A Worldwide Phylogenetic Analysis of *Scutellaria* (Lamiaceae)

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ABSTRACT

The plant genus *Scutellaria* L. (Lamiaceae) is comprised of approximately 350 cosmopolitan species. Twenty-four species are found in the southeastern United States. The genus has been used medicinally on multiple continents, predominantly for its anxiolytic, anti-inflammatory and analgesic properties. Recently, certain species in the genus have been investigated for their ability to inhibit cancer cell growth. Despite the vast interest in the group, a molecular phylogeny has never been performed on the genus as a whole. My project involves a preliminary phylogenetic analysis of *Scutellaria* with representatives from every continent except Antarctica. DNA from field, herbaria (MO, NY, VSC) and lab specimens were extracted for a total of 30 species including one outgroup taxon, *Holmskioldia sangunea.* Gene regions sequenced include *matK* and the *trnL* intron + *trnL-F* spacer. The resulting phylogeny will provide preliminary scaffolding upon which a more targeted approach to medicinal screening within the genus can be based.

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Chapter I

INTRODUCTION

Scutellaria L. (Lamiaceae) is one of the largest genera in the mint family with approximately 350 species (Paton, 1990). The scutellum, or dish-shaped structure present at the base of the upper calyx lip can identify *Scutellaria* morphologically from the rest of Lamiaceae (Paton, 1990). This structure is formed from a fold in the upper calyx lip that stands erect and enlarges during fruiting (Paton, 1990). Another morphological synapomorphy of the genus is a lack of endosperm in the mature seed (Paton, 1990). *Scutellaria* is found on every continent except Antarctica with Central/South America and North Africa/Eurasia being the most species rich areas (Paton, 1990). *Scutellaria* has been used for thousands of years in Chinese medicine for a variety of ailments (Tan and Vanitha, 2004; Shang et al., 2011). Currently, a total of 35 species have been studied and nearly 300 compounds have been identified (Shang et al., 2011). Some recent studies show that extracts from species of *Scutellaria* exhibit antioxidant properties (Gao et al., 1999), antimicrobial activity (Sato et al., 2000), anti-inflammatory activity (Lin and Shieh, 1995), neuroprotective effects (Yune et al., 2009), anxiolytic or anti-anxiety properties (Hui et al., 2002), and cancer cell inhibition (Zhang et al., 2003).

The objective of this project is to create a preliminary worldwide phylogeny of *Scutellaria*. The first goal is to determine if section *Scutellaria* is monophyletic and the second is to determine if geography is phylogenetically important in the genus.

Chapter II

MORPHOLOGY

Family Diagnosis

The mint family Lamiaceae or Labiatae includes annual or perennial herbs, shrubs or trees with quadrangular stems (Radford et al., 1964; Zomlefer, 1995). Leaves are simple, opposite, decussate and often aromatic (Radford et al., 1964; Zomlefer 1995). Lamiaceae infloresences are determinate paniculate thyrses, corymbs, capitulums, racemes or are reduced to solitary flowers (Radford et al.; Zomlefer, 1995). Flowers are zygomorphic to actinomorphic with five fused petals arranged in two lobes (bilabiate; Radford et al., 1964; Zomlefer, 1995). Two or four didynamous stamens make up the androecium while the gynoecium consists of two carpals (Radford et al., 1964; Zomlefer, 1995). The fruit is a schizocarp consisting of four mericarps (Radford et al., 1964; Zomlefer, 1995).

Scutellaria Diagnosis

Scutellaria consists of perennial herbs with a quadrangular stem (Radford et al., 1964). The inflorescence is a bracteate raceme or panicle (Radford et al., 1964). The calyx is bilabiate with acrested upper lobe (Radford et al., 1964). The calyx closes and expands when fruiting (Radford et al., 1964; Zomlefer, 1995). A peg-like gynophore, dimidate stamens with only the lower locule of the anther present and an exalbuminous seed with embryo slightly curved to curved to a right angle between the cotyledons and radicle are some distinctive characteristics that separate *Scutellaria* from the remainder of Lamiaceae (Paton, 1990). These characters, although rare, can be found individually in other Lamiaceae genera including *Renschia* Vatke*, Sideritis* L., and *Tinnea* Kotschy ex Hook. f. (Paton 1990). The combination of these characters however, is seen only within *Scutellaria* (Paton, 1990)

Chapter III

TAXONOMY

Family Classification

Lamiaceae has 236 genera with more than 7,000 species and is divided into seven subfamilies: Ajugoideae, Lamioidae, Nepetoideae, Prostantheroideae, Scutellarioideae, Symphorematoideae, and Viticoideae (Bendiksby et al., 2011). *Scutellaria* is located within Scutellarioidae and this subfamily is sister to Lamioidae, which contains common mint genera such as *Mentha* and *Salvia* (Wink and Kaufmann, 1996; Wagstaff and Olmstead, 1997). Within Scutellarioidae there are four genera: *Holmskioldia* Retz., *Scutellaria*, *Tinnea*, and *Wenchengia* (Cantino, 1993; Lei et al*.*, 2012). *Holmskioldia* is a relatively small genus consisting of approximately 20 species native to the Himalayan region of India, but the genus has been cultivated and become naturalized throughout the old and new worlds (Moldenke, 1981). *Tinnea* is a tropical African genus with 30 species (Third, 1976) and *Wenchengia* is a monotypic genus endemic to Hainan, an island off the southern coast of China (Lei et al*.*, 2012). *Scutellaria* has been shown to be a monophyletic group, albeit with extremely limited sampling (bootstrap (bt) = 92, Wink and Kaufmann, 1995; bt > 90, Bendiksby *et al*., 2011; bt = 91, Lei *et al*., 2012). It is sister to *Tinnea*, also with extremely limited sampling (bt > 90, Bendiksby et al., 2011; bt = 96, Wagstaff and Olmstead, 1999). *Scutellaria* + *Tinnea* are sister to *Holmskioldia* (bt = 71, Wagstaff and Olmstead, 1999; bt > 90, Bendiksby et al., 2011; bt = 98, Lei et al., 2012).

Scutellaria + *Tinnea* + *Holmskioldia* are in turn sister to *Wenchengia* (bt = 87, Lei et al., 2012).

