A phylogeny of Thermopsideae (Leguminosae: Papilionoideae) inferred from nuclear ribosomal internal transcribed spacer (ITS) sequences

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Based on nuclear ribosomal DNA internal transcribed spacer (ITS) sequences, Thermopsideae is phylogenetically studied within a genistoid background. Analysis reveals that the tribe is not supported as a monophyletic group. Some species of *Sophora s.s* are nested within it. The central Asian desert *Ammopiptanthus* forms an isolated clade but is relatively remote to other Thermopsideae members. *Piptanthus*, *Anagyris*, *Baptisia*, and *Thermopsis* are clustered together into a robust clade. We hence propose that the tribe could either be reduced to just the four 'core genera' with *Ammopiptanthus* excluded, or, as an alternative, that Thermopsideae could become part of a new Sophoreae *s.s.* if it is re-circumscribed in the future. Both *Piptanthus* and *Baptisia* appear as monophyletic. The genus *Anagyris* is closer to some east Asian *Thermopsis* species than to *Piptanthus*. The east Asian and North American disjunct *Thermopsis* is not monophyletic. The ITS results suggest a geographical division between the Old World and New World *Thermopsis*. The east Asian species are clustered with *Piptanthus* and *Anagyris*, whereas the North American species are allied to *Baptisia*. Nonetheless, the only two north-eastern east Asian native *Thermopsis* species appear to be more related to the North American group than to the east Asian one. The related biogeographical significance has therefore been additionally discussed. © 2006 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2006, **151**, 365–373.

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INTRODUCTION

Traditionally, the small tribe Thermopsideae (Leguminosae: Papilionoideae) includes six genera with a total of (43)–45–(46) species scattered through the Mediterranean and temperate regions of North America, the Mediterranean Basin, and central to north-east Asia (Turner, 1981; Lock, 2005). Recently, *Pickeringia*, a spinescent shrub of the coastal Chapparal vegetation of western North America, has been confirmed to be most closely related to the basal papilionoids *Cladrastis–Styphnolobium* and distant from Thermopsideae (Sousa & Rudd, 1993; Wojciechowski, Lavin & Sanderson, 2004). *Thermopsis* and *Baptisia* are two peren-

nial herbs producing rhizomes, the former being an east Asian and North American disjunct taxon, the latter an exclusively North American element. *Anagyris*, *Piptanthus*, and *Ammopiptanthus* are shrubby genera native to Eurasia. The two species of *Anagyris* occur around the Mediterranean Basin and in Macaronesia (Browicz, 1978). *Piptanthus* and *Ammopiptanthus* are Sino-Himalayan (Wu & Wu, 1996) and central Asian genera, respectively. The tribe as a whole is absent from the Southern Hemisphere. Free stamens have always marked Thermopsideae as distinct, and led to an association with Sophoreae. Early workers (e.g. Bentham, 1865) placed it in the largely Australian Podalyrieae. However, anatomical and phytochemical researches (summarized by Turner, 1981) point to a closer relationship with Genisteae (Lock, 2005).

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Molecular analyses (e.g. Kajita *et al.*, 2001; Pennington *et al.*, 2001; Wojciechowski *et al.*, 2004) and studies combining molecular and phytochemical data (e.g. Kaess & Wink, 1995; Wink & Mohamed Gamal, 2003) all related Thermopsideae with Genisteae and placed them as part of the 'core genistoids' group [Thermopsideae, Sophoreae *s.s.*, Euchresteae, Podalyrieae *s.l.* (including Liparieae), Crotalarieae and Genisteae] (Crisp, Gilmore & Van Wyk, 2000; Wojciechowski, 2003; Lock, 2005). Circumscription, as well as taxonomy and phylogeny, of the genistoid alliance (Polhill, 1976, 1981, 1994) has been the subject of intensive research over the last 20 years (Crisp *et al.*, 2000), and especially during the past 4–5 years (Pennington *et al.*, 2000, 2001; Kajita *et al.*, 2001; van der Bank *et al.*, 2002; Aïnouche *et al.*, 2003; van Wyk, 2003; Wojciechowski, 2003; Heenan, Dawson & Wagstaff, 2004; Wojciechowski *et al.*, 2004). Several genistoid tribes have been studied based on various molecular sequences (e.g. Kaess & Wink, 1995, 1997a; Thompson, Ladiges & Ross, 2001; van der Bank *et al.*, 2002; Aïnouche *et al.*, 2003). More individual genistoid taxa need to be studied to further understand the phylogeny of the whole genistoid group.

