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Chapter 6 Molecular Phylogeny of *Trigonostemon* and its relatives (Euphorbiaceae)

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Abstract *Trigonostemon*, *Dimorphocalyx*, *Tritaxis* and *Ostodes* are tropical Asian genera that are very similar in morphology. The former three genera have been treated either as a single genus *Trigonostemon sensu lato* or as separate genera. A molecular phylogeny based on the nuclear ITS and chloroplast *trnK* intron, *trnT-L* and *trnL-F* sequences reveals that *Trigonostemon* is a monophyletic group, and *Dimorphocalyx* and *Tritaxis* together form another monophyletic group separate from *Trigonostemon*. Within the genus *Trigonostemon* a strong correlation between the molecular phylogeny and pollen morphology is found. It demonstrates two potential evolutionary directions for the common 'croton pattern' ornamentation of pollen in the genus: the subunits that form the 'croton pattern' ornamentation tend to reduce to spines or they tend to merge together in the later developed groups. The correlation between the molecular phylogeny and macromorphology of *Trigonostemon* is discussed and a new infrageneric classification with four sections is provided. *Dimorphocalyx* is reduced to *Tritaxis*, the older generic name.

Key words

Dimorphocalyx
Euphorbiaceae
Ostodes
phylogeny
pollen
taxonomy
Trigonostemon
Tritaxis

INTRODUCTION

Trigonostemon Blume is a genus in the Euphorbiaceae containing about 60 species of monoecious small trees or shrubs growing in lowland rainforests. The plants are found from S India and Sri Lanka to S China, throughout continental SE Asia and Malesia to NE Australia and the W Pacific (Govaerts et al. 2000). The genus is morphologically recognised by the 5-merous flowers with colourful petals (purple, red, pink, orange, yellow, white and intermediate colours between these) and one whorl of three or five connate stamens (Figure 40a–f).

Trigonostemon is traditionally classified in the subfamily Crotonoideae, tribe Codiaeae, subtribe Trigonostemoniae (Webster 2014). In the molecular phylogeny of the uniovulate Euphorbiaceae (Wurdack et al. 2005) it is placed in the C2 clade of the subfamily Crotonoideae, close to *Ostodes* Blume and other genera.

Blume (1825) established the genus based on *Trigonostemon serratus* Blume, which has one whorl of three connate stamens forming a triangular shape when seen from ‘above’, hence the generic name. After Blume, over 140 scientific names were published within *Trigonostemon* (Govaerts et al. 2000), although the circumscription of the genus has remained unclear during the years. The previous studies diverged into two mainstream opinions: (1) Müller Argoviensis (1865, 1866), as opposed to Blume’s (1825) original definition, regarded the genus in a wider sense by including species with two or three whorls of stamens in *Trigonostemon*; as a result, a few genera including *Dimorphocalyx* Thwaites (1861; two whorls of stamens, Figure 40k) and *Tritaxis* Baillon (1858; three whorls of stamens, for illustration see Gagnepain 1925b) were merged with *Trigonostemon* into a new *Trigonostemon sensu lato*; (2) most other botanists, e.g., Bentham (1878, 1880) and Pax (1890), preferred Blume’s concept (1825), keeping *Trigonostemon* restricted to the species with only one whorl of stamens. Airy Shaw (1969) had an eclectic opinion between the two theories. He kept *Dimorphocalyx* separate but included *Tritaxis* in *Trigonostemon* despite the 13 stamens of *Tritaxis* being obviously aberrant among the other species. Unfortunately, none of these circumscriptions has yet been confirmed by a molecular phylogeny. In addition, *Ostodes* has 20–41 stamens, with the inner ones partly connate (van Welzen & Winkel 2015; Figure 40l), suggesting a potentially close relationship with *Trigonostemon* and *Dimorphocalyx*. Therefore, *Ostodes* should also be taken into account when assessing this whole complex. All these genera have petals in the pistillate flowers, which make them distinct from most other Euphorbiaceae.

Within *Trigonostemon sensu stricto*, a few infrageneric classifications have been made using different combinations of morphological characters (for a brief review, see Yu & van Welzen 2018): Bentham (1878, 1880) and Hooker (1887) emphasised the position of the inflorescences, the number and division of the anthers, and they designated three sections within the genus; Merrill (1924) and Pax & Hoffmann (1931) extended this system to include five sections; Jablonski (1963) did not rely on the number of stamens but focused on whether the stigmas were bifid or not, and recognised three sections (only for the species of the Malay Peninsula, Sumatra and Borneo). Unfortunately, these classifications are not generally applicable, because they were based on a limited number of species and contained confusing misidentifications and misplacements.

At present the main genera involved in the complex, i.e., *Trigonostemon*, *Dimorphocalyx* and *Ostodes*, are already morphologically revised for the Flora Malesiana project (Yu & van Welzen 2018; van Welzen & van Oostrum 2015; van Welzen & Winkel 2015, respectively) and other local floras (e.g., Chakrabarty & Balakrishnan 1990, 1993; Balakrishnan & Chakrabarty

1991; Chantaranonthai 2007; Phattarahirankanok & Chayamarit 2005, 2007; Li & Gilbert 2008). Six species have been treated within *Tritaxis* in the literature (also see Taxonomic treatment below), but these names are now all synonymised within *Trigonostemon* (Airy Shaw 1969, Yu & van Welzen 2018), *Dimorphocalyx* (van Welzen & van Oostrum 2015) and *Paracroton* Miq. (Balakrishnan & Chakrabarty 1993, Esser 2007). Additionally, the pollen morphology of all these genera was also studied (Yu et al. 2019c). Two major pollen types (including two subtypes; Figure 40m–t) were found in *Trigonostemon* (sensu Blume 1825), but the pollen characters could not yet be discussed in the light of evolution because a phylogeny was still lacking. Therefore, the aim of our study is to analyse the phylogenetic relationships between *Trigonostemon* and its closely resembling genera using several molecular markers (nuclear ITS and the chloroplast *trnK* intron, *trnT-L*, *trnL-F* and *rbcL*), in order to (1) confirm the systematic position of *Trigonostemon* in the family, (2) clarify the generic delimitation of *Trigonostemon* and its possible relatives, (3) interpret the evolutionary trends in *Trigonostemon* in reference to the pollen morphology and (4) revise the infrageneric classification of the genus.

MATERIAL AND METHODS

Leaf samples of 41 species (out of about 60, Yu & van Welzen 2018) and four varieties of *Trigonostemon*, 11 species (out of about 13, van Welzen & van Oostrum 2015) of *Dimorphocalyx*, one species (probably monotypic, Gagnepain 1925b) of *Tritaxis*, one species (out of two, van Welzen & Winkel 2015) of *Ostodes* and one species of *Jatropha* L. (used as outgroup; selection based on Wurdack et al. 2005, for details see discussion) were used in the analysis. *Trigonostemon* is here regarded in the strict sense (see Introduction) and *Tritaxis* is taken separately, not as part of *Trigonostemon*. The samples were collected in the field (dried in silica gel) or taken from herbarium collections. Taxa and voucher information are shown in Table 3.

Total genomic DNA was isolated from fresh and herbarium materials using the standard CTAB extraction protocol (Doyle & Doyle 1987).

The nuclear ribosomal internal transcribed spacer (nrITS) and chloroplast *trnK* intron, *trnT-L*, *trnL-F* (including the *trnL* intron and *trnL-F* spacer) and *rbcL* (of only a few species, see Table 3) were amplified from genomic DNA using the polymerase chain reaction (PCR). The primer information is shown in Table 4.

The PCR was performed with the following reaction components: 10–100 ng genomic DNA, 5 µl 5x PCR buffer (containing 15 mM MgCl₂; Thermo Fisher, Waltham, USA), 1 µl bovine serum albumin (BSA; Life), 1 µl dNTPs, 1 µl for both forward and reverse primers in 10 µM and 2.5 U *Taq* DNA polymerase (Thermo Fisher, Waltham, USA) and adjusted to a final volume of 25 µl by ultrapure water. The PCR program was: initialization at 98°C for 2.5 minutes, followed by 35–40 cycles of denaturation: 98°C for 15 seconds, annealing: 48–55°C for 15 seconds, extension: 72°C for 45–60 seconds, followed by a final extension for 5 minutes and eventually stopped at 12°C as a final hold. The PCR products were sequenced at BaseClear, Leiden, the Netherlands. The sequencing results were aligned using Geneious 8.1.8 (<https://www.geneious.com>) with the multiple sequence Geneious alignment algorithm and then manually adjusted within the same program. Some sequences were trimmed at the two ends to reduce missing data. Two matrices were made. In matrix 1 all sequences obtained from this study were aligned with the dataset of the Euphorbiaceae based on *trnL-F* and *rbcL* sequences (Wurdack et al. 2005); in total 5 markers were used: the nuclear ITS and the chloroplast *trnK* intron, *trnT-L*, *trnL-F* and *rbcL*. Matrix 2 included the nuclear ITS and the chloroplast *trnK* intron, *trnT-L* and *trnL-F* sequences for a restricted dataset of *Trigonostemon* and allies.

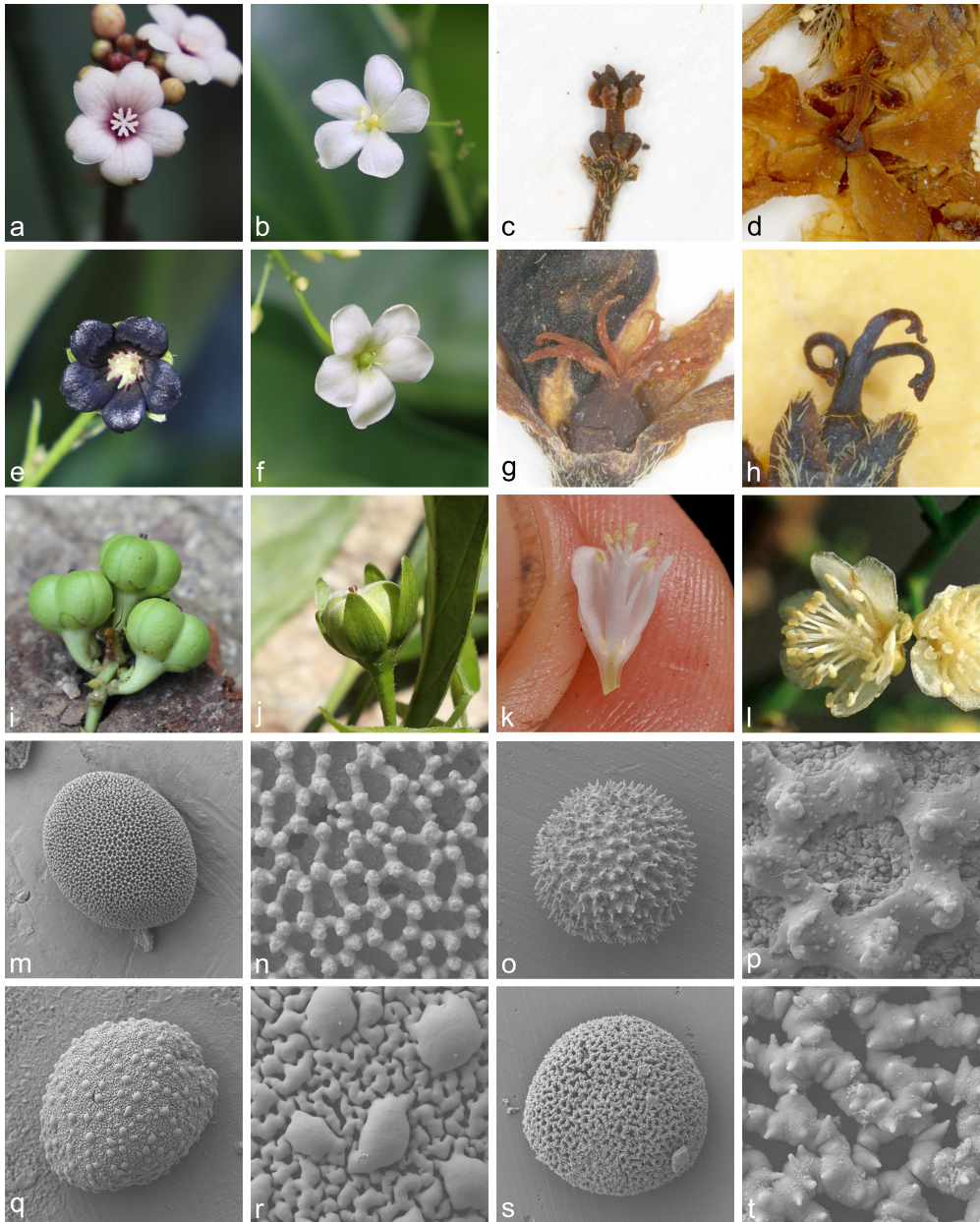


Figure 40 Illustrations of the key characters of *Trigonostemon*, *Dimorphocalyx* (now *Tritaxis*) and *Ostodes*. a. staminate flower, showing the divaricate stamens (*Trigonostemon pentandrus*); b. staminate flower, showing the non-divaricate stamens (*T. albiflorus*); c. androphore, showing the divaricate stamens and disc lobes (*T. philippinensis*); d. androphore, showing the non-divaricate stamens and an annular disc (*T. viridissimus* var. *viridissimus*); e. pistillate flowers, showing the deeply bifid stigmas (*T. filiformis*); f. pistillate flowers, showing the stigmas only very slightly bifid at apex (*T. albiflorus*); g. pistil, showing the deeply bifid stigmas and disc lobes (*T. villosus* var. *merrillianus*); h. top of a pistil, showing the stigmas only very slightly bifid at apex (*T. viridissimus* var. *elegantissimus*); i. young fruits, showing the non-acrescent sepals (*T. longipes*); j. a young fruit, showing the accrescent sepals (*T. victoriae*); k. staminate flowers of *Dimorphocalyx ixoroides* [= *Tritaxis ixoroides*];

The phylogenetic trees were reconstructed separately based on the two alignments under Maximum Parsimony (MP; matrices 1 and 2) using PAUP 4.a165 (Swofford 2002), Maximum Likelihood (ML; only matrix 2) using RaxML 8.2.10 and Bayesian Inference (BI; matrices 1 and 2) using MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001). The analyses were performed via the CIPRES Science Gateway (Miller et al. 2010; <http://www.phylo.org/>). In order to minimize bias in the comparison of results, analyses based on matrix 1 (MP and BI) were performed under the same parameters as Wurdack et al. (2005); the settings for analyses of matrix 2 were as follows:

In the MP analysis, all nucleotide characters were treated unordered and unweighted, polymorphisms were treated as uncertainties and gaps were treated as missing data. The heuristic search for the most parsimonious trees was performed using the tree-bisection-reconnection (TBR) swapping algorithm on starting trees obtained by a 1,000 random taxon additional sequence replicates (RAS), holding 10 trees at each step, with an unlimited maximum number of trees to be saved. The 50% majority consensus tree was calculated. A bootstrap analysis (Felsenstein 1985) of 5,000 replicates was made to evaluate the support for the clades, where each replicate of RAS (= 100) and TBR (holding 10 trees at each step) had a limit of maximum 100 trees saved.

The ML tree was reconstructed under General Time Reversible (GTR) model (Nei & Kumar 2000). A discrete Gamma distribution (+ Γ) was used to model evolutionary rate differences among sites (4 categories), and a certain fraction of sites are assumed evolutionarily invariable (+I). 100,000 replicates of bootstrap analysis were performed.

The BI analyses were conducted with two independent runs of four Markov-Monte Carlo chains (MCMC), using the General Time Reversible (GTR+ Γ +I) model (Nei & Kumar 2000) for a total of 100,000,000 generations. After every 1,000 generations a tree was sampled and the first 25% trees were discarded as burn-in. In the ML and BI analysis, the substitution model was selected based on the lowest Akaike Information Criterion scores detected by Modeltest-NG 0.1.5 (Darriba et al. 2019).

RESULTS

Sequence alignments

Sequences of 62 taxa were obtained. There are relatively fewer taxa with chloroplast sequences than those with ITS because of failure in PCR. Particularly, the amplifications of the *trnK* intron and *trnT-L* failed more often in *Dimorphocalyx* species. Properties of the sequences are shown in Table 5. Because the nuclear ITS and chloroplast *trnK* intron and *trnT-L* sequences were not included in Wurdack et al. (2005), the alignments of these three markers are the same in both matrices.

