Abstract

PARSING POLYPHYLETIC *PUERARIA:* DELIMITING DISTINCT EVOLUTIONARY LINEAGES THROUGH PHYLOGENY

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A taxon is defined as polyphyletic when it does not include the last common ancestor of all true members of the taxon, resulting in a number of subgroups not united by a common ancestor. Previous work has suggested *Pueraria* (Fabaceae) to be polyphyletic. Although several taxonomic treatments have recognized *Pueraria* as an unnatural grouping since its creation in 1825, and two have put forth taxonomic hypotheses, the polyphyly has never been resolved. The need for further biosystematic research has always been cited as the reason no changes were proposed. This project attempted to address this issue by sampling broadly across phaseoloid legumes with an initial target goal of 156 species including 15 species of *Pueraria*. Ultimately, 104 species across 69 genera were sampled for *AS2* and 116 species across 64 genera for *matK*. Phylogeny reconstruction was carried out using maximum likelihood and Bayesian inference. Both analyses yielded congruent tree topologies and similar support values. Both previous taxonomic hypotheses show some congruence with the data, but discrepancies do occur. This work provides strong support for the existence of five separate clades within the genus *Pueraria*, requiring the resurrection of the genus *Neustanthus* for *P. phaseoloides* along with the need to create a new genus each for *P. stricta*, *P. peduncularis*, and *P. wallichii*.

PARSING POLYPHYLETIC *PUERARIA:* DELIMITING DISTINCT EVOLUTIONARY LINEAGES THROUGH PHYLOGENY

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Though this work has only my name on it, I am not solely responsible for all the work herein. Without the help of others this project would not have accomplished what it has.

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INTRODUCTION

Pueraria montana var. *lobata* (Fabaceae), commonly known as kudzu, is a notoriously invasive species in the Southeast U.S. Kudzu was first introduced in the U.S. in 1876 during the Philadelphia Centennial Exposition as an ornamental vine (Britton et al., 2002; Shurtleff and Aoyagi, 1977; Ward, 1999) and then later displayed at the New Orleans Exposition in 1883 (Hill, 1985; Ward, 1999). The first person to experience and document the choking power of *P. montana* was David Fairchild, a botanist and field explorer for the USDA (Hill, 1985; Britton, 2002). The second major person to interact with and document *P. montana* was a Mr. C.E. Pleas, who championed kudzu as fodder. After observing local livestock aggressively eating *P. montana* he became convinced of its usefulness and proceeded to plant all 35 acres he owned with it in 1910 (Hill, 1985). For the next 40 years he would preach its redeeming qualities even going so far as to write a pamphlet extolling its virtues in 1925 (Hill, 1985; Britton et al., 2002).

Mr. Pleas was not the only one to jump on the proverbial bandwagon. Kudzu's high rate of photosynthesis, the ability to fix atmospheric nitrogen, and the ability to root at nodes in contact with the soil allows kudzu to grow and spread quickly (Forseth and Innis, 2004), making it an ideal candidate for soil erosion prevention. During the 1930's and 1940's the Soil Conservation Service promoted its planting to prevent soil erosion by distributing 73-85 million seeds and giving money to farmers to plant it (Britton et al., 2002; Hill, 1985; Ward, 1999). Eventually people would come to realize what an invasive monster kudzu could be. In 1953, the Soil Conservation Service removed it from the list of permissible cover crops (Britton et al., 2002; Hill, 1985) and then finally, in 1970 it was classified as a weed (Corley et al., 1997; Hill, 1985). The traits that had made it so ideal for soil erosion prevention had also made it an aggressive pest.

Invasive species are often introduced for good reasons ranging from use as a forage crop to being used for timber plantations (Sakai et al., 2001; Baker 1974, 1986). No mater how good the intentions, its important to remember that invasive species threaten biodiversity, ranking second only to habitat destruction in cause of biodiversity loss (Simberloff 2000). Invasive species can impact native biota through competitive exclusion as well as through hybridization with native species. This kind of event can lead to reduced fitness and potential extinction of native species (Mooney and Cleland, 2001; Rhymer and Simberloff 1996). The closest relative to kudzu here in the U.S. is *Glycine max* (Britton et al., 2002), the soybean. The soybean, like kudzu, is not a native plant but rather an introduced one from Asia. In terms of native North American relatives, the closest are *Amphicarpaea bracteata* and several species of *Cologania* (Britton et al., 2002).

Invasive species can affect more than just other organisms. Kudzu has been coined as a "polluting plant" due to its contributions to ozone pollution. Kudzu emits isoprene (Forseth and Innis, 2004), a photochemically reactive hydrocarbon that forms ozone and smog in the presence of nitrogen oxides. Kudzu also has the capability to fix nitrogen, 2/3 the rate of soybeans, which causes an increase in soil emission of nitrous oxide (Hickman et al., 2010). Coupled with its isoprene emissions, kudzu seems to be working to change the climate it inhabits by raising summer temperatures in the areas it resides. This process would allow it to push climate borders blocking its expansion further north. Therefore it is important we try to control its expansion by any means necessary.

Thanks to soil erosion-prevention planting, herbicide treatment is often difficult due to the closeness of waterways (Frye et al., 2012; Everest et al., 1999) and other means of control such as herbivory must be analyzed (Frye et al., 2012). Even with treatment of some kind,

complete removal may take many applications and many years along with cooperation between landowners to prevent kudzu reinvasion from neighboring land (Everest et al., 1999; Forseth and Innis, 2004).

Kudzu has been shown to have high levels of genetic diversity (Pappert et al., 2000), perhaps because of the combination of genotypes from separate sources, China and Japan, for the multiple introductions believed to have occurred (Pappert et al., 2000) with kudzu (Sun et al., 2005). It has been posited that species may not show the same level of susceptibility to biocontrol agents due to this high level of genetic diversity (Sun et al., 2005). Also of concern is making sure that biocontrol agents do not affect native plants such as soybean or native animals as well (Forseth and Innis, 2004). It is therefore important to carry out studies on kudzu specifically to determine what will safely work.

However, kudzu is not considered invasive in its natural habitat of Southeast Asia (Figure 1). A potential explanation for this is the presence of natural predators and diseases not found here in the U.S. In a previous study numerous insects were found feeding on the plant along with the presence of unknown rust and what researchers believed to be a mosaic virus of some sort on the lead blades (Pemberton, 1988). Some predation does occur here in the U.S. but mostly on the seeds (Forseth and Innis, 2004) which can number up to 20 per pod (Lackey, 1977; Van der Maesen, 1985; Ward, 1999; Britton, 2002). Unfortunately that is not the only way kudzu spreads. Kudzu can grow out from just one root crown (Ward, 1999; Britton, 2002) and then spread vegetatively (Pappert, 2000) across the ground and up any structure it can find. It is also capable of both sexual and asexual reproduction through underground runners, helping to maintain genetic variation within the species.

Pappert (2000) has proposed two possible explanations for the higher than average level of genetic diversity. The first, discussed previously, is that many individuals from multiple different sources established some populations. The second possible explanation is that populations start with a few founders but due to the pollen movement and seed dispersal new genetic material is introduced into the population. Based on his findings Pappert (2000) came to the conclusion that evolution may be favoring heterozygous plant expansion. It still remains a possibility that U.S. populations are the product of multiple introductions since previous studies have only focused on *P. montana* and *P. phaseoloides*, the two species most commonly referred to as kudzu.

Here in the U.S. the species *Pueraria montana* var. *lobata* and its varieties are commonly referred to as kudzu. In reality it is part of a much larger kudzu species complex that consists of *P. montana* and *P. edulis* and their varieties. The usage of the name kudzu can often times be confusing. It is used to reference different species depending on what part of the world you reside in. Here in the U.S. kudzu is the common name used for *Pueraria montana* var. *lobata*, whereas *Pueraria phaseoloides* is known as tropical kudzu, often shortened to kudzu. Confusion over the proper name for the species has also caused issue. Van der Maesen originally incorrectly referred to the species as *Pueraria lobata* in his monograph (1985). Upon review of literature he later corrected this based on the fact that *Dolichos montana* was merged into *Pueraria* in 1935 while *Pueraria lobata* was not until 1947 (Van der Maesen 1988). The name *Pueraria lobata* was originally used to refer to the presence of lobed leaflets while *Pueraria montana* was the designation for specimens collected in modern day Vietnam (Ward, 1998). Thus, the correctly designated species name is *Pueraria montana* var. *lobata*.

The genus *Pueraria* has been in existence since 1825 when De Candolle first described and named it after a colleague and friend (De Candolle, 1825). At its outset it consisted of only 2 species, *Pueraria tuberosa* and *P. wallichii.* Since that time, other species have been described, reduced to the variety level, or had their removal from the genus advocated. There are 20 currently accepted species today: *P. alopecuroides, P. bella, P. bouffordii, P. calycina, P. candollei, P. edulis, P. garhwalensis, P. imbricata, P. lacei, P. maclurei, P. montana, P. peduncularis, P. phaseoloides, P. pulcherrima, P. sikkimensis, P. stracheyi, P. stricta, P. tuberosa, P. wallichii,* and *P. xyzhui.* Traditionally, *Pueraria* species are generally described as twining vines or shrubs that have trifoliate leaves, and inflorescences in a pseudoraceme (Lackey, 1977; Van der Maesen, 1985; Ward, 1999; Pappert 2000). Its initial separation from *Hedysarum*, into *P. tuberosa* and *P. wallichii*, was based on non-articulating pods and monodelphous stamens. Remaining characteristics are variable in terms of appearance and taxonomically diagnostic importance.

A member of one of the most economically important subtribes, Glycininae, *Pueraria* is a genus whose versatility and range of uses throughout history knows almost no bounds. It has served as a simple ornamental to having important uses within the realms of medicine and agriculture. *Pueraria phaseoloides* is still used and advocated today in the use of soil loss prevention during crop rotation in some areas of the world (Salako et al., 2006). *Pueraria montana* can even be used as a high protein forage crop for livestock and baled as hay although harvesting can be difficult (Everest et al., 1999). *Pueraria montana* even shows potential as a valuable biofuel resource better than corn because of its high carbohydrate levels (Sage et al., 2009), fast growth, and high biomass. Extracts from kudzu have been shown to curb alcohol cravings while avoiding the dangerous side effects of more conventional medications (Keung et

al., 1995; Keung and Vallee, 1993). The large tubers of *P. tuberosa* can even be used as a food source for both humans and cattle during times of famine (Van der Maesen, 1994). For a genus that has so many uses, it's a mystery as to why so little is known about its true taxonomy.

Previous taxonomic treatments have recognized *Pueraria* as an unnatural grouping and suggested different hypotheses on how the genus should be divided up. In 1977, Lackey separated 20 recognized *Pueraria* species into four tentative groups (Figure 2) based on number of flowers per node, stipule type, calyx type, the presence of callosities on the vexillum, and the pod type (Lackey, 1977) (Table 1). Based on these morphological characteristics he argued that *P. wallichii* should be excluded from *Pueraria.* He also put forth the idea that *P. colletii* Prain, *P. brachycarpa, P. bella,* and *P. stricta* Kurz should be removed from *Pueraria* and coupled with *Neonotonia* and related genera such as *Shuteria*. He also suggested that the species *P. subspicata* Benth. and *P. phaseoloides* Benth. bore enough morphological differences from others that they should be given their own genus. However, he did not revise the genus in any way based on the groupings he came up with, noting the genus has not been the subject of a modern monograph since Benth's 1867 study.

Van der Maesen recently did monographic work based on Lackey's revision and outlined 17 species over the course of several botanical treatments (van der Maesen,1985; van der Maesen, 1995; van der Maesen, 2002; van der Maesen and Almeida, 1988). Instead of four groups, van der Maesen ended up with five, *P. pulcherrima* and *P. phaseoloides* each getting their own (Figure 2). It was the first time it had been the subject of a monograph since 1867. Van der Maesen also stated that "*Pueraria* has served more or less as a receptacle for species not easily classified elsewhere" but did not make any changes to the genus based on Lackey's

groups, claiming "further biosystematic research" was needed to relate *Pueraria* with other Glycininae taxa.

Previous phylogenetic studies have shown *Pueraria* to be polyphyletic (Doyle et al., 2003; Lee and Hymowitz, 2001; Stefanovic et al., 2009; Egan et al., unpublished data). A polyphyletic taxon is one that does not include the last common ancestor of all true members of the taxon, resulting in a number of subgroups not united by a common ancestor. Lee and Hymowitz (2001) found five species of *Pueraria* separating out into four distinct groups during the course of their analysis of the subtribe Glycininae. They found *P. stricta* to be allied with *Teyleria, P. montana* and *P. pulcherrima* allied with *Nogra,* and *P. phaseoloides* with *Pachyrhizus.* They agreed more with the classifications of Lackey (1977) than Van der Maesen (1985), though the results of their study pointed to *Pueraria* not being sister to *Glycine* (Lee and Hymowitz, 2001).

Five species is a poor sample with which to reconstruct the complete story of *Pueraria.* In order to obtain the full story behind the evolutionary relationships of *Pueraria,* we need to sample across the vast evolutionary and taxonomic landscape of legumes in the phaseoleae tribe. To do this we increased the taxonomic sampling of *Pueraria* and phaseoloid legumes in order to place the species of *Pueraria* in their proper evolutionary and taxonomic context. Understanding these relationships will provide the context necessary to begin examining the trait of invasiveness in kudzu.

Through the use of phylogenetic analysis we have strived to accomplish the following objectives. Firstly, we sought to determine the number of distinct evolutionary lineages in *Pueraria* and how those lineages are dispersed among phaseoloid legumes. Next, we compared the previous taxonomic hypotheses of Lackey (1977) and Van der Maesen (1985) concerning the

interspecific relationships within *Pueraria*. Finally, we use this information to inform future taxonomic revisions.

