	GGC	ATG	CGC	CAA	GGA	AAA	CTC
Pinnacles	TTG 1	_	_		AAC	GAA	CCC	CGG	CGT	GGC	ATG	CGC	CAA	GGA	AAA	CTC
Bear_Valley	TTG 1	_	_		AAC	GAA	CCC	CGG	CGT	ggG	ATG	CGC	CAA	GGA	AAA	CTO
Auberry_Road	TTG 1	TGC (_	CCT	AAC	GAA	CCC	CGG	CGT	GGC	ATG	CGC	CAA	GGA	AAA	CIC
Lake_Santa_Margarita	TTG 1) වවව	CI	AAC	GAA	CCC	SSS	CGT	Sec	ATG	CGC	CAA	GGA	AAA	CIO

H 0 0	9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	GGG
	166 161 161 161 161 161 161 161 161 161	TGT
	990 990 990 990 990 990 990 990 990 990	_
		GTG
	661 661 661 661 661 661 661 661 661 661	•
		S
	C GTT	
	1 1 2 2 2 3 3 4 4 5 6 5 6 5 6 5 6 5 6 5 6 5 6 5 6 5 6	•
	C CGT C CGT C CGT C CGT C CGT C CGT C CAT C CAT C CAT C CTT	
		_
	CT ACC	•
		G TCT
	444444444444444444444444444444444444 4	
	ACA GA ACA AC	ACA GA
L 44 R		K
	olia gilo	rita
	eridif eridif sa_A sa_B sa_B bata_A sbata_B rbata_B rilat ey ey ey	arga
	opter eosa eosa arba arba spri tr ch ablo ablo illey cos_P	ta_M
	Pcystopteridifolia_B Pcystopteridifolia_B Pbracteosa_A Psemibarbata_A Psemibarbata_B McBride_Springs Pinehurst Hobo_Gulch Inskip Weaver_Camp Hurles_Circle Briceland Mount_Diablo Missouri_Flat Round_Valley Paradise San_Marcos_Pass Pinnacles Bear_Valley Auberry Road	Lake_Santa_Margarita
	Pcys Pcys Pcys Pbra Psem Psem McBrid Pinehu Hobo_G Inskip Weaver Hurles Bricel Mount Missou Round Paradi San_Ma	Lake

▼5.8S Ribosomal DNA

240

TCT

TCT

ATA

TCT

ATA ATA

ATA

TCT TCT TCT TCT TCT TCT

ATA ATA ATA ATA ATA

ATA

TCT TCT TCT

TCT

ATA ATA ATA

ATA

TCT

TCT

ATA

ATA ATA ATA

ATA

CGG CAA CGG TCT TTT TTT TTT TCT LTT TTTTTT TTT TCT GAC AAC AAM AAC AAC AAC AAC AAC AAC AAC AAC CAT TGT IGI TGT TGT TGI TGT TGT TGT IGT IGT IGT CAA CAA CAA TAA TAA CAA CTA CTA CTC CTC CIC CTC CTC CTC CIC CIC CTC CIC CIC CTC CTC CTC CIC CIC CIC CTC CTY CTC TCT GCT TCT lCT ACG GCG GCG GCG ACG ACG ACG ACG GCG GCG GCG ACG CTG CTG CTG CTG CTG CTG CYG STG CTG CTG CTG CTG CTG CTG CTG SIG CTG CTG CTG CTG GAC GAC GAC GAC GAC GAC GAA GAC 99 _cystopteridifolia_A cystopteridifolia_B Lake_Santa_Margarita P. semibarbata McBride Springs San Marcos Pass P._bracteosa_B P._semibarbata_ P._bracteosa_A Weaver_Camp Hurles_Circle Missouri Flat Mount_Diablo Auberry_Road Round Valley Bear_Valley Hobo_Gulch Briceland Pinehurst Pinnacles Paradise Inskip

т т V	AAC GCA AGT TGC GCC	GCA AGT TGC
	GAG TCT TTG	GAG
	TGA ACC ATC	TGA ACC
	AGA ATC CCG	AGA ATC
01 00 OV	A AAT TGC	
	P. cystopteridifolia A P. cystopteridifolia B P. bracteosa A P. bracteosa B P. semibarbata A P. semibarbata B McBride Springs Pinehurst Hobo Gulch Inskip Weaver Camp Hurles Circle Briceland Mount Diablo Missouri Flat Round Valley Paradise San Marcos Pass Pinnacles Bear Valley Auberry Road	Lake_Santa_Margarita

_
Б
ί
H
con
•
$\overline{}$
_
13 (
13 (
٥
٥
ข

VITS2	8	80	4	GCC TGG GCG TCA CGC	GCC TGG GCG TCA CGC	GCC TGG GCG TCA CGC	T GCC TGG GCG TCA CGC ATC GCG	GCC TGG GCG TCA CGC	BCC	GCC TGG GCG TCA CGC	BCC		GCC TGG GCG TCA	GCC TGG GCG TCA CGC	GCC TGG GCG TCA CGC	T GCC TGG GCG TCA CGC ATC GCG	GCC TGG GCG TCA CGC	GCC TGG GCG TCA CGC	GCC TGG GCG TCA CGC ATC	GCC TGG GCG TCA CGC	GCC TGG GCG TCA CGC	GCC TGG GCG TCA CGC	GCC TGG GCG TCA CGC	GCC TGG GCG TCA CGC AT	GCC TGG GCG TCA CGC
	3	3	7	TCG GCC GAG GGC ACG T	AGC CAT TCG	CKA AGC CAT TCG GCC GAG GGC ACG TCT	AGC CAT TCG GCC GAG GGC ACG	CGA AGC CAT TCG GCC AAG GGC ACG TCT	MGC CAT TCG	\mathtt{TCG}	AGC	AGC CAT TCG GCC AAG	AGC CAT TCG GCC AAG GGC		AGC CAT TCG GCC AAG GGC ACG	CGA AGC CAT TCG GCC AAG GGC ACG CCT	TCG GCC AAG	AGC	CAT TCG GCC AAG GGC ACG	CGA AGC CAT TCG GCC AAG GGC ACG CCT	CAT TCG GCC AAG	CAT TCG GCC AAG	CGA AGC CAT TCG GCC AAG GGC ACG CCT	CGA AGC CAT TCG GCC AAG GGC ACG CCT	CGA AGC CAT TCG GCC AAG GGC ACG CCT
				P. cystopteridifolia A	Pcystopteridifolia_B	P. bracteosa A	Pbracteosa_B	Psemibarbata_A	Psemibarbata_B	McBride_Springs	Pinehurst	Hobo_Gulch	Inskip	Weaver_Camp	Hurles_Circle	Briceland	Mount_Diablo	Missouri_Flat	Round_Valley	Paradise	San_Marcos_Pass	Pinnacles	Bear_Valley	Auberry_Road	Lake_Santa_Margarita

ന

ν,)	7	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG
			GGG	GGG	GGG	GGG	GGG	GGG	GGG	GGG	GGG	GGG	GGG	GGG	TGG	\mathbf{TGG}	\mathbf{TGG}	TGG	\mathbf{TGG}	GGG	\mathbf{TGG}	TGG	GGG	\mathbf{TGG}
			AGG	AGG	CGT	CGT	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG	CGG
			$_{ m TGT}$	TGT	TGG	TGG	TGG	\mathbf{TGG}	TGG	TGG	TGG	TGG	TGG	TGG	TGG	TGG	TGG	TGG	TGG	TGG	TGG	TGG	TGG	TGG
			GGG	GGG				GGG													GGG	GGG	GGG	999
			ATT	ATT	ATC			ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC
			BCB	gcg	gcg			gcg		gag			gcg	gcg	gcg	ggg	gcg				gce			5005
			GGG	GGG	GGG	GGG	GGG	GGG		GGG		GGG	GGG	ggg	9999	GGG	999	999	999	GGG	999	999	999	999
			CAC	CAC	CAC	CAC	CAC	CAC	CAC	CAC								CAC	CAC	CAC	CAC	CAC	CAC	CAC
				ညည		CCC				CCC												CCC		מממ
			_	_	_	ACT	ACT			ACT											ACT			ACT
				ညည			CCC														ညည		•	CCC
							CCA												GCA			GCA		GCA
				CCC			CCA												CCA (CCA (CCA (CCA (CCA	CCA
			CCT	CCT	CCC	CCC	CCT	CCI	CCT	CCT	CCT	CCT	CCT	CCL	ACT	CCT	CCT	CCT	CCT	CCT	CCT	CCT	CCT	CCT
α)	ഹ	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG
			Pcystopteridifolia_A	P. cystopteridifolia_B	P. bracteosa A	P. bracteosa B	P. semibarbata A	P. semibarbata B	McBride_Springs	Pinehurst	Hobo_Gulch	Inskip	Weaver_Camp	Hurles_Circle	Briceland	Mount_Diablo	Missouri_Flat	Round_Valley	Paradise	San_Marcos_Pass	Pinnacles	Bear_Valley	Auberry_Road	Lake_Santa_Margarita

4 & 0	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC	AAC
	_	GAG		GAG														GAG .			
	•	AAT (_		_	_
	TTA 7		CTA 7												-			-		•	•
	ט כ	. 000 000				355 360							255						_	383	_
	•	פננו	_	_	_								CCC (_	_	_	_	_	_	_	229
					GCG (GTG (_
	CGC C		CGC																		
			ACT (
	CTA		CTA 7						-					•	•	•	•	•			•
		GYG		GCT																	
	CGT	_	_														_	_	_	_	-
			325																300	325	
		TTG															_	_	_	_	_
4 w w		AAA		AAA	AAA	AAA	AAA	AAA 1		AAA 1			AAA 1			AAA 1	AAA 1	AAA 1	AAA 1	AAA 1	AAA 1
	4	٩										Ť	Ť	·		·	·	•	·	•	·
	Pcystopteridifolia_	Fcystopteiluliolia_P. bracteosa A	Pbracteosa_B	P. semibarbata A	P. semibarbata B	McBride_Springs	Pinehurst	Hobo_Gulch	Inskip	Weaver_Camp	Hurles_Circle	Briceland	Mount_Diablo	Missouri_Flat	Round_Valley	Paradise	San_Marcos_Pass	Pinnacles	Bear_Valley	Auberry_Road	Lake_Santa_Margarita

ω 72 ω

1	A CCG T	cystopteridifolia_B CCG TGG CGA	bracteosa A CCG TGG CGA	bracteosa B CCG TGG CGA	semibarbata A CCG TGG CGA	_	_	_	_	_	Weaver_Camp CCG TGG CGA	le ccg rgg	CCG TGG	_	_	_	_	San_Marcos_Pass CCG TGG CGA	Pinnacles CCG TGG CGA	•	Auberry_Road CCG TGG CGA	Lake_Santa_Margarita CCG TGG CGA
	CTC GCG	CTC GCG	CTC GCG	CTC GCG	CTC GCG	BCG	BCB	GCG	GCG	GCG	CTC GCG	GCG	GCG	GCG	GCG	GCG	GCG	GCG	GCG	CIC GCG	CIC GCG	CTC GCG
	TCA CG	_	TCA CG		TCA CG						TCA CGA		_	_	_	_	_	_	_	TCA CGA	TCA CGA	TCA CGA
		CGA CCA	CGA CCA								A CCA									A CCA	<u> </u>	ia cca
	GTG	GTG 1	GTG 1								GTG								GTG	GTG	GTG	GTG
	GTG	GTG	GTG	GTG							GTG							GTG	GTG	GTG	GTG	GTG
	GTT (GTT (GTT (GTT (GTT (-	_	_	_	_	GTT (GTT	GTT (GTT (GTT (GTT (
	BAA (BAA (BAA (_	_	_				_	GAA (_	_	_	_	_	_	_	_	SAA (GAA (SAA (
	CTC TCA	CTC TCA	CAC TCA								CAC TCA											CAC TCA
	A ACT	A ACT	A ACT	A ACT	A ACT	A ACT	A ACT		A ACT		A ACT									A ACT	•	A ACT
ω	CIC	CIC	CIC	CIC	CIC	CIC	CIC				CIC								CTC	CIC	CIC	CIC

6 7 5	222 222 242	200	S S S	G G G	CCCA	
		AGA CAGA CAGA CAGA CAGA CAGA CAGA CAGA	AGA CAGA CAGA CAGA CAGA CAGA CAGA CAGA			
						1 CG1
	GTG GTG	999 900 000	999		666 666 7666	
						610 610 610 610 610
	300 300 300					
	TGC TGC CCG	CCG TCG TTG	TCG TCG	TCG	100 100 100 100	100 100 100 100 100
	GAG GAG GAG	GAG GAG GAG	GAG GAG GAG	GAG GAG GAG	GAG GAG GAG	GAG GAG GAG GAG GAG GAG
	TTA TTA TCC	TCC	700 700 700	100 100 100	NCC FCC	1100
						CGT
					16C 16C 16C	
	TCG TCG TCG				17CG 17CG 17CG 17CG 17CG 17CG 17CG 17CG	
	GTG C GTG C GTG C	GTG CGTG CGTG CGTG CGTG CGTG	GTG CTG CTG CTG CTG CTG CTG CTG			
n 0 0		0 0 0	000	0 0 0	0 0 0 0	
	Pcystopteridifolia_A Pcystopteridifolia_B Pbracteosa_A	Pbracteosa_B Psemibarbata_A Psemibarbata_B	McBride_Springs Pinehurst Hobo Gulch	Inskip Weaver_Camp Hurles Circle	Briceland Mount_Diablo Missouri_Flat	Paradise San_Marcos_Pass Pinnacles Bear_Valley Auberry_Road Lake_Santa_Margarita

q
cont,
13
Table

	2 7								90	
	7								m	
P. cystopteridifolia A	ATC	ggc	gcg	ATC	CTA	TCG	CGC	CTT	CGA	
P. cystopteridifolia B	ATC	GGC	GCG	ATC	CTA	\mathbf{T}^{CG}	CGC	CTT	CGA	
P. bracteosa A	AAC	GGC	BOB	ATC	CTA	\mathbf{TCG}	\mathbf{TGC}	CTT	CGA	
P. bracteosa B	AAC	GGC	gcg	ATC	CTA	${ m TCG}$	$^{\mathrm{TGC}}$	CTT	CGA	
P. semibarbata A	AAT	GGC	BOB	ATC	CCA	\mathbf{TCG}	CGC	CTT	CGA	
P. semibarbata B	AAT	GGC	BOB	ATC	CCA	\mathbf{TCG}	CGC	CTT	CGA	
McBride_Springs	AAC	GGC	BOB	ATC	CTA	TGG	CGC	CTT	CGA	
Pinehurst	AAC	GGC	GCG	ATC	CTA	\mathbf{TGG}	CGC	CTT	CGA	
Hobo Gulch	AAC	GGC	GCG	ATC	CTA	TGG	CGC	CTT	CGA	
Inskip	AAC	ggc	gag	ATC	CTA	TGG	CGC	CTT	CGA	
Weaver_Camp	AAC	GGC	BOB	ATC	CTA		CGC		TGA	
Hurles_Circle	AAC	CGC	BOB	ATC	CTA	TGG	CGC		CGA	
Briceland	ACG	GGG	gag	ATC	CTA		GNG	CTT	CGA	
Mount_Diablo		GGC	BOB	ATC	CTA		ည္သည	CTT	CGA	
Missouri_Flat	AAC	ggc	GCG	ATC	CTA	\mathbf{TGG}	CGC	CTT	CGA	
Round_Valley	AAC	GGC	GCG	ATC	CTA	\mathbf{TGG}	CGC	CTT	CGA	
Paradise	1 1 1	1 1 1	i i I	1	1	1	1 1 1	!	1	
San_Marcos_Pass	AAC	GGC	GCG	ATC	CTA	TGG	CGC	CIT	CGA	
Pinnacles	AAC	GGC	gag	ATC	CTA	TGG	CGC	CTT	CGA	
Bear_Valley	AAC	GGC	GCG	ATC	CTA	\mathbf{TGG}	CGC	CTT	CGA	
Auberry_Road	AAC	ggc	GCG	ATC	CTA	\mathbf{TGG}	CGC	CLT	CGA	
Lake_Santa_Margarita	AAC	GGC	BOB	ATC	ł !	! ! !	! ! !	1 1 1	1 1 1	

APPENDIX B

Aligned sequences of *Pedicularis*: intergenic region between *trn*L3' and *trn*F coding regions

Table 14. Aligned sequences of intergenic region between trnL3' and trnF coding regions

4 &

4 6

6 9

CCT

CCT CCT CCT CCT CCT CCT

CCT CCT CCT CCT

CCT

CCT

CCT CCT

CCT CCT

CCT

L 4 C

TCC AAA AAG GCA TAA AAT CCA ACA CAT ACA AAT TAG ATA GTG CAT TCA ICA ICA TCA ICA TCA TCA **LTA** LTA ITA ITA LTA LTA TTA TTA TTA TTA LTA TTA TTA TTA TTA TTA TTA TTA TTA ITA LTA cystopteridifolia_A cystopteridifolia_B Lake Santa Margarita P. semibarbata_A P._cystcr_ P. bracteosa_A McBride_Springs San Marcos Pass P._semibarbata_ bracteosa B Weaver_Camp Hurles_Circle Missouri Flat Mount Diablo Auberry_Road Round Valley Bear_Valley Hobo_Gulch Briceland Pinehurst Pinnacles Paradise Inskip

1 6 E	cystopteridifolia_A TA: ::: cystopteridifolia_B TA: ::: bracteosa A TAT :TT	bracteosa_B TAT : TT semibarbata_A TAT : TT	TAT	Pinehurst TAT ATT	TAT	_Camp TAT	rcle TAT	Briceland TAT ATT		t TAT	Round_Valley TAT ATT	Paradise TAT ATT	San_Marcos_Pass TAT ATT	Pinnacles TAT ATT	Bear_Valley TAT ATT	Auberry_Road TAT ATT	argarita TAT
	::: ::: ::: ::: GAA TGA															_	_
		TTC										TTC	$_{ m LLL}$	TTC			
	::: ACA												ACA			ACA	
	::: ::: ATC									-	-	-	-			•	•
	 AAT A																
		AAC A													-	-	-
		ATT A															
		ACT C															
	::: :: ::: :: CAT AC																
	•	ACT GA				CT GAA					••	•••			T GAA		
	::: ::: ::: ::: GAA ACT			GAA AC								•••					
	H			TCG		T TCG		••								T TCG	
040	ტ	ប្តូ ប្តូ ប្	ָטָ טָ	ָטָ טָ	ņ	ថ្ង	Ŋ	••	••	••	••	••	Ö	Ŋ	Ŋ	ָטַ	Ŋ

7 8 8

AAC AAC

AAC

AAC AAC

AAC

AAC

AAC AAC AAC

AAC

AAC AAC AAC AAC AAC AAC AAC AAC AAC AAC

	28 6	9 3 3	
	GTA ATC TCC CCC AGG	TTT AAT TGA CAT AGA CCC CAG TNA	r .
idifolia *	TCC CCC AGG	TTT AAT TGA CAT AGA CCC CAG TTA	F. F
F. Dracteosa B	GTA ATC TCC CCC	TTT AAT	
P. semibarbata A	GTA ATC TCC CCC AGG	TTT AAT TGA CAT AGA CCC CAG TTA	.
P. semibarbata B	ATC TCC CCC AGG	TTT AAT TGA CAT AGA CCC CAG TTA	٠.
McBride_Springs	ATC TCC CCC AGG	TTT AAT TGA CAT AGA CCC CAG TTA	.
Pinehurst	TCC CCC AGG	TIT AAT TGA CAT AGA CCC CAG TTA	٠.
Hobo_Gulch	ATC TCC CCC AGG	TTT AAT TGA CAT AGA CCC CAG TTA	٠.
Inskip	GTA ATC TCC CCC AGG	TTT AAT TGA CAT AGA CCC CAG TTA	.
Weaver_Camp	GTA ATC TCC CCC AGG	TTT AAT TGA CAT AGA CCC CAG TTA	.
Hurles_Circle	GTA ATT TCC CCC AGG	TIT AAT TGA CAT AGA CCC CAG TTA	
Briceland	GTA ATC TCC CCC AGG	TIT AAT TGA CAT AGA CCC CAG TIA	.
Mount_Diablo	GTA ATC TCC CCC AGG	TTT AAT TGA CAT AGA CCC CAG	•
Missouri_Flat	TCC CCC AGG	TIT AAT IGA CAT AGA CCC CAG ITA	
Round_Valley	GTA ATC TCC CCC AGG	TIT AAT IGA CAT AGA CCC CAG ITA	. .
Paradise	TCC CCC AGG	TTT AAT TGA CAT AGA CCC CAG TTA	
San_Marcos_Pass	GTA ATC TCC CCC AGG	TIT AAT TGA CAT AGA CCC CAG	
Pinnacles	CCC AGG	TIT AAT IGA CAT AGA CCC CAG ITA	•
Bear_Valley	TCC CCC AGG	TTT AAT TGA CAT AGA CCC CAG TTA	. .
Auberry_Road	AGG	TTT AAT TGA	
Lake_Santa_Margarita	AGG	TTT AAT TGA	

m	∞	4	1 1 1	CTC	CTC	CTC	CTC	CIC	CTC	CTC	CTC	CTC	CTC	CTC	CTC	CTC	CTC	CIC	1 1	CIC	CTC	CIC	! !	CIC
			 	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	1	TAG	TAG	TAG	1 1	TAG
			!	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	1	GGA
			 	TCG	\mathbf{T}^{CG}	$\mathbf{T}^{\mathbf{C}}$	\mathbf{TCG}	\mathbf{TCG}	$\mathbf{T}^{\mathbf{C}}$	TCG	TCG	$\mathbf{T}^{\mathbf{C}}$	\mathbf{I}^{CG}	$\mathbf{T}^{\mathbf{C}}$	\mathbf{T}^{CG}	\mathbf{TCG}	\mathbf{TCG}	TCG	TCG	\mathbf{T}^{CG}	TCG	TCG	! !	TCG
			 	TGG	TGG	TGG	\mathbf{TGG}	$_{\mathrm{TGG}}$	$_{\mathrm{TGG}}$	TGG	$^{\mathrm{TGG}}$	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	$_{\mathrm{TGG}}$	\mathbf{TGG}	\mathbf{TGG}	$^{\mathrm{TGG}}$	\mathbf{TGG}	1 1	\mathbf{TGG}
			! ! !	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	 	GAA
			1 1 1	TGG	TGG	TGG	\mathbf{TGG}	\mathbf{TGG}	TGG	\mathbf{TGG}	$_{\mathrm{TGG}}$	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	${\tt TGG}$	${ m TGG}$	\mathbf{TGG}	TGG	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	1	\mathbf{TGG}
			1 1 1	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	! !	AAT
			 	TAC	TAC	TAC	TAA	TAA	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	1 1	TAC
			1 1 1	TGC	TGC	\mathbf{TGC}	$\mathbf{I}\mathbf{G}\mathbf{C}$	$_{\rm IGC}$	$\mathbf{I}\mathbf{G}\mathbf{C}$	\mathbf{TGC}	$_{\mathrm{IGC}}$	\mathbf{TGC}	\mathbf{IGC}	TGC	TGC	$_{\mathrm{TGC}}$	TGC	IGC	TGC	TGC	TGC	TGC	1 1 1	TGC
			GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	 	GGA
			ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	1 1 1	ATA
			AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	1 1 1	AGC
			ATG	ATG	ATA	ATA	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	1	ATG
			AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	1 1 1	AAA
m	ന	7	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	1	AAT
			P. cvstopteridifolia A		P. bracteosa A	P. bracteosa B	P. semibarbata A	P. semibarbata B	McBride_Springs	Pinehurst	Hobo_Gulch	Inskip	Weaver_Camp	Hurles_Circle	Briceland	Mount_Diablo	Missouri_Flat	Round_Valley	Paradise	San_Marcos_Pass	Pinnacles	Bear_Valley	Auberry_Road	Lake_Santa_Margarita

_
ਰ
.0
٠.
بد
ㅁ
O
cont
$\overline{}$
14
e 14
υ
υ

4	0	4	 	CT	i	L	CI	CI	l	CH	CI	CI	CT	1	CI	CI	CŢ	I I	1	CI	CI	CI	1	1
			1 1 1	GGA	1	GGA	GGA	GGA	1 1	GGA	GGA	GGA	GGA	1	GGA	GGA	GGA	G	1	GGA	GGA	GGA	! ! !	G
			! !	AGA	AGA	AGA	AGA	AGA	1	AGA	AGA	AGA	AGA	1	AGA	AGA	AGA	AGA	1 1 1	AGA	AGA	AGA	 	AGA
			! !	AGC	AGC	AGC	AGC	AGC	 	AGC	AGC	AGC	AGC	1	AGC	AGC	AGC	AGC	1 1 1	AGC	AGC	AGC	1 1 1	AGC
			1	TAG	TAG	TAG	TAG	TAG	1	TAG	TAG	TAG	TAG	1 1 1	TAG	TAG	TAG	TAG	1	TAG	TAG	TAG	1	TAG
			1	\mathbf{TGG}	\mathbf{TGG}	TGG	TGG	\mathbf{TGG}	1	TGG	TGG	TGG	\mathbf{TGG}	l 1 1	TGG	TGG	TGG	\mathbf{TGG}	1 1	TGG	TGG	TGG	1 1 1	\mathbf{TGG}
က	œ	വ	! !	AGC	AGC	AGC	AGC	AGC	A	AGC	AGC	AGC	AGC	1	AGC	AGC	AGC	AGC	 	AGC	AGC	AGC	ł 	AGC
			P. cystopteridifolia A	P. cystopteridifolia B	P. bracteosa A	P. bracteosa B	P. semibarbata A	Psemibarbata_B	McBride Springs	Pinehurst	Hobo Gulch	Inskip		Hurles Circle	Briceland	Mount Diablo	Missouri Flat	Round_Valley	Paradise	San Marcos Pass	Pinnacles _	Bear_Valley	Auberry Road	Lake_Santa_Margarita

APPENDIX C

Aligned sequences of *Pedicularis*: intergenic region between *psbA* and *trnH* coding regions

Table 15. Aligned sequences of intergenic region between psbA and trnH coding regions from the taxa sampled in Pedicularis. Names correspond to vouchers listed in Table 1.

"-" GAC GAC GAC GAC GAC GAC GAC GAC 4 8 GAC Gaps indicated with ":", Ambiguities indicated with "N", Missing data indicated with TAA GGA GGG GGA GGG GGA GGA GGA GGA GGG GGA GGA AAT ACA CCA GCT GAA ATC ATC ATT ATT ATT ATT ATT ATT ATT ATT ATT ATC ATT ATT ATT ATT ATT ATT ATT ATT ATT GCT GCG GCT TTG TIG TTG ITG TTG TTG TTG TTG TTG GAC CTA CCT 1 TTC TTC TTC TTC TIC TIC TTC TTC TTC TTC TIC TTC TTC TIC TTC 1 TIC TTC P._cystopteridifolia_B P._bracteosa_A P._cystopteridifolia_A Lake Santa Margarita P._semibarbata_A McBride_Springs San Marcos Pass P. semibarbata bracteosa B Hurles Circle Missouri Flat Mount_Diablo Round Valley Auberry_Road Camp Bear_Valley Hobo_Gulch Pinnacles Pinehurst Briceland Paradise Weaver_ Inskip P. L

TAT	TAT	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
TAA	TAA	•••	••	••	••	••	••	••	••	••	••	••	••	••	••	•••	••	••	::	••	••
ATA	ATA	ATA	ATA	ATT	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA
AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT
AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT
TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG
AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA
TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT
AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG
AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG
TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT
GTG	\mathtt{GTG}	GTG	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	GTG	\mathtt{GTG}	GTG	\mathtt{GTG}	GTG	GTG	\mathtt{GTG}	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG
TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA
GLT	GLL	GIC	GIC	GIC	GIC	GTC	GIC	GTC	GTC	GIC	GTC	GTC	GIC	GTC	GTC	GIC	GIC	GIC	GTC	GTC	GTC
\mathtt{TTG}	TTG	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	TTG	TTG	TTG		TTG	TTG	TIG	TTG	TTG	TTG	TTG	$_{ m TTG}$	TTG	TTG	TTG	TTG
Pcystopteridifolia_A	Pcystopteridifolia_B	Pbracteosa_A	Pbracteosa_B	P. semibarbata A	P. semibarbata_B	McBride_Springs	Pinehurst	Hobo_Gulch	Inskip	Weaver_Camp	Hurles_Circle	Briceland	Mount_Diablo	Missouri_Flat	Round_Valley	Paradise	San_Marcos_Pass	Pinnacles	Bear_Valley	Auberry_Road	Lake_Santa_Margarita

P. cystopteridifolia A	AAG	GAG	CAA	TAA	ACT	CTT	$\mathbf{I}^{\mathbf{C}\mathbf{I}}$	TGG	ICI	ACC	AAG	AGG	ცვ	: TT	TTT	GCT
P. cystopteridifolia B	AAG G	GAG	CAA	TAA	ACT	CIT	\mathtt{TCT}	\mathbf{TGG}	TCT	ACC	AAG	AGG	GG:	: TI	TTT	GCT
P. bracteosa A	:AG	GAG	CAA	TAA	ACT	CTT	TCT	\mathbf{TGG}	\mathbf{TCT}	ACC	AAG	AGG	gg:	: TT	TTT	GCT
P. bracteosa B	:AG	GAG	CAA	TAA	ACC	: (၄:	TCT	\mathbf{TGG}	TAG	ACC	AAG	AAA	GAG	TTT	ATT	GCT
P. semibarbata_A	AAG	GAG	CAA	TAA	ACT	CTT	\mathtt{TCT}	\mathbf{TGG}	TCT	ACC	AAG	AGG	gg:	: TT	TTT	GCT
Psemibarbata_B	: AG	GAG	CAA	TAA	ACT	CTT	TCT	\mathbf{TGG}	\mathbf{ICI}	ACC	AAG	AGG	GG:	: TT	TTT	GCT
McBride_Springs	:AG	GAG	CAA	TAA	ACT	CTT	TCT	\mathbf{TGG}	TCI	ACC	AAG	AGG	gg:	: TT	TTT	GCT
Pinehurst	:AG	GAG	CAA	TAA	ACT	CTT	TCT	TGG	$\mathbf{I}\mathbf{C}\mathbf{I}$	ACC	AAG	AGG	gg:	: TT	TTT	GCT
Hobo_Gulch	:AG	GAG	CAA	TAA	ACT	CTT	TCT	${ m TGG}$	\mathbf{TCT}	ACC	AAG	AGG	GG:	: TT	TTT	GCT
Inskip	:AG	GAG	CAA	TAA	ACT	CTT	TCT	\mathbf{TGG}	TCT	ACC	AAG	AGG	gg:	: TT	TTT	GCT
Weaver_Camp	:AG	GAG	CAA	TAA	ACT	CTT	\mathbf{TCT}	\mathbf{TGG}	TCT	ACC	AAG	AGG	GG:	: TT	TTT	GCT
Hurles_Circle	:AG	GAG	CAA	TAA	ACT	CTT	TCT	${ m TGG}$	\mathtt{TCT}	ACC	AAG	AGG	GG:	: TT	TTT	GCT
Briceland	: AG	GAG	CAA	TAA	ACT	CTT	TCT	TGG	TCT	ACC	AAG	AGG	GG:	TT:	TTT	GCT
Mount_Diablo	: AG	GAG	CAA	TAA	ACT	CTT	$\mathbf{I}^{\mathbf{C}}$	${\tt TGG}$	\mathbf{ICI}	ACC	AAG	AGG	gg:	: TT	TTT	GCT
Missouri_Flat	:AG	GAG	CAA	TAA	ACT	CTT	TCT	TGG	\mathbf{TCT}	ACC	AAG	AGG	GG:	: TT	$\mathbf{T}\mathbf{T}\mathbf{I}$	GCT
Round_Valley	:AG	GAG	CAA	TAA	ACT	CTT	TCT	TGG	TCT	ACC	AAG	AGG	GG:	:TT	TTT	GCT
Paradise	:AG	GAG	CAA	TAA	ACT	CTT	TCT	\mathbf{TGG}	\mathbf{ICI}	ACC	AAG	AGG	GG:	:TT	TTT	GCT
San_Marcos_Pass	: AG	GAG	CAA	TAA	ACT	CTT	\mathbf{ICI}	TGG	\mathbf{ICT}	ACC	AAG	AGG	GG:	:TT	TTT	GCT
Pinnacles	: AG	GAG	CAA	TAA	ACT	CTT	\mathbf{ICI}	\mathbf{TGG}	\mathbf{ICI}	ACC	AAG	AGG	gg:	: TT	TTT	GCT
Bear_Valley	:AG	GAG	CAA	TAA	ACT	CTT	\mathbf{ICI}	\mathbf{TGG}	\mathbf{ICI}	ACC	AAG	AGG	GG:	:TT	TTT	GCT
Auberry_Road	:AG	GAG	CAA	TAA	ACT	CTT	\mathbf{ICI}	\mathbf{TGG}	TCT	ACC	AAG	AGG	GG:	: TT	TTT	GCT
Lake_Santa_Margarita	:AG	GAG	CAA	TAA	ACT	CTT	\mathbf{ICI}	\mathbf{TGG}	\mathbf{TCT}	ACC	AAG	AGG	gg:	: TT	TTT	GCT

