Phylogenetic reconstruction and divergence time estimation of Blumea DC. (Asteraceae: Inuleae) in China based on nrDNA ITS and cpDNA trnL-F sequences

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ABSTRACT

The genus *Blumea* DC. is one of the economically most important genera of Inuleae (Asteraceae) in China. It is particularly diverse in south China, where 30 species occur, more than half of which are used as herbal medicine or in the chemical industry. However, little is known about the phylogenetic relationships and molecular evolution of this genus in China. We used nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) and chloroplast DNA (cpDNA) trnL-F sequences to reconstruct the phylogenetic relationship and estimate the divergence time of *Blumea* DC. in China. Results indicated the genus of *Blumea* DC. was monophyletic and could be divided into two clades, the two clades were resolved that differ with respect to habitat, morphology, chromosome type and chemical composition, of their members. Furthermore, based on the two root time of Asteraceae, the divergence time of *Blumea* DC. were compare estimated. Results indicated the most recent common ancestor of Asteraceae maybe originated from 76–66 Ma, and *Blumea* DC. maybe originated from 72.71–52.42 Ma, and had an explosive expansion during Oligocene

and Miocene (30.09-11.44 Ma), two major clades were differentiated during the Palaeocene and Oligocene (66.29-46.72 Ma), also the evidence from paleogeography and paleoclimate also supported *Blumea* DC. had an differentiation and explosive expansion during Oligocene and Miocene.

Keywords: *Blumea* DC.; nrDNA ITS; cpDNA trnL-F; temporal origin; diversification time

INTRODUCTION

The genus *Blumea* DC. is one of the largest genera of Inuleae (Asteraceae), and contains approximately 100 specie(Anderberg & Eldenäs, 2007; Bremer & Anderberg, 1994; Randeria, 1960). It is most diverse in Asia, Africa, and Australia, and has more than one main center of diversity in Africa and South Asia (Randeria, 1960). China is one center of diversity of this genus, with 30 species distributed throughout South China, of which there are 5 species are endemic (Shi, Chen, Chen, Lin, Liu, Ge, Gao, Zhu, Liu, Yang, Humphries, Raab-Straube, Gilbert, Nordenstam, Kilian, Brouillet, Illarionova, Hind, Jeffrey, Bayer, Kirschner, Greuter, Anderberg, Semple, Štěpánek, Freire, Martins, Koyama, Kawahara, Vincent, Sukhorukov, Mavrodiev & Gottschlich, 2011; Zhang & Cheng, 1984). *Blumea* DC. has economic and ecological value in China. More than half of the species belonging to this genus have medical or ethnobotanical value (Jia & Li, 2005); for example, *B. balsamifera* DC. is an economic source for L-borneol or camphor extraction, and is widely cultivated in the Philippines and China (China, 2000; Huang, Di & Zhao, 2001; Perry & Metzger, 1980) . *B. megacephala* and *B. riparia* are important sources of the medical materials for “fuxuekang”(China, 2000). Other species such as *B. aromatica*, *B. formosana*, and *B. densiflora* are used to treat rheumatism, esophagitis, headache, hypertension, etc(Jia & Li, 2005). The taxonomy, plant morphology, and anatomy of the genus *Blumea* DC. were first described in China by Zhang and Cheng(Zhang & Cheng, 1984), the authors treated six subsections and 30 species. Since the first description of this genus, its phylogenetic analysis and molecular evolution have been vigorously disputed. Over the years, phylogenetic analysis using molecular markers has been

widely used to attempt to resolve this dispute. Molecular phylogenetics based on samples from Thailand, Burma, and Vietnam among other countries was used to assess the generic interrelationships and molecular evolution of *Blumea* DC. The results showed that *Blumea* is monophyletic, if the genera *Blumeopsis* and *Merritia* are included, and suggested this genus could be divided into two main clades, which differ in respect to habitat, ecology, and distribution (Pornpongrungrueng, Borchsenius, Englund, Anderberg & Gustafsson, 2007). In the present study, we have extended the molecular phylogenetic analysis of *Blumea* DC. by (a) adding several samples from China to provide a more thorough coverage of the overall distribution and diversity of *Blumea* DC., and (b) performing divergence time estimation. We aimed to (a) estimate the molecular evolution and phylogenetic relationships of *Blumea* DC. with a focus on the genus in China, and (b) offer the best hypothesis for the divergence time and evolutionary events of this genus in China.

MATERIALS AND METHODS

Plant materials

In this study, 16 Chinese samples, including 12 species belong to 3 sections of *Blumea* DC., were newly collected and sequenced, and the specimens of those samples were deposited in the herbarium of the Chinese Academy of Tropical Agricultural Sciences (herbarium code: CATCH). Moreover, 9 reference samples of *Blumea* DC. and 7 samples of outgroup (7 species, 6 genera) were downloaded from Genbank. The sequence information, locality, and other details of the accessions are given in Table 1.

DNA isolation

Genomic DNA was isolated from the above accessions by using the QIAGEN DNeasy Plant Minikit (QIAGEN, Düsseldorf, Germany), according to the manufacturer's instructions. The DNA was diluted to 30 ng/μL, and the ultraviolet absorption values at A260 were used.

DNA amplification and sequencing

ITS and trnL-F sequence amplification and sequencing: Internal transcribed spacer (ITS) and trnL-F sequence amplification and analysis were conducted according to previously established protocols (Gustafsson, Pepper, Albert & Källersjö, 2010; Taberlet, GiellyGuy & Bouvet, 1991). The PCR mixture consisted of 60 ng DNA, 1.0 μ M of each primer (Invitrogen Corp., Carlsbad, CA, USA), 25.0 μ L of 2 $\times$ Taq PCR Master Mix (0.1 U/ μ L Taq polymerase, 500 μ M/ μ L of each dNTP, 20 mM/ μ L Tris-HCl at pH 8.3, 100 mM/ μ L KCl, 3 mM/ μ L MgCl₂; Tiangen, China) in a 50- μ L volume. The PCR cycle protocol was based on previously established methods(Gustafsson et al., 2010; Taberlet et al., 1991). The PCR products obtained were separated on a 1.2% agarose gel, and then, bands of the expected size were excised from the gel and purified using the QIAquick Gel Extraction Kit (QIAGEN Inc., Valencia, CA, USA), according to the manufacturer's instructions. The purified PCR products were subjected to sequencing (Invitrogen, Shanghai, China).

