

Molecular phylogenetic analyses of Euphorbiaceae tribe Epiprineseae, with the description of a new genus, *Tsaiodendron* gen. nov., from south-western China

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A phylogenetic analysis based on 18S nuclear ribosomal DNA and four plastid markers (*atpB*, *matK*, *rbcL* and *trnL-F*) strongly supports the position of a new genus in Euphorbiaceae tribe Epiprineseae (subfamily Acalyphoideae). *Tsaiodendron dioicum* gen. nov., sp. nov. is described and illustrated here as a new genus and species from Yunnan province, south-western China. The phylogenetic position of the new taxon is also supported by morphological and palynological evidence. *Tsaiodendron* is a distinct lineage sister to *Cladogynos* in tribe Epiprineseae and is easily distinguished from related members of the tribe by the sexual system, inflorescence arrangement and presence of disc glands in the pistillate flower. Molecular divergence dating analysis based on *matK*, *rbcL* and *trnL-F* sequences suggests that *Tsaiodendron* originated in the late Miocene 10.42 Mya (95% HPD: 5.23–18.88 Mya). The origin of *Tsaiodendron* may be correlated with the Red River incision from south-western China. Tribe Epiprineseae are shown to be monophyletic and phylogenetic relationships and evolutionary trends in the sexual system are also discussed based on our phylogenetic results.

ADDITIONAL KEYWORDS: dioecy – phylogenetics – Yunnan – Yuanjiang dry-hot valley – *Tsaiodendron dioicum*.

INTRODUCTION

The Yuanjiang dry-hot valley lies in southern central Yunnan province, south-western China, with an elevation range of 327–2580 m. The valley is located on the south-eastern edge of the Hengduan Mountains and the southern centre of the Yunnan Plateau; the Red River (called the Yuanjiang River in China) runs within it. The rain shadow caused by (1) the Ailao-Wuliang Mountains (elevation > 3000 m) blocking south-western warm moist air from the Indian Ocean on the south-western side and (2) the Yunnan-Guizhou Plateau blocking south-eastern warm moist air from the Pacific Ocean on the north-eastern side gives the valley a distinctive hot and dry climate that contrasts with most of the

surrounding regions (Jin & Ou, 2000; Li *et al.*, 2016). The mean annual evaporation capacity (2700–3800 mm) is three to six times higher than the precipitation (600–800 mm), with 80–90% of the precipitation concentrated in the wet season (Jin, 2002). The vegetation in the valley is savanna-like (Jin, 1999), in contrast to the closed evergreen forest that covers most of the surrounding region. This type of savanna-like vegetation is also narrowly distributed in the dry-hot or dry-warm valleys of the Jinsha River, Mekong (Lancang) River and Salween (Nu) River basins in south-western China, but it is best preserved in the Yuanjiang region (Jin, 2002).

The dominant species from the Yuanjiang dry-hot valley are *Euphorbia neriiifolia* L., *Lannea coromandelica* (Houtt.) Merr., *Buchanania latifolia* Roxb., *Searsia paniculata* (Wall. ex G. Don) Moffett, *Woodfordia fruticosa* Kurz. and *Heteropogon contortus* Beauv.

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ex Roem. & Schult. (Zhang, Hao & Cao, 2009). About 835 genera and 2080 species of seed plants have been recognized from this distinctive vegetation, among which most genera are tropical and about ten species are endemic to this region, including *Clematis wissmanniana* Hand.-Mazz., *Capparis wui* B.S. Sun and *Zanthoxylum yuanjiangensis* C.C. Huang (Jin, 2002).

Floristically, the Yuanjiang dry-hot valley is considered to have a transitional flora from tropical to subtropical, but with evident characteristics of a tropical flora. The flora is also rich in endemism (three families endemic to East Asia, five genera and 647 species endemic to China) compared with the valleys in the other three river basins (Li, Du & Wang, 2008).

During our recent floristic explorations of the Yuanjiang valley, we collected an unusual species. The plant was easily identified as a member of Euphorbiaceae based on the three-lobed capsule and unisexual flowers. Euphorbiaceae are one of the larger families of angiosperms, with 218 genera and 6462 species currently recognized (WCSP, 2016). The family is most diverse in subtropical and tropical regions, although it has a cosmopolitan distribution (Webster, 2014). After the removal of several previously included families, such as the biovulate Phyllanthaceae, Picrodendraceae (Savolainen *et al.*, 2000b; Chase *et al.*, 2002; APG II, 2003) and Peraceae (Wurdack & Davis, 2009; APG IV, 2016), based on molecular phylogenetic studies, Euphorbiaceae *s.s.* (*sensu* APG IV, 2016) are monophyletic and entirely uniovulate and comprise four subfamilies: Cheilosoideae, Acalyphoideae, Crotonoideae and Euphorbioideae (Wurdack, Hoffmann & Chase, 2005; Tokuoka, 2007; Webster, 2014). The representation of Euphorbiaceae in China is modest, with 255 species, 55 of which are endemics (Li *et al.*, 2008). This may in part be due to the limited tropical regions in the country. The unusual plant we collected was originally identified as belonging to subfamily Acalyphoideae, based on the lack of milky or coloured latex, the presence of which defines Crotonoideae and Euphorbioideae, and the non-echinate pollen exine, which if present in combination with lack of latex defines Cheilosoideae. However, after a closer examination, the set of morphological characters, including the strictly dioecious sexual system, stellate indumentum and almost single staminate or pistillate flowers developed in the reduced inflorescence, did not match any genus of subfamily Acalyphoideae; this showed that it probably represented a new taxon. *Trigonostemon tuberculatus* F. Du & Ju He, a peculiar species of Euphorbiaceae, is also endemic to Yuanjiang dry-hot valley and was published recently (Du *et al.*, 2010). These two new taxa of Euphorbiaceae have sympatric distributions on open dry slopes with sparse secondary shrubs at elevations of 350–500 m, indicating that the particular dry-hot habitat in the Yuanjiang valley might be responsible for the origin of some endemic

plant taxa. Our newly discovered taxon had a slightly narrower distribution (elevation 400–500 m, not extending to the riverside) than *T. tuberculatus* (350–460 m, closer to the riverside).

To assess the phylogenetic position of this unusual plant, DNA sequence data were generated from 18S nuclear ribosomal DNA and four plastid markers (*atpB*, *matK*, *rbcL* genes and the *trnL-F* region) as used in previous molecular phylogenetic studies at the family level (Wurdack *et al.*, 2005; Tokuoka, 2007). In addition, we assessed the morphological characters of this new taxon, including the features of the indumentum, the sexual system and palynology. Based on phylogenetic and morphological analyses, we describe this plant as representing a new species *Tsaiodendron dioicum* in a monotypic new genus *Tsaiodendron*, nested in tribe Epiprineae of subfamily Acalyphoideae.

Epiprineae comprise a small tribe with six to ten Palaeotropical genera mainly distributed in Asia, the only exceptions being *Cephalocroton* Hochst. in tropical Africa and Madagascar to Socotra (Yemen) and *Rockinghamia* Airy Shaw in tropical Australia (Webster, 2014). The framework of the current circumscription of the tribe was initially proposed by Webster (1994) and accepted by Radcliffe-Smith (2001). Nine genera and two subtribes were recognized, with *Cephalomappa* Baill. forming a subtribe itself (Cephalomappinae) and the other eight genera forming another subtribe (Epiprininae). Webster (2014) slightly revised the composition of the tribe, with a broad concept of *Cephalocroton* (including *Adenochlaena* Boiss. ex Baill. and *Cephalocrotonopsis* Pax) and the tentative inclusion of *Rockinghamia*. The monophyly of Epiprineae was partly tested by previous molecular phylogenetic work (Wurdack *et al.*, 2005; Cervantes *et al.*, 2016), with *Adenochlaena*, *Cephalocroton*, *Cephalomappa* and *Koilocroton* Hassk. being sampled. The tribe appears to be monophyletic, with *Cephalocroton s.l.* (including *Adenochlaena*) being a sister to the clade comprising *Cephalomappa* and *Koilocroton*. However, previous sampling was incomplete. Several genera of Epiprininae are still lacking exemplars in the backbone phylogenetic tree for Euphorbiaceae (van Welzen, 2010) and the inclusion of *Rockinghamia* in the tribe is still unclear. In addition, the circumscription and monophyly of some constituent genera of the tribe has also not been adequately tested, such as for *Cephalocroton s.l.*, for which Radcliffe-Smith (2001) recognized the segregation of *Adenochlaena* and *Cephalocrotonopsis*, but Webster (2014) did not, and *Epiprinus* Griff. *s.l.*, for which Radcliffe-Smith (2001) and Webster (2014) both recognized the segregation of *Symphyllia* Baill., but Li *et al.* (2008) did not in the Flora of China. More molecular phylogenetic work focusing on Epiprineae is therefore needed.

We present the results of a molecular phylogenetic study focusing on Epiprineae. Based on this analysis,

we discuss the evolution and phylogenetic relationships in the tribe to which the new taxon belongs. We also carry out a molecular divergence dating analysis to estimate the timing of the split of this new taxon from its close relatives.

