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ORIGINAL ARTICLE

New insights into the phylogenetic relationships of tribe Astragaleae (Fabaceae subfamily Papilionoideae) and *Astragalus*—the largest genus of angiosperm

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Abstract

Astragaleae is one of the largest tribes in the legume family, harboring the most species-rich genus in Angiosperm, *Astragalus*. The tribal and generic delimitation of Astragaleae remains unclear. With samples from 19 genera/dubious genera of the tribe represented by 272 species, as well as with nuclear and chloroplast data, we re-delimited and described the tribe based on the *Erophaca*-Astragalean clade. We recognized 16 genera within Astragaleae: *Astragalus*, *Biserrula*, *Carmichaelia*, *Clianthus*, *Colutea*, *Eremosparton*, *Erophaca*, *Lessertia*, *Montigena*, *Oxytropis*, *Phyllolobium*, *Podlechiella*, *Smirnowia*, *Sphaerophysa*, *Streblorrhiza*, and *Swainsona*, and a generic key was proposed. Our results may lay a foundation for future studies on the taxonomy and biogeography of Astragaleae, and deepen the public's understanding of angiosperm diversity.

KEYWORDS
16 genera, Astragaleae, *Astragalus*, generic delimitation, the *Erophaca*-Astragalean clade

Highlights

- The molecular phylogenetic study resurrected and re-delimited the highly diversity leguminous tribe Astragaleae.
- Astragaleae is described and a generic key was proposed.
- We recognized 16 genera within Astragaleae: *Astragalus*, *Biserrula*, *Carmichaelia*, *Clianthus*, *Colutea*, *Eremosparton*, *Erophaca*, *Lessertia*, *Montigena*, *Oxytropis*, *Phyllolobium*, *Podlechiella*, *Smirnowia*, *Sphaerophysa*, *Streblorrhiza*, and Swainsona.

Lei Duan, Yu-Wen Ji and Yan Jiang contributed equally to this work.

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1 | INTRODUCTION

The morphologically diverse tribe Astragaleae Adans. belongs to the subfamily Papilionoideae of the legume family (Fabaceae) (Figure 1), and is widely distributed in temperate regions of every continent except for Antarctica and serves as an important vegetation component of desert, mountain, grassland, and forest[1–3] (also see Table 1). The most recent circumscribed Astragaleae[4] contains 15–20 genera with 3000–3500 species, and embraces the most species-rich genus in angiosperm, *Astragalus* L. Plants of Astragaleae comprise popular medicinal herbs, such as *Astragalus membranaceus* (Fisch.) Bunge from eastern and central Asia,[5] and *Sutherlandia frutescens* (L.) R. Br. from southern Africa.[6] Besides, the taxa of the tribe can be widely used as green manure, forage, and sand binders.[7]

Since the initial proposal of Astragaleae,[8] its tribal status was accepted only by fewer taxonomists[1–3,9]; most workers treated it as the subtribe Astragalinae within tribe Galegeae[5,10] or just decomposed Astragaleae and inserted its genera into Galegeae.[7,11,12] The abovementioned circumscriptions of Astragaleae and Astragalinae only narrowly contained *Astragalus*, *Oxytropis* DC. and their satellite genera, of which monophyly were questioned by following phylogenetic investigations. Since the last century, multiple molecular studies indicated that Astragaleae sensu Hutchinson,[1] Galegeae, as well as its subtribe Astragalinae, were all polyphyletic.[12–16] These results also found that the species-rich *Astragalus*, *Oxytropis*, *Colutea* L., *Swainsona* Salisb., *Carmichaelia* R. Br. and their satellite genera formed the Astragalean clade, as a core group of Galegeae, which covered Astragaleae, Carmichaelieae, and Coluteae sensu Hutchinson.[1] It is clear that the narrowly demarcated Astragaleae requires a re-delimitation. We recently reinstated the tribal status based on the clade comprising the Astragalean clade and the genus *Erophaca*[4]; however, due to lacking generic-level samples, our earlier result needs further verification.

Approximately 15–20 genera constituted our recently delimited Astragaleae[4] (see Table 1). Within the tribe, *Astragalus* embraced ca. 250 sections with 2500–3000 species[17] (also see Table 1; Figure 1A-B), and taxonomists split over 10 genera from *Astragalus* due to its highly infrageneric morphological diversity, of which six of them were relatively widely accepted[10–12] (Table 1). However, although most workers supported the generic statuses of *Biserrula* L., *Barnebyella* Podlech, and *Ophiocarpus* (Bunge) Ikonn., they are questioned by recent phylogenetic results.[18,19] As the largest genus of angiosperm, *Astragalus* has attracted much attention from botanists; great efforts were made as follow to resolve its infrageneric phylogenetic relationship. Although early molecular studies on *Astragalus* based on Sanger data biased either the Old World[18–22] or the New World,[23–26] two consensuses were gradually reached: 1. Most species of this genus formed a monophyletic core group, *Astragalus* s.s.; 2. The New World taxa grouped into a single clade, but the Old World taxa did not. Recently, our chloroplast (cp) genomic work resolved 10 clades among 55 *Astragalus* sections and reconfirmed the validation of *Astragalus* s.s.[17] Based on nuclear sequences obtained from the NGS (next-generation Sequencing) approach, the newest phylogenetic study of Folk et al.[27] further confirmed these two consensuses with over 800 *Astragalus* species, but their analyses mostly focused on biogeography and niche evolution of the very genus. Therefore to date, few molecular studies paid adequate attention to verifying the generic boundaries between *Astragalus* and its satellite genera.

Oxytropis is the second largest genus in the Astragalean clade, possessing ca. 25 sections with over 400 species[28] (also see Table 1; Figure 1C-D), and was regarded as a close relative of *Astragalus*,[29,30] while some other studies, based on cp and nuclear gene data, revealed a non-sister relationship between the two genera,[4,17] which was agreed by recent NGS-based results.[27] Thus, the generic boundaries of *Astragalus* and *Oxytropis* and their allies are still unclear.

