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

Wei Shi [1, 2] , **Pei-Liang Liu** [3] , **Lei Duan** [4] , **Bo-Rong Pan** [Corresp., 1, 2] , **Zhi-Hao Su** [1]

[1] Key Laboratory of Biogeography and Bioresource in Arid Land, Institute of Ecology and Geography in Xinjiang, The Chinese Academy of Sciences, Urumqi, Xinjiang, China

[2] Turpan Eremophytes Botanic Garden, The Chinese Academy of Sciences, Turpan, Xinjiang, China

[3] College of Life Sciences, Northwest A&F University, Yangling, Shaanxi, China

[4] Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, Guangdong, China

Corresponding Author: Bo-Rong Pan
Email address: brpan@ms.xjb.ac.cn

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 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.

1   **Evolutionary response to the Qinghai-Tibetan Plateau uplift: Phylogeny and biogeography**

2   **of *Ammopiptanthus* and tribe Thermopsideae (Fabaceae)**

3

4   Wei Shi[1,2*], Pei-Liang Liu[3*], Lei Duan[4*], Bo-Rong Pan[corresp., 1, 2], Zhi-Hao Su[1]

5   Correspondence to B.-R. Pan (brpan@ms.xjb.ac.cn)

6   [1] Key Laboratory of Biogeography and Bioresource in Arid Land, Institute of Ecology and

7   Geography in Xinjiang, The Chinese Academy of Sciences, 830011 Urumqi, China

8   [2] Turpan Eremophytes Botanic Garden, The Chinese Academy of Sciences, 838008 Turpan,

9   China

10 [3] College of Life Sciences, Northwest A&F University, Yangling, 712100, China

11 [4] Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China

12 Botanical Garden, Chinese Academy of Sciences, 510650 Guangzhou, China

13 [*]These authors contributed equally to this work.

14

15 **Abstract:**

16   Based on sequence data from the nuclear ITS and four cpDNA regions (*matK*, *trnH-psbA*,

17 *trnL-trnF*, *rbcL*), phylogeny of the tribe Thermopsideae was inferred. Our analyses supported

18 this tribe being merged into a monophyletic Sophoreae in a broad sense, with exclusion of

19 *Pickeringia*. Genera of Sophoreae were separated into the Thermopsoid clade and Sophoroid

20 clade. Monophyly of *Anagyris*, *Baptisia* and *Piptanthus* were basically supported in the

21 Thermopsoid clade. *Ammopiptanthus*, consisting of *A. mongolicus* and *A. nanus*, nested within

22 the Sophoroid clade, with *Salweenia* as its sister. *Ammopiptanthus* and *Salweenia* disjunctively

23 distributed in desert of Northwestern China and Hengduan Mountains, respectively. Divergence

24 age was estimated based on the ITS phylogenetic analysis. Emergence of the common ancestor

25 of *Ammopiptanthus* and *Salweenia*, divergence between these two genera, and split of

26 *Ammopiptanthus* species occurred at approximately 26.96 Ma, 4.74 Ma and 2.04 Ma,

27 respectively, which may be response to the second, third, fourth rapid uplift of the Qinghai-

28 Tibetan Plateau, respectively.

29

30   **Introduction:**

31   Thermopsideae (Yakovlev 1972) is a small tribe in Leguminosae, comprising six genera,

32   *Ammopiptanthus* S.H.Cheng, *Anagyris* L., *Baptisia* Vent., *Pickeringia* Nutt. ex Torr. & A.Gray,

33   *Piptanthus* Sweet, *Thermopsis* R.Br. ex W.T.Aiton, with a total of ca. 45 species. Thermopsideae

34   ranges from Mediterranean Basin, C and NE Asia to temperate N America (Lock 2005; Turner

35   1981; Wang 2001). Early phylogenetic works supported that Thermopsideae, except for

36   *Pickeringia*, was nested in the "core genistoids" group, which always contains quinolizidine

37   alkaloids (Crisp et al. 2000; Wojciechowski et al. 2004). Subsequent results of Wang et al. (2006)

38   resolved two unsisterly clades in this tribe: the genus *Ammopiptanthus* and the "core genera"

39   clade, consisting of *Anagyris*, *Baptisia*, *Piptanthus* and *Thermopsis*. Based on plastid marker

40   *matK*, some recent analyses conducted by Cardoso et al. (2012a, 2013) treated the five

41   abovementioned genera of Thermopsideae into Sophoreae in a broad sense. However, Zhang et

42   al. (2015a) accepted the concept of Thermopsideae without sampling of *Pickeringia*. The

43   monophyly and the tribal rank of Thermopsideae are thus controversial.

44   Within Thermopsideae, *Anagyris* (Ortega-Olivencia 2009), *Baptisia* (Larisey 1940a; Turner

45   2006), *Pickeringia* (Wojciechowski 2013), *Piptanthus* (Turner 1980; Wei 1998; Wei & Lock

46 2010) and *Thermopsis* (Chen et al. 1994; Czefranova 1970; Larisey 1940b; Peng 1992; Sa 1999;

47 Sa 2000) were studied taxonomically, phylogentically and biogeographically. The genus

48 *Ammopiptanthus* was established by Cheng (1959) on the basis of *A. mongolicus* (Maxim.)

49 Cheng. and *A. nanus* (M.Pop.) Cheng f., agreed by Yakovlev (1988), Yakovlev et al. (1996) and

50 Wei (1998), while Wei & Lock (2010) unified these two species. Although some phylogenetic

51 works indicated a well supported *Ammopiptanthus* (Cardoso et al. 2013; Wang et al. 2006), the

52 infra- and inter-generic phylogeny of this genus need further studies. Zhang et al. (2015a)

53 inferred a diverging time of *Ammopiptanthus* from the "core genera" clade, but some closely

54 related Sophoreae genera were not sampled (Cardoso et al. 2013; Wang et al. 2006), which may

55 affect the accuracy of dating.

56   As for phytogeography of *Ammopiptanthus*, various workers proposed different

57 speculations. Liu et al. (1996) suggested ancestor of this genus emerged in southern hemisphere,

58 dispersing northwards when the Tertiary forest expanded due to the uplift of the Qinghai-Tibet

59 Plateau (QTP) and the retreat of Tethys. Some following studies granted *Ammopiptanthus* a

60 southern laurasian origination, and regarded this genus as a relic of Tertiary flora (Sun 2002a;

61 Sun & Li 2003; Wang 2001). Based on molecular evidence, Wang et al. (2006) and Zhang et al.

62   (2015a) approved the relic status of *Ammopiptanthus*, holding its ancestral area as in central Asia.

63   However, the existing phylogeny-based biogeographic analyses were under sampled towards the

64   tribe Sophoreae, which is closely related to Thermopsideae (Cardoso et al. 2012a; Cardoso et al.

65   2013; LPWG 2013), leading to possible inaccuracy in their bioinformatical inference.