The name *scutellaria* originated from the Latin word *scutella*, or dish, in reference to the dish-like calyx feature seen in some European species (Paton, 1990). The group was first called *Scutellaria* by Cortuso (1591), and referred to as *Cassida* in subsequent years (Colonna, 1616). Linnaeus restored *Scutellaria* in publication (1753). *Scutellaria* was later classified into four genera, *Hatlanlewisia, Perilomia, Salazaria,* and *Scutellaria* in 1990 (Paton). This paper addresses the limits of *Scutellaria* as defined by Paton (1990).

Intrageneric Classification

Scutellaria has been separated into two subgenera: *Scutellaria* Paton and *Apeltanthus* (Nevski ex Juz.) Juz emend. Paton (Paton, 1990). Within the subgenus *Scutellaria* there are five sections: *Scutellaria* Paton*, Perilomia* (Kunth) Epling emend.*, Salazaria* (Torrey) Paton*, Salvifoliae* (Boiss.) Edmondson*,* and *Anaspis* (Rench. f.) Paton (Paton, 1990). Within subgenus *Apeltanthus* are two sections: *Apeltanthus* Nevski ex Juz. and *Lupulunaria* A. Hamilton (Paton, 1990). Section *Lupulunaria* is further broken into two subsections: *Lupulunaria* (A. Hamilton) Paton and *Cystaspis* Juz. (Paton, 1990).

Scutellaria is the largest section with about 240 species and the most widespread, found on every continent in both temperate and tropical locations (Paton, 1990). In comparison to the other sections, *Scutellaria* has a one-sided inflorescence while in other sections the inflorescence radiates out in all directions (Paton, 1990). Section *Perilomia* has eleven species found only in temperate South America (Paton, 1990). Two distinct morphological characters of *Perilomia* are equal corolla lobes and glabrous anthers

(Paton, 1990). Section *Salazaria* has one species and is found in the southwestern United States (Paton, 1990). A defining character of *Salazaria* is an inflated calyx that is membranous and bladder-like (Paton, 1990). Section *Anaspis* has 15 species located in southern Iran, Afghanistan, central Asia and Tibet (Paton, 1990). *Anaspis* has a testudinate calyx that expands to more than 5 mm when fruiting (Paton, 1990). Section *Salviifoliae* has five species located in the eastern Mediterranean through Turkey (Paton, 1990). *Salviifoliae* is noted as an intermediate between subgenera *Scutellaria* and *Apeltanthus*, exhibiting chracters derived from both subgenera (Paton, 1990). Subgenus *Apeltanthus* has nearly 150 species and of those 12 species located in the Middle East and Soviet central Asia are in section *Apeltanthus* (Paton, 1990).

Several characters that set sections *Apeltanthus* and *Lupulinaria* apart from one another include: testudinate calyx, scale-like lower leaves and longer calyx hairs on the upper lip than the lower lip (Paton, 1990). Section *Lupulinaria* has 130 species and is further broken into two subsections: *Lupulinaria* and *Cystaspis* (Paton, 1990). *Cystaspis* is the smaller of the two subsections with only 11 species found in the Himalaya and associated mountains (Paton, 1990). Subsection *Cystaspis* can be distinguished from subsection *Lupulinaria* by two calyx features absent in *Lupulinaria*: an inflated-membranous scutellum and longer calyx hairs on the upper lip than the lower lip (Paton, 1990). *Lupulinaria* is the larger subsection with approximately 120 species distributed throughout North Africa and Eurasia (Paton, 1990).

In his classification, Paton produces a UPGMA cluster analysis of *Scutellaria* based on morphological characters (Figure 1; 1990). In the UPGMA analysis Paton (1990) shows a pattern of evolution where section *Perilomia* is the first diverging branch.

Perilomia is sister to a large *Scutellaria* clade + a clade containing all other sections (Figure 1; Paton, 1990). The section *Scutellaria* clade is paraphyletic due to the inclusion of *Scutellaria ghorana* Hedge (section *Anapsis*) and *Scutellaria mexicana* Torrey (section *Salazaria*; Figure 1; Paton, 1990). Within the clade containing all other sections, a monophyletic group of section *Anapsis* is sister to a clade containing section *Apeltanthus* sister to section *Salvifoliae* + section *Lupulinaria* (Figure 1; Paton, 1990). Subsection *Lupulunaria* is paraphyletic with the inclusion of *Scutellaria pointica* Koch. (section *Salviifolia*) and subsection *Cytaspis* (*Scutellaria heydei* Hook. f. and *S. macrochlamys* Rench.f. and Fitz; Figure 1; Paton, 1990).

In his morphological treatment however, Paton suggests that *Scutellaria* is the most primitive section and as it spread into new niches, it became specialized thus forming the various sections positioning it at the base of all other sections (Figure 2a; 1990). Sections *Anaspis*, *Perilomia*, *Salazaria* and *Salviifolia* are shown in Paton's morphological hypothesis as being closely related to section *Scutellaria* with only a few (1-2) morphological characters separating them from the proposed primitive section *Scutellaria* (Figure 2a; Paton, 1990). Thus subgenus *Apeltanthus* is hypothesized to have evolved from the section *Salviifolia* branch (Figure 2a; Paton 1990). This relationship is due to a morphological character (nutlet hairs) found only in subgenus *Apeltanthus* and section *Salviifolia* (Figure 2a; Paton, 1990). In subgenus *Apeltanthus*, section *Lupulinaria*, subsection *Lupulinaria* is the most primitive with section *Apeltanthus* and section *Lupulinaria*, subsection *Cystaspis* further evolving from this branch (Figure 2a; Paton, 1990). Section *Apeltanthus* has the most character changes from the primitive section Scutellaria than any other section. Using the morphological characters in Paton's tree (Figure 2a; 1990), I created a more contemporary tree echoing what was being shown (Figure 2b). This tree shows section *Scutellaria* as sister to all other sections with no character changes from the ancestral state (Figure 2b). Section *Perilomia* has two character changes while sections *Salvifoliae* and *Anaspis* have one (Figure 2b). These three sections form an unresolved clade sister to a clade including: section *Salazaria* and a clade containing all the sections of subgenus *Apeltanthus* (Figure 2b). This clade is distinguished from the ancestor state by one morphological change (Figure 2b). Section *Salviifoliae* is sister to a clade contining all of subgenus *Apeltanthus* (Figure 2b). The clade containing sections of subgenus *Apeltanthus* is separated from section *Salviifoliae* by three morphological charcters (Figure 2b). Subsection *Lupulunaria* is sister to a clade containing subsection *Cystaspis* and section *Apeltanthus* (Figure 2b). These are separated based on one morphological trait (Figure 2b). Subsection *Cystaspis* and section *Apeltanthus* are separated from each other based on four characters (one for subsection *Cystaspis* and three for section *Apeltanthus*; Figure 2a).

