

Evolutionary response to the Qinghai-Tibetan Plateau uplift: Phylogeny and biogeography of *Ammopiptanthus* and tribe Thermopsideae (Fabaceae) (#15834)

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Evolutionary response to the Qinghai-Tibetan Plateau uplift: Phylogeny and biogeography of *Ammopiptanthus* and tribe Thermopsidae (Fabaceae)

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Based on sequence data from the nuclear ITS and four cpDNA regions (*matK*, *trnH-psbA*, *trnL-trnF*, *rbcL*), phylogeny of the tribe Thermopsidae was inferred. Our analyses supported this tribe being merged into a monophyletic Sophoreae in a broad sense, with exclusion of *Pickeringia*. Genera of Sophoreae were separated into the Thermopsoid clade and Sophoroid clade. Monophyly of *Anagyris*, *Baptisia* and *Piptanthus* were basically supported in the Thermopsoid clade. *Ammopiptanthus*, consisting of *A. mongolicus* and *A. nanus*, nested within the Sophoroid clade, with *Salweenia* as its sister. *Ammopiptanthus* and *Salweenia* disjunctively distributed in desert of Northwestern China and Hengduan Mountains, respectively. Divergence age was estimated based on the ITS phylogenetic analysis. Emergence of the common ancestor of *Ammopiptanthus* and *Salweenia*, divergence between these two genera, and split of *Ammopiptanthus* species occurred at approximately 26.96 Ma, 4.74 Ma and 2.04 Ma, respectively, which may be response to the second, third, fourth rapid uplift of the Qinghai-Tibetan Plateau, respectively.

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Abstract:

Based on sequence data from the nuclear ITS and four cpDNA regions (*matK*, *trnH-psbA*, *trnL-trnF*, *rbcL*), phylogeny of the tribe Thermopsideae was inferred. Our analyses supported this tribe being merged into a monophyletic Sophoreae in a broad sense, with exclusion of *Pickeringia*. Genera of Sophoreae were separated into the Thermopoid clade and Sophoroid clade. Monophyly of *Anagyris*, *Baptisia* and *Piptanthus* were basically supported in the Thermopoid clade. *Ammopiptanthus*, consisting of *A. mongolicus* and *A. nanus*, nested within the Sophoroid clade, with *Salweenia* as its sister. *Ammopiptanthus* and *Salweenia* disjunctively distributed in desert of Northwestern China and Hengduan Mountains, respectively. Divergence age was estimated based on the ITS phylogenetic analysis. Emergence of the common ancestor of *Ammopiptanthus* and *Salweenia*, divergence between these two genera, and split of *Ammopiptanthus* species occurred at approximately 26.96 Ma, 4.74 Ma and 2.04 Ma, respectively, which may be response to the second, third, fourth rapid uplift of the Qinghai-Tibetan Plateau, respectively. 

Introduction:

Thermopsideae (Yakovlev 1972) is a small tribe in Leguminosae, comprising six genera, *Ammopiptanthus* S.H.Cheng, *Anagyris* L., *Baptisia* Vent., *Pickeringia* Nutt. ex Torr. & A.Gray, *Piptanthus* Sweet, *Thermopsis* R.Br. ex W.T.Aiton, with a total of ca. 45 species. Thermopsideae ranges from Mediterranean Basin, C and NE Asia to temperate N America (Lock 2005; Turner 1981; Wang 2001). Early phylogenetic works supported that Thermopsideae, except for *Pickeringia*, was nested in the “core genistoids” group, which always contains quinolizidine alkaloids (Crisp et al. 2000; Wojciechowski et al. 2004). Subsequent results of Wang et al. (2006) resolved two ~~unsis~~ clades in this tribe: the genus *Ammopiptanthus* and the “core genera” clade, consisting of *Anagyris*, *Baptisia*, *Piptanthus* and *Thermopsis*. Based on plastid marker *matK*, some recent analyses conducted by Cardoso et al. (2012a, 2013) treated the five abovementioned genera of Thermopsideae into Sophoreae ~~in a broad sense~~. However, Zhang et al. (2015a) accepted the concept of Thermopsideae without sampling of *Pickeringia*. The monophyly and the tribal rank of Thermopsideae are thus controversial.

Within Thermopsideae, *Anagyris* (Ortega-Olivencia 2009), *Baptisia* (Larisey 1940a; Turner 2006), *Pickeringia* (Wojciechowski 2013), *Piptanthus* (Turner 1980; Wei 1998; Wei & Lock

2010) and *Thermopsis* (Chen et al. 1994; Czefranova 1970; Larisey 1940b; Peng 1992; Sa 1999; Sa 2000) were studied taxonomically, phylogenetically and biogeographically. The genus *Ammopiptanthus* was established by Cheng (1959) on the basis of *A. mongolicus* (Maxim.) Cheng. and *A. nanus* (M.Pop.) Cheng f. ~~agreed~~ by Yakovlev (1988), Yakovlev et al. (1996) and Wei (1998), while Wei & Lock (2010) unified ~~these~~ two species. Although some phylogenetic works indicated a well supported *Ammopiptanthus* (Cardoso et al. 2013; Wang et al. 2006), the infra- and inter-generic phylogeny of this genus need further studies. Zhang et al. (2015a) inferred a diverging time of *Ammopiptanthus* from the “core genera” clade, but some closely related Sophoreae genera were not sampled (Cardoso et al. 2013; Wang et al. 2006), which may affect the accuracy of dating.

As for ~~phyto~~phytogeography of *Ammopiptanthus*, various workers proposed different speculations. Liu et al. (1996) suggested ~~ancestor~~ ancestor of this genus emerged in ~~southern~~ southern hemisphere, dispersing northwards when the Tertiary forest expanded due to the uplift of the Qinghai-Tibet Plateau (QTP) and the retreat of ~~Tethys~~ Tethys. ~~Some following studies granted~~ Some following studies granted *Ammopiptanthus* a southern laurasian ~~origination~~ origination, and regarded this genus as a relic of ~~Tertiary~~ Tertiary flora (Sun 2002a; Sun & Li 2003; Wang 2001). Based on molecular evidence, Wang et al. (2006) and Zhang et al.

(2015a) approved the relic status of *Ammopiptanthus*, holding its ancestral area as in central Asia. However, the existing phylogeny-based biogeographic analyses were under sampled towards the tribe Sophoreae, which is closely related to Thermopsidae (Cardoso et al. 2012a; Cardoso et al. 2013; LPWG 2013), leading to possible inaccuracy in their bioinformatical inference.

We herein employ sequence data from nrDNA ITS and plastid *matK*, *rbcL*, *trnL-trnF* and *psbA-trnH*, with an extensive sampling for *Ammopiptanthus* and its allies, to a) test the monophyly and systematic status of Thermopsidae; b) infer the phylogeny and biogeography of *Ammopiptanthus*.