MATERIALS AND METHODS

Taxon Sampling—To understand the evolutionary history of *Pueraria,* we attempted to sample all currently recognized species of *Pueraria*. We were able to sample 14 of the 20 species recognized by Van der Maesen (1985). To place *Pueraria* lineages within the evolutionary context of related taxa, we sampled widely across core phaseoloid legumes. We need both a nuclear (maternally and paternally inherited) and chloroplast (maternally inherited) gene region for analysis in order to track lineages across more than one inheritance. For *AS2,* we included 69 genera (including *Pueraria*) representing 104 species with 64 *Pueraria* accessions. For *matK* we included 64 genera representing 116 species with 81 *Pueraria* accessions. Plant material was obtained from multiple sources: various herbaria located in Europe (Royal Botanic Gardens, KEW (K); Royal Botanic Garden Edinburgh (E), and the Muséum national d'Histoire naturelle (P) in Paris, France) and Asia (Botanical Survey of India (CAL) and the Herbarium of Thailand) as well as here in the U.S. (New York Botanical Garden and Missouri Botanical Garden), from field collections performed by Dr. Egan (China, and the U.S.), and from the previously extracted DNA generously shared by Jeff J. Doyle of Cornell University. Voucher specimens, source, and DNA accession numbers can all be found in Appendix A.

DNA Extraction, Amplification, and Sequencing—DNA extraction was carried out using the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA) for both herbarium and collected samples (dried in silica gel) following manufacture's instructions. Because *AS2* is a new marker for phylogenetic use, a brief description of it is provided below.

AS2 is a low copy nuclear gene region capable of being alternatively transcribed, allowing it to code for multiple proteins. Within the region there exist two motifs; one is a leucine-zipper –like motif while the other is a cysteine repeat that has been dubbed the C-motif

(Iwakawa et al., 2002) (Figure 3). *AS2* is expressed in all above ground portions of the plant except for the stem (Iwakawa et al., 2002, Xu et al., 2002). Its primary function is in the establishment of leaf polarity where it regulates the adaxial-abaxial axis, resulting in planar leaves. Iwakawa also suggested, based on his observations, that *AS2* might be involved in the development of the entire venation system. *AS2* is found in the plant nuclei even though it does not include an obvious nuclear localization signal implying that it could also be controlling the transcription of certain genes in the nucleus (Iwakawa et al., 2002). It is composed of one exon (the *AS2* domain; 1-293 bp), followed by the alternatively transcribed intron (position 305-534) and then ending with a 24 bp exon (Egan, unpublished data). This project is the first to use *AS2* as a phylogenetic marker, the primers for which were created by A.N. Egan based on comparison of multiple legume genomes and homoeologues (Egan and Doyle, 2010). The nuclear marker *AS2* was chosen for its ease and quality of amplification. Primers are presented here for the first time. *AS2* was amplified using primers AS2F (5'-CAC CAT GTG CAG CAT GCA AGT TCT-3') and AS2R (5'-AGT TGC CCT AAG CTG GCG GAT ATG-3') and the following conditions of 5 min at 94º followed by 35 cycles of 40 s at 94º, 1 min at 57º, 2 min at 72º and then ending with a final elongation of 7 min at 72º.

MatK has long been used as a molecular marker in plants, with wide application across angiosperms (Hilu et al, 2003). The *matK* region amplified in this study is a modified version of only 722 base pairs (roughly from position 1210-1932 in the full *matK* gene) to ensure consistent amplification in *Pueraria* and phaseoloid legumes. We shortened this region because we have many samples derived from herbarium material, where the DNA is often degraded. Short regions have been shown to work better with degraded DNA (Sarkinen et al 2012). The chloroplast marker *matK* was chosen for its ongoing usage in legume systematics so that our

work could contribute to global efforts to build the legume tree of life. The PCR protocol used followed the same conditions as those set in Hu et al (2000) using the primers 1210F (5'-CTA TCC ATC TGG AAA TCT TGG TTC-3') and 1932R (5'-CAG ACC GGC TTA CTA ATG GG-3').

Primers were added to a master mix that came in two varieties. The first was a premade master mix made by Promega that requires only 1 uL of forward and reverse 10 uM primers for each sample. The second consists of: 5 uL 5x Buffer, 1.5 uL 50mM MgCl2, 1.5 uL 10 mM dNTP, .15 uL DMSO, 1 uL 10 uM forward primer, 1 uL 10 uM reverse, and .125-.25 uL Taq. Four different types of taq were used during the course of this study: GoTaq Flexi DNA Polymerase (Promega, Fitchburg, Wisconsin), Maser Mix Taq DNA polymerase (Promega, Fitchburg, Wisconsin), Mango Taq (Bioline USA, Taunton, Massachusetts), and Platinum Taq (Life Technologies – Invitrogen, Carlsbad, California).

PCR products deemed worthy of sequencing, based on band quality, were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA). Sequencing was performed on the 3130 Genetic Analyzer from Applied Biosystems using BigDyeTerminator v3.1 chemistry. Some sequences were taken from GenBank and were downloaded (Appendix B) while others are available from past research (Dr. Ashley Egan, personal communication). Forward and reverse sequences were edited and aligned into contigs with Sequencher 4.7 (Gene Codes Corp., Ann Arbor, MI). All sequences for both *matK* and *AS2* will be uploaded to NCBI.

We initially attempted to sample 156 species across phaseoloid legumes, which included the following 15 species of *Pueraria* (not counting varieties): *P. alopecuroides, P. calycina, P. candollei, P. edulis, P. imbricata. P. lacei, P. montana, P. peduncularis, P. phaseoloides, P. pulcherrima, P. sikkimensis, P. stricta, P. tuberosa,* and *P. wallichii*. Ultimately we were only

able to amplify 14 of the 15 *Pueraria* species, *P. rigens* being the one exception. We were able to amplify three different species to serve as a consistent outgroup: *Clitoria ternatea, Clitoria mexicana, and Centrosema virginianum.* In the end 103 species and 60 genera for *AS2,* and 115 species and 65 genera for *matK* were actually sampled across phaseoloid legumes.

DNA Alignment, Phylogeny Reconstruction, and Network Analyses—Alignment of DNA sequences was carried out in MUSCLE (Edgar 2004) through the EMBL-EBI website. Alignments from MUSCLE were checked by eye in SE-AL (Rambaut, 2006). In order to ensure quality of alignment the amino acid alignment was honed and then matched to the DNA alignment. Both maximum likelihood and Bayesian inference analyses were performed. Maximum likelihood based methods have been shown to be more efficient at picking the right tree over both parsimony and distance based methods (Hasegawa, Kishino, and Saitou, 1991). Maximum likelihood can be defined as the probability of the data given a model of evolution (Posada and Buckley, 2004).

Because models play such an important role it is key that we select the best one. The two most often used methods of doing so are the Likelihood Ratio Test (LRT) and the Akaike Information Criterion (AIC). The goal is to pick the best fitting model, without under or over parameterizing. The LRT allows only the testing of nested models due to its reliance upon comparing across parameter distributions (Sullivan and Joyce, 2005). This is done by performing a pairwise comparison of log likelihoods to determine which of the two nested models is better (Posada and Buckley, 2004). AIC, on the other hand, does not require comparison between nested models, but instead measures the loss of information between two models through approximation (Joyce and Sullivan, 2005; Posada and Buckley, 2004). AIC is often considered to be the better of the two (Posada and Buckley, 2004). Modeltest 3.7 (Posada

and Crandall, 1998) was used to estimate models of evolution for each of our data sets or data partitions, with the best model chosen using AIC (Tables 2-5), where delta is the AIC score of the model minus the lowest AIC score and weight is the relative likelihood of the model. Maximum likelihood analysis was carried out using RAxML (Stamatakis, 2006) through the RAxML BlackBox server (Stamatakis, 2008) using the model of evolution allowed on the server that closely approximates the model chosen by AIC. 1000 bootstrap replicates were performed to estimate nodal support.

We performed Bayesian inference using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) for both *matK* and *AS2*. For the *AS2* dataset we coded our alignments with and without gaps into the following: gap coded introns, gap coded exons, full *AS2* partitioned into its intron and exon, and a total evidence alignment. Evolutionary models were determined for each partition and implemented in the accompanying data set's partitioned Bayesian analysis. Previous research has suggested that alignment gaps can be a valuable source of phylogenetic signal (Egan and Crandall, 2008; Simmons et al., 2007). In order to code for gaps we treated each indel as a simple binary character (Simmons and Ochoterena, 2000). The binary matrix was created using IndelCoder (Muller, 2006), a program wrapped inside SeqState (Muller, 2005).

Bayesian Inference was carried out on each data set with gaps treated as missing data and the variable model for gap coding used. All analyses started with a random tree. Bayesian inference runs consisted of four Markov chain Monte Carlo chains run for 10-25 million generations with trees sampled every 1000 generations. Priors for analyses were of equal probability. The amount of burnin was determined based on log likelihood scores found using the program Tracer (Rambaut and Drummond, 2004). Convergence was assessed by checking if the standard deviation of split frequencies was below 0.01, the Potential Scale Reduction Factor

(PSRF) approached 1, and the effective sample size (ESS) is above 100. Trees were summarized in a consensus tree with posterior probabilities as nodal support. However our *matK* analyses did not converge within 25 million generations and the best run was used in its place. Tracer was used to ensure that the best run had a good mixing of parameters and was heading towards convergence without any large jumps in the trace file.

Results from our analyses for different data sets were compared to determine whether we could combine data sets into a total evidence analysis. For those topologies with comparable taxa sets, we looked for strongly supported nodes (>70 bp or > 0.95 pp) in conflict between topologies (Mason-Gamer and Kellogg, 1996).

The historically used test for tree topology was the K-H test developed by Kishino and Hasegawa in 1989. This test is good for testing topologies from different data sets, but not for topologies derived from the same dataset; a common mistake in early phylogenetics (Goldman et al., 2000). For this reason, the Shimodaira-Hasegawa (SH) test was developed. By including a prior topology in the same set as the tree topology outputs of ones analysis (Goldman et al., 2000) we can compare tree topologies across genes. The SH test was instituted in PAUP 4.0 (Swofford, 2002).

Phylogenies assume bifurcation, resulting in completely resolved topologies. However, when speciation is ongoing, species may still be able to hybridize, resulting in a loop in the topology. Network analyses do not assume bifurcation, but allow the visualization of multipleunderlying evolutionary trajectories. This could be especially helpful in visualizing insipient species and those where hybridization may have occurred in the recent past. A split network allows us to separate taxa along parallel lines that represent the information that “splits” the taxa apart from each other into groups. These parallel lines signify the difference that divides

groups of taxa apart from others. Because of this, not all nodes will represent ancestral species, providing us with only an "implicit" view of the evolutionary history (Huson and Bryant, 2006). Network analysis was carried out using Splitstree 4 (Huson and Bryant, 2006) for the large *Pueraria* clade. *Nogra* was included to act as outgroup, but later removed to improve visualization due to its long branch length that condensed the shorter branches during visualization. *P. lacei* was removed due to shortness of sequence length that contributed to a lack of phylogenetic signal.

RESULTS

The results of the maximum likelihood analyses were largely congruent with our results from Bayesian inference analyses. Therefore, relationships are illustrated through Bayesian inference trees only. *AS2* saw better resolution with gaps coded than with gaps coded as missing. Coding gaps did not improve resolution for *matK*, likely because there were only 18 gaps in the alignment. All analyses show strong support for polyphyly in *Pueraria* and failure of *Pueraria* to produce a monophyletic clade. Both our *AS2* and *matK* trees suggest five distinct clades within the genus, spread all across the phaseoloid legumes. *P. phaseoloides, P. stricta, P. peduncularis,* and *P. wallichii* all represent their own distinct lineages. The clade containing multiple *Pueraria* species consists mainly of *P. montana* and its varieties along with *pulcherrima, P. alopecuroides, P. candollei, P. imbricata, P. sikkimensis. P. edulis* and *P. calycina.*

A total evidence analysis for *AS2* and *matK* was unable to be performed. This was because of strongly supported incongruence surrounding the inclusion of tribe Psoraleeae within subtribe Glycininae for *matK* but not for *AS2*. In order to test whether or not this was a significant difference in the topology we used the SH test. The SH test found the *AS2* and *matK* trees to be significantly different with $p < 0.05$

AS2 Results—Of the 156 *AS2* sequences included in our study, all 156 will be newly published. Unaligned sequences ranged from 325 base pairs (*Pueraria lacei*) to 568 base pairs (*Vigna radiata*). Length differences were due to variation in actual sequence length as well as to truncated sequences due to poor sequence quality. The alignment of these 156 accessions contained 669 base pairs in length due to the alignment of gaps. Coding for indels resulted in 81 binary characters. The alignment was also partitioned into an exon running from position 1-311

and an intron running from 312-669. AIC chose unequal-frequency Kimura 3-parameter plus Gamma (K81uf+G) for *AS2* intron, however MrBayes does not support this model. The next highest model supported is Hasegawa-Kishino-Yano plus Gamma (HKY+G). For the *AS2* exon, AIC chose the Tamura-Nei plus Invariance of Sites plus Gamma model. This too is unsupported by MrBayes thus we chose the highest scoring supported model, which was the General Time Reversible plus Invariance of Sites plus Gamma. *AS2* total evidence's highest scoring model was the same as the exon's. However, its next highest supported scoring model was the HKY+I+G.

In the Bayesian inference analysis of a partitioned *AS2* with gaps coded (Figures 4 & 5) we see support for a *Pueraria* clade consisting of many species of *Pueraria* in addition to a separate clade consisting of only accessions belonging to *P. phaseoloides* and its varieties; both are strongly supported with posterior probability (PP) of 1.0. However, some accessions of *P. phaseoloides* come out in the large *Pueraria* clade. These sequences are being rechecked for quality and error. The *P. phaseoloides* clade is shown to be sister to the genus *Sinodolichos* with moderate support (PP=0.92). *P. peduncularis* is allied with *Dumasia, Neorautanenia, Pachyrhizus, and Calopogonium.* It too sees a couple of its members scattered throughout the tree, these sequences and vouchers are being reviewed as well. *P. stricta* is strongly allied with *Teyleria* as well as *Neonotonia* with PP=1.0. Finally we see the *P. wallichii* clade coming out separate from everything else around it with posterior nodal support of 1, supporting it as a new genus.