Paton maintains that the morphological relationships he proposed are more accurate than the UPGMA tree because in this distance method, only sheer trait similarity is used for tree generation. This feature can cause species with similar traits to be closely related regardless of evolutionary history (Paton, 1990).

Chapter IV

ECOLOGY

Although *Scutellaria* is a widely distributed genus, other genera in the Scutellarioidae subfamily have limited native distributions (Atkins, 1996; Cantino, 1993).

Holmsioldia is found naturally throughout the sub-tropical Himalayas but has been cultivated since 1796 and can be found throughout the world (Atkins, 1996). Although found in cultivation, *Holmskioldia* grows best under tropical or subtropical conditions such as warm temperatures (>15° C or 60° F), moist nutrient rich soils and high humidity (Atkins, 1996).

Tinnea, much like *Holmskioldia* has a limited native range native to tropical Africa specifically central and southern Africa (Vollesen, 1976). *Tinnea* grows from sea level to 2500 m above sea level and is mostly abundant in grasslands and wooded grasslands but is also found growing in ravines and secondary forests (Vollesen, 1976). Likewise, *Wenchengia* has an extremely limited distribution (Cantino, 1993; Li et al., 2012). It is endemic to the island Hainan, the southernmost province of China (Li et al., 2012). *Wenchengia* can be found in dense rainforest habitats, along streambeds and in occasionally flooded valleys at altitudes ranging from 200-300m (Li et al., 2012).

Unlike *Holmskioldia, Tinnea* and *Wenchengia*, *Scutellaria* has a wide native range and is found in a plethora of environments ranging from xerophytic areas to frequently flooded areas (Paton, 1990). Although found in a wide range of environments on a variety of continents, *Scutellaria* is often found in disturbed areas (Radford et al., 1964; Flora of China). Some Western North American species live in xerophytic environments (Paton, 1990) while Eastern North American species are oftentimes found in low woodlands and along roadsides in moist habitats (Nelson, 2006; Radford et al., 1964). In Central and South America *Scutellaria* is also found in moist environments including alluvial woodlands and marshes (Paton, 1990). Species native to Northern China are found in similar habitats as those found in Eastern North America including disturbed roadsides and grasslands (Paton, 1990; Flora of China). *Scutellaria* found in New Zealand and Australia will is found growing in frequently flooded and somewhat disturbed areas (Paton, 1990; Williams, 1992)

Chapter IV

MATERIALS AND METHODS

DNA Extraction and Taxon Sampling

Total DNA was extracted using Qiagen DNeasy plant mini kit (Qiagen Inc., Valencia, CA) from herbarium material and silica-dried fresh samples, or obtained directly from the Olmstead lab (University of Washington, Seattle). Taxa sampled included 28 species of *Scutellaria*, one species of *Holmskioldia* and one species of *Tinnea* (see Table 1 in Appendix B). *Holmskioldia* is the outgroup for all analyses since it has been found to be, sister to *Scutellaria* + *Tinnea* (Cantino et al., 1999).

Gene Regions and DNA Sequencing

We sampled two chloroplast gene regions: *matK* and the *trnL* intron + *trnL-F* spacer. Primers are listed in Table 2 (see Appendix B). PCRs were performed in volumes of 50 µl using 49 µl of master mix including 29 µl sterile distilled deionized water, 25 µl GoTaq green, 0.5 µl each of forward and reverse primer (10 pmol) and 1 µl template DNA. Amplifications were performed on a BioRad MJ Mini Personal Thermal Cycler using a program consisting of 4 minutes at 90 ◦C followed by 30 cycles of 30 seconds denaturation (94° C), 30 seconds annealing (52-60° C), and 1 minute extension (72° C), with a final extension of 4 minutes (72° C).

Successfully PCR-amplified fragments were cleaned by Qiagen PCR cleanup kit (Qiagen Sample and Assay Technologies, Valencia, CA) or QIAEX II Gel Extraction Kit

(Qiagen Sample and Assay Technologies, Valencia, CA). DNA was sequenced by Eurofins MWG Operon in Huntsville, AL. Sequences were aligned manually in MacClade. Accession numbers for GenBank are included in Table 1.

Phylogenetic Analyses

Each region of chloroplast DNA was analyzed separately first and then combined into a matrix of total data if there was no strong conflict among the individual analyses (80% bootstrap support). Phylogenetic analyses were performed with PAUP* using maximum parsimony settings (Swofford, 1999). All characters are weighted equally, and only those that are parsimony-informative are included. Construction of the trees was performed using a heuristic search with 1000 replicates. Bootstrap analyses were used to determine support the phylogenetic trees using a heuristic search with 200 replications (Felsenstein, 1985).

Chapter V

RESULTS

MatK

The aligned *matK* data set includes 23 taxa (Table 1) with 664 nucleotide positions including 12 parsimony-informative characters (1.8%). There were five most parsimonious trees found (length [L] = 14; consistency index [CI] = 0.857; retention index [RI] = 0.956). This tree is rooted with *Tinnea.*

Scutellaria angustifolia Pursh. and *S. nana* A. Gray are sister taxa (Figure 3; bt = 69) and form a clade with *S. bolanderi* A. Gray and *S. brittonii* Porter(Figure 3; bt = 68). *Scutellaria alabamensis* Alexander, *S. multiglanduosa* (Kearney) Small ex Harper, and *S. racemosa* Torrey form an unresolved clade (Figure 3; bt = 90). *Scutellaria costaricensis* H. Wendl. is sister (Figure 3; bt = 53) to a large clade containing all previously mentioned species as well as the unresolved species: *S. barbata* D. Don*, S. indica* L.*, S. minor* Huds.*, S. galericulata* L.*, S. lateriflora* L.*, S. novae-zelandica* Hook. f. and *S. longituba.* Koidz. Two accessions of *Scutellaria baicalensis* Georgi*, S. viscidula* Bunge and *S. scordifolia* Fischer form a clade (Figure 3; bt = 64) that is sister to *S. rehderiana* Diels (Figure 3; bt = 84). *Scutellaria alpina* L. and *S. tournfortii* Benth. are unresolved outside of all other ingroup taxa (Figure 3).

