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New insights into the phylogeny of Burasaieae (Menispermaceae) with the recognition of a new genus and emphasis on the southern Taiwanese and mainland Chinese disjunction

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ABSTRACT

Taiwan is a continental island lying at the boundary between the Eurasian and the Philippine tectonic plates and possesses high biodiversity. Southern Taiwan, viz. Hengchun Peninsula, is notably floristically different from northern Taiwan. The floristic origin and relationships of the Hengchun Peninsula have been rarely investigated in a phylogenetic context. In this study, data from six plastid and nuclear sequences were used to reconstruct phylogenetic relationships within Burasaieae (Menispermaceae), which mainly inhabits tropical rainforests. The tree-based comparisons indicate that the position of *Tinospora* sensu stricto conflicts significantly between the cpDNA and ITS trees. However, alternative hypothesis tests from the ITS data did not reject the result of the cpDNA data, which suggests that tree-based comparisons might sometimes generate an artificial incongruence, especially when markers with high homoplasy are used. Based on the combined cpDNA and ITS data, we present an intergeneric phylogenetic framework for Burasaieae. Sampled species of *Tinospora* are placed in three different clades, including *Tinospora dentata* from southern Taiwan and *T. sagittata* from mainland China in an unresolved position alongside six lineages of Burasaieae. By integrating lines of evidence from molecular phylogeny, divergence times, and morphology, we recognize the three *Tinospora* clades as three different genera, including *Tinospora* sensu stricto, a new genus (*Paratinospora*) for *T. dentata* and *T. sagittata*, and *Hyalosepalum* resurrected. *Tinospora dentata*, now endemic to the Hengchun Peninsula, originated from the Late Eocene (*ca.* 39 Ma), greatly predating the formation of Taiwan. Our study suggests that the flora of the Hengchun Peninsula contains some ancient components that might have migrated from mainland China.

1. Introduction

Taiwan, one of the largest islands off the southeastern coast of mainland China, lies between Ryukyu Islands to the north and Philippines to the south. It possesses diverse vegetation types, and correspondingly harbors high biodiversity (Fang and Zhuo, 1995; Zhang, 1995; Cai and Shu, 2002; Hsieh, 2002; Lai, 2003). The flora of Taiwan comprises 1389 genera and 4216 species of vascular plants, of which approximately 26% are endemic (Hsieh, 2002). Owing to its botanical wealth and geographic position, the origin and relationships of the Taiwanese flora have fascinated botanists and biogeographers for more than a century (e.g., Henry, 1896; Hayata, 1908; Li, 1957; Zeng, 1994; Hsieh et al., 1994; Zhang, 1995; Lu, 2001; Hsieh, 2002; Ying and Hsu, 2002; Lai, 2003; Huang, 2011; Chen et al., 2012). Molecular phylogenetic analyses have shown that during the late Miocene to the Pleistocene, some plants migrated to Taiwan either from mainland China, such as *Cunninghamia* (Chung et al., 2004), *Pseudotsuga* (Wei et al., 2010), and *Sassafras* (Nie et al., 2007), or from the Japanese Archipelago, such as *Chamaecyparis* (Wang et al., 2003) and

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Trochodendron aralioides (Huang and Lin, 2006). These migrations occurred almost simultaneously with or after the formation of Taiwan, which began 5–9 Ma (Sibuet and Hsu, 2004). Geologically, Taiwan and the neighboring China continental margin belonged to the Eurasian plate and were part of a subaerially exposed landmass during the Late Cretaceous, the landmass has been subjected to multiple stretching and rifting since the Paleocene (Ye, 1982; Teng, 1992; Zhang, 1995; Lai, 2003; Suo et al., 2015). Whether much older migrations to Taiwan from other neighboring regions have occurred remains to be explored.

To date, phylogenetic analyses only included a few subtropical or temperate plants in northern Taiwan (e.g., Chung et al., 2004; Wang et al., 2003; Nie et al., 2007). The southernmost part of Taiwan, generally known as the Hengchun Peninsula, is notably different floristically from northern Taiwan (Hsieh et al., 1994; Wu and Wu, 1996; Lai, 2003). The vegetation in the Hengchun Peninsula is mainly subtropical evergreen broad-leaved forests with a few semi-tropical rainforests. The Hengchun Peninsula, together with Lanyu and Lutao islands, has been regarded either as an independent floristic region (Li and Keng, 1950) or a part of the Malesian floristic region (Zhang, 1995), whereas most of Taiwan (including the northern and central regions) have been included in the eastern Asiatic region (Takhtajan, 1986). Floristic comparisons between Taiwan and its neighboring regions indicate that the flora of the Hengchun Peninsula is a combined assemblage of both mainland China and Philippine elements (Li and Keng, 1950; Hsieh, 2002), but is more closely allied to the flora of mainland China (Hsieh et al., 1994; Ying and Hsu, 2002). However, no molecular study has been conducted to test the hypothesis so far.

Menispermaceae is a characteristic and structural component of modern tropical rainforests (Gentry, 1991; Richards, 1996; Wang et al., 2012). Molecular phylogenetics has contributed greatly to our understanding of relationships within the family. Phylogenetic studies of Menispermaceae have indicated that most tribes delimited by Diels (1910) and Kessler (1993) are not monophyletic (Ortiz et al., 2007, 2016; Wang et al., 2007, 2009, 2012; Hoot et al., 2009; Jacques et al., 2011; Wefferling et al., 2013). The classification of the family at the subfamilial and tribal levels has recently been revised (Ortiz et al., 2016), with recognition of two subfamilies: Menispermoideae (including seven tribes) and Chasmantheroideae (=Tinosporoideae *sensu* Wang et al., 2009) (including two tribes). In the classification of Ortiz et al. (2016), the delimitation of Burasaieae has been clarified, which includes Fibraureae, Peniantheae, and Tinosporeae of Diels (1910). However, generic circumscriptions in Burasaieae remain unresolved. For instance, *Jateorhiza* and *Tinospora* were included in *Chasmanthera* by Baillon (1872), a classification that was not followed by later authors (e.g., Diels, 1910; Troupin, 1962; Kessler, 1993). Troupin (1962) argued that *Kolobopetalum*, *Leptoterantha*, and *Syntriandrium* should also be included in *Chasmanthera* if the delimitation of Baillon (1872) was followed. Similarly, Barneby (1972) suggested that *Odontocarya* could also be included in *Chasmanthera* sensu Baillon (1872). Family-wide phylogenetic analyses indicate that all of these genera and *Rhigiocarya* form a monophyletic group (Jacques et al., 2011; Wang et al., 2012; Ortiz et al., 2016). Intergeneric relationships within Burasaieae are well-resolved except for the relationships among six lineages (Ortiz et al., 2007, 2016; Hoot et al., 2009; Jacques et al., 2011; Wefferling et al., 2013), which diverged rapidly near the Cretaceous-Paleogene (K-Pg) boundary (Wang et al., 2012). However, only chloroplast DNA (cpDNA) sequences have been employed in the phylogenetic analyses involved in the tribe so far.

