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# Molecular phylogenetic and morphological support for the recognition of *Friesodielsia lalisae* (*Annonaceae*), a new species from S Thailand

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**Abstract:** Thirteen species of *Friesodielsia* Steenis (*Annonaceae*) and 11 representatives of related genera were included in molecular phylogenetic analyses using up to six plastid DNA regions (*psbA-trnH*, *trnL-trnF* intergenic spacers; *trnL* intron; *matK*, *ndhF*, *rbcL* exons). The results support the recognition of a new species, *F. lalisae* Damth., Baka & Chaowasku from Narathiwat, S Thailand, as belonging to one of the two major clades of *Friesodielsia*. The members of this clade show outer petals that separate at anthesis and have a concave basal portion, while members in the other major clade exhibit outer petals that separate early during developmental stages and have a flat base. *Friesodielsia lalisae* is described and illustrated. It is most morphologically similar to *F. argentea* (J. Sinclair) Steenis and *F. glauca* (Hook. f. & Thomson) Steenis but differs from the two by having dissimilar sepal shape, higher proportion of inner petal to outer petal length and longer inner petals. The new species additionally differs from the former by having different leaf base and from the latter by having denser indumentum on young twigs and shorter flowering pedicels. Narathiwat, a province to which the new species is endemic, seems to be one of the most underexplored areas in Thailand as evidenced by a number of species described based on recent material. The conservation status of the new species is provisionally assessed as Critically Endangered.

Keywords: Annonaceae, Friesodielsia, molecular phylogeny, Narathiwat, new species, systematics, taxonomy, Thailand, Uvarieae

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#### Introduction

The pantropical family Annonaceae consists of trees, shrubs and lianas classified in 109 genera (Jaikhamseub & al. 2022 plus subsequent publications: Cheek & al. 2022 [Lukea Cheek & Gosline, a recently established genus]; Couvreur & al. 2022 [acceptance of Dennettia Baker f.]) and about 2550 species (Couvreur & al. 2022). According to Bangkomnate & al. (2021), the liana genera Pyramidanthe Miq. and Mitrella Miq. are considered congeneric, with the acceptance of the former name. Friesodielsia Steenis (Annonoideae, Uvarieae), comprising c. 48 species (Turner 2018; Saunders & al. 2020; Leeratiwong & al. 2021a, 2023), is also one of the genera with a liana habit (Turner 2012; Guo & al. 2017a). The genus underwent recent realignments based on molecular phylogenetic inferences, with the recombination of most African species names into Monanthotaxis Baill. and a few into Afroguatteria Boutique and Sphaerocoryne (Boerl.) Scheff. ex Ridl.; therefore, its distribution is restricted to tropical Asia plus New Guinea (Guo & al.

2017a; Saunders & al. 2020). In addition to the liana habit, Friesodielsia can also be circumscribed by the presence of (1) a more or less glaucous lower leaf surface, (2) a usually obvious pair of glands at the base of each leaf blade, (3) initially terminal inflorescences developing to become internodal (or sometimes leaf-opposed), (4) inner petals that are smaller than the outer petals and remain connivent at maturity and (5) usually single-seeded monocarps (e.g. Leeratiwong & al. 2021a). In *Uvarieae*, Friesodielsia and three related genera: Dasymaschalon (Hook. f. & Thomson) Dalla Torre & Harms, Desmos Lour. and Monanthotaxis are clustered in a strongly supported clade; Monanthotaxis is then a sister group of a strongly supported clade composed of the other three genera (e.g. Guo & al. 2017a, 2017b). In Thailand, 18 species of Friesodielsia were reported (Leeratiwong & al. 2023), but based on preliminary observations on recently collected specimens, several undescribed species seem to exist. In this study we determine the taxonomic status of an unidentifiable gathering from Narathiwat, one of the southernmost and inadequately explored prov-

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inces of Thailand by morphological investigations and comparisons in combination with molecular phylogenetic analyses.