Many molecular phylogenetic studies concerning Thermopsideae have been carried out (Kaess & Wink, 1995, 1996, 1997a, b; Doyle *et al.*, 1997, 2000; Crisp *et al.*, 2000; Kajita *et al.*, 2001; Pennington *et al.*, 2001; Heenan *et al.*, 2004; Wojciechowski *et al.*, 2004), but all of these analyses adopt a few samples and *Ammopiptanthus* is omitted. In some analyses, Thermopsideae (usually represented by *Anagyris foetida*, *Piptanthus nepalensis*, *Thermopsis fabacea*, and *Baptisia tinctoria*) is supported as monophyletic and sister to Sophoreae *s.s.* [Crisp *et al.*, 2000, based on internal transcribed spacer (ITS) and a combination of ITS and *rbc*L; Heenan *et al.*, 2004, based on ITS]; however, in other analyses (Kaess & Wink, 1995, on *rbc*L; Kajita *et al.*, 2001, on *rbc*L; Wojciechowski *et al.*, 2004, on *mat*K), the tribe is not supported as monophyletic. In the latter analyses, some of its elements are nested within a paraphyletic Sophoreae *s.s.* (e.g. some, but not all, species of *Maackia*, *Ammodendron*, and *Sophora*, etc.), and the combined Sophoreae *s.s.*–Thermopsideae clade is sister to a Podalyrieae–Crotalarieae–Genisteae clade.

Therefore, a re-circumscribed Thermopsideae, excluding *Pickeringia*, requires further study to ascertain its tribal composition and validity (Lock, 2005). Moreover, making a phylogenetic and biogeographical study of the typical Northern Hemisphere temperate member is also significant. Species of the tribe are distributed sparsely in Eurasia and North America, and materials for testing are difficult to obtain, especially samples of the endemic oligotypical taxa confined to narrow areas or those taxa close to extinction. The primary goals of this study are: (1) to test whether or not Thermopsideae is monophyletic and to assess the placement of *Ammopiptanthus*; (2) to estimate the related biogeographical significance, with special reference to the east Asian and North American disjunct *Thermopsis*.

MATERIAL AND METHODS

STUDY GROUP

A total of 53 species was sampled (Table 1), 18 of which were sequenced in the study; the others can be obtained from GenBank. Among them, 25 species represent five genera of the Thermopsideae group. Three small genera, including *Anagyris*, *Piptanthus*, and *Ammopiptanthus*, were completely sampled. Five species of *Baptisia* were sampled to represent the genus (about 35 species; Turner, 1981). Fourteen species of *Thermopsis* were selected so as to provide a balanced, representative sample of the recognized resolution and of the geographical range of the genus (about 21 species, ten in North America and 11 in east Asia; Chen, Mendenhall & Turner, 1994; Sa, Chen & Li, 2000). Among *Thermopsis*, *T. fabacea* (a native northeastern east Asian species, GenBank number: AY091573) has been detected having identical ITS sequences with that of the same-named species sampled previously by Kaess & Wink (1995, 1997a, 1997b), Doyle *et al.* (1997), Crisp *et al.* (2000), and Heenan *et al.* (2004) (GenBank numbers: Z72316 and Z72317). We additionally refer to the species for consideration as it has some biogeographical significance in the discussion. We investigated Thermopsideae within the 'core genistoids' in order to test the phylogeny of it as rigorously as possible. This analysis was similar to part of the work of Crisp *et al.* (2000), whereas some data were moderately altered (Table 1). Outside Thermopsideae, an additional 27 species representing five other genistoid tribes were included. The monogeneric Euchresteae was omitted because its ITS sequence is unavailable, but this will not influence the phylogenetic analysis of Thermopsideae. A single outgroup sequence of *Amorpha fruticosa* (tribe Amorpheae) was used as it has been shown to be basal to the genistoid alliance (Crisp *et al.*, 2000; Kajita *et al.*, 2001; Pennington *et al.*, 2001; Wojciechowski *et al.*, 2004). Accession information, including GenBank numbers, are listed in Table 1, and vouchers for the new sequences are deposited in the Herbarium of the Institute of Botany (PE), The Chinese Academy of Sciences and the Herbarium of Kunming Institute of Botany (KUN), The Chinese Academy of Sciences. The complete data sets are available upon request from the first author.