Of the three largest genera in the Burasaieae, namely *Disciphania*, *Odontocarya*, and *Tinospora*, (Kessler, 1993; Ortiz et al., 2016), only the circumscription of *Tinospora* has been questioned. Diels (1910) recognized *Desmonema* and *Tinospora* as two different genera. The former is restricted to Africa, whereas the latter is distributed in Asia and Australia, as well as Africa. Troupin (1962) synonymized *Desmonema* with *Tinospora*, which was followed by Kessler (1993). Based on the chloroplast *rbcL* and *atpB* sequences, Hoot et al. (2009) found that African *Tinospora caffra* (=*Desmonema caffra*) did not cluster together with Australian *Tinospora*, which is congruent with the result of Wefferling et al. (2013) who analyzed, in addition, the plastid *matK* sequences. Similarly, other phylogenetic analyses confirmed the result and further supported that Australian and Asian *Tinospora* species grouped together (Ahmad et al., 2009; Jacques et al., 2011; Ortiz et al., 2016). *Tinospora* contains 36 species (Ortiz et al., 2016), however less than six species of the genus have been included in phylogenetic analyses so far. Moreover, no other African species were sampled except for *T. caffra*.

Tinospora dentata, endemic to Taiwan, is restricted to tropical lowland forests of the Hengchun Peninsula (Fig. 1). Its tuberous root is known as "Ching-Zhong" and reported to be used as a bitter stomachic in Taiwan (Chen, 1975). Owing to its habitat destruction and over-harvesting of its roots, *T. dentata* has become endangered in the wild and is listed in China Species Red List. This species has not been sampled in any previous molecular study, however it presents an opportunity to explore the floristic relationships between the Hengchun Peninsula and other neighboring regions.

In this study, our objectives are (1) to investigate the phylogenetic relationships within Burasaieae using six markers from the plastid and nuclear genomes with a focus on *Tinospora*, hence with a more extensive sampling than in any previous study, (2) to examine the circumscription of *Tinospora* and the taxonomic status of *Desmonema*, and (3) to explore the floristic origin of the southern Taiwan by estimating the time of divergence between *T. dentata* and its sister taxa.

2. Materials and methods

2.1. Taxon sampling and DNA sequencing

The general sampling strategy was to include the majority of the genera of Burasaieae with increased sampling for *Tinospora*, whose delimitation has remained disputed in traditional classifications. A total of 41 accessions of Burasaieae were included in the present study, representing 33 species from 20 of the 24 currently recognized genera in the tribe (Ortiz et al., 2016). Our sampling encompasses 13 species of *Tinospora*, two from Africa, two from Australia, and nine from Asia, including the type of the genus *T. cordifolia* and the southern Taiwanese endemic *T. dentata*. Following the results of Wang et al. (2012), Wefferling et al. (2013), and Ortiz et al. (2016), we selected three species representing all three genera of Coscinieae (Chasmantheroideae), and one species of Menispermeae (Menispermoideae) as outgroups. Species, geographic origin of the sequenced vouchers, and GenBank accession numbers are listed in Table S1.

Six molecular markers, including plastid (*rbcL*, *atpB*, *matK*, *ndhF*, and *trnL-F*) and nuclear (ITS) loci were used in this study. Total genomic DNA was extracted from silica gel-dried leaf materials or herbarium specimens using DNeasy Mini Plant Kits (Tiangen Biotech, Beijing, China). The primers listed in Wang et al. (2012) were used to amplify and sequence the five cpDNA regions, and the primers ITS-1 and ITS-4 of White et al. (1990) were used to amplify and sequence the ITS region. PCR products were purified using the Tian quick Midi Purification Kit (TianGen Biotech) and directly sequenced. Sequencing reactions were performed using the ABI Prism Bigdye Terminator Cycle Sequencing Kit (Applied Biosystems, ABI). Sequences were analyzed using an ABI 3730xl DNA sequencer. PCR products of the ITS region were single bands,

