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PHYLOGENETIC ANALYSIS OF *MECONOPSIS* (PAPAVERACEAE) AND EVALUATION OF TWO CONTROVERSIAL TAXONOMIC SPECIES

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Abstract: *Meconopsis* is a genus native to the high elevation habitats that range from the western Himalaya eastward to the Hengduan Mountains (China). The genus has been the subject of several taxonomic treatments and monographs by generations of botanists, which has led to a long and confusing taxonomic history with inconsistent species concepts and conflicting interpretations of relationships among named taxa. In the present study, we reconstructed the evolutionary history of *Meconopsis* utilizing four chloroplast markers (*rbc*L, *mat*K, *ndh*F and the *trn*L-*trn*F intergenic spacer) and the nuclear ribosomal internal transcribed spacer (nrITS). Incongruence found between the cpDNA and nrITS trees was investigated to detect reticulate evolution, using the approximately unbiased (AU) method. Based on the evolutionary patterns revealed by our resultant phylogenies, we evaluated the species delimitations of the two most controversial "species" (*Meconopsis horridula* and *Meconopsis napaulensis*) in the genus and the inconsistency among their previously published treatments. As a result, we provide taxonomic suggestions for these species that include the proposal of a *M. horridula* species complex.

Keywords: Himalaya, blue poppies, phylogeny, taxonomy, species delimitation, species complex

Meconopsis Vig., also known as the Himalayan Poppy or Blue Poppy, is an Old World genus in the subfamily Papaveroideae of Papaveraceae. The genus occurs mainly at high altitudes (often exceeding 3500 meters) of the Himalaya, the Hengduan Mountains (southwest China), and the southeast Tibetan Plateau (Grey-Wilson, 2014). *Meconopsis* species are highly valued by indigenous cultures and some species are used in traditional herbal medicine (Kala, 2003). With delicate and exquisitely beautiful flowers, species of the genus have provided some of the most desirable horticultural plants in British gardens since they were first introduced in the early 20th Century (Taylor, 1934). The great morphological diversity in *Meconopsis* has been documented during a century of botanical exploration but translated into very different taxonomic systems. A series of early studies (Prain, 1895, 1906, 1915; Fedde, 1909, 1936; Kingdon-Ward, 1926, 1935) mostly focused on descriptions of new species. Taylor, in 1934, published the first monograph of *Meconopsis* in which he systematically examined a significant number of herbarium collections and reviewed previous species treatments in depth. Taylor's (1934) work was the first serious study of species delimitations and became the "standard" classification for *Meconopsis* that was widely followed until the recent taxonomic revisions were published by Grey-Wilson (2000, 2006, 2014). It is worth noting that Taylor (1934) accepted 41 species in his monograph while Grey-Wilson (2014) included 79. The large number discrepancy was primarily due to the authors' different philosophies of species concepts.

Grey-Wilson's disagreements with Taylor (1934) have mostly centered around *Meconopsis horridula* Hook.f. & Thomson (Grey-Wilson, 2000 & 2014) and *Meconopsis napaulensis* DC. (Grey-Wilson, 2006 & 2014). Taylor (1934) employed a broad concept of *M. horridula* from which Grey-Wilson (2000) segregated three species. In

LUNDELLIA 18:14–27. 2015

Grey-Wilson's recent monograph (2014), he included eleven species that are within the morphological range of *M. horridula sensu* Taylor (1934). Taylor (1934) stated he was unable to find a justifiable method to split his *M. horridula*. However, has Grey-Wilson's (2014) strategy led to several well-defined species? In this paper, we evaluate both Taylor's (1934) and Grey-Wilson's (2000, 2014) treatments of "*M. horridula*" in light of our phylogenetic results.

For *Meconopsis napaulensis*, Grey-Wilson (2006) concluded that Taylor (1934) did not sufficiently examine the type material and, therefore, mistakenly assigned specimens to *M. napaulensis*. Grey-Wilson (2006), consequently, proposed a new taxonomy for *M. napaulensis* and its allies that included descriptions of four new species and a reassignment of many of Taylor's "*napaulensis*" specimens to other species. This treatment (Grey-Wilson, 2006) was also kept in his recent book (Grey- Wilson, 2014). Egan (2011) described a new species *Meconopsis autumnalis* Egan that is morphologically similar to, and geographically overlapping with, *M. napaulensis sensu* Grey-Wilson (2006). *Meconopsis autumnalis* was also accepted and listed in Grey-Wilson's revision (2014) as well. These recently published species and the shuffling of epithets have caused a great deal of confusion and it has never been clear how these taxonomic entities relate to each other. Here, we clarify how Grey-Wilson's (2006, 2014) taxonomy of *M. napaulensis* differs from Taylor's (1934) by showing the phylogenetic relationships of the named taxa involved. We also provide an evaluation of Taylor (1934) and Grey-Wilson's species treatments for *M. napaulensis*.

The criterion we used for evaluating species circumscription is monophyly. To apply this criterion, we needed to understand the evolutionary history of *Meconopsis* which had not been resolved by an earlier preliminary molecular study (Yuan, 2002) that used only the *trn*L-*trn*F spacer and nrITS sequences. We provide here a robust phylogeny using sequences from four chloroplast markers and nrITS with more complete taxon sampling than that of Yuan's (2002) study. We use our phylogenetic results to examine species as treated in previous taxonomies and resolve historical taxonomic conflicts.

Material and Methods

Taxon Sampling. We sampled 70 *Meconopsis* accessions for this study that represent every proposed section and series (Taylor, 1934) in the genus. Nine outgroup species (accessions) were selected and sampled based on previous phylogenetic studies of *Meconopsis* (Yuan, 2002) and *Papaver* (Carolan & al., 2006). Samples were collected from the wild, from the living collection in the Royal Botanical Garden at Edinburgh, and (with permission) from specimens in various herbaria. Species names, authorities, collection information (including localities), and sequence information are listed in Appendix 1. In addition, we included 19 *Meconopsis* accessions from Yuan's (2002) study, and downloaded their *trn*L-*trn*F spacer and nrITS sequences from GenBank. Their vouchers and sequence information are also listed in Appendix 1. Genetic markers for our accessions that could not be successfully amplified or for Yuan's (2002) accessions that were not available in GenBank were coded as missing data (Appendix 1).