103

104 **Sequence processing, conversion, and analysis**

Single sequence: First, the raw sequences of the ITS and trnL-F fragments (Invitrogen, Shanghai, China) were edited to remove the low quality parts. The edited sequences were then submitted to GenBank (<http://www.ncbi.nlm.nih.gov>). The sequences of both loci were aligned using MEGA 7.0 (<https://www.megasoftware.net/>) (Kumar, Stecher & Tamura, 2016) with default options, and saved in the *.nexus and *.aln formats. The interleaved *.nexus files were converted to the non-interleaved *.nex format by using PAUP 4.0a164 (<https://paup.phylosolutions.com/>)(Swofford, 2003). The sequence saturation and best substitution model were analyzed using DAMBE(<http://dambe.bio.uottawa.ca/Include/software.aspx>) (Xia & Sudhir, 2018) and jmodeltest 2.1.7 (<http://evomics.org/learning/phylogenetics/jmodeltest/>)(Darriba, Taboada, Doallo & Posada, 2012), Then the poorly aligned positions and divergent regions in each aligned sequence were removed by Gblocks0.91b (http://molevol.cmima.csic.es/castresana/Gblocks/Gblocks_documentation.html) (Castresana, 2000; Talavera & Castresana, 2007), and the filtration parameters used with were described by Guillou (Talavera & Castresana, 2007).

120 **Combined sequence.** The datasets of ITS and trnL-F sequences were loaded into
121 PAUP 4.0 (<https://paup.phylosolutions.com/>) (Swofford, 2003) for compatibility
122 testing (ILD test) (Farris, Källersjö, Kluge & Bult, 2010), the compatibility testing
123 (ILD test) result reveals there is no incongruence between ITS and trnL-F sequences
124 ($p=0.19$). Then, the sequences were merged using SequenceMatrix1.8
125 (<https://www.softpedia.com/get/Science-CAD/Sequence-Matrix.shtml>)
126 (Vaidya, Lohman & Meier, 2011), and saved in the *.nex format.

127 **Phylogenetic tree construction**

128 In order to reconstruct a more accurate phylogeny of Blumea DC., first, the
129 single sequence or combined sequence were respectively used for construct a
130 phylogeny tree with four phylogenetic tree construction methods, include the
131 Neighbor-joining (NJ) method, Maximum Parsimony (MP) method, Maximum
132 likelihood (ML) method and Bayesian inference (BI) method in PAUP 4.0a164
133 (<https://paup.phylosolutions.com/>) (Swofford, 2003), IQ-TREE
134 (<http://www.iqtree.org/>) (Chernomor, Von & Minh, 2016; Lam-Tung, Schmidt, Arndt
135 & Bui Quang, 2015) and MrBayes 3.2.6
136 (<http://nbisweden.github.io/MrBayes/download.html>) (Huelsenbeck & Ronquist,
137 2001). Then the topology of phylogenetic tree with different methods were evaluated
138 by TreePuzzle 5.3.rc16 (<http://www.tree-puzzle.de/>) (Schmidt, Petzold, Vingron &
139 Haeseler, 2003) and CONSEL (<http://stat.sys.i.kyoto-u.ac.jp/prog/consel/>) software
140 (Shimodaira & Hasegawa, 2001). The phylogenetic trees with best topology were
141 edited with FigTree 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>) and Inkscape
142 (<https://inkscape.org/>).

143 **Neighbor-joining (NJ) method** :The NJ analysis with the PAUP 4.0a164 version
144 (Swofford, 2003) was performed with heuristic searches under the random option of
145 stepwise addition algorithm with equal weighting of all characters. Bootstrap 50%
146 majority-rule consensus trees were reconstructed by performing the heuristic searches
147 with 1,000 bootstrap replicates.

148 **Maximum Parsimony (MP) method:** The same with NJ method, an Maximum

149 Parsimony(MP) analysis were also reconstructed with the PAUP 4.0a164 (Swofford,
150 2003), and performing the heuristic searches with 1,000 bootstrap replicates.

151 **Maximum likelihood (ML) method:** Before Maximum likelihood (ML)
152 analysis with IQ-TREE(Chernomor et al., 2016; Lam-Tung et al., 2015). The best
153 substitution models of single or combined sequences were computed with
154 jmodeltest2.1.7(Darriba et al., 2012). Then the Phylogenetic tree with maximum
155 likelihood (ML) method were analyzed with IQ-TREE (Chernomor et al., 2016;
156 Lam-Tung et al., 2015)with default settings, and 1000 bootstrap (BP) values for tree
157 evaluation.

158 **Bayesian inference (BI) method:** With the best substitution models parameters
159 computed by jmodeltest 2.1.7 (Darriba et al., 2012), Bayesian inference analysis (BI)
160 analysis were performed with MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001). The
161 analysis parameters were set as four chains ran simultaneously for 10,000,000
162 generations or until the average standard deviation of split frequencies fell below 0.01.
163 And the trees were being sampled with every 100 generations, a total of 20,000 trees
164 were generated with the initial sample. Subsequently, the consensus tree were
165 summarized with LogCombiner 1.10.4 (<http://beast.community/logcombiner>) and
166 TreeAnnotator 1.10.4 (<http://beast.community/treeannotator>) , and the parameters
167 were set as first 10% were discarded as burn-in and 50% majority-rule consensus tree
168 with the posterior probability (PP) values.