Network analysis of the large *Pueraria* clade reveals a cluster of species undergoing an incipient speciation (Figure 6). Strongly supported species clusters are still conserved. Both *P. alopecuroides* and *P. pulcherrima* cluster along their own separate split partitions. *P. edulis* also

sees two of its four species clustering along another split partition. *P. montana* and its varieties along with *P. sikkimensis* and the other two *P. edulis* lie scattered around the network.

matK Results—Of the 184 *matK* sequences included in our study, 138 will be newly published. Unaligned sequences ranged from 365 base pairs (*Otholobium glandulosum*) to 736 base pairs (*Pueraria peduncularis).* The alignment of these 184 taxa was made up of 1128 positions due to the inclusion of GenBank accessions that are significantly longer than our truncated region. Coding for indels resulted in only 18 binary characters. This is due to our amplification of a truncated version of *matK* (1210-1932) and the conserved nature of this marker. Using the AIC, modeltest computed the highest scoring model for total *matK* to be GTR + gamma.

In the MrBayes consensus tree (Figures 7 & 8) the large *Pueraria* clade is structured similarly as in the *AS2* tree (inclusive of *Nogra*) however it does see a lower nodal support of PP=0.9. The *P. phaseoloides* clade comes out closely related to *Sinodolichos* again with a PP= 0.91. However, it is not the closest branch to our large *Pueraria* clade. The *P. stricta* clade has a posterior of 1 and shows close relationship with the genus *Teyleria* as well as *Neonotonia*. *P. peduncularis* and *P. yunnanensis* come out in a clade with a posterior of 1 allied with *Shuteria hirsuta*. *P. wallichii* also keeps its conserved clade with a posterior of 1 and a posterior of 0.98 separating it from other nearby tribal clades, once again presenting strong evidence for the need of it's own genus.

Network analysis of the large *Pueraria* clade sees a strong partitioning of species across network splits for both *AS2* (Figure 6) and *matK* (Figure 9). *P. montana* and its varieties lie grouped together in a split partition separate from the rest of clade. Their sequences contain a conserved area missing from the rest of the other species. *P. pulcherrima* partitions strongly as

well. *P. candollei, P. calycina, P. alopecuroides, P. imbricata,* and *P. sikkimensis* all partition separately from the rest of the clade along a common split. While still having unique enough sequences to justify separation of their species they all share sequence information that sets them apart from *P. montana* and its varieties. *P. calycina* is the most similar to the rest of the clade with a branch at the base of the split. *P. candollei* serves as a point of origin for the splits of the other members of the clade with two members of *P. alopecuroides* splitting off from the rest.

DISCUSSION

Phylogenetics can provide the means with which to reclassify and clean up the taxonomic quagmire that is *Pueraria*. By sampling genetic information we can backtrack across the evolutionary lineages currently classified within the genus. Our results paint a more complex picture of *Pueraria* with greater polyphyly than previously suggested (Lee and Hymowitz, 2001; Egan et al, unpublished data), with five distinct evolutionary lineages instead of four.

The subtribe Glycininae has been shown to not be monophyletic (Kajita et al., 2001). Polyphyletic members such as *Pueraria* can be strong contributors to polyphyly of a subtribe. Polyphyly is not a problem confined to the members of *Pueraria*, its subtribe, or even the legume family. Previous research has found polyphyly within other genera such as *Pleurospermum* (Apiaceae; Valiejo-Roman et al., 2012), *Polycarpon* (Caryophyllaceae; Kool et al., 2007), *Saxifraga* (Saxifragaceae; Soltis et al., 1996) and *Rhodomyrtus* (Myrtaceae; Snow et al., 2011), among others.

Chloroplast and nuclear genes have been shown to give slightly different topologies (Soltis and Kuzoff, 1995; Zhang et al., 2010) in previous studies of other taxa. Our chloroplast *matK* tree strongly favors the nesting of the Psoraleeae tribe within the Glycininae subtribe whereas our nuclear marker, *AS2*, supports a large Glycininae clade which excludes Psoraleeae. A potential explanation for this is chloroplast capture. Chloroplast capture is replacement of one plant's chloroplast genome with another (Tsitrone et al., 2003; Acosta and Premoli, 2010). Chloroplast capture is often the result of hybridization (Soltis and Kuzoff, 1995; Tsitrone et al., 2003), although recent research has shown capture through horizontal gene transfer via grafting (Stegemann et al, 2011). The exact mechanics of chloroplast capture has been one of much speculation. Regardless of mechanics, what we do have is a potential hypothesis that Psoraleeae

underwent an ancient hybridization event with Glycininae. Future works will attempt to look into this hypothesis further.

Both nuclear (*AS2*) and chloroplast *(matK*) markers gave similar trees, both indicating the presence of five clades of *Pueraria*. This congruence is key to our interpretation of how many separate evolutionary lineages are currently circumscribed within the genus *Pueraria*. The SH test found the two separate gene trees to be significantly different, likely due to the placement difference of the tribe Psoraleeae within Glycininae in the *matK* tree. Other than the placement of this one tribe all other tribes and subtribes see congruence across both gene regions. Because chloroplast DNA is maternally inherited while nuclear is a mix of paternal and maternal, congruence across both sets of data provides strong support for our parsing of a polyphyletic *Pueraria*.

As for previous hypotheses, the results of our analyses do agree with some of the observations of both Lackey (1977) and van der Maesen (1985) (Figure 10). The greatest agreement between their hypotheses and our research is the congruence with Lackey's largest grouping of *Pueraria.* His largest grouping matches our largest *Pueraria* clade. Van der Maesen on the other hand saw only partial congruence with our clade, specifically the species of *P. montana, P. imbricata, P. edulis. P. calycina,* and *P. lacei.* He instead places *P. pulcherrima* in its own group and separates *P. tuberosa, P. sikkimensis, P. candollei,* and *P. mirifica* into their own group within the genus *Pueraria*. Van der Maesen did however match our results with the separation of *P. phaseoloides* out from the rest of *Pueraria*, as did Lackey. Lackey was of the opinion however, that *P. subspicata* was a sister species rather than a variety of *P. phaseoloides*. Both did agree on a defining character that set *P. phaseoloides* apart, which was the presence of barrel shaped seeds. Van der Maesen grouped *P. peduncularis, P. stricta, and P. wallichii*

together as species that should be removed from the genus. Lackey's hypothesis agrees that *P. wallichii* and *P. stricta* should be removed from the genus. However, he does not call for the removal of *P. peduncularis*, even though he groups it with *P. wallichii*.

While these morphological analyses can give us some information on how species are related and divided within their own genus, the bigger picture cannot be achieved without the inclusion of molecular data as well. Morphological characteristics are important for identification in the field and the identification of fossil relationships (Wiens, 2004). Once we get the field sample back to the lab molecular data can allow us to see past morphological similarities that convergent evolution has given rise to in nonrelated species.

Morphological analyses can also be subjective, leading to differing opinions on what the proper classification of an organism is. Molecular data can allow us to validate and clarify these taxonomic hypotheses and help ascertain the diagnostic morphology (Martin et al., 2008). This is evident in the similarities and dissimilarities seen between the results of our research and the research of both Lackey and van der Maesen. We propose the need for taxonomical revision based on both our molecular data and the morphological descriptions of Lackey and van der Maesen.

Pueraria Clade— In 1867 when Bentham performed his monograph he described the constituents of *Pueraria* as being united by having the habit and pod of *Phaseolus* with a flower more like *Dioclea* (Bentham, 1867). At the time of his writing, the genus consisted of only nine members, and, despite ranges in morphological characteristics that he recognized as considerable, all of them were "most conveniently considered as congeners" (Bentham, 1867). Polyphyly within the genus *Pueraria* has arisen due to the use of variable convergent traits to justify the incorporation of a species into its genus. These traits include the presence of trifoliate

leaves, regardless of shape and other characteristics, a non-articulating seedpod, elongate ovaries, and leguminous inflorescences born in a pseudoraceme. An illustration tracing these traits among our five clades shows how they share and contract between the clades (Figure 11), providing evidence that their variability should not be ignored when distinguishing the correct members of *Pueraria*. Those finer differences within these variable categories have often been ignored due to the difficulty of placing the specimen being described to begin with.

Regardless of this polyphyly, one clade must retain the genus name *Pueraria*. When De Candolle first described *Pueraria* in 1825 he removed *Hedysarum tuberosum* from its genus and established it as *Pueraria tuberosa.* His defining character was the lack of an articulating pod (De Candolle 1825), which is a defining characteristic of *Hedysarum*. He also created the species *Pueraria wallichii* during the course of his morphological studies. He went on to describe the genus as having "a calyx bell somewhat elongated, five short obtuse teeth, the two upper more or less joined together forming a lip sometimes entire, sometimes two small teeth. Corolla much longer than the calyx, petals have short tabs, standard is obovate, with very small ear; wings oblong, with a headset, and parts of the hull welded except at their base. Stamens are 10 in number, all fertile, welded into a sheath, filament more or less split on the upper side, sometimes the tenth stamen is half separated, the anthers are small, oval. The ovary is linear. Style filiform. Stigma is finished as small, rounded, hairy, when viewed under the microscope. The fruit is a compressed pod, planar, linear or oblong, tapering at the base, slightly stalked, tapering from the base of the style, in continuous two valves. Stems are woody, climbing and cylindrical, their stipules are deciduous, non-welded petiole the stipels are sharp, very small, with winged leaves are odd, three leaflets, broad, oval, pointed, veined. Clusters of flowers are axillary, branched, almost paniculate, their pedicels born germinal or dull, each responsible for a

single flower, the petals appear yellowish after they dry." (Translated from French to English using Google Translate). De Candolle then goes on to say that the two species he relates this to are *Pueraria tuberosa* and *Pueraria wallichii*. *Pueraria tuberosa* is generally accepted as the type species (Hutchinson, 1964).

Pueraria tuberosa falls within the large *Pueraria* clade, which includes most of the species historically classified as *Pueraria*. For these two reasons we propose that these species should retain and define the genus *Pueraria*. The following general description of the genus is based of the most recent morphological descriptions of van der Maesen (van der Maesen, 1985). This general description fits all species within our large *Pueraria* clade: .

> *Pueraria* is a perennial woody climber with pubescent bark. Peltate stipules are conserved, with other varying characteristics. Leaves have striate to canaliculate or ribbed petioles, with leaflets being ovate, orbicular, rhomboid or lanceolate, lobed or not. However, side leaflets always obliquely express the general leaflet shape with the apex being long to acuminate and the leaflet being pubescent below. Every flower has only 2 bracteoles and stamens are monodelphous with the exception of *P. imbricata* and *P. calycina,* whose are diadelphous. The flattened seed pods always contain flattened-ovoid rarely reniform seeds.

The Splitstree network analysis allows us to look at the species in question in an unrooted tree with no forced bifurcation or assumption of no recombination or hybridization events. Nodes within the network are considered to be ancestral species, where nodes that originate from two lines correspond to hybridization or recombination events (Huson and Bryant, 2006).

Parallel lines serve as the indicators of splitting and collapse of those lines can be seen as removing the data that splits species. In both the *AS2* (Figure 6) and *matK* (Figure 9) networks we see a large cluster of all the varieties of *P. montana*. This points to a frequent occurrence of hybridization and recombination in ancestral species, some of which may still be extant. Our conserved clusters of species show a high level of robustness for their continued separation at the species level, an observation that is unattainable within the normal confines of our phylogenetic trees that assume a tree-like evolutionary history (Bryant and Moulton, 2004), which can oversimplify the evolutionary view (Lo et al., 2010). The tight grouping of reticulate events within varieties of *P. montana* also provides confidence in the their designation as varieties rather than separate species through the sharing of the same lineage.

P. phaseoloides* Clade—P. phaseoloides* comes out close to the large *Pueraria* clade and shares many characteristics with its members. Both our *Pueraria* clade species and *P. phaseoloides* are perennial climbers with pinnately trifoliate leaves with ovate to rhomboid leaflets that are pubescent below. Ribs are prominent with petiolules barely thickened. Both have elongated pubescent ovaries with terminal stigmas that are globular and pencillate at the base. There are a few key morphological differences between the two that forms the basis for their separation. Chief among them is the difference in pod and seed structure. Pods tend to be flattened and oblong in *Pueraria.* However *P. phaseoloides* has round cylindrical pods with rounded barrel shaped seeds as opposed to the flattened ovoid seeds found in *Pueraria. P. phaseoloides* also prefers a tropical low humidity and altitude environment while species like *P. montana* prefer warm to temperate high altitude environments (Heider et al., 2007). *Pueraria phaseoloides* started as Roxburgh's *Dolichos phaseoloides* (Bentham, 1867), however Bentham established the genus *Neustanthus* for *Dolichos phaseoloides* (Bentham, 1852). His rational for

creating the new genus *Neustanthus* rather than merging *Dolichos phaseoloides* with *Pueraria* was the presence of non-articulating pods on *D. phaseoloides.* In 1867 upon further review of many specimens of *P. tuberosa* he concluded that the original specimen drawing must have had the non-articulating pod added by the artist. Because of the lack of a non-articulating pod Bentham later merged his genus of *Neustanthus* into *Pueraria* (Bentham, 1867).