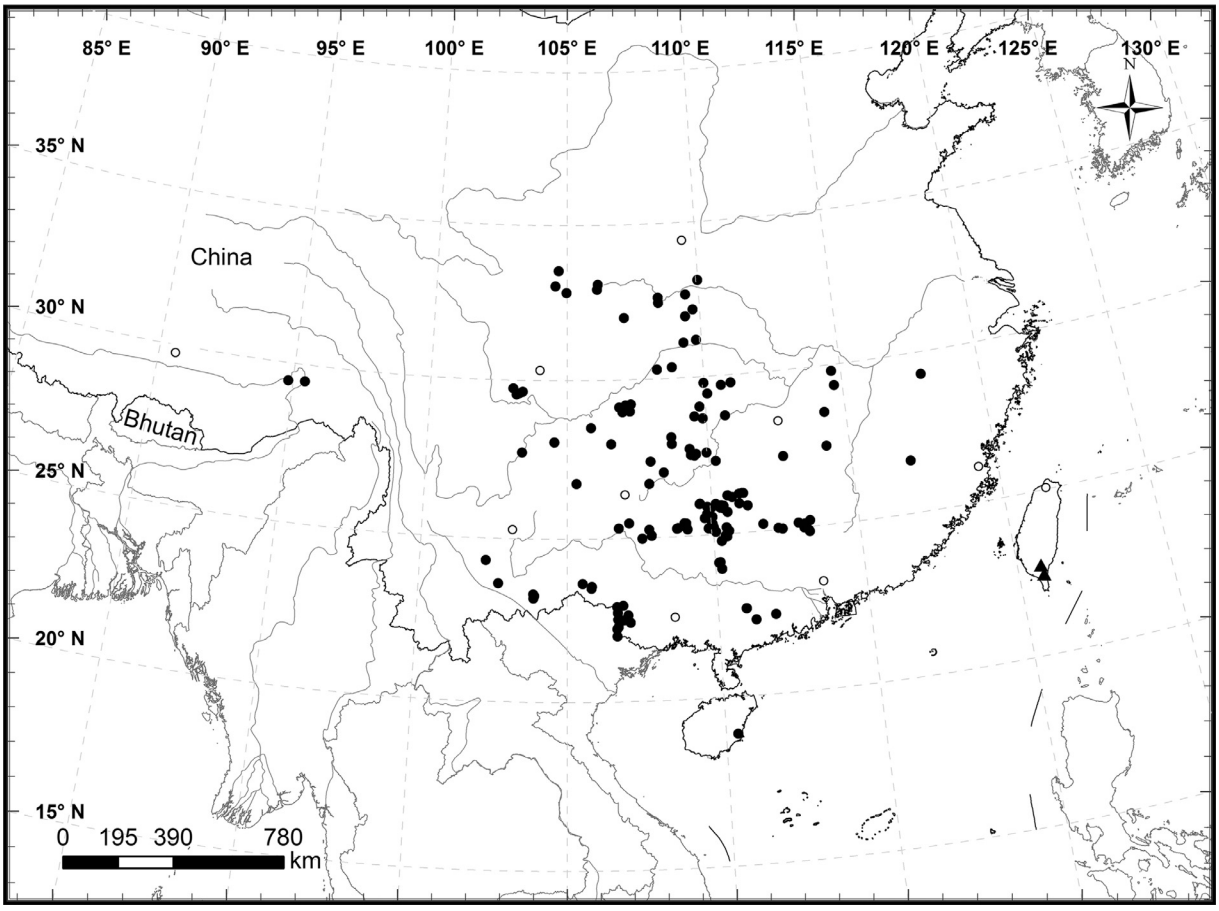

Fig. 1. Distribution of *Tinospora dentata* (filled triangles) and *T. sagittata* (filled circles).

and no double peaks or ambiguous base calls were found in electropherograms of ITS sequences. Most of the sequences used in this study were newly generated or from our previous study (Wang et al., 2012); a few were from GenBank (Table S1).

2.2. Phylogenetic analysis

The five cpDNA sequences (*rbcL*, *atpB*, *matK*, *ndhF*, and *trnL-F*) were all easily aligned manually with BioEdit v7.0 (Hall, 1999) using the previous data matrices of Wang et al. (2012) as the reference. ITS sequences were aligned using Clustal X v1.83 (Thompson et al., 1997) and subsequently adjusted in BioEdit. One polyAG region in *trnL-F* (representing 13 nucleotides) and four difficult-to-align regions in the ITS data (encompassing 121 nucleotides) were excluded from the analyses.

Phylogenetic analyses were carried out using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) methods for the combined sequences of *rbcL-atpB-matK-ndhF-trnL-F* from the cpDNA genome (referred to hereafter as the cpDNA data), ITS data, and combined sequences of all six regions (referred to as total evidence data). The MP, ML, and BI analyses were conducted in PAUP* v4.0b10 (Swofford, 2003), RAxML v7.0.4 (Stamatakis, 2006), and MrBayes v3.2.5 (Ronquist et al., 2012), respectively.

For MP analyses, heuristic searches were performed with 1000 replicates of random addition, tree-bisection-reconnection branch swapping, MulTrees in effect, and steepest descent off. Gaps were treated as missing data. Node support was estimated with 1000 bootstrap replicates as described above. For ML analyses, each partition was assigned a GTR + Γ model and all model parameters were estimated. Nodal support on the ML tree was estimated by nonparametric bootstrap (1000 replicates). Akaike Information Criterion via jModeltest v2.1.4 (Posada, 2008) was used to determine the best-fit model for each DNA region. For BI analyses, each DNA region was assigned its own model of nucleotide substitution. Two independent runs, each consisting of four Markov Chain Monte Carlo chains, were conducted with one tree sampled for every 1000 generations over 50 million generations, starting with a random tree. The stationarity of the runs was assessed using Tracer v1.5 (Rambaut and Drummond, 2009). Majority rule (>50%) consensus trees were constructed after removing the burn-in period samples (the first 25% of sampled trees). Based on Wang et al. (2014), the thresholds bootstrap support (BS) $\geqslant$ 70% and posterior probability (PP) $\geqslant$ 0.95 were used as an indication of significantly supported conflict between plastid and nuclear datasets.

2.3. Alternative hypothesis test

To assess the strength of the conflicts between the topologies generated from the cpDNA and ITS data and between the traditional *Tinospora* circumscriptions and those recovered from the two genomic data, we compared the likelihood values of the alternative hypotheses against those of the unconstrained ML tree using the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999), the Kishino-Hasegawa (KH) test (Kishino and Hasegawa, 1989), and the approximately unbiased (AU) test (Shimodaira, 2002). The constrained trees (with the taxa of interest constrained as monophyletic and the others left as a polytomy) were constructed using Mesquite v2.74 (Maddison and Maddison, 2010), and then optimized in RAxML (GTR + Γ model,

partitioned by DNA region). The optimized constrained tree was then used to test the alternative hypothesis. Site-wise log-likelihood values were calculated by Tree-Puzzle v5.2 (Schmidt et al., 2002), and then were used as input data for the Consel v0.1k analysis (Shimodaira and Hasegawa, 2001).

2.4. Molecular dating

Divergence times were estimated using BEAST v1.4.8 (Drummond and Rambaut, 2007) which takes into account phylogenetic and calibration uncertainties. We used the cpDNA data rather than ITS or total evidence data for age estimates. The cpDNA sequences are single-copy and uniparentally inherited (Soltis and Soltis, 1998), and thus can avoid the problems of recombination and incomplete concerted evolution that are common in ITS sequences (Álvarez and Wendel, 2003). Moreover, ITS sequences are highly divergent as compared with the other molecular markers used in this study.

We selected three fossils as the calibration points, all of which were constrained using the youngest age of the relevant geological stage based on the latest geological time scale from Cohen et al. (2013). The stem group age of *Jateorhiza* was constrained to be 47.8 Ma based on the fossil *Jateorhiza gilliami* from the London Clay, UK (Chandler, 1964). The stem group age of *Parabaena* was also constrained to be 47.8 Ma based on the fossil *Parabaena bognorensis*, also from the London Clay (Chandler, 1964). A fossil endocarp of *Anamirta* was used to constrain its stem age of 43.7 Ma (Manchester, 1994). The detailed justifications for the placements of these three fossils are presented in Wang et al. (2012).

We first ran analyses by sampling from the prior under exponential, uniform, lognormal, and normal distributions for the nodes constrained by fossils. To avoid overestimation of root age, we set a 109 Ma maximum age for the root in these four analyses, which is the estimated crown age of Menispermaceae (Wang et al., 2012), with a normal distribution and a standard deviation of three. The different prior distribution models for the three fossil calibrations did not show significant different ages and generated highly consistent likelihood values after convergence determined in Tracer v1.5. We therefore adopted a conservative way in which all fossil calibration points were enforced using uniform distributions. Dating analysis was performed with partitioned relaxed-clock models (one uncorrelated lognormal clock per marker), GTR substitution model, gamma site heterogeneity model, estimated base frequencies, Yule process of speciation, and a ML starting tree. Two distinct runs were conducted with 50 million generations and trees sampled every 5000 generations. Convergence of runs was evaluated in Tracer v1.5. After a burn-in of 25%, the effective sample size value of each parameter was >200. The maximum clade credibility tree with median branch lengths and a 95% highest posterior density (HPD) interval on nodes was compiled using TreeAnnotator v1.5.4 (Drummond and Rambaut, 2007).