169 **Divergence time estimation**

170 **Time node selection and correction:** Because there are no unequivocal fossils
171 for *Blumea* DC. and its relatives in Inuleae, the divergence and diversification times
172 were difficult to estimate. In order to have an accurate divergence time estimation
173 with *Blumea* DC., in this study, two root ages of Asteraceae were compared, First,
174 reference the recently results by Barreda et al (Barreda, Luis, Tellería, Olivero, J Ian
175 & Félix, 2015), the root age of Asteraceae as 76–66 Ma; Second, reference the most
176 received time by other researchers, the root age of Asteraceae were set as 49–42 Ma
177 (Kim, Choi & Jansen, 2005; TORICES, 2010; Wagstaff, Breitwieser & Ito, 2011).
178 Subsequently, the geological event time of Qiongzhou strait and Hainan island

179 5.8–3.7 Ma, were selected as the latest differentiation time of *B. aromatica* DC.
 180 samples from Guizhou and Hainan(Huang, Guo, Ho, Shi, Li, Li, Cai & Wang, 2013;
 181 Zhao, Wang & Yuan, 2007; Zhu, 2016). Subsequently, in order to investigate the
 182 speciation rates of *Blumea* DC. along with time, BAMM (<http://bamm-project.org/>)
 183 (Rabosky, 2014) and BAMMtools
 184 (<https://cran.rstudio.com/web/packages/BAMMtools/index.html>) (Rabosky, Grundler,
 185 Anderson, Title, Shi, Brown, Huang & Larson, 2014) were used for compare the
 186 speciation rates with the two hypothesis between two root age of Asteraceae.

187 **Divergence time estimation and evolutionary event hypothesis:** All
 188 divergence times and evolutionary events were hypothesized using BEAST 1.10.4
 189 (<http://beast.community/>) (Suchard, Lemey, Baele, Ayres, Drummond & Rambaut,
 190 2018). First, the Likelihood ratio test (LRT) (Felsenstein, 1988) were used to
 191 determine the ITS or trnL-F data were conformed to molecular clock hypothesis.
 192 Results indicates the *Blumea* data weren't suitable the molecular clock hypothesis (P
 193 = 0.03). Then the divergence times were analyzed with BEAST 1.10.4 (Suchard et al.,
 194 2018) incorporating with an uncorrelated lognormal clock model and a birth–death
 195 speciation process, and the nucleotide substitution model settings were with
 196 jmodeltest result. During the analysis process, the parameters were set as the similar
 197 parameters with MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001) , 10,000,000
 198 generations for four independent Markov chain Monte Carlo (MCMC) runs, sampling
 199 with every 10000 generations, with substitution and clock models unlinked between
 200 partitions. Convergence and effective sample size (> 200) of parameters were
 201 analyzed with Tracer 1.7 (<http://tree.bio.ed.ac.uk/software/tracer/>). LogCombiner
 202 1.10.4 (<http://beast.community/logcombiner>) and TreeAnnotator 1.10.4
 203 (<http://beast.community/treeannotator>) were used for summarized the trees, and
 204 parameters were set as first 10% were discarded as burn-in and 50% majority-rule
 205 consensus tree with the posterior probability (PP) values. Last, in order to reflect the
 206 divergence time of *Blumea* DC., especially the evolutionary time with geological time
 207 scale, a strap package(Bell & Lloyd, 2015) in R was used for visualization of the
 208 results of BEAST.

209 RESULTS

210 Features of nrDNA ITS and cpDNA trnL-F sequences in *Blumea* DC.

211 Based on the results of sequence features and substitution models of single
 212 sequence or combined sequence from MEGA 7.0 (Kumar et al., 2016), DAMBE(Xia
 213 & Sudhir, 2018), and jmodeltest 2.1.7 (Darriba et al., 2012) (Table 2.), results
 214 indicated: (1) the nrDNA ITS sequence ranged from 694 ~ 738 bp, after aligned the
 215 sequence contained 657 characters, including 230 constant characters (35.01%), 314
 216 informative characters (47.79%), 114 uninformative characters (17.20%). (2) The
 217 cpDNA trnL-F sequence ranged from 766~850 bp, after aligned the sequence
 218 contained 858 characters, including 730 constant characters (85.08%), 58 informative
 219 characters (6.76%), 70 uninformative characters (8.16%). (3) And the combined
 220 sequence ranged from 1423~1507 bp, including 960 constant characters (63.49%),
 221 370 informative characters (24.47%). And also the results from the compatibility
 222 testing (ILD test) with PAUP 4.0a164 (Swofford, 2003) indicated, the nrDNA ITS
 223 sequence and cpDNA trnL-F sequence can combined, and with $p=0.19$.

224 Also the results of best substitution models from jmodeltest 2.1.7 with AIC
 225 information criterion (Akaike, 1973) and BIC information criterion (Schwarz, 1978)
 226 indicated, the best substitution models of ITS , trnL-F or combined were SYM+I+G,
 227 TVM+G, and GTR+I+G (Table 2), other parameters could also find from Table 2.

228 Phylogenetic relationship of *Blumea* DC.

229 Based on the sequence of nrDNA ITS, the results of four different methods all
 230 indicated the genus of *Blumea* DC. was monophyletic, but the topology of four
 231 methods were somehow difference. For example, the topology of NJ method (Fig. 1A)
 232 indicated the genus of *Blumea* DC. could be divided into two clades, the phylogenetic
 233 relationship of *B. aromatica* clade maybe clear, but the phylogenetic relationship of
 234 others were disordered, also with very low posterior probabilities. The results from
 235 MP method (Fig. 1B) seem to mostly conform to the relationship of biological
 236 property in *Blumea* DC., the genus could be divided into three clades, clade B.
 237 balsamifera was the basal clade of this genus, the *B. aromatica* clade maybe the
 238 transitional clade of this genus, and others maybe the higher group, also with more