DNA Extraction, PCR and Sequencing. Genomic DNA was extracted from silica-dried leaf materials or herbarium specimens using the DNeasy Plant Minikit (Qiagen, Valencia, California, USA). We chose nrITS and the cpDNA marker *trn*L-*trn*F spacer that had been shown to be phylogenetically informative in previous studies of Papaveroideae (Yuan, 2002; Carolan & al., 2006). We also selected the cpDNA marker *rbc*L because it is commonly used for molecular dating in basal eudicot families (Wikström & al., 2001; Anderson & al., 2005; Bell & al., 2010) and also showed

sequence variations in the selected *Meconopsis* species we tested. Additionally, the cpDNA markers *mat*K and *ndh*F were tested and selected because they were easy to amplify and significantly contributed to the resolution of the relationships at the sectional level in *Meconopsis*. PCR amplification was carried out in 12 μL reaction volume with 1–20 ng DNA, 1.0 unit of Taq polymerase (labmade, The University of Texas at Austin), 0.5X Failsafe Buffer B (Epicentre Biotechnologies, Madison, WI, USA), and 2.0 μmol/L primers. Forty-five PCR cycles were performed at 95° C for 30 seconds, 50° C for 45 seconds, and 72° C for 45 seconds for each cycle. Internal primers were designed for amplifying herbarium samples. All the primer pairs used are listed in Appendix 2. All of the PCR products were visualized on agarose gel containing Syber Safe DNA gel stain (Invitrogen, Eugene, Oregon, USA). Successfully amplified products were cleaned using ExoSap (Exonuclease I: New England Biolabs Beverly, MA, USA; Shrimp Alkaline Phosphatase: Progema, Madison, WI, USA) following the manufacturers' protocols. Cleaned PCR products were sequenced using an ABI 3730 DNA Analyzer at the Institute for Cell and Molecular Biology Core Facility at The University of Texas at Austin. Amplifying primers were used for sequencing. In addition, internal primers were also used for sequencing if the amplicon was greater than 900 base pairs (i.e., *rbc*L, *mat*K and *ndh*F).

PHYLOGENETIC ANALYSIS. Sequences were assembled in Geneious 5.5 (Biomatters, New Zealand), and aligned by Geneious Alignment with the default setting and 5 refinement iterations. Alignments were then reviewed and refined manually. The partition heterogeneity test (ILD test, Farris & al., 1994, 1995) was used to test pairwise combinability for each pair of chloroplast markers and for the combined chloroplast sequences versus nrITS. The ILD test was implemented in PAUP* version 4.0b10 (Swofford, 2002) with the setting of simple taxon addition, TBR branch swapping, and 1000 heuristic searches of the datasets that only included variable sites to generate a null distribution. The results of the ILD test suggested that the four cpDNA markers (*rbc*L, *mat*K, *ndh*F and *trn*L-*trn*F spacer) are combinable (a $p > 0.32$ was found for the test of each pair), but the combined cpDNA dataset was not combinable with the nrITS dataset ($p < 0.01$). Therefore, we concatenated the four cpDNA markers.

Bayesian analyses were conducted for the nrITS and concatenated cpDNA data sets using MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2005). Partition analysis was conducted only for the combined cpDNA dataset with each cpDNA marker treated as a separate partition. The evolutionary models of nucleotide substitution were first selected by jModelTest (Posada, 2008) under the Akaike information criterion (AIC), and we applied the models available in MrBayes v3.1.2 and most similar to the best fit models estimated by jModelTest for each gene partition: GTR+I for nrITS, GTR+G for *rbc*L, GTR+I+G for *ndh*F, GTR+G for *mat*K, and GTR+G for *trn*L-*trn*F. Prior probability distributions on all parameters were set to the defaults. Twenty million generations were run using a Markov chain Monte Carlo (MCMC) method with four chains. Trees were collected every 100th generation. With 25% burn-in, both 50% and 80% majority-rule consensus trees were estimated to generate a posterior probability (pp) for each node.

TREE INCONGRUENCE TESTING. We tested the topological conflict between the nrITS and cpDNA trees in order to evaluate the probability that the observed discordance was due to stochastic errors (for instance, data sampling and tree estimating errors) rather than to different evolutionary histories. The testing of tree incongruence is a well-studied field with several established techniques that can be used to explain disagreement between tree topologies (Planet, 2006). Many of these tests are philosophically as well as algorithmically different from one another, allowing researchers to choose one or multiple tests depending on the research purpose and the knowledge of the accuracy of the test. In

this study, we were interested in detecting potential reticulate evolution by localizing significant disagreements between the cpDNA and nrITS phylogenies. We applied the approximately unbiased (AU) method (Efron, 1985; Efron & Tibshirani, 1998; Shimodaira & Hasegawa, 2001) to test competing hypotheses of different tree topologies. We selected this method for its type I error controls (Shimodaira, 2002). The AU test is a non-parametric method that calculates *p*-values for the candidate trees or hypotheses. A null distribution is generated based on bootstrap replicates of log-likelihoods with different replicate sizes.

In the AU test, the concatenated cpDNA dataset and nrITS datasets were treated as two independent partitions. Topologies (or nodes) subjected to testing were inferred from the tree outputs of the Bayesian analyses. PAUP* version 4.0b10 was used to generate the site likelihood scores of the unconstrained and constrained trees for the comparison. Two series of tests were completed: 1) an unconstrained nrITS tree was compared to a set of constrained nrITS trees, each of which was constrained by each of the recovered nodes found on the cpDNA consensus tree; 2) an unconstrained cpDNA tree was compared to a set of constrained cpDNA trees, each of which was constrained at each of the nodes on the nrITS consensus tree. The PAUP files of site likelihood scores for each tree were then reformatted for use in the program CONSEL (Shimodaira & Hasegawa, 2001) to perform the AU tests.

RESULTS

PHYLOGENETIC ANALYSIS. We obtained and analyzed 716 bp (432 variable) of nrITS, 1756 bp (358 variable) of *mat*K, 1648 bp (231 variable) of *ndh*F, 1085 bp (588 variable) of *trn*L-*trn*F, and 1395 bp (55 variable) of *rbc*L sequences. The phylogenies are illustrated in Fig. 1, in which we show only the most closely related outgroup species, *Papaver alpinum*. The specimens used in Fig. 1 were assigned to species by W. Xiao. The recovered nrITS tree (Fig. 1B) shows an unresolved basal polytomy that is well resolved in the cpDNA tree (Fig. 1A). We found that species or clades on the cpDNA tree (Fig. 1A) were frequently located at discordant positions in the nrITS tree (Fig. 1B). For example, *Meconopsis napaulensis* (circled in Fig. 1) is a sister taxon to *Meconopsis autumnalis* (pp 1.00) on the cpDNA tree; but most closely related to *Meconopsis ganeshensis* (pp 1.00) on the nrITS tree.