66   We herein employ sequence data from nrDNA ITS and plastid *matK*, *rbcL*, *trnL-trnF* and

67   *psbA-trnH*, with an extensive sampling for *Ammopiptanthu*s and its allies, to a) test the

68   monophyly and systematic status of Thermopsideae; b) infer the phylogeny and biogeography of

69   *Ammopiptanthus*.

70

71   **Materials and methods**

72   **Sampling scheme**

73   All the 9 haplotypes (A–H, 270) of the two species of *Ammopiptanthus* found by Su et al.

74   (2016) and Shi et al. (accepted for publication) were included in the present study. Both species

75   of *Salweenia* Baker f. were sampled (Yue et al. 2011). The nuclear internal transcribed spacer

76   (ITS) sequences for *Salweenia wardii* Baker f. and *Maackia amurensis* Rupr., and the plastid

77   *psbA-trnH* and *trnL-trnF* intergenic spacer sequences for *Maackia amurensis* were generated in

78   the present study. The DNA extraction, amplification and sequencing methods followed Su et al.

79   (2016). All other ITS, *matK*, *rbcL*, *trnL-trnF* and *psbA-trnH* sequences were obtained from

80   GenBank. According to the phylogenetic analyses of *Ammopiptanthus* by Wang *et al.* (2006) and

81   Zhang *et al*. (2015a), and phylogeny of the Genistoids s.l. (Cardoso et al. 2012b; Crisp et al.

82   2000; Pennington et al. 2001; Peters et al. 2010; Wojciechowski 2003), we selected 21 species in

83   *Thermopsis*, 7 species in *Piptanthus*, 2 species in *Anagryris*, 6 species in *Bapstisia*, 13 species in

84   *Sophora*, one or two species in *Ammodendron*, *Genista* and so on. The specific taxa including

85   their GenBank accession numbers were showed in Table 1.

86   **Phylogenetic analyses**

87   Multiple sequence alignments were performed using MUSCLE (Edgar 2004) in the

88   Geneious v.8.1.2 platform (Kearse et al. 2012) with default settings and manual adjustments. The

89   best-fit substitution models for the ITS1, 5.8S, ITS2, *matK*, *psbA-trnH*, *rbcL* and *trnL-trnF*

90   regions were determined separately using jModelTest v.2.1.7 (Darriba et al. 2012). Phylogenetic

91   relationships were inferred using Bayesian inference (BI) as implemented in MrBayes v.3.2.5

92   (Ronquist & Huelsenbeck 2003) and maximum likelihood (ML) analysis with RAxML v.8.2

93   (Stamatakis 2014). The nuclear ITS dataset was partitioned into ITS1, 5.8S and ITS2 partitions.

94   For the concatenated plastid dataset, partitions were done for the *matK*, *psbA-trnH*, *rbcL* and

95   *trnL-trnF* regions separately. In BI, two independent analyses with one cold and three

96   incrementally heated Markov chain Monte Carlo (MCMC) chains were run for 10,000,000

97   generations. Trees were sampled every 1,000 generations. All Bayesian analyses produced split

98   frequencies of less than 0.01, showing convergence between the paired runs. The first 2,500 trees

99   were discarded as burn-in, and the remaining trees were used to construct a 50% majority-rule

100   consensus tree and posterior probabilities (PP). In ML, the rapid bootstrap analysis was

101   performed with a random seed, 1,000 alternative runs, and the same partition scheme as in the

102   Bayesian analysis. The model parameters for each partition of the dataset were optimized by

103   RAxML with the GTRCAT command. Trees were visualized in FigTree v1.4.3

104   (http://tree.bio.ed.ac.uk/software/figtree/). The ML bootstrap support values (BS) were labeled

105   on the corresponding branches of the BI trees.

106   **Estimation of divergence times**

107   Divergence times were estimated by using the ITS dataset and the BEAST v.2.4.3 package

108   (Bouckaert et al. 2014). The ITS dataset was partitioned into the ITS1, 5.8S and ITS2 partitions,

109   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 Thermopsideae. 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 Crotalarieae, 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 Thermopsideae

169   The widely distributed legume tribe Thermopsideae 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   Thermopsideae. Our results confirm such view (Fig. 1). *Pickeringia* also differs from other

179   genera of Thermopsideae in basic chromsome 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   Thermopsideae 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 verified this hypothesis.

203

204   Phylogeny of the Thermopsoid clade

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

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

207   group.

208   The monophyletic *Anagyris* (also see Ortega-Olivencia & Catalan 2009) is endemic to

209   circum-Mediterranean region, and belongs to the Eurasian group (Figs 2 & 3). The Eurasian

210   group also includes the Hengduan-Himalaya-distributed genus *Piptanthus*, whose monophyly

211   was accepted by Wang et al. (2006) and supported by our ITS result (Fig. 2). *Baptisia* is

212   restricted to North America (central, northern and southern states of U.S.A.), embedding within

213   the Thermopsoid American group. Our analyses yielded robust support for this genus, following

214   Wang et al. (2006), Uysal et al. (2014) and Zhang et al. (2015a).

215   Previous (Uysal et al. 2014; Wang et al. 2006; Zhang et al. 2015a) and the present results

216   (Figs. 2 & 3) resolve a polyphyletic *Thermopsis*, with its species being assigned into both the

217   Eurasian and the American groups. It is obvious that this genus needs further taxonomic revision.

218   Noticeably, three Asian species, *Thermopsis fabacea* (Pall.) DC., *T. chinensis* Benth. ex S.Moore

219   and *T. turcica* Kit Tan, Vural & Küçük., nest in the American group, making biogeography of

220   this genus an attractive question in the future. Besides, our trees failed to support the generic

221   status of the monotypic *Vuralia* Uysal & Ertuğrul (= *Thermopsis turcica*), which was proposed

222   by Uysal et al. (2014) mainly based on some unique morphological characters such as 3-

223   carpellate ovary and indehiscent fruit.

224

225   Placing *Ammopiptanthus* within the Sophoroid clade

226   Within the Sophoroid clade, the monophyletic *Maackia* Rupr. diverges first, and the

227   remaining taxa are divided into two highly supported groups. The first group embraces a non-

228   monophyletic *Sophora* (also see (Cardoso et al. 2013; Kajita et al. 2001; Kass & Wink 1997; Lee

229   et al. 2004; Wink & Mohamed 2003), and some allied Sophoreae genera, i.e. *Ammodendron*

230   Fisch. ex DC., *Ammothamnus* Bunge, *Echinosophora* Nakai, *Euchresta* Benn. *Sophora* is a

231   widespread genus, and has been revised by various taxonomists (Bao 2010; Heenan et al. 2004;

232   Ma 1990; Ma 1994; Tsoong 1981a; Tsoong 1981b; Vasil'chenko 1945; Yakovlev 1996), whereas

233   its phylogeny and taxonomy are long-standing puzzles, which require unremitting efforts to

234   solve.

235   The former Thermopsideae member *Ammopiptanthus*, with a sister of *Salweenia*, constitute

236   another group in the Sophoroid clade (Figs. 2 & 3). Traditionally, *Ammopiptanthus* contains two

237   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 InnerMongolia, 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 Thermopsideae, 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).