extensive environmental adaptability, such as with one year herbal, diverse habitat, and others. The results with BI method (Fig. 1C.) seem to have same results with NJ method, also with high posterior probabilities, but pitched the *Caesulia axillaris* into *B. aromatica* clade. Also the topology with different methods were compared with TreePuzzle 5.3.rc16 (<http://www.tree-puzzle.de/>) (Schmidt et al., 2003) and CONSEL (<http://stat.sys.i.kyoto-u.ac.jp/prog/consel/>) (Shimodaira & Hasegawa, 2001), results indicated ML method were the most suitable tree-reconstructed method for ITS sequence, and with first Rank (Table 3). Based on the phylogenetic tree with ITS sequence and MP method (Fig. 1D), the genus of *Blumea* DC. could be divided into two major clades. Clade I were consisted with three species of *Macrophyllae* (including *B. aromatica*, *B. densiflora* and *B. balsamifera*) and one species of section *Paniculatae* (*B. lanceolaria*), but with very low bootstrap. Clade II, with a very perfect bootstrap, was consisted with three subclades and two single species, *B. virens* and *B. fistulosa*. Subclade I were consisted with three species of section *Paniculatae* (including *B. saussureoides*, *B. sinuata*, *B. oblongifolia*, and *B. lacera*), also very perfect bootstrap value. Subclade II were consisted with three species (*B. oxyodonta*, *B. mollis*, *B. hieraciifolia*) and two variants of *B. hieraciifolia* (*B. hieraciifolia* var. *hamiltoni* and *B. hieraciifolia* var. *macrostachy*). Subclade III were consisted with seven species, but with diverse inscape, including with two species of *Semivestitae* (*B. megacephala* and *B. riparia*), two species of *Macrophyllae* (*B. formosana* and *B. saxatilis*), two of *Semivestitae* (*B. napifolia* and *B. clarkei*), and also *Blumeopsis flava* also belong to this subclade.

With cpDNA trnL-F sequence, the results of four different methods were also indicated the genus of *Blumea* DC. were also divided into two clades, but the relationship of four methods were somehow difference, especially the species in clade II (Fig2.). Combined the results from topology compare indicated Bayesian inference (BI) method maybe was the most suitable tree-reconstructed method (Table 3). With BI method, *Blumea* DC. also could be divided into two clades, clade I, also with the same members in nrDNA ITS data set, was consisted with three species of *Macrophyllae* (including *B. aromatica*, *B. densiflora* and *B. balsamifera*) and one

species of section *Paniculatae* (*B. lanceolaria*), and the other species were combined into Clade II, but with low posterior probabilities (Fig.2D). Also the same results were observed from NJ, MP and ME method (Fig.2A, Fig.2B and Fig.2C), the genus could be divided into two clades, but with low bootstrap value, especially in the phylogenetic tree in NJ method and MP method, and this may be caused by the little informative characters in trnL-F data set.

Furthermore, the combined sequences were also analyzed with both four methods, results indicated Bayesian inference (BI) method was also the most suitable tree-reconstructed method for combined sequences (Table 3), also with the same topology based on the nrDNA ITS sequence (Fig 3.). And the genus of *Blumea* DC. could be divided into two clades, and clade II was also consisted with three subclades and two single species (Fig 3.D). From the results from NJ, MP and ME method (Fig.3A, Fig.3B and Fig.3C), the same results were observed, and also with better bootstrap posterior supported value. This means the combined sequence can improve the lower posterior probabilities or bootstrap caused by single sequence, also can solve the dispute caused by single sequence.

Divergence time estimation and evolutionary event hypothesis

Based on the compare of two calibration points of root age of Asteraceae and geography-species differentiation time, a series of evolutionary events and divergence times were estimated (Fig. 4). With the root age of Asteraceae as 49–42 Ma (Kim et al., 2005; TORICES, 2010; Wagstaff et al., 2011) (Fig. 4B), the time of genus *Blumea* DC. differentiated from *Inuleae* was estimated to be 45.16–33.73 Ma (the time between late of Eocene and early of Oligocene). The divergence time of two major clades in *Blumea* DC. was estimated to be 42.40–29.28 Ma. During the Oligocene and Miocene, this genus had an explosive expansion, and many species were speciated during the period of 20.05–5.75 Ma. With the root age of Asteraceae as 76–66 Ma (Barreda et al., 2015) (Fig. 4D), the time of genus *Blumea* DC. differentiated from *Inuleae* was estimated to be 72.71–52.42 Ma (the time between late of Upper and early of Paleocene), the divergence time of two major clades in *Blumea* DC. was further be estimated to be 66.29–46.72 Ma. During Oligocene and Miocene (30.09–11.44 Ma),

299 this genus had an explosive expansion, and many species were speciated during this
300 time. Furthermore, the speciation rate were also estimated with BAMM (Rabosky,
301 2014) and BAMMtools (Rabosky et al., 2014), and results indicated the speciation
302 rate were higher in the early time(Fig. 4A and Fig. 4D). Combined the speciation rate
303 estimation results and the compare divergence time estimation results with two
304 calibration points of root age of Asteraceae, I believed the with the root age of
305 Asteraceae as 76–66 Ma may be had an relative accuracy divergence time estimation
306 for *Blumea* DC.. First, based on the paleogeography and paleoclimate of Oligocene,
307 the temperature decline in Paleogene Period is interrupted by an Oligocene (~33.5
308 Ma), and an stepwise climate increase began 32.5 Ma and lasted through to 25.5
309 Ma(Lisiecki & Raymo, 2005; Miller, Browning, Aubry, Wade, Katz, Kulpecz &
310 Wright, 2008), also the same time is the most important period for the grasslands
311 expanded and forests extent, and the sunflower family had an explosive expansion
312 during this time (Barreda et al., 2015). Second, the early of Miocene (~20 Ma), is the
313 time of formation and differentiation time of Malaysian flora (Smedmark &
314 Anderberg, 2007). In the old Chinese mainland, because of the relative warm climate,
315 was the most vigorous area of the Earth, and numerous mammalian fossils have been
316 found in China (for example in Gansu Province and Qinghai Province).

317 With the evidence from paleogeography and paleoclimate, the time of genus
318 *Blumea* DC. maybe estimated to be 72.71-52.42 Ma, the divergence time of two
319 major clades was further be estimated to 66.29-46.72Ma, and during Oligocene and
320 Miocene (30.09-11.44 Ma), this genus had an explosive expansion .