TrnL intron + trnLF spacer

The aligned *trnL* intron + *trnLF* introgenic spacer includes 21 taxa (Table 1) with 663 nucleotides including 32 parsimony-informative characters (4.8%). There were five most parsimonious trees (L = 37; CI = 0.946; RI = 0.975) rooted with *Holmskioldia sanguinea* Retz. In this analysis there are four clades that are strongly supported as being a monophyletic group together, although their relationships to each other are unresolved (Figure 4; bt = 100). *Scutellaria costaricensis* makes up a monotypic clade (Figure 4). An unresolved clade including the southeastern United States species, *S. alabamensis* + *S. incana* Spreng.+ *S. multiglanduosa* + *S. racemosa* (Fig. 4; bt = 91) is present. A second unresolved clade including *S. amana* Bornm. + *S. bolanderi* + *S. brittonii* +*S. lateriflora* + *S. longituba* + *S. nana* + *S. novae-zelandica* (Figure 4; bt = 94) was also formed. Lastly, a resolved clade including *Scutellaria baicalensis, S. viscidula, S. rehderana, S. orientalis* L.*, S. hirta* Sm.*, S. seiberi* Benth.*,* and *S. tournfortii* was formed (Figure 4). Within the previously mentioned clade, *S. baicalensis and* S. viscidula are sister (Figure 4; bt = 53). Sister to the two of those is *S. rehderana* (Figure 4; bt = 60). Sister to the three previously mentioned taxa is *S. orientalis* (Figure 4; bt = 91). The above four taxa form a clade sister to an unresolved clade including *S. hirta*, *S. siberi*, and *S. tournfortii* (Figure 4; bt = 79). The two previously mentioned clades form a monophyletic group (Figure 4; bt = 69). *Scutellaria leucantha* Loes. is unresolved outside of all other ingroup taxa (Figure 4)

MatK and trnL intron + trnLF spacer combined

Species for whom both gene regions were available (Table 1) with no bootstrap conflict greater than 80% were combined into one matrix including 14 taxa with 1326 characters (23 parsimony informative; 1.7%). One unrooted most parsimonious tree was recovered (L = 25; CI = 1.00; RI = 1.00). In this analysis *Scutellaria alabamensis*, *S. multiglanduosa* and *S. racemosa* are unresolved in respect to all other taxa. The first clade includes an unresolved clade of *Scutellaria brittonii* + *S. bolanderi* + *S. nana* (Figure 5; bt = 62) sister to two unresolved taxa: *S. longituba* and *S. novae-zelandica* (Figure 5; bt = 66). *Scutellaria lateriflora* is sister to the previous five taxa (Figure 5; bt = 97). The second clade includes *S. baicalensis* + *S. viscidula* (bt = 87) sister to *S. rehderana* (bt = 100). *S. tournfortii* is sister to the previous three taxa (Figure 5; bt = 92) with *S. costaricensis* sister to the four taxa (Figure 5; bt = 79). These two larger clades are sister to each other with strong support (Figure 5; bt = 100).

Chapter VI

DISCUSSION

The two main questions addressed by this study are: is section *Scutellaria* monophyletic and is geography phylogenetically important in the genus? In Paton's (1990) UPGMA analysis section is not monophyletic because *Scutellaria ghorana* and *S. mexicana* are embedded within the section *Scutellaria* clade. In his morphological classification, however, Paton (1990) maintains that section *Scutellaria* is monophyletic. Our *matK* tree does not resolve this question with respect to *Scutellaria alpina* because the relationships are unresolved (Figure 3). *Scutellaria orientalis* in the *trnL* + *trnLF* tree does not make section *Scutellaria* monophyletic (Figure 4; bt = 91).

In both the *matK* and *trnL* + *trnLF* analyses, the Eastern United States taxa (*Scutellaria alabamensis, S. multiglandulosa,* and *S. racemosa*) are highly supported as being monophyletic (Figure 3; bt = 90 Figure 4; bt = 91). In the *trnL* + *trnLF* analysis an additional Eastern United States taxa is present in the highly supported clade: *Scutellaria incana* (Figure 4; bt = 91). Although a clade of Eastern United States taxa is highly supported in the individual analyses, these taxa (*Scutellaria alabamensis, S. multiglanduosa* and *S. racemosa*) are unresolved in respect to all other taxa in the combined tree (Figure 5). The monophyly of sampled Eastern United States taxa is seen in other genera including *Cornus, Tiarella* and *Trautvetteria* (Xiang et al*.,* 1998).

A Western United States clade including *Scutellaria brittonii, S. bolanderi* and *S. nana* was supported in both the *matK* and combined analyses (Figure 3; bt = 68: Figure 5;

bt = 62). In addition to the three taxa mentioned above, *Scutellaria angustifolia* is included in the *matK* analysis (Figure 3; bt = 68). In the *trnL* + *trnLF* analysis however, these taxa remained unresolved in a clade containing widespread taxa (Figure 4). This pattern is also seen in other genera such as *Boyknia, Cornus, Tiarella,* and *Tratvetteria* (Xiang et al*.,* 1998).

Lastly, a clade of Northern China taxa (*Scutellaria baicalensis*, *S. viscidula* and *S. rehderana*) is supported in all three analyses (Figure 3, bt = 84; Figure 4, bt = 60; Figure 5, bt = 79). In the *matK* analyses there are five taxa in this clade (two samples of *Scutellaria baicalensis,* and one each of *S. rehderana, S. scordifolia,* and *S. viscidula*: Figure 3, bt = 84). In the *trnL* + *trnLF* analyses there are four taxa (*Scutellaria baicalensis, S. orientalis, S. rehderana,* and *S. viscidula*). This trend has also been shown in genera including *Hamamelis* and *Weigela-Diervilla* (Donoghue et al., 2001).