7 6 7

:GT

AGT AGT AGT AGT AGT AGT AGT AGT AGT

AGT

AGT

::: :GT

:GT

	нов															040
Pcystopteridifolia_A Pcystopteridifolia_B	AGT AGT	ATT	GTA GTA	CTT	305 305	TAG TAG	ACT	$ ext{TTT}$	TTC	TGT TGT	TCA	TTA	CAT	AAA	AAA	GA: GA:
P. bracteosa A	AGT	ATT	GTA	CTT	GCC	TAG	ACT	TTT	TTC	TGI					AAA	GA:
rbracteosa_b Psemibarbata_A	AGT	ATT	GTA	CTT	325	TÀG	ACT	TTT	TTA	TGL	CC.			AAA	AAA	GA:
Psemibarbata_B	AGT	ATT	GTA	CTT	CCC	TAG	ACT	TTT	TTA					-	AAA	GA:
McBride_Springs	AGT	ATT	GTA	CTT	GCC	TAG	ACT	TTT	TTC	TGT				-	AAA	TAT
Pinehurst Hobo Gulch	AGT	ATT	GTA			TAG	ACT		TTC	TGI	CCA			-	AAA	TAT
Inskip	AGT	ATT	GTA	CTT	BCC	TAG	ACT	TTT	TTC	TGI					AAA	TAT
Weaver_Camp	AGT	ATT	GTA	CTT	GCC	TAG	ACT	TTT	TTC	TGI					AAA	TAT
Hurles_Circle	AGT	ATT	GTA	CTT	gcc	TAG	ACT	TTT	TTC	TGI				•	AAA	TAT
Briceland	AGT	ATT	GTA	CTT	gcc	TAG	ACT	TTT	$\mathbf{T}\mathbf{T}\mathbf{C}$	TGI					AAA	TAT
Mount_Diablo	AGT	ATT	GTA	CTT	gcc	TAG	ACT	TTT	$\mathbf{T}\mathbf{T}\mathbf{C}$	TGI					AAA	TAT
Missouri_Flat	AGT	ATT	GTA	CTT	gaa	TAG	ACT	TTT	$\mathbf{T}\mathbf{T}\mathbf{C}$	TGT					AAA	TAT
Round_Valley	AGT	ATT	GTA	CTT	CCC	TAG	ACT	TTT	TTC	TGT				-	AAA	\mathtt{TAT}
Paradise	AGT	ATT	GTA	CTT	gcc	TAG	ACT	TTT	$\mathbf{T}\mathbf{T}\mathbf{C}$	TGT				-	AAA	TAT
San_Marcos_Pass	AGT	ATT	GTA	CTT	gcc	TAG	ACT	TTT	$\mathbf{T}\mathbf{T}\mathbf{C}$	TGT	CCA		_		AA:	TAT
Pinnacles	AGT	ATT	GTA	CTT	gcc	TAG	ACT	TTT	$\mathbf{T}\mathbf{T}\mathbf{C}$	TGT			_		AAA	TAT
Bear_Valley	AGT	ATT	GTA	CTT	gcc	TAG	ACT	TTT	TTC	TGI	_	TTA	_	•	AAA	TAT
Auberry_Road	AGT	ATT	GTA	CTT	gcc	TAG	ACT	TTT	$\mathbf{T}\mathbf{T}\mathbf{C}$	TGI	CCA	TTA	_	•	AAA	TAT
Lake Santa Margarita	AGT	ATT	GTA	CTT	gcc	TAG	ACT	TTT	TTC	TGT	CCA	TTA	_	AAA	AAA	TAT

0 00 00	GAT		GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	\mathtt{GAT}
	GTT	- · · · · · · · · · · · · · · · · · · ·	AII	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	\mathtt{GTT}	GTT	GTT	GTT	GTT	GTT
	AGG		AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG
	TTA	¥ : :	TTA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
	TTC	¥ : !	TLL	:TA	:TA	: TT	:TA	:CA	:TA	:AA	:TA	:AA	:AA	:AA	TTA	:TA	TTA	TTA	TTA
	TTT TTT				•••	•••	••			••	••	•••	••	••	•••	••	TTT	••	••
	TAT	٠٠ ،		••	••	•••	••	••	••	••	••	••	••	••	•••	••	ATA	••	••
			AAT	••	••	••	••			••	••	••	••	•••	••	••	AAT	••	••
		¥ :: ;	AAA AAA	••	••	•••	••	•••	••	••	••	••	••	••	••	••	AAA	••	••
	AA:	£ ::	. 4	••	••	•••	••		••	••	••	••	••	••	•••	••	ATA	••	••
	•• •• •	• ••		••	••	•••	••	••	••	••	•••	••	••	••	••	••	TAC	••	••
	•• •• •	• ••		••	••	••	••	••	••	••	••	••	••	••	••	••	CAT	••	••
	•• •• •	• ••	•• ••	••	••	••	••	••	••	••	••	••	••	••	••	••	GIC	••	••
	•• ••	• ••	•• ••	••	••	••	••	••	••	••	••	••	••	••	••	••	TCT	••	••
	•• ••	• • •	•• ••	TT:	TT:	TT:	TT:	TT:	TT:	TT:	TT:	TT:	TT:	TT:	TT:	TT:	TTT	TT:	TT:
241	•• ••		•••••	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT
		bracteosa_B	Fsemibarbata_A Psemibarbata_B	ן מ		Hobo Gulch	Inskip	Weaver Camp	Circle		Mount Diablo	Missouri Flat	Round Valley	Paradise Paradise	San Marcos Pass	1	Bear Valley	ਯੋ	argarita

m m ७	CAA CAA		•••••	• ••	••	••		••	••	••	••	••	••	••	••	••	••	••
	TGT TGT	• •• ••	•• ••	•	••	•••	••	••	••	••	••	••	••	••	••	••	•••	••
	TTT TTT	· · · · · · · · · · · · · · · · · · ·	•• ••	• ••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
	ATT	• • •	•• ••	• ••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
	CTA 7		•• ••	•••		::	::	::	••			::			::	::	••	••
	TGT C	· · · ·	••••	• ••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
	111 111 	· · · ·	•• ••	• ••					••	••	••		••	••	••	••	••	••
	TC A'	· ·· ··	•• ••	•	••	••	••	••	••	••	••	••	••	••	••	••	••	••
	ט ט ט) ·· O	D F	_	Ö	T G	E G	T G	E E	O	Ö	Ŋ	Q	D H	Q	E E	Ö	Ŋ
	ATT	· ·· 🛏		LL	TTT			TTT,			TTT.	TLL		TLL	TTT	TTT	H	TTT
	TCT) [-	TTT	TTT	$_{ m LLL}$	TTT	TTI	TTT	TTT	$ ext{TTL}$	TTT	TTT	TTT	TTI	TTT	TTT	TTT	$\mathbf{T}\mathbf{T}\mathbf{T}$
	TAT TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT
	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT
	TAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG
	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT
	TAA TAA		TAA															TAA
01 00 O	TAC				TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC
	Pcystopteridifolia_A Pcystopteridifolia_B P_bracteoga_A	bracteosa_B .semibarbata A	_ _semibarbata_B Bride_Springs	Pinehurst	ulch		Weaver_Camp	le	Briceland	Mount_Diablo	Missouri_Flat	Round_Valley	Paradise	San Marcos Pass	Pinnacles	Bear_Valley	Auberry_Road	argarita

	7 M L															w 00 41	ν α 4.
Pcystopteridifolia_A	AAG	GTT	GGC	AAG	$_{ m LLL}$	TGC	CAA	TTC	TAT	$_{ m TTT}$	ALL	GAT	TAA	ATT	GAT	ATT	E.
P. cystopteridifolia_B	AAG	GTT	GGC	AAG	TTT	TGC	CAA	TTC	TAT	TTT	ATT	GAT	TAA	ATT	GAT	ATT	L
Pbracteosa_A	••	•••	•••	•••	••	••	•••	•••	••	••	••	::	•••	•••	••	••	
Pbracteosa_B	••	••	••	::	•••	••	••	••	••	••	••	••	••	••	••	••	••
Psemibarbata_A	••	•••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	
P. semibarbata_B	••	••	•••	•••	•••	•••	••	••	•••	••	••	••	::	••	•••	::	••
McBride_Springs	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
Pinehurst	••	••	••	••	••	••	::	••	••	••	••	••	••	••	••	::	••
Hobo_Gulch	••	••	••		••	••	•••	••	::	••	••	••	••	••	••	•••	
Inskip	••	••	•••	::	•••	•••	•••	••	::	•••	••	•••	•••	••	••	•••	••
Weaver_Camp	••	••	::	::	•••	••	•••	•••		::	••	•••	::	::	••	::	••
Hurles_Circle	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	::	••
Briceland	••	••	••	•••	•••	••	••	••		••	::	••	•••	••	••	::	••
Mount_Diablo	••	••	••	•••	•••	•••	::	••	••	••	••	••	••	••	••	::	••
Missouri_Flat	••	••	••	•••	••	••		•••	••	••	•••	•••	••	::	••	::	••
Round_Valley	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	::	
Paradise	••	••	•••	•••	••	••	••	••	••	••	::	••	::	::	::	::	
San_Marcos_Pass	••	••	••	••	•••	••	•••	•••		••	••	••	••	••	••	••	••
Pinnacles	••	••	••	•••	•••	••	::	••	•••	••	•••	••	••	•••	••	::	••
Bear_Valley	••	••	••	•••	•••	••	••	••	••	••	••	••	••	••	••	••	••
Auberry_Road	••	••	••	•••	••	••	••	••	•••	••	••	••	••	••	••	•••	••
Lake_Santa_Margarita	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	::	

2

Table 15 (cont'd)

i	••	ICC	••	••	••	••	•••	••	••	••	••	••	•••	••	••	••	••	••	••	•••	::
I	••	AA	••	::	••	••	::	••	••		••	••	••	••	••	••	••	••	••	••	••
			••		••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
i I	••	TAA	::	••	::	::	::	::	•••		::	::	::	::	••	::	•••	••	•••	::	••
l I	••	TA		••	••	••	•••	••	••	••	••	••	••	••	••	••	••	••	::		••
			••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
TT-	TT:	$_{ m LLL}$	••	••	••	••	••	••	••	••		••	••	••	••	••	•••	••	::	::	••
TTT	TT	$_{ m LLL}$	•••	•••	••	•••		••	••	••	••	••	••	••	••		••	••	••	••	••
				••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
AT	CAT	AT	••	••	••	••		••	••	••	••	••	••	••	•••	••	••	••	••	••	••
				••	••	••	••	••	••	••	••	••	••	••	••				••		••
GCT	Š	GCT	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
								••	••	••		••	••	••	••	••	••	••	••	••	••
H	TTT	E	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
																••	••	••	••	••	••
T	ATA	T		•••	::	••	•••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
				••	••				••	••	••	••	••	••	••	••	••	••	••	••	••
NAG	NAG	AAG	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
		-		••					••	••	••	••	••	••	••	••	••				••
NAC	NAG	¥¥	••	•••	••	••	•••	••	••	••	••	••	••	••	•••	••	::	•••	::	::	::
			••	••	••			••	••	••	••	••		••	••	••		••	••	••	••
NININ	NNN	AGA	••	••	••	••		••	••	••	••	••	••	••	••	••	••	••	••	••	••
3	Z	\$	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
G.P.	GAN	Ö	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
TCA	Ą	ICC	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
Ħ	H	H	••	••	••	••	••	••	••	••					••						
IC		ຕູ	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
AT	AT	AG	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••	••
Pcystopteridifolia_A	Pcystopteridifolia_B	osa_A	osa_B	rbata_A	rbata_B	prings		ď		đu	rcle		olo	flat	ley		s_Pass		Λέ	bad	Lake_Santa_Margarita
Pcystopt	Pcystopt	Pbracteosa_A	Pbracteosa_B	Psemibarbata_	Psemibarbata_B	McBride_Springs	Pinehurst	Hobo_Gulch	Inskip	Weaver_Camp	Hurles_Circle	Briceland	Mount_Diablo	Missouri Flat	Round_Valley	Paradise	San_Marcos_Pass	Pinnacles	Bear_Valley	Auberry_Road	Lake_Santa
									10	_											

(D) +1000/ 11 - 1																Ŋ
Table 15 (come c)	4															7
	+ ∞ ⊣															∞
P. cystopteridifolia A	!	! !	1	} ! !	! !	!!!	 	1	1 1	! !	!!	! 1	1 1	1	! !	1 1
Pcystopteridifolia_B	••	••								••	••	••				••
Pbracteosa_A	TAA	TGA				AAA				TAA	AAT	AAT	AAT		TAA	TAG
P. bracteosa B	•••	::	••	••	••		••	••	••	••	••	••		••		TAG
P. semibarbata_A	••	••	••						••	••	•••	••	•••	•••		•••
Psemibarbata_B	••	••	••							••	••	••	••	••	••	••
McBride Springs	••	••	••						••	••	•••	••	••	••		••
Pinehurst	••	••	••						••	••	••	••	••	••	••	••
Hobo_Gulch	••	••	••						••	••	••	••	••	••		
Inskip	••	••	••						••	••	••	••	••	••	••	••
Weaver_Camp	••	••	••						••	••	••	••	••	•••	••	•••
Hurles_Circle	••	••	•••						••	••	••	••	••	••	••	••
Briceland	::	••	••						••	••	••	••	••	••	••	••
Mount_Diablo	••	••								••	•••	••	••	••	•••	•••
Missouri_Flat	••	••	••						••	••	••	••	••	••	••	••
Round_Valley	::	••	••	••		••			••	••	••	••	••	••	••	••
Paradise	••	••	::						::	::		••		•••		•••
San_Marcos_Pass	••	••	••						::	••	•••	••	•••	•••	•••	•••
Pinnacles	••	••	••						::	••	::	••		•••	•••	
Bear_Valley	••		::						::	::	•••	••	•••		::	::
Auberry_Road	••	••	•••						::	••	••	••				
Lake_Santa_Margarita	::	::			••		••							•••	::	•••

6 7 5

Table 15 (cont'd)										9
	72 12									0
	, _									വ
Pcystopteridifolia_A	1	1 1	1	1] 	† 	1 1 1	1	1 1 1	1
Pcystopteridifolia_B	::	••	••	••	••	••	••	••	••	••
P. bracteosa A	AAA	ATG	AAT	GAA	ATA	GAA	ATC	GTA	GAG	gg
bracteos	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAG	GG
P. semibarbata A	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAA	GG
P. semibarbata B	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAA	ტე
McBride_Springs	AAA	ATG	AAT	GAA	ATA	NAA	ATC	ATA	GAG	gg
Pinehurst	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAG	GG
Hobo_Gulch	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAG	GG
Inskip	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAG	GG
Weaver_Camp	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAG	gg
Hurles_Circle	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAG	GG
Briceland	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	TAG	g
Mount_Diablo	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	TAG	GG
Missouri_Flat	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	TAG	gg
Round_Valley	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	TAG	GG
Paradise	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAG	gg
San_Marcos_Pass	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAG	GG
Pinnacles	AAA	ATG	AAT	GAN	ATA	GAA	ATC	ATA	GAG	GG
Bear_Valley	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAG	gg
Auberry_Road	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAG	GG
Lake_Santa_Margarita	AAA	ATG	AAT	GAA	ATA	GAA	ATC	ATA	GAG	GG

APPENDIX D

Aligned sequences of Cantua: intergenic region between trnT5' and trnL coding regions

with "N", Missing data indicated with "-". Specific insertion deletion events are labeled gap Table 16. Aligned sequences of the intergenic spacer region between the trnT5' and trnL epithets on vouchers listed in Table 4. Gaps indicated with ":", Ambiguities indicated coding regions for the taxa sampled in Cantua data set. Names correspond to specific Coding region at the end of the sequence, "C" represents gap presence, "A" represents in the alignment with insertion deletion coding at the end of the sequence. In Indel absence.

4 &

Н

GAT TTC TTC TIC TTC TIC TTC TIC TIC TIC TTC LTC TIC TIC TIC TTA ITA ITA TTA TTA TAC FAC FAC FAC FAC PAC **TAC** FAC TAT IAT TAT **LAT** TAT TAT **TAT** TAT FAT AGC CLL CLL CLI CTT AAT TGG TGG TGG TGG IGG TGG IGG TGG TGG TGG TGG **IGG** TGG TGG **I**GG IGG PGG PGG. TAT TAC IAT **IAT** TAT TAT AGA AAA AAA AAA ATA TTA TTA TTA ITA LTA TTA TTA LTA TTA TTA TTA TTA TTA LTA TTA LTA TTA LTA AAT AGG AGC AGC AGG AAG AGG AGG AGG AGC CAT TAT Bonplandia_geminiflora Cobaea_pachysepala quercifolia_B U quercifolia A buxifolia_B huxifolia_C candelilla A Cobaea_minor quercifolia buxifolia_A volcanica_B volcanica_A candelilla_ cuzcoensis pyrifolia flexuosa flexuosa bicolor n.sp.

CTA ATA ATA ATA ATA ATA ATA ATA ATA CTA CTA CTA CTA ATA ATA ATA CAT ATT AAT TAA ATA CTC ATA TCA ATA TTC TTC TTC TIC TTC TIC TTC TTC TTC ITC TTC TTC LTC TIC TTC GAA TCA TCA TCA TCA ICA TCA TCA ICA TCA TCA TCA TCA TCA TCA TCA TCA TCA AAT CAT AAT AAT AAT TIC TIC TTC TIC TTCTTC TIC TTC TTC TIC TIC TTC TTC TTC TTC CGA TTT TTT TTT TTT TTT TTT TTI TTT TTT TTT TTT TTT TTT TTT LTT TTT TTT IAT TAT IAT Bonplandia_geminiflora Cobaea_pachysepala quercifolia_B quercifolia_C quercifolia_A candelilla_A Cobaea_minor buxifolia C buxifolia_A volcanica B buxifolia_B volcanica_A candelilla flexuosa B cuzcoensis pyrifolia flexuosa bicolor n.sp.

flexuosa_A	GAA		AAT	TGC	AAA	TAA	ATA	\mathtt{TTG}	AAT	ATT	TTA	GAA	CAT	AAC	AAT	TAA
flexuosa_B	1 1	1	1	1 1	 	 	1	 	1	1 1	1	 	1 1	1 1 1	1	
cuzcoensis	GAA		AAT	\mathbf{TGC}	AAA	TAA	ATA	TTG	AAT	ATT	TTA	GAA	CAT	AAC	AAT	
bicolor	GAA		AAT	\mathbf{TGC}	AAA	TAA	ATA	TTG	AAT	ATT	TTA	GAA	CAT	AAC	AAT	
buxifolia_A	GAA		AAT	TGC	AAA	TAA	ATA	TTG	AAT	ATT	TTA	GAA	CAT	AAC	AAT	
buxifolia_B	GAA		AAT	\mathbf{TGC}	AAA	TAA	ATA	TTG	AAT	ATT	TTA	GAA	CAT	AAC	AAT	
buxifolia_C	GAA		AAT	\mathbf{TGC}	AAA	TAA	ATA	TTG	AAT	ATT	TTA	GAA	CAT	AAC	AAT	
candelilla_A	GAA		AAT	\mathbf{TGC}	AAA	TAA	ATA	TTG	AAT	ATT	TTA	GAA	CAT	AAC	AAT	
candelilla_B	GAA		AAT	\mathbf{TGC}	AAA	TAA	ATA	TTG	AAT	ATT	TTA	GAA	CAT	AAC	AAT	_
pyrifolia	GAA		AAT	\mathbf{TGC}	AAA	TAA	ATA	$\mathbf{T}\mathbf{T}\mathbf{G}$	AAT	$\mathbf{A}\mathbf{T}\mathbf{T}$	TTA	GAA	CAT	AAC	AAT	ъ.
n.sp.	GAA		AAT	IGC	AAA	TAA	ATA	$\mathbf{T}\mathbf{T}\mathbf{G}$	AAT	$\mathbf{A}\mathbf{T}\mathbf{T}$	TTA	GAA	CAT	AAC	AAT	•
volcanica_A	GAA		AAT	IGC	AAA	TAA	ATA	$\mathbf{T}\mathbf{T}\mathbf{G}$	AAT	$\mathbf{A}\mathbf{T}\mathbf{T}$	TTA	GAA	CAT	AAC	AAT	
volcanica_B	GAA		AAT	TGC	AAA	TAA	ATA	\mathbf{TTG}	AAT	ATT	TTA	GAA	CAT	AAC	AAT	
quercifolia_A	GAA		AAT	TGC	AAA	TAA	ATA	${\tt TGG}$	AAT	ATT	TTA	GAA	CAT	AAC	AAT	
quercifolia_B	GAA		AAT	TGC	AAA	TAA	ATA	TTG	AAT	ATT	TTA	GAA	CAT	AAC	AAT	-
quercifolia_C	GAA		AAT	TGC	AAA	TAA	ATA	TTG	AAT	ATT	TTA	GAA	CAT	AAC	AAT	-
Bonplandia_geminiflora	GAA		AAT	TGC	AAA	TAA	ATA	TTG	AAT	ATT	TTA	GAA	TAT	AAC	AAT	\sim
Cobaea_pachysepala	GAA	\mathtt{TCT}	AAT	TGC	AAA	TAA	ATA	TTG	AAT	ATT	TTA	GAA	CAT	AAC	AAT	TAA
Cobaea_minor	GAA		AAT	TGC	AAA	TAA	ATA	${ m TTG}$	AAT	TTT	TTA	GAA	CAT	AAC	AAT	

_
$\boldsymbol{\sigma}$
•
u
cont
0
Ŭ
_
9
H
, ,
Ø
ŭ
ap
ત
Ë

	L 4 2															9 9
						Ä	Indel	-								
flexuosa_A	TAT A	AGC	GAT	ATT	CTA	GAT	ATA	ATA	TAC	ATA	AAT	ATC	CGT	TTA	CAT	ATT
flexuosa B	1	!	!	1	1	1 1	1 1	1	! !	1	1 [1 1 1	1	1 1	!	1
cuzcoensis	TAT A	AGC	GAT	ATT	CTA	GAT	ATA	ATA	TAC	ATA	AAT	ATT	CGT	TTA	CAT	ATT
bicolor	TAT A	AGC	GAT	ATT	CTA	GAT	ATA	ATA	TAC	ATA	AAT	ATC	CGT	TTA	CAT	ATT
buxifolia A	TAT A	AGC	GAT	ATT	CTA	GAT	ATA	ATA	TAC	ATA	AAT	ATC	CGT	TTA	CAT	ATT
buxifolia_B		AGC	GAT	ATT	CTA	GAT	ATA	ATA	TAC	ATA	AAT	ATC	CGT	TTA	CAT	ATT
buxifolia_C		AGC	GAT	ATT	CTA	.: G	••	••	••	••	••	••	••	••	••	••
candelilla A		AGC	GAT	ATT	CTA	.: G	••	••	••	••	••	••	••	••	••	••
candelilla B	TAT A	AGC	GAT	ATT	CTA	.: G	••	••	••		••	••	••	••	::	••
pyrifolia _		AGC	GAT	ATT	CTA	GAT	ATA		TAC	ATA	AAT	ATC	CGT	TTA	CAT	ATT
n.sp.	TAT A		GAT	ATT	CTA	GAT	ATA	ATA		ATA	AAT	ATC	CGT	TTA	CAT	ATT
volcanica A		AGC	GAT	ATT	CTA	GAT	ATA	ATA	TAC	ATA	AAT	ATC	CGT	TTA	CAT	ATT
volcanica_B		AGC	GAT	ATT	CTA	GAT	ATA	ATA	TAC	ATA	AAT	ATC	CGT	TTA	CAT	ATT
quercifolia_A	TAT A	AGC	GAT	ATT	CTA	GAT	ATA	ATA	TAC	ATA	AAT	ATC	CGT	TTA	CAT	ATT
quercifolia_B	TAT A	AGC	GAT	ATT	CTA	GAT	ATA	ATA	TAC	ATA	AAT	ATC	CGT	TTA	CAT	ATT
quercifolia_C	TAT A	AGC (GAT	ATT	CTA	GAT	ATA	ATA	TAC	ATA	AAT	ATC	CGT	TTA	CAT	ALT
Bonplandia_geminiflora	TAG A	AGC	GAT	ATT	CGA	GAT	ATA	ATA	\mathbf{TGC}	ATA	AAT	ATC	CGT	TTA	CAT	ATT
Cobaea_pachysepala	TAT A	AGC	GAT	ATT	CTA	GAT	AGA	ATC	TAC	ATA	AAT	ATC	CGT	TTA	CAT	ATT
Cobaea_minor	TAT A	AGC (GAT	ATT	CTA	GAT	ATA	ATA	TAC	ATA	AAT	ATC	CGT	TTA	CAT	ATT

U 4 O		H	ı	H	H	H	E	Ħ	E	H	H	H	₽	⊣	H	H	H	⊣	⊣	H
		GTT	I	GTT		GTT						GTT							GTT	GTT
		AAG	1	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG
		TAA	 	TAA	TAA	TAA	TAA	.: A	: : A	::A	TAA	TAA	TAA	TAA	TAA	TAA	TAA	TAA	TAA	TAA
		ACA	1	ACA	ACA	ACA	ACA	••	••	••	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	AAA
		ATT	1	ATT	ATT	ATT	ATT	••	••	••	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	TAA
		TAT	1	TAT	TAT		TAT	••	••			TAT						TAT	TAT	ACA
		TIC :	!	_	TTC	_	TTC :	••	••			TTC						_		AAT 1
		GAT I	!	GAT I	GAT I	GAT I	GAT I	••			GAT I			GAT I	GAT I		GAT I	GAT I	GAT I	CAT A
		ATC G	1	-	_	_	_										_	_	TC G	:A C
			1		T ATC		T ATC	••	••	••	T ATC		T ATC	T ATC	T ATC	T ATC	T ATC	T ATC	Æ	••
	7	TCL	1	TCL					••			TCL							TCT	••
	Indel	TLI	1	TTT	TTT	TTI	TTT	••	••	••	TTT	TTT	TTT	TTT	TTT	TTT	$\mathbf{T}\mathbf{T}\mathbf{I}$	TTT	$\mathbf{T}\mathbf{T}\mathbf{I}$	••
	Jul	GTA	1	GTA	GTA	GTA	GTA	••	••	••	GTA	GTA	GTA	GTA	GTA	GTA	GTA	GTA	GTA	••
		ATT	1	ATT	ATT	ATT	ATT	••	::	••	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT
		TAT	! !	TAT	TAT	TAT	TAT	••	••	••	TAT	TAT			TAT	TAT	TAT	TAT	TAT	TAT
		TTC	1	TTC	$\mathbf{T}\mathbf{T}\mathbf{C}$	$\mathbf{T}\mathbf{T}\mathbf{C}$	TTC	••	•••	•••	TTC	TTC	TIC	TTC	TTC	TTC	$\mathbf{I}\mathbf{I}\mathbf{C}$	TIC	TIC	TTC
H 0 E		GLL	1 1	GLL	GTT	GLT	GLL	••	••	••	GTT	GTT	GTT	GTT			GTT	GLL		GTT
		flexuosa_A	flexuosa_B	cuzcoensis	bicolor	buxifolia_A	buxifoliaB	buxifolia_C	candelilla_A	a_B	pyrifolia		volcanica_A	volcanica_B	quercifolia A	quercifolia_B	quercifolia_C	Bonplandia geminiflora		