GENOMIC DNA EXTRACTION, POLYMERASE CHAIN
REACTION AMPLIFICATION AND SEQUENCING

Total DNA was extracted from leaves using a modified cetyl trimethyl ammonium bromide (CTAB) procedure (Doyle & Doyle, 1987). We used silica gel-dried or fresh leaves for all accessions, sterilizing the surface of the leaves prior to DNA isolation. Double-stranded DNA was directly amplified by symmetric polymerase chain reaction (PCR) amplification using the ITS5 and ITS4 primers of White et al. (1990). Reaction volumes were 20 μL and each contained 1.5 U AmpliTaq DNA polymerase, Replitherm TM buffer, 1.5 mmol/L MgCl$_2$, 0.4 mmol/L dNTP, 0.1 μmol/L primer, 5% dimethylsulphoxide (DMSO), and 25–60 ng sample DNA. PCR was performed in a GeneAmp 9600 thermal cycler (Perkin-Elmer) and consisted of 30 cycles of 1.5 min at 94 °C for template denaturation, 2 min at 55 °C for primer annealing, 1 min at 72 °C for primer extension, followed by a final extension of 7 min at 72 °C. PCR products were purified using Watson's purification kit prior to sequencing.

Double-stranded purified PCR products were sequenced using the dideoxy chain termination method employing an ABI PRISM Bigdye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase FS (Perkin-Elmer). Reactions and programs were chosen according to the recommendations of the handbook, with slight modification in some cases. Samples were electrophoresed in an ABI310 automated sequencer. Primers ITS5 and ITS4 were used to sequence all samples and, in the case of potential nucleotide site polymorphisms or ambiguous sequences, primer N18L18 (Wen, Jansen & Zimmer, 1996) was also used. Boundaries of both ITS1 and ITS2 genes were determined by comparison with the published sequences (Baldwin, 1992).

DNA sequences were aligned by Megalign (DNASTAR) and adjusted manually where necessary. In all phylogenetic analyses, characters were unordered and weighted equally. Maximum parsimony (MP) analysis was performed using PAUP version 4.0b10 (Swofford, 2003). Gaps were treated as missing data. Heuristic searches for MP analysis with 100 random taxon addition replicates, tree bisection–reconnection (TBR) branch swapping, and collapse of zero-length branches were conducted. The amount of support for the clades revealed in the maximum parsimony tree(s) [MPT(s)] was examined with 1000 bootstrap replicates with the heuristic search options using parsimony.

RESULTS

The ITS sequences varied in length from 589 to 608 nucleotides. The aligned sequences comprised 644 characters, 37 (5.74%) of which were indel positions. A total of 111 positions were parsimony-informative. During the sequence matrix alignment, we found just one or two regions in a few taxa that seemed a little ambiguous, and the result was insignificantly different when they were removed from the analysis. A heuristic search of the ITS data recovered 42 MPTs of 813 steps (CI = 0.519, excluding uninformative characters; RI = 0.810) in a single island. The strict consensus tree of 42 representative equally most parsimonious trees was resolved and is presented in Figure 1 with bootstrap values.

The core genistoid alliance was shown to form a strong monophyletic group (Clade A, 100% bootstrap) (Fig. 1). Despite a few discrepancies (e.g. the uncertain position of Liparia umbellifera), the major phylogenetic frame and relationships within the genistoid alliance were consistent with those of Crisp et al. (2000) as well as other workers (Kaess & Wink, 1995, 1996, 1997b; Doyle et al., 2000; Kajita et al., 2001; Pennington et al., 2001; van der Bank et al., 2002; Wojciechowski et al., 2004).

Within Thermopsideae, three main clades occur. First, the four genera, Piptanthus, Anagyris, Thermopsis, and Baptisia, called 'core Thermopsideae' members, cluster into a group (Clade C, 100% bootstrap); second, the two species of the central Asian Ammopiptanthus form a monophyletic group (Clade B, 100% bootstrap); third, however, a monophyletic Sophora s.s. clade (Clade D, 99% bootstrap), including two Eurasian species (Sophora davidii and S. flavescens) and a New Zealand endemic S. microphylla, is embedded within Thermopsideae. Sophora s.s. is sister weakly to the four core Thermopsideae genera, in agreement with the studies of Heenan et al. (2004) and Crisp et al. (2000). Ammopiptanthus is basal in Sophora s.s. plus other Thermopsideae members (89% bootstrap). Within core genistoids, all of them as a whole are allied to a Podalyrieae–Amphithalea (Liparieae) clade (99% bootstrap).