270   The third rapid uplift of QTP as a whole happened at 7-8 Ma (Harrison & Copeland 1992;

271   Liu et al. 2001; Wang et al. 2008; Zheng & Yao 2006), and followed by a main raising of NW

272   QTP at ca. 4.5 Ma (Zheng et al. 2000), leading to the "inlandization" of central Asia, as well as

273   cooler climate and aridification. The vicariance and environment change probably led to the

274   divergence between *Ammopiptanthus and Salweenia* (ca. 4.74 Ma, see Fig. 4: Node II). The

275   former kept the evergreen shrubby habit and obtained xerophytic characters, e.g. the pubescent,

276   coriaceous leaves, in the central Asian arid land; while the latter retained more Tertiary flora

277   traits in the less disturbed region of the Hengduan Mountains (Sun 2002a; Sun 2002b; Sun & Li

278   2003).

279   Split of the two *Ammopiptanthus* species (2.04 Ma; see Fig. 4: Node III) is possibly the

280   response to the last (fourth) rapid elevation of QTP, when aridification of Asian inner land

281   intensified (3.6-2.5 Ma; Chen et al. 1999; Li & Fang 1999; Li 2001; Tang & Liu 2001; Zheng &

282   Yao 2006). This estimated age is slightly older than that of Su et al. (2016), which shared the

283   view with us that the speciation of *Ammopiptanthus* was caused by climate oscillation and range

284   shifts. *A. nanua* grows in a dryer habitat than that of *A. mongolicus*, the former therefore

285   possesses more xerophytic apomorphy: shorter plants, usually 1-foliolate, unambiguous leaf

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

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

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

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

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

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

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

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

294

Acknowledgments

References

300   Akdeniz D, and Ozmen A. 2011. Antimitotic effects of the biopesticide oxymatrine. *Caryologia* 64:117-120.

301   Bao BJ, Vincent, M.A. 2010. Sophora. In: Wu ZY, Hong, D.Y. & Raven P.H., ed. *Flora of China*. Beijing &   St. Louis:
302   Science Press & Missouri Botanical Garden Press, 85-93.

303   Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, and Drummond AJ. 2014.
304   BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput Biol* 10:e1003537.

305   Cardoso D, de Lima HC, Rodrigues RS, de Queiroz LP, Pennington RT, and Lavin M. 2012a. The realignment of
306   Acosmium sensu stricto with the Dalbergioid clade (Leguminosae: Papilionoideae) reveals a proneness for
307   independent evolution of radial floral symmetry among early-branching papilionoid legumes. *Taxon*
308   61:1057-1073.

309   Cardoso D, De Queiroz LP, Pennington RT, De Lima HC, Fonty É, Wojciechowski MF, and Lavin M. 2012b. Revisiting
310   the phylogeny of papilionoid legumes: New insights from comprehensively sampled early-branching
311   lineages. *American Journal of Botany* 99:1991-2013.
312   Cardoso D, Harris DJ, Wieringa JJ, São-Mateus WM, Batalha-Filho H, Torke BM, Prenner G, and de Queiroz LP. 2016.
313   A molecular-dated phylogeny and biogeography of the monotypic legume genus Haplormosia, a missing
314   African branch of the otherwise American-Australian Brongniartieae clade. *Molecular Phylogenetics and*
315   *Evolution*.
316   Cardoso D, Pennington R, De Queiroz L, Boatwright J, Van Wyk B-E, Wojciechowski M, and Lavin M. 2013.
317   Reconstructing the deep-branching relationships of the papilionoid legumes. *South African Journal of*
318   *Botany* 89:58-75.
319   Chen CJ, Mendenhall MG, and Turner BL. 1994. Taxonomy of Thermopsis (Fabaceae) in North America. *Annals of*
320   *the Missouri Botanical Garden*:714-742.
321   Chen CJ, Zhu, X.Y., Yuan, Y.M. 1992. Cytological studies on the tribe Thermopsideae (Fabaceae) I: Report on
322   karyotypes of eleven species of four genera. *Cathaya* 4:103-116.
323   Chen LX, Liu JP, Zhou XJ, and Wang PX. 1999. Impact of uplift of Qinghai-Xizang Plateau and change of land-ocean
324   distribution on climate over Asia. *Quaternary Sciences* 4:314-329.
325   Cheng SH. 1959. Ammopiptanthus Cheng f., a new genus of Leguminosae from central Asia. *Botanicheskii Zhurnal*
326   44:1381-1386.
327   Crisp M, Gilmore S, and Van Wyk B. 2000. Molecular phylogeny of the Genistoid tribes of Papilionoid Leguminosae.
328   In: Herendeen P, and Bruneau A, eds. *Advances in Leguminosae systematics.* Kew, Richmond: Royal
329   Botanic Gardens, 249-276.
330   Czefranova Z. 1970. Series novae generis Thermopsis R.Br. *Novitates Systematicae Plantarum Vascularium* 7:213-
331   216.
332   Darriba D, Taboada GL, Doallo R, and Posada D. 2012. jModelTest 2: more models, new heuristics and parallel
333   computing. *Nature methods* 9:772-772.
334   Doyle J, Chappill J, Bailey C, and Kajita T. 2000. Towards a comprehensive phylogeny of legumes: evidence from
335   rbcL sequences and non-molecular data. In: Herendeen P, and Bruneau A, eds. *Advances in Leguminosae*
336   *systematics*. Kew, Richmond: Royal Botanic Gardens, 1-20.
337   Duan L, Yang X, Liu P, Johnson G, Wen J, and Chang Z. 2016. A molecular phylogeny of Caraganeae (Leguminosae,
338   Papilionoideae) reveals insights into new generic and infrageneric delimitations. *PhytoKeys*:111.
339   Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids*
340   *research* 32:1792-1797.
341   Feng WJ, Ou YF, Su YL, Li J, and Ji TF. 2011. Chemical constituents of Ammopiptanthus mongolicus. *China journal of*
342   *Chinese materia medica* 36:1040-1042.
343   Fu KT. 1987. Ammopiptanthus and Thermopsis. In: Liou YX, ed. *Flora in Desertis Reipublicae Populorum Sinarum*.
344   Beijing: Science Press, 230-232.
345   García N, Meerow AW, Soltis DE, and Soltis PS. 2014. Testing deep reticulate evolution in Amaryllidaceae tribe
346   Hippeastreae (Asparagales) with ITS and chloroplast sequence data. *Systematic Botany* 39:75-89.
347   Goldblatt P. 1981. Cytology and the phylogeny of Leguminosae. In: Polhill RM, Raven, P.H., ed. *Advances in legume*
348   *systematics*. Richmond: Royal Botanic Gardens, Kew, 427-463.
349   Harrison TM, and Copeland P. 1992. Raising tibet. *Science* 255:1663.