N

4

0 00 00

TTTTTTTTT TTT TTT TTT TTT TTT TTTTTT TTTTTT TTT TTT TAA TAA TCA TAA TIC TTC TTC ITC TTC TIC TIC TTC TTC TIC TTC TIC TTC TIC TTC TIC TAA TAA TAA TAA TAA TAA TAA TAA GAA TAA TAA TAA TAA TAA TAA TAA GAA AAT TCT TCTTCT TCT TCT TCT AAA CTA AAT TTT TTTTTT TTT TTTTTTTTT TTT TTT TTT TTTTTG TTG TTT TTT TTT TTT TAA AAT TTT TTT PTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTTLLI AAG TAT GAT GAA Bonplandia geminiflora Cobaea pachysepala quercifolia_B quercifolia_C quercifolia_A candelilla A Cobaea minor buxifolia_A buxifolia_B volcanica_B volcanica_A candelilla cuzcoensis buxifolia pyrifolia flexuosa flexuosa bicolor

	gcc	 	GCC	ದಿದ್ದ	gaa	gaa	gcc	CCC	gcc	gaa	ರಿದಿ	ದ್ದಿದ್ದ	CCC	GCC	GCC	೦೦೦	TCC	$\mathbf{I}^{\mathbf{CC}}$	TCC
	CAC	! !	CAC	CAC	CAC	CAC	CAC	CAC	CAC	CAC	CAC	CAC	CAC	CAC	CAC	CAC	CAC	CAC	CAC
	ATC	1	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC
	TAT	1	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT
	AAT	! !	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT
	ATT	! !	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT
	CCA	l l	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA
	AGA	! !	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA
	AAT	1	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT
	ATT	 	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT
	TTT	 	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT
	AAT	1 1	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT	AAT
	TAT	! !	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT
0, 00 v	TTG	! !	\mathbf{TTG}	\mathbf{TTG}	TTG	TTG	TTG	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	TTG	TTG	TTG	TTG	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	${ m TTG}$
	flexuosa_A	flexuosa_B	cuzcoensis	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla_A	candelilla_B	pyrifolia	n.sp.	volcanica_A	volcanica_B	quercifolia_A	quercifolia_B	quercifolia_C	Bonplandia_geminiflora	Cobaea_pachysepala	Cobaea_minor

m m 9

7 3 3															w
										Ä	ndel	m			
TTC ATT	CGT	AAA	GGT	GGA	AGT	TAA	GAT	GAA	AAA	AAG	AAT	_	-	TT	H
1 1 1 1	1	1 1	1 1	1	1 1	1	1	1	1	1	1	1	i	!	ı
TTC ATT	CGT	AAA	GGT	GGA	AGT	TAA	GAT	GAA	AAA	AAG	AAT	CGA	-	TT	E
TTC ATT	CGT	AAA	GGT	GGA	AGT	TAA	GAT	GAA	AAA	AAG	AAT	CGA		TT	Ħ
TTC ATT	CGT	AAA	GGT	GGA	AGT	TAA	GAT	GAA	AAA	AAG	AAT	CGA		TT	Ē
TTC ATT	CGT	AAA	GGT	GGA	AGT	TAA	GAT	GAA	AAA	AAG	AAT	CGA		TT	۲
TTC ATT	CGT	AAA	GGT	GGA	AGT	TAA	GAT	GAA	AAA	AAG	•	_		TT	Ę⊣
TTC ATT	CGT	AAA	GGT	GGA	AGT	TAA	GAT	GAA	AAA	AAG	•	_		TT	Ē
TTC ATT	CGT	AAA	ggT	GGA	AGT	TAA	GAT	GAA	AAA	AAG	•	_			[→
TTC ATT	CGT	AAA	ggT	GGA	AGT	TAA	GAT	GAA	AAA	AAG	•	_			Ē
TTC ATT	CGT	AAA	GGT	GGA	AGT	TAA	GAT	GAA	AAA	AAG	•	_		TT	Ħ
TTC ATT	CGT	AAA	ggT	CGA	AGT	TAA	GAT	GAA	AAA	AAG	•	_		TT	۲
TTC ATT	CGT	AAA	ggT	CGA	AGT	TAA	GAT	GAA	AAA	AAG	•	_		TL	Ē
TTC ATT	CGT	AAA	ggT	GGA	AGT	TAA	GAT	GAA	AAA	AAG	•	•		TI	Ę→
TTC ATT	CGT	AAA	GGT	AGA	AGT	TAA	GAT	GAA	AAA	AAG	•	_		TT	[H
TTC ATT	CGT	AAA	GGT	GGA	AGT	TAA	GAT	GAA	AAA	AAG	•	_		TT (Ē
TTC ATT	CGT	AAA	GGT	GGA	AGT	TAA	GAT	GAA	AAA	A:G		_		TT	٢
TTC ATT	CGT	AAA	GGT	GGA	AGT	TAA	GAT	GAA	AAA	AAG		CGA		TT	Ē
TTC ATT	CGT	AAA	GGT	GGA	AGT	TAA	GAT	GAA	AAA	AAG		•		TTT 5	Ē
		ATT	ATT CGT	ATT CGT AAA ATT CGT AAA	ATT CGT AAA GGT GGA	ATT CGT AAA GGT GGA	ATT CGT AAA GGT GGA AGT ATT CGT AAA GGT GGA AGT	ATT CGT AAA GGT GGA AGT TAA ATT CGT AAA GGT GGA AGT TAA	ATT CGT AAA GGT GGA AGT TAA GAT ATT CGT AAA GGT GGA AGT TAA GAT ATT CGT AAA GGT GGA AGT GAT ATT CGT AAA GGT GGA AGT GAT ATT	ATT CGT AAA GGT GGA AGT TAA GAT GAA	ATT CGT AAA GGT GGA AGT TAA GAT GAA AAA A	Libar	Indel	TT CGT AAA GGT GGA AGT TAA GAT GAA AAA AAG AAT CGA	Indel 3

() *	PHG !	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG
S C	454	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA
ָּ נ	166	TGG	\mathbf{TGG}	TGG	TGG	\mathbf{TGG}	TGG	\mathbf{TGG}	\mathbf{TGG}	TGG	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	TGG	\mathbf{TGG}	\mathbf{TGG}
K K	4	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
() 	146	TCG	TAG	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	TAG	TAG	TAG	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	TAG	TAG	TAG	TAG	TAG	TAG	GAG	TAG
£ £	777	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	$\mathtt{T}\mathtt{T}\mathtt{I}$
\$ \$ \$	HH	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	ATG	AAA	AAA
E S	145	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAG	GAG	GAG
E	ָרָאָן וֹיִין	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT
K E	HIT	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA
, ,	HAH	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
Ć	4)	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA
E K	THI	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT
നയവ ദ	HAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG
(1-6-4	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla_A		pyrifolia	n.sp.	volcanica_A	volcanica_B	quercifolia_A	quercifolia_B	quercifolia_C	Bonplandia_geminiflora	Cobaea_pachysepala	Cobaea_minor

4 8 0

Table 16 (cont'd)

٠,	•	.,				.,			r.,	L.,	.,	٠.			•	•	٠.	٠.
TGA	1	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA
AAA	1	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
CAG	1 1 1	CAG	CAG	CAG	CAG	CAG	CAG	CAG	CAG	CAG	CAG	CAG	CAG	CAG	CAG	CAG	CAG	CAG
ATA	1	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA
CGA	1 1 1	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA
$_{ m TTG}$	1	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	$_{ m TTG}$	TTG	TTG	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	TTG	TTG	TTG	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$
GAA	1	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA
ATT	1	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT
TAT	1 1	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT
ATC	1 1	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC
ICC	! !	TCC	ICC	TCC	TCC	ICC	TCC	TCC	TCC	$\mathbf{I}^{\mathbf{C}\mathbf{C}}$	TCC	TCC	$\mathbf{I}^{\mathbf{C}\mathbf{C}}$	ICC	TCC	TCC	TCC	TCC
ATA	1	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA
TAT	1	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT
TGG	 	TGG	TGG	TGG	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	$_{\mathrm{TGG}}$	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	\mathbf{TGG}	TGG	TGG	TGG
ATG	1 1	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG
flexuosa_A	flexuosa_B	cuzcoensis	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla A	candelilla_B	pyrifolia	n.sp.	volcanica_A	volcanica_B	quercifolia_A	quercifolia_B	quercifolia_C	Bonplandia_geminiflora	Cobaea_pachysepala	Cobaea_minor
							21	Λ										

8 2 2

AGA Indel GAA AGA GAA GAT GAA ATA CAT CCT CCT CCT CAT CAT CCT CCT CCT CCT CCT CCT CCT CCT TCT TTT ICT GGG GGG GGG GGG 366 GGG GGG GGG GGG 9999 GGG GGG GGG 9999 GGG TGG 9999 TGG ATA CAA AAG AAC AAG AAG AAG AAG TTG TTG TIG TTG TTG TTG TIG TTG TTG TTG TTG TTG TTG TTG TTG ITG TGA TGA IGA TGA TGA IGA IGA **IGA** IGA GA TGA GA TGA **IGA** TGA IGA **IGA** CTT CTT CTT CTT CTT CTT CTT CTT CIT CTT CTT CTT CTT CAT AAT Bonplandia geminiflora Cobaea pachysepala quercifolia_B quercifolia_C quercifolia A candelilla A Cobaea_minor บ volcanica B buxifolia_A flexuosa_A flexuosa_B volcanica_A candelilla cuzcoensis buxifolia buxifolia_ pyrifolia bicolor

5 7 9

GAT AGG GAT CGA LTT TTT TTT TTT TTT LTT TTT TTT LTT TTT TTI TTI LTT TTI TTT LLI AGG AGA AAC GAA AAA GAA GAA GAA GAA GAA GAA GAA AGA TGA IGA TGA TCA TCA TCA TCA TCA CCA TCA TCA TCA TCA TCA TCA TCA TCA TCA CCA CCA AAT AGA GGG GGG GGG 9999 GGG TAG :AG TAG TAG 1 AGA •• AGA AGA Bonplandia geminiflora Cobaea pachysepala quercifolia_C quercifolia_A quercifolia_B buxifolia_B buxifolia_C candelilla_B candelilla A Cobaea_minor volcanica_B buxifolia_A volcanica_A flexuosa_A flexuosa B cuzcoensis pyrifolia bicolor n.sp.

9 7 4

GGA GGA

AGG AGG AGG AGG

AGG

GGA GGA GGA

GGA

GGA

AGG AGG AGG AGG

1

GGA

AGG

1

GGA

GGA GGA

AGG AGG

AGG AGG AAG

1

A--

$\overline{}$
Ъ
•
Ħ
con
Ö
ŏ
೭
9
۲
Ø
ĕ
്റ
Tab
70

9	4	2 INDEL CODING	123 45	→ → → → → → S 1	ACA ATG AGA AAA		ACA ATG AGA AAA	ACA ATG AGA AAA	ACA ATA AGA AAA	ACA ATG	C-A	ACA ATG AGA C-A	C-A	C ACA ATA AGA AAA AA	ACA A AAA	ACA ATG AGA AAA	C ACA ATG AGA AAA AA	AAA A-	C ACA ATG AGA AAA AA	ACA ATG AGA AAA	AAC C-	AAA A-	ACA A-	
				Indel	GAC ATC	1			C ATC		1	C ATC	1	C ATC			C ATC			C ATC	1	1	1	
				→		1	G GAC	G G:C		G GAC	1	G GAC	1 1 1	G GAC		G GAC	G GAC	1 1 1	G GAC	G GAC	1	1	1	
9	7	2			AAG	1	AAG	AAG	AAG	AAG	1 1	AAG	l 	AAG	AAG	AAG	AAG	1	AAG	AAG	1	1	l l	
					flexuosa_A	flexuosa B	cuzcoensis	bicolor	buxifolia A	buxifolia_B	buxifolia_C	candelilla A		pyrifolia _	n.sp.	volcanica_A	volcanica B	quercifolia A	quercifolia_B	quercifolia C	Bonplandia_geminiflora	Cobaea_pachysepala	Cobaea_minor	

APPENDIX E

Aligned sequences of *Cantua*: intergenic region between *trn*L3' and *trn*F coding regions

with "N", Missing data indicated with "-". Specific insertion deletion events are labeled gap Table 17. Aligned sequences of the intergenic spacer region between the trnL3' and trnF epithets on vouchers listed in Table 4. Gaps indicated with ":", Ambiguities indicated Coding region at the end of the sequence, "C" represents gap presence, "A" represents coding regions for the taxa sampled in Cantua data set. Names correspond to specific in the alignment with insertion deletion coding at the end of the sequence. In Indel absence

GCA ω GCA GCT 4 AAA ATA CCT ••• ••• .. ••• TAT ••• ATT .. ATA ••• :: ••• TTA TCC ••• ••• TAC ••• :: :: :: ATT ••• ••• ••• TTGTTG.. ••• Indel 1 TAT TAT GCA GCA AAA CCA CCA CCA CCA CCA CCA CCA CCA CCM CCA CCA CCA ! 1 1 Bonplandia_geminiflora Cobaea pachysepala ָט quercifolia_A quercifolia B candelilla A candelilla_B quercifolia_ Cobaea_minor U volcanica_B buxifolia_A volcanica_A cuzcoensis buxifolia buxifolia pyrifolia flexuosa flexuosa bicolor

σ	9		TCC	TCC	TCC	TCC	TCC	TCC	TCC	TCC	TCC	TCC	TCC	TCC	TCC	TCC	TCC	TCC	ATT	••	••
		7												TTA		TTA		TTA	CTT	••	••
		Indel	Н	ы	TAT	ы	TAT								TAT		TAT	TAT	$_{ m LLL}$	••	••
		¤I ↑—	$_{ m TCT}$		TCT	\mathbf{ICI}	$_{ m LCI}$	TCT			TCT		TCT		TCT	TCT	.: H::	.: H	. :.	•••	••
		 	TTT		TTI	TTT	TTT	TTT			TTT				TTT	TTT	••	••	••	••	••
				ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC
				CCT	CCT		CCI		CCI					CCL		CCI		CCT	GCT	CCT	CCI
			TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TCT	TCT
			ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT			ATT	ATT	ATT
			ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	AMA	ATA	ATA	ATA	ATA	ATA
			TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA
			TCC	TCC	TCC	$\mathbf{I}^{\mathbf{CC}}$	$\mathbf{I}^{\mathbf{C}\mathbf{C}}$	$\mathbf{I}^{\mathbf{C}\mathbf{C}}$	ICC	TCC	TCC	$\mathbf{I}^{\mathbf{CC}}$	TCC	GCC	ದಿದ್ದ	GCC	ညည	CCC	TCC	ICC	ICC
			TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC	TAC
			ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT
			TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	-TG	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	ATG	\mathtt{TTG}	$\mathbf{T}\mathbf{T}\mathbf{G}$	\mathbf{TTG}	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$
4	σ		TAT	TAT	TAT	1	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT
			flexuosa A	flexuosa_B	cuzcoensis	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla A	candelilla_B	pyrifolia	n.sp.	volcanicaA	volcanica_B	quercifolia_A	quercifolia_B	quercifoliaC	Bonplandia_geminiflora	Cobaea_pachysepala	Cobaea_minor

σ	ı

	Indel		→	Inde										
flexuosa_A		TIC GI	r Acc	AAT	TCA	AAA	TGC	GCT	ATC	TTT	CTC	ATT	TAG	
flexuosa_B	TAT CCT TTT	TTC GT	r Acc	AAT	TCA	AAA	TGC	GCT	ATC	TTT	CTC	ATT	TAG	
cuzcoensis		TTC GTT	_ •	AAT	TCA	AAA	$\mathbf{T}\mathbf{G}\mathbf{C}$	GCT	ATC	TTT	CTC	ATT	TAG	
bicolor		TTC GTT	r Acc	AAT	TCA	AAA	TGC	GCT	ATC	TTT	CTC	ATT	TAG	
buxifolia_A	TAT CCT TTT	TTC GTT		AAT	TCA	AAA	TGC	GCT	ATC	TTT	CTC	ATT	TAG	
buxifolia_B	CCT	TTC GTT	r ACC	AAT	TCA	AAA	TGC	GCT	ATC	TTT	CTC	ATT	TAG	
buxifolia_C	CCL	TTC GT			TCA	AAA	TGC	GCT	ATC	TTT	CIC	ATT	TAG	
candelilla_A	CCT	TTC GT			TCA	AAA	TGC	GCT	ATC	TTT	CTC	ATT	TAG	
candelilla_B		TTC GTT			TCA	AAA	TGC	GCT	ATC	TTT	CTC	ATT	TAG	
pyrifolia	CCT	TTC GTT			TCA	AAA	TGC	GCT	ATC	TTT	CTC	ATT	TAG	
nsp.	CCT				TCA	AAA	\mathbf{TGC}	GCT	ATC	TTT	CTC	ATT	TAG	
volcanica_A	CCT				TCA	AAA	TGC	GCT	ATC	TTT	CTC	ATT	TAG	
volcanica_B	CCT				TCA	AAA	TGC	GCT	ATC	TTT	CTC	ATT	TAG	
quercifolia_A	CCT	TTT GGT			TCA	AAA	\mathbf{TGC}	GCT	ATC	TTT	CTC	ATT	TAG	
quercifolia_B			r Acc	AAT	TCA	AAA	TGC	GCT	ATC	TTT	CTC	ATT	TAG	
quercifolia_C		TTT GGT			TCA	AAA	\mathbf{TGC}	GCT	ATC	TTT	CTC	ATT	TAG	
Bonplandia_geminiflora	TCT CCT TTT	TTC GTT	r AC:	AGT	TCA	AAA	TGC	ACT	ATC	TTT	CTC	ATT	\mathbf{I}^{CG}	
Cobaea_pachysepala	::: :CT TTT	TTC GTT	. ¬	AGT	TCA	AAA	IGC	GCT	ATC	TTT	CIC	ATT	TAG	
Cobaea_minor	::: :CT TTT	TTC GT	r ACT	AGT	TCA	AAA	TGC	GCT	ATC	TTT	CTC	ATT	TAG	

1 6 2

Table 17 (cont'd)	,																c	
	-1 ത ന																740	
			u i →	Indel	7													
			→	†Inde	31 8											Inde	le1 9	•
flexuosa A	AAG G		G	••	TT:	m TTT	AGG	AAG	_		CCA		r gaa			c Acg	Ď	
flexuosa_B		H			TT:	TTT	AGG	AAG	_		CCA		r GAA	_		•	Ď	
cuzcoensis		GAA T	: CG:	••	: TT	TTT	AGG	AAG	GAA	TCC 1	CCA	Y TTT	_	A TGA	A TTC	CAC	ស្ត	
bicolor			TCG:	••	: TT	TTT	AGG	AAG	_		CCA		r GAA			C ACG	ស្ត	
buxifolia_A			TCG:	••	: TT	TTT	AGG	AAG	_		CCA		r GAA			C ACG	ស្ត	
buxifolia_B			 gg	••	:TT	TTT	AGG	AAG	_		CCA					C ACG	ស្ត	
buxifolia_C			TCG:	••	: TT	TTT	AGG	AAG			CCA					C ACG	ស្ត	
candelilla_A			TCG:	••	:TT	TTT	AGG	AAG	_		CCA					C ACG	ភ្ជ	
candelilla_B			 gg	••	: TT	TTT	AGG	AAG	_		CCA						ស្ត	
pyrifolia			 წე	••	:TT	$_{ m LLL}$	AGG	AAG	_				r GAA				ស្ត	
nsp.			 წე	••	: TT	TTT	AGG	AAG	_								ជ	
volcanica_A				TTT :	LTT	TTT	AGG	AAG	_		CCA					C ACA	Ą	
volcanica_B			_	-	LTT	TTT	AGG	AAG	_							-	Ķ	
quercifolia_A				TTT .	LTJ	TLL	AGG	AAG	_		CCA					C ACG	Ď	
quercifolia_B				TTT :	lTT	TTT	AGG	AAG	_		CCA					C ACG	ស្	
quercifolia_C				TTT .	$_{ m LLL}$	TTT	AGG	AAG	_		CCA		C GAA				ស្	
Bonplandia_geminiflora			AG:	•••	: TT	$\mathbf{T}\mathbf{T}\mathbf{G}$	AGG	AAG	_		CCA					::	••	
Cobaea_pachysepala	AAG G	GAA T	TAA :		: TT	$\mathbf{T}\mathbf{T}\mathbf{G}$	AGG	AAG	GAA		CCA		_			C ACG	ស្ត	
Cobaea_minor		GAA T	 \	••	: TT	TGG	AGG	AAG	_		CCA		r GAA				ស្ត	

				-	11																				
	7	80	œ	Indel	Indel	⊋	:GA	:GA	:GA	TGA	:GA	:GA	:GA	:GA	:GA	:GA	:GA	:GA	:GA	:GA	:GA	:GA	••	::	::
				H →	\rightarrow	el 1	TTT	TTT	TTT	TTT	TTT	TTT	TT:	TLL	TTT	TT:	TT:	TT:	TT:	TT:	TT:	TTT	:: H	:: H	:: H
						Indel	TTT	$\mathbf{T}\mathbf{L}\mathbf{L}$	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	$_{ m LLL}$	TTT	TTT
							TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	${f TTT}$
							GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GLL	GLL	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT
				10			AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	: AA	AAA
				del			GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	•••	GAC
				[Inde]	•		ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACG	A::	ACT
							GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA
							ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT
							CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT		CAT	CAT	CAT
							ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT
							TTT	TTT	TTT	TTT	_	TTT	TTT			TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT
							CTC	CIC	CIC		CIC	CIC	CIC		CTC		CIC	CIC	CIC	CIC	CIC	CIC	CIC	CIC	CIC
							CAT	_	CAT	CAT	_	CAT	CAT			L						CAT			
	7	4	н				GTC	GTC	GTC	GIC	GTC	GIC	GIC	GTC	GIC	GTC	GTC	GTC	GIC	GTC	GTC	GIC	GIC	GIC	GIC
(cont'd)							Ą	, ^{eq} ,	is		a_A	a_B	ລືເ	la_A	la_B	æ		a_A	a B	lia_A	lia_B		ia_geminiflora	Cobaea_pachysepala	inor
Table 17							flexuosa	flexuosa	cuzcoensis	bicolor	buxifolia	buxifolia	buxifolia	candelilla	candelilla	pyrifolia	n.sp.	volcanic	volcanica	quercifolia	quercifolia	quercifolia	Bonplandia_	Cobaea_p	Cobaea_minor
												:	221												

7	ω	σ

flexuosa_A	AAA	_			-			_	GAT	AAG	ACT	TTG	_	TAG	CLL	ATC
flexuosa_B	AAA	_		-	-			_	GAT	AAG	ACT	$\mathbf{T}\mathbf{T}\mathbf{G}$	_	TAG	CIT	ATC
cuzcoensis	AAA	_			-			_	GAT	AAG	ACT	TTG	-	TAG	CTT	ATC
bicolor	AAA	_			_			_	GAT	AAR	ACT	TTG	_	TAG	CTT	ATC
buxifolia_A	AAA	_			-			_	GAT	AAG	ACT	TTG	_	TAG	CTT	ATC
buxifolia_B	AAA	_		-	_			_	GAT	AAG	ACT	TTG	_	TAG	CTT	ATC
buxifolia_C	AAA	_		-	-			_	GAT	AAG	ACT	TTG	_	TAG	CTT	ATC
candelilla_A	AAA	_		-	-			_	GAT	AAG	ACT	$\mathbf{T}\mathbf{T}\mathbf{G}$	_	TAG	CTT	ATC
candelilla_B	AAA	_		-	_			_	GAT	AAG	ACT	TTG	_	TAG	CTT	ATC
pyrifolia	AAA	_			_			_	GAT	AAG	ACT	TTG	_	TAG	CTT	ATC
nsp.	AAA	_			-			_	GAT	AAG	ACT	TTG	_	TAG	CTT	ATC
volcanica_A	AAA	_			_			_	GAT	AAG	ACT	TTG	_	TAG	CTT	ATC
volcanica_B	AAA	_			_			_	GAT	AAG	ACT	TTG	_	TAG	CTT	ATC
quercifolia_A	AAA	-		•	-			_	GAT	AAG	ACT	TTG	_	TAG	CTT	ATC
quercifolia_B	AAA	-		•	_			_	GAT	AAG	ACT	TTG	_	TAG	CTT	ATC
quercifolia_C	AAA	_		•	-		-	•	GAT	AAG	ACT	$_{ m LLC}$	_	TAG	CTT	ATC
Bonplandia_geminiflora	:AA	TCC	AAG	AAA	TTC	CAA	AGT	CIT	ATC	CAG	GCT	TTG	GAA	TAG	CTT	ATC
Cobaea_pachysepala	:AA	-		•	-			_	GAT	AAG	ACT	TIG	AAA	TAG	CIT	ATC
Cobaea_minor	:AA	TCC	. •	AAA	TTC	CAA	-	CTG	GAT	AAG	ACT	$\mathbf{T}\mathbf{T}\mathbf{G}$	•	TAG	CTT	ATC

w a 4		AGA	GA	GA	GA	GA	GA	GA	GA	GA	AGA	GA	GA	GA	GA	GA	GA	GA	GG	99	
	14				-		-	-		-	TT A	-	-					TT A	IT A	rt A	
	ž					•	•										••	••			
	Ä				::			::		::					:: ::					:: 	
		AA		-						•	AAA		•	•	•	•	•	AA	AA	AA	
		GTA	GTA	GTA	GTA	GTA	GTA	GTA	GTA	GTA	GTA	GTA	GTA	GTA	GTA	GIC	GTA	ATA	GTA	GTA	
		CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	CGA	
		CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	_	_	_	CAT	
		AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	
		CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA				CCA	
		GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	
		ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	
		GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	GAC	
		ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	
											TTA							•	•	LTA .	
		CLL	CLL	CLL	CLL	CLL	CLL	CLL	CLL	CTT	CIT	CIL	CTT	CTT	CTT	CTT	CIL	CIL	LIL	CTT	
4 3 3		_	_	_		_	_	_	_	_	GTT	_	_	•	_	•	•	•	•	GTT (
		flexuosa_A	flexuosa_B	cuzcoensis	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla_A	candelilla_B	_ pyrifolia	nsp.	volcanica_A	volcanica_B	quercifolia_A	quercifolia_B	quercifolia_C	Bonplandia_geminiflora	Cobaea_pachysepala	Cobaea_minor	

Table 17 (cont'd)														
	സയവ													
flexuosa A	ATG A	_	EH	ICT	GAA	TGG	TCG	GGA	TAG	CTC	AGC	•	; ;	;
flexuosa_B				TCT	GAA	TGG	$\mathbf{T}^{\mathbf{C}}$	GGA	TAG	CTC	AGC	${ m TGG}$	TAG	AG
cuzcoensis	ATG 1	ATG	Н	$_{ m LCT}$	GAA	\mathbf{TGG}	\mathbf{T}^{CG}	GGA	TAG	CTC	AGC		TAG	AG
bicolor	ATG A			TCT	GAA	\mathbf{TGG}	$\mathbf{T}^{\mathbf{G}}$	GGA	TAG	CTC	AGC	\mathbf{TGG}	TAG	AG
buxifolia A	ATG A			ICT	GAA	TGG	\mathbf{T}^{CG}	GGA	TAG	CTC	AGC		TAG	AG
buxifolia_B	ATG 1			TCT	GAA	\mathbf{TGG}	${ m TCG}$	GGA	TAG	CTC	AGC		TAG	AG
buxifolia_C				TCT	GAA	\mathbf{TGG}	\mathbf{TCG}	GGA	TAG	CIC	AGC		TAG	AG
candelilla_A	ATG A			\mathbf{rcr}	GAA	\mathbf{TGG}	$\mathbf{T}^{\mathbf{G}}$	GGA	TAG	CTC	AGC		TAG	AG
candelilla_B				$_{ m LCT}$	GAA	TGG	${ m TCG}$	GGA	TAG	CTC	AGC	TGG	TAG	AGC
pyrifolia				TCT	GAA	\mathbf{TGG}	\mathbf{T}^{CG}	GGA	TAG	CTC	AGC		1	i
n.sp.	ATG A			$_{ m LCL}$	GAA	\mathbf{TGG}	$\mathbf{T}^{\mathbf{G}}$	GGA	TAG	CTC	AGC		TAG	AGC
volcanica_A				Γ CT	GAA	TGG	${ m TCG}$	GGA	TAG	CIC	AGC		TA-	i
volcanica_B	ATG 7			\mathbf{ICT}	GAA	\mathbf{TGG}	$\mathbf{T}^{\mathbf{C}}$	GGA	TAG	CTC	AGC	\mathbf{TGG}	TAG	AG
quercifolia_A	ATG A			Γ CT	GAA	\mathbf{TGG}	\mathbf{TCG}	GGA	TAG	CTC	AGT		TAA	AGC
quercifolia_B			TAT	ICT	GAA	\mathbf{TGG}	TCG	GGA	TAG	CTC	AGT		TAG	AGC
quercifolia_C				Γ CT	GAA	\mathbf{TGG}	$\mathbf{T}^{\mathbf{G}}$	GGA	TAG	CTC	AGT		TAG	AGO
Bonplandia_geminiflora	ATG A			Γ CT	GAA	\mathbf{TGG}	$\mathbf{T}^{\mathbf{G}}$	GGA	TAG	CTC	AGC		TAG	AGO
Cobaea_pachysepala	ATG 7	_	TAT	ICT	GAA	\mathbf{T} GG	\mathbf{TCG}	GGA	TAG	CTC	AGC		TAG	AGO
Cobaea_minor	ATG 7	ATG	TAT	Γ CT	GAA	\mathbf{TGG}	TCG	GGA	TAG	CTC	AGC	$^{\mathrm{TGG}}$	TAG	AGC

			[11	234	$\stackrel{\rightarrow}{\rightarrow}$	₹CC	ACC	₹CC	AAC	ACA	ACA	ე-ე	ACC	ACC	ე-ე	ე-ე	<u>2-</u> C	ე-ე	<u>-</u> 2	ر ا ک	ACC	<u>ل</u>	ပ 	ر
			911 1	01	$\stackrel{\rightarrow}{\Rightarrow}$												AA		AA	AA O		AC .	ນູ	AAC -
		CODING	œ		→ → → →															-CA A	CCA A	AAC C		AAC A
		Ö	5 67																			•	•	•
		EL	34		$\stackrel{\rightarrow}{\Rightarrow}$	AA	AA	AA	AA	AA	AA					AA	AAC	AAC	AA	AA	AA	ACZ	CAA	CAA
	4	4 INDEL	812		\Rightarrow	-AA	TAA	TCA	T-A	-CA	TCA	TCA	-CA	TCA	-CA	T-A	-CA	TCA	TCA	ICC	TCC	TCC	TC-	TC-
						1	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	1	GKG	GTG	1	\mathtt{GTG}	1	GTG	! !	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	GTG
						1 1 1	CTC	CTC	CTC	1	CTC	CIC	 	CTC	1 1 1	CTC	1 1 1	CIC	MTC	CIC	CIC	CIC	CTC	CTC
						1 1 1	ATC	ATC	ATC	1	ATC	ATC	1 1	ATC	1	ATC	1	ATC	ATC	ATC	ATC	ATC	ATC	ATC
						1 1	AAA	AAA	AAA	1	AAA	AAA	1	AAA	1	AAA	1 1	AAA	AAA	AAA	AAA	AAA	AAA	AAA
	4	က	က			1	CTG	CTG	CTG	1	CTG	CTG	1	CTG	1 1 1	CTG	1	CTG	CTG	CTG	CTG	CTG	CTG	CTG
Table 17 (cont'd)						flexuosa_A	flexuosa_B	cuzcoensis	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla_A	candelilla_B	pyrifolia	nsp.	volcanica_A	volcanica_B	quercifolia_A	quercifolia_B	quercifoliaC	Bonplandia_geminiflora	Cobaea_pachysepala	Cobaea_minor
															_									

APPENDIX F

Aligned sequences of *Cantua*: 938 base pairs of the *ndh*F coding region

sampled in the Cantua data set. Names correspond to specific epithets on vouchers listed Aligned sequences of 938 basepairs of the ndhF coding region from the taxa in Table 4. Gaps indicated with ":", Ambiguities indicated with "N", Missing data Table 18.

indicated with "-".