MATERIAL AND METHODS

PLANT MATERIAL

Specimens and silica-dried leaf material of *Tsaiodendron dioicum* were collected from the only known population (Fig. 1). The preliminary results, based only on *rbcL* and *trnL-F* sequences in accordance with the family framework of Wurdack *et al.* (2005), showed that the new taxon was nested in Epiprineae and sister to *Cephalocroton mollis* Klotzsch (data not shown). To improve taxon sampling, we sampled six additional accessions of this tribe in addition to *Tsaiodendron*, including one accession of *Cladogynos orientalis* Zipp., one of *Cleidiocarpon cavalieriei* (H.Lév.) Airy Shaw, one of *Cephalomappa sinensis* (Chun & F.C.How) Kosterm., one of *Koilodepas hainanense* (Merr.) Croizat and two of *Epiprinus siletianus* (Baill.) Croizat (= *Symphyllia siletiana* Baill.). The genera *Cladogynos* Zipp. ex Span., *Cleidiocarpon* Airy Shaw and *Epiprinus* are first sampled here.

Voucher information and GenBank accession numbers for sequences included in this study are listed in Supporting Information, Appendix S1.

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Total genomic DNA was extracted from silica-gel-dried leaf material, using Tiangen DNA extraction kits (Tiangen Biotechnology Company, Beijing, China) following the manufacturer's protocol. To infer the phylogenetic position of *Tsaiodendron* in the Acalyphoideae, we conducted amplifications of 18S rDNA and four plastid markers (*atpB*, *matK* and *rbcL* genes and the *trnL-F* region) based on previous family-level phylogenetic work (Wurdack *et al.*, 2005; Tokuoka, 2007). The *matK* gene was amplified and sequenced using primers *matK-5F* and *C12MR* (Haberle *et al.*, 2009), and the other four markers were amplified and sequenced in two or three segments with the following primer pairs: *25e/SR3* and *SF3/1769R* for 18S rDNA (Nickrent & Starr, 1994; Tokuoka & Tobe, 2006); *2F/766R*, *AF3/AR3* and *1022F/1494R* for *atpB* (Savolainen *et al.*, 2000a; Tokuoka & Tobe, 2006); *1F/724R* and *636F/1368R* for *rbcL* (Lledó *et al.*, 1998); and *c/d* and *e/f* for *trnL-F* (Taberlet *et al.*, 1991).

All PCRs were conducted in a 25- μ L volume consisting of *c.* 10–20 ng sample DNA, 1 \times buffer, 2 mM

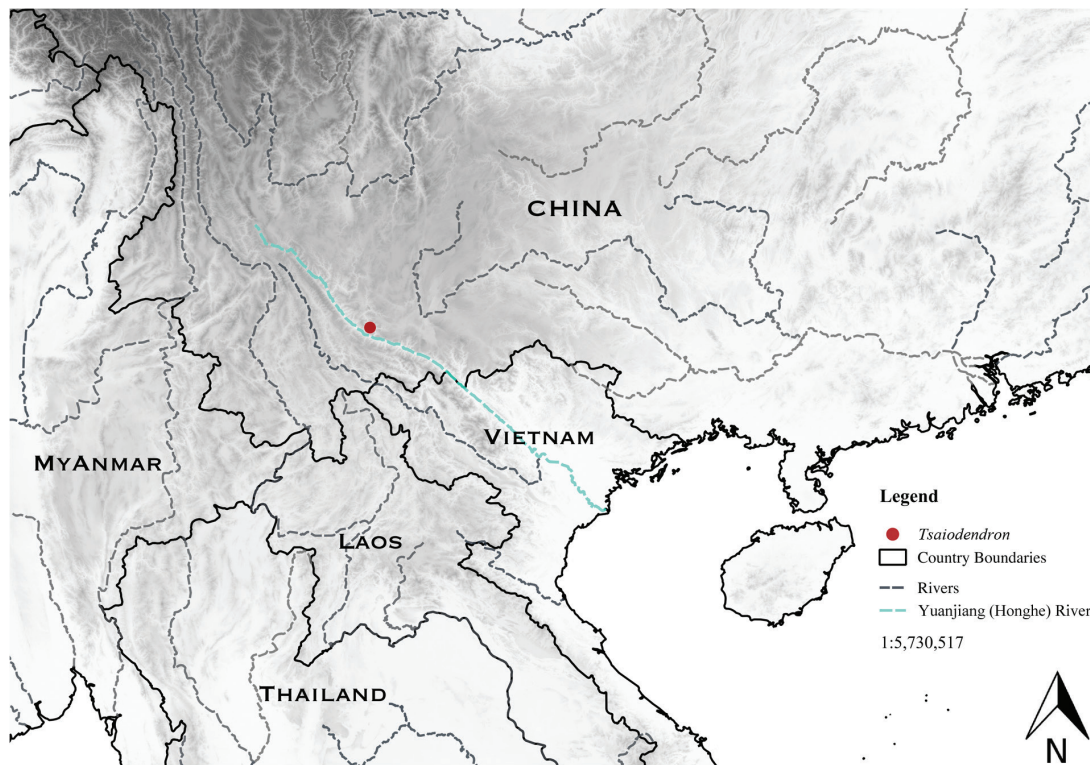


Figure 1. Geographical distribution of *Tsaiodendron dioicum* gen. nov., sp. nov.

MgCl₂, 200 µM dNTPs, 0.6 µM each primer and 1.5 U of Taq polymerase (BioLine, Randolph, MA, USA). The PCR amplification profiles for 18S rDNA, *rbcL* and *trnL-F* consisted of 3 min at 95 °C, followed by 37 cycles of 20 s at 94 °C, 30 s at 50 °C and 40 s at 72 °C, and then a final 5 min extension at 72 °C. The PCR protocol used for *atpB* and *matK* followed Tokuoka & Tobe (2006).

The PCR products were purified using the polyethylene glycol (PEG) precipitation procedure (Hiraishi, Kamagata & Nakamura, 1995). The cycle sequencing reactions were conducted in 10-µL volumes which contained 0.25 µL BigDye 3.1, 0.5 µL primers, 2.0 µL purified PCR products and 1.75 µL sequencing buffer. The sequencing reactions were run on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA).

PHYLOGENETIC ANALYSES

Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA) was used to evaluate chromatograms for base confirmation and to edit contiguous sequences. Sequences were initially aligned with MUSCLE 3.8.31 (Edgar, 2004), followed by manual adjustments with Se-Al v2.0a11 by similarity (Rambaut, 2007). Manual correction was straightforward in the case of the less variable nuclear ribosomal 18S and coding *atpB*, *matK* and *rbcL*, but *trnL-F* is indel-rich across the family and created many ambiguously aligned regions. We did not exclude any ambiguous regions in the *trnL-F* alignment. Multiple sequence alignments, data matrices and a strict consensus tree are available in TreeBASE (<http://purl.org/phylo/treebase/phyloWS/study/TB2:S20489>). Phylogenetic analyses were conducted on each DNA region and the combined data sets. The program jModelTest 2.0 (Guindon & Gascuel, 2003; Durrin *et al.*, 2012) was run to select the appropriate model of nucleotide evolution based on the program PhyML, using the Akaike information criterion (AIC) (Akaike, 1974), and the results were the basis of the Bayesian analysis.

We inferred relationships from the nucleotide data using maximum parsimony (MP) and Bayesian inference (BI). Fifty-two accessions from all major clades of Acalyphoideae were selected for the phylogenetic analysis. MP analyses were performed in PAUP* 4.0b10 (Swofford, 2002), with heuristic searches of 1000 replicates with random stepwise addition using tree bisection-reconnection (TBR) branch swapping, MulTrees and Collapse option selected. All character states were treated as unordered and equally weighted, with gaps as missing data. To evaluate the relative robustness of clades in the MP trees, bootstrap analysis (Felsenstein, 1985) was performed with 1000 replicates using the

same options as above. Those clades that received > 50% bootstrap support (BP) were thought to be supported by a typical convention. Bootstrap values of 95% or greater was considered statistically significant and indicated a well-supported clade, whereas 70–95% corresponded to a moderately supported 'real' clade (Soltis & Soltis, 2003).

BI was performed in the program MrBayes 3.1 (Ronquist & Huelsenbeck, 2003), based on the appropriate nucleotide substitution model as determined by jModelTest. The Markov chain Monte Carlo (MCMC) algorithm was run for 2000 000 generations with one cold and three heated chains, starting from random trees. Trees were sampled every 100 generations. The convergence among chains was assessed in TRACER 1.5 (effective sample size > 200) (Drummond & Rambaut, 2007). We also used the online tool AWTY (Nylander *et al.*, 2008) to check for stationarity. The first 10% of runs were discarded as burn-in. A majority rule consensus of the remaining trees from the four runs was produced in PAUP* 4.0 and used as the Bayesian inference tree with posterior probabilities (PPs).

MOLECULAR DIVERGENCE DATING

To estimate the divergence time of *Tsaiodendron*, we employed data sets of three plastid markers (*matK*, *rbcL*, *trnL-trnF*) for the dating analysis, which was to adjust to the markers used by previous phylogenetic studies at the family and subfamily (Acalyphoideae) levels, as implemented by Wurdack *et al.* (2005) and Cervantes *et al.* (2016), respectively. We did not include the other two markers (18S, *atpB*) to minimize missing data, as the family framework of Tokuoka (2007) with four markers (18S, *atpB*, *matK*, *rbcL*) did not cover taxa so comprehensively.