350   Heenan PB, Dawson MI, and Wagstaff SJ. 2004. The relationship of Sophora sect. Edwardsia (Fabaceae) to Sophora
351   tomentosa, the type species of the genus Sophora, observed from DNA sequence data and morphological
352   characters. *Botanical Journal of the Linnean Society* 146:439-446. 10.1111/j.1095-8339.2004.00348.x
353   Kajita T, Ohashi H, Tateishi Y, Bailey CD, and Doyle JJ. 2001. rbcL and legume phylogeny, with particular reference
354   to Phaseoleae, Millettieae, and allies. *Systematic Botany* 26:515-536.
355   Kass E, and Wink M. 1994. Molecular phylogeny of the papilionoideae (family leguminosae) - rbcl gene-sequences
356   versus chemical taxonomy. *Botanica Acta* 108:149-162.
357   Kass E, and Wink M. 1997. Phylogenetic relationships in the papilionoideae (family leguminosae) based on
358   nucleotide sequences of cpDNA (rbcL) and ncDNA (ITS 1 and 2). *Molecular Phylogenetics and Evolution*
359   8:65-88. 10.1006/mpev.1997.0410
360   Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, and Duran C.
361   2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and
362   analysis of sequence data. *Bioinformatics* 28:1647-1649.
363   Larisey MM. 1940a. A monograph of the genus Baptisia. *Annals of the Missouri Botanical Garden* 27:119-244.
364   Larisey MM. 1940b. A revision of the North American species of the genus Thermopsis. *Annals of the Missouri*
365   *Botanical Garden* 27:245-258.
366   Lavin M, Herendeen PS, and Wojciechowski MF. 2005. Evolutionary rates analysis of Leguminosae implicates a
367   rapid diversification of lineages during the Tertiary. *Systematic biology* 54:575-594.
368   Lazkov G. 2006. Generis Ammopiptanthus S.H.Cheng (Fabaceae) species nova e Kyrgyzstania. *Novitates*
369   *Systematicae Plantarum Vascularium* 38:134-138.
370   Lee WK, Tokuoka T, and Heo K. 2004. Molecular evidence for the inclusion of the Korean endemic genus
371   "Echinosophora" in Sophora (Fabaceae), and embryological features of the genus. *Journal of plant*
372   *research* 117:209-219.
373   Li J, and Fang X. 1999. Uplift of the Tibetan Plateau and environmental changes. *Chinese Science Bulletin* 44:2117-
374   2124.
375   Li JJ, Fang, X.M., Pan, B.T., Zhao, Z.J., Song, Y.G. 2001. Late Cenozoic intensive uplift of Qinghai-Xizang Plateau and
376   its impacts on environments in surrounding area. *Quaternary Sciences* 21:381-391.
377   Li PQ, and Ni CC. 1982. The formation and differentiation of the Leguminosae flora in Xizang (Tibet). *Acta*
378   *Phytotaxonomica Sinica* 20:142-156.
379   Li XY, and Yan P. 2011. Leguminosae. In: Sheng GM, ed. *Flora Xinjiangensis*. Xinjiang: Xinjiang Science & Technology
380   Publishing House, 11-12.
381   Liu S, Chi X, Li C, and Yang R. 2001. The summarizing for the forming and uplifted mechanism of Qinghai-Tibet
382   Plateau. *World Geology* 20:105-112.
383   Liu YH, Wang SM, and Wang HS. 1996. A study on the chromosomal geography of *Ammopiptanthus* genus.
384   *Geographical Research* 15.
385   Lock J. 2005. Thermopsideae. In: Lewis G, Schrire B, Mackinder B, and Lock M, eds. *Legumes of the world*. Kew,
386   Richmond: Royal Botanic Gardens, 263-265.
387   LPWG (Legume Phylogeny Working Group). 2013. Legume phylogeny and classification in the 21st century:
388   progress, prospects and lessons for other species-rich clades. Taxon 62:217-248.
389   Ma CY. 1990. Review of the classifical system on the genus Sophora. *Bulletin of Botanical Research* 10:77-86.
390   Ma CY. 1994. Sophora. In: Wei Z, ed. *Flora Reipublicae Popularis Sinicae*. Beijing: Science Press, 64-95.

391 Ortega-Olivencia A. 2009. Systematics and evolutionary history of the circum-Mediterranean genus Anagyris
392   L.(Fabaceae) based on morphological and molecular data. *Taxon* 58:1290-1306.
393 Ortega-Olivencia A, and Catalan P. 2009. Systematics and evolutionary history of the circum-Mediterranean genus
394   Anagyris L. (Fabaceae) based on morphological and molecular data. *Taxon* 58:1290-1306.
395 Pan BR, and Huang SP. 1993. A cytological studies of genus Ammopiptanthus. *Acta Botanica Sinica* 35.
396 Peng ZX, Yuan, Y.M. 1992. Systematic revision on Thermopsideae (Leguminosae) of China. *Acta Botanica Boreali-*
397   *Occidentalia Sinica* 12:158-166.
398 Pennington RT, Lavin M, Ireland H, Klitgaard B, Preston J, and Hu J-M. 2001. Phylogenetic relationships of basal
399   papilionoid legumes based upon sequences of the chloroplast trnL intron. *Systematic Botany* 26:537-556.
400 Peters WS, Haffer D, Hanakam CB, van Bel AJ, and Knoblauch M. 2010. Legume phylogeny and the evolution of a
401   unique contractile apparatus that regulates phloem transport. *American Journal of Botany* 97:797-808.
402 Polhill RM. 1994. Classification of the Leguminosae. In: Southon IW, Bisby, F.A., Buckingham, J., Harborne, J.B., ed.
403   *Phytochemical dictionary of the Leguminosae*. London: Chapman & Hall, XXXV-LVII.
404 Ronquist F, and Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models.
405   *Bioinformatics* 19:1572-1574. 10.1093/bioinformatics/btg180
406 Sa R. 1999. Systematics of Thermopsis (Leguminosae) Ph.D. Chinese Academy of Sciences.
407 Sa R, Sudebilige, Chen, J.R. 2000. Epidermal characters of leaves in Thermopsis and their biolocal Significances.
408   *Acta Agrestia Sinica* 8:66-76.
409 Shi W, Pan, B.R., Zhang, Q. 2009. Comparison of element contents in habitat soil and plant leaves of
410   *Ammopiptanthus nanus* and *A. mongolicus*. *Chinese journal of Applied and Environmental Biology* 15:660-
411   665.
412 Shi W, Su ZH, Liu PL, Pan BR, Zhao Y, and Wang J. 2017. Molecular, karyotypic and morphological evidence for
413   Ammopiptanthus (Fabaceae) taxonomy. Annals of the Missouri Botanical Garden 102. (Accepted,
414   unpulished)
415 Shi Y, Li J, and Li B. 1999. Uplift of the Qinghai-Xizang (Tibetan) plateau and east Asia environmental change during
416   late Cenozoic. *ACTA GEOGRAPHICA SINICA-CHINESE EDITION*- 54:20-28.
417 Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies.
418   *Bioinformatics* 30:1312-1313.
419 Su Z, Pan B, Zhang M, and Shi W. 2016. Conservation genetics and geographic patterns of genetic variation of
420   endangered shrub Ammopiptanthus (Fabaceae) in northwestern China. *Conservation Genetics* 17:485-496.
421   10.1007/s10592-015-0798-x
422 Sun H. 2002a. Tethys retreat and Himalayas-Hengduanshan Mountains uplift and their significance on the origin
423   and development of the Sino-Himalayan elements and alpine flora. *Acta Botanica Yunnanica* 24:273-288.
424 Sun H. 2002b. Evolution of arctic-tertiary flora in Himalayan-Hengduan Mountains. *Acta Botanica Yunnanica*
425   24:671-688.
426 Sun H, and Li Z. 2003. Qinghai-Tibet Plateau uplift and its impact on Tethys flora. *Advances in Earth Science* 18:852-
427   862.
428 Tang M, and Liu Y. 2001. On causes and environmental consequences of the uplift of Qinghai-Xizang Plateau.
429   *Quaternary Sciences* 21:500-507.
430 Teng JW, ZHang ZJ, Zhang BM, and Zhang H. 1997. Environmental change and the uplift of Tibetan Plateau. *EARTH*
431   *SCIENCE FRONTIERS* 4:247-254.