P. phaseoloides exhibits a close alliance with the genus *Sinodolichos.* It is interesting to note that *Sinodolichos* means "China"-*Dolichos* (Allen et al., 1981) because *Pueraria phaseoloides* was originally *Dolichos phaseoloides* (Roxburgh, 1832). *Sinodolichos* can be described as a perennial twining herb, with axillary racemes, ovate bracts, a campanulate calyx, an orbicular standard, an obovate-oblong keel, and linear-oblong legumes. *P. phaseoloides* on the other hand is an herbaceous vine, with solitary racemes, linear-lanceolate bracts, a pilose calyx, a suborbicular standard, a falcate keel, and cylindrical legumes. Based on these morphological differences and considerable molecular distance based on the fairly long branch lengths on the phylogenies (Figures 5 & 8), which separate these two clades, we propose the creation of a separate genus for *P. phaseoloides.* The work of Bentham sets the precedence for the name of that genus to be *Neustanthus.* This genus will accommodate *N. phaseoloides, N. phaseoloides var. subspicata,* and *N. phaseoloides var. javanica.*

***P. stricta Clade*—**Kurz first described *Pueraria stricta* in 1873 along with two other *Pueraria* specimens *P. hirsuta* and *P. brachycarpa.* Both of the latter epithets are now recognized as synonyms of *P. stricta. P. stricta* is unique within *Pueraria* because it is a straggling shrub with flattened pods containing 5-10 seeds per pod, with soft hooked bracts that are more or less pubescent. It is thought that *P. colletii* might be the closest relative (Prain, 1897), however, under the most recent treatment by van der Maesen (2002), it too is recognized

as a synonym of *P. stricta.* Lackey proposed that *P. colletii* may be allied to *Neonotonia* based on the presence of Canavanine (Lackey, 1977), and as a synonym this would mean the same for *P. stricta.* However it differs in the areas of calyx shape, flower size and shape, pod size and shape, and inflorescence size (van der Maesen, 1985). In *P. stricta* inflorescences are axillary, many flowered with one main branch. It bears 4-6 flowers supported by soft bracts. Pods are flattened, oblong like *Pueraria* with failed ovules rarely constricting, diagonally striate, with 5-10 seeds and valves curling when ripe, with an interior lined with a thin papery layer.

P. stricta at the molecular level, appears to have a close relationship for *Teyleria.* However, there are morphological differences that would point to keeping them apart. *P. stricta* is a shrub while *Teyleria* is considered an herb, *P. stricta* also has a single branch axial inflorescence as opposed to having irregular branches at the lower parts of the inflorescence. *P. stricta* also has elongated ovaries instead of sessile. Based on the morphology and the nodal support values for *P. stricta* we propose the creation of an entirely new and separate genus. Since the *P. stricta* type was initially described in *Pueraria,* there is no precedence for the genus name.

***P. peduncularis Clade*—**Bentham first described *Pueraria peduncularis* in 1867. In figures 5 & 8, *P. peduncularis* is shown as strongly grouping with *P. yunnanensis*, a species long recognized as a synonym of *P. peduncularis* (e.g. Lackey 1977 and van der Maesen 1985) while others support its rank as a species (Le and Zhu, 2009) based on microscopic analyses of leaves and seeds. Like the rest of *Pueraria* it is a woody perennial climber with peltate stipules, striated petioles, ovate to rhomboid leaflets with the side ones obliquely so and an apex that is long to acuminate. It also has 2 bracteoles per flower, flattened oblong pods and flattened ovoid seeds. By all means it looks very much like a *Pueraria,* yet it forms its own clade with a long branch

length and very strong nodal support near the base of subtribe Glycininae. What does set it apart from its former peers is a corolla that is 2-3 times as long as the calyx, with slender and long pedicels. The flowers are not crowded with 4-7 per node and inflorescences unbranched with 1-2 per axil and seedpods that are flat and papery. *P. peduncularis* does have diadelphous stamens, a morphology only found in *P. imbricata* and *P. calycina.* There are other characteristics that combined with the small morphological differences begins to clarify the separation. Unlike other *Pueraria* species *P. peduncularis* lacks paraveinal mesophyll, a trait it shares with *P. wallichii* another species separate from *Pueraria* (Lackey, 1977). This suite of morphological differences relegate *P. peduncularis* be classified as a new genus.

P. wallichii Clade—*Pueraria wallichii* was first described in 1825 during the creation of *Pueraria* as the second member of the genus. *P. wallichii* shares with *Pueraria* striated petioles, leaflets that are pubescent below and whose apex are long-acuminate. It also has 2 bracteoles per flower, with monodelphous stamens. Chief among its differences is the fact that *P. wallichii* is a shrub instead of a climbing vine. Its stipules are very caducous rather than peltate. The corolla is less than twice as long as the calyx with the calyx lobes short to obtuse. Inflorescences can be either axillary or terminal and seedpods have a somewhat S shape. Like *P. phaseoloides* it has a distinctly different seed shape, having brown with black mosaic reniform shaped seeds.

P. wallichii is morphologically distinct from *Pueraria,* and it's interesting how things might have been if de Candolle had designated it as the type rather than *P. tuberosa.* The removal of *P. wallichii* along with *P. stricta* from *Pueraria* helps to reinforce the description that *Pueraria* is strictly a climbing vine genus. Seed shape also serves as a reliable distinguishable characteristic as it is one of the main differences between *P. wallichii* and *P. phaseoloides. P. wallichii* also sees what is probably the strongest clade among our *Pueraria* species. Across all

trees it sees maximum nodal support for both RAxML and MrBayes. Coupled with morphological data, it becomes the strongest example for revision. Due to its creation stemming from the differences between *Hedysarum* and what would become *Pueraria* along with the results of our research we propose the creation of a new genus for this one species.

Thoughts on missing species—It is unfortunate that we were unable to sample all the species of *Pueraria*. *P. bella* is a very rare specimen found only in hard to reach places. Lackey suggested possibly transferring it to *Neonotonia* (Lackey 1977b) but van der Maesen (1985) presumed an alliance with *P. candollei* even though he stated that it keyed out with *P. montana.* Because of these diverging hypotheses, we reserve judgment as to the placement of *P. bella*. *P. bouffordiii* is a relatively new species described only recently (Ohashi, 2005), that shares a considerable number of morphological traits with members of the *Pueraria* clade where we are confident that it would be placed phylogenetically. *P. xyzhui* was also recently described in the Journal of Japanese Botany (Ohashi et al, 2006); pollen morphology places it within the *Pueraria* clade. *Pueraria maclurei* was first described in a Technical Bulletin of the U.S. Department of Agriculture (Hermann, 1962). Based on the fact that its basionym is *Glycine maclurei*, it seems likely that it would stay a member of *Pueraria*. *P. stracheyi* is described as herbaceous, flower pedicels nearly or quite as long as the calyx, having branches with short deciduous hairs, flowers in a raceme, leaflets membranous and very thin, and a reddish corolla that is distinctly spurred (Hooker, 1876). Lackey considered the possibility of it being a *Shuteria* (Lackey, 1977) while van der Maesen simply noted Lackey's consideration (van der Maesen, 1985). Due to the brevity of the description and the lack of resources, we reserve judgment on where this species might place in our tree. *P. garhwalensis* was described in the Journal of the Bombay Natural History Society (Dangwal and Rawat, 1996). Specimens of this taxon were

formerly united with *Pueraria ferruginea*, a synonym of *Shuteria hirsuta.* Because of this, it is possible that this species could be united with either *Shuteria* or the *P. phaseoloides* clade. We reserve judgment as to its placement until we can examine specimens therefrom.

***Conclusion*—**In conclusion, our phylogenetic study shows strong support for the polyphyly of the genus *Pueraria* as it is currently described. Lackey and van der Maesen both had some of their classifications correct, but ultimately neither one was entirely accurate (Figure 10). Lackey did correctly classify the relatedness of the species that would come to form what we propose is the correct makeup of the genus *Pueraria.* We propose the need to resurrect *Neustanthus* to include *P. phaseoloides* and its varieties, and to create three new genera to accommodate *P. stricta, P. peduncularis, and P. wallichii.* The newly circumscribed *Pueraria* will contain *P. montana* and its varieties along with *P. pulcherrima, P. alopecuroides, P. candollei, P. imbricata, P. sikkimensis, P. edulis* and *P. calycina.*

Table 1. Recognized *Pueraria* species from 3 treatments; varietal epithets are not listed.

Classification according to Lackey (1977b) with spelling and authorships therefrom:
Group A: *P. calycina* Franchet; *P. mirifica* Airy Shaw & Suvatabandhu; *P. lobata* (Willd.) Ohwia; *P. edulus* Pampan; *P. montana* (Lour.) Merr.; *P. candollei* Grah.; *P. tuberosa* DC.; *P. lacei* Craib; *P. alopecuroides* Craib; *P. sikkimensi* Prain; *P. pulcherrima* (Merr.) Merr.
Group B: *P. subspicata* Benth. *P. phaseoloides* (Roxb.) Benth.
Group C: *P. colletii* Prain; *P. brachycarpa* Kurz; *P. bella* Prain; *P. stricta* Kurz
Group D: *P. wallichii* DC.; *P. peduncularis* Grah.; *P. stracheyi* Bak.
Additional species recognized by either van der Maesen (1994) or Wu & Thulin (2010) (some may be synonyms): *P. imbricata* van der Maesen; *P. rigens* Craib; *P. maesenii* Niyomdham; *P. bouffordii* H. Ohashi; *P. xyzhui* H. Ohashi & Iokawa.

Table 2. Modeltest results for the AS2 exon partition.

* MODEL SELECTION UNCERTAINTY : Akaike Weights

Model	-lnL	K	AIC	delta	weight	cumWeight
TrN+I+G	3383.4871	7	6780.9741	0.0000	0.5474	0.5474
TIM+I+G	3383.3533	8	6782.7065	1.7324	0.2302	0.7776
GTR+I+G	3382.2148	10	6784.4297	3.4556	0.0973	0.8748
HKY+I+G	3386.4460	6	6784.8921	3.9180	0.0772	0.9520
K81uf+I+G	3386.3120	7	6786.6240	5.6499	0.0325	0.9845
TVM+I+G	3385.0500	9	6788.1001	7.1260	0.0155	1.0000
TVMef+I+G	3397.9580	6	6807.9160	26.9419	7.73e-07	1.0000
SYM+I+G	3397.6340	7	6809.2681	28.2939	3.93e-07	1.0000
K80+I+G	3405.2346	3	6816.4692	35.4951	1.07e-08	1.0000
TrNef+I+G	3404.2712	4	6816.5425	35.5684	1.03e-08	1.0000
TIMef+I+G	3404.1218	5	6818.2437	37.2695	4.42e-09	1.0000
K81+I+G	3405.1335	4	6818.2671	37.2930	4.37e-09	1.0000
GTR+G	3409.2361	9	6836.4722	55.4980	4.86e-13	1.0000
TrN+G	3412.2451	6	6836.4902	55.5161	4.82e-13	1.0000
TVM+G	3411.0181	8	6838.0361	57.0620	2.23e-13	1.0000
TIM+G	3412.2395	7	6838.4790	57.5049	1.78e-13	1.0000
HKY+G	3414.4473	5	6838.8945	57.9204	1.45e-13	1.0000
K81uf+G	3414.4451	6	6840.8901	59.9160	5.34e-14	1.0000
SYM+G	3423.8318	6	6859.6636	78.6895	4.48e-18	1.0000
TVMef+G	3424.8801	5	6859.7603	78.7861	4.27e-18	1.0000
TrNef+G	3430.9829	3	6867.9658	86.9917	7.05e-20	1.0000
TIMef+G	3430.9414	4	6869.8828	88.9087	2.70e-20	1.0000
K80+G	3432.9695	2	6869.9390	88.9648	2.63e-20	1.0000
K81+G	3432.9314	3	6871.8628	90.8887	1.00e-20	1.0000
HKY+I	3488.4351	5	6986.8701	205.8960	1.40e-45	1.0000
TVM+I	3485.5750	8	6987.1499	206.1758	1.40e-45	1.0000
TrN+I	3488.4353	6	6988.8706	207.8965	0.00e+00	1.0000
K81uf+I	3488.4412	6	6988.8823	207.9082	0.00e+00	1.0000
GTR+I	3485.5410	9	6989.0820	208.1079	0.00e+00	1.0000
TIM+I	3488.4189	7	6990.8379	209.8638	0.00e+00	1.0000
F81+I+G	3501.2205	5	7012.4409	231.4668	0.00e+00	1.0000
SYM+I	3503.8784	6	7019.7568	238.7827	0.00e+00	1.0000
TrNef+I	3507.6565	3	7021.3130	240.3389	0.00e+00	1.0000
TIMef+I	3507.6294	4	7023.2588	242.2847	0.00e+00	1.0000
TVMef+I	3509.5403	5	7029.0806	248.1064	0.00e+00	1.0000
K80+I	3513.2354	2	7030.4707	249.4966	0.00e+00	1.0000

Table 3. Modeltest results for the AS2 intron partition.