BEAST v.1.8.3 was used to estimate the divergence times (Drummond & Rambaut, 2007; <http://beast.bio.ed.ac.uk>). We applied a general time reversible (GTR) nucleotide-substitution model with gamma + invariant sites, gamma shape distribution (with four categories) and proportion of invariant sites. A lognormal relaxed molecular clock model and a Yule pure birth model of speciation prior set were used to estimate the divergent time and their credibility intervals. Posterior distributions of parameters were based on MCMC analysis of 40 000 000 generations with 10% burn-in. The log file was then checked for convergence of the chains in Tracer 1.3. The samples from post-burn-in posterior distributions were summarized on the maximum clade credibility (MCC) tree, which has the maximum sum of posterior probabilities on its internal nodes using TreeAnnotator v.1.5.4 (Drummond & Rambaut, 2007) with the posterior probability limit set to 0.5 summarizing mean node heights. The MCC tree was visualized

using FigTree v.1.3.1 and the means and 95% higher posterior densities (HPDs) could be obtained from it.

Our molecular divergence dating was implemented in the framework of Acalyphoideae, with 66 taxa selected, which covered the major clades in the subfamily. Dating analysis was based on two fossil constraints (1 and 2, below) and one secondary calibration (3, below). (1) [Cervantes *et al.* \(2016, appendix S2\)](#) summarized the reliability of fossil selection in the dating analyses of Euphorbiaceae. According to the review, the most reliable pollen fossil in Acalyphoideae is *Acalyphapollenites acalyphoides* M.R.Sun from the early Palaeocene of Jiangsu, China ([Sun *et al.*, 1989](#)), which could be undoubtedly associated with the extant members of *Acalypha* L. [Van Ee *et al.* \(2008\)](#) and [Cervantes *et al.* \(2016\)](#) both used this record as the minimum age constraint for the stem node of *Acalypha*. We followed this method in the present study. A lognormal prior of 60 was therefore taken as zero offset; the default lognormal mean of 1 and default lognormal standard deviation of 1 were used to constrain the node containing the *Acalypha* spp. and their sister *Mareya micrantha* Müll. Arg., giving a 95% confidence interval of 60.4–79.3 Mya. (2) Among the macrofossils in the subfamily, fossil leaves, flowers, fruits and pollen from the early Miocene (c. 23 Ma) of Otago, southern New Zealand ([Lee *et al.*, 2010](#)), were selected in the current study. These fossils were assigned to the *Mallotus* Lour. – *Macaranga* Thouars. clade. [Nucete, van Konijnenburg-van Cittert & van Welzen \(2012\)](#) linked them to an extant Australian species, *Mallotus nesophilus* Müll. Arg., and qualified the fossils as reliable. [Van Welzen *et al.* \(2014\)](#) used these fossils to constrain the crown age of the node containing *M. nesophilus*, but [Cervantes *et al.* \(2016\)](#) used the same records as the minimum age constraint for the stem node of the *Mallotus*–*Macaranga* clade. We followed the approach used by [Cervantes *et al.* \(2016\)](#). A lognormal prior of 23 was taken as zero offset with the default lognormal mean and lognormal standard deviation of 1, used to constrain the node containing the *Mallotus*–*Macaranga* clade and their closest relative *Hancea* Seem., giving a 95% confidence interval of 23.4–42.3 Mya for the node. (3) The root age of Acalyphoideae was estimated to be 92.5 Mya (95% HPD: 84.2–101.6 Mya) in [Cervantes *et al.* \(2016\)](#). We used this age to calibrate the root age. A normal distribution was taken with a mean at 92.5 Mya, and a standard deviation of 4.5, giving a 95% confidence interval of 83.7–101.3 Mya as our prior for the root node.

INDUMENTUM AND PALYNOLOGY

Leaf indumentum and pollen grains were observed in *T. dioicum* by using scanning electron microscopy

(SEM). For indumentum observations, mature leaves were chosen and cut into pieces (0.3 × 0.4 cm) from the middle–upper part. Leaf pieces were mounted on double-sided sticky conducting tape, fixed on labelled stubs and sputter-coated with gold palladium. Trichomes on both abaxial and adaxial surfaces were observed. For palynological observations, anthers were removed from the specimen of *T. dioicum* we collected in the field. To prepare specimens for SEM examination, anthers were mounted on specimen stubs with double-sided sticky tape and then carefully dissected to expose the pollen. The stubs were then sputter-coated with gold. Indumentum and pollen morphology were examined and photographed using a Zeiss (Oberkochen, Germany) EVO LS10 scanning electron microscope at 10 kV (XTBG).

The terminology of indumentum morphology follows [Webster, Del-Arco-Aguilar & Smith's \(1996\)](#) trichome classification system for *Croton* L. The terminology of palynology follows that of [Punt *et al.* \(2007\)](#). The measurements given in the descriptions were made via SEM, and represent the mean and the range of dimensions of 15 pollen grains.

RESULTS

INDUMENTUM MORPHOLOGY AND PALYNOLOGY

Morphological comparisons of *Tsaiodendron* and related genera in Epiprineae are listed in [Table 1](#). The indumentum of *T. dioicum* is the stellate type ([Fig. 2A–D](#)). [Webster *et al.* \(1996\)](#) defined some subclasses in the stellate type in studying the large genus *Croton*. According to their classification system for trichomes, the indumenta on the adaxial leaf surface of *Tsaiodendron* are simple to stipitate-stellate with three to six radii, sparsely distributed ([Fig. 2A, B](#)); on the abaxial surface, the stellate trichomes belong to the multiradiate type, with eight to 14 radii, are stipitate and densely distributed ([Fig. 2C, D](#)). Trichomes on both surfaces are spreading. The stomata of *T. dioicum* occur only on the abaxial surface and belong to the paracytic type ([Fig. 2E, F](#)), in which the stoma is accompanied on either side by one or more subsidiary cells parallel to the long axis of the guard cells ([Evert, 2006](#)). The stomata are sunken, which has generally been linked to arid habitats as an adaptation to preserve water ([Jordan *et al.*, 2008](#)) and these are consistent with the habitat of the Yuanjiang dry-hot valley where *Tsaiodendron* occurs. The guard cells of stomata are oval. The pollen grains of *T. dioicum* are oblate to prolate spheroidal in equatorial view. Pollen characteristics were as follows: size, P (polar) × E (equatorial) = (26.9–41.7) × (22.2–32.8) µm, P/E = 1.23;

Table 1. Morphological comparison of *Tsaiodendron dioicum* gen. nov., sp. nov. with other genera in Epiprineeae according to Radcliffe-Smith (2001) and Webster (2014)

	<i>Tsaiodendron</i>	<i>Cladogynos</i>	<i>Cleidiocarpon</i>	<i>Epiprinus</i> s.l.	<i>Cephalocroton</i> s.l.	<i>Cephalomappa</i>	<i>Koilolepas</i>
Life form	Deciduous shrub	Evergreen shrub	Evergreen tree	Evergreen tree or shrub	Semi-deciduous shrub	Evergreen tree or shrub	Evergreen tree
Leaf vein	Triplinerved	Palmate or triplinerved	Pinnate	Pinnate	Palmate or triplinerved	Pinnate	Pinnate
Leaf margin	Crenate-dentate	Double-dentate	Entire	Subentire	Crenate-dentate	Entire or dentate	Entire or crenate-serrulate
Sexual system	Dioecy	Monoecy	Monoecy	Monoecy	Monoecy	Monoecy	Monoecy
Inflorescences	Unisexual, male and female on different individuals	Bisexual, axillary	Bisexual or unisexual, terminal	Bisexual, terminal	Bisexual, terminal or axillary	Bisexual, axillary	Bisexual, axillary
Staminate flowers	Only one developed in a reduced inflorescence, terminal on short shoot, calyx 2- to 4-lobed	In a capitulum on the terminal of inflorescence, calyx 2- to 4-lobed	In glomerules widely spaced along the axis, calyx 4- or 5-lobed	In glomerules widely spaced along the axis, calyx 3- or 4-lobed	In a capitulum on the terminal of inflorescence, calyx 3- to 6-lobed	In glomerules on the terminal of inflorescence, calyx connate at base	In glomerules sparsely spaced along the axis, calyx 3- or 4-lobed
Stamen numbers	(5)6–9	(3)4(5)	4–5	4–15	(4)6–8(10)	(2)3–4	4–5(8)
Pistillate flowers	Only one developed in a reduced inflorescence, terminal on short shoot, pedicellate	One proximal female flower on inflorescence, pedicellate	Female flowers 1–6 along lower part of inflorescence, subsessile	Several female flowers at the base of inflorescence, subsessile to long pedicellate	Several proximal female flowers, separated by a long internode with male flowers, pedicellate	Female flowers 1–3 at proximal axils, pedicellate	Few female flowers at the base of inflorescence, sessile
Pistillate disc	Disc glands present, sepal-like and alternise-palous	Disc glands present, alternise-palous	Absent	Absent	Annular disc in some species	Absent	Absent
Fruit	3-lobed capsular, pistillate flower sepals persistent	3-lobed capsular, pistillate flower sepals persistent	1- or 2-lobed drupe, beaked	3-lobed capsular, pistillate flower sepals persistent	3-lobed capsular, pistillate flower sepals persistent	3-lobed capsular, pistillate flower sepals deciduous	3-lobed capsular, pistillate flower sepals persistent
Distribution	Endemic in tropical China	Widespread in Southeast Asia	Northern Burma, China, western Thailand and Vietnam	India to Malaya, widespread in Southeast Asia	Tropical Africa, Madagascar, Socotra Island and Sri Lanka	Southern China, Malay Peninsula, Sumatra and Borneo	India to New Guinea, widespread in Southeast Asia

tricolporate, the colpi 22.1–30.9 µm long, inoperculate and scarcely marginate; sexine tectate-perforate, tectum almost complete, shallowly punctate; smooth in the junction between muri (Fig. 2G–I).