Materials and methods

Sampling scheme

All the 9 haplotypes (A-270) of the two species of *Ammopiptanthus* found by Su et al. (2016) and Shi et al. (accepted for publication) were included in the present study. Both species of *Salweenia* Baker f. were sampled (Yue et al. 2011). The nuclear internal transcribed spacer (ITS) sequences for *Salweenia wardii* Baker f. and *Maackia amurensis* Rupr., and the plastid *psbA-trnH* and *trnL-trnF* intergenic spacer sequences for *Maackia amurensis* were generated in

the present study. The DNA extraction, amplification and sequencing methods followed Su et al. (2016). All other ITS, *matK*, *rbcL*, *trnL-trnF* and *psbA-trnH* sequences were obtained from GenBank. According to the phylogenetic analyses of *Ammopiptanthus* by Wang et al. (2006) and Zhang et al. (2015a), and phylogeny of the Genistoids s.l. (Cardoso et al. 2012b; Crisp et al. 2000; Pennington et al. 2001; Peters et al. 2010; Wojciechowski 2003), we selected 21 species in *Thermopsis*, 7 species in *Piptanthus*, 2 species in *Anagryris*, 6 species in *Baptisia*, 13 species in *Sophora*, one or two species in *Ammodendron*, *Genista* and so on. The specific taxa including their GenBank accession numbers were showed in Table 1.

Phylogenetic analyses

Multiple sequence alignments were performed using MUSCLE (Edgar 2004) in the Geneious v.8.1.2 platform (Kearse et al. 2012) with default settings and manual adjustments. The best-fit substitution models for the ITS1, 5.8S, ITS2, *matK*, *psbA-trnH*, *rbcL* and *trnL-trnF* regions were determined separately using jModelTest v.2.1.7 (Darriba et al. 2012). Phylogenetic relationships were inferred using Bayesian inference (BI) as implemented in MrBayes v.3.2.5 (Ronquist & Huelsenbeck 2003) and maximum likelihood (ML) analysis with RAxML v.8.2 (Stamatakis 2014). The nuclear ITS dataset was partitioned into ITS1, 5.8S and ITS2 partitions.

For the concatenated plastid dataset, partitions were done for the *matK*, *psbA-trnH*, *rbcL* and *trnL-trnF* regions separately. In BI, two independent analyses with one cold and three incrementally heated Markov chain Monte Carlo (MCMC) chains were run for 10,000,000 generations. Trees were sampled every 1,000 generations. All Bayesian analyses produced split frequencies of less than 0.01, showing convergence between the paired runs. The first 2,500 trees were discarded as burn-in, and the remaining trees were used to construct a 50% majority-rule consensus tree and posterior probabilities (PP). In ML, the rapid bootstrap analysis was performed with a random seed, 1,000 alternative runs, and the same partition scheme as in the Bayesian analysis. The model parameters for each partition of the dataset were optimized by RAxML with the GTRCAT command. Trees were visualized in FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>). The ML bootstrap support values (BS) were labeled on the corresponding branches of the BI trees.

Estimation of divergence times

Divergence times were estimated by using the ITS dataset and the BEAST v.2.4.3 package (Bouckaert et al. 2014). The ITS dataset was partitioned into the ITS1, 5.8S and ITS2 partitions, and nucleotide substitution models were unlinked across the three partitions. Models were those

110 determined by jModelTest. The log normal relaxed clock model was used, and clock model was
 111 linked across partitions. The birth-death model was employed, and was linked across partitions.
 112 Two independent MCMCs were each run for 50,000,000 generations, and samples were stored
 113 every 1,000 generations. The effective sample size (ESS) of each sampled parameter and the
 114 convergence between runs were checked by using Tracer v.1.6 (<http://beast.bio.ed.ac.uk/Tracer>).
 115 The ESSs of all the parameters exceeded 200, and the two independent runs were convergent.
 116 After removing a 25% burn-in of each run, the trees from the two runs were combined by using
 117 LogCombiner (Bouckaert et al. 2014). The maximum clade credibility tree was found and
 118 annotated by using TreeAnnotator (Bouckaert et al., 2014), and only the branches with posterior
 119 probability greater than 0.5 were annotated. The dating tree was visualized in FigTree v.1.4.3.
 120 Calibration points were chosen from the molecular dating analysis of the Fabaceae Family of
 121 Lavin et al. (2005). In the *matK* phylogeny of Lavin et al. (2005), the essential Genistoid crown
 122 clade (excluding *Ormosia* Jacks.) had been set to a minimum of 56 million years ago (Ma)
 123 according to fossil records. This clade was equal to our clade of ingroups, therefore the crown
 124 age of our ingroups was set as an exponential distribution with a mean of 1 and an offset of 56
 125 Ma. The Genistoid crown age had been estimated as 56.4 ± 0.2 Ma (Lavin et al. 2005); this age

126 was used to set the age of the root of our tree as a normal distribution with a mean of 56.4 Ma
127 and a standard deviation of 0.2 Ma. The age of the most recent common ancestor (MRCA) of
128 *Bolusanthus speciosus* Harms and *Spartium junceum* Linn. was set as a normal distribution with
129 a mean of 45.2 Ma and a standard deviation of 2.2 Ma, and the age of the MRCA of *Piptanthus*
130 *nepalensis* Sweet and *Baptisia australis* R.Br. was set as a normal distribution with a mean of
131 26.5 Ma and a standard deviation of 3.4 Ma, according to the ages of the equivalent nodes that
132 had been estimated by Lavin et al. (2005).

133 **Results**

134 Phylogenetic analyses

135 Because the plastid sequences putatively evolve as a single molecule, sequences of the four
136 plastid markers (*matK*, *rbcL*, *psbA-trnH* and *trnL-trnF*) were concatenated. Phylogenetic
137 analyses were conducted on both of the nuclear and combined four plastid data (Figs. 1-3: Fig.1
138 emphasized the position of *Pickeringia*; Figs. 2-3 intensified the sampling for Sophoreae). The
139 models used in the Bayesian analyses were listed as follow: *matK*: GTR+G; *psbA-trnH*: HKY+G;
140 *rbcL*: HKY+I+G; *trnL-trnF*: GTR+G; ITS1: GTR+G; 5.8S: K80+G; ITS2: GTR+G. The ITS and
141 plastid tree topology were distinct with regard to some key groups, we thus analyzed them

142 separately.

143 Our analysis (Fig. 1) displayed that *Pickeringia* was phylogenetically far from the rest
 144 genera of Thermopsidae. According to the detailed trees (Figs. 2 & 3), all genera of this tribe,
 145 except for *Pickeringia*, belonged to the well supported “Core Genistoids” (PP = 1/BS = 100%
 146 and PP = 1/BS = 94% in Figs 2 and 3, respectively). Four genera, *Anagyris*, *Baptisia*, *Piptanthus*
 147 and *Thermopsis*, clustered into the “Thermopsoid clade” (1/100% for ITS tree; 1/94% for plastid
 148 tree), within which *Anagyris* (1/100% & 1/99%) and *Baptisia* (1/100% & 0.95/95%) were shown
 149 to be monophyletic. *Piptanthus* was strongly supported by the ITS tree (1/99%).
 150 *Ammopiptanthus*, showing a sistership with *Salweenia* (1/100% in both trees), was monophyletic
 151 (1/100% & 0.99/89%). This genus was not related to the Thermopsoid clade. It nested in the
 152 “Sophoroid clade” (0.99/83% & 0.71/74%), which in turn form a robustly supported group (1/96%
 153 & 1/100%, the tribe Sophoreae, see Discussion) with the Thermopsoid clade.