3. Results

3.1. Sequence characteristics

With a few exceptions, we obtained sequence data for all six DNA regions of all sampled species (Table S1). We were unable to obtain materials of two species and for these sequences are missing as follows: *T. cordifolia* (lacking *ndhF*) and *T. malabarica* (lacking *matK*, *ndhF*, and *trnL-F*). In addition, we encountered difficulties in obtaining *atpB* sequence for *T. sagittata* (Voucher: Yang GH 56874), and *ndhF* and ITS sequences for *T. caffra*. The total evidence dataset included 45 taxa, in which the amount of missing data was: *rbcL* = 0, *atpB* = 2.2% (1/45), *matK* = 2.2% (1/45), *ndhF* = 6.7% (3/45), *trnL-F* = 2.2% (1/45), and ITS = 2.2% (1/45).

3.2. Phylogenetic analyses

The cpDNA dataset comprised 7240 aligned nucleotides: *rbcL*,1386 bp; *atpB*, 1413 bp; *matK*, 1242 bp; *ndhF*, 2079 bp; and *trnL-F*, 1120 bp. Table 1 summarizes the numbers of variable and parsimony informative sites and tree statistics for the MP analysis and the best-fit model estimated by jModeltest for the five chloroplast datasets. ML and BI analyses produced identical trees that are highly congruent with those from MP analysis except for five nodes with poor support (Fig. 2A). Within the Burasaieae, *Calycocarpum* is the earliest-diverging lineage, followed by the clade containing *Aspidocarya*, *Disciphania*, and *Parabaena*, and then *Tinomiscium*. *Jateorhiza* is sister to the monophyletic group containing clade I (*Kolobopetalum* and *Rhigiocarya*), *Tinospora* 1 (*Tinospora* sensu stricto), and clade II. *Tinospora* 1 is sister to Clade I (MP-BS 90%, ML-BS 83%, PP 1.0). African *T. bakis* is embedded within *Tinospora* 1. Within clade II, *Chasmanthera* is sister to the remainders. *Tinospora* 3 (*T. caffra*) is sister to *Leptoterantha* (MP-BS < 70%, ML-BS 95%, PP 0.95). *Syntriandrium* and *Odontocarya* are grouped together with strong support. *Tinospora* 2 clade (including *T. dentata* and *T. sagittata*) is in an unresolved position in relation to *Fibraurea*, *Borismene*, *Penianthus*, *Sphenocentrum*, *Orthogynium-Burasaia*, and *Dioscoreophyllum-Jateorhiza*.

The aligned matrix of ITS sequences was 662 characters in length. The numbers of variable and parsimony informative sites, tree statistics for the MP analysis and the best-fit model determined by jModeltest are summarized in Table 1. ML and BI analyses yielded highly similar trees that are largely congruent with those from the MP analysis (Fig. 2B). Comparing to the cpDNA topology, the ITS topology has lower resolution, but a distant relationship between *Tinospora* 1 and 2 is also recovered, as is the African *T. bakis*, as a member of *Tinospora* 1. *Tinospora* 1 is strongly supported as sister to Clade II (MP-BS 95%, ML-BS 90%, PP 1.0).

The total evidence dataset consisted of 7902 characters, of which 1466 were variable and 755 parsimony-informative sites. ML and BI analyses resulted in identical trees that are highly consistent with the tree of MP analysis except for three nodes with weak support (Fig. 3). The topology of Burasaieae phylogeny resulting from the total evidence analyses (Fig. 3) was identical to the cpDNA tree (Fig. 2) with the exception that in the cpDNA tree *Sphenocentrum* is recovered as sister to *Burasaia* and *Orthogynium*, with poor support.

3.3. Alternative hypothesis test

The results of alternative hypothesis tests are shown in Table 2. All three alternative hypothesis tests show that constraining *Tinospora* 1 as sister to Clade II always resulted in topologies that did not fit the cpDNA data significantly better ($P < 0.05$) than the unconstrained ML topology. However, none of the test sets rejected *Tinospora* 1 as sister to Clade I based on the ITS data. Constraining *Tinospora* as monophyletic in any of the two clades (1 + 2, 1 + 3, 2 + 3 in the cpDNA data; 1 + 2 in the ITS data), or all three clades (in cpDNA data) was rejected under any test. However, none of the tests rejected *Tinospora* 1 as sister to Clade I based on the ITS data.

3.4. Divergence-time estimates

Divergence time estimates for Burasaieae are presented in Fig. 4. The extant Burasaieae dated to the middle Cretaceous (node 1: 99.55 Ma) (95% HPD: 88.82–109.23). The seven Burasaieae lineages started to diverge rapidly near the K-Pg boundary (node 2:

Table 1
Statistics from the analyses of the various datasets. CI = consistency index; RI = retention index; RC = rescaled consistency index.

Dataset	No. taxa	Total length	No. variable characters	No. informative characters	No. trees	Length of trees	CI	RI	RC	Model
rbcL	45	1386	133	74	14,641	221	0.66	0.80	0.53	TVM + I + Γ
atpB	44	1413	124	58	130	159	0.83	0.91	0.76	TVM + I
matK	44	1242	259	104	44	364	0.79	0.87	0.69	TVM + I + Γ
ndhF	42	2079	406	193	36	642	0.73	0.83	0.61	TVM + I + Γ
trnL-F	44	1120	196	89	4	251	0.86	0.92	0.80	TVM + Γ
cpDNA	45	7240	1118	518	72	1652	0.76	0.86	0.65	–
ITS	44	662	348	237	291	1130	0.55	0.73	0.40	GTR + Γ + I
cpDNA + ITS	45	7902	1466	755	5	2807	0.67	0.80	0.53	–