The aligned ITS sequences contain 693 nucleotide sites, of which 311 are variable and 169 are parsimony informative. The non-coding parts (ITS1 and ITS2) present most variation.

←
l, staminate flowers of *Ostodes paniculata*; m, n. *T. reidioides* type and *T. reidioides* subtype pollen (*T. reidioides*); o, p. *T. reidioides* type *T. longifolius* subtype pollen (*T. sandakanensis*); q, e. *T. verrucosus* type pollen (*T. laevigatus* var. *laevigatus*); s, t. Pollen of *T. inopinatus*. Photos a–j by Ren-Yong Yu; photo k © P.B. Pelser & J.F. Barcelona (Pelser et al. 2011 onwards); photo l © N. Pattharahirantricin, reproduced with permission from the Editorial Board of the Flora of Thailand project; Photos m–t by Ren-Yong Yu, reproduced from Grana 58: 114–128.

Table 3 Voucher information. * = cultivated. *Trigonostemon* sp. 1 will be described as *T. palustris* R.Y.Yu & Welzen (Yu et al. 2019a); *Trigonostemon* sp. 2 will be described as *T. montanus* R.Y.Yu & Welzen (2019b); we were unable to identify *Trigonostemon* sp. 3 for the time being.

Taxa	Collector	Field No.	Location	Barcode	ITS	trnK intron	trnT-L	trnL-F	rbcL
<i>Dimorphocalyx australiensis</i>	Gray	7859	Australia	L.2211804	MK876508	MK876670	MK876568	MK876614	
<i>D. beddomei</i>	Ridsdale	388	India	L.2211814	MK876509	MK876671		MK876615	
<i>D. cumingii</i>	Mendoza	PNH 42406	Philippines	L.2211815	MK876519				
<i>D. denticulatus</i>	Church	1819	W. Kalimantan	L.2204228	MK876510	MK876672		MK876616	
<i>D. glabellus</i>	Kosterman	26317	India	L.2204358	MK876511			MK876617	
<i>D. ixoroides</i>	Ruffo	PNH 41828	Philippines	L.2204362	MK876512			MK876618	
<i>D. malayanus</i>	Sidsunthom	ST 1640	Thailand	L.2204317	MK876513			MK876619	
<i>D. moluccensis</i>	Ramlanto	905	Moluccas	L.2204173				MK876620	
<i>D. muricatus</i>	Pooma	4499	Thailand	L.2204330	MK876514			MK876621	
<i>D. pauciflorus</i>	Forman	906	Brunei	L.2211818	MK876516				
<i>D. sp.</i>	Yu	SAN 158453	Sabah	L	MK876515			MK876622	
<i>D. trichocarpus</i>	Anderson	20974	Sarawak	L.2182719	MK876517	MK876673	MK876569	MK876623	
<i>Jatropha gossypifolia</i>	Snellius	11150	Lesser Sunda Islands	L.2236164	MK876567	MK876717	MK876613	MK876669	
<i>Ostodes paniculata</i>	Yu	172	Java	L	MK876520	MK876675	MK876571	MK876625	
<i>Trigonostemon adenocalyx</i>	Huang	H 09427	Guangxi, China	IBK 00214246	MK876547	MK876699	MK876594	MK876651	
<i>T. albiflorus</i>	Gillespie	7405	Vietnam	L.2260160	MK876527	MK876682	MK876578	MK876632	
<i>T. auranitacus</i>	Yu	160	Java*	L	MK876529	MK876683	MK876579	MK876634	
<i>T. balgooyi</i>	Hisham	FRI 73820	Malay Peninsula	KEP 217032	MK876530	MK876684	MK876580	MK876635	
<i>T. beccarii</i>	Yu	169	Java*	L	MK876531	MK876685	MK876581	MK876636	
<i>T. bonianus</i>	Huang	NG 293	Guangxi, China	IBK 00214243	MK876541	MK876694	MK876590	MK876646	
<i>T. capillipes</i>	Gardner	ST 2836	Thailand	L.2260155	MK876535	MK876689	MK876585	MK876640	
<i>T. cherrieri</i>	Veillon	7420	New Caledonia	L.2260157	MK876536	MK876690	MK876586	MK876641	
<i>T. detritiferus</i>	Yu	91	Brunei	L	MK876538	MK876692	MK876588	MK876643	

Table 3 (cont.)

Taxa	Collector	Field No.	Location	Barcode	ITS	trnK intron	trnT-L	trnL-F	rbcl
<i>T. diffusus</i>	Pureseglove	P 4702	Sarawak	L.2260164	MK876539			MK876644	
<i>T. filiformis</i>	Yu	243	Philippines	L	MK876522	MK876677	MK876573	MK876627	MK876719
<i>T. sp. aff. filiformis</i>	Yu	SAN 158462	Sabah	L	MK876561	MK876711	MK876607	MK876663	
<i>T. flavidus</i>	Yu	264	Guangdong, China*	L	MK876540	MK876693	MK876589	MK876645	
<i>T. hartleyi</i>	Hartley	TGH 11087	New Guinea	L 0043309	MK876542	MK876695		MK876647	
<i>T. inopinatus</i>	Forster	PIF 30012	Australia	L.2260532	MK876543	MK876696	MK876591	MK876648	MK876723
<i>T. laevigatus</i> var. <i>laevigatus</i>	Yu	SAN 158478	Sabah	L	MK876544	MK876697	MK876592	MK876649	
<i>T. lili</i>	Huang	NG 306	Guangxi, China	IBK 00214245	MK876533	MK876687	MK876583	MK876638	
<i>T. longifolius</i>	Yu	SAN 158474	Brunei	L	MK876545	MK876698	MK876593	MK876650	
<i>T. longipes</i>	Yu	227	Philippines	L	MK876521	MK876676	MK876572	MK876626	MK876718
<i>T. lychnos</i>	Ali Ahmad	BRUN 19908	Brunei	SING 0179065	MK876546				
<i>T. magnificus</i>	de Wilde	19441	Sumatra	L.2260380	MK876548	MK876700	MK876595	MK876652	
<i>T. malaccanus</i>	Yu	176	Malay Peninsula	L	MK876549	MK876701	MK876596	MK876653	
<i>T. merrillii</i>	Yu	255	Philippines	L	MK876524	MK876679	MK876575	MK876629	MK876721
<i>T. murtonii</i>	Webb	WA 207	Cambodia	L.2260393	MK876551	MK876703	MK876598	MK876655	
<i>T. pentandrus</i>	Yu	FRI 86653	Malay Peninsula	L	MK876552	MK876704	MK876599	MK876656	
<i>T. philippinensis</i>	Bouman	RWB 67	Yunnan, China*	XTBG	MK876553	MK876705	MK876600	MK876657	
<i>T. polyanthus</i>	Lagrimas	PNH 39433	Philippines	L.2258802	MK876554				
<i>T. quocensis</i>	Middleton	4046	Thailand	L.2258834	MK876555	MK876706	MK876601	MK876658	
<i>T. reidioides</i>	Cheng	CL 1238	Cambodia	L.3784729	MK876556	MK876707	MK876602	MK876659	
<i>T. rufescens</i>	Yu	FRI 86664	Malay Peninsula	L	MK876557	MK876708	MK876603	MK876660	
<i>T. sandakanensis</i>	Yu	SAN 158473	Sabah	L	MK876559	MK876710	MK876605	MK876662	
<i>T. semperflorens</i>	Koelz	27866	India	L.2258764	MK876560		MK876606		
<i>T. sp. 1</i>	Yu	260	Philippines	L	MK876526	MK876681	MK876577	MK876631	

Table 3 (cont.)

Taxa	Collector	Field No.	Location	Barcode	ITS	trnK intron	trnT-L	trnL-F	rbcL
<i>T. sp. 2</i>	Coelz	27903	India	L.2260526	MK876532	MK876686	MK876582	MK876637	
<i>T. sp. 3</i>	Leong-Skornickova	OS 6243	Laos	SING 0190181	MK876528			MK876633	
<i>T. verticillatus</i> var. <i>salicifolius</i>	Worthington	13073	Malay Peninsula	L.2258554	MK876558	MK876709	MK876604	MK876661	
<i>T. verticillatus</i> var. <i>verticillatus</i>	Hai	HN-NY 503	Vietnam	L	MK876562	MK876712	MK876608	MK876664	
<i>T. verrucosus</i>	Chase	1274	Java*	K	MK883479	MK883481	MK883480	AY794703	
<i>T. victoriae</i>	Yu	258	Philippines	L	MK876525	MK876680	MK876576	MK876630	MK876722
<i>T. villosus</i> var. <i>borneensis</i>	Yu	SAN 158461	Sabah	L	MK876534	MK876688	MK876584	MK876639	
<i>T. villosus</i> var. <i>cordatus</i>	Yu	SAN 158479	Sabah	L	MK876537	MK876691	MK876587	MK876642	
<i>T. villosus</i> var. <i>merrillianus</i>	Yu	254	Philippines	L	MK876523	MK876678	MK876574	MK876628	MK876720
<i>T. villosus</i> var. aff. <i>merrillianus</i>	Yu	107	Brunei	L	MK876550	MK876702	MK876597	MK876654	
<i>T. villosus</i> var. <i>villosus</i>	Yu	178	Malay Peninsula	L	MK876564	MK876714	MK876610	MK876666	
<i>T. viridissimus</i> var. <i>viridissimus</i>	Julius	FRI 56285	Malay Peninsula	L.3795794	MK876563	MK876713	MK876609	MK876665	
<i>T. wui</i>	Yu	266	Guangdong, China*	L	MK876565	MK876715	MK876611	MK876667	
<i>T. xyphophylloides</i>	Yu	265	Guangdong, China*	L	MK876566	MK876716	MK876612	MK876668	
<i>Tritaxis gaudichaudii</i>	Soejarto	DDS 14143	Vietnam	L.3784654	MK876518	MK876674	MK876570	MK876624	

Table 4 Molecular primers used in the study. For ITS, the primers ITS5 + ITS4 were used to amplify the whole sequence, and two pairs of primers were designed for degraded or fungus-contaminated templates: for ITS1, ITS66F + ITS408R and for ITS2, ITS386F + ITS749R. For the *trnK* intron, the primers trnK 570F + 190R were used for the amplification. For *trnT-L* and *trnL-F*, the primers a + b and c + f were used to amplify the two sequences respectively, and two pairs of primers c + d and e + f were used for degraded templates to amplify the *trnL* intron and *trnL-F* spacer separately. In addition, two extra primers were designed to use together with c and f for greatly degraded templates to amplify the *trnL* intron in two pieces: c + t262R and t191F + d. For *rbcL*, the primers 1F + 724R and 636F + 1368R were used to amplify the gene in two parts.

Primer	Sequence	DNA marker	Reference
ITS5	5'-GGA AGT AAA AGT CGT AAC AAG G-3'	ITS	White et al. 1990
ITS4	5'-TCC TCC GCT TAT TGA TAT GC-3'	ITS	White et al. 1990
ITS66F	5'-CGA CCA GCG AAC ATG TTA TTC-3'	ITS	self-designed
ITS408R	5'-GGG ATT CTG CAA TTC ACA CCA AG-3'	ITS	self-designed
ITS386F	5'-CTT GGT GTG AAT TGC AGA ATC CC-3'	ITS	self-designed
ITS749R	5'-TTA AAC TCA GCG GGT GTT CCC-3'	ITS	self-designed
trnK570F	5'-TCC AAA ATC AAA AGA GCG ATT GG-3'	<i>trnK</i> intron	Samuel et al. 2005
190R	5'-CGA TCT ATT CAT TCA ATA TTT C-3'	<i>trnK</i> intron	Samuel et al. 2005
a	5'-CAT TAC AAA TGC GAT GCT CT-3'	<i>trnT-L</i>	Taberlet et al. 1991
b	5'-TCT ACC GAT TTC GCC ATA TC-3'	<i>trnT-L</i>	Taberlet et al. 1991
c	5'-CGA AAT CGG TAG ACG CTA CG-3'	<i>trnL-F</i>	Taberlet et al. 1991
d	5'-GGG GAT AGA GGG ACT TGA AC-3'	<i>trnL-F</i>	Taberlet et al. 1991
e	5'-GGT TCA AGT CCC TCT ATC CC-3'	<i>trnL-F</i>	Taberlet et al. 1991
f	5'-ATT TGA ACT GGT GAC ACG AG-3'	<i>trnL-F</i>	Taberlet et al. 1991
t262R	5'-CCT TTA CTT TAT CCT TTC TGG AG-3'	<i>trnL-F</i>	self-designed
t191F	5'-GGA GTT GAC TGC GTT GCA TTA G-3'	<i>trnL-F</i>	self-designed
1F	5'-ATG TCA CCA CAA ACA GAA AC-3'	<i>rbcL</i>	Lledo et al. 1998
636F	5'-GCG TTG GAG AGA TCG TTT CT-3'	<i>rbcL</i>	Lledo et al. 1998
724R	5'-TCG CAT GTA CCT GCA GTA GC-3'	<i>rbcL</i>	Lledo et al. 1998
1368R	5'-CTT CCA AAT TTC ACA AGC AGC A-3'	<i>rbcL</i>	Lledo et al. 1998

Table 5 Summary of sequence and alignment properties. The data of *trnL-F* and *rbcL* presented here only include the sequences obtained from this study. Matrix 1 includes sequences of all 5 markers obtained from this study aligned with the dataset of Euphorbiaceae based on *trnL-F* and *rbcL* sequences (Wurdack et al. 2005); matrix 2 includes the sequences of four markers obtained from this study: ITS, *trnK* intron, *trnT-L* and *trnL-F*. Note: the number before the slash (/) refers to matrix 1 and after the slash (/) to matrix 2.

DNA marker	No. taxa	Sequence length	Alignment length	Polymorphic sites	Variable sites	Parsimonious informative sites
ITS	61	282–754	693	0–25	311	169
<i>trnK</i> intron	49	335–498	570	0–1	98	31
<i>trnT-L</i>	47	323–528	640	0–1	99	30
<i>trnL-F</i>	57	372–1070	2327/1310	0–4	1310/196	888/55
<i>rbcL</i>	8	1390	1428	0–1	504	371
matrix 1	291	—	5658	—	2322	1489
matrix 2	62	—	3213	—	704	285

No significant indels (longer than 20 bp) are found in the major groups. The *trnK* intron alignment has 570 sites, including 98 variable and 31 parsimonious informative sites. A parsimony informative insertion of 25–40 bp is found at c. 195 bp upstream of the *matK* gene in 5 taxa (*Dimorphocalyx beddomei* (Benth.) Airy Shaw, *D. denticulatus* Merr., *Trigonostemon albiflorus* Airy Shaw, *T. wui* H.S.Kiu and *Tritaxis gaudichaudii*). The *trnT-L* alignment has 640 sites, of which 99 are variable and 30 are parsimonious informative. A parsimony informative insertion of 19 bp is found at c. 140 bp downstream of the *trnT* gene in *Trigonostemon longifolius* Wall. ex Baill. and *T. sandakanensis* Jabl. The alignment of the *trnL-F* sequences obtained from this study (part of matrix 2) contains 1310 sites, including 196 variable and 55 parsimony informative sites. A major parsimonious informative deletion of c. 29 bp is found in the *trnL* gene intron in 15 *Trigonostemon* taxa (*T. detritiferus* R.I.Milne, *T. diffusus* Merr., *T. filiformis* Quisumb., *T. flavidus* Gagnep., *T. longipes* (Merr.) Merr., *T. malaccanus* Müll.Arg., *T. murtonii* Craib, *Trigonostemon* sp. 1, *T. verticillatus* (Jack) Pax, *T. victoriae* R.Y.Yu & Welzen and some varieties of *T. villosus* Hook.f.). Also, an insertion of c. 62 bp was found in the *trnL-F* spacer of the outgroup (*Jatropha gossypifolia* L.). When the *trnL-F* sequences are further aligned with Wurdack et al.'s (2005) dataset, the matrix (part of matrix 1) includes 2327 sites with 1310 variable sites and 888 of them are parsimony informative. In addition, *rbcL* sequences of seven *Trigonostemon* taxa were also aligned with the data of Wurdack et al. (2005). The *rbcL* matrix has 1428 sites, of which 504 are variable and 371 are parsimony informative. However, the eight *Trigonostemon* taxa (seven from our lab and *T. verrucosus* J.J.Sm. from Wurdack et al. 2005) in this matrix display very little variation, with only 34 variable and 11 parsimony informative sites and no indels.