In the *trnL* + *trnLF* and combined analyses, the Western United States taxa are embedded within a clade of widespread taxa from the Pacific clade (Japan, New Zealand and South America: Figure 4, bt = 94; Figure 5, bt = 97). In the *trnL* + *trnLF* analysis this clade is unresolved and includes: *Scutellaria amana, S. bolanderi, S. brittonii, S. lateriflora, S. longituba, S. nana,* and *S. novae-zealandica* (Figure 4, bt = 94). In the combined analyses the Pacific clade has more resolution including a Western United States clade (*Scutellaria bolanderi, S. brittonii,* and *S. nana* [Figure 5, bt = 62]) within the larger cosmopolitan clade including *S. lateriflora, S. longituba* and *S. novae-zealandica* (Figure 5, bt = 97). This Western United States + Japan clade is also seen in *Hamamilis* (Donoghue et al., 2001).

In Paton's (1990) UPGMA analysis, species generally did not group together by geography. In our analysis, however, several geographically informative clades were found (Eastern North American clade in both *matK* and *trnL* + *trnLF* [Figure 3; bt = 90: Figure 4; bt = 91]; Western North American clade in *matK*, *trnL* + *trnLF*, and combined [Figure 3; bt = 68: Figure 4; bt = 94: Figure 5; bt = 62]; a Pacific clade in both *trnL* + trnLF and combined analyses [Figure 4; bt = 94: Figure 5; bt = 97]; and a Northern China clade in *matK*, *trnL* + *trnLF*, and combined [Figure 3; bt = 84: Figure 4; bt = 91: Figure 5; bt = 100]).

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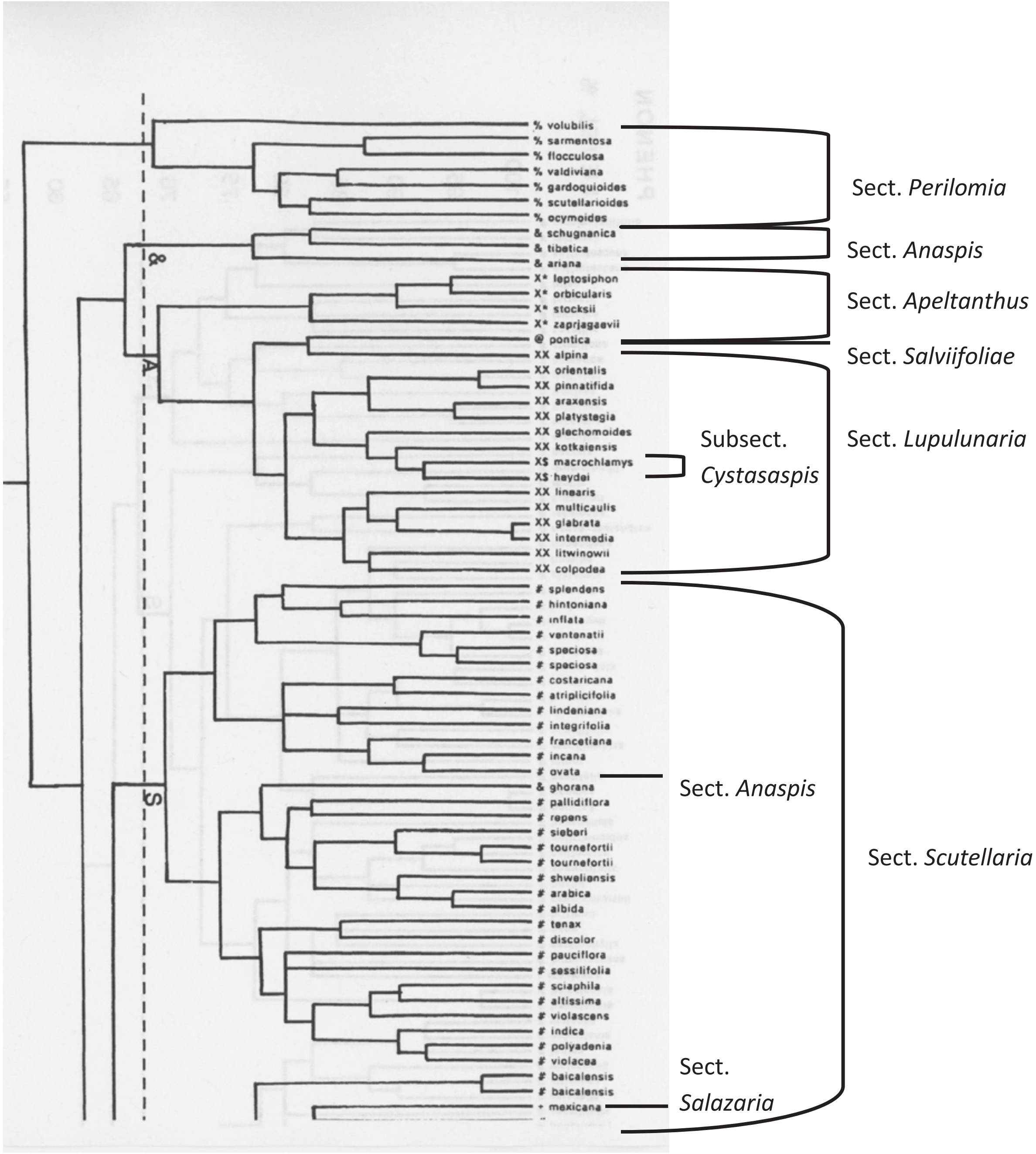
APPENDIX A

Figures 1 through 5

Figure 1: from Paton (1990). Fig. 5. Dendrogram produced by cluster analysis (UPGMA) showing relationships between representative sample of species of *Scutellaria*. Each species' position in the proposed classification is indicated by the symbol preceding the species name: # Sect. *Scutellaria*, % Sect. *Perilomia*, + Sect. *Salazaria*, & Sect. *Anaspis*, @ Sect. *Salviifoliae*, X* Sect. *Apeltanthus*, XX Sect. *Lupulinaria*, X$ Sect. *Lupulunaria* Subsect. *Cystaspis*. The broken line represents the 67% phenon level.