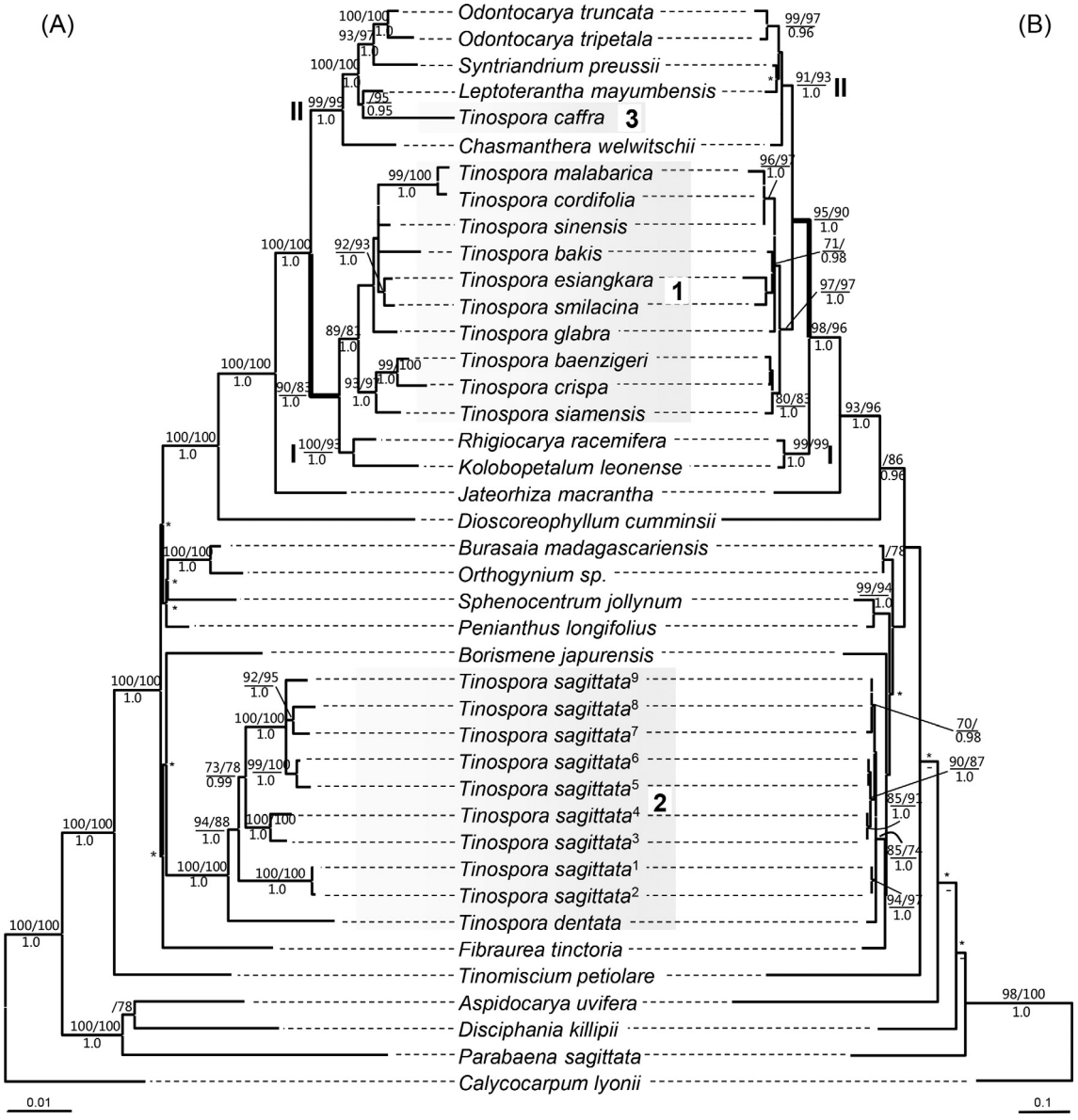

Fig. 2. ML trees inferred from the cpDNA (A) and nuclear ITS (B) data (outgroups removed but the same as in Fig. 3). Numbers above and below branches are bootstrap percentages (MP/ML) and Bayesian posterior probabilities, respectively. Supports with BS ⩾ 70% and BS ⩾ 0.95 are indicated. "∗" and "-" indicates the node not supported in the MP and BI analyses, respectively. The thick branches represent the significant incongruence between the cpDNA and ITS trees based on tree-based comparisons.

67.84 Ma) (95% HPD: 59.58–77.32). The stem age of *Tinospora* 2 was estimated to be 59.96 Ma (95% HPD: 46.97–71.75; node 3). The split between Taiwanese *T. dentata* and mainland Chinese *T. sagittata* is estimated to be 38.97 Ma (95% HPD: 26.45–52.52; node 4), and the latter began to diversify at 31.84 Ma (95% HPD: 20.64–44.31; node 5). *Tinospora* 1 diverged from its close relatives at 31.22 Ma (95% HPD: 22.85–39.73; node 6), and became differentiated at 23.67 Ma (95% HPD: 16.26–31.95; node 7). The split of *T. caffra* and *Leptoterantha* occurred at 17.02 Ma (95% HPD: 7.95–26.62; node 13).