#### Material and methods

#### Phylogenetic reconstructions

The ingroup was composed of 24 accessions, divided into four genera: Dasymaschalon (four accessions representing four species), Desmos (five accessions representing five species), Friesodielsia (13 accessions representing 12 species plus an unidentifiable accession from Narathiwat, S Thailand [= Friesodielsia sp. TH]) and Monanthotaxis (two accessions representing two species). Outgroups consisted of two species in the tribe Uvarieae: Pyramidanthe elegans (Hook. f. & Thomson) Bangk. & Chaowasku and Uvaria dasoclema L. L. Zhou & al. The information of voucher specimens and GenBank accession numbers used in this study is shown in Appendix 1. Up to six plastome regions (psbA-trnH, trnL-trnF intergenic spacers; trnL intron; matK, ndhF, rbcL exons) were included. The methods for DNA extraction, amplification and sequencing used in the present study, including primer information, followed Chaowasku & al. (2018a, 2018b, 2020). Sequences were edited using the Staden package (Staden & al. 2000) and the data matrix was aligned by Multiple Sequence Comparison by Log-Expectation (MUSCLE; Edgar 2004) in MEGA7 (with default settings; Kumar & al. 2016). The aligned data matrix was subsequently manually checked and realigned where necessary using the similarity criterion (Simmons 2004). In total, there were 5496 nucleotide plus six binary-coded indel characters. The simple method for indel coding of Simmons & Ochoterena (2000) was used, with the emphasis on nonautapomorphic and less homoplastic indel structures.

Parsimony analysis was performed in TNT version 1.5 (Goloboff & Catalano 2016). All characters were equally weighted and unordered. The setting concerning collapsing rules was set to "max. length = 0". Incongruence among plastid DNA regions was evaluated by analysing each region individually to detect if there was any significant topological conflict (e.g. Wiens 1998). Most parsimonious trees were generated by a heuristic search of the combined data, with 9000 replicates of random sequence addition, saving 10 trees per replicate and using the tree bisection and reconnection (TBR) branch-swapping algorithm. Clade support was measured by symmetric resampling (SR; Goloboff & al. 2003), with default change probability (P=33). Two hundred thousand replicates were run, each with four replicates of random sequence addition, saving four trees per replicate. A clade with SR  $\geq$  85%, 70–84% or 50–69% was considered strongly, moderately or weakly supported, respectively.

Maximum likelihood analysis was carried out in IQ-TREE version 2.1.2 (Minh & al. 2020) under partition

models (Chernomor & al. 2016) employed with the "-p" command, whereas Bayesian Markov chain Monte Carlo (MCMC; Yang & Rannala 1997) phylogenetic analysis was conducted in MrBayes version 3.2.7a (Ronquist & al. 2012). Both methods of phylogenetic reconstruction were analysed via the CIPRES Science Gateway version 3.3 (Miller & al. 2010). The aligned data matrix was divided into five partitions based on DNA-region identity (the trnL intron and adjacent trnL-trnF intergenic spacer were combined into a single partition). The most suitable model of sequence evolution for each DNA partition was selected using the Akaike Information Criterion (AIC; Akaike 1974) scores calculated in jModelTest version 2.1.10 (Darriba & al. 2012), with the following selections: +F, +G (nCat 4), ML optimized (base tree for likelihood calculations) and Best (base tree search). The General Time Reversible (GTR; Tavaré 1986) substitution model with a gamma distribution for among-site rate variation was selected for matK and ndhF partitions, whereas the Hasegawa-Kishino-Yano (HKY; Hasegawa & al. 1985) substitution model with a gamma distribution for amongsite rate variation was chosen for the remaining partitions (psbA-trnH, rbcL and trnL-trnF [= trnL intron + trnL-trnF intergenic spacer]). In the maximum likelihood analysis, the model "JC2+FQ+ASC" was selected using the corrected AIC scores for the binary indel partition. Clade support was assessed by a non-parametric bootstrap resampling (BS; Felsenstein 1985) with 2000 replicates. A clade with BS  $\geq$  85%, 70–84% or 50–69% was considered strongly, moderately or weakly supported, respectively. In the Bayesian analysis, the "coding=variable" setting was assigned to the binary indel partition, which was implemented under a simple F81-like model without a gamma distribution for among-site rate variation. Four independent runs, each with four MCMC chains, were simultaneously performed; each run was set for 10 million generations. The default prior settings were used except for the prior parameter of rate multiplier ("ratepr" [=variable]). The temperature parameter was set to 0.08. Trees and all parameter values were sampled every 1000th generation. Convergence was assessed by checking the standard deviation of split frequencies of the runs with values < 0.01interpreted as indicative of a good convergence and by checking for adequate effective sample sizes (ESS > 200) using Tracer version 1.7.1 (Rambaut & al. 2018). The first 25% of all trees sampled were removed as burn-in and the 50% majority-rule consensus tree was produced from the remaining trees. A clade with posterior probabilities (PP)  $\geq$  0.95, 0.9–0.94 or 0.5–0.89 was considered strongly supported, weakly supported or unsupported, respectively.