321 **DISCUSSION**

322 The genus *Blumea* DC. is one of the largest genera of Inuleae (Asteraceae),
323 widely distribute ancient tropical region of around the Pacific, and South China is one
324 center of diversity of this genus, contains 30 species. Because of the variety of its
325 morphology, since the first description of this genus, its phylogenetic analysis and
326 molecular evolution have been vigorously disputed. Over the years, phylogenetic
327 analysis using molecular markers has been widely used to attempt to resolve this
328 dispute (Pornpongrungrueng et al., 2007; Pornpongrungrueng, Borchsenius &

Gustafsson, 2009), the results showed that *Blumea* (included *Blumeopsis flava*) is monophyletic, and suggested this genus could be divided into two main clades and one single species, *B. balsamifera*, which differ in terms of habitat, ecology, and distribution (Pornpongrungrueng et al., 2007). In this study, both nrDNA ITS and cpDNA trnL-F sequences were used to estimate the phylogenetic relationship between the members of this genus. And results indicated this genus could be divided into two clades, Clade I included with *B. aromatica*, *B. densiflora*, *B. balsamifera* and *B. lanceolaria*, the others belong to the Clade II. Compare with habit, distribution, chromosome number, chemical composition and etc., we found the two main clades differ in habit, ecology, distribution, chromosome number and chemical composition, the *Blumea* DC. clade contains mostly perennial shrubs or subshrubs, and mostly distributed from evergreen forests, and also with the same chromosome number ($2n = 18$) and chromosome structure ($6M+8m+4sm$) (Lu, 1996), also the chemical composition are similar (Pang, Wang, Fan, Chen, Yu, Hu, Wang & Yuan, 2014). And clade II, which is a widespread paleotropical group that comprises mostly annual, weedy herbs of open forests and fields.

Divergence time estimation and evolutionary event hypothesis is an important content of phylogenetic, but because of the fossil record, it is very difficult to estimate the evolutionary event and divergence time. Choose some geography events as calibration points, could provide more evidence for the process of phylogenetic evolution and divergence event hypothesis (Lanyon & Omland, 1999). But because the fossil record information of Inuleae is very little, most of the families have no fossil record, and several fossils clearly identifiable as members of the Asteraceae and Goodeniaceae from Oligocene and later and seeds of the Menyanthaceae and Campanulaceae from Oligocene and Miocene, respectively (Benton, 1993). There are also several records of Eocene pollen of Asteraceae were found from South America or China (Graham, 1996; Zhu, Wu & Xi, 1985). Because Oligocene pollen of the Asteraceae is of a comparatively specialized type and is found on several continents, it is reasonable to assume that the root time dates back at least to the Oligocene-Eocene boundary 42–49 Ma. Also the same time consulted by Hind, et al.

(Hind, Jeffrey & Pope, 1995). But according to a more recent paper by Barreda et al.(Barreda et al., 2015), the most recent common ancestor of Asteraceae maybe originated from 76–66 Ma. In this research, combined the form time of Hainan island and Qiongzhou strait (5.8-3.7 Ma)(Huang et al., 2013; Zhao et al., 2007; Zhu, 2016), the divergence time of Blumea DC. were compare estimated, and results indicated Blumea DC. maybe originated from 72.71-52.42 Ma, and had an explosive expansion during Oligocene and Miocene (30.09-11.44 Ma), two major clades were differentiated during the Paleocene and Oligocene (66.29-46.72 Ma), also the evidence from paleogeography and paleoclimate also supported Blumea DC. had an differentiation and explosive expansion during Oligocene and Miocene (Lisiecki & Raymo, 2005; Miller et al., 2008).

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Reference

- Akaike H. 1973.** Information Theory and an Extension of the Maximum Likelihood Principle. In: Csáki F and Petrov BN, eds. *Proceedings of the 2nd International Symposium on Information Theory*. Budapest, Budapest: Akadémiai Kiado. 5.
- Anderberg AA, Eldenäs P. 2007.** XVII. Tribe Inuleae Cass. . In: Kadereit JW and Jeffrey C, eds. *Flowering Plants. Eudicots: Asterales*. Berlin, Germany: Springer. 374-390.
- Barreda VD, Luis P, Tellería MC, Olivero EB, J Ian R, Félix F. 2015.** Early evolution of the angiosperm clade Asteraceae in the Cretaceous of Antarctica. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 10989-10994.
- Bell MA, Lloyd GT. 2015.** strap: an R package for plotting phylogenies against stratigraphy and assessing their stratigraphic congruence. *Palaeontology* **58**: 379-389.
- Benton M. 1993.** *Fossil Record 2*. Springer Netherlands.
- Bremer K, Anderberg AA. 1994.** *Asteraceae: Cladistics & Classification*. Timber Press.

389 **Castresana J. 2000.** Selection of conserved blocks from multiple alignments for their use in
390 phylogenetic analysis. *Molecular Biology & Evolution* **17**: 540-552.

391 **Chernomor O, Von HA, Minh BQ. 2016.** Terrace Aware Data Structure for Phylogenomic Inference
392 from Supermatrices. *Systematic Biology* **65**: 997-1008.

393 **China TSPCoPsR. 2000.** *Pharmacopoeia of the People's Republic of China* Chemical Industry Press:
394 Beijing,china.

395 **Darriba D, Taboada GL, Doallo R, Posada D. 2012.** jModelTest 2: more models, new heuristics and
396 parallel computing. *Nature Methods* **9**: 772.

397 **Farris JS, Källersjö M, Kluge AG, Bult C. 2010.** Testing Significance of Incongruence.
398 *Cladistics-the International Journal of the Willi Hennig Society* **10**: 315-319.

399 **Felsenstein J. 1988.** Phylogenies from Molecular Sequences: Inference and Reliability. *Annual Review*
400 *of Genetics* **22**: 521-565.

401 **Graham A. 1996.** A contribution to the geologic history of the Compositae. In: Hind DJN and Beentje
402 H, eds. *Proceedings of the International Compositae Conference*. Kew: Royal Botanic Gardens.
403 123-140.

404 **Gustafsson MHG, Pepper ASR, Albert VA, Källersjö M. 2010.** Molecular phylogeny of the
405 Barnadesioideae (Asteraceae). *Nordic Journal of Botany* **21**: 149-160.

406 **Hind DJN, Jeffrey C, Pope GV. 1995.** Advances in compositae systematics. In: Hind DJN, Jeffrey C
407 and Pope GV, eds. *Royal Botanic Gardens, Kew*: Royal Botanic Gardens.

408 **Huang T, Di Z, Zhao S. 2001.** *Modern Ben Cao Gang Mu*. Chinese medical science press: Beijing,
409 China.