432   Tsoong PC, Ma, C.Y. 1981a. A study on the genus Sophora Linn. *Acta Phytotaxonomica Sinica* 19:1-22.

433   Tsoong PC, Ma, C.Y. 1981b. A study on the genus Sophora Linn. (Cont.). *Acta Phytotaxonomica Sinica* 19:143-167.

434   Turner B. 1981. Thermopsodeae. In: Polhill RM RP, ed. *Advances in Legume Systematics*. Richmond: Royal Botanic
435   Garden, Kew, 403-407.

436   Turner B. 2006. Overview of the genus Baptisia (Leguminosae). *Phytologia* 88:253-268.

437   Turner BL. 1980. REVISION OF THE GENUS PIPTANTHUS (FABACEAE, THERMOPSIDEAE). *Brittonia* 32:281-285.
438   10.2307/2806715

439   Uysal T, Ertuğrul K, and Bozkurt M. 2014. A new genus segregated from Thermopsis (Fabaceae: Papilionoideae):
440   Vuralia. *Plant systematics and evolution* 300:1627-1637.

441   Vasil'chenko IT. 1945. Sophora. In: Komarov VL, ed. *Flora of the USSR*. Leningrad: Izdatel'stvo Akademii Nauk SSSR,
442   20-24.

443   Wang C, Zhao X, Liu Z, Lippert PC, Graham SA, Coe RS, Yi H, Zhu L, Liu S, and Li Y. 2008. Constraints on the early
444   uplift history of the Tibetan Plateau. *Proceedings of the National Academy of Sciences* 105:4987-4992.

445   Wang H. 2001. A preliminary study on phytogeography of the tribe Thermopsideae (Papilionaceae). *Acta Botanica*
446   *Yunnanica* 23:17-28.

447   Wang HC, Sun H, Compton JA, and Yang JB. 2006. A phylogeny of Thermopsideae (Leguminosae : Papilionoideae)
448   inferred from nuclear ribosomal internal transcribed spacer (ITS) sequences. *Botanical Journal of the*
449   *Linnean Society* 151:365-373. 10.1111/j.1095-8339.2006.00512.x

450   Wang Y, Jiao P, Li B, and Liu C. 2010. Tissue Culture and Regeneration of Ammopiptanthus nanus(M.Pop.)Cheng f.
451   *Plant Physiology Communications* 46:375-376.

452   Wei H, Wu P, Ge X, Liu M, and Wei X. 2007. Chemical constituents of the seeds of Ammopiptanthus (Leguminosae)
453   and their systematic and ecological significance. *Biochemical Systematics and Ecology* 35:274-280.

454   Wei KH, Gao SL, and Huang HP. 2010. Tissue culture and generation of autotetraploid plants of Sophora flavescens
455   Aiton. *Pharmacognosy Magazine* 6:286-292. 10.4103/0973-1296.71793

456   Wei SQ. 1998. Thermopsideae. In: Cui HB, ed. *Flora Reipublicae Popularis Sinicae*. Beijing: Science Press, 88-411.

457   Wei Y, and Shi QH. 1995. Spectrum analysis on the esterase isozymes of Ammopiptanthus. *Arid Zone Research*
458   12:36, 53-54.

459   Wei Z, and Lock JM. 2010. Fabaceae Tribe Thermopsideae. In: Wu ZY, Hong, D.Y. & Raven P.H., ed. *Flora of China*.
460   Beijing &   St. Louis: Science Press & Missouri Botanical Garden Press, 100-104.

461   Wink M, and Mohamed G. 2003. Evolution of chemical defense traits in the Leguminosae: mapping of distribution
462   patterns of secondary metabolites on a molecular phylogeny inferred from nucleotide sequences of the
463   rbcL gene. *Biochemical Systematics and Ecology* 31:897-917.

464   Wojciechowski MF. 2003. Reconstructing the phylogeny of legumes (Leguminosae): an early 21st century
465   perspective. *Advances in legume systematics, part* 10:5-35.

466   Wojciechowski MF. 2013. The Origin and Phylogenetic Relationships of the Californian Chaparral 'Paleoendemic'
467   Pickeringia (Leguminosae). *Systematic Botany* 38:132-142. 10.1600/036364413x662024

468   Wojciechowski MF, Lavin M, and Sanderson MJ. 2004. A phylogeny of legumes (Leguminosae) based on analyses of
469   the plastid matK gene resolves many well-supported subclades within the family. *American Journal of*
470   *Botany* 91:1846-1862. 10.3732/ajb.91.11.1846

471   Xie YP, Meng Y, Sun H, and Nie ZL. 2016. Molecular Phylogeny of Gueldenstaedtia and Tibetia (Fabaceae) and Their
472   Biogeographic Differentiation within Eastern Asia. *PloS one* 11:e0162982.