154 Presently sampled taxa from the tribes Crotalariae, Genisteae and Podalyrieae formed a
 155 clade (the PCG clade; 0.89/80% & 0.92/79%), while *Bolusanthus* and *Dicraeopetalum* clustered
 156 together (the BOD clade; 1/100% in both trees). These two clades occupied different position
 157 with relation to Sophoreae (0.92/88% & 0.99/56%).

158

159 Estimating divergence time

160 A phylogenetic dating was conducted based on the ITS dataset (Fig. 4). The estimated mean
 161 ages and their 95% highest posterior density intervals (in parentheses) ~~of the interested clades~~
 162 were ~~put as below:~~ 41.24 (35.2, 46.93) Ma for the Sophoreae plus PCG clade, 35.59 (28.88,
 163 42.44) Ma for the Sophoroid plus Thermopsoid clade, 30.61 (22.91, 38.28) Ma for the *Maakia*
 164 plus its sister clade, 26.96 (19.36, 34.62) Ma for Node I, 4.74 (1.72, 8.77) Ma for Node II and
 165 2.04 (0.67, 3.73) Ma for Node III.

166

167 Discussion

168 Phylogenetic position of Thermopsidae

169 The widely distributed legume tribe Thermopsidae containing six genera, was proposed by
 170 Yakovlev (1972), and was accepted by most of subsequent studies (Lock 2005; Polhill 1994;
 171 Turner 1981; Wang 2001; Wei et al. 2010; Wei 1998; Yakovlev 1972). Phylogenetic works
 172 indicated most genera of this tribe are members of the “core genistoids”, which in turn belongs to
 173 the Genistoid clade in a broad sense (Cardoso et al. 2012b; Cardoso et al. 2016; Cardoso et al.

174 2013; Crisp et al. 2000; Pennington et al. 2001; Peters et al. 2010; Wojciechowski 2003).

175 However, the western North American endemic genus *Pickeringia* was an outlier from the core

176 genistoids (Fig. 1; ~~also see~~ Lavin et al. 2005; Wojciechowski 2013; Wojciechowski et al. 2004;

177 LPWG 2013). Therefore, Lock (2005) suggested that this genus ~~may be ruled out~~ from

178 Thermopsidae. Our results confirm such ~~view~~ (Fig. 1). *Pickeringia* also differs from other

179 genera of Thermopsidae in basic ~~chromosome~~ number ($x = 7$ vs. $x = 8$; Chen 1992; Goldblatt

180 1981; Pan & Huang 1993) and ~~quinolizidine alkaloids (absence vs. presence; see Turner 1981;~~

181 Käss & Wink 1994; Crisp et al. 2000; Doyle et al. 2000).

182 With the exclusion of *Pickeringia*, Cardoso et al. (2012b, 2013) proposed to merge

183 Thermopsidae into Sophoreae sensu Cardoso, which is characterized by free stamens, to render

184 it monophyletic. Such ~~treatment is basically~~ verified by our results (Figs. 2 & 3). A more

185 inclusive Sophoreae sensu Cardoso can ~~avoid~~ taxonomic over-fragmentation of the core

186 Genistoids taxa and the establishments of new tribes based on many small clades. On the other

187 ~~side, one clade, constituted of~~ *Bolusanthus speciosus* Harms and *Dicraeopetalum mahafaliense*

188 (M.Peltier) Yakovlev (the BOD clade), was ~~involved~~ in Sophoreae by Cardoso et al. (2013) with

189 weak support. Such ~~relationship~~ is not validated by our ITS tree (Fig. 2; also not supported by

190 the likelihood bootstrap value of plastid tree, see Fig. 3). The newly circumscribed Sophoreae,
 191 equal to Sophoreae sensu Cardoso with exclusion of the BOD clade, is further divided into the
 192 Thermopsoid clade and Sophoroid clade (Figs. 2 & 3). ~~Besides~~, Cardoso et al. (2013) elevated
 193 *Ormosia* from Sphoroeae as tribe Ormosieae, yet our results do not confirm the affiliation of
 194 *Clathrotropis* with this tribe (Figs. 2 & 3).

195 The Core Genistoids is composed of three robust groups: Sophoreae, the BOD clade and
 196 PCG clade. Our ITS and plastid tree topologies are incongruent with regard to these clades.
 197 Sophoreae forms a clade with the PCG clade in the ITS tree (Fig. 2), whereas it is sister to the
 198 BOD clade in the plastid tree (Fig. 3). Although not all of the support values are significant (BI
 199 posterior probability > 0.95, ML bootstrap value > 70%), the current case of topological
 200 discordance is similar to Xu et al. (2012), García et al. (2014) and Duan et al. (2016), which
 201 likely implied a chloroplast capture event in the origin of Sophoreae. Nevertheless, highly
 202 supported analyses are required to further verify this hypothesis.

203

204 Phylogeny of the Thermopsoid clade

205 The Thermopsoid clade possesses four genera: *Anagyris*, *Baptisia*, *Piptanthus* and

Thermopsis, and is divided into two well supported groups: the Eurasian group and the American group.

The monophyletic *Anagyris* (also see Ortega-Olivencia & Catalan 2009) is endemic to circum-Mediterranean region, and belongs to the Eurasian group (Figs 2 & 3). The Eurasian group also includes the Hengduan-Himalaya-distributed genus *Piptanthus*, whose monophyly was accepted by Wang et al. (2006) and supported by our ITS result (Fig. 2). *Baptisia* is restricted to North America (central, northern and southern states of U.S.A.), embedding within the Thermopsoid American group. Our analyses yielded robust support for this genus, following Wang et al. (2006), Uysal et al. (2014) and Zhang et al. (2015a).

Previous (Uysal et al. 2014; Wang et al. 2006; Zhang et al. 2015a) and the present results (Figs. 2 & 3) resolve a polyphyletic *Thermopsis*, with its species being assigned into both the Eurasian and the American groups. It is obvious that this genus needs further taxonomic revision. Noticeably, three Asian species, *Thermopsis fabacea* (Pall.) DC., *T. chinensis* Benth. ex S.Moore and *T. turcica* Kit Tan, Vural & Küçük, nest in the American group, making biogeography of this genus an attractive question in the future. Besides, our trees failed to support the generic status of the monotypic *Vuralia* Uysal & Ertuğrul (= *Thermopsis turcica*), which was proposed

by Uysal et al. (2014) mainly based on some unique morphological characters such as 3-
carpellate ovary and indehiscent fruit.

Placing *Ammopiptanthus* within the Sophoroid clade

Within the Sophoroid clade, the monophyletic *Maackia* Rupr. diverges first, and the
remaining taxa are divided into two highly supported groups. The first group embraces a non-
monophyletic *Sophora* (also see (Cardoso et al. 2013; Kajita et al. 2001; Kass & Wink 1997; Lee
et al. 2004; Wink & Mohamed 2003), and some allied Sophoreae genera, i.e. *Ammodendron*
Fisch. ex DC., *Ammothamnus* Bunge, *Echinosophora* Nakai, *Euchresta* Benn. *Sophora* is a
widespread genus, and has been revised by various taxonomists (Bao 2010; Heenan et al. 2004;
Ma 1990; Ma 1994; Tsoong 1981a; Tsoong 1981b; Vasil'chenko 1945; Yakovlev 1996), whereas
its phylogeny and taxonomy are long-standing puzzles, which require unremitting efforts to
solve.