Н

CTA	CTA TTT	CTA	CTA	CTA	CTA	CTA	CTA	CTA	CTA	CTA	ATA '	ATA	CTA	CTA	CIA	TTA	CTA	. 4TC
CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	ד אס
ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT
\mathtt{TGT}	\mathtt{TGT}	$_{\mathrm{IGT}}$	\mathbf{TGT}	\mathtt{TGT}	TGT	TGT	\mathbf{TGT}	\mathbf{TGT}	\mathtt{TGT}	$_{\mathrm{IGT}}$	\mathbf{TGT}	\mathbf{TGT}	\mathbf{TGT}	\mathbf{TGT}	TGT	\mathbf{TGT}	\mathbf{TGT}	ТÇТ
GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	ggT	GGT	ggT	GGT	TCC.
GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GCT	GCT	GTT	GTT	GTT	GTT	GTT	CTT
ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA
ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG
TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	TTA	ATT.
TTC	TIC	$\mathbf{T}\mathbf{T}\mathbf{C}$	TIC	TTC	$\mathbf{T}\mathbf{I}\mathbf{C}$	TTC	TTC	TIC	TIC	TTC	TIC	$\mathbf{T}\mathbf{T}\mathbf{C}$	TIC	$\mathbf{T}\mathbf{T}\mathbf{C}$	TIC	ည 	TIC	TTC
AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	1 1 1	AGA	AGA
ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	! ! !	ATG	1
AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	NAG	AAG	AAG	AAG	AAG	1	AAG	1
CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CNA	CCA	CCA	CCA	CCA	1	CCA	1
GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	1	GGT	1
flexuosa A	flexuosa_B	cuzcoensis	bicolor	buxifolia A	buxifolia_B	buxifolia_C	candelilla A	candelilla_B	pyrifolia _	n. sp.	volcanica A	volcanica_B	quercifolia A	quercifolia_B	quercifolia_C	Bonplandia geminiflora	Cobaea pachysepala	Cobaea minor

CAG GAT TAA CCG CAT TTT ATA	GAT TAA CCG CAT TTT	GAT TAA CCG CCT TTT	GAT TAA CCG CCT TTT														
r cta cag r cta cag	CTA	CTA (CTA (CTA (
TAG CTT CGT	CTT	CLL	CLL	CLL	CLL	CLL	CLL	CTT	CTT	CLL	CLL	CLL	CLL	CLL	CTT (CTT	rag ctt ggt
TAA	•	TAA	TAA	TAA '	TAA '	TAA.	•	TAA	TAA	TAA	TAA	TAT	•	TAT	TAA		
TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG	TCG
flexuosa_A flexuosa_B	cuzcoensis	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla_A	candelilla_B	pyrifolia	n.sp.	volcanica_A	volcanica_B	quercifolia_A	quercifolia_B	quercifolia_C	Bonplandia_geminiflora	Cobaea pachysepala	Cobaea_minor

flexuosa_A	TTC G	ĞĀ	TTT	ATT	_	_		TTG	AGG	_			ATG	TTA	ATT	TTA
flexuosa_B	TTC G	GGA	TTT	ATT	TAC	TTA	CTT	\mathtt{TTG}	AGG	GAC	ATT	TTA	ATG	TTA	ATT	TTA
cuzcoensis	TTC G	GA	TTT	ATT	-	_		${ m TTG}$	AGG				ATG	\mathtt{TTA}	ATT	TTA
bicolor	TTC G	GA	TTT	ATT	-	_		TTG	AGG				ATG	TTA	ATT	TTA
buxifolia_A	TTC G	GA	TTT	ATT	-			$\mathbf{T}\mathbf{T}\mathbf{G}$	AGG				ATG	TTA	ATT	TTA
buxifolia_B	TTC G	GA	TTT	ATT	-	_		${ m TTG}$	AGG	_			ATG	TTA	ATT	TTA
buxifolia_C	TTC G	GA	TTT	ATT	-			TTG	AGG	_			ATG	TTA	ATT	TTA
candelilla_A	TTC G	GA	TTT	ATT	-	_		${f TTG}$	AGG				ATG	TTA	ATT	TTA
candelilla_B	TTC G	GA	TTT	ATT	-			TTG	AGG	-		_	ATG	TTA	ATT	TTA
pyrifolia	TTC G	GA	TTT	ATT	-	-		$\mathbf{T}\mathbf{T}\mathbf{G}$	AGG	_		_	ATA	\mathtt{TTA}	ATT	TTA
nsp.	TTC G	GA	TTT	ATT	-	-		$\mathbf{T}\mathbf{T}\mathbf{G}$	AGG	_		_	ATA	TTA	ATT	TTA
volcanica_A	TTC G	GN	TTT	ATT	-	_		TTG	AGG	_		_	NTG	TCA	ATT	TTA
volcanica_B	TTC G	GA	TTT	ATT	-	_		TTG	AGG	_		-	\mathtt{GTG}	TCA	ATT	TTA
quercifolia_A	TTC G	GA	TTT	ATT	•	-		TTG	AGG	_		_	ATG	TTA	ATT	TTA
quercifolia_B	TTC G	GA	TTT	ATT	-	_		TTG	AGG	_		_	ATG	TTA	ATT	TTA
quercifolia_C	TTC G	GA	TTT	ATT	•	-		$\mathbf{T}\mathbf{T}\mathbf{G}$	AGG	_		-	ATG	TTA	ATT	TTA
Bonplandia_geminiflora	TTC G	GA	TTT	ATT	•	-		TTG	AGG	_		-	ATG	TTA	ATT	TAA
Cobaea_pachysepala	TTC G	GA	TTT	ATT	•	_		TTG	AGG	_		_	ATG	TTA	ATT	TTA
Cobaea minor	TIC G	ĞA	TTT	ATT	-	_		TTG	AAG	GAC		_	ATG	TTA	ATT	TTA

7 6 7

TAT
TAT
TAT
TAT
TAT
TAT
TAT

CTC

TTT

CTC

TTT

CTC

CAA ACT TCT TCT TAT TAT TCT TCT TCT TCT TCT TCT ICT TCT TCT TCT TCT TCT TCT CAG TAG CAA CAG ACT GTA AAA NAA AAA GCA GTA ACG ATT AAA Bonplandia geminiflora Cobaea_pachysepala volcanica_B quercifolia_A quercifolia_B quercifolia_C candelilla_A buxifolia_B buxifolia_C quercifolia_ Cobaea_minor buxifolia_A volcanica_A candelilla Д cuzcoensis pyrifolia flexuosa flexuosa bicolor n.sp.

TAT
TAT
TAT
TAT
TAT
TAT
TAT
TAT

CIC

CTC

CTC

CIC

CTC

TAT TAT TAT

TTT

TTT

CTC

TTT

TTT

н о н

4 0

TAA

TAA TAA TAA TAA TAA TAA

TAA

LTT PTT TTT TTT TTT LLL TTT TTT TTT TTT TTTTGT TGT TGT TTT LTT GTT GTTTTT TTT TTT TTT TTT TTT TTT LTT TTT TTT TTT LTT TTI TTT III GTT GTG GTG GTTGTT GTT AAA ACA AAA ACA ACA ACA ACA ACA ACA TTA GAA TAA GAA GAA GAA GAA GAA GAA GAA GAA TAA GAA GAA GAA GAA GAA GAA GAA AAA TGA TGA TGA TGA TGA TGA IGA TGA TGA TGA TGA TGA TGA TGA TGA TGA GTT CAG GAG GAG AGG GAC GGC GGC GGC GGG 9999 GGG GGG GGG GGG GGG 366 366 Bonplandia_geminiflora Cobaea_pachysepala quercifolia_A quercifolia_B candelilla A quercifolia Cobaea_minor U volcanica B buxifolia A buxifolia_B volcanica A candelilla cuzcoensis buxifolia pyrifolia flexuosa flexuosa bicolor

TAA TAA TAA TAA AGA

TTT

CAA

TTT

AGA AGA

CAA CAA CAA

TTT

TTT TTT TTT TTT

AGA AGA AGA AGA AGA AGA AGA

> CAA CAA

TTT TTT TTT AGA AGA AGA AGA

TTT

TTT TTT TTT TTT TTT

TTT

4 -

TTT TTT TTT TTT TTT TTT $_{
m LLL}$ $_{
m LLL}$ $_{
m LLL}$ TTT TTT TTTTTT TTT TTT TGT TGT TGT TGT TGT TGT TGI TGT TGT TGT TGT TGT TGT CIC CIC TGT TGI CTA CTT GTT CTT CTT GTA GTC GTA GTA GTA GTA AAA AAA AAA AAA AAA AAA AAA AAA ACA ACA AAA AAA AAA AAA AAA AAA AAA AAA ATG ATA TGA TGA TGA TGA TGA TAA TGA TTA GAA AAA GAA GAA GAA TAA TAT CCT GCT CCT CCT CCT Bonplandia_geminiflora Cobaea_pachysepala quercifolia_B quercifolia_C quercifolia A candelilla_A Cobaea minor quercifolia บ buxifolia_B М buxifolia_A volcanica_A candelilla buxifolia_ flexuosa B cuzcoensis flexuosa A volcanica pyrifolia bicolor sp.

GAC TAC TAC TAC TAC TAA TAC TAC TAC TAC TAC TAC TAA TAC TAC TAC IAC TGA IGA TGA TGA TGA TGA TGA AGA AGA AGC AGT AGT AGT AGA GAA TAA ATG ATA ATG ATT LTA TTA TTA ITA TTA TTA TTA ITA TTA TTA TTA TTA TTA TTA LTA LTA TTA GAA AAA GAA GAA GAA GAA ATC CGT CAT CAT CAT CGT CGT CCT AGC GGC AGC AGC 0 00 0 Bonplandia geminiflora Cobaea pachysepala quercifolia_B quercifolia_C quercifolia_A candelilla_A Cobaea_minor buxifolia_A buxifolia_B buxifolia C volcanica_B volcanica A candelilla cuzcoensis pyrifolia flexuosa_ flexuosa bicolor n. sp. 233

TTA

TTA TTA TTA

633

717 717 717 717 717 717 717 717

CTT

CTT

TTA TTA TTA

CTT

CTT

CTT

7 3 3

8 4

ATG ATG

CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT

ATG ATG

ATC CCT CCTCCT CCT CCT CAT CCT CCT CCT CCT CCT CCT CAT CCTCCT CCT TCT CCT CCT ATT GTT GTT GTT GTT GTTGTG GTT ATA AAA AAA AAA AAA AAA AAA AAA AAA AGA AGA AAA AAA AAA AAA AAA AAA AAA AAA AAA ACA GCA GCA GCA GCA GTA GCA ITG TTG TTG TTG TTG ITG TTG LTG [TG ATT ATC TTA CTA TTA LTA LTA LTA LTA Bonplandia_geminiflora Cobaea_pachysepala quercifolia_B U quercifolia_A candelilla_A quercifolia_ Cobaea_minor volcanica_B buxifolia_A buxifolia_B volcanica_A candelilla_ cuzcoensis buxifolia_ flexuosa_ pyrifolia flexuosa bicolor n.sp.

CTT CTT

CTT

CTT CTT CTT

CTT CTT TGA **IGA** IGA

TAT TAT TAT TAT TAT TAT TAT

TAT

വയവ

TAC CAC CAC TGG **IGG** IGT TAT TTT TTT TTT LTT TTT LTT LTT TTT TTT TTTTTT ICT LLL LTI LL LL TAC CIC CTC CTC CIC CIC CIC CIC CIC CIC CIC CIC CTC CIC CIC CIC CTT CIC CTT TIC TIC TIC TIC TIC TTC TTC TIC TIC TIC TTC TIC TTC TAT TGT TGT TGT TGT TGT TGT TGT IGT TGT TGT TGT TGT IGT TGT IGT IGI IGT [GT IGT CTA ATA CAG AAT Bonplandia_geminiflora Cobaea pachysepala quercifolia_B quercifolia_C quercifolia_A candelilla A Cobaea minor buxifolia_C buxifolia_B volcanica_B buxifolia_A flexuosa_B cuzcoensis volcanica_A candelilla pyrifolia flexuosa bicolor sp.

TAT

TAT TAT TAT TAT TAT TAT TAT TAT TAT TAT 4 8 0

	\circ	\circ	\circ	\circ	\circ	\circ	\circ	\circ	\circ	\circ	\circ	\circ	\circ	\circ	\circ	\mathbf{C}	0	O	0	
	AAG	AAG	AAG	AAG	AAA	AAA	AAG	AAG	AAG	AAA	AAA	AAG	AAG							
	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	AAG	
	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	
	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	
	CTT	CTT	CTT	CTT	CTT	CTT	CII	CTT	CLT	CLT	CTT	CTT	CTT							
	TTC	TTC	TTC	TTC	TTC	TTC	TIC	TIC	TTC	TTC	TTC	$_{ m LLC}$	$_{ m LLC}$	TTC	TTC	TTC	TTT	TTC	TTC	
	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	
	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	
	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TTA	
	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	
	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	$_{ m TTG}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	${ m TTG}$								
	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$_{ m ITG}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	$_{ m TTG}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$_{ m LLG}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	TKG	
	TCT	TCT	TCT	TCT	TCT	TCT	TCT	TCT	TCT	TCT	TCT	TCT	TCT	TCT	TCT	TCT	TTT	TCT	TCT	
# M M	CIC	CIC	CIC	CTC	CIC	CTC	CIC	CIC	CIC	CTC	CTC	CTC	CTC	CIC	CTC	CIC	CTC	CTC	CTC	
	flexuosa_A	flexuosa_B	cuzcoensis	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla_A	candelilla_B	⁹ pyrifolia	nsp.	volcanica_A	volcanica_B	quercifolia_A	quercifolia_B	quercifolia_C	Bonplandia_geminiflora	Cobaea_pachysepala	Cobaea_minor	
										-										

 1AG

 1AG

· & -

8 2 2

TAC IAC TTT TTT TTTTTT TTTTTT TTT TTT TTT TTT TTT TTT TTT TTT TTT LTT TTT ATC TAA IAA TAA TAA TAA TAA TAA TAA TAA TAA CTA CTG TI CTT CIC CIC CTC CIC CTC CIC CIC CTC CTC CIC TGC TGC CTC CIC CIC CIC CTC TAA GGT AAT CTA CTA CCA CAA CAA CCA CCA TAT TTT LTT lTT TTT TTT TTT TTT ATA TGG TGG TGG TGG TGG TGG TGG \mathbf{TGG} TGG ATT Bonplandia geminiflora Cobaea pachysepala quercifolia_B quercifolia_C quercifolia_A candelilla A Cobaea_minor ט volcanica B buxifolia_B buxifolia_A volcanica_A candelilla cuzcoensis flexuosa_A buxifolia_ pyrifolia flexuosa_ bicolor n.sp.

	ιο (Λ φ	ATC AAA ATT TGA	AAA ATT	AAA	ATC AAA ATT TGA	ATC CCA ATT TGA	ATC CCA ATT TGA	ATC CAA ATT TGC	ATC CAA ATT TGC	ATC CAA ATT TGC	A ATC CAA ATT TGA	ATC AAA ATT GGA	ATC CAA ATT GGA								
iable is (coll u)		flexuosa_A	flexuosa_B	cuzcoensis	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla_A	candelilla_B	pyrifolia	nsp.	volcanica_A	volcanica_B	quercifolia_A	quercifolia_B	quercifolia_C	Bonplandia_geminiflora	Cobaea_pachysepala	Cobaea_minor	

TAA TAA TAA

AAT

ATG ATG ATG ATG ATG ATG ATG

GGG

ATG

ATT ATT ATT ATT ATT ATT

> 100 100 100 100 100 100

TCG

ACA ACA ACA ACA ACA ACA ACA

ATG

AAT

GGG

TAA NAA TAA TAA TAA TAA

AAT AAT AAT AAT AAT

GGG

GGG

ATT

999 999 999 999

AGT AGT AGT AGT AGT ATT

ATT ATT ATT ATT ATT

ACA AAA AAA AAA

TCG TCG TCG

AAG AAG AAG AAG AAG AAG AAG

AAG AAG AAG AAG

> TAA TAA TAA TAA

AAT AAT AAT AAT

ATG

9999 9999 9999

TCG

CTT

ATT

ATT ATT ATT ATT ATT ATT

ATG

666 666 666 666

TCG

TTT

ATG ATG ATG

AAT

GGT

GGT

AAA

AAA

(a)	5 7 7	CAA TIT TIT CAG	CAA TTT TTT CAG	CAA TTT TTT CAG	CAA TIT TIT CAG	CAA TTT TTT CAG	CAA TTT TTT CAG	$_{ m LLL}$	CAA TTT TTT CAG	CAA TTT TTT CAG	CAA TTT TTT CAG	TTT		TTT	CAA TTT TTT CAG	CAA TTT TTT CAG	CAA TTT TTT CAG	geminiflora CAA TTT TTT CAG	pala CAA TTT TTT CAG
rabie 18 (cont.u)		flexuosa A		cuzcoensis	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla_A	candelilla_B	pyrifolia	nsp.	volcanica_A	volcanica_B	quercifolia_A	quercifolia_B	quercifolia_C	Bonplandia_gem	Cobaea pachysepa

TTA TTA

TAG TAG TAG TAG TAG NAG TAG TAG

TAG

GAA GGA

lTT LTT

TCG

TAG TAG

TAT TAT TAT TAT TAT TAT

TCG

TTT TTTTTT TTT

TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG

TTA

TAG

TTA TTA TTA

TAT TAT

GAA

GAA

LTT TTT 292 292 292 292

TTA TTA TTA

CGT CGT CAC CAC CAC

TTA

TAT TAT TAT

TTT LLI LTT TTT TTT

TAT

TTT TTT TTA TTA

TCT TCT TCT

TAG

TTA

TAT

TAG

TTA TTA

TAT

TAG

TAT

TCG

CTT

GAA

LTT

TCT

CAC CGC



Table 18 (cont'd)											
	3 7 8										
flexuosa A	ATT	CAT	TTG	TTA	AAA	TGG	GCT	CTA	AGA	GAA	TTT
flexuosa_B	ATT	CAT	TTG	TTA		\mathbf{TGG}	GCI	CTA	AGA	GAA	$_{ m LLL}$
cuzcoensis	ATT	CAT	TTG	TTA		\mathbf{TGG}	gCT	CTA	AGA	TAA	TTT
bicolor	ATT	CAT	TTG	TTA		TGG	GCI	CTA	AGA	GAA	TTT
buxifolia A	ATT	CAT	TTG	TTA		\mathbf{TGG}	GCT	CTA	AGA	GAA	TTT
buxifolia_B	ATT	CAT	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTA		\mathbf{TGG}	GCT	CTA	AGA	GAA	TTT
buxifolia_C	ATT	CAT	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTA		\mathbf{TGG}	GCT	CTA	AGA	GAA	TTT
candelilla A	ATT	CAT	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTA		\mathbf{TGG}	GCT	CTA	AGA	GAA	TTT
candelilla_B	ATT	CAT	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTA		\mathbf{TGG}	GCT	CTA	AGA	GAA	TTT
pyrifolia	ATT	CAT	TTG	TTA	AAA	\mathbf{TGG}	GCT	CTA	AGA	GAA	TTT
n.sp.	ATT	CAT	TTG	TTA		\mathbf{TGG}	GCT	CTA	AGA	GAA	$_{ m LLL}$
	E	Ę		É		ב ב	Ę	ETE	F	KKIK	E

0 7 7

TAA TAA TAA TAA

ACA ATA ATA

GGG GGG

TAG

TAT TAG TAG TAG

TAG

TAA ACA ATA GGG TAG TTT GAA AGA CTA GCT TGG AAA TTA TTG CAT 1 ATT Bonplandia_geminiflora Cobaea pachysepala volcanica_A volcanica_B quercifolia_A quercifolia_B quercifolia_B Cobaea_minor

TAA TAA TAA TAA TAA TAA

ACA ACA

ACA ACA ACA ACA

GGG

GGG GGG GGG GGG

TAG TAG TAG TAG

GGG

ACA ACA

GGG

TAA TAA TAA

ACA

GGG

TAG

GAA GAA GAA

AGA

ACA

GGG

TAG TAG

AGA AGA

CTA CTA

CTA

AAA

TTG

TTA TTA

TTG

ATT ATT ATT

TTA

ACA

GGG

TTT

ACA

GGG

TTT TTT TTT

AGA

CNA

GCT GCT GCT GCT

TGG TGG TGG TGG

AAA AAA AAA

TTA

TTG $_{
m TTG}$

CAT CAT CAT CAT

ATT

GGG

TAG TAG

flexuosa A AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC GTG GTT ACA TAG ATG cuzcoensis Bicolor AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC GTG GTT ACA TAG ATG bicolor Bicolor AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC GTG GTT ACA TG ATG bicolor AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC GTG GTT ACA TAG ATG buxifolia B AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC GTG GTT ACA TAG ATG candelilla A AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC GTG GTT ACA TAG ATG ATG candelilla B AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC GTG GTT ACA TAG ATG candelilla B AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC GTG GTT ACA TAG ATG ATG ATG ATG ATG ATG ATG ATG AT	zh	Zh.	rh	Zh.	Zh.	rh	rh	rh	Zh.	Zh.	zh.	٥	<u>.</u>	rh.	77	rh		,	
AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC GTG GTT ACA AGA CTT TCA TAT AT																	1	1	
AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC GTG GTT AGA CTT TCA TAT ATG GTG CAT ATA ATC GTG GTT AGA CTT TCA TAT ATG GTG CAT ATA ATC GTG GTT AGA CTT TCA TAT ATG GTT CAT ATA ATC GTG GTT AGA CTT TCA TAT ATG GTT CAT ATA ATC GTG GTT AGA CTT TCA TAT ATG GTT CAT ATA ATC GTG GTT AGA CTT TCA TAT ATG GTT CAT ATA ATC GTG GTT CAT ATT ATC GTG GTT CAT ATT GTG GTG GTG CTT CAT ATT CA	TAG	TAG	\mathbf{T}^{CG}	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG	! !	1	
AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC GTG AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC GTG	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	1 1 1	! ! !	
AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC AGA CTT TCA TAT ATG ATT GGT CAT ATA ATC	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	i 1 1	1	
AGA CTT TCA TAT ATG ATT GGT CAT ATA AGA CTT TCA TATA ATG ATT GGT CAT ATA ATA AGA CTT TCA TATA ATG ATT GGT CAT ATA ATA AGA CTT TCA TAT ATG	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	GTG	GTG	GTG	\mathtt{GTG}	GTG	\mathtt{GTG}	\mathtt{GTG}	GTG	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	1 1	 	
AGA CTT TCA TAT ATG ATT GGT CAT	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ANC	ATC	ATC	ATC	ATC	1 1	1 1	
AGA CTT TCA TAT ATG ATT GGT AG	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	ATA	1 1	i !	
AGA CTT TCA TAT ATG ATT AGA CT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	! !	 	
AGA CTT TCA TAT ATG	ggT	GGT	GGT	GGT	GGT	GGT	GGT	GGT	ggT	GGT	GGT	ggT	GGT	GGT	GGT	GGT	1	! !	
AGA CTT TCA TAT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ALT	ATT	ATT	ATT	ATT	1 1	1	
AGA CTT TCA TAT	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	1 1	 	
AGA CTT																	1 1	1 1 1	
AGA AGA AGA AGA AGA AGA AGA AGA AGA AGA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	TCA	1 1	1 1 1	
niflora	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CTT	 	 	
flexuosa_A flexuosa_B cuzcoensis bicolor buxifolia_A buxifolia_B buxifolia_C candelilla_A candelilla_A candelilla_A candelilla_A candelilla_B pyrifolia n. sp. volcanica_A quercifolia_A quercifolia_B Guercifolia_A Cobaea_pachysepala	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	AGA	1 1 1	1 1	
		В	is							ı							niflora	Cobaea_pachysepala	•

9 1 9

	Table 18 (cont'd)															
		2 9 9														
		'n														
	flexuosa A	TTT	ATG	CAG	CAT	CCT	TAA	CTG	TCG	GGA	TAA	GAG	GAT	${ m TGG}$	CTG	
	flexuosa_B	TTT	ATG	CAG	CAT	CCT	TAA	CTG	\mathbf{T}^{CG}	GGA	TAA	GAG	GAT	\mathbf{TGG}	CTG	
	cuzcoensis	TTT	ATG	CAG	CAT	CCT	TAA	CTG	\mathbf{TCG}	GTA	TAA	GAG	GAT	TGG	CTG	
	bicolor	TTT	ATG	CAG	CAT	CCT	TAA	CTG	\mathbf{TCG}	GGA	TAA	GAG	GAT	TGG	CTG	
	buxifolia_A	TTT	ATG	CAG	CAT	CCT	TAA	CTG	\mathbf{T}^{CG}	GGA	TAA	GAG	GAT	$^{\mathrm{TGG}}$	CTG	
	buxifolia_B	TTT	ATG	CAG	CAT	CCT	TAA	CTG	\mathbf{TCG}	GGA	TAA	GAG	GAT	$^{\mathrm{TGG}}$	CTG	
	buxifolia_C	TTT	ATG	CAG	CAT	CCT	TAA	CTG	\mathbf{T}^{CG}	GGA	TAA	GAG	GAT	TGG	CTG	
	candelilla_A	TTT	ATG	CAG	CAT	CCT	TAA	CTG	TCG	GGA	TAA	GAG	GAT	\mathbf{TGG}	CTG	
24	candelilla_B	TTT	ATG	CAG	CAT	CCT	TAA	CTG	TCG	GGA	TAA	GAG	GAT	\mathbf{TGG}	CTG	
3	pyrifolia	TTT	ATG	CAG	CAT	CCT	TAA	CTG	$\mathbf{T}^{\mathbf{C}}$	GGA	TAA	GAG	GAT	\mathbf{TGG}	CTG	
	nsp.	TTT	ATG	CAG	CAT	CCI	TAA	CTG	TCG	GGA	TAA	GAG	GAT	\mathbf{TGG}	CTG	
	volcanica_A	TTT	ATG	CAG	CAT	CCI	TAA	CTG	TCG	GGA	TAA	GAG	\mathtt{TAT}	\mathbf{TGG}	CTG	
	volcanica_B	TLL	ATG	CAG	CAT	CCI	TAA	CTG	\mathbf{T}^{CG}	GGA	TAA	GAG	TAT	\mathbf{TGG}	CTG	
	quercifolia_A	TTT	ATA	CAG	CAT	TCT	TAA	CTG	TCG	GGA	TAA	GAG	GAT	\mathbf{TGG}	CTG	
	quercifolia_B	TTT	ATA	CAG	CAT	\mathbf{ICI}	TAA	CTG	\mathbf{T}^{CG}	GGA	TAA	GAG	GAT	TGG	CTG	
	quercifolia_C	TTT	ATA	CAG	CAT	TCT	TAA	CTG	$\mathbf{T}^{\mathbf{C}}$	GGA	TAA	GAG	GAT	\mathbf{TGG}	CTG	-
	Bonplandia_geminiflora	1	 	1	i ! !	! ! !] 	1 1	! ! !	! ! !	1	1 1	 	1 1 1	1 1 1	
	Cobaea_pachysepala	1	1 1	1	!	1 1	1	1 1 1	1	 	1 1	! !	i ! !	 	1 1	
	Cobaea_minor	1 1	1 1	1	1 1	1 1	 	1 1	1] 	 	1 	1 1 1	1 1	 	

1 1 2

8 9 4

717G 717G 717G 717G 717G

GAG GAG GAG CAG GAG ATG CAA CAA CAA CAA CAA CGA CGA CAA CAA CAA CAA CAA CGA CGA CGA TTA GAA ATG TAG TAA GAG TAC TAC TAC GAC TAC TAC GAC GAC TAC GAC GAC GAC GAC GAC GAC ATA TTG TTG TTG TTG ITG LTG TTG ITG TTG lTG TTG TTG lTG ITG ITG TTT TTT TTT TTT TTT TTT TTT TTTTTT TTTTTT TTT TTT TTT LTT ATT 1 CTC CTC CIC CTC CTG CTG CTC CTC CIC CTT CTC CTC CTT CTT CTC Bonplandia geminiflora Cobaea pachysepala quercifolia_B quercifolia_C quercifolia_A candelilla A quercifolia_ Cobaea minor buxifolia C buxifolia_B volcanica B buxifolia A volcanica_A candelilla_ flexuosa A М cuzcoensis flexnosa pyrifolia bicolor n. sp.

TTG TTG TTG TTG TTG TTG

	& &															б Н
	2															1 73
flexuosa_A	GTT	TGA	TGA	GTT	TTT	TTG	TAG (GAG	AGG	GCA	TCA	AAT	ATG	TAG	GGA	GTG
flexuosa_B	GTT	TGA	! ! !	1	1	1 1 1	1 1	1	1	1	1	! ! !	1	1 1 1	1 1	1
cuzcoensis	GTT	TGA	TGA	GTT	TTT	$\mathbf{T}\mathbf{T}\mathbf{G}$	_	3AG	AGG	GCA	TCA	AAT	$\mathbf{A}\mathbf{T}\mathbf{G}$	TAG	GGG	GGG
bicolor	GTT	\mathbf{TGA}	TGA	GTT	$_{ m LLL}$	TTG	TAG (GAG	AGG	GCA	\mathbf{TCA}	AAT	ATG	TAG	GGG	GTG
buxifolia_A	GTT	TTA	TGA	GTT	TTT	TTG		SAG	AGG	GCA	TCA	AAT	ATG	TAG	GGG	GTG
buxifolia_B	GTT	TTA	TGA	GTT	TTT	$\mathbf{T}\mathbf{T}\mathbf{G}$	_	3AG	AGG	GCA	TCA	AAT	ATG	TAG	GGG	GTG
buxifolia_C	GTT	TGA	TGA	GTT	TTT	TTG	_	SAG	AGG	GCA	TCA	AAT	$\mathbf{A}\mathbf{T}\mathbf{G}$	TAG	GGG	GTG
candelilla_A	GTT	TGA	TGA	GTT	TTT	$\mathbf{T}\mathbf{T}\mathbf{G}$		3AG	AGG	GCA	TAA	AAT	$\mathbf{A}\mathbf{T}\mathbf{G}$	TAG	GGG	GTG
candelilla_B	GTT	TGA	TGA	GTT	TTT	TTG	TAG (GAG	AGG	GCA	TAA	AAT	ATG	TAG	GGG	GTG
pyrifolia	GTT	TTA	TGA	GTT	TTT	$_{ m TTG}$	_	BAG	AGG	GCA	TCA	AAT	ATG	TAG	GGG	GTG
n.sp.	GTT	TTA	TGA	GTT	TTT	\mathtt{TTG}	_	3AG	AGG	GCA	TCA	AAT	ATG	TAG	GGG	GTG
volcanica_A	GTT	TTA	TGA	GTT	TTT	TTG	_	3AG	AGG	GCA	NNA	AAT	ATG	GAG	GGN	NNG
volcanica_B	GTT	TTA	TGA	GTT	TTT	TTG	_	3AG	AGG	GCA	TCA	AAT	ATG	GAG	GGG	GTG
quercifolia_A	GTT	TTA	TGA	GCT	TTT	TTG	_	TG	AGG	GCA	TCA	AAT	ATG	GAG	GGG	GTG
quercifolia_B	GTT	TTA	TGA	GCT	TTT	$_{ m TTG}$	TAG	3TG	AGG	GCA	TCA	AAT	ATG	GAG	GGG	GTG
quercifolia_C	GTT	TTA	TGA	GCT	TTT	TTG	TAG	3TG	AGG	GCA	TCA	AAT	ATG	GAG	GGG	GTG
Bonplandia_geminiflora	!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!	! ! !	 	 	1	 	! !	1	1	 	† 	! !	 	1	 	
Cobaea_pachysepala	1	 	1	1 1	1 1	1 1	! ! !	1	1	1 1	 	 	 	 	1 1	1 1
Cobaea_minor	i i i	1 1 1	1 1 1	1 ! !	1 1 1	 	!!!	1	!	1 1 1	! ! !	1 ! !	1 1	1 1 1	1 1	! !