Besides the larger genera *Astragalus*, *Oxytropis* and their allies, a subclade was found within the Astragalean clade, containing Eurasian *Eremosparton* Fisch. and C.A.Mey., *Phyllolobium* Fisch., *Smirnowia* Bunge, *Sphaerophysa* DC (Figure 1G), *Colutea* (Figure 1F) and its ally *Oreophysa* (Bunge ex Boiss.) Bornm., as well as Southern African endemic *Lessertia* DC (Figure 1H) and *Sutherlandia* B.Br. Ex W.T. Aiton.[4,30] Another subclade of the Astragalean clade was restricted within the temperate Oceania continent, including *Swainsona* (Figure 1I), *Streblorrhiza* Endl (Figure 1K), *Carmichaelia* (Figure 1J), *Montigena* Heenan, and *Clianthus* Sol. Ex Lindl.[1,10,12] (see Table 1). The two subclades confronted a similar problem, that is, controversy over the generic boundary. For instance, should *Sutherlandia* be treated within *Lessertia*?[31,32] Is *Montigena* an independent genus from *Swainsona* or not?[33,34] What is the phylogenetic position of the extinct genus *Streblorrhiza*? Due to under-sampling or biased sampling issues,[30,34–36] few studies have thoroughly explored the phylogeny of the Astragalean clade, especially the generic delimitation.

In this study, based on more thorough sampling on the generic level, as well as using both cp (*matK*) and nuclear (ITS) genes, we aimed to 1. further validate the tribe Astragaleae sensu Duan et al.[4] and 2. test the generic boundaries within Astragaleae.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling

For the purpose of familiarizing taxonomic revisions and key morphological traits in the classification of Astragaleae, we carried out a thorough literature search on main revisions[1,10,12] and checklists[11,37–39] of this tribe, as well as most florae that record the genera of Astragaleae.[2,7,40–44] Samples and specimens of Astragaleae were collected in the field from Turkey, Russia, USA, and Northern China where this tribe is distributed. In addition, we viewed all the Astragaleae specimens deposited in the herbaria IBSC, KUN, LE, MO, NY, PE, US, WUK, and XJBI, in addition to all the online specimen images from E, G, K, LINN, and P, to further reinforce our understanding of the morphology of Astragaleae taxa.

We used a more thorough generic-level sampling of Astragaleae for molecular phylogenetic analyses up to date, represented by 19 genera/dubious genera with 272 species (the extinct *Streblorrhiza* not included; see Table 1) following several prior studies,[4,15,17] including 104 species of *Astragalus* s.s. We also selected 40 other genera of the IRLC (54 spp.) and three genera of robinioids as outgroups. Most samples for the study were obtained from fresh field collections or preserved herbarium specimens (see Table S1 in Supporting Information S1 for details).

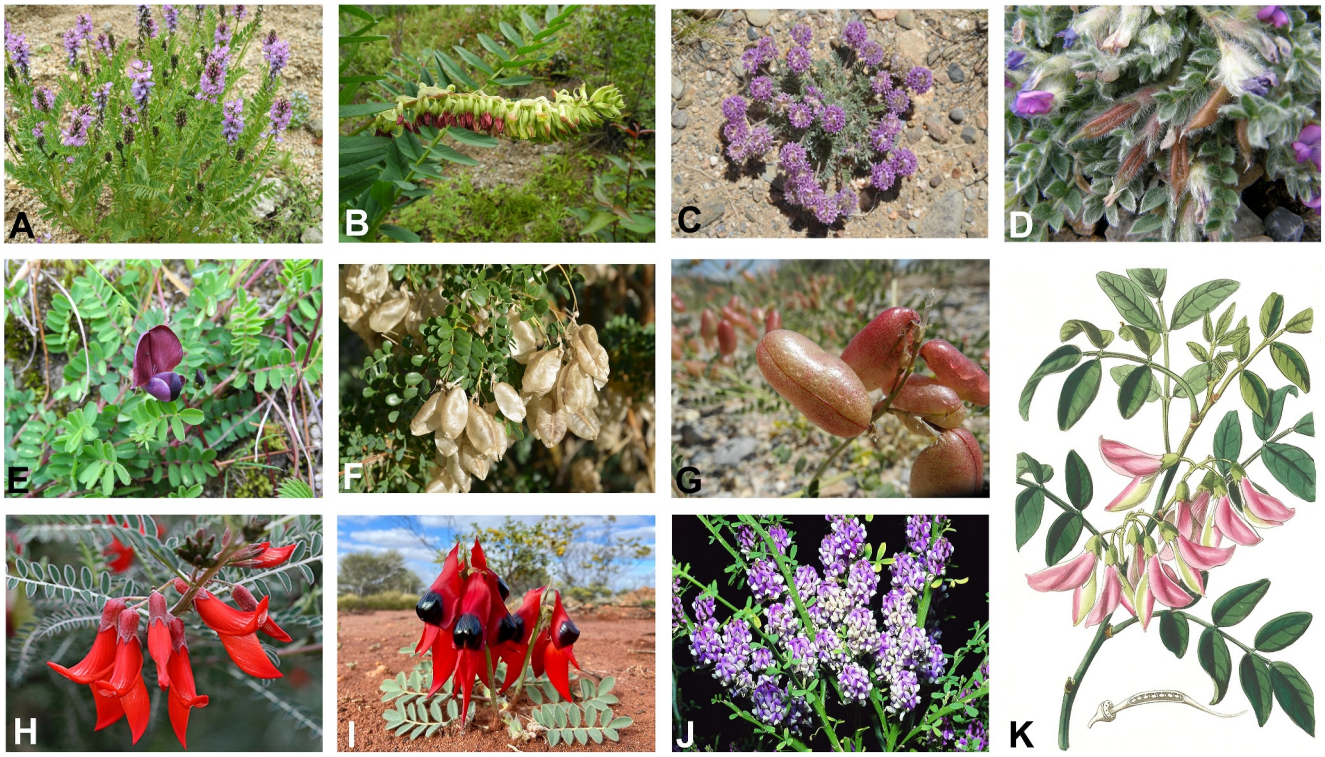


FIGURE 1 Representatives of Astragaleae. A: *Astragalus strictus*; B: *Astragalus stipulatus*; C: *Oxytropis tatarica*; D: *Oxytropis latibracteata*; E: *Phyllolobium donianum*; F: *Colutea nepalensis* (photograph by Pei-Liang Liu); G: *Sphaerophysa salsula*; H: *Lessertia montana* (photograph by Kelly Kilpatrick); I: *Swainsona formosa* (photograph downloaded from iNaturalist); J: *Carmichaelia odorata* (photograph by Gwilym Lewis); and K: *Streblorrhiza speciosa* (Illustration by Sarah Drake).