#### Morphology

The morphological information of relevant *Friesodiel-sia* species was derived from literature (Sinclair 1955; Leeratiwong & al. 2021a; Johnson & al. 2022) as well as

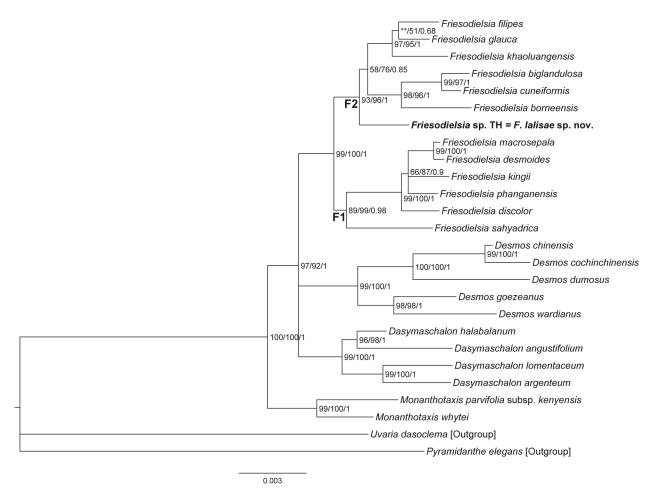


Fig. 1. Phylogram derived from Bayesian inference. Parsimony symmetric resampling values (SR), maximum likelihood bootstrap values (BS) and Bayesian posterior probabilities (PP) are indicated: SR/BS/PP. \*\* = SR < 50%. Scale bar unit = substitutions per site.

their type specimens at the herbaria K, L and SING (herbarium codes according to Thiers 2022+). Some floral organs of the unidentifiable gathering from Narathiwat, S Thailand (*Friesodielsia* sp. TH) were measured from spirit material (in square brackets in the description of the new species below). The indumentum terminology used followed Hewson (1988).

#### **Results and Discussion**

The parsimony analysis resulted in 18 most parsimonious trees with 689 steps. The consistency and retention indices (CI and RI) were 0.84 and 0.8, respectively. There was no strong incongruence (SR ≥ 85%) among the analysis of each plastome region. The ingroup, comprising four genera: *Dasymaschalon*, *Desmos*, *Friesodielsia* and *Monanthotaxis*, received maximal support (Fig. 1). Accessions of *Monanthotaxis* formed a strongly supported (SR 99%, BS 100%, PP 1) clade sister to a larger strongly supported (SR 97%, BS 92%, PP 1) clade embracing the remaining accessions. In the latter clade, there was a polytomy consisting of three genera (*Dasymaschalon*, *Desmos* and *Friesodielsia*), each receiving strong support (SR 99%, BS 100%, PP

1). In *Friesodielsia*, two major clades were retrieved, each with strong support (Fig. 1): clades F1 (SR 89%, BS 99%, PP 0.98) and F2 (SR 93%, BS 96%, PP 1). In clade F1, *F. sahyadrica* N. V. Page & Survesw. was sister to a strongly supported (SR 99%, BS 100%, PP 1) clade composed of the remaining accessions in this clade. In clade F2, *Friesodielsia* sp. TH was recovered as a sister group of an unsupported to moderately supported (SR 58%, BS 76%, PP 0.85) clade consisting of the rest of this clade.