* MODEL SELECTION UNCERTAINTY : Akaike Weights

Model	-lnL	K	AIC	delta	weight	cumWeight
K81uf+G	6539.8955	6	13091.7910	0.0000	0.3327	0.3327
TIM+G	6539.6611	7	13093.3223	1.5312	0.1547	0.4874
HKY+G	6541.8931	5	13093.7861	1.9951	0.1227	0.6101
K81uf+I+G	6539.8955	7	13093.7910	2.0000	0.1224	0.7324
TIM+I+G	6539.6611	8	13095.3223	3.5312	0.0569	0.7894
TrN+G	6541.7002	6	13095.4004	3.6094	0.0547	0.8441
HKY+I+G	6541.8931	6	13095.7861	3.9951	0.0451	0.8892
TVM+G	6539.8955	8	13095.7910	4.0000	0.0450	0.9343
GTR+G	6539.6470	9	13097.2939	5.5029	0.0212	0.9555
TrN+I+G	6541.7002	7	13097.4004	5.6094	0.0201	0.9756
TVM+I+G	6539.8955	9	13097.7910	6.0000	0.0166	0.9922
GTR+I+G	6539.6470	10	13099.2939	7.5029	0.0078	1.0000
SYM+G	6552.6484	6	13117.2969	25.5059	9.63e-07	1.0000
SYM+I+G	6552.6484	7	13119.2969	27.5059	3.54e-07	1.0000
TIMef+G	6557.4502	4	13122.9004	31.1094	5.84e-08	1.0000
TrNef+G	6558.8511	3	13123.7021	31.9111	3.91e-08	1.0000
TIMef+I+G	6557.4502	5	13124.9004	33.1094	2.15e-08	1.0000
TrNef+I+G	6558.8511	4	13125.7021	33.9111	1.44e-08	1.0000
TVMef+G	6558.7734	5	13127.5469	35.7559	5.72e-09	1.0000
TVMef+I+G	6558.7734	6	13129.5469	37.7559	2.11e-09	1.0000
K81+G	6563.8262	3	13133.6523	41.8613	2.70e-10	1.0000
K80+G	6565.2173	2	13134.4346	42.6436	1.83e-10	1.0000
K81+I+G	6563.8262	4	13135.6523	43.8613	9.95e-11	1.0000
K80+I+G	6565.2173	3	13136.4346	44.6436	6.73e-11	1.0000
GTR+I	6835.0352	9	13688.0703	596.2793	0.00e+00	1.0000
TVM+I	6836.5615	8	13689.1230	597.3320	0.00e+00	1.0000
TIM+I	6841.3730	7	13696.7461	604.9551	0.00e+00	1.0000
K81uf+I	6842.5347	6	13697.0693	605.2783	0.00e+00	1.0000
TrN+I	6843.5181	6	13699.0361	607.2451	0.00e+00	1.0000
HKY+I	6844.6094	5	13699.2188	607.4277	0.00e+00	1.0000
TIMef+I	6875.3892	4	13758.7783	666.9873	0.00e+00	1.0000
TrNef+I	6876.6504	3	13759.3008	667.5098	0.00e+00	1.0000
SYM+I	6874.8750	6	13761.7500	669.9590	0.00e+00	1.0000
F81+G	6880.2744	4	13768.5488	676.7578	0.00e+00	1.0000
F81+I+G	6879.9658	5	13769.9316	678.1406	0.00e+00	1.0000
K81+I	6887.0317	3	13780.0635	688.2725	0.00e+00	1.0000

Table 4. Modeltest results for the AS2 total gene region. Model selected for analysis highlighted.

* MODEL SELECTION UNCERTAINTY : Akaike Weights

Model	-lnL	K	AIC	delta	weight	cumWeight
TrN+I+G	3395.4790	7	6804.9580	0.0000	0.4490	0.4490
TIM+I+G	3395.4114	8	6806.8228	1.8647	0.1768	0.6258
HKY+I+G	3397.5176	6	6807.0352	2.0771	0.1589	0.7847
GTR+I+G	3393.8411	10	6807.6821	2.7241	0.1150	0.8997
K81uf+I+G	3397.4563	7	6808.9126	3.9546	0.0622	0.9619
TVM+I+G	3395.9463	9	6809.8926	4.9346	0.0381	1.0000
TVMef+I+G	3409.6025	6	6831.2051	26.2471	8.97e-07	1.0000
SYM+I+G	3408.9829	7	6831.9658	27.0078	6.13e-07	1.0000
K80+I+G	3416.5081	3	6839.0161	34.0581	1.81e-08	1.0000
TrNef+I+G	3415.6079	4	6839.2158	34.2578	1.63e-08	1.0000
K81+I+G	3416.4102	4	6840.8203	35.8623	7.33e-09	1.0000
TIMef+I+G	3415.5139	5	6841.0278	36.0698	6.60e-09	1.0000
TVM+G	3420.1436	8	6856.2871	51.3291	3.21e-12	1.0000
GTR+G	3419.3833	9	6856.7666	51.8086	2.52e-12	1.0000
HKY+G	3423.8469	5	6857.6938	52.7358	1.59e-12	1.0000
TrN+G	3423.2800	6	6858.5601	53.6021	1.03e-12	1.0000
K81uf+G	3423.8469	6	6859.6938	54.7358	5.84e-13	1.0000
TIM+G	3423.2769	7	6860.5537	55.5957	3.80e-13	1.0000
SYM+G	3433.6123	6	6879.2246	74.2666	3.35e-17	1.0000
TVMef+G	3435.7456	5	6881.4912	76.5332	1.08e-17	1.0000
TrNef+G	3441.1030	3	6888.2061	83.2480	3.76e-19	1.0000
TIMef+G	3441.0830	4	6890.1660	85.2080	1.41e-19	1.0000
K80+G	3444.2959	2	6892.5918	87.6338	4.20e-20	1.0000
K81+G	3444.2551	3	6894.5103	89.5522	1.61e-20	1.0000
TVM+I	3492.4153	8	7000.8306	195.8726	1.32e-43	1.0000
HKY+I	3495.5210	5	7001.0420	196.0840	1.18e-43	1.0000
GTR+I	3492.3472	9	7002.6943	197.7363	5.18e-44	1.0000
TrN+I	3495.4429	6	7002.8857	197.9277	4.76e-44	1.0000
K81uf+I	3495.5308	6	7003.0615	198.1035	4.34e-44	1.0000
TIM+I	3495.4429	7	7004.8857	199.9277	1.68e-44	1.0000
SYM+I	3511.0344	6	7034.0688	229.1108	0.00e+00	1.0000
F81+I+G	3512.0730	5	7034.1460	229.1880	0.00e+00	1.0000
TrNef+I	3515.2075	3	7036.4150	231.4570	0.00e+00	1.0000
TIMef+I	3515.2061	4	7038.4121	233.4541	0.00e+00	1.0000
TVMef+I	3517.4927	5	7044.9854	240.0273	0.00e+00	1.0000
K80+I	3521.5559	2	7047.1118	242.1538	0.00e+00	1.0000

Table 5. Modeltest results for the matK total gene region. Model selected for analysis highlighted.

* MODEL SELECTION UNCERTAINTY : Akaike Weights

Model	-lnL	K	AIC	delta	weight	cumWeight
GTR+G	10223.0791	9	20464.1582	0.0000	0.3110	0.3110
TVM+G	10224.1973	8	20464.3945	0.2363	0.2764	0.5874
GTR+I+G	10222.4209	10	20464.8418	0.6836	0.2210	0.8084
TVM+I+G	10223.5635	9	20465.1270	0.9688	0.1916	1.0000
TIM+G	10249.5068	7	20513.0137	48.8555	7.66e-12	1.0000
TIM+I+G	10248.8721	8	20513.7441	49.5859	5.31e-12	1.0000
K81uf+G	10251.4805	6	20514.9609	50.8027	2.89e-12	1.0000
K81uf+I+G	10250.8223	7	20515.6445	51.4863	2.05e-12	1.0000
TrN+G	10309.4131	6	20630.8262	166.6680	2.00e-37	1.0000
TrN+I+G	10308.8281	7	20631.6562	167.4980	1.32e-37	1.0000
HKY+G	10311.3555	5	20632.7109	168.5527	7.80e-38	1.0000
HKY+I+G	10310.7363	6	20633.4727	169.3145	5.33e-38	1.0000
GTR+I	10315.8691	9	20649.7383	185.5801	1.57e-41	1.0000
TVM+I	10318.0508	8	20652.1016	187.9434	4.80e-42	1.0000
TIM+I	10343.8330	7	20701.6660	237.5078	0.00e+00	1.0000
K81uf+I	10345.5605	6	20703.1211	238.9629	0.00e+00	1.0000
F81+G	10391.7295	4	20791.4590	327.3008	0.00e+00	1.0000
F81+I+G	10391.1621	5	20792.3242	328.1660	0.00e+00	1.0000
TVMef+G	10402.6270	5	20815.2539	351.0957	0.00e+00	1.0000
TVMef+I+G	10402.0918	6	20816.1836	352.0254	0.00e+00	1.0000
SYM+G	10402.4365	6	20816.8730	352.7148	0.00e+00	1.0000
SYM+I+G	10401.8994	7	20817.7988	353.6406	0.00e+00	1.0000
K81+G	10406.6670	3	20819.3340	355.1758	0.00e+00	1.0000
K81+I+G	10406.1465	4	20820.2930	356.1348	0.00e+00	1.0000
TIMef+G	10406.3896	4	20820.7793	356.6211	0.00e+00	1.0000
TIMef+I+G	10405.8652	5	20821.7305	357.5723	0.00e+00	1.0000
TrN+I	10410.0723	6	20832.1445	367.9863	0.00e+00	1.0000
HKY+I	10411.5488	5	20833.0977	368.9395	0.00e+00	1.0000
TVM	10423.9902	7	20861.9805	397.8223	0.00e+00	1.0000
GTR	10423.4414	8	20862.8828	398.7246	0.00e+00	1.0000
K80+G	10442.2939	2	20888.5879	424.4297	0.00e+00	1.0000
K80+I+G	10441.7803	3	20889.5605	425.4023	0.00e+00	1.0000
TrNef+G	10442.0156	3	20890.0312	425.8730	0.00e+00	1.0000
TrNef+I+G	10441.4961	4	20890.9922	426.8340	0.00e+00	1.0000
K81uf	10481.0908	5	20972.1816	508.0234	0.00e+00	1.0000
TIM	10480.1992	6	20972.3984	508.2402	0.00e+00	1.0000

Figure 1. Native (black) and introduced (green; primarily *P. lobata* and *P. phaseoloides*) range of *Pueraria*. Modified from van der Maesen 1985.

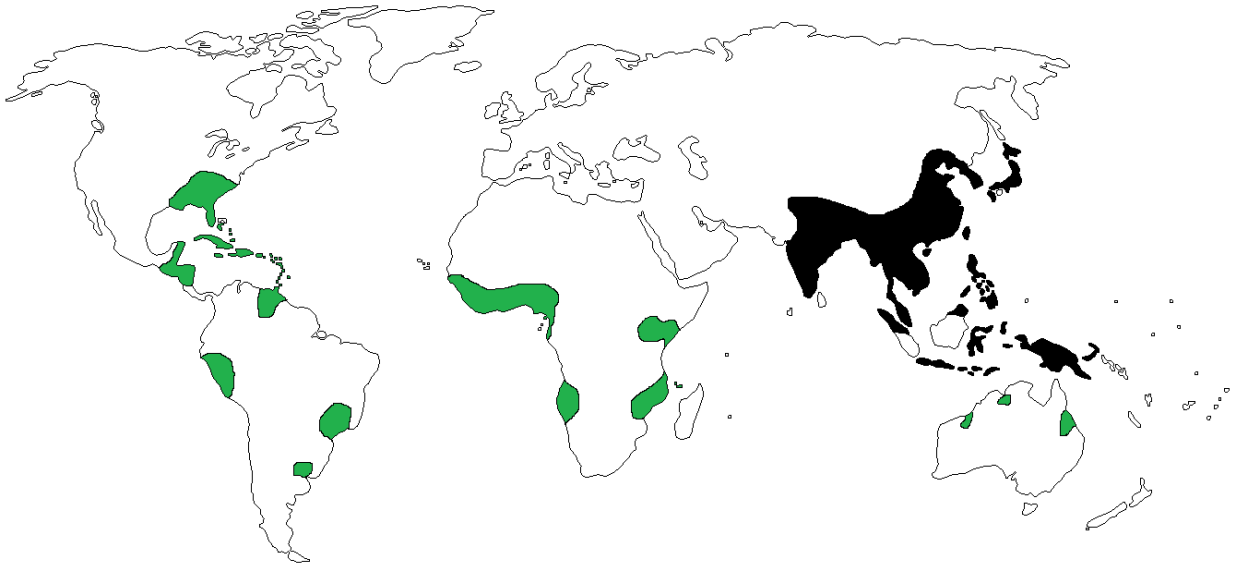

Figure 2. Lackey's (1977) morphologically based groups (left) vs. van der Maesen's (1985) (right).

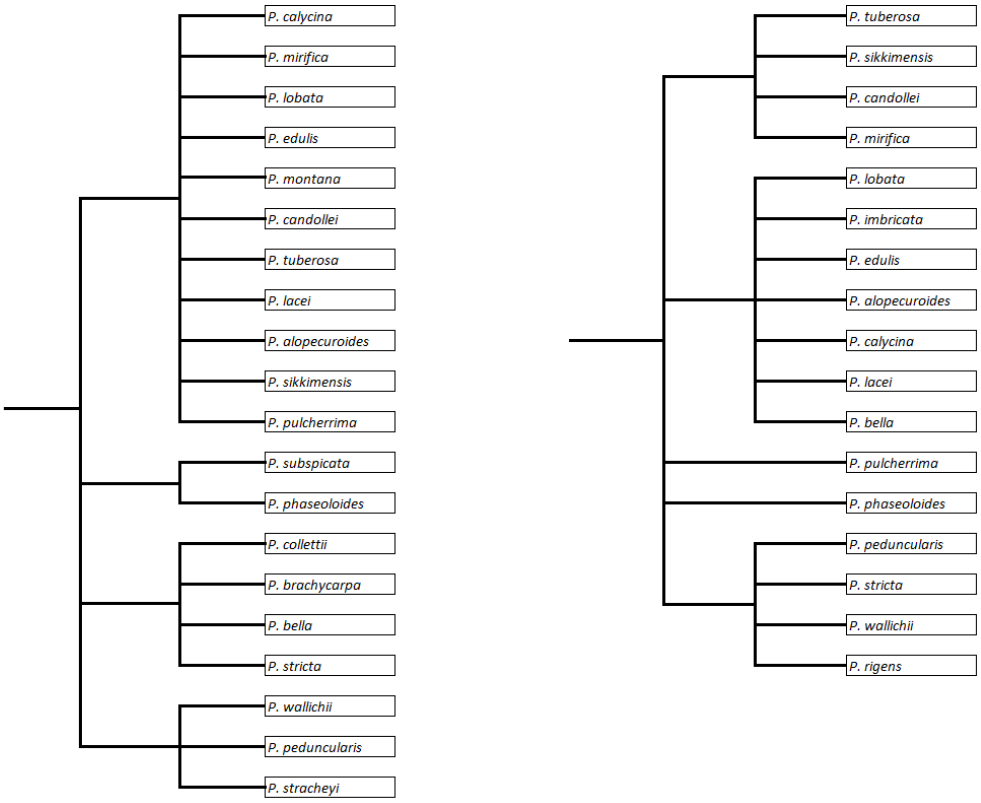

Figure 3: Domain organization and characteristic features of *AS2*. The exons are color coded in green, the intron in red, the C-motif in blue, and the zipper-like-motif in purple.