A significant number of polymorphisms are found in the ITS sequences, particularly in *Trigonostemon albiflorus* (25 sites) and *Dimorphocalyx* sp. (15 sites). For other markers, polymorphic sites are always fewer than 4 per taxon.

Analyses of the combined dataset (matrix 1)

The MP and BI analyses result in the same topology (Figure 41) for the main clades as Wurdack et al. (2005), although the bootstrap supports and posterior probabilities of some clades are relatively lower. This is probably because of the large amount of absent data in the taxa obtained from Wurdack et al. (2005), because they did not analyse the nuclear ITS and chloroplast *trnK* intron and *trnT-L* sequences.

The species of *Trigonostemon* form a monophyletic group in the C2 clade (99 MP bootstrap credibility and 1.0 posterior probability; Figure 41); so do the *Dimorphocalyx* species (including *Tritaxis gaudichaudii*, discussed below), but the group has relatively lower support (66 MP bootstrap credibility and 0.89 posterior probability). *Ostodes paniculata* Blume (two samples) appears to be sister to *Vernicia montana* Lour. with a posterior probability of 0.72 (Figure 41). The relationships between *Trigonostemon*, *Dimorphocalyx* and *Ostodes* are still unresolved.

Analyses of *Trigonostemon* and its close relatives only (matrix 2)

In general, the ITS yields more variation than the chloroplast markers (*trnK* intron, *trnT-L* and *trnL-F*), but the phylograms (Figures 43–46) obtained separately from the nuclear and chloroplast markers have similar topologies for the main clades. In both phylograms *Trigonostemon* forms a monophyletic group and includes three main clades (less resolved in the chloroplast phylogram). However, in the BI phylogram (Figure 45) based only on the chloroplast markers, *Dimorphocalyx malayanus* Hook.f. is oddly placed as a sister taxon of

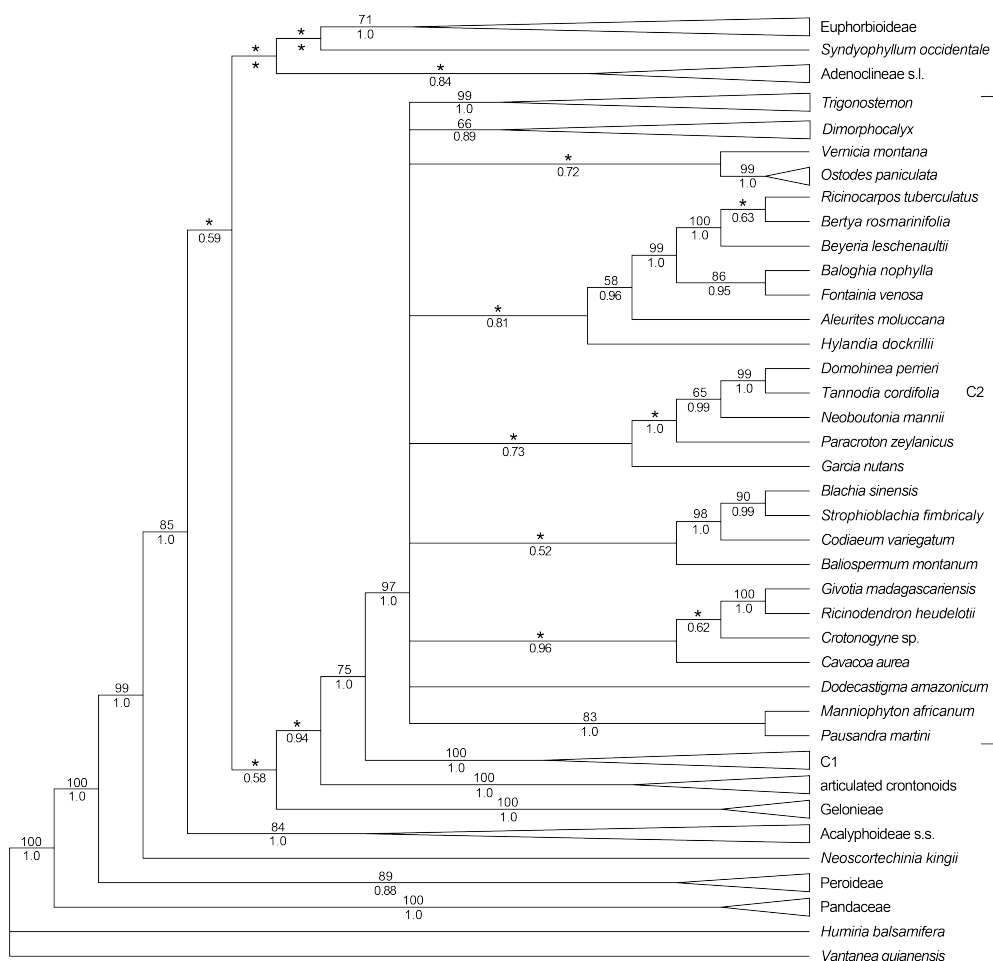


Figure 41 Phylogenetic position of *Trigonostemon*, *Dimorphocalyx* (now *Tritaxis*) and *Ostodes* in the Euphorbiaceae, resulting from the Maximum Parsimony and Bayesian Inference analyses of combined nuclear ITS and chloroplast *trnK* intron, *trnT-L*, *trnL-F* and *rbcL* data obtained from this study and Wurdack et al. (2005). The number above the clades is the bootstrap credibility from the MP analysis, and the number below the clades is the posterior probability from the BI analysis. Clades are omitted when the bootstrap support is below 50 or the posterior probability is below 0.5, except where marked with an asterisk. The figure is a summary of Wurdack et al. (2005) and the triangles represent groups of species (or specimens in the case of *Ostodes*).

Ostodes, but this is likely because the chloroplast data of *D. malayanus* is largely missing (only part of the *trnL-F* sequence was included; see Table 3); *Dimorphocalyx australiensis* C.T.White forms a polytomy with all other clades because of a low posterior probability (but still within the *Dimorphocalyx* clade in the maximum clade credibility tree). The four markers are combined as one dataset for analyses and for the discussion below.

The trees made by the three methods (MP, ML and BI) have the same topology for the main clades in the majority rule consensus trees (Figures 42, 47, 48), except in the MP analysis, *Ostodes paniculata* is branching outside *Dimorphocalyx* and *Trigonostemon* (Figure 47). This discrepancy, however, does not change the monophyly of the *Dimorphocalyx* and *Trigonostemon* clades.

The MP analysis resulted in a total of 732,240 trees as the best score. In the 50% majority-rule consensus tree, the frequency of occurrence is always 100, except in some minor clades within the T1 group. Two major parsimony informative indels appear to support the phylogeny (Figure 47): 1. the insertion in the *trnT* gene of *Trigonostemon longifolius* and *T. sandakanensis*; 2. the deletion in the *trnL-F* spacer of several *Trigonostemon* taxa in the T1 clade. The insertion in the *matK* gene in 5 taxa (*Dimorphocalyx beddomei*, *D. denticulatus*, *Trigonostemon albiflorus*, *T. wui* and *Tritaxis gaudichaudii*) does not support the phylogeny.

A noteworthy discrepancy in the results among the different methods is in the placement of three *Trigonostemon* species collected from China (*T. adenocalyx* Gagnep., *T. bonianus* Gagnep. and *T. lii* Y.T.Chang): they always cluster as one group with strong support in all analyses (100 both MP and ML credibility, 1.0 posterior probability), but in the MP and ML analyses this group is branching at the base of the T3 clade (71 both MP and ML bootstrap credibilities for the ingroup of T3, Figures 47, 48), whereas in the BI analysis, it is one node higher inside the T3 clade, leaving four species, *T. aurantiacus* (Kurz ex Teijsm. & Binn.) Boerl., *T. hartleyi* Airy Shaw, *T. philippinensis* Stapf and *T. xyphophylloides* (Croizat) L.K.Dai & T.L.Wu branching at the base of the T3 group (1.0 posterior probability for the ingroup, Figure 42).

In the phylogram (Figure 42), *Trigonostemon* and *Dimorphocalyx* form their own monophyletic groups with strong support (100 both MP and ML bootstrap credibility, 1.0 posterior probability for *Trigonostemon*; 96 both MP and 97 ML bootstrap credibility, 0.99 posterior probability for *Dimorphocalyx*). *Tritaxis gaudichaudii* is embedded in the *Dimorphocalyx* clade, therefore, the two genera are merged together in the taxonomic treatment (discussed below). *Ostodes* (only one taxon sampled) does not fit in either *Trigonostemon* or *Dimorphocalyx* and is regarded as a separate genus.

Within *Trigonostemon*, three well supported major clades (T1, T2, T3) can be recognised, of which the T1 and T2 clades are sister clades. Several morphological and pollen characters are also mapped on the phylogram (Figure 42).

DISCUSSION

Generic delimitations of *Trigonostemon*, *Dimorphocalyx* and *Ostodes*

Only one taxon of *Trigonostemon* (*T. verrucosus*) was included in the phylogenetic analyses of the uniovulate Euphorbiaceae by Wurdack et al. (2005). The taxon was branching in the C2 clade of the Crotonoideae. Our results (Figures 41, 42), with 47 more taxa of *Trigonostemon*, confirm its phylogenetic position, and clearly show monophyly of the genus.

Dimorphocalyx (c. 13 species mainly occur in SE Asia, van Welzen & van Oostrum 2015) was established based on *D. glabellus* Thwaites (1861), a species with two whorls of stamens (5+5) of which only the inner whorl has connate stamens. The genus is also monophyletic and is separate from *Trigonostemon* in both molecular analyses (Figures 41, 42). The precise relationship between *Dimorphocalyx* and *Trigonostemon* is still unclear (Figure 41), as the relationships of most genera in the C2 clade are still unresolved. Moreover, *Dimorphocalyx* can be morphologically distinguished from *Trigonostemon* by its predominantly dioecious sexual system (vs. always monoecious in the latter), the stamens being more than 7 and often arranged in two (or three) whorls (Figure 40k; vs. 3 or 5 connate stamens in a single whorl in *Trigonostemon*, Figure 40a–d), and the white petals (Figure 40k; vs. petals of various colours including white in *Trigonostemon*, Figure 40a, b, e, f). Therefore, following most botanists

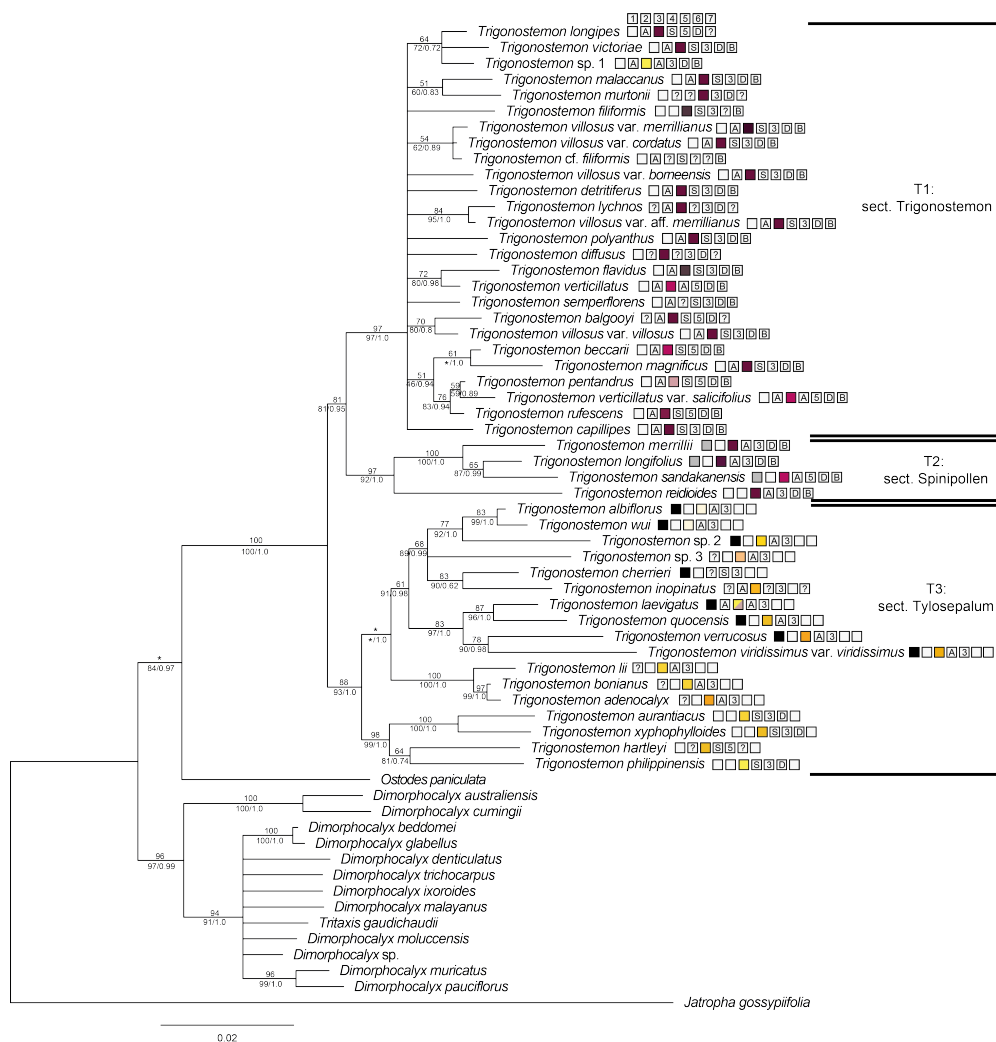


Figure 42 Phylogenetic relationship of selected species of *Trigonostemon*, *Dimorphocalyx* (now *Tritaxis*), *Ostodes* and *Tritaxis gaudichaudii* based on nuclear ITS and chloroplast *trnK* intron, *trnT-L* and *trnL-F* sequences. The branch lengths are based on the BI method. The number above the clades is the bootstrap credibility from the MP analysis, and the numbers below the clades are the bootstrap credibility from the ML analysis and the posterior probability from the BI analysis. The clades are omitted if the MP or ML bootstrap credibility is below 50. Asterisks indicate a different topology of the relevant clade in the MP or ML analysis. Next to each taxon of *Trigonostemon* are seven characters of the pollen and macromorphology (from left to right): 1. pollen type: □ = *Trigonostemon reidioides* type and subtype pollen, ■ = *Trigonostemon reidioides* type *Trigonostemon longifolius* subtype pollen, ■ = *Trigonostemon verrucosus* type pollen; 2. pistillate sepals: ▣ = accrescent when fruiting, □ = not accrescent; 3. the colour of petals; 4. disc shape (both sexes): ▣ = scales, ▣ = annular; 5. number of stamens; 6. anthers: ▣ = divaricate at the top, □ = not divaricate; 7. stigmas: ▣ = deeply bifid, □ = not obviously bifid, ▣ = unknown.

(e.g., Bentham 1878, 1880 and Pax 1890), we keep *Trigonostemon* and *Dimorphocalyx* as two separate genera.

Tritaxis was described in 1858 by Baillon, three years earlier than *Dimorphocalyx*, mainly on the basis of the three whorls of stamens in the type species, *T. gaudichaudii* Baill. (hence its name; for illustration see Gagnepain 1925b). In the following years, a few more species were published under *Tritaxis*, but they were then all transferred to other genera (see Introduction and Taxonomic treatment). The type species of *Tritaxis*, *T. gaudichaudii*, is embedded in the *Dimorphocalyx* clade (Figure 42), indicating the congeneric status of the two genera. Furthermore, by the modern generic definition of *Dimorphocalyx*, species with three whorls of stamens are included in the genus (van Welzen & van Oostrum 2015), which also lends weight to merging these two genera together. Because *Tritaxis* is an older name, *Dimorphocalyx* is now treated as its synonym.