$\overline{}$
d
ú
≒
con
\preceq
ω
18
le 1
le 1
e 1

iable 18 (collc.u)									
	ο, ι								σ (
	- m								nω
flexuosa_A	GCC	GCA	TTT	CGT	CTT	ATC	$_{ m TTT}$	TCT	 H
flexuosa_B	1 1	1 1	 	 	 	1 1 1	1	 	!
cuzcoensis	GGC	GCA	TTT	CGT	CTT	ATC	$_{ m LLL}$	$_{ m TCT}$	GT
bicolor	GGC	GCA	TTT	CAT	CTT	ATC	TTT	TCT	ŢĞ
buxifolia_A	ggc	GCA	$_{ m LLL}$	CGT	CTT	ATC	TTT	\mathbf{TCT}	ŢĠ
buxifolia_B	SGC	GCA	$\mathbf{T}\mathbf{T}\mathbf{I}$	CGT	CTT	ATC	TTT	TCT	ŢĠ
buxifolia_C	GGC	GCA	TTT	CGT	CTT	ATC	TTT	\mathbf{TCT}	TG
candelilla_A	299	GCA	TTT	CGT	CTT	ATC	TTT	TCT	ŢĠ
candelilla_B	GGC	GCA	$_{ m LLL}$	CGT	CTT	ATC	TTT	TCT	TG
pyrifolia	GGC	GCA	$_{ m TTT}$	CGT	CTT	ATC	TTT	TCT	TG
n.sp.	GGC	GCA	TTT	CGT	CTT	ATC	TTT	TCT	ŢĠ
volcanica_A	GGC	GCA	TTT	CGN	CTT	ATC	TTT	TCG	ŢĠ
volcanica_B	CCC	GCA	TTT	CGT	CTT	ATC	TTT	\mathbf{TCG}	ΤĠ
quercifolia_A	GTC	GCA	TTT	CGT	CIT	ATC	TTT	\mathbf{T}^{CG}	ŢĠ
quercifolia_B	GTC	GCA	TTT	CGT	CTT	ATC	TTT	$\mathbf{T}^{\mathbf{C}}$	ŢĠ
quercifolia_C	GTC	GCA	TTT	CGT	CTT	ATC	TTT	\mathbf{TCG}	TG
Bonplandia_geminiflora	1	1 1 1	t 1 1	1	 	; ! !	1	! ! !	l I
Cobaea_pachysepala	 	1 1	i i i	i i	1 1 1	! ! !	1	1	1
Cobaea_minor	1		! ! !	1 	 	! !	1	1 1	!

APPENDIX G

Aligned sequences of *Cantua*: nuclear ribosomal internal transcribed spacer regions and 5.8S ribosomal RNA gene

of Table 19. Aligned sequences of the nuclear ribosomal internal transcribed spacer regions deletion events are labeled in the alignment with insertion deletion coding at the end correspond to specific epithets on vouchers listed in Table 4. Gaps indicated with ":" and the 5.8S subunit of ribosomal RNA from the taxa sampled in Cantua data set. Names Ambiguities indicated with "N", Missing data indicated with "-". Specific insertion the sequence. In Indel Coding region at the end of the sequence, "C" represents gap presence, "A" represents gap absence.

▼ITS 1

	\rightarrow	[Indel	Н _	$\stackrel{\cdot\cdot}{\rightarrow}$	Indel	ر ا						[Inde]	le1 4		→
		∫Ind	del ;	~1										Indel	→
flexuosa_A	TCG AAA	_		CAG	CAG	CAC	GAC	CCG	CGA	ACT	\mathbf{TGT}	AAT	CTC	TAT	CTC
flexuosa B	TCG AAA		ggg	CAG	CAG	CAC	GAC	CCG	CGA	ACT	\mathbf{TGT}	AAT	CTC	TAT	CIC
cuzcoensis	TCG AAA			CAG	CAG	CAC	GAC	CCG	CGA	ACT	$_{\mathrm{TGT}}$	AAT	CTC	TAT	CIC
bicolor	-CG AA:			cc:	:AG	CAC	GAC	CCG	CGA	ACT	\mathbf{TGT}	AAT	CTT	TAT	CTC
buxifolia_A	TCG AAA	CCI	_	CAG	CAG	CAC	GAC	CCG	CGA	ACT	\mathbf{TGT}	AAT	CTT	TAT	CIC
buxifolia_B	TCG AAA		GSC	CAG	CAG	CAC	GAC	CCG	CGA	ACT	\mathbf{TGT}	AAT	CTT	TAT	CTC
buxifoliaC	TCG AAA			CAG	CAG	CAC	GAC	CCG	CGA	ACT	\mathbf{TGT}	AAT	CTT	TAT	CTC
candelilla A	1 1 1 1		1 1 1	1 1 1	1	1	i	1	 	1 1 1	\mathbf{TGT}	AAT	CTT	TAT	CTC
candelilla_B	TCG AAA	CCI	_	CAG	CAG	CAC	GAC	CCG	YGA	ACT	TGT	AAT	CTT	TAT	CIC
pyrifolia	AA	_	ညည	CAG	CAG	CGC	GAC	CCG	CGA	ACT	\mathbf{TGT}	AAT	CTT	TAT	CTC
n.sp.	TCG AAA	_	_	CAG	CAG	ည္သည	GAC	CCG	CGA	ACT	TGT	AAT	CTT	TAT	CTC
volcanica_A	1 1 1 1 1	1	!	1	1	1	!	1	-GA	ACT	\mathbf{TGT}	ATT	CTT	ATA	TT:
volcanica_B	TCG AAA		GTC	CCG	AAA	CAC	GAC	CCG	CGA	ACT	TGT	ATT	CTT	ATA	TT:
quercifolia A	TCG AAA	: CI	GTC	SCG	AAA	CAC	GAC	CCG	TGA	ACT	TGT	ATT	CIC	ATA	TC:
quercifolia_B	TCG AAA		GTC	SCG	AAA	CAC	GAC	CCG	TGA	ACT	$_{\mathrm{IGT}}$	ACT	CIC	ACA	IC:
quercifolia_C	TCG AAA		GTC	SCG	AAA	CAC	GAC	CCG	TGA	ACT	\mathtt{TGT}	ATT	CTC	ATA	TC:
Bonplandia_geminiflora	TCG AAA	_	BCC	TAG	CAG	AAC	AAC	CCG	CGA	ACT	CGT	:TT	AAC	ATA	IC:
Cobaea_pachysepala	TCG AAA	_	GCA	TAG	CAG	AAT	GAC	CTG	TGA	ACT	TAT	: TT	ACA	AAC	TA:
Cobaea_minor	TCG AAA	-	GCA	TAG	CAG	AAT	GAC	CCG	CGA	ACT	\mathtt{TGT}	: TT	CCA	AAC	TC:

	4 O														01 0	
	Indel	5		\rightrightarrows	Indel	7	Tnde	6					Ĭ	Indel	10	
	İ			•	\Ind	del	80						H	Indel	11	
flexuosa A	ලලල ල	333	GCG ATC	C GAT	r : CG	A:C		GGT	CCT	CGG	TCG	$_{ m TTG}$	CCA	CCG	CGI	.
flexuosa_B	ලලල ලැ	333	GCG ATC	C GA	. C	A:0	TCC	GGT	CCT	CGG	TCG	TTG	CCA	SCG	CGJ	.
cuzcoensis	ලලල ලැ	3GC G	GCG ATC	C GAT	r : CG	A:0	TCC	GGT	CCI	CGG	TCG	TTG	CCA	CCG	CGI	. .
bicolor	ලලල ල	3GC G	GCG ATC	C GAT	r : CG	A:0	TCC	GGT	CCI	CGG	TCG		CCA	\mathbf{T}^{CG}	CG	. .
buxifolia_A	ලලල ල	3 3 3 3 3	GCG ATC	C GAT	r : CG	A:0	TCC	GGT	CCT	CGG	TCG	TTG	CCA	CCG	CGJ	.
buxifolia_B	ලලල ල	33C G	GCG AT	C GAT	r : CG	A:0	TCC	GGT	_	CGG			CCA	CCG	CG	.
buxifolia_C	ලලල ල	3 3 3 3	GCG ATC	C GAT	r : CG	A:0	TCC						CCA	CCG		٠.
candelilla_A	ලලල ල	3 3 3 3 3	GCG AT	C GAT	r : CG	A:0	TCC						CCA	CCG		. .
candelilla_B	ලලල ල		GCG AT			A: 0	TCC						CCA	SCG		٠.
pyrifolia _	පියල ය		GCG ATC		r : CG	A:0	TCC	GGT	CCC	_			CCA	CCG		.
n. sp.	ලලල ල		GCG AT	C GAT		A:0	ICC			_			CCA	CCG		٠.
volcanica A	GGG TO		ACG GI	C GAT		G:0	TI						CTC	CCT	_	
volcanicaB	GGG TO		ACG GI	C GAT	_	G:0	TI			_			CTC	CCI	_	
quercifolia_A	GGG TC		ATG GI	C GAT		0:5	TTC	GGT					CTA	CCC		
quercifoliaB	GGG TO		ATG GI	C GAT	r : CG	G:0	TT	GGT	CCI.	CGG	CCA		CTA	CCC		
quercifolia_C	GGG TC	TGC A	ATG GTC	C GAT		G:0	TT	GGT	CCT	CGG	CCA	$\mathbf{T}\mathbf{T}\mathbf{G}$	CTA	CCC		
Bonplandia_geminiflora	GG GG	GGC A	ACT CGG	G CCT	r TCG	AAC	TCC	GGT	CCI	ggg	\mathbf{TCG}	TTT	:: ::	CCC	CIC	٠.
Cobaea_pachysepala	GGT G	GGC A	ACA ATT	F.G.	: : CG	GAC	-	GGT	CCI	CAT	TTG	$\mathbf{T}\mathbf{T}\mathbf{C}$:: ປ່	CCT	TT	
Cobaea_minor	GGT G	GGC A	ACG AT	ຕ ຕ	: TA	GAC	TT	GGT	CCT	CGG	TCG	${ m TTG}$:: ::	CCC	TCA	_

GCA TTG ATT TTG ATT Indel 20 Indel 21 TTG TTG TTG ATG TIG ATG (Indel 19 GCA GTG GTG GTG C: GG: G C: G G: G G: G C:GG: G C:G G: G Ω:Ω C:G G: G CTG CTG GCG CTG T:G CLL CTT CTT CTT CIL CTT CTT CTT CIC CTC CTT CTT CTT CTT CTT CTT GAG GAG GAG GAG GAG GG CGA GAG [Indel 16 Indel 18 CGA CGA CGA CGA CGA CGA CGA CGA CGA CGT CGA CGA CGT CGT CGT CGT Indel 17 : GG : GG :GG S.G.G. . წ gg 9 CGG CGG CGG CGG : AG ATA . G CAG :: :: $\Gamma\Gamma$ CCT CCT CCT CTT CCT CCT CCT CCT CCT CCI CCT CTT CCC CCC CCC CCG Indel 13 Indel 15 TTG TTG TTG TTG TTG TCG GCA TTG CAC CAC YGT AGT CAC CAC CAC CAC CAC CAC CAC CAC CAC AGT CAC CAC CAC CAC CAC AGT AGT TGC GAC AGT AGT AGT GAC GAC AGT AGT AGT AGT AGI TG: $_{
m AGL}$ CGT CGT TGT TGI GA TGA TGT CGT CGT CGI TGI CGI CGI TGI Indel12 TGG CGG S.G. CGG TGG TGG TGG G:T 9 5 Bonplandia geminiflora Cobaea pachysepala Table 19 (cont'd) quercifolia_B quercifolia_C quercifolia_A buxifolia_B buxifolia_C candelilla A Cobaea_minor buxifolia_A volcanica_B volcanica_A candelilla flexuosa_A flexuosa_B cuzcoensis pyrifolia bicolor n.sp.

ATT ATT

GTJ

TTG

ATT

Table 19 (cont'd)

H 4 C

) In	Indel 2	2				
flexuosa A	909	CLL	CCC	SGC	ACA	TAA	ACG	AAC	CCC	: GG	CGC	GGT	ATG	TGC	CAA	GGA
flexuosa_B	929	CLL	CCC	SGC	ACA	TAA	ACG	AAC	CCC	:GG	CGC	GGT	ATG	\mathbf{TGC}	CAA	GGA
cuzcoensis	929	CLL	CCC	SGC	ACA	TAA	ACG	AAC	CCC	:GG	CGC	GGT	ATG	$^{\mathrm{TGC}}$	CAA	GGA
bicolor	929	CLL	CCC	SGC	ACA	TAA	ACG	AAC	CCC	:GG	CGC	GGT	ATG	TGC	CAA	GGA
buxifolia_A	909	CLL	CCC	SGC	ACA	TAA	ACG	AAC	CCC	GG	CGC	GGT	ATG	$^{\mathrm{TGC}}$	CAA	GGA
buxifolia_B	929	CTT	CCC	SGC	ACA	TAA	ACG	AAC	CCC	: GG	CGC	GGT	ATG	\mathbf{TGC}	CAA	GGA
buxifolia_C	909	CTT	CCC	SGC	ACA	TAA	ACG	AAC	CCC	: GG	CGC	GGT	ATG	\mathbf{TGC}	CAA	GGA
candelilla_A	909	CTT	CCC	SGC	ACA	TAA	ACG	AAC	CCC	:GG	CGC	GGT	ATG	\mathbf{TGC}	CAA	GGA
candelilla_B	909	CTT	CCC	SGC	ACA	TAA	ACG	AAC	CCC	:GG	CGC	GGT	ATG	$^{\mathrm{TGC}}$	CAA	GGA
pyrifolia	909	CLL	CCC	SGC	ACA	CAA	ACG	AAC	CCC	: GG	CGC	ggT	ATG	\mathbf{TGC}	CAA	GGA
nsp.		CLL	CCC	SGC	ACA	CAA	ACG	AAC	CCC	: GG	CGC	GGT	ATG	TGC	CAA	GGA
volcanica_A	TTG	CLL	CCC	GAC	ACA	CTA	ACG	AAC	CCC	:GG	CGC	ggG	ATG	TGC	CAA	GGA
volcanica_B		CLL	CCC	GAC	ACA	CTA	ACG	AAC	CCC	:GG	CGC	ggc	ATG	$^{\mathrm{TGC}}$	CAA	GGA
quercifolia_A	909	CLL	ညည	GAC	AAA	CCA	ACG	AAC	CCC	:GG	CAC	ggc	ATG	$^{ m TGC}$	CAA	GGA
quercifolia_B		CLL	CCC	GAC	AAA	CCA	ACG	AAC	CCC	:GG	CAC	GGC	ATG	\mathbf{TGC}	CAA	GGA
quercifolia_C		CLL	CCC	GAC	GAA	CCA	ACG	AAC	CCC	:GG	CAC	GGC	ATG	\mathbf{TGC}	CAA	GGA
Bonplandia_geminiflora	GTA	LTT	CIC	SGC	AAC	CGA	ACA	AAC	CCC	CGG	CGC	GGC	ACG	CGC	CAA	GGA
Cobaea_pachysepala	GCA	$_{ m LLL}$	TTT	AGC	ACA	CTA	ACG	AAC	CCC	: GG	CGC	GGT	ATG	\mathbf{TGC}	CAA	GGA
Cobaea_minor	GCA	TTT	CCC	SGC	AAA	TAA	ACG	AAC	CCC	: GG	CGC	SGC	ATG	CRC	CAA	GGA

(cont'd)
19
Je J
lab

7	4	0		25	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG	3TG	GT:	GTG	3TG
			4	ndel	CGG	Seg	CGG	GGG	CGG	GGG	Sgg	CGG	ggg	CGG	ggg	ggg	SSS	gg	CGG	CGG	ggg (ggg ·	992
			91 2	In		_	_												_	_	_	TTT (•
			Indel		-	:GT 1							-			_	_	_	_	-	_	ATT I	-
																			_			CCT A	
					_	_	_	_	_	_	_	_	•	•	•	•	_	_	_	_	_	TGC C	•
																					•	_	_
					•	_	_	_	_	_	_	_	C CCA	_	_	_	_	_	_	_	c cee	•	•
						FCC																	CCA
					•	TAG	•													SCG.	_	_	TTG
			23		GGC	_	GGC	gg	ggo				ggc				AGC	-		GGI	AGC	TGC	TGC
			le1		: AG	:AG	:AG	:AG	:AG	:AG	:AG	:AG	:AG	:AG	:AG	:AG	:AG	:AT	GAT	:AT	: AG	:AG	:AG
			Indel		••	::	••	••	::	••	••	••	••	•••	••	••	••	••	GAA	••	••	••	••
					GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	GAG	AAG	AAG	AAG	AAC	AAG	AAG	AAG	AAG
					ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ATG	ATG	ACG	ACG	ACG	ACA	ACA	ACA
					ATA	ATA	RTA	GTA	GTA	GTA	GTA	3TA	STA	3TA	STA	STA	STA	STA	STA	GTA	rgA	AAA	FAA
	•					ATT 7			ATT (ATT (ATT (ATT (ATT (ATT (ATT (ATT (ATT (ATT (ACT	ATA i	TCA
1	6	E			flexuosa A	flexuosa_B A	cuzcoensis	bicolor	buxifolia A A	buxifolia_B P	buxifolia_C A	candelilla_A A	candelilla_B A	pyrifolia	n.sp.	ica_A	volcanica_B A	quercifolia A A	quercifolia_B A	quercifolia_C A	Bonplandia_geminiflora A	Cobaea_pachysepala A	Cobaea_minor T

					SGC	SGC	ggG	299	CGC	GGC	SGC	SGC	SGC	SGC	SGC	SGC	200	3GC	3gc	380	3gc	1 1	!
					CIC	CIC	CIC	CIC	CIC	_		_			_		_		_	_	CIC	!!!	! !
					ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	! ! !	
					ACG	ACG	ACG	ATG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ATG	ATG	ATG	ACG	! !	1 1 1
					AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	TGA	TGA	GAA	TAA	GAA	TAA	1 1 1	1 1 1
					AAC	AAC	AMC	AAC	AMC	ACC	AAC	AAC	AAC	AAC	AAC	AAT	AAT	AAT	AAT	AAT	TAC	TAC	TAC
					CGG	CGG	CGG	CGA	CGG	CGG	CGG	CGG	CGG	CGG	CGG	TGA	TGA	CGA	CGA	CGA	CAA	CAA	CAA
				58	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACA	ACG	ACG	ACG	ACT	ACA	ACA
			27	del	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CTT	CIT	CTC	CIC	CTG	CTG	CTG	CTT	CIT	CTT
			ndel	Ħ	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT	CAT						CRT	CAT	$_{ m TGT}$	T:T
					G:C	ດ:	ບ:	ບ:ບ	G:C	ບ:			ບ:	G: C			Sec	ggg	ggg	ggg	\mathbf{TGC}	TGC	AGC
			ndel		AGA	AGA	•	AGA		AKA	•	•	-								CAA	CAA	AAA
			Ĥ		_	•	GAC	_	Ŭ	•	•	•	•	•	•			999	999	_	_	_	ტ: ტ:
					AAG	• •	•	AAG	•			-		-	-								AAG
N	4	н			CXG	SCG	SCC	SCG	SCC	SCG	CCG	SCC	CCG	SCC	SCC	CTG	CTG	TCG	TCG	TCG	ဗည	TCA	CTG
					flexuosa_A	flexuosa_B	cuzcoensis	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla_A	candelilla_B	pyrifolia	n.sp.	volcanica_A	volcanica_B	quercifolia_A	quercifolia_B	quercifolia_C	Bonplandia_geminiflora	Cobaea_pachysepala	Cobaea_minor
	N	4	4. 1.	\undel26 \indel	↓Indel26 ↓Indel 27	2 4 1 Lindel26 lindel 27 Lindel 28 A CYG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC	2 4 1 Lindel26 Lindel 27 Lindel 28 CYG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC B CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC	LIndel26 Indel 27 Indel 28 CYG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCC AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCC AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCC AAG GAC AGA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCC	a_B CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC a_B CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC Sis CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGA AAA ACG ACT CTC	Lindel26 Lindel 27	Lindel26 Lindel 27 Lindel 28 CYG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AXA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CTC CTC CTC CTC CTC CTC CTC CTC	Lindel26 Lindel 27 Lindel 28 CYG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AYA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AYA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AYA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AYA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AYA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AYA ACG ACT CTC CCC CCG AAG GAC AYA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCC CCC CACC CA	Lindel26 Indel 27 Lindel26 Lindel 27 Lindel26 Lindel 27 Lindel 28 Lindel26 Lindel 27 Lindel 28 CYG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC ATA G:C CAT CTT ACA CGG AMC AAA ACG ACT CTC CCG AAG GAC AKA G:C CAT CTT ACA CGG ACC AAA ACG ACT CTC CCG AAG GAC AKA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AAG G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AAG G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AAG G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AAG G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AAG G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AAG G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AAG G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AAG ACG ACT CTC CTC CTC CTC CTC CTC CTC CTC CTC	2 4 1 ↓Indel26 ↓Indel 27 ↓Indel 28 □ CYG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ATA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ATA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ATA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC ATA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC ATA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG ACC AAG ACC CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG ACC AAG ACC CAT CTC ACG AAC AAA ACG ACT CTC ACT ACT	LINGel26 Indel 27 Indel 28 CYG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ATA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ATA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ATA G:C CAT CTT ACA CGG AAC AAA ACG ACT CTC AAA ACG ACT CTC AAA ACG ACT CTC AAA ACG AAC AAA ACG AAC AAA ACG ACT CTC AAA ACG AAC AAA ACG ACT CTC AACG AACG	Sa_A CYG AAG GAC AGA G: CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G: CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G: CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G: CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G: CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ACA G: CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ATA G: CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ATA G: CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ATA G: CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ATA G: CAT CTT ACA CGG AAC AAA ACG ACT CTC IIIa_B CCG AAG GAC AGA G: CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AAG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGG GC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGG CCT CTT ACA CGG AAC AAA ACG ACT CTC CTC CTC CTC CTC CTC CTC CTC CTC	Lindel26 Indel 27	Lindel26 Lindel 27 Lindel 28 CYG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC CG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ACA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC ACA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC ACA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC ACA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC AGA G: C CAT CTT ACA CGG AAC AAA ACG ACT CTC ACG AAG GAC GCG ACG AAC ACA ACG ACT CTC ACG AAG GAC GCG ACC AAA ACG ACT CTC ACG AAG GAC GCG ACC AAA ACG ACT CTC ACG AAG GAC GCG ACC AAA ACG ACT CTC ACG AAG GAC GCG ACC AAA ACG ACT CTC ACG AAG GAC GCG ACC AAA ACG ACT CTC ACG AAG GAC GCG GCC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GCG GCC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GCG CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GCG CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GCG CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GCG CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GAC GCG CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GCC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GCC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GCC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GCC CAT CTT ACA CGG AAC AAA ACG ACT CTC CCG AAG GCC CAT CTC CTC CCG AAG GCC CAT CTC CTC CTC CTC ACA ACG ACT CTC CTC CTC CTC ACT CTC ACA TGA AAT TGA ACG ACT CTC CTC CTC CTC ACT CTC ACT TGA AAT TGA ACG ACT CTC CTC CTC ACT TGA ACT TGA ACT CTC CTC CTC CTC CTC CTC ACT TGA AAT TGA ACG ACT CTC CTC CTC CTC CTC ACT TGA ACT TGA ACG ACT CTC CTC CTC ACT TGA ACT TGA ACT CTC CTC CTC ACT TTC ACT TGA ACT TGA ACT CTC CTC CTC ACT TTC ACT TGA ACT TTC CTC CTC CTC CTC ACT CTC CTC ACT TTC ACT T	Lindel26 Jindel 27 ↓Indel26 Jindel 27 ↓Indel26 Jindel 27 ↓Indel26 Jindel 28 □ CSG AAG GAC AGA G:C CAT CTT ACA GG AAC AAA ACG ACT CTC AGA GAC AAG GAC AGA G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CG AAG GAC AGA G:C CAT CTT ACA GG AMC AAA ACG ACT CTC CC CAGA G:C CAT CTT ACA GG AMC AAA ACG ACT CTC CC CAG AG G:C CAT CTT ACA GG AMC AAA ACG ACT CTC CC CAG AG G:C CAT CTT ACA GG AMC AAA ACG ACT CTC AAA GG CC AAG GC CAT CTT ACA GG AMC AAA ACG ACT CTC AAA GG CC AAG GC AAG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CAG AG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC AAA GG CC AGG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GAC AGG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GAC GG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GAC GG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GAC GG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GAC GG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GAC GG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GAC GG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GAC GG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GAC GG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GAC GG G:C CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GG CG GC CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GG CC CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GG CC CAT CTT ACA GG AAC AAA ACG ACT CTC CC CC AAG GG CC CAT CTC ACA TGA AAT TGA ACG ACT CTC CTC CC CC AAG GG CC CAT CTC ACA TGA AAT TGA ACG ACT CTC CTC CTC CTC CTC CTC CTC CTC CTC	1 ↓Indel26 ↓Indel 27 ↓Indel 28 ↓Indel 26 ↓Indel 27 ↓Indel 28 ↓Indel 29 ↓Indel 28 ↓Indel 29 ↓Ind	Lindel26 Indel 27	Lindel26 Indel 27	Lindel26 Indel 27

0 8 8

ммю		CGA TAC CGA TAC															1 1 1 1 1	
	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG	1	
	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	1 1 1	
	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	AGC	!	
	•	CGT	_		_	_	_	_	_	_	_	•	•	_	CGT	CGT	1	
	_	GAA	_		_	_	_	_	_	_	_	_	_	_	<u> </u>	GAA	1	
	•	r gaa r gaa	_	_		_	•	_	_	•	_	•	_	_	GAA	GAA	1	
		C GAT															:	
	CATC		CATC									-	-			•	i i	
		r raga	_	_	_	_	_	_		_	_	•	_	_	_	r cgc	i	
		TCT						_	_	_	_	_	_	_	_	-	i	
	333	999 999	Sec	GGG	Sec	Sec	GGC	Sec	Sec	Sec	Sec	Seg	Seg	969	Sec	Sec	1	
	CTC	CTC	CTC	CTC	CTC	CTC	CTC	CTC	CTC	CIC	CIC	CTC	CIC	CTC	CIC	CTC	1	
	TAT	TAT TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	i 1 1	
0/ 00 0/	GGA	GGA GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	GGA	1	
		flexuosa_B cuzcoensis	bicolor	buxifolia A	buxifolia_B	buxifolia_C	candelilla_A	candelilla_B	pyrifolia	n. sp.	volcanica A	volcanica_B	quercifolia A	quercifolia B	quercifolia_C	Bonplandia_geminiflora	Cobaea pachysepala	יין איניין א

ж 8 4

	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	ACG	1	! ! !
	TGA	TGA	TGA	TGA	TGA	\mathbf{TGA}	TGA	\mathbf{TGA}	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	 	1
	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	$\mathbf{M}\mathbf{L}\mathbf{L}$	TTT	TTT	TTT	1	1 1
	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	AGT	1	1 1 1
	\mathbf{TCG}	\mathbf{TCG}	$\mathbf{T}^{\mathbf{G}}$	\mathbf{TCG}	\mathbf{TCG}	\mathbf{T}^{CG}	\mathbf{TCG}	\mathbf{ICG}	\mathbf{TCG}	\mathbf{I}^{CG}	\mathbf{TCG}	$\mathbf{T}^{\mathbf{C}}$	$\mathbf{T}^{\mathbf{C}\mathbf{G}}$	\mathbf{TCG}	$\mathbf{T}^{\mathbf{C}\mathbf{G}}$	$\mathbf{T}^{\mathbf{C}\mathbf{G}}$	\mathbf{TCG}	1	1 1 1
	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	CCA	1 1	1 1 1
	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	1	1 1 1
	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	1	1
	TCC	TCC	TCC	TCC	TCC	TCC	TCC	TCC	\mathbf{ICC}	TCC	TCC	ICC	$\mathbf{I}^{\mathbf{C}\mathbf{C}}$	TCC	$\mathbf{I}^{\mathbf{C}}$	ICC	TCC	1	1 1
	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	1	1
	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	1	1 1
	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	1	1
	TGA	TGA	TGA	TGA	\mathbf{TGA}	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	1	
	GTG	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	GTG	ATG	ATG	\mathtt{GTG}	\mathtt{GTG}	GTG	\mathtt{GTG}	GTG	\mathtt{GTG}	\mathtt{GTG}	\mathtt{GTG}	1 1	1
w w r	TTG	TTG	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	TTG	TTG	TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$	TTG	TTG	 - 	1
	flexuosa_A	flexuosa_B	cuzcoensis	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla_A	candelilla_B	pyrifolia	n.sp.	volcanica_A	volcanica_B	quercifolia_A	quercifolia_B	quercifolia_C	Bonplandia_geminiflora	Cobaea_pachysepala	Cobaea_minor

4 m 0

CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	TGT	$\mathbf{T}\mathbf{G}\mathbf{I}$	TGT	CGT	1 1	
පලල	GGG	GGG	GGG	GGG	GGG	999	999	GGG	ggg	GGG	GGG	ggg	999	999	GGG	GGG	1 1	1 1 1
CCT	CCT	CCT	CTT	CCT	CCT	CCL	CCI	CCT	CCI	CCL	CCI	CCT	CTT	CTT	CCI	CCT	1 1	1 1
CTG	CTG	CTG	CTG	CTG	CTG	CTG	CTG	CTG	CTG	CIG	CTG	CTG	CTG	CTG	CTG	CTG	! !	
CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGT	CGC	CGC	CGC	CGC	CGC	CGC	i	1 1 1
GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	GCA	1 1	1 1
AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	1 1	
SCG	CCG	CCG	SCG	CCG	CCG	CCG	CCG	CCG	SCG	CCG	CCG	CCG	CCG	CCG	CCG	SCG	1 1	! !
AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	1 1 1	
ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ACT	t t 1	! !
ညဗ	BCC	gcc	gcc	gaa	ggg	gcc	ညည	ggg	ದ್ದಿದ್ದ	gcc	ညည	gcc	ದ್ದಿದ್ದ	ညည	ರ್ಧ	gcc	1	1 1 1
GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	1 1 1	1 1
SSS	CCC	CCC	CCC	CCC	CCC	CCC	CCC	CCC	CCC	CCC	CCC	CCC	CCC	CCC	CCC	CCC	1	1
gcg	GCG	GCG	gag	gag	gcg	gag	gag	gcg	gag	gcg	gcg	gag	gcg	gag	gcg	gag	1 1 1	1 1
3 8 5 GTT	GTT	GTT	GTT	GTT	GLL	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	GTT	l l	! !
flexuosa A	flexuosa_B	cuzcoensis	bicolor	buxifolia_A	buxifolia_B	buxifolia_C	candelilla_A	candelilla_B	pyrifolia	nsp.	volcanica_A	volcanica_B	quercifolia_A	quercifolia_B	quercifolia_C	Bonplandia_geminiflora	Cobaea_pachysepala	Cobaea_minor