PHYLOGENETIC ANALYSES AND MOLECULAR DATING

Thirty-five sequences from seven samples were newly generated in the present study. In some cases the

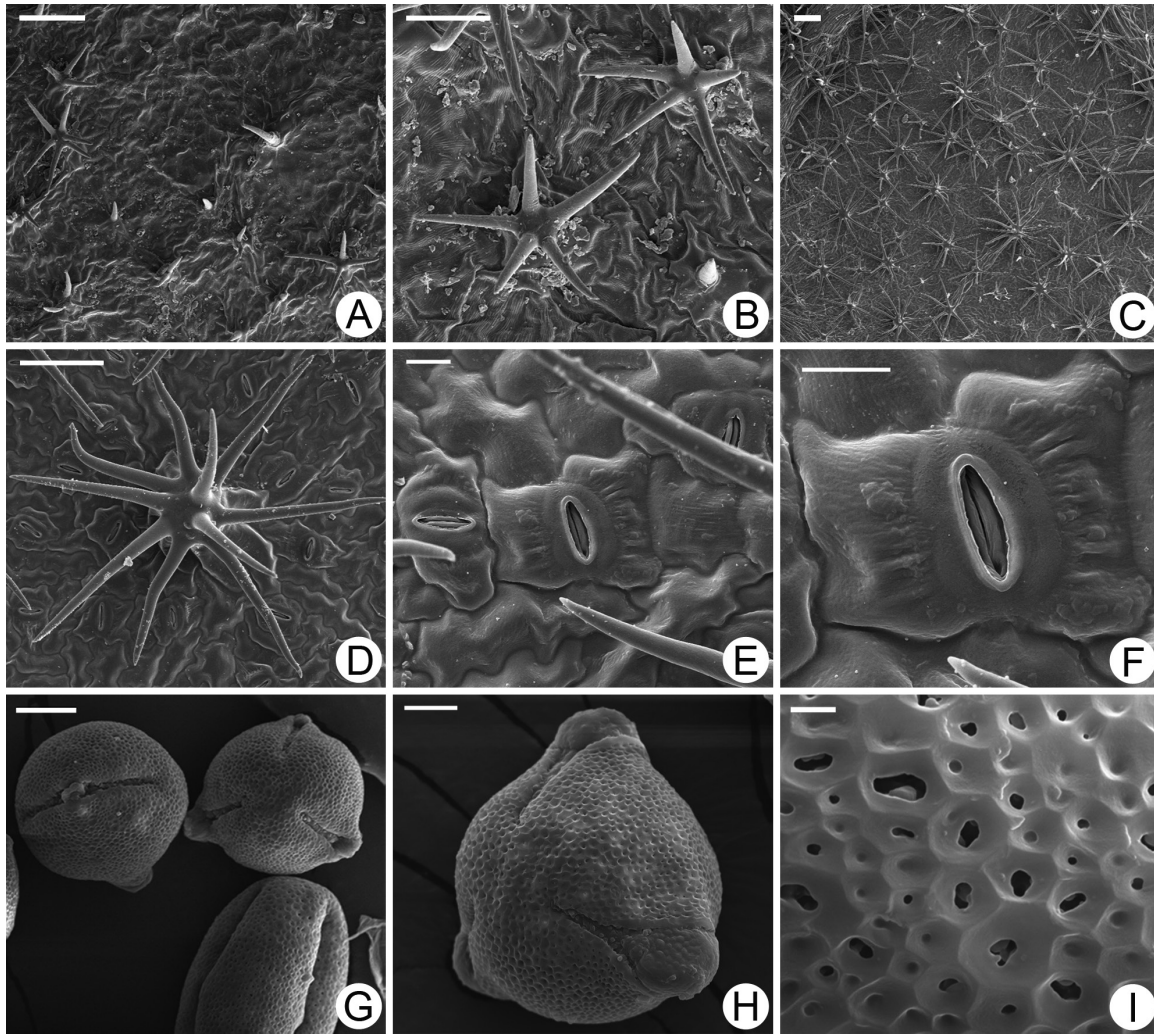


Figure 2. Micromorphological characters of *Tsaiodendron dioicum* gen. nov., sp. nov. (A–B) adaxial surface of leaves under SEM, displaying the simple to stipitate-stellate indumentum; (C–D) abaxial surface of leaves under SEM, displaying the densely distributed multiradiate stellate indumentum; (E–F) stomata from abaxial surface of leaves under SEM; (G–I) external pollen morphology and ultrastructure of pollen surface under SEM (scale bars = 100 μ m in (A and C), 50 μ m in (B and D), 10 μ m in (E, F and G), 5 μ m in (H) and 1 μ m in (I)). Voucher *Bo-Jian Gu 0001* (HITBC).

trnL-F and the other four markers were from two different species in the same genus, combined as one genus-level accession. For example, we combined the *trnL-F* sequence of *Lasiocroton microphyllus* (A.Rich.) Jestrow and the other four markers of *L. macrophyllus* Griseb. into a single accession, representative of the genus *Lasiocroton* Griseb. (Fig. 3). We only did this for taxa presumed monophyletic in previous studies (Jestrow, Rodríguez & Francisco-Ortega, 2010; Cardinal-McTeague & Gillespie, 2016; Cervantes *et al.*, 2016) with systematic positions distant from our focal tribe, Epiprineseae, to minimize the influence of missing data.

The statistics for the aligned matrix of the individual and combined data sets are presented in Table 2.

The MP trees based on individual plastid regions were at different levels of resolution (not shown), but all showed that Epiprineseae (excluding *Rockinghamia*) were a monophyletic group with two subclades (BP 90, 100, 97 and 75 in *atpB*, *matK*, *rbcL* and *trnL-F*, respectively). *Tsaiodendron* was sister to *Cladogynos* in the Epiprineseae clade (BP 95, 100 and 96 in *atpB*, *matK* and *rbcL*, respectively), but collapsed in the polytomy subclade from the MP tree based on *trnL-F*. The MP tree based only on 18S was marked by a large polytomy due to the low proportion of potentially parsimony-informative sites (Table 2), but *Tsaiodendron* was also found to form a clade with *Cladogynos* and this clade was sister to *Cleidiocarpon*. Relationships among all other genera of Epiprineseae were not resolved in the MP tree