473   Xu B, Wu N, Gao X-F, and Zhang L-B. 2012. Analysis of DNA sequences of six chloroplast and nuclear genes suggests
474   incongruence, introgression, and incomplete lineage sorting in the evolution of Lespedeza (Fabaceae).
475   *Molecular Phylogenetics and Evolution* 62:346-358.
476   Yakovlev GP. 1972. A contribution to the system of the order Fabales Nakai (Leguminales Jones). *Botanicheskii*
477   *Zhurnal* 57.
478   Yakovlev GP. 1988. *Thermopsis*. In: Yakovlev GP, ed. *Plants of Central Asia*. Enfield & Plymouth Science Publishers,
479   Inc.
480   Yakovlev GP. 1996. Thermopsideae. In: Yakovlev GP, Sytin, A.K., Roskov, Y.R., ed. *Legumes of Northern Eurasia: a*
481   *checklist*. Richmond: Royal Botanic Gardens, Kew, 461-465.
482   Yin LK, and Zhang J. 2004. Change of the protein amino acid content in Ammopiptanthus Cheng f. under the
483   different habitats. *Arid Zone Research* 21:269-274.
484   Yuan YM, and Chen JR. 1993. Anatomical evidence for phylogeny of the tribe Thermopsideae (Fabaceae). *Journal of*
485   *Lanzhou University Natural Sciences*:97-104.
486   Yue XK, Yue JP, Yang LE, Li ZM, and Sun H. 2011. Systematics of the genus Salweenia (Leguminosae) from
487   Southwest China with discovery of a second species. *Taxon* 60:1366-1374.
488   Zhang J, Liao K, Li D, Yan Z, and Zhang J. 2010. Distribution pattern and characteristics of Ammopiptanthus
489   mongolicus in several different habitat conditions. *Journal of Arid Land Resources and Environment*
490   24:151-154.
491   Zhang ML, Wen ZB, Fritsch PW, and Sanderson SC. 2015b. Spatiotemporal Evolution of Calophaca (Fabaceae)
492   Reveals Multiple Dispersals in Central Asian Mountains. *PloS one* 10:e0123228.
493   Zhang ML, Wen ZB, Hao XL, Byalt VV, Sukhorukov AP, and Sanderson SC. 2015c. Taxonomy, phylogenetics and
494   biogeography of Chesneya (Fabaceae), evidenced from data of three sequences, ITS, trnS-trnG, and rbcL.
495   *Biochemical Systematics and Ecology* 63:80-89.
496   Zhang ML, Huang JF, Sanderson SC, Yan P, Wu YH, and Pan BR. 2015a. Molecular Biogeography of Tribe
497   Thermopsideae (Leguminosae): A Madrean-Tethyan Disjunction Pattern with an African Origin of Core
498   Genistoides. *BioMed research international* 2015:864804. 10.1155/2015/864804
499   Zhang Q, Pan BR, Zhang YZ, and Duan SM. 2007. Analysis on the Characteristics of Communities of
500   Ammopiptanthus nanus and A . mongolicus. *Arid Zone Research* 24:487-494.
501   Zheng D, and Yao TD. 2006. Uplifting of tibetan plateau with its Environmental effects. *Advances in Earth Science*
502   21:451-458.
503   Zheng H, Powell CM, An Z, Zhou J, and Dong G. 2000. Pliocene uplift of the northern Tibetan Plateau. *Geology*
504   28:715-718.

505

507 **Figure 1.** Bayesian tree of the concatenated nuclear ITS (Left) and the concatenated plastid data

508 of *matK*, *rbcL*, *trnL-trnF* and *psbA-trnH* sequences (Right) data. Bayesian posterior probabilities

509 and maximum likelihood bootstrap are given above branches.

510

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

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

513

514 **Figure 3.** Bayesian tree of the concatenated plastid data of *matK*, *rbcL*, *trnL-trnF* and *psbA-trnH*

515 sequences, showing Sophoreae and its allies. Bayesian posterior probabilities and maximum

516 likelihood bootstrap are given above branches.

517

518 **Figure 4.** Divergence times estimated by using BEAST based on the ITS dataset. Calibration

519 points are marked by A-D. Nodes labels and bars represent the estimated mean ages (in Ma) and

520 their 95% highest posterior density intervals. Node I, II, and III represented the divergence ages

521 of 26.96 Ma, 4.74 Ma and 2.04 Ma, respectively.

522

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*

526   *wardii.*

527

528

529

530

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

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

Species						Voucher
Maackia amurensis	*	Z70137	AY386944	*	*	China: Jilin, Fusong, *Sun s.n.* (NENU)
Maackia amurensis subsp. *buergeri*	-	AB127041	-	-	-	
Maackia chinensis	EF457721	-	-	-	-	
Maackia floribunda	-	AB127042	-	-	AB127034	
Maackia tashiroi	-	AB127043	-	-	AB127035	
Ormosia amazonica	EF457724	GQ981820	-	GQ982307	AF309484	
Ormosia fordiana	KP092737	KP094453	KP093527	KP095377	-	
Ormosia coccinea	-	JQ625915	GQ982055	GQ982308	-	
Ormosia costulata	-	-	JX295887	-	JX275917	
Pickeringia montana	*	-	*	*	*	Mexico: Tecate, *Moran r.* 13982 (US)
Ormosia arborea	-	KF981227	JX295939	-	-	
Piptanthus laburnifolius	KP636565	-	-	KP636579	KP636630	
Piptanthus nepalensis	AF215922	Z70123	AY386924	-	-	
Piptanthus nepalensis1	FJ482250	-	-	KP636581	KP636631	
Piptanthus tomentosus	AY091570	-	-	-	-	
Piptanthus concolor	KP636564	-	-	KP636578	KP636629	
Piptanthus leiocarpus	AY091569	-	-	KP636580	-	
Piptanthus leiocarpus	KP636566	-	-	-	-	
Poecilanthe itapuana	KJ028462	AB045818	KJ028458	-	-	
Poecilanthe parviflora	KJ028463	-	KJ028459	-	AF208897	
Salweenia wardii	*	U74251	-	JF725689	JF725659	China: Tibet, Qamdo, *Chang et al. QZ-491* (WUK)
Salweenia bouffordiana	-	-	-	JF725692	JF725662	
Sophora davidii	AY773352	Z70138	AY386958	JF725695	JF725665	

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

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

					AF385937
Ulex europaeus	AY263686	KM361025	JQ669586	-	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.

