Molecular Biogeography of Tribe Thermopsideae (Leguminosae): A Madrean-Tethyan Disjunction Pattern with an African Origin of Core Genistoides

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Thermopsidaceae has 45 species and Exhibits a series of interesting biogeographical distribution patterns, such asMadrean-Tethyan disjunction and East Asia-North America disjunction, with a center of endemism in the Qinghai-Xizang Plateau (QTP) and Central Asia. Phylogenetic analysis in this paper employed maximum likelihood using ITS, rps16, psbA-trnH, and trnL-F sequence data; biogeographical approaches included BEAST molecular dating and Bayesian dispersal and vicariance analysis (S-DIVA). The results indicate that the core genistoides most likely originated in Africa during the Eocene to Oligocene, ca. 55-30 Ma, and dispersed eastward to Central Asia at ca. 33.47 Ma. The origin of Thermopsidaceae is inferred as Central Asian and dated to ca. 28.81 Ma. Ammopiptanthus is revealed to be a relic. Birth of the ancestor of Thermopsidaceae coincided with shrinkage of the Paratethys Sea at ca. 30 Ma in the Oligocene. The Himalayan motion of QTP uplift of ca. 20 Ma most likely drove the diversification between Central Asia and North America. Divergences in East Asia, Central Asia, the Mediterranean, and so forth, within Eurasia, except for Ammopiptanthus, are shown to be dispersals from the QTP. The onset of adaptive radiation at the center of the tribe, with diversification of most species in Thermopsis and Piptanthus at ca. 4-0.85 Ma in Tibet and adjacent regions, seems to have resulted from intense northern QTP uplift during the latter Miocene to Pleistocene.

1. Introduction

In the Leguminosae, the so-called core genistoides includes tribes Crotalarieae, Genisteae, Podalyrieae, Thermopsidaceae, Euchresteae, and Sophoreae sensu strictu [1–8]. Tribe Thermopsidaceae includes seven genera and about (43)-45-(46) species and occurs in the temperate regions of Eurasia and North America [6, 9]. Of them, Pickeringia, with one species endemic to western North America, has been transformed into Cladrastis-Styphnolobium [5, 10]. Thermopsis and Baptisia are two perennial herbaceous genera, respectively, in distributions of an East Asian-North American disjunction and North American endemism. Anagyrus, Piptanthus, and Ammopiptanthus are shrubby and in Eurasia. Anagyrus includes two species and occurs around the Mediterranean Basin [11, 12]. Piptanthus and Ammopiptanthus mainly occur in China, the former in Sino-Himalayan [13] and the latter in Central Asian regions [14]. New monotypic genus Vuralia recently is segregated from Thermopsis in Turkey [15].

Molecular evidence above the rank of genus has provided a foundation for Thermopsidaceae systematics and biogeography [4, 5, 16–19]. However, due to a lack of sufficient
species sampling, a dense addition of species at generic level is necessary. Wang et al. [14] carried out a comprehensive systematic study of Thermopsideae on the basis of dense species addition and ITS sequences. Biogeographically, the fossil record indicates that the three legume subfamilies appeared in the early Eocene, and extensive diversification and origin of most of the woody legume lineages occurred in the middle Eocene [20]. Schrire et al. [21] divided the distribution patterns of ca. 730 legume genera into four biomes, that is, succulent, grass, rainforest, and temperate, with temperate groups possessing the largest numbers. From macrofossils of leaves and pods, the origin of legumes appears unlikely to have been much before 60 Ma, and, from that time, a rapid diversification among major clades took place [22]. In contrast with a proposed West Gondwana origin of the family [23, 24] or a "moist equatorial megathermal" origin, recent studies favor an origin in the seasonally dry to arid tropical Tethyan seaway corridor [21]. Lavin et al. [22] established a comprehensive schematic chronogram of legumes based on sequence data and fossil constraint, employing a total of 324 species. However, only three species of Thermopsideae were sampled. Lavin et al. [22] estimated ca. 26.5 Ma for the time of origin of Thermopsideae, and Ortega-Olivencia and Catalán [12] dated the appearance of Anagyris to late Miocene (8.2 ± 4.5 Ma). Xie and Yang [25] estimated Ammopiptanthus to have originated in early Miocene ca. 20–21 Ma.