2.2 | DNA extraction, sequencing, and gene selection

We extracted the total genomic DNA following a modified CTAB protocol.[45] Yield and integrity (size distribution) of genomic DNA extracts were quantified by fluorometric quantification on a Qubit (Invitrogen, Carlsbad, California, USA) using a dsDNA HS kit, as well as by visual assessment on 1% agarose gels. Subsequently, all samples were used to build blunt-ended DNA libraries via the NEBNext Ultra II DNA library Prep kit for Illumina (New England Biolabs) according to the protocol of the manufacturer. The final indexed libraries were then pooled in equimolar ratios and were sequenced in a single lane of an Illumina X Ten sequencing system (Illumina Inc.).

We filtered out adapters and low-quality reads using Trimmomatic[46] from the raw reads. The quality of the remaining reads was checked using FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc/). As per the ITS assembly, we first screened and excluded all the plastid-like reads by employing a customized Python script[47] with its default parameters to apply BLAST and a built-in library. Based on the remaining reads, de novo assemblies were performed with the complete ITS sequence (including ITS1, 18 S, and ITS2 ribosomal RNA genes) of *Astragalus mongholicus* Bunge (GenBank Accession #: MT923547), and with a mapping approach following Ji et al.[48] The ITS regions were annotated and defined by comparison with the annotated reference within Geneious Prime.[49]

So far, released NGS data cannot support a reliable phylogenetic analysis to clarify the generic boundary of Astragaleae,[17,27,50] until denser sampling for the whole tribe, rather than *Astragalus* only, was achieved. In contrast, published sequences of the nuclear ITS and cp *matK* already covered most genera in the tribe, and are thought to be a better data source for a generic study at the current stage. Herein, we used 16 newly obtained ITS sequences in this study, and 258 ITS and *matK* markers assembled from our published NGS raw data,[4,17] as well as 175 sequences downloaded from NCBI (https://www.ncbi.nlm.nih.gov/genbank/) (see Table S1 in Supporting Information S1for details). The assembling process of *matK* was similar to that of ITS sequences mentioned above.

2.3 | Phylogenetic analysis

We aligned the ITS and *matK* sequences independently with MAFFT.[51] Accordingly, we carried out separate phylogenetic analyses using Bayesian inference (BI)[52,53] implemented in the program MrBayes[54,55] by applying default prior settings. Each BI was performed by applying two independent runs of the Markov chain Monte Carlo (MCMC) with 15 and 10 million generations for ITS and *matK*, respectively. The trees were sampled every 1000 generations, and the first 20% of trees were discarded as burn-in. The analysis stationarities were verified with Tracer v.1.6 (http://tree.bio.ed.ac.uk/software/tracer) by ensuring that all ESS values exceeded 200, and the convergence was confirmed between independent runs. In addition to BI, we also performed maximum likelihood (ML) analyses for ITS and *matK*, respectively, using IQ-TREE[56] with the following settings: rapid bootstrap analysis with 1000 replicates, followed by a search for the best-scoring ML tree starting with a random seed.

TABLE 1 Genus information of Astragaleae sensu Duan et al.[4]

Genus	Species number	Distribution	Main taxonomic treatments
Astragalus (Figure 1A–B)	2500–3000	Temperate region of every continent, except for Oceania	Genera *Astracantha*, *Didymopelta*, *Neodielsia*, *Phaca*, *Sewerzowia*, *Tragacantha* were treated within *Astragalus*
Erophaca	1	Circum-Mediterranean region	Established based on *Astragalus lusitanicus*
Phyllolobium (Figure 1E)	21	Northern and Southwestern China, Himalayan region, and Tajikistan	Sometimes as *Astragalus* sect. *Phyllolobium*
Podlechiella	1	From Central Asia to North and East Africa	Established based on *Astragalus* sect. *Herpocaulos*
Biserrula	1	Circum-Mediterranean region, Canary Islands, and Eastern and Northeastern Africa	Sometimes as *Astragalus* sect. *Biserrula*
Barnebyella	1	Iran, Afghanistan, and Pakistan	Established based on *Astragalus* sect. *Mirae*
Ophiocarpus	1	From Iraq to Northwestern India	Established based on *Astragalus* sect. *Ophiocarpus*
Oxytropis (Figure 1C–D)	300–400	Temperate Eurasia and North America	Genus *Aragallus* were treated within *Oxytropis*
Colutea (Figure 1F)	28	From Eastern Asia, through Central and Southern Europe, to Northeastern Africa	
Oreophysa	1	Northern Iran	Established based on *Colutea* sect. *Oreophysa*
Smirnowia	1	Iran, Afghanistan, Uzbekistan, and Turkmenistan	
Eremosparton	3	From Xinjiang, China, to Southeastern Russia	
Sphaerophysa (Figure 1G)	2	Turkey, Southeastern Russia, Caucasus, Central Asia, Mongolia, and Northern China	
Lessertia (Figure 1H)	50	South Africa, Namibia, Botswana, Zimbabwe, Western Zambia, Southern Angola, and Southern Tanzania	
Sutherlandia	2	South Africa, Namibia, and Botswana	Treated into *Lessertia* by multiple taxonomists
Swainsona (Figure 1I)	84	Australia	Genera *Cyclogyne*, *Diplolobium*, *and Willdampia* were treated within *Swainsona*
Montigena	1	New Zealand	Split from *Swainsona*
Clianthus	2	North Island of New Zealand	
Carmichaelia (Figure 1J)	23	New Zealand	Genera *Chordospartium*, *Corallospartium*, *Notospartium* were treated within *Carmichaelia*
Streblorrhiza (Figure 1K)	1	Phillip Island of Australia (extinct)	

3 | RESULTS

Our phylogenetic analyses included 275 newfound or reproduced sequences[4,17] (see Table S1 in Supporting Information S1). The lengths of ITS and *matK* alignments for the MrBayes and IQ-TREE analyses were 795 and 1624 bp, respectively (see alignments in Supplementary files).