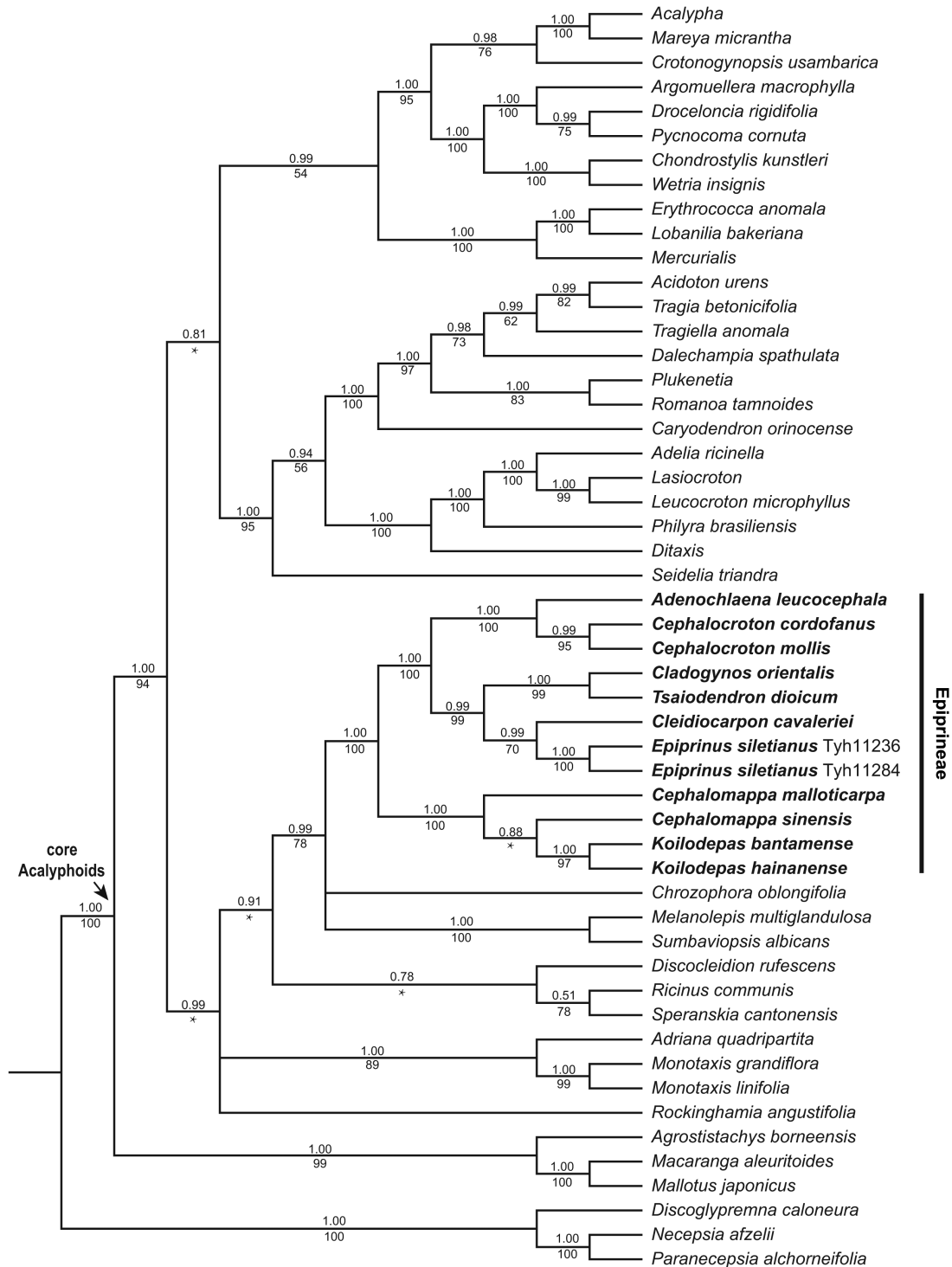


Figure 3. Bayesian majority-rule consensus tree based on combined 18S and four plastid marker datasets. Posterior probabilities are noted above branches. Bootstrap percentage values are indicated below branches, with an asterisk indicating clades collapsed in the maximum parsimony tree. Taxa in Epiprineae are in bold type.

based only on the 18S gene. Since the plastid genome is generally considered as one unit without recombination or conflict (Nie *et al.*, 2012), MP trees based only

on 18S rDNA or individual plastid regions were much less resolved and supported than the strict consensus phylogenetic tree based on the combined data set and

Table 2. Statistics for the alignments of nuclear ribosomal 18S, *atpB*, *matK*, *rbcL*, *trnL-F* and combined datasets

Parameter	18S	<i>atpB</i>	<i>matK</i>	<i>rbcL</i>	<i>trnL-F</i>	Combined	
						plastid DNA	18S+plastid DNA
Number of sequences (number of ingroup sequences)	43 (7)	43 (7)	48 (12)	50 (10)	42 (12)	52 (12)	52 (12)
Aligned length (bp)	1736	1469	1257	1323	1668	5717	7453
Variable characters (%)	130 (7.5)	296 (20.1)	553 (44.0)	274 (20.7)	502 (30.1)	1625 (28.4)	1755 (23.5)
Parimony-informative characters (%)	54 (3.1)	156 (10.6)	300 (23.9)	147 (11.1)	241 (14.4)	844 (14.8)	898 (12.0)
Variable characters within ingroup	21	41	62	54	54	211	232
Parimony-informative characters within ingroup	9	15	66	31	55	167	176

comparisons among those trees revealed no significant incongruence, we combined all five markers in our analysis.

Treating gaps as missing data, with *Discoglyprena* Prain, *Necepsia* Prain and *Paranecepsia* Radcl.-Sm. as outgroups, the MP analysis from combined datasets produced 26 maximally parsimonious trees (MPTs) of 3353 steps, with a consistency index (CI) of 0.66, CI excluding uninformative characters of 0.52 and a retention index (RI) of 0.68.

For BI analysis, AIC identified the optimal models as GTR+I+G for combined datasets. The topology of the Bayesian majority-rule tree and the MP tree showed no conflicts, but some different levels of resolution (Fig. 3, some clades collapsed in the MP tree). The topology of Epiprineae was nearly identical in both analyses, but *Cephalomappa sinensis* collapsed in the MP tree, whereas it was sister to *Koilocarpus* with BI with low support values (PP = 88, Fig. 3). The sister relationship between *Cleidiocarpon* and *Epiprinus* was strongly supported in the Bayesian half-compatible consensus tree (PP = 99), but was only weakly supported (BP = 70) in the MP tree (Fig. 3). *Cleidiocarpon*, with drupaceous fruits, collapsed in the single-partition MP tree of *atpB*, *matK* and *trnL-F*, and was sister to *Epiprinus* only in the MP tree from *rbcL* with a moderate bootstrap support value (BP = 87). We speculated that the Bayesian PPs might overestimate the node support, as shown by several empirical and theoretical studies (for a review, see Alfaro & Holder, 2006). All other critical nodes in Epiprineae were strongly supported in both analyses and our results suggested that (1) the new genus *Tsaiodendron* is sister to the tropical Asian monotypic genus *Cladogynos*; and (2) Epiprineae are monophyletic (Fig. 3).

The MCC tree derived from BEAST was identical to the phylogenetic tree from parsimony and Bayesian analyses. *Tsaiodendron* diverged from its closest relative *Cladogynos* at 10.42 Mya (95%

HPD: 5.23–18.88 Mya) and Epiprineae originated at 50.23 Mya (95% HPD: 33.73–59.10 Mya) and the two subclades began to separate at 39.86 Mya (95% HPD: 27.08–50.17 Mya) (Fig. 4).

DISCUSSION

PHYLOGENETIC POSITION AND ORIGIN OF THE NEW GENUS *TSAIODENDRON*

Tsaiodendron is sister to another East Asian endemic monotypic genus *Cladogynos* nested in the clade of tribe Epiprineae with high support values in our analysis (BP = 99, PP = 1.00; Fig. 3). The closest relatives of Epiprineae are some members of the polyphyletic tribe Chrozophoreae (Müll. Arg.) Pax & K.Hoffm. (e.g. *Chrozophora* Necker ex A.Juss., *Melanolepis* Rchb.f. & Zoll. and *Sumbaviopsis* J.J.Sm.) (Wurdack *et al.*, 2005; Tokuoka, 2007). In comparison with its closest allies, the synapomorphies of Epiprineae include monoecious trees or shrubs, indumentum stellate, petals or disc nearly lacking in both staminate and pistillate flowers, stamens 4–15 (vs. monoecious or dioecious, diverse indumentum, petals usually 4–5, no disc or disc annular or dissected, stamens five-many in Chrozophoreae) (Webster, 2014). Except for the dioecious sexual system and disc segments present in the pistillate flower, *Tsaiodendron* possesses all the other synapomorphies of Epiprineae with usually 6–9 stamens. Moreover, pollen grains of all genera previously included in the tribe Epiprineae share certain characteristics: three-colporate apertures, sexine tectum perforate, punctate to microreticulate (vs. sexine reticulate and usually distinctly heterobrochate in Chrozophoreae) (Takahashi *et al.*, 2000). All these shared characteristics were also demonstrated in our palynological analysis of *Tsaiodendron* (Fig. 2G–I). Thus, the molecular, morphological and palynological results all support the new genus as a member of the Epiprineae.