Exceptionally, in the Wang et al. [14] study, although there was strong support for the tribal clade, the systematic position of Ammopiptanthus was suspected as not being a member of Thermopsideae because of the nesting of three Sophora species with it, resulting in Ammopiptanthus being placed in a basally branching position with respect to the rest of the tribe. Many studies have speculated that Piptanthus, Ammopiptanthus, Thermopsis, and so forth in the tribe originated in the Tertiary [14, 26–29], but the exact time and place of origin have remained poorly understood.

In summary, Thermopsideae contains many attractive biogeographical topics, Central Asia, East Asia, and QTP endemism and Madrean-Tethyan disjunction, East Asia-North America disjunction, Tertiary origin, and so forth. Therefore, this paper attempts to reconstruct the phylogeny of the tribe using four genes and, afterward, focuses on the tribe Thermopsideae biogeography by employing biogeographical molecular dating and S-DIVA approaches to explore the spatiotemporal origin and evolution of Thermopsideae and its evolutionary dynamics; to confirm the Madrean-Tethyan disjunction using Thermopsideae; and to discuss the East Asia-North America disjunction, Central Asian endemism, QTP endemism, and so forth.

2. Materials and Methods

2.1. Taxon Sampling. We sampled 32 individuals of 20 species, mainly from China, belonging to three genera, Thermopsis, Piptanthus, and Ammopiptanthus of Thermopsideae; see Table 1. Outgroups were selected from Sophora (S. davidii, S. flavescens, and S. microphylla), Podalyria (Podalyriaceae), and Cytisus (Genistaeae); see Supplementary Material S 1 available online at http://dx.doi.org/10.1155/2015/864804. More out-group species were used in ITS phylogeny; also see S 2.

2.2. DNA Sequencing. Total genomic DNA was extracted using the CTAB method [30]. The polymerase chain reaction (PCR) was used for amplification of double stranded DNA. A 25 μL reaction system contained 0.25 μL of Ex Taq, 2.5 μL of 10× Ex Taq buffer (Mg²⁺ concentration of 25 mM), 2.0 μL of dNTP mix (2.5 mM concentration for each dNTP), 1 μL of the forward and reverse primers at 5 μmol/μL, and 0.5 μL of template DNA. The following primers were used: for ITS, ITSI-F (5'-AGA AGT CGT AAC AAG GTT TCC GTA GC-3') and ITS4-R (5'-TCC TCC GTT CTA TTA TAT GC-3') [31], for trnL-F, trnLF (5'-CGA ATT GAG TAG ACG CTA CG-3') and trnFR (5'-ATT TGA ACT GCT GAC ACG AG-3') [32], for psbA-trnH, psbAF (5'-GTT ATG CAT GAA CGT AAT GCT C-3') [33] and trnHR (5'-CGC GCA TGG ATT CAC AAT CC-3') [34], and, for the intron of rps16, rps16F (5'-CGT GTA GAA AGC GTG CGA CTT-3'), and for rps16R (5'-TCG GGA TCG AAC ATC TAC TGC AAC-3') [35].

PCR amplifications were carried out using the following procedures: there was pre-denaturation at 94°C for 3 min., followed by 30 cycles of (1) denaturation at 94°C for 30 s, (2) annealing at 48°C–54°C for 30 s, and (3) extension at 72°C for 1 min.; at the end of these cycles, there was a final extension at 72°C for 10 min. PCR products were purified using the PEG precipitation procedure [36]. Sequencing reactions were performed by a company specializing in the procedure (Beijing Sanbo Biological Engineering Technology and Service Corporation, China). Sequences were aligned using CLUSTAL X software [37] and then adjusted by hand in BioEdit ver. 5.0.9 [38].

2.3. Phylogenetic Analyses. Two datasets consisting of ITS and the 4-gene sequences combined (ITS+3cpDNA) were prepared for phylogenetic analysis. The 4-gene dataset was examined using the incongruence length difference (ILD) tests [39], implemented in PAUP version 4.0b10 [40], with 100 homogeneity replicates, 10 random addition sequences, tree-bisection-reconnection (TBR) branch swapping on best only, and MULTREES on, and this was performed to test whether the four datasets could be combined. The data partitions of four genes were not significantly incongruent on the basis of the ILD tests (P = 0.01).

Phylogenetic analysis by Maximum Likelihood (ML) of the 4-gene combined sequences was conducted using PAUP* 4.0b10 [40].