The former Thermopsidae member *Ammopiptanthus*, with a sister of *Salweenia*, constitute
another group in the Sophoroid clade (Figs. 2 & 3). Traditionally, *Ammopiptanthus* contains two
species: *A. mongolicus* and *A. nanus* (Cheng 1959; Fu 1987; Li & Yan 2011; Wei 1998;

238 Yakovlev 1996;), while Wei & Lock (2010) merged the latter into the former. Our results (Figs.
239 2 & 3) confirmed the specific status of *A. nanus*, which is confined in SW Xinjiang of China and
240 E Kirgizstan, compared to a non-overlapping range of *A. mongolicus* in N Inner Mongolia, N
241 Gansu, E Xinjiang of China and S Mongolia (Fig. 5). Besides, taxonomic separation of the two
242 species is also supported by morphological (Cheng 1959; Wei 1998), anatomical (Yuan & Chen
243 1993; Shi et al. unpublished), cytological (Chen 1992; Liu et al. 1996; Pan & Huang 1993) and
244 biochemical (Feng et al. 2011; Shi 2009; Wei et al. 2007; Wei & Shi 1995; Yin & Zhang 2004)
245 evidence. Recently, Lazkov (2006) described a new species in Kirgizstan: *Ammopiptanthus*
246 *kamelinii* Lazkov. Yet its type specimen is not significantly distinct from *A. nanus*, plus its type
247 locality is overlapped with *A. nanus*, we thus suspend the recognition of *A. kamelinii*.

248

249 Biogeography of *Ammopiptanthus* and *Salweenia*

250 The abovementioned *Ammopiptanthus*-*Salweenia* group displays a disjunctive distribution.
251 *Ammopiptanthus* is recorded from arid lands of NW China, S Mongolia and E Kirgizstan (Fig.
252 5A - C). contrastively, *Salweenia* is endemic to the Hengduan Mountains in E Qinghai-Tibetan
253 Plateau (QTP) (Fig. 5A & D). Several hypotheses have been proposed for the evolutionary

254 history of *Ammopiptanthus*, most of which ~~believe~~ that this genus is a relic survivor of the
255 Tertiary flora (Sun 2002a; Sun & Li 2003; Wang 2001; Wang et al. 2006; Zhang et al. 2015a).
256 Yet these studies were conducted in a context of Thermopsidae, ~~which is already treated~~ into
257 Sophoreae (see Discussion above). Furthermore, ~~none of them paid attention to the sister~~
258 relationship between *Ammopiptanthus* and *Salweenia*.

259 ~~Central~~ Asian origination for *Ammopiptanthus*, as suggested by Wang et al. (2006) and
260 Zhang et al. (2015a) may be valid ~~due to its~~ unique habit in the NW desert of China: it is the only
261 evergreen broadleaf shrub ~~therein~~, which can be regarded as a symplesiomorphy ~~characterized~~
262 ~~by~~ the Tertiary flora. However, due to the monophyly of the *Ammopiptanthus-Salweenia* group,
263 the ancestral range of *Salweenia* is probably not in Gondwana as depicted in Li & Ni (1982) and
264 Yue et al. (2011). Thus, we may hypothesize the evolution ~~process for~~ this group as below (see
265 Fig. 4). The second main uplift of QTP occurred at ca. 25 Ma, triggering the E Asian monsoon
266 (Chen et al. 1999; Li 2001; Shi et al. 1999; Teng et al. 1997). ~~Common~~ ancestor of
267 *Ammopiptanthus* and *Salweenia* ~~arose~~ in the Tertiary evergreen forest of ancient central Asia at
268 ca. 26.96 Ma (Fig. 4: Node I). ~~Their~~ common ancestor dispersed southwards with the forest
269 expansion after Tethys retreat (as in Sun 2002b).

The third rapid uplift of QTP as a whole happened at 7-8 Ma (Harrison & Copeland 1992; Liu et al. 2001; Wang et al. 2008; Zheng & Yao 2006), and followed by a main raising of NW QTP at ca. 4.5 Ma (Zheng et al. 2000), leading to the “inlandization” of central Asia, as well as cooler climate and aridification. The vicariance and environment change probably led to the divergence between *Ammopiptanthus* and *Salweenia* (ca. 4.74 Ma, see Fig. 4: Node II). The former kept the evergreen shrubby habit and obtained xerophytic characters, e.g. the pubescent, coriaceous leaves, in the central Asian arid land; while the latter retained more Tertiary flora traits in the less disturbed region of the Hengduan Mountains (Sun 2002a; Sun 2002b; Sun & Li 2003).

Split of the two *Ammopiptanthus* species (2.04 Ma; see Fig. 4: Node III) is possibly the response to the last (fourth) rapid elevation of QTP, when aridification of Asian inner land intensified (3.6-2.5 Ma; Chen et al. 1999; Li & Fang 1999; Li 2001; Tang & Liu 2001; Zheng & Yao 2006). This estimated age is slightly older than that of Su et al. (2016), which shared the view with us that the speciation of *Ammopiptanthus* was caused by climate oscillation and range shifts. *A. nanua* grows in a dryer habitat than that of *A. mongolicus*, the former therefore possesses more xerophytic apomorphy: shorter plants, usually 1-foliolate, unambiguous leaf

nerves, thicker root cortex, more complex karyotype, more vulnerable phytocommunities, etc.

(Cheng 1959; Pan & Huang 1993; Wei 1998; Zhang et al. 2007; Shi et al. unpublished).

Such disjunction resulting from QTP uplift also takes place in other Legume taxa, e.g.

infra-generic biogeography of some genera in the tribe Caraganeae (QTP-NW China/C Asia

disjunction; see Zhang et al. 2010; Zhang et al. 2015b; Zhang et al. 2015c); inter-generic

evolutionary history of *Gueldenstaedtia* and *Tibetia* (mesic E Asia-QTP disjunction; see Xie et

al., 2016). Unlike neither of the cases above, our results may provide a new insight into the

evolutionary pattern of an inter-generic QTP-NW China/C Asia disjunctive distribution.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 41271070) and the grants from the Special Service Project of Chinese Academy of Sciences (no. TSS-2015-014-FW-4-1). We thank Dr. Sun Ming-Zhou for kindly providing samples.

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Figure 1. Bayesian tree of the concatenated nuclear ITS (Left) and the concatenated plastid data of *matK*, *rbcL*, *trnL-trnF* and *psbA-trnH* sequences (Right) data. Bayesian posterior probabilities and maximum likelihood bootstrap are given above branches.

Figure 2. Bayesian tree of the concatenated nuclear ITS data, showing *Sophoreae* and its allies. Bayesian posterior probabilities and maximum likelihood bootstrap are given above branches.

Figure 3. Bayesian tree of the concatenated plastid data of *matK*, *rbcL*, *trnL-trnF* and *psbA-trnH* sequences, showing *Sophoreae* and its allies. Bayesian posterior probabilities and maximum likelihood bootstrap are given above branches.

Figure 4. Divergence times estimated by using BEAST based on the ITS dataset. Calibration points are marked by A-D. Nodes labels and bars represent the estimated mean ages (in Ma) and their 95% highest posterior density intervals. Node I, II, and III represented the divergence ages of 26.96 Ma, 4.74 Ma and 2.04 Ma, respectively.