Sect. *Scutellaria*

Figure 2a: Paton's Morphological Hypothesis

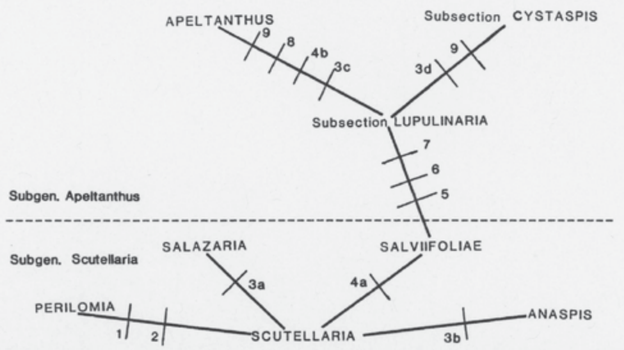


FIG. 6. The proposed phylogenetic relationships between the infrageneric taxa of *Scutellaria*. The numbered bars refer to the following apomorphies: **1** Equal corolla lobes; **2** Glabrous anthers; **3a** Calyx inflated, membranous and bladder-like; **3b** Calyx testudinate, greater than 5 mm in fruit; **3c** Calyx testudinate, less than 4 mm in fruit; **3d** Calyx with a membranous-inflated scutellum; **4a** Nutlet hairs completely covering the nutlet surface; **4b** Nutlet hairs partially covering the nutlet surface; **5** Cucullate bracts; **6** 4-sided inflorescence; **7** Strongly flattened, erect pedicels; **8** Scale-like lower leaves; **9** Calyx hairs on upper lip longer than those on lower.

Figure 2b: My Interpretation of Paton's Morphological Classification

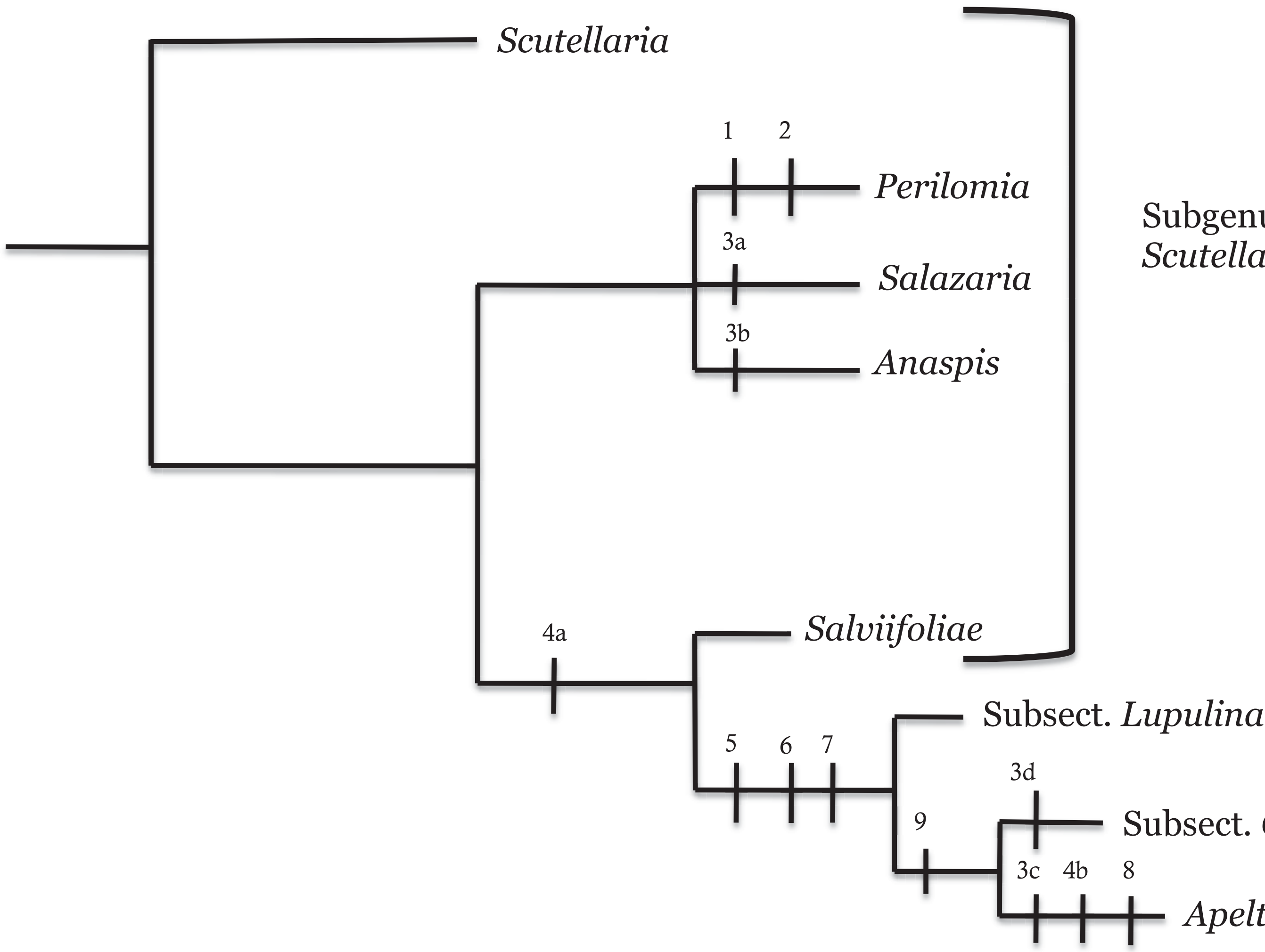

Scutellaria
1
2
Perilomia
3a
Salazaria
3b
Anaspis
Subgenu
Scutella
4a
Salviifoliae
Subsect. Lupulina
5
6
7
3d
9
Subsect.
3c
4b
8
Apelt

Figure 3: *matK* phylogenetic analysis of 23 taxa with 664 nucleotides (12 parsimony informative characters)