Our cpDNA phylogeny (Fig. 1A), for the first time, resolved the relationships between different sub-groups of *Meconopsis*. This recovered phylogenetic structure is not consistent with any previously published *Meconopsis* infrageneric classifications. For discussion, we divided the cpDNA tree into five clades (Clades 1–4 and Group H) (Fig. 1A). The Clades 1–4 somewhat correspond to known chromosome numbers (Ratter, 1968; Ying & al., 2006; Kumar & al., 2013). In Clade 1, the only known chromosome number (*M. bella*) is $2n=22$. In Clade 2, the most frequent chromosome number is $2n= 84$ with others varying from $2n=74$ up to 120. In Clade 3, the chromosome number is commonly $2n=56$, rarely $2n=28$. In Clade 4, the chromosome number is normally $2n=56$ or, rarely, $2n=14$. Each clade also represents a section in our new infrageneric revision for the genus (Xiao, 2013). Group H (Fig. 1) contains all the *M. horridula* (*sensu* Taylor 1934) accessions we sampled in this study. Because Grey-Wilson (2000, 2014) and other authors (Ohba & al., 2009; Yoshida & Boufford, 2010; Yoshida & al., 2011) favored subdivisions of Taylor's *M. horridula* (1934), we show this clade in detail using the species names of Grey-Wilson (2000) in Fig. 2B. A few accessions could not be identified with certainty using Grey-Wilson's (2000) concepts (our determinations are given in Fig. 1) because some of his supposedly key characters overlap between different "species." The phylogenetic structures (Fig. 2) show

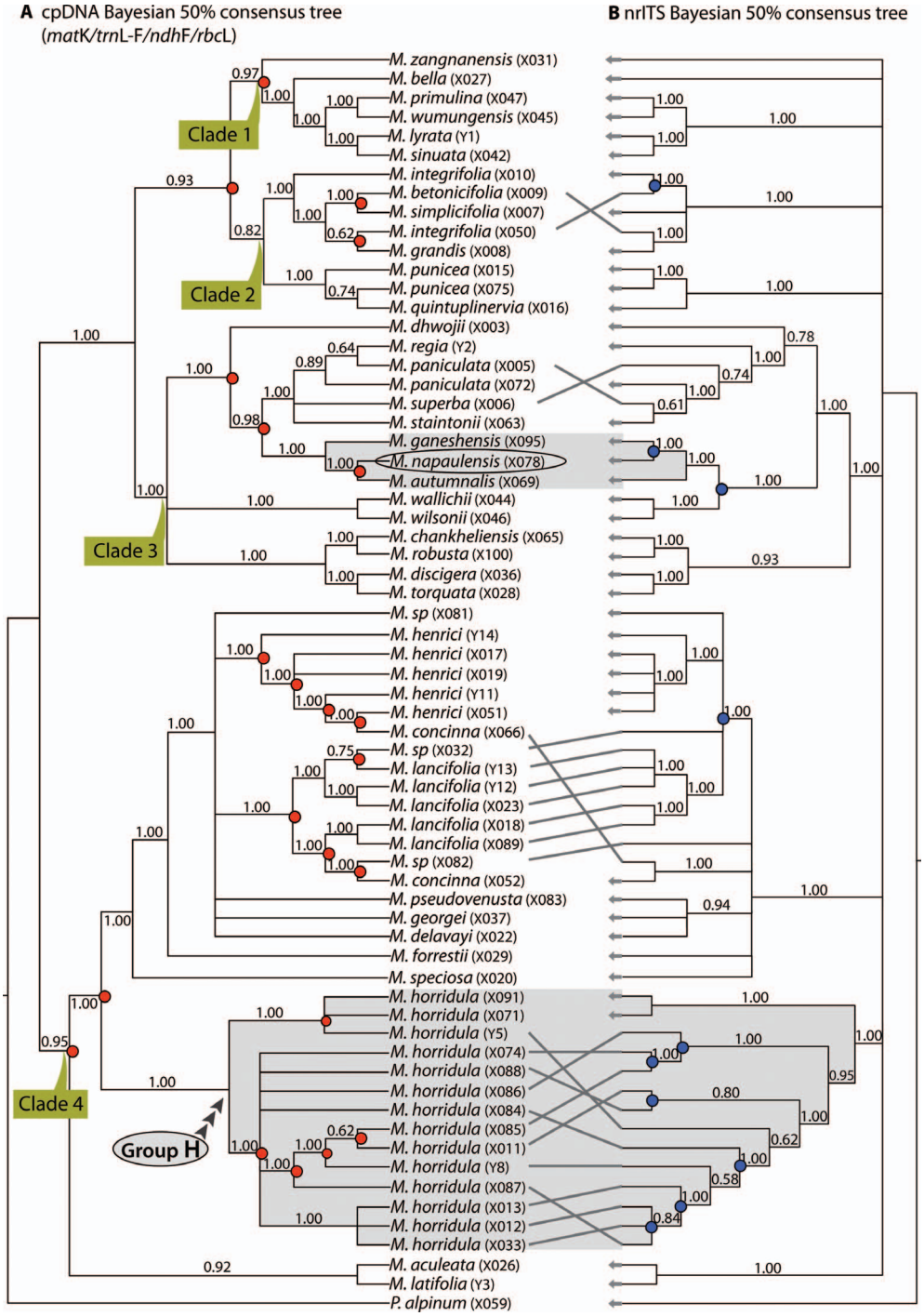

FIG. 1. cpDNA and nrITS Bayesian phylogenies of *Meconopsis* and tree incongruence test results. Names used for accessions were determined by W. Xiao and can differ from annotations of previous authors. All nodes (one by one) in both nrITS and cpDNA Bayesian trees were subjected to AU tests. Each red dot indicates a clade in which the monophyly of all its descendants disagrees significantly with the recovered nrITS tree topology (i.e., the unconstained nrITS tree had a significantly higher likelihood

that Grey-Wilson's (2000) *M. horridula* is not monophyletic in either the cpDNA or nrITS tree (Fig. 2A, D). Our samples No.1, 2, 3, 8, 9, 10 and 11 (Fig. 2 A), determined as *M. horridula sensu* Grey-Wilson (2014), do not represent a monophyletic taxon on either cpDNA or ITS tree. However, all of the accessions in Fig. 2 which correspond to Taylor's (1934) broad circumscription of *M. horridula* form a monophyletic group in both the cpDNA and nrITS trees (Fig. 1, Group H).

We illustrate the main taxonomic discrepancies between Taylor (1934) and Grey-Wilson's (2006) treatment of *Meconopsis napaulensis* in Fig. 3. The phylogenetic relationships among the included species (or accessions) are inferred from Fig. 1. According to either the cpDNA or nrITS phylogeny, Taylor's (1934) *M. napaulensis* does not reflect a monophyletic group while Grey-Wilson's (2006, 2014) treatment is consistent with our phylogenetic results.

TREE INCONGRUENCE TESTING. Our results testing if the disagreements between the cpDNA and nrITS tree were statistically significant (indicated by the colored dots on the Bayesian trees in Fig. 1), showed that when constraining to monophyly all the taxa derived from a red node (labeled on the cpDNA tree, Fig. 1A), the constrained nrITS tree had a significant lower likelihood score ($p < 0.01$) than the unconstrained nrITS tree; and similarly, by constraining all the taxa derived from a blue node on the nrITS tree (Fig. 1B), a significant difference of likelihood score resulted between the unconstrained and constrained cpDNA trees. These results localized the taxa that caused the significant incongruence between the cpDNA and nrITS trees.