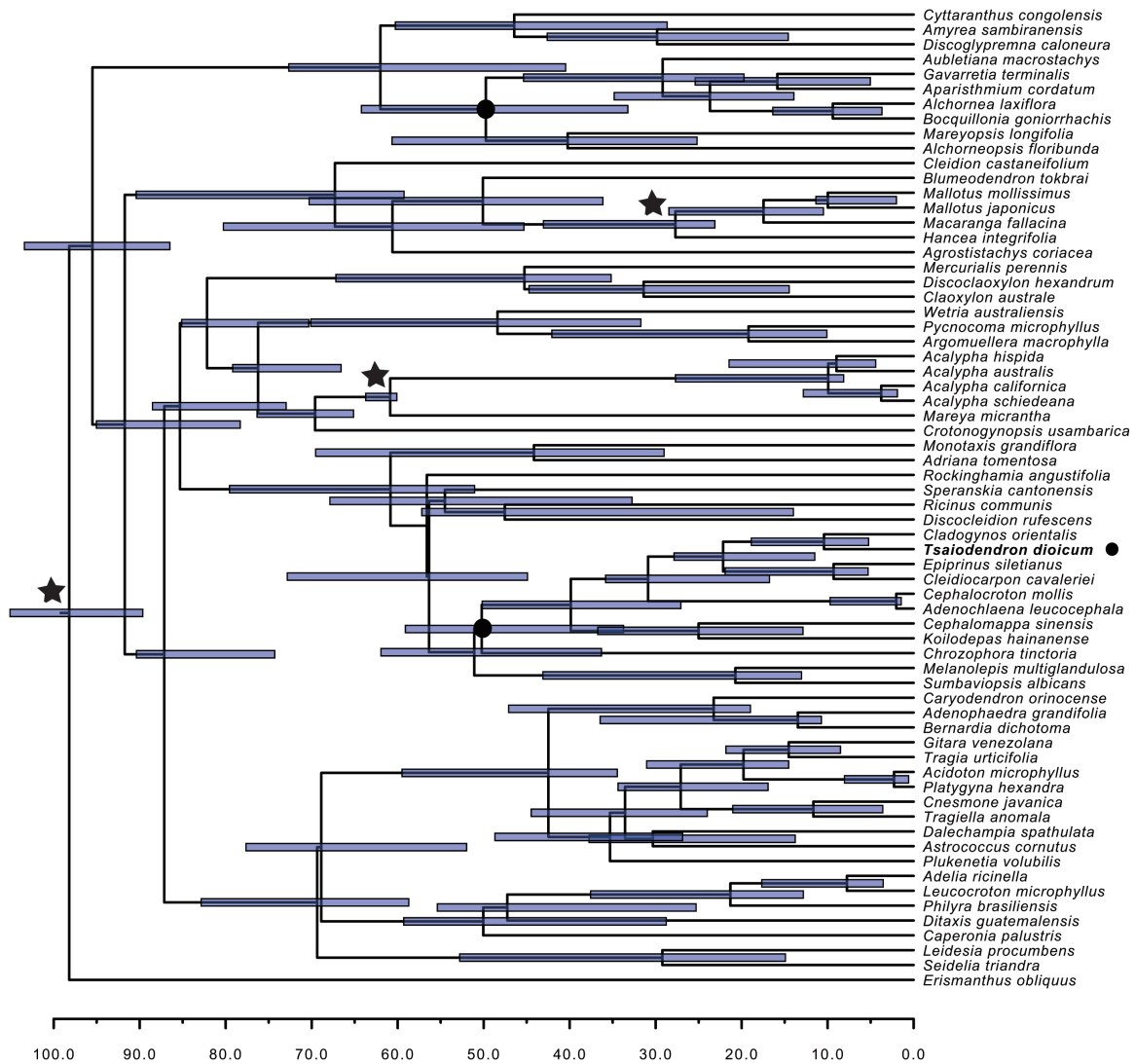


Figure 4. The maximum clade credibility (MCC) tree derived from BEAST based on three plastid markers (*matK*, *rbcL* and *trnL-F*). Grey bars represent the 95% highest posterior density intervals for node ages. The fossil calibration and the root constraint are marked by asterisks. Round symbols indicate the position of *Tsaiodendron dioicum* gen. nov., sp. nov. and the stem clade of Epiprineae and Alchorneae.

However, *Tsaiodendron* also possesses some characteristics that are unusual for the tribe: it is a drought-deciduous shrub (some *Cephalocroton* species may also be drought-deciduous, although we are not certain of this), with a strictly dioecious sexual system, only one staminate or pistillate flower developed in a reduced inflorescence that is terminal on a short shoot (Figs 5, 6), and disc segments present (f in Fig. 6; see Supporting Information Appendix S2) in the pistillate flower (vs. usually evergreen tree or shrub, monoecious, inflorescences usually bisexual and staminate flowers in capitula or other type of inflorescences, and disc nearly absent from the other members of this tribe) (Table 1; Figs 5, 6). This makes the new taxon

a morphologically distinct genus in the Epiprineae. A pistillate disc is almost absent throughout the Epiprineae, only in some species of *Cephalocroton* is there an annular and subentire disc (Radcliffe-Smith, 2001), while in *Tsaiodendron* and *Cladogynos* disc segments are present (Radcliffe-Smith, 2001; present study, Supporting Information Appendix S2). Such sepal-like and alternisepalous structures are always termed disc glands, but in general this is a vague term for structures that roughly share the same position in the flowers of Euphorbiaceae. The homology of disc glands is not clear and probably varies across the family; it might be a segmented disc, glandular in nature or a reduced petal/sepal. Disc segments present in the

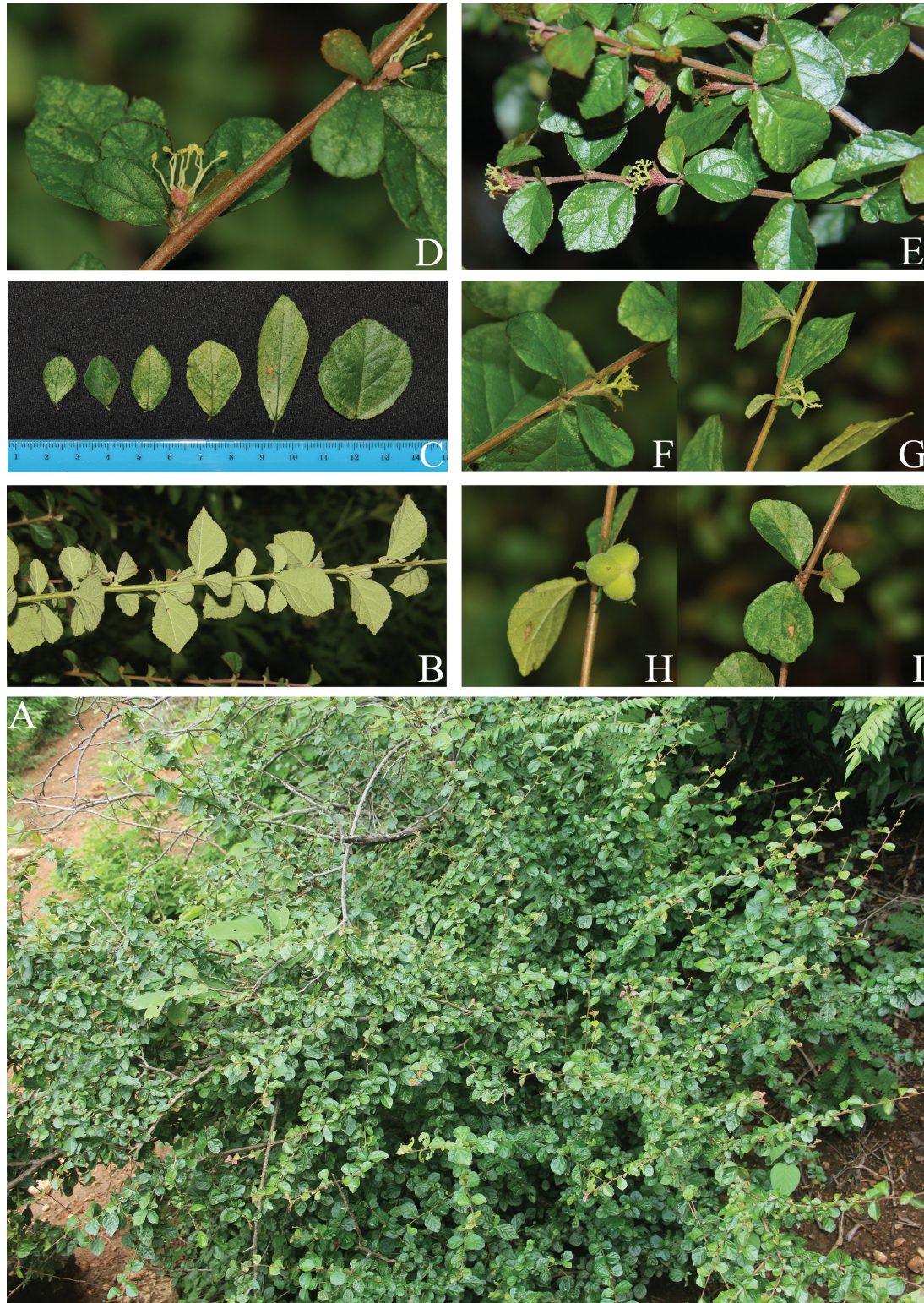


Figure 5. *Tsaiodendron dioicum* Y.H.Tan, Z.Zhou & B.J.Gu **gen. nov., sp. nov.** (A) habit; (B) twig, showing lower surface of leaves; (C) leaf blade; (D) male flower; (E–G) female flower; (H–I) fruit. Photographs by Yun-Hong Tan and Bo-Jian Gu.