410 **Huang Y, Guo X, Ho SYW, Shi H, Li J, Li J, Cai B, Wang Y. 2013.** Diversification and Demography
411 of the Oriental Garden Lizard (*Calotes versicolor*) on Hainan Island and the Adjacent Mainland.
412 *Plos One* **8**: e64754.

413 **Huelsenbeck JP, Ronquist F. 2001.** MRBAYES: Bayesian inference of phylogenetic trees.
414 *Bioinformatics* **17**: 754-755.

415 **Jia M, Li X. 2005.** *Chinese national pharmacology*. Chinese medical science press: Beijing, China.

416 **Kim K, Choi K, Jansen RK. 2005.** Two chloroplast DNA inversions originated simultaneously during
417 the early evolution of the sunflower family (Asteraceae). *Molecular Biology and Evolution* **22**:
418 1783-1792.

419 **Kumar S, Stecher G, Tamura K. 2016.** MEGA7: Molecular Evolutionary Genetics Analysis Version
420 7.0 for Bigger Datasets. *Molecular Biology & Evolution* **33**: 1870.

421 **Lam-Tung N, Schmidt HA, Arndt VH, Bui Quang M. 2015.** IQ-TREE: a fast and effective
422 stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology &*
423 *Evolution* **32**: 268-274.

424 **Lanyon SM, Omland KE. 1999.** A molecular phylogeny of the blackbirds (Icteridae): Five lineages
425 revealed by cytochrome-B sequence data. . *Auk* **116**: 629-639.

426 **Lisiecki LE, Raymo ME. 2005.** A Pliocene-Pleistocene stack of 57 globally distributed benthic $\delta^{18}\text{O}$
427 records. *Paleoceanography*: PA1003.

428 **Lu W. 1996.** A systematic study of Blumea DC. (Asteraceae) of Taiwan. Unpublished Doctor, National
429 Taiwan University.

430 **Miller KG, Browning JV, Aubry M-P, Wade BS, Katz ME, Kulpecz AA, Wright JD. 2008.**
431 Eocene–Oligocene global climate and sea-level changes: St. Stephens Quarry, Alabama.
432 *Geological Society of America Bulletin* **120**: 34-53.

433 **Pang Y, Wang D, Fan Z, Chen X, Yu F, Hu X, Wang K, Yuan L. 2014.** Blumea balsamifera--a
434 phytochemical and pharmacological review. *Molecules* **19**: 9453-9477.

435 **Perry LM, Metzger J. 1980.** *Medicinal plants of East and Southeast Asia: attributed properties and*
436 *uses*. Massachusetts Institute of Technology: Cambridg.

437 **Pornpongrueng P, Borchsenius F, Englund M, Anderberg AA, Gustafsson MHG. 2007.**
438 Phylogenetic relationships in Blumea (Asteraceae: Inuleae) as evidenced by molecular and
439 morphological data. *Plant Systematics & Evolution* **269**: 223-243.

440 **Pornpongrueng P, Borchsenius F, Gustafsson MHG. 2009.** Relationships within Blumea
441 (Inuleae, Asteraceae) and the Utility of the 5S-NTS in Species-Level Phylogeny Reconstruction.
442 *Taxon* **58**: -.

443 **Rabosky DL. 2014.** Automatic Detection of Key Innovations, Rate Shifts, and Diversity-Dependence
444 on Phylogenetic Trees. *Plos One* **9**: e89543.

445 **Rabosky DL, Grundler M, Anderson C, Title P, Shi JJ, Brown JW, Huang H, Larson JG. 2014.**
446 BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees.
447 *Methods in Ecology & Evolution* **5**: 701-707.

448 **Randeria AJ. 1960.** The Compositae genus Blumea: A taxonomic revision. *Blumea*: 176-317.

449 **Schmidt HA, Petzold E, Vingron M, Haeseler Av. 2003.** Molecular phylogenetics: parallelized
450 parameter estimation and quartet puzzling. *Journal of Parallel and Distributed Computing* **63**:
451 719-727.

452 **Schwarz GE. 1978.** Estimating the Dimension of a Model. *Annals of Statistics*: 461-464.

453 **Shi Z, Chen Y, Chen Y, Lin Y, Liu S, Ge X, Gao T, Zhu S, Liu Y, Yang Q, Humphries CJ,**
454 **Raab-Straube Ev, Gilbert MG, Nordenstam B, Kilian N, Brouillet L, Illarionova ID, Hind**
455 **DJN, Jeffrey C, Bayer RJ, Kirschner J, Greuter W, Anderberg AA, Semple JC, Štěpánek J,**
456 **Freire SE, Martins L, Koyama H, Kawahara T, Vincent L, Sukhorukov AP, Mavrodiev EV,**
457 **Gottschlich G. 2011.** Asteraceae (Compositae) [family introduction, glossary, systematic list, and
458 key to tribes]. In: Wu ZY, Raven PH and Hong DY, eds. *Flora of China*. Beijing, China: Science
459 Press (Beijing) & Missouri Botanical Garden Press (St. Louis). 1-8.

460 **Shimodaira H, ., Hasegawa M, . 2001.** CONSEL: for assessing the confidence of phylogenetic tree
461 selection. *Bioinformatics* **17**: 1246-1247.

462 **Smedmark JEE, Anderberg AA. 2007.** Boreotropical migration explains hybridization between
463 geographically distant lineages in the pantropical clade Sideroxyleae (Sapotaceae). *American*
464 *Journal of Botany* **94**: 1491-1505.

465 **Suchard M, Lemey P, Baele G, Ayres D, Drummond AJ, Rambaut A. 2018.** Bayesian phylogenetic
466 and phylodynamic data integration using BEAST 1.10. *Virus Evolution* **4**: vey016.

467 **Swofford DL. 2003.** PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods) Version 4.
468 Sunderland, Massachusetts: Sinauer Associates.

469 **Taberlet P, GiellyGuy L, Bouvet P. 1991.** Universal primers for amplification of three non-coding
470 regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105-1109.

471 **Talavera G, Castresana J. 2007.** Improvement of phylogenies after removing divergent and
472 ambiguously aligned blocks from protein sequence alignments. . *Systematic Biology*: 564-577.

473 **TORICES R. 2010.** Adding time-calibrated branch lengths to the Asteraceae supertree. *Journal of*
474 *Systematics & Evolution* **48**: 271-278.