523 **Figure 5.** Distribution (A) and representative plants of *Ammopiptanthus* (B & C) and *Salweenia*
 524 (D). A: red - *Ammopiptanthus* (I: distribution of *A. mongolicus*; II: distribution of *A. nanus*),
 525 green - *Salweenia*; B: *Ammopiptanthus mongolicus*; C: *Ammopiptanthus nanus*; D: *Salweenia*
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Table 1(on next page)

Table 1. Taxa names, sources and GenBank accession numbers of DNA sequences.

New sequences generated in this study are indicated by an asterisk (*), Missing sequences are indicated by a dash (-).

- 1 **Table 1. Taxa names, sources and GenBank accession numbers of DNA sequences.** New sequences generated in this study are indicated by an asterisk (*).
- 2 Missing sequences are indicated by a dash (-).

Species Pop.	GenBank Accession Number					Sources
	ITS	<i>rbcL</i>	<i>matK</i>	<i>psbA-trnH</i>	<i>trnL-trnF</i>	
<i>Ammopiptanthus nanus</i>	KP636563	-	JQ820170	KP636577	KP636626	
<i>Ammopiptanthus nanus</i> A	KU178932	-	-	KU178934	KU178937	39.66° N, 74.75° E, 2290 m
<i>Ammopiptanthus nanus</i> B	KU178932	-	-	KU178935	KU178937	39.49° N, 74.88° E, 2512 m
<i>Ammopiptanthus nanus</i> C	KU178932	-	-	KU178934	KU178937	39.76° N, 76.39° E, 2350 m
<i>Ammopiptanthus mongolicus</i>	KP636562	-	JQ820168	KP636576	KP636624	
<i>Ammopiptanthus mongolicus</i> D	KU178933	-	-	KU178936	KU178938	41.63° N, 103.22° E, 1010 m
<i>Ammopiptanthus mongolicus</i> E	KU178933	-	-	KU178936	KU178939	40.49° N, 106.86° E, 1039 m
<i>Ammopiptanthus mongolicus</i> F	KU178933	-	-	KU178936	KU178940	38.98° N, 105.87° E, 1762 m
<i>Ammopiptanthus mongolicus</i> G	KU178933	-	-	KU178936	KU178941	37.99° N, 105.25° E, 1323 m
<i>Ammopiptanthus mongolicus</i> H	KU178933	-	-	KU178936	KU178940	37.93° N, 105.26° E, 1355 m
<i>Ammopiptanthus mongolicus</i> 270	KU178933	-	-	*	*	China: Turpan, Turpan Eremophytes Botanic Garden, <i>Pan b. r.</i> (TURP)
<i>Ammodendron conollyi</i>	EF457705	-	-	-	-	
<i>Ammodendron argenteum</i>	-	-	AY386957	-	-	
<i>Ammothamnus lehmannii</i>	EF457706	-	-	-	-	
<i>Anagyris foetida</i>	AY091571	Z70122	KP230735	-	FJ499429	
<i>Anagyris latifolia</i>	FJ482248	-	-	-	FJ499419	
<i>Anarthrophyllum desideratum</i>	-	-	AY386923	-	-	
<i>Anarthrophyllum rigidum</i>	FJ839488	-	-	-	FJ839594	
<i>Baptisia alba</i>	AY773348	KP126860	KP126860	-	-	
<i>Baptisia cinerea</i>	AY773350	-	-	-	-	
<i>Baptisia tinctoria</i>	Z72314 & Z72315	Z70120	-	-	AJ890964	

<i>Baptisia sphaerocarpa</i>	AY773351	-	-	-	-	
<i>Baptisia australis</i>	AY091572	KF613006	AY386900	-	FJ499421	
<i>Baptisia bracteata</i>	AY773349	KP126854	KP126854	-	-	
<i>Bolusanthus speciosus</i>	EF457708	U74243	AF142685	-	AF310994	
<i>Bowdichia nitida</i>	JX124478	-	JX124419	-	JX124432	
<i>Cadia purpurea</i>	KF850559	U74192	JX295932	-	AF309863	
<i>Castanospermum australe</i>	*	-	*	*	*	USA: Sri Lanka, kandy, Rudd v.e.3339 (US)
<i>Calpurnia aurea</i>	CAU59887	U74239	AY386951	-	AF310993	
<i>Clathrotropis brachypetala</i>	EF457714	-	-	-	AF309827	
<i>Clathrotropis macrocarpa</i>	-	-	JX295930	-	JX275957	
<i>Crotalaria incana</i>	JQ067262	JQ591662	GQ246141	JQ067481	KP691137	
<i>Cyclolobium nutans</i>	AF467041	-	AF142686	-	AF309857	
<i>Cytisus scoparius</i>	AF351120	KM360746	AY386902	-	KJ746350 & AF352216	
<i>Dicraeopetalum mahafaliense</i>	EF457716	-	-	-	-	
<i>Dicraeopetalum stipulare</i>	-	-	GQ246142	-	AF310995	
<i>Diploctropis purpurea</i>	JX124507	JQ625878	JX124418	GQ428691	JX124441	
<i>Echinosophora koreensis</i>	-	AB127036	-	-	AB127028	
<i>Euchresta formosana</i>	-	AB127039	-	-	AB127031	
<i>Euchresta japonica</i>	-	AB127040	-	-	AB127032	
<i>Genista monspessulana</i>	JF338307	KM360800	AY386862	-	JF338219 & JF338559	
<i>Guianodendron praeclarum</i>	JX124489	-	JX124403	-	JX124443	
<i>Lupinus argenteus</i>	AY338929	-	AY386956	-	AY618502 &AF538706	

<i>Maackia amurensis</i>	*	Z70137	AY386944	*	*	China: Jilin, Fusong, <i>Sun s.n.</i> (NENU)
<i>Maackia amurensis</i> subsp. <i>buergeri</i>	-	AB127041	-	-	-	
<i>Maackia chinensis</i>	EF457721	-	-	-	-	
<i>Maackia floribunda</i>	-	AB127042	-	-	AB127034	
<i>Maackia tashiroi</i>	-	AB127043	-	-	AB127035	
<i>Ormosia amazonica</i>	EF457724	GQ981820	-	GQ982307	AF309484	
<i>Ormosia fordiana</i>	KP092737	KP094453	KP093527	KP095377	-	
<i>Ormosia coccinea</i>	-	JQ625915	GQ982055	GQ982308	-	
<i>Ormosia costulata</i>	-	-	JX295887	-	JX275917	
<i>Pickeringia montana</i>	*	-	*	*	*	Mexico: Tecate, <i>Moran r.</i> 13982 (US)
<i>Ormosia arborea</i>	-	KF981227	JX295939	-	-	
<i>Piptanthus laburnifolius</i>	KP636565	-	-	KP636579	KP636630	
<i>Piptanthus nepalensis</i>	AF215922	Z70123	AY386924	-	-	
<i>Piptanthus nepalensis</i> 1	FJ482250	-	-	KP636581	KP636631	
<i>Piptanthus tomentosus</i>	AY091570	-	-	-	-	
<i>Piptanthus concolor</i>	KP636564	-	-	KP636578	KP636629	
<i>Piptanthus leiocarpus</i>	AY091569	-	-	KP636580	-	
<i>Piptanthus leiocarpus</i>	KP636566	-	-	-	-	
<i>Poecilanthus itapua</i>	KJ028462	AB045818	KJ028458	-	-	
<i>Poecilanthus parviflora</i>	KJ028463	-	KJ028459	-	AF208897	
<i>Salweenia wardii</i>	*	U74251	-	JF725689	JF725659	China: Tibet, Qamdo, <i>Chang et al.</i> QZ-491 (WUK)
<i>Salweenia bouffordiana</i>	-	-	-	JF725692	JF725662	
<i>Sophora davidii</i>	AY773352	Z70138	AY386958	JF725695	JF725665	