It is noteworthy that the two major clades of *Frieso-dielsia* correspond well with their morphology, i.e. the outer petals of members of clade F1, including the recently described *F. macrosepala* Leerat. & Aongyong and *F. phanganensis* Leerat., separate early during developmental stages and have a flat base, whereas those of members of clade F2, including *Friesodielsia* sp. TH and the recently described *F. khaoluangensis* Leerat. & Aongyong, separate at anthesis and have a concave basal portion, appearing as an excavation (Fig. 2B [left]; Guo & al. 2017a; Leeratiwong & al. 2021a). Upon morphological comparisons, *F. argentea* (J. Sinclair) Steenis native to Peninsular Malaysia and *F. glauca* (Hook. f. & Thomson) Steenis native to S Thailand and Peninsular Malaysia are most morphologically similar to *Friesodielsia* sp. TH; the three can

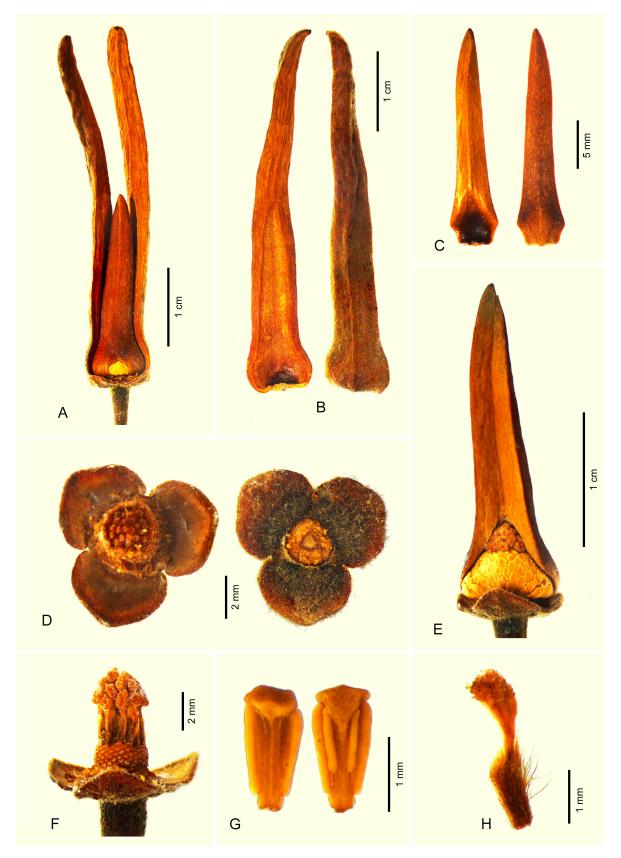


Fig. 2. Flower and floral organs of *Friesodielsia lalisae* – A: flower with one outer petal removed showing connivent inner petals; B: adaxial (left) and abaxial (right) sides of outer petal; C: adaxial (left) and abaxial (right) sides of inner petal; D: flower with petals, stamens and carpels removed showing adaxial (left) and abaxial (right) sides of sepals; E: flower with outer petals and one inner petal removed showing stamens surrounding carpels; F: flower with petals and stamens removed showing carpels and torus (side view); G: stamen, adaxial (left) and abaxial (right) sides; H: carpel. – All from *Aongyong & Baka 57* (CMUB – spirit material).



Fig. 3. Holotype of  $Friesodielsia\ lalisae$ :  $Aongyong\ \&\ Baka\ 57\ (CMUB)$ .