475 **Vaidya G, Lohman DJ, Meier R. 2011.** SequenceMatrix: concatenation software for the fast assembly
476 of multi-gene datasets with character set and codon information. *Cladistics* **27**: 171-180.

477 **Wagstaff SJ, Breitwieser I, Ito M. 2011.** Evolution and biogeography of Pleurophyllum (Astereae,
478 Asteraceae), a small genus of megaherbs endemic to the subantarctic islands. *American Journal of*

479 *Botany* **98**: 62-75.

480 **Xia X, Sudhir K. 2018.** DAMBE7: New and Improved Tools for Data Analysis in Molecular Biology
481 and Evolution. *Molecular Biology and Evolution*: 2.

482 **Zhang Z, Cheng Y. 1984.** The Asteraceae: Tribe of Inuleae-Helenieae. In: Science CFEBocAo, ed.
483 *Flora Reipublicae Popularis Sinica*. Beijing, China: Science Press. 11-44.

484 **Zhao H, Wang L, Yuan J. 2007.** Origin and time of Qiongzhou strait. *Marine & Quaternary Geology*
485 **27**: 7.

486 **Zhu H. 2016.** Biogeographical Evidences Help Revealing the Origin of Hainan Island. *Plos One* **11**:
487 e0151941.

488 **Zhu Z, Wu L, Xi P. 1985.** *A research on Tertiary Palynology from the Qaidam Basin, Qinghai*
489 *Province*. The Petroleum Industry Press: Beijing.

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491 **Table 1.** Localities and samples used in the present study

Section	Code	Latin name	Locality	GenBank accession number		Reference
				ITS	trnL-F	
Semivestitae (2 spp.)	<i>B. megacephala</i>	<i>Blumea megacephala</i> (Randeria) Chang & Tseng	Guangxi, China	KP052666	KP052682	This research
	<i>B. riparia</i>	<i>B. riparia</i> (Bl.) DC.	Yunnan, China	KP052668	KP052685	This research
Macrophyllae (6 spp.)	<i>B. balsamifera</i> sp1	<i>B. balsamifera</i> (L.) DC.	Guizhou, China	KP052658	KP052674	This research
	<i>B. balsamifera</i> sp2	<i>B. balsamifera</i> (L.) DC.	Yunnan, China	KP052659	KP052675	This research
	<i>B. balsamifera</i> sp3	<i>B. balsamifera</i> (L.) DC.	Hainan, China	KP052660	KP052676	This research
	<i>B. aromatic</i> sp1	<i>B. aromatica</i> DC.	Guizhou, China	KP052656	KP052672	This research
	<i>B. aromatic</i> sp2	<i>B. aromatica</i> DC.	Hainan, China	KP052657	KP052673	This research
	<i>B. oxydonta</i>	<i>B. oxydonta</i> DC.	Thailand	EU195665	EU195630	Pornpongrueng et al. 2009
	<i>B. densiflora</i>	<i>B. densiflora</i> DC.	Thailand	EF210934	EF211029	Pornpongrueng et al. 2007
	<i>B. saxatilis</i>	<i>B. saxatilis</i> Zoll. & Mor.	Australia	EF210945	EF211040	Kanis 1993
	<i>B. formosana</i>	<i>B. formosana</i> Kitam	Zhejiang, China	KP052665	KP052678	This research
Paniculatae (9 spp.and 2 varieties)	<i>B. clarkei</i>	<i>B. clarkei</i> Hook. f.	Thailand	EF210974	EF211069	Pornpongrueng et al. 2007
	<i>B. fistulosa</i>	<i>B. fistulosa</i> (Roxb.) Kurz.	Yunnan, China	KP052661	KP052677	This research
	<i>B. hieraciifolia</i>	<i>B. hieraciifolia</i> (D. Don) DC.	Yunnan, China	KP052662	KP052679	This research
	<i>B. hieraciifolia</i> var. hamiltonii	<i>B. hieraciifolia</i> var. hamiltonii	Burma	EF210972	EF211067	Pornpongrueng et al. 2007

	<i>B. hieraciifolia</i> var. <i>macrostachya</i>	<i>B. hieraciifolia</i> var. <i>macrostachya</i>	Thailand	EF210937	EF211032	Pornpongrungrueng et al. 2007
	<i>B. lanceolaria</i>	<i>B. lanceolaria</i> (Roxb.) Druce.	Guangxi, China	KP052664	KP052681	This research
	<i>B. lacera</i>	<i>B. lacera</i> (Burm. f.) DC.	Zhejiang, China	KP052663	KP052680	This research
	<i>B. mollis</i>	<i>B. mollis</i> (D. Don) Merr.	Hainan, China	KP052670	KP052683	This research
	<i>B. napifolia</i>	<i>B. napifolia</i> DC.	Thailand	EF210959	EF211054	Pornpongrungrueng et al. 2007
	<i>B. oblongifolia</i>	<i>B. oblongifolia</i> Kitam	Hainan, China	KP052667	KP052684	This research
	<i>B. saussureoides</i>	<i>B. saussureoides</i> Chang & Tseng	Yunan, China	KP052669	KP052686	This research
	<i>B. sinuata</i>	<i>B. sinuata</i> (Lour.) Merr.	Thailand	EF210948	EF211043	Pornpongrungrueng et al. 2007
	<i>B. virens</i>	<i>B. virens</i> DC.	Thailand	EF210957	EF211052	Pornpongrungrueng et al. 2007
Uncertainty	<i>Blumeposis. flava</i>	<i>Blumeposis. flava</i> Gagnep.	Thailand	EF210960	EF211055	Pornpongrungrueng et al. 2007
Outgroup	<i>Caesulia axillaris</i>	<i>Caesulia axillaris</i> Roxb.	India	EF210949	EF211044	Pornpongrungrueng et al. 2007
	<i>Pluchea carolinensis</i>	<i>Pluchea carolinensis</i> (Jacq.) G. Don.	Taiwan, China	AF437850	EU385104	Panero& Funk. 2008
	<i>Laggera alata</i>	<i>Laggera alata</i> (D. Don) Sch.-Bip. ex Oliv.	Thailand	EF210930	EF211025	Pornpongrungrueng et al. 2007
	<i>L. pterodonta</i>	<i>L. pterodonta</i> (DC.) Sch. Bip. ex Oliv.	Thailand	EF210929	EF211024	Pornpongrungrueng et al. 2007
	<i>Schlechtendalia luzulifolia</i>	<i>Schlechtendalia luzulifolia</i>	Australia	KF989506	KF989612	Funk et al. 2014
	<i>Barnadesia caryophylla</i>	<i>Barnadesia caryophylla</i>	Australia	AY504686	AY504768	Funk et al. 2014
	<i>Elephantopus scaber</i>	<i>Elephantopus scaber</i> L.	Hainan, China	KP052671	KP052687	This research

Table 2. Features of nrDNA ITS and cpDNA trnL-F sequences of *Blumea* DC.