DISCUSSION

MECONOPSIS HORRIDULA COMPLEX. In the first monograph of *Meconopsis*, Taylor (1934) treated *Meconopsis horridula* as a polymorphic species by aggregating seven previously described species, among which *Meconopsis racemosa*, *Meconopsis rudis*, *Meconopsis prattii*, and *Meconopsis horridula* had been the most widely recognized taxa. Taylor's (1934) treatment was based on his observations that a wide range of intermediate forms bridge the extreme forms across his concept of *M. horridula*, with no satisfactory distinctions. In light of our phylogenetic results, Taylor's treatment (1934) cannot be rejected because all of the accessions of what he recognized as *M. horridula* were monophyletic in both the cpDNA and nrITS trees (Group H, Fig. 1).

Grey-Wilson (2000) divided the Taylor's *horridula* into two widely distributed species, *M. horridula* and *M. prattii*, and a narrowly endemic species *M. rudis*. In his treatment, *M. horridula* was described as a high altitude species of short stature ($<$ 40 cm) with predominantly scapose flowers and *M. prattii* as a tall species (30–70 cm) growing at relatively low elevations with all flowers arising along a central stem. Grey-Wilson (2000) diagnosed *M. rudis* by its bluish green leaf blades and a unique dark purple color at the bases of the leaf spines. Defined as such, *M. rudis* occurs only in northwestern Yunnan Province (China) centered on the Yulong Mountain. We found that neither the cpDNA nor nrITS phylogeny supports (Fig. 2) the monophyly of *M. horridula sensu* Grey-Wilson (2000). In addition, closely related specimens within the same clade (in both the cpDNA or nrITS trees,

←
score than the nrITS trees constrained to monophyly of taxa derived from a red node); and each blue dot indicates a clade, in which, the monophyly of all its descendants disagrees significantly with the recovered cpDNA tree topology (the unconstained cpDNA tree had a significantly higher likelihood score than the cpDNA trees constrained to monophyly of taxa derived from a blue node).

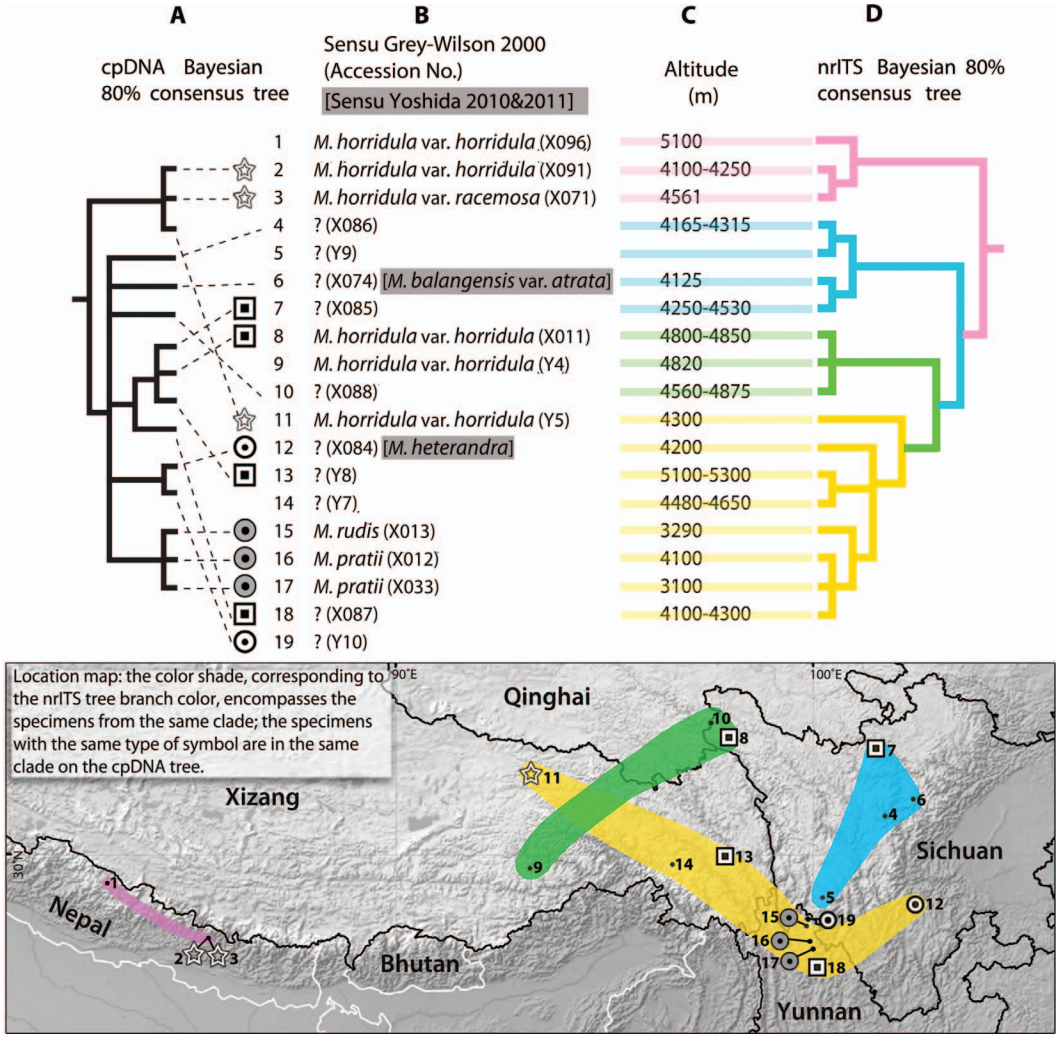

FIG. 2. Phylogenies of the *Meconopsis horridula* complex. Top figures: (A) Tree topology of the *M. horridula* complex from the Bayesian analysis of the cpDNA markers. (B) The taxa included are given names as they would be applied following Grey-Wilson's (2000) treatment and arranged according to their position in the nrITS tree shown in Column D. A "?" indicates that the specimen could not be determined with certainty using the descriptions and geographic ranges provided by Grey-Wilson (2000). [Note: *Our* proposed names for these accessions are given in Fig. 1, Group H]. The dashed lines in Column A connect the terminal taxa on the cpDNA tree to their accessions in Column B. The symbols in Column A are used to plot the accessions on the map (bottom). Specimens marked by the same symbol come from the same clade in the cpDNA tree. (C) Elevations of the accessions in Column B. (D) Tree topology of nrITS Bayesian analysis. The clades in this tree are color coded and mapped in the bottom figure. The map also shows the location of each accession using their associated numbers in Column A.