Our ML results (see Trees S1 and S2 in Supporting Information S1) were congruent in topology with the corresponding BI trees (see Trees S3 and S4 in Supporting Information S1), and the support values of the ML analysis (as "LBS" hereafter) were thus labeled on the corresponding branches of the BI trees (as "PP" hereafter). Phylogenetic reconstructions according to the ITS (Figure 2A) and *matK* (Figure 2B) data supported the monophyly of the IRLC (ITS: LBS = 73%; *matK*: LBS = 100%), within which Adinobotryeae, Glycyrrhizeae (ITS: PP = 1, LBS = 95%; *matK*: PP = 1, LBS = 100%), Wisterieae (ITS: PP = 1, LBS = 75%; *matK*: PP = 1, LBS = 100%), Vicioids (ITS: PP = 1, LBS = 98%; *matK*: PP = 1, LBS = 95%), Caraganeae subtr. Caraganinae (ITS and *matK*: PP = 1, LBS = 100%), Hedysareae (ITS: PP = 1, LBS = 82%; *matK*: PP = 1, LBS = 92%), Caraganeae subtr. Chesneyinae (ITS: PP = 1, LBS = 75%; *matK*: PP = 1, LBS = 99%), and Astragaleae (ITS and *matK*: PP = 1, LBS = 100%) were each well supported.

Within Astragaleae, our trees (Figure 2) indicate the generic statuses of *Erophaca*, *Oxytropis* (ITS: PP = 1, LBS = 99%; *matK*: PP = 1, LBS = 100%), *Astragalus* s.s. (ITS: PP = 1, LBS = 98%; *matK*: weakly supported), and *Phyllolobium* (ITS: PP = 1, LBS = 100%; *matK*: PP = 1, LBS = 97%). On the other hand, our *matK* tree (Suppl. Trees 2 and 4) showed highly similar main clades of *Astragalus* s.s. With our previous result based on cp genomes,[17] both our ITS (Figure 3) and *matK* analyses (Trees S2 and S4 in Supporting Information S1) indicated that the genera *Barnebyella* and *Ophiocarpus* are nested within *Astragalus* s.s. Because our *matK* tree topologies were nearly identical to our previous result,[17] we only made the ITS tree of *Astragalus* s.s. into a figure herein (Figure 3).