For ML analysis, the best fitting DNA substitution model was found employing Modeltest 3.6 [41], of which the Akaike information criterion (AIC) was selected on the basis of the log likelihood scores of 56 models [41]. For the dataset, the TrN+G model was selected as the most appropriate in Modeltest 3.5, with the nucleotide frequencies A = 0.3283, C = 0.1676, G = 0.1985, T = 0.3055, the shape parameter = 0.6264, and an assumed proportion of invariant (PIV) sites = 0. Clade support for the phylogenetic tree was estimated,
<table>
<thead>
<tr>
<th>Species</th>
<th>Voucher</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ammopiptanthus</em></td>
<td></td>
<td></td>
</tr>
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<td><em>A. mongolicus</em></td>
<td>S.H.Cheng</td>
<td>Wuda, Inner Mongolia, China</td>
</tr>
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<td>(Maxim.) S.H.Cheng</td>
<td>Turpan Eremophytes Botanic Garden, China</td>
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<td>M.L.Zhang s.n.</td>
<td>Wuqi, Xinjiang, China</td>
</tr>
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<td></td>
<td>SAF21592</td>
<td>Yadan, Tibet, China</td>
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<td>Wuqia, Xinjiang, China</td>
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<td>Wuqia, Xinjiang, China</td>
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<tr>
<td><em>Piptanthus</em></td>
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<td>H.T.Wang H.C.</td>
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<td><em>P. leiocarpus</em></td>
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<td>Tibet, China</td>
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<tr>
<td><em>Thermopsis</em></td>
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<td><em>T. lanceolata</em></td>
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<tr>
<td><em>T. licentiana</em></td>
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<td><em>T. mongolica</em></td>
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<td><em>T. yushuensis</em></td>
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</tr>
</tbody>
</table>

*HNWP (Herbarium, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, Qinghai); PE (China National Herbarium, Institute of Botany, Chinese Academy of Sciences, Beijing); SHI (Herbarium, Shandong University, Shandong, China).*
employing bootstrap values in PAUP and posterior probability values in MrBayes software.

In order to obtain comprehensive molecular dating, ITS sequence data covered broad outgroups including the four core genistoides tribes and seven fossil genera, which came from our data and from GenBank; see S 2. The final dataset comprised 107 species and 722 bps.

2.4. Estimating Divergence Times

2.4.1. Fossil Constraints. Legumes have rich fossil records [20, 42], but there are fewer fossils assignable to the core genistoides and Thermopsideae. Most fossils of legume genera date to the Miocene, with Bauhinia and Cercis extending even to the Late Cretaceous [42, 43]. Seven fossil genera are used as outgroups; see Table 2. The occurrence of Sophora in the Oligocene-Miocene is credible, since its fossils are known from the Eocene of eastern Siberia and North America [42, 43]. In China, Sophora fossils have been found in Oligocene strata from Heilongjiang province, the Miocene from Shandong and Yunnan provinces, and Pliocene from Shanxi province [42, 43]. Acacia in the Eocene is also well represented in museum collections. Dalbergia fossils, including leaves and fruit, have been recorded at the Eocene-Miocene boundary in North America, the Oligocene-Miocene boundary in Europe, and in the Miocene in Yunnan Province, China. Fossil leaves of Pueraria appeared in the Miocene in Shandong and Yunnan provinces, China [42, 43], and Cladrastis has been dated to middle Eocene [22].

In terms of the ancient fossil record and the phylogenetic tree, the root taxon was considered as Cercis. In the southern China Guangdong province, Cercis fossils have been found from the Late Cretaceous to Eocene, in the Oligocene of Yunnan province, and the Miocene of Shandong and Qinghai provinces. Therefore, this genus is regarded as the root taxon and the age of its ancestor is constrained at 60 Ma. This root constraint is in agreement with Lavin et al. [22], by whom the ancestor of Polygala and Cercis was constrained at 60 Ma. Detailed information are described in Table 2.

The outgroup fossil dates were used as the constraint minimum ages; that is, the maximum fossil dates were selected as the generic minimum age of the most recent common ancestor (MRCA).

2.4.2. Dating Implementation. Currently, phylogenetic dating approaches include r8s, PAML, and BEAST. Of them, BEAST has an advantage for practical applications because of its non-dependence on a phylogenetic tree, and convenient implementation software (BEAST v1.46, http://beast.bio.ed.ac.uk). Moreover, a relaxed molecular clock and Bayesian MCMC search optima are available within it [44, 45].