Fig. 2A, D) are frequently found across a wide elevational range, indicating that there is no clear altitudinal boundary to subdivide Taylor's broad concept of *M. horridula* (1934) in contrast to Grey-Wilson's (2000) assertion.

Grey-Wilson (2014) included eleven species of *Meconopsis* referable to Taylor's

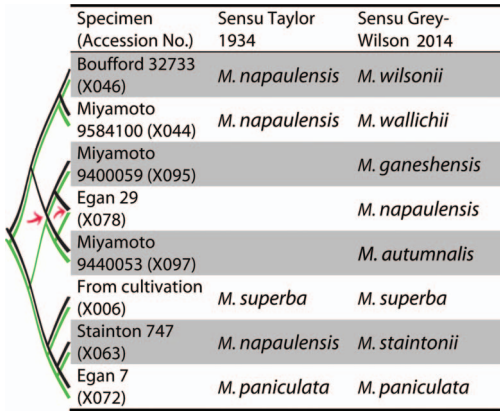

FIG. 3. The superimposed cpDNA (green) and nrITS (black) trees showing discrepancies in the placements of *Meconopsis napaulensis* and its allies. The right two columns show the probable identifications of the accessions using either the key provided by Taylor (1934) or following Grey-Wilson (2014). The red arrows point to putative hybridization events.

(1934) *M. horridula*, and organized them into two series under his section *Racemosae*: series *Heterandrae* includes only *Meconopsis heterandra* and *Meconopsis balangensis*; and series *Racemosae* includes the rest of the section. In Fig. 2B, it is shown that *M. heterandra* and *M. balangensis* are not related. Thus, neither of the series *Heterandrae* nor series *Racemosae* is monophyletic. Our samples 1, 2, 3, 8, 9, 10 and 11 (Fig. 2) were determined as *M. horridula sensu* Grey-Wilson (2014) which appears as a polyphyletic group on either the cpDNA or ITS tree.

One plausible reason for the difficulty in dividing Taylor's *M. horridula* is revealed in Fig. 2. There is frequent incongruence between the two trees for these accessions, suggesting a history of frequent reticulation within this widely distributed group of taxa. Such a pattern of gene flow could explain Taylor's recognition of a broadly defined *M. horridula* in which he stated that it is difficult to assign a large range of intergrading morphologies into meaningful subdivisions. We, therefore, propose that Taylor's (1934) concept of *M. horridula* is best considered as a species complex which can serve as a guide for future phylogeographic studies: this species complex with its wide geographic distribution, remarkable ecological plasticity and morphological diversity (Taylor, 1934) could be a model for understanding the evolutionary and biogeographic history of many plant taxa that extend across the Himalaya through the Tibetan Plateau to the Hengduan Mountains. This *M. horridula* complex would then include the following named taxa:

Meconopsis horridula Hook.f. & Thomson, Fl. Ind. [Hooker f. & Thomson] 1: 252 (1855). *Meconopsis racemosa* Maxim., Bull. Acad. Imp. Sci. Saint-Pétersbourg 23: 310 (1877). *Meconopsis rudis* Prain, Ann. Bot. (Oxford) 20: 347 (1906). *Meconopsis prattii* Prain, Curtis's Bot. Mag. 140: Tab. 8568 (1914). *Meconopsis prainiana* Kingdon-Ward, Garden (London 1871–1927) 90: 115 (1926). *Meconopsis rigidiuscula* Kingdon-Ward, Gard. Chron. 79: 308 (1926). *Meconopsis calciphila* Kingdon-Ward, Gard. Chron. 82: 506 (1927). *Meconopsis pseudohorridula* C.Y. Wu & H. Chuang, Fl. Xizang. 2: 234 (1985). *Meconopsis bijiangensis* H. Ohba, Tosh. Yoshida & H. Sun, J. Jap. Bot. 84: 294 (2009). *Meconopsis castanea* H. Ohba, Tosh. Yoshida & H. Sun, J. Jap. Bot. 84: 300 (2009). *Meconopsis heterandra* Tosh. Yoshida, H. Sun & Boufford, Acta Bot. Yunnan. 32 (6): 505 (2010). *Meconopsis balangensis* Tosh. Yoshida, H. Sun & Boufford, Pl. Diversity Resources 33 (4): 409 (2011). *Meconopsis lhasaensis* Grey-Wilson, Gen. *Meconopsis* 248 (2014). *Meconopsis zhongdianensis* Grey-Wilson, Gen. *Meconopsis* 258 (2014).

MECONOPSIS NAPAULENSIS. A series of taxonomic conflicts has also centered around *Meconopsis napaulensis*. Unlike the situation in the *Meconopsis horridula* complex, the problems in *M. napaulensis* are not simply a matter of how to establish species boundaries but rather different interpretations of the identity of *M. napaulensis*. The species was described by Augustin Pyramus de Candolle in 1824, however, the type specimen is fragmentary and has no flowers. The lack of important characteristics and detailed information on the label, as well as a cursory description, have led to different

opinions about assigning specimens to *M. napaulensis*. Between the two opinions of *M. napaulensis* from Taylor (1934) and Grey-Wilson (2006, 2014), Grey-Wilson (2006) justified his interpretation of *M. napaulensis* by arguing that he matched the locality, the morphology of the fruit, and indumentum of the type of *M. napaulensis* to some specimens collected from Ganesh Himal (a sub-range of the Himalaya in north-central Nepal). Taylor (1934), in contrast, presented little detail of his reason for assigning specimens to *M. napaulensis*. Thus, Grey-Wilson's (2006) work provides a more convincing interpretation of the circumscription and identity of *M. napaulensis* than Taylor's (1934).

We selected a few specimens representing both Taylor's (1934) and Grey-Wilson's (2006, or 2014) species concepts of *Meconopsis napaulensis*. The phylogenetic relationships of these specimens and how they would have been identified by Taylor and Grey-Wilson are shown in Fig. 3. We can see that Taylor's *M. napaulensis* is split with specimens re-assigned to *M. staintonii, M. wilsonii* and *M. wallichii* by Grey-Wilson (2006, or 2014). The latter two, accommodating the majority of the specimens Taylor annotated as *M. napaulensis*, are not related to *M. staintonii* (Fig. 3), which indicates that Taylor's (1934) *M. napaulensis* does not represent a monophyletic taxon.

Taylor (1934) had access to only a limited number of specimens, but he placed plants with "non-yellow (e.g., red, blue) petals" into two species: *Meconopsis napaulensis* (a widely distributed species) and *M. violacea* (a species of restricted distribution). Taylor's classification cannot accommodate some recent collections (collected after 1935), as indicated in Fig. 3, but it had a lasting impact: most (if not all) of the red-flowered plants (e.g., *M. staintonii*, *M. ganeshensis, M. wallichii*, and *M. chankheliensis*) were originally collected and first determined as *M. napaulensis*, and/or introduced to British gardens as such. These plants, however, are not members of the same clade (Fig. 1). Grey-Wilson's treatment (2006, or 2014) corrected the classification by assigning these red-flowered plants to different taxa. Given that Grey-Wilson's taxonomy of *M. napaulensis* and its allies (2006) is compatible with our phylogenetic results, we have followed it in our work.