The rare species *Dimorphocalyx cumingii* (Müll.Arg.) Airy Shaw, endemic to the Philippines, was originally proposed under *Trigonostemon* sect. *Anisotaxis*, but was later treated under *Tritaxis* (Bentham 1878; Pax 1910; Merrill 1923) or *Dimorphocalyx* (Airy Shaw 1969), because different delimitations of *Trigonostemon* were applied. The species has fewer stamens (8–9, in two whorls) and more condensed inflorescences than most *Dimorphocalyx* species, but the characters are still well in line with *Dimorphocalyx*. This is also supported by the molecular phylogeny, as it is embedded in the *Dimorphocalyx* clade (Figure 42) sister to *D. australiensis* C.T.White. As a result of the synonymisation discussed above, the name *Tritaxis cumingii* (Müll.Arg.) Benth. is the accepted name.

Ostodes is a small genus with two species characterised by a relatively bigger tree size (up to 16 m tall and a diameter at breast height up to 40 cm) and more stamens (20–41, Figure 40l) compared to *Dimorphocalyx* and *Trigonostemon*. The pistillate calyx does not enlarge when mature, which is also obviously different from the other two genera. In the molecular analyses (Figure 41), *Ostodes* is separate from both *Dimorphocalyx* and *Trigonostemon* as a unique clade, potentially sister to *Vernicia montana* (0.77 posterior probability). *Vernicia* Lour. is also a small genus with three morphologically similar species (Stuppy et al. 1999, 2005). The genus is quite different from *Ostodes*, as *Vernicia* often has lobed leaves with different basilaminar glands (also in the sinuses when lobed), much larger flowers, different disc glands in both sexes, the stamens are much more united (also the outer whorl) than *Ostodes*, and the fruits are much larger, tardily dehiscent and up to 5-locular. *Vernicia* was not included in our matrix 2 and the phylogeny by Wurdack et al. (2005) does not show enough resolution, thus more molecular analyses are needed to solve the relationships. As both genera are easy to separate and recognize, we refrain from uniting them.

Evolution of the ‘croton pattern’ ornamentation in pollen morphology

Within the *Trigonostemon* group, three major clades can be recognised, noted as T1, T2 and T3 (Figure 42). These clades correlate well with the pollen morphology of the genus (Yu et al. 2019c; Figure 42 character 1). Generally, the species in clade T1 have *Trigonostemon reidioides* type and *reidioides* subtype pollen (Figure 40m, n), whereas the T2 clade represents the *Trigonostemon reidioides* type *longifolius* subtype pollen (Figure 40o, p) and the T3 clade the *Trigonostemon verrucosus* type pollen (Figure 40q, r).

It is noteworthy that the taxa that basally branch in the T2 and T3 clades, i.e., *T. reidioides* (T2), and *T. aurantiacus*, *T. hartleyi*, *T. philippinensis* and *T. xyphophylloides* (T3), also have

the same pollen as the T1 clade, while their sister groups are both monophyletic with good supporting values (97 MP and 92 ML bootstrap credibility and 1.0 BI posterior probability in the T2 clade, 88 MP and 93 ML bootstrap credibility and 1.0 BI posterior probability in the T3 clade). Moreover, the *reidioides* subtype pollen bears the same tectum ornamentation as in the outgroups *Dimorphocalyx*, *Ostodes* and *Jatropha*; thus the 'croton pattern' ornamentation with clearly discernible obtuse 'subunits' is plesiomorphic, except that the pollen is considerably smaller in the average size (Yu et al. 2019c). On the other hand, the *longifolius* subtype pollen and the *Trigonostemon verrucosus* type pollen are probably never found in any other taxa in the Euphorbiaceae. This potentially reveals an evolutionary trend in which the common 'croton pattern' ornamented pollen in the Euphorbiaceae starts specializing in *Trigonostemon* in two directions: in the T2 clade, the 'croton pattern' ornamentation remains but the subunits reduce to spines; in the T3 clade, the 'croton pattern' ornamentation collapses by merging the subunits together into bigger 'lumps' supported by more collumellae.

A difference in the position of three species in the T3 clade was found between the trees reconstructed by different methods (see Results), but the pollen morphology of these three species (*T. adenocalyx*, *T. bonianus* and *T. lili*) is unknown. Thus, they are not considered in the discussion here for the moment. Pollen of *Trigonostemon inopinatus* Airy Shaw (Figure 40s, t) appears aberrant among all the other species as it has lost the 'croton pattern' ornamentation but has many 'spines' on the muri of the tectum. It was temporarily classified in the *Trigonostemon reidioides* type *longifolius* subtype, but molecular phylogeny shows that it belongs to the T3 clade, together with species with the *Trigonostemon verrucosus* type pollen. This also conforms with our discussion of the loss of 'croton pattern' ornamentation in the T3 clade.

Infrageneric classification of Trigonostemon

Morphologically, species of the T3 clade are easiest to distinguish from the others, irrespective of the discrepancy in the placement of *T. adenocalyx* Gagnep., *T. bonianus* Gagnep. and *T. lili* Y.T.Chang (see above): they have lighter colours in petals, often white, yellow to orange (Figure 40b, f; Figure 42 character 3) compared to often dark reddish purple (Figure 40e) in the other two clades; the stigmas are not bifid or sometimes only with a very shallow groove at the top (Figure 40f, h; Figure 42 character 7), whereas in the other species, the stigmas are always deeply divided (Figure 40e, g). In addition, the sepals of pistillate flowers are often not much accrescent (for example of accrescent/non-acrescent sepals, see Figure 40i, j; Figure 42 character 2) except for *T. inopinatus*. Two sub-groups of the T3 clade are distinguished from each other by the stamens: divaricate at apex (for example of divaricate stamens, see Figure 40a, c; Figure 42 character 6) forming a V-shaped structure with an often conical appendix (see the description of *Prosartema* Gagnep. [= *Trigonostemon*], Gagnepain 1924b) or numerous cells of secretion on the connective (discussed in Yu & van Welzen 2018) in *T. aurantiacus*, *T. philippinensis* and *T. xyphophylloides* (unknown for *T. hartleyi*), which is also present in the T1 and T2 clades; in the other species, the anthers are short and round without splitting at the top (for example, see Figure 40b, d; stamens sometimes very slightly divaricate as in *T. capitellatus*).

The T1 and T2 clades have a closer relationship: these clades ally with each other as a sister of the T3 clade in the phylogram (supported by 81 both MP and ML bootstrap credibility and 0.95 BI posterior probability). This is reflected in their closer pollen ('croton pattern' ornamentation, see above) and macromorphology: often dark reddish purple petals (except for *Trigonostemon* sp. 1), divaricate stamens (for example, see Figure 40a, c) and bifid stigmas (for example, see

Figure 40e, g). However, they are different in the pistillate sepals and disc shape: in the T1 clade the pistillate sepals are often obviously accrescent when fruiting (they are at least twice the size as those in the staminate flowers; for example, see Figure 40j; Figure 42 character 2) and the disc comprises separate lobes or glands (except in *T. verticillatus*; for example, see Figure 40c, g; Figure 42 character 4), whereas in the T2 clade, the pistillate sepals are not much accrescent (for example, see Figure 40i) and the disc is annular (for example, see Figure 40d). Moreover, the division in the stigma in the T2 clade is often shallower than in the T1 clade.

The number of stamens (either 3 or 5) is considered an important character for species delimitation (Yu & van Welzen 2018), because it provides a reliable evidence of morphological discontinuity. However, this character does not strictly correspond to any of the clades (Figure 42 character 5), therefore, it is not used in the infrageneric classifications.

Among the previous infrageneric classifications, Jablonski's (1963) seems the closest to the molecular phylogeny, as he precisely recognised the importance of the division of the stigmas and the variation in stamens, but he wrongly designated *T. longifolius* as the type for sect. *Trigonostemon* (because the autonomous section has the same type as the genus, i.e., | *T. serratus*, even though the latter was not included in his revision), resulting in misplacements of species in sect. *Telogyne* Baill., which should be sect. *Trigonostemon*. Also, *T. laevigatus* Müll.Arg., according to his criteria, should be placed in sect. *Tylosepalum* (Kurz) Benth. not sect. *Trigonostemon*, but this was because of lack of material.

The molecular phylogeny suggests three sections for the genus. Although the type species of *Trigonostemon*, *T. serratus*, is not included in the molecular phylogeny (no suitable material available), its morphology, e.g., the accrescent pistillate sepals, glandular disc, divaricate stamens and bifid stigmas strongly indicates a position within the T1 clade, which therefore, forms the autonomous sect. *Trigonostemon*. A new sect. *Spinipollen* R.Y.Yu & Welzen is proposed for the T2 clade with *T. longifolius* as type, and the T3 clade represents a third sect. *Tylosepalum* with *T. aurantiacus* as type. In addition, Benth (1878) proposed the sect. *Pycnanthera* Benth. for *T. diplopetalus* Thwaites and *T. nemoralis* Thwaites, which are two rare species characterised by the sessile stamens adnate to a greatly thickened connective (or joined connectives). These two species have a strictly limited distribution: *T. diplopetalus* is endemic to Sri Lanka and *T. nemoralis* is only distributed in Travancore (S India) and Sri Lanka. No material was available for the molecular analyses (for more descriptions and illustrations of the species, see Trimén 1898, Balakrishnan & Chakrabarty 1991 and Philcox 1997), but their greatly thickened connective, among others, certainly displays a morphological discontinuity and therefore sets them apart from other species as a separate sect. *Pycnanthera* (not included in the phylogeny; Figure 42). *Trigonostemon diplopetalus* is selected as the type of this section. There is one exception in the literature citations in the taxonomic treatment: Pax & Hoffmann (1911) also included *Trigonostemon lanceolatus* (S.Moore) Pax in sect. *Pycnanthera*, but based on its morphology it most likely belongs to sect. *Trigonostemon*.

Taxonomic treatment of *Trigonostemon*

The accepted species are based on Yu & van Welzen (2018), Balakrishnan & Chakrabarty (1991), Phattarahirankanok & Chayamarit (2005) and Li & Gilbert (2008); only the protologue of each taxon is given.

Trigonostemon Blume, Bijdr. Fl. Ned. Ind. (1825) 600 ('*Trigostemon*'); Fl. Javae (1828) Preface 8 (name corrected in note), nom. et orth. cons. — Type: *Trigonostemon serratus* Blume.

Enchidium Jack, Malayan Misc. 2 (1822) 89, nom. rej. — Type: *Enchidium verticillatum* Jack [= *Trigonostemon verticillatus* (Jack) Pax].

Key to the sections

1. Stigmas deeply bifid (at least 1/2 the length of stigma) 2
1. Stigmas not or slightly (less than 1/2 the length of stigma) divided (often bifid; but twice divided in *T. diplopetalus*) 3
2. Pistillate sepals accrescent when fruiting (at least twice the size as those in staminate flowers), disc (both staminate and pistillate flowers) of five scales or glands (except annular with 5-toothed protrusions in *T. verticillatus*) sect. 1. ***Trigonostemon***
2. Pistillate sepals not accrescent when fruiting, disc (both staminate and pistillate flowers) annular sect. 2. ***Spinipollen***
3. Petals white, yellow, orange, occasionally light pinkish red; filaments connate as an androphore sect. 3. ***Tylosepalum***
3. Petals red or dull purple; filaments absent, anthers sessile, clustered on a greatly thickened connective (or joined connectives) sect. 4. ***Pycnanthera***

1. ***Trigonostemon*** sect. ***Trigonostemon*** — Type: *Trigonostemon serratus* Blume.

Silvaea Hook. & Arn., Bot. Beechey Voy. (1837) 211. — *Trigonostemon* Blume sect. *Silvaea* (Hook. & Arn.) Müll.Arg., Linnaea 34 (1865) 214. — Type: *Silvaea semperflorens* (Roxb.) Müll.Arg. Hook. & Arn. [= *Trigonostemon semperflorens* (Roxb.) Müll.Arg.].

Telogyne Baill., Étude Euphorb. (1858) 327, pl. 11, f. 13. — *Trigonostemon* Blume sect. *Telogyne* (Baill.) Müll.Arg., Linnaea 34 (1865) 214. — Type: *Telogyne indica* Baill. [= *Trigonostemon verticillatus* (Jack) Pax].

1. ***Trigonostemon balgooyi*** R.Y.Yu & Welzen, Blumea 62 (2018) 188, f. 2. — Type: *van Balgooy 7102* (holo L, barcode L.2258686; iso K, KEP, barcode KEP222394, L, barcode L.2258685, SAN, accession 051745), Malaysia, Johor, Endau-Rompin, Kuala Jasin, 2°32'N, 103°22'E, 100 m, lowland rainforest on clay soil, 1 Mar 1996.
2. ***Trigonostemon beccarii*** Ridl., Bull. Misc. Inform. Kew (1925) 89. — Type: *Beccari PS 965* (BM, K, barcode K000959300, L, barcode L0016478), Indonesia, Sumatra, Padang, Sungei Buluh, Sep 1878.
3. ***Trigonostemon capillipes*** (Hook.f.) Airy Shaw, Kew Bull. 20 (1967) 413. — Type: *Lobb s.n.* (K, barcode K000894763), Singapore.
4. ***Trigonostemon detritiferus*** R.I.Milne, Kew Bull. 49 (1994) 446, f. 2. — Type: *Dransfield 6913* (holo K, barcode K000959297; iso BRUN, accession B008840, K, barcode K000959298), Brunei, Temburong, Selapon, village margin, 4°40'N, 115°12'E, 15 m, old secondary forest on alluvial flat, 18 Nov 1990.
5. ***Trigonostemon diffusus*** Merr., Sarawak Mus. J. 3 (1928) 525. — Type: *Mjöberg 145* (BM, K, barcode K000959296, UC, barcode UC231637), Malaysia, Sarawak, foot of Mt. Poi, 1924.

6. *Trigonostemon filiformis* Quisumb., Philipp. J. Sci. 41 (1930) 328, f. 7. 1930. — Type: *Clemens 16751* (A, barcode A00048894, NY, barcode NY00273349, UC, barcode UC347527), Philippines, Luzon, Isabela, Mt. Moises, Apr 1926.
7. *Trigonostemon flavidus* Gagnep., Bull. Soc. Bot. France 69 (1922) 749. — Type: *Harmand 3273* (P, barcodes P00717104, P00717105), Laos, in Mount Lakhon near Me-Kong, Sep 1877.
8. *Trigonostemon longipes* (Merr.) Merr., Philipp. J. Sci. 11 (1916b) 191. — Lectotype (designated by Yu & van Welzen, 2018): *FB (Whitford) 1066* (lecto K, barcode K000959374; isolecto P, barcode P00648673), Philippines, Luzon, Bataan, Mount Mariveles, Jan 1905.
9. *Trigonostemon lychnos* (R.I.Milne) R.Y.Yu & Welzen, Blumea 62 (2018) 201. — Type: *Coode 6766* (holo K, barcode K000959280; iso BRUN, accession B013567), Brunei, Belait, Andulau Forest Reserve, compartment 7, 4°35'N, 114°33'E, forest, 14 Apr 1990.
10. *Trigonostemon magnificus* R.I.Milne, Kew Bull. 50 (1995b) 51, f. 1, ('*magnificum*'). — Type: *de Wilde & de Wilde-Duyfjes 19441* (holo K, barcode K000959284; iso BO, accession BO1835092, BO, no accession number, K, barcode K000959283, KLU, accession J2349, L, barcode L.2260380, P, barcode P04804057), Indonesia, Sumatra, Aceh, c. 75 km WNW of Medan, Gunung Leuser National Park, Sikunder Forest Reserve, Besitang River, c. 3°55'N, 98°05'E, 50–100 m, marshy forest, 6 Aug 1979.
11. *Trigonostemon malaccanus* Müll.Arg., Flora 47 (1864a) 482. — Type: *Griffith KD 4782* (K, barcode K000959325), Malaysia, Malacca.
12. *Trigonostemon murtonii* Craib, Bull. Misc. Inform., Kew (1911) 464, ('*murtoni*'). — Type: *Murton 18* (K, barcode K000959311), Thailand, west coast, Koh Klone.
13. *Trigonostemon pentandrus* Pax & K.Hoffm. in Engl., Pflanzenr. IV.147 vii (1914) 406. — Neotype (designated by Yu & van Welzen, 2018): *KEP FRI (Yu) 86653* (neo L; isoneo KEP), Malaysia, Negeri Sembilan, Gunung Angsi, Bukit Putus to Angsi summit, before the first rest gazebos, mixed forest, understorey on sandy soil, 16 Dec 2018.
14. *Trigonostemon polyanthus* Merr., Philipp. J. Sci. 9 (1914) 492. — Type: *BS (Ramos) 1645* (BM, BO, accession BO1722340, BRI, barcode BRI-AQ0342683, CAL, barcode CAL0000023675, G, barcode G00435098, GH, barcode GH00048899, L, barcode L.2258801, NY, barcode NY00273354, P, barcode P00717156, SING), Philippines, Samar, Cauayan Valley, Apr 1914.
15. *Trigonostemon rufescens* Jabl., Brittonia 15 (1963) 152, f. 2. — Type: *SFN (Corner) 29428* (holo SING, barcode SING0051354; iso A, barcode A00048887, BO, K, barcode K000959319, L, barcode L.2258748, SAN, accession 0106235), Malaysia, Johore, 13.5 miles on Mawai – Jemaluang Road, altitude low, 13 May 1935.
16. *Trigonostemon semperflorens* (Roxb.) Müll.Arg. in A.DC, Prodr. 15 (1866) 1110. — Lectotype (designated here): Roxburgh's *Flora Indica* drawing, no. 2401 (CAL).