BEAST was implemented [46] using a Yule process speciation prior to an uncorrelated lognormal model of rate variation and a normal distribution. Tracer v1.4 was used to measure the effective sample size of each parameter and mean and 95% credibility intervals. Two separate MCMC analyses were run for 20,000,000 generations and sampled every 1000 generations. After discarding as burn-in the first 10% of trees searched, the mean and 95% credibility intervals of MRCA nodes were calculated by TreeAnnotator v1.4.8. and visualized by FigTree v1.2.4 [46].

2.5. Biogeographic S-DIVA. DIVA is used to infer mainly ancestral distributions and biogeographical events [47]; it is an event-based method that optimizes ancestral distributions by assuming a vicariance explanation, while incorporating the potential contributions of dispersal and extinction, despite minimizing these under a parsimony criterion [47, 48]. Nylander et al. [49] proposed a modified approach to DIVA naming it Bayes-DIVA because it integrates biogeographical reconstructions of DIVA over the posterior distribution of a Bayesian MCMC sample of tree topologies. Bayes-DIVA is also referred to as S-DIVA [50].

The BEAST dating tree (Figure 2) was treated as a fully resolved phylogram for use as a basis for S-DIVA, and 791 post burnin trees derived from the BEAST analysis were used for ancestral area reconstruction in the program S-DIVA beta version 1.9. S-DIVA was performed with constraints of maximum areas 2 at each node, to infer possible ancestral areas and potential vicariance and dispersal events.

Geographic areas were chosen to cover the distributions of the four core genistoides tribes, especially tribe Thermopsideae. Seven geographic endemic areas were defined in this

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Time (Ma)</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cercis</td>
<td>60–11 Late Cretaceous-Miocene</td>
<td>China</td>
<td>Tao, 1992 [58]; Tao et al., 2000 [42]</td>
</tr>
<tr>
<td>Cercis</td>
<td>Eocene</td>
<td>N America</td>
<td>Lavin et al., 2005 [22]</td>
</tr>
<tr>
<td>Acacia</td>
<td>47–42 Eocene</td>
<td>Liaoning, China; Tanzania</td>
<td>Tao et al., 2000 [42]; Lavin et al., 2005 [22]</td>
</tr>
<tr>
<td>Acacia</td>
<td>15 Miocene</td>
<td>Dominican Rep.</td>
<td>Lavin et al., 2005 [22]</td>
</tr>
<tr>
<td>Bauhinia</td>
<td>ca. 65 later Cretaceous</td>
<td>Heilongjiang, China,</td>
<td>Tao et al., 2000 [42]</td>
</tr>
<tr>
<td>Cladrastis</td>
<td>Miocene</td>
<td>Inner Mongolia</td>
<td>Tao et al., 2000 [42]</td>
</tr>
<tr>
<td>Pueraria</td>
<td>17–5 Miocene</td>
<td>Yunnan, Shandong, China</td>
<td>Tao, 1992 [58]; Tao et al., 2000 [42]</td>
</tr>
<tr>
<td>Dalbergia</td>
<td>19.5–5 Miocene</td>
<td>China, N America, Europe</td>
<td>IB &amp; NIGP, 1978 [43]; Tao, 1992 [58]; Tao et al., 2000 [42]</td>
</tr>
</tbody>
</table>

IB and NIGP: Institute of Botany and Nanjing Institute of Geology and Palaeontology, Academia Sinica.
study: East Asia, Central Asia, the Mediterranean, Africa, Russia (including Central East, Caucasus, and northeastern Russia), North America, and Tibet. Because of its species richness and endemism, the QTP, Tibet is regarded as an area separated from the East Asian floristic region [13].

3. Results

3.1. Phylogenetic Analyses

3.1.1. 4-Gene Combined Analysis. The 4-gene combined dataset included 38 samples and 3099 bps; 496 variable characters were parsimony-uninformative and 421 were parsimony-informative. ML analysis resulted in three optimum trees, topologically almost equivalent; one of them is shown in Figure 1. Bootstrap support from PAUP and Bayesian posterior probability are labeled on the nodes in Figure 1.

Thermopsidae and Ammopiptanthus, respectively, formed a monophyletic group with high support, near 100% bootstrap (BT) and posterior probability values (PP). Piptanthus did not form a monophyletic group, since P. nepalense was placed outside of the genus (Figure 1). Even though the samples of Thermopsis came only from China (Figure 1), the results show that section Thermopsis sensu Sa et al. [28] can apparently be divided into two clades (Figure 1).