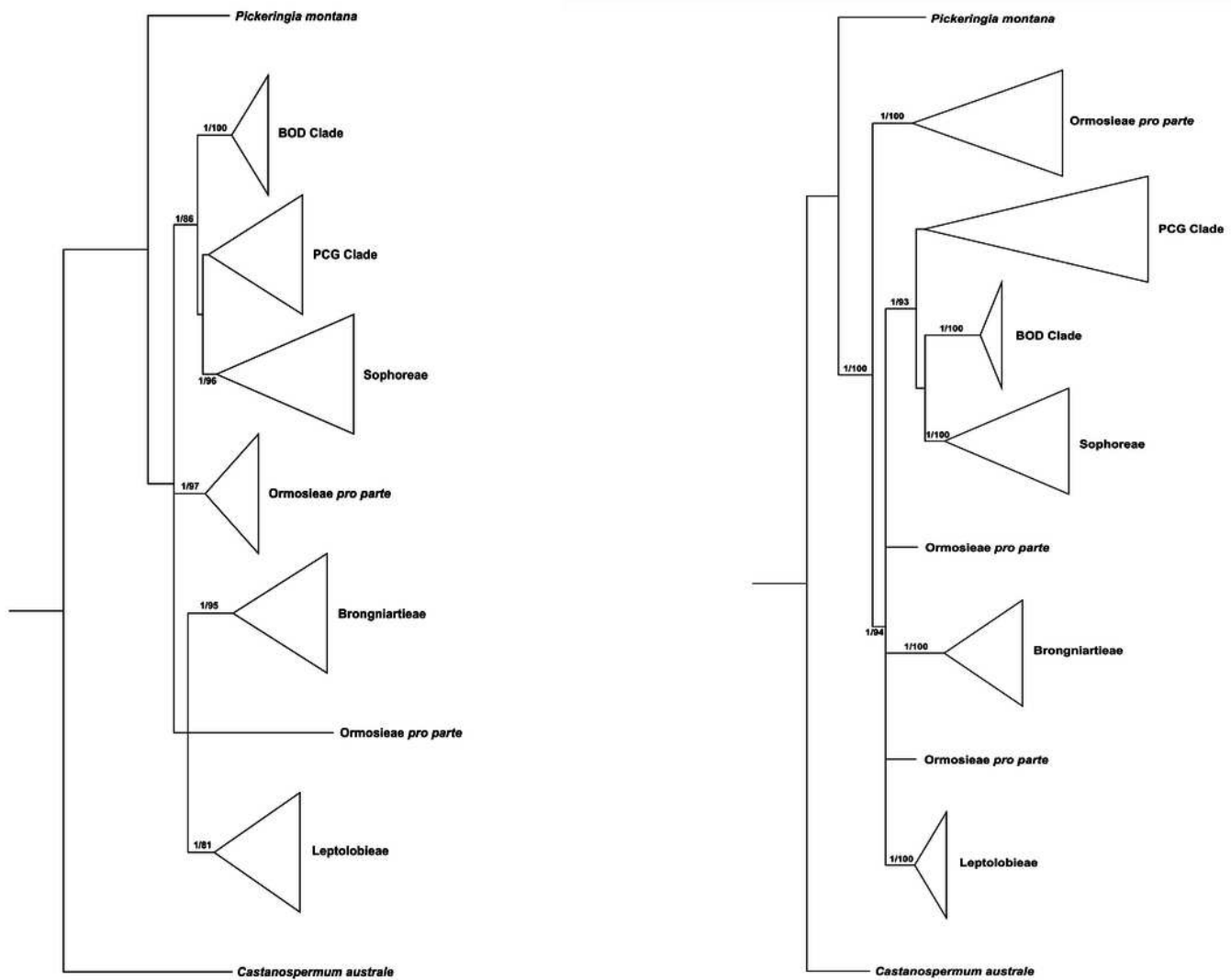Bayesian posterior probabilities and maximum likelihood bootstrap are given above branches.

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.
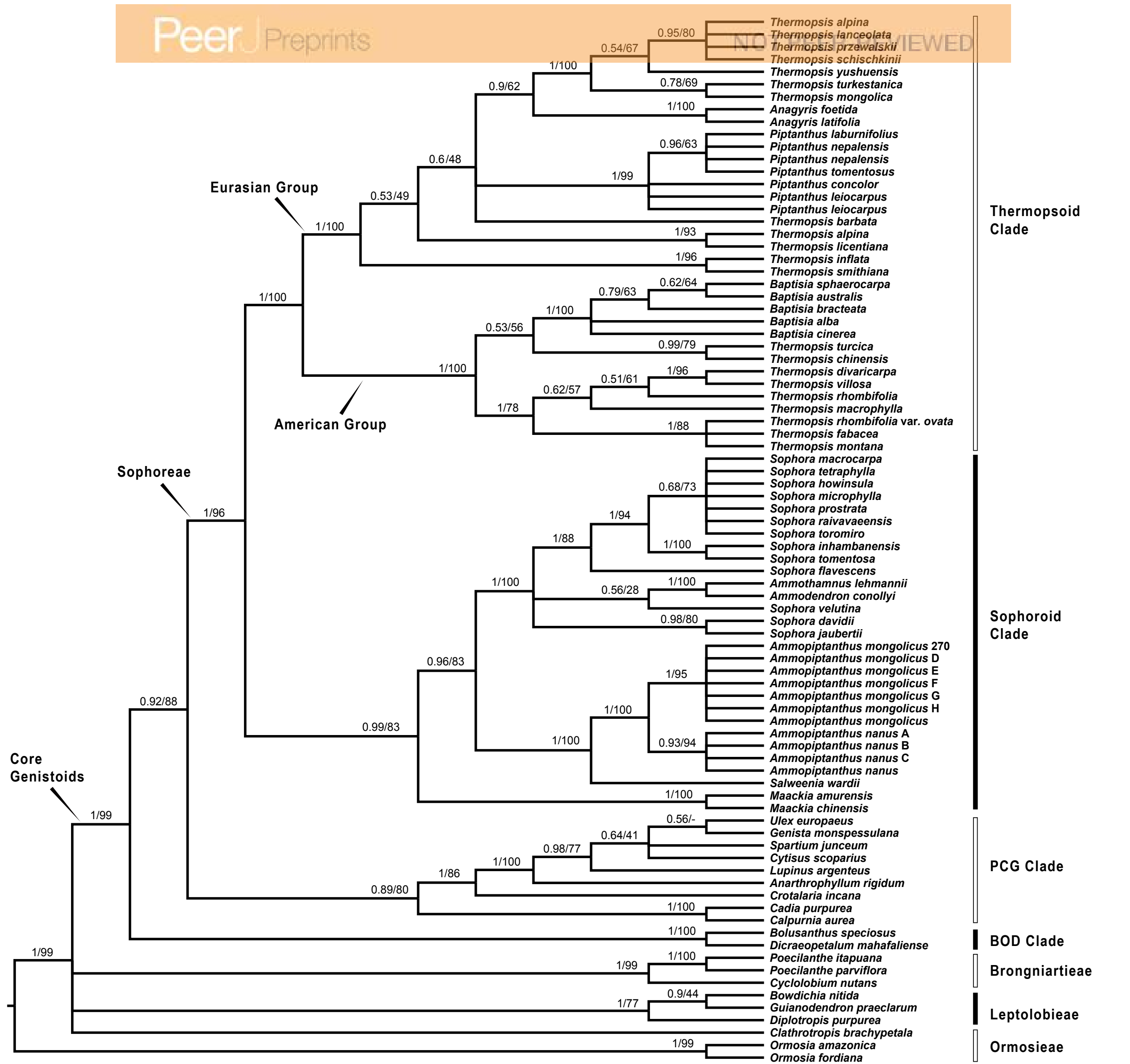
Figure 3(on next page)