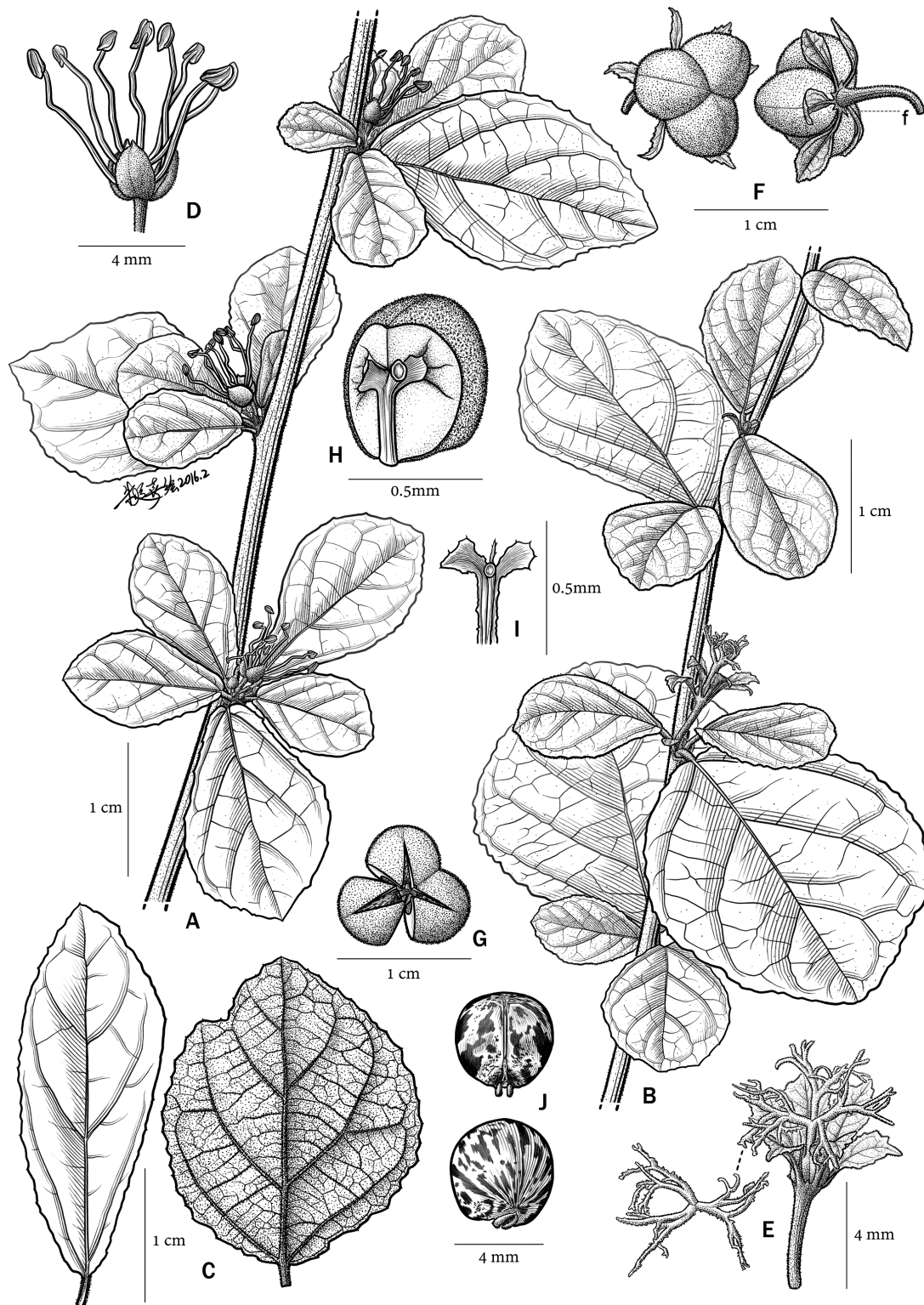


Figure 6. *Tsaiodendron dioicum* Y.H.Tan, Z.Zhou & B.J.Gu gen. nov., sp. nov. (A–B) Habit; (C) leaf blade; (D) staminate flower; (E) pistillate flower, showing elongate stigma; (F) fruit (f, disc segments); (G) dehiscent fruit (apical view); (H) mericarp, exposing column with axile placentation; (I) columella; (J) seed. Drawn by Yun-xi Zhu from *Yun-Hong Tan 11205* (HITBC).

pistillate flower is a synapomorphy of *Tsaiodendron* and *Cladogynos*. We speculate that the structures might be homologous (compared to the annular structure in *Cephalocroton*) in the two sister genera, and reduced petal/sepals would best explain the origin of the segments. In *Cladogynos*, the disc segments are smaller and more reduced.

In the Euphorbiaceae, with strictly unisexual flowers, monoecious and dioecious genera are nearly equal in number (Webster, 1994). Monoecy and dioecy also can coexist in the same tribe (e.g. Chrozophoreae) and the same genus (e.g. *Bernardia* Houston ex Mill.). In many monoecious Euphorbiaceae, the basic inflorescence is a complicated cyme, with spatial (e.g. terminal pistillate flower subtended by lateral staminate axes in *Jatropha* L.) and temporal (e.g. protogynous) separation of the staminate and pistillate flowers (Webster, 2014). However, in many other genera, the dichasia are reduced to glomerules, and separation of the sexes to prevent autogamy is commonly achieved by further spatial separation in the same plant (e.g. several proximal female flowers separated by a long internode with terminal male flowers in *Cephalocroton*) or by dioecy. *Tsaiodendron* is an example of the latter separation mechanism with dioecy but a much more reduced inflorescence, whereas other members in the tribe Epiprineseae show the former, with monoecy but further spatial separation in a rather more complicated inflorescence than in *Tsaiodendron*. Therefore, *Tsaiodendron* appears to be a distinctive taxon in the Epiprineseae.

Tsaiodendron diverged from the tropical Asian widespread genus *Cladogynos* in the late Miocene at 10.42 Mya (95% HPD: 5.23–18.88 Mya; Fig. 4) according to our divergence dating analysis. Some tropical taxa with an East Asian distribution have been similarly estimated to have originated or diverged from their sister groups around the middle to late Miocene; for example, the East Asian lineage [*Breynia retusa* (Dennst.) Alston] of *Breynia* J.R.Forst. & G.Forst. (Phyllanthaceae) diverged from its Southeast Asian relatives at 9.03 Mya (van Welzen *et al.*, 2015) and *Pogostemon* Desf. (Lamiaceae) diversified at 14.87 Mya (95% HPD: 12.09–17.99 Mya) (Yao *et al.*, 2016), although some other taxa with similar patterns have much older estimated ages, such as the East Asian lineage of *Mallotus* Lour. which diverged from the tropical Asian lineage at 34.31 Mya (95% HPD: 32.35–44.79 Mya) (van Welzen *et al.*, 2014). We suggest that the geological events in the Red River and adjacent regions may have played an important role in the origin of *Tsaiodendron*. These events include: (1) In the Oligocene and early Miocene (22–17 Mya), collision of India with Asia displaced Indochina south-eastward along the Ailao Shan–Red River shearzone, which initiated extensive uplift of the Ailao Mountains (Harrison *et al.*, 1996; Wang *et al.*, 2006; Che *et al.*, 2010); (2) incision of the

Red River system is thought to have begun in the mid to late Miocene (13–9 Mya), which shaped the embryonic form of the mountain range in this region, accompanying the formation of the South Asian monsoon in this period (Allen *et al.*, 1984; Clark *et al.*, 2005; Wang *et al.*, 2006). The uplift of the Ailao Mountains blocked the subsequent South Asian monsoon from the Indian Ocean, and the incision of the Red River system further shaped the Yuanjiang dry-hot valley as a region with a unique climate and habitat. The divergence of *Tsaiodendron* may be correlated to incision of the Red River, since the emergence time estimated by our dating analysis coincides with the geological timetable. However, the uncertainty in molecular divergence dating analysis and a rather broad 95% HPD range for the emergence time of *Tsaiodendron* should also be acknowledged. Studies based on more molecular markers and more potential reliable fossil constraints will make the divergence time more accurate.

PHYLOGENETIC RELATIONSHIPS AND EVOLUTION IN TRIBE EPIPRINEAE

The monophyly of the Epiprineseae was partly tested by Wurdack *et al.* (2005). Their results showed the sampled genera of tribe appeared to form a monophyletic group, except for the enigmatic Australian tropical genus *Rockinghamia*, which was then tentatively added to the tribe by Webster (2014). Our results, based on all the genera (in the broad sense), strongly support (BP = 100, PP = 1.00; Fig. 3) the monophyly of Epiprineseae *sensu* Webster (1994) and Radcliffe-Smith (2001), including the new genus *Tsaiodendron*. The more distant relationship of *Rockinghamia* from Epiprineseae suggests this genus should be excluded from this tribe. Epiprineseae originated in the middle-late Eocene, at about 50.23 Mya (95% HPD: 33.73–59.10 Mya; Fig. 4). This period is consistent with the origin of some other tribes in Acalyphoideae, such as Alchorneae (Hurus.) Hutch. (49.8 Mya, 95% HPD: 33.2–64.2 Mya; see Fig. 4), and also coincides with the onset of diversification in some large genera of the subfamily, such as *Mallotus* (van Welzen *et al.*, 2015).

Two subclades of Epiprineseae were recognized by our analyses with strong support (Fig. 3), but did not exactly correspond to Cephalomappinae (only including *Cephalomappa*) and Epiprininae (including all other genera), as recognized by Webster (1994) and Radcliffe-Smith (2001). *Cephalomappa* is closely related to *Koiloceras* rather than being a sister group to all other taxa. These two genera form one clade, which is sister to the clade containing all other genera. Both have pinnate leaf venation and axillary bisexual inflorescences (Table 1). In the Epiprininae clade, taxa with staminate flowers in racemoid or spiciform inflorescences (*Cleidocarpon*

and *Epiprinus s.l.*) form a clade, whereas taxa with staminate flowers in capitula (*Cephalocroton* and *Cladogynos*) are not monophyletic, with the tropical African to Socotran genus *Cephalocroton s.l.* sister to the rest of the subtribe (Fig. 3). Thin (1995) proposed a new subtribe Cleidiocarpaceae for *Cleidiocarpon* with its unusual drupaceous fruit. Our results also demonstrate this monotypic subclade, sister to *Epiprinus*, but with weak support in the MP analysis (BP = 70). After *Tsaiodendron* is included, the synapomorphies of the sexual system in Epiprineae need to be expanded.