For future studies, more precise delimitations between *Meconopsis napaulensis* and its allies is desirable, for even Grey-Wilson's latest treatment (2014) does not clearly address the potential of gene flow between the species he recognizes. For example, *Meconopsis regia*, *Meconopsis paniculata*, and *Meconopsis staintonii* are known to hybridize freely with each other in the garden (Grey-Wilson, 2006). Taylor (1934) treated *Meconopsis wallichii* (*sensu* Grey-Wilson 2006) and *Meconopsis wilsonii* (*sensu* Grey-Wilson 2006) as a single species (i.e., his *M. napaulensis*) and pointed out a flux of continuous forms that cannot be satisfactorily assigned to species. Moreover, the placement of *M. napaulensis* (i.e., accession X078) caused significant disagreement between the nrITS and cpDNA trees (Fig. 1), and this *M. napaulensis* accession grows in the same area as *M. autumnalis* and *M. ganeshensis*. This pattern of nuclear and organelle tree incongruence indicates a need for further study to resolve whether each of the three named taxa is a distinct lineage.

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APPENDICES

APPENDIX 1. Voucher and sequence information (Accession number; ***species name***; (Collecting) COUNTRY: Subdivision; voucher (herbarium); GenBank ID for nrITS, *mat*K, *ndh*F, *trn*L-*trn*F, *rbc*L. Accessions beginning with "X" were analyzed and sequenced in this study, and accessions beginning with "Y" were published by Yuan (2002). "-", denotes a missing sequence).

X001; ***Argemone albiflora*** Hornem.; USA: Texas; *W. Xiao 090515* (TEX); JX078976, JX087885, JX087848, -, JX087687. X002; ***Chelidonium majus*** L.; CHINA: Shaanxi; *W. Xiao 090814* (TEX); JX079037, JX087914, JX087828, -, JX087694. X003; ***Meconopsis dhwojii*** G. Taylor; UK (cultivated); *W. Xiao RICB9* (E); JX079001, JX087915, JX087815, JX087755, JX087699. X004; ***Meconopsis wallichii*** Hook.; UK (cultivated); *W. Xiao RICB10* (E); JX078975, JX087895, JX087821, -, JX087711. X005; ***Meconopsis paniculata*** Prain; UK (cultivated); *W. Xiao RICB5* (E); JX079022, JX087868, JX087830, JX087743, JX087720. X006; ***Meconopsis superba*** King ex Prain; UK (cultivated); *W. Xiao RICB7* (E); JX079006, JX087858, JX087851, JX087735, JX087683. X007; ***Meconopsis simplicifolia*** (D. Don) Walp.; NEPAL: Bagmati; *Egan 4* (private collection); JX079040, JX087891, JX087803, JX087751, JX087700. X008; ***Meconopsis grandis*** Prain; UK (cultivated); *W. Xiao RICB6* (E); JX079010, JX087873, JX087832, -, JX087695. X009; ***Meconopsis betonicifolia*** Franch.; UK (cultivated); *W. Xiao RICB2* (E); JX079034, JX087871, JX087806, -, JX087716. X010; ***Meconopsis integrifolia*** (Maxim.) Franch.; CHINA: Yunnan; *W. Xiao 080620* (TEX); JX079030, JX087901, JX087804, -, JX087701. X011; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Sichuan; *Boufford 33724* (GH); JX078978, JX087905, JX087812, JX087770, JX087712. X012; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Yunnan; *W. Xiao 080616* (TEX); JX078988, JX087898, JX087826, -, JX087729. X013; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Yunnan; *ACE 1773* (E); JX079044, JX087852, JX087801, JX087783, JX087713. X014; ***Papaver cambricum*** L.; UK (cultivated); *W. Xiao RICB1* (E); JX078996, JX087883, JX087835, -, JX087689. X015; ***Meconopsis punicea*** Maxim.; CHINA: Sichuan; *Boufford 33684* (GH); JX079003, JX087862, JX087849, -, JX087718. X016; ***Meconopsis quintuplinervia*** Regel; CHINA: Sichuan; *W. Xiao RICB8* (E); JX079007, JX087865, JX087831, -, JX087706. X017; ***Meconopsis henrici*** Bureau & Franch.; CHINA: Sichuan; *W. Xiao 090726-3* (TEX); JX078974, JX087916, JX087809, JX087763, JX087728. X018; ***Meconopsis lancifolia*** Franch. ex Prain; CHINA: Yunnan; *W. Xiao 080621-1* (TEX); JX079008, JX087857, JX087818, JX087750, JX087731. X019; ***Meconopsis henrici*** Bureau & Franch.; CHINA: Sichuan; *W. Xiao 090722-1* (TEX); JX078987, JX087913, JX087797, JX087739, JX087724. X020; ***Meconopsis speciosa*** Prain; CHINA: Yunnan; *W. Xiao 090703-2* (TEX); JX078993, JX087920, JX087829, JX087781, JX087682. X021; ***Meconopsis simplicifolia*** (D. Don) Walp.; NEPAL: Bagmati; *Egan 6* (private collection); JX079028, JX087877, JX087842, -, JX087684. X022; ***Meconopsis delavayi*** Franch.