DNA region	Number of characters	Number of constant characters	Number of variable characters	Number of informative characters	Percent of informative Sites (%)	Best substitution model	Model matrix			
							Rates	Ncat	P-invar	Gamma shape
nrDNA ITS	657	230	427	314	47.79	SYM+I+G	Gamma	4	0.2390	2.1770
cpDNA trnL-F	858	700	158	70	8.16	TVM+G	Gamma	4	0.0	0.9240
Combined sequence	1545	960	551	375	24.27	-	-	-	-	-

Table 3. The topology compare with four phylogenetic tree construction method

DNA region	Tree method	Rank	obs ^a	au ^b	np ^c	bp ^d	pp ^e	kh ^f	sh ^g	wkh ^h	wsh ⁱ
nrDNA ITS	NJ	3	2.6	0.358	0.331	0.095	0.067	0.337	0.557	0.285	0.635
	MP	4	26.5	0.002	0.002	0.002	3E-012	0.013	0.025	0.013	0.019
	ML	1	-2.6	0.681	0.669	0.674	0.866	0.662	0.859	0.662	0.885
	BI	2	2.6	0.357	0.331	0.229	0.067	0.338	0.558	0.338	0.648
cpDNA trnL-F	NJ	4	0.4	0.337	0.274	0.275	0.176	0.300	0.309	0.300	0.309
	MP	3	0.0	0.410	0.213	0.210	0.274	0.326	0.743	0.326	0.620
	ML	2	0.0	0.503	0.357	0.351	0.274	0.453	0.682	0.453	0.655
	BI	1	-0.0	0.731	0.163	0.164	0.275	0.547	0.997	0.547	0.992

Combined sequence	NJ	4	8.5	0.097	0.077	0.077	1E-004	0.125	0.229	0.125	0.187
	MP	3	0.0	0.328	0.293	0.301	0.009	0.297	0.435	0.297	0.413
	ML	2	0.0	0.165	0.055	0.056	0.495	0.085	0.753	0.085	0.691
	BI	1	-0.0	0.867	0.578	0.566	0.496	0.915	0.982	0.915	0.986

Notes: Obs^a = observed log-likelihood difference to the best topology; au^b = approximately unbiased; np^c = bootstrap probability of the topology (i.e., the probability that the given topology has the largest likelihood in 10 scaled sets of 10,000 bootstrap replicates); bp^d = np with 10 non-scaled sets of 10,000 bootstrap replicates; pp^e = Bayesian posterior probabilities of the model; kh^f = Kishino-Hasegawa; sh^g = Shimodeira-Hasegawa; wkh^h = weighted KH; wshⁱ = weighted SH.

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Fig. 1. Phylogenetic tree based on the nrDNA ITS sequence, and numbers

above branches indicate bootstrap value or posterior probabilities. (A)

Phylogenetic tree determined by NJ method with using PAUP program, and numbers

above branches indicate bootstrap value. (B) The phylogenetic tree with best topology,

determined by MP method with using PAUP program, and numbers above branches

indicate bootstrap value. (C) Phylogenetic tree determined by BI method with using

MrBayes program, and numbers above branches indicate posterior probabilities. (D)

Phylogenetic tree determined by ML method with using IQtree program, and numbers

above branches indicate bootstrap value.

Fig. 2. Phylogenetic tree based on the cpDNA trnL-F sequence, and numbers

above branches indicate bootstrap value or posterior probabilities. (A) Phylogenetic tree determined by NJ method with using PAUP program, and numbers above branches indicate bootstrap value. (B) The phylogenetic tree with best topology, determined by MP method with using PAUP program, and numbers above branches indicate bootstrap value. (C) Phylogenetic tree determined by ML method with using IQtree program, and numbers above branches indicate bootstrap value. (D) Phylogenetic tree determined by BI method with using MrBayes program, and numbers above branches indicate posterior probabilities.

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522 **Fig. 3. Phylogenetic tree based on the combined sequence, and numbers**
523 **above branches indicate bootstrap value or posterior probabilities. (A)**
524 **Phylogenetic tree determined by NJ method with using PAUP program, and numbers**
525 **above branches indicate bootstrap value. (B) Phylogenetic tree determined by MP**
526 **method with using PAUP program, and numbers above branches indicate bootstrap**
527 **value. (C) Phylogenetic tree determined by ML method with using IQtree program,**
528 **and numbers above branches indicate bootstrap value.(D) Phylogenetic tree**
529 **determined by BI method with using MrBayes program, and numbers above branches**
530 **indicate posterior probabilities.**

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Fig. 4. Divergence time estimation and speciation rate analysis based on the combined sequence and unlike model. (A) The speciation rate analysis performed with the root age of Asteraceae as 49–42 Ma. (B) The divergence time estimation phylogenetic tree with geological time scale, and with the root age of Asteraceae as 49–42 Ma. The internal nodes of the tree are indicated with circles, where circles mark nodes with posterior probability: ● > 0.95, 0.95 > ● > 0.75, 0.75 > ●. (C) The speciation rate analysis performed with the root age of Asteraceae as 76–66 Ma. (D) The divergence time estimation phylogenetic tree with geological time scale, and with the root age of Asteraceae as 76–66 Ma. The internal nodes of the tree are indicated with circles, where circles mark nodes with posterior probability: ● > 0.95, 0.95 > ● > 0.75, 0.75 > ●.