The evolutionary trend of sexual systems in Epiprineae is from monoecy to dioecy, with the only dioecious member *Tsaiodendron* deeply nested in the clade. This pattern supports the hypothesis that flowering plants have evolved from hermaphrodites to separate sexes (dioecious), which also has been validated in many taxa (Richards, 1997; Barrett, 2002). Transitions from monoecy to dioecy may have happened numerous times in Euphorbiaceae, as both types can coexist in the same tribe or genus. In Epiprineae, *Tsaiodendron* is the only genus that grows exclusively in the dry-hot valley habitat (some *Cephalocroton* spp. also occur in similar habitats, but this genus has a wider distribution). We speculate that the drought stress in its original habitat might favour the breeding system shift. Harsh habitats with low resource availability (soil, water, nutrients) could be one of the drivers for the evolution from hermaphroditism to dioecy, although the exact mechanism remains unclear (Ashman, 2006; Sakai & Weller, 1999). One possible hypothesis supposes that hermaphrodites allocate resources to both pollen and seeds, whereas female mutants (male sterile) invest only in seeds; consequently, in low-resource environments some hermaphrodites may be unable to maintain both sex functions, instead allocating resources preferentially to the male function (Ashman, 2006). Thus, resource stress may promote the spread of unisexual individuals in a population. The association of arid habitat with evolution of sexual dimorphism has also been reported in some other plant taxa. For example, all sexually dimorphic species of *Schiedea* Cham. & Schltdl. occur only in dry habitats (Weller, Wagner & Sakai, 1995). A few other taxa from south-western China in similar habitats also exhibit the same transition of sexual system. For example, the dry-hot valley species *Bauhinia brachycarpa* Wall. ex Benth. (Z.Z., pers. observ.) and *Oxyria sinensis* Hemsl. (Li et al., 2003) represent the rare dioecious and subdioecious systems in their genus and family, respectively. Ecological studies suggest the higher nitrogen-use efficiency in unisexual individuals of *O. sinensis* might have contributed to the evolution of dioecy from the hermaphrodite to some degree (Yang et al., 2014).

TAXONOMIC TREATMENT

***TSAIODENDRON* Y.H.TAN, H.ZHU
& H.SUN GEN. NOV. (希陶木属)**

Type: *Tsaiodendron dioicum* Y.H.Tan, Z.Zhou & B.J.Gu sp. nov.

Diagnosis: The new taxon differs from the rest of Epiprineae in being: deciduous shrubs *in situ*, dioecious, inflorescences terminal on leafy spur (short lateral) shoots; staminate inflorescences sessile, one- or several-flowered, flowers emerging sequentially; pistillate inflorescences one-flowered, flowers with disc glands.

Description: Deciduous shrubs, dioecious. Latex absent. Indumentum stellate, dense on most parts. Stems with sparingly branched erect principal axes, and extremely short, lateral spur stems. Stipules minute, usually caducous. Leaves alternate, simple, mostly clustered on axillary short shoots; blade ovate to obovate, margin crenate-dentate with glandular teeth, nervation pinnate with the basal nerve pair acute, glandular tissue absent. Inflorescences terminal on spur (short lateral) shoots; unisexual, male and female on different individuals; staminate inflorescences sessile, one- or several-flowered, flowers emerging sequentially; pistillate inflorescences one-flowered. Flowers actinomorphic; petals absent. Staminate flowers densely hairy, pedicellate; sepals two to four (five), valvate; disc absent; stamens (five) six to nine, filaments free; pistillate flowers pedicellate; sepals five or six, foliaceous, disc-glands sepal-like, alternisepalous; ovary trilocular, tomentose; ovules single per locule; style present, stigmas elongate, split at the apex. Fruits trilobed capsular, tomentose, sepals persistent, dehiscing into two-valved cocci; columella persistent. Seeds subglobose, marbled, caruncle or aril absent.

Distribution: Monotypic genus, endemic to China.

Etymology: The genus is named in honour of the late Prof. Cai Xitao (Tsai Hse-Tao, 1911–1981), who devoted all his life to the study of Chinese plants and to the Xishuangbanna Tropical Botanical Garden (XTBG) and Kunming Institute of Botany (KIB). The specific epithet indicates the dioecious sexual system, which is the most obvious unique character of the new taxon in the tribe.

***TSAIODENDRON DIOICUM* Y.H.TAN,
Z.ZHOU & B.J.GU SP. NOV. (希陶木)**

Holotype: China, Yunnan, Yuanjiang, Pupiao, elevation 497 m, 23°28'17"N, 102°10'58"E (Fig. 1), dry-hot valley with savanna vegetation, 5 August 2014, *Yun-Hong Tan 11205* (HITBC; isotypes: KUN, IMDY). Figures 5, 6.

Deciduous shrub, dioecious, 0.5–1.5 m tall, much branched; bark dark greyish brown, slightly striate;

branches slender, brownish, densely stellate puberulent when young, internodes 1–3 cm long, bearing numerous axillary leafy spur shoots. Leaves densely crowded on spur shoots, few spread on primary axes, alternate, simple, leathery. Stipules minute, *c.* 0.8–1.0 mm long, usually caduceous. Petiole short, 1.5–2.5 mm long, hairy. Leaf blades rhombic-elliptic, rhombic-ovate, obovate or ovate, 0.50–3.50 × (0.23–) 0.30–2.30 (–2.90) cm, apex rounded, base obtuse, rounded to subcordate, margin crenate with glandular teeth, secondary nerves three to five, basally three-nerved, equally developed, basal pair of nerves arising near the petiole apex; nerves arising from the midrib at *c.* 45°, arching upwards towards the margin; glandular tissue completely absent from the leaf blade; lower surface densely stellate puberulent, upper surface sparsely so. Inflorescences terminal on leafy spur shoots, sessile, often reduced to solitary or a few flowers. Staminate inflorescences solitary or several-flowered, flowers emerging sequentially in the multi-flowered inflorescences; pistillate inflorescences solitary or two- or three-flowered, flowers emerging sequentially in the multi-flowered inflorescences. Flowers actinomorphic, densely stellate puberulent; petals absent. Staminate flowers, pedicels 2.0–2.6 mm long; sepals two to four (five), ovate, *c.* 2.0 mm long, 2.0–2.5 mm wide, valvate; disc absent; stamens (five) six to nine, filaments free, 6–7 mm long, anthers pale yellow, dorsiventrally flattened, 0.8–1.0 × 0.5 mm, dehiscing laterally, the two cells opposed, connective dilated, flattened; pistillode absent; pistillate flowers, pedicels 4.0–4.5 mm long; sepals five or six, foliaceous, pedicellate, obovate to oblanceolate, 3–5 mm long, 1.5–2.0 mm wide, apex acute, base cuneate, margin dentate with glandular teeth; disc glands sepal-like, alternisepalous but smaller than sepals, *c.* 1 mm long; ovary tri- (tetra-)locular, tomentose, 1.8–2.0 mm long; ovules single per locule; styles three (four), connate at the base, present 1.8–2.0 mm long, stigmas elongate, papillose, branched or multifid at the apex, up to 4.5–5.0 mm long. Fruit tridymous, 5.0–5.5 × 8.5–9.0 mm, tomentose, dehiscing into three two-valved cocci; the mericarp valves fully split, exocarp thin; endocarp crustaceous, columella persistent. Seeds subglobose, 4.0–4.5 × 3.5 mm, testa crustaceous, black and white marbled, smooth; caruncle or aril absent.

Paratype: China, Yunnan, Yuanjiang, Pupiao, elevation 430 m, 13 August 2014, *Bo-Jian Gu 0001* (HITBC, KUN, IMDY).

Distribution: Endemic to China, only found in Yuanjiang dry-hot valley, Yunnan, China.

Habitat and phenology: In dry-hot valley with savanna vegetation. Elevation 400–500 m. Flowering and fruiting: June to October by our observations in 2014 and 2015. Deciduous time: November–December to March of the second year, also depending on when the rainy season ends. In 2014, the rainy season ended earlier in September and we saw the deciduous status

in mid-October; in 2015, the rainy season ended in late October and the leaves were still green in November. We have observed new leaves beginning to appear in March. The deciduous status of *Tsaiodendron* is shown in Appendix S3.

In general, the rainy season in Yuanjiang dry-hot valley is from May to October, and the dry season can be further divided into a cool dry season (November to February) and a hot dry season (March to April). Vegetation in this region is dominated by deciduous plants with leaves falling in the cool dry season (Zhang *et al.*, 2009), with *Tsaiodendron* also belonging to this major phenological type. This may be due to: (1) total precipitation is low and drought stress occurs easily; and (2) the temperature in the early dry season is low in this region, but most of the plants here are of tropical origin and are sensitive to low temperatures in the cold-dry season.

IUCN Conservation Status: VU B1ab (i,iii,iv,v) (IUCN, 2012). Only one population and *c.* 500 individuals were found in the type locality, Yuanjiang dry-hot valley, Yunnan Province. It is threatened due to increasing habitat loss and human activities.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Voucher and GenBank accession numbers for taxa used in phylogenetic and dating analysis.

Appendix S2. Disc glands in *Tsaiodendron dioicum* gen. nov. sp. nov. (A–C) and *Cladogynos orientalis* (D), indicated by arrows.

Appendix S3. Deciduous status of *Tsaiodendron dioicum* gen. nov. sp. nov.