3.1.2. ITS Analysis. The ITS BEAST implementation yielded a phylogenetic tree and dating chronogram; see Figure 2. This topology of tree is in rough agreement with that of the previous ITS tree [14] and our 4-gene tree (Figure 1). It indicates that Ammopiptanthus and Thermopsidae are monophyletic groups, respectively. Importantly, this chronogram has a temporal evolutionary significance for the Thermopsidae, Podalyriaceae, Crotalariaeae, Genistaeae, and so forth.

In contrast with previous phylogenies, especially Wang et al. [14], this ITS tree places Ammopiptanthus within Thermopsidae rather than outside of the tribe, and this is the same as in our 4-gene tree (Figure 1). The topological structure of the four tribes of core genistoides is also somewhat different from previous studies [19]; Thermopsidae is related to the cluster of Genistaeae and Podalyriaceae, while Crotalariaeae is more isolated.

3.1.3. Estimating Divergence Times. Using seven fossil genera as constraints and outgroups, for 107 species and ITS dataset, the estimated root age of the four tribes of core genistoides was ca. 54.43 Ma and that of Thermopsidae was ca. 28.81 Ma, as presented in Figure 2. The estimated crown ages of the four tribes range from later Eocene 39.45 Ma (Crotalariaeae) to Miocene 11.89 Ma (Podalyriaceae).

Within Thermopsidae, five genera are well monophyletic, respectively, with credible crown and stem ages excluding Thermopsis. Ammopiptanthus has stem age ca. 28.81 Ma, namely, crown age of Thermopsidae. In order to discuss the origin and evolution of taxa, a geological scale was appended to the BEAST diagram in Figure 2.

3.1.4. Biogeographic S-DIVA. Reconstruction of ancestral areas with S-DIVA (Figure 2) suggested that the ancestral distribution area of core genistoides is Africa (A) and that of Thermopsidae is possibly Central Asia (C) and that Ammopiptanthus is directly derived from Thermopsidae. Extant taxa of North America and the QTP are shown to be dispersals from Central Asia; several events of dispersal and vicariance are illustrated in Figure 2. The most distinct dispersal for the core genistoides is from Africa to Central Asia. From the QTP, a dispersal was westward via the Himalayas to the Mediterranean for the genus Anagyrus, and dispersal and adaptive radiation to East Asia and Central Asia. The eastward line is via the Bering Strait to North America (Figures 2 and 3).

4. Discussion

4.1. Systematics of Thermopsidae. In the previous ITS phylogenetic tree [14], the five genera of Thermopsidae formed a well resolved monophyly, except for the fact that Ammopiptanthus fell outside of Thermopsidae due to the nesting of a few species of Sophora. In addition, diversification into East Asian and North American groups is observed in Thermopsis. From our 4-gene combined (Figure 1) and ITS trees (Figure 2), the monophyly of Thermopsidae and Ammopiptanthus is confirmed once more, and Ammopiptanthus is entirely included within Thermopsidae with high confidence support. Consequently, our enhanced species sampling and 4-gene tree (Figure 1) yield a distinct result compared with Wang et al. [14], mainly, that Thermopsidae is monophyletic, since the three Sophora species (S. davidii, S. flavescens, and S. microphylla) are out of the tribe; two phylogenetic clades are recognized, where the previous tree only had one (see Figure 1 of Wang et al. [14]; our ITS tree also has one clade see Figure 2). This probably will be useful for the revision of classification [28, 51, 52], especially for section Thermopsis sensu Sa et al. [28], of which most species occur in Asia. In addition, the previous taxonomic opinion of Ammopiptanthus being morphologically related to Piptanthus [26, 53] should be considered as reflecting a convergence, since our results (Figures 1 and 2) illustrate that they are separated in the tree. Vuralia has only one species V. turcica (Kit Tan et al.) and has a narrowed distribution (marshy side of Aksehir in Turkey) Uysal et al. [15], even though it had not been joined into the present dataset of Thermopsidae; however, together with Thermopsis chimensis and Th. fabacea, all are shown to be included into North America clade node 9 (Figure 2) [15].