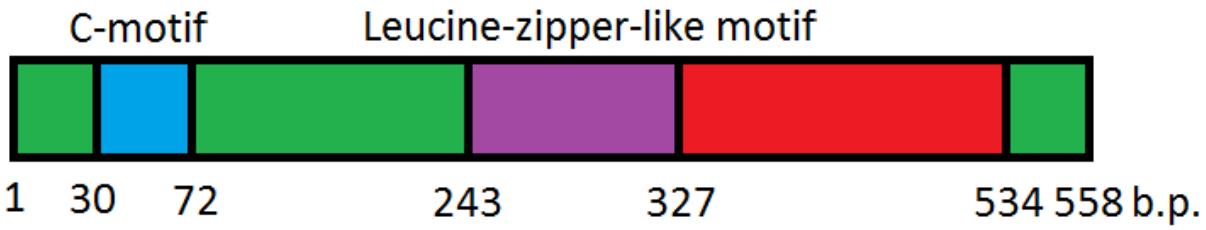

Figure 4. Phylogenetic relationships of *Pueraria* in the context of phaseoloid legumes based on Bayesian Inference with simple indel coding and partitioning for the *AS2* exon and intron. Posterior probabilities shown near each node. *P. wallichii* clade highlighted in blue. Subtribe Glycininae (Figure 5) connects to the top of this figure.

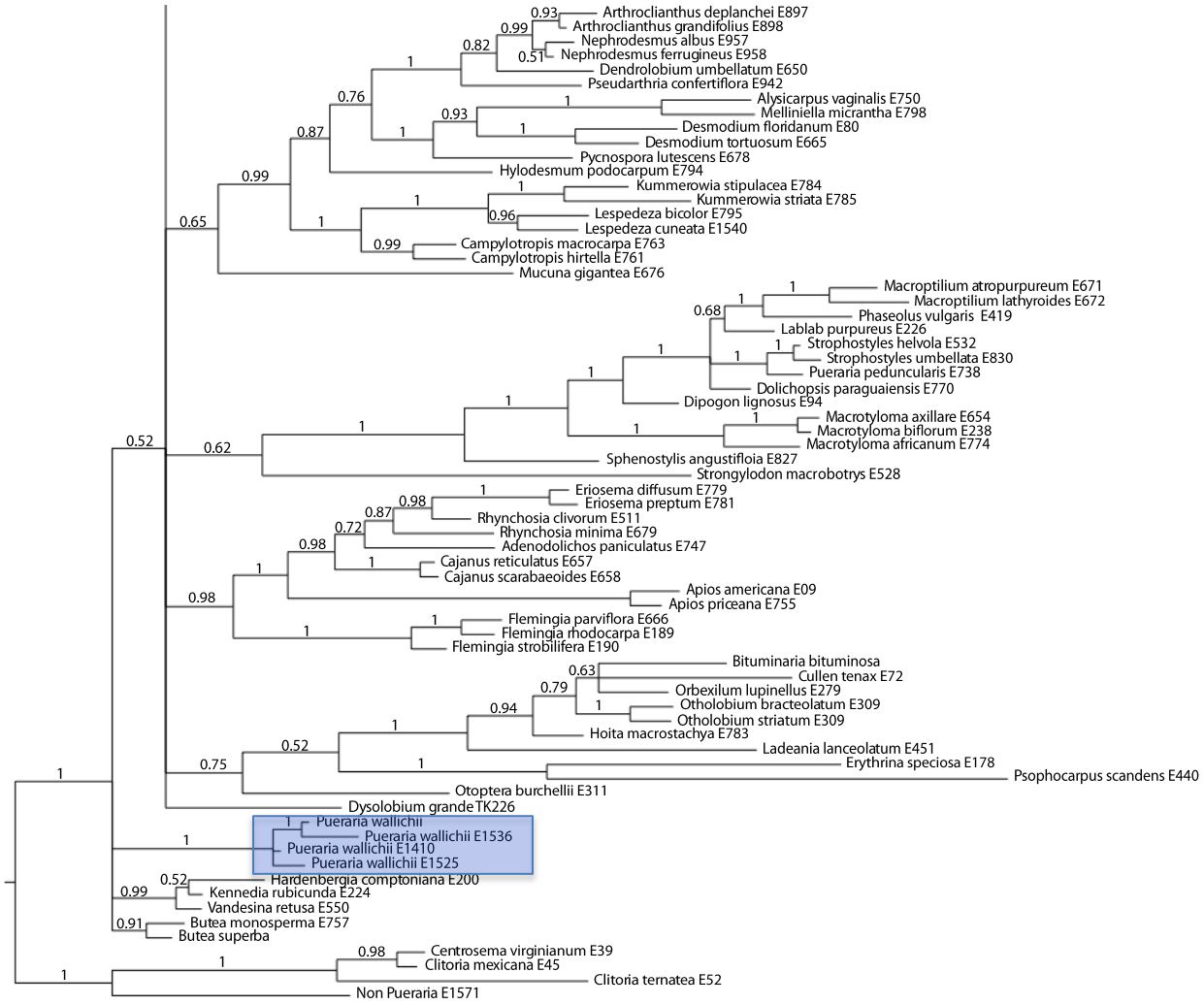

Figure 5. Phylogenetic relationships of *Pueraria* in the context of phaseoloid legumes based on Bayesian Inference with simple indel coding and partitioning for the *AS2* exon and intron. Subtribe Glycininae is shown here with posterior probabilities shown near each node. The phylogeny continues by connecting at the bottom to Figure 4. The *Pueraria* clade is highlighted in green, the *P. phaseoloides* clade in teal, *P. stricta* clade in red, and the *P. peduncularis* clade in purple.

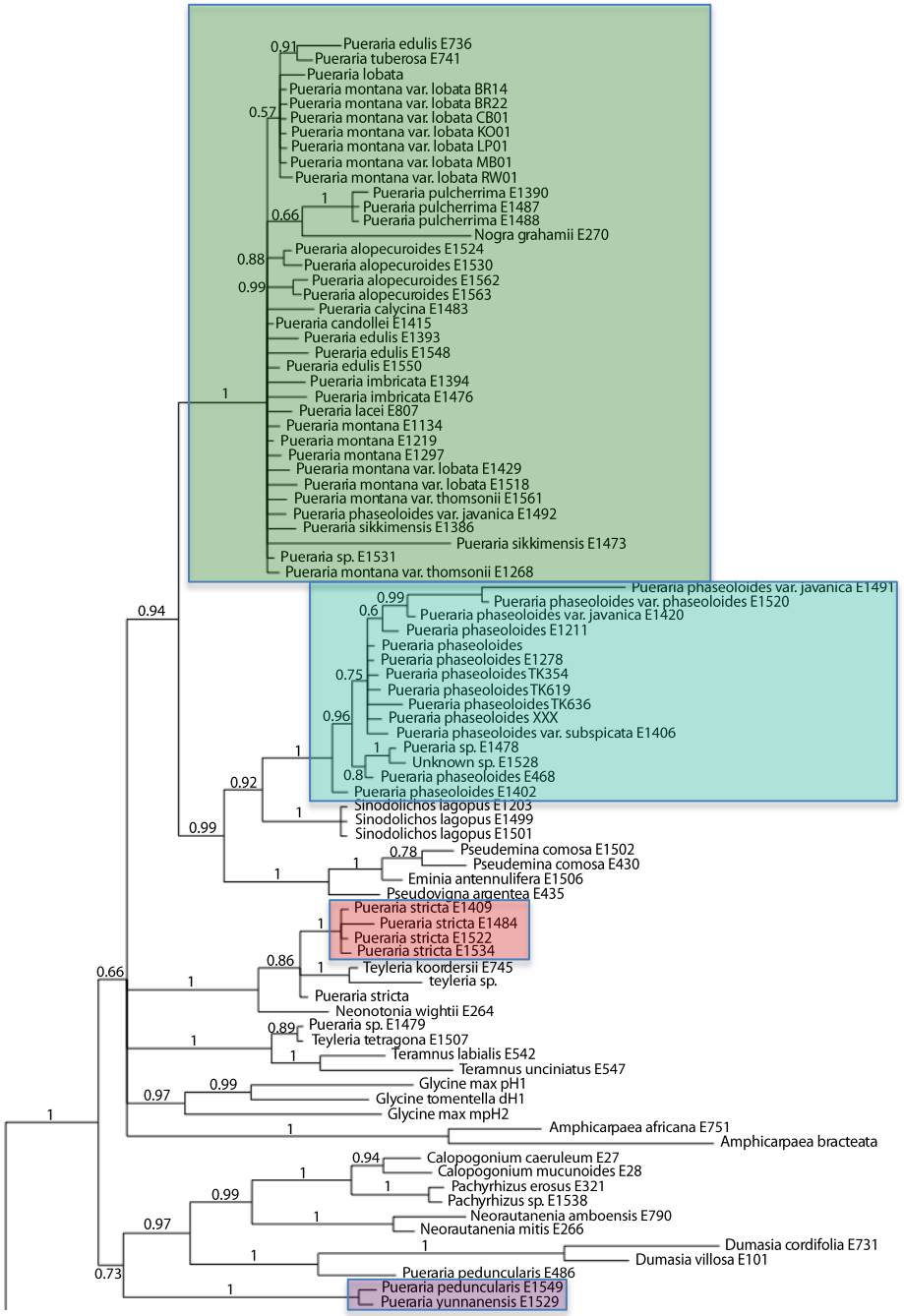

Figure 6. Network analysis via Splitstree of the *AS2 Pueraria* clade.

Figure 7. Phylogenetic relationships of *Pueraria* within the context of phaseoloid legumes based on Bayesian Inference of the best *matK* run with total evidence and no gap coding. Posterior probability shown near each node. *P. wallichii* clade highlighted in blue. Subtribe Glycininae (Figure 8) connects to the top of this figure.

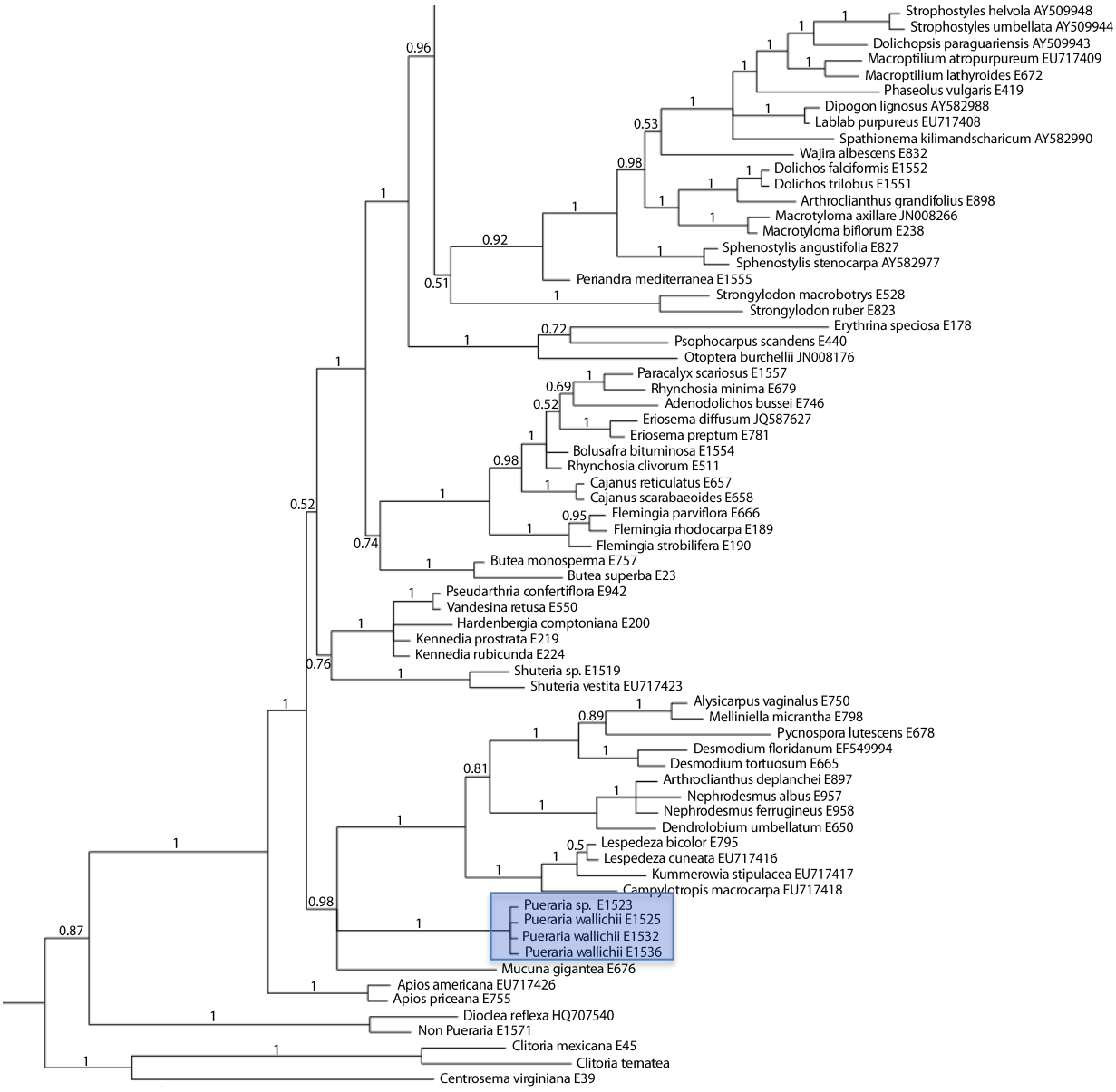

Figure 8. Phylogenetic relationships of *Pueraria* in the context of phaseoloid legumes based on Bayesian Inference of the best *matK* run with total evidence and no gap coding. Subtribe Glycininae and tribe Psoraleeae are shown here with posterior probabilities shown near each node. The phylogeny continues by connecting at the bottom to Figure 7. The *Pueraria* clade is highlighted in green, the *P. phaseoloides* clade in teal, *P. stricta* clade in red, and the *P. peduncularis* clade in purple.