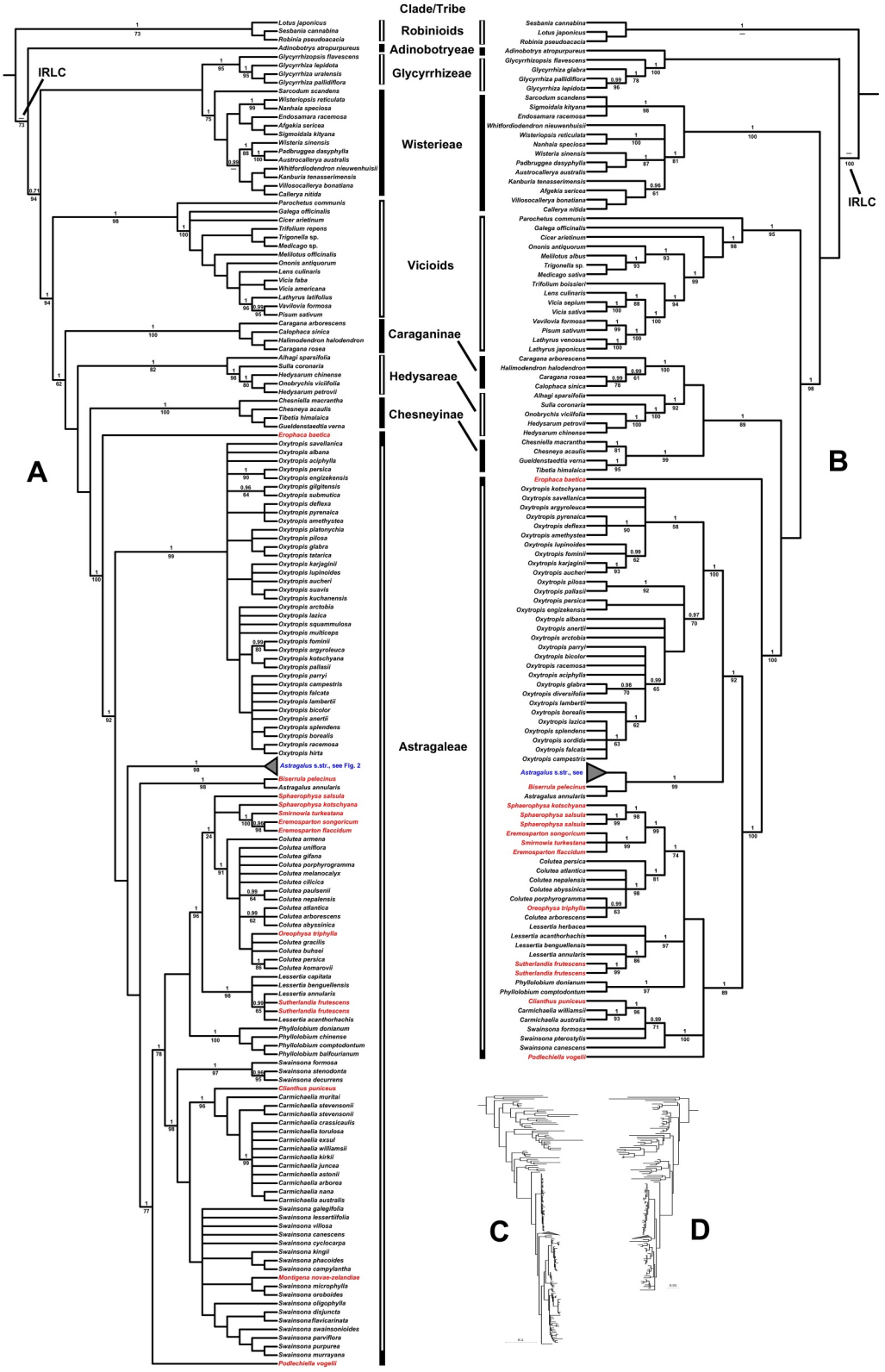


FIGURE 2 Bayesian trees of Astragaleae and outgroups based on nuclear (A; ITS) and chloroplast DNA regions (B; *matK*). Bayesian posterior probabilities (≥0.95) are given above branches, and maximum likelihood bootstrap values (≥50%) are below branches. The labeled tribes and clades largely followed Duan et al.[4] The ITS and *matK* results are shown in trees C and D with branch lengths, respectively. The Latin names in red indicate the genera with a species number less than ten.

4 | DISCUSSION

The present phylogenetic analyses (Figure 2), that the tribe Galegeae is polyphyletic, and Astragaleae should be resurrected and re-circumscribed based on the well-supported *Erophaca*-Astragalean clade, were largely in line with our earlier results.[4] Although Astragaleae is still a morphologically diverse tribe in this case, but given its monophyly, it might be a more plausible tribal circumscription than either the non-monophyletic, narrowly demarcated Astragaleae sensu Hutchinson,[1] or the polyphyletic Galegeae subtribe

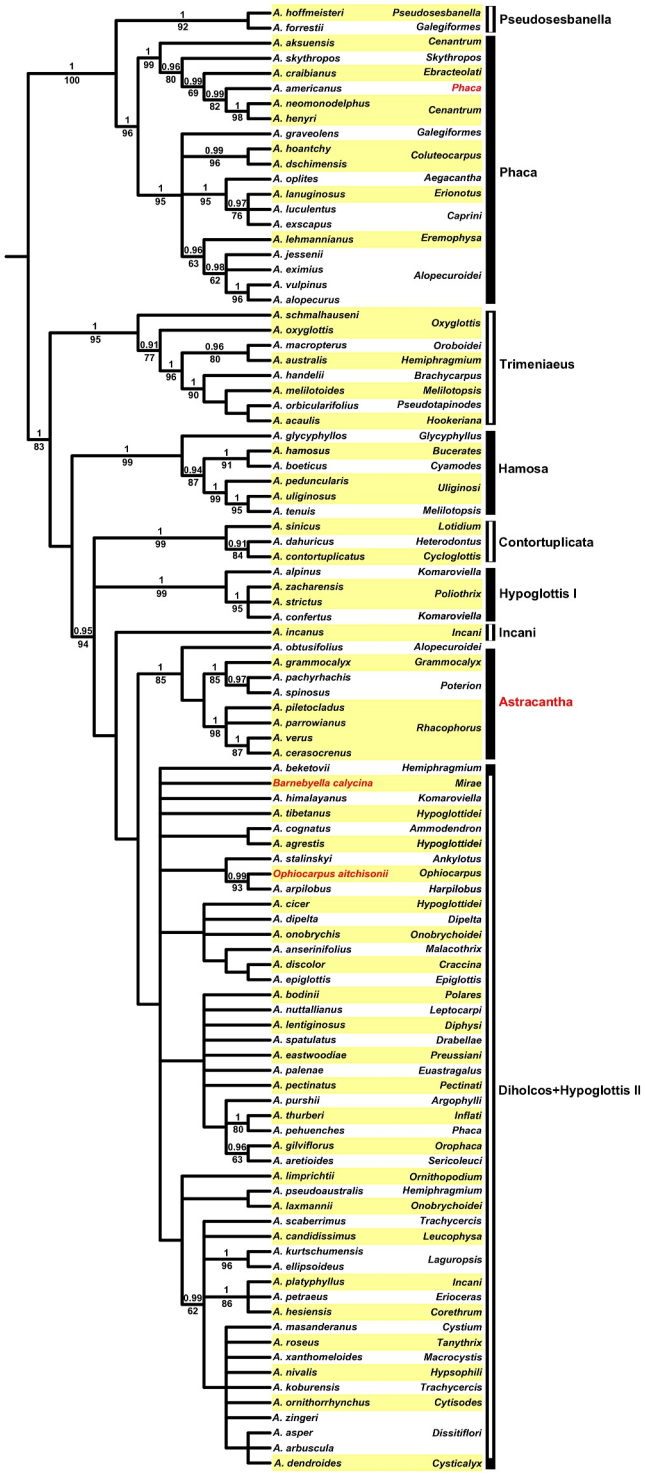


FIGURE 3 Bayesian trees of *Astragalus* based on ITS. Bayesian posterior probabilities (≥0.95) are given above branches, and maximum likelihood bootstrap values (≥50%) are below branches. The labeled tribes and clades largely followed Su et al.[17] The Latin names in red indicate the dubious genera.

Astragalinae sensu Polhill[10] (see below for the taxonomic treatment). However, the relationship of Astragaleae remains uncertain in relation to Hedysareae, or to subtribes Caraganinae and Chesneyinae of tribe Caraganeae sensu Ranjbar et al.[57] (Figure 2). Denser sampling is required to resolve the intertribal position of Astragaleae, especially referring to the larger genera such as *Astragalus*, *Oxytropis*, and *Swainsona*.