ex Prain; UK (cultivated); *W. Xiao 090526* (TEX); JX079017, JX087866, JX087816, JX087736, JX087688. X023; ***Meconopsis lancifolia*** Franch. ex Prain; CHINA: Yunnan; *ACE 568* (E); JX079021, JX087917, JX087794, JX087746, JX087722. X024; ***Cathcartia oliveriana*** (Franch. ex Prain) W. Xiao; CHINA: Shaanxi; *J.Z. Xiao 1* (TEX); JX079016, JX087907, JX087791, JX087765, -. X026; ***Meconopsis aculeata*** Royle; UK (cultivated); *C5255* (E); JX079029, JX087912, JX087820, -, JX087709. X027; ***Meconopsis bella*** Prain; NEPAL: Kone Khola; *McBeath 1496* (E); JX078982, JX087919, JX087823, -, JX087723. X028; ***Meconopsis torquata*** Prain; CHINA: Xizang; *Ludlow 9904* (E); JX078999, JX087875, -, JX087737, JX087696. X029; ***Meconopsis forrestii*** Prain; CHINA: Yunnan; *Fang1154* (Xiang Ge Li La Alpine Garden); JX079041, JX087853, JX087807, JX087734, -. X030; ***Meconopsis dhwojii*** G. Taylor; NEPAL: Pokhara; *C5257* (E); -, JX087855, -, JX087745, -. X031; ***Meconopsis zangnanensis*** L.H. Zhou; CHINA: Xizang; *Chen 25-960* (KUN); JX079018, JX087884, JX087799, -, JX087705. X032; ***Meconopsis* sp**; CHINA: Sichuan; *Boufford 33308* (GH); JX079002, JX087903, JX087837, JX087749, JX087710. X033; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Yunnan; *W. Xiao 080623-2* (TEX); JX079039, JX087896, JX087846, JX087758, JX087717. X034; ***Cathcartia chelidonifolia*** (Bureau & Franch.) W. Xiao; UK (cultivated); *W. Xiao RICB4* (E); JX079013, JX087897, JX087840, -, JX087690. X035; ***Meconopsis argemonantha*** Prain; CHINA: Xizang; *Bowes Lyon 11101* (E); -, -, JX087814, JX087778, -. X036; ***Meconopsis discigera*** Prain; BHUTAN: Upper Mo Chu District; *Bowes Lyon15045* (E); JX079038, JX087918, JX087824, JX087774, JX087686. X037; ***Meconopsis georgei*** G. Taylor; CHINA: Yunnan; *Forrest 30595* (E); JX078989, JX087856, JX087792, JX087768, JX087693. X042; ***Meconopsis sinuata*** Prain; INDIA: Sikkim; *ESK 683* (E); JX078991, JX087890, JX087785, -, JX087725. X044; ***Meconopsis wallichii*** Hook.; NEPAL: Sagarmatha Zone; *Miyamoto 9584100* (E); JX079025, JX087867, JX087810, JX087747, JX087732. X045; ***Meconopsis wumungensis*** K.M. Feng; CHINA: Yunnan; *Liu 1990July* (KUN); JX078997, JX087922, -, -, JX087707. X046; ***Meconopsis wilsonii*** Grey-Wilson; CHINA: Sichuan; *Boufford 32733* (GH); JX078995, JX087924, JX087838, JX087740, JX087691. X047; ***Meconopsis primulina*** Prain; BHUTAN: Upper Mo Chu District; *Sargent170* (E); JX079035, JX087887, JX087843, -, JX087685. X050; ***Meconopsis integrifolia*** (Maxim.) Franch.; CHINA: Yunnan; *ACE 705* (E); JX078984, JX087878, -, JX087756, JX087703. X051; ***Meconopsis henrici*** Bureau & Franch.; CHINA: Sichuan; *Boufford 35710* (GH); JX079043, JX087886, JX087802, JX087762, JX087730. X052; ***Meconopsis concinna*** Prain; CHINA: Yunnan; *Boufford 35133* (GH); JX079031, JX087889, JX087841, JX087759, JX087721. X054; ***Meconopsis* x *cookei*** G. Taylor; CHINA: Qinghai; *Long 696* (E); JX079042, JX087869, JX087827, -, JX087726. X055; ***Cathcartia villosa*** Hook.f.; INDIA: Sikkim; *ESK 205* (E); JX078972, -, JX087847, -, JX087708. X058; ***Papaver* sp**; UK (cultivated); *W. Xiao 090527-1* (TEX); JX079012, JX087880, JX087844, JX087752, JX087727. X059; ***Papaver alpinum*** L.; UK (cultivated); *W. Xiao 090527-2* (TEX); JX079023, JX087879, JX087836, JX087766, JX087719. X060; ***Papaver lateritium*** K. Koch; UK (cultivated); *W. Xiao 090527-3* (TEX); JX078983, JX087900, JX087813, JX087776, JX087697. X061; ***Stylophorum diphyllum*** Nutt.; UK (cultivated); *W. Xiao 090527-4* (TEX); JX079036, JX087859, JX087793, JX087757, JX087704. X063; ***Meconopsis staintonii*** Grey-Wilson; NEPAL: Larjung; *Stainton 747* (E); JX079027, JX087893, -, -, -. X064; ***Meconopsis florindae*** Kingdon-Ward; CHINA: Xizang; *Kingdon-Ward 6206* (E); -, JX087870, JX087839, -, -. X065; ***Meconopsis chankheliensis*** Grey-Wilson; NEPAL: Chanke-Lekh; *Bailey 1936June* (E); JX078973, JX087904, JX087787, JX087753, JX087702. X066; ***Meconopsis concinna*** Prain; CHINA: Yunnan; *Forrest 12670* (E); JX079020, JX087902, JX087819, -, -. X067; ***Argemone subfusiformis*** G.B. Ownbey; PERU; *Ortiz 2302* (TEX); -, JX087874, -, JX087775, -. X069; ***Meconopsis autumnalis*** P.A. Egan; NEPAL: Bagmati; *Egan 17* (private collection); JX078977, JX087872, JX087822, JX087748, JX087714. X070; ***Meconopsis autumnalis*** P.A. Egan; NEPAL: Bagmati; *Egan 25* (private collection); JX079011, JX087861,