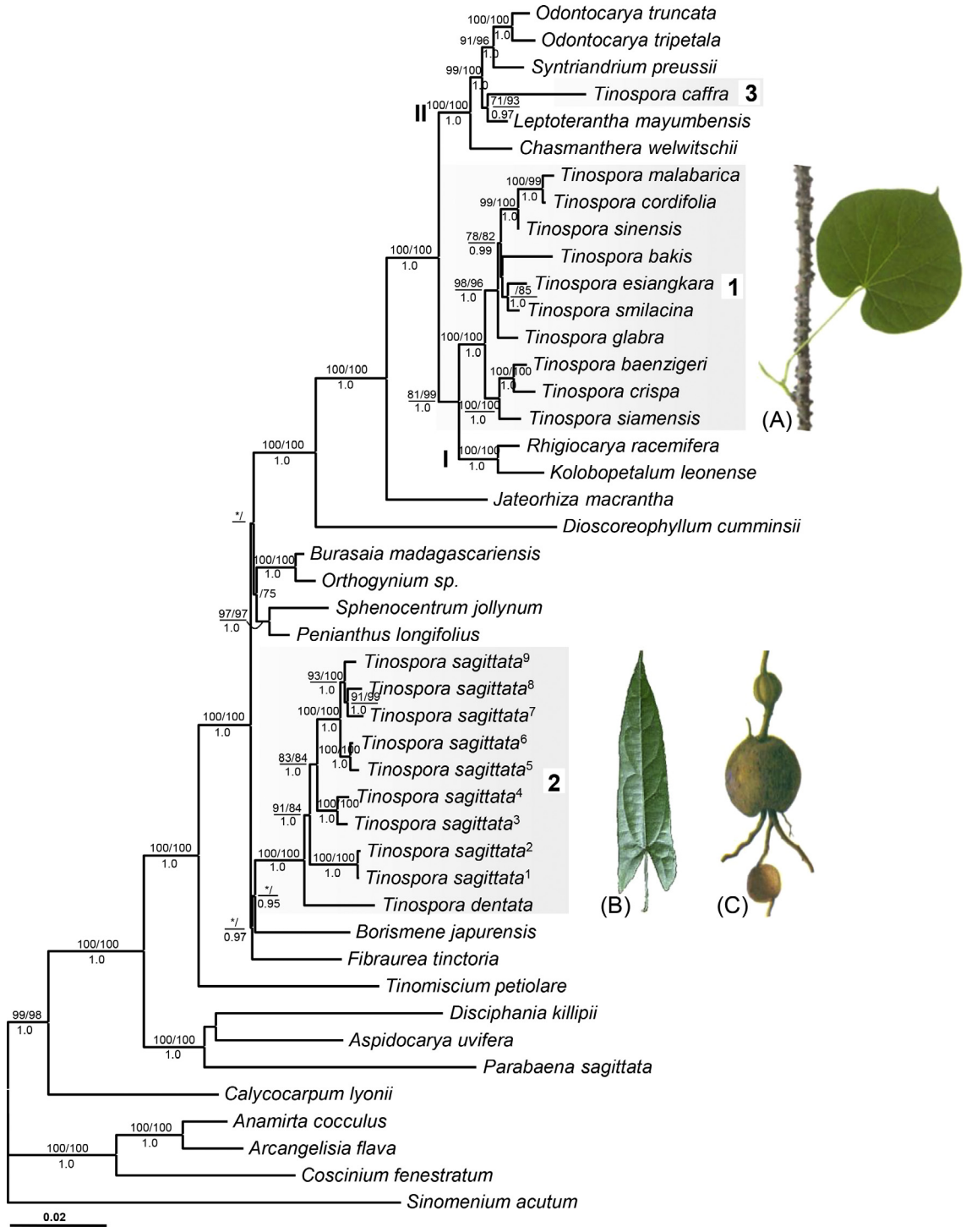

Fig. 3. ML tree inferred from the total evidence data. Numbers above and below branches are bootstrap percentages (MP/ML) and Bayesian posterior probabilities, respectively. Supports with BS ⩾ 70% and BS ⩾ 0.95 are indicated. "*" indicates the node not supported in the MP analysis. The pictures to the right of the cladogram: (A) stem and leaf of *Tinospora cordifolia*, (B) leaf of *T. sagittata*, and (C) root of *T. sagittata*.

4. Discussion

4.1. Phylogeny of Burasaieae

Nuclear DNA sequences were used for the first time to reconstruct the phylogeny of Burasaieae. Previously, inter-generic relationships were recovered based only on cpDNA data (Ortiz et al., 2007, 2016; Wang et al., 2012; Hoot et al., 2009; Jacques et al., 2011; Wefferling et al., 2013). Although resolution and support at deep nodes is lower in ITS than in cpDNA trees, the ITS data recovered some clades with moderate to strong supports, such as clades I and II, and *Tinospora* 1 (Fig. 2B). Interestingly, based on the tree-based comparisons, the position of *Tinospora* 1 conflicts significantly between the cpDNA and ITS trees (Fig. 2). Our alternative hypothesis tests indicate that the cpDNA data strongly reject the result of ITS data (*Tinospora* 1 as sister to clade II), whereas

Table 2
Comparison between the alternative hypotheses. *P*-values were estimated with Tree-Puzzle and Consel. The sign "+" indicates the alternative topology differing significantly (*P*-value at 0.05) from the ML tree, and should be rejected.

Data	Hypothesis	Δ logL	S. E.	SH	KH	AU
cpDNA	*Tinospora* 1 + Clade II	34.51	17.88	0.031+	0.031+	0.021+
	Tinospora monophyletic (1 + 2 + 3)	406.62	41.35	0.000+	0.000+	0.000+
	Tinospora 1 + *Tinospora* 2	332.37	37.10	0.000+	0.000+	0.000+
	Tinospora 1 + *Tinospora* 3	66.97	21.38	0.001+	0.002+	0.001+
	Tinospora 2 + *Tinospora* 3	153.90	26.95	0.000+	0.000+	0.000+
ITS	*Tinospora* 1 + Clade I	2.25	12.45	0.429	0.427	0.445
	Tinospora monophyletic (1 + 2)	137.25	21.76	0.000+	0.000+	0.000+