Biological diversity is a scientific research focus nowadays, and the subjects of highly diverse plant groups are always eye-catching to botanists, for example, the complicated generic relationships among *Astragalus* and its allies within Astragaleae. *Astragalus* is the most species-rich genus of flowering plants, comprising almost 3000 species and more than 250 sections,[58] widely distributed in temperate Eurasia, North Africa, and Americas.[12] For a long period, phylogeneticists have reached a consensus that *Astragalus* s.l. was not monophyletic; most taxa formed the clade of *Astragalus* s.s., the core group of the genus.[18,20,26,59] In this case, the star genus *Astragalus* can be possibly re-delimited based on *Astragalus* s.s., but few studies so far included adequate in-groups for the genus to confirm this point. Our present results also recovered the clade of *Astragalus* (Figure 2 & 3), and the infrageneric topology and subclades are almost identical to those in our previous study.[17] However, *Astragalus* is still weakly supported by our chloroplast *matK* results (Figure 2B), but our recent cp genome-based analyses already verified its monophyly.[17] Besides, we herein agree with most prior phylogenetic investigations,[17–20] that the satellite genera *Astracantha* Podlech, *Barnebyella* Podlech, *Ophiocarpus* (Bunge) Ikonn. and *Phaca* L. are well nested within *Astragalus* (Figure 3, Trees S2 and S4 in Supporting Information S1), and their diagnosable features are not beyond the morphological variation range of *Astragalus*. Their generic statuses are thus rejected, which was in accordance with a newly published phylogenomic analysis.[27]

As per another ally genera of *Astragalus*, the circum-Mediterranean endemic, monospecific genus *Erophaca* Boiss.[60] was erected based on *A. lusitanicus* L. with a set of morphological features, for example, stipellate leaflets and minute stigma that is hardly distinguishable from style. The genus has been accepted by most taxonomists.[12,61,62] Moreover, prior molecular phylogenetic studies indicated a sistership of *Erophaca* to the rest Astragaleae taxa,[16,63] consistent with our current results (Figure 2), which further favors its generic rank. *Biserrula* L. was proposed by Linnaeus[64] and accepted by most workers[10,12,42,43] mainly based on its characteristic fruits with toothed wings, but sometimes it was controversially merged into *Astragalus*.[65] Recent molecular results showed that *Biserrula* belonged to the Glottis clade along with *Astragalus* sect. *Epiglottis* Bunge and *A.* sect. *Annularis* DC., which is outside *Astragalus*.[18,19,21,22,27,66] Our trees revealed a similar topology, with this clade being represented by *Biserrula pelecinus* L. and *A. annularis* Forssk (Figure 2). Due to its unique fruit morphology and outlying position, we agree on the generic status of *Biserrula*. While in consideration of the distinct morphologies of the two species within the Glottis clade and its uncertain phylogenetic positions between our ITS (Figure 2A) and *matK* (Figure 2B) trees, *Astragalus annularis* cannot be either treated in *Biserrula* nor remained in *Astragalus* at this stage; further data are thus needed to test its exact generic affiliation. Similar to *Erophaca*, the main distribution of the Glottis clade is also in the circum-Mediterranean area,[18] but its biogeographic implication requires further inference.

Two decades ago, Kazempour Osaloo et al.[20] elevated *Astragalus* sect. *Herpocaulos* Bunge to the generic rank as *Podlechiella* Maassoumi et Kazempour Osaloo according to its extremely distant relationship with *Astragalus*, concurred by multiple subsequent molecular phylogenetic investigations[18,19,21,22,27,30,66] and the present analyses (Figure 2). However, the only species of this genus,

Podlechiella vogelii (Webb) Maassoumi et Kazempour Osaloo, has a suspiciously wide range from central Asia to North and East Africa and occupies various types of habitats.[20] In the near future, denser sample collections from different areas may uncover whether the *P. vogelii* should be actually split into multiple species. On the other hand, Fischer[67] established *Phyllolobium* with penicillately hairy stigma, which was then transferred into Astragalus as sect. *Phyllolobium* (Fisch.) Bunge.[68] A series of molecular analyses from Zhang et al.[69,70] resolved a distant relationship between this genus and *Astragalus*, and recovered the generic status of *Phyllolobium* accordingly, which is consented by our results (Figure 2) and the new NGS-based analyses from Folk et al.[27] The genus was also taxonomically revised based on morphological and molecular evidence by Zhang et al.[71,72] Herein, we accept the establishments of the genera *Podlechiella* and *Phyllolobium*.

With ca. 400 species, *Oxytropis* is the second largest genus in Astragaleae, distributed in temperate Eurasia and North America.[10] The genus morphologically resembles *Astragalus* but is recognizable from its pointed keel. Traditionally, taxonomists and phylogeneticists took the two genera as sister groups, even taking them as an *Oxytropis-Astragalus* complex.[29,73,74] Recent molecular analyses approve the monophyly of *Oxytropis*, however, disagreed on its sister relationship with *Astragalus*.[4,27,73] Although the exact phylogenetic position of *Oxytropis* is still unclear, we herein reiterated based on a denser sampling that *Astragalus* and *Oxytropis* are clearly two separated, monophyletic genera; in addition, they are neither sister groups nor a complex (Figure 2). On the other hand, we agreed with Lock and Schrire[12] to treat *Aragallus* Neck. within *Oxytropis*: the former *Aragallus* species *Oxytropis campestris* (L.) DC., *Oxytropis falcata* (Greene) A. Nelson, *Oxytropis lambertii* Pursh, *Oxytropis parryi* A. Gray, and *Oxytropis splendens* Douglas ex Hook. are well nested within *Oxytropis* (Figure 2). At the infrageneric level, unfortunately, few studies resolved the phylogeny of *Oxytropis* with high support up to date[27,28,74] (also see Figure 2). It is probably because *Oxytropis* may commit rapid radiation and reticulated evolution.