JX087850, JX087754, -. X071; ***Meconopsis horridula*** Hook.f. & Thomson; NEPAL: Bagmati; *Egan 15* (private collection); JX078971, JX087910, JX087790, JX087742, JX087692. X072; ***Meconopsis paniculata*** Prain; NEPAL: Bagmati; *Egan 7* (private collection); JX079004, JX087860, JX087789, JX087777, -. X073; ***Meconopsis lyrata*** (H.A. Cummins & Prain) Fedde; BURMAR: N.E. upper Burma; *Forrest 25047* (E); -, -, JX087800, -, -.X074; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Sichuan; *Boufford 38460* (GH); JX078980, JX087921, -, JX087771, -. X075; ***Meconopsis punicea*** Maxim.; CHINA: Sichuan; *Boufford 40141* (GH); JX079019, JX087876, JX087834, -, -. X078; ***Meconopsis napaulensis*** DC.; NEPAL: Bagmati; *Egan 29* (private collection); JX078979, JX087906, JX087798, JX087760, JX087698. X079; ***Meconopsis napaulensis*** DC.; NEPAL: Bagmati; *Egan 16* (private collection); JX079024, JX087909, JX087788, JX087733, JX087715. X080; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Yunnan; *W. Xiao 090707-1* (TEX); JX078985, -, -, JX087784, -. X081; ***Meconopsis* sp**; CHINA: Yunnan; *W. Xiao 090707-2* (TEX); JX079033, JX087888, JX087805, JX087744, -. X082; ***Meconopsis* sp**; CHINA: Yunnan; *W. Xiao 090705-1* (TEX); JX078998, JX087892, JX087808, JX087782, -. X083; ***Meconopsis pseudovenusta*** G. Taylor; CHINA: Yunnan; *W. Xiao 090705-2* (TEX); JX079009, JX087894, JX087796, JX087741, -. X084; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Sichuan; *Boufford 32738* (GH); JX079005, JX087911, JX087786, JX087767, -. X085; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: SiChuan; *Boufford 39222* (GH); JX079032, JX087923, JX087817, JX087764, -. X086; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Sichuan; *Boufford 38099* (GH); JX079000, JX087854, JX087825, JX087773, -. X087; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Yunnan; *Boufford 35132* (GH); JX078992, JX087908, JX087795, JX087738, -. X088; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Sichuan; *Boufford 33530* (GH); JX078981, JX087864, JX087833, JX087761, -. X089; ***Meconopsis lancifolia*** Franch. ex Prain; CHINA: Sichuan; *Boufford 34065* (GH); JX078994, JX087881, JX087811, JX087779, -. X090; ***Meconopsis wallichii*** Hook.; UK (cultivated); *W. Xiao 090522* (TEX); JX079026, JX087863, JX087845, JX087780, -. X091; ***Meconopsis horridula*** Hook.f. & Thomson; NEPAL: Bagmati; *Miyamoto 9420086* (E); JX078986, JX087882, -, JX087769, -. X095; ***Meconopsis ganeshensis*** Grey-Wilson; NEPAL: Bagmati; *Miyamoto 9400059* (E); JX079014, JX087899, -, JX087772, -. X096; ***Meconopsis horridula*** Hook.f. & Thomson; NEPAL: Dolpo; *Grey-Wilson 434* (K); JX079015, -, -, -, -. X097; ***Meconopsis autumnalis*** P.A. Egan; NEPAL: Bagmati; *Miyamoto 9440053* (E); JX078990, -, -, -, -. X100; ***Meconopsis robusta*** Hook.f. & Thomson; NEPAL: Bajhang; *Nepal Bajhang 2009 Expedition 20913119* (E); KF777122, KF777124, KF777123, KF777120, KF777121. Y1; ***Meconopsis lyrata*** (H.A. Cummins & Prain) Fedde; NEPAL: Bagmati; *Miyamoto 9484087* (E); AY328267.1, -, -, AY328215.1, -. Y2; ***Meconopsis regia*** G. Taylor; NEPAL: above Doadi Khola; *Stainton 4627* (E); AY328273.1, -, -, AY328224.1, -. Y3; ***Meconopsis latifolia*** Prain; INDIA: Kashimir; *Stewart 22563a* (unknown); AY328264.1, -, -, AY328226.1, -. Y4; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Xizang; *Boufford 30022* (GH); AY328258.1, -, -, -, -. Y5; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Xizang; *Boufford 30011* (GH); AY328261.1, -, -, AY328208.1, -. Y6; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Yunnan; *Yuan 2000635* (SYS); AY328262.1, -, -, AY328207.1, -. Y7; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Xizang; *Boufford 29724* (GH); AY328260.1, -, -, -, -. Y8; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Xizang; *Boufford 29486* (GH); AY328257.1, -, -, AY328206.1, -. Y9; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Sichuan; *Yuan 2000668* (SYS); AY328259.1, -, -, -, -. Y10; ***Meconopsis horridula*** Hook.f. & Thomson; CHINA: Yunnan; *Yuan 2000655* (SYS); -, -, -, AY328205.1, -. Y11; ***Meconopsis henrici*** Bureau & Franch.; CHINA: Sichuan; *Yuan 2000682* (SYS); AY328281.1, -, -, AY328209.1, -. Y12; ***Meconopsis lancifolia*** Franch. ex Prain; CHINA: Yunnan; *Yuan 2000657* (SYS); AY328282.1, -, -, AY328212.1, -. Y13; ***Meconopsis lancifolia***

Franch. ex Prain; CHINA: Sichuan; *Yuan 2000667* (SYS); AY328284.1, -, -, AY328213.1, -. Y14; ***Meconopsis henrici*** Bureau & Franch.; CHINA: Sichuan; *Yuan 2000712* (SYS); AY328280.1, -, -, AY328210.1, -. Y15; ***Meconopsis lancifolia*** Franch. ex Prain; CHINA: Yunnan; *Boufford 29191* (GH); AY328283.1, -, -, -, -. Y16; ***Meconopsis gracilipes*** G. Taylor; NEPAL: South of Annapurna; *Troth 980* (unknown); AY328270.1, -, -, -, -. Y17; ***Meconopsis wilsonii*** Grey-Wilson; CHINA: Yunnan; *Gong 20020611* (unknown); AY328269.1, -, -, AY328228.1, -. Y18; ***Meconopsis taylorii*** L.H.J. Williams; NEPAL: Annapurna Himalaya; *Stainton 6593* (E); AY328275.1, -, -, -, -. Y19; ***Cathcartia smithiana*** Hand.-Mazz.; CHINA: Yunnan; *GSE97 9592* (E); AY328301.1, -, -, AY328247.1, -.

APPENDIX 2. Primer list (Primer name, primer sequences (source or reference). "*" indicates the primer designed by this study).

ITS forward primer sequence, 5′-GGAAGGAGAAGTCGTAACAAGG-3′ (Blattner, 1999); ITS reverse primer sequence, 5′-TCCTCCGCTTATTGATATGC-3′ (White & al., 1990); trnL-trnF forward primer sequence, 5′-CGAAATCGGTAGACGCTACG-3′ (Taberlet & al., 1991); trnL-trnF reverse primer sequence, 5′-ATTTGAACTGGTGACACGAG-3′ (Taberlet & al., 1991); matK forward primer sequence, 5′-ACTGTATCGCACTATGTATCA-3′ (Sang & al., 1997); matK reverse primer sequence, 5′-GAACTAGTCGGATGGAGTAG-3′ (Sang & al., 1997); matK internal forward primer sequence*, 5′-GGAGCATCCTTTAGTAGTGTTTAG-3′; matK internal reverse primer sequence*, 5′-ATTTATTCATMAAAAGAGGACTTCC-3′; ndhF forward primer sequence, 5′-CTGTCTATTCAGCAAATAAAT-3′ (shared by R.K. Jansen); ndhF reverse primer sequence, 5′-CGATTATAGGACCAATCATATA-3′ (shared by R.K. Jansen); ndhF internal forward primer sequence*, 5′-ATGGGATCATATCGAGCTG-3′; ndhF internal reverse primer sequence*, 5′-CCCATAAGAGCCATATTCTGG-3′; rbcL forward primer sequence, 5′-ATGTCACCACAAACAGARACTAAAGC-3′ (designed by R. Beaman); rbcL reverse primer sequence, 5′-CTTTTAGTAAAAGATTGGGCCGAG-3′ (designed by R. Beaman); rbcL internal forward primer sequence F*, 5′-CCCTTTATGCGTTGGAGAGA-3′; rbcL internal reverse primer sequence*, 5′-CTCTGGCAAATACAGCCCTT-3′.