17. *Trigonostemon verticillatus* (Jack) Pax in Engl., Pflanzenfam. IV.147 iii (1911) 87. — Neotype (designated by Yu & van Welzen, 2018): *Maingay 1403* (neo L, barcode L.2258683; isoneo BM, CAL, barcode CAL0000031928, K), Malaysia, Penang.
 18. *Trigonostemon victoriae* R.Y.Yu & Welzen in Blumea 62 (2018) 215, f. 15. — Type: *PNH (Sulit) 12317* (holo L, barcode L.2260416; iso L, barcode L.2260417, PNH, on 3 sheets), Philippines, Palawan Province, Aborlan, Panacan, SE base of Victoria Mountains, partially open at the bank of Karaniogan river, 10 May 1950.
 19. *Trigonostemon villosus* Hook.f., Fl. Brit. India 5: 397. 1887. — Syntypes: *King's collector s.n.* (K, barcode K000959324), Malaysia, Perak; *King's collector 4819* (CAL, barcode CAL0000023668), Malaysia, Perak, Larut, Gopeng, 500–800 ft, Aug 1883; *King's collector 2400* (CAL, barcode CAL0000023669), Malaysia, Perak, Larut, 1000–2000 ft, Sep 1881.
- The following species are not included in the molecular phylogeny but also belong to this section based on morphology:
20. *Trigonostemon calcicolus* (R.I.Milne) R.Y.Yu & Welzen, Blumea 62 (2018) 189. — Type: *Chew 679* (holo K, barcode K000959281), Malaysia, Sarawak, 1st Division, Kuching District, Tiang Bekap, Mt. Mentawa, 1°12' N, 110°23'E, 250 ft, on limestone.
 21. *Trigonostemon dipteranthus* Airy Shaw, Kew Bull. 20 (1966) 47. — Type: *Korthals s.n. ('853')* (L, barcode L0160131), Sumatra, West Coast Res., Padang.
 22. *Trigonostemon kerrii* Craib, Bull. Misc. Inform., Kew (1924) 97. — Lectotype (designated by Chantaranothai 2005): *Kerr 5871* (lecto BK, barcode BK239811; isolecto A, barcode A00048870, P, barcode P04810877), Thailand, Nakawn Tai, c. 200 m, evergreen forest, 17 Apr 1922.
 23. *Trigonostemon laetus* Baill. [Étude Euphorb. (1858) 341, nom. nud.] ex Müll.Arg. in A.DC., Prodr. 15 (1866) 1109. — Type: *Wallich 7740B* (syn G-DC, barcodes G00319757, on 2 sheets, G00319769, P, barcodes P00717111, P00717112; isosyn: CAL, barcode CAL0000023673), Myanmar, Amherst.
 24. *Trigonostemon lanceolatus* (S.Moore) Pax in Engl., Pflanzenr. IV.147 iii (1911) 93. — Type: *Beddome s.n.* (BM, barcode BM000951502), Myanmar, Tenasserim, Mooleyit slopes.
 25. *Trigonostemon pierreii* Gagnep., Bull. Soc. Bot. France 69 (1922) 752. — Type: *Pierre 1530* (A, barcode A00048875, K, barcode K000959307, P, barcodes P00717121, P00717122, P00717123, US, barcode US00096541), Vietnam, in insula Phu Quoc ad montibus Ong Chao, Apr 1874.
 26. *Trigonostemon phyllocalyx* Gagnep, Bull. Soc. Bot. France 69 (1922) 753. — Type: *Poilane 2700* (A, barcode A00048879, P, barcodes P00717119, P00717120), Vietnam, Annam, Nhatrang, versant grande montagne de Đông-Bô, versant de la mer NE, 5 Mar 1922.

27. *Trigonostemon scopulatus* R.Y.Yu & Welzen, *Blumea* 62 (2018) 210, f. 14. — Type: *KEP FRI (Cockburn) 7859* (holo L, barcode L.2258747; iso K, KEP, barcode KEP157633, SING, accession 531533), Malaysia, Johore, Ulu Endau, Labis FR, compartment 277, disturbed forest near logging road, 22 Mar 1968.
28. *Trigonostemon serratus* Blume, *Bijdr. Fl. Ned. Ind.* (1825) 600, ('*serratum*'). — Lectotype (designated by Yu & van Welzen, 2018): *Blume 1648* (lecto L, barcode L.2258717; isolecto L, barcodes L.2258713, L.2258714, L.2258716), Indonesia, Java.
29. *Trigonostemon sinclairii* Jabl., *Brittonia* 15 (1963) 154, f. 3, 4. — Type: *SFN (Sinclair & Kiah bin Salleh) 40418* (holo SING), Malaysia, Terengganu, Block 3B, Gunong Tebu F.R., 51st mile Kuala Terengganu – Berut Road, 10 Nov 1954.
30. *Trigonostemon wetriifolius* Airy Shaw & Ng, *Malaysian Forester* 41 (1978) 237, f. 1–3. — Type: *KEP FRI (F.S.P. Ng) 27157* (holo K; iso KEP), Malaysia, S Pahang, Lesong Forest Reserve in proposed Endau-Rompin National Park, primary forest under logging, 6 Sep 1977.
31. *Trigonostemon wildeorum* R.Y.Yu & Welzen, *Blumea* 62 (2018) 222, f. 20. — Type: *de Wilde & de Wilde-Duyfjes 20274* (holo L, barcode L.2258570; iso L, barcode L.2258569, BO, accessions BO000954, BO000955), Indonesia, Sumatra, Aceh, Middle Alas River (Lae Sauraya) area, c. 15 km N of Gelombang, S of Bengkong River, 2°55' N, 99°57' E., c. 50 m, foot hill forest, on yellow-red loamy soil, 23 Jul 1985.
2. *Trigonostemon* sect. *Spinipollen* R.Y.Yu & Welzen, *sect. nov.* — Type: *Trigonostemon longifolius* Wall. ex Baill.

Diagnosis: Pistillate sepals not accrescent; petals in both staminate and pistillate flowers spatulate, base claw-like, disc annular; stigmas apically bifid into 2 arms.

Description: Shrubs or small trees. *Stipules* subulate to falcate, often pubescent. *Leaves*: petiole up to 3.5 cm long, often less than 1/7 the length of the blade, hairy; blade oblanceolate, oblong to elliptic, 4–46 by 1–15 cm, glabrous or hairy. *Inflorescences* unisexual or bisexual, axillary or terminal, paniculate or thyrsoid, flowers arranged in racemes or clustered in very short cymes or glomerules along the main rachis. *Staminate flowers*: pedicel slender, pubescent, base articulate; sepals elliptic, margin entire or undulate, apex acute to rounded, glabrous or pubescent outside, glabrous inside; petals often spatulate, pink, red, dark purple or rarely white, base slightly claw-like, outer surface glabrous, inner surface glabrous or very rough (*T. sandakanensis*), sometimes with 2 gland lobes (*T. longifolius*); disc annular; stamens 3 or 5, connate in a column, anthers divaricate, connective often apically forming an appendage with few expanded cells of secretion; pollen grains with 'croton pattern' ornamentation, subunits reduced to spines. *Pistillate flowers*: pedicel longer and broader than staminate ones; sepals very slightly or not accrescent when fruiting; petals and disc as staminate flowers; ovary slightly to densely pubescent, sometimes warty (*T. longifolius*); style short, stigmas bifid into 2 arms. *Fruits* glabrous, warty or hairy; exocarp often partly detaching.

1. *Trigonostemon longifolius* Wall. ex Baill., Étude Euphorb. Atlas (1858) 23. pl. 11. f. 12, ('*longifolium*'). — Lectotype (designated by Yu & van Welzen, 2018): *Wallich* 7717 (lecto K, barcode K000959328; isolecto BR, barcode BR0000005105366, G-DC, barcode G00319787, GH, barcode GH00048885, K, barcode K000959327), Malaysia, Penang.
2. *Trigonostemon merrillii* Elmer, Leaflet. Philipp. Bot. 4: 1304. 1911. — Type: *Elmer* 12819 (BISH, barcode BISH1001684, BM, BO, accession BO1722342, F, barcode F0057180F, G, barcodes G00435100, G00435101, HBG, barcode HBG515778, L, barcode L0160280, MO, barcode MO260397, NY, barcode NY00273350, US, barcode US00096538), Philippines, Palawan, Puerto Princesa (Mt. Pulgar), Mar 1911.
3. *Trigonostemon reidioides* (Kurz) Craib, Bull. Misc. Inform., Kew (1911) 464. — Syntypes: *Teijsmann* HB 5981 (K, barcode K000959313, U, barcode U.1260354), Thailand, Kanburi [= Kanchanaburi]; *Teijsmann* s.n. (CAL, barcode CAL0000023659), Thailand, Kanburi [= Kanchanaburi].
4. *Trigonostemon sandakanensis* Jabl., Brittonia 15: 159, f. 5, 6. — Type: *SAN* (Wood) 16018 (holo SING, barcode SING0051356; iso KEP, barcode KEP222404, L, barcode L.2258760), Malaysia, Sabah, Kabili-Sepilok Forest Reserve, 15 miles W of Sandakan, compartment 10, 15 ft above sea level, 21 Apr 1955.

The following species are not included in the molecular phylogeny but also belong to this section based on morphology:

5. *Trigonostemon hybridus* Gagnep., Bull. Soc. Bot. France 69 (1922) 750. — Type: *Pierre* 6282 (P, barcode P00717109, other duplicates are syntypes of *T. rubescens*, see below), Vietnam, Phu-Quoc, Mar 1874.
6. *Trigonostemon oblongifolius* Merr., Philipp. J. Sci. 7 (1912) 409. — Type: *BS* (Ramos) 13965 (A barcode A00048898, US, barcode US00096540), Philippines, Luzon, Cagayan, Abulug River, Feb 1912.
7. *Trigonostemon rubescens* Gagnep., Bull. Soc. Bot. France 69 (1922) 754. — Syntypes: *Pierre* 6281 (P, barcodes P00717139, P00648668, P00648669, P00648670), Cambodia, Kompong Spen, Mont Ramcon, Jan 1870; *Pierre* 6282 (K, barcode K000959312, P, barcodes P00717143, P00717144, P00717145), Vietnam, Phu-Quoc, Mar 1874; *Thorel* 2290 (NY, barcode NY00273346, P, barcodes P00717140, P00717141, P00717142, US, barcode US00096544), Laos, Kong et île de Khon, 1866–1868.

3. ***Trigonostemon* sect. *Tylosepalum*** (Kurz) Benth. in Benth. & Hook.f., Gen. Pl. 3 (1880) 298.

Tylosepalum Kurz ex Teijsm. & Binn., Natuurk. Tijdschr. Ned.-Indië 27 (1864) 50. 1864. — *Codiaeum* A.Juss. sect. *Tylosepalum* (Kurz ex Teijsm. & Binn.) Müll.Arg. in A.DC., Prodr. 15 (1866) 1117. — *Trigonostemon* Blume sect. *Tylosepalum* (Kurz ex Teijsm. & Binn.) Pax & K.Hoffm. in Engl & Harms in Nat. Pflanzenfam. ed. 2, 19c (1931) 169. — Type: *Tylosepalum aurantiacum* Kurz ex Teijsm. & Binn. [= *Trigonostemon aurantiacus* (Kurz ex Teijsm. & Binn.) Boerl.].

Trigonostemon Blume sect. *Dichotomae* Merr., Univ. Calif. Publ. Bot. 10 (1924) 425. — Type: *Trigonostemon petelotii* Merr. [= *Trigonostemon laevigatus* Müll.Arg.].

Prosartema Gagnep., Bull. Soc. Bot. France 71 (1924b) 875. — Type: *Prosartema stellaris* Gagnep. [= *Trigonostemon stellaris* (Gagnep.) Airy Shaw].

Neotrigonostemon Pax & K.Hoffm., Notizbl. Bot. Gart. Berlin-Dahlem 10 (1928) 385. — Type: *Neotrigonostemon diversifolius* Pax & K.Hoffm. [= *Trigonostemon viridissimus* (Kurz) Airy Shaw].

Kurzi dendron N.P.Balakr., Bull. Bot. Surv. India 8 (1966) 68. — Type: *Kurzi dendron viridissimum* (Kurz) N.P.Balakr. [= *Trigonostemon viridissimus* (Kurz) Airy Shaw].

1. ***Trigonostemon albiflorus*** Airy Shaw, Kew Bull. 25 (1971) 547. — *Trigonostemon leucanthus* Airy Shaw var. *siamensis* H.S.Kiu in H.S.Kiu & X.X.Chen, Guihaia 12 (1992) 211. — Type: *Winit 1704* (syn K, barcodes K000959303, K000959304, isosyn BK, barcode BK257891, BM, barcode BM000951503), Thailand, Lampang, Mê Pêng, 110–200 m, evergreen forest, by stream, 19 Jun 1926.
2. ***Trigonostemon aurantiacus*** (Kurz ex Teijsm. & Binn.) Boerl., Handl. Fl. Ned. Ind. 3 (1900): 284. — Lectotype (designated by Yu & van Welzen, 2018): *Teijsmann s.n.* (lecto L, barcode L.2260196), Indonesia, Java, cultivated in Bogor Botanical Garden.
3. ***Trigonostemon bonianus*** Gagnep., Bull. Soc. Bot. France 69 (1922) 747. — Lectotype (designated by Li et al., 2006): *Bon 718* (lecto P, barcode P00717086), Vietnam, Mount Trui, 12 Sep 1881.
4. ***Trigonostemon cherrieri*** J.M.Veillon, Bull. Mus. Natl. Hist. Nat., B, Adansonia, sér. 4, 14 (1992) 55. — Type: *Veillon 7385* (holo P, barcode P00057693; iso K, barcode K000959366, L, barcode L0016479, P, barcodes P00057694, P00057695, P00057696, MO, barcode MO260396, NOU, barcodes NOU005872, NOU005874), New Caledonia, Poya, sud de Mepouiri, non loin de la côte, 10 m, Forêt sclerophylle, en sous-bois, argiles noires tropicales, 25 Jun 1991.
5. ***Trigonostemon hartleyi*** Airy Shaw, Kew Bull. 33 (1979) 535. — Type: *Hartley 11087* (holo BRI, barcode BRI-AQ0342687; iso L, barcode L0043309), New Guinea, Morobe, Busu, S of the Busu River, c. 12 miles N of Lae, 500 ft, rainforest on slope, 8 Jan 1963.
6. ***Trigonostemon inopinatus*** Airy Shaw, Kew Bull. 31 (1976) 396. — Type: *Webb & Tracey 7762* (holo BRI, barcode BRI-AQ0205473, on 2 sheets), Australia, Queensland, Cawley State Forest west of Cathu between Mackay & Proserpine, 2000 ft, rainfall 70 inch, mixed notophyll vine forest on soils derived from granite, Jun 1965.