Within the newly circumscribed Astragaleae, we included the former tribe Coluteae,[1] which was equal to Galegeae subtribe Coluteinae Benth. and Hook. f.,[10] represented here by *Clianthus*, *Colutea* (Figure 1F), *Eremosparton*, *Lessertia* (Figure 1H), *Oreophysa*, *Sphaerophysa* (Figure 1G), *Smirnowia*, *Sutherlandia*, and *Swainsona* (Figure 1I). This group is not monophyletic in our trees (Figure 2) or in previous studies.[75,76] *Colutea* (see Table 1) is an understudied genus; nearly all the existing phylogenetic analyses suffered from under-sampling problem. While *Oreophysa*, a genus established based on *Colutea* sect. *Oreophysa*, was found to be embedded in a well-supported *Colutea*[30] (also see Figure 2). Browicz[77] revised the infrageneric taxonomy of *Colutea*, which waits for further verification and renewal based on molecular evidence. *Eremosparton*, *Smirnowia*, and *Sphaerophysa* are all species-poor, shrubby genera that are distributed in the temperate Asia interior.[11] Our *matK* results were consistent with LPWG[75] and Moghaddam et al.[30] that *Sphaerophysa* is well supported, but *Smirnowia* is nested within *Eremosparton* (Figure 2B), while our ITS analyses indicated a sister relationship between *Smirnowia* and *Eremosparton* (Figure 2A). We herein temporarily admit the three genera based on the ITS results (Figure 1A) and their distinct morphologies, especially those of leaves and fruits.[1,10]

Lessertia and *Sutherlandia* were endemic to the sub-Sahara region in Africa, and the latter is recognizable from the former mainly by large red flowers and ballooned fruits.[78] Taxonomically, researchers controversially regarded them as two separated genera[1,10,12,79] or merged *Sutherlandia* within *Lessertia*,[78,80] whereas, recent Sanger-based molecular phylogenetic results inclined to support the latter scenario,[30,32] in accordance with our analyses (Figure 2). Apart from the taxonomic issue, what is more interesting is its spatiotemporal evolution question: as the only representative of Astragaleae in the sub-Sahara area, when and how *Lessertia* reached there? How did the species diversity of *Lessertia* (ca. 50 spp.) develop? These problems need further studies with more intensive samples at the species level.

Among the genera of Astragaleae, *Carmichaelia*, *Clianthus*, *Montigena*, *Streblorrhiza*, and *Swainsona* are confined in temperate Oceania: *Swainsona* and *Streblorrhiza* in Australia and the other three genera in New Zealand.[10,12] The five genera formed a clade[34,36] (Figure 2), within which *Swainsona* is the most species-rich genus, but it was less concerned by phylogeneticists. We herein consented to Wagstaff et al.[34] on the polyphyly of *Swainsona*, and *Montigena* tangled with species of *Swainsona* (Figure 2). However, due to limited informative sites, the supports of our and Wagstaff et al.'s trees were weakly supported[34]; while based on the morphological and anatomical difference between *Swainsona* and *Montigena*,[33] their generic statuses are better to be retained at this stage. On the other hand, New Zealand endemic *Clianthus* is sister to a monophyletic *Carmichaelia* according to our trees (weakly supported in ITS trees), consistent with previous molecular[36] and morphological[35] analyses, a significant indication to support the two genera although the infrageneric phylogenies of *Swainsona* and *Carmichaelia* were unsolved herein. In this study, we did not find a sample for the Phillip Island endemic, extinct genus *Streblorrhiza*, while Heenan et al.[36] contributed a valuable molecular phylogenetic work, and discovered that this genus is the sister to a clade formed by all the other Oceanian Astragaleae taxa. Such topology implied a highly noticeable evolutionary and biogeographic pattern for Astragaleae that the common ancestor of the whole Oceanian clade may originate from this tiny island, or the island is an important path for the common ancestor's migration. Given its distinguishable morphological features (pulvinate leaves and a series of anatomical features) and an outlying phylogenetic position with respect to other Oceanian genera of Astragaleae,[36,81] also for the purpose of calling people's attention to this "lost genus", we herein keep *Streblorrhiza* within this tribe, following most taxonomists' treatments.[1,10,12,81] However, the evolutionary history of the Oceanian group can hardly be further studied until the sample of *Streblorrhiza* is collected; we still cherish the hope that "this plant may still survive somewhere in cultivation" as stated by Schrire.[82]

5 | TAXONOMIC TREATMENT

5.1 | Astragaleae adan., tr. emend. - Type: *Astragalus* L.

Adanson, Fam. Pl. 2: 324. 1763; Torrey and Gray, Fl. N. Amer. 328. 1838; Hutchinson, Gen. Fl. Pl. 1: 407. 1964; Rechinger, Fl. Iran. 157: 81. 1984; Castroviejo, Fl. Iber. VII(I): 266. 1999.

Galegeae subtr. Astragalinae—Benth. Et Hook. f., Gen. Pl. 1: 446. 1865; Polhill in Polhill et Raven, Adv. Legum. Syst. 1: 360. 1981; Fu, Fl. Reipubl. Popul. Sin. 42 (1): 11. 1993.

Description—small trees, herbs, or shrubs; hairs basifixed or biramous; sometimes glandular or punctate; leaves epulvinate or puvinus reduced, usually distichous, estipellate, imparipinnate, or paripinnate, rarely 1–3-foliolate; stipules free or joined abaxial, usually adnate to the petiole; flowers in axillary racemes or clusters; bracteoles present or absent; calyx campanulate to tubular; standard clawed or narrowed at the base; wings usually with a fold connecting with the keel; keels blunt to apiculate with claws joined, sometimes abaxially connate; vexillary filament free; anthers usually uniform, separated from thecae; pollen generally small, three to four colporate; ovules (1)-2-numerous, usually biseriate; style tapered, sometimes bearded, with a terminal, lateral, or introrse stigma; fruits compressed or inflated, dehiscent or indehiscent; seeds oblong-reniform, with a small hilum.