	Table 19 (cont'd)		
			•
		T. T	4
		m	ω
		8	0
		\langle Indel29_\tau\indel 30 \tau \indel 32	
		→ Ti	Indel 3
	flexuosa_A	CGT CGC CCC AAA : CC TAT :: C CCT ACG GGA ATA TCG G:T	ATG
	flexuosa_B	'CGC CCC AAA :CC TAT ::C CCT ACG GGA ATA TCG G:T	ATG
	cuzcoensis	ACA TCG CGT CGC CCC AAA : CC TAT :: C CCT ACG GGA ATA TCG G:T]	MTG
	bicolor	ACA TCG CGT CGC CCC AAA : CC TAT :: C CCT ACG GGA ATA TCG G:T	ATG
	buxifolia_A	:CC TAT ::C CCT ACG GGA ATA TCG G:T	CTG
	buxifolia_B	TCG CGT CGC CCC AAA : CC TAT :: C CCT ACG GGA ATA TCG G:T	CTG
	buxifolia_C	CCC AAA : CC TAT :: C CCT ACG GGA ATA TCG G:T	CTG
	candelilla_A	CGC CCC AAA : CC TAT :: C CCT ACG GGA ATA TCG G:T	CTG
257	candelilla_B	TCG CGT CGC CCC AAA : CC TAT :: C CCT ACG GGA ATA TCG G:T	CTG
7	pyrifolia	CGT CGC CCC AAA : CC CAT :: C CCT ACG GGA ATA TCG G:T	CTG
	nsp.	CGC CCC AAA :CC CAT ::C CCT ACG GGA ATA TCG G:T	CTG
	volcanica_A	TGC CCC AGC : CC TAT :: C TCC CCG GGG ATA TGG G:T	$_{ m LLG}$
	volcanica_B	CGT TGC CCC AGC : CC TAT :: C TCC CCG GGG ATA TGG G:T	$\mathbf{T}\mathbf{T}\mathbf{G}$
	quercifolia_A	CCC AAA CCC TGT :: C CCC TCG GGG AAA TCA G:A	TTG
	quercifolia_B	CGC CCC AAA CCC TGT :: C CCC TCG GGG AAA TCA G:A	$\mathbf{T}\mathbf{T}\mathbf{G}$
	quercifolia_C	TGT :: C CCC TCG GGG AAA TCA G:A	TTG
	Bonplandia_geminiflora	ACA TCG CGT CGC CCC CAA CCC CTT ACC CCT TCG GGC GTG ACG GGC	TTG
	Cobaea_pachysepala	ATT : TC CAT TAG GAA TCA TTA TTG	$\mathbf{T}\mathbf{T}\mathbf{G}$
	Cobaea_minor	CAA CCC TTT :TC CTT AAG GAA ::T TGT CGT	${ m TTG}$

8 7 2

4	æ .⊣		pui	flexuosa A GGG : CG	flexuosa_B GGG : CG	1.8	bicolor GGG :CG		buxifolia_B GGG : CG	999	candelilla A GGG : CG	999	pyrifolia GGG : CG		ica_A GGG		quercifolia_A GGG : CG			miniflora	Cobaea_pachysepala G:A GCG	Cobaea_minor GGA GCG
		34	idel 35	GAA	•	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAA	GAG	GAG	GAG	GAA	GAA	GAA
				T GGC	_	T GGT	_	_	•	_	_	•	_	•	_	_	_	_	•	_	T GGT	•
				_	CIC															CTC		_
				SSS	CCG	SCG	_	SCG		_	_	_	SCG	_	_	_	_	_	_	CCG	_	SCG
				CGC	CGC	CGC	CGC	CGC	CGC	CGC	CGC	CGC	CGC	CGC	\mathbf{TGC}	\mathbf{TGC}	\mathbf{TGC}	\mathbf{TGC}	\mathbf{TGC}	\mathbf{IGC}	\mathbf{TGC}	TGC
				ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	AAA	AAA	ACA	ACA	ACA	BCC	ACA	ACT
				gcg	gcg	gcg	gcg	RCG	RCG	ACG	ACG	ACG	ACG	ACG	TTA	TTA	TCG	TCG	TCG	TAG	TTG	TTG
				TTG ;		TTG 1															TTG	
				AGC G														_		TGC G	•	•
				GGT I												_	_	-	-	_	GGT I	GGT T
				_	•	_	_	_				-				_	_	_	_	_	rgg c	_
				מממ	ນ	CCY	CCC	CCY	CY	SC	CT	CI	E D	Į.	CI	E U	Ę	IJ	IJ	CCT	CCL	Ę

 AAA

 | rv 64 ev | | | | | | | | | | | | | | | | 6 7 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | - | Indel | 36 | | | | | | | ui
→ | Indel | 37 |
| | | | | | | | | | | | | | | Ind | Indel 38 | →8 | |
| | | | | | | | | | | | | | | Н | Inde] | 39 | → |
| flexuosa_A | AAT | GAG | \mathbf{TGG} | ACG | \mathbf{TGG} | ACA | ATT | GGC | GTC | ACA | ACG | TGT | GGT | GGT | | | |
| flexuosa_B | AAT | GAG | \mathbf{TGG} | ACG | \mathbf{TGG} | ACA | ATT | GGC | \mathtt{GTC} | ACA | ACG | \mathbf{TGT} | GGT | GGT | | | •• |
| cuzcoensis | AAT | GAG | \mathbf{TGG} | ACG | \mathbf{TGG} | ACA | ATT | GGC | GTC | ACA | ACG | \mathbf{TGT} | GGT | GGT | _ | | •• |
| bicolor | AAT | GAG | \mathbf{TGG} | ACG | \mathbf{TGG} | ACA | ATT | GGG | \mathtt{GTC} | ACA | ACG | \mathbf{TGT} | GGT | GGT | _ | | •• |
| buxifolia_A | AAT | GAG | TGG | ACG | \mathbf{TGG} | ACA | ATT | GGC | GTC | ACA | ACG | \mathtt{TGT} | GGT | GGT | _ | | •• |
| buxifolia_B | AAT | GAG | \mathbf{TGG} | ACG | TGG | ACA | ATT | GGC | GTC | ACA | ACG | \mathbf{TGT} | GGT | | CG. | | •• |
| buxifolia_C | AAT | GAG | \mathbf{TGG} | ACG | \mathbf{TGG} | ACA | ATT | ggc | \mathtt{GTC} | ACA | ACG | \mathbf{TGT} | GGT | | | | |
| candelilla_A | AAC | GAG | \mathbf{TGG} | ACG | \mathbf{TGG} | ACA | ATT | GGC | \mathtt{GTC} | ACA | ACG | \mathtt{TGT} | GGT | GGT | CGT | CG: | |
| candelilla_B | AAC | GAG | \mathbf{TGG} | ACG | ${ m TGG}$ | ACA | ATT | GGC | \mathtt{GTC} | ACA | ACG | \mathtt{TGT} | GGT | | | | •• |
| pyrifolia | AAM | GAG | CGG | ACG | \mathbf{TGG} | ACA | ATT | GGC | GTC | ACA | ACG | \mathtt{TGT} | GGT | | | | •• |
| nsp. | AAC | GAG | CGG | ACG | \mathbf{TGG} | ACA | ATT | ggc | GTC | ACA | ACG | \mathbf{TGT} | GGT | | | | •• |
| volcanica_A | TAA | GAG | \mathbf{TGG} | ATG | \mathbf{TGG} | AC: | ::
!: | GGC | GTC | ACG | ATA | \mathtt{TGT} | GGT | | | | •• |
| volcanica_B | TAA | | \mathbf{TGG} | ATG | \mathbf{TGG} | AC: | <u>ຕ</u> | GGC | GTC | ACG | ATA | \mathtt{TGT} | GGT | | | | •• |
| quercifolia_A | TAA | | \mathbf{TGG} | ACG | $\mathbf{T}\mathbf{T}\mathbf{G}$ | AT: | <u>ຕ</u> | GGC | GTC | ACG | ACA | \mathbf{TGT} | GGT | | | | H |
| quercifolia_B | TAA | | \mathbf{TGG} | ACG | TTG | AT: | <u>ຕ</u> | GGC | GTC | ACG | ACA | \mathtt{TGT} | GGT | | | | ບ |
| quercifolia_C | TAA | GAG | \mathbf{TGG} | ACG | $\mathbf{T}\mathbf{Y}\mathbf{G}$ | AT: | <u>ຕ</u> | GGC | GTC | ACG | ACA | \mathbf{TGT} | GGT | GGT | | | ບ |
| Bonplandia_geminiflora | AAC | GAG | \mathbf{TGC} | ACG | \mathbf{TGG} | AC: | .:
G | GAC | GTC | ACA | ACA | \mathbf{TGT} | GGT | GGT | | | Æ |
| Cobaea_pachysepala | ATT | GAG | TAA | ACG | \mathbf{TGG} | AT: | .:
G | AGA | GCC | GTA | ACA | \mathtt{TGT} | GGT | GGT | TGA | CA: | Æ |
| Cobaea_minor | ATA | GAG | TTC | ACG | \mathtt{TGT} | AC: | .:
G | AGA | GIC | ACA | ACA | AGT | GGT | GGT | :GT | | Æ |

	5 7 7 2	64 60 60 60 60 60 60 60 60 60 60 60 60 60
flexuosa_A	TCG GAA A:C	-
18	CGT TGC GTT GTG CGC TCG TCG TCG GAA R:C	
bicolor buxifolia A	GIT GIG CGC T:G TCG	ATA S
buxifolia_B buxifolia_C	GTT GTG CGC Y:G TCG TCC TCG GAA R:C GTT GTG CGC T:G TCG TCC TCG GAA A:C	ATA
candelilla_A	CGT TGC TIT GTC GTT GTG CAC T:G TCG TCC TCG GAA A:C	
Œ	CGT TGC TIT GTC GTT GTG CRC T:G TCG TCG GAA A:C	
pyrifolia n. sp.	GTT GTG CGC T:G TCG GTT GTG CGC T:G TCG	ATA
volcanicaA	CGT TGC GTT GCT ATT GTG CGT C:G TCG TTC TCG GAA	
volcanica_B	CGT TGC GTT GCT ATT GTG CGT C:G TCG TTC TCG GAA :GC	TTA
quercifolia_A	CKT TGC GTT GCC GTT GTG CGT C:G TCG TCT CCG GAA AGC	
quercifolia_B	CGT TGC GTT GCG GTT GTG CGT C:G TCG TCT CCG GAA AGC	
quercifolia_C	AAC CGT TGC GTT GCC GTT GTG CGT C:G TCG TCT CCG GAA	
Bonplandia_geminiflora	AAC CGT TGC GTT CCT GTT GTT GGT C:G TTC TCC TCG GTT AGC	
Cobaea_pacnysepala	CGI' TGC GITI GIT' GIG TGA T':C TIG TCC TC: AAA	
Cobaea_minor	CC: GAA AAC	ATT

	9 7			99	INDEL		CODING	
	S			0	123 4	2 951	89 1	111
								12
					$\overrightarrow{\rightarrow}$	→ → →	$\rightarrow \uparrow \uparrow$	$\stackrel{\rightarrow}{\Rightarrow}$
flexuosa_A	:: G ACC CTG TCG TGT CGC	Sec	GCC	TCG ACC		•		AA
flexuosa_B		299	GAT GCC T	TCG ACC				AA
cuzcoensis	\mathbf{YGT}	GGC						AAA
bicolor	CTG TTG TGT	GGC	GCC	TCG ACC				AAA
buxifolia_A	CTG TTG	TCC GGC	BCC	TCG ACC	AAA 1		ACC A	AA
buxifolia_B	TTG CGT	GGC	225					AA
buxifolia_C	TTG CGT	GGC						AA
candelilla_A	CTG TTG CGT	GGC	BCC					AA
candelilla_B	TTG CGT	GGC		TCG ACC				AA
pyrifolia	CTG TTG CGT	GGC	BCC		AAA 1			AA
nsp.	CTG TTG CGT	GGC	BCC	CG ACC				AA
volcanica_A			!	!	<i></i>			AA
volcanica_B	CTG CAC	GGT		TCG ACT	ACA 7			AA
quercifolia_A	CT: CTG CGC	AGC	GCC		ACA 7		-	AA
quercifolia_B	CTG CGC	TTC AGC C	GCC	TCG ATC				AA
quercifolia_C	_	AGC		TCG ATC				AA
Bonplandia_geminiflora	ATG CGT	_		TCG ACC	AAA (AC
Cobaea_pachysepala	:: G ACC CTA TTG CAT TGT	GAA TGT G		TCG ACT	AAA (CAC C	C-A C	CA
Cobaea_minor	G ACC CTG TTG CGT TGC	GCA AGT C	GAC GCC T(TCG ACC	AAA (CACC	_	CAA

Table 19 (cont'd)

	IND	EL C	INDEL CODING	CO CO	CON'T						
	111	111	122	222	222	223	333	333	333	444	444
	345	678	901	234	567	890	123	456	789	012	345
	→ → →	→ →		→ → →	→ →	→ → →	→ →	→ →	→ →	→ → →	→ →
flexuosa_A	AAA	C-A	_	CCC	AAC	ACC	-AC	ACA	AAC	CAA	CCA
flexuosa_B	AAA	C-A		CCC	AAC	ACC	-AC	ACA	AAC	CAA	CCA
cuzcoensis	AAA	C-A		CCC	AAC	ACC	-AC	ACA	AAC	AAA	CCA
bicolor	AAA	C-A		CCC	AAC	ACC	-AC	ACA	AAC	CAA	CCA
buxifolia_A	AAA	C-A		CCC	AAC	ACC	-AC	ACA	AAC	CAA	CCA
buxifolia_B	AAA	C-A		CCC	AAC	ACC	-AC	ACA	AAC	CAA	CCA
buxifolia_C	AAA	C-A		CCC	AAC	ACC	-AC	ACA	AAC	CAA	CCA
candelilla_A	AAA	C-A		CCC	AAC	ACC	-AC	ACA	AAC	CAA	CCA
candelilla_B	AAA	C-A		CCC	AAC	ACC	-AC	ACA	AAC	CAA	CCA
pyrifolia	AAA	C-A	CAA	CCC	AAC	ACC	-AC	ACA	AAC	CAA	CCA
nsp.	AAA	C-A		CCC	AAC	ACC	-AC	ACA	AAC	CAA	CCA
volcanica_A	AAA	C-A		CCC	AAA	ACC	-AC	ACC	AAC	CA-	1
volcanica_B	AAA	C-A		CCC	AAA	ACC	-AC	ACC	AAC	CAC	ACA
quercifolia_A	AAA	AAA		CCC	AAA	AAC	-AC	ACC	AAA	CAA	ACC
quercifolia_B	AAA	ACA	AAA	CAA	AAA	AAC	-AC	ACC	AAA	CAA	ACC
quercifolia_C	AAA	ACA	AAA	CCC	AAA	AAC	-AC	ACC	AAA	CAC	ACC
Bonplandia_geminiflora	ACC	ACA	ACC	ACC	CCA	AAA	AAA	AAC	AAA	CAA	AAA
Cobaea_pachysepala	CAA	AAC	CAA	CCA	ACA	AAA	CAA	CAC	ACA	CCA	ACA
Cobaea_minor	CAA	AAC	CAA	CCC	ACA	CAA	CCA	AAC	CCA	CCA	ACA

APPENDIX H

Aligned sequences of *Brassicaceae*: 771 base pairs of the *ndh*F coding region

ed in Idicated Table 20. Aligned sequences or //1 pasepairs or the main country region inching

sted indj	4	ω		æ	A	Æ	Ø	Æ	Ø	Ø	Æ	Æ	Ø	Æ	Æ	Æ	A	Æ	Æ	Æ	Æ	Æ	Æ	Æ	Æ	Æ	Æ	Æ
.⊣ .q				•	•	_											. AAA				-						•	
dat				_	\mathbf{TCT}	_	TAT	TAT	TAT	Į.	TAT	Ţ	TGT	TAT	TAT	TAT	TAT	TAT	ŢĊŢ	TCI	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT
cimens sing d				TII	$\mathbf{T}\mathbf{T}\mathbf{I}$	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTG	TTT	$\mathbf{T}\mathbf{T}\mathbf{I}$	$\mathbf{L}\mathbf{L}\mathbf{L}$	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT
a) a				ĮĮ.	ITA		ITA	ITA	LTA	LTA	LTA	LTA	TTA	LTA	TAA	LTA	TTA	ΓTA	FTA	ITA	LTA	LTA	TTA	LTA	LTA	LTA	ITA	LTA
				ט	CAC	CAC	CAC	CAC	_	_	CAC	_	CAC		CAC					CAC		CAC	CAC	CAC	CAC	CAC	CAC	CAC
voucher th "N",				-		AAA O	_		_								AAA O						AAA O			AAA C	AAA O	AAA C
o vou with				_	-		•	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•					_
1)				_	•	Ť.	•	-									A TTT						_		A TTT	A TITT	A TTT	A TTT
ond ate				ACA	ACA	ACA	ACA	ACA	•	ACA	ACA	ACA	ACA		ACA		ACA		ACA	ACA		S	ACA	S	ACA	ACA	្	ACA
rrespond indicated				AGG	AGG	AGG	1	- GG	AGG	- GG	- G	ტ-	1	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	AGG	1	AGG
0				TGA	TGA	TGA	! !	1 1	1 1	1	1	1 1 1	1	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	TGA	CGA	4	1	TGA
es cies				$\mathbf{T}\mathbf{L}\mathbf{L}$	TTT	TTT	1	1 1	1	1 1	† 	1	1	TTT	TIL	TTT	$\mathbf{T}\mathbf{T}\mathbf{I}$	TTT	$\mathbf{T}\mathbf{T}\mathbf{I}$	TTI	LLL	$\mathbf{T}\mathbf{T}$	TLL	LLL	$\mathbf{T}\mathbf{T}\mathbf{T}$	1	1	TTI
et. Names c Ambiguities					TAC	TAC	1	1	!	1	1	1	1	TAC			TAC	FAC	TAC	TAC		TAC	TAC	TAC	TAC	!	!	CAC
t. Ambi					ACT	ACT 7	:	:	1	1	!	1	1		ACT		ACT		_	ACT 1	ACT 1	ACT 1	ACT 1	ACT 1	ACT 1	!	!	ACT
ຜຸ				_ `	_	TTT A	!	!	!	!	1	1	1				TTT A				•				TTT A	!	!	TTT A
data .":"				-A T	_	-	1	i	i 1	i 1	i	1	1	-A T	_	_	-	_	_	-	-	_	_	_	-A T	i	i	_
				!	r TTA	r TTA	1	!	1	1	!	1	1	1	A		r TTA	_		•	-	r TTA	r TTA	-	-	:	1	r GTA
ace d ≪	Н			i	GAT	GAT	1	i	İ	i	1	i	i	1	i	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	GAT	i	į	i	GAT
sampled in the Brassicaceae Table 8. Gaps indicated with with "-".			,	Cleome_spinosa	Aethionema_arabicum	Aethionema_grandiflorum	Brassica_nigra	Raphanus_raphanistrum	Raphanus_sativus	Lobularia maritima	Hesperis_matronalis	Arabidopsis_lyrata	Arabis_hirsuta	Iberis	Cakile_edentula	Sisymbrium_altissimum	Sisymbrium_pinnatum	Barbarea_vulgaris	Arabidopsis_thaliana	Capsella_bursa-pastoris	Nasturtium_officinale	Brassica_oleracea	Barbarea_verna	Brassica_napus	Alliaria petiolata	Cardamine_concatenata	Brassica_rapa	Matthiola

6 9

999

999 GGG 999 999 999 GGG GGG 9999 GGG 999 999 9999 GGG GGG 999 999 999 GGG

CGT CTT CLL CTT CLL CTT CTT CTT CGI TTT CTT CTT CTT CLL CTT CGT CTT CTT CIT CTC TTC CTC CTC TTC CTC CIC CIC CTC CTC CTC CIC CTC CIC CIC CIC CTC TAG TAG TCG TCG TCG TAG TAG TAG TGG CAG TAG $\mathbf{T}^{\mathbf{C}\mathbf{G}}$ TAG CAG TAG TCG TAG TAG TCG JCG TAG TAG CAG TAG AAG AAA TGG TGG \mathbf{TGG} TGG TAG TAG TAG CAG CAG CAG CAG CAG CAG CAG CAG TAG CAG TAG CAG CAG CAG CAG TAG TAG CAG TTA Aethionema grandiflorum Capsella bursa-pastoris Raphanus_raphanistrum Sisymbrium_altissimum Nasturtium officinale Cardamine_concatenata Arabidopsis_thaliana Aethionema_arabicum Hesperis matronalis Sisymbrium pinnatum Alliaria_petiolata Lobularia maritima Arabidopsis_lyrata Barbarea vulgaris Brassica_oleracea Raphanus_sativus Cakile edentula Brassica nigra Arabis_hirsuta Barbarea_verna Brassica_napus Cleome spinosa Brassica_rapa Iberis

ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG GTG ATG ATG ATG ATG ATG ATG ATG TTT TTT TIT TTT TTT TII TTT TLL TTT TTT TTT TIT TTT TTT TIL TTT TTT TTT TIL TII TTT CTC CTC CTC CIC CTC CIC CTC CTC TTC CTC CTC CTC AAT AAT AAT AAT AAT AAT AAT AAT AAT AAT ACT AAT AAT ACT AAT AAT AAT AAT AAT AAT ACT ACT TIC TTC TIC CIC TTC TTC TTC TIC TTC TIC TIC TTC TIC TTC TIC TTC TIC TTC TIC TIC TIC CTA CTA CTA CTA ATA CTA CIA

9999 GGG TTT AAT TTC CTA ATA TAG AAG AAA TGG

9999

Matthiola

AGA TAA TAA TAA TAA TAA TAA TAA TAA GAA TAA TAA TAA GAA TAA TAA TAA TAA TAA TAA TAA TAA Aethionema_arabicum Aethionema_grandiflorum Capsella bursa-pastoris Raphanus_raphanistrum Sisymbrium_altissimum Nasturtium officinale Cardamine_concatenata Arabidopsis thaliana Sisymbrium pinnatum Hesperis matronalis Lobularia maritima Arabidopsis_lyrata Alliaria petiolata Barbarea vulgaris Brassica_oleracea Raphanus sativus Cakile_edentula Cleome spinosa Brassica nigra Arabis_hirsuta Barbarea_verna Brassica_napus Brassica_rapa Iberia

ATT ATT ATT ATT ATT ATT ATT GTT ATT ACC AGT GTT GTT GTT GTT ATT GLL GTT GTT GTT GTT GTT ATT GTT GTT GTT TTT GTT GIT GTT GTT TGG TGA TGG IGG TGG TGG TGG TGG TGG TTG TGG TGG වි TGG CGGG TGG TGG TGG TGG TGG TGG IGA TII TII TTT TTT TTT TTT TTT TTT TTT TTT TI TTT TII TII TIT LIT TII TTT TII TII AAA AAA CAA AAA AAA AAA AAA AAA AAA AAA AAA AAA GAA AAA GAA AAA AAA AAA AAA AAA AAA AAA GAA TAG TAG TAG TAA TAG TCG TAG TAG TAA TAG TAG TAG TCG TAG TAA TAA TAG TAG TAG TAG TAG TAG TAG CAG TAA TAA TAA TAA TAA TAA TAA TAA TAA TAA TAA TAA AAA TAA TAA GAA GAA TAA TAA TAA TAA TAA TAA TAA AAT AAT ACT CCT ACT AAA AAA CAA AAA AAA AAA AAA TAA AAA TAA TAA TAA TAA TAA TAA AAA AAA ACA AA TAA AAA AAA AAA AAA TAA AAA AAA TAA AAA AGA AGA CGG AGA TAA

Matthiola

1	4	വ

AAC AAT GAA TAA GAA AAG AGC TTC TTG TTT TTG TAA GAA	ATT AAC AAT GAA TAA GAA AAG AGC TTC TTT TTT TTC CAA GAA AAC	AAC AAT GAA TAA TAA GAA AGG AGC TTC TTT TTT TTC CAA NAA	AAC AAT GAA TAA TAC GAA AAG AGC TTC TTT TTT TGG CAA TAA	AAC AAT GAA TAA TAC GAA AAG AGC TTC TTT TTT TGG CAA TAA	AAT GAA TAA TAC GAA AAG AGC TTC TTT TTT TGG CAA TAA	AAT AAC GAA TAA TAC GAA AAG AGC TTC TGT TTT TGG CAA GAA	AAC AAT GAA TAC GAA ACG AGC TTC TTT TGG CAA TAA	AAT GAA TAA TAC AAA AAG AGC TTC TTT TTT CAA TAA	AAC AAT GAA TAC GAA AAG AGT TTC TTT TGG CAA GAA	AAC AAT GAA TAA TAT AAA AAG AGC TTC TTT TTG CAA GAA	AAC AAT GAA TAC GAA AAG AGC TTC TTT TTT TGG CAA TAA	AAC AAT GAA TAA TAC GAA AAG AGC TTC TTT TTT TGG CAA TAA	AAT GAA TAA TAC GAA AAG AGC TTC TTT TTT TGG CAA TAA	AAC AAT GAA TAC GAA AAG AGC TTC TTT TTT TTA CAA GAA	AAT GAA TAA TAC AAA AAG AGC TTC TTT TTT TTG CAA TAA	AAC AAT GAA TAA TAC AAA AAG ATC CTC TTT TTT TTG CAA TAA	AAC AAT GAA TAA TAC GAA AAG AGC TTC TTT TTG TAA GAA	AAC AAT GAA TAA TAC GAA AAG AGC TTC TTT TTT TGG CAA TAA	AAC AAT GAA TAA TAC GAA AAG AGC TTC TTT TTT CAA GAA	AAT GAA TAA TAC GAA AAG AGC TTC TTT TTT TGG CAA TAA	AAC AAT GAA TAC GAA AAG AGC TTC TTT TTT TGG CAA TAA	AAT GAA TAA TAC GAA AAG AGC TTC TTT TTG CAA GAA	AAC AAT GAA TAC GAA AAG AGC TTC TTT TTT TGG CAA TAA	
		_				-			•		•		•	•	•	•	•	•	•	•	_	•	•	
Cleome spinosa	bicum	mnic	Brassica_nigra	Raphanus_raphanistrum	Raphanus_sativus	ma	Ø		Arabis_hirsuta	Iberis	Cakile_edentula	Sisymbrium_altissimum_	Stanleya pinnata	Barbarea_vulgaris	ana	Capsella_bursa-pastoris 1	Nasturtium_officinale 1	Brassica_oleraceae			lata	ata	Brassica_rapa 1	

Ø 6

4 0

TGC TGC TGA TGA TGA TGA TGA TGA TGA TGA TGA TAA TGA
TGT

TGT TGT TGT TGT

TGT

TGI TGT TGT \mathbf{TGT} TGT TGT TGT TGT TGT GGT

ATA Aethionema grandiflorum Capsella bursa-pastoris Sisymbrium altissimum Raphanus_raphanistrum Nasturtium officinale Cardamine_concatenata Arabidopsis thaliana Aethionema_arabicum Hesperis_matronalis Lobularia maritima Arabidopsis lyrata Brassica oleraceae Alliaria petiolata Barbarea_vulgaris Raphanus sativus Stanleya pinnata Cakile edentula Brassica nigra Arabis_hirsuta Barbarea_verna Brassica_napus Brassica_rapa Iberis

TAC TAT TAT GAT TAT GAT TAT TAT TAT TTT TTT LLI TTT TTI TIL TTI TTT TTT TIT TTT TTT TTT TTT TTT TTI LLI TTI AAC AAC AAC AAC AAC AAC AAC AAC AAC AAC AAC AAT AAT AAC AAT AAT AAC AAC AAC AAC AAC AAC AAC AAC TCA TCA TCA TCA TCA TAA TCA TCA TGA TAA TAA TCA TCA TCA TCA TCA TCA TAA TCA TAA TCA TCA AAA AAG AAG AAG AAG AAG AAG AAG AAG AAG AAC AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT IGI TGT TGT TGT TGT TGT TGT TGT TGI TGI TGT TGT TAA TAG TAA TAA TAG TAG TAG TAG TAG AAG TAG AAT CAT TAA TCA TAA TCA TAA TAA TAA TAA TAA Cleome spinosa Matthiola

TGT TGT TGT

TGT IGI IGI IGT TGT

7	4

N & &

Cleome_spinosa	GAA	TTT	TGG	CCT	TAA	TAC	AAG	AAC		CTC	TTA	TCC	CCA	TGA	ATC	GGA
Aethionema_arabicum aethionema_grandiflorum	AAA	TTG	757	ACT	TAA	TAA	AAA	AAC	TTA	TTA	TTA			TGA	ATC	AGA
Brassica nigra	AAA	TIT	TGG	ACT	TAA	TAC	AAG	AAC		CTA	TTA	TCC	SCA	TGA	ATC	AGA
Raphanus raphanistrum	AAA	TTT	TGG	ACT	TAA	TAC	AAG	AAC		CTA	TTA	TCC	CCA	TGA	ATC	AGA
Raphanus sativus	AAA	TTT	TGG	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	TCC	CCA	TGA	ATC	AGA
Lobularia maritima	AAA	TTT	TGG	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	TCC	CCA	GGA	ATC	AGA
Hesperis matronalis	AAA	$\mathbf{T}\mathbf{T}\mathbf{G}$	TGG	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	TCC	CCA	TGA	ATC	AGA
Arabidopsis lyrata	AAA	TTT	TGG	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	TCC	CCA	TGA	ATC	AGA
Arabis hirsuta	AAA	TTT	999	ACT	TAA	TAC	ACG	AAC	۲.	CTA	TTA	TCC	CCA	TGA	ATC	AGA
Iberis_	AAA	CH	TGG	ACT	TAA	TAA	AAG	AAC	٠.	TTA	TTA	TCC	CCA	TGA	ATC	AGA
Cakile edentula	AAA	$\mathbf{T}\mathbf{T}\mathbf{T}$	TGG	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	TCC	SCA	TGA	ATC	AGA
Sisymbrium altissimum	AAA	TTT	TGG	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	TCC	CAA	TGA	ATC	AGA
Stanleya pinnata	AAA	TTT	TGG	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	TCC	CAA	TGA	ATC	AGA
Barbarea vulgaris	AAA	TTT	\mathbf{TGG}	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	\mathbf{TCC}	CCA	AGA	ATC	AGA
Arabidopsis thaliana	AAA	TTT	TGG	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	TCC	GCA	TGA	ATC	AGA
Capsella bursa-pastoris	AAA	TTT	TGG	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	TCC	CCA	TGA	ATC	AGA
Nasturtium officinale	AAA	TTT	TGG	ACT	TAA	TCC	AAG	AAC	٠.	CTA	TTA	$\mathbf{I}^{\mathbf{CC}}$	CCA	CGA	ATC	AGA
Brassica oleraceae	AAA	TTT	TGG	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	TCC	CCA	TGA	ATC	AGA
Barbarea_verna	AAA	TTT	\mathbf{TGG}	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	\mathbf{TCC}	SCA	AGA	ATC	AGA
Brassica napus	AAA	TTT	TGG	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	$\mathbf{I}^{\mathbf{C}\mathbf{C}}$	SC	TGA	ATC	AGA
Alliaria petiolata	AAA	TTT	TGG	ACT	TAA	TAC	AAG	AAC	۲.	CTA	TTA	\mathbf{ICC}	SCA	TGA	ATC	AGA
Cardamine concatenata	AAA	TII	TGG	ACT	TAA	\mathbf{I}^{CC}	AAG	AAC	٠.	CTA	TTA	TCC	SCA	TGA	ATC	AGA
Brassica rapa	AAA	TII	\mathbf{TGG}	ACT	TAA	TAC	AAG	AAC	٠.	CTA	TTA	$\mathbf{I}^{\mathbf{C}\mathbf{C}}$	CCA	TGA	ATC	AGA
Matthiola	AAA	$\mathbf{T}\mathbf{L}\mathbf{L}$	\mathbf{TGG}	ACT	TAA	TAC	AAA	AAC	TTT	CTA	TTA	ICC	CCA	TGA	ATC	AGA