DISCUSSION

The present ITS analysis reveals that Thermopsideae (excluding Pickeringia but including Ammopiptanthus) may not be a monophyletic group, as some members of Sophora s.s. are nested within it. The four core Thermopsideae genera are closer to Sophora s.s. than to Ammopiptanthus. This indicates that the placement of Ammopiptanthus may be problematic and that Thermopsideae needs further re-circumscription. Obviously, it is not fitting that some members of Sophoreae, e.g. Sophora s.s and relatives, should be included as part of a wider Thermopsideae. We hence propose that the tribe could either be reduced to just Thermopsis, Piptanthus, Baptisia, and Anagyris, with

Table 1. Terminal taxa used in the phylogenetic analyses, with identifiers of vouchers (collector's name, number, and herbarium acronym) for the authors' collections and those donated by others, and accession numbers for those sequences lodged in GenBank

Taxon	Voucher specimen	Voucher location	ITS identifier	Author, year
Ammopiptanthus nanus (M. Pop.) Cheng f.	Yin LK, 248 (KUN)	Xinjiang, China	AY091567	Wang HC, 2002
Ammopiptanthus mongolicus (Maxim. ex Kom.) Cheng f.	Yin LK, 247 (KUN)	Inner Mongolia	AF209784	Wang HC, 1999
Piptanthus leiocarpus Stapf	Sunhang-01 (KUN)	Tibet, China	AY091569	Wang HC, 2002
Piptanthus tomentosus Franch.	Wang HC, 0132 (KUN)	Yunnan, China	AY091570	Wang HC, 2002
Piptanthus nepalensis (Hook.) D. Don	Wang HC, 0121 (KUN)	Yunnan, China	AF215922	Wang HC, 1999
Thermopsis lanceolata R. Br.	Saren 010 (PE)	Qinghai, China	AF123448	Saren, 2000
Thermopsis smithiana Peter-Stibal.	Sun Hang BM-1 (KUN)	Yunnan, China	AY773354	Wang HC, 2004
Thermopsis alpina (Pallas) Ledeb.		Tibet, China	AF123447	Saren, 2000
Thermopsis chinensis Benth. ex Moore	Yuan YM, s.n. (PE)	Shanxi, China	AF123443	Saren, 2000
Thermopsis barbata Benth.	David Boufford 105849 (KUN, A)	Tibet, China	AY773353	Wang HC, 2004
Thermopsis turkestanica Gand.	Saren 051 (PE)	Xinjiang, China	AF123446	Saren, 2000
Thermopsis inflata Camb.	Liu JQ s.n.	Qinghai, China	AF123451	Saren, 2000
Thermopsis licentiana Pet.-Stib.	Liu JQ s.n.	Qinghai, China	AF123449	Saren, 2000
Thermopsis fabacea (Pall.) DC.	Wang HC, KBG-063 (KUN)	North-east China	AY091573	Wang HC, 2002
Thermopsis montana Nutt. ex Torrey & A. Gray	Jones PD, NCBG-07 (KUN)	North America	AY091574	Wang HC, 2002
Thermopsis rhombifolia (Nutt. ex Pursh) Richardson var. *ovata*		North America	AF007468	Ainouche & Bayer, 1999
Thermopsis divaricarpa Nelson	Jones PD, NCBG-01 (KUN)	North America	AY091575	Wang HC, 2002
Thermopsis macrophylla Hook. ex Arn.	Turner BL s.n. (TEX)	California, USA	AF123450	Saren, 2000
Thermopsis villosa (Walt.) Fern. & Schub		North America	AY773355	Wang HC, 2004
Baptisia alba (L.) Vent.	Jones PD, NCBG-3-00 (KUN)	North America	AY773348	Wang HC, 2004
Baptisia bracteata Muhl. ex Ell.	Jones PD, NCBG-5-00 (KUN)	North America	AY773349	Wang HC, 2004
Baptisia cinerea (Raf.) Fern. & Schub.	Jones PD, NCBG-4-00 (KUN)	North America	AY773350	Wang HC, 2004
Baptisia australis (L.) R. Br.	Jones PD, NCBG-05 (KUN)	North America	AY091572	Wang HC, 2002
Baptisia sphaerocarpa Nutt.	Jones PD, NCBG-1-00 (KUN)	North America	AY773351	Wang HC, 2004
Anagyris foetida L.	Wang HC, KBG-127 (KUN)	Yunnan, China	AY091571	Wang HC, 2002
Sophora microphylla Aiton			AY056075	Mitchell & Heenan, 2002
Sophora flavescens Alt. Hort	Saren Garden 001 (PE)	Beijing, China	AF123452	Saren, 1999
Sophora davidii (Franch.) Skeels	Sun Hang 9001 (KUN)	Kunming, China	AY773352	Wang HC, 2004