<i>Sophora flavescens</i>	FJ528290	Z70139	HM049520	JF725696	JF725666
<i>Sophora velutina</i>	FN813569	-	-	-	AF309828
<i>Sophora jaubertii</i>	Z72342 & Z72343	Z70140	-	-	-
<i>Sophora macrocarpa</i>	Z95563 & Z95577	AY725479	JQ619975	-	-
<i>Sophora inhambanensis</i>	FN813570	KM894237	KM896910	-	-
<i>Sophora tomentosa</i>	HQ207666	AB127038	-	JX495463	AB127030
<i>Sophora tetraphylla</i>	AJ310734	-	-	-	-
<i>Sophora howinsula</i>	AY046514	-	-	-	-
<i>Sophora microphylla</i>	AY056075	AY725480	JQ619976	GQ248391	-
<i>Sophora prostrata</i>	AY056077	-	-	-	-
<i>Sophora raivavaeensis</i>	AY056080	-	-	-	-
<i>Sophora toromiro</i>	AY056079	GQ248696	GQ248201	GQ248392	-
<i>Sophora viciifolia</i>	-	KP088855	KP089313	-	-
<i>Spartium junceum</i>	DQ524327	KM360993	AY386901	HE966833	JF338264 & JF338600
<i>Thermopsis inflata</i>	AF123451	-	-	-	-
<i>Thermopsis inflata</i> 1	-	-	-	KP636586	KP636638
<i>Thermopsis inflata</i> 2	-	-	-	-	KP636639
<i>Thermopsis inflata</i> 3	-	-	-	KP636587	KP636640
<i>Thermopsis smithiana</i>	KP636573	-	-	KP636597	KP636650
<i>Thermopsis turkestanica</i>	KP636574	-	-	KP636598	KP636651
<i>Thermopsis mongolica</i>	KP636570	-	-	KP636594	KP636647
<i>Thermopsis alpina</i>	KP636567	-	JQ669594	KP636582	KP636632

<i>Thermopsis alpina</i> 1	AF123447	-	-	--	KP636633
<i>Thermopsis alpina</i> 2	-	-	-	KP636583	KP636634
<i>Thermopsis alpina</i> 3	-	-	-	KP636584	KP636635
<i>Thermopsis alpina</i> 4	-	-	-	KP636585	KP636636
<i>Thermopsis lanceolata</i>	AF123448	-	JQ669595	KP636589	KP636642
<i>Thermopsis lanceolata</i> 1	-	-	-	KP636590	KP636643
<i>Thermopsis przewalskii</i>	KP636571	-	-	-	KP636648
<i>Thermopsis schischkinii</i>	KP636572	-	-	KP636596	KP636649
<i>Thermopsis yushuensis</i>	KP636575	-	-	KP636599	KP636652
<i>Thermopsis barbata</i>	KP636568	-	-	-	KP636637
<i>Thermopsis licentiana</i>	KP636569	-	-	-	-
<i>Thermopsis licentiana</i> 1	-	-	-	KP636591	KP636644
<i>Thermopsis licentiana</i> 3	-	-	-	KP636592	KP636645
<i>Thermopsis licentiana</i> 4	-	-	-	KP636593	KP636646
<i>Thermopsis turcica</i>	JQ425645	KT175217	KT175216	KT175218	-
<i>Thermopsis chinensis</i>	AF123443	-	-	GU396777	-
<i>Thermopsis macrophylla</i>	AF123450	-	-	-	-
<i>Thermopsis divaricarpa</i>	AY091575	-	-	-	-
<i>Thermopsis villosa</i>	AY773355	-	-	-	AF311384
<i>Thermopsis rhombifolia</i>	KP861904	JX848468	AY386866	KP861905	AY618487
<i>Thermopsis rhombifolia</i> var. <i>ovata</i>	AF007468	-	-	-	-
<i>Thermopsis fabacea</i>	AY091573	Z70121	-	-	-
<i>Thermopsis kaxgarica</i>	-	-	-	KP636588	KP636641
<i>Thermopsis montana</i>	AY091574	-	-	-	AF385411 &

<i>Ulex europaeus</i>	AY263686	KM361025	JQ669586	-	AF385937
					AF385427
					&AY264062

3 * I will added the Genebank number after accepted

4

Figure 1

Figure 1. Bayesian tree of the concatenated nuclear ITS (Left) and the concatenated plastid data of matK, rbcL, trnL-trnF and psbA-trnH sequences (Right) data.

Bayesian posterior probabilities and maximum likelihood bootstrap are given above branches.