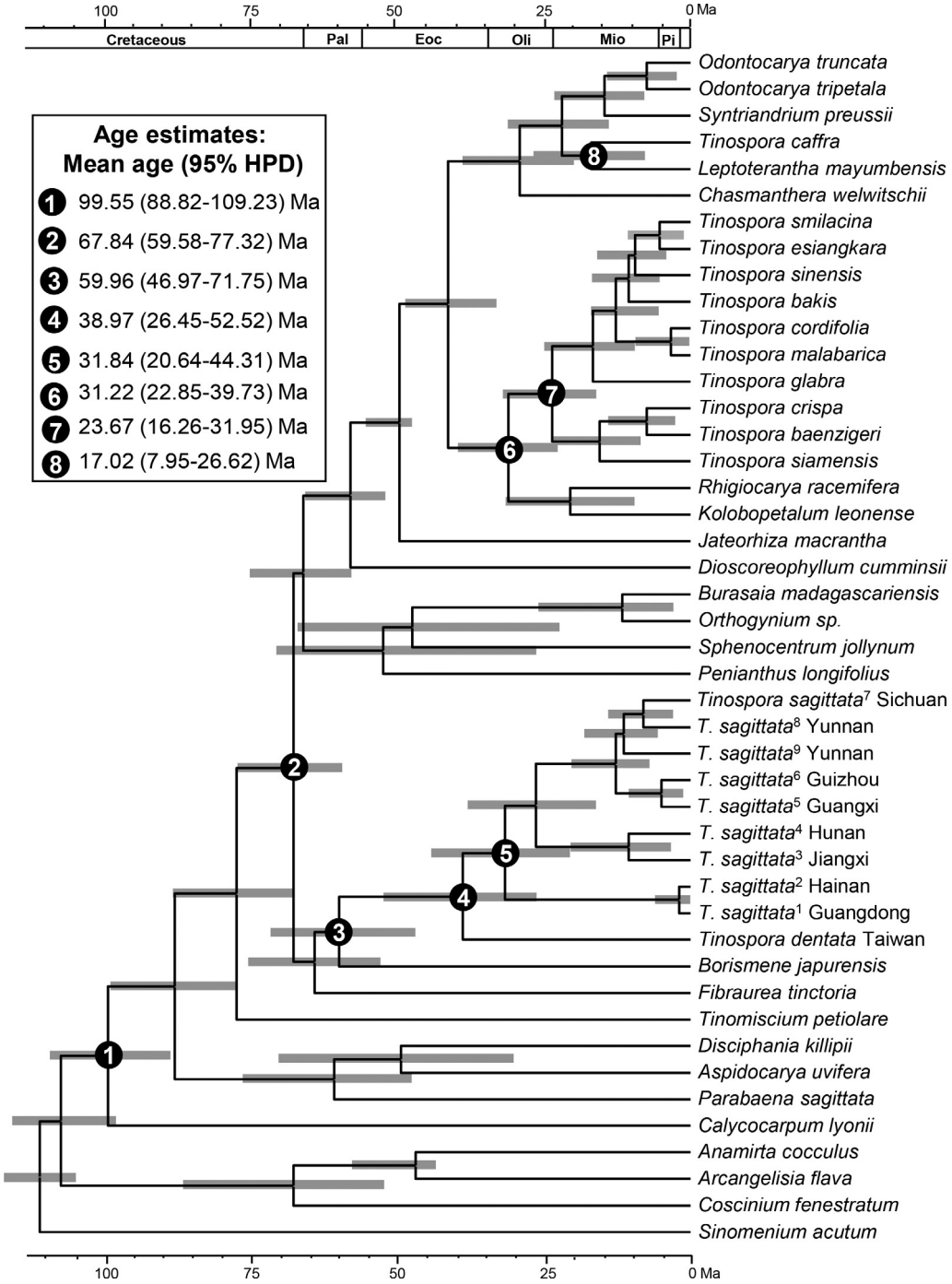

Fig. 4. Chronogram of Tinosporeae based on the BEAST analysis using the cpDNA data. Gray bars indicate 95% highest posterior density intervals. Nodes of interests were marked as 1–8.

the ITS data do not reject the result of the cpDNA data (*Tinospora* 1 as sister to clade I) (Table 2). In Burasaieae, substitution rates of the ITS sequences are by far the highest among the six markers (Table 1): 52.6% for ITS (348/662), 9.6% for *rbcL* (133/1386), 9.8% for *atpB* (124/1413), 20.9% for *matK* (259/1242), 19.5% for *ndhF* (406/2079), and 17.5% for *trnL-F* (196/1120). As shown by the data in Table 1, ITS data yielded lower CI, RI, and RC values than any of the five cpDNA markers. These suggest that the ITS dataset has a higher level of homoplasy than the plastid datasets, which is in agreement with previous reports (Álvarez and Wendel, 2003; Guzmán and Vargas, 2005).

The trees resulting from the total evidence data are congruent with those of the cpDNA data. The MB-BS value supporting *Tinospora* 1 as sister to clade I was only 9% lower (from 90% in the cpDNA data to 81% in total evidence data), however, the ML-BS value was 16% higher (83–99%). Tree-based comparisons are often used to identify incongruence between phylogenies obtained from plastid and nuclear datasets (Wang et al., 2014). However, our results indicate that such comparisons might sometimes give an artificial incongruence, especially when using markers with high levels of homoplasy.

Contrary to the cpDNA markers, ITS sequences have faster evolutionary rates and higher levels of homoplasy in Burasaieae. However, the combined cpDNA and ITS dataset increased support values for many nodes (Fig. 3 *vs*. Fig. 2A). As was pointed out by Jian et al. (2008), a rapidly evolving marker, when combined with a slowly evolving one, can enhance the resolution. Phylogenetic relationships within Burasaieae generated from the total evidence analyses (Fig. 3) are highly congruent with those obtained from the cpDNA (Fig. 2A). Similar results were also obtained by previous studies (Wang et al., 2012; Wefferling et al., 2013; Ortiz et al., 2016), suggesting that we are converging on a robust intergeneric phylogenetic reconstruction for Burasaieae.

Based on our phylogenetic analyses, at least seven genera, *Jateorhiza*, *Kolobopetalum*, *Leptoterantha*, *Rhigiocarya*, *Syntriandrium*, *Tinospora*, and *Odontocarya* would need to be reduced to synonymy if *Chasmanthera* sensu Baillon (1872) is accepted (Figs. 2 and 3). As *Tinospora* and *Odontocarya* contain >30 species each (Kessler, 1993; Ortiz et al., 2016), a broadly circumscribed *Chasmanthera* will require many species names to be changed. This approach may not be convenient. Thus, we are in agreement with earlier authors (e.g., Diels, 1910; Troupin, 1962; Kessler, 1993; Ortiz et al., 2016) and retain these eight genera as separate.

4.2. Non-monophyletic Tinospora

In agreement with other studies (Hoot et al., 2009; Jacques et al., 2011; Wefferling et al., 2013; Ortiz et al., 2016), we also found a non-monophyletic *Tinospora* (Figs. 2 and 3). Based on alternative hypothesis tests, the cpDNA and ITS data also rejected a monophyletic *Tinospora* (Table 2). Notably, by a more extensive *Tinospora* sampling than in any previous study, our analyses placed *Tinospora* species in three different clades, *Tinospora* 1–3 (Figs. 2A and 3), instead of two clades, as found before.

4.2.1. A new generic name for T. dentata and T. sagittata

Our phylogenetic analyses indicate that Asian *T. dentata* and *T. sagittata* (*Tinospora* 2) do not cluster with other Asian/Australian *Tinospora* (*Tinospora* 1; Figs. 2A and 3). These two species have always been placed in *Tinospora* in all traditional classifications (e.g., Diels, 1910; Forman, 1981, 1986; Kessler, 1993; Luo, 1996; Luo et al., 2008). However, all data matrices, cpDNA, ITS, and total evidence data, strongly support that *Tinospora* 2 clade has a distant relationship with *Tinospora* 1. The alternative hypothesis tests based on the cpDNA and ITS data strongly rejected to place *Tinospora* 1 and *Tinospora* 2 together ($P < 0.001$), and the cpDNA data also rejected the grouping of *Tinospora* 2 with African *T. caffra* (Table 2). Our time estimates indicate that *Tinospora* 2 originated earlier than *Tinospora* 1 (59.96 Ma *vs.* 31.22 Ma). *Tinospora dentata* and *T. sagittata* are markedly different from other *Tinospora* in lacking aerial roots (*vs.* present) and having sagittate to hastate leaf base (*vs.* truncate to cordate), herbaceous stems (*vs.* woody), lens-shaped lenticels (*vs.* cross-shaped), and roots with tuberous swellings (*vs.* lacking) (Fig. 3A–C; Diels, 1910; Forman, 1986; Luo et al., 2008).

Our phylogenetic analyses show that *Tinospora* 2 is in an unresolved position in relation to six Burasaieae lineages (Fig. 3). These seven lineages diverged rapidly near the K-Pg boundary (Fig. 4). It is not appropriate to include the two species in any of the already recognized genera, thus we propose a new generic name for the two species:

Paratinospora Wei Wang, gen. nov. TYPE: *Paratinospora sagittata* (Oliv.) Wei Wang.