4.2. Age and Distribution Pattern of Thermopsidae. The age of Thermopsidae has been estimated several times by the molecular dating approach. On the basis of fossil data, the genistoides crown node was constrained at 56.42 ± 0.2 Ma. Lavin et al. [22] dated Thermopsidae to ca. 26.5 Ma, but only three species were sampled, that is, Piptanthus nepalense, Baptisia australis, and Thermopsis rhombifolia. This node of the genistoides is placed at ca. 54.43 Ma (Figure 2), near this fossil constraint, confirming the validity of our dating.
To estimate the age of *Anagyris*, Ortega-Olivencia and Catalán [12] employed numerous samples of the two species *A. foetida* and *A. latifolia* and added three other species in the tribe. Their results indicated that an estimated age of Thermopsidae was 27.2 ± 4.1 Ma and of *Anagyris* was 8.2 ± 4.5 Ma. The present paper dates Thermopsidae to ca. 28.81 Ma, which approaches the dates from previous studies by Lavin et al. [22] and Ortega-Olivencia and Catalán [12]. Therefore, the middle Oligocene ca. 28.81 Ma should be treated as the diversification age of the tribe.

Along with the significant global climate cooling and increased aridity from Eocene to Oligocene, seven distinctive biomes have been recognized for the Oligocene (38–24 Ma) [54]. At ca. 30 Ma, one of seven is the warm/cool temperate biome, with a wide band of broadleaved evergreen and deciduous woodland throughout central Eurasia and North America. This biome in its northernmost part just covers the distribution range of Thermopsidae. These woodlands and forests replaced the dominantly evergreen paratropical rainforest of the middle Paleocene and much of the Eocene [54]. Therefore, we can determine that the original accompanying vegetation of Thermopsidae was woodlands and forest, with broadleaved evergreen and deciduous plants. From the Oligocene ca. 30 Ma to middle-to-late Miocene, shrinkage of the Paratethys played an important role in causing transformation of the Central Asian climate from an oceanic to a continental condition [55]. As Hrbek and Meyer [56] have reviewed, the closing of the sea near

**Figure 1**: Phylogenetic tree resulted from maximum likelihood analysis of the combined dataset of 4 genes (ITS, *trn*L-*F*, *psb*A-*trn*H, and *rps*16). Bootstrap support values > 50% above branches and posterior probability support > 0.5 below branches are indicated.
the Oligocene/Miocene boundary had a major impact on the distribution of organism diversity. Therefore, origin and diversification of the Thermopsideae at ca. 30 Ma could therefore have been driven by the closing of the Paratethys, which resulted in a series of changes of environmental and ecological factors and profoundly affected the evolution of the tribe.

The Oligocene environment in Kazakhstan, with broad-leaved forest and swamps, was indicated to be a wet climate [57]. However, the Paleogene floristics of northwestern and central China evidenced by fossil data was dry and subtropical [58]. During the Oligocene, the climate of middle China is speculated to have been an arid-semiarid belt [59]. Therefore, climate in Oligocene Central Asia should have changed from western wet (relic locations of Paratethys shrinkage) in Kazakhstan to eastern dry in northwestern China. These wet environments and climates of western parts of Central Asia most likely fit the emergence of the ancestor of Ammopiptanthus and Thermopsideae, with a broad-leaved forest and a wet to arid climate.

Therefore, from these perspectives of time and place of origin, paleovegetation, and paleoclimate, we can confirm a balanced Oligocene Central Asian origin of Thermopsideae.

4.3. Central Asian Origin and Diversifications among Central Asia, the QTP, and North America. In terms of our inferences of dispersal and vicariance in Thermopsideae (Figures 2 and 3), we can speculate that, after origination in Central Asia, most of its broad-leaved evergreen and deciduous ancestors probably soon became extinct.
Only a few survived, a case being Ammopiptanthus, which evolved from the ancestor of Thermopsidae (Figure 2).

These events strikingly influenced the ecology and environment of the QTP and adjacent regions, especially northern China. These ecological and environmental settings, consequently, can hold temporally the dispersal from Central Asia (see Figure 2, node 8) into the QTP.

As mentioned above, shrinkage of the Paratethys starting from the Oligocene [55] was a dynamic influence for Thermopsidae and most likely also drove the dispersal to North America (dispersal from node 7 Figures 2 and 3). The diversification age between Central Asia and QTP of ca. 20.32 Ma implies a response to QTP uplift and extension as a geological event. QTP uplift is presumed to have initiated very early but had a major phase near the Oligocene-Miocene boundary when loess deposition began and strengthened thereafter. The first phase (Gangdese motion ca. 40 Ma, 45–38 Ma) is characterized by the Indian plate subducting under the Eurasian plate, resulting in the rise of the Gangdise Mountains. High altitude conifers became abundant in the QTP starting at 38 Ma [60]. The second phase (Himalayan motion ca. 21 Ma, 25–17 Ma) is characterized by westward withdrawal of the Paratethys Sea and aridification of interior Asia. The rise or expansion of the QTP became sufficient for the initiation of dust deposition due to the Asian winter monsoon [59, 61, 62]. The third phase, uplift of northern and eastern parts of the QTP at many intervals during the late Neogene to Pleistocene [63–65] is correlated with appearance of ocean upwelling connected to development of the Asian summer monsoons. A particularly intense geologic uplift during this period was recorded in parts of the northernmost QTP at ca. 3.6 Ma [63, 66–68], which was accompanied by the intensification of monsoons to present levels.