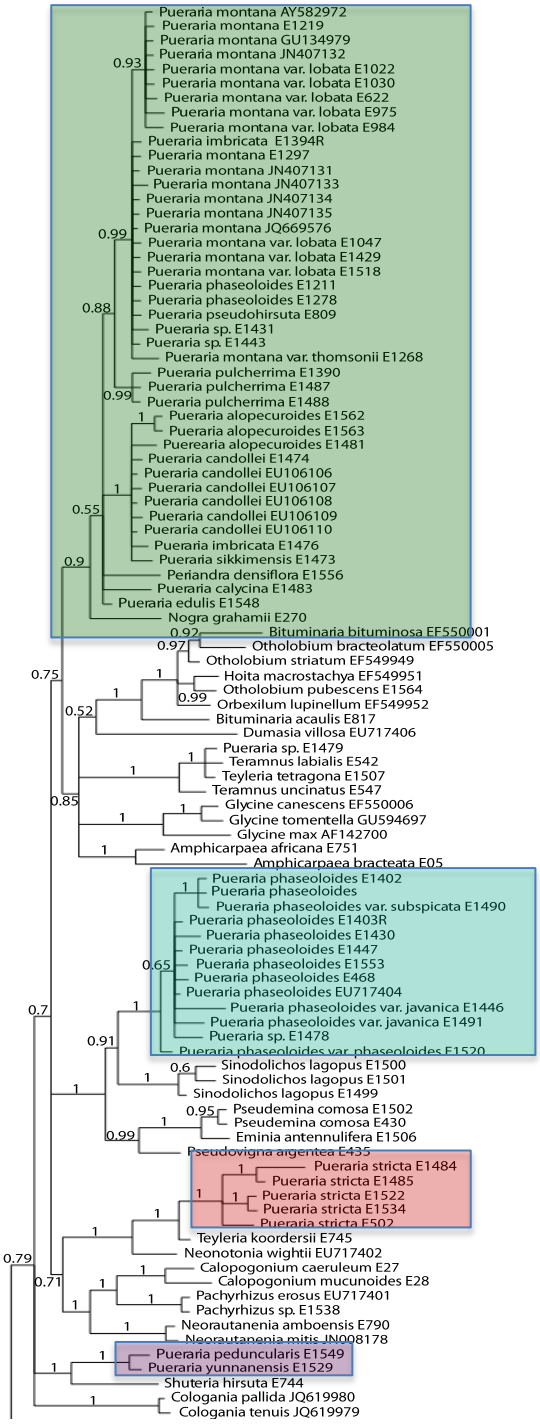

Figure 9. Network analysis via Splitstree of the *matK Pueraria* clade.

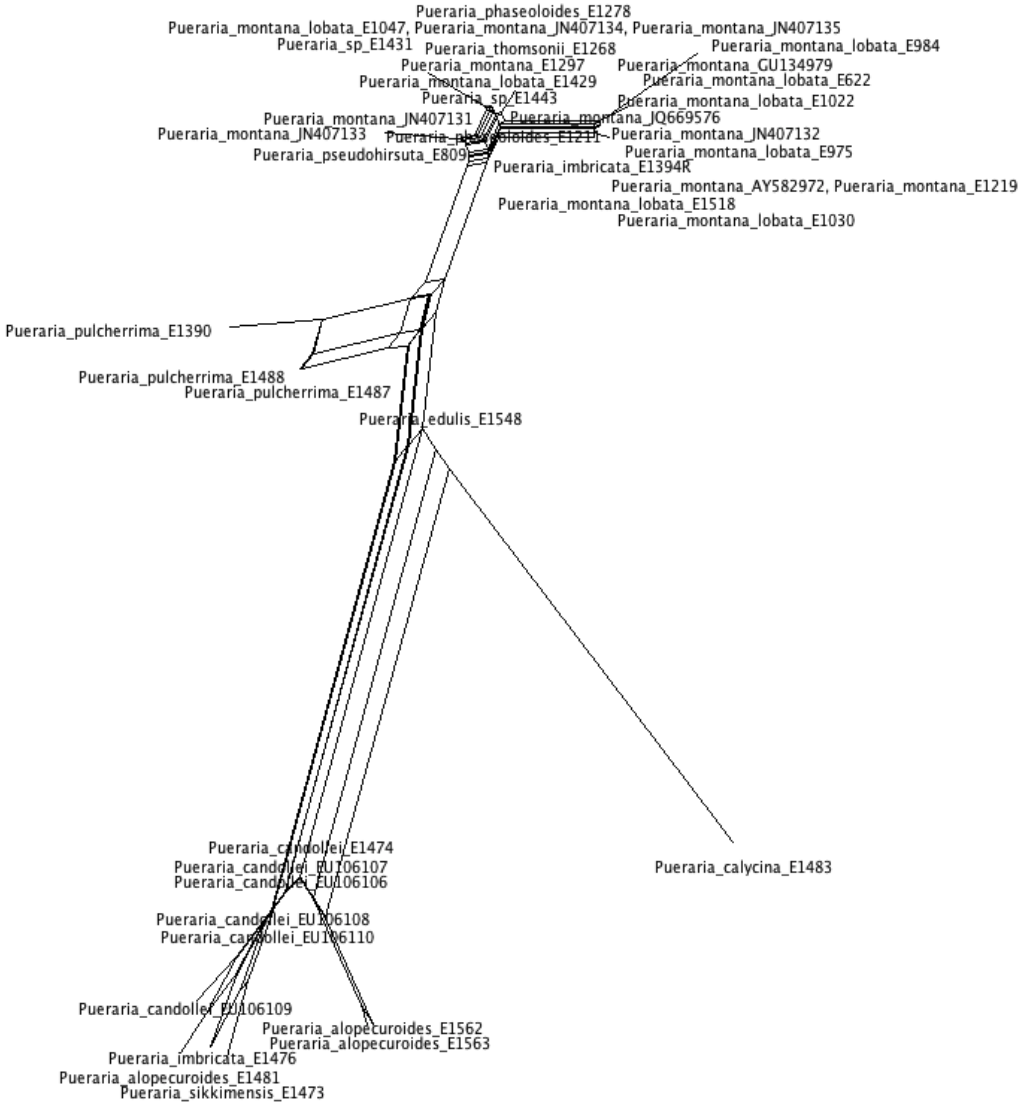

Figure 10. Graphical comparisons between the hypotheses of Lackey (1977), van der Maesen (1985), and our research results (shown from left to right in that order). Clades from our tree are highlighted in their corresponding colors.

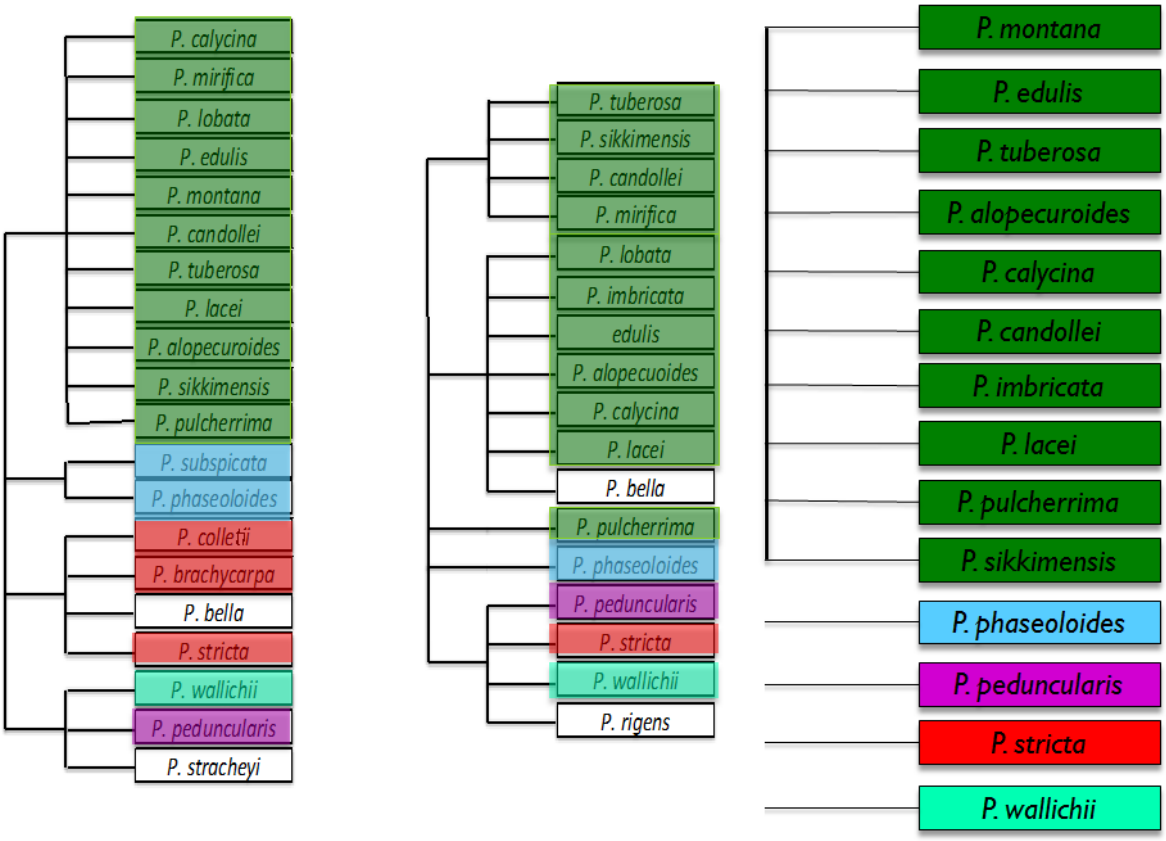

Fig 11. A map of the distinct variable morphological characteristics within the current description of the genus *Pueraria*. The top box lists the common characteristics that all members of our *Pueraria* analysis share. Colored lines matching the species or clade they correspond to trace the shared convergent evolutionary traits that have helped pave the way to the polyphyly present in the genus today.

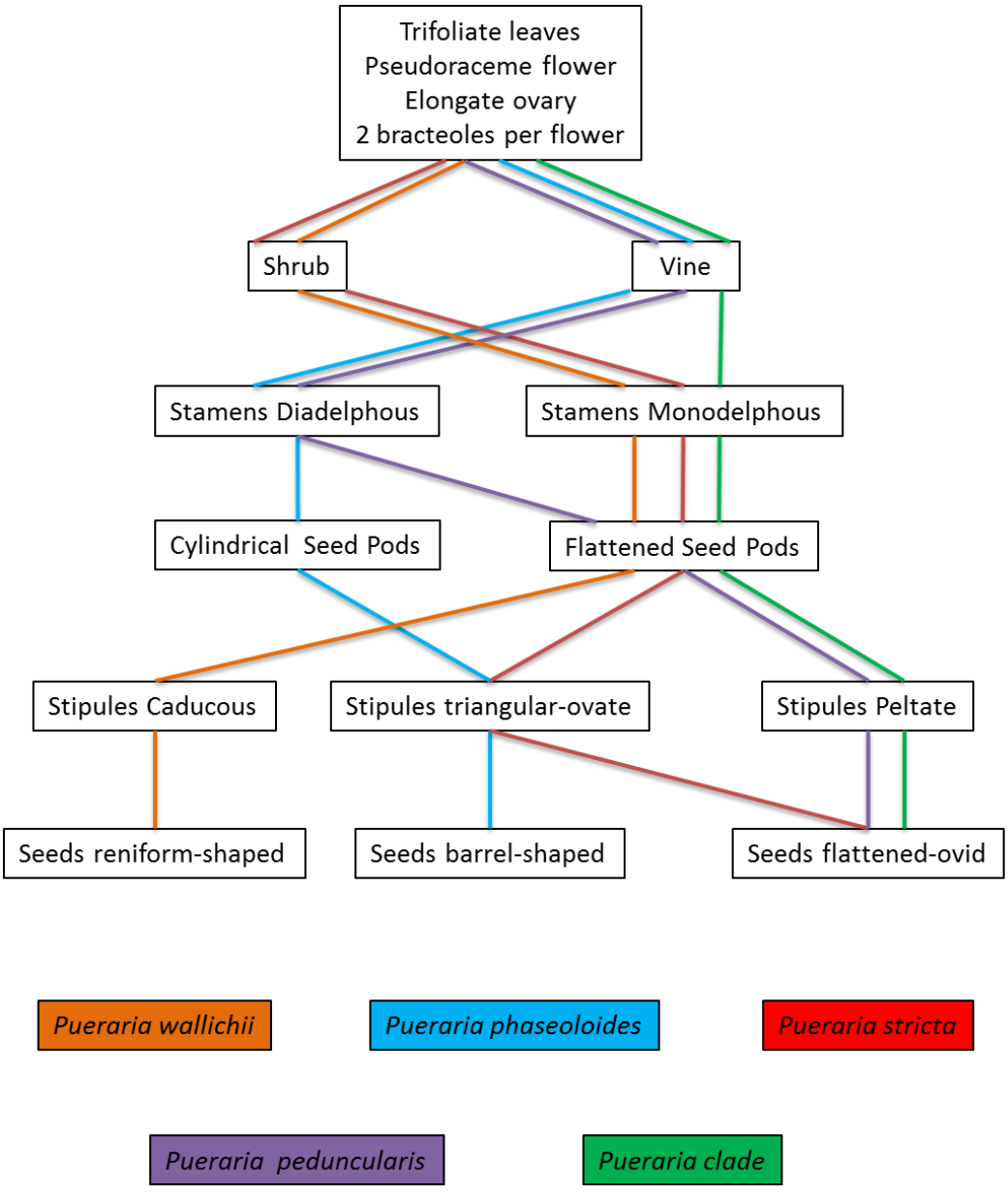

Fig ?. A map of the distinct variable morphological characteristics within the current description of the genus *Pueraria*. The top box lists the common characteristics that all members of our *Pueraria* analysis share. Colored lines matching the species or clade they correspond to trace the shared convergent evolutionary traits that have helped pave the way to the monophyly present in the genus today.