1. *Paratinospora sagittata* (Oliv.) Wei Wang, comb. nov. with all three varieties currently recognized. *Limacia sagittata* Oliv. In Hooker's Icon. Pl. 18: t. 1749. 1888. *T. sagittata* (Oliv.) Gagnep. in Bull. Soc. Bot. France 55: 45. 1908. *T. capillipes* Gagnep. in Bull. Soc. Bot. France 55: 44. 1908. *T. imbricata* S. Y. Hu in J. Arn. Arb. 35: 195. 1954. *T. sagittata* var. *leucocarpa* Y. Wan & C. Z. Gao in Guihaia 10: 178. 1990. *T. szechuanensis* S. Y. Hu. in J. Arn. Arb. 35: 196. 1954. TYPE: CHINA. Hubei, Yichang, Ichang and Immediate Neighborhood, Oct 1887, A. Henry 3431 (lectotype designated here, K000644596, digital image!; isolectotype, K000644594, K000644595, E00386198, E00386199, P00744856).
2. *Paratinospora dentata* (Diels) Wei Wang, comb. nov. *Tinospora dentata* Diels in Engler Pflanzenreich 46 (IV. 94): 139. 1910. TYPE: CHINA. Taiwan, Bankinsing, 13 March (no year), A. Henry 152 (holotype, K000644592; isotype, B100294288, K000644591, NY00320672).

4.2.2. The resurrection of Hyalosepalum

In 1867, Miers established the genus *Desmonema* to include the sole species *D. caffra* endemic to Africa. Later, Diels (1910) expanded the genus and included seven species, all of which are distributed in Africa. Assuming whether filaments are free or not could not be used to separate the genera, Troupin (1962) reduced *Desmonema* to *Tinospora*, and divided his broadly conceived *Tinospora* into two subgenera: *Tinospora* (including Asian and Australian species) and *Africana* (including African species). Hoot et al. (2009) included *T. caffra* (=*D. caffra*) in their phylogenetic analyses and found that this species was not allied with other sampled *Tinospora*, a finding corroborated by later studies (Ahmad et al., 2009; Jacques et al., 2011; Wefferling et al., 2013; Ortiz et al., 2016). Our analyses also found the same result, and further supported *T. caffra* as sister to *Leptoterantha* (MP-BS 71, ML-BS 93, PP 0.97), in agreement with the result of Ortiz et al. (2016). *Tinospora caffra* is distinguished from *Leptoterantha* by its three stamens (*vs.* 6), anthers with a longitudinal dehiscence (*vs.* transverse), and triporate pollen (*vs.* cryptoporate) (Diels, 1910; Thanikaimoni, 1984; Kessler, 1993).

A close relationship between *T. caffra* and *Tinospora* 1 was strongly rejected by the alternative hypothesis tests (Table 2). Our phylogenetic analyses do not support a close relationship of African *T. bakis* and *T. caffra* (Figs. 2 and 3). *Tinospora caffra* is inferred to have originated in late Early Miocene, whereas *Tinospora* 1 is inferred to have diverged earlier, in the Early Oligocene (Fig. 4). Besides having different stamen features (filaments connate *vs*. free), *T. caffra* also differs from the species of *Tinospora* 1 in having endocarps with one abaxial ridge, one adaxial ridge, and one lateral ridge on each side (*vs.* only one abaxial; endocarps

of African *T. oblongifolia*, a species not sampled here, have three to four slightly developed lateral ridges on each side) (Jacques, 2009), and triporate pollen (*vs.* tricolporate) (Thanikaimoni, 1984). The herbaceous habit distinguishes *Tinospora caffra* from all other African species of *Tinospora* which are woody. *Tinospora caffra*, as well as African *T. oblongifolia* and *T. penninervifolia*, has three stamens, whereas other four African species of *Tinospora* have six stamens (Troupin, 1962). Because the generic name *Desmonema* was already used in Euphorbiaceae by Rafinesque (1833), Troupin (1949) proposed *Hyalosepalum* as a new generic name for the African species. Our data support *Hyalosepalum* as a segregate genus, but its circumscription needs to be further examined by sampling more African *Tinospora*.

4.3. Southern Taiwanese and mainland Chinese disjunction

Our phylogenetic analyses indicate that *T. dentata*, endemic to the Hengchun Peninsula, is sister to *T. sagittata* (Figs. 2 and 3), which is mainly distributed in the tropical and subtropical forests of southern and southwestern China and northern Vietnam (Fig. 1). The distribution patterns of the two species thus represent a disjunction between southern Taiwan and mainland China (Fig. 1). Based on our time estimates, Taiwanese *T. dentata* dates back to the Late Eocene, *ca.* 39 Ma (node 4 in Fig. 4), which greatly predated the formation of Taiwan, beginning in the Late Miocene (Sibuet and Hsu, 2004). A similar disjunction predating the formation of Taiwan is shown by the Taiwanese *Hynobius* (Hynobiidae), which split from its Japanese relatives in the Early Oligocene, 32.79 Ma (95% HPD: 25.94–40.04) (Li et al., 2011).

Taiwan is located at the eastern edge of the Eurasian plate and the Taiwan Strait could first have occurred in the Late Mesozoic (Ye, 1982; Suo et al., 2015). Owing to the intense tectonic movements, contacts between Taiwan and mainland China occurred during the Late Cretaceous-Early Paleocene and Late Eocene-Early Oligocene (Teng, 1992; Zhang, 1995), and thereby may have facilitated the floristic exchanges between the two regions. It has been suggested that tropical rainforests in Indo-Malayan region may have appeared near the K-Pg boundary (Wang et al., 2012). Menispermaceae, as an ancient lineage with members in tropical rainforests, could have dispersed to Taiwan during that period. Macrofossils of some plants, closely related to extant subtropical species, have been reported from the Early Miocene of Taiwan, whereas pollen fossils can date to the Oligocene (Li, 2000), although their geographic sources remains disputed (Huang, 2011). Owing to the landmass subjected to stretching and rifting (Ye, 1982; Teng, 1992; Suo et al., 2015) and an increasingly cooler climate in the Late Eocene in Asia (Zachos et al., 2001), a vicariance event may have occurred between Taiwan and mainland China around that time. The part of the mountain ranges in Taiwan is a relict area of the "Taiwan-Sinzi Folded Zone" (Ye, 1982; Teng, 1992), and the Hengchun Peninsula is part of the Central Range (Teng, 1992). Thus, our data from *Tinospora* suggest that the flora of the Hengchun Peninsula might contain some relatively ancient components.

It has been acknowledged that the flora of mainland China and the Philippines have contributed to the floristic assembly of the Hengchun Peninsula (Li and Keng, 1950; Hsieh, 2002). However, it is only in the Late Miocene (9–6 Ma) that the Luzon arc began to collide with the Eurasian plate (Sibuet et al., 2002). Thus, we hypothesize that the relatively young components of the flora of the Hengchun Peninsula might have originated from mainland China and the Philippines, whereas more ancient components were from mainland China alone. This hypothesis remains to be further tested by studying additional plant groups, especially ancient lineages, through integration of phylogenetic and molecular dating methods.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.12.038.

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