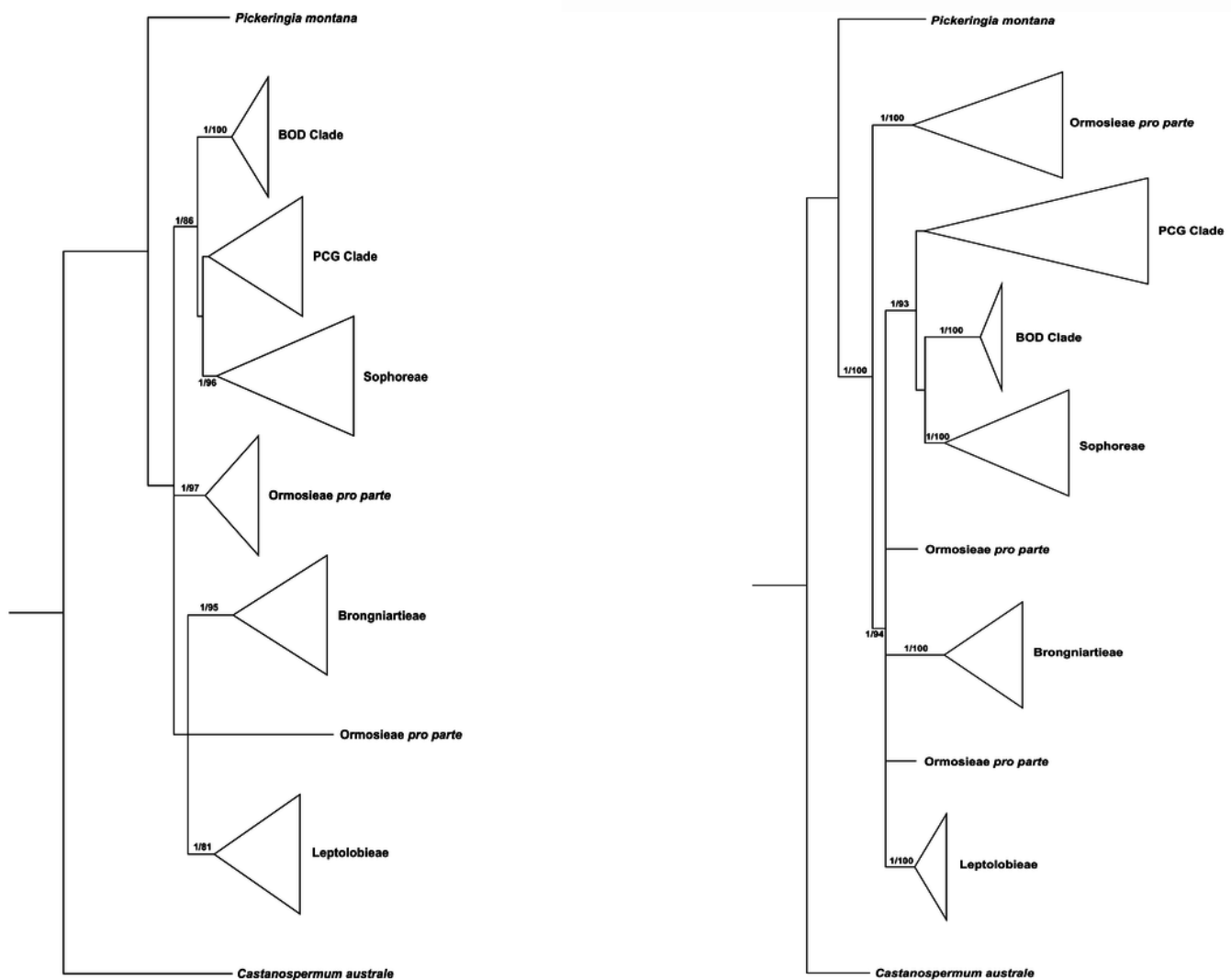


Figure 2 (on next page)

Figure 2. Bayesian tree of the concatenated nuclear ITS data, showing Sophoreae and its allies.

Bayesian posterior probabilities and maximum likelihood bootstrap are given above branches.

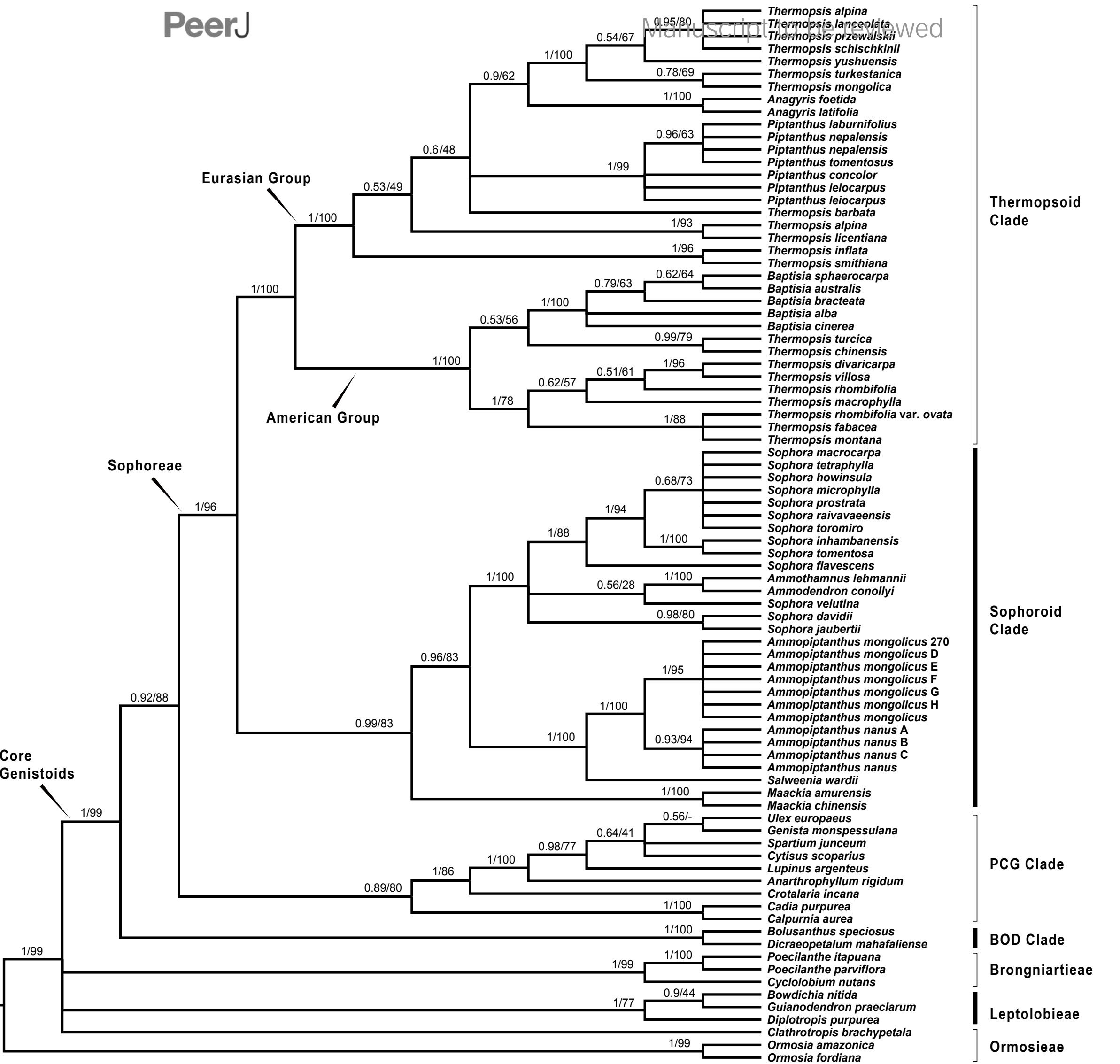


Figure 3(on next page)

Figure 3. Bayesian tree of the concatenated plastid data of *matK*, *rbcL*, *trnL-trnF* and *psbA-trnH* sequences, showing Sophoreae and its allies.

Bayesian posterior probabilities and maximum likelihood bootstrap are given above branches.