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To explain evolutionary process of Thermopylae using uplift is reasonable. For instance, divergence of Piptanthus and other taxa in the QTP and North China (node 11 in Figure 2) is dated to ca. 4 Ma, during the third phase (intensive northern QTP uplift ca. 3.6 Ma). Piptanthus is estimated to be young, with a diversification age of ca. 0.85 Ma, which falls into the period of QTP maximum iceosphere (cryosphere) during the third phase of uplift [66, 68]. Many people have discussed the East Asia-North America disjunction in regard to Thermopsis [14, 28, 51, 52, 69]. Yuan et al. [69–71] and Peng and Yuan [51] considered Thermopsis section Archithemopsis, C. J. Chen, R. Sa, and P. C. Li, to be occurring in Sino-Japanese
regions, as the primitive group in the genus. Sa et al. [28] concluded that Thermopsis originated from the Sino-Japanese flora and then dispersed to North America via the Bering Strait. However, our analysis shows the ancestor of North American species to have come from Central Asia (node 8 in Figure 2) rather than East Asia and that the East Asia clade also on the whole evolved in Central Asia, which is different from previous hypotheses. In addition, a new genus Vuralia is erected from Thermopsis and related to North American node in the ITS phylogenetic tree [15], whereas Thermopsis chinensis and Th. fabacea (Figure 2) are also included in North American node; therefore, these three species in Eurasia can be regarded as the dispersals from North America and recent event in Pliocene-Pleistocene from North America crown root 5 Ma, as shown in Figures 2 and 3.

Meanwhile, our estimated diversification age of the Eurasia (Central Asia)-North America disjunction within Thermopsis is ca. 20.32 Ma (node 8 in Figure 2). This early Miocene time is similar to that of most other genera showing East Asia-North America disjunctions, for example, Cercis ca. 15.41 Ma, Torreya ca. 16.7 Ma [72], Cornus 13.1 Ma [73], Calycanthus 16 Ma [74], Epimedium-Vancouveria 9.7 Ma [75], and Hamamelis 7.7-71 Ma [76], but is different from Kelloggia with 5.42 Ma [77] and Phryma 5.23-3.68 Ma [78]. As mentioned above, another diversification between Eurasia-North America disjunction which originates from North America at ca. 5 Ma Pliocene-Pleistocene (node 9, Figure 2) resembles Kelloggia and Phryma.

From Central Asia, a western dispersal route via the Caucasus arriving at the Mediterranean, is illustrated with Anagyris in Figures 2 and 3. Our dating of Anagyris is 3.08 Ma, which is different from the suggested age of 8.2 ± 4.5 Ma [12]. The Anagyris estimate of Ortega-Olivencia and Catalán [12] probably lacks denser sampling from Thermopsideae, since only 5-6 species were selected in total. In general, sufficient samples are necessary in dating. Vuralia, another Mediterranean genus that belongs to the North America clade (node 9 in Figure 2), as well as Thermopsis chinensis and Th. fabacea, probably dispersed from North America in Pliocene-Pleistocene, since our estimated crown age of North America clade node 9 is about 5 Ma; see Figures 2 and 3.

4.4. Origin and Geographic Diversification of Ammopiptanthus. In view of the unique evergreen broadleaf habit of Ammopiptanthus in the desert region of northwestern China and Kyrgyzstan, many people have speculated that Ammopiptanthus is a relic of the evergreen broadleaf forest of this region from the Tertiary period [14, 26–29, 79, 80]. The two species form an obvious disjunction pattern, A. mongolicus distributed in western Inner Mongolia and the south Gobi desert and A. nanus in the western Tianshan Mts. restricted to the borders between China and Kyrgyzstan [29]. Both species of Ammopiptanthus are diploid [81]. Genetic diversity from ISSR analysis [29] indicated that differentiation of the two species was significant. In view of its high genetic diversity, a vicariance possibly resulted from the fragmentation of the ancestor range.