7. *Trigonostemon laevigatus* Müll.Arg., Flora 47 (1864b) 538. — Lectotype (designated by Yu & van Welzen, 2018): *Motley* 686 (lecto K, barcode K000959291), Indonesia, South Kalimantan, Bangarmassing [= Banjarmasin], 1857–1858.
8. *Trigonostemon lii* Y.T.Chang, Guihaia 3 (1983) 175. — Type: *Li* 4576 (holo KUN, barcode KUN1294357), China, Yunnan, Xishuangbanna Botanical Garden, on limestone hill, 25 Jun 1963.
9. *Trigonostemon philippinensis* Stapf, Leaflet. Philipp. Bot. 1 (1907) 206, ('*philippinense*'). — Type: *Elmer* 8326 (BO, accession BO1298668, G, barcode G00435099, K, barcode K000959370, L, barcode L.2260355), Philippines, Luzon, Laguna, Los Baños, May 1906.
10. *Trigonostemon quocensis* Gagnep., Bull. Soc. Bot. France 69 (1922) 753. — Syntypes: *Pierre* s.n. (K, barcode K000959314, P, barcodes P00717135, P00717136, P00717138), Vietnam, Phu Quoc, Mar 1874; *Godefroy* 739 (P, barcode P00717131, P00717132), Vietnam, Ha-tien, 20 Sep 1875; *Godefroy* 740 (P, barcode P00717130), Vietnam, Ha-tien, 20 Sep 1875; *Pierre* 6232 (G, barcode G00435097, P, barcodes P00717133, P00717134, P00717137, MPU, barcodes MPU015001, MPU015002, NY barcode NY00273345), Vietnam, Chaudoc, Mount Pell, Dec 1867; *Harmand* 632 (P, barcodes P00717128, P00717129), Vietnam, Nui Cam, Jun 1876.
11. *Trigonostemon viridissimus* (Kurz) Airy Shaw, Kew Bull. 25 (1971) 545. — Type: *Kurz* s.n. (K, barcodes K000246871, K000246871), India, South Andaman, Port Mouat.
12. *Trigonostemon verrucosus* J.J.Sm., Bull. Jard. Bot. Buitenzorg ser. 3, 6 (1924) 97. — Type: *Bogor Botanical Garden VIII.E.16* (BO, accessions BO1298241, BO1298242, BO1298243, IBSC, barcode IBSC0306957, K, barcode K000959299, L, barcode L.2258669, SING, U, barcode U0002105), Cultivated in Bogor Botanical Garden, 20 May 1896.
13. *Trigonostemon wui* H.S.Kiu, J. Trop. & Subtrop. Bot. 3 (1995) 19, f. 2. — Type: *Kiu* 451 (holo IBSC, not found; iso IBK, barcode IBK00190788), China, Guangdong, Fengkai, Yulao, 80 m, foot of limestone hill, 12 May 1995.
14. *Trigonostemon xyphophylloides* (Croizat) L.K.Dai & T.L.Wu, Acta Phytotax. Sin. 8 (1963) 277. — Type: *Wang* 34005 [cited as '34006' in Croizat 1940, probably a typo; marked as holotype in A by L.C.M. Croizat] (A, barcode A00045961, IBK, barcode IBK00169539, IBSC, K, barcode K000959334), China, Hainan, Ya County, in dense forest, 17 Sep 1933.

The following species are not included in the molecular phylogeny but also belong to this section based on morphology:

15. *Trigonostemon adenocalyx* Gagnep., Bull. Soc. Bot. France 69 (1922) 747. — Type: *Unknown collector* (P, barcode P00717084), Indochina?
16. *Trigonostemon apetalogyne* Airy Shaw, Kew Bull. 33 (1979) 534. — Type: *Kostermans & Soegeng* 340 (holo K; iso BO), Indonesia, West Papua, limestone hills E of Sukarnapura [= Hollandia = Djajapura], Polima I, c. 100 m, 13 Aug 1966.

17. ***Trigonostemon birmanicus*** Chakrab. & N.P.Balakr., J. Econ. Taxon. Bot. 5 (1984a) 175. — Type: *Chin* [collector's name uncertain] 5849 (holo CAL, barcode CAL0000023654), Myanmar, Upper Chindwin, Numpakom drainage, 1400 ft, on loamy soil, 29 Apr. 1911.
18. ***Trigonostemon capitellatus*** Gagnep., Bull. Soc. Bot. France 69 (1922) 748. — Syntypes: *Thorel s.n.* (P, barcode P00717089), Laos, Île de Khon, 1866–1868; *Pierre* 472 (P, barcode P00717093), Vietnam, ad flumen Dongnai prope Tri Huyen in prov. Bien Hoa, Sep 1869; *Pierre* 1323 (K, barcode K000959315, P, barcodes P00717090, P00717091, P00717092, US00433339), Vietnam, ad flumen Dongnai prope Tri Huyen in prov. Bien Hoa, Mar 1873.
19. ***Trigonostemon cochinchinensis*** Gagnep., Bull. Soc. Bot. France 69 (1922) 748. — Type: *Pierre* 1869 (K, barcode K000959316, P, barcodes P00717094, P00717095, P00717096), Vietnam, Bao Chiang, Jul 1877.
20. ***Trigonostemon chinensis*** Merr., Philipp. J. Sci., Bot. 21 (1922) 498. — Type: *Tsoong* (PE, barcode PE01110916, PNH, accession 88948), China, Guangxi, Tung Sing [= Dongxing], Dongjingshan, 21 [22 in protologue] Jun 1918.
21. ***Trigonostemon eberhardtii*** Gagnep., Bull. Soc. Bot. France 69 (1922) 749. — Syntypes: *Bon* 5239 (P, barcode P00717103), Vietnam, Annam, Thanh-hoa; *Bon* 5465 (P, barcodes P00717099, P00717100), Vietnam, Annam, Son-thôn; *Eberhardt* 4293 (P, barcodes P00717101, P00717102), Vietnam, Tonkin, Hoa-binh, Mai-ha.
22. ***Trigonostemon fragilis*** (Gagnep.) Airy Shaw, Kew Bull. 32 (1978): 415. — Type: *Poilane* 2927 (A, barcode A00106974, P, barcodes P00712172, P00712173, P00712174), Vietnam, Annam, Île Tre près de Nhatrang, 10 Apr 1922.
23. ***Trigonostemon harmandii*** Gagnep., Bull. Soc. Bot. France 69 (1922) 750. — Type: *Harmand* 2956 (P, barcodes P00648665, P00648666, P00648667), Cambodia, 1875–1877.
24. ***Trigonostemon heteranthus*** Wight, Icon. Pl. Ind. Orient. 5 (1852) 24, t. 1890. — Syntypes: *Griffith s.n.* (K, barcode K000246861), Myanmar, Mergui; *Griffith* KD 4796 (K, barcode K000246910, P, barcode P00717107), Myanmar, Mergui; *Helper* KD 4796 (K, barcode K000246862), Myanmar, Mergui.
25. ***Trigonostemon nigrifolius*** N.P.Balakr. & Chakrab., J. Econ. Taxon. Bot. 5 (1984b) 173. — Type: *Po Khaut* 12434 (holo DD), Myanmar, Maymyo, Gokteik viaduct, 16 May 1929.
26. ***Trigonostemon poilanei*** Gagnep., Bull. Soc. Bot. France 69 (1922) 753. — Type: *Poilane* [*Chevalier* & *Poilane* in protologue] 40807 (A, barcode A00048878, P, barcode P00717127), Vietnam, Bienhoa, Giaray, 22 May 1919.
27. ***Trigonostemon thorelii*** Gagnep., Bull. Soc. Bot. France 69 (1922) 755. — Type: *Thorel* 2264 (A, barcode A00048877, P, barcodes P00648671, P00648672, P00717148), Laos, Stung Tréng à Kong, 1866–1868.

28. *Trigonostemon tuberculatus* F.Du & Ju He, Kew Bull. 65 (2010) 111, f. 1, 2. — Type: *Du, He & Zhang 200401* (holo SWFC), China, Yunnan, Yuanjiang, 460 m, in secondary shrub on slope of dry hill, 1 May 2004.

4. *Trigonostemon* Blume sect. *Pycnanthera* Benth., J. Linn. Soc., Bot. 17 (1878) 225. — Lectotype (designated by Yu & van Welzen 2018): *Trigonostemon diplopetalus* Thwaites.

Nepentandra S.Moore in J. Bot. 43 (1905) 149. — Type: *Nepentandra lanceolata* S.Moore [= *Trigonostemon lanceolatus* (S.Moore) Pax].

1. *Trigonostemon diplopetalus* Thwaites, Enum. Pl. Zeyl. (1861) 277. — Type: *Thwaites CP 578* (A, barcode A00048867, BR, barcode BR0000005105045, CAL, barcodes CAL0000023676, CAL0000023677, FR, barcode FR0036073, G, barcode G00435106, G-DC, barcode G00319791, GH, barcode GH00048868, P, barcodes P00717097, P00717098, K, barcode K000246865), Sri Lanka, Reigam Corle, Palahattoo, at no great elevation, Sep 1858.

2. *Trigonostemon nemoralis* Thwaites, Enum. Pl. Zeyl. (1861) 277. — Types: *Thwaites CP 3570* (A, barcode A00048874, CAL, barcodes CAL0000023670, CAL0000023671, FR, barcode FR0036074, G-DC, barcodes G00319812, G00319788, GH, barcode GH00048873, K, barcodes K000246866, K000246867, K000246868, K000246869, NY, barcode NY00273343, P, barcodes P00717116, P00717117, P00717118), Sri Lanka, Central Province, Madamahanewera, 2000 ft.

Taxonomic treatment of *Tritaxis*

Taxonomic treatment of *Dimorphocalyx* spp. is based on van Welzen & van Oostrum (2015), Chakrabarty & Balakrishnan (1990), Venkata Raju & Pullaiah (1994), Gagnepain (1924a) and Pax & Hoffmann (1924); only the protologue of each taxon is given.

Tritaxis Baill., Étude Euphorb. (1858) 342. — Type: *Tritaxis gaudichaudii* Baill.

Dimorphocalyx Thwaites, Enum. Pl. Zeyl. (1861) 278. — *Trigonostemon* Blume sect. *Dimorphocalyx* (Thwaites) Müll.Arg., Linnaea 34 (1865) 212. — Type: *Dimorphocalyx glabellus* Thwaites.

1. *Tritaxis australiensis* S.Moore, J. Linn. Soc., Bot. 45 (1920) 218. — Type: *Damel s.n.* (BRI, barcode BRI-AQ0202253, K, barcode K000894750), Australia, Queensland, Cape York, Cook pastoral district, Mar 1868.

Dimorphocalyx australiensis C.T.White, Proc. Roy. Soc. Queensl. 47 (1936) 80. — Type: *Brass 2019* (holo BRI, barcode BRI-AQ0342526; iso A, barcode A00047607, K, barcode K000894749, US, barcode US01108415), Australia, Queensland, Mowbray River, Rainforest, 23 Jan 1932.

2. ***Tritaxis balakrishnanii*** (Chakrab. & Premanath) R.Y.Yu & Welzen, *comb. nov.* — *Dimorphocalyx balakrishnanii* Chakrab. & Premanath, J. Econ. Taxon. Bot. 4 (1983) 1013. — Type: *Ansari 1368A* (holo CAL), India, Andaman-Nicobar Islands, Havelock Island, 40 m, 15 May 1874; *Ansari 1368B* (iso PBL), India, Andaman-Nicobar Islands, Havelock Island, 40 m, 15 May 1874; *Ansari 1368C* (iso PBL), India, Andaman-Nicobar Islands, Havelock Island, 40 m, 15 May 1874; *Ansari 1368D* (iso PBL), India, Andaman-Nicobar Islands, Havelock Island, 40 m, 15 May 1874; *Ansari 1368E* (iso PBL), India, Andaman-Nicobar Islands, Havelock Island, 40 m, 15 May 1874.
Dimorphocalyx dilipianus N.P.Balakr. & Chakrab., J. Econ. Taxon. Bot. 4 (1983) 1017. — Type: *Nair 498A* (holo CAL), India, Andaman-Nicobar Islands, South Andaman Island, Carbyn's Cove, c. 20 m, 10 Oct 1973; *Nair 498B* (iso PBL), India, Andaman-Nicobar Islands, South Andaman Island, Carbyn's Cove, c. 20 m, 10 Oct 1973; *Nair 498C* (iso PBL), India, Andaman-Nicobar Islands, South Andaman Island, Carbyn's Cove, c. 20 m, 10 Oct 1973; *Nair 498D* (iso PBL), India, Andaman-Nicobar Islands, South Andaman Island, Carbyn's Cove, c. 20 m, 10 Oct 1973; *Nair 498E* (iso PBL), India, Andaman-Nicobar Islands, South Andaman Island, Carbyn's Cove, c. 20 m, 10 Oct 1973.
3. ***Tritaxis beddomei*** Benth., J. Linn. Soc., Bot. 17 (1878) 221. — *Dimorphocalyx beddomei* (Benth.) Airy Shaw in Kew Bull. 23 (1969) 124. — Type: *Beddome 37* (K, barcode K000246899), India, Tinnevely.
4. ***Tritaxis cumingii*** (Müll.Arg.) Benth., J. Linn. Soc., Bot. 17 (1878) 221. — *Trigonostemon cumingii* Müll.Arg., Linnaea 34 (1865) 213. — *Dimorphocalyx cumingii* (Müll.Arg.) Airy Shaw, Kew Bull. 23 (1969) 124. — Type: *Cuming 1693* (holo G-DC, barcode G00319795; iso BM, barcodes BM000951496, BM000951497, E, barcode E00570192, G, barcode G00435094, K, barcodes K000959395, K000959396, L, barcodes L0016166, L0016167, P, barcode P00640260), Philippines, 1841.
5. ***Tritaxis denticulatus*** (Merr.) R.Y.Yu & Welzen, *comb. nov.* — *Dimorphocalyx denticulatus* Merr., Philipp. J. Sci. 4 (1909) 278. — Type: *FB (Whitford & Hutchinson) 9033* (K, barcode K000959394, US, barcode US00096562), Philippines, Mindanao, Zamboanga, Port Banga, 30 m, in dipterocarp forests, Nov–Dec 1907.
Dimorphocalyx murinus Elmer, Leaflet Philipp. Bot. 4 (1911) 1285, ('*murina*'). — Lectotype (designated by van Welzen & van Oostrum, 2015): *Elmer 12844* (lecto L, barcode L0245421; islecto BM, G, barcode G00434828, GH, barcode GH00047605, HBG, barcode HBG516375, K, barcode K000959390, L, barcode L0245421, NY, barcode NY00804228, P, barcode P00712200, U, barcode U0226764, US, barcode US00731069), Philippines, Palawan, Puerto, Princesa (Mt. Pulgar), Mar 1911.
Dimorphocalyx loheri Merr., Philipp. J. Sci. 27 (1925) 30. — Lectotype (designated by van Welzen & van Oostrum, 2015): *Loher 12467* (lecto UC, barcode UC241187; islecto A, barcode A00047604), Philippines, Luzon, Rizal, Montalban., Jun 1909.
6. ***Tritaxis gaudichaudii*** Baill., Étude Euphorb. (1858) 343. — Lectotype (designated here): *Gaudichaud 278* (lecto P, barcode P00712262; islecto P, barcodes P00712260, P00712261), Vietnam, 1836–1837. Other syntypes: *Gaudichaud 296* (P, barcodes P00712258, P00712259), Vietnam, 1836–1837.

7. ***Tritaxis glabellus*** (Thwaites) R.Y.Yu & Welzen, *comb. nov.* — *Dimorphocalyx glabellus* Thwaites, Enum. Pl. Zeyl. (1861) 278. — Type: *Thwaites C.P. 1046* (BR, barcode BR0000005101849, FR, barcode FR0031457, G, barcode G00434940, P, barcodes P00712195, P00712196, P00712197), Sri Lanka.