Species	Accession	Reference
Podalyria calyptrate (Retz.) Willd.	AF287670	Crisp *et al.*, 2000
Podalyria speciosa Eckl. & Zeyh	AF287671	Crisp *et al.*, 2000
Virgilia oroboides (P.J.Bergius) TM.Salter	AF287669	Crisp *et al.*, 2000
Cyclopia glabra (Hofmeyr & E.Phillips) A.L.Schutte	AF287666	Crisp *et al.*, 2000
Amphithalea ericifolia (L.) Eckl & Zeyh	AF287673	Crisp *et al.*, 2000
Liparia umbellifera Thunb	AF287665	Crisp *et al.*, 2000
Genista tournefortii Spach	AY263669	Cubas & Pardo, 2005
Genista versicolor (L'Her.) Poiret	AY263677	Cubas & Pardo, 2005
Lupinus polyphyllus Lindley	AY338945	Ree *et al.*, 2004
Echinospartum ibericum Rivas Mart	AY263629	Cubas & Pardo, 2005
Retama monosperma (L.) Boiss.	AY263681	Cubas & Pardo, 2005
Retama sphaerocarpa (L.) Boiss.	AY263683	Cubas & Pardo, 2005
Stauracanthus boivinii (Webb) Samp	AY263684	Cubas & Pardo, 2005
Ulex micranthus Lange	AY263687	Cubas & Pardo, 2005
Melolobium microphyllum (L.f) Eckl. & Zeyh	AF287683	Crisp *et al.*, 2000
Aspalathus cordata (L.) R. Dahlgren	AF287681	Crisp *et al.*, 2000
Aspalathus corrudifolia Bergius	AF287682	Crisp *et al.*, 2000
Lebeckia sessilifolia Benth.	AF287678	Crisp *et al.*, 2000
Rafnia ovata Schinz	AF287680	Crisp *et al.*, 2000
Rafnia perfoliata E. Mey.	AF287679	Crisp *et al.*, 2000
Pearsonia sessilifolia (Harvey) Dümmer	AF287675	Crisp *et al.*, 2000
Lotononis laxa Eckl. & Zeyh.	AF287677	Crisp *et al.*, 2000
Lotononis lotononoides (Scott-Elliot)	AF287676	Crisp *et al.*, 2000
Crotalaria capensis Baker	AF287674	Crisp *et al.*, 2000
Amorpha fruticosa L.	U59890	Doyle *et al.*, 1997