Feature	F. lalisae	F. argentea	F. glauca
Young twigs	densely hairy	densely hairy	glabrous to sparsely hairy
Leaf base	obtuse to rounded	usually cuneate	obtuse to rounded
Pedicel length (mm, in flower)	9–10	c. 10	(14-)20-28
Sepal shape	transversely ovate	triangular	triangular
Proportion of inner petal to outer petal length	C. ½	c. ½3	c. <sup>1</sup> / <sub>3</sub>
Inner petal length (mm)	19 [23–25]	6–10	5–10

Table 1. Morphological comparisons between *Friesodielsia lalisae*, *F. argentea* and *F. glauca*. Square brackets indicate measurements from spirit material.

be distinguished by several features as shown in Table 1. It should be noted that, following Leeratiwong & al. (2021a) and Johnson & al. (2022), we consider the morphological features of F. glauca as based on material that only corresponds to the type of Oxymitra glauca Hook. f. & Thomson (the basionym of F. glauca), not the type of its several heterotypic synonyms, including O. argentea J. Sinclair (the basionym of *F. argentea*) (see Turner 2018). Based on morphological differences between F. argentea and F. glauca as indicated in Table 1, we believe the former should be regarded as distinct from F. glauca. Because F. argentea, F. glauca and two other species closely related to F. glauca: F. filipes (Hook. f. & Thomson) Steenis and F. khaoluangensis (Fig. 1) all have rather similar petal traits, i.e. proportion of inner petal to outer petal length (c. 1/3 or lower) and inner petal length (not exceeding 10 mm) (Table 1; Leeratiwong & al. 2021a; Johnson & al. 2022), it is hypothesized that F. argentea is also phylogenetically allied to F. glauca, which is distantly related to Friesodielsia sp. TH (Fig. 1). On the basis of these findings, Friesodielsia sp. TH deserves recognition as a new species, which is described below. It is worthwhile to note that Narathiwat constitutes one of the most insufficiently explored areas in Thailand as deduced from several species of Annonaceae described based on recently collected material (e.g. Jongsook & al. 2020; Bunchalee & al. 2021; Leeratiwong & al. 2021b; Wiya & al. 2021).

*Friesodielsia lalisae* Damth., Baka & Chaowasku, **sp. nov.** – Fig. 2–4.

Holotype: Thailand, Narathiwat Province, Chanae District, May 2022 [in flower], *Aongyong & Baka 57* (CMUB [barcode CMUB003997901]; isotypes: B, CMUB, QBG).

Diagnosis — Friesodielsia lalisae is most morphologically similar to F. argentea and F. glauca. The new species differs from these two species by having a different sepal shape, longer inner petals and a higher proportion of inner petal to outer petal length. Furthermore, F. lalisae differs from F. argentea by having an obtuse to rounded (vs usually cuneate) leaf base and from F. glauca by having denser indumentum on young twigs and shorter flowering pedicels.

Description (square brackets indicate measurements from spirit material) — Woody climbers; young twigs tomentose with erect and appressed hairs. Petiole 3-5 mm long, tomentose with erect and appressed hairs, slightly grooved above; leaf blade chartaceous, 7.7-16.2 x 2.8–5.8 cm, elliptic to elliptic-obovate, seldom obovate, puberulous-tomentose with erect and appressed hairs above, puberulous-tomentose with erect hairs below, base obtuse to rounded, apex ± cuspidate, acute to acute-acuminate, rarely obtuse or rounded; midrib slightly sunken above, tomentose with mostly erect hairs, raised below, puberulous-tomentose with erect and appressed hairs; secondary veins prominent below, 12-15 per side, angle with midrib 37°-46° (at middle part of leaf blade). Flowers solitary, terminal developing to internodal, fragrant in vivo; pedicel 9–10 mm long, curly-tomentose, bearing 1 bract near pedicel midpoint (but a bit lower), ovate-triangular. Sepals free,  $[3-3.1 \times 4.5-4.6]$  mm, transversely ovate, without visible veins on both sides, outside curlytomentose on basal half, more sparsely so on apical half, margin curly-tomentose, inside tomentose with appressed hairs only near margin, remaining area glabrous. Petals  $\pm$  yellow in vivo; outer petals 34 [46–47]  $\times$  6 [8] mm, narrowly ovate-triangular, outside puberulous-tomentose with mostly appressed hairs, margin tomentose with appressed hairs, inside glabrous, each outer petal with an excavation on  $\pm$  basal half, apex of outer petals  $\pm$  acute; inner petals 19  $[23-25] \times [5-5.5]$  mm, narrowly ovate, c. ½ as long as outer petals, outside puberulous with appressed hairs only along bilateral midline, remaining area glabrous, margin and inside glabrous, apex acute. Torus depressed subglobose, villous intermixed with tomentose (both with erect hairs) on area surrounding each carpel. Stamens c. 132 per flower, [1.5-2.1] mm long, connective apex ± truncate or with a slanted prolongation, covering thecae. Carpels c. 22 per flower, [2.6–3.8] mm long; stigmas ± elongated and irregular-shaped; ovaries villous with mostly appressed hairs; ovule 1 per ovary, basal. Fruit unknown.