- 7a. ***Tritaxis glabellus*** (Thwaites) R.Y.Yu & Welzen var. ***glabellus***
- 7b. ***Tritaxis glabellus*** (Thwaites) R.Y.Yu & Welzen var. ***lawianus*** (Nimmo) R.Y.Yu & Welzen, *comb. nov.* — *Croton lawianus* Nimmo, Cat. Pl. Bombay (1839) 251. — *Trigonostemon lawianus* (Nimmo) Müll.Arg., Linnaea 34 (1865) 212. — *Dimorphocalyx lawianus* (Nimmo) Hook.f., Fl. Brit. India 5 (1887) 404. — *Dimorphocalyx glabellus* Thwaites var. *lawianus* (Nimmo) Chakrab. & N.P.Balakr., Proc. Indian Acad. Sci (Plant Sci) 100 (1990) 296. — Type: *Gibson 19* (K, barcode K000246856), India, Bombay, Bheema Snnker.

8. ***Tritaxis ixoroides*** (C.B.Rob.) R.Y.Yu & Welzen, *comb. nov.* — *Ostodes ixoroides* C.B.Rob., Philipp. J. Sci. 6 (1911) 332. — *Dimorphocalyx ixoroides* (C.B.Rob.) Airy Shaw, Kew Bull. 20 (1967) 412. — Type: *FB (Klemme) 13426* (US, barcode US00096560), Philippines, Luzon, Ilocos Norte, Bangui, Apr 1909.

9. ***Tritaxis kurnoolensis*** (R.R.V.Raju & Pull.) R.Y.Yu & Welzen, *comb. nov.* — *Dimorphocalyx kurnoolensis* R.R.V.Raju & Pull., Bot. Bull. Acad. Sin. 35 (1994) 201. — Type: *Venkata Raju 6661* (holo MH; iso SKU), India, Andhra Pradesh, Kurnool, Owk stream.

10. ***Tritaxis malayanus*** (Hook.f.) R.Y.Yu & Welzen, *comb. nov.* — *Dimorphocalyx malayanus* Hook.f., Fl. Brit. India 5 (1887) 404. — Lectotype (designated by van Welzen & van Oostrum, 2015): *Griffith KD 4785* (lecto K, barcode K000894751; isolecto A, barcode A00139220, P, barcode P00648681), Malaysia, Malacca.
Dimorphocalyx kunstleri King ex Hook.f., Fl. Brit. India 5 (1887) 405. — Type: *King's collector 1455* (K, barcode K000894756), Malaysia, Penang, Mar 1881.
Dimorphocalyx luzoniensis Merr., Philipp. J. Sci. 5 (1910) 192. — Lectotype (designated by van Welzen & van Oostrum, 2015): *FB (Tamesis) 11907* (lecto K, barcode K000959392; isolecto US, barcode US00096564), Philippines, Luzon, Prov. of Laguna, Los Baños, 22 Jan, 1910.
Dimorphocalyx beccarii Gagnep., Bull. Soc. Bot. France 71 (1924a) 621. — Lectotype (designated by van Welzen & van Oostrum, 2015): *Beccari PB 2215* (lecto P, barcode P00648685; isolecto A, barcode A00047603, K, barcodes K000959398, K000959400), Malaysia, Sarawak.
Trigonostemon bulusanensis Elmer, Leaflet Philipp. Bot. 10 (1939) 3735. — *Dimorphocalyx bulusanensis* (Elmer) Airy Shaw, Kew Bull. 27 (1972b) 92. — Type: *Elmer 17296* (G, barcode G00435102, HBG, barcode HBG515781, L, barcode L.2204370, MO, barcode MO260399, NY, barcode NY00179801, S, accession S07-17047, U, barcode U0226748), Philippines, Luzon, Sorsogon, Irosin (Mt. Bulusan), 2000 ft, in well shaded woods.

11. ***Tritaxis meeboldii*** (Pax & K.Hoffm.) R.Y.Yu & Welzen, *comb. nov.* — *Dimorphocalyx meeboldii* Pax & K.Hoffm. in Engl., Pflanzenr. IV. 147 xvi (1924) 190. — Type: *Meebold 16837* (?), Myanmar, Moulmein, Papun.

12. ***Tritaxis moluccensis*** (Welzen & Oostrum) R.Y.Yu & Welzen, *comb. nov.* — *Dimorphocalyx moluccensis* Welzen & Oostrum, *Blumea* 59 (2015) 198. — Type: *de Vogel 3134* (holo L, barcode L.2204209), Indonesia, N Moluccas, Halmahera, Ekor, side of Gunung Panjang, c. 0°49' N, 127°52'E, 15 m, rather dense primary forest 35 m high, with little undergrowth, rather steep hill side on deep clay soil, 27 Sep 1974.

13. ***Tritaxis muricatus*** (Hook.f.) R.Y.Yu & Welzen, *comb. nov.* — *Ostodes muricata* Hook.f., *Fl. Brit. India* 5 (1887) 401. — *Ostodes muricata* Hook.f. var. *genuina* Pax in Pax & K.Hoffm. in Engl., *Pflanzenr.* IV.147 iii (1911) 21, nom. inval. — *Dimorphocalyx muricatus* (Hook.f.) Airy Shaw, *Kew Bull.* 20 (1967) 412. — Type: *King's collector 3162* (K, barcode K000894757), Malaysia, Perak, Larut, within 500 ft, dense jungle, Aug 1882.
Trigonostemon asahanensis Croizat, *J. Arnold Arbor.* 23 (1942a) 54. — Type: *Rahmat Si Boeea 9872* (holo A, barcode A00048880; iso L, barcode L.2204342), Indonesia, Sumatra, Asahan, vicinity of Tomoean Dolok, c. 1000m, 1–20 Aug 1936.
Ostodes muricata Hook.f. var. ? *minor* Hook.f., *Fl. Brit. India* 5 (1887) 401. — *Dimorphocalyx muricatus* (Hook.f.) Airy Shaw var. *minor* (Hook.f.) Airy Shaw, *Kew Bull.* 20 (1967) 412. — Lectotype (designated by van Welzen & van Oostrum, 2015): *Lobb 304* (lecto BM, barcode BM000541792; isolecto E, barcode E00314237, GH, barcodes GH00139221, GH00139222, K, barcode K000894761, L, barcode L0245384), Singapore.

14. ***Tritaxis pauciflorus*** (Merr.) R.Y.Yu & Welzen, *comb. nov.* — *Ostodes pauciflorus* Merr., *Philipp. J. Sci.* 11 (1916a) 72. — *Dimorphocalyx pauciflorus* (Merr.) Airy Shaw, *Kew Bull.* 20 (1967) 413. — Type: *Hose 244* (A, barcode A00048886, K, barcodes K000959402, K000959403, L, barcode L0016394, P, barcode P00648684), Malaysia, Sarawak, Baram District, Baram, Mar 1895.

15. ***Tritaxis poilanei*** (Gagnep.) R.Y.Yu & Welzen, *comb. nov.* — *Dimorphocalyx poilanei* Gagnep., *Bull. Soc. Bot. France* 71 (1924a) 622. — Type: *Poilane 4786* (A, barcode A00135094, K, barcode K000959404, P, barcodes P00648682, P00648683), Vietnam, Annam, Nui Han Heo près de Nhatrang, sol rocheux, forêt, 300 m, 28 Sep 1922.

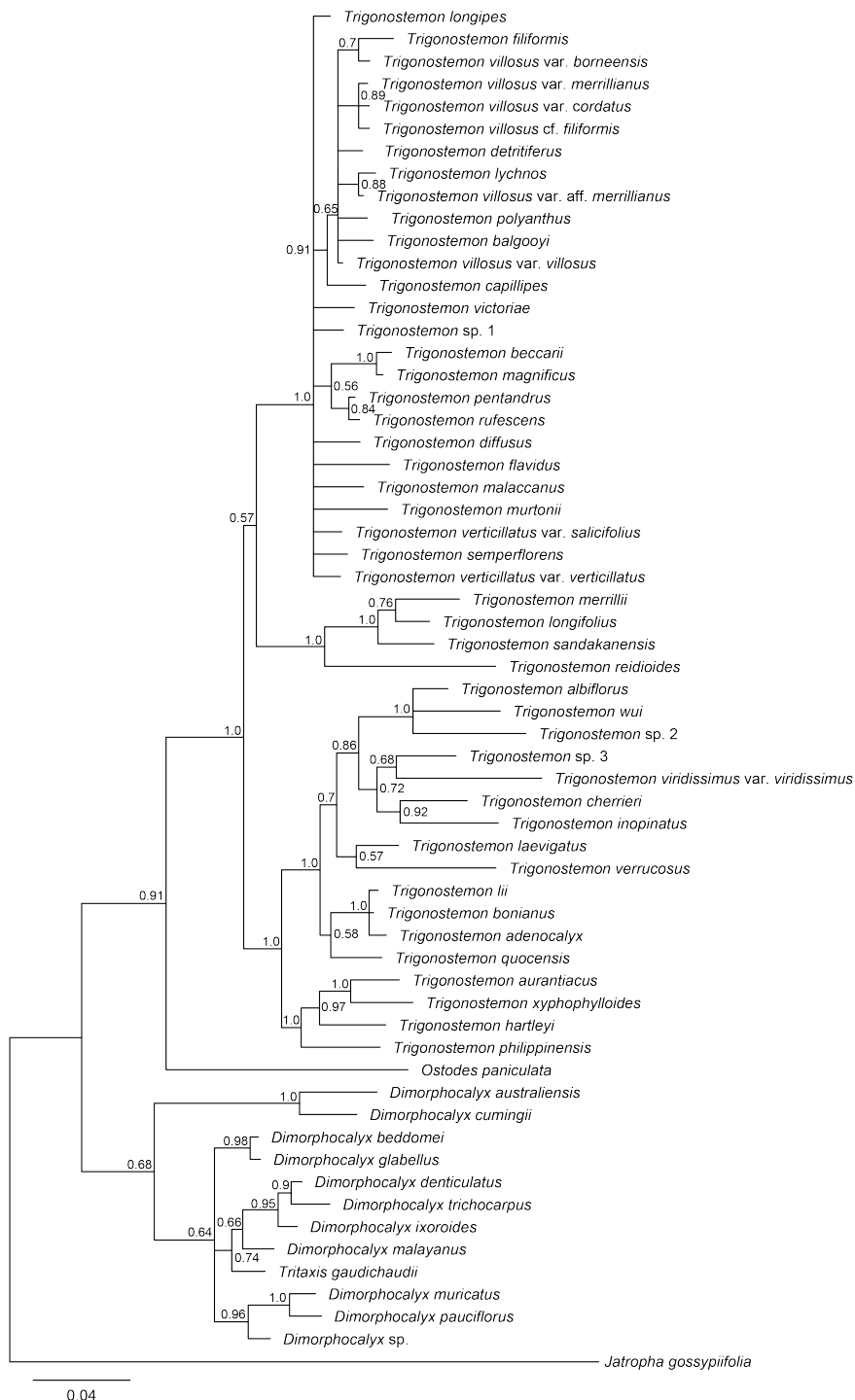
16. ***Tritaxis trichocarpus*** (Airy Shaw) R.Y.Yu & Welzen, *comb. nov.* — *Dimorphocalyx trichocarpus* (Airy Shaw) Welzen & Oostrum, *Blumea* 59 (2015) 199. — *Dimorphocalyx luzoniensis* Merr. var. *trichocarpus* Airy Shaw, *Kew Bull.*, *Addit. Ser.* 4 (1975) 96. — Type: *S (Anderson) 20974* (holo K, barcode K000959401; iso L, barcode L.2182719), Malaysia, Sarawak, First Division, Bidi, Bau, 300 ft, on limestone rocks, with intervening igneous derived soil, at base of limestone hill, 12 Apr 1965.

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Research Institute Malaysia), Maribel E. Agoo (De La Salle University, Manila, the Philippines) and John Rey Callado (National Museum of the Philippines) are thanked for their assistance with the field work. The last author thanks the Treub-Maatschappij for supporting the Ornstein chair in Tropical Plant Biogeography.

Supplementary material



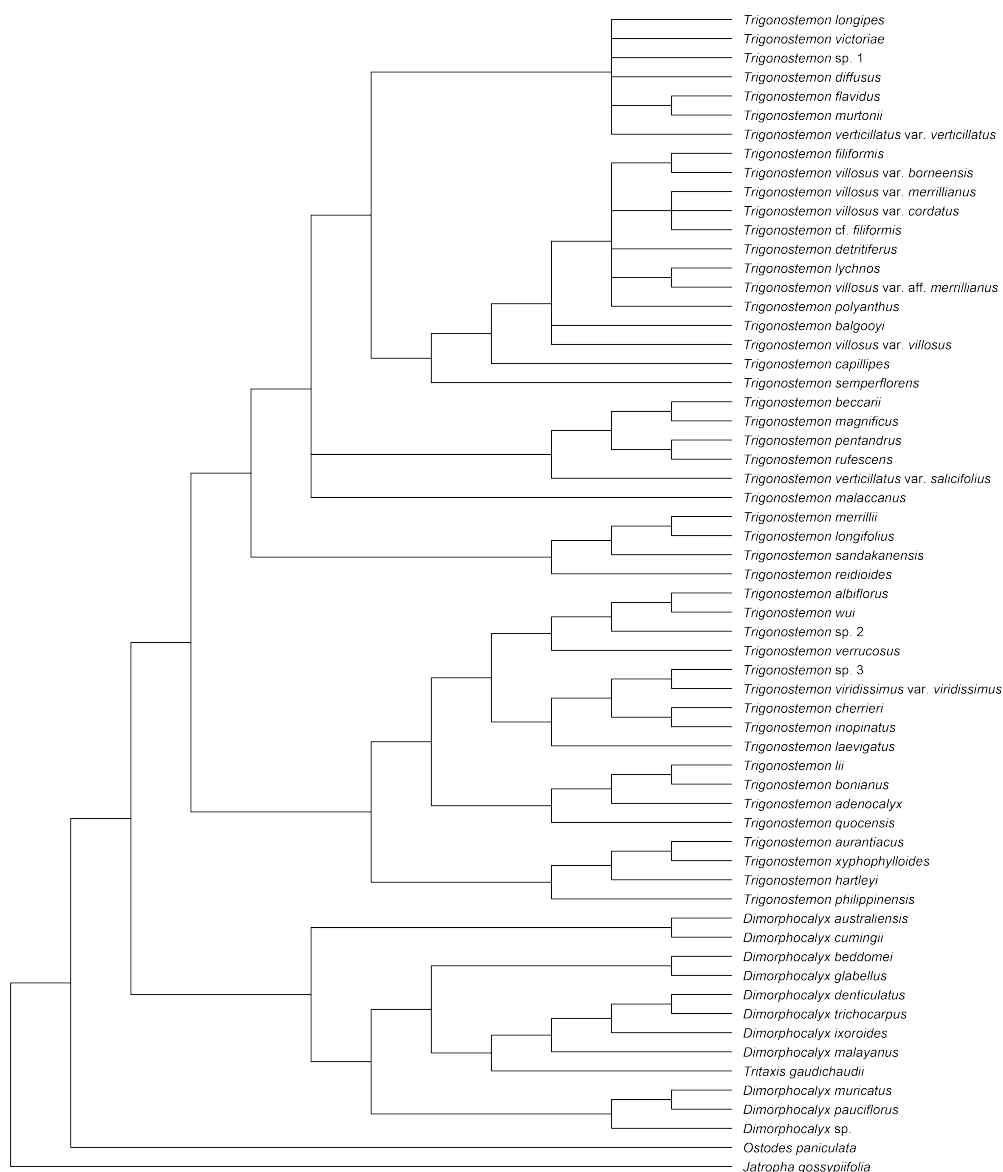
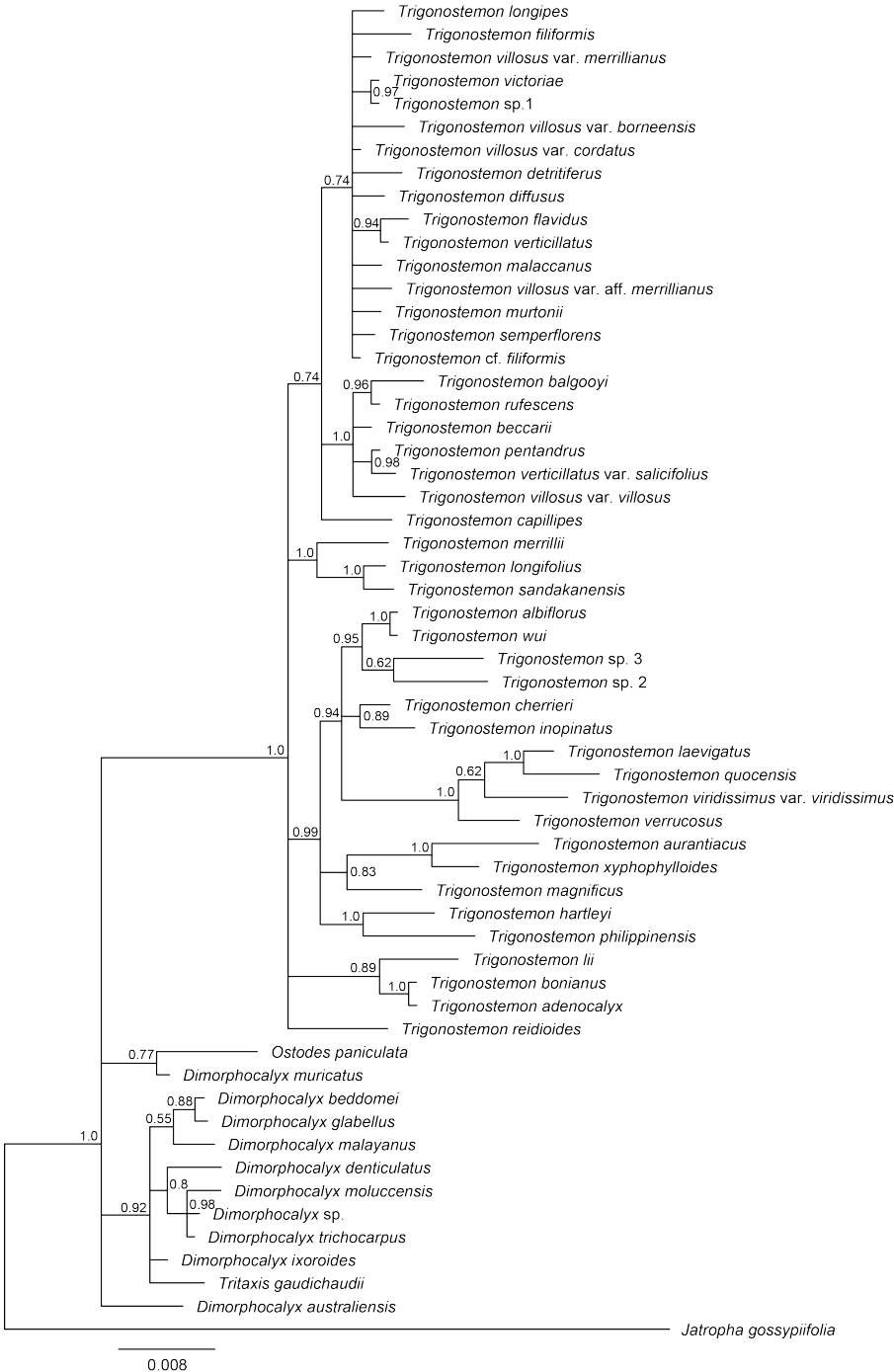