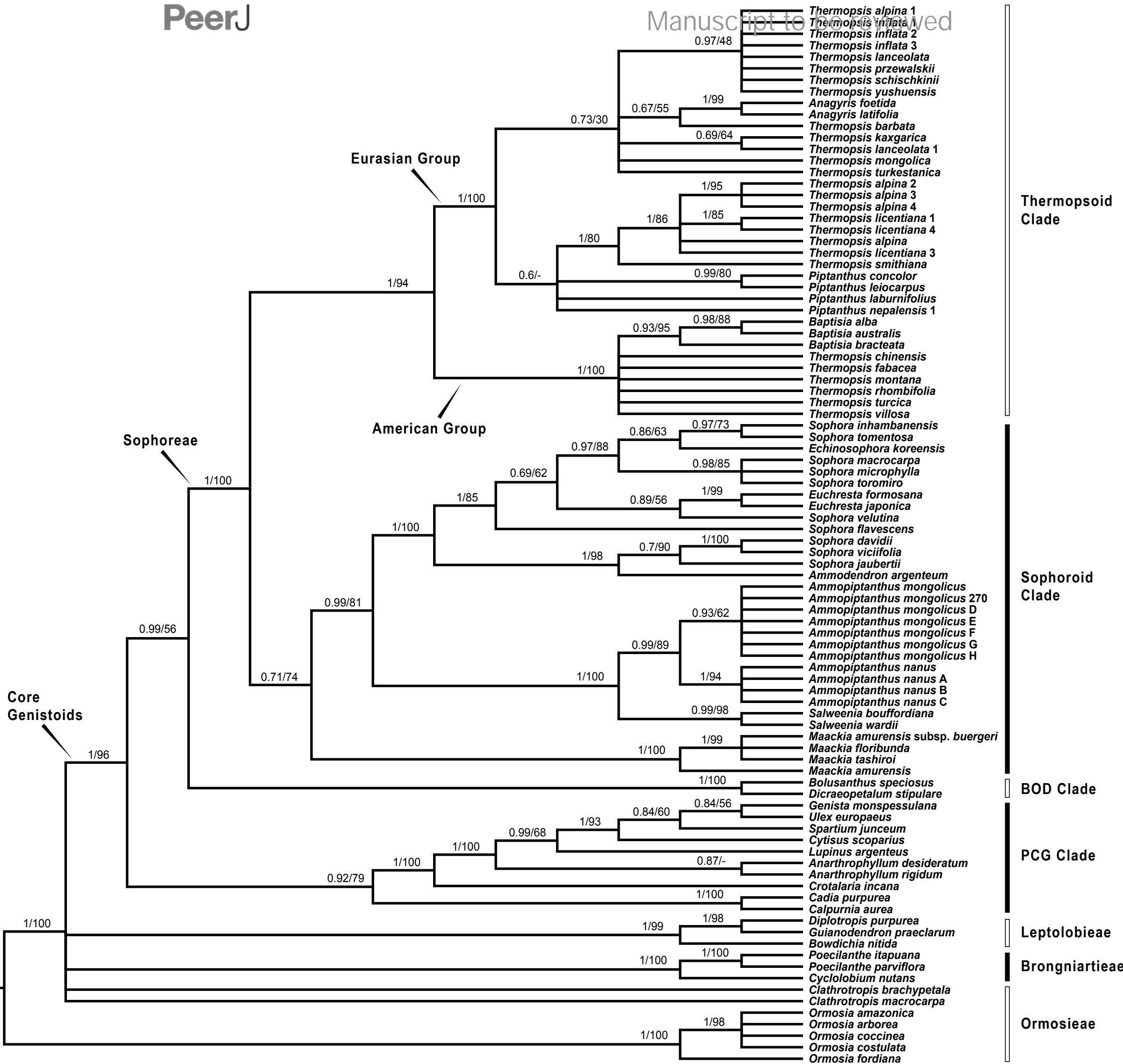


Figure 4(on next page)

Figure 4. Divergence times estimated by using BEAST based on the ITS dataset. Calibration points are marked by A-D.

Nodes labels and bars represent the estimated mean ages (in Ma) and their 95% highest posterior density intervals. Node I, II, and III represented the divergence ages of 26.96 Ma, 4.74 Ma and 2.04 Ma, respectively.

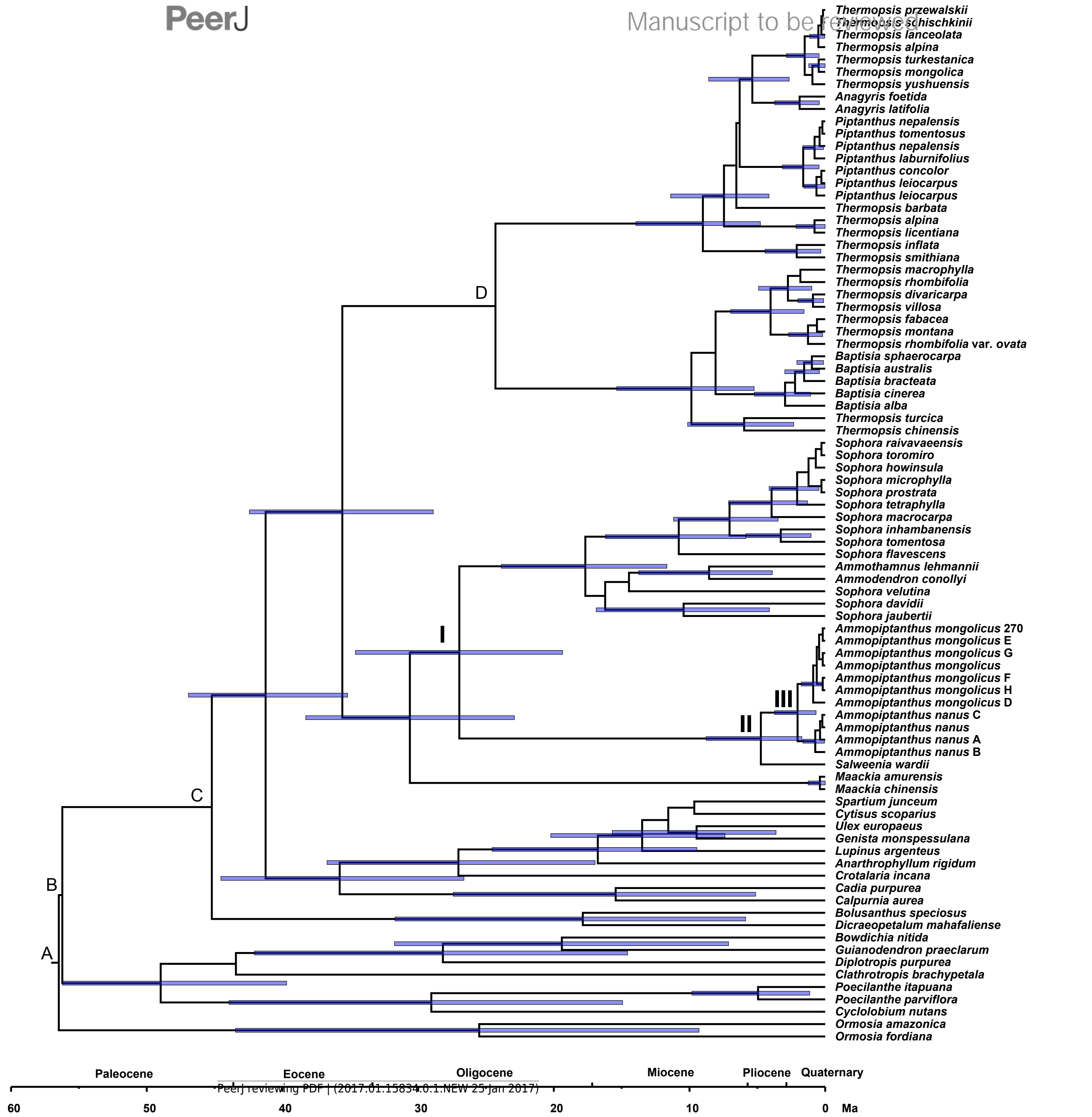


Figure 5(on next page)

Figure 5. Distribution (A) and representative plants of *Ammopiptanthus* (B & C) and *Salweenia* (D).

A: red - *Ammopiptanthus* (I: distribution of *A. mongolicus*; II: distribution of *A. nanus*), green - *Salweenia*; B: *Ammopiptanthus mongolicus*; C: *Ammopiptanthus nanus*; D: *Salweenia wardii*.