	œ															m	
	თ															9	
Cleome_spinosa	CAA	TAA	TAT	TCT	ATT	TCC		GCI	TGT	ATT	GGT	TTT		TAC		GTT	
Aethionema_arabicum	CAA	TAC	TAT	TTT	ATT	TCC		GCI	\mathtt{TGT}		ACT	TTT	TTT	TAC		GTT	
Aethionema_grandiflorum	CAA	TAC	TAT	TTT	ATT	TCC		GCT	TGT	ATT	ACT	TTT	ATT	TAC		GTT	
Brassica_nigra	CAA	TAC	TAT	\mathbf{TCT}	ATT	TCC			TGT		GCT	TTT	ATT	TAC		ATT	
Raphanus_raphanistrum	CAA	TAC	TAT	TCT	ATT	TCC		GCT	TGT		GCT	TTT	ATT	TAC		GTT	
Raphanus_sativus	CAA	TAC	TAT	TCT	ATT	TCC		GCT	TGT	ATT	GCT	TTA	ATT	TAC		GTT	
Lobularia_maritima	CAA	TAC	TAT	\mathbf{TCT}	ATT	TCC		GCT	TCT		GCT	$\mathbf{T}\mathbf{L}\mathbf{L}$		TAC		GTT	
Hesperis_matronalis	CAA	TAC	TAT	GCT	ATT	TCC		GCI	TGT		GCT	$\mathbf{T}\mathbf{T}\mathbf{T}$		TAC		ATT	
Arabidopsis_lyrata	CAA	TAC	TAT	\mathbf{TCT}	ATT	TCC			TAT		GGT	$\mathbf{T}\mathbf{L}\mathbf{L}$		TAC		GTT	
Arabis_hirsuta	CAA	TAC	TAT	TCT	ATT	TCC					ACT	$\mathbf{T}\mathbf{T}\mathbf{I}$		TAC		GTT	
Iberis	CAA	TAC	TAT	TCT	ATT	TCC					GCT	TTT	ATT	TAC		GTT	
Cakile_edentula	CAA	TAC	TAT	\mathbf{TCT}	ATT	TCC					GCT	TTT		TAC		GTT	
Sisymbrium_altissimum	CAA	TAC	TAT	TCI	ATT	TCC					GCT	TTT	ATT	TAC		GTT	
Stanleya pinnata	TAA	TAC	TAT	TCI	ATT						GCT	TTT		TAC		GTT	
Barbarea_vulgaris	CAA	TAC	TAT	TCT	ATT						GCT	TTT		TAC		ATT	
Arabidopsis_thaliana	CAA	TAC	TAT	TCI	ATT			GCT		TII	GGT	TTT	ATT	TAC		GTT	
Capsella_bursa-pastoris	CAA	TAC	TAT	TCT	ATT		_				GCT	TTT		TAC		GTT	
Nasturtium_officinale	CAA	TAC	TAT	TCI	ATT		_				GCT	$\mathbf{T}\mathbf{T}\mathbf{I}$		TAC		GTT	
Brassica_oleraceae	CAA	TAC	TAT	TCT	ATT	TCC	_				GCT	TTT		TAC		GTT	
Barbarea_verna	CAA	TAC	TAT	\mathbf{ICI}	ATT	TCC	_	GCT	TGT	ATT	GCT	TTT	ATT	TAC		GTT	
Brassica_napus	CAA	TAC	TAT	\mathbf{ICI}	ATT	TCC	_		TGT		GCT	$\mathbf{T}\mathbf{T}\mathbf{I}$		TAC		GTT	
Alliaria_petiolata	CAA	TAC	TAT	TCI	ATT	TCC	_		TGT		GCT	TIT		TAC		GTT	
Cardamine_concatenata	CAA	TAC	TAT	TCI	ATT	TCC	TAT	GCT	TGT		GCT	TTT	•	TAC	TTT	GTT	
Brassica_rapa	CAA	TAC	TAT	TCT	ATT	TCC	_	GCT	TGT	ATT	GCT	TTT	ATT	TAC	TTT	GTT	
Matthiola	CAA	TAC	TAT	GCT	CTT	TCC	_	GCT	TGT	ATT	GCT	TTT	ATT	TAC	TTT	GTT	

K & 4	11GA 11GA 11GA 11GA 11GA 11GA 11GA 11GA	IGA
	STATE COLD COLD COLD COLD COLD COLD COLD COLD	CLL
	HER AGA AGA AGA AGA AGA AGA AGA AGA AGA AG	AGA
	AAT AAT AAT AAT AAT AAT AAT AAT AAT AAT	
	AGG AGG AGG AGG AGG AGG AGG AGG AGG AGG	
	AAAA AGA AGA AGA AGA AGA AGA AGA AGA AG	AGA
	555555555555555555555555555555555555555	TCA
	C C C C C C C C C C C C C C C C C C C	
	1717 1717 1717 1717 1717 1717 1717 171	CTT
		TCC
	AATT AAAT AAAT AAAT AAAT AAAT AAAT AAA	AAT
	AGG AGG AGG AGG AGG AGG AGG AGG AGG AGG	AGG
	CAT CAT CAT CAT CAT CAT CAT CAT CAT CAT	CAT
	AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC	AGC
	1166 1166 1166 1166 1166 1166 1166 116	TGG
7 3 3	TGT TAT TAT TAT TAT TAT TAT TAT TAT TAT	_
	Cleome_spinosa Aethionema_arabicum Aethionema_grandiflorum Brassica_nigra Raphanus_raphanistrum Raphanus_sativus Lobularia_maritima Hesperis_matronalis Arabidopsis_lyrata Arabidopsis_lyrata Arabidopsis_lyrata Barbarea_vulgaris Arabidopsis_thaliana Capsella_bursa-pastoris Barbarea_verna Brassica_oleraceae Barbarea_verna Brassica_napus Alliaria_petiolata Brassica_raba	•

4 E 2

TAA

TIT

GAT

AAA AAA

	7	7	נז	נז	۲)	(ع	7)	ر ع	7)	נז	7)	7)	7	7)	E)	۲)	E)	7)	r)	E)	7)	2)	7)	71	7)
	BCC	ညည	ggg	ညည	ညည	ပ္သည္ဟ	CC	ggg	gcc	gcc	ACC	gcc	ညည	ညည	ggg	GCC	gcc	ACC	gcc	ညည	ggg	gcc	ACC	gcc	ggg
	GAC	CAC	CAC	AAC	AAC	AAC	CAT	CAC	CAC	CAC	CAC	AAC	AAC	AAC	CAC	CAC	CAC	CAC	AAC	CAC CAC	AAC	CAC	CAC	AAC	CAC
	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	$\mathbf{T}\mathbf{L}$	ATT	ATT	AGT	ATT	ATT	ATT	ATT	ATT	ATT
	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC	ATC
	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT	ATT
വയവ	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT	TAT
	Cleome_spinosa	Aethionema_arabicum	Aethionema_grandiflorum	Brassica_nigra	Raphanus_raphanistrum	Raphanus_sativus	Lobularia maritima	Hesperis matronalis	Arabidopsis_lyrata	Arabis_hirsuta	Iberis	Cakile_edentula	Sisymbrium_altissimum	Stanleya pinnata	Barbarea_vulgaris	Arabidopsis_thaliana	Capsella_bursa-pastoris	Nasturtium_officinale	Brassica_oleraceae	Barbarea_verna	Brassica_napus	Alliaria_petiolata	Cardamine_concatenata	Brassica_rapa	Matthiola

TTT

AAA AAA AAA

CCT

AAA AAA AAA TAA TAA

475 475 475

TTT TTT TTT TTT

TAA TAA

TTT

AAA

TIT

AAA AAA AAA

GAT GAT GAT GAT

TAC

TAC

TTT

LTT

TAC TAC TAA TAA TAA TAA TAA TAC TAC

TTT TTT TTT

AAT
AAT
GAT
GAT
GAT
GAT
GAT
GAT
AAT
AAT

TTT

AAA AAA AAA AAA

> GAT GAT AAT

TTT

TTT

4 & 0

	4
	٣
	m
Cleome spinosa	TT
Aethionema arabicum	Ţ
Aethionema_grandiflorum	H
Brassica_nigra	TI
Raphanus_raphanistrum	TT
Raphanus_sativus	H
Lobularia_maritima	ŢĞ
Hesperis_matronalis	H
Arabidopsis_lyrata	H
Arabis_hirsuta	H
Iberis	Ē
Cakile_edentula	Ē
Sisymbrium_altissimum	IJ
Stanleya pinnata	H
Barbarea_vulgaris	TI
Arabidopsis_thaliana	TI
Capsella_bursa-pastoris	II
Nasturtium_officinale	H
Brassica_oleraceae	H
Barbarea_verna	H
	H
Alliaria_petiolata	H
Cardamine_concatenata	Ħ
Brassica rapa	H

AAC AAT AAT AAT AAC AAC AAT AAT AAC AAC AAC AAC AAC AAT AAC AAC AAC TGC TGC TGC TGC TGT TGC TGC TGC TGC $\mathbf{I}\mathbf{G}\mathbf{C}$ TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC AAA GAG TAA GAA GAG GAG TAG TAG TAG GCA GAA GAA GAG GCG GAA GAG GAA AAA GAG GAA GAA TAA TTT TTT TTT TLL TTT TTT TTT TTTTTT TTT TTT TTTTTT TTT TTT TTT TTTTTT TTT TTT ATT TGA GTA TTG TTG GTG TTG TTG TTG TTG TTG TTG $\mathbf{T}\mathbf{T}\mathbf{G}$ TTG TTG TTG TTG TTG TTG TTG TTG TTG AGA GGA AGA AGA GGA AGA TGT IGT TGT TTT TTT TTT TTT TTT TTT TTT TTT TTTTTT TTTTII TTT TTT TTT TTT TIL TTT AAA AAA AAT AAA AAA AAA AAA AAA AAA AAG AAA AAG AAA AAG AAA AAA AAG AAA AAA AAA AAA AAA ACA CCA AGA AGA GCA AGA ACA ACA ACA ACA ACA AGA GCA ACA ACA AGA ACA ACA AGA GCA biassica_iapa Matthiola

4 8 -

8 2 2

KTW GTT TTT TTT

CTG TTK CTG CTG

ATA ATA ATA ATA

TAT TAT

AAT AAT AAT AAT

AGC AGC AGC AGC AGC

ATT ATT ATT ATT ATT

TTTTTTTTT

TTT

AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC

TAT

TAT TAT TAT TAT

ATT ATT ATT ATT

AAT AAT AAT AAT AAT AAT AAT AAT AAT AAT

CTG CTG CTG CTG CTG

ATA ATA ATA ATA ATA ATA ATA

TAT

		Н								
	Cleome_spinosa	TTC	AGT	CAG	TAT	AGC	TTT	TTT	\mathbf{TGG}	•
	Aethionema_arabicum	$\mathbf{T}\mathbf{T}\mathbf{C}$	AGT	CAG	TAT	AGC	TTT	TTT	\mathbf{TGG}	•
	Aethionema_grandiflorum	TIC	AGT	CAG	TAT	AGC	TTT	TTT	\mathbf{TGG}	•
	Brassica_nigra	TTC	AGT	CAG	TAT	AGC	TTT	GTT	\mathbf{TGG}	•
	Raphanus_raphanistrum	TTC	AGT	CAG	TAT	AGC	TTT	GTT	\mathbf{TGG}	•
	Raphanus_sativus	$\mathbf{T}\mathbf{T}\mathbf{C}$	AGT	CAG	TAT	AGC	TTT	GTT	$\overline{1}$	•
	Lobularia_maritima	$\mathbf{T}\mathbf{T}\mathbf{C}$	AGT	CAG	TAT	AGC	TTT	GTT	TGG	•
	Hesperis_matronalis	TTC	AGT	CAG	TAT	AGC	TTT	TTT	${ m TGG}$	•
	Arabidopsis_lyrata	TTC	AGT	GAG	TAT	AGC	TTT	TTT	${ m TGG}$	•
	Arabis hirsuta	$\mathbf{T}\mathbf{T}\mathbf{C}$	AGT	CAG	TAT	AGC	TTT	GTT	\mathbf{TGG}	•
	Iberis	TTC	AGT	CAG	TAT	AGC	TTT	GTT	TGG	
•	Cakile_edentula	TTC	AGT	CAG	TAT	AGC	TTT	GTT	TGG	•
	Sisymbrium_altissimum	TIC	AGT	CAG	TAT	AGC	TTT	GTT	\mathbf{TGG}	
	Stanleya pinnata	TTC	AGT	CAG	TAT	AGC	$\mathbf{T}\mathbf{L}\mathbf{L}$	GTT	\mathbf{TGG}	
	Barbarea_vulgaris	TTC	AGT	CAG	TAT	AGC	TTT	$\mathbf{T}\mathbf{T}\mathbf{I}$	\mathbf{TGG}	
	Arabidopsis_thaliana	TIC	GGT	CAG	TAT	AGC	CTT	TTT	TGG	
	Capsella_bursa-pastoris	$\mathbf{T}\mathbf{T}\mathbf{C}$	AGT	CAG	TAT	AGC	TTT	TTT	\mathbf{TGG}	•
	Nasturtium_officinale	TTC	AGT	TAG	TAT	AGC	TTT	TTT	\mathbf{TGG}	
	Brassica_oleraceae	$\mathbf{T}\mathbf{T}\mathbf{C}$	AGT	CAG	TAT	AGC	TTT	GTT	\mathbf{TGG}	•
	Barbarea_verna	TTC	AGT	CAG	TAT	AGC	TTT	TTT	TGG	
	Brassica_napus	TTC	AGT	CAG	TAT	AGC	$\mathbf{T}\mathbf{L}$	GTT	TGG	
	Alliaria_petiolata	TTC	AGT	CAG	TAT	AGC	TTT	GTT	TGG	
	Cardamine_concatenata	$\mathbf{T}\mathbf{T}\mathbf{C}$	AGT	CAG	TAT	AGC	TTT	$\mathbf{T}\mathbf{L}$	\mathbf{TGG}	
	Brassica_rapa	$\mathbf{T}\mathbf{T}\mathbf{C}$	AGT	CAG	TAT	AGC	TTT	GLT	\mathbf{TGG}	
	Matthiola	TTC	AGT	CAG	TAT	AGC	TTT	$\mathbf{T}\mathbf{T}\mathbf{I}$	\mathbf{TGG}	. •

TTT TTT TTTTTT

CTG CTG CTG CTG CTG CTG

TAT

ATA ATA

TAT TAT

ATT ATT

TTTTTT TTT

TAT TAT TAT

ATT

ATA ATA ATA

ATT

ATT

TTT TTT TTT

ATA

ATA

TAT

CTG

AGC AGC AGC AGC AGC AGC

ATA

TAT TAT

ATT ATT ATT ATT ATT ATT

AAT AAT AAT AAT AAT AAT

TTT

ATA

TAT

CTG CTG CTG

TTT

TAT

TAT

TTT

TTT

ATA ATA

TAT

9 2 2

5 7

	١										
Cleome spinosa	TAA	သည	$\mathbf{T}\mathbf{T}\mathbf{T}$	TTA	TTC	ATC	TTT	ATT	AAA	TTT	
Aethionema arabicum	TAA	೮೮೮	TTT	TAA	TTC	ATC	TTT	ATT	AAA	TTT	
Aethionema_grandiflorum	TAA	သည	TTT	TTA	TIC	ATC	TTT	ATT	AAA	TIL	
Brassica_nigra	TAA	gcc	TLL	TTA	TTC	ATC	TAA	ATT	AAA	TTT	
Raphanus_raphanistrum	TAA	ညည	TTT	TTA	TTC	ATC	TAA	ATT	AAA	TTT	
Raphanus_sativus	TAA	ggg	TTT	TTA	TTC	ATC	TAA	ATT	AAA	TTT	
Lobularia_maritima	TAA	ညည	TTT	TTA	TTC	ATC	TTT	ATT	AAA	TTT	
Hesperis_matronalis	TAA	ညည	LLL	TTA	TTC	ATC	TTT	ATT	AAA	TII	
Arabidopsis_lyrata	TAA	ggg	TTT	TTA	TTC	ATC	TTT	ATT	AAA	TTT	
Arabis_hirsuta	TAA	ggg	TTT	TTA	TIC	ATC	TTT	ATT	AAA	TTT	
Iberis	TAA	ညည	TTT	TTA	TTC	ATC	GTT	ATT	AAA	TTT	
Cakile_edentula	TAA	ပ္သင္ဟ	TII	TTA	TTC	ATC	TAA	ATT	AAA	TTT	
 Sisymbrium_altissimum	TAA	ACC	TIL	TTA	TIC	ATC	TAA	ATT	AAA	TTT	
Stanleya pinnata	TAA	ggg	TTT	TTA	TIC	ATC	TAA	ATT	AAA		_
Barbarea_vulgaris	TAA	ညည		TTA	TTC	ATC	TTT	ATT	AAA		
Arabidopsis_thaliana	TAA	ညည		TTA	TTC	ATC	TTT	ATT	AAA		
Capsella_bursa-pastoris	TAA	ညည		TTA	TTC	ATC	TTT	ATT	AAA	TTT	
Nasturtium_officinale	TAA	ပ္သည္ဟ		TTA	TTC	ATC	TTT	ATT	AAA	TTT	
Brassica_oleraceae	TAA	ညည	$\mathbf{T}\mathbf{L}$	TTA	TIC	ATC	TAG	ATT	AAA	TTT	_
Barbarea_verna	TAA	ညည	TTT	TTA	TTC	ATC	TTT	ATT	AAA	TTT	
Brassica_napus	TAA	ದ್ದಿದ್ದ	TII	TTA	TTC	ATC	TAG	ATT	AAA	TTT	
Alliaria_petiolata	TAA	ညည	$\mathbf{T}\mathbf{L}$	TTA	TTC	ATC	\mathbf{LLL}	ATT	AAA	TTT	
Cardamine_concatenata	TAA	ပ္ပင္သ	\mathbf{I}	TTA	TTC	ATC	TTT	ATT	AAA	TTT	
Brassica_rapa	TAA	ညည	$\mathbf{T}\mathbf{T}\mathbf{I}$	TTA	TTC	ATC	TAG	ATT	AAA	TTT	
Matthiola	TAA	ပ္ပင္ပ	$\mathbf{T}\mathbf{L}$	TTA	TIC	ATC	TTT	ATT	AAA	TIT	

ACT

ACT

111 C 111 C

ACT

GAA

ACT ACT ACT ACT

TAA TAA TAA TAA

TAA

ACT ACT ACT

ACT

AAC AAC AAC AAC AAC AAC AAC CAC AAC AAC AAC AAC CAC AAC AAC AAC AAC

ACT

AAC

CTT CTT CGT CTT CTTCTT CTT CTT CTT CTT CTT CTT CTT CTT TTT CIT TTTCTT

TAA GAA TAA TAA

AGT ACT ACT ACT

CTT

AAC AAC AAC TAA TAA TAA GAA TAA

ACT

ACT

ACT

ACT

9 7 4

TTT

AAT

AAT

AAT

AAT

ACT ACC ACT ACT ACT ACT ACT ACT ACT ACT ACC ACT ACT ACC ACT ACC ACT ACT ACT ACT ACC AAG AAA GGA GTG GTG TTG TIG TTG GTG GTG GTG GTG GTG TTG GTG GTG GTG TTG GTG TTG GTG GTG TTG GTG TAG TAG TCG TGG TAG TCG TCG TAG TTG TAG TCG TCG TAG TCA TCG TCG TAG TAG TCG TAG TAG TAG AAT AAT AAT AAT AAT AAT AAT AAT AAT AAT AAT AAT AAT AAT AAT AAT ACT AAT AAT AAT AAT AAG AAG AAG AAG ACG ACG AAG AAG ACC AAG AAG AAG AAG AAG CCG AAG ACG ACG ACG AAG ACG AAG AAG CCC TAA TTC TTC TTC TIC TIC TIC TIC TTC TTA TIC TTC TTC TIC TIC TIC TTC TIC TTC TTC TIC $\mathbf{T}\mathbf{T}\mathbf{C}$ TIC GAG GAG GAG GAG GAG GAG GAG GAA GAG GAG GAA GAG GAG GAG GAA GAA GAA GAA GAA GAA GAG GTG ATG GTG ATG ATG GTG ATG ATG GTG GTG ATG ATG ATT GTG ATG GTG ATG GTG CTG CTG ATT AAA AAA AAA AAA AAA AAA AAA AAA AAC AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA TCA TCA TCA TCA TCA TCA TCA TAA TCA TGA TCA IG P Aethionema grandiflorum Capsella bursa-pastoris Sisymbrium altissimum Raphanus_raphanistrum Nasturtium officinale Cardamine_concatenata Arabidopsis_thaliana Aethionema arabicum Hesperis_matronalis Lobularia maritima Arabidopsis lyrata Brassica_oleraceae Alliaria petiolata Barbarea_vulgaris Raphanus sativus Stanleya pinnata Cakile edentula Cleome spinosa Brassica nigra Barbarea_verna Brassica_napus Arabis_hirsuta Brassica rapa Matthiola Iberis

TTT TAT TTT TTT

AAT

AAT AAT AAT

AAT

AAT

TTT
TTT
TTT
TTT
TTT

AAT

AAA AAA AAA

AAT

AAT

WAA

AAT

TAT

AAT

CAA

AAT

AAT

AAT

2 7 6

27 0

J
TCT ATA
-
-
•
IGT
ľGT
ľĠŢ
ГGТ
-
-
•
IGT.
ľGT
IGT

TAA AAA AAAA AAA AAAA TAAA TAAA TAAA AAAA TAAA TAAA TAAA TAAA

117.4
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7
117.7

3 7

0 2

TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTTTTT TTT TTT TTT

ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATA ATC ATC ATC ATA ATC ATC AAA AAC AAA AAA AAC AAA AAN AAC AAC AAC AAC AAA AAA AAA AAA AAC AAA AAC AAC AAC AAC AAC AAA Aethionema grandiflorum Capsella bursa-pastoris Sisymbrium altissimum Raphanus raphanistrum Nasturtium officinale Cardamine_concatenata Arabidopsis thaliana Aethionema arabicum Hesperis matronalis Alliaria petiolata Lobularia maritima Arabidopsis lyrata Brassica_oleraceae Barbarea vulgaris Raphanus sativus Stanleya pinnata Cakile_edentula Brassica nigra Cleome_spinosa Arabis hirsuta Brassica_napus Barbarea_verna Iberis

GAC TCA TAA GAA GAA GAA AAA TAA GAC GAA AAA GAA AAA AAA GAA GAA TAA GAA GAA GAA GAA GCA GAA TAA AAC AAC AAC AAC AAC AAC AAC AAC AAC AAC AAC AAC AAC AAC AAT AAT AAC AAC AAC AAC AAC AAC AAC AAC ACA ACA ACA ACA ACA ACA ACA ACA ACA ACA CA ACT ACA ACA ACA ACA ACA ACA ACA ACT ACA ACA ACA ACA ACA AAA AGC AGC AGC AGC AGC AGC AGC AGC AGC AGG AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC ATT ATT ATT ATT ATT ATT ATT ATT ATT ATT ATT ATT ATT ATT ATT ATT GTT ATT ATT ATT ATT ATT ATT ATT AAG AAG AAG AAG AAA AAA AAG AAG AAG AAG AAG AAA AAC AGG AAA AAA AAA AGG AAG AAG AAA AAC AAA AAA AAG AAG AAG AAG AAG AAT AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG NAG AAG AAG TAT AAG AAG AAA AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG AAA AAA AAG AAG AAG AAG TGA AAC AAC AAT AAT AAT AAT AAC AAT AAC AAC AAT AAC AAC AAC AAC AAC AAT AAC AAC AAT AAC AAC AAC AAC AAC TIT CTT TIT LLL TTT TTT LLL TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT GTT TTT CLL TII TII TIL ATC ATC AAC AAA Brassica_rapa

TTT

Matthiola

TTT TTT

7 7 7

Cleome_spinosa	TTT	TGA	_	ACG	AAT	AAT	TGA	TGG	AAT	TAC	AAA	CGC	AGT	AGG	L	1
Aethionema_arabicum	TTT	NGA	-	ACG	AAT	AAT	TGA	TGG	AAT	TAC	AAA	\mathbf{TGG}	AGT	AGG	TAT	TAC
Aethionema_grandiflorum	$\mathbf{T}\mathbf{T}\mathbf{I}$	TGA	-	ACG	AAT	AAT	TGA	$\overline{1}GG$	AAT	TAC	AAA	\mathbf{TGG}	AGT	AGG	TAT	TAC
Brassica_nigra	$\mathbf{T}\mathbf{T}\mathbf{T}$	TGA	TAA	ACG	AAT	CAT	TGA	$\overline{1}GG$	AAT	TAC	AAA	$\overline{1}$ GG	AGT	AG-	 	1
Raphanus_raphanistrum	TTT	TGA	-	ACG	AAT	CAT	TGA	\mathbf{TGG}	AAT	TAC	AAA	\mathbf{TGG}	AGT	1	! !	: :
Raphanus_sativus	TTT	TGA	_	ACG	AAT	CAT	TGA	\mathbf{TGG}	AAT	TAC	AAA	$^{\mathrm{TGG}}$	AGT	A	 	1
Lobularia_maritima	TTT	TGA	-	ACG	AAT	CAT	TGA	${ m TGG}$	AAT	TAC	AAA	\mathbf{TGG}	AGT	A	1	1
Hesperis_matronalis	TTT	TGA	-	ACG	AAT	\mathbf{TGT}	TGA	\mathbf{TGG}	AAT	TAC	AAA	TG-	1	1	1 1	1
Arabidopsis_lyrata	TTT	TGA	-	ACA	AAT	CAT	TGA	\mathbf{TGG}	AAT	TAC	AAA	\mathbf{TGG}	AGT	AGG	 L	1
Arabis_hirsuta	TTT	TGA	-	AAA	AAT	CAT	TGA	\mathbf{TGG}	AAT	TAC	AAA	TG-	!	1	1	1
Iberis	TTT	TGA	-	ACG	AAT	CAT	TGA	\mathbf{TGG}	AAT	TAC	AAA	\mathbf{TGG}	AGT	AGG	TAT	TAC
Cakile_edentula	TTT	TGA	-	ACG	AAT	CAT	TGA	\mathbf{TGG}	AAT	TAC	AAA	TG-	1 1	1	1	1 1
Sisymbrium_altissimum	$\mathbf{T}\mathbf{T}\mathbf{I}$	TGA	-	ACG	AAT	CAT	TGA	\mathbf{TGG}	AAT	TAC	AAA	\mathbf{TGG}	AGT	AGG	TAT	TAC
Stanleya pinnata	TTT	TGA	•	ACG	AAT	CAT	TGA	\mathbf{TGG}	AAT	TAC	AAA	\mathbf{TGG}	AGT	AGG	TAT	TAC
Barbarea_vulgaris	TTT	TGA	•	ACG	AAT	CAT	TGA	$\overline{1}GG$	AAT	TAC	AAA	\mathbf{TGG}	GGT	AGG	TAT	TAC
Arabidopsis_thaliana	TTT	TGA	•	ACG	AAT	CAT	TGA	${\tt TGG}$	AAT	TAC	AAA	\mathbf{TGG}	AGT	AGG	TAT	TAC
Capsella_bursa-pastoris	TGT	TGA	•	ACG	AAT	CAT	TGA	TGG	AAT	TAC	AAA	\mathbf{TGG}	AGT	AGG	TAT	TAC
Nasturtium_officinale	TTT	TGA	•	ACG	AAT	CAT	TGA	$\overline{166}$	AAT	TAC	AAA	\mathbf{TGG}	GGT	AGG	TAT	TAC
Brassica_oleraceae	TLT	TGA	•	ACG	AAT	CAT	TGA	$\overline{1}$ GG	AAT	TAC	AAA	\mathbf{TGG}	AGT	AGG	TAT	TAC
Barbarea_verna	TTT	TGA	•	ACG	AAT	CAT	TGA	\mathbf{TGG}	AAT	TAC	AAA	\mathbf{TGG}	GGT	AGG	TAT	TAC
Brassica_napus	$\mathbf{T}\mathbf{T}\mathbf{T}$	TGA	-	ACG	AAT	CAT	TGA	TGG	AAT	TAC	AAA	$\mathbf{T}GG$	AGT	AGG	TAT	1
Alliaria_petiolata	\mathbf{TTT}	TGA	•	ACG	AAT	CAT	TGA	TGG	AAT	TAC	AAA	TGG	AGT	AGG	TAT	TAC
Cardamine_concatenata	TTT	NGA	•	ACG	AAT	CAT	TGA	TGG	AAT	TAC	NAA	TING	!!!	1 1	1	1
Brassica_rapa	TTT	TGA	•	ACG	AAT	CAT	TGA	TGG	AAT	TAC	AAA	\mathbf{TGG}	AGT	AG-	1 1	1
Matthiola	$\mathbf{T}\mathbf{T}\mathbf{I}$	TGA	•	ACG	AAT	CAT	TGA	\mathbf{TGG}	AAT	TAC	AAA	\mathbf{TGG}	AGT	AGG	TAT	TAC

	7 7	6 7			AAG	AA-	1	! !	!	1	1	1 1	1 1	AAG	!!	AAG	AAG	AAG	AAG	AAG	AAG	AAG	CAG	1	CAG	! ! !	1	AAG
Table 20 (cont.d)				Cleome_spinosa	Aethionema_arabicum	Aethionema_grandiflorum	Brassica_nigra	Raphanus_raphanistrum	Raphanus_sativus	Lobularia maritima	Hesperis matronalis	Arabidopsis_lyrata	Arabis_hirsuta	Iberis_	Cakile_edentula	Sisymbrium_altissimum	Stanleya pinnata	Barbarea_vulgaris	Arabidopsis_thaliana	Capsella_bursa-pastoris	Nasturtium officinale	Brassica oleraceae	Barbarea_verna	Brassica napus	Alliaria_petiolata	Cardamine_concatenata	Brassica rapa	Matthiola -

APPENDIX I

Pollen data for *Cantua* (Polemoniaceae): Light microscopy pollen diameter and style length analysis

Table 21: Pollen grain diameter and style length mean, sample size (N), standard deviation, and range for Cantua herbarium specimens used in light microscopy pollen analysis.