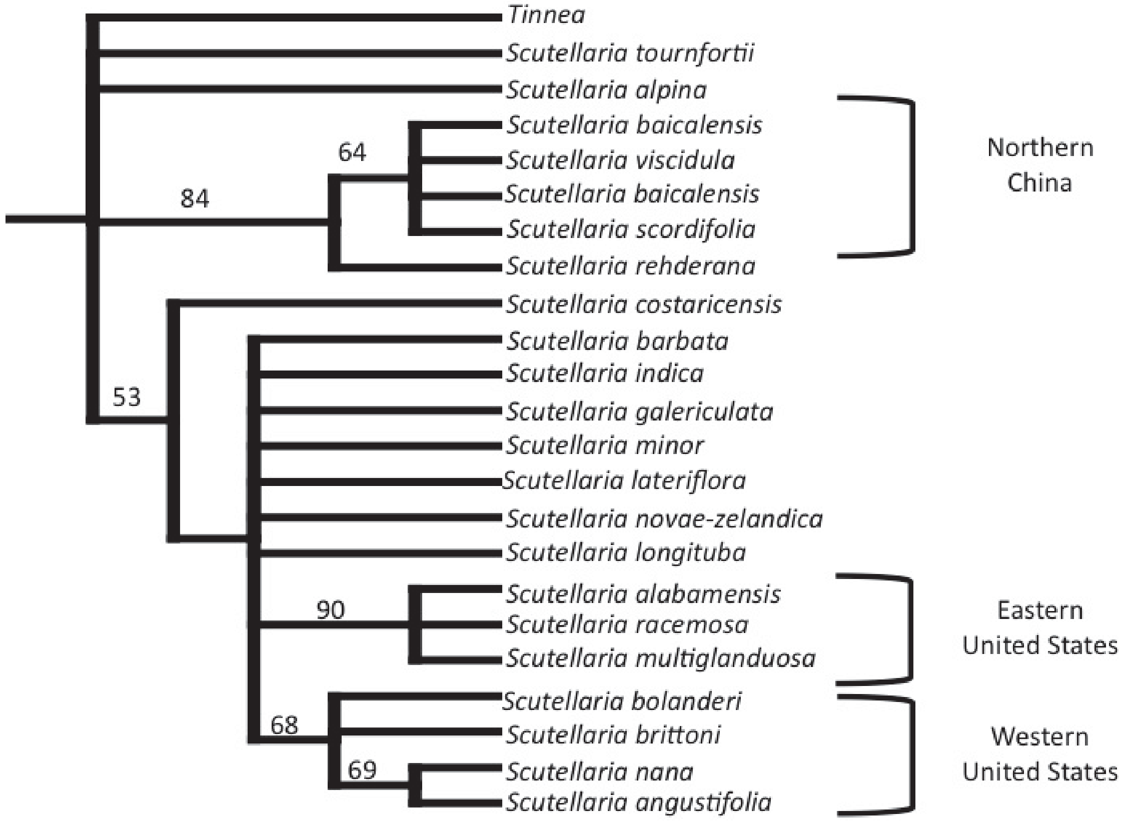


Figure 4: *trnL* + *trnLF* Maximum parsimony analysis including 21 taxa with 663 nucleotides (32 parsimony informative characters)

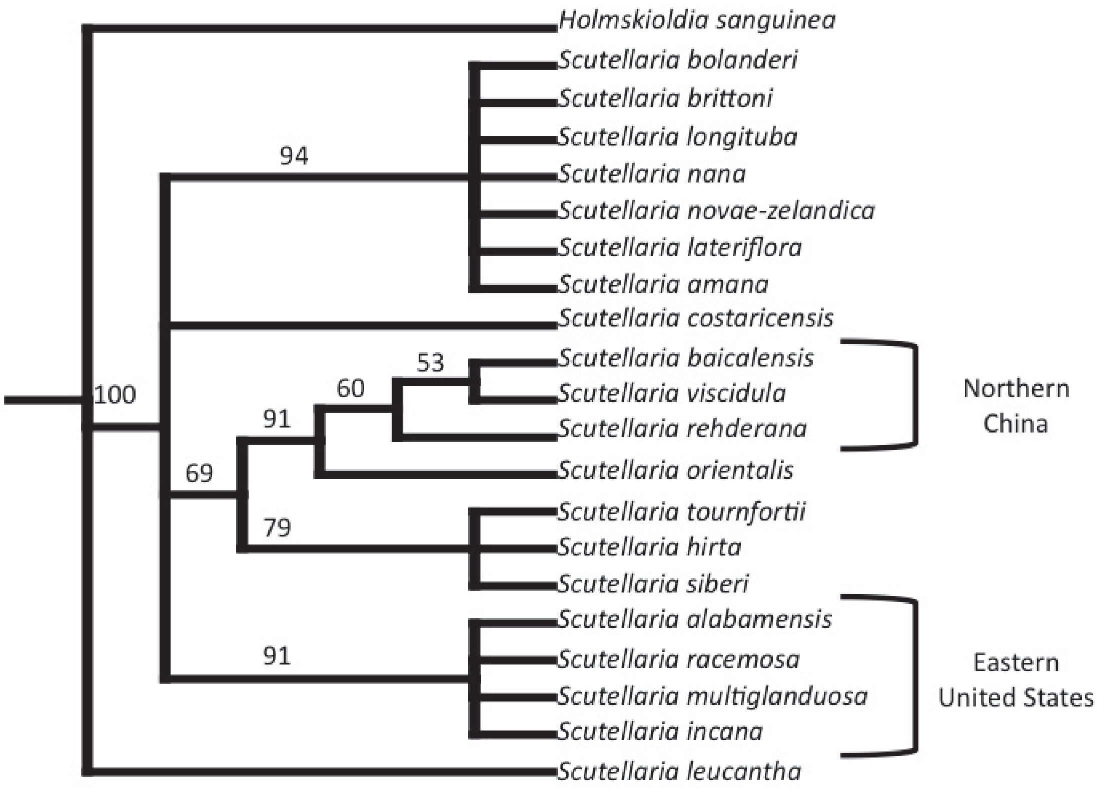
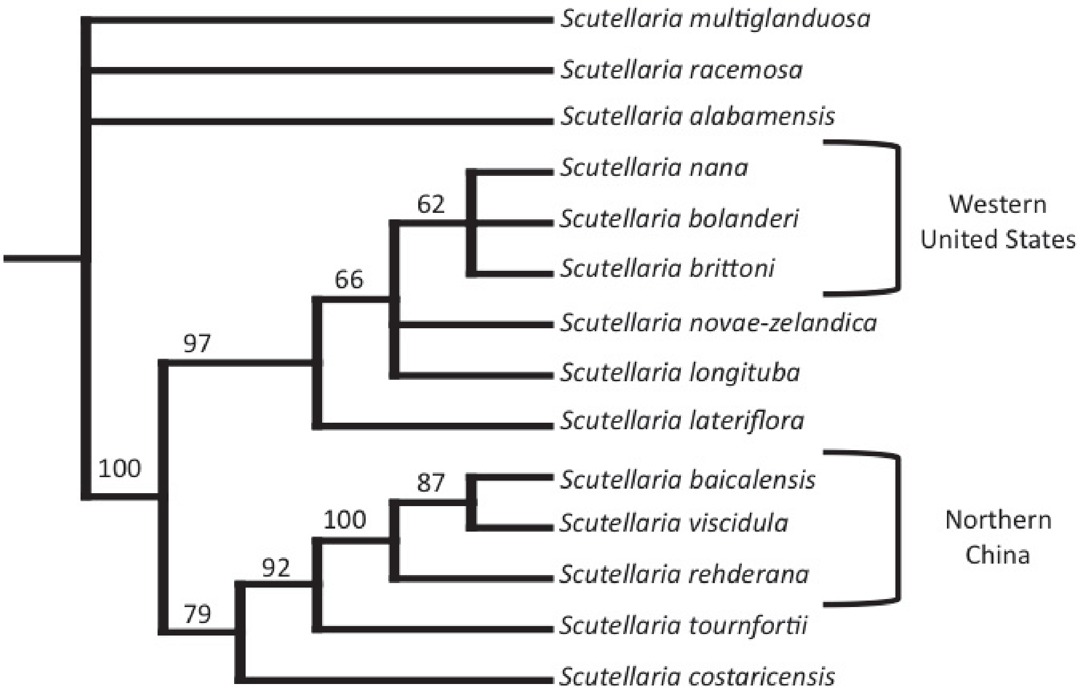