Phenology and ecology — Flowering material was collected in May. The species appears to grow near streams in secondary forests adjacent to rubber-tree plantations at an elevation of c. 90 m.



Fig. 4. *Friesodielsia lalisae* – A: flower bud; B: flower at anthesis; both from *Aongyong & Baka 57* (CMUB). – Photographs taken at type locality: Thailand, Narathiwat Province, Chanae District, May 2022, by A. Baka.

Distribution — Endemic to Narathiwat, S Thailand.

Preliminary conservation assessment — So far, Frieso-dielsia lalisae is only known to occur in secondary forests adjacent to rubber-tree plantations. Its habitat is highly threatened by agricultural activities. Only two individuals in a single location were observed, one of which has been cut recently. The AOO (area of occupancy) based on this single location is estimated to be less than 10 km². Although more exploratory data seem crucial, we believe the category Critically Endangered: CR B2ab(iii) based on IUCN Standards and Petitions Committee (2022) is appropriate for now and any conservation effort should be immediately initiated.

Etymology — The new species is named in honour of Lalisa Manobal, a famous Thai rapper, singer and dancer, whose motivation has greatly inspired the first author to overcome any obstacles during her Ph.D. study.

# **Author contributions**

T.C. conceived and coordinated the study, obtained the research grant and performed molecular phylogenetic analyses; A.D. performed morphological investigations and comparisons, as well as DNA amplification; N.K. performed DNA amplification; S.K., C.S., C.W. and P.U. assisted with morphological investigations and comparisons, as well as with manuscript preparations; A.B. and K.A. provided crucial plant specimens; all authors drafted every version of the manuscript.

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### **Supplemental content online**

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Original alignments and alignments for phylogenetic analyses in FASTA format.

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Appendix 1. Specimens for molecular phylogenetic analyses and their GenBank accession numbers. Unavailable sequences are denoted with —, whereas newly generated sequences are denoted with \*\*.