KUN, Kunming Institute of Botany, Kunming, China; PE, Institute of Botany, Beijing, China. Nomenclature follows ILDIS (http://www.ildis.org/LegumeWeb/) and Tropicos (http://www.mobot.org/Pick/Search/pick.html).
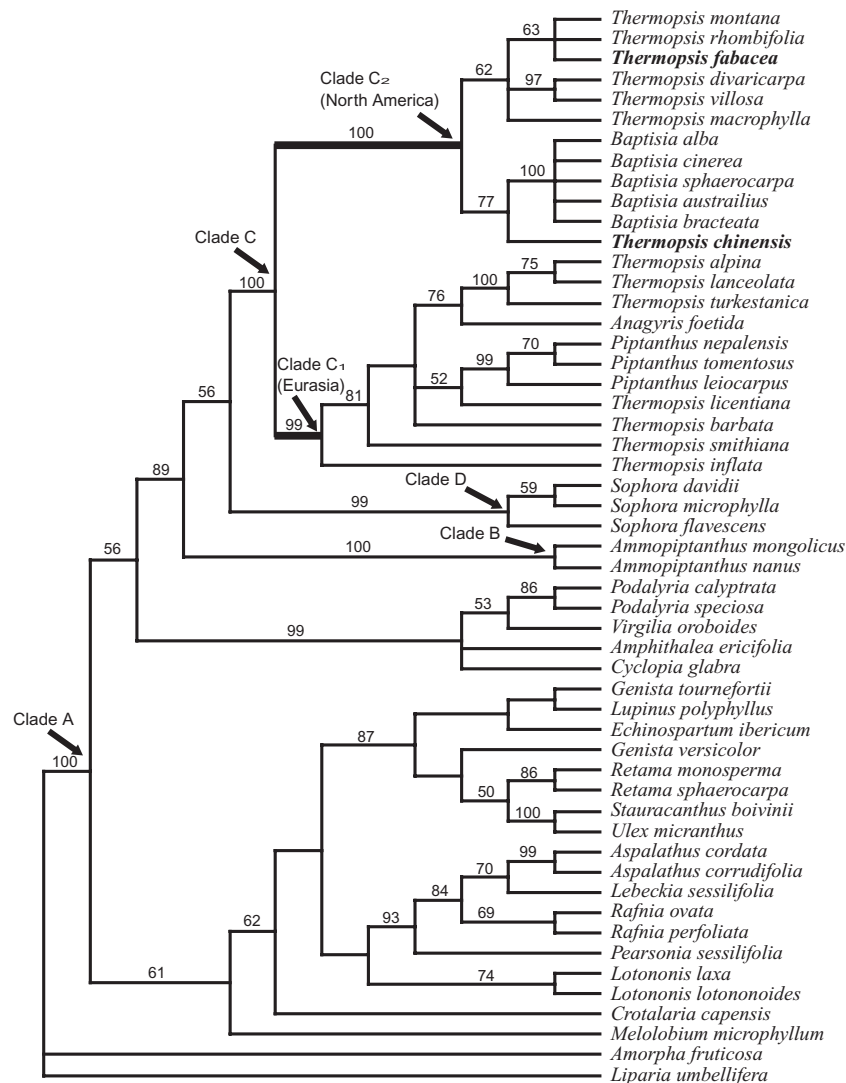
Figure 1. Strict consensus tree resulting from nuclear ribosomal DNA internal transcribed spacer (ITS) sequence data. Forty-two maximum parsimony trees of 813 steps (CI = 0.519, excluding uninformative characters; RI = 0.810) were recovered and these were in a single island. Numbers on the branches indicate the bootstrap values where these are 50% or more. The two north-eastern east Asian endemic species of *Thermopsis*, *T. fabacea* and *T. chinensis*, are marked in bold.

Ammopiptanthus excluded, or perhaps, as an alternative, it could be treated as a subclass (e.g. treated as a subtribe) of a new Sophoreae *s.s.* if this tribe is re-circumscribed in future (Lock, 2005).

Nevertheless, we must still be careful in ascertaining whether or not the new reduced Thermopsideae is monophyletic. Although the cladogram indicates that it forms a robust clade (Fig. 1, Clade C, 100% bootstrap), and Crisp *et al.* (2000) and Heenan *et al.* (2004) have revealed that it is a strong monophyletic group also, information from other phylogenetic analyses should not be ignored. Wojciechowski *et al.* (2004) (based on *mat*K) not only indicated that *Pikeringia* is remote from the tribe, but also showed that *Piptan-* *thus* is closely allied with *Maackia*, and *Baptisia* plus *Thermopsis* is allied with *Ammodendron* and *Sophora s.s.* In an unweighted MPT, Kaess & Wink (1995: 158) (based on *rbc*L) also revealed that the *Anagyris–Piptanthus* clade is more related to *Sophora s.s.* than to the *Thermopsis–Baptisia* clade. We are now virtually certain that the main Thermopsideae members are always sister to some Sophoreae *s.s.* species. However, some important genera of Sophoreae *s.s.*, such as *Maackia*, *Ammodendron*, *Bolusanthus*, etc., shown by other phylogenetic analyses (mentioned above) to be close to *Sophora s.s.*, have not been included in this analysis. To elucidate the phylogeny of Thermopsideae, sampling of these taxa would be very desirable.