Figure 5: Combined *matK* and *trnL + trnLF* maximum parsimony analysis including 14 taxa with 1326 nucleotides (23 parsimony informative characters).



APPENDIX B

Tables 1 and 2

Table 1: List of Species Used, Locations, Sources, GenBank Numbers, and Species Included in each Analysis.

SPECIES	LOCATION	SOURCE	SECTION	VOUCHER SPECIMEN	GEN BANK NUMBERS	matK	trnL intron + trnLF spacer
Holmskioldia	Himalayas	NY		NY1183720	NA	X	√
S. alabamensis	Alabama, USA	VSC	Scutellaria	R KRAL 90104	NA	√	√
S. alpina	Europe	Olmstead lab	Lupulunaria	NA	NA	√	X
S. amoena	South/Central America	Gen Bank	Scutellaria	NA	JN675930.1	X	√
S. angustifolia	W. USA	Olmstead lab	Scutellaria	RGO 551	NA	√	X
S. baicalensis	China	Olmstead lab	Scutellaria	RGO 98-54	NA	√	√
S. barbata	S. China	Gen Bank	Scutellaria	NA	JX981420	√	X
S. bolanderi	W. USA	Olmstead Lab	Scutellaria	RGO 803	NA	√	√
S. brittonii	W. USA	Olmstead lab	Scutellaria	RGO 5/26/95	NA	√	√
S. costaricensis	Costa Rica	Olmstead lab	Scutellaria	RGO 5/17/94	NA	√	√
S. galericulata	Widespread USA	Gen Bank	Scutellaria	NA	JN894430	√	X
S. hirta	Crete	Gen Bank	Scutellaria	NA	EF546927	X	√
S. incana	SE, USA	VSC	Scutellaria	R. KRAL & V. BATA 88544	NA	X	√
S. indica	Japan	Olmstead lab	Scutellaria	RGO 94-07	NA	√	X
S. lateriflora	Widespread USA	Gen Bank	Scutellaria	NA	JX981411.1	√	√
S. leucanthia	S. America	NY	Scutellaria	NY0050532	NA	X	√
S. longituba	China/Japan	Olmstead lab	Scutellaria	RGO 92-214	NA	√	√
S. minor	Europe	Gen Bank	Scutellaria	NA	HM850804	√	X
S. multiglandulosa	Florida, USA	Fresh	Scutellaria	BUSH 505		√	√
S. nana	W. USA	Olmstead lab	Scutellaria	RGO 584	NA	√	√
S. novae-zealandica	New Zealand	Olmstead lab	Scutellaria	RGO 8/28/92	NA	√	√
S. orientalis	Eurasia	NY	Lupulunaria	NY1041079	NA	X	√

S. racemosa	Florida, USA: Native to South America	Fresh	Scutellaria	BUSH 504	NA	√	√
S. rehderiana	N. China	Gen Bank	Scutellaria	NA	JN675928	√	√
S. scordifolia	N. China	Gen Bank	Scutellaria	NA	HQ839713.1	√	X
S. sieberi	Siberia	Gen Bank	Scutellaria	NA	EF546848.1	X	√
S. tournfurtii	Middle East	Olmstead lab	Scutellaria	NA	NA	√	√
S. viscidula	N. China	Gen Bank	Scutellaria	NA	JN675929	√	√
Tinnea rhodesiana	Africa	Gen Bank		NA	JX981418.1	√	X

Table 2: Primers Utilized

Primer	Sequence (5’-3’)
matK-3F (Bendiksby et al. 2011)	CATGTGGAAATCTTGGTTCAAATC
matK-5Ra (Bendiksby et al. 2011)	CAAGAAAGTCGAAGTATATACTTTA
trnL-F spacer B49873 (Taberlet et al., 1991)	GGTTCAAGTCCCTCTATCCC
trnL-F spacer A50272 (Taberlet et al., 1991)	ATTTGAACTGGTGACACGAG
trnL intron B49317 (Taberlet et al., 1991)	CGAAATCGGTAGACGCTACG
trnL intron A49855 (Taberlet et al., 1991)	GGGGATAGAGGGACTTGAAC