Taxon	Location	Collector and number (herbarium)	matK	ndhF	psbA-trnH	rbcL	trnL-trnF
Dasymaschalon angustifolium Jing Wang & R. M. K. Saunders	Thailand	Chaowasku 145 (CMUB)	MT264029	MT263997	MT264005	MT264013	MT264021
Dasymaschalon argenteum (Griff.) Jongsook & Chaowasku	Thailand	Aongyong 14 (CMUB)	MT264030	MT263998	MT264006	MT264014	MT264022
Dasymaschalon halabalanum Jongsook & Chaowasku	Thailand	Chaowasku 180 (CMUB)	MT264033	MT264001	MT264009	MT264017	MT264025
Dasymaschalon lomentaceum Finet & Gagnep.	Thailand	Chaowasku 182 (CMUB)	MT264034	MT264002	MT264010	MT264018	MT264026
Desmos chinensis Lour.	Hong Kong	Pang N2 (HKU)	JQ768567	JQ768603	JQ768646	JQ768687	JQ768727
Desmos cochinchinensis Lour.	China	Wang 0612 (HKU)	JQ768568	JQ768604	JQ768647	JQ768688	JQ768728
Desmos dumosus (Roxb.) Saff.	China	Wang 068 (HKU)	JQ768570	JQ768606	JQ768649	JQ768689	JQ768730
Desmos goezeanus (F. Muell.) Jessup	Australia	Ford & Cinelli 04780 (BRI)	JQ768572	JQ768607	JQ768651	JQ768691	JQ768732
Desmos wardianus (F. M. Bailey) Jessup	Australia	Sankowsky 2664 (BRI)	JQ768574	30768608	JQ768653	JQ768693	JQ768734
Friesodielsia biglandulosa (Blume) Steenis	Indonesia	Slik 3809 (L)	KX786592	JQ768610	JQ768655		JQ768736
Friesodielsia borneensis (Miq.) Steenis	Indonesia	Kessler 2018 (A)	KX786593	KX786608	KX786616		KX786633
Friesodielsia cuneiformis (Blume) Steenis	cultivated (Bogor Bot. Gard.)	Ardi 54 (HKU)	JQ768576	JQ768611	I	JQ768695	JQ768737
Friesodielsia desmoides (Craib) Steenis	cultivated (Queen Sirikit Bot. Gard.)	Khunarak I (CMUB)	0Q505965**	0Q505974**	OQ505965** OQ505974** OQ505983**	0Q505992**	0Q506001**
Friesodielsia discolor (Craib) D. Das	Thailand	Aongyong & Roopkom 48 (CMUB)	0Q505964**	0Q505973**	0Q505982**	0Q505991**	00506000**
Friesodielsia filipes (Hook. f. & Thomson) Steenis	Thailand	Aongyong & Baka 46 (CMUB)	0Q505966**	0Q505975**	OQ505966** OQ505975** OQ505984**	0Q505993**	0Q506002**
Friesodielsia glauca (Hook. f. & Thomson) Steenis	Thailand	Aongyong & Samae 49 (CMUB)	00505968**	0Q505977**	0Q505986**	0Q505995**	0Q506004**
Friesodielsia khaoluangensis Leerat. & Aongyong	Thailand	Aongyong 20 (CMUB)	0Q505961**	0Q505970**	0Q505979**	0Q505988**	0Q505997**
Friesodielsia kingii (J. Sinclair) Steenis	Thailand	Aongyong & Sama-ae 43 (CMUB)	0Q505963**	0Q505972**	0Q505981**	00505990**	0Q505999**

Тахоп	Location	Collector and number (herbarium)	matK	ndhF	psbA-trnH	rbcL	trnL-trnF
Friesodielsia lalisae Damth., Baka & Chaowasku	Thailand	Aongyong & Baka 57 (CMUB)	0Q505969**	0Q505978**	0Q505987**	OQ505969** OQ505978** OQ505987** OQ505996**	00506005**
Friesodielsia macrosepala Leerat. & Aongyong	Thailand	Aongyong 23 (CMUB)	0Q505962**	0Q505971**	00505980**	OQ505962** OQ505971** OQ505980** OQ505989**	00505998**
Friesodielsia phanganensis Leerat.	Thailand	Sinbumroong 09082021 (CMUB)	0Q505967**	0Q505976**	OQ505967** OQ505976** OQ505985** OQ505994**	0Q505994**	0Q506003**
Friesodielsia sahyadrica N. V. Page & Survesw.	India	Page 110949 (CAL)	KC933936	KC933942	KC933940	KC933934	KC933938
Monanthotaxis parvifolia subsp. kenyensis Verdc.	Kenya	Luke 7299 (EA)	KX761312	KX787032	KX786971	KX761343	KX787001
Monanthotaxis whytei (Stapf) Verdc.	cultivated (Utrecht Univ. Bot. Gard.)	Chatrou 475 (U)	EF179278	EF179304	EF179315	AY841635	AY841713
Pyramidanthe elegans (Hook. f. & Thomson) Bangk. & Chaowasku	Thailand	Aongyong & Baka 37 (CMUB)	OL546479	OL546490	OL546506	OL546522	OL546538
Uvaria dasoclema L. L. Zhou & al.	Thailand	Damthongdee AD I (BKF)	OL546465	OL546481	OL546492	OL546508	OL546524