Species	Voucher		Pollen (µm)			Styles (mm)	
		Mean (N)	SD	range	Mean (N)	SD	range
Cantua bicolor Lem.	Cardenas 5479 (HUH)	61.76 (29)	5.25	51.45-73.50	36.12 (4)	0.73	35.21-36.93
Cantua bicolor	Beck, Kiesling & Metzing 22154 (MSC)	72.85 (30)	3.75	63.70-78.40	42.36 (3)	0.30	42.12-42.69
Cantua buxifolia Lam.	Gentry, Smith & Tredwell 37515 (MO)	74.32 (15)	3.16	68.60-80.85	73.89 (6)	5.31	67.56-80.30
Cantua buxifolia	Lopez & Sagastegui 8023 (LL)	72.48 (24)	4.21	61.25-78.40	60.24(5)	16.02	35.02-73.85
Cantua buxifolia	Plowman & Davis 4653 (HUH)	72.68 (30)	5.51	51.45-78.40	72.10 (8)	5.65	63.08-77.53
Cantua buxifolia	Bohs 2053A(HUH)	77.75 (30)	4.12	71.05-85.75	69.67 (3)	3.49	65.65-71.97
Cantua candelilla Brand	Hutchison 1252 (UC)	81.34 (30)	3.72	73.50-90.65	65.01 (8)	7.12	56.86-73.95
Cantua candelilla	Hutchison & Wright 7235 (UC)	82.57 (30)	3.16	73.50-85.75	66.36 (4)	2.79	62.92-68.72
Cantua cuzcoensis Infantes	Vargas 9785 (MO)	75.87 (30)	3.55	63.70-80.85	55.40 (5)	1.68	53.54-57.96
Cantua cuzcoensis	Balls B6903 (UC)	69.73 (30)	3.85	63.70-78.40	51.68 (6)	2.96	48.50-55.88
Cantua flexuosa Pers.	Gentry, Smith & Tredwell 37518 (NY)	77.01 (30)	3.00	71.05-83.30	30.92(1)	One style	One style
Cantua flexuosa	Beck 1349 (MSC)	(01) 99:59	4.87	58.80-73.50	37.28 (5)	2.04	35.16-39.67
Cantua n. sp.	Knapp & Alcom 7556 (NY)	75.34 (4)	5.43	68.60-80.85	42.78 (6)	2.93	38.27-45.95
Cantua n. sp.	Leiva, Chuna & Cadle 1419 (F)	76.47 (19)	3.33	71.05-80.85	No style	No style	No style
Cantua pyrifolia Juss.	Hitchcock 21580 (HUH)	75.46 (30)	3.61	63.70-80.85	26.03 (2)	1.51	24.96-27.10
Cantua pyrifolia	Sagástegui & Cabanillas 8560 (MO)	72.96 (9)	6.46	66.15-83.30	27.51 (1)	One style	One style
Cantua pyrifolia	Van der Werff & Palacios 8891 (NY)	80.69 (30)	4.02	73.50-85.75	36.71 (6)	3.45	33.07-41.14
Cantua pyrifolia	Woytkowski 7670 (HUH)	82.08 (30)	2.86	75.95-85.75	41.68 (4)	5.90	33.17-46.77
Cantua pyrifolia	Camp 4280 (NY)	74.97 (6)	2.19	73.50-78.40	No style	No style	No style
Cantua quercifolia	Wrigley 2 (MSC)	87.22 (30)	2.92	80.85-93.10	87.22 (1)	One style	One style
Cantua quercifolia	Hutchison & Wright 3482 (HUH)	84.65 (20)	3.13	78.40-90.65	45.00 (5)	4.61	39.87-52.13
Cantua volcanica (Brand) J. M. Porter & Prather	West 7139 (MO)	62.64 (30)	2.55	58.80-68.60	20.08 (3)	4.17	16.42-24.62
Cantua volcanica	Varoas 19918 (US)	62.26 (29)	2.58	53.90-66.15	22.23 (4)	1.35	20.79-23 48

LITERATURE CITED

LITERATURE CITED

Ackermann, R.R., and J.M. Cheverud. 2000. Phenotypic covariance structure in Tamarins (Genus *Saguinus*): A comparison of variation patterns using matrix correlation and common principal component analysis. 111:489-501.

Adams, R.J., and J.K. Morton. 1972. An improved technique for examining pollen under the scanning electron microscope. Pollen et Spores 14:203-212.

Aguilar, R., G. Bernadello, and L. Galetto. 2002. Pollen-pistil relationships and pollen size-number trade-off in species of the tribe Lycieae (Solanaceae). J. Plant Res. 115:335-340.

Albert, V.A., M.H.G. Gustaffson, and L. Dilaurenzio. 1998. Ontogenetic systematics, molecular developmental genetics, and the angiosperm petal. Pp. 349-374 in Molecular systematics of plants II: DNA sequencing, Soltis, D.E., P.S. Soltis, and J.J. Doyle (eds.). Norwell, Massachusetts.

Al-Shehbaz, I.A. 1984. The tribes of the cruciferae (Brassicaceae) in the southeastern United States. Journal of the Arnold Arboretum 65:85-111.

Ambrosini, M., L.R. Ceci, S. Fiorella, and R. Gallerani. 1992. Comparison of regions coding for tRNA(His) genes of mitochondrial and chloroplast DNA in sunflower: a proposal concerning the classification of "CP-like" tRNA genes. Plant Mol. Biol. 20(1):1-4.

Angiosperm Phylogeny Group. 1998. An ordinal classification for the families of flowering plants. Annals of the Missouri Botanical Garden 85:531-553.

Angiosperm Phylogeny Group. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society 141:399-436.

Armbruster, W.S. 1993. Evolution of plant pollination systems: hypotheses and tests with the neotropical vine *Dalechampia*. Evolution 47:1480-1505.

Arnold, S.J. 1992. Constraints on phenotypic evolution. Am. Nat. 140:S85-S107.

Arnold, S.J., M.E. Pfrender, and A.G. Jones. 2001. The adaptive landscape as a conceptual bridge between micro- and macroevolution. Genetica 112-113:9-32.

Arnold, S.J., and P.C. Phillips. 1999. Hierarchical comparison of genetic variance-covariance matrices. II. Coastal-inland divergence in the Garter Snake, *Thamnophis elegans*. Evolution 53:1516-1527.

Badyaev, A.V., and K.R. Foresman. 2000. Extreme environmental change and evolution: stress-induced morphological variation is strongly concordant with patterns of evolutionary divergence in shrew mandibles. Proc. R. Soc. Lond. B 267:371-379.

Badyaev, A.V., and G.E. Hill. 2000. The evolution of sexual dimorphism in the House finch. I. Population divergence in morphological covariance structure. Evolution 54:1784-1794.

Baker, H.G., and I. Baker. 1979. Starch in angiosperm pollen grains and its evolutionary significance. American Journal of Botany. 66:591-600.

Baker, R.H., and G.S. Wilkinson. 2003. Phylogenetic analysis of correlation structure in stalk-eyed flies (*Diasemopsis*, Diopsidae). 57:87-103.

Beardsley, P.M., and R.G. Olmstead. 2002. Redefining Phrymaceae: the placement of *Mimulus*, tribe Mimuleae, and *Phryma*. American Journal of Botany 87(7):1093-1102.

Bruneau, A. 1997. Evolution and homology of bird pollination syndromes in *Erythrina* (Leguminosae). American Journal of Botany 84:54-71.

Camara, M.D., and M. Pigliucci. 1999. Mutational contributions to genetic variance-covariance matrices: an experimental approach using induced mutations in *Arabidopsis thaliana*. Evolution 53:1692-1703.

Cheverud, J.M. 1988. A comparison of genetic and phenotypic correlations. Evolution 42:958-968.

Cheverud, J.M. 1996. Quantitative genetic analysis of cranial morphology in cotton-top (Saguinus oedipus) and saddle-back (S. fuscicollis) tammarins. J. Evol. Biol. 9:5-42.

Conner, J.K. 1997. Floral evolution in wild radish: the roles of pollinators, natural selection, and genetic correlations among traits. International Journal of Plant Science 158:S108-S120.

Conner, J.K., R. Franks, and C. Stewart. 2003. Expression of additive genetic variances and covariances for wild radish floral traits: comparison between field and greenhouse environments. Evolution 57:487-495.

Conner, J.K., and A. Sterling, 1995. Testing hypothesis of functional relationships: a comparative survey of correlation patterns among floral and vegetative traits in five insect-pollinated plants. American Journal of Botany 82:1399-1406.

Conner, J.K., and A. Sterling, 1996. Selection for independence of floral and vegetative traits: evidence from correlation patterns in five species. Canadian Journal of Botany 74: 642-644.

Conner, J.K., and S. Via. 1993. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. Evolution 47:704-711.

Cruden, R.W., and D.L. Lyon. 1985. Correlations among stigma depth, style length, and pollen grain size. Do they reflect function or phylogeny? Botanical Gazette 146:143-149.

Cruzan, M.B. 1990. Variation in pollen size, fertilization ability, and post fertilization siring ability in *Erythronium grandiflorum*. Evolution 44:843-856.

Darwin, C. 1877. The various contrivances by which orchid are fertilized by insects, 2nd ed. John Murray, London (1st ed.1863).

Darwin, C. 1896. The different forms of flowers on plants of the same species. Appleton, New York.

Day, A.G., and R. Moran. 1986 *Acanthogilia*, a new genus of Polemoniaceae from Baja California, Mexico. Proceedings of the California Academy of Science 44:111-126.

De-Yaun, H. 1983. The distribution of Scrophulariaceae in the holoarctic with special reference to the floristic relationships between Eastern Asia and Eastern North America. Ann. Missouri Bot. Gard. 70:701-712.

Dodd, R.S., Z.A. Rafii, and A. Bousquet-Mélou. 2000. Evolutionary divergence in the pan-Atlantic mangrove *Avicennia germinans*. New. Phytol. 145:115-125.

Elisens, W.J. 1986. Pollen morphology and systematic relationships among new world species in the tribe Antirrhineae (Scrophulariaceae). American Journal of Botany 73: 1298-1311.

Faegri, K. and L. van der Pijl. 1966. The principles of pollination ecology, 1st ed. Pergamon Press, Oxford.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

Flury, B. 1988. Common principal components and related multivariate models. Wiley, New York.

Fujii, N., K. Ueda, Y. Watano, and T. Shimizu. 1995. Intraspecific sequence variation in chloroplast DNA of *Primula cunaifolia* Ledeb. (Primulaceae). J. Phytogeogr. & Taxon. 43:15-24.

Fujii, N., K. Ueda, Y. Watano, and T. Shimizu. 1997. Intraspecific sequence variation of chloroplast DNA in *Pedicularis chamissonis* Steven (Schrophulariaceae) and geographic structuring of the Japanese "Alpine" Plants. J. Plant Res. 110:195-207.

Futuyma, D.J., M.C. Keese, and D.J. Funk. 1995. Genetic constraints on macroevolution: The evolution of host affiliation in the leaf beetle genus *Ophraella*. Evolution 49:797-809.

Galloway, G.L., R.L. Malmberg, and R.A. Price. 1998. Phylogenetic utility of the nuclear gene argenine decarboxylase: An example from Brassicaceae. Mol. Biol. Evol. 15:1312-1320.

Grant, K.A. 1966. A hypothesis concerning the prevalence of red coloration in California hummingbird flowers. Am. Nat. 100:85-97.

Grant, K.A., and V. Grant. 1967. Effects of hummingbird migration on plant speciation in the California flora. Evolution 21:457-465.

Grant, K.A., and V. Grant. 1968. Hummingbirds and their flowers. Columbia University Press. New York.

Grant, P.R., and B.R. Grant. 1995. Predicting microevolutionary responses to directional selection on heritable variation. Evolution 49:241-251.

Gomez-Campo C. 1999. Taxonomy. Pp. 3-32 in Biology of Brassica coenospecies, Gomez-Campo, C. (ed.). Elseveir Science B.V., Amsterdam.

Hall, J.C., K.J. Sytsma and H.H. Iltis. 2002. Phylogeny of the Capparaceae and Brassicaceae based on chloroplast sequence data. American Journal of Botany 89:1826-1842.

Harvey, P.H. and M.D. Pagel. 1991 The comparative method in evolutionary biology. Oxford University Press, Oxford.

Hayek, A. 1911. Von. Entwurf eins Cruciferen-Systems auf phylogenetischer Grundlage. Beih. Bot. Centralbl. 27:127-335. pls. 8-12.

Hennig, W. 1950. Grundzüge einer theorie der phylogenetischen systematic. Deutscher Zentralverlag, Berlin.

Hooker, J.W. 1838. Flora Boreali-Americana. Vol. 2. NewYork: Hafner Publishing Co. P. 110.

Houle, D.H., J. Mezey, and P. Galpern. 2002. Interpretation of the results of common principal components analysis. Evolution 56:433-440.

Janchen, E. 1942. Das Sytem der Cruciferen. Österr. Bot. Zeitscher. 91:1-28.

Jansen, R.K. 1992. Current research. Plant Molecular Evolution Newsletter 2:13-14.

Jepson, W.L. 1925. *Pedicularis* L. Lousewort. Pp. 948-949 in A manual of the flowering plants of California. Associated Student Store, University of California, Berkeley, California.

Johnson, L.A., J. L. Schultz, D.E. Soltis, and P.S. Soltis. 1996. Monophyly and generic relationships of Polemoniaceae based on *matK* sequences. American Journal of Botany 83:1207-1224.

Johnson, L.A., D.E. Soltis, and P.S. Soltis. 1999. Phylogenetic relationships of Polemoniaceae inferred from 18S ribosomal DNA sequences. Plant Systematics and Evolution 214:65-68.

Johnson, R.A., and D.W. Wichern. 2002. Applied Multivariate Statistical Analysis, 5th ed. Prentice Hall, Upper Saddle River, New Jersey.

Judd, W.S., R.W. Sanders, and M.J. Donoghue. 1994. Angiosperm family pairs: Preliminary phylogenetic analyses. Harvard Papers on Botany 5:1-51.

Kaiser, H.F. 1958. The varimax criterion for analytical rotation in factor analysis. Psychometrika 23:187-200

Kercher, S., and J.K. Conner. 1996. Patterns of genetic variability within and among populations of wild radish, Raphanus Raphanistrum (Brassicaceae). American Journal of Botany 83:1416-1421.

Koch, M., B. Haubold, and T. Mitchell-Olds. 2001. Molecular systematics of the Brassicaceae: evidence from coding plastic *mat*K and nuclear *Chs* sequences. American Journal of Botany 88:534-544.

Inaba, R., and T. Nishio. 2002. Phylogenetic analysis of *Brassiceae* based on the nucleotide sequences of the *S*-locus related gene, *SLR1*. Theor. Appl. Genet. 105:1159-1165.

Lande, R. 1976. Natural selection and random genetic drift in phenotypic evolution. Evolution 30:314-334.

Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. Evolution 33:402-416.

Lande, R. 1985. The dynamics of peak shifts and the patterns of morphological evolution. Paleobiology 12:34-354.

Lande, R. and S.J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210-1226.

Lee, S. 1978. A factor analysis study of the functional significance of angiosperm pollen. Systematic Botany 3:1-19.

Lee, S. 1989. Some functional aspects of angiosperm pollen. Kor. J Plant Tax. 19: 289-301.

Lofsvold, D. 1986. Quantitative genetics of morphological differentiation in *Peromyscus*. I. Test of the homogeneity of genetic covariance structure among species and subspecies. Evolution 40:559-573.

Loockerman, D.J., and R.K. Jansen. 1996. The use of herbarium material for DNA studies. Pp. 205-220 in Sampling the green world, Stuessy, T.F., and S.H. Sohmer (eds.). Columbia University Press, New York.

Macior, L.W. 1973. The pollination ecology of *Pedicularis* on Mount Rainier. American Journal of Botany. 60(9):863-871.

Macior, L.W. 1977. The pollination ecology of *Pedicularis* (Scrophulariaceae) in the Sierra Nevada of California. Bulletin of the Torrey Botanical Club 104:148-154.

Macior, L.W. 1982. Plant community and pollinator dynamics in the evolution of pollination mechanisms in *Pedicularis* (Scrophulariaceae). Pp. 29-45 in Pollination and evolution, Armstrong, J.A., J.M. Powell, and A.J. Richards (eds.). Royal Botanic Gardens, Sydney, Australia.

Macior, L.W. 1983. The pollination dynamics of sympatric species of *Pedicularis* (Scrophulariaceae). Amer. J. Bot. 70(6):844-853

Macior, L.W. 1984. Behavioral coadaptation of *Bombus* pollinators and *Pedicularis* flowers. V^{èrne} Symposium International sur la Pollinisation. Versailles 27-30 Septembre 1983. Ed. INRA Publ. (Les Colloques de l'INRA, n-21).

Macior, L.W. 1986a. Floral resource sharing by bumblebees and hummingbirds in *Pedicularis* (Scrophulariaceae) pollination. Bull. Torrey Bot. Club. 113:101-109.

Macior, L.W. 1986b. Pollination ecology and endemic adaptation of *Pedicularis howellii* Gray (Scrophulariaceae). Pl. Sp. Biol. 1:163-172.

Macior, L.W. 1995a. Pollination ecology of *Pedicularis* in the Teton Mountain region. Plant Species Biol. 10:77-82.

Macior, L.W. 1995b. Pollination ecology of *Pedicularis parryi* ssp. *purpurea* (Parry) Carr (Scrophulariaceae). Plant Species Biol. 10:163-168.

Macior, L.W. 1996. Pollination ecology of *Pedicularis bracteosa* in the montane-subalpine ecotone. Plant Species Biol. 11:165-171.

Macior, L.W., and T. Ya. 1997. A preliminary study of the pollination ecology of *Pedicularis* in the Chinese Himilaya. Plant Species Biol. 12:1-7.

McDade, L.A. 1992. Pollinator relationships, biogeography, and phylogenetics. Bioscience 42:21-26.

Mitchell, A.D., and P.B. Heenan. 2000. Systematic relationships of New Zealand endemic Brassicaceae inferred from nrDNA ITS sequence data. Systematic Botany 25:98-105.

Monson, R.K. 1996. The use of phylogenetic perspective in comparative plant physiology and development biology. Ann. Missouri Botanical Garden 83:3-16.

Morell, P.L. and L.H. Reisberg. 1998. Molecular tests of the proposed diploid hybrid origin of *Gillia achilleifolia* (Polemoniaceae). American Journal of Botany 85:1439-1453.

Moritz, C. 1995. Uses of molecular phylogenies for conservation. Philosophical Transactions of the Royal Society of London 349:113-118.

Olmstead, R.G., C.W. DePamphilis, A.D. Wolfe, N.D. Young, W.J. Elisons, and P.A. Reeves. 2001. Disintegration of the Schrophulariaceae. American Journal of Botany 88(2):348-361.

Olmstead, R.G., and J.A. Sweere. 1994. Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. Syst. Biol. 43:467-481.

Olmstead, R.G., J. A. Sweere, and K.H. Wolfe. 1993. Ninety extra nucleotide in the *ndh*F gene of tobacco chloroplast DNA: A summary of revisions to the 1986 genome sequence. Plant Molecular Biology 22:1191-1193.

Pagel, M. 1993. Seeking the evolutionary regression coefficient: an analysis of what comparative methods measure. Journal of Theoretical Biology 164:191-205.

Phillips, P.C. 1998. CPCRand: randomization test of the CPC hierarchy. Univ. of Oregon Software available at <darkwing.uoregon.edu/~pphil/software.html>.

Phillips, P.C., and S.J. Arnold. 1999. Hierarchical comparison of genetic variance-covariance matrices. I. Using the Flury hierarchy. Evolution 53:1506-1515.

Plitmann, U., and D.A. Levin. 1983. Pollen-pistil relationships in the Polemoniaceae. Evolution 37:957-967.

Porter, J.M. 1997. Phylogeny of the Polemoniaceae based on nuclear ribosomal internal transcribed spacer DNA sequences. Aliso 15:57-77.

Porter, J.M., and L.A. Johnson. 1998. Phylogenetic relationships of Polemoniaceae: inferences from *nad*1B intron sequences. Aliso 17:157-188.

Porter, J.M., and L.A. Johnson. 2000. A phylogenetic classification of Polemoniaceae. Aliso 19:55-91.

Pradham A.K., S. Prakash, A. Mukhopadhyay, and D. Pental. 1992. Phylogeny of *Brassica* and allied genera based on variation in chloroplast and mitochondrial DNA patterns: Molecular and taxonomic classifications are incongruous. Theo. Appl. Genet. 85:331-340.

Prather, L.A. 1999. Systematics of *Cobaea* (Polemoniaceae). Systematic Botany Monographs 57:1-81.

Prather, L.A., C.J. Ferguson, and R.K. Jansen. 2000. Polemoniaceae phylogeny and classification: Implications of sequence data from the chloroplast gene *ndh*F. Am. J. Bot. 87:1300-1308.

Prather, L.A., and R.K. Jansen. 1998. The phylogeny of *Cobaea* (Polemoniaceae) based on sequence data from the ITS region of nuclear ribosomal DNA. Systematic Botany 23:55-70.

Prather, L.A., A.K. Monfils, A.L. Posto, and R.A. Williams. 2002. Monophyly and phylogeny of *Monarda* (Lamiaceae): Evidence from the internal transcribed spacer (ITS) region of Nuclear Ribosomal DNA. Systematic Botany 27:127-137.

Price, R.A., J.D. Palmer, and I.A. Al-Shehbaz. 1994. Systematic relationships of *Arabidopsis*: Molecular and morphological perspective. Pp. 7-19 in Arabidopsis, Meyerowitz, E.M., and C.R. Somerville (eds.). Cold Spring Harbor Press, New York, New York, USA.

Price, T.D., and P.R. Grant. 1985. The evolution of ontogeny in Darwin's finches: A quantitative genetic approach. Am. Nat. 125:169-188.

Purvis, A., and A. Rambaut. 1995. Comparative analysis by independent contrasts (CAIC): An Apple Macintosh application for analyzing comparative data. Cabios 11:247-251.

Ree, R.H. 2001. Homoplasy and the Phylogeny of *Pedicularis*. Ph. D. dissertation. Harvard University Graduate School of Arts and Sciences, Cambridge, Massachusetts.

- Reisberg, L.H., M.A. Hanson, and C.T. Philbrick. 1992. Androdioecy is derived from dioecy in Datiscaceae: evidence from restriction site mapping of PCR-amplifies chloroplast DNA fragments. Systematic Botany 17:324-326.
- Reisberg, L.R. and N.C. Ellstrand. 1993. What can molecular and morphological markers tell us about plant hybridization. Crit. Rev. Plant Sci. 12:213-241.
- Robart, B.W. 2000. The systematics of *Pedicularis bracteosa*: Morphometrics, development, pollination ecology, and molecular phylogenetics. Illinois State University Department of Biological Science, Bloomington, Illinois.
- Rodman, J.E., K.G. Karol, R.A. Price, and K.J. Sytsma. 1996. Molecules, morphology, and Dahlgren's expanded order of the Capparales. Systematic Botany 21:289-307.
- Rodman, J.E., P.S. Soltis, D.E. Soltis, K.J. Sytsma, and K.G. Karol. 1998. Parallel evolution of glucosinilate biosynthesis inferred from congruent nuclear and plastid gene phylogenies. American Journal of Botany 85:997-1006.
- Roff, D.A. 2000. The evolution of the G-matrix: selection or drift? Heredity 84:135-142.
- Roff, D.A., and T.A. Mousseau. 1999. Does natural selection alter genetic architecture? An evaluation of quantitative genetic variation among populations of *Allonemobius socius* and *A. fasciatus*. J. Evol. Biol. 12:361-369.
- Roff, D.A., T.A. Mousseau, and D.J. Howard. 1999. Variation in genetic architecture of calling song among populations of *Allonemobius socius*, *A. fasciatus*, and a hybrid population: Drift or selection? Evolution 53:216-224.
- Sakai, A.K., S.G. Weller, W.L. Wagner, P.S. Soltis, and D.E. Soltis. 1997. Phylogenetic perspective on the evolution of dioecy: Adaptive radiation in the Hawaiian genera *Schiedea* and *Alsinidendron* (Charyophylaceae: Alisinoideae). Pp. 455-473 in Molecular evolution and adaptive radiation, Givnish, T.J., and K.J. Sytsma (eds.). Cambridge University Press, New York.
- Sang, T., D.J. Crawford, and T.F. Stuessy. 1995. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: Implications for biogeography and concerted evolution. Proceedings of the National Academy of Sciences 92:6813-6817.
- Sang, T., D.J. Crawford, and T.F. Stuessy. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). American Journal of Botany 84(8):1120-1136.
- Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766-1776.

Schulz, O.E. 1936. Cruciferae. In A. Engler & K. Prantl. Nat.Pflanzenfam. ed. 2. 17b: 227-658.

Simmons, M.P., and H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analysis. Systematic Biology 49:369-381.

Soltis, P.S., and M.A. Gitzendanner. 1999. Review: Molecular systematics and the conservation of rare species. Conservation Biology 13:471-483.

Soltis, P.S., and D.E. Soltis. 2000. The role of genetic and genomic attributes in the success of polyploids. Proceedings of the National Academy of Sciences 97:7051-7057.

Song, K.M., and T.C. Osborn. 1992. Polyphyletic origins of *Brassica napus*: new evidence based on organelle and nuclear RFLP analyses. Genome 35:992-1001.

Song, K.M., T.C. Osborn, and P.H. Williams. 1988. *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs): I. Genome evolution of diploid and amphidiploid species. Thero. Appl. Genet. 75:784-794.

Song, K.M., T.C. Osborn, and P.H. Williams. 1990. *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs): III Genome relationships in *Brassica* and related genera and the origin of *B. oleracea* and *B. rapa* (syn. *campestris*). Thero. Appl. Genet. 79:497-506.

Sprague, E.F. 1958. A new subspecies of *Pedicularis densiflora*. Aliso 4:130.

Sprague, E.F. 1960. Ecological life history of California species of *Pedicularis*. Claremont Graduate School, Claremont, California.

Sprague, E.F. 1961. Parasitism in *Pedicularis*. Madrono 16:192-200.

Sprague, E.F. 1962. Pollination and evolution in *Pedicularis* (Schrophulariaceae). Aliso 5:181-209.

Steele, K.P., and R. Vigalys. 1994. Phylogenetic analysis of Polemoniaceae using nucleotide sequences of the plastid gene *mat*K. Systematic Botany 19:126-142.

Steppan, S.J. 1997a. Phylogenetic analysis of phenotypic covariance structure. I Contrasting results from matrix correlation and common principal component analysis. Evolution 51:571-586.

Steppan, S.J. 1997b. Phylogenetic analysis of phenotypic covariance structure. II Reconstructing Matrix evolution. Evolution 51:587-594.

Steppan, S.J., P.C. Phillips, and D. Houle. 2002. Comparative quantitative genetics: evolution of the G matrix. Trends in Ecology and Evolution 17:320-327.

Stroo, A. 2000. Pollen morphological evolution in bat pollinated plants. Plant Syst. Evol. 222:225-242.

Stuchlik, L. 1967a. Pollen morphology in the Polemoniaceae. Grana Palynol. 7:146-240.

Stuchlik, L. 1967b. Pollen morphology and taxonomy of the family Polemoniaceae. Rev. Paleobot. Palynol. 4:325-333.

Swofford, D.L. 2000. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, Massachusetts, Sinauer Associates.

Swofford, D.L., and W. P. Maddison. 1987. Reconstructing ancestral character states under Wagner parsimony. Mathematical biosciences 87:199-229.

Taberlet, P.L., L. Gielly, L. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Pl. Mol. Bio. 17:1105-1109.

Taylor, T.N., and D.A. Levin. 1975. Pollen morphology of Polemoniaceae in relation to systematics and pollination: Scanning electron microscopy. Grana 15:91-112.

Thorman, C.E., M.E. Ferreira, L.E.A. Camargo, J.G. Tivang, and T.C. Osborn. 1994. Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theor. Appl. Genet. 88:973-980.

Vorobik, L.A. 1993. *Pedicularis* (Scrophulariaceae). Pp. 1049-1051 in The Jepson manual: Higher plants of California, Hickman, J.C. (ed.). University of California Press, Berkeley, California.

Waitt, D.E., and D.A. Levin. 1998. Genetic and phenotypic correlations in plants: A botanical test of Cheverud's conjecture. Heredity 80:310-319.

Warwick, S.I., and L.D. Black. 1991. Molecular systematics of *Brassica* and allied genera (Subtribe Brassicinae, Brassiceae) – chloroplast genome and cytodeme congruence. Theor. Appl. Genet. 82:81-92.

Warwick, S.I., and L.D. Black. 1993. Molecular relationships in subtribe Brassicinae (Cruciferae, tribe Brassiceae). Canadian Journal of Botany 71:906-918.

Warwick, S.I., and L.D. Black. 1997. Phylogentic implications of chloroplast DNA restriction site variation in subtribes Raphaninae and Cakilinae (Brassicaceae, tribe Brassiceae). Canadian Journal of Botany 75:960-973.

Webb, C.O., D.D. Ackerly, M.A. McPeek, and M.J. Donoghue. 2002. Phylogenies and community ecology. Annu. Rev. Ecol. Syst. 33:475-505.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 in PCR protocols: A guide to methods and applications, Innis, M., D. Gelfand, J. Sninsky, and T. White (eds.). Academic Press, San Diego.

Willis, J.H., J.A. Coyne, and M. Kirkpatrick. 1991. Can one predict the evolution of quantitative characters without genetics? Evolution 45:441-444.

Wroblewski, T., S. Coulibaly, J. Sadowski and C.F. Quiros. 2000. Variation and the phylogenetic utility of the *Arabidopsis thaliana Rps2* homolog in various aspects of the tribe Brassiceae. Mol. Phylog. Evol. 16:440-448.

Yang, Y.W., K.N. Lai, P.Y. Tai, and W.H. Li. 1999. Rates of nucleotide substitution in angiosperm mitochondrial DNA sequences and dates of divergence between *Brassica* and other angiosperm lineages. Journal of Molecular Evolution. 74:597-604.