Figure 44 Phylogenetic relationship of selected species of *Trigonostemon*, *Dimorphocalyx*, *Ostodes* and *Tritaxis* using the maximum parsimony method. The phylogram was reconstructed based on the nuclear ITS sequence. The 50% consensus of the best-scored trees is shown.

Figure 43 Phylogenetic relationship of selected species of *Trigonostemon*, *Dimorphocalyx*, *Ostodes* and *Tritaxis* using the Bayesian inference method. The phylogram was reconstructed based on the nuclear ITS sequence. The numbers next to the clades are the posterior probability from the BI analysis. The clades are omitted if the posterior probability is below 0.55.



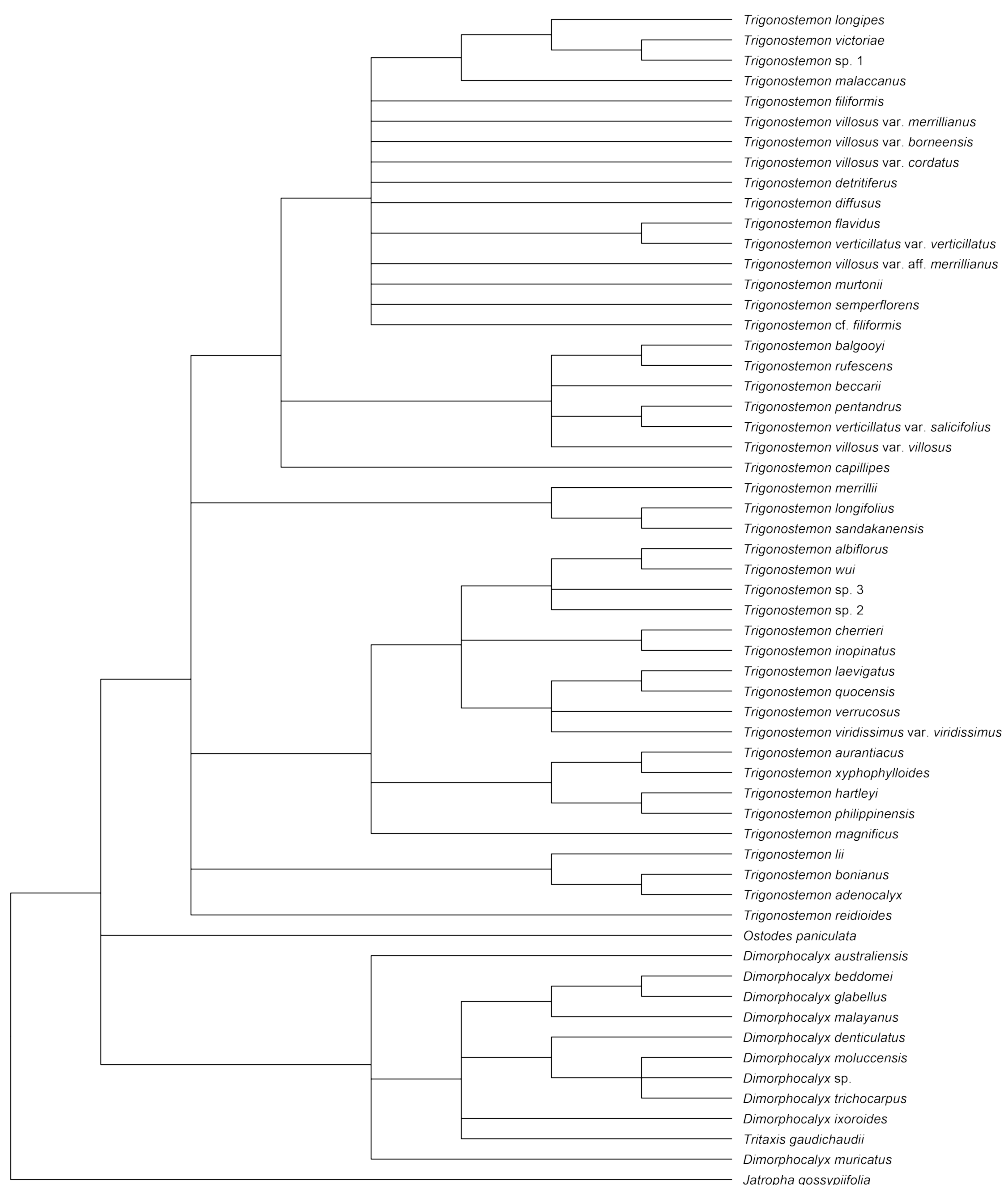
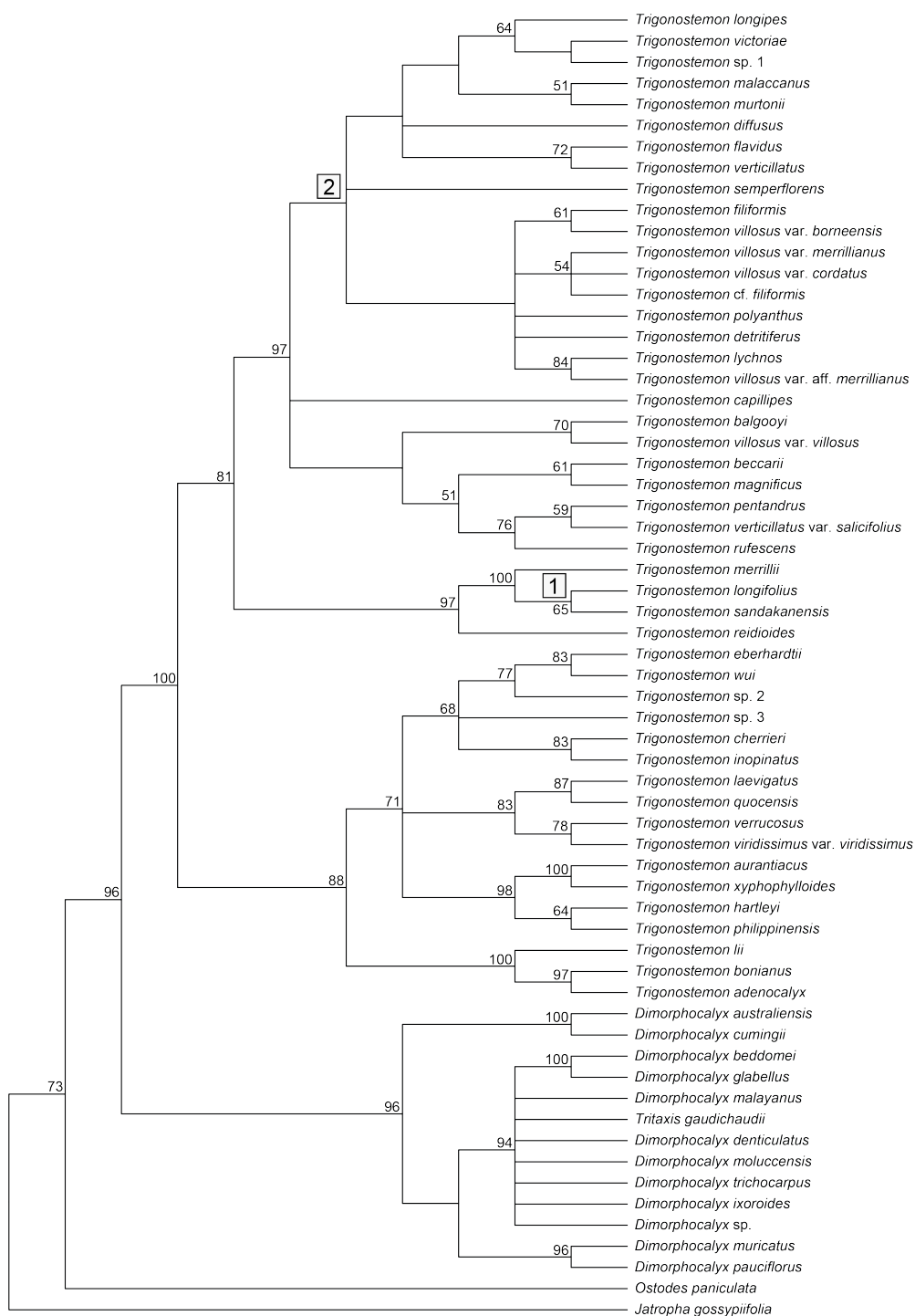


Figure 46 Phylogenetic relationship of selected species of *Trigonostemon*, *Dimorphocalyx*, *Ostodes* and *Tritaxis* using the maximum parsimony method. The phylogram was reconstructed based on the chloroplast *trnK* intron, *trnT-L* and *trnL-F* sequences. The 50% consensus of the best-scored trees is shown.

Figure 45 Phylogenetic relationship of selected species of *Trigonostemon*, *Dimorphocalyx*, *Ostodes* and *Tritaxis* using the Bayesian inference method. The phylogram was reconstructed based on the chloroplast *trnK* intron, *trnT-L* and *trnL-F* sequences. The numbers next to the clades are the posterior probability from the BI analysis. The clades are omitted if the posterior probability is below 0.55.



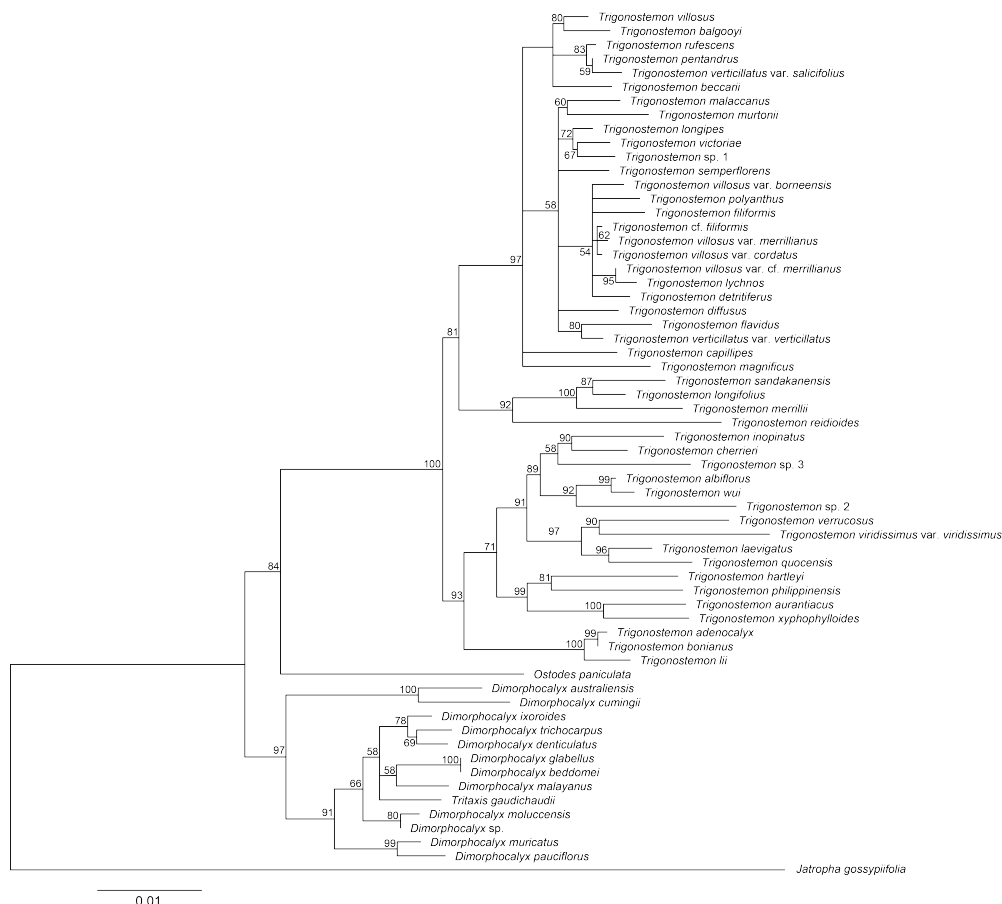


Figure 48 Phylogenetic relationship of selected species of *Trigonostemon*, *Dimorphocalyx*, *Ostodes* and *Tritaxis* based on the nuclear ITS and the chloroplast *trnK* intron, *trnT-L* and *trnL-F* sequences. The phylogram was reconstructed using the maximum likelihood method. The numbers next to the clades are the bootstrap supports. The clades are omitted if the bootstrap support is below 50.

Figure 47 Phylogenetic relationship of selected species of *Trigonostemon*, *Dimorphocalyx*, *Ostodes* and *Tritaxis* using based on the nuclear ITS and the chloroplast *trnK* intron, *trnT-L* and *trnL-F* sequences. The phylogram was reconstructed using the maximum parsimony method. The 50% consensus of the best-scored trees is shown. The bootstrap support are shown next to nodes if the value is above 50. Two major parsimony informative indels are marked on the phylogram.