From the present analysis (Figure 2), Ammopiptanthus is shown to be directly derived from the common ancestor of Thermopsideae, and its divergence time is estimated at ca. 28.81 Ma of middle Oligocene. As mentioned above, during this period, the climate was cooling and increasing in aridity and the vegetation was broadleaved evergreen and deciduous woodland. Therefore, Ammopiptanthus spatiotemporally should be speculated to be a relict survivor of the evergreen broadleaf forest at the Tertiary Oligocene.

Much evidence indicates that CO₂ decline promoted the origin of C₃ photosynthesis in grasses in the middle Oligocene ca. 30 Ma [82–84]. Similar to C₃ grass plants, emergence of Ammopiptanthus just falls into this period, and since it favors cold and arid climates in an arid region, it can be regarded as a plant case of response to CO₂ decline. In view of the unique evergreen broadleaf habit of Ammopiptanthus s.l. (Platanaceae) ca. 20.5–21.9 Ma [91]; it presents a Madrean-Tethyan disjunction. From the temporal dimension, our dating of Thermopsis section Thermia and Baptisia in North America and Thermopsis sections excluding section Thermia and Piptanthus in Eurasia, mainly in the QTP and its adjacent regions and Ammopiptanthus in Central Asia and Anagyrus in the Mediterranean. Clearly, Thermopsideae presents a Madrean-Tethyan disjunction. From the temporal dimension, our dating of Thermopsis to Oligocene ca. 28.81 Ma, is just consistent with Axelrod [89] time range of early Tertiary Oligocene. This is different from the ages of origin of Madrean-Tethyan disjunctions (see review of Wen and Ickert-Bond [90]), for instance, Platanus orientalis-P. racemosa s.l. (Platanaceae) ca. 20.5–21.9 Ma [91]; Juniperus was at 43.66 Ma [92]. Since Thermopsideae is illustrated to be derived from Central Asia, the evolutionary pattern of this tribe would be migration from Eurasia to North America via the Bering Strait. Moreover, types of Thermopsideae, except for members of Thermopsis and Baptisia, are perennial herbs producing rhizomes. The rest of these taxa, especially Ammopiptanthus, are shrubby and likely to be relics of the Tertiary dry broadleaf evergreen sclerophyllous vegetation [89]. This, in fact, provides a relict status of dry broadleaf
evergreen sclerophyllous vegetation of Madrean-Tethyan disjunction. Therefore, from perspectives of distribution, dated age, and vegetation of Thermopsideae, it fits as a good case of Madrean-Tethyan disjunction.

4.6. African Origin and Dispersal of Core Genistoides. The so-called core genistoides are defined on the basis of molecular phylogeny [1, 3–5, 16–19]. This clade has four tribes taxonomically [19], namely, Crotalarieae, Podalyrieae, Genisteae, and Thermopsideae. Except for Thermopsideae, the tribes occur mainly in Africa, and only a few species expand to the Mediterranean, southern Europe, the Middle East, the Caucasus, and Russia [6–8].

Schrire et al. [21] stated that the derived genistoides, including Crotalarieae, Podalyrieae, and Genisteae, have their basal branching elements in warm temperate southern Africa, an ancestral crown in the southern warm temperate biome [22]. From there, they would have migrated northwards through montane tropical Africa to the Mediterranean and Macaronesian regions and sequentially to the New World, or have secondarily invaded the tropics.

Our molecular dating and S-DIVA results (Figures 2 and 3) indicate that the core genistoides originated from Africa, probably warm temperate southern Africa as mentioned above [21], from Eocene to Oligocene ca. 54.43–33.47 Ma (Nodes 1,3). The four tribes not only dispersed to the Mediterranean, West Asia, the Caucasus, northwestern Russia, Central Asia, East Asia, and North America, but also continuously diversified in Africa in situ until middle Miocene ca. 12 Ma, which is fundamentally due to the diversification of the three tribes Crotalarieae, Podalyrieae, and Genisteae in that continent (Figure 2). The exact place of origin of these three tribes will probably become less ambiguous due to discovery of fossil records and increased taxon sampling and sequencing and so forth. However, an African origin is affirmed; furthermore, the biome warm temperate southern Africa, sensu Schrire et al. [21], is accepted here. This also illuminates the origin of Thermopsideae.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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