Note—Astragaleae includes 16 genera with 3000–3500 species, widely distributed in temperate Eurasia and North America, extending to mountainous and/or drier places in sub-Sahara Africa, temperate regions of Australia, New Zealand, and South America. This tribe generally contains canavanine.

5.2 | Key to genera of Astragaleae

1a. Leaves pulvinate (endemic to Philip Is., Australia)	*Streblorrhiza*
1b. Leaves epulvinate or pulvinus reduced	2
2a. Branches ribbed; leaves small, sparse to lacking on flowering branches (endemic to New Zealand)	*Carmichaelia*
2b. Branches unribbed	3
3a. Styles bearded	4
3b. Styles glabrous	11
4a. Standards long-acuminate; flowers large and showy, pendulous (endemic to North Island of New Zealand)	*Clianthus*
4b. Standards not long-acuminate	5
5a. Leaves reduced to scales style bearded below stigma; fruits thin, not inflated (temperate and arid Eurasian interior)	*Eremosparton*
5b. Leaves imparipinnate, rarely 1-foliolate	6
6a. Leaves 1-foliolate; fruits inflated (Afghanistan, Iran, Turkmenistan, and Uzbekistan)	*Smirnowia*
6b. Leaves imparipinnate	7
7a. Styles apically hooked, bearded along the adaxial side; fruits membranous, inflated, and mostly indehiscent (from N China to Mediterranean S Europe and NE Africa)	*Colutea*
7b. Styles not hooked at the tip	8
8a. Styles bearded on the adaxial side; fruits subglobose, seed-bearing suture slightly intruded into the fruit (from N China and Mongolia to SE Russia and Turkey)	*Sphaerophysa*
8b. Styles bearded only around the stigma	9
9a. Fruits mostly compressed, seed-bearing suture not intruded into the loculus (endemic to Southern Africa)	*Lessertia*
9b. Fruits cylindrical to inflated, seed-bearing suture not intruded into the loculus	10
10a. Plants strongly rhizomatous; pith persistent (endemic to South Island of New Zealand)	*Montigena*
10b. Plants not or slightly rhizomatous; pith impersistent; flowers often showy (endemic to Australia)	*Swainsona*
11a. Plants perennial; keel apex with one apiculus (northern Eurasia and North America)	*Oxytropis*
11b. Plants annual or perennial; apex of the keels without apiculus	12
12a. Fruits flattened, with toothed wings (Mediterranean region and Canary Islands, E and NE Africa)	*Biserrula*
12b. Fruits rarely winged	13
13a. Styles with a brush of straight, rigid, and short hairs just below glabrous stigma (the Himalayas, Xizang, SW, NW, and N China)	*Phyllolobium*
13b. Styles without brushy hair	14
14a. Plants with medifixed hairs; fruit unilocular fruits (subtropical deserts of N Africa and SW Asia)	*Podlechiella*
14b. Lacking the combination of medifixed hairs and unilocular fruits	15
15a. Leaflets without powder and without nectaries at the base; symmetric inflorescences at the axils of the leaves; stigma humid (widely distributed in N Temperate and temperate S America)	*Astragalus*
15b. Leaflets with pulvinule, with two nectaries at the base; inflorescences in multiflorous racemes, with most of the flowers arranged unilaterally; stigma dry (Mediterranean region: Spain, NW Africa, Turkey, and Lebanon)	*Erophaca*

6 | CONCLUSIONS

Astragaleae is one of the largest tribes in the legume family and includes *Astragalus*, the most species-rich genus in angiosperm. The phylogenetic relationships of the tribe would be attractive to a vast range of botanists and pharmaceutical scientists, and a profounder understanding of Astragaleae would promote the hotspot research on plant diversity. In this study, the tribal concept of Astragaleae is accepted, re-delimited and described based on the *Erophaca*-Astragalean clade. We herein recognize 16 genera within the tribe: *Astragalus*, *Biserrula*, *Carmichaelia*, *Clianthus*, *Colutea*, *Eremosparton*, *Erophaca*, *Lessertia*, *Montigena*, *Oxytropis*, *Phyllolobium*, *Podlechiella*, *Smirnowia*, *Sphaerophysa*, *Streblorrhiza*, and *Swainsona*. However, due to Sanger data and under-sampling problems toward the larger genera such as *Astragalus*, *Oxytropis*, *Lessertia*, and *Swainsona*, not all the genera listed above are strongly supported herein; the present study can be considered as a positive attempt to clarify the generic boundaries within Astragaleae. In principle, we provisionally retain the dubious generic statuses for *Montigena*, *Smirnowia*, and *Swainsona*, but an expectant thorough taxonomic treatment is still premature for them. We have committed to having a denser sample collection, including the extinct *Streblorrhiza*, to further resolve their

phylogenies based on next-generation sequencing. The corresponding taxonomic revision is promising in the near future accordingly.

AUTHOR CONTRIBUTIONS

Lei Duan: Conceptualization; funding acquisition; supervision; writing - review and editing; visualization. **Chun Su**: Resources. **Jun Wen**: Conceptualization; project administration. **Yu-Wen Ji**: Methodology; formal analysis; writing - original draft. **Yan Jiang**: Methodology; formal analysis; writing - original draft. **Ting Zhang**: Software. **Joseph L. M. Charboneau**: Resources. **Lin Fu**: Investigation. **Qi-Fei Yi**: Investigation. **Fa-Guo Wang**: Resources; software. **Tie-Yao Tu**: Resources; validation. **Shi-Jin Li**: Resources. **Kai-Wen Jiang**: Resources. **Yu-Han Fang**: Visualization. **Hong-Feng Chen**: Funding acquisition; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Genetic data: data available in a publicly accessible repository in GenBank.

ETHICS STATEMENT

This article does not include human or animal study. Sample collection activities from the field and herbarium were approved and abided by the law and local regulations.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

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