The systematic position of *Ammopiptanthus* needs to be clarified in future. Its two species can be distinguished by the shape of their leaves (trifoliate in *A. mongolicus* compared with simple leaves in *A. nanus*). Both species are narrowly distributed; *A. mongolicus* is endemic to the south Gobi desert and *A. nanus* is restricted to the borders between China and Kyrgyzstan, growing in a narrow altitudinal strip between 1800 and 2800 m (Liu *et al*., 1995; Liu, 1998; Ge *et al*., 2005). In the north-western desert of China, *Ammopiptanthus* is the only evergreen broadleaf shrub, which has been viewed as an ancestral trait that identifies it as a Tertiary relict taxon (Liu *et al*., 1995). Morphologically, the genus is different from other Thermopsideae members in, for example, stipules small, subulate, not united, arising on either side of the petiole and possessing two bracteoles (Turner, 1981). Liu & Qiu (1982) and Yuan, Peng & Chen (1991) investigated its special anatomical and ecological characteristics as adaptations to an arid environment, and Yuan & Peng (1991) observed its distinct pollen structure. Yuan & Chen (1993) therefore thought of the genus as an isolated taxon. In our analysis, *Ammopiptanthus* may not be a member of Thermopsideae, but it forms a single clade well outside *Sophora s.s.* plus core Thermopsideae species (89% bootstrap). This implies that it has a more or less close affinity with the Sophoreae *s.s.*–Thermopsideae clade, and hence it is not possible to be a member of any other core genistoid, e.g. Podalyrieae, Crotalarieae or Genisteae, etc. Combining its morphological and ecological features as well as its geographical distribution with the present molecular analysis, we believe that the genus may be a remnant survivor of the evergreen broadleaf forest of the central Asian desert from the Tertiary period, when the vegetation in north-western China was dominated by evergreen and/or deciduous broadleaf forest (Geng, Tao & Xie, 2001). To sum up, *Ammopiptanthus* is an interesting but controversial taxon and its phylogeny requires further study.

The remaining four genera of Thermopsideae are clustered together to form a strong clade (Clade C). Within the group, the three species of *Piptanthus* and five species of *Baptisia* form two monophyletic groups (99% and 100% bootstrap, respectively). However, the phylogeny of *Thermopsis* is relatively complex, and has no support as a monophyletic group. *Anagyris*, proposed previously to be a relative of *Piptanthus* (Turner, 1981; Kaess & Wink, 1995, 1997b; Crisp *et al*., 2000; Kajita *et al*., 2001; Heenan *et al*., 2004), is nested within some species of east Asian *Thermopsis*.

Thermopsis can generally be divided into two parts, with the east Asian group allied with *Anagyris* as well as *Piptanthus* (Fig. 1, Clade C_1) and the North American group with *Baptisia* (Clade C_2). Obviously, the ITS results suggest a geographical division between the Old World and New World *Thermopsis*. There is a rich morphological diversity in *Thermopsis* and the genus occurs in a wide range of eco-geographical conditions in both east Asia and North America (Larisey, 1940; Isely, 1981; Peng & Yuan, 1992; Chen *et al*., 1994; Sa *et al*., 2000). The east Asian species are primarily distributed in Sino-Himalayan regions (Wu & Wu, 1996) at elevations of 1500–4000 m, west as far as India and northward into central Asia, and east as far as the Bering Strait regions. The North American parts are mainly distributed throughout the western states (seven endemic species) and in the southern Appalachian foothills (three endemic species) (Sa *et al*., 2000). Sa *et al*. (2000) have classified *Thermopsis* into five sections including *Archithermopsis*, *Thermia*, *Thermopsis*, *Thermopsidella*, and *Pseudothermia*. The first two, representing the North American species, are generally taller with free bracts, whereas the last three, representing the east Asian parts, are relatively dwarf, pubescent with connate bracts. Combining the above molecular results, we suspect that the derivatives of *Thermopsis* in east Asia and in North America might each have their own unique evolutionary history.

In addition, the only two north-eastern east Asian endemic species (*T. fabacea* and *T. chinensis*, sampled in this analysis) may exhibit profound phytogeographical significance. They are restricted to north-east China, Japan, Korean Peninsula to Far Eastern Russia (Wei, 1998), and are the typical Sino-Japanese floristic elements (Wu & Wu, 1996). Nevertheless, our analysis shows that the two species are instead clustered with the North American parts. Sa *et al*. (2000) have also included them with six North American species to comprise the sect. *Archithermopsis*. This phylogenetic relationship may reveal corresponding morphological convergence as well as divergence between east Asian and North American *Thermopsis*. Consider that, in *Thermopsis*, some intercontinental exchanges of species could have taken place around the Bering Strait regions in earlier geological history, and such floristic connections consequently have enriched *Thermopsis* diversity. This is a preliminary molecular systematic study on Thermopsideae. In order to resolve the phylogeny of the tribe more clearly, more evidence and more samplings are needed.

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