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.
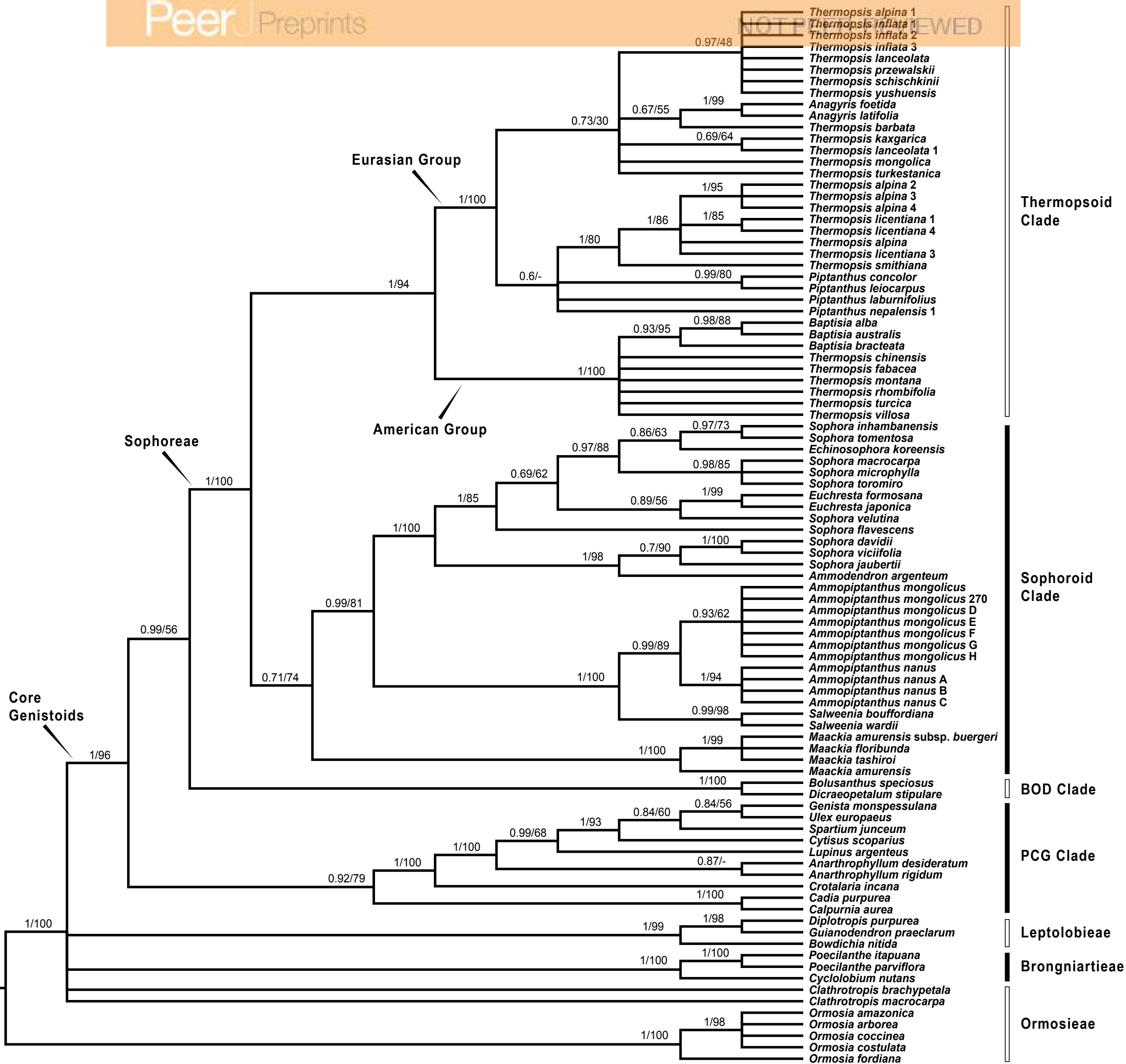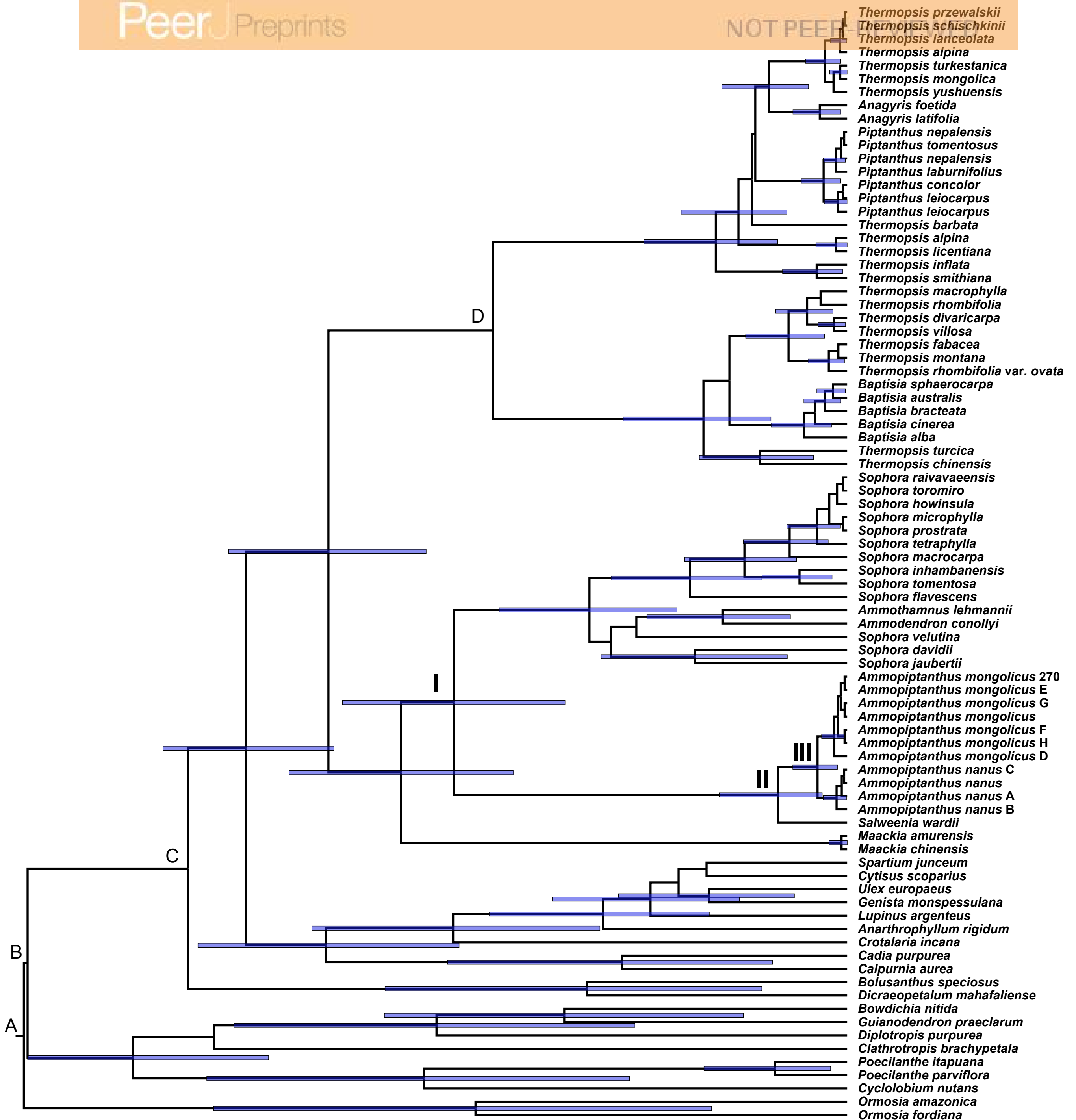
Figure 4(on next page)

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.

Paleocene	Eocene	Oligocene	Miocene	Pliocene	Quaternary		

| 60 | 50 | 40 | 30 | 20 | 10 | 0 Ma |

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.*