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Appendix A. Sample Collection Information.

Genus	Species	Sample	Voucher
Adenodolichos	*bussei*	E746	E.A. Robinson 6064
	paniculatus	E747	P.K. Rwaburindore 1500
Alysicarpus	*vaginalis*	E750	D.H. Lorence 9830
Amphicarpaea	*bracteata*	E05	ANE 326
	africana	E751	D. Arusho 24654
Apios	*americana*	E755	Unknown
	priceana	E755	A. Bruneau 254
Arthroclianthus	*deplanchei*	E897	J.N. Labat 3911
	grandifolius	E898	J.N. Labat 3918
Bituminaria	*acaulis*	E817	W.T. Stearn (K)
	bituminosa	E20	Unknown
Bolusafra	*bituminosa*	E1554	Egan
Butea	*monosperma*	E757	D. Neill 5220
	superba	E23	Ho 77.639
Cajanus	*scarabaeoides*	E658	ANE_11_57
	reticulatus	E657	ANE_11_37
Calopogonium	*caeruleum*	E27	Unknown
	mucunoides	E28	Unknown
Campylotropis	*hirtella*	E761	D.E. Boufford et al. 29343
	macrocarpa	E763	Wang Zhon-tao et al. 375
Centrosema	*virginianum*	E39	Unknown
Clitoria	*ternatea*	E52	VZ-1
	mexicana	E45	Bonet 53
Cullen	*tenax*	E72	246747
Dendrolobium	*umbellatum*	E650	ANE_11_48
Desmodium	*floridanum*	E80	Unknown
	tortuosum	E665	ANE_11_39
Dipogon	*lignosus*	E94	Doyle 1297
Dolichopsis	*paraguariensis*	E770	S.A. Renvoize 3552
Dolichos	*falciformis*	E1552	ANE 13-7
	trilobus	E1551	ANE 13-3
Dumasia	*cordifolia*	E731	Unknown
	villosa	E101	Unknown
Dysolobium	*grande*	TK226	Kajita
Eminia	*antennulifera*	E1506	S. Bidgood et al. 5302(K)
Eriosema	*diffusum*	E779	R. Aguilar 6667
	preptum	E781	H.J. Venter&A. Venter 10237
Erythrina	*speciosa*	E178	Anne Bruneau
Flemingia	*rhodocarpa*	E189	Zaire 9-11-87

	strobilifera	E190	Unknown
	parviflora	E666	PIF38257 - P.I. Forster
Glycine	*max*	GmpH2	
	max	GmpH1	
	tomentella	GtdH1	
Hardenbergia	*comptoniana*	E200	CHIL 664
Hoita	*macrostachya*	E783	L. Ahart 10420
Hylodesmum	*podocarpum*	E794	H. Ohashi & Y. Ohashi 61828
Kennedia	*prostrata*	E219	CHIL740
	rubicunda	E224	CBG-1
Kummerowia	*stipulacea*	E784	Guocheng-yong 20065-436-4
	striata	E785	W.P. Longbottom 14077
Lablab	*purpurues*	E226	Unknown
Ladeania	*lanceolata*	E451	Hartman 13554
Lespedeza	*bicolor*	E795	Guocheng-yong 200065-404-4
	cuneata	E1540	AN Egan
Macroptilium	*atropurpureum*	E671	ANE_11_5
	lathyroides	E672	ANE_11_58
Macrotyloma	*africanum*	E774	Z.L. Magombo et al. 72
	biflorum	E238	Seydel 2803
Melliniella	*micrantha*	E798	J.E. Madsen 5875
Mucuna	*gigantea*	E676	Holland_3002
Mysanthus	*uleanus*	E789	N.G. Jesus 858 et al.
Neonotonia	*wightii*	E264	VI 4
Neorautanenia	*amboensis*	E790	R. Seydel 1328 a
	mitis	E266	Belsky 505
Nephrodesmus	*albus*	E957	J.N. Labat 3932
	ferrugineus	E958	J.N. Labat 3910
Nogra	*grahamii*	E270	Unknown
Non Pueraria		E1571	12-278
Orbexilum	*lupinellus*	E279	Unknown
Otholobium	*bracteolatum*	E296	LL-Tex.
	pubescens	E1564	Salas 16136
	striatum	E309	LL-Tex.
Otoptera	*burchellii*	E311	Leistner 594
Pachyrhizus	*erosus*	E321	AE 511
	sp.	E1538	AN Egan 12-240
Paracalyx	*scariosus*	E1557	V.d. Maesen 2357
Periandra	*densiflora*	E1556	Jrwin et al 17057
	mediterranea	E1555	Jrwin eta 30424
Phaseolus	*vulgaris*	E419	CIAT 616798
Pseudarthria	*confertiflora*	E942	Kenya Chyulu hills
Pseudeminia	*comosa*	E430	Unknown

	comosa	E1502	Pocs, T. & Orban S. 89157/EK
Pseudovigna	*argentea*	E435	Unknown
Psophocarpus	*scandens*	E440	Unknown
Pueraria	*alopecuroides*	E1481	Sorenson, Th. Et al. 1651 K
	alopecuroides	E1524	PA1 AN Egan & Xubo JP1
	alopecuroides	E1530	AN Egan 12_273 Xubo JP1
	alopecuroides	E1562	JP10
	alopecuroides	E1563	PA5
	calycina	E1483	Forrest, G. 15312 K
	candollei	E1415	Phengkhlai 361 P02752679
	candollei var. mirifica	E1400	Maxwell 89-1075 1542*51
	edulis	E736	J.F. Rock 5412
	edulis	E1392	McLaren AA239 1542*48
	edulis	E1393	Groerspm 2689 1542*47
	edulis	E1548	ANE&Xubo 12*219 ML1
	edulis	E1550	Egan&Xubo 12*229
	imbricata	E1394	Maxwell 89-1349 1542*52
	imbricata	E1395	Maxwell 89-1284 1542*53
	imbricata	E1476	Larsen, K. & S.S. 34073 K
	montana	E1134	Kajita 56
	montana	E1219	Kajita 141
	montana	E1297	Kajita 219
	montana var. lobata	E622	ANE_11_108
	montana var. lobata	E809	G.Z. Li 214
	montana var. lobata	E975	ANE_12_15
	montana var. lobata	E984	ANE_12_24
	montana var. lobata	E1022	ANE_12_62
	montana var. lobata	E1030	ANE_12-70
	montana var. lobata	E1047	ANE_12-92
	montana var. lobata	E1429	Larsen 43761 P03065960
	montana var. lobata	E1518	Clark, R.P. 103 K
	montana var. thomsonii	E1268	Kajita 190
	montana var. thomsonii	E1561	DPS
	peduncularis	E486	Kajita
	peduncularis	E738	F. Kingdon-Ward 18838
	peduncularis	E1549	12-220
	phaseoloides	E468	Unknown
	phaseoloides	E1211	Kajita 133
	phaseoloides	E1278	Kajita 200
	phaseoloides	E1401	Croat 18299 1542*2
	phaseoloides	E1402	Pendry DNEP2 B55 1542*26
	phaseoloides	E1403	Mikage 9554138154225
	phaseoloides	E1430	Rudd 3315 P02961678

phaseoloides	E1447	Chan 124 P02961373
phaseoloides	E1533	AN Egan 12-254
phaseoloides	TK354	Kajita
phaseoloides	TK619	Kajita
phaseoloides	TK636	Kajita
phaseoloides	XXX	Unknown
phaseoloides var. javanica	E1404	Unknown
phaseoloides var. javanica	E1405	Unknown
phaseoloides var. javanica	E1420	Matras 29 P02752658
phaseoloides var. javanica	E1446	Larsen 32862 P02961679
phaseoloides var. javanica	E1491	Powell, D.A. & H'ng Kim Chey 655 K
phaseoloides var. javanica	E1492	Cramer, L.H. 5257 K
phaseoloides var. phaseoloides	E1520	David et al. CL729K
phaseoloides var. subspicata	E1416	Jasima s.n. P01733265
phaseoloides var. subspicata	E1407	Henry 13626 1542*6
phaseoloides var. subspicata	E1490	Yandall, T. 331 K
pulcherrima	E1389	Sayers 13281 1542*21
pulcherrima	E1390	Womersly 17807 1542*20
pulcherrima	E1487	Takeuchi, W. 7391 K
pulcherrima	E1488	Takeuchi, W. 7391 K
pulcherrima	E1489	Forster, P.I. & Liddle, D.J. PIF8672 K
rigens	E1385	Maxwell 91-700 1542*54
sikkimensis	E1386	Grierson 3625 1542*50
sikkimensis	E1473	Grierson, A.J.C. & Long, D.G. 3328 K
sp.	E1387	Poema 5839 1542*14
sp.	E1431	McKee 44875 P03065965
sp.	E1443	McKee 43488 P03065926
sp.	E1478	Sorenson et al. 5766 K
sp.	E1479	van Beusekom, C.F. et al 4183 K
sp.	E1523	Clark, R. P. 223
sp.	E1531	AN Egan 12-264
sp.	E1399	Cunningham 105 1542*39
stricta	E502	AE 509
stricta	E1534	AN Egan 12-255
stricta	E1409	Henry 10575 1542*30
stricta	E1484	McKee, H.S. 5891 K
stricta	E1485	Vogt, G.B. s.n. BU-445 K
stricta	E1522	Clark, R.P. 210 K
stricta	E1534	AN Egan 12-255
tuberosa	E741	(NY)

	wallichii	E1410	Stainton 8237 1542*35
	wallichii	E1525	AN Egan & Xubo 12*270
	wallichii	E1532	AN Egan 12-253
	wallichii	E1536	AN Egan & Xubo 12-256 MK3
	yunnanensis	E1529	AN Egan 12-262
Pycnospora	*lutescens*	E678	R. Jensen 1920
Rhynchosia	*clivorum*	E511	Unknown
	minima	E679	ANE_11_56
Shuteria	*hirsuta*	E744	F. Kingdon-Ward 17785
	sp.	E1519	Clark, R.P. 231 K
Sinodolichos	*lagopus*	E1203	Kajita 125
	lagopus	E1499	Collins, D.J. 1699 K
	lagopus	E1500	Larsen, K & S.S. 34479 K
	lagopus	E1501	Christensen, H. 481 K
Sphenostylis	*angustifolia*	E827	H.J. Venter & A. Venter 9880
Strongylodon	*macrobotrys*	E528	PTBG
	ruber	E823	V.J. Krajina 611028251
Strophostyles	*helvola*	E532	Doyle 1601
	umbellata	E830	D.M. Ferguson et al. 1120
Teramnus	*labialis*	E542	Unknown
	uncinatus	E547	322671 01 SD
Teyleria	*tetragona*	E1507	Garret, H.B.G. 1226 K
	koordersii	E745	K.S. Chow et al. 78227
	sp.	E549	CV-92
Unknown	*sp.*	E1528	AN Egan 12-262
Vandasina	*retusa*	E550	NWCL 602
Wajira	*albescens*	E832	Pasquet 1057

Appendix B. Sample information for *matK* sequences retrieved from GenBank.

Genus	Species	Genbank Acession #
Apios	*americana*	EU717426
Bituminaria	*bituminosa*	EF550001
Campylotropis	*macrocarpa*	EU717418
Cologania	*pallida*	JQ619980
Cologania	*tenuis*	JQ619979
Desmodium	*floridanum*	EF549994
Dioclea	*reflexa*	HQ707540
Dipogon	*lignosus*	AY582988
Dolichopsis	*paraguariensis*	AY509943
Eriosema	*diffusum*	JQ587627
Glycine	*canescens*	EF550006
Glycine	*max*	AF142700
Glycine	*tomentella*	GU594697
Hoita	*macrostachya*	EF549951
Kumerowia	*stipulacea*	EU717417
Lablab	*purpureus*	EU717408
Lespedeza	*cuneata*	EU717416
Macroptillium	*atropurpureum*	EU717409
Macrotyloma	*axillare*	JN008266
Neonotonia	*wightii*	EU717402
Neorautanenia	*mitis*	JN008178
Orbexilum	*lupinellum*	EF549952
Otholobium	*bracteolatum*	EF550005
Otholobium	*striatum*	EF549949
Otoptera	*burchellii*	JN008176
Pachyrhizus	*erosus*	EU717401
Pueraria	*candollei*	EU106106
Pueraria	*candollei*	EU106107
Pueraria	*candollei*	EU106108
Pueraria	*candollei*	EU106109
Pueraria	*candollei*	EU106110
Pueraria	*montana*	GU134979
Pueraria	*montana*	JN407131
Pueraria	*montana*	JN407132
Pueraria	*montana*	JN407133
Pueraria	*montana*	JN407134
Pueraria	*montana*	JN407135
Pueraria	*montana*	JQ669576
Shuteria	*vestita*	EU717423
Spathionema	*kilimandscharicum*	AY582990

Sphenostylis	*stenocarpa*	AY582977
Strophostyles	*helvola*	AY509948
Strophostyles	*umbellata*	AY509944